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Analysis of the Rubbing Behaviour of Psammophiids: A Methodological Approach

Dissertation

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1. INTRODUCTION

The objective of this study is to analyse the behavioural and functional aspects of the rubbing behaviour found only in snakes belonging to the family Psammophiidae. This behaviour consists of the smearing of the nasal gland secretion of the snake on its own belly and flanks by sinusoidal head movements. The species *Malpolon insignitus fuscus*, *Psammophis mossambicus*, *P. schokari*, *Psammophylax acutus acutus* and *Scutophis moilensis* were tested on their rubbing behaviour, how they perform it and on which occasion it takes place. In regards of the functional aspects of this behaviour, the morphology of the scales of these snakes was also examined thanks scanning electron microscopy, as well as the chemical composition of the secretion by gas chromatography/ mass spectrometry.

1.1 PSAMMOPHIIDS- GENERALITIES

Psammophiids (sand snakes) are characteristic snakes of Africa. Widely distributed throughout the deserts and savannas of this continent, they are also present in the Mediterranean basin. Some species reach farther region of Asia, as Thailand and Cambodia (BROADLEY, 1962; BRANDSTÄTTER, 1996; CHIPPAUX, 1999; DE HAAN, 1999; SPAWLS et al., 2002; BRANCH, 2005; TRAPE and MANÉ, 2006) (**Fig.1.1**).

These fast moving diurnal snakes morphologically present a head clearly separated from the body and with large eyes. Moreover, a very long tail compared to the total body length make these snakes easily to recognise (BOGERT, 1940; DOWLING and SAVAGE, 1960; BRANDSTÄTTER, 1995; GREEN et al., 1997; CHIPPAUX, 1999; SPAWLS et al., 2002; BRANCH, 2005; TRAPE and MANÉ, 2006). The autotomy of this long tail has been reported several times in literature, presumably as defensive mechanism (WERNER, 1902; BROADLEY, 1987; BRANCH, 2001; AKANI et al., 2002; SPAWLS et al., 2002). Some psammophiids have enlarged rostral shield allowing them to burrow in sand or loose earth, whilst other species have slender bodies better adapted to the arboreal life.

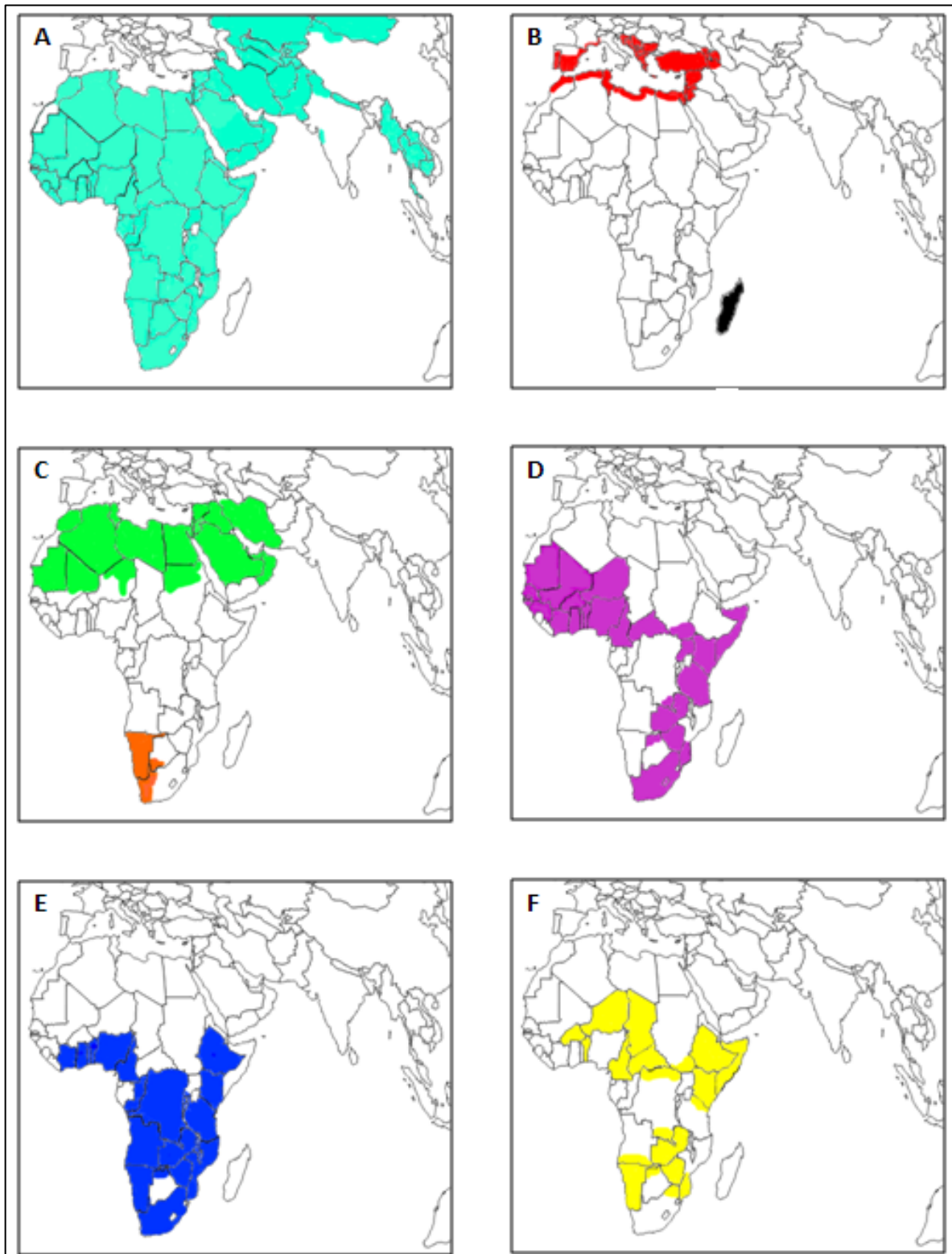


Fig.1.1: Geographical distribution of psammophiid genera: **(A)** *Psammophis*, with around 30 species. Five of them are only present in Asia; **(B)** *Malpolon* (red), the sole psammophiid genus also distributed in Europe with two species and *Mimophis mahfalensis*, the sole Madagascan genus (black). The status of a subspecies, *M. m. madagascariensis* is uncertain (APREA et al., 2003); **(C)** *Scutophis moilensis* (green) (one species) and *Dipsina multimaculata* (orange)-only one species known in South Africa; **(D)** *Rhamphiophis* (3 species), essentially in Africa. **(E)** *Psammophylax* with four recorded species inhabits; **(F)** *Hemirhagerrhis*, also with four species mainly. (Map: <http://alecks.free.fr>).

Psammophiids belongs to the rear-fanged (opisthognath) snakes, having enlarged venom teeth situated slightly behind or directly under the eyes (BROADLEY et al., 2003; BRANCH, 2005) (**Fig.1.2**). The venom is used to kill their preys, mainly fast moving lizards (GREEN et al., 1997; CHIPPAUX, 1999; BRANCH, 2001; SPAWLS et al., 2002; COTTONE and BAUER, 2008b). Often the snakes maintain their prey by constriction until the venom shows its properties (RIEPEL, 1979; DE HAAN, 1999).

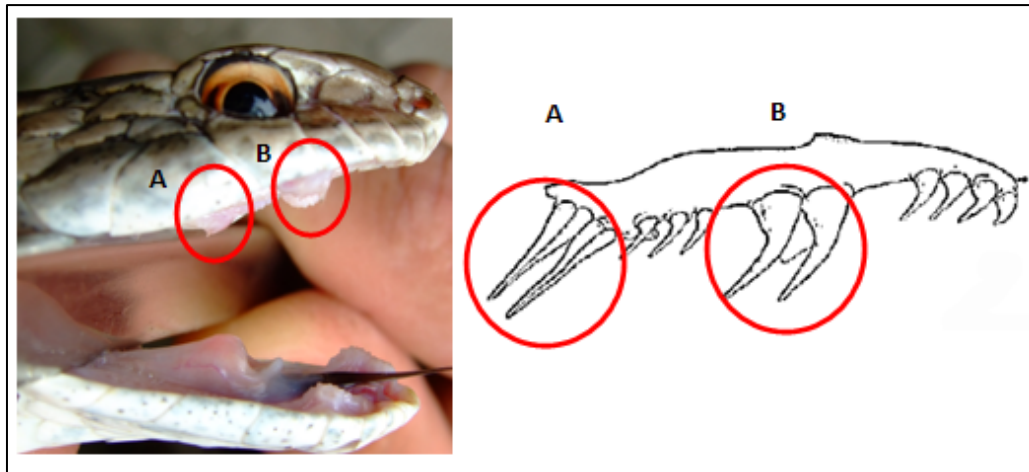


Fig.1.2: Pictures showing the position of the teeth in *Psammophis mossambicus*. **Left:** Picture showing the position of the venomous teeth (**A**) and the enlarged non-venomous maxillary teeth (**B**). **Right:** Drawing (after Bogert, 1940) of the maxillary teeth showing the clear differences between the grooved venomous teeth (**A**) situated in the back of the mouth and (**B**) the enlarged maxillary teeth. When bitten by a larger individual the venomous teeth may inject some venom.

The anatomy of the venom apparatus of opisthognath snakes makes it generally not appropriate to deliver dangerous bites to human. The venom properties of psammophiids on human seem to depend on the species and the size of the specimen considered, but altogether the venom of these snakes is classified as mildly toxic (ISEMONGER, 1962; STEEHOUDER, 1987; BROADLEY et al., 2003; BRANCH, 2005). The general consequences of a psammophiid bite would then be nausea, pain and itching (KRAMER and SCHNURRENBERGER, 1958; ISEMONGER, 1962; SPAWLS et al., 2002; BROADLEY ET AL., 2003; BRANCH, 2005; and TRAPE and MANÉ, 2006). Some studies analysed psammophiids' venom as in *Rhamphiophis* (LUMSDEN et al., 2005) or *Malpolon monspessulanus* (SLAVTCHEV, 1985; ROSENBERG et al., 1992; ARIKAN et al., 2005; LARRÉCHÉ et al., 2008). The venom of this latest species seems to be particularly

toxic to human (BRANCH, 1988; CHIPPAUX, 1999; DE HAAN, 1999). Severe cases of envenomation were reported, describing the effects of the bite as similar to those of *Vipera aspis* –occurring sympatrically with *M. monspessulanus* in the South of France (GONZALES, 1979; POMMIER and DE HARO, 2007). More or less severe envenomation was also reported for *Scutophis moilensis* (KRAMER and SCHNURRENBERGER, 1958; PERRY, 1988). On the other hand, GRUBER (1989) described *Malpolon* and *Scutophis* as harmless. Although *Psammophis* sp. are known to bite when disturbed, their venom has been described to be harmless on human (BROADLEY and COCK, 1975).

1.2 PHYLOGENY OF PSAMMOPHIIDS

The systematics of psammophiids has been uncertain for a long time. Until 2008, the eight genera known as psammophiids (*Dipsina*, *Dromophis*, *Hemirhagerrhis*, *Malpolon*, *Mimophis*, *Psammophis*, *Psammophylax*, *Rhamphiophis*) were either considered as subfamily, as tribe of diverse subfamilies, or as single species of different families or subfamilies (**Table 1.1**).

However, the affiliation of these eight genera to one “group” –regardless of which family- has been clear since BOGERT (1940). He placed them without specific systematic commentary but their small unornamented hemipenes in its “Group XVI” (**Chapter 1.6**) -a systematic criteria proposed by COPE (1900).

The monomorphism for sexual characters, the long tails, the large eyes or even the same prey class are found in unrelated taxa of colubrids called “whip snakes” (SHINE, 1980; BAHHA EL DIN and ATTUM, 1998; AKANI et al., 2003; LUISELLI, 2006; SHINE et al., 2006; COTTONE and BAUER, 2008a), so that psammophiids were also often considered (and/or called) as whipsnakes (COBORN, 1991; LUISELLI, 2006). These similarities lead some authors to speak about convergence between psammophiids and whipsnakes (DITMARS, 1952; STEWARD, 1971; VITT and VANGILDER, 1983; COBORN, 1991; LUISELLI, 2006; COTTONE and BAUER, 2009b). Well-known “whip snakes” are e.g. the genus *Coluber* in Europe, North America and Asia, *Demansia* in Australia and *Masticophis* in North America.

UNDERWOOD (1967) affiliated them to the subfamily Colubrinae due to their retinal character, whilst BOURGEOIS (1968) considered them as being an own subfamily of the Colubridae - the Psammophidinae - because of cranial character being different from those of other colubrids. Later on, MINTON and SALANITRO (1972) analysed serological relationships within colubrids, and found another argument to clearly separate the sand snakes from the other colubrids. Nevertheless, the mtDNA sequences analysis of the last years supported the psammophiids as a well established monophyletic group (GRAVLUND, 2001; VIDAL and HEDGES, 2002; KELLY and al., 2003; NAGY et al., 2003).

The study of KELLY et al. (2008 and published 2009) was the first detailed investigation of psammophiids. This allowed them not only to define psammophiids as the family Psammophiidae, but they could also clarify some relationships within and between the genera: The common phylogeny until this date divided the psammophiids into eight genera: *Dipsina*, *Dromophis*, *Hemirhagerrhis*, *Malpolon*, *Mimophis*, *Psammophis*, *Psammophylax* and *Rhamphiophis*. The authors synonymised *Dromophis* with *Psammophis* (see HUGHES, 2004) and affiliated the species *Rhamphiophis acutus acutus* to *Psammophylax acutus acutus*. Problems still remained concerning the taxonomy of the so-called “*Psammophis sibilans* complex”, grouping several species with similar colouration and habitus (**Chapter 1.3**). Alike, the authors recorded divergence between *Malpolon monspessulanus* and “*Malpolon*” *moilensis*, supporting the assignation of the latest to an own genus (BRANDSTÄTTER, 1995) (**Chapter 1.5**).

Table 1.1
Previous and recent phylogeny of psammophiids.

	Fam. Colubridae	Fam. Lamprophiidae	Fam. Psammophiidae
Bogert (1940)	Subfam. Colubrinae		
Dowling (1967)	Subfam. Colubrinae Tribe Psammophini		
Bourgeois (1968)	Subfam. Psammophidinae		
Schmidt (1923) Coborn (1991)	Subfam. Boiginae		
McDowell (1987)	Subfam. Boaedontinae		
Phelps (1989)	Single species of Colubrinae		
Brandstätter (1995)	Subfam. Lycodontinae Tribe Psammophini		
Smith et al., (1977) Heymans (1981) Branch (1988) Zaher (1999) Broadley and Hughes (2000)	Subfam. Psammophi(i)nae		
Kelly et al., (2008)			✓
Vidal et al., (2008)		Subfam. Psammophiinae	

1.3 THE GENUS *PSAMMOPHIS*

This genus is the most speciose of psammophiids with around 30 species. It is mainly distributed through Africa except five species that occur in Asia (**Fig.1.1A**). The uncertainties existing between the boundaries of several *Psammophis* species make a correct taxonomic classification of this genus difficult, and therefore the revision of the genus had been undertaken several times (LOVERIDGE, 1940; BROADLEY, 1966, 1977, 2002; BRANDSTÄTTER, 1996; HUGHES, 1999; RATO et al., 2007; KELLY et al., 2008).

Already WERNER (1902) pointed out the great variability in colour and pattern within this genus challenging the exact identification of species as for example for *P. schokari* (KRAMER and SCHNURRENBERGER, 1963; KARK et al., 1997) or *P. phillipsi* (HUGHES, 1999; LUISELLI et al., 2004). Furthermore, hybrids have been reported for some given populations (BROADLEY, 1962, 1966; 2002; SPAWLS et al., 2002; KELLY and al., 2008).

The *Psammophis* species had been classified into two complexes: the “*schokari*-complex” (considered as primary) and the “*sibilans*-complex” (BROADLEY, 1977). BRANDSTÄTTER (1996) also considered a third “advanced-complex” grouping the snakes by reason of the arrangement of their scales (**Table 1.2**). The investigation of KELLY et al. (2008) using phylogenetic network made them split the “*sibilans*-complex” into the “*phillipsi*-complex” and the “*subtaeniatus*-complex” (**Fig.1.3**) so that roughly three complexes are known for psammophiids: the *schokari*-complex, the *phillipsi*-complex and the *subtaeniatus*-complex. The “advanced-complex” of BRANDSTÄTTER is not mentioned by KELLY et al..

Table 1.2

Species-complexes in psammophiids after BRANDSTÄTTER (1996). The species are assigned to the advanced-complex depending on their scale number (elevated in more developed species).

<i>schokari</i> -complex	<i>sibilans</i> -complex	advanced-complex
<i>schokari</i>	<i>sibilans</i>	<i>angolensis</i>
<i>linoleatus</i>	<i>phillipsi</i>	<i>ansorgi</i>
<i>leithii</i>	<i>rukwaë</i>	<i>condanarus</i>
<i>longifrons</i>	<i>pulcher</i>	
<i>trigrammus</i>	<i>tanganicus</i>	
<i>jallae</i>	<i>biseriatus</i>	
<i>notostictus</i>	<i>crucifer</i>	
<i>leightoni</i>	<i>brevirostris</i>	
<i>elegans</i>	<i>subtaeniatus</i>	
<i>punctulatus</i>		

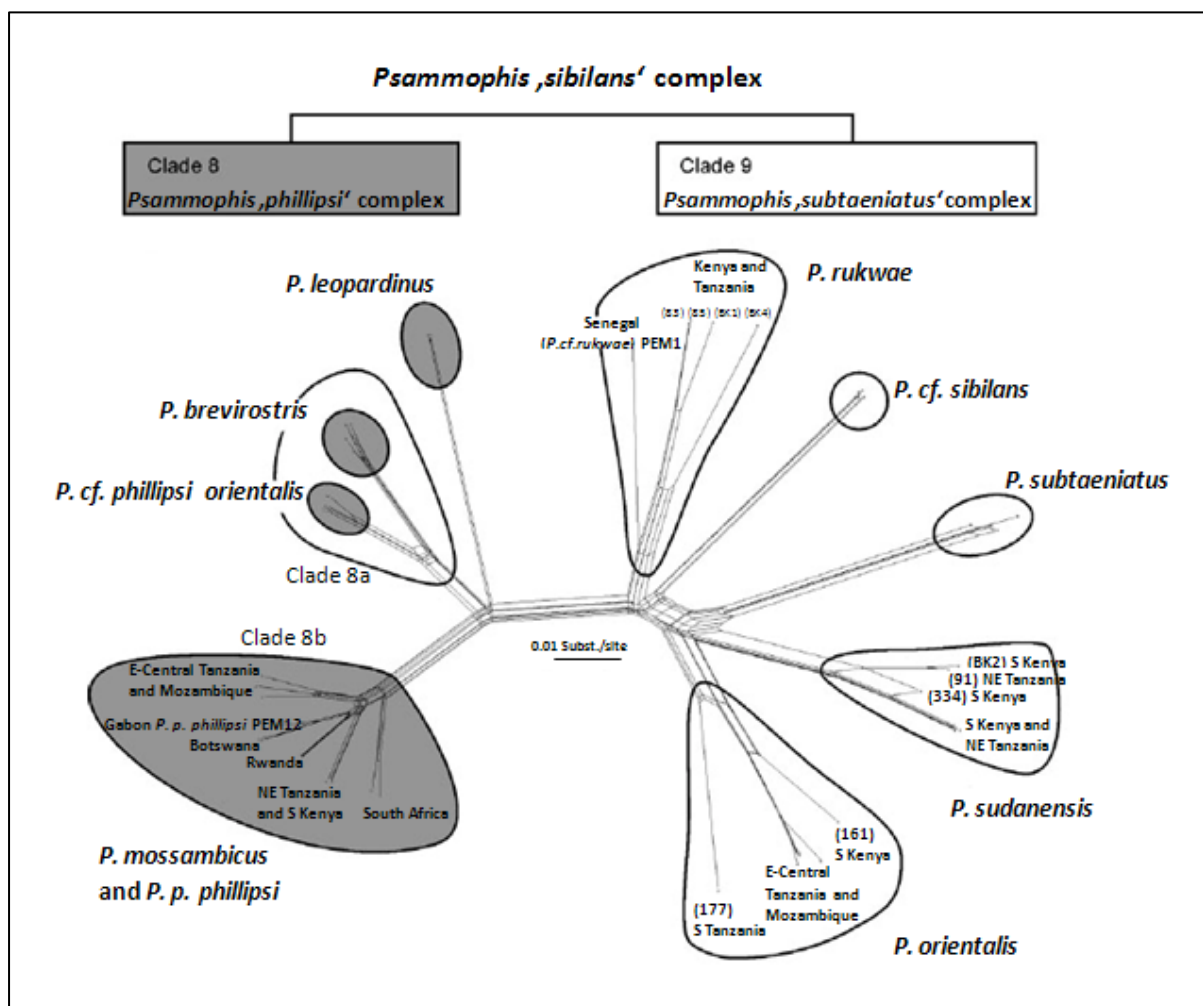


Fig.1.3: New phylogenetic network for the *Psammophis sibilans* complex showing the splitting of this complex into the “*phillipsi*” and the “*subtaeniatus*” one (after Kelly et al., 2008).

The main remaining confusion concerns the species *phillipsi* and *mossambicus*. *P. phillipsi* itself occurs with two forms in southern Nigeria: One with an entire, the other one with a divided anal shield (LUISELLI et al., 2004). HUGHES (1999) proposed to consider them as two distinct species but LUISELLI et al. (2004) could not notice any differences in their ecology and behaviour. In their “*Snakes of Zambia*”, BROADLEY et al. (2003) pointed out that *P. mossambicus* had previously been confused with *P. sibilans* from Egypt to be then identified as a form of *P. phillipsi* of West Africa. Hybrids of *phillipsi* and *mossambicus* seem to occur in some areas (BROADLEY 2002). The sole haplotype of *P. phillipsi* analysed by KELLY et al. (2008) was grouped within *P. mossambicus* so that the species status of this snake could not be considered as exclusive. So far, the occurrence of intermediate forms between *P. mossambicus*, *sibilans*, *phillipsi*, *orientalis* and *rukwa*e are known in East Africa rendering the correct assignation of these species more than uncertain (BROADLEY et al., 2003).



Fig.1.4: *Psammophis mossambicus* kept at the Museum Koenig.

Although the small size of the hemipenes leads to the absence of clear sexual dimorphism in *Psammophis* (CORTI et al., 2001; AKANI et al., 2002, 2003; SHINE et al., 2006; COTTONE and BAUER, 2009bcd) (**Chapter 1.6**), males seem to be larger and heavier than females in some species (*P. sibilans* (STEEHOUDER, 1992), *P. brevirostris* (BRANDSTÄTTER, 1995) and *P. condanarus* (SHINE, 1978)). The minor dimorphism recorded in tail length makes the determination of sex of living snakes difficult (BUTLER, 1993; CORTI et al., 2001; SHINE et al., 2006; COTTONE and BAUER, 2009b). Male-male combats (PITMAN, 1974; SHINE, 1978; HAAGNER and MORGAN, 1991) and the

marking of the female by the male with nasal gland secretion (**Chapter 1.6**) during mating period in *P. brevisrostris* have been reported (BRANDSTÄTTER, 1996).

1.4 THE GENUS *MALPOLON*

The genus *Malpolon* is the only genus being present in Europe besides the Middle-East and Africa (BRUNO, 1977; VINCENT, 1991; BRANDSTÄTTER, 1995; CARRANZA et al., 2006; GENIEZ et al., 2006; POTTIER et al., 2006) (**Fig.1.1B**).

Until 1995 one species, *Malpolon monspessulanus*, with the subspecies *M. m. monspessulanus* and *M. m. insignitus* was recognised. CARRANZA et al. (2006) split the European population of *Malpolon monspessulanus* in two species based on different mitochondrial gene fragments and morphological aspects: the nominal form, *Malpolon monspessulanus* (Hermann, 1804) in West Europe and *Malpolon insignitus* (Geoffroy St-Hilaire, 1827) in East Europe (stat. nov.), with the subspecies *Malpolon insignitus fuscus* (Fleischmann, 1831) (comb. nov.). The morphological traits as colouration and size of the eastern forms had already been subject of discussion in the past (VEITH, 1991). GENIEZ et al. (2006) described a new subspecies for the nominal form distributed in North-West Africa: *Malpolon monspessulanus saharatlanticus* (**Table 1.3**).

Table 1.3
Species and subspecies of the genus *Malpolon*.

Genus	Species	Subspecies	Geographical distribution
<i>Malpolon</i>	<i>monspessulanus</i>	<i>monspessulanus</i>	South-East France, Spain, Portugal, Morocco, Northern Italy (Piedmont and Liguria)
<i>Malpolon</i>	<i>monspessulanus</i>	<i>saharatlanticus</i>	North-western Atlantic coast of Morocco
<i>Malpolon</i>	<i>insignitus</i>	<i>insignitus</i>	Eastern Europe
<i>Malpolon</i>	<i>insignitus</i>	<i>fuscus</i>	Easter-Europe/ Middle-East, entering North Africa (Egypt, Libya)

The sexual dimorphism in *M. m. monspessulanus* is the most conspicuous in Palaearctic snakes studied until now (FERICHE et al., 1993): Males are not only larger than females and but they are also differently coloured when adults whilst the females keep the juvenile colouration (DE HAAN, 1999, 2003a). As for *Vipera berus* (ANDRÉN, 1986) and *V. aspis* (SAINT-GIRONS, 1996), territoriality, male-male combat and mate-guarding have been reported (CAPULA and LUISELLI, 1997; DE HAAN, 1999). Unlike other snakes, *M. m. monspessulanus* has been reported to have a kind of social system during the mating period (DE HAAN and CLUCHIER, 2006): Males do not only guard their female and nest, but also hunt for their female or accompany it when it leaves the nest. This couple tolerates next to the nest other males called “vassals”. They are reported to help the “dominant” male to defend its territory, but do not reproduce with the female. This forms a little group, those affiliation seems to be due to a scent mark given by the secretion of the nasal gland of the dominant male of the group, rubbed on the bodies of the vassals and the female.



Fig.1.5: *Malpolon monspessulanus saharatlanticus*.

1.5 THE GENUS *SCUTOPHIS*

A brief commentary will be given here concerning the denomination of *Scutophis moilensis*, whose correct assignation seems to be problematic: It has been designed to the genus *Malpolon* (PARKER, 1931) with which it is sensed to share the grooved dorsal scale (GRUBER, 1989), whilst MARX and RABB (1972) affiliated “*moilensis*” to *Rhamphiophis* because of its prominent rostral shield.

BRANDSTÄTTER (1995) was the first to propose to clearly separate *moilensis* from *Malpolon* to transfer it to its own genus, *Scutophis*, referring to its ability to flatten its neck (literally translated it means “shielded snake”; the name is derived from *scutum*, the Latin word for “shield”) (**Fig.1.6**). This transfer was criticised by BROADLEY (2005), questioning whether the name had been adequately diagnosed, since BRANDSTÄTTER had put it only at the end of his commentaries on *Scutophis* and not at the beginning. The new genus name did not found unanimity and was only used sporadically in the literature (GENIEZ et al., 2004; SCHLÜTER, 2005; PADIAL, 2006).

The study of KELLY et al. (2008) allowed to clarify the situation thanks to the molecular data analysed (bases in mitochondrial and nuclear genome). They noted clear divergence between *Malpolon* and *moilensis* supporting BRANDSTÄTTER’S assignation. Their study showed that *Malpolon*, *Rhamphiophis*, and *Scutophis moilensis* form a sister group of the remaining psammophiids, so that the previous authors mentioned were not completely wrong with their assumptions concerning the affiliation of *moilensis* to *Rhamphiophis*.



Fig.1.6: Neck flattening in *Scutophis moilensis*.

1.6 CHARACTERISTICS OF PSAMMOPHIIDS

The form of the hemipenes described as small, filiform or even qualified as vestigial (SHINE et al., 2006) is one peculiar characteristic of psammophiids (BOGERT, 1940; DOWLING and SAVAGE, 1960) (**Fig.1.7**). This leads to the absence of clear sexual dimorphism in most genera of this family, since the basis of the tail is not thickened by the presence of large hemipenes as in other snake species (MATTISON, 2007).

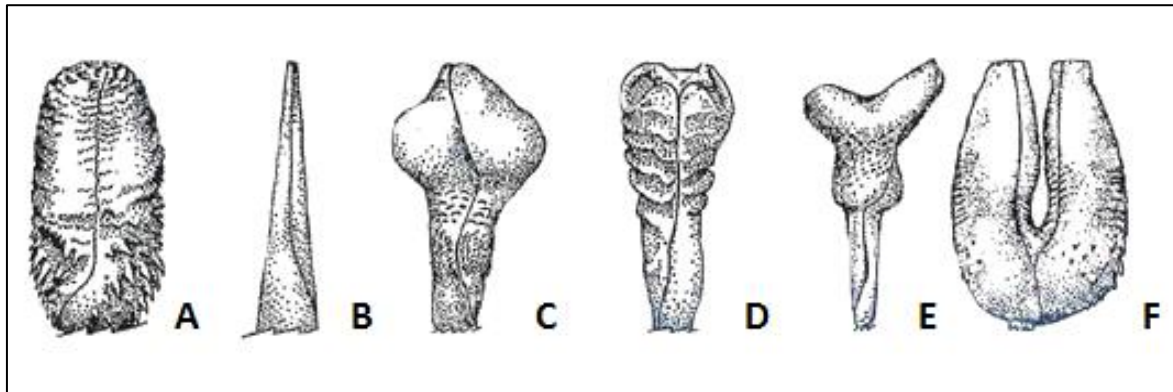


Fig.1.7: Hemipenial morphology showing the unornamented and filiform hemipenis of *Psammophis sibilans* (**B**) compared to the ornamented and structured one of: (**A**) *Spalerosophis diadema* (**C**) *Spilotes pullatus* (**D**) *Charina bottae* (**E**) *Pantherophis obsoletus* (**F**) *Crotalus viridis*. (After Dowling and Savage, 1960).

However, besides this morphological aspect, the most important characteristic that makes psammophiids unique among snakes is that these snakes smear the secretion of their nasal glands situated on each side of the head between eye and nostril on their scales (**Fig.1.8A**): this is done by little fast and tight zigzagging head movement along the belly and flanks (**Fig.1.9**).

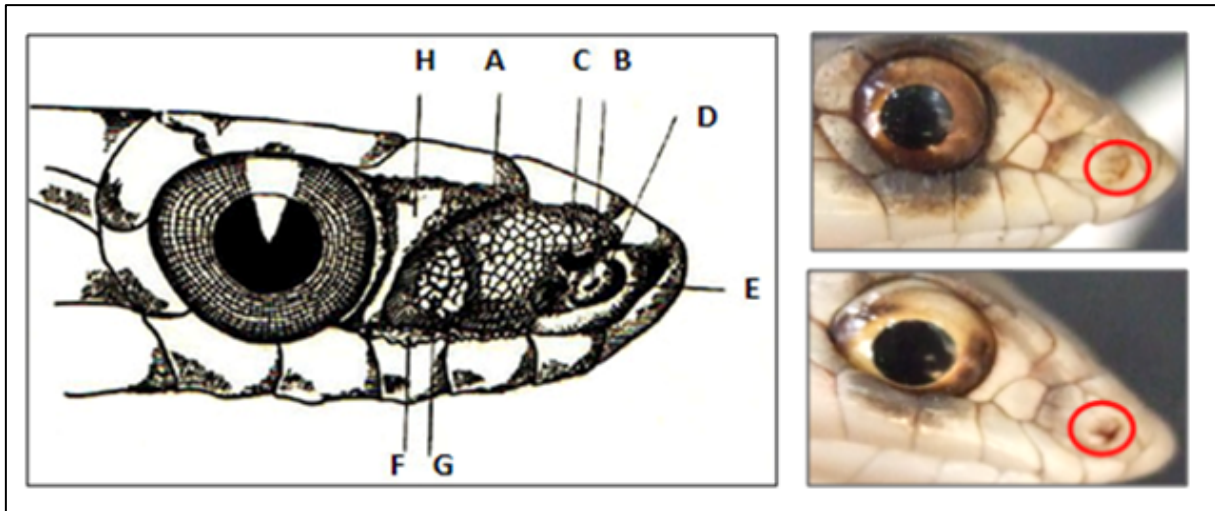


Fig.1.8: Left: Head of *M. monspessulanus* (after Darevsky, 1956) showing the enlarged nasal gland (A) and the narial valve (D). (B) Opening of the duct; (C) Duct; (E) Naris; (F) Part of nasal gland covered by connective tissues; (G) Maxillary bone; (H) Prefrontal scale. Right: Head of *Scutophis moilensis* showing the narial valve closed (above) and open (below).



Fig.1.9: Rubbing of *Scutophis moilensis*. (© M. Aymerich).

This behaviour is called “rubbing behaviour” (RB) in the present study. In the literature, the terms “groom themselves” (BRANCH, 1988), “polish themselves” (GREEN et al., 1997; SPAWLS et al., 2002; MATTISON, 2007), “comportement de frottement” (DE HAAN, 1982), “frotamiento” (PLEGUEZUELOS, 2009), “putzen” (RIEPEL, 1973; BRANDSTÄTTER, 1995, 1996) or “self-rubbing” (DE HAAN and CLUCHIER, 2006; COTTONE and BAUER, 2009a) can also be found. The secretion is composed of lipids, proteins, fatty acids and K^+ and Na^+ ions (DUNSON et al., 1978). Until now, RB was reported in six genera: *Hemirhagerrhis*, *Mimophis* (DE HAAN, 2003a); *Malpolon* (DE GRIJS, 1898); *Scutophis* (DE HAAN, 1982); in several species of *Psammophis* (DUNSON et al., 1978; DE

HAAN, 1982; STEEHOUDER, 1992; BRANCH, 1988; BRANDSTÄTTER, 1995); in *Psammophylax* (STEEHOUDER, 1987; HARTMANN, 1998; HALL et al., 1994; COTTONE and BAUER, 2009a). For the genera *Dipsina* and *Rhamphiophis* RB has not been observed until now.

DE HAAN (1982; 2003a) described two “rubbing systems”: the one refers to the genus *Psammophis* and is called the “P-system”. The second, the “M-system”, refers to the genus *Malpolon*. The P-system is used by snakes whose habitat can also be extended to trees and allows them to perform RB on branches as well; the M-system is performed by more terrestrial species (see **Fig.1.9**).

The function itself of this RB left place to several speculations. First, considering that the secretion applied is rich in lipids (DUNSON et al., 1978), several authors suggested that RB would be a protective way against water loss of the skin (DAREVSKY, 1956; DUNSON et al., 1978; BRANCH, 1988; LAHAV and DMI’EL, 1996; GREEN et al., 1997; WELDON et al., 2008). In this sense, *Malpolon monspessulanus* (DUNSON et al., 1978) and *Psammophis schokari* (LAHAV and DMI’EL, 1996) have extremely low rates of evaporative water loss compared to other snakes living in the same habitat. Indeed, lipids are known to have a protective function in reptiles’ skin: Situated in the stratum corneum in the mesos layer (IRISH et al., 1988; WELDON et al., 2008) (**Fig.1.10**), they provide an efficient barrier for water permeation (BURKEN et al., 1985a; ROBERTS and LILLYWHITE, 1980, 1983; BAEYENS and ROUNTREE, 1983) and retard efficiently the water loss of the skin (LANDMANN, 1986, 1988; KATTAN and LILLYWHITE, 1989; EYNAN and DMI’EL, 1993; LAHAV and DMI’EL, 1996; DMI’EL, 1998).

Relating to this protective function against water loss, some anecdotal reports mentioned that RB frequency was lower when the snakes were exposed to higher relative humidity (STEEHOUDER, 1992; BRANDSTÄTTER, 1996), or higher when the snakes were exposed to high temperatures (DAREVSKY, 1956; RIEPPEL, 1973; DE HAAN, 1982; STEEHOUDER, 1987, 1992).

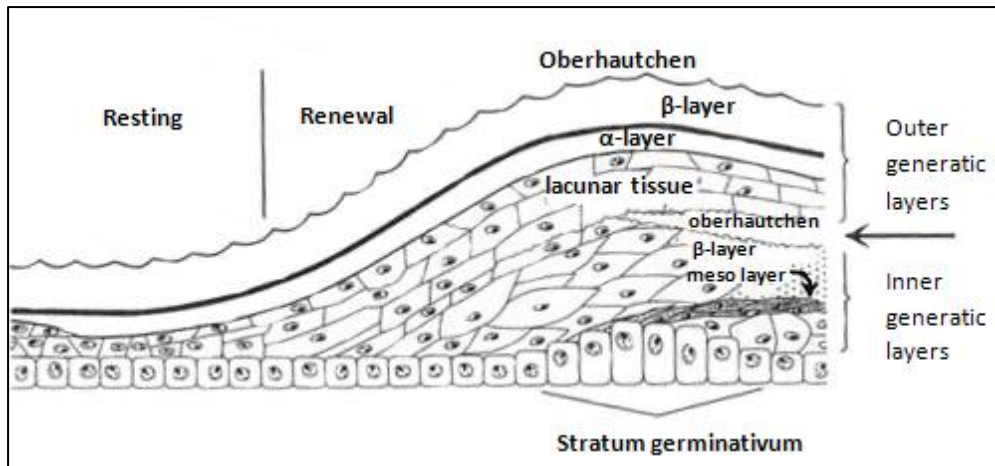


Fig.1.10: Skin layers in reptiles. The lipids are produced in the stratum corneum of the mesos layer. The cells of the β -layer lose their cell borders at maturity forming a stratum of beta keratin; the lipid-rich cells layers defined as mesos layer are extremely thin whilst the cells of the α -layer are thicker. In the renewal phase of shedding, the overlying clear layer of the old generation and the oberhautchen of the new generation mature at the same time, followed then by the β -, mesos and initial α -layers of the inner generation. (After Zug et al., 2001).

During the last years, basing on observations of captive specimen of *Malpolon monspessulanus*, DE HAAN and CLUCHIER (2006) proposed that RB is used to mark hunting routes, territories, nests and conspecifics. Marking routes or territories occurs automatically when the snakes crawls on the substrate after RB. In captivity, the secretion seems to be applied to the terrarium walls as well (DE HAAN, 1982; BRANDSTÄTTER, 1995). Congeners' marking occurs if one individual crawls on the other one after RB, where the secretion is presumably collected in the grooved scales of the snake (DE HAAN, 2003a; DE HAAN and CLUCHIER, 2006). This would be particularly important during the mating period in which the secretion is supposed to be a chemical marker of the female and other conspecifics used by a dominant male (**Chapter 1.4**). In regard to this, lipids are also known to play a role of sexual attractant during the mating period in different snakes (as in *Thamnophis sirtalis parietalis* GARSTKA and CREWS, 1981; LEMASTER and MASON, 2001; O'DONNELL et al., 2004; HUANG et al., 2006 or in *Boiga irregularis*, GREENE et al., 2001): The pheromones are extruded on the dorsal skin of the female and so deposited on the substrate (GARSTKA and CREWS, 1981; O'DONNELL et al., 2004).

However, the question of territory in snakes remains quite delicate. This would imply that a given area is defended throughout the activity period of the snake against conspecifics (**Chapter 4.3.2**). At present, territoriality in snakes is only known during the mating period as in *Vipera berus* (ANDRÉN, 1986) or *V. aspis* (SAINT-GIRONS, 1996), and a surface defended throughout the year as we know it e.g. in mammals, is not known in snakes until now (GILLINGHAM, 1987; RIVAS and BURGHARDT, 2005). RB is also performed in juveniles few hours after hatching (BRANDSTÄTTER, 1996).

1.7 OBJECTIVES OF THE STUDY

Until now, despite its uniqueness among snakes, RB has never been the subject of behavioural studies. The focus of the present study is set on a detailed analysis of this RB of psammophiids, to determine under which condition it is performed and for which purpose. All experiments were conducted under laboratory, reproducible conditions in order to obtain qualified conclusions. Following working hypotheses were considered to investigate RB:

- a) THE SCALE ULTRASTRUCTURE OF PSAMMOPHIIDS DIFFERS FROM THOSE OF OTHER SNAKES
- b) THE COMPOSITION OF THE NASAL GLAND SECRETION OF *PSAMMOPHIS MOSSAMBICUS* AND *SCUTOPHIS MOILENSIS* IS SIMILAR TO THE ONE OF *MALPOLON MONSPESSULANUS*
- c) RUBBING BEHAVIOUR IS NOT A MARKING BEHAVIOUR
- d) RUBBING BEHAVIOUR DEPENDS ON EXTERNAL FACTORS AS TEMPERATURE
- e) MORPHOLOGICAL DIVERGENCES SUPPORT THE DIFFERENTIATION OF THE GENERA *MALPOLON* AND *SCUTOPHIS*

a) The scale ultrastructure of psammophiids differs from those of other snakes

Lipids have to be extruded by the skin to play a role against desiccation. CHIASSON et al. (1989) showed that the dorsal scales of snakes have a porous pattern that works as a “[...] *surface opening for generalized epidermal sebaceous glands* [...]”. They argued that this porous structure is present on dorsal scales of all snakes -this would include the psammophiids as well. However, if psammophiids have a porous structure to allow the exit of lipids (CHIASSON and LOWE, 1989), why should they additionally smear a secretion containing lipids on their body? This question led to a detailed analysis of the scale structure of psammophiids. Snakes presenting the same habitus as psammophiids were used as control taxa.

The working hypothesis was that psammophiids do not have porous structure on their scales and therefore have to protect their skin by another way –by smearing of the nasal gland secretion.

b) The composition of the nasal gland secretion of the *Psammophis mossambicus* and *Scutophis moilensis* is similar to the one of *Malpolon monspessulanus*

The composition of the nasal gland secretion of *Malpolon monspessulanus*, known to perform rubbing behaviour was analysed by DUNSON et al. (1978). Since RB has also been mentioned for other psammophiids, it was verified whether the nasal gland secretion of two other species, *Psammophis mossambicus* and *Scutophis moilensis* also contains lipids.

The working hypothesis is that the secretion would contain lipids in both species.

c) Rubbing behaviour is not a marking behaviour

DE HAAN and CLUCHIER (2006) stated that in *Malpolon monspessulanus monspessulanus* RB is used to mark the territory of the snake, hunting routes and conspecifics rather than to be a protection against desiccation. After smearing the secretion on its body, the snake will scrap it off by crawling on the substrate and in

this way mark its territory. The decisive argument against the marking of a territory is given by juvenile psammophiids, also performing RB after the first shedding (BRANDSTÄTTER, 1996), a period of time certainly too short for the snake to have founded a territory.

The working hypothesis of the present study is that RB is not a marking behaviour.

d) Rubbing behaviour depends on external factors as temperature

Anecdotal reports mentioned that RB was done more frequently at higher temperatures but so far there is no experiment that evidences this. In the present study, temperature is varied in an experimental setup to test the tendency of the snakes to perform RB.

The working hypothesis is that the frequency of RB will increase with increasing temperature.

e) Morphological divergences support the differentiation of the genera *Malpolon* and *Scutophis*

The taxonomic status of *Scutophis moilensis* has been clarified thanks to the study of KELLY et al. (2008). Morphologically, both species share the form of their nostrils (**Fig.1.5**). They differ in the form of their rostral shield which is more prominent in *S. moilensis* and especially in the ability of this species to flatten the neck when threatened –*M. monspessulanus* is unable to do this. To see whether this flattening is due to an elongation of the neck ribs -as known for cobras, the ribs of both genera were analysed with X-ray. This analysis was completed by dissection.

The working hypothesis is that the neck ribs in *S. moilensis* will be longer than in *M. monspessulanus*.

2. MATERIAL AND METHODS

For the behavioural experiments, 17 psammophiids (six *Psammophis mossambicus*, four *P. schokari*, one *Malpolon insignitus fuscus*, four *S. moilensis* and two *Psammophylax acutus acutus*) were studied (**Table 2.1**). These species are not on the IUCN Red List of Threatened Species. All specimens were kept at the Museum Koenig Bonn, Germany.

The snakes were housed individually or in pairs in glass terraria (100cm x 80cm x 70cm), with a mixture of sand and earth for the mesic species and sand for the desert species, all with climbing and hiding possibilities. Water was provided *ad libitum*. The terraria had a 12/12 light cycle, air temperature varied between 25°C and 33°C; both, light and temperature were given by a 75W light bulb. All snakes were fed once weekly with a diet of rodent.

Studies were conducted throughout the year with *Psammophis mossambicus* and *Psammophylax a. acutus*. *Psammophis schokari*, *Malpolon insignitus fuscus* and *S. moilensis* had a short hibernation from November to January.

Table 2.1
Genera and species examined in this study; SVL= snout vent length.

Genus	Species	Subspecies	Mean SVL [cm]	Nb. of snake observed	Habitat
<i>Malpolon</i>	<i>insignitus</i>	<i>fuscus</i>	130	1	semi-arid/ mesic
<i>Scutophis</i>	<i>moilensis</i>		100	4	arid
<i>Psammophis</i>	<i>elegans</i>		135	1	mesic
<i>Psammophis</i>	<i>mossambicus</i>		140	6	mesic
<i>Psammophis</i>	<i>schokari</i>		95	4	arid
<i>Psammophylax</i>	<i>acutus</i>	<i>acutus</i>	90	2	mesic
<i>Rhamphiophis</i>	<i>rostratus</i>		150	3	semi-arid
<i>Rhamphiophis</i>	<i>rubropunctatus</i>		150	1	semi-arid

2.1 SCANNING ELECTRON MICROSCOPY

Additionally to the 17 psammophiids mentioned above, 10 other snakes presenting the same habitus as psammophiids (see whipsnakes, **Chapter 1.2**) were used as control taxa for scanning electron microscopy: *Hemorrhois hippocrepis*, *H. algirus*, *Masticophis mentovarius* and *M. flagellum* (subfamily Colubrinae); *Philodryas psammophidea* and *Liophis typhlus* (subfamily Xenodontinae); *Demansia psammophis* (family Hydrophiidae); *Nerodia rhombifera*, *Natrix natrix* and *Thamnophis sirtalis sirtalis* (subfamily Natricinae). The voucher numbers of the snakes used can be found on **Table 8.2**.

Oberhautchen¹ samples of preserved specimen and/or from shed skins of live specimens was analysed (as in BURSTEIN et al., 1974; IRISH et al, 1988). The museum samples were from species known to perform RB (**Chapter 1.6**) and from some taxa for which RB had not been described yet. Two or three vertebral scales and one lateral scale (first row of scales in contact with the ventral scales, slightly larger than the dorsal scales) were analysed, removed about 7cm before the cloaca (**Fig.2.1(I)**). The middle of the scale was analysed (**Fig.2.1(II)**).

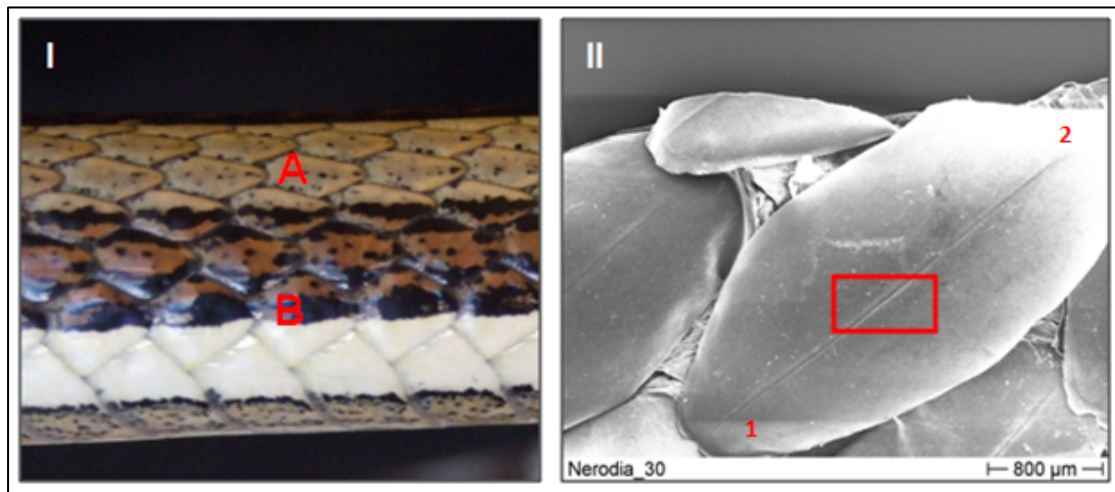


Fig.2.1: (I) Lateral view of *Psammophis elegans*: (A) Dorsal scale, (B) lateral scale. (II) Overview of a scale for SEM (here *Nerodia rhombifera*, 40x). The square indicates the area studied; (1) is the apical part of the scale; (2) is the basal part of the scale, “attached” to the skin.

¹ „[...] called the Oberhäutchen since Maderson’s early work. Hereafter, we shall consider this German word to be anglicized and, hence, requiring neither an umlaut nor initial capitalization.” (IRISH et al., 1988)

The oberhautchen of at least two species of each of the seven psammophiids genera were examined, except for *Mimophis* (the genus is monophyletic), *Hemirhagerrhis* (the specimen of the second species was too small) and *Psammophis* since (two African and one Asiatic species were studied). Oberhautchen specimens were mounted on aluminium stubs (5mm diameter) with their external (dorsal) surface exposed. The clear layer (internal surface of the oberhautchen) was analysed as well. The samples were coated into a Hummer VII Scanning Electron Microscope Sputtering System (Anatech LTD) with 20-30nm gold (4A) during 5 minutes. All samples were viewed at 0° tilt, at 25kV and 70-90µA with an S-2460N Scanning Electron Microscope (Hitachi Scientific Instrument) (see JOSEPH et al., 2007).

Specimen examination was at low (600x-1250x) and medium (2000x and 5000x) magnification for the description of the general scale pattern. Studies of the scales in the literature showed that pores appear at a magnification of 4000x-5000x and have a dimension inferior to $\leq 1\mu\text{m}$ (STILLE, 1987; CHIASSON and LOWE, 1989; CHIASSON et al., 1989; PRICE and KELLY, 1989; HAZEL et al., 1999; GOWER, 2003; JOSEPH et al., 2007).

2.2 GAS CHROMATOGRAPHY

To analyse the composition of the nasal secretion of *Psammophis mossambicus* and *Scutophis moilensis* the secretion of two snakes of each species was collected during one week to be analysed by gas chromatography-mass spectrometry (GC/MS). This method allows the separation of the diverse components of a given solution.

The middle ventral and lateral part of the body of the snakes was scrapped off with a cover glass to collect dried secretion (DE HAAN and CLUCHIER, 2006). The slides were then placed in 3ml hexane to solve the nasal gland secretion. Each solution was stored in the freezer until analysis by GC/MS. Before analysis the hexane was removed by rotary evaporation so that a thin residue was deposited on the recipient. This residue was solved with 50µl methanol. 1µl of the 50µl was used for GC/MS. Gas chromatography was carried out on a Perkin Elmer AutoSystem XL gas chromatograph with a Perkin Elmer Turbo Mass mass spectrometer. The column was a fused silica PE-1 column (30m x 0.32mm x 0.25µm). The carrier gas was helium

(2ml, split 1:30). Injection temperature was 230°C. The oven was held at 80°C for 3min isotherm and the programmed to ramp up to 320°C by steps of 10°C/min. Then the temperature of 320°C was maintained during 8min.

Two additional controls, once only hexane and one hexane plus glass slides were also measured to assure no contamination was inside the solvent or glass.

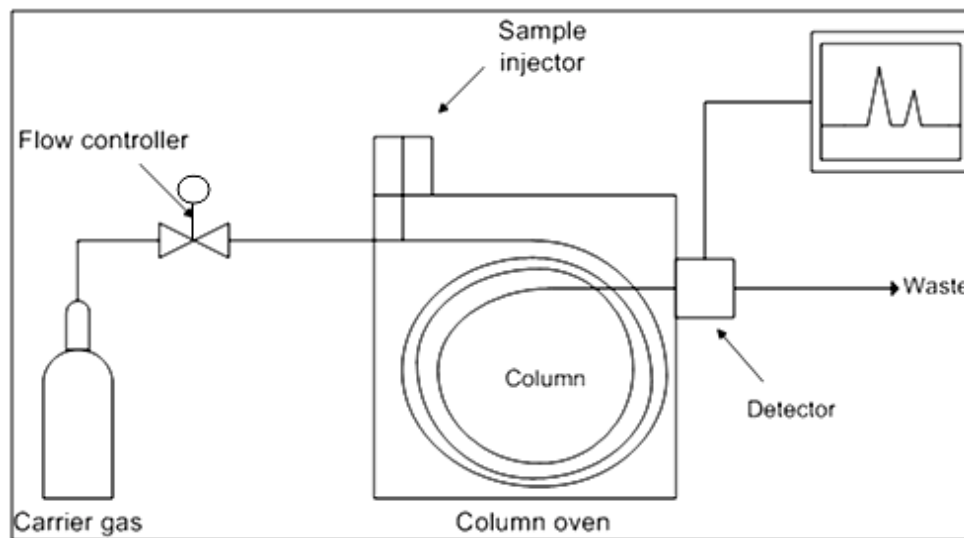


Fig.2.2: Schematic representation of a gas chromatograph. The carrier gas is pressed through the column where it interacts with the sample to be analysed. Depending on polarity and vapour pressure of a substance, each compound needs a different time through the column (retention time). *Picture after <http://en.wikipedia.org>.*

2.3 RUBBING BEHAVIOUR IN PSAMMOPHIIDS

Preliminary observations were done to determine the period of activity of the snakes, whether they perform RB in presence of an observer, the location they perform it, how much time they need to perform RB when placed into a new terrarium; moreover, it was analysed whether rubbing the snakes with 70% alcohol or with (hand) cream had an effect on their RB.

“Continuous recording” with cameras was chosen as method to record RB (MARTIN and BATESON, 1993). This method has the advantage to leave the snake undisturbed so that it can perform its behaviour without disturbance of an observer.

Two webcams (Philips SPCI 300NC) and two CCTV Panasonic cameras (models WV-BL200/G and WV-BL600/G) were positioned to give a dorsal and/ or lateral

perspective of the body during RB. The resulting tapes were analysed to count the number of RB occurring during the recorded time. The snakes were filmed 8h/day.

Two observation terraria (with dimensions 114cm x 80cm x 120cm and 100cm x 80cm x 70cm) were used for the experiments. All terraria contained a bedding of earth or sand about 2cm deep. During the shedding cycle or when a snake was ill, no experiments were conducted with this snake. All terraria were cleaned and disinfected (with Sterilium) between each experiment.

RB itself was defined as follow: application of the nostril on the belly or flank followed by zigzagging movements of the head along the entire body at least until the cloaca.

2.3.1 ANALYSIS OF RUBBING BEHAVIOUR IN PSAMMOPHIIDS

The RB of *Psammophis mossambicus*, *P. schokari*, *Psammophylax a. acutus*, *Malpolon insignitus fuscus* and *Scutophis moilensis* was analysed as follows:

- 1) The method of rubbing: if only one or both nostrils were used;
- 2) The RB duration;
- 3) The total number of head movements to apply the secretion on the body.

One head movement was defined as: pulling the nostril along the body, starting on the belly, pulling it along the flank to finish of the top of the back. The video tapes were analysed on slow-motion to describe RB.

The acquisition of three *Rhamphiophis rostratus*, one *R. rubropunctatus* and one *Psammophis elegans* made possible to observe whether these snakes perform RB as well since RB had not been described previously for these three species.

2.3.2 RUBBING BEHAVIOUR AS MARKING BEHAVIOUR

In the literature it had been stated that the secretion, after application on the belly, is scrapped off on the substrate to leave a scent trail marking the territory and/ or hunting routes in *Malpolon monspessulanus* (DE HAAN, 1999). In view of this previous

observation it was important to test RB's marking properties on an experimental way since it had never been done before.

The first part of the present experiment consisted on determining the rubbing frequency when the snake was placed into a new, unknown terrarium during five days. 15 of the psammophiids at disposal (one *M. m. insignitus*; six *P. mossambicus*; four *P. schokari*; two *S. moilensis*; two *P. a. acutus*) were used for this experiment, to determine whether "territoriality" is also present in these species. It was expected that the rubbing frequency would be higher on the first day since the snake would mark its territory. The frequency of the first day was then compared with the mean frequency of the four following days with a paired sample t-test. The data were analysed using SPSS Statistics 18 Software (SPSS INC., USA). Light and warmth was given by a 75W light bulb. The snakes were filmed 8h/day. Between each snake the terrarium was cleaned and disinfected.

The second part of this experiment was done to determine whether the scrapped off secretion of one snake would elicit any reaction in a second conspecific snake. For this experiment, a glass plate was placed into the middle of the terrarium: DE HAAN (1999) and BRANDSTÄTTER (1995) had written that the secretion can be seen on the terrarium's glass walls as whitish or yellowish trace. Blotting paper was additionally stuck on two walls to see whether the secretion could also be made visible on it.

During two weeks, four adult *P. mossambicus* and four adult *S. moilensis* were used (**Table 2.2**). The gender of only two snakes of each species was known. For each species, one snake was chosen randomly to be placed at first into the cleaned terrarium during four hours. Preliminary experiments showed that RB is mainly performed during the first 60-90 minutes after being placed into a new terrarium. After four hours, the first snake was removed. It was checked, wearing gloves, whether some traces of secretion could be seen on the glass plate and on the blotting paper. Then the second snake was placed for the same period of time into the terrarium, but without cleaning the terrarium between both snakes. On the video

tapes it was analysed whether the second snake used the same way as the first one, whether it behave differently, trying to avoid the way used by the first snake.

Each snake was tested into both situations, once being the snake placed at first into the terrarium, once at second. Both snakes were placed on the same “starting” place into the terrarium (**Fig.2.3**). To record the way used by the snakes, a transparent grid (16x12cm) was applied on the screen whilst analysing video tapes (**Fig.2.3**). A control run was done for each snake to see how it behaved when no snake had been placed before it.

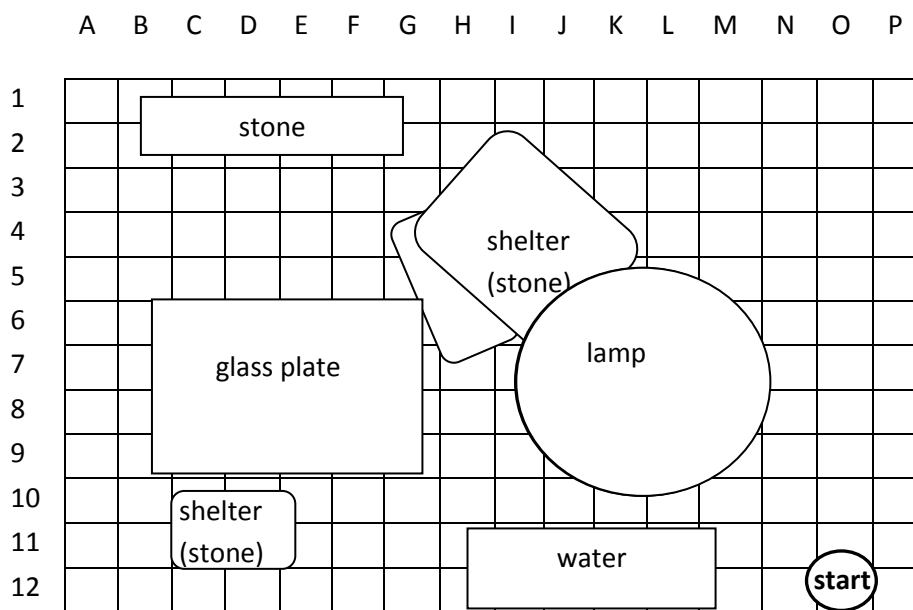


Fig.2.3: Schematic representation of the grid to analyse trails. The grid (16x12cm) was applied on the screen and the direction used by the snake was recorded. The objects in the terrarium as stones or the lamp are represented for more clarity. Dot: place where the snake was placed into the terrarium. Bold lines: Blotting paper.

Table 2.2

Snakes used in part 2. Four *Scutophis moilensis* (*S.m.*) and four *Psammophis mossambicus* (*P.m.*) were used. Snakes no 1 and 11 were males, snakes no 2 and 13 were females.

		Snake placed at first into the terrarium (also control run)							
		<i>S. m.</i> no11 ♂	<i>S. m.</i> no12	<i>S. m.</i> no13 ♀	<i>S. m.</i> no14	<i>P. m.</i> no1 ♂	<i>P. m.</i> no2 ♀	<i>P. m.</i> no3	<i>P. m.</i> no4
Snakes placed at second into the terrarium	<i>S. m.</i> no11			✓					
	<i>S. m.</i> no12				✓				
	<i>S. m.</i> no13	✓							
	<i>S. m.</i> no14		✓						
	<i>P. m.</i> no1						✓		
	<i>P. m.</i> no2					✓			
	<i>P. m.</i> no3								✓
	<i>P. m.</i> no4							✓	

2.3.3 FREQUENCY OF RUBBING BEHAVIOUR DEPENDING ON TEMPERATURE

The 17 snakes were analysed to test whether their RB depends on temperature. To induce RB, the snake was exposed to four different temperatures during a period of one week each. Temperature was given by light bulbs of 40W, 75W, 100W, 80W (reflector OSRAM), mounted 30cm above the ground. They gave respectively the mean temperature of 27,24°C; 31,72°C; 38,36°C and 67,16°C on the ground under the bulb (**Table 2.3**). The temperatures were measured with a Lucky Reptile Thermo Recorder ($\pm 1^\circ\text{C}$ when temperature superior to 20°C).

The temperatures were assigned randomly to each snake. 31,72°C was the temperature the snake had in their housing terrarium. All snakes had the possibility to escape heat. The air temperature and the relative humidity in the observation terrarium were recorded (TFA Hygro-Therm, $\pm 1^\circ\text{C}$; $\pm 5\%$).

To obtain frequency of rubbing behaviour (number of RB performed per hour), the total number of rubbing behaviour exhibited by each snake for the given condition was divided by the time of observation for this condition. These frequencies were analysed with the rank Spearman correlation coefficient test, to test whether they depend on temperature (SPSS Statistics 18 Software (SPSS INC., USA, 2009)).

Table 2.3
Conditions for the frequency of rubbing behaviour for the 17 snakes observed.

Name	Condition	Maximal Temperature (under bulb)	Mean Temperature (under bulb)
Temp. 1	40W bulb	28,62°C	27,24° C
Temp. 2	75W bulb	32,4° C	31,72° C
Temp. 3	100W bulb	39,4° C	38,36° C
Temp. 4	80W heater	69,4° C	67,16° C

2.4 MORPHOLOGICAL DIVERGENCES BETWEEN *MALPOLON MONSPESSULANUS* AND *SCUTOPHIS MOILENSIS*

Within both genera, *Scutophis moilensis* is the only one able to spread the ribs of the neck. The morphology of the neck ribs of both genera was analysed with X-ray to clarify whether the flattening of *S. moilensis* can be due to the elongation of the ribs.

The chest region, defined here as being about 15cm of the body measured behind the head of four *S. moilensis* and four *Malpolon monspessulanus* was removed for analysis. This region comprised about 40 ribs whereof the 20 first were measured. The *S. moilensis* had unfortunately died previously and had been frozen until analysis; the four *Malpolon monspessulanus* were of the Museum's collection (ZFMK no 23060, 23061, 23062 and 89766). All specimens had total body length of 60-80cm. In a first time, the snakes were analysed with an x-ray apparatus (Faxitron LX-60) to see whether morphological differences could be made visible with this method. Then for a more complete analysis, the snakes were dissected to remove the ribs in order to measure them.

To not damage the Museum specimens by complete dissection, only about 15cm of the trunk region, without skin, was removed carefully so that the skin of the preserved specimen could still maintain the body in "one piece". The trunks of the eight snakes were boiled to detach the flesh and to facilitate the removal of the ribs. After dissection, the ribs were detached to be measured as linear connection

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between their attachment points to the vertebral column to their tip. They were deposited on graph paper to be measured, since measuring them with a calliper damaged the tip. The width of the ribs, on its part, could be measured with the calliper on the first third of the length of the ribs (measuring error: $\pm 0,01\text{mm}$). The width of each rib was measured three times to obtain a mean value.

3. RESULTS

3.1 SCANNING ELECTRON MICROSCOPY

The dorsal scale surface of psammophiids was smooth to the naked eye except for *Malpolon insignitus fuscus* and *M. m. monspessulanus* which have a visible medial groove. Since the structure of the lateral scales was less pronounced than the one of the dorsal scale, only the dorsal scales were further analysed.

The microstructure of the dorsal scales was different, depending whether the basal or apical end was considered (see **Fig.2.1II**): The attachment zone of the scale on the skin comprises highly porous cells of the stratum corneum (ENGELMANN and OBST, 1981) responsible for the metabolite exchange (POCKRANDT, 1936), clearly visible at 2000x magnification (**Fig.3.1**). The microstructure at the basal part can be considered as a “draft” of the following microstructure (**Fig.3.2a-f**). Progressing then along the scale, the proper microstructure was revealed, generally at a magnification of 600-800x. It consists of longitudinal ridges described as “alignment of indentation” by PAUWELS et al. (2000), orientated parallel in the basal to apical direction. These ridges are bounded, forming little cups (called in this study “cup-like” formation). These cups are “filled” with a structure described in this study as vermicular, more or less dense depending on the species considered (**Fig.3.2f and 3.3A**).

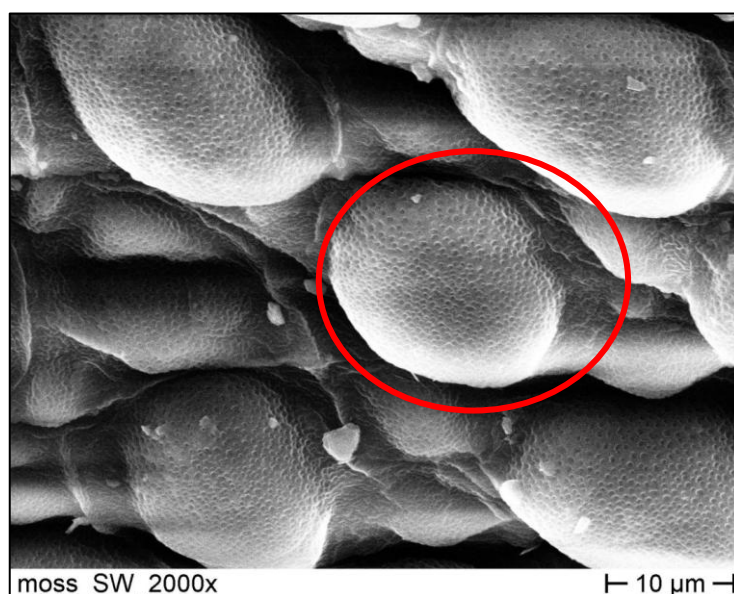


Fig.3.1: Porous cells of the stratum corneum in *P. mossambicus*. The red circle designs on of those porous cells (2000x).

The basis of the oberhautchen itself consists of several parallel stripes those anterior fringes are slightly denticulate (**Fig.3.2b-d**). The surface of these stripes is sprinkled with little “pits” forming a kind of grid which corresponds posteriorly to the vermicular structure between the striations (**Fig.3.2f**). Progressing along the oberhautchen to the centre of the scale, the denticulation becomes more and more specified to form little spines. These spines become more acute and overlap slightly the spines of the next stripe (**Fig.3.2f**). The spines are bounded to form the cup-like structure previously described. Through the elongation of the “pits”, the vermicular structure appears (**Fig.3.2a-f**). The definite structure of the microornamentation is reached in the first few millimetres after the porous cells of the stratum corneum (**Fig.3.2a**). Apical pits visible to the naked eye as those described by CHIASSON (1981) or BRANDSTÄTTER (1995) could not be seen on all species analysed.

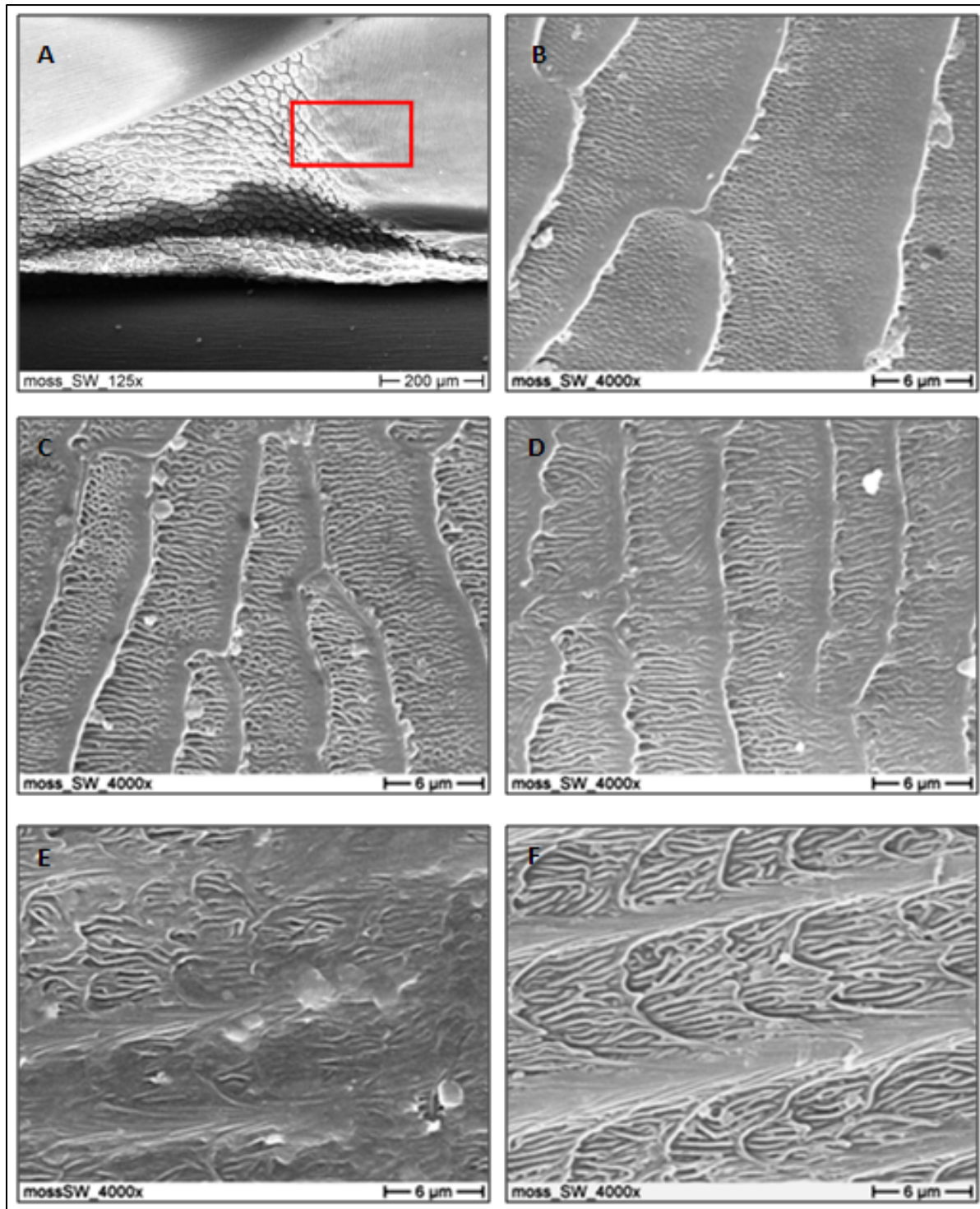


Fig.3.2a-f: Development of the microornamentation in *P. mossambicus*. **(A)** The square indicates the part of the scale analysed (125x); **(B)** Slightly denticulate anterior fringes; the “pits” are visible; **(C)&(D)** Determination of the spines and elongation of the “pits”; **(E)** Spines becoming more acute; **(F)** “Final” microstructure showing the dentition separated by cup-like formation and filled with the vermicular structure. **(B)-(F):** 4000x magnification.

At 2000x magnification, four major scale patterns for psammophiids could be identified: “scalar”, “caniculate”, “cup-like flat” and “cup-like ridge” (see **Table 3.1** for description and **Fig.3.3, 3.5 and 3.6**). On psammophiid scales, a structure similar to the one described as “porous” in CHIASSON and Lowe (1989) and CHIASSON et al. (1989) was only present at the basal end: No “pores” could be identified on the median dorsal surface scale, neither at 5000x, nor at higher magnifications (**Table 3.2**).

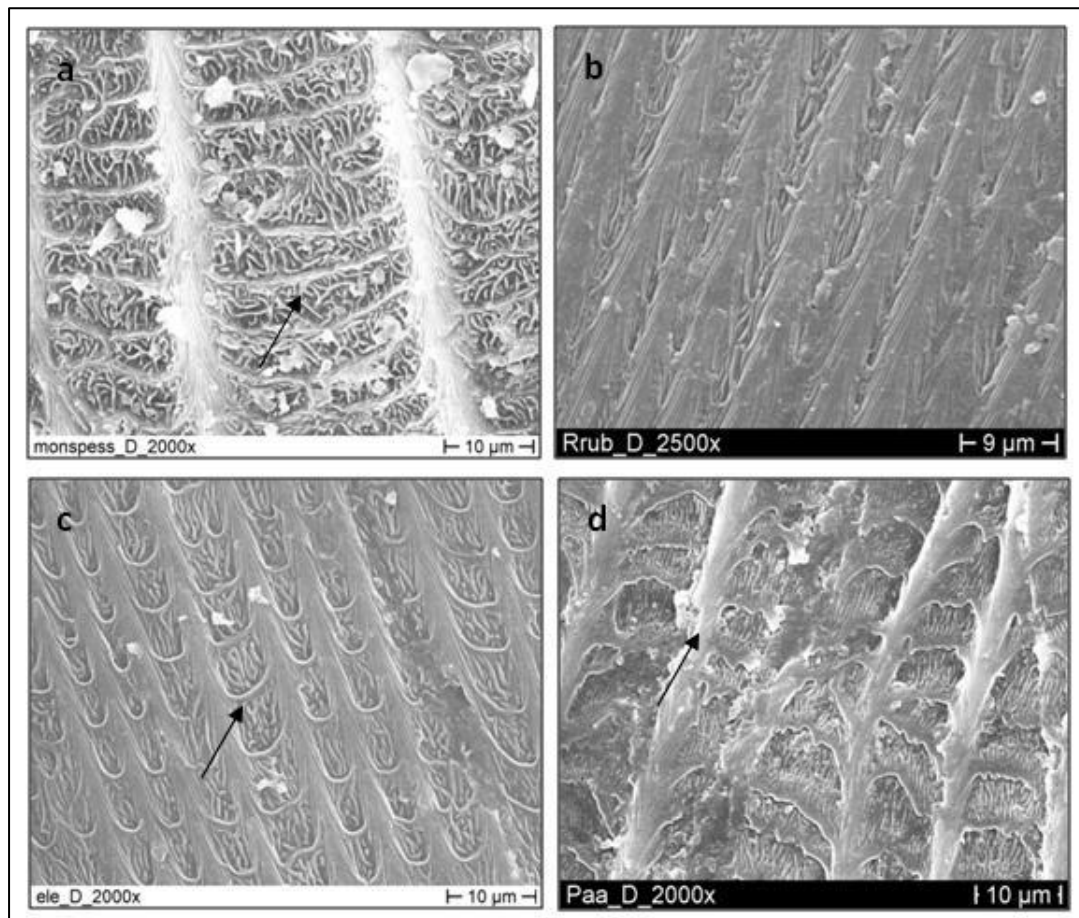


Fig.3.3: The four main types of microornamentation in psammophiids: **(a)** scalar (*M. i. fuscus*, 2000x), **(b)** caniculate (*R. rubropunctatus*, 2500x), **(c)** cup-like flat (*P. elegans*, 2000x) and **(d)** cup-like ridge (*P. a. acutus*, 2000x). The arrows show in **(a)** the linear junction between the ridges, in **(c)** the curved junction between the ridges and in **(d)** the upraised ridges.

The dorsal scales of the control genera were also smooth to the naked eye, except the scales of *Natrix natrix* and *Nerodia rhombifera* having a medial ridge. The basal structure of the microornamentation was similar to the one found in psammophiids being a “draft” of the future microornamentation.

The 600x magnification showed mainly transversal ridges. 2000x magnification revealed also a “cup-like ridge” and a “caniculate” pattern as well as a “papillate”, an “echinate” and an “echinate-canaliculate” pattern (**Fig.3.4** and **Table 3.1**) (for the nomenclature of the microornamentation see PRICE, 1982).

The structure between the ridges had “pits” in *C. algirus*, *C. hippocrepis*, *L. typhlus*, *N. natrix* and *N. rhombifera* (**Fig.3.7 and 3.8**) and was vermicular, although to a lesser extent than in psammophiids, in *D. psammophis*, *M. flagellum*, *M. mentovarius* and in *P. psammophidea* (**Fig.3.7-3.8** and **Table 3.1**).

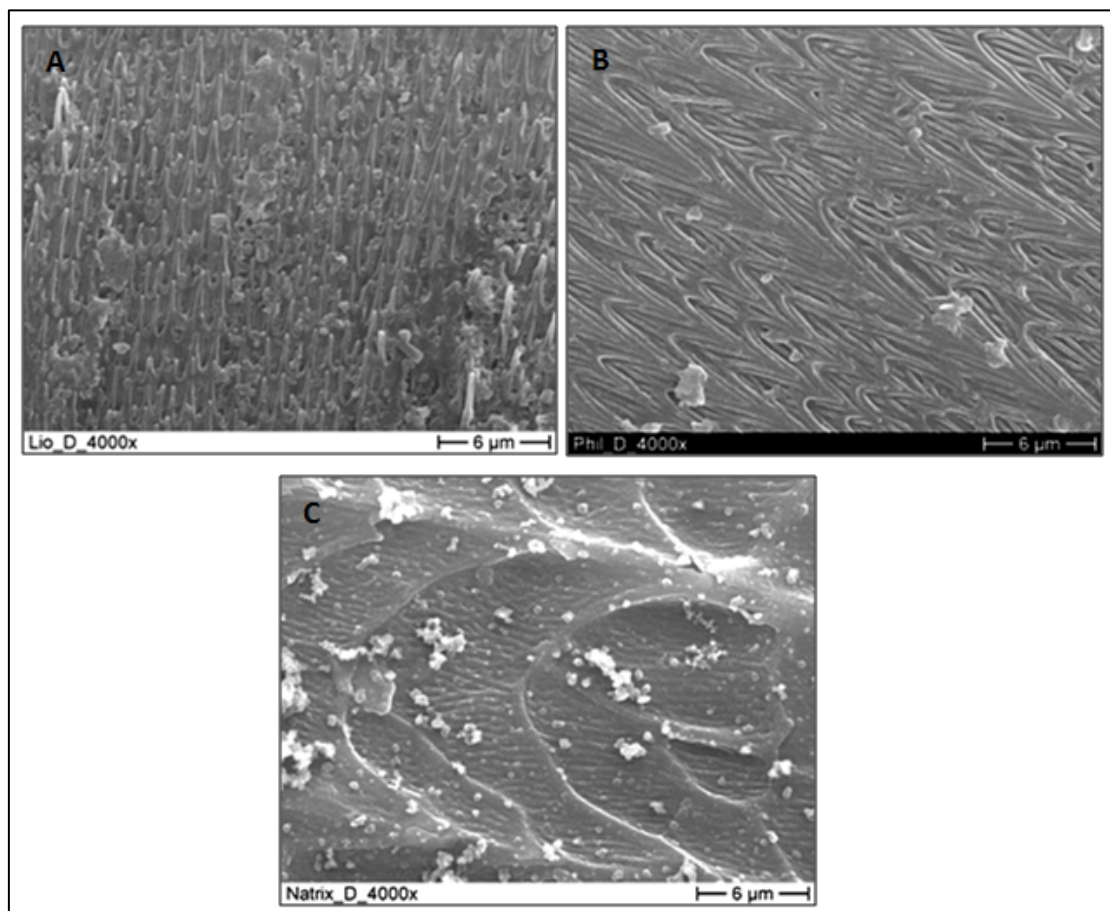


Fig.3.4: The main types of microornamentation in the control group: **(A)** In *Liophis*, a papillate microornamentation was found, **(B)** an echinate in *Philodryas*, **(C)** an echinate-canaliculate in *Natrix*. The white particles are dirt. Magnification: 4000x.

Table 3.1
Description of the ultrastructure of psammophiid scales. Control taxa are indicated in bold letters.

Pattern	Description	Species
Caniculate	Ridges are at close quarters forming channels between them	<i>Rhamphiophis oxyrhynchus</i> , <i>R. rostratus</i> and <i>R. rubropunctatus</i> <i>Masticophis flagellum</i>, <i>M. mentovarius</i>
Cup-like flat	Junction between the ridges is curved, ridges are flat	<i>Mimophis mahfalensis</i> , <i>Psammophis elegans</i> , <i>P. condanarus</i> , <i>P. mossambicus</i> , <i>P. phillipsi</i> , <i>P. sudanensis</i> , <i>Psammophylax rhombeatus</i>
Cup-like ridge	Junction between the ridge is curved, ridges are upraised	<i>Hemirhagerrhis hildebrandti</i> , <i>Psammophylax a. acutus</i> , <i>P. tritaeniatus</i> <i>Demansia psammophis</i>
Echinate	Cup-like formation is flat	<i>Coluber algirus</i>, <i>Philodryas psammophidea</i>
Echinate-canicate	Cup-like formation is flat and ridges upraised	<i>Natrix natrix</i>, <i>Nerodia rhombifera</i>
Papillate	Spines ending with a little bulb	<i>Coluber hippocrepis</i>, <i>Liophis typhlus</i>
Scalar	Junction between the ridges are linear	<i>Dipsina</i> , <i>Malpolon spc.</i> , <i>Psammophis schokari</i> , <i>Scutophis moilensis</i>

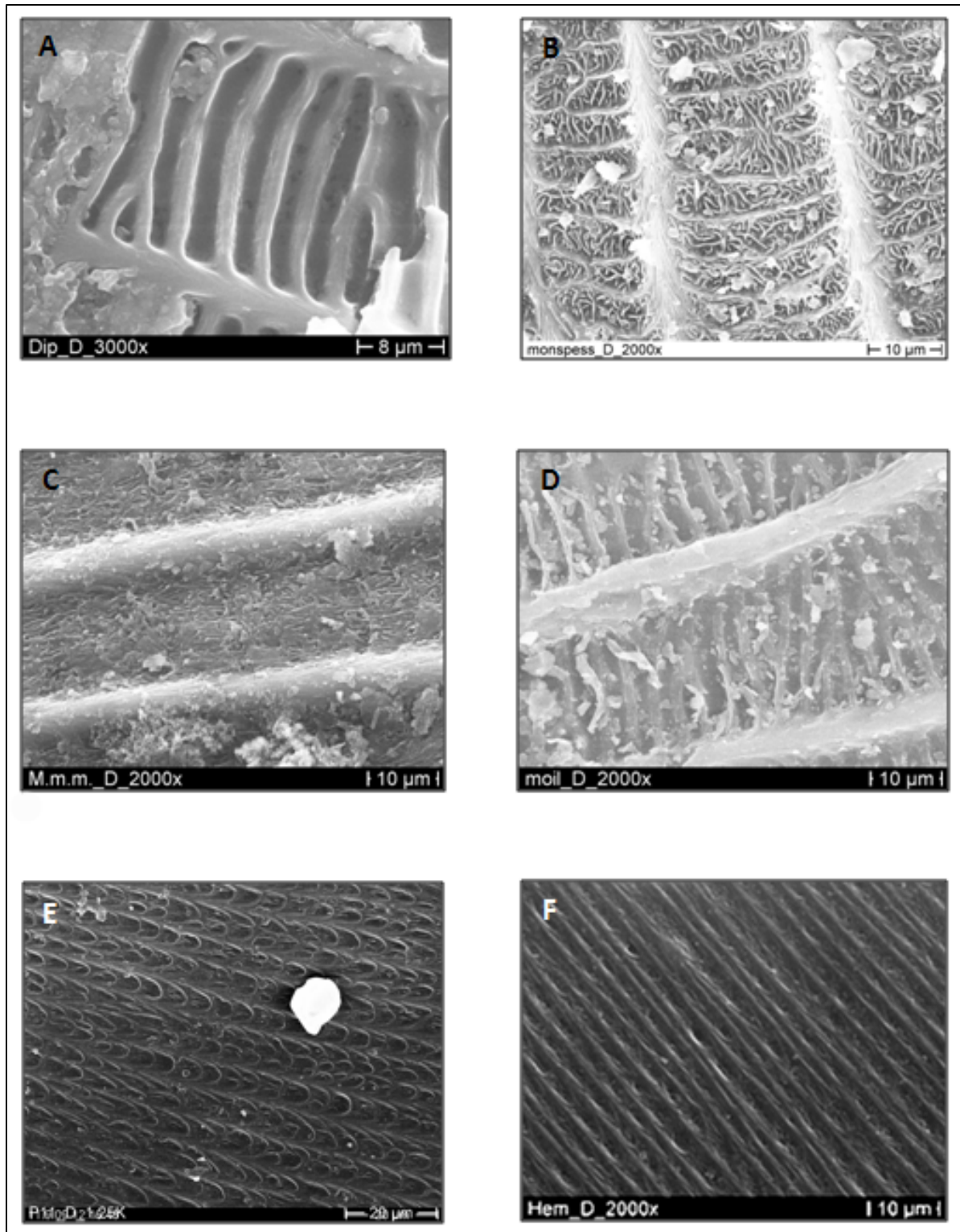


Fig.3.5: SEM picture of the psammophiids examined. **(A)** *Dipsina multimaculata* (3000x) **(B)** *Malpolon insignitus fuscus* **(C)** *Scutophis moilensis* **(D)** *Malpolon monspessulanus monspessulanus* **(E)** *Mimophis mahfalensis* (1250x) **(F)** *Hemirhagerris hildebrandti*. The white particles seen are dirt or little grain of sand. Magnification: 2000x.

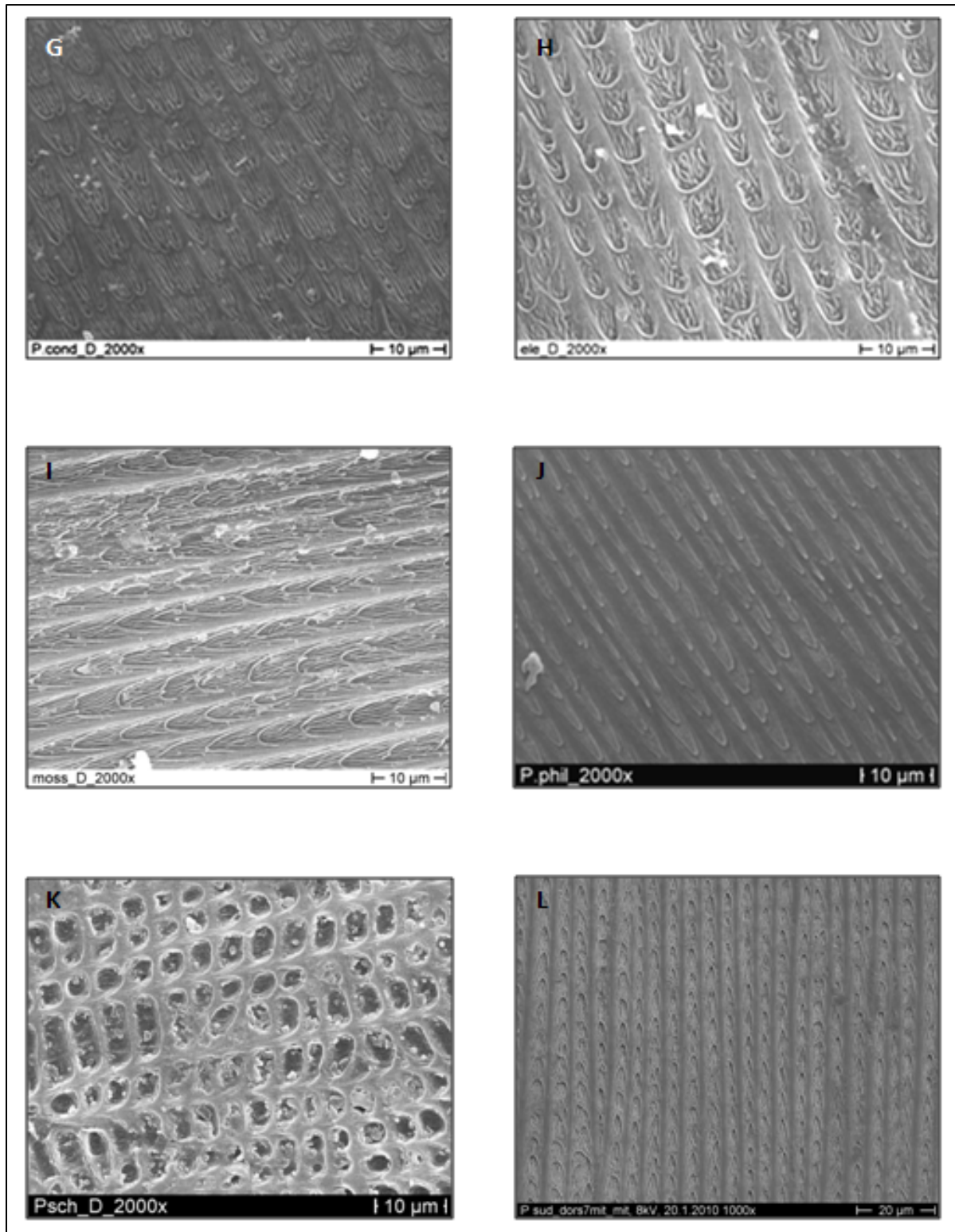


Fig.3.6: SEM picture of the psammophiids examined. (G) *Psammophis condanarus* (H) *Psammophis elegans* (I) *Psammophis mossambicus* (J) *Psammophis phillipsi* (K) *Psammophis schokari* (L) *Psammophis sudanensis* (1000x)(©M. Spinner). Magnification: 2000x.

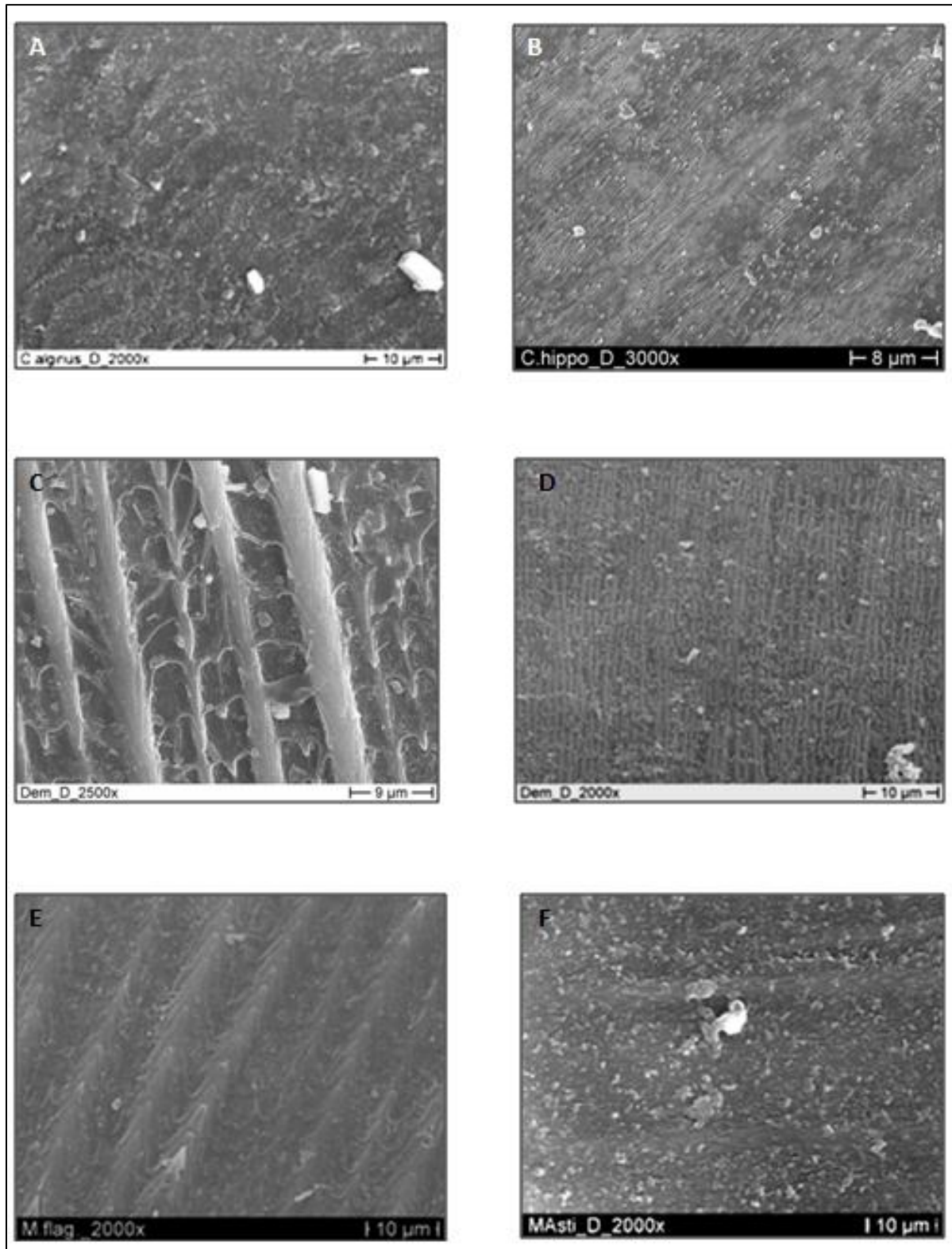


Fig.3.7: SEM picture of the control taxa examined. **(A)** *Coluber algirus* (2000x) **(B)** *Coluber hippocrepis* (3000x) **(C)** *Demansia psammophis* (2500x) **(D)** *Liophis typhlus* (2000x) **(E)** *Masticophis flagellum* (2000x) **(F)** *Masticophis mentovarius* (2000x).

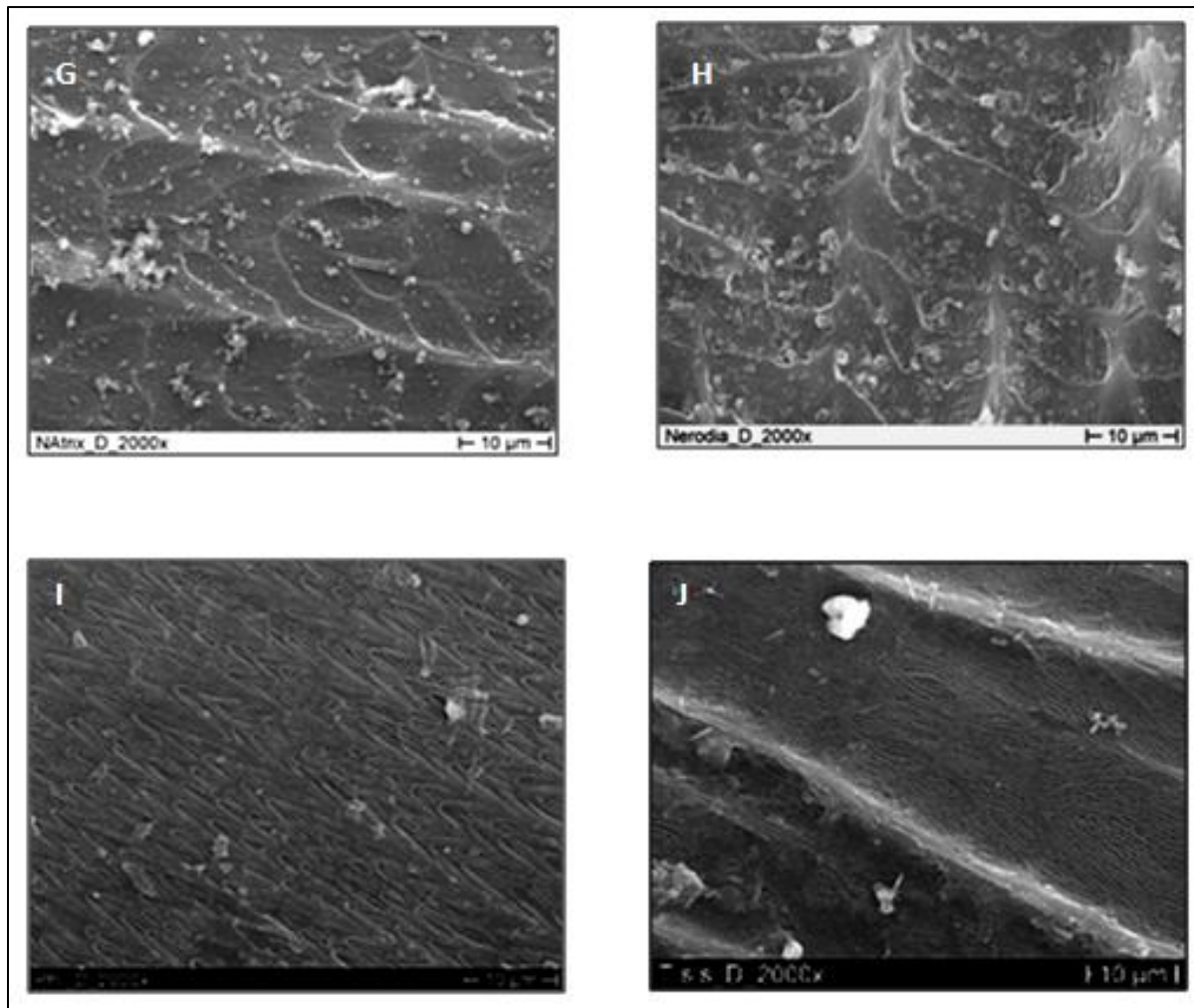


Fig.3.8: SEM picture of the control taxa examined. **(G)** *Natrix natrix* **(H)** *Nerodia rhombifera* **(I)** *Philodryas psammophidea* **(J)** *Thamnophis sirtalis sirtalis*. Magnification: 2000x.

Table 3.2
Shape of the ultrastructure of psammophiids and control genera examined with SEM.

Species	Control taxon	Ultrastructure (2000x)	Pore-like structure on scale surface
<i>Coluber algirus</i>	✓	Echinate	✓
<i>Coluber hippocrepis</i>	✓	Papillate	✓
<i>Demansia Psammophis</i>	✓	Cup-like ridge	
<i>Dipsina multimaculata</i>		Scalar	
<i>Hemirhagerrhis hildebrandti</i>		Cup-like ridge	
<i>Liophis typhlus</i>	✓	Papillate	✓
<i>Scutophis moilensis</i>		Scalar	
<i>Malpolon monspessulanus monspessulanus</i>		Scalar	
<i>M. insignitus fuscus</i>		Scalar	
<i>Masticophis mentovarius</i>	✓	Caniculate	
<i>Masticophis flagellum</i>	✓	Caniculate	
<i>Mimophis mahfalensis</i>		Cup-like flat	
<i>Natrix natrix</i>	✓	Echinate-canicate	✓
<i>Nerodia rhombifera</i>	✓	Echinate-canicate	✓
<i>Philodryas psammophidea</i>	✓	Echinate	
<i>Psammophis condanarus</i>		Cup-like flat	
<i>P. elegans</i>		Cup-like flat	
<i>P. mossambicus</i>		Cup-like flat	
<i>P. phillipsi</i>		Cup-like flat	
<i>P. schokari</i>		Scalar	
<i>P. sudanensis</i>		Cup-like flat	
<i>Psammophylax acutus acutus</i>		Cup-like ridge	
<i>P. rhombeatus</i>		Cup-like ridge	
<i>P. tritaeniatus</i>		Cup-like ridge	
<i>Rhamphiophis oxyrhynchus</i>		Caniculate	
<i>R. rostratus</i>		Caniculate	
<i>R. rubropunctatus</i>		Caniculate	

3.2 GAS CHROMATOGRAPHY

GC/MS analysis of the control samples, hexane and hexane with glass slides showed one peak of high intensity at 12,40 minutes(**Fig.3.9a**). For a better overview, only the graph of hexane alone is showed here. The chromatograms of *S. moilensis* no1 (**Fig.3.9b**) and *S. moilensis* no2 (**Fig.3.9c**) were similar when compared together.

In *S. moilensis*, the only peak appearing at the same time was the one at 17,47min. This peak was analysed to see whether the same masses are present in both snakes. The scan of this peak showed different masses-to charge (m/z) for both snakes (**Fig.3.10** and **Fig.3.11**): The scan of *S. moilensis* no2 showed also (m/z) of 300amu (atomic mass unit) and more, which are absent from the scan of *S. moilensis* no1. It could not be defined with certitude whether these masses were the molar mass of a given substance (lipid) or whether they were fragment ions of a lipid with a higher molecular mass. Because of this imprecision, no “definite” lipid-type could be determined for this species.

The chromatograms of *P. mossambicus* no1 and *P. mossambicus* no2 showed similar retention times (**Fig.3.9d** and **Fig.3.9e**). A peak appearing at 17,77min in both snakes was detected (**Fig.3.12** and **Fig.3.13**). The scans of this peak were more similar to each other as when compared with *S. moilensis*. In the scan of snake no1 an additional (m/z) of 296,8amu appeared. As in *S. moilensis* it could not be clearly defined whether the masses obtained were the molar mass of a given lipid or of a fragment ion of a lipid.

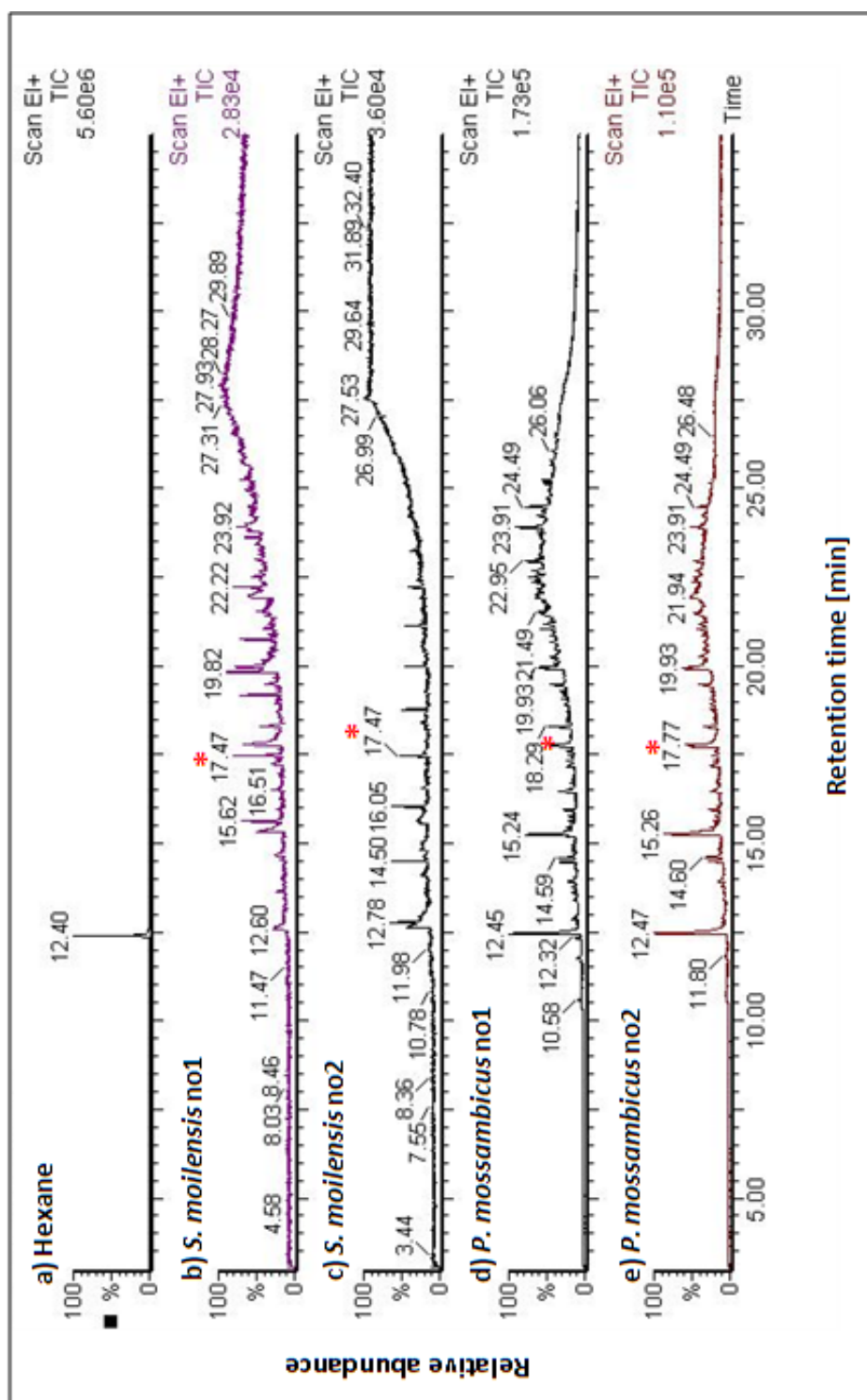


Fig.3.9: Gas chromatograms of: **(a)** the control sample (hexane), the same as for hexane with glass slides. Only one graph is represented here; **(b)** *S. moilensis* no1; **(c)** *S. moilensis* no2 **(d)** *P. mossambicus* no1 and **(e)** *P. mossambicus* no2. The retention times in minutes are indicated on the x-axis. The relative ion abundance (or intensity) is on the y-axis. The peaks indicated in red were analysed for their composition. TIC: total ion chromatogram.

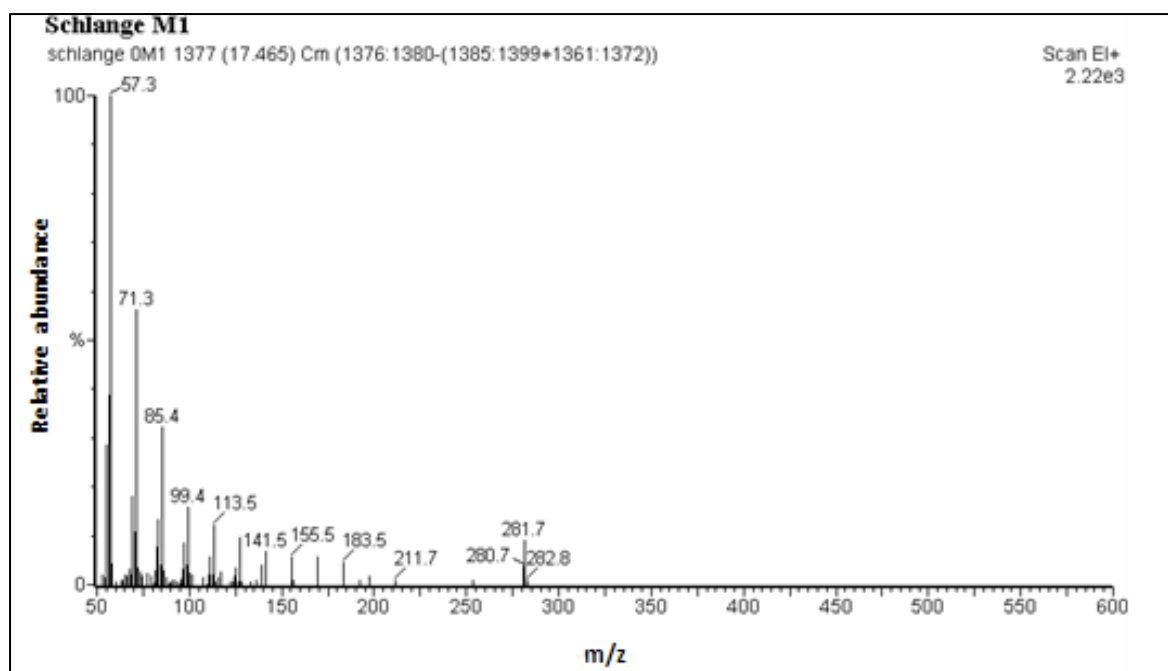


Fig.3.10: Mass spectrum (m/z) of the peak at a retention time of 17,47min in *S. moilensis* no1. Four mass-to-charge superior to 200amu were determined.

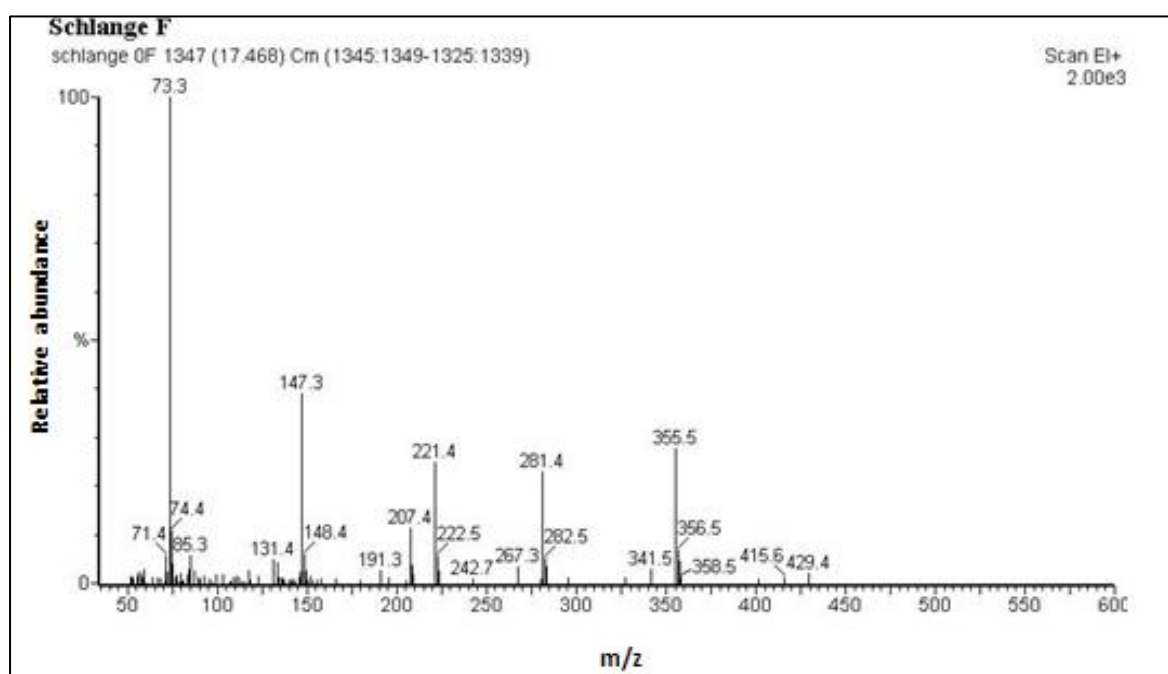


Fig.3.11: Mass spectrum of *S. moilensis* no2 at 17,47min. 13 fragments with a mass superior to 200amu were detected, although it is the scan at the same retention time as for *S. moilensis* no1.

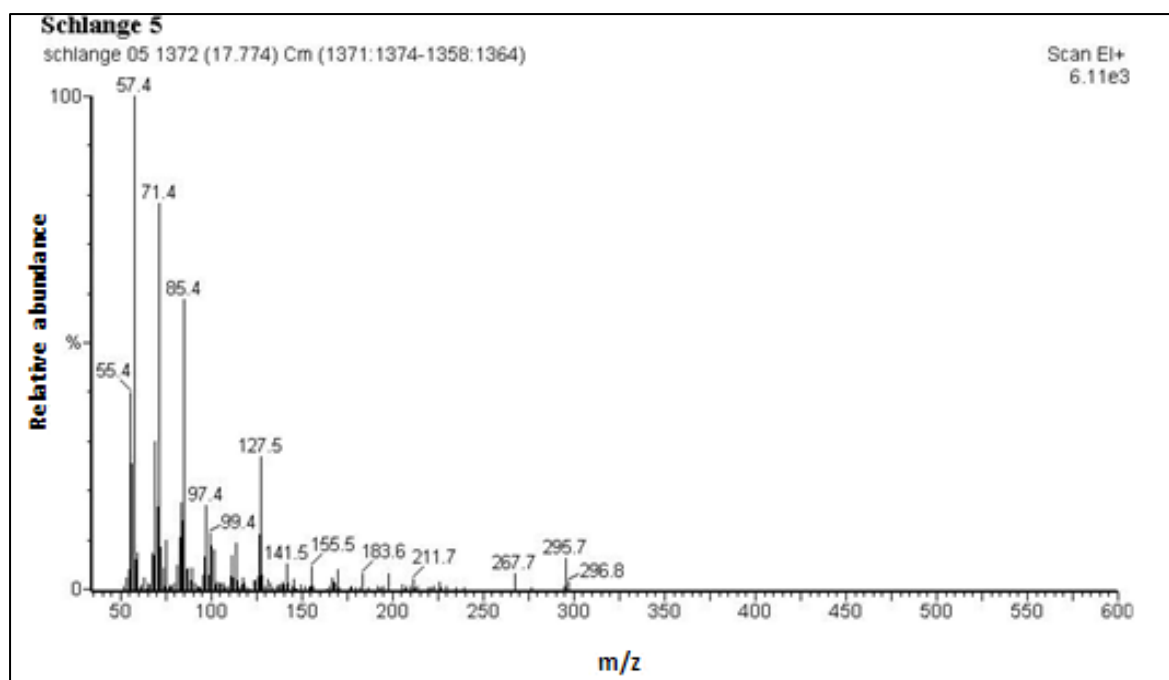


Fig.3.12: Mass spectrum of the peak at retention time 17,77min of *P. mossambicus* no1. Only three fragments with a mass-to-charge superior to 200amu were detected. Mass-to-charge 267,7 and 295,7 were found in both snakes (See below).

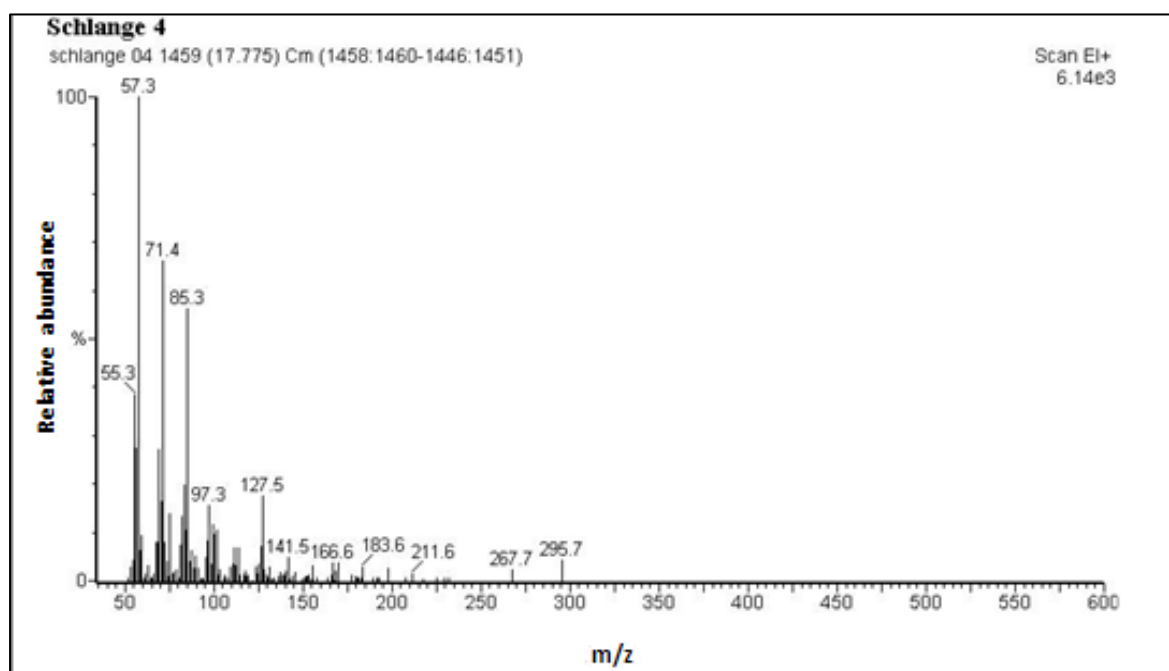


Fig.3.13: Mass spectrum of the secretion of *P. mossambicus* no2 at 17,77min. The fragments with the mass-to-charge (m/z) 267,7 and 295,7amu were also found in *P. mossambicus* no1.

3.3 RUBBING BEHAVIOUR IN PSAMMOPHIIDS

A total of 509 RB could be analysed. All RBs were performed by all snakes on the ground except once by a *S. moilensis* and once by a *P. schokari*, each one on a branch. For all snakes, RB began in a similar way: the snake stretched its body, turned its head in an almost right angle to its body and began with rubbing its throat.

The preliminary observations showed that rubbing the snakes with 70% alcohol or hand cream did not affect their rubbing behaviour. It had been expected, that the alcohol would have removed the fatty nasal gland secretion so that the snake would immediately perform RB but it was not the case for all 17 snakes. Rubbing them with hand cream seemed to elicit discomfort in the snakes, since sand and earth adhered to their body. So far, they rubbed their body against stones and branches to remove the particles of dirt.

3.3.1 ANALYSIS OF RUBBING BEHAVIOUR IN PSAMMOPHIIDS

The method of rubbing was analysed in all 17 psammophiids. Preliminary observations showed specific and individual differences. In the following the species are grouped according to their rubbing method. Only the species for which RB had not been described before are separated.

3.3.1.1 PSAMMOPHIS MOSSAMBICUS AND P. SCHOKARI

Rubbing behaviour was analysed in *P. mossambicus* and *P. schokari*. Individuals of both species used both nostrils by turns, to spread the secretion on their body. In both species rubbing usually began with 2-7 movements only on their throat, with only one nostril (**Fig.3.14A**). After this initial sequence they continued to rub their body with both nostrils alternately.

In both species one rubbing movement can be divided into three steps:

- 1) Application of the left (or right) nostril on the ventralia; back- and forwards movements on 2-3cm (**Fig.3.14B**); the belly is lifted up;
- 2) The nostril is “dragged” on the right (or left) flank (**Fig.3.14C**);
- 3) The chin has contact with the dorsum (**Fig.3.14D**) before the snake uses the other nostril (**Fig.3.14C**).

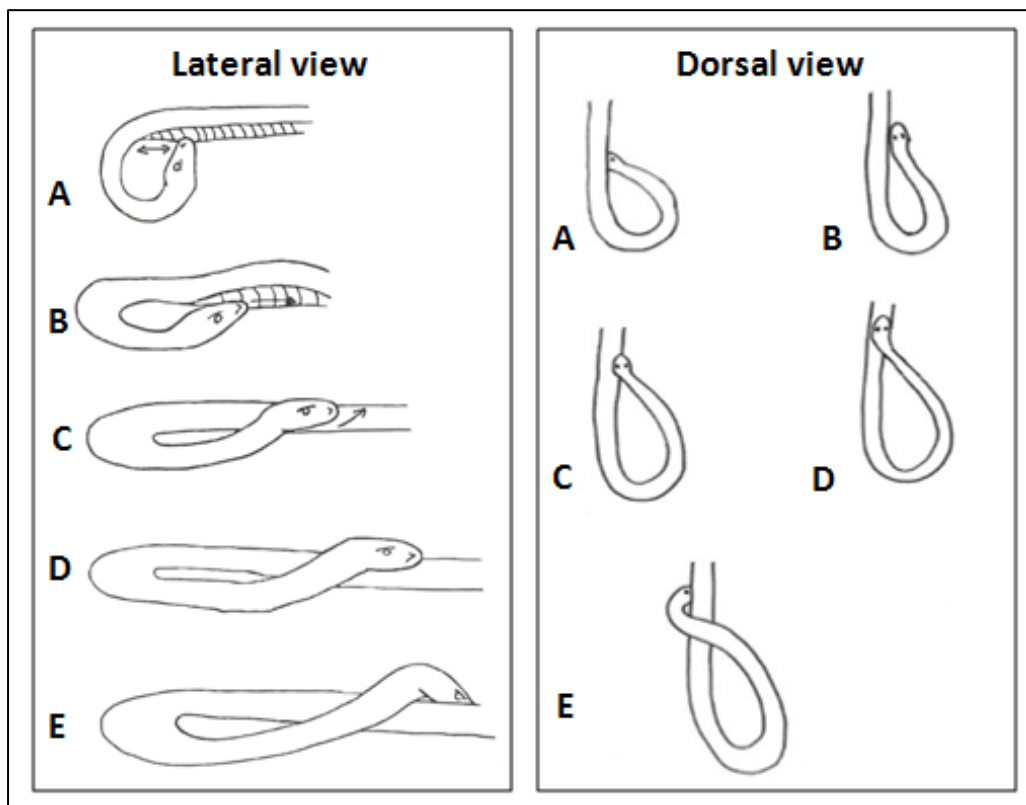


Fig.3.14: Rubbing sequence of *Psammophis elegans*, *P. mossambicus* and *P. schokari*. (I) Lateral view, (II) dorsal view. (A) First sequence, rubbing of the throat only (B) The secretion is applied on the ventralia (C) The nare is dragged along the flank (D) The chin has contact with the back (E) the second nare is used to apply the secretion on the ventralia and the flanks on the other side. Drawing based on studied videos.

Then steps 1-3 are repeated either until the cloaca or the tip of the tail. When changing the nostril, the snake “re-rubs” the end of the trace left by the other nostril just before.

Since the tail is thinner, the snake rather pulls its nostril along the last centimetres. Following a pause of 2-3 seconds, all individuals observed rubbed their throat again 1-2 times in both species (**Fig.3.14B**).

The mean duration \pm standard deviation (SD) of RB in *P. mossambicus* was $122 \pm 45,00$ seconds (n=31) and $79 \pm 21,00$ seconds (n=15) in *P. schokari*. *P. mossambicus* had a mean \pm SD of $33 \pm 5,35$ head movements (n=31) to spread the secretion on their body (**Table 3.3**) *P. schokari* a mean \pm SD of $34,07 \pm 3,10$ (n=15) (**Table 3.3**). If the snake was next to an obstacle as stone, branch, rubbing was interrupted there and not continued.

3.3.1.2 MALPOLON INSIGNITUS FUSCUS AND SCUTOPHIS MOILENSIS

Individuals of both species used only one nostril to apply the secretion on the body (**Fig.3.15**). They rubbed throat and belly (and/ or flanks) in one sequence consisting of rapid sinusoidal head movements mostly until the tip of the tail. The flanks were more or less rubbed depending on the individual observed. The nostril was also pulled along the tail since it becomes thinner. To allow rubbing, the part of the belly being rubbed was slightly lifted up (**Fig.3.15**).

Only *M. i. fuscus* rubbed sometimes its body using both nostrils one by one. There was a pause of 2-10 seconds between changing the side. The use of both nostrils was counted as one RB if the pause did not exceed 30 seconds. The entire body was rubbed within a mean \pm SD of $79 \pm 35,00$ seconds (n=8) in *M. i. fuscus* with a mean \pm SD of $119,22 \pm 41,32$ head movements (n=8) (**Table 3.3**). The mean duration \pm SD of RB in *S. moilensis* was $33 \pm 07,00$ seconds (n=24) with a mean \pm SD of $72,21 \pm 7,48$ head movements (n=24) (**Table 3.3**). As for *Psammophis*, rubbing was interrupted if there was an obstacle along the body.



Fig.3.15: Rubbing behaviour of *S. moilensis*: zigzagging head movements (black line) spread the nasal secretion mainly on the belly. This is similar in *M. insignitus fuscus*, *Psammophylax acutus acutus*, *Rhamphiophis oxyrhynchus* and *R. rubropunctatus*. (© M. Aymerich).

3.3.1.3 *PSAMMOPHIS ELEGANS*, *PSAMMOPHYLAX ACUTUS ACUTUS*, *RHAMPHIOPHIS ROSTRATUS* AND *R. RUBROPUNCTATUS*

Rubbing behaviour could be observed for the first time in *Rhamphiophis rubropunctatus*, in *R. rostratus*, in *P. a. acutus* and in *P. elegans*. *Rhamphiophis* and *Psammophylax* performed RB with only one nostril in a movement similar to the one found in *Malpolon* and *Scutophis* (Fig.3.15), but the distance between the wavelengths of the sinusoidal head movement was larger. As in *M. i. fuscus*, the individual of *R. rostratus* observed rubbed sometimes its body twice using one by one each nostril until the tip of the tail (Table 3.3). In both species rubbing of the flanks was more distinctive than in *Malpolon*. RB lasted about $154 \pm 39,00$ seconds in *R. rubropunctatus* made with a mean \pm SD head movements of $66,11 \pm 17,07$ (n=9); in *R. rostratus* it lasted $134 \pm 36,00$ seconds made with a mean \pm SD head movements of $51 \pm 17,89$ (n=5) (Table 3.3).

In *P. a. acutus* the mean \pm SD duration of RB was 125 ± 88 seconds and the mean \pm SD head movements was $25,11 \pm 6,85$ (n=9). The head movements were less distinct than in the other mentioned genera (Table 3.3). Both individuals rubbed very often their body not completely. They rubbed e.g. 5cm of their midbody and two hours later another part of their body. This little rubbing sequences were not

considered as RB, since they did not rubbed their body in one movement at least until their cloaca.

RB in *P. elegans* was done on the same way as in *P. mossambicus* and *P. schokari* with both nostrils alternating to apply the nasal gland secretion (see **Fig.3.14**). The mean \pm SD duration of RB was $170 \pm 10,00$ seconds with a mean \pm SD head movements of $44,60 \pm 8,35$ (n=8) (**Table 3.3**).

Table 3.3
Analysis of RB: Duration, number of head movements and methods used in psammophiids.

Species	Nb. of individual observed	Nb. of observations	Mean duration (sec.)	Standard deviation (sec.)	Mean nb. of head movements	Standard deviation (sec.)	Method	RB until
<i>Psammophis elegans</i>	1	8	170	± 10	44,60	± 8,35	A	1
<i>Psammophis mossambicus</i>	6	31	122	± 45	32,85	± 5,54	A	1&2
<i>Psammophis schokari</i>	4	15	79	± 21	34,07	± 3,10	A	2
<i>Scutophis moilensis</i>	4	24	33	± 07	72,21	± 7,48	B	1
<i>Malpolon insignitus fuscus</i>	1	8	79	± 35	119,22	± 41,32	C	1
<i>Psammophylax acutus acutus</i>	1	9	65	± 88	25,11	± 6,85	C	2
<i>Rhamphiophis rubropunctatus</i>	1	9	154	± 39	64,11	± 17,07	B	1&2
<i>Rhamphiophis rostratus</i>	1	5	134	± 36	51,00	± 17,89	C	1&2

Method: A= both nostrils alternate; B= only one nostril; C= each nostril once, one by one.
RB until: 1= Tip of the tail; 2= Cloaca.

3.3.2 RUBBING BEHAVIOUR AS MARKING BEHAVIOUR

In the first part of this experiment, the frequency of rubbing of the first day of an experimental week (five days) was compared to the mean frequency of the following days. The rubbing frequency was not significantly higher on the first day than on the following ones in the 15 psammophiids tested (overall paired sample t-test, $T_{14} = -0,158$; $N=15$, $p=0,877$).

The second part of the experiment was conducted to see whether the secretion leave an informative trail to conspecifics. On the glass plate previously placed into the middle of the terrarium to see whether the secretion could be seen, nothing was visible for all individuals of both species studied, even with back light.

The analysis of the video tapes showed that all snakes of both species performed at least one RB during their control run and during their second test. Only *S. moilensis* no13 did never perform RB. All snakes crawled mainly along the terrarium's walls but no traces of secretion were seen on the blotting paper. All snakes crossed at least once the glass plate, and the snake placed at second did also cross the plate without paying any attention to it, although the first snake had cross the plate.

For the both "couple" studied it made no difference which snake was placed first. The second snake did not behaved differently than in its control run and no differences in the behaviour of the males when placed after the females. When the females were placed after the males, no differences could be seen either.

Table 3.4

Number of RB performed by the snake when placed at first or at second into the terrarium and main direction taken first or after another snake had been previously placed. The last position recorded is where the snake was when the experiment was finished. Most of them were under the stone (J5).

Snake no	Nb. of RB when placed at first	Nb. of RB when placed at second	Mean crawling direction when at first	Mean crawling direction when at second
<i>S. moilensis</i> no11	2	2	P12-A1-P1-P12-A12-A1-P1-P12-J4	P12-P1-A1-P12-P1-A1-A12-P12
<i>S. moilensis</i> no12	1	2	P12-A1-P1-P12-A1-P1-P12-A1-J5	P12-A12-A1-P1-A12-P12-A1-M8
<i>S. moilensis</i> no13	-	-	P12-A1-P1-P12-A12-A1-P1-A12-A1-J4	P12-P1-A1-P1-A12-P12-P1-J5
<i>S. moilensis</i> no14	1	1	P12-A1-P1-P12-A1-P1-P12-A1-F7	P12-A12-A1-P1-P12-P1-A1-P1-J5
<i>P. mossambicus</i> no1	1	1	P12-P1-A1-P12-A12-A1-P1-A12-A1	P12-A1-A12-P12-P1-A1-H12
<i>P. mossambicus</i> no2	1	2	P12-P1-A1-A12-P12-P1-O5	P12-P1-A12-A1-P1-P12-A12-A7
<i>P. mossambicus</i> no3	1	-	P12-A12-A1-P12-P1-A1-A12-P12-P1	P12-A12-A1-P1-P12-A1-P1-J5
<i>P. mossambicus</i> no4	1	1	P12-P1-A1-A12-P12-P1-A1-A12-L12	P12-P1-A1-P12-A12-A1-P12

3.3.3 FREQUENCY OF RUBBING BEHAVIOUR DEPENDS ON TEMPERATURE

The dependency of temperature on RB frequency was tested. The rubbing frequency increased linearly with increasing temperature in psammophiids (rank Spearman correlation test: $r_2=0,315$; $N=354$; $p=0,000$) (Fig. 3.16).

The within species analysis showed a correlation between temperature and rubbing frequency in *P. mossambicus* ($r_2=0,418$; $N=136$; $p=0,000$) and *P. schokari* ($r_2=0,363$; $N=78$; $p=0,000$) (Fig. 3.17A and B). No dependence of temperature on rubbing frequency was seen in *M. insignitus fuscus* ($r_2=0,222$; $N=27$; $p=0,266$), *S. moilensis* ($r_2=0,161$; $N=66$; $p=0,196$) (Fig. 3.17C and D) and *P. acutus acutus* ($r_2=0,100$; $N=47$; $p=0,504$) (Fig 3.17E).

A dependence of temperature on rubbing frequency was seen in snakes no2, no3, no4, no5, no6, no12 (**Table 3.5**); snake no8 and no9 showed p-values of $p=0,061$ and $p=0,054$ respectively, indicative of a tendency for the dependence of rubbing frequency on temperature (**Table 3.5**).

No dependence of temperature on rubbing frequency was seen in snakes no1, no7, no10, no11, no13, no14, no15, no16 and no17 (**Table 3.6**). All individual graphs can be seen in **Appendix 8.5**.

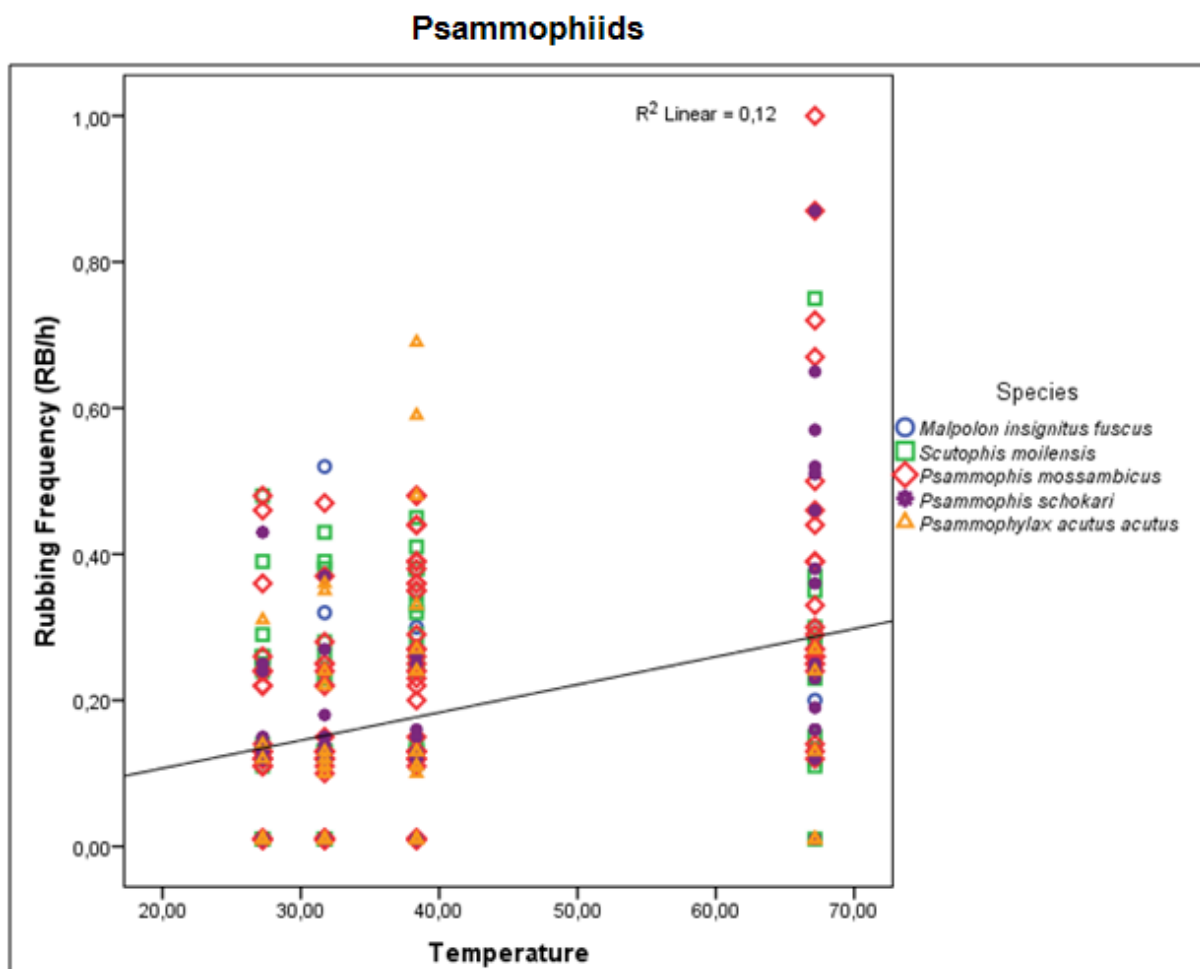


Fig. 3.16: Dependency of temperature on rubbing behaviour in psammophiids studied. The overall analysis of the 17 snakes studied showed that frequency of rubbing behaviour was depending on temperature.

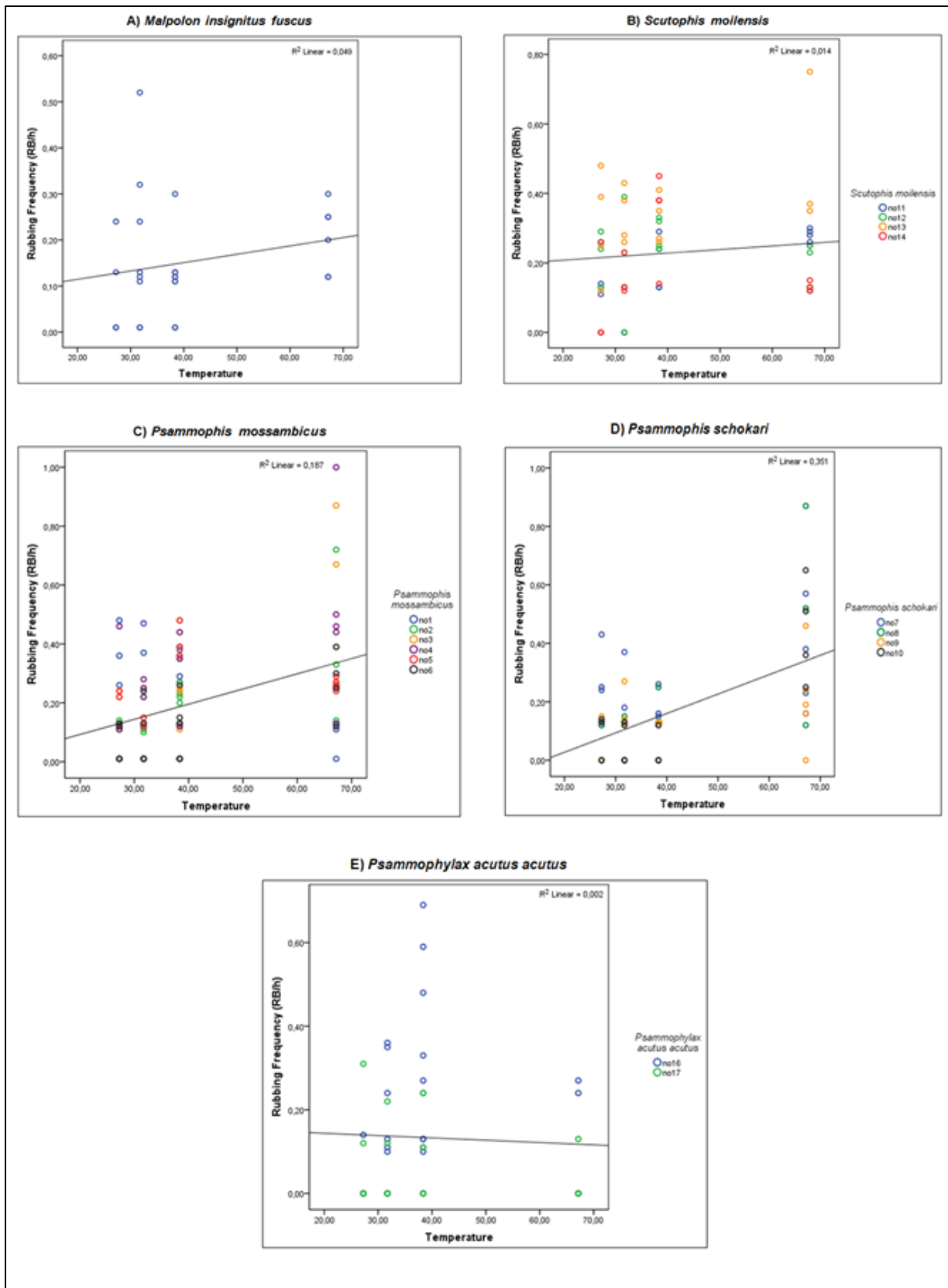


Fig.3.17A-E: Dependency of temperature of rubbing behaviour in psammophiids. **(A)** *Malpolon insignitus fuscus* **(B)** *Scutophis moliensis* **(C)** *Psammophis mossambicus* **(D)** *Psammophis schokari* **(E)** *Psammophylax acutus acutus*. All species but *Psammophylax acutus acutus* showed dependency of temperature. The graphs more detailed can be consulted in **Appendix 8.5**.

Table 3.5

Rank Spearman correlation test showing the dependence of temperature on rubbing frequency in eight individuals of psammophiids. Whilst in these individuals of *Psammophis mossambicus* and *Scutophis moilensis* the dependence is clear, a tendency to dependence could be noted in these individuals of *Psammophis schokari*.

Species	Snake no	$r_2=$	N=	p=
<i>Psammophis mossambicus</i>	2	0,696	26	0,000
	3	0,553	23	0,006
	4	0,547	24	0,006
	5	0,585	22	0,004
	6	0,476	21	0,029
<i>Scutophis moilensis</i>	12	0,623	16	0,010
<i>Psammophis schokari</i>	8	0,438	19	0,061
	9	0,437	20	0,054

Table 3.6

Rank Spearman correlation test showing no dependence of temperature on rubbing frequency in nine individuals of psammophiids. In all individual, the p-values clearly indicate no dependence on temperature.

Species	Snake no	$r_2=$	N=	p=
<i>Psammophis mossambicus</i>	1	-0,250	20	0,287
<i>Psammophis schokari</i>	7	0,175	20	0,460
	10	0,382	19	0,106
	11	-0,213	17	0,412
<i>Scutophis moilensis</i>	13	0,318	16	0,229
	14	0,059	17	0,822
	15	0,222	27	0,266
<i>Malpolon insignitus fuscus</i>	15	0,222	27	0,266
<i>Psammophylax acutus acutus</i>	16	0,279	25	0,177
	17	-0,104	22	0,644

3.4 MORPHOLOGICAL DIVERGENCES BETWEEN *MALPOLON MONSPESSULANUS* AND *SCUTOPHIS MOILENSIS*

The preparation of the *Malpolon monspessulanus* should be done more carefully than the one of the *Scutophis moilensis* since they were rigid due to their stay in alcohol; an adequate stretching of the skin was not possible. The X-ray analysis showed that the ribs in the neck region of *Scutophis moilensis* seemed thinner and longer than in *M. monspessulanus*. The tuning fork shaped form visible on **Fig.3.18** is the hyoideum (lingual bone) (see **Fig.3.29**). It does not possess any articulation to other bones and is in snakes rather cartilaginous than bony (LÜDICKE, 1962).

To confirm this, the 20 first ribs were removed in both species and prepared by dissection to be measured (**Fig.3.18** to **Fig.3.25**). This showed that the left and right neck ribs in *S. moilensis* were about 3mm longer than in *M. monspessulanus* (**Table 3.7** and **Table 3.8**). These neck ribs in *S. moilensis* were also a bit wider than in *M. monspessulanus* (**Table 3.9** and **Table 3.10**). Whilst sampling of the ribs of *M. monspessulanus*, the bones seemed to be more solid than those of *S. moilensis*.

Furthermore, careful examination of the individuals of *Scutophis moilensis* showed that most of them had a central little depression on their frontal plate or on the parietal ones, corresponding to the pits described by UNDERWOOD (1967) and DE HAAN (2003b) (**Fig.3.27**).

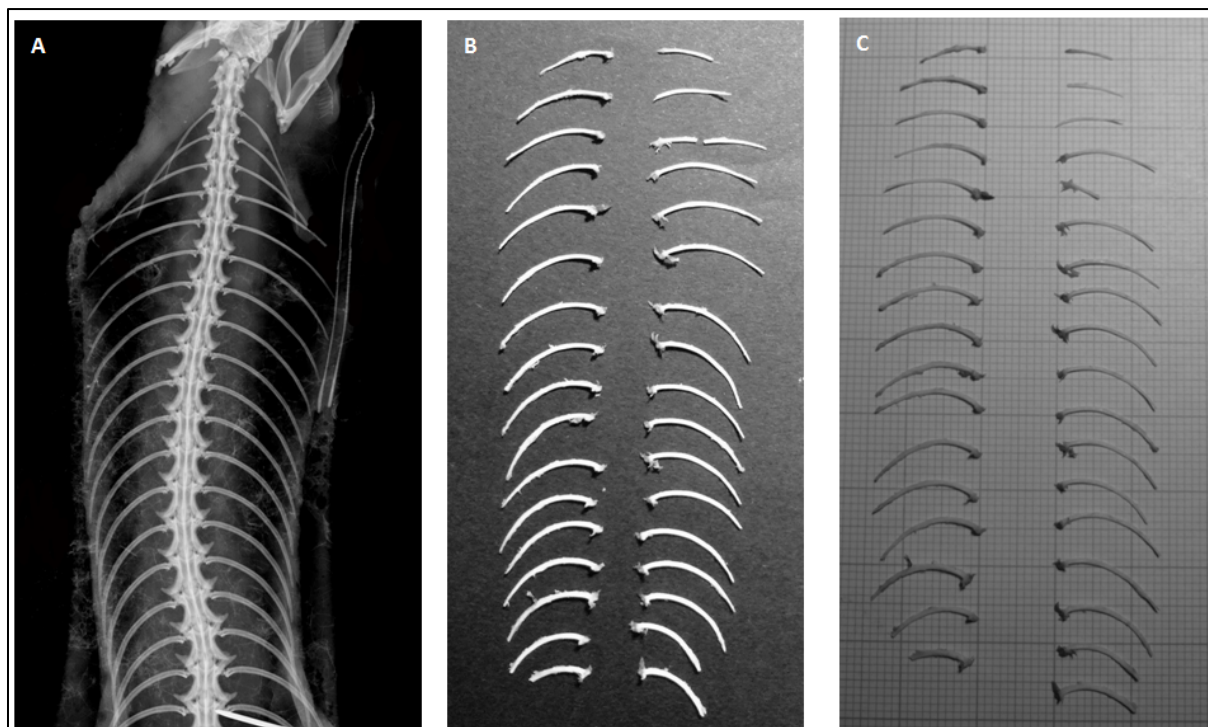


Fig.3.18: *S. moilensis* no1. **(A)** Radiography of the upper part of the body; **(B)** prepared ribs; **(C)** prepared ribs for measurement on graph paper.

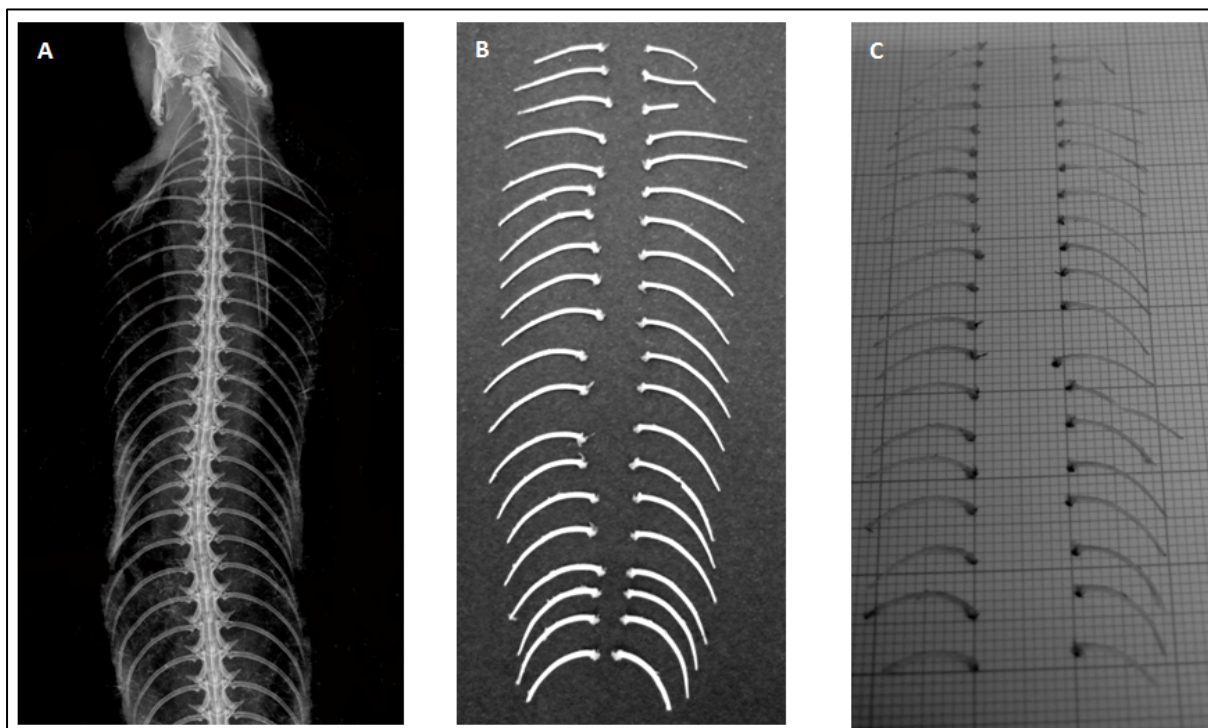


Fig.3.19: *S. moilensis* no2, with the radiography on **(A)** the prepared ribs **(B)** and the prepared ribs for measurement on graph paper **(C)**.

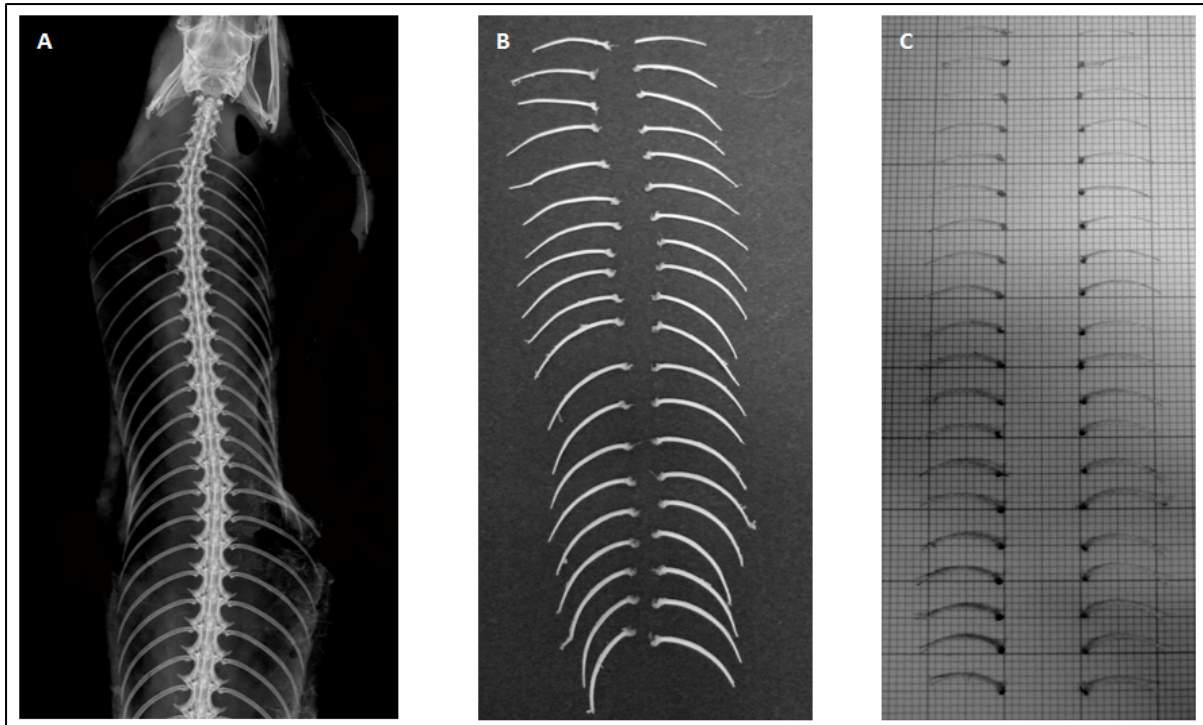


Fig.3.20: *S. moilensis* no3, with the radiography of the forepart of the body on (A). The ribs were prepared (B) and measured on graph paper (C).

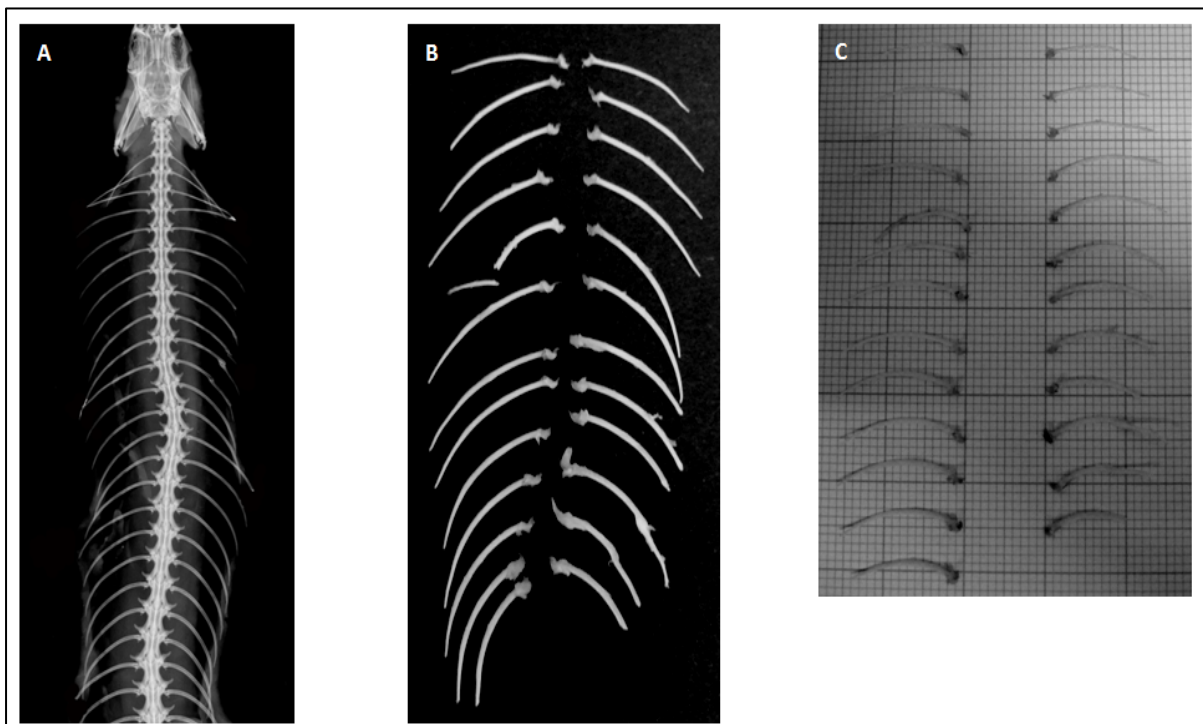


Fig.3.21: *S. moilensis* no4, with the radiography on (A). (B) prepared ribs; (B) same ribs measured on graph paper (C).

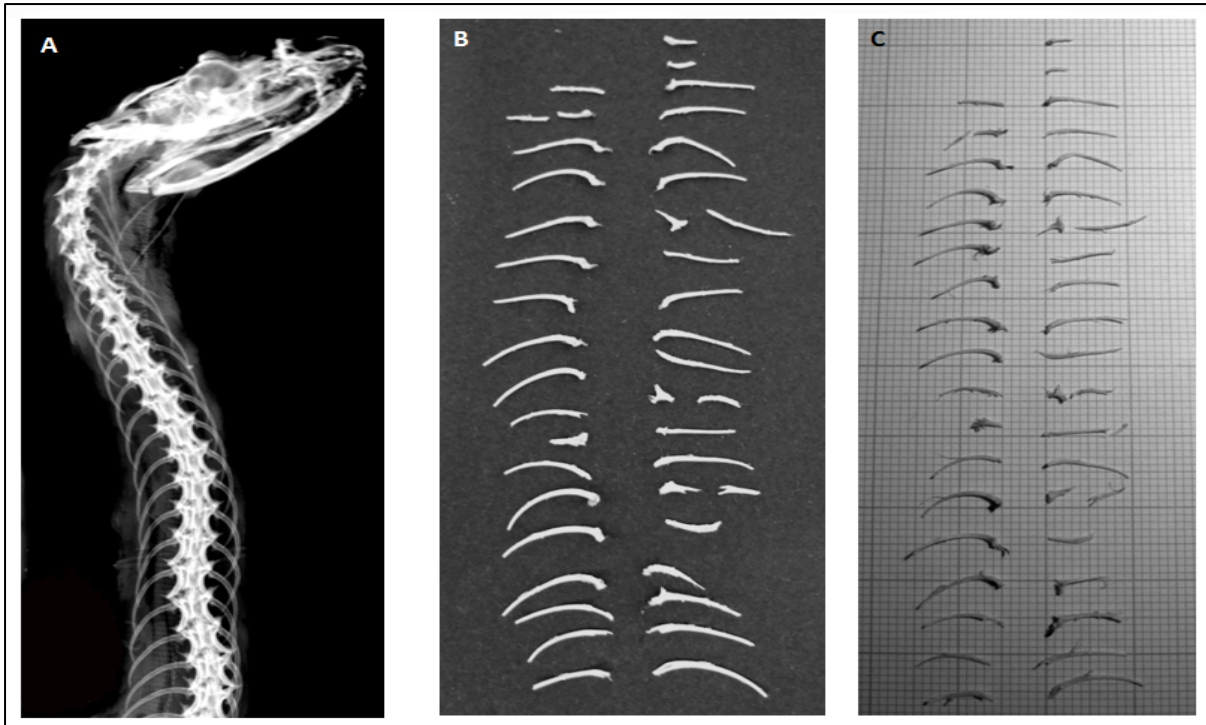


Fig.3.22: *M. monspessulanus* ZFMK 23060. **(A)** X-ray picture. Due to the conservation in alcohol, it was not possible to “stretch” the snake adequately for the picture. **(B)** Prepared ribs: the first and second ribs on the left side are missing; **(C)** measurement of the ribs.

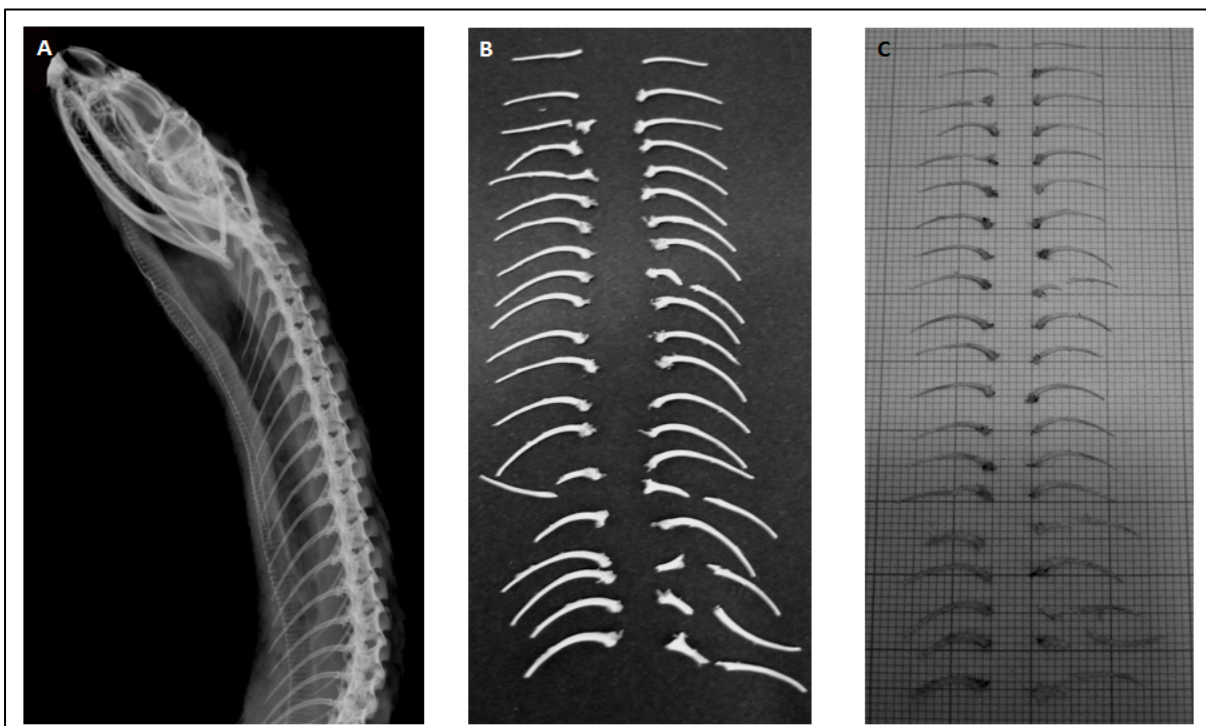


Fig.3.23: *M. monspessulanus* ZFMK 23061. **(A)** Radiography; **(B)** the prepared ribs; **(C)** ribs placed on graph paper for measurement.

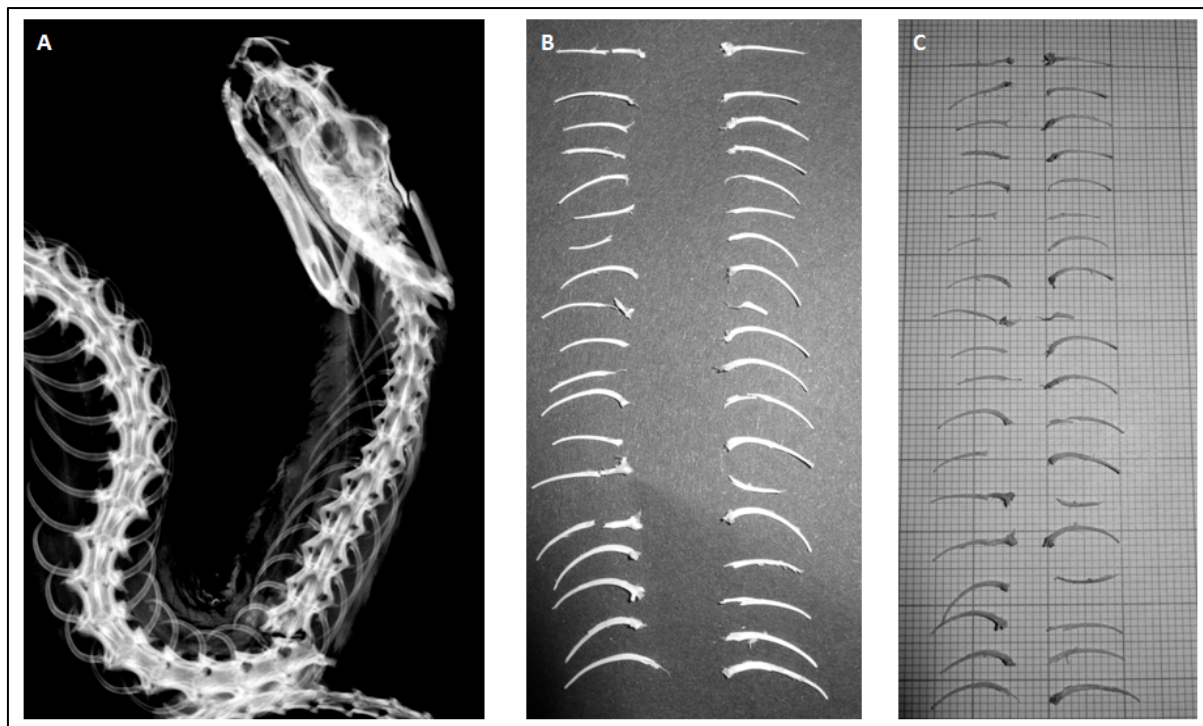


Fig.3.24: *M. monspessulanus* ZFMK 23062. **(A)** Radiography; **(B)** prepared ribs for the measurement on **(C)**.

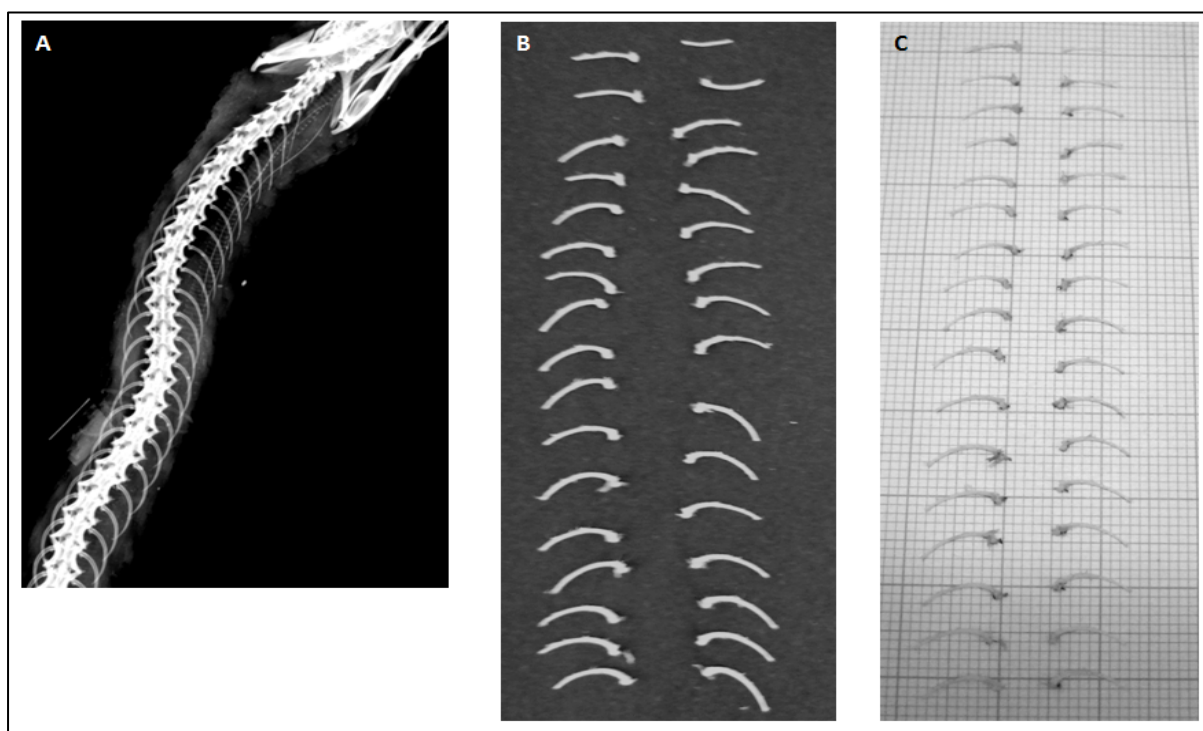


Fig.3.25: *M. monspessulanus* ZFMK 89766. **(A)** Radiography; **(B)** prepared ribs **(C)** measurement.

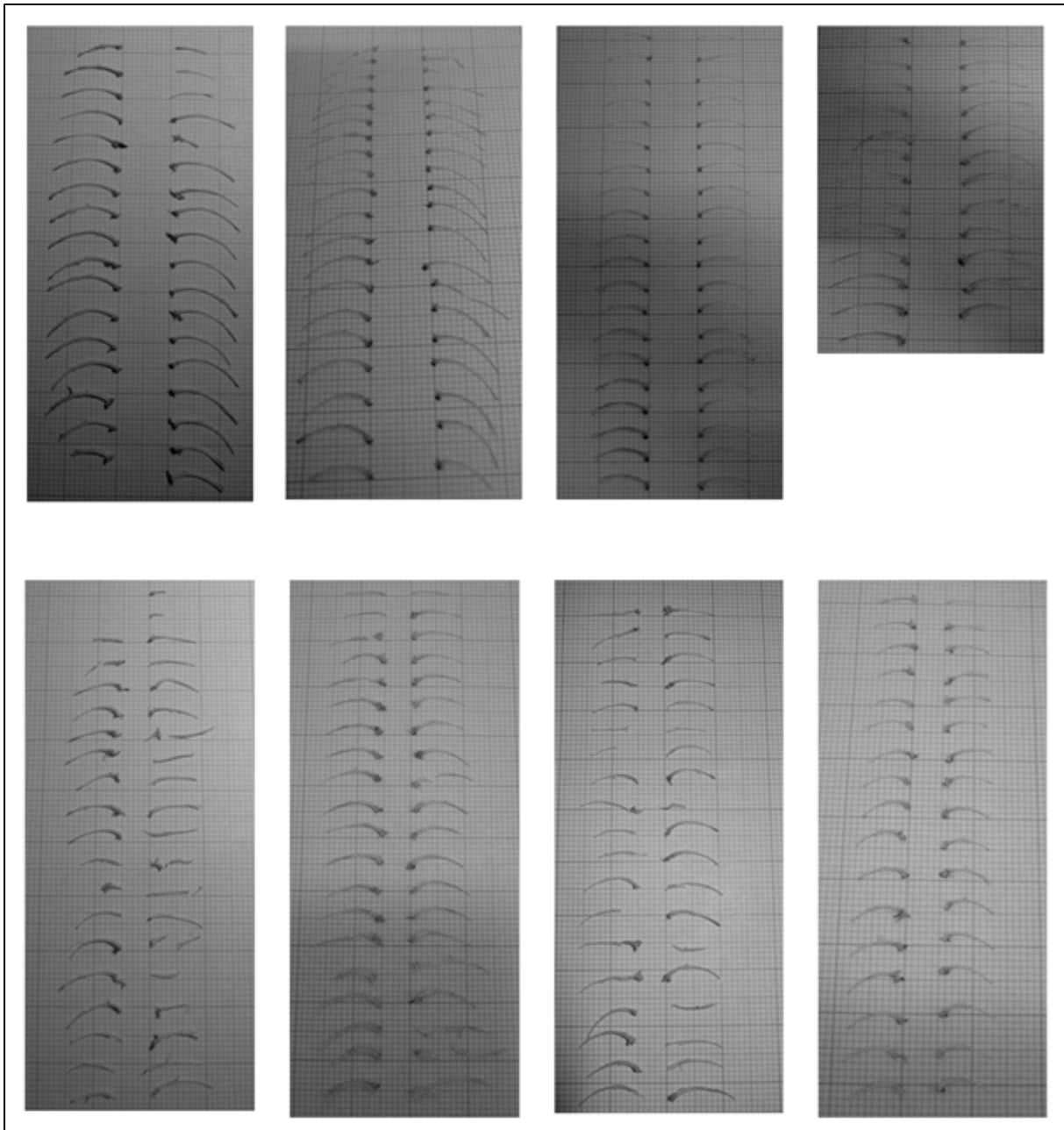


Fig.3.26: Direct comparison showing that the ribs of *Scutophis moilensis* (above) are longer than those of *Malpolon monspessulanus* (below) thanks graph paper.

Table 3.7
Mean value of the length of the neck ribs of *Scutophis moilensis*. SD= Standard deviation.

Snake	Mean value left neck ribs (mm) ± SD	Mean value right neck ribs (mm) ± SD
<i>Scutophis moilensis</i> no1	13,19 ± 1,22	13,13 ± 1,36
<i>Scutophis moilensis</i> no2	11,42 ± 1,12	11,35 ± 1,35
<i>Scutophis moilensis</i> no3	10,81 ± 0,83	10,64 ± 0,93
<i>Scutophis moilensis</i> no4	14,55 ± 1,21	13,78 ± 1,72
Overall mean value ± SD for all <i>Scutophis moilensis</i>	12,27 ± 1,76	12,03 ± 1,76

Table 3.8
Mean value of the length of the neck ribs of *Malpolon monspessulanus*. SD= Standard deviation.

Snake	Mean value left neck ribs (mm) ± SD	Mean value right neck ribs (mm) ± SD
<i>Malpolon monspessulanus</i> ZFMK23060	10,74 ± 1,28	10,65 ± 0,99
<i>Malpolon monspessulanus</i> ZFMK 23061	10,00 ± 0,73	10,10 ± 0,72
<i>Malpolon monspessulanus</i> ZFMK 23062	9,74 ± 0,56	10,21 ± 0,54
<i>Malpolon monspessulanus</i> ZFMK 89766	7,29 ± 0,69	7,53 ± 0,62
Overall mean value ± SD for all <i>Malpolon monspessulanus</i>	9,51 ± 1,52	9,70 ± 1,40

Table 3.9

Mean value of the width of the neck ribs of *Scutophis moilensis*. SD= Standard deviation.

	Mean value left neck ribs (mm) ± SD	Mean value right neck ribs (mm) ± SD
<i>Scutophis moilensis</i> no1	0,61 ± 0,04	0,52 ± 0,01
<i>Scutophis moilensis</i> no2	0,41 ± 0,04	0,35 ± 0,02
<i>Scutophis moilensis</i> no3	0,41 ± 0,03	0,38 ± 0,05
<i>Scutophis moilensis</i> no4	0,50 ± 0,02	0,51 ± 0,03
Overall mean value ± SD for all <i>Scutophis moilensis</i>	0,47 ± 0,10	0,43 ± 0,12

Table 3.10

Mean value of the width of the neck ribs of *Malpolon monspessulanus*. SD= Standard deviation.

	Mean value left neck ribs (mm) ± SD	Mean value right neck ribs (mm) ± SD
<i>Malpolon monspessulanus</i> ZFMK 23060	0,51 ± 0,03	0,47 ± 0,04
<i>Malpolon monspessulanus</i> ZFMK 23061	0,44 ± 0,02	0,42 ± 0,02
<i>Malpolon monspessulanus</i> ZFMK 23062	0,37 ± 0,02	0,36 ± 0,02
<i>Malpolon monspessulanus</i> ZFMK 89766	0,46 ± 0,02	0,48 ± 0,05
Overall mean value ± SD for all <i>Malpolon monspessulanus</i>	0,45 ± 0,09	0,44 ± 0,09

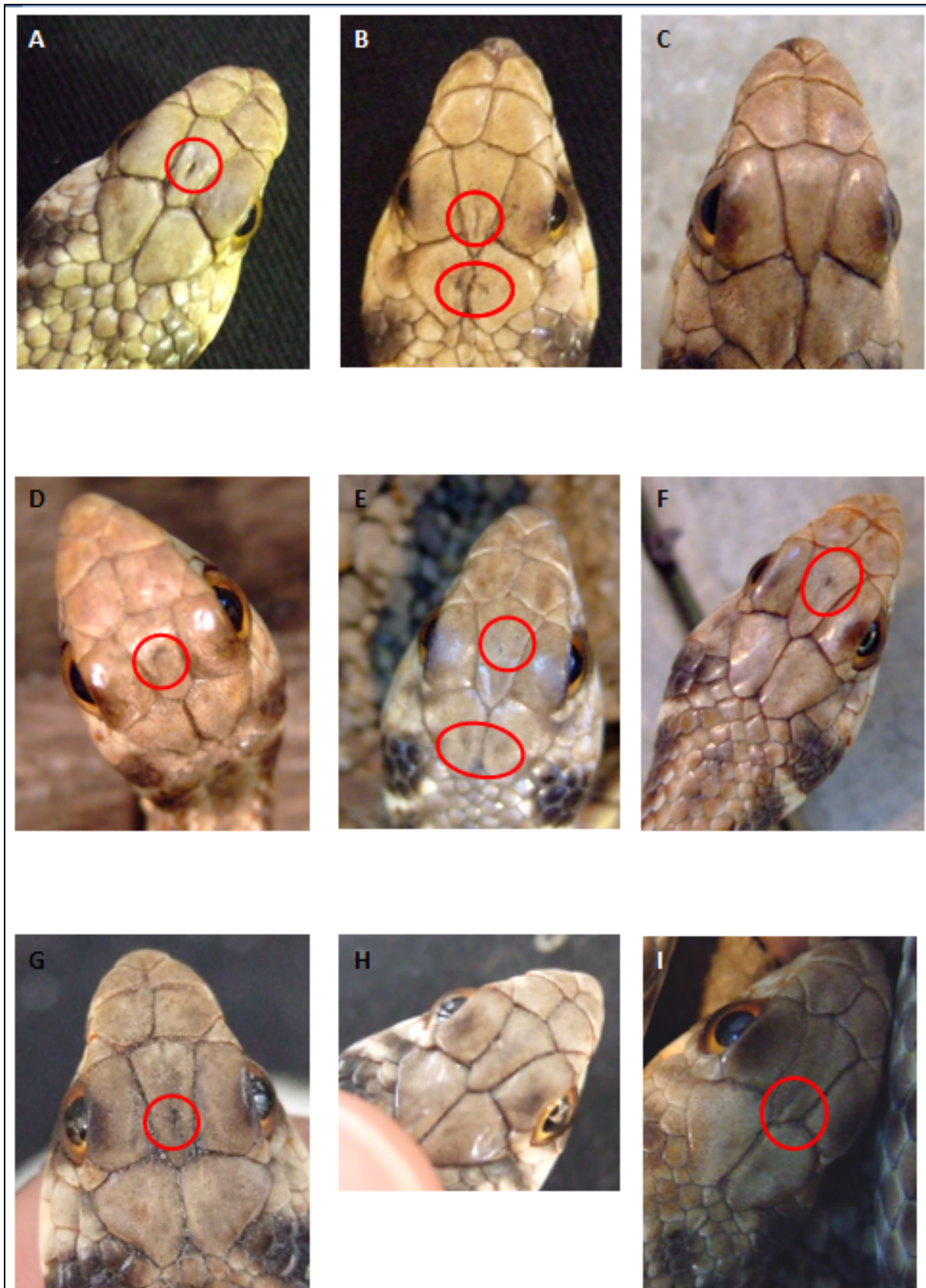


Fig.3.27: Pits (red circles) found on the frontal scales of several individuals of *Scutophis moilensis*. Snakes **C** and **H** did not present these depressions. Snakes **B** and **E** also have pits on their parietal shields.

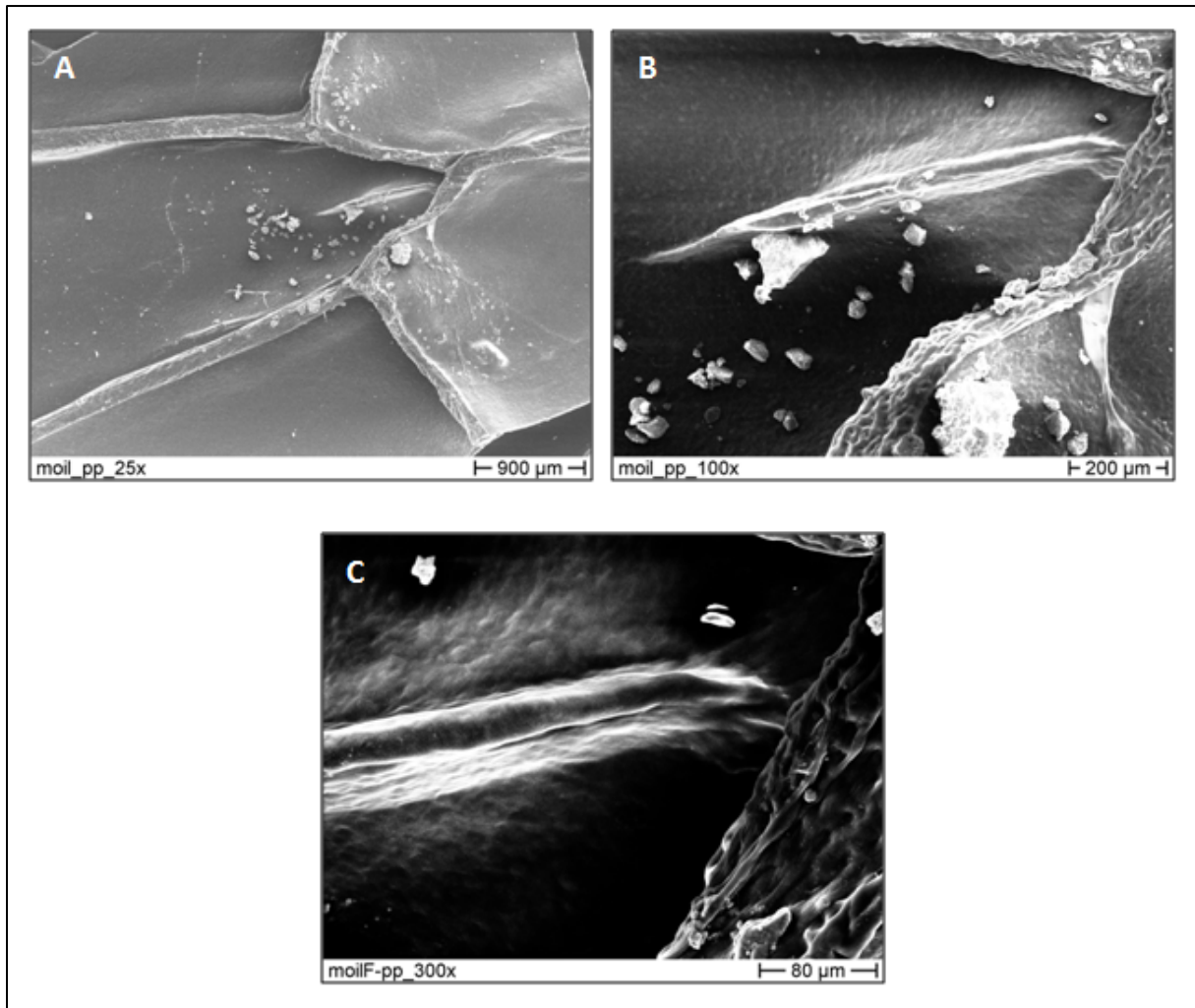


Fig. 3.28: SEM picture of the pit on the frontal scale of the shed skin of the *Scutophis moilensis* I on **Fig. 3.27**. The part being on the side of the skull has been photographed. UNDERWOOD (1967) saw the head pits serving as radiation receptors.

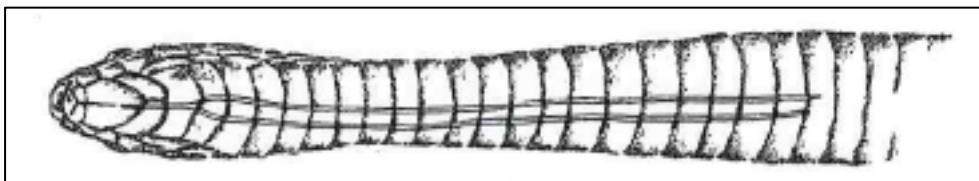


Fig. 3.29: Position of the hyoideum in *Dasypeltis scabra* (after Lüdicke, 1962).

4. DISCUSSION

4.1 SCANNING ELECTRON MICROSCOPY

Hypothesis: The scale ultrastructure of psammophiids differs from those of other snakes.

The SEM analysis of the scale structure revealed that pores are absent of the surface of dorsal scales of psammophiids and of control taxa. So far, the statement of CHIASSON et al. (1989) that all snakes have pores on their dorsal scales allowing the passage of lipids to the surface could not be verified in this study. Only *Nerodia rhombifera* and *Natrix natrix* showed a structure similar to a porous one. Several remarks have to be added to these results:

- When removing the oberhautchen from the scale, no imprints of “pores” were present on the scale surface speaking for a connection between the epidermal layers and the surface of the skin.
- Ultrathin sections for transmission electron microscopy showed clearly that there is no connection (e.g. channels) between the pores and the lipid secreting layers of the dermis (AMEMIYA et al., 1996; ALIBARDI, 2005; EBERT, 2007).
- Studies done with scaleless snakes showed that even these snakes are optimally protected against desiccation although they do not have scales (LICHT and BENNETT, 1972; BENNETT and LICHT, 1975; ROBERTS and LILLYWHITE, 1980).

GARSTKA and CREWS (1981) showed that lipid-rich areas are (also) situated in vesicles located in the dermal muscle, “[...] concentrated in the regions between the scales and adjacent to the dermal vascular bed” or within the mesos layer. They proposed that lipids reach the surface by little ruptures in the skin between the scales. Muscular contraction would press the lipids outwards, a mechanism described in *Natrix tigrina* (NAKAMURA, 1935), in *Diplodactylus* (ROSENBERG and RUSSELL, 1980) and *Macropisthodon* (SMITH, 1938) allowing these snakes to extrude secretions of ductless dermal glands.

IRISH et al. (1988) also wrote that lipids would reach the surface through pores present in the epidermis, but 11 years later, HAZEL et al. (1999) showed that some *pits* found on the scales of *Boa constrictor* and *Morelia spilotes* were in fact *continuous pores* since they permitted in an experiment the passage of viscous liquid.

Therefore the morphology of these “depressions” or “pits” themselves should be clearly defined since HAZEL et al. (1999) and EBERT (2007) already noted morphological differences for the “pores” analysed. On the one hand we would have the *pores*, larger in diameter, for the passage of substances (as described by HAZEL et al.) and on the other hand we would have the *pits* (or micropits to avoid any confusion with the *pits* described by UNDERWOOD (1967), **Chapter 3.4**), parts of the microornamentation of the scale as analysed by AMEMIYA et al., ALIBARDI and EBERT (op. cit. above) (**Fig.4.1**).

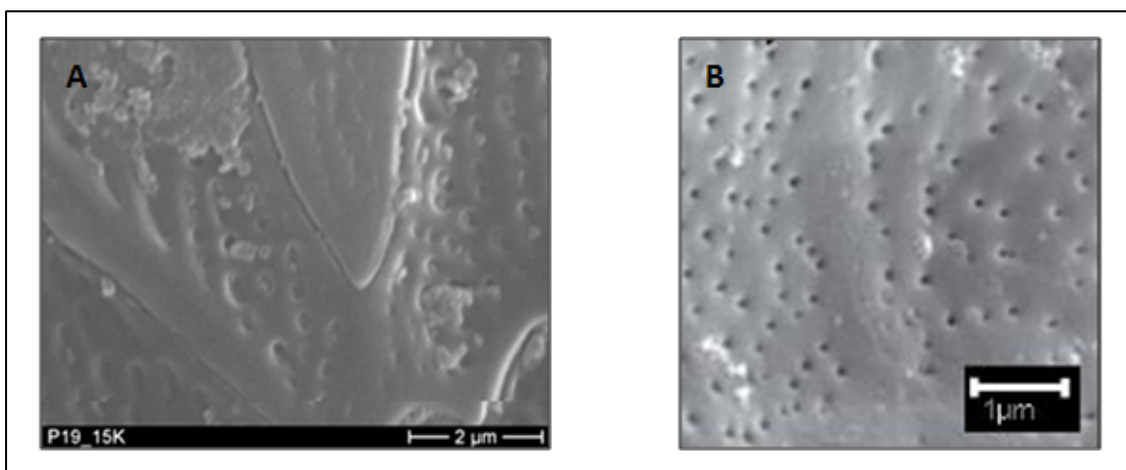


Fig.4.1: (A) Scale of *Natrix natrix* (15000x), (B) Scale of *Corallus hortulanus* (similar magnification). On scale (A), the depressions would correspond to pits, on scale (B) they would correspond to pores. ((B) ©Ebert et al., 2006).

Possibly we would have then two excretive mechanisms existing conjointly: The first would allow the excretion of substances through *visible pores* on the scale surface, the second through little skin ruptures for snakes having *pits* on their scales.

The question of the function of the microdermatoglyphics (or microstructures) of snakes' scales has been subject of earlier discussions and has never been clearly resolved. They have been proposed:

- To be a facilitation in shedding (MADERSON, 1966);
- To be a minimisation of friction (STEWART and DANIEL, 1973);
- To allow a smooth gliding (HAZEL et al., 1999);
- To help the conversion of harmful solar radiation to heat (PORTER, 1967);
- To be important for the detection of infrared radiation in pit organs (CAMPBELL et al., 1999);
- To serve in retention or dispersal of pheromones (SMITH et al., 1982; CHIASSON and LOWE, 1989);
- That they create air layer on the scale surface improving the resistance against water loss (LILLYWHITE and MADERSON, 1982);
- To have a taxonomical value (PICADO, 1931; DOWLING et al., 1972; BEA, 1978, 1987; PRICE, 1982; PETERSON, 1984; PETERSON and BEZY, 1985; BEA and FONTARNAU, 1986; BRANDSTÄTTER, 1995; JOSEPH et al., 2007);
- To be an indicator for the snake's ecology (JOSEPH et al., 2007);
- And BEA (1978) noted that the more meridional a species, the more complex its microornamentation, likely a high adaptation to evaporation.

Following the results of the present study, microdermatoglyphics in psammophiids seem rather to be an indicator for the habitat of the species studied than being used only for taxonomy since two or three genera share here a similar pattern (see **Table 3.1**):

- 1) The scalar pattern: *Psammophis schokari*, *Scutophis moilensis*, *Malpolon insignitus fuscus*, *M. monspessulanus*, and *Dipsina multimaculata* are inhabitant of arid and semi-arid habitat (for *Malpolon* see DE HAAN, 1999);
- 2) The cup-like flat pattern: *Mimophis mahfalensis*, diverse species of *Psammophis*, and *Psammophylax rhombeatus* are inhabitant of moist and drier savannas;
- 3) The caniculate pattern: *Rhamphiophis sp.* often burrowing in sandy ground;

- 4) The cup-like ridge pattern: *Hemirhagerrhis sp.* and *Psammophylax sp.* living in drier regions.

Already HOGE and SOUZA-SANTOS (1953) noted differences in the shape of microdermatoglyphics among species of the same genus and that different genera shared the same pattern: Within their studied genera, *Constrictor* and *Xenoboa* showed the same scale pattern, *Eunectes* and *Epicrates* as well.

In regards of the RB, the delicate sculptured microornamentation would then serve to the retention of the nasal gland secretion on the scales' surface. Sand Snakes would then be protected "passively" thanks to the lipids extruded through the skin and "actively" through to the nasal gland secretion. The use of RB will then enable the snake to determine the appropriate moment when the protective action of the secretion can be used. This double protection would not only confer them their extraordinary resistance to water loss but also be an advantage to the snake allowing being active during the hottest hours of the day, when other predators escape heat.

Only the scale structure of *M. i. fuscus* and *M. m. monspessulanus* was different from the dorsal scales of the other psammophiids analysed since they had a medial groove. This medial groove could not be seen in *Scutophis moilensis* although it has been reported for this species by GRUBER (1989). SEM pictures showed that this surface is composed of several parallel ridges similar to the other ones on the scale surface (**Fig.4.2**).

It is known for *Nerodia rhombifera* (and other aquatic snake species) that the medial ridge of their scales improve their traction (MATTISON, 2007). The possible function of the grooves in *Malpolon* -the retention of the secretion allowing conspecific recognition- (DE HAAN, 2003a) was never experimentally studied.

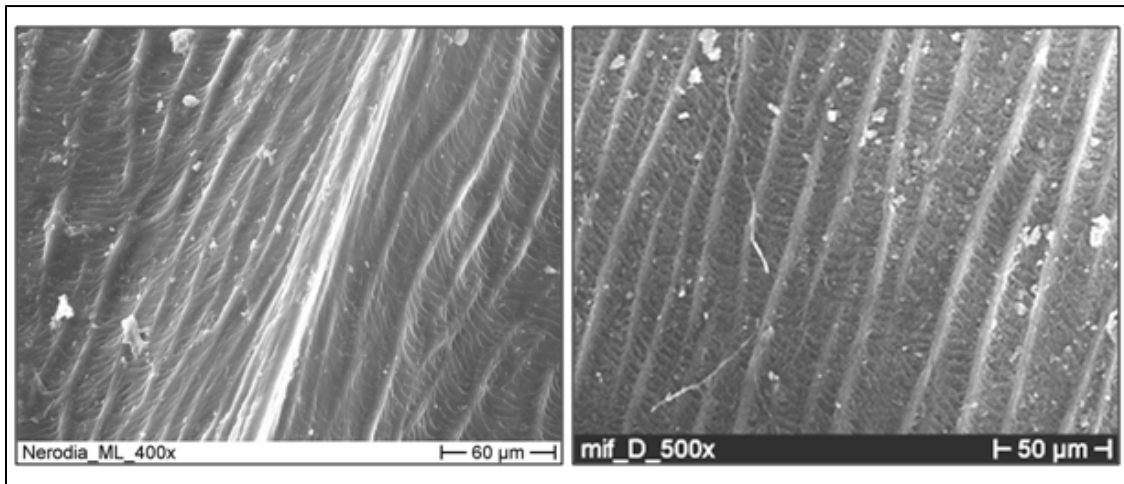


Fig.4.2: Similarities of the structure of the dorsal medial ridge of *Nerodia rhombifera* (A) and the dorsal medial groove of *Malpolon insignitus fuscus* (B). Contrary to *Nerodia* the medial groove of *Malpolon spc.* has not a different structure. Whilst the medial ridge in *Nerodia* and aquatic species is known to make them more hydrodynamic, it is still unclear which role the groove has in *Malpolon ssp.*

Even if previously cleaned, some scales were covered by a thin layer of “dirt” (Fig.4.3). This layer correspond to the one described by several authors as being the lipid layer covering the scales and protecting them against desiccation (IRISH et al., 1988; BEYERLEIN, 1993; BRANDSTÄTTER, 1995). BRANDSTÄTTER (1995) wrote that this layer of lipids was more distinct in species occurring in arid regions as *P. schokari* and *D. multimaculata*, confirming in his eyes the hypothesis of the protection against desiccation.

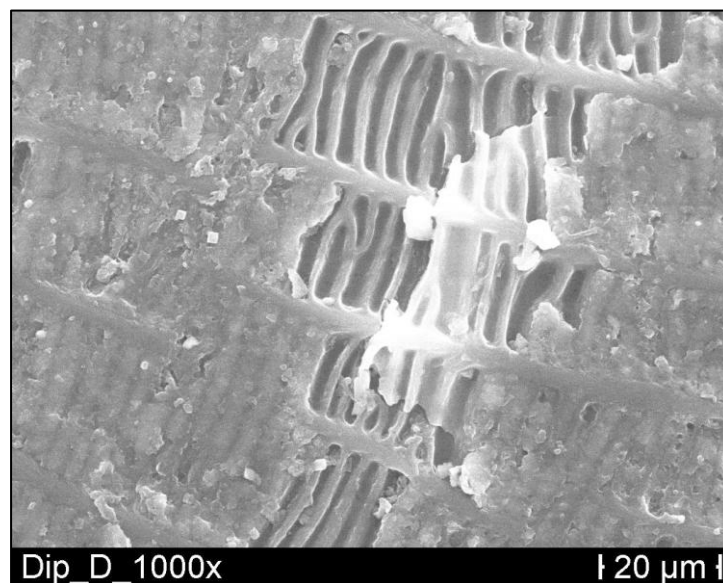


Fig .4.3: Scale of *Dipsina multimaculata*. The scale surface is covered by a thin layer of lipids.

Through the sinusoidal head movement done during RB, it is evident that not all scales are covered by nasal gland secretion. Possibly the microornamentation of the scales has a capillary function, dispersing the secretion on adjacent scales. A comparative analysis of e.g. the space between the ridges, the form of the cups and the form of the vermicular structure of the diverse species should be done in order to see if microdermatoglyphics can be used for taxonomy of psammophiids.

So far the working hypothesis could be confirmed: the scale ultrastructure of psammophiids differs from those of other species. However, the analysis of the scale ultrastructure of the control taxa showed that they do not have any porous pattern either. Therefore, the statement of CHIASSON et al. (1989) that snakes have a porous pattern is refuted. Moreover it is important to make a clear difference between *pits* and *pores* and give a precise definition of them to allow a correct determination.

4.2 GAS CHROMATOGRAPHY

Hypothesis: the composition of the nasal gland secretion of the genera Psammophis mossambicus and Scutophis moilensis is similar to that of Malpolon monspessulanus.

The analysis of the nasal secretion of *P. mossambicus* and *S. moilensis* by GC/MS showed that the chromatograms are similar for the individual of one species and different between the species. The presence of lipids in the nasal secretion of both species cannot be determined clearly by means of this GC/MS analysis. The peaks can only be assigned with certainty to substances with the help of reference substances (often the “pure” substance itself). This, in turn, can only be done when it is known which substance classes are comprised in the secretion; the spectra of these substances have then to be compared with the chromatograms of pure substances to determine precisely the mass found under the peaks and to attribute them with certainty to a given substance class. Since no comparison substances were at disposal, a clear assignation of the masses-to-charge found could not be done. So in the present case it can only be hypothesised that lipids are present in the secretion of both species since they also perform RB as *Malpolon monspessulanus* for which lipids were found in the secretion, but it cannot be said whether they are e.g. fatty acids or

aldehydes. It was rather curious to obtain a peak at 12,40min in the control samples (hexane alone and hexane with glass slides), since actually no peak is expected for this pure substance and the glass cannot solve in hexane. So far it is evident that an impurity was present in the solution.

Ideally, each substance has the same retention time in each sample, when the parameters of GC/MS are the same. When the retention time and the spectrum are identical, it is the same substance. However, particularly for such a complex "mixture" as it is the case for the snake's skin, it is high probable that diverse substances have the same or a similar retention time. In that way, since the scan of the peak of *S. moilensis* no1 does not show the same masses as the scan of *S. moilensis* no2 at the same time, it has to be assumed that two different substances are present, with a similar retention time.

This can maybe be explained by the fact that *S. moilensis* no1 was a male and *S. moilensis* no2 was a female. Indeed, it had been shown by lipid extraction from the skin of males and females *Thamnophis sirtalis parietalis* that there are differences at the level of the composition of the skin lipid, depending on the sex of the individual analysed (MASON et al., 1987). This allows the males to make the differences between males and females e.g. during the mating/ breeding period and thus, to court the right snake.

The scan of the peak at 17,77min in *P. mossambicus* should have been similar in both individuals since it was the same retention time, but in *P. mossambicus* an additional mass-to-charge of 296,8amu appeared. As for *S. moilensis* this means that it is not the same substance although it is the same retention time. It could not be determined here whether it is a question of differences in the sex of the individuals analysed since it could not be determined precisely whether these snakes were males or females.

All studies conducted until now about the presence of lipids in snakes used extracts of or shed skin themselves. The results indicated often masses between 394amu and 532amu (ROBERTS and LILLYWHITE, 1980), much higher than those of the present study, so that the results cannot be efficiently compared. The presence of

free fatty acids, methyl ketones, neutral lipids, cholesterol, hydrocarbons or fatty alcohol was often detected (AHERN and DOWNING, 1974; TSUMITA et al., 1979; ROBERTS and LILLYWHITE, 1980; BURKEN et al. 1985b; MASON et al., 1989).

It is of course delicate to state that the substance analysed here is effectively the dried nasal gland secretion of the body, since it was not possible to collect nasal gland secretion directly at the nare, as done by DUNSON et al. (1978). It is probable that the chromatograms are also those of the substances present in the substrate of the terraria or those of the lipids produced by the skin. The eventuality to prepare the nasal gland itself for GC/MS had been considered, but the analysis would also have given the peaks of the compounds present in the cells of the nasal gland itself and would have then given imprecise results.

To make a complete and precise analysis of this nasal secretion different steps have to be considered: 1) it has to be determined precisely if there are lipids on the psammophiids' skin and if yes, which ones by comparison with pure substances; 2) it has to be determined precisely which lipids are present in the psammophiids' secretion by comparison with pure substances; 3) it has to be compared whether these "nasal" substances are identical in to those of the skin; 4) it has to be tested whether the composition of the skin changes before and/ after rubbing.

So far, the present analysis showed that the chromatograms are different between the species and similar, at the first glance, for the individuals of a given species. Only the comparison with the chromatogram and mass spectrum of a pure substance would help to determine the substance precisely. By virtue of these findings, the role of RB functioning as mechanism of isolation between the species has to be reconsidered (BRANDSTÄTTER, 1996). The results of the present study showed that different species or even genera use the same method (either both nares alternating or one nare one-by-one) to spread the secretion on their belly: it cannot be species-specific. However, since it has been proven that the skin secretions not only between males and females of *T. sirtalis parietlis* but even between the populations can be different, the species-specific differences in psammophiids has to be search at the level of the lipids. And as written above, it is now a question whether

the lipids allowing recognition are present only in the secretion of the nasal gland, only in the skin or in both.

Indeed, beyond their role in retarding water loss of the skin, it shall be shortly added that lipids in snakes play the role of pheromones in the chemical communication in reptiles. It was shown that females of *Thamnophis sirtalis parietalis* secrete a pheromone eliciting courtship behaviour in males (MASON et al., 1987; MASON et al., 1989; LEMASTER and MASON, 2001) giving information about the mating status of the female (O'DONNELL et al., 2004). Pheromones are known to inhibit male courtship behaviour in *Boiga irregularis* (GREENE and MASON, 2003). Moreover it was shown that the lipid composition can vary between the populations of a same species (*T. s. parietalis*) so that males prefer to courtship the females of their own population (LEMASTER and MASON, 2003) leading to the assumption that a sexual isolation exist between certain populations of *T. s. parietalis*. It could be shown that also lizards are able to make differences between mates or conspecific and non-conspecific females thanks pheromones (BULL and LINDLE, 2002; COOPER and PEREZ-MELLADO, 2002).

The working hypothesis could only partially be confirmed: Lipids are present in the nasal secretion of *P. mossambicus* and *S. moilensis*, but at the moment it can not be determine precisely which type of lipids they are. But since they also perform RB as *Malpolon monspessulanus*, and that the secretion of this latest species is known to be lipid-rich, is has to be expected, that similar lipid-classes are also present in the secretion of *P. mossambicus* and *S. moilensis*.

4.3 RUBBING BEHAVIOUR IN PSAMMOPHIIDS

The objective of the behavioural analysis was to determine the functional aspects of the RB of psammophiids, how they perform it and when it takes place. The method of “continuous recording” with cameras was advantageous, since it left the snake undisturbed.

4.3.1 ANALYSIS OF RUBBING BEHAVIOUR IN PSAMMOPHIIDS

The analysis of RB showed differences between the genera studied concerning the method and the number of head movements necessary to spread the secretion on the body, and the duration of RB. To spread the secretion two methods were used: either both nostrils apply the secretion in alternation (*Psammophis elegans*, *P. mossambicus* and *P. schokari*) or each nostril is used only once (*Malpolon*, *Psammophylax* and *Rhamphiophis*).

RB always implied the rubbing of the belly and, to a more or less extent, the flanks as well. The video analysis showed that in some species (especially *Psammophis* sp.) it is rather the body which is pulled along the nostril(s) than the nostrils along the body. In several individuals it could be observed that the snake stopped to rub its body when the body was next to a stone or branch. Interestingly the snake did not move forward to continue rubbing where it had been interrupted but left this part of the body “unrubbed”. It was rubbed the next time snake performed RB.

The number of head movements in the genus *Malpolon* and *Scutophis* did correspond quite exactly to the total body length of the snake (**Table 2.1 and Table 3.3**). This would mean that one head movement spread the secretion on one centimetre body. Although *P. acutus acutus* and *Rhamphiophis* sp. use the same method, the number of head movements did not correspond to the total body length of the snake. This is certainly due to the fact that in both genera the wavelengths were larger than in *Malpolon* and *Scutophis*, covering more of the body surface. In *Psammophis* the number of head movements did either not correspond to the total body length, since their method, both nostrils used in alternation, covered also more of the body surface -about 2cm per head movement.

It was always recorded with which nostril the snake began to rub their body but the snakes seemed not to show any preference to initiate rubbing with the left or the right nostril. “Right-handedness” and “left-handedness” seems to be present in cats or fishes (TAN and KUTLU, 1991; BURT DE PERERA and BRAITHWAITE, 2005) so that it can also be present in psammophiids.

In this study, RB could not be observed in branches although it has been reported that the method used by *Psammophis sp.* would allow them to perform RB in trees (DE HAAN, 2003a). Even if the snakes basked in the branches at their disposal in the experimental terraria, they performed always RB on the ground. Only once, a RB was observed on a branch in a *S. moilensis*: according to DE HAAN'S observation (2003a) this species should not be able to perform RB in trees by virtue of its rubbing method.

Since the four genera *Malpolon*, *Scutophis*, *Rhamphiophis sp.* and *P. a. acutus* in this study rubbed their body with only one nostril, this method cannot be considered as peculiar to one genus to allow specific differentiation (BRANDSTÄTTER, 1996). If RB serves to interspecific communication (BRANDSTÄTTER, 1996) it is rather due to the lipids present in the secretion acting as pheromones (SCHELL and WELDON, 1987; MASON et al., 1990; MASON, 1992) rather than the method itself used to apply the secretion on the body. These pheromones are in turn species-specific, allowing distinction not only between the individuals (GREENE et al., 2001; LEMASTER and MASON, 2003; O'DONNELL et al., 2004) but also between the species (DEVINE, 1977 in HALPREN, 1992; MASON, 1992).

The RB of *P. mossambicus* of this study was not treated a part as that of *P. elegans*, although it has not been (explicitly) mentioned in the literature yet: Indeed, there had still be confusion in the past about the correct assignation of psammophiids to the species *P. phillipsi*, *P. mossambicus* and *P. sibilans* (**Chapter 1.3**). So RB may have already been described for *P. mossambicus* but under the name of *P. phillipsi* or *P. sibilans* and was therefore not described as new observation in this study.

Although DE HAAN and CLUCHIER (2006) described the narial valve as being a particular morphological trait of psammophiids and indispensable to perform and enabling RB, the narial valve was only clearly visible in *Malpolon insignitus fuscus* and in *Scutophis moilensis*. It was not seen in *Psammophis mossambicus*, *P. schokari*, *P. elegans*, *Psammophylax acutus acutus*, and in *Rhamphiophis rubropunctatus* and *R. rostratus*, but all these species were able to perform RB.

In his “Everyone’s guide to the snakes of Southern Africa” BRANCH (2001) wrote: “Some desert species have a nasal gland; these species groom themselves, spreading the nasal secretion evenly on the body, which helps to reduce water loss”. The results of the present study show that RB is not only present in desert species as *Psammophis schokari* also in species of more mesic habitats as *Psammophylax acutus acutus* or *Psammophis mossambicus*.

4.3.2 RUBBING BEHAVIOUR AS MARKING BEHAVIOUR

Hypothesis: Rubbing behaviour is not a marking behaviour.

In the first part of this experiment, the assumption that the rubbing frequency would be more elevated on the first day since the snake has to “mark” its new environment could not be confirmed. The results of the t-test ($T_{14}=-0,158$; $N=15$, $p=0,877$) showed no significant difference between the frequency of RB on the first day compared to the mean frequency of RB of the following four days.

The second part of the experiment based on DE HAAN’s (1999) observations, that the snakes rub two or three times before crawling through a less well known environment. A similar behaviour was expected here since the snakes were placed into an unknown terrarium. However no traces of secretion could be identified neither on the blotting paper of the walls of the terrarium, nor on the glass plate situated in the middle of the terrarium. The videos showed that all snakes crawled along the walls of the terrarium, regardless whether another snake had been placed previously into the terrarium or not. Different reasons can be given to this:

- 1) The snakes behaved differently due to the transport between the home and the experimental terrarium. They wanted to escape the new terrarium independently of the previous presence of another snake.
- 2) The sex of both snake was identical –no interest to follow the scent trail.
- 3) Following of either males or females take place but only during the mating season and this experiment was conducted at the false time of the year.

- a. The first snake was a male the second a female -either no interest to follow the trail.
 - b. The first snake was a female, the second a male -no interest for the male to follow the female being beyond the mating period.
- 4) The second snake did react to the previous presence of the first one but on a different manner not identified by the observer.

As for the first part of the experiment (see above), the snakes did not rub more frequently when placed into the new environment (cleaned terrarium). All snakes always returned to the shelter situated at the middle of the terrarium although two shelters were at disposal (**Fig.2.3**). Since this cover was situated half under the lamp it may have been an agreeable warm place to conceal. Two possibilities can be given why the snake placed in second also sheltered under this stone: because it was an agreeable warm place for the snake placed at first; and secondly, MASON (1992) noted that when a shelter is used permanently, the substrate might contain chemical cues facilitating the aggregation of snakes (habitat conditioning): in our case, the snake placed in second would have preferred to shelter where the first one was, since the first one would have left chemical cues under this stone.

NOBLE and CLAUSEN (1936) showed that beyond the mating period of *Steroria dekayi*, males and females follow chemical cues of conspecifics regardless of their sex. On the contrary, during the mating period, males follow uniquely the trails of females, whilst females follow furthermore the trails of males and females. This led the authors to suppose, that the females produce additional substances attracting the males during the mating period. This could at least be confirmed by GARSTKA and CREWS (1981) and LEMASTER and MASON (2001) in *Thamnophis sirtalis parietalis*. So possibly it is the same in the psammophiids used here: that the important cues are produced passively through the skin whilst the secretion applied on the body has another purpose - the avoidance of desiccation of the body. Possibly the secretion has another composition during the mating period serving then as sexual attractant whilst it has the function to reduce water loss besides the mating period. For this the

exact period of reproduction of all species should be known and secretion analysed during and beyond the mating period.

No really satisfactory explanation could be found why there was no trace of secretion neither on the glass plate in the middle of the terrarium, nor on the blotting paper applied on the terrarium's walls. It may be possible that the secretion could not be deposited on a substrate as "smooth" as glass, although it can be deposited on the "rough" substrate of sand or earth. Maybe the secretion is not fatty enough, as for human finger prints, to be seen on the blotting paper or even on glass.

So far, field studies should complete this experiment to test the snakes adequately as detailed for *Thamnophis sirtalis parietalis* (LEMASTER and MASON, 2001; O'DONNELL et al., 2004), *Boiga irregularis* (GREENE et al., 2001) and other snakes (GEHLBACH et al., 1971) to determine on one side trailing behaviour in the wild and on the second one, to report these field observations under standardised laboratory conditions.

Although RB had been proposed to be used to mark the territory of the snake, the question of territoriality of snakes has to be considered carefully. The territorial defence reported for adders was observed only during the reproductive period and the "space" defended was the immediate area around the reproductive female (CARPENTER, 1977; ANDREN, 1986). Past and recent debate criticised the occurrence of territoriality in any snake (GILLINGHAM, 1987; FORD and HOLLAND, 1990; RIVAS and BURGHARDT, 2005). An alternative term was proposed, the "successive female defensive polygyny" considering that males *Vipera* defend only their females and not eventual resources (DUVALL et al., 1992), since it does not match with the definition given by BROWN and ORIANI (1970) „(...) a territory can be defined explicitly as any defended area that meets the following three conditions: it is a fixed area; it is defended with behavioral acts that cause escape or avoidance by intruders; and such behavioral acts results in exclusive use with respect to rivals“.

The observations on mate guarding in *Malpolon* (DE HAAN, 1999; DE HAAN and CLUCHIER, 2006) match with those in *Vipera* (ANDREN, 1986). However, the literature

not describes evidently whether conspecifics are also chased out of the mating period or whether females are -by virtue of their RB, territorial as well.

For psammophiids, additionally to the term of successive female defensive polygyny, I would suggest to speak rather about a “home range”. This term has been already used successfully several times in literature as in studies on other snakes as *Coronella austriaca* (SPELLERBERG, 1988; ZIMMERMANN, 1988; STRIJBOSCH and VAN GELDER, 1993; LARSSON, 1995; ALFERMANN and BÖHME, 2009). According to its definition, it is an area associated with one or more resource(s) in which foraging and social activities can take place, as accurately described by PITMAN (1974) for *Psammophis sibilans*: “[...] habitually basking day after day same place same time in brilliant sunshine on bushes, pendant branches small trees, shrubs and other vegetation [...]” and not defended against conspecifics (ZUG et al., 2001).

It could be observed several times in all snakes studied, that they rubbed their nose directly on artefacts as branches and/ or stones -as known for cats. This method would obviously be a more efficient marking than first covering the entire body with nasal gland secretion and then crawling on the substrate. Basing on own experience, the secretion is rapidly scrapped off of the belly (**Chapter 2.3**): This implies that if the snake effectively use RB as marking, it has to rub itself every 2-3 body length to obtain a (continuous) marking trail.

In relation to this, traces of secretion could be seen on the terrarium’s walls and doors of the home terraria of *M. i. fuscus* and of two *S. moilensis*. These traces could be similar to the ones described by DE HAAN (1999) and BRANDSTÄTTER (1995).

The traces were sinusoidal corresponding to the head movements of the snake during RB (**Chapter 3.3.1.2**). There were smeared on 30cm length in *M. i. fuscus* (**Fig.4.4A**). However, although the body height of *M. i. fuscus* is about 2-3cm, the height of the secretion in *M. i. fuscus* was larger, 7-10cm. One trace of *M. i. fuscus* had been applied bottom-up (**Fig.4.4B**). In *S. moilensis* the secretion was smeared on 5cm length in *S. moilensis*; the height of the secretion did correspond approximately to their body height, about 1cm (**Fig.4.4C**).

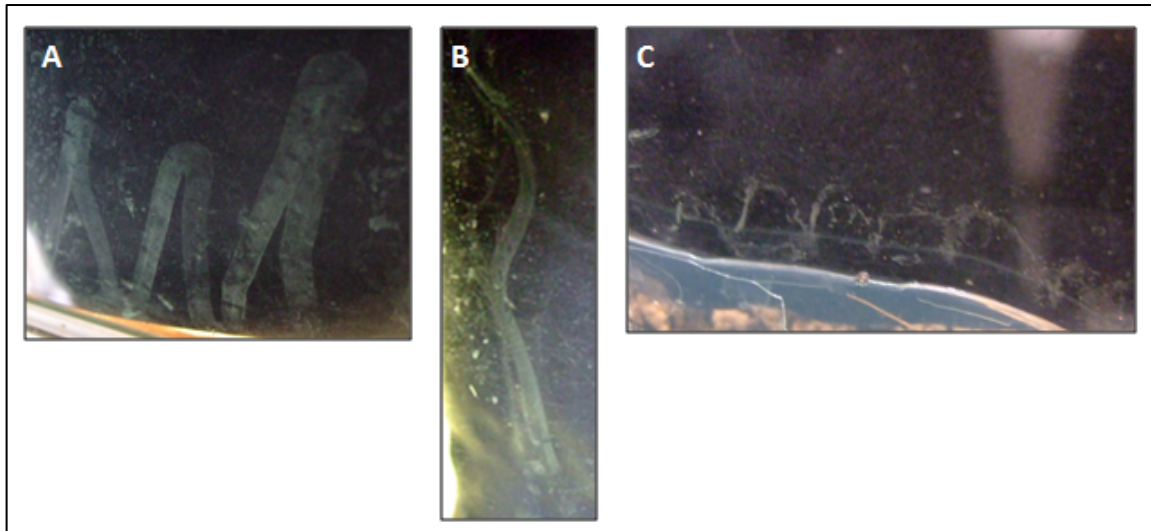


Fig.4.4: Traces of nasal gland secretion on the terrarium's walls of *M. i. fuscus* (**A and B**). **A:** 7cm length and 5cm height, **B:** 8cm length. **(C):** *S. moilensis* (5cm length and 2cm height). Black paper has been applied behind the traces to bring them out.

If the snake use effectively the secretion to mark a territory it should renew after removal. The traces reappeared exactly seven days after removal. The occurrence of the traces high above the ground can easily be explained: *M. i. fuscus* as other psammophiids often erects its head to observe its environment. Since its terrarium was situated in front of another, in which it could see other snakes or lizards, it is to presume that the snake entered in contact with the glass whilst observing. Since glass is a smooth and polished surface, in that way similar to the snake's scales, it would have act as an oversized stimulus when the snake entered in contact with the glass, eliciting RB.

Interestingly, these traces were water-repellent (**Fig.4.5**) speaking for the protective function against desiccation of the secretion caused by the hydrophobic compounds (lipids) of the secretion. However, the use of the secretion to mark a territory or to leave a scent trail to conspecifics could not be clearly demonstrated in this experiment.

The results showed that the RB frequency of the 17 snakes observed is not significantly elevated when the snake is placed into an unknown terrarium so that in this case, RB cannot be considered as being used to mark a new environment. Moreover, when nasal secretion traces were seen on the glass door and removed,

the traces were not renewed. In this study, individuals of *P. mossambicus* and *S. moilensis* did not react to the previous passage of a conspecific. So far it could not clearly be demonstrated here whether RB serves to leave an informative trail to conspecifics.



Fig.4.5: Visible traces of secretion being hydrophobic. This argues for the protective function against desiccation of the secretion.

4.3.3 FREQUENCY OF RUBBING BEHAVIOUR IS TEMPERATURE DEPENDENT

Hypothesis: Rubbing behaviour depends on external factors as temperature.

The present study confirmed the increasing of rubbing frequency with increasing temperature. The overall analysis of the pooled data of the psammophiids of this study (n=17) gave a significant correlation of dependency of temperature on rubbing behaviour frequency ($r_2=0,315$; $N=354$; $p=0,000$). Due to the low number of specimens for each species observed (*P. mossambicus* (n=6), *P. schokari* (n=4), *M. i. fuscus* (n=1), *S. moilensis* (n=4) and *P.a. acutus* (n=2)) it was not possible to determine a significance correlation of dependency of temperature for the individual species. Only the tendency that a significant correlation exists of the dependency of temperature on RB could be seen.

The increase of RB frequency with temperature in the present study confirms the observations done in the past (DAREVSKY, 1956; RIEPPEL, 1973; DE HAAN, 1982;

STEEHOUDER, 1987) and speaks for the hypothesis emitted by DUNSON et al. (1978) that RB has a protective function against desiccation. This could explain the great increase of rubbing frequency in *P. mossambicus* since this species occurs in equatorial and eastern Africa, living in a more mesic habitat than in the desert. A dramatic increase of temperature (Temp.4 almost 70°C) lead the snakes to perform more RB than snakes adapted to heat -as *P. schokari*. As described be GREEN et al. (1997), the temperature has an influence on the physiological function of ectoterm animals. They described that high temperatures are dangerous to slender bodied animals since the surface /mass rate leads to rapid cool down or warm up. Protected by a lipid-rich secretion would prevent the psammophiids to be dependent from the external temperature, so that they cannot only be active in the hottest hours of the day. Several authors described *Malpolon* as active during midday, when other animals escape heat (STEMMLER, 1962; KRAMER and SCHNURRENBERGER, 1963) or as the snake with the greatest tolerance to hot temperatures (BRUNO, 1977), basking directly whilst other snake prefer indirect heat (BRANDSTÄTTER, 1996).

Relative humidity was not varied intentionally in this experiment, but the values at the beginning and at the end of the experiment were recorded (**Chapter 2.3.3**). For each value of relative humidity the frequency of RB was determined as done for the temperature, but no significant relationship between relative humidity and rubbing frequency was detected for the 17 snakes studied (**Appendix 8.6**).

Since it is known that increasing temperature elevates the activity of the animal during the day (MORENO-RUEDA and PLEGUEZUELOS, 2007), a greater activity lead certainly the snake to sweat more, causing then a higher rate of water loss (in reference to WARBURG, 1965). To avoid then large amount of water loss, the snake would rub more frequently.

In addition to the other studies about rates of water loss and skin resistance to desiccation involving psammophiids the present results clearly show that psammophiids are perfectly adapted to high temperatures in part due to an efficient protective system -the rubbing behaviour. However, this study cannot be concluded without stating that RB bears an analogy to a similar behaviour found in frogs.

In regards to water loss and protection against desiccation, the attention was paid some years ago to frogs living under arid climatic conditions. The majority of amphibian species cannot “control” the rates of their evaporative water loss and rely therefore on proximity of water (BARBEAU and LILLYWHITE, 2005), but these frogs have found a solution which is redolent of the rubbing behaviour of psammophiids: Some frogs of the genera *Chiromantis* (DREWES et al., 1977), *Hyla* (BARBEAU and LILLYWHITE, 2005), *Hyperolius* (WITHERS et al., 1982), *Litoria* (CHRISTIAN et al., 1988), *Phyllomedusa* (BLAYLOCK et al., 1976; MC CLANAHAN et al., 1978) and *Polypedates* (LILLYWHITE et al., 1997) spread the secretion of dorsal glands over their entire body by complex stereotyped “[...] but not rigidly fixed movements” (Fig.4.6) (BLAYLOCK et al., 1976).

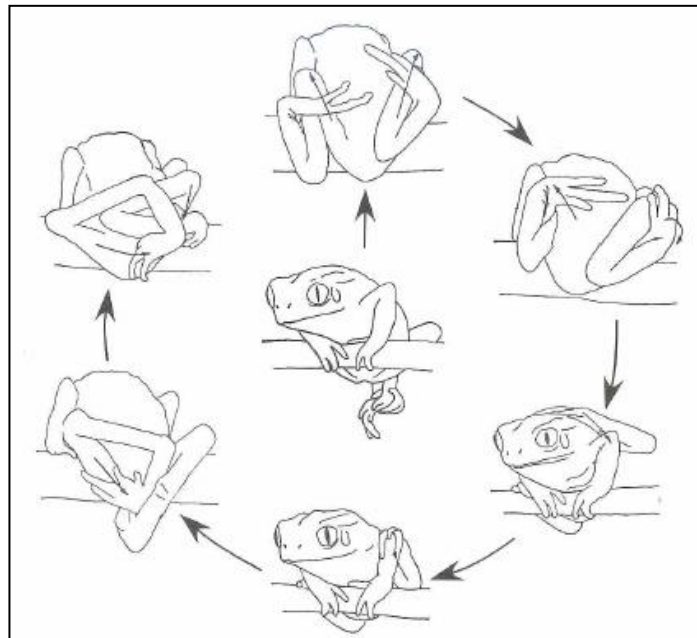


Fig.4.6: Wiping behaviour of *Phyllomedusa sauvagei* (Blaylock et al., 1976).

The secretion is either mucus as in *Chiromantis* and *Hyperolius* (DREWES et al., 1977; WITHERS et al., 1982) or a mixture of wax ester, oleic acids and long chain alcohol produced by lipid glands as in four species of *Phyllomedusa* (BLAYLOCK et al., 1976). CHRISTIAN and PARRY (1997) showed that the secretion of *Litoria caerulea* and *Cyclorana australis* contains in fact a relatively low concentration of lipids, 5-10% whereas the concentration of proteins of 78-85% is very high. CHRISTIAN and PARRY

suggested that the retardation of water loss is given by the proteins present in the secretion, in addition to the lipids. This could also be similar in psammophiids, since proteins were also found in the secretion of *Malpolon monspessulanus* by DUNSON et al. (1978).

SEM analysis of the skin structure of *Phyllomedusa sauvagei*, *Litoria caerulea* and *Polypedates maculatus* revealed a high structured surface, similar to the vermicular structure between the ridges of psammophiid scales (**Fig.4.7**).

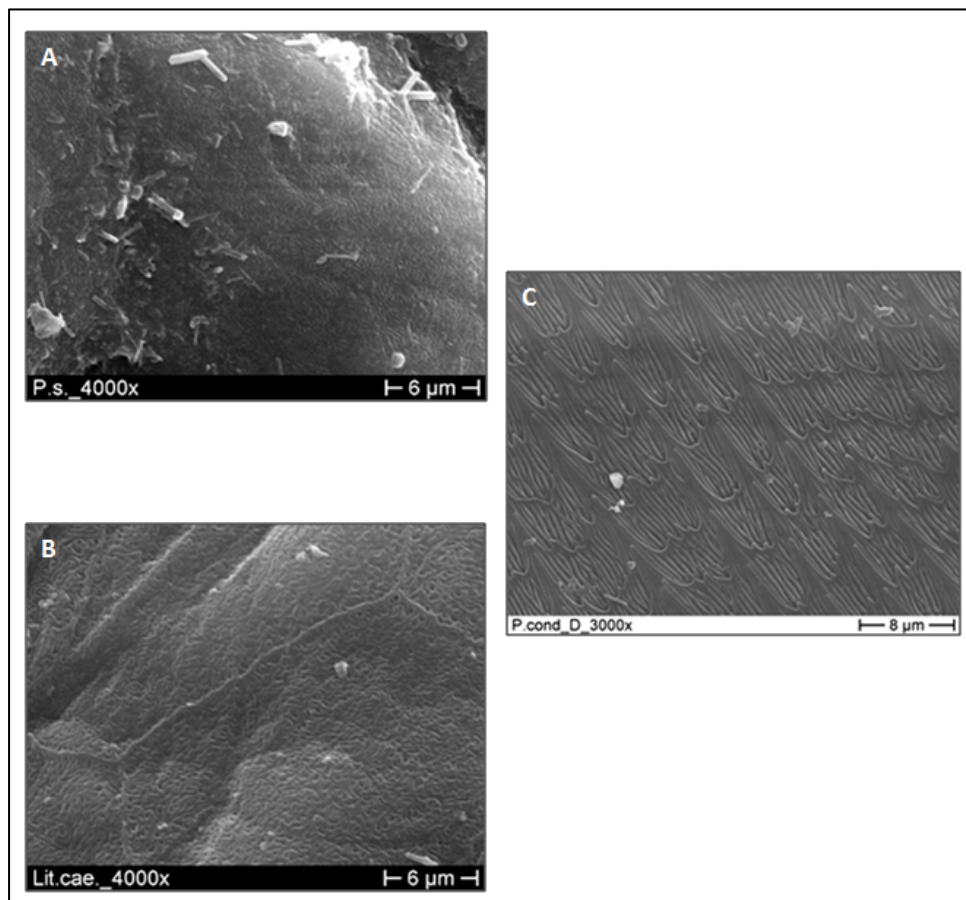


Fig.4.7: SEM picture of the frogs *Phyllomedusa sauvagei* (A) and *Litoria caerulea* (B) showing the highly structured skin of these frogs similar to the vermicular structure of the inter-ridges space of psammophiids, here *Psammophis condanarus* (C).

The origin of the wiping behaviour seems to be derived from movements used to remove shed skin or debris from the body (BLAYLOCK et al., 1976), whilst the desiccation “[...] appears to be the principal selective force which has coupled the evolution of lipid secretion with wiping behaviour” (LILLYWHITE et al., 1997). This

enabled for example *Phyllomedusa sauvagei* to live in semi-arid condition over a wide geographic range -as the arid regions of Argentina (BLAYLOCK et al., 1976). This wiping behaviour could also be observed in newly metamorphosed frogs of *P. hypochondrialis* (ALAMILLO and PRAMUK, 2002).

Different snakes of different species could be observed drinking the water running from their head to their mouth or directly from their body. This latest method of drinking could be observed by chance once in *P. a. acutus*: the movements of the head and its position along the flanks and back whilst drinking were strongly redolent of the movement of the head during RB (**Fig.4.8**). Possibly, a similar evolutive way as in *Phyllomedusa* could have take place in psammophiids: the rubbing movement is derivated from the drinking of water droplets of the body and the dehydration of the skin coupled it with RB.



Fig.4.8: Drinking in *Psammophylax acutus acutus*. The head movements are very similar to the head movements of rubbing behaviour.

The overall analysis of the 17 psammophiids studied confirmed the working hypothesis that RB depends on external factors as temperature. The similarity to the wiping behaviour of frogs, known to reduce water loss, has to be taken in consideration that the main role of RB is to protect the snake against desiccation.

4.4 MORPHOLOGICAL DIVERGENCES BETWEEN *MALPOLON MONSPESSULANUS* AND *SCUTOPHIS MOILENSIS*

Hypothesis: Morphological divergences support the differentiation between the genera Malpolon and Scutophis.

GRUBER (1989) wrote that both, *Malpolon monspessulanus* and “*Malpolon*” *moilensis* have grooved dorsal scales. This could not be seen neither in live specimens observed, nor in the specimens of the Museum collection. But interestingly, in his list of synonyms, BRANDSTÄTTER (1995) recorded that *Scutophis* had previously been called “*Coelopeltis*” by diverse authors. “*Coelopeltis*” had been previously given to *Malpolon* in reference to its grooved scales (*coelo-* being the Greek word for “hollow” and *pelta* the Latin word a little for “little crescent-shaped shield”), but it is clear that this name has then been erroneously applied to “*Malpolon*” *moilensis*. The only common morphological trait is the valvular nostril (Fig.1.8).

Completed by x-ray analysis the dissection showed that the ribs in the neck region of *S. moilensis* are slightly longer and wider than in *M. monspessulanus*. This speaks for an additional difference between both species and support the affiliation of *moilensis* to its own genus *Scutophis*. However, due to the low specimens’ number further individual should be dissected in order to make an adequate statistical analysis. Since the specimens of *M. monspessulanus* had been a long time in alcohol, their ribs were extremely fragile and did incline to break during preparation.

Curiously it could be observed several times in different individuals that *S. moilensis* also apparently use their shield whilst basking, although it is not outspread as during defensive behaviour.

Another morphological particularity should be discussed here. In its article about parietal pits (little pits on the parietal plates) found sporadically in some psammophiids, DE HAAN (2003b) wrote that they were absent from *Scutophis moilensis*. However, all the *S. moilensis* observed in the present study had pits but not only on their parietal plates but also on the frontal one. MIRALLES and INEICH (2006) found parietal pits in *Atretium schistosum*, another colubrid species. UNDERWOOD

(1967) wrote that pits on the head of snakes are rarely mentioned in literature and that if present, they would act as receptor for radiation.

The measurements of the neck ribs' length of *S. moilensis* and *M. monspessulanus* could confirm the differentiation between both genera, and state the affiliation of "*moilensis*" to its own genus *Scutophis*.

5. SUMMARY

Several snakes belonging to the eight genera of the family Psammophiidae are the sole known snakes to smear the secretion of their nasal gland on their own belly and flanks by little rapid head movements. The main objective of the present study is to determine behaviourally the function of this “rubbing behaviour”. The rubbing behaviour itself and its possible functions have been mentioned several times in literature, but most of them without specific experimental analysis. This is the first behavioural study focussing on the rubbing behaviour of psammophiids in order to examine its function with more clarity. Psammophiids belonging to the four genera *Malpolon*, *Psammophis*, *Psammophylax* and *Scutophis* were studied.

Since it had been stated that snakes have pores on their scales allowing the passage of lipids through the skin to protect it against desiccation, it was important to verify this feature in psammophiids. The scanning electron microscopic analysis of the dorsal scale ultrastructure of several psammophiids and control species was undertaken: the results showed that neither psammophiids, nor control taxa have pores on their scale surface.

Some years ago, the nasal gland secretion of *Malpolon monspessulanus*, a psammophiid known to do rubbing behaviour, had been analysed. This analysis showed the presence of lipids, known to protect the skin against desiccation. Since other species of psammophiids also perform rubbing behaviour, it was verified whether their nasal secretion also contains lipids. The presence of lipids in the nasal gland secretion of *Psammophis mossambicus* and *Scutophis moilensis* could only be presumed by gas chromatographic analysis. But it has to be assumed that these species also have a lipid-rich nasal secretion since they also perform rubbing behaviour -as *Malpolon monspessulanus*.

One hypothesis on the function of rubbing behaviour is that it serves to mark an unknown environment. To verify this, the snakes were placed into a terrarium unknown to the snakes: The frequency of its rubbing behaviour on the first day was compared to the mean frequency of rubbing of the following days. If the rubbing behaviour serves to mark, its frequency would be more elevated on the first day. This

frequency was however not significantly higher, so that rubbing behaviour cannot be considered as marking behaviour.

In a further experiment, it was tested whether nasal secretion is used to leave an informative trail to conspecifics. Two couples of *Psammophis mossambicus* and *Scutophis moilensis* were studied. The snakes were placed one by one into a terrarium. No changes in behaviour (e.g. elevated tongue flicking) could be detected in the snakes placed at second in the terrarium, regardless of their sex or the sex of the snake introduced at first.

Finally, in order to determine whether rubbing behaviour depends on external factors, temperature was varied in an experimental setup. There was a significant correlation between increasing temperature and the number of rubbing behaviour in all psammophiids studied. This states the hypothesis that rubbing behaviour is primarily dependent on external factors and that it is used to avoid desiccation. This is done by means of the nasal secretion known to be lipid-rich as in *Malpolon monspessulanus* and it has then to be considered that this secretion is also lipid-rich in the other psammophiid species since their number of rubbing behaviour also increased with temperature. This allows these snakes to be active even in the hottest hours of the day, e.g. minimising concurrence with other predators.

Additionally to the behavioural experiments, for the first time the rubbing behaviour of the genus *Rhamphiophis* could be verified in *R. rubropunctatus* and *R. rostratus*. This elevates the number of genera performing rubbing behaviour on seven, of the eight comprised in this family. The “last” genus in which rubbing behaviour has not been recorded yet is *Dipsina*. The rubbing behaviour could additionally be documented for a further *Psammophis* species, *P. elegans* and for a further *Psammophylax* species, *P. acutus acutus*.

A further morphological analysis should confirm the affiliation of the species *moilensis* to its own genus. It had been affiliated long time to the genus *Malpolon*. Its attribution to its own genus, *Scutophis*, had been proposed but not unanimously adopted in the literature. The affiliation was primarily based on the ability of *moilensis* to spread the neck when threatened -*Malpolon* species are unable to do

this. The comparative morphological analysis between the neck ribs of *Malpolon monspessulanus* and *Scutophis moilensis* confirmed differences at the level of the length of these ribs, those in *S. moilensis* being obviously longer. This difference reinforces the affiliation of both species to different genera.

6. ZUSAMMENFASSUNG

Die Schlangen der acht Gattungen der Psammophiidae zeigen ein einzigartiges Verhalten auf: Sie reiben durch kleine schnelle Kopfbewegungen das Sekret ihrer Nasendrüse auf ihren Bauch und Flanken. Das Hauptziel dieser Studie ist dieses „Putzverhalten“ mittels Verhaltensexperimente zu analysieren. Die möglichen Funktionen des Putzverhaltens wurden mehrmals in der Literatur erwähnt und selten wurden sie experimentell erprobt. Es handelt sich hier um die erste Studie über das Putzverhalten von Psammophiiden um seine Funktion zu beschreiben. Schlangen der vier Gattungen *Malpolon*, *Psammophis*, *Psammophylax* und *Scutophis* wurden untersucht.

In der Literatur wird behauptet, dass die Rückenschuppen der Schlangen Poren aufweisen, welche das Austreten von Lipiden begünstigen und somit die Haut vor Austrocknung schützen, war es wichtig diese Aussage in Psammophiiden nachzugehen. Die rasterelektronenmikroskopischen Untersuchungen verschiedener Psammophiiden und Kontroll-Arten ergab, dass weder die einen noch die anderen Poren auf ihre Rückenschuppen haben.

Vor einigen Jahren wurde das Nasendrüsensekret von *Malpolon monspessulanus*, die für sein Putzverhalten bekannt ist, auf seine Zusammensetzung untersucht. Die Ergebnisse zeigten das Vorhandendasein von Lipiden -die die Haut gegen Austrocknung schützen. Es war demnach wichtig das Sekret von anderen sich putzenden Arten auf seine Zusammensetzung zu untersuchen. Das Vorkommen von Lipiden im Sekret von *Psammophis mossambicus* und *Scutophis moilensis* konnte mittels gas chromatographischer Untersuchungen nicht eindeutig festgestellt werden. Anhand der erzeugten Ergebnisse liegt der Verdacht jedoch nahe, dass diese Arten auch ein Lipid-reiches Sekret haben, weil sie auch das Putzverhalten zeigen - wie *Malpolon monspessulanus*.

Eine der Hypothesen zum Putzverhalten ist, dass es zum Markieren einer unbekanntes Umgebung dient. Um dies zu untersuchen wurden die Schlangen in ein von ihnen unbekanntes Terrarium eingesetzt. Die Frequenz des Putzverhaltens am ersten Tag wurde mit dem Mittelwert der Frequenz der darauffolgenden Tage

verglichen. Wenn das Putzverhalten dem Markieren dient, sollte seine Frequenz am ersten Tag höher sein. Diese Frequenz war jedoch nicht signifikant höher, so dass das Putzverhalten nicht als Markierverhalten gedeutet werden kann.

In einem weiteren Experiment wurde untersucht, ob das Sekret benutzt wird, um eine informative Duftspur für Artgenossen zu hinterlassen. Zwei Pärchen der Arten *Psammophis mossambicus* und *Scutophis moilensis*. Die Schlangen jedes Paares wurden nacheinander in ein Terrarium gesetzt. Die zweitplazierte Schlange zeigte jedoch keine Änderung in ihrem Verhalten (z.B. erhöhtes Züngeln), unabhängig von ihrem Geschlecht oder von dem der ersten Schlange.

Abschließend, um zu bestimmen ob das Putzverhalten von äußerlichen Faktoren abhängig ist, wurde die Temperatur experimentell variiert. Eine signifikante Korrelation zwischen der steigenden Temperatur und die Anzahl des Putzverhaltens ergaben sich für die Gesamtheit der untersuchten Psammophiiden. Dies beweist die Hypothese, dass das Putzverhalten von äußerlichen Faktoren abhängig ist, und dass es als Schutz gegen die Austrocknung der Haut dient. Dies geschieht anhand des Nasendrüsensekrets, reich an Lipiden wie es für *Malpolon monspessulanus* bekannt ist, und es muss davon ausgegangen werden, dass das Sekret anderer Psammophiiden auch Lipid-haltig ist, da sie auch eine Erhöhung ihres Putzverhaltens bei steigender Temperatur zeigten. Dies erlaubt diesen Schlangen auch an den heißen Stunden des Tages aktiv zu sein und somit z.B. die Konkurrenz mit anderen Prädatoren zu verringern.

Zusätzlich zu den Verhaltensexperimenten konnte zum ersten Mal das Putzverhalten der Gattung *Rhamphiophis* bei *R. rubropunctatus* und *R. rostratus* nachgewiesen werden. Dies bringt die Anzahl der sich „putzenden“ Gattungen auf sieben von acht, welcher dieser Familie zugehörig sind. Bei der somit „letzten“ Gattung *Dipsina* konnte bis jetzt das Putzverhalten noch nicht dokumentiert werden. Bei einer weiteren *Psammophis*-Art, *P. elegans*, und bei einer weiteren *Psammophylax*-Art, *P. acutus acutus* konnte das Putzverhalten dokumentiert werden.

Eine weiter morphologische Untersuchung sollte die Zuordnung der Art *moilensis* zu ihrer eigenen Gattung bestätigen; sie wurde lange Zeit der Gattung

Malpolon zugeordnet. Ihre Zuordnung zu einer eigenen Gattung, *Scutophis*, wurde vorgeschlagen jedoch nicht einstimmig in der Literatur angenommen. Sie beruht auf die Fähigkeit dieser Tiere bei Bedrohung die Halsrippen zu spreizen -die *Malpolon*-Arten können das nicht. Der anatomische Vergleich der Halsrippen von *Scutophis moilensis* und *Malpolon monspessulanus* zeigte, dass die Rippen von *S. moilensis* länger sind. Dies verstärkt die Zuordnung von *moilensis* zu ihrer eigenen Gattung *Scutophis*.

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APPENDIX



8. APPENDIX

8.1 RUBBING BEHAVIOUR BEFORE AND AFTER SHEDDING

In *Psammophis mossambicus* no1 and no3 and in *P. schokari* no10 it could be observed that RB almost completely ceased few days before shedding, diagnosed thanks to the greyish colouration of the scales and the milky eyes.

On the day of shedding itself, after the loss of the old skin, the snakes performed more RB than on the previous and following days (**Fig.8.1**). Contrary to other observed snakes as *Python regius* or *Acranthophis dumerili* (pers. obs.), psammophiids did not try to escape heat during the shedding period: all observed snakes stayed all the hours before shedding directly under the light bulb whilst RB ceased.

On its shedding day, snake no1 did 14 RB. The four previous days it had a mean number of 0,75 RBs. Its number of RB was still elevated on the day following shedding (6 RBs). The mean number of RB the four days after shedding was 3,75 (**Table 8.1**). Snake no3 had nine RBs the day of shedding, a mean number of RBs of 0,5 the four days before, and a mean of 2,75 for the four following days (**Table 8.1**). Snake no10 shed two days after begin of the observation so that there is only one day before the shedding day. It did five RBs on the day of shedding whilst the day before the mean number of RB was 2,0; for the four days after shedding it was 2,5 (**Table 8.1**).

The mean number of RBs of the four following days corresponds to the “normal” number of RBs beyond the shedding period. Noteworthy in snake no10 was that the five RBs were all performed within the first 25 minutes after the loss of the skin. The first RB took place before the skin was completely lost. Both *P. mossambicus* rubbed their new skin regularly but less frequently, about twice to thrice per hour after shedding. Beyond the shedding period RB took place once about once each two hours.

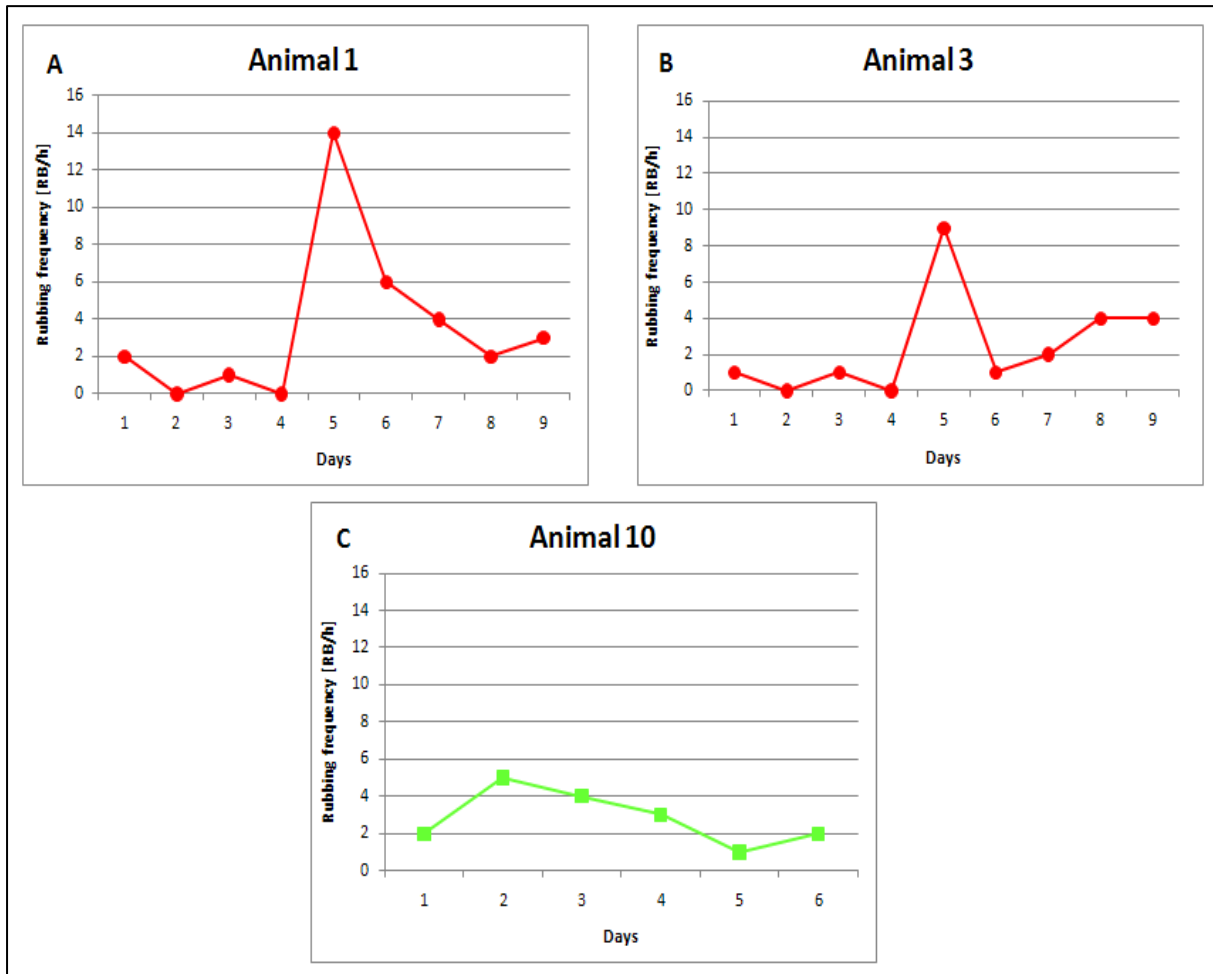


Fig.8.1: **(A)** Snake no1: on the day of shedding (day 3), after the loss of the old skin, the snake performed 14 RB. The days before, the mean number of RB is 0,75, the mean number of RB after shedding is 3,75. **(B):** In snake no3, nine RB were done on the day of shedding (day 3). The days before, the mean number of RB is 0,5. The mean number of RB after shedding is 2,75. **(C):** Snake no10 performed six RB on the day of shedding (day 2). The days before, the mean number of RB is 2,0. The mean number of RB after shedding is 2,5.

Table 8.1
Frequency of RB on the days before, beneath and after shedding in snakes no1, no3 and no10.

Snake	Mean number of RB		
	four days before shedding	on the shedding day	four days after shedding
no1	0,75	14	3,75
no3	0,5	9	2,75
no10	2	5	2,5

The variability of water loss during the shedding period has been reported several times in literature for snake and lizards (BOGERT and COWLES, 1947; CLAUSSEN, 1967; GANS et al., 1968; MINNICH, 1970; COHEN, 1975; ZUCKER and MADERSON, 1980; LANDMANN, 1981). So far it seems evident that the snakes pay a particular attention to the new skin by rubbing it more frequently to avoid a greater loss of water.

This decrease of RB frequency before shedding remains unclear. Possibly the fatty secretion would retain the old skin on the snake's body so that RB has to be ceased to allow the shedding of the old skin. The increase of RB on the new skin after shedding can be explained with regards on the function of protection of the lipids for the skin (**Chapter 1.6**). With such a presumption it can be supposed that the new skin is extremely sensitive to desiccation and therefore has to be especially protected during the first hours and/ or even days after shedding.

8.2 BREEDING BEHAVIOUR IN *PSAMMOPHYLAX ACUTUS ACUTUS*

Two *Psammophylax acutus acutus* were kept and for all psammophiids, the identification of sex of these specimens could not be determined with precision. For the keeping conditions see **Chapter 2.1**.

On 10th August 2009, eleven eggs were found under a bark in the terrarium, with the smallest snake coiled on them. Since it was known that the females of *P. tritaeniatus* (SWEENEY, 1971; HALL et al., 1994; HARTMANN, 1998), *P. multisquamis* (SPAWLS et al., 2002; BRANCH, 2005) and *P. rhombeatus* (BRANCH, 1988) lay on their eggs to guard them it was decided to not remove the eggs in order to observe the behaviour of the snake. This behaviour allowed determining the sex of these snakes: the female was coiled around the eggs. Moss was placed around the nest to increase humidity and sprayed twice a day.

To make sure the female lay on the eggs to protect them and not because the bark was the best hiding possibility of the terrarium, a similar hiding was placed into the terrarium. This was ignored by the female but accepted by the male. The female ignored food during all the time it protected the eggs.

The incubation time of *P. tritaeniatus* was about 60 days (HALL et al., 1994; HARTMANN, 1998) and 74 for *Rhamphiophis oxyrhynchus rostratus* (WALSH AND DAVIS, 1978); the approximate same time was expected for *P. acutus acutus*. After three to four week, four of the eggs began to chime in, supposing they were sterile. After 80 days, the seven other eggs began to mould, indicating they were dead. Opening them revealed perfectly formed snake babies. The yolk bag was hard, indicating the death of the embryos for a couple of days.

During breeding, the female became only alert when spraying or cleaning the terrarium. This was shown by increased tongue flicks; hissing as for *P. tritaeniatus* (HARTMANN, 1998) was not heard and active defence of the eggs was not seen. So far it is preferable in the present case to speak about a “nest or egg attendance”, implicating remaining with the eggs or nest but without nest defence rather than a “nest or egg guarding”, which implies an active defence of the nest (after ZUG et al., 2001). The skin of the little snakes which did not have the possibility to hatch was analysed with SEM. The ultrastructure seen was identical to the one of the adults (Fig.8.3).



Fig.8.2: Female *P. acutus acutus* with its eggs.

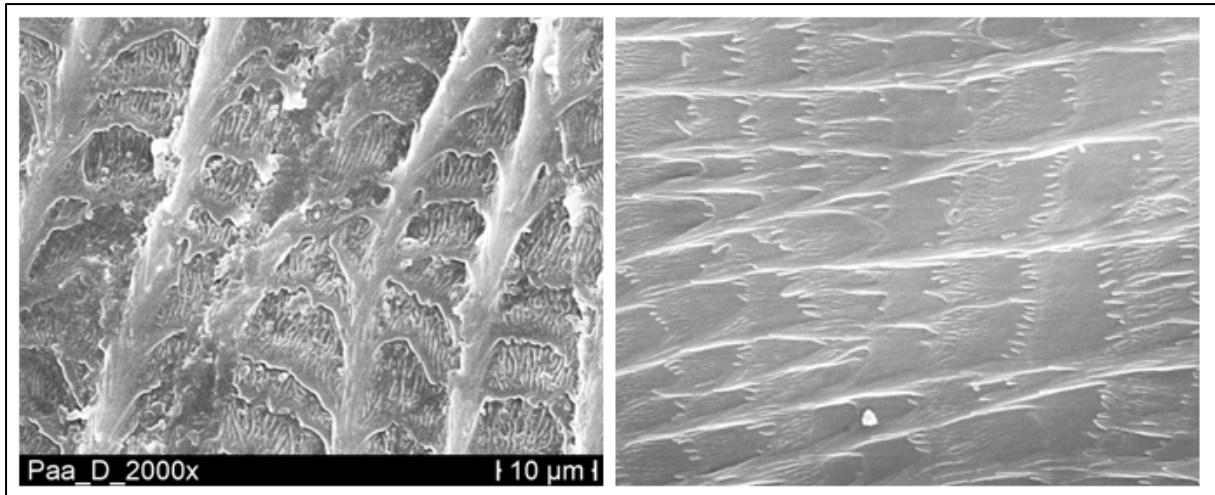


Fig.8.3: SEM picture of the ultrastructure of an adult (**left**) and unborn (**right**) *P. a. acutus*.

8.3 CURIOUS BEHAVIOURAL ASPECTS IN SOME OBSERVED PSAMMOPHIIDS

WAVE-LIKE MOVEMENTS

On some video tapes of *Psammophis elegans*, some *P. mossambicus* and *S. moilensis* curious body movements were noticed: after crawling the snake more or less “froze” and did undulating movements with the forepart of its body. MERTENS (1965) and RIEPPEL (1972) already recorded this behaviour for some species of *Boiga*, *Chironius*, *Coluber*, *Elaphe*, *Oligodon*, *Thelotornis*, *Telescopus* and *Malpolon monspessulanus* but without giving a satisfactory explanation.

MERTENS (1965) described these movements as little metameric contractions of the muscles, giving the appearance of waves. He thought these waves could be either the results of a possible frightening of the snake or that it is a kind of mimesis, giving the snake the appearance of a branch or liana. In the present study there was no reason why the snakes should frighten -nothing was seen on the video tapes. These undulating movements were observed when the snake was on the ground so that it is rather impossible to consider it as a kind of mimesis of a branch or liana.

SPREADING THE RIBS

Once, while feeding the female *Psammophylax acutus acutus*, it could be observed that it spread a little bit the ribs of its neck. It had just killed its prey and crawled around the mouse with the ribs spread.

VILLIERS (1963) and SWEENEY (1971) described a little hood as being not exclusive to *S. moilensis* but also present in the genus *Rhamphiophis*. In the present case it can not be assumed whether it was a kind of defensive behaviour as it is known for *S. moilensis*.

NASTY SMELLING

When handling both *Psammophylax acutus acutus* these snakes produced a nasty odour probably produced by their anal gland: The scent of this gland is known to have a repellent role towards enemies (OLDAK, 1976). Similar behaviour was reported in the related species *Psammophylax rhombeatus* (STEEHOUDER, 1987).

8.4 GENERA AND SPECIES FOR SEM

Table 8.2

Voucher of genera and species of Psammophiidae and control taxa examined with SEM.

Taxon	Control taxon	N°	RB known
<i>Coluber algirus</i>	✓	ZFMK 23155	
<i>Coluber hippocrepis</i>	✓	ZFMK 31716	
<i>Demansia Psammophis</i>	✓	ZFMK 36396	
<i>Dipsina multimaculata</i>		ZFMK 29480	
<i>Hemirhagerrhis hildebrandti</i>		ZFMK 21442	✓
<i>Liophis typhlus</i>	✓	ZFMK 85437	
<i>Scutophis moilensis</i>		shed skin	✓
<i>M. monspessulanus monspessulanus</i>		ZFMK 52175	✓
<i>M. insignitus fuscus</i>		shed skin	✓
<i>Masticophis mentovarius</i>	✓	ZFMK 57786	
<i>Masticophis flagellum</i>	✓	MHNG 2414.61	
<i>Mimophis mahfalensis</i>		ZFMK 83415	✓
<i>Natrix natrix</i>	✓	ZFMK 62405	
<i>Nerodia rhombifera</i>	✓	ZFMK 52968	
<i>Philodryas psammophidea</i>	✓	ZFMK 53244	
<i>Psammophis condanarus</i>		ZFMK 16658	✓
<i>P. elegans</i>		shed skin	✓
<i>P. mossambicus</i>		shed skin	✓
<i>P. phillipsi</i>		ZFMK 77028	✓
<i>P. schokari</i>		shed skin	✓
<i>P. sudanensis</i>		shed skin	✓
<i>Psammophylax acutus acutus</i>		shed skin	✓
<i>P. rhombeatus</i>		ZFMK 53455	✓
<i>P. tritaeniatus</i>		ZFMK 18906	✓
<i>Rhamphiophis oxyrhynchus</i>		ZFMK Kenya 1975	
<i>R. rostratus</i>		shed skin	✓
<i>R. rubropunctatus</i>		ZFMK 29006	✓
<i>Thamnophis sirtalis sirtalis</i>	✓	ZFMK 83421	
<i>Litoria caerulea</i>	✓	MHNG 1212.87	<i>Wiping behaviour</i>
<i>Phyllomedusa sauvagei</i>	✓	MHNG 2245.21	<i>Wiping behaviour</i>
<i>Polypedates maculatus</i>	✓	MHNG 2414.61	<i>Wiping behaviour</i>

8.5 RUBBING FREQUENCY DEPENDING ON TEMPERATURE

Table 8.3

Frequency on rubbing depending on temperature in psammophiids (number of RB ÷ number of observation hours).

Species	Snake no	Temp. 1 (27.24°C)	Temp.2 (31.72°C)	Temp. 3 (38.36°C)	Temp. 4 (67.16°C)
<i>Psammophis mossambicus</i>	1	0,26	0,21	0,29	0,13
	2	0,07	0,09	0,17	0,34
	3	0,17	0,11	0,21	0,49
	4	0,14	0,17	0,24	0,46
	5	0,13	0,08	0,32	0,26
	6	0,05	0,08	0,08	0,26
<i>Psammophis schokari</i>	7	0,23	0,19	0,14	0,32
	8	0,08	0,08	0,11	0,50
	9	0,03	0,15	0,08	0,21
	10	0,08	0,05	0,05	0,44
<i>Scutophis moilensis</i>	11	0,23	0,19	0,27	0,17
	12	0,16	0,04	0,20	0,27
	13	0,07	0,15	0,34	0,13
	14	0,31	0,33	0,33	0,39
<i>Malpolon insignitus fuscus</i>	15	0,10	0,19	0,12	0,20
<i>Psammophylax acutus acutus</i>	16	0,03	0,16	0,31	0,14
	17	0,07	0,07	0,07	0,03

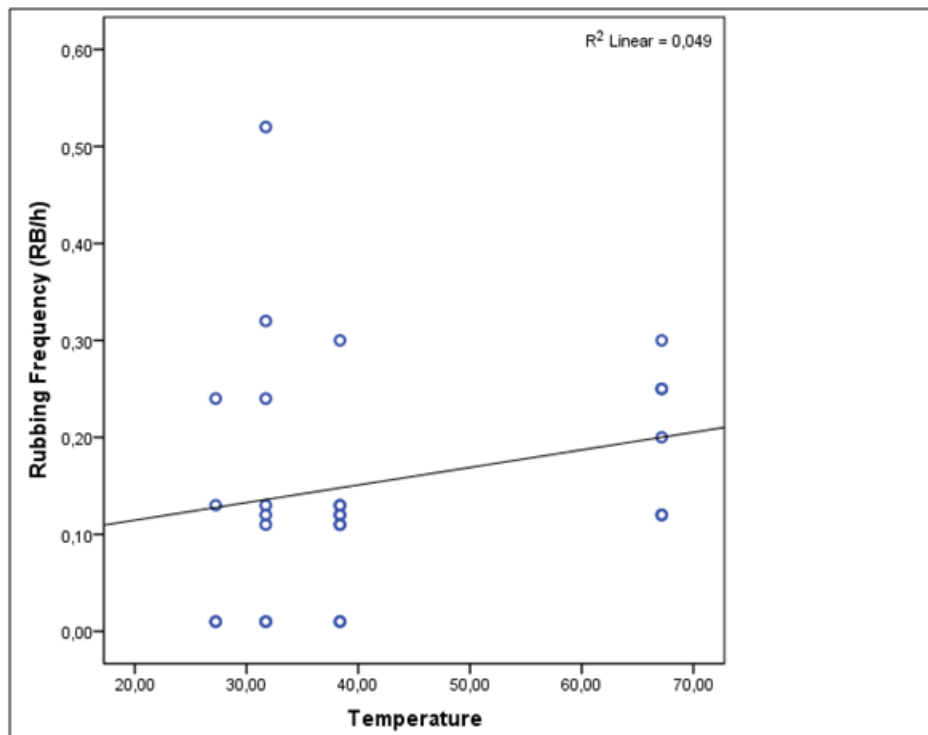
A) *Malpolon insignitus fuscus*

Fig.8.4: Correlation between temperature and rubbing behavior in *Malpolon insignitus fuscus*.

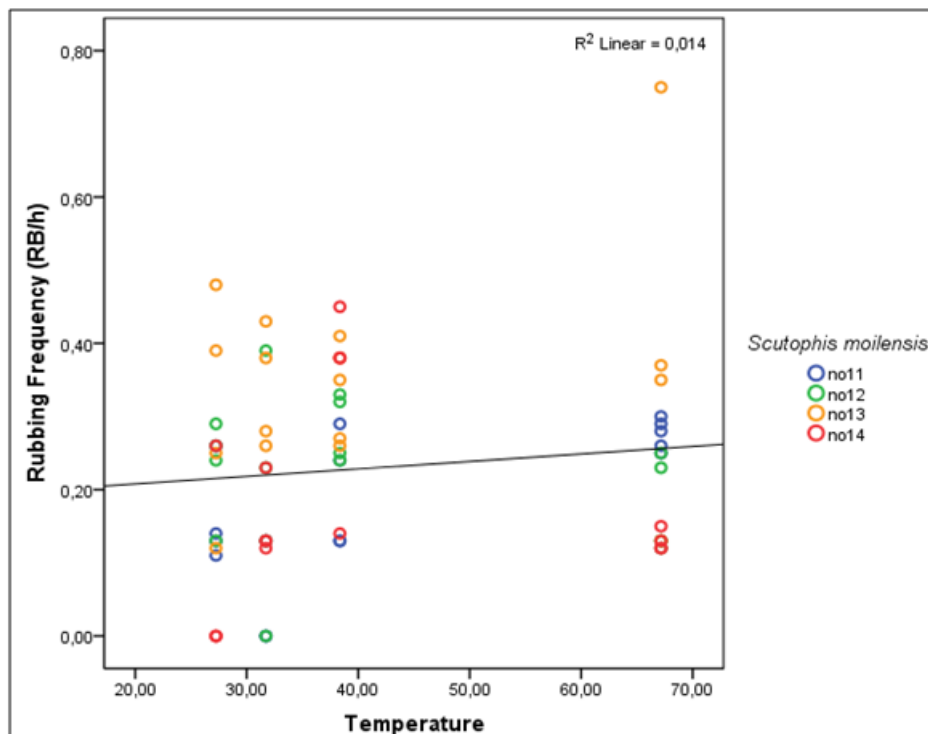
B) *Scutophis moilensis*

Fig.8.5: Correlation between temperature and rubbing behavior in *Scutophis moilensis*.

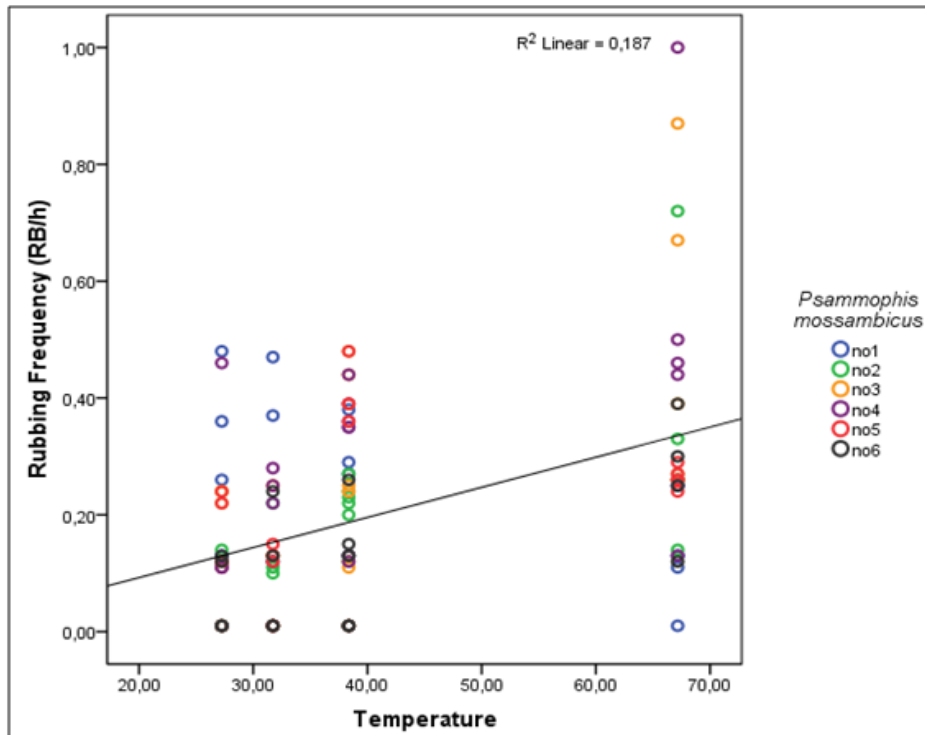
C) *Psammophis mossambicus*

Fig.8.6: Correlation between temperature and rubbing behavior in *Psammophis mossambicus*.

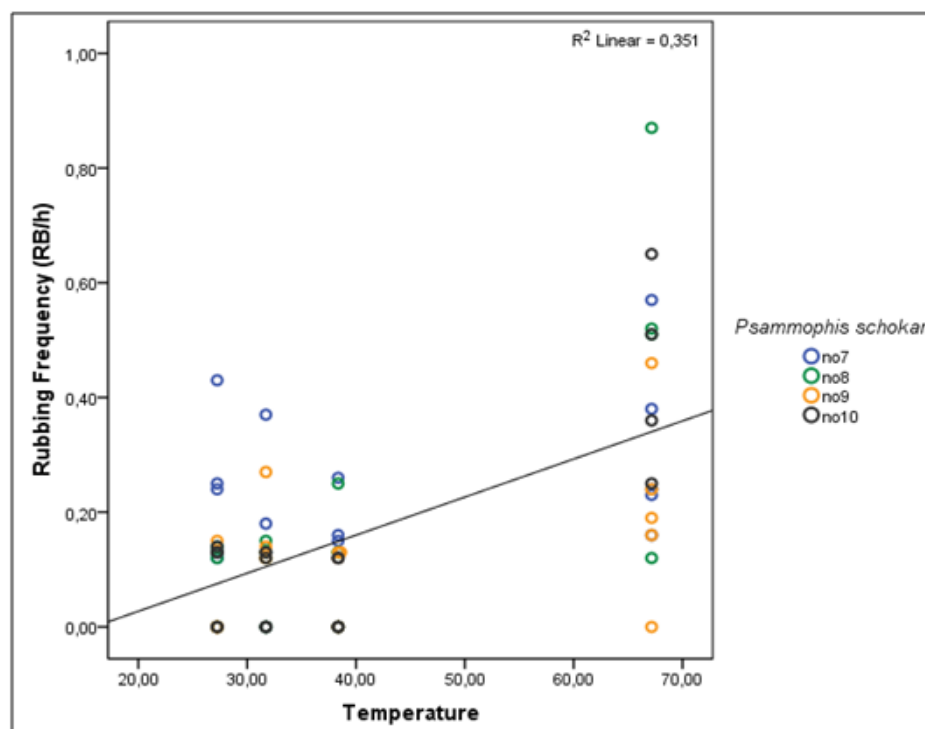
D) *Psammophis schokari*

Fig.8.7: Correlation between temperature and rubbing behavior in *Psammophis schokari*.

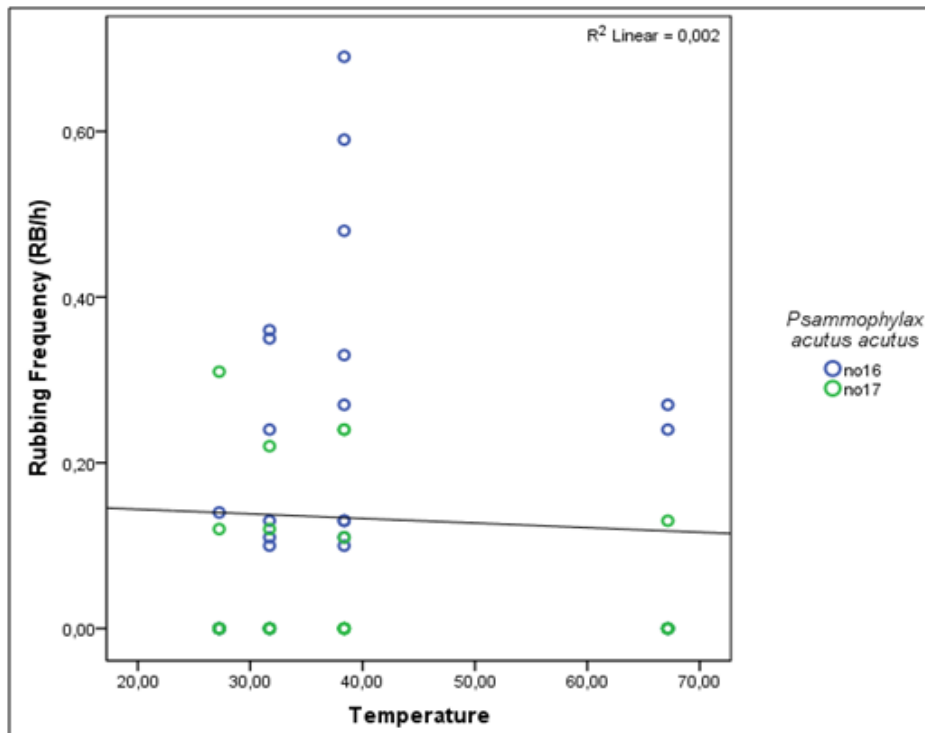
E) *Psammophylax acutus acutus*

Fig.8.8: Correlation between temperature and rubbing behavior in *Psammophylax acutus acutus*.

8.6 RUBBING FREQUENCY DEPENDING ON RELATIVE HUMIDITY

Table 8.4

Rank Spearman correlation test for the dependence of relative humidity on rubbing frequency in individuals of psammophiids. No dependence could be seen except for snakes no 12 and no 17.

Species	Snake no	$r_2=$	N=	p=
<i>Psammophis mossambicus</i>	1	0,115	10	0,751
	2	-0,360	13	0,227
	3	-0,174	14	0,553
	4	-0,505	7	0,248
	5	0,519	7	0,233
	6	0,354	12	0,259
<i>Psammophis schokari</i>	7	-0,467	9	0,205
	8	-0,416	11	0,203
	9	0,292	10	0,413
	10	0,192	11	0,572
<i>Scutophis moilensis</i>	11	-0,182	10	0,614
	12	0,669	10	0,035
	13	-0,182	10	0,614
	14	-0,015	14	0,958
<i>Malpolon insignitus fuscus</i>	15	-0,311	11	0,353
<i>Psammophylax acutus acutus</i>	16	-0,110	13	0,720
	17	-0,623	11	0,041

8.7 GEOGRAPHICAL DISTRIBUTION

Note: All information about the habitat of the snakes was taken from field guides specific to some African region. There was no guide about snakes throughout Africa. Therefore, some snakes have twice or more habitat, depending on in which country they were recorded.

Table 8.5
Geographical distribution and habitats of psammophiids.

Species	Country/ Region	Habitat
<i>Dipsina multimaculata</i> SMITH 1847	Namibia, S Botswana, C/W Republic of South Africa	Rocky sandy area (BRANCH, 1988)
<i>Hemirhagerrhis hildebrandtii</i> PETERS 1878	SE Sudan, Ethiopia, Somalia, Kenya, NE Tanzania	Savannah, coastal ticket (BRANCH, 1988)
<i>Hemirhagerrhis kelleri</i> BOETTGER 1893	S Sudan, Ethiopia, Somalia, Kenya, N Tanzania	
<i>Hemirhagerrhis nototaenia</i> GÜNTHER 1864	Namibia, N Botswana, NE Republic of South Africa (Transvaal, Natal), Zimbabwe, S/C Mozambique, S Angola, S Sudan, Somalia, Kenya, Tanzania, Malawi, Zambia, N/S Democratic Republic of the Congo (Zaire), Central African Republic, Ethiopia, Cameroon, Chad, Niger, Benin, Burkina Faso	Savannah, woodlands (BRANCH, 1988); semi-desert, dry and moist savannas (SPAWLS et al., 2002); wooded savannah (BROADLEY ET AL, 2003)
<i>Hemirhagerrhis viperina</i> BOCAGE 1873	SW Angola, NW Namibia	
<i>Malpolon monspessulanus</i> HERMANN 1804	Italy (W Liguria), SE France, Spain, Portugal, Morocco; along north- western Atlantic coast from Bou Izakarn (Morocco to Dakhla (Western Sahara); inland extensions up to Aoulouz and Tafraoute on southern slopes of western Atlases including the lower Drâa valley and upper Souss valley	
<i>Malpolon insignitus</i> GEOFFROY DE ST-HILAIRE 1809	Syria, E. Morocco, Algeria, Tunisia, Libya, N Egypt, Palestine, Israel, Jordan, Syria, Turkey, Greece (Corfou), Cyprus, Iraq, Iran, Lebanon	

<i>Scutophis moilensis</i> REUSS 1834	Morocco, Algeria, Tunisia, Libya, Egypt, Israel, Sinai, Jordan, Syria, Iraq, SW Iran, Saudi Arabia, Oman, United Arab Emirates (UAE), Kuwait, Mauritania, Niger, Mali, Sudan, Eritrea, Ghana(?)	Arid areas (TRAPE and MANÉ, 2006)
<i>Mimophis mahfalensis</i> GRANDIDIER 1867	Madagascar	
<i>Psammophis aegyptius</i> MARX 1958	SE Algeria, S Egypt, Libya, Israel, Niger, Chad	Desert (TRAPE and MANÉ, 2006)
<i>Psammophis angolensis</i> BOCAGE 1872	Zimbabwe, N Botswana, NE Republic of South Africa, S/C Mozambique, NE Namibia, S Democratic Republic of the Congo (Zaire), Tanzania, Zambia, Angola, Malawi, Ethiopia (relict population)	Moist and dry wooded savannah (BRANCH, 1988); high grassland, coastal ticket (SPAWLS et al., 2002); woodland (BRAODLEY, 2003)
<i>Psammophis ansorgii</i> BOULENGER 1905	Angola (central highlands)	
<i>Psammophis biseriatus</i> PETERS 1881 <i>Psammophis condanarus</i> MERREM 1820	S Somalia, Kenya, NE Tanzania, S Libya, Sudan, Ethiopia, Uganda N India, Pakistan, Nepal	Dry savannah, semi-desert, coastal ticket (SPAWLS et al., 2002)
<i>Psammophis crucifer</i> DAUDIN 1803	Republic of South Africa, E Zimbabwe, Swaziland, Mozambique	Highveld, montane grassland entering fynbos (BRANCH, 1988)
<i>Psammophis elegans</i> SHAW 1802	Senegal, Guinea-Bissau, Mali, Burkina Faso, Ghana, Benin, Togo, Nigeria, Central African Republic, Gambia, Ivory Coast, Cameroon, S Mauritania, Guinea, Burkina Faso, Togo, S Niger	Savannas, forest (TRAPE and MANÉ, 2006)
<i>Psammophis indochinensis</i> SMITH 1943	Thailand, Myanmar (Burma), Cambodia	
<i>Psammophis jallae</i> PERACCA 1896	NE Namibia, Botswana, NE Republic of South Africa, Zimbabwe, SE Angola, W Zambia, S Democratic Republic of the Congo (Zaire)	Grassland and savannah woodland (BRANCH, 1988); grassland and woodland on Kahlari sand (BROADLEY ETAL., 2003)
<i>Psammophis leightoni</i> BOULENGER 1902	Namibia, Botswana, Republic of South Africa, S Angola	Coastal fynbos, (semi)-desert, entering savannah (BRANCH, 1988); Kahlari thornveld, dry mixed savannah/ grassland (BROADLEY, 1962)
<i>Psammophis leithii</i> GÜNTHER 1869	Pakistan, Afghanistan, western India (Maharashtra (Poona) district)	

<i>Psammophis leopardinus</i> BOCAGE 1887	SW Angola, NW Namibia	
<i>Psammophis lineatus</i> DUMÉRIL, BIBRON & DUMÉRIL 1854	W Zimbabwe (Caprivi Strip), Democratic Republic of the Congo (Zaire), Congo, Central African Republic, Sudan, Senegal, S Mali, Guinea, Guinea-Bissau, Burkina Faso, Nigeria, Chad, Ethiopia, Angola, Uganda, Cameroon, Kenya [HR 12: 65], Botswana, Tanzania, Gambia, Ghana, N Malawi, Zambia	
<i>Psammophis lineatus</i>	W Zimbabwe (Caprivi Strip), Democratic Republic of the Congo (Zaire), Congo, Central African Republic, Sudan, Senegal, S Mali, Guinea, Guinea-Bissau, Burkina Faso, Nigeria, Chad, Ethiopia, Angola, Uganda, Cameroon, Kenya, Botswana, Tanzania, Gambia, Ghana, N Malawi, Zambia	Low, waterside vegetation (CHIPPAUX, 1999; BRANCH, 1988); swamp vegetation (BROADLEY ET AL., 2003); marshy area, secondary forest (TRAPE and MANÉ, 2006)
<i>Psammophis lineolatus</i> BRANDT 1836	East shore of Caspian Sea to S/E Kazakhstan, Azerbaijan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Afghanistan, Iran, Pakistan, Mongolia, NW China (Gansu, Ningxia, Xinjiang)	
<i>Psammophis longifrons</i> BOULENGER 1896	India (Maharashtra)	
<i>Psammophis mossambicus</i> PETERS 1882	Mozambique, Zambia	Waterside vegetation (SPAWLS et al., 2002; BROADLEY ET AL., 2003)
<i>Psammophis namibensis</i> BROADLEY 1975	Namibia	
<i>Psammophis notostictus</i> PETERS 1867	Namibia, S Angola, Republic of South Africa (Cape, S OFS), Botswana	Arid scrubland and karroid region (BRANCH, 1988; BROADLEY, 1962)
<i>Psammophis orientalis</i> BROADLEY 1977	E Kenya, Tanzania, Malawi, Mozambique, E Zimbabwe. May extend north to S Sudan and Ethiopia	Coastal and mesic ticket (BRANCH 2005)
<i>Psammophis phillipsi</i> HALLOWELL 1844	N Namibia, N Botswana, Zimbabwe, NE Republic of South Africa, Natal, S Mozambique, Tanzania, Sudan, Kenya, Senegal (near border of Guinea-Bissau), Gambia (HÅKANSSON 1981), Mauritania, Mali, Guinea, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Central	Moist savannah, low-dry grassland (BRANCH, 1988); forest (CHIPPAUX, 1999); moist savannah, riparian habitats, swamps, reedbeds, cultivated areas (BROADLEY, 1962)

	African Republic, Democratic Republic of the Congo (Zaire), Congo, Uganda, Equatorial Guinea, Gabon, Congo, Angola, Swaziland, Zambia	
<i>Psammophis praeornatus</i> SCHLEGEL 1837	Senegal, Guinea-Bissau, Mali, Burkina Faso, Ivory Coast, Ghana, Togo, Benin, S Niger, Nigeria, Cameroon, Central African Republic	Savannah (TRAPE and MANÉ, 2006)
<i>Psammophis pulcher</i> BOULENGER 1895	Ethiopia, S Kenya	
<i>Psammophis punctulatus</i> DUMÉRIL, BIBRON & DUMÉRIL 1854	Sudan, N Ethiopia, Eritrea, Djibouti, Uganda, N Somalia, NE Kenya, N Tanzania	Dry savanna, semi-desert (SPAWLS et al., 2002)
<i>Psammophis rukwae</i> BROADLEY 1966	Senegal, Cameroon, Algeria, Mali, Tanzania (“extending west to Senegal” fide BROADLEY & HOWELL 1991), Ghana	Moist savannah (SPAWLS et al., 2002)
<i>Psammophis schokari</i> FORSKAL 1775	NW India, Afghanistan, Pakistan, S Turkmenistan, Western Sahara (?), Morocco, Algeria, Libya, Egypt, Sinai, Israel, Mali, Niger, Mauritania, Nigeria, Sudan, Ethiopia, Eritrea, Somalia, Saudi Arabia, Oman, United Arab Emirates (UAE), Kuwait, Syria, Iraq, Iran (Kavir desert), Yemen	Arid areas, desert (CHIPPAUX, 1999; TRAPE and MANÉ, 2006)
<i>Psammophis sibilans</i> LINNAEUS 1758	Algeria, W Libya, Egypt, Eritrea, Ethiopia, Somalia, Namibia, SE Botswana, NE Republic of South Africa, Swaziland, E Zimbabwe, Angola, Zaire (?), Congo, Equatorial Guinea, Cameroon, Nigeria, Benin, Togo, Ghana, Ivory Coast, Liberia, Sierra Leone, Guinea-Bissau, Guinea, Senegal, Burkina Faso, Gambia, Mauritania, Central African Republic, Chad, Tanzania, “virtually pan-African”, Senegal	Highveld, montane grassland or rocky arid savannah (BRANCH, 1988)
<i>Psammophis subtaeniatus</i> PETERS 1881	N Namibia, N/E Botswana, Zimbabwe, Mozambique, E Democratic Republic of the Congo (Zaire), NE Republic of South Africa, Swaziland, S Angola, S/SE Zambia, E Kenya, Tanzania, Malawi, Sudan, Central African Republic, Ethiopia	Open dry savannah, thornveld, bushveld (BRANCH, 1988; CHIPPAUX, 1999; BROADLEY ET AL., 2003)

<i>Psammophis sudanensis</i> WERNER 1919	Central African Republic	Dry savannah (SPAWLS et al., 2002)
<i>Psammophis tanganicus</i> LOVERIDGE 1940	S Libya through Sudan, Eritrea, Ethiopia and Somalia, Kenya, Uganda, to C Tanzania	
<i>Psammophis trigrammus</i> GÜNTHER 1865	Namibia, S Angola, Republic of South Africa (Namaqualand)	Arid scrubland (BRANCH, 1988)
<i>Psammophis trinasalis</i> WERNER 1902	Namibia, Botswana, Republic of South Africa, S Angola	
<i>Psammophis zambiensis</i> HUGHES 2002	Zambia	
<i>Psammophylax acutus</i> GÜNTHER 1888	Angola, N/S Democratic Republic of the Congo (Zaire), Congo, W Tanzania, Burundi, Zambia, W Uganda, Cameroon, Nigeria, Benin, Togo, Ghana, Ivory Coast, W Zambia	Moist savannah and woodland, marshy area, floodplain (SPAWLS et al., 2002)
<i>Psammophylax rhombeatus</i> LINNAEUS 1758	S Namibia, Republic of South Africa, Lesotho, Swaziland, SW Angola	Highveld grasslands, fynbos, entering karroid area (BRANCH, 1988)
<i>Psammophylax tritaeniatus</i> GÜNTHER 1868	NE Namibia, N Botswana, Zimbabwe, NE Republic of South Africa, Angola, S Tanzania, Zambia, Malawi, S Democratic Republic of the Congo (Zaire), Zambia, Mozambique	Open grassland (BRANCH, 1988); moist savannah (SPAWLS et al., 2002); open grassland (BROADLEY ET AL., 2003).
<i>Psammophylax variabilis</i> GÜNTHER 1893	N Botswana (Chobe floodplain), N/S Democratic Republic of the Congo (Zaire), Tanzania, Burundi, Rwanda, Uganda, Kenya, Ethiopia, Malawi, Zambia, Mozambique; Highlands of Ethiopia, Kenya and N Tanzania, with a relict population in N Rwanda	Floodplain grassland (BRANCH, 1988); moist savannah (SPAWLS et al., 2002); montane grassland (BROADLEY ET AL, 2003)
<i>Rhamphiophis oxyrhynchus</i> REINHARDT 1843	N Botswana, Zimbabwe, S Mozambique, Zambia, N Democratic Republic of the Congo (Zaire), Rwanda, Burundi, Uganda, Ghana, Sudan, Central African Republic, Cameroon, Benin, Nigeria, Togo, Burkina Faso, Mali, Ivory Coast, Guinea, Senegal, Guinea-Bissau, Mali, Mauritania, Ethiopia, Somalia, Kenya, Tanzania, Malawi	Sandy ground, savannah (TRAPE and MANÉ, 2006); sandy thornveld or bushveld (BRANCH, 1988); moist savannah (SPAWLS et al., 2002)
<i>Rhamphiophis rostratus</i> PETERS 1854	Republic of South Africa (E Transvaal), E Africa from S Sudan and Ethiopia to moazmbique,	Semi-desert, dry and moist savannah, coastal ticket and woodland (SPAWLS et al., 2002);

<i>Rhamphiophis rubropunctatus</i> FISCHER 1884	through Somalia, Kenya, Uganda, Tanzania, Malawi, SE Zaire, Zimbabwe S Sudan, Ethiopia, Somalia, E Kenya, N Tanzania	sandy bush, termitoria (BROADLEY ET AL, 2003) Coastal ticket, woodland, semi- desert, dry savannah (SPAWLS et al., 2002); and scrublands (BROADLEY ET AL, 2003)
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