DISSERTATION

Investigations on Marine Natural Products from Indo-Pacific Nudibranchia (Mollusca: Gastropoda): Chemoecology, Medicinal Potential & Toxin Resistance

CORA HERTZER

2022

Investigations on Marine Natural Products from Indo-Pacific Nudibranchia (Mollusca: Gastropoda): Chemoecology, Medicinal Potential & Toxin Resistance

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Cora Hertzer

aus

Berlin-Lichtenberg

Bonn, 2022

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

Diese Arbeit wurde am Institut für Pharmazeutische Biologie und in Zusammenarbeit mit dem Zoologischen Forschungsmuseum Alexander König in Bonn durchgeführt.

1. Gutachterin: Prof. Dr. Gabriele M. König
   Institut für Pharmazeutische Biologie

2. Gutachterin: Prof. Dr. Heike Wägele
   Zoologisches Forschungsmuseum Alexander König

Tag der Promotion: 11.03.2022
Erscheinungsjahr: 2022

Einige Teile dieser Arbeit wurden bereits in ähnlicher Form als wissenschaftliche Fachartikel veröffentlicht oder sind für eine zukünftige Veröffentlichung vorgesehen. Entsprechende Texte und Abbildungen wurden als solche gekennzeichnet und zitiert.

KAPITEL I: „Investigations of the natural products and chemical ecology of *Phyllodesmium longicirrum* (Nudibranchia: Aeolidioidea)“ war Teil meiner Masterarbeit, die unter der Betreuung von Prof. Gabriele M. König und Prof. Heike Wägele durchgeführt und geschrieben wurde. KAPITEL I hat zu den folgenden zwei Publikationen beigetragen:[1,2]

1. Bogdanov A, Hertzer C, Kehraus S, Nietzer S, Rohde S, Schupp PJ, Wägele H, König GM. Defensive Diterpene from the Aeolidoidean Phyllodesmium longicirrum. *J Nat Prod*. 2016;79(3):611-615. doi:10.1021/acs.jnatprod.5b00860

2. Bogdanov A, Hertzer C, Kehraus S, Nietzer S, Rohde S, Schupp PJ, Wägele H, König GM. Secondary metabolome and its defensive role in the aeolidoidean Phyllodesmium longicirrum, (Gastropoda, Heterobranchia, Nudibranchia). *Beilstein J Org Chem*. 2017;13:502-519. doi:10.3762/bjoc.13.50

KAPITEL II: „Antibacterial scalarane from *Doriprismatica stellata* nudibranchs (Nudibranchia: Chromodorididae), egg ribbons, and their dietary sponge *Spongia* cf. *agaricina* (Demospongiae: Dictyoceratida) “ wurde in ähnlicher Form als folgender Fachartikel publiziert:[3]

3. Hertzer C, Kehraus S, Böhringer N, Kaligis F, Bara R, Erpenbeck D, Wörheide G, Schäberle TF, Wägele H, König GM. Antibacterial scalarane from Doriprismatica stellata nudibranchs (Gastropoda, Nudibranchia), egg ribbons, and their dietary sponge Spongia cf. agaricina (Demospongiae, Dictyoceratida). *Beilstein J Org Chem*. 2020;16(1):1596-1605. doi:10.3762/bjoc.16.132

Teile der folgenden Einleitung und KAPITEL III sind für eine zukünftige Veröffentlichung in einem Fachartikel vorgesehen.

Für meine Schwester.

CONTENTS

ABBREVIATIONS

$[\alpha]_D^{20}$	Specific optical rotation
δ	NMR chemical shift [ppm]
ε	Molar attenuation coefficient
λ_{max}	Wavelength at which the maximum fraction of light is absorbed
ν_{max}	Maximum frequency
1D	One dimensional
2D	Two dimensional
ATR	Attenuated Total Reflection
c	Concentration [mg/mL]
BLAST	Basic Local Alignment Search Tool
$CaCl_2$	Calcium chloride
$CHCl_3$	Chloroform
$CDCl_3$	Deuterated chloroform
COSY	Correlation spectroscopy
DAD	Diode array detector
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DEPT	Distortionless enhancement by polarization transfer
ESI	Electrospray ionization
EtOAc	Ethyl acetate
EtOH	Ethanol
H_2O	Water
HESI	Heated electrospray ionization
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography

HPLC-MS	High performance liquid chromatography-mass spectrometry
HR-ESI-MS	High-resolution-electrospray ionization-mass spectrometry
HR-APCI-MS	High-resolution-atmospheric pressure chemical ionization-mass spectrometry
HSQC	Heteronuclear single quantum coherence
IR	Infrared
J	Coupling constant
KCl	Potassium chloride
LC-ESI-MS	Liquid chromatography-electrospray ionization- mass spectrometry
m/z	Mass-to-charge ratio
$[M+H]^+$	Protonated molecular ion
MALDI MSI	Matrix-assisted laser desorption/ionization mass spectrometry imaging
MDF	Mantle dermal formation
MeOH	Methanol
MeOD/MeOH-d_4	Tetradeuteromethanol
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
MNPs	Marine natural products
MS	Mass spectrometry
NaCl	Sodium chloride
NaHCO3	Sodium bicarbonate
NCBI	National Center for Biotechnology Information
NH_4Ac	Ammonium acetate
NMR	Nuclear magnetic resonance
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser enhancement spectroscopy
NP	Natural product

OD	Optical density
PCR	Polymerase chain reaction
pH	Potential/power of hydrogen
ppm	Parts per million
ROE	Rotating-frame nuclear overhauser effect
ROESY	ROE correlation spectroscopy
RP	Reversed phase
sp.	Specimens
UV/VIS	Ultraviolet-visible
UHPLC	Ultra high-performance liquid chromatography
UPLC-HR-MS	Ultra-performance liquid chromatography-high-resolution mass spectrometry
VLC	Vacuum liquid chromatography
WoRMS	World Register of Marine Species
^{13}C NMR:	s = qC; d =CH, t = CH_2, q = CH_3
^{1}H NMR:	s = Singulet, d = Doublet, dd = Doublet of doublets, t = Triplet, m = Multiplet, br = Broad signal, ^{b} = Overlapping signals

GENERAL INTRODUCTION

Marine Natural Products

The interdisciplinary project 'INDOBIO Indonesian Opisthobranchs and associated microorganisms – From Biodiversity to drug lead discovery', funded by the German Federal Ministry of Education and Research (BMBF), had the aim to document the state of the art biodiversity in North Sulawesi, Indonesia,[4–7] with an emphasis on heterobranch sea slugs, their food sources, associated microorganisms and their potential for drug lead discovery.[3,8–10] This dissertation is a part of the INDOBIO project and focuses on the marine natural products (MNPs) from nudibranchs of the Central Indo-Pacific Ocean.

Marine habitats cover more than 70% of the Earth, provide vital ecosystem services,[11–15] and are home to a multitude of organisms capable of the production of invaluable MNPs.[16–22] In habitats with exceptional biodiversity, such as the coral reefs of the Indo-Pacific Ocean,[23] intense competition and feeding pressure lead to a vast chemical diversity and a variety of bioactive structures, ranging from small molecules to large and complex proteins.[24] Bioactive MNPs are of high interest for pharmacological applications, as they can become important lead structures in the drug development process.[8,9,16,22,25–40] These metabolites may provide evolutionary advantages and are found particularly in exposed, sessile, and slow-moving organisms, like invertebrates, that use them to adapt to abiotic and biotic factors of their complex environments and ecological niches.[18,29,41,42] Due to their diversity in structure and function, they play key roles as semiochemicals for inter- and intra-specific communication, reproduction and development, in the competition for space, as venom to capture prey, or as a defence against predators, pathogens and overgrowth by fouling organisms.[42–59] Furthermore, prophylactic or therapeutic self-medication behaviour (zoopharmacognosy) has been observed, for example in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), which regularly rub on gorgonians (*Rumphella* sp.), that are covered in mucus with bioactive, antimicrobial, antioxidant and anti-inflammatory MNPs.[60–63] Similar to other invertebrates, marine gastropods present multiple fascinating and advantageous molecules.

Gastropods: Mucus, Natural Products & Medicinal Potential

All organisms from all kingdoms of life produce mucus, which can have a wide range of functions. It is often an essential barrier against biotic and abiotic factors of the surrounding environment and a first line of defence against pathogenic organisms and predators.[64,65]

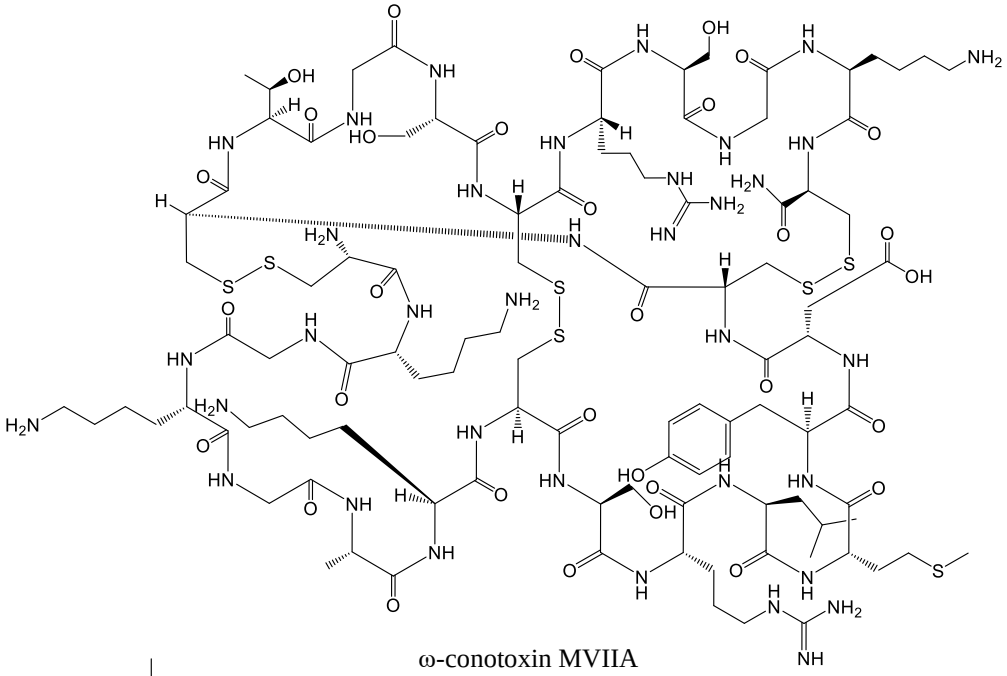
In gastropods, mucus composition varies according to species, its role and formulation, which can be adjusted to serve multiple functions and needs. Typically, the mucus consists of around 90-99% water, mucopolysaccharides, a mucin-like glycoprotein complex, antimicrobial peptides, metal ions and may contain further bioactive small molecules. Epithelial goblet cells in mucus glands and the pedal gland secrete the mucus with lubricant and adhesive properties. It is produced for locomotion, reproduction, adhesion to any type of surface, and to minimize body desiccation, the effects of physical damage or harmful substances.[66–75]

For centuries, terrestrial gastropods and their mucus have been used in folkloric and traditional medicine.[76–78] More recently, anticancer, antioxidant, anti-inflammatory, antimicrobial, and skin-regenerating properties of slug mucus have been studied and confirmed.[66–69,78–80] Furthermore, gastropod mucus continues to inspire the development of medicinal adhesives and further biomimetic fibers.[70,71,81,82]

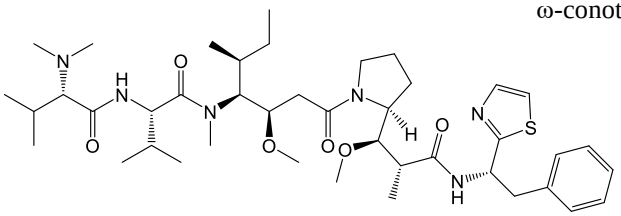
Marine gastropods have equally great medicinal potential as their terrestrial counterparts, but are lesser known from ethnomedical reports, due to the difficulty of collecting marine organisms.[16,83] Nonetheless, with increasing interest in the marine environment and improved technological capabilities allowing for better accessibility, several drugs based on MNPs isolated from marine gastropods have been developed. For example: the first "directly from the sea" drug PRIALT®, a treatment for severe pain derived from a small peptide ω-conotoxin MVIIA venom (ziconotide, Figure 1.1) produced by the fish hunting snail *Conus magus*,[84,85] the anticancer drug Adcetris®,[86,87] which is based on the cytotoxic peptide dolastatin 10 (Figure 1.1) isolated from *Dolabella auricularia*,[88] and the anti-tumour depsipeptide kahalalide F (Figure 1.1), which was isolated from *Elysia* species.[89–92]

Some heterobranch gastropods are capable of *de novo* biosynthesis. For example, the biosynthesis of polyproprionates by sacoglossans and biosynthesis of terpenoids by nudibranchs have liberated some of these slugs from dependance on a particular food source.[45,93–98] However, more frequently they sequester, accumulate and store, with or without biotransformation, MNPs produced by their preferred food sources (mainly algae, Porifera, Bryozoa, Tunicata and Cnidaria), or their symbiotic microorganisms.[45,57,95,99–104] For example, dolastatin 10 is produced by cyanobacteria of the genus *Symploca* [105] and kahalalides are produced by the bacterium "*Candidatus* Endobryopsis kahalalidefaciens" as part of a complex tripartite marine symbiosis between the sacoglossan sea slug *Elysia rufescence*, the algae *Bryopsis* sp. and their bacterial symbiont.[106]

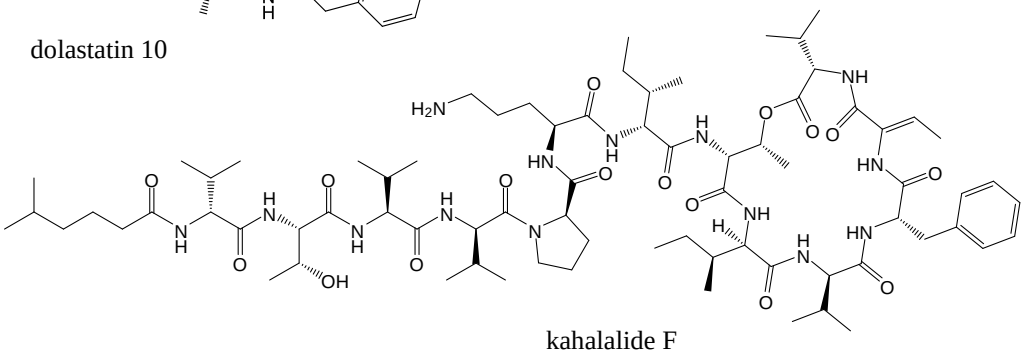
Hence, MNPs are not only pharmacologically interesting, but can also reveal important ecological and coevolutionary relationships and mechanisms.



ω-conotoxin MVIIA



dolastatin 10



kahalalide F

Conus magus Linnaeus, 1758

Dolabella auricularia Lightfoot, 1786

Elysia rufescens Pease, 1871

Figure 1.1 Bioactive natural products from marine gastropods used as drug lead structures. Pictures of *C. magus* (© Ashton Williams), *D. auricularia* (© ivansls) and *E. rufescens* (© uwkwaj) were taken from @iNaturalist.org.

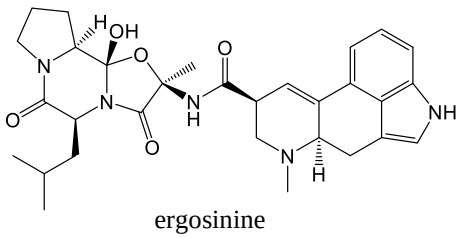
Heterobranchia: Taxonomy, Key Characters & Survival Strategies

Heterobranchia is a large, highly diverse and important group of gastropods with ~ 44 000 described species, that show the greatest morphological and ecological disparity among all gastropod clades. This group currently comprises two infraclasses: the so-called 'lower Heterobranchia' and Euthyneura, including a variety of snails, slugs, false limpets, and even bivalved gastropods with clam-like shells.[107,108] Heterobranchs have evolved to colonize virtually all habitats, from marine, to freshwater, to terrestrial areas, due to numerous adaptations.[108,109] For more than a century, heterobranch gastropods were divided into the para- and polyphyletic subtaxa "Opisthobranchia" (sea slugs and related snails) and "Pulmonata" (aquatic or terrestrial slugs and snails), that reflected researchers' preferences for a particular fauna or habitat, rather than monophyly.[101,110,111] Therefore, these approaches were abandoned and merged into the concept of Heterobranchia, as originally established by Burmeister in 1837 and revived by Haszprunar in 1985.[112] Since then, much research has been dedicated to resolving phylogenetic relationships among the taxon and to support or reject previous considerations of monophyly, as for example in works on Nudibranchia,[113–120] Sacoglossa,[121] Cephalaspidea,[122] Aplysiidae,[123] Stylommatophora,[124] Pteropoda,[125] and Runcinida.[126] In recent years, many of these taxonomic groupings have undergone systematic rearrangements and are continuously rearranged, based on the advancements in molecular techniques and computational analyses.[111,117,118,127–129] However, the phylogeny of the major heterobranch clade, Euthyneura, still remains partially unresolved and heavily discussed.[108,129–131] Currently, among Euthyneura two major clades are accepted: Tectipleura (Euopisthobranchia + Panpulmonata), and Ringipleura [Ringiculoidea + Nudipleura (Pleurobranchida + Nudibranchia)].[129,130] A coherent understanding of the evolutionary relationships between this group of molluscs and the factors that drive evolution between their clades is not only relevant, but a necessity for further scientific considerations.

Several heterobranchs serve as model organisms and lay the foundation for important research, such as the investigation on chloroplast incorporation (kleptoplasty),[132–135] chemical interactions and defence mechanisms by incorporation of MNPs (kleptochemistry),[24,104] neurobiological and behavioural studies,[136,137] ecotoxicology,[138] ecosystem wellbeing and climate change,[139] host-parasite interactions,[140] and pharmaceutical research.[26,35,40,141] Members of the Nudipleura clade can be described as marine gastropods, that have undergone partial and in some cases complete detorsion of the visceral mass and display some of the most spectacular and diverse body forms, patterns and colors found in nature.[101,109] Their intriguing phenotypes are the result of adaptations, coping with a reduced, internalized, or completely lost shell.[101,142]

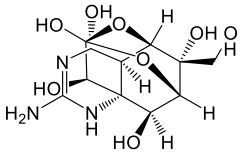
The consequence of shell reduction is certainly a loss of physical protection. However, it also enables the exploration and exploitation of new habitats and various food sources, leading to the evolution of unique key characters and survival strategies, which in turn increases radiation within these taxa.[101,111,143] Some of these key characters and survival strategies involve the sequestration, retention and maintenance of photosynthetic units. This can either be the sequestration of functional chloroplasts from siphonaceous algae, as done by the herbivorous taxon Sacoglossa,[133,135,144–147] or the ingestion and further mutualistic symbiosis with zooxanthellae of the genus *Symbiodinium*, mostly obtained from soft corals. The latter is described for the Cladobranchia, one of the two diverse suborders of Nudibranchia.[145,148–150] The incorporation of chloroplasts or zooxanthellae provides color camouflage, but more importantly, it enables the host to survive periods of food shortage, due to additional nutrients, gained as photosynthetic products from the symbionts.[133,135,145,151,152] One might assume that shell-less sea slugs are easy prey for predators like fish and decapods, especially in habitats characterized by intense feeding pressure, such as coral reefs. However, reports of their predation are scarce and have only recently been reported and summarized in the scientific literature.[44,56,153–159] The lack of a shell and this surprising immunity against predation is correlated with alternative defence mechanisms.[2,160,161] Besides mineral-based subepidermal spicules,[113,162–164] aposematic or cryptic coloration, and mimicry,[120,165–169] many sea slugs of the Nudipleura clade evolved the ability to steal, incorporate and utilize the defence system of their prey.[95,153,160,170,171] Although members of the order Pleurobranchida are known to defend themselves with acid secretions, produced by their epithelial cells and subepithelial glands of the epidermis,[172] some species have been found to additionally accumulate neurotoxic alkaloids, such as the ergot alkaloid ergosinine,[173] tetrodotoxin,[174–176] and paralytic shellfish toxins, like saxitoxin and its analogues (Figure 1.2).[177] Yet, the exact origins of these neurotoxins in pleurobranchids are unclear,[175] and it is unknown how these gastropods protect themselves from autotoxicity (i.e., self-intoxication).

Pleurobranchus forskalii Rüppell & Leuckart, 1828

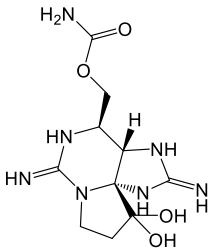


ergosinine

Pleurobranchaea maculata Quoy & Gaimard, 1832



tetrodotoxin (TTX)



saxitoxin (STX)

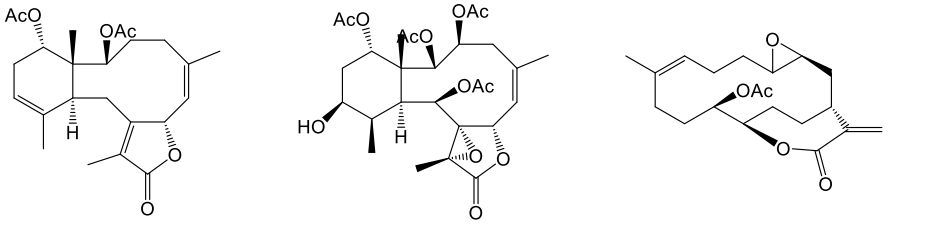
Figure 1.2 Neurotoxic alkaloids isolated from Pleurobranchida species. Pictures of *Pleurobranchus forskalii* (© Mike Krampf) and *Pleurobranchaea maculata* (© Simon Franicevic) were taken from @iNaturalist.org.

Alternative defence mechanisms have been hypothesized as the main driver for the diversification of Nudibranchia (~ 2500 species, as currently listed in WoRMS;[178] World Register of Marine Species).[101,111,142] Cladobranchia and Doridina, the two suborders of Nudibranchia, share ancestors that switched from algal food sources to feeding on sponges (Porifera). Sponges are repellent to most other predators due to mechanical (spicules) and chemical (metabolites) defences. The ancestors of Cladobranchia and Doridina nudibranchs were able to detoxify, sequester and use these metabolites; an ability, which is inherited by their progeny.[95,179] Nevertheless, within the different groups of Nudibranchia there have been multiple shifts to feed on different food sources.

The Cladobranchia (~ 1000 species, as listed in WoRMS) are a highly diverse group in which there has been a shift in prey preference, from feeding on Porifera to a variety of animal taxa, including Crustacea, Bryozoa, Hydrozoa, and Anthozoa (e.g. Hexacorallia & Octocorallia).[95,128,142,180] Traditionally, Cladobranchia consisted of three taxonomic divisions: Dendronotoidea (~ 250 species), Arminoidea (~ 100 species), and Aeolidioidea (~ 600 species). Yet, the evolutionary history and majority of phylogenetic relationships among Cladobranchia remain unclear, although recent studies have provided a solid foundation and framework for future work on their taxonomy.[118,128] Currently, cladobranchs are divided into the seven

superfamilies: Aeolidioidea, Arminoidea, Dendronotoidea, Doridoxoidea, Fionoidea, Proctonotoidea, and Tritonioidea.[181] The Aeolidioidea outnumber the other clades, which is most likely the result of an enhanced speciation and radiation, due to their ability to incorporate and utilize nematocysts (stinging capsules) from their cnidarian prey, primarily hydrozoans.[101,113,142,180,182] Although the sequestration and incorporation of nematocysts is unique to cladobranchs, especially aeolids, it needs to be noted that this ability originated at least twice within the Cladobranchia.[182] These "kleptocnides" are ingested along with the prey, pass through the digestive system into the terminal branches of the digestive glands (cerata), and are stored within the cnidosac, at the tip of the cerata, where they mature by acidification.[182–185] Cnidosacs may function as a storage and disposal organ, to protect the nudibranchs digestive system from possible harm; however, many studies support their function as an important additional defence mechanism.[168,184–188]

Less is known about chemical defences in the Aeolidioidea.[142] Almost all members of the genus *Phyllodesmium* Ehrenberg, 1831 (Aeolidioidea: Myrrhinidae) have switched and specialized from feeding on Hydrozoa to feeding on Octocorallia, particularly Alcyonacea.[1,2,151,189] Chemical investigation of *P. briareum* and its prey organism *Briareum* sp. revealed cytotoxic briarane diterpenes, such as brianthein W and excavatolide C, and several cembrane diterpenes, such as the cytotoxic 11-episinulariolide acetate, were isolated from *P. magnum* (Figure 1.3).[189]



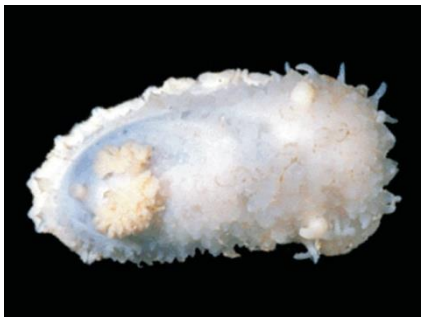
brianthein W excavatolide C 11-episinulariolide acetate

Figure 1.3 Selected cytotoxic metabolites from *Phyllodesmium* species.

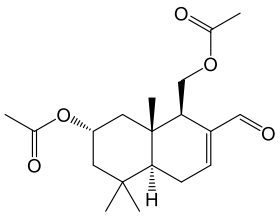
Most *Phyllodesmium* species do not store nematocysts, despite having cerata with cnidosacs.[113,152,189,190] This begs the question of whether they use a chemical defence strategy instead to protect themselves from predation. Further detailed exploration of this question is the aim of the following "Chapter I: Investigations of the natural products and chemical ecology of *Phyllodesmium longicirrum* (Nudibranchia: Aeolidioidea)".

Chemistry of Doridina

Development of a chemical defence was proposed to be a preadaption in the Nudipleura clade, enabling them to dispense their shell in the first place, leading to enhanced speciation and radiation, especially in the suborder Doridina.[160,191] The Doridina (~ 1500 species, currently listed in WoRMS) are by far the best-studied group of heterobranchs with regard to defensive chemical compounds. They consist of two infraorders, Bathydoridoidei and Doridoidei. Even the most basal species, such as the Antarctic *Bathydoris hodgsoni* and *Prodoris* (*Bathydoris*) *clavigera* are protected by bioactive chemical compounds, as for example by drimane sesquiterpenes, such as hodgsonal (Figure 1.4), from potential predators like the sea star *Odontaster validus* or the anemone *Epiactis* sp.[104,192,193]



Bathydoris hodgsoni Eliot, 1907



hodgsonal

Figure 1.4 Feeding deterrent sesquiterpene hodgsonal, isolated from the Antarctic nudibranch *Bathydoris hodgsoni*. The picture of *B. hodgsoni* was taken from @sealifebase.ca, © Wolf E. Arntz.[194]

While Bathydoridoidei includes only one family with two genera (Bathydorididae; *Bathydoris* and *Prodoris*), the highly diverse Doridoidei currently consist of 19 families, divided into the five superfamilies: Chromodoridoidea, Doridoidea, Onchidoridoidea, Phyllidioidea, and Polyceroidea. Over the years, there have been a number of excellent reviews about the chemistry of molluscs and nudibranchs,[9,95,104,160,195–203] and each year there are new bioactive MNPs isolated and reported in the scientific literature and annual review of marine natural products.[204] Listing all of them would be beyond the scope of this introduction. Nonetheless, the chemistry of specific related genera relevant to this thesis, such as *Doriprismatica* and *Chromodoris*, are investigated in "CHAPTER II: Antibacterial scalarane from *Doriprismatica stellata* nudibranchs (Nudibranchia: Chromodorididae), egg ribbons, and their dietary sponge *Spongia* cf. *agaricina* (Demospongiae: Dictyoceratida)" and "CHAPTER III: Protection from self-intoxication: A novel actin isoform in *Chromodoris* nudibranchs supports sequestration and storage of the cytotoxin latrunculin A", respectively.

Although chemotaxonomic approaches have proven useful, it must be noted that the chemistry of nudipleuran gastropods is strongly influenced by their respective food source and can vary inter- and intra-specifically depending on geographic location and available prey.[205,206] Many MNPs, that are sequestered by dorid nudibranchs, are terpenoids, especially oxygenated sesqui-, di-, and sesterterpenes. Terpenes and terpenoids belong to the largest class of natural products, with more than 80,000 naturally occurring compounds reported.[207–209] All terpenoids are built by successive addition of isoprene units (C_5), composed from the precursors: isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are either derived from the mevalonate or deoxyxylulose phosphate pathway.[207–211] Terpenes are simple hydrocarbons, whereas terpenoids are modified terpenes with additional functional groups. They are categorized according to the number of carbons and isoprene units: hemiterpene/isoprene (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}, e.g., steroids and saponins) and tetraterpenes (C_{40}, e.g., carotenoids). Terpenoids have significant biological activities, such as antimicrobial, anticancer, anti-inflammatory, antioxidant, antiallergic, antidiabetic, immunomodulatory, anti-insecticidal, and as skin permeation enhancer.[212–224] They are known to have anti-fouling properties and serve as allelopathic agents in the inter-specific competition for space.[225–227] Furthermore, they play important roles in communication, reproduction and defence.[225–228] Some examples of terpenoids from dorid nudibranchs are the spongian-type furanoterpenoids, diterpenoids, and sesquiterpenoids; and scalarane-type sesqui- and sesterterpenoids (Figure 1.5), which can be found in the genera *Cadlina*, *Chromodoris*, *Doriprismatica*, *Felimare*, *Felimida*, *Glossodoris* and *Goniobranchus*.[95,198,205,229–235] *Cadlina* and *Phyllidia* species may further contain nitrogenous terpenes, such as isocyanides and isothiocyanates,[179,236,237] and a *Phylidiella pustulosa* clade was found to contain rare dichloroimidic sesquiterpene derivatives (Figure 1.5).[10]

Further fascinating MNPs isolated from dorids are isoquinoline alkaloids and macrolides with antitumoral activity (Figure 1.6), such as jorumycin from *Jorunna funebris*, which led to the development of PM00104 (Zalypsis®),[238–241] or the trisoxazol macrolides kabiramides, halichondramides and ulapualides from *Hexabranchus sanguineus*,[43,242–244] sphinxolide from an unknown nudibranch,[245] and latrunculins, such as latrunculin A, from *Chromodoris* species.[246–248]

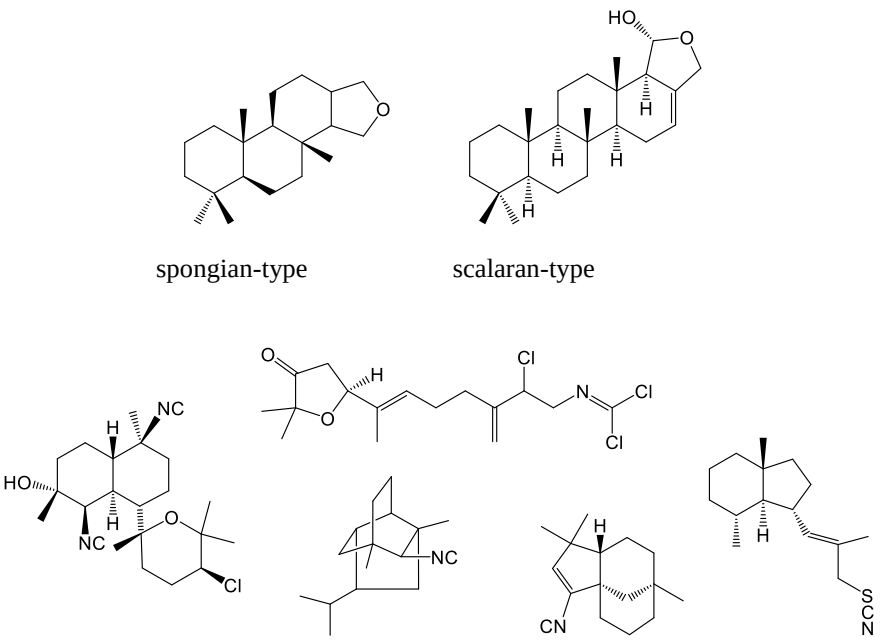


Figure 1.5 Examplary types of terpenoids found in dorid nudibranchs. Spongian- and scalaran-type terpenoids are frequent in the genera *Chromodoris*, *Doriprismatica*, *Felimare*, *Felimida*, *Glossodoris* and *Goniobranchus*, whereas *Cadlina*, *Phyllidia* and *Phylidiella* may also contain terpenoids with nitrogen and chloride functionalities, such as isocyanides, isothiocyanates and dichloroimidic sesquiterpenes.

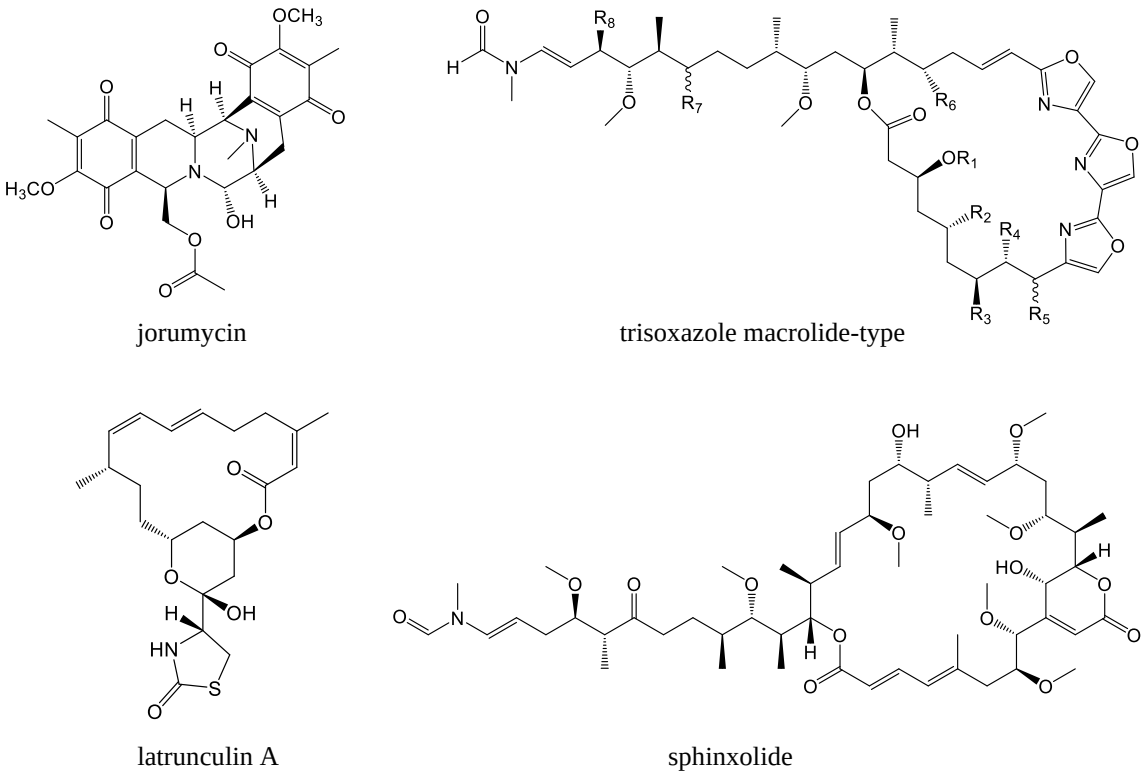


Figure 1.6 Examples of an alkaloid (jorumycin) and macrolides with antitumoral activity isolated from dorid nudibranchs.

Mantle Dermal Formations (MDFs) and Chemical Transformation

Nudibranchs of the Chromodorididae family store high concentrations of sequestered compounds in the heavily glandular, exposed mantle tissue, especially in specialized spherical storage glands in the subepithelial layer, which consist of cells with large, non-staining vacuoles: the so-called mantle dermal formations (MDFs).[99,101,113,249–252] These MDFs are highly concentrated chemical packages, deterring predators which might not have been deterred by lower concentrations of the metabolites uniformly distributed in the mantle.[253] Furthermore, MDFs are situated in the often brightly and aposematically colored mantle rim, drawing the predators' attention towards this well-defended area.[143,167,248,252] The evolution of defensive mantle glands and MDFs is considered a key innovation that has contributed to the extensive radiation and speciation of the Chromodorididae.[143] However, storage of secondary metabolites and a defensive role of MDFs might have evolved secondarily to their primary function as excretory or detoxification organs, expelling toxic substances and avoiding autotoxicity.[101,113,251,253,254] The secretion of toxic waste material by mantle glands or its accumulation in MDFs is an additional selective advantage against predation in combination with the prevention of autotoxicity. Hence, localization and storage of toxic compounds in MDFs can be considered a form of compartmentalization strategy in Chromodorididae. Chemical transformation is another common detoxification mechanism among marine heterobranch molluscs.[104,203] In Chromodorididae, this was investigated for *Felimare* and *Glossodoris* nudibranchs, where the biotransformation of dietary scalaranes and sesquiterpenes, such as scalaradial, into related molecules, in particular 6-keto or 12-keto derivatives, was demonstrated.[57,100,195,203,232,233,251,255,256] Injection of scalaradial into *G. pallida* proved to be non-toxic to the nudibranch and resulted in rapid conversion of this compound in less than 24 h. These biotransformed molecules are also further stored in the mantle tissue and are used as feeding deterrents against crabs and reef fish.[57]

Coevolution: Toxicity & Resistance

Predator-prey relationships between chemically protected organisms, like nudibranchs and their prey, are the result of a complicated evolutionary history that requires a tandem origin of toxin production or acquisition, unique genetic, physiological and morphological adaptations, and ecological transformations. Therefore, these antagonistic coevolutionary processes can have profound effects on macroevolutionary patterns and are considered to be main drivers of genotypic adaptations that can lead to the development of diverse resistance mechanisms.[257–260] Nonetheless, novel adaptations must originate and function within an already established

genome and the underlying mechanisms of resistance evolve under the influence of diversifying and purifying (i.e., stabilizing) selection in connection with increased toxin exposure.[261–271]

These coevolutionary processes and the evolution of toxin resistance have been investigated well in plant-herbivore interactions,[272–286] especially for cardiac glycosides from plants, bufonid toads and fireflies, and the widespread convergent evolution of resistant sodium-potassium-pumps in insects, amphibians, reptiles, and mammals.[274,278,281,284,287–295] Further examples can be found in the anthropogenic use of insecticides, herbicides, antimicrobials and resistances evolved by the targeted organisms,[265,296–312] as well as the evolution of self-resistant enzymes in microbial NP producers to prevent self-harm,[313] or grazers that evolved resistance to harmful algal blooms.[263,268,314–318] Among vertebrates, dendrobatid poison frogs and poisonous *Pitohui* and *Ifrita* birds are able to sequester and use alkaloids as a defensive 'toxin mantle'.[269,319–326] Grasshopper mice, that prey on bark scorpions, can even use the scorpion venom as an analgesic to their own advantage, as they have developed resistant voltage-gated sodium channels.[327] Opossums, that prey on vipers, have evolved a resistant hemostatic von Willebrand factor blood protein,[328,329] and various organisms, that coevolve with venomous snakes using α-neurotoxins, have convergently evolved resistant alpha-1 nicotinic acetylcholine receptors.[259,330–333] Furthermore, extensive research on the complex and diverse predator-prey interactions using the neurotoxins tetrodotoxin (TTX) and paralytic shellfish toxins (PSTs), revealed the widespread convergent evolution of resistant voltage-gated sodium channels as a resistance mechanism against TTX and PSTs.[50,159,177,265,267,271,302,334–357]

These studies, among many more, have taught us that there are a few general mechanisms, which enable these antagonistic coevolutionary processes of developing toxicity, resistance to these toxins, and the prevention of autotoxicity (i.e., self-intoxication). To survive and gain an advantage, organisms that produce or acquire toxic natural products must be resistant to the action of these substances.[258,271,313,358,359] For this purpose, seven major underlying strategies have established, which can be used alone or in synergy and in addition to behavioural aspects: (1) Compartmentalization, or (sub)cellular localization, where toxins are concentrated away from any vulnerable targets in vesicles, vacuoles, compartments or glands; (2) efflux pump transport proteins, like ATP-binding cassette (ABC) transporters, which are one of the most common mechanisms for the extrusion of toxic compounds; (3) production or storage of an inactive prodrug, which converts to the active defence only after excretion or uptake by a predator/competitor; (4) chemical transformations, that inactivate a toxic metabolite either by addition of a chemical entity or degradation; (5) toxin scavenging, where molecules, often proteins, are produced to bind to a toxin with a higher affinity than the target and inactivate it

in a ‘self-sacrifice’; (6) off-target repurposing, where another target is produced with enhanced binding affinities, but altering the physiological effect of the toxin in a beneficial way; (7) and target-site insensitivity modifications, where the gene sequence of a target is altered to inhibit toxin binding to that target.[258,264,271,358–361]

Several Heterobranchia, especially many dorid nudibranchs, acquire and use toxins from their prey. They must be able to protect themselves from autotoxicity to survive and gain an advantage. Even though a vast number of chemical investigations provide us with fascinating and invaluable MNPs, little is known about the underlying mechanisms that allow for the sequestration and accumulation of these toxins in nudibranchs. Further exploration of this topic and a possible resistance mechanism in *Chromodoris* nudibranchs are the aims of “CHAPTER III: Protection from self-intoxication: A novel actin isoform in *Chromodoris* nudibranchs supports sequestration and storage of the cytotoxin latrunculin A”.

Biodiversity: The Indo-Malay Archipelago and Coral Triangle

The tropical Central Indo-Pacific Ocean is the region of greatest biodiversity in the marine world, including a high diversity of predators and presumably the highest level of species competititon.[362–366] It is the largest biogeographical realm, spanning more than half of the world. At the heart of this area lies the Indo-Malay Archipelago (also referred to as Nusantara, Indonesian Archipelago, Indo-Australian Archipelago and Coral Triangle, depending on the respective delineation) with more than 25,000 islands. Especially Indonesia, which consists of around 7,000 islands and 70% sea, is the largest archipelago country in the world. Additionally, as a maritime bottleneck, Indonesia is of particular importance for global ocean circulation, climate variability and biogeochemistry.[367,368] Its inter-oceanic currents provide the only connection between the world's oceans at low latitudes and connect the tropical Pacific and Indian Ocean via the Indonesian Throughflow (ITF).

The exceptional biodiversity of this region is the result of several unique factors and was only able to develop in this area due to a large and extreme diversity of habitats, created by the extensive and complex coastlines of the archipelago and its key ecosystems: the coral reefs, mangrove forests, and seagrass meadows.[363,364,369] These coastal and oceanic ecosystems have the highest diversity in the Indo-Malay region, which has the greatest concentration of tropical shallow water habitat on Earth. They are tightly connected and interdependant for the exchange of organisms, food and nutrients. Furthermore, they provide crucial ecosystem services, such as blue carbon storage, coastal protection, nursery habitats and are an important source of

income for local communities.[370,371] However, this vital region is also vulnerable and currently threatened by a multitude of direct human-induced pressures (e.g. pollution, overfishing, poaching and habitat destruction), rising temperatures, the climate crisis, deoxygenation and acidification. These stressors can drive mass mortality and have a dramatic impact on the diversity and integrity of the key coastal ecosystems (mangrove forests, seagrass beds and coral reefs), making them a top priority for conservation.[12,14,363,366,370–375]

A factor that contributes to the high diversity of this region is that it has been tectonically unstable for at least 38 million years, therefore creating ever-changing environments and disturbances that lead to an extreme habitat heterogeneity of complex shallow habitats alongside deep (~ 150 m) ocean. The shallow ecosystems are further affected by rapid changes (over geologic time) in sea level, resulting in more localized differences in oceanographic patterns and isolation or linkage of populations.[363,365] According to fossil records, it has been suggested that corals of the Coral Triangle (CT) are the world's youngest, which have either evolved in this region or survived there while going extinct in other places.[363,376]

Many reef-dwelling species are also represented by a free-living phase (such as larvae), in which the dispersal of these organisms is determined by the duration of their free-living phase and the speed and direction of the currents. This is especially true for bottom-dwelling marine invertebrates, which rely on dispersal and long-distance transport of their planktonic larvae by currents.[363,364,369] The CT acts as a 'catch basin' for all larvae that move towards the region via both the South Equatorial Current (SEC) and North Equatorial Current (NEC). Dispersion away from the CT occurs northward to mainland Japan via the Kuroshio Current, southward along the west Australian coast via the ITF and Leeuwin Current, and southward along the east Australian coast (to the Great Barrier Reef and into the Tasman Sea) via the East Australian Current (EAC).[363,364,369] Currents can act as both genetic barriers (in vicariance) and as paths of genetic connectivity. All of these conditions promote reticulate evolution in the CT (sometimes referred to as 'evolutionary cauldron'), enhanced by the ever-changing habitat diversity and the complexity of ocean surface currents, which lead to the great biodiversity of the CT and Indo-Malay coastlines.[363]

Species diversity attenuates away from the CT at progressively increasing distance according to ocean temperature. Heterobranch sea slugs also show such a pattern of rapid attenuation of species diversity from ~ 1,000 species in the northern Great Barrier Reef to ~ 500 species in central New South Wales.[377,378] Climate change can influence this pattern and southern range extension with a poleward shift has been described for many taxa, including sea slugs.[378–381]

However, there is a lack of baseline data for marine invertebrates and sampling bias may further affect our knowledge and perception of species richness, which also strongly depends on sampling strategy.[369,382] Recently though, as part of the INDOBIO project, considerable sampling effort has led to several publications on Heterobranchia species in North Sulawesi, Indonesia, increasing our knowledge for this group in this important region.[4–7] Furthermore, joint initiatives like the Sea Slug Forum (seaslugforum.net) or iNaturalist (inaturalist.org) and citizen scientist projects including volunteers in data collection for marine ecosystems, such as the 'sea slug census', can provide valuable data for research and may help to reinforce the importance of marine ecosystem health.[383] As most sea slugs are specialists that depend on specific food sources, documenting their species numbers and distribution can reveal valuable information on other important taxa and the state of the ecosystem as well.

AIMS OF THE THESIS

This dissertation is part of the interdisciplinary project INDOBIO, in which it focusses on the investigation of MNPs from nudibranchs and their food sources of the Central Indo-Pacific Ocean, namely *Phyllodesmium longicirrum* from Lizard Island, northern Great Barrier Reef, Australia, *Doriprismatica stellata* and five *Chromodoris* species from North Sulawesi, Indonesia. Furthermore, isolated MNPs were examined for their possible chemoecological role and medical potential. A mechanism for the prevention of autotoxicity was explored.

CHAPTER I focusses on the chemical investigation of MNPs from *P. longicirrum*. Unlike other Aeolidioidea, members of the highly diverse genus *Phyllodesmium* changed their food preference from Hydrozoa to Octocorallia and most of them do not store nematocysts for protection, despite having cerata. Instead, they incorporate symbiotic zooxanthellae and may sequester terpenoid metabolites from their prey. After extraction, chromatographic isolation and structure elucidation, pure NPs were examined in a fish feeding deterrence assay with the generalist pufferfish *Canthigaster solandri*, to test for a possible ecological function in defence. Additionally, isolated NPs were compared to metabolites from alcyonacean sources to determine the most likely octocorallian prey.

CHAPTER II investigates the biochemical relationship between *D. stellata* nudibranchs, their egg ribbons, and the associated dietary sponge, with an emphasis on a structurally new specialized metabolite, shared by all samples. The sponge was identified as *Spongia* cf. *agaricina* to increase knowledge on the specialized predator-prey relationship for this hardly investigated species of nudibranchs. Lipophilic extracts and the shared isolated pure NP were tested for antibacterial activity to identify a possible ecological function and its potential as drug lead structure.

CHAPTER III focusses on four key objectives: (1) chemical investigation of five closely related chromodorid nudibranchs: *C. annae*, *C. dianae*, *C. lochi*, *C. strigata* and *C. willani*, and their spongian food source, identified as *Cacospongia mycofijiensis*; (2) chemical analysis of the distribution of the shared main metabolite LatA within the nudibranchs' body and mucus and its visualization in cross-sections of *Chromodoris* specimen using MALDI MSI; (3) evalutation of the toxicity and mode of action of the isolated LatA using HEK-293 cells, *Chromodoris* and *Elysia viridis* heterobranchs; (4) investigation of a potential molecular resistance mechanism of *Chromodoris* nudibranchs, by sequencing and comparison of the obtained actin nucleotide and amino acid sequences from different heterobranch sea slugs.

CHAPTER I: Investigations of the natural products and chemical ecology of *Phyllodesmium longicirrum* (Nudibranchia: Aeolidioidea)

Large parts of this chapter are from the master thesis written by the authoress of this thesis, supervised by Prof. Gabriele M. König and Prof. Heike Wägele. The results contributed to the publications Bogdanov et al. 2016, 2017.[1,2]

Abstract

The constant struggle of prey organisms against predation led to the evolution of remarkable defence mechanisms to avoid detection or fight off predators. Aeolid nudibranchs lack a protective shell. Instead, they are well known for the ability to sequester nematocysts from their hydrozoan prey and use them for their own defence. Yet, there is one genus within this taxon, which does not store and utilize nematocysts. Members of *Phyllodesmium* have specialized to feed on alcyonacean soft corals, which house symbiotic zooxanthellae. Furthermore, they are a rich source of terpenoid MNPs. However, besides being camouflaged to avoid detection, little is known about the defence mechanisms of *Phyllodesmium*.

Here we show that one of the largest species, *P. longicirrum*, incorporates terpenoid compounds, especially cembranoid diterpenes. These metabolites are acquired from its prey, species of the soft coral genus *Sarcophyton*. Investigation of the lipophilic extract of a single *P. longicirrum* specimen led to the isolation of nine terpenoid metabolites: the cembranoid diterpene (2*R*,11*R*,12*R*)-isosarcophytoxide (**1**), its epimer (2*S*,11*R*,12*R*)-isosarcophytoxide (**2**), the related (3*R*,4*S*,11*R*,12*R*)-bisepoxide (**3**), and the γ-lactone bearing cembranes sarcophytonin B (**4**) and 13-dehydroxysarcoglaucol-16-one (**5**); the two polycyclic diterpenes 4-oxo-chatancin (**6**) and 1-*O*-methyl-4-oxo-chatancin (**7**); and the two biscembranes bisglaucumlide L (**8**) and bisglaucumlide M (**9**).

All of these compounds were either previously isolated from species of the genus *Sarcophyton*, or show a strong similarity to their compounds, supporting that *P. longicirrum* sequestered these metabolites from its prey. Furthermore, metabolites **6** and **2** significantly deterred feeding by the generalist pufferfish *Canthigaster solandri*, which emphasizes their ecological role in defence. Compound **1**, the epimer of **2**, exhibited no significant deterrence, suggesting that feeding deterrence might be conformation-dependant. *Phyllodesmium* is the species-richest genus within the Aeolidioidea. This is most likely because of an enhanced radiation and

speciation due to the shift from a hydrozoan to an alcyonacean food source, and the successful scavenging of their prey. The incorporation of symbiotic zooxanthellae provides additional nutrition, and the sequestration of defensive metabolites is a metabolically cheap and effective protection from predators, leading to a higher viability.

Keywords: chemical defence; chemoecology; natural compounds; Nudibranchia; *Phyllodesmium longicirrum*

Introduction

Ecology of the Genus Phyllodesmium

The genus *Phyllodesmium* Ehrenberg, 1831 (Aeolidioidea: Myrrhinidae) is unique among aeolids, because its members switched and specialized from feeding on Hydrozoa to feeding on Octocorallia, particularly Alcyonacea (Figure 2.1).[1,2,151,189] They are distributed in the Indo-Pacific Ocean, including coral reefs close to Japan, New Zealand and South Africa, where they live as stenophagous predators in a close ecological relationship with their octocoral prey.[151,190] The more primitive species, e.g. *Phyllodesmium serratum*, feed on corals that lack photosynthetic zooxanthellae, e.g. *Clavularia* and *Carijoa*, and show minimal or no branching of their digestive tract. While more derived species, with exceedingly branched digestive glands, have further evolved to participate in a mutualistic symbiotic relationship with photosynthetic zooxanthellae of the genus *Symbiodinium*.[132,151,384] This relationship is quite fascinating, since these slugs feed on the primary host, consume and digest the coral, while selectively preserving and translocating the entire symbiont into cells of the cerata, where exposure to sunlight is at its highest.[132,151,384] It was hypothesized that the acquisition of a photosynthetic ability is one of several important traits, which enhances speciation.[132] Indeed, *Phyllodesmium* is the species-richest genus (~30 species), compared to the other myrrhinid genera, which all comprise only one to five species each (see WoRMS). However, it should be noted that there is a high variation in the ability to retain zooxanthellae among different species of *Phyllodesmium*; with some aposymbiotic species, which completely digest the symbiont along with their prey, some retaining them for a short time, and others, being able to preserve the zooxanthellae for significant periods of food shortage.[132,384]

Unlike other aeolids, most members of *Phyllodesmium* do not incorporate and use nematocysts, but instead favour the symbiosis with *Symbiodinium* and an astounding mimicry (= crypsis) with their home coral for protection.[151,385] A likely explanation is that alcyonacean corals either completely lack or possess only a few, small nematocysts of the atrichous isorhiza type.[226,385-387]

However, a study conducted by Yoffe et al. 2012,[389] revealed that *Heteroxenia fuscescens* (Alcyonacea: Xeniidae) possesses an abundant array of nematocysts, challenging the general perception on stinging cells in this group. Indeed, there is one species, *Phyllodesmium jakobsenae*, which feeds on xeniid soft corals and has two different types of cerata, with the smaller ones storing nematocysts of its prey.[152] Yet, it is uncertain whether these kleptocnides are effectively used by *P. jakobsenae* as an additional defence, or are merely stored in the cerata to be excreted later on. Interestingly, around half of the *Phyllodesmium* species form a monophyletic clade, which have all specialized on feeding upon soft corals of the family Xeniidae, with the only exception of *P. koehleri*, which has specialized on feeding upon *Lemnalia*, members of the family Nephtheidae (Figure 2.1).[132,189] This additional "mini-radiation" on the Xeniidae was proposed to be triggered by the alternative composition of natural products in this family, which might enhance their defence.[132,390] The other zooxanthellate species of *Phyllodesmium* either feed on different genera of the family Alcyoniidae, e.g. *Sarcophyton* and *Sinularia*, or have specialized on members of the Anthotheidae, Briareidae or Tubiporidae (Figure 2.1).[132] To date, only five species of *Phyllodesmium* have been chemically investigated: *P. guamensis*, *P. lizardensis*, *P. briareum*, *P. magnum* and *P. longicirrum*,[1,2,189,390–393] revealing a large variety of terpenes, especially sesquiterpenes, cembranes and briaranes, derived from their octocoral food source.

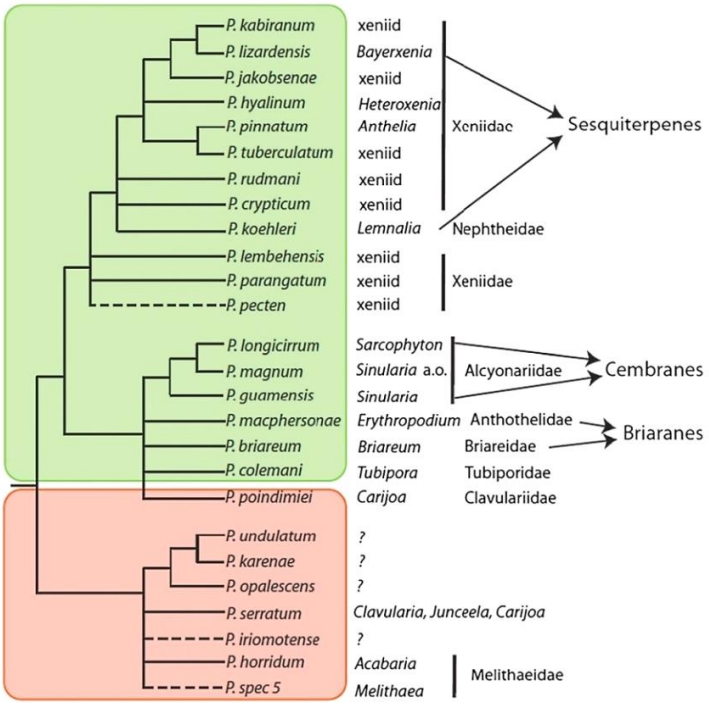


Figure 2.1 Cladogram of the genus *Phyllodesmium*, with zooxanthellate (green) and azooxanthellate (red) species and their respective alcyonacean food source (genus and family), if known. Arrows indicate relevant secondary metabolites isolated from the respective coral family, taken from Bogdanov et al., 2014.[189]

Chemical Ecology of Alcyonacea Soft Corals Associated with Phyllodesmium

Since the early Cretaceaous, hard corals (Hexacorallia: Scleractinia) and soft corals (Octocorallia: Alcyonacea) have been on separate evolutionary paths, diverging greatly in morphology, physiology, and chemical composition.[394,395] Coral reefs are mainly associated with scleractinians, but alcyonaceans dominate many reefs of the Indo-Pacific Ocean, with the most prolific genera being *Lobophytum*, *Nepthea*, *Sinularia*, and *Sarcophyton*.[227,387,396,397] Usually, soft corals are not considered as reef builders, however, a study by Jeng et al. 2011,[398] has shown that colonies of *Sinularia* are able to cement their calcium carbonate sclerites and consolidate them at their base into spiculite, laying a new foundation for future corals.

Octocorals are sessile throughout their adult life phase and cannot escape biotic and abiotic stress factors of their habitat by means of running away, or having a physical protection, like scleractinians.[44,387] Instead, they are known to possess high levels of MNPs, which are attributed to be the reason for their evolutionary success in the Indo-Pacific.[227,399–403] The highest diversity of toxic or deterrent MNPs is found in coral reefs, which are characterized by intense competition and feeding pressure due to carnivorous predators.[44,227] Since the beginning of marine natural product research in the mid-1960's, more than 3000 compounds have been isolated from Octocorallia.[401–407] These secondary metabolites, mainly diterpenoids, sesquiterpenoids and steroids, serve multiple functions, which are not mutually exclusive.[226,227] Most of them have been investigated for important pharmacological activities, showing antimicrobial, antiviral, anti-inflammatory, antitumor, HIV-inhibitory and cytotoxic activities, as well as cardiac and vascular responses.[224,397,408–411] Especially terpenoids from alcyonaceans have been shown to provide notable bioactivities.[224,397,409]

Although much research was dedicated to octocorals, their symbionts and metabolites, determining the true origin of a certain compound (soft coral or microbial), as well as its true ecological function is challenging and searching for a single role may be too simplistic.[226,405] Studies by Ne'eman et al. 1974,[412] Tursch 1976,[400] and Kashman & Groweiss 1977,[413] have been the first to confirm the defensive role of terpenoids in predation. They revealed that lobolide (Figure 2.2; **1**, extracted from *Lobophytum* sp.), lobophytolide (**2**, from *L. cristagalli*), crassolide (**3**, from *L. crissum*), africanol (**4**, from *Lemnalia africana*), and sarcophine (**5**, from *Sarcophyton glaucum*) are toxic to fish (*Gambusia affinis* and *Lebistes reticulatus*). Palustrol (**6**, from *Cespitularia* sp. aff. *subviridis*) and sarcophytonone (**7**, from *S. crassocaule*) exhibited toxicity against a variety of marine crustaceans, for example *Artemia salina*.[414,415] Sarcophytoxide (**8**), another cembranoid diterpene extracted from the genus *Sarcophyton*, showed high fish feeding deterrence against *G. affinis*.[228,391,397] Dihydroflexibilide (**9**),

11,12-deoxyflexibilide, and 11b-acetoxypukalide (**10**) were isolated from different species of *Sinularia* (e.g. *S. flexibilis* and *S. maxima*) and displayed feeding deterrent and ichthyotoxic activities against the mosquitofish *G. affinis* and pufferfish *Canthigaster solandri*.[46,392,416,417] Denticulatolide (**11**) and lobophynin C (**12**), both extracted from the genus *Lobophytum*, were ichthyotoxic against the killifish *Oryzias latipedes* and crustaceans of the genus *Artemia*.[228]

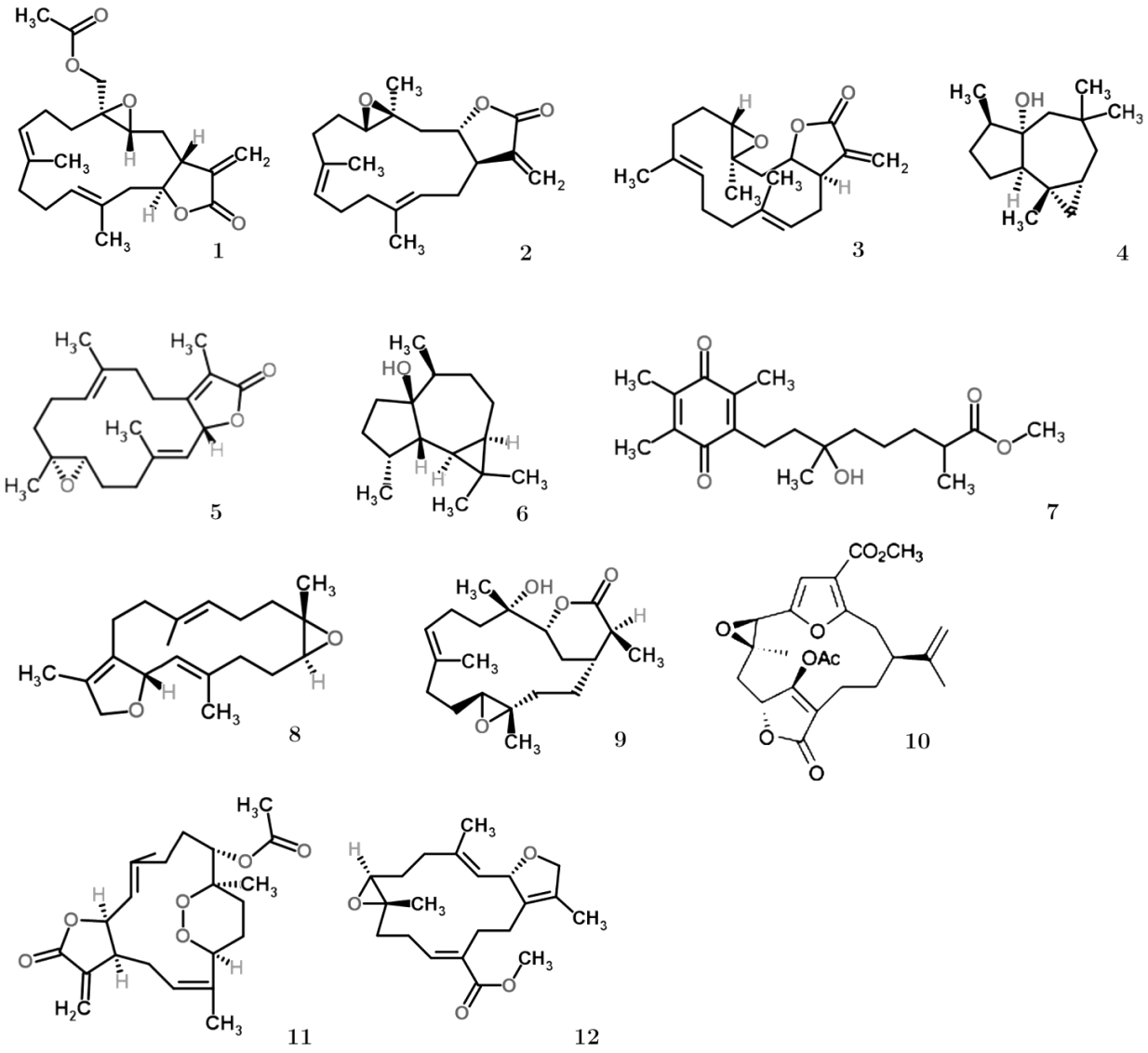


Figure 2.2 MNPs isolated from alcyonacean soft corals, the food source of the genus *Phyllodesmium*, ascertained to have defensive properties, such as feeding deterrence, ichthyotoxicity, or toxicity to crustaceans.

Further tests for defensive properties were conducted using the complete crude extracts of various soft corals, without determining specific compounds responsible for this action. Crude extracts of the xeniid soft corals *Ovabunda crenata* and *Heteroxenia ghardagensis*, were shown to be feeding deterrent against natural populations of Red Sea reef fishes.[418] The crude extracts

of *S. glaucum*, *Lemnalia* sp. and *Sinularia* sp. each inhibited predation by *G. affinis*.[226,419] Though toxicity is expected to be highest in coral reefs, crude extracts of the antarctic alcyonaceans *Alcyonium paessleri* and *Gersemia antarctica* have also been shown to be predator deterrent against the sea star *Odontaster validus*.[228,420,421]

Indeed, tests with crude extracts revealed that more than 60% of soft corals are ichthyotoxic, with the most toxic groups being *Lemnalia* and *Sarcophyton*, and around 75% of soft corals possess feeding deterrent activities.[226,227,399,419] Furthermore, presence of ichthyotoxicity was negatively correlated with the occurrence of physical morphological attributes specifically associated with anti-predator defence, emphasizing the trade-off from physical to a highly effective chemical defence in alcyonacean soft corals.[422]

It is expected that members of *Phyllodesmium* contain some of the previously mentioned metabolites, respectively to the according food source. Chemical investigation of five *Phyllodesmium* species, *P. guamensis*, *P. lizardensis*, *P. briareum*, *P. magnum* and *P. longicirrum* by Coll et al. 1985,[391] Slattery et al. 1998,[392] Affeld et al. 2009,[390] Mao et al. 2011,[393] and Bogdanov et al. 2014,[189] revealed a large variety of different terpenes, sequesterd from their prey. Yet, none of the alcyonacean compounds tested in feeding deterrence assays were retrieved from any *Phyllodesmium* species. Nevertheless, Bogdanov et al. 2014,[189] found at least the molecular mass of 317.2110 Da (M+H), which may indicate the presence of sarcophine (Figure 2.2; **5**). Further studies are necessary to determine the entire secondary metabolome of *Phyllodesmium* species, as well as of their alcyonacean food source.

Secondary Metabolites of Phyllodesmium longicirrum

P. longicirrum (Figure 2.3) is considered to be one of the most derived species of the genus *Phyllodesmium*.[384] This species can grow to a size of more than 12 cm and is therefore one of the largest species, besides *P. magnum*, with whom it forms a moderately supported clade.[132,151,384] *P. longicirrum* is especially notable, since it is able to retain communities of symbiotic zooxanthellae for more than 5 months, therefore having a most efficient photosynthesis.[132] This is due to its highly ramified digestive gland, housing the symbionts, and huge flattened cerata, which provide a maximum of sunlight, maximising the photosynthetic output.[132,151,384] This unique adaptation has most likely enabled the host to grow to such an exceptional size (Figure 2.3).

Figure 2.3 *In vivo* habitus of *P. longicirrum* feeding on a *Sarcophyton* soft coral (© B. Rudman, @seaslugforum.com).

Though being large might have advantages, it also increases the risk of being spotted by predators, making alternative defences crucial for survival. *P. longicirrum* is at least protected by crypsis on its respective food coral, but investigations towards additional defence mechanisms have been scarce. This species is often found on soft corals of the genus *Sarcophyton* and *Lobophytum*, but actual feeding has only been observed on *S. trocheliophorum*, supporting that *P. longicirrum* is a specialized feeder on alcyoniidaen species of the genus *Sarcophyton*, one of the most toxic soft coral genera.[151,226,391] Therefore, the assumption that *P. longicirrum* acquires toxic MNPs from *Sarcophyton* and uses them for its own defence is plausible.

So far only one study has been conducted, showing that the cembrane diterpenes thunbergol (Figure 2.4; **1**), epoxythunbergol (**2**), and a diterpene alcohol (**3**) are dietary derived and incorporated by *P. longicirrum*.[391] However it was not investigated whether these compounds deter feeding. Another study by Bogdanov et al. 2016,[1] revealed that a specimen of *P. longicirrum* from the Great Barrier Reef (Australia) also contained four rare cembrane-based polycyclic diterpenes 4-oxo-chatancin (Figure 2.4; **4**), 4-acetoxy-chatancin (**5**), 1-*O*-methyl-4-oxo-chatancin (**6**), and 1-oxo-9-hydro-isochatancin (**7**). Though these compounds have not yet been isolated from any other organism, they share a striking resemblance with chatancin (**8**) and sarcophytin (**9**), which were isolated from soft corals of the genus *Sarcophyton*, supporting that *P. longicirrum* acquires these metabolites from its prey. Furthermore, it was shown in a chemical defence assay with the generalist predator *C. solandri*, that 4-oxo-chatancin (**4**) significantly deters feeding, emphasizing that these molecules might be used as additional chemical defence.[1]

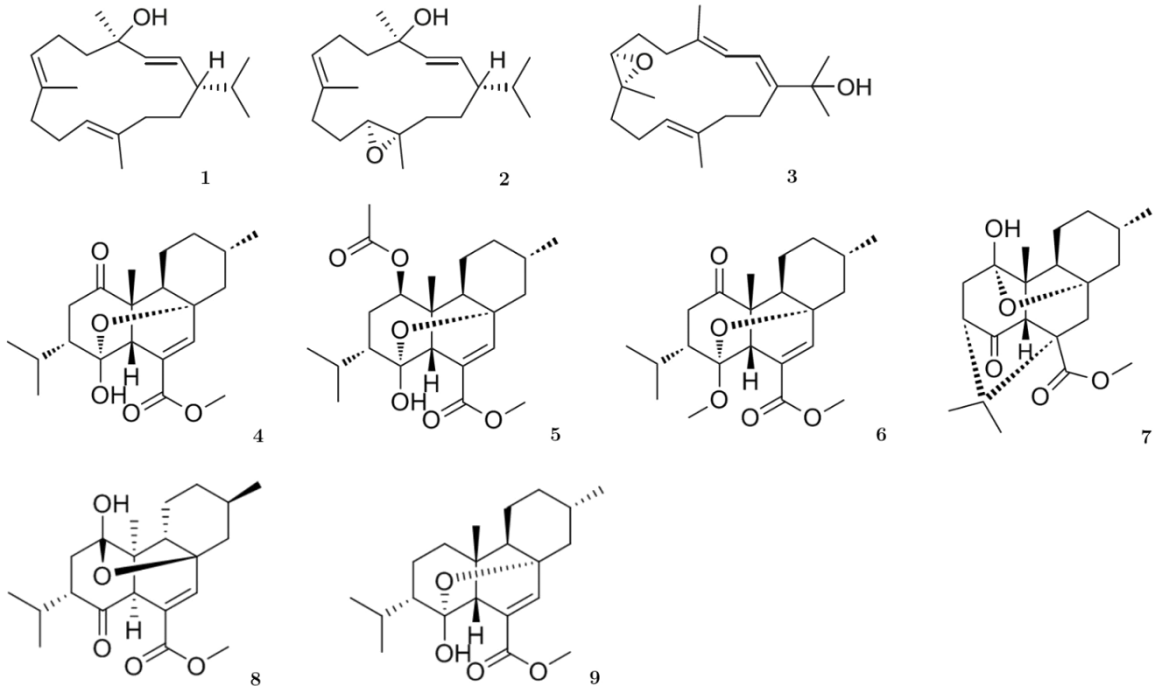


Figure 2.4 Metabolites isolated from *P. longicirrum* (**1-7**). Compounds (**1-3**) were also isolated from *Sarcophyton*. Compounds (**4-7**) resemble chatancin (**8**) and sarcophytin (**9**), which were isolated from *Sarcophyton* as well.

Aim of the Study

This study was conducted to investigate the composition of MNPs, especially terpenoids, in the aeolid nudibranch *Phyllodesmium longicirrum*. For this purpose, an ethyl acetate-extract of one *P. longicirrum* specimen was chromatographically separated and its compounds were isolated, analysed and elucidated by means of mass spectrometry, UV/Vis and IR spectroscopy, optical rotation measurements, but primarily by extensive analysis of 1D and 2D NMR spectra (SI). Elucidated metabolites were compared to compounds from alcyonacean soft corals to assign a possible dietary origin and gather further information on the predator-prey relationship between *P. longicirrum* and soft corals of this family. Feeding assays with the pufferfish *Canthigaster solandri* were carried out under laboratory conditions to evaluate defensive properties of the compounds isolated from *P. longicirrum*.

Results

In this study nine terpenoids were isolated by successive fractionation of previously obtained fractions, from the ethyl acetate (EtOAc) extract of a single *P. longicirrum* specimen (Table 2.1**)**. All compounds were isolated as colorless oils.

Table 2.1 Isolated compounds from the respective EtOAc fractions of *P. longicirrum*, their total amount and percentage share of the EtOAc extract.

Fraction	Isolated Secondary Metabolite	Total Amount	% of EtOAc Extract
VLC 7.2	**1**	41.0 mg	1.9
	2	42.0 mg	1.9
	4	17.8 mg	0.8
	5	3.7 mg	0.2
VLC 6 S 7	**3**	28.0 mg	1.3
	6	150.0 mg	7.0
	7	3.4 mg	0.2
VLC 6 S 5	**8**	4.4 mg	0.2
	9	2.3 mg	0.1

Cembranoid Diterpenes

Diterpenoids are a versatile class of compounds with significant biological activities, which can be isolated from various natural sources.[212] The most common diterpenoids reported from alcyoniidaen soft corals, the prey of *P. longicirrum*, are cembranoid diterpenes, which are based on a 14-membered carbocyclic ring.[400,404,423] The isolated metabolites **1**, **2**, **3**, **4**, and **5** belong to this abundant group (Figure 2.5)**.** Their structures are known and they have previously been isolated from different species of the soft coral genus *Sarcophyton* and the closely related genus *Lobophytum*, as in the case of compounds **1** and **2.**[213,400,424–428] Until now, only the planar structure of compound **3** was known. Its relative configuration was determined in this study by extensive analysis of the ^{1}H-^{1}H NOESY correlations.

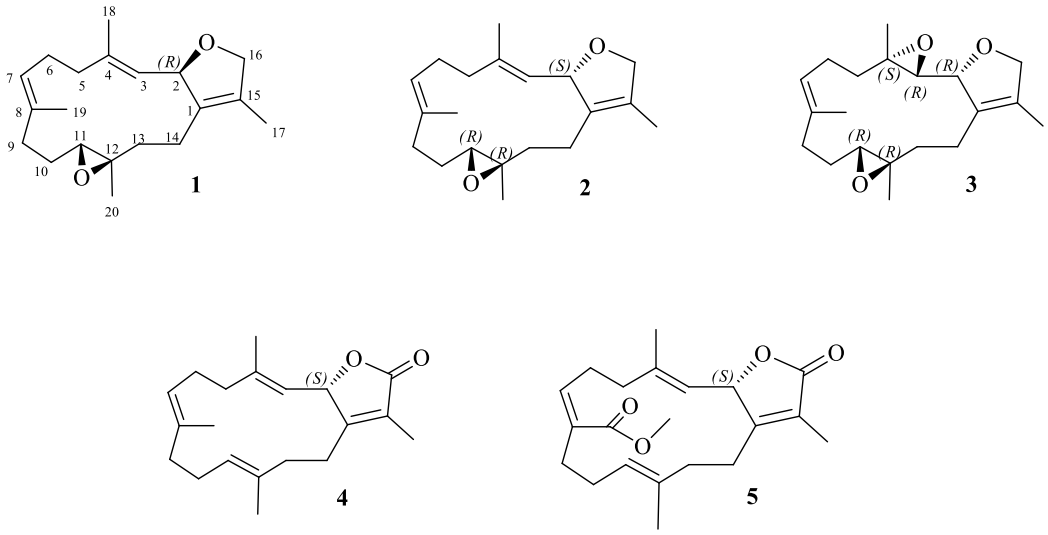


Figure 2.5 Cembranoid diterpenes (**1-5**) isolated in this study from *P. longicirrum*.

*Structure Elucidation of Compounds **1** and **2***

Mass spectral analysis of **1** and **2** indicated the formula $C_{20}H_{30}O_2$ (*m/z* 302.2, M^+) for both compounds. ^{1}H- and ^{13}C NMR spectral data are depicted in Figure S1.1 and Figure S1.2 for compound **1**, and in Figure S1.3, and Figure S1.4 for compound **2**.

The ^{13}C NMR spectra (Figure S1.2, Figure S1.4) showed 20 resonances for four methyl groups, seven sp^3 methylene groups, two sp^3 and two sp^2 methine groups and five quaternary carbon atoms as indicated by the DEPT135 measurement. Three double bonds are indicated by the four singlets and two doublets in the sp^2 region. The presence of a dihydrofuran ring was confirmed by a methine signal at δ 84.8 and a methylene signal at δ 79.0 in the ^{13}C-NMR spectrum. Literature research revealed a striking similarity of **1** and **2** with the monoepoxy dihydrofuran containing cembrane sarcophytoxide and the isomeric isosarcophytoxide.[400,424,426,429] Comparison of the ^{1}H-NMR spectra with those reported for sarcophytoxide and isosarcophytoxide, revealed that **1** and **2** were indeed isosarcophytoxide, since the major observable difference was in the signal attributed to the epoxy methine proton (H-11: sarcophytoxide: δ 2.74, triplet, J = 4 Hz; isosarcophytoxide: δ 2.81, doublet of doublets, J = 9.3 Hz), which resonated at δ 2.88 (**1**) and at δ 2.85 (**2**) as a doublet of doublets in both cases. Furthermore, the two compounds exhibited large specific rotations, which differed in the sign of rotation, as described by Bowden et al. 1987,[427] identifying compound **1** as (2*R*,11*R*,12*R*)-isosarcophytoxide ($[\alpha]_D^{20}$ = - 156, $CHCl_3$) and compound **2** as its epimer (2*S*,11*R*,12*R*)-isosarcophytoxide ($[\alpha]_D^{20}$ = + 196, $CHCl_3$).

Though we were able to isolate 12 mg of relatively pure compound **1** and 13 mg of **2**, most of them were combined in an inseparable mixture (58 mg). Simultaneous occurrence of **1** and **2** is consistent with previous findings of Bowden et al. 1987,[427] and Kashman et al. 1974,[424] which stated that the doubly allylic oxygenated position of the dihydrofuran ring may epimerize under certain conditions, leading to co-occurrence of isosarcophytoxides **1** and **2**, which only differ in configuration at the doubly allylic C-2 position. In total compounds **1** and **2** (Figure 2.5) were the second and third major metabolites isolated from *P. longicirrum*. The related sarcophytoxide is known as an allelochemical with cytotoxic and ichthyotoxic properties,[397] and isosarcophytoxides (Figure 2.5; **1** and **2**) are known to be at least cytotoxic.[214] Therefore, we hypothesized that compounds **1** and **2** might be used in chemical defence, and measured their ability to deter feeding by *C. solandri*.

*Structure Elucidation of Compound **3***

The molecular formula $C_{20}H_{30}O_3$ (*m/z* 318.2, M^+) of metabolite **3** was ascertained by LC-ESI-MS. ^{1}H- and ^{13}C NMR spectral data of **3** are depicted in Figure S1.5, Figure S1.6. The compound showed a high spectral similarity to compounds **1** and **2**, indicating that it could be the related bisepoxy dihydrofuran derivative of sarcophytoxide (Figure 2.5; **3**).

The ^{13}C NMR spectrum (Figure S1.6) displayed 20 resonances for four methyl groups, seven methylene groups, three sp^3 methine groups and one sp^2 methine, and five non-protonated carbons. Only two double bonds are indicated (C-8: δ 135.8; C-15: δ 131.8; C-1: δ 130.9; C-7: δ 127.1, H-7: δ 5.26), one trisubstituted (C-7 to C-8) and one tetrasubstituted (C-1 to C-15), with the latter being in a dihydrofuran ring. This was also confirmed by the presence of the methine signal (C-2: δ 87.9) and the methylene signal (C-16: δ 79.7), as expected for a dihydrofuran moiety. Furthermore, two trisubstituted epoxides were indicated by ^{13}C NMR signals at δ 66.4 (C-3), δ 63.1 (C-11), δ 62.6 (C-12), and δ 62.3 (C-4). The broad doublet centred at δ 2.80 (H-3, J = 8.2 Hz), the doublet of doublets at δ 2.88 (H-11, J = 3.3, 9.8 Hz), and the two methyl singlets at δ 1.30 (H_3-20) and δ 1.46 (H_3-18) support that compound **3** is the (3,4;11,12)-bisepoxide related to sarcophytoxide and the isosarcophytoxides **1** and **2** (Figure 2.5). Optical rotation ($[\alpha]_D^{20}$ = -44.6, $CHCl_3$) also coincides with the optical rotation value of (3,4;11,12)-bisepoxide ($[\alpha]_D^{20}$ = -46.7, $CHCl_3$[426]). Compound **3** was first isolated by Bowden et al. 1979,[426] from an unknown species of the soft coral genus *Sarcophyton*.

Though the planar structure of metabolite **3** has been known for more than 35 years, there was no published investigation of its stereochemistry. Hence, a detailed analysis of the ^{1}H-^{1}H NOESY correlations of **3** was conducted to propose the possible relative

configuration (Figure S1.7). An NOE correlation of H-2 to H_3-18 indicated the relative configuration of C-2 to be *R**. Since H_3-18 and H-2 point into the same direction, the relative configuration of C-4 was established as *S**. Relative configuration of C-3 was determined as *R**, due to the NOE correlation of H-3 to H-7 and H_3-20. NOE correlations of H_3-19 only to H_2-6 and H_2-9 emphasize that this methyl group points outwards. The relative configuration of C-11 and C-12 was established as *R** in both cases, which is the same as described for the related isosarcophytoxides **1** and **2** (Figure 2.5),[427] indicated by NOE correlations of H_3-20 to H-3 and H-11 (Figure S1.7). Until now, no bioassays or feeding deterrence experiments have been conducted for metabolite **3.** Therefore, it is unknown what impact the second epoxide group might have. Further experiments regarding its biological activities and ecological function are needed.

*Structure Elucidation of Compound **4***

The molecular formula of compound **4** was established by HRMS to be $C_{20}H_{28}O_2$ (*m/z* 301.2160, M+H). ^{1}H- and ^{13}C NMR spectral data are shown in Figure S1.8 and Figure S1.9.

The ^{13}C NMR spectrum (Figure S1.9) exhibited 20 resonances for four methyl groups, six sp^3 methylene groups, one sp^3 and three sp^2 methine groups and six quaternary carbon atoms as suggested by the DEPT135 measurement. The carbon signals indicate the presence of four double bonds (C-1 to C-15; C-3 to C-4, C-7 to C-8, and C-11 to C-12) and a γ-lactone moiety (C-1: δ 166.1; C-2: δ 81.1; C-15: δ 122.9; C-16: δ 177.5; C-17: δ 8.8). Comparison of the obtained ^{1}H- and ^{13}C NMR data of compound **4** (Figure S1.8 and Figure S1.9) and the optical rotation measurement ($[\alpha]_D^{20}$ = +158.2, $CHCl_3$) with values from the literature showed that they fit to sarcophytonin B (**4**). Compound **4** was first isolated by Kobayashi & Hirase 1990[428] from an unknown species of the soft coral genus *Sarcophyton*. They determined the absolute configuration at C-2 to be *S* from the CD spectrum, which showed a negative Cotton curve (247 nm, Δ ε -3.3) due to the chiral butenolide ring.

*Structure Elucidation of Compound **5***

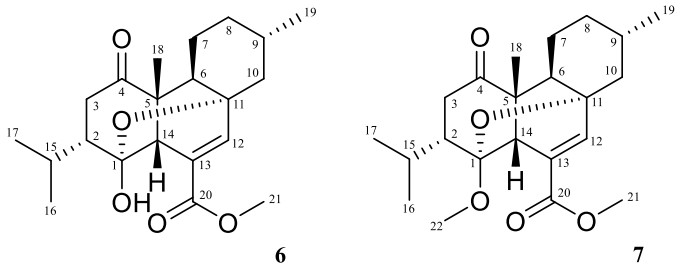
Mass spectral analysis of compound **5** determined the molecular formula to be $C_{21}H_{28}O_4$ (*m/z* 344.199, M^+). ^{1}H- and ^{13}C NMR spectral data are represented in Figure S1.10 and Figure S1.11.

The ^{13}C NMR spectrum (Figure S1.11) showed 21 resonances for four methyl groups, one of them being a methoxy (OCH_3-21), six methylene groups, three sp^2 and one sp^3 methine groups

and seven non-protonated carbons as indicated by the DEPT135 measurement. A γ-lactone moiety (C-1: δ 166.0; C-2: δ 81.1; C-15: δ 123.0; C-16: δ 177.5; C-17: δ 8.8) was present in this metabolite as well. Though the overall NMR spectra showed great similarity to compound **4**, there was one conspicuous difference, being that **5** contained a carboxylate ester (C-19: δ 169.7 (s), H_3-21: δ 3.78). Therefore, metabolite **5** represents an example of rare cembranoids, which are fuctionalized at C-19. Comparison with literature data revealed that the obtained ^{1}H-, ^{13}C NMR (Figure S1.10, Figure S1.11) and optical rotation value of **5** ($[\alpha]_D^{20}$ = + 81.4, $CHCl_3$) match those of 13-dehydroxysarcoglaucol-16-one,[213] previously isolated from the soft coral *Sarcophyton cherbonnieri*.

Polycyclic Diterpenes

Compounds **6** and **7** are two rare cembrane-based polycyclic diterpenes (Figure 2.6). Only 6 members of this group have been described to date, which were isolated from soft corals of the genus *Sinularia* and *Sarcophyton*.[430,431] These complex molecules have been shown to be platelet-activating factor (PAF) antagonists, useful against numerous diseases, including those of the respiratory and cardiovascular system.[432,433]



6 **7**

Figure 2.6 Chemical structures of compounds **6** and **7** isolated from *P. longicirrum*.

*Structure Elucidation of Compounds **6** and **7***

The molecular formula of compound **6** was established by HRMS to be $C_{21}H_{30}O_5$ (*m/z* 385.1977, M+Na). ^{1}H- and ^{13}C NMR spectral data of **6** are depicted in Figure S1.12 and Figure S1.13, and summarized in Table S1.1.

Its ^{13}C NMR spectrum (Figure S1.13) showed 21 resonances for five methyl groups, five sp^3 methine groups, one sp^2 methine group, four methylene groups and six non-protonated carbon atoms as indicated by a DEPT135 measurement. The ^{1}H-^{1}H COSY spectrum enabled the determination of two main fragments. The first reached from proton H-6 to H-10, including the methyl group CH_3-19, while the second composed an isopentyl moiety with the methyl groups

CH_3-16 and CH_3-17, methine groups CH-15 and CH-2 and methylene group CH_2-3. ^{1}H-^{13}C HMBC correlations from H_2-3 to carbonyl carbon C-4, from H-2 to C-1, and from H-14 to both C-1 and C-5 indicated the presence of an isopropylcyclohexanone moiety. HMBC correlations from H-6 and H_2-10 to C-11 identified the second ring as a methylcyclohexane fragment. The formation of a phenanthrene ring, with the double bond $\Delta^{12,13}$ and the connection between C-6 and C-5 was confirmed by HMBC correlations between H-6 and C-5, H-14 and C-13, as well as between H-12 and C-11 and C-13 both. HMBC correlations between the remaining methyl group CH_3-18 and C-5 enabled its correct positioning, attached to C-5. The methoxycarbonyl group was attached to C-13, due to HMBC correlations between H-14 and C-20. An additional ring closure was introduced between C-1 and C-11 by an oxygen bridge, supported by the downfield shifts of both C-1 (δ 98.8) and C-11 (δ 77.1). Hence, the planar structure of compound **6** is the same as isosarcophytin.[434]

However, the optical rotation of compound **6** was quite different from isosarcophytin ($[\alpha]_D^{20}$ **6**: - 14.6; isosarcophytin: - 65.0). An exhaustive analysis of the NOESY spectrum showed that the difference between both compounds had to be at C-2. NOE correlation of H-2 to H-14 indicated the relative configuration of C-2 in **6** to be opposite to that of isosarcophytin. The absolute configuration was determined from an ECD spectrum of compound **6**, which showed a small negative Cotton effect Δε at 300 nm (- 0.13). This suggests that the absolute configuration is that of chatancin,[435] (Figure 2.4) on the basis of the octant rule using the energy minimized conformation model of compound **6**. Structural elucidation of this compound was mainly conducted by A. Bogdanov and S. Kehraus, and the trivial name 4-oxo-chatancin was proposed for compound **6**.[1]

^{1}H- and ^{13}C NMR spectral data of **7** are depicted in Figure S1.14 and Figure S1.15, and summarized in Table S1.2. Its molecular formula was established by HRMS to be $C_{22}H_{32}O_5$ (*m/z* 399.2140, M+Na). Analysis of the 1D and 2D NMR spectra showed that compound **7** has the same planar as well as relative configuration as **6**, and differs only by the presence of an additional methoxy group (^{1}H-22: δ 3.16; ^{13}C-22: δ 51.5). Its connection to C-1 was evident due to the HMBC correlation of H_3-22 to C-1. It is assumed that compound **7** has the same absolute configuration as compound **6**, due to comparable optical rotation values ($[\alpha]_D^{20}$ **7**: -10.0; **6**: - 14.6). It cannot be excluded, that compound **7** is an artefact, as methanol was used several times in the isolation procedure. For metabolite **7**, 1-*O*-methyl-4-oxo-chatancin was proposed as the trivial name.[1,2]

Biscembranes

The soft coral genus *Sarcophyton* is also known to be the source of unusual tetraterpenoids (biscembranes), which are formed by coupling two cembranoid units through a Diels-Alder reaction.[436] They are characterized by a 14-6-14-membered tricyclic skeleton, with the structural variation occurring most often in ring C (Figure 2.7), through high oxygenation, and tri-, penta-, and hexaepoxy cyclization.[437]

In this study, two biscembranes were isolated from *P. longicirrum* (Figure 2.7). Compounds **8** and **9** exhibited great similarity to the already known bisglaucumlide B and bisglaucumlide C (Figure 2.7), which were isolated from *S. glaucum*.[438] However, several ^{1}H- and ^{13}C NMR shifts were mismatched and optical rotations differed from the reported values.

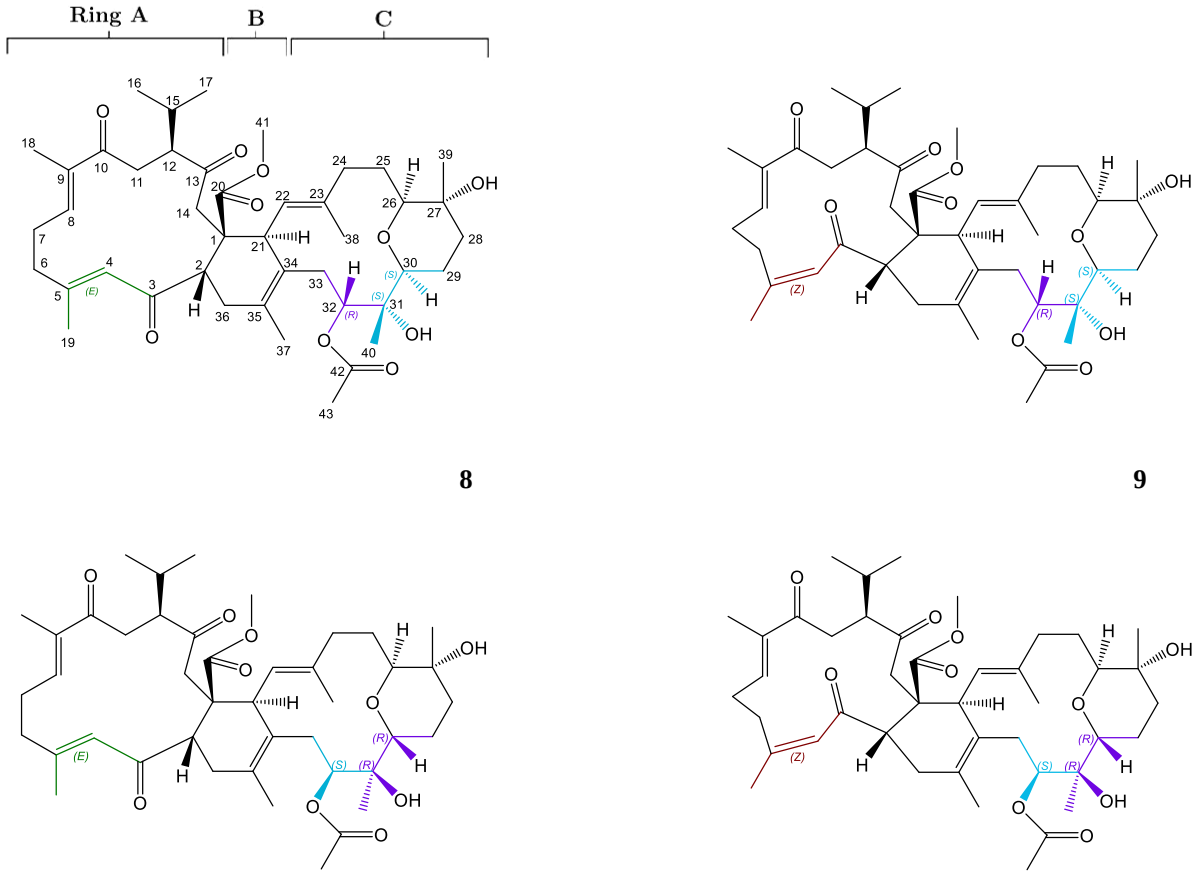


Figure 2.7 Relative planar structures of the isolated biscembranes **8** and **9** from *P. longicirrum* in comparison to bisglaucumlide B and C isolated from *S. glaucum*.[438]

Though it is difficult to determine the stereochemistry of biscembranes by 2D NMR analysis alone, an exhaustive analysis of the ^{1}H-^{1}H ROESY spectrum was conducted to propose the possible variations in the C-ring stereochemistry of compounds **8** and **9**, in comparison to the already published bisglaucumlide B and bisglaucumlide C (Figure S1.20).

*Structure Elucidation of Compounds **8** and **9***

Mass spectral analysis of **8** and **9** indicated the molecular formula $C_{43}H_{62}O_{10}$ (*m/z* 738.4, M^{+}) for both compounds. ^{1}H- and ^{13}C NMR spectroscopic data are summarized in Table S1.3 (**8**), and Table S1.4 (**9**). They are depicted in Figure S1.16 and Figure S1.17 (**8**), and in Figure S1.18 and Figure S1.19 (**9**).

The NMR spectra of both compounds were very similar. They showed 43 resonances for ten methyl groups, two of them being methoxy groups, one as part of a methyl ester ($COOCH_3$-41) and the other one being part of an acetyl ($COCH_3$-43), as indicated by the ^{1}H NMR spectra (C-41: δ 3.65 (**8**), δ 3.58 (**9**); C-43: δ 2.10 (**8** and **9**)). Furthermore, ten methylene groups, seven sp^3 and three sp^2 methine groups, and thirteen quaternary carbons, three of them as ketone groups (C-3, C-10, and C-13) and two being part of the methyl ester (C-20$OOCH_3$) and the acetyl (C-42OCH_3), were found. The spectra indicated the presence of 4 double bonds (C-4 to C-5, C-8 to C-9, C-22 to C-23, and C-34 to C-35) and one oxygen bridge in ring C, between C-26 and C-30. The occurrence of an isopropyl moiety in both compounds was validated by two doublets at δ 0.86 (d, *J* = 7.0 Hz, H_3-16) and δ 0.90 (d, *J* = 7.0 Hz, H_3-17) for compound **8**, and δ 0.90 (d, *J* = 6.8 Hz, H_3-16) and δ 0.96 (d, *J* = 6.8 Hz, H_3-17) for compound **9**. The isopropyl group was placed at C-1, due to HMBC correlations of C-14 to C-20 and C-41. The acetyl group was determined to be positioned at C-32, because of an HMBC correlation of C-43 to C-32.

Despite the remarkable similarity of **8** and **9**, different structural features were evident when comparing the chemical shifts of H-4 (δ 6.18 (**8**) to δ 6.29 (**9**)), and H_3-19 (δ 2.12 (**8**) to δ 2.00 (**9**)), which were shifted downfield and upfield by 0.11 and 0.12 ppm, respectively. A more drastically shift upfield by 8.6 ppm was observed for C-6 (δ 40.3 (**8**) to δ 31.7 (**9**)), and downfield by 6.3 ppm for C-19 (δ 18.6 (**8**) to δ 24.9 (**9**)), suggesting that compound **9** was a geometrical isomer of **8** with regard to the geometry of the C-4 olefin. The bond was assigned the absolute configuration *E* for compound **8** and *Z* for compound **9**.

A comparison of the obtained data to literature values showed, that compound **8** bears a striking resemblance to the known biscembrane bisglaucumlide B, whereas compound **9** shows the highest similarity to bisglaucumlide C (Figure 2.7). Both molecules were previously isolated

from the soft coral *Sarcophyton glaucum* by Iwagawa et al. 2006.[438] They are formed through a Diels-Alder reaction of the geometric isomers of methyl sarcophytoate (left part of the biscembrane) with the unknown $\Delta^{1,3,15}$-cembratriene (right part of the biscembrane).

However, optical rotations of **8** ($[\alpha]_D^{20}$ = -5.2, MeOH) and **9** ($[\alpha]_D^{20}$ = -14.4, MeOH) did not match the published values (bisglaucumlide B: $[\alpha]_D^{20}$ = +126, MeOH; bisglaucumlide C: $[\alpha]_D^{20}$ = +32, MeOH), and several chemical shift deviations were observed, especially in ring C (e.g., C-30: δ 82.0 (**8**,**9**); δ 69.2 (bisglaucumlide B and C); C-38: δ 16.4 and 16.6 (**8**,**9**); δ 20.0 and 19.7 (bisglaucumlide B and C)). This indicates that compounds **8** and **9** differ in their stereochemistry in ring C. Therefore, ^{1}H-^{1}H ROESY correlations of **8** and **9** were analysed to determine the stereostructural variation (Figure S1.20). The ROESY was used instead of NOESY, since NOE correlations become vanishingly small for mid-sized molecules like biscembranes **8** and **9**, and the ROESY provides an alternative solution in this case.

The ROE correlation of H_3-38 to H-32 indicated the relative configuration of C-32 to be *R** instead of *S**, as in bisglaucumlide B and C. Furthermore, relative configuration of C-31 and C-30 were determined to be *S** instead of *R**, due to the ROE correlations of H-32 to H_3-40 and H-30 (Figure S1.20). Thus, **8** and **9** are stereoisomers of bisglaucumlide B and C, respectively (Figure 2.7). Until now, 11 bisglaucumlides (A-K) have been described.[438,439] Six of them exhibited weak cytotoxic activity against proliferation of human promyelocytic leukemia cells (HL-60), but none of the metabolites were tested for feeding deterrence or other ecological functions. Following the previous naming, the trivial names bisglaucumlide L (**8**) and bisglaucumlide M (**9**) are proposed.

Chemical Defence

Compounds **1**, **2**, and **6** were tested for the ability to deter feeding by the fish predator *C. solandri* (Figure 2.8, Figure 2.9). The major metabolite **6** showed a significant deterrent effect (p = 0.015) at a concentration as low as 0.5% in the dry pellet mass, which is an order of magnitude lower than the estimated abundance in *P. longicirrum*. The deterrent effect intensified with increasing concentration of compound **6** (1.0%: p = 0.006; 2.0%: p = 0.0001; Figure 2.8). A similar result was obtained for metabolite **2**, which showed a significant deterrence at a concentration of 1.0% (p = 0.023), and 2.0% (p = 0.001). Interestingly, compound **1**, which is the enantiomer of compound **2**, exhibited no deterrent effect at a concentration of 1.0% (p = 0.37; Figure 2.9). Further experiments are needed to determine whether feeding deterrence of **1** and **2** is truly configuration-dependant, as might be expected from the obtained results.

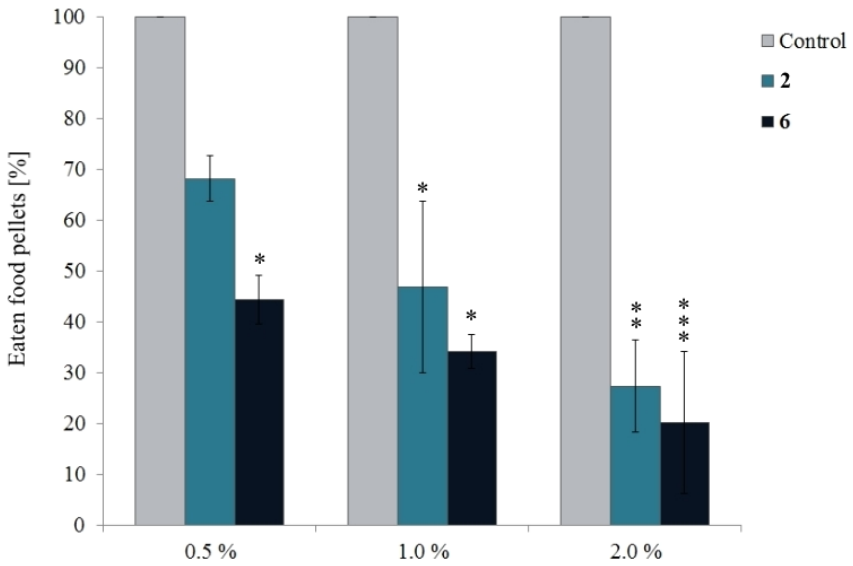


Figure 2.8 Feeding deterrence of the isolated compounds **2** and **6** at different concentrations (0.5%, 1.0%, and 2.0% of dry mass). Percentage of food pellets eaten by *C. solandri* was significantly decreased by both compounds at 1.0% and 2.0%. Compound **6** was also significantly deterrent in the lowest concentration of 0.5%. Significance was determined with the *Fisher's exact test* ($p < 0.05$ = *; $p < 0.005$ = **; $p < 0.0005$ = ***).

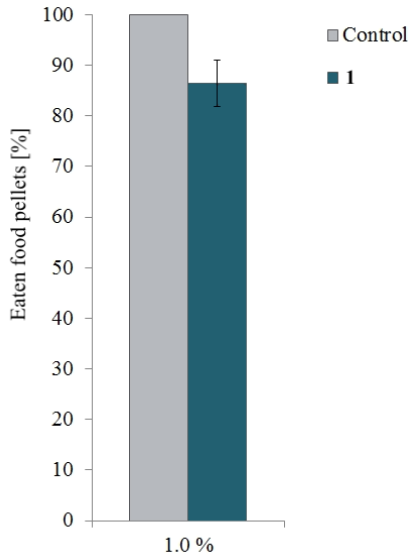


Figure 2.9 Feeding deterrence of the isolated compound **1** at 1.0% concentration of dry mass. No significant decrease of the percentage of food pellets eaten by *C. solandri* was observed. ($p = 0.37$).

Discussion and Conclusion

Terpenoids Isolated from Phyllodesmium longicirrum

Chemical investigation of a single specimen of *P. longicirrum* led to the isolation of nine terpenoid metabolites, revealing that this aeolid nudibranch contains a larger diversity of chemical entities, than previously known (Figure 2.10). Thunbergol (**1**), epoxythunbergol (**2**), and a diterpene alcohol (**3**), the first cembrane diterpenes isolated from *P. longicirrum* by Coll et al. 1985,[391] could not be retrieved in the current study. Instead, five other cembranoid diterpenes were found, representing the second largest share of the EtOAc-extract (132.5 mg).

These five metabolites were: the co-occurring isomers of sarcophytoxide, (2*R*,11*R*,12*R*)-isosarcophytoxide (**8**) and its epimer (2*S*,11*R*,12*R*)-isosarcophytoxide (**9**), as well as the related bisepoxy dihydrofuran (3*R*,4*S*,11*R*,12*R*)-bisepoxide (**10**); plus, the two γ-lactone bearing cembranes sarcophytonin B (**11**) and 13-dehydroxysarcoglaucol-16-one (**12**), a rare cembranoid with a carboxylate ester at C-19.

Two further compounds were elucidated in this study, belonging to the rare group of cembrane-based polycyclic diterpenes: 4-oxo-chatancin (**4**) and 1-*O*-methyl-4-oxo-chatancin (**6**). 4-oxo-chatancin (**4**), 4-acetoxy-chatancin (**5**) and 1-oxo-9-hydro-isochatancin (**7**) were previously isolated from different fractions of the same *P. longicirrum* specimen by A. Bogdanov,[1,2] and combined results revealed, that 4-oxo-chatancin (**4**) was the major compound (150 mg) contained by this specimen.

Furthermore, two unusual biscembranes, bisglaucumlide L and M (**13** and **14**) were isolated, differing in their stereostructure in ring C from the otherwise very similar bisglaucumlide B and C, isolated from *Sarcophyton glaucum* by Iwagawa et al. 2006.[438]

Overall, combined with the results obtained from the same specimen by A. Bogdanov (personal communication),[1,2] it was discovered that *P. longicirrum* contains a broad spectrum of terpenoids, belonging to the class of cembranoid diterpenes, secogorgosterols, unusual tetracyclic and pentacyclic diterpenes, and biscembranes. These foundational results enable to raise and investigate further questions, regarding the origin, biosynthesis, transport, storage, ecological function and relevance of these metabolites, as well as the necessary protective mechanisms applied by *P. longicirrum* to feed upon its toxic prey without having to suffer from self-intoxication.

Additionally, these compounds may have pharmacological activity, offering a chance to find and develop new drugs, e.g. antitumor drugs and compounds with high activity against multiresistant bacteria, which have become an increasing global problem.[397,409] Terpenoids are known to have many important biological, especially antitumor and antibacterial activities; hence they are of major medical, economic and scientific interest.[224,409]

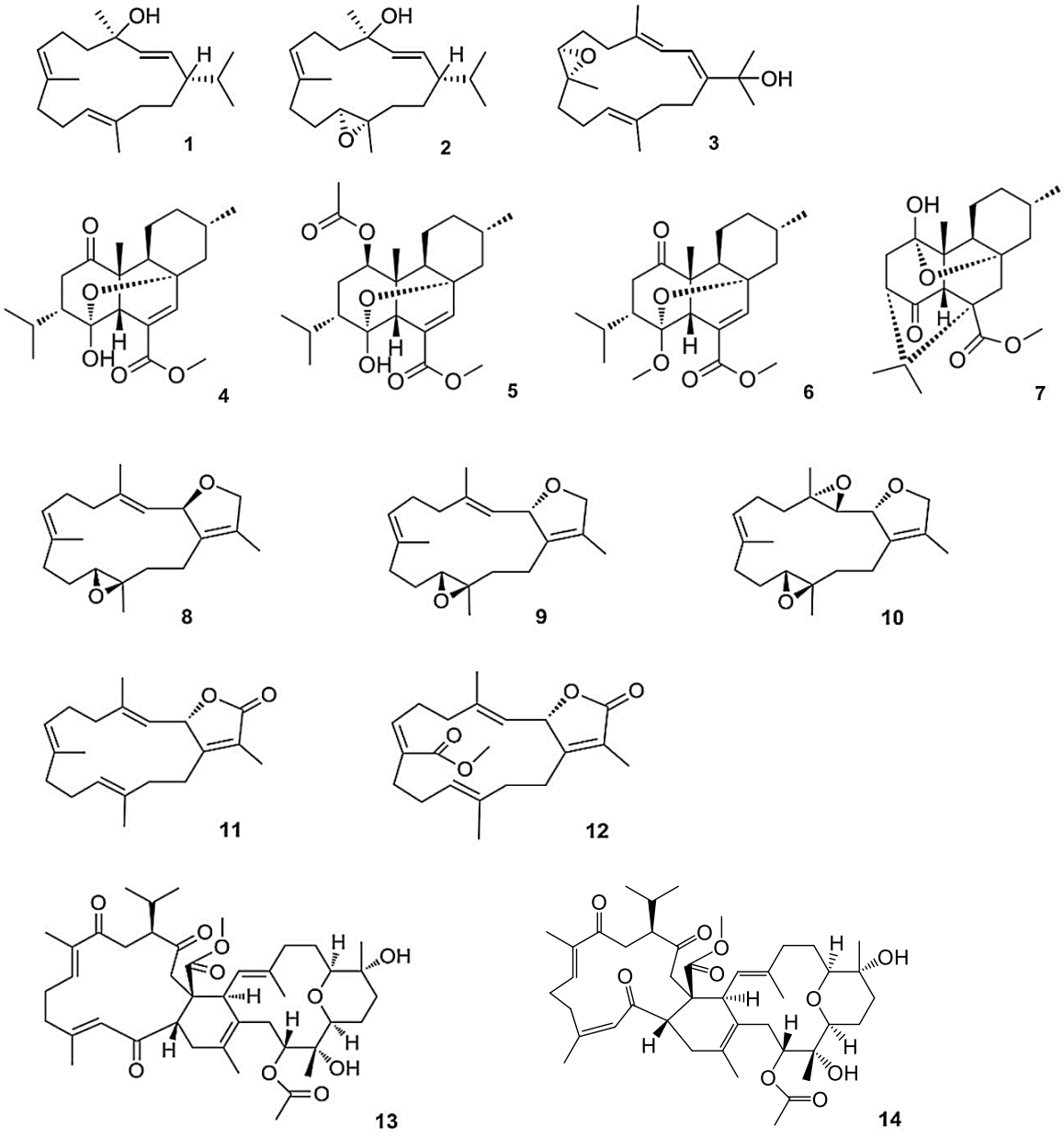


Figure 2.10 Terpenoids isolated from *P. longicirrum*. Compounds (**1-3**) were elucidated by Coll et al. 1985,[391] but were not retrieved in this study. Compounds (**4-7**), (**11**) and (**12**) were previously extracted from the same specimen by A. Bogdanov as well.[1,2] Compounds (**4**), (**6**) and (**8-14**) were isolated in the current study.

Predator-Prey Relationship between P. longicirrum and Alcyoniidaen Soft Corals

Most of the secondary metabolites isolated from *P. longicirrum* belong to the class of cembrane diterpenoids, which are present both in the plant and animal kingdom.[440] The first cembrane structures, e.g. (+)-cembrene from pine oleoresins and two epimeric cembratriene-diols obtained from tobacco were reported in 1962.[440,441] Since then, more than 300 members of this class have been isolated from terrestrial, and especially from marine sources, uncovering that they are the most common and widely distributed of all diterpene families.[397] From the marine habitat, anthozoans, especially of the soft coral genera *Lobophytum*, *Nephthea*, *Sinularia*, and *Sarcophyton* have proven to be a particularly rich source of cembrane natural products, displaying a wide range of biological activities.[397,431]

All of the cembranoid diterpenes isolated so far from *P. longicirrum* (Figure 2.10; **1-3** and **8-12**), were previously found in different species of the alcyoniidaen genus *Sarcophyton*.[213,400,424–428] The polycyclic diterpenes and biscembranes elucidated in the current study have not yet been isolated from any other organism. However, 4-oxo-chatancin and 1-*O*-methyl-4-oxo-chatancin (**4** and **6**) strongly resemble chatancin and sarcophytin (Figure 2.4; **8** and **9**), and bisglaucumlide L and M (Figure 2.10; **13** and **14**) show a striking similarity to bisglaucumlide B and C (Figure 2.7), which have all been isolated from different species of the genus *Sarcophyton* as well, e.g. *S. elegans* and *S. glaucum*.[434,435,438]

These results strongly support the hypotheses that *P. longicirrum* has specialized on feeding upon alcyoniidaen soft corals of the genus *Sarcophyton*,[391] which is one of the most prevalent and most toxic soft coral genera in the Indo-Pacific Ocean,[387,399] and that this aeolid nudibranch derives the secondary metabolites from its prey, rather than by *de novo* biosynthesis.[142]

In 2005, a study by Tanaka et al.,[419] has shown that each *Sarcophyton* species may have a distinct pattern of chemotypes, with the largest diversity of secondary metabolites found for the *S. glaucum*/*cinereum* species complex, and only a moderate diversity for *S. trocheliophorum*. The specimen investigated by Coll et al. 1985,[391] was found feeding on *S. trocheliophorum* and only three cembrane diterpenoids were extracted, which were not rediscovered in the *P. longicirrum* specimen investigated in the current study. The most likely explanation for this is the varying diversity of chemical contents in the different *Sarcophyton* species. The presence of biscembranes in the analysed specimen is another clue pointing towards this explanation, since biscembranes of this type have, until now, only been isolated from *S. glaucum*. This indicates that the investigated *P. longicirrum* specimen has fed at least upon *S. glaucum*, which also showed the largest diversity of compounds in the study by Tanaka et al. 2005.[419] However,

it needs to be considered that the chemical content of soft corals varies not only between species, but also between individuals, due to various reasons, like seasonal changes, reproductive stage, genetic differences, hybridization between species, and induction of certain biosynthetic pathways by environmental factors including surrounding organisms.[419] Furthermore, there has been some debate regarding the contributions of symbionts in terpene biosynthesis, which still remains unresolved,[442–444] but receives growing support, with at least some metabolites being produced by the symbionts.[445] Therefore, genetic differences of symbiotic microorganisms and zooxanthellae might further influence the chemical composition of each soft coral, hence of each *P. longicirrum* specimen feeding upon these corals.

Dietary Chemicals as Defensive Weapons

The chemical defence assay employed in this study confirmed the hypothesis that *P. longicirrum* is chemically defended by storing alcyoniidaen-derived terpenoids, at least with regard to the generalist fish predator *C. solandri.*

Especially the major metabolite, the polycyclic diterpene 4-oxo-chatancin (Figure 2.10: **4**), exhibited a strongly significant deterrent activity (Figure 2.8), supporting its ecological function as defensive chemical. A similar deterrent effect was evoked by the cembranoid diterpene (2*S*,11*R*,12*R*)-isosarcophytoxide (**9**) (Figure 2.8), indicating that multiple compounds stored by the nudibranch have the ability to act as feeding deterrents.

The mechanisms by which these metabolites discourage predation are still unknown, since many are not very poisonous, but instead seem to be effective as deterrent olfactory or gustatory cues.[46,53,446] However, the low-solubility of terpenoids in water determines that they are used for short-range or contact communication by preventing or strongly limiting the dilution of the signal in the medium. This is plausible, since interactions with predators or conspecifics of sessile or slow-moving marine benthic organisms occur at extremely close range and are therefore often mediated by lipophilic compounds.[53]

Many fish show a conspicuous behaviour when feeding: they repeatedly take food into their oral cavity and reject it, before either swallowing or completely refusing it.[53] This behaviour was also observed and taken into consideration in the conducted feeding deterrence assay of the current study. It shows the crucial need of the fish to detect lipophilic substances via contact, as these MNPs cannot be perceived from a distance. Therefore, a likely explanation for the feeding deterrent activity of non-toxic terpenoids would be that they are able to bind to receptors in the oral cavity of the fish.

Interestingly, (2*R*,11*R*,12*R*)-isosarcophytoxide (**8**), the epimer of (2*S*,11*R*,12*R*)-isosarcophytoxide (**9**), did not deter feeding (Figure 2.9). This suggests that the ability to bind to certain receptors and promotion of feeding deterrence depends on the configuration of the metabolite at each chiral centre. Experiments regarding structural modifications on antifeedant activity are seldomly conducted and only some reports are known from terrestrial chemical ecology, most often using insects. Some of these studies support the importance of the configuration of chiral centres for feeding deterrent activities,[447,448] while others find no difference in feeding deterrence for enantiomeric compounds.[449] Therefore, studies concerning configuration-dependant feeding deterrence, especially in the marine habitat are needed to understand the underlying mechanisms promoting predation avoidance. Furthermore, it needs to be considered that some metabolites only show the ability to deter feeding, when they act in synergy or additive with other compounds. Hence, MNPs which show no effect when tested as single chemicals could enhance a feeding deterrent effect, when in combination with other metabolites.[450]

Moreover, even if some of the terpenoids do not show feeding deterrent activities, they might play a role in other important ecological tasks.[404] For example, studies by Tursch et al. 1978,[451] have shown that the cembranoid diterpene sinulariolide from the soft coral *Sinularia flexibilis* is an algicidal molecule, a key chemical responsible for antifouling properties. Other diterpenes have also been shown to be effective in the control of algal overgrowth.[452] The diterpene flexibilide, isolated also from *S. flexibilis*, causes necrotic effects in neighbouring organisms.[404] It is therefore an important allelopathic compound in the inter-specific competition for space, one of the most important factors determining the distribution of species on a coral reef.[404] Furthermore, sinulariolide and flexibilide have also been shown to exhibit marked antimicrobial activity, inhibiting overgrowth of Gram-positive bacteria.[215] To increase our understanding about the way oganic molecules and organisms interact in nature, further studies are needed to improve our knowledge on the functions of NPs.

Concluding, we found that *P. longicirrum* feeds on alcyoniidaen species of the genus *Sarcophyton*, such as *S. glaucum*, incorporates their MNPs and uses at least some of them for defence against potential predatory fish. These defensive metabolites in addition to its highly efficient symbiotic relationship with the photosynthetic *Symbiodinium*, allow this species to grow to an exceptional size, without having to fear intense predation. Furthermore, the exceeding species richness of the genus *Phyllodesmium* can be attributed to the shift from feeding on hydrozoans to feeding on octocorals and the exploitation of this unusual ecological

niche, which most likely enhanced radiation and speciation within this genus. Incorporation of the corals' symbiotic zooxanthellae provides additional nutrition in periods of food shortage, and sequestered defensive MNPs protect these aeolid nudibranchs from predation. These abilities are crucial advantages, which lead to a higher viability.

CHAPTER II: Antibacterial scalarane from *Doriprismatica stellata* nudibranchs (Nudibranchia: Chromodorididae), egg ribbons, and their dietary sponge *Spongia* cf. *agaricina* (Demospongiae: Dictyoceratida)

This chapter has previously been published in a similar form as Hertzer et al. 2020,[3] written by the authoress of this thesis and supervised by Prof. Gabriele M. König and Prof. Heike Wägele.

Abstract

Investigations on the biochemical relationship between *Doriprismatica stellata* (Nudibranchia: Doridina: Chromodorididae) nudibranchs, their egg ribbons, and the associated dietary sponge *Spongia* cf. *agaricina* (Porifera: Demospongiae: Dictyoceratida) led to the isolation of the structurally new scalarane-type sesterterpene 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin, with an unprecedented position of the cyclopropane ring annelated to the ring A. Unlike other scalaranes, which are most often functionalized at C-12 of ring C, it bears two acetoxy groups at C-11 and C-24 instead. The compound was present in all three samples, supporting the dietary relationship between chromodorid nudibranchs of the genus *Doriprismatica* and scalarane-containing dictyoceratid sponges of the Spongiidae family. The results also indicate that *D. stellata* passes the scalarane metabolite on to its egg ribbons, most likely for protective purposes. The scalarane showed antibacterial activity against the Gram-positive bacteria *Arthrobacter crystallopoietes* (DSM 20117) and *Bacillus megaterium* (DSM 32).

Keywords: antibacterial; Dictyoceratida; Nudibranchia; scalarane; sesterterpene

Introduction

In habitats with intense competition and feeding pressure, such as coral reefs, sessile or slow-moving organisms commonly defend themselves with toxic or deterrent molecules.[44,106,153,251,256,453–455] Sponges (Porifera), for example, represent one of the main sources of marine bioactive natural products, due to their impressive chemical armoury.[44] These MNPs can be produced either by the sponge itself or by associated microbial symbionts.[42,48,456–461] Their production is assumed to be useful against numerous environmental stress factors, such as predation, pathogens, overgrowth by fouling organisms, or competition for space.[42-44,48]

Though defensive metabolites are effective against most predators, some also attract nudibranchs of the family Chromodorididae (Mollusca: Gastropoda). These colorful, shell-less sea slugs are specialized to live and feed on noxious demosponges (Porifera: Demospongiae). They evolved the ability to sequester, accumulate, and store spongian metabolites to their own advantage.[9,45,57,95,101,160,195,202,203,251,256,456,462–468] Besides, specific MNPs can be passed on from the sea slugs to their similarly conspicuous and physically defenceless eggs. This has been shown exemplarily for the egg ribbons of certain nudipleuran taxa, such as *Hexabranchus sanguineus*,[43] *Pleurobranchaea maculata*,[176] *Cadlina luteomarginata*,[469] and the two *Dendrodoris* species *D. grandiflora* and *D. limbata*.[470] The passing on of special metabolites from sea slugs to their egg ribbons suggests an additional biological role in the reproductive cycle or as protection of the eggs against predation or fouling.

Chemotaxonomic approaches have shown that chromodorid nudibranchs of the genera *Chromodoris*, *Doriprismatica*, *Felimare*, *Felimida*, *Glossodoris/Casella*, and *Goniobranchus* sequester and reuse spongian-type furanoterpenoids, diterpenoids, and sesquiterpenoids, or scalarane-type sesquiterpenoids and sesterterpenoids from their sponge prey.[95,198,205,229–235] However, confusion in the chemotaxonomy of Chromodorididae arose by multiple changes in species names, including splitting and synonymizations, and the inclusion of species that have since been discovered to be members of other genera. Additionally, a splitting of generic groups into several genera and resurrection of old names increased the confusion.[116,117,231,233,471,472] To classify specialized metabolites in the Chromodorididae in a meaningful way, a solid understanding of their taxonomy, biology, and prey is essential.

Members of *Glossodoris/Casella* and *Doriprismatica* represent such a case of complex systematic challenges and complicated taxonomic histories.[472] Previous work on *Doriprismatica* (former *Glossodoris*) *sedna*,[231] and *Doriprismatica* (former *Glossodoris* or *Casella*) *atromarginata*,[205,230,234,235,473] reported the isolation of scalaranes, homoscalaranes, norscalaranes, spongian diterpenoids and furanoditerpenoids. A dietary origin of these molecules was inferred and attributed to dictyoceratid sponges of the genera *Hyrtios* and *Carteriospongia* (Thorectidae), as well as *Hyattella* and *Spongia* (Spongiidae). Geographical variation was described between *D. atromarginata* populations from Sri Lanka and Australia, containing furanoditerpenes, and a *D. atromarginata* population from India, containing scalarane sesterterpenes as a consequence of sponge prey availability.[205] The isolated metabolites showed various biological activities, such as cytotoxicity, antimicrobial, antiviral and antitumor activities, inhibition of transactivation for the farnesoid X receptor, inhibition of mammalian phospholipase A_2, and ichthyotoxicity against the mosquitofish

Gambusia affinis.[216,218,231,467,468,474–477] Furthermore, a Vietnamese collection of *D. atromarginata* was found on the gorgonian *Menella woodin* (Alcyonacea: Plexauridae). Instead of spongian- or scalarane-type metabolites, they contained steroidal compounds, presumably sequestered from *M. woodin*. [478]

Here, we report the first investigation on the biochemical relationship between *Doriprismatica* (former *Glossodoris*) *stellata* (Doridina: Chromodorididae) of the Indo-West Pacific (Figure 3.1), their egg ribbons, and the associated dietary sponge, identified as *Spongia* cf. *agaricina* (Demospongiae: Spongiidae). We describe the structure elucidation of the new scalarane sesterterpene 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin (Figure 3.2), isolated from all our *Doriprismatica stellata* nudibranch, egg ribbon and *Spongia* cf. *agaricina* samples (Figure 3.3). It is the first scalarane sesterterpene reported with a cyclopropane ring bridging the carbons C-3, C-22 and C-4 in ring A, and an acetoxy group at C-11 instead of C-12 in ring C (Figure 3.2). All ethyl acetate extracts, as well as the isolated new scalarane, showed antibacterial activity against the Gram-positive bacteria *Arthrobacter crystallopoietes* (DSM 20117) and *Bacillus megaterium* (DSM 32), in a screening approach.

Figure 3.1 *Doriprismatica stellata* nudibranch, egg ribbon, and *Spongia* cf. *agaricina* specimen, taken from Hertzer et al. 2020.[3]

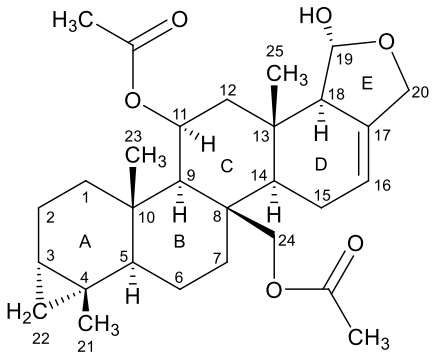


Figure 3.2 The structurally new 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin (relative stereochemistry depicted), isolated from *Doriprismatica stellata* nudibranchs, their egg ribbons and the dietary sponge *Spongia* cf. *agaricina*, taken from Hertzer et al. 2020.[3]

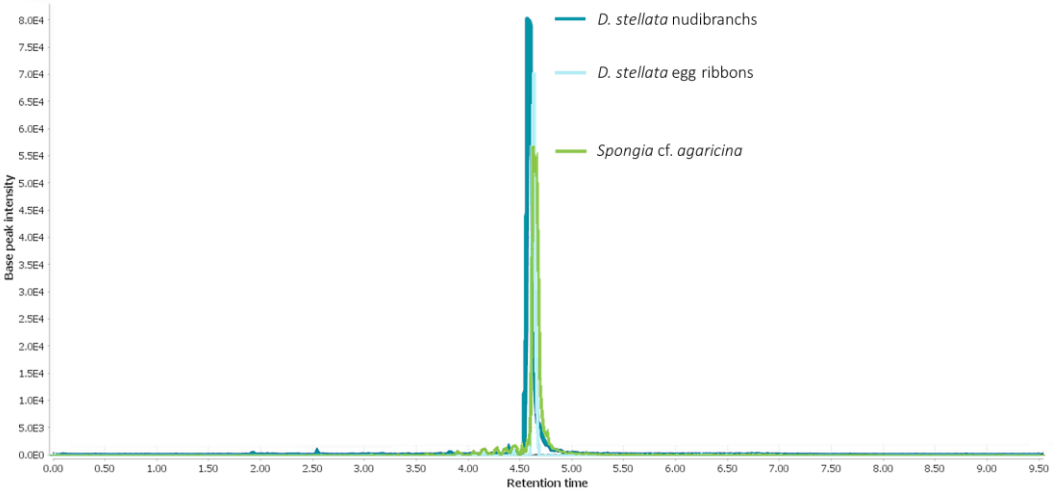


Figure 3.3 Superimposed HPLC–MS chromatogram of *Doriprismatica stellata* nudibranch, egg ribbon, and *Spongia* cf. *agaricina* extracts, showing the presence of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in all three samples, taken from Hertzer et al. 2020.[3]

Results

Chemical investigation on Doriprismatica stellata nudibranchs, egg ribbons and Spongia cf. agaricina

The new molecule was isolated as a white amorphous solid from *D. stellata* nudibranchs (11 mg, 0.3% wet weight). Specific optical rotation was measured in chloroform (c = 0.6), giving $[\alpha]_D$ +40.5. The molecular formula $C_{29}H_{42}O_6$ was established based on ^{13}C NMR data and HRAPCIMS measurements, yielding *m/z* 487.3054 $[M + H]^+$ (SI).[3] The double bond equivalent (DBE) was calculated to be nine and together with the ^{13}C NMR data, giving evidence for one C–C and two C–O double bonds, thus suggested a structure with six rings. The presence of a hydroxy group and ester functionalities was deduced from characteristic IR absorptions at 3416, 1732 and 1234 cm^{-1} (SI).[3,231,232,475]

The planar structure of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin was established by extensive 1D and 2D NMR experiments (^1H, ^{13}C, ^1H,^1H-COSY, DEPT, HSQC and HMBC, see Table 3.1, SI). The ^{13}C NMR spectrum showed 29 resonances attributable to five methyl groups, nine methylene and eight methine moieties (one olefin: C-16 (δ 117.5), and two oxygen bearing groups: C-11 (δ 68.4) and C-19 (δ 98.9)), and seven quaternary carbons, as obvious from a DEPT135 spectrum. The ^1H NMR spectrum showed unusual upfield resonances, diagnostic for a cyclopropyl ring H_2-22 (δ −0.06 brt, J = 4.8 Hz, δ 0.43 dd, J = 3.9, 9.2 Hz). Furthermore, this spectrum proved the presence of the olefinic proton H-16 (δ 5.49 brs), the downfield shifted methine proton H-11 (δ 5.49 brs), and the hemiacetal hydrogen atom H-19 (δ 5.24 d, J = 4.4 Hz). The ^1H NMR spectrum also featured two downfield shifted methylene systems H_2-20 (δ 4.44, δ 4.15 d, J = 12.2 Hz) and H_2-24 (δ 4.91, δ 4.81 d, J = 12.9 Hz), as well as two acetoxy groups H_3-11-OAc (δ 2.06 s) and H_3-24-OAc (δ 2.08 s), and three methyl groups H_3-21 (δ 0.94 s), H_3-23 (δ 0.95 s), and H_3-25 (δ 0.98 s).

Table 3.1 NMR spectroscopic data of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin ($CDCl_3$), taken from Hertzer et al. 2020.[3]

C	δ_H (mult. *J* in Hz)	δ_C	COSY	HMBC	NOESY
1β	1.73, m	35.3, CH_2	H-1α, H-2α/β	C-2, C-3, C-5, C-9, C-10, C-23	H-1α, H-11
1α	0.53, m		H-1β, H-2α/β	C-2, C-3, C-5, C-9, C-10, C-23	H-1β, H-22b
2β	1.96, m	19.0, CH_2	H-3, H-1α/β, H-2α	C-1, C-2, C-22	H-2α, H-3, H_3-23
2α	1.70, m		H-3, H-1α/β, H-2β	C-1, C-2, C-22	H-2β
3	0.55, m	17.9, CH	H-22α/β, H-2α/β	C-1, C-2, C-4, C-5, C-10, C-21, C-22	H_3-21, H_3-23, H-22a
4		16.1, C			
5	0.92, m	53.2, CH	H-6a/b	C-1, C-6, C-7, C-10, C-21, C-22, C-23	H-9, H-22b
6a	1.68, m	22.1, CH_2	H-5, H-6b, H-7α/β	C-5, C-7, C-8, C-10	H-6b
6b	1.49, m		H-5, H-6a, H-7α/β	C-5, C-7, C-8, C-10	H-6a
7β	2.42, m	36.5, CH_2	H-6a/b, H-7α	C-6, C-8, C-9, C-14, C-24	H-7β
7α	0.81, m		H-6a/b, H-7β	C-6, C-8, C-9, C-14, C-24	H-7α, H-14
8		41.2, C			
9	1.01, s	57.4, CH	H-11	C-8, C-10, C-23, C-24	H-5, H-11, H-14
10		36.5, C			

11	5.49, brs	68.4, CH	H-9, H-12α/β	C-8, C-9, C-10, C-11-OAc (21.9, 170.2), C-12, C-13	H-1α, H-9, H-12α/β
12β	2.19, m	44.2, CH_2	H-11, H-12α	C-11, C-13, C-25	H-12α
12α	1.51, m		H-11, H-12β	C-11, C-13, C-25	H-12β
13		32.5, C			
14	1.40, brt (8.5)	55.0, CH	H-15α/β	C-8, C-9, C-13, C-15, C-18, C-24	H-9, H-18
15a/b	2.27, m	24.0, CH_2	H-14, H-16		H-16
16	5.49, brs	117.5, CH	H-20α/β, H-18, H-15α/β		H-20b, H-15a/b
17		135.6, C			
18	2.15, m	62.7, CH	H-16, H-19	C-13, C-14, C-19, C-25	H-12α, H-14
19	5.24, d (4.4)	98.9, CH	H-18	C-13, C-17, C-18, C-20	H-12β, H3-25
20a	4.44, d (12.2)	68.8, CH_2	H-16, H-20b		H-20b
20b	4.15, d (12.2)		H-16, H-20a	C-16, C-17, C-18, C-19	H-16, H-20a
21	0.94, s	23.3, CH_3		C-3, C-4, C-5, C-22	H-3, H-22a
22a	0.43, dd (3.9, 9.2)	22.7, CH_2	H-3, H-22b	C-2, C-5, C-21	H-3, H-22b

22b	-0.06, brt (4.8)		H-3, H-22a	C-2, C-5, C-21	H-1α, H-5, H-22a
23	0.95, s	14.0, CH_3		C-1, C-5, C-9, C-10	H-3, H-24a
24a	4.91, d (12.9)	64.2, CH_2	H-24b	C-7, C-8, C-9, C-14, 24-OAc (21.3, 170.9)	H_3-23
24b	4.81, d (12.9)		H-24a	C-7, C-8, C-9, C-14, 24, 24-OAc (21.3, 170.9)	H_3-25
25	0.98, s	16.1, CH_3		C-12, C-13, C-14, C-18	H-19, H-24b
11-OAc	2.06, s	21.9, CH_3		C-11	
		170.2, C			
24-OAc	2.08, s	21.3, CH_3		C-24	
		170.9, C			

[a] ^{1}H (600 MHz), ^{13}C NMR (150 MHz), all δ in ppm relative to $CDCl_3$ = 7.26/77.0.

[b] Multiplicities determined by DEPT.

The analysis of the 2D NMR data and comparison to literature values [475] suggested that the compound belongs to the family of scalarane sesterterpenoids, with similarities to the deoxoscalarin-like molecule 12,24-diacetoxydeoxoscalarin, previously isolated from a Korean sponge of the genus *Spongia*.[475] The two acetoxy groups were located at the C-11 (δ 68.4) and the C-24 (δ 64.2) carbon atoms based on HMBC cross peaks between the methine proton H-11 (δ 5.49 brs) and the carbon atoms C-11-OAc (δ 21.9, 170.2), as well as the methylene protons H_2-24 (δ 4.91, δ 4.81 d, J = 12.9 Hz) and the carbon atoms C-24-OAc (δ 21.3, 170.9). The location of C-24 was apparent from HMBC cross peaks between the methylene protons H_2-24 (δ 4.91, δ 4.81 d, J = 12.9 Hz) and the carbon atoms C-7 (δ 36.5) and C-14 (δ 55.0). The cyclopropyl group was assigned to the C-3 (δ 17.9) and the C-4 (δ 16.1) carbon atoms, based on a ^{1}H,^{1}H-COSY correlation between the methylene protons H_2-22 (δ −0.06 brt, J = 4.8 Hz, δ 0.43 dd, J = 3.9, 9.2 Hz), and the methine proton H-3 (δ 0.55 m), based on HMBC cross peaks between the protons H_2-22 and the carbon atoms C-2 (δ 19.0), C-5 (δ 53.2) and C-21 (δ 23.3). The entire assignment of all NMR data is given in Table 3.1. The relative configuration was determined from proton coupling constants and NOE data (Table 3.1, Figure 3.4).[3] NOESY cross peaks between H-3 (δ 0.55 m), and H-22a (δ 0.43 dd, J = 3.9, 9.2 Hz), H_3-21 (δ 0.94 s), and H_3-23 (δ 0.95 s), as well as between H_3-23 and H-24a (δ 4.91 d, J = 12.9 Hz), H-24b (δ 4.81 d, J = 12.9 Hz) and H_3-25 (δ 0.98 s), and between H_3-25 and H-19 (δ 5.24 d, J = 4.4 Hz), indicated that these protons share the same orientation on the molecular plane. The chemical shifts of the angular methyl groups CH_3-23 (δ 14.0) and CH_3-25 (δ 16.1) suggested that all ring junctions are *trans*.[479–481] This was supported by NOESY cross peaks between H-22b (δ −0.06 brt, J = 4.8 Hz) and H-5 (δ 0.92 m), angular methines H-5 and H-9 (δ 1.01 s), H-9 and H-14 (δ 1.40 brt, J = 8.5 Hz), and between H-14 and H-18 (δ 2.15 m), from which a shared α-orientation can be inferred. Moreover, the cross peak between H-19 (δ 5.24 d, J = 4.4 Hz) and H_3-25 (δ 0.98 s), and a coupling constant of J = 4.4 Hz between H-19 and H-18, further confirm the *trans* relationship between these protons. Hence, the structure and relative configuration of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin was determined. It needs to be noted that the molecule was unstable over time, especially in ring E, and a variety of degradation products formed by, inter alia, hydrolysis of the hemiacetal and loss of the acetoxy groups.

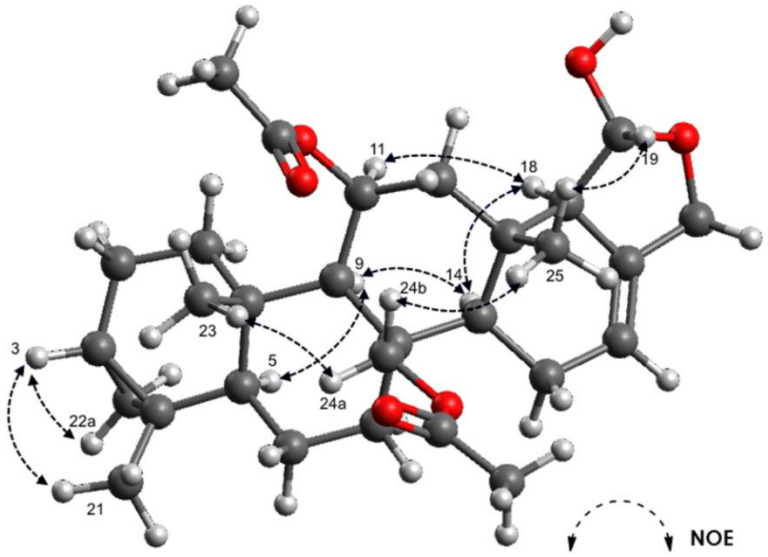


Figure 3.4. Proposed relative configuration of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin. Selected NOE correlations are indicated with arrows. The model was obtained using Avogadro, an open-source molecular builder and visualization tool, version 1.2.0. Taken from Hertzer et al. 2020.[3]

The new scalarane was also detected in *Doriprismatica stellata* egg ribbons and *Spongia* cf. *agaricina* (Figure 3.4).[3] It was isolated from both samples (egg ribbons: 1 mg, 0.1% wet weight; sponge: 0.7 mg, 0.02% wet weight) and the identity was validated by comparison of the MS and NMR spectra.

Antibacterial activity

All ethyl acetate extracts from *Doriprismatica stellata* nudibranchs, egg ribbons and *Spongia* cf. *agaricina* showed antibacterial activity against the Gram-positive *Arthrobacter crystallopoietes* (DSM 20117) in a first screening approach. The pure compound 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin, isolated from all three extracts, was active against the Gram-positive *Bacillus megaterium* (DSM 32) (SI).[3]

Discussion

In this study, the new scalarane-type sesterterpene 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin was isolated from *Doriprismatica stellata* nudibranchs (Mollusca: Gastropoda), their egg ribbons, and the associated sponge *Spongia* cf. *agaricina* (Porifera: Demospongiae), collected from Bunaken National Park (BNP, North Sulawesi, Indonesia). Nudibranchs and their egg ribbons revealed higher concentrations of the scalarane in comparison to the sponge, likely due to a continuous accumulation of this compound.

In general, scalarane sesterterpenes are bioactive metabolites, mainly isolated from marine sources, such as Dictyoceratida sponges and the nudibranchs that feed on them.[203,455,465,468,477] Most of them can be attributed into two main groups, according to their sesterterpenoid skeleton: scalaranes either with the characteristic 6/6/6/6-tetracarbocyclic framework, or with a 6/6/6/6/5-pentacyclic fused ring system, containing an additional lactone, acetal, or furan type ring. Usually, they possess a conserved trans-configuration of the A/B/C/D ring junctions.[467] Scalaranes can be nor- or alkylated, commonly at C-19, C-20 and/or C-24, and contain oxygenated functions, like alcohols, ketones, aldehydes and acetates, at different positions, but particularly at C- and D-ring carbons C-12 and C-16. Exceptions are scalaranes, which have a nitrogen atom in the fifth ring, forming a pyrrole or a lactam unit, with an oxocycle, or which differ in their structure by the presence of cyclobutane or cyclopropane rings.[220,468,477] Until now, only six scalaranes containing cyclopropane rings constructed of C-4, C-19 and C-20, have been identified.[220,482] Honu'enone was isolated from an Indonesian *Strepsichordaia aliena* sponge,[482] and five more scalaranes with a cyclopropane were isolated from a *Dysidea granulosa* sponge, collected in the South China Sea.[220] The first member of the scalarane family, scalarin, was isolated in 1972 by Fattorusso et al. from *Cacospongia* (= *Scalarispongia*) *scalaris*.[483] However, they are also commonly found within genera of the Thorectidae family, like *Carteriospongia*,[484–487] *Collospongia*,[488] *Hyrtios*,[62-67] *Lendenfeldia*,[494–496] *Phyllospongia*,[497–502] *Scalarispongia*,[503] *Smenospongia*,[504] and *Strepsichordaia*.[482,505,506] Furthermore, they occur within the Dysideidae family, in *Dysidea*,[220] and *Lamellodysidea*,[507] and in the Irciniidae genus *Ircinia*.[508] In addition, scalarane sesterterpenes have been isolated from the Spongiidae genera *Coscinoderma*,[509] *Hyatella*,[474,510] and *Spongia*.[475,511–515] Noteworthy, a new cytotoxic scalarane, Perisomalien A, was isolated from *Periploca somaliensis* fruits,[516] and two new antiprotozoan scalaranes were discovered in *Pleurotus ostreatus* and *Scleroderma areolatum* mushrooms,[219] as the first scalaranes from terrestrial sources.

The new 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin shared high similarities with 12,24-diacetoxydeoxoscalarin, a farnesoid X-activated receptor antagonist, isolated by Nam et al. from a Korean sponge of the genus *Spongia*.[475] However, differing from the previously reported scalaranes,[203,220,233,465,468,477,482] the new metabolite is functionalized at C-11 instead of C-12 and has a cyclopropane ring bridging C-3, C-22 and C-4 of ring A.

Scalarane sesterterpenes are considered as chemotaxonomic markers for the sponge families Thorectidae, Dysideidae and Spongiidae.[486,517] In Spongiidae, they have been isolated from the genera *Coscinoderma*, [509] *Hyattella*,[474,510] and *Spongia*.[475,511–515] Our results further support this chemotaxonomic classification, by the presence of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin within *Spongia* cf. *agaricina*, (Dictyoceratida: Spongiidae). Primordially, chromodorid nudibranchs feed upon a broad range of sponges, however, more derived genera like *Glossodoris* and *Doriprismatica* have taken to feeding upon a narrow range of sponges.[95,117,466] As the first chemical investigation of *D. stellata* nudibranchs, our results indicate that these sea slugs live and feed upon the dictyoceratid sponge *Spongia* cf. *agaricina*. This, among other investigations on *Doriprismatica atromarginata* [205,230,234,235,473] and *D. sedna*,[231] supports the idea of a stenophagous dietary relationship between nudibranchs of the genus *Doriprismatica* and scalarane-containing dictyoceratid sponges of the families Thorectidae and Spongiidae. This relationship is further reflected by their shared specialized metabolite 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin, as proven in this study.

Sesterterpenes are a rare terpene class, accounting for less than 2% of all known terpenoids, with only a few reports on their biosynthesis.[518–522] Their structural and functional diversity is likely built upon a complex biosynthetic network, involving multiple gene clusters.[521] However, their frequent occurrence in marine organisms is striking and sponges are considered as the prime source of these terpenoids.[465] Yet determining the origin and *in vivo* production of these metabolites is anything but trivial. So far, only few experiments have been performed to prove biosynthesis of terpenoids and sterols in sponges, with partly inconclusive results.[523–525] A biosynthesis by the mevalonate-independant pathway, as discovered in bacteria, algae and higher plants, could be implied.[526,527] It has even been suggested that sponges might not be capable of *de novo* sesterterpene synthesis.[479,524] Nevertheless, a study by Silva et al. has shown that sponges are capable of *de novo* sterol synthesis,[528] indicating that they have multiple means of obtaining sterols: de novo sterol biosynthesis, in addition to dealkylation of dietary sterols, and incorporation of dietary sterols from marine plankton.

However, sesterterpene synthesis requires sesterterpene cyclases, which are considered as the next frontier for terpenoid cyclase structural biology and protein engineering.[529] So far, six known bifunctional sesterterpene synthases (STSs), containing prenyltransferase (PT) and terpene synthase (TPS) domains, were discovered in fungi of the order Eurotiales, like *Aspergillus clavatus*,[518] *Neosartorya* (= *Aspergillus*) *fischeri*,[519] *Aspergillus ustus*,[521] *Emericella variecolor*,[520] *Penicillium brasilianum* and *Penicillium verruculosum*.[522] Nonetheless, convergent evolution of plant and fungal STSs has been suggested, based on colocalized PT-TPS gene pairs found in the Brassicaceae *Arabidopsis thaliana*, *Capsella rubella* and *Brassica oleracea*, yielding fungal-type sesterterpenes with tri-, tetra-, and pentacyclic scaffolds.[221]

Sponges are known to host complex symbiont communities, with up to 30–60% as microbial biomass.[459,530] These highly species-specific communities are most probably vertically transmitted,[531] and were shown to share and cover various core functions of sponge metabolism by functionally equivalent symbionts, analogous enzymes, or biosynthetic pathways.[461,532,533] Although terpenes and terpenoids are mostly regarded as of fungal or plant origin, biosynthesis by bacteria is not uncommon and attracting increasing research interest.[534] Another *Spongia* species, *S. officinalis*, was shown to harbour bacteria with terpenoid cyclases/protein prenyltransferases responsible for a wide chemodiversity of terpenoid natural products.[460,529] Besides, the marine fungi *Penicillium* spp. and *Aspergillus* spp. are often associated with sponge hosts and were found to produce various terpenoids as well.[42,535,536] Hence, if sponges are not the original producers of these MNPs, it is tempting to argue that the sesterterpene biosynthesis could be performed or mediated by their microbial symbionts. This further indicates a close association, interconnectedness, and probable coevolution between microorganisms, sponges and nudibranchs.[456]

D. stellata was not only found to sequester and accumulate 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin from *Spongia* cf. *agaricina*, but to pass it on to the egg ribbons as well. This, in addition to its bioactivity, might suggest a biological role, either as protection against predation, fouling, or in the reproductive cycle, as mentioned in previous studies on nudibranch egg ribbons.[43,176,469,470] The antibacterial activity of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin could point towards a potential protective role against bacterial biofilm formation. Unfortunately, the metabolite was unstable over time and it was not possible to conduct further assays. Future studies on scalarane sesterterpenes could reveal their full potential and true biological and ecological functions in these complex, co-evolved communities.

CHAPTER III: Protection from self-intoxication: A novel actin isoform in *Chromodoris* nudibranchs supports sequestration and storage of the cytotoxin latrunculin A

Parts of the general introduction and CHAPTER III are planned to be published in a manuscript currently in preparation, written by the authoress of this thesis and supervised by Prof. Gabriele M. König and Prof. Heike Wägele.

Abstract

Marine natural products are important leads in the drug discovery process. Biological and chemical diversity evolve as adaptations to conditions and challenges in a habitat. *Chromodoris*, a genus of colorful nudibranchs (Mollusca: Gastropoda), live and feed on noxious sponges, from which they sequester deterrent and toxic molecules as a chemical defence. Here we investigate sequestration of a cytotoxic marine natural product by five *Chromodoris* species (Doridina: Chromodorididae) from the Central Indo-Pacific Ocean. Comparison of the individual extracts led to HPLC isolation of the highly cytotoxic 2-thiazolidinone macrolide latrunculin A (LatA), identified by HR-ESI-MS, 1D and 2D NMR spectroscopy. LatA was the major metabolite in all examined *Chromodoris* species. Additionally, the macrolide was isolated from the associated sponge *Cacospongia mycofijiensis* (Porifera: Thorectidae), supporting a dietary origin of LatA. Furthermore, LatA was secreted with the mucus trail, where it possibly serves in short-range chemical communication as a semiochemical deterring predators and attracting mating partners.

This study is one of the first to visualize the distribution of LatA within the body of two *Chromodoris* species using MALDI MS-Imaging. LatA was accumulated and stored specifically throughout the mantle tissue, mucus glands, and especially in vacuoles of the mantle dermal formations (MDFs), emphasizing their importance as subcellular toxin repository. Using HEK-293 cells and fluorescence microscopy, we show that the isolated LatA is bioactive and its cytotoxicity results from binding to one of the most essential eukaryotic proteins, G-actin monomers. LatA prohibits polymerization of G-actins and causes severing of the F-actin network, ultimately resulting in the collapse and death of the cell.

To survive and gain an advantage, organisms that produce or acquire toxic natural products must be resistant to the action of these substances. *In vivo* toxicity experiments with direct administration of LatA showed 100% mortality in *Elysia viridis*, but 0% mortality in

Chromdoris heterobranchs. These results led us to investigate a possible underlying molecular resistance mechanism to LatA in the genus *Chromodoris*. Examination and comparison of heterobranch actin genes revealed a novel actin isoform in all investigated *Chromodoris* species with two crucial amino acid substitutions, D187G and R206T, at the 'nucleotide binding' cleft, the binding site of LatA.

We propose that these substitutions lead to target-site modifications, interfering with LatA binding and causing insensitivity. The novel, resistant D187G/R206T actin isoform is suggested to be the prerequisite for *Chromodoris* nudibranchs to sequester LatA from sponges. Furthermore, this would allow them to store LatA in the mantle tissue and use it for their defence, without having to suffer from its cytotoxicity.

Keywords: actin, *Chromodoris*, cytotoxin, latrunculin, Nudibranchia, resistance

Introduction

Nudibranchia, the Genus Chromodoris and Their MNPs

Like all members of the monophyletic Nudibranchia (Mollusca: Gastropoda), sea slugs of the family Chromodorididae have completely reduced their shell.[101] At first glance, a loss of the protective shell as a defensive organ may seem like a disadvantage. However, from an evolutionary perspective, it represents several advantages such as less energetic costs of developing and transporting a shell, as well as other respiratory and excretory benefits.[57,160] Yet, little is known about common predators of heterobranch sea slugs and records of their predation are scarce, although it needs to be noted that significant observations and summaries have recently been published in the scientific literature.[155–159] The scarcity of predation on sea slugs has been attributed to the efficiency of their defences. For example, the recently radiated genus *Chromodoris*,[6,117,120,169] has evolved elaborate mechanisms to protect itself from most predators, such as the selective uptake, sequestration and storage of toxic chemicals from their sponge prey,[9,45,57,95,104,202] and the display of aposematic colors and patterns, as part of Müllerian and quasi-Batesian mimicry.[6,120,169,248,537] So far, the only observed predation on *Chromodoris* nudibranchs has been by members of the carnivorous dorid nudibranch genus *Gymnodoris*.[159,538]

Currently, there are 22 described and further putative 18 undescribed *Chromodoris* species distributed throughout the Indo-Pacific Ocean and the Red Sea.[169] However, it must be noted that species delimitation for this genus is subject to ongoing research, due to exceptional challenges, such as extraordinary cryptic diversity, mimicry and recent radiation with described

introgression, mitochondrial capture and hybridization, and could therefore undergo further revisions in the future.[6,117,120,169] Of these putative 40 *Chromodoris* species, 12 have been chemically investigated and were included in several reviews.[9,57,104,202,203] All of them were found to contain diet-derived, bioactive, often cytotoxic, MNPs with variations in relation to prey availability. Analysis of Japanese *C. willani* revealed the sesterterpenes deoxymanoalide and deoxysecomanoalide,[539] and Japanese *C. aspersa* showed a mixture of sesquiterpenoid inorolides and scalaranes.[540] Indonesian *C. lochi* was found to sequester the polyketides laulimalide and isolaulimalide,[541] whereas *C. lochi* from Vanuatu contained hybrid PKS-NRPS derived mycothiazole.[542] Investigation of a *C. inopinata* specimen from Sri Lanka revealed the terpenoids aplyroseol, γ-lactone 9, and spongian-16-one.[543] Indian *C. mandapamensis* was found to contain the diterpenoid spongiadiol and a mixture of related spongiane compounds.[255] Extracts of *C. hamiltoni* from South Africa contained unusual chlorinated homo-diterpenes hamiltonin A-D and the sesterterpene hamiltonin E,[544] and extracts of *C. hamiltoni* specimens from East Africa showed spongian diterpene lactones 7β,11β-diacetoxy-16-oxospongian-17-al and 7β,11β-diacetoxy-16-oxospongi-12-en-17-al.[545] *C. africana,* from the Red Sea, contained the furanoterpene kurospongin.[546] Most notably, though, is the selective uptake and storage of the cytotoxins latrunculin A (LatA, Figure 4.1) and latrunculin B (LatB) by closely related *Chromodoris* species.[248] Until now, the incorporation of latrunculins has been reported for Australian *Chromodoris annae*, *C. elisabethina*, *C. kuiteri*, *C. lochi* and *C. magnifica*,[246–248] South and East African *C. hamiltoni*,[544,545] as well as *C. quadricolor* [547,548] and *C. africana* from the Red Sea.[546]

Sponges Containing Latrunculins

Chromodoris nudibranchs acquire latrunculins from their sponge food sources. In the Red Sea and around Africa sponges that contain latrunculins are *Negombata* (previously *Latrunculia*) *magnifica*, *N. corticata* ,[547–552] *Diacarnus erythraeanus* and *D. ardoukobae* (all four species belong to the Porifera: Demospongiae: Poecilosclerida: Podospongiidae),[553] whereas the unrelated *Cacospongia* (previously *Spongia*) *mycofijiensis* (Porifera: Demospongiae: Dictyoceratida: Thorectidae) is a known spongian source of latrunculins in the Indo-Pacific Ocean.[247,552,554] *Dactylospongia* sp., *Hyattella* sp., and *Fasciospongia rimosa* from the Indo-Pacific have also been reported to contain latrunculins, however, it was suggested that these samples were misidentified.[552] Even so, the taxonomically unrelated sponge genera *Negombata* and *Cacospongia* are reliable sources of latrunculins, especially LatA,[552] and serve as food sources for *Chromodoris* nudibranchs.

Latrunculin A

In 1980, after observing the ichthyotoxic effect of exudates from the Red Sea sponge *Negombata magnifica*,[549] Kashman and coworkers isolated and elucidated the structures of LatA and LatB for the first time. Latrunculins are cytotoxic 14- and 16-membered macrolides with an attached rare 2-thiazolidinone moiety (Figure 4.1), derived from mixed polyketide synthase/non-ribosomal peptide synthetase (PKS-NRPS) biogenesis and are unique to the marine environment.[552,554]

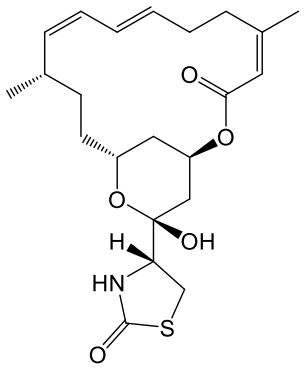


Figure 4.1 Chemical structure of latrunculin A.

Although there is much evidence indicating that sponge symbionts are the true producers of various MNPs, so far, it was not possible to find the biosynthetic gene cluster of LatA and more extensive studies are needed to solve this question.[48,459,532,555–562] In the following 40 years, latrunculins inspired, and continue to do so, the curiosity of researchers from diverse fields, investigating further marine organisms as sources, their biological activities, biosynthesis, total syntheses, the synthesis of analogues and epimers, and especially their mechanism of action and potential for therapeutic applications.[27,551–553,561,563–603] This substantial interest in latrunculins is primarily based on their actin filament depolymerizing effect and LatA has become the most widely used small molecule to study actin-based processes, microfilament organization, cytoskeleton dynamics, mechanisms of cellular function and the potential of latrunculins as a treatment against cancer, neurological disorders and infectious diseases.[27,573,576,600,603–605] The multiple applications of LatA stem from the myriad of biological functions of actin, which are critical for the maintenance of eukaryotic life.

Actin

Actin is one of the most abundant proteins on Earth and above all in all eukaryotes, from yeast to plants to humans. This essential and highly conserved protein of around 375 amino acids is ubiquitously expressed in every eukaryotic cell.[606–612] Actin exists as a 'universal pool' of globular monomers (G-actin), which can be tapped into by many processes to dynamically nucleate and polymerize G-actin into helical filaments (F-actin), and to reversibly depolymerize and reorganize F-actin, depending on the interacting partners and required cellular processes. ATP-hydrolysis to ADP-pi, as a source of energy, and binding of Mg^{2+} and Ca^{2+} ions are essential components of the polymerization-depolymerization process. In short, ATP binds to the central 'nucleotide binding' cleft of G-actin, this ATP-actin is then incorporated into the barbed end of F-actin, the ATP is then hydrolyzed, phosphate is released, the resulting ADP-actin is released at the pointed end of F-actin and undergoes nucleotide exchange to generate ATP-actin, which can then be used for another round of polymerization. This process is called actin treadmilling.[606,610,612–623] Actin filaments can be further assembled into a wide variety of at least 15 distinct, higher-order cellular structures in metazoan cells. Each of these structures performs specific functions and actins participate in more protein-protein interactions than any other known protein, with numerous partners, of which more than 60 classes are currently known. Actin-binding proteins modulate actin filament dynamics by sequestration of G-actin monomers, increase of ATP-ADP-pi exchange, and capping and severing of F-actin.[600,606–610,612,614,615,620,621,623–625] The G-actin monomer folds into two major α/β-domains, similar to all proteins of the structural superfamily of the sugar kinases, hexokinases and Hsp70.[608] Each large domain consists of two subdomains, and a four-subdomain nomenclature has traditionally been adopted (I-IV, Figure 4.2).[614]

Actin Isoforms: Evolution and Functions

Although often thought of as a single protein, actin consists in most eukaryotes as different isoforms encoded by a multigene family, a set of structurally related genes that descended by duplication and divergence from common ancestral genes.[606,607,609–612,622,626–636] These isoforms (i.e., isoproteins), or isoactins, serve overlapping, but non-redundant functions and their number can vary greatly between different lineages. As cells became more specialized and architecturally complex, the compositional diversity of actin and tropomyosin isoforms provided the opportunity for an extraordinary diversity of functions.[607,612,622,633–636] Expansion and diversification of actin genes, as seen in many plants, or in anticorrelation, the expansion and diversification of actin regulators like tropomyosin, as seen in various animals, allowed for

an enhancement of the functional repertoire in a multicellular environment.[612,622,635] Post-translational modifications, such as arginylation and acetylation, and 'silent' code regulations, by synonymous changes at the gene and mRNA level, add an additional layer of complexity.[612,622,635–638] In Mammalia, 6 actin isoforms are known, 4 related to muscular functions (α-skeletal, α-cardiac, α-smooth, and γ-smooth muscles) and 2 related to the cytoskeleton (β- and γ-actin).[607,612,626,630] Little is known about the diversity, classification, expression and molecular evolution of actin isoforms in marine molluscs and nothing is known for the Nudipleura group. Some studies have described multiple isoforms for molluscs. For example, the planorbid snails *Biomphalaria glabrata* and *Helisoma trivolvis* contain at least 5-10 actin isoform genes.[639,640] The presence of several actins was also inferred for scallops like *Chlamys farreri*,[641] with 12 to 15 actins in the sea scallop *Placopecten magellanicus*,[642] and 8 actins in the great scallop *Pecten maximus*.[643] In the Pacific oyster *Crassostrea gigas* 13 distinct actin genes were annotated.[644] Various coleoid Cephalopoda have at least 3 actin isoforms.[645] Marine sea snails of the genus *Haliotis* contain 3 to 8 isoforms,[646,647] 1 actin gene was described from the marine snail *Rapana venosa*,[648] and the sea hare *Aplysia californica* was found to contain at least 3 distinct actin isoforms, but a much larger gene family is suspected.[649–651] Most invertebrate actin isoforms are ubiquitously expressed and have been reported to share the highest similarity to vertebrate cytoplasmic β-actin, even when isolated from the muscle tissue, and it was speculated that cytoplasmic actin might be the ancestral form, or evolutionary archetype, from which muscle actins have evolved.[647,648,652]

The origin and evolution of actin, one of the most conserved gene families, is a subject of ongoing research, examining the origin of the eukaryote branch of life.[609,611,614,622,635,653–661] Nevertheless, it has been suggested, that the high similarity between the structures and functions of actins and actin modulators in eukaryotes, Asgard archaea, and the bacterial actin-like homologs MreB, FtsA, and ParM, derives from an ancestral polymer-forming actin-like protein, presumably already present in an RNA-based 'urkaryote', hence, preceding the divergence of eukaryotes, archaea and bacteria more than two billion years ago.[622,653,654,656–658,661–663] Keeping this in mind, it is not surprising that actin plays such a crucial role in nearly all cellular processes and that it has remained almost unchanged, due to negative selection pressure imposed by the various interactants and large numbers of interactions it must preserve.[612,622,629,653] A partial, but not nearly comprehensive, list of its functions includes a main role in the cytoskeleton formation, providing structural support and shape to the cell, transport of proteins and vesicles within the cell, cell proliferation, junction formation, cell migration, chromatin remodeling, transcriptional regulation and DNA replication.[607,609–612,622,624,664,665]

Actin Targeting Toxins: Mode of Action, Resistance and Disorders

The vital importance, evolutionary age and high conservation of the actin protein family makes it a perfect target for toxins. Several organisms have evolved the ability to produce or acquire toxins that address actin, thus ensuring effectiveness against a broad spectrum of organisms and often lethality.[27,603,605,666] Some heterobranch sea slugs were found to either produce or acquire actin-binding toxins. For example, the macrolide aplyronine A was isolated from the sea hare *Aplysia kurodai* [27,603,667–670] and the trisoxazole-bearing macrolides kabiramides and ulapualides were isolated from the chromodorid nudibranch *Hexabranchus sanguineus* and its egg ribbons.[27,43,242,243,603,666,671,672] Aplyronine A, as well as the trisoxazole macrolides, are highly cytotoxic due to their binding to G-actin in a molar ratio of 1:1 and they act as small molecule biomimetics of actin-capping proteins like gelsolin, additionally severing F-actin. Until now, it is unknown how heterobranch sea slugs can tolerate the toxicity of actin-binding macrolides like aplyronine A, kabiramides, and latrunculins.

Although trisoxazole macrolides and aplyronine A have a similar mode of action as latrunculins, they interact with actin residues between subdomains I and III (Figure 4.2) and bind to the hydrophobic 'target binding' cleft, mimicking actin-capping proteins. Indeed, most actin-binding proteins and all actin-depolymerizing macrolides bind to the 'target binding' cleft between subdomains I and III, except latrunculins.[27,559,608,671,673] Because of that, latrunculins are unique as they are the only currently known toxins that bind to the 'nucleotide binding' cleft of G-actin, close to the ATP-binding pocket, between subdomains II and IV (Figure 4.2).[27,573,576,581,600,608] However, like aplyronine A and kabiramides, cytotoxicity of LatA is a direct result of its binding to G-actin monomers in a 1:1 molar ratio, impeding polymerization and dissociating phosphate from F-actin,[600] and it was shown that one amino acid mutation of actin can be enough to cause resistance. Several actin amino acid positions were identified, that lead to latrunculin resistance when mutated (Table 4.1).[573,574,576,581,674–680] However, the precise changes and mechanisms leading to a loss of latrunculin binding, as well as how specific mutations of the 'nucleotide binding' cleft affect ATP-binding and actin polymerization as a whole require further investigation. There is evidence suggesting basic physiochemical changes, strengthened G-actin monomer-monomer interactions, altered F-actin dynamics and overall increased F-actin stability.[675,677,679,681,682]

Table 4.1 Amino acid mutations of actin proteins that inhibit binding of latrunculins.

Amino Acid Mutations	Subdomain	References
Tyr 69 (Y)	II	576,674
Glu 117 (E)	III	675
Asp 157 (D)	III	574,576,674
Val 159 (V)	III	676
Arg 183 (R)	IV	573,581,674,677
Asp 184 (D)	IV	573,581,674
Thr 186 (T)	IV	576,674
Asp 187 (D)	IV	678
Arg 196 (R)	IV	679
Arg 210 (R)	IV	573,576,674
Asp 211 (D)	IV	573
Lys 213 (K)	IV	573
Glu 214 (E)	IV	573,576,674
Lys 215 (K)	IV	573
Arg 335 (R)	I	680

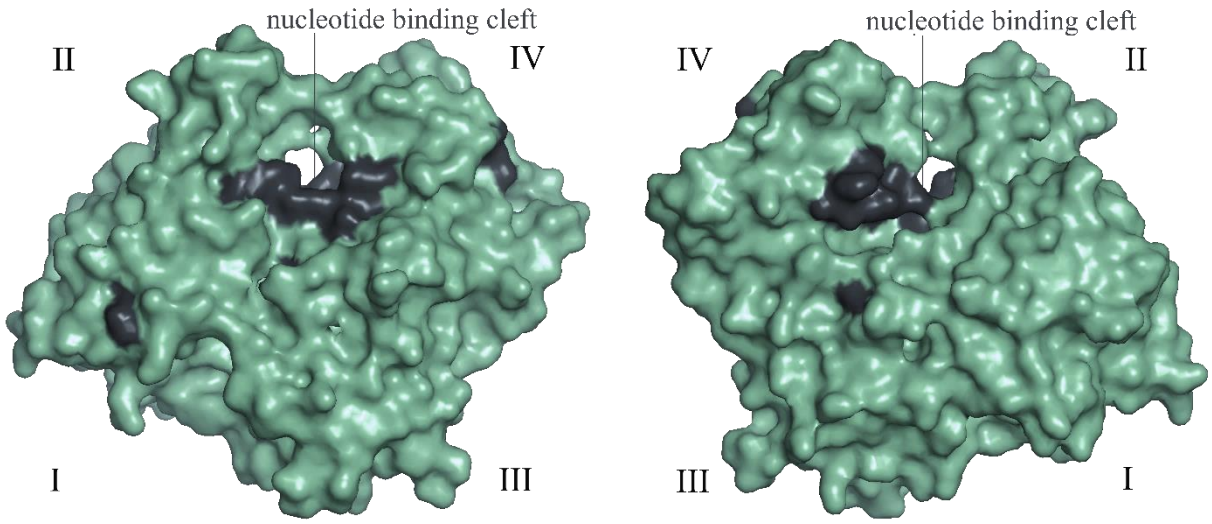


Figure 4.2 Surface-model of native G-actin and its subdomains I-IV (green, PDB: 3HBT[683]) with reported sites of amino acid mutations (black), each leading to the inhibition of latrunculin binding (see Table 4.1). The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC. was used to display and color the model.

These changes can have far-reaching consequences, especially in organisms with only a limited number of genes for cytoplasmic actins, like humans. Deregulation of the cytoplasmic β-actin gene is associated with several types of cancer and immunodeficiency, and heterozygous β-actin gain-of-function and loss-of-function mutations were found to cause several diseases and disorders in humans, in particular: developmental disorders and the Baraitser-Winter syndrome,[675,677,679,684–695] which is characterized by distinct facial features, structural brain malformation, dystonia, hearing loss, seizures, ocular coloboma, cardiac defects, short stature, developmental delay and intellectual disabilities. In two of these studies, cells of the patients were treated with LatA and found to be resistant, due to increased F-actin content and stability.[675,679] Given the significance of LatA and its effect on actins, our knowledge regarding how *Chromodoris* nudibranchs, as well as *Negombata* and *Cacospongia* sponges, resist their toxic defence is quite limited. Compartmentalization of cytotoxic compounds has frequently been observed in sponges.[551,696–704] Gillor and colleagues investigated the localization of LatB in *N. magnifica* by immunolabeling, which revealed that LatB is produced either by the sponge or by its symbionts in choanocytes, but is stored in high concentrations within membrane-bound, actin-free, vacuoles in archeocytes.[551] This shows that *N. magnifica* uses at least compartmentalization of LatB to prevent autotoxicity. However, other resistance mechanisms have not yet been investigated. Similarly, predators of *N. magnifica* and *C. mycofijiensis*, such as *Chromodoris* nudibranchs, must also be able to prevent the toxic effects of latrunculins and autotoxicity to feed on these sponges and survive.

Aim of the Study

Here, we investigate the sequestration and distribution of a toxic MNP by five closely related nudibranch species: *Chromodoris annae*, *C. dianae*, *C. lochi*, *C. strigata* and *C. willani* (Figure 4.3). Additionally, we examine a possible underlying molecular resistance mechanism in these nudibranchs, a prerequisite that would allow them to sequester, survive and store the cytotoxin LatA. Our key objectives in this study were to: (1) chemically investigate and characterize the main metabolite in all five *Chromodoris* species, their dissected body and mantle tissue, secreted mucus, and prey sponge *Cacospongia mycofijiensis*; (2) visualize the cross-sectional distribution of the main metabolite LatA in *C. annae* and *C. dianae* nudibranchs by MALDI MSI; (3) examine and compare LatAs toxicity and mode of action in HEK-293 cells, and *in vivo* in *Chromodoris* and *Elysia viridis* heterobranchs; (4) investigate a possible molecular resistance mechanism in *Chromodoris* nudibranchs, by comparison of actin nucleotide and amino acid sequences from *Chromodoris* species, *Elysia viridis*, *Aplysia californica*, *Flabellina affinis*, *Embletonia pulchra* and *Armin tigrina*.

Results

Chemical Investigation of Chromodoris Nudibranchs and Cacospongia mycofijiensis.

We investigated extracts from five closely related nudibranch species: *Chromodoris annae*, *C. dianae*, *C. lochi*, *C strigata* and *C willani* (Figure 4.3). The nudibranchs were dissected into body and mantle tissue prior to separate extraction. Furthermore, we collected and investigated mucus collected from their notum and trails, and samples of the associated sponge prey *Cacospongia mycofijiensis* (Porifera: Thorectidae). Comparison of the individual extracts, using HPLC-MS, revealed that all of the *Chromodoris* nudibranchs, as well as their food source *C. mycofijiensis*, contained a shared metabolite with protonated ion fragments and adducts typical for the 2-thiazolidinone macrolide latrunculin A (LatA; *m/z* 386 $[M+H-2H_2O]^+$, 404 $[M+H-H_2O]^+$, 422 $[M+H]^+$ and 444 $[M+Na]^+$, SI). The metabolite was isolated by HPLC and conclusively identified as LatA (0.4 mg/specimen, Figure 4.1), by further 1D and 2D NMR spectroscopy (SI), optical rotation measurements, and comparison to the literature.[550(p198),568,569,584,588,705] HPLC-MS analysis revealed especially high concentrations of LatA in the extracts of the dissected mantle tissue, in comparison to the dissected body tissue (SI). LatA was also secreted in small amounts with the mucus trail and mucus collected directly from the notum (SI).

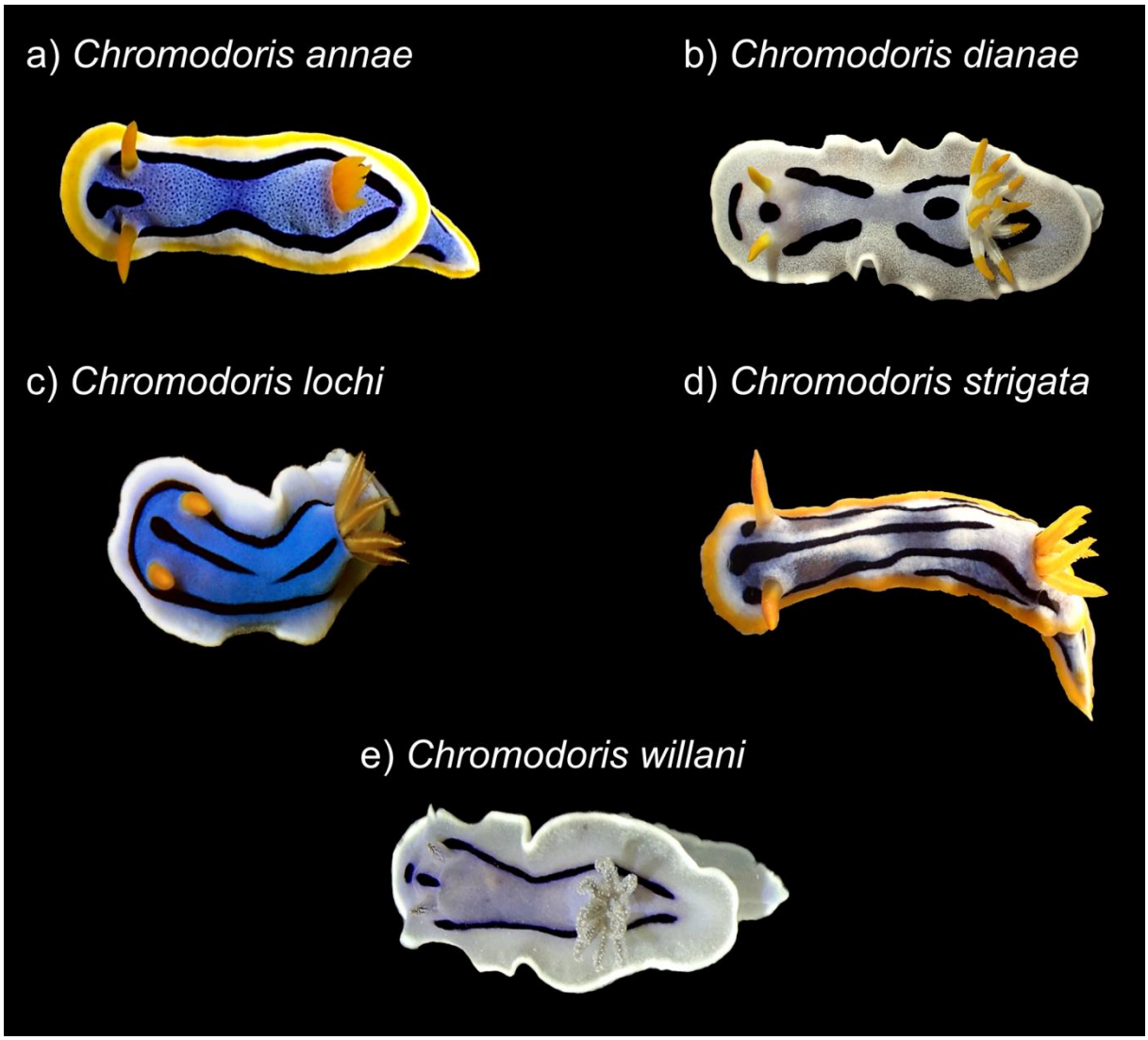


Figure 4.3 Photographs of the investigated *Chromodoris* species.

MALDI-MS Imaging.

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) has become a fundamental analytical tool for analysing substances in biological specimens. This method allows the correlation of spatial ion distribution with histological features. Here, it was used to map the distribution of the cytotoxin LatA (LatA; *m/z* 444.1813 $[M+Na]^+$, 10 µm/pixel, green) within cross-sections of *C. annae* and *C. dianae* tissue (Figure 4.4). MALDI MSI revealed that LatA is not randomly distributed in the nudibranch's body. Instead, it is explicitly stored in the mantle tissue, especially in vacuoles of the mantle dermal formations (MDFs) (Figure 4.4 A/B3, SI) and mucus glands. For the first time, these results directly visualize the long-proposed hypothesis that the mantle tissue of dorid nudibranchs, their mucus glands, and especially the vacuoles of MDFs, store large quantities of toxins,[93,101,113(p200),248–250,706–710] in this case, LatA.

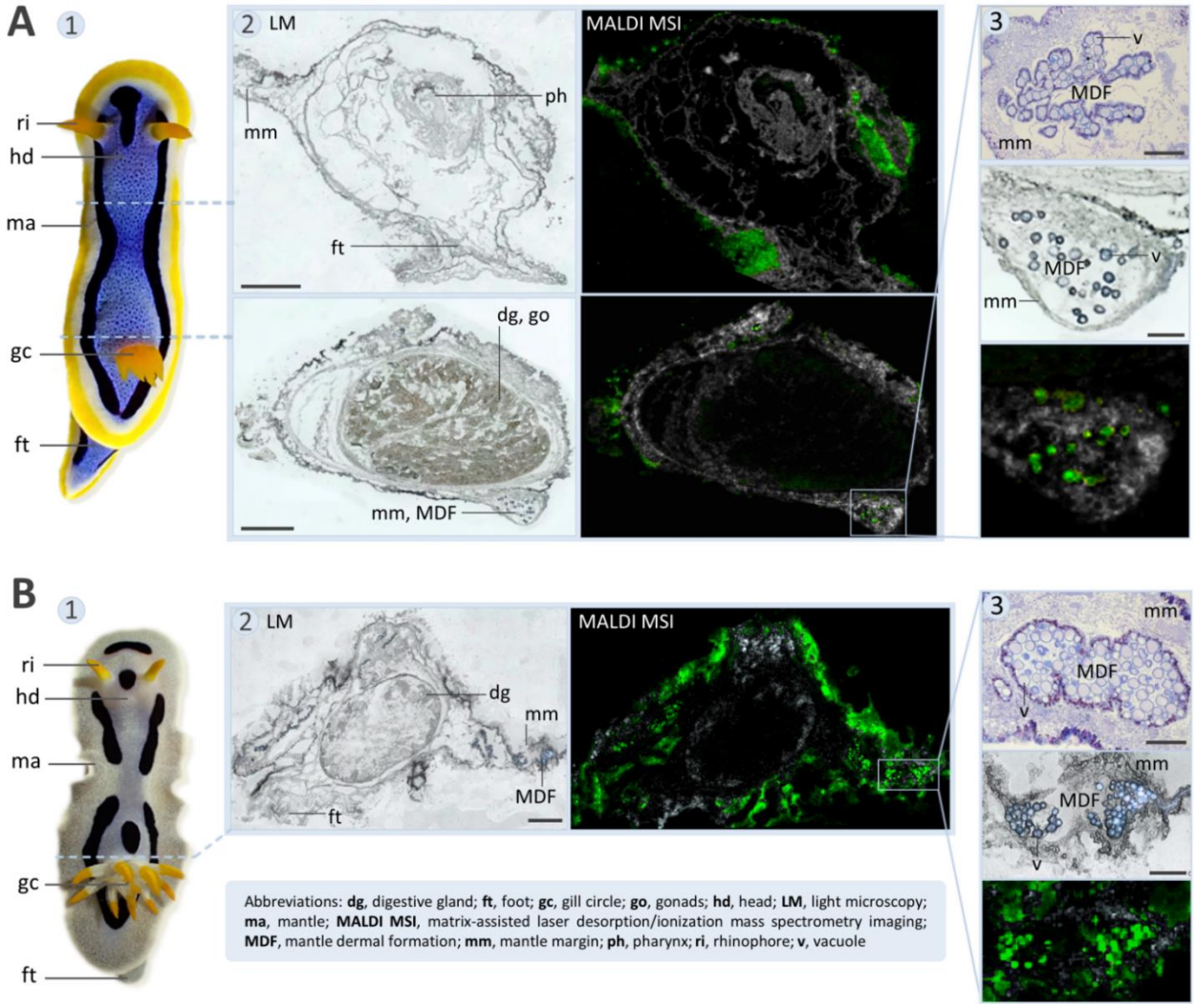


Figure 4.4 Comparison of *Chromodoris* optical and MALDI mass spectrometry images, revealing the distribution of LatA (green). **A1** Dorsal overview of a *C. annae* specimen (top). **A2** Overview of the anterior and middle cross-sections (20 µm, dotted lines) of *C. annae* by light microscopy and MALDI MSI, showing the distribution of LatA (*m/z* 444.1813 $[M+Na]^+$, 10 µm/pixel) in green. **A3** Close-up view of a *C. annae* MDF histological section (top), optical (middle), and MALDI image (bottom). **B1** Dorsal overview of a *C. dianae* specimen (bottom). **B2** Overview of the posterior cross-section (20 µm, dotted line) of *C. dianae* by light microscopy and MALDI MSI, showing the distribution of LatA (*m/z* 444.1813 $[M+Na]^+$, 10 µm/pixel) in green. **B3** Close-up view of a *C. dianae* MDF histological section (top), optical (middle) and MALDI image (bottom).

Abbreviations: **dg**, digestive gland; **ft**, foot; **gc**, gill circle; **go**, gonads; **hd**, head; **LM**, light microscopy; ma, mantle; **MALDI MSI**, matrix-assisted laser desorption/ionization mass spectrometry imaging; **MDF**, mantle dermal formation; **mm**, mantle margin; **ph**, pharynx; **ri**, rhinophore; **v**, vacuole. Scale bars: **A/B2** = 1000 µm; **A/B3** = 200 µm.

Fluorescence Microscopy.

Bioactivity of LatA, isolated from *Chromodoris* nudibranchs, was examined for its effect on the F-actin cytoskeleton of human embryogenic kidney cells (HEK-293), using fluorescence microscopy. Cytotoxic activity of LatA is a direct result of its binding to globular actin-monomers (G-actin), impeding their polymerization to filamentous actin (F-actin).[576,581] In addition to G-actin sequestration, LatA depolymerizes F-actin, by severing and rapidly dissociating phosphate from its ends, ultimately resulting in the collapse of the cytoskeleton.[600] HEK-293 cells, incubated with 50 µM of the *Chromodoris*-LatA, collapsed, deformed and rounded up, due to the destruction of the filamentous actin (Figure 4.5). This indicates, that LatA is stored unaltered and active in the body of *Chromodoris* nudibranchs. Thus, raising the question of how *Chromodoris* specimens survive and store the active LatA, without having to suffer from self-intoxication (i.e., autotoxicity).

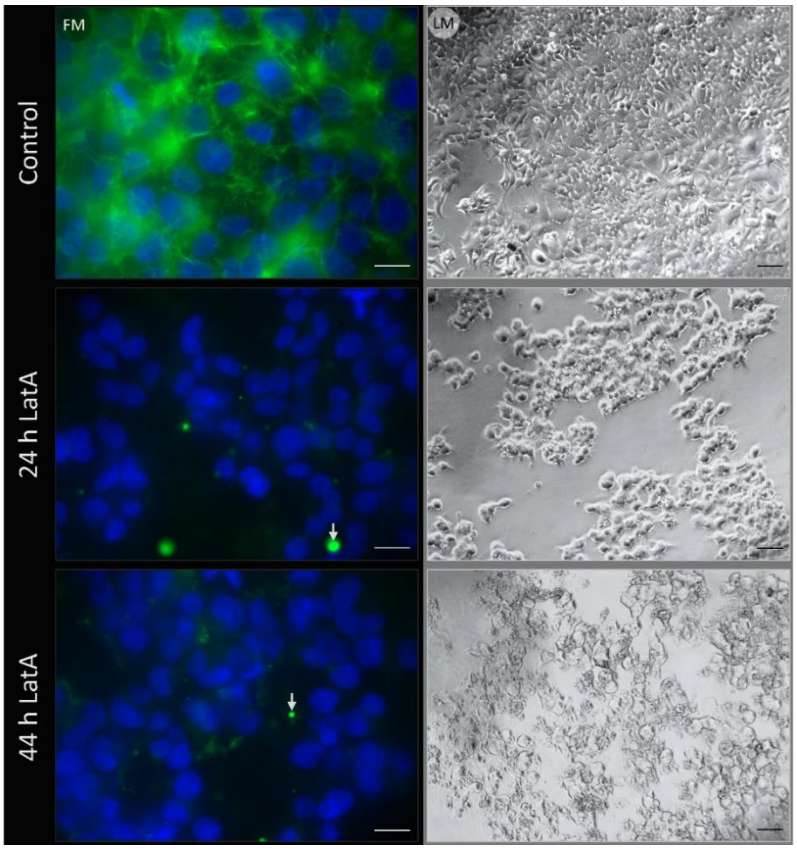


Figure 4.5 Cytoskeletal visualization of HEK-293 cells using fluorescent microscopy (**FM**, left) with Phalloidin staining filamentous actin (green), and Hoechst staining DNA (blue), in comparison to light microscopy images (**LM**, right). Cells were incubated in DMEM medium, either with 50 µM LatA, isolated from *Chromodoris* nudibranchs, for 24 h (middle) and 44 h (bottom) or without LatA (control, top). Arrows mark condensed actin. Scale bars: **FM** = 20 µm; **LM** = 50 µm.

Comparative Analysis of Heterobranchia Actin Genes.

So far, only nudibranchs of the genus *Chromodoris* are known to store latrunculins,[246–248,544,584,708] indicating an exceptional resistance mechanism within this group. LatA's toxicity is a direct result of G-actin sequestration and F-actin severing,[576,581,600] therefore we isolated DNA from *C. annae* (4 sp.), *C. dianae* (2 sp.), *C. lochi* (2 sp.), *C. strigata* (3 sp.), *C. willani* (2 sp.), and *E. viridis* (1 sp.) and used PCR with primers specifically designed to amplify fragments of the coding region (~ 885 bp) of putative actin genes, including the binding site of LatA. The amplified actin gene fragments were isolated, sequenced and aligned with cladobranch transcriptome sequence data, kindly provided by D. Karmeinski,[118] from *A. tigrina*, *E. pulchra*, and *F. affinis* and an actin sequence from *A. californica* (GenBank, NCBI). The actin sequences were compared and examined for mutations (Figure 4.6), which have been reported to cause LatA resistance (see Table 4.1 and references cited therein).

The amino acids threonine T186 and arginine R210, which directly bind to latrunculins, are highly conserved in all heterobranchs. However, several differences in the latrunculin-binding region can be detected in *Chromodoris* nudibranchs, of which most are representing substitutions of amino acids with similar chemical properties, such as lysine (pKa 10.79, pI 9.87) → arginine (pKa 12.48, pI 10.76, alkaline, polar), serine (pI 5.68) → asparagine (pI 5.41, neutral, polar), or threonine (pI 5.60) → serine (pI 5.68, neutral, polar). Nonetheless, *Chromodoris* actins show crucial substitutions of aspartic acid D187 (pKa 3.86, pI 2.98, acidic, polar) to glycine (pI 5.97) and arginine R206 (pKa 12.48, pI 10.76) to threonine (pI 5.60). Aspartic acid D187 and arginine R206 usually form a salt-bridge with a strong positive density, essential for the stabilization of the tertiary structure of actin and binding of latrunculins (see Figure 4.7).[576,678,682] Their substitutions to the neutral amino acids glycine D187G and threonine R206T disable the formation of this salt-bridge, possibly altering latrunculin binding, hence, resulting in LatA resistance. However, additional studies regarding heterobranch actins and full characterization of the novel *Chromodoris* actin isoform, as well as the affinity of its binding sites are needed to further investigate this hypothesis.

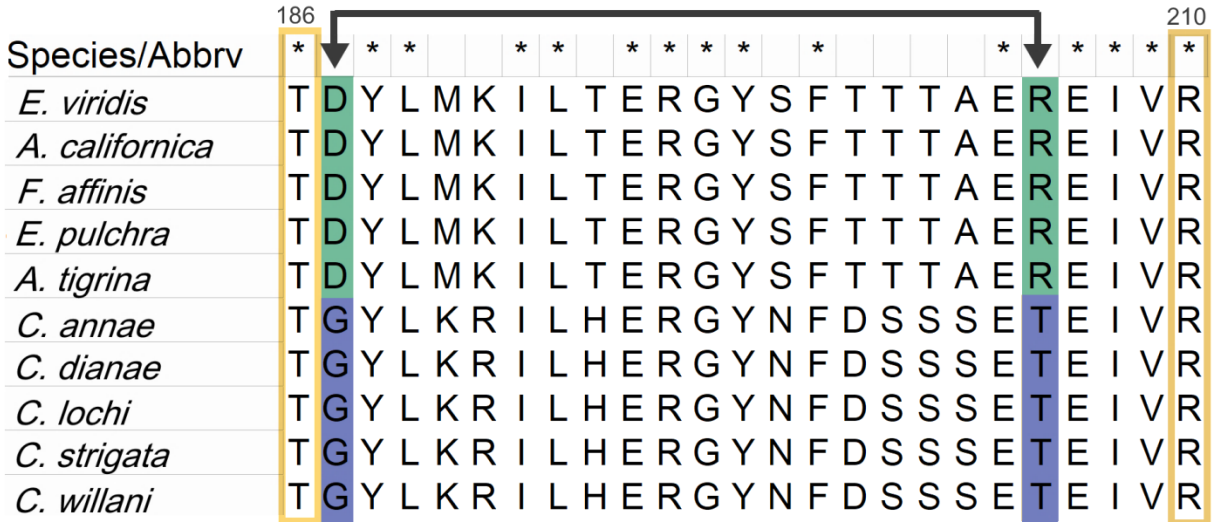


Figure 4.6 Multiple consensus amino acid sequence alignments of the latrunculin-binding region of heterobranch actins. Threonine T186 and arginine R210, directly interacting with latrunculins, are framed with yellow rectangles. Aspartic acid D187 and arginine R206 forming a salt bridge (indicated by arrows) are highlighted in green. The substitutions D187G and R206T are highlighted in blue. Highly conserved amino acids are marked with an asterisk.

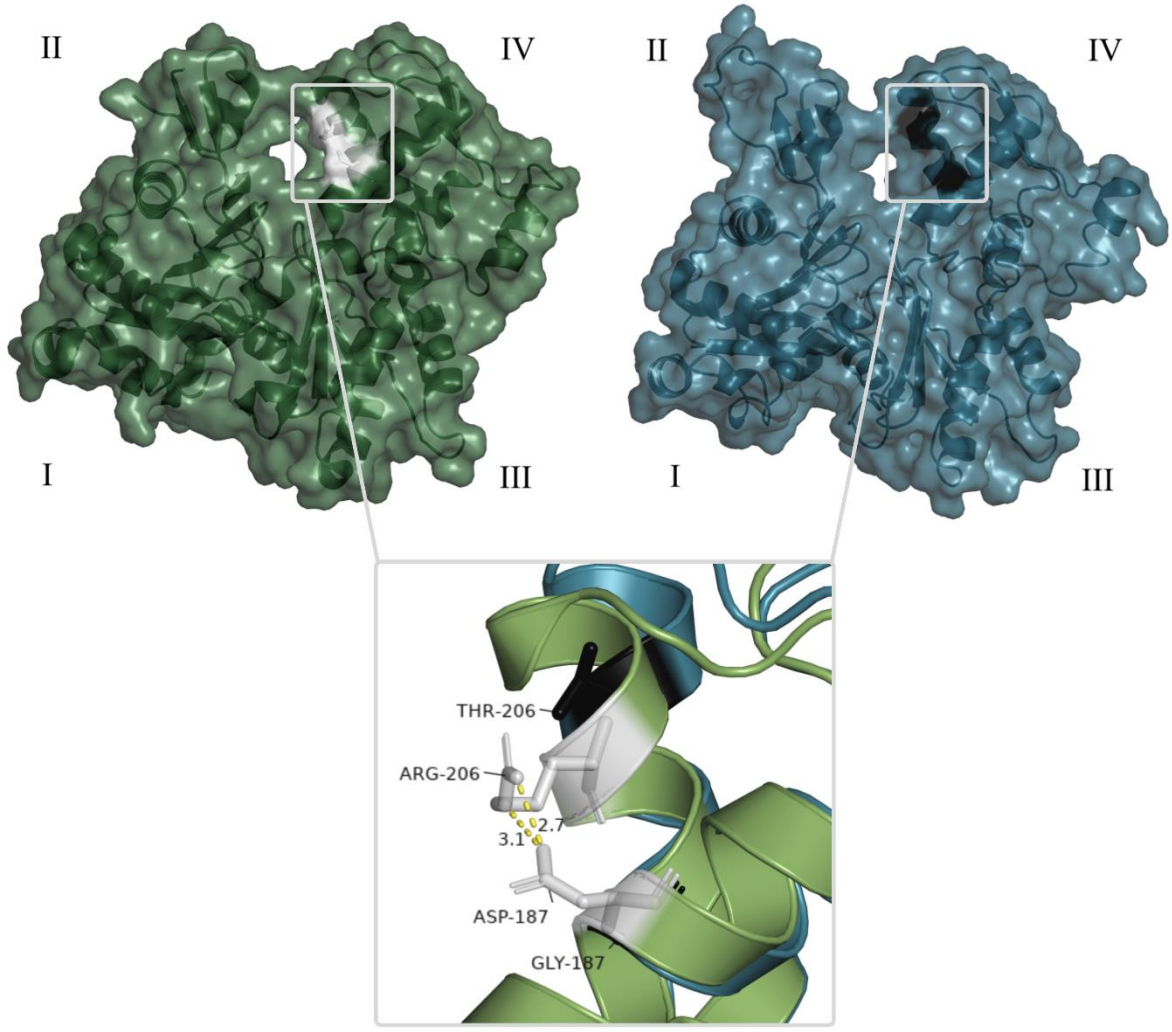


Figure 4.7 Overall and close-up comparison of a model of native G-actin (green, left, PDB: 3HBT,[683] 375 aa) and an *in silico* hybrid-model of a combination of the amplified and sequenced *Chromodoris* actin (286 aa) and subsequently added parts of an *A. tigrina* actin sequence (89 aa), to approximate missing amino acids (SI, blue, right, 375 aa. The model was created using the Phyre2 web portal for protein modelling, prediction and analysis[711]). Native aspartic acid D187 (ASP) and arginine R206 (ARG), shown in white, form a salt bridge (yellow lines, 3.1 and 2.7 Å), whereas the substitutions glycine G187 (GLY) and threonine T206 (THR), shown in black, present in *Chromodoris* actin, are unpolar and do not interact. Both substitutions are adjacent to the nucleotide binding cleft and are part of the latrunculin-binding region. A mutation of D187 has previously been reported experimentally to inhibit latrunculin binding (see Table 4.1 and Figure 4.2). The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC. was used to display and color the models.

Genetic and BLAST® [711] analyses (Figure 4.8, SI), and percent sequence identity comparison (Table 4.2) of the newly obtained *Chromodoris* sequences showed that they shared ≥ 68% identity at the DNA level and ≥ 75% identity at the amino acid level with other heterobranch actin sequences, while being near-identical to each other with ≥ 98% DNA sequence identity. Cladobranchia actin sequences showed higher similarity to *A. californica* and *E. viridis*, ≥ 82% identity at the DNA level and ≥ 92% identity at the amino acid level than to *Chromodoris* actin isoform sequences (Figure 4.8, Table 4.2). BLAST® comparison showed, that the newly sequenced *Elysia viridis* actin shared highest similarity, ≥ 98%, to the recently published actin amino acid sequences from *Plakobranchus ocellatus* and *Elysia marginata*,[712] whereas its nucleotide sequence was most similar to the published *A. californica* actin nucleotide sequences (SI). Overall, the *A. californica* actin sequence, obtained from GenBank (NCBI),[711] showed higher sequence identity to other Gastropoda sequences available in the GenBank database, than to the newly sequenced isoform from *Chromodoris* sea slugs (SI), which instead matched highest with lancelet actins, *Branchiostoma floridae* ≤ 79.5% amino acid identity, and predicted squirrel actins, *Urocitellus parryii* ≤ 72.4% DNA sequence identity (SI). Of course, this peculiar bias does not mean that lancelet, squirrel and *Chromodoris* actins would be more homologous. Instead, it emphasizes a lack in availability for many Gastropoda, Heterobranchia, and especially Nudibranchia actin isoform sequences in current databases. Though actin is encoded by a single gene in yeast, some protozoans and single-celled green algae,[622,627,628,631] in all other eukaryotes actin proteins are encoded by a set of structurally related genes, a multigene family, that descended by duplication and divergence from a common ancestral gene.[622,628] The number of actin isoforms varies highly in different lineages, with 8-44 isoforms in plants,[713–715] ≥ 6 actin isoforms in mammals,[716] ≥ 9 isoforms in teleost fishes,[717] ≥ 8 isoforms in echinoderms,[718,719] ≥ 6 actins in insects ,[720] ≥ 8 isoforms in *Pecten maximus*,[643] 12-15 isoforms in *Placopecten magellanicus*,[642] and at least 3-5 actin isoforms in *Haliotis* species,[647] coleoid Cephalopods,[645] and *Aplysia californica*.[649–651] Divergence of the new *Chromodoris* actin isoform to other heterobranch actins by ≥ 23% (Figure 4.8, Table 4.2) could be attributable to at least one more duplication event and further speciation in chromodorid nudibranchs due to different levels of selective pressure imposed by its function and interacting partners.[264,622,721] So far, there are no other actin sequences available for the Nudipleura group. Future studies could reveal further actin isoforms, allowing for a more detailed analysis regarding the evolution of actin proteins in this group and their implications for toxin resistance and chemical defence.

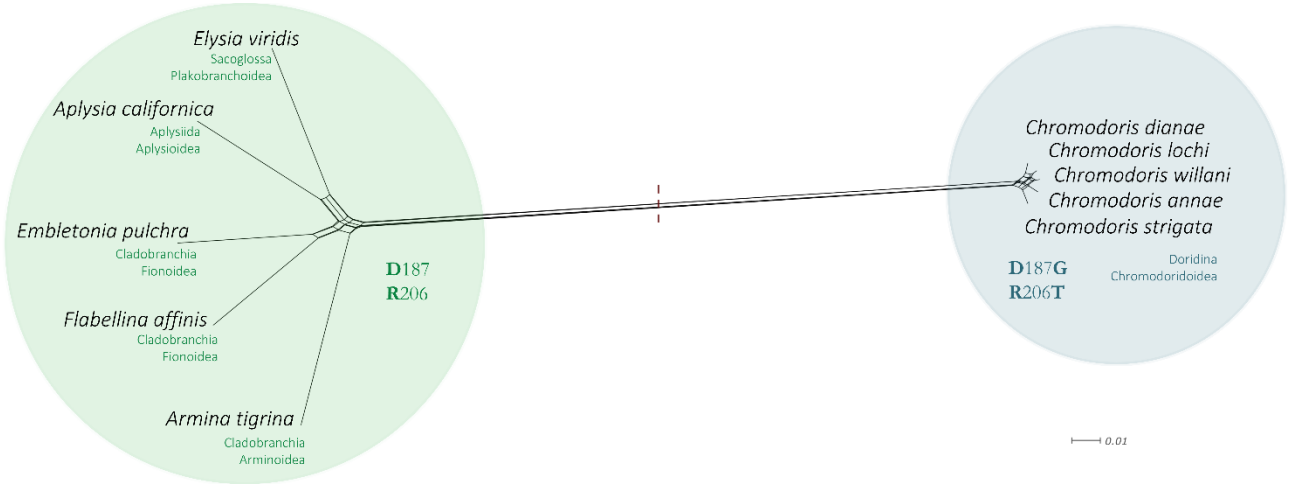


Figure 4.8 Unrooted phylogenetic split network of heterobranch actins, showing two distinct actin groups. The network is based on the core nucleotide coding region (808 bp) of combined genomic gDNA (*Chromodoris* and *Elysia*) and transcriptomic cDNA (*Armina*, *Embletonia*, and *Flabellina*) actin sequences. The *Aplysia californica* actin sequence was obtained from GenBank (SI). Sequences were aligned using the MUSCLE algorithm in MEGA X version 10.0.5.[723] The split network was generated using SplitsTree4 v4.16.2. and the NeighborNet method.[724] The scale bar indicates 0.01 substitutions/site.

Table 4.2 Percent identity matrices of heterobranch actin sequences. (1) Identity matrix based on 808 nucleotide positions. (2) Identity matrix based on 269 amino acid positions. Values > 80% are shown in bold.

(1)	*C. annae*	*C. dianae*	*C. strigata*	*C. lochi*	*C. willani*	*F. affinis*	*A. tigrina*	*E. pulchra*	*A. californica*	*E. viridis*
C. annae		**99**	**99**	**99**	**99**	70	69	70	69	69
C. dianae	**99**		**98**	**99**	**99**	70	69	70	69	69
C. strigata	**99**	**98**		**98**	**99**	70	69	70	70	69
C. lochi	**99**	**99**	**98**		**99**	69	68	70	69	69
C. willani	**99**	**99**	**99**	**99**		69	69	70	69	69
F. affinis	70	70	70	69	69		**82**	**87**	**85**	**87**
A. tigrina	69	69	69	68	69	**82**		**87**	**87**	**85**
E. pulchra	70	70	70	70	70	**87**	**87**		**88**	**89**
A. californica	69	69	70	69	69	**85**	**87**	**88**		**91**
E. viridis	69	69	69	69	69	**87**	**85**	**89**	**91**	

(2)	*C. annae*	*C. dianae*	*C. strigata*	*C. lochi*	*C. willani*	*F. affinis*	*A. tigrina*	*E. pulchra*	*A. californica*	*E. viridis*
C. annae		**99**	**99**	**99**	**100**	76	77	77	76	77
C. dianae	**99**		**99**	**100**	**100**	76	77	76	75	77
C. strigata	**99**	**99**		**99**	**100**	76	77	77	76	77
C. lochi	**99**	**100**	**99**		**100**	76	77	76	75	77
C. willani	**100**	**100**	**100**	**100**		76	77	77	76	77
F. affinis	76	76	76	76	76		**92**	**93**	**93**	**93**
A. tigrina	77	77	77	77	77	**92**		**95**	**95**	**96**
E. pulchra	77	76	77	76	77	**93**	**95**		**97**	**97**
A. californica	76	75	76	75	76	**93**	**95**	**97**		**96**
E. viridis	77	77	77	77	77	**93**	**96**	**97**	**96**	

In Vivo Toxicity Assay

Direct toxicity of administered LatA, previously isolated from *Chromodoris* nudibranchs, and resistance against it, was tested in living heterobranchs. For this purpose, 75 µM LatA solved in isotonic solution (SI) with 5% DMSO, or just the isotonic solution with 5% DMSO as a control, were injected into living *C. annae* (1 sp.), *C. dianae* (2 sp.) and *E. viridis* (12 sp.; 6 sp. with LatA, 6 sp. as control). All *Chromodoris* specimen injected with LatA, and control group *E. viridis* heterobranchs survived the injections, showing defensive responses during the insertion of the needle, similar to the behavioural descriptions of *Hexabranchus* and aeolid*s* [722,723] and other nudibranchs,[724] by mantle flexing, expanding the mantle area, retracting the foot and contracting the rest of the body. This behaviour likely deflects attacks towards the unpalatable, defensive tissue and away from the vulnerable body parts. When an additional light source was switched on, the animals exhibited defence and escape behaviour, by either contracting their body or quickly moving away from the light. No LatA intoxication or further defensive responses were observed for the *Chromodoris* or control *Elysia* group after the injection and the heterobranchs returned to 'normal' behaviour in captivity,[725] e.g. all control *E. viridis* specimens returned to their provided food source *Codium fragile* and continued feeding. However, all of the six *E. viridis* specimens injected with the isotonic solution, containing 75 µM LatA died. During the insertion of the needle, they showed the same defensive response as the control group. Yet afterwards, they did not return to 'normal' behaviour. Instead, their movement was highly constrained within minutes, they were not able to return to their food source and showed an impaired response to the light stimulus. The six

E. viridis specimen were declared dead when no general movement and no response towards touch or light stimuli could be observed. Therefore, injection of LatA caused 100% mortality in *E. viridis* and 0% mortality in *C. annae* and *C. dianae*. These results are in accordance with previous toxicity assays of LatA, also causing 100% mortality in mosquitofish and brine shrimp.[248,549]

Discussion

In this study, we investigated the sequestration and storage of the highly cytotoxic 2-thiazolidinone macrolide LatA (Figure 4.1) in closely related nudibranch species from Indonesia: *Chromodoris annae*, *C. dianae*, *C. lochi*, *C. strigata* and *C. willani* (Figure 4.3). Furthermore, we explored a possible underlying resistance mechanism in *Chromodoris* nudibranchs that could prevent LatAs toxic effects and self-intoxication.

Every eukaryotic cell depends on the many structures and functions provided by actin, an evolutionary ancient protein, or its variant isoactins.[607,609–612,622,624,634–636,653,660,661,664,665] LatAs toxicity is based on its binding to G-actin monomers, the building blocks of the cytoskeleton, and severing of the F-actin networks, ultimately resulting in a collapsed cytoskeleton and death of the cell.[576,581,600] Therefore, LatA is an immensely effective defence against every possible eukaryotic predator, if the producer or sequestering organism is self-resistant to its cytotoxic activity. All of the *Chromodoris* species, investigated in this study, were found to selectively sequester and store LatA, similar to previous reports on *Chromodoris annae, C. elisabethina, C. kuiteri, C. lochi* and *C. magnifica* from Australia, by Cheney and colleagues.[248]

On site, we observed several *Chromodoris* species feeding on *Cacospongia mycofijiensis* and isolated LatA from the collected nudibranch and sponge samples (SI). This further supports a dietary relationship between *Chromodoris* nudibranchs and *C. mycofijiensis* in the Indo-Pacific Ocean. However, not all specimens were found invariably living and feeding on *C. mycofijiensis* and it has previously been proposed that *Chromodoris* sea slugs might have less specialized alimentary habits than thought.[248,466] Being resistant to LatAs cytotoxic effects would open up *C. mycofijiensis* as a new food source, simultaneously providing a toxic defence. Furthermore, the ability to store LatA in the mantle tissue and MDfs for a longer period would enable *Chromodoris* nudibranchs to additionally seek and use other food sources, while remaining to be chemically defended in the meantime.

For the first time, we were able to visualize the long-proposed hypothesis that MDFs of the mantle tissue act as important storage compartments for toxic compounds and potent defensive toxin packages,[99,101,113,161,229,249–251,253,706,726] by using MALDI-IMS, which shows the

distribution of LatA in cross-sections of *C. annae* and *C. dianae* (Figure 4.4). LatA was not randomly distributed, but stored specifically throughout the mantle tissue, mucus glands, and especially in high concentrations in the vacuoles of MDFs, emphasizing their importance as subcellular toxin repository. Additionally, the bright and contrasting coloration of the mantle tissue can draw the attention of predators to this chemically well defended area.[161,167,206,252,254] These results show that *Chromodoris* nudibranchs compartmentalize LatA, a mechanism that is commonly used to prevent autotoxicity, indicating that the initial role of these vacuoles and accumulation structures might have been that of excretion or autoprotection and evolved later into a defensive organelle.[254] A similar compartmentalization was also described in a prey sponge *N. magnifica* in the Red Sea, storing high concentrations of LatB within membrane-bound, actin-free vacuoles in archeocytes.[551] Future studies might reveal that compartmentalization and storage of latrunculins in actin-free vacuoles may be a common mechanism to cope with these toxic substances among organisms producing or sequestering them.

Furthermore, we found that LatA is secreted in small amounts with the mucus trail (SI). This could be a remnant kind of excretion of toxic substances from a previous detoxification process, but there is evidence suggesting that small amounts of chemical cues, in this case LatA, are secreted with the mucus trail for additional purposes, such as chemical communication.[18,53,248] For gastropods, the production of mucus is vital but costly and several behavioral adaptations, such as trail following, have evolved to compensate for energy losses.[51,538,727] However, leaving a trail poses a risk, as it reveals the position not only to mating partners, but also to predators. It has been shown, that gastropod conspecifics and predators alike can pick up various cues, allowing them to determine the polarity (i.e., direction) of the trail and follow it to the mucus producing organism.[51,538] LatA has low solubility in water (~ 0.02 mg/mL), allowing for short-range chemical communication in aqueous environments through taste. Therefore, addition of small amounts of LatA as a semiochemical to the mucus mixture by *Chromodoris* nudibranchs could simultaneously inform and attract mating partners, while deterring predators trying to follow the trail. Chromodorid sea slugs are known to display bright visual signals, such as aposematic coloration and use mimicry to advertise their toxicity.[6,120,248,252,537] The addition of LatA to the mucus would further deter organisms with poor vision or when visibility is not guaranteed, therefore playing an important role in the enhancement of warning signals.

Biotransformation of dietary substances is a described detoxification mechanism among heterobranchs and has been reported in Chromodorididae, especially for the genera *Felimare* and *Glossodoris*.[57,100,195,203,232,233,251,255,256] However, LatA was not biotransformed by

Chromodoris nudibranchs, but instead stored actively within the mantle rim, mucus glands and MDFs. LatA, isolated from *Chromodoris* slugs, retained its cytotoxic activity, due to the binding to G-actin monomers and severing of F-actin, resulting in the collapse of the cytoskeleton, as shown by fluorescence microscopy experiments with HEK-293 cells (Figure 4.5). *In vivo* toxicity experiments with direct administration of LatA showed 100% mortality in *Elysia viridis* heterobranchs, but 0% mortality in *Chromdoris* specimen. These results indicate that *Chromodoris* nudibranchs employ additional resistance mechanisms protecting them from LatAs cytotoxicity.

Considering LatAs mode of action,[576,581,600] known established mechanisms for the evolution of toxin resistances,[258,271,359–361] and studies reporting individual cases of resistance to LatA,[573,574,576,581,674–680] led us to sequence and compare heterobranch actin genes for a possible molecular resistance mechanism in *Chromodoris* nudibranchs (Figure 4.6). This is the first time that an underlying resistance mechanism in heterobranch sea slugs toward sequestered toxic molecules was investigated. We identified a novel actin isoform in all examined *Chromodoris* species containing two crucial amino acid substitutions, of aspartic acid D187 (pKa 3.86, pI 2.98, acidic, polar) to glycine (pI 5.97) and arginine R206 (pKa 12.48, pI 10.76) to threonine (pI 5.60), at the 'nucleotide binding' cleft, the binding site of LatA (Figure 4.7). Usually, aspartic acid D187 and arginine R206 form a salt-bridge with a strong positive density, which is essential for the stabilization of the tertiary structure of actin and binding of latrunculins.[576,678,682] The substitutions to the neutral amino acids glycine D187G and threonine R206T prevent the formation of this salt-bridge, possibly altering latrunculin binding, which could therefore result in LatA resistance. There have been studies suggesting strenghtened G-actin monomer-monomer interactions and overall increased F-actin content and stability by mutations of the actin 'nucleotide binding' cleft, that could be responsible for increased resistance against the depolymerizing activity of LatA.[675,677,679,681,682] Therefore, we propose that the substitutions D187G and R206T of the 'nucleotide binding' cleft lead to target-site modifications, interfering with LatA binding, hence causing insensitivity to LatA. The D187G/R206T isoactin could enable *Chromodoris* nudibranchs to sequester LatA from *C. mycofijiensis* and compartmentalize it in their mantle tissue for defence, without having to suffer from its cytotoxicity. Nevertheless, these results are only a first step toward improving our understanding of toxin resistance in chromodorid nudibranchs and they do not rule out other possible resistance mechanisms that may be used in synergy. Additional studies regarding heterobranch actins and their expression, as well as a full characterization of the novel

Chromodoris actin isoform, its physiochemical properties and the affinity of its binding sites, are needed to further investigate this hypothesis.

Gene duplications are ubiquitous and a major driver of phenotypic diversity, functional innovation and genomic adaptations to changing environmental conditions.[264,622,721,728] Most genes encoding venom protein toxins have arisen from significant gene duplication events and subsequent modification, promoting rapid evolution.[265,729] Furthermore, convergent toxin resistance, by gene duplications and substitutions, is commonly observed among organisms in chemically defended antagonistic predator-prey relationships. For example, duplications and convergent molecular changes to the sodium-potassium pump (Na^+/K^+-ATPase) in insects, amphibians, reptiles, and mammals, have been described to affect resistance against toxic cardiac glycosides.[284,291–293] Likewise, widespread convergent substitutions in alpha-1 nicotinic acetylcholine receptors have been shown to cause resistance against α-neurotoxins of venomous snakes,[259,331–333] and the convergent evolution of resistant voltage-gated sodium channels allows for widespread predator-prey interactions among diverse organisms using neurotoxic TTX and PSTs.[267,334,343,351,356]

Although actin is regularly thought of as a single protein, in most eukaryotes it consists of multiple isoforms with overlapping but non-redundant functions, encoded by structurally related genes that evolved by duplication and divergence from a common ancestral gene.[607,612,622,634–636] These isoforms are among the most essential, abundant and conserved eukaryotic proteins at the level of the amino acid sequence.[612,622,636] Actin gene duplications with followed diversification are common and known from freshwater and marine gastropods.[640,646–651] However, in contrast to vertebrate actins that have specialized into muscle and cytoplasmic isoforms, all gastropod actin genes are expressed across all tissues and are most similar to cytoplasmic actins, [640,646–651] suggesting the cytoplasmic form as an evolutionary archetype. In cells and organisms with only a limited number of genes for cytoplasmic actins, like humans, mutations of these genes can have serious consequences and lead to diseases and disorders, such as the Baraitser-Winter syndrome.[675,677,679,684–695] Further research investigating the exact changes caused by mutations and overall effects on actin expression, ATP-binding and polymerization dynamics could improve our understanding of these mechanisms and their consequences from a cell biological and medical point of view.

Another intriguing question would be whether cells could cope better with these mutations, if they have acquired additional cytoplasmic actin isoforms by gene duplication events. Cytoplasmic isoactins can copolymerize and cooperate in an unknown fashion to endow microfilament networks with diverse features.[607,634,635,730] Complex F-actin properties, such as

polymerization dynamics and stability, vary according to the mixture of isoactins in the filament, their geometrical organization, covalent modifications, and collaborative or competitive binding of multiple actin-binding proteins.[607,634,635,730] Actin, as a building block of the cytoskeleton, can be interpreted as cellular alloy.[607] The ratio of isoactins and their organization determine the properties of the filament network, enabling and adapting the cytoskeleton to a variety of functions. Additionally, a recent study by Vedula et al. revealed, that cytoplasmic actins are not only affected and regulated by changes in the amino acid sequence, but also at the gene and mRNA level, by 'silent' substitutions of the nucleotide sequence, adding even more complexity to these considerations.[636]

So far, we do not know the exact number of actin genes, their isoforms, the compositional diversity, or their substitution rates in Chromodorididae, Nudibranchia, or Heterobranchia. However, with advancing technologies, especially next-generation sequencing and whole genome analyses, these questions could be adressed in the future. Comparison of Cladobranchia, *Elysia* and *Aplysia* actin sequences (Table 4.2) showed, that they shared ≥ 82% identity at the DNA level and ≥ 92% identity at the amino acid level. *Chromodoris* actin sequences were near-identical to each other, with ≥ 98% DNA sequence identity, however, they only shared ≥ 68% identity at the DNA level and ≥ 75% identity at the amino acid level to other heterobranch actin sequences. The increased divergence of the newly described *Chromodoris* actin isoform in comparison to other heterobranch actins by ≤ 25% at the amino acid level and ≤ 32% at the gene level (Table 4.2, Figure 4.8) may be explained by at least one more actin gene duplication event, relaxing purifying (i.e., stabilizing) selection and allowing for faster evolution of the isoactin. This could then be followed by further adaptation and speciation in chromodorid nudibranchs, due to different levels of selective pressure imposed by its functions, interacting partners, and toxic latrunculins in the food sources *N. magnifica* and *C. mycofijiensis*.[264,622,721,728] In this case, mutations of the novel isoactin that lead to resistance against latrunculins would provide an inheritable evolutionary advantage to *Chromodoris* nudibranchs.

The coevolutionary predator-prey relationship between the chemically defended *Chromodoris* nudibranchs and their prey *C. mycofijiensis* and *N. magnifica*, requires tandem origin of toxin acquisition and the development of resistance to prevent self-intoxication. An essential question to investigate would be whether the mutations that led to latrunculin resistance developed before the division of the infraorder Doridoidei into the superfamilies Polyceroidea, Chromodoridoidea, Phyllioidea, Doridoidea, and Onchidoridoidea. If other dorid nudibranchs, like the predatory genus *Gymnodoris* (Nudibranchia: Polyceroidea), whose members have been

observed feeding on *Chromodoris* nudibranchs,[159,538] would already carry a latrunculin resistant isoactin, these carnivorous sea slugs would also be able to prey on the genus *Chromodoris* without having to fear their cytotoxic defence. Another chromodorid nudibranch, *Hexabranchus sanguineus*, is known to sequester and store the cytotoxic, actin-binding kabiramides and ulapualides,[27,43,242,243,603,666,671,672] and the euopisthobranch *Aplysia kurodai* sequesters the toxic macrolide aplyronine A.[27,603,667–670] Kabiramides, ulapualides and aplyronine A have a similar mode of action as latrunculins, however, they bind like all other actin-depolymerizing macrolides, to the hydrophobic 'target binding' cleft between subdomains I and III of G-actin monomers.[27,559,608] Future studies may reveal additional actin isoforms in heterobranch sea slugs, which would allow for more detailed analyses of the evolution of actin proteins in this group of marine molluscs, their implications for toxin resistances, chemical defence and the predator-prey relationships that they might enable.

In conclusion, we found that nudibranchs of the genus *Chromodoris* sequester the highly cytotoxic macrolide LatA from their food source *Cacospongia mycofijiensis*. They accumulate LatA untransformed in their mantle tissue and mucus glands. In particular, LatA was compartmentalized in high amounts in the vacuoles of MDFs, emphasizing their importance as subcellular toxin repository. LatA was secreted with the mucus, possibly as a semiochemical to attract partners and deter predators. Cytotoxicity of LatA is a direct result of its binding to one of the most essential eukaryotic proteins, G-actin monomers, prohibiting their polymerization and severing of the F-actin network, ultimately resulting in the collapse and death of the cell. However, in vivo experiments with direct administration of LatA lead to a mortality of 0% in *Chromodoris* nudibranchs and 100% mortality in *Elysia viridis* sea slugs.

We amplified, sequenced, and described a novel actin isoform from all investigated *Chromodoris* species, carrying crucial amino acid substitutions at the 'nucleotide binding' cleft of actin, the binding site of LatA. Especially the amino acid substitutions D187G and R206T, which usually form an important salt-bridge, have been identified as possible underlying molecular mechanism resulting in LatA resistance. This is the first study investigating a molecular mechanism of resistance in a group of heterobranch sea slugs. The results are a first step to improve our understanding of a possible gene-based resistance mechanism in *Chromodoris* sea slugs against the cytotoxin LatA. The resistance to LatA, caused by a novel D187G/R206T isoactin, would enable *Chromodoris* nudibranchs to sequester the toxin from their prey *C. mycofijiensis*. Furthermore, this would allow them to store LatA for a longer period

in the mantle tissue and use it for their own defence, without having to suffer from its cytotoxicity.

We hope our work emphasizes the potential of MNP and resistance research in heterobranch sea slugs, with combined insights from a chemical, molecular, ecological, and cell biological perspective. Many questions remain to be resolved and we hope to inspire future studies to investigate marine gastropod isoactins, their evolution, expression, physiochemical properties and possible implications for cytotoxin resistance.

GENERAL CONCLUSION AND OUTLOOK

The herein presented studies were part of the interdisciplinary project INDOBIO and aimed to contribute foundational results on marine natural products from the nudibranch *Phyllodesmium longicirrum*, *Doriprismatica stellata* and five *Chromodoris* species of the Central Indo-Pacific Ocean. Each chapter had a respective focus on particular food sources, possible chemoecological roles and relationships, medical potential or an underlying mechanism of resistance for the prevention of autotoxicity.

Nudibranch sea slugs have reduced, internalized or completely lost their protective shell. Instead, various adaptations enabled these gastropods to explore and exploit new niches and food sources, which led to unique survival strategies and increased radiation within these taxa.[101,111,143] The exceptional biodiversity, intense competition and feeding pressure in coral reefs of the Central Indo-Pacific Ocean lead to a vast chemical diversity and variety of bioactive MNPs, especially in exposed, sessile, and slow-moving organisms.[2324] As an overarching conclusion of this thesis, it can be inferred that the herein investigated species of nudibranchs were rich sources of MNPs, especially of terpenoids, like cembranoid and polycyclic diterpenes, biscembranes, a scalarane sesterterpene, and of the cytotoxic macrolide LatA (Figure 5.1). The sea slugs acquired and accumulated all of these compounds in larger quantities by feeding on a specific type of prey that produces or contains these compounds. Therefore, targeting shell-less gastropods may result in the discovery of accumulated unexpected natural products. However, it must be noted that the limiting factor for elucidation of a novel compound, let alone testing its bioactivity, is its quantity when isolated. This has been, and continues to be, one of the longstanding challenges in natural product drug discovery. All of the investigated nudibranchs contained numerous metabolites, as evident from mass spectrometry data, yet isolation of amounts large enough for their elucidation and bioactivity tests proved difficult. New techniques and technologies with increased sensitivity might allow for their elucidation in the future. Currently, this field is developing rapidly and there are several fascinating approaches to make natural product data more accessible by open-access knowledge bases,[731–737] and increase the speed of discovery by computer aided structure elucidation,[738–742] which can help with the flood of data produced in spectrometry and spectroscopy laboratories.

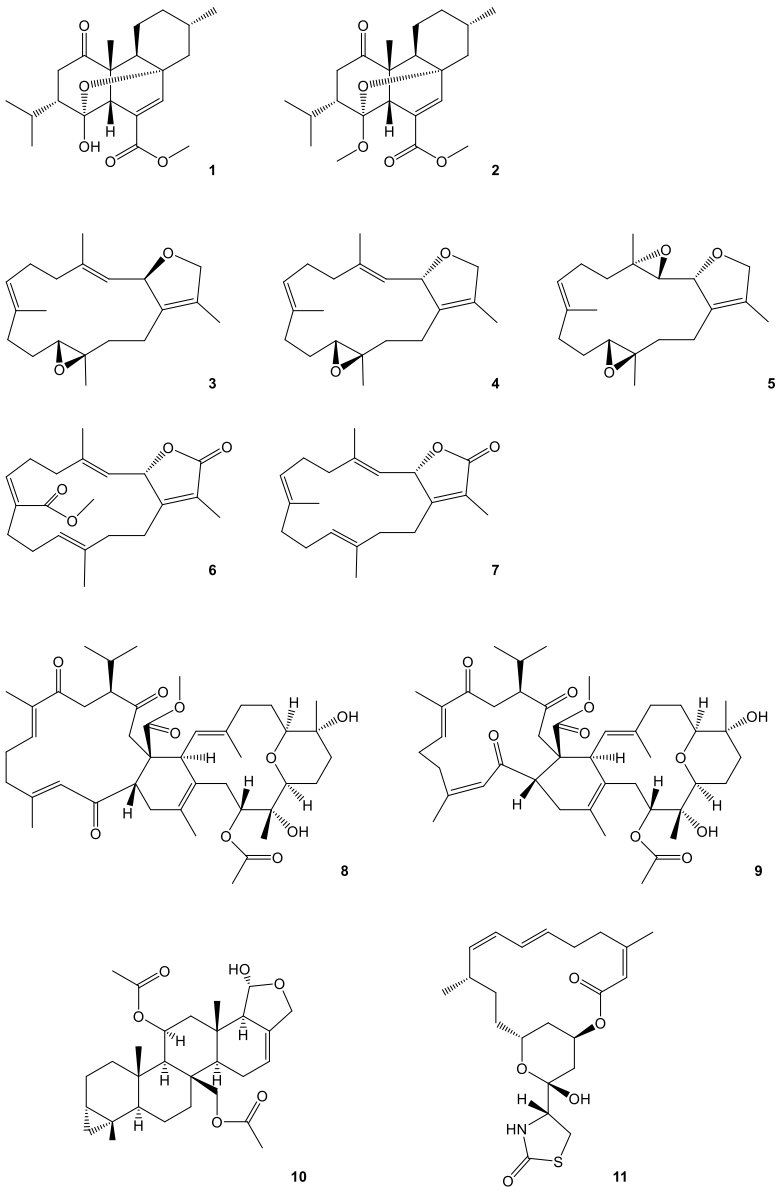


Figure 5.1 MNPs that were isolated, identified and elucidated in the current thesis. Compounds (**1**-**9**) are polycyclic (**1**-**2**) and cembranoid (**3**-**7**) diterpenes and biscembranes (**8**-**9**) isolated from *Phyllodesmium longicirrum*, which were sequestered and accumulated most likely by feeding on an alcyoniidaen soft coral of the genus *Sarcophyton*. Compound (**10**) is a structurally new scalarane sesterterpene isolated from *Doriprismatica stellata* nudibranchs, which was sequestered from the dictyoceratid sponge *Spongia* cf. *agaricina* and was further passed on to the egg ribbons. Compound (**11**) was isolated from all five investigated *Chromodoris* species and identified as the highly cytotoxic macrolide LatA, which they sequestered from their thorectid food source *Cacospongia mycofijiensis*.

In this thesis, we found that *Phyllodesmium longicirrum* and *Doriprismatica stellata* are rich sources of terpenoid MNPs, while all of the *Chromodoris* species accumulated especially high amounts of the cytotoxic 2-thiazolidinone macrolide latrunculin A (LatA, Figure 5.1). All of the investigated species sequestered these MNPs from their respective food sources, which suggests specialized alimentary habits. Therefore, we additionally investigated and identified their food sources. However, enrichment and storage of a particular bioactive MNP, such as LatA, could also enable *Chromodoris* nudibranchs to seek and use other food sources in addition, while remaining to be chemically defended in the meantime.

Although aeolid nudibranchs are well known to sequester and store nematocysts from their hydrozoan prey, most members of the genus *Phyllodesmium* specialized to feed on alcyonacean soft corals and sequester the symbiotic, photosynthetic zooxanthellae. This raised the question, whether these *Phyllodesmium* species rely on a chemical defence strategy instead. Our investigation has shown that *P. longicirrum*, one of the largest species (12-15 cm), feeds on alcyoniidaen soft corals of the genus *Sarcophyton*, from which it sequesters and accumulates terpenoids. Some of these terpenoid compounds showed feeding deterrent activity, as for example tested in this study against the pufferfish *Canthigaster solandri* (e.g., Figure 5.1: 4-oxo-chatancin (**1**) and (2*S*,11*R*,12*R*)-isosarcophytoxide (**4**)), which indicates that at least some of the terpenoids are used as chemical defence. Interestingly, (2*R*,11*R*,12*R*)-isosarcophytoxide (**3**), the epimer of (**4**), did not deter feeding, suggesting that the ability to bind to specific chemoreceptors and induce feeding deterrence may depend on the configuration of the molecule at each chiral center.[1–7] The highly efficient symbiotic relationship with photosynthetic *Symbiodinium*, cryptic shape and coloration, and the accumulated defensive terpenoids allow *P. longicirrum* to grow to an exceptional size, without having to fear intense predation. The shift from feeding on hydrozoans to feeding on toxic octocorals and exploitation of this unusual niche likely enhanced radiation and speciation within the genus *Phyllodesmium*. Incorporation of the corals' symbiotic zooxanthellae provides additional nutrition in periods of food shortage, while stored MNPs protect these aeolid nudibranchs from predators. These crucial advantages can lead to a higher viability.

Dorid nudibranchs live and feed on noxious sponges, from which they sequester deterrent and toxic molecules as a chemical defence.[45,57,95,101,160,195,251,256,456,464–466,468] As the first chemical investigation on *Doriprismatica stellata*, we found that these nudibranchs feed upon the dictyoceratid sponge *Spongia* cf. *agaricina*, from which it sequesters the novel scalarane-type sesterterpene 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin (Figure 5.1). This MNP showed promising antibacterial activity against Gram-positive bacteria.

The nudibranchs and their egg ribbons contained higher quantities of this compound than the sponge, most likely due to a continuous accumulation of this sesterterpene. The results indicate that *D. stellata* passes the scalarane metabolite on to its egg ribbons, likely for protective purposes against overgrowth. Our results further support scalarane sesterterpenes as a chemotaxonomic marker for the sponge genus *Spongia* (Spongiidae, Dictyoceratida), among the families Thorectidae, Dysideidae and Spongiidae, which are a major source of this otherwise considered rare class of terpenes, accounting for less than 2% of all known terpenoids.[465]

Similar to other dorid nudibranchs, *Chromodoris* sea slugs have specialized to feed on demosponges. While *Chromodoris* species in the Red Sea have been found to feed on *Negombata* (previously *Latrunculia*) *magnifica* (Porifera: Demospongiae: Poecilosclerida: Podospongiidae), the five investigated species in this study: *C. annae*, *C. dianae*, *C. lochi*, *C. strigata* and *C. willani* from the Central Indo-Pacific Ocean, were found to feed on *Cacospongia* (previously *Spongia*) *mycofijiensis* (Porifera: Demospongiae: Dictyoceratida: Thorectidae). Intestestingly, even though *N. magnifica* and *C. mycofijiensis* are taxonomically unrelated and occur at distant geographic locations, they are both reliable sources of toxic latrunculins, unique 14- or 16-membered macrolides that are attached to a rare 2-thiazolidinone moiety, especially LatA,[552] and serve as food sources for *Chromodoris* nudibranchs. Similar to previous reports by Cheney et al. on *C. annae*, *C. elisabethina*, *C. kuiteri*, *C. lochi* and *C. magnifica* from Queensland, Australia,[248] we found that all five investigated *Chromoris* species (*C. annae*, *C. dianae*, *C. lochi*, *C. strigata* and *C. willani*) from North Sulawesi, Indonesia, also selectively accumulate the cytotoxin LatA.

Diversity and variation of MNPs between Heterobranchia species, populations at different locations or individuals of the same species, may reflect a more varied diet than previously thought, as for example observed in studies on *Doriprismatica* nudibranchs containing either norsesterterpenes, spongian diterpenes, scalarane sesterterpenes or steroids, depending on the location and prey eaten.[3,234,478] Further variation may occur due to detoxification processes, such as biotransformation of dietary compounds into related molecules, as for example described for *Felimare* and *Glossodoris* nudibranchs.[57,100,232,233,251,255,256] However, in the current study investigating *P. longicirrum*, *D. stellata* and *Chromodoris* sea slugs, we did not find any indications for biotransformation of the sequestered molecules.

While *P. longicirrum* accumulates the acquired terpenoids in their exposed cerata, the dorid nudibranchs *D. stellata* and *Chromodoris* species accumulated and stored the sequestered

chemicals along the exposed and often conspicuously colored mantle border and mantle dermal formations (MDFs). Storing diet-derived chemicals in MDFs has three potential advantages: (1) concentrating the MNPs near the surface to facilitate excretion into the mucus; (2) maximizing the defensive effects by concentration of the compounds; and (3) avoiding autotoxicity. These advantages do not have to be mutually exclusive and can work in synergy. We used matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) to provide deeper insight into the storage site of sequestered MNPs, in this case LatA, and the long-proposed hypothesis that the mantle tissue of dorid nudibranchs, their mucus glands, and especially the vacuoles of MDFs, store larger quantities of toxins.[93,101,113,248–250,706–710] To the best of our knowledge, this is the first time MALDI MSI was used to investigate the distribution of a MNP in nudibranchs. We found that *Chromodoris* nudibranchs store large amounts of LatA in the mantle tissue and vacuoles of MDFs. Additionally, LatA was present in the mucus glands and was also secreted along with the mucus trail. Secretion of LatA could be a remnant form of excretion from a previous detoxification process, however, it might also serve as a chemical cue in chemical communication, simultaneously informing and attracting mating partners, while deterring predators that try to follow the mucus trail. Furthermore, using LatA as a semiochemical might play an important role in the enhancement of warning signals, as it could deter organisms with poor vision, or when visibility of the aposematic coloration is not guaranteed.

LatAs toxicity is based on its binding to G-actin monomers, the building blocks of the cytoskeleton, and severing of the F-actin networks. Since every eukaryotic cell depends on the numerous structures and functions provided by actin or its variant isoactins, binding of LatA to these proteins results in a collapsed cytoskeleton and death of the cell.[576,581,600] Therefore, LatA is an immensely effective chemical for defence, if the producer or organism sequestering it is resistant to its cytotoxic activity. Compartmentalization, as for example in the vacuoles of MDFs in *Chromodoris* species, or in membrane-bound, actin-free vacuoles in the archeocytes of *N. magnifica*,[551] might be one common mechanism to prevent autotoxicity. Furthermore, we identified a novel actin isoform in the examined *Chromodoris* species, containing two crucial amino acid substitutions, D187G and R206T, at the ‘nucleotide binding’ cleft, the binding site of LatA, leading to target-site modifications that may affect latrunculin binding, hence, presumably resulting in LatA resistance. The D187G/R206T isoactin may enable *Chromodoris* nudibranchs to sequester and accumulate LatA from *C. mycofijiensis* and *N. magnifica* sponges and compartmentalize it in the MDFs for defence, without having to suffer from its cytotoxicity. However, little is known about heterobranch actin isoforms and this study is only a first step

towards improving our knowledge on toxin resistance in chromodorid nudibranchs. When we investigated and sequenced further heterobranch actins to determine a possible origin of the D187G/R206T isoform, we found that not only the genus *Chromodoris* but also several other chromodorid nudibranchs contain and express this isoactin, as evident from genomic and transcriptomic data (SI). However, nudibranchs that belong to the suborder Cladobranchia were void of this isoactin (SI). Future studies might be able to further delimit the origin, occurrence and evolution of this isoactin, as well as its properties, functions and implications for toxin resistance and predator-prey relationships of heterobranch sea slugs.

SUMMARY

This thesis is part of the interdisciplinary project INDOBIO and focusses on the investigation of marine natural products (MNPs) from nudibranchs and their food sources of the Central Indo-Pacific Ocean, namely *Phyllodesmium longicirrum* from Lizard Island, northern Great Barrier Reef, Australia, *Doriprismatica stellata* and five *Chromodoris* species from North Sulawesi, Indonesia. Furthermore, isolated MNPs were examined for their possible chemoecological role and medical potential and a mechanism for the prevention of autotoxicity was explored.

MNPs are important lead structures in the drug discovery process, as they can provide important chemical scaffolds with advantageous pharmacological properties. Marine gastropods can produce or accumulate such compounds and several drugs have been developed as treatments against cancer and severe pain that use gastropod MNPs as lead structures.[84–92] The exceptional biodiversity, intense competition and feeding pressure in coral reefs of the Central Indo-Pacific Ocean lead to a vast chemical diversity and variety of bioactive MNPs, especially in exposed, sessile, and slow-moving organisms.[23,24] Nudipleuran sea slugs are a clade of charismatic gastropods that have reduced, internalized or completely lost their protective shell. They display some of the most spectacular and diverse body forms, patterns and colors found in nature.[101,109] To protect themselves from predation, several defence mechanisms have evolved, including cryptic or aposematic coloration and mimicry,[120,165–169] acid secretions,[172] subepidermal spicules,[113,162–164] and the ability to steal, incorporate and use the defence system of their prey, as for example stinging nematocysts or MNPs, which can be used for chemical defence.[95,153,160,170,171]

Aeolid nudibranchs are well known to sequester and store nematocysts (kleptocnides) from their hydrozoan prey, however, most members of the species-rich genus *Phyllodesmium* have specialized to feed on alcyonacean soft corals and sequester their symbiotic, photosynthetic zooxanthellae. Here we show that one of the largest species, *Phyllodesmium longicirrum* (Nudibranchia: Aeolidioidea: Myrrhinidae), acquires and accumulates terpenoid compounds from their prey, especially cembranoid diterpenes. Investigation of the lipophilic extract of a single *P. longicirrum* specimen led to the isolation of nine terpenoid metabolites: the cembranoid diterpene (2*R*,11*R*,12*R*)-isosarcophytoxide (**1**), its epimer (2*S*,11*R*,12*R*)-isosarcophytoxide (**2**), the related (3*R*,4*S*,11*R*,12*R*)-bisepoxide (**3**), and the γ-lactone bearing cembranes sarcophytonin B (**4**) and 13-dehydroxysarcoglaucol-16-one (**5**); the two polycyclic

diterpenes 4-oxo-chatancin (**6**) and 1-*O*-methyl-4-oxo-chatancin (**7**); and the two biscembranes bisglaucumlide L (**8**) and bisglaucumlide M (**9**). *P. longicirrum* sequestered these metabolites from its prey, most likely alcyonacean soft corals of the genus *Sarcophyton*. Metabolites (**6**) and (**2**) significantly deterred feeding by the generalist pufferfish *Canthigaster solandri*, emphasizing an ecological role in defence for at least some of these terpenoids. Interestingly, compound (**1**), the epimer of compound (**2**), showed no significant deterrent activity, which suggests that feeding deterrence might be conformation-dependant. The shift from hydrozoan to alcyonacean food sources and the successful scavenging of this prey likely enhanced radiation and speciation in the genus *Phyllodesmium*. Sequestration of photosynthetic zooxanthellae and defensive metabolites provides additional nutrition and a metabolically cheap and effective protection, which can lead to a higher viability.

The structurally new scalarane-type sesterterpene 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin was isolated from *Doriprismatica stellata* (Nudibranchia: Doridina: Chromodorididae) nudibranchs, their egg ribbons, and the associated dietary sponge *Spongia* cf. *agaricina* (Porifera: Demospongiae: Dictyoceratida). The scalarane showed antibacterial activity against the Gram-positive bacteria *Arthrobacter crystallopoietes* (DSM 20117) and *Bacillus megaterium* (DSM 32). Structural elucidation revealed that the cyclopropane ring had an unprecedented position annelated to ring A and, unlike previously reported scalaranes,[203,220,233,465,468,477,482] it bears two acetoxy groups at C-11 and C-24 of ring C, instead of being functionalized at C-12. The occurrence of this scalarane in all three samples further supports the dietary relationship between chromodorid nudibranchs of the genus *Doriprismatica* and scalarane-containing dictyoceratid sponges of the Spongiidae family. Furthermore, the presence of this molecule in the egg ribbons of *D. stellata* suggests that the nudibranch passes the scalarane on to its offspring, most likely for protective purposes.

Chromodoris sea slugs, a genus of aposematically colorful nudibranchs (Nudibranchia: Doridina: Chromodorididae), live and feed on noxious sponges, from which they sequester deterrent and toxic molecules. Here we show that five closely related species of *Chromodoris* sea slugs (*C. annae*, *C. dianae*, *C. lochi*, *C. strigata* and *C. willani*) from North Sulawesi, Indonesia, selectively accumulate the cytotoxin LatA from their food source *Cacospongia mycofijiensis* (Porifera: Demospongiae: Dictyoceratida). The distribution of LatA was visualized for the first time within the body of two *Chromodoris* species using MALDI MS-Imaging (Figure 4.4**)**, showing that LatA was accumulated and stored specifically throughout the mantle tissue, mucus glands, and especially in vacuoles of the mantle dermal formations (MDFs). These results emphasize the importance of MDFs as repository for the

storage of highly concentrated amounts of toxic molecules, in this case LatA. Furthermore, LatA was secreted along with the mucus and mucus trail, where it possibly serves as a semiochemical in short-range chemical communication, deterring predators and attracting mating partners. Using HEK-293 cells and fluorescence microscopy, we show that the isolated LatA is bioactive. Its cytotoxicity results from binding to G-actin monomers, one of the most essential eukaryotic proteins, which prohibits their polymerization to F-actin and additionally severs the F-actin network, which leads to the collapse of the cytoskeleton and death of the cell. *In vivo* toxicity experiments with direct administration of LatA showed 100% mortality in *E. viridis*, but 0% mortality in *Chromodoris* heterobranchs. Investigation of an underlying molecular resistance mechanism against the cytotoxic activity of LatA led us to amplify, sequence, examine and compare heterobranch actin genes, revealing a novel actin isoform in all investigated *Chromodoris* species. This isoactin carries two crucial amino acid substitutions, D187G and R206T, at the the 'nucleotide binding' cleft, the binding site of LatA. These substitutions likely lead to target-site modifications, interfering with LatA binding, hence, causing LatA insensitivity. Isoactin D187G/R206T is suggested to be a prerequisite for *Chromodoris* nudibranchs to sequester latrunculins from *Negombata magnifica* and *Cacospongia mycofijiensis* sponges. It would allow *Chromodoris* sea slugs to store and utilize LatA for their own defence, without having to suffer from its cytotoxicity.

MATERIAL AND METHODS

General Experimental Procedures

Mass spectra were either recorded by E. Egereva (Institute of Pharmaceutical Biology, Bonn) on an Agilent 1100 system, including a DAD (205 nm) and an API 2000 Triple Quadrupole LC/MS/MS with ESI source (Applied Biosystems/MDS Sciex), a C_{18} column (Macherey-Nagel Nucleodur 100, 125 x 2mm, 5 µm) and a gradient elution (from MeOH:H_2O 10:90 to MeOH:H_2O 100:0 in 20 min, MeOH 100% for 10 min, with added NH_4Ac, 2 mM) or by M. Crüsemann (Institute of Pharmaceutical Biology, Bonn) on a micrOTOF-Q mass spectrometer (Bruker) with ESI-source coupled with an HPLC Dionex Ultimate 3000 (Thermo Scientific) using an Agilent Zorbax Eclipse Plus C_{18} column (2.1 x 50 mm, 1.8 µm) at a temperature of 45 °C. MS data were acquired over a range from 100-3000 *m/z* in positive mode. Auto MS/MS fragmentation was achieved with rising collision energy (35-50 keV over a gradient from 500 - 2000 *m/z*) with a frequency of 4 Hz for all ions over a threshold of 100. UHPLC started with 90% H_2O containing 0.1% acetic acid. The gradient began after 0.5 min to 100% acetonitrile (0.1% acetic acid) in 4 min. 2 µL of a 1 mg/mL sample solution was injected to a flow of 0.8 mL/min. Further HR-ESI-MS and UPLC-HR-MS measurements were conducted by M. Sylvester (Institute of Biochemistry and Molecular Biology, Bonn) on a LTQ Orbitrap mass spectrometer or on a Thermo Scientific Qexactive with HESI source (Phenomenex Kinetex C_{18} column, 150 mm x 4.6 mm, 2.6 µm, 100 Å). Mass spectra were evaluated using Analyst ® software 1.5 (© 2010-2015 AB Sciex). HPLC was carried out on a Waters Breeze HPLC system equipped with a 1525µ dual pump, a 2998 DAD detector, and a Rheodyne 7725i injection system and with a Waters Alliance HPLC system equipped with a Waters 2695 separation module and a Waters 996 DAD detector. A Macherey-Nagel Nucleodur C_{18} Pyramid column (250 mm x 10 mm; 5 µm) and a Phenomenex Kinetex C_{18} column (250 mm x 4.6 mm, 5 µm) were used for separation.

Biological Material

In CHAPTER I extractions were conducted using a single specimen of *Phyllodesmium longicirrum* Bergh, 1905 (Mollusca: Gastropoda: Nudibranchia: Aeolidioidea). The sample was collected in 2008 by H. Wägele, during a field trip to Lizard Island (Great Barrier Reef, Australia) and stored in ethanol (96%) until further processing.

In CHAPTER II samples of *Doriprismatica stellata* Rudman, 1986 nudibranchs (Mollusca: Gastropoda: Nudibranchia: Chromodorididae), their egg ribbons and pieces of the sponge, on which they were found (1.2 g, 0.7 g, and 3.5 g wet weight, respectively) were collected via scuba diving in August 2015 during a field trip to Bunaken National Park (BNP, North Sulawesi, Indonesia, 1° 37' 51" N, 124° 45' 05" E) at the coral reef drop off. Four additional *D. stellata* sea slugs (2.5 g wet weight) were collected in October 2016 during another field trip to BNP. The nudibranchs and associated egg ribbons were identified as *D. stellata* by H. Wägele and N. Undap at the Zoological Research Museum Alexander Koenig, Bonn, Germany.[4,5] The sponge displayed a foliose habit with brownish - violett pigmentation and was identified as *Spongia* cf. *agaricina* Pallas, 1766 (Porifera: Demospongiae: Dictyoceratida) using methods as described in Ackers et al. 2007,[743] see also Erpenbeck et al. 2020,[744] (SI). Specimens were stored in ethanol (96%) at -20 °C until further extraction and processing in the laboratories at the University of Bonn. Part of the collected sea slug- and substrate material will be finally stored at the Sam Ratulangi University, Manado, Indonesia, in the Reference Collection under the numbers SRU2015/01 und SRU2016/02. A fraction of the sponge material is stored the Bavarian State Collection for Paleontology and Geology under collection number SNSB-BSPG.GW41291.

In CHAPTER III the following specimens (sp.) were collected via scuba diving in August 2015 and October 2016 during field trips to Bunaken National Park (BNP, North Sulawesi, Indonesia, 1° 37' 51" N, 124° 45' 05" E), at the coral reef drop off, dissected and extracted for chemical analyses: *Chromodoris annae* Bergh, 1877 (58 sp.), *C. dianae* Gosliner & Behrens, 1998 (56 sp.), *C. lochi* Rudman, 1982 (31 sp.), *C. willani* Rudman, 1982 (32 sp.) (Mollusca: Gastropoda: Nudibranchia: Chromodorididae), and pieces of the sponges, on which they were found.[4,5] Additional *C. annae* (8 sp.) and *C. dianae* (5 sp.) were collected in August 2016 at Sangihe Island,[6] for chemical comparison of the dissected mantle and body tissues. Specimens and separated tissues were stored in ethanol (96%) at -20 °C until further extraction and processing in the laboratories at the University of Bonn. Part of the material will be finally stored at the Sam Ratulangi University in the Reference Collection under the numbers SRU2015/01, SRU2016/02, SRU2017/1. The nudibranchs were identified by H. Wägele and N. Undap at the Zoological Research Museum Alexander Koenig, Bonn, Germany.[4,5] The sponge was identified as *Cacospongia mycofijiensis* Kakou, Crews & Bakus, 1987 (Porifera: Demospongiae: Dictyoceratida) using methods as described in Ackers et al. 2007,[743] see also Erpenbeck et al. 2020.[744] Furthermore, *C. annae* (2 sp.), collected at BNP in May 2017, were kindly provided by F. Kaligis. These samples were frozen in seawater and stored at -80°C until further

processing. Additionally, ethanolic storage solutions of *C. strigata* Rudman, 1982 (2 sp., collected in 2017, at Banka Island, Indonesia, 1° 44' 09.88" N, 125° 09' 06.33" E), and mucus collected from the notum and foot of alive *C. annae* specimen (collected in 2019 by A. Papu), were examined using mass spectrometry. Also, living *C. annae* (1 sp.) and *C. dianae* (3 sp.), were kindly provided by A. Papu and kept in aerated aquaria matching the temperature, salinity and density of the Indo-Pacific Ocean, until further conduction of experiments. One of the living *C. dianae* specimens was snap-frozen with liquid nitrogen and stored at -80°C. The snap-frozen *C. dianae* and two of the *C. annae* specimens, frozen in seawater, were used for further MALDI-MS imaging experiments at the Institute of Inorganic and Analytical Chemistry, Justus-Liebig-University, Giessen, Germany. Furthermore, living *Elysia viridis* Montagu, 1804 (Mollusca: Gastropoda: Sacoglossa: Plakobranchoidea; 12 sp., collected in Figueira da Foz, Portugal, in November 2018), were kindly provided by C. Greve and kept in aerated aquaria matching the temperature, salinity and density of the Mediterranean Sea until further conduction of experiments. Living *C. annae* (1 sp.), *C. dianae* (2 sp.) and *E. viridis* (12 sp.) were used in the *in vivo* toxicity assay.

Extraction and Isolation

In CHAPTER I, an extract of *P. longicirrum* was produced by crushing and washing of the specimen three times in 100 mL MeOH each, and once in 100 mL DCM. The extracts were combined with the ethanol storage solution and the solvents were evaporated at 40 °C, using a rotary evaporator (Heidolph Laborota 4000). The resulting crude extract (4.52 g) was separated three times in a separation funnel between 100 mL H_2O and EtOAc each. The lipophilic phase yielded 2.16 g and was fractionated by vacuum liquid chromatography (VLC) over Polygoprep 60-50 C_{18} stationary phase (Macherey-Nagel). A gradient elution from 20:80 (MeOH:H_2O) to 100% MeOH was used, resulting in 11 fractions (VLC 1-11). VLC fraction 6 (763.3 mg) and 7 (695.8 mg) were further separated on Sephadex LH-20 material (MeOH). Separation of VLC 6 resulted in 11 fractions (S 1-11), and 7 fractions were obtained from VLC 7 (VLC 7.1 - 7.7). VLC 7.2 (530 mg), being the largest fraction, was further divided into 5 subfractions (VLC 7.2.1-7.2.5) by a repeated separation on Sephadex LH-20 material (MeOH). Subsequent separation and isolation were either conducted on a Grace Reveleris X_2 flash chromatography system, on a Merck Hitachi HPLC system equipped with a L-6200A pump, a L-4500A photodiode array detector, a D-6000A interface with D-7000A HSM software, a Rheodyne 7725i injection system or on a Waters Breeze HPLC system equipped with a 1525µ dual pump, a 2998 DAD detector, and a Rheodyne 7725i injection system, Macherey-Nagel Nucleoshell

C_{18} column (250 mm x 4.6 mm, 5 µm) or Knauer Eurospher C_{18} column (250 mm x 8 mm, 5 µm). Fractions with a sufficient amount (≥ 1.0 mg) were analysed as described in the following sections. A large part of the previously obtained fractions was further separated and analysed by A. Bogdanov.[189] However, this chapter focuses on the isolation of compounds from the fractions VLC 6 S 5 (60.3 mg), S 7 (412.9 mg) and from subfractions VLC 7.2.2 (180 mg), 7.2.3 (100 mg), and 7.2.4 (65 mg). The results were combined and reported in Bogdanov et al. 2016, 2017.[1,2] Fraction VLC 6 S 5 (60.3 mg) was separated with RP-HPLC (MeOH:H_2O 70:30, Macherey-Nagel Nucleoshell column, 1 mL/min), resulting in 4 compounds (VLC 6 S 5.1-5.4). Of these, S 5.2 (4.4 mg) and S 5.4 (2.3 mg) were subsequently analysed. Fraction VLC 6 S 7 (412.9 mg) was further fractionated with RP flash chromatography (MeOH:H_2O 60:40, 30mL/min; Reveleris C_{18} column, 12 g) to yield 21 fractions (VLC 6 S 7 Fl 1-21). Fractions Fl 11, 12 and 13 were combined (54.1 mg), since they contained the same compounds, confirmed by ^{1}H-NMR analysis, and were submitted to RP-HPLC (MeOH:H_2O 72:28, Macherey-Nagel Nucleoshell column, 1 mL/min), resulting in 6 compounds (Fl 11-13.1 - 11-13.6), which were analysed in the following. Fraction VLC 7.2.3 (100 mg) was divided into 8 compounds (VLC 7.2.3.1-7.2.3.8) by RP-HPLC (MeOH:H_2O 75:25, Macherey-Nagel Nucleoshell column, 0.9 mL/min), of which 4 fractions (7.2.3.3, 7.2.3.4, 7.2.3.6 and 7.2.3.7) yielded a sufficient amount for further analysis. Fractions VLC 7.2.2 (180 mg) and 7.2.4 (65 mg) were submitted to RP flash chromatography (MeOH:H_2O 80:20, 30 mL/min; Reveleris C_{18} column, 12 g) to yield 4 semi-pure compounds (VLC 7.2.2.3, 7.2.2.4, 7.2.2.6 and 7.2.4.5), which were subsequently analysed.

(2*R*,11*R*,12*R*)-isosarcophytoxide (1): colorless oil (41.0 mg); $[\alpha]_D^{20}$ = -156 (*c* 0.2, $CHCl_3$); ^{13}C NMR (75 MHz, MeOH-*d*$_4$) δ 141.4 (s), 134.4 (s), 133.5 (s), 129.5 (s), 127.6 (d), 126.8 (d), 84.8 (d), 79.0 (t), 64.0 (d), 63.1 (s), 39.9 (t), 38.5 (t), 37.5 (t), 25.2 (t), 24.7 (t), 23.3 (t), 15.8 (q), 14.9 (q), 14.8 (q), 9.8 (q); ^{1}H NMR (300 MHz, MeOH-*d*$_4$) δ 5.46 (m, H-2), 5.08 (m, H-3), 5.04 (m, H-7), 4.48 (m, H_2-16), 2.88 (dd, *J* = 3.3, 9.1 Hz, H-11), 2.35 (m, H_2-6), 1.81 (m, H_2-10), 1.73 (s, H_3-17), 1.69 (s, H_3-19), 1.67 (s, H_3-18), 1.27 (s, H_3-20)]; LC-ESI-MS *m/z* 302.2 [M^+] (calcd for $C_{20}H_{30}O_2$, 302.22).

(2*S*,11*R*,12*R*)-isosarcophytoxide (2): colorless oil (42.0 mg); $[\alpha]_D^{20}$ = +196 (*c* 0.2, $CHCl_3$); ^{13}C NMR (75 MHz, MeOH-*d*$_4$) δ 141.4 (s), 134.4 (s), 133.5 (s), 129.5 (s), 127.6 (d), 126.8 (d), 84.8 (d), 79.0 (t), 64.0 (d), 63.1 (s), 39.9 (t), 38.5 (t), 37.5 (t), 25.2 (t), 24.7 (t), 23.3 (t), 15.8

(q), 14.9 (q), 14.8 (q), 9.8 (q); ^{1}H NMR (300 MHz, MeOH-*d*$_4$) δ 5.43 (m, H-2), 5.08 (m, H-3), 5.02 (m, H-7), 4.48 (m, H$_2$-16), 2.85 (dd, *J* = 3.5, 9.2 Hz, H-11), 2.33 (m, H$_2$-6), 1.82 (m, H$_2$-10), 1.71 (2s, H$_3$-17 and H$_3$-19), 1.64 (s, H$_3$-18), 1.31 (s, H$_3$-20); LC-ESI-MS *m/z* 302.2 [M$^+$] (calcd for $C_{20}H_{30}O_2$, 302.22).

(3*R*,4*S*;11*R*,12*R*)-bisepoxide (3): colorless oil (28.0 mg); $[\alpha]_D^{20}$ = -44.6 (*c* 3.3, $CHCl_3$); ^{13}C NMR (75 MHz, MeOH-*d*$_4$) δ 135.8 (s), 131.8 (s), 130.9 (s), 127.1 (d), 87.9 (d), 79.7 (t), 66.4 (d), 63.1 (d), 62.6 (s), 62.3 (s), 38.4 (t), 37.5 (t), 35.4 (t), 25.4 (t), 24.8 (t), 22.0 (t), 18.8 (q), 18.5 (q), 15.8 (q), 10.0 (q); ^{1}H NMR (300 MHz, MeOH-*d*$_4$) δ 5.26 (m, H-7), 4.58 (m^b, H-2), 4.52 (m^b, H-16), 2.88 (dd, *J* = 3.3, 9.8 Hz, H-11), 2.80 (brd, *J* = 8.2 Hz, H-3), 2.28 (m^b, H$_2$-9), 2.27 (m^b, H$_2$-6), 2.12 (m^b, H$_2$-14), 1.72 (s, H$_3$-17), 1.69 (s, H$_3$-19), 1.46 (s, H$_3$-18), 1.30 (s, H$_3$-20); LC-ESI-MS *m/z* 318.2 [M$^+$] (calcd for $C_{20}H_{30}O_3$, 318.22).

Sarcophytonin B (4): colorless oil (17.8 mg); $[\alpha]_D^{20}$ = +158.2 (*c* 0.7, $CHCl_3$); ^{13}C NMR (75 MHz, MeOH-*d*$_4$) δ 177.5 (s), 166.1 (s), 146.5 (s), 135.3 (s), 134.8 (s), 126.3 (s), 125.5 (d), 122.9 (d), 120.8 (d), 81.1 (d), 40.9 (t), 39.7 (t), 37.3 (t), 28.0 (t), 25.6 (t), 24.5 (t), 16.0 (q), 15.9 (q), 15.4 (q), 8.8 (q); ^{1}H NMR (300 MHz, MeOH-*d*$_4$) δ 5.52 (dd, *J* = 1.5, 10.0 Hz, H-2), 5.16 (brdd, *J* = 6.3, 7.4 Hz, H-7), 4.92 (brd, *J* = 10.3 Hz, H-3), 1.86 (s, H$_3$-17), 1.85 (s, H$_3$-18), 1.69 (s, H$_3$-19), 1.65 (s, H$_3$-20); HRMS *m/z* 301.2160 [M+H] (calcd for $C_{20}H_{28}O_2$, 300.21).

13-Dehydroxysarcoglaucol-16-one (5): colorless oil (3.7 mg); $[\alpha]_D^{20}$ = +81.4, *c* 0.4, $CHCl_3$); ^{13}C NMR (75 MHz, MeOH-*d*$_4$) δ 177.5 (s), 169.7 (s), 166.0 (s), 146.2 (s), 144.0 (d), 136.4 (s), 132.0 (s), 124.7 (d), 123.0 (s), 121.1 (d), 81.1 (d), 51.7 (q), 39.6 (t), 37.2 (t), 36.2 (t), 27.7 (t), 27.2 (t), 25.7 (t), 16.1 (q), 16.0 (q), 8.8 (q); ^{1}H NMR (300 MHz, MeOH-*d*$_4$) δ 5.75 (m, H-2), 5.75 (m, H-7), 5.12 (t, *J* = 6.6 Hz, H-11), 4.98 (d, *J* = 9.5 Hz, H-3), 3.78 (s, H$_3$-21), 2.96 (m, H$_2$-6), 2.51 (m, H$_2$-9), 1.89 (s, H$_3$-18), 1.85 (t, *J* = 1.5 Hz, H$_3$-17), 1.66 (s, H$_3$-20); LC-ESI-MS *m/z* 344.2 [M$^+$] (calcd for $C_{21}H_{28}O_4$, 344.20).

4-Oxo-chatancin (6): colorless oil (150.0 mg); $[\alpha]_D^{20}$ = -14.6 (*c* 1.0, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 214sh (3.79) nm; ECD (CH_3CN): λ_{max} (Δ ε) 209 (+1.45), 300 (-0.13) nm; IR (ATR) ν_{max} 3418, 2953, 2870, 1705, 1437, 1365, 1265, 1151, 1076, 992 cm^{-1}; ^{1}H and ^{13}C NMR (SI); HR-ESI-MS *m/z* 385.1977 [M+Na]$^+$ (calcd for $C_{22}H_{31}O_5Na$, 385.1985).

1-*O*-methyl-4-oxo-chatancin (7): colorless oil (3.4 mg); $[\alpha]_D^{20}$ = -10.0 (*c* 0.3, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 216sh (3.77) nm; ECD (CH_3CN): λ_{max} (Δ ε) 214 (+1.56), 296 (-0.12) nm; IR (ATR) ν_{max} 3431, 2925, 2854, 1711, 1457, 1378, 1267, 1247, 1205, 1142, 995 cm^{-1}; ^1H and ^{13}C NMR (SI); HR-ESI-MS *m/z* 399.2140 $[M+Na]^+$ (calcd for $C_{22}H_{32}O_5Na$, 399.2142).

Bisglaucumlide L (8): colorless oil (4.4 mg); $[\alpha]_D^{20}$ = -5.2 (c 0.3, MeOH); ^1H and ^{13}C NMR (SI); LC-ESI-MS *m/z* 738.4 $[M^+]$ (calcd for $C_{43}H_{62}O_{10}$, 738.43).

Bisglaucumlide M (9): colorless oil (2.3 mg); $[\alpha]_D^{20}$ = -14.4 (c 0.2, MeOH); ^1H and ^{13}C NMR (SI); LC-ESI-MS *m/z* 738.4 $[M^+]$ (calcd for $C_{43}H_{62}O_{10}$, 738.43).

In CHAPTER II, six *D. stellata* nudibranchs (3.7 g wet weight), their egg ribbons (0.7 g wet weight) and pieces of the associated sponge (3.5 g wet weight) were separately frozen, crushed and ultrasonicated for a total of 3 minutes (30 sec. intervals) on ice, while submerged in a minimum of first acetone (Ac) and consecutively methanol (MeOH). The ethanolic storage solutions of *D. stellata* nudibranch-, egg ribbon- and *S.* cf. *agaricina* samples were each combined with the respective Ac/MeOH extracts of the samples and dried under vacuum to give the crude extracts. After liquid-liquid separation of the three crude extracts (0.9 g, 0.3 g, and 0.2 g, respectively) between 50 mL water (H_2O) and three times 50 mL ethyl acetate (EtOAc), EtOAc solubles (223 mg, 35 mg, and 81 mg) were separated by RP-HPLC. A Macherey-Nagel Nucleodur C_{18} Pyramid column (250 mm x 10 mm; 5 µm) and a Phenomenex Kinetex C_{18} column (250 mm x 4.6 mm, 5 µm), with a linear gradient elution from 70:30 (MeOH:H_2O) to 100% MeOH in 25 min, and a flow of 1.5 mL/min were used for separation. The isolated metabolite had a retention time around 13 minutes.

12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin: white amorphous solid (12.4 mg); $[\alpha]^{20}_D$ +40.5 (*c* 0.6, $CHCl_3$; IR (ATR) ν_{max} 3416, 2922, 2861, 1732, 1234 cm^{-1}; ^1H and ^{13}C NMR (SI); HRAPCIMS *m/z* 487.3054 $[M+H]^+$ (calcd. for $C_{29}H_{43}O_6$, 487.3060).

In CHAPTER III, preserved *C. annae*, *C. dianae*, *C. lochi* and *C. willani* specimens were separated into species and location groups. They were separately frozen, crushed and ultrasonicated (30 sec. intervals) on ice, while submerged in a minimum of first acetone (AC) and consecutively methanol (MeOH). Ethanolic storage solutions were each combined with the respective AC/MeOH extracts and dried under vacuum to give the crude extracts. Crude extracts were analysed using HR-ESI-LCMS. Afterwards, crude extracts were separated, using liquid-liquid separation, between water (H_2O) and three times ethyl acetate (EtOAc). EtOAc-solubles were again analysed by HR-ESI-LCMS. Location groups, of the same species, with a similar MS-profile, were combined and further separated by RP-HPLC. A Macherey-Nagel Nucleodur C_{18} Pyramid column (250 mm x 10 mm; 5 µm), with isocratic gradient elution, 82:18 (MeOH:H_2O), and a flow of 1.0 mL/min were used for separation. The isolated main metabolite (LatA) had a retention time of around 41 minutes. Furthermore, eight of the *C. annae* and five of the *C. dianae* specimens, collected at Sangihe Island, August 2016, were carefully dissected in the laboratory of Politeknik Nusa Utara, Sangihe, Indonesia, to separate the mantle tissue with the mantle rim, incorporating mantle dermal formations (MDFs), from the body with viscera (internal organs). Dissected tissues were separately extracted as described above. Crude- and EtOAc-extracts of the mantle and body tissues were analysed using HR-ESI-LCMS. Additionally, ethanolic storage solutions of *C. strigata*, and mucus collected with cellulose from the notum and foot of living *C. annae* specimen, were examined using HR-ESI-LCMS.

Latrunculin A: white amorphous solid (22.4 mg, ~ 0.4 mg/specimen); $[\alpha]^{20}_{D}$ +273.5 (*c* 0.1, $CHCl_3$); ^{1}H and ^{13}C NMR (SI); HR-ESI-MS *m/z* 444.0638 $[M+Na]^+$ (calcd. for $C_{22}H_{31}NO_5S$ + Na, 444.1821).

UV/Vis and IR Spectroscopy

A Perkin-Elmer FT-IR Spectrum BX spectrometer with Spectrum software (v3.01, ©1998-2015 PerkinElmer Inc) was used for the recording of IR spectra. UV spectra of the compounds were recorded in MeOH on a Perkin-Elmer Labda 40 with a 1.0 cm quartz cell, using the UV WinLab software (v2.80.03, ©1998-2015 PerkinElmer Inc). The molar attenuation coefficient ε was determined in accordance with the Lambert-Beer law:

$$A = \varepsilon \; x \; c \; x \; b \Leftrightarrow \varepsilon \left[\frac{L}{mol \; x \; cm}\right] = \frac{A}{c \left[\frac{mol}{L}\right] \; x \; b \; [cm]}$$

A: Absorption at peak maximum

ε: Molar attenuation coefficient

c: Concentration

b: Layer thickness of solution

Optical Rotation

Optical rotations were measured with a Jasco DIP 140 polarimeter (1 dm, 1 cm^3 cell). Samples were dissolved either in 1.2 mL MeOH or $CHCl_3$ and measured at λ = 589 nm, corresponding to the sodium D line, at room temperature. At least 10 measurements were recorded for each compound. The average was calculated and assigned as *α*.

Specific optical rotation $[\alpha]_D^{20}$ was calculated as:

$$[\alpha]_D^T = \frac{100 \; x \; \alpha}{c \; x \; l}$$

α: Optical rotation

T: Temperature [°C]

D: Sodium D line [λ = 589]

c: Concentration [g/mL]

l: Cell length [dm]

NMR Spectroscopy

Structures were elucidated by extensive spectroscopic analyses, especially 1D (^{1}H, ^{13}C, DEPT-135) and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{1}H NOESY and ^{1}H-^{1}H ROESY) NMR techniques. NMR spectra were either recorded in MeOH-*d*$_4$ or in $CDCl_3$ on a Bruker Avance 300 DPX operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). To obtain a higher sensitivity and better resolution, certain compounds were additionally recorded by S. Kehraus (Institute of Pharmaceutical Biology, Bonn) on a Bruker Ascend 600 spectrometer, equipped with a CryoProbe™ Prodigy, operating at 600 MHz (^{1}H) and 150 MHz (^{13}C). External calibration was attained by referencing the spectra to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0 for MeOH-*d*$_4$ and $\delta_{H/C}$ 7.26/77.0 for $CDCl_3$. NMR spectra were analysed using Topspin v3.1.3 (©2004 BRUKER Biospin) and MestReNova v14.2.0-26256 (©2020 Mestrelab Research S.L.) software. Obtained results from the spectroscopic analyses were compared to published data, whenever possible. For this purpose searches for published structures and substructures were carried out using the databases SciFinder® (©2015 American Chemical Society), AntiBase (©2015 Wiley-VCH Verlag GmbH & Co.), and MarinLit™(©Royal Society of Chemistry 2015). Structures were drawn with ChemBioDraw Ultra v12.0 (©1986-2009 CambridgeSoft.)

MALDI-MS-Imaging

Chemicals. Trifluoroacetic acid (TFA), water (HPLC grade), and 2,5-dihydroxybenzoic acid (DHB) were purchased from Fluka (Neu Ulm, Germany), tragacanth from Sigma-Aldrich (Steinheim, Germany), and acetone from Merck (Darmstadt, Germany).

Sample Preparation, Instrumentation, and Data Processing. Specimens *Chromodoris annae* (2 sp.) and *Chromodoris dianae* (1 sp.) were embedded in 5% (w/v) gelatin aqueous solution and frozen at −80°C for 60 min to form solid blocks. Afterwards, tissue sections of 20 µm thickness were cut using a cryomicrotome (HM 525 cryostat, Thermo Scientific, Dreieich, Germany) at −20°C. The embedding material was removed carefully with a painting brush to prevent tissue distortion. Sections were thaw mounted on microscope glass slides (ground edges frosted, VWR International GmbH, Darmstadt, Germany) and stored at −80°C before analysis. The samples were brought to room temperature, using a desiccator to avoid condensation of humidity. Optical microscopic images of the sections were captured before matrix application (Olympus BX-41, Olympus Europa GmbH, Hamburg, Germany).

High spatial resolution MALDI-MS imaging requires a uniform coating of tissue sections with a microcrystalline matrix material. For this purpose, a dedicated matrix preparation system (SMALDIPrep, TransMIT GmbH, Giessen, Germany) was used to spray 100 µl (10 µl/min) of the matrix solution, 30 mg/mL of 2,5-dihydroxybenzoic acid in 50:50 (*v*/*v*) acetone:H_2O (0.1% TFA) for low molecular weight compounds in positive ion mode, on top of the tissue sections.[745] Homogeneity and crystal sizes were controlled after matrix application by microscopy before fixing the sample on the sample holder of the imaging source.

MALDI-MS imaging experiments were performed with a high spatial-resolution MS imaging ion source (AP-SMALDI10®, TransMIT GmbH, Giessen, Germany) operating at atmospheric pressure.[746] The minimum laser beam focus results in an ablation spot diameter of 5 µm.[747–749] However, for the experiment described here, the laser focus size was set to 10 µm. The samples were scanned by the movement of the x-, y-, and z-stages placed in front of the transfer capillary of the mass spectrometer. For desorption/ionisation, a diode-pumped solid-state laser at 343-nm wavelength, operating at 100 Hz was used. Generated ions were co-axially transferred to a high mass-resolution mass spectrometer (Q Exactive™, Thermo Fisher Scientific GmbH, Bremen, Germany, mass resolution, R = 140,000 at *m/z* 200). Mass spectra in the mass range of *m/z* 250–850 were generated and the analyzer was operated in positive ion mode. For internal calibration of mass spectra, a ubiquitous signal of the MALDI matrix was used as a lock mass, providing a mass accuracy better than 2 ppm root mean square error. High-quality MS ion images were generated using the Mirion software package.[750] A narrow image bin width of $\Delta m/z = \pm 5$ ppm was used for image generation. MS images were normalized to the highest signal intensity per image for each imaged analyte ion species. No additional data processing steps, such as smoothing, interpolation, or normalization to matrix signals, were employed. RGB (Red–green–blue) overlay images were generated for the selected analyte ion signals to demonstrate the distribution of LatA in *Chromodoris* cross-sections. The red ion signals were later edited to grey for better accessibility.

Fluorescence Microscopy

Cell culture. To investigate and visualize the activity of latrunculin A, isolated from *Chromodoris* sea slugs, we conducted fluorescence microscopy experiments with human embryogenic kidney cells (HEK293, obtained from Leibniz Institute, DSMZ GmBH, Braunschweig, Germany, reference number ACC 305). The HEK293 cells were cultivated in Dulbecco's modified Eagle's medium (DMEM), low glucose, pyruvate (Life Technologies Ltd.; Paisley, UK) supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin and 10% fetal calf serum. HEK293 cells and all generated clones were maintained by ten-fold dilutions with fresh medium every 3-4 days in 10 cm dishes. All cells were cultured at 37° C and 5% CO_2.

Experimental setup. Fluorescence imaging was conducted with an Axiovert® 200 M microscope, equipped with a Colibri.2® LED system including a 365 nm LED, LD Achroplan 40x, NA 0.60 Korr. objective, AxioCamMR3® camera, and filter set 49 (Excitation: G 365, Beam Splitter: FT 395 Emission: BP 445/50). The system was operated with Axiovision® Rev. 4.8. All parts mentioned were from Carl Zeiss Microscopy GmbH, Jena, Germany.

Cell measurements. HEK293 cells were seeded in a density of 12500 cells per cm^2 on 18 mm glass coverslips in DMEM (Life Technologies Ltd.; Paisley, UK), supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin and 10% fetal calf serum, two days before the experiment. Glass coverslips were coated using 50 µl of 0.1 mg/mL PDL solution for 30 min at 37 °C and were afterwards washed three times with 50 µl PBS. After the cells attached to the coverslips, either only DMSO as control or 50 µM latrunculin A (LatA) solved in DMSO, was added to the medium. Pictures of the stained cells were taken 24h and 44h after the addition of DMSO/LatA. On the day of the experiment, cells of the control group reached a density of 75-90%. The medium was exchanged with 1 mL methanol free 4% paraformaldehyde/10% sucrose solution in PBS buffer and kept for 10 min at room temperature. This solution was exchanged with 1 mL 0.1% Triton in PBS buffer and was kept for 2 min at room temperature. Afterwards, the cells were washed two times with PBS buffer. Coverslips were incubated on parafilm, each with 50 µL Phalloidin-Atto 488-solution (1:500 in PBS/1% BSA) and Hoechst stain (500 ng/mL), in darkness, for 45 min at room temperature. Subsequently, coverslips were washed two times with PBS, one time with water and were then mounted on a slide using Mowiol as a mounting medium.

PCR Amplification, Sequencing and Alignment

Comparative Analysis of Heterobranchia Actin Genes. Genomic DNA was extracted from the foot, notum, or whole body of the specimens, depending on the size of the nudibranch, with the Qiagen DNeasy Blood & Tissue Kit, following the manufacturer's instructions. Degenerated and subsequently, specific primers were designed based on available mollusc actin gene data from GenBank, NCBI (SI).[711] Putative actin gene fragments of the coding region (~ 885 bp) of *Chromodoris* specimens were amplified by polymerase chain reaction (PCR) using the specific forward primer "Act1F": 5'-CAG GGT GTT GGA GAA GAT CTG GCA TC-3', and the reverse primer "Act1R": 5'-TAG AAG CAC TTC CTG TGG ACA ATG GA-3' (Table M1.1). A putative actin gene fragment of the coding region (~ 827 bp) of *Elysia viridis* was amplified by PCR using the specific forward primer "F19_1": 5'-GGA GAA GAT CTG GCA TC-3', and the reverse primer "R19_1": 5'-GAT CCA CAT CTG CTG G -3' (Table M1.1). PCR products were separated using gel electrophoresis (1% agarose, 110 V, 45 min.) and stained with ethidium bromide. The fragments were isolated from the gel and purified using either the Zymoclean ™ Large Fragment DNA Recovery Kit (Zymo Research Europe GmbH, Freiburg, Germany) or the FastGene® Gel/PCR Extraction Kit (NIPPON Genetics Europe, Düren, Germany), according to the manufacturer's instructions. Purified PCR products were sent to Eurofins Genomics and sequenced by Sanger sequencing (Eurofins Genomics Germany GmbH, Ebersberg, Germany). All obtained gDNA sequences for each species were compared to each other and consensus sequences were created for further analyses. Furthermore, these sequences were compared to available nucleotide sequences of the NCBI GenBank database using BLAST® (SI), and to transcriptomic cDNA sequences of Cladobranchia species, kindly provided by D. Karmeinski.[118] Obtained heterobranchia sequences were aligned using the MUSCLE algorithm and compared using MEGA X version 10.0.5,[751] SplitsTree4 v4.16.2.,[752] and ClustalX2.1.[753] Sequence identity was calculated based on 808 nucleotide and 269 amino acid positions, using ClustalX2.1.[753] The six new actin isoform sequences reported in this study, five from *Chromodoris* species and one from *Elysia viridis*, were deposited at the NCBI GenBank database under accession numbers: OK074000, OK074001, OK074002, OK074003, OK074004 and OK074005.

Table M1.1 Specimens, primer sequences and PCR conditions used for the amplification of putative actin gene fragments within the coding region.

Species (specimen number)	Primer	PCR Conditions
Chromodoris annae (Chan16Sa-9, Chan16Sa-3, Chan16Bu-6, Chel16Sa-1) ***Chromodoris dianae*** (Chdi16Sa-6, Chdi16Bu-6) ***Chromodoris lochi*** (Chlo16Bu-1, Chlo16Bu-2) ***Chromodoris strigata*** (Chmi16Bu-1, Chst16Sa-1, Chst17Ba-1) ***Chromodoris willani*** (Chwi16Bu-1, Chwi16Bu-2)	**Forward** Act1F: 5'-CAG GGT GTT GGA GAA GAT CTG GCA TC-3' **Reverse** Act1R: 5'-TAG AAG CAC TTC CTG TGG ACA ATG GA-3' ~ 885 bp	2.-4. 32 cycles 98°C, 30 sec. 98°C, 10 sec. 61°C, 30 sec. 72°C, 30 sec. 72°C, 2min. 4°C, ∞
Elysia viridis (gDNA kindly provided by G. Christa)	**Forward** F19_1: 5'-GGA GAA GAT CTG GCA TC-3' **Reverse** R19_1: 5'-GAT CCA CAT CTG CTG G -3' ~ 827 bp	2.-4. 32 cycles 98°C, 30 sec. 98°C, 10 sec. 58°C, 30 sec. 72°C, 30 sec. 72°C, 2min. 4°C, ∞

Antibacterial Activity

In CHAPTER I, compound **6** and **3** were tested for antibacterial activity against *Arthrobacter crystallopoietes* (DSM 20117). The assay was conducted by H. Harms (Institute of Pharmaceutical Biology, Bonn). Antibacterial activity of the compounds was measured as growth-inhibition of *Arthrobacter crystallopoietes* (DSM 20117), which was previously cultivated on a Corynebacterium Agar Medium with pH 7.2-7.4 (SI). The bacterial culture was diluted until OD600 = 0.1. Of the bacterial suspension, 200 µL were inserted in all vials, except in row 8 (control). Additional 200 µL of the bacterial suspension were added to column A. Antibiotics (0.4 µL of the stock solution, SI) were added to column A row 2-7, as additional positive control. Compound **6** (0.5 mg) and compound **3** (0.1 mg) were dissolved in 20 µL DMSO each. Of these, 0.4 µL were inserted into a vial containing 200 µL of the bacterial solution, respectively. Compound **6** was added to row 10 and compound **3** to row 12.

A serial dilution was conducted in four steps, using 200 µL of the previous vial in each step:

	Compound **6**	Compound **3**
	(Row 10)	(Row 12)
1. Vial	0.500 µg/µL	0.100 µg/µL
2. Vial	0.250 µg/µL	0.050 µg/µL
3. Vial	0.125 µg/µL	0.025 µg/µL
4. Vial	0.0625 µg/µL	0.0125 µg/µL

Vials were closed with a lid, sealed with parafilm and kept overnight at room temperature on an orbital shaker at low speed (140 - 350 rpm). Measurements were performed two days after insertion of the compounds and extract, using a Tecan plate reader (SUNRISE; Serial number: 605000077; Firmware: V 3.31 25/08/05; XFLUOR4 Version: V 4.51). Absorbance was measured at 560 nm (SI).

For CHAPTER II, all ethyl acetate extracts of *D. stellata* nudibranchs, egg ribbons and *S.* cf. *agaricina* were tested as described above, in a first screening approach for antibacterial activity against the Gram-positive *Arthrobacter crystallopoietes* (DSM 20117). The pure compound 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin, isolated from all three extracts, was tested for antibacterial activity against the Gram-positive *Bacillus megaterium* (DSM 32) (SI).

Chemical Defence Assay

Feeding deterrence of compound **1**, **2** and **6**, isolated from *P. longicirrum*, was measured in order to investigate, whether they act as a chemical defence against fish predators. For this purpose, laboratory feeding assays with the pufferfish *Canthigaster solandri* Richardson, 1845 were conducted. *C. solandri* is a very common predator, known to feed on various invertebrates and benthic algae.[754,755] Sharing the same habitat with *P. longicirrum*, the tropical sublitoral coral reefs of the Indo-Pacific, makes *C. solandri* a useful model predator, which has previously been used in several studies concerning chemical feeding deterrence.[41,754] 11 individuals were kept separately in 70 L flow-through tanks. They were regularly fed a day prior to the feeding assays to avoid changing the feeding preference patterns. Compound **1**, **2**, and **6** were individually incorporated into the artificial diet, at concentrations below estimated natural occurrence (**6** and **2**: 0.5%, 1.0% and 2.0%; **1**: 1.0% of dry mass). To create the artificial diet, 0.3 g alginate and 0.5 g squid powder were filled up with purified water to 10 g. The mixture was vigorously stirred and heated in a microwave oven. Compounds were each dissolved in a drop of EtOH in an Eppendorf tube and homogenized with 1 mL of the artificial diet. A drop of EtOH was added to the artificial diet to produce a comparable control. Since control and treated pellets showed the same coloration, additional coloring was not used. The semi-liquid diet was poured into 0.25 M $CaCl_2$ solution to solidify, using a disposable syringe, which gave the artificial diet a vermiform appearance. After solidification, it was cut into suitable pieces. Each day, two test series were conducted with two of the three compounds. The first test series with one of the compounds was conducted in the morning, while the second series with one of the other compounds was performed in the late afternoon. In each test series, one control and one treated food pellet were offered sequentially to *C. solandri*. A second control pellet was offered if the treated pellet was rejected completely or spit out at least three times, to ensure that the refusal was due to the incorporated compound and not because the fish generally ceased to feed. A rejection was only counted as such, if both controls were eaten. The results were analysed using *Fisher's exact test* to statistically confirm the reduced palatability of treated pellets compared to control pellets. Significance was determined as following: $p < 0.05$ = significant (*), $p < 0.005$ = very significant (**) and $p < 0.0005$ = strongly significant (***).

In Vivo Toxicity Assay

Living *Chromodoris annae* (1 sp.) and *C. dianae* (2 sp.) were kept in glass aquaria with artificial seawater (Instant Ocean®, synthetic sea salt, added according to instructions), salinity around 33 – 34.5 PSU, relative density ~ 1.025 – 1.026, at 26 – 28 °C, aerated via an oxygenation pump and air stone bubblers. Also, living *Elysia viridis* (12 sp.) were kept in glass aquaria, with artificial seawater (Instant Ocean®, synthetic sea salt, added according to instructions), salinity around 36 – 37.5 PSU, relative density ~ 1.0275 – 1.0286, at 17 – 19 °C, aerated via an oxygenation pump and air stone bubblers. The 12 *E. viridis* specimen were separated into two groups. As a control, 6 *E. viridis* were injected into the muscular foot with an isotonic solution (0.01 mL, 5% DMSO, SI), the other 6 *E. viridis*, 1 *C. annae* and 2 *C. dianae* were injected with an isotonic solution containing 75 µM LatA (0.01 mL, 5% DMSO). Since only 3 *Chromodoris* specimens were kept, all of them were injected with LatA and there was no additional control *Chromodoris* group. Each injection was carried out with a 3-piece single-use, fine dosage syringe (Omnifix®-F Luer Duo, Braun, 1 mL, 0.01 mL graduation, DIN EN ISO-Norm 7886-1) and a sterile, hypodermic Sterican®needle (25 G/0.5 x 26 mm, DIN EN ISO-Norm 7864). After injection, all animals were kept in separate groups and were observed over several hours. Symptoms, behavioural changes and deaths, if occurring, were noted.

REFERENCES

1. Bogdanov A, Hertzer C, Kehraus S, Nietzer S, Rohde S, Schupp PJ, Wägele H, König GM. Defensive Diterpene from the Aeolidoidean Phyllodesmium longicirrum. *J Nat Prod*. 2016;79(3):611-615. doi:10.1021/acs.jnatprod.5b00860

2. Bogdanov A, Hertzer C, Kehraus S, Nietzer S, Rohde S, Schupp PJ, Wägele H, König GM. Secondary metabolome and its defensive role in the aeolidoidean Phyllodesmium longicirrum, (Gastropoda, Heterobranchia, Nudibranchia). *Beilstein J Org Chem*. 2017;13:502-519. doi:10.3762/bjoc.13.50

3. Hertzer C, Kehraus S, Böhringer N, Kaligis F, Bara R, Erpenbeck D, Wörheide G, Schäberle TF, Wägele H, König GM. Antibacterial scalarane from Doriprismatica stellata nudibranchs (Gastropoda, Nudibranchia), egg ribbons, and their dietary sponge Spongia cf. agaricina (Demospongiae, Dictyoceratida). *Beilstein J Org Chem*. 2020;16(1):1596-1605. doi:10.3762/bjoc.16.132

4. Eisenbarth JH, Undap N, Papu A, Schillo D, Dialao J, Reumschüssel S, Kaligis F, Bara R, Schäberle TF, König GM, et al. Marine Heterobranchia (Gastropoda, Mollusca) in Bunaken National Park, North Sulawesi, Indonesia—A Follow-Up Diversity Study. *Diversity*. 2018;10(4):127. doi:10.3390/d10040127

5. Kaligis F, Eisenbarth JH, Schillo D, Dialao J, Schäberle TF, Böhringer N, Bara R, Reumschüssel S, König GM, Wägele H. Second survey of heterobranch sea slugs (Mollusca, Gastropoda, Heterobranchia) from Bunaken National Park, North Sulawesi, Indonesia - how much do we know after 12 years? *Mar Biodivers Rec*. 2018;11(1):2. doi:10.1186/s41200-018-0136-3

6. Undap N, Papu A, Schillo D, Ijong FG, Kaligis F, Lepar M, Hertzer C, Böhringer N, König GM, Schäberle TF, et al. First Survey of Heterobranch Sea Slugs (Mollusca, Gastropoda) from the Island Sangihe, North Sulawesi, Indonesia. *Diversity*. 2019;11(9):170. doi:10.3390/d11090170

7. Papu A, Undap N, Martinez NA, Segre MR, Datang IG, Kuada RR, Perin M, Yonow N, Wägele H. First Study on Marine Heterobranchia (Gastropoda, Mollusca) in Bangka Archipelago, North Sulawesi, Indonesia. *Diversity*. 2020;12(2):52. doi:10.3390/d12020052

8. Böhringer N, Fisch KM, Schillo D, Bara R, Hertzer C, Grein F, Eisenbarth JH, Kaligis F, Schneider T, Wägele H, et al. Antimicrobial Potential of Bacteria Associated with Marine Sea Slugs from North Sulawesi, Indonesia. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.01092

9. Fisch KM, Hertzer C, Böhringer N, Wuisan ZG, Schillo D, Bara R, Kaligis F, Wägele H, König GM, Schäberle TF. The Potential of Indonesian Heterobranchs Found around Bunaken Island for the Production of Bioactive Compounds. *Mar Drugs*. 2017;15(12). doi:10.3390/md15120384

10. Bogdanov A, Papu A, Kehraus S, Cruesemann M, Wägele H, König GM. Metabolome of the Phyllidiella pustulosa Species Complex (Nudibranchia, Heterobranchia, Gastropoda) Reveals Rare Dichloroimidic Sesquiterpene Derivatives from a Phylogenetically Distinct and Undescribed Clade. *J Nat Prod*. 2020;83(9):2785-2796. doi:10.1021/acs.jnatprod.0c00783

11. Daily GC, Söderqvist T, Aniyar S, Arrow K, Dasgupta P, Ehrlich PR, Folke C, Jansson A, Jansson BO, Kautsky N, et al. The Value of Nature and the Nature of Value. *Science*. 2000;289(5478):395-396. doi:10.1126/science.289.5478.395

12. Geange S, Townsend M, Clark D, Ellis JI, Lohrer AM. Communicating the value of marine conservation using an ecosystem service matrix approach. *Ecosyst Serv*. 2019;35:150-163. doi:10.1016/j.ecoser.2018.12.004

13. Townsend M, Lohrer AM. Empirical Validation of an Ecosystem Service Map Developed From Ecological Principles and Biophysical Parameters. *Front Mar Sci*. 2019;6. doi:10.3389/fmars.2019.00021

14. Macreadie PI, Anton A, Raven JA, Beaumont N, Connolly RM, Friess DA, Kelleway JJ, Kennedy H, Kuwae T, Lavery PS, et al. The future of Blue Carbon science. *Nat Commun*. 2019;10(1):3998. doi:10.1038/s41467-019-11693-w

15. Vieira H, Leal MC, Calado R. Fifty Shades of Blue: How Blue Biotechnology is Shaping the Bioeconomy. *Trends Biotechnol*. 2020;38(9):940-943. doi:10.1016/j.tibtech.2020.03.011

16. Carté BK. Biomedical Potential of Marine Natural Products. *BioScience*. 1996;46(4):271-286. doi:10.2307/1312834

17. Allsopp M, Pambuccian SE, Johnston P, Santillo D. *State of the World's Oceans*. Springer Science & Business Media; 2008.

18. Sotka EE, Forbey J, Horn M, Poore AGB, Raubenheimer D, Whalen KE. The emerging role of pharmacology in understanding consumer–prey interactions in marine and freshwater systems. *Integr Comp Biol*. 2009;49(3):291-313. doi:10.1093/icb/icp049

19. Calado R, Costa Leal M, Gaspar H, Santos S, Marques A, Nunes M, Vieira H. How to Succeed in Marketing Marine Natural Products for Nutraceutical, Pharmaceutical and Cosmeceutical Markets. In: ; 2018:317-403. doi:10.1007/978-3-319-69075-9_9

20. Leal MC, Anaya-Rojas JM, Munro MHG, Blunt JW, Melian CJ, Calado R, Lürig MD. Fifty years of capacity building in the search for new marine natural products. *Proc Natl Acad Sci*. 2020;117(39):24165-24172. doi:10.1073/pnas.2007610117

21. R. Carroll A, R. Copp B, A. Davis R, A. Keyzers R, R. Prinsep M. Marine natural products. *Nat Prod Rep*. 2021;38(2):362-413. doi:10.1039/D0NP00089B

22. Esteban M, Thompson F, Rotter A, Am B, Ml C, Mf C, Ar DM, Ot E, Rj F, Lm G, et al. The Essentials of Marine Biotechnology. *Front Mar Sci*. 2021;8:629629. doi:10.3389/fmars.2021.629629

23. Edie SM, Jablonski D, Valentine JW. Contrasting responses of functional diversity to major losses in taxonomic diversity. *Proc Natl Acad Sci*. 2018;115(4):732-737. doi:10.1073/pnas.1717636115

24. Avila C. Chemical War in Marine Animal Forests: Natural Products and Chemical Interactions. In: Rossi S, Bramanti L, eds. *Perspectives on the Marine Animal Forests of the World*. Springer International Publishing; 2020:239-307. doi:10.1007/978-3-030-57054-5_9

25. König GM, Wright AD. Marine Natural Products Research: Current Directions and Future Potential. *Planta Med*. 1996;62(3):193-211. doi:10.1055/s-2006-957861

26. Proksch P, Edrada-Ebel R, Ebel R. Drugs from the Sea - Opportunities and Obstacles. *Mar Drugs*. 2003;1(1):5-17. doi:10.3390/md101005

27. Saito S ya. Toxins Affecting Actin Filaments and Microtubules. In: Fusetani N, Kem W, eds. *Marine Toxins as Research Tools*. Vol 46. Progress in Molecular and Subcellular Biology. Springer Berlin Heidelberg; 2009:187-219. doi:10.1007/978-3-540-87895-7_7

28. Hughes CC, Fenical W. Antibacterials from the Sea. *Chem Weinh Bergstr Ger*. 2010;16(42):12512-12525. doi:10.1002/chem.201001279

29. Benkendorff K. Molluscan biological and chemical diversity: secondary metabolites and medicinal resources produced by marine molluscs. *Biol Rev*. 2010;85(4):757-775. doi:10.1111/j.1469-185X.2010.00124.x

30. Montaser R, Luesch H. Marine natural products: a new wave of drugs? *Future Med Chem*. 2011;3(12):1475-1489. doi:10.4155/fmc.11.118

31. Tohme R, Darwiche N, Gali-Muhtasib H. A Journey Under the Sea: The Quest for Marine Anti-Cancer Alkaloids. *Mol Basel Switz*. 2011;16:9665-9696. doi:10.3390/molecules16119665

32. Gogineni V, Hamann MT. Marine natural product peptides with therapeutic potential: Chemistry, biosynthesis, and pharmacology. *Biochim Biophys Acta BBA - Gen Subj*. 2018;1862(1):81-196. doi:10.1016/j.bbagen.2017.08.014

33. Raimundo I, Silva SG, Costa R, Keller-Costa T. Bioactive Secondary Metabolites from Octocoral-Associated Microbes—New Chances for Blue Growth. *Mar Drugs*. 2018;16(12):485. doi:10.3390/md16120485

34. Nathani N, Mootapally CS, Gadhvi IR, Maitreya B, Joshi CG, eds. *Marine Niche: Applications in Pharmaceutical Sciences : Translational Research*. Springer Singapore; 2020. doi:10.1007/978-981-15-5017-1

35. Patra S, Praharaj PP, Panigrahi DP, Panda B, Bhol CS, Mahapatra KK, Mishra SR, Behera BP, Jena M, Sethi G, et al. Bioactive compounds from marine invertebrates as potent anticancer drugs: the possible pharmacophores modulating cell death pathways. *Mol Biol Rep*. 2020;47(9):7209-7228. doi:10.1007/s11033-020-05709-8

36. Conte M, Fontana E, Nebbioso A, Altucci L. Marine-Derived Secondary Metabolites as Promising Epigenetic Bio-Compounds for Anticancer Therapy. *Mar Drugs*. 2021;19(1):15. doi:10.3390/md19010015

37. Wang C, Tang S, Cao S. Antimicrobial compounds from marine fungi. *Phytochem Rev*. 2021;20(1):85-117. doi:10.1007/s11101-020-09705-5

38. Dinarvand M, Spain M. Identification of Bioactive Compounds from Marine Natural Products and Exploration of Structure—Activity Relationships (SAR). *Antibiotics*. 2021;10:337. doi:10.3390/antibiotics10030337

39. D. Sigwart J, Blasiak R, Jaspars M, Jouffray JB, Tasdemir D. Unlocking the potential of marine biodiscovery. *Nat Prod Rep*. Published online 2021. doi:10.1039/D0NP00067A

40. Zhang H, Zou J, Yan X, Chen J, Cao X, Wu J, Liu Y, Wang T. Marine-Derived Macrolides 1990–2020: An Overview of Chemical and Biological Diversity. *Mar Drugs*. 2021;19(4):180. doi:10.3390/md19040180

41. Rohde S, Gochfeld DJ, Ankisetty S, Avula B, Schupp PJ, Slattery M. Spatial Variability in Secondary Metabolites of the Indo-Pacific Sponge Stylissa massa. *J Chem Ecol*. 2012;38(5):463-475. doi:10.1007/s10886-012-0124-8

42. Petersen LE, Kellermann MY, Schupp PJ. Secondary Metabolites of Marine Microbes: From Natural Products Chemistry to Chemical Ecology. In: Jungblut S, Liebich V, Bode-Dalby M, eds. *YOUMARES 9 - The Oceans: Our Research, Our Future: Proceedings of the 2018 Conference for YOUng MArine RESearcher in Oldenburg, Germany*. Springer International Publishing; 2020:159-180. doi:10.1007/978-3-030-20389-4_8

43. Pawlik JR, Kernan MR, Molinski TF, Harper MK, Faulkner DJ. Defensive chemicals of the Spanisch dancer nudibranch Hexabranchus sanguineus and its egg ribbons: macrolides derived from a sponge diet. *J Exp Mar Biol Ecol*. 1988;119(2):99-109. doi:10.1016/0022-0981(88)90225-0

44. Proksch P. Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs. *Toxicon*. 1994;32(6):639-655. doi:10.1016/0041-0101(94)90334-4

45. Avila C. Natural products of opisthobranch molluscs: A biological review. In: Ansell AD, Gibson RN, Barnes M, eds. *Oceanography and Marine Biology - an Annual Review, Vol 33*. Vol 33. U C L Press Ltd; 1995:487-559.

46. McClintock JB, Baker BJ. *Marine Chemical Ecology*. CRC Press; 2001.

47. Mebs D. Toxicity in animals. Trends in evolution? *Toxicon*. 2001;39(1):87-96. doi:10.1016/S0041-0101(00)00155-0

48. Taylor MW, Radax R, Steger D, Wagner M. Sponge-Associated Microorganisms: Evolution, Ecology, and Biotechnological Potential. *Microbiol Mol Biol Rev*. 2007;71(2):295-347. doi:10.1128/MMBR.00040-06

49. Taylor MW, Hill RT, Piel J, Thacker RW, Hentschel U. Soaking it up: the complex lives of marine sponges and their microbial associates. *ISME J*. 2007;1(3):187-190. doi:10.1038/ismej.2007.32

50. Williams BL. Behavioral and Chemical Ecology of Marine Organisms with Respect to Tetrodotoxin. *Mar Drugs*. 2010;8(3):381-398. doi:10.3390/md8030381

51. Ng TPT, Saltin SH, Davies MS, Johannesson K, Stafford R, Williams GA. Snails and their trails: the multiple functions of trail-following in gastropods. *Biol Rev*. 2013;88(3):683-700. doi:10.1111/brv.12023

52. Puglisi MP, Sneed JM, Sharp KH, Ritson-Williams R, Paul VJ. Marine chemical ecology in benthic environments. *Nat Prod Rep*. 2014;31(11):1510-1553. doi:10.1039/C4NP00017J

53. Mollo E, Fontana A, Roussis V, Polese G, Amodeo P, Ghiselin MT. Sensing marine biomolecules: smell, taste, and the evolutionary transition from aquatic to terrestrial life. *Chem Biol*. 2014;2:92. doi:10.3389/fchem.2014.00092

54. Rohde S, Nietzer S, Schupp PJ. Prevalence and Mechanisms of Dynamic Chemical Defenses in Tropical Sponges. *PLOS ONE*. 2015;10(7):e0132236. doi:10.1371/journal.pone.0132236

55. Venuleo M, Raven JA, Giordano M. Intraspecific chemical communication in microalgae. *New Phytol*. 2017;215(2):516-530. doi:https://doi.org/10.1111/nph.14524

56. Putz A, Proksch P. Chemical Defence in Marine Ecosystems (From APR Volume 39). In: *Annual Plant Reviews Online*. American Cancer Society; 2018:162-213. doi:10.1002/9781119312994.apr0419

57. Avila C, Núñez-Pons L, Moles J, Núñez-Pons L, Moles J. From the Tropics to the Poles : Chemical Defense Strategies in Sea Slugs (Mollusca: Heterobranchia). Chemical Ecology. doi:10.1201/9780429453465-3

58. Puglisi MP, Becerro MA, eds. *Chemical Ecology*. 1st edition. CRC Press; 2020.

59. Müller C, Caspers BA, Gadau J, Kaiser S. The Power of Infochemicals in Mediating Individualized Niches. *Trends Ecol Evol*. 2020;35(11):981-989. doi:10.1016/j.tree.2020.07.001

60. Ziltener A, Kreicker S, Gross S. Selective self-rubbing behaviour in Indo-Pacific bottlenose dolphins off Hurghada, Northern Red Sea, Egypt. In: *21st Biennial Conference on the Biology of Marine Mammals.* ; 2015.

61. Chang YC, Chiang CC, Chang YS, Chen JJ, Wang WH, Fang LS, Chung HM, Hwang TL, Sung PJ. Novel Caryophyllane-Related Sesquiterpenoids with Anti-Inflammatory Activity from Rumphella antipathes (Linnaeus, 1758). *Mar Drugs*. 2020;18(11):554. doi:10.3390/md18110554

62. Sung PJ, Chuang LF, Kuo J, Fan TY, Hu WP. Rumphellatin A, the first chloride-containing caryophyllane-type norsesquiterpenoid from Rumphella antipathies. *Tetrahedron Lett*. 2007;48(23):3987-3989. doi:10.1016/j.tetlet.2007.04.040

63. Li G, Li P, Tang X. Natural Products from Corals. In: Li Z, ed. *Symbiotic Microbiomes of Coral Reefs Sponges and Corals*. Springer Netherlands; 2019:465-504. doi:10.1007/978-94-024-1612-1_16

64. Dash S, Das SK, Samal J, Thatoi HN. Epidermal mucus, a major determinant in fish health: a review. *Iran J Vet Res*. 2018;19(2):72-81.

65. Bansil R, Turner BS. The biology of mucus: Composition, synthesis and organization. *Adv Drug Deliv Rev*. 2018;124:3-15. doi:10.1016/j.addr.2017.09.023

66. Cilia G, Fratini F. Antimicrobial properties of terrestrial snail and slug mucus. *J Complement Integr Med*. 2018;15(3). doi:10.1515/jcim-2017-0168

67. Hayashida PY, da Silva Junior PI. Insights into Antimicrobial Peptides from Limacus flavus Mucus. *Curr Microbiol*. 2021;78(8):2970-2979. doi:10.1007/s00284-021-02552-3

68. Onzo A, Pascale R, Acquavia MA, Cosma P, Gubitosa J, Gaeta C, Iannece P, Tsybin Y, Rizzi V, Guerrieri A, et al. Untargeted analysis of pure snail slime and snail slime-induced Au nanoparticles metabolome with MALDI FT-ICR MS. *J Mass Spectrom*. 2021;56(5):e4722. doi:10.1002/jms.4722

69. Tachapuripunya V, Roytrakul S, Chumnanpuen P, E-kobon T. Unveiling Putative Functions of Mucus Proteins and Their Tryptic Peptides in Seven Gastropod Species Using Comparative Proteomics and Machine Learning-Based Bioinformatics Predictions. *Molecules*. 2021;26(11):3475. doi:10.3390/molecules26113475

70. Zhong T, Min L, Wang Z, Zhang F, Zuo B. Controlled self-assembly of glycoprotein complex in snail mucus from lubricating liquid to elastic fiber. *RSC Adv*. 2018;8(25):13806-13812. doi:10.1039/C8RA01439F

71. Newar J, Verma S, Ghatak A. Effect of Metals on Underwater Adhesion of Gastropod Adhesive Mucus. *ACS Omega*. 2021;6(24):15580-15589. doi:10.1021/acsomega.0c06132

72. Landauer MR, Chapnick SD. Responses of Terrestrial Slugs to Secretions of Stressed Conspecifics. *Psychol Rep*. 1981;49(2):617-618. doi:10.2466/pr0.1981.49.2.617

73. Denny M. 10 - Molecular Biomechanics of Molluscan Mucous Secretions. In: Hochachka PW, ed. *Metabolic Biochemistry and Molecular Biomechanics*. Academic Press; 1983:431-465. doi:10.1016/B978-0-12-751401-7.50017-X

74. Deyrup-Olsen I, Luchtel DL, Martin AW. Components of mucus of terrestrial slugs (Gastropoda). *Am J Physiol-Regul Integr Comp Physiol*. 1983;245(3):R448-R452. doi:10.1152/ajpregu.1983.245.3.R448

75. Bretz DD, Dimock RV. Behaviorally important characteristics of the mucous trail of the marine gastropod Ilyanassa Obsoleta (Say). *J Exp Mar Biol Ecol*. 1983;71(2):181-191. doi:10.1016/0022-0981(93)90072-V

76. Bonnemain B. Helix and Drugs: Snails for Western Health Care From Antiquity to the Present. *Evid Based Complement Alternat Med*. 2005;2(1):25-28. doi:10.1093/ecam/neh057

77. Meyer-Rochow VB. Therapeutic arthropods and other, largely terrestrial, folk-medicinally important invertebrates: a comparative survey and review. *J Ethnobiol Ethnomedicine*. 2017;13(1):9. doi:10.1186/s13002-017-0136-0

78. Dhiman V, Pant D. Human health and snails. *J Immunoassay Immunochem*. 2021;42(3):211-235. doi:10.1080/15321819.2020.1844751

79. Liu L, Sood A, Steinweg S. Snails and Skin Care—An Uncovered Combination. *JAMA Dermatol*. 2017;153(7):650-650. doi:10.1001/jamadermatol.2017.1383

80. Vassilev NG, Simova SD, Dangalov M, Velkova L, Atanasov V, Dolashki A, Dolashka P. An 1H NMR- and MS-Based Study of Metabolites Profiling of Garden Snail Helix aspersa Mucus. *Metabolites*. 2020;10(9):360. doi:10.3390/metabo10090360

81. Li J, Celiz AD, Yang J, Yang Q, Wamala I, Whyte W, Seo BR, Vasilyev NV, Vlassak JJ, Suo Z, et al. Tough adhesives for diverse wet surfaces. *Science*. 2017;357(6349):378-381. doi:10.1126/science.aah6362

82. Cerullo AR, Lai TY, Allam B, Baer A, Barnes WJP, Barrientos Z, Deheyn DD, Fudge DS, Gould J, Harrington MJ, et al. Comparative Animal Mucomics: Inspiration for Functional Materials from Ubiquitous and Understudied Biopolymers. *ACS Biomater Sci Eng*. 2020;6(10):5377-5398. doi:10.1021/acsbiomaterials.0c00713

83. Voultsiadou E. Therapeutic properties and uses of marine invertebrates in the ancient Greek world and early Byzantium. *J Ethnopharmacol*. 2010;130(2):237-247. doi:10.1016/j.jep.2010.04.041

84. Olivera BM, Cruz LJ. Conotoxins, in retrospect. *Toxicon*. 2001;39(1):7-14. doi:10.1016/S0041-0101(00)00157-4

85. Hannon HE, Atchison WD. Omega-Conotoxins as Experimental Tools and Therapeutics in Pain Management. *Mar Drugs*. 2013;11(3):680-699. doi:10.3390/md11030680

86. Senter PD, Sievers EL. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. *Nat Biotechnol*. 2012;30(7):631-637. doi:10.1038/nbt.2289

87. Newman DJ, Cragg GM. Current Status of Marine-Derived Compounds as Warheads in Anti-Tumor Drug Candidates. *Mar Drugs*. 2017;15(4). doi:10.3390/md15040099

88. Pettit GR, Kamano Y, Herald CL, Tuinman AA, Boettner FE, Kizu H, Schmidt JM, Baczynskyj L, Tomer KB, Bontems RJ. The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10. *J Am Chem Soc*. 1987;109(22):6883-6885. doi:10.1021/ja00256a070

89. Hamann MT, Otto CS, Scheuer PJ, Dunbar DC. Kahalalides: Bioactive Peptides from a Marine Mollusk Elysia rufescens and Its Algal Diet Bryopsis sp.1. *J Org Chem*. 1996;61(19):6594-6600. doi:10.1021/jo960877+

90. Martín-Algarra S, Espinosa E, Rubió J, López JJL, Manzano JL, Carrión LA, Plazaola A, Tanovic A, Paz-Ares L. Phase II study of weekly Kahalalide F in patients with advanced malignant melanoma. *Eur J Cancer*. 2009;45(5):732-735. doi:10.1016/j.ejca.2008.12.005

91. Salazar R, Cortes-Funes H, Casado E, Pardo B, Lopez-Martın A, Cuadra C, Tabernero J, Coronado C, Garcıa M, Matos-Pita AS, et al. Phase I study of weekly kahalalide F as prolonged infusion in patients with advanced solid tumors. *Cancer Chemother Pharmacol*. Published online 2013:9.

92. Tilvi S, Devi P, Majik MS. Quick elucidation of cyclodepsipeptide sequence from sacoglossan Elysia grandifolia using electrospray ionisation-tandem mass spectrometry. *Eur J Mass Spectrom*. 2017;23(3):92-97. doi:10.1177/1469066717699218

93. Cimino G, Rosa SD, Stefano SD, Sodano G, Villani G. Dorid Nudibranch Elaborates Its Own Chemical Defense. *Science*. 1983;219(4589):1237-1238. doi:10.1126/science.219.4589.1237

94. Faulkner DJ, Molinski TF, Andersen RJ, Dumdei EJ, De Silva ED. Geographical variation in defensive chemicals from pacific coast dorid nudibranchs and some related marine molluscs. *Comp Biochem Physiol Part C Comp Pharmacol*. 1990;97(2):233-240. doi:10.1016/0742-8413(90)90133-T

95. Cimino G, Ghiselin MT. Chemical defense and evolutionary trends in biosynthetic capacity among dorid nudibranchs (Mollusca: Gastropoda: Opisthobranchia). *CHEMOECOLOGY*. 1999;9(4):187-207. doi:10.1007/s000490050052

96. Barsby T, Linington RG, Andersen RJ. De Novo terpenoid biosynthesis by the dendronotid nudibranch Melibe leonina. *CHEMOECOLOGY*. 2002;12(4):199-202. doi:10.1007/PL00012669

97. Putz A, Kehraus S, Diaz-Agras G, Waegele H, Koenig GM. Dotofide, a Guanidine-Interrupted Terpenoid from the Marine Slug Doto pinnatifida (Gastropoda, Nudibranchia). *Eur J Org Chem*. 2011;(20-21):3733-3737. doi:10.1002/ejoc.201100347

98. Pawlik JR. Antipredatory Defensive Roles of Natural Products from Marine Invertebrates. In: Fattorusso E, Gerwick WH, Taglialatela-Scafati O, eds. *Handbook of Marine Natural Products*. Springer Netherlands; 2012:677-710. doi:10.1007/978-90-481-3834-0_12

99. Avila C, Cimino G, Fontana A, Gavagnin M, Ortea J, Trivellone E. Defensive strategy of twoHypselodoris nudibranchs from Italian and Spanish coasts. *J Chem Ecol*. 1991;17(3):625-636. doi:10.1007/BF00982131

100. Cimino G, Fontana A, Giménez F, Marin A, Mollo E, Trivellone E, Zubía E. Biotransformation of a dietary sesterterpenoid in the Mediterranean nudibranchHypselodoris orsini. *Experientia*. 1993;49(6):582-586. doi:10.1007/BF01955168

101. Wägele H, Klussmann-Kolb A. Opisthobranchia (Mollusca, Gastropoda) – more than just slimy slugs. Shell reduction and its implications on defence and foraging. *Front Zool*. 2005;2:3. doi:10.1186/1742-9994-2-3

102. Bhushan A, Peters EE, Piel J. Entotheonella Bacteria as Source of Sponge-Derived Natural Products: Opportunities for Biotechnological Production. In: Müller WEG, Schröder HC, Wang X, eds. *Blue Biotechnology: From Gene to Bioactive Product*. Progress in Molecular and Subcellular Biology. Springer International Publishing; 2017:291-314. doi:10.1007/978-3-319-51284-6_9

103. Uria AR, Piel J, Wakimoto T. Biosynthetic Insights of Calyculin- and Misakinolide-Type Compounds in "Candidatus Entotheonella sp." In: *Methods in Enzymology*. Vol 604. Elsevier; 2018:287-330. doi:10.1016/bs.mie.2018.02.017

104. Avila C, Angulo-Preckler C. Bioactive Compounds from Marine Heterobranchs. *Mar Drugs*. 2020;2020:657. doi:10.3390/md18120657

105. Luesch H, Harrigan GG, Goetz G, Horgen FD. The cyanobacterial origin of potent anticancer agents originally isolated from sea hares. *Curr Med Chem*. 2002;9(20):1791-1806. doi:10.2174/0929867023369051

106. Zan J, Li Z, Tianero MD, Davis J, Hill RT, Donia MS. A microbial factory for defensive kahalalides in a tripartite marine symbiosis. *Science*. 2019;364(6445). doi:10.1126/science.aaw6732

107. Wong NLWS, Sigwart JD. Natural history clues to the evolution of bivalved gastropods (Mollusca: Gastropoda: Sacoglossa: Juliidae). *Mar Biodivers*. 2019;49(4):1997-2007. doi:10.1007/s12526-019-00960-0

108. Ponder WF, Lindberg DR, Ponder JM. Gastropoda III – The Heterobranchia. In: *Biology and Evolution of the Mollusca*. CRC Press; 2020.

109. Klussmann-Kolb A, Dinapoli A, Kuhn K, Streit B, Albrecht C. From sea to land and beyond – New insights into the evolution of euthyneuran Gastropoda (Mollusca). *BMC Evol Biol*. 2008;8(1):57. doi:10.1186/1471-2148-8-57

110. Wägele H, Klussmann-Kolb A, Vonnemann V, Medina M. Heterobranchia I: The Opisthobranchia. Published online January 1, 2008:385-408.

111. Wägele H, Klussmann-Kolb A, Verbeek E, Schrödl M. Flashback and foreshadowing—a review of the taxon Opisthobranchia. *Org Divers Evol*. 2013;14(1):133-149. doi:10.1007/s13127-013-0151-5

112. Haszprunar G. The Heterobranchia –a new concept of the phylogeny of the higher Gastropoda. *J Zool Syst Evol Res*. 1985;23(1):15-37. doi:10.1111/j.1439-0469.1985.tb00567.x

113. Wägele H, Willan RC. Phylogeny of the Nudibranchia. *Zool J Linn Soc*. 2000;130(1):83-181. doi:10.1111/j.1096-3642.2000.tb02196.x

114. Wollscheid E, Wägele H. Initial Results on the Molecular Phylogeny of the Nudibranchia (Gastropoda, Opisthobranchia) Based on 18S rDNA Data. *Mol Phylogenet Evol*. 1999;13(2):215-226. doi:10.1006/mpev.1999.0664

115. Wollscheid-Lengeling E, Boore J, Brown W, Wägele H. The phylogeny of Nudibranchia (Opisthobranchia, Gastropoda, Mollusca) reconstructed by three molecular markers. *Org Divers Evol*. 2001;1(4):241-256. doi:10.1078/1439-6092-00022

116. Turner LM, Wilson NG. Polyphyly across oceans: a molecular phylogeny of the Chromodorididae (Mollusca, Nudibranchia). *Zool Scr*. 2008;37(1):23-42. doi:10.1111/j.1463-6409.2007.00310.x

117. Johnson RF, Gosliner TM. Traditional Taxonomic Groupings Mask Evolutionary History: A Molecular Phylogeny and New Classification of the Chromodorid Nudibranchs. *PLOS ONE*. 2012;7(4):e33479. doi:10.1371/journal.pone.0033479

118. Karmeinski D, Meusemann K, Goodheart JA, Schroedl M, Martynov A, Korshunova T, Wägele H, Donath A. Transcriptomics provides a robust framework for the relationships of the major clades of cladobranch sea slugs (Mollusca, Gastropoda, Heterobranchia), but fails to resolve the position of the enigmatic genus Embletonia. *bioRxiv*. Published online September 23, 2020:2020.09.22.307728. doi:10.1101/2020.09.22.307728

119. Korshunova T, Fletcher K, Picton B, Lundin K, Kashio S, Sanamyan N, Sanamyan K, Padula V, Schroedl M, Martynov A. The Emperor's Cadlina, hidden diversity and gill cavity evolution: new insights for the taxonomy and phylogeny of dorid nudibranchs (Mollusca: Gastropoda). *Zool J Linn Soc*. Published online February 20, 2020. doi:10.1093/zoolinnean/zlz126

120. Layton K, Carvajal J, Wilson N. Mimicry and mitonuclear discordance in nudibranchs: New insights from exon capture phylogenomics. *Ecol Evol*. 2020;10. doi:10.1002/ece3.6727

121. Jensen KR. Phylogenetic systematics and classification of the Sacoglossa (Mollusca, Gastropoda, Opisthobranchia). *Philos Trans R Soc Lond B Biol Sci*. 1996;351(1335):91-122. doi:10.1098/rstb.1996.0006

122. Mikkelsen P. The evolutionary relationships of Cephalaspidea S.L. (Gastropoda: Opisthobranchia): A phylogenetic analysis. *Malacologia*. 1996;37:375-442.

123. Klussmann-Kolb A. Phylogeny of the Aplysiidae (Gastropoda, Opisthobranchia) with new aspects of the evolution of seahares. *Zool Scr*. 2004;33(5):439-462. doi:10.1111/j.0300-3256.2004.00158.x

124. Wade CM, Mordan PB, Clarke B. A phylogeny of the land snails (Gastropoda: Pulmonata). *Proc R Soc Lond B Biol Sci*. 2001;268(1465):413-422. doi:10.1098/rspb.2000.1372

125. Klussmann-Kolb A, Dinapoli A. Systematic position of the pelagic Thecosomata and Gymnosomata within Opisthobranchia (Mollusca, Gastropoda) – revival of the Pteropoda. *J Zool Syst Evol Res*. 2006;44(2):118-129. doi:10.1111/j.1439-0469.2006.00351.x

126. Araujo AK, Pola M, Malaquias MAE, Ballesteros M, Vitale F, Cervera JL. Molecular phylogeny of European Runcinida (Gastropoda, Heterobranchia): the discover of an unexpected pool of complex species, with special reference to the case of Runcina coronata. *Zool J Linn Soc*. 2021;(zlab041). doi:10.1093/zoolinnean/zlab041

127. Carmona L, Pola M, Gosliner TM, Cervera JL. A Tale That Morphology Fails to Tell: A Molecular Phylogeny of Aeolidiidae (Aeolidida, Nudibranchia, Gastropoda). *PLoS ONE*. 2013;8(5):e63000. doi:10.1371/journal.pone.0063000

128. Goodheart JA, Bazinet AL, Collins AG, Cummings MP. Relationships within Cladobranchia (Gastropoda: Nudibranchia) based on RNA-Seq data: an initial investigation. *R Soc Open Sci*. 2015;2(9):150196. doi:10.1098/rsos.150196

129. Moles J, Giribet G. A polyvalent and universal tool for genomic studies in gastropod molluscs (Heterobranchia). *Mol Phylogenet Evol*. 2021;155:106996. doi:10.1016/j.ympev.2020.106996

130. Pabst E, Kocot K. Phylogenomics confirms monophyly of Nudipleura (Gastropoda: Heterobranchia). *J Molluscan Stud*. 2018;84:259-265. doi:10.1093/mollus/eyy013

131. Varney RM, Brenzinger B, Malaquias MAE, Meyer CP, Schrödl M, Kocot KM. Assessment of mitochondrial genomes for heterobranch gastropod phylogenetics. *BMC Ecol Evol*. 2021;21(1):6. doi:10.1186/s12862-020-01728-y

132. Heike Wägele MJR. Solar powered seaslugs (Opisthobranchia, Gastropoda, Mollusca): incorporation of photosynthetic units: a key character enhancing radiation? In: Glaubrecht M (ed) Evolution in action. Springer, Berlin. *Evol Action Case Stud Adapt Radiat Speciat Orig Biodivers*. 2010;263-283:263-282. doi:10.1007/978-3-642-12425-9

133. Laetz EMJ, Wägele H. Chloroplast digestion and the development of functional kleptoplasty in juvenile Elysia timida (Risso, 1818) as compared to short-term and non-chloroplast-retaining sacoglossan slugs. *PLOS ONE*. 2017;12(10):e0182910. doi:10.1371/journal.pone.0182910

134. Melo Clavijo J, Frankenbach S, Fidalgo C, Serôdio J, Donath A, Preisfeld A, Christa G. Identification of scavenger receptors and thrombospondin-type-1 repeat proteins potentially relevant for plastid recognition in Sacoglossa. *Ecol Evol*. 2020;10(21):12348-12363. doi:10.1002/ece3.6865

135. Frankenbach S, Luppa Q, Serôdio J, Greve C, Bleidissel S, Melo Clavijo J, Laetz EMJ, Preisfeld A, Christa G. Kleptoplasts are continuously digested during feeding in the plastid-bearing sea slug Elysia viridis. *J Molluscan Stud*. 2021;87(3). doi:10.1093/mollus/eyab022

136. KANDEL ER. Behavioral biology of Aplysia. *Contrib Comp Study Opisthobranch Molluscs*. 1979;463. https://ci.nii.ac.jp/naid/10004770212/en/

137. Gillette R, Brown JW. The Sea Slug, Pleurobranchaea californica : A Signpost Species in the Evolution of Complex Nervous Systems and Behavior. *Integr Comp Biol*. 2015;55(6):1058-1069. doi:10.1093/icb/icv081

138. Amorim J, Abreu I, Rodrigues P, Peixoto D, Pinheiro C, Saraiva A, Carvalho AP, Guimarães L, Oliva-Teles L. Lymnaea stagnalis as a freshwater model invertebrate for ecotoxicological studies. *Sci Total Environ*. 2019;669:11-28. doi:10.1016/j.scitotenv.2019.03.035

139. Keul N, Peijnenburg KTCA, Andersen N, Kitidis V, Goetze E, Schneider RR. Pteropods are excellent recorders of surface temperature and carbonate ion concentration. *Sci Rep*. 2017;7(1):12645. doi:10.1038/s41598-017-11708-w

140. Nelwan M. *Intermediate Host and Host-Parasite Interactions*. Social Science Research Network; 2020. doi:10.2139/ssrn.3697037

141. Kijjoa A, Sawangwong P. Drugs and Cosmetics from the Sea. *Mar Drugs*. 2004;2(2):73-82. doi:10.3390/md202073

142. Putz A, König GM, Wägele H. Defensive strategies of Cladobranchia (Gastropoda, Opisthobranchia). *Nat Prod Rep*. 2010;27(10):1386-1402. doi:10.1039/B923849M

143. Wägele H. Potential key characters in Opisthobranchia (Gastropoda, Mollusca) enhancing adaptive radiation. *Org Divers Evol*. 2004;4(3):175-188. doi:10.1016/j.ode.2004.03.002

144. Händeler K, Grzymbowski YP, Krug PJ, Wägele H. Functional chloroplasts in metazoan cells - a unique evolutionary strategy in animal life. *Front Zool*. 2009;6(1):28. doi:10.1186/1742-9994-6-28

145. Wägele H, Raupach MJ, Burghardt I, Grzymbowski Y, Händeler K. Solar Powered Seaslugs (Opisthobranchia, Gastropoda, Mollusca): Incorporation of Photosynthetic Units: A Key Character Enhancing Radiation? In: Glaubrecht M, ed. *Evolution in Action*. Springer Berlin Heidelberg; 2010:263-282. doi:10.1007/978-3-642-12425-9_13

146. de Vries J, Christa G, Gould SB. Plastid survival in the cytosol of animal cells. *Trends Plant Sci*. 2014;19(6):347-350. doi:10.1016/j.tplants.2014.03.010

147. Gould S, Rauch C, Jahns P, Tielens A, Martin W. MINI REVIEW On Being the Right Size as an Animal with Plastids. *Front Plant Sci*. 2017;1. doi:10.3389/fpls.2017.01402

148. Marin A, Ros J. Presence of Intracellular Zooxanthellae in Mediterranean Nudibranchs. *J Molluscan Stud*. 1991;57:87-101.

149. Burghardt I, Stemmer K, Wägele H. Symbiosis between Symbiodinium (Dinophyceae) and various taxa of Nudibranchia (Mollusca: Gastropoda), with analyses of long-term retention. *Org Divers Evol*. 2008;8(1):66-76. doi:10.1016/j.ode.2007.01.001

150. Moore E, Gosliner T. Additions to the Genus Phyllodesmium, with a Phylogenetic Analysis and its Implications to the Evolution of Symbiosis. *Veliger*. 2014;51(4):237-251. doi:10.5281/zenodo.20947

151. Rudman W. Further-Studies on the Taxonomy and Biology of the Octocoral-Feeding Genus Phyllodesmium Ehrenberg, 1831 (nudibranchia, Aeolidoidea). *J Molluscan Stud*. 1991;57:167-203. doi:10.1093/mollus/57.2.167

152. Burghardt I, Wägele H. A new solar powered species of the genus Phyllodesmium Ehrenberg, 1831 (Mollusca : Nudibranchia : Aeolidoidea) from Indonesia with analysis of its photosynthetic activity and notes on biology. *Zootaxa*. 2004;(596):1-18.

153. Karuso P. Chemical Ecology of the Nudibranchs. In: Scheuer PPJ, ed. *Bioorganic Marine Chemistry*. Bioorganic Marine Chemistry. Springer Berlin Heidelberg; 1987:31-60. doi:10.1007/978-3-642-72726-9_2

154. Valdes A, Blanchard L, Marti W. Caught naked: First report a nudibranch sea slug attacked by a cone snail. *Am Malacol Bull*. 2013;31(2):337-338.

155. Dietz L, Dömel JS, Leese F, Lehmann T, Melzer RR. Feeding ecology in sea spiders (Arthropoda: Pycnogonida): what do we know? *Front Zool*. 2018;15(1):7. doi:10.1186/s12983-018-0250-4

156. Mehrotra R, Monchanin C, Scott CM, Phongsuwan N, Gutierrez MC, Chavanich S, Hoeksema BW. Selective consumption of sacoglossan sea slugs (Mollusca: Gastropoda) by scleractinian corals (Cnidaria: Anthozoa). *PLOS ONE*. 2019;14(4):e0215063. doi:10.1371/journal.pone.0215063

157. Anker A, Ivanov Y. First record of the predation upon sea slugs (Cephalaspidea and Nudibranchia) by the peculiar elbow crab Lambrachaeus ramifer Alcock, 1895 (Decapoda: Parthenopidae). *Mar Biodivers*. 2020;50(2):24. doi:10.1007/s12526-020-01047-x

158. Battini N, Bravo G. Unexpected meal: first record of predation upon a potentially neurotoxic sea slug by the European green crab Carcinus maenas. *N Z J Zool*. Published online December 2, 2020. doi:10.1080/03014223.2020.1848889

159. Battini N, Giachetti C, Castro K, Bortolus A, Schwindt E. Predator–prey interactions as key drivers for the invasion success of a potentially neurotoxic sea slug. *Biol Invasions*. 2021;23:1-23. doi:10.1007/s10530-020-02431-1

160. Faulkner D, Ghiselin M. Chemical Defense and Evolutionary Ecology of Dorid Nudibranchs and Some Other Opisthobranch Gastropods. *Mar Ecol Prog Ser*. 1983;13(2-3):295-301. doi:10.3354/meps013295

161. Haber M, Cerfeda S, Carbone M, Calado G, Gaspar H, Neves R, Maharajan V, Cimino G, Gavagnin M, Ghiselin MT, et al. Coloration and Defense in the Nudibranch Gastropod Hypselodoris fontandraui. *Biol Bull*. 2010;218(2):181-188. doi:10.1086/BBLv218n2p181

162. Ehrlich H. Spicular Structures in Molluscs. In: Ehrlich H, ed. *Marine Biological Materials of Invertebrate Origin*. Biologically-Inspired Systems. Springer International Publishing; 2019:133-157. doi:10.1007/978-3-319-92483-0_10

163. Penney BK, Ehresmann KR, Jordan KJ, Rufo G. Micro-computed tomography of spicule networks in three genera of dorid sea-slugs (Gastropoda: Nudipleura: Doridina) shows patterns of phylogenetic significance. *Acta Zool*. 2020;101(1):5-23. doi:10.1111/azo.12266

164. Nikitenko E, Ereskovsky A, Vortsepneva E. Ontogenetic dynamics of the subepidermal spicule complex in Nudibranchia (Gastropoda): the case of Onchidoris muricata. *Zool Jena Ger*. 2021;144:125886. doi:10.1016/j.zool.2020.125886

165. Edmunds M. Color in Opisthobranchs. *Am Malacol Bull*. 1987;5(2):185-196.

166. Cortesi F, Cheney KL. Conspicuousness is correlated with toxicity in marine opisthobranchs. *J Evol Biol*. 2010;23(7):1509-1518. doi:10.1111/j.1420-9101.2010.02018.x

167. Winters AE, Green NF, Wilson NG, How MJ, Garson MJ, Marshall NJ, Cheney KL. Stabilizing selection on individual pattern elements of aposematic signals. *Proc R Soc B*. 2017;284(1861):20170926. doi:10.1098/rspb.2017.0926

168. Aguado, F. M A. Warning coloration associated with ne matocyst-based defences in Aeolidiodean nudibranchs. *J Molluscan Stud*. 2007;73(1). doi:10.1093/mollus/eyl026

169. Layton KKS, Gosliner TM, Wilson NG. Flexible colour patterns obscure identification and mimicry in Indo-Pacific Chromodoris nudibranchs (Gastropoda: Chromodorididae). *Mol Phylogenet Evol*. 2018;124:27-36. doi:10.1016/j.ympev.2018.02.008

170. Cimino G, De Rosa S, De Stefano S, Morrone R, Sodano G. The chemical defense of nudibranch molluscs: Structure, biosynthetic origin and defensive properties of terpenoids from the dorid nudibranch dendrodoris grandiflora. *Tetrahedron*. 1985;41(6):1093-1100. doi:10.1016/S0040-4020(01)96477-4

171. Paul VJ, Van Alstyne KL. Use of ingested algal diterpenoids by Elysia halimedae Macnae (Opisthobranchia : Ascoglossa) as antipredator defenses. *J Exp Mar Biol Ecol*. 1988;119(1):15-29. doi:10.1016/0022-0981(88)90149-9

172. Wägele H, Knezevic K, Moustafa AY. Distribution and morphology of defensive acid-secreting glands in Nudipleura (Gastropoda: Heterobranchia), with an emphasis on Pleurobranchomorpha. *J Molluscan Stud*. 2017;83(4):422-433. doi:10.1093/mollus/eyx030

173. Wakimoto T, Tan KC, Abe I. Ergot alkaloid from the sea slug Pleurobranchus forskalii. *Toxicon*. 2013;72:1-4. doi:10.1016/j.toxicon.2013.05.021

174. McNabb P, Selwood AI, Munday R, Wood SA, Taylor DI, MacKenzie LA, van Ginkel R, Rhodes LL, Cornelisen C, Heasman K, et al. Detection of tetrodotoxin from the grey side-gilled sea slug - Pleurobranchaea maculata, and associated dog neurotoxicosis on beaches adjacent to the Hauraki Gulf, Auckland, New Zealand. *Toxicon*. 2010;56(3):466-473. doi:10.1016/j.toxicon.2010.04.017

175. Wood SA, Casas M, Taylor DI, McNabb P, Salvitti L, Ogilvie S, Cary SC. Depuration of Tetrodotoxin and Changes in Bacterial Communities in Pleurobranchea maculata Adults and Egg Masses Maintained in Captivity. *J Chem Ecol*. 2012;38(11):1342-1350. doi:10.1007/s10886-012-0212-9

176. Salvitti LR, Wood SA, Winsor L, Cary SC. Intracellular Immunohistochemical Detection of Tetrodotoxin in Pleurobranchaea maculata (Gastropoda) and Stylochoplana sp. (Turbellaria). *Mar Drugs*. 2015;13(2):756-769. doi:10.3390/md13020756

177. Farias NE, Goya AB, Schwindt E, Obenat S, Dhanji-Rapkova M, Turner AD. The invasive sea slug Pleurobranchaea maculata is a vector of two potent neurotoxins in coasts of Argentina. *Mar Biol*. 2019;166(7):82. doi:10.1007/s00227-019-3529-x

178. WoRMS - World Register of Marine Species. Accessed October 20, 2021. http://www.marinespecies.org/index.php

179. Cimino G, De Rosa S, De Stefano S, Sodano G. The chemical defense of four Mediterranean nudibranchs. *Comp Biochem Physiol Part B Comp Biochem*. 1982;73(3):471-474. doi:10.1016/0305-0491(82)90061-X

180. Goodheart JA, Bazinet AL, Valdés Á, Collins AG, Cummings MP. Prey preference follows phylogeny: evolutionary dietary patterns within the marine gastropod group Cladobranchia (Gastropoda: Heterobranchia: Nudibranchia). *BMC Evol Biol*. 2017;17(1):221. doi:10.1186/s12862-017-1066-0

181. Bouchet P, Rocroi JP, Hausdorf B, Kaim A, Kano Y, Nützel A, Parkhaev P, Schroedl M, Strong E. Revised Classification, Nomenclator and Typification of Gastropod and Monoplacophoran Families. *Malacologia*. 2017;61:1-526. doi:10.4002/040.061.0201

182. Goodheart JA, Bleidißel S, Schillo D, Strong EE, Ayres DL, Preisfeld A, Collins AG, Cummings MP, Wägele H. Comparative morphology and evolution of the cnidosac in Cladobranchia (Gastropoda: Heterobranchia: Nudibranchia). *Front Zool*. 2018;15:43. doi:10.1186/s12983-018-0289-2

183. Kälker H, Schmekel L. Bau und Funktion des Cnidosacks der Aeolidoidea (Gastropoda Nudibranchia). *Zoomorphologie*. 1976;86(1):41-60. doi:10.1007/BF01006712

184. Greenwood PG. Acquisition and use of nematocysts by cnidarian predators. *Toxicon*. 2009;54(8):1065-1070. doi:10.1016/j.toxicon.2009.02.029

185. Obermann D, Bickmeyer U, Wägele H. Incorporated nematocysts in Aeolidiella stephanieae (Gastropoda, Opisthobranchia, Aeolidoidea) mature by acidification shown by the pH sensitive fluorescing alkaloid Ageladine A. *Toxicon*. 2012;60(6):1108-1116. doi:10.1016/j.toxicon.2012.08.003

186. Miller JA, Byrne M. Ceratal autotomy and regeneration in the aeolid nudibranch Phidiana crassicornis and the role of predators. *Invertebr Biol*. 2000;119(2):167-176. doi:10.1111/j.1744-7410.2000.tb00005.x

187. Martin R. Management of nematocysts in the alimentary tract and in cnidosacs of the aeolid nudibranch gastropod Cratena peregrina. *Mar Biol*. 2003;143(3):533-541. doi:10.1007/s00227-003-1078-8

188. Frick KE. Nematocyst complements of nudibranchs in the genus Flabellina in the Gulf of Maine and the effect of diet manipulations on the cnidom of Flabellina verrucosa. *Mar Biol*. 2005;147(6):1313-1321. doi:10.1007/s00227-005-0034-1

189. Bogdanov A, Kehraus S, Bleidissel S, Preisfeld G, Schillo D, Piel J, Brachmann AO, Wägele H, König GM. Defense in the Aeolidoidean Genus Phyllodesmium (Gastropoda). *J Chem Ecol*. 2014;40(9):1013-1024. doi:10.1007/s10886-014-0496-z

190. Daniel Wagner SEK. Observations on the life history and feeding ecology of a specialized nudibranch predator (Phyllodesmium poindimiei), with implications for biocontrol of an invasive octocoral (Carijoa riisei) in Hawaii. *J Exp Mar Biol Ecol*. 2009;372(1-2):64-74. doi:10.1016/j.jembe.2009.02.007

191. Thompson TE. Defensive adaptations in opisthobranchs. *J Mar Biol Assoc U K*. 1960;39(1):123-134. doi:10.1017/S0025315400013163

192. Iken K, Avila C, Ciavatta ML, Fontana A, Cimino G. Hodgsonal, a new drimane sesquiterpene from the mantle of the Antarctic nudibranch Bathydoris hodgsoni. *Tetrahedron Lett*. 1998;39(31):5635-5638. doi:10.1016/S0040-4039(98)01095-8

193. Avila C, Iken K, Fontana A, Cimino G. Chemical ecology of the Antarctic nudibranch Bathydoris hodgsoni Eliot, 1907: defensive role and origin of its natural products. *J Exp Mar Biol Ecol*. 2000;252(1):27-44. doi:10.1016/S0022-0981(00)00227-6

194. Rauschert M, Arntz W. *Antarctic Macrobenthos: A Field Guide of the Invertebrates Living at the Antarctic Seafloor*. Arntz & Rauschert Selbstverlag; 2015.

195. Wahidullah S, Guo YW, Fakhr IMI, Mollo E. Chemical Diversity in Opisthobranch Molluscs from Scarcely Investigated Indo-Pacific Areas. In: Cimino G, Gavagnin M, eds. *Molluscs: From Chemo-Ecological Study to Biotechnological Application*. Progress in Molecular and Subcellular Biology. Springer Berlin Heidelberg; 2006:175-198. doi:10.1007/978-3-540-30880-5_8

196. Avila C. Molluscan Natural Products as Biological Models: Chemical Ecology, Histology, and Laboratory Culture. In: Cimino PDG, Gavagnin PDM, eds. *Molluscs*. Progress in Molecular and Subcellular Biology. Springer Berlin Heidelberg; 2006:1-23. doi:10.1007/978-3-540-30880-5_1

197. Cimino G, Gavagnin M, eds. *Molluscs: From Chemo-Ecological Study to Biotechnological Application*. Springer-Verlag; 2006. doi:10.1007/978-3-540-30880-5

198. Mudianta IW, White AM, Suciati, Katavic PL, Krishnaraj RR, Winters AE, Mollo E, Cheney KL, Garson MJ. Chemoecological studies on marine natural products: terpene chemistry from marine mollusks. *Pure Appl Chem*. 2014;86(6):995-1002. doi:10.1515/pac-2013-1111

199. Zhukova NV. Lipid Classes and Fatty Acid Composition of the Tropical Nudibranch Mollusks Chromodoris sp. and Phyllidia coelestis. *Lipids*. 2007;42(12):1169-1175. doi:10.1007/s11745-007-3123-8

200. Dang VT, Benkendorff K, Green T, Speck P. Marine Snails and Slugs: a Great Place To Look for Antiviral Drugs. *J Virol*. 89(16):8114-8118. doi:10.1128/JVI.00287-15

201. Bornancin L, Bonnard I, Mills SC, Banaigs B. Chemical mediation as a structuring element in marine gastropod predator-prey interactions. *Nat Prod Rep*. 2017;34(6):644-676. doi:10.1039/C6NP00097E

202. J. Dean L, R. Prinsep M. The chemistry and chemical ecology of nudibranchs. *Nat Prod Rep*. 2017;34(12):1359-1390. doi:10.1039/C7NP00041C

203. Avila C. Terpenoids in Marine Heterobranch Molluscs. *Mar Drugs*. 2020;18(3):162. doi:10.3390/md18030162

204. Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR. Marine natural products. *Nat Prod Rep*. 2020;37(2):175-223. doi:10.1039/C9NP00069K

205. Somerville MJ, Mollo E, Cimino G, Rungprom W, Garson MJ. Spongian Diterpenes from Australian Nudibranchs: An Anatomically Guided Chemical Study of Glossodoris atromarginata. *J Nat Prod*. 2006;69(7):1086-1088. doi:10.1021/np060002i

206. Winters AE, White AM, Cheney KL, Garson MJ. Geographic variation in diterpene-based secondary metabolites and level of defence in an aposematic nudibranch, Goniobranchus splendidus. *J Molluscan Stud*. 2019;85(1):133-142. doi:10.1093/mollus/eyy057

207. Eisenreich W, Schwarz M, Cartayrade A, Arigoni D, Zenk MH, Bacher A. The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. *Chem Biol*. 1998;5(9):R221-R233. doi:10.1016/S1074-5521(98)90002-3

208. Perveen S, Al-Taweel A. *Terpenes and Terpenoids*.; 2018. doi:10.5772/intechopen.71175

209. Rudolf JD, Alsup TA, Xu B, Li Z. Bacterial terpenome. *Nat Prod Rep*. 2021;38(5):905-980. doi:10.1039/D0NP00066C

210. Beytia ED, Porter JW. Biochemistry of Polyisoprenoid Biosynthesis. *Annu Rev Biochem*. 1976;45(1):113-142. doi:10.1146/annurev.bi.45.070176.000553

211. Dewick PM. The biosynthesis of C 5 –C 25 terpenoid compounds. *Nat Prod Rep*. 1999;16(1):97-130. doi:10.1039/A708935J

212. Perveen S. *Introductory Chapter*. IntechOpen; 2018. doi:10.5772/intechopen.79683

213. Gross H, Kehraus S, Nett M, König GM, Beil W, Wright AD. New cytotoxic cembrane based diterpenes from the soft corals Sarcophyton cherbonnieri and Nephthea sp. *Org Biomol Chem*. 2003;1(6):944-949.

214. Wu YC, Hsieh PW, Duh CY, Wang SK, Soong K, Fang LS. Studies on the Formosan Soft Corals I-Cytotoxic Cembrane Diterpenes from Sarcophyton Trocheliophorum. *J Chin Chem Soc*. 1992;39(4):355-357. doi:10.1002/jccs.199200062

215. Aceret TL, Coll JC, Uchio Y, Sammarco PW. Antimicrobial activity of the diterpenes flexibilide and sinulariolide derived from Sinularia flexibilis Quoy and Gaimard 1833 (Coelenterata: Alcyonacea, Octocorallia). *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol*. 1998;120(1):121-126.

216. Betancur-Galvis L, Zuluaga C, Arnó M, González MA, Zaragozá RJ. Cytotoxic Effect (on Tumor Cells) and in Vitro Antiviral Activity against Herpes Simplex Virus of Synthetic Spongiane Diterpenes. *J Nat Prod*. 2002;65(2):189-192. doi:10.1021/np010206t

217. Youssef DTA, Shaala LA, Emara S. Antimycobacterial Scalarane-Based Sesterterpenes from the Red Sea Sponge Hyrtios erecta. *J Nat Prod*. 2005;68(12):1782-1784. doi:10.1021/np0502645

218. Evidente A, Kornienko A, Lefranc F, Cimmino A, Dasari R, Evidente M, Mathieu V, Kiss R. Sesterterpenoids with Anticancer Activity. *Curr Med Chem*. 2015;22(30):3502-3522.

219. Annang F, Pérez-Victoria I, Appiah T, Pérez-Moreno G, Domingo E, Martín J, Mackenzie T, Ruiz-Pérez L, González-Pacanowska D, Genilloud O, et al. Antiprotozoan sesterterpenes and triterpenes isolated from two Ghanaian mushrooms. *Fitoterapia*. 2018;127:341-348. doi:10.1016/j.fitote.2018.03.016

220. Wang Q, Sun Y, Yang L, Luo X, de Voogd NJ, Tang X, Li P, Li G. Bishomoscalarane Sesterterpenoids from the Sponge Dysidea granulosa Collected in the South China Sea. *J Nat Prod*. 2020;83(2):516-523. doi:10.1021/acs.jnatprod.9b01202

221. Huang AC, Kautsar SA, Hong YJ, Medema MH, Bond AD, Tantillo DJ, Osbourn A. Unearthing a sesterterpene biosynthetic repertoire in the Brassicaceae through genome mining reveals convergent evolution. *Proc Natl Acad Sci*. 2017;114(29):E6005-E6014. doi:10.1073/pnas.1705567114

222. Paruch E, Ciunik Z, Nawrot J, Wawrzeńczyk C. Lactones. 9. Synthesis of Terpenoid LactonesActive Insect Antifeedants. *J Agric Food Chem*. 2000;48(10):4973-4977. doi:10.1021/jf991307o

223. Wonganuchitmeta S ngam, Yuenyongsawad S, Keawpradub N, Plubrukarn A. Antitubercular Sesterterpenes from the Thai Sponge Brachiaster sp. *J Nat Prod*. 2004;67(10):1767-1770. doi:10.1021/np0498354

224. Yang B, Liu J, Wang J, Liao S, Liu Y. Cytotoxic Cembrane Diterpenoids. In: Kim SK, ed. *Handbook of Anticancer Drugs from Marine Origin*. Springer International Publishing; 2015:649-672. doi:10.1007/978-3-319-07145-9_30

225. Bernstein J, Shmeuli U, Zadock E, Kashman Y, Neeman I. Sarcophine, a New Epoxy Cembranolide from Marine Origin. *Tetrahedron*. 1974;30(16):2817-2824. doi:10.1016/S0040-4020(01)97451-4

226. Sammarco PW, Coll JC. Lack of predictability in terpenoid function Multiple roles and integration with related adaptations in soft corals. *J Chem Ecol*. 1990;16(1):273-289. doi:10.1007/BF01021284

227. PW Sammarco JC. Chemical adaptations in the Octocorallia: evolutionary considerations. Mar Ecol Prog Ser. *Mar Ecol-Prog Ser - MAR ECOL-PROGR SER*. 1992;88(1):93-104. doi:10.3354/meps088093

228. Maia LF, Fleury BG, Lages BG, Creed JC, de Oliveira LFC. Chapter 10 - New Strategies for Identifying Natural Products of Ecological Significance from Corals: Nondestructive Raman Spectroscopy Analysis. In: Atta-ur-Rahman, ed. *Studies in Natural Products Chemistry*. Vol 43. Elsevier; 2014:313-349. doi:10.1016/B978-0-444-63430-6.00010-2

229. Fontana A, Mollo E, Ricciardi D, Fakhr I, Cimino G. Chemical Studies of Egyptian Opisthobranchs: Spongian Diterpenoids from Glossodoris atromarginata. *J Nat Prod*. 1997;60(5):444-448. doi:10.1021/np960690d

230. Fontana A, Cavaliere P, Ungur N, D'Souza L, Parameswaram PS, Cimino G. New Scalaranes from the Nudibranch Glossodoris atromarginata and Its Sponge Prey. *J Nat Prod*. 1999;62(10):1367-1370. doi:10.1021/np9900932

231. Fontana A, Mollo E, Ortea J, Gavagnin M, Cimino G. Scalarane and Homoscalarane Compounds from the Nudibranchs Glossodoris sedna and Glossodoris dalli: Chemical and Biological Properties. *J Nat Prod*. 2000;63(4):527-530. doi:10.1021/np990506z

232. Gavagnin M, Mollo E, Docimo T, Guo YW, Cimino G. Scalarane Metabolites of the Nudibranch Glossodoris rufomarginata and Its Dietary Sponge from the South China Sea. *J Nat Prod*. 2004;67(12):2104-2107. doi:10.1021/np040087s

233. Manzo E, Gavagnin M, Somerville MJ, Mao SC, Ciavatta ML, Mollo E, Schupp PJ, Garson MJ, Guo YW, Cimino G. Chemistry of Glossodoris nudibranchs: specific occurrence of 12-keto scalaranes. *J Chem Ecol*. 2007;33(12):2325-2336. doi:10.1007/s10886-007-9387-x

234. Yong KWL, Mudianta IW, Cheney KL, Mollo E, Blanchfield JT, Garson MJ. Isolation of Norsesterterpenes and Spongian Diterpenes from Dorisprismatica (= Glossodoris) atromarginata. *J Nat Prod*. 2015;78(3):421-430. doi:10.1021/np500797b

235. Li XL, Li SW, Yao LG, Mollo E, Gavagnin M, Guo YW. The chemical and chemo-ecological studies on Weizhou nudibranch Glossodoris atromarginata. *Magn Reson Chem*. 2019;n/a(n/a). doi:10.1002/mrc.4949

236. Meinwald J, Thompson JE, Walker RP, Wratten SJ, Faulkner DJ. The Organic Chemistry of Animal Defense MechanismsA chemical defense mechanism for the nudibranch cadlina luteomarginata. *Tetrahedron*. 1982;38(13):1865-1873. doi:10.1016/0040-4020(82)80035-5

237. Sim DCM, Wayan Mudianta I, White AM, Martiningsih NW, Loh JJM, Cheney KL, Garson MJ. New sesquiterpenoid isonitriles from three species of phyllidid nudibranchs. *Fitoterapia*. doi:10.1016/j.fitote.2017.10.003

238. Fontana A, Cavaliere P, Wahidullah S, Naik C, Cimino G. A New Antitumor Isoquinoline Alkaloid from the Marine Nudibranch Jorunna funebris. *Tetrahedron*. 2000;56. doi:10.1016/S0040-4020(00)00629-3

239. He WF, Li Y, Feng MT, Gavagnin M, Mollo E, Mao SC, Guo YW. New isoquinolinequinone alkaloids from the South China Sea nudibranch Jorunna funebris and its possible sponge-prey Xestospongia sp. *Fitoterapia*. 2014;96:109-114. doi:10.1016/j.fitote.2014.04.011

240. Petek BJ, Jones RL. PM00104 (Zalypsis®): A Marine Derived Alkylating Agent. *Molecules*. 2014;19(8):12328-12335. doi:10.3390/molecules190812328

241. Wang YJ, Li YY, Liu XY, Lu XL, Cao X, Jiao BH. Marine Antibody–Drug Conjugates: Design Strategies and Research Progress. *Mar Drugs*. 2017;15(1):18. doi:10.3390/md15010018

242. Matsunaga S, Fusetani N, Hashimoto K, Koseki K, Noma M, Noguchi H, Sankawa U. Bioactive marine metabolites. 25. Further kabiramides and halichondramides, cytotoxic macrolides embracing trisoxazole, from the Hexabranchus egg masses. *J Org Chem*. 1989;54(6):1360-1363. doi:10.1021/jo00267a024

243. Parrish SM, Yoshida W, Yang B, Williams PG. Ulapualides C–E Isolated from a Hawaiian Hexabranchus sanguineus Egg Mass. *J Nat Prod*. 2017;80(3):726-730. doi:10.1021/acs.jnatprod.6b00896

244. Wu L, Ye K, Jiang S, Zhou G. Marine Power on Cancer: Drugs, Lead Compounds, and Mechanisms. *Mar Drugs*. 2021;19(9):488. doi:10.3390/md19090488

245. Guella G, Mancini I, Chiasera G, Pietra F. Sphinxolide, a 26-Membered Antitumoral Macrolide Isolated from an Unidentified Pacific Nudibranch. *Helv Chim Acta*. 1989;72(2):237-246. doi:10.1002/hlca.19890720207

246. Okuda RK, Scheuer PJ. Latrunculin-A, ichthyotoxic constituent of the nudibranchChromodoris elisabethina. *Experientia*. 1985;41(10):1355-1356. doi:10.1007/BF01952094

247. Kakou Y, Crews P, Bakus GJ. Dendrolasin and Latrunculin A from the Fijian Sponge Spongia mycofijiensis and an Associated Nudibranch Chromodoris lochi. *J Nat Prod*. 1987;50(3):482-484. doi:10.1021/np50051a023

248. Cheney KL, White A, Mudianta IW, Winters AE, Quezada M, Capon RJ, Mollo E, Garson MJ. Choose Your Weaponry: Selective Storage of a Single Toxic Compound, Latrunculin A, by Closely Related Nudibranch Molluscs. *PLOS ONE*. 2016;11(1):e0145134. doi:10.1371/journal.pone.0145134

249. García-Gómez JC, Cimino G, Medina A. Studies on the defensive behaviour ofHypselodoris species (Gastropoda: Nudibranchia): Ultrastructure and chemical analysis of mantle dermal formations (MDFs). *Mar Biol*. 1990;106(2):245-250. doi:10.1007/BF01314807

250. Fontana A, Giménez F, Marin A, Mollo E, Cimino G. Transfer of secondary metabolites from the spongesDysidea fragilis andPleraplysilla spinifera to the mantle dermal formations (MDFs) of the mudibranchHypserlodoris webbi. *Experientia*. 1994;50(5):510-516. doi:10.1007/BF01920760

251. Avila C, Paul VJ. Chemical ecology of the nudibranch Glossodoris pallida: is the location of diet-derived metabolites important for defense? *Mar Ecol Prog Ser*. 1997;150(1/3):171-180.

252. Winters AE, White AM, Dewi AS, Mudianta IW, Wilson NG, Forster LC, Garson MJ, Cheney KL. Distribution of Defensive Metabolites in Nudibranch Molluscs. *J Chem Ecol*. Published online March 19, 2018:1-13. doi:10.1007/s10886-018-0941-5

253. Carbone M, Gavagnin M, Haber M, Guo YW, Fontana A, Manzo E, Genta-Jouve G, Tsoukatou M, Rudman WB, Cimino G, et al. Packaging and Delivery of Chemical Weapons: A Defensive Trojan Horse Stratagem in Chromodorid Nudibranchs. *PLoS ONE*. 2013;8(4):e62075. doi:10.1371/journal.pone.0062075

254. Wägele H, Ballesteros M, Avila C. Defensive Glandular Structures In Opisthobranch Molluscs — From Histology To Ecology. *Oceanogr Mar Biol Annu Rev*. 2006;44:197-276. doi:10.1201/9781420006391.ch5

255. Fontana A, Ciavatta ML, DeSouza L, Mollo E, Naik CG, Parameswaran PS, Wahidullah S, Cimino G. Selected chemo-ecological studies of marine opisthobranchs from Indian coasts. Published online 2001. Accessed November 1, 2021. https://drs.nio.org/xmlui/handle/2264/398

256. Rogers SD, Paul VJ. Chemical defenses of three Glossodoris nudibranchs and their dietary Hyrtios sponges. *Mar Ecol Prog Ser*. 1991;77(2/3):221-232.

257. Arbuckle K, Speed MP. Antipredator defenses predict diversification rates. *Proc Natl Acad Sci*. 2015;112(44):13597-13602. doi:10.1073/pnas.1509811112

258. Arbuckle K, Rodríguez de la Vega RC, Casewell NR. Coevolution takes the sting out of it: Evolutionary biology and mechanisms of toxin resistance in animals. *Toxicon*. 2017;140:118-131. doi:10.1016/j.toxicon.2017.10.026

259. Jones L, Harris RJ, Fry BG. Not Goanna Get Me: Mutations in the Savannah Monitor Lizard (Varanus exanthematicus) Nicotinic Acetylcholine Receptor Confer Reduced Susceptibility to Sympatric Cobra Venoms. *Neurotox Res*. Published online March 20, 2021. doi:10.1007/s12640-021-00351-z

260. Arbuckle K. Special Issue: Evolutionary Ecology of Venom. *Toxins*. 2021;13(5):310. doi:10.3390/toxins13050310

261. Jacob F. Evolution and Tinkering. *Science*. 1977;196(4295):1161-1166.

262. Price GR. The nature of selection. *J Theor Biol*. 1995;175(3):389-396. doi:10.1006/jtbi.1995.0149

263. Jiang X, Lonsdale DJ, Gobler CJ. Rapid gain and loss of evolutionary resistance to the harmful dinoflagellate Cochlodinium polykrikoides in the copepod Acartia tonsa. *Limnol Oceanogr*. 2011;56(3):947-954. doi:https://doi.org/10.4319/lo.2011.56.3.0947

264. Kondrashov FA. Gene duplication as a mechanism of genomic adaptation to a changing environment. *Proc R Soc B Biol Sci*. 2012;279(1749):5048-5057. doi:10.1098/rspb.2012.1108

265. Nelsen DR, Nisani Z, Cooper AM, Fox GA, Gren ECK, Corbit AG, Hayes WK. Poisons, toxungens, and venoms: redefining and classifying toxic biological secretions and the organisms that employ them. *Biol Rev*. 2014;89(2):450-465. doi:https://doi.org/10.1111/brv.12062

266. Sunagar K, Moran Y. The Rise and Fall of an Evolutionary Innovation: Contrasting Strategies of Venom Evolution in Ancient and Young Animals. *PLOS Genet*. 2015;11(10):e1005596. doi:10.1371/journal.pgen.1005596

267. McGlothlin JW, Kobiela ME, Feldman CR, Castoe TA, Geffeney SL, Hanifin CT, Toledo G, Vonk FJ, Richardson MK, Brodie ED, et al. Historical Contingency in a Multigene Family Facilitates Adaptive Evolution of Toxin Resistance. *Curr Biol*. 2016;26(12):1616-1621. doi:10.1016/j.cub.2016.04.056

268. Jiang X, Gao H, Zhang L, Liang H, Zhu X. Rapid evolution of tolerance to toxic Microcystis in two cladoceran grazers. *Sci Rep*. 2016;6(1):25319. doi:10.1038/srep25319

269. Tarvin RD, Santos JC, O'Connell LA, Zakon HH, Cannatella DC. Convergent Substitutions in a Sodium Channel Suggest Multiple Origins of Toxin Resistance in Poison Frogs. *Mol Biol Evol*. 2016;33(4):1068-1080. doi:10.1093/molbev/msv350

270. Tarvin RD, Borghese CM, Sachs W, Santos JC, Lu Y, O'Connell LA, Cannatella DC, Harris RA, Zakon HH. Interacting amino acid replacements allow poison frogs to evolve epibatidine resistance. *Science*. 2017;357(6357):1261-1266. doi:10.1126/science.aan5061

271. Ogawara H. Comparison of Strategies to Overcome Drug Resistance: Learning from Various Kingdoms. *Mol J Synth Chem Nat Prod Chem*. 2018;23(6):1476. doi:10.3390/molecules23061476

272. Wilson TG. Resistance of Drosophila to Toxins. *Annu Rev Entomol*. 2001;46(1):545-571. doi:10.1146/annurev.ento.46.1.545

273. Després L, David JP, Gallet C. The evolutionary ecology of insect resistance to plant chemicals. *Trends Ecol Evol*. 2007;22(6):298-307. doi:10.1016/j.tree.2007.02.010

274. Agrawal AA, Petschenka G, Bingham RA, Weber MG, Rasmann S. Toxic cardenolides: chemical ecology and coevolution of specialized plant–herbivore interactions. *New Phytol*. 2012;194(1):28-45. doi:10.1111/j.1469-8137.2011.04049.x

275. Leimu R, Muola A, Laukkanen L, Kalske A, Prill N, Mutikainen P. Plant-herbivore coevolution in a changing world. *Entomol Exp Appl*. 2012;144(1):3-13. doi:10.1111/j.1570-7458.2012.01267.x

276. Moore BD, Andrew RL, Külheim C, Foley WJ. Explaining intraspecific diversity in plant secondary metabolites in an ecological context. *New Phytol*. 2014;201(3):733-750. doi:10.1111/nph.12526

277. Speed MP, Fenton A, Jones MG, Ruxton GD, Brockhurst MA. Coevolution can explain defensive secondary metabolite diversity in plants. *New Phytol*. 2015;208(4):1251-1263. doi:10.1111/nph.13560

278. Bramer C, Dobler S, Deckert J, Stemmer M, Petschenka G. Na+/K+-ATPase resistance and cardenolide sequestration: basal adaptations to host plant toxins in the milkweed bugs (Hemiptera: Lygaeidae: Lygaeinae). *Proc R Soc B Biol Sci*. 2015;282(1805):20142346. doi:10.1098/rspb.2014.2346

279. Heidel-Fischer HM, Vogel H. Molecular mechanisms of insect adaptation to plant secondary compounds. *Curr Opin Insect Sci*. 2015;8:8-14. doi:10.1016/j.cois.2015.02.004

280. Schuman MC, Baldwin IT. The Layers of Plant Responses to Insect Herbivores. *Annu Rev Entomol*. 2016;61(1):373-394. doi:10.1146/annurev-ento-010715-023851

281. Bramer C, Friedrich F, Dobler S. Defence by plant toxins in milkweed bugs (Heteroptera: Lygaeinae) through the evolution of a sophisticated storage compartment. *Syst Entomol*. 2017;42(1):15-30. doi:10.1111/syen.12189

282. Jander G. Revisiting Plant-Herbivore Co-Evolution in the Molecular Biology Era. In: *Annual Plant Reviews Online*. American Cancer Society; 2018:361-384. doi:10.1002/9781119312994.apr0515

283. Zhang X, van Doan C, Arce CCM, Hu L, Gruenig S, Parisod C, Hibbard BE, Hervé MR, Nielson C, Robert CAM, et al. Plant defense resistance in natural enemies of a specialist insect herbivore. *Proc Natl Acad Sci*. 2019;116(46):23174-23181. doi:10.1073/pnas.1912599116

284. Yang L. THE DYNAMICS OF EVOLUTION OF TOXIN RESISTANCE IN INSECTS AND VERTEBRATES. Published online 2020. Accessed April 13, 2021. https://dataspace.princeton.edu/handle/88435/dsp01nc580q72n

285. Xia J, Guo Z, Yang Z, Han H, Wang S, Xu H, Yang X, Yang F, Wu Q, Xie W, et al. Whitefly hijacks a plant detoxification gene that neutralizes plant toxins. *Cell*. 2021;184(7):1693-1705.e17. doi:10.1016/j.cell.2021.02.014

286. Pokharel P, Steppuhn A, Petschenka G. Dietary cardenolides enhance growth and change the direction of the fecundity-longevity trade-off in milkweed bugs (Heteroptera: Lygaeinae). *bioRxiv*. Published online March 30, 2021:2021.03.29.437508. doi:10.1101/2021.03.29.437508

287. Aardema ML, Zhen Y, Andolfatto P. The evolution of cardenolide-resistant forms of Na^+,K^+-ATPase in Danainae butterflies: EVOLUTION OF CARDENOLIDE-RESISTANT FORMS. *Mol Ecol*. 2012;21(2):340-349. doi:10.1111/j.1365-294X.2011.05379.x

288. Petschenka G, Fandrich S, Sander N, Wagschal V, Boppré M, Dobler S. Stepwise Evolution of Resistance to Toxic Cardenolides Via Genetic Substitutions in the Na+/K+-Atpase of Milkweed Butterflies (lepidoptera: Danaini). *Evolution*. 2013;67(9):2753-2761. doi:10.1111/evo.12152

289. Petschenka G, Pick C, Wagschal V, Dobler S. Functional evidence for physiological mechanisms to circumvent neurotoxicity of cardenolides in an adapted and a non-adapted hawk-moth species. *Proc R Soc B Biol Sci*. 2013;280(1759):20123089. doi:10.1098/rspb.2012.3089

290. Petschenka G, Agrawal AA. Milkweed butterfly resistance to plant toxins is linked to sequestration, not coping with a toxic diet. *Proc R Soc B Biol Sci*. 2015;282(1818):20151865. doi:10.1098/rspb.2015.1865

291. Ujvari B, Casewell NR, Sunagar K, Arbuckle K, Wüster W, Lo N, O'Meally D, Beckmann C, King GF, Deplazes E, et al. Widespread convergence in toxin resistance by predictable molecular evolution. *Proc Natl Acad Sci U S A*. 2015;112(38):11911-11916. doi:10.1073/pnas.1511706112

292. Dalla S, Dobler S. Gene duplications circumvent trade-offs in enzyme function: Insect adaptation to toxic host plants. *Evolution*. 2016;70(12):2767-2777. doi:10.1111/evo.13077

293. Karageorgi M, Groen SC, Sumbul F, Pelaez JN, Verster KI, Aguilar JM, Hastings AP, Bernstein SL, Matsunaga T, Astourian M, et al. Genome editing retraces the evolution of toxin resistance in the monarch butterfly. *Nature*. 2019;574(7778):409-412. doi:10.1038/s41586-019-1610-8

294. Yoshida T, Ujiie R, Savitzky AH, Jono T, Inoue T, Yoshinaga N, Aburaya S, Aoki W, Takeuchi H, Ding L, et al. Dramatic dietary shift maintains sequestered toxins in chemically defended snakes. *Proc Natl Acad Sci*. 2020;117(11):5964-5969.

295. Medina-Ortiz K, Lopez-Alvarez D, Navia F, Hansen T, Fierro L, Castaño S. Identification of Na+/K + -ATPase α/β isoforms in Rhinella marina tissues by RNAseq and a molecular docking approach at the protein level to evaluate α isoform affinities for bufadienolides. *Comp Biochem Physiol A Mol Integr Physiol*. 2021;254:110906. doi:10.1016/j.cbpa.2021.110906

296. Abraham EP, Chain E. An Enzyme from Bacteria able to Destroy Penicillin. *Nature*. 1940;146(3713):837-837. doi:10.1038/146837a0

297. Georghiou GP, Wirth MC. Influence of Exposure to Single versus Multiple Toxins of Bacillus thuringiensis subsp. israelensis on Development of Resistance in the Mosquito Culex quinquefasciatus (Diptera: Culicidae). *Appl Environ Microbiol*. 1997;63(3):1095-1101. doi:10.1128/aem.63.3.1095-1101.1997

298. Tabashnik BE, Liu YB, Finson N, Masson L, Heckel DG. One gene in diamondback moth confers resistance to four Bacillus thuringiensis toxins. *Proc Natl Acad Sci*. 1997;94(5):1640-1644.

299. REBOUD X, MAJERUS N, GASQUEZ J, POWLES S. Chlamydomonas reinhardtii as a model system for pro-active herbicide resistance evolution research. *Biol J Linn Soc*. 2007;91(2):257-266. doi:10.1111/j.1095-8312.2007.00787.x

300. Collins S, Gardner A. Integrating physiological, ecological and evolutionary change: a Price equation approach. *Ecol Lett*. 2009;12(8):744-757. doi:10.1111/j.1461-0248.2009.01340.x

301. Baxter SW, Badenes-Pérez FR, Morrison A, Vogel H, Crickmore N, Kain W, Wang P, Heckel DG, Jiggins CD. Parallel Evolution of Bacillus thuringiensis Toxin Resistance in Lepidoptera. *Genetics*. 2011;189(2):675-679. doi:10.1534/genetics.111.130971

302. Zakon HH. Adaptive evolution of voltage-gated sodium channels: The first 800 million years. *Proc Natl Acad Sci*. 2012;109(Supplement 1):10619-10625.

303. Jochumsen N, Marvig RL, Damkiær S, Jensen RL, Paulander W, Molin S, Jelsbak L, Folkesson A. The evolution of antimicrobial peptide resistance in Pseudomonas aeruginosa is shaped by strong epistatic interactions. *Nat Commun*. 2016;7(1):13002. doi:10.1038/ncomms13002

304. Alyokhin A, Chen YH. Adaptation to toxic hosts as a factor in the evolution of insecticide resistance. *Curr Opin Insect Sci*. 2017;21:33-38. doi:10.1016/j.cois.2017.04.006

305. Peyser RD, Lanno SM, Shimshak SJ, Coolon JD. Analysis of cytochrome P450 contribution to evolved plant toxin resistance in Drosophila sechellia. *Insect Mol Biol*. 2017;26(6):715-720. doi:10.1111/imb.12329

306. MacLean RC, Millan AS. The evolution of antibiotic resistance. *Science*. 2019;365(6458):1082-1083. doi:10.1126/science.aax3879

307. Kairigo P, Ngumba E, Sundberg LR, Gachanja A, Tuhkanen T. Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments. *Sci Total Environ*. 2020;720:137580. doi:10.1016/j.scitotenv.2020.137580

308. Christaki E, Marcou M, Tofarides A. Antimicrobial Resistance in Bacteria: Mechanisms, Evolution, and Persistence. *J Mol Evol*. 2020;88(1):26-40. doi:10.1007/s00239-019-09914-3

309. Papkou A, Hedge J, Kapel N, Young B, MacLean RC. Efflux pump activity potentiates the evolution of antibiotic resistance across S. aureus isolates. *Nat Commun*. 2020;11(1):3970. doi:10.1038/s41467-020-17735-y

310. Stanton IC, Murray AK, Zhang L, Snape J, Gaze WH. Evolution of antibiotic resistance at low antibiotic concentrations including selection below the minimal selective concentration. *Commun Biol*. 2020;3(1):1-11. doi:10.1038/s42003-020-01176-w

311. Gurney J, Pradier L, Griffin JS, Gougat-Barbera C, Chan BK, Turner PE, Kaltz O, Hochberg ME. Phage steering of antibiotic-resistance evolution in the bacterial pathogen, Pseudomonas aeruginosa. *Evol Med Public Health*. 2020;2020(1):148-157. doi:10.1093/emph/eoaa026

312. Bottery MJ, Pitchford JW, Friman VP. Ecology and evolution of antimicrobial resistance in bacterial communities. *ISME J*. 2021;15(4):939-948. doi:10.1038/s41396-020-00832-7

313. Yan Y, Liu N, Tang Y. Recent developments in self-resistance gene directed natural product discovery. *Nat Prod Rep*. Published online January 8, 2020. doi:10.1039/C9NP00050J

314. Jiang X, Zhang L, Liang H, Li Q, Zhao Y, Chen L, Yang W, Zhao S. Resistance variation within a Daphnia pulex population against toxic cyanobacteria. *J Plankton Res*. 2013;35(5):1177-1181. doi:10.1093/plankt/fbt062

315. Prince EK, Poulson-Ellestad KL. Predator–Prey Interactions in the Marine Plankton: The Role of Signals, Cues, and Defensive Compounds. In: *Chemical Ecology*. CRC Press; 2018.

316. Matthews B, Jokela J, Narwani A, Räsänen K, Pomati F, Altermatt F, Spaak P, Robinson CT, Vorburger C. On biological evolution and environmental solutions. *Sci Total Environ*. 2020;724:138194. doi:10.1016/j.scitotenv.2020.138194

317. Isanta-Navarro J, Hairston NG, Beninde J, Meyer A, Straile D, Möst M, Martin-Creuzburg D. Reversed evolution of grazer resistance to cyanobacteria. *Nat Commun*. 2021;12(1):1945. doi:10.1038/s41467-021-22226-9

318. Turner AD, Lewis AM, Bradley K, Maskrey BH. Marine invertebrate interactions with Harmful Algal Blooms – Implications for One Health. *J Invertebr Pathol*. Published online February 16, 2021:107555. doi:10.1016/j.jip.2021.107555

319. Dumbacher JP, Spande TF, Daly JW. Batrachotoxin alkaloids from passerine birds: A second toxic bird genus (Ifrita kowaldi) from New Guinea. *Proc Natl Acad Sci*. 2000;97(24):12970-12975.

320. Jønsson KA, Bowie RCK, Norman JA, Christidis L, Fjeldså J. Polyphyletic origin of toxic Pitohui birds suggests widespread occurrence of toxicity in corvoid birds. *Biol Lett*. 2008;4(1):71-74. doi:10.1098/rsbl.2007.0464

321. Dumbacher JP, Deiner K, Thompson L, Fleischer RC. Phylogeny of the avian genus Pitohui and the evolution of toxicity in birds. *Mol Phylogenet Evol*. 2008;49(3):774-781. doi:10.1016/j.ympev.2008.09.018

322. A. Saporito R, F. Spande T, Martin Garraffo H, A. Donnelly M. Arthropod Alkaloids in Poison Frogs: A Review of the 'Dietary Hypothesis.' *HETEROCYCLES*. 2009;79(1):277. doi:10.3987/REV-08-SR(D)11

323. Menon GK, Dumbacher JP. A 'toxin mantle' as defensive barrier in a tropical bird: evolutionary exploitation of the basic permeability barrier forming organelles. *Exp Dermatol*. 2014;23(4):288-290. doi:10.1111/exd.12367

324. Ligabue-Braun R, Carlini CR. Poisonous birds: A timely review. *Toxicon*. 2015;99:102-108. doi:10.1016/j.toxicon.2015.03.020

325. Santos JC, Tarvin RD, O'Connell LA. A Review of Chemical Defense in Poison Frogs (Dendrobatidae): Ecology, Pharmacokinetics, and Autoresistance. In: Schulte BA, Goodwin TE, Ferkin MH, eds. *Chemical Signals in Vertebrates 13*. Springer International Publishing; 2016:305-337. doi:10.1007/978-3-319-22026-0_21

326. Abderemane-Ali F, Rossen ND, Kobiela ME, Craig RA, Garrison CE, O'Connell LA, Bois JD, Dumbacher JP, Minor DL. Sodium channel toxin-resistance mutations do not govern batrachotoxin (BTX) autoresistance in poison birds and frogs. *bioRxiv*. Published online October 29, 2020:2020.10.29.361212. doi:10.1101/2020.10.29.361212

327. Rowe AH, Xiao Y, Rowe MP, Cummins TR, Zakon HH. Voltage-Gated Sodium Channel in Grasshopper Mice Defends Against Bark Scorpion Toxin. *Science*. 2013;342(6157):441-446. doi:10.1126/science.1236451

328. Jansa SA, Voss RS. Adaptive Evolution of the Venom-Targeted vWF Protein in Opossums that Eat Pitvipers. *PLoS ONE*. 2011;6(6). doi:10.1371/journal.pone.0020997

329. Drabeck DH, Rucavado A, Hingst-Zaher E, Cruz YP, Dean AM, Jansa SA. Resistance of South American opossums to vWF-binding venom C-type lectins. *Toxicon*. 2020;178:92-99. doi:10.1016/j.toxicon.2020.02.024

330. Drabeck DH, Dean AM, Jansa SA. Why the honey badger don't care: Convergent evolution of venom-targeted nicotinic acetylcholine receptors in mammals that survive venomous snake bites. *Toxicon*. 2015;99:68-72. doi:10.1016/j.toxicon.2015.03.007

331. Khan MA, Dashevsky D, Kerkkamp H, Kordiš D, de Bakker MAG, Wouters R, van Thiel J, op den Brouw B, Vonk FJ, Kini RM, et al. Widespread Evolution of Molecular Resistance to Snake Venom α-Neurotoxins in Vertebrates. *Toxins*. 2020;12(10):638. doi:10.3390/toxins12100638

332. Harris RJ, Nekaris KAI, Fry BG. Coevolution between primates and venomous snakes revealed by α-neurotoxin susceptibility. *bioRxiv*. Published online January 29, 2021:2021.01.28.428735. doi:10.1101/2021.01.28.428735

333. Harris R. Electrostatic resistance to alpha- neurotoxins conferred by charge reversal mutations in nicotinic acetylcholine receptors. *Proc R Soc B Biol Sci*. 2021;288. doi:10.1098/rspb.2020.2703

334. Daly JW, Gusovsky F, Myers CW, Yotsu-Yamashita M, Yasumoto T. First occurrence of tetrodotoxin in a dendrobatid frog (Colostethus inguinalis), with further reports for the bufonid genus Atelopus. *Toxicon*. 1994;32(3):279-285. doi:10.1016/0041-0101(94)90081-7

335. Jeziorski MC, Greenberg RM, Anderson PA. Cloning of a putative voltage-gated sodium channel from the turbellarian flatworm Bdelloura candida. *Parasitology*. 1997;115 (Pt 3):289-296. doi:10.1017/s0031182097001388

336. Geffeney S, Brodie ED, Ruben PC, Brodie ED. Mechanisms of Adaptation in a Predator-Prey Arms Race: TTX-Resistant Sodium Channels. *Science*. 2002;297(5585):1336-1339. doi:10.1126/science.1074310

337. Williams BL, Brodie ED Jr, Brodie ED III. COEVOLUTION OF DEADLY TOXINS AND PREDATOR RESISTANCE: SELF-ASSESSMENT OF RESISTANCE BY GARTER SNAKES LEADS TO BEHAVIORAL REJECTION OF TOXIC NEWT PREY. *Herpetologica*. 2003;59(2):155-163. doi:10.1655/0018-0831(2003)059[0155:CODTAP]2.0.CO;2

338. Bricelj VM, Connell L, Konoki K, MacQuarrie SP, Scheuer T, Catterall WA, Trainer VL. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. *Nature*. 2005;434(7034):763-767. doi:10.1038/nature03415

339. Geffeney SL, Fujimoto E, Brodie ED, Brodie ED, Ruben PC. Evolutionary diversification of TTX-resistant sodium channels in a predator–prey interaction. *Nature*. 2005;434(7034):759-763. doi:10.1038/nature03444

340. Soong TW, Venkatesh B. Adaptive evolution of tetrodotoxin resistance in animals. *Trends Genet*. 2006;22(11):621-626. doi:10.1016/j.tig.2006.08.010

341. Jost MC, Hillis DM, Lu Y, Kyle JW, Fozzard HA, Zakon HH. Toxin-Resistant Sodium Channels: Parallel Adaptive Evolution across a Complete Gene Family. *Mol Biol Evol*. 2008;25(6):1016-1024. doi:10.1093/molbev/msn025

342. Lee CH, Jones DK, Ahern C, Sarhan MF, Ruben PC. Biophysical costs associated with tetrodotoxin resistance in the sodium channel pore of the garter snake, Thamnophis sirtalis. *J Comp Physiol A*. 2011;197(1):33-43. doi:10.1007/s00359-010-0582-9

343. Hanifin CT, Gilly WF. Evolutionary history of a complex adaptation: Tetrodotoxin resistance in salamanders. *Evol Int J Org Evol*. 2015;69(1):232-244. doi:10.1111/evo.12552

344. Jal S, Khora SS. An overview on the origin and production of tetrodotoxin, a potent neurotoxin. *J Appl Microbiol*. 2015;119(4):907-916. doi:10.1111/jam.12896

345. Feldman CR, Durso AM, Hanifin CT, Pfrender ME, Ducey PK, Stokes AN, Barnett KE, Brodie Iii ED, Brodie Jr ED. Is there more than one way to skin a newt? Convergent toxin resistance in snakes is not due to a common genetic mechanism. *Heredity*. 2016;116(1):84-91. doi:10.1038/hdy.2015.73

346. Li Y, Sun X, Hu X, Xun X, Zhang J, Guo X, Jiao W, Zhang L, Liu W, Wang J, et al. Scallop genome reveals molecular adaptations to semi-sessile life and neurotoxins. *Nat Commun*. 2017;8(1):1-11. doi:10.1038/s41467-017-01927-0

347. Magarlamov TYu, Melnikova DI, Chernyshev AV. Tetrodotoxin-Producing Bacteria: Detection, Distribution and Migration of the Toxin in Aquatic Systems. *Toxins*. 2017;9(5):166. doi:10.3390/toxins9050166

348. Shen H, Li Z, Jiang Y, Pan X, Wu J, Cristofori-Armstrong B, Smith JJ, Chin YKY, Lei J, Zhou Q, et al. Structural basis for the modulation of voltage-gated sodium channels by animal toxins. *Science*. 2018;362(6412). doi:10.1126/science.aau2596

349. Turner AD, Fenwick D, Powell A, Dhanji-Rapkova M, Ford C, Hatfield RG, Santos A, Martinez-Urtaza J, Bean TP, Baker-Austin C, et al. New Invasive Nemertean Species (Cephalothrix Simula) in England with High Levels of Tetrodotoxin and a Microbiome Linked to Toxin Metabolism. *Mar Drugs*. 2018;16(11):452. doi:10.3390/md16110452

350. Dell'Aversano C, Tartaglione L, Polito G, Dean K, Giacobbe M, Casabianca S, Capellacci S, Penna A, Turner AD. First detection of tetrodotoxin and high levels of paralytic shellfish poisoning toxins in shellfish from Sicily (Italy) by three different analytical methods. *Chemosphere*. 2019;215:881-892. doi:10.1016/j.chemosphere.2018.10.081

351. Geffeney SL, Williams BL, Rosenthal JJC, Birk MA, Felkins J, Wisell CM, Curry ER, Hanifin CT. Convergent and parallel evolution in a voltage-gated sodium channel underlies TTX-resistance in the Greater Blue-ringed Octopus: Hapalochlaena lunulata. *Toxicon*. 2019;170:77-84. doi:10.1016/j.toxicon.2019.09.013

352. Hague MTJ, Stokes AN, Feldman CR, Brodie ED, Brodie ED. The geographic mosaic of arms race coevolution is closely matched to prey population structure. *Evol Lett*. 2020;4(4):317-332. doi:https://doi.org/10.1002/evl3.184

353. Reimche JS, Brodie ED, Stokes AN, Ely EJ, Moniz HA, Thill VL, Hallas JM, Pfrender ME, Brodie ED, Feldman CR. The geographic mosaic in parallel: Matching patterns of newt tetrodotoxin levels and snake resistance in multiple predator–prey pairs. *J Anim Ecol*. 2020;89(7):1645-1657. doi:10.1111/1365-2656.13212

354. Vaelli PM, Theis KR, Williams JE, O'Connell LA, Foster JA, Eisthen HL. The skin microbiome facilitates adaptive tetrodotoxin production in poisonous newts. Baldwin IT, Robert CA, Robert CA, González Montoya MC, eds. *eLife*. 2020;9:e53898. doi:10.7554/eLife.53898

355. Whitelaw BL, Cooke IR, Finn J, Zenger K, Strugnell JM. The evolution and origin of tetrodotoxin acquisition in the blue-ringed octopus (genus Hapalochlaena). *Aquat Toxicol*. 2019;206:114-122. doi:10.1016/j.aquatox.2018.10.012

356. Whitelaw BL, Cooke IR, Finn J, da Fonseca RR, Ritschard EA, Gilbert MTP, Simakov O, Strugnell JM. Adaptive venom evolution and toxicity in octopods is driven by extensive novel gene formation, expansion, and loss. *GigaScience*. 2020;9(giaa120). doi:10.1093/gigascience/giaa120

357. Gendreau K, Hornsby A, Hague M, McGlothlin J. *Gene Conversion Facilitates the Adaptive Evolution of Self-Resistance in Highly Toxic Newts*.; 2021. doi:10.1101/2021.03.25.437018

358. Singh HP, Batish DR, Kohli RK. Autotoxicity: Concept, Organisms, and Ecological Significance. *Crit Rev Plant Sci*. 1999;18(6):757-772. doi:10.1080/07352689991309478

359. Almabruk KH, Dinh LK, Philmus B. Self-Resistance of Natural Product Producers: Past, Present, and Future Focusing on Self-Resistant Protein Variants. *ACS Chem Biol*. 2018;13(6):1426-1437. doi:10.1021/acschembio.8b00173

360. Kistler HC, Broz K. Cellular compartmentalization of secondary metabolism. *Front Microbiol*. 2015;6. doi:10.3389/fmicb.2015.00068

361. Blanco J. Accumulation of Dinophysis Toxins in Bivalve Molluscs. *Toxins*. 2018;10(11):453. doi:10.3390/toxins10110453

362. Briggs JC. Extinction and replacement in the Indo-West Pacific Ocean. *J Biogeogr*. 1999;26(4):777-783. doi:10.1046/j.1365-2699.1999.00322.x

363. Veron JEN, Devantier LM, Turak E, Green AL, Kininmonth S, Stafford-Smith M, Peterson N. Delineating the Coral Triangle. *Galaxea J Coral Reef Stud*. 2009;11(2):91-100.

364. Hoeksema BW. In search of the Asian-Pacific Centre of Maximum Marine Biodiversity: Explanations from the past and present. *Galaxea J Coral Reef Stud*. 2013;15(Supplement):1-8. doi:10.3755/galaxea.15.1

365. Sanciangco JC, Carpenter KE, Etnoyer PJ, Moretzsohn F. Habitat Availability and Heterogeneity and the Indo-Pacific Warm Pool as Predictors of Marine Species Richness in the Tropical Indo-Pacific. *PLoS ONE*. 2013;8(2):e56245.

366. Förderer M, Rödder D, Langer MR. Patterns of species richness and the center of diversity in modern Indo-Pacific larger foraminifera. *Sci Rep*. 2018;8(1):8189. doi:10.1038/s41598-018-26598-9

367. Sprintall J, Gordon AL, Koch-Larrouy A, Lee T, Potemra JT, Pujiana K, Wijffels SE. The Indonesian seas and their role in the coupled ocean–climate system. *Nat Geosci*. 2014;7(7):487-492. doi:10.1038/ngeo2188

368. Lee T, Fournier S, Gordon AL, Sprintall J. Maritime Continent water cycle regulates low-latitude chokepoint of global ocean circulation. *Nat Commun*. 2019;10(1):2103. doi:10.1038/s41467-019-10109-z

369. Hoeksema BW. Delineation of the Indo-Malayan Centre of Maximum Marine Biodiversity: The Coral Triangle. *Top Geobiol*. Published online 2007:117-178.

370. Watanabe A, Nakamura T. Carbon Dynamics in Coral Reefs. In: Kuwae T, Hori M, eds. *Blue Carbon in Shallow Coastal Ecosystems: Carbon Dynamics, Policy, and Implementation*. Springer; 2019:273-293. doi:10.1007/978-981-13-1295-3_10

371. Kasim M. Measuring Vulnerability of Coastal Ecosystem and Identifying Adaptation Options of Indonesia's Coastal Communities to Climate Change: Case Study of Southeast Sulawesi, Indonesia. In: Djalante R, Jupesta J, Aldrian E, eds. *Climate Change Research, Policy and Actions in Indonesia: Science, Adaptation and Mitigation*. Springer Climate. Springer International Publishing; 2021:149-172. doi:10.1007/978-3-030-55536-8_8

372. Garçon V, Laffoley D, Baxter J. Ocean deoxygenation: everyone's problem. Causes, impacts, consequences and solutions. In: ; 2019. doi:10.2305/iucn.ch.2019.13.en

373. Hughes DJ, Alderdice R, Cooney C, Kühl M, Pernice M, Voolstra CR, Suggett DJ. Coral reef survival under accelerating ocean deoxygenation. *Nat Clim Change*. 2020;10(4):296-307. doi:10.1038/s41558-020-0737-9

374. Laffoley D, Baxter JM, Amon DJ, Claudet J, Hall-Spencer JM, Grorud-Colvert K, Levin LA, Reid PC, Rogers AD, Taylor ML, et al. Evolving the narrative for protecting a rapidly changing ocean, post-COVID-19. *Aquat Conserv*. Published online November 25, 2020:10.1002/aqc.3512. doi:10.1002/aqc.3512

375. Jennerjahn TC, Rixen T, Irianto HE, Samiaji J. 1 - Introduction—Science for the Protection of Indonesian Coastal Ecosystems (SPICE). In: Jennerjahn TC, Rixen T, Irianto HE, Samiaji J, eds. *Science for the Protection of Indonesian Coastal Ecosystems (SPICE)*. Elsevier; 2022:1-11. doi:10.1016/B978-0-12-815050-4.00013-4

376. Wang G, Percival IG, Zhen YY. The youngest Ordovician (latest Katian) coral fauna from eastern Australia, in the uppermost Malachis Hill Formation of central New South Wales. *Alcheringa Australas J Palaeontol*. 2020;44(3):356-378. doi:10.1080/03115518.2020.1747540

377. Rudman WB, Willan RC. *Opisthobranchia. The Southern Synthesis. Fauna of Australia*. CSIRO, Melbourne; 1998.

378. Nimbs MJ, Larkin M, Davis TR, Harasti D, Willan RC, Smith SDA. Southern range extensions for twelve heterobranch sea slugs (Gastropoda: Heterobranchia) on the eastern coast of Australia. *Mar Biodivers Rec*. 2016;9(1):27. doi:10.1186/s41200-016-0027-4

379. Nimbs MJ, Smith SDA. Welcome strangers: Southern range extensions for seven heterobranch sea slugs (Mollusca: Gastropoda) on the subtropical east Australian coast, a climate change hot spot. *Reg Stud Mar Sci*. 2016;8:27-32. doi:10.1016/j.rsma.2016.08.008

380. Nimbs MJ, Smith SDA. Beyond Capricornia: Tropical Sea Slugs (Gastropoda, Heterobranchia) Extend Their Distributions into the Tasman Sea. *Diversity*. 2018;10(3):99. doi:10.3390/d10030099

381. Gervais CR, Champion C, Pecl GT. Species on the move around the Australian coastline: A continental-scale review of climate-driven species redistribution in marine systems. *Glob Change Biol*. 2021;27(14):3200-3217. doi:10.1111/gcb.15634

382. Larkin MF, Smith SDA, Willan RC, Davis TR. Diel and seasonal variation in heterobranch sea slug assemblages within an embayment in temperate eastern Australia. *Mar Biodivers*. 2018;48(3):1541-1550. doi:10.1007/s12526-017-0700-9

383. Smith SDA, Davis TomR. Slugging it out for science: volunteers provide valuable data on the diversity and distribution of heterobranch sea slugs. *Molluscan Res*. 2019;39(3):214-223. doi:10.1080/13235818.2019.1594600

384. Elizabeth Moore TMG. Additions to the genus Phyllodesmium, with a phylogenetic analysis and its implications to the evolution of symbiosis. *Veliger -Berkeley-*. 2014;51(4):237-251.

385. Burghardt I, Gosliner TM. Phyllodesmium rudmani (Mollusca : Nudibranchia : Aeolidoidea), a new solar powered species from the Indo-West Pacific with data on its symbiosis with zooxanthellae. *Zootaxa*. 2006;(1308):31-47.

386. Sammarco PW, Coll JC. The Chemical Ecology of Alcyonarian Corals. In: Scheuer PPJ, ed. *Bioorganic Marine Chemistry*. Bioorganic Marine Chemistry. Springer Berlin Heidelberg; 1988:87-116. doi:10.1007/978-3-642-48346-2_3

387. Fabricius K, Alderslade P. *Soft Corals and Sea Fans*. Australian Institute of Marine Science; 2001.

388. Fautin DG. Structural diversity, systematics, and evolution of cnidae. *Toxicon Off J Int Soc Toxinology*. 2009;54(8):1054-1064. doi:10.1016/j.toxicon.2009.02.024

389. Yoffe C, Lotan T, Benayhau Y. A Modified View on Octocorals: Heteroxenia fuscescens Nematocysts Are Diverse, Featuring Both an Ancestral and a Novel Type. *PLoS ONE*. 2012;7(2):e31902. doi:10.1371/journal.pone.0031902

390. Affeld S, Kehraus S, Wägele H, König GM. Dietary derived sesquiterpenes from Phyllodesmium lizardensis. *J Nat Prod*. 2009;72(2):298-300. doi:10.1021/np800583e

391. Coll JC, Bowden BF, Tapiolas DM, Willis RH, Djura P, Streamer M, Trott L. Studies of australian soft corals—XXXV. *Tetrahedron*. 1985;41(6):1085-1092. doi:10.1016/S0040-4020(01)96476-2

392. Slattery M, Avila C, Starmer J, Paul VJ. A sequestered soft coral diterpene in the aeolid nudibranch Phyllodesmium guamensis Avila, Ballesteros, Slattery, Starmer and Paul. *J Exp Mar Biol Ecol*. 1998;226(1):33-49. doi:10.1016/S0022-0981(97)00240-2

393. Mao SC, Gavagnin M, Mollo E, Guo YW. A new rare asteriscane sesquiterpene and other related derivatives from the Hainan aeolid nudibranch Phyllodesmium magnum. *Biochem Syst Ecol*. 2011;39(4-6):408-411. doi:10.1016/j.bse.2011.05.018

394. Stanley GD, Schootbrugge B van de. The Evolution of the Coral–Algal Symbiosis. In: Oppen MJH van, Lough JM, eds. *Coral Bleaching*. Ecological Studies. Springer Berlin Heidelberg; 2009:7-19. doi:10.1007/978-3-540-69775-6_2

395. Sammarco PW, Strychar KB. Responses to High Seawater Temperatures in Zooxanthellate Octocorals. *Plos One*. 2013;8(2):e54989. doi:10.1371/journal.pone.0054989

396. Dinesen ZD. Patterns in the distribution of soft corals across the central Great Barrier Reef. *Coral Reefs*. 1983;1(4):229-236. doi:10.1007/BF00304420

397. Liang XT, Fang WS. *Medicinal Chemistry of Bioactive Natural Products*. John Wiley & Sons; 2006.

398. Jeng MS, Huang HD, Dai CF, Hsiao YC, Benayahu Y. Sclerite calcification and reef-building in the fleshy octocoral genus Sinularia (Octocorallia: Alcyonacea). *Coral Reefs*. 2011;30(4):925-933. doi:10.1007/s00338-011-0765-z

399. Coll J, Labarre S, Sammarco P, Williams W, Bakus G. Chemical Defenses in Soft Corals (coelenterata, Octocorallia) of the Great Barrier-Reef - a Study of Comparative Toxicities. *Mar Ecol Prog Ser*. 1982;8(3):271-278. doi:10.3354/meps008271

400. Tursch B. Some Recent Developments in the Chemistry of Alcyonaceans. *Pure Appl Chem*. 1976;48(1):1-6. doi:10.1351/pac197648010001

401. Faulkner DJ. Marine natural products. *Nat Prod Rep*. 2001;18(1):1R-49R. doi:10.1039/B006897G

402. Berrue F, Kerr RG. Diterpenes from gorgonian corals. *Nat Prod Rep*. 2009;26(5):681-710. doi:10.1039/b821918b

403. Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR. Marine natural products. *Nat Prod Rep*. 2015;32(2):116-211. doi:10.1039/C4NP00144C

404. Coll JC. The chemistry and chemical ecology of octocorals (Coelenterata, Anthozoa, Octocorallia). *Chem Rev*. 1992;92(4):613-631. doi:10.1021/cr00012a006

405. Jr JFW, Torres MS. *Defensive Mutualism in Microbial Symbiosis*. CRC Press; 2009.

406. Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep*. 2004;21(1):1-49. doi:10.1039/b305250h

407. Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep*. 2011;28(2):196-268. doi:10.1039/C005001F

408. Correa H, Aristizabal F, Duque C, Kerr R. Cytotoxic and antimicrobial activity of pseudopterosins and seco-pseudopterosins isolated from the octocoral Pseudopterogorgia elisabethae of San Andrés and Providencia Islands (Southwest Caribbean Sea). *Mar Drugs*. 2011;9(3):334-343. doi:10.3390/md9030334

409. Rocha J, Peixe L, Gomes NCM, Calado R. Cnidarians as a Source of New Marine Bioactive Compounds—An Overview of the Last Decade and Future Steps for Bioprospecting. *Mar Drugs*. 2011;9(10):1860-1886. doi:10.3390/md9101860

410. Wang SK, Duh CY. New Cytotoxic Cembranolides from the Soft Coral Lobophytum michaelae. *Mar Drugs*. 2012;10(2):306-318. doi:10.3390/md10020306

411. Li C, Jiang M, La MP, Li TJ, Tang H, Sun P, Liu BS, Yi YH, Liu Z, Zhang W. Chemistry and Tumor Cell Growth Inhibitory Activity of 11,20-Epoxy-3Z, 5(6)E-diene Briaranes from the South China Sea Gorgonian Dichotella gemmacea. *Mar Drugs*. 2013;11(5):1565-1582. doi:10.3390/md11051565

412. Neeman I, Fishelso.l, Kashman Y. Sarcophine - New Toxin from Soft Coral Sarcophyton-Glaucum (alcyonaria). *Toxicon*. 1974;12(6):593-. doi:10.1016/0041-0101(74)90192-5

413. Kashman Y, Groweiss A. Lobolide: A new epoxy cembranolide from marine origin. *Tetrahedron Lett*. 1977;18(13):1159-1160. doi:10.1016/S0040-4039(01)92858-8

414. Li L, Wang CY, Shao CL, Han L, Sun XP, Zhao J, Guo YW, Huang H, Guan HS. Two new metabolites from the Hainan soft coral Sarcophyton crassocaule. *J Asian Nat Prod Res*. 2009;11(10):851-855. doi:10.1080/10286020902867060

415. Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep*. 2010;27(2):165. doi:10.1039/b906091j

416. Aceret TL, Sammarco PW, Coll JC, Uchio Y. Discrimination between several diterpenoid compounds in feeding by Gambusia affinis. *Comp Biochem Physiol Part C Toxicol Pharmacol*. 2001;128(1):55-63. doi:10.1016/S1532-0456(00)00182-4

417. Kim SK. *Springer Handbook of Marine Biotechnology*. Springer; 2015.

418. Hoang BX, Sawall Y, Al-Sofyani A, Wahl M. Chemical versus structural defense against fish predation in two dominant soft coral species (Xeniidae) in the Red Sea. *Aquat Biol*. 2015;23(2):129-137. doi:10.3354/ab00614

419. Tanaka J, Yoshida T, Benayahu Y. Chemical diversity of Sarcophyton soft corals in Okinawa. *J Jpn Coral Reef Soc*. 2005;2005(7):1-9. doi:10.3755/jcrs.2005.1

420. Slattery M, McClintock JB, Heine JN. Chemical defenses in Antarctic soft corals: evidence for antifouling compounds. *J Exp Mar Biol Ecol*. 1995;190(1):61-77. doi:10.1016/0022-0981(95)00032-M

421. Slattery M, McClintock JB, Bowser SS. Deposit feeding: a novel mode of nutrition in the Antarctic colonial soft coral Gersemia antarctica. *Oceanogr Lit Rev*. 1997;9(44):996.

422. Sammarco P, Labarre S, Coll J. Defensive Strategies of Soft Corals (coelenterata, Octocorallia) of the Great-Barrier-Reef .3. the Relationship Between Ichthyotoxicity and Morphology. *Oecologia*. 1987;74(1):93-101. doi:10.1007/BF00377351

423. Krebs HChr. Recent Developments in the Field of Marine Natural Products with Emphasis on Biologically Active Compounds. In: Hill RA, Krebs HChr, Verpoorte R, Wijnsma R, Herz W, Grisebach H, Kirby GW, Tamm Ch, eds. *Fortschritte Der Chemie Organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products*. Fortschritte der Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products. Springer; 1986:151-363. doi:10.1007/978-3-7091-8846-0_3

424. Kashman Y, Zadock E, Neeman I. Some New Cembrane Derivatives of Marine Origin. *Tetrahedron*. 1974;30(19):3615-3620. doi:10.1016/S0040-4020(01)97044-9

425. Bowden B, Coll J, Hicks W, Kazlauskas R, Mitchell S. Studies of Australian Soft Corals .10. Isolation of Epoxyisoneocembrene-a from Sinularia-Grayi and Isoneocembrene-a from Sarcophyton-Ehrenbergi. *Aust J Chem*. 1978;31(12):2707-2712.

426. Bowden B, Coll J, Mitchell S, Stokie G. Studies of Australian Soft Corals .11. 2 New Cembranoid Diterpenes from a Sarcophyton-Species. *Aust J Chem*. 1979;32(3):653-659.

427. Bowden BF, Coll JC, Heaton A, König G, Bruck MA, Cramer RE, Klein DM, Scheuer PJ. The Structures of Four Isomeric Dihydrofuran-Containing Cembranoid Diterpenes from Several Species of Soft Coral. *J Nat Prod*. 1987;50(4):650-659. doi:10.1021/np50052a013

428. Kobayashi M, Hirase T. Marine Terpenes and Terpenoids. XI. : Structures of New Dihydrofuranocembranoids Isolated from a Sarcophyton sp. Soft Coral of Okinawa. *Chem Pharm Bull (Tokyo)*. 1990;38(9):2442-2445. doi:10.1248/cpb.38.2442

429. Coll J, Bowden B, Alino P, Heaton A, Konig G, Denys R, Willis R, Sammarco P, Clayton M. Chemically Mediated Interactions Between Marine Organisms. *Chem Scr*. 1989;29(4):383-388.

430. Li Y, Pattenden G. Perspectives on the structural and biosynthetic interrelationships between oxygenated furanocembranoids and their polycyclic congeners found in corals. *Nat Prod Rep*. 2011;28(7):1269-1310. doi:10.1039/C1NP00023C

431. Liang LF, Guo YW. Terpenes from the Soft Corals of the Genus Sarcophyton: Chemistry and Biological Activities. *Chem Biodivers*. 2013;10(12):2161-2196. doi:10.1002/cbdv.201200122

432. Singh P, Singh IN, Mondal SC, Singh L, Garg VK. Platelet-activating factor (PAF)-antagonists of natural origin. *Fitoterapia*. 2013;84:180-201. doi:10.1016/j.fitote.2012.11.002

433. Zhao YM, Maimone TJ. Short, Enantioselective Total Synthesis of Chatancin. *Angew Chem Int Ed*. 2015;54(4):1223-1226. doi:10.1002/anie.201410443

434. Anjaneyulu ASR, Venugopal MJRV, Sarada P, Rao GV, Clardy J, Lobkovsky E. Sarcophytin, A Novel Tetracyclic Diterpenoid From The Indian Ocean Soft Coral Sarcophyton elegans. *Tetrahedron Lett*. 1998;39(1–2):135-138. doi:10.1016/S0040-4039(97)10469-5

435. Sugano M, Shindo T, Sato A, Iijima Y, Oshima T, Kuwano H, Hata T. Chatancin, a PAF antagonist from a soft coral, Sarcophyton sp. *J Org Chem*. 1990;55(23):5803-5805. doi:10.1021/jo00310a001

436. Zhou Z fang, Guo Y wei. Bioactive natural products from Chinese marine flora and fauna. *Acta Pharmacol Sin*. 2012;33(9):1159-1169. doi:10.1038/aps.2012.110

437. Nam NH, Tung PT, Ngoc NT, Hanh TTH, Thao NP, Thanh NV, Cuong NX, Thao DT, Huong TT, Thung DC, et al. Cytotoxic Biscembranoids from the Soft Coral Sarcophyton pauciplicatum. *Chem Pharm Bull (Tokyo)*. 2015;63(8):636-640.

438. Iwagawa T, Hashimoto K, Okamura H, Kurawaki J ichi, Nakatani M, Hou DX, Fujii M, Doe M, Morimoto Y, Takemura K. Biscembranes from the Soft Coral Sarcophyton glaucum. *J Nat Prod*. 2006;69(8):1130-1133. doi:10.1021/np058115+

439. Iwagawa T, Hashimoto K, Yokogawa Y, Okamura H, Nakatani M, Doe M, Morimoto Y, Takemura K. Cytotoxic biscembranes from the soft coral Sarcophyton glaucum. *J Nat Prod*. 2009;72(5):946-949. doi:10.1021/np8003485

440. Weinheimer AJ, Chang CWJ, Matson JA. Naturally Occurring Cembranes. In: Herz W, Grisebach H, Kirby GW, eds. *Fortschritte Der Chemie Organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products*. Fortschritte der Chemie Organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products. Springer Vienna; 1979:285-387. doi:10.1007/978-3-7091-3265-4_3

441. Wahlberg I, Eklund AM. Cembranoids, Pseudopteranoids, and Cubitanoids of Natural Occurrence. In: Herz PW, Kirby PGW, Moore PRE, Steglich PDW, Tamm PDC, eds. *Fortschritte Der Chemie Organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products*. Fortschritte der Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products. Springer Vienna; 1992:141-294. doi:10.1007/978-3-7091-9150-7_2

442. Kokke WC, Epstein S, Look SA, Rau GH, Fenical W, Djerassi C. On the origin of terpenes in symbiotic associations between marine invertebrates and algae (zooxanthellae). Culture studies and an application of 13C/12C isotope ratio mass spectrometry. *J Biol Chem*. 1984;259(13):8168-8173.

443. Ciereszko LS. Sterol and Diterpenoid Production by Zooxanthellae in Coral Reefs: A Review. *Biol Oceanogr*. 1989;6(3-4):363-374. doi:10.1080/01965581.1988.10749538

444. Michalek-Wagner K, Bourne DJ, Bowden BF. The effects of different strains of zooxanthellae on the secondary-metabolite chemistry and development of the soft-coral host Lobophytum compactum. *Mar Biol*. 2001;138(4):753-760. doi:10.1007/s002270000505

445. Mydlarz LD, Jacobs RS, Boehnlein J, Kerr RG. Pseudopterosin biosynthesis in Symbiodinium sp., the dinoflagellate symbiont of Pseudopterogiorgia elisabethae. *Chem Biol*. 2003;10(11):1051-1056. doi:10.1016/j.chembiol.2003.10.012

446. Núñez-Pons L, Avila C. Deterrent activities in the crude lipophilic fractions of Antarctic benthic organisms: chemical defences against keystone predators. *Polar Res*. 2014;33(0). doi:10.3402/polar.v33.21624

447. Paruch E, Nawrot J, Wawrzeńczyk C. Lactones: Part 11. Feeding-deterrent activity of some bi- and tricyclic terpenoid lactones. *Pest Manag Sci*. 2001;57(9):776-780. doi:10.1002/ps.353

448. Dancewicz K, Gabrys B, Dams I, Wawrzenczyk C. Enantiospecific effect of pulegone and pulegone-derived lactones on Myzus persicae (Sulz.) settling and feeding. *J Chem Ecol*. 2008;34(4):530-538. doi:10.1007/s10886-008-9448-9

449. Streibl M, Nawrot J, Herout V. Feeding deterrent activity of enantiomeric isoalantones. *Biochem Syst Ecol*. 1983;11(4):381-382. doi:10.1016/0305-1978(83)90041-8

450. Calcagno MP, Coll J, Lloria J, Faini F, Alonso-Amelot ME. Evaluation of Synergism in the Feeding Deterrence of Some Furanocoumarins on Spodoptera littoralis. *J Chem Ecol*. 2002;28(1):175-191. doi:10.1023/A:1013575121691

451. Scheuer PJ. *Marine Natural Products: Chemical and Biological Perspectives*. Academic Press; 2013.

452. Coll JC, Price IR, König GM, Bowden BF. Algal overgrowth of alcyonacean soft corals. *Mar Biol*. 1987;96(1):129-135. doi:10.1007/BF00394846

453. Pawlik JR. Marine invertebrate chemical defenses. *Chem Rev*. 1993;93:1911-1922. doi:10.1021/cr00021a012

454. Lindquist N. Tridentatols D−H, Nematocyst Metabolites and Precursors of the Activated Chemical Defense in the Marine Hydroid Tridentata marginata (Kirchenpauer 1864). *J Nat Prod*. 2002;65(5):681-684. doi:10.1021/np010339e

455. Gershenzon J, Dudareva N. The function of terpene natural products in the natural world. *Nat Chem Biol*. 2007;3(7):408-414. doi:10.1038/nchembio.2007.5

456. Guyot M. Intricate aspects of sponge chemistry. *Zoosystema*. 2000;22(2):419-431.

457. Wilson MC, Mori T, Rückert C, Uria AR, Helf MJ, Takada K, Gernert C, Steffens UAE, Heycke N, Schmitt S, et al. An environmental bacterial taxon with a large and distinct metabolic repertoire. *Nature*. 2014;506(7486):58-62. doi:10.1038/nature12959

458. Kiran GS, Sekar S, Ramasamy P, Thinesh T, Hassan S, Lipton AN, Ninawe AS, Selvin J. Marine sponge microbial association: Towards disclosing unique symbiotic interactions. *Mar Environ Res*. Published online April 27, 2018. doi:10.1016/j.marenvres.2018.04.017

459. Thomas T, Moitinho-Silva L, Lurgi M, Björk JR, Easson C, Astudillo-García C, Olson JB, Erwin PM, López-Legentil S, Luter H, et al. Diversity, structure and convergent evolution of the global sponge microbiome. *Nat Commun*. 2016;7(1):1-12. doi:10.1038/ncomms11870

460. Karimi E, Ramos M, Gonçalves JMS, Xavier JR, Reis MP, Costa R. Comparative Metagenomics Reveals the Distinctive Adaptive Features of the Spongia officinalis Endosymbiotic Consortium. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.02499

461. Freeman CJ, Easson CG, Matterson KO, Thacker RW, Baker DM, Paul VJ. Microbial symbionts and ecological divergence of Caribbean sponges: A new perspective on an ancient association. *ISME J*. Published online March 20, 2020:1-13. doi:10.1038/s41396-020-0625-3

462. Faulkner DJ. Marine natural products: metabolites of marine invertebrates. *Nat Prod Rep*. 1984;1(6):551-598. doi:10.1039/NP9840100551

463. Faulkner DJ. Marine natural products. *Nat Prod Rep*. 1988;5(6):613-663. doi:10.1039/NP9880500613

464. Faulkner DJ. Marine natural products. *Nat Prod Rep*. 1992;9(4):323-364. doi:10.1039/NP9920900323

465. Gross H, König GM. Terpenoids from Marine Organisms: Unique Structures and their Pharmacological Potential. *Phytochem Rev*. 2006;5(1):115-141. doi:10.1007/s11101-005-5464-3

466. Rudman, W.B., Bergquist, P.R. A review of feeding specificity in the sponge-feeding Chromodorididae (Nudibranchia: Mollusca). *Molluscan Research*. Published 2007. https://www.mapress.com/mr/content/v27/2007f/n2p088.htm

467. Kamel HN, Kim YB, Rimoldi JM, Fronczek FR, Ferreira D, Slattery M. Scalarane Sesterterpenoids: Semisynthesis and Biological Activity. *J Nat Prod*. 2009;72(8):1492-1496. doi:10.1021/np900326a

468. A. Gonzalez M. Scalarane Sesterterpenoids. *Curr Bioact Compd*. 2010;6(3):178-206. doi:10.2174/157340710793237362

469. Dumdei EJ, Kubanek J, Coleman JE, Pika J, Andersen RJ, Steiner JR, Clardy J. New terpenoid metabolites from the skin extracts, an egg mass, and dietary sponges of the Northeastern Pacific dorid nudibranch Cadlinaluteomarginata. *Can J Chem*. 1997;75(6):773-789. doi:10.1139/v97-094

470. Avila C, Cimino G, Crispino A, Spinella A. Drimane sesquiterpenoids in MediterraneanDendrodoris nudibranchs: Anatomical distribution and biological role. *Experientia*. 1991;47(3):306-310. doi:10.1007/BF01958169

471. Matsuda SB, Gosliner TM. Molecular phylogeny of Glossodoris (Ehrenberg, 1831) nudibranchs and related genera reveals cryptic and pseudocryptic species complexes. *Cladistics*. Published online February 1, 2017:n/a-n/a. doi:10.1111/cla.12194

472. Matsuda SB, Gosliner TM. Glossing over cryptic species: Descriptions of four new species of Glossodoris and three new species of Doriprismatica (Nudibranchia: Chromodorididae). *Zootaxa*. 2018;4444(5):501-529. doi:10.11646/zootaxa.4444.5.1

473. Dilip de Silva E, J. Scheuer P. Furanoditerpenoids from the Dorid Nudibranch Casella atromarginata. *HETEROCYCLES*. 1982;17(1):167. doi:10.3987/S-1982-01-0167

474. Somerville MJ, Hooper JNA, Garson MJ. Mooloolabenes A−E, Norsesterterpenes from the Australian Sponge Hyattella intestinalis. *J Nat Prod*. 2006;69(11):1587-1590. doi:10.1021/np060244i

475. Nam SJ, Ko H, Shin M, Ham J, Chin J, Kim Y, Kim H, Shin K, Choi H, Kang H. Farnesoid X-activated receptor antagonists from a marine sponge Spongia sp. *Bioorg Med Chem Lett*. 2006;16(20):5398-5402. doi:10.1016/j.bmcl.2006.07.079

476. Wu SY, Sung PJ, Chang YL, Pan SL, Teng CM. Heteronemin, a Spongean Sesterterpene, Induces Cell Apoptosis and Autophagy in Human Renal Carcinoma Cells. *BioMed Res Int*. 2015;2015:e738241. doi:10.1155/2015/738241

477. Máximo P, Lourenço A. Marine sesterterpenes: an overview. *Curr Org Chem*. 2018;22(24):2381-2393. doi:10.2174/1385272822666181029101212

478. Kolesnikova SA, Lyakhova EG, Diep CN, Tu VA, Huong PT, Kalinovskii AI, Dmitrenok PS, Nam NH, Stonik VA. Steroidal Metabolites from the Vietnamese Nudibranch Mollusk Doriprismatica atromarginata. *Chem Nat Compd*. 2017;1(53):194-195. doi:10.1007/s10600-017-1948-9

479. Crews P, Naylor S. Sesterterpenes: An Emerging Group of Metabolites from Marine and Terrestrial Organisms. In: Fortschritte der Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products. Springer, Vienna; 1985:203-269. doi:10.1007/978-3-7091-8815-6_3

480. Crews P, Bescansa P. Sesterterpenes from a Common Marine Sponge, Hyrtios erecta. *J Nat Prod*. 1986;49(6):1041-1052. doi:10.1021/np50048a012

481. Roy MC, Tanaka J, de Voogd N, Higa T. New Scalarane Class Sesterterpenes from an Indonesian Sponge, Phyllospongia sp. *J Nat Prod*. 2002;65(12):1838-1842. doi:10.1021/np020311i

482. Jiménez JI, Yoshida WY, Scheuer PJ, Kelly M. Scalarane-Based Sesterterpenes from an Indonesian Sponge Strepsichordaia aliena. *J Nat Prod*. 2000;63(10):1388-1392. doi:10.1021/np0000771

483. Fattorusso E, Magno S, Santacroce C, Sica D. Scalarin, a new pentacyclic C-25 terpenoid from the sponge Cacospongia scalaris. *Tetrahedron*. 1972;28(24):5993-5997. doi:10.1016/0040-4020(72)88132-8

484. Baekman JC, Daloze D, Kaisin M, Moussiaux B. Ichthyotoxic sesterterpenoids from the neo guinean sponge carteriospongia foliascens. *Tetrahedron*. 1985;41(20):4603-4614. doi:10.1016/S0040-4020(01)82355-3

485. Barron PF, Quinn RJ, Tucker DJ. Structural Elucidation of a Novel Scalarane Derivative by Using High-Field (14.1T) N.M.R. Spectroscopy. *Aust J Chem*. 1991;44(7):995-999. doi:10.1071/ch9910995

486. Jaspars M, Jackson E, Lobkovsky E, Clardy J, Diaz MC, Crews P. Using Scalarane Sesterterpenes To Examine a Sponge Taxonomic Anomaly. *J Nat Prod*. 1997;60(6):556-561. doi:10.1021/np960147x

487. Cao F, Wu ZH, Shao CL, Pang S, Liang XY, Voogd NJ de, Wang CY. Cytotoxic scalarane sesterterpenoids from the South China Sea sponge Carteriospongia foliascens. *Org Biomol Chem*. 2015;13(13):4016-4024. doi:10.1039/C4OB02532F

488. Bergquist PR, Cambie RC, Kernan MR. Scalarane sesterterpenes from Collospongia auris, a new thorectid sponge. *Biochem Syst Ecol*. 1990;18(5):349-357. doi:10.1016/0305-1978(90)90008-4

489. Tsuchiya N, Sato A, Hata T, Sato N, Sasagawa K, Kobayashi T. Cytotoxic Scalarane Sesterterpenes from a Sponge, Hyrtios erecta. *J Nat Prod*. 1998;61(4):468-473. doi:10.1021/np970462z

490. Elhady SS, El-Halawany AM, Alahdal AM, Hassanean HA, Ahmed SA. A New Bioactive Metabolite Isolated from the Red Sea Marine Sponge Hyrtios erectus. *Molecules*. 2016;21(1):82. doi:10.3390/molecules21010082

491. Alahdal AM, Asfour HZ, Ahmed SA, Noor AO, Al-Abd AM, Elfaky MA, Elhady SS. Anti-Helicobacter, Antitubercular and Cytotoxic Activities of Scalaranes from the Red Sea Sponge Hyrtios erectus. *Mol Basel Switz*. 2018;23(4). doi:10.3390/molecules23040978

492. Pettit GR, Tan R, Cichacz ZA. Antineoplastic Agents. 542. Isolation and Structure of Sesterstatin 6 from the Indian Ocean Sponge Hyrtios erecta. *J Nat Prod*. 2005;68(8):1253-1255. doi:10.1021/np0402221

493. Yu ZG, Bi KS, Guo YW. Hyrtiosins A–E, Five New Scalarane Sesterterpenes from the South China Sea Sponge Hyrtios erecta. *Helv Chim Acta*. 2005;88(5):1004-1009. doi:10.1002/hlca.200590070

494. Rao ChB, Kalidindi RSHSN, Trimurtulu G, Rao DV. Metabolites of Porifera, Part III. New 24-Methylscalaranes from Phyllospongia dendyi of the Indian Ocean. *J Nat Prod*. 1991;54(2):364-371. doi:10.1021/np50074a002

495. Alvi KA, Crews P. Homoscalarane Sesterterpenes from Lendenfeldia frondosa. *J Nat Prod*. 1992;55(7):859-865. doi:10.1021/np50085a004

496. Chill L, Rudi A, Aknin M, Loya S, Hizi A, Kashman Y. New sesterterpenes from Madagascan Lendenfeldia sponges. *Tetrahedron*. 2004;60(47):10619-10626. doi:10.1016/j.tet.2004.09.022

497. Chee Chang L, Otero-Quintero S, Nicholas GM, Bewley CA. Phyllolactones A–E: new bishomoscalarane sesterterpenes from the marine sponge Phyllospongia lamellosa. *Tetrahedron*. 2001;57(27):5731-5738. doi:10.1016/S0040-4020(01)00515-4

498. Ponomarenko LP, Kalinovsky AI, Stonik VA. New Scalarane-Based Sesterterpenes from the Sponge Phyllospongia madagascarensis. *J Nat Prod*. 2004;67(9):1507-1510. doi:10.1021/np040073m

499. Li HJ, Amagata T, Tenney K, Crews P. Additional Scalarane Sesterterpenes from the Sponge Phyllospongia papyracea. *J Nat Prod*. 2007;70(5):802-807. doi:10.1021/np070020f

500. Zhang HJ, Yi YH, Yang F, Chen WS, Lin HW. Sesterterpenes and a New Sterol from the Marine Sponge Phyllospongia foliascens. *Molecules*. 2010;15(2):834-841. doi:10.3390/molecules15020834

501. Hassan MHA, Rateb ME, Hetta M, Abdelaziz TA, Sleim MA, Jaspars M, Mohammed R. Scalarane sesterterpenes from the Egyptian Red Sea sponge Phyllospongia lamellosa. *Tetrahedron*. 2015;71(4):577-583. doi:10.1016/j.tet.2014.12.035

502. Zhang H, Dong M, Wang H, Crews P. Secondary Metabolites from the Marine Sponge Genus Phyllospongia. *Mar Drugs*. 2017;15(1):12. doi:10.3390/md15010012

503. Lee YJ, Kim SH, Choi H, Lee HS, Lee JS, Shin HJ, Lee J. Cytotoxic Furan- and Pyrrole-Containing Scalarane Sesterterpenoids Isolated from the Sponge Scalarispongia sp. *Molecules*. 2019;24(5):840. doi:10.3390/molecules24050840

504. Rho JR, Lee HS, Shin HJ, Ahn JW, Kim JY, Sim CJ, Shin J. New Sesterterpenes from the Sponge Smenospongia sp. *J Nat Prod*. 2004;67(10):1748-1751. doi:10.1021/np040103l

505. Bowden BF, Coll JC, Li H, Cambie RC, Kernan MR, Bergquist PR. New Cytotoxic Scalarane Sesterterpenes from the Dictyoceratid Sponge Strepsichordaia lendenfeldi. *J Nat Prod*. 1992;55(9):1234-1240. doi:10.1021/np50087a009

506. Jahn T, König GM, Wright AD. Three New Scalarane-Based Sesterterpenes from the Tropical Marine Sponge Strepsichordaia lendenfeldi. *J Nat Prod*. 1999;62(2):375-377. doi:10.1021/np980389s

507. Kashman Y, Zviely M. New alkylated scalarins from the sponge dysidea herbacea. *Tetrahedron Lett*. 1979;20(40):3879-3882. doi:10.1016/S0040-4039(01)95551-0

508. Lai YY, Lu MC, Wang LH, Chen JJ, Fang LS, Wu YC, Sung PJ. New Scalarane Sesterterpenoids from the Formosan Sponge Ircinia felix. *Mar Drugs*. 2015;13(7):4296-4309. doi:10.3390/md13074296

509. Kimura J, Hyosu M. Two New Sesterterpenes from the Marine Sponge, Coscinoderma mathewsi. *Chem Lett*. 1999;28(1):61-62. doi:10.1246/cl.1999.61

510. Hernández-Guerrero CJ, Zubía E, Ortega MJ, Carballo JL. Sesterterpene metabolites from the sponge Hyatella intestinalis. *Tetrahedron*. 62(23):5392-5400.

511. Terem B, Scheuer PJ. Scalaradial derivatives from the nudibranch chromodoris youngbleuthi and the sponge spongia oceania. *Tetrahedron*. 1986;42(16):4409-4412. doi:10.1016/S0040-4020(01)87279-3

512. Davis R, Capon RJ. Two New Scalarane Sesterterpenes: Isoscalarafuran-A and -B, Epimeric Alcohols From a Southern Australian Marine Sponge, Spongia hispida. *Aust J Chem*. 1993;46(8):1295-1299. doi:10.1071/ch9931295

513. Tsukamoto S, Miura S, van Soest RWM, Ohta T. Three New Cytotoxic Sesterterpenes from a Marine Sponge Spongia sp. *J Nat Prod*. 2003;66(3):438-440. doi:10.1021/np020497l

514. Phan CS, Kamada T, Hamada T, Vairappan CS. Cytotoxic Sesterterpenoids from Bornean Sponge Spongia sp. *Rec Nat Prod.* 2018;12(6):643-647. doi:10.25135/rnp.69.18.01.209

515. Yang I, Lee J, Lee J, Hahn D, Chin J, Won DH, Ko J, Choi H, Hong A, Nam SJ, et al. Scalalactams A–D, Scalarane Sesterterpenes with a γ-Lactam Moiety from a Korean Spongia Sp. Marine Sponge. *Molecules*. 2018;23(12). doi:10.3390/molecules23123187

516. Jabal KA, Abdallah HM, Mohamed GA, Shehata IA, Alfaifi MY, Elbehairi SEI, Koshak AA, Ibrahim SRM. Perisomalien A, a new cytotoxic scalarane sesterterpene from the fruits of Periploca somaliensis. *Nat Prod Res*. 2019;0(0):1-6. doi:10.1080/14786419.2019.1577842

517. Erpenbeck D, Soest RWM van. Status and Perspective of Sponge Chemosystematics. *Mar Biotechnol*. 2007;9(1):2. doi:10.1007/s10126-005-6109-7

518. Chiba R, Minami A, Gomi K, Oikawa H. Identification of Ophiobolin F Synthase by a Genome Mining Approach: A Sesterterpene Synthase from Aspergillus clavatus. *Org Lett*. 2013;15(3):594-597. doi:10.1021/ol303408a

519. Ye Y, Minami A, Mandi A, Liu C, Taniguchi T, Kuzuyama T, Monde K, Gomi K, Oikawa H. Genome Mining for Sesterterpenes Using Bifunctional Terpene Synthases Reveals a Unified Intermediate of Di/Sesterterpenes. *J Am Chem Soc*. 2015;137(36):11846-11853. doi:10.1021/jacs.5b08319

520. Matsuda Y, Mitsuhashi T, Quan Z, Abe I. Molecular Basis for Stellatic Acid Biosynthesis: A Genome Mining Approach for Discovery of Sesterterpene Synthases. *Org Lett*. 2015;17(18):4644-4647. doi:10.1021/acs.orglett.5b02404

521. Chai H, Yin R, Liu Y, Meng H, Zhou X, Zhou G, Bi X, Yang X, Zhu T, Zhu W, et al. Sesterterpene ophiobolin biosynthesis involving multiple gene clusters in Aspergillus ustus. *Sci Rep*. 2016;6(1):1-11. doi:10.1038/srep27181

522. Mitsuhashi T, Rinkel J, Okada M, Abe I, Dickschat JS. Mechanistic Characterization of Two Chimeric Sesterterpene Synthases from Penicillium. *Chem – Eur J*. 2017;23(42):10053-10057. doi:10.1002/chem.201702766

523. Garson MJ. Biosynthesis of the novel diterpene isonitrile diisocyanoadociane by a marine sponge of the amphimedon genus: incorporation studies with sodium [14C]cyanide and sodium [2-14C]acetate. *J Chem Soc Chem Commun*. 1986;(1):35-36. doi:10.1039/C39860000035

524. Garson MJ, Partali V, Liaaen-Jensen S, Stoilov IL. Isoprenoid biosynthesis in a marine sponge of the Amphimedon genus: Incorporation studies with [1-14C] acetate, [4-14C] cholesterol and [2-14C] mevalonate. *Comp Biochem Physiol Part B Comp Biochem*. 1988;91(2):293-300. doi:10.1016/0305-0491(88)90145-9

525. Kerr RG, Stoilov IL, Thompson JE, Djerassi C. Biosynthetic studies of marine lipids 16. De novo sterol biosynthesis in sponges. Incorporation and transformation of cycloartenol and lanosterol into unconventional sterols of marine and freshwater sponges. *Tetrahedron*. 1989;45(7):1893-1904. doi:10.1016/S0040-4020(01)80054-5

526. Rohmer M. The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants†. *Nat Prod Rep*. 1999;16(5):565-574. doi:10.1039/A709175C

527. Genta-Jouve G, Thomas OP. Chapter four - Sponge Chemical Diversity: From Biosynthetic Pathways to Ecological Roles. In: Becerro MA, Uriz MJ, Maldonado M, Turon X, eds. *Advances in Marine Biology*. Vol 62. Advances in Sponge Science: Physiology, Chemical and Microbial Diversity, Biotechnology. Academic Press; 2012:183-230. doi:10.1016/B978-0-12-394283-8.00004-7

528. Silva CJ, Wünsche L, Djerassi C. Biosynthetic studies of marine lipid 35. The demonstration of de novo sterol biosynthesis in sponges using radiolabeled isoprenoid precursors. *Comp Biochem Physiol Part B Comp Biochem*. 1991;99(4):763-773. doi:10.1016/0305-0491(91)90140-9

529. Christianson DW. Structural and Chemical Biology of Terpenoid Cyclases. *Chem Rev*. 2017;117(17):11570-11648. doi:10.1021/acs.chemrev.7b00287

530. Mehbub MF, Lei J, Franco C, Zhang W. Marine Sponge Derived Natural Products between 2001 and 2010: Trends and Opportunities for Discovery of Bioactives. *Mar Drugs*. 2014;12(8):4539-4577. doi:10.3390/md12084539

531. Schmitt S, Tsai P, Bell J, Fromont J, Ilan M, Lindquist N, Perez T, Rodrigo A, Schupp PJ, Vacelet J, et al. Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *ISME J*. 2012;6(3):564-576. doi:10.1038/ismej.2011.116

532. Thomas TRA, Kavlekar DP, LokaBharathi PA. Marine Drugs from Sponge-Microbe Association—A Review. *Mar Drugs*. 2010;8(4):1417-1468. doi:10.3390/md8041417

533. Fan L, Reynolds D, Liu M, Stark M, Kjelleberg S, Webster NS, Thomas T. Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. *Proc Natl Acad Sci*. 2012;109(27):E1878-E1887. doi:10.1073/pnas.1203287109

534. Yamada Y, Kuzuyama T, Komatsu M, Shin-ya K, Omura S, Cane DE, Ikeda H. Terpene synthases are widely distributed in bacteria. *Proc Natl Acad Sci*. 2015;112(3):857-862. doi:10.1073/pnas.1422108112

535. Wiese J, Ohlendorf B, Blümel M, Schmaljohann R, Imhoff JF. Phylogenetic Identification of Fungi Isolated from the Marine Sponge Tethya aurantium and Identification of Their Secondary Metabolites. *Mar Drugs*. 2011;9(4):561-585. doi:10.3390/md9040561

536. E. Rateb M, Ebel R. Secondary metabolites of fungi from marine habitats. *Nat Prod Rep*. 2011;28(2):290-344. doi:10.1039/C0NP00061B

537. Padula V, Bahia J, Stöger I, Camacho-García Y, Malaquias MAE, Cervera JL, Schrödl M. A test of color-based taxonomy in nudibranchs: Molecular phylogeny and species delimitation of the Felimida clenchi (Mollusca: Chromodorididae) species complex. *Mol Phylogenet Evol*. 2016;103:215-229. doi:10.1016/j.ympev.2016.07.019

538. Nakano R, Hirose E. Field Experiments on the Feeding of the Nudibranch Gymnodoris spp. (Nudibranchia: Doridina: Gymnodorididae) in Japan. *Veliger*. 2011;51(2):66-75.

539. Uddin MH, Otsuka M, Muroi T, Ono A, Hanif N, Matsuda S, Higa T, Tanaka J. Deoxymanoalides from the Nudibranch *Chromodoris willani*. *Chem Pharm Bull (Tokyo)*. 2009;57(8):885-887. doi:10.1248/cpb.57.885

540. Miyamoto T, Sakamoto K, Amano H, Arakawa Y, Nagarekawa Y, Komori T, Higuchi R, Sasaki T. New cytotoxic sesterterpenoids from the nudibranch Chromodoris inornata. *Tetrahedron*. 1999;55(30):9133-9142. doi:10.1016/S0040-4020(99)00477-9

541. Corley DG, Herb R, Moore RE, Scheuer PJ, Paul VJ. Laulimalides. New potent cytotoxic macrolides from a marine sponge and a nudibranch predator. *J Org Chem*. 1988;53(15):3644-3646. doi:10.1021/jo00250a053

542. Crews Phillip, Kakou Yao, Quinoa Emilio. Mycothiazole, a polyketide heterocycle from a marine sponge. *J Am Chem Soc*. 1988;110(13):4365-4368. doi:10.1021/ja00221a042

543. de Silva ED, Morris SA, Miao S, Dumdei E, Andersen RJ. Terpenoid Metabolites from Skin Extracts of Four Sri Lankan Nudibranchs in the Genus Chromodoris. *J Nat Prod*. 1991;54(4):993-997. doi:10.1021/np50076a011

544. Pika J, John Faulkner D. Unusual chlorinated homo-diterpenes from the South African nudibranch Chromodoris hamiltoni. *Tetrahedron*. 1995;51(30):8189-8198. doi:10.1016/0040-4020(95)00440-J

545. McPhail K, Davies-Coleman MT. New spongiane diterpenes from the East African nudibranch Chromodoris hamiltoni. *Tetrahedron*. 1997;53(13):4655-4660. doi:10.1016/S0040-4020(97)00198-1

546. Guo Y. Chemical studies of the novel bioactive secondary metabolites from the benthic invertebrates: isolation and structure characterization. Published online 1997.

547. Mebs D. Chemical defense of a dorid nudibranch,Glossodoris quadricolor, from the red sea. *J Chem Ecol*. 1985;11(6):713-716. doi:10.1007/BF00988300

548. Ilan M. Reproductive Biology, Taxonomy, and Aspects of Chemical Ecology of Latrunculiidae (Porifera). *Biol Bull*. 1995;188(3):306-312. doi:10.2307/1542307

549. Nèeman I, Fishelson L, Kashman Y. Isolation of a new toxin from the sponge Latrunculia magnifica in the Gulf of Aquaba (Red Sea). *Mar Biol*. 1975;30(4):293-296. doi:10.1007/BF00390634

550. Kashman Y, Groweiss A, Shmueli U. Latrunculin, a new 2-thiazolidinone macrolide from the marine sponge latrunculia magnifica. *Tetrahedron Lett*. 1980;21(37):3629-3632. doi:10.1016/0040-4039(80)80255-3

551. Gillor O, Carmeli S, Rahamim Y, Fishelson Z, Ilan M. Immunolocalization of the Toxin Latrunculin B within the Red Sea Sponge Negombata magnifica (Demospongiae, Latrunculiidae). *Mar Biotechnol*. 2000;2(3):213-223. doi:10.1007/s101260000026

552. Amagata T, Johnson TA, Cichewicz RH, Tenney K, Mooberry SL, Media J, Edelstein M, Valeriote FA, Crews P. Interrogating the Bioactive Pharmacophore of the Latrunculin Chemotype by Investigating the Metabolites of Two Taxonomically Unrelated Sponges. *J Med Chem*. 2008;51(22):7234-7242. doi:10.1021/jm8008585

553. Al-Tarabeen M, El-Neketi M, Albohy A, Müller WEG, Rasheed M, Ebrahim W, Proksch P, Ebada SS. Isolation and Molecular Docking of Cytotoxic Secondary Metabolites from Two Red Sea Sponges of the Genus Diacarnus. *ChemistrySelect*. 2021;6(2):217-220. doi:https://doi.org/10.1002/slct.202003114

554. Sonnenschein RN, Johnson TA, Tenney K, Valeriote FA, Crews P. A Reassignment of (–)-Mycothiazole and the Isolation of a Related Diol. *J Nat Prod*. 2006;69(1):145-147. doi:10.1021/np0503597

555. Fenical William. Chemical studies of marine bacteria: developing a new resource. *Chem Rev*. 1993;93(5):1673-1683. doi:10.1021/cr00021a001

556. Kobayashi Junichi, Ishibashi Masami. Bioactive metabolites of symbiotic marine microorganisms. *Chem Rev*. 1993;93(5):1753-1769. doi:10.1021/cr00021a005

557. Bewley CA, Holland ND, Faulkner DJ. Two classes of metabolites fromTheonella swinhoei are localized in distinct populations of bacterial symbionts. *Experientia*. 1996;52(7):716-722. doi:10.1007/BF01925581

558. Piel J. Metabolites from symbiotic bacteria. *Nat Prod Rep*. 2009;26(3):338-362. doi:10.1039/B703499G

559. Ueoka R, Uria AR, Reiter S, Mori T, Karbaum P, Peters EE, Helfrich EJN, Morinaka BI, Gugger M, Takeyama H, et al. Metabolic and evolutionary origin of actin-binding polyketides from diverse organisms. *Nat Chem Biol*. 2015;11(9):705-712. doi:10.1038/nchembio.1870

560. Wakimoto T, Egami Y, Abe I. Calyculin: Nature's way of making the sponge-derived cytotoxin. *Nat Prod Rep*. 2016;33(6):751-760. doi:10.1039/C5NP00123D

561. Silva SCGP da. Multi-approach analysis of the metagenome of a marine sponge containing latrunculin A. Published online November 30, 2017. Accessed May 3, 2021. https://repositorio.ul.pt/handle/10451/36088

562. McCauley EP, Piña IC, Thompson AD, Bashir K, Weinberg M, Kurz SL, Crews P. Highlights of marine natural products having parallel scaffolds found from marine-derived bacteria, sponges, and tunicates. *J Antibiot (Tokyo)*. 2020;73(8):504-525. doi:10.1038/s41429-020-0330-5

563. Spector I, Shochet NR, Kashman Y, Groweiss A. Latrunculins: novel marine toxins that disrupt microfilament organization in cultured cells. *Science*. 1983;219(4584):493-495. doi:10.1126/science.6681676

564. Kashman Y, Groweiss A, Lidor R, Blasberger D, Carmely S. Latrunculins: NMR study, two new toxins and a synthetic approach. *Tetrahedron*. 1985;41(10):1905-1914. doi:10.1016/S0040-4020(01)96553-6

565. de Oliveira CA, Mantovani B. Latrunculin A is a potent inhibitor of phagocytosis by macrophages. *Life Sci*. 1988;43(22):1825-1830. doi:10.1016/0024-3205(88)90282-2

566. Spector I, Shochet NR, Blasberger D, Kashman Y. Latrunculins—novel marine macrolides that disrupt microfilament organization and affect cell growth: I. Comparison with cytochalasin D. *Cell Motil*. 1989;13(3):127-144. doi:10.1002/cm.970130302

567. Gulavita NK, Gunasekera SP, Pomponi SA. Isolation of Latrunculin A, 6,7-Epoxylatrunculin A, Fijianolide A, and Euryfuran from a New Genus of the Family Thorectidae. *J Nat Prod*. 1992;55(4):506-508. doi:10.1021/np50082a019

568. Smith AB, Leahy JW, Noda I, Remiszewski SW, Liverton NJ, Zibuck R. Total synthesis of the latrunculins. *J Am Chem Soc*. 1992;114(8):2995-3007. doi:10.1021/ja00034a036

569. White JD, Kawasaki M. Total synthesis of (+)-latrunculin A, an ichthyotoxic metabolite of the sponge Latrunculia magnifica and its C-15 epimer. *J Org Chem*. 1992;57(20):5292-5300. doi:10.1021/jo00046a008

570. Braet F, De Zanger R, Jans D, Spector I, Wisse E. Microfilament-disrupting agent latrunculin A induces and increased number of fenestrae in rat liver sinusoidal endothelial cells: Comparison with cytochalasin B. *Hepatology*. 1996;24(3):627-635. doi:10.1053/jhep.1996.v24.pm0008781335

571. Tanaka J ichi, Higa T, Bernardinelli G, Jefford CW. New Cytotoxic Macrolides from the Sponge Fasciospongia rimosa. *Chem Lett*. 1996;25(4):255-256. doi:10.1246/cl.1996.255

572. Oliveira CA, Kashman Y, Mantovani B. Effects of latrunculin A on immunological phagocytosis and macrophage spreading-associated changes in the F-actin/G-actin content of the cells. *Chem Biol Interact*. 1996;100(2):141-153. doi:10.1016/0009-2797(96)03695-2

573. Ayscough KR, Stryker J, Pokala N, Sanders M, Crews P, Drubin DG. High Rates of Actin Filament Turnover in Budding Yeast and Roles for Actin in Establishment and Maintenance of Cell Polarity Revealed Using the Actin Inhibitor Latrunculin-A. *J Cell Biol*. 1997;137(2):399-416. doi:10.1083/jcb.137.2.399

574. Belmont LD, Patterson GM, Drubin DG. New actin mutants allow further characterization of the nucleotide binding cleft and drug binding sites. *J Cell Sci*. 1999;112 (Pt 9):1325-1336.

575. Cai S, Liu X, Glasser A, Volberg T, Filla M, Geiger B, Polansky JR, Kaufman PL. Effect of latrunculin-A on morphology and actin-associated adhesions of cultured human trabecular meshwork cells. *Mol Vis*. 2000;6:132-143.

576. Morton WM, Ayscough KR, McLaughlin PJ. Latrunculin alters the actin-monomer subunit interface to prevent polymerization. *Nat Cell Biol*. 2000;2(6):376-378. doi:10.1038/35014075

577. Yarmola EG, Somasundaram T, Boring TA, Spector I, Bubb MR. Actin-latrunculin A structure and function. Differential modulation of actin-binding protein function by latrunculin A. *J Biol Chem*. 2000;275(36):28120-28127. doi:10.1074/jbc.M004253200

578. Baluška F, Jasik J, Edelmann HG, Salajová T, Volkmann D. Latrunculin B-Induced Plant Dwarfism: Plant Cell Elongation Is F-Actin-Dependent. *Dev Biol*. 2001;231(1):113-124. doi:10.1006/dbio.2000.0115

579. Wakatsuki T, Schwab B, Thompson NC, Elson EL. Effects of cytochalasin D and latrunculin B on mechanical properties of cells. *J Cell Sci*. 2001;114(5):1025-1036.

580. Pring M, Cassimeris L, Zigmond SH. An unexplained sequestration of latrunculin A is required in neutrophils for inhibition of actin polymerization. *Cell Motil Cytoskeleton*. 2002;52(2):122-130. doi:10.1002/cm.10039

581. Fujita M, Ichinose S, Kiyono T, Tsurumi T, Omori A. Establishment of latrunculin-A resistance in HeLa cells by expression of R183A D184A mutant beta-actin. *Oncogene*. 2003;22(4):627-631. doi:10.1038/sj.onc.1206173

582. Vilozny B, Amagata T, Mooberry SL, Crews P. A New Dimension to the Biosynthetic Products Isolated from the Sponge Negombata magnifica. *J Nat Prod*. 2004;67(6):1055-1057. doi:10.1021/np0340753

583. Ethier CR, Read AT, Chan DWH. Effects of latrunculin-B on outflow facility and trabecular meshwork structure in human eyes. *Invest Ophthalmol Vis Sci*. 2006;47(5):1991-1998. doi:10.1167/iovs.05-0327

584. Houssen WE, Jaspars M, Wease KN, Scott RH. Acute actions of marine toxin latrunculin A on the electrophysiological properties of cultured dorsal root ganglion neurones. *Comp Biochem Physiol Toxicol Pharmacol CBP*. 2006;142(1-2):19-29. doi:10.1016/j.cbpc.2005.09.006

585. Khalifa S, Ahmed S, Mesbah M, Youssef D, Hamann M. Quantitative determination of latrunculins A and B in the Red Sea sponge Negombata magnifica by high performance liquid chromatography. *J Chromatogr B*. 2006;832(1):47-51. doi:10.1016/j.jchromb.2005.12.028

586. El Sayed KA, Youssef DTA, Marchetti D. Bioactive Natural and Semisynthetic Latrunculins. *J Nat Prod*. 2006;69(2):219-223. doi:10.1021/np050372r

587. Sierra-Paredes G, Oreiro-García T, Núñez-Rodriguez A, Vázquez-López A, Sierra-Marcuño G. Seizures induced by in vivo latrunculin A and jasplakinolide microperfusion in the rat hippocampus. *J Mol Neurosci*. 2006;28(2):151-160. doi:10.1385/JMN:28:2:151

588. Fürstner A, De Souza D, Turet L, Fenster MDB, Parra-Rapado L, Wirtz C, Mynott R, Lehmann CW. Total syntheses of the actin-binding macrolides latrunculin A, B, C, M, S and 16-epi-latrunculin B. *Chem Weinh Bergstr Ger*. 2007;13(1):115-134. doi:10.1002/chem.200601135

589. Fürstner A, Kirk D, Fenster MDB, Aïssa C, De Souza D, Nevado C, Tuttle T, Thiel W, Müller O. Latrunculin Analogues with Improved Biological Profiles by "Diverted Total Synthesis": Preparation, Evaluation, and Computational Analysis. *Chem – Eur J*. 2007;13(1):135-149. doi:10.1002/chem.200601136

590. Meadows JC, Millar J, Solomon M. Latrunculin A Delays Anaphase Onset in Fission Yeast by Disrupting an Ase1-independent Pathway Controlling Mitotic Spindle Stability. *Mol Biol Cell*. 2008;19(9):3713-3723. doi:10.1091/mbc.e08-02-0164

591. Konishi H, Kikuchi S, Ochiai T, Ikoma H, Kubota T, Ichikawa D, Fujiwara H, Okamoto K, Sakakura C, Sonoyama T, et al. Latrunculin A Has a Strong Anticancer Effect in a Peritoneal Dissemination Model of Human Gastric Cancer in Mice. *Anticancer Res*. 2009;29(6):2091-2097.

592. Kudrimoti S, Ahmed SA, Daga PR, Wahba AE, Khalifa SI, Doerksen RJ, Hamann MT. Semisynthetic Latrunculin B Analogs: Studies of Actin Docking Support a Proposed Mechanism for Latrunculin Bioactivity. *Bioorg Med Chem*. 2009;17(21):7517-7522. doi:10.1016/j.bmc.2009.09.012

593. Ketelaar T, Meijer HJG, Spiekerman M, Weide R, Govers F. Effects of latrunculin B on the actin cytoskeleton and hyphal growth in Phytophthora infestans. *Fungal Genet Biol*. 2012;49(12):1014-1022. doi:10.1016/j.fgb.2012.09.008

594. Moscatelli A, Idilli AI, Rodighiero S, Caccianiga M. Inhibition of actin polymerisation by low concentration Latrunculin B affects endocytosis and alters exocytosis in shank and tip of tobacco pollen tubes. *Plant Biol*. 2012;14(5):770-782. doi:10.1111/j.1438-8677.2011.00547.x

595. Terashita Y, Yamagata K, Tokoro M, Itoi F, Wakayama S, Li C, Sato E, Tanemura K, Wakayama T. Latrunculin A Treatment Prevents Abnormal Chromosome Segregation for Successful Development of Cloned Embryos. *PLOS ONE*. 2013;8(10):e78380. doi:10.1371/journal.pone.0078380

596. Mallol A, Santaló J, Ibáñez E. Improved Development of Somatic Cell Cloned Mouse Embryos by Vitamin C and Latrunculin A. *PLOS ONE*. 2015;10(3):e0120033. doi:10.1371/journal.pone.0120033

597. Kopecká M, Yamaguchi M, Kawamoto S. Effects of the F-actin inhibitor latrunculin A on the budding yeast Saccharomyces cerevisiae. *Microbiol Read Engl*. 2015;161(7):1348-1355. doi:10.1099/mic.0.000091

598. Ebrahim HY, El Sayed KA. Discovery of Novel Antiangiogenic Marine Natural Product Scaffolds. *Mar Drugs*. 2016;14(3):57. doi:10.3390/md14030057

599. Varghese S, Rahmani R, Drew D, Williams M, Huang J, Wilkinson M, Tan YH, Tonkin C, Beeson J, Baum J, et al. Truncated Latrunculins as Actin Inhibitors Targeting Plasmodium falciparum Motility and Host-Cell Invasion. *J Med Chem*. 2016;59. doi:10.1021/acs.jmedchem.6b01109

600. Fujiwara I, Zweifel ME, Courtemanche N, Pollard TD. Latrunculin A accelerates actin filament depolymerization in addition to sequestering actin monomers. *Curr Biol CB*. 2018;28(19):3183-3192.e2. doi:10.1016/j.cub.2018.07.082

601. Würtemberger J, Tchessalova D, Regina C, Bauer C, Schneider M, Wagers AJ, Hettmer S. Growth inhibition associated with disruption of the actin cytoskeleton by Latrunculin A in rhabdomyosarcoma cells. *PLOS ONE*. 2020;15(9):e0238572. doi:10.1371/journal.pone.0238572

602. Varghese S, Rahmani R, Drew DR, Beeson JG, Baum J, Smith BJ, Baell JB. Structure-Activity Studies of Truncated Latrunculin Analogues with Antimalarial Activity. *ChemMedChem*. 2021;16(4):679-693. doi:https://doi.org/10.1002/cmdc.202000399

603. Lenz KD, Klosterman KE, Mukundan H, Kubicek-Sutherland JZ. Macrolides: From Toxins to Therapeutics. *Toxins*. 2021;13(5):347. doi:10.3390/toxins13050347

604. Foissner I, Wasteneys GO. Wide-Ranging Effects of Eight Cytochalasins and Latrunculin A and B on Intracellular Motility and Actin Filament Reorganization in Characean Internodal Cells. *Plant Cell Physiol*. 2007;48(4):585-597. doi:10.1093/pcp/pcm030

605. Risinger AL, Du L. Targeting and extending the eukaryotic druggable genome with natural products: cytoskeletal targets of natural products. *Nat Prod Rep*. Published online November 25, 2019. doi:10.1039/C9NP00053D

606. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. The Actin Cytoskeleton. *Mol Cell Biol 4th Ed*. Published online 2000. Accessed June 14, 2021. https://www.ncbi.nlm.nih.gov/books/NBK21493/

607. Perrin BJ, Ervasti JM. The Actin Gene Family: Function Follows Isoform. *Cytoskelet Hoboken Nj*. 2010;67(10):630-634. doi:10.1002/cm.20475

608. Dominguez R, Holmes KC. Actin Structure and Function. *Annu Rev Biophys*. 2011;40(1):169-186. doi:10.1146/annurev-biophys-042910-155359

609. Pollard TD. Actin and Actin-Binding Proteins. *Cold Spring Harb Perspect Biol*. 2016;8(8). doi:10.1101/cshperspect.a018226

610. Pollard TD. What We Know and Do Not Know About Actin. *Handb Exp Pharmacol*. 2017;235:331-347. doi:10.1007/164_2016_44

611. Stoddard PR, Williams TA, Garner E, Baum B. Evolution of polymer formation within the actin superfamily. *Mol Biol Cell*. 2017;28(19):2461-2469. doi:10.1091/mbc.e15-11-0778

612. Vedula P, Kashina A. The makings of the 'actin code': regulation of actin's biological function at the amino acid and nucleotide level. *J Cell Sci*. 2018;131(9). doi:10.1242/jcs.215509

613. Oosawa F, Asakura S, Hotta K, Imai N, Ooi T. G-F transformation of actin as a fibrous condensation. *J Polym Sci*. 1959;37(132):323-336. doi:10.1002/pol.1959.1203713202

614. Kabsch W, Mannherz HG, Suck D, Pai EF, Holmes KC. Atomic structure of the actin: DNase I complex. *Nature*. 1990;347(6288):37-44. doi:10.1038/347037a0

615. Kabsch W, Vandekerckhove J. Structure and Function of Actin. *Annu Rev Biophys Biomol Struct*. 1992;21(1):49-76. doi:10.1146/annurev.bb.21.060192.000405

616. Selden LA, Estes JE, Gershman LC. The tightly bound divalent cation regulates actin polymerization. *Biochem Biophys Res Commun*. 1983;116(2):478-485. doi:10.1016/0006-291x(83)90548-x

617. Gershman LC, Selden LA, Kinosian HJ, Estes JE. Actin-Bound Nucleotide/Divalent Cation Interactions. In: Estes JE, Higgins PJ, eds. *Actin: Biophysics, Biochemistry, and Cell Biology*. Advances in Experimental Medicine and Biology. Springer US; 1994:35-49. doi:10.1007/978-1-4615-2578-3_4

618. Zigmond SH. Beginning and ending an actin filament: control at the barbed end. *Curr Top Dev Biol*. 2004;63:145-188. doi:10.1016/S0070-2153(04)63005-5

619. Winder SJ, Ayscough KR. Actin-binding proteins. *J Cell Sci*. 2005;118(4):651-654. doi:10.1242/jcs.01670

620. Holmes KC. Actin in a twist. *Nature*. 2009;457(7228):389-390. doi:10.1038/457389a

621. Lee SH, Dominguez R. Regulation of actin cytoskeleton dynamics in cells. *Mol Cells*. 2010;29(4):311-325. doi:10.1007/s10059-010-0053-8

622. Gunning PW, Ghoshdastider U, Whitaker S, Popp D, Robinson RC. The evolution of compositionally and functionally distinct actin filaments. *J Cell Sci*. 2015;128(11):2009-2019. doi:10.1242/jcs.165563

623. Chou SZ, Pollard TD. Mechanism of actin polymerization revealed by cryo-EM structures of actin filaments with three different bound nucleotides. *Proc Natl Acad Sci*. 2019;116(10):4265-4274. doi:10.1073/pnas.1807028115

624. Chhabra ES, Higgs HN. The many faces of actin: matching assembly factors with cellular structures. *Nat Cell Biol*. 2007;9(10):1110-1121. doi:10.1038/ncb1007-1110

625. Erickson HP. Evolution of the cytoskeleton. *BioEssays*. 2007;29(7):668-677. doi:10.1002/bies.20601

626. Vandekerckhove J, Weber K. Chordate muscle actins differ distinctly from invertebrate muscle actins: The evolution of the different vertebrate muscle actins. *J Mol Biol*. 1984;179(3):391-413. doi:10.1016/0022-2836(84)90072-X

627. Cupples CG, Pearlman RE. Isolation and characterization of the actin gene from Tetrahymena thermophila. *Proc Natl Acad Sci U S A*. 1986;83(14):5160-5164. doi:10.1073/pnas.83.14.5160

628. Hightower RC, Meagher RB. The Molecular Evolution of Actin. *Genetics*. 1986;114(1):315-332.

629. Rubenstein PA. The functional importance of multiple actin isoforms. *BioEssays*. 1990;12(7):309-315. doi:10.1002/bies.950120702

630. Herman IM. Actin isoforms. *Curr Opin Cell Biol*. 1993;5(1):48-55. doi:10.1016/S0955-0674(05)80007-9

631. Reece KS, Siddall ME, Burreson EM, Graves JE. Phylogenetic Analysis of Perkinsus Based on Actin Gene Sequences. *J Parasitol*. 1997;83(3):417-423. doi:10.2307/3284403

632. Wahlberg MH, Johnson MS. Isolation and Characterization of Five Actin cDNAs from the Cestode Diphyllobothrium dendriticum: A Phylogenetic Study of the Multigene Family. *J Mol Evol*. 1997;44(2):159-168. doi:10.1007/PL00006132

633. Vedula P, Kurosaka S, Leu NA, Wolf YI, Shabalina SA, Wang J, Sterling S, Dong DW, Kashina A. Diverse functions of homologous actin isoforms are defined by their nucleotide, rather than their amino acid sequence. *eLife*. 2017;6. doi:10.7554/eLife.31661

634. Skruber K, Read TA, Vitriol EA. Reconsidering an active role for G-actin in cytoskeletal regulation. *J Cell Sci*. 2018;131(jcs203760). doi:10.1242/jcs.203760

635. Boiero Sanders M, Antkowiak A, Michelot A. Diversity from similarity: cellular strategies for assigning particular identities to actin filaments and networks. *Open Biol*. 10(9):200157. doi:10.1098/rsob.200157

636. Vedula P, Kurosaka S, MacTaggart B, Ni Q, Papoian G, Jiang Y, Dong DW, Kashina A. Different translation dynamics of β- and γ-actin regulates cell migration. Singer RH, Akhmanova A, Mullins RD, Gunning PW, eds. *eLife*. 2021;10:e68712. doi:10.7554/eLife.68712

637. Terman JR, Kashina A. Post-translational modification and regulation of actin. *Curr Opin Cell Biol*. 2013;25(1):30-38. doi:10.1016/j.ceb.2012.10.009

638. Varland S, Vandekerckhove J, Drazic A. Actin Post-translational Modifications: The Cinderella of Cytoskeletal Control. *Trends Biochem Sci*. 2019;44(6):502-516. doi:10.1016/j.tibs.2018.11.010

639. Adema CM. COMPARATIVE STUDY OF CYTOPLASMIC ACTIN DNA SEQUENCES FROM SIX SPECIES OF PLANORBIDAE (GASTROPODA: BASOMMATOPHORA). *J Molluscan Stud*. 2002;68(1):17-23. doi:10.1093/mollus/68.1.17

640. Adema CM, Hillier LW, Jones CS, Loker ES, Knight M, Minx P, Oliveira G, Raghavan N, Shedlock A, do Amaral LR, et al. Whole genome analysis of a schistosomiasis-transmitting freshwater snail. *Nat Commun*. 2017;8(1):15451. doi:10.1038/ncomms15451

641. Ma H, Mai K, Liufu Z, Xu W. Cloning and characterization of an actin gene of Chlamys farreri and the phylogenetic analysis of mollusk actins. *Chin J Oceanol Limnol*. 2007;25(3):304-309. doi:10.1007/s00343-007-0304-5

642. Patwary MU, Reith M, Kenchington E. Isolation and characterization of a cDNA encoding an actin gene from sea scallop (Placopecten magellanicus). *J Shellfish Res*. 1996;15:265-270.

643. Artigaud S, Lavaud R, Thébault J, Jean F, Strand Ø, Strohmeier T, Milan M, Pichereau V. Proteomic-based comparison between populations of the Great Scallop, Pecten maximus. *J Proteomics*. 2014;105:164-173. doi:10.1016/j.jprot.2014.03.026

644. Zhang G, Wang X, Song X, Li L. An Improved Method of DNA Extraction from the Shell of the Pacific Oyster, Crassostrea gigas. *Israeli Journal of Aquaculture*. 2012;64:20608. doi:10.46989/001c.20608

645. Carlini DB, Reece KS, Graves JE. Actin Gene Family Evolution and the Phylogeny of Coleoid Cephalopods (Mollusca: Cephalopoda). *Mol Biol Evol*. 2000;17(9):1353-1370. doi:10.1093/oxfordjournals.molbev.a026419

646. Bryant MJ, Flint HJ, Sin FYT. Isolation, characterization, and expression analysis of three actin genes in the New Zealand black-footed abalone, Haliotis iris. *Mar Biotechnol N Y N*. 2006;8(2):110-119. doi:10.1007/s10126-005-5139-5

647. Sin FYT, Bryant MJ, Johnstone A. Molecular Evolution and Phylogeny of Actin Genes in Haliotis Species (Mollusca: Gastropoda). *Zool Stud*. Published online 2007:12.

648. Ivanov M, Todorovska E, Radkova M, Georgiev O, Dolashki A, Dolashka P. Molecular cloning, characterization and phylogenetic analysis of an actin gene from the marine mollusk Rapana venosa (class Gastropoda). Published online 2015:14.

649. DesGroseillers L, Auclair D, Wickham L. Nucleotide sequence of an actin cDNA gene from Aplysia californica. *Nucleic Acids Res*. 1990;18(12):3654.

650. DesGroseillers L, Auclair D, Wickham L, Maalouf M. A novel actin cDNA is expressed in the neurons of Aplysia californica. *Biochim Biophys Acta*. 1994;1217(3):322-324. doi:10.1016/0167-4781(94)90293-3

651. Zappulla JP, Angers A, Barbas D, Castellucci VF, DesGroseillers L. A novel actin isoform is expressed in the ovotestis of Aplysia californica. *Comp Biochem Physiol B Biochem Mol Biol*. 2005;140(3):403-409. doi:10.1016/j.cbpc.2004.11.005

652. Mounier N, Gouy M, Mouchiroud D, Prudhomme JC. Insect muscle actins differ distinctly from invertebrate and vertebrate cytoplasmic actins. *J Mol Evol*. 1992;34(5):406-415. doi:10.1007/BF00162997

653. Doolittle RF, Gerhart J, Hunt RT, Kirschner MW, Wolpert L. The origins and evolution of eukaryotic proteins. *Philos Trans R Soc Lond B Biol Sci*. 1995;349(1329):235-240. doi:10.1098/rstb.1995.0107

654. Ent F van den, Amos LA, Löwe J. Prokaryotic origin of the actin cytoskeleton. *Nature*. 2001;413(6851):39-44. doi:10.1038/35092500

655. Ayscough KR, Winder SJ. Two billion years of actin. *EMBO Rep*. 2004;5(10):947-952. doi:10.1038/sj.embor.7400252

656. Koonin EV. The origin and early evolution of eukaryotes in the light of phylogenomics. *Genome Biol*. 2010;11(5):209. doi:10.1186/gb-2010-11-5-209

657. Eme L, Spang A, Lombard J, Stairs CW, Ettema TJG. Archaea and the origin of eukaryotes. *Nat Rev Microbiol*. 2017;15(12):711-723. doi:10.1038/nrmicro.2017.133

658. Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU, et al. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature*. 2017;541(7637):353-358. doi:10.1038/nature21031

659. Akıl C, Robinson RC. Genomes of Asgard archaea encode profilins that regulate actin. *Nature*. 2018;562(7727):439-443. doi:10.1038/s41586-018-0548-6

660. Akıl C, Tran LT, Orhant-Prioux M, Baskaran Y, Manser E, Blanchoin L, Robinson RC. Insights into the evolution of regulated actin dynamics via characterization of primitive gelsolin/cofilin proteins from Asgard archaea. *Proc Natl Acad Sci*. 2020;117(33):19904-19913. doi:10.1073/pnas.2009167117

661. Akıl C, Kitaoku Y, Tran LT, Liebl D, Choe H, Muengsaen D, Suginta W, Schulte A, Robinson RC. Mythical origins of the actin cytoskeleton. *Curr Opin Cell Biol*. 2021;68:55-63. doi:10.1016/j.ceb.2020.08.011

662. Erickson HP. Evolution in bacteria. *Nature*. 2001;413(6851):30-30. doi:10.1038/35092655

663. Hartman H, Fedorov A. The origin of the eukaryotic cell: A genomic investigation. *Proc Natl Acad Sci U S A*. 2002;99(3):1420-1425. doi:10.1073/pnas.032658599

664. Skau CT, Waterman CM. Specification of Architecture and Function of Actin Structures by Actin Nucleation Factors. *Annu Rev Biophys*. 2015;44(1):285-310. doi:10.1146/annurev-biophys-060414-034308

665. Parisis N, Krasinska L, Harker B, Urbach S, Rossignol M, Camasses A, Dewar J, Morin N, Fisher D. Initiation of DNA replication requires actin dynamics and formin activity. *EMBO J*. 2017;36(21):3212-3231. doi:10.15252/embj.201796585

666. Wada S ichi, Matsunaga S, Saito S ya, Fusetani N, Watabe S. Actin-Binding Specificity of Marine Macrolide Toxins, Mycalolide B and Kabiramide D. *J Biochem (Tokyo)*. 1998;123(5):946-952. doi:10.1093/oxfordjournals.jbchem.a022029

667. Yamada K, Ojika M, Ishigaki T, Yoshida Y, Ekimoto H, Arakawa M. Aplyronine A, a potent antitumor substance and the congeners aplyronines B and C isolated from the sea hare Aplysia kurodai. *J Am Chem Soc*. 1993;115(23):11020-11021. doi:10.1021/ja00076a082

668. Saito S ya, Watabe S, Ozaki H, Kigoshi H, Yamada K, Fusetani N, Karaki H. Novel Actin Depolymerizing Macrolide Aplyronine A1. *J Biochem (Tokyo)*. 1996;120(3):552-555. doi:10.1093/oxfordjournals.jbchem.a021449

669. Ojika M, Kigoshi H, Yoshida Y, Ishigaki T, Nisiwaki M, Tsukada I, Arakawa M, Ekimoto H, Yamada K. Aplyronine A, a potent antitumor macrolide of marine origin, and the congeners aplyronines B and C: isolation, structures, and bioactivities. *Tetrahedron*. 2007;63(15):3138-3167. doi:10.1016/j.tet.2007.02.011

670. Kigoshi H, Kita M. Antitumor Effects of Sea Hare-Derived Compounds in Cancer. In: Kim SK, ed. *Handbook of Anticancer Drugs from Marine Origin*. Springer International Publishing; 2015:701-739. doi:10.1007/978-3-319-07145-9_33

671. Klenchin VA, Allingham JS, King R, Tanaka J, Marriott G, Rayment I. Trisoxazole macrolide toxins mimic the binding of actin-capping proteins to actin. *Nat Struct Biol*. 2003;10(12):1058-1063. doi:10.1038/nsb1006

672. Tanaka J, Yan Y, Choi J, Bai J, Klenchin VA, Rayment I, Marriott G. Biomolecular mimicry in the actin cytoskeleton: Mechanisms underlying the cytotoxicity of kabiramide C and related macrolides. *Proc Natl Acad Sci*. 2003;100(24):13851-13856. doi:10.1073/pnas.2233339100

673. Dominguez R. Actin-binding proteins – a unifying hypothesis. *Trends Biochem Sci*. 2004;29(11):572-578. doi:10.1016/j.tibs.2004.09.004

674. Roopa L, Pravin KR, M. MSM. Molecular Dynamics Simulation Reveal the Mechanism of Resistance of Mutant Actins to Latrunculin A – Insight into Specific Modifications to Design Novel Drugs to Overcome Resistance. *Curr Comput Aided Drug Des*. 2016;12(2):107-118.

675. Johnston JJ, Wen KK, Keppler-Noreuil K, McKane M, Maiers JL, Greiner A, Sapp JC, NIH Intramural Sequencing Center, Demali KA, Rubenstein PA, et al. Functional analysis of a de novo ACTB mutation in a patient with atypical Baraitser-Winter syndrome. *Hum Mutat*. 2013;34(9):1242-1249. doi:10.1002/humu.22350

676. Belmont LD, Drubin DG. The Yeast V159N Actin Mutant Reveals Roles for Actin Dynamics In Vivo. *J Cell Biol*. 1998;142(5):1289-1299.

677. Procaccio V, Salazar G, Ono S, Styers ML, Gearing M, Davila A, Jimenez R, Juncos J, Gutekunst CA, Meroni G, et al. A Mutation of β-Actin That Alters Depolymerization Dynamics Is Associated with Autosomal Dominant Developmental Malformations, Deafness, and Dystonia. *Am J Hum Genet*. 2006;78(6):947-960. doi:10.1086/504271

678. Schwarzerová K, Vondráková Z, Fischer L, Bořík̆ová P, Bellinvia E, Eliášová K, Havelková L, Fišerová J, Vágner M, Opatrný Z. The role of actin isoforms in somatic embryogenesis in Norway spruce. *BMC Plant Biol*. 2010;10:89. doi:10.1186/1471-2229-10-89

679. Rivière JB, van Bon BWM, Hoischen A, Kholmanskikh SS, O'Roak BJ, Gilissen C, Gijsen S, Sullivan CT, Christian SL, Abdul-Rahman OA, et al. De novo mutations in the actin genes ACTB and ACTG1 cause Baraitser-Winter syndrome. *Nat Genet*. 2012;44(4):440-S2. doi:10.1038/ng.1091

680. Filipuzzi I, Thomas JR, Pries V, Estoppey D, Salcius M, Studer C, Schirle M, Hoepfner D. Direct Interaction of Chivosazole F with Actin Elicits Cell Responses Similar to Latrunculin A but Distinct from Chondramide. *ACS Chem Biol*. 2017;12(9):2264-2269. doi:10.1021/acschembio.7b00385

681. Johannes F j., Gallwitz D. Site-directed mutagenesis of the yeast actin gene: a test for actin function in vivo. *EMBO J*. 1991;10(12):3951-3958. doi:10.1002/j.1460-2075.1991.tb04965.x

682. Wertman KF, Drubin DG, Botstein D. Systematic mutational analysis of the yeast ACT1 gene. *Genetics*. 1992;132(2):337-350.

683. Wang H, Robinson RC, Burtnick LD. The structure of native G-actin. *Cytoskeleton*. 67(7):456-465. doi:10.1002/cm.20458

684. Di Donato N, Rump A, Koenig R, Der Kaloustian VM, Halal F, Sonntag K, Krause C, Hackmann K, Hahn G, Schrock E, et al. Severe forms of Baraitser-Winter syndrome are caused by ACTB mutations rather than ACTG1 mutations. *Eur J Hum Genet EJHG*. 2014;22(2):179-183. doi:10.1038/ejhg.2013.130

685. Hundt N, Preller M, Swolski O, Ang AM, Mannherz HG, Manstein DJ, Müller M. Molecular mechanisms of disease-related human β-actin mutations p.R183W and p.E364K. *FEBS J*. 2014;281(23):5279-5291. doi:10.1111/febs.13068

686. Verloes A, Di Donato N, Masliah-Planchon J, Jongmans M, Abdul-Raman OA, Albrecht B, Allanson J, Brunner H, Bertola D, Chassaing N, et al. Baraitser–Winter cerebrofrontofacial syndrome: delineation of the spectrum in 42 cases. *Eur J Hum Genet*. 2015;23(3):292-301. doi:10.1038/ejhg.2014.95

687. Verloes A, Drunat S, Pilz D, Donato ND. *Baraitser-Winter Cerebrofrontofacial Syndrome*. University of Washington, Seattle; 2015. Accessed June 14, 2021. https://www.ncbi.nlm.nih.gov/sites/books/NBK327153/

688. Conboy E, Vairo F, Waggoner D, Ober C, Das S, Dhamija R, Klee EW, Pichurin P. Pathogenic Variant in ACTB, p.Arg183Trp, Causes Juvenile-Onset Dystonia, Hearing Loss, and Developmental Delay without Midline Malformation. *Case Rep Genet*. 2017;2017:9184265. doi:10.1155/2017/9184265

689. Cuvertino S, Stuart HM, Chandler KE, Roberts NA, Armstrong R, Bernardini L, Bhaskar S, Callewaert B, Clayton-Smith J, Davalillo CH, et al. ACTB Loss-of-Function Mutations Result in a Pleiotropic Developmental Disorder. *Am J Hum Genet*. 2017;101(6):1021-1033. doi:10.1016/j.ajhg.2017.11.006

690. Freitas JL, Vale TC, Barsottini OGP, Pedroso JL. Expanding the Phenotype of Dystonia-Deafness Syndrome Caused by ACTB Gene Mutation. *Mov Disord Clin Pract*. 2019;7(1):86-87. doi:10.1002/mdc3.12854

691. Choi GJ, Kim MS, Park H, Kim JY, Choi JM, Lee SM, Jang JH, Cho SY, Jin DK. The First Korean Case of Baraitser-Winter Cerebro-Fronto-Facial Syndrome with a Novel Mutation in ACTB Diagnosed Via Targeted Gene Panel Sequencing and Literature Review. *Ann Clin Lab Sci*. 2020;50(6):818-824.

692. Parker F, Baboolal TG, Peckham M. Actin Mutations and Their Role in Disease. *International Journal of Molecular Sciences*. 2020;21(9):3371. doi:10.3390/ijms21093371

693. Baumann M, Beaver EM, Palomares-Bralo M, Santos-Simarro F, Holzer P, Povysil G, Müller T, Valovka T, Janecke AR. Further delineation of putative ACTB loss-of-function variants: A 4-patient series. *Hum Mutat*. 2020;41(4):753-758. doi:10.1002/humu.23970

694. Hampshire K, Martin PM, Carlston C, Slavotinek A. Baraitser–Winter cerebrofrontofacial syndrome: Report of two adult siblings. *Am J Med Genet A*. 2020;182(8):1923-1932. doi:10.1002/ajmg.a.61637

695. Sprenkeler EGG, Webbers SDS, Kuijpers TW. When Actin is Not Actin' Like It Should: A New Category of Distinct Primary Immunodeficiency Disorders. *J Innate Immun*. 2021;13(1):3-25. doi:10.1159/000509717

696. Thompson JE, Barrow KD, Faulkner DJ. Localization of Two Brominated Metabolites, Aerothionin and Homoaerothionin, in Spherulous Cells of the Marine Sponge Aplysina fistularis (=Verongia thiona). *Acta Zool*. 1983;64(4):199-210. doi:10.1111/j.1463-6395.1983.tb00801.x

697. Müller WE, Diehl-Seifert B, Sobel C, Bechtold A, Kljajić Z, Dorn A. Sponge secondary metabolites: biochemical and ultrastructural localization of the antimitotic agent avarol in Dysidea avara. *J Histochem Cytochem*. 1986;34(12):1687-1690. doi:10.1177/34.12.3782777

698. Uriz MJ, Becerro MA, Tur JM, Turon X. Location of toxicity within the Mediterranean sponge Crambe crambe (Demospongiae: Poecilosclerida). *Mar Biol*. 1996;124(4):583-590. doi:10.1007/BF00351039

699. Uriz MJ, Turon X, Galera J, Tur JM. New light on the cell location of avarol within the sponge Dysidea avara (Dendroceratida). *Cell Tissue Res*. 1996;285(3):519-527. doi:10.1007/s004410050668

700. Garson MJ, Thompson JE, Larsen RM, Battershill CN, Murphy PT, Bergquist PR. Terpenes in sponge cell membranes: Cell separation and membrane fractionation studies with the tropical marine spongeAmphimedon sp. *Lipids*. 1992;27(5):378-388. doi:10.1007/BF02536153

701. Garson MJ, Flowers AE, Webb RI, Charan RD, McCaffrey EJ. A sponge/dinoflagellate association in the haplosclerid sponge Haliclona sp.: cellular origin of cytotoxic alkaloids by Percoll density gradient fractionation. *Cell Tissue Res*. 1998;293(2):365-373. doi:10.1007/s004410051128

702. Turon X, Becerro MA, Uriz MJ. Distribution of brominated compounds within the sponge Aplysina aerophoba: coupling of X-ray microanalysis with cryofixation techniques. *Cell Tissue Res*. 2000;301(2):311-322. doi:10.1007/s004410000233

703. Gerçe B, Schwartz T, Voigt M, Rühle S, Kirchen S, Putz A, Proksch P, Obst U, Syldatk C, Hausmann R. Morphological, Bacterial, and Secondary Metabolite Changes of Aplysina aerophoba upon Long-Term Maintenance Under Artificial Conditions. *Microb Ecol*. 2009;58(4):865-878.

704. Tianero MD, Balaich JN, Donia MS. Localized production of defence chemicals by intracellular symbionts of Haliclona sponges. *Nat Microbiol*. 2019;4(7):1149-1159. doi:10.1038/s41564-019-0415-8

705. Groweiss A, Shmueli U, Kashman Y. Marine toxins of Latrunculia magnifica. *J Org Chem*. 1983;48(20):3512-3516. doi:10.1021/jo00168a028

706. Crozier WJ, Crozier WJ. The nature of the conical bodies on the mantle of certain nudibranchs. *The Nautilus*. 1917;30:103-106.

707. Joandomènec 1946-Ros. Sistemas de defensa en los Opistobranquios. *Oecologia Aquat*. 1976;(2):41-77.

708. Okuda RK. Chemical ecology of some opisthobranch mollusks. Published online 1983. Accessed December 6, 2020. http://scholarspace.manoa.hawaii.edu/handle/10125/9493

709. García J, Medina A, Coveñas R. Study of the anatomy and histology of the mantle dermal formations (MDFs) of Chromodoris and Hypselodoris (Opisthobranchia: Chromodorididae). *Malacologia*. 1991;32:233-240.

710. Fontana A, Avila C, Martinez E, Ortea J, Trivellone E, Cimino G. Defensive allomones in three species ofHypselodoris (gastropoda: Nudibranchia) from the Cantabrian sea. *J Chem Ecol*. 1993;19(2):339-356. doi:10.1007/BF00993700

711. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res*. 2018;46(Database issue):D8-D13. doi:10.1093/nar/gkx1095

712. Maeda T, Takahashi S, Yoshida T, Shimamura S, Takaki Y, Nagai Y, Toyoda A, Suzuki Y, Arimoto A, Ishii H, et al. Chloroplast acquisition without the gene transfer in kleptoplastic sea slugs, Plakobranchus ocellatus. *eLife*. 10:e60176. doi:10.7554/eLife.60176

713. McLean BG, Huang S, McKinney EC, Meagher RB. Plants contain highly divergent actin isovariants. *Cell Motil*. 1990;17(4):276-290. doi:10.1002/cm.970170403

714. Reece KS, McElroy D, Wu R. Function and Evolution of Actins. In: Hecht MK, Wallace B, Macintyre RJ, eds. *Evolutionary Biology: Volume 26*. Evolutionary Biology. Springer US; 1992:1-34. doi:10.1007/978-1-4615-3336-8_1

715. Šlajcherová K, Fišerová J, Fischer L, Schwarzerová K. Multiple Actin Isotypes in Plants: Diverse Genes for Diverse Roles? *Front Plant Sci*. 2012;3. doi:10.3389/fpls.2012.00226

716. Vandekerckhove J, Weber K. At least six different actins are expressed in a higher mammal: An analysis based on the amino acid sequence of the amino-terminal tryptic peptide. *J Mol Biol*. 1978;126(4):783-802. doi:10.1016/0022-2836(78)90020-7

717. Venkatesh B, Tay BH, Elgar G, Brenner S. Isolation, Characterization and Evolution of Nine Pufferfish (Fugu rubripes) Actin Genes. *J Mol Biol*. 1996;259(4):655-665. doi:10.1006/jmbi.1996.0347

718. Lee JJ, Shott RJ, Rose SJ, Thomas TL, Britten RJ, Davidson EH. Sea urchin actin gene subtypes: Gene number, linkage and evolution. *J Mol Biol*. 1984;172(2):149-176. doi:10.1016/S0022-2836(84)80035-2

719. Fang H, Brandhorst BP. Evolution of actin gene families of sea urchins. *J Mol Evol*. 1994;39(4):347-356. doi:10.1007/BF00160267

720. Fyrberg EA, Kindle KL, Davidson N, Sodja A. The actin genes of drosophila: a dispersed multigene family. *Cell*. 1980;19(2):365-378. doi:10.1016/0092-8674(80)90511-5

721. Kondrashov FA, Rogozin IB, Wolf YI, Koonin EV. Selection in the evolution of gene duplications. *Genome Biol*. 2002;3(2):research0008.1. doi:10.1186/gb-2002-3-2-research0008

722. Edmunds M. Protective mechanisms in the Eolidacea (Mollusca Nudibranchia). *Zool J Linn Soc*. 1966;46(308):27-71. doi:10.1111/j.1096-3642.1966.tb00082.x

723. Edmunds M. On the swimming and defensive response of Hexabranchus marginatus (Mollusca, Nudibranchia). *Zool J Linn Soc*. 1968;47(313):425-429. doi:10.1111/j.1096-3642.1968.tb00550a.x

724. Ghazali SR. Displays of Defense : Behavioral Differences in Antagonist Avoidance in Four Opisthobranch Mollusks. Published online December 1, 2006. Accessed April 5, 2018. https://escholarship.org/uc/item/9s6740fr

725. Alqudah A, Saad S, Susanti D, Hadry F, Fikri M, Khodzori FA, Yusof M, Rani H. Jurnal Teknolgi Full Paper OBSERVATIONS ON NUDIBRANCH BEHAVIOUR PATTERNS UNDER LABORATORY CONDITIONS. *J Teknol*. 2016;78:11-2016. doi:10.11113/.v78.6639

726. Avila C, Durfort M. Histology of epithelia and mantle glands of selected species of Doridacean molluscs with chemical defensive strategies. *Veliger -Berkeley-*. 1996;39:148-163.

727. Davies MS, Blackwell J. Energy saving through trail following in a marine snail. *Proc R Soc Lond B Biol Sci*. 2007;274(1614):1233-1236. doi:10.1098/rspb.2007.0046

728. David KT, Oaks JR, Halanych KM. Patterns of gene evolution following duplications and speciations in vertebrates. *PeerJ*. 2020;8:e8813. doi:10.7717/peerj.8813

729. Casewell NR, Wüster W, Vonk FJ, Harrison RA, Fry BG. Complex cocktails: the evolutionary novelty of venoms. *Trends Ecol Evol*. 2013;28(4):219-229. doi:10.1016/j.tree.2012.10.020

730. Bergeron SE, Zhu M, Thiem SM, Friderici KH, Rubenstein PA. Ion-dependent Polymerization Differences between Mammalian β- and γ-Nonmuscle Actin Isoforms. *J Biol Chem*. 2010;285(21):16087-16095. doi:10.1074/jbc.M110.110130

731. Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, Nguyen DD, Watrous J, Kapono CA, Luzzatto-Knaan T, et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nat Biotechnol*. 2016;34(8):828-837. doi:10.1038/nbt.3597

732. Aron AT, Gentry EC, McPhail KL, Nothias LF, Nothias-Esposito M, Bouslimani A, Petras D, Gauglitz JM, Sikora N, Vargas F, et al. Reproducible molecular networking of untargeted mass spectrometry data using GNPS. *Nat Protoc*. 2020;15(6):1954-1991. doi:10.1038/s41596-020-0317-5

733. Nothias LF, Petras D, Schmid R, Dührkop K, Rainer J, Sarvepalli A, Protsyuk I, Ernst M, Tsugawa H, Fleischauer M, et al. Feature-based molecular networking in the GNPS analysis environment. *Nat Methods*. 2020;17(9):905-908. doi:10.1038/s41592-020-0933-6

734. Schmid R, Petras D, Nothias LF, Wang M, Aron AT, Jagels A, Tsugawa H, Rainer J, Garcia-Aloy M, Dührkop K, et al. Ion identity molecular networking for mass spectrometry-based metabolomics in the GNPS environment. *Nat Commun*. 2021;12(1):3832. doi:10.1038/s41467-021-23953-9

735. van Santen JA, Poynton EF, Iskakova D, McMann E, Alsup TA, Clark TN, Fergusson CH, Fewer DP, Hughes AH, McCadden CA, et al. The Natural Products Atlas 2.0: a database of microbially-derived natural products. *Nucleic Acids Res*. Published online October 28, 2021:gkab941. doi:10.1093/nar/gkab941

736. Sorokina M, Merseburger P, Rajan K, Yirik MA, Steinbeck C. COCONUT online: Collection of Open Natural Products database. *J Cheminformatics*. 2021;13(1):2. doi:10.1186/s13321-020-00478-9

737. Rutz A, Sorokina M, Galgonek J, Mietchen D, Willighagen E, Gaudry A, Graham J, Stephan R, Page R, Vondrasek J, et al. *The LOTUS Initiative for Open Natural Products Research: Knowledge Management through Wikidata*.; 2021. doi:10.1101/2021.02.28.433265

738. Bremser W. Structure Elucidation and Artificial Intelligence. *Angew Chem Int Ed Engl*. 1988;27(2):247-260. doi:10.1002/anie.198802471

739. Howarth A, Ermanis K, Goodman JM. DP4-AI automated NMR data analysis: straight from spectrometer to structure. *Chem Sci*. 2020;11(17):4351-4359. doi:10.1039/D0SC00442A

740. Reher R, Kim HW, Zhang C, Mao HH, Wang M, Nothias LF, Caraballo-Rodriguez AM, Glukhov E, Teke B, Leao T, et al. A Convolutional Neural Network-Based Approach for the Rapid Annotation of Molecularly Diverse Natural Products. *J Am Chem Soc*. 2020;142(9):4114-4120. doi:10.1021/jacs.9b13786

741. Pesek M, Juvan A, Jakoš J, Košmrlj J, Marolt M, Gazvoda M. Database Independent Automated Structure Elucidation of Organic Molecules Based on IR, 1H NMR, 13C NMR, and MS Data. *J Chem Inf Model*. 2021;61(2):756-763. doi:10.1021/acs.jcim.0c01332

742. Skinnider MA, Wang F, Pasin D, Greiner R, Foster LJ, Dalsgaard PW, Wishart DS. A deep generative model enables automated structure elucidation of novel psychoactive substances. *Nat Mach Intell*. 2021;3(11):973-984. doi:10.1038/s42256-021-00407-x

743. Ackers RG, Moss D, Picton BE, Bt B, Stone SMK, Morrow CC. Sponges of the British Isles - A colour guide and working document, 1992 Edition. *Mar Conserv Soc*. Published online 2007:165.

744. Erpenbeck D, Galitz A, Ekins M, Cook S de C, Soest RWM van, Hooper JNA, Wörheide G. Soft sponges with tricky tree: On the phylogeny of dictyoceratid sponges. *J Zool Syst Evol Res*. 2020;58(1):27-40. doi:10.1111/jzs.12351

745. Bouschen W, Schulz O, Eikel D, Spengler B. Matrix vapor deposition/recrystallization and dedicated spray preparation for high-resolution scanning microprobe matrix-assisted laser desorption/ionization imaging mass spectrometry (SMALDI-MS) of tissue and single cells. *Rapid Commun Mass Spectrom*. 2010;24(3):355-364. doi:10.1002/rcm.4401

746. Koestler M, Kirsch D, Hester A, Leisner A, Guenther S, Spengler B. A high-resolution scanning microprobe matrix-assisted laser desorption/ionization ion source for imaging analysis on an ion trap/Fourier transform ion cyclotron resonance mass spectrometer. *Rapid Commun Mass Spectrom RCM*. 2008;22(20):3275-3285. doi:10.1002/rcm.3733

747. Römpp A, Guenther S, Schober Y, Schulz O, Takats Z, Kummer W, Spengler B. Histology by Mass Spectrometry: Label-Free Tissue Characterization Obtained from High-Accuracy Bioanalytical Imaging. *Angew Chem Int Ed*. 2010;49(22):3834-3838. doi:10.1002/anie.200905559

748. Römpp A, Spengler B. Mass spectrometry imaging with high resolution in mass and space. *Histochem Cell Biol*. 2013;139(6):759-783. doi:10.1007/s00418-013-1097-6

749. Guenther S, Koestler M, Schulz O, Spengler B. Laser spot size and laser power dependence of ion formation in high resolution MALDI imaging. *Int J Mass Spectrom*. 2010;294(1):7-15. doi:10.1016/j.ijms.2010.03.014

750. Paschke C, Leisner A, Hester A, Maass K, Guenther S, Bouschen W, Spengler B. Mirion—A Software Package for Automatic Processing of Mass Spectrometric Images. *J Am Soc Mass Spectrom*. 2013;24(8):1296-1306. doi:10.1021/jasms.8b04589

751. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol*. 2018;35(6):1547-1549. doi:10.1093/molbev/msy096

752. Huson DH, Bryant D. Application of Phylogenetic Networks in Evolutionary Studies. *Mol Biol Evol*. 2006;23(2):254-267. doi:10.1093/molbev/msj030

753. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. Clustal W and Clustal X version 2.0. *Bioinformatics*. 2007;23(21):2947-2948. doi:10.1093/bioinformatics/btm404

754. Rohde S, Schupp PJ. Allocation of chemical and structural defenses in the sponge Melophlus sarasinorum. *J Exp Mar Biol Ecol*. 2011;399(1):76-83. doi:10.1016/j.jembe.2011.01.012

755. Amesbury S, Myers R. The Fishes. A Guide to the Coastal Resources of Guam. Vol. 1. *Guam Univ Guam Press P*. 1982;141.

APPENDIX

SUPPLEMENTARY INFORMATION FOR CHAPTER I

SI for CHAPTER I was partly also provided in Bogdanov et al. 2016, 2017.[1,2]

Table of contents

Figure S1.1 ^{1}H-NMR spectrum of compound **1** in MeOH-d_4.

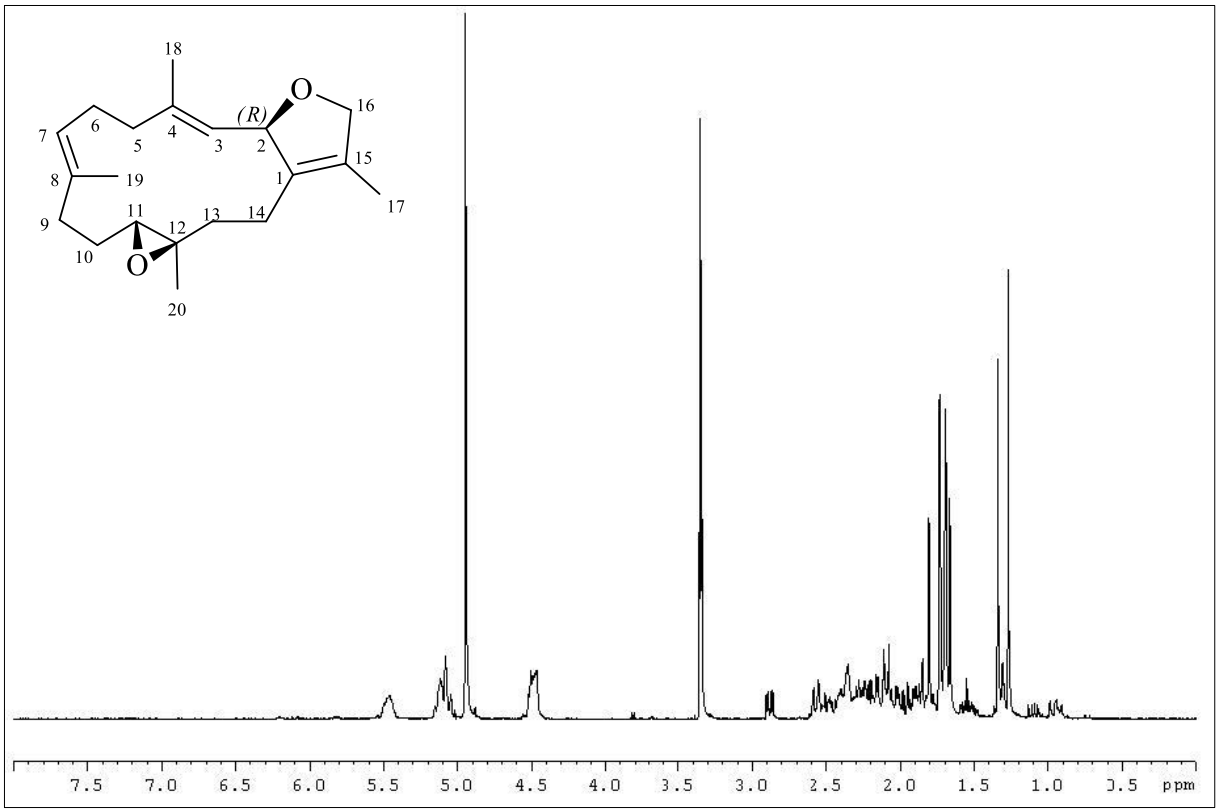


Figure S1.2 ^{13}C-NMR spectrum of compound **1** in MeOH-d_4.

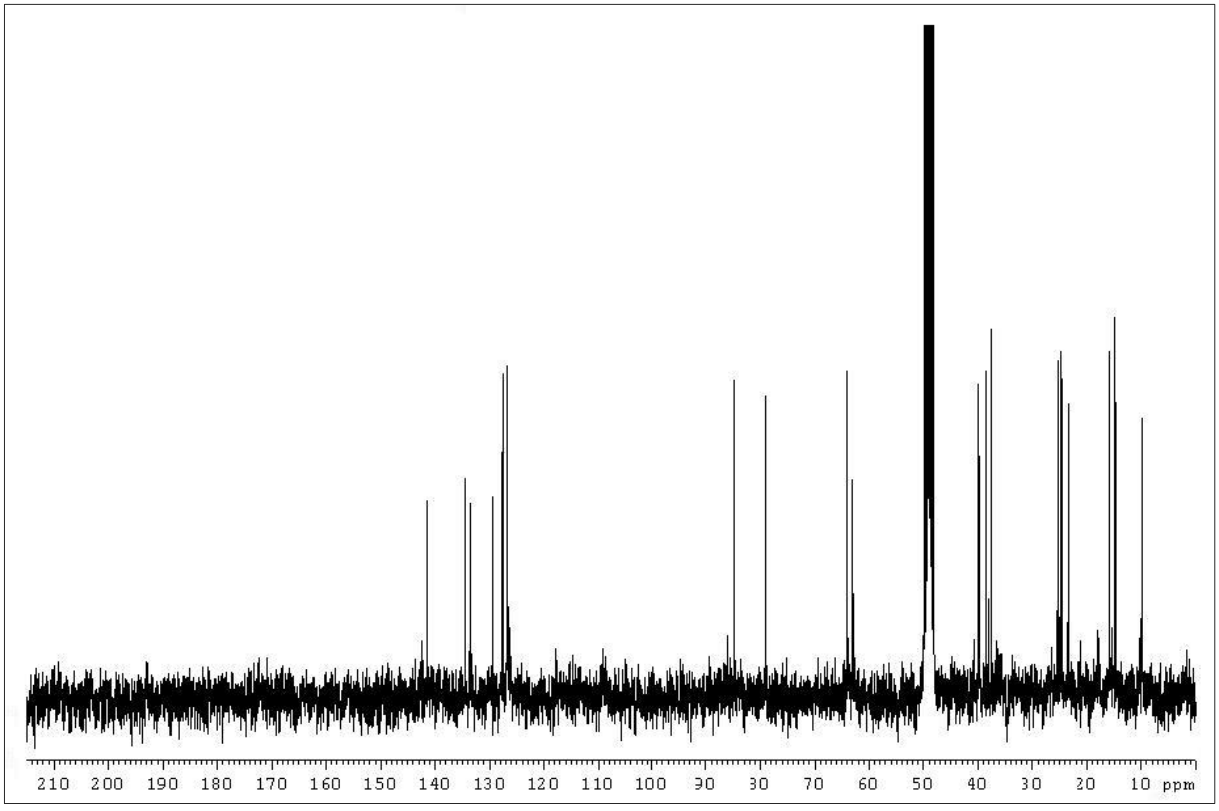


Figure S1.3 ^{1}H-NMR spectrum of compound 2 in MeOH-d_4.

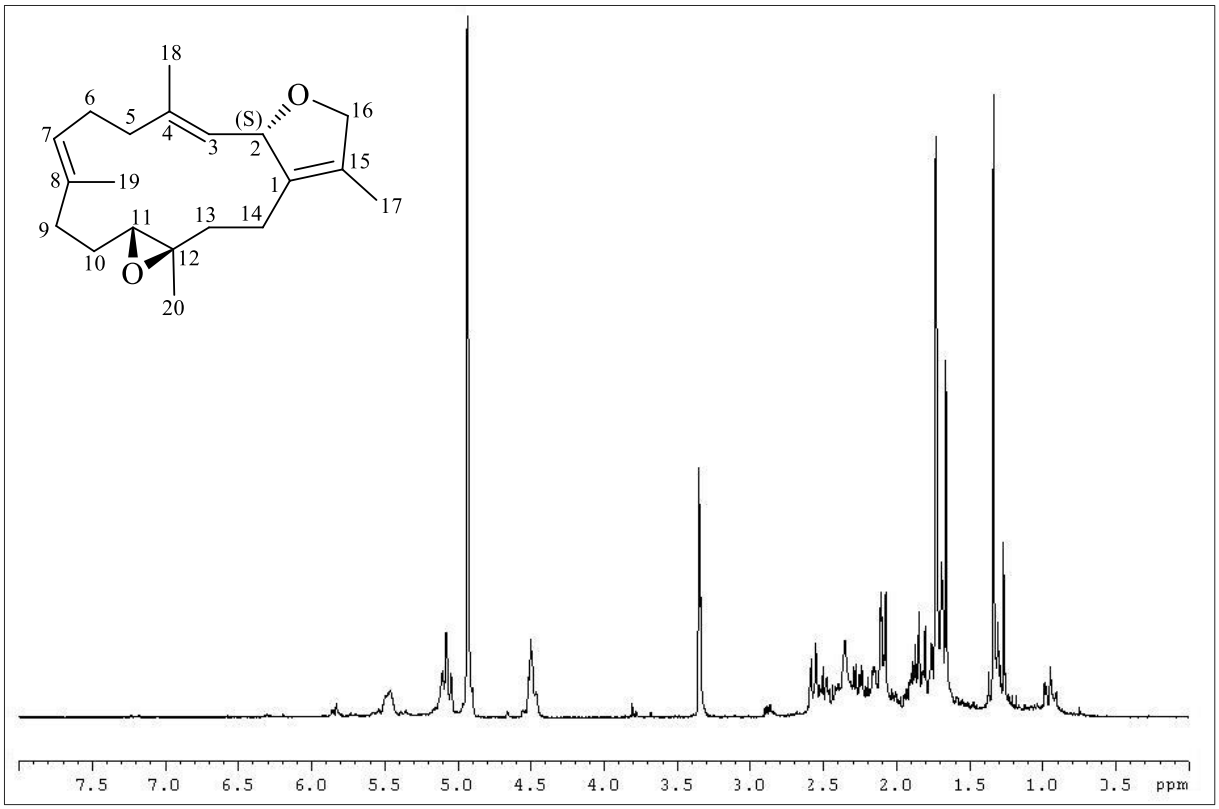


Figure S1.4 ^{13}C-NMR spectrum of compound **2** in MeOH-d_4.

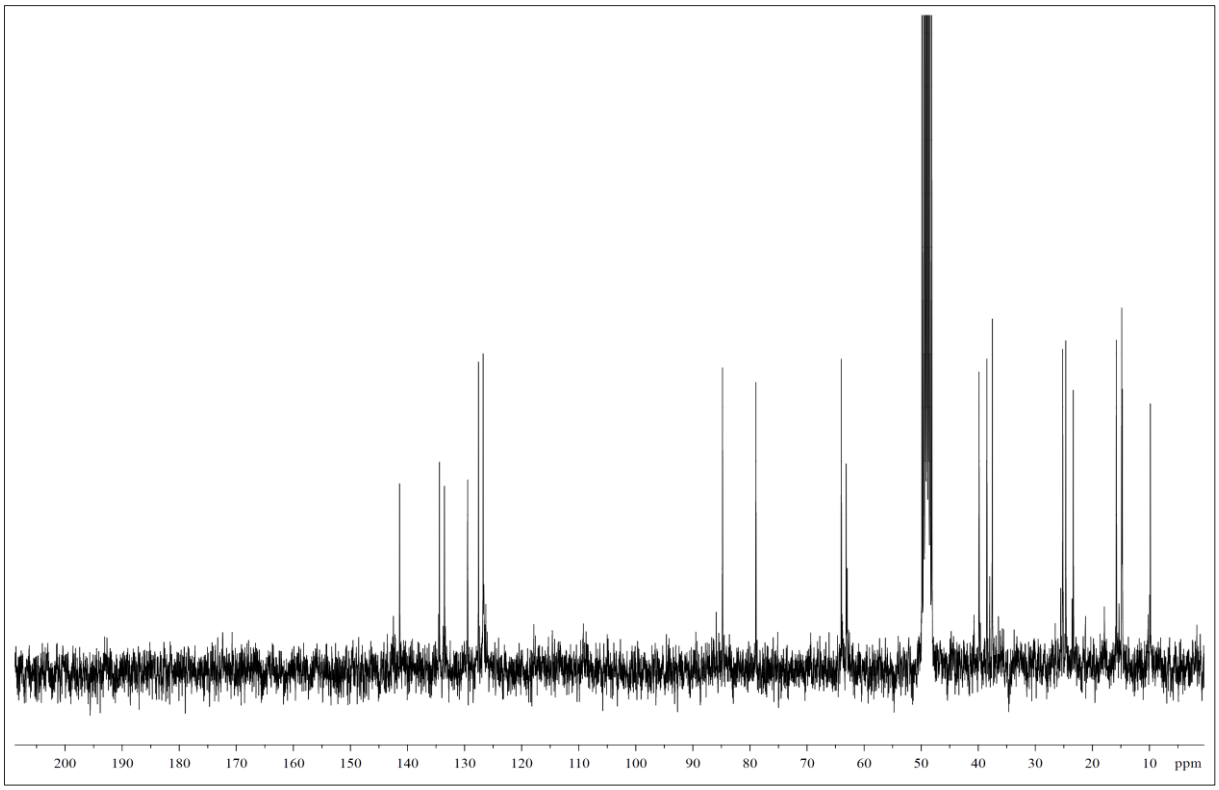


Figure S1.5 ^{1}H-NMR spectrum of compound **3** in MeOH-d_4.

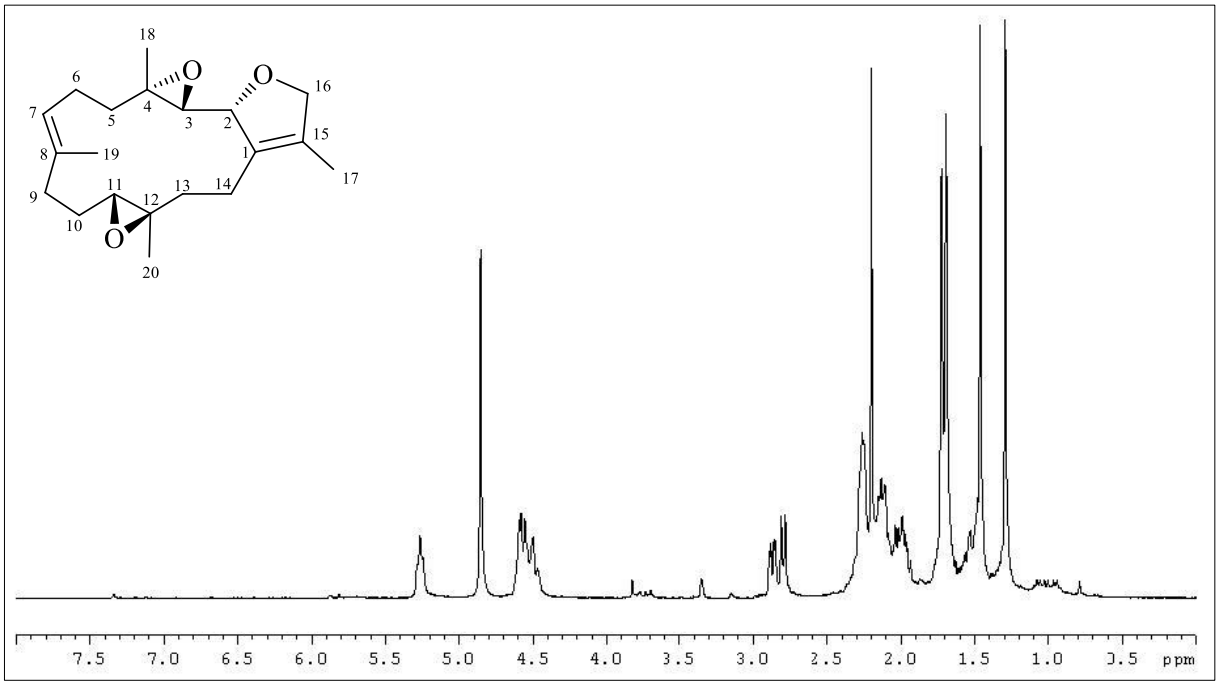


Figure S1.6 ^{13}C-NMR spectrum of compound **3** in MeOH-d_4.

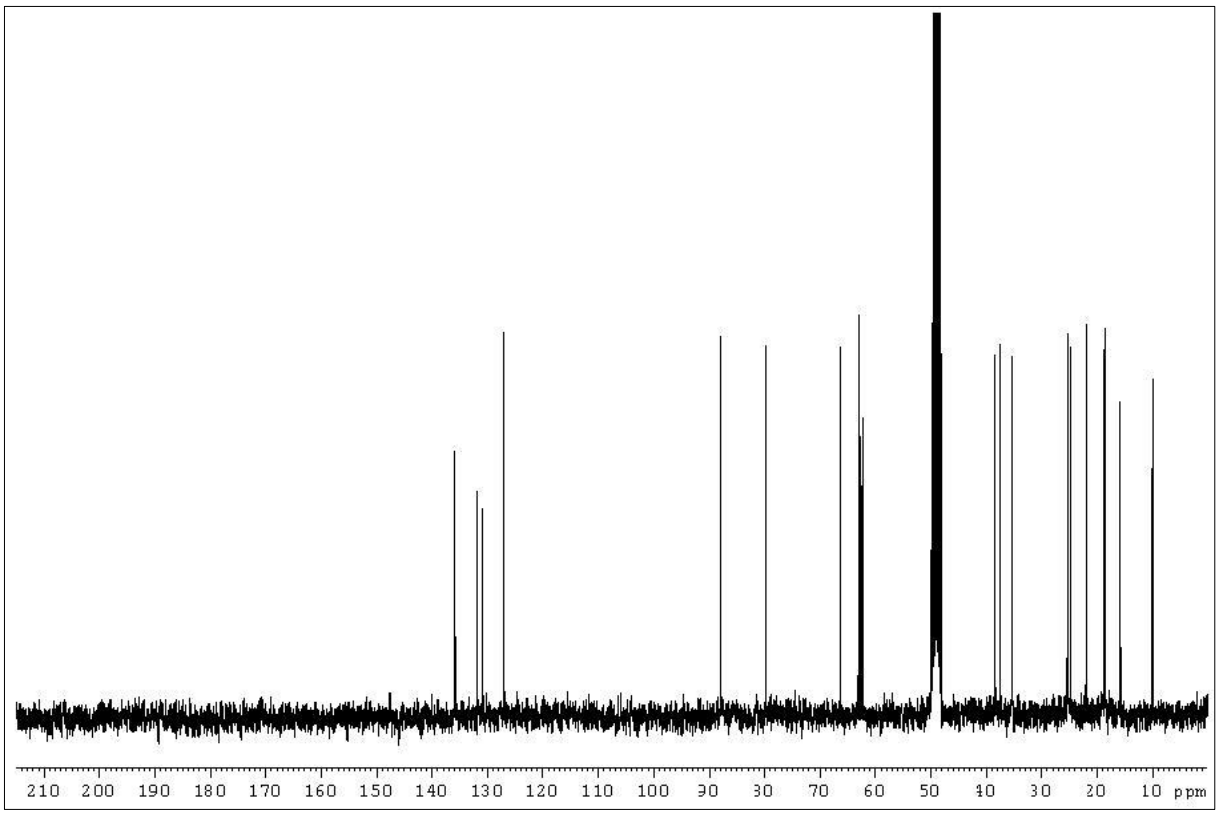


Figure S1.7 Proposed stereostructure of compound **3** with selected key NOESY correlations.

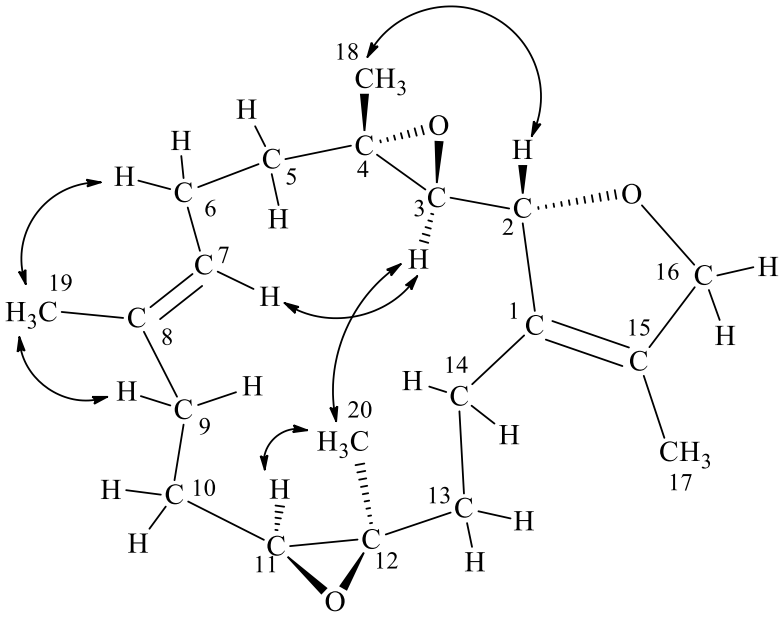


Figure S1.8 ^{1}H-NMR spectrum of compound **4** in MeOH-d_4.

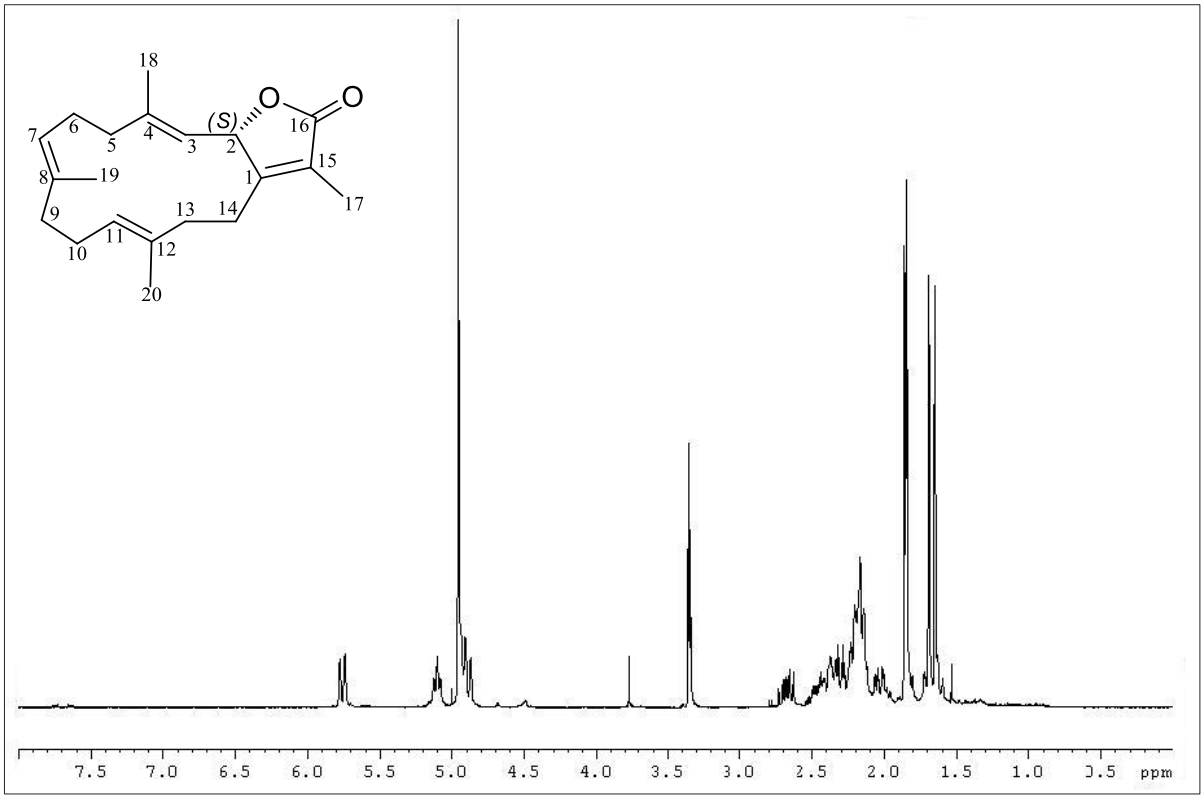


Figure S1.9 ^{13}C-NMR spectrum of compound **4** in MeOH-d_4.

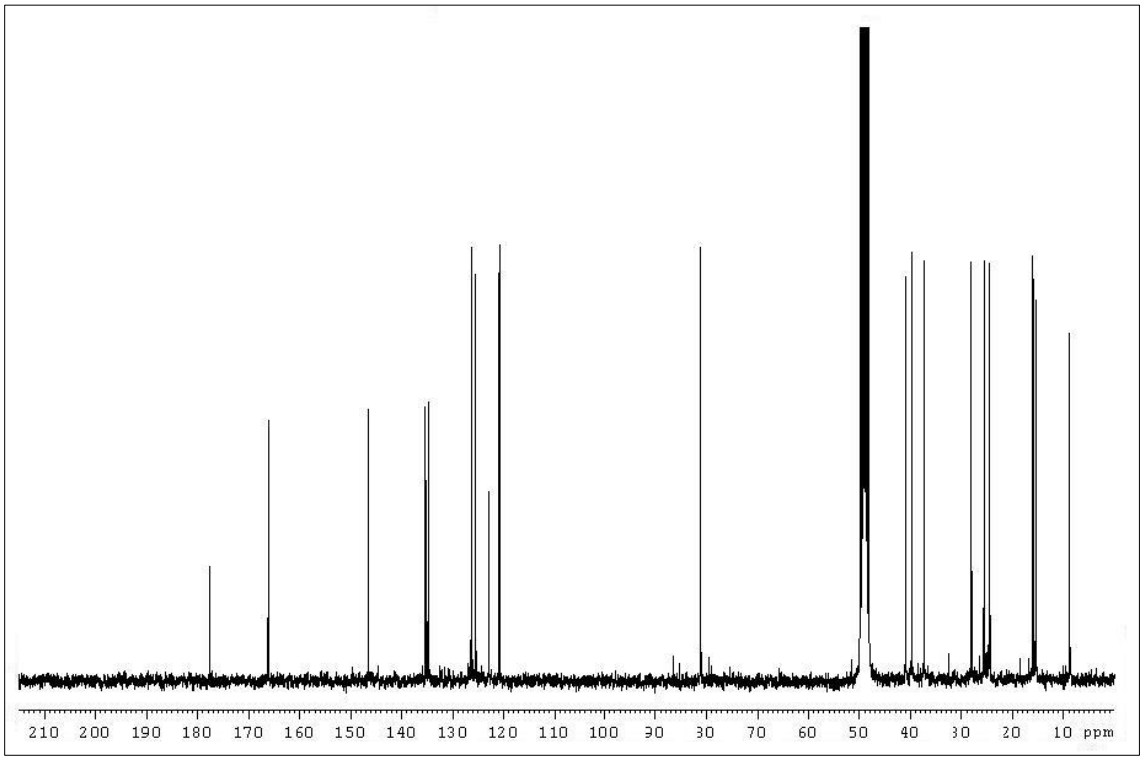


Figure S1.10 ^{1}H-NMR spectrum of compound **5** in MeOH-d_4.

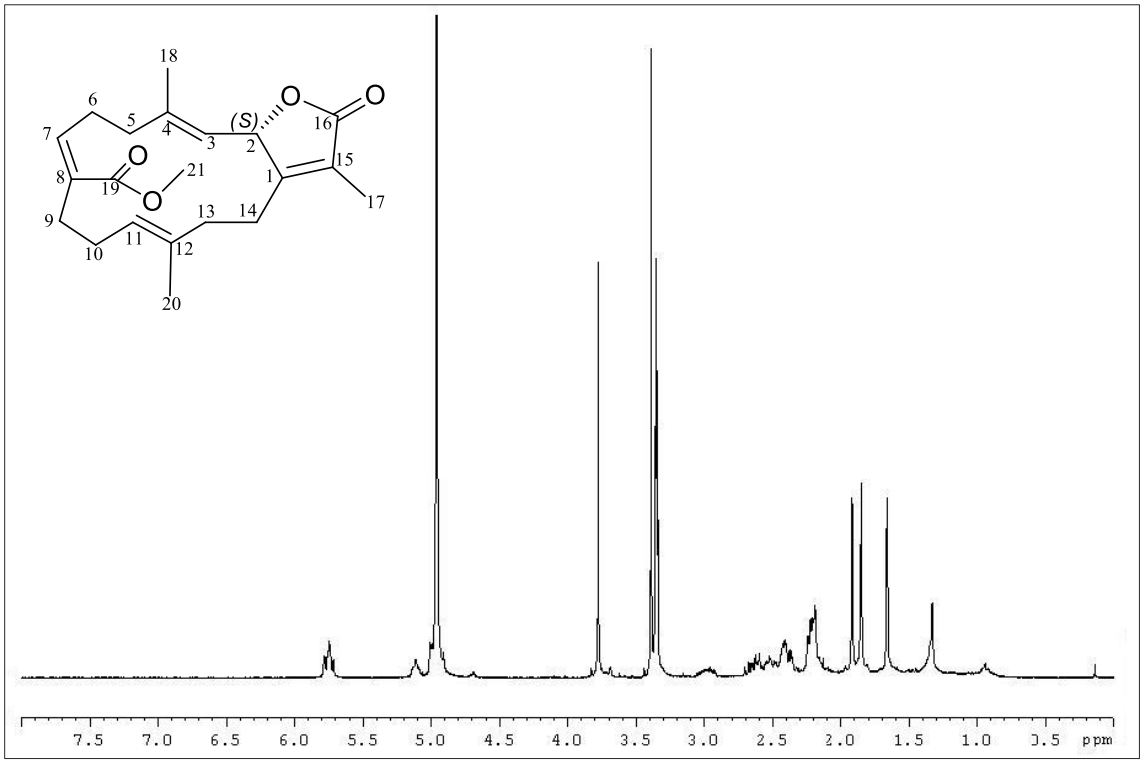


Figure S1.11 ^{13}C-NMR spectrum of compound **5** in MeOH-d_4.

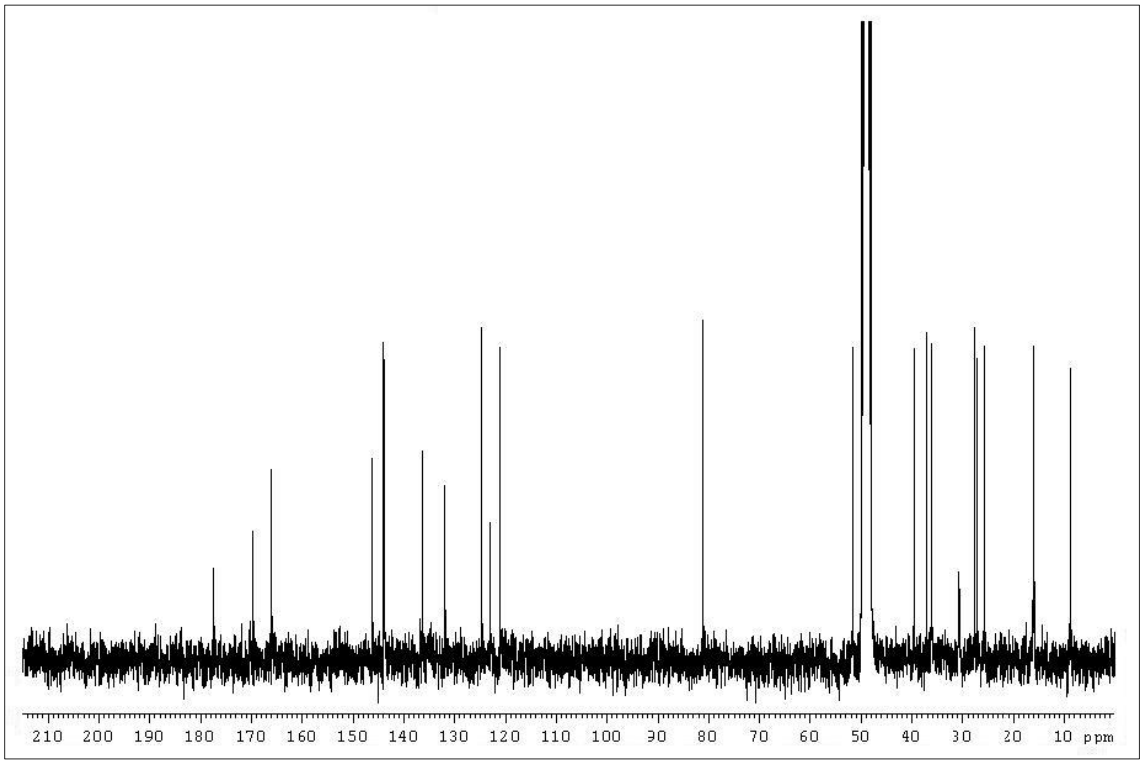


Figure S1.12 ^{1}H-NMR spectrum of compound **6** in MeOH-d_4.

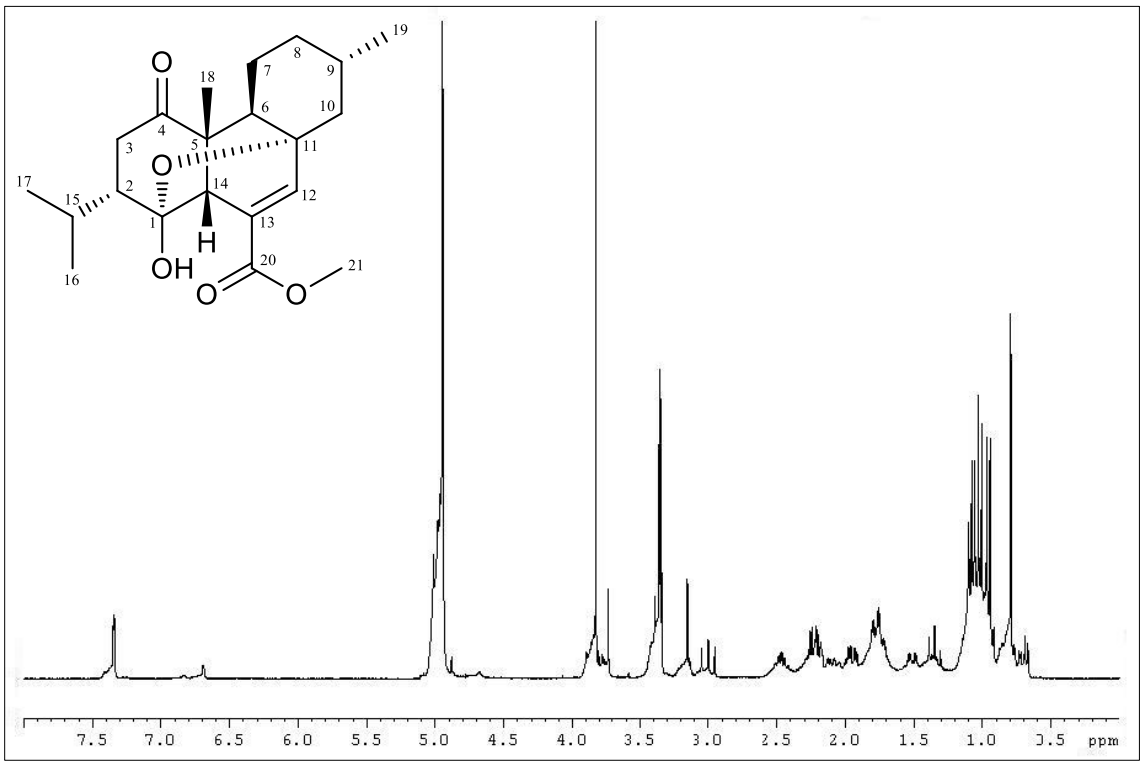


Figure S1.13 ^{13}C-NMR spectrum of compound **6** in MeOH-d_4.

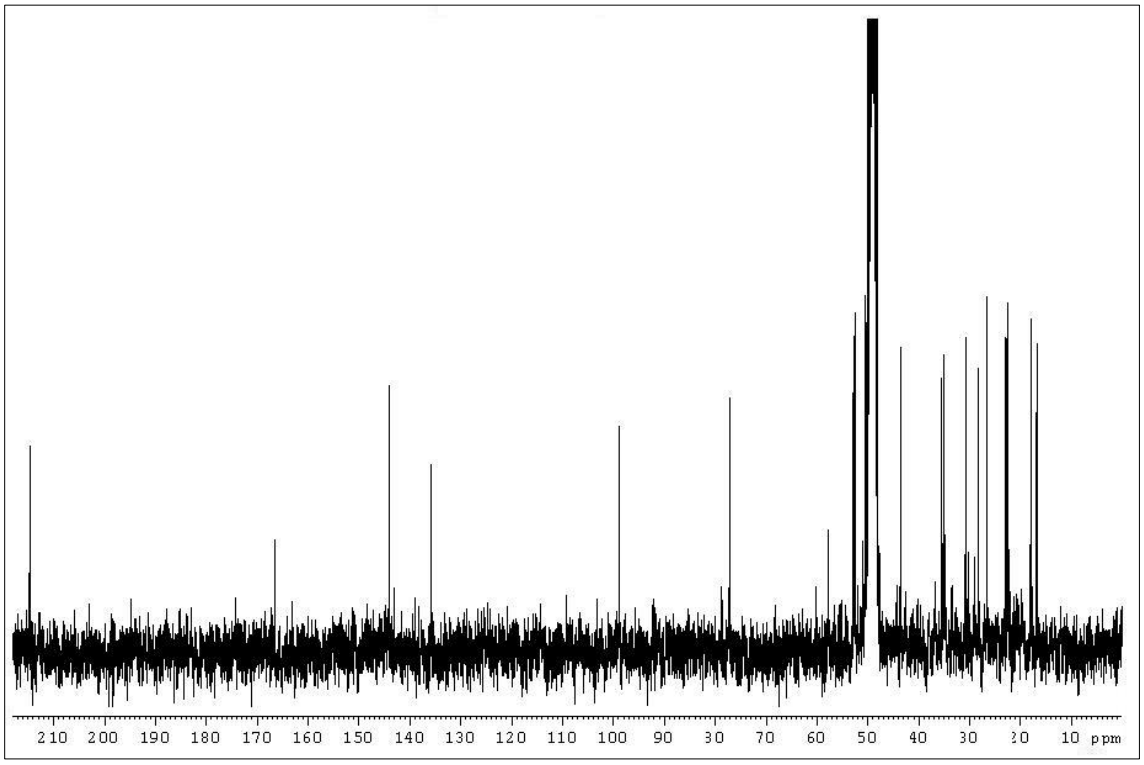


Table S1.1 NMR spectroscopic data of compound **6** in MeOH-d_4.

C	δ_H (mult, *J* in Hz)	δ_C	COSY	HMBC
1		98.8, C		
2	1.95, ddd (2.6, 5.1, 13.9)	49.9, CH	H_2-3, H-15	C-1, C-3, C-4, C-15, C-16, C-17
3	a 2.23, dd (5.1, 13.9)	35.0, CH_2	H-2	C-1, C-2, C-4, C-15
	b 3.00, t (13.9)		H-2	
4		214.6, C		
5		50.3, C		
6	1.78, dd (4.0, 13.2)	50.5, CH	H_2-7	C-4, C-5, C-7, C-8, C-10, C-11, C-14
7	a 0.75, m	28.2, CH_2	H-6, H_2-8	C-5, C-6, C-8, C-9, C-11
	b 1.51, dq (13.2, 3.7)		H-6, H_2-8	
8	a 1.00, m	35.5, CH_2	H-7b, H-9	C-7, C-9

	b 1.74, m		H-7a, H-9	
9	1.76, m	30.8, CH	H_2-8, H_2-10, H_3-19	
10	a 1.35, t (12.4) b 2.20, m	43.5, CH_2	H-9	C-6, C-8, C-9, C-11, C-12, C-13
11		77.1, C		
12	7.34, d (1.8)	144.0, CH	H-14	C-10, C-11, C-13, C-14, C-20,
13		135.7, C		
14	3.15, d (1.5)	52.8, CH	H-12	C-1, C-5, C-6, C-11, C-12, C-13, C-18, C-20
15	2.47, m	26.6, CH	H-2, H_3-16, H_3-17	C-1, C-2, C-3, C-16, C-17
16	1.02, d (7.0)	17.9, CH_3	H-15	C-2, C-15, C-17
17	0.96, d (7.0)	22.8, CH_3	H-15	C-2, C-15, C-16
18	0.79, s	16.8, CH_3		C-1, C-4, C-5, C-6, C-14
19	1.07, d (6.2)	22.5, CH_3	H-9	C-8, C-9, C-10, C-11
20		166.5, C		
21	3.83, s	52.5, CH_3		C-20

[a] All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC). [b] Multiplicities determined by DEPT.

Figure S1.14 ^{1}H-NMR spectrum of compound **7** in MeOH-d_4.

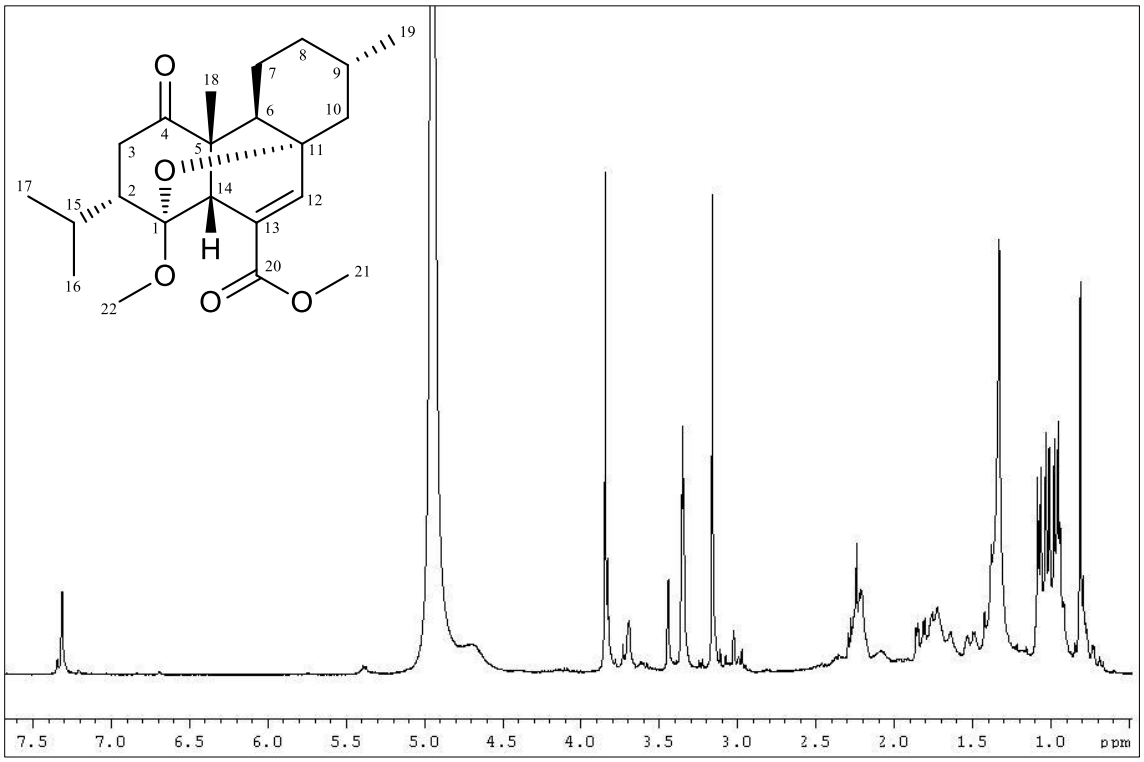


Figure S1.15 ^{13}C-NMR spectrum of compound **7** in MeOH-d_4.

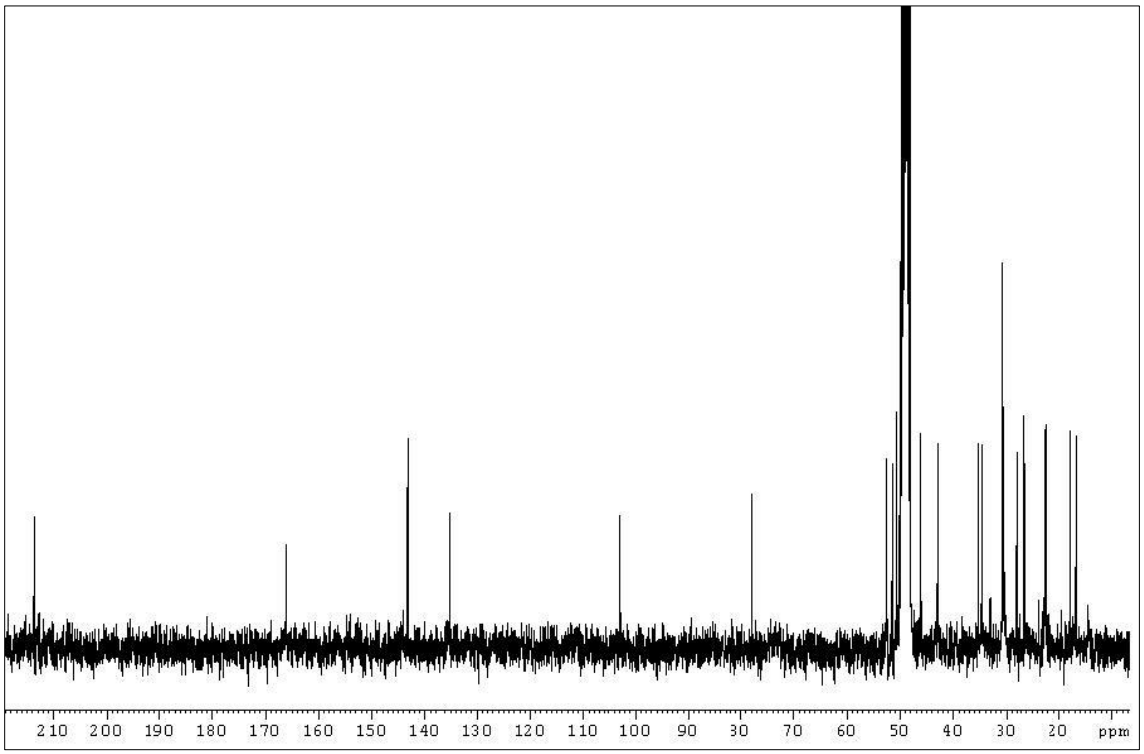


Table S1.2 NMR spectroscopic data of compound **7** in MeOH-d_4.

C	δ_H (mult, *J* in Hz)	δ_C	COSY	HMBC
1		102.9, C		
2	2.22, m	46.1, CH	H_2-3, H-15	C-1, C-3, C-4, C-15, C-16, C-17
3	a 2.26, m b 3.03, m	34.7, CH_2	H-2 H-2	C-1, C-2, C-4, C-15
4		213.7, C		
5		50.1, C		
6	1.84, dd (4.0, 13.2)	50.7, CH	H_2-7	C-4, C-5, C-7, C-8, C-10, C-11, C-14
7	a 0.76, m b 1.52, dq (13.2, 3.7)	28.1, CH_2	H-6 H-6	C-5, C-6, C-8, C-9, C-11
8	a 1.02, m b 1.75, m	35.4, CH_2	H-7b, H-9 H-7a, H-9	C-7, C-9
9	1.78, m	30.8, CH	H_2-8, H_2-10, H_3-19	
10	a 1.39, m b 2.25, m	43.0, CH_2	H-9	C-6, C-8, C-9, C-11, C-12, C-13
11		77.9, C		
12	7.32, d (1.8)	143.3, CH	H-14	C-10, C-11, C-13, C-14, C-20,
13		135.1, C		
14	3.44, d (1.8)	48.9, CH	H-12	C-1, C-5, C-6, C-11, C-12, C-13, C-18, C-20
15	2.25, m	26.5, CH	H-2, H_3-16, H_3-17	C-1, C-2, C-3, C-16, C-17
16	1.03, d (7.0)	17.8, CH_3	H-15	C-2, C-15, C-17
17	0.97, d (7.0)	22.6, CH_3	H-15	C-2, C-15, C-16
18	0.82, s	16.8, CH_3		C-1, C-4, C-5, C-6, C-14
19	1.08, d (6.2)	22.5, CH_3	H-9	C-8, C-9, C-10, C-11

20		166.1, C	
21	3.85, s	52.6, CH_3	C-20
22	3.16, s	51.5, CH_3	C-1

[a] All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC). [b] Multiplicities determined by DEPT.

Figure S1.16 ^{1}H-NMR spectrum of compound **8** in MeOH-d_4.

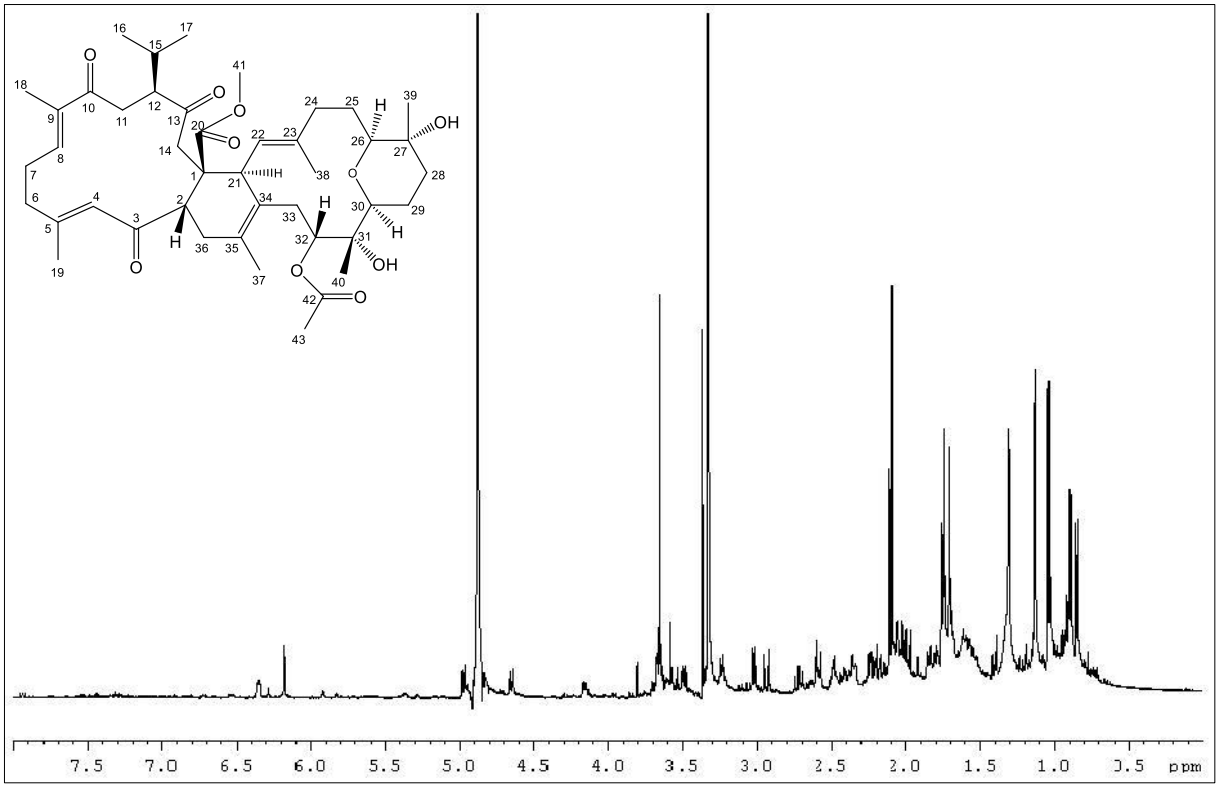


Figure S1.17 ^{13}C-NMR spectrum of compound **8** in MeOH-d_4.

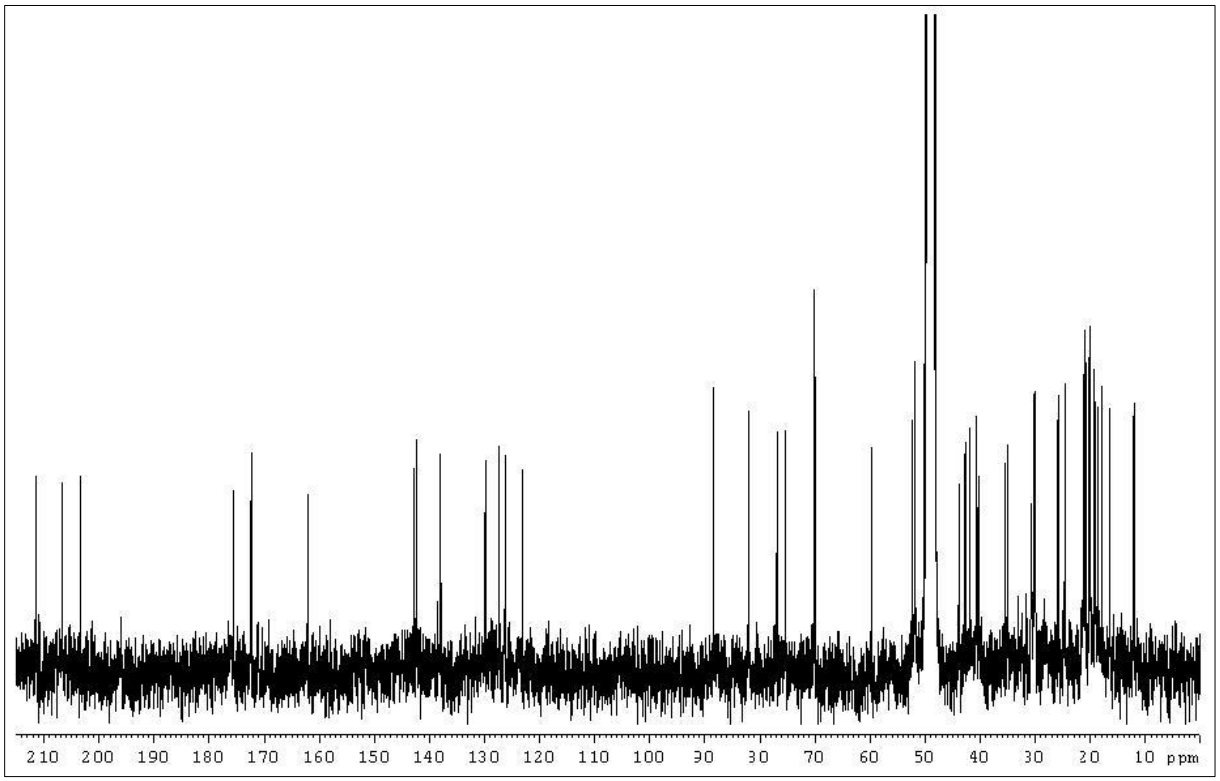


Table S1.3 NMR spectroscopic data of compound **8** in MeOH-d_4.

C	δ_H (mult, *J* in Hz)	δ_C	COSY	HMBC
1		50.2, C		
2	3.50 m	52.4, CH	H-36 a	C-4
3		203.5, C		
4	6.18 s	126.3, CH	H_3-19	C-6
5		162.3, C		
6	a 2.48[b]	40.3, CH_2		C-4, C-8, C-19
	b 2.42 m[b]			C-4, C-8, C-19
7	a 2.64[b]	25.9, CH_2	H-8	
	b 2.49[b]			C-5
8	6.36 br dd (7.0, 13.0)	142.4, CH	H-7 a, H_3-18	

9		138.0, C		
10		206.9, C		
11	a 3.35 dd (7.5, 20.0)	35.5, CH_2	H-12	C-9, C-13, C-15
	b 2.24 m[b]			C-9, C-13, C-15
12	2.60 br dd (6.9, 18.0)	59.7, CH	H_2-11, H-14 a	C-10, C-15, C-16, C-17
13		211.6, C		
14	a 2.94 d (18.0)	43.9, CH_2	H-12	C-20, C-21, C-41
	b 2.59 dd (7.0, 18.0)			C-2, C-15, C-20, C-21, C-41
15	2.26 m[b]	30.0, CH	H-11 a, H-12, H_3-16, H_3-17	C-11, C-16, C-17
16	0.86 d (7.0)	18.0, CH_3		C-12, C-15, C-17
17	0.90 d (7.0)	21.3, CH_3		C-12, C-15, C-17
18	1.74 s	12.1, CH_3		C-8, C-10
19	2.12 s	18.6, CH_3		C-4, C-6
20		175.7, C		
21	4.16 d (11.4)	42.7, CH	H-22, H_3-37	
22	4.65 d (11.4)	123.2, CH	H-21, H-24 a, H_3-38	C-24, C-34, C-38
23		142.9, C		
24	a 2.35[b]	41.9, CH_2		C-22
	b 2.07[b]			
25	a 1.81 m	25.8, CH_2	H-24 a	C-27, C-30, C-39
	b 1.62[b]			
26	3.02 d (9.9)	88.5, CH	H-25 b, H_2-28 b	C-24, C-30, C-39
27		70.1, C		
28	a 1.85[b]	40.7, CH_2		C-30
	b 1.60[b]		H-26	C-26, C-30
29	a 1.72 m	24.6, CH_2		

	b 1.55[b]		H-30	
30	3.24 dd (2.0, 13.9)	82.1, CH	H-29 b	C-31
31		75.4, C		
32	4.97 dd (2.0, 13.9)	76.9, CH	H_2-33	C-34, C-42
33	a 2.72 m[b]	30.2, CH_2	H-32	C-21, C-31, C-35
	b 2.16[b]		H-32	
34		127.4, C		
35		129.9, C		
36	a 2.37 m [b]	35.1, CH_2	H-2	C-1, C-34, C-37
	b 2.21 m [b]		H-2	C-34
37	1.76 s	20.3, CH_3		C-34, C-36
38	1.71 s	16.6, CH_3		C-22, C-24
39	1.13 s	20.0, CH_3		C-25, C-26
40	1.04 s	19.3, CH_3		C-30, C-32
41	3.65 s	51.8, CH_3		C-20
42		172.5, C		
43	2.10 s	20.9, CH_3		C-32, C-42

[a] All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC). [b] Multiplicities determined by DEPT.

Figure S1.18 ^{1}H-NMR spectrum of compound **9** in MeOH-d_4.

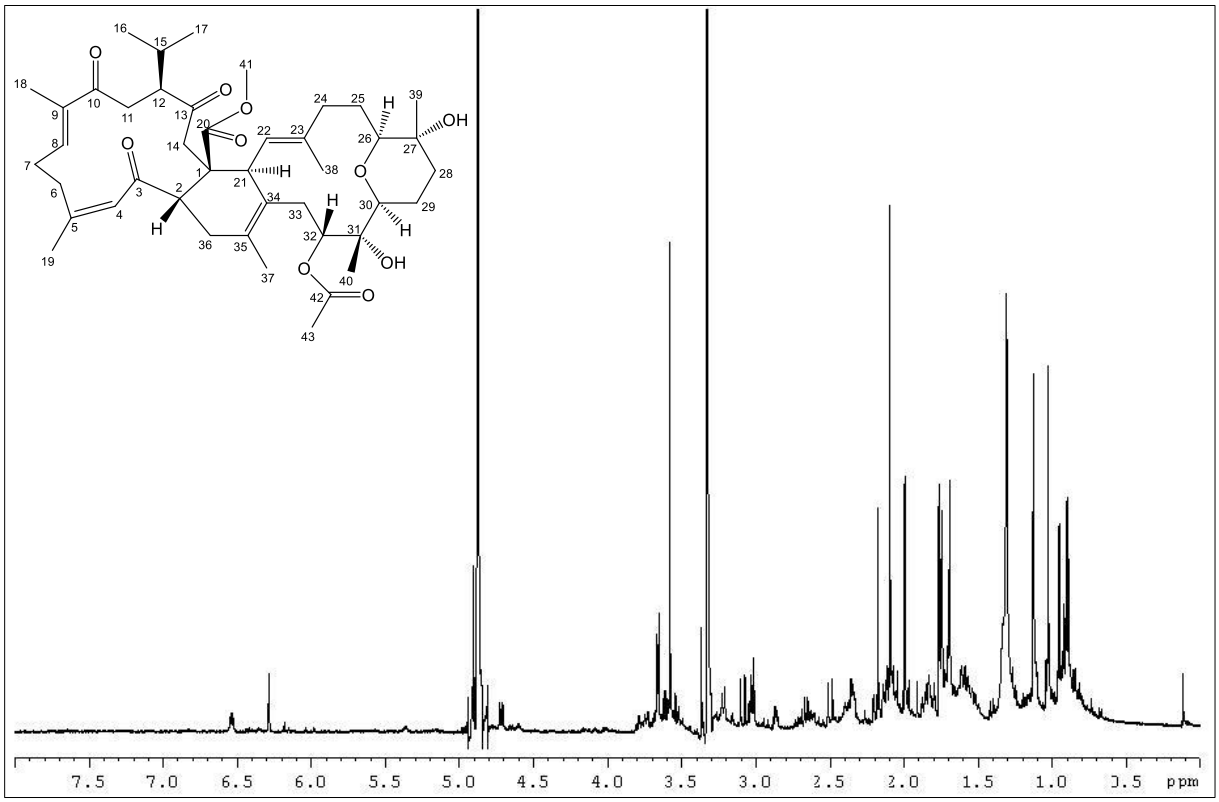


Figure S1.19 ^{13}C-NMR spectrum of compound **9** in MeOH-d_4.

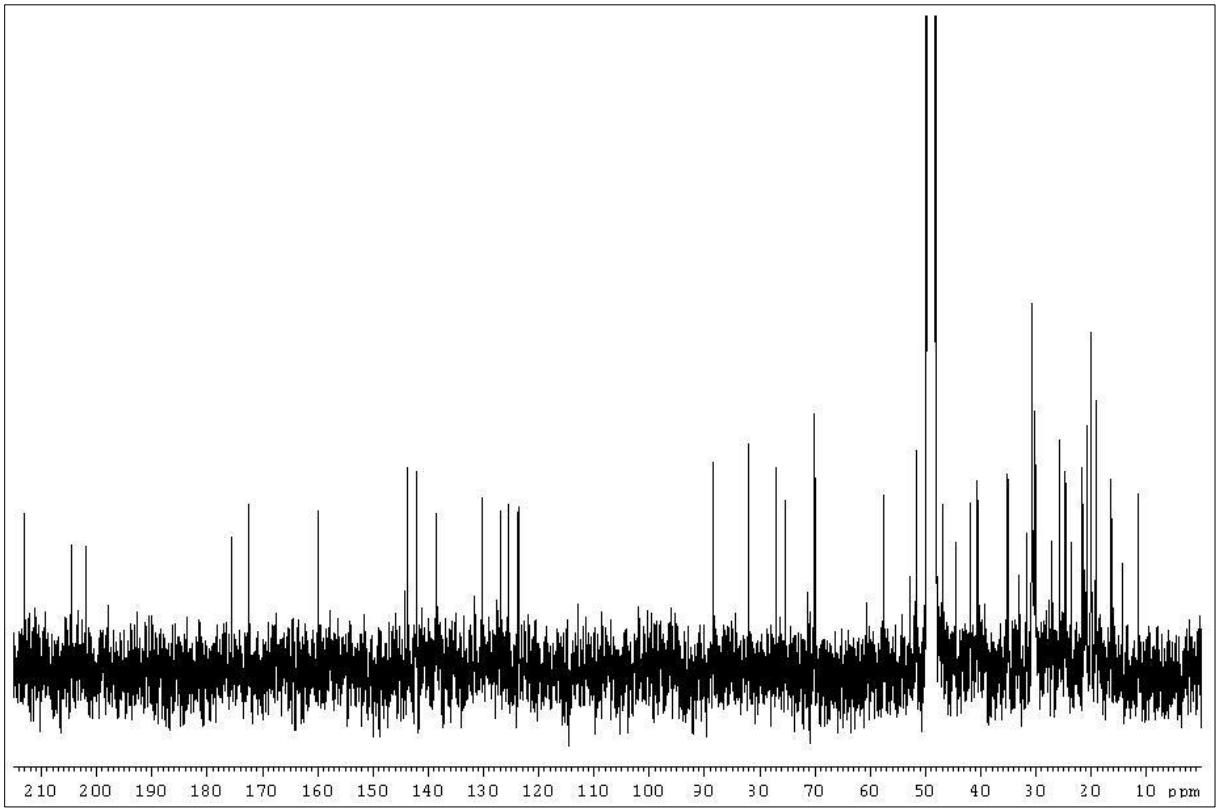


Table S1.4 NMR spectroscopic data of compound **9** in MeOH-d_4.

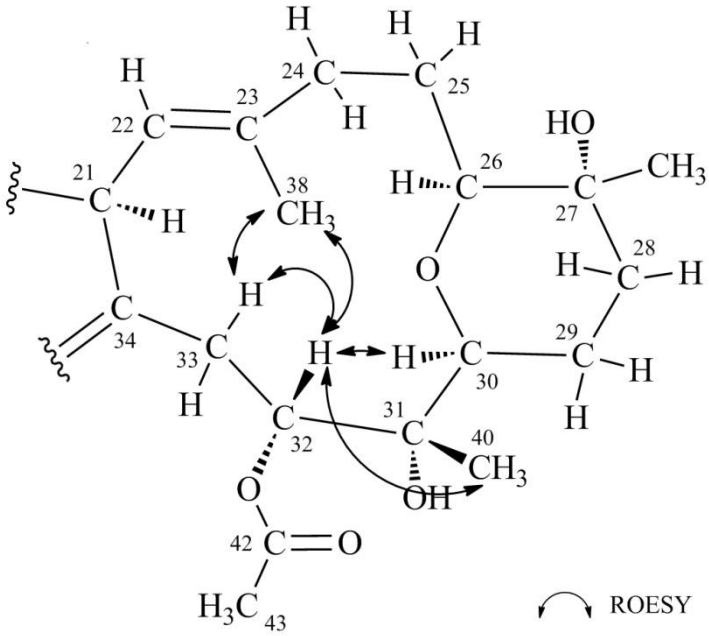
C	δ_H (mult, *J* in Hz)	δ_C	COSY	HMBC
1		49.5, C		
2	3.62 dd (8.1, 10.8)	52.8, CH	H_2-36	C-1, C-3, C-14 (w), C-20, C-21 (w), C-36
3		202.0, C		
4	6.29 s	125.6, CH	H_3-19	C-3, C-5, C-6, C-19
5		159.9, C		
6	a 3.79 m	31.7, CH_2		
	b 2.14 m[b]		H-7a	C-4, C-5
7	a 2.65 m	27.1, CH_2		C-6, C-8, C-9
	b 2.41 m		H-8, H-6a	
8	6.54 m	143.8, CH	H-7a	
9		138.5, C		
10		204.5, C		
11	a 3.04 m[b]	35.3, CH_2	H-12	C-10, C-12, C-13, C-15
	b 2.19 m[b]		H-12	C-10, C-12, C-13, C-15
12	2.87 dd (6.4, 10.5)	57.6, CH	H_2-11	C-11, C-13, C-15, C-16, C-17
13		213.0, C		
14	a 3.09 brd (18.0)	46.9, CH_2		C-1, C-2, C-13, C-20, C-21
	b 2.50 brd (18.0)			C-1, C-2, C-13, C-20, C-21
15	2.13 m[b]	30.2, CH		
16	0.90 d (6.8)	19.2, CH_3	H-15	C-12, C-15, C-17
17	0.96 d (6.8)	21.7, CH_3	H-15	C-12, C-15, C-16
18	1.78 s	11.5, CH_3	H-8	C-8, C-9, C-10,
19	2.00 s	24.9, CH_3	H-4	C-4, C-5, C-6
20		175.5, C		

21	3.75 m	44.5, CH	H-22, H_3-37 (w)	
22	4.72 d (11.7)	123.7, CH	H-21, H_3-38	C-24, C-38
23		142.0, C		
24	a 2.39 m	41.8, CH_2	H-25a	
	b 2.09 m[b]		H-25b	C-22 (w), C-23, C-26 (w), C-38 (w)
25	a 1.81 m[b]	25.8, CH_2	H-24a	C-23, C-24, C-26, C-27, C-30
	b 1.61 m[b]		H-26	
26	3.03 m[b]	88.4, CH	H-25b, H-28b	C-24, C-25, C-27, C-28, C-30, C-39
27		70.1, C		
28	a 1.85 m	40.6, CH_2	H_2-29	C-26, C-27, C-30, C-39
	b 1.60 m		H-26	C-27, C-29
29	a 1.72 m	24.6, CH_2		
	b 1.54 m		H-30, H-28a	
30	3.23 m	82.0, CH	H-33a (w), H-29b, H_3-38	C-26 (w), C-28 (w), C-31, C-40
31		75.3, C		
32	4.91 m	77.0, CH	H_2-33	C-33 (w), C-34, C-40 (w), C-42
33	a 2.69 m	30.0, CH_2	H-32	C-21, C-31, C-32, C-34, C-35
	b 2.25 m			C-34
34		127.0, C		
35		130.2, C		
36	a 2.36 m	35.1, CH_2	H-2	
	b 2.36 m		H-2	
37	1.75 s	20.2, CH_3	H-21	C-34, C-35, C-36
38	1.70 s	16.4, CH_3	H-22	C-22, C-23, C-24
39	1.13 s	20.0, CH_3		C-26, C-27, C-28

40	1.03 s	19.2, CH_3	C-30, C-31, C-32
41	3.58 s	51.6, CH_3	C-20
42		172.5, C	
43	2.10 s	20.9, CH_3	C-32, C-42

[a] All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC). [b] Multiplicities determined by DEPT.

Figure S1.20 Proposed stereostructural variation in ring C of the biscembranes **8** and **9** with selected key ROESY correlations.



SUPPLEMENTARY INFORMATION FOR CHAPTER II

SI for CHAPTER II was also provided in Hertzer et al. 2020.[3]

Table of contents

Figure S2.1 ^{1}H NMR spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in $CDCl_3$.

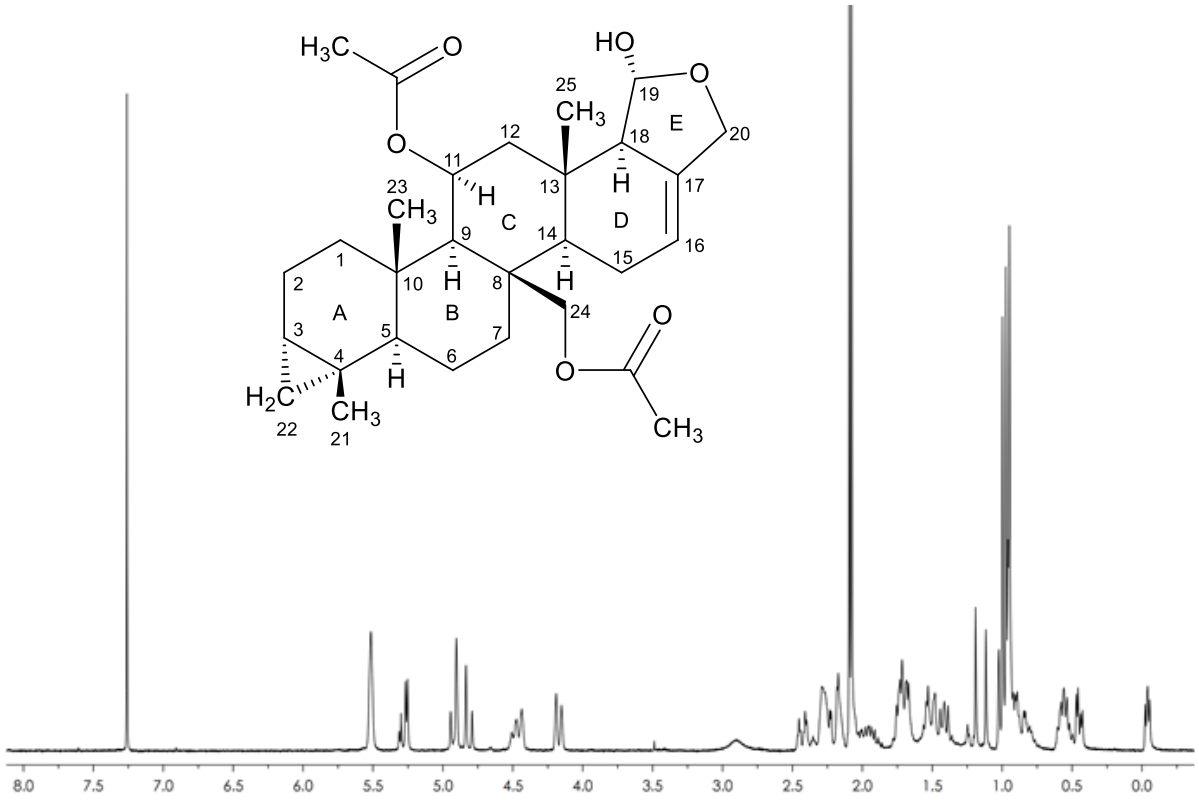


Figure S2.2 ^{13}C NMR spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in $CDCl_3$.

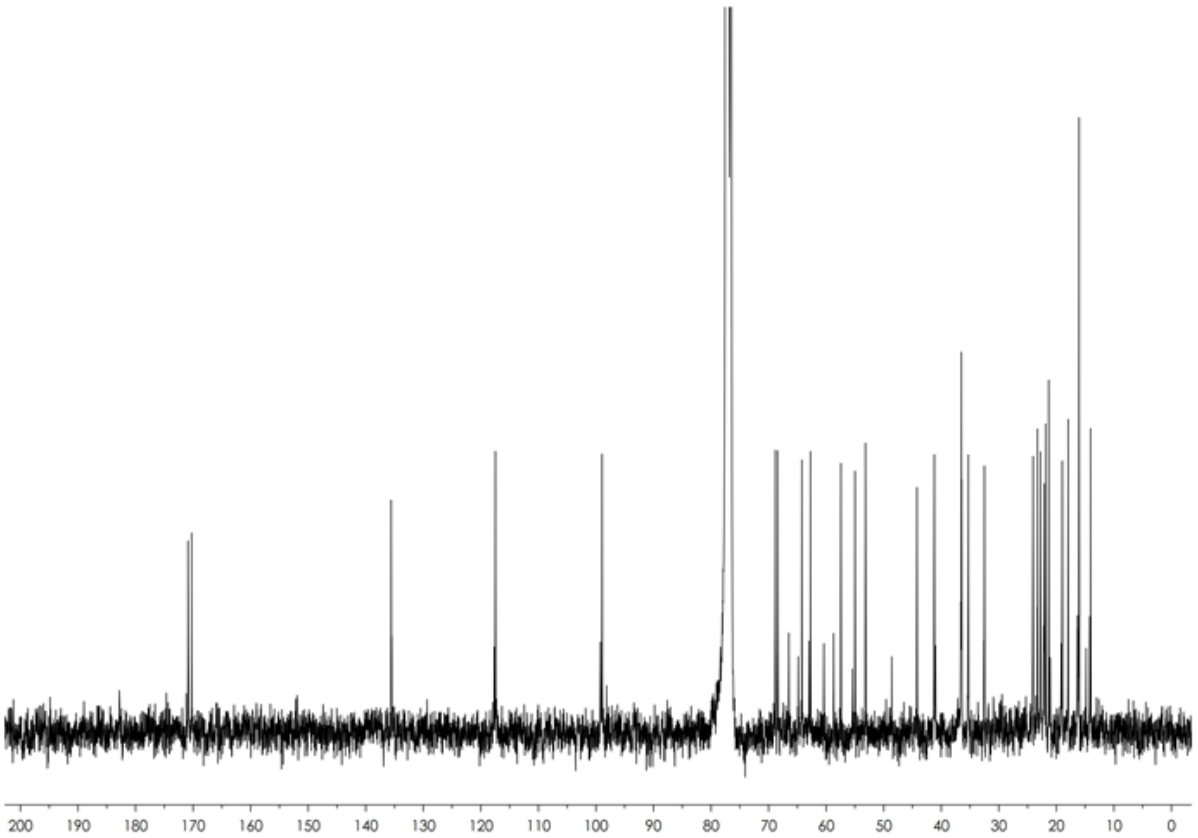


Figure S2.3 DEPT spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in $CDCl_3$.

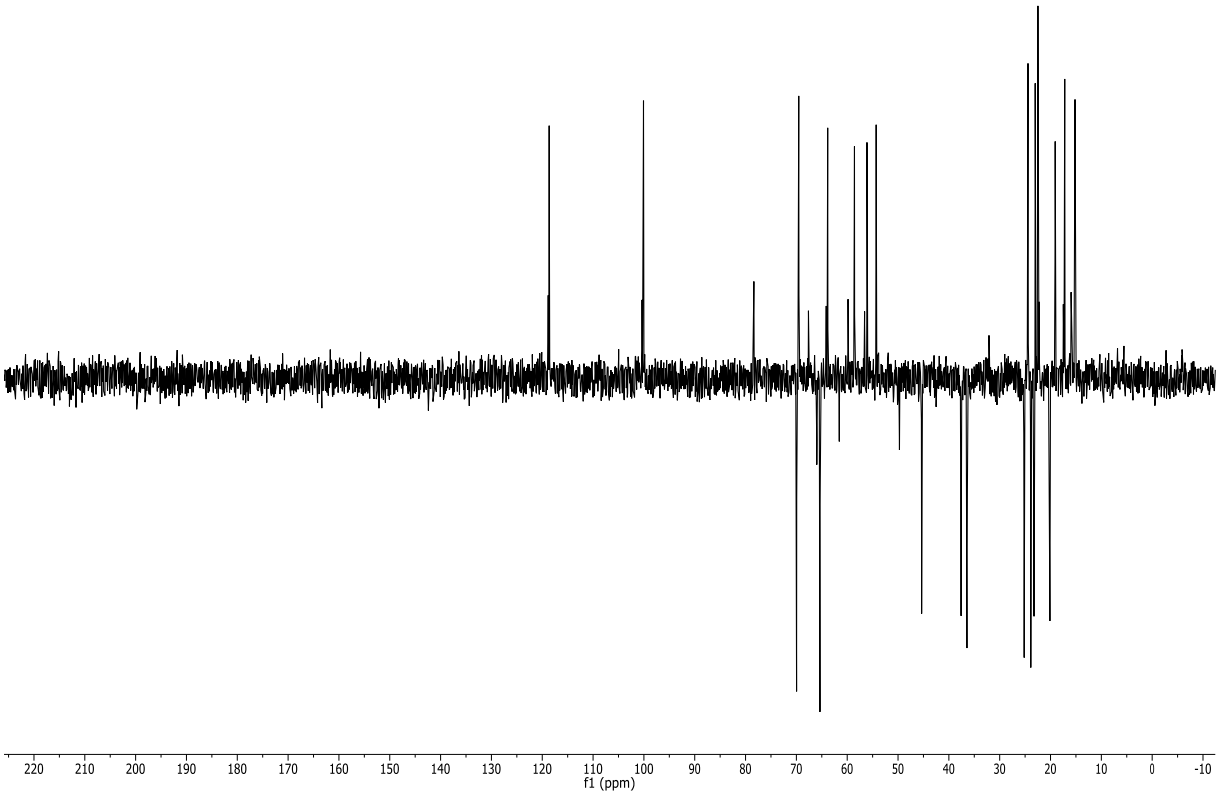


Figure S2.4 HSQC spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in $CDCl_3$.

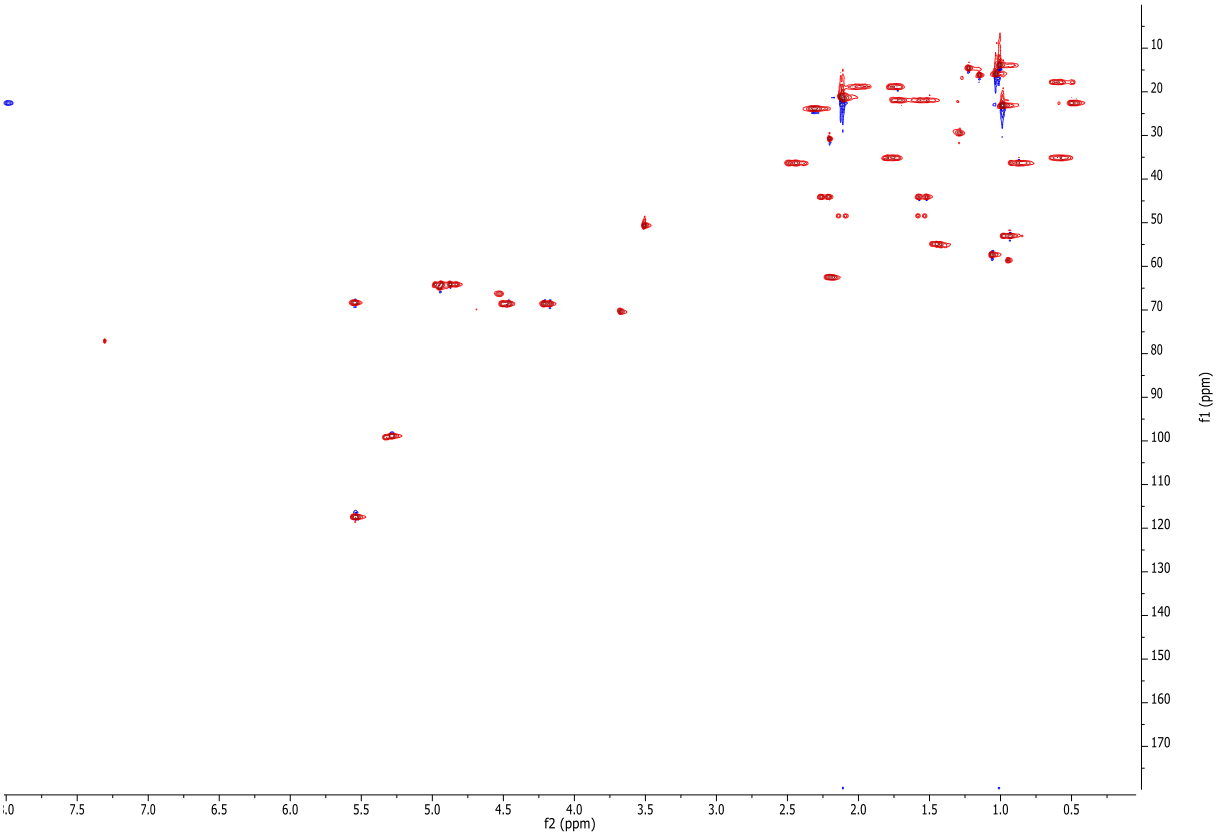


Figure S2.5 HMBC spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in $CDCl_3$.

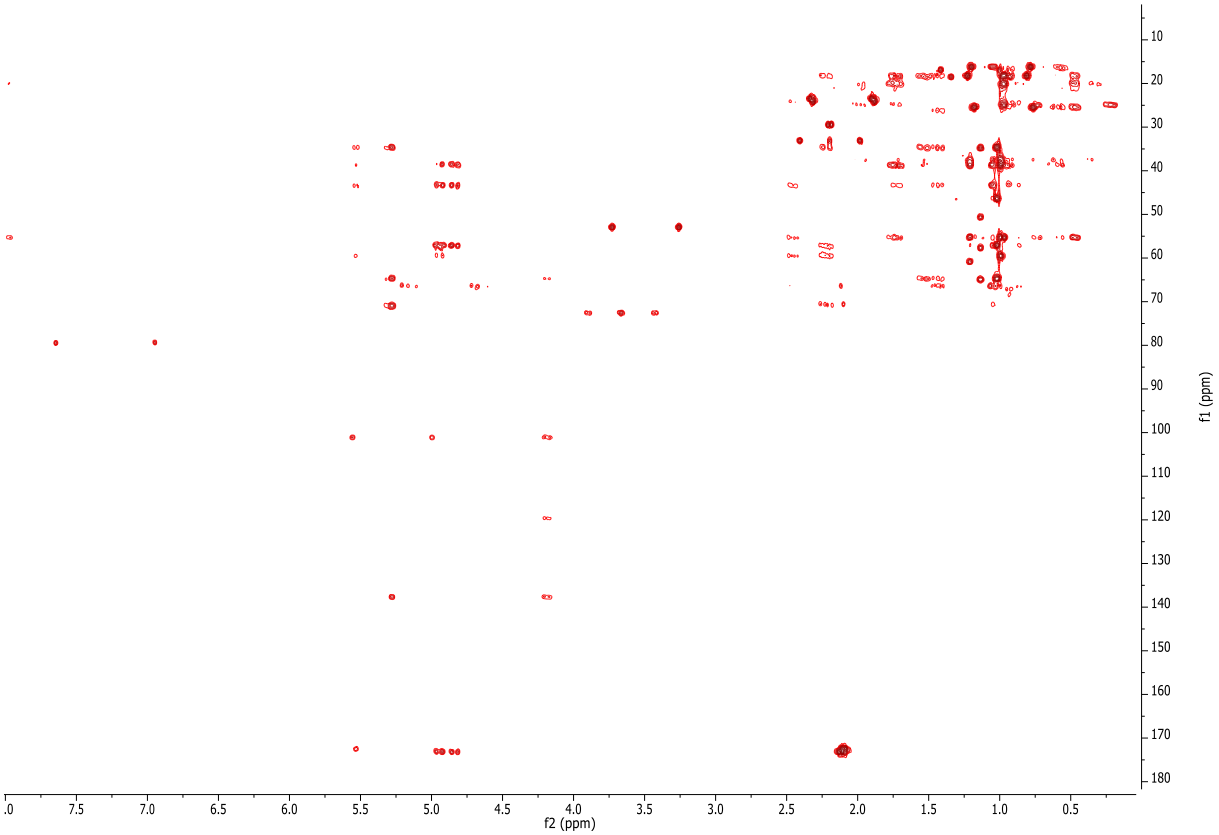


Figure S2.6 COSY spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in $CDCl_3$.

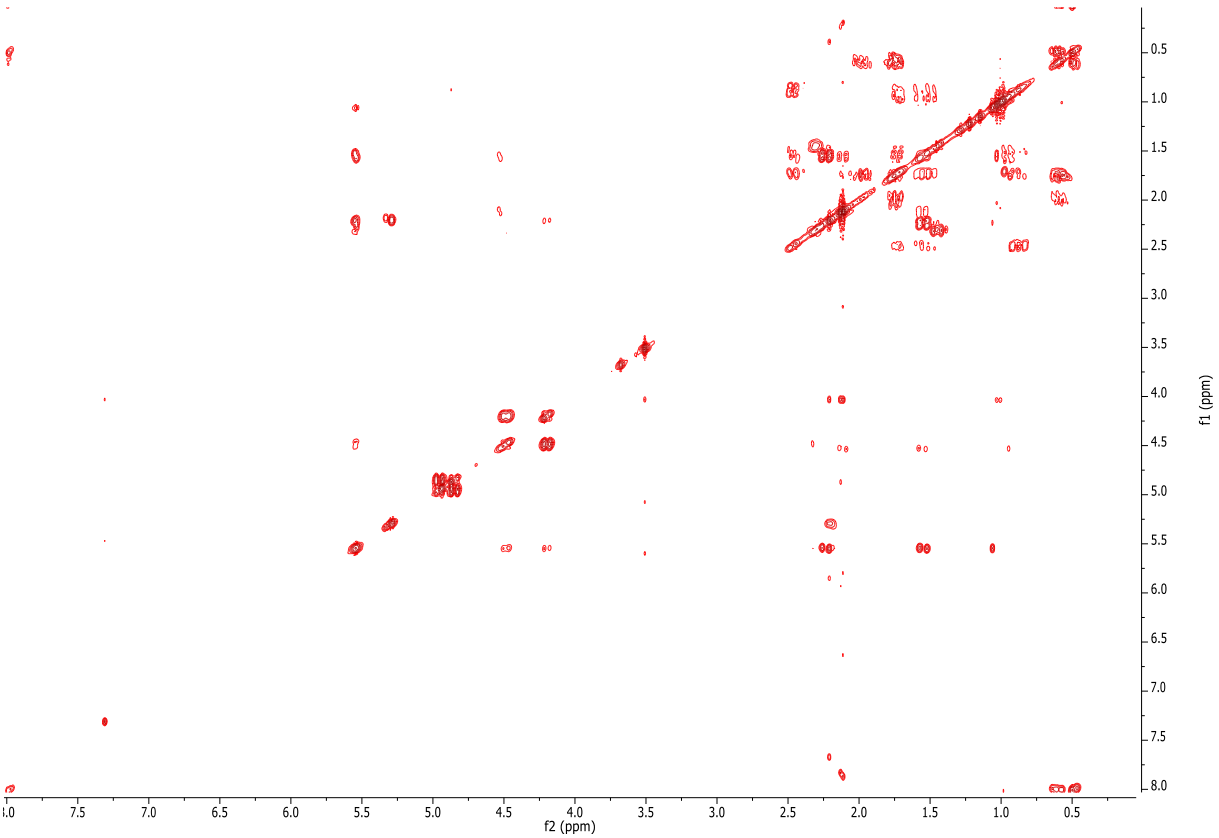


Figure S2.7 NOESY spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in $CDCl_3$.

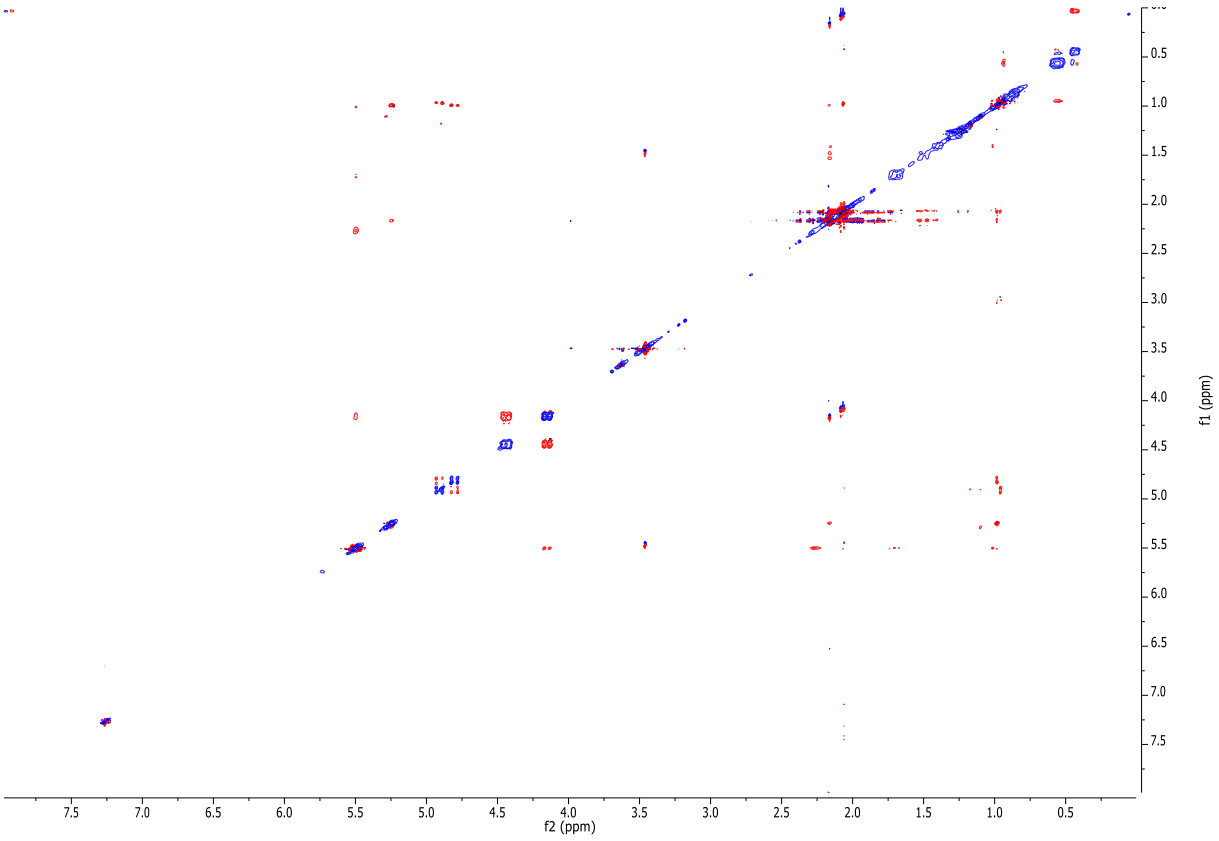


Figure S2.8 Key COSY and HMBC correlations of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin.

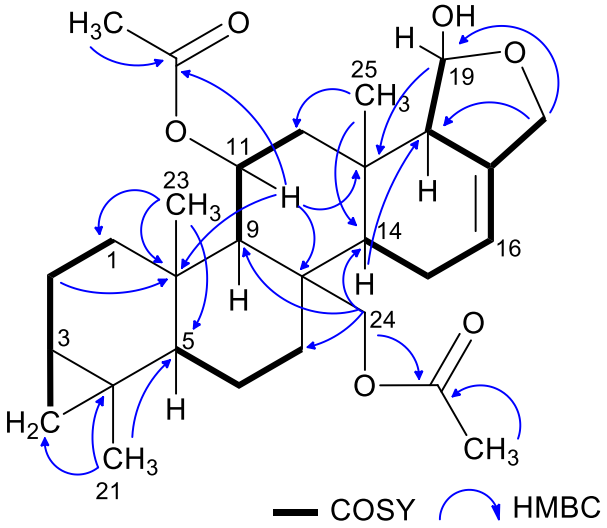


Figure S2.9 Key NOE correlations of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin.

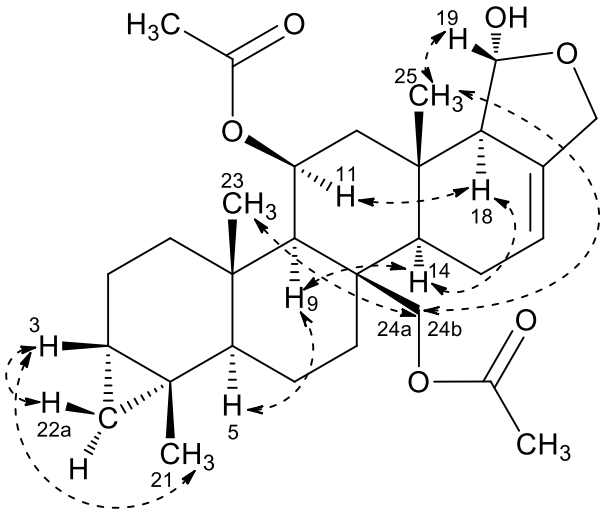


Figure S2.10 UV spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin in ACN.

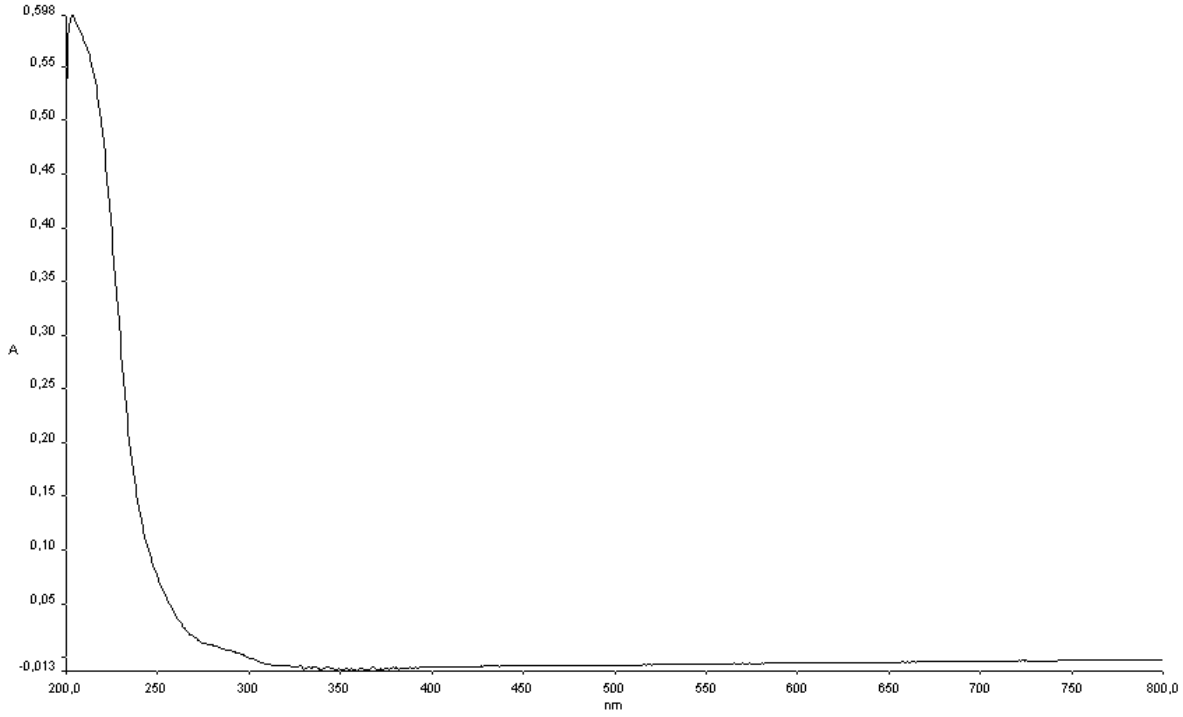


Figure S2.11 IR (ATR) spectrum of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin.

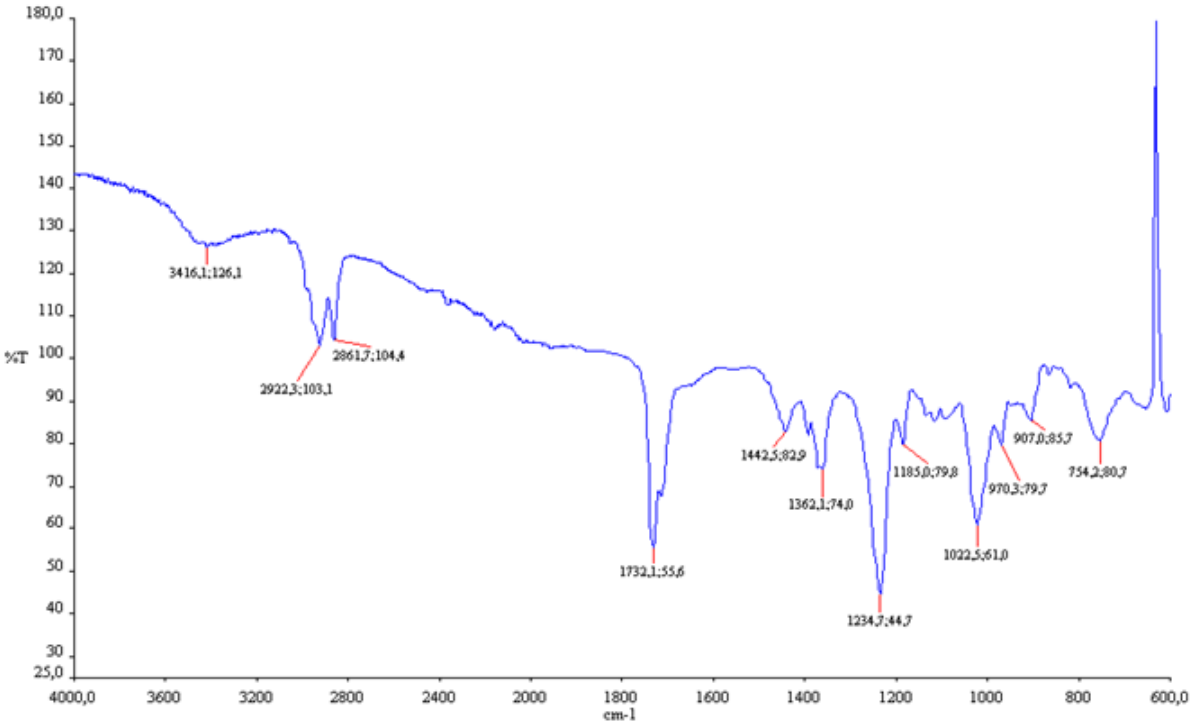


Figure S2.12 HRAPCIMS measurement of 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin, yielding *m/z* 487.3054 $[M+H]^+$, calcd. 487.3060.

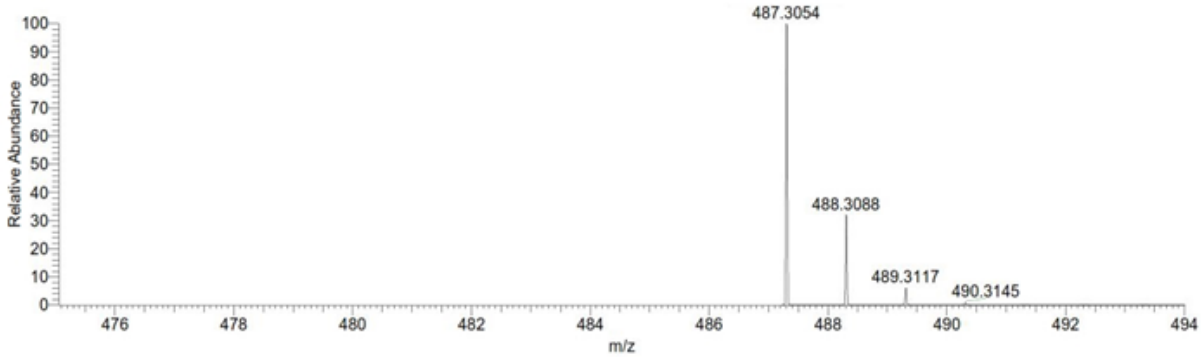


Figure S2.13 Reticulate spongin fibre skeletal arrangement of *S.* cf. *agaricima*

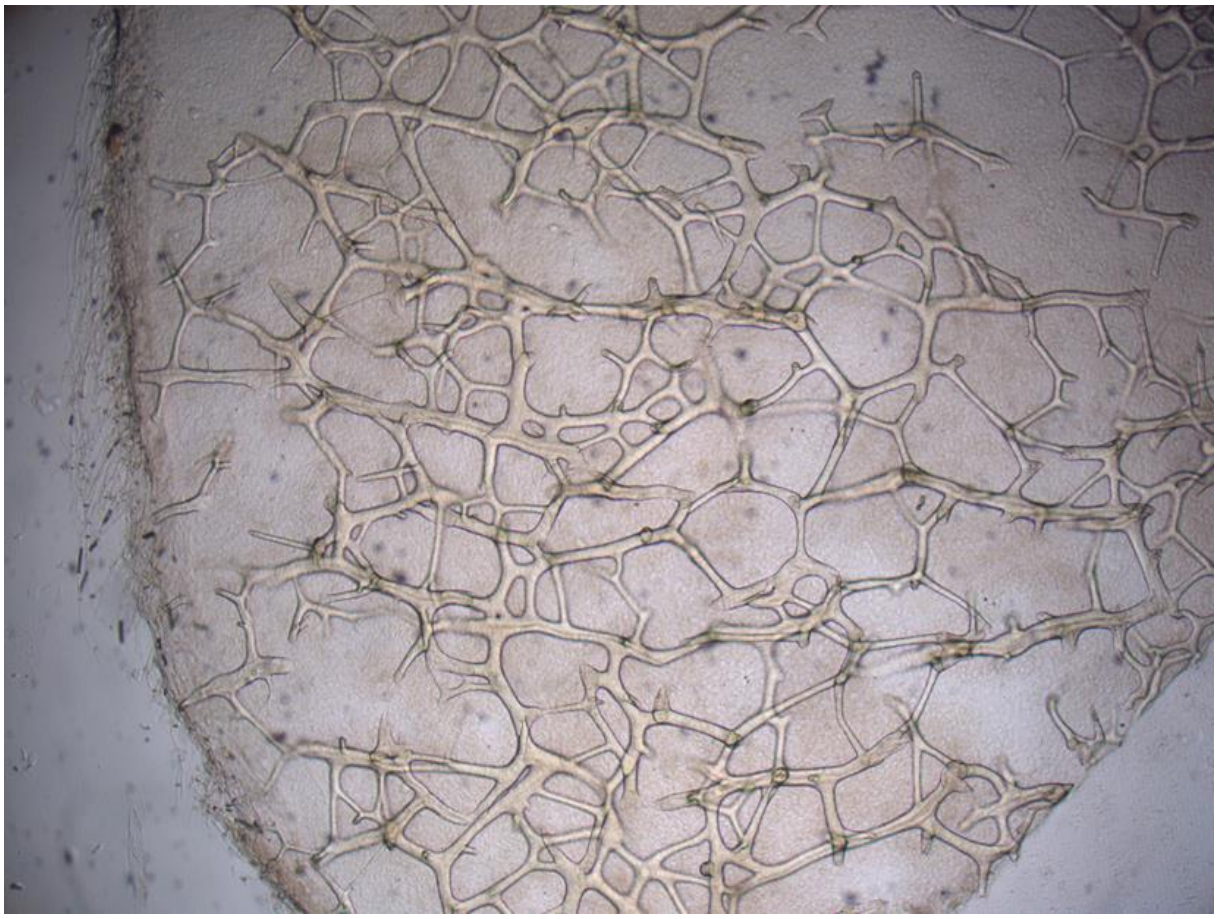


Table S2.1 Recipe for 53. Corynebacterium Liquid Medium

Casein peptone, tryptic digest	10 g
Yeast extract	5 g
Glucose	5 g
NaCl	5 g
Distilled water	1000 mL

Adjust pH to 7.2 – 7.4

Table S2.2 Raw data OD measurements of antibacterial activity assays.

EtOAc-fractions of *D. stellata* nudibranchs, egg ribbons and the dietary sponge *Spongia* cf. *agaricina* tested against *Arthrobacter crystallopoietes* (DSM 20117). Concentrations of 50, 100 and 200 µg/mL per well, respectively, were used. Antibacterial activity was measured as bacterial growth inhibition in liquid media. Mean OD values (λ 560 nm) of negative DMSO controls were set as 100% (maximal bacteria growth and OD). Percentages for individual sample wells were calculated as individual sample OD*100/mean OD negative DMSO controls. Sample values marked in bold.

SUNRISE; Serial number: 605000077; Firmware: V 3.31 25/08/05; XFLUOR4 Version: V 4.51

Date:	25.1.16
Time:	15:38
Measurement mode:	Absorbance
Measurement wavelength:	560 nm
Read mode:	Center

Rawdata

<>												
A	1,0980	0,9630	0,1780	0,1070	0,1090	0,1100	0,1330	0,1890	0,1800	1,0970	1,2330	1,1000
B	1,1370	0,9620	0,9870	0,1360	1,3260	1,4470	**0,3050**	**0,1420**	**0,1380**	1,2430	1,1960	1,2100
C	0,8790	0,9660	1,0300	0,9270	1,1040	1,1640	**0,3620**	**0,2880**	**0,2180**	1,2630	1,1710	1,0580
D	0,9750	1,0450	1,0330	0,1280	0,2420	0,2980	**0,2854**	**0,2000**	**0,1930**	1,2180	1,2680	1,1360
E	0,1380	0,9210	0,8040	0,2070	1,2720	1,1440	1,3250	1,2970	0,9140	1,2780	1,2620	1,2680
F	0,9490	0,9830	1,0000	0,2160	1,1080	1,1380	0,9910	1,0190	0,9070	1,2760	1,1750	1,1450
G	0,1840	0,2110	0,3920	1,1270	0,9280	1,0510	0,9750	1,0780	1,2420	0,1500	0,1650	0,1390
H	0,1170	0,1240	0,1200	0,1680	1,1360	1,1940	1,2770	1,2920	1,2800	1,0570	0,8860	1,3270

Calculated percentages of *Arthrobacter crystallopoietes* (DSM 20117) bacterial growth in comparison to the negative DMSO controls:

	50 µg/mL	**100 µg/mL**	**200 µg/mL**
D. stellata nudibranchs	28,0%	13,0%	12,7%
D. stellata egg ribbons	33,2%	26,4%	20,0%
S. cf. *agaricina*	26,2%	18,3%	17,7%
(+) Control Carbenicillin	15,1%	13,8%	12,8%

After complete structure elucidation the isolated new scalarane 3 cyclopropyl-12-deacetoxy-11,24-diacetoxy-deoxoscalarin was tested against *Bacillus megaterium* (DSM 32). Concentrations of 100 µg/mL (205 µM), 50 µg/mL (103 µM) and 25 µg/mL (51 µM) per well, respectively, were used. Sample values marked in bold.

SUNRISE; Serial number: 605000077; Firmware: V 3.31 25/08/05; XFLUOR4 Version: V 4.51

Date: 12.5.17

Time: 10:39

Measurement mode: Absorbance

Measurement wavelength: 560 nm

Read mode: Center

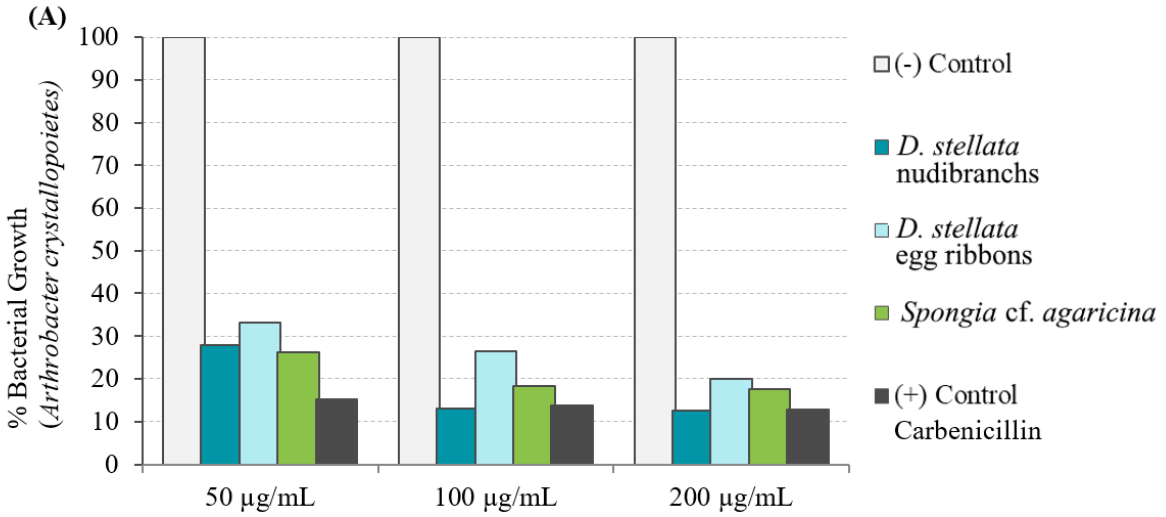
Rawdata

<>												
A	0,9180	0,7280	0,7790	1,1300	0,5990	0,7090	0,8250	0,6990	0,7560	0,7370	0,8910	0,8870
B	0,6060	0,7630	0,8890	0,8690	0,6480	0,7100	1,0170	0,6910	0,7520	0,7330	0,6500	0,9720
C	1,0320	0,6760	0,7790	0,7090	0,5460	0,5600	0,7590	0,7360	0,6200	0,5920	0,7860	1,0220
D	0,6220	0,8480	0,5930	0,6950	1,4880	1,0860	0,9800	0,7400	0,6990	0,7230	0,6230	0,9240
E	1,0280	0,9770	0,7980	0,7550	0,7930	0,9210	0,8050	0,7770	0,6840	0,7660	1,1460	0,8380
F	0,6140	0,5580	0,5580	0,6640	1,0300	1,3960	1,5200	1,2160	0,0610	**0,0030**	**0,1040**	**0,1650**
G	0,6940	0,8100	0,9600	1,1440	0,9830	1,0780	1,3600	1,0600	1,1430	1,0820	1,2730	1,1320
H	0,1470	0,1450	0,1520	0,0960	0,1400	0,2160	0,6570	1,0870	1,3260	1,1350	1,0830	1,1520
MW	0,7663	0,7707	0,9477	1,1803	0,7663	0,7707	0,9477	1,1803	0,7663	0,7707	0,9477	1,1803
<>												
A	119,79	94,46	82,20	95,74	78,16	92,00	87,06	59,22	98,65	95,63	94,02	75,15
B	79,08	99,01	93,81	73,62	84,56	92,13	107,32	58,54	98,13	95,11	68,59	82,35
C	134,67	87,72	82,20	60,07	71,25	72,66	80,09	62,36	80,90	76,82	82,94	86,59
D	81,17	110,03	62,57	58,88	194,17	140,92	103,41	62,69	91,21	93,81	65,74	78,28
E	134,15	126,77	84,21	63,96	103,48	119,51	84,95	65,83	89,26	99,39	120,93	71,00
F	80,12	72,40	58,88	56,26	134,41	181,14	160,39	103,02	7,96	**0,39**	**10,97**	**13,98**
G	90,56	105,10	101,30	96,92	128,27	139,88	143,51	89,81	149,15	140,40	134,33	95,91
H	5,48	7,14	8,13	9,60	18,27	28,03	69,33	92,09	173,03	147,28	114,28	97,60

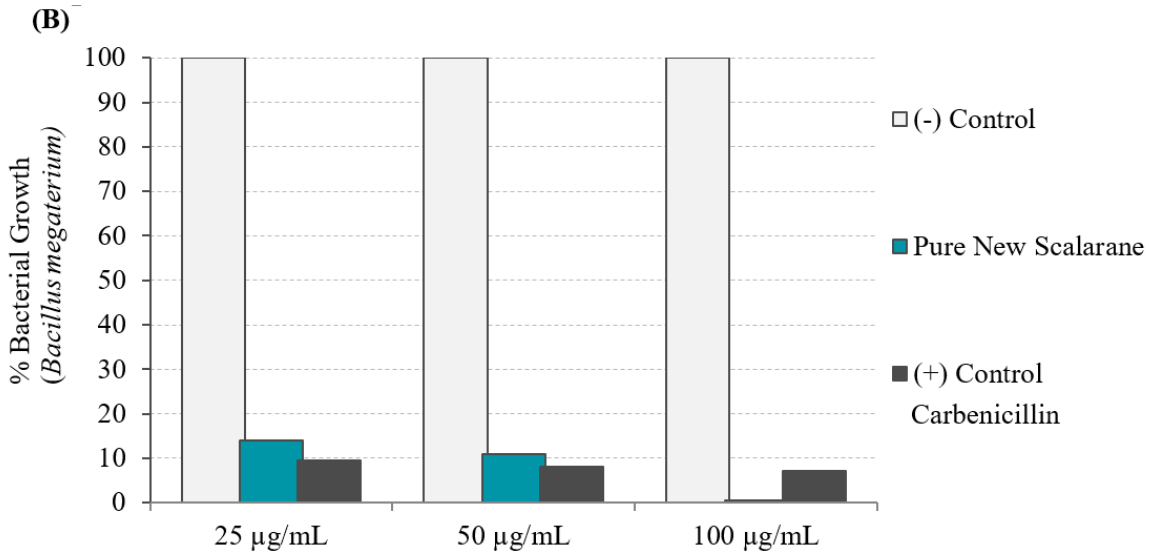
Calculated percentages of *Bacillus megaterium* (DSM 32) bacterial growth in comparison to the negative DMSO controls:

	25 µg/mL	**50 µg/mL**	**100 µg/mL**
Pure new Scalarane	14,0%	11,0%	0,4%
(+) Control Carbeicillin	9,6%	8,1%	7,1%

Figure S2.14 Antibacterial activity assay of extracts and the pure compound 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin against Gram-positive bacteria



EtOAc-fractions of *D. stellata* nudibranchs, egg ribbons and the dietary sponge *Spongia* cf. *agaricina* tested against *Arthrobacter crystallopoietes* (DSM 20117). The bacterial growth was inhibited and reduced to 28.0% (nudibranchs), 33.2% (eggs) and 26.2% (sponge) at 50 µg/mL, to 13.0% (nudibranchs), 26.4% (eggs), and 18.3% (sponge) at 100 µg/mL, and to 12.7% (nudibranchs), 20.0% (eggs), and 17.7% (sponge) at 200 µg/mL, in comparison to the negative control (DMSO).



Isolated new scalarane 12-deacetoxy-4-demethyl-11,24-diacetoxy-3,4-methylenedeoxoscalarin tested against *Bacillus megaterium* (DSM 32). The pure scalarane reduced bacterial growth to 0.4% at 100 µg/mL (205 µM), 11.0% at 50 µg/mL (103 µM), and 14.0% at 25 µg/mL (51 µM) in comparison to the negative control (DMSO). Antibacterial activity measured as bacterial growth inhibition in a liquid medium. Mean OD values (λ 560 nm) of negative DMSO controls set as 100% (maximal bacteria growth and OD). Percentages for individual sample wells calculated as individual sample OD*100/mean OD negative DMSO controls.

SUPPLEMENTARY INFORMATION FOR CHAPTER III

Table of contents

Figure S3.1 ^{1}H-NMR spectrum of latrunculin A in $CDCl_3$.

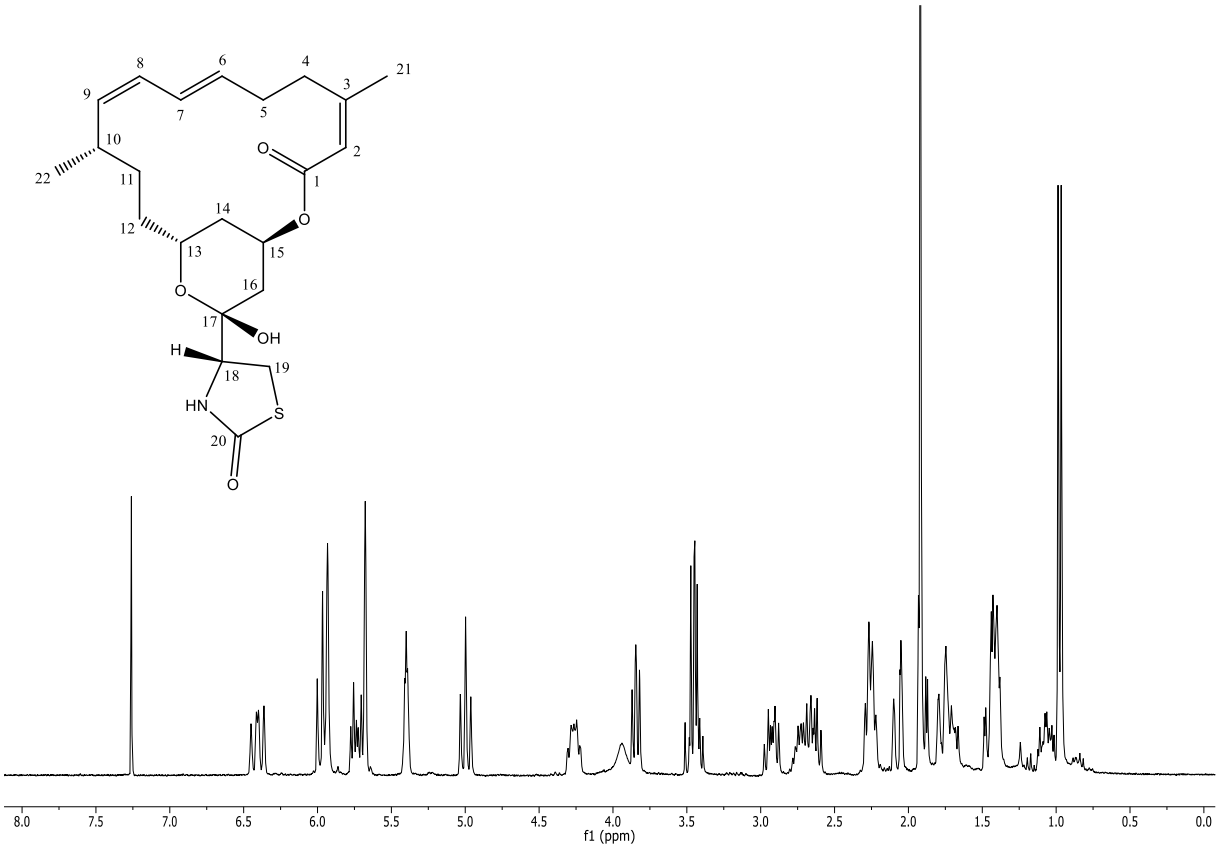


Figure S3.2 ^{13}C-NMR spectrum of latrunculin A in $CDCl_3$.

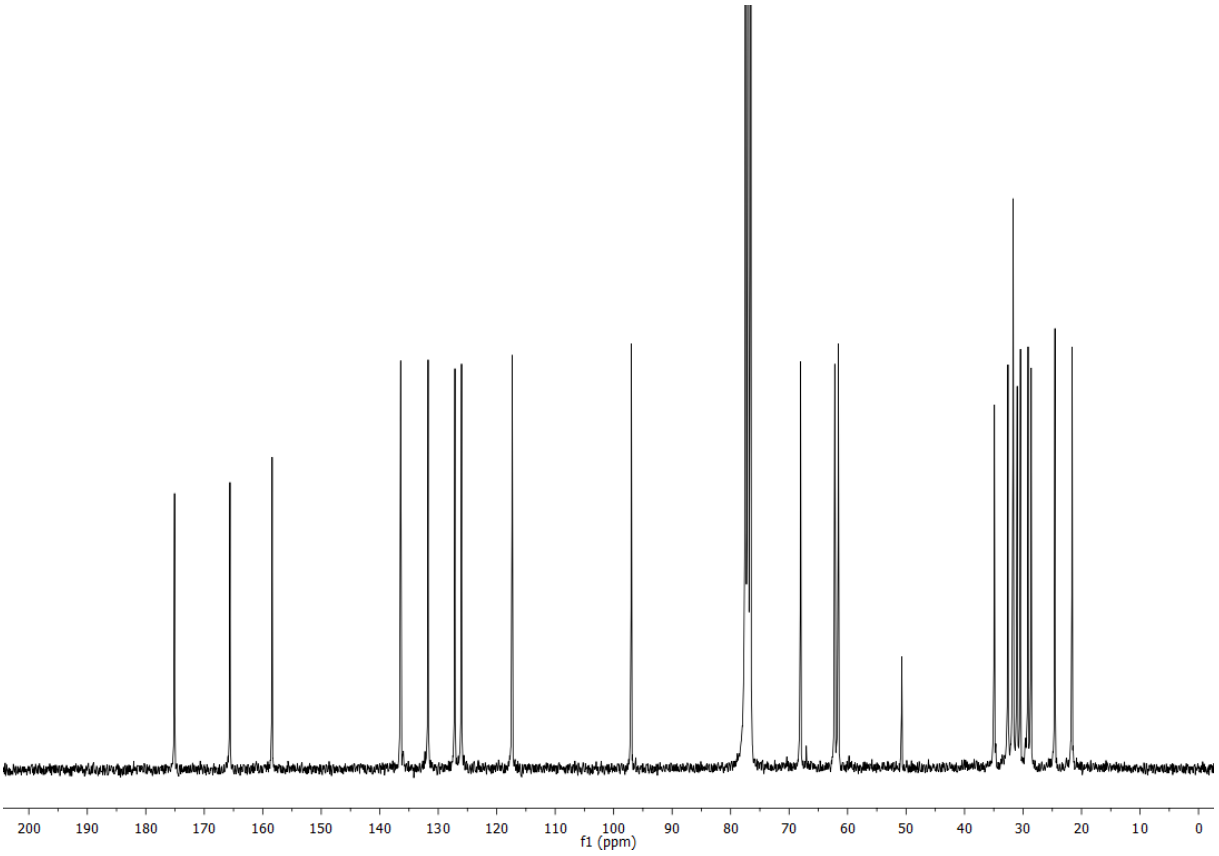


Table S3.1 A comparison of the ^{1}H-NMR Data reported for latrunculin A

C	Experimental Data (MeOD) δ_H	Experimental Data ($CDCl_3$) δ_H	Kashman et al. 1980,[550] ($CDCl_3$) δ_H	Groweiss et al., 1983,[705] ($CDCl_3$) δ_H	Smith et al. 1992,[568] ($CDCl_3$) δ_H	White et al. 1992,[569] ($CDCl_3$) δ_H	Houssen et al. 2006,[584] (DMSO- d_6) δ_H	Fürstner et al. 2007,[588] ($CDCl_3$) δ_H
1								
2	5.62 brs	5.68 brs	5.69 d	5.69 d	5.62 s	5.62 s	5.54 d	5.65-5.69 m
3								
4 α	3.43 - 3.35 m	3.48 - 3.39 m		3.00 dt	3.39-3.43 m		3.18 dt	
4 β	2.52 – 2.34 m	2.72 m	2.60 m	2.60 dt	2.64-2.74 m	2.74-2.63 m	2.30 dt	2.62-2.77 m
5 α	2.32 m	2.66 m	2.26 m	2.26 m	2.23-2.28 m	2.30-2.26 m	2.20 m	2.23-2.34 m
5 β	2.24 m	2.26 m		2.26 m			2.12 m	
6	5.76 ddd	5.73 dt	5.74 dt	5.74 dt	5.74 dt	5.73 dt	5.69 ddd	5.74 s
7	6.56 dd	6.41 dd	6.41 dd	6.41 dd	6.40 dd	6.40 dd	6.46 dd	6.40 dt
8	6.01 t	5.97 t	5.98 t	5.98 t	5.97 dd	5.97 t	5.93	5.97 dd
9	4.98 m	5.00 t	5.02 t	5.02 t	5.01 dd	5.01 t	4.91	5.01 dd
10	2.91 m	2.92 m	2.83 m	2.83 m	2.86-2.92 m	2.91-2.85 m	2.80	2.86-2.95 m
11 α	2.10 dt	2.08 dt			2.04-2.07 m	2.06 dt	1.69	2.04 - 2.07 m

11 β	1.79 dd	1.75 m					0.91	
12	1.08 - 1.02 m	1.12 - 1.01 m			1.06-1.11 m	1.12-1.03 m	1.22	1.01-1.14 m
13	3.83 t	3.85 t	4.29 m	4.29 m	4.22-4.26 m	4.25 m	4.28	4.2-4.3 m
14 α	1.65 dd	1.68 m				1.71 m	1.42 bs	
14 β	1.48 m	1.48 d				1.49-1.39 m	1.32 ddd	
15	5.17 brt	5.40 p	5.43 bt	5.43 bt	5.43 m	5.43 p	4.99 bs	5.42 m
16	1.41 dq	1.42 m			1.25-1.96 m	1.82 d	1.97 bd	1.24-1.98 m
						1.79 d	1.53 dd	
17								
18	4.46 dt	4.26 dt	3.87 dd	3.87 dd	3.83-3.86 m	3.89 s	3.63 dd	3.82-3.93 m
						3.85 dd		
19	3.47 m	3.45 m	3.51 dd	3.51 dd	3.46-3.51 m	3.46 dd	3.48 dd	3.37-3.52 m
			3.48 dd			3.44 dd	3.32 dd	
20								
21	1.94 s	1.92 s	1.92 d	1.92 d	1.93 s	1.92 dd	1.83 d	1.93 s
22	0.99 d	0.98 d	0.98 d	0.98 d	0.99 d	0.98 d	0.87 d	0.98 d
NH			6.3				7.99	
OH			5.8	5.80 bs	5.69 s	5.69 s	5.66	5.69 brs

[a] ^{1}H (600 MHz), all δ in ppm relative to MeOD = 3.35/49.0, or $CDCl_3$ = 7.26/77.0.

Table S3.2 A comparison of the ^{13}C-NMR Data reported for latrunculin A

C	Experimental Data (MeOD) δ_C	Experimental Data ($CDCl_3$) δ_C	Kashman et al. 1980,[550] ($CDCl_3$) δ_C	Groweiss et al., 1983,[705] ($CDCl_3$) δ_C	Smith et al. 1992,[568] ($CDCl_3$) δ_C	White et al. 1992,[569] ($CDCl_3$) δ_C	Houssen et al. 2006,[584] (DMSO-d_6) δ_C	Fürstner et al. 2007,[588] ($CDCl_3$) δ_C	
1	168.5	165.6	166.0	166.0	165.3	165.3	166.3	165.4	qC
2	118.9	117.4	117.6	117.6	117.3	117.3	118.7	117.3	CH
3	160.1	158.4	158.3	158.3	158.5	158.4	157.8	158.3	qC
4	33.5	32.6	32.7	32.7	32.7	32.7	32.7	32.7	CH_2
5	31.8	30.4	30.6	30.6	30.4	30.4	30.8	30.5	CH_2
6	133.0	131.7	131.8	131.8	131.8	131.8	132.6	131.8	CH
7	127.6	126.0	126.3	126.3	126.0	126.0	126.8	126.0	CH
8	128.8	127.2	127.3	127.3	127.1	127.2	128.1	127.2	CH
9	137.1	136.4	136.5	136.5	136.5	136.5	136.5	136.5	CH
10	30.3	29.1	29.2	29.2	29.2	29.2	29.2	29.2	CH
11	32.1	31.7	31.2	31.2	31.0	31.0	31.6	31.0	CH_2
12	32.4	31.0	31.8	31.8	31.4	31.4	32.1	31.5	CH_2
13	64.5	62.2	62.3	62.3	62.3	62.3	61.6	62.3	CH

14	36.4	34.9	35.1	35.1	34.9	34.9	35.7	34.9	CH_2
15	69.3	68.1	68.1	68.1	68.2	68.2	67.6	68.2	CH
16	33.0	31.7	32.1	32.1	31.7	31.8	32.7	31.8	CH_2
17	97.9	97.0	96.9	96.9	97.3	97.3	96.4	97.3	qC
18	63.2	61.6	62.1	62.1	62.3	62.3	62.9	62.4	CH
19	29.6	28.6	28.7	28.7	28.7	28.7	28.5	28.7	CH_2
20	177.8	175.1	175.5	175.5	174.6	174.6	173.9	174.8	qC
21	25.0	24.5	24.7	24.7	24.5	24.5	25.0	24.5	CH_3
22	22.3	21.6	21.8	21.8	21.6	21.6	22.6	21.6	CH_3

[a] ^{13}C NMR (150 MHz), all δ in ppm relative to MeOD = 3.35/49.0, or $CDCl_3$ = 7.26/77.0.

[b] Multiplicities determined by DEPT

.

Figure S3.3 Comparison of HPLC-MS chromatograms of the investigated *Chromodoris* species, mucus collected from the back (notum) and the trail of *C. annae*, and the associated sponge *Cacospongia mycofijiensis*, showing the presence of LatA (1) in all samples.

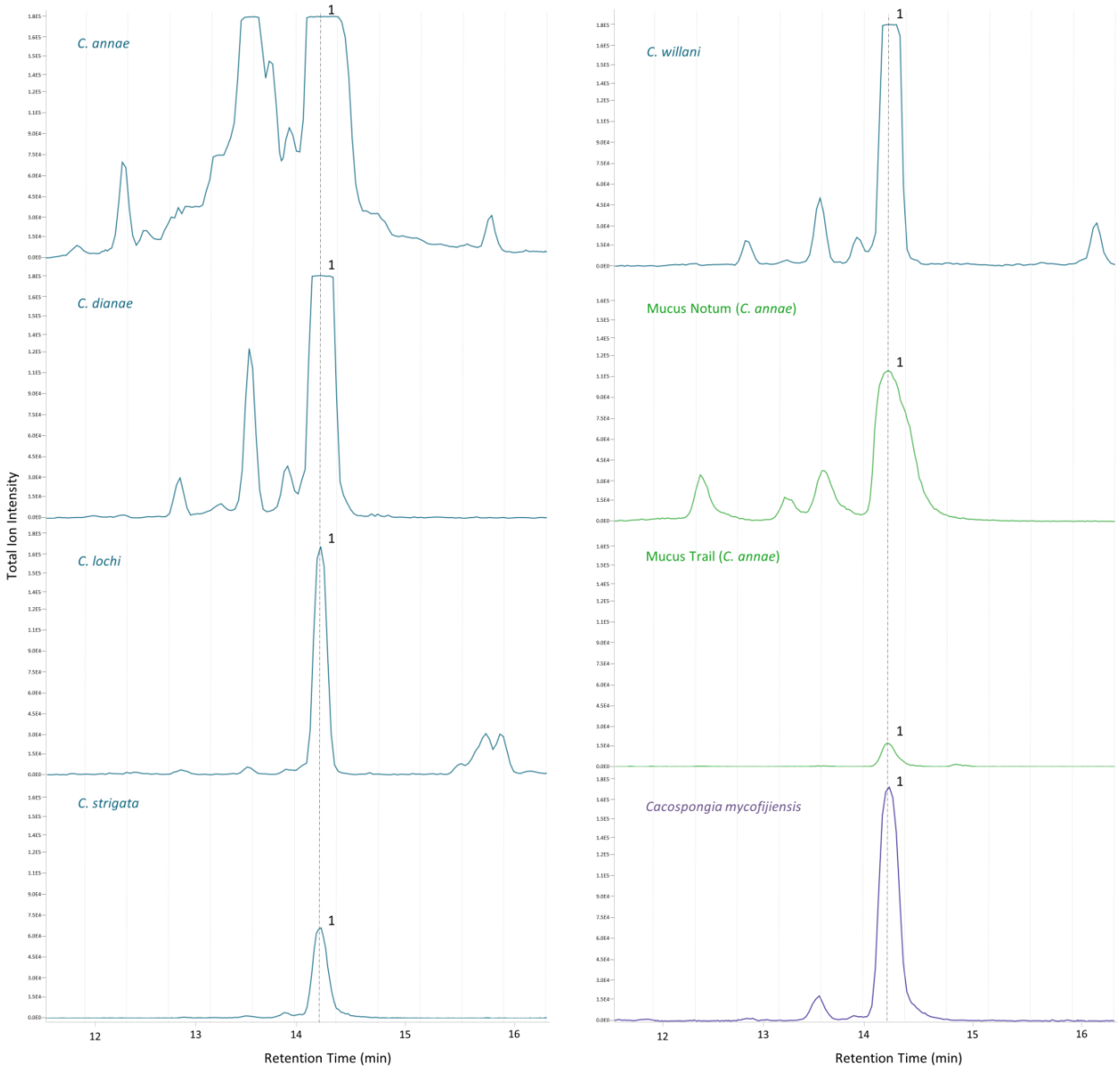


Figure S3.4 Comparison of superimposed HPLC-MS chromatograms of *C. annae* and *C. dianae* mantle and body extracts, showing a higher presence of LatA (1) in the mantle tissues.

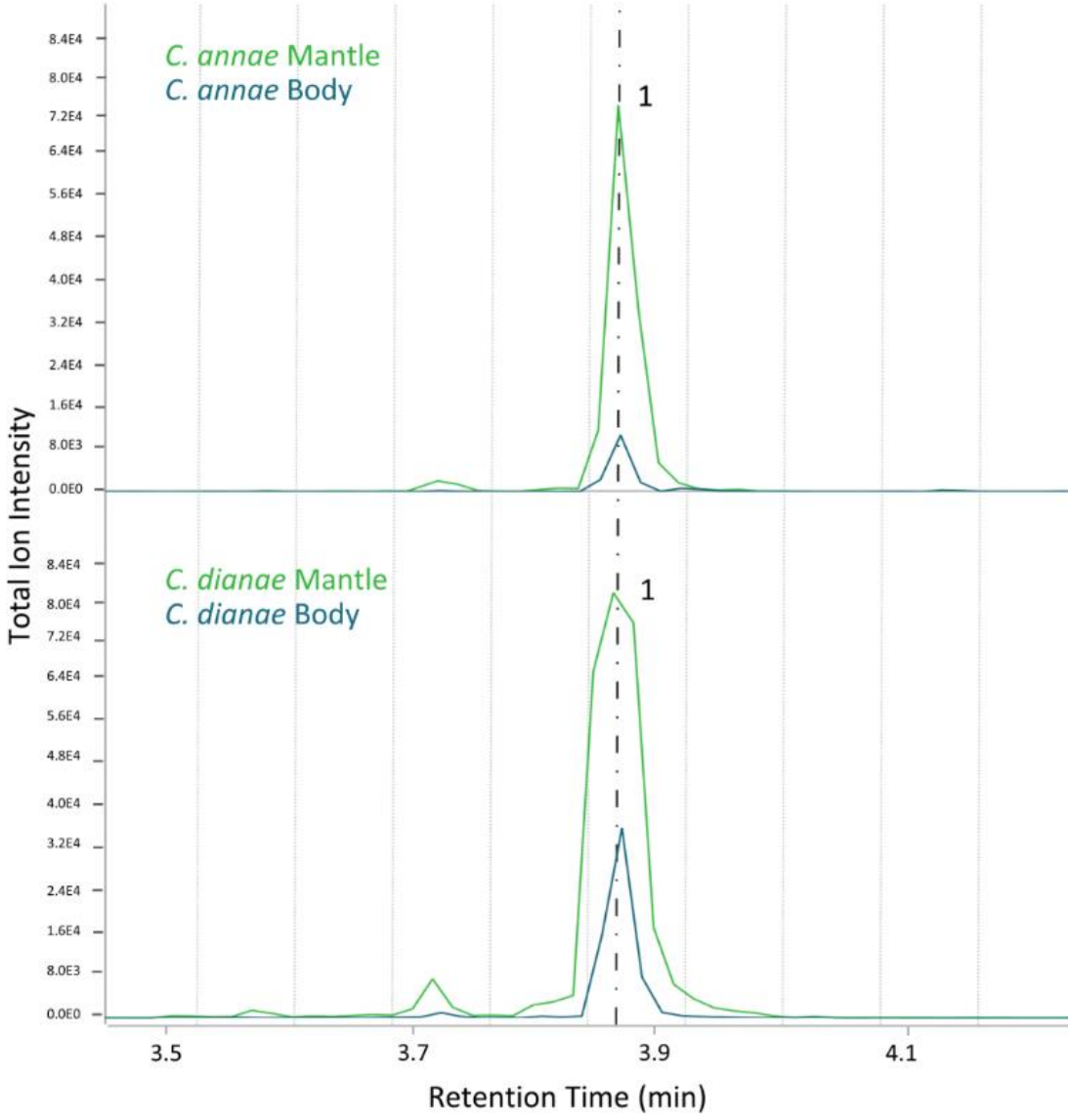


Figure S3.5 Exemplary mass spectrum from the chromatographic peaks marked as (1) in Figure S3.3 and Figure S3.4., showing characteristic protonated ion fragments and adducts for latrunculin A (*m/z* 386 $[M+H-2H_2O]^+$, 404 $[M+H-H_2O]^+$ and 444 $[M+Na]^+$). Spectra were obtained in the positive ion mode.

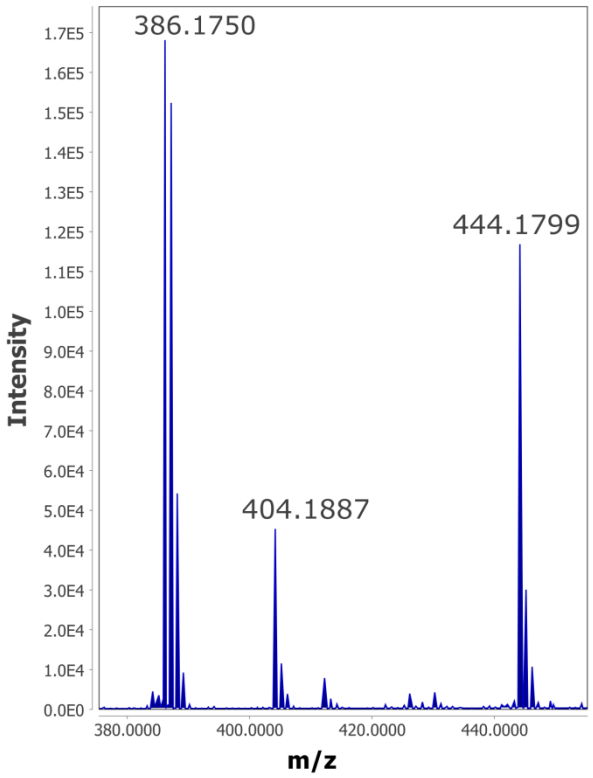


Figure S3.6 Single-pixel (10 µm) mass spectrum of the MALDI MSI from one of the *Chromodoris* MDF-vacuoles, showing protonated ion fragments and adducts typical for LatA (*m/z* 386 $[M+H-2H2O]^+$ and 444 $[M+Na]^+$) as main signals.

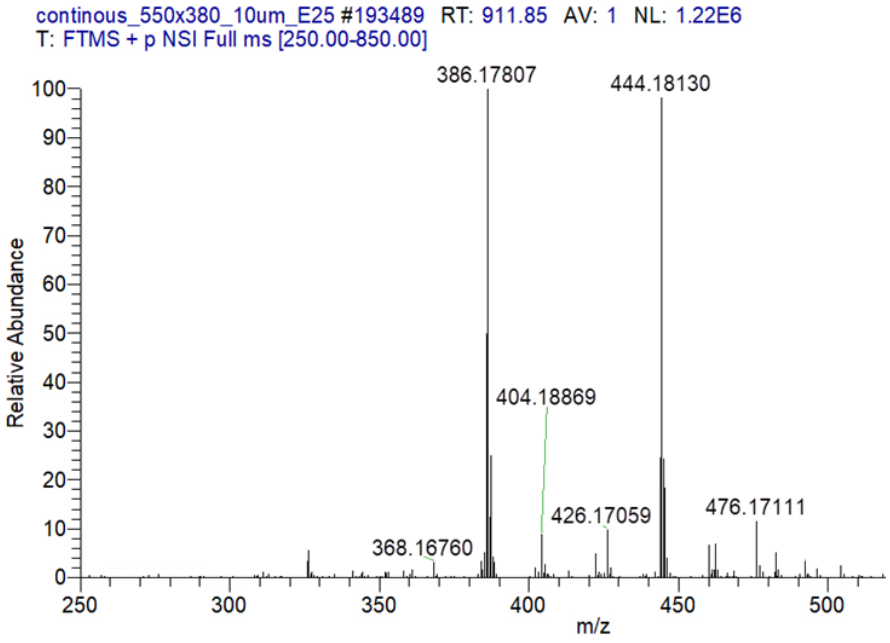


Figure S3.7 Exemplary gel-electrophoresis image of *Chromodoris* putative actin gene fragments (~ 885 bp), amplified by polymerase chain reaction (PCR) and stained with ethidium bromide.

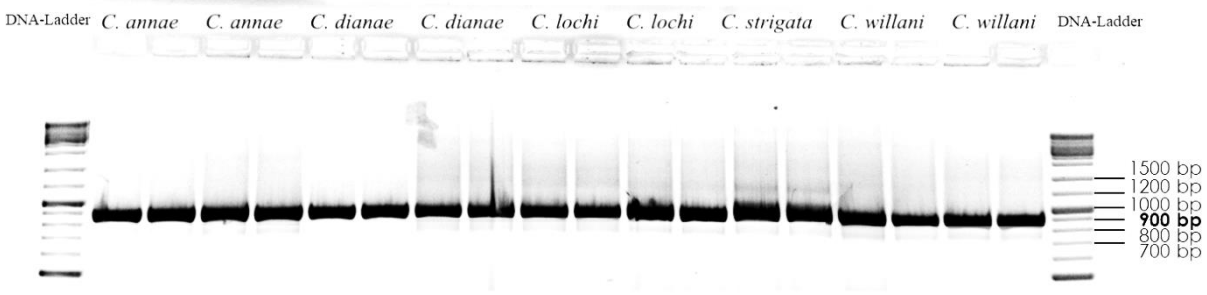


Table S3.3 Recipe for 10 mL isotonic solution (~ 1000 mOsmoles).

H_2Odest	10 mL
NaCl	125.0 mg
Glucose	62.4 mg
$MgSO_4*7H_2O$	62.4 mg
KCl	3.44 mg
$NaHCO_3$	1.92 mg
$MgCl_2*6H_2O$	57.0 mg
$CaCl_2$	14.88 mg

Table S3.4 NCBI BLAST result for *Chromodoris annae* actin isoform nucleotide sequence

Description	Scientific Name	Query Cover	Per. Ident	Accession
PREDICTED: Urocitellus parryii actin, alpha 1, skeletal muscle (Acta1), transcript variant X2, mRNA	*Urocitellus parryii*	99%	72.4%	XM_026412460.1
PREDICTED: Urocitellus parryii actin, alpha 1, skeletal muscle (Acta1), transcript variant X1, mRNA	*Urocitellus parryii*	99%	72.4%	XM_026412451.1
PREDICTED: Ictidomys tridecemlineatus actin, alpha 1, skeletal muscle (Acta1), transcript variant X2, mRNA	*Ictidomys tridecemlineatus*	99%	72.1%	XM_005320387.2
PREDICTED: Ictidomys tridecemlineatus actin, alpha 1, skeletal muscle (Acta1), transcript variant X1, mRNA	*Ictidomys tridecemlineatus*	99%	72.1%	XM_005320386.2
PREDICTED: Pantherophis guttatus actin, gamma-enteric smooth muscle (LOC117673363), mRNA	*Pantherophis guttatus*	99%	71.9%	XM_034430717.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-16	*Artemia franciscana*	99%	71.7%	AJ269582.1
Dictyocaulus viviparus actin variant 2 mRNA, complete cds	*Dictyocaulus viviparus*	99%	71.6%	EU169822.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-14	*Artemia franciscana*	99%	71.5%	AJ269586.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-3	*Artemia franciscana*	99%	71.5%	AJ269583.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-13	*Artemia franciscana*	99%	71.5%	AJ269580.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-9	*Artemia franciscana*	99%	71.5%	AJ269579.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-11	*Artemia franciscana*	99%	71.5%	AJ269578.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-18	*Artemia franciscana*	99%	71.5%	AJ269581.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-19	*Artemia franciscana*	99%	71.4%	AJ269577.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-15	*Artemia franciscana*	99%	71.4%	AJ269575.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-17	*Artemia franciscana*	99%	71.4%	AJ269574.1
Artemia franciscana mRNA for actin (actin 302 gene), clone RT-PCR-1	*Artemia franciscana*	99%	71.4%	AJ269567.1
Artemia mRNA for actin (clone pArAct302)	*Artemia sp.*	99%	71.4%	X52604.1

PREDICTED: Drosophila suzukii actin-87E (LOC118877550), mRNA	*Drosophila suzukii*	99%	71.4%	XM_036816658.1
PREDICTED: Lepidothrix coronata actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA	*Lepidothrix coronata*	99%	71.4%	XM_017803871.1
PREDICTED: Chiroxiphia lanceolata actin alpha 2, smooth muscle (ACTA2), mRNA	*Chiroxiphia lanceolata*	99%	71.4%	XM_032695678.1
PREDICTED: Corapipo altera actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA	*Corapipo altera*	99%	71.2%	XM_027648332.1
PREDICTED: Denticeps clupeoides actin, alpha skeletal muscle 2 (LOC114795339), mRNA	*Denticeps clupeoides*	99%	71.2%	XM_028988485.1
PREDICTED: Erinaceus europaeus actin, alpha skeletal muscle (LOC103127961), transcript variant X2, mRNA	*Erinaceus europaeus*	99%	71.1%	XM_007538841.2
PREDICTED: Erinaceus europaeus actin, alpha skeletal muscle (LOC103127961), transcript variant X1, mRNA	*Erinaceus europaeus*	99%	71.1%	XM_007538840.1
PREDICTED: Chinchilla lanigera actin, alpha 1, skeletal muscle (Acta1), mRNA	*Chinchilla lanigera*	99%	71.1%	XM_005405008.1
PREDICTED: Opisthocomus hoazin actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA	*Opisthocomus hoazin*	99%	71.0%	XM_009935662.1
PREDICTED: Opisthocomus hoazin actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA	*Opisthocomus hoazin*	99%	71.0%	XM_009935660.1
PREDICTED: Pan troglodytes actin, alpha 1, skeletal muscle (ACTA1), mRNA	*Pan troglodytes*	99%	71.0%	XM_016940594.1
PREDICTED: Pterocles gutturalis actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA	*Pterocles gutturalis*	99%	70.9%	XM_010082746.1
PREDICTED: Pterocles gutturalis actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA	*Pterocles gutturalis*	99%	70.9%	XM_010082745.1
PREDICTED: Catharus ustulatus actin alpha 2, smooth muscle (ACTA2), mRNA	*Catharus ustulatus*	99%	70.9%	XM_033065747.1
PREDICTED: Aptenodytes forsteri actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA	*Aptenodytes forsteri*	99%	70.9%	XM_009277749.2
PREDICTED: Pygoscelis adeliae actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA	*Pygoscelis adeliae*	99%	70.8%	XM_009320191.1
PREDICTED: Balearica regulorum gibbericeps actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA	*Balearica regulorum gibbericeps*	99%	70.7%	XM_010305821.1
PREDICTED: Balearica regulorum gibbericeps actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA	*Balearica regulorum gibbericeps*	99%	70.7%	XM_010305820.1
PREDICTED: Egretta garzetta actin alpha 2, smooth muscle (ACTA2), transcript variant X3, mRNA	*Egretta garzetta*	99%	70.7%	XM_035897268.1

PREDICTED: Egretta garzetta actin alpha 2, smooth muscle (ACTA2), transcript variant X2, mRNA	*Egretta garzetta*	99%	70.7%	XM_035897267.1
PREDICTED: Egretta garzetta actin alpha 2, smooth muscle (ACTA2), transcript variant X1, mRNA	*Egretta garzetta*	99%	70.7%	XM_035897266.1
PREDICTED: Perca fluviatilis actin, alpha cardiac muscle 1 (LOC120549587), mRNA	*Perca fluviatilis*	99%	70.7%	XM_039786604.1
PREDICTED: Orbicella faveolata actin, cytoplasmic 1 (LOC110065202), mRNA	*Orbicella faveolata*	99%	70.6%	XM_020772311.1
PREDICTED: Fulmarus glacialis actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA	*Fulmarus glacialis*	99%	70.6%	XM_009576330.1
PREDICTED: Fulmarus glacialis actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA	*Fulmarus glacialis*	99%	70.6%	XM_009576329.1
PREDICTED: Gymnodraco acuticeps actin, alpha skeletal muscle (LOC117544084), mRNA	*Gymnodraco acuticeps*	99%	70.5%	XM_034213253.1
PREDICTED: Hipposideros armiger actin, alpha 1, skeletal muscle (ACTA1), transcript variant X2, mRNA	*Hipposideros armiger*	99%	70.5%	XM_019626360.1
PREDICTED: Hipposideros armiger actin, alpha 1, skeletal muscle (ACTA1), transcript variant X1, mRNA	*Hipposideros armiger*	99%	70.5%	XM_019626358.1
PREDICTED: Sturnus vulgaris actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA	*Sturnus vulgaris*	99%	70.4%	XM_014875386.1
PREDICTED: Pseudochaenichthys georgianus actin, alpha skeletal muscle (LOC117468060), mRNA	*Pseudochaenichthys georgianus*	99%	70.4%	XM_034112020.1
PREDICTED: Trematomus bernacchii actin, alpha skeletal muscle (LOC117478450), mRNA	*Trematomus bernacchii*	99%	70.4%	XM_034125513.1
PREDICTED: Vombatus ursinus actin, alpha skeletal muscle (LOC114049606), mRNA	*Vombatus ursinus*	99%	70.4%	XM_027870867.1
PREDICTED: Vombatus ursinus actin, alpha skeletal muscle (LOC114049602), mRNA	*Vombatus ursinus*	99%	70.4%	XM_027870861.1
PREDICTED: Phascolarctos cinereus actin, alpha 1, skeletal muscle (ACTA1), mRNA	*Phascolarctos cinereus*	99%	70.3%	XM_021000483.1
PREDICTED: Charadrius vociferus actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X2, mRNA	*Charadrius vociferus*	99%	70.3%	XM_009888704.1
PREDICTED: Charadrius vociferus actin, alpha 2, smooth muscle, aorta (ACTA2), transcript variant X1, mRNA	*Charadrius vociferus*	99%	70.3%	XM_009888702.1
Clupea harengus actin alpha 1, skeletal muscle a (acta1a), mRNA	*Clupea harengus*	99%	70.3%	NM_001309834.1

Table S3.5 NCBI BLAST result for *Chromodoris annae* actin isoform amino acid sequence.

Description	**Scientific Name**	**Query Cover**	**Per. Ident**	**Accession**
actin, cytoplasmic-like [Branchiostoma floridae]	*Branchiostoma floridae*	99%	79.5%	XP_035697441.1
unnamed protein product [Dimorphilus gyrociliatus]	*Dimorphilus gyrociliatus*	99%	79.1%	CAD5124374.1
actin-2-like [Amblyraja radiata]	*Amblyraja radiata*	99%	79.1%	XP_032876180.1
ACTB_G1 [Mytilus coruscus]	*Mytilus coruscus*	99%	79.1%	CAC5391235.1
actin A3 [**Haliotis iris**]	***Haliotis iris***	99%	**78.7%**	**AAX19288.1**
actin isoform X2 [Salvelinus namaycush]	*Salvelinus namaycush*	99%	78.7%	XP_038842271.1
hypothetical protein HELRODRAFT_96235 [Helobdella robusta]	*Helobdella robusta*	99%	78.7%	XP_009030330.1
actin [Crotalaria spectabilis]	*Crotalaria spectabilis*	99%	78.7%	CEO86982.1
actin, non-muscle 6.2-like [Salvelinus alpinus]	*Salvelinus alpinus*	99%	78.7%	XP_023997352.1
cytoplasmic actin [Hirudo medicinalis]	*Hirudo medicinalis*	99%	78.7%	ABC60434.1
hypothetical protein HELRODRAFT_185075 [Helobdella robusta]	*Helobdella robusta*	99%	78.7%	XP_009025437.1
cytoplasmic actin [Hirudo medicinalis]	*Hirudo medicinalis*	99%	78.7%	ABC60436.1
actin, non-muscle 6.2 isoform X1 [Salvelinus namaycush]	*Salvelinus namaycush*	99%	78.7%	XP_038842264.1
non-muscle actin II [Hydractinia echinata]	*Hydractinia echinata*	99%	78.7%	ADR10434.1
actin, cytoplasmic 2 [Pseudoalteromonas sp. BMB]	*Pseudoalteromonas sp. BMB*	99%	78.7%	WP_069019001.1
actin, cytoplasmic-like [Branchiostoma floridae]	*Branchiostoma floridae*	99%	78.7%	XP_035695124.1

hypothetical protein FO519_010338 [Halicephalobus sp. NKZ332]	*Halicephalobus sp. NKZ332*	99%	78.4%	KAE9546450.1
actin A2 [**Haliotis iris**]	***Haliotis iris***	99%	**78.4%**	**AAX19287.1**
actin-2-like isoform X1 [Mizuhopecten yessoensis]	*Mizuhopecten yessoensis*	99%	78.4%	XP_021356756.1
predicted protein [Hordeum vulgare subsp. vulgare]	*Hordeum vulgare subsp. vulgare*	99%	78.4%	BAJ97607.1
Actin protein [Sycon ciliatum]	*Sycon ciliatum*	99%	78.4%	CCQ18644.1
actin A1 [**Haliotis iris**]	***Haliotis iris***	99%	**78.4%**	**AAX19286.1**
ACTB_G1 [Mytilus coruscus]	*Mytilus coruscus*	99%	78.4%	CAC5420182.1
actin [Euagrus chisoseus]	*Euagrus chisoseus*	99%	78.4%	ABZ91664.1
Actin-4 [Caenorhabditis elegans]	*Caenorhabditis elegans*	99%	78.4%	NP_001368079.1
unnamed protein product [Spirometra erinaceieuropaei]	*Spirometra erinaceieuropaei*	99%	78.4%	VZI50703.1
actin, non-muscle 6.2 [Thalassophryne amazonica]	*Thalassophryne amazonica*	99%	78.4%	XP_034027240.1
PREDICTED: actin, non-muscle 6.2 [Hydra vulgaris]	*Hydra vulgaris*	99%	78.4%	XP_002154462.1
hypothetical protein LOTGIDRAFT_193218 [Lottia gigantea]	*Lottia gigantea*	99%	78.4%	XP_009060604.1
actin [Thecamoeba similis]	*Thecamoeba similis*	99%	78.4%	AAQ55801.1
actin [Euagrus chisoseus]	*Euagrus chisoseus*	99%	78.4%	ABZ91662.1
PREDICTED: actin, adductor muscle [Octopus bimaculoides]	*Octopus bimaculoides*	99%	78.4%	XP_014771927.1
Bm9237, isoform b [Brugia malayi]	*Brugia malayi*	99%	78.4%	CDP93363.1
non-muscle actin 6.2 [Malo kingi]	*Malo kingi*	99%	78.4%	ACY74447.1

actin, cytoplasmic 2 [Escherichia coli]	*Escherichia coli*	99%	78.4%	WP_126755788.1
unnamed protein product [Onchocerca ochengi]	*Onchocerca ochengi*	99%	78.4%	VDM97777.1
Actin [Oesophagostomum dentatum]	*Oesophagostomum dentatum*	99%	78.4%	KHJ95400.1
hypothetical protein LOTGIDRAFT_208208 [Lottia gigantea]	*Lottia gigantea*	99%	78.4%	XP_009043796.1
actin [Bursaphelenchus xylophilus]	*Bursaphelenchus xylophilus*	99%	78.4%	BAI52958.1
PREDICTED: actin, non-muscle 6.2-like [Hydra vulgaris]	*Hydra vulgaris*	99%	78.4%	XP_002154696.1
actin [Stemonaria longa]	*Stemonaria longa*	99%	78.0%	AFY23984.1
beta-actin [Cepaea nemoralis]	*Cepaea nemoralis*	99%	78.0%	AXI69344.1
beta-actin 2 [**Haliotis diversicolor**]	***Haliotis diversicolor***	99%	**78.0%**	**ABY87412.1**
actin [**Haliotis diversicolor**]	***Haliotis diversicolor***	99%	**78.0%**	**ABU86741.1**
actin-2 isoform X1 [Pecten maximus]	*Pecten maximus*	99%	78.0%	XP_033737032.1
actin-3-like [Mizuhopecten yessoensis]	*Mizuhopecten yessoensis*	99%	78.0%	XP_021356754.1
actin [Mesenchytraeus solifugus]	*Mesenchytraeus solifugus*	99%	78.0%	AOR07101.1
actin [**Haliotis tuberculata**]	***Haliotis tuberculata***	99%	**78.0%**	**CAJ85786.1**
actin [Eisenia fetida]	*Eisenia fetida*	99%	78.0%	AUS83928.1
Actin actin domain containing protein [Meloidogyne graminicola]	*Meloidogyne graminicola*	99%	78.0%	KAF7632952.1
hypothetical protein L596_016279 [Steinernema carpocapsae]	*Steinernema carpocapsae*	99%	78.0%	TKR82582.1
actin-2-like [Mizuhopecten yessoensis]	*Mizuhopecten yessoensis*	99%	78.0%	XP_021356759.1

ACTB_G1 [Mytilus coruscus]	*Mytilus coruscus*	99%	78.0%	CAC5419237.1
hypothetical protein EGW08_007646 [**Elysia chlorotica**]	***Elysia chlorotica***	99%	**78.0%**	**RUS84619.1**
uncharacterized protein MONBRDRAFT_37852 [Monosiga brevicollis MX1]	Monosiga brevicollis MX1	99%	78.0%	XP_001747496.1

[a] Gastropoda shown in bold

Table S3.6 NCBI BLAST result for *Elysia viridis* actin isoform nucleotide sequence.

Description	**Scientific Name**	**Query Cover**	**Per. Ident**	**Accession**
Aplysia californica actin (LOC100533345), mRNA	**Aplysia californica**	100%	**89.69%**	**NM_001204640.1**
PREDICTED: **Aplysia californica** actin, cytoplasmic (LOC106013368), mRNA	**Aplysia californica**	100%	**88.70%**	**XM_013088898.1**
PREDICTED: Acipenser ruthenus actin, beta 1 (actb1), mRNA	Acipenser ruthenus	100%	88.34%	XM_034052523.2
PREDICTED: Epinephelus lanceolatus actin, beta 2 (actb2), mRNA	Epinephelus lanceolatus	100%	87.98%	XM_033645256.1
PREDICTED: Manis pentadactyla actin, cytoplasmic 1 (LOC118933670), mRNA	Manis pentadactyla	100%	87.98%	XM_036928225.1
Danio rerio bactin2, mRNA (cDNA clone MGC:85665 IMAGE:6960309), complete cds	Danio rerio	100%	87.98%	BC067566.1
Danio rerio actin, beta 2 (actb2), mRNA	Danio rerio	100%	87.85%	NM_181601.5
Epinephelus lanceolatus beta-actin mRNA, complete cds	Epinephelus lanceolatus	100%	87.85%	KU200949.2
Epinephelus akaara beta-actin mRNA, complete cds	Epinephelus akaara	100%	87.85%	HQ007251.1
Danio rerio bactin2, mRNA (cDNA clone MGC:172104 IMAGE:7912932), complete cds	Danio rerio	100%	87.85%	BC154531.1
Danio rerio beta actin mRNA, complete cds	Danio rerio	100%	87.85%	AF025305.1

PREDICTED: Astatotilapia calliptera actin beta (actb), mRNA	Astatotilapia calliptera	100%	87.73%	XM_026165516.1
PREDICTED: Oreochromis niloticus actin beta (actb), mRNA	Oreochromis niloticus	100%	87.73%	XM_003443127.5
PREDICTED: Pundamilia nyererei actin, beta (actb), mRNA	Pundamilia nyererei	100%	87.73%	XM_005743477.1
PREDICTED: Simochromis diagramma actin, beta 2 (actb2), mRNA	Simochromis diagramma	100%	87.73%	XM_040006209.1
PREDICTED: Neolamprologus brichardi actin, beta 2 (actb2), mRNA	Neolamprologus brichardi	100%	87.73%	XM_006797985.2
Danio rerio bactin2, mRNA (cDNA clone MGC:192911 IMAGE:100061397), complete cds	Danio rerio	100%	87.73%	BC165823.1
Danio rerio bactin2, mRNA (cDNA clone MGC:56040 IMAGE:3820122), complete cds	Danio rerio	100%	87.73%	BC045879.1
Epinephelus merra beta-actin mRNA, partial cds	Epinephelus merra	98%	87.70%	EU555181.1
Argyrosomus regius beta-actin (B-Act) mRNA, partial cds	Argyrosomus regius	99%	87.64%	KM402038.1
PREDICTED: Alosa sapidissima actin, beta 2 (actb2), mRNA	Alosa sapidissima	100%	87.62%	XM_042085004.1
Oreochromis mossambicus partial mRNA for beta-actin (actb gene)	Oreochromis mossambicus	100%	87.61%	FN673689.1
Squaliobarbus curriculus beta-actin mRNA, complete cds	Squaliobarbus curriculus	100%	87.61%	MT119965.1
PREDICTED: Notolabrus celidotus actin, beta 2 (actb2), mRNA	Notolabrus celidotus	100%	87.61%	XM_034711156.1
PREDICTED: Maylandia zebra actin beta (actb), mRNA	Maylandia zebra	100%	87.61%	XM_004560737.3
PREDICTED: Haplochromis burtoni actin, beta 2 (actb2), mRNA	Haplochromis burtoni	100%	87.61%	XM_005946208.3
Epinephelus awoara beta-actin mRNA, complete cds	Epinephelus awoara	100%	87.61%	JX110447.1
Epinephelus coioides beta actin mRNA, complete cds	Epinephelus coioides	100%	87.61%	AY510710.2
PREDICTED**: Biomphalaria glabrata** actin, adductor muscle (LOC106058166), mRNA	**Biomphalaria glabrata**	98%	**87.55%**	**XM_013215543.1**

Larimichthys polyactis beta-actin mRNA, complete cds	Larimichthys polyactis	99%	87.52%	MT330378.1
Morulius calbasu beta-actin mRNA, complete cds	Labeo calbasu	100%	87.50%	AF393832.1
PREDICTED: Acipenser ruthenus actin, cytoplasmic 2 (LOC117418240), mRNA	Acipenser ruthenus	100%	87.48%	XM_034031052.2
PREDICTED: Pangasianodon hypophthalmus actin, beta 2 (actb2), mRNA	Pangasianodon hypophthalmus	100%	87.48%	XM_026929614.2
PREDICTED: Esox lucius actin, beta 2 (actb2), mRNA	Esox lucius	100%	87.48%	XM_010905274.4
PREDICTED: Cottoperca gobio actin beta (actb), mRNA	Cottoperca gobio	100%	87.48%	XM_029438524.1
Mylopharyngodon piceus beta-actin mRNA, complete cds	Mylopharyngodon piceus	100%	87.48%	KP185128.1
PREDICTED: Colius striatus actin, cytoplasmic 2-like (LOC104553370), mRNA	Colius striatus	100%	87.48%	XM_010195987.1
PREDICTED: Plectropomus leopardus actin, beta 2 (actb2), mRNA	Plectropomus leopardus	100%	87.48%	XM_042505881.1
PREDICTED: Thunnus maccoyii actin, beta 2 (actb2), mRNA	Thunnus maccoyii	100%	87.48%	XM_042393876.1
PREDICTED: Polyodon spathula actin, cytoplasmic 2 (LOC121300962), mRNA	Polyodon spathula	100%	87.48%	XM_041229999.1
PREDICTED: Polyodon spathula actin, cytoplasmic 2 (LOC121294181), mRNA	Polyodon spathula	100%	87.48%	XM_041217804.1
Thunnus maccoyii beta actin mRNA, partial cds	Thunnus maccoyii	100%	87.48%	JX157141.1
PREDICTED: Oreochromis aureus actin, beta 2 (actb2), mRNA	Oreochromis aureus	100%	87.48%	XM_031749543.2
Spinibarbus denticulatus beta-actin mRNA, complete cds	Spinibarbus denticulatus	100%	87.48%	DQ656598.1
Tilapia mossambica mRNA for beta-actin, complete cds	Oreochromis mossambicus	100%	87.48%	AB037865.1
PREDICTED: Gymnodraco acuticeps actin, cytoplasmic 1-like (LOC117534622), mRNA	Gymnodraco acuticeps	100%	87.39%	XM_034198869.1
PREDICTED: Gymnodraco acuticeps actin, cytoplasmic 1-like (LOC117534613), mRNA	Gymnodraco acuticeps	100%	87.39%	XM_034198865.1

PREDICTED: Gymnodraco acuticeps actin, cytoplasmic 1-like (LOC117534613), mRNA	Gymnodraco acuticeps	100%	87.39%	XM_034198858.1
PREDICTED: Larimichthys crocea actin beta (actb), mRNA	Larimichthys crocea	99%	87.39%	XM_027284923.1
PREDICTED: Gouania willdenowi actin beta (actb), mRNA	Gouania willdenowi	100%	87.38%	XM_028454717.1
PREDICTED: Betta splendens actin, beta 2 (actb2), mRNA	Betta splendens	100%	87.38%	XM_029158727.2
Osmerus mordax clone omor-eva-519-037 Actin, cytoplasmic 1 putative mRNA, complete cds	Osmerus mordax	100%	87.38%	BT074966.1
PREDICTED: Ailuropoda melanoleuca actin, cytoplasmic 1 (LOC117800265), mRNA	Ailuropoda melanoleuca	100%	87.36%	XM_034652793.1
PREDICTED: Hippoglossus hippoglossus actin, beta 2 (actb2), mRNA	Hippoglossus hippoglossus	100%	87.36%	XM_034593147.1
PREDICTED: Sparus aurata actin beta (actb), mRNA	Sparus aurata	100%	87.36%	XM_030406939.1

[a] Gastropoda shown in bold

Table S3.7 NCBI BLAST result for *Elysia viridis* actin isoform amino acid sequence.

Description	**Scientific Name**	**Query Cover**	**Per. Ident**	**Accession**
actin [**Plakobranchus ocellatus**]	***Plakobranchus ocellatus***	100%	**98.52%**	**GFN80144.1**
actin [**Elysia marginata**]	***Elysia marginata***	100%	**98.52%**	**GFS25392.1**
RecName: Full=Actin, cytoplasmic, intermediate form; [**Biomphalaria pfeifferi**]	***Biomphalaria pfeifferi***	100%	**97.79%**	**Q964E2.1**
actin [**Elysia marginata**]	***Elysia marginata***	100%	**97.79%**	**GFR57762.1**
actin 1 [Halisarca dujardinii]	*Halisarca dujardinii*	100%	97.79%	QSX72278.1
actin [**Elysia marginata**]	***Elysia marginata***	100%	**97.79%**	**GFR57757.1**

hypothetical protein EGW08_006629 [**Elysia chlorotica**]	***Elysia chlorotica***	100%	**97.79%**	**RUS85617.1**
act protein isoform X1 [Ciona intestinalis]	*Ciona intestinalis*	100%	97.42%	XP_009861333.1
actin, cytoplasmic [Asterias rubens]	*Asterias rubens*	100%	97.42%	XP_033640031.1
actin, cytoplasmic [Orbicella faveolata]	*Orbicella faveolata*	100%	97.42%	XP_020600429.1
hypothetical protein LDENG_00240370 [Lucifuga dentata]	*Lucifuga dentata*	100%	97.42%	KAF7643397.1
actin 4 [Halisarca dujardinii]	*Halisarca dujardinii*	100%	97.42%	QSX72281.1
beta-actin isotype 2 [**Lymnaea stagnalis**]	***Lymnaea stagnalis***	100%	**97.42%**	**AOV18887.1**
beta actin [Doryteuthis pealeii]	*Doryteuthis pealeii*	100%	97.42%	AAU11523.1
cytoplasmic actin [Dreissena polymorpha]	*Dreissena polymorpha*	100%	97.42%	AAC32224.1
actin [**Rapana venosa**]	***Rapana venosa***	100%	**97.42%**	**AGZ87937.1**
unnamed protein product [**Candidula unifasciata**]	***Candidula unifasciata***	100%	**97.42%**	**CAG5123311.1**
actin, cytoplasmic-like [Actinia tenebrosa]	*Actinia tenebrosa*	100%	97.05%	XP_031571867.1
Actin, cytoplasmic, intermediate form; [**Biomphalaria tenagophila**]	***Biomphalaria tenagophila***	100%	**97.05%**	**Q964E0.1**
PREDICTED: actin, cytoplasmic [Pundamilia nyererei]	*Pundamilia nyererei*	100%	97.05%	XP_005754021.1
cytoplasmic actin 1 [Botryllus schlosseri]	*Botryllus schlosseri*	100%	97.05%	CAX48981.1
actin, cytoplasmic 1 [Nematostella vectensis]	*Nematostella vectensis*	100%	97.05%	XP_001637076.1
actin, cytoplasmic [Exaiptasia diaphana]	*Exaiptasia diaphana*	100%	97.05%	XP_020916414.1
hypothetical protein BaRGS_009049 [**Batillaria attramentaria**]	***Batillaria attramentaria***	100%	**97.05%**	**KAG5710333.1**

actin, cytoplasmic [Nematostella vectensis]	*Nematostella vectensis*	100%	97.05%	XP_001621292.2
actin, cytoplasmic 1-like [Sphaeramia orbicularis]	*Sphaeramia orbicularis*	100%	97.05%	XP_029985695.1
actin, cytoplasmic [Cheilinus undulatus]	*Cheilinus undulatus*	100%	97.05%	XP_041642816.1
PREDICTED: actin, cytoplasmic 1-like [Poecilia reticulata]	*Poecilia reticulata*	100%	97.05%	XP_008417682.1
beta-actin [Meretrix meretrix]	*Meretrix meretrix*	100%	97.05%	AEK81538.1
hypothetical protein BaRGS_009048 [**Batillaria attramentaria**]	***Batillaria attramentaria***	100%	**97.05%**	**KAG5710332.1**
beta actin [Exaiptasia diaphana]	*Exaiptasia diaphana*	100%	97.05%	AAQ62633.1
actin-like protein [Stylophora pistillata]	*Stylophora pistillata*	100%	97.05%	AGG36337.1
actin, cytoplasmic [Patiria miniata]	*Patiria miniata*	100%	97.05%	XP_038044475.1
actin, cytoplasmic isoform X1 [Stylophora pistillata]	*Stylophora pistillata*	100%	97.05%	XP_022795459.1
Actin, cytoplasmic [Stylophora pistillata]	*Stylophora pistillata*	100%	97.05%	PFX22595.1
actin, cytoplasmic [Sebastes umbrosus]	*Sebastes umbrosus*	100%	97.05%	XP_037633840.1
actin, non-muscle 6.2 [Salarias fasciatus]	*Salarias fasciatus*	100%	97.05%	XP_029961458.1
unnamed protein product [Darwinula stevensoni]	*Darwinula stevensoni*	100%	97.05%	CAD7248021.1
beta-actin [**Cepaea nemoralis**]	***Cepaea nemoralis***	100%	**97.05%**	**AXI69344.1**
Oidioi.mRNA.OKI2018_I69.PAR.g11455.t1.cds [Oikopleura dioica]	*Oikopleura dioica*	100%	97.05%	CAG5087094.1
unnamed protein product [Oikopleura dioica]	*Oikopleura dioica*	100%	97.05%	CBY24839.1
actin [**Plakobranchus ocellatus**]	***Plakobranchus ocellatus***	100%	**97.05%**	**GFN80142.1**

Actin, cytoplasmic, intermediate form; [**Biomphalaria alexandrina**]	***Biomphalaria alexandrina***	100%	**96.68%**	**Q964E3.1**
beta-actin [Nothobranchius furzeri]	*Nothobranchius furzeri*	100%	96.68%	ABR86936.1
actin, cytoplasmic 1 [Oreochromis niloticus]	*Oreochromis niloticus*	100%	96.68%	XP_003444532.1
actin, cytoplasmic [Oryzias latipes]	*Oryzias latipes*	100%	96.68%	XP_011477986.1
actin [Cerebratulus lacteus]	*Cerebratulus lacteus*	100%	96.68%	ANC90245.1
non-muscle actin 6.2 [Malo kingi]	*Malo kingi*	100%	96.68%	ACY74447.1
PREDICTED: actin, cytoplasmic 1-like [Nothobranchius furzeri]	*Nothobranchius furzeri*	100%	96.68%	XP_015815812.1
Actin, cytoplasmic, intermediate form; Flags: Precursor [**Planorbella trivolvis**]	***Planorbella trivolvis***	100%	**96.68%**	**Q964D9.1**
beta-actin isotype 1 [**Lymnaea stagnalis**]	***Lymnaea stagnalis***	100%	**96.68%**	**AOV18885.1**
Actin, cytoplasmic, intermediate form; Flags: Precursor [**Biomphalaria glabrata**]	***Biomphalaria glabrata***	100%	**96.68%**	**P92179.2**
hypothetical protein LOTGIDRAFT_205506 [**Lottia gigantea**]	***Lottia gigantea***	100%	**96.68%**	**XP_009065998.1**
hypothetical protein CCH79_00016215 [Gambusia affinis]	*Gambusia affinis*	100%	96.68%	PWA24173.1
hypothetical protein OJAV_G00095600 [Oryzias javanicus]	*Oryzias javanicus*	100%	96.68%	RVE68811.1

[a] Gastropoda shown in bold

Table S3.8 NCBI BLAST result for *Aplysia californica* actin nucleotide sequence

Description	Scientific Name	Query Cover	Per. Ident	Accession
Aplysia californica actin (LOC100533345), mRNA	***Aplysia californica***	100%	**100.0%**	**NM_001204640.1**
PREDICTED: **Aplysia californica** actin, cytoplasmic (LOC106013368), mRNA	***Aplysia californica***	100%	**96.4%**	**XM_013088898.1**
Littorina littorea actin (Act1) mRNA, complete cds	***Littorina littorea***	100%	**88.9%**	**KM892438.1**
PREDICTED: Megalops cyprinoides actin, beta 2 (actb2), mRNA	*Megalops cyprinoides*	100%	88.3%	XM_036552203.1
PREDICTED: Acipenser ruthenus actin, beta 1 (actb1), mRNA	*Acipenser ruthenus*	100%	88.3%	XM_034052523.2
Rapana venosa actin (Act1) mRNA, complete cds	***Rapana venosa***	100%	**88.3%**	**KF410817.1**
Acipenser dabryanus beta actin mRNA, partial cds	*Acipenser dabryanus*	100%	88.0%	MF536662.1
Haliotis midae clone Hdd.c148 microsatellite sequence	***Haliotis midae***	100%	**88.0%**	**GU263794.1**
Haliotis diversicolor clone HDr4CJ470 beta-actin 2 mRNA, complete cds	***Haliotis diversicolor***	100%	**88.0%**	**EU244396.1**
PREDICTED: Acipenser ruthenus actin, cytoplasmic 2 (LOC117418240), mRNA	*Acipenser ruthenus*	100%	87.9%	XM_034031052.2
PREDICTED: Pangasianodon hypophthalmus actin, beta 2 (actb2), mRNA	*Pangasianodon hypophthalmus*	100%	87.9%	XM_026929614.2
Danio rerio actin, beta 2 (actb2), mRNA	*Danio rerio*	100%	87.9%	NM_181601.5
Danio rerio bactin2, mRNA (cDNA clone MGC:172104 IMAGE:7912932), complete cds	*Danio rerio*	100%	87.9%	BC154531.1
PREDICTED: Esox lucius actin, beta 2 (actb2), mRNA	*Esox lucius*	100%	87.8%	XM_010905274.4
PREDICTED: Cyprinus carpio actin, cytoplasmic 1 (LOC109051881), mRNA	*Cyprinus carpio*	100%	87.8%	XM_019069375.1
Spinibarbus denticulatus beta-actin mRNA, complete cds	*Spinibarbus denticulatus*	100%	87.8%	DQ656598.1
Danio rerio bactin2, mRNA (cDNA clone MGC:85665 IMAGE:6960309), complete cds	*Danio rerio*	100%	87.8%	BC067566.1
Danio rerio beta actin mRNA, complete cds	*Danio rerio*	100%	87.8%	AF025305.1

Haliotis diversicolor actin mRNA, complete cds	***Haliotis diversicolor***	100%	**87.8%**	**EF587284.1**
PREDICTED: **Biomphalaria glabrata** actin, adductor muscle (LOC106058166), mRNA	***Biomphalaria glabrata***	99%	**87.7%**	**XM_013215543.1**
Haliotis tuberculata mRNA for actin (actin gene) from haemocyte cells	***Haliotis tuberculata***	100%	**87.6%**	**AM236595.1**
Urechis unicinctus beta-actin mRNA, complete cds	*Urechis unicinctus*	100%	87.6%	GU592178.1
PREDICTED: Esox lucius actin, beta 1 (actb1), mRNA	*Esox lucius*	100%	87.5%	XM_010903121.4
Acipenser dabryanus beta-actin mRNA, complete cds	*Acipenser dabryanus*	100%	87.5%	MH790260.1
Carassius auratus B-actin mRNA for beta-actin, complete cds	*Carassius auratus*	100%	87.5%	LC382464.1
Danio rerio bactin2, mRNA (cDNA clone MGC:192911 IMAGE:100061397), complete cds	*Danio rerio*	100%	87.5%	BC165823.1
Danio rerio bactin2, mRNA (cDNA clone MGC:56040 IMAGE:3820122), complete cds	*Danio rerio*	100%	87.5%	BC045879.1
Danio rerio beta-actin mRNA, complete cds	*Danio rerio*	100%	87.5%	AF057040.1
Morulius calbasu beta-actin mRNA, complete cds	*Labeo calbasu*	100%	87.4%	AF393832.1
Danio rerio actin, beta 1 (actb1), mRNA	*Danio rerio*	100%	87.4%	NM_131031.2
PREDICTED: Cyprinus carpio actin, cytoplasmic 1 (LOC109073280), transcript variant X2, mRNA	*Cyprinus carpio*	100%	87.4%	XM_019089433.1
PREDICTED: Cyprinus carpio actin, cytoplasmic 1 (LOC109073280), transcript variant X1, mRNA	*Cyprinus carpio*	100%	87.4%	XM_019089432.1
Sinocyclocheilus anshuiensis actin, cytoplasmic 1 (LOC107692666), mRNA	*Sinocyclocheilus anshuiensis*	100%	87.4%	XM_016491832.1
Sinocyclocheilus anshuiensis actin, cytoplasmic 1-like (LOC107702535), transcript variant X2, mRNA	*Sinocyclocheilus anshuiensis*	100%	87.4%	XM_016504885.1
Sinocyclocheilus anshuiensis actin, cytoplasmic 1-like (LOC107702535), transcript variant X1, mRNA	*Sinocyclocheilus anshuiensis*	100%	87.4%	XM_016504884.1
Danio rerio bactin1, mRNA (cDNA clone MGC:77623 IMAGE:6996683), complete cds	*Danio rerio*	100%	87.4%	BC063950.1
Aplysia californica actin, muscle (LOC100533357), mRNA	***Aplysia californica***	100%	**87.4%**	**NM_001204651.1**
Placopecten magellanicus actin mRNA, complete cds	*Placopecten magellanicus*	100%	87.3%	U55046.1
PREDICTED: Anguilla anguilla actin, cytoplasmic 2 (LOC118216518), mRNA	*Anguilla anguilla*	100%	87.2%	XM_035397752.1

PREDICTED: Sinocyclocheilus anshuiensis actin, cytoplasmic 1 (LOC107703420), mRNA	*Sinocyclocheilus anshuiensis*	100%	87.2%	XM_016506040.1
Crassostrea gigas genome assembly, linkage group: LG7	*Crassostrea gigas*	99%	87.2%	LR761640.1
PREDICTED: Carassius auratus actin, cytoplasmic 1 (LOC113044540), mRNA	*Carassius auratus*	100%	87.1%	XM_026204620.1
PREDICTED: Sinocyclocheilus rhinocerous actin, cytoplasmic 1 (LOC107725373), mRNA	*Sinocyclocheilus rhinocerous*	100%	87.1%	XM_016534610.1
PREDICTED: Sinocyclocheilus rhinocerous actin, cytoplasmic 1 (LOC107722944), mRNA	*Sinocyclocheilus rhinocerous*	100%	87.1%	XM_016531442.1
PREDICTED: Cyprinodon variegatus actin, beta (actb), mRNA	*Cyprinodon variegatus*	100%	87.1%	XM_015378596.1
Rhodeus uyekii beta-actin mRNA, complete cds	*Rhodeus uyekii*	100%	87.1%	KJ867513.1
Onychostoma macrolepis beta-actin mRNA, complete cds	*Onychostoma macrolepis*	100%	87.1%	JN254630.1
PREDICTED: Colossoma macropomum actin, beta 1 (actb1), mRNA	*Colossoma macropomum*	100%	87.1%	XM_036557681.1
Danio rerio bactin1, mRNA (cDNA clone MGC:192419 IMAGE:100060865), complete cds	*Danio rerio*	100%	87.1%	BC165331.1
Danio rerio bactin1, mRNA (cDNA clone MGC:55989 IMAGE:3819668), complete cds	*Danio rerio*	100%	87.1%	BC045846.1
PREDICTED: Pangasianodon hypophthalmus actin, beta 1 (actb1), transcript variant X2, mRNA	*Pangasianodon hypophthalmus*	100%	87.1%	XM_026928833.2
PREDICTED: Pangasianodon hypophthalmus actin, beta 1 (actb1), transcript variant X1, mRNA	*Pangasianodon hypophthalmus*	100%	87.1%	XM_026928832.2
PREDICTED: **Pomacea canaliculata** actin, adductor muscle (LOC112575079), mRNA	***Pomacea canaliculata***	99%	**87.1%**	**XM_025256639.1**
PREDICTED: Merops nubicus actin, cytoplasmic 1 (LOC103775609), transcript variant X5, mRNA	*Merops nubicus*	99%	87.1%	XM_008943076.1
PREDICTED: Merops nubicus actin, cytoplasmic 1 (LOC103775609), transcript variant X1, mRNA	*Merops nubicus*	99%	87.1%	XM_008943072.1

[a] Gastropoda shown in bold.

Table S3.9 NCBI BLAST results for *Aplyisa californica* actin amino acid sequence

Description	**Scientific Name**	**Query Cover**	**Per. Ident**	**Accession**
actin [**Aplysia californica**]	***Aplysia californica***	100%	**100.0%**	**NP_001191569.1**
actin, cytoplasmic [**Aplysia californica**]	***Aplysia californica***	100%	**98.1%**	**XP_012944352.1**
actin [**Littorina littorea**]	***Littorina littorea***	100%	**97.8%**	**AJA37852.1**
actin, cytoplasmic [**Pomacea canaliculata**]	***Pomacea canaliculata***	100%	**97.8%**	**XP_025110090.1**
actin, cytoplasmic [Octopus sinensis]	*Octopus sinensis*	100%	97.8%	XP_029639042.1
Actin, cytoplasmic, intermediate form [**Biomphalaria tenagophila**]	***Biomphalaria tenagophila***	100%	**97.4%**	**Q964E0.1**
Actin, cytoplasmic, intermediate form [**Biomphalaria obstructa**]	***Biomphalaria obstructa***	100%	**97.4%**	**Q964E1.1**
actin [**Rapana venosa**]	***Rapana venosa***	100%	**97.4%**	**AGZ87937.1**
Actin, cytoplasmic, intermediate form [**Biomphalaria pfeifferi**]	***Biomphalaria pfeifferi***	100%	**97.4%**	**Q964E2.1**
beta-actin isotype 2 [**Lymnaea stagnalis**]	***Lymnaea stagnalis***	100%	**97.4%**	**AOV18887.1**
hypothetical protein LOTGIDRAFT_227913 [Lottia gigantea]	*Lottia gigantea*	100%	97.4%	XP_009043798.1
actin, cytoplasmic isoform X1 [Lingula anatina]	*Lingula anatina*	100%	97.4%	XP_013402367.1
Actin, cytoplasmic, intermediate form [**Biomphalaria alexandrina**]	***Biomphalaria alexandrina***	100%	**97.0%**	**Q964E3.1**
actin, cytoplasmic-like [Actinia tenebrosa]	*Actinia tenebrosa*	100%	97.0%	XP_031571867.1
Actin, cytoplasmic, intermediate form [**Planorbella trivolvis**]	***Planorbella trivolvis***	100%	**97.0%**	**Q964D9.1**

actin, cytoplasmic [Orbicella faveolata]	*Orbicella faveolata*	100%	97.0%	XP_020600429.1
cytoplasmic actin [Dreissena polymorpha]	*Dreissena polymorpha*	100%	97.0%	AAC32224.1
actin [Diplodon chilensis]	*Diplodon chilensis*	100%	97.0%	AVN67034.1
PREDICTED: actin, cytoplasmic [Octopus bimaculoides]	*Octopus bimaculoides*	100%	97.0%	XP_014780347.1
cytoplasmic actin [Pinctada fucata]	*Pinctada fucata*	100%	97.0%	BAE80701.1
beta-actin [**Cepaea nemoralis**]	***Cepaea nemoralis***	100%	**97.0%**	**AXI69344.1**
actin, cytoplasmic 1 [Nematostella vectensis]	*Nematostella vectensis*	100%	96.7%	XP_001637076.1
actin, cytoplasmic [Asterias rubens]	*Asterias rubens*	100%	96.7%	XP_033640031.1
act protein isoform X1 [Ciona intestinalis]	*Ciona intestinalis*	100%	96.7%	XP_009861333.1
hypothetical protein LOTGIDRAFT_205506 [Lottia gigantea]	*Lottia gigantea*	100%	96.7%	XP_009065998.1
actin, cytoplasmic [Nematostella vectensis]	*Nematostella vectensis*	100%	96.7%	XP_001621292.2
actin, cytoplasmic 1 [Nematostella vectensis]	*Nematostella vectensis*	100%	96.7%	XP_032239984.1
beta-actin isotype 1 [**Lymnaea stagnalis**]	***Lymnaea stagnalis***	100%	**96.7%**	**AOV18885.1**
Actin, cytoplasmic, intermediate form **[Biomphalaria glabrata**]	***Biomphalaria glabrata***	100%	**96.7%**	**P92179.2**
beta-actin [Sinanodonta woodiana]	*Sinanodonta woodiana*	100%	96.7%	AMR60408.1
beta-actin [Meretrix meretrix]	*Meretrix meretrix*	100%	96.7%	AEK81538.1
actin-2 [Nematostella vectensis]	*Nematostella vectensis*	100%	96.7%	XP_001630583.1
actin beta/gamma 1 [Paragonimus westermani]	*Paragonimus westermani*	100%	96.7%	KAA3669848.1

Actin, cytoplasmic [Stylophora pistillata]	*Stylophora pistillata*	100%	96.7%	PFX22595.1
actin-like protein [Stylophora pistillata]	*Stylophora pistillata*	100%	96.7%	AGG36337.1
actin, cytoplasmic isoform X1 [Stylophora pistillata]	*Stylophora pistillata*	100%	96.7%	XP_022795459.1
beta actin [Doryteuthis pealeii]	*Doryteuthis pealeii*	100%	96.7%	AAU11523.1
Actin-2 isoform 1 [Schistosoma japonicum]	*Schistosoma japonicum*	100%	96.7%	TNN13940.1
PREDICTED: actin, cytoplasmic [Pundamilia nyererei]	*Pundamilia nyererei*	100%	96.3%	XP_005754021.1
actin, cytoplasmic 2-like [Petromyzon marinus]	*Petromyzon marinus*	100%	96.3%	XP_032817105.1
actin, cytoplasmic 1-like [Sphaeramia orbicularis]	*Sphaeramia orbicularis*	100%	96.3%	XP_029985695.1
hypothetical protein LDENG_00240370 [Lucifuga dentata]	*Lucifuga dentata*	100%	96.3%	KAF7643397.1
hypothetical protein LOTGIDRAFT_202971 [Lottia gigantea]	*Lottia gigantea*	100%	96.3%	XP_009043797.1
predicted protein [Nematostella vectensis]	*Nematostella vectensis*	100%	96.3%	EDO42646.1
PREDICTED: actin, cytoplasmic [Acropora digitifera]	*Acropora digitifera*	100%	96.3%	XP_015753791.1
PREDICTED: actin, cytoplasmic 1-like [Poecilia formosa]	*Poecilia formosa*	100%	96.3%	XP_007573219.1
actin, cytoplasmic-like [Monopterus albus]	*Monopterus albus*	100%	96.3%	XP_020450872.1
actin [Galaxea fascicularis]	*Galaxea fascicularis*	100%	96.3%	BAC44866.1
actin, cytoplasmic 1 [Oreochromis niloticus]	*Oreochromis niloticus*	100%	95.9%	XP_003444532.1
actin, cytoplasmic [Oryzias latipes]	*Oryzias latipes*	100%	95.9%	XP_011477986.1
PREDICTED: actin, cytoplasmic 1-like [Nothobranchius furzeri]	*Nothobranchius furzeri*	100%	95.9%	XP_015815812.1

beta-actin [Nothobranchius furzeri]	*Nothobranchius furzeri*	100%	95.9%	ABR86936.1
actin, cytoplasmic 2 [Oryzias melastigma]	*Oryzias melastigma*	100%	95.9%	XP_024116512.1
actin [Cerebratulus lacteus]	*Cerebratulus lacteus*	100%	95.9%	ANC90245.1
PREDICTED: actin, cytoplasmic 1-like [Poecilia reticulata]	*Poecilia reticulata*	100%	95.9%	XP_008417682.1

[a] Gastropoda shown in bold

Table S3.10 List of Heterobranchia actin sequences analysed and discussed in CHAPTER III and their NCBI Genbank accession numbers. Transcriptomic data for Cladobranchia was kindly provided by D. Karmeinski.

```
LOCUS       OK074000        803 bp    DNA     linear   INV 05-SEP-2021
DEFINITION  Chromodoris annae actin isoform, partial CDS.
ACCESSION  OK074000
SOURCE      Chromodoris annae
ORGANISM  Chromodoris annae
Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda;
Heterobranchia; Euthyneura; Nudipleura; Nudibranchia; Doridina;
Eudoridoidea; Chromodorididae; Chromodoris.
REFERENCE   1  (bases 1 to 803)
AUTHORS   Hertzer,C., Undap,N., Bhandari,D.R., Spengler,B., Aatz,S.,
Franken,S., Haeberlein,H., Kehraus,S., Kaligis,F., Bara,R.,
Ijong,F.G., Schaeberle,T.F., Waegele,H. and Koenig,G.M.
TITLE     Protection From Self-Intoxication: A Novel Actin Isoform in
Chromodoris Nudibranchs Supports Sequestration and Storage of the
Cytotoxin Latrunculin A
JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 803)
AUTHORS   Hertzer,C. and Undap,N.
TITLE     Direct Submission
JOURNAL   Submitted (05-SEP-2021) Institute of Pharmaceutical Biology,
University of Bonn, Nussallee 6, Bonn, NRW 53115, Germany
FEATURES             Location/Qualifiers
source          1..803
/organism="Chromodoris annae"
/mol_type="genomic DNA"
/db_xref="taxon:508118"
CDS             <1..>803
/codon_start=1
```

/product="actin"

/translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAFYVSIQAV

LALYASGRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHERG

YNFDSSSETEIVRDVKEKLAYVALDFEQEMDASAKSSTVERSYELPDGQVITLGSERF

RCPEVLFQPSFIGMETVGIHEMIYNSVTKCDIDLRRELYYNIVLSGGTTMFPGIADRL

HKELESVAPASNKIKIIAPPERKYSVWIGGSILGSLSTFQQMWITKQEY"

BASE COUNT 219 a 200 c 196 g 188 t

ORIGIN

1 gtggcacccg aagagcaccc cgtattattg acagaagctc cactaaaccc caaggccaac

61 cgtgagaaaa tgacccagat tatgttcgag actttcaact caccagcttt ttacgtcagt

121 atccaggcgg tacttgctct gtacgcatca gggaggacaa cgggcgtggt gctggacgcg

181 ggagacgggg ttactcatat cattccaata tacgagggct acgccctgcc ccacgctatt

241 gaaaagatga acctggccgg ccgcgaccta actggctatc tgaagcgaat tctccatgag

301 cgaggctaca actttgattc gtcctcggag accgaaatag tgcgagatgt gaaggaaaag

361 ctggcttacg tcgctctgga ctttgaacag gagatggacg catcggccaa gtcgtcaact

421 gtcgagagat cttacgaact acccgatggt caagtgatta ctttgggctc cgaacgattc

481 aggtgccccg aggttttatt tcagccgtct ttcataggaa tggaaaccgt ggggatccac

541 gaaatgatct acaactctgt taccaaatgt gacattgatc tcagaagaga attgtactac

601 aacatcgtcc tttctggcgg aacaacaatg ttcccaggta tagctgatcg gttacataaa

661 gagctggaat ccgttgctcc agccagtaac aagatcaaaa tcattgcccc tcccgaacgc

721 aaatactctg tttggattgg aggatctatc ctgggatctt tgtcaacctt ccagcagatg

781 tggatcacca aacaagagta tga

//

LOCUS OK074001 803 bp DNA linear INV 05-SEP-2021

DEFINITION Chromodoris dianae actin isoform, partial CDS.

ACCESSION OK074001

SOURCE Chromodoris dianae

ORGANISM Chromodoris dianae

Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda;

Heterobranchia; Euthyneura; Nudipleura; Nudibranchia; Doridina;

Eudoridoidea; Chromodorididae; Chromodoris.

REFERENCE 1 (bases 1 to 803)

AUTHORS Hertzer,C., Undap,N., Bhandari,D.R., Spengler,B., Aatz,S.,

Franken,S., Haeberlein,H., Kehraus,S., Kaligis,F., Bara,R.,

Ijong,F.G., Schaeberle,T.F., Waegele,H. and Koenig,G.M.

TITLE Protection From Self-Intoxication: A Novel Actin Isoform in

Chromodoris Nudibranchs Supports Sequestration and Storage of the

Cytotoxin Latrunculin A

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 803)

AUTHORS Hertzer,C. and Undap,N.

TITLE Direct Submission

JOURNAL Submitted (05-SEP-2021) Institute of Pharmaceutical Biology,

University of Bonn, Nussallee 6, Bonn, NRW 53115, Germany

FEATURES Location/Qualifiers

source 1..803

/organism="Chromodoris dianae"

/mol_type="genomic DNA"

/db_xref="taxon:508128"

CDS <1..>803

/codon_start=1

/product="actin"

/translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAFYVSIQAV

LALYASGRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHERG

YNFDSSSETEIVRDVKEKLAYVALDFEQEMDASAKSSTVERSYELPDGQVITLGSERF

RCPEVLFQPSFIGMETVGIHEMIYNSVTKCDIDLRRELYHNIVLSGGTTMFPGIADRL

HKELESVAPASNKMKIIAPPERKYSVWIGGSILGSLSTFQQMWITKQEY"

BASE COUNT 215 a 200 c 199 g 189 t

ORIGIN

1 gtggcacccg aagagcaccc cgtattattg acagaagctc cactgaaccc caaggccaac

61 cgtgagaaaa tgacccagat tatgttcgag actttcaact cgccagcttt ttacgtcagt

121 atccaggcgg tacttgctct gtacgcttca gggaggacaa cgggggtggt gctggacgcg

181 ggagacgggg ttactcatat cattccaata tacgagggct acgccctgcc ccacgctatt

241 gaaaagatga acctggccgg ccgcgacctc actggctatc tgaagcgaat tctccatgag

301 cgaggctaca actttgattc gtcctcggag accgaaatag tgcgagatgt gaaggaaaag

361 ctggcttacg tcgctctgga ctttgaacag gagatggacg catcggccaa gtcgtcaact

421 gtcgaaagat cttacgaact acccgatggt caagtgatta ctttgggctc cgaacgcttc

481 aggtgccccg aggttttatt tcagccgtct ttcataggaa tggaaactgt ggggatccac

541 gaaatgatct acaactctgt taccaaatgt gacattgatc tcagaagaga attgtaccac

601 aacattgtcc tttctggcgg aacaacaatg ttcccaggta tagctgatcg gttacataaa

661 gagctggaat ccgttgctcc agccagcaac aagatgaaaa tcattgcccc tcccgaacgc

721 aaatactctg tttggattgg aggatctatc ctgggatctt tgtcaacctt ccagcagatg

781 tggatcacca aacaagagta tga

//

LOCUS OK074002 803 bp DNA linear INV 05-SEP-2021

DEFINITION Chromodoris lochi actin isoform, partial CDS.

ACCESSION OK074002

SOURCE Chromodoris lochi

ORGANISM Chromodoris lochi

Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia; Doridina; Eudoridoidea; Chromodorididae; Chromodoris.

REFERENCE 1 (bases 1 to 803)

AUTHORS Hertzer,C., Undap,N., Bhandari,D.R., Spengler,B., Aatz,S., Franken,S., Haeberlein,H., Kehraus,S., Kaligis,F., Bara,R., Ijong,F.G., Schaeberle,T.F., Waegele,H. and Koenig,G.M.

TITLE Protection From Self-Intoxication: A Novel Actin Isoform in Chromodoris Nudibranchs Supports Sequestration and Storage of the Cytotoxin Latrunculin A

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 803)

AUTHORS Hertzer,C. and Undap,N.

TITLE Direct Submission

JOURNAL Submitted (05-SEP-2021) Institute of Pharmaceutical Biology, University of Bonn, Nussallee 6, Bonn, NRW 53115, Germany

FEATURES Location/Qualifiers

source 1..803

/organism="Chromodoris lochi"

/mol_type="genomic DNA"

/db_xref="taxon:262607"

CDS <1..>803

/codon_start=1

/product="actin"

/translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAFYVSIQAV LALYASGRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHERG

YNFDSSSETEIVRDVKEKLAYVALDFEQEMDASAKSSTVERSYELPDGQVITLGSERF

RCPEVLFQPSFIGMETVGIHEMIYNSVTKCDIDLRRELYHNIVLSGGTTMFPGIADRL

HKELESVAPASNKMKIIAPPERKYSVWIGGSILGSLSTFQQMWITKQEY"

BASE COUNT 214 a 203 c 199 g 187 t

ORIGIN

1 gtggcacccg aagagcaccc cgtattattg acagaagctc cactgaaccc caaggccaac

61 cgtgagaaaa tgacccagat tatgttcgag actttcaact cgccagcttt ttacgtcagt

121 atccaggcgg tacttgctct gtacgcttca gggaggacaa cgggcgtggt gctggacgcg

181 ggagacgggg ttactcatat cattccaata tacgagggct acgccctgcc ccacgctatt

241 gaaaagatga acctggccgg ccgcgacctc actggctatc tgaagcgaat tctccatgag

301 cgaggctaca actttgattc gtcctcggag accgaaatag tgcgagatgt gaaggaaaag

361 ctggcttacg tcgctctgga ttttgaacag gagatggacg catcggccaa gtcgtcaact

421 gtcgagagat cttacgaact acccgatggc caagtgatta ctttgggctc cgaacgcttc

481 aggtgccccg aggttttatt tcagccgtct ttcataggaa tggaaaccgt ggggatccac

541 gaaatgatct acaactctgt taccaaatgt gacattgatc tcagaagaga attgtaccac

601 aacatcgtcc tttctggcgg aacaacaatg ttcccaggta tagctgatcg gttacataaa

661 gagctggaat ccgttgctcc agccagcaac aagatgaaaa tcattgcccc tcccgaacgc

721 aaatactctg tttggattgg aggatctatc ctgggatctt tgtcaacctt ccagcagatg

781 tggatcacca aacaagagta tga

//

LOCUS OK074003 803 bp DNA linear INV 05-SEP-2021

DEFINITION Chromodoris strigata actin isoform, partial CDS.

ACCESSION OK074003

SOURCE Chromodoris strigata

ORGANISM Chromodoris strigata

Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda;

Heterobranchia; Euthyneura; Nudipleura; Nudibranchia; Doridina;

Eudoridoidea; Chromodorididae; Chromodoris.

REFERENCE 1 (bases 1 to 803)

AUTHORS Hertzer,C., Undap,N., Bhandari,D.R., Spengler,B., Aatz,S.,

Franken,S., Haeberlein,H., Kehraus,S., Kaligis,F., Bara,R.,

Ijong,F.G., Schaeberle,T.F., Waegele,H. and Koenig,G.M.

TITLE Protection From Self-Intoxication: A Novel Actin Isoform in

Chromodoris Nudibranchs Supports Sequestration and Storage of the

Cytotoxin Latrunculin A

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 803)

AUTHORS Hertzer,C. and Undap,N.

TITLE Direct Submission

JOURNAL Submitted (05-SEP-2021) Institute of Pharmaceutical Biology,

University of Bonn, Nussallee 6, Bonn, NRW 53115, Germany

FEATURES Location/Qualifiers

source 1..803

/organism="Chromodoris strigata"

/mol_type="genomic DNA"

/db_xref="taxon:262613"

CDS <1..>803

/codon_start=1

/product="actin"

/translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAFYVSIQAV

LALYASGRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHERG

YNFDSSSETEIVRDVKEKLAYVALDFEQEMDASAKSSTVERSYELPDGQVITLGSERF

RCPEVLFQPSFMGMETVGIHEMIYNSVTKCDIDLRRELYHNIVLSGGTTMFPGIADRL

HKELESVAPASNKIKIIAPPERKYSVWIGGSILGSLSTFQQMWITKQEY"

BASE COUNT 217 a 201 c 197 g 188 t

ORIGIN

1 gtggcacccg aagagcaccc cgtattattg acagaagctc cactgaaccc caaggctaac

61 cgtgagaaaa tgacccagat tatgttcgag actttcaact caccagcttt ttacgtcagt

121 atccaggcgg tacttgctct gtacgcttca gggaggacaa cgggcgtggt gctggacgcg

181 ggagacgggg tcactcatat cattccaata tacgagggct acgccctgcc ccacgctatt

241 gaaaagatga acctggccgg ccgcgaccta actggctatc tgaagcgaat tcttcatgag

301 cgaggctaca actttgattc gtcctcggag accgaaatag tgcgagatgt gaaggaaaag

361 ctggcttacg tcgctctgga ctttgaacag gagatggacg catcggccaa gtcgtcaact

421 gtcgagagat cttacgaact acccgatggt caagtgatta ctttgggctc cgaacgattc

481 aggtgccccg aggtcttatt tcagccgtct ttcatgggaa tggaaaccgt gggaatccac

541 gaaatgatct acaactctgt taccaaatgt gacattgatc tcagaagaga attgtaccac

601 aacatcgtcc tttctggcgg aacaacaatg ttcccaggta tagctgatcg gttacataaa

661 gagctggaat ccgttgctcc agccagtaac aagatcaaaa tcattgcccc tcccgaacgc

721 aaatactctg tttggattgg aggatctatc ctgggatctt tgtcaacctt ccagcagatg

781 tggatcacca aacaagagta tga

//

LOCUS OK074004 803 bp DNA linear INV 05-SEP-2021

DEFINITION Chromodoris willani actin isoform, partial CDS.

ACCESSION OK074004

SOURCE Chromodoris willani

ORGANISM Chromodoris willani

Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda;

Heterobranchia; Euthyneura; Nudipleura; Nudibranchia; Doridina;

Eudoridoidea; Chromodorididae; Chromodoris.

REFERENCE 1 (bases 1 to 803)

AUTHORS Hertzer,C., Undap,N., Bhandari,D.R., Spengler,B., Aatz,S.,

Franken,S., Haeberlein,H., Kehraus,S., Kaligis,F., Bara,R.,

Ijong,F.G., Schaeberle,T.F., Waegele,H. and Koenig,G.M.

TITLE Protection From Self-Intoxication: A Novel Actin Isoform in

Chromodoris Nudibranchs Supports Sequestration and Storage of the

Cytotoxin Latrunculin A

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 803)

AUTHORS Hertzer,C. and Undap,N.

TITLE Direct Submission

JOURNAL Submitted (05-SEP-2021) Institute of Pharmaceutical Biology,

University of Bonn, Nussallee 6, Bonn, NRW 53115, Germany

FEATURES Location/Qualifiers

source 1..803

/organism="Chromodoris willani"

/mol_type="genomic DNA"

/db_xref="taxon:508140"

CDS <1..>803

/codon_start=1

/product="actin"

/translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAFYVSIQAV

LALYASGRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHERG

YNFDSSSETEIVRDVKEKLAYVALDFEQEMDASAKSSTVERSYELPDGQVITLGSERF

RCPEVLFQPSFIGMETVGIHEMIYNSVTKCDIDLRRELYHNIVLSGGTTMFPGIADRL

HKELESVAPASNKIKIIAPPERKYSVWIGGSILGSLSTFQQMWITKQEY"

BASE COUNT 215 a 202 c 198 g 188 t

ORIGIN

1 gtggcacccg aagagcaccc cgtattattg acagaagctc cactgaaccc caaggccaac

61 cgtgagaaaa tgacccagat tatgttcgag actttcaact cgccagcttt ttacgtcagt

121 atccaggcgg tacttgctct gtacgcttca gggaggacaa cgggcgtggt gctggacgcg

181 ggagacgggg ttactcatat cattccaata tacgagggct acgccctgcc ccatgctatt

241 gaaaagatga acctggccgg ccgcgaccta actggctatc tgaagcgaat tctccatgag

301 cgaggctaca actttgattc gtcctcggag accgaaatag tgcgagatgt gaaggaaaag

361 ctggcttacg tcgctctgga ctttgaacag gagatggacg catcggccaa gtcgtcaact

421 gtcgagagat cttacgaact acccgatggc caagtgatta ctttgggctc cgaacgcttc

481 aggtgccccg aggttttatt tcagccgtct ttcataggaa tggaaaccgt ggggatccac

541 gaaatgatct acaactctgt taccaaatgt gacattgatc tcagaagaga attgtaccac

601 aacatcgtcc tttctggcgg aacaacaatg ttcccaggta tagctgatcg gttacataaa

661 gagctggaat ccgttgctcc agccagtaac aagatcaaaa tcattgcccc tcccgaacgc

721 aaatactctg tttggattgg aggatctatc ctgggatctt tgtcaacctt ccagcagatg

781 tggatcacca aacaagagta tga

//

LOCUS OK074005 803 bp DNA linear INV 05-SEP-2021

DEFINITION Elysia viridis actin isoform, partial CDS.

ACCESSION OK074005

SOURCE Elysia viridis

ORGANISM Elysia viridis

Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda;

Heterobranchia; Euthyneura; Panpulmonata; Sacoglossa;

Placobranchoidea; Plakobranchidae; Elysia.

REFERENCE 1 (bases 1 to 803)

AUTHORS Hertzer,C., Undap,N., Bhandari,D.R., Spengler,B., Aatz,S.,

Franken,S., Haeberlein,H., Kehraus,S., Kaligis,F., Bara,R.,

Ijong,F.G., Schaeberle,T.F., Waegele,H. and Koenig,G.M.

TITLE Protection From Self-Intoxication: A Novel Actin Isoform in

Chromodoris Nudibranchs Supports Sequestration and Storage of the

Cytotoxin Latrunculin A

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 803)

AUTHORS Hertzer,C. and Undap,N.

TITLE Direct Submission

JOURNAL Submitted (05-SEP-2021) Institute of Pharmaceutical Biology,

University of Bonn, Nussallee 6, Bonn, NRW 53115, Germany

FEATURES Location/Qualifiers

source 1..803

/organism="Elysia viridis"

/mol_type="genomic DNA"

/db_xref="taxon:71494"

CDS <1..>803

/codon_start=1

/product="actin"

/translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAV

LSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTERG

YSFTTTAEREIVRDIKEKLCYVALDFEQEMQTAATSSSLEKSYELPDGQVITIGNERF

RCPEAVFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYANTVVSGGSTMFPGIADRM

QKEISSLAPPTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQEY"

BASE COUNT 185 a 254 c 190 g 174 t

ORIGIN

1 gtggccccg aggagcaccc agtcttgctc acagaggctc ccctcaaccc caaagccaac

61 agagagaaga tgacacagat catgttcgaa accttcaaca cacccgccat gtacgtcgcc

121 atccaggctg tgctgtccct gtacgcctct ggtcgtacca ctggtattgt gctcgactct

181 ggtgatggtg tttcccacac tgtccccatc tacgagggat atgcccttcc ccacgccatc

241 ctgaggttgg acttggctgg ccgtgacctc acagattacc tcatgaagat cctcacagag

301 aggggctact ccttcacaac cacagctgag agggagattg tccgtgacat caaggagaag

361 ctgtgctacg tcgccctcga cttcgagcag gagatgcaga cagctgccac atcctcctcc

421 ctggagaaga gctacgagct tcccgacggc caggtcatca ccatcggcaa cgagcgtttc

481 aggtgccctg aagcagtgtt ccagccatcc ttcctgggta tggaatctgc tggcatccac

541 gagaccacct acaactccat catgaagtgt gatgtggaca tccgtaagga tctctacgcc

601 aacacggttg tgtctggtgg ctccaccatg tttccaggca ttgctgaccg tatgcagaaa

661 gaaatctcat ccttggcacc tcccaccatg aagatcaaga tcatcgctcc ccctgagcgt

721 aaatactctg tctggatcgg aggctcaatc cttgcctccc tgtccacctt ccaacagatg

781 tggatctcca agcaagagta cga

//

LOCUS Armina tigrina 803 bp mRNA linear

DEFINITION Armina tigrina actin isoform, partial CDS.

ACCESSION not yet submitted to GenBank

SOURCE Armina tigrina

ORGANISM Armina tigrina

Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda;

Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;

Cladobranchia; Arminoidea; Arminidae; Armina

FEATURES Location/Qualifiers

source 1..803

/organism="Armina tigrina"

/mol_type="mRNA"

CDS <1..>803

/codon_start=1

/product="actin"

/translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNAPAMYVAIQAV

LSLYASGRTTGIVLDSGDGVTHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTERG

YSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNERF

RCPESLFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYANTVLSGGTTMFPGIADRM

QKEITSLAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQEY"

BASE COUNT 185 a 254 t 190 g 174 c

ORIGIN

1 gttgcacccg aggagcaccc cgtcctcctg acagaggccc cccttaaccc caaagccaac

61 agagaaaaga tgacccagat catgttcgag accttcaacg cccccgccat gtacgtcgcc

121 atccaagccg tgctctccct gtatgcttcc ggtcgtacca caggtatcgt cctcgactcc

181 ggtgacggtg tcacccacac tgtccccatc tacgagggtt acgctctccc ccatgccatc

241 ctccgtcttg acttggccgg cagagatctt actgattacc tcatgaagat cctgactgag

301 agaggttact cattcaccac caccgccgag agagagattg tccgtgacat caaggagaag

361 ctctgctacg tcgccttgga cttcgagcag gagatggcca ccgccgcctc ctcctcctcc

421 ctggagaaga gctacgagct tcccgacgga caggtcatca ccatcggaaa cgaaagattc

481 aggtgccccg agtctctctt ccagccatcc ttcttgggta tggaatctgc cggtatccat

```
541 gaaaccacat acaactccat catgaagtgc gacgtcgaca tccgtaagga tctgtacgcc
601 aacactgtct tgtccggagg taccaccatg ttccctggta ttgccgatcg tatgcagaag
661 gaaatcacct ccctggcccc aagcaccatg aagatcaaga tcattgctcc tcccgagagg
721 aaatactccg tatggatcgg tggctccatc ttggcctctc tgtccacctt ccaacagatg
781 tggatctcca aacaggaata cga
//
```

```
LOCUS       Embletonia pulchra                 803 bp    mRNA     linear
DEFINITION  Embletonia pulchra actin isoform, partial CDS.
ACCESSION  not yet submitted to GenBank
SOURCE      Embletonia pulchra
ORGANISM  Ebletonia pulchra
Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda;
Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
Cladobranchia; Fionoidea; Embletoniidae; Embletonia
FEATURES             Location/Qualifiers
source          1..803
/organism="Embletonia pulchra"
/mol_type="mRNA"
CDS             <1..>803
/codon_start=1
/product="actin"
/translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAV
LSLYASGRTTGIVLDSGDGVTHTVPIYEGYALPHAIIRLDLAGRDLTDYLMKILTERG
YSFTTTAEREIVRDIKEKLCYVALDFEQEMQTAATSSSLEKSYELPDGQVITIGNERF
RTPEAMFQPSFLGMESSGVHETTYNSIMKCDVDIRKDLYANTVLSGGTTMFPGIADRM
QKEISALAPPTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQEY"
BASE COUNT      185 a    254 t    190 g    174 c
```

ORIGIN

1 gttgcccccg aagagcaccc cgtccttctc acagaggctc ccctcaaccc caaagccaac

61 agggaaaaga tgacccagat catgttcgaa accttcaaca ccccagccat gtacgtcgcc

121 atccaggctg tgctctctct gtacgcctct ggacgtacca ccggaattgt cctcgattcc

181 ggagatggtg tcacccacac cgtccccatc tacgagggat atgcccttcc ccacgccatc

241 atccgtcttg atcttgctgg ccgtgacctc acagactacc tcatgaagat cctcactgag

301 agaggttact ctttcaccac caccgctgag agagaaatcg tccgtgacat caaggagaag

361 ctctgctacg ttgccctcga cttcgagcag gaaatgcaaa ccgccgccac atcctcttcc

421 ctcgagaaga gctacgagct tcccgacgga caagtcatca ccatcggaaa cgagcgcttc

481 aggacccccg aggccatgtt ccagccatcc ttcctcggaa tggaatcctc tggcgtccac

541 gagaccacct acaactccat catgaagtgt gacgtcgaca tccgtaaaga cttgtacgcc

601 aacactgtct tgtccggagg caccaccatg ttccccggaa ttgctgaccg tatgcagaaa

661 gaaatctccg ccctcgcccc accaaccatg aagatcaaga tcattgcccc accagagcgt

721 aaatactccg tatggatcgg aggctccatc ttggcttccc tctccacctt ccaacagatg

781 tggatctcca aacaagaata cga

//

LOCUS Flabellina affinis 803 bp mRNA linear

DEFINITION Flabellina affinis actin isoform, partial CDS.

ACCESSION not yet submitted to GenBank

SOURCE Flabellina affinis

ORGANISM Flabellina affinis

Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca; Gastropoda;

Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;

Cladobranchia; Flabellinoidea; Flabellinidae; Flabellina

FEATURES Location/Qualifiers

source 1..803

/organism="Flabellina affinis"

/mol_type="mRNA"

CDS <1..>803

/codon_start=1

/product="actin"

/translation=" VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAV

LSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTERG

YSFTTTAEREIVRDIKEKLSYVALDFEQEMQTAASSSSLEKSYELPDGQVITIGNERF

RCPETMFQPSFIGMESSGIHETTYNSIMKCDVDIRKDLYANTVLSGGSTMFPGIADRM

QKEISALAPPTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQEY"

BASE COUNT 185 a 254 t 190 g 174 t

ORIGIN

1 gttgcccccg aagagcaccc cgtccttctc acagaggctc ccctcaaccc caaagccaac

61 agagaaaaga tgacccagat catgtttgaa accttcaaca ccccagccat gtacgtcgcc

121 atccaggctg tgctttccct gtacgcctct ggacgtacca ccggaatcgt tcttgactct

181 ggtgatggtg tctcccacac tgtccccatc tacgagggtt atgcccttcc ccacgccatc

241 atgaggttag acttggccgg ccgtgacctt accgattacc tcatgaagat cctcactgag

301 agaggctact cattcaccac caccgccgag agagaaatcg tccgtgacat caaggaaaag

361 ctcagctacg tcgcccttga cttcgaacag gaaatgcaga ctgctgcttc ttcatcctcc

421 ctcgagaaga gctacgaatt gcctgacgga caggtcatca ccatcggaaa cgagcgtttc

481 agatgccccg agaccatgtt ccagccatcc ttcattggaa tggaatcttc tggtatccac

541 gaaaccacct acaactccat catgaagtgt gacgttgaca tccgtaagga cttgtacgcc

601 aacaccgtct tgtccggtgg atctaccatg ttccccggta ttgctgaccg tatgcagaag

661 gaaatcagtg cccttgcccc accaaccatg aagatcaaga tcattgcccc accagagcgt

721 aaatactccg tatggatcgg tggctccatc ctcgcctctc tgtccacctt ccaacagatg

781 tggatctcga aacaggaata cga

//

Table S3.11 List of further Heterobranchia actin sequences obtained during this thesis. Transcriptomic data for *Hypselodoris emma* was kindly provided by A. Donath.

```
LOCUS       Caloria_elegans_iso1 803 bp     mRNA
DEFINITION  Caloria elegans actin isoform 1, partial CDS.
ACCESSION  not yet submitted to GenBank
SOURCE      Caloria elegans
  ORGANISM  Caloria elegans
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Cladobranchia; Aeolidioidea; Facelinidae; Caloria
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Caloria elegans"
                     /mol_type="mRNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"

               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAATSSSLEKSYELPDG
                     QVITIGNERFRCPESLFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMYPGIADRMQKEITALAPPTMKIKIIAPPERKYSVWIGGSI
                     LASLSTFQQMWISKQEY"

ORIGIN
        1 GTTGCCCCCG AAGAGCACCC CGTCCTTCTC ACAGAGGCTC CCCTCAACCC CAAAGCCAAC
       61 AGAGAAAAGA TGACCCAGAT CATGTTTGAA ACCTTCAACA CCCCAGCTAT GTACGTCGCC
      121 ATCCAGGCTG TGCTTTCTCT GTACGCCTCT GGTCGTACCA CCGGTATTGT CCTCGACTCT
      181 GGTGATGGTG TCACCCACAC TGTCCCCATC TACGAAGGTT ATGCCCTCCC CCACGCCATC
      241 ATGAGGTTGG ATTTGGCTGG TCGAGATCTC ACAGATTACC TCATGAAGAT CCTCACTGAG
      301 AGAGGATACT CTTTCACCAC CACAGCTGAG AGAGAAATCG TCCGTGACAT CAAGGAGAAA
      361 CTCTGCTACG TCGCCCTCGA CTTCGAACAG GAAATGGCCA CCGCTGCCAC CTCCTCCTCC
      421 CTGGAGAAGA GCTACGAGCT TCCCGACGGT CAAGTCATCA CCATCGGAAA CGAGAGATTC
      481 CGTTGCCCCG AGTCTCTCTT CCAGCCATCC TTCTTGGGTA TGGAATCTGC CGGTATCCAT
      541 GAAACCACCT ACAACTCCAT CATGAAGTGT GACGTCGACA TCCGTAAAGA CTTGTACGCC
```

```
      601 AACACCGTCC TGTCTGGTGG CTCCACCATG TACCCCGGTA TTGCTGACCG TATGCAGAAG
      661 GAAATCACCG CCCTTGCCCC ACCCACCATG AAGATCAAGA TCATTGCTCC ACCAGAGCGT
      721 AAATACTCCG TATGGATCGG AGGCTCCATC CTTGCATCCC TCTCCACCTT CCAACAGATG
      781 TGGATCTCCA AGCAAGAGTA CGA
//

LOCUS       Caloria_elegans_iso2  803 bp     mRNA
DEFINITION  Caloria elegans actin isoform 2, partial CDS.
ACCESSION  not yet submitted to GenBank
SOURCE      Caloria elegans
  ORGANISM  Caloria elegans
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Cladobranchia; Aeolidioidea; Facelinidae; Caloria
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Caloria elegans"
                     /mol_type="mRNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"

                /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                     RGYSFTTSAEREIVRDIKEKLCYVALDFENEMQTAASSSSLEKSYELPDG
                     QVITIGNERFRCPEALFQPNFLGMETVGVHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMYPGIADRMQKEITALAPPTMKIKIIAPPERKYSVWIGGSI
                     LASLSTFQQMWISKQEY"
```

```
ORIGIN
        1 GTTGCCCCCG AAGAGCACCC CGTCCTTCTC ACAGAGGCTC CCCTCAACCC CAAAGCCAAC
       61 AGAGAAAAGA TGACCCAGAT CATGTTTGAA ACCTTCAACA CCCCAGCTAT GTACGTCGCC
      121 ATCCAGGCTG TGCTTTCTCT GTACGCCTCT GGTCGTACCA CCGGTATTGT CCTCGACTCT
      181 GGTGATGGTG TCACCCACAC TGTCCCCATC TACGAAGGTT ATGCCCTCCC CCACGCCATC
      241 ATGAGGTTGG ATTTGGCTGG TCGAGATCTC ACAGATTACC TCATGAAGAT CCTCACTGAG
      301 AGAGGATACT CTTTCACCAC CTCAGCTGAG AGAGAAATCG TCCGTGACAT CAAGGAGAAA
      361 CTCTGCTACG TCGCTCTTGA CTTTGAAAAC GAAATGCAGA CAGCTGCATC ATCATCCTCT
      421 CTGGAGAAGA GCTACGAATT GCCTGATGGA CAGGTCATCA CCATTGGAAA CGAGCGTTTC
      481 AGGTGCCCCG AGGCTCTCTT CCAGCCCAAC TTCTTGGGAA TGGAAACTGT TGGAGTCCAC
      541 GAGACCACAT ACAACTCTAT CATGAAGTGT GACGTCGACA TCCGTAAAGA CTTGTACGCC
      601 AACACCGTCC TGTCTGGTGG CTCCACCATG TACCCCGGTA TTGCTGACCG TATGCAGAAG
      661 GAAATCACCG CCCTTGCCCC ACCCACCATG AAGATCAAGA TCATTGCTCC ACCAGAGCGT
      721 AAATACTCCG TATGGATCGG AGGCTCCATC CTTGCATCCC TCTCCACCTT CCAACAGATG
      781 TGGATCTCCA AACAAGAATA CGA
//
```

```
LOCUS       Caloria_indica_(ZFMKDNAFD02298395)    803 bp    DNA
DEFINITION  Caloria_indica_(ZFMKDNAFD02298395) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Caloria indica
  ORGANISM  Caloria indica
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Cladobranchia; Aeolidioidea; Facelinidae; Caloria
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Caloria indica"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAHEQHPVLLTEAPLNPKANREKMTQIMFESFNAPAFYVGIQAVLALYAS
                     GRTTGIVMDSGDGVTHAVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                     RGYSFATTAEREIVRDIKENLCYTALDFESEMDTAATSSALEKCYELPDG
                     QIITIGNESFRRPEALFQPSFLGMEIPGVHEMLYNSIMKCDMDIRKDMYA
                     ATVLSGGTTLFSGMADRLQKEMSALAPPSNKVKVIAPPERKYSVWIGRSI
                     LASLSTFQQMWISKQEY"
ORIGIN
        1 GTGGCCCATG AGCAGCACCC TGTTCTTCTA ACGGAAGCTC CTCTGAACCC GAAAGCAAAC
       61 CGCGAGAAGA TGACCCAAAT CATGTTCGAA TCTTTCAACG CCCCGGCTTT CTACGTGGGG
      121 ATTCAGGCAG TTCTGGCTCT GTATGCATCT GGTCGTACGA CAGGAATCGT CATGGACTCT
      181 GGAGACGGAG TGACGCACGC CGTGCCCATC TACGAAGGTT ACGCCCTCCC CCACGCCATC
      241 ATGAGGTTGG ATCTGGCTGG CCGTGATCTC ACCGATTACC TTATGAAAAT CCTCACTGAG
      301 AGAGGATACT CTTTCGCCAC CACCGCCGAG CGCGAGATTG TGCGAGACAT CAAAGAGAAC
      361 CTCTGCTACA CCGCGCTTGA TTTTGAGTCG GAAATGGACA CTGCCGCCAC CTCATCCGCC
      421 CTCGAAAAAT GTTACGAGCT GCCGGACGGC CAGATTATCA CCATTGGAAA CGAGAGTTTC
      481 AGACGCCCCG AGGCTCTGTT CCAGCCGTCT TTCTTGGGAA TGGAGATTCC AGGGGTCCAT
      541 GAAATGCTGT ACAACTCCAT CATGAAGTGT GACATGGATA TAAGGAAGGA TATGTATGCC
      601 GCCACTGTTC TTTCAGGGGG CACCACTCTC TTCTCTGGCA TGGCTGACAG GTTGCAAAAA
      661 GAGATGTCTG CCTTGGCACC TCCTTCTAAC AAGGTCAAGG TCATTGCCCC ACCGGAGAGG
      721 AAATACTCGG TGTGGATCGG CCGGTCCATC CTTGCATCCC TCTCCACCTT CCAACAGATG
      781 TGGATCTCCA AGCAAGAGTA CGA
//
```

```
LOCUS       Ceratosoma_sp__1_(Cesp1_17Bu1/NU344)    755 bp    DNA
DEFINITION  Ceratosoma_sp._1_(Cesp1_17Bu1/NU344) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Ceratosoma sp.1
  ORGANISM  Ceratosoma sp.1
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Ceratosoma
FEATURES             Location/Qualifiers
     source          1..755
                     /organism="Ceratosoma sp.1"
                     /mol_type="genomic DNA"
     CDS             <1..>755
                     /codon_start=1
                     /product="actin"
              /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                   GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                   RGYSFTTTAEREIVRDIKEKLCYIALDFEQEMQTAATSSSLEKSYELPDG
                   QVITIGNERFRAAEAMLQPSFIGMESAGVHETTYNSIMKCDVDIRKDLYA
                   NTVLSGGSTMYSGIADRMQKEITALAPQTMKIKIIAPPERKYSVWIGGSPL"
ORIGIN
        1 GTCGCCCCAG AGGAACACCC AGTCCTTCTT ACAGAAGCTC CCCTTAACCC TAAGGCCAAC
       61 AGAGAAAAGA TGACTCAAAT TATGTTTGAA ACTTTTAACT CACCGGCTAT GTACGTTGCT
      121 ATTCAAGCTG TGCTCTCTCT CTATGCTTCG GGTCGCACAA CGGGTATCGT GCTTGATTCT
      181 GGAGATGGTG TTACTCATAC TGTTCCCATC TACGAAGGAT ACGCCCTTCC CCACGCCATC
      241 ATGAGACTGG ACTTGGCAGG ACGTGACCTT ACGGATTACC TTATGAAGAT CCTCACGGAG
      301 AGAGGCTACA GTTTTACAAC GACAGCTGAG AGGGAAATTG TCCGTGATAT TAAAGAAAAG
      361 CTTTGCTATA TTGCTTTGGA CTTCGAGCAA GAAATGCAAA CGGCCGCCAC TTCCTCATCC
      421 CTTGAGAAAA GTTACGAACT GCCTGATGGA CAAGTAATCA CCATTGGAAA TGAACGTTTC
      481 AGGGCAGCTG AAGCCATGCT GCAACCGTCT TTCATTGGTA TGGAATCTGC TGGTGTTCAT
      541 GAAACCACCT ACAACTCTAT CATGAAGTGT GATGTTGATA TCCGTAAAGA CTTGTATGCT
      601 AACACTGTCT TGTCTGGAGG TTCCACAATG TACTCTGGTA TTGCTGACCG CATGCAAAAG
      661 GAGATAACAG CTTTAGCTCC TCAGACCATG AAGATCAAAA TCATTGCGCC TCCAGAACGT
      721 AAGTATTCTG TATGGATCGG AGGTTCTCCG CTCCC //
```

```
LOCUS       Doriprismatica_atromarginata_1_(Doat17Ba1/NU379)    803 bp    DNA
DEFINITION  Doriprismatica atromarginata_1_(Doat17Ba1/NU379) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Doriprismatica atromarginata
  ORGANISM  Doriprismatica atromarginata
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Doriprismatica
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Doriprismatica atromarginata"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPILLTEAPLNPKSNREKMTQILFETFNAPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFDSSSETEIVRDVKEKLAYVALDFEQEIDVAAKSSTIEKSYELPDG
                     QVITVGSERFRCPEVLFQPSFTGMETVGIHEMIYNSITKCDIDLRKELYY
                     NIVLSGGTTMFPGIADRLHKELESLAPASNKIKIIAPPERKYSVWIGGSI
                     LGSLTTFQQMWINKQEY"
ORIGIN
        1 GTGGCACCCG AAGAACACCC CATCTTATTG ACAGAAGCTC CCTTAAACCC GAAGTCCAAC
       61 CGTGAGAAAA TGACCCAAAT TTTGTTCGAG ACATTCAATG CACCAGCTTT TTATGTCAGC
      121 ATCCAGGCCG TACTTGCTTT GTACGCTTCA GGGAGGACGA CAGGTGTCGT GTTGGACGCA
      181 GGAGACGGGG TCACTCATAT CATTCCAATA TATGAGGGTT ATGCTCTTCC CCATGCTATT
      241 GAAAAAATGA ACCTGGCTGG ACGGGACCTA ACTGGTTATC TGAAGCGAAT CCTCCATGAA
      301 CGAGGCTATA ATTTTGATTC TTCTTCGGAG ACTGAAATAG TGCGAGATGT AAAAGAAAAG
      361 TTGGCTTATG TTGCTCTTGA CTTTGAACAG GAGATAGACG TAGCAGCCAA GTCTTCAACC
      421 ATCGAAAAAT CTTATGAGCT ACCAGATGGT CAAGTGATTA CTGTGGGTTC CGAACGATTC
      481 AGGTGTCCTG AGGTTCTATT TCAGCCGTCT TTCACAGGAA TGGAAACCGT GGGCATCCAC
      541 GAAATGATCT ACAACTCCAT TACCAAATGT GACATTGATC TCAGAAAAGA ACTCTACTAC
      601 AACATTGTCC TATCTGGTGG AACCACCATG TTCCCAGGTA TAGCCGATAG GTTACATAAA
      661 GAGCTGGAAT CCTTAGCCCC AGCTAGCAAC AAGATCAAGA TCATTGCCCC TCCCGAACGT
      721 AAATACTCAG TTTGGATTGG AGGATCCATC TTGGGGTCTT TGACAACCTT CCAGCAGATG
      781 TGGATCAACA AACAGGAGTA TGA
//
```

```
LOCUS       Doriprismatica atromarginata_2_(Doat17Ba2/NU380)    803 bp    DNA
DEFINITION  Doriprismatica atromarginata_2_(Doat17Ba2/NU380) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Doriprismatica atromarginata
  ORGANISM  Doriprismatica atromarginata
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Doriprismatica
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Doriprismatica atromarginata"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPILLTEAPLNPKSNREKMTQILFETFNAPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFDSSSETEIVRDVKEKLAYVALDFEQEIDVAAKSSTIEKSYELPDG
                     QVITLGSERFRCPEVLFQPSFTGMETVGIHEMIYNSITKCDIDLRKELYY
                     NIVLSGGTTMFPGIADRLHKELESLAPASNKIKIIAPPERKYSVWIGGSI
                     LGSLSTFQQMWINKQEY"
ORIGIN
        1 GTGGCACCCG AAGAACACCC CATCTTATTG ACAGAAGCTC CCTTAAACCC GAAGTCCAAC
       61 CGTGAGAAAA TGACCCAAAT TTTGTTCGAG ACATTCAATG CACCAGCTTT TTATGTCAGC
      121 ATCCAGGCCG TACTTGCTTT GTACGCTTCA GGGAGGACGA CAGGTGTCGT GTTGGACGCA
      181 GGAGACGGGG TCACTCATAT CATTCCAATA TATGAGGGTT ATGCTCTTCC CCATGCTATT
      241 GAAAAAATGA ACCTGGCTGG ACGGGACCTA ACTGGTTATC TGAAGCGAAT CCTCCATGAA
      301 CGAGGCTATA ATTTTGATTC TTCTTCGGAG ACTGAAATAG TGCGAGATGT AAAAGAAAAG
      361 TTGGCTTATG TTGCTCTTGA CTTTGAACAG GAGATAGACG TAGCAGCCAA GTCTTCAACC
      421 ATCGAAAAAT CTTATGAGCT ACCAGATGGT CAAGTGATTA CTCTGGGTTC CGAACGATTC
      481 AGGTGTCCCG AGGTTCTATT TCAGCCGTCT TTCACAGGAA TGGAAACCGT GGGCATCCAC
      541 GAAATGATCT ACAACTCCAT TACCAAATGT GACATTGATC TCAGAAAAGA ACTCTACTAC
      601 AACATTGTCC TATCTGGTGG AACCACCATG TTCCCAGGTA TAGCCGATAG GTTACATAAA
      661 GAGCTGGAAT CCTTAGCCCC AGCTAGCAAC AAGATCAAGA TCATTGCCCC TCCCGAACGT
      721 AAATACTCAG TTTGGATTGG AGGATCCATC TTGGGGTCTT TGTCAACCTT CCAGCAGATG
      781 TGGATCAACA AACAGGAGTA TGA
//
```

```
LOCUS       Doriprismatica_sibogae_(Dosi17Ba1/NU381)    803 bp    DNA
DEFINITION  Doriprismatica_sibogae_(Dosi17Ba1/NU381) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Doriprismatica sibogae
  ORGANISM  Doriprismatica sibogae
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Doriprismatica
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Doriprismatica sibogae"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPILLTEAPLNPKSNREKMTQIFFETFNAPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFDSSSETEIVRDVKEKLAYVALDFEQEIDVAAKSSTIEKSYELPDG
                     QVITVGSERFRCPEVLFQPSFTGMETVGIHEMIYNSITKCDIDLRKELYY
                     NIVLSGGTTMFPGIADRLHKELESLAPASNKIKIIAPPERKYSVWIGGSI
                     LGSLSTFQQMWINKQEY"
ORIGIN
        1 GTGGCACCCG AAGAACACCC CATCTTATTG ACAGAAGCTC CCTTAAACCC GAAGTCCAAC
       61 CGTGAGAAAA TGACCCAAAT TTTTTTCGAG ACATTCAATG CACCAGCTTT TTATGTCAGC
      121 ATCCAGGCCG TACTTGCTTT GTACGCTTCA GGGAGGACGA CAGGTGTCGT GTTGGACGCA
      181 GGAGACGGGG TCACTCATAT CATTCCAATA TATGAGGGTT ATGCTCTTCC CCATGCTATT
      241 GAAAAAATGA ACCTGGCTGG ACGGGACCTA ACTGGTTATC TGAAGCGAAT CCTCCATGAA
      301 CGAGGCTATA ATTTTGATTC TTCTTCGGAG ACTGAAATAG TGCGAGATGT AAAAGAAAAG
      361 TTGGCTTATG TTGCTCTTGA CTTTGAACAG GAGATAGACG TAGCAGCCAA GTCTTCAACC
      421 ATCGAAAAAT CTTATGAGCT ACCAGATGGT CAAGTGATTA CTGTGGGTTC CGAACGATTC
      481 AGGTGTCCTG AGGTTCTATT TCAGCCGTCT TTCACAGGAA TGGAAACCGT GGGCATCCAC
      541 GAAATGATCT ACAACTCCAT TACCAAATGT GACATTGATC TCAGAAAAGA ACTCTACTAC
      601 AACATTGTCC TATCTGGTGG AACCACCATG TTCCCAGGTA TAGCCGATAG GTTACATAAA
      661 GAGCTGGAAT CCTTAGCCCC AGCTAGCAAC AAGATCAAGA TCATTGCCCC TCCCGAACGT
      721 AAATACTCAG TTTGGATTGG AGGATCCATC TTGGGGTCTT TGTCAACCTT CCAGCAGATG
      781 TGGATCAACA AACAGGAGTA TGA
//
```

```
LOCUS       Doriprismatica_stellata_(Dost17Bu3/NU346)    803 bp    DNA
DEFINITION  Doriprismatica_stellata_(Dost17Bu3/NU346) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Doriprismatica stellata
  ORGANISM  Doriprismatica stellata
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Doriprismatica
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Doriprismatica stellata"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPILLTEAPLNPKSNREKMTQIIFETFNAPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFDSSSETEIVRDVKEKLAYVALDFEQEIDVAAKSSTIEKSYELPDG
                     QVITLGSERFRCPEVLFQPSFTGMETVGIHEMIYNSITKCDIDLRKELYY
                     NIVLSGGTTMFPGIADRLHKELESLAPASNKIKIIAPPERKYSVWIGGSI
                     LGSLSTFQQMWINKQEY"
ORIGIN
        1 GTGGCACCCG AAGAACACCC CATCTTATTG ACAGAAGCTC CCTTAAACCC GAAGTCCAAC
       61 CGTGAGAAAA TGACCCAAAT TATTTTCGAG ACATTCAATG CACCAGCTTT TTATGTTAGC
      121 ATTCAGGCCG TACTTGCTTT GTACGCTTCA GGGAGGACGA CAGGTGTCGT GTTGGACGCA
      181 GGAGACGGGG TCACTCATAT CATTCCAATA TATGAGGGTT ATGCTCTTCC CCATGCTATT
      241 GAAAAAATGA ACCTGGCAGG ACGGGACCTA ACTGGTTATC TGAAGCGAAT CCTCCATGAA
      301 CGAGGCTATA ATTTTGATTC TTCTTCGGAG ACTGAAATAG TGCGAGATGT AAAAGAAAAG
      361 TTGGCTTATG TTGCTCTTGA CTTTGAACAG GAGATAGACG TAGCAGCCAA GTCTTCAACC
      421 ATCGAAAAAT CTTATGAGCT ACCAGATGGT CAAGTGATTA CTCTGGGCTC CGAACGATTC
      481 AGGTGTCCCG AGGTTCTATT TCAACCGTCT TTCACAGGAA TGGAAACCGT GGGCATCCAC
      541 GAAATGATCT ACAACTCCAT TACCAAATGT GACATTGATC TCAGAAAAGA ACTCTACTAC
      601 AACATTGTCC TATCTGGTGG AACCACCATG TTCCCAGGTA TAGCTGATAG GTTACATAAA
      661 GAGCTGGAAT CCTTAGCCCC AGCTAGCAAC AAGATCAAGA TCATTGCCCC TCCCGAACGT
      721 AAATACTCAG TTTGGATTGG AGGATCCATC TTGGGGTCTT TGTCAACCTT CCAGCAGATG
      781 TGGATCAACA AACAGGAGTA TGA
//
```

```
LOCUS       Felimare_tricolor_(F3_provided by C. Greve)    747 bp    DNA
DEFINITION  Felimare_tricolor_(F3_provided by C. Greve) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Felimare tricolor
  ORGANISM  Felimare tricolor
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Felimare
FEATURES             Location/Qualifiers
     source          1..747
                     /organism="Felimare tricolor"
                     /mol_type="genomic DNA"
     CDS             <1..>747
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSTLEKSYELPDG
                     QVITIGNERFRTPEAMFQPSFLGMESAGVHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGS"
ORIGIN
        1 GTTGCCCCAG AGGAACATCC AGTCCTCCTC ACAGAGGCTC CTCTCAACCC TAAAGCCAAT
       61 AGGGAAAAGA TGACTCAGAT CATGTTTGAA ACCTTCAACT CACCGGCCAT GTACGTGGCT
      121 ATCCAAGCCG TGCTTTCTCT CTACGCTTCA GGCCGCACCA CGGGTATCGT GCTCGATTCC
      181 GGGGACGGTG TCACGCACAC AGTCCCCATC TACGAAGGGT ACGCCCTTCC CCACGCAATC
      241 ATGAGACTGG ATCTGGCGGG ACGCGACCTC ACCGACTACC TCATGAAAAT CCTGACCGAG
      301 AGAGGTTACA GTTTCACCAC CACCGCGGAG CGAGAGATCG TCCGCGACAT CAAAGAAAAG
      361 CTCTGCTACG TCGCGCTGGA CTTCGAGCAA GAGATGGGCA CCGCGGCGTC CTCCTCTACC
      421 CTCGAGAAAA GCTACGAGCT TCCGGACGGC CAGGTCATCA CCATCGGCAA CGAGCGTTTC
      481 AGAACTCCCG AGGCGATGTT CCAGCCCTCG TTCTTGGGCA TGGAATCGGC GGGCGTCCAC
      541 GAGACGACGT ACAACTCCAT CATGAAATGC GACGTCGACA TCCGGAAAGA TCTTTACGCC
      601 AACACGGTTC TCTCCGGCGG ATCCACTATG TATCCGGGCA TCGCGGACCG CATGCAGAAG
      661 GAGATAACGG CCCTCGCACC CAGTACGATG AAAATCAAGA TCATCGCGCC TCCGGAGCGG
      721 AAGTACTCGG TCTGGATCGG AGGCTCC
//
```

```
LOCUS       Felimare_orsinii_1_(F1_provided by C. Greve)    747 bp    DNA
DEFINITION  Felimare_orsinii_1_(F1_provided by C. Greve) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Felimare orsinii
  ORGANISM  Felimare orsinii
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Felimare
FEATURES             Location/Qualifiers
     source          1..747
                     /organism="Felimare orsinii"
                     /mol_type="genomic DNA"
     CDS             <1..>747
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSTLEKSYELPDG
                     QVITIGNERFRTPEAMFQPSFLGMESAGVHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGS"
ORIGIN
        1 GTTGCCCCAG AGGAACATCC AGTCCTCCTC ACAGAGGCTC CTCTTAACCC TAAAGCCAAT
       61 AGGGAAAAGA TGACTCAGAT CATGTTTGAA ACCTTCAACT CACCGGCCAT GTACGTGGCT
      121 ATCCAAGCCG TGCTTTCTCT CTACGCTTCA GGCCGCACCA CGGGTATCGT GCTCGATTCC
      181 GGGGACGGTG TCACGCACAC AGTCCCCATC TACGAAGGAT ACGCCCTTCC CCACGCAATC
      241 ATGAGACTGG ATCTGGCGGG ACGCGACCTT ACCGACTACC TGATGAAAAT CCTGACGGAG
      301 AGGGGTTACA GCTTCACCAC CACCGCGGAG CGAGAAATCG TCCGCGACAT CAAAGAAAAG
      361 CTCTGCTACG TTGCGCTGGA TTTTGAACAA GAGATGGGCA CCGCGGCGTC CTCCTCTACC
      421 CTCGAGAAAA GCTACGAGCT TCCCGACGGT CAGGTCATCA CCATCGGCAA CGAGCGTTTC
      481 AGAACTCCCG AGGCGATGTT CCAGCCCTCG TTCTTGGGCA TGGAATCGGC GGGCGTCCAC
      541 GAGACGACGT ACAACTCCAT CATGAAATGC GACGTCGACA TCCGGAAAGA TCTTTACGCC
      601 AACACGGTTC TCTCCGGCGG ATCCACTATG TATCCGGGCA TCGCGGACCG CATGCAGAAG
      661 GAGATAACGG CCCTCGCACC CAGTACGATG AAAATCAAGA TCATCGCGCC TCCGGAGCGG
      721 AAGTACTCGG TCTGGATCGG AGGCTCA
//
```

```
LOCUS       Felimare_orsinii_2_(F2_provided by C. Greve)    747 bp    DNA
DEFINITION  Felimare_orsinii_2_(F2_provided by C. Greve) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Felimare orsinii
  ORGANISM  Felimare orsinii
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Felimare
FEATURES             Location/Qualifiers
     source          1..747
                     /organism="Felimare orsinii"
                     /mol_type="genomic DNA"
     CDS             <1..>747
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                    GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                    RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSTLEKSYELPDG
                    QVITIGNERFRTPEAMFQPSFLGMESAGVHETTYNSIMKCDVDIRKDLYA
                    NTVLSGGSTMYPCISDRMQKEITALAPSTMKIKIIAPPERKYSVWIGGS"
ORIGIN
        1 GTTGCCCCAG AGGAACATCC AGTCCTCCTC ACAGAGGCTC CTCTTAACCC TAAAGCCAAT
       61 AGGGAAAAGA TGACTCAGAT CATGTTTGAA ACCTTCAACT CACCGGCCAT GTACGTGGCT
      121 ATCCAAGCCG TGCTTTCTCT CTACGCTTCA GGCCGCACCA CGGGTATCGT GCTCGATTCC
      181 GGGGACGGTG TCACGCACAC AGTCCCCATC TACGAAGGAT ACGCCCTTCC CCACGCAATC
      241 ATGAGACTGG ATCTGGCGGG ACGCGACCTT ACCGACTACC TGATGAAAAT CCTGACGGAG
      301 AGGGGTTACA GCTTCACCAC CACCGCGGAG CGAGAAATCG TCCGCGACAT CAAAGAAAAG
      361 CTCTGCTACG TTGCGCTGGA TTTTGAACAA GAGATGGGCA CCGCGGCGTC CTCCTCTACC
      421 CTCGAGAAAA GCTACGAGCT TCCCGACGGT CAGGTCATCA CCATCGGCAA CGAGCGTTTC
      481 AGAACTCCCG AGGCGATGTT CCAGCCCTCG TTCTTGGGCA TGGAATCGGC GGGCGTCCAC
      541 GAGACGACGT ACAACTCCAT CATGAAATGC GACGTCGACA TCCGGAAAGA TCTTTACGCC
      601 AACACGGTTC TCTCCGGCGG ATCCACTATG TATCCGTGCA TCTCGGACCG CATGCAGAAG
      661 GAGATAACGG CCCTCGCACC CAGTACGATG AAAATCAAGA TCATCGCGCC TCCGGAGCGG
      721 AAGTACTCGG TCTGGATCGG AGGCTCA
//
```

```
LOCUS       Felimida_krohni_(F5_provided by C. Greve)    783 bp    DNA
DEFINITION  Felimida_krohni_(F5_provided by C. Greve) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Felimida krohni
  ORGANISM  Felimida krohni
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Felimida
FEATURES             Location/Qualifiers
     source          1..783
                     /organism="Felimida krohni"
                     /mol_type="genomic DNA"
     CDS             <1..>783
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLNYVALDFEQEMQTAATSSSLEKSYELPDG
                     QVITIGNERFRCPEAFFQPSFLGMEAAGIHETTYNSIMKCDVDIRKDLYS
                     NTVLSGGSTMFPGIADRMQKEITALAPPTMKIKIIAPPERKYSVWIGGSI
                     LASLSTFQQMW"
ORIGIN
        1 GTGGCCCCAG AGGAGCACCC AGTCCTGCTC ACAGAGGCTC CCCTCAACCC CAAGGCCAAC
       61 AGAGAGAAGA TGACACAGAT CATGTTCGAG ACCTTCAACA CACCCGCCAT GTACGTCGCT
      121 ATCCAGGCTG TGTTGTCCCT GTACGCCTCT GGTCGTACCA CCGGTATTGT TCTCGACTCC
      181 GGAGACGGTG TTTCCCACAC TGTCCCCATC TACGAGGGAT ACGCCCTCCC CCACGCCATC
      241 CTCCGTCTTG ACCTGGCCGG CCGTGACCTC ACAGACTACC TCATGAAGAT CCTCACCGAG
      301 AGGGGGTACT CCTTCACCAC CACCGCCGAG AGGGAGATCG TCCGCGACAT CAAGGAGAAG
      361 CTCAACTACG TCGCCCTGGA CTTCGAGCAG GAGATGCAGA CAGCCGCCAC GTCGTCATCC
      421 CTGGAGAAGA GCTACGAGCT GCCCGACGGT CAGGTCATCA CCATTGGCAA CGAGCGGTTC
      481 AGGTGCCCTG AAGCATTCTT CCAGCCATCC TTCCTGGGTA TGGAGGCTGC TGGCATCCAC
      541 GAGACCACGT ACAACTCCAT TATGAAGTGT GATGTGGACA TCCGTAAGGA CCTGTACTCC
      601 AACACCGTGT TGTCTGGCGG CTCCACCATG TTCCCAGGCA TCGCTGACCG TATGCAGAAG
      661 GAGATCACAG CCCTGGCACC TCCCACGATG AAGATCAAGA TCATCGCTCC CCCCGAGAGG
      721 AAATACTCTG TCTGGATCGG AGGCTCCATC TTGGCTTCCC TGTCCACCTT CCAGCAGATG
      781 TGG
//
```

```
LOCUS       Glossodoris_cincta_/_hikuriensis_(Glci17Bu3/NU349)    803 bp    DNA
DEFINITION  Glossodoris_cincta_/_hikuriensis_(Glci17Bu3/NU349) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Glossodoris cincta/hikuriensis
  ORGANISM  Glossodoris cincta/hikuriensis
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Glossodoris
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Glossodoris cincta/hikuriensis"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPILLTEAPLNPKSNREKMTQIMFETFNTPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFDSSSETEIVRDVKEKLAYVALDFEQEIDASAKSSTIEKSYELPDG
                     QVITLGSERFRCPEVLFQPSFTGMETVGIHEMIYNSITKCDIDLRKELYY
                     NIVLSGGTTMFPGIADRLHKELESLAPASNKIKIIAPPERKYSVWIGGSI
                     LGSLSTFQQMWITKQEY"
ORIGIN
        1 GTGGCACCCG AAGAACACCC CATCTTATTG ACAGAAGCTC CCTTAAACCC GAAGTCCAAC
       61 CGTGAGAAAA TGACCCAGAT TATGTTTGAG ACTTTCAACA CACCAGCTTT TTATGTCAGT
      121 ATCCAGGCGG TACTTGCTTT GTACGCTTCA GGGAGGACGA CAGGAGTGGT GTTAGACGCA
      181 GGAGATGGAG TTACACACAT CATTCCAATA TATGAGGGTT ACGCTCTGCC CCATGCTATT
      241 GAAAAGATGA ACCTGGCTGG ACGGGACCTC ACAGGTTATT TGAAGCGAAT TCTCCATGAG
      301 CGAGGCTACA ATTTCGATTC GTCTTCAGAG ACAGAAATAG TGCGAGACGT GAAGGAAAAG
      361 TTGGCTTACG TAGCATTAGA CTTTGAACAG GAGATAGACG CATCAGCCAA GTCTTCAACT
      421 ATCGAAAAAT CTTATGAACT GCCTGATGGT CAAGTGATTA CTCTGGGCTC TGAACGATTC
      481 AGGTGTCCCG AGGTTTTGTT TCAACCGTCT TTCACAGGAA TGGAAACTGT GGGCATCCAC
      541 GAAATGATCT ACAACTCCAT TACCAAATGT GACATTGATC TCAGAAAAGA GCTGTACTAT
      601 AACATTGTCC TTTCTGGTGG AACCACAATG TTCCCAGGTA TAGCTGATCG CTTACATAAA
      661 GAACTGGAAT CCTTAGCTCC AGCCAGTAAC AAGATCAAGA TCATTGCCCC TCCTGAACGC
      721 AAATACTCTG TTTGGATTGG AGGGTCTATC CTGGGTTCTT TGTCAACTTT CCAGCAGATG
      781 TGGATCACCA AACAGGAGTA TGA
//
```

```
LOCUS       Glossodoris_cincta_1_(Glci16Sa2/NU23)    803 bp    DNA
DEFINITION  Glossodoris_cincta_1_(Glci16Sa2/NU23) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Glossodoris cincta
  ORGANISM  Glossodoris cincta
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Glossodoris
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Glossodoris cincta"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPILLTEAPLNPKSNREKMTQIMFETFNTPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFDSSSETEIVRDVKEKLAYVALDFEQEIDASAKSSTIEKSYELPDG
                     QVITLGSERFRCPEVLFQPSFTGMETVGIHEMIYNSITKCDIDLRKELYY
                     NIVLSGGTTMFPGIADRLHKELESLAPASNKIKIIAPPERKYSVWIGGSI
                     LGSLSTFQQMWITKQEY"
ORIGIN
        1 GTGGCACCCG AAGAACACCC CATCTTACTG ACAGAAGCTC CCCTAAACCC GAAGTCCAAC
       61 CGTGAGAAAA TGACCCAGAT TATGTTTGAG ACTTTCAACA CACCAGCTTT TTATGTCAGT
      121 ATCCAGGCGG TACTTGCTTT GTACGCTTCA GGGAGGACGA CAGGAGTGGT GTTAGACGCA
      181 GGAGATGGAG TTACACACAT CATTCCAATA TATGAGGGTT ACGCTCTGCC CCATGCTATT
      241 GAAAAGATGA ACCTGGCTGG ACGGGACCTC ACCGGTTATC TGAAGCGAAT TCTCCATGAG
      301 CGAGGCTACA ATTTCGATTC GTCTTCAGAG ACAGAAATAG TGCGAGACGT GAAGGAAAAG
      361 TTGGCTTACG TCGCCTTAGA CTTTGAACAG GAGATAGACG CATCAGCCAA GTCTTCAACT
      421 ATCGAAAAGT CTTATGAACT GCCTGATGGT CAAGTGATTA CTCTGGGCTC TGAACGATTC
      481 AGGTGTCCTG AGGTTTTGTT TCAACCGTCT TTCACAGGAA TGGAAACTGT GGGCATCCAT
      541 GAAATGATCT ACAACTCCAT TACCAAATGT GACATTGATC TCAGAAAAGA GCTGTACTAT
      601 AACATTGTCC TTTCTGGTGG AACCACAATG TTCCCAGGTA TAGCTGATCG CTTACATAAA
      661 GAACTGGAAT CCTTAGCTCC GGCCAGTAAC AAGATCAAGA TCATTGCCCC TCCTGAACGC
      721 AAATACTCTG TTTGGATTGG AGGGTCTATT CTGGGTTCTT TGTCAACTTT CCAGCAGATG
      781 TGGATCACCA AACAGGAGTA TGA
//
```

```
LOCUS       Glossodoris_cincta_2_(Glci17Bu2/NU348)    803 bp    DNA
DEFINITION  Glossodoris_cincta_2_(Glci17Bu2/NU348) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Glossodoris cincta
  ORGANISM  Glossodoris cincta
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Glossodoris
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Glossodoris cincta"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPILLTEAPLNPKSNREKMTQIMFETFNTPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFDSSSETEIVRDVKEKLAYVALDFEQEIDASAKSSTIEKSYELPDG
                     QVITLGSERFRCPEVLFQPSFTGMETVGIHEMIYNSITKCDIDLRKELYY
                     NIVLSGGTTMFPGIADRLHKELESLAPASNKIKIIAPPERKYSVWIGGSI
                     LGSLSTFQQMWITKQEY"
ORIGIN
        1 GTGGCACCCG AAGAACACCC CATCTTACTG ACAGAAGCTC CCCTAAACCC GAAGTCCAAC
       61 CGTGAGAAAA TGACCCAGAT TATGTTTGAG ACTTTCAACA CACCAGCTTT TTATGTCAGT
      121 ATCCAGGCGG TACTTGCTTT GTACGCTTCA GGGAGGACGA CAGGAGTGGT GTTAGACGCA
      181 GGAGATGGAG TTACACACAT CATTCCAATA TATGAGGGTT ACGCTCTGCC CCATGCTATT
      241 GAAAAGATGA ACCTGGCTGG ACGGGACCTC ACCGGTTATC TGAAGCGAAT TCTCCATGAG
      301 CGAGGCTACA ATTTCGATTC GTCTTCAGAG ACAGAAATAG TGCGAGACGT GAAGGAAAAG
      361 TTGGCTTACG TCGCCTTAGA CTTTGAACAG GAGATAGACG CATCAGCCAA GTCTTCAACT
      421 ATCGAAAAGT CTTATGAACT GCCTGATGGT CAAGTGATTA CTCTGGGCTC TGAACGATTC
      481 AGGTGTCCTG AGGTTTTGTT TCAACCGTCT TTCACAGGAA TGGAAACTGT GGGCATCCAT
      541 GAAATGATCT ACAACTCCAT TACCAAATGT GACATTGATC TCAGAAAAGA GCTGTACTAT
      601 AACATTGTCC TTTCTGGTGG AACCACAATG TTCCCAGGTA TAGCTGATCG CTTACATAAA
      661 GAACTGGAAT CCTTAGCTCC GGCCAGTAAC AAGATCAAGA TCATTGCCCC TCCTGAACGC
      721 AAATACTCTG TTTGGATTGG AGGGTCTATT CTGGGTTCTT TGTCAACTTT CCAGCAGATG
      781 TGGATCACCA AACAGGAGTA TGA
//
```

```
LOCUS       Goniobranchus_cavae_(Goca16S2/NU25)    803 bp    DNA
DEFINITION  Goniobranchus_cavae_(Goca16S2/NU25) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Goniobranchus cavae
  ORGANISM  Goniobranchus cavae
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Goniobranchus
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Goniobranchus cavae"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPILLTEAPLNPKSNREKMTQIMFETFNAPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFESSSETEIVRDVKEKLAYVALDFEQEMDAAAKSSTIERSYELPDG
                     QVITLGSERFRCPEVLFQPSFMGVETAGIHEMIYNSITKCDIDLRRELYH
                     NIVLSGGTTMFPGIADRLHRELESIAPASNKIKIIAPSERKYSVWIGGSI
                     LGSLSTFQQMWITKQEY"
ORIGIN
        1 GTGGCACCAG AAGAACATCC CATCTTGTTG ACAGAAGCTC CACTAAATCC CAAATCCAAC
       61 CGTGAGAAAA TGACCCAGAT CATGTTCGAA ACTTTCAACG CGCCAGCTTT TTATGTCAGT
      121 ATCCAGGCGG TCTTAGCTCT GTACGCTTCA GGCAGGACGA CAGGCGTGGT GTTAGACGCA
      181 GGAGACGGCG TCACTCATAT CATTCCAATA TACGAGGGCT ATGCTCTGCC CCACGCTATT
      241 GAAAAGATGA ATTTGGCCGG TCGGGACCTC ACTGGCTATC TCAAGCGAAT CCTTCACGAA
      301 CGAGGTTACA ATTTTGAATC GTCTTCCGAG ACGGAAATAG TCCGAGACGT GAAGGAAAAG
      361 CTGGCCTACG TCGCTCTGGA TTTTGAACAG GAGATGGACG CAGCAGCCAA GTCGTCTACC
      421 ATCGAAAGAT CTTACGAACT TCCCGATGGT CAAGTGATTA CTCTGGGCTC CGAACGATTT
      481 AGGTGTCCTG AAGTTTTGTT CCAACCGTCT TTCATGGGAG TGGAAACTGC CGGCATTCAC
      541 GAAATGATCT ACAACTCCAT AACCAAGTGT GACATTGATC TCCGAAGAGA ATTGTACCAT
      601 AACATCGTCC TGTCCGGGGG AACCACAATG TTCCCAGGTA TCGCCGATCG GTTACACAGA
      661 GAGTTGGAAT CCATTGCCCC AGCTAGTAAC AAGATCAAGA TCATTGCTCC TTCCGAGCGC
      721 AAATACTCGG TTTGGATTGG TGGATCAATC TTGGGTTCTT TGTCAACTTT CCAGCAGATG
      781 TGGATCACCA AACAGGAGTA TGA
//
```

```
LOCUS       Goniobranchus_coi_(Gocho17Ba2/NU384)    784 bp    DNA
DEFINITION  Goniobranchus_coi_(Gocho17Ba2/NU384) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Goniobranchus coi
  ORGANISM  Goniobranchus coi
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Goniobranchus
FEATURES             Location/Qualifiers
     source          1..784
                     /organism="Goniobranchus coi"
                     /mol_type="genomic DNA"
     CDS             <1..>784
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNVPAMYVAFQAVLSLYAS
                     GRTTGFVMDSGDGVSHTVPIYKGYALPHAILRLDLAGRDLTDWMVKLLTE
                     RGYSFTTTAEREIVRDIKEKLAYVALDFDQEMQTAASSSALEKSYELPDG
                     QIITIGNERFRCPEALFQPSFLGMESPGVHETTYNSIMKCDVDIRKDLYA
                     NVVLSGGTTMYAGIADRMSKEITALAPASMKVKIIAPPERKYSVWIGGSI
                     LSSLSTFQQMW"
ORIGIN
        1 GTGGCGCCTG AAGAGCATCC CGTCTTGCTC ACCGAGGCCC CGCTCAACCC GAAGGCCAAT
       61 CGCGAGAAGA TGACCCAGAT CATGTTCGAG ACTTTCAATG TCCCCGCCAT GTATGTCGCC
      121 TTCCAGGCCG TACTTTCGCT CTACGCTTCG GGCCGTACCA CAGGCTTTGT CATGGACTCT
      181 GGCGACGGGG TCTCGCACAC CGTCCCGATC TACAAGGGCT ACGCGCTGCC GCACGCCATC
      241 CTCCGCCTCG ACCTGGCCGG CCGCGACCTC ACCGACTGGA TGGTCAAGCT GCTCACCGAG
      301 CGCGGCTACT CCTTCACCAC CACCGCCGAG CGCGAGATTG TGCGCGACAT CAAAGAGAAG
      361 CTCGCCTACG TCGCGCTTGA CTTTGACCAG GAGATGCAGA CCGCCGCCTC CTCGTCGGCC
      421 CTCGAGAAGT CGTACGAGCT GCCGGACGGC CAGATCATCA CCATTGGCAA CGAGCGCTTC
      481 CGCTGCCCCG AGGCCCTCTT CCAGCCGTCC TTCCTCGGCA TGGAGTCGCC GGGCGTGCAC
      541 GAAACGACGT ACAACTCGAT CATGAAGTGC GACGTCGACA TCAGGAAGGA CCTGTACGCG
      601 AACGTCGTGC TGTCAGGCGG CACCACAATG TACGCCGGCA TTGCCGACCG CATGTCCAAG
      661 GAGATCACTG CCCTCGCGCC CGCCTCGATG AAGGTCAAGA TCATCGCGCC GCCCGAGCGC
      721 AAGTACTCGG TCTGGATCGG CGGCTCCATC CTCTCGTCGC TCTCCACCTT TCAGCAGATG
      781 TGGA
//
```

```
LOCUS       Goniobranchus_fidelis_(Gofi15Ba1/NU385)    803 bp    DNA
DEFINITION  Goniobranchus_fidelis_(Gofi15Ba1/NU385) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Goniobranchus fidelis
  ORGANISM  Goniobranchus fidelis
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Goniobranchus
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Goniobranchus fidelis"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEKHPILSTEPPLNPKANREKMTQIMFETFNTPAFYVGIQAVLALYAF
                     GRTTGVVLDAGDGVTHVVPIYQGYVLPHAIEKMDLAGRDLTGYMKRILPE
                     RGYSFDTSSETEIVRDIKEKLAYVALDFDQEMHTAASSSSLERSYELPDG
                     QMITMGSERFRCPEALFQPSFIGMESVGIHKMIFNSIMKCDVDIRKDLYA
                     NIVLSGGTTMFPGIADRMHKEISSLAPPTMKIKIIAAPERKHLVWICSSI
                     FVSFPNFYRSWITKQEY"
ORIGIN
        1 GTGGCACCAG AGAAGCATCC CATCTTGTCG ACAGAACCTC CACTGAACCC GAAGGCCAAC
       61 CGTGAGAAAA TGACCCAGAT TATGTTCGAA ACTTTTAACA CGCCAGCTTT TTACGTCGGA
      121 ATTCAGGCGG TGCTTGCTTT GTACGCTTTT GGGAGGACCA CTGGCGTGGT GCTGGACGCC
      181 GGGGACGGTG TTACCCATGT CGTTCCAATC TACCAAGGCT ATGTTCTGCC CCATGCTATC
      241 GAAAAGATGG ACTTGGCCGG ACGGGACCTC ACTGGCTATA TGAAGCGAAT CCTCCCTGAG
      301 AGAGGATACA GTTTTGACAC CTCCTCCGAG ACGGAAATTG TGCGAGACAT CAAGGAAAAG
      361 CTCGCCTACG TCGCTCTTGA TTTCGACCAG GAGATGCACA CTGCCGCCTC GTCGTCCTCT
      421 CTCGAGAGAT CTTACGAACT GCCCGATGGT CAGATGATCA CCATGGGATC TGAGCGATTC
      481 AGGTGTCCCG AGGCTTTGTT TCAACCGTCT TTCATCGGTA TGGAATCCGT GGGCATCCAC
      541 AAGATGATCT TCAATTCTAT CATGAAGTGT GATGTGGATA TCCGTAAAGA CTTGTACGCC
      601 AACATCGTCC TCTCTGGCGG AACCACAATG TTCCCAGGTA TCGCCGACCG CATGCATAAG
      661 GAAATCTCGT CTCTTGCTCC ACCCACCATG AAGATCAAGA TCATTGCTGC TCCGGAACGC
      721 AAACACTTGG TTTGGATTTG CAGTTCAATC TTTGTTTCTT TTCCCAATTT CTACCGAAGT
      781 TGGATCACCA AACAGGAGTA TGA
//
```

```
LOCUS       Goniobranchus_geometricus_(Goge16S4/NU29)    765 bp    DNA
DEFINITION  Goniobranchus_geometricus_(Goge16S4/NU29) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Goniobranchus geometricus
  ORGANISM  Goniobranchus geometricus
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Goniobranchus
FEATURES             Location/Qualifiers
     source          1..765
                     /organism="Goniobranchus geometricus"
                     /mol_type="genomic DNA"
     CDS             <1..>765
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNAPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMHTAASSSSLEKSYEVPDG
                     QVITIGNERFRCPEALFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSI
                     LASLS"
ORIGIN
        1 GTTGCGCCCG AGGAGCACCC AGTCCTGCTC ACAGAGGCCC CCCTCAACCC TAAGGCCAAC
       61 AGGGAGAAGA TGACCCAGAT CATGTTCGAG ACCTTCAACG CCCCCGCCAT GTATGTCGCC
      121 ATCCAGGCTG TGCTGTCCCT GTACGCTTCT GGTCGTACCA CCGGTATTGT GCTGGACTCC
      181 GGAGACGGCG TCACCCACAC CGTCCCCATC TATGAAGGTT ATGCCCTGCC CCACGCCATC
      241 CTGCGTCTGG ACTTGGCTGG CCGTGATCTC ACTGACTACC TGATGAAGAT CCTTACTGAG
      301 CGAGGCTACA GCTTCACCAC CACTGCCGAG CGAGAGATTG TTCGTGACAT CAAGGAAAAA
      361 CTCTGCTACG TGGCCCTGGA CTTTGAACAA GAAATGCACA CCGCCGCCTC CTCCTCCTCC
      421 CTGGAGAAGA GCTACGAAGT GCCCGATGGA CAAGTCATCA CCATTGGAAA CGAGCGTTTC
      481 AGGTGTCCCG AGGCTCTGTT CCAGCCATCC TTCCTGGGTA TGGAATCTGC TGGTATCCAT
      541 GAAACCACCT ACAACTCCAT CATGAAGTGT GATGTTGACA TCCGTAAAGA CTTGTACGCC
      601 AACACAGTAT TGTCTGGTGG CACCACCATG TACCCCGGTA TCGCTGACCG CATGCAGAAG
      661 GAGATCACTG CCCTGGCTCC TAGCACCATG AAGATCAAGA TCATTGCTCC TCCAGAGCGC
      721 AAGTACTCCG TATGGATCGG TGGCTCCATC TTGGCTTCTC TCTCC
//
```

```
LOCUS       Goniobranchus_kuniei_(Goku16Sa1/NU30)    803 bp    DNA
DEFINITION  Goniobranchus_kuniei_(Goku16Sa1/NU30) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Goniobranchus kuniei
  ORGANISM  Goniobranchus kuniei
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Goniobranchus
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Goniobranchus kuniei"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAFYVAIQAVLSLYAF
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMKLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMETASTSSSLEKSYEFPDG
                     QVITLGNERFRCPEVLFQPSFLGMETAGIHETIYNSIMKCDVEFGKDLYA
                     NTVLSGGSTMFPAIGDRMQKEITALAPPTMKIKIIAPPERKYSVWICSSI
                     FVSFPNFYRSWITKQEY"
ORIGIN
        1 GTTGCGCCCG AGGAGCACCC AGTCCTGCTC ACAGAGGCCC CCCTCAACCC TAAGGCCAAC
       61 AGGGAGAAGA TGACCCAAAT CATGTTTGAA ACTTTCAACT CCCCAGCTTT TTATGTCGCT
      121 ATCCAGGCTG TACTTTCTCT GTATGCTTTC GGTCGTACCA CAGGTATTGT GCTGGACTCC
      181 GGAGACGGCG TCACCCACAC CGTTCCTATC TATGAAGGAT ACGCCCTTCC CCATGCTATC
      241 ATGAAACTGG ACTTGGCCGG ACGTGACCTC ACTGACTACC TCATGAAAAT CCTTACAGAA
      301 AGAGGTTACA GTTTTACAAC AACTGCTGAG AGAGAAATTG TCCGAGACAT CAAAGAAAAG
      361 CTGTGCTACG TTGCTCTTGA TTTTGAACAA GAAATGGAAA CTGCTTCCAC GTCCTCATCC
      421 CTTGAAAAAT CTTATGAATT TCCCGATGGT CAAGTGATTA CTCTTGGAAA CGAACGATTT
      481 AGGTGTCCTG AAGTTTTGTT TCAACCGTCT TTCTTGGGAA TGGAAACGGC TGGTATCCAC
      541 GAAACCATCT ACAATTCCAT TATGAAATGT GATGTGGAAT TCGGAAAAGA CTTGTACGCC
      601 AACACAGTTC TTTCTGGTGG TTCCACTATG TTCCCTGCTA TTGGCGACCG CATGCAGAAA
      661 GAAATAACAG CCCTTGCTCC ACCCACCATG AAGATCAAAA TCATTGCCCC TCCGGAACGG
      721 AAATACTCGG TTTGGATTTG CAGTTCAATC TTTGTTTCTT TTCCCAATTT CTACCGAAGT
      781 TGGATCACCA AACAGGAGTA TGA
//
```

```
LOCUS       Goniobranchus_reticulatus_(Gore16Sa1/NU31)    803 bp    DNA
DEFINITION  Goniobranchus_reticulatus_(Gore16Sa1/NU31) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Goniobranchus reticulatus
  ORGANISM  Goniobranchus reticulatus
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Goniobranchus
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Goniobranchus reticulatus"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMKMDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLSYVALDFEQEMETAANSSSLEKTYEFPDG
                     QVITFGNERFRCPEALFQPSFLGMGTAGVHETTYNSIMKCDVDIRKELYA
                     NTVLSGGSTMFPGIADRMQKEITSLAPPTMKIKIIAPPEGKYSVWICSSI
                     FVSFPNFYRSWITKQEY"
ORIGIN
        1 GTTGCGCCCG AGGAGCACCC AGTCCTGCTC ACAGAGGCCC CCCTCAACCC TAAGGCCAAC
       61 AGGGAGAAGA TGACTCAAAT CATGTTTGAA ACCTTCAACT CTCCGGCCAT GTATGTCGCT
      121 ATTCAGGCTG TGCTTTCCCT CTATGCTTCA GGTCGTACCA CAGGTATTGT GCTGGACTCT
      181 GGAGATGGTG TTACCCACAC CGTTCCAATC TACGAAGGTT ACGCTCTGCC CCATGCTATC
      241 ATGAAGATGG ATTTGGCCGG GCGTGACCTC ACTGATTATC TTATGAAAAT CCTTACTGAG
      301 AGAGGTTACA GTTTTACCAC AACTGCTGAG AGAGAAATTG TCCGTGACAT CAAAGAAAAG
      361 CTCTCCTACG TCGCTCTTGA TTTTGAGCAG GAAATGGAAA CTGCTGCCAA TTCTTCATCC
      421 CTGGAAAAAA CTTACGAATT TCCCGATGGC CAGGTTATTA CATTTGGAAA CGAACGTTTC
      481 AGATGCCCTG AGGCTTTGTT TCAGCCCTCT TTCTTGGGAA TGGGAACGGC GGGCGTCCAT
      541 GAAACCACCT ACAATTCTAT TATGAAATGT GATGTGGATA TCCGTAAAGA ATTGTACGCC
      601 AACACTGTCC TGTCTGGGGG ATCAACAATG TTTCCAGGCA TTGCTGACCG CATGCAGAAA
      661 GAAATTACAT CTCTTGCCCC ACCCACCATG AAGATCAAGA TCATTGCCCC TCCGGAAGGG
      721 AAATACTCGG TTTGGATTTG CAGTTCAATC TTTGTTTCTT TTCCCAATTT CTACCGAAGT
      781 TGGATCACCA AACAGGAGTA TGA
//
```

```
LOCUS       Hypselodoris_emma_BINPACKER_14_10 isoform 1    803 bp    mRNA
DEFINITION  Hypselodoris_emma_BINPACKER_14_10 isoform 1 actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris emma
  ORGANISM  Hypselodoris emma
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Hypselodoris emma"
                     /mol_type="mRNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVSHTVPIYEGYALPHAIIRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLDYVALDFEQEMQTASSSSSLEKSYELPDG
                     QVITIGNERFRCPEAMFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMFPGIADRMQKEISALAPPTMKIKIIAPPERKYSVWIGGSI
                     LASLSTFQQMWISKQEY"
ORIGIN
        1 GTTGCCCCAG AAGAACATCC TGTCCTTCTC ACAGAAGCTC CACTCAACCC TAAAGCTAAT
       61 AGAGAAAAAA TGACCCAAAT CATGTTTGAA ACCTTCAACT CCCCAGCCAT GTATGTTGCT
      121 ATTCAAGCCG TGCTTTCTCT CTATGCTTCA GGTCGTACCA CAGGTATTGT GCTTGATTCT
      181 GGAGATGGTG TCAGCCACAC AGTTCCAATT TATGAGGGCT ATGCTCTTCC TCATGCTATC
      241 ATCAGATTGG ATTTGGCTGG AAGGGACCTT ACAGATTACC TTATGAAGAT CCTTACCGAG
      301 AGAGGTTACA GCTTCACCAC AACTGCTGAA AGAGAAATTG TTCGTGATAT CAAAGAAAAA
      361 TTAGACTACG TTGCACTTGA CTTTGAGCAA GAAATGCAGA CTGCATCCTC ATCCTCATCT
      421 CTGGAAAAGA GCTACGAATT GCCAGATGGT CAGGTTATTA CTATTGGAAA TGAACGATTT
      481 AGGTGCCCGG AGGCCATGTT CCAGCCATCT TTCTTGGGTA TGGAATCAGC TGGTATTCAC
      541 GAAACTACCT ACAACTCCAT CATGAAATGT GATGTTGATA TCCGTAAAGA CTTGTATGCT
      601 AACACCGTCC TCTCTGGTGG CTCCACCATG TTCCCTGGTA TCGCTGATCG TATGCAGAAA
      661 GAAATTTCAG CACTTGCCCC ACCCACAATG AAGATCAAGA TCATTGCACC TCCTGAGCGT
      721 AAATACTCTG TATGGATTGG AGGTTCTATT TTGGCATCTT TGTCTACATT TCAACAGATG
      781 TGGATCTCCA AACAAGAATA TGA
//
```

```
LOCUS       H_emma_BINPACKER_187_31 isoform 2    803 bp    mRNA
DEFINITION  H_emma_BINPACKER_187_31 isoform 2 actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris emma
  ORGANISM  Hypselodoris emma
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Hypselodoris emma"
                     /mol_type="mRNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYAS
                     GRTTGIVFDSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMQTAASSSSMEKSYELPDG
                     QVITIGNERFRCPEALFQPSFLGMESAGIHETCYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMYPGIADRMQKEITALAPPTMKIKIIAPPERKYSVWIGGSI
                     LASLSTFQQMWISKQEY"
ORIGIN
        1 GTTGCACCAG AAGAGCACCC AGTATTGCTG ACAGAGGCTC CCCTCAACCC CAAGGCCAAC
       61 AGAGAAAAGA TGACCCAGAT CATGTTTGAG ACCTTCAACA CACCAGCCAT GTATGTTGCC
      121 ATCCAGGCTG TGTTGTCATT GTATGCATCT GGTCGTACCA CTGGAATCGT GTTCGACTCT
      181 GGAGATGGAG TGTCCCACAC TGTTCCCATC TATGAAGGTT ATGCCCTCCC TCACGCCATC
      241 CTCCGTTTGG ATTTGGCAGG ACGTGATCTA ACTGACTACC TGATGAAGAT CTTGACCGAG
      301 AGAGGTTACT CTTTCACCAC CACTGCTGAA CGTGAAATTG TCCGTGACAT CAAGGAGAAG
      361 TTGTGCTACG TTGCATTGGA CTTTGAACAA GAGATGCAGA CTGCTGCTAG CAGCTCAAGC
      421 ATGGAGAAGA GCTATGAGCT TCCTGATGGT CAAGTGATCA CCATTGGCAA CGAGCGATTC
      481 CGATGCCCTG AGGCTCTCTT CCAACCATCC TTCTTGGGAA TGGAATCAGC TGGTATCCAC
      541 GAGACCTGCT ACAACTCCAT CATGAAGTGT GACGTAGACA TCCGTAAAGA TCTGTATGCC
      601 AACACTGTGT TGTCTGGAGG CAGCACCATG TACCCAGGAA TTGCTGACAG AATGCAGAAG
      661 GAGATCACAG CTTTGGCACC ACCCACAATG AAGATTAAGA TCATTGCTCC ACCAGAGAGG
      721 AAATACTCTG TATGGATTGG TGGCTCCATC TTGGCTTCCC TGTCAACCTT CCAACAGATG
      781 TGGATCTCCA AACAGGAGTA CGA
//
```

```
LOCUS       H_emma_Single_97563 isoform 3   803 bp    mRNA
DEFINITION  H_emma_Single_97563 isoform 3 actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris emma
  ORGANISM  Hypselodoris emma
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Hypselodoris emma"
                     /mol_type="mRNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPVLLTEAPLNPKSNREKMTQIMFETFNAPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFESSSETEIVRDVKEKLAYIALDFEQEMDAAAKSSTIERSYELPDG
                     QMITLGSERFRCPEVLFQPSFVGVETAGIQEMIYNSITKCDIDLRRELYN
                     NIVLSGGTTMFPGIADRLHKELESIAPASNKIKIIAPPERKYSVWIGGSI
                     LGSLSTFQQMWITKQEY"
ORIGIN
        1 GTGGCACCTG AAGAACATCC CGTCTTGTTG ACAGAAGCTC CACTAAACCC CAAGTCCAAT
       61 CGTGAGAAAA TGACCCAGAT AATGTTCGAA ACTTTCAACG CGCCAGCTTT TTACGTCAGT
      121 ATTCAGGCGG TCCTAGCTCT GTACGCTTCA GGCAGGACGA CAGGCGTGGT GTTAGACGCA
      181 GGAGACGGCG TCACCCATAT CATTCCAATA TACGAAGGCT ATGCTCTTCC CCACGCTATT
      241 GAAAAGATGA ACTTGGCCGG TCGGGACCTC ACTGGCTATC TCAAGCGAAT CCTTCACGAA
      301 CGAGGTTACA ATTTTGAATC GTCTTCGGAG ACCGAAATAG TCCGAGACGT AAAGGAAAAG
      361 CTCGCCTACA TCGCTCTGGA TTTTGAACAG GAGATGGACG CAGCAGCCAA GTCGTCAACC
      421 ATCGAAAGAT CATACGAACT TCCCGATGGT CAAATGATTA CTCTAGGCTC CGAACGATTC
      481 AGGTGCCCTG AAGTTTTGTT CCAACCGTCT TTCGTGGGAG TAGAAACTGC CGGCATTCAA
      541 GAAATGATCT ACAACTCCAT AACCAAGTGT GACATTGATC TCCGAAGAGA ATTATACAAT
      601 AACATTGTCC TCTCTGGGGG AACCACAATG TTCCCAGGTA TCGCCGATCG GTTACACAAA
      661 GAGTTGGAAT CCATTGCCCC AGCGAGTAAC AAAATCAAGA TCATTGCTCC TCCCGAACGC
      721 AAATACTCGG TTTGGATTGG TGGATCAATT TTGGGATCTT TGTCAACTTT CCAACAGATG
      781 TGGATCACTA AACAAGAGTA TGA
//
```

```
LOCUS       Hexabranchus_sanguineus/Hypselodoris_(Hysp16Bu1/NU88)    803 bp    DNA

DEFINITION  Hexabranchus_sanguineus/Hypselodoris_(Hysp16Bu1/NU88) actin, partial
CDS

ACCESSION  not yet submitted to GenBank

SOURCE      Hexabranchus sanguineus

  ORGANISM  Hexabranchus sanguineus

            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;

             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;

             Doridina; Chromodoridoidea; Hexabranchidae; Hexabranchus

FEATURES             Location/Qualifiers

     source          1..803

                     /organism="Hexabranchus sanguineus"

                     /mol_type="genomic DNA"

     CDS             <1..>803

                     /codon_start=1

                     /product="actin"

               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNAPAFYVGIQAVLALYSS

                     GRTTGVVLDAGDGVSHVIPIYKGYALPHAIEKMNLAGRDLTGYLKRILHE

                     RGYNFETSSETEIVRDVKEKLAYVAFDFEKEMDTASQSSAVEKSYELPDG

                     QMVTLGSERFRCPEVLFQPSFIGMESVGIHEMIYNSVMKCDIDLRRDLYN

                     NIVLSGGTTMFPGISNRLHREIEAIAPSGNKIKIVAPHERKYSVWIGGSI

                     MGSLSTFQQMWISKQEY"

ORIGIN

        1 GTGGCACCTG AAGAGCACCC AGTCTTGCTG ACCGAGGCAC CCCTGAACCC CAAAGCCAAT

       61 CGAGAGAAAA TGACACAAAT CATGTTTGAA ACATTCAATG CGCCAGCCTT TTACGTCGGT

      121 ATTCAGGCAG TCCTGGCTTT GTATTCATCT GGGAGGACAA CTGGCGTTGT GTTGGATGCG

      181 GGAGATGGCG TCAGCCACGT CATTCCAATC TACAAAGGCT ACGCCCTTCC CCATGCCATA

      241 GAAAAGATGA ATCTAGCCGG CCGAGACCTT ACGGGTTATT TGAAACGCAT CCTTCATGAA

      301 CGAGGCTACA ACTTTGAAAC GTCGTCTGAG ACAGAGATTG TGCGTGATGT CAAGGAAAAG

      361 TTGGCATATG TCGCTTTTGA CTTTGAAAAG GAGATGGACA CTGCCAGTCA GTCATCAGCT

      421 GTCGAAAAAT CTTATGAGTT GCCTGATGGT CAAATGGTTA CCTTGGGCTC TGAACGATTC

      481 AGGTGTCCAG AGGTTTTGTT CCAGCCATCT TTCATTGGGA TGGAGTCCGT GGGAATCCAT

      541 GAAATGATCT ATAATTCTGT TATGAAATGT GACATTGATC TGCGGCGGGA CTTGTACAAC

      601 AACATTGTCT TGTCGGGAGG AACGACCATG TTTCCAGGCA TCTCCAATCG TTTGCATAGG

      661 GAAATTGAAG CCATTGCCCC ATCTGGCAAT AAGATTAAAA TCGTTGCACC TCATGAACGC

      721 AAATATTCCG TATGGATTGG AGGATCTATT ATGGGATCTT TGTCAACGTT CCAGCAGATG

      781 TGGATCAGCA AACAGGAATA TGA

//
```

```
LOCUS       Hexabranchus_sanguineus_(Glsp1_17Ba1/NU382)    803 bp    DNA
DEFINITION  Hexabranchus_sanguineus_(Glsp1_17Ba1/NU382) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hexabranchus sanguineus
  ORGANISM  Hexabranchus sanguineus
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Hexabranchidae; Hexabranchus
FEATURES             Location/Qualifiers
     source          1..803
                     /organism="Hexabranchus sanguineus"
                     /mol_type="genomic DNA"
     CDS             <1..>803
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPVLLTEAPLNPKANREKMIQIMFETFNAPAFYVGIQAVLALYSS
                     GRTTGVVLDAGDGVSHVIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                     RGYNFETSSETEIVRDVKEKLAYVALDFEKEMDTASQSSAVEKSYELPDG
                     QMVTLGSERFRCPEVLFQPSFIGMESVGIHEMIYNSVMKCDIDLRRDLYN
                     NIVLSGGTTMFPGISNRLHREIEAIAPSGNKIKIVAPHERKYSVWIGGSI
                     MGSLSTFQQMWISKQEY"
ORIGIN
        1 GTGGCACCTG AAGAGCACCC AGTCTTGCTG ACCGAGGCAC CCCTGAACCC CAAAGCCAAT
       61 CGAGAGAAAA TGATACAAAT CATGTTTGAA ACATTCAATG CGCCAGCCTT TTACGTCGGT
      121 ATTCAGGCAG TCCTGGCTTT GTATTCATCT GGGAGGACAA CTGGCGTTGT GTTGGATGCG
      181 GGAGATGGCG TCAGCCATGT CATTCCAATC TACGAAGGCT ACGCCCTTCC CCATGCCATA
      241 GAAAAGATGA ATCTAGCCGG CCGAGACCTT ACGGGTTATT TGAAACGCAT CCTTCATGAA
      301 CGAGGCTACA ACTTTGAAAC GTCGTCTGAG ACAGAGATTG TGCGTGATGT CAAGGAAAAG
      361 TTGGCATATG TCGCTCTTGA CTTTGAAAAG GAGATGGACA CTGCCAGTCA GTCATCAGCT
      421 GTCGAAAAAT CCTATGAGCT GCCCGATGGT CAAATGGTTA CCTTGGGCTC TGAACGATTC
      481 AGGTGTCCAG AGGTTTTGTT CCAGCCATCT TTCATTGGGA TGGAGTCCGT GGGAATCCAT
      541 GAAATGATCT ATAATTCTGT TATGAAATGT GACATTGATC TGCGGCGGGA CTTGTACAAC
      601 AACATTGTCT TGTCGGGAGG AACGACCATG TTTCCAGGCA TCTCCAATCG TTTGCATAGG
      661 GAAATTGAAG CCATTGCCCC ATCTGGCAAT AAGATTAAAA TCGTTGCACC TCATGAACGC
      721 AAATATTCCG TATGGATTGG AGGATCTATT ATGGGATCTT TGTCAACGTT CCAGCAGATG
      781 TGGATCAGCA AACAGGAATA TGA
//
```

```
LOCUS       Hypselodoris_apolegma_(Hyap17Ba1/NU387)    787 bp    DNA
DEFINITION  Hypselodoris_apolegma_(Hyap17Ba1/NU387) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris apolegma
  ORGANISM  Hypselodoris apolegma
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..787
                     /organism="Hypselodoris apolegma"
                     /mol_type="genomic DNA"
     CDS             <1..>787
                     /codon_start=1
                     /product="actin"
              /translation="VAPEEHPVLLTEAPLNPKANREKMTQNMFETFNAPAFYVSIQAVLALYAS
                   GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                   RGYNFDTSSETEIVRDVKEKLAYVALDFEQEMDTAVKSSTVERSYELPDG
                   QVITLGSERFRCPEVLFQPSFTGVETAGIHEMIYNSVTKCDIDLRRELYH
                   NVVLSGGTTMFPGIADRLHKELESIAPASNKIKIIAPPERKYSVWIGGSI
                   LVSLSTFQQMWI"
ORIGIN
        1 GTTGCTCCAG AAGAACACCC AGTGCTCCTT ACGGAAGCTC CCCTTAACCC TAAAGCCAAC
       61 CGTGAGAAAA TGACCCAGAA TATGTTCGAA ACTTTCAACG CGCCAGCTTT TTACGTCAGT
      121 ATTCAGGCGG TGCTAGCTCT GTACGCTTCA GGCAGGACGA CGGGCGTGGT GTTAGACGCA
      181 GGCGACGGTG TCACTCATAT CATTCCAATA TATGAGGGCT ATGCTCTGCC TCATGCTATT
      241 GAAAAGATGA ACTTGGCCGG CCGGGATCTT ACTGGCTACC TCAAGCGAAT CCTTCACGAG
      301 CGAGGTTACA ATTTTGATAC GTCTTCGGAG ACCGAAATAG TTCGAGACGT GAAGGAAAAG
      361 CTGGCCTACG TCGCTCTGGA TTTTGAGCAG GAGATGGACA CAGCCGTCAA GTCGTCAACA
      421 GTCGAAAGGT CTTACGAACT TCCCGATGGT CAAGTGATTA CTCTGGGCTC CGAACGATTT
      481 AGGTGCCCCG AAGTTCTCTT CCAACCCTCT TTCACAGGAG TGGAAACTGC CGGCATCCAC
      541 GAAATGATCT ACAACTCTGT AACCAAGTGT GACATTGATC TACGAAGAGA ATTGTACCAT
      601 AACGTTGTCC TGTCTGGGGG AACCACAATG TTCCCAGGTA TAGCCGATCG GTTACACAAA
      661 GAGTTGGAAT CCATTGCCCC AGCCAGTAAC AAAATCAAAA TCATTGCCCC TCCCGAGCGC
      721 AAATACTCTG TTTGGATTGG AGGATCCATC TTGGTGTCTT TGTCAACCTT CCAGCAGATG
      781 TGGATCA
//
```

```
LOCUS       Hypselodoris_bullockii_(Hybu16Bu1/NU86)    786 bp    DNA
DEFINITION  Hypselodoris_bullockii_(Hybu16Bu1/NU86) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris bullockii
  ORGANISM  Hypselodoris bullockii
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..786
                     /organism="Hypselodoris bullockii"
                     /mol_type="genomic DNA"
     CDS             <1..>786
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLSYVALDFEQEMKTAAESSSLEKSYELPDG
                     QVITIGNERFRCPEAMLQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMFPGIADRMQKEITALAPPTMKIKIIAPPEREYSVWIGGSIL
                     ASLSTFQQMWI"
ORIGIN
        1 GTTGCTCCAG AAGAACACCC AGTGCTCCTT ACGGAAGCTC CCCTTAACCC TAAAGCCAAC
       61 AGAGAAAAGA TGACTCAAAT CATGTTTGAA ACCTTCAACT CACCGGCTAT GTACGTCGCT
      121 ATCCAAGCTG TGCTTTCTCT TTATGCTTCA GGTCGTACCA CAGGTATCGT GCTTGACTCT
      181 GGAGATGGCG TCAGCCATAC GGTTCCAATC TATGAAGGTT ACGCACTTCC TCATGCCATC
      241 CTAAGATTGG ATCTGGCGGG ACGTGATCTC ACAGACTATT TGATGAAAAT CCTAACAGAG
      301 AGAGGTTACA GTTTCACTAC AACTGCTGAA AGAGAAATTG TCAGAGACAT TAAAGAAAAA
      361 TTATCCTACG TCGCTCTTGA TTTTGAACAG GAAATGAAAA CCGCAGCCGA ATCTTCCTCC
      421 CTGGAAAAGA GTTACGAATT ACCCGACGGG CAGGTGATCA CAATTGGCAA CGAGCGGTTC
      481 AGATGTCCAG AAGCCATGCT CCAGCCCTCT TTCTTGGGTA TGGAATCAGC TGGTATCCAT
      541 GAAACCACGT ACAATTCTAT TATGAAGTGT GATGTGGATA TCCGTAAAGA CTTGTATGCA
      601 AACACTGTCC TGTCTGGAGG CTCCACTATG TTCCCTGGCA TCGCTGATCG TATGCAGAAG
      661 GAAATAACTG CGCTTGCTCC ACCTACCATG AAGATCAAAA TCATTGCACC TCCCGAGCGT
      721 GAATACTCTG TATGGATTGG AGGATCTATC CTGGCATCTT TGTCAACATT CCAACAGATG
      781 TGGATC
//
```

```
LOCUS       Hypselodoris_maculosa_1_(Hyma17Ba1/NU388)    787 bp    DNA
DEFINITION  Hypselodoris_maculosa_1_(Hyma17Ba1/NU388) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris maculosa
  ORGANISM  Hypselodoris maculosa
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..787
                     /organism="Hypselodoris maculosa"
                     /mol_type="genomic DNA"
     CDS             <1..>787
                     /codon_start=1
                     /product="actin"
              /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNAPAFYVSIQAVLALYAS
                   GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTGYLKRILHE
                   RGYNFESSSETEIVRDVKEKLAYVALDFEQEMDAAAKSSTIERSYELPDG
                   QVITLGSERFRCPEVLFQPSFVGVETAGIHEMIYNSITKCDIDLRRELYN
                   NIVLSGGTTMFPGIADRLHKELESIAPASNKIKIIAPPERKYSVWIGGSI
                   LGSLSTFQQMWI"
ORIGIN
        1 GTTGCTCCAG AAGAACACCC AGTGCTCCTT ACGGAAGCTC CCCTTAACCC TAAAGCCAAC
       61 CGTGAGAAAA TGACCCAGAT AATGTTCGAA ACTTTCAACG CGCCAGCTTT TTACGTCAGT
      121 ATCCAGGCGG TCCTAGCTCT GTACGCTTCA GGCAGGACGA CAGGCGTGGT GTTAGACGCA
      181 GGAGACGGCG TCACCCATAT CATTCCAATA TACGAGGGCT ATGCTCTGCC CCACGCTATT
      241 GAAAAGATGA ACTTGGCCGG TCGGGATCTC ACTGGCTATC TCAAGCGAAT CCTTCACGAA
      301 CGAGGTTACA ATTTTGAATC GTCTTCCGAG ACCGAAATAG TCCGAGACGT GAAGGAAAAG
      361 CTGGCCTACG TCGCTCTGGA TTTTGAACAG GAGATGGACG CAGCAGCCAA GTCGTCAACC
      421 ATCGAAAGAT CTTACGAACT TCCCGATGGT CAAGTGATTA CTCTGGGCTC CGAACGATTT
      481 AGGTGCCCTG AAGTTTTGTT TCAACCGTCT TTCGTGGGAG TGGAAACTGC CGGCATTCAC
      541 GAAATGATCT ACAACTCCAT AACCAAGTGT GACATTGATC TCCGAAGAGA ATTGTACAAT
      601 AACATTGTCT TGTCTGGAGG AACCACAATG TTCCCAGGTA TCGCCGATCG GTTACACAAA
      661 GAGTTGGAAT CCATTGCCCC AGCCAGTAAC AAAATCAAGA TCATTGCTCC TCCCGAACGC
      721 AAATACTCGG TTTGGATTGG TGGATCAATC TTGGGATCTT TGTCAACCTT CCAGCAGATG
      781 TGGATCA
//
```

```
LOCUS       Hypselodoris_maculosa_2_(Hyma17Ba2/NU389)    787 bp    DNA
DEFINITION  Hypselodoris_maculosa_2_(Hyma17Ba2/NU389) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris maculosa
  ORGANISM  Hypselodoris maculosa
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..787
                     /organism="Hypselodoris maculosa"
                     /mol_type="genomic DNA"
     CDS             <1..>787
                     /codon_start=1
                     /product="actin"
              /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNAPAFYVSIQAVLALYAS
                   GRTTGVVLDAGDGVTHIIPIYEGYALPHAVEKMNLAGRDLTGYLKRILHE
                   RGYNFESSSETEIVRDVKEKLAYVALDFEQEMDAAAKSSTIERSYELPDG
                   QMITLGSERFRCPEVLFQPSFIGVETAGIHEMIYNSITKCDIDIRRELYN
                   NIVLSGGTTMFPGIADRLHKELESVAPASNKIKIIAPPERKFSVWIGGSI
                   LGSLSTFQQMWI"
ORIGIN
        1 GTTGCTCCAG AAGAACACCC AGTGCTCCTT ACGGAAGCTC CCCTTAACCC TAAAGCCAAC
       61 CGTGAGAAAA TGACCCAGAT AATGTTCGAA ACTTTCAACG CGCCAGCTTT TTACGTCAGT
      121 ATCCAGGCGG TCCTAGCTCT GTACGCTTCA GGCAGGACGA CAGGCGTGGT GTTAGACGCA
      181 GGAGACGGCG TCACCCATAT CATTCCAATA TACGAGGGCT ATGCTCTGCC CCACGCTGTT
      241 GAAAAGATGA ACTTGGCCGG TCGGGACCTC ACTGGCTATC TCAAGCGGAT CCTTCACGAA
      301 CGAGGTTACA ATTTTGAATC GTCTTCGGAG ACCGAAATAG TCCGAGACGT GAAGGAAAAG
      361 CTGGCCTACG TCGCTCTGGA TTTTGAACAG GAGATGGACG CAGCAGCCAA GTCGTCAACC
      421 ATCGAAAGAT CTTACGAACT TCCCGATGGT CAAATGATTA CTCTGGGCTC CGAACGATTT
      481 AGGTGCCCTG AAGTTTTGTT CCAACCGTCT TTCATTGGAG TGGAAACTGC CGGCATTCAC
      541 GAAATGATCT ACAACTCCAT AACCAAGTGT GACATTGATA TCCGAAGAGA ACTGTACAAT
      601 AACATTGTCC TGTCTGGGGG AACCACAATG TTCCCAGGTA TCGCCGATCG GTTACACAAA
      661 GAGTTGGAAT CCGTCGCCCC AGCCAGTAAC AAAATCAAGA TCATTGCTCC TCCCGAGCGC
      721 AAATTCTCGG TTTGGATTGG TGGATCAATC TTGGGATCTT TGTCAACCTT CCAGCAGATG
      781 TGGATCA
//
```

```
LOCUS       Hypselodoris_maridadilus_(Hymari17Ba1/NU390)    784 bp    DNA
DEFINITION  Hypselodoris_maridadilus_(Hymari17Ba1/NU390) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris maridadilus
  ORGANISM  Hypselodoris maridadilus
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..784
                     /organism="Hypselodoris maridadilus"
                     /mol_type="genomic DNA"
     CDS             <1..>784
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSTLEKSYELPDG
                     QVITIGNERFRTPEAMFQPSFLGMESAGVHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMYPGIADRMQKEITALAPSTMKIKIIAPPEREYSVWIGGSI
                     LASLSTFQQMW"
ORIGIN
        1 GTTGCTCCAG AAGAACACCC AGTGCTCCTT ACGGAAGCTC CCCTTAACCC TAAAGCCAAC
       61 AGAGAAAAGA TGACCCAAAT CATGTTTGAA ACCTTCAACT CACCGGCTAT GTATGTGGCT
      121 ATTCAAGCTG TGCTTTCTCT CTATGCCTCA GGCCGTACCA CAGGTATCGT GCTTGACTCC
      181 GGAGACGGTG TTACCCATAC GGTCCCAATC TATGAAGGAT ACGCCCTTCC CCATGCAATC
      241 ATGAGACTGG ATTTGGCGGG ACGTGACCTC ACGGACTACC TTATGAAAAT CCTGACAGAG
      301 AGAGGTTACA GTTTCACCAC CACCGCGGAG AGAGAAATTG TCCGTGACAT TAAAGAAAAG
      361 CTCTGCTACG TCGCTCTTGA TTTTGAACAA GAAATGGGCA CTGCGGCATC TTCCTCTACC
      421 CTTGAGAAAA GCTACGAGCT TCCCGACGGG CAAGTCATCA CCATTGGAAA CGAGCGATTC
      481 AGAACTCCGG AAGCGATGTT CCAGCCATCG TTTTTGGGAA TGGAATCGGC AGGCGTCCAT
      541 GAGACTACGT ACAATTCCAT CATGAAATGT GATGTTGACA TCCGGAAAGA TCTGTACGCC
      601 AATACCGTTC TCTCTGGTGG TTCTACCATG TACCCAGGCA TTGCTGACCG TATGCAAAAA
      661 GAGATAACGG CCCTTGCTCC AAGCACCATG AAGATCAAGA TCATTGCACC TCCCGAGCGT
      721 GAATACTCTG TATGGATTGG AGGATCTATC CTGGCATCTT TGTCAACATT CCAACAGATG
      781 TGGA
//
```

```
LOCUS       Hypselodoris_tryoni_(Hytr17Ba1/NU90)    747 bp    DNA
DEFINITION  Hypselodoris_tryoni_(Hytr17Ba1/NU90) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Hypselodoris tryoni
  ORGANISM  Hypselodoris tryoni
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Hypselodoris
FEATURES             Location/Qualifiers
     source          1..747
                     /organism="Hypselodoris tryoni"
                     /mol_type="genomic DNA"
     CDS             <1..>747
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYAS
                     GRTTGIVMDSGDGVSHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKIMTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMQTASSSSSLEKTYELPDG
                     QVITIGNERFRCPEAMFQPSFLGMESAGVHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMFPGIADRMQKEITALAPPTMKIKIIAPPERKYSVWIGGS"
ORIGIN
        1 GTTGCCCCAG AAGAACATCC AGTTCTTCTC ACAGAAGCTC CTCTCAACCC TAAAGCCAAC
       61 AGAGAAAAGA TGACTCAAAT CATGTTTGAA ACCTTCAACA CACCAGCAAT GTATGTAGCC
      121 ATTCAAGCGG TACTTTCTCT TTATGCTTCG GGTCGTACCA CAGGTATTGT GATGGACTCT
      181 GGAGATGGTG TTTCTCACAC AGTCCCTATC TATGAAGGAT ACGCTCTTCC TCATGCAATA
      241 ATGAGACTGG ATTTAGCTGG ACGTGACCTT ACCGATTACC TCATGAAAAT CATGACAGAG
      301 AGAGGCTATA GCTTTACAAC CACTGCTGAG AGAGAAATTG TACGTGATAT AAAAGAAAAA
      361 CTATGCTATG TTGCTCTTGA TTTTGAACAA GAGATGCAAA CGGCTTCATC GTCCTCATCT
      421 CTTGAAAAGA CCTATGAATT GCCCGATGGA CAAGTTATCA CCATTGGAAA CGAGCGATTC
      481 AGGTGTCCAG AAGCTATGTT TCAGCCATCT TTCTTGGGCA TGGAAAGTGC TGGTGTCCAT
      541 GAAACCACGT ACAATTCTAT AATGAAATGT GACGTAGACA TCCGTAAAGA CCTGTACGCA
      601 AACACTGTTT TGTCTGGAGG TTCCACAATG TTTCCTGGCA TTGCTGACCG CATGCAGAAG
      661 GAAATTACAG CTCTTGCTCC TCCCACCATG AAAATCAAAA TCATTGCACC TCCAGAGCGT
      721 AAGTACTCTG TATGGATTGG AGGCTCT //
```

```
LOCUS       Miamira_spec_(Misp17Ba_1/NU395) isoform 1   756 bp    DNA
DEFINITION  Miamira_spec.(Misp17Ba_1/NU395) isoform 1 actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Miamira spec. Misp17Ba_1/NU395
  ORGANISM  Miamira spec. Misp17Ba_1/NU395
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Miamira
FEATURES             Location/Qualifiers
     source          1..756
                     /organism="Miamira spec. Misp17Ba_1/NU395"
                     /mol_type="genomic DNA"
     CDS             <1..>756
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPTLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLNLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMQTAASSSSLENSYELPDG
                     QVITIGNERFRCPEAMFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMFPGIADRMQKEISALAPPTMKIKIIAPPERKYSVWIGSSILV"
ORIGIN
        1 GTGGCACCTG AGGAACATCC CACCTTATTG ACGGAGGCTC CTCTCAACCC AAAAGCCAAC
       61 AGAGAAAAGA TGACCCAAAT CATGTTTGAG ACCTTTAACT CACCCGCCAT GTACGTGGCT
      121 ATTCAAGCTG TGCTTTCTCT CTATGCTTCA GGTCGTACCA CAGGTATTGT GCTGGATTCC
      181 GGAGATGGTG TCACACACAC TGTTCCCATC TATGAGGGAT ATGCTCTTCC CCATGCCATC
      241 ATGAGATTGA ATTTGGCTGG ACGTGACCTA ACCGATTACC TTATGAAGAT CCTTACGGAG
      301 AGAGGCTACA GTTTCACCAC CACTGCTGAA AGGGAAATTG TCCGTGATAT TAAAGAAAAG
      361 CTCTGCTACG TTGCTCTTGA TTTTGAACAA GAAATGCAAA CAGCTGCATC TTCTTCTTCT
      421 CTTGAAAATA GTTACGAATT ACCTGATGGC CAAGTCATCA CCATTGGAAA CGAGCGATTC
      481 AGGTGTCCTG AAGCCATGTT TCAACCATCT TTCTTGGGTA TGGAATCTGC TGGTATCCAT
      541 GAAACCACTT ACAATTCTAT TATGAAGTGT GATGTGGACA TCCGTAAAGA TTTGTATGCA
      601 AACACTGTCT TGTCTGGAGG CTCCACCATG TTCCCTGGTA TTGCTGACCG CATGCAGAAG
      661 GAAATCTCAG CTCTTGCTCC ACCCACCATG AAGATCAAAA TCATCGCACC TCCAGAGCGT
      721 AAATATTCTG TATGGATCGG AAGCTCCATC CTCGTC //
```

```
LOCUS       Miamira_spec__(Misp17Ba_1/NU395) isoform 2   756 bp    DNA
DEFINITION  Miamira_spec._(Misp17Ba_1/NU395) isoform 2 actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Miamira spec. (Misp17Ba_1/NU395)
  ORGANISM  Miamira spec. (Misp17Ba_1/NU395)
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Miamira
FEATURES             Location/Qualifiers
     source          1..756
                     /organism="Miamira spec. (Misp17Ba_1/NU395)"
                     /mol_type="genomic DNA"
     CDS             <1..>756
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPTLLTEAPLNPKANREKMTQIMFETFNTPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMQTAASSSSLERSYELPDG
                     QVITIGNERFRCPEAMFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMFPGIADRMQKELESLAPASNKIKIIAPPERKYSVWIGGSILV"
ORIGIN
        1 GTGGCACCTG AGGAACATCC CACCTTATTG ACAGAAGCTC CCCTGAATCC AAAGGCTAAC
       61 CGTGAGAAAA TGACCCAGAT TATGTTCGAG ACTTTCAACA CGCCAGCTTT CTACGTCAGT
      121 ATCCAGGCGG TTCTTGCTTT GTACGCTTCA GGGAGGACAA CAGGCGTGGT GTTGGATGCA
      181 GGAGATGGGG TGACTCATAT CATTCCAATA TACGAGGGCT ACGCTCTGCC CCATGCTATT
      241 GAAAAGATGA ACTTGGCTGG ACGTGACCTC ACCGATTACC TTATGAAGAT CCTTACGGAG
      301 AGAGGCTACA GTTTCACCAC CACTGCTGAA AGGGAAATTG TCCGTGATAT TAAAGAAAAG
      361 CTCTGCTACG TTGCTCTTGA TTTTGAACAA GAAATGCAAA CAGCTGCATC TTCTTCTTCT
      421 CTTGAGAGAT CTTACGAACT GCCCGATGGT CAAGTGATCA CCATTGGAAA CGAGCGATTC
      481 AGGTGTCCTG AAGCCATGTT TCAACCGTCT TTCTTGGGTA TGGAATCTGC TGGTATCCAC
      541 GAAACCACTT ACAATTCTAT TATGAAGTGT GATGTGGACA TCCGTAAAGA TTTGTATGCA
      601 AACACTGTCT TGTCTGGAGG CTCCACCATG TTCCCTGGTA TTGCTGACCG CATGCAGAAG
      661 GAGCTGGAAT CCCTAGCCCC AGCCAGTAAC AAAATCAAGA TCATTGCCCC TCCCGAGCGC
      721 AAATATTCTG TTTGGATTGG AGGATCAATC CTCGTC //
```

```
LOCUS       Miamira_sinuata_(Misi16Bu1/NU92)    756 bp    DNA
DEFINITION  Miamira_sinuata_(Misi16Bu1/NU92)
ACCESSION  not yet submitted to GenBank
SOURCE      Miamira sinuata
  ORGANISM  Miamira sinuata
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Miamira
FEATURES             Location/Qualifiers
     source          1..756
                     /organism="Miamira sinuata"
                     /mol_type="genomic DNA"
     CDS             <1..>756
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPALLTEAPLNPKANREKMTQIMFETFNTPAFYVSIQAVLALYAS
                     GRTTGVVLDAGDGVTHIIPIYEGYALPHAIEKMNLAGRDLTDYLMKILTH
                     RGYSFTTTAEREIVRDIKEKLCYIALDFEQEMMTSSISSSLERSYELPDG
                     QVITIGNERFRCPEAMFQPSFLGMENAGIHEMIYNSITKCDIDLRRDLYQ
                     NIVLSGGTTMFPGIADRLHKEMESIAPPSNKIKVIAPPERKYSVWIGGSILV"
ORIGIN
        1 GTGGCACCTG AGGAACATCC CGCCTTATTA ACAGAGGCAC CCTTAAACCC AAAGGCCAAC
       61 CGTGAGAAAA TGACCCAGAT AATGTTCGAA ACTTTCAACA CGCCAGCTTT TTACGTCAGC
      121 ATTCAAGCAG TACTTGCTTT GTACGCTTCA GGGAGAACGA CAGGTGTGGT GTTGGACGCA
      181 GGAGATGGTG TGACTCACAT CATTCCTATA TACGAGGGCT ACGCTCTTCC CCATGCTATT
      241 GAAAAGATGA ACCTGGCTGG CCGTGATCTC ACAGATTACC TTATGAAAAT CCTTACGCAT
      301 AGAGGCTACA GCTTCACAAC TACTGCCGAG AGGGAAATTG TCCGTGATAT AAAGGAAAAG
      361 CTCTGCTACA TTGCTCTTGA TTTTGAACAA GAAATGATGA CATCTTCAAT ATCTTCATCT
      421 CTTGAAAGAT CCTATGAACT GCCAGATGGA CAAGTTATCA CCATAGGGAA CGAGCGATTC
      481 AGATGTCCTG AAGCCATGTT TCAACCGTCT TTCTTGGGTA TGGAAAACGC CGGTATCCAC
      541 GAAATGATCT ACAACTCCAT AACCAAATGT GACATTGATC TTAGAAGAGA TCTGTACCAA
      601 AATATCGTCT TGTCTGGCGG AACTACAATG TTCCCAGGTA TAGCTGATCG ATTACATAAA
      661 GAAATGGAAT CCATCGCTCC ACCAAGTAAC AAGATCAAGG TCATTGCTCC TCCCGAGCGC
      721 AAATACTCTG TTTGGATCGG AGGATCCATC CTCGTC //
```

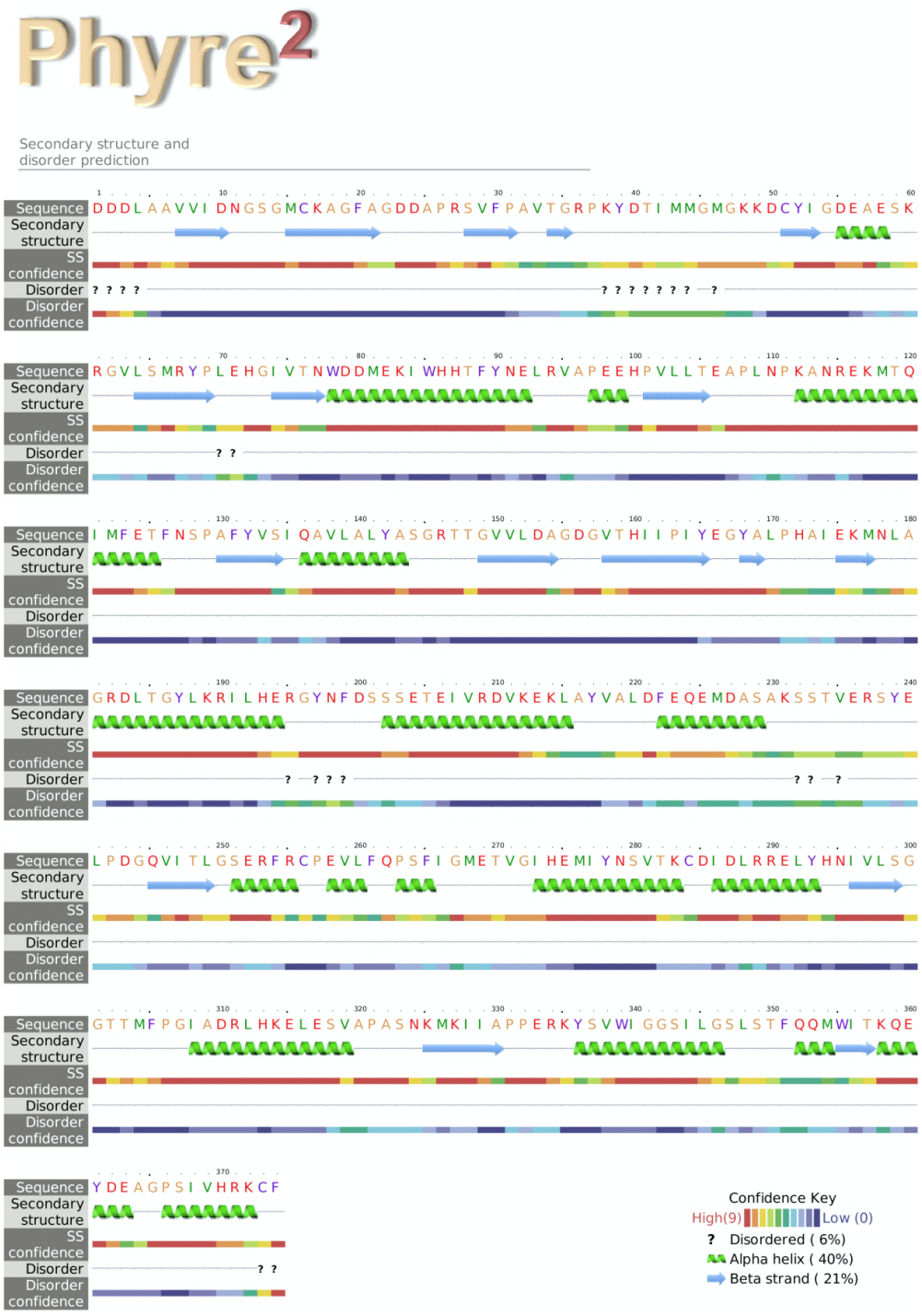
```
LOCUS       Phyllidia_coelestis_(ZFMKDNAFD02298087)    747 bp    DNA
DEFINITION  Phyllidia_coelestis_(ZFMKDNAFD02298087) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Phyllidia coelestis
  ORGANISM  Phyllidia coelestis
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Phyllidioidea; Phyllidiidae; Phyllidia
FEATURES             Location/Qualifiers
     source          1..747
                     /organism="Phyllidia coelestis"
                     /mol_type="genomic DNA"
     CDS             <1..>747
                     /codon_start=1
                     /product="actin"
               /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVSHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMQTAASSSSLEKSYELPDG
                     QVITIGNERFRCPEAMFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMFPGIADRMQKEISALAPPTMKIKIIAPPERKYSVWIGGS"
ORIGIN
        1 GTTGCCCCAG AAGAACATCC TGTCCTTCTC ACAGAGGCTC CTCTCAACCC CAAGGCCAAC
       61 AGAGAAAAGA TGACCCAAAT TATGTTTGAA ACCTTCAACT CACCAGCCAT GTACGTTGCT
      121 ATCCAAGCCG TACTGTCTCT GTACGCTTCA GGTCGTACCA CAGGTATCGT TCTGGACTCA
      181 GGAGATGGTG TCAGTCATAC TGTCCCCATC TATGAAGGTT ATGCTCTGCC TCATGCCATC
      241 ATGAGATTGG ATTTGGCCGG ACGTGATCTC ACAGATTACC TTATGAAAAT CCTCACTGAA
      301 AGAGGCTACA GCTTCACCAC CACTGCTGAA AGAGAAATTG TCCGTGACAT CAAGGAAAAG
      361 CTCTGTTATG TCGCTCTTGA CTTCGAACAA GAAATGCAAA CTGCAGCCTC ATCATCATCC
      421 CTTGAGAAGA GCTACGAATT GCCCGATGGA CAAGTCATCA CAATTGGAAA CGAGCGTTTC
      481 CGATGCCCCG AAGCCATGTT CCAACCATCT TTCTTGGGTA TGGAATCTGC TGGCATTCAT
      541 GAAACCACTT ACAACTCTAT CATGAAGTGT GACGTCGATA TCCGTAAAGA CTTGTATGCC
      601 AACACCGTCC TTTCTGGTGG CTCCACCATG TTCCCTGGCA TTGCTGATCG TATGCAAAAG
      661 GAAATCTCAG CTCTTGCTCC ACCCACCATG AAGATCAAGA TTATCGCTCC ACCAGAACGT
      721 AAATACTCTG TATGGATCGG AGGCTCA //
```

```
LOCUS       Pleurobranchus peronii    737 bp    DNA
DEFINITION  Pleurobranchus_peronii actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Pleurobranchus peronii
  ORGANISM  Pleurobranchus peronii
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Pleurobranchida;
             Pleurobranchdoidea; Pleurobranchidae; Pleurobranchus
FEATURES             Location/Qualifiers
     source          1..737
                     /organism="Pleurobranchus peronii"
                     /mol_type="genomic DNA"
     CDS             <1..>737
                     /codon_start=1
                     /product="actin"
                /translation="VAPERHPVHLTEAPLNPKRNREKMTQILFETFNTPAFYVSIQAVLSLYSS
                     GRTTGIVLDSGDGVTHTVPIYEGYTLPHALNRIDLAGRDLTSYLQRILNE
                     RGYSLTTTSEKEIVRDIKEKLCYTALDFDAEMNAASQSSSIEKSYELPDG
                     QIVTIGNERFRCTEVLFQPSFIGREDGGISELLYNSVMKCDIDIRRDLLT
                     NSVISGGTTMFPGLADRLQKELTTLAPSSNKVRVIAPPERKYSVW"
ORIGIN
        1 GTTGCACCCG AGAGACATCC TGTGCATCTG ACAGAAGCTC CACTTAACCC TAAGCGTAAC
       61 AGAGAAAAAA TGACACAAAT CCTCTTTGAG ACATTCAATA CCCCAGCTTT CTACGTGAGT
      121 ATCCAGGCTG TGCTGAGTTT GTATTCTTCC GGTCGAACGA CCGGAATTGT TTTGGATTCT
      181 GGGGACGGCG TTACACACAC AGTTCCTATA TACGAAGGTT ATACCCTCCC CCATGCACTC
      241 AATCGAATAG ATCTGGCTGG AAGAGACCTG ACCAGCTATC TTCAAAGGAT TCTTAACGAG
      301 AGAGGGTACA GTCTTACGAC TACGTCAGAA AAGGAAATCG TTAGAGACAT TAAAGAGAAA
      361 CTGTGCTATA CTGCTTTGGA CTTCGACGCT GAAATGAACG CTGCATCCCA ATCATCGTCG
      421 ATAGAAAAGT CATACGAACT GCCCGATGGA CAAATCGTTA CCATTGGCAA TGAACGCTTC
      481 CGCTGCACAG AGGTACTTTT TCAACCTTCC TTCATAGGGC GAGAAGACGG TGGAATTAGC
      541 GAGCTTCTGT ACAATTCAGT AATGAAATGC GACATTGACA TCCGTCGGGA CTTGCTCACT
      601 AATTCTGTGA TCTCAGGGGG AACGACCATG TTTCCAGGGC TTGCAGATCG ACTGCAGAAA
      661 GAACTCACCA CATTGGCTCC TTCCTCAAAC AAAGTACGCG TTATTGCTCC CCCTGAACGG
      721 AAGTATTCCG TGTGGAT //
```

```
LOCUS       Thorunna_furtiva_(Thfu16Bu1/NU93)    784 bp    DNA
DEFINITION  Thorunna_furtiva_(Thfu16Bu1/NU93) actin, partial CDS
ACCESSION  not yet submitted to GenBank
SOURCE      Thorunna furtiva
  ORGANISM  Thorunna furtiva
            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;
             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;
             Doridina; Chromodoridoidea; Chromodorididae; Thorunna
FEATURES             Location/Qualifiers
     source          1..784
                     /organism="Thorunna furtiva"
                     /mol_type="genomic DNA"
     CDS             <1..>784
                     /codon_start=1
                     /product="actin"
                /translation="VAPEEHPVLLTEAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYAS
                     GRTTGIVLDSGDGVTHTVPIYEGYALPHAIMRLDLAGRDLTDYLMKILTE
                     RGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSTLEKSYELPDG
                     QVITIGNERFRTPEAMFQPSFLGMESAGVHETTYNSIMKCDVDIRKDLYA
                     NTVLSGGSTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSI
                     LASLSTFQQMW"
ORIGIN
        1 GTTGCCCCTG AGGAACATCC AGTTCTCCTC ACAGAGGCTC CTCTCAACCC TAAAGCCAAC
       61 AGAGAAAAGA TGACCCAAAT TATGTTTGAA ACCTTCAACT CACCAGCTAT GTACGTGGCT
      121 ATCCAAGCCG TGCTGTCTCT CTACGCTTCA GGCCGTACCA CCGGTATCGT GCTGGATTCT
      181 GGGGACGGCG TGACCCACAC AGTCCCCATC TATGAAGGGT ACGCCCTTCC CCACGCTATC
      241 ATGAGGCTGG ACCTGGCAGG ACGGGACCTC ACCGACTACC TCATGAAAAT CCTCACGGAG
      301 AGAGGCTACA GCTTCACCAC CACCGCGGAG AGAGAGATCG TTCGCGACAT CAAAGAGAAG
      361 CTCTGCTACG TGGCCTTGGA TTTCGAACAA GAAATGGGCA CCGCGGCGTC CTCGTCCACC
      421 CTCGAGAAAA GCTACGAGCT CCCGGACGGC CAGGTCATCA CCATCGGGAA CGAGAGGTTC
      481 AGAACTCCCG AGGCCATGTT CCAGCCCTCG TTTCTGGGAA TGGAGTCGGC AGGGGTCCAC
      541 GAAACGACGT ACAATTCCAT CATGAAGTGC GACGTGGACA TTCGGAAAGA CCTGTACGCC
      601 AACACGGTGC TCTCCGGCGG CTCGACCATG TACCCGGGCA TTGCGGACCG CATGCAAAAG
      661 GAAATCACGG CCCTTGCCCC GAGCACCATG AAGATTAAAA TCATCGCACC GCCCGAGAGG
      721 AAGTATTCGG TTTGGATTGG AGGCTCCATC TTGGCCTCTC TGTCGACCTT CCAGCAGATG
      781 TGGA
//
```

```
LOCUS      Verconia_simplex_NU396_(Nosi17Ba1/NU396_formerly_Noumea_simplex)    762
bp    DNA

DEFINITION  Verconia_simplex_NU396_(Nosi17Ba1/NU396_formerly_Noumea_simplex) actin,
partial CDS

ACCESSION  not yet submitted to GenBank

SOURCE      Verconia simplex

  ORGANISM  Verconia simplex

            Eukaryota; Metazoa; Spiralia; Lophotrochozoa; Mollusca;

             Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia;

             Doridina; Chromodoridoidea; Chromodorididae; Verconia

FEATURES             Location/Qualifiers

     source          1..762

                     /organism="Verconia simplex"

                     /mol_type="genomic DNA"

     CDS             <1..>762

                     /codon_start=1

                     /product="actin"

               /translation="VAPEEHPVLLTEAPLNLKANREKMTQIMFETFNAPAMYVAIQAVLSLYAS

                     GRTTGMVLDSGDGVTHIIPIYEGYALPHAIDKLDLAGRDLTDYLKRILHE

                     RGYNFASSSETEIVRDVKEKLAYVALDFEQEMDTAAKSSCMEKSYEIPDG

                     QVITLGSERFRCPEAMFQPSFLGMESAGFHEIMYNSIMKCDVDIRRDLYG

                     NIVLSGGSTMFPGITDRMQKEITTRAPSTMKIKMIASPEHKYSVWISSSI

                     RVLL"

ORIGIN

        1 GTGGCCCCGG AAGAGCATCC AGTCCTACTC ACAGAGGCCC CCCTGAACCT CAAGGCCAAC

       61 AGAGAAAAGA TGACGCAGAT CATGTTCGAG ACCTTCAATG CACCCGCCAT GTATGTCGCC

      121 ATTCAGGCTG TGCTGTCCCT TTACGCGTCC GGTAGGACAA CAGGAATGGT GTTGGACTCT

      181 GGTGATGGGG TAACTCACAT TATCCCAATC TATGAGGGAT ACGCTCTTCC TCATGCTATT

      241 GATAAGTTGG ATTTGGCCGG GCGTGACCTT ACTGATTACC TGAAGCGAAT CCTTCACGAG

      301 AGGGGCTATA ATTTTGCCTC GTCCTCTGAA ACCGAAATCG TCAGAGACGT TAAGGAAAAG

      361 CTTGCGTACG TGGCTCTTGA TTTTGAACAG GAAATGGACA CAGCAGCCAA ATCGTCCTGT

      421 ATGGAGAAGT CCTACGAAAT ACCAGATGGT CAAGTGATCA CCCTGGGATC TGAGCGATTC

      481 AGGTGTCCAG AAGCCATGTT TCAACCGTCT TTCTTAGGCA TGGAATCTGC GGGATTTCAT

      541 GAAATCATGT ACAATTCTAT CATGAAATGT GATGTTGATA TCAGAAGAGA TTTGTACGGC

      601 AACATCGTCT TGTCTGGGGG ATCCACGATG TTCCCAGGCA TCACCGACAG GATGCAGAAG

      661 GAGATCACCA CCCGGGCACC TAGCACCATG AAGATCAAGA TGATCGCATC CCCAGAGCAC

      721 AAGTACTCCG TGTGGATCAG CAGCTCCATC CGGGTTCTAC TA

//
```

Figure S3.8 Phyre2 prediction of secondary structure and disorder of the *in silico* *Chromodoris*/*Armina*-actin-hybrid-model.



PUBLICATIONS, PARTICIPATION IN OTHER STUDIES, TALKS & CONFERENCE CONTRIBUTIONS

PUBLICATIONS & PARTICIPATION IN OTHER STUDIES

(1) **Hertzer, C.; Kehraus, S.; Böhringer, N.; Kaligis, F.; Bara, R.; Erpenbeck, D.; Wörheide, G.; Schäberle, T. F.; Wägele, H.; König, G. M.** Antibacterial Scalarane from *Doriprismatica Stellata* Nudibranchs (Gastropoda, Nudibranchia), Egg Ribbons, and Their Dietary Sponge *Spongia* Cf. *Agaricina* (Demospongiae, Dictyoceratida). *Beilstein J. Org. Chem.* **2020**, *16* (1), 1596–1605. https://doi.org/10.3762/bjoc.16.132.

(2) **Undap, N.; Papu, A.; Schillo, D.; Ijong, F. G.; Kaligis, F.; Lepar, M.; Hertzer, C.; Böhringer, N.; König, G. M.; Schäberle, T. F.; et al.** First Survey of Heterobranch Sea Slugs (Mollusca, Gastropoda) from the Island Sangihe, North Sulawesi, Indonesia. *Diversity* **2019**, *11* (9), 170. https://doi.org/10.3390/d11090170.

(3) **Fisch, K. M.; Hertzer, C.; Böhringer, N.; Wuisan, Z. G.; Schillo, D.; Bara, R.; Kaligis, F.; Wägele, H.; König, G. M.; Schäberle, T. F.** The Potential of Indonesian Heterobranchs Found around Bunaken Island for the Production of Bioactive Compounds. *Mar Drugs* **2017**, *15* (12). https://doi.org/10.3390/md15120384.

(4) **Böhringer, N.; Fisch, K. M.; Schillo, D.; Bara, R.; Hertzer, C.; Grein, F.; Eisenbarth, J.-H.; Kaligis, F.; Schneider, T.; Wägele, H.; et al.** Antimicrobial Potential of Bacteria Associated with Marine Sea Slugs from North Sulawesi, Indonesia. *Front. Microbiol.* **2017**, *8*. https://doi.org/10.3389/fmicb.2017.01092.

(5) **Bogdanov, A.; Hertzer, C.; Kehraus, S.; Nietzer, S.; Rohde, S.; Schupp, P. J.; Wägele, H.; König, G. M.** Secondary Metabolome and Its Defensive Role in the Aeolidoidean *Phyllodesmium Longicirrum*, (Gastropoda, Heterobranchia, Nudibranchia). *Beilstein J Org Chem* **2017**, *13*, 502–519. https://doi.org/10.3762/bjoc.13.50.

(6) **Bogdanov, A.; Hertzer, C.; Kehraus, S.; Nietzer, S.; Rohde, S.; Schupp, P. J.; Wägele, H.; König, G. M.** Defensive Diterpene from the Aeolidoidean *Phyllodesmium Longicirrum*. *J. Nat. Prod.* **2016**, *79* (3), 611–615. https://doi.org/10.1021/acs.jnatprod.5b00860.

INVITED TALKS

27th January 2020	Seminar at *The Zoological Research Museum Alexander Koenig, Leibniz-Association for Animal Biodiversity*, Bonn, Germany
22nd August 2018	Seminar at LOEWE *Center for Insect Biotechnology & Bioresources*, Giessen, Germany

CONFERENCE CONTRIBUTIONS

1st – 5th September 2019	XVI MaNaPro & XI ECMNP, Marine Natural Products, Peniche, Portugal (Poster)
25th – 27th February 2019	BIOPROSP_2019, Unlocking the potential of biomolecules from marine environment, Tromsø, Norway (Poster & Award Winner)
2nd – 5th September 2018	3rd European Conference on Natural Products, DECHEMA, Frankfurt, Germany (Poster)
3rd – 7th September 2017	10th European Conference on Marine Natural Products, Kolymbari, Greece (Talk)

CURRICULUM VITAE

University Education

2009 – 2012 **Bachelor of Science**, in Biology, *Philipps-University*, Marburg

Supervisor: Prof. Dr. Roland Brandl, *General and Animal Ecology*

Thesis: „Chemical Ecology of Tannins and Other Phenolics. New Approach – Old Results. No Support for Tannin Oxidation Hypothesis among European Temperate Tree Genera" (Grade 1.0)

2013 – 2015 **Master of Science**, in OEP-Biology, *Rheinische Friedrich-Wilhelms-University*, Bonn (Final Grade 1.2)

Supervisors: Prof. Dr. Gabriele M. König and Prof. Dr. Heike Wägele

Thesis: „Nude but Not Defenceless: Isolation and Structure Elucidation of Terpenoids from *Phyllodesmium longicirrum* (Aeolidida, Nudibranchia, Gastropoda) and Their Role in Defence" (Grade 1.0)

2016 – 2020 **PhD in Drug Sciences**, *Rheinische Friedrich-Wilhelms-University*, Bonn
Institute of Pharmaceutical Biology, AG König

Supervisors: Prof. Dr. Gabriele M. König and Prof. Dr. Heike Wägele

Thesis: „Investigations on Marine Natural Products From Indo-Pacific Nudibranchia (Mollusca: Gastropoda): Chemoecology, Medicinal Potential & Toxin Resistance"

ACKNOWLEDGEMENTS / DANKSAGUNG

Ich möchte mich bei vielen Menschen bedanken, ohne die es diese Arbeit so nicht geben würde.

Zuerst möchte ich mich ganz herzlich bei Prof. Gabriele M. König und Prof. Heike Wägele für die jahrelange Betreuung und Unterstützung, während der Masterarbeit und der anschließenden Doktorarbeit, bedanken. Sie haben mir die Möglichkeit gegeben das spannende Thema der Meeresschnecken, insbesondere Nudibranchia, und ihrer Naturstoffe kennenzulernen und weiter zu erforschen, gaben mir stets den Freiraum eigene Ideen auszutesten und standen mir mit gutem Rat und Vorschlägen zur Seite.

Weiterhin möchte ich mich bei Prof. Evi Kostenis und Prof. Gabriele Bierbaum dafür bedanken, dass Sie sich dazu bereit erklärt haben die weiteren Gutachten dieser Arbeit zu übernehmen.

Außerdem möchte ich mich bei Prof. Till F. Schäberle, sowie allen Mitgliedern, Beteiligten und Kooperationspartnern des INDOBIO-Projekts bedanken. Dafür, dass ich Teil dieses internationalen Projekts sein und an einer der Forschungsreisen nach Indonesien teilnehmen durfte. Terima kasih.

Ganz besonders möchte ich mich auch bei Dr. Alexander Bogdanov und Dr. Stefan Kehraus bedanken, deren Anleitung, Hilfe und Humor mir die Durchführung dieser Arbeit, sowie die Betreuung der Kurse und Exkursionen, sehr erleichtert haben.

Ich möchte mich bei Ekaterina Egereva, Dr. Max Crüsemann und Dr. Mark Sylvester für die LC-MS-, HR-MS- und UPLC-HR-MS-Messungen bedanken. Außerdem gilt Ekaterina Egereva und Emilie Goralski auch mein Dank für all ihre Antworten und Hilfe in den Laboren.

Ebenfalls möchte ich mich sehr bei Dr. Dhaka Ram Bhandari und Prof. Bernhard Spengler dafür bedanken, dass ich bei und mit Ihnen die MALDI-MS-Imaging-Experimente durchführen durfte, sowie bei Dr. Stefan Aatz, Dr. Sebastian Franken und Prof. Hanns Häberlein dafür, dass ich bei und mit Ihnen die Zellkultur- und Fluoreszenzmikroskopie-Experimente durchführen konnte.

Allen Mitgliedern der Arbeitsgruppen AG König und AG Wägele gilt mein Dank für das tolle Arbeitsumfeld und dafür, dass sie mich so freundlich aufgenommen und unterstützt haben.

Besonderer Dank geht an Nani Undap, Adelfia Papu, Dario Karmeinski, Gregor Christa, Carola Greve, Fatemeh Maniei, Elise Laetz und Juan Moles, für die ansteckende und geteilte Begeisterung für die Erforschung der Heterobranchia.

Ganz besonders möchte ich hierbei auch nochmal Sophie Klöppel, Wiebke Hanke, Benjamin Libor, Raphael Reher, Alexander Bogdanov, Paul Barac, Daniel Wirtz und Cornelia Hermes erwähnen und mich bei ihnen für die vielen schönen Abende, Feiern, gemeinsamen Konferenzen, Spielerunden, kreative Ideen und Gespräche, für die vielen fröhlichen und aufmunternden Momente im Labor und Büro bedanken.

Ein besonderer Dank gilt auch Isabel Kilian und allen Mitgliedern unserer „Scrum“-Gruppe, die mir gerade während den schwierigen Phasen der Pandemie geholfen haben, nicht die Motivation zu verlieren.

Vor allem möchte ich mich aber bei meinen Freunden, meiner Familie, meinem Partner, Komplizen, Fellnasen und allen, die wie Familie für mich sind, bedanken. Für all die Unterstützung und Ermutigung, gemeinsame Spielerunden, Reisen, Abenteuer, gefeierten Feste, gelöste Rätsel, jedes „2. Zuhause“, Kaffee-Gespräche, „Bier-Tage“, gemeinsames Tanzen, Klettern und Sinnieren. Ohne euch wäre ich nicht, wo ich heute bin.

Ihr wisst, wer gemeint ist. Danke!