# Systematics, Taxonomy, and Evolution of *Urtica* L. (Urticaceae)

## Dissertation

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*Urtica dioica* subsp. *dioica* var. *hispida* in den Schweizer Alpen.

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# CHAPTER 1

## General Introduction

### 1.1 The family Urticaceae

The Urticaceae consist of 54 genera with more than 2000 species (Wu et al. 2013, Kim et al. 2015) and have a subcosmopolitan distribution with most genera and species found in the moist tropics (Fig. 1.1). The by far largest concentration is found in tropical Asia which seems to indicate an origin of the family in the humid regions of the palaeotropics (Takhtajan 1969, Friis 1989, 1993). Most of the genera and species prefer humid habitats, e.g. along streams, on moist soils of tropical montane forests at medium altitudes, or rarely of tropical lowland forests (Friis 1993). The Urticaceae were described by Antoine-Laurent de Jussieu in 1789 under the name "Urticae". He subdivided the family into three groups based on inflorescence morphology. "Group II" which he circumscribed as having flowers arranged in separate, commonly many-flowered receptacles, or flowers arranged in a capitate involucre of bracts, or flowers distinct and scattered (Conn & Hadiah 2009) contained the genera *Elatostema* Forst., *Boehmeria* Jacq., *Procris* Commers., *Urtica* L., *Forsskaolea* L. (as *"Forskalea"*) and *Parietaria* L. as well as *Cannabis* L., *Cecropia* L., *Artocarpus* Forst., *Morus* L., *Pteranthus* Forssk., *Humulus* L. and *Theligonum* L. which are however circumscribed in distinct families today [Moraceae, Urticaceae, (Cecropiaceae)]. Gaudichaud (1830: 491) revised the family as "La famille des urticées" and classified the genera into five tribes or subfamilies ("cinq tribus ou sous-familles"). He circumscribed the first unit (defined as "Urticées vraies") as having rectified ovules, initially attached at both ends, and with reversed, straight embryos (Conn & Hadiah 2009). He further subdivided these "Urticées vraies" into six "subdivisions": Elatostemateae (as "Elatostemeae"), Urereae, Boehmerieae, Parietarieae, Forsskaoleae (as "Forskalieae") and Cecropieae and thus used the currently accepted format for tribal names (suffix "–eae") for the first time (Conn & Hadiah 2009). Weddell (1854, 1856, 1869) attempted to subdivide the genera into natural units based on gender distribution and adopted the "subdivisions" as proposed by Gaudichaud (1830) and recognized these as tribes within the Urticaceae. Most importantly, he was the first who regarded the today's

Cecropiaceae, Moraceae and Urticaceae as distinct families and all subsequent taxonomists (e.g. Berg 1977, 1989, Friis 1989, 1993) followed this idea (Hadiah et al. 2008). Weddell used the same tribal names for Boehmerieae, Parietarieae, and Forsskaoleeae (as "Forskohleae") and modified the name Urereae to Urticeae. It is however unclear why Weddell changed the name of Gaudichaud's subdivision "Elatostemeae" to Lecantheae when he published the description of the genus *Lecanthus* in 1854. In his subsequent publication (Weddell 1856), he again changed the tribal name from Lecantheae to Procrideae when he defined the genus *Procris* as a member of this tribe. Friis (1989 and 1993) confirmed the tribal classification proposed by Weddell and supported it by the analysis of a broad spectrum of morphological characters, but did not adopt the name Procrideae but retained the older name Lecantheae as originally proposed by Weddell (1854). Conn and Hadiah (2009) revised the nomenclature of tribes within the Urticaceae and proposed that the "subdivisions" originally defined by Gaudichaud (1830) should be regarded as validly published (with the Cecropieae reduced to tribal rank again). Conn and Hadiah (2009) thus proposed the following tribal names: Boehmerieae Gaudich., Elatostemateae Gaudich., Forsskaoleeae Gaudich., Parietarieae Gaudich., Urticeae Gaudich., and Cecropieae Gaudich. Especially the taxonomic rank of Cecropieae/Cecropiaceae is still controversially discussed. Recent studies based on molecular data show that Urticaceae (including Cecropieae/Cecropiaceae) are monophyletic and that

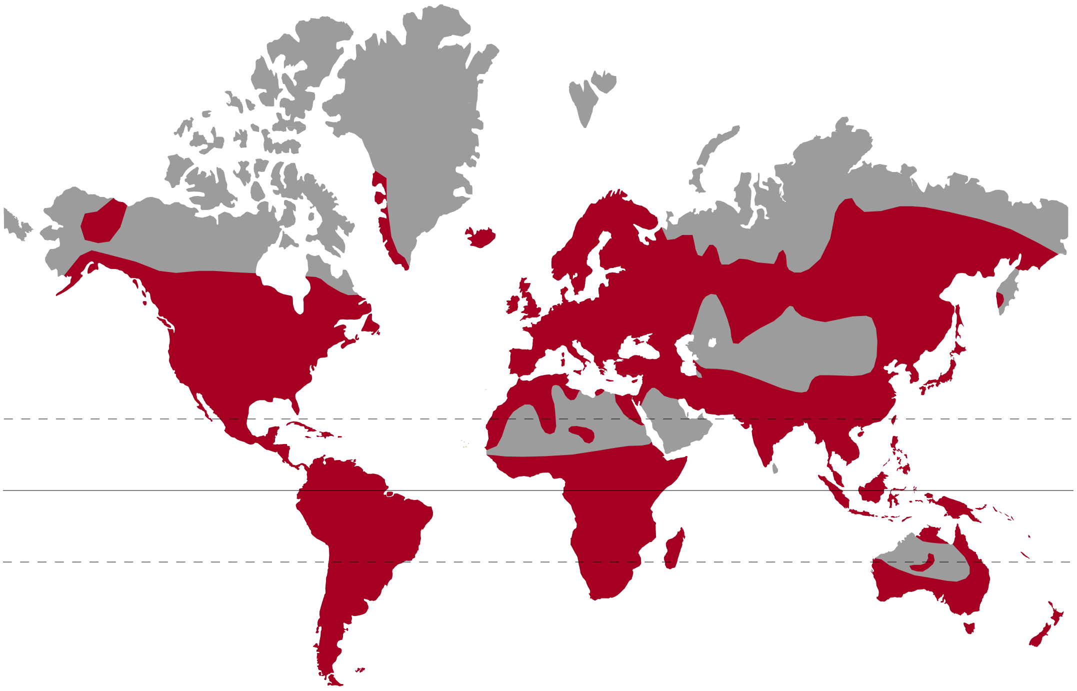

**Figure 1.1** Distribution map of Urticaceae. Solid line, equator; dashed lines, Tropics of Cancer and Capricorn. Map provided by C. Poretschkin, Bonn.

Cecropieae/Cecropiaceae are biphyletic with both lineages nested within the Urticaceae which indicates that Cecropiaceae should not be recognized as a distinct family (e.g. Hadiah et al. 2008, Deng et al. 2013, Wu et al. 2013, Kim et al. 2015). Currently, the family thus includes six tribes (Boehmerieae, Elatostemateae, Forsskaoleeae, Parietarieae, Urticeae, Cecropieae) which have demonstrated to be monophyletic each based on DNA sequence data (Wu et al. 2013, Kim et al. 2015).

## 1.2 The tribe Urticeae

The Urticeae comprise 12 genera and ca. 220 species. *Urtica* (63 spp.) is the largest genus within the Urticeae, followed by *Dendrocnide* (37 spp.), *Urera* (35 spp.) and *Laportea* (28 spp.; Friis 1993, Grosse-Veldmann et al. 2016b). Urticeae often occur in humid habitats under forest canopies or at forest margins in both the Old and the New World (Friis 1993, Hadiah et al. 2008, Deng et al. 2013, Kim et al. 2015). Some genera (e.g. *Obetia* and *Forsskaolea*) are adapted to dry habitats and some species of *Pilea* grow in the alpine vegetation of New Guinea (Friis 1988, 1993). Urticeae are economically important for its fibers (Woodland 1989). Beyond that, species of *Pilea* and *Urtica* serve as pot herbs, some *Pilea* species are used as ornamentals in horticulture and various members (especially *Urtica*) are used as medicinal plants (Woodland 1989, Friis 1993). Urticeae show a remarkable diversity of habits – from annual and perennial herbs (e.g. *Nanocnide*, *Girardinia*, *Urtica*) to shrubs, woody climbers, and trees (e.g. *Dendrocnide, Urera, Urtica*), with the majority of species being herbaceous perennials. All Urticeae have stinging hairs on their leaves and/or stems except for *Poikilospermum* (Kim et al. 2015). Moreover, two types of leaf arrangement can be found within the Urticeae: opposite leaves (*Hesperocnide, Urtica*) and alternate leaves (e.g. *Dendrocnide, Girardinia, Laportea, Nanocnide, Obetica, Urera, Zhengyia*). Stipule numbers range from one (e.g. *Zhengyia*), to two (e.g. *Nanocnide*) up to four (e.g. *Urtica*; Friis 1993, Kim et al. 2015). Male flowers usually have 4-5 tepals, female flowers 4 tepals, frequently with one pair larger than the other (Friis 1993).

All genera within the Urticeae are monophyletic except for *Urtica*, *Laportea* and *Urera*. *Urtica* is paraphyletic caused by two *Hesperocnide* species that are nested within *Urtica*. *Hesperocnide* has previously been separated from *Urtica* due to their tubular female flowers (Woodland et al. 1976), but this feature seems to have evolved several times independently within the Urticeae (see Kim et al. 2015). *Laportea* and *Urera* are polyphyletic and are both divided into three groups (Hadiah et al. 2008, Deng et al. 2013, Kim et al. 2015). Recent molecular studies (especially Deng et al. 2013, Kim et al. 2015) infer five clades within the

Urticeae: clade A (*Urtica* + *Hesperocnide, Zhengyia, Laportea I, Nanocnide*), clade B (*Dendrocnide, Discocnide*), clade C (*Girardinia*), clade D (*Laportea II*), and cade E (*Obetia, Urera, Poikilospermum*).

## 1.3 The genus *Urtica*

*Urtica* is of subcosmopolitan distribution, found on all continents, except the Antarctica, and most extratropical islands and ranges from Alaska to Patagonia, Spitzbergen to the Cape and Camtschatka to the subantarctic islands. Over most of its range *Urtica* is frequently an extremely common genus, often found in anthropogenic habitats. *Urtica* is commonly found as a weed, but can occupy a range of natural habitats (Figs. 1.2, 1.3). It is found in montane forests in Macaronesia, East Africa and in the Andes, but also in High Andean paramo and puna habitats at over 4500 m a.s.l. (Weigend et al. 2005). Many species are relatively widespread – e.g. the South American species *U. magellanica* (38–54° S) and *U. leptophylla* (6–11° N), other taxa are very narrowly endemic in the same overall region (*U. lalibertadensis, U. peruviana, U. urentivelutina;* Mutke et al. 2014). Additionally, island endemics (Fig. 1.4) are very common in the genus with endemic species found on Cyprus (*U. dioica* L. subsp. *cypria* H. Lindb.), Sicily (*U. rupestris* Guss.), Corsica and Sardinia (*U. atrovirens* Req. ex Loisel.), the Canary Islands (e.g. *U. stachyoides* Webb & Berthel.), Madeira (e.g. *U. portosanctana* Press), Mallorca (*U. bianorii* (Knoche) Paiva), Hispaniola (*U. domingensis* Urb.), Juan Fernández Islands (*U. glomerulaeflora* Steud.), Indonesia (*U. grandidentata* Miq.), Taiwan (*U. taiwaniana* S.S. Ying), Papua New Guinea (*U. papuana* Zandee), and New Zealand (including the Auckland and Chatham Islands; *U. perconfusa* Grosse-Veldmann & Weigend) which represents a degree of island colonization probably unique amongst flowering plants.

*Urtica* is highly associated with nutrient-rich sites (Olsen 1921, Pigott & Taylor 1964) and is known to be a nitrophilous plant with great N and P requirement and prefers to grow at high water availability sites (Pigott & Taylor 1964; Hejcman et al. 2012). However, it is also able to grow on dry and exposed calcareous grounds as well as on moist spring swamps, in scree forests, and on acidic raw humus in the understory of shady deciduous and coniferous forests (Reif et al. 1985). Under natural competition, *Urtica* prefers to grow on soils with a medium to high N and P content (Pigott & Taylor 1964). Due to the increasing anthropogenic influence of subnatural vegetation units, especially by additional nutrient input, *Urtica* has penetrated into plant communities whose environmental conditions show considerable

**Figure 1.2** *Urtica* in its natural habitat, **a**, *U. cannabina,* Mongolia*,* **b***, U. dioica* subsp. *dioica* var. *dioica*, Germany, Ostallgäu, **c**, *U. flabellata* on sheep excrement, Peruvian High Andes, **d**, *U. dioica* subsp. *dioica* var. *holosericea,* coniferous forest, Switzerland*,* **e**, *U. leptophylla,* Peruvian High Andes. Fotos: H. H. Hilger (a), M. Weigend (b, c, e).

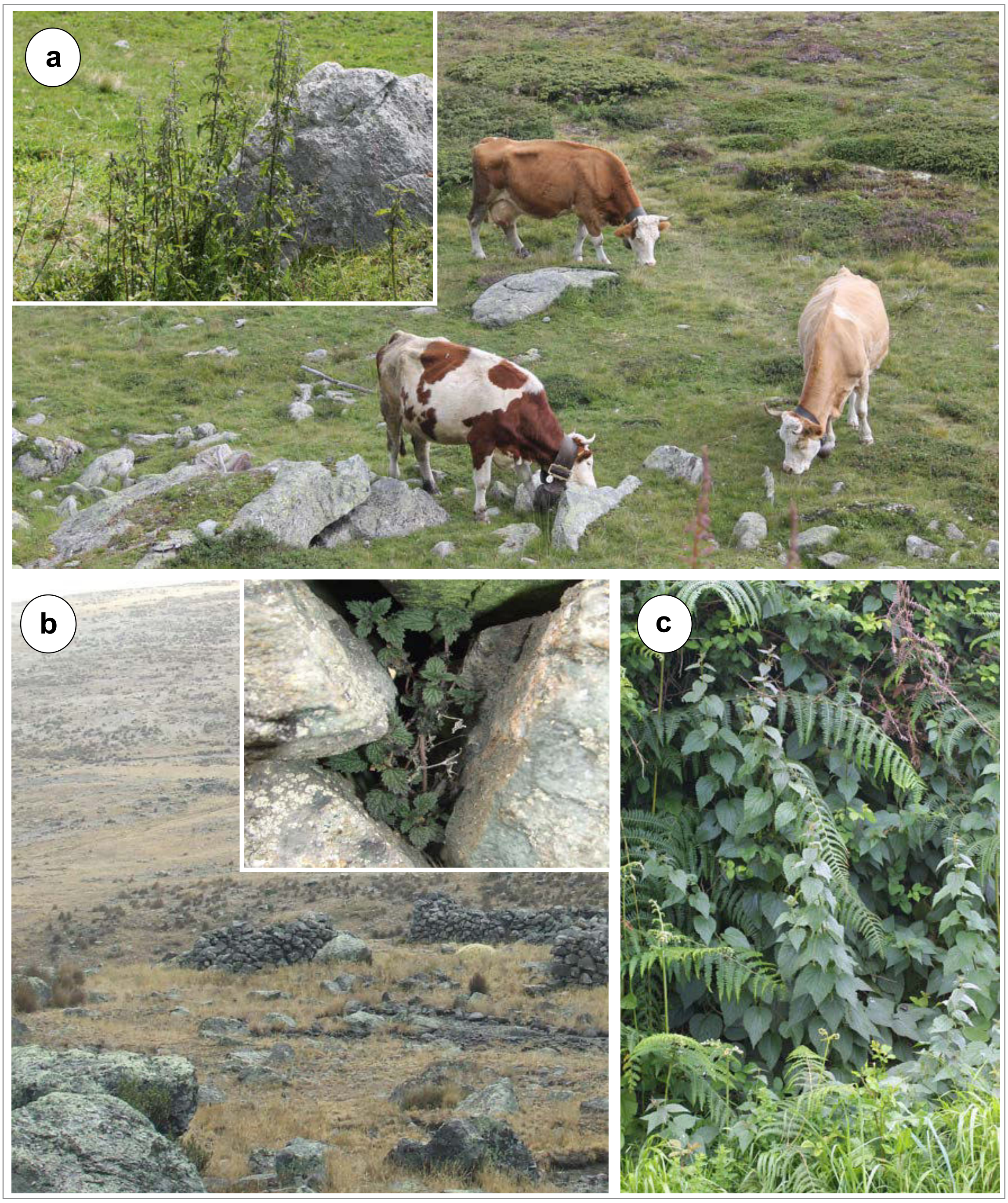

**Figure 1.3** *Urtica* in its natural habitat, **a**, *U. dioica* subsp. *dioica* var. *hispida,* Swiss Alps, landscape and detail*,* **b***, U. echinata*, Peruvian High Andes, landscape and detail, **c**, *U. morifolia,* Spain, Tenerife. Fotos: H. H. Hilger (c), M. Weigend (b).

differences to its natural habitat (Reif et al. 1985). However, *Urtica* thrives in these conditions and profits from the changes humans have made to its environment and is therefore a typical hemerophilic species. *U. dioica* subsp. *subinermis* from western European riparian habitats for example is not only able to build up steady populations in regularly streamed flood plains by its expanded and stable rhizome network, but is also able to actively influence flood plains by an efficient fixation of soil material. The subspecies usually forms spatially limited but dense populations on river banks, where even the neophyte *Impatiens glandulifera* (Balsaminaceae) rarely succeeds (Frank 2008).

## 1.4 Dispersal and diversification

Seed dispersal is crucial for gene flow and colonization and affects changes in gene frequencies within populations and geographic plant distributions on a broader scale. Most dispersal is local, but long-distance dispersal events are probably the factors which determine the selection of dispersal structures since they enable plants to colonize unoccupied habitats and thus increase the fitness of the population (Webb 1998). In general, selection favours a mixture of local and long-distance dispersal which is mostly achieved with a single type of dispersal structure (Webb 1998). In a study on the evolution of seed dispersal in the weed *Crepsis sancta* in an urban environment, Cheptou et al. (2008) showed that the dispersal mode evolves very fast. Species that were faced with changes in land use by humans (e.g. deforestation, urbanization, and agriculture) and the loss of habitats, reduced dispersal quickly. Consequently, the selection against dispersal in a fragmented landscape will reduce gene flow among populations, and thus exacerbate the isolation created by fragmentation, which could endanger the population persistence of the species.

Plants have several dispersal vectors to achieve dispersal on a local or a larger scale (long-distance dispersal) which are basically water dispersal (hydrochory), wind dispersal (anemochory), and animal dispersal (zoochory; Berg 1983, Czarnecka 2005). Dispersal by water is quite effective since it transports seeds to other wet places which have thus a high probability of being a suitable habitat. Wind dispersal in contrast is rather undirected and transports seeds to a variety of habitats which are probably not exactly suitable. Animal dispersal is suitable for local and long-distance dispersal and can be further differentiated according to the mechanism the seeds are distributed – endozoochory (transport within an animal) or epizoochory (transport on the outside of an animal). Soons et al. (2008) stated that especially mammals and birds are effective long-distance dispersal vectors, which is particularly important for plants living in dynamic habitats. They investigated the dispersal of wetland plants by ducks and found out that most seeds were dispersed up to 780 km, and the smallest seeds up to 3000 km during migration. 21 of the 23 species investigated could be dispersed by ducks, and up to 32 % of the ingested seeds germinated successfully. Species with the smallest seeds passed fastest through the digestive tract, were retrieved in the greatest numbers and germinated best. Seed coat thickness seems to play a minor role. Tabacchi et al. (2005) investigated seeds distributed along a transverse successional gradient of a riparian zone in France on an area of 16 qm. In total, 117 000 seeds belonging to 296 species were collected and sixty plant species represented 95% of all seeds trapped. *Urtica dioica* (55 899 seeds) was among the ten most abundant species. Mouissie et al. (2005) showed in a simulation study, that sheep were the best long-distance dispersal vectors and suitable for seeds of any morphology. Cattle and fallow deer dispersed bristly and hooked seeds over long distances, but not smooth seeds. Wood mice generated only short distance dispersal and dispersed seeds no further than 12 m.

The genus *Urtica* seems to have undergone numerous dispersal-establishment events both between continents and onto different islands. The high dispersability of *Urtica* is already indicated by the widespread occurrence of island endemics. Between the major landmasses and islands typically two independent colonisations are observed. Hawaii and the Juan Fernández Islands are the only exception. Three recent species radiations are inferred in *Urtica*, one in America centered in the Andes, one in New Zealand, and one in northern Eurasia. Both South Africa and New Zealand were apparently colonized twice independently. The Americas comprise two different clades of *Urtica*, and there appears to have been repeated dispersal between North and South America in both of these clades (see Grosse-Veldmann et al. 2016b).

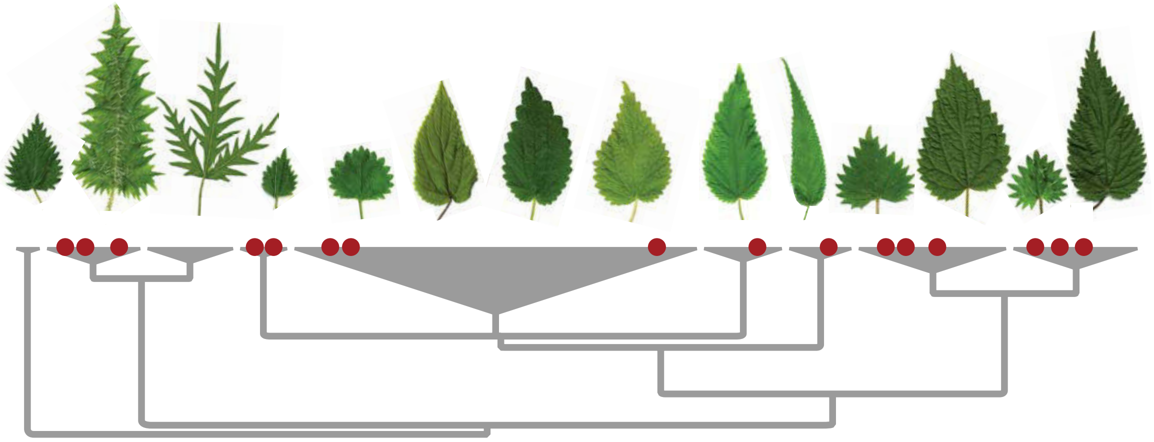

**Figure 1.4** Distribution of island endemics (red dots) across the genus *Urtica*. Figure published as “graphical abstract” in the online version of Grosse-Veldmann et al. (2016b) in *Molecular Phylogenetics and Evolution*.

Urticaceae fruits are achenes, i.e. they are monocarpellate and indehiscent. Focussing on the tribe Urticeae, the achenes are either dispersed without perianth (some species of *Urtica* and *Laportea*), enclosed in a dry, basically unaltered (sometimes accrescent) perianth (other species of *Urtica*, *Laportea* and *Hesperocnide*), in an accrescent dry perianth forming wings (*Obetia*), or in an accrescent fleshy perianth (*Urera*; Friis 1993). The achenes are small and dry and apparently without specialized features for dispersal. In some genera of the Urticeae, e.g. *Urera* and in the Boehmerieae, the perianth is persisting and becomes red and fleshy, possibly acting as an attraction for animal respectively bird dispersal (Friis 1993). Especially in *Urtica*, the dispersal of the fruits with or without perianth seems to be species-specific and represents the only substantial difference between species.

Mainly three different dispersal mechanisms are known in *Urtica*: wind dispersal, water dispersal and animal dispersal (endo- and epizoochory). The dispersal by humans is here regarded as a form of animal dispersal. Within these mechanisms, *Urtica* fruits have developed a broad spectrum of different dispersal structures depending on their specific habitats. Dispersal on the mainland is mainly accomplished by water and larger mammals. One-island-endemics are mostly dispersed by larger mammals. Endemics occurring on several islands, e.g. Macaronesia (*U. stachyoides*, *U. portosanctana*, *U. morifolia*) have fruits that are perfected for bird-dispersal and are probably not qualified for the dispersal by mammals. As soon as the fruits are humidified by the air, they produce sweet mucilage (myxocarpy), and are thus able to adhere to the feathering of birds. Alternatively, the sweet mucilage is probably a sugar source for birds which consume the sweetened seeds and thus disperse them. Species occurring on e.g. Mallorca/Menorca (*U. bianorii*) or Sardinia/Corsica (*U. atrovirens*) have fruits that are mainly adapted to water dispersal. Fruits of high montane species (e.g., *U. trichantha* from the Peruvian High Andes or *Hesperocnide tenella* from the Sierra Nevada in California and Hawaii) have a dense cover of large trichomes, which are able to adhere to the coat of small mammals and are thus specialized for the dispersal by small mammals. Wind dispersal seems to be inefficient both on the mainland and between islands and is otherwise only possible with the presence of a perianth.

An analysis of the endozoochorous seed dispersal by the European bison (*Bison bonasus*) revealed, that *U. dioica* is an important food source even for bisons. Nearly half of all seedlings recorded from bison dung samples belonged to *U. dioica* (Jaroszewicz et al. 2009). Even higher values were obtained for the donkey (*Equus asinus asinus). U. dioica* was the by far most abundantly germinating species (70 % of the seedlings in the endozoochory samples

(Couvreur et al. 2005). Also, Pakeman et al. (1999), Gill & Beardall (2001) and Eycott et al. (2007) investigated dung samples of various European wild animals, e.g. the western Eurasian fallow deer (*Dama dama),* the red deer (*Cervus elaphus),* the roe deer *(Capreolus capreolus),* the Muntjac *(Muntiacus reevesi),* and common rabbits *(Oryctolagus cuniculus),* and found that more than half (56 %) of all seedlings recorded, belonged to three plants species (*Urtica dioica, Chenopodium album* and *Agrostis stolonifera)*, with *Urtica dioica* the most abundant species. Even brown bears (*Ursus arctos*) and occasionally also grizzly bears (*Ursus arctos horribilis*) eat considerable amounts of *U. dioica* including their seeds (28 % of their herbal food; MacHutchon et al. 1993, Lagalisse 2002) and thus contribute to the dispersal of *Urtica*. Kuiters & Huiskes (2010) investigated sheep (*Ovis orientalis)* dung samples and showed that *U. dioica* seeds were found in 80 % of all recorded samples. Beyond that, *U. dioica* plays a major role in the nutrition of the nutria (*Myocastor coypus*; Prigioni et al. 2005) as well as the European beaver (*Castor fiber*; Krojerová-Prokešová 2010). Grazing animals prefer stinging nettles in a withered and dried condition; wood mouses (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) particularly eat the fruits and thus disperse them albeit on a more local scale (Watts 1968). Especially in winter, the seeds of *Urtica* are a common food source for birds, e.g. the bullfinch (*Pyrrhula pyrrhula nesa*; Newton 1969). Besides, seeds make up to 10 % of the food source of passerine birds as for example the cirl bunting *(Emberiza cirlus)* or the corn bunting *(Miliaria calandra;* Holland et al. 2006). Heinken & Raudnitschka (2002) investigated the diaspore diversity of vascular plants that attached to coat and hooves of roe deer (*Capreolus capreolus*) and wild boars (*Sus scrofa*) and found out that 10 % of all diaspores found, belonged to *U. dioica*. The nuts may also be dispersed by the attachment to lamb's wool (Fischer et al. 1996). In general, most dispersal mechanisms in *Urtica* are dependent on mammals.

## 1.5 Breeding systems

Urticaceae are wind-pollinated. Pollen grains are ca. 10–29 µm in diameter (e.g. Woodland et al. 1982, Friis 1993) and are actively ejected by sudden reflexion of the stamens in the male flowers (Friis 1993). According to Friis (1993), some species of *Elatostema* and *Boehmeria* are apomictic, i.e. they reproduce asexually (production of seeds without pollination). Apart from that, little is known about the reproductive systems in the family. Individual flowers of *Urtica* are always unisexual. This condition is quite rare in angiosperms with only 10 % of all angiosperms having unisexual flowers (Barrett 2002). There is no evidence for bisexual (hermaphroditic) flowers in *Urtica* so far.

The genetic mechanisms of sex determination in sexually dimorphic flowering plant species are diverse (e.g. Dellaporta & Calderon-Urrea 1993, Ainsworth et al. 1995, Barrett 2002, Glawe & de Jong 2009, Grant 1999). In most dimorphic species, the mechanisms of sex determination are however largely unknown (Shanon & Holsinger 2007). In dioecious plants, males mostly represent the heterogametic sex (producing two types of gametes), while females are homogametic (producing only one gamete type; Glawe & de Jong 2009). Moreover, Glawe & de Jong (2009) showed that monoecious plants are heterogametic as well. There is evidence for a maternal influence on sex determination and for the possibility of gynodioecy as an intermediate stage in the evolutionary pathway to dioecy (Shanon & Holsinger 2007). Shanon & Holsinger (2007) found out that multiple genes are involved in sex determination in *U. dioica* which is also supported by the observation that different genetic mechanisms for producing male and female individuals are obtained by different progeny segregations from male and female parents of different ancestry. Glawe & de Jong (2009) confirmed these observations and pointed out the dominance of the male allele over the female allele in unisexual *Urtica* species (especially *U. dioica*). In monoecious species however, the male and female alleles were co-dominant. The monoecious plants were heterozygous at a major sex determination locus. Their crossing experiments showed that after self-pollination, the offspring of monoecious plants segregated in monoecious, female and male individuals. Monoecious individuals were frequently produced in crosses between male and female individuals and this character was passed on to the offspring which has also been reported for *U. dioica* in Shannon & Holsinger (2007). Older literature (Strasburger 1910, Meurman 1925) refers to differentiated sex chromosomes, which could not be confirmed by Glawe & de Jong (2009) who did not observe morphological differences within the chromosome pairs.

Most chromosome counts for *Urtica* were carried out before 1970 (e.g. Funabiki 1958, Sokolovskaya 1966, Zhukova 1967), few of which were based on counts from several locations or on a broader geographical area (Woodland et al. 1982). The small size and stickiness of the chromosomes additionally complicate the counting. Earlier counts of European taxa reported a polyploid series based on x=12. Subsequent studies of Löve & Löve (1975a, b) revealed x=13. Woodland et al. (1982) therefore assumed that chromosomes have been lost during preparation. Studies of perennial *Urtica* in North America by Woodland et al. (1982) as well revealed a base chromosome number of x=13 with diploid and tetraploid levels. In general, chromosome counts only exist for few *Urtica* species (see Tab. 1.1 for additional information). Different chromosome numbers have been reported (2n=24, 26, 32, 48, 49, 52). Artificial hybridization showed that diploid and tetraploid chromosome races were infertile within their particular chromosome level. *U. dioica* (n=26) was crossable with other tetraploid taxa, but incompatible with diploid taxa. The tetraploid crosses resulted in dioecious hybrids.

**Table 1.1** Compilation of chromosome numbers of different *Urtica* species found in the literature.

| Taxon | Chromosome number | Reference |
|---|---|---|
| "Asiatic taxa" *(U. platyphylla* Wedd.*, U. angustifolia* Fisch. ex Hornem.) | 2n=52 (tetraploid); 2n=48 (aneuploid); 2n=76-78 (polyploid/aneuploid) | Funabiki (1958), Sokolovskaya (1966), Woodland (1982), Zhukova (1967) |
| *U. dioica* | 2n=52 (tetraploid) | Mowforth (1986) |
| *U. dioica* L. subsp. *gracilis* Ait (Selander) | 2n=26 (diploid); 2n=52 (tetraploid) | Woodland et al. (1982) |
| *U. dioica* L. subsp. *dioica* | 2n=52 (tetraploid) | Woodland et al. (1982) |
| *U. dioica* L. subsp. *holosericea* (Nutt.) R.F.Thorne | 2n=26 (diploid) | Woodland et al. (1982) |

Estimates of genome sizes in *Urtica* range from 597 to 1540 Mbp for *U. dioica* (Mowforth 1986, Barow & Meister 2003, Bainard et al. 2011) and from 318 to 523 Mbp for *U. urens* (Benneth & Smith, 1976, Barow & Meister 2003). Genome sizes of other species have not been investigated so far.

## 1.6 The keystone species concept

The concept of keystone species was coined by Robert T. Paine in 1969. He originally introduced this concept by his work on a rocky shore community in California. The removal of the dominant starfish *Pisaster ochraceus* from a section of the shore produced significant changes in population density and species composition. The original 15 species assemblage in this section was reduced to eight species. Indirect evidence suggested that equivalent changes did not appear with the exclusion of other consumers. The collapse of a system as a result of the removal of one of its elements promoted the idea of the architectural analogy with the keystone to an arch. Subsequently, numerous species in a wide range of communities throughout the world have been claimed to have keystone species status (Payton et al. 2002). Various definitions of keystone species have been attempted. The probably most useful one is given by Power et al. (1996) which is an expanded definition from the original usage of Paine (1969). She defined an ecological keystone species as a species that has a disproportionally large effect on the biodiversity of its ecosystem relative to its abundance. A keystone species plays a major role in maintaining the structure of an ecological community, affecting many other organisms in an ecosystem and helping to determine the types and numbers of various other species in the community. The role that a keystone species plays in its ecosystem is analogous to the role of a keystone in an arch. While the keystone is under the least pressure of any of the stones in an arch, the arch still collapses without it. The same effect can be observed for a keystone species. An ecosystem may experience a dramatic shift if a keystone species is removed, even though that species was a small part of the ecosystem by measures of biomass or productivity.

### 1.6.1 *Urtica dioica* – a keystone species?

Since *Urtica*, especially the widely distributed and weedy species *U. dioica,* harbours quite many animals relative to its biomass, it is probably an ecological keystone species and thus highly significant and even responsible for the biodiversity in its ecosystem (Davis 1989; Bryant et al. 1997, 2000; Hardy & Dennis 1999; Stefanescu 2001).

Stinging nettles are an essential food plant for a broad range of invertebrates and vertebrates and also provide an important habitat for them. Many species of butterflies (e.g. *Aglais urticae*, *Inachis io*, *Vanessa atalanta*) and cicada (e.g. *Eupteryx urticae*, *E. cyclops* and *E. aurata)* use *U. dioica* as main food source, some of them are even exclusively dependent on that species. Tuberville et al. (1996) showed, that stinging hairs of *U. dioica* did not interfere with feeding by the investigated invertebrates (*Vanessa atalanta*, *Popillia japonica*, *Chortophaga).* In a study of Cates & Orians (1975), two species of slugs (*Agriolimax reticulatus* and *Cepaea nemoralis*) showed strong preferences for their leaves. Not only invertebrates, but also a broad range of mammals and birds use *U. dioica* as their food source as e.g. European bisons (*Bison bonasus*; Jaroszewicz et al. 2009), donkeys (*Equus asinus asinus*; Couvreur et al. 2005), western Eurasian fallow deer (*Dama dama),* red deer (*Cervus elaphus),* roe deer *(Capreolus capreolus),* Muntjacs *(Muntiacus reevesi),* common rabbits *(Oryctolagus cuniculus;* Pakeman et al. 1999, Gill & Beardall 2001, Eycott et al. 2007) : brown bears (*Ursus arctos*) and grizzly bears (*Ursus arctos horribilis*; MacHutchon et al. 1993, Lagalisse 2002), sheep (*Ovis orientalis;* Kuiters & Huiskes 2010), nutria (*Myocastor coypus*; Prigioni et al. 2005), European beaver (*Castor fiber*; Krojerová-Prokešová 2010), wood mouses (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*; Watts 1968) as well as birds, e.g. the bullfinch (*Pyrrhula pyrrhula nesa*; Newton 1969), and passerine birds (*Emberiza cirlus, Miliaria calandra;* Holland et al. 2006). *U. dioica* however is not only an indispensable food plant, but also an important habitat for many animals. Large *Urtica* populations are an indicator for disturbed habitats and nutrient input and may replace an otherwise diverse herbal layer (Lethmate 2005). Davis (1989) investigated the insect fauna of *U. dioica* in Central- and Southern Europe and identified over 100 insect species in six orders, of which ca. 48 herbivorous and several predatory species usually occur together with *U. dioica a*nd 24 species were even exclusively restricted to *U. dioica*, respectively strongly associated with it. Judd & Hodkinson (1998) investigated in particular the association of floor bugs with *U. dioica* and found out, that the two bug species *Heterogaster urticae* and *Scolopostethus thomsoni* very frequently occurred together with *U. dioica.*

## 1.7 Economic significance and commercial use

Besides its enormous ecological significance, *Urtica* is also essential for humans. For though, stinging nettles usually are unwelcome guests, they are extensively used by humans especially as food plant and for medicinal use – and that all over the world. Several species of the genus *Urtica* (especially *U. dioica*) are used in one way or the other – a Google search mentions *Urtica* on more than one million websites; most of them dealing with its medicinal use, but also with its use in human nutrition (as green vegetable; Kavalali 2003), as animal fodder (Garber 1950, Bogachkov & Morozov 1990, Wetherilt 1992), in cosmetics, in horticulture as fertilizer, in the extraction of chlorophyll, in fiber production (Dreyer 1999) as well as with the ecological importance in different habitats up to phytoremediation (Khan & Joergensen 2006). *U. dioica* is so far the only species of *Urtica* to be cultivated commercially for pharmaceutical purposes (El Haouari et al. 2006, Exarchou et al. 2006, Nahata & Dixit 2012), for the commercial extraction of chlorophyll (known as the food additive E140) and for fiber production (Dreyer et al. 1999). In Europe, *U. dioica* was cultivated during the 19th century, and has a long history as a fiber plant. Especially the agricultural management of the plant is therefore relatively well documented (propagation, fertilization, harvest, etc.). *U. dioica* is well suitable for fiber production (Franke 1997, Dreyer 1999, Kavalali 2003): In May, seeds are sown or rhizomes respectively seedlings are planted outdoors and at the end of the flowering period which is usually in September, plants are harvested, whereat up to ten tons per hectare can be achieved. Numerous valuable metabolites have been described from *U. dioica*, including flavonoids, phenylpropanoids, scopoletin, β-sitosterol, , silicates and ascorbic acid (Chaurasia & Wichtl 1987, Bucar et al. 2006, Pinelli et al. 2008, Roschek et al. 2009). Caffeic acid analogues and fatty acids have been found in *U. dioica* and *U. urens* (Budzianowski 1991, Gansser & Spiteller 1995, Guil-Guerrero et al. 2003). The study of Farag et al. (2013) represents the first attempt to explore variation in secondary metabolites from a wide range of *Urtica* species. A total of 43 *Urtica* taxa (including subspecies and varieties) have been investigated, representing a broad geographic and taxonomical sampling. Overall, metabolite profiles indicate considerable phytochemical diversity in the genus. 43 metabolites were identified, with phenolic compounds and hydroxy fatty acids as the dominant substance groups. The irritant fluid of *Urtica* contains, amongst others, histamine, acetylcholine and serotonin. Pharmaceutical studies confirmed the positive effect of these substances in the therapy of arthritis, rheumatism, eczema, and allergic rhinitis as well as anaphylactic inflammations and prostate diseases (Hirano et al. 1994, Chrubasik et al. 1997, Legssyer et al. 2002, Kavalali 2003, Takahashi et al. 2008, Roschek et al. 2009).

## 1.8 Cryptic species – a result of molecular phylogenies

Cryptic or hidden species are usually defined as two or more distinct species that are erroneously classified (and hidden) under one species name (Bickford et al. 2007). Species are regarded as cryptic if they are classified as a single nominal species because they are at least superficially morphologically indistinguishable. Other authors state that cryptic species should be recently diverged, separable only with molecular data, occur in sympatry, or be reproductively isolated (Stebbins 1950). Since cryptic species are nowadays frequently detected by molecular data, Bickford et al. (2007) propose that DNA sequence analyses should be incorporated in the research of taxonomists as a routine technique and genetic material should be preserved for possible subsequent molecular analyses. Molecular data have expanded the ability to define and describe biological diversity dramatically. Especially next generation sequencing data seem to be a promising tool to uncover cryptic species (e.g. Leavitt et al. 2015, Zhan et al. 2015, Shen et al. 2016). Also the combination of molecular data with other types of data, especially data using non-morphological features such as e.g. chemical signals to distinguish otherwise undistinguishable species, are very promising and increasingly published (e.g. Brízová et al. 2013, Ocasio-Torres et al. 2014, Ruther 2014). Funk et al. (2011) regard the uncovering of cryptic diversity as important for the understanding and hence the conservation of biodiversity.

## 1.9 Aims and scope of the study

### 1.9.1 Research questions

1. Which “real” morphotypes can be retrieved for European *Urtica dioica* based on field and herbarium studies and the cultivation of material, and which names can be assigned to them?
2. Which species do really occur in Australia and New Zealand based on a critical re-examination of the specimens of “*Urtica dioica*” and “*Urtica incisa*” from Australia and

New Zealand and a comparison to authentic material of *U. dioica* and *U. gracilis?* Does true introduced *U. dioica* and *U. gracilis* exist in New Zealand?

3. How can the eastern Asian species "*Urtica laetevirens*" as well as the Southeast Asian *Urtica fissa*-clade be delimited and what are the infraspecific relationships within these species based on morphological and molecular data?
4. What is the overall phylogeny of *Urtica* based on a comprehensive sampling of the species and subspecific entities including the numerous local and regional endemics in the genus?
5. Does authentic dioecy exist in *Urtica* and which types of gender distribution can be found in the genus based on herbarium and literature studies and cultivated plants?
6. Do growth habit, leaf shape and gender distribution as classical morphological characters have phylogenetic information and are growth habit and gender distribution correlated with each other?
7. Is it possible to resolve infraspecific relationships within the Eurasian *Urtica dioica s.str.* clade based on highly resolving genotyping-by-sequencing-data?

### 1.9.2 Overview

The contents of the following chapters have been published, submitted or are in preparation to be submitted to a variety of peer-reviewed scientific journals. They are presented as the author's version and publication information is provided in the beginning of each chapter. All references are given in a combined reference list after chapter 8, and supplementary data for individual chapters are provided in Appendix A and B of this work.

Chapters 2 and 3 are dedicated to the taxonomy of *Urtica dioica sensu lato* which is the most problematic complex in the genus. In Europe, over 70 infrasubspecific names referable to the weedy *U. dioica* subsp. *dioica* have been used in one form or another, and over 40 of them are formally described. Many of these names are however invalid and/or superfluous, but five stable morphotypes could be identified based on field studies, literature and herbarium studies as well as the cultivation of material. Chapter 2 thus represents a characterization of these five morphotypes of *U. dioica* subsp. *dioica* and synonymizes all other infrasubspecific taxon names with one of them. In chapter 3, species limits of "*U. dioica*", – respectively "*U. incisa*" are redefined for Australia and New Zealand. There, these two names have been confused in the past. This study is based primarily on the investigation of herbarium material, and additionally results in the description of two new species, *U. sykesii* Grosse-Veldmann & Weigend and *U. perconfusa* Grosse-Veldmann & Weigend.

Chapters 4 and 5 are two taxonomic studies which aim at clarifying the relationships of two problematic Asian *Urtica* clades based on morphological as well as molecular data. Chapter 4 thus represents a characterization of the eastern Asian species *U. thunbergiana* and also includes the description of one new taxon from Taiwan, leading to the recognition of four subspecies, which are subsp. *thunbergiana,* subsp. *dentata* (Hand.-Mazz.) K.Becker & Weigend, subsp. *silvatica* (Hand.-Mazz.) K.Becker & Weigend, and subsp. *perserrata, subspec. nov.* Chapter 5 examines a monophyletic group of taxa around *Urtica fissa* from East-Southeast Asia and aims at resolving their confused taxonomy. A total of five species and two subspecies are recognized within the *Urtica fissa*-clade.

Chapter 6 is a molecular study based on a four-marker-analysis, representing the first comprehensive phylogeny of the genus *Urtica* and including 61 of the 63 species currently recognized. The study, moreover, comprises samples from all over the world, which makes it possible to analyse the biogeography sorting of the genus.

Chapter 7 is dedicated to the evolution of sexual systems in *Urtica* and is based on both extensive morphological studies and the phylogenetic data retrieved in chapter 6. Since no comparable patterns of gender distribution exist in other plant groups, the chapter also defines technical terms for all unique sexual systems found in *Urtica* (and closely related genera) and lists those terms that are already established.

The study presented in chapter 8 uses highly resolving genotyping-by-sequencing (GBS) data in an attempt to investigate relationships within the Eurasian *U. dioica s.str.* clade, including the morphotypes redefined in chapter 2. Classical marker studies failed to resolve these satisfactorily (see chapter 6). A remarkable amount and quality of data was retrieved (over 4000 loci and over 30000 SNPs). However, the GBS data also failed to resolve the issue.

### 1.9.3 Contribution to Chapters

Chapter 2: Grosse-Veldmann, B. & Weigend, M. (2015) Weeding the nettles III: Named nonsense versus named morphotypes in European *Urtica dioica* L. (Urticaceae). *Phytotaxa* 208(4): 239-260.

Own contributions: Revision of literature, herbarium specimens and cultivated plants, and writing the manuscript (together with M. Weigend), preparation of tables and figures

Chapter 3: Grosse-Veldmann, B., Conn, B. J., Weigend, M. (2016) Weeding the nettles IV: A redefinition of *Urtica incisa* and allies in New Zealand and Australia, including the segregation of two new species *Urtica sykesii* and *U. perconfusa*. *Phytotaxa* 245(4): 251-261.

Own contributions: Revision of literature, herbarium specimens and cultivated plants, and writing the manuscript (together with M. Weigend), preparation of tables and figures

Chapter 4: Becker, K., Grosse-Veldmann, B., Weigend, M. (2016). Weeding the nettles V: Taxonomic and phylogenetic studies of the eastern Asian species *Urtica thunbergiana* Sieb. & Zucc. (Urticaceae). *submitted to Phytotaxa, 23/08/2016.*

Own contributions: Co-supervision of the M.Sc. thesis of Karin Becker, which resulted in two manuscripts (here shown as chapters 4 and 5)

Chapter 5: Becker, K., Grosse-Veldmann, B., Weigend, M. (2016). Weeding the nettles VI: Taxonomic and phylogenetic studies of the Southeast Asian *Urtica fissa*-clade (Urticaceae). *submitted to Phytotaxa, 23/08/2016.*

Own contributions: Co-supervision of the M.Sc. thesis of Karin Becker, which resulted in two manuscripts (here shown as chapters 4 and 5)

Chapter 6: Grosse-Veldmann, B., Nürk, N. M., Smissen, R., Breitwieser, I., Quandt, D., Weigend, M. (2016) Pulling the Sting out of Nettle Systematics – a Comprehensive Phylogeny of the Genus *Urtica* L. (Urticaceae). *Molecular Phylogenetics and Evolution* 102, 9–19.

Own contributions: Performance of phylogenetic analyses, parts of the lab work, writing the manuscript (together with M. Weigend and contributions of the other co-authors), preparation of tables and figures

Chapter 7: Grosse-Veldmann, B., Weigend, M. (2016) The geometry of gender–hyper-diversification of sexual systems in *Urtica* L. (Urticaceae). *submitted to Cladistics, 16/08/2016.*

Own contributions: Investigation of herbarium specimens, literature, and (to a minor degree) cultivated plants, performance of phylogenetic and ACR analyses, writing the manuscript (together with M. Weigend), preparation of tables and figures

Chapter 8: Limits of infraspecific differentiation of *Urtica dioica* L. (Urticaceae) based on genotyping-by-sequencing (GBS)-data, *in preparation.*

Own contributions: Collection of large parts of the plant material used, performance of phylogenetic and population structure analyses (if not stated otherwise), parts of the lab work, preparation of text, tables and figures

# CHAPTER 2

## Weeding the nettles III: Named nonsense versus named morphotypes in European *Urtica dioica* L. (Urticaceae)*

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### Abstract

*Urtica dioica* L. in its wider sense is reported from nearly all temperate zones of the world, from throughout Eurasia, from North America and South America, from S Africa and New Zealand. In Europe, over 70 infrasubspecific names referable to the widespread and often weedy *U. dioica* subsp. *dioica* have been used in one form or another; over 40 of them are formally described. Many of these names are invalid and/or superfluous. However, several identifiable morphotypes can be found, which are stable in cultivation and usually occur in several different regions of Europe and/or are characterized by some type of habitat preference. These morphotypes in their most characteristic expression are quite well differentiated, but are connected by a continuous series of intermediates in nature. As a working hypothesis we here propose the tentative recognition of the following five morphotypes: *U. dioica* subsp. *dioica* var. *dioica*, -var. *hispida,* -var. *sarmatica,* -var. *holosericea,* and -var. *glabrata*. A characterization of each morphotype and corresponding synonyms are presented here based on extensive field studies, literature and herbarium studies and cultivation of material. Lecto-and neotypes are designated where necessary for some of the more important names.

*Keywords:* infraspecific taxa, stinging nettle, taxonomy

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## 2.1 Introduction

The genus *Urtica* is taxonomically difficult and the bulk of the problems are centred around the perennial, rhizomatous taxa which are loosely associated with the name *Urtica dioica* Linnaeus (1753: 984) and its plethora of named infraspecific entities (Weddell 1856, 1869, Schreiber 1981). The past years have brought considerable progress in the taxonomical re-definition of individual components of this complex (Weigend 2005, 2006, Weigend & Luebert 2009, Henning *et al.* 2014). Especially the molecular studies in Farag *et al.* (2013) and Henning *et al.* (2014) for the first time permitted the identification of a monophyletic entity around *Urtica dioica* in the strict sense and the exclusion of numerous, superficially similar taxa from Australasia, E Asia, and the Americas from a more narrowly re-defined *Urtica dioica* s.l. This *Urtica dioica* s.l. is thus re-defined as a complex largely restricted to central and western Eurasia and Africa. As circumscribed by the phylogeny of Henning *et al.* (2014), these taxa are characterized by perennial, rhizomatous habit, ovate achenes and polygamy: The bulk of a clone is unisexual, but up to 10% of the clone can have both male and female flowers on the same plant (Heemskerk *et al.* 1998). The vast majority of other taxa in *Urtica* are strictly monoecious, including the American taxa that until recently were included in *U. dioica* (Henning *et al.* 2014). Molecular and morphological analysis permitted the removal of several taxa from *U. dioica*, somewhat simplifying taxonomy: *Urtica dioica* subsp. *gracilis*, *U. dioica* subsp. *holosericea* and closely allied *U. mollis* and *U. aquatica* have all been removed as subspecies to *U. gracilis* (Henning *et al.* 2014). Similarly, New Zealand specimens of *U. dioica* have been shown to belong to *U. incisa* Poir. (1816: 224) and Chinese material previously assigned to *U. dioica* has been shown to actually belong to an entirely unrelated complex around *U. cannabina* L. (1753: 984). Also, several morphologically well-characterized western Eurasian taxa were elevated to subspecies rank: *Urtica dioica* subsp. *afghanica* and *U. dioica* subsp. *kurdistanica*, both from Iran, Iraq and Afghanistan; *U. dioica* subsp. *pubescens* from southern and eastern Europe; *U. dioica* subsp. *cypria* from Cyprus, *U. dioica* subsp. *subinermis* from western European riparian habitats; and *U. dioica* subsp. *sondenii* from Scandinavia. These infraspecific entities are morphologically, ecologically and geographically defined and capture a considerable part of the morphological diversity in western Eurasian *Urtica dioica*. The crucial problems remain in the widespread and often weedy "type" subspecies *U. dioica* subsp. *dioica* in western Eurasia. This study is concerned with the large number of additional, so far unclarified names in the literature, mostly at the level of "variety", "subvariety" and "form". Weddell (1869) recognized 7 infraspecific taxa (1 variety and 6 subvarieties) overall, Ascherson & Graebner (1911) recognized 27 infraspecific

**Table 2.1** Overview over the infrasubspecific taxa of *U. dioica* L. recognized in selected floras. [§]Type variety not explicitly named, [*]superfluous or invalid name for type variety var. *dioica,* [+]incorrect for var. *subinermis*. [$]Probably meant to be a system of varieties, subvarieties and forms, but taxonomic levels not explicit.

| Source | Weddell 1869 | Opiz 1852 | Fiek 1881 | Pospichal 1897 | Ascherson & Graebner 1911[$] | Becherer 1956 | Fiori 1969 | Schreiber 1981 | Zapałowiecz 1908 |
|---|---|---|---|---|---|---|---|---|---|
| Region | worldwide | Czech Republic | Silesia (Poland) | „Austrian Coast“ (Croatia, Serbia) | Germany | Wallis/ Switzerland | Italy | Central Europe | Galicia (SE Poland, Ucrainia) |
| subsp. *dioica* | var. *vulgaris* subvar. *vulgaris*[*]<br>var. *vulgaris* subvar. *umbrosa*<br>var. *vulgaris* subvar. *hispida*<br>var. *vulgaris* subvar. *horrida*<br>var. *vulgaris* subvar. *duplicato-serrata*<br>var. *vulgaris* subvar. *glabrata*<br>var. *sicula* | (var. *dioica*)[§]<br>var. *monoica*<br>var. *hispida*<br>var. *umbrosa*<br>var. *latifolia*<br>var. *angustifolia* | (var. *dioica*)[§]<br>var. *microphylla*<br>var. *hispida*<br>var. *monoica* | var. *typica*[*]<br>var. *hispida*<br>var. *monoica* | var. *curvidens*<br>var. *vulgaris*[*]<br>var. *latifolia*<br>var. *hispida*<br>var. *hispidula*<br>var. *horrida*<br>var. *glabrata*<br>var. *carpatica*<br>var. *sarmatica*<br>var. *subsetosa*<br>var. *duplicato-serrata*<br>var. *trilobescens*<br>var. *macrodonta*<br>var. *lamiifolia*<br>var. *spicata*<br>var. *umbrosa* | (var. *dioica*)[§]<br>var. *hispida* | var. *typica*[*]<br>var. *galeopsifolia*<br>var. *hispida*<br>var. *glabrata*<br>var. *monoica*<br>var. *microphylla*<br>var. *sicula* | var. *dioica*<br>var. *androgyna*<br>var. *carpatica*<br>var. *elegans*<br>var. *hispida*<br>var. *hispidula*<br>var. *microphylla*<br>var. *mitissima*<br>var. *pilosa*<br>var. *spicata* | (var. *dioica* forma *dioica*)[§]<br>var. *dioica* forma *carpatica*<br>var. *dioica* forma *czarnohorensis*<br>var. *dioica* forma *parvifolia*<br>var. *dioica* forma *androgyna*<br>var. *mirabilis*<br>var. *hispida*<br>var. *sarmatica*<br>var. *sarmatica* forma *subsetosa* |
| Other subspecies (present in the area) | var. *galeopsifolia*<br>var. *pubescens* | | | | var. *subinermis* | | var. *galeopsifolia* | *var. subinermis* | |
| Other subspecies (not present in the area) | | | var. *subinermis* | var. *subinermis* | var. *xiphodon* | var. *subinermis* | | | var. *inermis*[+] |
| Other species (not present in the area) | var. *angustifolia* | | var. *angustifolia* | var. *angustifolia* | var. *angustifolia* | var. *angustifolia* | | var. *angustifolia* | |

taxa for Germany alone taxonomic levels not explicit, but probably meant to be a system of varieties, subvarieties and forms) and Schreiber (1981) recognized 11 different varieties within *Urtica dioica* subsp. *dioica* (compare Tab. 2.1). An additional complication arises from the fact that until recently infraspecific names were not routinely captured in taxonomic databases and are not included in the International Plant Names Index (IPNI 2014). This makes it very difficult and onerous to verify whether names found on herbarium specimens have been validly published. The purpose of this paper is to address the following two questions: 1) which "real" morphotypes can be retrieved based on field and herbarium studies and the cultivation of material, and, 2) which names can be assigned to them.

## 2.2 Material and methods

For this study ca. 200 living collections of European *Urtica dioica* were investigated in Berlin and Bonn. Plants were either taken into cultivation from rhizome cuttings or seeds and cultivated in borders under common garden conditions under identical watering and fertilization regimes. The character states expressed were compared to those observed in the wild-collected source material. In addition, a large number of herbarium specimens were revised, including those type specimens that could be located. In many cases unequivocal type material could not be traced in any of the herbaria investigated. Material was examined from the following herbaria: B, BM, BONN, BR, BSB, E, FI, FR, G, GB, JENA, K, KIEL, KRAM, LE, M, MA, MO, MSB, NY, P, PR, S, USM, W, herb. Hügin, Lang private herb. A large, but probably not exhaustive literature research was undertaken and the descriptions analyzed. Specimens and cultivated plants were subsequently revised and compared to the descriptions and characterizations of infrasubspecific taxa in the literature and organized into the morphotypes as delimited below. Morphological assignment of the often very scantily described taxa was not always straightforward and so geographical and ecological information was also considered for assigning the taxa to the varities recognized below. Approximately 100 old floras and floristic accounts from across Europe were evaluated for infraspecific names in *U. dioica*, ca 50 of them yielded relevant taxonomic information (see literature cited). Additionally, infraspecific names found on annotated herbarium specimens were recorded and—wherever possible—matched with names cited in the literature. In this study, all of the names located have been cited, even if they were only found as an annotation on a herbarium sheet: It would be beyond our resources to verify whether or not these taxa were validly published somewhere or whether potential type material exists. In addition, we refrained from assigning neotypes to validly published names where we could not trace type specimens: On the one hand, it was impossible to make an exhaustive investigation of especially Eastern European herbaria, where some of the types may be housed, on the other hand, the vast majority of these names are not relevant since they represent additional names for morphotypes which already have a name. We therefore limit ourselves to ensuring that the names of the five tentatively recognized morphotypes are here stabilized by type specimens and provide neotypes where necessary. Conservation assessments were undertaken using IUCN Red List Categories and Criteria (2001).

## 2.3 Results

Most modern floras refer only to ssp. *dioica* without further subdivision (e.g. Butcher 1961, Edmondson 1992, Wisskirchen 1998), but this does not signify that the numerous names for infraspecific taxa have been satisfactorily resolved: Several infrasubspecific taxa are still recognized in more than one national or local flora (see Tab. 1) and these appear to refer to identifiable, albeit not necessarily highly distinct or stable ecotypes. Most of these infrasubspecific taxa have to date not been formally assigned to a particular subspecies, have not been typified and they appear to include a number of synonyms. In addition to the problems of assigning these taxa to recognized species and subspecies, several of the names are also incorrectly interpreted. These putatively unpublished names are here provided with the potential type material so that they can be formally synonymised; a valid publication should be established at a later date.

One crucial problem in the identification of the infrasubspecific taxa is that the types were rarely assigned and in many cases it is doubtful whether a specimen was at all prepared. In general individuals of *Urtica dioica* not conforming to the "typical" morphological delimitation of the species have been assigned to various infraspecific names. The "typical" morphological delimitation of *Urtica dioica* is of a plant with unbranched shoots, broad ovate leaves along the entire stem and including the upper part of the inflorescence, a dense cover of stinging hairs and only one sex on each individual plant ("*dioica*"). This latter character has long been known to be variable with the polygamy of the species established by Heemskerk *et al.* (1998). We categorize all atypical morphotypes into one of the five distinct classes.

### 2.3.1 Names for the typical form

A range of *"nomina superflua"* have been created for the typical form of *Urtica dioica*, amongst them subsp. *eudioeca* Selander (1947: 271), var. *typica* Pospich. (1897: 339), var.

*latifolia* Ledeb. (1833: 240) and var. *vulgaris* Wedd. in DC. (1869: 50). These names are formally redundant.

### 2.3.2 Names for sexual morphs

*Urtica dioica* is known to have monoecious individuals in otherwise morphologically orthodox populations (Heemskerk *et al.* 1998); names for the monoecious forms are therefore superfluous. Names for these monoecious forms include var. *androgyna* Beck (1890) from the Austrian Alps, var. *hermaphrodita* Čelak. (1867) and var. *monoica* Tausch ex Ott (1851) both from Bohemia (Czech Republic) and var. *mirabilis* Zapał. from Galicia (Zapałowiecz 1908). Monoecious forms also occur in the other morphotypes of *Urtica dioica,* e.g. they are common in the forest form and in *Urtica dioica* subsp. *subinermis* (Uechtr.) Weigend.

### 2.3.3 Names for ontogenetic variants

Initially unbranched, *Urtica dioica* typically branches late in the season and loses its large leaves on the main axis. The lateral branches then have atypically small, often narrow leaves with very few stinging hairs, irrespective of the morphology of the leaves on the main stem. Several varietal names have been assigned to these ontogenetic variants, most of which refer to "forms" of var. *dioica* and are synonymous with that taxon. Thus the small-leaved, branched, and basally woody form of *Urtica dioica* has been described several times from: eastern Germany (var. *microphylla* Baen. 1862); Slovakia (var. *ramosissima* L.Richt. 1872); Hungary (var. *parvifolia* Wierzb. ex Heuff. 1858); Sweden (forma *ramosa* L.Neumann 1901); Italy [var. *sicula* (Guss.) Wedd. 1856]; and Turkey (var. *haussknechtii* Boiss. 1879). A late season plant of *U. dioica* subsp. *pubescens* has been described as var. *microphylla* Hausmann (1852, see Weigend 2006). Even if the main shoot fails to branch, it is common for it to lose most of its leaves, both basally and apically and this "leafless form" has been described as var. *spicata* Asch. & Graeb. (1898).

### 2.3.4 Names misapplied to European forms

There is one name that has been used with considerable confusion: *Urtica angustifolia* Fisch. ex Hornem. (Hornemann 1819: 107) which was described from material originally collected in northeastern Asia (Transbaikalia), but cultivated in Copenhagen. This taxon was included in recent molecular studies with material from NE Asia and comes out on a separate clade, unrelated to *Urtica dioica* in the strict sense (Henning *et al.* 2014). This species was, however, reduced to a variety as *Urtica dioica* var. *angustifolia* (Fisch. ex Hornem.) Ledeb. by Ledebour (1833) who concurrently created the superfluous name *Urtica dioica* var. *latifolia* Ledeb. to accommodate "typical" *Urtica dioica* (see above). Independently of Ledebour, Wimmer & Grabowski (1829: 336) had previously described a narrow-leaved form of *Urtica dioica* (see above) from Silesia (southern Poland) rendering Ledebour's name an illegitimate homonym. *Urtica dioica* var. *angustifolia* has been widely reported from Europe always as Ledebour's illegitimate combination that was based on the Asian taxon (Weddell 1856, 1869, Heuffel 1858, Fiek 1881, Ascherson & Graebner 1911, Garcke 1922, 1972, Becherer 1956, Schreiber 1981). The valid name *Urtica dioica* L. var. *angustifolia* Wimm. & Grab. (1829) was adopted by Schlechtendal (1832), but misapplied to a Mexican taxon (Hennig *et al.* 2014). This error was further compounded by Maire (1961) who cited the name incorrectly as "*Urtica dioica* var. *angustifolia* Schltdl." and the locality as North Africa—where the taxon does not occur. Wimmer & Grabowski's (1829) valid name *Urtica dioica* var. *angustifolia* Wimm. & Grab. referred to the European forest form of *U. dioica* (see below) which is synonymised with var. *holosericea* Fries (1828: 281) below.

### 2.3.5 Morphotypes and infrasubspecific variation that is stable in cultivation

Based on the analysis of herbarium specimens protologues, observations in the field and of cultivated material it is possible to assign morphotypes of *Urtica dioica* subsp. *dioica* which are stable in cultivation to valid infrasubspecific names. Based on this "taxonomy of morphotypes" we propose a system of weakly but consistently differentiated varieties, usually with clear differences in habitat preference and distributed across several different regions of Europe. These morphotypes represent the extremes of an essentially continuous variation in indument (simple trichomes, bristles and stinging hairs), leaf shape (broadly to narrowly ovate, base rounded to deeply cordate) and leaf margin morphology (shallowly and simply mucronate-serrate to more deeply and irregularly serrate, sometimes doubleserrate). These morphotypes and their corresponding names and synonyms are given below.

## 2.4 Discussion

The present study identifies a total of five morphotypes which show moderate degrees of morphological divergence. The morphology appeared to be stable overall when vegetative material was cultivated under controlled conditions. Future studies should be based on investigating the stability of these morphotypes when raised from seed under uniform and standardized conditions, including their stability across generations. The present study identifies the names available for these morphotypes; these should be recognized in the context of floristic or ecological studies. However, the present study does not address the phylogenetic relationships or monophyly of these taxa. Future studies should be directed towards identifying the infrasubspecific relationships in *Urtica* based on highly-resolving molecular markers. We consider it highly likely that morphologically similar populations arose several times independently in *U. dioica* and that some of the varieties here identified do not represent monophyletic entities, but the data available at present do not permit to address this question adequately.

## 2.5 Formal taxonomy

### 2.5.1 Key to the varieties of *Urtica dioica* subsp. *dioica* from Europe

1 Plants ca. 60–80 cm tall, densely covered with stinging hairs and bristles; appearing whitish; leaf margins coarsely and deeply serrate, some of the proximal teeth divided; inflorescences strongly branched and spreading in flower; perianth with one to several stinging hairs (on calcareous ground, mainly southern Alps and Pyrenees) ............................................................................*Urtica dioica* subsp. *dioica* var. *hispida*

\- Plants ca. 60–200 cm tall, more or less pubescent with few to numerous stinging hairs and bristles; never appearing whitish; leaf margins coarsely and regularly serrate, proximal teeth rarely divided; inflorescences rarely to strongly branched and pendulous to spreading in flower; perianth no, or only individual stinging hairs ..............................................................2

2 Plants densely covered with stinging hairs, short, stiff bristles and simple trichomes; median leaves broadly ovate with a cordate base; leaf margins coarsely and regularly serrate; inflorescences strongly branched; plants 60–150 cm tall (lowland and coastal weedy form) ............................................................................*Urtica dioica* subsp. *dioica* var. *dioica*

\- Plants sparsely covered with stinging hairs, bristles usually absent, simple trichomes sparse to very dense; median leaves broadly to narrowly ovate with marginally to distinct cordate base; leaf margins shallowly crenate to serrate; inflorescences sparsely to strongly branched; plants often up to 200 cm tall ..........................................................................................3

3 Female inflorescences densely branched, initially spreading, deflexed to pendulous in fruit; male inflorescences strongly branched and horizontally spreading; plants with dense and soft pubescence, sparsely covered with stinging hairs; leaves dark green, broadly ovate with distinctly cordate base; leaf margins coarsely and regularly serrate (dry forests in eastern Germany and Poland) ............................................*Urtica dioica* subsp. *dioica* var. *sarmatica*

\- Female inflorescences sparsely branched and lax, initially spreading, soon pendulous; male inflorescences typically very sparsely branched and pendulous; plants with more or less dense pubescence, very few stinging hairs and no bristles; leaves narrowly ovate, only marginally cordate; leaf margins shallowly crenate to serrate ..................................................4

4 Plants more or less densely pubescent, sometimes very densely so and velvety to the touch (less pubescent and with very narrow leaves in central and eastern Europe), never appearing

shiny, vivid green and lustrous; few stinging hairs and virtually no bristles (deciduous, coniferous and mountain forests) .........................*Urtica dioica* subsp. *dioica* var. *holosericea*

- Plants very sparsely pubescent, appear shiny, vivid green and lustrous, never velvety to the touch; very few stinging hairs and no bristles (montane forests in the southern Alps and on the Balkan peninsula) ...............................................*Urtica dioica* subsp. *dioica* var. *glabrata*

### 2.5.2 ***Urtica dioica*** Linnaeus subsp. **dioica** var. **dioica**. Fig. 2.1

≡ *Urtica dioica* Linnaeus (1753: 984). Lectotype (designated by Woodland 1982: 283):—*herb. Linnaeus 1111.8* (LINN!, photographs in AUB, DAO, M, MTMG).

= *Urtica sicula* Gasp. ex Guss. (1844: 580). ≡ *Urtica dioica* L. var. *sicula* (Guss.) Wedd. (1856: 78). Lectotype (designated by Corsi *et al.* 1999):—ITALY. Sicily: "in umbrosis montosis Sicilia, Madonie alla Pietá", *Gasparrini s.n.* (herb. Gasparrini, PAV).

Both the historical material and the plants collected at the type locality which were taken into cultivation in Berlin [Weigend 7801 (B, BM, KRAM, M, LE)] coincide with typical *U. dioica*:—ITALY. Sicily: Palermo al Parco, Polizzi all´acqua della Pietà, leg. *Gasparrini s.n.,* in herb. Gussone (FI!); Mt. Etna, San Nicoló del Bosco, *Parlatore s.n.* (ex herb. Gussone, FI!), see Corsi *et al.* (1999) and Weigend (2005).

= *Urtica dioica* L. var. *vulgaris* Wedd. subvar. *montana* Wedd. (1856: 77). Holotype:—SWITZERLAND. Wallis: "in Valesia regione alpina, juxta monticolarum domos", ad. 2400 m, *J. Gay s.n.* (not located).

= *Urtica dioica* L. var. *parvifolia* Wierzb. ex Heuff. (1858: 157). Holotype:—HUNGARY. No additional locality data (not located).

Small-leaved, late-season form (see Weigend 2005).

= *Urtica dioica* L. var. *curvidens* Schur (1866: 601). Holotype:—ROMANIA [?]. Transylvania: Sibiu, in shady montane forests, Arpaser-Kerzesorer-Gebirge, July, *Schur sert. n. 2513* (not located).

= *Urtica dioica* L. var. *montana* Schur (1866: 601). Holotype:—ROMANIA. "Auf Felsen und Mauern, Kalk, bei Kronstadt", *Schur s.n.* (not located).

Small individual on a dry stone wall (see Weigend 2005).

= *Urtica dioica* L. subsp. *dioica* var. *lamiifolia* Schur (1866: 601). Holotype:—ROMANIA [?]. Transylvania: Sibiu, "var. *alpina*", "auf Triften und Sennhütten der Alpen", 6000´, July, Aug., *Schur, sert. n. 2513* (not located). Neotype (here designated):—SLOWAKIA. Brünn, 8 Juli 1871, *Schur 3639A* (FI!).

= *Urtica dioica* L. var. *hermaphrodita* Čelak. (1867: 146). Holotype:—CZECH REPUBLIC. Hradec Králové Region: Gradlitz, *Preissler s.n.* (not located).

Monoecious form (see Weigend 2005).

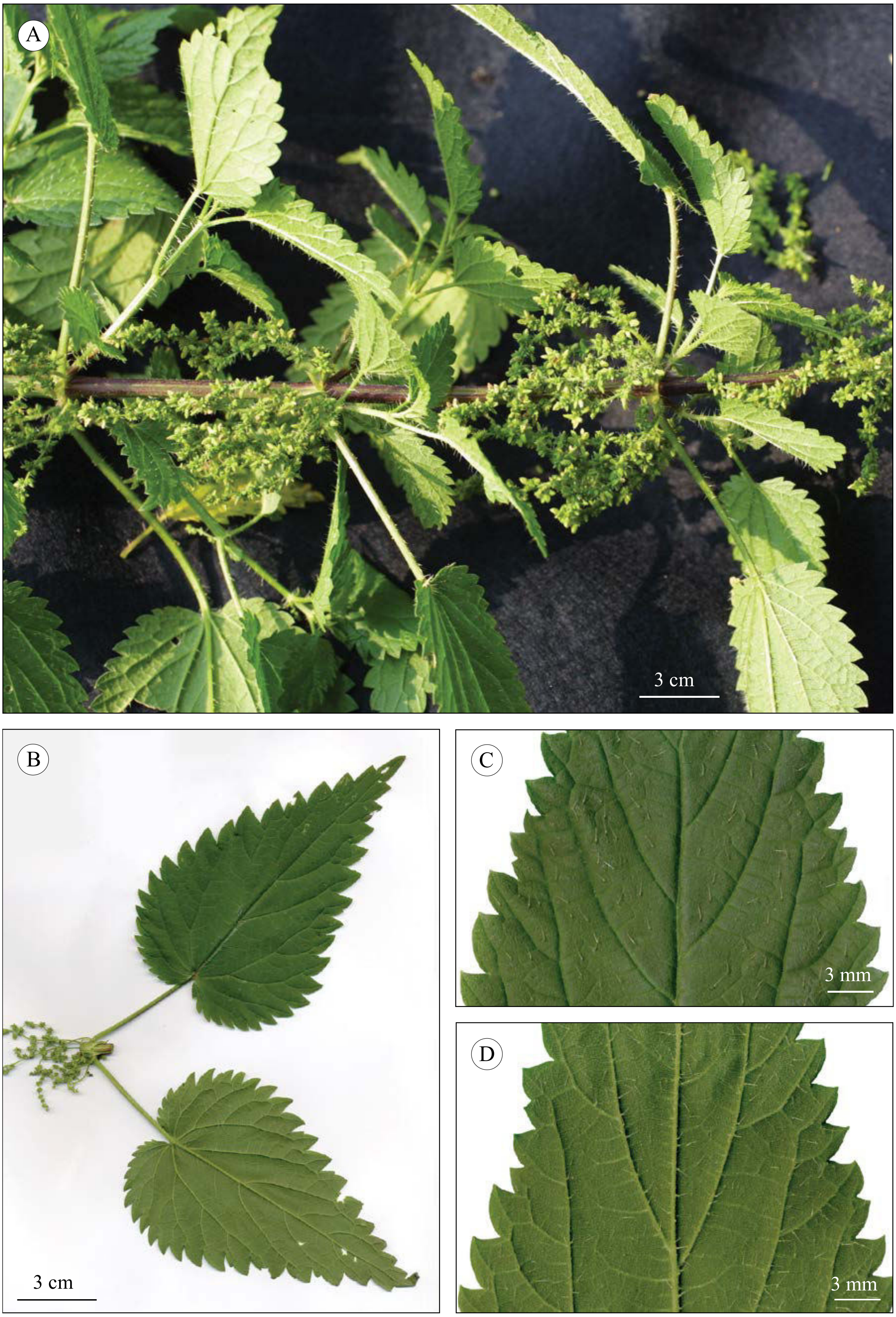

**Figure 2.1** *Urtica dioica* L. subsp. *dioica* var. *dioica*: (A: *N. Dostert s.n.*, B–D: *M. Weigend 9388*), **A.** Habit with female inflorescences, **B.** Node with broadly ovate leaves with a cordate base and female inflorescences, leaf margin coarsely serrate, **C.** Adaxial leaf surface, **D.** Abaxial leaf surface; both sides densely covered with stinging hairs.

= *Urtica dioica* L. var. *ramosissima* L.Richt. (1872: 167). Holotype:—HUNGARY [?]. Nyitra county: near Zala, near the river Waag, August [year?], *L. Richter s.n.* (not located). Numerous specimens of Ludwig (Lajos) Richter have been seen but none is annotated as "*ramosissima*". From the description, material was evidently of a late-season form with extensive branching and small leaves (Weigend 2005).

= *Urtica haussknechtii* Boiss. (1879: 1146). Holotype:—TURKEY [?]. Iter Syriaco-Armeniacum: Hab. ad maenia urbis Eski Malatia Catatoniae, 18 Sept. 1865, *Ch. Haussknecht s.n.* (JE!).

From the type specimens and description this represents a small-leaved, late-season form (Weigend 2005).

= *Urtica dioica* L. var. *trilobescens* Ullep. (1888: 20). ≡ *Urtica dioica* L. f. *trilobescens* (Ullep.) Sagorski & G.Schneid. (1891: 450). Neotype (here designated):—HUNGARY [?]. Tatra: Im Belaer Kalkgebirge heisst eine Lehne seit undenklichen Zeiten "*Nesselblösse*", June 1892, *Ullepitsch s.n.* (W!–1959–7171 , isolectotype: FR!).

= *Urtica dioica* var. *androgyna* Beck (1890: 306). Holotype:—AUSTRIA. Lower Austria: near Raxalpe-Gaislochsteig, *Beck s.n.* (not located).

Monoecious form.

= *Urtica dioica* L. var. *dioica* f. *ramosa* L.Neumann (1901: 587). Holotype:—no locality or collection given, not located. Neotype (here designated):—SWEDEN. Scania: Ystad, Aug. 1901, *H. Nilsson s.n.* (PR! No. 57910).

Typical small-leaved, branched late-season from (see Weigend 2005).

= *Urtica dioica* L. var. *mitissima* Hausskn. (1901: 134). Lectoype (designated by Weigend 2005):—GERMANY. Bavaria: Upper Palatinate, Fichtelgebirge, Katharinenberg near Wunsiedel, 3 Aug. 1900, *Ch. Haussknecht s.n.* (JE!, 4 sheets).

Additional material:—Several collections from type locality, M. & K. Weigend 7779-C (♀: B, BM, HBG, JE, KRAM, M, NY, LE, W; ♂: B, BM, HBG, JE, KRAM, M, NY, LE, W), compare Weigend (2005).

= *Urtica dioica* L. var. *pseudo-balearica* Lojac. (1904: 350). Holotype:—ITALY. Sicily: Palermo, Castelbuono a Bocca di Cava, „sub *U. balearica* in herb. Pan. serv." (not seen).

From the description not to be distinguished from typical *U. dioica,* our own collections from the type locality confirm this [Sicily: Palermo, Parco delle Madonie, above Castelbuono, 1000–1100 m, Oct. 2003, ♂, *M. & K. Weigend 8082* (M, W, LE, KRAM, BM); same locality and date, ♀, *M. & K. Weigend 8083* (M, W, KRAM, BM)].

= *Urtica dioica* L. (var. *dioica*) f. *czarnohorensis* Zapał. (1908: 91). Holotype:—UKRAINE. E Carpathians: in the subalpine (dwarf pine) belt of Cernohora, Breskuł, near Pożyżewska, appr. 1700 m, 4 Aug. 1908, *Zapałowicz 221.598* (♂, KRAM, No. 153157!).

Smaller, more coarsely dentate form typical of more exposed habitats and higher elevations.

= *Urtica dioica* L. var. *carpatica* (Zapał.) A.Schreib., in Hegi (1981: 301). ≡ *Urtica dioica* f. *carpatica* Zapał. (1908: 92). Holotype:—POLAND. Zawoja: Babia Góra, Polish Western Carpathians, Western Beskidy, 30 km NW from Tatras, in mixed forests and beech forests, very abundantly at "Czarna Hala" pasture, 19 July 1906, *ZapałowieczZapalowiecz s.n.* (♂, KRAM, No. 153156!).

= *Urtica dioica* L. var. *mirabilis* Zapał. (1908: 91). Holotype:—POLAND. Foothills of Polish Eastern Carpathians: Strzyżów in the region of Jasło, *Holzer 51.102* (KRAM!, KRAM No. 152746).

Monoecious form, with some male flowers with moderately developed (but evidently abortive) ovary (see Weigend 2005).

= *Urtica dioica* L. var. *dioica* f. *parvifolia* Zapał. (1908: 91). Holotype:—UKRAINE. Podolia: W Ukraine, Dniestr valley, Rozwadów in the region of Żydaczów, 6 Aug. 1896, *Paczoski 102.304* (♂, KRAM!, KRAM No. 153165).

Typical small-leaved, branched late-season form (see Weigend 2005).

= *Urtica dioica* L. var. *sarmatica* Zapał. f. *subsetosa* Zapał. (1908: 93). Lectotype (designated by Weigend 2005):—POLAND. Biecz: Eastern Polish Carpathians, 28 June 1876, *Pospolita s. n.* (♂, KRAM!, KRAM No. 152743).

= *Urtica dioica* L. var. *macrodonta* Borb. (1900: 337). Lectotype (here designated):—Anno 1892, *Baenitz* herb. Europ. No. 6996 (B!, isotypes: BM!, H!, PRC!).

Often erroneously cited from Ascherson & Graebner (1911) and based on the exsiccat „Borb. ex Baenitz", but clearly first published with a latin diagnosis in Borbás 1900.

- *Urtica dioica* L. var. α *latifolia* Ledeb. (1833: 240), *nom. superfl.*

Refers explicitly to *U. dioica* L. and is used to differentiate it from (illegitimate) var. *angustifolia* (Fisch. ex Hornem.)Ledeb., erroneously taken up in Ascherson & Graebner (1911: 608) and Hayek (1927: 93).

- *Urtica dioica* L. var. *latifolia* Peterm. ex Opiz (1852: 100–101), *nom. illeg.* & superfl. (non var. *latifolia* Ledeb. 1833).

- *Urtica dioica* L. var. *vulgaris* Wedd., in DC. (1856: 77), *nom. superfl.*

- *Urtica dioica* L. var. *microphylla* Baen. (1862: 233), *nom. illeg.* (non var. *microphylla* Hausmann 1852). Original material:—GERMANY. Görlitz, *Baenitz s.n.* (B!).

- *Urtica dioica* L. var. *typica* Pospich. (1897: 339), *nom. superfl.*

Refers explicitly to *U. dioica* L., erroneously taken up in e.g. Ascherson & Graebner (1911: 608).

- *Urtica dioica* L. var. *latifolia* Farw. (1930: 57), nom. illeg. (non var. α *latifolia* Ledeb. (1833). Lectotype (designated by Woodland 1982: 283):—USA. Michigan Lake Linden, 29 July 1929, *Farwell 8513* (MICH).

- *Urtica diocia* L. subsp. *eu-dioeca* Selander (1947: 271), *nom. superfl.*

Refers explicitly to *U. dioica* L. and is used to differentiate it from subsp. *sondenii* (Simmons) Hylander.

- *Urtica dioica* L. var. *setosa* A. Żumday, sensu A. Żumday, ined. in herb. KRAM:—POLAND. Kraków: Podgórze, Ludwinów, 29 July 1908, *A. Żumday s.n.* (KRAM, 041369!, 152899!).

Female plants, evidently typical *dioica* (strongly urticating).

- *Urtica intermedia* Guss., ined. in herb. FI:—ITALY. Sicily: "Boccadisalto", herb. *Gussone s.n.* (FI).

- *Urtica dioica* L. var. *amoena* Henby, ined. in herb. M:—GERMANY. Bavaria: Middle Franconia, Rothenburg, above the Tauber, in the garden, Sept.1953, *D.J. Henby s.n.* (M).

  Typical var. *dioica*, with unusually neat and symmetrical leaves.

- *Urtica dioica* L. var. *aberrata* Termonia, ined. in herb. B:—FRANCE. Charente-Inferieure: Saintes, haies, pies des murs, Aug. 1897, *Termonia 4156* (B!, possibly published by the Societé Rochelaise).

  Small-leaved late-season form, otherwise typical var. *dioica*.

- *Urtica dioica* L. var. *androgyna* Beck f. *mixta* Dybowski, ined. in herb. KRAM:—LITHUANIA [?] (Litwa). Niánków: “im Obstgarten, sehr selten”, 27 July 1894, *Dybowski 218.1418* (KRAM, 153145, 153146).

  Monoecious plants, sexes completely mixed on branches, otherwise typical var. *dioica*.

- *Urtica dioica* L. var. *dioica* var. *gynandra* Beck (f.) *androgyna* Dyb., ined. in herb. KRAM:—LITHUANIA [?] (Litwa). Niánków, 15 July 1894, *Dybowski 218.1417* (KRAM, 152737, 152738).

  Monoecious specimen, with female flowers basally, male flowers apically, otherwise typical var. *dioica*.

- *Urtica dioica* L. subsp. *hermaphroditica* Lonatschewskij, ined. in herb. PR, KIEL:—RUSSIA (Moldavia). Kiev, “in ruderatis”, 29 July 1909, *A. Lonatschewskij s.n.* (PR, 57912, without number); dito, *A. Lonatschewskij 817* (KIEL!).

  Monoecious specimen, but typical var. *dioica*.

- *Urtica dioica* L. (var. *dioica*) f. *czarnohorensis* Zapał. subf. *verticillata* Olson, ined. in herb. GB:—SWEDEN. Vestrogothia: Toarp, Trollhättar, 10 July 1922, *A.O. Olson s.n.* (GB).

  Typical var. *dioica,* but with three (instead of two) leaves per node.

- *Urtica dioica* var. *diversifolia* Ullep., ined. in herb. B:—POLAND. Opole: Zips, Villa Lers, July 1892, *Ullpeitsch s.n.* (B!) und Littmann gegen Leschnitz, June 1891, *Ullepitsch s.n.* (B!). MS on sheet: “*Urtica dioica* L. var. *longifolia* Michi”.

  Typical form from moist places with widely ovate lower and median leaves.

- *Urtica dioica* L. var. *longifolia* Ullep., ined. in herb. B:—POLAND. Opole: Zips, June 1891, *Ullepitsch s.n.* (B!).

  Female, MS on sheet “*Urtica dioica L. var. longifolia* Michi”.

- *Urtica drepanodonta* Ullep., ined. in herb. B:—SLOVAKIA. Zips: Villa Lers, July 1893, *Ullepitsch s.n.* (B!).

- *Urtica longifolia* Ullep., ined. in herb. B:—POLAND. Wólka wieś, *A. Rehmann s.n.* (herb. Ulleptisch, B!); Pogórze Przemyskie, Dobromil olszynka nad Wyrwa, *A. Rehman s.n.* (B!).

A comparison with the type specimen at LINN shows that this is indeed the typical form of *Urtica dioica* and can therefore be formally (if redundantly) called: *Urtica dioica* subsp. *dioica* var. *dioica*. This is the common, lowland and coastal, weedy form: robust, often over

150 cm tall, median leaves broadly ovate with a cordate base, coarsely and regularly serrate leaf margins, teeth usually undivided even on the largest leaves, but sometimes the proximal ones again with two or three teeth. The entire plant is more or less densely covered with stinging hairs usually interspersed with short, stiff bristles and simple trichomes. The perianth has no, or only individual stinging hairs (usually only in some female flowers). Female inflorescences are initially spreading, densely and strongly branched, but deflexed to pendulous in fruit, male inflorescences are much branched, and horizontally spreading. The characters of the inflorescence, habit and gross leaf morphology are shared by the subsequent form (var. *hispida*), which differs mainly in details of leaf serration and indument. Montane forms have been segregated under a range of names, but show no consistent morphological differences. There is a trend for particularly coarse leaf teeth, these more frequently lobed, and the plants are also often slightly smaller, but these differences are not stable in cultivation.

**Distribution and Ecology:**—Common mostly in open habitats, on roadsides, waste grounds, disturbed sites, pastures at low and intermediate elevations, also commonly found in the mountains of eastern, central and southern Europe, especially in alpine pastures and on nutrient rich and permanently moist, but not wet sites.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica dioica* subsp. *dioica* var. *dioica* is considered "Least Concern" (LC).

**Representative specimens:**—**BULGARIA. Haskovo:** Dolno Lukovo, Eastern Rhodopen, 19 June 2006, *W. Lang 48* (private herb.); same locality and date, *W. Lang 46* (private herb.); **Sofia:** E Sofia, Koprivschtiza, brookside, *W. Lang 1* (private herb.);—**GERMANY. Baden-Württemberg:** Neckargemünd, Wiesenbachertal, entrance from Herrenweg, left wayside, 211 m, 20 Aug. 2007, *T. Camenzind 23* (♀, BONN); **Bavaria:** Northern Upper Palatinate, Katharinenberg near Wunsiedel, from type locality of *var. mitissima* Hausskn., cultivated in Berlin July 2003, *M. & K. Weigend 7779-C* (♂ and ♀, BONN, E, K, MA); **Berlin:** Friedrichshain, Corinthstraße parking lot, N 52°29'58,46", E 13°27'42,33", 39 m, cultivated in Berlin July 2010, *M. Weigend 9388* (♂, BONN); same locality and date, cultivated in Berlin July 2010, *M. Weigend 9389* (♀, BONN); Dahlwitzer Landstr. N of Friedrichshagen, branch to Ravensburg, across from 60 km/h sign 47 m, 15 Aug. 2007, *T. Camenzind 26* (<♂♀>, BONN); Friedrichshagen, Müggelseedamm E of "Bundesamt für Strahlenschutz" right into path, at small bath meadow, 32 m, 15 Aug. 2007, *T. Camenzind 09* (♂, BONN); same locality and date, *T. Camenzind 10* (♂, BONN); FU

Berlin, compost near green houses of the “Institut für Pflanzenphysiologie”, 53 m, 17 Aug. 2007, *T. Camenzind 20* (♂, BONN); Krumme Lake, Berlin-Müggelheim, corner Staudenheimer Str./Duchrother Str. right into path, at 2. road junction, 17 Sept. 2007, cultivated in Berlin August 2008, *T. Camenzind 29-C* (<♂♀>, BONN); Teltow-Fläming, Grossbeeren, road from Grossbeeren to Diedersdorf, near Diedersdorf at an old transformer building, 40 m, 12 July 2006, *N. M. Nürk 320* (<♂♀>, BONN); **Brandenburg:** Pichelswerder, near eastern bank, Oct. 2001, cultivated in Berlin June 2003, *K. Weigend 2001/3* (♀, BONN, LE, M, W); same locality and date, cultivated in Berlin June 2003, *K. Weigend 2001/4* (♀, BM, BONN, KRAM, LE, M, W); same locality and date, cultivated in Berlin June 2003, *K. Weigend 2001/5b* (♀, BONN, KRAM, LE, M, W); Reinickendorf, Tegel, Tegeler See, 10 October 2001, cultivated in Berlin July 2005, *T. Henning & Ch. Schneider 313* (♀, BONN, LE, M); Bleyen near Küstrin, banks of the River Oder, immediately next to the water in seasonally inundated region, Oct. 2001, cultivated in Berlin July 2003, *M. & K. Weigend 5658-C* (♂, BONN); Großer Wannsee, Street “Am Postfenn”, 20 May 2002, *M. & K. Weigend 7102* (♂, and <♂♀>, BONN); Märkisch-Oderland, Neuenhagen near Berlin on road towards B1, 10 Oct. 2001, cultivated in Berlin July 2004, *T. Henning & Ch. Schneider 312* (♀, BM, BONN, KRAM, LE, M, NY, P, W); Müggelheim, Spreewald near Müggelspree (Großer Müggelsee E of Berlin), Str. 35, near Ferryport F24.0, Oct. 2001, cultivated in Berlin June 2003, *K. Weigend 2001/2a* (♂, BONN, KRAM, M); same locality and date, cultivated in Berlin June 2003, *K. Weigend 2001/2c* (<♂♀>, BONN, M); N of Berlin, Landkreis Barnim, Blumberg near Berlin, 10 October 2001, cultivated in Berlin July 2003, *T. Henning & Ch. Schneider 310* (♀, BONN, LE, M, W); N of Berlin, Landkreis Barnim, Blumberg near Berlin, 10 Oct. 2001, cultivated in Berlin July 2003, *T. Henning & Ch. Schneider 311* (♀, BONN, M, W); Berlin-Dahlem, grounds of the “Institut für Biologie, FU Berlin”, Altensteinstr. 6, 40 m, 19 Sept. 2006, *N. M. Nürk & J. Devers 337* (♀, BONN);—**GREECE. Imathias:** Vrisaki (little village 2 km W of Alexandria); alluvial plain of the river Aliakmonas, Leg. Th. Franke & P. Iosifidou Juli 9 2006, 06-01 (♂, BONN); same locality and date, 06-02 (♀, BONN); same locality and date, 06-03 (♂, BONN);—**HUNGARY. Pest:** “In fossis ad Szabadszállás”, 25 June 1918, *A . de Degen s.n.* (BM); In pratis silvaticis Hohe Tatra Carpathorum, Julio 1892, F. Schultz–herbarium normale, nov. ser. cent. 29, *Ullepitsch 2885* (BM, PR, W);—**ITALY. Sicily:** Siracusa, Valle dell ‘ Anapo (nature preserve) near to Sortino, Road SP 28 from Sortino to Ferla (in the valley of Anapo), ca. 425 m, 10 May 2007, *N. M. Nürk 342* (♀, BONN); same locality and date*, N.M. Nürk 343* (♂, BONN); Spiaggia, 26 July 1851, *leg. Parlatore s.n.* (FI); Panormi al flumine? Areto, June 1835, *Da Parlatore s.n.* (FI); Isola di ???

vers Spiagiia, 09 Aug. 1851, *Da Parlatore s.n.* (FI);—**LITHUANIA. Nowogródek:** Nianków, anno 1894, *W. Dybowski 236/11172* (KRAM);—**NETHERLANDS. Limburg:** Neercanne castle near Maastricht, 19 Aug. 1950, *R.C. Bakhuizen & v.D. Brink 6907* (BM);—**POLAND. Lower Silesia:** Wrocław, at river Oder in front of the zoological garden, July 1866, *R. v. Uechtritz s.n.* (JENA); Wrocław, Uhlauer Vorstadt, Kleine Lepsingbrücke, 17 Aug. 1877, *R. v. Uechtritz s.n.* (JENA); **Podlaskie:** Osowiec Twierdza, at northern entrance to the town, ca. 100 m of town sign on the right side in understory, cultivated in Berlin Aug. 2008, *M. Weigend 9145* (♂, BONN); **Cieszyn:** Harbutowice, Beskid Średni, 26 July 1965, *H. & T. Tacik s.n.* (KRAM); Beskid Niski, 25 July 1956, *T. Tacik s.n.* (KRAM); **Tatry:** Mala Kosista, 29 July 1953, *T. Tacik s.n.* (KRAM); 6 September 1926, *Anon. s.n.* (KRAM 221896);—**SLOVAKIA.** Villa Lers (Hungary), August 1892, *Ullepitsch s.n.* (2 x B); without legible locality and date, *Zips & Ulleptisch s.n.* (B);—**SWEDEN. Gotland:** Klinteham, 26 Aug. 1882, *M. Lönnroth s.n.* (GB); **Halland:** Släp parish, shore of Kyviken, 08 July 1980, *C.I. Sahlin s.n.* (♂, GB); **Jämtland:** Bräcka, 11 July 1918, *K.B. Nordstrom s.n.* (GB); **Norrbotten:** between Gammelstaden and Luleâ, original collection by H. H. Hilger August 2005, cultivated in Berlin July 2006, *M. Weigend 8717* (♀, B, BONN, M); Frösön, Storsjön, 29 Sept. 1916, *G. Öhrstedt s.n.* (FI!BM); **Öland:** Eastcoast, Tribergaläge between Triberga and Hultenstad, 23 November 2001, cultivated in Berlin July 2003, *S. Kipka s.n.* (♀, BM, BONN, KRAM, LE, M, NY, P, W); Borgholm, July 1907, *G.? Johannson s.n.* (2 x BM); Westrogothia, par. Toarp ad Lindås, 20 Aug. 1913, *A.D.Olson s.n.* (BM fem. blühend); **Samland** [?]: Namjö [?], 07 Aug. 1909, *A. Hälphers s.n.* (fr., fem., GB); **Skåne Iän:** Ö. Vram, annot 1910, *G.E. Nihlen 165* (BM); Toarp, Trollhättar, 10 July 1922, *A.O. Olson s.n.* (GB); Malmö, September 1894, *G. Johannsson s.n.* (BM); **Sodermanland:** Nikolai, Lifsholmen, 06 July 1902, *C. Blom s.n.* (GB); **Värmland:** 30 June 1921, *A. Binning s.n.* (GB); **Västergötland:** Skövde & Hjo, at 194, forest, N 58 20,68, E 14 09,063, original collection by H.H. Hilger Aug. 2005, cultivated in Berlin July 2006, *M. Weigend 8719-C,* (♀, B, BONN, M); par. Toarp, ad Bråthule, 11 August 1913, *A. Olson s.n.* (BM); **Västernorrlandslän:** Solberg, N 63 47,064, E 17 39,098, original collection by H. H. Hilger Aug. 2005, cultivated in Berlin July 2006, *M. Weigend 8718-C* (♀, B, BONN, M);—**SWITZERLAND. Wallis:** Zeneggen near Visp, Moosalp, parking lot next to restaurant, ca. 2000 m, 29 Aug. 2004, *M. & K. Weigend 8105* (<♂♀>, BONN, FI, M, NY, P, S, USM);—**UKRAINE. Kiew:** near Kiew, city of Uman, July 1907, *N. Schibika s.n.* (JENA); Kiew, Aug. 1907, *A. Lonatschewksi s.n.* (JENA, KIEL, KRAM); Kiew, 20 July 1907, *A. Lonatschewksi s.n.* (BM, JENA, KRAM); **Lwów** [Lviv], *Rehman 69.1244* (KRAM, 163076, B); Kleparów na wale kolejowym, *A. Rehmann s.n.* (B); **Ternopil:** Ostapie near Skałat, SE from Ternopil, W Ukraine, *Paczoski 102.303*, (KRAM, 23.7.1896); **UNITED KINGDOM. Devon:**—Star Point, Aug. 1899, *Druce s.n.* (BM); Countisbury, Coombe Farm, 06 Sept. 1917, *W.C. Barton 282* (BM); E. Norfolk, Sheringham, Aug. 1916, *F. Long 1843* (BM); N Devon, Brendon, roadside, 25 Aug. 1917, *W.C. Barton 281* (BM, pro parte); **Westerness:** Arisaig, Aug. 1903, *H.J. Riddendell s.n.* (2 x BM, 1x with G.C. Druce s.n. as collector!); **Cheshire:** Edge Quarry, 15 Aug. 1908, *Lex 1483c* (2 x BM); **Herefordshire:** near Ross, 27 Aug. 1901, *A. Ley 380* (BM); Weston-under-Penyard, 30 Aug. 1906, *A. Ley* 1380 (BM); Hereford, Breinton, 31 August 1905, *A. Ley 1380* (2 x BM); riverbank, Carey, 5 Sept. 1902, *A. Ley 1380* (2 x BM); Mouns [?], wood, 25 Aug. 1891, *A. Ley 1266c* (BM); Hanwell Tip, 23 September 1952, *D.H. Kent 21* (BM); Little Birch, 12 Aug. 1888, *A. Bromwich s.n.* (BM); **Leicestershire:** Quorn, 03 Aug. 1905, *F.L. Foord-Kelcey 1483c* (BM); **Buckshire:** Ditch, Little Kimble, 13 Sept. 1910, *F.L. Foord-Kelcey 711* (BM); **Sussex:** W Sussex, Shorney Island, near W Shorney, 06 Sept. 1903, *R.S. Standen s.n.* (BM). **Somerset:** N Somerset?, Portishead, 03 Aug. 1922, *I.M. Berger 1483* (3 x BM); Leicester, Newstead road, 29 July 1903, *W. Bell s.n.* (1 x BM); **Yorkshire:** NW Yorkshire, Conistone, 29 Aug. 1930, *T.J. Foggit s.n.* (BM); **Middlesex:** between Devon and Harefiled, 19 Sept. 1910, *Hast? s.n.* (BM); **Brecon**: Glen Collwng, 850 ft., 24 Sept. 1908, *A. Ley 1483b* (BM); **Perthshire:** Glen Fallock, 04 Aug. 1946, *J.T.I. Boswell-Syme s.n.* (BM); **Wiltshire:** S Wiltshire, roadside near Keevil, 18 Aug. 1903, *E.S. Marshall 2716* (BM).

**Intermediate specimens:—GERMANY. Brandenburg:** Müggelheim, Spreewald near Müggelspree (Großer Müggelsee E of Berlin), Str. 35, near Ferryport F24.0, Oct. 2001, cultivated in Berlin June 2003, transition to var. *holosericea*, *K. Weigend 2001/1* (BONN); Pichelswerder, near eastern bank, Oct. 2001, cultivated in Berlin July 2003, transition to var. *holosericea*, *K. Weigend 2001/5a* (♀, BONN); Bleyen near Küstrin, banks of the River Oder, parking lot ca. 50 m from the water near Oder river, Oct. 2001, cultivated in Berlin July 2003, transition to var. *sarmatica*, *M. & K. Weigend 5660-C* (♀, BONN);—**SWEDEN. Värmland:** Nordmarken, between Lake Nedre Blomsjörn and Lake Lelang, next to old watermill, 59°16'38.17"N, 11°57'49.73"E, cultivated in Berlin July 2008, transition to var. *holosericea*, *J. Schulz 02-C* (♂ and ♀, BONN).

### 2.5.3 ***Urtica dioica*** L. subsp. ***dioica*** var. ***hispida*** (Lam. ex DC.) Tausch ex Ott (1851: 41). Fig. 2.2

Basionym: *Urtica hispida* Lam. ex DC. (1815: 355). Lectotype (here designated):—FRANCE. Départ. Pyrénées-Orientales: Region Languedoc-Roussillon, Eastern Pyrenees, Prades, anno 1814, *M. Coder 182* (G–DC!). Syntype:—same locality, *M. Coder s.n.* (G–DC!).

≡ *Urtica dioica* L. var. *vulgaris* Wedd. subvar. *hispida* (Lam. ex DC.) Wedd., in DC. (1869: 50).

≡ *Urtica dioica* L. f. *hispida* (Lam. ex DC.) Sagorski & G.Schneid. (1891: 450).

≡ *Urtica dioica* L. subsp. *hispida* (Lam. ex DC.) Chrtek (1981: 212).

= *Urtica dioica* L. var. *hispida* (Lam. ex DC.) Wedd. subvar. *horrida* Wedd. (1856). Holotype:—"In Corsica necnon in Gallia meridionalis frequens" (not located).

≡ *Urtica dioica* L. var. *vulgaris* Wedd. subvar. *horrida* (Wedd.) Wedd., in DC. (1869: 51).

≡ *Urtica dioica* L. var. *horrida* (Wedd.) Rouy (1910: 273).

= *Urtica dioica* L. var. *hispida* (Lam. ex DC.) Wedd. subvar. *duplicato-serrata* Wedd. (1856). Holotype:—(not located).

≡ *Urtica dioica* L. var. *vulgaris* Wedd. subvar. *duplicato-serrata* (Wedd.) Wedd., in DC. (1869: 51).

= *Urtica hispidula* Cariot (1865: 505) ≡ *Urtica dioica* L. var. *hispidula* (Cariot) Hegi (1911: 139). Holotype:—Paturages et rocailles des hautes montagnes. Ain. Le Colombier du Jura et le Reculet, Is. La Grande Chartreuse, entre Grande Vache et Chalais (not located).

Var. *hispida* is similar to var. *dioica*, but is always robust and generally low-growing (typically 60–80 cm tall). Like var. *dioica* it has leaf margins which are coarsely and deeply serrate and some of the proximal teeth are usually divided. However, the plants are extremely densely covered with stinging hairs and bristles, often appearing nearly white. The perianth, both of the female and male flowers, always has one to several stinging hairs. Female inflorescences are initially spreading, very densely and strongly branched, deflexed in fruit, male inflorescences are much branched, and horizontally spreading.

**Distribution and Ecology:**—Typically found on open habitats in alpine pastures on calcareous ground, preferably between rocks, in the southern Alps and the Pyrenees, also on Corsica and possibly elsewhere.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica dioica* subsp. *dioica* var. *hispida* is considered "Least Concern" (LC).

**Representative specimens:—ALBANIA.** Without legible locality, anno 1894, *A. Baldacci 156* (FI);—**FRANCE. Ain**: 14 July 1871, *F. Lacroix s.n.* (FR); **Hautes Pyrenees:** Gèdre,

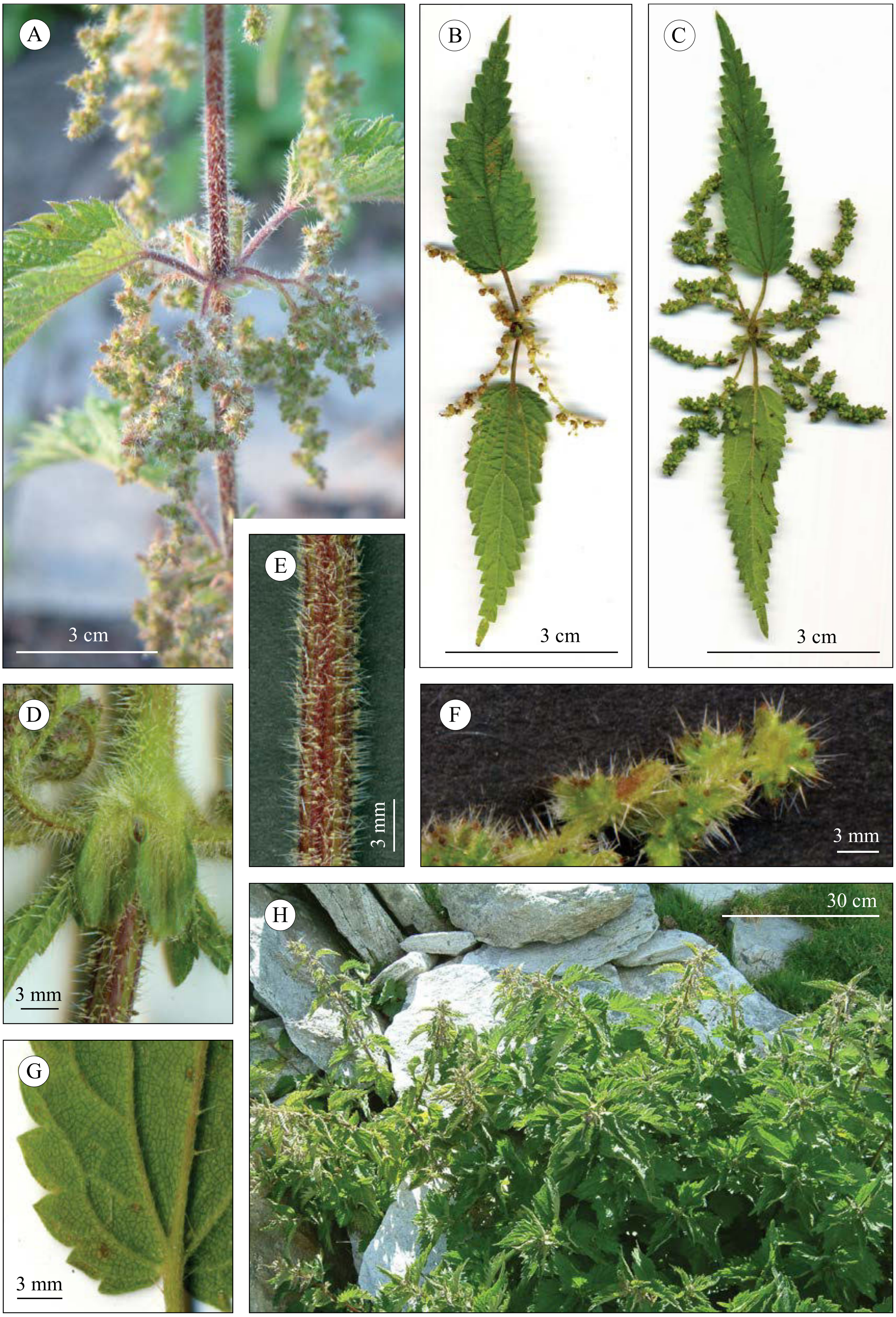

**Figure 2.2** *Urtica dioica* L. subsp. *dioica* var. *hispida*: (A, D–F: *M. Weigend 8109*, B, C, G: *M. Weigend 8108*), **A.** Habit with dense indument appearing nearly white, **B.** Node with leaf pair and male inflorescences, **C.** Node with leaf pair and female inflorescences, **D.** Node with stipules, **E.** Stem densely covered with stinging hairs and bristles, **F.** Female inflorescence: perianth with stinging hairs, **G.** Abaxial leaf surface with stinging hairs, leaf margins coarsely and deeply serrate, **H.** Typical open habitat on calcareous ground between rocks in the Swiss Alps.

Brada, Aug. 1885, *Bordére s.n.* (PR, M); Estaubé, anno 1872, *Bordére s.n.* (B), Camp [*illegible*], anno 1872, *Bordére s.n.* (B); D´Estaubé, 1800 m, 28 July 1882, *Bordére s.n.* (FR); Aug. 1887, *Bordére s.n.* (M);—**ITALY. Treviso:** Alpi Trevigiane, Bosco Cansiglio, R. Palazzo, suolo dolomitico, 1030 m, 04 Aug. 1917, *A. Fiori s.n.* (FI); **Turin:** Giaveno, near D´Alpe, 31 July 1928, *Fontana s.n.* (M); **Tuscany:** Firenze a San Gervasio, June 1904, *A. Fiori s.n.* (FI); **Venetia**: Prov. de Treviso, Bosco Cansiglio, circa habitationis et secus vicus frequens, 1000 m, sol. calc., 10 July 1922, *A. Fiori 2645* (B, BM, PR, 53041); same locality and date, Flora Italica Exsiccata *A. Fiori & A. Béguinot No. 2645, A. Fiori s.n.* (FI);—**SPAIN. Soria:** Carrascosa de la Sierra, camino a La Dehesa, 1190 m, 07 July 1980, *Granzow & Zaballos 45* (M); Estepa de San Juan, Puerto de Oncala, 1465 m, 07 Sept. 1980, *Zaballos 296* (M);—**SWITZERLAND. Wallis**: above Gletscheralp, near Saas Fee, 2400 m, 12 July 1911, *Ruppert 857* (M); Gärsthorn, above Visp, near Mund, 2200 m, 03 Sept. 2004, *M. & K. Weigend 8113* (♀, BONN); same locality and date, *M. & K. Weigend 8114* (♂, BM, BONN, M, P, W); specimens prepared from cultivated plants in June 2005, *M. & K. Weigend 8114-C* (♂, BONN); Gärsthorn, above Visp, near Mund, 2500–2600 m, 03 Sept. 2004, *M. & K. Weigend 8111* (BONN, FI, KRAM, M); specimens prepared from cultivated plants in June 2005, *M. & K. Weigend 8111-C* (♀, BONN); same locality and date, *M. & K. Weigend 8112* (♀, BONN, FI, LE, M, S, W); specimens prepared from cultivated plants in June 2005, *M. & K. Weigend 8112-C* (♀, BONN); Sion, Col du Sanetsch, 2200 m, Aug. 2004, *M. & K. Weigend 8108* (♀ and <♂♀>, BONN, LE, M); specimens prepared from cultivated plants in June 2005, *M. & K. Weigend 8108-C* (♀ and <♂♀>, BONN, M, NY, P, W); same locality and date, *M. & K. Weigend 8109* (♀, BONN, LE, M); specimens prepared from cultivated plants in June 2005, *M. & K. Weigend 8109-C* (♀, BONN, M, P, W); same locality, 03 Sept. 2004, cultivated in Berlin June 2005, *M. & K. Weigend 8190* (♀, B, BM, BONN, FI, M, MO, P); same locality, 30 Aug. 2004, cultivated in Berlin June 2005, *M. & K. Weigend 8179* (♂, B, BONN, M, cult: M); same locality, Sept. 2004, cultivated in Berlin Aug. 2005, *M. Weigend 8200* (♂, BONN, K, M, MO, P).

### 2.5.4 ***Urtica dioica*** L. subsp. **dioica** var. ***sarmatica*** Zapał. (1908: 93). Fig. 2.3

Lectotype (designated by Weigend 2005):—UKRAINE. Podolia: W Ukraine: Niwra in the region Borszczów, between Tarnopol and Czerniowce, *Lenz 93.255* (♀: KRAM!, KRAM No. 153161).

This taxon was generally overlooked and was considered as synonymous with *Urtica dioica* subsp. *pubescens* (Ledeb.) Domin (1944: 71, Weigend 2005). More collections in recent years and cultivation of this form indicate that var. *sarmatica* represents a distinct ecotype. This

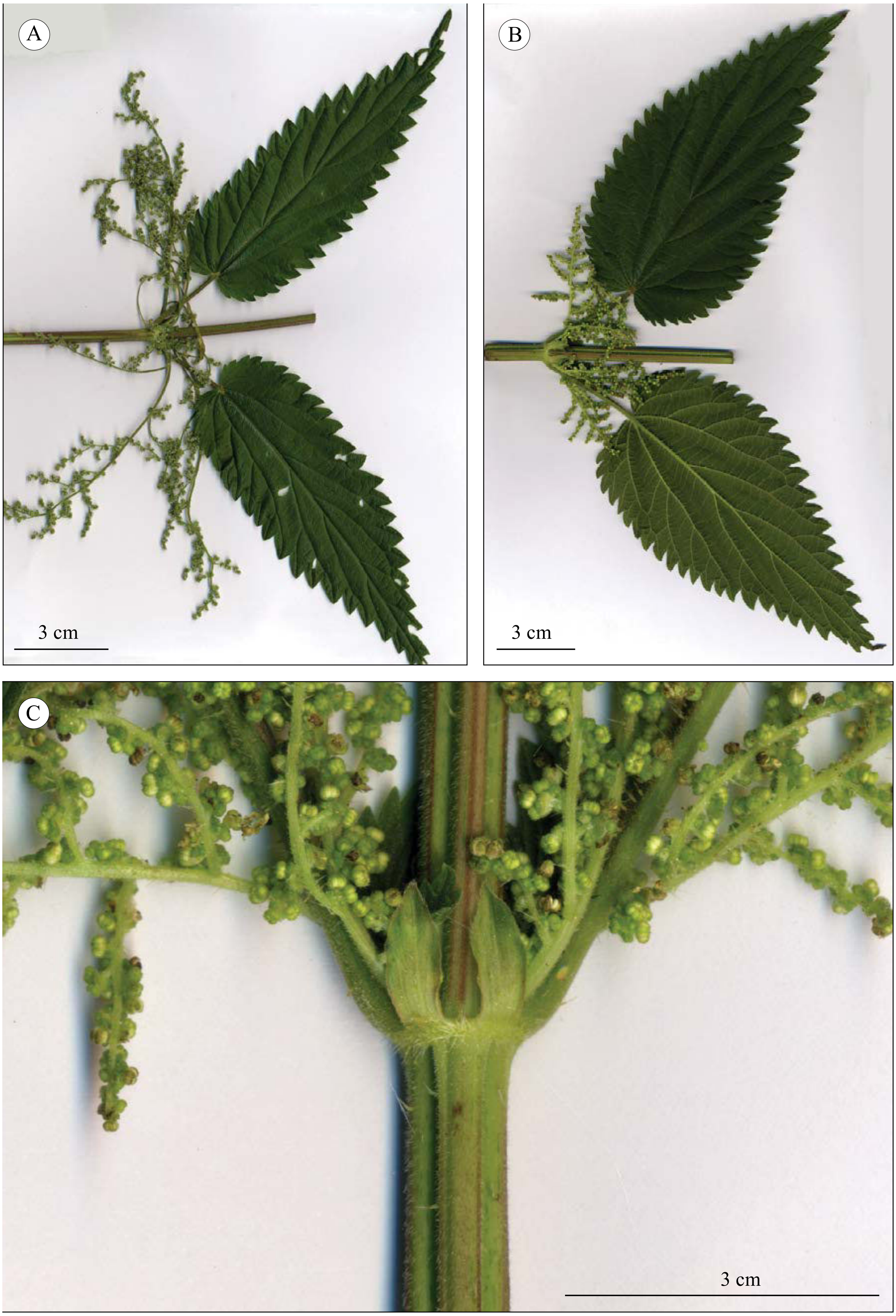

**Figure 2.3** *Urtica dioica* L. subsp. *dioica* var. *sarmatica*: (A: *M. Weigend 9328*, B, C: *M. Weigend 9327*), **A.** Node with leaf pair and female inflorescences, **B.** Node with leaf pair and male inflorescences, leaf surface is weakly covered with stinging hairs, **C.** Node with stipules and male inflorescences.

variety is quite similar to var. *dioica*, but very robust ( up to 200 cm tall) and differs in its dense and soft, albeit not white pubescence, relatively much weaker cover with stinging hairs. In inflorescence morphology it cannot be differentiated from var. *dioica*.

**Distribution and Ecology:**—Typically found in dry forests, e.g., under oaks in eastern Germany and Poland, probably wide-spread in eastern Europe at least to the Black Sea.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica dioica* subsp. *dioica* var. *sarmatica* is considered "Least Concern" (LC).

**Representative specimens:—GERMANY. Berlin:** FU Berlin, compost near greenhouses of the "Institut für Pflanzenphysiologie" 53 m, 17 Aug. 2007, *T. Camenzind 21* (♂, BONN); same locality and date, *T. Camenzind 22* (♂, BONN); **Brandenburg:** Küstrin, directly E of village next to German-Polish Borderpost (last roadbend before bridge, E of road towards the River Oder), 23 July 2002, *M. & K. Weigend 7130* (♀, BM, BSB, GB, M, W).

**Intermediate specimens to var. *dioica*:—GERMANY. Brandenburg:** Küstrin, just before the Polish border post, Oct. 2001, cultivated in Berlin July 2003, *M. & K. Weigend 5662-C* (<♂♀>, BONN); near Oderberg (near River Oder), hill called Pimpinellenberg, 21 April 2002, cultivated in Berlin July 2003, *M. & K. Weigend 7087-C* (♀, BONN, BR, E, MA, MO); Neukölln, Castle Britz, Park, under oaks and maples, 20 Nov. 2001, cultivated in Berlin July 2003, *M. & K. Weigend 5671-C* (♂, BONN, MA, P);—**POLAND. Bialystok:** Bielowicza, Oct. 2001, *I. Polunin s.n.*, cultivated in Berlin July 2002, *M. & K. Weigend 5648-C* (♀, ♂, and <♂♀>, BM, BONN, KRAM, LE, M, NY, P); same locality and date, cultivated in Berlin July 2002, *M. & K. Weigend 5653-C* (♀, BONN).

### 2.5.5 ***Urtica dioica*** L. subsp. ***dioica*** var. ***holosericea*** Fries (1828: 281). Fig. 2.4

Holotype:—SWEDEN (not located). Neotype (*here designated*):—GERMANY. Brandenburg: Müggelheimer Damm E of Köpenick, 1. bus stop left into path, 1. road junction right, right wayside, 38 m, 15 Aug. 2007, *T. Camenzind 01* (♂, BONN).

= *Urtica dioica* L. subsp. *dioica* var. *angustifolia* Wimm. & Grab. (1829: 336). Holotype:—POLAND. Silesia (not located).

= *Urtica dioica* L. var. *elegans* Chenevard (1904: 806). Lectotype (here designated):—SWITZERLAND. Tessin: Terrains vagues près la gare de Locarno, 24 Aug. 1903, *P. Chenevard s.n.* (Z-000053938!); Syntypes:—same data, *P. Chenevard s.n.* (Z-000053939!), *P. Chenevard s.n.* (G-00354326!).

= *Urtica dioica* L. var. b. *spicata* Asch. & Graeb. (1898: 262). Holotype:—RUSSIA, Eastern Prussia, formerly Germany. Insterburger Stadtwald (not located).

Described as a late-season form without leaves in the distal part, this is very common in this variety.

= *Urtica dioica* L. var. *monoica* Tausch ex Ott (1851: 41). Lectotype (here designated):—CZECH REPUBLIC. Prague: at roadsides near Prague, [no date], *Tausch 1342b* (PR, 99502!).

Monoecious form of the forest variety.

= *Urtica dioica* L. var. *vulgaris* Wedd. subvar. *umbrosa* Wedd. (1856: 77). Holotype:—"*in Sylvis Europae passim"* (not located).

≡ *Urtica dioica* L. var. *umbrosa* (Wedd.) Rouy (1910: 273).

= *Urtica dioica* L. var. *pilosa* Aschers. & Graeb. (1898: 262). Holotype:—GERMANY. Brandenburg: "*Selten auf Mooren*" (not located).

- *Urtica dioica* L. var. *holosericea* Fries ex Saelán (1889: 130). Holotype:—SWEDEN (not located).

- *Urtica dioica* L. (var.) *angustata* Schur, ined. in herb. FI:—SLOVAKIA. Brünn, 3 Aug. 1871[?], *Schur 6947* (FI).

Male plant, annotated "*Urtica dioica* L. *angustata* = *Urtica umbrosa* Schur".

- *Urtica dioica* L. var. *angustifolia* Peterm. ex Opiz (1852: 100–101), nom. illeg. (non var. *angustifolia* Wimm. & Grab. 1829).

- *Urtica dioica* L. var. *β angustifolia* A.Blytt (1869: 188), nom. illeg. (non var. *angustifolia* Wimm. & Grab. 1829).

- *Urtica dioica* L. var. *β angustifolia* Heuff. (1858: 157), *nom. illeg.* (*non* var. *angustifolia* Wimm. & Grab. 1829).

- *Urtica dioica* L. var. *ternata* Girth, ined. in herb. W:—GERMANY. Hessia: Kreis Groß-Gerau, "In Wäldern bei Büttelborn, vom Rande der Landstraße nach Darmstadt", 28 June 1928, *Girth 1103* (W, 1970-10921!).

- *Urtica dioica* L. var. *umbrosa* Opiz (1852: 100–101). Holotype:—not located.

- *Urtica umbrosa* Schur, ined. in herb. FI:—SLOVAKIA. Brünn, 3 Aug. 1871 [?], *Schur 6947* (FI).

Male plant, evidently the common forest form, annotated "*Urtica dioica* L. *angustata* = *Urtica umbrosa* Schur".

- *Urtica dioica* L. var. *umbraticola* Schur, ined. in herb. FI:—SLOVAKIA. Brünn, 11 August 1871 [?], *Schur 1233* (FI).

Male plant, evidently the common forest form.

≠ *Urtica dioica* L. var. *angustifolia* (Fisch. ex. Hornem.) Ledeb. (1833: 241), *nom. illeg.* (*non* var. *angustifolia* Wimm. & Grab. (1829). = *Urtica angustifolia* Fisch. ex. Hornem**.** (1819: 107).

This combination is illegitimate, since it post-dates Wimmer & Grabowski 1829.

≠ *Urtica dioica* L. var. *holosericea* (Nutt.) C.L.Hitchc. (1964: 91), *nom. illeg.* (*non* var. *holosericea* Fries ex Saelán 1889). = *Urtica gracilis* Ait. subsp. *holosericea* (Nutt.)—Weigend (compare Henning *et al.* 2014).

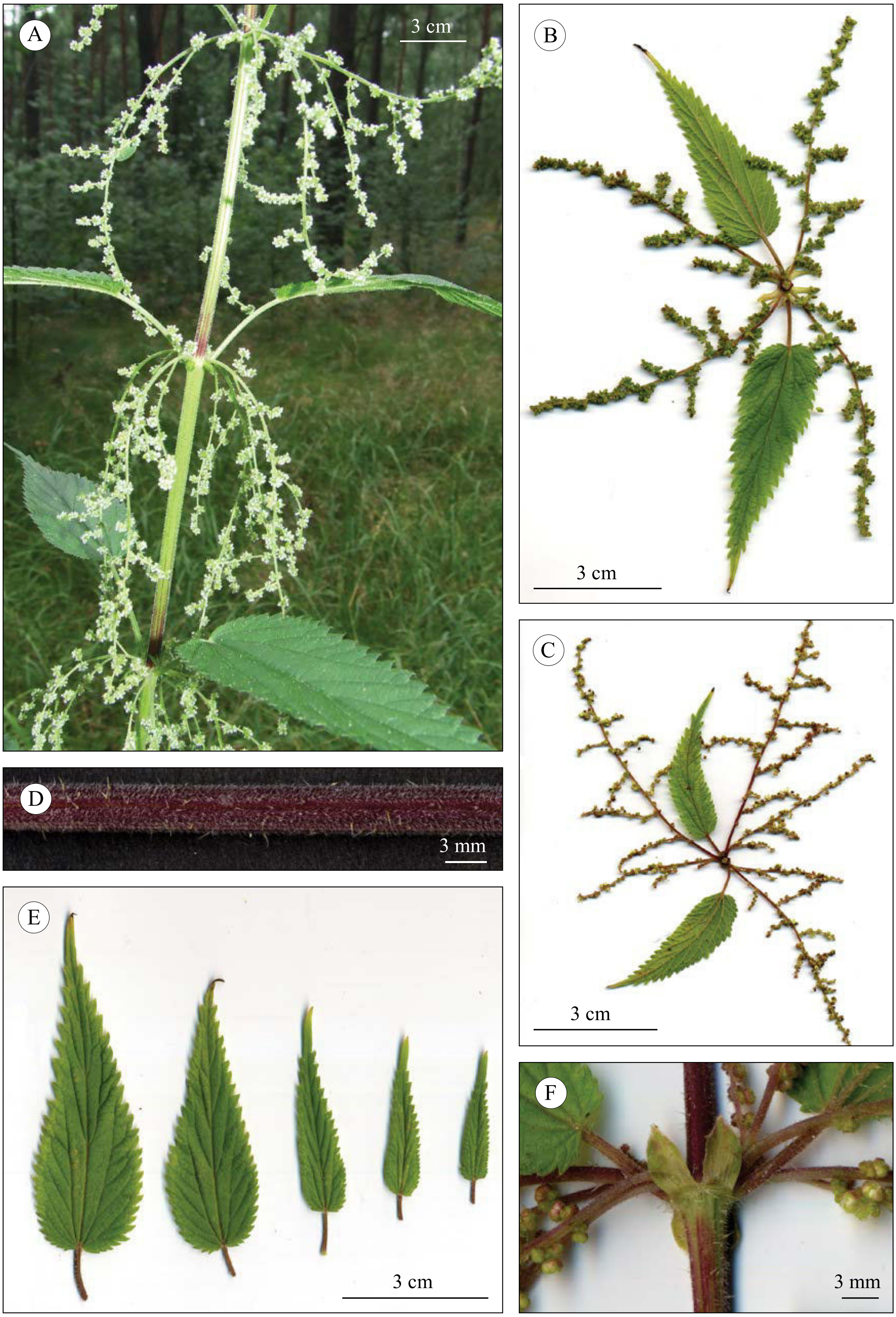

**Figure 2.4** *Urtica dioica* L. subsp. *dioica* var. *holosericea*: (B, E: *Opf. 8100*, C, D, F: *Opf. 8099*), **A.** Habit with typical beaded female inflorescences, **B.** Node with leaf pair and female inflorescences, **C.** Node with leaf pair and male inflorescences, **D.** Stem fine pubescent with only few stinging hairs, **E.** Leaves of the upper and median portion of the stem narrowly ovate, only marginally cordate, leaf margins shallowly crenate to serrate, **F.** Node with stipules.

This varietal name has also been used twice in *Urtica dioica*, the oldest use is that for the pilose forest form in southern Sweden, the varietal name used in North America is formally illegitimate and refers to a taxon now treated as subspecies of *U. gracilis* Aiton (1789: 341, see Henning *et al.* 2014). This form is a slender plant, often 150–200 cm tall with the leaves on the upper and median portion of the stems narrowly ovate, only marginally cordate, with shallowly crenate to serrate leaf margins. Female inflorescences are initially spreading, sparsely branched and lax, soon pendulous, male inflorescences are very sparsely branched and pendulous. Generally with few stinging hairs and virtually no bristles. The perianth is always free of stinging hairs, very rarely with individual stinging hairs. Typically, this form is quite pubescent in northern and north-eastern Europe and may be densely soft pubescent and velvety to touch. A slightly younger name is var. *angustifolia* Wimmer & Grabowski 1829, which could be considered as referring to the generally less pubescent central and eastern European plants with occasionally very narrow leaves. However, a clear dividing is definitely absent and there are distinctly pubescent forms across the range of this form. We therefore advocate recognizing only one sparsely setose and generally narrow-leaved forest form across Europe, with the only exception of distinctly glabrescent var. *glabrata* (see below). Intermediate individuals between the two varieties are commonly found where var. *dioica* grows in the immediate vicinity, e.g. along forest roads (see below for intermediate specimens).

**Distribution and Ecology:**—Forest form found in nutrient rich, humid to wet soils in both deciduous and coniferous forests across, also on steep rocky slopes in mountain forests. Europe including humid mountain ranges in the Mediterranean, but overall distribution is poorly known. The pubescent form is particularly common found in the swampy forests in Brandenburg and Mecklenburg-Vorpommern and apparently also in southern Scandinavia.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica dioica* subsp. *dioica* var. *holosericea* is considered "Least Concern" (LC).

**Representative specimens:**—**AUSTRIA. Carinthia:** Spittal a.d. Drau, Gemeinde Flattern, Fraganter Hütte, 1400 m, Hochstaudenflur, MTB 9044/1431, 10 Aug. 1994, *F. Schuhwerk 94/627* (M);—**CZECH REPUBLIC.** Elbe Sandstone Mountains, road Prague-Dresden, ca. 3 km above Borislav, ca. 700 m, 22 Oct. 2001, cultivated in Berlin July 2003, *M. & K. Weigend 5666-C* (BONN);—**DENMARK. Zealand:** between Stampen and Rådvad in Jaegersborg, 18 Aug. 1971, *J. Svendsen 487* (M); same date and locality, *J. Svendsen 486* (M);—**FINLAND. Satakunta:** Par. Nakkila, Soinila, 17 Aug. 1970, *P.S. Jokela s.n.* (M);—

**FRANCE. Haute-Marne:** Eclaron, 12 Aug. 1993, *B. du Retz 91102* (MSB); **Haute-Rhin:** Tête des Peches, > 1100 m, MTB 8107/2, 18 July 2004, *G. Hügin 18 092* (herb. Hügin: 2 n = 24/ oder 26, counted 2004/05);—**GERMANY. Baden-Württemberg:** Schwarzwald, Feldberg, Grafenmatt, Ruckenhütte, ca. 1200 m, 27 June 2006, MTB 8114/3, *G. Hügin* 19696 (19696b: Berlin, 19696, 19696a, 19696c: herb. Hügin); **Bavaria:** Berchtesgadener Land, national park Berchtesgaden, path from St. Bartholomä-Schreinbachhütte, ca. 900 m, 22 July 1998, cultivated from *W. Lippert 3417* in July 2003, *W. Lippert 3417-C* (♂, B, BONN, E, K, M, MA, MO, P); Regensburg, Oberbachtal N Sulzbach a.d. Donau, along hiking path E of Danube towards Hammermühle to Unterlichtenwald, 28 June 1998, cultivated in Berlin July 2003, *W. Lippert 3376-C* (♂, BONN, M); Upper Bavaria, Munich, Pullach, Förther, 1998, cultivated in Berlin July 2003, *W. Lippert 3288-C* (♂, BONN, M); Upper Palatinate, Kallmünz between Schwandorf and Regensburg, castle ruins above village, 30 April 2002, cultivated in Berlin July 2003, *M. & K. Weigend 7782-C* (♂, BONN); Upper Palatinate, Weiden, Flossenbürg, ruins of Haselstein castle, 680–705 m, 29 June 1997, cultivated in Berlin July 2003, *W. Lippert 3258b* (♀, BM, BONN, KRAM, LE, M, NY, P, W); **Berlin:** Dahlwitzer Landstr. N of Friedrichshagen, at town sign left into bridle path, left at wayside, 38 m, 17 Aug. 2007, *T. Camenzind 13* (♂, BONN); same locality and date, *T. Camenzind 14* (<♂♀>, BONN); same locality and date, *T. Camenzind 18* (♂, BONN); Dahlwitzer Landstr. N of Friedrichshagen, branch to Ravensburg, across from 60 km/h sign 47 m, 15 Aug. 2007, *T. Camenzind 12* (<♂♀>, BONN); Friedrichshagen, Müggelseedamm E of "Bundesamt für Strahlenschutz" right into path, at small bath meadow, 32 m, 15 Aug. 2007, *T. Camenzind 08* (♂, BONN); same locality and date, *T. Camenzind 11* (♂, BONN); Müggelheim, corner Staudernheimer Str./Duchrother Str. right into path, 2. road junction right across from wayside, 43 m, 30 Aug. 2007, *T. Camenzind 25* (♂, BONN); same locality and date, *T. Camenzind 24* (<♂♀>, BONN); Müggelheimer Damm E of Köpenick, 1. bus stop left into path, 1. road junction right, right wayside, 38 m, 15 Aug. 2007, *T. Camenzind 04* (♂, BONN); same locality and date, *T. Camenzind 05* (♀, BONN); same locality and date, *T. Camenzind 02* (♂, BONN); **Brandenburg:** near Krumme Laake at the Müggelsee, 15 June 2002, cultivated in Berlin July 2003, *M. & K. Weigend 7783-C* (<♂♀>, BONN); Dahlwitzer Landstr. N of Friedrichshagen, at town sign left into bridle path until wood, right side behind shrubs, 38 m, 17 Aug. 2007, *T. Camenzind 19* (<♂♀>, BONN); Dahlwitzer Landstr. N of Friedrichshagen, at town sign left into bridle path, left at wayside, 17 Sept. 2007, *T. Camenzind 17* (<♂♀>, BONN); Müggelheimer Damm E of Köpenick, 1. bus stop left into path, 1. road junction right, right wayside, 38 m, 15 Aug. 2007, *T. Camenzind 06* (♂, BONN); Müggelheim, Spreewald near Müggelspree (Großer Müggelsee E of Berlin), Str. 35, near Ferryport F24.0, Oct. 2001, cultivated in Berlin June 2003, *K. Weigend 2001/2* (♂, BONN); FU Berlin, Garden of Institut für Biologie—Systematische Botanik und Pflanzengeographie, cultivated in Berlin July 2004, *M. Weigend 8101* (♀, BONN);—**ITALY. Tuscany:** Lucca, Mte Procinto, 1177 m, N. Expos., 3 June 1979, *Amann & Gottanka s.n.* (M); Apennin between Forli and Bibiena, Passo del Mandrioli, 1170–1180 m, April 2002, cultivated in Berlin July 2003, *M. & K. Weigend 7092-C* (♂, BONN);—**LITHUANIA. Siedlce:** Wojnów, *W. Dybowski 218.1415* (KRAM);—**POLAND. Bialystok:** Bielowicza, Aug. 2005, original collection by A. K. Pietrcyk & D. Ullmann, cultivated in Berlin Aug. 2006, *M. Weigend 8698* (♀, B, BONN, M); **Lesser Poland:** Podgórze, near Ludwinowa near Trawil, *A Żunday 176.500* (2 x KRAM); Ludwinowa, 01 Aug. 1908, *A. Zunday 176.499* (KRAM); **Nowogródek:** Nianków, 05 July 1894, *W. Dybowski 201.887* (KRAM); **Podtarze:** Carna Góra, 27 Sept. 1954, *T. Tacik s.n.* (KRAM); **Silesia:** Obernik, in moist wood, Aug. 1867, *R. v. Uechtritz s.n.* (JENA); 29 August 1897, *I. Kalinowska s.n.* (KRAM); Wrocław, Ufer der Osla bei Zedlitz, July 1863, *C. v. Haussknecht s.n.* (JENA);—**NORWAY. Porsanger:** Porsangerfjord, Laksely, anno 1930, *N.V. Polunin 683.XIX. a* (BM);—**RUSSIA. Moscow:** Moscow area, Station Bitza, along railroad, 27 Aug. 1980, *O.W. Shurba s.n.* (JENA, KRAM); **Saint Petersburg:** Saint Petersburg area, near Daschnoe suburb, 1920, *R. Roshewitz s.n.* (JENA);—**SPAIN. Burgos:** Peñas de Cervera, Contreras, Alto de San Carlos, 1400 m, 18 July 1979, *Muñoz Garmendia et al. 534* (M);—**SWEDEN. Skåne län:** Scania, Bingsgården, August 1903, *E. Ohlsson s.n.* (B); Helsingborg, 08 Aug. 1909, *Th. Sjövall s.n.* (GB); **Stockholms Iän:** Stockholm, 01 Aug. 1912, *A. Hülphers s.n.* (GB); **Västra Götaland:** Dalia, par. Åmål, Buxbol, Aug. 1899, *P.A. Larsson s.n.* (GB); Toarp, anno 1886, A.O. Olson s.n. (GB); **Vermland:** Råda, Ritberg [?], 10 Sept. 1908, *H.A. Fröding s.n.* (2 x BM); Råda, 18 Sept. 1897, *H.A. Fröding s.n.* (B);—**TURKEY. Kahramanmaraş:** Çaðlayancerit, dere kenan, 1159 m, N37°44'25" E37°16'47", 09 June 2007, *B. Tankahya 2498* (♀, BONN);—**UNITED KINGDOM. Berkshire:** near Radley, Sept. 1889, *G.C. Druce s.n.* (BM); **Cambridgeshire:** Wicken Fen, 09 Aug. 1947, *A.H.G. Alston* (BM); **Devon:** N Devon, Brendon, roadside, 25 Aug. 1917, *W.C. Barton 281* (BM, pro parte); N Devon, South Twaton, 02 Sept. 1919, *W.C. Barton 460b & 460b* (BM); **Hampshire:** N Hampshire, Ripley, bank of the Whitewater River, 13 June 1948, *E.Wallace 6113b* (BM—very silky, but completely sterile); **Herefordshire:** Weston, 30 Aug. 1906, *W.C. Barton s.n.* (BM); Aldenham School, 27 Aug. 1973, *K. Wheeler s.n.* (BM); **Leicester:** Newstead road, 29 July 1903, *W. Bell s.n.* (BM); **Leicestershire:** Knighton Spinneys, Sept.1904, *W. Bell s.n.* (BM); Nyor [?], 31 October 1893, *H.J. Riddelsdell s.n.* (BM); **Norfolk:**

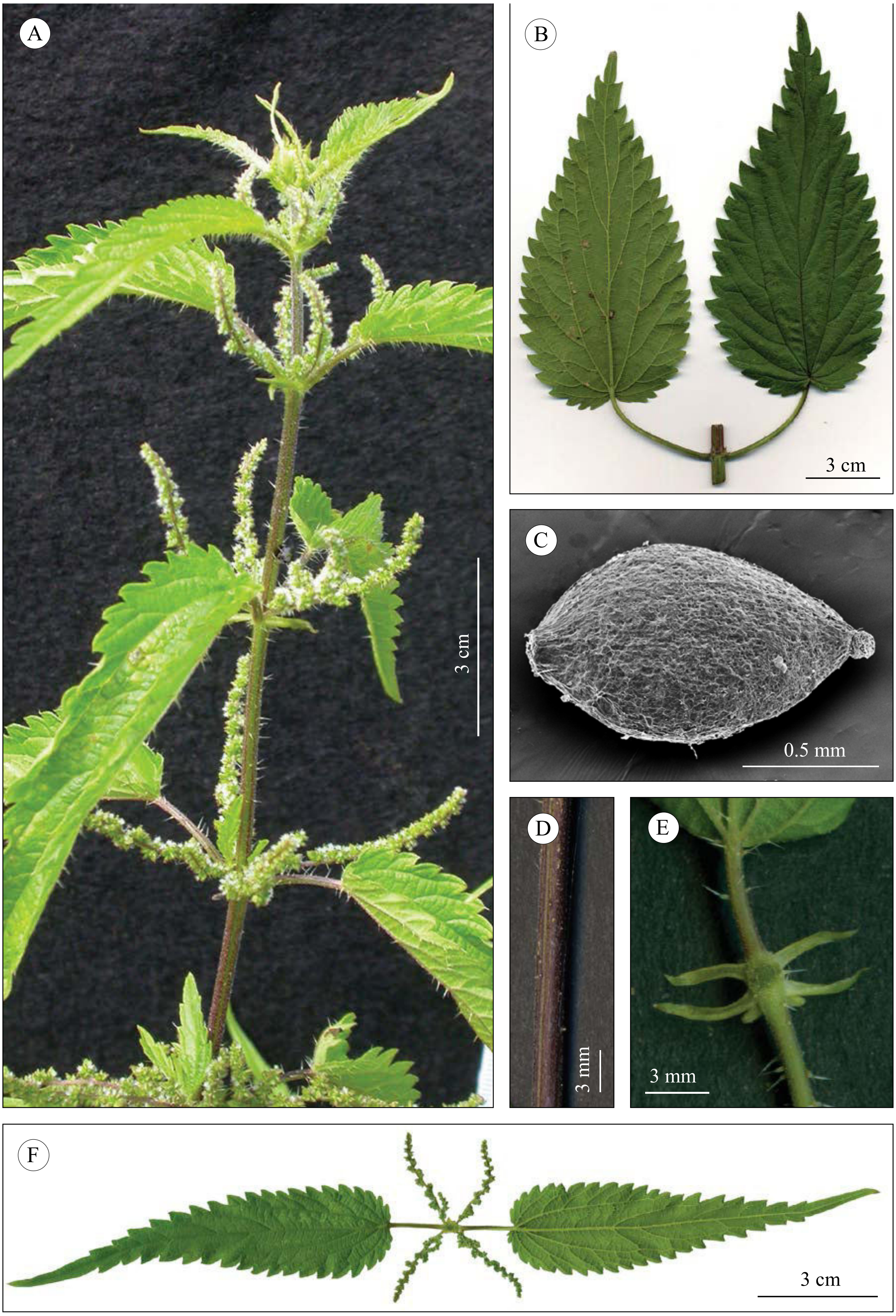

**Figure 2.5** *Urtica dioica* L. subsp. *dioica* var. *glabrata*: (all *M. Weigend 7097*), **A.** Habit, **B.** Node with leaf pair with abaxial (left) and adaxial (right) surface, **C.** Achene, **D.** Stem only sparsely pubescent, **E.** Node with stipules, **F.** Leaf pair with female inflorescences.

Strumshaw Fen Nature Reserve, cultivated at The Natural History Museum´s Wildlife Garden, fen area, 10 July 2002, *A.R. Vickery 883* (BM, BSB particularly pilose form!); South Bank of River Kennet, 25 km W of footbridge over river, 51°24'N, 01°10'W, 03 Sept. 1994, *L. C. & S.L. Jury 358* (B); **Oxfordshire:** Oxon, North Stoke, Sept. 1912, *G.C. Druce 2250* (BM); Oxon, Island, Goring, 26 June 1900, *A.J. Riddelsdell s.n.* (BM); Derby, Alverston, 13 Aug. 1943*, D.P. Young s.n.* (BM); **Surrey:** West Barnes, 30 July 1908, *C.E. Britton s.n.* (BM, sparsly covered with stinging hairs); West Barens, Morton, 13 Aug. 1908, *C.E. Britton s.n.* (BM); Wimbledon Common, Sept. 1920, *H.S. Redgrove s.n.* (BM); **Sussex:** Forest Row, 23 Sept. 1916, *F.J. Hanbury s.n.* (2 x BM); **Western Lancaster** [?]: near Inskip, 5 Aug. 1895, *E.S. Marshall s.n.* (BM).

**Intermediate specimens to var. *dioica*:—GERMANY. Brandenburg:** Dahlwitzer Landstr. N of Friedrichshagen, at town sign left into bridle path, again left, left wayside, 44 m, 17 Aug. 2007*, T. Camenzind 15* (♂, BONN); same locality and date, *T. Camenzind 16* (♂, BONN); Großer Wannsee, Street “Am Postfenn”, 20 May 2002, *M. & K. Weigend 7103* (♀, BONN); W of Müncheberg, in a Carpinus-Quercus-forest, June 2002, cultivated in Berlin July 2004, *M. & K. Weigend 5647-C* (♀ and <♂♀>, BONN); Havelländisches Luch, N of Brandenburg near Seelensdorf, 9 June 2002, *M. & K. Weigend 7120* (♀, BM, BSB, GB, M, W);—**POLAND. Bialystok:** Bielowicza, Aug. 2005, original collection by A. K. Pietrcyk & D. Ullmann, cultivated in Berlin Aug. 2006, *M. Weigend 8702* (<♂♀>, B, BONN, FR, M, UPS); same collection and date, original collection by A. K. Pietrcyk & D. Ullmann, cultivated in Berlin Aug. 2006, *M. Weigend 8701* (♀, B, BONN, G, M); Bielowicza, October 2001, *I. Polunin s.n.*, cultivated in Berlin July 2002, *M. & K. Weigend 5655-C* (♀, BONN, M);—**SWITZERLAND. Wallis:** Baltschiedertal, near Raaft, above Visp, ca. 1000 m, 02 Sept. 2004, M. *& K. Weigend 8115* (♀, BONN); Zeneggen near Visp, between Zeneggen and Castel, 1500–1800 m, 01 Sept. 2004, , *M. & K. Weigend 8110* (♀, BM, BONN, LE, M, P, W), specimens prepared from cultivated plants in June 2005, *M. & K. Weigend 8110-C* (♀, BONN).

### 2.5.6 ***Urtica dioica*** L. subsp. ***dioica*** var. ***glabrata*** (Clem. ex Visiani) Asch. & Graeb. (1911: 609). Fig. 2.5

Basionym: *Urtica glabrata* Clem. ex Visiani (1842: 217). Holotype:—CROATIA. Southern Croatia: Dinaric Alps, Mount Biokovo Mountains, “Habitat frequens in nomoroviis lateris orientials montis Biokovo” (not located). Neotype (*here designated*):—ITALY. Southern Tyrolia: Trentino, Monte Stivo NW of Arco (N of Pass over Monte Velo), ca. 1100 m, April 2002, cultivated in Berlin July 2003, *M. & K. Weigend 7097-C* (♂, BONN!, isoneotypes: BM!, KRAM!, LE!, M!, MA! , W!).

Kanitz (1872) searched for the type and was convinced that the specimen was either never prepared by Visiani, or already lost then.

≡ *Urtica dioica* L. var. *vulgaris* Wedd. subvar. *glabrata* (Clem. ex Vis.) Wedd., in DC. (1869: 51).

This variety has been little cited, but it may be more or less widespread in the southern Alps. We recollected this form in northern Italy and brought it into cultivation, where it turned out to be stable. This form is essentially identical to var. *holosericea*, but differs in the very sparse pubescence, so that the whole plant appears shiny, vivid green and lustrous. There are very few stinging hairs and no bristles.

**Distribution and Ecology:**—Forest form found in nutrient rich, humid montane forests in the southern Alps and on the Balkan peninsula.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica dioica* subsp. *dioica* var. *glabrata* is considered “Not Evaluated” (NE), since it is known from very few localities.

**Representative specimens:—ITALY. Southern Tyrolia:** Trentino, Monte Stivo NW of Arco (N of Pass over Monte Velo), ca. 1100 m, April 2002, cultivated in Berlin July 2003, *M. & K. Weigend 7097-C* (<♂♀>, BONN, M).

## 2.6 Acknowledgements

We would like to express our sincere gratitude to a range of colleagues who participated in the early stages of this study, most importantly W. Lippert (Munich) and N.M. Nürk (Heidelberg). Numerous additional colleagues also provided plant material for our studies, which is here gratefully acknowledged. The most important contributions came from T. Camenzind, J. Devers, T. Henning, H.H. Hilger, G. Parolly, T. Duerbye, S. Kipka, J. Schulz, Ch. Schneider, B. Tarikahya (Berlin), D. V. Geltman (St. Petersburg), Th. Franke & P. Iosifidou and F. Schuhwerk (Munich), G. Hügin (Denzlingen), N.M. Nürk (Heidelberg), F. Selvi (Florence), K. Weigend (Bonn), A.R. Vickery (London).

# CHAPTER 3

## Weeding the nettles IV: A redefinition of *Urtica incisa* and allies in New Zealand and Australia, including the segregation of two new species *Urtica sykesii* and *U. perconfusa**

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### Abstract

Taxon differentiation in *Urtica* from Australia and New Zealand initially appears to be uncomplicated, with taxa being easy to distinguish. However, a revision of the type material, more recent collections and a comparison of Australian and New Zealand material shows that three of the names are misapplied. *Urtica gracilis* (as *U. dioica* subsp. *gracilis,* North America) has been reported as introduced to New Zealand, but molecular data retrieve the corresponding specimens with the other NZ species and we argue that they belong to the polygamous Australian species *Urtica incisa*. A critical revision of the protologues and type collections reveals that the names *Urtica incisa*, originally described from mainland Australia, and *U. incisa* var. *linearifolia* from Tasmania, have been misapplied to New Zealand taxa. Both New Zealand "*Urtica linearifolia*" and "*U. incisa*" represent unnamed taxa and are here formally described as *Urtica perconfusa* and *Urtica sykesii*, respectively. *Urtica perconfusa* corresponds to what is erroneously known as *U. linearifolia*. *Urtica sykesii* is an overlooked species, erroneously interpreted as *U. incisa* in New Zealand. It may be differentiated from *U. incisa* Poir. by its smaller, deltoid leaf lamina with a truncate to

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subcordate base (versus truncate to cuneate), fewer leaf teeth (9–12 on each side rather than 14–20 in *U. incisa*) and smaller plant size (20–60 cm rather than 60–200 cm in *U. incisa*). We found evidence for the presence of true introduced *U. dioica* subsp. *dioica* in New Zealand, but not for *U. gracilis*. Rather, New Zealand specimens assigned to the putatively introduced northern hemisphere *U. gracilis* belong to *U. incisa* as described from Australia. Typifications for the species treated here are provided, including an updated key to the Australian and New Zealand taxa. There are thus six native species of *Urtica* in New Zealand, four of them endemic, and two also indigenous in Australia.

*Keywords:* endemic, taxonomy, Urticaceae, *Urtica dioica*, *Urtica gracilis*

## 3.1 Introduction

The genus *Urtica* is subcosmopolitan and has numerous problems in taxon delimitation. Species definitions are complicated by the overall highly conserved morphology, with fruits and flowers virtually uniform across the genus and species delimitations essentially based on growth habit, leaf and stipule morphology and details of the indumentum. All these character complexes are subject to phenotypic plasticity and/or difficult to reliably recognize in herbarium specimens (Weigend & Luebert 2009, Grosse-Veldmann & Weigend 2015). Critical morphological studies based on cultivated plants and especially molecular data have helped in the past few years to resolve some of the long-standing problems in the systematics of *Urtica*. The most problematic complex, the one including the perennial taxa within the *Urtica dioica* group, has now been largely resolved (Henning *et al.* 2014). One important insight was the independent origin of American "*Urtica dioica*" (now redefined as *U. gracilis* Ait.). The data also showed that the New Zealand material considered as belonging to "*Urtica dioica* subsp. *gracilis"* (= *U. gracilis* Ait. subsp. *gracilis*) by Webb *et al.* (1988) groups with specimens of *Urtica incisa* from both Australia and New Zealand, representing a different clade (Farag *et al.* 2013, Henning *et al.* 2014). This is a first indication that the *Urtica* taxonomy in Allan (1961) and Webb *et al.* (1988) is problematic and that their reports of *U. dioica* from New Zealand require critical re-examination. At the same time, Allan (1961) and Morris & Baker (2009) already indicated that the NZ material of "*Urtica incisa*" was "somewhat different" from the Australian material of *U. incisa*. These issues motivated a critical re-examination of the specimens of "*Urtica dioica*" and "*Urtica incisa*" from New Zealand and a comparison to authentic material of *U. dioica* and *U. gracilis*.

## 3.2 Material and methods

Material was examined from the following herbaria: AD, AK, B, BONN, CANB, CHR, E, FI, FR, HO, K, KIEL, M, NSW, P, PR, SP, W. *Urtica sykesii* (i.e., New Zealand "*U. incisa*") was taken into cultivation in Berlin in 2006, *Urtica incisa* was taken into cultivation at Bonn University Botanical Gardens in 2011. Conservation assessments were undertaken using the New Zealand Threat Classification System (de Lange *et al.* 2013).

## 3.3 Results

### 3.3.1 Characterization of *Urtica incisa*

*Urtica incisa* was originally described based on specimens collected from mainland Australia ("Nouvelle Hollande"), without any details on the locality known. Of the original material now housed at Paris (P) and Florence (FI, see formal taxonomy), only the Florence specimen is well-preserved (the Paris specimen is only a tiny fragment). The Florencetype corresponds to a very narrow-leaved, stiffly erect, unbranched herb with female flowers only. This material is virtually indistinguishable from the Tasmanian holotype of *U. lucifuga* var. *linearifolia* Hook.f., now treated as *Urtica linearifolia* (Hook.f.) Cockayne, which only differs in having marginally wider leaves and male shoots only. The stiffly erect, virtually unbranched, narrow-leaved, unisexual Tasmanian-Australian taxon is thus *Urtica incisa* in the narrowest sense and *Urtica linearifolia* has to be synonymized with *U. incisa*. *Urtica lucifuga* var. *linearifolia* came to be identified with a monoecious New Zealand taxon and considered as a distinct species. The reduction of the name *U. linearifolia* as a synonym under *U. incisa* leaves the New Zealand taxon lacking a name. *Urtica lucifuga* Hook.f. is consistently treated as a synonym of *Urtica incisa* in the literature. The Tasmanian types represent monoecious individuals of a more broad-leaved form, with the male flowers in the lower part of the stem and the female flowers in the upper part of the stem. However, after revising herbarium material and comparing it to the type specimens, and keeping in mind the plasticity of certain leaf characters in *Urtica* (e.g., Grosse-Veldmann & Weigend 2015), we conclude that the differences in leaf shape and the monoecious nature of the type of *U. lucifuga* in—usually dioecious—*U. incisa* fall well within the range of variability of a single species. We therefore propose the redefinition of *Urtica incisa* as outlined above and the synonymization of both *Urtica linearifolia* and *U. lucifuga*. Monoecy is quite a constant character in *Urtica*, but dioecy is not: Widespread and common *Urtica dioica* and all its subspecies are polygamous, as has been frequently documented (Henning *et al.* 2014). The predominantly dioecious, but actually polygamous species *U. incisa* as here defined based on the type material, is widely distributed in southern and eastern Australia and Tasmania and is also found in New Zealand, where it is probably naturalized based on the habitats and locations it has been collected from.

### 3.3.2 “*Urtica dioica*” and “*U. incisa*” in New Zealand

Using the key in Webb *et al.* (1988), the plants that fall under *U. incisa* as here defined key out as *U. dioica* subsp. *dioica* (for the wider leaved form) or *U. dioica* subsp. *gracilis* (≡ *U. gracilis* subsp. *gracilis*) for the narrow-leaved form (i.e., typical *U. incisa*). The molecular data provided in Henning *et al.* (2014) in combination with a morphological comparison underscore that most of this material can be referred to *U. incisa*. However, we can confirm that true northern hemisphere *U. dioica* is present in New Zealand based on the material seen (CHR 474966, CHR 495175, CHR 515018, CHR 554345, CHR 605119) and on DNA sequence data of the same numbers which retrieve the specimens with other *U. dioica* sequences. We have no evidence for the presence of introduced *U. gracilis* (R. Smissen, pers. comm.). Cheeseman (1925), Allan (1961) and Webb *et al.* (1988) also report *Urtica incisa* from New Zealand, characterizing it as a fairly small, strictly monoecious herb with triangular-ovate leaves with cordate leaf bases. This characterization was followed by Chew (1989) in the *Flora of Australia*, evidently based on the definition in Cheeseman (1925) and Allan (1961), who also provided an illustration of this plant. Morris & Baker (2009) indicate that the description of Chew (1989) does not correspond to the material identified as *Urtica incisa* occurring in Tasmania. A revision of the available names and the plant material of *Urtica incisa sensu* Cheeseman (1925) shows that this plant is widespread in New Zealand and also found in Australia, and that it is a distinct species currently lacking a name. Both unnamed species are formally described below.

## 3.4 Formal taxonomy

### 3.4.1 Key to the species of *Urtica* from New Zealand and Australia

1 Annual herbs with fibrous root.

2 All inflorescences bisexual, sessile or shortly pedunculate, at least median and lower ones much shorter than petioles; leaves ovate, dark green; achenes ovoid, 1.5–2 mm long……………………………………………………………*U. urens*

2* Most inflorescences unisexual, distinctly pedunculate, male ones flattened, with flowers only on one side, usually longer than petioles; leaves ovate, bright green; achenes broadly ovoid, 1–1.2 mm long……………………………………………*U. membranacea*

1* Perennial herbs with some type of underground shoot system, often rhizomatous, sometimes shrubs.

3 Shrubs to 2 m and more; stems stiff and woody; stinging hairs (setae) 0.5–1 mm on very long, pluricellular pedestal to 6 mm long; lamina triangular-ovate……………………*U. ferox*

3* Herbs to 1.5 (rarely 2) m; stems herbaceous, sometimes lignescent at base, rarely to 2 m then plant scrambling to lianescent, but usually much shorter; stinging hairs (setae) 1–1.5 mm or much shorter, pluricellular pedestal < 1mm long; lamina ovate to subcircular, narrowly ovate to linear or triangular-ovate.

4 Plant with very slender, weak, openly branched shoots, scrambling or lianoid; lamina narrowly ovate from rounded base; inflorescence 3–10 mm, glomerulous, much shorter than petiole……………………………………………………………………………………………*U. perconfusa*

4* Plant with erect shoots, usually not or sparsely branched; lamina usually ovate or triangular ovate, rarely narrowly obovate or oblong, then inflorescences more than 20 mm long.

5 Leaves widely ovate to subcircular in outline, 100–200 mm long; plant sparsely setose……………………………………………………………………………………………*U. australis*

5* Leaves narrowly ovate or triangular-ovate, usually less than 120 mm long; plant usually densely setose.

6 Plants bisexual with male flowers below and female flowers above; lamina triangular ovate from subcordate base, 20–60 mm long……………………………………………………*U. sykesii*

6* Plants unisexual; rarely bisexual with individual inflorescences male or female, then lamina not triangular-ovate and/or larger; inflorescence often longer than petiole.

7 Herb to 0.4 m; lamina 20–40 mm long, about as long as wide; petioles usually more than 2/3 of overall length of leaf lamina………………………………………………………………*U. aspera*

7* Herb more than 0.4 m, often more than 1 m; lamina 60–150 mm long, longer than wide; petioles usually up to 1/3–1/2 of overall length of leaf lamina.

8 Leaves triangular-ovate to narrowly oblong with a cuneate to truncate base, much longer than wide; stinging hairs few; inflorescences usually weakly branched; achene subcircular, rounded at base and tip, c. 1.2–1.5(–2.0) × 0.8–1.2 mm…………………………………*U. incisa*

8* Leaves broadly ovate with a cordate base, usually not much longer than wide; stinging hairs many; inflorescences strongly branched; achene ovoid with a narrowed base and apex, widest below the middle, c. 1–1.5 × 0.6–0.8 mm…………………………………………………*U. dioica*

The formal taxonomy is here provided for a redefined *Urtica incisa*, the two species lacking a name and the introduced *Urtica dioica*. The formal taxonomy for other NZ-species of *Urtica* (*U. aspera* and *U. australis*) is provided in the Appendix 1.

### **3.4.2 *Urtica incisa*** Poir. in Lamarck (1816: 224)

Lectotype (here designated):—[AUSTRALIA. Nouvelle Hollande]. *Urtica incisa* (*n*), nov. holl., labill. [*J. J. H. de Labillardière s.n.*]

(P-DESF 00601927!). Syntype:—[AUSTRALIA. Nouvelle Hollande]. n. holl., *bill.* [*J. J. H. de Labillardière s.n.*] (FI-WEBB 169977!).

= *Urtica lucifuga* Hook.f. var. *lucifuga* Hook.f. (1847: 285). Lectotype (here designated):—[AUSTRALIA. Tasmania.] *Urtica incisa* Poir. *Urtica lucifuga n.sp*. V.D. Land [Van Diemen´s Land], *"541"*, *Gunn s.n.* (K 000351867!); Isolectotype: *"541"* [*Gunn s.n.*], *Urtica incisa* Poir. *Urtica lucifuga* n.sp. common—chiefly in damp shady ravines and creeks [?*illegible*] V. D. Land (K 000351868!).

= *Urtica linearifolia* (Hook.f.) Cockayne (1914 [1915]: 111). Basionym: *Urtica lucifuga* Hook.f. var. *linearifolia* Hook.f. (1847: 285).

Holotype:—[AUSTRALIA. Tasmania.] V. D. Land [Van Diemen´s Land], *Urtica incisa* Poir., *Urtica lucifuga* n.sp. var. *β*., Circular Head, 1842, 4/11/37, "*541 ", Gunn s.n.*(K 000351869!).

≡ *Urtica incisa* Poir. var. *linearifolia* (Hook.f.) Cheeseman (1906: 636).

non *Urtica incisa* B.Heyne ex Wall. (1831), illeg. homonym [= *Girardinia diversifolia* (Link) Friis (1981: 145)].

Erect, perennial, rhizomatous herb (0.25–)0.43–2 m, usually unbranched. Stem indumentum of few stinging hairs with pluricellular base c. 0.2–0.3(–0.5) mm overall and erect setae 0.2–0.3(–0.4) mm long and few simple trichomes 0.2–0.3 mm long. Leaf lamina 60–120(–150) × (10–)15–30(–60) mm triangular-ovate to narrowly oblong; surface sparsely pubescent with short simple trichomes 0.2–0.5 mm long and few stinging hairs (abaxially only on the veins), adaxially with punctiform cystoliths; leaf base cuneate to truncate; margins regularly dentate, rarely doubly dentate, with 14–20 teeth on each side; leaf apex acute to acuminate; lamina light greenish; stipules free (4 per node) 2–4 mm long; petioles (10–)15–25(–40) mm long. Plants mostly unisexual, rarely male and female flowers on the same plant. Staminate flowers with tepals c. 1.3–1.8 mm long. Pistillate flowers with short tepals 0.5–0.8 mm long and long tepals 0.9–1.2 mm long, sparsely pubescent, esetulose. Inflorescence 24–40(–60) mm long, often longer than petioles. Mature fruit with longer tepals 1.3–1.5 mm long, achenes subcircular in outline, rounded at base and at the tip, laterally flattened, c. 1.2–1.5(–2.0) × 0.8–1.2 mm.

**Notes:—***Urtica incisa* is a perennial rhizomatous herb with tall and mostly unbranched flowering shoots. Like *U. dioica* this species is polygamous and the vast majority of clones are male or female. There are a few collections with both male and female flowers on the same shoot (E.g. *F. Hood 137, A. M. Buchanan 2592*, *M. D. Crisp 3495*, *V. Klaphake s.n.* NSW 797263 & 797264, *S. W. L. Jacobs 2781*). The leaves are generally much longer than wide and triangular-ovate to narrowly oblong and the inflorescences are comparatively long, often longer than the petioles. In overall appearance, it is very similar to northern hemisphere *Urtica dioica* and *U. gracilis*, but molecular data clearly show it to be only remotely related.

**Distribution:—**The species is widely distributed in (south-)eastern Australia and Tasmania.

**Habitat and ecology:—***Urtica incisa* is found at disturbed sites, forest edges, in swamps and on river banks from sea-level to c. 250 m.

**Phenology:—***Urtica incisa* flowers throughout the year.

**Conservation status:—**According to the New Zealand Threat Classification System (de Lange *et al.* 2013), *Urtica incisa* is considered “Not Threatened”.

**Selected additional specimens examined:—AUSTRALIA. New South Wales:** North Coast: 470 Bulli Creek Road, SSE of Byabarra, S 31° 34’ 6”, E 152° 31’ 54”, 12 May 2009, *V. Klaphake s.n.* (NSW 797270–797274!); Central Coast: Bents Basin, Nepean River, S 33° 55’, E 150° 37’, 5 Oct. 1964, *B. G. Briggs s.n.* (NSW 656029!); Leacocks Lane, below Glenfield Farm, near railway line, Casula, S 33° 57’ 38”, E 150° 54’ 21”, 3 July 2009, *V. Klaphake s.n.* (NSW 797263–797267!); Glenbrook Creek, [4/j/1959], *H. S. McKee 6776* (P 06820929!); S side of Mogo Creek Road, 20 km S of Bucketty towards St Albans, S 33° 12’ 3”, E 151° 1’ 37”, 5 Oct. 1996, *P. S. Jobson 4407* (NSW!); South Coast: 20 km SW of Eden, Mount Imlay, near trig, S 37° 11’, E 149° 44’, 25 Oct. 1977, *M. D. Crisp 3495* (NSW!); Kiah (or Towamba) River, c. 2.5 km NE of Kiah, S 37° 8’, E 149° 52’, 27 Nov. 1976, *S. W. L. Jacobs 2781* (NSW!); Northern Tablelands: Alongside Barrington Trail at either end of bridge over Tugalow Creek, Barrington Tops State Conservation Area, S31° 54’ 31”, E 151° 26’ 41”, 24 Feb. 2008, *J. R. Hosking 3073* (CANB!, NSW!); Central Tablelands: Mt Tomah (Blue Mts), 10 Feb. 1978, collector unknown, legit G. Sag, Feb. 1978 (P 06456160!); Newnes, Wolgan River Valley, NW of Lithgow, S 33° 12’, E 150° 14’, 30 Mar. 1983, *T. A. James 278 & W. Bishop* (NSW!); Southern Tablelands: Cave Creek, 0.25 mile below The Blue Waterholes, 11 miles NE of Rules Point, S 36° 33’, E 148° 49’, 11 Dec. 1969, *A. N. Rodd 912 & R. G. Coveny* (NSW!); North Western Slopes: 17 km from Bingara along highway towards Warialda, S 29° 43’ 46”, E 150° 32’ 26”, 16 Nov. 2000, *J. J. Bruhl 1976 & I. R. Telford* (NE, NSW!); Central Western Slopes: 4.4 km S of Mumbil, S 32° 45’ 52”, E 149° 3’ 12”, 20 Nov. 1999, *A. R. Bean 15814* (BRI, NSW!); South Western Slopes: Murrumbidgee River, off the Leeton-Narrandera Road, S 34° 42’, E 146° 28’, 30 Sept. 1989, *T. A. James 1129* (NSW!); **Victoria:** East Gippsland: Croajingolong National Park, Kingfisher Point, Bottom Lake, Mallacoota, S 37° 31’ 5”, E 149° 46’ 20”, 20 Oct. 1991, *J. H. Ross 3488 & C. A. Coles* (MEL, NSW!); **South Australia:** Murray River: Second swamp S of Blanchetown, S 34° 22’ 26”, E 139° 37’ 23”, 21 Mar. 1973, *J. Z. Weber 3475* (AD, NSW!); Wellington, c. 30 km south-southeast of Murray bridge, at east bank of the River Murray, 01 Feb. 1970, *L. D. Williams* 3499 (AD 97007330!); Kangaroo Island: Near Cape Borda, 24 Sept. 1908, *R. S. Rogers s.n.* (AD, NSW 656026!); **Tasmania:** Without locality data, *W. Archer s.n.* (P 06456147!); without locality data, *King s.n.* (P-DESF 00601928!); without locality data, *King s.n.* (P-DESF 006456159!); without locality data and collector (CHR 524242!); Central Highlands Region, Serpentine Rivulet, Gully 250 m W of Serpentine Rivulet, approximately 2.5 km NE of where it crosses Bronte Canal, S 42° 06' 20.9", E 146° 31' 35.4", 745 m, 27 Feb. 2014, *M. F. de Salas & L. Cave 779* (HO 574971!); Central Highlands Region, 2 km from junction with Tarraleah Canal No. 1 and Lyell Highway, S 42° 19', E 146° 25', 02 Feb. 1981, *A. Brown 193* (HO 407037!); East Coast Region, St. Patricks Head, S 41° 34' E 148° 13', 400 m, 5 Feb. 1982, *A. M. Buchanan 911* (HO 50265!); East Coast Region, Mt. Wellington, S 42° 54', E 147° 17', Oct. 1873, *S. G. Hannaford s.n.* (HO 23589!); East Coast Region, Orford, S 42° 34', E 147° 52', 27 Nov. 1973, *F. Hood 137* (HO 569876!); East Coast Region, Tunnack, S 42° 27', E 147° 28', Sept. 1977, collector unknown, ex Tasmanian Department of Agriculture Herbarium (HO 569053!); Furneaux Region, East Flinders Island, behind Planter Beach, S 40° 09', E 148° 17', 0 m, 26 Sept. 1989, *P. Collier 4198* (HO 119905!); Furneaux Region, Big Dog [Great Dog] Island, S 40° 15', E 148° 15', 08 Oct. 1996, *S. Harris s.n.* (HO 534769!); Furneaux Region, Logan Lagoon Conservation Area [Cameron Inlet], Flinders Island, S 40° 06' 41.9", E 148° 17' 31.7", 26 Mar. 2014, *M. L. Baker & M. F. de Salas 2931* (HO 574022!); Midlands Region, Bessels Road, below northern slope of Western Tiers, S 41° 43' E 146° 36', 630 m, 16 Oct. 1984, *W. F. Pataczek 033* (HO 86026!); Midlands Region, Tunbridge Tier, Tunbridge Tier Rd., S 42° 07', E 147°, 16', 800 m, 26 Sept. 2001, A. *C. Rozefelds 2072* (HO 531053!); North East Region, near Thomas Plain, S 41° 12', E 147° 54', 05 Feb. 1877, *A. Simpson s.n.* (HO 504805!); North West Region, Nettle Bay, west of Marrawah, S 40° 55' 02", E 144° 39' 13", 20 m, 24 Oct. 1992, *A. C. Rozefelds 577* (HO 324652!); South West Region, Cox Bight, west of Freney Lagoon exit, S 43° 29' E 146° 13', 0 m, 30 Nov. 1982, *D. I. Morris 82115* (HO 68047!); North West Region, Hellyer Gorge Valley, c. 350 m, 7 Jan. 1977, *H. D. Wilson s.n.* (CHR 310840!); Mouth of Oxley Creek, Spero Bay, S 42° 37', E 145° 19', 2 m, 16 Jan. 1984, *A. M. Buchanan 2592* (HO 98479!); West Coast Region, Sundown Point, south of Arthur River, S 41° 07' 12", E 144° 40' 25", 5 m, 24 Oct. 1997, *A. C. Rozefelds 569* (HO 324646!); **Victoria.** County of Follett, "Nine mile creek", 03 Mar. 1904, *F. M. Reader s.n.* (P 06456149!);—**NEW ZEALAND. Canterbury (South Island):** Waimate Distr., Waimate, South Canterbury, 30 Apr. 1979, *J. Oliver s.n.* (CHR 355708!); **Greater Wellington (North Island):** Wairarapa Distr., Ruamahanga River, SW of Martinborough, 3 m, 15 Nov. 1970, *B. H. Macmillan 10/254* (CHR 525260!); Wellington

Land Distr., Martinborough-Pirinoa Rd, 21 Sept. 2006, *B. Winder s.n.* (CHR 585611!); Wellington, Haywards Hutt Valley, 04 Jan. 1953, *A. J. Healy 53/11* (CHR 88531B!); **Manawatu-Wanganui (North Island):** Ruapehu-Distr., near Raetihi, Dec. 1940, *J. S. Attwood s.n.* (CHR 48288!); **Marlborough (South Island):** Marlborough Land Distr., Lake Rotorua, Kaikoura Lakes near Peketa, 30 m, 28 Sept. 1998 *C. Jones CJ01: 17* (CHR 525311!); **Taranaki (North Island):** Taranaki Land Distr., Taranaki, Eltham, S 39° 25', E 174° 18', 243 m, 19 Nov. 1982, *C. J. Webb s.n.* (CHR 404138A!, CHR 404138B!); **Waikato (North Island):** Cambridge Domain, Cambridge, 17 Mar. 1964, *R. K. Ward s.n.* (CHR 234516A!).

### 3.4.3 ***Urtica sykesii*** Grosse-Veldmann & Weigend, *spec. nov.* Fig. 3.1

Type:—NEW ZEALAND. Otago (South Island): Waipori Falls, approx. 25 km south of Dunedin, *Nothofagus menziesii* forest, original collection by Th. Franke, Mar. 2005, cultivated in Berlin May 2006, *M. Weigend 8212* (holotype: CHR!, isotypes: B!, BONN!, E 00267313!).

Erect, perennial, rhizomatous herb (0.15–)0.25–0.30(–0.60) m with elongating rhizomes, aerial stems usually unbranched. Stem indumentum of very few stinging hairs with pluricellular base c. 0.2–0.3(–0.5) mm overall and erect setae (1.3–)1.8–2.2 mm long and very few simple trichomes 0.2–0.3 mm long. Leaf lamina 20–60 × 20–50 mm triangular to triangular-ovate; surface very sparsely pubescent with short simple trichomes 0.2–0.3(–0.5) mm long and very few stinging hairs (abaxially only on the veins), adaxially with punctiform cystoliths; leaf base truncate to subcordate; margins regularly dentate with 9–10(–12) teeth on each side; leaf apex acute to acuminate; lamina light greenish; stipules free (4 per node) 2–4(–10) mm long; petioles 30–45(–70) mm long. Plants monoecious; lowest inflorescences pure male, upper ones pure female. Staminate flowers with tepals c. 1.2–1.8 mm long. Pistillate flowers with short tepals 0.5–0.7 mm long and long tepals 0.9–1.1 mm long, sparsely pubescent, esetulose. Inflorescence 10–20 mm. Mature fruit with longer tepals 1.3–1.5 mm long, achenes subcircular in outline, rounded at base and at the tip, laterally flattened, c. 1.2–1.5 × 0.8–1 mm.

**Additional specimens examined:—AUSTRALIA. Victoria:** Casterton, 16 Mar. 1908, F. M. Reader s.n. (P06456150!);—**NEW ZEALAND.** Without locality data, W. Colenso 3 (P 06456153!); Southern Alps mountain range (South Island), Dec. 1873, Julius von Haast 224 (FI!); **Auckland (North Island):** Hunua, Auckland, 1850–1860, A. Sinclair s.n. (NSW 810012); without locality data, 1850–1860, A. Sinclair s.n. (NSW 810013); **Canterbury**

**(South Island):** Canterbury Land Distr., Banks Peninsula, track to Mt. Sinclair near Whatarangi Scenic Reserve, S 43° 43’, E 172° 52.2’, 26 Jan. 1997, C. J. Webb & L. F. Delph 97/3 (CHR 511586!); same locality, Banks Peninsula, upper Kaituna Valley, 24 Nov. 1991, W. R. Sykes 416/91 (CHR 474045!); same locality, Organ Range, Organ Stream tributary, Island Hills Station, S 42° 40’, E 172° 33’, 560 m, 13 Mar. 1991, B. H. Macmillan 91/53 (CHR 472639!). **Greater Wellington (North Island):** Karori, Wellington, 3 Mar. 1948 T.W. Rawson 62012 (NSW); Wellington, Dec. 1908, c. 130 m, H. H. Travers s.n. (M!, PR!); same locality, wet places—sea level, Mar. 1909, H. H. Travers s.n.(P 06456146!); Pakuratahi forest, 1875, M. Filhol s.n. (P 06456144!); Pakuratahi, without date, T. Kirk s.n. (NSW 810016); **Marlborough (South Island):** Marlborough Land Distr., Lake Rotorua, Kaikura Lakes near Peketa, 30 m, 10–20 km from lake shore, 01 Oct. 1997, *C. Jones CJ01:4C* (CHR 525300!); **Waikato (North Island):** Hamilton Ecological Distr., Hamilton City, Hammond Bush, S 37° 48’, E 175° 19’, 20 Sept. 1992, *P. J. De Lange 1631* (CHR 480432!); Rangitoto Ecological Distr., Rangitoto Station, Rangitoto Range, 20 Nov. 1972, *R. O. Gardner 300* (NSW); Waitomo Distr., Waitomo Caves, Nov. 1956, *E. J. McBarron 6618* (NSW); **West Coast (South Island):** NE of Karamea Finian Trail, 15 Feb. 1983, *M. M. J. van Balgooy 4481* (NSW).

**Notes:**—*Urtica sykesii* Grosse-Veldmann & Weigend corresponds to *Urtica incisa sensu* Cheeseman (1906). That *Urtica sykesii* came to be confused with *U. incisa* is very surprising since, by the standards of *Urtica,* it is very distinct (see above and Tab. 3.1 for comparison). *Urtica sykesii* diverges from *U. incisa* in the much smaller growth with the aerial shoots reaching rarely more than 40 cm, densely and shortly rhizomatous stems forming dense, low stands; or the basal inflorescences are always male and the upper one always female; and the leaves are distinctly triangular to triangular-ovate with a subcordate base. In contrast, *U. incisa* is a robust plant, typically 60–120 cm tall, individual plants are usually unisexual, and the leaves are larger and usually much narrower. Virtually all illustrations of *U. incisa* from New Zealand show *Urtica sykesii.*

**Distribution:**—*Urtica sykesii* Grosse-Veldmann & Weigend is known from both the North and South Islands of New Zealand and from Victoria (Australia) and is probably more widespread in eastern Australia.

**Habitat and ecology:**—*Urtica sykesii* Grosse-Veldmann & Weigend is found at forest margins growing in rich, moist soil between rocks e.g. on streamsides, near swamps, on lakeshores and waterfalls at 0–560 m.

**Phenology:—***Urtica sykesii* Grosse-Veldmann & Weigend flowers throughout the year.

**Etymology:—***Urtica sykesii* Grosse-Veldmann & Weigend is named after the eminent New Zealand botanist William Russell Sykes (*1927 in Christchurch).

**Conservation status:—**According to the New Zealand Threat Classification System (de Lange *et al.* 2013), *Urtica sykesii* (referred to as *U. incisa*) is considered "Not Threatened". (factsheet NZPCN_Species_2282.pdf downloaded from www.nzpcn.org.nz on 30 Nov. 2015).

### 3.4.4 ***Urtica perconfusa*** Grosse-Veldmann & Weigend, *spec. nov.*

Type:—NEW ZEALAND. [Greater Wellington (North Island): Wellington Land Distr.] Wellington, Waiwiri Stream, at bases of *Carex secta* amongst *Phormium* swamp, Alt: 1, Map 260: S 25 Grid: 934619, Lat: 40° 37' S, Long: 175° 10' E, *P. J. De Lange 2094* (holotype: CHR 497596!).

Lax, slender, perennial, rhizomatous herb 0.45–2.0 m, usually much-branched, scrambling or climbing. Stem indumentum of few stinging hairs with pluricellular base c. 0.2–0.5 mm overall and erect setae 0.2–0.4 mm long and few simple trichomes 0.2–0.3 mm long. Leaf lamina 40–80(–100) × 4–10 mm narrowly ovate; surface sparsely pubescent with short simple trichomes 0.2–0.5 mm long and few stinging hairs (abaxially only on the veins), adaxially with punctiform cystoliths; leaf base rounded; margins regularly dentate, rarely doubly dentate, with 12–20 teeth on each side; leaf apex acuminate; lamina thinly membranaceous; stipules free (4 per node) 2–4 mm long; petioles thin, flexuose, 15–45 mm long. Plants monoecious. Staminate flowers with tepals c. 1.3–1.8 mm long. Pistillate flowers with short tepals 0.5–0.8 mm long and long tepals 0.8–1.2 mm long, sparsely pubescent, esetulose. Inflorescence glomerulous, 3–10 mm long, usually shorter than petioles. Mature fruit with longer tepals 1.2–1.5 mm long, achenes subcircular in outline, rounded at base and at the tip, laterally flattened, c. 1.2–1.5(–2.0) × 0.8–1.3 mm.

**Additional specimens examined:—NEW ZEALAND.** Without locality data, *Jung 226* (SP 023898!); without locality data, *W. Colenso s.n.* (SP 023899); **Greater Wellington (North Island):** Wellington Land Distr., Ranigtikei, Turakina, Makirikiri Stream catchment, "Tunnel Hill Farm", S 40° 04', E 175° 12.2', 05 Mar. 1995, *C. C. Ogle 2926* (CHR 506008!); same locality, Lake Horowhenua, Hokio Stream, S 40° 36', E 175° 14.2', *P. J. De Lange 2141* (CHR 497643!); **Marlborough (South Island):** Marlborough Land Distr., Opawa River, Blenheim, 5 m, rough pasture and river banks, 30 July 2000, *J. Clayton-Greene CJ01:14* (CHR 525620!); **North Canterbury (South Island):** Canterbury Land

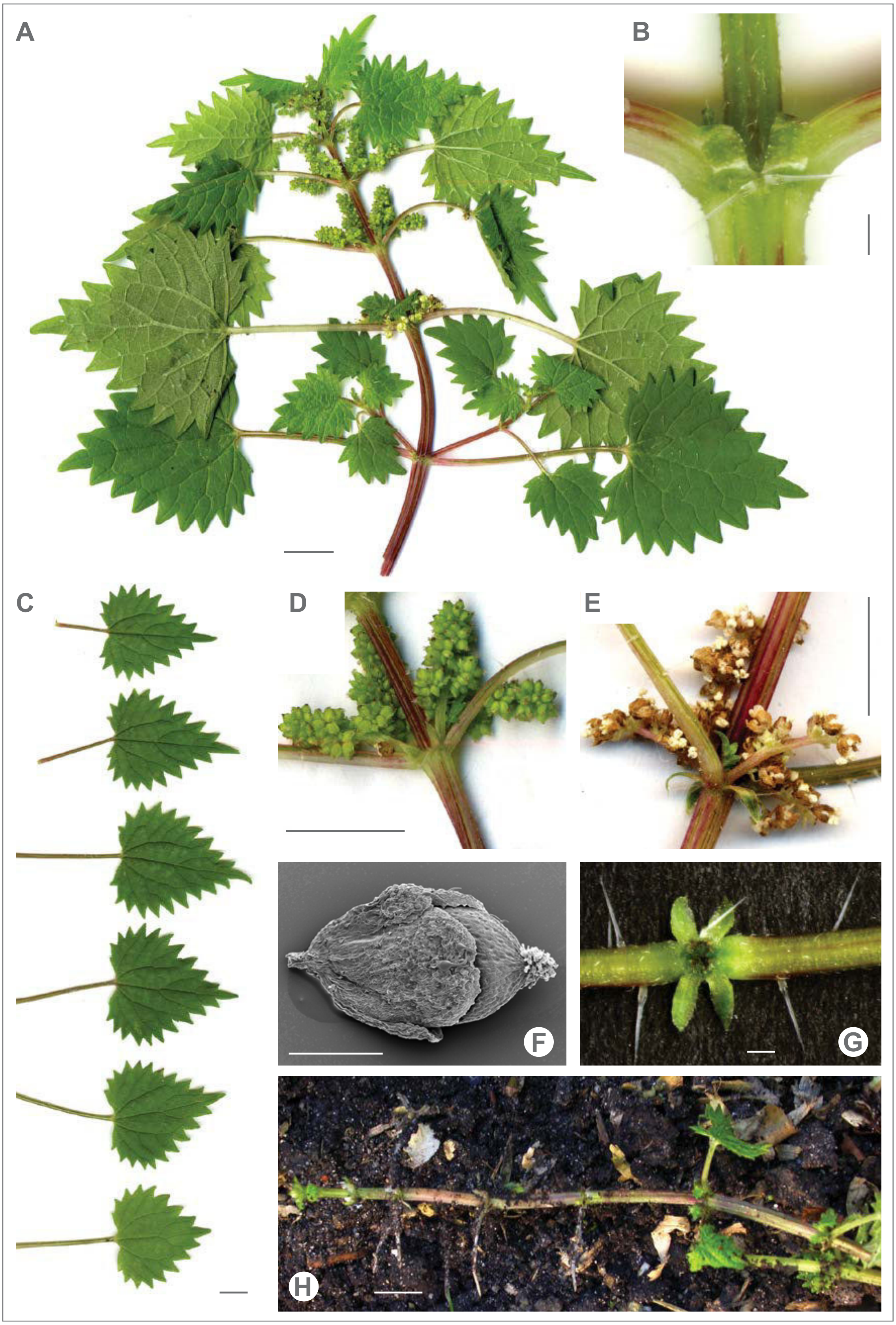

**Figure 3.1** *Urtica sykesii* (M. Weigend 8212), photographic plate. A, Habit with first flowering node male and higher nodes female; B, Node with stipules; C, Leaf series from basal to apical, abaxial; D, Female inflorescence; E, Male inflorescence; F, Achene; G, Petioles with stipules and stinging hairs; H, Elongating rhizome. Scale bar: A, 0.5 mm, B–H, 10 mm. Photos: H. J. Ensikat, N. M. Nürk, M. Weigend.

Distr., Low Plains, Amberley Beach, S 43° 09', E 172° 47', 28 July 1999, *R. Pender s.n.* (CHR 536516!); Canterbury Plains, "common", Oct. 1872, *J. von Haast s.n.* (FI!, FR!); Hannover plains, *J. von Haast s.n.* (FR!).

**Notes:—***Urtica perconfusa* Grosse-Veldmann & Weigend corresponds to *Urtica linearifolia* (Hook.f.) Cockayne *sensu* Cockayne (1915). It has been mistakenly identified as *U. incisa* subsp. *linearifolia* (see discussion under *U. incisa*) in the past (Cheeseman 1906, 1925, Allan 1961). It is very surprising that this confusion has persisted, given that the type of this New Zealand endemic was from Tasmania! *Urtica perconfusa* Grosse-Veldmann & Weigend is a lax, much-branched, scrambling or climbing species up to 2 m high; the inflorescences are glomerulous and very short, usually much shorter than the petioles, sometimes with both sexes mixed on the same branch or at least always with inflorescences of both sexes on the same plant; leaf morphology does resemble that of the narrow-leaved form of *U. incisa*, but the leaves of *Urtica perconfusa* are narrowly ovate with a rounded base, thinly membranaceous and with thin, flexuose petioles. *Urtica perconfusa* is densely and shortly rhizomatous, very similar to *U. sykesii* (see Tab. 3.1 for comparison).

**Distribution:—***Urtica perconfusa* Grosse-Veldmann & Weigend is known from both the North and South Island of New Zealand, but apparently rare. It has not been reported from Australia and was considered as endemic to NZ already in its previous incarnation as "*U. linearifolia*" (but then with a type from Tasmania—Allan 1961).

**Habitat and ecology:—***Urtica perconfusa* Grosse-Veldmann & Weigend is found in lowland swamps and along lake and river margins, often growing over old tree stumps and shrubs.

**Phenology:—***Urtica perconfusa* Grosse-Veldmann & Weigend flowers throughout the year.

**Etymology:—***Urtica perconfusa* Grosse-Veldmann & Weigend means "the thoroughly confused nettle" and commemorates its taxonomic history.

**Conservation status:—**According to the New Zealand Threat Classification System (de Lange et al. 2013), *Urtica perconfusa* is considered "At Risk—declining" (factsheet NZPCN_Species_211.pdf downloaded from www.nzpcn.org.nz on 30 Nov. 2015).

**Table 3.1** Diagnostic comparison of *Urtica incisa, U. sykesii* and *U. perconfusa*.

| | ***U. incisa*** | ***U. sykesii*** | ***U. perconfusa*** |
|---|---|---|---|
| **Leaf lamina** | triangular-ovate to narrowly oblong | triangular to triangular-ovate | narrowly ovate |
| **Leaf lamina (mm)** | 60–120–(150) × 15–30(–60) | 20–60 × 20–50 | 40–80(–100) × 4–10 |
| **Leaf base** | cuneate to truncate | (truncate to) subcordate | rounded |
| **Number of teeth per side** | 14–20 | 9–10(–12) | 12–20 |
| **Inflorescence (mm)** | 24–60 | 10–20 | 3–10 |
| **Petioles (mm)** | (10–)15–25(–40) | 30–45(–70) | 15–45 |
| **Gender distribution** | mostly dioecious | monoecious, male flowers below, female flowers above | monoecious |

### 3.4.5 *Urtica dioica* L. (1753: 984)

Lectotype (designated by Woodland 1982: 283):—herb. Linnaeus 1111.8 (LINN!, photographs in AUB, DAO, M, MTMG).

Synonyms are listed in Grosse-Veldmann & Weigend (2015).

Erect, perennial, rhizomatous herb 0.6–1.5(–2) m, unbranched, but forms branches late in the season. Stem indumentum of many stinging hairs with pluricellular base c. (0.1–)0.3–0.5 mm overall and erect setae 0.2–0.5 mm long, short stiff bristles and numerous simple trichomes 0.1–0.5 mm long. Leaf lamina 60–120(–150) × 45–85(–100) mm broadly ovate; surface densely pubescent with short simple trichomes 0.1–0.5 mm long and many stinging hairs, adaxially with punctiform cystoliths; leaf base cordate; margins coarsely and regularly serrate, with 14–25 teeth on each side, teeth usually undivided, sometimes with two or three teeth; leaf apex acute to acuminate; stipules free (4 per node) (2–)5–10 mm long; petioles 20–50 mm long. Plants mostly dioecious, sometimes monoecious. Staminate flowers with tepals c. 0.5 mm long. Pistillate flowers with short tepals 0.2 mm long and long tepals 0.3 mm long, sparsely pubescent, esetulose. Inflorescence (20–)30–50 mm long and strongly branched. Mature fruit with longer tepals c. 1.3–1.5 mm long, achenes ovoid with a narrowed base and apex, widest below the middle, c. 1–1.5 × 0.6–0.8 mm.

**Notes:—**The weedy form of *Urtica dioica* L. (subsp. *dioica*) which is found in New Zealand is of unbranched shoots, broad ovate leaves with a cordate base, a dense cover of stinging hairs interspersed with short stiff bristles and simple trichomes and mostly one sex on each individual plant. Like all of the widespread forms of *Urtica, U. dioica* is also variable in appearance. A very broadly-leaved form which is morphologically similar to the weedy form, but which has only few stinging hairs and a dense and soft pubescence occurs in dry forests in eastern Germany and Poland. The weedy form is absent from undisturbed, humid and nutrient rich forests (e.g. in Europe, in the Southern Alps and on the Balkan pensinsula) where it is replaced by narrow-leaved "forest forms" of *U. dioica* with only few stinging hairs and a more or less dense pubescence. Robust and low-growing plants with a very dense cover of stinging hairs, deeply serrate leaf margins and strongly branched and dense inflorescences are typically found in alpine pastures on calcareous ground e.g. in the Southern Alps and the Pyrenees. Plants with high moisture demands are restricted to river banks in central and eastern Europe and are characterized by a stiff, erect and very robust habit, narrow leaves, few stinging hairs and a sparse pubescence (see Weigend 2005, Grosse-Veldmann & Weigend 2015). In overall appearance, typical *U. dioica* is very similar to native *U. incisa*, but molecular data clearly show it to be only remotely related. Morphologically it can be differentiated from *U. incisa* by its broader leaf shape, a dense cover of stinging hairs, strongly branched inflorescences and ovoid achenes.

**Distribution:—***Urtica dioica* L. occurs throughout Eurasia and China and has been introduced to eastern USA and Canada, as well as to Chile and New Zealand. In New Zealand it is known from both the North and South Islands. It has not (yet) been reported from Australia.

**Habitat and ecology:—**The common lowland and coastal, weedy form occurs in nutrient rich open habitats, on road sides, waste grounds and disturbed sites at low and intermediate elevations. In New Zealand it is found on river terraces, open meadows, roadsides, disturbed sites, on pasture and on beaches in a variety of substrates.

**Phenology:—***Urtica dioica* L. flowers throughout the year.

**Conservation status:—**According to the New Zealand Threat Classification System (de Lange *et al.* 2013), *Urtica dioica* L. is considered "Exotic".

**Additional specimens examined:—NEW ZEALAND. Greater Wellington (North Island):** Wellington Ecological Distr., Somes (Matiu) Island, S 41° 15.5', E 174° 52.0', 20 m, 19 Sept. 1991, *P.J. de Lange 1050* (CHR 474966!); **Manawatu-Wanganui (North Island):** Wanganui Distr., Wanganui city, Papaiti, Whanganui River, S 39° 51', E 175° 5', 20 m, 7 Nov. 1993, *C. C. Ogle 2654* (CHR 495175!); Wellington Land Distr., Wanganui Distr., Fordell, No. 2 Line, "Aird" farm, S 39° 57 ', E 175° 11', 160 m, 2 Nov. 1997, *C. C. Ogle 3298* (CHR 515018!).

**Specimens from cultivation:**—Original collection: **NEW ZEALAND. Canterbury (South Island):** Canterbury Land Distr., Hope, north of Lewis Pass, 13 Jan. 2004, collector unknown, cultivated in: Canterbury (South Island): Canterbury Land Distr., Christchurch, St. Albans, 115 Packe Str., 13 Jan. 2004, *W. R. Sykes 18/04* (CHR 605119!); **Hawke's Bay (North Island):** Hawke's Bay Land Distr., Weleda (NZ) Limited Gardens, Havelock North, 29 Nov. 2011, *QA Technician s.n.* (CHR 554345A!, CHR 554345B!).

## 3.5 Acknowledgements

We would like to express our sincere gratitude to various colleagues who contributed to this study, most importantly R. D. Smissen (Landcare Research, Christchurch, New Zealand) for his numerous helpful comments and additions to the manuscript. Thanks are also due to T. Franke (Munich), I. Breitwieser (Allan Herbarium, Landcare Research, New Zealand), and Miguel de Salas (Tasmanian Herbarium, Tasmanian Museum and Art Gallery, Australia) for help and access to data and plant material.

## 3.6 Appendix 1: Representative specimens of other NZ-species of *Urtica*.

### *3.6.1 Urtica aspera* Petrie (1918 [1919]: 107)

Type:—NEW ZEALAND. Waikato (North Island): Firewood Creek, Dunstan Range, *D. Petrie* (WELT, not seen).

**Additional specimens examined:—NEW ZEALAND. North Canterbury (South Island):** Canterbury Land Distr., S. Canterbury, Simons Hill, 9 km E of Lake Pukaki, 20 Jan. 1968, *G. I. Collett s.n.* (CHR 184386!); Canterbury Land Distr., S. Canterbury, Lawrence Valley, S 43° 24', E 170° 54', 11 Mar. 1977, *D. R. Given 9793* (CHR 478561!); **Otago (South Island):** Otago Land Distr., Dunstan Mtns on Dunstan Gorge side with Sonora Basin to the west and leaning rock to the east, main track to Leaning Rock, S 45° 05', E 169° 18', 550 m, 15 Dec. 1996, *B. H. Patrick & J. Douglas s.n.* (CHR 511575!); Otago Land Distr., Upper Manuherikia Valley, Fiddlers Flat Road, Falls Dam, S 44° 52', E 169° 54.2', 06 Dec. 1993, *B. H. Patrick s.n.* (CHR 506539!); Otago Land Distr., Otago, Hakdun Range, Blue Duck Creek, S 44° 54', E 170° 11', 1035 m, 14 Mar. 1985, *C. C. Ogle 1213* (CHR 418236!).

### *3.6.2 Urtica australis* Hook.f. (1844: 68)

Type:—NEW ZEALAND. Lord Auckland's Island, In woods near the sea, Nov. 1840, [s. coll.]; 1489 [coll. no.] Lord Aucklands Groups; Herbarium Hookerianum 1867 (K 000351871!).

= *Urtica aucklandica* Hook.f. (1844: 68). Type:—NEW ZEALAND. Lord Auckland's group, [Auckland Islands], J. D. H. [*J. D. Hooker s.n.],* Better specimen—Brit. Mus. [*sic*!] (K 000351873!, BM?).

**Additional specimens examined:—NEW ZEALAND. Greater Wellington (North Island):** Wellington Land Distr., Chatham Islands, Chatham Island, Waitangi-Tuku road 3–4 km from Waitangi, 50 m, 27 Nov. 1993, *W. R. Sykes 368/93* (CHR 496662A!); Wellington Land Distr., Chatham Islands, Chatham Island, Waitangi-Tuku road 3–4 km from Waitangi, 50 m, 27 Nov. 1993, *W. R. Sykes 368/93* (CHR 496662B!); Wellington Land Distr., Chatham Islands, Chatham Island, Waitangi-Tuku road 3–4 km from Waitangi, 50 m, 27 Nov. 1993, *W. R. Sykes 368/93* (CHR 496662C!); **Southland (South Island):** Southland Land Distr., Auckland Islands, Enderby Island, 12 Feb. 1987, *W. R. Sykes 16/87* (CHR 437022A!); Southland Land Distr., Auckland Islands, Enderby Island, 12 Feb. 1987, *W. R. Sykes 16/87* (CHR 437022B!); Southland Land Distr., Fiordland, Resolution Island, Woodhen Cove Te Anau, S 45° 39', E 166° 33.1', 2 m, 16 Feb. 1984, *P. N. Johnson 173* (CHR 415667!).

# CHAPTER 4

## Weeding the nettles V: Taxonomic and phylogenetic studies of the eastern Asian species *Urtica thunbergiana* Sieb. & Zucc. (Urticaceae)*

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### Abstract

*Urtica* L. (Urticaceae) is a subcosmopolitan genus, which is also common throughout Asia. Taxon differentiation in the Asian *Urtica* species however is difficult due to the limited diversity of taxonomically useful characters combined with a range of phenotypic variation. The present study investigates the species *Urtica thunbergiana* Sieb. & Zucc. (incl. *U. laetevirens*) from eastern Asia, based on herbarium collections, including most type specimens. The delimitation of *U. thunbergiana* is expanded to include all three currently recognized subspecies of *U. laetevirens* Maxim. Also, one new infraspecific taxon is described from Taiwan. Based on our revision the recognition of a total of four subspecies of *Urtica thunbergiana* is proposed: subsp. *thunbergiana,* subsp. *dentata* (Hand.-Mazz.) K.Becker & Weigend, subsp. *silvatica* (Hand.-Mazz.) K.Becker & Weigend, and subsp. *perserrata, subspec. nov.* The systematic re-arrangements are based on morphological analyses and a highly resolved phylogeny based on the molecular markers ITS1–5.8S–ITS2, *psb*A–*trn*H, *trn*L–*trn*F and *trn*S–*trn*G.

*Keywords:* chloroplast markers, Himalayas, infraspecific taxa, nuclear marker, phylogeny, stinging nettle, taxonomy, *Urtica laetevirens*, Urticaceae

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*manuscript submitted to *Phytotaxa*, 23/08/2016

## 4.1 Introduction

The subcosmopolitan genus *Urtica* L. (Urticaceae) is a very common genus over much of its range. It is often found in anthropogenic habitats, but can also occupy a range of natural habitats. In spite of its abundance and the visibility of the genus, its taxonomy remains problematic and its alpha-taxonomy is still very incompletely understood (Friis, 1993). The difficulties are mainly caused by a lack of taxonomically useful characters in a combination with a broad range of phenotypic variation. The past years have brought considerable progress in the taxonomical re-definition of individual components within the genus *Urtica*, especially in the European, American and Australian/New Zealand taxa (e.g. Weigend, 2005, 2006; Weigend et al., 2005; Weigend & Luebert, 2009; Farag et al., 2013; Henning et al., 2014; Grosse-Veldmann & Weigend, 2015; Weigend & Monro, 2015; Grosse-Veldmann et al., 2016a). Most notably, a near-comprehensive phylogeny was recently published (Grosse-Veldmann et al., 2016b), for the first time providing a clear picture of the relationships and lineages in the genus. The phylogeny resolves six clades which contain or are exclusively composed of Asian taxa. Two East Asian to Southeast Asian clades representing taxa with mainly fused stipules (e.g., *U. laetevirens* and *U. mairei*) are taxonomically particularly intractable and riddled with taxonomic problems. Recent taxonomic treatments (LIT) indicate that there are only few taxonomically useful characters combined with a broad range of phenotypic variation. In the present study we address this problem based on a morphological and molecular approach, in order to identify the delimitation of "*U. laetevirens*" and clarify the infraspecific relationships within the species.

## 4.2 Material and methods

### 4.2.1 Plant material

During the preparation of the sampling for Grosse-Veldmann et al. (2016b) it became apparent that species limits in *U. laetevirens* and *U. thunbergiana* are anything but straightforward and taxon delimitation in the literature appeared to be at odds with the actual variability. Especially, typical *U. laetevirens* and *U. thunbergiana*, both described from Japan, turned out to be extremely difficult to differentiate. Therefore, all material referable to these species and the putative subspecies of *U. laetevirens* was critically revised. Material from the following herbaria was examined either as physical specimens or as herbarium scans (abbreviations following Holmgren & Holmgren 1998 ff): B, BONN, CDBI, E, HENU, HUH, IFP, K, KUN, L, LE, M, MO, NY, P, PE, TAIF, TAIM, US and WU. Original protologues and type material were studied and compared to taxon delimitations of current floras [(e.g. Flora of China (Chen et al., 2003), Flora of U.S.S.R. (Komarov, 1936)] and compared to original protologues and type specimens.

Plant material used for the phylogenetic analyses was silica-dried or taken from herbarium material. In total, 13 ingroup and 7 outgroup accessions were sampled. Representatives of other *Urtica* species within the genus covering a broad geographical and phylogenetic range (compare Grosse-Veldmann et al. 2016b) were chosen as outgroup. A complete voucher list of the plant material used in this study including voucher information and GenBank accession numbers is given in Table 1.

Conservation assessments were undertaken using IUCN Red List Categories and Criteria (2001).

### 4.2.2 DNA extraction, amplification, and sequencing

DNA extraction, amplification, purification, and sequencing followed standard protocols as described in Gottschling & Hilger (2001) and Weigend et al. (2010). Samples were sequenced for four genomic regions: the nuclear ribosomal ITS1–5.8S–ITS2 (thereafter ITS), and three plastid regions: the *psb*A–*trn*H intergenic spacer (IGS), *trn*L–*trn*F (including the *trn*L group I intron and the *trn*L–*trn*F IGS), and *trn*S–*trn*G (including the *trn*S–*trn*G IGS and the *trn*G group II intron). The same primers were used for amplification and for sequencing. The primers used were ITS5 and ITS4 for ITS (White et al. 1990), *psb*AF and *trn*HR for *psb*A–*trn*H (Sang et al. 1997), C and F for *trn*L–*trn*F (Taberlet et al. 1991) and trn_S$_{(GCU)}$ and trn_G$_{(UCC)}$ for *trn*S–*trn*G (Hamilton 1999). Amplicons were sequenced by either Macrogen Inc., South Korea (http://www.macrogen.com) or GATC Biotech AG, Konstanz, Germany (http://www.gatc-biotech.com). All sequences generated in this study have been submitted to the GenBank genetic sequence database (see Tab. 4.1 for accession numbers).

### 4.2.3 Alignment and phylogenetic analyses

The alignment was conducted using the MAFFT algorithm in the Geneious software package ver. R8 with default settings, followed by manual adjustments using PhyDE® ver. 1 (Müller et al. 2005) in order to build a motif alignment, based on the criteria laid out in Kelchner (2000). The combined data set (ITS, *trn*S–*trn*G, *trn*H–*psb*A and *trn*L–*trn*F) contains 2473 aligned positions (ITS: 625, *trn*S–*trn*G: 750, *psb*A–*trn*H: 272, *trn*L–*trn*F: 837).

**Table 4.1** List of taxa included in the phylogenetic study of *Urtica thunbergiana* Sieb. & Zucc.

| **Taxon** | **Country of origin** | **Herbarium voucher** | **DNA No.** | **ITS** | ***trn*S-*trn*G** | ***psb*A-*trn*H** | ***trn*L-*trn*F** |
|---|---|---|---|---|---|---|---|
| *U. cannabina* L. | China | Q. R. Wu 322 (MO) | W 2038 | KX271370 | KX271525 | KX271601 | KX271451 |
| *U. dioica L.* subsp. *dioica* | Spain | E. Zippel 2002/2b (B) | W 2232 | KF558920 | KF559101 | KF558980 | KF559040 |
| *U. echinata* Benth. | Ecuador | Loejtnant & Molau 11657 (GB) | W 1863 | KX271427 | KX271577 | KX271657 | KX271501 |
| *U. incisa* Poir. | New Zealand | Ward CHR 234516A (NZ Landcare Research) | W 2264 | KF971218 | KF971185 | KF971284 | KF971251 |
| *U. massaica* Mildbr. | Uganda | M. Ackermann 1050 (B) | ED 841 | KX271388 | KX271539 | KX271619 | KX271464 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *perserrata* K.Becker & Weigend | China (Taiwan) | S. T. Chiu et al. 02924 (TAIM) | W 2212 | KF558912 | KF559093 | KF558972 | KF559032 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *dentata* (Hand.-Mazz.) C.J.Chen | China (Hupheh) | Dr. Aug. Henry's Collections from Central China 5859 (HUH) | W 4214 | KX271408 | KX271559 | KX271639 | KX271483 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *dentata* (Hand.-Mazz.) C.J.Chen | China (Sichuan) | Zheng-yu 15565 (MO) | W 2013 | KX271409 | KX271560 | KX271640 | KX271484 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *dentata* (Hand.-Mazz.) C.J.Chen | China (Sichuan) | Zheng-yu 15572 (MO) | W 2042 | KX271410 | KX271561 | KX271641 | KX271485 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *silvatica* | China (Henan) | D. E. Boufford et al. 26323 (MO) | W 2014 | KX271412 | KX271563 | KX271643 | KX271487 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *silvatica* (Hand.-Mazz.) K.Becker & Weigend | China (Tibet) | D. E. Boufford et al. 30067 (HUH) | W 4209 | KX271413 | KX271564 | KX271644 | KX271488 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *silvatica* (Hand.-Mazz.) K.Becker & Weigend | China (Yunnan) | C. W. Wang 73915 (HUH) | W 4542 | to be submitted | to be submitted | to be submitted | to be submitted |
| *U. thunbergiana* Sieb. & Zucc. subsp. *silvatica* (Hand.-Mazz.) K.Becker & Weigend | China (Yunnan) | C. W. Wang 70702 (HUH) | W 4545 | to be submitted | to be submitted | to be submitted | to be submitted |
| *U. thunbergiana* Sieb. & Zucc. subsp. *thunbergiana* | China (Mandschurei) | B. V. Skvortzov s.n. (HUH) | W 4230 | KX271411 | KX271562 | KX271642 | KX271486 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *thunbergiana* | Russia (Kamchtka) | D. Geltman 326/1 (LE) | W 2963 | KX271407 | KX271558 | KX271638 | KX271482 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *thunbergiana* | Russia (Primorye) | P. Gorovoi et al. 6868 (B, HUH) | W 1868 | KX271406 | KX271557 | KX271637 | KX271481 |
| *U. thunbergiana* Sieb. & Zucc. subsp. *thunbergiana* | Japan | S. Suzuki 250 (HUH) | W 4543 | to be submitted | to be submitted | to be submitted | to be submitted |
| *U. thunbergiana* Sieb. & Zucc. subsp. *thunbergiana* | Japan | T. Jossberger s.n. (BONN) | W 3112 | KX271405 | KX271556 | KX271636 | KX271480 |

The data set contains two hairpin-associated inversions, one situated in P8 of the *trn*L intron (compare Borsch et al. 2003) and the second approximately 90 nt upstream of the *trn*F gene. Both inversions were positionally isolated in the alignment and included as reverse complement in the nexus files used for phylogenetic analyses (see Quandt et al. 2003, Borsch & Quandt 2009). Each data set was analysed independently to identify potential incongruences. No hard incongruences between the separate plastid and ITS trees were observed (data not shown). Phylogenetic analyses were conducted on a concatenated dataset employing maximum likelihood (ML) and Bayesian inference (BI).

ML analyses were conducted with the standard settings in RAxML Version 8 (Stamatakis 2006, Stamatakis et al. 2008). The node support under ML is based on 1000 bootstrap replicates. BI analyses were conducted in MrBayes vers. 3.2.2 (Ronquist & Huelsenbeck 2003) with six independent runs of 2,000,000 generations each under the GTR + Γ + I model with partitions unlinked. Chains were sampled every 1000th generation. Log likelihoods were examined using Tracer v1.5 (Rambaut & Drummond 2009) in order to determine the burn-in and to ensure that an adequate effective sample size (ESS) was attained. The consensus tree and the posterior probability (PP) of clades were calculated based upon the trees sampled after the burn-in set at 500,000 generations. TreeGraph2 (Stöver & Müller 2010) was used for tree drawing. We used *Urtica cannabina* to root the final tree.

## 4.3 Results

### 4.3.1 Molecular data

Representative specimens for the entire geographical and morphological range of *U. laetevirens* and *U. thunbergiana* were selected, considerably expanding the sampling of Grosse-Veldmann et al. (2016b). As a result, a highly resolved phylogeny of four well-supported clades was retrieved (Fig. 4.1). Typical *U. thunbergiana* from Japan is retrieved in a clade with typical *U. laetevirens* from Japan and *U. cyanescens* from E Russia (PP 1, ML-BS 98%). This expanded *U. thunbergiana (U. thunbergiana subsp. thunbergiana* in the tree*)* is retrieved as sister to the Taiwanese accession of the group (PP 1, ML-BS 99%), and this clade is sister to two clades of what used to be called *U. laetevirens* in Chen (1983), corresponding to *U. laetevirens* subsp. *silvatica (U. thunbergiana subsp. silvatica* in the tree) and *U. laetevirens* subsp. *dentata (U. thunbergiana subsp. dentata* in the tree) as the basally branching clade of the complex (both nodes PP 1, ML-BS 100%).

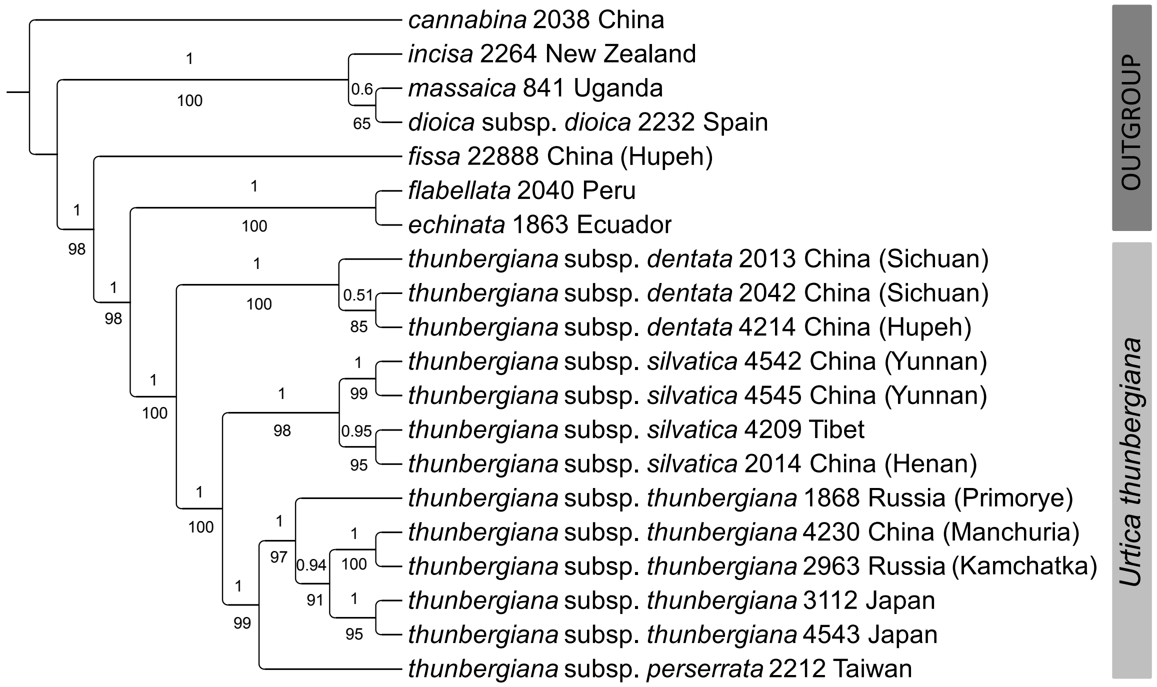

**Figure 4.1** *U. thunbergiana* in maximum likelihood tree based on concatenated data set (ITS, *trn*S–*trn*G, *psb*A–*trn*H and *trn*L–F). Bayesian posterior probabilities are indicated above branches; bootstrap support under likelihood is indicated below.

### 4.3.2 Morphology

A critical revision of numerous collections including type collections and living plants indicated that the taxa *U. laetevirens* (–subsp. *perserrata*, –subsp. *dentata*, –subsp. *silvatica*, –subsp. *thunbergiana*) variously recognized since the early 19th century (Blume, 1849; Handel-Mazzetti, 1937, and still accepted until recently (Chen et al., 2003), represent a more or less continuous range of characters such as an ovate leaf lamina (45–110 × 30–90 mm) with regularly or doubly dentate to lobulate leaf margins, long petioles of 30–100 mm length, an indumentum of stinging hairs 0.8–3.5 mm long overall, and plant populations gynodioecious consisting of female and monoecious individuals.

Further critical studies on the material referable to this species complex reveal four identifiable morphological subgroups. Material from Taiwan could not be assigned to any previously described taxon, but clearly belongs to this complex. It is described as subsp. *perserrata, subsp. nov.* below.

The main characters to distinguish the different subspecies of *U. thunbergiana* are mainly found in the details of the leaf margin. Leaves of subspecies *thunbergiana* are triangular-ovate and deeply serrate to lobulate with very long and pointy teeth, frequently with each lobe/tooth having one small tooth on either one side or on both (Fig. 4.2 A, F, G). Leaves of subspecies *dentata* and subsp. *silvatica* are very broadly triangular-ovate and simply serrate to crenate. Subspecies *dentata* has sharply pointed, triangular teeth (Fig. 4.3 A, F, G) and occasionally exhibits heteroblasty with the basal leaves nearly as long as with and with a cordate base contrasting with the apical leaves nearly three times longer than broad and with a rounded to attenuate base (Fig. 4.3 F, G). Subspecies *silvatica* has crenate leaf margins with rounded teeth (Fig. 4.4 A, F) and shows not marked heteroblasty. The newly described subspecies *perserrata* is most similar to subspecies *thunbergiana* in leaf morphology, but the leaves are broadly ovate and the leaf margins narrowly lobulate, the lobules with one or two sharp and narrow teeth (Fig. 4.5 A, F). Also, subsp. *perserrata* is strikingly densely setose, especially on the stem and the adaxial leaf surfaces (Fig. 4.5 A–F), whereas the other subspecies, and especially subsp. *thunbergiana*, are sparsely setose. Stipule morphology is also differentiated, with subsp. *thunbergiana* and subsp. *silvatica* having four free, usually narrowly oblong to ovate stipules per node or two fused pairs of stipules which are occasionally incised distally (Fig. 4.2 B, C, 4 B, C). The four narrowly oblong stipules of subsp. *dentata* are always free (Fig. 4.3 B). Subsp. *perserrata* has two fused stipule pairs per node, that are usually incised for 1/3rd or ½ of their length (Fig. 4.5 B, C).

## 4.4 Discussion

The morphological analyses clearly retrieve the *U. thunbergiana* complex as a well supported monophylum with clear internal structure. *Urtica laetevirens, U. thunbergiana* and *U. cyanescens* are retrieved in a single terminal clade, confirming that traditional species delimitation on putative minor differences in leaf dissection are spurious. The specimens of the samples with the DNA-numbers 1868, 4230 and 2963 closely correspond to the type specimens of *U. laetevirens* subsp. *laetevirens*, and the specimens of the samples with the DNA-numbers 3112 and 4543 closely correspond to the type specimens of *U. thunbergiana* subsp. *thunbergiana*. All three taxa represent a single taxon, here formalized as a single subspecies *U. thunbergiana* subsp. *thunbergiana*. The Taiwanese accession is clearly retrieved as sister and due to its clear and consistent morphological differences is here formalized as *U. thunbergiana* subsp. *perserrata* K.Becker & Weigend. The two subspecies of *U. laetevirens* recognized in the flora of China (Chen et al., 2003) are successive sisters to this terminal clade and the necessary new combinations *U. thunbergiana* subsp. *silvatica* and *U. thunbergiana* subsp. *dentata* are here provided.

Morphological characters are highly problematic in *Urtica* and this is nicely underscored by the fact, that Chen (1983) placed *U. thunbergiana* into the Section *Ardentia* while placing *U. laetevirens* in Section *Urtica* – whereas we here show that they belong to the same subspecies.

Geographical distribution seems to be a poor predictor for infraspecific relationships within *U. thunbergiana*. According to the specimens seen, *U. thunbergiana* subsp. *thunbergiana* occurs throughout China (except on the islands), Japan, Korea and southeast Russia. *U. thunbergiana* subsp. *silvatica* is also found throughout mainland China and *U. thunbergiana* subsp. *dentata* appears to be restricted to central China (Guizhou, Hubei, Hunan, Sichuan and Yunnan). *U. thunbergiana* subsp. *perserrata* is the only subspecies occurring on Taiwan and appears to be endemic to that island. Overall, these three subspecies have a relatively large overlapping distribution, and thus, only morphological and molecular data can be used for classification. In conclusion, infraspecific relationships within the species *U. thunbergiana* could be fully resolved by morphological and phylogenetic data. Most useful morphological characters to identify the different subspecies are the structure of the leaf margin and the tooth shape.

## 4.5 Formal Taxonomy

The formal taxonomy is here provided for redefined *Urtica thunbergiana* and corresponding subspecies.

### 4.5.1 ***Urtica thunbergiana*** Sieb. & Zucc. (1846: 214)

Erect, perennial herb 0.25–1.50 m, from perennial root and with compact pleiokorm of perennating underground stems; stem with few to numerous stinging hairs with a pluricellular base, c. 1.0–4.0 mm long overall and setae 0.8–2.7 mm long, and simple trichomes ca. 0.1–0.2 mm long; leaf lamina 45–110 × 30–90 mm, widely ovate to narrowly ovate (basal leaves usually broader), leaf margins regularly or doubly dentate to lobulate with 9–20 large teeth on each side, 2–15 mm long, occasionally each large tooth with one small tooth, ca. 1 mm long, on each side, leaf base cordate, rounded or attenuate, leaf apex apiculate to aristate; leaf indumentum of stinging hairs with pluricellular base c. 1.0–3.5 mm long overall, setae 0.8–2.8 mm, and with simple trichomes 0.1–0.3 mm long; leaf surface with punctiform to elliptical cystoliths; petioles 30–100 mm long; stipules free (4 per node) or 2 fused pairs of stipules per node, occasionally incised distally, 5–15 mm long, elongated; plant populations gynodioecious, if both genders on one plant, usually staminate flowers basal, carpellate flowers apical, male inflorescence usually clearly longer than female inflorescence; staminate flowers with all tepals 1.0–2.0 mm long, pubescent; pistillate flowers with tepals ca. 0.2 mm long, pubescent; infructescence 15–80 mm; mature fruits with longer tepals ca. 1.0–2.0 mm long, achenes subcircular in outline, laterally flattened, ca. 1.0–2.0 × 1.0–1.5 mm.

### 4.5.2 Key to the subspecies of *Urtica thunbergiana*

1. Leaf margin regularly dentate……………………………………………………………………………………2

\- Leaf margin doubly dentate, usually each large tooth with one small tooth on each side (one or both of the small teeth may be missing on some teeth)………………………………………………………4

2. Teeth rounded, 2–5 mm long………………………………………*Urtica thunbergiana* subsp. *silvatica*

\- Teeth pointed, 2–15 mm long……………………………………………………………………………………3

3. Teeth simple, widely triangular; leaf base rounded to cordate; occasionally heteroblastic, with the basal leaves nearly as long as broad with a cordate base and the apical leaves nearly three times longer than broad with a rounded to attenuate base……………………………………………………………………*Urtica thunbergiana* subsp. *dentata*

\- Teeth oblong, often leaves lobulate with lobules with one small tooth, ca. 1 mm long, on each side (the small teeth may be missing); leaf base rounded, cordate or attenuate; not or only weakly heteroblastic……………………………………………*Urtica thunbergiana* subsp. *thunbergiana*

4. Stinging hairs few, 1–3 mm long; at least basal leaves broadly ovate with cordate base………………………………………………………………………*Urtica thunbergiana subsp. thunbergiana*

\- Stinging hairs numerous, up to 4 mm long; all leaves ovate……………………………………………………………………*Urtica thunbergiana* subsp. *perserrata*

### 4.5.3 *Urtica thunbergiana* Sieb. & Zucc. (1846: 214) subsp. ***thunbergiana***. Fig. 4.2

Lectotype (here designated):—[JAPAN] Japonia, 1842, Herbarium Zuccarinii*, P.F. von Siebold s.n.* (M!). Syntypes:—[JAPAN] Japonia, Herbarium Regium Monacense, *H. Bürger s.n.* (M!); Japonia, Herbarium Zuccarinii, 1843, *P.F. von Siebold s.n.* (M!).

= *Urtica cyanescens* Kom. (1936: 714). ≡ *Urtica laetevirens* Maxim. subsp. *cyanescens* (Kom.) C.J.Chen (1983: 115). Lectotype (here designated):—12 Jun. 1913, *Komarov s.n.* (LE!), Isolectotype: 13 Jun. 1913, *Komarov s.n.* (LE!)

= *Urtica laetevirens* Maxim. (1877: 236). Syntypes: Japonia [Japan], Hakodate, 1861, *Maximowicz s.n.* (FI-4444 38!, NY_00284488!, P_06855912!, P_06855918!, K-000694837!, L_1629729!).

= *Urtica macrorrhiza* Hand.-Mazz. (1929: 115). Holotype: [China] Yunnan: On the eastern flank of the Tali Range, May–Jul. 1906, *G. Forrest 4671* (K_000694841!)

Leaves coarsely (bi-)serrate to lobulate, teeth oblong and pointed, occasionally each large tooth with one small tooth, ca. 1 mm long, on each side (one or both of the small teeth may be missing on some large teeth); leaf indumentum of few stinging hairs with pluricellular base 1.0–2.5 mm long overall, setae 0.8–2.4 mm (stinging hairs on the upper surface often shorter than on the lower surface, especially the pluricellular base on the upper surface is often conspicuously shorter than on the lower surface); stem indumentum of few stinging hairs with pluricellular base 1.0–3.0 mm long overall, setae 0.8–2.8 mm.

**Distribution and Habitat:**—*U. thunbergiana* subsp. *thunbergiana* is widespread in eastern Asia and is here reported from China, Japan, Korea and eastern Russia. The subspecies occurs on roadsides, in the understory of forests growing in rich and moist soil, near streams, and is usually found at intermediate and high elevations.

**Phenology:—***U. thunbergiana* subsp. *thunbergiana* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica thunbergiana* Sieb. & Zucc. subsp. *thunbergiana* is considered “Least Concern” (LC).

**Representative specimens:—CHINA. Guizhou:** 22 May 1902, *M. Cavalerie 1296* (P_06750070!); **Heilongjiang**: Raohe, 10 Sep. 1952, *G. Wang s.n.* (IFP_03906004x0001!); Raohe, 10. Sep. 1952, *G. Wang s.n.* (IFP_03906004x0002!); Yichun, Cuiluan, in the forest, 10 Apr. 1950, S. Liu & *Y. Zang s.n.* (IFP_03906004x0003! & IFP_03906004x0004); Yichun, Dailing, hillside, roadside, 25 Jul. 1954, *S. Liu 6052* (IFP_03906004x0005! & IFP_03906004x0006!); Yichun, East Mountain near Yichun Wuying, in a Korean pine forest, 03. Aug. 1956, *S. Liu et al. 7898* (IFP_03906004x0008!); Yichun, Min River, 1935, *S. Liu et al. s.n.* (IFP_03906004x0009!); Yichun, the fifth battalion, east mountain, Korean pine forest, 03 Aug. 1966, *S. Liu et al. 7898* (IFP_03906004x0007!); **Henan:** Xinxiang, Funiu Mountain, Wetland in a valley, 08. Aug. 1956, *L. Shen 0012* (HENU_0010822!); **Jilin:** Hillside in the forest, 14 Sep. 1959, *Collector unknown* (IFP_03906004x0010!); **Manchuria [nowadays: Heilongjiang, Jilin, Liaoning and the northern part of Inner Mongolia]:** W Manchuria, *D. Litvinov 2161* (HUH!); NE Manchuria, *D. Litvinov 2690* (HUH!); N Manchuria, *B.V. Skvortzov s.n.* (HUH!); Korea Septentrionalis, Districtus Oze-song, 27 Aug. 1897, *Komaòv 513* (P_06855913!); **Shanxi:** Heshun, Yangqu Mountain, Aug. 1985, *T. Liu & Z. Zeng 21* (MO_04730599!); Yuanqu, Tongshan, Longmiaogou, margin of woods, 11 Jul. 1984, *Yellow Plateau Team 02174* (MO_04755586!); **Sichuan:** Nanchang, Shikong, around stony wall, Liang-ho-kup, Jul.–Aug. 1939, *S.Y. Hu 1464* (HUH_00278052!); Nanping, Jiuzhaigou, under some *Picea*, 2300 m, 26 Sep. 1986, *Z. Wu et al. 986* (KUN_0524936!); West of Wen-chuan Hsien, road side, 2500 m, 2 Apr. 1930, *F.T. Wang 21118* (HUH_00278019!); **Sikang [nowadays: eastern Tibet & western Sichuan]:** Djer-mai, Tsa-wa-rung, under forest, 3200 m, Aug. 1935, *C.W. Wang 65689* (HUH_00278007!); **Xinjiang:** Fukang Xian, Tian Shan, in vicinity of Hainan, S of Tianchi lake, 1910 m, 43°52’7”N, 88°8’44”E, Picea forest and open slopes, 21 Jul. 2001, *B. Bartholomew 8567* (MO_5724302!); **Yunnan:** Tali Range, Lat. 25°40’N, Alt. 2133–2743 m, May–Jul. 1906, *G.Forrest 4671* (K_000694840! & K_000694841!); 19 Jun. 1887, *M. Delavay 2839* (P_06749814!); 1934, *H.T. Tsai 51409* (HUH_00278004!); Bai-mar-shan, A-tun-tze, ravine, 2500 m, Sep. 1935, *C.W. Wang 69792* (HUH_00278024!); *M. Delavay 2839* (P_06822169!); O-shan Hsien, by stream, 1650 m, 09 Jun. 1933, *H.T. Tsai 53485* (HUH_00278013!); Ping-pien Hsien, on road side, 1200 m, 17 May 1934, *H.T. Tsai 55162* (HUH_00278021!); plaine de Za-Kou, 2400 m, Jun., E.E. Maire s.n. (P_06750042! & P_06750052!);—**JAPAN.** 1842, *Siebold s.n.* (M!); 1843, *Siebold s.n.* (M!); *Bürger & Siebold s.n.* (M!); *Siebold s.n.* (L_0175969!); **Hakodate:** 1861, *Maximowicz s.n.* (FI_444438! & K_000694837! & L_1629729! & NY_00284488! & P_06855912! & P_06855918!); **Hokkaido:** Hidaka, Shizunai-co. ca 14 km. ENE of Shizunai, off highway 235, mixed deciduous streamside forest along tributary of Shizunai River, 19 Aug. 1977, *D.E. Boufford& E.E. Wood 19684* (MO_4019140!); Ikahe in Kotsuke, 06 Sep. 1950, *J. Ohwi 83* (P_06855930! & MO_1617896!); Ikahe in Kotsuke, 06 Sep. 1950, *J. Ohwi 84* (L_1629481!); Aizawa foot of Mt. Arafuna, Nishimoku-mura, Shimonita-machi, Kita-kamra-gun, 03 Sep. 1956, *M. Furuse 31561* (PE_00097330!); Kuasawa Oku-tama-choo, Nishi-tama-gun, Tokyo-to, 13 Aug. 1971, *M. Furuse 50546* (PE_00097340!); Mt. Kiyo-sumi, Amatsu-kominato-choo, Awa-gun, 383 m, 31 Oct. 1967, *M. Furuse 46162* (PE_00096844!); **Hondo:** Shidzuoka, about Suzaki Shimoda-choo Kamo-gun, 14 Nov. 1969, *Furuse 48570* (PE_0097339!); Shidzuoka, Suzaki Shimoda-choo Kamo-gun, 14 Nov. 1969, *M. Furuse 48570* (PE_00097339!); **Honshu:** Kyoto-pref.: Mt. Ponpon-yama, Oharano-Ishidzukuri-cho, Nishigyo-ku, Kyoto-shi, 430 m alt., 25 Sep. 1997, *S. Tsugaru & T. Takahashi 25630* (MO_04940294!); Mt. Kiyosumi, Boso-pen. Prefecture Chiba, Hondo, N.Lat.: 35°09’, E.Long.: 140°09’, in forest, 17 Oct. 1938, *S. Suzuki 250* (HUH!); Pref. Shizuoka: The Shiraitonotaki Fall, Kami-ide, Fujinomiya city, ca. 500 m alt., moist weedy place, in the basin of waterfall, 28 Oct. 1976, *F. Kanta, E. Hayakawa & Y. Ishida 11690* (HUH!); Sagami, 4 May

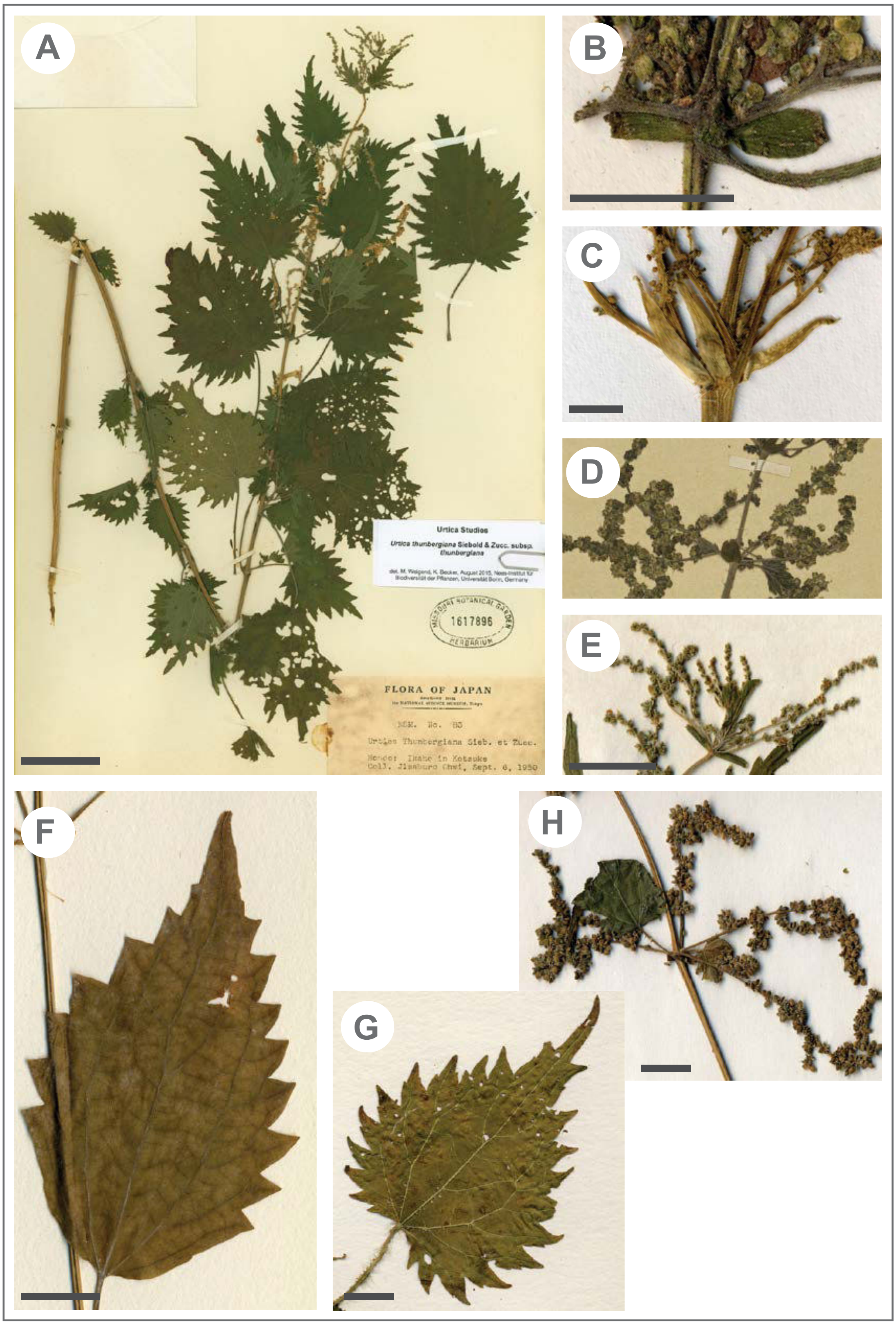

**Figure 4.2** *Urtica thunbergiana* subsp. *thunbergiana*: **A.** Habit (*J. Ohwi 83*), **B.** Node with fused stipules (*Shiota 7952*), **C.** Node with free stipules (*Wang 21118*), **D.** Male inflorescences (P_06855932), **E.** Female inflorescences (*J. Ohwi 83*), **F.** Regularly dentate leaf (*Boufford & Wood 19684*), **G.** Doubly dentate leaf (*Tsugaru & Takahashi 25630*), **H.** Infructescence (MO_3535031). Scale bars: A = 5 cm; B–H = 1 cm.

1934, *K. Shiota 7952* (HUH!); **Kanagawa:** Yokohama, 1862, *Maximowicz s.n.* (K_06855931! & K_000694839!); Yokosuka, in Lepibus, 1866–1871, *Savatier 1120* (P_06822174! & P_06855927! & P_06855928! & P_06855934!); **Kumamoto:** Nakamatsu-staion near Hakusui-son, Aso-gun, 420 m, 17 Sep. 1984, *M. Furuse 52942* (PE_00097341!); **Musashi [nowadays mainly parts from Tokio und Saitama]:** Mt. Mitake, 19. Jul. 1932, *Collector unknown* (KUN_0523753!); Tsubakuro-iwa, Mt. Temso-zan Nippara Hikawa-machi, Nishi-tama-gun, Tokyo-to, Hondo, about 1100 m, by forest, 15 Jul. 1958, *M. Furuse s.n.* (HUH!); **Tokyo:** 27 Sep. 1913, *T. Makino 119445* (L_1629483!); **Without legible locality**: Oct. 1898, *Collector not legible* (MO_3535031!);—**KOREA.** Without legible locality information, 28 Sep. 1906, M. *Faurie 912* (P_06750066!);—**RUSSIA**. **Far East:** The pass Suputinka, Maikhe, Novo-Khotunichi village, mixed forest at the ridge in the South Ussuri territory 12 Jun. 1913 [jul. Calender], *Komarov 201* (2 x LE!); The pass Suputinka, Maikhe, Novo-Khotunichi village, mixed forest at the ridge in the South Ussuri territory 13 Jun. 1913 [jul. Calender], *Komarov 201* (LE!); **Primorja:** In viciniis opp. Ussurijsk, in valle fl. Komarovka, silva mixta, 12 Jul. 1986, *P. Gorovoi, E. Boyko & N. Ustimenko 6868* (HUH!); In viciniis opp. Ussurijsk, in valle fl. Komarovka, silva mixta, 12 Jul. 1968, *P. Gorovoi, E. Boyko & N. Ustimenko1990* (P_06821123!);—**Unidentified localities:** *Drake 25* (P_06855933!); *Collector not legible* (P_06855932!)

### 4.5.4 ***Urtica thunbergiana*** Sieb. & Zucc. subsp. ***dentata*** (Hand.-Mazz.) K.Becker & Weigend, comb. nov. & stat. nov., Fig. 4.3

≡ *Urtica dentata* Hand.-Mazz. (1929: 112) = *Urtica laetevirens* Maxim. subsp. *dentata* (Hand.-Mazz.) C.J. Chen (1983: 115). Lectotype (here designated):—CHINA. Hupeh: Mar. 1889, *A. Henry 5859* (HUH!, isolectotype: K_000694846!). Syntypes:—CHINA. Hupeh: 1885–1888, *A. Henry 5401* (NY_00284487!, US_01107870!, HUH!, K_000694845!); Hunan: In monte Yün-schan prope urbem Wukang, Apr. 1919, *Handel-Mazzetti 262* (P_06855739! & K000694844!).

= *Urtica pachyrrhachis* Hand.-Mazz. (1929: 113). Holotype:—[China] Hunan austro-occ.: In monte Yün-schan prope urbem Wukang, 12 Jun.1918, *Handel-Mazzetti 12099* (WU_0035448!)

Nodes with 4 free stipules; leaf lamina 45–90 × 20–50 mm, teeth 2–4 mm long, teeth pointed and detached, leaf base rounded to cordate; heteroblasty occasionally developed with the basal leaves nearly as long as broad and with a cordate base and the apical leaves nearly three times longer than wide and with a rounded to attenuate base.

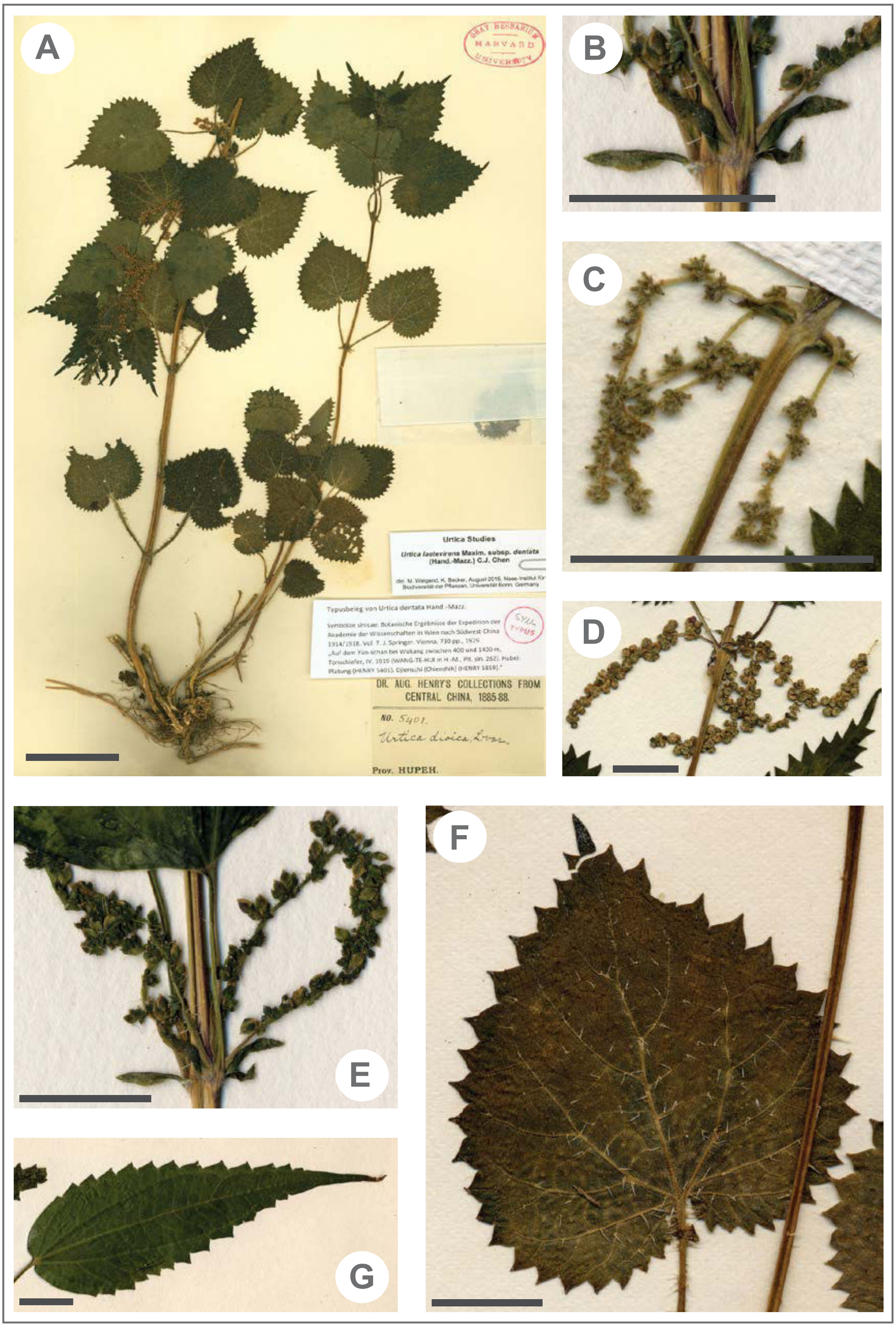

**Figure 4.3** *Urtica thunbergiana* subsp. *dentata*: **A.** Habit (*Henry 5401*), **B.** Node with free stipules ( *Luo 1347*), **C.** Female inflorescences (*Boufford et al. 28908*), **D.** Male inflorescences (*Boufford et al. 28908*), **E.** Infructescence (*Luo 1347*), **F.** Basal leaf with a cordate base, nearly as long as broad (*Henry 5859*), **G.** Apical leaf with a rounded to attenuate base, nearly three times longer than broad (*Henry 5401*). Scale bars: A = 5 cm; B–G = 1 cm.

**Distribution and Habitat:**—*U. thunbergiana* subsp. *dentata* is restricted to central China and is here reported from Guizhou, Hunan, Hupeh, Sichuan and Yunnan. The subspecies occurs on roadsides, in the understory of forests growing in rich and moist soil, near streams, and is usually found at intermediate elevations.

**Phenology:—***U. thunbergiana* subsp. *dentata* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica thunbergiana* Sieb. & Zucc. subsp. *dentata* (Hand.-Mazz.) K.Becker & Weigend is considered "Least Concern" (LC).

**Representative specimens:—CHINA. Guizhou**: Leijinag, E118°9'40", N26°23'30", 24 May 1965, *W. Zhang & Y. Ma 50358* (KUN_0523685!); **Hunan:** In monte Yün-schan prope urbem Wukang, 400–1400 m, Mar. 1919 *Wang-Te-Hui s.n.* (WU_0035446! & K_000694844!); In monte Yün-shan prope urbem Wukang, 400–1400 m, 1974, *Wang-Te-Hui 262* (P_06855739!); In monte Yün-shan prope urbem Wukang, in silva elata frondosa umbrosa in valle ad septentr. templi Gwanyin-gp versus Wulingan descendente, ca. 1000 m, 12 Jun. 1918, *H.R.E. Handel-Mazzetti 2499* (WU_0035448!); Shangpai, Yangming Mountain National Forestpark, Yangming Mountain, 1400 m, shady place in open forest in a valley, 10 to 12 Apr. 1942, *S.H. Chun 685* (MO_04154107!); Xining Co. 26.4N, 110.8E, in woods, 1200 m, 10 May 1996, *L. Chong-chun 1347* (MO_05087041!, HUH!, P_06820975!, PRE!); Yongshun, Brook Nature Reserve, riverside in evergreen broad-leaved forest 480–520 m, 27 May 1988, *Beijing Team 0173* (KUN_0571676!); Yongzhou, Lingling, Mt. Yangming, in the spare woods of a valley, 1400 m, 12 Apr. 1942, *S. Chen 685* (MO_04119815!); **Hupeh:** 1885–1888, *A. Henry 5401* (HUH! & NY_00284487! & US_01107870!); 1885–1888, *A. Henry 5859* (HUH!); Enshi Prefecture, Lichuan, Mao Peak, in grooves, 14 Apr. 1973, *H.G. Li 11110* (MO_04503446!); Mar. 1889, *A. Henry 5401* (K_000694845!); Mar. 1889, *A. Henry 5859* (K_000694846!); **Sichuan:** Between rocks, 1400 m, 20. Jun. 1958, *T. Dai 100932* (MO_04476384!); Chengtu, At the side of forest, 09 May 1928, *W.P. Fang 13566* (KUN_0523682!); Kuan-hsien, Mt. Tsing-chen, 09 May 1939, *W.P. Wang 13565* (HUH_00278008!); Luding, Moxi, roadside, 3200 m, 13 Sep. 1980, *Chengdu Institute of Biology, Chinese Academy of Science 23542* (CDBI_0017408!); Nanchuan Co. 29.1° N, 107.0° E, 730 m, by the river, 11 Apr. 1996, *L. Zheng-yu 15565* (MO_5309133!, HUH_00240985!, P_06820976!); Nanchuan Co. 29.1° N, 107.0° E, 750 m, by the road, 11 Apr. 1996, *L. Zheng-yu 15572* (MO_5309129! & HUH_00240986!); South of Kuan Hsien, in ravine, 1130 m, 19 Apr. 1930, *F.T. Wang 20549* (HUH_00278017!); Tchen Keou Tin [nowadays: Chengkou], 1895–1897, *Fargens s.n.* (P_06855888!); Xiangchen Xian, Niding:

Base of Rizhou Mountain behind village of Niding, in west-opening gorge, 20°5’26”N, 99°40’29”E, 3450 m, cut over mixed conifer forest in limestone gorge, boulder field on slope in mixed sun-shade of shrubs and Picea, 16 Jul. 1998, *D.E. Boufford, B. Bartholomew, C.Y. Chen, M.J. Donoghue, R.H. Ree, H. Sun & S.K. Wu 28908* (MO_5766519! & HUH_00240988!); **Yunnan:** Ngaala, Tsa-wa-rung, stream side, 3600 m, Aug. 1935, *C.W. Wang 66066* (HUH_00278010!);—**Unidentified localities:** 1887, *Drake 2838* (P_06855915!)

### 4.5.5 ***Urtica thunbergiana*** Sieb. & Zucc. subsp. ***silvatica*** (Hand.-Mazz.) K.Becker & Weigend, comb. nov. & stat. nov., Fig. 4.4

Lectotype (here designated):—[CHINA], Yunnan, Inter pagum Dschungdien ("Chungtien") et vicum Djitsung, in regionis frigide temperatae abietetis jugi Ngukala, ad rivulos. 3750–3800 m, 25 Aug. 1915, *Handel-Mazzetti 7797* (WU_0035449!). Isolectotypes:—[CHINA], Yunnan, Inter pagum Dschungdien ("Chungtien") et vicum Djitsung, in regionis frigide temperatae abietetis jugi Ngukala, ad rivulos. 3750–3800 m, 25 Aug. 1915, *Handel-Mazzetti 7797* (E_00275393! & K_000694838!). Syntypes:—[CHINA, Sichuan] Dongrergo bei Sungpan, im Tannenurwalde, 3900 m, 10 Aug. 1922, *H. Smith 3486* (not localized); Karlong im Tannenurwalde, 3400 m, 27 Aug. 1922, *H. Smith 4229* (not localized); Drogotschi, im buschigen Tale, 3600m, 26 Sep. 1922, *H. Smith 4705* (not localized); [Shaanxi] S-Shenhsi, Giraldi 1345 (B); [Gansu] SE-Kansu, Vers Pei la hia, 28 Apr. 1919, *Licent 5166* (not localized).

≡ *Urtica silvatica* Hand.-Mazz. (1929: 113). Syntypes:—[CHINA], Yunnan, Inter pagum Dschungdien ("Chungtien") et vicum Djitsung, in regionis frigide temperatae abietetis jugi Ngukala, ad rivulos. 3750–3800 m, 25 Aug. 1915, *Handel-Mazzetti 7797* (E_00275393!, K_000694838!, P_06855873!, WU_0035449!); [Sichuan] Dongrergo bei Sungpan, im Tannenurwalde, 3900 m, 10 Aug. 1922, *H. Smith 3486* (not localized); Karlong im Tannenurwalde, 3400 m, 27 Aug. 1922, *H. Smith 4229* (not localized); Drogotschi, im buschigen Tale, 3600m, 26 Sep. 1922, *H. Smith 4705* (not localized); [Shaanxi] S-Shenhsi, *Giraldi 1345* (B); [Gansu] SE-Kansu, Vers Pei la hia, 28 Apr. 1919, *Licent 5166* (not localized).

Herb 0.25–1.00 m; stem stinging hairs 1.0–2.0 mm long overall, setae 0.8–1.5 mm; leaf lamina 50–85 × 25–50 mm, teeth 2–5 mm long, teeth rounded, leaf base rounded to attenuate; leaf stinging hairs 1.5–2.0 mm long overall, setae 0.8–1.2 mm; petioles 20–50 mm long; nodes with 4 free stipules 3–4 mm long.

**Distribution and Habitat:**—*U. thunbergiana* subsp. *silvatica* is restricted to Tibet and central China and is here reported from Anhui, Beijing, Congqing, Gansu, Guizhou, Hebei, Henan, Hubei, Hunan, Shaanxi, Shanxi, Sichuan and Yunnan. The subspecies mainly occurs in the understory of forests growing in rich and moist soil, on roadsides, in wetlands, near streams, and is usually found at intermediate and high elevations.

**Phenology:—***U. thunbergiana* subsp. *silvatica* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica thunbergiana* Sieb. & Zucc. subsp. *silvatica* (Hand.-Mazz.) K.Becker & Weigend is considered "Least Concern" (LC)

**Representative specimens:—CHINA. Anhui:** Ch'ienshan, Fengtien, 29 Jun. 1930, *H.W. Kung K.794* (PE_00509551!); **Beijing:** 500 km in the south of Peking, 1903–1935, *L. Chanet & J.H. Serre 2066* (P_06822183!); Mentougou District, Xiaolongmen Forest Farm, Aug. 1888, *Z. Wang s.n.* (PE_00514239!); **Congqing:** Nanchuan District, Jinfo Mountain, Yangyuping, Road side, 02 Sep. 1994, *Z. Liu 14546* (MO_04539240!); **Gansu:** Chine Septentrionale, 28 Apr. 1919, *R.P. Licent 5166* (P_06855872!); **Guizhou:** 28 May 1936, *S. Deng 90389* (MO_04176242!); **Hebei:** Neigin, Xiaolingdi, Wuzhi Kiln, 23 Jul. 1950, *Y. Liu 13160* (MO_04719097!); **Henan**: Wetland in a valley, 09 Jul. 1962, *S.Han 20514* (HENU_0010820!); Xinxiang, Funiu Mountain, Wetland in a valley, 08 Aug. 1956, *Y. Lu 002* (HENU_0010821!); Xinxiang, Funiu Mountain, Wetland in a valley, 08 Aug. 1956, *L. Shen 099* (HENU_0010823!); **Hubei:** Shennongjia, riverside, near woods, 2360 m, 23 Sep. 1958, *R.Y. Huang 184* (MO_04500020!); **Hunan:** In monte Yün-shan prope urbem Wukang, 400–1400 m, 1974, *Wang-Te-Hui 262* (WU_0035466!); **Gansu:** Vicinity of Labrang, 3000–4000 m, 17–20 Aug. 1923, *R.C. Ching 810* (HUH_00240987!); **Shaanxi:** Liuba, Miaotaizi Dam groove, Lat. 33.6177643 Long. 106.9212306, Hillside, Jungle, 17 Oct. 1952, *K. Fu 6290* (MO_04486877!); Foping, Hetaoping, 1600 m, 18 Jun. 1952, *K. Fu 4610* (KUN_0523689!); **Shanxi:** Jiexiu, Main Mountain, Jiezi Temple neigbourhood, 11 Jun. 1957, *J. Liu s.n.* (MO_04486398!); **Sichuan:** Jiuzhaigou (conservation area), Lajiao forestry, 1300 m, hillside, 18 Jul. 1993, *L. Xu & J. Zhang 2006* (MO_04482826!); Kangding, Gongga Temple, under some oaks, 3600 m, 20 Jul. 1974, *X. Hu 20695* (CDBI_0017409!); Kangding, Mountainous area, 15 Jul. 1963, *K. Huan & W. Wang 121* (MO_04526644!); Kangding, Zheduo Pond, Grassland near thickets on hillside, 3100 m, 01 Aug. 1963, *K. Guan & W. Wang 1230* (MO_04735716!); Kangtin, Minya Konka, Konka Temple, under an oak tree, 3600 m, 20 Jul. 1979, *X. Hu 20643* (CDBI_0017410!); Luding, Moxi, roadside, 3200 m, 14 Sep. 1980 *Chengdu Institute of Biology, Chinese Academy of Science 23542* (CDBI_0017407!); Mt. Omei, on watery rock, 2400 m, Jul. 1935, *F.T. Wang 23383* (HUH_00278016!); reg. bor.-occid, Harlong in silva abientina, muscosa, ca. 3400 m, 27 Aug. 1922, *H. Smith 4229* (PE_00509585!); West of Wen-chaun Hsien, ravine, 2900 m, 27 May 1930, *F.T. Wang 20990* (HUH_00278002!); **Yunnan:** 1938, *T.T. YÜ 9937* (HUH_00278005!); *C.W. Wang 69792* (KUN_0523671!); Deqin, Benzilan, Baimang Snow Mountain, near dragon spruce and

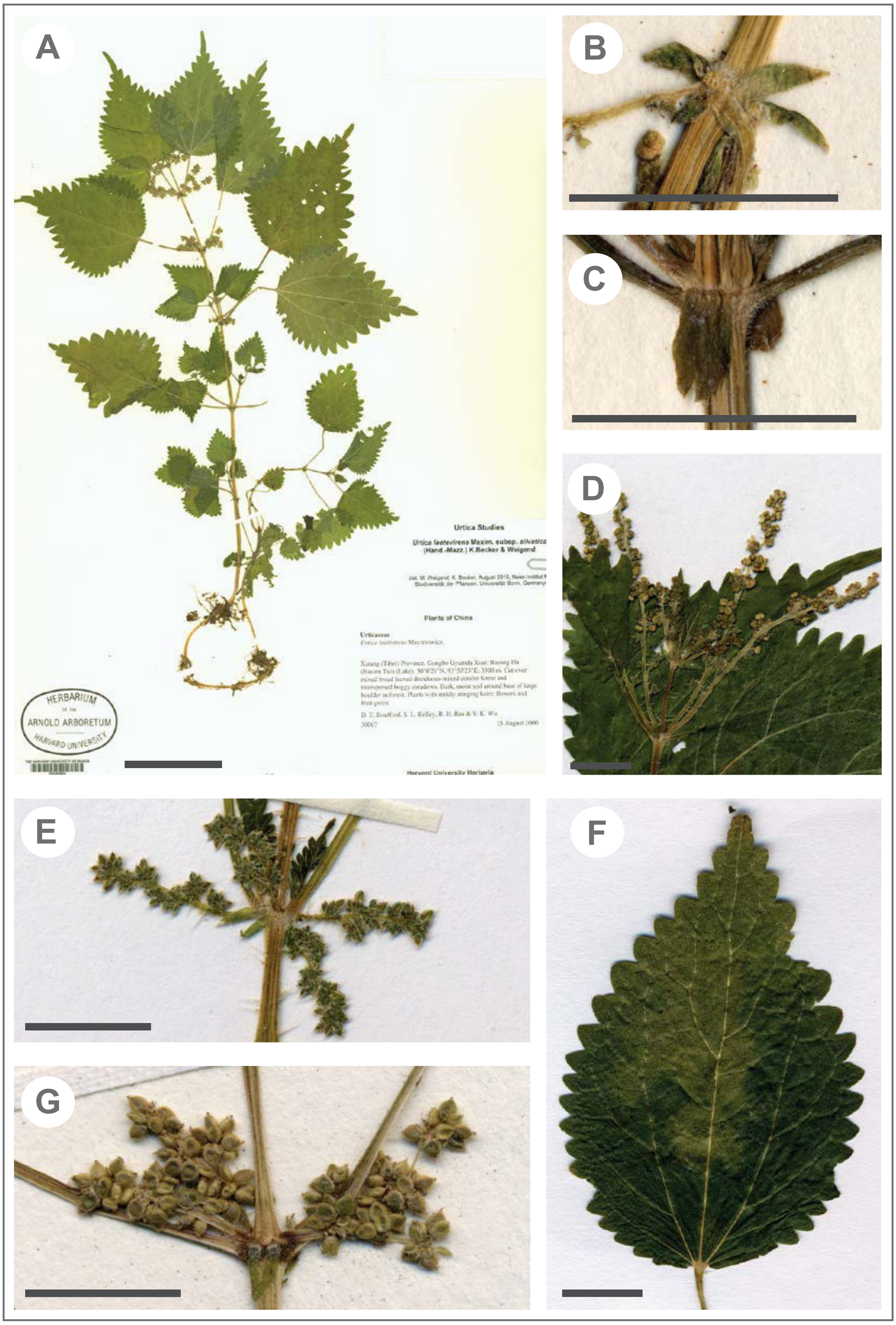

**Figure 4.4** *Urtica thunbergiana* subsp. *silvatica*: **A.** Habit (*Boufford et al. 26323)*, **B.** Node with free stipules (*Wang 70702)*, **C.** Node with fused and incised stipules (*Liu 13160)*, **D.** Male inflorescences (*Rushforth & McAllister 5327)*, **E.** Female inflorescence (*Rushforth & McAllister 5327)*, **F.** Typical leaf (*Wang 70702)*, **G.** Infructescence (*Boufford et al. 30067)*. Scale bars: A = 5 cm; B–F = 1 cm.

fir woods, 3200–3400 m, 06 Jul. 1981, *Tibet Team2182* (CDBI_0017414!); Fo-Hai, Thickets, 1540 m, Jun. 1936, *C.W. Wang 74402* (HUH_00278012!); Hillside in the forest, 3500 m, 26 Aug. 1962, *Middle Team 1357* (KUN_0523673!); Inter pagum Dschungdien ("Chungtien") et vicum Djitsung, in regionis frigide temperatae abietetis jugi Ngukala, ad rivulos. 3750–3800 m, 25 Aug. 1915, *Handel-Mazzetti 7797* (E_00275393!, K_000694838!, P_06855873!, WU_0035449!); Li-kiang Hsien, ravine, 2500 m, Jul. 1935, *C.W. Wang 70702* (HUH_00278003!); Li-kiang Hsien, Ravine, Jul. 1935, *C.W. Wang 70912* (KUN_0523672!); *M. Delavay 2838* (P_06822165!); Muli, shady forest, 3200 m, 24 May 1937, *T.T. Yü 5686* (KUN_0523678!); Vallées des mount á Tong-Tchouan, 2600 m, Sep., *E.E. Maire s.n.* (P_06749817!); Wei-si Hsien, ravine side, 3500 m, Jun. 1935, *C.W. Wang 73915* (HUH_00278026!);—**TIBET. Gongbo Gyamda Xian:** Basong Hu (Basum Tso) ( Lake), 30°0'21"N,93°55'23"E, 3500 m, cut over mixed broad leaved deciduous-mixed forest and interspersed boggy meadows, dark, moist soil around base of large boulder in forest, 15 Aug. 2000, *D.E. Boufford, S.L. Kelly, R.H. Ree, S.K. Wu 30067* (P_06855914!, HUH_00240984!); **Kongbo:** Nyima La (SE Tibet), 29 33 36.3 N, 94 40 47.8 E, Mixed *Picea-Abies*-broadleaved forest, dense patch about 25 m tall on moist soil in deep shade under shrubs on steep bank, Common at low altitudes in temperate rain forest in very wet, shaded situation, often occurring in sheets as the dominant ground cover in such habitats with *Meconopsis betonicifolia* and *Primula chungensis*, 12 Oct. 1997, Cultivated specimens collected in University of Liverpool Botanik Gardens, Ness, *K. Rushforth & Hugh McAllister 5327* (BONN!); Kongbo: Tamnyen Chu (SE Tibet), Tsangpo Valley, Lat. N.29 27', Long. E.94 38', 10,000 ft, Along path in Abies-forest, 24 Jun. 1938, *F. Ludlow, G. Sherriff & G. Taylor 4971* (HUH_00278022!);—**Unidentified localities:** *Drake 2838* (P_06855916!); In the forest, 2600m, *Collector unknown* (KUN_0523663!).

### 4.5.6 *Urtica thunbergiana* Sieb. & Zucc. subsp. *perserrata* K.Becker & Weigend, subspec. nov., Fig. 4.5

Holotype:—TAIWAN. Taitung, Hsiangyangshan, 3493–3600 m, 23 Jun. 1995, *S.T. Chiu, C.H. Chu & C.M. Chao 02924* (TAIM!).

Isotype:—TAIWAN. Taitung, Hsiangyangshan, 3493–3600 m, 23 Jun. 1995, *S.T. Chiu, C.H. Chu & C.M. Chao 02924* (TAIF_082883!)

Perennial herb 0.25–1.50 m, stems densely setose with stinging hairs 2.5–4.0 mm long overall, setae 1.5–3.0 mm; leaf margin with 10–20 large strongly pointed teeth on each side,

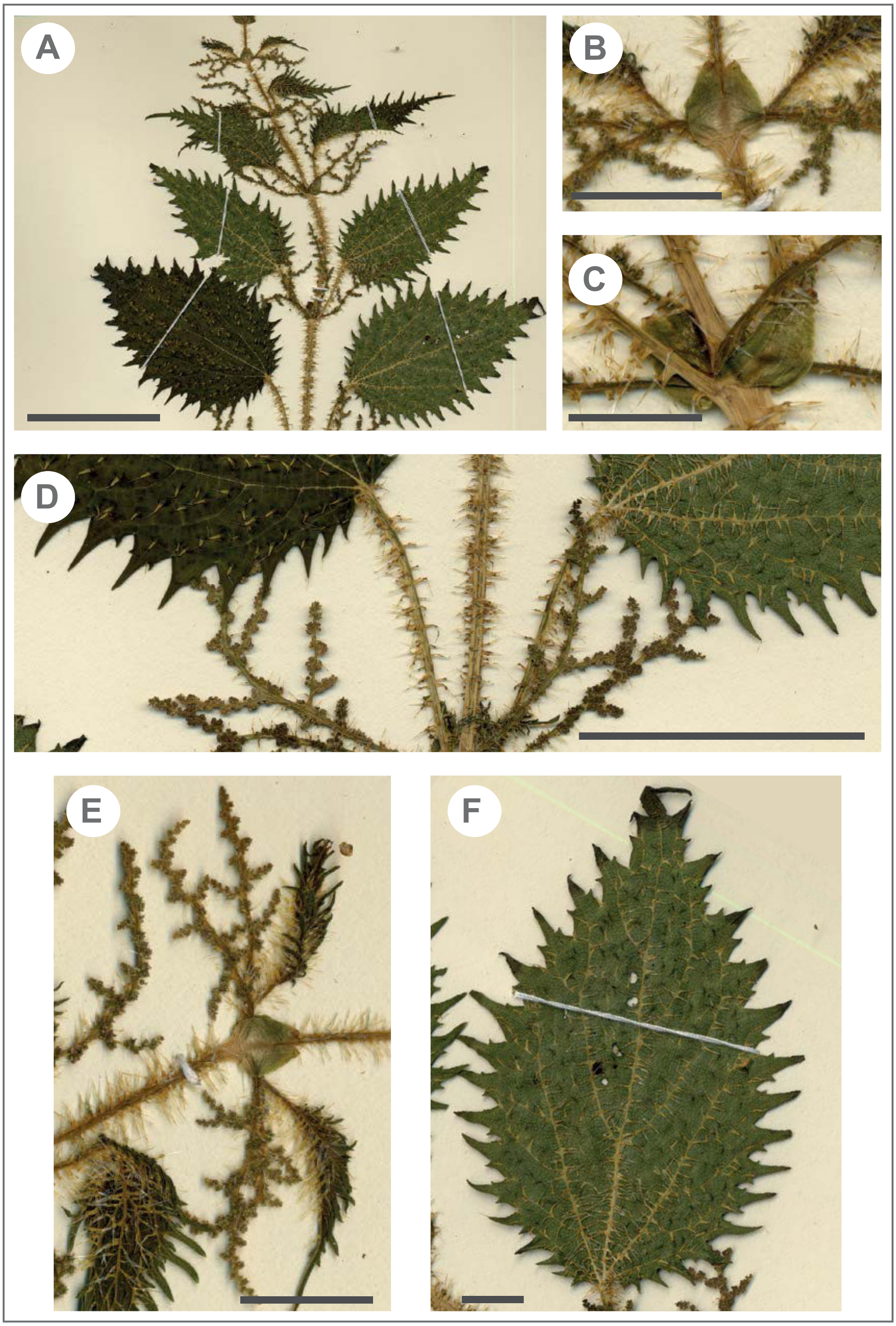

**Figure 4.5** *Urtica thunbergiana* subsp. *perserrata* (A–F: Chiu et al. *02924*): **A.** Habit, **B.** Node with fused and incised stipules, **C.** Node with fused stipules, **D.** Male inflorescences, **E.** Female inflorescence, **F.** Typical leaf. Scale bars: A, D = 5 cm; B–C, E-F= 1 cm.

5–10 mm long; leaf base rounded; leaf stinging hairs 3.0–3.5 mm long overall, setae 1.5–2.5 mm; petioles 30–50 mm long; 4 stipules forming 2 fused pairs per node, occasionally incised distally.

**Distribution and Habitat:**—*U. thunbergiana* subsp. *perserrata* is only known from Taiwan so far.

**Phenology:—***U. thunbergiana* subsp. *perserrata* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica thunbergiana* subsp. *perserrata* K.Becker & Weigend is considered "Not Evaluated" (NE).

**Representative specimens:—TAIWAN. Taitung:** Hsiangyangshan, 3494–3600 m, 23 Jun. 1995, *S.T. Chiu, C.H. Chu & C.M. Chao 02924* (TAIF_082883! & TAIM!).

## 4.6 Acknowledgements

We are grateful to the gardeners of the Bonn Botanical Gardens, especially J. Manner and his colleagues for maintaining the *Urtica* living collection. We are particularly grateful for some valuable recent collections facilitated by K. Rushforth and H. McAllister from Liverpool and by David Boufford from Harvard University.

# CHAPTER 5

## Weeding the nettles VI: Taxonomic and phylogenetic studies of the Southeast Asian *Urtica fissa*-clade (Urticaceae)*

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### Abstract

*Urtica* L. (Urticaceae) is a subcosmopolitan genus, also common throughout temperate Asia. Species delimitation is notoriously difficult and the present study investigates a group of taxa around *Urtica fissa* from East-Southeast Asia, based mainly on herbarium collections, including most type specimens, and cultivated plants. In herbaria, but also in floristic treatments species limits especially of *U. mairei*, *U. fissa* and *U. himalayensis* have been consistently confused. In order to resolve the confused taxonomy, we additionally generated a molecular phylogeny of the group based on the markers ITS1-5.8S-ITS2, *psb*A–*trn*H, *trn*L–*trn*F and *trn*S–*trn*G. Based on our revision, a total of five species and two subspecies are here recognised: *U. fissa* E.Pritz ex Diels, *U. grandidentata* Miq. subsp. *grandidentata, U. grandidentata* Miq. subsp. *lombok* K.Becker & Weigend, *U. himalayensis* Kunth & C.D.Boché, *U. mairei* Lév. and *U. parviflora* Roxb. The requisite typifications and descriptions are provided.

*Keywords:* Asia, chloroplast markers, Himalayas, infraspecific taxa, nuclear marker, phylogeny, stinging nettle, taxonomy, Urticaceae

*manuscript submitted to *Phytotaxa*, 23/08/2016

## 5.1 Introduction

*Urtica* L. (Urticaceae) is a common genus over most of its range, often found in anthropogenically altered habitats and naturally disturbed sites. In spite of its abundance and the visibility of the genus, its systematics remains problematic and its alpha-taxonomy is still incompletely understood (Friis 1993). The past years have brought considerable progress in the taxonomical re-definition of individual components within the genus *Urtica*, especially in the European, American and Australian/New Zealand taxa (e.g. Weigend 2005, 2006, Weigend et al. 2005, Weigend & Luebert 2009, Farag et al. 2013, Henning et al. 2014, Grosse-Veldmann & Weigend 2015, Weigend & Monro 2015, Grosse-Veldmann et al. 2016a, 2016b). Most notably, the first near-comprehensive phylogeny was published in Grosse-Veldmann et al. (2016b) which provides a clear picture of the overall taxonomy within the genus. One of the clades retrieved in Grosse-Veldmann et al. (2016b) comprises tall, late-flowering species with largely fused and often very large stipules, namely the species complex around the taxa *U. fissa* E.Pritz ex Diels and *U. parviflora* Roxb. This group ranges across the Himalayas to western China and into Indonesia. In the present study we address this problematic group based on a morphological and molecular approach, in order to identify and clarify the delimitation and relationships of the taxa.

## 5.2 Material and methods

### 5.2.1 Plant material

A large number of herbarium specimens, including type specimens, was revised for the present study. Material was examined from the following herbaria (abbreviations following Holmgren & Holmgren 1998 ff): B, BONN, CDBI, E, HENU, HUH, IFP, K, KUN, L, LE, M, MO, NY, P, PE, TAIF, TAIM, US and WU. Two species, *Urtica fissa* and *U. parviflora,* were taken into cultivation at Bonn University Botanical Gardens in 2014 and 2015. All protologues and type specimens were studied and compared to current floristic treatments [e.g., Flora of Bhutan (Grierson & Long, 1983), Flora of China (Chen et al., 2003), Flora of Pakistan (Ghafoor, 1981), Flora of Xizangica (Wu, 1983)]. Plant material used for the phylogenetic analyses was silica-dried or taken from herbarium material. In total, 12 ingroup and 7 outgroup accessions were sampled. Representatives of other *Urtica* species within the genus covering a broad geographical and phylogenetic range (compare Grosse-Veldmann et al., 2016b) were chosen as outgroup. A complete voucher list of the plant material used in this study including voucher information and GenBank accession numbers is given in Table 1.

Conservation assessments were undertaken using IUCN Red List Categories and Criteria (2001).

### 5.2.2 DNA extraction, amplification, and sequencing

DNA extraction, amplification, purification, and sequencing followed standard protocols as described in Gottschling & Hilger (2001) and Weigend et al. (2010). Samples were sequenced for four genomic regions: the nuclear ribosomal ITS1–5.8S–ITS2 (thereafter ITS), and three plastid regions: the *psb*A–*trn*H intergenic spacer (IGS), *trn*L–*trn*F (including the *trn*L group I intron and the *trn*L–*trn*F IGS), and *trn*S–*trn*G (including the *trn*S–*trn*G IGS and the *trn*G group II intron). The same primers were used for amplification and for sequencing. The primers used were ITS5 and ITS4 for ITS (White et al., 1990), *psb*AF and *trn*HR for *psb*A–*trn*H (Sang et al., 1997), C and F for *trn*L–*trn*F (Taberlet et al., 1991) and trn_S$_{(GCU)}$ and trn_G$_{(UCC)}$ for *trn*S–*trn*G (Hamilton 1999). Amplicons were sequenced by either Macrogen Inc., South Korea (http://www.macrogen.com) or GATC Biotech AG, Konstanz, Germany (http://www.gatc-biotech.com). All sequences generated in this study have been submitted to the GenBank genetic sequence database (see Tab. 5.1 for accession numbers).

### 5.2.3 Alignment and phylogenetic analyses

The alignment was conducted using the MAFFT algorithm in the Geneious software package ver. R8 with default settings, followed by manual adjustments using PhyDE® ver. 1 (Müller et al., 2005) in order to build a motif alignment, based on the criteria laid out in Kelchner (2000). The combined data set (ITS, *trn*S–*trn*G, *trn*H–*psb*A and *trn*L–*trn*F) contains 2473 aligned positions (ITS: 624, *trn*S–*trn*G: 740, *psb*A–*trn*H: 282, *trn*L–*trn*F: 837).

The data set contains two hairpin associated inversions, one situated in P8 of the *trn*L intron (compare Borsch et al. 2003) and the second approximately 90 nt upstream of the *trn*F gene. Both inversions were positionally isolated in the alignment and included as reverse complement in the nexus files used for phylogenetic analyses (see Quandt et al., 2003; Borsch & Quandt, 2009). Each data set was analyzed independently to identify potential incongruences. No hard incongruences between the separate plastid and ITS trees were observed (data not shown). Phylogenetic analyses were conducted on a concatenated dataset employing maximum likelihood (ML) and Bayesian inference (BI).

**Table 5.1** List of taxa included in the phylogenetic study of the *Urtica fissa*-clade.

| **Taxon** | **Country of origin** | **Herbarium voucher** | **DNA No.** | **ITS** | ***trn*S-*trn*G** | ***psb*A-*trn*H** | ***trn*L-*trn*F** |
|---|---|---|---|---|---|---|---|
| *U. cannabina* L. | China | Q. R. Wu 322 (MO) | W 2038 | KX271370 | KX271525 | KX271601 | KX271451 |
| *U. dioica* L. subsp. *dioica* | Spain | E. Zippel 2002/2b (B) | W 2232 | KF558920 | KF559101 | KF558980 | KF559040 |
| *U. echinata* Benth. | Ecuador | Loejtnant & Molau 11657 (GB) | W 1863 | KX271427 | KX271577 | KX271657 | KX271501 |
| *U. fissa* E.Pritz ex Diels | China | C.Y. Wang 7438 (HUH) | W 4540 | to be submitted | to be submitted | to be submitted | to be submitted |
| *U. fissa* E.Pritz ex Diels | China | W. Li s.n. (MO) | W 4553 | to be submitted | to be submitted | to be submitted | to be submitted |
| *U. fissa* E.Pritz ex Diels | Taiwan | M. Weigend 8129 (B) | W 1880 | KX271397 | KX271548 | KX271628 | KX271473 |
| *U. fissa* E.Pritz ex Diels | China | Hsiu-Lan Ho 951 (MO) | W 2011 | KF558905 | KF559086 | KF558965 | KF559025 |
| *U. fissa* E.Pritz ex Diels | China | Sino-American Guizhou Botanical Expedition 1135 (HUH) | W 4211 | KX271395 | KX271546 | KX271626 | KX271471 |
| *U. fissa* E.Pritz ex Diels | China | A. Henry 2900 (K) | K 22888 | KX271396 | KX271547 | KX271627 | KX271472 |
| *U. grandidentata* Miq. subsp. *grandidentata* | Indonesia | S. H. Koorders 37901B (K) | K 22882 | KX271401 | KX271552 | KX271632 | to be submitted |
| *U. grandidentata* Miq. subsp. *grandidentata* | Indonesia | H. N. Ridley s.n. (K) | K 22883 | KX271402 | KX271553 | KX271633 | KX271477 |
| *U. himalayensis* Kunth & Bochè | India | H. Collett 590 (K) | K 22889 | to be submitted | to be submitted | to be submitted | - |
| *U. incisa* Poir. | New Zealand | Ward CHR 234516A (NZ Landcare Research) | W 2264 | KF971218 | KF971185 | KF971284 | KF971251 |
| *U. mairei* Lévl. | Tibet | K. Rushforth & H. McAllister 5247 (BSB) | W 2910 | KX271398 | KX271549 | KX271629 | KX271474 |
| *U. massaica* Mildbr. | Uganda | M. Ackermann 1050 (B) | ED 841 | KX271388 | KX271539 | KX271619 | KX271464 |
| *U. parviflora* Roxb. | China | B. Dickoré s.n. (B) | W 2238 | KX271400 | KX271551 | KX271631 | KX271476 |
| *U. parviflora* Roxb. | Indien | H. Binski s.n. (BONN) | W 4538 | to be submitted | to be submitted | to be submitted | to be submitted |

ML analyses were conducted with the standard settings in RAxML Version 8 (Stamatakis, 2006; Stamatakis et al., 2008). The node support under ML is based on 1000 bootstrap replicates. BI analyses were conducted in MrBayes vers. 3.2.2 (Ronquist & Huelsenbeck 2003) with six independent runs of 2,000,000 generations each under the GTR + $\Gamma$ + I model with partitions unlinked. Chains were sampled every 1000th generation. Log likelihoods were examined using Tracer v1.5 (Rambaut & Drummond 2009) in order to determine the burn-in and to ensure that an adequate effective sample size (ESS) was attained. The consensus tree and the posterior probability (PP) of clades were calculated based upon the trees sampled after the burn-in set at 500,000 generations.

TreeGraph2 (Stöver & Müller, 2010) was used for tree drawing. We used *Urtica cannabina* to root the final tree.

## 5.3 Results

### 5.3.1 Molecular data

The sampling of Grosse-Veldmann et al. (2016b) was significantly expanded and resulted in a well resolved phylogeny of the species *U. fissa* and allied taxa based on the markers ITS, *trn*S–*trn*G, *psb*A–*trn*H and *trn*L–F (Fig. 5.1). The ITS and chloroplast trees were calculated separately and the well-supported nodes are all congruent between the ITS-tree and the chloroplast trees (data not shown). *U. grandidentata* subsp. *lombok* was only available as a specimen scan and was therefore not included into the phylogenetic study. *U. fissa* and closely allied taxa are retrieved as monophyletic (Fig. 5.1). Five species are recognized by the present phylogenetic study: *U. mairei, U. himalayensis, U. fissa, U. parviflora* and *U. grandidentata*. The relationships between the species could however not be fully resolved with the standard molecular markers used.

### 5.3.2 Morphology

A critical revision of numerous collections including type collections and living plants leads to the recognition of six morphological entities. The taxa of this clade are all robust, perennial herbs, with large, often subcircular leaves and the four stipules fused in two, so that each node bears one pair of broadly interpetiolar stipules. These interpetiolar stipules are subcircular to widely ovate in *U. grandidentata, U. mairei, U. himalayensis* and *U. fissa*. *Urtica parviflora* has ovate to oblong-acuminate stipules. The main characters to distinguish the species are found in the details of the leaf margin. *U. fissa* is the only species with palmately lobed leaves (Fig. 5.2 A–C), there are 4–6 lobes on each side, the leaves lobes are

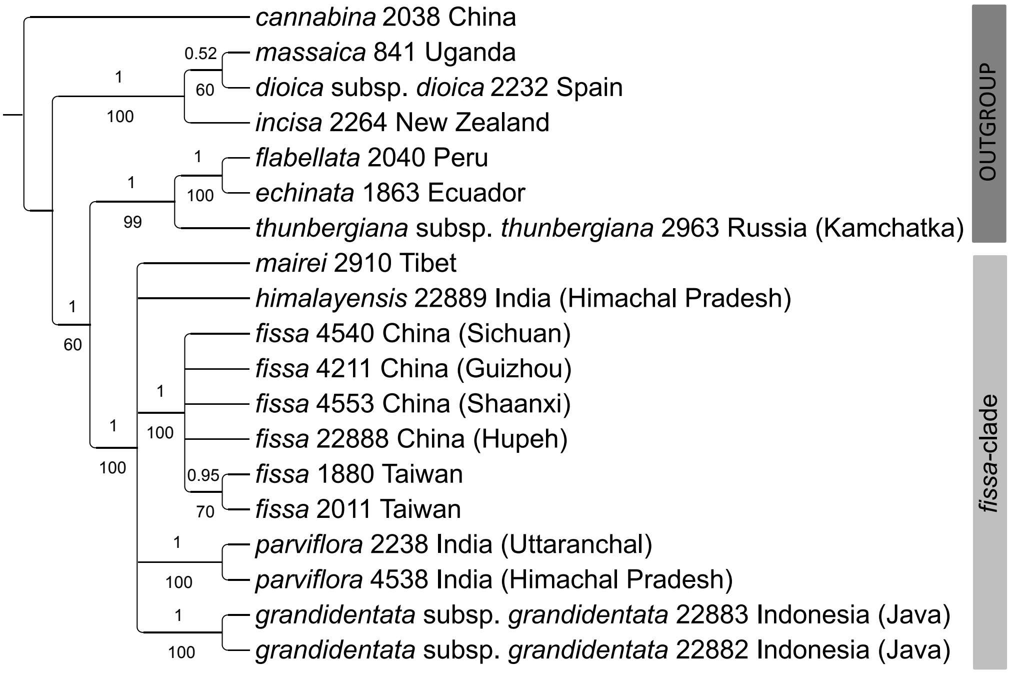

**Figure 5.1** Maximum likelihood tree based on concatenated data set (ITS, *trn*S–*trn*G, *psb*A–*trn*H and *trn*L–F) of the *U. fissa*-clade. Bayesian posterior probabilities are indicated above branches; bootstrap support under likelihood is indicated below.

themselves coarsely and irregularly serrate to lobulate and incisions are 1/3$^{rd}$ to 2/3$^{rds}$ of the leaf diameter. Leaves of *U. grandidentata* subsp. *grandidentata* are widely ovate to subcircular with lobulate margin, with 12–20 irregularly and coarsely dentate large lobules (Fig. 5.3 A, E). The leaf margin of *U. grandidentata* subsp. *lombok* is similar, but the leaves are triangular-ovate and have ca. 15–25 lobules on each side (Fig. 5.4). Both subspecies have a shallowly cordate leaf base. *U. mairei* has widely ovate to subcircular leaves (Fig. 5.6 C; Fig. 5.7), superficially similar to those of *U. grandidentata* subsp. *grandidentata*. Leaf margin is also lobulate, with 8–15 more narrowly triangular and irregularly and coarsely dentate large with more pointed teeth. Especially the apex of each lobe is protracted into a narrowly triangular apex. *Urtica himalayensis* has finely and irregularly doubly serrate leaf margins on widely triangular-ovate leaves, distally they are doubly serrate (Fig. 5.5 D). *Urtica parviflora* is the only species of this group with a regularly dentate leaf margin (occasionally some teeth from older leaves are doubly dentate with small teeth, Fig. 5.8 C). *Urtica himalayensis* is here redefined to include both *U. mairei* var. *oblongifolia* and *U. zayuensis,* because consistent morphological differences between these two taxa could not be found. *U. parviflora*, *U. ardens* and *U. virulenta* are also considered as synonymous and the name *U. parviflora* takes priority. This deviates from the Flora of China (Chen et al., 2003), where *U. ardens* is erroneously considered as the valid name for *U. himalayensis*. And it also differs from the Flora of Pakistan (Ghafoor, 1981), where *U. parviflora* and *U. himalayensis* are listed incorrectly as synonyms for *U. ardens*. Similarly, in the Flora of Bhutan (Grierson & Long, 1983) *U. parviflora* and *U. ardens* are listed as two separate species which we believe to be incorrect. In total, five distinct species can be morphologically differentiated for this SE Asian clade of *Urtica*: *U. fissa, U. grandidentata, U. himalayensis, U. mairei and U. parviflora*. Within *U. grandidentata* two subspecies are proposed, the typical subspecies *U. grandidentata* subsp. *grandidentata* and *U. grandidentata* subsp. *lombok*.

## 5.4 Discussion

The overall morphological diversity of the relevant characters across the taxa studied is very limited and it is not always easy to distinguish the species, as evidenced by the highly inconsistent treatment of the group in the past. Especially the recently published comprehensive phylogeny of the genus by Grosse-Veldmann et al. (2016b) provided first insights into the relationships among the East-Southeast Asian *Urtica* species retrieved as a monophyletic group ("*fissa*-clade"). All species within this clade have four stipules fused in two. Chen (1983) already concluded a close relationship in this group based on morphological studies. Due to a lack of designate types and the morphological similarity, the names *U. ardens, U. himalayensis, U. mairei* var. *oblongifolia*, *U. parviflora, U. virulenta* and *U. zayuensis* haven been frequently mixed up in the past [see Flora of Pakistan (Ghafoor, 1981), Flora of China (Chen et al., 2003), Chen (1983), Grierson & Long (1983), Wu (1983), The Plant List (2013)]. Morphological differences between *U. grandidentata* subsp. *grandidentata*, *U. bullata* and *U. bullata var. contracta* could not be identified and the three taxa are here summarized under the oldest name *U. grandidentata* (subsp. *grandidentata*). Furthermore we segregate a new subspecies from eastern Java and Lombok under the name *U. grandidentata* subsp. *lombok*. So far we have only seen a few specimens from Java and Lombok and no recent material was available for phylogenetic studies. *U. fissa* is clearly distinguishable from the other species by its palmately lobed leaves. Geographical distribution is mostly a poor predictor of relationships of different species within the *fissa*-clade. According to the specimens seen, *U. himalayensis*, *U. mairei* and *U. parviflora* largely have the same distribution in and around Tibet. *U. fissa* is found in central China, Taiwan and the Philippines, nevertheless the distribution area of this species overlaps partially with *U. mairei* and *U. himalayensis*. *U. grandidentata* occurs in contrast occurs on Indonesia; *U.*

*grandidentata* subsp. *grandidentata is* found on Java and *U. grandidentata* subsp. *lombok* on eastern Java and Lombok.

## 5.5 Formal Taxonomy

The formal taxonomy is here provided for redefined *Urtica fissa*-clade.

### 5.5.1 Key to the species of the *Urtica fissa*-clade present in Asia

1. Leaf margin regularly dentate, occasionally some teeth of the older leaves doubly dentate with small teeth ca. 0.5–1 mm long; vegetative basal part of the plant with conspicuously more stinging hairs than the generative part……………………………………………………*Urtica parviflora*

\- Leaf margin doubly dentate, doubly serrate, or palmately lobed; vegetative basal part of the plant not with conspicuously more stinging hairs than the generative part…………………………2

2. Leaf margin doubly serrate, large teeth 1–2 mm long, small teeth 0.5–1 mm long…………………………………………………………………………………………*Urtica himalayensis*

\- Leaf margin doubly dentate or palmately lobed, large teeth or lobes more than 2 mm long…….3

3. Leaf base ovate to cordate, leaf margin doubly dentate with 10–20 large teeth on each side, large teeth dentate with 1–3 small teeth ca. 1–2 mm long………………………*Urtica grandidentata*
   a. Large teeth 5–15 mm long, irregularly and coarsely dentate with 1–3 small teeth on each side, leaf base cordate…………………………………………………………………subsp. *grandidentata*
   b. Large teeth ca. 5 mm long, regularly dentate with 1–2 small teeth on each side, leaf base ovate……………………………………………………………………………………………subsp. *lombok*

\- Leaf base subcircular, leaf margin doubly dentate with 8–15 large teeth on each side or palmately lobed with 2–6 lobes on each side, lobe margins irregularly and coarsely regularly or doubly dentate………………………………………………………………………………………………4

4. Leaf margin doubly dentate with 8–15 large teeth on each side, 2–10 mm long, large teeth irregularly and coarsely dentate with 2–4 small teeth on each side, small teeth 0.5–2 mm…………………………………………………………………………………………………*Urtica mairei*

\- Leaf margin palmately lobed with 2–6 large lobes on each side, lobes 5–50 mm long, lobe margins irregularly and coarsely regularly or doubly dentate with 2–15 teeth on each lobe side, teeth 1–15 mm……………………………………………………………………………………*Urtica fissa*

### 5.5.2 *Urtica fissa* E.Pritz ex Diels (1900: 301). Fig. 5.2

Holotype:—[CHINA, Chongqing] Nan ch´uan, Lung mo ai, *B. v. Rosthorn 866* (WU † burned)

Neotype (here designated):—CHINA. Zhejiang: Hangzhou, Longjing Village, SE of Beili Lake, valley from Longjing to Jiuxicun, stream-sides in a valley with tea plantages and bamboo forest, 40 m, N 30,20183 E 120,10825; source collection by N. M. Nürk 2012, cultivated in the

Botanical Gardens of the University of Bonn, *B. Große-Veldmann et al. 134-C* (BONN!, isoneotypes in B!, HUH!, K!, MO!)

= *Urtica pinfaensis* H. Lév. & Blin in H. Lév. (1912: 371). Holotype: [China, Kouy-Tchéou = Guizhou] 9 Oct. 1902, *J. Cavalerie 771* (E_00275397!)

Erect, perennial herb 0.7–2.5 m, form perennial root and with pleiokorm of perennating underground stems; stem indumentum of stinging hairs with pluricellular base 2.0–4.0 mm long overall, setae 1.8–3.3 mm and simple trichomes ca. 0.1 mm long; leaf lamina subcircular, 70–130 × 50–110 mm, palmately lobed margin with 2–6 large lobes on each side, lobes 5–50 mm long; lobe margins coarsely regularly or doubly dentate with 2–15 teeth on each lobe side, teeth 1–15 mm, leaf base subcordate to rounded, leaf apex apiculate; leaf indumentum of stinging hairs with pluricellular base 2.0–3.5 mm long overall, setae 1.6–3.2 mm, simple trichomes 0.1–0.5 mm long; leaf surface with punctiform cystoliths; petioles 25–70 mm long; 4 stipules forming 2 fused pairs per node, 10–15 mm long, elongated; plant monoecious, usually staminate flowers basal, carpellate flowers apical.; staminate flowers with all tepals ca. 1.0 mm long, pubescent; pistillate flowers with tepals ca. 0.5 mm long, pubescent; infructescence 40–70 mm; mature fruits with longer tepals 1.0–1.2 mm long, achenes subcircular in outline, laterally flattened, ca. 1.0 × 1.0 mm.

**Distribution and Habitat**:—*U. fissa* is widespread in central to eastern China, and is here reported from the provinces of Chongqing, Guangxi, Guizhou, Hubei, Hunan, Shaanxi, Sichuan, and Zhejiang. It is also found in northeast Vietnam, on Taiwan and the Philippines, thus replacing *U. himalayensis* essentially on the eastern side down to the Philippenes, whereas the latter describes a more south-easterly pattern ranging from Kashmir south to Sumatra. *U. fissa* is commonly found on roadsides, waste grounds and disturbed sites, in the understory of forests growing in rich and moist soil, near streams, on fields and meadows, and is usually found at low and intermediate elevations.

**Phenology:—***U. fissa* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica fissa* E.Pritz ex Diels is considered “Least Concern” (LC).

**Representative specimens:—CHINA. Guangxi:** Jinxiu, in a valley, sparse woods, wet soil, 1000 m, 24 Sep. 1981, *Collector unknown* (IBK_00193189!); Lingle, Gangle, in hilly

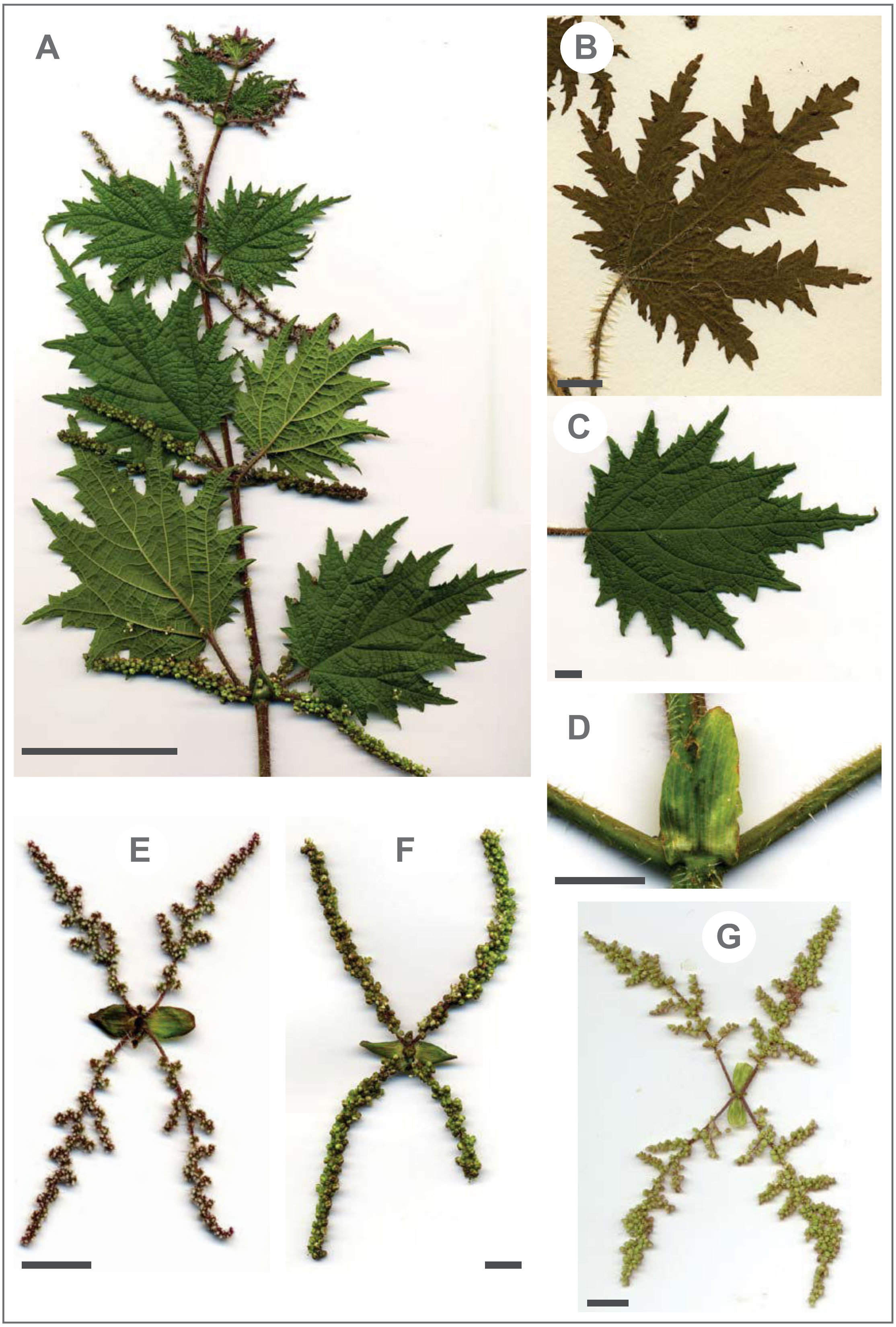

**Figure 5.2** *Urtica fissa* (A, C-G: *B. Große-Veldmann et al. 134-C*): **A.** Habit, **B.** Typical deeply incised palmately lobed leaf (*Smith, 4595)*, **C.** Typical weakly incised palmately lobed leaf, **D.** Node with fused stipules, **E.** Female inflorescence, **F.** Male inflorescence, **G.** Infructescence. Scale bar: A = 5 cm; B–G = 1 cm.

woods as understory, in the shade, 22 Jun. 1959, *Z. Li 603489* (IBK_00130574!); Lingle, riverside, hillside, in the shade, 04 May 1960, *C. Liang 32952* (IBK_00130573!); Longjin, Gebu, Riverside in a forest with hills and valleys, 800 m, 20. Oct. 1953, *Z. Zhang 10229* (IBK_00130571!); Roadside, in the shade, 28 Apr. 1956, *Collector unknown* (IBK_00130575!); Yao Shan, 8 Dec. 1936, *C. Wang 40582* (HUH!); **Guizhou:** [Kouy-Tchéou = Guizhou] 9 Oct. 1902, *J. Cavalerie 771* (E_00275397!); Bieyang, Luho, 333 m, *Y. Tsiang 7202* (K_000229280!); Daozhen, Huilong Town, back river, in the grass by a stream, 720 m, 16 Oct.1995, *L. Zhengyu 16123* (MO_04485995!); Jiangkou Xian, Daiyenpeng along the Kaitu River on the SW side of the Fanjing Shan mountain range, Elevation ca. 750–1000 m, on moist slope in farmyard, 11 Sep. 1986, *Sino-American Guizhou Botanical Expedition 1135* (HUH!); Songtao Xian, vicinity of Lengjiaba in the vicinity of the confluence of the Xiaohe and Dahe rivers, NE side of Fanging Shan mountain range, Elevation 820–1120 m, 5–9 Oct. 1986, *Sino-American Guizhou Botanical Expedition 2239* (HUH!); Zheng'an, Dalou Mountains, Qingding, in the grass by a stream, 560 m, 20 Aug. 1996, *L. Zhengyu 20237* (MO_04485996!); Hunan: Baojing, Kapeng, a valley in mountainous regions, 500 m, *L. Linhan 9735* (MO_04732502!); Dongkon, Fulong Zhou, by the roadside of flatland in the hill, 300 m, 11 Nov. 1963, *L. Linhan & H. Guanzhou 016628* (MO_04732142!)**;** Ma-Ling-Tung, Sinning Hsien, 600 m, shade, 24 Jun. 1935, *C.S. Fan & Y.Y. Li 704* (L_1640100!); **Hupeh:** Feb. 1887, A. *Henry 2900* (K_000229279! & P_06854464!); Shaanxi: Baocheng (now Nanzheng), Bao's Shop, by the roadside, 11 Oct. 1952, *F. Kunjun s.n.* (MO_04486901!); Shaanxi: Foping, Lianghe Township, a shady wet place in the field, 500 m, 15 Aug. 1998, *L. Weiqing s.n.* (MO_04563053!); **Sichuan:** Chengtu, 12 Nov. 1938, *W.P. Fang 13173* (HUH_00240969!); Chengtu, 21 Sep. 1943, *C.Y. Wang 7438* (HUH_00240974!)**;** Kiating, 1 Oct. 1943, *H.H. Chung 3* (HUH_00240970!); Kiating, by the roadside, 12 Oct. 1939, *H.H. Tai 269* (HUH_00240971!); Kiating, outside of the city, by roadside, 23 Nov. 1938, *H.C. Chow 8868* (HUH_00240973!); Lifan: Mung-twin-ko, 7,500 ft. alt.; abundant in waste place, 14 Aug. 1941, *S.Y. Hu 2108* (HUH_00278063!)**;** Mt. Omei, by the slope of hill, 14 Oct. 1939, *S.C. Sun & K. Chang 1551* (HUH_00240972!); Omei-hasien: Mt. Omei, alt. 1700 m, 29 Sep. 1941, *W.P. Fang 17820* (HUH_00240967!)**;** reg. bor.-occid.: Hsu-Tsing, in rupibus supra Ta-chin-ho, ca. 2100 m, 9 Oct. 1922, *H. Smith 4595* (MO_4383339!); W. Sichuan, By the roadside, 30 Sep. 1942, *L.Y. Tai 1420* (HUH_00240968!); Hangzhou, Pearl Temple, in the grass by the roadside, 09 Oct. 1981, *P.L. Chiu 2224* (MO_04491527!); Mu-gan Shan, 25 Sep. 1998, *S.Y. Hu ZJ 43* (MO_5307228! & HUH!);—**PHILIPPINES. Benguet:** Between Mts. Abbocot & Libbung, Kabayan, Luzon, 14 Dec. 1960, *D.R. Mendoza* (L_1629152!); **Cordillera:** Mt. Polis, Bontoc Sub-Provinve, Luzon, Feb. 1920, *M. Ramos & G.E. Edaño 37725* (P_06456161!); Mt. Pulog, 2200 m, clearing, mossy forest, 14 Mar. 1961, *M.L. Steiner 2096* (L_1629153!); **Davao:** Mindanao, 4 Sep. 1946, *G.E. Edaño 1084* (L_1629154!); Mindanao, Aug. 1909, *A.D.E. Elmer 11583* (K_000229284!, L_1629155!, MO_3535020!);—**TAIWAN. Musya**, 30 Sep. 1929, *T. Tanaka 355* (P_06855929!); Ilan County, Nanhu Shan Mt., 2500 m, on the way from central highway to Nanhu Peak, near Nanhu River Cabin in a dense Abies forest. Seeds from original collection from T. Henning (Apr. 2005), cultivated in Berlin, 02 Oct. 2006, *M. Weigend 8129* (B!); Kaohsiung Hsien: Taoyuan Hsiang. Paoshan Village, Chuyunshan Forest Trail: en route from Shelter to the Yunshan Bridge. 23°02' N, 120° 46' E, Elev. Ca. 740 m, On shady and wet forest trail with abundant herbs, 30 Dec. 1992, *H.L. Ho 951* (MO_4327087! & HUH!); Natou, 2295 m, Nov. 1997, *J. Chen 91453* (PE_00509587!);—**VIETNAM. Northeast:** Tonkin, 20 Dec. 1887, B. Balansa 2527 (K_000229278!, P_06855808!, P_06855802!);—**Unidentified localities.** 24 Oct. 1985, *Q.S. Wang 2924* (MO_04512800!).

### 5.5.3 *Urtica grandidentata* Miq. (1853: 27)

Erect, perennial herb ca. 1.5 m, form perennial root and with pleiokorm of perennating underground stems; stem indumentum of stinging hairs with pluricellular base 2.0–3.0 mm long overall, setae 2.0–1.0 mm, and with simple trichomes ca. 0.1 mm long; leaf lamina ovate, 90–180 (–300) × 70–130 (–300) mm, leaf margin doubly dentate with 10–20 large pointed teeth on each side, 5–15 mm long, large teeth dentate with 1–3 small teeth, 1–2 mm long, leaf base subcordate to rounded, leaf apex apiculate; leaf indumentum of stinging hairs with pluricellular base 1.5–3.0 mm long overall, setae 1.0–2.5 mm, and with simple trichomes 0.1–0.5 mm long; petioles 30–100 (–250) mm long; 4 stipules, forming 2 fused pairs per node, 10–15 mm long, elongated to rounded; plant monoecious, mature fruits with tepals ca. 1.2 mm long, achenes subcircular in outline, laterally flattened, ca. 1.2 × 1.0 mm.

#### 5.5.3.1 *Urtica grandidentata* Miq. subsp. *grandidentata*. Fig. 5.3

Holotype:—[INDONESIA, Java] Wonosari, *F.W. Junghuhn, s.n.* (L_0356531!).

= *Urtica bullata* Blume (1856: 145). Holotype:—[INDONESIA, Java] *F.W. Junghuhn, s.n.* (L_0356531!)

= *Urtica bullata* Blume var. *contracta* Hochr. (1936: 20). Holotype: [INDONESIA, Java, 19 Jan. 1905] *Hochreutiner 2680* (L_1629158!)

- non Urtica grandidentata Moris, Stirp. Sard. Elench. Fasc. II: 9 (1828)

- *non Urtica grandidentata* Liebm. Vidensk. Selsk. Skr. V. ii. (1851) 296

Leaf lamina subcircular, 90–130 (–300) × 90–130 (–300) mm; lobes irregularly and coarsely dentate; petioles 40–100 (–250) mm long.

**Note:** —The most recent collection seen of this species is from 1938. It is likely restricted to montane forests on Java and may be on the brink of extinction.

**Distribution and Habitat:**—*U. grandidentata subsp. grandidentata* is restricted to Java and is usually found at intermediate elevations.

**Phenology:—***U. grandidentata subsp. grandidentata* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica grandidentata* Miq. subsp. *grandidentata* is considered "Least Concern" (LC).

**Representative specimens:—INDONESIA. Java:** [Klakah] *Zollinger 2539* (P_06456163!); 1835–1863, *Junghuhn 493* (L_1629143!); anno 1903–1905, *Hochreutiner 2680* (L_1629158!); *Collector unknown* (L_0356530!); Besoeki [nowadays: Besuki], 22 Jun. 1918, *Backer 25241* (L_1629141!); Besoeki [nowadays: Besuki], Westzijde Jang Plateu, Djentor-Sekassor 2100–2600 m, 14 Jul. 1938, *v. Steenis 10875* (L_1629140!); *Junghuhn 483* (K_000229282!); *Junghuhn s.n.* (L_1629150!); Klakah, *Zollinger 2539* (P_06456162!); *Leschenault 605* (P_06456164!); Near Ngadisari, 2200 m, 18 Oct. 1899, *S.H. Koorders 37901B* (K_000229283!); Near Ngadisari, 2200 m, 18 Oct. 1899, *S.H. Koorders 37901B* (L_1629145!); Near Ngadisari, 2200 m, 18 Oct. 1899, *S.H. Koorders s.n.* (L_1629144!); Without legible locality information, 20 Jan. 1915, *H.N. Ridley s.n.* (K_000229281!); Without legible locality information, 4 to 5 Jul. 1913, *Backe 8361* (L_1629142!); Without legible locality information, 6 Jun. 1927, *Backe 37519* (L_1629138!); Wonosari, *Junghuhn s.n.* (L_0356531!).

### 5.5.3.2 *Urtica grandidentata* Miq. subsp. *lombok* K.Becker & Weigend, *subspec. nov.*, Fig. 5.4

Holotype(here designated):—[INDONESIA] Oost Java**:** *J.H. Coert 1174* (L_1629139!)

Leaf lamina triangular-ovate, 100–180 × 70–130 mm; leaf lobes regularly dentate with 1–2 small teeth on each side, each ca. 1 mm long; petioles 30–50 mm long.

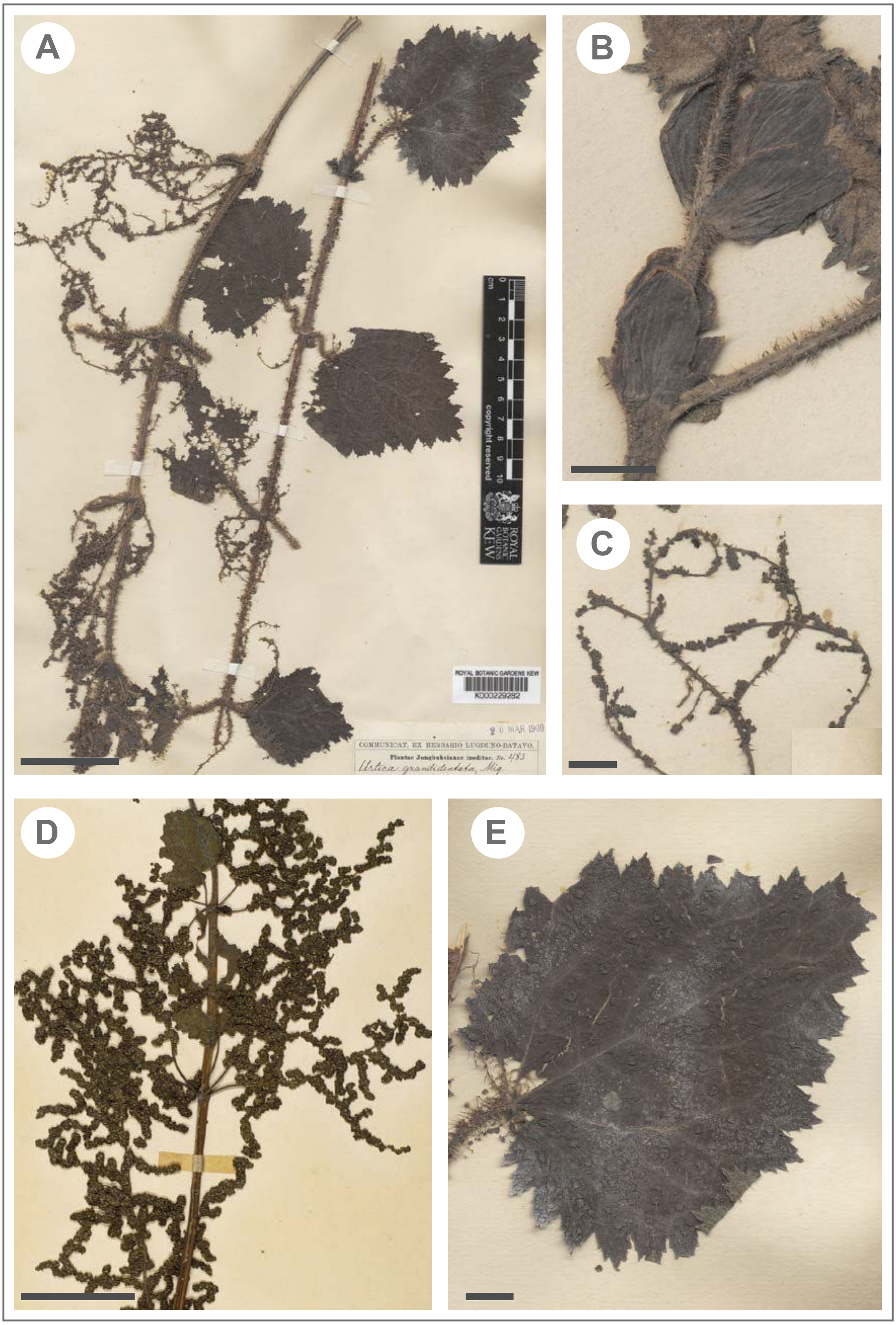

**Figure 5.3** *Urtica grandidentata* subsp. *grandidentata*: **A.** Habit (*Junghuhn, 483*), **B.** Node with fused stipules (*Koorders 37901B*), **C.** Female inflorescence (*Koorders 37901B*), **D.** Male inflorescence (*Junghuhn s.n.*), **E.** Typical leaf (*Junghuhn, 483*). Scale bar: A, D = 5 cm; B, D, E = 1 cm.

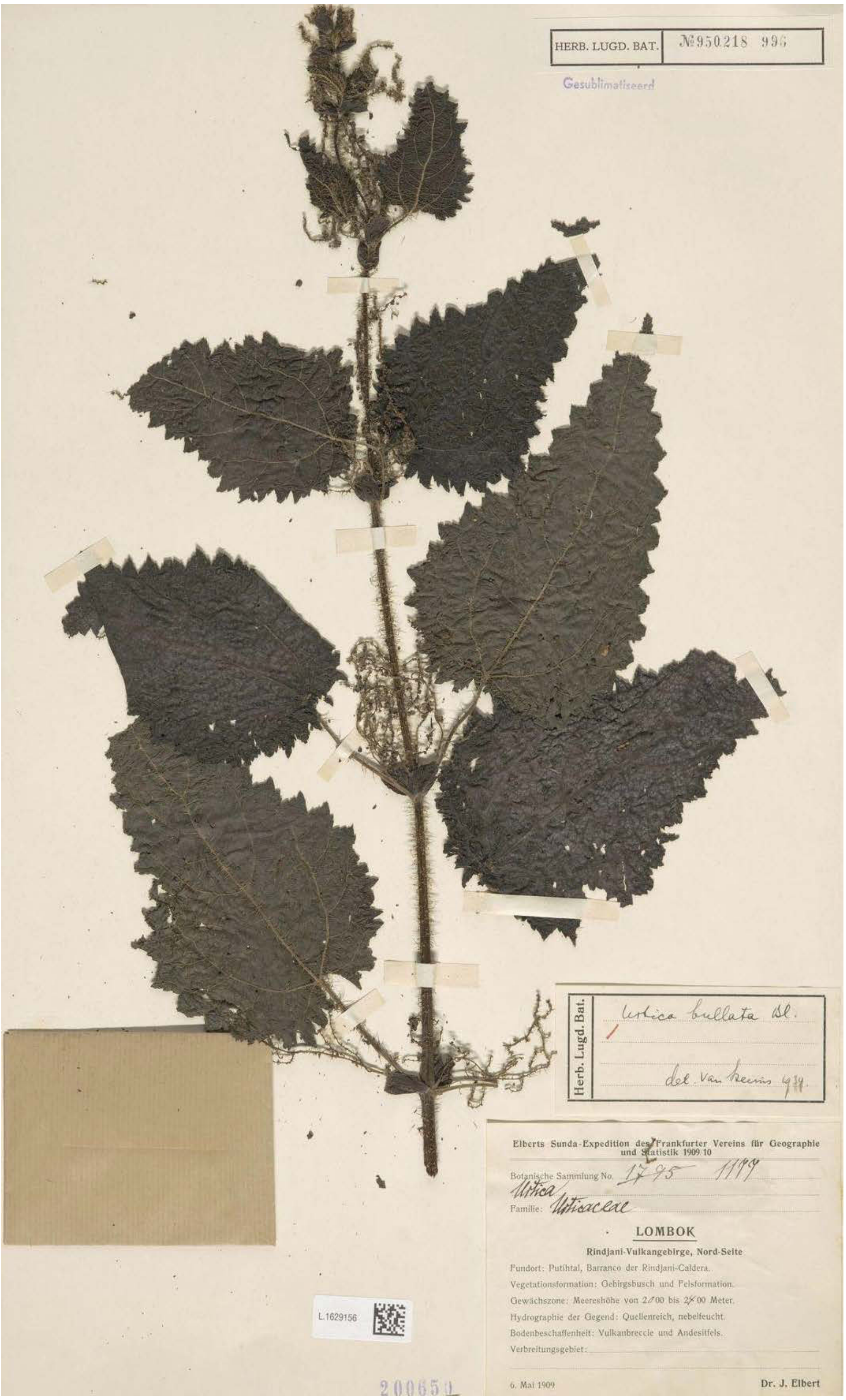

**Figure 5.4** Habit of *Urtica grandidentata* subsp. *lombok* (*Elbert 1177*).

**Note:**—This very poorly understood subspecies is currently differentiated by leaf type only – but the differences are very distinctive by the standards of *Urtica* systematics. Clearly more material is desirable of this taxon, which was last collected in 1909 and which is apparently restricted to montane forests.

**Distribution and Habitat:**—*U. grandidentata subsp. lombok* is restricted to Indonesia and is here reported from the islands of Java and Lombok. The subspecies is known from volcanic mountains at intermediate elevations and grows on moist soil.

**Phenology:—***U. grandidentata subsp. lombok* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica Urtica grandidentata* Miq. subsp. *lombok* K.Becker & Weigend is considered "Not Evaluated" (NE).

**Representative specimens:—INDONESIA. Java:** Oost-Java (Jawa Timur), *J.H. Coert 1174* (L_1629139!); **Lombok:** Rindjani volcanic mountains, north side, Putihtal, Barranco Rindjani-Caldera, 2100–2600 m, many springs, moist from the fog, 06 May 1909, *J. Elbert 1121* (L_1629157!); Rindjani volcanic mountains, north side, Putihtal, Barranco Rindjani-Caldera, 2000–2400 m, many springs, moist from the fog, 06 May 1909, *J. Elbert 1177* (L_1629156!).

### 5.5.4 *Urtica himalayensis* Kunth & C.D.Boché (1846: 11). Fig. 5.5

Holotype:—Himalaya, Oct. 1844, *A. Braun 13* (B100088731!).

= *Urtica mairei* Lévl. var. *oblongifolia* C.J. Chen (1983: 122). Holotype:—[CHINA] Yunnan: Gingtung: Ban-ma, 2200 m, on ditch side, 17 Jan. 1940, *M.K. Li 2985* (Y, isotype: PE-_00565202!). Paratypes:—CHINA. Guangxi: 13 Jan. / 3 Jun. 1940, *M.K. Li 2782* (KUN_0523743! & KUN_0523742!).

= *Urtica zayuensis* C.J. Chen (1983: 123). Holotype:—TIBET. Chayu, evergreen forest, 1500 m, 7.7.1980, *Z. Ni, Y. Wang, Ciduo, Cidan 0494* (PE-00509715!). Paratypes:—CHINA. Yunnan: A. *Henry 11197* (K000229275! & MO_3535025!); 11 Jul. 1973, *Qinghai-Xizang-Exped. 402* (not localized).

Erect, perennial herb ca. 1.5 m, form perennial root and with pleiokorm of perennating underground stems; stem indumentum of stinging hairs with pluricellular base 1.0–2.0 mm long overall, setae 0.8–1.5 mm, and with simple trichomes 0.1–0.2 mm long; leaf lamina subcircular to ovate-lanceolate, 5–150 (–230) × 35–100 (–160) mm, leaf margin doubly serrate with 30–55 (–70) large pointed teeth on each side, large teeth 1–2 mm long, each large tooth with 0–3 small teeth 0.5–1 mm long, leaf base subcordate to rounded, leaf apex apiculate; leaf indumentum of stinging hairs with pluricellular base 1.4–1.8 mm long overall, setae 1.0–1.6 mm, and with simple trichomes 0.1–0.5 mm long; leaf surface with punctiform cystoliths; petioles 10–60 (–150) mm long; 4 stipules, forming 2 fused pairs per node, 5–15 mm long, elongated to rounded in the upper part of the plant; plant monoecious, usually staminate flowers proximal, carpellate flowers distal; staminate flowers with all tepals ca. 1.0 mm long, pubescent; pistillate flowers with tepals ca. 0.2 mm long, pubescent; infructescence 40–80 mm.; mature fruits with longer tepals ca. 1.5 mm long, achenes subcircular in outline, laterally flattened, ca. 1.5 × 1.0 mm.

**Note:**—The type specimen for this species is from cultivation in Berlin Botanical Garden, it is unknown where the seed was obtained from. *Urtica mairei* var. *oblongifolia* is here synonymized with *U. himalayensis* since the isotype seen (*M.K. Li 2985*) clearly corresponds to this species. However, the paratype (*M.K. Li 2782*) has to be referred to *U. mairei*, indicating a certain degree of confusion in the description of this taxon The presence of *U. himalayensis* on Sumatra is somewhat surprising, since its distribution is otherwise exclusively Himalayan. Clearly, more and better material from Indonesia would be highly desirable. As now defined, the species ranges down to Sumatra and is replaced by *U. grandidentata* on Java. The latter differs by clearly lobed and much larger, subcircular leaves.

**Distribution and Habitat:**—*U. himalayensis* is widespread in the Himalayas and is reported from Yunnan (China), India (Sikkim, Punjab and Himachal Pradesh), Nepal and Tibet and with one additional specimen collected in Indonesia, from the highest peak of Sumatra. *U. himalayensis* is commonly found on roadsides, near streams, in the understory of forests growing in rich and moist soil, and is usually found at intermediate elevations.

**Phenology:—***U. himalayensis* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica himalayensis* Kunth & C.D.Boché is considered “Least Concern” (LC).

**Representative specimens:—CHINA. Yunnan:** Feng chen, 2133 m, *A. Henry 11197* (K_000229275!); Gingtung: Ban-ma, 2200 m, on ditch side, 17 Jan. 1940, *M.K. Li 2985* (PE_00565202!); Gongshan, Dulong River, Ba Slope, shrubland in a valley, 1300 m, 18 Nov. 1990, *Dulong River Expedition Team 512* (KUN_523797! & KUN_523798!); Gongshan, Dulong River, Meiqiewang, shrubland in a valley, 1620 m, 10 Jan. 1991, *Dulong River Expedition Team 1804* (KUN_523794! & KUN_523795!); Gongshan, Dulong River, secondary shrubland, 1500 m, 20 Dec. 1990, *Dulong River Expedition Team 1260* (KUN_523796! & PE_00509719!); Gongshan, Dulong River, secondary shrubland, 2000 m, 26 Mar. 1991, *Dulong River Expedition Team 5208* (KUN_523793! & PE_00509718!); Dulongjiang, Moquiwang Cun, ca 3.6 direkt km NW of Moquiwang in the vicinity of the bridge across the Moquiqang river on the road from Gonshang to Kongdang, W side of Gaoligong Shan, 2240 m, S facing 10–30° slope, 27° 54'38" N, 98° 24' 39.8" E, subtropical evergreen broadleaf forest dominated by *Lithocarpus* and *Pinus bhutanensis*, disturbed by clearing, 09 Jan. 2006, Gaoligong *Shan Biodiversity Survey 34461* (HUH_00285255!); Guangxi, Naop, shrubland in a valley, 1100 m, 09 Dec. 1958, *Z. Zhang 13088* (IBK_00130578!); Lushui, Yaojiaping, forest farm, roadside of theropencedrymion [forest type], 2300 m, 02 Aug. 1981, *Hengduan Mountain Team, Institute of Botany, The Academy of Science 379* (PE_00509713!); *A. Henry 11197* (MO_3535025!); –**INDIA. Himachal Pradesh:** Lahol [Lahaul], Kardong to Dartse in the Bhaga valley, 15 to 18 Jun. 1856, *Schlagintweit 2767* (PR!); Simla, 2133 m, 01 Sep. 1880, *Collett 590* (K_000229276!); Simla, Himalaya occ. 10 Oct. 1888, *Harmand s.n.* (P_06750057! & P_06750058!); **Punjab:** Bhogarmanly, 1524 m, *Barrett 21997* (K_000229287!); **Sikkim:** *no locality data*, 1524 m, *J.D. Hooker & Thompson 1855* (P_06855893!); 1828–2438 m, *J.D. Hooker & Thompson s.n.* (P_06855895!); 1828–2438 m, *J.D. Hooker & Thompson s.n.* (P_06855896! & P_06855895!); 2133 m, *J.D. Hooker & Thompson s.n.* (L_1629647!); India, *no province, Jacquemont 1247* (P_06855898!); *no collector, s.n.* (P_06855900!); –**INDONESIA. Sumatra:** Kerinchi, Sep. 1915, *Jacobson 2503* (L_1629137!);–**NEPAL. Janakpur:** Ramechhap, Choarma (2750 m) – Kyama (2600 m), 03 Aug. 1985, *H. Ohba, T. Kikuchi, M. Wakabayashi, M. Suzuki, N. Kurosaki, K.R. Rajbhandari & S.K. Wu 8571187* (E_00148746!);–**TIBET. Chayu:** evergreen forest, 1500 m, 07 Jul. 1980, *Z. Ni, Y. Wang, Ciduo, Cidan 0494* (PE_00509715!); hillside, grassland, 2400 m, 25 Jun. 1980, *Z. Ni, Y. Wang, Ciduo, Cidan 0185* (PE_00509714!)**;** Shangzay, humid valley, evergreen forest, 2200 m, 11 Jul. 1996, *Collector unknown* (PE_00509716! & PE_00509717!);–**Unidentified localities:** Himalayas, Oct. 1844, *Kunth s.n.* (B_100088731!); Kashmir [India, Pakistan or China]. 2133 m, *J.D. Hooker & Thompson s.n.* (P_06855897!).

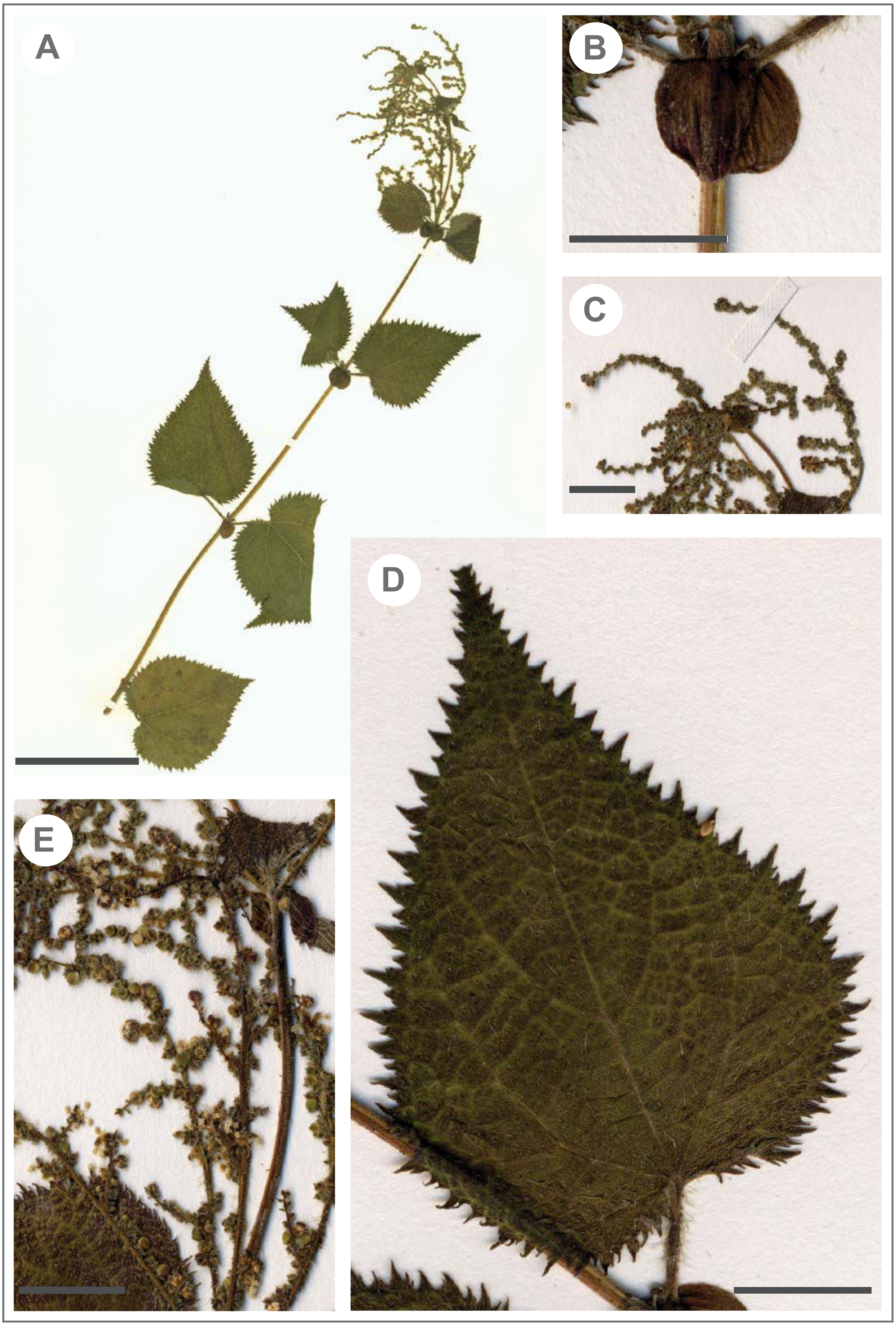

**Figure 5.5** *Urtica himalayensis* (all *Gaoligong Shan Biodiversity Survey 34461*): **A.** Habit, **B.** Node with fused stipules, **C.** Female inflorescences, **D.** Typical leaf, **E.** Infructescence and male flowers. Scale bar: A = 5 cm; B–E = 1 cm.

### 5.5.5 *Urtica mairei* Lév. (1913: 183). Figs. 5.6 and 5.7

Holotype:—[China. Yunnan] pied des murs-plaine de Tong-Tchouan, 2500 m, Sep. 1913, *E.E. Maire s.n.* (E_00275395!).

Paratypes:—[China, Yunnan] pied des murs-plaine de Tong-Tchouan, 2500 m, *E.E. Maire s.n.* (E_00275394! & E_00275396! & WSY_0092153!)

Erect, perennial herb ca. 1.5 m, form perennial root and with pleiokorm of perennating underground stems; stem indumentum of stinging hairs with pluricellular base 1.0–1.5 mm long overall, setae 0.8–1.0 mm, and with simple trichomes 0.1 mm long; leaf lamina subcircular, 70–120 × 50–90 mm, margin doubly dentate with 8–15 large teeth on each side, 2–10 mm long, large teeth irregularly and coarsely dentate with 2–4 small teeth on each side, small teeth 0.5–2 mm, leaf base subcordate to rounded, leaf apex apiculate; leaf indumentum of stinging hairs with pluricellular base 1.3–2.0 mm long overall, setae 1.1–1.5 mm, and with simple trichomes 0.1–0.2 mm long; leaf surface with punctiform cystoliths; petioles 40–90 mm long; 4 stipules, forming 2 fused pairs per node, 100–150 mm long, elongated; plant monoecious, usually staminate flowers basal, carpellate flowers apical; staminate flowers with all tepals 1.0 mm long, pubescent; pistillate flowers with tepals 0.2–0.5 mm long, pubescent; infructescence 30–90 mm; mature fruits with longer tepals 1.0–1.3 mm long, achenes subcircular in outline, laterally flattened, ca. 1.0–1.3 × 1.0 mm.

**Note:**—Amongst the numerous collections of *U. mairei* none was exactly identical to the type specimen, all o them differ in marginally smaller stipules (compare Figs. 5.6 and 5.7) and slightly less sharp teeth. However, we consider this part of the natural variation within one species.

**Distribution and Habitat:**—*U. mairei* is restricted to southern Asia and is here reported from Northeast-Vietnam, Taiwan, Tibet and the Chinese provinces Guangxi, Sichuan, and Yunnan. *U. mairei* is commonly found on roadsides, pastures, in the understory of forests growing in rich and moist soil, near streams, and is usually found at intermediate to high elevations.

**Phenology:**—*U. mairei* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica mairei* Lév. is considered "Least Concern" (LC).

**Representative specimens:—CHINA. Guangxi:** Longjin, in a valley, 560 m, 18 Nov. 1957, *Collector unknown* (IBK_00130570!)**;** Longjin, in a valley, roadside, 1000 m, 09 Nov. 1957, *Collector unknown* (IBK_00130572!)**;** Longlin, in a valley, slow slope, 1570 m, 12 Mar. 1956, *Collector unknown* (IBK_00130576!); **Sichuan:** Linggu, back mountain, hillside, in a groove under a tree, 50–80 m, 18 Jul. 1976, *Collector unknown* (CDBI_0017400! & CDBI_0017401!); Riverside, understory, 2600 m, 19 Aug. 1978, *Zhao, Yang, Kehua Nian 2600* (CDBI_0017432!, CDBI_0017433!, CDBI_0017434!); **Yunnan:** 21. Jul. 1898, *F. Ducloux 659* (P_06749810!); Dry shady situatios in pine and mixed forests on the eastern flank of the Lichiang Range, Lat. 27°20'N, 2743–3048 m, Aug. 1910, *G.Forrest 63931* (P_06855806!); 1934, *H.T. Tsai 57550* (HUH_00240989!); Central-Yunnan Wuding Sheshan, Pinus-Castanopsis mixed forest, 2000–2400 m, 25 Dec. 1984, *T. Deding 840035* (HUH_00278031! & HUH_00278030!); Chungtien, Haba, Altitude: 2600 m, Habitat: Margin of garden, 25 Nov. 1937, *T.T. Yü 14978* (HUH_00278006!); Gingtung A-Lo-De, 2200 m, on road side, 13 Jan. 1940, *M.K. Li 2782* (KUN_0523742! & KUN_0523743!); Jingdong, evergreen broad-leaf forest, 2300 m, 21 Oct. 1993, *Dai Peng 1408* (KUN_0523718!); Kunming, West Mountain, Sanqing Pavilion, 01 Jun. 1957, *W. Yin 881* (KUN_0523724!); Likiang city, open pasture, 23 Sep. 1939, *R.C. Ching 21696a* (HUH_00240990!); Luna, Stone Forest, 1800 m, 07 Sep. 1977, *S. Qiu s.n.* (CDBI_0017436!); Muli, Tongtian River, Riverside, sunny slope, 04 Oct. 1959, *Wu 3493* (CDBI_0017435!); Plain de Tong-Tchouan,2500 m, Aug. 1913, *E.E. Maire s.n.* (P_06750044!); Plain de Tong-Tchouan, 2500 m, Oct. 1913, *E.E. Maire s.n.* (P_06822139!); Plain de Tong-Tchouan, beneath walls, 2500 m, Sep. 1913, *E.E. Maire 1184* (E_00275395!, E_00275394!, E_00275396!); Wei-si Hsien, Yeh-Chih, Altidude: 3200 m, Habitat: Mountain slope, Aug. 1935, *C.W. Wang 68073* (HUH_00278027!);—**TAIWAN.** 1500m, Dec. 1914, *M. Faurie 1511* (HUH! & P_06855803!); Mt. Taiha, Izawayamasitn, 04 Aug. 1934, *Suzuki s.n.* (PE_00509586!); Nokosan (Mt. Noko), between Noko Police Station (alt. 9437 ft.) and the Prefectural Boundary, 10,200 ft., west of the divide, 05 Oct. 1926, *H.H. Bartlett 6235* (HUH!);—**TIBET. Kongbo:** near Tripe (SE Tibet), roadside climbing out of Tripe towards Gyala, Shady cleft in roadside cliff by track, Near 29 36 54.4 N 94 56 26.8 E, 3000 m, 08 Oct. 1997, cultivated specimens collected in University of Liverpool Botanic Gardens, Ness, *K. Rushforth & H. McAllister 5247* (BONN!); **Nacuo**, in the forest, 2750 m, 26 Aug. 1975, *Tibet Team 751685* (KUN_0523739!, KUN_0523740!);—**VIETNAM.** 17 Sep. 1888, *Anon. 3251* (P_06855805!); **Northeast:** Tonkin, 06 Jul. 1909, *C. Alleizette s.n.* (L_1629818!).

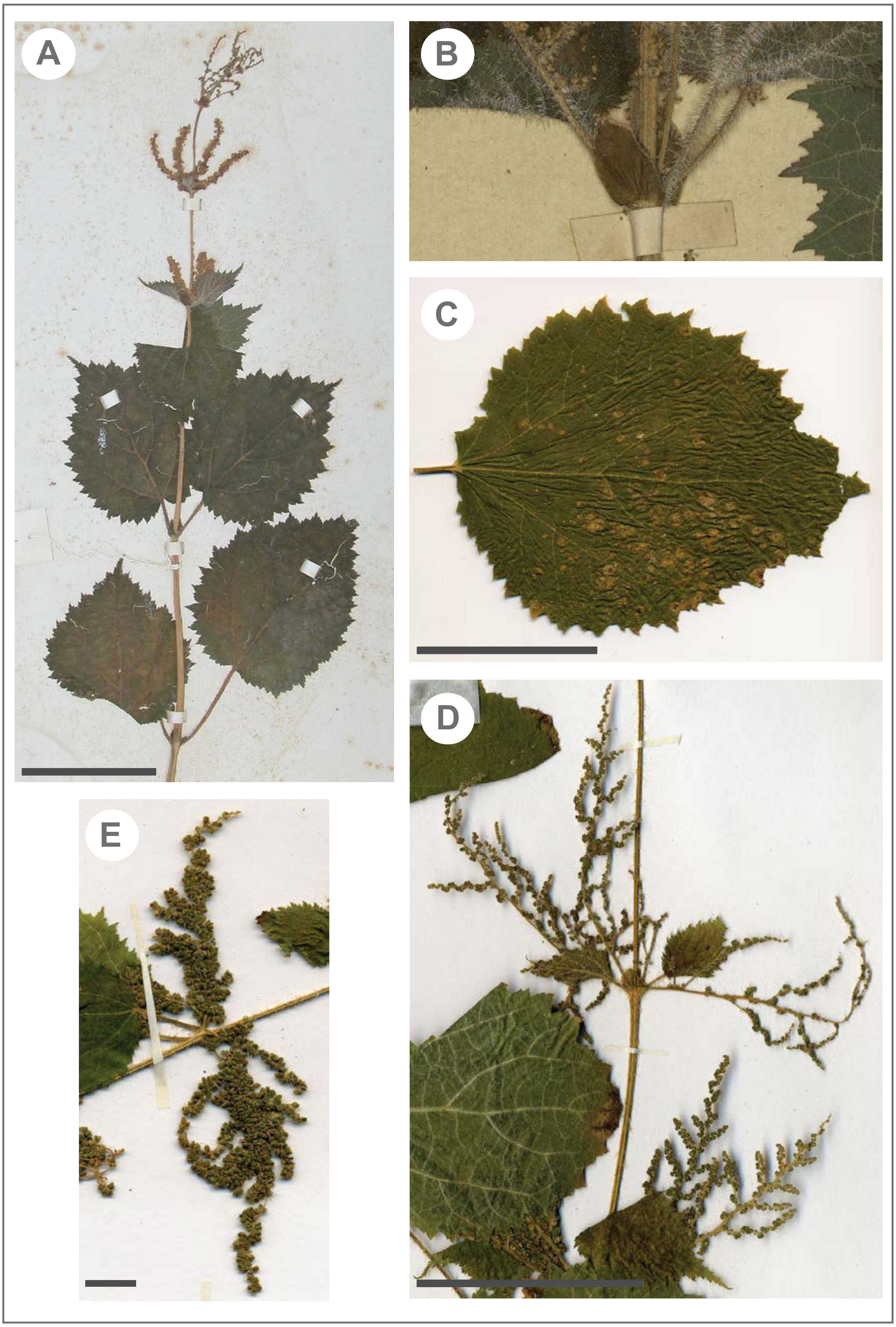

**Figure 5.6** *Urtica mairei*: **A.** Habit (*Qiu s.n.*), **B.** Node with fused stipules (*Ducloux 659*), **C.** Typical leaf *(Rushforth & McAllister 5247)*, **D.** Male inflorescence *(Rushforth & McAllister 5247)*, **E.** Infructescence (*Rushforth & McAllister 5247*). Scale bar: A, C, D = 5 cm; B, E = 1 cm.

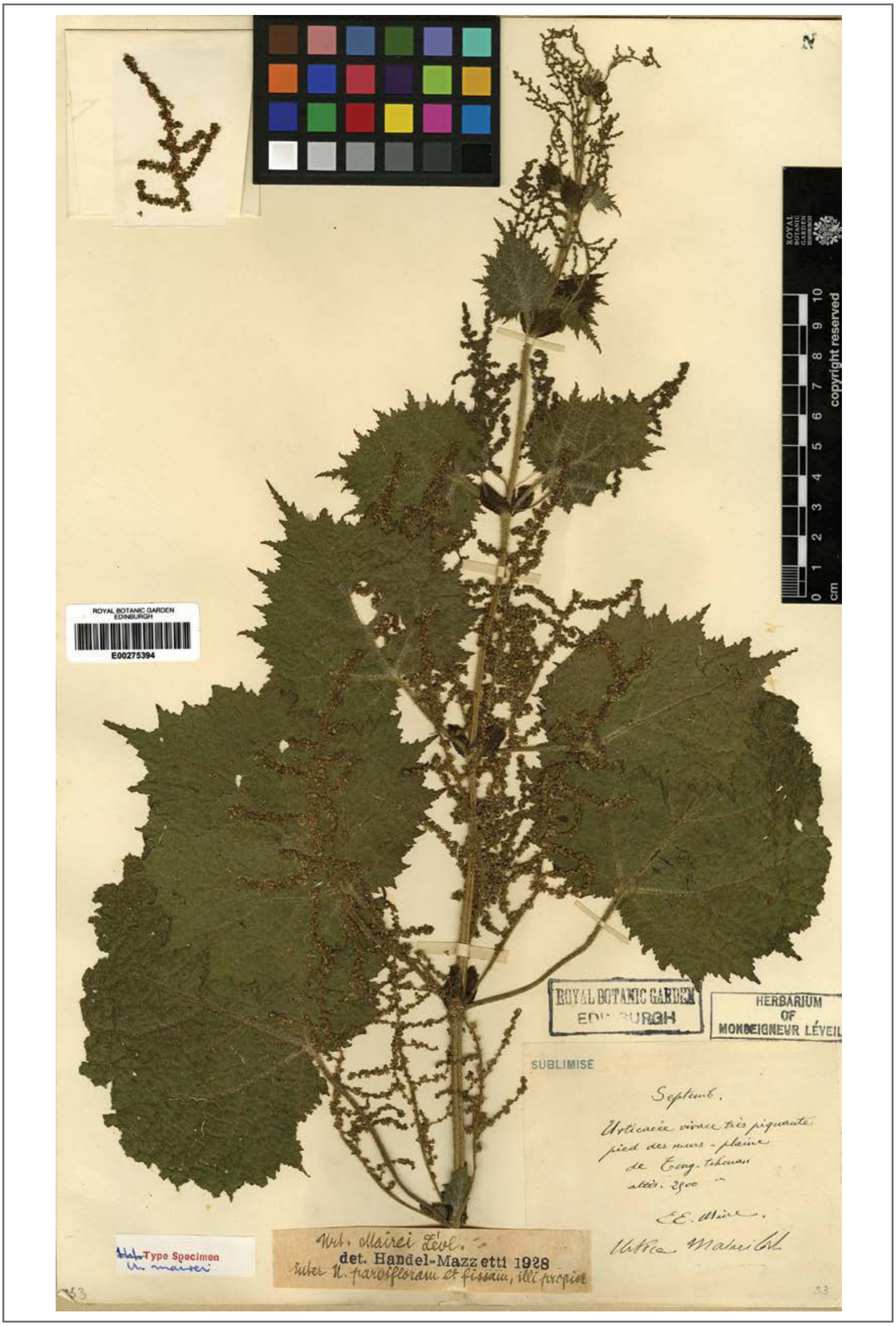

Figure 5.7 Habit of a type specimen of *Urtica mairei* (*Maire s.n., E_00275394*).

### 5.5.6 *Urtica parviflora* Roxb. (1814: 67). Fig. 5.8

Holotype:—INDIA. Uttar Pradesh: Rohilcund [=Rohilkhand], 1803, *A. Gott s.n.* (not localized).

Iconotype:—R. [Roxburgh] No. 1909.

Epitype (here designated):—INDIA. Himachal Pradesh: Upper Dharamsala, ca. 2000 m, 25 Mar. 2013, source collection by *H. Binski* 25 March 2013, cultivated in the Botanical Gardens of the University of Bonn, *B. Große-Veldmann et al. 136-C* (BONN!, isoepitypes in B!, HUH!, K!, MO!)

= *Urtica ardens* Link (1822: 385). *Type not named*. Neotype (here designated): NEPAL. Bagmati: Along side of road below Royal Drug Research Laboratory, Godavari, Kathmandu Valley, 28 June 1987, *W. Codon & A. Codon* (MO_4320803!).

= *Urtica virulenta* Wall. (1831: 4586). Holotype:—Nepal. 1821, *Wallich 4586* (K_001039385!)

Erect, perennial herb 0.7–1.5 m, form perennial root and with pleiokorm of perennating underground stems; stem indumentum of stinging hairs with pluricellular base 3.0–4.5 mm long overall, setae 1.8–3.5 mm, and with simple trichomes ca. 0.1 mm long; indumentum is ontogenetically variable: vegetative basal part of the plant with conspicuously more and shorter stinging hairs than the generative part; leaf lamina ovate-lanceolate, 50–80 × 35–15 mm, leaf margin dentate with 15–30 teeth on each side, teeth 1–3 mm long, occasionally some teeth doubly dentate with small teeth ca. 0.5–1 mm long, leaf base rounded, leaf apex aristate; leaf indumentum of stinging hairs with pluricellular base 2.0–3.5 mm long overall, setae 1.6–3.0 mm, and with simple trichomes 0.1–0.2 mm long; leaf surface with punctiform cystoliths; petioles 20–60 mm long; 4 stipules, forming 2 fused pairs per node, occasionally incised distally, 5–10 mm long, elongated; plant monoecious, usually staminate flowers basal, carpellate flowers apical; staminate flowers with tepals ca. 1.0 mm long, pubescent; pistillate flowers with tepals ca. 0.2 mm long, pubescent; infructescence 50–100 mm; mature fruits with longer tepals 1.2–1.5 mm long, achenes subcircular in outline, laterally flattened, ca. 1.2 × 1.5 mm.

**Note:**—*Urtica ardens* and *U. virulenta* are here treated as synonyms of *U. parviflora*, this is clear from the type material (in *U. virulenta*) respectively the description (in *U. ardens*). The taxon considered as *U. ardens* in Chen et al. (2003) therefore has to be correctly called *U. himalayensis*.

**Distribution and Habitat:**—*U. parviflora* is restricted to the Himalayas and is here reported from Nepal, the Indian provinces Sikkim, Uttar Pradesh and Himachal Pradesh,

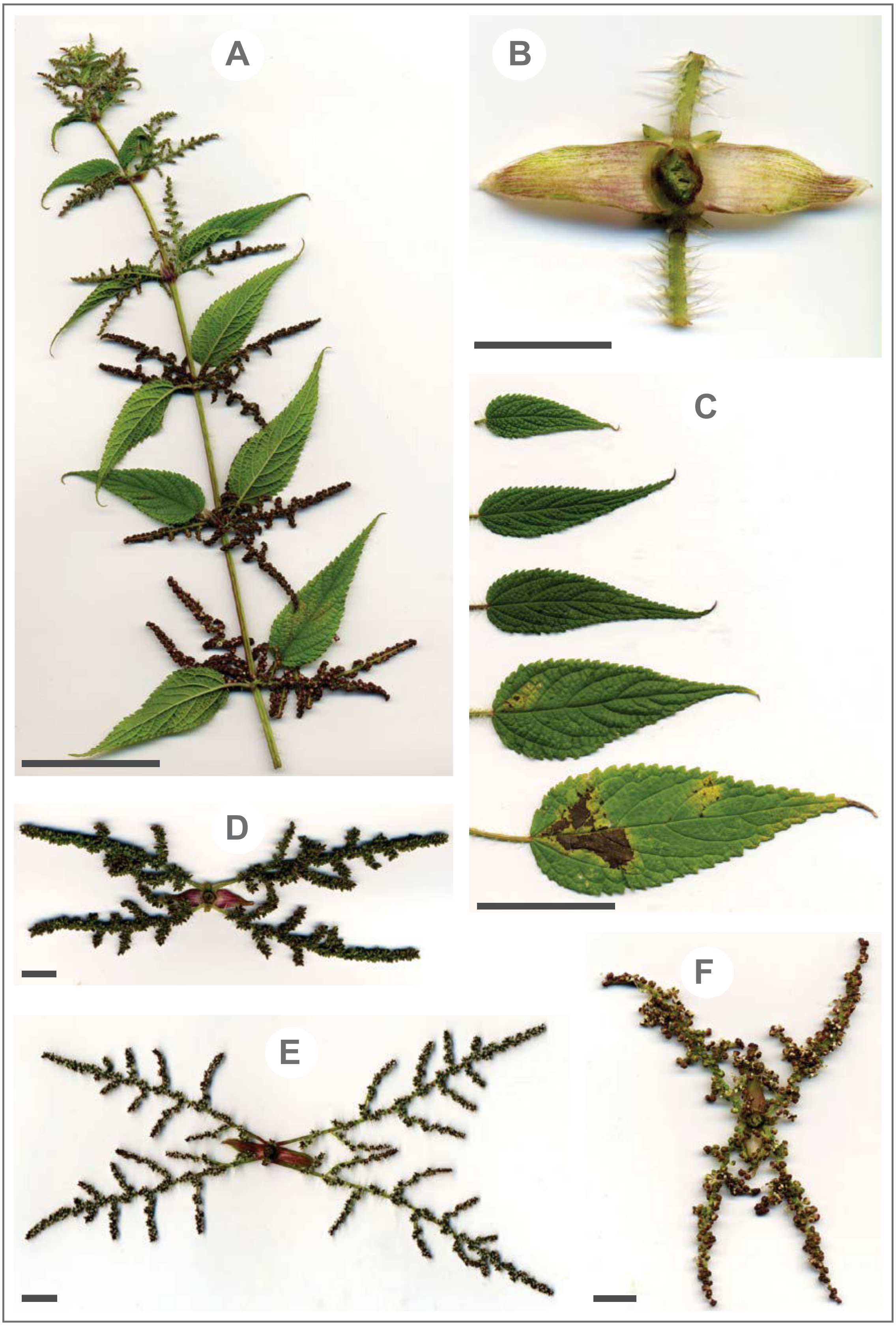

**Figure 5.8** *Urtica parviflora* (all *B. Große-Veldmann et al. 136-C*): **A.** Habit, **B.** Node with fused stipules, **C.** Typical leaves, **D.** Infructescence, **E.** Female inflorescence, **F.** Male inflorescence. Scale bar: A, C = 5 cm; B, D–F = 1 cm.

Tibet and Kashmir (nowadays parts of northern India, eastern Pakistan and western China). *U. parviflora* is found on roadsides and in the understory of forests at intermediate elevations.

**Phenology:—***U. parviflora* flowers throughout the year.

**Conservation status:**—Using IUCN criteria (IUCN 2001), *Urtica parviflora* Roxb. is considered "Least Concern" (LC).

**Representative specimens:—INDIA. Sikkim**: 1855, *J.D. Hooker s.n.* (P_06855887!);—**KASHMIR [nowadays parts from India, Pakistan & China]. Jammu and Kashmir [India]:** Drained lake basin of Kashmir, environs of Srinagger, within a circle of 8 miles radius, 10 Aug. to 30 Sep. 1856, *Schlagintweit 4367* (P_06855889!);—**NEPAL.** *Wallich 4586* (K_001039385!); **Bagmati:** Along side of road below Royal Drug Research Laboratory, Godavari, Kathmandu Valley, 28 June 1987, *W. Codon & A. Codon* (MO_4320803!);—**TIBET. Nyalam:** Nepal friendship bridge, 18 Aug. 1992, *J. Chen s.n.* (PE_00565204! & PE_00565205!);—**Unidentified localities:** *Collector unknown* (P_06855892!)

## 5.6 Acknowledgements

We are grateful to the gardeners of the Bonn Botanical Gardens, especially J. Manner and his colleagues for maintaining the *Urtica* living collection. We are particularly grateful for some valuable recent collections facilitated by K. Rushforth and H. McAllister from Liverpool and by David Boufford from Harvard University.

# CHAPTER 6

## Pulling the sting out of nettle systematics–A comprehensive phylogeny of the genus *Urtica* L. (Urticaceae)*

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### Abstract

The genus *Urtica* L. is subcosmopolitan, found on all continents (except Antarctica) and most extratropical islands and ranges from Alaska to Patagonia, Spitzbergen to the Cape and Camtschatka to the subantarctic islands. However, throughout its geographical range morphologically nearly indistinguishable species are found alongside morphologically quite disparate species, with the overall diversity of morphological characters extremely limited. The systematics of *Urtica* have puzzled scientists for the past 200 years and no single comprehensive attempt at understanding infrageneric relationships has been published in the past, nor are species delimitations unequivocally established. We here provide the first comprehensive phylogeny of the genus including 61 of the 63 species recognized, represented by 144 ingroup accessions and 14 outgroup taxa. The markers ITS1–5.8S–ITS2, psbA–trnH intergenic spacer, trnL–trnF and trnS–trnG are used. The phylogeny is well resolved. The eastern Asian *Zhengyia shennongensis* T. Deng, D.G. Zhang & H. Sun is retrieved as sister to *Urtica*. Within *Urtica*, a clade comprising the western Eurasian species *U. pilulifera* L. and *U. neubaueri* Chrtek is sister to all other species of the genus. The phylogenetic analyses retrieve

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numerous well-supported clades, suggesting previously unsuspected relationships and implying that classically used taxonomic characters such as leaf morphology and growth habit are highly homoplasious. Species delimitation is problematical, and several accessions assigned to *Urtica dioica* L. (as subspecies) are retrieved in widely different places in the phylogeny. The genus seems to have undergone numerous dispersal-establishment events both between continents and onto different islands. Three recent species radiations are inferred, one in America centered in the Andes, one in New Zealand, and one in northern Eurasia which includes *Urtica dioica s.str.* sensu Henning et al. (2014). The present study provides the basis of a critical re-examination of species limits and taxonomy, but also of the dispersal ecology of this widespread plant group and an in-depth study of the three clades with recent radiations.

*Keywords:* Chloroplast markers, Nuclear marker, Phylogeny, Stinging nettle, *Urtica*, Urticaceae

## 6.1 Introduction

The genus *Urtica* (Urticaceae) is of subcosmopolitan distribution and its taxonomy has been incompletely understood (Friis, 1993). Over most of its range *Urtica* is frequently an extremely common genus, often found in anthropogenic habitats and extensively used by humans for food and other purposes, e.g., as medicinal plants, for fiber production, as animal food, for phytoremediation, etc. (Bogachkov and Morozov, 1990; Dreyer, 1999; Garber, 1950; Kavalali, 2003; Khan and Joergensen, 2006; Wetherilt, 1992). *Urtica* is commonly found as a weed, but can occupy a range of natural habitats and is found in montane forests in Macaronesia, East Africa and in the Andes, but also in High Andean paramo and puna habitats at over 4500 m a.s.l. (Weigend et al., 2005). Many species are relatively widespread – e.g. the South American species *U. magellanica* Juss. ex Poir. (38–54° S) and *U. leptophylla* Kunth (6–11° N), other taxa are very narrowly endemic in the same overall region (*U. lalibertadensis* Weigend, *U. peruviana* Geltman, *U. urentivelutina* Weigend; Mutke et al., 2014).

In spite of its abundance and the visibility of the genus, its systematics and taxonomy remain problematic. Estimates of the number of *Urtica* species range from 30 to 80 (e.g. Cheeseman, 1925; Chrtek, 1969/79; Friis, 1993; Geltman, 1998; Juárez, 1991; Weddell, 1856, 1869). This divergence is primarily caused by a scarcity of taxonomically useful characters in this small-flowered, wind-pollinated genus with fairly homogeneous fruit morphology in combination with remarkable phenotypic plasticity (Figs. 6.1 and 6.2). This problem is nicely demonstrated by the fact that "*U. dioica*" has been reported from most parts of the range of the genus and over 150 infraspecific names have been applied to local and seasonal forms from New Zealand to Kamchatka, and from Alaska to Cape Horn (Weigend, 2005). Several recent taxonomic studies, some using phylogenetic analyses of DNA sequence data, resolved a range of taxonomic problems, especially in European and American *Urtica* (Grosse-Veldmann et al., 2016; Henning et al., 2014; Weigend, 2005, 2006; Weigend et al., 2005; Weigend and Luebert, 2009; Weigend and Monro, 2015). Most importantly, Henning et al. (2014) showed that *Urtica dioica* is virtually restricted to (mostly northern) Eurasia, with outliers in northern Africa, South Africa and Iran, and most specimens assigned to that species from China, South America and New Zealand belong to different species. Figs. 6.1 and 6.2 illustrate a range of taxa from *Urtica*, showing the limited range of morphological diversity in the genus. An early attempt at subdividing the genus into natural units was undertaken by Weddell (1856, 1869), based on gender distribution (see Appendix A1). He recognized 51 species (1856) then later 37 species (1869) and several infraspecific taxa, including "varieties" and "forms". Some of the taxa he recognized are still valid (e.g. Deng et al., 2013; Farag et al., 2013; Henning et al., 2014; Kim et al., 2015; Wu et al., 2013), some of these were assigned to another rank, but the bulk have been synonymised later. After the two classical revisions by Weddell, there has been little systematic progress overall. Chrtek (1969/79), Geltman (1982) and Chen (1983), Table 6.1, proposed classifications including only some of the currently recognized species in their schemes. Chrtek (1969/79) and Geltman (1982) both assigned 11 species to explicit infrageneric units, corresponding to ca 12–30% of the overall species numbers, depending on the number of taxa recognized. Chrtek (1969/79) defined three subgenera, whereas Geltman (1982) defined two subgenera, which he again subdivided into sections and subsections and which are mostly monotypical.

In recent years, several phylogenetic studies including representatives of the genus *Urtica* were published (Deng et al., 2013; Kim et al., 2015; Wu et al., 2013), sampling ca 10–12 species of *Urtica* each. They provide some insights into *Urtica*-phylogeny, but allow few general conclusions, due to limited sampling. Farag et al. (2013) and especially Henning et al. (2014) provide a much broader sampling resolving the backbone of the genus fairly well. The analyses in these two phylogenetic studies recovered several clades in a highly stratified phylogeny. The analyses of Henning et al. (2014) and Farag et al. (2013) thus provide a highly resolved framework for understanding *Urtica* phylogeny, but exclude the bulk of the narrowly endemic species. The present study addresses the overall phylogeny of *Urtica* based on comprehensive sampling of the species and subspecific entities in the genus. We place special emphasis on sampling the numerous local and regional endemics in the genus, e.g., from Macaronesia (*U. stachyoides* Webb & Berthel., *U. portosanctana* Press, *U. morifolia* Poir), Corsica and Sardinia (*U. atrovirens* Req. ex Loisel.), Cyprus (*U. dioica* L. subsp. *cypria* H. Lindb.), Hispaniola (*U. domingensis* Urb.), Sicily (*U. rupestris* Guss.), Indonesia (*U. grandidentata* Miq.), Mallorca (*U. bianorii* (Knoche) Paiva), Papua New Guinea (*U. papuana* Zandee), New Zealand and the subantarctic islands (*U. perconfusa* Grosse-Veldmann & Weigend), Juan Fernández Islands (*U. glomerulaeflora* Steud.), Syria (*U. fragilis* J. Thiébaut) and Taiwan (*U. taiwaniana* S.S. Ying).

**Figure 6.1** Leaf and inflorescence morphology of selected species of *Urtica* from the *U. pilulifera* clade and clades I and IIIa and IIIb (from cultivation, vouchers at BONN unless otherwise indicated). A, *U. ferox* (New Zealand, voucher M. Weigend 9213 – B); B, *U. pilulifera* (M. Weigend 8153); C, *U. membranacea* (Spain, Mallorca, voucher M. Weigend 8154); D, *U. urens* (N.M. Nürk 287); E, *U. cannabina* (Mongolia, voucher M. Weigend 8118); F, *U. portosanctana* (Spain, Teneriffe, voucher M. Weigend 8234); G, *U. fragilis* (Turkey, voucher B. Tarikahya & B. Özüdogru 2410); H, *U. morifolia* (Spain, Tenerife, voucher M. & K. Weigend 8240); I, U. *circularis* (Brazil, voucher, M. Weigend 9311); J, *U. flabellata* (Peru, M. Weigend 9111). Photos: M. Weigend, N.M. Nürk.

**Figure 6.2** Leaf and inflorescence morphology of selected species of *Urtica* from the clades IIIb, IIIc, IV, V and VI (from cultivation, vouchers at BONN unless otherwise indicated). A, *U. gracilis* subsp. *gracilis* (USA, California, voucher A. Liston 1221); B, *U. leptophylla* (Peru, voucher M. Weigend 7763); C, *U. fissa* (Japan, voucher M. Weigend 8129); D, *U. ardens* (China, voucher M. Weigend 8684-4 – B); E, *U. echinata* (Peru, voucher M. Weigend 7706); F, *U. platyphylla* (Japan, voucher T. Azuma s.n.); G, *U. atrovirens* (Italy, Sardinia, voucher M. Weigend 7800); H, *U. bianorii* (Spain, Mallorca, voucher M. & K. Weigend 8155); I, *U. dioica* subsp. *cypria* (Cyprus, voucher M. Weigend 8229); J, *U. dioica* subsp. *dioica* var. *holosericea* (Germany, voucher M. & K. Weigend 8100). Photos: M. Weigend, N.M. Nürk.

**Table 6.1** Conspectus of previous classifications of the genus *Urtica* L. [1]= *U. leptophylla* Kunth, [2]= *U. dioica* subsp. *pubescens* (Ledeb.) Domin, [3]= *U. dioica* subsp. *sondenii* (Simmons) Hyl., [4]= *U. dioica* L. subsp. *dioica*, [5]=*U. laetevirens* Maxim subsp. *laetevirens*, [6]=*U. dioica* L. subsp. *afghanica* Chrtek, [7]=*U. thunbergiana* Siebold & Zucc., [8]=*U. ardens* Link.

| Chrtek (1969/79) | Geltman (1982) | Chen (1983) |
|---|---|---|
| SUBGENUS *URTICA* | SUBGENUS 1. *URTICA* | 1. SECTIO *URTICA* |
| ***U. dioica*** L. | SECTIO 1. *URTICA* | 1. SERIES *URENTES* C.J.CHEN |
| ***U. urens*** L. | SUBSECTIO 1. *URTICA* | 1. ***U. atrichocaulis*** (Hand.-Mazz.) C.J.Chen |
| ***U. cannabina*** L. | 1. ***U. dioica*** L. | 2. ***U. urens*** L. |
| ***U. magellanica*** Poir. | 2. *U. galeopsifolia* Wierzb. ex Opiz[2] | 2. SERIES *DIOICAE* C.J.CHEN |
| ***U. stachyoides*** Webb & Berthel. | 3. ***U. pubescens*** Ledeb.[2] | 3. ***U. triangularis*** Hand.-Mazz. |
| ***U. neubaueri*** Chrtek | 4. ***U. sondenii*** (Simmons) Avrorin[3] | 3a. ***U. triangularis*** Hand.-Mazz. subsp. ***triangularis*** |
| *U. ballotaefolia* Wedd.[1] | 5. ***U. angustifolia*** Fisch. ex Hornem. | 3b. ***U. triangularis*** Hand.-Mazz. subsp. ***pinnatifida*** (Hand.-Mazz.) C.J.Chen |
| SUBGENUS *SARCOURTICA* CHRTEK | 6. ***U. platyphylla*** Wedd. | 3c. ***U. triangularis*** Hand.-Mazz. subsp. ***trichocarpa*** C.J.Chen |
|  | SUBSECTIO 2. *KIOVIENSES* GELT. | 4. ***U. cannabina*** L. |
| ***U. pilulifera*** L. | 7. ***U. kioviensis*** Rogow. | 5. *U. tibetica* W.T.Wang ex C.J.Chen[4] |
| (*U. dodartii* L.) | SUBSECTIO 3. *CANNABINAE* GELT. | 6. ***U. hyperborea*** Jacquem. ex Wedd. |
| SUBGENUS *DENDROURTICA* CHRTEK | 8. ***U. cannabina*** L. | 7. ***U. laetevirens*** Maxim. |
|  | SECTIO 2. *LAETEVIRENTES* GELT. | 7a. ***U. laetevirens*** Maxim. subsp. ***laetevirens*** |
| ***U. morifolia*** Poir. | 9. ***U. laetevirens*** Maxim. | 7b. *U. laetevirens* Maxim. subsp. *cyanescens* (Kom.) C.J.Chen |
| ***U. rupestris*** Guss. | SECTIO 3. *URENTES* GELT. | 7c. ***U. laetevirens*** Maxim. subsp. ***dentata*** (Hand.-Mazz.) C.J.Chen[5] |
|  | 10. ***U. urens*** L. | 8. ***U. angustifolia*** Fisch. ex Hornem. |
|  | SUBGENUS 2. *SARCOURTICA* CHRTEK | 9. *U. dioica* L. |
|  | 11. ***U. pilulifera*** L. | 9a. ***U. dioica*** L. subsp. ***dioica*** |
|  |  | 9b. ***U. dioica*** L. subsp. ***afghanica*** Chrtek |
|  |  | 9c. *U. dioica* L. subsp. *xingjiangensis* C.J.Chen[6] |
|  |  | 9d. ***U. dioica*** L. subsp. ***gansuensis*** C.J.Chen |
|  |  | 2. SECTIO *ARDENTIA* C.J.CHEN |
|  |  | 10. *U. membranifolia* C.J.Chen |
|  |  | 11. *U. macrorrhiza* Hand.-Mazz.[7] |
|  |  | 12. ***U. thunbergiana*** Siebold & Zucc. |
|  |  | 13. ***U. fissa*** E.Pritz. ex Diels |
|  |  | 14. ***U. mairei*** H.Lév. |
|  |  | 14a. ***U. mairei*** H.Lév. var. ***mairei*** |
|  |  | 14b. *U. mairei* H.Lév. var. *oblongifolia* C.J.Chen[8] |
|  |  | 15. *U. zayuensis* C.J.Chen[7] |
|  |  | 16. ***U. ardens*** Link |

## 6.2 Material and methods

### 6.2.1 Plant material and taxon sampling

Several extensive field trips to various parts of the globe and the cultivation of a wide range of taxa provided much of the plant material used for the present study. Plant material from the field and cultivation was silica-dried. The sampling was further complemented by plant material provided by colleagues and several large herbarium loans from different herbaria (see Acknowledgements). All plant material included in our analysis was checked for correct determination by both comparing it to original protologues and type specimens and by identifying it with the current floras (e.g. Flora of China, Chen et al., 2003), wherever possible. The European varietal names of *U. dioica* are based on the circumscriptions in Grosse-Veldmann and Weigend (2015). The identifications and sampling provided here are the result of 15 years of critical taxonomic work, study of type specimens, correspondence with colleagues worldwide, processing of herbarium loans and our own collections in a range of countries on three continents.

The sampling from Farag et al. (2013) and Henning et al. (2014) was significantly expanded resulting in a nearly complete set of *Urtica* species. Widely distributed species were sampled from multiple parts of their range wherever possible. We currently recognize 63 species based on the studies of Farag et al. (2013), Grosse-Veldmann and Weigend (2015), Grosse-Veldmann et al. (2016), Henning et al. (2014), Weigend (2005, 2006), Weigend et al. (2005), Weigend and Luebert (2009). In total, 158 individuals were sampled for the phylogenetic analyses including 144 ingroup taxa in 61 *Urtica* species (see Appendix A2). Overall, we obtained near-comprehensive sampling with 61 of the 63 species we recognize, but were ultimately unable to include two doubtful taxa, namely *Hesperocnide sandwicensis* Wedd. from Hawaii (probably synonymous to *H. tenella* Torr. from California) and *U. lilloi* (Hauman) Geltman from Argentina (probably synonymous to *U. leptophylla*). 14 outgroup taxa from the Urticaceae are included (representatives of *Hesperocnide* Torr., *Laportea* Gaudich., *Nanocnide* Blume, *Obetia* Gaudich., *Urera* Gaudich. as well as the recently described *Zhengyia shennongensis* from central China, compare Hadiah et al., 2008; Deng et al., 2013, and Wu et al., 2013). Sequences of *Zhengyia shennongensis* were downloaded from GenBank.

A complete list of the plant material used in this study including voucher information and GenBank accession numbers is provided in Appendix A2.

### 6.2.2 DNA extraction, amplification, and sequencing

DNA extraction was possible from most samples, including several collections from the 19th century (see Appendix A2). DNA extraction, amplification, purification, and sequencing followed standard protocols as described in Gottschling and Hilger (2001) and Weigend et al. (2010). Samples were sequenced for four genomic regions: the nuclear ribosomal ITS1–5.8S–ITS2 (henceforth ITS), and three plastid regions: the *psb*A–*trn*H intergenic spacer (IGS), *trn*L–*trn*F (including the trnL group I intron and the *trn*L–*trn*F IGS), and *trn*S–*trn*G (including the *trn*S–*trn*G IGS and the *trn*G group II intron). The same primers were used for amplification and for sequencing. The primers used were ITS5 and ITS4 for ITS (White et al., 1990), *psb*AF and *trn*HR for *psb*A–*trn*H (Sang et al., 1997), C and F for *trn*L–*trn*F (Taberlet et al., 1991) and *trn*_S(GCU) and *trn*_G(UCC) for *trn*S–*trn*G (Hamilton, 1999). Amplicons were sequenced by either Macrogen Inc., South Korea (http://www.macrogen.com), GATC Biotech AG, Konstanz, Germany (http://www.gatc-biotech.com), or Massey Genome Service, New Zealand. All sequences generated in this study have been submitted to the GenBank genetic sequence database (see Appendix A2 for accession numbers).

### 6.2.3 Alignment and phylogenetic analyses

The alignment was conducted using the MAFFT algorithm in the Geneious software package ver. R8 with default settings, followed by manual adjustments using PhyDE® ver. 1 (Müller et al., 2005) in order to build a motif alignment, based on the criteria laid out in Kelchner (2000). The combined data set (ITS, *trn*S–*trn*G, *psb*A–*trn*H and *trn*L–*trn*F) contains 3826 aligned positions (ITS: 734, *trn*S–*trn*G: 1159, *psb*A–*trn*H: 811, *trn*L–*trn*F: 1122).

The data set contains two hairpin associated inversions, one situated in P8 of the *trn*L intron (compare Borsch et al., 2003) and the second approximately 90 nt upstream of the *trn*F gene. Both inversions were positionally isolated in the alignment and included as reverse complement in the nexus files used for phylogenetic analyses (see Borsch and Quandt, 2009; Quandt et al., 2003). Each data set was analyzed independently to identify potential incongruences. No hard incongruences between the separate plastid and ITS trees were observed (data available in the online version). Phylogenetic analyses were conducted on a concatenated dataset employing maximum likelihood (ML) and Bayesian inference (BI).

ML analyses were conducted with the standard settings in RAxML Version 8 (Stamatakis, 2006; Stamatakis et al., 2008). The node support under ML is based on 1,000 bootstrap replicates. BI analyses were conducted in MrBayes vers. 3.2.2 (Ronquist and Huelsenbeck,

2003) with six independent runs of 2,000,000 generations each under the GTR + Γ + I model with partitions unlinked. Chains were sampled every 1000th generation. Log likelihoods were examined using Tracer v1.5 (Rambaut and Drummond, 2009) in order to determine the burn-in and to ensure that an adequate effective sample size (ESS) was attained. The consensus tree and the posterior probability (PP) of clades were calculated based upon the trees sampled after the burn-in set at 500,000 generations. TreeGraph2 (Stöver and Müller, 2010) and Figtree vers. 1.4.2 (Rambaut, 2014) were used for tree drawing. We used the species *Obetia radula* (Bak.) B.D. Jackson to root the final tree. In order to identify changes in topology and support, an additional tree without external outgroup, but with *U. pilulifera* as internal outgroup was generated.

## 6.3 Results

The phylogeny of the genus is based on the markers ITS, *trn*S–*trn*G, *psb*A–*trn*H and *trn*L–F and has near-comprehensive sampling of species (Figs. 6.3–6.6). The ITS and chloroplast trees were calculated separately and the well-supported nodes are congruent between the ITS-tree and the chloroplast trees (data available in the online version). The chloroplast tree is completely congruent to the combined tree with regard to the backbone and the sequence and support of the clades, including the terminal clades. The only exception is the *dioica s.l.*-clade, which is retrieved with good support, but little internal resolution in the chloroplast tree (only the *dioica s.str.*-clade supported; Fig. 6.6). Conversely, the ITS-tree shows an entirely unresolved backbone and fails to retrieve the basal sister relationships, probably due to considerable sequence divergence and large indels, especially in the outgroups and the pilulifera-clade. However, the ITS-tree retrieves most terminal clades as shown and named in Fig. 6.3 with good support, with only two exceptions: The *Urtica dioica* complex is retrieved as a polytomy at the base of *Urtica*, with all other groups (including the pilulifera-clade, *U. cannabina*-clade) united on one essentially unsupported clade (ML BS 50) in this polytomy. The *urens*-clade also breaks down, with *U. membranacea* Poir. and the remainder retrieved separately and their relationships to *U. ferox* G. Forst./*U. lobulata* E. Mey. and the *cannabina*-clade were not retrieved.

The combined dataset reflects essentially the chloroplast topology, with the ITS-data evidently contributing considerably to the resolution in the terminal clades, especially in the *dioica s.l.*-clade and the American clade (Figs. 6.5 and 6.6). Fig. 6.3 shows a largely resolved

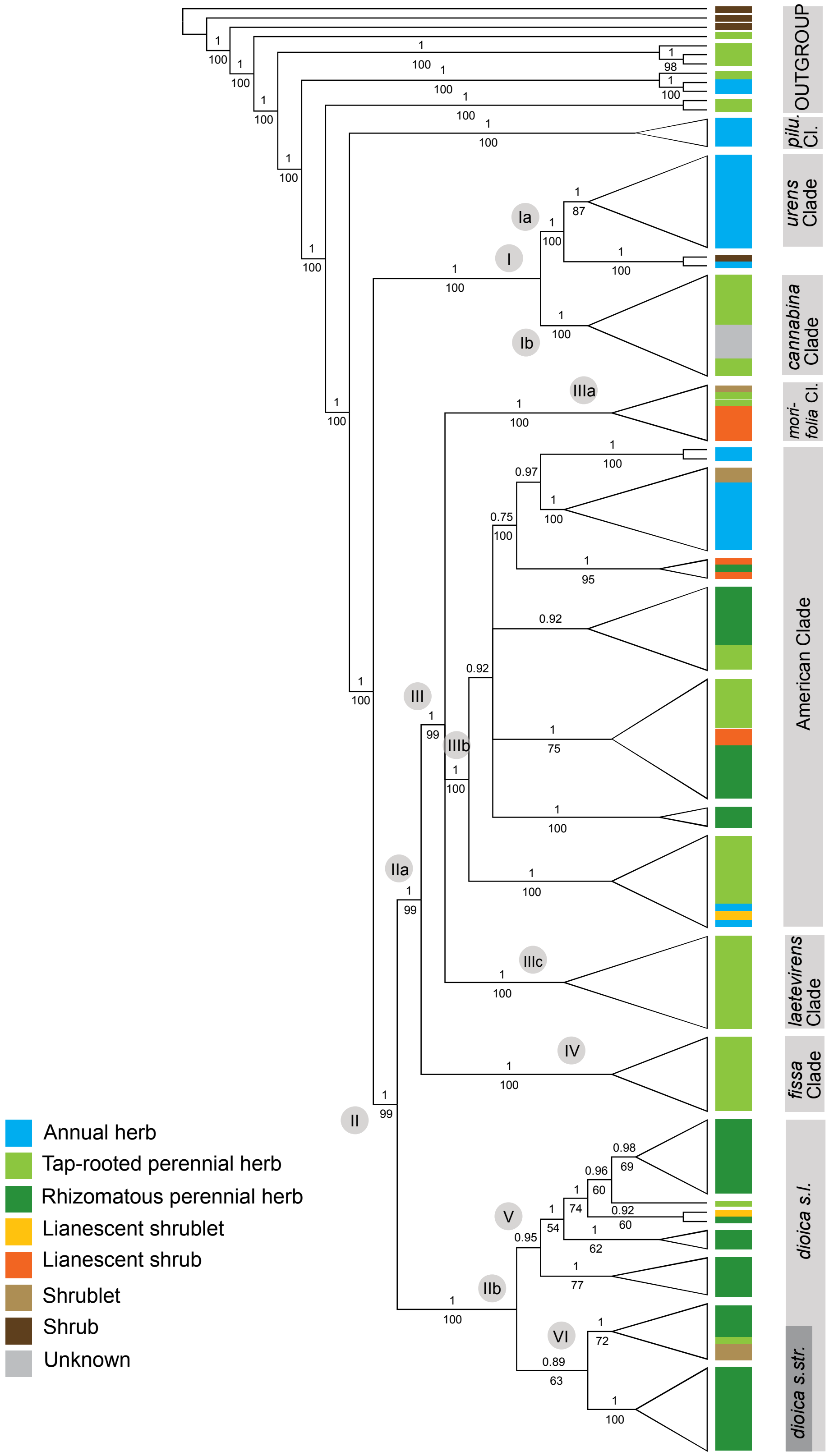

**Figure 6.3** Maximum likelihood tree based on a concatenated data set (ITS, *trn*S–*trn*G, *psb*A–*trn*H, *trn*L–F) indicating growth habits as one classical morphological character. Bayesian posterior probabilities are indicated above branches; bootstrap support under likelihood is indicated below. Color codes: dark brown – shrub, light brown – shrublet, orange – lianescent shrub, yellow – lianescent shrublet, dark green – rhizomatous perennial, light green – tap-rooted perennial, blue – annual herb (see Appendix A3 for character states).

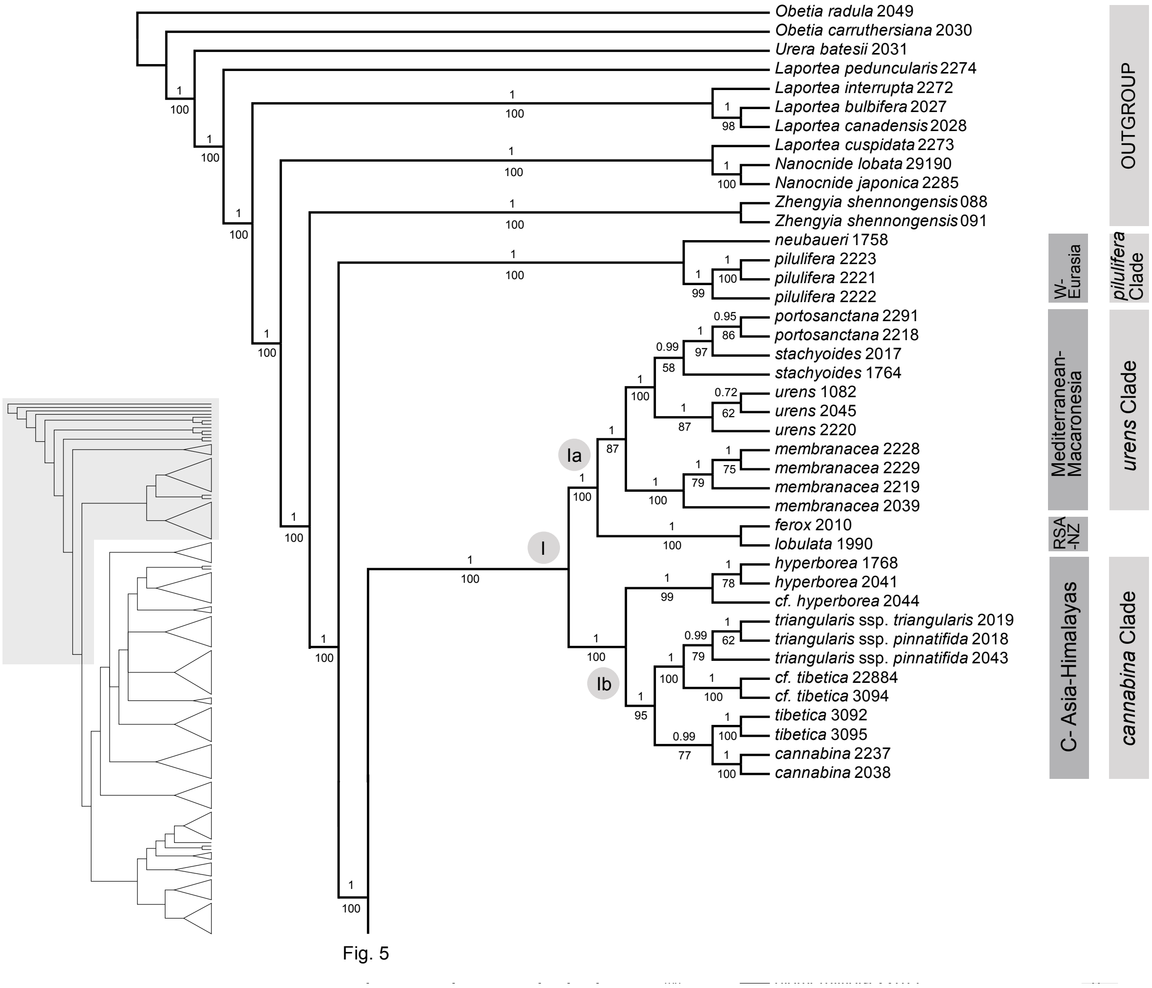

**Figure 6.4** Basally branching groups in maximum likelihood tree based on a concatenated data set (ITS, *trn*S–*trn*G, *psb*A–*trn*H, *trn*L–F). Bayesian posterior probabilities are indicated above branches; bootstrap support under likelihood is indicated below. Geographic distribution is displayed in gray bars on the right side. RSA, Republic of South Africa; NZ, New Zealand.

phylogeny with numerous well-resolved clades based on the combined dataset. Figs. 6.4–6.6 show a detailed view of the clades including taxon names and support values. Standard molecular markers are thus able to provide a highly resolved phylogeny of the genus. Topology and support do not significantly change by excluding the outgroup (data not shown). Recently described *Zhengyia* is confirmed as sister to the genus *Urtica* (Fig. 6.4). Within *Urtica*, the Western Eurasian annuals *U. pilulifera* and *U. neubaueri* (*pilulifera*-clade) comprise a sister clade to the remaining species of the genus (Fig. 6.4). The remainder of the genus segregates into two well-supported major clades (Figs. 6.4–6.6, clades I and II).

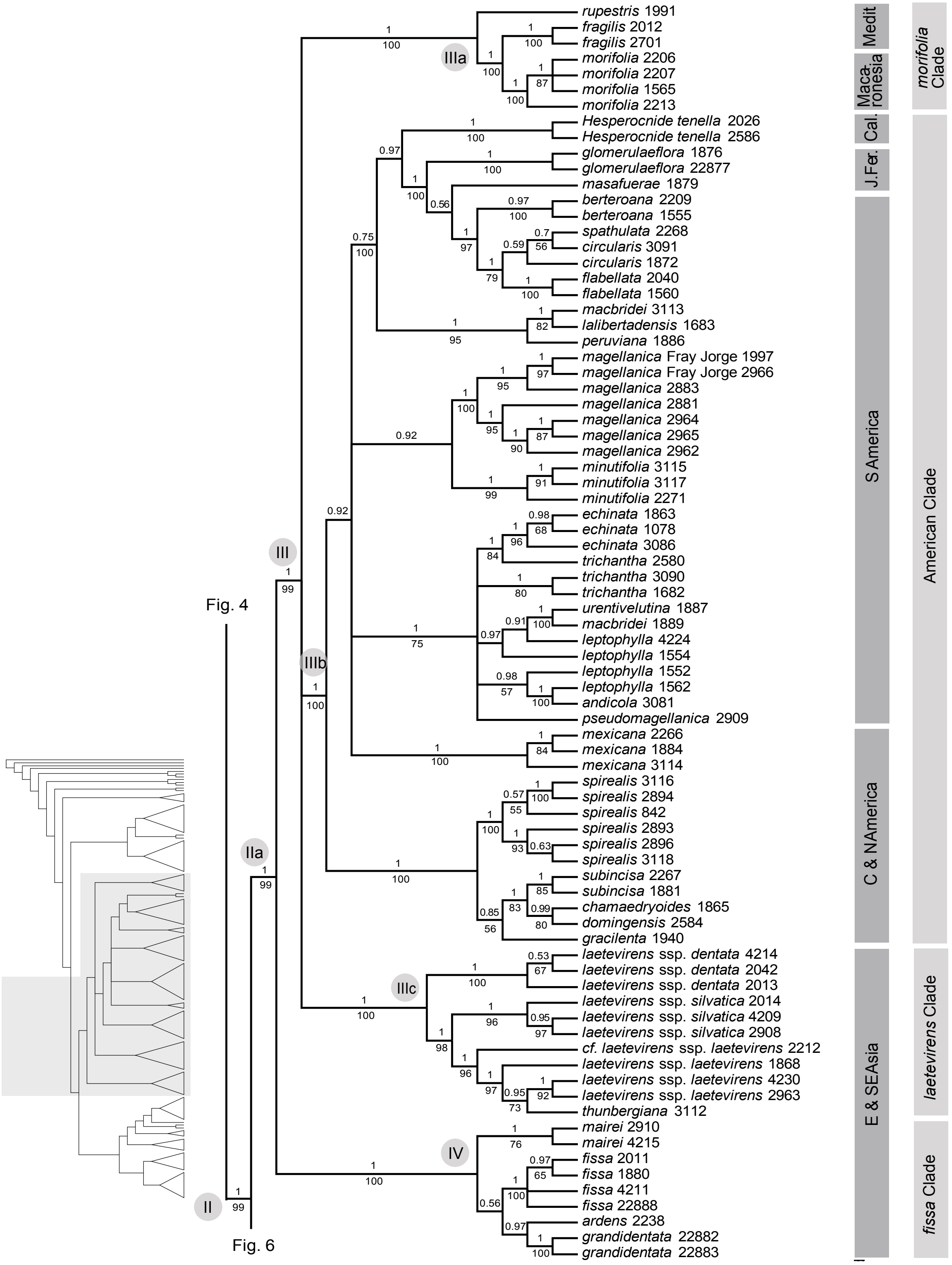

**Figure 6.5** American clade and allied clades from Asia and Macronesia and the Mediterranean. Maximum likelihood tree based on a concatenated data set (ITS, *trn*S–*trn*G, *psb*A–*trn*H, *trn*L–F). Bayesian posterior probabilities are indicated above branches; bootstrap support under likelihood is indicated below. Geographic distribution is displayed in gray bars on the right side. RSA, Republic of South Africa; NZ, New Zealand; Medit., Mediterranean; Cal., California; J. Fer., Juan Fernández Islands.

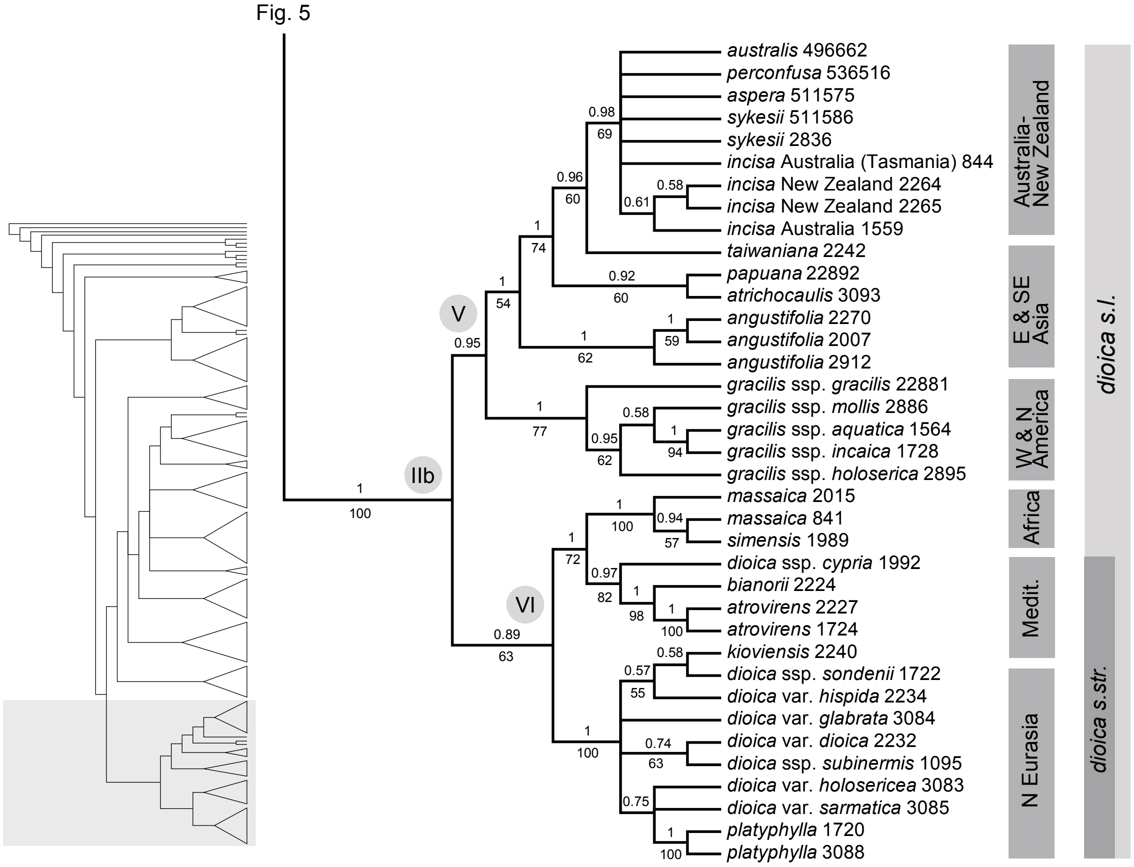

**Figure 6.6** *Dioica*-clade in maximum likelihood tree based on a concatenated data set (ITS, *trn*S–*trn*G, *psb*A–*trn*H, *trn*L–F). Bayesian posterior probabilities are indicated above branches; bootstrap support under likelihood is indicated below. Geographic distribution is displayed in gray bars on the right side. Medit., Mediterranean.

The first major clade (Fig. 6.4, clade I) divides into two smaller clades (Ia and Ib) – one includes a clade of Mediterranean-Macaronesian annuals (the *urens*-clade) + *U. ferox* (from New Zealand) and *U. lobulata* (from South Africa) which is sister to a Central Asian clade comprising *U. cannabina* L., *U. hyperborea* Jacquem. ex Wedd. and allies.

The second major clade (Fig. 6.5, clade II) again divides into a clade (IIa) essentially containing the American taxa and one (Fig. 6.6, clade IIb) essentially containing *Urtica dioica s.l.* Clade IIa is resolved with the fissa-clade (IV) as sister to a range of Southeast Asian taxa, followed by a polytomy of three clades: the *morifolia*-clade (IIIa) with Macaronesian-Mediterranean taxa [*U. rupestris* (Sicily), *U. fragilis* (Syria, SE Turkey, Lebanon) as sister to Macaronesian *U. morifolia*], the American clade (IIIb), comprising all native American taxa apart from *U. gracilis* Ait. and its subspecies (retrieved in the *U. dioica* clade – Fig. 6.6) and the *laetevirens*-clade (IIIc) as another East Asian clade (*U. laetevirens* Maxim. and Japanese *U. thunbergiana* Siebold & Zucc.).

Relationships among species of the "American clade" (Fig. 6.5, clade IIIb) are only partly resolved and relationships between taxa remain partly ambiguous. This clade includes all the widespread Andean species (*U. flabellata* Kunth, *U. leptophylla*, *U. echinata* Benth.), but also species endemic to Mexico (*U. spirealis* Blume, *U. subincisa* Benth.), the Juan Fernández-Islands (*U. glomerulaeflora*), central Chile (*U. berteroana* Phil.), northern Peru (*U. urentivelutina*, *U. peruviana*, *U. lalibertadensis*) and Hispaniola (*U. domingensis*). Within the American clade, there is some degree of resolution, albeit not always with high support. The North American clade including *U. spirealis*, *U. subincisa*, *U. chamaedryoides*, *U. gracilenta* and the only Caribbean endemic (*U. domingensis*) is well-supported and retrieved as sister to the other American taxa.

The main clade of American *Urtica* is retrieved only with low support and divides into four smaller clades. One well-supported clade includes two branches – Northern Andean *U. macbridei* Killip, *U. peruviana* and *U. lalibertadensis* as sister to the western North American-Hawaiian genus *Hesperocnide*, traditionally considered as a different genus, in a clade with the Juan Fernández-endemic (*U. glomerulaeflora*), followed by central Chilean *U. berteroana*, the only SE South American species (*U. circularis* (Hicken) Sorarú, *U. spathulata* Sm.) and widespread Andean *U. flabellata*. Patagonian *U. magellanica* forms a well resolved clade together with *U. minutifolia* Griseb. from the southern Andes. The Central Andean species (*U. echinata*, *U. trichantha* (Wedd.) Acevedo & L.E. Navas, *U. leptophylla, U. urentivelutina*) are retrieved on a moderately supported clade, but species limits are evidently in need of critical revision, with some species not monophyletic as here defined.

The final clade (IIb) comprises *Urtica dioica s.l.* and the details are shown in Fig. 6.6. It also falls into two well-supported clades (V and VI). The first clade (V) has western American *U. gracilis* as sister to an E Asian clade, with all Australasian taxa (excluding *U. ferox*) nested within the E Asian clade. Relationships among the taxa from Australia and New Zealand were not resolved. The situation is mirrored in the second clade (VI), where the Mediterranean endemics from Cyprus, Corsica and Mallorca are sister to the two E African species and this group retrieved as sister to U. dioica in the strict sense, an exclusively Eurasian group including *U. kioviensis* Rogow. from western Eurasia and *U. platyphylla* Wedd. from northeastern Eurasia.

## 6.4 Discussion

Analysis of DNA sequence data provides a comprehensive phylogeny of the genus *Urtica*, displaying a well-resolved backbone of the tree and numerous well supported clades (Figs. 6.3–6.6). The molecular phylogeny is divergent from existing classifications based on morphological characters and none of the published classifications (Chen, 1983; Chrtek, 1969/79; Geltman, 1982; Weddell, 1856, 1869) are supported by our study. The groups proposed by Weddell (1856, 1869) could not be confirmed by the molecular data. He placed *U. hyperborea* (Asian), *U. stachyoides* (Mediterranean), *U. atrovirens* (Mediterranean), *U. spirealis* (Central American) and *U. magellanica* (South American) into the same morphological group, but our molecular analyses suggest no two of them are closely related. Similarly, Chen (1983) placed the Chinese species *U. atrichocaulis* (Hand.-Mazz.) C.J. Chen and *U. urens* L. into series Urentes C.J. Chen and considered them as closely related. The molecular data are not congruent with this: *U. atrichocaulis* appears with *U. papuana* from Papua New Guinea (Fig. 6.6, clade V) and both are retrieved in *Urtica dioica s.l.* (IIb), whereas *U. urens* is retrieved near the base of the phylogeny with other Mediterranean annuals (Fig. 6.4, clade Ia). A plethora of further contradictions between classifications based on morphology and those based on molecular data can be seen comparing Figs. 6.3–6.6 to Appendix A1.

Growth habit, leaf shape and gender distribution have classically been used as characters to classify *Urtica*, but all these characters are highly homoplasious and transitions in, e.g., leaf shape and growth habit appear to have been frequent. Most notably, growth habit has little phylogenetic information (Fig. 6.3). Many clades contain rhizomatous herbs and tap-rooted herbs in nearly equal numbers. From these two widespread conditions, shrublets, shrubs, lianescent shrublets and lianescent shrubs have arisen several times independently, with none of these growth habits characterizing any specific monophyletic group. Taxa such as *U. morifolia* (Fig. 6.5, clade IIIa), *U. dioica* subsp. *dioica* (Fig. 6.6, clade VI), *U. dioica* subsp. *cypria* (VI), *U. platyphylla* (VI), *U. leptophylla* (Fig. 6.5, clade IIIb) and *U. gracilis* (Fig. 6.6, clade V) are virtually indistinguishable at first glance, and are yet retrieved in widely different clades. Conversely, *U. bianorii* and *U. atrovirens* (Fig. 6.6, clade VI) are morphologically highly divergent from closely allied *U. dioica s.l.*, and *U. flabellata* and *U. leptophylla* bear no similarity to each other in either leaf morphology or habit (Fig. 6.5, clade IIIb). Possibly the most surprising result is the sister-relationship of the small, annual South African *U. lobulata* to the tall, shrubby New Zealand species *U. ferox* (Fig. 6.4, clade Ia) and these two taxa are closely allied to a group of Macaronesian-Mediterranean annuals (e.g., *U. membranacea*; Fig. 6.4, clade Ia). The sister relationship of *U. ferox* and *U. lobulata* represents the only case of southern hemisphere disjunctions in the genus *Urtica*, a phenomenon widely reported from other plant groups (Berry et al., 2004: Fuchsia; Cosacov et al., 2009: Calceolaria; Nylinder et al., 2012: Jovellana). The divergent – and indeed unique – vegetative morphology of *U. ferox* does not indicate any different or contradictory relationships and is likely explained by island woodiness (Carlquist, 1974; Darwin, 1859: 392; Lens et al., 2013). Additionally, the morphologically divergent evolution of *U. ferox* has been hypothesized to have taken place in the ecological context of moa browsing (Batcheler, 1989), an ecological determinant not experienced by its South African sister species – which consequently retained an overall morphology similar to the annual, Mediterranean sister group.

The widespread occurrence of island endemics already indicates high dispersability of *Urtica*, with endemic species found on Cyprus, Sicily, Corsica, the Canary Islands, Madeira, Hispaniola, Juan Fernández Islands, Hawaii, Taiwan, Papua New Guinea, the Philippines, Malaysia, and New Zealand (including the Auckland and Chatham Islands) – a degree of island colonization probably unrivalled amongst flowering plants. Furthermore, between the major landmasses and islands typically two independent colonisations are observed. Hawaii and the Juan Fernández Islands are the only exception. Both South Africa and New Zealand were apparently colonized twice independently. The Americas comprise two different clades of *Urtica*, and there appears to have been repeated dispersal between North and South America in both of these clades (Fig. 6.5, clade IIIb; Fig. 6.6, clade V).

Overall, both morphology and geographical range are thus very poor predictors of relatedness in this genus. Even morphological species limits are anything but straightforward, as previously shown (Henning et al., 2014) and only molecular data can resolve a whole range of taxonomic problems. Several taxa which are barely distinguishable by their morphology and have therefore been regarded as one and the same in earlier morphological studies are here clearly shown to be only distantly related. *Urtica tibetica* W.T. Wang ex C.J. Chen is synonymised with *U. dioica* subsp. *dioica* in the Flora of China (Chen et al., 2003), since it is morphologically similar to that species. However, our molecular data retrieve the corresponding specimens on two separate clades (Fig. 6.4, clade Ib; Fig. 6.6, clade VI) on a branch separated from *U. dioica* – together with *U. triangularis* Hand.-Mazz., *U. hyperborea* and *U. cannabina*. This clade clearly requires critical revisionary studies. Similarly, morphologically weakly differentiated taxa such as *U. papuana, U. dioica* subsp. *cypria*, and

*U. angustifolia* Fisch. ex Hornem. are retrieved as distinct lineages in the phylogeny (Fig. 6.6). The taxonomically complex group of East-Southeast Asian taxa with fused stipules (e.g., *U. laetevirens* and *U. mairei* H. Lév.) is also retrieved in two separate clades (Fig. 6.5, clades IIIc and IV). Overall, several species groups which have been difficult to distinguish from some other species by morphological characters alone in the past are now well resolved. There have been three minor radiations in the genus, one in America, one in Australia-New Zealand, and one in northern Eurasia which includes *U. dioica* s.str. sensu Henning et al. (2014), but relationships within each of these were not resolved by analysis of the current molecular data set. The American clade, with mostly Andean, Mexican and south-temperate groups, and the taxa from Australia-New Zealand are the two most striking cases (Fig. 6.5, clade III; Fig. 6.6, clade V). In spite of the apparently high dispersability of *Urtica*, there are several cases of notable biogeographical sorting: Australian and New Zealand species (excepting *U. ferox*) form a well-supported clade, with Taiwanese endemic *U. taiwaniana* as sister, this clade is then sister to *U. papuana* from Papua New Guinea and *U. atrichocaulis* from China, and this southeast Asian group is in turn sister to northeast Asian *U. angustifolia*. This entire clade is then again sister to the *Urtica gracilis*-clade, where the two North American subspecies represent the basal grade, indicating a north-temperate origin of the group as a whole (Fig. 6.6, clade V) and subsequent southward colonization in both the Americas and South East Asia– - Australasia. A similar highly structured pattern is found in the western Eurasian-African clade around *U. dioica s.str.*: The two African species *U. massaica* Milbr. and *U. simensis* Hochst. ex A. Rich. are closely related to the Mediterranean island endemics *U. atrovirens* from Sardinia (Italy), *U. bianorii* from Mallorca (Spain) and *U. dioica* subsp. *cypria* from Cyprus and this entire clade is then sister to Eurasian *Urtica dioica s.str.*, which, however, remains largely unresolved (Fig. 6.6, clade VI).

## 6.5 Conclusions

The present study provides a well-supported, near comprehensive phylogeny for the genus *Urtica*. This well-resolved phylogeny contrasts with the difficulties in recognizing species limits and natural groupings based on morphology or distribution. The phylogenetic data suggest that the (limited) morphological characters used to distinguish and group species in the genus do not reflect phylogenetic relatedness. Based on the phylogenetic data here presented, a critical re-examination of some promising morphological character complexes, especially indumentum, inflorescence morphology, and gender distribution should be carried out in order to identify possible synpomorphies and/or diagnostic characters for the clades, but also in order to resolve and improve classification of the species and assign species names to the cryptic taxa revealed in the analysis. There is also a need to study the groups that appear to have experienced recent radiations, such as Eurasian *U. dioica s.str.*, the American clade and the Australasian taxa, with more highly resolving molecular tools. The complex geographical patterns found also invite a study of the dispersal ecology of *Urtica*, about which little is known.

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*Supplementary data associated with this chapter can be found in Appendix A.*

# CHAPTER 7

## The geometry of gender–hyper-diversification of sexual systems in *Urtica* L. (Urticaceae)*

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### Abstract

*Urtica* L. (Urticaceae) is mostly reported as a genus of monoecious and dioecious taxa. However, the gender information found in the literature does not at all reflect the actual diversity of gender patterns in *Urtica*. Dioecy appears to be truly absent from *Urtica*, but otherwise there has been a major diversification in the geometry of gender and no comparable patterns exist in other plant groups. Thus, we here define technical terms for all unique architectural types of monoecy found in *Urtica* and closely related genera and reconstruct the ancestral gender states in a Bayesian framework. Our studies are based on a near-comprehensive sampling, including 61 of the 63 *Urtica* species recognized. We found five different architectural types of monoecy, two types of gynodioecy, and polygamy. A total of 15 switches appear to have taken place within the genus. Although gender characteristics have strongly diversified, they are relatively conserved within clades. Monoecy is the predominating sexual system within *Urtica* and specifically basiandrous monoecy (i.e. basal inflorescences of each individual male only, apical inflorescences pure female) is the most widespread type, found in 11 different clades. It especially characterizes the basally branching *pilulifera*-clade and the sister group *Zhengyia*, and may thus represent the plesiomorphic condition. Gender distribution and gross morphology thus appear to evolve largely independently from each other and gender distribution is largely independent of growth habit. However, polygamous taxa are most common amongst rhizomatous perennials.

*Keywords:* bisexual, dioecy, monoecy, polygamy, unisexual, *Urtica dioica*

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## 7.1 Introduction

Various types of sexual systems can be found in angiosperms and refer to features of flowers, individuals and populations. An overview of sexual systems, their frequency, their suggested relationship to dioecy, and selected references is provided by Renner (2014). The most frequent sexual systems are monoecy (each individual with male and female flowers), dioecy (populations with male and female individuals) and polygamy (populations with bisexual/hermaphroditic individuals, male individuals, and female individuals). The variety of mixed sexual systems (e.g. androdioecy, andromonoecy, gynodioecy, gynomonoecy) probably evolved via a spatial separation of gender-specific flowers occurring in monoecious or dioecious plants (Guibert et al. 2009). Renner (2014) suggests that the relatively few reports of e.g. gynodioecy (populations with bisexual/hermaphroditic individuals and female individuals) and other mixed sexual systems, compared to monoecy, may be an artefact of scarce fieldwork on tropical plants. Monoecy is considered as the crucial step in the evolution to dioecy (Renner & Ricklefs 1995) and is much more common in angiosperms than dioecy - only 7 % of all angiosperm genera contain dioecious species (Guibert et al. 2009). Dioecy, however, seems to be advantageous in an environment with changing conditions (Bertin 2007) and correlations appear to exist between dioecy and island habitats as well as between dioecy and plant size, i.e. trees and shrubs have the highest incidence of dioecy and herbs the lowest (Bawa 1980). Urticaceae – among other plant families such as the Anacardiaceae, Euphorbiaceae and Moraceae – have almost equal numbers of monoecious and dioecious taxa. Conversely, other families such as Daphniphyllaceae and Myristicaceae are exclusively dioecious, while others, such as Bombacaceae and Dipterocarpaceae, have no known dioecious species (Bawa 1980).

More complicated patterns of gender distribution are reported in several plant taxa. Soza et al. (2012) reported that dioecy, andromonoecy (each individual with bisexual/hermaphroditic and male flowers) and gynomonoecy (each individual with bisexual/hermaphroditic and female flowers) evolved at least twice from hermaphroditism in *Thalictrum* (Ranunculaceae). Within the genus *Schiedea* (Caryophyllaceae), dioecious, hermaphroditic, gynodioecious, and subdioecious (trioecious/polygamous) species occur (Willyard et al. 2011) and within the annual herb *Mercurialis perennis* (Euphorbiaceae), dioecy, monoecy, and androdioecy (populations with bisexual/hermaphroditic individuals and male individuals) occur, which are moreover correlated with different parts of its geographic range (Pannell et al. 2008). Further, Zhou et al. (2016) showed that the species *Tapiscia sinensis* (Tapisciaceae) is androdioecious and the woodland herb *Acalypha rhomboidea* (Euphorbiaceae) produces both male and female inflorescences at each leaf axil of an individual plant (Cid-Benevento 1987). The most complex distribution patterns of unisexual flowers on and between plants reported so far are found in *Carex* (Cyperaceae). Especially in the Core-*Carex*-clade containing about 1500 species and the *Vignea*-clade containing about 300 species (Escudero et al. 2012) there are a wide range of different arrangements of male and female flowers in the inflorescence (Molina et al. 2012). The patterns found in *Carex* are most likely comparable with those found in *Urtica*.

Individual flowers of *Urtica* are always unisexual - this condition is quite rare in angiosperms with only 10 % of all angiosperms having unisexual flowers (Barrett 2002). There is no evidence for bisexual (hermaphroditic) flowers in *Urtica* so far. *Urtica* is mostly reported as a genus of monoecious and dioecious taxa and most studies refer to *U. dioica* L. as the best-known and most widespread member of the genus (e.g. Greig-Smith 1948, Zuk 1970, Freeman et al. 1980, Heemskerk et al. 1998, de Jong et al. 2005, Glawe & de Jong 2005, 2009, Shannon & Holsinger 2007). Monoecy is often overlooked (especially in the taller species) due to fragmentary nature of herbarium specimens, e.g., in "*Urtica pseudodioica*" (Navas 1961), where specimens represent either apical sections of the inflorescence, which are purely female, or young plants where only the male flowers are visibly developed (Weigend 2009). Thus, most putatively "dioecious" specimens are actually monoecious, as was correctly identified by Taylor (2003). Specimens of monoecious species may also be apparently female, since male flowers are shed after anthesis and are thus absent from fruiting plants (Henning et al. 2014). Numerous names for the monoecious forms especially of *U. dioica* have been assigned, e.g. var. *androgyna* Beck (1890) from the Austrian Alps, var. *hermaphrodita* Čelak. (1867) and var. *monoica* Tausch ex Ott (1851) both from Bohemia (Czech Republic) and var. *mirabilis* Zapał. from Galicia (Zapałowiecz 1908), which are however superfluous (see Grosse-Veldmann & Weigend, 2015) since *U. dioica* is known to have monoecious individuals in otherwise morphologically orthodox populations (Heemskerk *et al.* 1998, de Jong et al. 2005, Weigend 2009).

Critical morphological studies based on cultivated plants, herbarium specimens and especially molecular data have helped in the past few years to resolve some of the long-standing problems in the systematics of *Urtica* and provided a stable basis for more detailed studies of evolutionary patterns (Weigend 2005, Weigend 2006, Weigend & Luebert 2009, Farag et al. 2013, Henning et al. 2014, Grosse-Veldmann & Weigend 2015, Grosse-Veldmann et al. 2016a, Grosse-Veldmann et al. 2016b). They also indicated the presence of some novel architectural types of monoecy such as sandwich monoecy (Henning et al. 2014).

Starting from the paradigm that *Urtica* falls into monoecious and dioecious taxa, we aim at answering the following questions: 1) Does authentic dioecy exist in *Urtica*? 2) Which types of gender distribution can be found in *Urtica* based on herbarium and literature studies and cultivated plants?

## 7.2 Material and methods

### 7.2.1 Data collection

61 of the 63 *Urtica* species currently recognized (see Grosse-Veldmann et al. 2016b) are included in our studies. For each species (plus outgroup species), several herbarium specimens were investigated (more than 1800 specimens in total), 1/3 of the species was studied in detail from plants cultivated in the Botanical Gardens of Berlin and Bonn, and for all species (if possible), protologues, floras and other literature data were analysed (Table 7.1). We made sure that all investigated herbarium specimens were correctly identified. Herbarium material was obtained from the following herbaria (abbreviations following Holmgren and Holmgren, 1998 ff): AAH, AD, AK, B, BM, BONN, BR, BSB, CANB, CDBI, CHR, CONC, E, F, FI, FR, G, G-DC, GB, GH, HAL, HAST, HBG, HENU, HO, herb. Hügin, HUH, HUSA, HUT, IBK, IFP, INB, JE, K, KIEL, KRAM, KUN, herb. Kürschner, L, Lang private herb., LE, LINN, LL, M, MA, MADJ, MO, MSB, NSW, NY, P, PAL, PE, PR, PRE, PRC, QCNE, S, SGO, SP, TAIF, TAIM, TEX, UPS, US, USM, W, WSY, WU. For the culture studies, 20 individuals per species were investigated. Each node of the main shoot of an individual plant was analysed from basal to apical. Flower sexes have been recorded at each node. If one inflorescence consisted of both male and female flowers, the distribution of male and female flowers within the inflorescence has been recorded as well.

### 7.2.2 Technical definitions

The gender distribution of *Urtica* has two different dimensions and concerns the differential distribution of unisexual flowers between individual plants and different architectural types within monoecious individuals. Since no comparable patterns of gender distribution exist in other plant groups, we here define technical terms for all unique sexual systems found in *Urtica* (and closely related genera) and list those terms that are already established (Tab.

7.2). Figs. 7.1-7.4 illustrate the different gender patterns with photographs and scans of living plants.

Monoecy is defined as the presence of male and female flowers on an individual plant. This is a common phenomenon in *Urtica*, but the distribution of male and female flowers on individual plants respectively between individuals is highly diversified across the genus, but it is usually quite conserved within species. The following conditions were recorded:

*Basiandrous monoecy*, i.e. basal inflorescence branches are male and the distal ones female, usually with 1-2 mixed nodes intercalating between them.

*Proxiandrous monoecy*, i.e. the basal inflorescence branches are female, median and apical inflorescences have male flowers proximally and female flowers distally on each inflorescence branch. Proxiandrous and basiandrous monoecy sometimes show transitional forms, especially in taxa of the American clade with very short inflorescence branches.

*Basigynous monoecy*, i.e. the basal inflorescences female, and median and apical inflorescence branches with both male and female flowers mixed randomly on each inflorescence.

*Sandwich monoecy*, i.e. individuals with basal inflorescences female, median inflorescences male and distal inflorescences again female.

*Variegated monoecy*, i.e. both male and female flowers mixed randomly on each inflorescence of an individual.

The majority of taxa in *Urtica* show some type of monoecy, but a range of taxa show more complex gender distribution. Gynodioecy is found in two different types:

*Basigynous gynodioecy*, i.e. populations consist of pure female individuals and monoecious individuals showing basigynous monoecy.

*Basiandrous gynodioecy*, i.e. populations consist of female individuals and monoecious individuals showing basiandrous monoecy.

*Polygamy*, i.e. the occurrence of male, female and monoecious individuals in a population, is also observed. As far as could be established, polygamy is usually associated with basiandrous monoecy in the monoecious individuals, apart from *Urtica dioica*, where it is by far the most common type, but others are also found (e.g., variegated monoecy).

**Table 7.1** Taxa used for the ancestral state reconstruction of *Urtica* L. comprising gender information, number of herbarium specimens investigated, plants cultivated, and literature data studied – including growth habits as another classical morphological character.

***U. andicola*** Wedd., proxiandrous monoecy, specimens (17), cultivation, literature (Weddell 1852: 198, Weddell 1856: 60) – rhizomatous perennial

***U. angustifolia*** Fisch. ex Hornem., polygamy, specimens (9), literature (Ledebour 1833: 241, Kunth 1847: 182, Henning et al. 2014) – rhizomatous perennial

***U. aspera*** Petrie, polygamy, specimens (8), literature (Petrie 1918 [1919]: 107, Allan 1961) – rhizomatous perennial

***U. atrichocaulis*** (Hand.-Mazz.) C.J.Chen, basiandrous monoecy, specimens (3), literature (Chen et al. 2003) – rhizomatous perennial

***U. atrovirens*** Req. ex Loisel., proxiandrous monoecy, specimens (5), cultivation, literature (Loiseleur-Deslongchamps 1827: 432, Weddell 1856: 69, Kavalali 2003) – shrublet

***U. australis*** Hook.f., basiandrous monoecy, specimens (8), literature (Hooker 1844: 68, Weddell 1856: 88, Allan 1961, Webb et al. 1988, Grosse-Veldmann et al. 2016a) – rhizomatous perennial

***U. berteroana*** Phil., proxiandrous monoecy, specimens (7), literature (Philippi 1864: 235) – annual herb

***U. bianorii*** (Knoche) Paiva, proxiandrous monoecy, specimens (3), cultivation – tap-rooted perennial

***U. cannabina*** L., basiandrous monoecy, specimens (7), cultivation, literature (Linnaeus 1753: 984, Blume 1856: 145, Weddell 1856: 76) – tap-rooted perennial

***U. chamaedryoides*** Pursh, proxiandrous monoecy, specimens (13), literature (Weddell 1856: 60, Coile 1999) – annual herb

***U. circularis*** (Hicken) Sorarú, proxiandrous monoecy, specimens (7), cultivation – annual herb

***U. dioica*** L. subsp. ***cypria*** H. Lindb., basiandrous monoecy, specimens (17), cultivation, literature (Weigend 2006) – rhizomatous perennial

***U. dioica*** L. subsp. ***dioica*** var. ***dioica***, polygamy, specimens (221), literature (Linnaeus 1753: 984, Grosse-Veldmann & Weigend 2015) – rhizomatous perennial

***U. dioica*** L. subsp. ***dioica*** var. ***glabrata*** Clem. ex Visiani, polygamy, specimens (7), literature (Ascherson 1911: 609, Grosse-Veldmann & Weigend 2015) – rhizomatous perennial

***U. dioica*** L. subsp. ***dioica*** var. ***hispida*** Wedd., polygamy, specimens (73), literature (Ott 1851: 41, Grosse-Veldmann & Weigend 2015) – rhizomatous perennial

***U. dioica*** L. subsp. ***dioica*** var. ***holosericea*** Fries, polygamy, specimens (132), literature (Fries 1828: 281, Grosse-Veldmann & Weigend 2015) – rhizomatous perennial

***U. dioica*** L. subsp. ***dioica*** var. ***sarmatica*** Zapał., polygamy, specimens (25), literature (Zapałowicz 1908: 93, Grosse-Veldmann & Weigend 2015) – rhizomatous perennial

***U. dioica*** L. subsp. ***sondenii*** (Simmons) Hyl., polygamy, specimens (9), literature (Simmons 1910: 78) – rhizomatous perennial

**U. dioica** L. subsp. ***subinermis*** (Uechtr.) Weigend, polygamy, specimens (102), literature (Uechtritz 1863: 146, Weigend 2005) – rhizomatous perennial

***U. domingensis*** Urb., proxiandrous monoecy, specimens (5) – lianescent shrublet

***U. echinata*** Benth., proxiandrous monoecy, specimens (33), literature (Blume 1856: 150, Weddell 1856: 64) – tap-rooted perennial

***U. ferox*** G.Forst., polygamy, specimens (10), literature (Weddell 1856: 89) – shrub

***U. fissa*** E.Pritz ex Diels, basiandrous monoecy, specimens (51), cultivation, literature (Pritzel 1900: 301) – tap-rooted perennial

***U. flabellata*** Kunth, proxiandrous monoecy, specimens (8), literature (Kunth 1817: 40, Blume 1856: 150, Weddell 1856: 65) – annual herb

***U. fragilis*** J.Thiébaut, basigynous gynodioecy, specimens (9), cultivation, literature (Thiébaut 1935: 192, Weigend 2006) – tap-rooted perennial

***U. glomerulaeflora*** Steud., proxiandrous monoecy, specimens (4), literature (Weddell 1856: 94) – shrublet

***U. gracilenta*** Greene, basiandrous monoecy, specimens (5), literature (Greene 1881: 122) – annual herb

***U. gracilis*** Ait. subsp. ***aquatica*** (Liebm.) Weigend, sandwich monoecy, specimens (38), literature (Liebmann 1851: 291, Weddell 1856: 80, Henning et al. 2014) – rhizomatous perennial

**Table 7.1 continued.**

***U. gracilis*** Ait. subsp. ***holosericea*** (Nutt.) Weigend, sandwich monoecy, specimens (21), literature (Nuttal 1848: 25, Henning et al. 2014) – rhizomatous perennial

***U. gracilis*** Ait. subsp. ***incaica*** Weigend, sandwich monoecy, specimens (13), literature (Henning et al. 2014) – rhizomatous perennial

***U. gracilis*** Ait. subsp. ***mollis*** (Steud.) Weigend, sandwich monoecy, specimens (40), cultivation, literature (Steudel 1850: 258, Henning et al. 2014) – rhizomatous perennial

***U. grandidentata*** Miq., basiandrous monoecy, specimens (23), literature (Miquel 1851: 27, Liebmann 1851: 296, Blume 1856: 145, Weddell 1856: 90) – tap-rooted perennial

***U. himalayensis*** Kunth & C.D.Boché, basiandrous monoecy, specimens (40), literature (Kunth 1847: 182) – tap-rooted perennial

***U. hyperborea*** Jacquem. ex Wedd., basiandrous monoecy, specimens (6), literature (Weddell 1856: 68) – tap-rooted perennial

***U. incisa*** Poir., polygamy, specimens (57), literature (Lamarck 1816: 224, Grosse-Veldmann et al. 2016a) – rhizomatous perennial

***U. kioviensis*** Rogow., basiandrous monoecy, specimens (3), literature (Rogowicz 1843: 324, Danin 2006) – rhizomatous perennial

***U. lalibertadensis*** Weigend, proxiandrous monoecy, specimens (38), literature (Weigend et al. 2005) – rhizomatous perennial

***U. leptophylla*** Kunth, proxiandrous monoecy, specimens (77), cultivation, literature (Kunth 1817: 39, Weigend et al. 2005) – rhizomatous perennial

***U. lobulata*** E.Mey., basiandrous monoecy, specimens (10), literature (Weddell 1856: 84) – annual herb

***U. macbridei*** Killip, proxiandrous monoecy, specimens (12), cultivation, literature (Killip 1925: 49, Weigend et al. 2005) – lianescent shrub

***U. magellanica*** Juss. ex Poir., basiandrous monoecy, specimens (69), literature (Lamarck 1816: 223, Weddell 1856: 70) – rhizomatous perennial

***U. mairei*** H.Lév., basiandrous monoecy, specimens (40), literature (Chen et al. 2003) – tap-rooted perennial

***U. masafuerae*** Phil., proxiandrous monoecy, specimens (7) – annual herb

***U. massaica*** Milbr., polygamy, specimens (8), literature (Mildbraed 1923: 275, Friis 1989) – rhizomatous perennial

***U. membranacea*** Poir., basigynous gynodioecy, specimens (6), cultivation, literature (Lamarck 1798: 638, Webb & Berthelot 1836: 259, Blume 1856: 147, Weddell 1856: 93, Kavalali 2003) – annual herb

***U. mexicana*** Liebm., basiandrous monoecy, specimens (12), literature (Liebmann 1851: 291, Weddell 1856: 67) – rhizomatous perennial

***U. minutifolia*** Griseb., proxiandrous monoecy, specimens (6), literature (Grisebach 1874: 112) – tap-rooted perennial

***U. morifolia*** Poir., basigynous gynodioecy, specimens (5), cultivation, literature (Lamarck 1816: 223, Webb & Berthelot 1836: 260, Weddell 1856: 91) – lianescent shrub

***U. neubaueri*** Chrtek, basiandrous monoecy, specimens (4), literature (Chrtek 1974 [105]: 6) – annual herb

***U. papuana*** Zandee, proxiandrous monoecy, specimens (2), literature (Zandee 1969: 444) – lianescent shrublet

***U. parviflora*** Roxb., basiandrous monoecy, specimens (10), cultivation, literature (Link 1822, Weddell 1856: 85) – tap-rooted perennial

***U. perconfusa*** Grosse-Veldmann & Weigend, basiandrous monoecy, specimens (10), literature (Grosse-Veldmann et al. 2016a) – rhizomatous perennial

***U. peruviana*** Geltman, proxiandrous monoecy, specimens (21), literature (Geltman 1998: 15, Weigend et al. 2005) – lianescent shrub

***U. pilulifera*** L., basiandrous monoecy, specimens (5), cultivation, literature (Blume 1856: 152, Weddell 1856: 74, Kavalali 2003) – annual herb

***U. platyphylla*** Wedd., polygamy, specimens (3), cultivation, literature (Weddell 1856: 86) – rhizomatous perennial

***U. portosanctana*** Press, basigynous monoecy, specimens (4), cultivation, literature (Press 1988) – annual herb

***U. pseudomagellanica*** Geltman, proxiandrous monoecy, specimens (3), literature (Geltman 1998: 16) – rhizomatous perennial

**Table 7.1 continued.**

***U. simensis*** Hochst. ex A.Rich., polygamy, specimens (7), literature (Richard 1850: 260, Weddell 1856: 87, Friis 1989) – rhizomatous perennial

***U. spathulata*** Sm., proxiandrous monoecy, specimens (6), literature (Weddell 1856: 66) – annual herb

***U. spirealis*** Blume, basiandrous monoecy, specimens (18), literature (Blume 1856: 152, Weddell 1856: 97) – tap-rooted perennial

***U. stachyoides*** Webb & Berthel., basigynous monoecy, specimens (5), literature (Webb & Berthelot 1836: 259, Weddell 1856: 72) – annual herb

***U. subincisa*** Benth., proxiandrous monoecy, specimens (7), literature (Bentham 1848: 293, Weddell 1856: 62) – tap-rooted perennial

***U. sykesii*** Grosse-Veldmann & Weigend, basiandrous monoecy, specimens (22), cultivation, literature (Grosse-Veldmann et al. 2016a) – rhizomatous perennial

***U. taiwaniana*** S.S.Ying, proxiandrous monoecy, specimens (6), cultivation, literature (Chen et al. 2003) – tap-rooted perennial

***U. thunbergiana*** Sieb. & Zucc. subsp. ***dentata*** (Hand.-Mazz.) C.J.Chen, basiandrous gynodioecy, specimens (52), literature (Handel-Mazzetti 1929: 112) – tap-rooted perennial (inferred)

***U. thunbergiana*** Sieb. & Zucc. subsp. ***silvatica***, basiandrous gynodioecy, specimens (53), literature (Handel-Mazzetti 1929: 113) – tap-rooted perennial (inferred)

***U. thunbergiana*** Sieb. & Zucc. subsp. ***thunbergiana***, basiandrous gynodioecy, specimens (90), literature (Siebold & Zuccarini 1846: 214) – tap-rooted perennial (inferred)

***U. tibetica*** W.T.Wang ex C.J.Chen, basiandrous monoecy, specimens (15), literature (Wu 1983: 526) – habit unknown

***U. triangularis*** Hand.-Mazz. subsp. ***pinnatifida*** (Hand.-Mazz.) C.J.Chen, basiandrous monoecy, specimens (6), literature (Chen et al. 2003) – tap-rooted perennial

***U. triangularis*** Hand.-Mazz. subsp. ***triangularis***, basiandrous monoecy, specimens (9), literature (Chen et al. 2003) – tap-rooted perennial

***U. trichantha*** (Wedd.) Acevedo & L.E.Navas, proxiandrous monoecy, specimens (10), literature (Weddell 1869: 42) – tap-rooted perennial

***U. urens*** L., proxiandrous monoecy, specimens (11), cultivation, literature (Linnaeus 1753: 984, Weddell 1856: 58, Kavalali 2003) – annual herb

***U. urentivelutina*** Weigend, proxiandrous monoecy, specimens (3), literature (Weigend et al. 2005) – lianescent shrub

***Hesperocnide tenella*** Torr., proxiandrous monoecy, specimens (8), literature (Torrey 1857: 139, Woodland et al. 1976, Calflora 2016) – annual herb

***Laportea bulbifera*** (Siebold & Zucc.) Wedd., basiandrous monoecy, specimens (6), literature (Chen et al. 2003) – tap-rooted perennial

***Laportea canadensis*** Gaudich., basiandrous monoecy, specimens (5), literature Gaudichaud-Beaupré 1830: 498) – tap-rooted perennial

***Laportea cuspidata*** (Wedd.) Friis, basiandrous monoecy, specimens (5), literature (Chen et al. 2003) – tap-rooted perennial

***Laportea interrupta*** (L.) Chew, variegated monoecy, specimens (14), literature (Friis 1989, Chen et al. 2003) – tap-rooted perennial

***Laportea peduncularis*** (Wedd.) Chew, basiandrous monoecy, specimens (6), literature (Friis 1989) – tap-rooted perennial

***Nanocnide japonica*** Blume, basigynous monoecy, specimens (10), literature (Blume 1856: 155, Chen et al. 2003) – annual herb

***Nanocnide lobata*** Wedd., variegated monoecy, specimens (4), literature (Weddell 1869: 69, Chen et al. 2003) – annual herb

***Obetia carruthersiana*** (Hiern.) Rendle, dioecy, specimens (2), literature (Rendle 1917, Friis 1983, 1989) – shrub

***Obetia radula*** (Bak.) B.D. Jackson, dioecy, specimens (6), literature (Friis 1983, 1989) – shrub

***Urera batesii*** Rendle, dioecy, specimens (8), literature (Rendle 1916: 368, Friis 1989, 1991) – shrub

***Zhengyia shennongensis*** T.Deng, D.G.Zhang & H.Sun, basiandrous monoecy, literature (Deng et al. 2013) – tap-rooted perennial

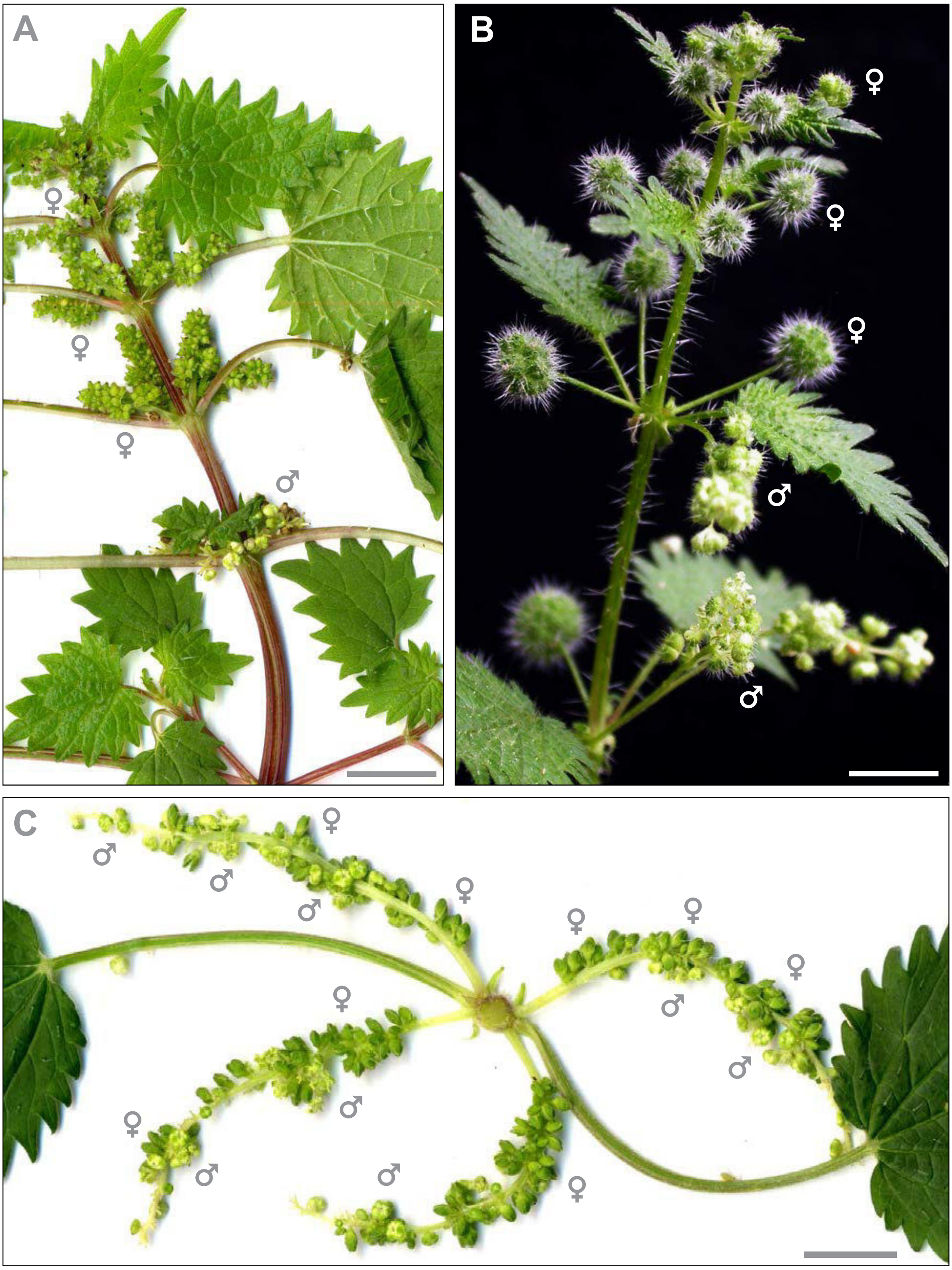


**Figure 7.1** Basiandrous (A, B) and basigynous (C) monoecy, **A**, *U. sykesii* (M. Weigend 8212), flowering stem part; **B**, *U. pilulifera* (M. Weigend 8210), flowering stem part; **C**, *U. portosanctana* (M. Weigend 8234), inflorescence at median stem part. Scale bar = 10 mm.

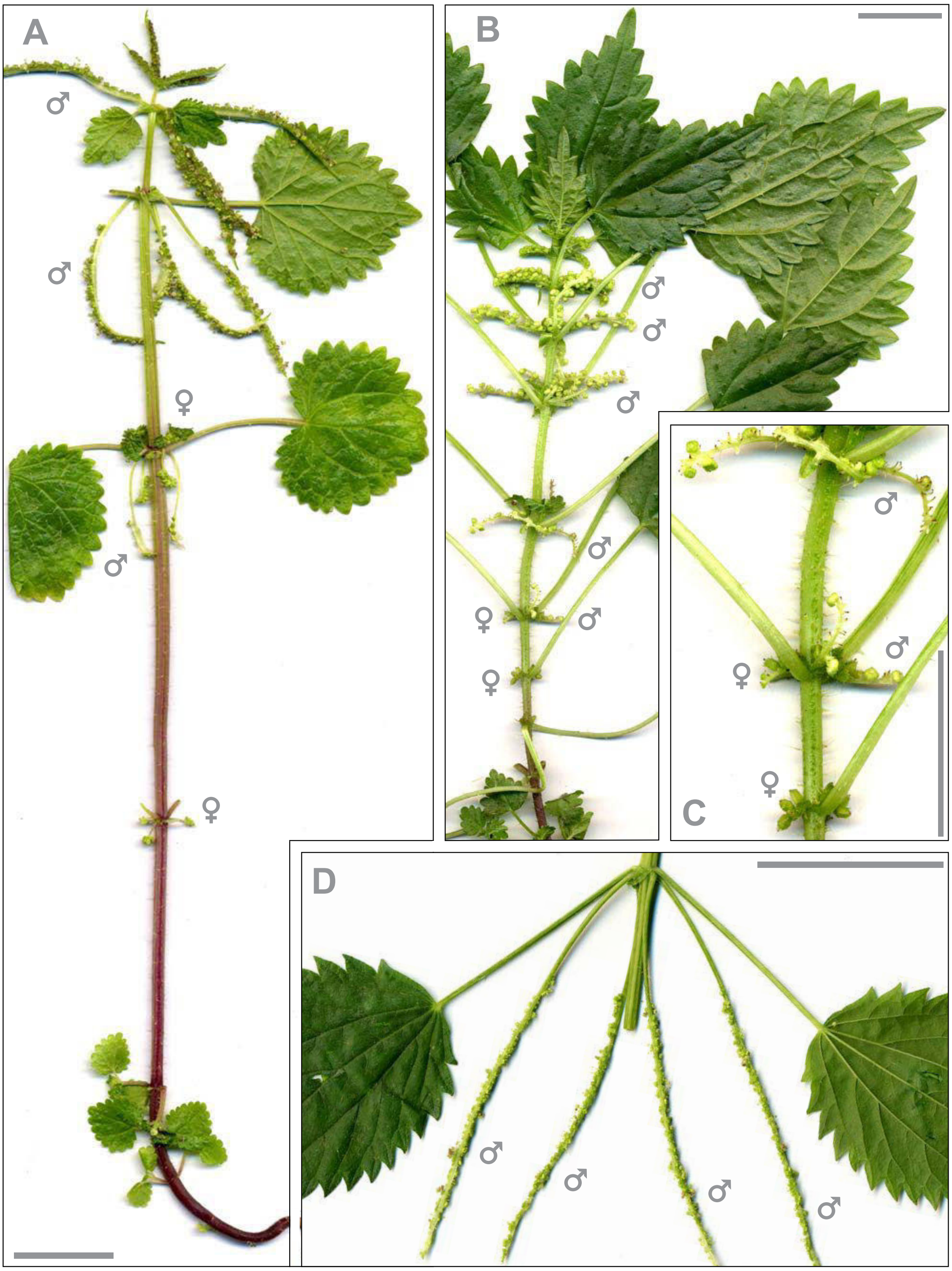


**Figure 7.2** Basigynous gynodioecy **A**, *U. membranacea* (M. Weigend 8154), monoecious individual; **B**, *U. fragilis*, monoecious individual; **C**, *U. fragilis* (B. Tarikahya & B. Özüdogru 2410), median stem part in detail; **D**, *U. membranacea*, male inflorescence. Scale bar = 20 mm.

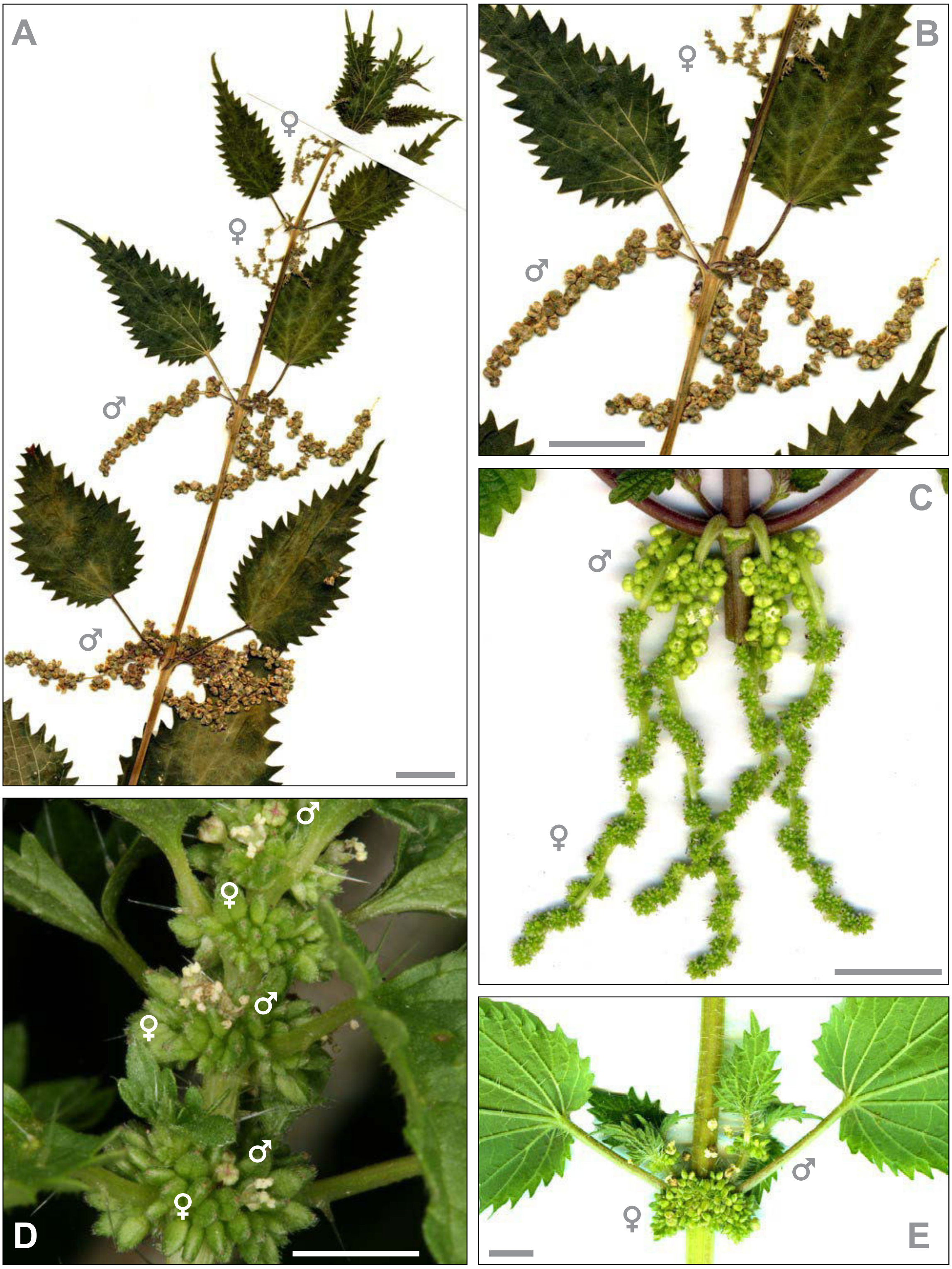


**Figure 7.3** Basiandrous gynodioecy (A, B) and proxiandrous monoecy (C–E), **A**, *U. thunbergiana* subsp. *dentata* (D.E. Boufford et al. 28908), monoecious individual, **B**, *U. thunbergiana* subsp. *dentata,* median stem part in detail; **C**, *U. macbridei* M. Weigend 9106), inflorescence at median stem part; **D**, *U. circularis* (M. Weigend 9311), median stem part; **E**, *U. echinata* (M. Weigend 7706), inflorescence at median stem part. Scale bar: A–C, E = 10 mm, D = 5 mm.

**Figure 7.4** Sandwich monoecy, **A**, *U. gracilis* subsp. *gracilis* (M. Weigend 9332), median and apical stem part; **B**, *U. gracilis* subsp. *incaica* (Ch. Schwarzer 14), sandwich monoecy, median and apical stem part. Scale bar = 20 mm.

**Table 7.2** Technical definitions of the gender distribution in *Urtica* and closely related genera. Colours: dark grey, female flowers; light grey, male flowers.

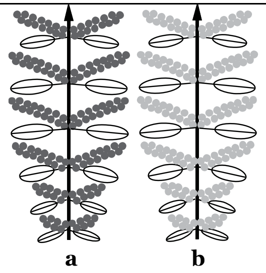
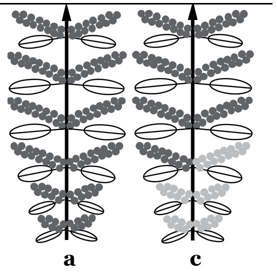
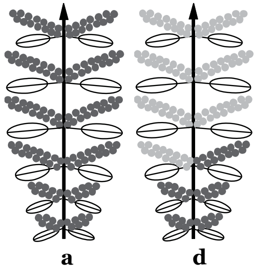
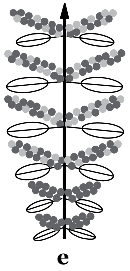
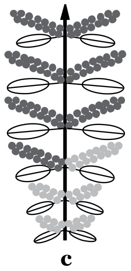
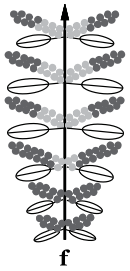
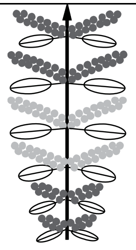
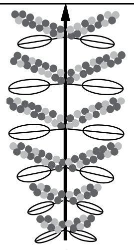
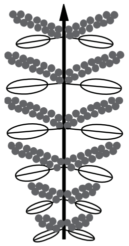
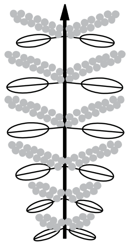
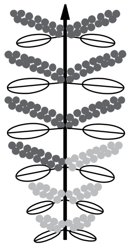
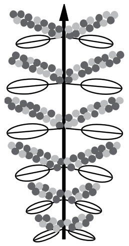
| Technical term | Definition | | Technical term | Definition | |
|---|---|---|---|---|---|
| **Dioecy** | Populations with male individuals and female individuals |  | **Basiandrous Gynodioecy** | Female individuals and monoecious individuals with basal inflorescences of one individual pure male and apical inflorescences pure female, usually with 1-2 mixed nodes in between (Fig. 7.3A, B) |  |
| **Basigynous Gynodioecy** | Female individuals and monoecious individuals with basal inflorescences of one individual pure female and apical inflorescences pure male, usually with 1-2 mixed nodes in between (Fig. 7.2) |  | **Basigynous Monoecy** | Basal inflorescences of each individual female only, median and apical inflorescences with both male and female flowers mixed randomly on each inflorescence (Fig. 7.1C) |  |
| **Basiandrous Monoecy** | Basal inflorescences of each individual male only, apical inflorescences pure female, usually with 1-2 mixed nodes in between (Fig. 7.1A, B) |  | **Proxiandrous Monoecy** | Usually basal inflorescences of each individual female, median and apical inflorescences mixed with male flowers proximally and female flowers distally situated on each inflorescence branch (Fig. 7.3C-E) |  |

**Table 7.2 continued** Technical definitions of the gender distribution in *Urtica* and closely related genera. Colours: dark grey, female flowers; light grey, male flowers.

| Technical term | Definition | Technical term | Definition |
|---|---|---|---|
| **Sandwich Monoecy** | Basal inflorescences of each individual female only, median inflorescences male only, apical inflorescences pure female (Fig. 7.4)  **g** | **Variegated Monoecy** | Both male and female flowers mixed randomly on each inflorescence  **h** |
| **Polygamy** | Populations with male individuals, female individuals and monoecious individuals  **a**  **b**  **c**  **h** | | |

### 7.2.3 Phylogenetic analyses

The basis for the present study is the comprehensive *Urtica* phylogeny provided by Grosse-Veldmann et al. (2016b) including 61 of the 63 species recognized (represented by 144 *Urtica* taxa and 14 outgroup taxa). To analyse the gender evolution in *Urtica* and closely related genera, we reduced this dataset to one accession per taxon resulting in 75 *Urtica* taxa and 12 outgroup taxa. All sequences are deposited to GenBank genetic sequence database (see Grosse-Veldmann *et al.,* 2016b: Supplementary Tab. S2 for accession numbers + *U. himalayenis* K22889, Genbank No. XXXX). Phylogenetic analyses were conducted on a concatenated dataset employing Bayesian inference, maximum likelihood and maximum parsimony (see Fig. 7.5 for support values). Bayesian inferences (BI) were conducted in MrBayes vers. 3.2.2 (Ronquist & Huelsenbeck 2003) with six independent runs of 2,000,000 generations each under the GTR + Γ + I model (nst = 6; rates = invgamma) with partitions unlinked. Chains were sampled every 1000th generation with trees written to a tree file (.t). Log likelihoods obtained from the MrBayes parameter files (.p) were examined using Tracer v1.5 (Rambaut & Drummond, 2009) in order to determine the burn-in and to ensure that an adequate effective sample size (ESS) was attained. The consensus tree and the posterior probability (PP) of clades were calculated based upon the trees sampled after the burn-in set at 500,000 generations (burnin = 500). Maximum likelihood analyses were conducted with the standard settings in RAxML Version 8 (Stamatakis, 2006; Stamatakis et al., 2008). The node support under ML is based on 1000 bootstrap replicates. Parsimony analyses were conducted in PAUP 4.0b10 (Swofford 2002) using the heuristic search option. A bootstrap analysis (criterion=parsimony) was estimated based on 1000 replicates (addseq=random, nreps=10, swap=tbr, MaxTrees=1000). The species *Obetia radula* was used to root the final tree.

### 7.2.4 Ancestral state reconstruction

Ancestral states were reconstructed for 25 well-supported nodes (PP ≥ 0.95) using BayesTraits V2.0 (Pagel & Meade 2013) selecting the "MultiState" model of evolution and the "MCMC" (Markov chain Monte Carlo) analysis method (Fig. 7.6). The MCMC method has the advantage of taking into account uncertainty in both phylogenetic topology and character mapping (Pagel et al. 2004). Reconstructions were based on 1000 randomly selected post-burnin Bayesian trees from the phylogenetic analysis in MrBayes 3.2.2. A reversible-jump (RJ) MCMC with a hyperprior approach was chosen and the interval of 0–30 (rjhp 0 30) for the RJ-hyperprior implementing an exponential distribution was applied. The posterior distribution of ancestral states was calculated for each selected node by the MRCA (Most Recent Common Ancestor)-MCMC analysis (AddMRCA). MCMC chains were run for 10 million iterations (it 10,000,000) with samples taken every 100 iterations (sample 100) after a burnin of 500,000 iterations (burnIn 500,000). In order to ensure that an adequate effective sample size (ESS) was attained, the results of the MCMC runs were analysed in Tracer V1.5 (Rambaut & Drummond, 2009). Ancestral states for each reconstructed node were evaluated by taking the arithmetic means of the sampled PPs for each character state (Tab. 7.3). Mean PPs, shown as proportions on pie charts, were plotted onto the phylogeny drawn with TreeGraph2 (Stöver & Müller, 2010).

## 7.3 Results

A careful examination of herbarium specimens and the cultivation of many species could not confirm the presence of truly dioecious species in *Urtica*. The species in the *U. dioica* clade, which have been referred to as dioecious in the past, are all polygamous or show different types of monoecy. *Urtica ferox*, another promising candidate for true dioecy, was taken into cultivation in 2009 and 2010. One of the five individuals developed branches with male flowers on an otherwise female individual, indicating that this species is also polygamous. Authentic dioecy with populations consisting of male and female individuals only, could not be confirmed for any species of *Urtica* and is here only found in the basal outgroup genera *Obetia* and *Urera*. This could not be verified in cultivation and the data are here taken from the literature only (Rendle 1916, Rendle 1917, Friis 1983, 1989, 1991). Overall, a broad range of gender distributions were observed, shown in Figures 7.1-7.4, corresponding to the gender types defined in Table 7.2. Gender characteristics are strongly diversified in *Urtica*, but are relatively conserved within clades. Monoecy is the predominating sexual system within *Urtica* and to smaller proportions gynodioecy and polygamy. The ancestral state reconstruction was based on a near-comprehensive sampling of species (61 of the 63 *Urtica* species recognized; Fig. 7.6). Basiandrous monoecy is the most widespread type of monoecy in *Urtica* and found in 11 different clades. It characterizes the basally branching *pilulifera*-clade (Ia) and the sister group *Zhengyia* and may thus represent the plesiomorphic character state. Basiandrous monoecy may have arisen several times independently within *Urtica* and is also found in the two Asian clades *cannabina* + *fissa*, where all members of the respective clade exhibit this form of monoecy, as well as in South African *U. lobulata* (IIa), and scattered in the American clade (Vb) and in *dioica s.l.* (IV). Proxiandrous monoecy is the other frequent type of monoecy in *Urtica* found in nine different clades and predominates in the American

**Table 7.3** Posterior probability values of ancestral gender states calculated in BayesTraits for selected nodes in the *Urtica* phylogeny. The highest probability value for each node is highlighted in bold.

| **Node** | **Dioecy** | **Basiandrous Gynodioecy** | **Basigynous Gynodioecy** | **Basiandrous Monoecy** | **Basigynous Monoecy** | **Proxiandrous Monoecy** | **Sandwich Monoecy** | **Variegated Monoecy** | **Polygamy** |
|---|---|---|---|---|---|---|---|---|---|
| **OI** | **0.989** | 0.002 | 0.001 | 0.000 | 0.002 | 0.000 | 0.002 | 0.002 | 0.001 |
| **OII** | **0.944** | 0.009 | 0.008 | 0.002 | 0.010 | 0.002 | 0.010 | 0.009 | 0.006 |
| **OIII** | **0.529** | 0.067 | 0.058 | 0.040 | 0.072 | 0.029 | 0.072 | 0.068 | 0.064 |
| **OIV** | 0.089 | 0.087 | 0.083 | **0.220** | 0.091 | 0.132 | 0.090 | 0.092 | 0.116 |
| **OV** | 0.080 | 0.080 | 0.076 | **0.267** | 0.088 | 0.116 | 0.083 | 0.099 | 0.112 |
| **OVa** | 0.049 | 0.059 | 0.053 | **0.333** | 0.077 | 0.046 | 0.063 | 0.227 | 0.094 |
| **OVI** | 0.073 | 0.074 | 0.071 | **0.312** | 0.084 | 0.113 | 0.078 | 0.085 | 0.111 |
| **OVIa** | 0.073 | 0.079 | 0.073 | **0.268** | 0.112 | 0.089 | 0.082 | 0.110 | 0.113 |
| **OVII** | 0.071 | 0.074 | 0.071 | **0.325** | 0.074 | 0.117 | 0.076 | 0.075 | 0.116 |
| **I** | 0.079 | 0.092 | 0.096 | **0.221** | 0.084 | 0.099 | 0.091 | 0.085 | 0.153 |
| **Ia** | 0.036 | 0.037 | 0.034 | **0.627** | 0.038 | 0.077 | 0.039 | 0.039 | 0.071 |
| **II** | 0.030 | 0.094 | 0.121 | 0.273 | 0.046 | 0.026 | 0.057 | 0.048 | **0.306** |
| **IIa** | 0.039 | 0.053 | 0.062 | **0.396** | 0.060 | 0.049 | 0.056 | 0.054 | 0.231 |
| **III** | 0.032 | 0.143 | 0.185 | 0.111 | 0.051 | 0.025 | 0.064 | 0.054 | **0.336** |
| **IIIa** | 0.028 | 0.206 | **0.291** | 0.150 | 0.054 | 0.054 | 0.049 | 0.053 | 0.115 |
| **IV** | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.052 | 0.001 | **0.940** |
| **V** | 0.020 | 0.285 | **0.441** | 0.005 | 0.049 | 0.035 | 0.041 | 0.048 | 0.076 |
| **Va** | 0.008 | 0.011 | **0.941** | 0.003 | 0.009 | 0.002 | 0.009 | 0.010 | 0.007 |
| **Vb** | 0.003 | 0.006 | 0.005 | 0.027 | 0.007 | **0.901** | 0.006 | 0.005 | 0.040 |
| **Vc** | 0.003 | **0.978** | 0.004 | 0.001 | 0.004 | 0.000 | 0.004 | 0.003 | 0.003 |
| **VI** | 0.001 | 0.001 | 0.001 | **0.991** | 0.001 | 0.002 | 0.001 | 0.001 | 0.002 |
| **VII** | 0.001 | 0.002 | 0.002 | 0.009 | 0.003 | **0.958** | 0.002 | 0.002 | 0.022 |
| **VIII** | 0.015 | 0.022 | 0.021 | 0.252 | 0.028 | **0.517** | 0.023 | 0.023 | 0.100 |
| **IX** | 0.004 | 0.007 | 0.004 | 0.002 | 0.007 | 0.004 | 0.317 | 0.007 | **0.648** |
| **X** | 0.001 | 0.002 | 0.001 | 0.003 | 0.002 | 0.007 | 0.004 | 0.002 | **0.978** |

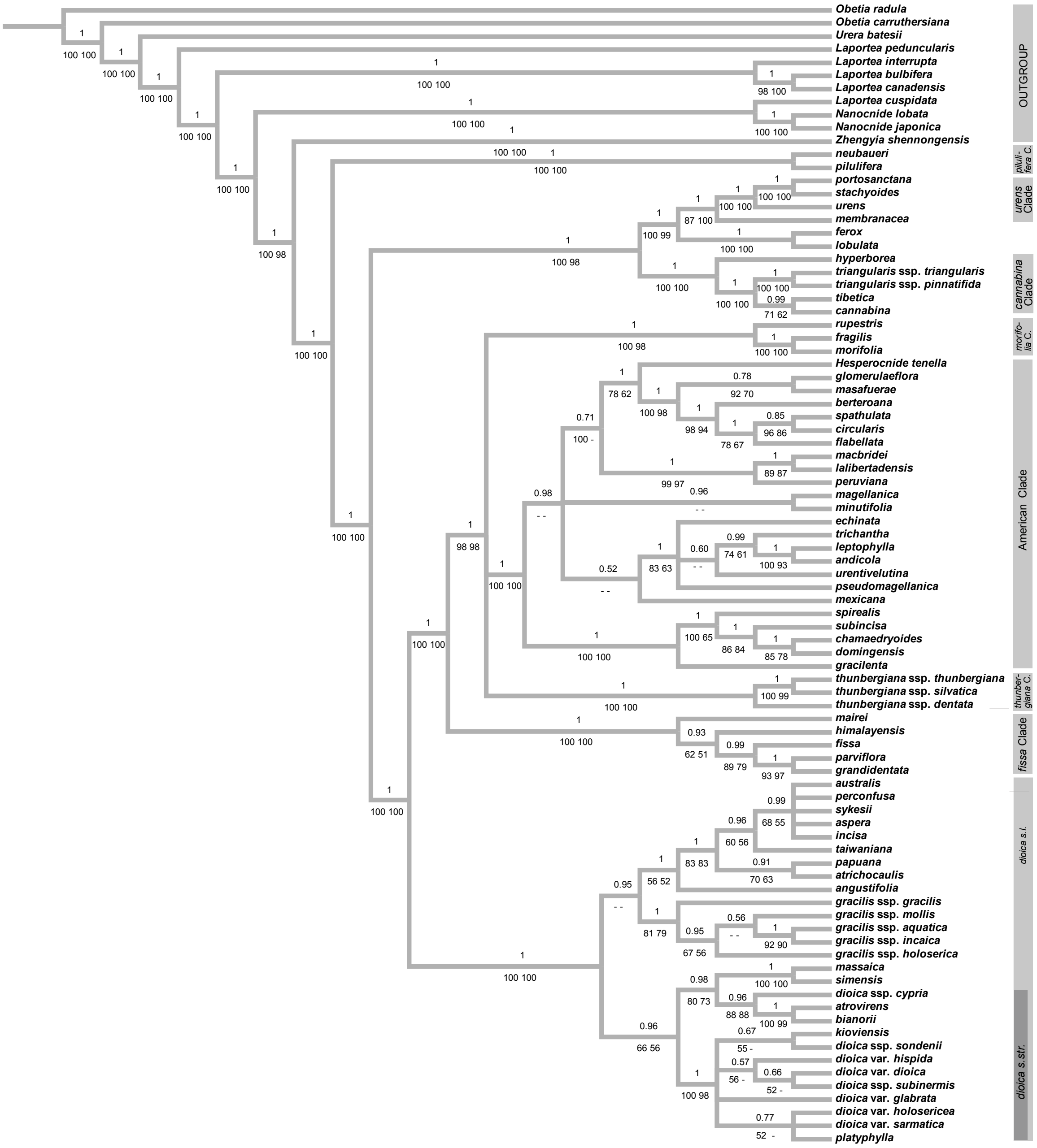


**Figure 7.5** *Urtica* phylogeny obtained from a Bayesian analysis of ITS, *trn*S–*trn*G, *psb*A–*trn*H, and *trn*L–F. Bayesian posterior probabilities are indicated above branches; bootstrap support is indicated below. The first value refers to the bootstrap support under likelihood, and the second to the parsimony analysis.

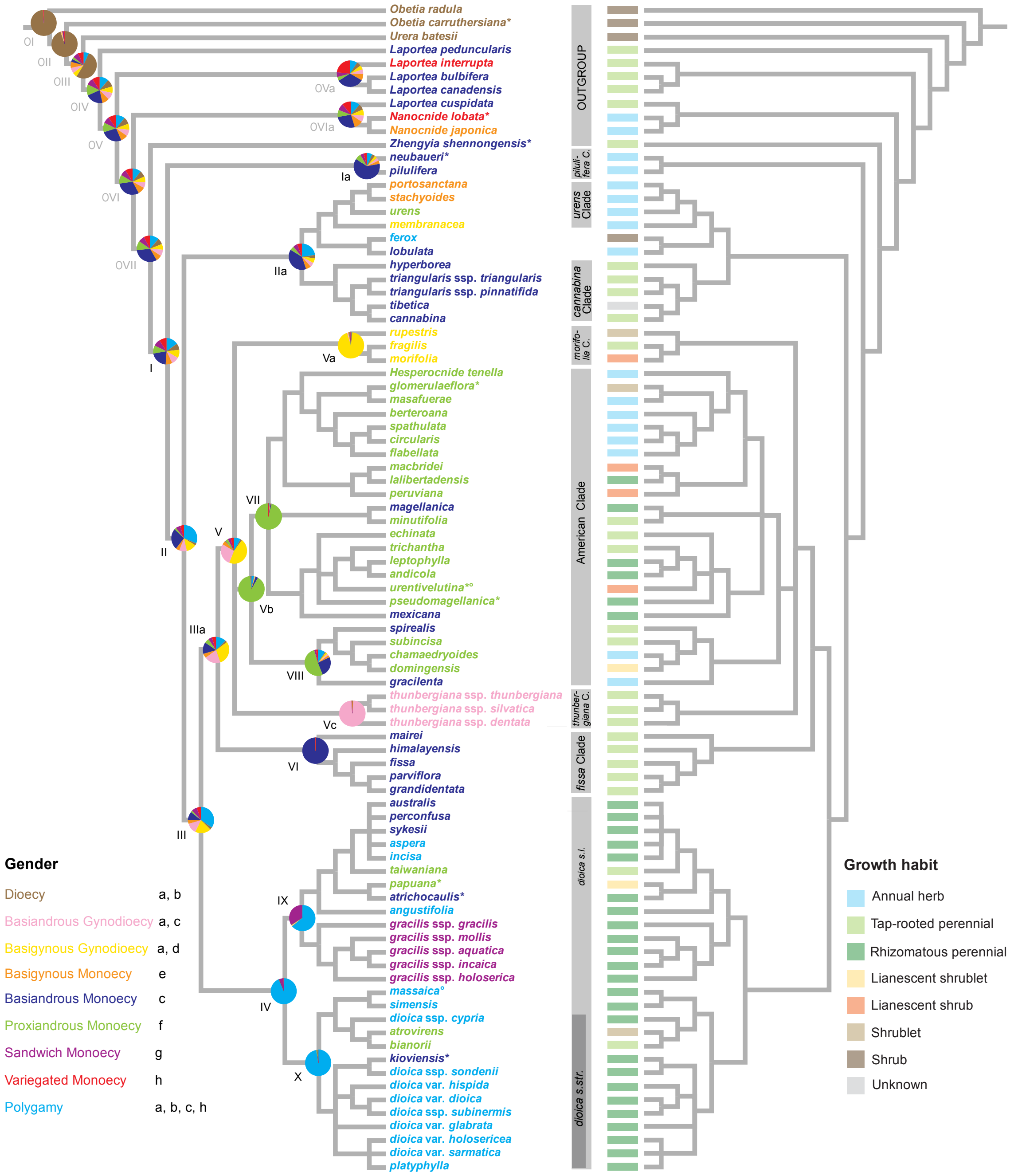


**Figure 7.6** Left: Ancestral state reconstruction of gender distribution based on the phylogenetic tree for *Urtica* and closely related genera from Grosse-Veldmann et al. (2016b). Pie charts at nodes indicate the ancestral states of the nodes. Asterisks, based on less than five herbarium specimens and no living plants analysed. Circle, gender inferred; Right: Growth habit as another classical morphological character plotted onto the mirrored tree. Color codes: dark brown – shrub, light brown – shrublet, orange – lianescent shrub, yellow – lianescent shrublet, dark green – rhizomatous perennial, light green – tap-rooted perennial, blue – annual herb (see also Tab. 7.1).

clade (Vb), but is also found in clades IIa, IX and X and is generally associated with sister taxa displaying polygamy or – more commonly – basiandrous monoecy. The two other types of monoecy are unique to one clade each: Basigynous monoecy is only recorded in the two Macaronesian species *U. portosanctana* and *U. stachyoides* (IIa) and sandwich monoecy only in *U. gracilis* (clade IX). Variegated monoecy is found in the outgroup genera *Laportea* and *Nanocnide*, and was observed in individual collections of polygamous *Urtica dioica*. Gynodioecy is found on three clades in *Urtica*, but is much rarer than monoecy. Basigynous gynodioecy is found in the *morifolia*-clade (Va) as well as in *U. membranacea* (IIa). Basiandrous gynodioecy is exclusively found in the Asian *thunbergiana*-clade (Vc). Polygamy, i.e. the occurrence of male, female and monoecious individuals in a population, is restricted to clade IV, *U. dioica s.l.* It is widely found in *U. dioica* (X), but also found in the African species *U. massaica* and *U. simensis*, as well as in northeast Asian *U. angustifolia* and the two Australian/New Zealand species *U. aspera* and *U. incisa*. There is however one earlier switch towards polygamy in one of the basal *Urtica* clades (IIa) represented by the New Zealand species *U. ferox*, where additional field observations would be highly desirable (monoecy only observed in one cultivated individual). In cultivation, populations of polygamous *U. dioica* subsp. *dioica* consist of predominantly unisexual individuals (ca. 80-90 %), ca. 10 % of the individuals are monoecious with basal inflorescences male and apical inflorescences female, and rarely (up to 5 %) male and female flowers are mixed randomly on each inflorescence.

## 7.4 Discussion

Based on the herbarium specimens analysed, our findings from cultivation and the literature data evaluated, the gender information found in the literature does not at all reflect the actual diversity of gender patterns in *Urtica*: Species cited as “dioecious”, are actually polygamous or show some type of monoecy (e.g. *U. aspera, U. fissa, U. gracilenta, U. stachyoides*) and conversely, species reported as “monoecious” in the literature, are actually gynodioecious or polygamous (e.g., *U. fragilis, U. incisa, U. platyphylla*) (e.g. Weddell 1856, 1869, Greene 1881, Thiébaut 1935, Allan 1961, Chen et al. 2003). Moreover, the characteristic expression of architectural types of monoecy, i.e., the differential distribution of male and female flowers on the flowering shoot, has been completely overlooked. Gender distribution, growth habit and leaf shape have classically been used as characters to subdivide the genus *Urtica*. But especially earlier attempts to subdivide the genus into natural units based on gender distribution (Weddell 1856, 1869; see also Grosse-Veldmann et al. 2016b: Supplementary

Tab. S1) do not reflect the actual pattern of gender distribution found in this study and are also not correlated with growth habit, which has little general phylogenetic information (see Grosse-Veldmann et al. 2016b).

Gross morphology and gender distribution appear to have evolved largely independently from each other: Virtually indistinguishable taxa such as *U. morifolia* (clade Va), *U. dioica* subsp. *dioica*, *U. platyphylla* (both clade X), *U. leptophylla* (clade VII) and *U. gracilis* (clade IX) are retrieved in widely different clades and also show divergent patterns of gender distribution – from basigynous gynodioecy (*U. morifolia*) and polygamy (*U. dioica* subsp. *dioica* and *U. platyphylla*) to proxiandrous monoecy (*U. leptophylla*) and sandwich monoecy (*U. gracilis*). Conversely, *U. bianorii* and *U. atrovirens* (clade X) are morphologically highly divergent from closely allied *U. dioica s.l.* (especially clade X), which is also reflected in their different gender patterns (proxiandrous monoecy: *U. bianorii* and *U. atrovirens* versus polygamy: e.g. *U. dioica*). *U. flabellata* and *U. leptophylla* (both clade VII) bear no similarity to each other in either leaf morphology or habit, but exhibit the same gender distribution (proxiandrous monoecy). The most surprising result in the phylogeny is the sister-relationship of the small, annual South African *U. lobulata* to the tall, shrubby New Zealand species *U. ferox* (clade IIa) which is accompanied with the gender distribution of both (basiandrous monoecy: *U. lobulata* and polygamy: *U. ferox*). Moreover, these two taxa are closely allied to a group of Macaronesian-Mediterranean annuals (clade IIa) which again have completely divergent and diverse gender distribution.

Dioecy appears to be truly absent from *Urtica*, but otherwise there has been a major diversification in the geometry of gender. Unisexual individuals are only found in polygamous taxa, respectively as female plants in basiandrous and basigynous gynodioecy. Apart from that, there have been 15 switches between different types of monoecy (basigynous, basiandrous, proxiandrous and sandwich), polygamy and gynodioecy within this genus and both predominantly annual clades (especially *urens* clade) and perennial clades (especially *dioica* clade) show four respectively five shifts. The only major clade that is relatively conserved is the American clade, where there are only very few shifts between basiandrous and proxiandrous monoecy. However, there are some general trends (compare Tab. 7.1): Most annuals (12 out of 13 taxa, including species and subspecies) across the genus are monoecious, and only one species (*U. membranacea*) is found to show gynodioecy. Tap-rooted perennials are mostly monoecious (15 out of 19 taxa), but may also be gynodioecious (4 out of 19 taxa). All lianescent shrubs (3 taxa) are monoecious. Lianescent shrublets as well as shrublets show either monoecy (2 out of 3 taxa each) or basigynous gynodioecy (1 taxon each). Even the bulk of the clonal, rhizomatous perennials (17 out of 26 taxa) are monoecious, but about one third of them (9 out of 26 taxa) are polygamous, as is the only “real” shrub found in *Urtica, U. ferox*.

There are two entirely different dimensions to the evolution of *Urtica* sexual systems – the distribution of different sexes between plants versus the geometry of their distribution on individual plants. Numerous theories have been proposed for the former (e.g. Greig-Smith 1948, Zuk 1970, Freeman et al. 1980, Heemskerk et al. 1998, de Jong et al. 2005, Glawe & de Jong 2005, 2009, Shannon & Holsinger 2007), but latter phenomenon has escaped the attention of scientists. No theories have so far been proposed for the later, since a similarly complex integration of the segregation of unisexual flowers within the inflorescence has not before been documented in flowering plants.

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# CHAPTER 8

## Limits of infraspecific differentiation of *Urtica dioica* L. (Urticaceae) based on genotyping-by-sequencing (GBS)-data

### 8.1 Introduction

*Urtica* L. (Urticaceae) is a wind-pollinated genus of nearly world-wide distribution and is commonly found at disturbed sites, but can occupy a range of natural habitats (e.g. montane forests in Macaronesia, East Africa and the Andes, or High Andean paramo and puna habitats; Friis 1993, Weigend et al., 2005). *Urtica* is quite plastic, tolerating a wide range of overall humidity and temperature conditions as well as different degrees of seasonality (Mutke et al. 2014). The pioneer genus seems to have undergone numerous dispersal-establishment events both between continents and onto different islands. The widespread occurrence of island endemics already indicates the high dispersability of *Urtica* and represents a degree of island colonization, probably unique amongst flowering plants (see Grosse-Veldmann et al. 2016b). Dispersal mechanisms in *Urtica* are diverse and range from wind dispersal (anemochory), and water dispersal (hydrochory) to animal dispersal (zoochory) which may be either epizoochorous (transport of the fruits on the outside of an animal) or even endozoochorous (transport of the fruits within an animal). The dispersal by humans is here regarded as a form of animal dispersal. Within these mechanisms, the fruits, which are small (e.g. 1–1.5 × 0.6–0.8 mm in *U. dioica*) and dry achenes, have developed a broad spectrum of different dispersal structures depending on their specific habitat (Friis 1993, Grosse-Veldmann & Weigend 2015). Fruits of high montane species (e.g., *U. trichantha* from the Peruvian High Andes or *Hesperocnide tenella* from the Sierra Nevada in California) have a dense cover of large trichomes, which are able to adhere to the coat of small mammals. Heinken & Raudnitschka (2002) investigated the diaspore diversity of vascular plants that attached to coat and hooves of roe deer (*Capreolus capreolus*) and wild boars (*Sus scrofa*) and found out that 10 % of all diaspores belonged to *U. dioica*. Fischer et al. (1996) also observed the fruits being attached to lamb's wool. An investigation of the endozoochorous seed dispersal by the European bison (*Bison bonasus*) revealed that nearly half of all seedlings recorded from dung samples belonged to *U. dioica* (Jaroszewicz et al. 2009). Even higher values (70 % of all seedlings recorded) were obtained for the donkey (*Equus asinus asinus;* Couvreur et al. 2005). Also Pakeman et al. (1999), Gill & Beardall (2001) and Eycott et al. (2007) investigated dung samples of various European wild animals (e.g. red deer, *Cervus elaphus*), and found that more than half (56 %) of all seedlings recorded belonged to three plants species (*Urtica dioica, Chenopodium album* and *Agrostis stolonifera)*, with *U. dioica* the most abundant species. Kuiters & Huiskes (2010) investigated sheep (*Ovis orientalis)* dung samples and showed that *U. dioica* seeds were found in 80 % of all recorded samples. Beyond that, *U. dioica* plays a major role in the nutrition of the nutria (*Myocastor coypus*; Prigioni et al. 2005) as well as the European beaver (*Castor fiber*; Krojerová-Prokešová 2010). Wood mouses (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) use the fruits as main food source and thus disperse them, albeit on a more local scale (Watts 1968). *Urtica* fruits are also a common food source (up to 10 %) for birds, e.g. bullfinches (*Pyrrhula pyrrhula nesa*) or passerine birds (e.g. *Emberiza cirlus;* Newton 1969, Holland et al. 2006). Some endemic *Urtica* species, e.g. from Macaronesia (*U. stachyoides*, *U. portosanctana*, *U. morifolia*) have fruits that are perfected for bird-dispersal: as soon as the fruits are humidified by the air, they produce sweet mucilage (myxocarpy) and are thus able to adhere to the feathering of birds or probably serve as a sugar source. Others, occurring on, e.g., Mallorca/Menorca (*U. bianorii*) or Sardinia/Corsica (*U. atrovirens*) have fruits that are adapted to water dispersal. *Urtica* fruits are also dispersed by wind, but wind dispersal is undirected and thus rather inefficient (Soons et al. 2008). The dispersal with or without perianth seems to be species-specific and represents the only substantial carpological difference between species. Seed dispersal is crucial for gene flow and colonization and affects changes in gene frequencies within populations and geographic plant distributions on a broader scale. Most dispersal is local, but long-distance dispersal events are probably the factors which determine the selection of dispersal structures since they enable plants to colonize unoccupied habitats and thus increase the fitness of the population (Webb 1998).

Many species and populations experience changes in environment stimulated by, e.g., long-distance dispersal, host switches, rapid climate change or the invasion of their habitat by exotic species. Thus, the expression of a flexible phenotype may be required in order to promote population persistence in changing environments (Hollander et al. 2014, Lande 2015). Phenotypic plasticity is the ability of the genotype to produce different phenotypes depending on the respective environmental conditions and requires heterogeneity in time and space to evolve (Agrawal 2001, Hollander et al. 2014). A high dispersal rate favours the evolution of phenotypic plasticity and consequently enhances gene flow (de Jong 2005, Hollander 2008, Hollander et al. 2014). Rate and distance of dispersal cause a dynamic continuum between the evolution of local adaptation and phenotypic plasticity (Hollander et al. 2014). *Urtica*, especially the widely distributed and weedy species *U. dioica*, exhibits a remarkable phenotypic plasticity, and combined with the existence of only few taxonomically useful characters, the systematics and taxonomy are problematic (Grosse-Veldmann et al. 2016b). Several recent taxonomic studies, some using phylogenetic analyses of DNA sequence data, resolved a range of taxonomic problems, especially in Eurasian and American *U. dioica* (Farag et al. 2013, Grosse-Veldmann & Weigend 2015, Grosse-Veldmann et al. 2016a, Grosse-Veldmann et al. 2016b; Henning et al., 2014; Weigend, 2005, 2006; Weigend et al., 2005; Weigend and Luebert, 2009; Weigend & Monro, 2015). Relationships at subspecies or variety level, however, remain largely unresolved with standard molecular markers. Inferring phylogenetic relationships among very closely related taxa is still challenging, especially due to the difficulty of obtaining genetic markers with enough phylogenetic signal (Escudero et al. 2014). The genotyping-by-sequencing (GBS) approach (Elshire et al. 2011) allows a targeted fraction of the genome (which is a reduced representation library) to be sequenced with next-generation sequencing (NGS) technologies, rather than the entire genome (Narum et al. 2013). The development of new sequencing technologies greatly increased the number of SNPs in many species (Poland et al. 2912) and established new possibilities to analyse phylogenetic relationships among very closely related species and the patterns of lineage sorting and historical hybridization (Escudero et al. 2014).

Due to the fact that *Urtica* apparently seems to overcome most natural barriers by a variety of dispersal mechanisms, the ability to easily establish in a changing environment, abiotic pollination, and the existence of ploidy levels up to (at least) tetraploidy, we hypothesize that *Urtica* undergoes an immense gene flow resulting in low genetic structure among populations and on species level. Consequently, morphological differences especially at subspecies or variety level might be due to phenotypic plasticity, rather than genetic divergence. The present study aims at investigating relationships within Eurasian *U. dioica s.str.* sensu Grosse-Veldmann et al. (2016b) with the help of highly resolving genotyping-by-sequencing (GBS) data. We aim at answering the following questions, 1) which subspecies and varieties identified on the basis of morphological studies can be supported by molecular data and 2) which of them represent monophyletic entitites?

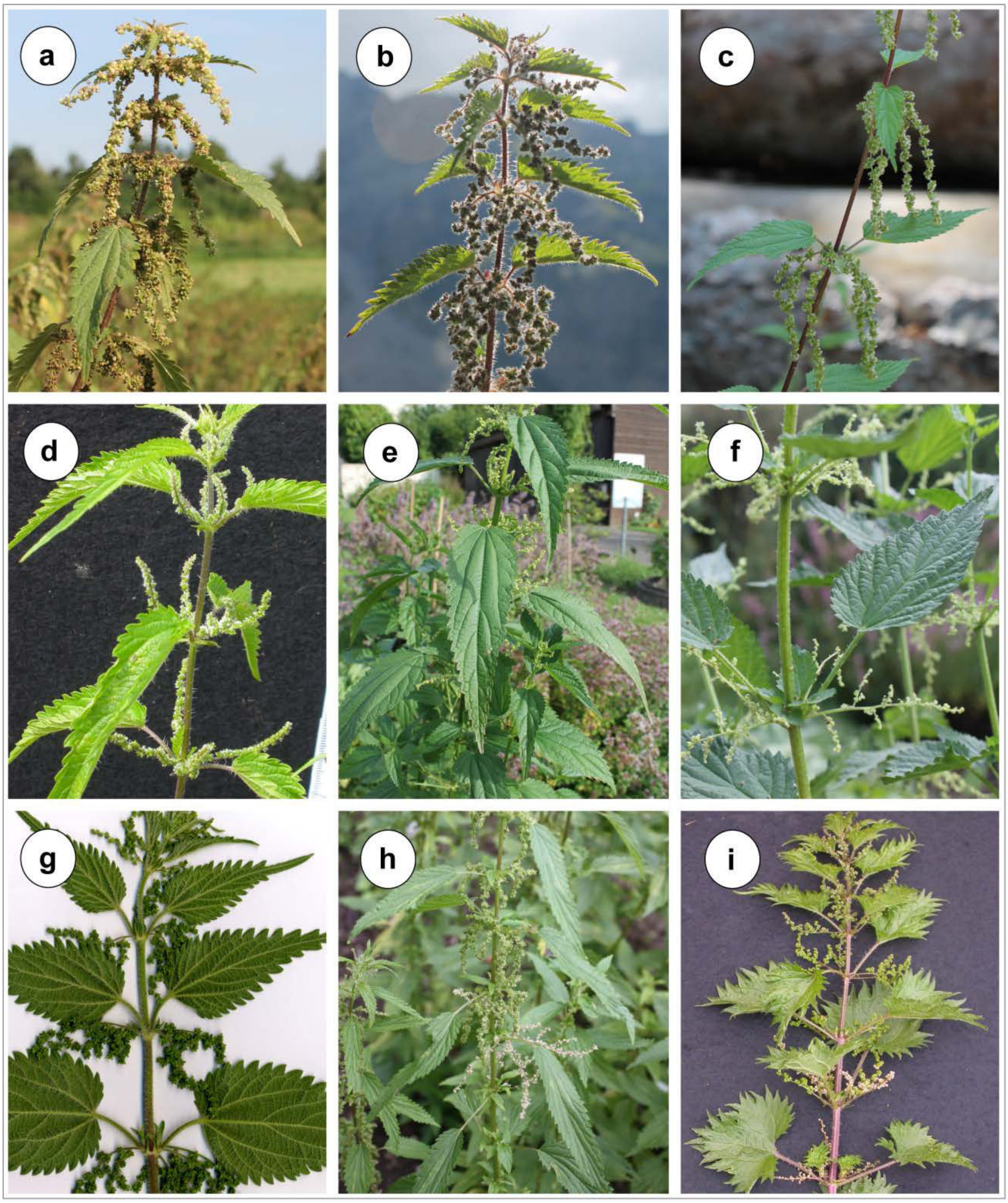


**Figure 8.1** **a**, *U. dioica* subsp. *dioica* var. *dioica*, **b**, *U. dioica* subsp. *dioica* var. *hispida*, **c**, *U. dioica* subsp. *dioica* var. *holosericea*, **d**, *U. dioica* subsp. *dioica* var. *glabrata*, **e**, *U. dioica* subsp. *subinermis*, **f**, *U. dioica* subsp. *dioica* var. *sarmatica*, **g**, *U. dioica* subsp. *pubescens*, **h**, *U. gracilis* subsp. *gracilis*, **i**, *U. atrovirens*. Plant sizes, a, c-h: up to 200 cm, b, i: up to 80 cm. Fotos: N. M. Nürk (d, i), M. Weigend (h).

## 8.2 Material and methods

### 8.2.1 Plant material and taxon sampling

Field collections carried out in various parts of Europe and the cultivation of material provided most of the plant material used for the present study. The sampling was further complemented by plant material provided by several colleagues. Plant material from the field and cultivation was silica-dried. Herbarium specimens of the respective plant material included in our analysis was checked for correct determination by both comparing it to original protologues and type specimens and by identifying it with the current floras. Representatives of taxa belonging to *U. dioica s.str.* sensu Grosse-Veldmann et al. (2016b) were sampled (Fig. 8.1). The subspecific and varietal names of *U. dioica* are based on the circumscriptions in Weigend (2005), Weigend (2006) and Grosse-Veldmann & Weigend (2015). Species were sampled from multiple parts of their range wherever possible. In total, 53 individuals were sampled including 50 ingroup taxa and 3 outgroup taxa. A complete list of the plant material used in this study including voucher information, DNA and GBS numbers as well as the respective barcodes for each sample is provided in Tab. 8.1.

### 8.2.2 GBS library preparation and high-throughput sequencing

DNA extraction was carried out using a standard DNA extraction kit (NucleoSpin® Plant II, Macherey-Nagel, Düren, Germany). DNA quantity was measured with a Qubit® 2.0 fluorometer (dsDNA HS Assay Kit, Invitrogen by Life Technologies, Carlsbad, CA, USA) and visualized on a 0.7 % agarose gel. The initial DNA-concentration was 20 ng/µl dissolved in TE-buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Genotyping-by-sequencing (GBS)-analysis was carried out at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, following the two-enzyme GBS protocol of Poland et al. (2012). Detailed instructions are given by Wendler et al. (2014), and are summarized here. Genomic DNA (200 ng per sample) was digested over night at 37°C using the rare-cutting enzyme *Pst*I-HF® (recognition site: CTGCA'G) and the methylation-sensitive enzyme *Msp*I (recognition site: C'CGG). A reverse Y-adapter with a *Pst*I restriction overhang and a forward adapter with an *Msp*I overhang containing a 7-bp-barcode for de-multiplexing in the pyRAD pipeline was ligated (22°C 60 min.) to the digested samples. As the Y-adapter contained the exact match to the reverse primer but no complement, only *Msp*I–*Pst*I fragments were amplified during the PCR (Polymerase chain reaction) step. The products were purified using SPRI (Solid Phase

**Table 8.1** List of taxa included in the phylogenetic study of Eurasian *U. dioica s.str.*

| Taxon | Country of origin | Herbarium voucher | DNA-No. | GBS-No. | Barcode |
|---|---|---|---|---|---|
| *U. atrovirens* Req. ex Loisel. | Italy (Sardinia) | M. Weigend 7800 (B) | W2227 | 886881 | GGATCAAA |
| *U. gracilis* Ait. subsp. *gracilis* | Canada | M. Weigend 9332 (B) | W4306 | 886887 | ACCAACTA |
| *U. kioviensis* Rogow. | Israel | H. Kürschner 7164 (Herb. Kürschner) | GBoL3438 | 886939 | CTCGCGCA |
| *U. platyphylla* Wedd. | Japan | T. Azuma s.n. (B) | GBoL3439 | 886940 | CTGCGACA |
| *U. dioica* L. subsp. *cypria* H. Lindb. | Cyprus | M. Weigend 8229 (B) | GBoL3423 | 886928 | GTACCGGA |
| *U. dioica* L. subsp. *cypria* H. Lindb. | Cyprus | M. Weigend 9652 (B) | W4305 | 886886 | GCTCGAAA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Italy | B. Große-Veldmann 83 (BONN) | GBoL3386 | 886889 | AACTCCGA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Italy | B. Große-Veldmann 92 (BONN) | GBoL3387 | 886890 | TTGAAGTA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Italy | B. Große-Veldmann 95 (BONN) | GBoL3388 | 886891 | ACTATCAA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Switzerland | B. Große-Veldmann 59 (BONN) | GBoL3389 | 886892 | TTGGATCA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Germany | B. Große-Veldmann & T. Henning 108 (BONN) | GBoL3406 | 886911 | CAGTACTA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Germany | B. Große-Veldmann & T. Henning 98 (BONN) | GBoL3407 | 886912 | AATAGTAA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Switzerland | B. Große-Veldmann 84 (BONN) | GBoL3409 | 886914 | TCATGGTA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Switzerland | B. Große-Veldmann 87 (BONN) | GBoL3410 | 886915 | AGAACCGA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Switzerland | B. Große-Veldmann 81 (BONN) | GBoL3411 | 886917 | TGGAATAA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Switzerland | B. Große-Veldmann 86 (BONN) | GBoL3413 | 886918 | CAGGAGGA |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Italy (Sicily) | M.& K. Weigend 7807 (B) | GBoL3426 | 886931 | AGTCAGAA |
| *U. dioica* L. subsp. *dioica* | Spain | E. Zippel 2002/2b (B) | GBoL3405 | 886910 | ATGCCGCA |
| *U. dioica* L. subsp. *dioica* | France (Corsica) | M. Weigend 8122 (B) | GBoL3385 | 886888 | CCGGTACA |
| *U. dioica* L. subsp. *dioica* | Greece | Th. Franke & P. Iosifidou 06-01 (BONN) | GBoL3415 | 886919 | AATACCTA |
| *U. dioica* L. subsp. *dioica* | Sweden | M. Weigend 8717 (BONN) | GBoL3427 | 886932 | AACTAGAA |
| *U. dioica* L. subsp. *dioica* | Poland | M. Weigend 8698 (BONN) | GBoL3429 | 886933 | CTATGGCA |
| *U. dioica* L. subsp. *kurdistanica* | Tajikistan | B. Große-Veldmann et al. 117-C (BONN) | GBoL3440 | 886941 | ACGTATGA |
| *U. dioica* L. subsp. *dioica* var. *glabrata* Clem. ex Visiani | Italy | M. Weigend 7097 (B) | GBoL3430 | 886934 | CGACGGTA |
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Switzerland | B. Große-Veldmann 65 (BONN) | GBoL3396 | 886900 | ACGCAACA |
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Switzerland | B. Große-Veldmann 71 (BONN) | GBoL3397 | 886901 | GCATTGGA |

**Table 8.1 continued.**

| Taxon | Country of origin | Herbarium voucher | DNA-No. | GBS-No. | Barcode |
|---|---|---|---|---|---|
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Switzerland | B. Große-Veldmann 74 (BONN) | GBoL3398 | 886902 | GATCTCGA |
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Switzerland | B. Große-Veldmann 88 (BONN) | GBoL3399 | 886903 | CAATATGA |
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Switzerland | B. Große-Veldmann 89 (BONN) | GBoL3400 | 886904 | TGACGTCA |
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Switzerland | B. Große-Veldmann 66 (BONN) | GBoL3421 | 886925 | ACTGGACA |
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Switzerland | B. Große-Veldmann 77 (BONN) | GBoL3422 | 886926 | AGCAGGTA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Germany | B. Große-Veldmann & T. Henning 107 (BONN) | GBoL3390 | 886893 | CGACCTGA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Germany | B. Große-Veldmann & T. Henning 99 (BONN) | GBoL3391 | 886895 | TAATGCGA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 68 (BONN) | GBoL3392 | 886896 | AGGTACCA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 80 (BONN) | GBoL3393 | 886897 | TGCGTCCA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 57 (BONN) | GBoL3394 | 886898 | GAATCTCA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 62 (BONN) | GBoL3416 | 886920 | CGAATGCA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 76 (BONN) | GBoL3417 | 886921 | TTCGCAAA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 85 (BONN) | GBoL3418 | 886922 | AATTCAAA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 61 (BONN) | GBoL3419 | 886923 | CGCGCAGA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 58 (BONN) | GBoL3420 | 886924 | AAGGTCTA |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Switzerland | B. Große-Veldmann 91 (BONN) | W4303 | 886884 | ATGGAGAA |
| *U. dioica* L. subsp. *dioica* var. *sarmatica* Zapał. | Germany | B. Große-Veldmann & T. Henning 103 (BONN) | GBoL3401 | 886906 | GATGCCAA |
| *U. dioica* L. subsp. *dioica* var. *sarmatica* Zapał. | Germany | B. Große-Veldmann & T. Henning 109 (BONN) | GBoL3402 | 886907 | CAATTACA |
| *U. dioica* L. subsp. *dioica* var. *sarmatica* Zapał. | Germany | B. Große-Veldmann & T. Henning 106 (BONN) | GBoL3403 | 886908 | AGATAGGA |
| *U. dioica* L. subsp. *pubescens* (Ledeb.) Domin | Georgia | A. Gröger et al. 208-9 (M) | ED863 | 886878 | TCGCAGGA |
| *U. dioica* L. subsp. *pubescens* (Ledeb.) Domin | Azerbaijan | G. Parolly et al. 13020 (B) | GBoL3433 | 886935 | AACCAAGA |
| *U. dioica* L. subsp. *pubescens* (Ledeb.) Domin | Georgia | A. Gröger et al. 211-17 (M) | ED858 | 886879 | CTCTGCAA |
| *U. dioica* L. subsp. *pubescens* (Ledeb.) Domin | Italy | M. Weigend 7089-C (B) | W2239 | 886880 | CCTAGGTA |
| *U. dioica* L. subsp. *sondenii* (Simmons) Hyl. | Norway | T. Alm s.n. (B) | GBoL3434 | 886936 | CGGCGTAA |
| *U. dioica* L. subsp. *subinermis* (Uechtr.) Weigend | Germany | B. Große-Veldmann & T. Henning 105 (BONN) | GBoL3404 | 886909 | CCGATTGA |
| *U. dioica* L. subsp. *subinermis* (Uechtr.) Weigend | Germany | M. & K. Weigend 8210 (B) | GBoL3424 | 886929 | GGTCAAGA |
| *U. dioica* L. subsp. *subinermis* (Uechtr.) Weigend | Germany | M. & K. Weigend 8208 (B) | GBoL3436 | 886937 | GCAGTCCA |

Reversible Immobilisation) and quantified using the Quant-iT Picogreen dsDNA assay kit (Invitrogen by Life Technologies, Carlsbad, CA, USA) and a Synergy HT microplate reader (BioTek, Bad Friedrichshall, Germany). The indexed samples were pooled in equimolar ratios. A sequencing library was constituted by the amplified sample pools, which was purified, and evaluated for fragment size and concentration. Cluster formation and 1 x 100 bp single-end sequencing-by-synthesis using the Illumina HiSeq2000 was performed according to the protocols provided by the manufacturer (Illumina Inc.).

### 8.2.3 GBS data assembling

GBS raw data (Illumina FASTQ output files) were processed for phylogenetic analyses on the Linux cluster system using the software pipeline pyRAD v.3.0.5 (available online at http://dereneaton.com/software). PyRAD (written in Python) is designed for phylogenetic analysis of any type of restriction-site associated DNA, e.g. RADseq or GBS data, allowing for the inclusion of indel variation in the alignment of loci which improves the identification of homology across highly divergent samples (Eaton 2014). PyRAD is basically composed of seven sequential steps which are summarized in Tab. 8.2. The set of parameters which yielded the highest number of loci and SNPs is here presented. Alternative settings are given after the respective parameter. Sequences were de-multiplexed allowing for one base mismatch in barcodes (maxM). Each sequence read was then quality checked and trimmed (removing barcodes, restriction sites and adapters, sequences with ambiguous ‘N’ nucleotides, and elimination of low quality reads). We tolerated 4 sites with a Phred score below 20 (NQual). A clustering similarity threshold of 90 % (=0.90) and alternatively 85 % (0.85) was applied and the minimum coverage for a cluster (MinDepth) was set to 4 (6). We tolerated 5 undetermined sites (maxN) as well as 5 heterozygous sites (maxH) in the individual consensus sequences. Due to the presence of tetraploid taxa (besides diploids), ploidy level was set to 4 (=tetraploid). We alternatively set the default value to 2 (diploid) which allows only 2 haplotypes in a consensus sequence after correcting for sequence errors and excludes consensus sequences with more than 2 alleles. The minimum number of samples in a final locus (MinCov) was set to 40 (4, 10, 15, 30). The maximum number of shared heterozygous sites (MaxSH) was set to a proportional value of 47 (3, 45, 50) which is 90 % across all samples for each locus. Reads were trimmed to shortest sequence on either side of the final locus (alternatively we allowed overhanging ends of reads in the final dataset). Finally, we made use of the “exclude taxa” option implemented in step 2 and have performed various analyses with different compositions of taxa. We excluded taxa with a low number of loci or taxa belonging to the outgroup according to Grosse-Veldmann et al. (2016b).

**Table 8.2** Overview of the seven sequential steps performed in PyRAD. Detailed descriptions to each step can be found in Eaton (2014) as well as in the corresponding user's manual available at http://dereneaton.com/software/pyrad/

| Step | Process |
|---|---|
| 1 | De-multiplexing: separates raw sequence data into separate files for each barcode; a maximum number of mismatches in a barcode can be defined |
| 2 | Quality filtering and removal of barcodes, cut sites and adapters |
| 3 | Clustering within samples and alignment: replicate sequences are collapsed into individual records and clustering is performed; the resulting clusters (stacks) are assigned to loci and aligned |
| 4 | Joint estimation of sequencing error rate and heterozygosity from the base frequencies at each site across all clusters in an individual |
| 5 | Creates consensus sequences for each cluster using error rate and heterozygosity estimated in step 4 |
| 6 | Consensus sequences are clustered across samples |
| 7 | The resulting clusters are aligned and filtered for paralogs; output files are created in a variety of readable formats (e.g. fasta, phylip, nexus, snps, loci) |

### 8.2.4 Phylogenetic inference

To infer a phylogeny from our GBS data set, we applied a supermatrix approach in which all GBS loci were concatenated into a single alignment. Missing data (Ns) were included as needed for loci with incomplete taxon sampling (de Queiroz and Gatesy, 2007). The data set was analysed employing maximum likelihood (ML), maximum parsimony (MP), Bayesian inference (BI), as well as Neighbour-joining (NJ). ML analyses were conducted with the standard settings in RAxML Version 8 (Stamatakis, 2006; Stamatakis et al., 2008). The node support under ML is based on 1000 bootstrap replicates. Parsimony analyses were conducted in PAUP 4.0b10 (Swofford 2002) using the heuristic search option. A bootstrap analysis (criterion=parsimony) was estimated based on 1000 replicates (addseq=random, swap=tbr, MaxTrees=1000). Neighbour-joining analysis was conducted in PAUP 4.0b10 (Swofford 2002) performing 1000 bootstrap replicates. Bayesian inference analyses were conducted in MrBayes vers. 3.2.2 (Ronquist and Huelsenbeck, 2003) with six independent runs of 2,000,000 generations each under the GTR + $\Gamma$ + I model with partitions unlinked. Chains were sampled every 1000th generation. Log likelihoods were examined using Tracer v1.5

(Rambaut and Drummond, 2009) in order to determine the burn-in and to ensure that an adequate effective sample size (ESS) was attained. The consensus tree and the posterior probability (PP) of clades were calculated based upon the trees sampled after the burn-in set at 500,000 generations (data not shown). TreeGraph2 (Stöver and Müller, 2010) was used for tree drawing. We used the species *U. gracilis* Ait. subsp. *gracilis* to root the final tree. The exclusion of the outgroup species (*U. gracilis* subsp. *gracilis*, *U. atrovirens*, *U. dioica* subsp. *cypria*) did not influence the result of the ingroup species, so we used the complete data set for illustration (Figs. 8.2-8.5).

The data set was also analysed employing a simple indel coding approach as advocated by Simmons & Ochoterena (2000) using the PAUP command file generated by Seqstate (Müller, 2004). According to this approach, indels were coded as binary characters in a matrix with 1 = nucleotides present, 0 = gap present, and ? = unclear if indel is present (Müller and Borsch 2005). Phylogenetic analyses were performed on bases alone (Fig. 8.2a, 8.3a), indels alone (data not shown) and with both character types combined (Figs. 8.2b, 8.3b). The combined base/indel matrix was analysed under Maximum likelihood using RaxML.

Additionally, we analysed the alleles for each sample at each locus separately (only diploid analyses are supported by pyRAD). We used the .alleles output file obtained from pyRAD and converted it to a nexus file with the help of a self-written Perl script (M. Krug, Bonn, unpublished). Allele phylogenies were generated with and without indel coding (Fig. 8.3) under Maximum likelihood using the same RaxML settings as described above.

Since traditional phylogenetic methods often fail to resolve infraspecific relationships, we additionally constructed a haplotype network using TCS (Clement et al. 2002) implemented in PopART (Leigh and Bryant 2015). TCS uses statistical parsimony to connect haplotypes based on a 95 % confidence interval. We used the default settings, with the exception that gaps were treated as missing data. We identified haplotype networks for different data sets (alleles separated, alleles not separated, with indel coding, without indel coding and with several taxa excluded). All data sets yielded essentially the same result. We used the data set with the alleles separated, without indel coding and with the outgroup species (*U. gracilis* subsp. *gracilis*, *U. atrovirens*, *U. dioica* subsp. *cypria*, *U. platyphylla* and *U. kioviensis*) excluded for illustration (Fig. 8.6).

### 8.2.5 Population structure analysis

To infer the population structure, we used tess3r which is an R package implementing TESS3, a popular algorithm for estimating spatial population structure (Caye et al. 2016; see http://membres-timc.imag.fr/Olivier.Francois/tess.html). In TESS3, ancestry populations are continuously distributed over geographic space and are estimated from genetic and geographic data. For choosing the number of ancestral populations (K), the cross-entropy criterion was applied, which is based on the prediction of a fraction of masked genotypes and on the cross-validation approach. The ploidy level was set to 4 = tetraploid. We performed runs for 16 values of K and have chosen the value of K for which the cross-entropy curve exhibited the lowest value (K=2, Fig. 8.7a). To visualize the spatial estimates of admixture coefficients for K=2, we plotted the predictions on a geographic map using a raster grid file representing Europe and the function "maps" (Jay et al. 2012; Fig. 8.7b). For the fastStructure as well as the TESS3 analysis, we considered a subset of taxa comprising European *Urtica dioica s.str*. To compare the results obtained from TESS3, we used the algorithm fastStructure (written in Python) which is able to handle large SNP genotype data rapidly based on a variational Bayesian framework (Raj et al. 2014; see also https://rajanil.github.io/fastStructure/). We ran fastStructure on our data set for each value of K (=number of populations) from 1 to 15 using the standard model with a logistic prior which should be used when population structure is difficult to resolve as well as with a simple prior for comparison (Fig. 8.8). To get an idea of the appropriate number of model components that explain structure in the data set, we additionally applied the chooseK.py algorithm which is able to calculate heuristic scores for identifiying the range of K. Results were illustrated using the Distruct package (Rosenberg 2004).

### 8.2.6 Following analyses

In order to make sure that our results are free from software artefacts, we will be reanalysing our dataset with the software Stacks (Catchen et al. 2011, 2013) and/or the recently published GIbPSs (Hapke et al. 2016) as alternatives to pyRAD. PyRAD differs from Stacks through its use of a global alignment clustering algorithm, which permits the incorporation of indel variation while identifying homology (Eaton 2014). The analysis will be conducted from step one (de-multiplexing of the raw sequence data). To the extent possible we are going to use analogous parameter settings for both programs. Additionally we are going to further analyse our data set obtained from pyRAD by varying the settings and combining them with geographical distribution data.

## 8.3 Results

### 8.3.1 GBS data and processing

Illumina sequencing produced an average of 1.20 x $10^6$ reads per sample, ranging from 4.55 x $10^5$ to 4.11 x $10^6$, which after filtering (ca. 90 % of the data passed the quality filter) was reduced to an average of 1.10 x $10^6$, ranging from 4.11 x $10^5$ to 3.77 x $10^6$ and after clustering at 90 % similarity to an average of 87145, ranging from 27622 to 393114 sequence clusters per individual. Mean depth of clusters was in average 13.168, ranging from 2.328 to 29.277. Trimming the reads to shortest sequence on either side of the final locus or allowing overhanging ends of reads did not influence the results. After removing paralogs, final filtering, and applying a minimum coverage depth of 4 with a minimum number of 40 samples in a final locus and a maximum number of 90 % shared heterozygous sites across all samples for each locus, the phylogenetic dat set contained 4013 loci and a total of 30840 SNPs (single nucleotide polymorphisms). The statistics to the data set here presented is summarized in Appendix B. The use of an alternative minimum coverage depth for a cluster of 6 as well as the use of a minimum coverage of samples in a final locus lower than 40 and a proportion of shared heterozygous sites lower than 90 % resulted in a lower number of loci and SNPs. A proportion of shared heterozygous sites higher than 90 % did not increase the number of loci and SNPs. Clustering at 85 % similarity and using the alternative depths of coverage for a cluster of 4 and 6 as well as a minimum coverage of samples in a final locus lower than or equal 40 and a proportion of shared heterozygous sites lower than or equal 90 % always resulted in data sets with a lesser number of loci and SNPs compared to the data set showing 90 % similarity. Varying ploidy levels (diploid or tetraploid) did not influence the number of loci and SNPs and did not change the position of taxa in the final phylogeny. Various combinations of taxa in the final data set were tested, e.g. by excluding taxa with a low number of loci or taxa belonging to the outgroup according to Grosse-Veldmann et al. (2016b), which slightly improved the results by increasing the support values in the final phylogeny. Thus, for a better understanding of the relationships within *U. dioica s.str.* we did not exclude the outgroup species in the phylogenetic analyses.

### 8.3.2 Phylogenetic reconstruction

We used the GBS data set from pyRAD with 4 as minimum depth coverage and clustering at 90 % similarity, as well as a minimum number of samples in a final locus of 40 and a maximum number of shared heterozygous sites of 47 which corresponds to 90 % across all samples for each locus. We included all 53 taxa (containing the outgroup species already identified by standard molecular markers in Grosse-Veldmann et al. 2016b and Henning et al. 2014) in order to be able to root the final tree and since there were no changes in topology when those taxa were excluded. The topology of the outgroup species, which are *U. gracilis* subsp. *gracilis* (Canada), *U. atrovirens* (Italy, Sardinia) and *U. dioica* subsp. *cypria* (Cyprus), could be confirmed by the means of phylogenetic inference of GBS data using ML, MP, BI and NJ (Figs. 8.2-8.5).

However, no method was able to unravel the infraspecific relationships within Eurasian *U. dioica s.str.* and resolve the ingroup. The additional inclusion of indel information as well as the separated treatment of the different alleles did not improve resolution. Also, ML analyses based on indels alone did not show increased resolution (data not shown), but was otherwise congruent with the other analyses here presented (Figs. 8.2-8.5). Only two taxa, *U. kioviensis* (Israel) and *U. platyphylla* (Japan), which were nested in Eurasian *U. dioica* in previous studies, could be separated by all phylogenetic methods here employed with high support (BS 100 %). *U. kioviensis* appears as direct sister species to the Eurasian *U. dioica s.str.* clade while *U. platyphylla* is sister to *U. kioviensis* plus the Eurasian *U. dioica s.str.* clade. Otherwise, only few tendencies of infraspecific relationships within the Eurasian *U. dioica s.str.* clade can be recorded, which however vary between the phylogenetic methods used. There is moderate ML and MP support (BS 68 resp. 69 %) of *U. dioica* subsp. *sondenii* (Norway) forming a clade with *U. dioica* subsp. *subinermis* (Germany; Figs. 8.2, 8.4), which is however not supported by the additional use of indel characters, by the separation of alleles, or by NJ (Figs. 8.2-8.5). Indel coding (with and without separation of the alleles) excludes *U. dioica* subsp. *sondenii* from the Eurasian *U. dioica s.str.* clade and unifies *U. dioica* subsp. *subinermis* with two Italian taxa (*U. dioica* subsp. *dioica* var. *dioica* and *U. dioica* subsp. *pubescens*; Figs. 8.2, 8.3). NJ does not detect any similarities between *U. dioica* subsp. *subinermis*, *U. dioica* subsp. *sondenii* and the two Italian taxa. A close relationship of all samples of *U. dioica* subsp. *subinermis* is however highly supported by all phylogenetic methods.

The topology of *U. kurdistanica* from Tajikistan and *U. dioica* subsp. *pubescens* from Azerbaijan and Georgia seems to be unclear and different phylogenetic approaches show them to be either a member of the Eurasian *U. dioica s.str.* clade or not, or retrieve them outside *U. dioica s.str.* (both with weak support). According to the MP (Fig. 8.4) and simple ML analysis (Fig. 8.2a), both taxa do not belong to the Eurasian *U. dioica s.str.* clade, while the other analyses retrieve in the clade. In the ML allele tree without indel coding (Fig. 8.3a), *U. kurdistanica* clusters together with *U. dioica* subsp. *pubescens* from Azerbaijan and Georgia, which is highly supported. Additionally, one accession of *U. dioica* subsp. *dioica* var. *sarmatica* (Germany, 886906) and one accession of *U. dioica* subsp. *dioica* var. *dioica* (Italy, 886931) appear as sister species in all analyses and occasionally two Italian accessions of *U. dioica* subsp. *dioica* var. *dioica* (886890 + 886891) cluster together. All other sister relationships seem to occur more or less random and highly method dependent. Additional ML, MP, BI and NJ analyses using alternative data sets from pyRAD analyses (data not shown) displayed largely identical topologies but lower support values (if any).

We additionally constructed a haplotype network, since these are often more suitable for the inference of infraspecific relationships among samples in contrast to phylogenetic analyses. We conducted haplotype networks for different data sets from pyRAD as well as with different approaches, i.e. with indel information, with alleles separated, and with various taxa excluded. All data sets yielded essentially the same result. We used the same data set from pyRAD as for the phylogenetic inferences combined with separate information for each allele, but without indel coding. The outgroup species (*U. gracilis* subsp. *gracilis*, *U. atrovirens*, *U. dioica* subsp. *cypria*, *U. platyphylla* and *U. kioviensis*) were excluded for data illustration (Fig. 8.6). Each of the sequences is retrieved as a single population and a clear pattern is not visible.

### 8.3.3 Population structure

For the analysis of population structure by TESS3, we used the same data set from pyRAD as for the phylogenetic analyses using a value of 4 as minimum depth coverage and clustering at 90 % similarity, as well as a minimum number of samples in a final locus of 40 and a maximum number of shared heterozygous sites of 47 which is 90 % across all samples for each locus. We excluded the extra-European taxa for the population structure analyses in order to achieve a better signal of the mainly European ingroup taxa. However, this did not change the patterns retrieved compared to an analysis including the extra-European taxa. Also, the use of alternative data sets from pyRAD analyses (data not shown) showed largely identical results. The cross-entropy curve (Fig. 8.7a) retrieves two main clusters (ancestral populations, K=2) in Europe, which are illustrated in Fig. 8.7b. One cluster contains *U. atrovirens* from Sardinia and *U. dioica* subsp. *cypria* from Cyprus, which are both inferred as closely related to Eurasian *U. dioica s.str.* in the phylogenetic studies presented here, and the second cluster contains all other accessions included in this analysis. The analysis of population structure inferred from fastStructure (not including geographical information of the accessions), retrieved an identical result of two populations (K=2). The use of different priors (simple or logistic) did not have any effect on the result.

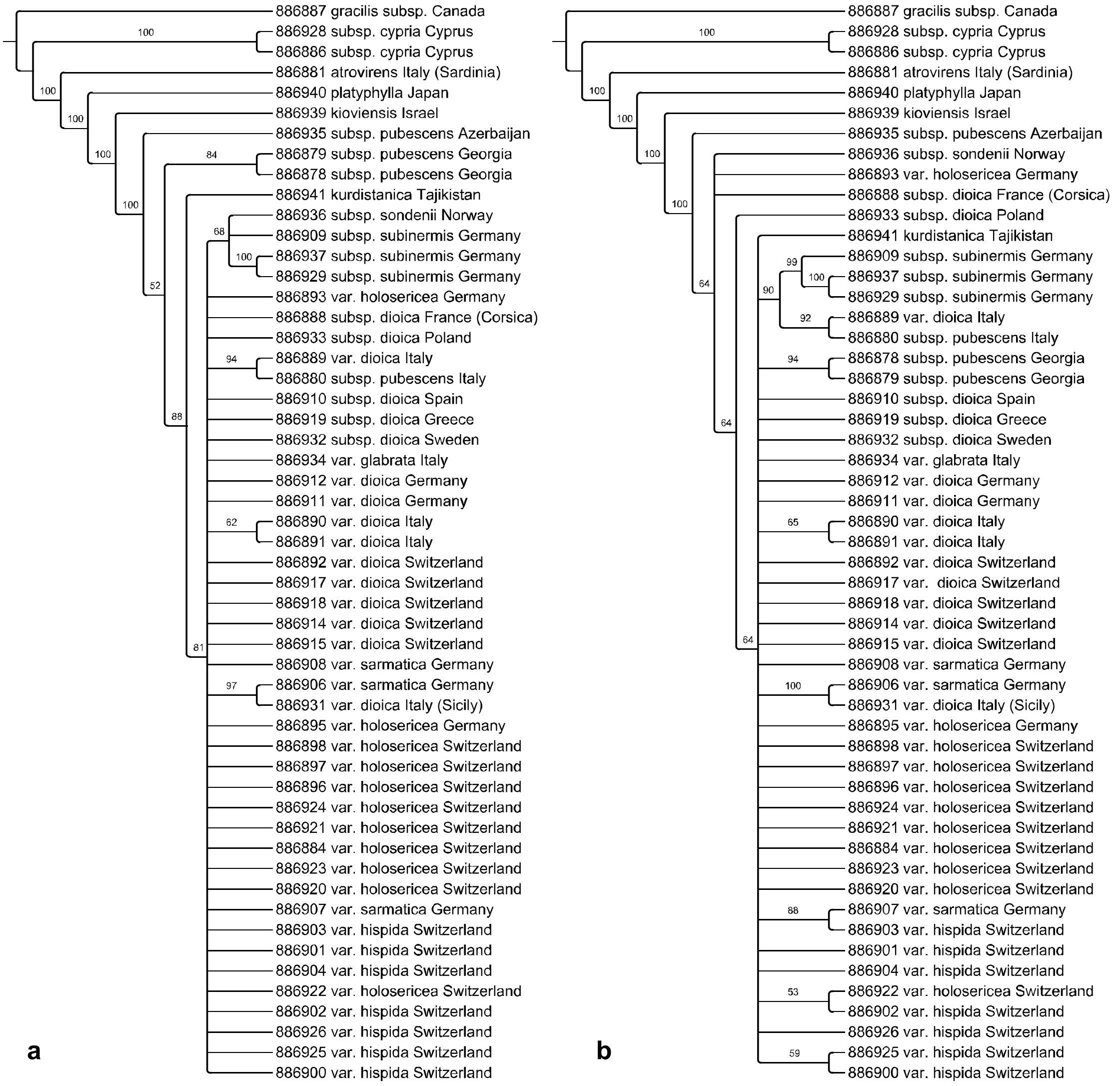
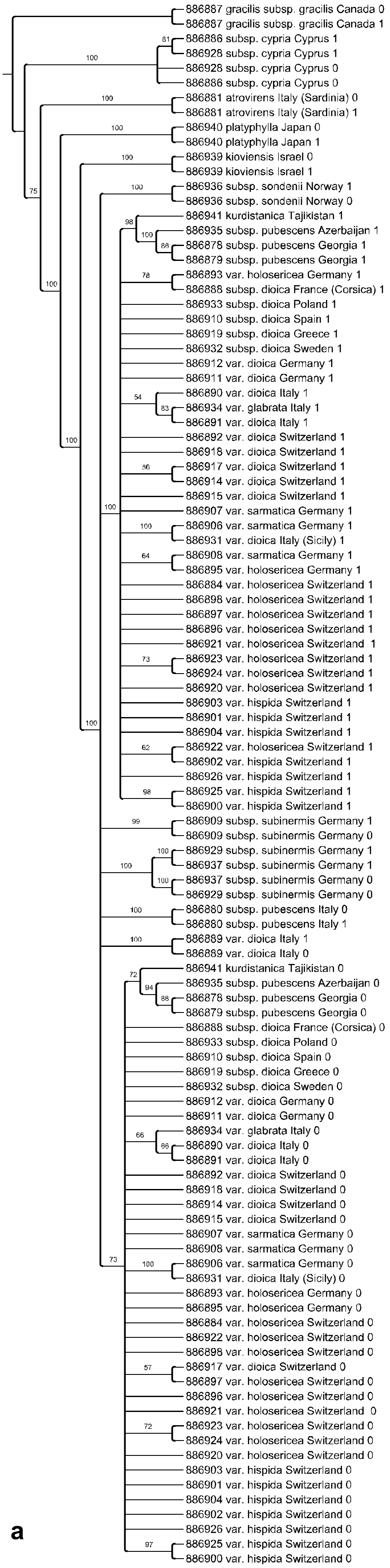


**Figure 8.2** Maximum likelihood trees based on a GBS data set from pyRAD with 4 as minimum depth coverage, clustering at 90 %, a minimum number of samples in a final locus of 40 and a maximum number of shared heterozygous sites of 90 % across all samples for each locus. Bootstrap support under likelihood is indicated above branches, **a**, simple tree without indel information, **b**, with additional indel information.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886886 subsp. cypria Cyprus 0
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886881 atrovirens Italy (Sardinia) 1
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886940 platyphylla Japan 1
886939 kioviensis Israel 0
886939 kioviensis Israel 1
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886936 subsp. sondenii Norway 0
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886935 subsp. pubescens Azerbaijan 1
886878 subsp. pubescens Georgia 1
886879 subsp. pubescens Georgia 1
886893 var. holosericea Germany 1
886888 subsp. dioica France (Corsica) 1
886933 subsp. dioica Poland 1
886910 subsp. dioica Spain 1
886919 subsp. dioica Greece 1
886932 subsp. dioica Sweden 1
886912 var. dioica Germany 1
886911 var. dioica Germany 1
886890 var. dioica Italy 1
886934 var. glabrata Italy 1
886891 var. dioica Italy 1
886892 var. dioica Switzerland 1
886918 var. dioica Switzerland 1
886917 var. dioica Switzerland 1
886914 var. dioica Switzerland 1
886915 var. dioica Switzerland 1
886907 var. sarmatica Germany 1
886906 var. sarmatica Germany 1
886931 var. dioica Italy (Sicily) 1
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886895 var. holosericea Germany 1
886884 var. holosericea Switzerland 1
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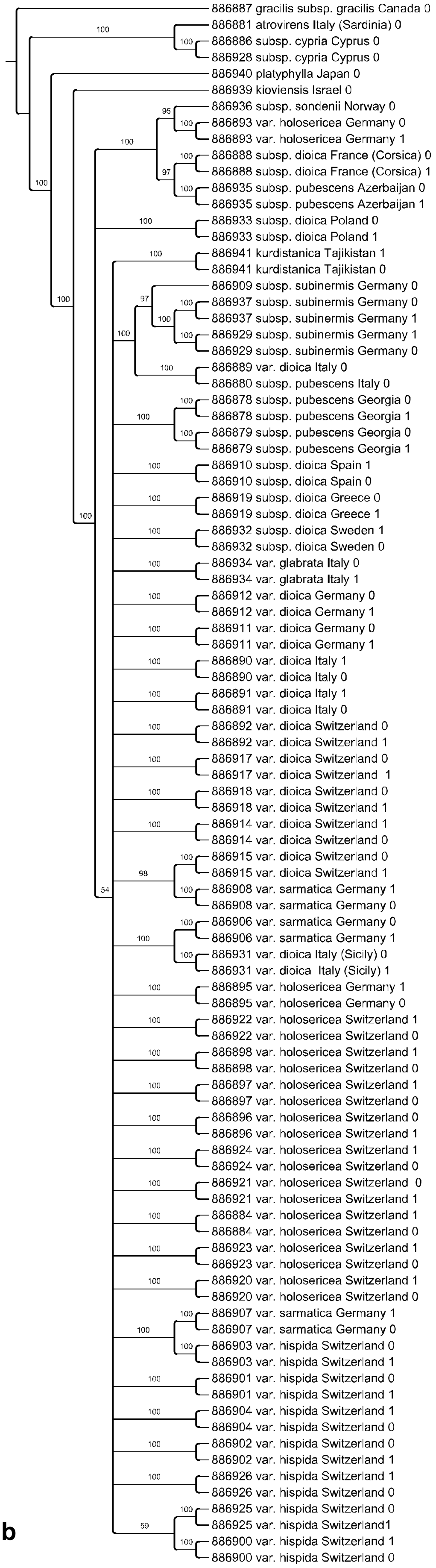
a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886925 var. hispida Switzerland 0
886925 var. hispida Switzerland1
886900 var. hispida Switzerland 1
886900 var. hispida Switzerland 0

b

**Figure 8.3** (p. 174) Maximum likelihood trees based on a GBS data set from pyRAD with 4 as minimum depth coverage, clustering at 90 %, a minimum number of samples in a final locus of 40, a maximum number of shared heterozygous sites of 90 % across all samples for each locus and the different alleles separated (= 0 & 1). Bootstrap support under likelihood is indicated above branches, **a**, tree without indel information, **b**, tree with additional indel information.

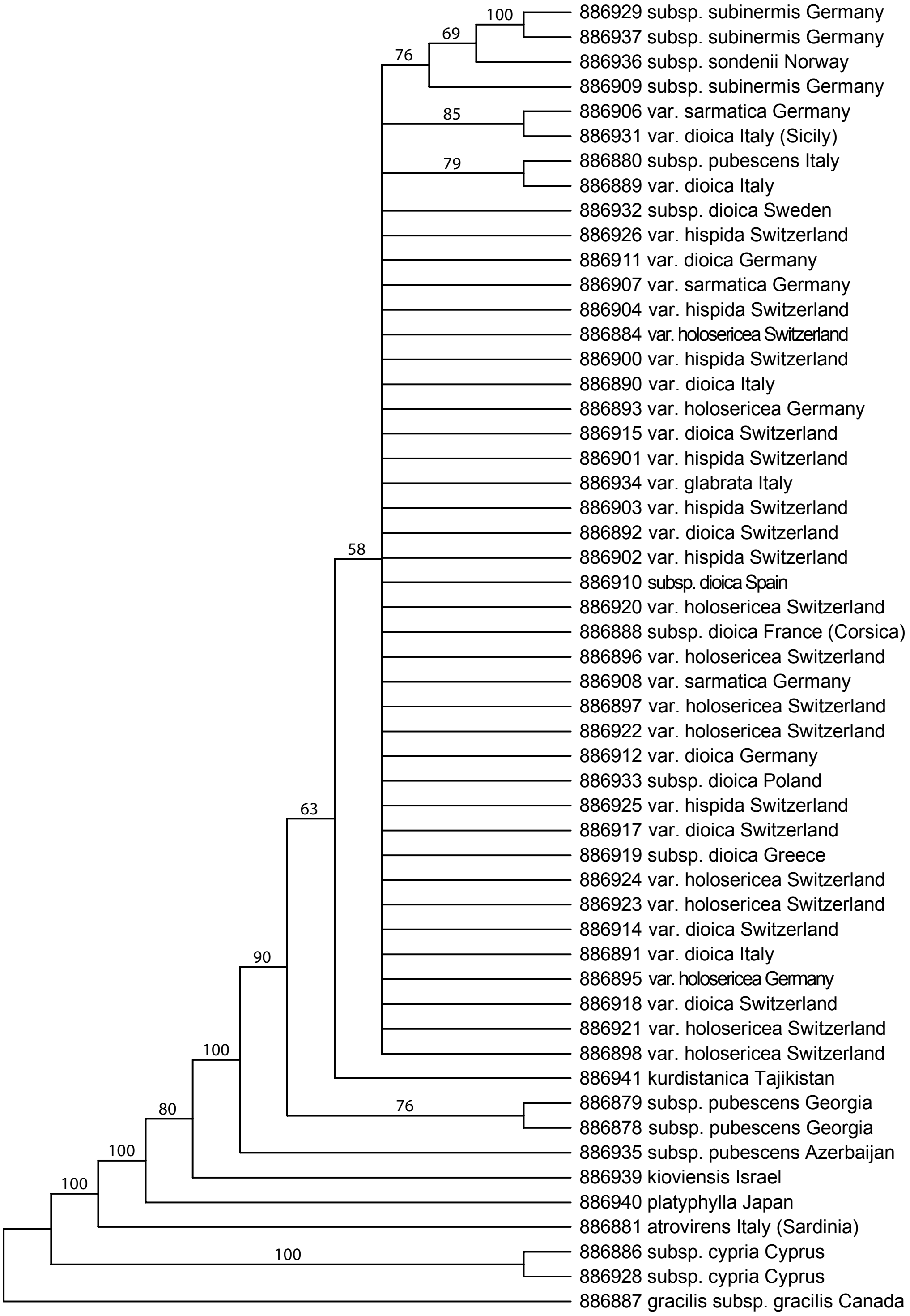


**Figure 8.4** Maximum parsimony tree based on a GBS data set from pyRAD with 4 as minimum depth coverage, clustering at 90 %, a minimum number of samples in a final locus of 40 and a maximum number of shared heterozygous sites of 90 % across all samples for each locus. Bootstrap support under parsimony is indicated above branches (conducted by F. R. Blattner).

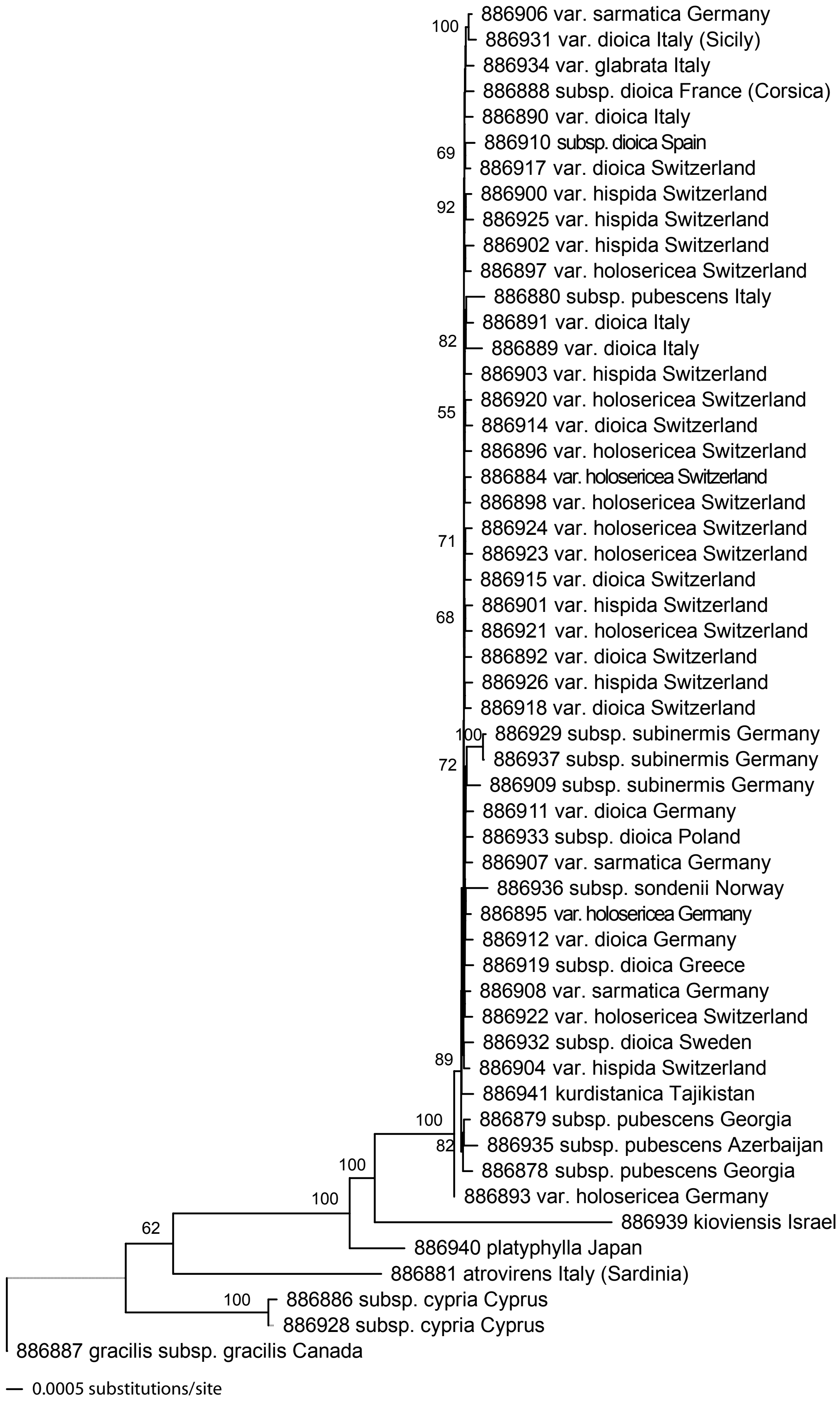


**Figure 8.5** Neighbour-joining tree based on a GBS data set from pyRAD with 4 as minimum depth coverage, clustering at 90 %, a minimum number of samples in a final locus of 40 and a maximum number of shared heterozygous sites of 90 % across all samples for each locus. Bootstrap support values are indicated above branches (conducted by F. R. Blattner).

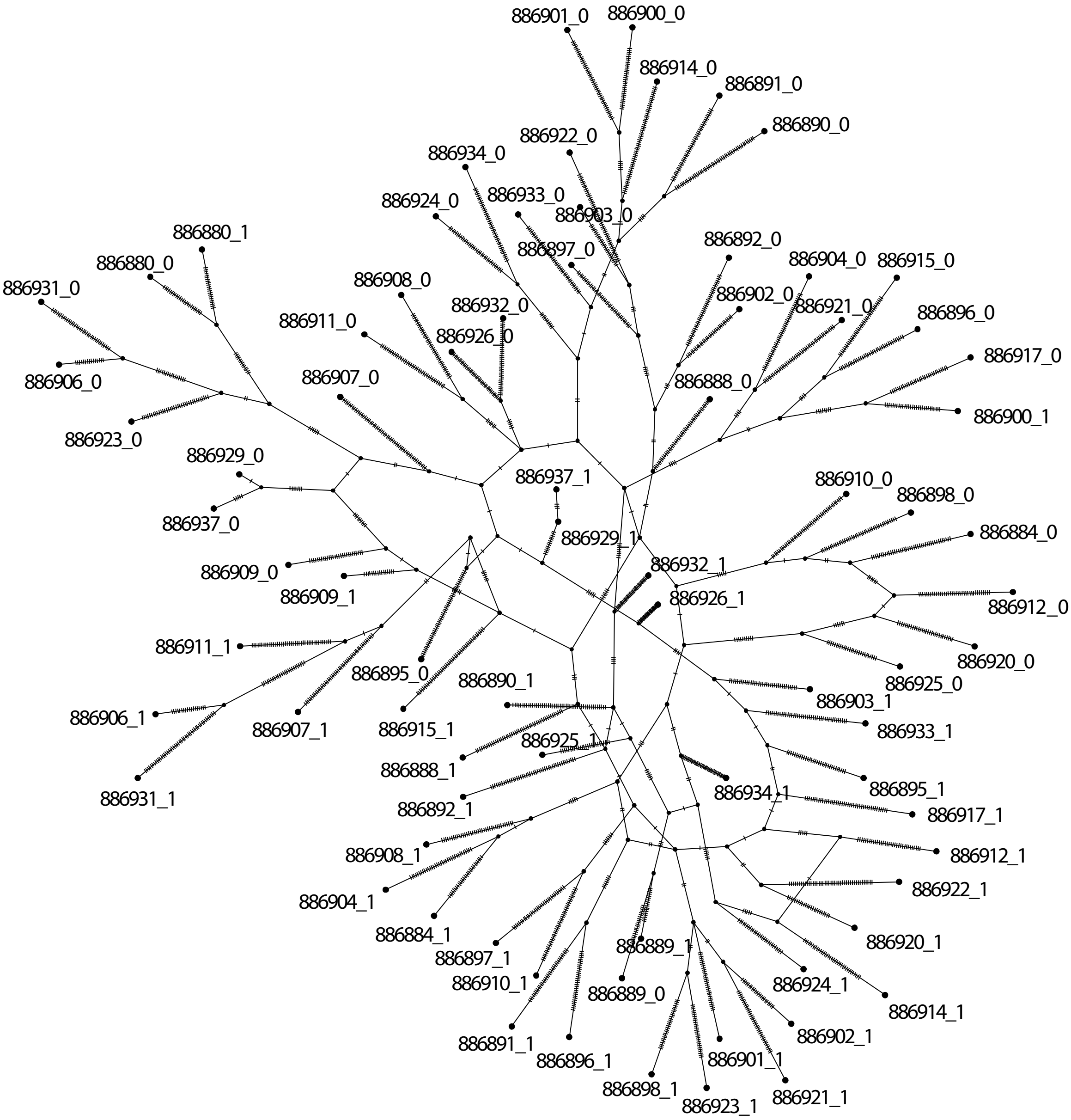
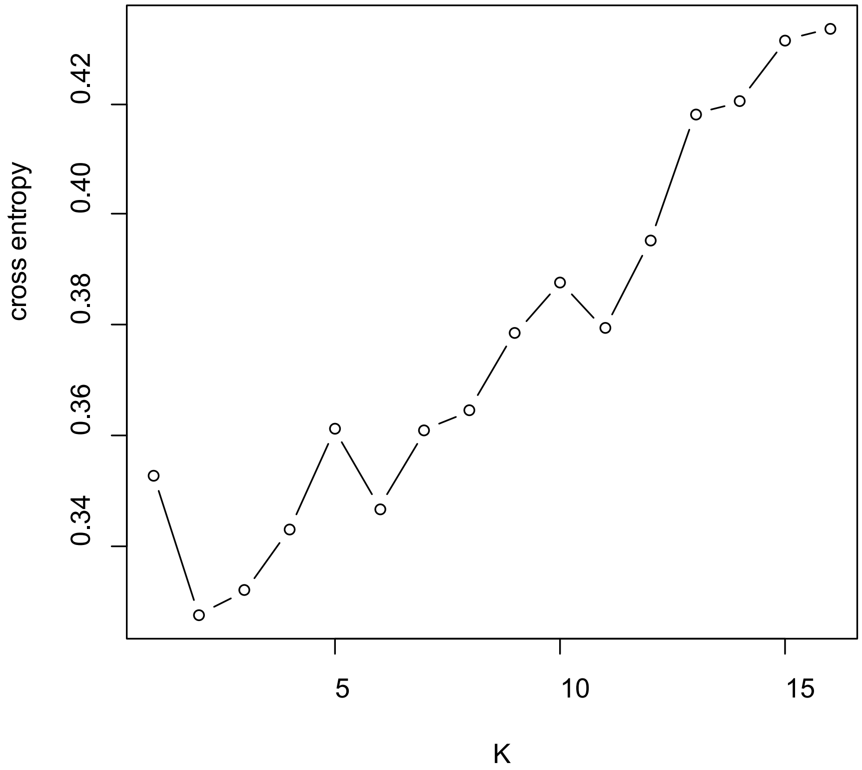


**Figure 8.6** Haplotype network using TCS implemented in PopART based on a GBS data set from pyRAD with 4 as minimum depth coverage, clustering at 90 %, a minimum number of samples in a final locus of 40 and a maximum number of shared heterozygous sites of 90 % across all samples for each locus. Alleles were separated and outgroup species excluded.

**a** **Cross-entropy curve**



**b** **Population structure (K=2)**

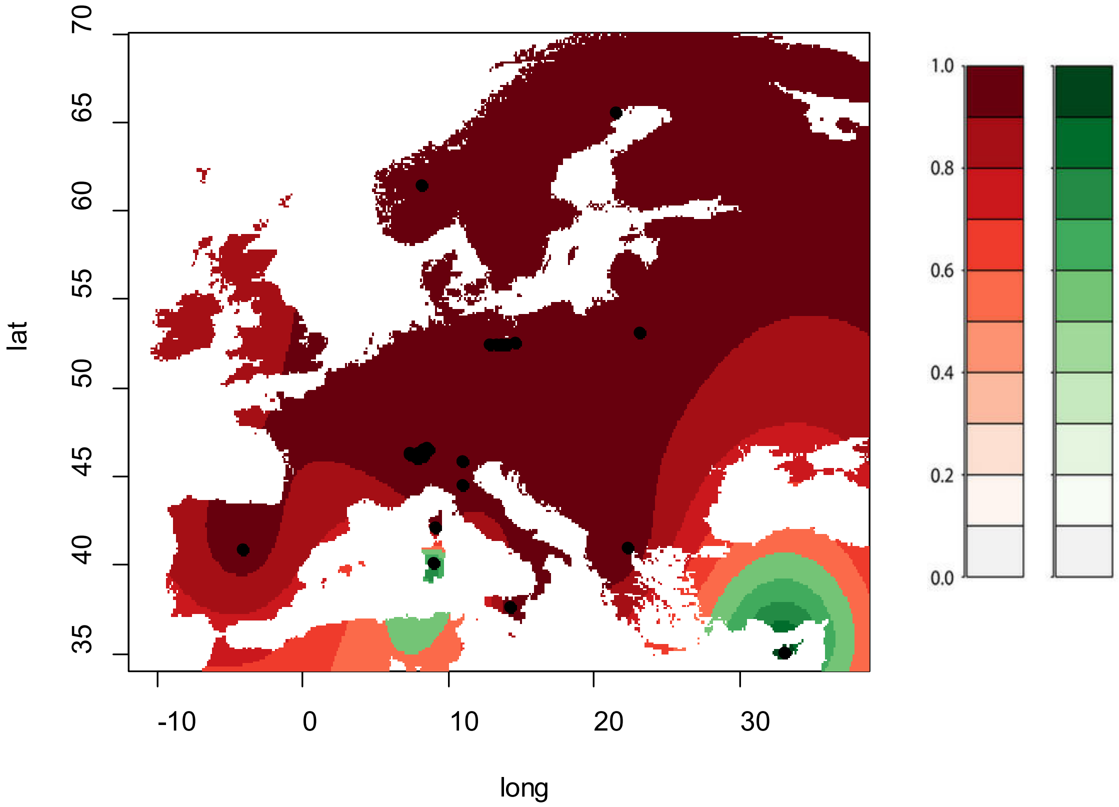


**Figure 8.7** Results of the population structure analysis with TESS3, **a**, Cross-entropy plot for the number of cluster K=1-16. The retained value of K is 2, **b**, Geographic map of ancestry coefficients using K=2 ancestral populations. Red = population 1, green = population 2.

**Population structure (K=2)**

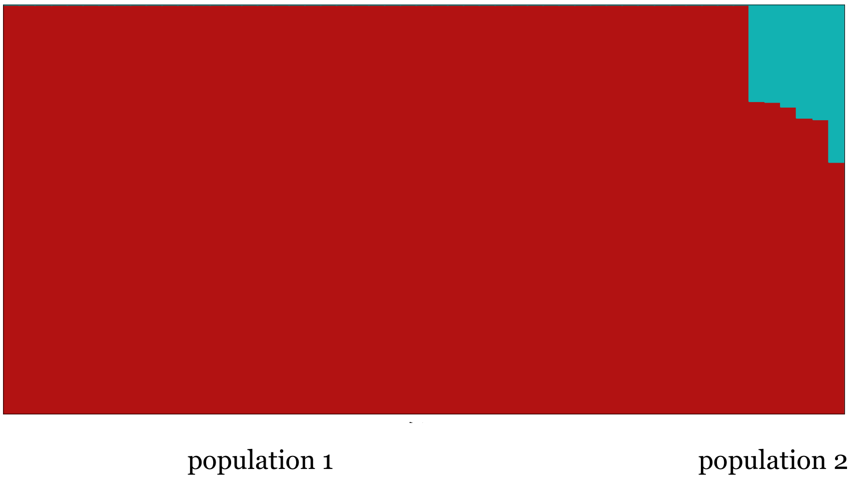


**Figure 8.8** Result of the population structure analysis with fastStructure. The retained value of K is 2. Red = population 1, blue = population 2.

## 8.4 Discussion

Our results show that a remarkable amount and quality of data through high-throughput Illumina sequencing was retrieved (over 4000 loci and over 30000 SNPs). However, the genotyping-by-sequencing (GBS) approach failed to resolve the infraspecific relationships of Eurasian *U. dioica s.str.* (Urticaceae). Previous results of phylogenetic relationships among these taxa based on standard molecular markers including the nuclear marker ITS as well as three chloroplast markers (*trn*S–*trn*G, *psb*A–*trn*H, *trn*L–*trn*F; see Farag et al. 2013, Grosse-Veldmann et al. 2016b, Henning et al. 2014) yielded essentially the same results. One notable difference is that two taxa could be reliably separated from the Eurasian *U. dioica s.str.* clade, namely *U. kioviensis* and *U. platyphylla*. This result was consistent in all ML, MP, BI and NJ phylogenies, while our four-marker analysis failed to resolve this relationship (see Grosse-Veldmann et al. 2016b). The additional use of indel information did not influence the results significantly, although Freudenstein & Chase (2001) state that indels can have a significant effect on the results of a phylogenetic analysis. *Urtica* is distributed nearly world-wide and seems to overcome most natural barriers by a variety of dispersal mechanisms, and as a weed, it is able to easily establish in a changed environment. Moreover, *Urtica* is wind-pollinated and shows ploidy levels up to (at least) tetraploidy. Chromosome counts in *Urtica* range from 2n=24, 26, 32, 48, 49, 52, 76, 78 (Funabiki 1958, Sokolovskaya 1966, Woodland et al. 1982, Zhukova 1967). The results presented here support our hypothesis that *Urtica*, especially *U. dioica*, undergoes an immense gene flow resulting in a high recombination rate, and that morphological differences traditionally recognized at subspecies and variety level go back both to local directional selection and owed phenotypic plasticity. It has long been known that the various subspecific entities proposed, especially in *U. dioica*, are connected by a more or less continuous series of morphological intermediates in nature (Grosse-Veldmann & Weigend 2015). It may therefore not be too surprising that the varieties and subspecies of Eurasian *U. dioica s.str.* provisionally identified by morphological data in previous studies (e.g. Grosse-Veldmann & Weigend 2015, Weigend 2005, Weigend 2006) cannot be confirmed by the molecular data here retrieved it is impossible to detect population structures (see Figs. 8.6-8.8). Morphologically similar populations – the "named ecotypes" of Grosse-Veldmann & Weigend (2015) – thus seem to arise in response to local environmental conditions, but fail to diverge from the common gene pool nor do they develop any isolation mechanisms. In contrast to *Urtica*, GBS analyses succeeded in some other plant taxa: Escudero et al. (2014) resolved the phylogenetic relationships of seven closely related species in the genus *Carex* sect. *Ovales* (Cyperaceae) based on >3000 loci and > 1300 SNPs using ML and BI inference. They received a highly supported phylogeny, but found limited evidence of interspecific gene flow. *Carex* exhibits several paralleles to *Urtica,* e.g., it is also wind-pollinated, distributed worldwide with a center of diversity in the temperate zones of the northern hemisphere and is able to colonize a wide range of habitats (Escudero et al. 2009, Hipp et al. 2006). The fruits are also achenes which may be distributed by wind, water and animals (Escudero et al. 2009, 2014). Beyond that, *Carex* exhibits a remarkable chromosomal diversity with ploidy levels up to polyploidy (Hipp et al. 2007, 2009). Wendler et al. (2014) investigated the genetic diversity of three diploid introgression lines of cultivated barley containing chromosomal segments of its close relative *Hordeum bulbosum* and retrieved large numbers of SNPs enabling the precise allocation of *H. bulbosum* introgressions and the identification of recombinant plants. Narum et al. (2013) provide an overview of further studies using the GBS approach in ecological and conservation genomics, most of them however examining marine invertebrates, small mammals and fungi. Apart from Eurasian *U. dioica s.str.* which has been analysed here, two other groups within *Urtica* appear to have experienced recent radiations, the American clade and the Australasian taxa. Future studies must reveal, whether these groups are subject to large-scale genetic exchange to similar degree.

*Supplementary data associated with this chapter can be found in Appendix B.*

# SUMMARY

**Große-Veldmann, Bernadette. 2016.** Systematics, Taxonomy, and Evolution of *Urtica* L. (Urticaceae). Ph.D. thesis, Mathematisch-Naturwissenschaftliche Fakultät, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany.

The genus *Urtica* L. (Urticaceae) is of subcosmopolitan distribution, found on all continents (except the Antarctica) and most extratropical islands and ranges from Alaska to Patagonia, Spitzbergen to the Cape and Camtschatka to the subantarctic islands. *Urtica* is commonly found in anthropogenic habitats as a weed, but can occupy a range of natural habitats and is found in montane forests in Macaronesia, East Africa and in the Andes, but also in High Andean paramo and puna habitats at over 4500 m a.s.l. The systematics of *Urtica* have puzzled scientists for the past 200 years and no single comprehensive attempt at understanding infrageneric relationships has been published in the past, nor have species delimitations been unequivocally established.

The major aims of this study are to clarify some taxonomic problems in morphologically difficult taxa with a special emphasis placed on the European, Australian/New Zealand and Asian species. In addition, the overall phylogeny of *Urtica* based on a comprehensive sampling of the species and subspecific entities in the genus was addressed and based on this, a re-examination of the gender distribution as an important and probably unique morphological feature within angiosperms.

In Europe, over 70 infrasubspecific names referable to the widespread and often weedy *U. dioica* subsp. *dioica* have been used in one form or another. Many of these names are however invalid and/or superfluous. The present study identifies a total of five morphotypes of *U. dioica* subsp. *dioica* (-var. *dioica*, -var. *hispida,* -var. *sarmatica,* -var. *holosericea,* and -var. *glabrata*) which are stable in cultivation and usually occur in several different regions of Europe and/or are characterized by some type of habitat preference. These morphotypes in their most characteristic expression are quite well differentiated, but are connected by a continuous series of intermediates in nature (Chapter 2).

Taxon differentiation in *Urtica* from Australia and New Zealand initially appears to be uncomplicated, with taxa being easy to distinguish. However, a revision of the type material, more recent collections and a comparison of Australian and New Zealand material shows that three of the names are misapplied. The third chapter thus addresses a redefinition of *U. incisa* and allies in New Zealand and Australia, including the segregation of two new species *U. sykesii* and *U. perconfusa*. In addition, evidence for the presence of true introduced *U. dioica* subsp. *dioica* in New Zealand was found, but not for *U. gracilis* (as *U. dioica* subsp. *gracilis,* North America). Rather, New Zealand specimens assigned to the putatively introduced northern hemisphere *U. gracilis* belong to polygamous Australian *U. incisa*, which is also retrieved by molecular data. There are thus six native species of *Urtica* in New Zealand, four of them endemic, and two also indigenous in Australia.

*Urtica* from Asia is also plagued with problems in taxon differentiation due to the limited diversity of taxonomically useful characters combined with a broad range of phenotypic variation. Based on a critical re-examination of morphological characters combined with molecular data, a total of four subspecies of the eastern Asian species *Urtica thunbergiana* is proposed: subsp. *thunbergiana,* subsp. *dentata* (Hand.-Mazz.) K.Becker & Weigend*,* subsp. *silvatica* (Hand.-Mazz.) K.Becker & Weigend*,* and subsp. *perserrata, subspec. nov.* (Chapter 4). Moreover, a total of five species and two subspecies are recognised within the East-Southeast Asian *Urtica fissa*-clade: *U. fissa* E.Pritz ex Diels*, U. grandidentata* Miq. subsp. *grandidentata, U. grandidentata* Miq. subsp. *lombok* K.Becker & Weigend*, U. himalayensis* Kunth & C.D.Boché*, U. mairei* Lév. and *U. parviflora* Roxb. (Chapter 5). The systematic re-arrangements are based on morphological analyses and a highly resolved phylogeny based on a four marker-analysis including ITS1–5.8S–ITS2, *psb*A–*trn*H, *trn*L–*trn*F and *trn*S–*trn*G.

Chapter 6 provides the first comprehensive phylogeny of the genus *Urtica* including 61 of the 63 species recognized, represented by 144 ingroup accessions and 14 outgroup taxa. The phylogeny is based on a four marker analysis comprising one nuclear and three plastid regions: ITS1–5.8S–ITS2, *psb*A–*trn*H intergenic spacer (IGS), *trn*L–*trn*F (including the *trn*L group I intron and the *trn*L–*trn*F IGS), and *trn*S–*trn*G (including the *trn*S–*trn*G IGS and the *trn*G group II intron). The phylogenetic analyses retrieve numerous well-supported clades and suggest that the (limited) morphological characters used to distinguish and group species in the genus (e.g. leaf morphology and growth habit) do not reflect phylogenetic relatedness. Eastern Asian *Zhengyia shennongensis* is retrieved as sister to *Urtica* and western Eurasian *U. pilulifera* and *U. neubaueri* are sister to all other species of the genus. A widespread occurrence of island endemics indicates a high dispersability of *Urtica*. The genus seems to have undergone numerous dispersal-establishment events, both between continents and onto different islands. Three recent species radiations are inferred, one in America centered in the Andes, one in New Zealand, and one in western Eurasia.

The evolution of *Urtica* sexual systems has so far been treated very generically in the literature; our studies reveal two entirely different dimensions to this phenomenon – the distribution of different sexes between plants versus the complex geometry of their distribution on individual plants (Chapter 7). Numerous theories have been proposed for the former phenomenon, but the geometry of gender distribution on individual plants has escaped the attention of scientists. We here provide a descriptive and evolutionary framework for gender distribution in *Urtica*. The analyses are based on both extensive morphological studies (based on the investigation of more than 1800 herbarium specimens, the cultivation of 1/3 of the species recognized, and literature studies) and a near-comprehensive phylogeny of the genus retrieved in chapter 6. We found five different architectural types of monoecy and two types of gynodioecy, apart from polygamy. A total of 15 switches appear to have taken place within the genus. Gender distribution and gross morphology appear to evolve largely independently from each other and gender distribution is largely independent of growth habit.

The potential of highly resolving next-generation-sequencing-data turned out to be limited in *Urtica* (Chapter 8). Even though a remarkable amount and quality of data was retrieved (over 4000 loci and over 30000 SNPs), genotyping-by-sequencing (GBS) failed to resolve the infraspecific relationships of Eurasian *U. dioica s.str*. Previous results of phylogenetic relationships among these taxa (Chapter 6) based on standard molecular markers including the nuclear marker ITS as well as three chloroplast markers (*trn*S–*trn*G, *psb*A–*trn*H, *trn*L–*trn*F) yielded essentially the same results. One notable difference is that two taxa which could not be separated from *U. dioica s.str.* in previous analyses, could be reliably separated from the Eurasian *U. dioica s.str.* clade by GBS data, namely *U. kioviensis* and *U. platyphylla*.

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# APPENDIX

## Appendix A to Chapter 6

**Appendix A1.** Conspectus of Weddell's "classifications" (1856 & 1869) of the genus *Urtica* L.

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**Weddell (1856)**

§ I. Paniculae androgynae
A. Stipulae inter petiolos utrinque geminae, omnino liberae vel ima basi tantum coadunatae.
a. Cymae fructiferae petiolo plerumque breviores.
1. ***U. urens*** L.
α. stimulosa
β. prorsus inermis
2. ***U. andicola*** Wedd.
3. ***U. chamaedryoides*** Pursh
α. *angustifolia*
β. *latifolia*
4. *U. glandulifera* Liebm.[1]
5. ***U. subincisa*** Benth.
β. *angustifolia*
6. *U. falcicrenata* Liebm.
7. *U. meyeri* Wedd.[2]
8. ***U. echinata*** Benth.
9. ***U. flabellata*** Kunth
10. ***U. spathulata*** Sm.
11. ***U. mexicana*** Liebm.
12. *U. sandwicensis* Wedd.[3]
b. Cymae fructiferae petiolo longiores.
13. ***U. hyperborea*** Jacquem. ex Wedd.
14. ***U. atrovirens*** Req. ex Loisel.
α. *floribunda*
γ. *angustifolia*
15. ***U. magellanica*** Poir.
α. foliis glabriusculis
β. foliis pubescentibus
16. *U. nicaraguensis* Liebm.[4]
17. ***U. stachyoides*** Webb & Berthel.
B. Stipulae utrinque inter petiolos solitariae, nempe duae in unam coalitae.
18. *U. ballotaefolia* Wedd.[4]

**Weddell (1869)**

§ 1. Flores masc. et fem. in inflorescentiis omibus aut rarius in intermediis solum intermixti.
* Inflorescentiae petiolo breviores, saepe glomeruliformes
1. ***U. urens*** L.
β. *parvifolia*
γ. *iners*
2. ***U. flabellata*** Kunth
3. ***U. spathulata*** Sm.
4. ***U. echinata*** Benth.
α. foliis ovatis grosse dentatis [...]
β. *echinata*
γ. ***berteroana***[11]
δ. ***trichantha***[12]
5. ***U. masafuerae*** Phil.
6. ***U. chamaedryoides*** Pursh
α. foliis plerisque ovato-lanceolatis [...]
β. *latifolia*
γ. *parvifolia*
δ. *orizabae*
7. *U. glandulifera* Liebm.[1]
8. ***U. subincisa*** Benth.
β. *angustifolia*
γ. *floribunda*
9. *U. meyeri* Wedd.[2]
α. foliis late cordato-ovatis [...]
β. *lobulata*
** Inflorescentiae saltem fructiferae petiolo longiores.
10. ***U. hyperborea*** Jacquem. ex Wedd.
11. ***U. stachyoides*** Webb & Berthel.
12. ***U. atrovirens*** Req. ex Loisel.
β. *floribunda*
γ. *angustifolia*
13. ***U. spirealis*** Blume

## Appendix A1 continued.

**Weddell (1856)**

α. foliis vix acuminates […]
β. *macrostachya* […]
§ II. Inflorescentiae unisexuales
A. Stipulae utrinque inter petiolos geminate, omnino liberae vel basi vix connatae.
a. Glomeruli feminei capitati, saepe solitarii.
19. ***U. pilulifera*** L.
(20. *U. dodartii* L.)
b. Glomeruli omnes spicati vel paniculati.
21. ***U. cannabina*** L.
22. ***U. dioica*** L.
α. *vulgaris*
subvar. *umbrosa*[5]
subvar. *montana*
β. ***hispida***
subvar. *horrida*
subvar. *duplicato*-serrata
γ. ***pubescens***[6]
δ. *angustifolia*
ε. *subincisa*
ζ. *sicula*
η. ***mollis***[7]
θ. ***capensis***
ι. *procera*[8]
κ. ***kioviensis***[9]
23. ***U. aquatica*** Liebm.[10]
24. ***U. thunbergiana*** Siebold & Zucc.
25. ***U. incisa*** Poir.
α. foliis lineari-lanceolatis, basi acutis
β. foliis oblongo-ovatis, basi truncatis
γ. foliis cordato-ovatis
26. *U. bracteata* Steud.
27. ***U. rupestris*** Guss.
28. ***U. lobulata*** E.Mey.
B. Stipulae utrinque inter petiolos solitariae, integerrimae vel apice bifidae.
29. ***U. parviflora*** Roxb.
α. foliis ovatis, subduplicato-serratis
β. foliis ovatis-lanceolatisve […]
30. ***U. platyphylla*** Wedd.
31. ***U. simensis*** Hochst. ex A.Rich.
32. ***U. australis*** Hook.f.
33. ***U. ferox*** G.Forst.

**Weddell (1869)**

α. foliis inferioribus ovalis […]
β?. *ligulata*
14. ***U. magellanica*** Poir.
β. *glomeruliflora*
γ. *bracteata*
15. *U. ballotaefolia* Wedd.[4]
β. *macrostachya*[13]
§ 2. Inflorescentiae unisexuales.
* Stipulae inter petiolos utrinque binae, omnino liberae aut basi vix connatae.
16. ***U. pilulifera*** L.
subvar. *balearica*
subvar. *chesneyana*
β. *dodartii*
17. ***U. cannabina*** L.
18. ***U. rupestris*** Guss.
19. ***U. dioica*** L.
α. *vulgaris*
subvar. *umbrosa*[5]
subvar. ***hispida***[14]
subvar. *horrida*
subvar. *duplicato-serrata*
subvar. ***glabrata***[15]
β. *eckloniana*[16]
γ. ***platyphylla***[17]
δ. ***kioviensis***[9]
ε. *galeopsifolia*[6]
ζ. ***pubescens***[6]
η. ***mollis***[7]
θ. *diplothricha*
ι. *pycnantha*
κ. *steudelii*
λ. ***angustifolia***
μ. *subincisa*
ν. *procera*[8]
subvar. *duplicato-serrata*
ξ. *sicula*
20. ***U. incisa*** Poir.
α. foliis lanceolatis […]
β. foliis oblongo-ovatis […]
γ. foliis triangulari-ovatis […]
** Stipulae inter petiolos utrinque solitariae (s. duae in unam interpetiolarem coalitae), integerrimae v. apice bifidae.
21. ***U. australis*** Hook.f.
22. *U. aucklandica* Hook.f.

## Appendix A1 continued.

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**Weddell (1856)**

34. ***U. grandidentata*** Miq.
35. *U. aucklandica* Hook.f.
36. ***U. morifolia*** Poir.
    α. dentibus foliorum plerumque rotundatis [...]
    β. dentibus foliorum triangularibus acutis
37. ***U. membranacea*** Poir.

Species quoad sectionem dubiae.

38. ***U. glomeruliflora*** Steud.
39. *U. trachycarpa* Wedd.[5]
40. *U. orizabae* Liebm.

Species mihi non satis notae.

41. *U. parvula* Blume
42. *U. atlantica* Blume
43. ***U. spirealis*** Blume
44. *U. fastigiata* Blume
45. *U. micrantha* Kunth & C.D.Bouché
46. *U. pseudodioica* Steud.
47. *U. serra* Blume
48. *U. galeopsifolia* Jacq.f.[6]
49. *U. foliosa* Blume

Species valde dubiae e genere fortassis removendae.

50. *U. tenuis* Steud.
51. *U. freireaeformis* Steud.

**Weddell (1869)**

23. ***U. simensis*** Hochst. ex A.Rich.
24. ***U. morifolia*** Poir.
    α. foliis grosse crenatis [...]
    β. *elevata*
25. ***U. parviflora*** Roxb.
    β. foliis ovatis lanceolatisve [...]
26. ***U. thunbergiana*** Siebold & Zucc.
27. *U. grandidentata* Miq.[11]
28. ***U. ferox*** G.Forst.
29. ***U. membranacea*** Poir.
    α. caule parce stimuloso, foliis acute dentatis [...]
    β. *crenata*
    γ. *horrida*
    δ. *neglecta*

Species dubiae affinitatis.

30. ***U. glomeruliflora*** Steud.
31. *U. trachycarpa* Wedd.[5]
32. *U. nicaraguensis* Liebm.[4]
33. ***U. mexicana*** Liebm.
34. *U. micrantha* Kunth & C.D.Bouché
35. *U. foliosa* Blume
36. *U. fastigiata* Blume
37. *U. stipulacea* Bertol.

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[1]= *U. chamaedryoides* Pursh, [2]= *U. lobulata* E.Mey., [3]= *Hesperocnide sandwicensis* Wedd., [4]= *U. leptophylla* Kunth, [5]=*U. dioica* L. subsp. *dioica* var. *holosericea* Fries, [6]= *U. dioica* L. subsp. *pubescens* (Ledeb.) Domin, [7]= *U. gracilis* Ait. subsp. *mollis* (Steud.) Weigend, [8]= *U. gracilis* Ait. subsp. *gracilis*, [9]= *U. kioviensis* Rogow., [10]= *U. gracilis* Ait. subsp. *aquatica* (Liebm.) Weigend, [11]= *U. berteroana* Phil., [12]= *U. trichantha* (Wedd.) Acevedo & L.E.Navas, [13]= *U. macbridei* Killip, [14]= *U. dioica* L. subsp. *dioica* var. *hispida* Wedd., [15]= *U. dioica* L. subsp. *dioica* var. *glabrata* Clem. ex Visiani, [16]= *U. dioica* L. var. *capensis* Wedd., [17]= *U. platyphylla* Wedd.

**Appendix A2.** List of taxa included in the phylogenetic study of *Urtica* L.

| **Taxon** | **Country of origin** | **Herbarium voucher** | **DNA-Nr.** | **ITS** | ***trn*S-*trn*G** | ***psb*A-*trn*H** | ***trn*L-*trn*F** |
|---|---|---|---|---|---|---|---|
| *Hesperocnide tenella* Torr. | USA | L. S. Rose 57086 (B) | W 2026 | KF558907 | KF559088 | KF558967 | KF559027 |
| *Hesperocnide tenella* Torr. | USA | L. Ahart 12535-C (BSB) | W 2586 | KF558930 | KF559111 | KF558990 | KF559050 |
| *Laportea bulbifera* (Sieb. & Zucc.)Wedd. | Japan | M. Tamura & H. Okada 26812 (B) | W 2027 | KX271355 | KX271510 | KX271586 | KX271436 |
| *Laportea canadensis* Gaudich. | USA | N. C. Coile 2959 (B) | W 2028 | KF971188 | KF971155 | KF971254 | KF971221 |
| *Laportea cuspidata* (Wedd.) Friis | India | M. Richter s.n. (BSB) | W 2273 | KX271353 | KX271508 | KX271584 | KX271434 |
| *Laportea interrupta* (L.) Chew | India | M. Richter s.n. (BSB) | W 2272 | KX271354 | KX271509 | KX271585 | KX271435 |
| *Laportea peduncularis* (Wedd.) Chew | South Africa | M. Weigend 8713 (BSB) | W 2274 | KF558927 | KF559108 | KF558987 | KF559047 |
| *Nanocnide japonica* Blume | China | D. E. Boufford et al. 25403 (E) | W 2285 | KF971190 | KF971157 | KF971256 | KF971223 |
| *Nanocnide lobata* Wedd. | Japan | M. Furuse 2091 (K) | K 29190 | KF971189 | KF971156 | KF971255 | KF971222 |
| *Obetia carruthersiana* (Hiern.) Rendle | Namibia | H. & E. Walter 1112 (B) | W 2030 | KF971187 | KF971154 | KF971253 | KF971220 |
| *Obetia radula* (Bak.) B.D. Jackson | Tanzania | Ch. Schlage & M. Heinrich 341 (B) | W 2049 | KX271352 | KX271507 | KX271583 | KX271433 |
| *Urera batesii* Rendle | Equatorial Guinea | A.M. Carvalho 3412 (B) | W 2031 | KF971186 | KF971153 | KF971252 | KF971219 |
| *U. andicola* Wedd. | Peru | M. Ackermann & D. Kollehn 276 (BSB) | W 3081 | KF558940 | KF559121 | KF559000 | KF559060 |
| *U. angustifolia* Fisch. ex Hornem. | Mongolia | Student Group 30 (MO) | W 2007 | KF558902 | KF559083 | KF558962 | KF559022 |
| *U. angustifolia* Fisch. ex Hornem. | Khazachstan | M. Weigend 8694 (B) | W 2270 | KX271379 | KX271530 | KX271610 | KX271456 |
| *U. angustifolia* Fisch. ex Hornem. | Mongolia | H.H. Hilger 1603 (B) | W 2912 | KF971217 | KF971184 | KF971283 | KF971250 |
| *U. ardens* Link | China | M. Weigend 8684-4 (B) | W 2238 | KX271400 | KX271551 | KX271631 | KX271476 |
| *U. aspera* Petrie | New Zealand | B.H. Patrick & J. Douglas s.n. (CHR) | CHR 511575 | KX271374 | - | KX271605 | - |
| *U. atrichocaulis* (Hand.-Mazz.) C.J.Chen | China | S.W. Teng 90389 (HUH) | W 3093 | KX271378 | KX271529 | KX271609 | KX271455 |
| *U. atrovirens* Req. ex Loisel. | Italy (Sardinia) | M. Weigend 7798 (BSB) | W 1724 | KF558895 | KF559076 | KF558956 | KF559016 |
| *U. atrovirens* Req. ex Loisel. | Italy (Sardinia) | M. Weigend 7800 (B) | W 2227 | KX271390 | KX271541 | KX271621 | KX271466 |
| *U. australis* Hook.f. | New Zealand | W.R. Sykes 368/93 (CHR 496662) | CHR 496662 | KX271371 | - | KX271602 | - |
| *U. berteroana* Phil. | Chile | R. Philippi s.n. (B) | W1555 | KX271384 | KX271535 | KX271615 | KX271460 |
| *U. berteroana* Phil. | Chile | J. Anderson s.n. (MO) | W 2209 | KX271383 | KX271534 | KX271614 | KX271459 |
| *U. bianorii* (Knoche) Paiva | Spain (Mallorca) | M. & K. Weigend 8155 (B) | W 2224 | KF558917 | KF559098 | KF558977 | KF559037 |
| *U. cannabina* L. | China | Q.R. Wu 322 (MO) | W 2038 | KX271370 | KX271525 | KX271601 | KX271451 |
| *U. cannabina* L. | Mongolia | M. Weigend 8678 (BSB) | W 2237 | KF558923 | KF559104 | KF558983 | KF559043 |
| *U. chamaedryoides* Pursh | Mexico | R. Rosas 299 (BM) | W 1865 | KF971202 | KF971169 | KF971268 | KF971235 |
| *U. circularis* (Hicken) Sorarú | Brazil | R.E. Fries 135 (GB) | W 1872 | KX271386 | KX271537 | KX271617 | KX271462 |
| *U. circularis* (Hicken) Sorarú | Brazil | M. Weigend 9311 (B) | W 3091 | KF971200 | KF971167 | KF971266 | KF971233 |

## Appendix A2 continued.

| Taxon | Country of origin | Herbarium voucher | DNA-Nr. | ITS | *trn*S-*trn*G | *psb*A-*trn*H | *trn*L-*trn*F |
|---|---|---|---|---|---|---|---|
| *U. dioica* L. subsp. *cypria* H. Lindb. | Cyprus | R. Hand 4723 (B) | W 1992 | KF558900 | KF559081 | KF558961 | KF559021 |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Spain (Segovia) | E. Zippel 2002/2b (B) | W 2232 | KF558920 | KF559101 | KF558980 | KF559040 |
| *U. dioica* L. subsp. *dioica* var. *glabrata* Clem. ex Visiani | Italy | M. Weigend 7097 (B) | W 3084 | KF971210 | KF971177 | KF971276 | KF971243 |
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Switzerland | M. & K. Weigend 8112-C (BSB) | W 2234 | KF558922 | KF559103 | KF558982 | KF559042 |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Germany | M. & K. Weigend 8100 (B) | W 3083 | KF558942 | KF559123 | KF559002 | KF559062 |
| *U. dioica* L. subsp. *dioica* var. *sarmatica* Zapał. | Germany | M. Weigend 9328 (BSB) | W 3085 | KF558943 | KF559124 | KF559003 | KF559063 |
| *U. dioica* L. subsp. *sondenii* (Simmons) Hyl. | Norway | T. Alm s.n. (B) | W 1722 | KF558894 | KF559075 | KF558955 | KF559015 |
| *U. dioica* L. subsp. *subinermis* (Uechtr.) Weigend | Austria | M. & K. Weigend 5665 (B) | W 1095 | KF558891 | KF559072 | KF558952 | KF559012 |
| *U. domingensis* Urb. | Dominican Republic | T. Clase 3820 (B) | W 2584 | KX271414 | - | - | - |
| *U. echinata* Benth. | Peru | M. Weigend & Skrabal 5852 (B) | W 1078 | KF558886 | KF559067 | KF558947 | KF559007 |
| *U. echinata* Benth. | Ecuador | Loejtnant & Molau 11657 (GB) | W 1863 | KX271427 | KX271577 | KX271657 | KX271501 |
| *U. echinata* Benth. | Peru | M. Weigend et al. 7279 (B) | W 3086 | KF558944 | KF559125 | KF559004 | KF559064 |
| *U. ferox* G.Forst. | New Zealand | M. Weigend 8211 (B) | W 2010 | KF558904 | KF559085 | KF558964 | KF559024 |
| *U. fissa* E.Pritz ex Diels | Taiwan | M. Weigend 8129 (B) | W 1880 | KX271397 | KX271548 | KX271628 | KX271473 |
| *U. fissa* E.Pritz ex Diels | China | Hsiu-Lan Ho 951 (MO) | W 2011 | KF558905 | KF559086 | KF558965 | KF559025 |
| *U. fissa* E.Pritz ex Diels | China | Sino-American Guizhou Botanical Expedition 1135 (HUH) | W 4211 | KX271395 | KX271546 | KX271626 | KX271471 |
| *U. fissa* E.Pritz ex Diels | China | A. Henry 2900 (K) | K 22888 | KX271396 | KX271547 | KX271627 | KX271472 |
| *U. flabellata* Kunth | Peru | M. Weigend et al. 7728 (B) | W 1560 | KF971199 | KF971166 | KF971265 | KF971232 |
| *U. flabellata* Kunth | Peru | M. Weigend et al. 8819 (B) | W 2040 | KF558908 | KF559089 | KF558968 | KF559028 |
| *U. fragilis* J.Thiébaut | Lebanon | G. Samuelson 5216 (MO) | W 2012 | KX271403 | KX271554 | KX271634 | KX271478 |
| *U. fragilis* J.Thiébaut | Turkey | B. Tarikahya & B. Özüdogru 2410-C(B) | W 2701 | KX271404 | KX271555 | KX271635 | KX271479 |
| *U. glomerulaeflora* Steud. | Chile (J. Fernández) | C. Skottsberg 478 (GB) | W 1876 | KX271381 | KX271532 | KX271612 | KX271457 |
| *U. glomerulaeflora* Steud. | Chile (J. Fernández) | Moseley s.n. (K) | K 22877 | KX271382 | KX271533 | KX271613 | KX271458 |
| *U. gracilenta* Greene | Mexico | P. Tonorio et al. 6277 (BM) | W 1940 | KF971201 | KF971168 | KF971267 | KF971234 |

## Appendix A2 continued.

| Taxon | Country of origin | Herbarium voucher | DNA-Nr. | ITS | *trn*S-*trn*G | *psb*A-*trn*H | *trn*L-*trn*F |
|---|---|---|---|---|---|---|---|
| *U. gracilis* Ait. subsp. *aquatica (*Liebm.) Weigend | Guatemala | Schneider et al. 31 (B) | W 1564 | KF971214 | KF971181 | KF971280 | KF971247 |
| *U. gracilis* Ait. subsp. *gracilis* | USA (California) | Ewan 9916 (K) | K 22881 | KF971216 | KF971183 | KF971282 | KF971249 |
| *U. gracilis* Ait. subsp. *holosericea* (Nutt.) Weigend | Mexico (Sonora) | Reina 2002-330 (TEX) | W 2895 | KF971215 | KF971182 | KF971281 | KF971248 |
| *U. gracilis* Ait. subsp. *incaica* Weigend | Peru | M. Weigend et al. 5847 (B) | W 1728 | KF558896 | KF559077 | KF558957 | KF559017 |
| *U. gracilis* Ait. subsp. *mollis* (Steud.) Weigend | Chile | M. Kalin Arroyo 81901 (CONC) | W 2886 | KF558935 | KF559116 | KF558995 | KF559055 |
| *U. grandidentata* Miq. | Indonesia | S.H. Koorders 37901 (B) | K 22882 | KX271401 | KX271552 | KX271632 | - |
| *U. grandidentata* Miq. | Indonesia | H.N. Ridley s.n. (K) | K 22883 | KX271402 | KX271553 | KX271633 | KX271477 |
| *U. hyperborea* Jacquem. ex Wedd. | China | Tafel 140 (M) | W 1768 | KX271364 | KX271519 | KX271595 | KX271445 |
| *U. hyperborea* Jacquem. ex Wedd. | China | Ho et al. 716 (MO) | W 2041 | KX271365 | KX271520 | KX271596 | KX271446 |
| *U.* cf. *hyperborea* Jacquem. ex Wedd. | China | Ho et al. 2807 (MO) | W 2044 | KF971194 | KF971161 | KF971260 | KF971227 |
| *U. incisa* Poir. | Australia (Tasmania) | T. Jossberger s.n (BONN) | ED 844 | KX271375 | KX271526 | KX271606 | KX271452 |
| *U. incisa* Poir. | Australia | Eichler 17739 (B) | W 1559 | KX271376 | KX271527 | KX271607 | KX271453 |
| *U. incisa* Poir. | New Zealand | R.K. Ward (CHR 234516A) | W 2264 | KF971218 | KF971185 | KF971284 | KF971251 |
| *U. incisa* Poir. | New Zealand | J.S. Attwood (CHR 48288) | W 2265 | KF558926 | KF559107 | KF558986 | KF559046 |
| *U. kioviensis* Rogow. | Germany | N.M. Nürk & J. Devers 333 (BSB) | W 2240 | KF558924 | KF559105 | KF558984 | KF559044 |
| *U. laetevirens* Maxim. subsp. *dentata* (Hand.-Mazz.) C.J.Chen | China | Dr. Aug. Henry's Collections from Central China 5859 (HUH) | W 4214 | KX271408 | KX271559 | KX271639 | KX271483 |
| *U. laetevirens* Maxim. subsp. *dentata* (Hand.-Mazz.) C.J.Chen | China | Zheng-yu 15565 (MO) | W 2013 | KX271409 | KX271560 | KX271640 | KX271484 |
| *U. laetevirens* Maxim. subsp. *dentata* (Hand.-Mazz.) C.J.Chen | China | Zheng-yu 15572 (MO) | W 2042 | KX271410 | KX271561 | KX271641 | KX271485 |
| *U. laetevirens* Maxim. subsp. *laetevirens* | China | B.V. Skovortzov s.n. (HUH) | W 4230 | KX271411 | KX271562 | KX271642 | KX271486 |
| *U. laetevirens* Maxim. subsp. *laetevirens* | Russia (Kamchatka) | D. Geltman 326/1 (LE) | W 2963 | KX271407 | KX271558 | KX271638 | KX271482 |
| *U. laetevirens* Maxim. subsp. *laetevirens* | Russia | P. Gorovoi et al. 6868 (B) | W 1868 | KX271406 | KX271557 | KX271637 | KX271481 |
| *U.* cf. *laetevirens* Maxim. subsp. *laetevirens* | Taiwan | S.T. Chiu et al. 02924 (TAIWAN) | W 2212 | KF558912 | KF559093 | KF558972 | KF559032 |
| *U. laetevirens* Maxim. subsp. *silvatica* | China | D.E. Boufford et al. 26323 (MO) | W 2014 | KX271412 | KX271563 | KX271643 | KX271487 |
| *U. laetevirens* Maxim. subsp. *silvatica* (Hand.-Mazz.) | China | D.E. Boufford et al. 30067 (HUH) | W 4209 | KX271413 | KX271564 | KX271644 | KX271488 |

## Appendix A2 continued.

| Taxon | Country of origin | Herbarium voucher | DNA-Nr. | ITS | ***trn*S-*trn*G** | ***psb*A-*trn*H** | ***trn*L-*trn*F** |
|---|---|---|---|---|---|---|---|
| *U. laetevirens* Maxim. subsp. *silvatica* (Hand.-Mazz.) | Tibet | K. Rushforth & H. McAllister 5252 (B) | W 2908 | KF558938 | KF559119 | KF558998 | KF559058 |
| *U. lalibertadensis* Weigend | Peru | M. Weigend & Ch. Schwarzer 7896 (B) | W 1683 | KF558893 | KF559074 | KF558954 | KF559014 |
| *U. leptophylla* Kunth | Peru | M. Weigend et al. 7706 (B) | W 1552 | KF558892 | KF559073 | KF558953 | KF559013 |
| *U. leptophylla* Kunth | Ecuador | A. Rimbach 139 (B) | W 1554 | KX271424 | KX271574 | KX271654 | KX271498 |
| *U. leptophylla* Kunth | Peru | M. Weigend et al. 7763 (B) | W 1562 | KF971208 | KF971175 | KF971274 | KF971241 |
| *U. leptophylla* Kunth | Venezuela | J.G. Wessels Boer 2177 (MO) | W 4224 | KX271428 | KX271578 | KX271658 | KX271502 |
| *U. lobulata* E.Mey. | RSA | Groenewald 8709 (PRE) | W 1990 | KX271363 | KX271518 | KX271594 | KX271444 |
| *U. macbridei* Killip | Peru | Ch. Schwarzer 05 (B) | W 1889 | KF558899 | KF559080 | KF558960 | KF559020 |
| *U. macbridei* Killip | Ecuador | M. Weigend & G. Brokamp 9106 (B) | W 3113 | KX271423 | KX271573 | KX271653 | KX271497 |
| *U. magellanica* Juss. ex Poir. | Chile (Fray Jorge) | F. Luebert & C. Becker 2912 (SGO) | W 1997 | KF971207 | KF971174 | KF971273 | KF971240 |
| *U. magellanica* Juss. ex Poir. | Chile | P.W. James 2260 (BM) | W 2881 | KF558933 | KF559114 | KF558993 | KF559053 |
| *U. magellanica* Juss. ex Poir. | Chile | F. Schlegel 2873 (CONC) | W 2883 | KX271421 | KX271571 | KX271651 | KX271495 |
| *U. magellanica* Juss. ex Poir. | Chile | M. O´Brien 38418 (CONC) | W 2962 | KX271422 | KX271572 | KX271652 | KX271496 |
| *U. magellanica* Juss. ex Poir. | Chile | C. Jiles 4741 (CONC) | W 2964 | KF971205 | KF971172 | KF971271 | KF971238 |
| *U. magellanica* Juss. ex Poir. | Chile | Gunckel 14.883 (CONC) | W 2965 | KF971204 | KF971171 | KF971270 | KF971237 |
| *U. magellanica* Juss. ex Poir. | Chile (Fray Jorge) | C. Jiles 4678 (CONC) | W 2966 | KF971206 | KF971173 | KF971272 | KF971239 |
| *U. mairei* H.Lév. | China | Gaoligong Shan B. Surv. 34442 (HUH) | W 4215 | KX271399 | KX271550 | KX271630 | KX271475 |
| *U. mairei* H.Lév. | Tibet | K. Rushforth & H. McAllister 5247 (BSB) | W 2910 | KX271398 | KX271549 | KX271629 | KX271474 |
| *U. masafuerae* Phil. | Chile (J. Fernández) | T. Germain s.n. (GB) | W 1879 | KX271380 | KX271531 | KX271611 | - |
| *U. massaica* Milbr. | Uganda | M. Ackermann 1050 | ED 841 | KX271388 | KX271539 | KX271619 | KX271464 |
| *U. massaica* Milbr. | Tanzania | K. Kibni 52 (B) | W 2015 | KX271389 | KX271540 | KX271620 | KX271465 |
| *U. membranacea* Poir. | Spain (Teneriffa) | M. & K. Weigend 8252 (B) | W 2039 | KX271362 | KX271517 | KX271593 | KX271443 |
| *U. membranacea* Poir. | Italy | M. Ackermann & C. Knödler s. n.(BSB) | W 2219 | KF558913 | KF559094 | KF558973 | KF559033 |
| *U. membranacea* Poir. | Spain (Mallorca) | M. Weigend 8154-C (B) | W 2228 | KX271361 | KX271516 | KX271592 | KX271442 |
| *U. membranacea* Poir. | Portugal | M. Gottschling 1 (B) | W 2229 | KF558918 | KF559099 | KF558978 | KF559038 |
| *U. mexicana* Liebm. | Mexico | C.G. Pringle 4821 (BM) | W 1884 | KX271420 | KX271570 | KX271650 | KX271494 |
| *U. mexicana* Liebm. | Mexico | T.B. Croat 45624 (MO) | W 2266 | KX271419 | KX271569 | KX271649 | KX271493 |
| *U. mexicana* Liebm. | Mexico | Santiz Ruíz 791 (MO) | W 3114 | KF971195 | KF971162 | KF971261 | KF971228 |

## Appendix A2 continued.

| Taxon | Country of origin | Herbarium voucher | DNA-Nr. | ITS | *trn*S-*trn*G | *psb*A-*trn*H | *trn*L-*trn*F |
|---|---|---|---|---|---|---|---|
| *U. minutifolia* Griseb. | Argentina | A.T. Hunziker 19695 (MO) | W 2271 | KX271432 | KX271582 | KX271662 | KX271506 |
| *U. minutifolia* Griseb. | Argentina | Eyerdam & Beetle 22338 (MO) | W 3115 | KX271430 | KX271580 | KX271660 | KX271504 |
| *U. minutifolia* Griseb. | Argentina | R. Sobils & E. Moscone (MO) | W 3117 | KX271431 | KX271581 | KX271661 | KX271505 |
| *U. morifolia* Poir. | Spain (Tenerife) | H.H. Hilger s.n. (B) | W 1565 | KX271393 | KX271544 | KX271624 | KX271469 |
| *U. morifolia* Poir. | Spain (Tenerife) | M. & K. Weigend 8242 (B) | W 2206 | KF558910 | KF559091 | KF558970 | KF559030 |
| *U. morifolia* Poir. | Spain (Tenerife) | M. & K. Weigend 8240 (B) | W 2207 | KF558911 | KF559092 | KF558971 | KF559031 |
| *U. morifolia* Poir. | Portugal (Madeira) | Fernandes 8711 (MADJ) | W 2213 | KX271394 | KX271545 | KX271625 | KX271470 |
| *U. neubaueri* Chrtek | Afghanistan | Breckle 1610 (M) | W 1758 | KX271356 | KX271511 | KX271587 | KX271437 |
| *U. papuana* Zandee | Papua New Guinea | L.J. Brass 9129 (K) | K 22892 | KX271377 | KX271528 | KX271608 | KX271454 |
| *U. perconfusa* Grosse-Veldmann & Weigend | New Zealand | R. Pender s.n. (CHR 536516) | CHR 536516 | KX271372 | - | KX271603 | - |
| *U. peruviana* Geltman | Peru | M. Weigend 7625 (B) | W 1886 | KF558897 | KF559078 | KF558958 | KF559018 |
| *U. pilulifera* L. | Spain (Mallorca) | M.&. K. Weigend 8153-C (B) | W 2221 | KF558915 | KF559096 | KF558975 | KF559035 |
| *U. pilulifera* L. | Italy (Sardinia) | M. Weigend 8120 (B) | W 2222 | KX271357 | KX271512 | KX271588 | KX271438 |
| *U. pilulifera* L. | Malta | H.H. Hilger s.n. (B) | W 2223 | KF558916 | KF559097 | KF558976 | KF559036 |
| *U. platyphylla* Wedd. | Japan | T. Azuma s.n. (B) | W 1720 | KX271391 | KX271542 | KX271622 | KX271467 |
| *U. platyphylla* Wedd. | Japan | N. M. Nürk 357 (B) | W 3088 | KF558945 | KF559126 | KF559005 | KF559065 |
| *U. portosanctana* Press | Spain (Tenerife) | M. & K. Weigend 8234 (B) | W 2218 | KX271360 | KX271515 | KX271591 | KX271441 |
| *U. portosanctana* Press | Portugal (Madeira) | H. Kürschner 13279 (HERBKürschner) | W 2291 | KF558929 | KF559110 | KF558989 | KF559049 |
| *U. pseudomagellanica* Geltman | Bolivia | B. Schlumpberger & G. Brokamp 728-C (BSB) | W 2909 | KX271425 | KX271575 | KX271655 | KX271499 |
| *U. rupestris* Guss. | Italy (Sicily) | G. Certa et al. s.n. (PALERMO) | W 1991 | KX271392 | KX271543 | KX271623 | KX271468 |
| *U. simensis* Hochst. ex A.Rich. | Ethiopia | O. Ryding 1995 (GB) | W 1989 | KX271387 | KX271538 | KX271618 | KX271463 |
| *U. spathulata* Sm. | Brazil | Ahumada 1338 (MO) | W 2268 | KX271385 | KX271536 | KX271616 | KX271461 |
| *U. spirealis* Blume | Guatemala | M. Véliz 99.7516 (MO) | ED 842 | KX271417 | KX271567 | KX271647 | KX271491 |
| *U. spirealis* Blume | Mexico | Ford Smith M 27 (TEX) | W 2893 | KF971196 | KF971163 | KF971262 | KF971229 |
| *U. spirealis* Blume | Mexico | McDonald 1819 (TEX) | W 2894 | KF971198 | KF971165 | KF971264 | KF971231 |
| *U. spirealis* Blume | Mexico | T. Wendt & D. Riskind 901 (TEX) | W 2896 | KF971197 | KF971164 | KF971263 | KF971230 |
| *U. spirealis* Blume | Mexico | G.B. Hinton et al. 23160 (MO) | W 3116 | KX271416 | KX271566 | KX271646 | KX271490 |
| *U. spirealis* Blume | Mexico | T. Wendt & D. Riskind 901 (MO) | W 3118 | KX271418 | KX271568 | KX271648 | KX271492 |

## Appendix A2 continued.

| Taxon | Country of origin | Herbarium voucher | DNA-Nr. | ITS | *trn*S-*trn*G | *psb*A-*trn*H | *trn*L-*trn*F |
|---|---|---|---|---|---|---|---|
| *U. stachyoides* Webb & Berthel. | Spain (Teneriffa) | A. Bresinsky 261 (M) | W 1764 | KX271358 | KX271513 | KX271589 | KX271439 |
| *U. stachyoides* Webb & Berthel. | Spain (Teneriffa) | M. & K. Weigend 8230 (B) | W 2017 | KF558906 | KF559087 | KF558966 | KF559026 |
| *U. subincisa* Benth. | Mexico | E.K. Balls 4901 (BM) | W 1881 | KX271415 | KX271565 | KX271645 | KX271489 |
| *U. subincisa* Benth. | Mexico | Pérez-Caliz 459 (MO) | W 2267 | KF971203 | KF971170 | KF971269 | KF971236 |
| *U. sykesii* Grosse-Veldmann & Weigend | New Zealand | C.J. Webb L.F. Delph 97/3 (CHR) | CHR 511586 | KX271373 | - | KX271604 | - |
| *U. sykesii* Grosse-Veldmann & Weigend | New Zealand | M. Weigend 8212 (BSB) | W 2836 | KF558932 | KF559113 | KF558992 | KF559052 |
| *U. taiwaniana* S.S.Ying | Taiwan | J.J. Chen et al. 00573-C (BSB) | W 2242 | KF558925 | KF559106 | KF558985 | KF559045 |
| *U. thunbergiana* Siebold & Zucc. | Japan | T. Jossberger s.n. (BONN) | W 3112 | KX271405 | KX271556 | KX271636 | KX271480 |
| *U. tibetica* W.T.Wang ex C.J.Chen | China | W.P. Fang 4279 (HUH) | W 3092 | KF971191 | KF971158 | KF971257 | KF971224 |
| *U. tibetica* W.T.Wang ex C.J.Chen | China | T.N. Ho et al. 219 (HUH) | W 3095 | KF971192 | KF971159 | KF971258 | KF971225 |
| *U.* cf. *tibetica* W.T.Wang ex C.J.Chen | China | J. F. Rock 14024 (HUH) | W 3094 | KF971193 | KF971160 | KF971259 | KF971226 |
| *U.* cf. *tibetica* W.T.Wang ex C.J.Chen | China | B. Dickoré 3647 (K) | K 22884 | KX271366 | KX271521 | KX271597 | KX271447 |
| *U. triangularis* Hand.-Mazz. subsp. *pinnatifida* (Hand.-Mazz.) C.J.Chen | China | T.N. Ho et al. 631 (MO) | W 2018 | KX271369 | KX271524 | KX271600 | KX271450 |
| *U. triangularis* Hand.-Mazz. subsp. *pinnatifida* (Hand.-Mazz.) C.J.Chen | China | T.N. Ho et al. 2354 (MO) | W 2043 | KX271368 | KX271523 | KX271599 | KX271449 |
| *U. triangularis* Hand.-Mazz. subsp. *triangularis* | China | T.N. Ho et al. 2576 (MO) | W 2019 | KX271367 | KX271522 | KX271598 | KX271448 |
| *U. trichantha* (Wedd.) Acevedo & L.E.Navas | Peru | M. Weigend & Ch. Schwarzer 7821 (B) | W 1682 | KX271429 | KX271579 | KX271659 | KX271503 |
| *U. trichantha* (Wedd.) Acevedo & L.E.Navas | Peru | M. Weigend 8848 (BSB) | W 2580 | KX271426 | KX271576 | KX271656 | KX271500 |
| *U. trichantha* (Wedd.) Acevedo & L.E.Navas | Peru | F. Cáceres 3326 (B) | W 3090 | KF558946 | KF559127 | KF559006 | KF559066 |
| *U. urens* L. | Germany | M. Weigend 5667 (B) | W 1082 | KF558889 | KF559070 | KF558950 | KF559010 |
| *U. urens* L. | Spain (Teneriffa) | M. & K. Weigend 8231 (B) | W 2045 | KX271359 | KX271514 | KX271590 | KX271440 |
| *U. urens* L. | Peru | M. Weigend et al. 8317 (B) | W 2220 | KF558914 | KF559095 | KF558974 | KF559034 |
| *U. urentivelutina* Weigend | Peru | M. Weigend 7907 (B) | W 1887 | KF558898 | KF559079 | KF558959 | KF559019 |
| *Zhengyia shennongensis* T.Deng, D.G.Zhang & H.Sun | China | Shennongjia Exp. 20110904001 (KUN) | Dt 088 | KC284948 | - | KC285026 | KC285000 |
| *Zhengyia shennongensis* T.Deng, D.G.Zhang & H.Sun | China | Shennongjia Exp. 20111107001 (KUN) | Dt 091 | KC284949 | - | KC285027 | KC285001 |

**Appendix A3.** Growth habit of the taxa included in this study (* inferred).

| **Taxon** | **Growth habit** |
|---|---|
| *Hesperocnide tenella* Torr. | Annual herb |
| *Laportea bulbifera* (Siebold & Zucc.) Wedd. | Tap-rooted perennial |
| *Laportea canadensis* Gaudich. | Tap-rooted perennial |
| *Laportea cuspidata* (Wedd.) Friis | Tap-rooted perennial |
| *Laportea interrupta* (L.) Chew | Tap-rooted perennial |
| *Laportea peduncularis* (Wedd.) Chew | Tap-rooted perennial |
| *Nanocnide japonica* Blume | Annual herb |
| *Nanocnide lobata* Wedd. | Annual herb |
| *Obetia carruthersiana* (Hiern.) Rendle | Shrub |
| *Obetia radula* (Bak.) B.D. Jackson | Shrub |
| *Urera batesii* Rendle | Shrub |
| *U. andicola* Wedd. | Rhizomatous perennial |
| *U. angustifolia* Fisch. ex Hornem. | Rhizomatous perennial |
| *U. ardens* Link | Tap-rooted perennial |
| *U. aspera* Petrie | Rhizomatous perennial |
| *U. atrichocaulis* (Hand.-Mazz.) C.J.Chen | Rhizomatous perennial |
| *U. atrovirens* Req. ex Loisel. | Shrublet |
| *U. australis* Hook.f. | Rhizomatous perennial |
| *U. berteroana* Phil. | Annual herb |
| *U. bianorii* (Knoche) Paiva | Tap-rooted perennial |
| *U. cannabina* L. | Tap-rooted perennial |
| *U. chamaedryoides* Pursh | Annual herb |
| *U. circularis* (Hicken) Sorarú | Annual herb |
| *U. dioica* L. subsp. *cypria* H. Lindb. | Rhizomatous perennial |
| *U. dioica* L. subsp. *dioica* var. *dioica* | Rhizomatous perennial |
| *U. dioica* L. subsp. *dioica* var. *glabrata* Clem. ex Visiani | Rhizomatous perennial |
| *U. dioica* L. subsp. *dioica* var. *hispida* Wedd. | Rhizomatous perennial |
| *U. dioica* L. subsp. *dioica* var. *holosericea* Fries | Rhizomatous perennial |
| *U. dioica* L. subsp. *dioica* var. *sarmatica* Zapał. | Rhizomatous perennial |
| *U. dioica* L. subsp. *sondenii* (Simmons) Hyl. | Rhizomatous perennial |
| *U. dioica* L. subsp. *subinermis* (Uechtr.) Weigend | Rhizomatous perennial |
| *U. domingensis* Urb. | Lianescent shrublet |
| *U. echinata* Benth. | Tap-rooted perennial |
| *U. ferox* G.Forst. | Shrub |
| *U. fissa* E.Pritz ex Diels | Tap-rooted perennial |
| *U. flabellata* Kunth | Annual herb |
| *U. fragilis* J.Thiébaut | Tap-rooted perennial |
| *U. glomerulaeflora* Steud. | Shrublet |
| *U. gracilenta* Greene | Annual herb |
| *U. gracilis* Ait. subsp. *aquatica* (Liebm.) Weigend | Rhizomatous perennial |
| *U. gracilis* Ait. subsp. *gracilis* | Rhizomatous perennial |
| *U. gracilis* Ait. subsp. *holosericea* (Nutt.) Weigend | Rhizomatous perennial |
| *U. gracilis* Ait. subsp. *incaica* Weigend | Rhizomatous perennial |
| *U. gracilis* Ait. subsp. *mollis* (Steud.) Weigend | Rhizomatous perennial |
| *U. grandidentata* Miq. | Tap-rooted perennial |
| *U. hyperborea* Jacquem. ex Wedd. | Tap-rooted perennial |
| *U. cf. hyperborea* Jacquem. ex Wedd. | Tap-rooted perennial |
| *U. incisa* Poir. | Rhizomatous perennial |

## Appendix A3 continued.

| Taxon | Growth habit |
|---|---|
| *U. kioviensis* Rogow. | Rhizomatous perennial |
| *U. laetevirens* Maxim. subsp. *dentata* (Hand.-Mazz.) C.J.Chen | *Tap-rooted perennial |
| *U. laetevirens* Maxim. subsp. *laetevirens* | *Tap-rooted perennial |
| *U. cf. laetevirens* Maxim. subsp. *laetevirens* | *Tap-rooted perennial |
| *U. laetevirens* Maxim. subsp. *silvatica* (Hand.-Mazz.) | *Tap-rooted perennial |
| *U. lalibertadensis* Weigend | Rhizomatous perennial |
| *U. leptophylla* Kunth | Rhizomatous perennial |
| *U. lobulata* E.Mey. | Annual herb |
| *U. macbridei* Killip | Lianescent shrub |
| *U. magellanica* Juss. ex Poir. | Rhizomatous perennial |
| *U. mairei* H.Lév. | Tap-rooted perennial |
| *U. masafuerae* Phil. | Annual herb |
| *U. massaica* Milbr. | Rhizomatous perennial |
| *U. membranacea* Poir. | Annual herb |
| *U. mexicana* Liebm. | Rhizomatous perennial |
| *U. minutifolia* Griseb. | Tap-rooted perennial |
| *U. morifolia* Poir. | Lianescent shrub |
| *U. neubaueri* Chrtek | Annual herb |
| *U. papuana* Zandee | Lianescent shrublet |
| *U. perconfusa* Grosse-Veldmann & Weigend | Rhizomatous perennial |
| *U. peruviana* Geltman | Lianescent shrub |
| *U. pilulifera* L. | Annual herb |
| *U. platyphylla* Wedd. | Rhizomatous perennial |
| *U. portosanctana* Press | Annual herb |
| *U. pseudomagellanica* Geltman | Rhizomatous perennial |
| *U. rupestris* Guss. | Shrublet |
| *U. simensis* Hochst. ex A.Rich. | Rhizomatous perennial |
| *U. spathulata* Sm. | Annual herb |
| *U. spirealis* Blume | Tap-rooted perennial |
| *U. stachyoides* Webb & Berthel. | Annual herb |
| *U. subincisa* Benth. | Tap-rooted perennial |
| *U.* sykesii Poir. | Rhizomatous perennial |
| *U. taiwaniana* S.S.Ying | Tap-rooted perennial |
| *U. thunbergiana* Siebold & Zucc. | Tap-rooted perennial |
| *U. tibetica* W.T.Wang ex C.J.Chen | Unknown |
| *U. cf. tibetica* W.T.Wang ex C.J.Chen | Unknown |
| *U. triangularis* Hand.-Mazz. subsp. *pinnatifida* (Hand.-Mazz.) C.J.Chen | Tap-rooted perennial |
| *U. triangularis* Hand.-Mazz. subsp. *triangularis* | Tap-rooted perennial |
| *U. trichantha* (Wedd.) Acevedo & L.E.Navas | Tap-rooted perennial |
| *U. urens* L. | Annual herb |
| *U. urentivelutina* Weigend | Lianescent shrub |
| *Zhengyia shennongensis* T.Deng, D.G.Zhang & H.Sun | Tap-rooted perennial |

# Appendix B to Chapter 8

## Assembled 90% data set at mindepth 4

```
4013      ## loci with > minsp containing data
4013      ## loci with > minsp containing data & paralogs removed
4013      ## loci with > minsp containing data & paralogs removed & final filtering
## number of loci recovered in final data set for each taxon.
taxon                                                   nloci
886878_TCGCAGGA                                         3535
886879_CTCTGCAA                                         3529
886880_CCTAGGTA                                         3735
886881_GGATCAAA                                         2677
886884_ATGGAGAA                                         3602
886886_GCTCGAAA                                         2641
886887_ACCAACTA                                         2967
886888_CCGGTACA                                         3039
886889_AACTCCGA                                         3662
886890_TTGAAGTA                                         3617
886891_ACTATCAA                                         3664
886892_TTGGATCA                                         3633
886893_CGACCTGA                                         2711
886895_TAATGCGA                                         3659
886896_AGGTACCA                                         3687
886897_TGCGTCCA                                         3606
886898_GAATCTCA                                         3645
886900_ACGCAACA                                         3656
886901_GCATTGGA                                         3632
886902_GATCTCGA                                         3647
886903_CAATATGA                                         3644
886904_TGACGTCA                                         3699
886906_GATGCCAA                                         3584
886907_CAATTACA                                         3670
886908_AGATAGGA                                         3617
886909_CCGATTGA                                         3821
886910_ATGCCGCA                                         3628
886911_CAGTACTA                                         3555
886912_AATAGTAA                                         3597
886914_TCATGGTA                                         3682
886915_AGAACCGA                                         3648
886917_TGGAATAA                                         3619
886918_CAGGAGGA                                         3597
886919_AATACCTA                                         3439
886920_CGAATGCA                                         3625
886921_TTCGCAAA                                         3623
886922_AATTCAAA                                         3655
886923_CGCGCAGA                                         3620
886924_AAGGTCTA                                         3668
886925_ACTGGACA                                         3646
886926_AGCAGGTA                                         3639
886928_GTACCGGA                                         2595
886929_GGTCAAGA                                         3746
886931_AGTCAGAA                                         3563
886932_AACTAGAA                                         3592
886933_CTATGGCA                                         3478
886934_CGACGGTA                                         3568
```

## Appendix B continued.

### Assembled 90% data set at mindepth 4

```
886935_AACCAAGA                                    2791
886936_CGGCGTAA                                    2983
886937_GCAGTCCA                                    3806
886939_CTCGCGCA                                    2741
886940_CTGCGACA                                    2446
886941_ACGTATGA                                    3615

## nloci = number of loci with data for exactly ntaxa
## ntotal = number of loci for which at least ntaxa have data
ntaxa                                              nloci   saved   ntotal
1                                                  -
2                                                  -               -
3                                                  -               -
4                                                  -               -
5                                                  -               -
6                                                  -               -
7                                                  -               -
8                                                  -               -
9                                                  -               -
10                                                 -               -
11                                                 -               -
12                                                 -               -
13                                                 -               -
14                                                 -               -
15                                                 -               -
16                                                 -               -
17                                                 -               -
18                                                 -               -
19                                                 -               -
20                                                 -               -
21                                                 -               -
22                                                 -               -
23                                                 -               -
24                                                 -               -
25                                                 -               -
26                                                 -               -
27                                                 -               -
28                                                 -               -
29                                                 -               -
30                                                 -               -
31                                                 -               -
32                                                 -               -
33                                                 -               -
34                                                 -               -
35                                                 -               -
36                                                 -               -
37                                                 -               -
38                                                 -               -
39                                                 -               -
40                                                 316     *       4013
41                                                 293     *       3697
42                                                 344     *       3404
43                                                 349     *       3060
44                                                 350     *       2711
```

## Appendix B continued.

### Assembled 90% data set at mindepth 4

```
45                336    *        2361
46                301    *        2025
47                332    *        1724
48                277    *        1392
49                310    *        1115
50                275    *        805
51                231    *        530
52                198    *        299
53                101    *        101

## nvar = number of loci containing n variable sites (pis+autapomorphies).
## sumvar = sum of variable sites (SNPs).
## pis = number of loci containing n parsimony informative sites.
## sumpis = sum of parsimony informative sites.
                  nvar   sumvar   PIS    sumPIS
0                 45     0        233    0
1                 70     70       511    511
2                 179    428      702    1915
3                 260    1208     703    4024
4                 316    2472     600    6424
5                 414    4542     424    8544
6                 416    7038     313    10422
7                 400    9838     215    11927
8                 385    12918    149    13119
9                 341    15987    80     13839
10                297    18957    41     14249
11                240    21597    28     14557
12                184    23805    6      14629
13                144    25677    3      14668
14                102    27105    4      14724
15                75     28230    0      14724
16                54     29094    1      14740
17                22     29468    0      14740
18                28     29972    0      14740
19                17     30295    0      14740
20                12     30535    0      14740
21                9      30724    0      14740
22                0      30724    0      14740
23                2      30770    0      14740
24                0      30770    0      14740
25                1      30795    0      14740
total var= 30795
total pis= 14740
sampled unlinked SNPs= 3968
sampled unlinked bi-allelic SNPs= -1233
sampled unlinked SNPs= 3968
sampled unlinked bi-allelic SNPs= -1233
sampled unlinked SNPs= 3968
sampled unlinked bi-allelic SNPs= -1233
```

# PUBLICATION LIST

## Books

Barthlott, W., **Grosse-Veldmann, B.**, Korotkova, N. (2014) Orchid seed diversity: A scanning electron microscopy survey. *Englera* 32: 1-244.

## Research papers

Becker, K., **Grosse-Veldmann,** B., Weigend, M. (2016). Weeding the nettles VI: Taxonomic and phylogenetic studies of the Southeast Asian *Urtica fissa*-clade (Urticaceae). *submitted to Phytotaxa, 23/08/2016.*

Becker, K., **Grosse-Veldmann,** B., Weigend, M. (2016). Weeding the nettles V: Taxonomic and phylogenetic studies of the eastern Asian species *Urtica thunbergiana* Sieb. & Zucc. (Urticaceae). *submitted to Phytotaxa, 23/08/2016.*

**Grosse-Veldmann, B.,** Weigend, M. (2016) The geometry of gender–hyper-diversification of sexual systems in *Urtica* L. (Urticaceae). *submitted to Cladistics, 16/08/2016.*

**Grosse-Veldmann, B.,** Nürk, N. M., Smissen, R., Breitwieser, I., Quandt, D., Weigend, M. (2016) Pulling the Sting out of Nettle Systematics – a Comprehensive Phylogeny of the Genus *Urtica* L. (Urticaceae). *Molecular Phylogenetics and Evolution* 102, 9–19.

**Grosse-Veldmann, B.**, Abrahamczyk, S., Mutke, J., Barthlott, W., Weigend, M. (2016). *Rhipsalis* (Cactaceae) - loss and gain of floral rewards is mirrored in range sizes and distribution patterns of species. *Botanical Journal of the Linnean Society* 180(4): 491-503.

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Henning, T., Quandt, D., **Grosse-Veldmann, B.**, Monro, A., Weigend, M. (2014) Weeding the Nettles II: A delimitation of "*Urtica dioica* L." (Urticaceae) based on morphological and molecular data, including a rehabilitation of *Urtica gracilis* Ait. *Phytotaxa* 162(2): 61-83.

**Grosse-Veldmann, B.**, Korotkova, N., Reinken, B., Lobin, W. & Barthlott, W. (2011) *Amborella trichopoda* – Cultivation of the most ancestral angiosperm in Botanic Gardens. *Sibbaldia* 9: 143-155.