Phylogenetic analyses in St. John's wort (*Hypericum*)

Inferring character evolution and historical biogeography

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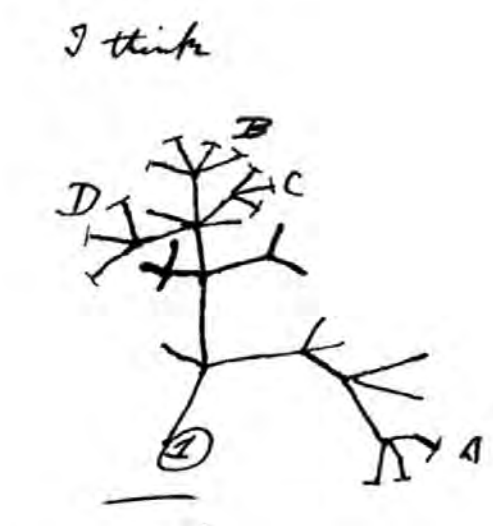


C. Darwin
(Notebook № B, 1837)

Nothing in biology makes sense except in the light of evolution

T. Dobzhansky
(1973)

Contents

1 Introduction

1.1 The genus *Hypericum*

The flowering plant genus of St. John's wort (*Hypericum*, Hypericaceae) consists of about 500 species of shrubs, herbs and a few trees. Members of *Hypericum* are distributed worldwide, with a main center of species richness in the temperate regions of the Northern Hemisphere. In cold temperate regions *Hypericum* is native mainly to lowland and upland areas, while in the tropics and warm temperate areas it is almost always confined to high elevation mountain habitats, *e.g.,* the Andes. *Hypericum* occurs in almost all kind of temperate habitats, but is rarely found in water other than in very shallow depths.

Hypericum is one of nine genera forming the family Hypericaceae (Stevens 2007; APG III 2009), which belongs to the clusioid clade of the Malpighiales (Gustafsson *et al.* 2002; Wurdack & Davis 2009). The clusioid clade includes five families (Bonnetiaceae, Calophyllaceae, Clusiaceae s.str. Podostemaceae, and Hypericaceae) represented by 94 genera and *c.* 1 900 species (Ruhfel *et al.* 2011). The eudicot order Malpighiales contains *c.* 16 000 species and is among the most diverse rosid clades (Korotkova *et al.* 2009; Wurdack & Davis 2009). Malpighiales constitute a large percentage of species in the shrub and small tree layer in tropical rain forests (Davis *et al.* 2005). Most lineages within the Malpighiales remained restricted to tropical climates. Only a few lineages made it out of the tropics and have been successful in the northern temperate zone, including Violaceae (violets), Salicaceae (willows), and *Hypericum* (Donoghue 2008).

Within the Hypericaceae, three tribes – Cratoxyleae (7 species, classified into *Cratoxylum* and *Eliea*), Hypericeae (*c.* 494 species, classified into *Hypericum*, *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum*), and Vismieae (*c.* 102 species, classified into *Harungana* and *Vismia*) – are recognized (Stevens 2007), which is in accordance with molecular findings (Ruhfel *et al.* 2011). *Hypericum*, together with the genus *Triadenum* and the monotypic genus *Lianthus*, is the only group of the clusioid clade occurring in temperate regions. All other members are native to pantropical or subtropical lowland regions of the world.

In order to investigate reasons responsible for the high diversity within *Hypericum* – 80 % of the family members belong to the genus – a phylogenetic hypothesis is needed to uncover insights into biogeographic patterns and character evolution. The aim of this thesis is to investigate the phylogeny of the genus and its close relatives in a comprehensive and comparative way by incorporating both molecular and morphological approaches.

1.1.1 Origin of the name, phytochemistry & economic importance

Origin of the name

Hypericum has been associated with pharmacy and folklore for many centuries. The perennial herb *H. perforatum* (Common St. John's wort) is due to its use in traditional and modern medicine as a mild antidepressant (Beerhues 2011) one of the best selling herbal ingredients worldwide (Crockett & Robson 2011). This traditional use of the herb, interestingly, is the source of origin of its name, *Hypericum*.

Despite the attempts of some lexicographers to derive it from *hypo-* or *hyper-* (beneath or above) and *ereikon* (the heath), *i. e.* a 'herb that is growing in the heath' (Robson 2003), the meaning and derivation of *Hypericum* is quite clear. The name υπερεικον (upereikon) was given by the ancient Greeks to a plant that they hung above their religious figures to ward off evil spirits (υπερ = above, εικων = image). Nikander (Alexipharmaka V, line 603) first mentioned the name in the second century bc, followed *c.* 250 years later by Dioscorides (Mat. Med., III, cap. 171), and the illustration of *Uperikon* in *De codicis Dioscuridei Aniciae Iulianae*: 357r, representing the traditional use of the name, portrays *H. empetrifolium* (Robson 1977). In regions away from the Aegean, where *H. empetrifolium* did not occur, other species and particularly *H. perforatum* were used for decoration (Robson 2003).

But why were the ancient Greeks confident in the power of the plant to ward off evil spirits? It has been stated by other authors (*e. g.*, Guiley 1991; Jurk 2005) that, since ancient times, depressions (or similar conditions) had been explained by evil demons inhabiting the sick person. *Hypericum* was a common remedy for what we now call a depression, that is, was considered to have 'the power to ward off evil spirits' in humans. Hence a medieval name was *fuga daemonum* ('flight of the demons' or, more loosely, 'make the demons flee'), as mentioned by Leonhard Fuchs (New Kreüterbuch. Cap. CCCXXIII, 1543; Robson 2003). It seems convincing that the people in ancient times used the plant that proved effective in humans to 'banish demons' to protect their

family altars as well. Thus, the name of the plant derives from its initial benefit in the treatment of depressions or similar conditions.

The power to ward off evil spirits was especially important at times when such spirits were believed to be most abundant, for example on Midsummer's Eve (21st of June), and *Hypericum* was picked at this date to decorate religious images. The pagan feast celebrated on Midsummer's Day was eventually christianized and dedicated to St. John the Baptist (and changed to his birthday, 3 days later), and the plant used on that day was subsequently named St. John's wort (*Johanniskraut*, *herbe de la Saint-Jean*, *hierba de San Juan*, *Erba di San Giovanni*, etc.; Robson 2003).

Phytochemistry & pharmacology

A complex mixture of bioactive secondary metabolites in several *Hypericum* species makes them valuable as herbal drugs (Crockett *et al.* 2005; Mártonfi *et al.* 2006; Crockett *et al.* 2010). *Hypericum perforatum* (Common St. John's wort), certainly the best-known and worldwide most abundant representative, is today the most investigated species of the genus (Nahrstedt & Butterweck 2010). Since the early 90s, *H. perforatum* has been clinically studied from the perspective both of its chemical constituency and of its biological activity (Röder *et al.* 2004). Around 2 500 studies on *Hypericum* have been published to date (thereof *c.* 950 without *H. perforatum*; S. Crockett, pers. com.), including several reviews focused on the phytochemistry of *H. perforatum* (*e.g.,* Nahrstedt & Butterweck 1997; Hölzl & Petersen 2003; Beerhues 2011), its pharmacology (*e.g.,* Butterweck & Schmidt 2007; Linde 2009), or both aspects (*e.g.,* Roth 1990; Avato 2005; Müller 2005; Nahrstedt & Butterweck 2010).

The antidepressant activity of *H. perforatum* is certainly the main reason for the general public's enthusiasm for this herbal medication (Linde *et al.* 2008). The classical use of (dried alcoholic) extracts of *H. perforatum* for the treatment of mild to moderate depression has been demonstrated to be effective in several trials and meta-analyses (*e.g.,* Linde *et al.* 2008; Kasper *et al.* 2010). The antidepressant activity of *H. perforatum*-based formulations can be attributed to several classes of secondary metabolites, which exhibit additive, synergetic and partly antagonistic effects (Butterweck & Schmidt 2007). According to the actual state of scientific knowledge, the total extract must be considered as the active principle (Beerhues 2011).

Approximately nine groups of bioactive natural product classes have been identified from *H. perforatum* (Hölzl & Petersen 2003), summarized in Table S1. The best known and probably main principles responsible for the antidepressant activity are (i) the *naphthodianthrones* hypericin and pseudohypericin, which are red pigments accumulated in the dark glands (Zobayed *et al.* 2006; Karppinen *et al.* 2008), (ii) the light-sensitive and unstable *phloroglucinol derivatives* hyperforin and adhyperforin, which accumulate in pale glands (Soelberg *et al.* 2007) mainly in the generative parts (Butterweck & Schmidt 2007), as well as (iii) *xanthones*, a class of substances produced in higher amounts in the roots of *Hypercium* (Eggelkraut-Gottanka *et al.* 2002), and (iv) *flavonoids* (Fig. S1.).

Photosensitivity (sensitivity to sunlight) following the ingestion of *Hypericum* or hypericin, a phenomenon known as Hypericism, was first observed in animals that had consumed the phototoxic plant. It has later been shown to be effective in several studies involving cell cultures and humans (Barnes *et al.* 2001). From these findings, however, it has been estimated that the usual

therapeutic doses of *Hypericum* extracts are about 30 to 50 times below the level required for phototoxicity (Siegers *et al.* 1993).

Economic importance

In most countries, *Hypericum* products are marketed as dietary supplements, and therefore not subjected to stringent drug regulations. In the European community, however, *Hypericum* products are available both as food supplements and as drugs (Linde 2009). *Hypericum perforatum* was among the top ten best-selling herbal dietary supplements sold in the USA in 2008, with sales estimated at *c.* 8.2 million US$ (American Botanical Council 2009), and it represented nearly 13 % of all European herbal product sales in 2004, valued at more than 70 million € in Germany alone (Bäcker *et al.* 2005; Crockett & Robson 2011). The main European production regions are in Germany (with more than 600 ha in 2003; Gärber & Schenk 2004), Poland and Mallorca (Roth 1990; Schempp *et al.* 2002; Gaudin *et al.* 2003).

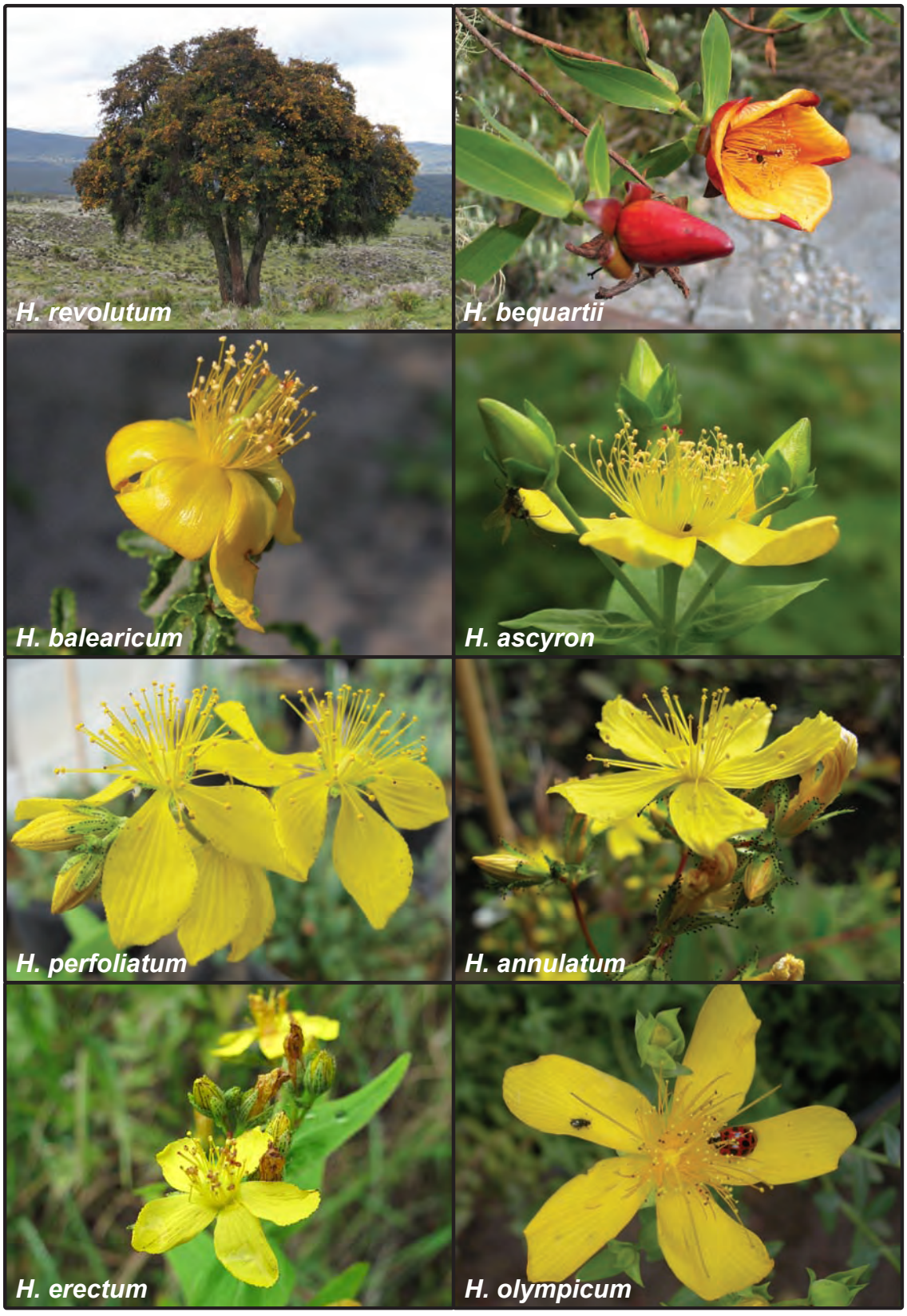
Numerous *Hypericum* species are cultivated as ornamentals, and various hybrids and cultivars have been developed for use in horticulture, such as *Hypericum* x *moserianum* (*H. calycinum* x *H. patulum*), *H. hookerianum* 'Charles Rogers', and *Hypericum* x 'Hidcote' (*Hypericum* x *cyathiflorum* 'Gold Cup' x *H. calycinum*).

Hypericum perforatum, *H. canariense* and *H. androsaemum* are recognized as invasive species in the USA, Australia and New Zealand (Lane 1979; Zouhar 2004; Dlugosch & Parker 2007; Groenteman *et al.* 2011). In the USA, *Chrysolina quadrigemina* (Klamath weed beetle, Chrysomelidae) has been considered an effective biological control for range populations of *H. perforatum*, and has been imported from Australia where it has been used as a successful biocontrol agent (Buckley *et al.* 2003; Sirvent *et al.* 2003, and citations within).

The worldwide rise of *H. perforatum* production has been accompanied by a documented increase in a plant disease called anthracnose. Caused by a fungal plant pathogen dispersed together with the seeds, anthracnose in St. John's wort fields in Europe is known as *Colletotrichum*-wilt since 1995 (Gaudin *et al.* 2003). The responsible fungus *Colletotrichum gloeosporioides* Penz. (Melanconiales, Coleomycetes) is a facultative parasite (Gärber & Schenk 2004). Several cultivars of *H. perforatum* differing considerably in susceptibility by *C. gloeosporioides* have been examined (in order of decreasing infestation they are: 'Hyperiflor', 'Hyperisol', 'Topaz', 'Hyperivo 7' and 'Taubertal'; Trautwein & Garber 2006). Other studies investigated the potential use of this fungus for the biocontrol of St. John's wort in Canada (Hildebrand & Jensen 1991).

Fig. 1.1-A Species of *Hypericum*. *H. hircinum* is a variable species that hybridizes with its close relatives (flower *c.* 1.5–3.0 cm in diameter). *H. aegypticum* (sect. *Adenotrias*) is a small leaved shrub that possesses staminodes ('fasciclodes') between the stamen fascicles (flower *c.* 1.0 cm in diameter). *H. canariense* (sect. *Webbia*) is native to the Canary Islands, but is an inversive species in California (flower *c.* 2–3 cm in diameter). *Tiadenum japonicum* has been excluded from *Hypericum* by some authors due to the color of the petals that is not yellow and the occurence of staminodes (flower *c.* 1.0 cm in diameter). *H. prolificum* (sect. *Myriandra*), *H. quitense, H. goyanense*, and *H strictum* (sect. *Brathys*) are native to the New World, the three later in South America. All species shown in this figure do not possess dark glands.


H. hircinum x cf. androsaemum
H. aegypticum
H. canariense
Triadenum japonicum
H. prolificum
H. quitense
H. goyanesii
H. strictum


H. revolutum
H. bequartii
H. balearicum
H. ascyron
H. perfoliatum
H. annulatum
H. erectum
H. olympicum

1.1.2 Biology of *Hypericum*

In the following, an overview on the biology of *Hypericum* is given, focusing on selected morphological characters and anatomical features, on floral development, cytology and on reproductive systems. A formal genus description is given in the Appendix 1.1. Species of the genus can be typically recognized by their leaves (opposite, simple and entire, lacking stipules), yellow flowers with petals free and several stamens in 3 or 5 fascicles, styles free, and the presence of pale and sometimes reddish to black glandular secretions (glands). The fruit is, in general, a dehiscent capsule, containing small cylindrical light brown to black seeds. For further and more comprehensive information about characters and descriptions of the genus, the reader is particularly referred to Robson (1981) and Stevens (2007), or to Ernst (2003) and Judd *et al.* (2008).

Habit

The typical habit in *Hypericum* is a shrub or a herb, each accounting for roughly 47 % of the species of the genus. A tree-like habit has been described for *c.* 5 % of the species, but true trees growing up to 12 m high and developing a single trunk, as observed in the Rwenzori Mountains of Central Africa for *H. bequaertii* and in the Ethiopian highlands for *H. revolutum* (Robson 1993), are rare and have been found in disturbed habitats (Berit Gerke, pers. com.). A perennial lifecycle is dominant for the herbaceous habit of *Hypericum*, an annual one being described only for *c.* 9 species from the New Word (*e. g., H. gentianoides* from Canada, or *H arenarioids* from lowland Cuba).

Characteristic for most representatives of *Hypericum* are 2–4 (–6) stem lines raised along each internode, often visible only when young, or expanded to from narrow wings (*e. g., H. tetrapterum*). In some species (*H. perforatum* and relatives) 'stolons' or 'runners' have developed, which allows these plants to vegetatively propagate in an effective way.

Fig. 1.1-B Species of *Hypericum*. *H. revolutum* and *H. bequartii* (sect. *Campylosporus*) can grow like trees, but in their natural (undisturbed) habitats they are instead tall shrubs (flower *c.* 4–8 cm in diameter). *H. balearicum* (sect. *Psorophytum*) is a small shrub with characteristic protuberances at stem and leaves (flower *c.* 1.4–4.0 cm in diameter). *H. ascyron* (sect. *Roscyna*) is an erect herb native to East Asia and with one subspecies it occurs in North America (flower *c.* 3–7 cm in diameter). *H. perfoliatum* (sect. *Drosocarpium*), *H. annulatum* (sect. *Adenosepalum*), *H. erectum* (sect. *Hypericum*), and *H. olympicum* (sect. *Olympia*) are herbs, which produce dark glands (flowers *c.* 1.5–4.0 cm in diameter).

Glands

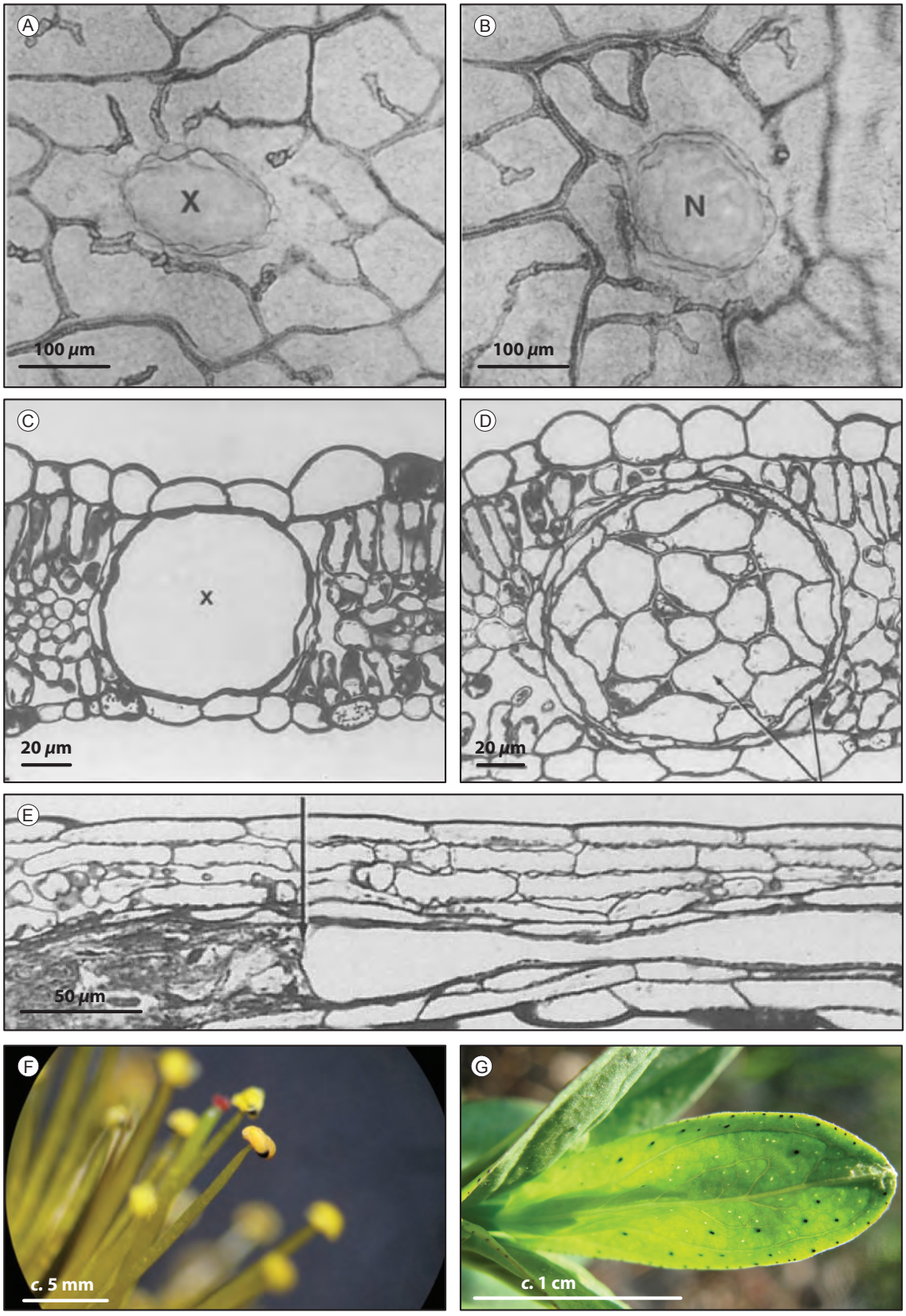
Secretory structures in *Hypericum* has been suggested to be involved in the plant's response to herbivore attack (Sirvent *et al.* 2003) and have been anatomically studied since the 19th century (Green 1884). Several types of secretory structures have been described, including spheroidal pale glands ('translucent cavities'), spheroidal dark glands ('red to black multicellular nodules') and pale and/or dark secretory canals of different types (Curtis & Lersten 1990; Ciccarelli *et al.* 2001; Maggi *et al.* 2004; Lotocka & Osinska 2010). The distribution and appearance of the glands and canals (*i. e.* as dots, streaks or lines, on leaves, stem or in flower organs) have been used in the subgeneric classification of the genus (Keller 1895; Robson 1977).

Pale glands are ubiquitous in *Hypericum* at least on leaves, making this organ often look perforated (Fig. 1.2). They consist of a schizogenous cavity surrounded by one or more layers of epithelial cells (Lotocka & Osinska 2010), and are the site of hyperforin accumulation (Soelberg *et al.* 2007). They stain positively for essential oils (Ciccarelli *et al.* 2001). Dark glands, in contrast, occur in just about ⅓ of the species. It has been shown, that the red pigment hypericin is mainly located in these dark glands, with the highest concentration in flower tissues, especially in stamens (Zobayed *et al.* 2006). Dark glands consist of a mono- or biseriate sheath of flat cells surrounding isodiametric and bigger inner cells (Lotocka & Osinska 2010; Fig. 1.2). They might be elongated as tubular nodules (Curtis & Lersten 1990) and may also be described as 'streaks' or 'lines' (Robson 1981).

Secretory canals of different types have been reported for *Hypericum*. Ciccarelli and colleagues (2001) have counted three types of translucent canals. Type A canals are associated with veins and may serve as a transport medium for photosynthates. Type B canals resemble anatomically and ontogenetically elongated pale glands, and type C canals are located on the ovary, and contain resins. The latter enlarge in fruit and have been described as amber 'vitae' or 'vesicles' (Robson 1981).

Curtis and Lersten (1990) report the observation of 'chimerical' canals, which change color abruptly at some point from black to translucent (Fig. 1.2-E). They conclude that the dark glands (nodules) are a modification of common pale glands (translucent secretory reservoirs), which is in accordance with Robson's observations (Robson 1981: 80 f., and pers. com.).

Fig. 1.2 Glands of *Hypericum perforatum*. **A**) Pale gland or 'translucent cavity' (X) seen in a cleared leaf in top view, surrounded by minor veins and vein endings. **B**) Dark gland or 'black nodule' (N) seen in a cleared leaf in top view, surrounded by minor veins and vein endings. **C**) Median view of pale gland with flattened epithelium surrounding the schizogenous cavity (X) in a leaf cross section. **D**) Median view of dark gland with clusters of interior cells and sheath of flattened cells (arrows) in a leaf cross section. **E**) Median longisection of a 'hybrid' streak showing nodule portion at left and cavity portion at right. The arrow indicates the boundary. **F**) Dark gland at the connective of the anther. **G**) Pale and dark glands in the leaf. A–D Modified from Curtis & Lersten (1990).


A
X
100 µm
B
N
100 µm
C
X
20 µm
D
20 µm
E
50 µm
F
c. 5 mm
G
c. 1 cm

Flowers and pollination

The bisexual flower of *Hypericum* is normally stellate, (3–) 20–40 (–70) mm in diameter, tetra- to pentamerous and homostylous with a general flower formula of K4–5 C4–5 A5–∞ G(3–5) (for details see Appendix 1.1). Flowers of *Hypericum* are generally nectarless. They are typical 'pollen-flowers' visited by less-specialized insects, of which the Syrphidae (Diptera) are the most common to *Hypericum* (Robson 1981: 119 ff.).

The yellow (flavonoid) colored petals are characteristic for *Hypericum* and are used to define borders of the genus (Robson 1977: 301). The petal color is white in *Lianthus*, pink to purple or white in *Triadenum*, and pink or pink and white in *Thornea*, but yellow in *Santomasia* (Stevens 2007). Within *Hypericum*, however, such modifications also occur. Pure white forms are very rare but have been recorded in *H. geminiflorum* from Taiwan and the Philippines (Luzon) and in *H. albiflorum* from Turkey. The red tinges (anthocyanin) usually occurring in dorsal parts of the petal are either confined to veins (*e.g.,* in *H. trichocoulon* from Crete), or are more or less diffused (Robson 1981: 94), resulting in a 'red-spotted' flower, *e.g.,* in *H. revolutum*, or in a 'crimson-flowered' *Hypericum*, as recorded in *H. capitatum* var. *capitatum* from Turkey and Syria (*cf.* Fig. 1.1).

Androecia

The specific arrangement of stamens in bundles, in the so-called stamen fascicles, is a characteristic feature for *Hypericum* and has been used for subgeneric classification (Keller 1895; Robson 1977, 1981). According to Leins & Erbar (2000) the polyadelphus androecia of *Hypericum* are antepetalous and of centrifugal development. The arrangement in bundles results from a primordial burgeon, on which several primordial stamens form, until the burgeon is fully occupied. Connate filaments of a fascicle are the result from the primordial burgeon that is developing (growing) together with the stamens (Leins & Erbar 2000).

Stamen fascicles

In a pentamerous flower one would also expect five stamen fascicles, as it is the case in the species of *Hypericum* sections *Campylosporus*, *Psorophytum*, *Ascyreia*, *Takasagoya*, *Androsaemum*, *Inodora* and *Roscyana*. In several other species of *Hypericum*, however, the number of fascicles appears to be smaller than that of the petals. This phenomenon results from the merging of adjacent pairs of fascicles, with the result that the 4 visible fascicles are really 1 + 1 + 1 + (2) and the 3 fascicles most commonly found in *Hypericum* are 1 + (2) + (2). These double fascicles are always opposite sepals, *i.e.* between petals (Robson 1977: 304). Several other androecial configurations are present in *Hypericum*, resulting from modifications of the fasciculate arrangement, *e.g.,* the union of fascicles to form a narrow or broad ring, or the reduction of each fascicle to a single stamen, as well as the elimination of one androecial member, resulting in tetramery (Robson 1981: fig. 20).

Staminodes

The occurrence of conical or ligulate bodies between the stamen fascicles, the staminodes (*i.e.* vestigial fascicles, also called 'fasciclodes'), is a common floral structure and present in all genera

of the Hypericaceae (Robson 1981: 64, 102 f.). They act like lodicules of grasses and help to expand the petals and sepals from the bud (Robson 1977: 302). Within *Hypericum*, however, they are present in four species only, namely in *H. elodes* (*H.* sect. *Elodes* from West Europe and the Azores), *H. aegypticum*, *H. russeggeri*, and *H. aciferum* (all *H.* sect. *Adenotrias* from the Mediterranean). The occurrence of staminodes in these species has been named an 'evolutionary recall' (Robson 1972). Thus, the hypothesis is that absence of such staminodes is an apomorphic character state for *Hypericum*.

Gynoecium

The ovary in *Hypericum* is superior, with connate carpels, and surmounted by (2–) 3–5 elongate and distinct to (basally) connate styles, terminated by minute, punctate stigmas. The gynoecium is typically syncarpous with an axile placentation, or sometimes paracarpous and parietal with deeply intruded placentas. The ovules are generally numerous per carpel and with a thin megasporangium.

Fruit and seeds

The fruit is commonly a septicidal capsule, or rarely tardily dehiscent or indehiscent with ± fleshy valves (*e.g., H. androsaemum*), which has been described as berry- or drupe-like (Robson 1981: 109). Glands, described as 'vittae' or 'vesicles', are commonly present on the pericarp (*e.g., H. perforatum*), but occasionally also absent (*e.g., H. hookerianum*). The tiny seeds are not arillate, and have a straight embryo and a scanty endosperm. The testa sculpturing has been described as reticulate, scalariform or papillose, with modification of all of these states (Robson 1981: 112 ff.). It is used as an important characteristic to distinguish between larger groups in the sectional key (Robson 1977: 342 ff., 2001: 43 f.).

Chromosome numbers

Within *Hypericum* karyology is quite diverse, as basic haploid chromosome numbers of x = 6–10 and 12 have been reported (Robson 1981; Kogi 1984). Robson (1981) suggested that n = 12 is the ancestral chromosome number within *Hypericum*. Aberrations of these numbers are frequent, as polyploidization is a common phenomenon in the genus (Robson 1981: 167 ff.). Tetraploidy is reported on the base of n = 6 and n = 8–10. Higher degrees of polyploidy are confined to the *H.* sect. *Hypericum*, and are associated with the largely apomictic *H. perforatum* aggregate.

Hypericum perforatum is supposed to be an allopolyploid hybrid, with *H. maculatum* subsp. *maculatum* and *H. attenuatum* as parents (Campbell & Delfosse 1984), and with the basic chromosome number of x = 8 (Robson & Adams 1968; Brutovská *et al.* 2000b). Results of cytological (Brutovská *et al.* 2000a) and mainly molecular studies (Scheriau & Koch in prep.), however, point instead to one or more origins of the polyploid populations out of diploid, *i.e.* suggest autopolyploidy for *H. perforatum*. In wild populations the tetraploid form (2n = 4x = 32) occurs most frequently. Although both diploid (2n = 2x = 16) and hexaploid (2n = 6x = 48) individuals can be found (Robson 1981; Matzk *et al.* 2001). Moreover, individuals of all three ploidy forms hybridize

with the diplo- and tetraploid subspecies of *H. maculatum*, resulting in the morphologically extremely plastic *Hypericum* x *desetangii* complex (Robson 1981, 2002).

Reproduction biology

The reproductive biology of *Hypericum* has been studied for almost a century. Apomixis, a form of asexual reproduction in which a seed is still developed, was first described for *H. perforatum* by Noack (1939), and for *H. virginicum* L. (*Triadenum virginicum* Raf.) by Myers (1964). Since Matzk and colleagues (2001) determined eleven different routes of reproduction in *H. perforatum*, the species has become a model plant for apomixis research (Matzk *et al.* 2003; Pank *et al.* 2003; Barcaccia *et al.* 2006; Matzk *et al.* 2007; Qu *et al.* 2010; Schallau *et al.* 2010). It has been shown that *H. perforatum* is a facultative apomict with the gametophytic mode of parthenogenesis (where a meiotically unreduced, non-fertile egg develops into an embryo). It includes apospory (the embryo sac develop from cell(s) adjacent to the megaspore mother cell), and the pseudogamous mode (fertilization of the polar nucleus is required) of endosperm development (for review see Schallau *et al.* 2010, and citations within), even though autonomous endosperm formation has also been reported (Matzk *et al.* 2001).

Within *Hypericum*, as many as 16 apomictic species have been described, one of which (*H. scabrum* from the Eastern Mediterranean to West Asia) is an obligate apomict (Matzk *et al.* 2003).

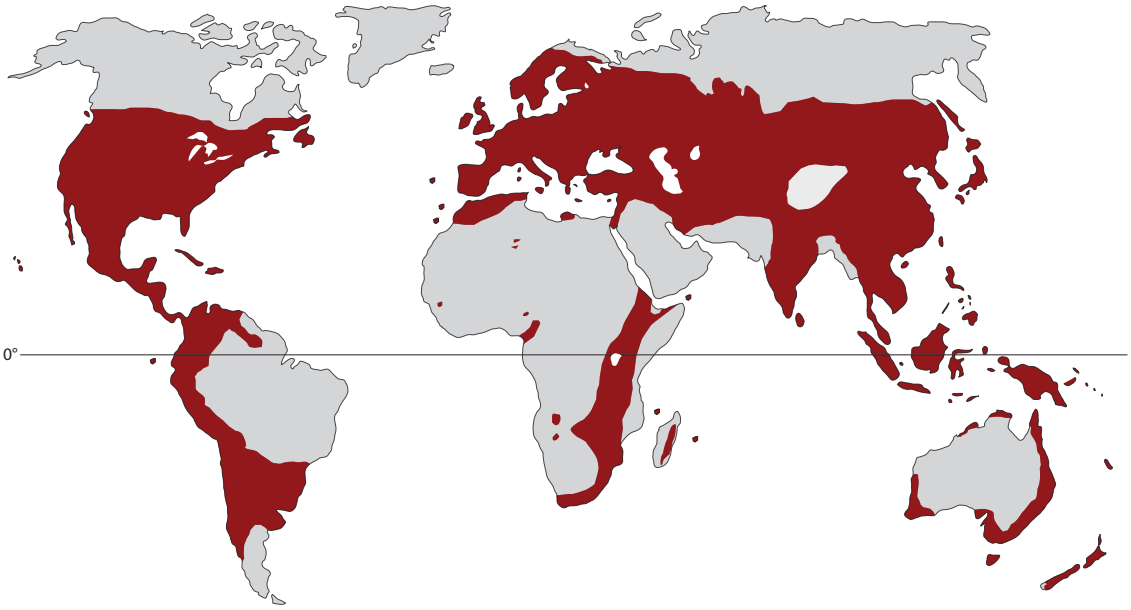


Fig. 1.3 Distribution of *Hypericum* (modified from Robson 1977 onwards).

1.1.3 Distribution and biogeography

Hypericum is distributed all over the Northern Hemisphere, in Andean South America, tropical and Southern Africa, Madagascar, Southeast Asia, and a few species also occur in Australasia and Oceania. The main center of the diversity of *Hypericum* can be found in the Palaearctic area, where more than 45 % of the described species are native. A second center is located in the Neotropic, with *c.* 30 % of the species. Compared to these numbers, the Indo-Malayan, Nearctic and Afrotropic regions harbor much less diversity, with 10 %, 8.5 % and 6.4 % of the known species, respectively.

As mentioned before, in tropical regions *Hypericum* is confined to high mountain habitats. Robson (1977, 1993) assumed that the equatorial species/sections of *Hypericum* were of early descent within the genus and, therefore, hypothesized a Gondwanan origin for *Hypericum* (specifically: Central Africa). As closely related genera (*Thornea*, *Vismia*, *Harungana*, *Cratoxylum* and *Eliea*) are tropical flora elements, this assumption is plausible.

The time frame for the break-up of South Gondwana (*i. e.* South America and Africa) more than 105 million years (Ma) ago (McLoughlin 2001), however, contradicts this vicariance hypothesis for *Hypericum*, as the family Hypericaceae has been estimated to be around 60–70 Ma old (Davis *et al.* 2005). Thus, Robson's biogeographic 'out of Africa' scenario would imply several long distance dispersal events at an early stage during the evolution of the genus. These issues will be investigated and discussed in the following chapters of this thesis.

Classification

The first generic description of *Hypericum* is that of Tournefort (1700: 254, t.131). The first treatment of the whole genus, however, was done by Choisy (1821), whose synaptic monograph of the 'Hypericineae' contained seven genera, of which three (*Androsaemum*, *Ascyrum* and *Hypericum*) together represent *Hypericum* in its current sense, except that Choisy included the species placed by Robson (1977: 294) in *Triadenum*. Robson (1977, 1981, 1985, 1987, 1990, 1996, 2001, 2002, 2006, 2010a, b) published in eight parts the most comprehensive monograph of *Hypericum* currently available (species names and corresponding authors etc. used in this thesis are listed in Table S1.2). The monograph includes a revised infrageneric classification and a review of previously published classifications of the genus (Spach 1836b, a; Jaubert & Spach 1842–1843; Keller 1895, 1925; Kimura 1951). Currently, 486 species have been recognized based on morphology, distribution and, to a certain amount, cytology (Robson [part 9] in prep.) and classified into 36 sections (Table 2.1). Based on several supposed evolutionary trends of certain characters, a genealogical scheme was developed, showing suggested relationships of the sections (Fig. 1.4).

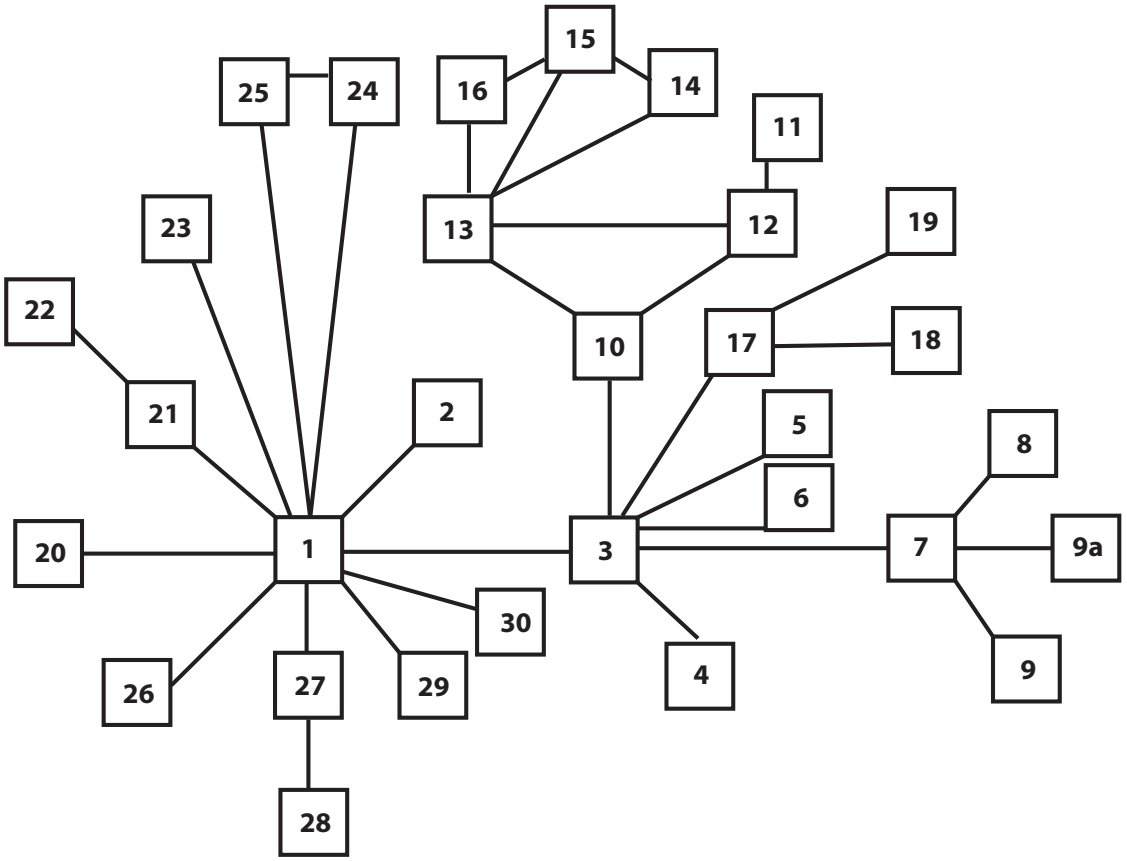


Fig. 1.4 Relationships of sections within *Hypericum* suggested by Robson (1977: fig. 1; 1981: fig. 2).

The central position of *Hypericum* sect. *Campylosporus* (section number 1), containing Afrotropic mountain species, reflects the evolutionary concept on which Robson's classification is based, *i. e.* allowing several taxa to be derived from centrally placed (paraphyletic) taxa, which some researchers refer to as an 'evolutionary' classification. The accuracy of this evolutionary hypothesis has not been comprehensively investigated yet by means of phylogenetics.

1.2 Objectives of this study

The aim of this dissertation is to provide new insights into the evolutionary history of the genus *Hypericum*. And to identify reasons that might have contributed to the observed species richness within this flowering plant group.

Results attained from investigations using morphological data and molecular phylogenetic inference will be presented. A phylogenetic hypothesis will be formulated first in order to position the analysis of evolution of the morphological and ecological characteristics, the reconstruction of historical biogeography as well as the identification of diversification events into a testable framework, *i. e.* into an explicit phylogenetic context.

1.2.1 Phylogeny

Phylogenetic analyses within *Hypericum* and related taxa are necessary to place subsequent analysis into a testable framework. To take into account the diversity within a large genus like *Hypericum*, a cladistic approach by analyzing morphological characters (Chapter 2) was used. With this conception the extensive data on species description available in the *Hypericum* monograph (Robson, 1977, onward) can be incorporated and processed. Thus, almost all described taxa of the genus are considered. In the second step, a molecular phylogenetic approach using nuclear DNA sequence analysis is undertaken. Incorporation of the results of both approaches makes it possible to circumscribe monophyletic units and ascertain their interrelationships (Chapter 3).

Based on a comprehensive genus' phylogeny, it is possible to investigate questions regarding character evolution and ecology (Chapter 3), historical biogeography and the reasons for the high species number in *Hypericum* (Chapter 4). The focus lies on the subjects listed in the following paragraphs.

1.2.2 Character evolution and ecology

A tree-like growth form is characteristic for most genera of Hypericaceae, but only for few species of *Hypericum*. Within *Hypericum*, around half of the species are shrubs, and the others are herbs. Questions here are: when did different life forms evolve and what is the ancestral character state within the genus? Did 'woodiness' evolve only once within the family and become lost within *Hypericum* later? Is this trait correlated to a certain niche, *e. g.,* occurrence in tropical habitats? Or similarly, is a herbaceous habit correlated to wet and swampy areas?

Some of the further traits investigated here are the evolution of dark glands, as these have been suggested to be involved in plant defense against herbivores, the evolution of fruit types, what might open new dispersal modes, and the occurrence of apomixis. The aim of these investigations is to recognize possible key innovations, that is, trait novelties correlated to radiation events.

1.2.3 Historical biogeography

Biogeography, the study of the distribution and evolution of organisms through space and time (Ball 1975; Wiley 1981), allows further insight into the evolutionary history of *Hypericum*. In this study I develop a general scenario of area colonization, as the proposed 'out of Africa' origin of *Hypericum* is in conflict with the supposed age of the family Hypericaceae and the break-up sequence of Gondwana (see 1.1.3). To be able to incorporate the climatic and geological background, divergence time estimations of lineages based on DNA sequence information (age estimations of certain clades) are conducted.

In detail, it is focused on dispersal *versus* vicariance between temperate and tropical regions, Eurasia and America, West and East Eurasia and North and South America.

1.2.4 Diversification

More than 80 % of the species described for the family Hypericaceae belong to the genus *Hypericum*. *A priory* one would expect that the phylogenetic tree imbalance for the family result from higher net diversification rates within *Hypericum*. Species richness, however, might also be an outcome of generally increased species turnover, as diversification is the product of speciation minus extinction (Vamosi & Vamosi 2011).

In this work, I present the results of analyses aiming to detect diversification rate shifts, either for the entire genus or for certain clades within *Hypericum*. The aim is to identify reasons for the species richness within the genus. It is possible to test for evolutionary events by asking whether potential rate shifts are more significantly correlated to the evolution of certain morphological innovations or to niche shifts (novel ecological characteristics) or to dispersal and colonization of new areas.

1.3 Methods

The next sections will give an overview on the methods employed to investigate the evolutionary history of *Hypericum*.

Since "only with a phylogeny can we begin to understand diversification, regularities in patterns of evolution, or simply suggest individual evolutionary changes within a clade" (Stevens 2001 onwards), it starts with the methods for reconstructing a phylogeny, that is the evolutionary history and relationships of biological taxa.

1.3.1 Phylogenetic inference

The classical way of estimating the relationships between species/taxa is to compare the variation across their characters and to classify the identified entities in a hierarchical system (Linnaeus 1758; Vandamme 2009). In the past two decades molecular data, mainly nucleotide or amino acid sequences, have revolutionized phylogenetics (Savolainen & Chase 2003), and have had a major impact on our understanding of evolution (Álvarez & Wendel 2003; Stech & Quandt 2010).

The main challenge in phylogenetic reconstructions is the enormous amount of possible tree topologies that can explain the data. The number of possible solutions is dependent on the number of included terminal taxa (or operational taxonomic units, OTUs), being (2n–3)!/2n–2(n–2))! for rooted, bifurcating tree topologies (Edwards & Cavalli-Sforza 1964).

Phylogenetic methods

Today, there are many statistical methods that can be used for reconstructing phylogenetic trees from different sources of data (Huelsenbeck *et al.* 2002; Felsenstein 2004). They can be classified according to the kind of data they use (discrete characters *versus* distance matrix of pairwise dissimilarities) or by the algorithmic approach of the method (optimality criterion *versus* clustering algorithm). Commonly they are grouped into three major classes: (A) distance, (B) parsimony and (C) model based methods. The first calculates a distance matrix and uses a clustering algorithm to find the single 'best' tree. The two latter use any kind of discrete character states and an optimality criterion (maximum parsimony, maximum likelihood, or Bayesian inference). Character-state methods analyze character positions independently by comparing their states.

Beside maximum likelihood that ends up with the statistically evaluated best tree, character-state methods sample a landscape of possible tree topologies (the 'tree-space'). Normally they end up with several hundred possible tree topologies and consensus trees are the common way in which to summarize the results.

(A) Distance method: NJ

Distance methods such as Neighbor-joining (NJ) calculate the dissimilarities of each pair of sequences (OTUs) usually employing an evolutionary model, and then infer the phylogenetic relationships of the OTUs from the distance matrix. They avoid the problem of evaluating different trees by gradually clustering OTUs into a single tree (Saitou & Nei 1986, 1987). However, as the original character state of the taxa is discarded, the information required to reconstruct the character states of ancestral nodes is lost.

The main advantage of distance methods is that they are computationally inexpensive, which makes these cluster analysis algorithms practical for fast data evaluation.

(B) Parsimony method: MP

Maximum parsimony (MP) aims to find a tree topology that minimizes the amount of evolutionary change required to explain the data, that is, to find the tree that can be explained with the smallest number of character changes (Fitch 1971). MP starts with a randomly produced tree and calculates a parsimony score (the 'tree length'), *i. e.* the sum of all character state changes required to explain a certain topology. When a reasonable number of topologies have been evaluated, the most parsimonious tree(s) is (are) selected (Swofford & Sullivan 2009). The MP method itself is based on William of Occam's (*c.* 1320) parsimony principle "*Pluralitas non est ponenda sine necessitate*", by which hypotheses should be kept as simple as possible (Posada & Buckley 2004).

The challenge about this method is that all possible tree topologies have to be searched to find the most parsimonious one, which can be time consuming depending on the amount of OTUs included. Algorithms implementing different kind of search methods, like an exhaustive search, or approximate methods such as stepwise addition, branch-swapping (tree bisection and reconnection, TBR) have been developed and implemented in software packages like PAUP* (Swofford 1993, 2002).

The critical feature in this approach is that one must demonstrate a direct relationship between the number of character-state changes required by a tree topology and the complexity of the corresponding hypothesis (Swofford & Sullivan 2009). This connection often implies an *ad hoc* hypothesis, such as the identification of shared traits derived from a common ancestor, called (syn-) apomorphies (Hennig 1953, 1966).

Maximum parsimony always assumes that a common character is inherited directly from a common ancestor. Thus, when homoplasy (*i. e.* the sharing of identical characters that cannot be explained by inheritance from a common ancestor of a group of taxa) is high in a dataset, parsimony misleadingly (and uncorrected; see below: model based methods) interprets these similarities as synapomorphies (Felsenstein 1978).

This trend can result in a phenomenon called long-branch attraction (LBA; Hendy & Penny 1989), which is especially critical when using DNA sequence data, for which each character has only four possible states. Thus, when DNA substitution rates are high, the probability that two lineages will independently evolve the same nucleotide (character state) at the same site increases (Siddall & Whiting 1999), and MP (and other phylogenetic approaches) will falsely join such long branches. However, some authors argue that LBA is mainly a sampling problem and may be al-

leviated by including taxa that break up the terminal long branches (for a review see Bergsten 2005). This approach is, of course, only feasible when the cause for sequence differences (*i. e.* long branches) between related taxa in a clade is not extinction.

To summarize one can conclude, that MP is a relatively fast method that allows one good exploration of alternative tree topologies, and that its attractiveness also originates from the use of a fundamental method in science, the parsimony approach.

(C) Model based methods: ML and BI

Model based methods incorporate, as prior to the analysis, ideas about the probability of character state changes, like nucleotide substitution models correcting for misinterpreted synapomorphies (see above: parsimony methods, LBA).

Maximum likelihood (ML)

Maximum likelihood methods (Fischer 1922) have the advantage of using a statistical criterion. ML is a method of estimating the parameters of a statistical model by using standard techniques (such as for coin-tossing problems) for inferring probability distributions in order to assign likelihoods to certain tree topologies and corresponding branch length (measured in units of the expected number of substitutions per side). This allows us to compare the relative support for different phylogenetic hypotheses in a statistical framework. ML algorithms search for the phylogeny that maximizes the probability of observing the data, given a certain topology and a defined model of evolution (Huelsenbeck & Crandall 1997).

Exploring all possible tree topologies that can explain the data is usually not feasible. Various heuristic strategies have been developed to tackle this drawback (*e. g.,* Lewis 1998), and are implemented in programs such as RAxML (Stamatakis 2006), which can analyze extensive datasets in a reasonable time (Smith *et al.* 2011; Soltis *et al.* 2011). However, none of the algorithms guarantee to find the best tree under the specified model in the 'tree space' (Siddall & Kluge 1997).

Bayesian inference (BI)

Bayesian phylogenetic methods also employ the concept of likelihood, but by targeting a posterior probability distribution of trees and priors explaining the data. The method is mathematically formulated in Thomas Bayes publication "Essay towards solving a problem in the doctrine of changes" (Bayes 1763).

Unlike ML, Bayesian methods incorporate prior information through the specification of a prior probability distribution on the substitution model parameters that vary with the details of the analysis (Huelsenbeck *et al.* 2002). The relative evidence present in the data is then used to adjust the prior beliefs, which results in a posterior probability for each tree, given the model, the prior, and the data. As some priors of the substitution models are not known (substitution model parameters, *e. g.,* base frequencies, rate variation) the Bayesian approach is that all trees are given equal weight, that is, *a priori* uniform priors are used and subsequently adjusted according to the data (Huelsenbeck *et al.* 2001). Thus, BI generates confidence multiplied by evidence.

To calculate the posterior probabilities in Bayesian phylogenetics a method called Markov chain Monte Carlo (MCMC) is used. It is a sampling technique that is based on Markov chains of the first order (*i. e.* the next state depends on the current state), which compares at each step (*i. e.* a set of parameters) the likelihood ratio and prior ratio for the new state relative to the previous state. When the combined product is better, the parameters are accepted and, relative to them, a new step is proposed (hill climbing approach). This procedure is stochastically repeated thousands or millions of times. After an initial convergence to a set of probable tree/model solutions, the algorithm is supposed to sample the 'posterior' distribution on the parameters explaining the data. The frequency by which a particular topology is sampled is then proportional to its posterior probability (Vandamme 2009). These methods have been incorporated into programs like MrBayes (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) and extended by parallel Metropolis coupled MCMC (MC^3) algorithms (Altekar *et al.* 2004), which allow multiple peaks in the landscape of trees to be more readily explored. Current developments especially aim at a cost-effective usage of computational power (Suchard & Rambaut 2009; Zhou *et al.* 2011).

The main advantage of Bayesian MCMC is that estimation of phylogeny is based on a posterior probability distribution of trees, what allows accommodating uncertainties in the topology in comparative studies (Huelsenbeck *et al.* 2000). Furthermore, the posterior probability (pp) concurrently calculated with the tree topology is an intuitive measure of support for trees (Huelsenbeck *et al.* 2002).

Statistic support methods: bootstrapping

Unlike Bayesian approaches, distance, maximum parsimony and maximum likelihood methods lack statistic support of results and need independent calculations to provide confidence limits on phylogenetic trees. The bootstrap method (Efron 1979) is a computer-based resampling technique for assessing the accuracy of almost any kind of statistical estimate (Efron & Tibshirani 1993). Felsenstein (1985) first introduced bootstrapping in phylogenetics by analyzing randomly generated 'resampled datasets'. The results (trees) of the resampled data are summarized in a consensus tree with percentage support values for the nodes reconstructed in *n* trees.

Model selection

Several methods have been proposed for the selection of nucleotide or amino acid substitution models necessary for distance, maximum likelihood and Bayesian estimation of phylogenies. The difficulty is to decide how complex a model should be for a given problem (Pybus 2006). Classically MP was used for model selection, later the hierarchical likelihood ratio test (LRT; Huelsenbeck & Crandall 1997; Posada & Crandall 1998) and at last a procedure based on the information criterion described by Akaike (AIC; Akaike 1974). The LRT and the AIC are implemented in programs like Modeltest (Posada & Buckley 2004; Posada 2006) or websites like FindModel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html).

1.3.2 Character evolution

The reconstruction of ancestral character states for a given phylogeny allows insights into character evolution in a group under study. Classically, Fitch's parsimony (Fitch 1971) was the method of choice, and was implemented in software packages like MacClade (Maddison & Maddison 1989; Maddison & Maddison 2003) or Mesquite (Maddison & Maddison 2010). The latter software also incorporates likelihood approaches, which aim at finding the ancestral states that maximize the probability the observed states would evolve under a stochastic model of evolution (Pagel 1999; Lewis 2001).

The Bayesian approach to reconstruct ancestral character states targets a probability distribution that samples ancestral states in proportion to their probable accuracy (see 1.3.1), and was implemented in the program BayesTraits (Pagel *et al.* 2004). BayesTraits allows one to explore a variety of models or to integrate over models with the use of reversible-jump Markov chain Monte Carlo (a variant of MC^3).

To account for uncertainties in the topology of the phylogeny, the Bayesian reconstruction of ancestral character states can be simulated over a posterior distribution of possible tree topologies (Smith 2009; Smith & Donoghue 2010), represented by 1 000 or more randomly sampled trees obtained by Bayesian phylogenetic analysis. The probabilities obtained for a certain character state at a certain node can be directly tested by bootstrapping (see 1.3.1), or by the likelihood ratio test (LRT, see 1.3.3) and variants of the LRT including the AIC (Akaike 1974; Felsenstein 2004).

1.3.3 The molecular clock

In the early 1960s, when protein sequences became available, it was observed that the rate of evolution for proteins, such as hemoglobin, were relatively constant among different evolutionary lineages of mammals (Zuckerkandl & Pauling 1962). This observation lead to the proposal of the molecular clock hypothesis by Emile Zuckerkandl and Linus Paulin (1965), who asserted that the rate of evolution of a certain DNA or protein sequence is constant over time or among lineages (which has been termed the 'strict' or 'global' molecular clock). Shortly after this, Motoo Kimura postulated the neutral theory of evolution (Kimura 1968; Kimura & Ohta 1971), and the molecular clock hypothesis was seen as a major piece of supporting evidence (Kimura 1983). These initial findings raised much excitement about using the molecular clock to estimate evolutionary dates, such as the divergence time of lineages (node ages).

Age estimations

Divergence times can be estimated by measuring the genetic distances between lineages and by using a calibration rate (the amount of genetic changes expected per unit time), or independent calibration points (paleontological or biogeographic dates, like fossil ages or availability of

land connections) to convert the distance to absolute time (Bromham & Penn 2003). However, molecular evolutionary rates depend on a combination of factors such as population size, generation time, replication and repair mechanisms, and the degree to which mutations are beneficial or deleterious (*i. e.* differences in natural selection), all of which may vary among lineages or species (Bromham & Penn 2003; Hedges & Kumar 2004). Thus, most genes or species groups violate the strict molecular clock model (Kumar 2005; Pybus 2006).

Recent developments have focussed on a variety of methods to 'relax' the molecular clock assumption, including 'penalized' clocks (Sanderson 1997, 2002), local clocks (Yoder & Yang 2000), and several Bayesian parametric model approaches (*e. g.,* Thorne *et al.* 1998; Huelsenbeck *et al.* 2000; Drummond *et al.* 2006). The Bayesian framework offers the opportunity of exploring a wide diversity of alternative modes and to examine corresponding parameters (see 1.3.1; Lepage *et al.* 2007). Choosing appropriate priors, however, is crucial, and model and parameter selection (Pybus 2006; Lepage *et al.* 2007; Morrison 2008) as well as methods to account for uncertainty in calibration dates (Ho & Phillips 2009) are at present under study.

Likelihood ratio test (LRT)

The LRT is a general statistical test method, used to evaluate whether the evolutionary rate is homogeneous among all branches of a phylogenetic tree (Huelsenbeck & Crandall 1997; Huelsenbeck & Rannala 1997). To test for rate constancy among lineages (*i. e. a* strict clock), likelihood scores (L) for a given tree are calculated with a molecular clock enforced (the simpler model, H0) and without (the more complex model, allowing rate heterogeneity among branches, H1). The likelihood ratio (LR) can be calculated by LR = 2 (–ln L0–(–ln L1)) and compared to a χ^2 distribution (with n-2 degrees of freedom; n = number of taxa) to assess significance. If the p-value is less than 0.05, one can reject the simpler model, in this case, the strict molecular clock (Felsenstein 2004).

Uncorrelated lognormal (UCLN) model

The UCLN model belongs to a class of parametric relaxed clock models, in which the rate at each branch is drawn from an underlying distribution such as an exponential, a gamma, or a lognormal distribution (Drummond *et al.* 2006). The model explicitly assumes the independence of branch rates, *i. e.* substitution rates between branches are not 'inherited' (autocorrelated; Lepage *et al.* 2007). Morrison (2008) has shown that treating divergence time as a lognormal variable is an accurate way to summarize the estimates and their confidence intervals. The UCLN model has recently been included in the software package BEAST (Drummond & Rambaut 2007).

Calibration of the tree

To convert the calculated distances into absolute time, calibration via external calibration points is essential. If available, the temporal information contained in fossils can be used to constrain a certain node using a soft minimum bound or a lognormal distribution (Ho & Phillips 2009). Biogeographic events are appropriate constraints in the absence of fossils clearly belonging to a living

relative. Depending on the geological history, biogeographic dates should be constrained via a normal or lognormal distribution. Age estimations and their confidence intervals derived from other studies are less accurate, but still helpful calibration techniques. Multiple calibration points avoid biases in estimates of rates and dates if a calibration is inaccurate, and decrease the difficulties of estimating the rates of nodes that are too distant from the constrained one (Ho & Phillips 2009).

1.3.4 Biogeography

Since the onset of cladistic theory (Hennig 1950, 1965), Hennig's progression rule argued that the branching order of lineages could contain information on their geographic origin (Hennig 1966). In the past four decades, phylogenies have become essential for historical biogeographical inference. They are being applied to reconstruct general area relationships and to infer the cause of common distribution patterns (cladistic biogeography), as well as to reconstruct ancestral ranges and biogeographic events on branches of the tree of life (taxon biogeography; Ree & Sanmartín 2009).

As biogeography deals with historical events that can neither been observed directly nor manipulated experimentally (Crisp *et al.* 2011), it is crucial to discern between true congruence, *i. e.* a common cause of common patterns, and pseudo-congruence, *i. e.* lineage specific causes for common patterns (Donoghue & Moore 2003). Moreover, classical biogeographic methods tend to generate, rather than test, hypotheses (Ball 1975).

The implementation of additional data, such as temporal information on divergence of lineages, or external data from fossils, palaeogeology, and climatology offer solutions to overcome the problem of inductivism (Donoghue & Moore 2003; Moore & Donoghue 2007; Moore *et al.* 2008). The development of synergies between these fields of study is providing novel data and hypothesis-testing opportunities (Smith & Donoghue 2010; Crisp *et al.* 2011).

Biogeographic inference methods

A diverse amount of biogeographic methods has been developed, and these can be categorized either into pattern-based (Nordlander *et al.* 1996; Nylander *et al.* 2008) or event-based biogeography (Sanmartín & Ronquist 2002; Ree & Sanmartín 2009). They can also differ by the method used to infer evidence, such as parsimony (Maddison & Maddison 2003; Maddison & Maddison 2010), non-parametric model based parsimony, known as dispersal-vicariance analysis (DIVA; Ronquist 1997; Yu *et al.* 2010), and parametric model based approaches using maximum likelihood (Ree *et al.* 2005; Ree & Smith 2008) and Bayesian inference (Sanmartín *et al.* 2008; Lemey *et al.* 2009). Recent studies compare (*e. g.,* Clark *et al.* 2008; Buerki *et al.* 2011; Emadzade *et al.* 2011) and review these methods (*e. g.,* Avise 2009; Lamm & Redelings 2009).

Within this work the parametric model-based approach described by Ree *et al.* (2005) was employed to locate dispersal events in *Hypericum*. This approach provides a hypothesis-testing framework based on tree topologies and likelihoods of alternative parameterized scenarios.

The method aims at identifying the biogeographic history that maximizes the likelihood that the observed geographic distribution is realized under a specified biogeographic model (Moore & Donoghue 2007).

The dispersal, extinction and cladogenesis (DEC) model

The analysis is based on the program package Lagrange (Likelihood analysis of geographic range evolution; Ree & Smith 2008) that implements a model for geographic range evolution by dispersal, extinction and cladogenesis (DEC). The DEC model (Ree *et al.* 2005) estimates ancestral ranges (areas) and biogeographic parameters (dispersal and extinction rates) based on current geographic distribution of species. It defines a matrix of probabilities of lineage dispersal between areas and extinction within an area based on the assumption that only a single dispersal/extinction event can occur in an instant of time (Ree & Sanmartín 2009; reviewed in Buerki *et al.* 2011). In this study, analyses were run over a subsample of trees randomly chosen from the posterior distribution of dated trees generated by a BEAST analysis (see 1.3.2, 1.3.3), to avoid conditioning results on any particular tree topology and branch length.

1.3.5 Diversification rates

The evolution of morphological characters (*e.g.,* key innovations) and ecological characteristics (*e.g.,* niche shifts) has been hypothesized to promote shifts in diversification rate by altering the possibility of speciation and/or extinction (Moore & Donoghue 2007). A comprehensive understanding of the causes of diversification (d = speciation minus extinction) thus requires incorporation of both biotic and abiotic factors. To identify correlation between possible key innovations and diversification events, I analyzed diversification rate shifts in *Hypericum* and related genera.

To access insights into diversification rates within *Hypericum*, I used an extension of the approach described by Rabosky *et al.* (2007) which uses phylogenetic and taxonomic information to estimate birth and death rates for an incompletely resolved phylogenetic tree. The method was recently implemented in the program MEDUSA (modeling stepwise diversification using stepwise AIC; Santini *et al.* 2009). MEDUSA uses a stepwise procedure to add rate shifts to a tree until there is no substantial improvement in the AIC score, *i.e.* $\Delta AIC \geq 4$ (Burnham & Anderson 2002). The detected shifts are then confirmed by a backward elimination process, where individual shifts are removed and the model reevaluated. In other words, MEDUSA takes a phylogeny where the tips of the phylogeny may represent stems of unresolved clades and a list of taxonomic richness (species number) for each tip clade and fits a series of birth-death models to each branch in the tree (Alfaro *et al.* 2009; Santini *et al.* 2009). To account for uncertainty in tree topology, the procedure is applied over a posterior distribution of possible tree topologies (see 1.3.2). The output, *i.e.* the detected diversification rate shifts can be tested for normal distribution using the Shapiro-Wilk Normality test and for significance using the t-test (for normal distributed data) and the Sign-test (for non-normal distributed data), respectively.

2 Cladistic analysis of morphological characters in *Hypericum* (Hypericaceae)

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2.1 Introduction

Hypericum and eight other genera have been treated as subfamily Hypericoideae Engl. within Clusiaceae Lind. (Guttiferae Juss.). Molecular studies, however, indicate that such a broadly circumscribed Clusiaceae is paraphyletic as a result of a sister group relationship between Hypericoideae and Podostemaceae Kunth. (Chase *et al.* 2002; Gustafsson *et al.* 2002). Thus, the current classification of flowering plants (APG III 2009) splits Clusiaceae into three families, one of which (Hypericaceae) matches the former Hypericoideae.

Keller (1895, 1925) first attempted a comprehensive classification of the genus *Hypericum*, followed by Kimura (1951). Both classifications were, however, unsatisfactory in several ways (Robson 1977, 2003). Robson (1977) provided a revision of the genus, and proposed a new classification, defining 30 sections. This publication was the first in a series of monographs of subgroups of *Hypericum* in which detailed information on characters for species descriptions are given (Robson 1981), as well as the formal taxonomy of sections and species (Robson 1985, 1987, 1990, 1996, 2001, 2002, 2006, 2010a, b). Thirty-six sections have been to date described and 472 species have been recognized (Table 2.1). Thus, the genus is one of the few big plant genera where

Classification Section Subsect. Series		Statistic support [bs|pp]	Number of species	Distribution	Systematic treatment
1.	*Campylosporus* (Spach) R.Keller	| 0.58	**10**	Tropical & SE Africa + adjacent isl., SW Iran	Robson 1985: 178
2.	*Psorophytum* (Spach) Nyman		**1**	Spain (Balearic Isl.)	Robson 1985: 202
3.	*Ascyreia* Choisy		**43**	SE Europe, W to SE Asia, S China	Robson 1985: 206, 2001: 49
4.	*Takasagoya* (Y.Kimura) N.Robson		**5**	Japan (Ryuku Isl.), Taiwan, Philippines	Robson 1985: 288
5.	*Androsaemum* (Duhamel) Gordon	51 | 0.99	**4**	Macaronesia, W & S Europe to Iran & Yemen	Robson 1985: 297
6.	*Inodora* Stef.		**1**	NE Turkey, Georgia	Robson 1985: 314
6a.	*Umbraculoides* N.Robson		**1**	Mexico (Oaxaca)	Robson 1985: 317
7.	*Roscyna* (Spach) R.Keller	57 | 1.00	**2**	Central to E Asia, NE America	Robson 2001: 52
8.	*Bupleuroides* Stef.		**1**	NE Turkey, Georgia	Robson 2001: 49
9.	*Hypericum*		**42**	Europe, NW Africa, Asia, NW America; introduced (*H. perforatum*) into many other parts of the world	Robson 2002: 66
	1. *Hypericum*		19		Robson 2002: 66
	1. *Hypericum*		*12*		Robson 2002: 66
	2. *Senanensia* N.Robson		*7*		Robson 2006: 28
	2. *Erecta* N.Robson		23		Robson 2006: 42
9a.	*Concinna* N.Robson		**1**	USA (northern California)	Robson 2001: 61
9b.	*Graveolentia* N.Robson		**9**	SE Canada, eastern USA to Guatemala	Robson 2006: 79
9c.	*Sampsonia* N.Robson	57 | 1.00	**2**	NE India to S Japan	Robson 2001: 63
9d.	*Elodeoida* N.Robson		**5**	E & SE Asia (China to Kashmir)	Robson 2001: 66
9e.	*Monanthema* N.Robson		**7**	E & SE Asia (China to Sri Lanka)	Robson 2001: 75
10.	*Olympia* (Spach) Nyman	| 0.71	**4**	S Balkan peninsula, W Turkey, Aegean Isl.	Robson 2010a: 18
11.	*Campylopus* Boiss.		**1**	S Bulgaria, NE Greece, NW Turkey	Robson 2010a: 30
12.	*Origanifolia* Stef.	| 1.00	**13**	Turkey, Georgia, Syria	Robson 2010a: 34
13.	*Drosocarpium* Spach	| 0.69	**11**	Madeira, Mediterranean to W Caucasus	Robson 2010a: 54
14.	*Oligostema* (Boiss.) Stef.		**6**	Europe, Macaronesia, Mediterranean	Robson 2010a: 90
15.	*Thasia* Boiss.		**1**	Greece, Bulgaria, Turkey	Robson 2010a: 109
16.	*Crossophyllum* Spach	| 0.99	**3**	N Aegean region, Turkey, Caucasus	Robson 2010a: 109
17.	*Hirtella* Stef.		**30**	W Mediterranean & S Europe to Altai	Robson 2010b: 135
	1. *Stenadenum* N.Robson		12		Robson 2010b: 139
	2. *Platyadenum* N.Robson		18		Robson 2010b: 162
	1. *Lydia* Sennikov		*5*		Robson 2010b: 162
	2. *Scabra* N.Robson		*3*		Robson 2010b: 170
	3. *Abbreviata* Sennikov		*10*		Robson 2010b: 175
18.	*Taeniocarpium* Jaub. & Spach		**28**	Europe, Mediterranean to Iran & Mongolia	Robson 2010b: 193
19.	*Coridium* Spach	| 1.00	**6**	Mediterranean, Alps, Caucasus	Robson 2010b: 239
20.	*Myriandra* (Spach) R.Keller	56 | 1.00	**29**	E & central North America to Honduras, Bermuda & Caribbean Isl.; introduced (?) into the Azores	Robson 1996: 92
	1. *Centrosperma* R.Keller		14		Robson 1996: 94
	2. *Pseudobrathydium* R.Keller		1		Robson 1996: 112
	3. *Suturosperma* R.Keller		7		Robson 1996: 113
	4. *Brathydium* (Spach) R.Keller		2		Robson 1996: 122
	5. *Ascyrum* (L.) N.Robson		5		Robson 1996: 124
21.	*Webbia* (Spach) R.Keller		**1**	Canary Isl., Madeira	Robson 1996: 133
22.	*Arthrophyllum* Jaub. & Spach		**5**	S Turkey, Syria, Lebanon	Robson 1996: 137
23.	*Triadenioides* Jaub. & Spach		**5**	S Turkey, Syria, Lebanon, Socotra [Yemen Isl.]	Robson 1996: 141
24.	*Heterophylla* N.Robson		**1**	Turkey (NW & W-central Anatolia)	Robson 1996: 146
25.	*Adenotrias* (Jaub. & Spach) R.Keller	96 | 1.00	**3**	S Morocco to Mediterranean	Robson 1996: 147
26.	*Humifusoideum* R.Keller		**12**	Tropical & S Africa, Madagascar, SE to E Asia	Robson 1996: 153
27.	*Adenosepalum* Spach		**25**	Canary Isl., Madeira, Europe, Africa, SW Asia	Robson 1996: 170
	1. *Aethiopica* N.Robson		7		Robson 1996: 172
	2. *Pubescentes* N.Robson		6		Robson 1996: 181
	3. *Caprifolia* N.Robson		3		Robson 1996: 189
	4. *Adenosepalum*		9		Robson 1996: 193
28.	*Elodes* (Adans.) W.Koch		**1**	Azores & W Europe	Robson 1996: 208
29.	*Brathys* (Mutis ex L.F.) Choisy		**87**	Central & South America, Caribbean Isl., SE Canada & eastern USA (south to Florida)	Robson 1987: 12, 1990: 12
	1. *Styphelioides* N.Robson	74 | 1.00	2		Robson 1990: 16
	2. *Phellotes* N.Robson		32		Robson 1990: 16
	3. *Brathys*		39		Robson 1990: 27
	4. *Spachium* R.Keller		14		Robson 1990: 29
30.	*Trigynobrathys* (Y.Kimura) N.Robson		**52**	South America to S Canada, E to SE Asia, the Hawaiian Isl., Australia, New Zealand, Africa; introduced into Europe	Robson 1990: 47
	1. *Connatum* (R.Keller) N.Robson		27		Robson 1990: 51
	2. *Knifa* (Adans.) N.Robson		25		Robson 1990: 95

alpha taxonomy will soon be complete. The work of Robson (1977 onwards) attempts to arrive at "a more natural system for the genus" (Robson 1977: 306). It has included data from studies on morphology, distribution, floral anatomy, and to certain extent cytology. Based on hypothesized evolutionary trends for 26 major characters (Table 2.2), an evolutionary scenario was proposed. The resulting classification was presented in a genealogical scheme showing suggested relationships of the sections (Robson 1977: fig. 1, 1981: fig. 2) and the distribution of certain characters among these groups (Robson 1981: figs. 6, 12, 16, 19, 22, 25, 27, 28, 29, 54). Based on this genealogical network, it was hypothesized that *Hypericum* could have evolved in Africa and spread to America, Asia and Australia before break-up of Gondwana (Robson 1977). This vicariance hypothesis, however, is in conflict with a probable age of Hypericaceae of about 74 million years (Ma) according to molecular phylogenies (Stevens 2001 onwards; Davis *et al.* 2005), as the final break-up of West Gondwana (South America and Africa) took place in the lower Cretaceous about ≥105 Ma ago (McLoughlin 2001).

Table 2.1 Classification of the genus *Hypericum* L. (*sensu* Robson) in numerical order of sections, subsections and series. In the second column statistic support values for sections are given. In case parsimony (MP) and Bayesian (BI) analysis revealed different phylogenetic status, two notifications are separated by MP | BI. The amount of species per section (bold), subsections (regular) and series (italic), general distribution and citation for the systematic treatment of the section is given in the remaining columns. Four hundred and fifty-seven species in thirty-six sections have so far been described by Robson (1977 onwards), plus one unnamed species (17. *H.* species) from section *Brathys*, which has not been included in the analyses due to missing character descriptions (Robson 1990: 22). Furthermore, 14 other species have been described: *H. dogobadanicum* Assadi (section *Campylosporus*, Iran), *Iran. Journ. Bot.* 2:89 (1984); *H. fosteri* N.Robson (section *Ascyreia*, China), *Acta Phytotax. Sin.* 43: 271 (2005); *H. wardianum* N.Robson (section *Ascyreia*, China), *Acta Phytotax. Sin.* 43: 273 (2005); *H. enshiense* L.H. Wu & F.S. Wang (section *Hypericum*, China), *Acta Phytotax. Sin.* 42: 76 (2004); *H. chejuense* S.-J. Park & K.-J. Kim (section *Hypericum* subsection *Erecta*, Korea), *Novon* 15: 258 (2005); *H. jeongjocksanense* S.-J. Park & K.-J. Kim (section *Hypericum* subsection *Erecta*, Korea), *Novon* 15: 260 (2005); *H. hubeiense* L.H. Wu & D.P. Yang (section *Elodeoida*, China), *Acta Phytotax. Sin.* 42: 74 (2004); *H. austroyunnanicum* L.H. Wu & D.P. Yang (section *Elodeoida*, China), *Acta Phytotax. Sin.* 40: 77 (2002); *H. haplophylloides* Halácsy & Bald. (section "24a." *Hyplophylloides* N.Robson [in prep.: *Hypericum* monograph part 9], Albania), *Verh. Zool.-Bot. Ges. Wien* 42: 576 (1893); *H. huber-morathii* N.Robson (section *Adenosepalum*, Turkey), *Notes Roy. Bot. Gard. Edinburgh* 27: 197 (1967); *H. minutum* Davis & Poulter (section *Adenosepalum*, Turkey), *Notes Roy. Bot. Gard. Edinburgh* 21: 182 (1954); *H. formosissimum* Takht. (section *Adenosepalum*, Turkey, Armenia, Iran), *Not. Syst. Bot. Tiflis = Zametki po Sistematike i Geografii Rastenii* 9 (1940); *H. rubicundulum* Heenan (section *Trigynobrathys*, New Zealand), *New Zealand J. Bot.* 46: 555 (2008); *H. minutiflorum* Heenan (section *Trigynobrathys*, New Zealand), *New Zealand J. Bot.* 46: 556 (2008). These names are not included in Robson's monograph yet (but will be in Robson [part 9] in prep.), nor in this analysis.

Hypericum perforatum (Common St. John's wort) is known as a source of hypericin, and extracts of this plant are sold as a treatment of mild to moderate depression. A considerable amount of research on the occurrence of secondary compounds in this species in particular, and in members oft the genus in general has been conducted (Avato 2005; Butterweck & Schmidt 2007). Over the last decade, *H. perforatum* has also become a subject of interest as a model plant for apomixis (asexual seed formation) research (Matzk *et al.* 2001; Barcaccia *et al.* 2006; Barcaccia *et al.* 2007; Schallau *et al.* 2010). Reproductive biology in *Hypericum* is quite diverse and at least 16 facultative pseudogamous apomictic species occurring in three different sections have been described (Matzk *et al.* 2003).

Comparative analysis of reproductive modes in the genus, as well as character evolution and historical biogeography necessitates knowledge of the phylogenetic relationships of *Hypericum*. Few studies using molecular approaches have been published so far (Crockett *et al.* 2004; Park & Kim 2004; Heenan 2008), all including relatively few species, and few or distantly related outgroup representatives. The work of Crockett *et al.* (2004) was based on nuclear rDNA spacer (ITS) sequences from 50 species, representing eleven sections with focus on *H.* sect. *Ascyreia* (12 species) and sect. *Myriandra* (24 species plus one undefined sample), and used *Clusia rosea* as outgroup. Heenan (2008) used the dataset of Park & Kim (2004) plus ITS sequences of three taxa native to New Zealand. Park & Kim (2004) included 36 species from ten sections, with focus on species from Korea and Japan, and used the two *Thornea* species occurring in Central America as outgroup (*T. matudae* and *T. calcicola*). Phylogenetic trees from these studies are not comparable due to different species sampling. Furthermore, the small and unrepresentative number of taxa included in these analyses does not allow one to infer the direction of character evolution, or to reconstruct the historical biogeography of the genus.

As a first step to understand the evolutionary history of the genus, we generated a phylogenetic hypothesis of *Hypericum* based on morphological characters analyzed with cladistic and Bayesian methods. We coded characters used in species descriptions for these analyses. In *Hypericum*, a complete revision done by one person is available, which makes description of characters largely comparable. We compared the obtained phylogenetic trees against the current infrageneric classification of *Hypericum*, and generated hypotheses for character evolution, the origin of apomixis and the historical biogeography to be tested in future analyses with molecular methods.

2.2 Materials & methods

A dataset was assembled for all 591 taxa of *Hypericum* described in the monograph (Robson 1977 onwards), including 457 species, 70 subspecies, 13 varieties, 11 formae and 40 hybrids (Table 2.1, and quotations within). For the cladistic analysis, only the 457 species were used. Hybrids were excluded because cladistic methods produce only divergently branching phylogenetic trees and thus cannot represent the reticulate structures if an analysis includes hybrids (McDade 1990), or may cause major topological changes if hybrids between distantly related parents are included (McDade 1992).Taxa below species level were excluded to keep the number of accessions to an

amount that allowed analysis to be run in a reasonable time. Nine outgroup taxa were included (*Santomasia steyermarkii*, *Lianthus ellipticifolius*, *Triadenum japonicum*, *T. breviflorum*, *Thornea matudae*, *Vismia cayennensis*, *Harungana madagascariensis*, *Cratoxylum arborescens*, and *C. celebicum*) representing eight of the nine genera of Hypericaceae (missing: *Eliea*), from all three accepted tribes (Hypericeae, Vismieae, Cratoxyleae) according to Stevens (2007).

2.2.1 Character coding

Eighty-nine characters consistently used in species descriptions in the *Hypericum* monograph (Robson 1977 onwards) and in descriptions of other genera of Hypericaceae (Li & Robson 2007; Stevens 2007) were chosen (see Appendix 2.1). We concentrated mainly on characters that were defined as discrete by Robson. Only three numeric characters were included (characters 1, 65 and 74), which were arbitrarily but consistently coded. The 86 remaining characters are discrete (*i. e.*, consistently used by Robson in the species descriptions, see Discussion). Of those, 43 are binary (absent or present), and 46 are multistate. The large number of multistate characters is due to the different character states in the species diagnoses used to describe these characters. Polymorphic characters were coded as ambiguities (*i. e.*, allowing variable taxa to have multiple character states). This method is least bias-causing according to Kornet & Turner (1999), and to be preferred if ancestral states are unknown. Characters described as multistate, but in practice non-additive (*e. g.,* pollen grain types) were coded as uncertain (character numbers 63, 70 and 89). The data matrix cells scored as missing data comprised 5.7 % of the entire matrix.

2.2.2 Phylogenetic inference

Phylogenetic analyses were performed to test the monophyly of *Hypericum* and of the sections within the genus, as well as to establish a hypotheses of sectional relationships. All analyses were performed on a dataset containing 466 species (457 *Hypericum* and 9 outgroup species). Two *Cratoxylum* species were defined as outgroup in all analyses following Wurdack & Davis (2009), who showed this genus to be sister to other Hypericaceae. All character states were treated as unordered.

Parsimony analyses were performed in PAUP* v.4.0b10 (Swofford 2002), Bayesian analysis with MrBayes v.3.1.2p (Ronquist & Huelsenbeck 2003; Altekar *et al.* 2004). Analyses were run on a Linux cluster. The parsimony analyses (MP) followed a two-step heuristic search approach modified from Blattner (2004), with equal weights for all characters, and multistate character interpretation varying depending on whether a state is 'uncertain' or 'polymorphic'. In an initial MP analysis (1st run), starting trees were obtained via 50 000 stepwise and random taxon additions, with only five trees held at each step, using tree bisection-reconnection (TBR) for branch-swap-

ping, swapping on best trees only, and saving only one optimal tree from each repetition, even if it was not optimal overall. The 50 000 saved trees obtained were afterwards ordered according to tree-length (scores), and the trees with the ten lowest scores (most parsimonious trees; normally more than one tree was found for each of those lowest scores) were used as starting trees for ten separate second analyses (2nd run). In the 2nd run only best trees were saved in the TBR search, which was limited to finding 100 000 trees. The strict consensus tree (Fig. 2.1) was calculated from that run revealing trees with the lowest scores. Statistical support of the branches was tested with 100 000 bootstrap re-samples (Felsenstein 1985), using the 'fast and stepwise' procedure of PAUP*. Tree lengths, consistency (CI) and retention index (RI) were calculated in PAUP* v.4.0b10 on a Macintosh OS 9 computer.

For Bayesian inference (BI), two runs were done with eight chains each for 5×10^7 generations under the Mk model for morphological data (Markov k model; Lewis 2001), using 0.05 as temperature, and sampling a tree every 1 000 generations. The initial 35 000 trees per chain were discarded as burn-in, and posterior probabilities were calculated from the remaining 30 002 trees. Character state changes were analyzed using the parsimony criterion in the program MacClade v.4.06 (Maddison & Maddison 2003). Visualization of results was done using FigTree v.1.2.3 (Rambaut 2006–2009). The data matrix and the MP consensus tree (Fig. S2.1, in Appendix S2) can be obtained from the corresponding author.

2.3 Results

The dataset of 466 species (457 *Hypericum* and 9 outgroup species) revealed that all 89 characters were variable and parsimony informative. The two runs of the BI analysis did not converge completely during one month of calculation. Therefore, only the last 30 % of trees, where chains closed in, were used to calculate the posterior probabilities. The BI analysis resulted in a phylogenetic tree (not shown) with completely resolved placement of outgroups (Fig. 2.2), but with a large polytomy within *Hypericum*. Several well-supported clades were placed along this backbone polytomy (Table 2.1). These clades were also found in the consensus tree of the MP analyses, where resolution within *Hypericum* is generally higher.

For the MP analyses, the consensus trees produced from the 100 000 trees of each of the ten analyses were all inspected, and the 100 000 trees with shortest lengths were chosen to calculate the consensus tree shown in Figures 2.1 and S2.1. The most parsimonious trees had a length of 1 677steps (CI 0.1094, RI 0.7958). Eight of the ten MP analyses placed *Lianthus* as sister to *Hypericum*, although with <50 % bootstrap support. *Santomasia* groups in all analyses within *Hypericum* (close to or within *H.* sect. *Ascyreia*). This non-monophyly of *Hypericum* is also seen in the results of the BI analysis, where *Santomasia* is also nested within *H.* sect. *Ascyreia*.

Table 2.2 Characters used for classifying the genus *Hypericum* listing the hypothesized character evolution (Robson, 1977) vs. direction of character evolution revealed in the cladistic analysis. Characters states in italic font highlight evolutionary directions being incongruent in the two columns.

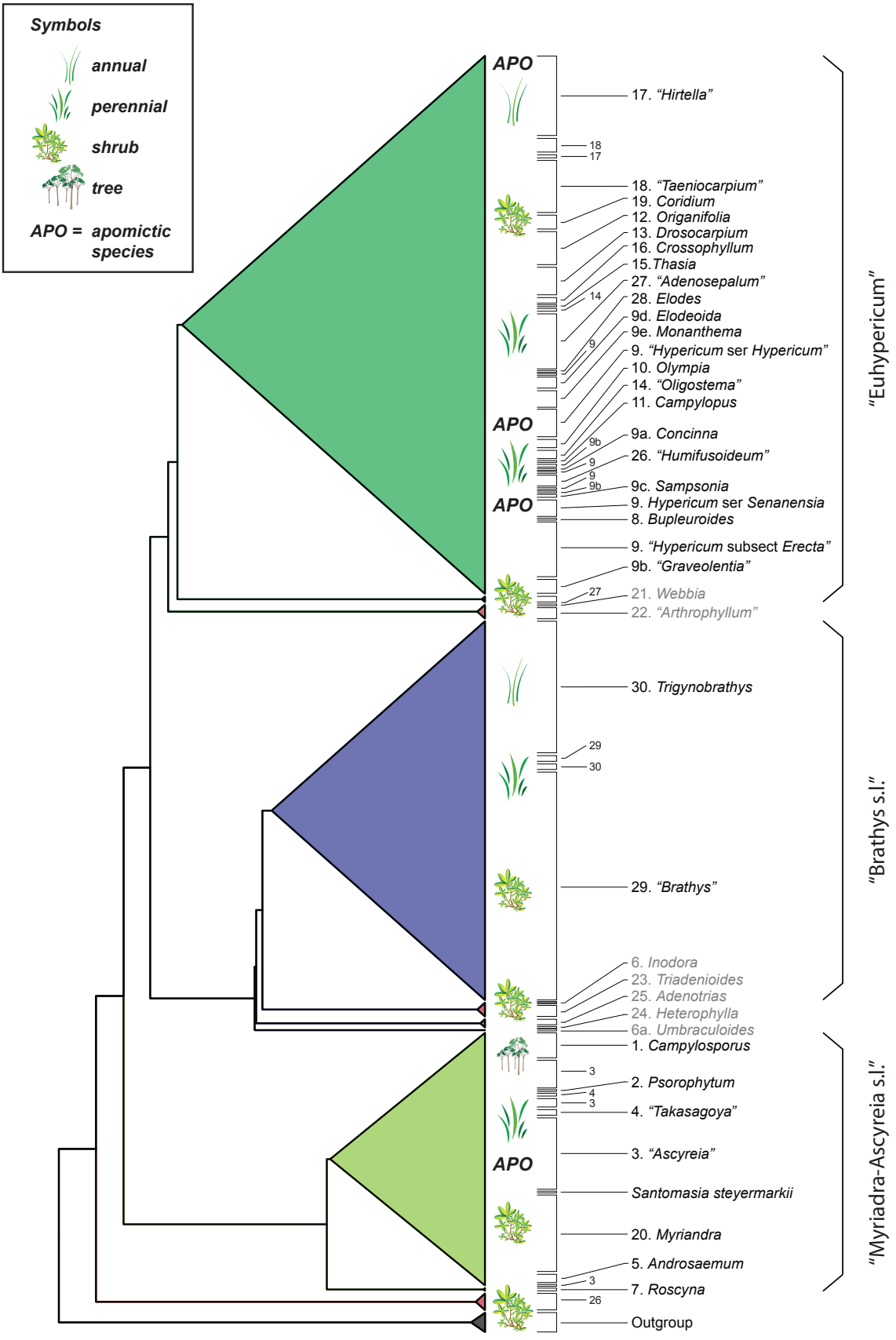
	Evolutionary direction used for classification (Robson 1977: 306 ff)			Evolutionary direction revealed in the cladistic analysis (Nürk & Blattner 2010)		
	Plesiomorphic character state		Apomorphic character state	Plesiomorphic character state		Apomorphic character state
Habit	*trees*	→	*shrubs → perennial → annuals*	*shrubs*	→	*herbs*
					→	*trees*
Indumentum	absent	→	present	absent	→	present
Glands	pale	→	dark	pale	→	dark
	pale channels	→	pale dots	pale channels	→	pale dots
	dark dots	→	dark streaks or lines	dark dots	→	dark streaks or lines
	increase of dark secretory tissue				?	
Stem	*4-lined*	→	*2-lined → terete*	*terete*	→	*2-lined → 4-lined*
Leaves	sessile	→	shortly petiolate	sessile	→	shortly petiolate
		→	amplexicaul → perfoliate		→	amplexicaul or perfoliate
	deciduous	→	persistent	deciduous	→	persistent → deciduous (?)
	opposite	→	3-whorled → 4-whorled	opposite	→	3-whorled or 4-whorled
	parallel venation	→	*reticulate venation*	*pinnate*	→	*parallelodromus*
Perianth	5-merous	→	4-merous	5-merous	→	3- or 4-merous
Sepals	persistent	→	deciduous	persistent	→	deciduous
	unequal	→	*equal*	*equal*	→	*unequal*
	free	→	united	free	→	united
	margin entire	→	dentate → ciliate → fimbriate	margin entire	→	margin not entire
Petals	persistent	→	deciduous	persistent	→	deciduous
	asymmetric	→	symmetric	asymmetric	→	symmetric
Stamen fascicles	persistent	→	deciduous	persistent	→	deciduous → persistent
	5	→	*4*	*2+2+1*	→	*5 → broad ring (?)*
	free	→	*variously united*		→	*narrow ring → reduction up to 5 single stamina*
Placentation	*loosely axile*	→	*definitely axile*	*axile*	→	*loosely axile → parietal*
		→	*parietal*			
Ovules per placenta	∞	→	2 → (?1)	∞	→	2
Seeds	*narrowly winged*	→	*carinate → cylindrical*	*cylindrical*	→	*carinate, or winged*
Basic chromosome numbers	*12*	→	*7 (? 6)*	*9 or 10*	→	*8*
		→	*14*		→	*14*

In both analyses (MP and BI), relationships between the remaining outgroups are completely resolved and statistically supported (Fig. 2.2). The placement of *Lianthus* as sister to *Hypericum* had little support (<50 % bs, 0.94 pp), as well as *Hypericum* itself (<50 % bs, but 0.97 pp).

Within *Hypericum* four major groups can be recognized that were present in all MP analyses but with <50 % bootstrap support (Fig. 2.1):

(1) A "Mediterranean grade" containing the monotypic sections *Inodora*, *Umbraculoides*, *Webbia* and probably *Heterophylla*, as well as sections *Arthrophyllum*, *Triadenioides* and *Adenotrias*. These seven sections had no fixed positions (in the different MP analyses), but were always placed on initial splits in the genus or as sister to one or several of the big clades.

(2) A clade comprising mainly Indo-Malayan species from sections *Ascyreia* and *Takasagoya* together with Afrotropical species from section *Campylosporus*, and the Mediterranean species from section *Psorophytum* in a clade (here named "Ascyreia s. l."). The monophyletic, Nearctic section *Myriandra*, together with the Palaearctic section *Androsaemum* is sister to "Ascyreia s. l.", and the Holarctic section *Roscyna* is placed as sister to all of them. This clade is named the "Myriandra-Ascyreia s. l." group.


Symbols
annual
perennial
shrub
tree
APO = apomictic species
APO
APO
APO
APO
17. "Hirtella"
18
17
18. "Taeniocarpium"
19. Coridium
12. Origanifolia
13. Drosocarpium
16. Crossophyllum
15. Thasia
27. "Adenosepalum"
14
28. Elodes
9d. Elodeoida
9e. Monanthema
9
9. "Hypericum ser Hypericum"
10. Olympia
14. "Oligostema"
11. Campylopus
9a. Concinna
9b
26. "Humifusoideum"
9
9
9b
9c. Sampsonia
9. Hypericum ser Senanensia
8. Bupleuroides
9. "Hypericum subsect Erecta"
9b. "Graveolentia"
27
21. Webbia
22. "Arthrophyllum"
30. Trigynobrathys
29
30
29. "Brathys"
6. Inodora
23. Triadenioides
25. Adenotrias
24. Heterophylla
6a. Umbraculoides
1. Campylosporus
3
2. Psorophytum
4
3
4. "Takasagoya"
3. "Ascyreia"
Santomasia steyermarkii
20. Myriandra
5. Androsaemum
3
7. Roscyna
26
Outgroup
"Euhypericum"
"Brathys s.l."
"Myriadra-Ascyreia s.l."

(3) A clade comprising the Neotropic section *Brathys* and the mainly Neotropic section *Trigynobrathys*, named the "Brathys s.l." group.

(4) A mainly Palaearctic clade including section *Hypericum* and sections 8–19 (section numbers refer to Table 2.1), apart of section *Humifusoideum* and sections *Adenosepalum* and *Elodes*. This group is named "Euhypericum". In the most parsimonious tree (Fig. 2.1) an Indo-Malayan/Australasian part of section *Humifusoideum* is placed as sister to all other species of the genus. In other, less parsimonious trees, this part of section *Humifusoideum* is included in the "Euhypericum" group.

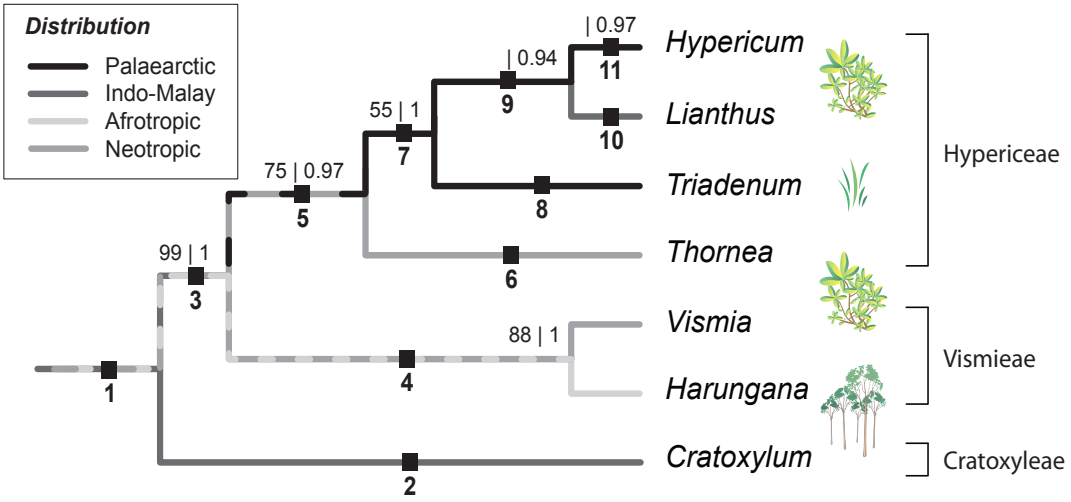
Due to the large polytomy within *Hypericum*, there is less resolution among the major clades in the BI tree. Twelve of the thirty-six constituent sections within the polytomy, however, are monophyletic and statistically supported (Table 2.1; Fig. S2.1).

Both the MP and BI analyses identified the same sections as monophyletic (Table 2.1). Only *H.* sect. *Crossophyllum* and sect. *Arthrophyllum* were unclear or polyphyletic in MP, and monophyletic in BI. That is, 69 % (MP) and 91 % (BI) of the sections were monophyletic or paraphyletic, and can so be said to agree with their recognition by Robson, who expected and accepted paraphyletic taxa (Robson 1981: 66: "The published sectional classification […] in Fig. 2, shows examples of sections with multiple derivates and more than one hidden example of paraphyly […]").

A comparison between the presumed direction of evolution of characters (Robson 1977) and the evolutionary direction derived from the cladistic analyses revealed good agreement (Table 2.2). However, several of the character states described by Robson (1977) as being 'primitive' (*i. e.*, plesiomorphic) appear apomorphic or ambiguous in the phylogenetic trees. As an example, within *Hypericum* evolution from trees to shrubs to herbs was postulated (Robson 1977: 306). However, from the phylogenetic tree (MP) the shrubby habit is plesiomorphic within *Hypericum*, and real trees occur only in *H.* sect. *Campylosporus* that is nested within the "Ascyreia s.l." group. According to the results of our analysis, we hypothesize that perennial herbs evolved from the shrubby habit at least four times independently within the three major clades.

Apomixis was expected to have evolved several times independently, as apomictic species have been recognized in three sections (*H.* sects. *Ascyreia*, *Hypericum*, *Hirtella*). In both the MP and the BI tree, apomictic species occur in three different clades.

Fig. 2.1 Scheme of the strict consensus of MP trees, showing phylogenetic relationships of 457 *Hypericum* species and 9 outgroup representatives (with one species from *Lianthus*, *Santomasia*, *Thornea*, *Vismia*, and *Harungana*, and two from *Triadenum* and *Cratoxylum*). For relationship among outgroups see Fig. 2.2. Square brackets and section names mark the position of sections in the tree. Section names in gray highlight the sections belonging to the "Mediterranean grade". Section names in quotation marks are polyphyletic in the tree. Small numbers to the left of section names and numbers mark the position of parts of polyphyletic sections. Symbols depict the general distribution of growth habit, and the occurrence of apomixis within *Hypericum*.



Apomorphies

1 Trees, glabrous or hairs unicellular; leaves petiolate, persistent, with dark glands, stomata paracytic; sepals 5(4), free, eglandular, veins unbranched, margin entire; petals 5(4), white, pink or greenish, symmetrical, deciduous, with dark glands, aestivation imbricate; (5-) ∞ stamens arranged in 3 fascicles (2+2+1), deciduous, with 3 fasciclodes and interstaminal glands between the fascicles, filaments united above middle, anthers eglandular; G 3, placentation axile, styles free; fruit a capsule, surface smooth (i.e. not vittate or vesiculate); seeds carinate and terminally winged.

2 Stem with dark glands, 4-lined; flowers tubular, sepal vains sometimes branched, petals with fringed ligula, capsules loculicidal; seeds flattened and elongated.

3 Stem without lines; seeds cylindrical.

4 Hairs multicellular; sepals united at base, with dark glands; petals adaxially pubescent; stamens arranged in 5 free fascicles (*1+1+1+1+1*), with 5 fasciclodes in between, anthers with black glands; G 5; fruit a berry.

4.1 *Harungana*: stem eglandular; flowers tubular, stamens persistent; seeds carinate.

4.2 *Vismia*: shrubs; stem with dark glands; flowers stellate; sepal margin not entire; seeds not winged.

5 Shrubs; stem eglandular; leaves with pale glands, stomata (cyclo- or) anomocytic; flowers stellate; interstaminal glands absent; capsules septicidal.

6 Sepal margin not entire; petals eglandular; stamens persistent, filaments shortly united; styles united.

7 Stem branches rooting, if decumbent; leaves sessile, deciduous; sepals with pale glands; petals with pale glands; anthers with amber glands; capsule with vertical vittae; seeds terminally not winged.

8 Herbs (perennials); flowers campanulate; aestivation contorted or cochleate; styles united; seeds carinate.

9 Wood parenchyma absent (? not known from *Lianthus*); sepal veins branched; aestivation contorted; filaments shortly united.

10 Seeds fusiform.

11 Stem 2-lined; petals yellow to red-tinged, asymmetrical, persistent; stamens persistent, fasciclodes absent; seeds often carinate.

Fig. 2.2 Scheme of a part of the strict consensus of MP trees, showing phylogenetic relationships of *Hypericum* with regard to the other genera of Hypericaceae included in the study. Distribution of morphological characters supporting the branches is marked on the tree by numbered black rectangles, and the corresponding character states (apomorphies) are given in the text below. Numbers above the branches indicate bootstrap values (bs in %) and posterior probabilities (pp) supporting the branches [bs|pp]. Rooting of the tree is that in Wurdack & Davis (2009).

2.4 Discussion

2.4.1 Character coding

The coding of characters followed Robson (1981). Several character states are defined in this publication as semantic discontinuities and are consistently used in species descriptions (Robson 1985 onwards). We studied these descriptions and extracted the character states given within to describe a species, using the code as defined in the Appendix 2.1. Therefore, several *de facto* continuous characters (Wagner 2001) were treated as discrete characters (19 or even more of the 86 'discrete' characters) – because they were already 'coded' by Robson (by giving them a certain term) – while examining the specimens. We excluded quantitative numeric characters (except for three characters, see Materials and Methods) from the cladistic analyses. The main problem was the lack of these measurements in the newly published parts of the *Hypericum* monograph (at the time we accumulated the data) that would lead to a huge amount of missing character states in the dataset. Furthermore, by excluding these characters we dismissed the issues of (1) within-taxon variation, and (2) comparability of numeric data between far related groups (Fristrup 2001), what might be problematic in a genus containing so many species as it is in *Hypericum*.

2.4.2 Phylogeny of Hypericaceae

Phylogenetic analyses could not confirm the monophyly of *Hypericum* (Fig. 2.1). Parsimony (MP) and Bayesian (BI) approaches showed that the monotypic genus *Santomasia* was included within *Hypericum*. The grouping of the remaining genera of Hypericaceae, with *Cratoxylum* as outgroup (*Eliea* was not included in the analysis), followed by *Vismia* and *Harungana* together in a clade, followed by *Thornea* and *Triadenum* in a grade and *Lianthus* as sister to *Hypericum* (Fig. 2.2) is in accordance with the most recent classification of Hypericaceae (Stevens 2007), and reflects the grouping of genera included in the molecular analysis of Wurdack & Davis (2009). *Santomasia* appears in the phylogenetic trees (MP and BI) always in close relationship to *H.* sect. *Ascyreia*. The main reason to exclude *Santomasia* from *Hypericum* has been the occurrence of staminodes (vestigial fascicles, 'fasciclodes') between the five free stamen fascicles (Robson 1981). The absence of such staminodes is probably an apomorphy for *Hypericum*, as five staminodes are found in *Vismia* and *Harungana* and three staminodes in *Lianthus*, *Triadenum*, *Thornea*, *Cratoxylum* and *Eliea*. In three species of *Hypericum* sect. *Adenotrias* and in the monotypic sect. *Elodes*, however, three staminodes are present between the stamen fascicles. These sections are separated from the other taxa showing three staminodes according to the classification of Robson (1977) and the results of this analysis. In addition, the staminodes in *H.* sect. *Adenotrias* and sect. *Elodes*

have no connection to the vascular cylinder (stele), whereas they do have a vascular connection in *Cratoxylum*.

The missing vascular connection, and mainly the position of these taxa in the tree (Figs. 2.1, S2.1), indicating that the occurrence of staminodes within *Hypericum* is a case of parallel evolution. Similarly, *Vismia* and *Harungana* with five staminodes are separated from *Hypericum* by clades with three staminodes. *Santomasia* is certainly not closely related to *Vismia* and *Harungana* (Robson 1981: table 2), and the occurrence of (five) staminodes in *Santomasia* might also have evolved in parallel. It is not known if the staminodes of *Santomasia* are connected to the stele or not.

Santomasia and the "Ascyreia s.l." group within *Hypericum* share several other characters such as the occurrence of five free stamen fascicles, loosely axile placentation, and cyathiform yellow flowers. Vegetatively, *Santomasia* is most similar to *H. roeperianum* (*H.* sect. *Campylosporus*) (Norman Robson, pers. comm.). If this resemblance indicates a close relationship, then floral similarities (*i. e.*, the staminodes) between *Santomasia* and *Hypericum* sect. *Adenotrias* and sect. *Elodes* would have evolved separately, as indicated by the cladistic analyses.

2.4.3 Phylogenetic inference within *Hypericum*

The parsimony analyses revealed four groups within *Hypericum*. These groups are present in all strict consensus trees of the MP analyses, although with <50 % bootstrap support.

(1) A "Mediterranean grade" (sections 6, 6a, and 21–25) containing 17 in part local endemic species mainly distributed in the Mediterranean Basin, the Canary Islands and on Socotra (with the exception of the monotypic section 6a *Umbraculoides* from Mexico). They are characterized by a deciduous shrubby habit, the occurrence of (only) punctiform pale glands on leaves, stamens and petals that are persistent after flowering (except *H.* sect. *Umbraculoides* having deciduous petals), and axile placentation. According to the results of this cladistic analysis, all these character states are plesiomorphic within the genus.

(2) The "Myriandra-Ascyreia s.l." group (containing sections 3 p.p. and 7 as sister to a clade containing sections 20 and 5 as sister to "Ascyreia s.l.", containing section 1, 2, 3 p.p., and 4) comprises 94 species, that is around 20 % of the genus. This clade is not characterized by uncontradicted apomorphies. Several apomorphic characters support the monophyly of *H.* sect. *Myriandra* (*e. g.*, sepals that are deciduous after flowering, androecial elements arranged in a broad continuous ring, and pollen type VII), or connect this section with the "Ascyreia s.l." group (*e. g.,* deciduous petals and stamens, and loosely axile placentation). Some species of *H.* sect. *Myriandra*, however, have developed parietal placentation, which also occurs in *H.* sect. *Brathys* and sect. *Trigynobrathys*, and some species of the "Ascyreia s.l." group have late deciduous (or nearly persistent) petals and stamens (Norman Robson, pers. comm.). Thus, the association between the monophyletic *H.* sect. *Myriandra* and the other sections of this clade is uncertain. The possession of five free fascicles is a synapomorphy for the "Ascyreia s.l." group.

(3) The mainly Neotropic "Brathys s.l." group (containing section 29 p.p. in a grade, followed by section 30, including three species from section 29), comprises 139 species, *i. e.* 30 % of the genus.

Several characteristics separate this clade from other members of *Hypericum*, such as a tendency towards modification/reduction of androecial elements from (a) an arrangement in a narrow continuous ring to (b) 5 or 3 obscure fascicles to (c) 5 free fascicles or 5 single stamens. They have parietal placentation (that otherwise occurs only in several species of *H.* sect. *Myriandra*, in one species of section *Monanthema*, and in the monotypic *H.* sect. *Elodes*), and have pollen type VIII.

(4) The "Euhypericum" group (sections 8–19 and 26–28) contains more than 45 % of the diversity of *Hypericum* – 207 species belonging to 20 sections, most of which are native to the Old World. The possession of dark glands, the dominance of the herbaceous habit, and the arrangement of stamens in a 2 + 2 + 1 configuration (resulting in three visible stamen fascicles) characterize members of this clade.

The existence of the "Mediterranean grade" and the monophyly of the three big groups mentioned above must be further confirmed by molecular data as they do not get convincing statistical support in our analysis (although preliminary phylogenetic data of the nuclear rDNA ITS region support most of these groups; (Nürk *et al.* submitted). The sections recognized by Robson (2003) are either monophyletic or paraphyletic in our analysis, but our results do not reflect the sectional relationships presented in Robson (1977, 1981, 2003). *Hypericum s*ect. *Euhypericum* Boiss. (Keller 1925) does, however, include most of the members of the "Euhypericum" group.

Comparing our results with previously published molecular approaches based on ITS sequences (Crockett *et al.* 2004) reveals some congruency. "Euhypericum" is nearly identical to clade A in Crockett *et al.* (2004), in which the monotypic *H.* sect. *Triadenioides* is included and placed as sister to "Euhypericum" taxa. Members of our "Myriandra-Ascyreia s.l." group are not monophyletic in Crockett *et al.* (2004), where *H.* sect. *Myriandra* (named clade C) is sister to two clades (named A and B) and where B that is mostly identical to our "Ascyreia s.l.". In their analysis, however, no taxa from *H.* sect. *Brathys* or sect. *Trigynobrathys* were included and, therefore, putative relationships cannot be clarified between sect. *Myriandra* and "Brathys s.l." and sect. *Myriandra* and "Ascyreia s.l.", respectively. Some species of the "Brathys s.l." group were included in Park & Kim (2004). Their analysis of ITS sequences focused mainly on East Asian species. Due to sparse sampling, the MP tree presented in Park & Kim (2004) is neither really comparable nor congruent with in Crockett *et al.* (2004), nor the results presented in this work.

2.4.4 Character evolution

The absence of dark glands is characteristic for most members of the "Mediterranean grade", the entire "Brathys s.l." group and "Myriandra-Ascyreia s.l.", and the presence of dark glands is characteristic for "Euhypericum". Dark glands do occur, however, in some species of *H.* sect. *Campylosporus* (nested within the "Ascyreia s.l." group; Fig. S2.1). Some other orphological characters are unique to this section, *e.g.,* they have a tree-like habit or grow as real trees (*H. bequaertii* De Wild.). *Hypericum bequaertii* was assumed to be the "most primitive" species of *Hypericum* (Robson 1981: 73, 1985: 164), showing character states that were assumed to be plesiomorphic, such as the possession of only pale glands (Robson 1985: 182). In this phylogenetic analysis, however, several of

these character states appear to be apomorphic or, at least, homoplastic (Table 2.2). Dark glands are present in *Vismia*, *Harungana* and *Cratoxylum*, but absent in *Lianthus*, *Triadenum* and *Thornea*.

Thus, presence of only pale glands (and absence of dark glands) seems to be a plesiomorphic character for *Hypericum* and the development of dark glands has apparently evolved in parallel in *Vismia*, *Harungana*, *Cratoxylum* and several times independently in *Hypericum*. Thus, the absence of dark glands does not necessarily indicate that *H. bequaertii* is particularly "primitive" within the genus (*i. e.*, has only/mostly plesiomorphic character states). In addition, the large flower of *H. bequaertii*, which is described as cyathiform or campanulate and has extensively fused stamen filaments, "could be either primitive or [display] specializations associated with high-altitude conditions" (Robson 1985: 182). The comparison with outgroup species having campanulate flowers and connate filaments, like *Triadenum* with small campanulate flowers, indicates that these characters evolved independently in both genera.

The evolution of the arrangement of stamens in fascicles (Robson 1981: fig. 20) was hypothesized to have taken place from five free fascicles towards various aggregations and reductions (*e. g.,* towards a 2 + 2 + 1 arrangement, or the reduction towards five single stamens). In our analysis, however, the comparison with outgroup species indicates the 2 + 2 + 1 arrangement to be the plesiomorphic character state within *Hypericum*.

The present reconstruction of the evolution of habit (Table 2.2) differs from previous hypotheses (Robson 1977). A shrub habit appears in this analysis to be plesiomorphic within *Hypericum*, and plants with a tree-like or herbaceous habit evolved several times from shrubby ancestors. The tree-like habit seems to dominate in the tropics, as also shown in other plant families (*e. g.,* Blattner & Kadereit 1995). Annuals are postulated to have evolved from perennials in the South American "Brathys s. l." group.

2.4.5 Biogeography

The position of African *Campylosporus* in the MP tree (Figs. 2.1, S2.1), embedded in a clade containing mainly Palaearctic or Indo-Malayan species, contradicts the hypotheses that it constitutes the most early diverging group of the genus. In the MP analyses the Mediterranean sections (*H.* sect. *Androsaemum* [Macronesia, Mediterranean and one species in western Europe as far north as Scotland], sect. *Inodora* [NE Turkey and Georgia], sect. *Webbia* [Canary Islands], sect. *Arthrophyllum* [Turkey, Lebanon], sect. *Triadenioides* [Socotra to the Levant], sect. *Heterophylla* [Turkey], and sect. *Adenotrias* [circum-Mediterranean]), some of which are local endemics, are always separated by initial splits from the reminder of the genus, and are placed within *Hypericum* as sister to one (or more than one) of the three big groups. The sister of *Hypericum*, *Lianthus ellipticifolius*, occurs in Yunnan, China. These phylogenetic relationships indicate a geographical origin of *Hypericum* in the area of the Mediterranean basin (and/or eastwards thereof), perhaps as part of the late Tethys Ocean.

The present distribution pattern of several species of *Hypericum* is puzzling, and requires further investigation using molecular tools. In detail, *H. umbraculoides* N.Robson (*H.* sect. *Umbraculoides*)

from Oaxaca, Mexico is placed in the "Mediterranean grade" and is not related to other American taxa in our analysis, nor in the scheme of sectional interrelationships given in Robson (2003).

Five species from *H.* sect. *Trigynobrathys* (*H. lalandii*, *H. globuliferum*, *H. humbertii*, *H. scioanum* and *H. oligandrum*) that are nested within the mainly Neotropic "Brathys s.l." group are native to Africa, and *H. japonicum* Thunb. ex Murray (*H.* sect. *Trigynobrathys*) occurs in Asia and Australasia. Section *Roscyna* (nested within the "Myriandra-Ascyreia s.l." group) comprises two mainly Palaearctic distributed species, one subspecies of which (*H. ascyron* subsp. *pyramidatum*) is native to Canada and Eastern USA. In *H.* sect. *Humifusoideum*, nine species are native to Southeastern Asia, but three species occur in Africa. No evidence for the monophyly of this section, however, is revealed by our analysis. *Hypericum* sect. *Graveolentia*, comprising nine species native to North or Central America is placed within the "Euhypericum" group, which comprises mostly Palaearctic taxa. Finally, *H. scouleri* from *H.* ser. *Hypericum* occurs in western North America extending south to Mexico and is placed in our analysis in a clade containing most of the species of this series, which all occur in the Palaearctic (Fig. S2.1).

During most of the Tertiary, the landmasses recognized today as the African and South American continents were much closer than at present and this might have facilitated interchange between them (McLoughlin 2001). If an origin subsequent to the break-up of Gondwana were assumed for *Hypericum*, several long-distance dispersal events must be invoked in order to explain the present distribution pattern. Growing evidence exists, however, (*e.g.,* for Malpighiaceae, Davis *et al.* 2002; *Hordeum* [Poaceae], Blattner 2006) that relatively recent long-distance dispersals rather than old vicariance of western Gondwana biotas might play a major role in the formation of biogeographic disjunctions. The tiny seeds with a sculptured testa, which are typical for all *Hypericum* species, might easily be attached to dispersal vectors as migrating birds (Robson 1981).

2.5 Conclusions

The present analysis yielded several testable hypotheses regarding relationships between *Hypericum* and the other genera of Hypericaceae, sectional relationships within the genus, and character evolution and biogeography of *Hypericum*. (1) *Hypericum* is monophyletic, if the monotypic genus *Santomasia* is included. (2) Either *Lianthus* or a clade containing *Triadenum* and *Lianthus* is sister to *Hypericum*. (3) Phylogenetically, *Hypericum* is made up of a Mediterranean grade and three big groups, although relationships among these groups are not yet clearly resolved as these groups had <50 % bootstrap support. Of special interest is the unclear affiliation of taxa of *H.* sect. *Myriandra* with the "Ascyreia s.l." and the "Brathys s.l." groups, respectively. (4) Character state evolution is often identical to evolutionary trends postulated earlier by Robson (1977), but exceptions exist. For example, habit seems to have changed several times from shrubs to herbs, and once from shrubs to trees, within *H.* sect. *Campylosporus* alone. Thus, shrubs and not trees are probably the ancestral state in *Hypericum*. (5) The historical biogeography of the genus has to be newly re-evaluated, as the postulated center of origin in Africa as part of the Gondwana continent does not fit the proposed age of the family (~74 Ma) according to molecular data, nor the time frame for the break-up of Gondwana (>105 Ma ago). The position of *H.* sect. *Campylosporus* in the "Ascyreia s.l."

group contradicts the hypothesis that the species of this section are sister to the reminder of the genus. Thus, our results imply a non-African origin for *Hypericum* (probably in the area of today's Mediterranean region). (6) Intercontinental long-distance dispersals may have occurred frequent within the genus, and would provide explanations for the distribution of particular taxa, namely in *H.* sects. *Umbraculoides*, *Humifusoideum* and *Trigynobrathys* they provide explanations for taxon distribution. (7) Immigration into montane habitats in the tropics results in the evolution of trees in *Hypericum*.

Open questions for which we cannot formulate hypotheses derived from our dataset are the ages of the genus and its infrageneric entities. To answer these questions and to test the hypotheses formulated above, future analyses employing molecular markers are necessary.

3 Molecular phylogeny and character evolution in St. John's wort (*Hypericum*)

The content of this chapter has been submitted to *Molecular Phylogenetics and Evolution* in August 2011 by Nicolai M. Nürk, Santiago Madriñán, Mark A. Carine, Mark W. Chase & Frank R. Blattner.[1]
http://www.journals.elsevier.com/molecular-phylogenetics-and-evolution

3.1 Introduction

During the last two decades, molecular phylogenies have become a common method to understand the relationships of taxa and have had a major impact in our understanding of evolution (Stech & Quandt 2010). To generate a phylogenetic hypothesis of species relationships within *Hypericum* and closely related taxa, we sequenced the nuclear ribosomal DNA (nrDNA) internal transcribed spacer region (ITS), including ITS-1, 5.8S rDNA, and ITS-2 (Baldwin 1992; Baldwin *et al.* 1995), of accessions representing the genus and five other genera of the Hypericaceae. Crocket *et al.* (2004), Park & Kim (2004), and Heenan (2008) have already demonstrated the utility of the ITS region for phylogenetic inference at the species level in *Hypericum*. However, none of these studies was based on a representative sampling for the entire genus or the family, and all included few and/or relatively far related outgroup representatives. The possibility to PCR-amplify ITS-1 and ITS-2 separately using internal primers annealing in the conserved 5.8S rDNA in between the two spacers (Blattner

[1] Contributions to the ITS dataset: 230 accessions were sequenced by NMN at the IPK Gatersleben, 55 by SM at Kew Gardens, and 25 by MAC at the Natural History Museum London.

1999), allowed us to use poorly preserved plant tissue from old herbarium specimens for amplification of this locus to extend our sampling to otherwise not accessible species.

The main objectives of this study are (i) to provide a molecular phylogeny based, wherever possible, on several accessions per species covering almost all sections of the genus and several related genera to test the monophyly of *Hypericum*, (ii) to compare the phylogeny with the current classification of the genus, and (iii) to reconstruct the evolution of characters and identify morphological character support for major groupings.

3.2 Materials & methods

3.2.1 Taxon sampling

Our approach aimed at extensive sampling within *Hypericum,* with multiple accessions included of as many species as possible from almost all sections. Taxon sampling is generally recognized to be important to recover the correct phylogeny by reducing branch length and homoplasy, both factors that can produce misleading phylogenies (Huelsenbeck 1995). In fact, adding additional taxa seems more valuable than adding more genes to improve the resolution of a phylogeny (Zwickl & Hillis 2002).

Samples were obtained from herbarium collections (ANDES, B, BM, GH, HEID, K, KYO, TI), from freshly collected silica-dried material from Colombia and Japan, as well as from living collections cultivated at the UK National Council for the Conservation of Plants and Gardens – National Plant Collection of *H.* sect. *Androsaemum* & *Ascyreia* at Wakehurst Place (WAK), the IPK Gatersleben (GAT) and the University of Heidelberg (HEID). Additionally, the DNA sequence database (GenBank, NCBI/EMBL) was queried for Hypericaceae ITS sequences. Fifty-six selected sequences from the database were included in the final alignment together with 310 newly generated ITS sequences. Twenty sequences published by Crockett *et al.* (2004) were re-edited from the original chromatogram files, resulting in longer ITS sequences, which were resubmitted to GenBank as updated versions.

In total, *Hypericum* currently comprises 486 accepted species (N. Robson, pers. com.) of which 194 were included in our analysis (*c.* 40 %). Thirty-four of the 36 sections recognized by Robson (1977 onwards) were sampled; we were unable to sample the two monotypic *H.* sect. *Umbraculoides* (*H. umbraculoides* from Oaxaca, Mexico), and sect. *Thasia* (*H. thasium* from Greece, Bulgaria, and Turkey). Outgroup representatives from *Triadenum*, *Thornea*, *Vismia*, *Harungana*, and *Cratoxylum* were included (Appendix S3.2), representing five of the eight remaining genera of the Hypericaceae, covering all three of the tribes (Hypericeae, Vismieae, Cratoxyleae.) recognized by Stevens (2007).

3.2.2 Molecular methods

Genomic DNA was extracted from fresh material, herbarium exsiccatae or silica dried samples. For DNA extraction, different amounts of plant tissue and several extraction methods were tested. Two methods provided the best results in *Hypericum*: a CTAB (cetyltrimethyl ammonium bromide) approach modified from Doyle and Doyle (1987, 1990), and the Invisorb® Spin Plant Mini Kit (Invitek, Berlin, Germany) following the manufacturer's protocol. For old and poorly preserved tissues from herbarium sheets the CTAB method, including 2 % PVP40 (polyvinylpyrolidone), was used. No more than 10 mg of plant tissue were included per extraction to avoid decrease of DNA quality and yield.

The entire ITS region was amplified with primers ITS-A and ITS-B (Blattner 1999) in 50 µl reactions using 1 U Taq DNA polymerase (QIAGEN, Hilden, Germany), 5 µl of the supplied buffer (10x) and additionally 5 mM MgCl2, 100 µM of each dNTP, 5 pmol of each primer and approximately 20 ng of total DNA. In order to weaken DNA secondary structures, Q-solution (QIAGEN) was added to the reactions with a final concentration of 20 %. In case of degraded herbarium material ITS-1 and ITS-2 were amplified separately using the initial amplification primers in combination with internal primers (*i. e.* ITS-A/ITS-C and ITS-B/ITS-D) binding within the 5.8S rDNA (Blattner 1999).

PCR profiles consisted of an initial denaturation at 95 °C for 3 min, followed by 38 cycles of 95 °C for 30 sec, 53 °C for 45 sec, 68 °C for 1 min and a final step at 70 °C for 8 min. PCR products were checked on 1.5 % agarose gels. Amplicons were cut out and purified using the QIAquick gel extraction kit (QIAGEN). About 20–40 ng of PCR product were directly sequenced on the ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using the respective dye-terminator sequencing technology. Samples were sequenced using either two nested sequencing primers, ITS-SF and ITS-SR (Blattner *et al.* 2001), or these primers together with primers ITS-C(F) and ITS-D(R). Forward and reverse sequences from each template were manually edited and combined in single consensus sequences with Sequencher v4.7 (Gene Codes of Ann Arbor, MI, USA). Polymorphic positions were coded as ambiguities.

To test for multiple ITS copies within individuals (intra-individual ITS polymorphism; Álvarez & Wendel 2003) PCR products of ten selected accessions belonging to *H.* sect. *Ascyreia*, *Hypericum*, *Olympia*, *Hirtella* and *Adenosepalum* (selection criteria: more than one polymorphic position in the chromatogram files obtained by direct sequencing) were cloned in the pGEM-T Easy vector (Promega, Madison, WI, USA). Between five and eight clones per individual were sequenced with the Templi-Phi DNA Sequencing Template Amplification Kit (GE Healthcare Life Science, Chalfont St. Giles, GB). All cloned sequences were carefully examined for mosaic sequence (chimaeric) patterns, which may be the results of recombination between different ITS copies after hybridization (Koch *et al.* 2003; Nieto Feliner *et al.* 2004; Robba *et al.* 2005).

To identify possible paralogous loci, the highly conserved 5.8S region was visually scanned for sequences considerably differing in variation compared to the entire dataset. Sequences will be deposited in the EMBL nucleotide database (for accession numbers see Appendix S3.2).

3.2.3 Phylogenetic inference

Sequences were aligned initially using the multiple alignment mode implemented in ClustalX v2 (Thompson *et al.* 1997; Larkin *et al.* 2007) and manually refined. Two datasets were aligned: the first contained only the sequences obtained by direct sequencing; the second contained additionally the cloned sequences.

Phylogenetic analyses were designed to test the monophyly of *Hypericum* and the groups described in chapter 2, to test for hybridization events between major groups, and to establish a general hypothesis of lineage relationships within the genus. Bayesian, likelihood and parsimony analyses were performed on a dataset containing 366 sequences as obtained by direct sequencing representing 192 Hypericum species and twelve outgroup representatives (Appendix S3.2). A likelihood analysis was performed on the dataset additionally containing 69 sequences obtained by cloning ten selected accessions (results not shown). Two *Cratoxylum* species were defined as outgroup, following Wurdack and Davis (2009) who showed this genus to be the sister to the remainder of Hypericaceae.

Different models of sequence evolution were tested in Modeltest 3.7 (Posada & Crandall 1998), and the GTR + I + Γ model was chosen according to the Akaike information criterion (Akaike 1974). Bayesian phylogenetics was performed using the Metropolis-coupled Markov chain Monte Carlo algorithm implemented in MrBayes v3.1.2p (Ronquist & Huelsenbeck 2003), likelihood analyses in RAxML v7.2.4 (Stamatakis 2006) and parsimony analyses in PAUP* v4.0b10 (Swofford 2002).

For Bayesian inference (BI) two simultaneous runs each with four chains and starting from a random starting tree were performed under the GTR + I + Γ model, for 14 x 10^6 generations, setting temperature to 0.1 and sampling a tree every 1 000 generations. Likelihood values appeared stationary after 3.5 x 10^6 generations and 25 % (the first 3 500 trees/run) were discarded as burn-in. Posterior probabilities were calculated on the basis of the remaining 21 002 trees.

Maximum likelihood (ML) analyses were run for 1 000 rapid bootstrappings and a subsequent ML search (RAxML was called with the GTRCAT model). These settings were also used to run the ML analysis for the dataset containing the sequences obtained by cloning (results not shown).

The parsimony analyses (MP) followed a two-step heuristic search approach modified from Blattner (2004) and described in detail in chapter 2 with the following modifications: the 1st analysis was conducted calculating 1 000 random additions with 20 trees held at each step and saving five trees from each repetition (even if they were not optimal overall). These trees were used as starting trees in the 2nd analysis, a TBR search that was limited to find 50 000 best trees. Statistical support of the branches was tested with 100 000 bootstrap re-samples (Felsenstein 1985), using the 'fast and stepwise' procedure in PAUP*. Tree lengths, consistency (CI) and retention index (RI) were calculated in PAUP* v4.0b10 on a Macintosh OS 9 computer. Visualization of results was done using FigTree v1.3.1 (Rambaut, 2006–2009).

3.2.4 Character reconstruction

Character states defined in chapter 2 were optimized on the topology shown in Figure 3.1 using Fitch parsimony (Fitch 1971) in Mesquite (Maddison & Maddison 2010). To count for phylogenetic uncertainties we also analyzed character state changes for eight selected characters (Appendix S3.2) over a posterior distribution of 1 000 trees using reversible-jump MCMC methods (Pagel 1999) in the program BayesMultiState (Pagel *et al.* 2004) as implemented in the software package BayesTraits (www.evolution.reading.ac.uk/BayesTraits.html).

The Bayesian reconstruction of ancestral character states was calculated for well supported nodes in the phylogeny (*i. e.*, the backbone nodes; cf. Figs. 3.1, 3.3) using the default settings and over a posterior distribution of possible tree topologies, represented by 1 000 randomly sampled trees (after discarding 25 % as burn-in) as obtained by the MrBayes analysis. A bootstrap analysis of 10 000 replicates was performed on the p values obtained by BayesTraits using a python script. Visualization of results was done in R (R Development Core Team 2011) using density plots and pie charts, respectively (results exemplarily shown only for the analysis of growth forms; Fig. 3.1). The character states reconstructed for ancestral nodes in both parsimony and Bayesian methods were taken to define apomorphic traits for certain nodes of the phylogeny of Hypericeae (Fig. 3.3).

3.3 Results

The final dataset included 366 sequences of the ITS region as obtained by direct sequencing. After introducing the necessary gaps the alignment comprised 780 base pairs (bp), of which 485 were variable and 410 were parsimony informative. Only one additional gap (one bp length) was necessary to align the cloned sequences, resulting in a dataset additionally including 69 sequences obtained by cloning (Appendix S3.2).

Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) analyses of directly sequenced ITS resulted in trees with an identical topology for the main clades, and minor differences at terminal clades/tips only. The most parsimonious trees had a length of 2 342 steps, a CI of 0.3911, and a RI of 0.9213. One polytomy occurring only in the BI tree (*H.* sect. *Adenotrias* and *Elodes*; see below, Fig. 3.1) was resolved in the ML tree (with *H.* sect. *Adenotrias* and *Elodes* in a sister group relationship and together as sister to the remainder; not shown) and in the MP consensus tree (with *H.* sect. *Adenotrias* as sister to the remainder; not shown), but both without bootstrap support values ≥50 %.

Each of the three tribes of Hypericaceae – Cratoxyleae, Vismieae and Hypericeae – is monophyletic and supported with maximum support values (sv; Figs. 3.1, 3.2). Within Hypericeae, *Thornea* and *Triadenum* are monophyletic (each with sv of: 1.0 Bayesian posterior probability (pp)|100 % bootstrap support (bs) for ML|100 bs for MP = sv max). A deep split is evident dividing Hypericeae into: (**A**) a grade comprising *Thornea*, followed by *H.* sect. *Adenotrias* and *Elodes* (sv 1 pp|75 bs ML|– (not resolved) ML), followed by a clade (sv 1 pp|92 bs ML|54 bs MP) that

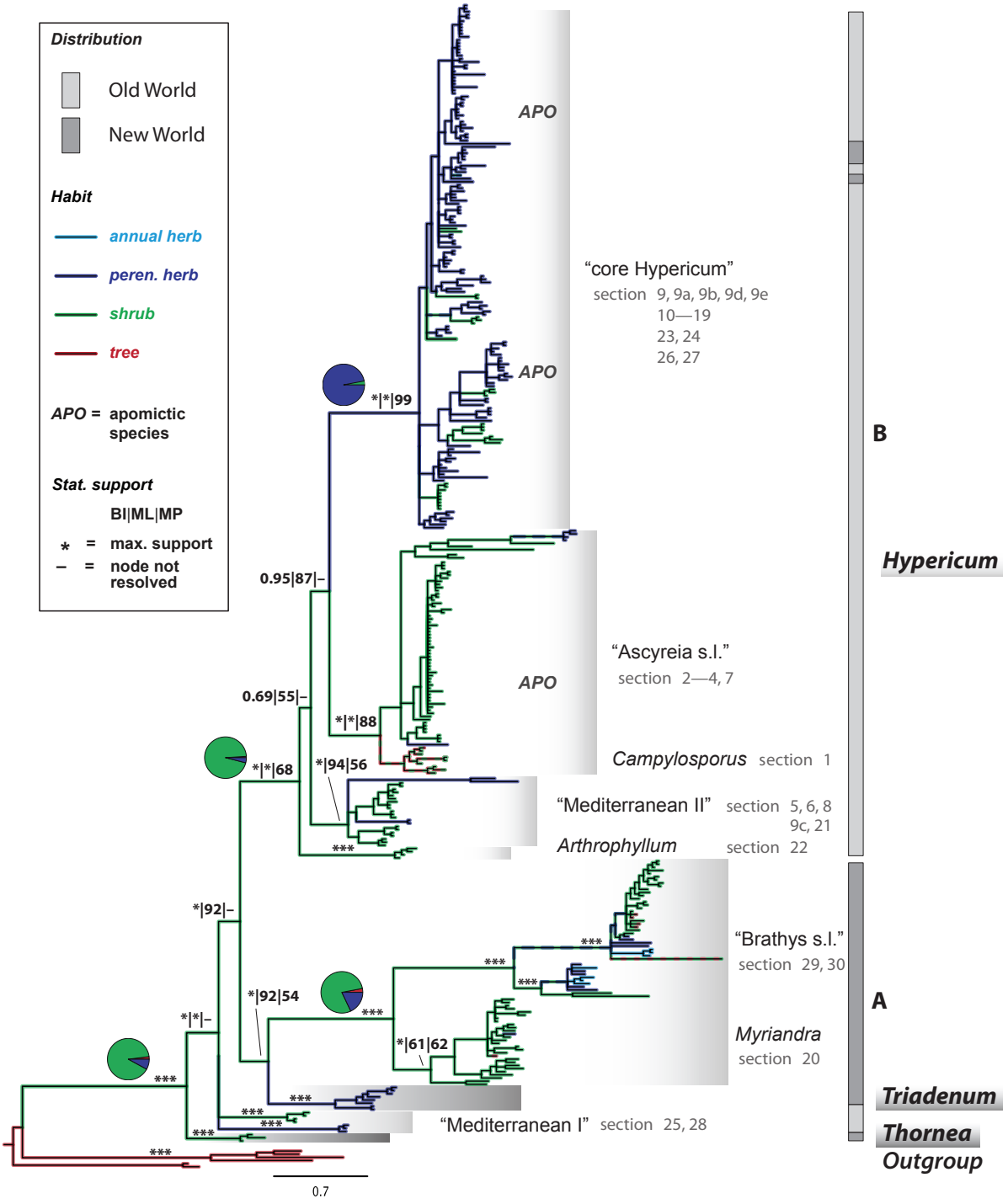


Fig. 3.1 Consensus tree obtained by the Bayesian analysis of ITS sequence data, showing the relationships of 194 *Hypericum* and 12 outgroup species, represented by 366 accessions. A light-grey background highlights clades containing taxa belonging to *Hypericum*, a dark-grey background the other taxa of Hypericeae. An asterisk marks maximum statistic support values (BI|ML|MP). The occurrence of shrubs (in green), trees (red), perennial (dark blue) and annual herbs (light blue) is highlighted by the color of branches. The four pie charts exemplarily display bootstrap values of the character states reconstructed in 1 000 trees at a certain node. Rooting of the tree is that in Wurdack & Davis (2009).

includes *Triadenum* as sister to the New World *H.* sect. *Myriandra*, *Brathys* and *Trigynobrathys* (sv 1|100|97), and (**B**) the remainder of *Hypericum* in a clade (sv 1|100|68). The position of *Triadenum* within *Hypericum* makes the latter paraphyletic in its current circumscription (referred to as *Hypericum* s.l.).

Within **A**, the (mainly) New World sections of *Hypericum* s.l. are placed in a clade (sv 1|100|98; here called "Brathys s.l." + *Myriandra*) with *H.* sect. *Myriandra* (sv 1|61|62) as sister to a clade comprising taxa of *H.* sect. *Brathys* and *Trigynobrathys* (sv max). Most of the accessions of *H.* sect. *Trigynobrathys* are resolved in a clade (sv 1|93|85) that is sister to a clade containing all accessions of *H.* sect. *Brathys* plus four species of *H.* sect. *Trigynobrathys* (sv max). The two Old World sections of *Hypericum* s.l. belonging to **A** (*H.* sect. *Adenotrias* and *Elodes*) are resolved in a polytomy in the BI tree (here called "Mediterranean I"), subsequent to *Thornea* and as sister to the remainder of Hypericeae (sv 0.99|75|–).

Group **B**, comprises the mostly Old World sections of *Hypericum* s.l. (*H.* sect. 1–19 & 21–24 & 26, 27, section numbers refer to Table 3.1). A grade here called "Mediterranean II" + *Arthrophyllum* comprises the Mediterranean *H.* sect. *Arthrophyllum*, *Androsaemum*, *Inodora*, *Bupleuroides*, *Webbia* and *H. sampsonii* Hance from China (*H.* sect. *Sampsonia*). The two clades forming this grade received high to moderate support (Fig. 3.1), but moderate to low support for their backbone nodes (sv 0.69| 55|– & 0.95|87|–). A clade called "Ascyreia s.l." + *Campylosporus* (sv 1|100|86) contains two subclades, one that includes Afrotropical species from *H.* sect. *Campylosporus* (sv 1|100|84) and a second that contains Mediterranean *H.* sect. *Psorophytum* together with Asian *H.* sect. *Ascyreia*, *Takasagoya* and *Roscyna*. The crown clade, here called "core Hypericum" (sv 1|100|99), contains the type species *H. perforatum*, and comprises taxa of *H.* sect. 9–9e, 10–19, 23–24, 26, and 27.

Within the named clades and grades identified in Figures 3.1 and 3.2 ("Mediterranean I", "Brathys s.l." + *Myriandra*, "Mediterranean II" + *Arthrophyllum*, "Ascyreia s.l." + *Campylosporus*, and "core Hypericum"), relationships between clades and species are often well resolved but sometimes not well supported by statistic values (Fig. 3.2, A–C). We do not discuss these terminal relationships here, because we feel that extended taxon sampling for these groups would be necessary to arrive at sound phylogenetic hypotheses for the species within these clades.

Multiple ITS copies were found in some individuals. For example, six different ITS copies were found in seven clones of *H. reflexum* and these differed by up to 10 nucleotide positions. Patterns consistent with hybridization between closely related species and/or polyploidization event were also detected, notably in *H. lancasteri* (*H.* sect. *Ascyreia*) wherein cloned ITS sequences grouped in two clades, but without statistic support ≥50 % bs. In all cases, cloned sequences derived from one individual grouped only within one of the major clades and they were resolved in a position consistent with the sectional placement of the species concerned (results not shown). No chimaeric ITS types consistent with ITS hybridization between the major clades were identified. Also sequences were amplified directly or by cloning, we found no evidence for paralogous ITS copies, as the 5.8S region was equally conserved across all accessions.

The reconstruction of ancestral states aimed to define apomorphic traits for well-supported nodes found by the ITS analysis. Both parsimony and reversible MCMC methods resulted in similar character states for a certain node. Although morphology is highly plastic and diverse within a genus containing almost 500 species like *Hypericum* s.l., it is possible to define characters

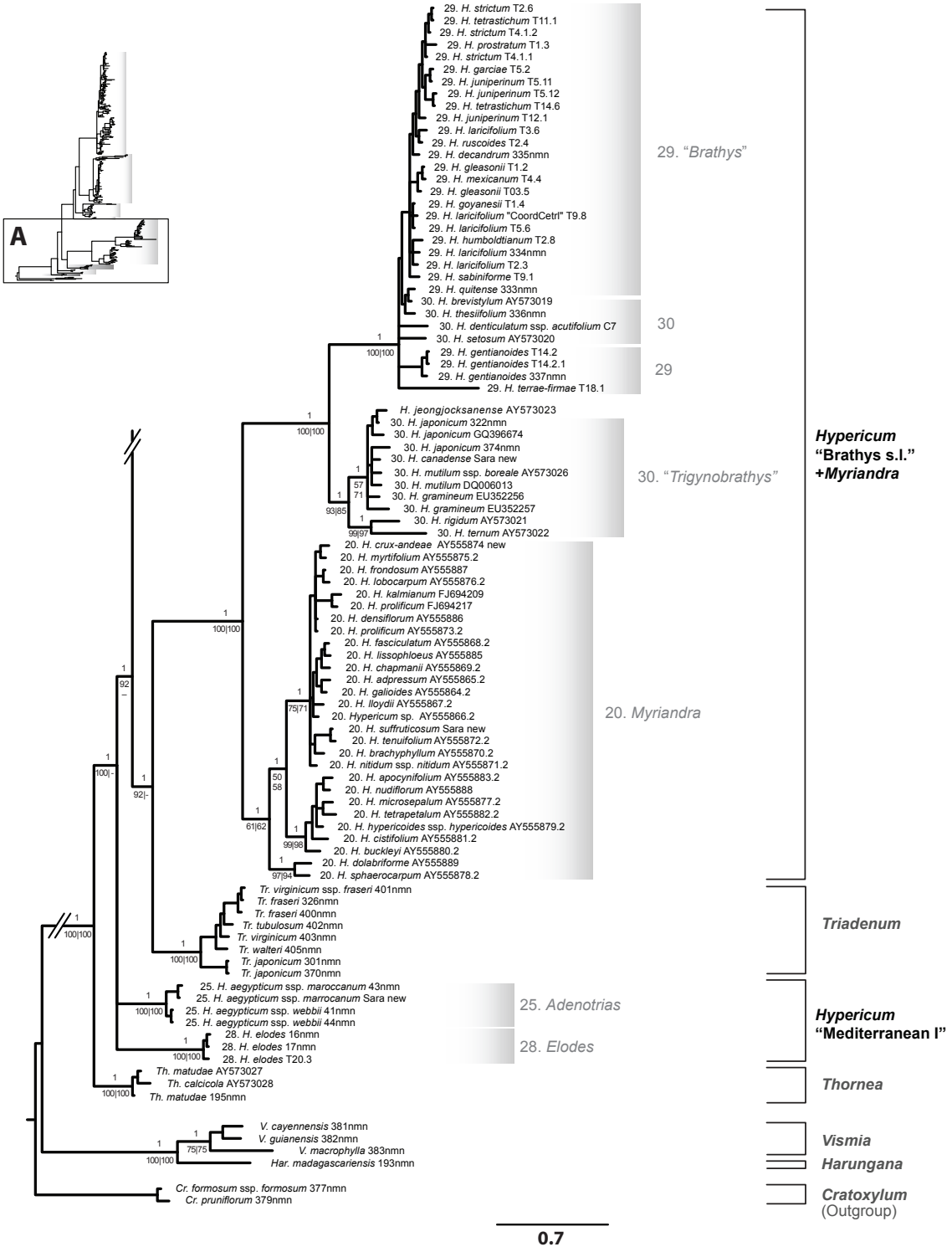


Fig. 3.2-A–C (part A) Phylogenetic tree obtained by the BI analysis of ITS sequence data, showing the relationships of accessions belonging to *Hypericum*, *Triadenum*, *Thornea*, and representatives of *Vismia*, *Harungana* and *Cratoxylum*. Genus names (in dark grey), clade/grade names within *Hypericum* (black) and section names (light grey) are given. Section numbers refer to the classification (see Table 3.1). Statistic support values above the branches depict posterior probabilities from BI, and below bootstrap support from ML|MP.

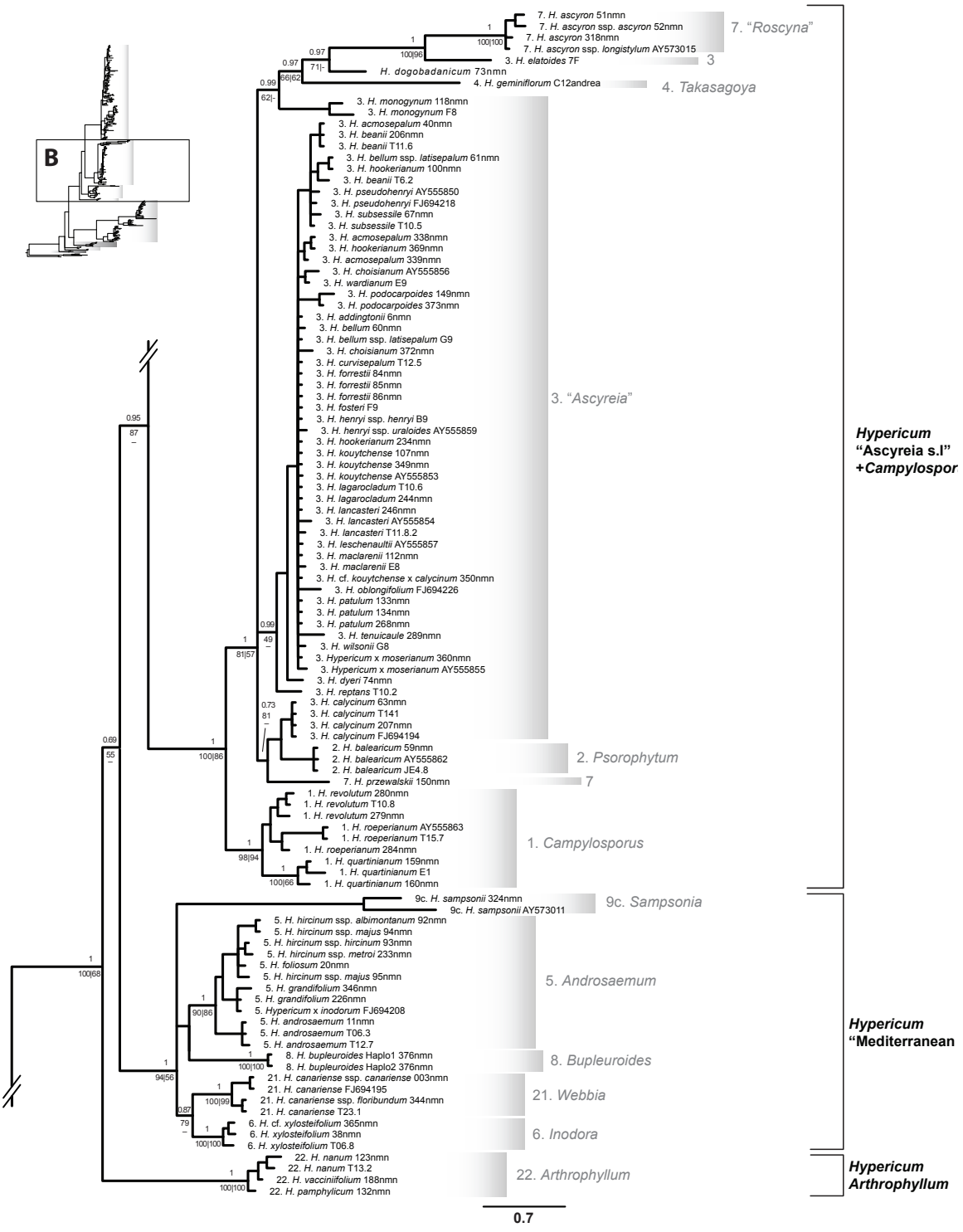


Fig. 3.2-A–C (part B) continued.

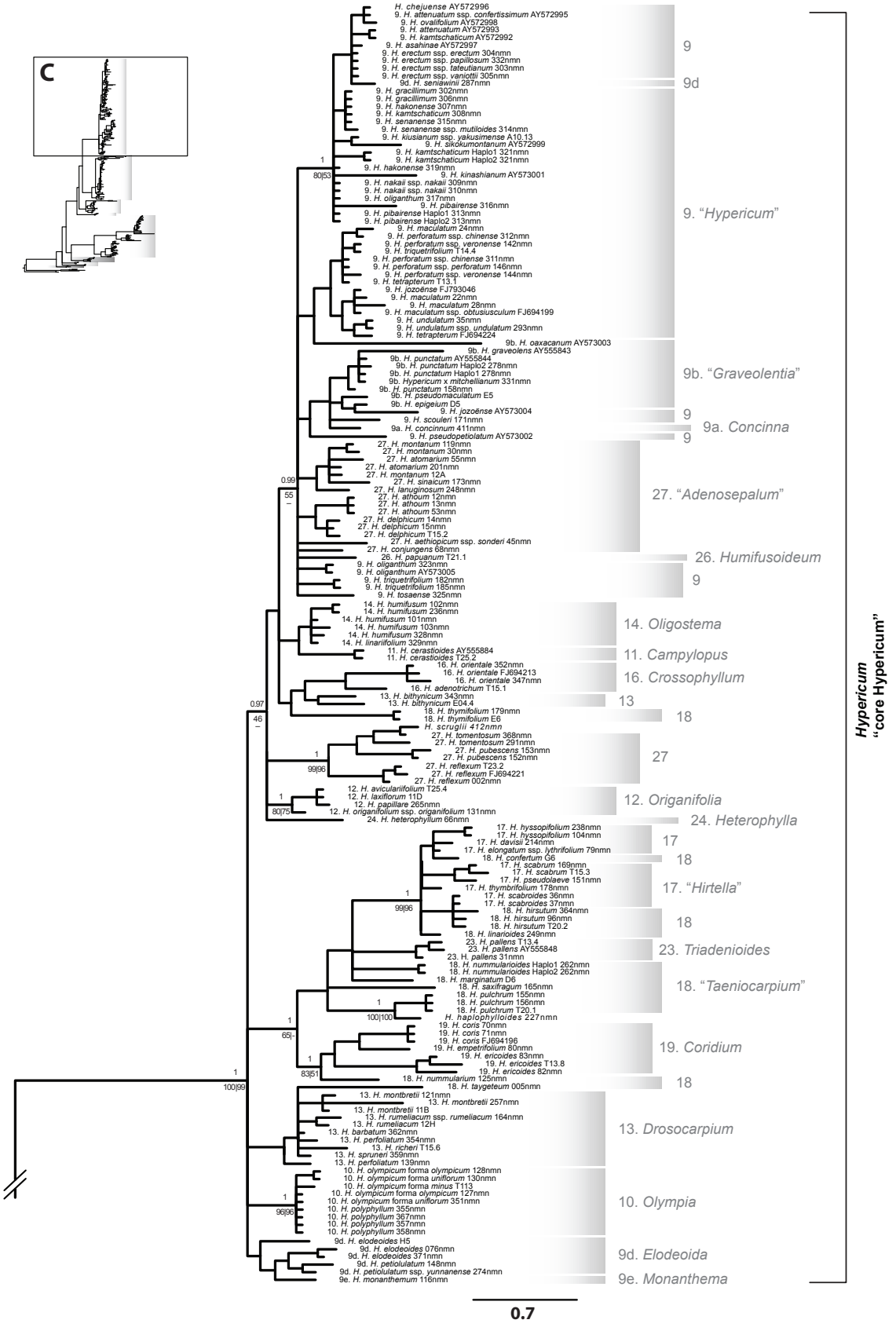


Fig. 3.2-A–C (part C) continued

supporting major clades (summarized in Fig. 3.3). As evident in part 3.4.1 and discussed in part 3.4.2 of this study, recognition of homology *versus* multiple origins (homoplasy) of certain characters can be challenging and has to be based on an explicit phylogenetic hypothesis. As an example, the reconstruction of states of the character 'habit' is given in Figure 3.1.

3.4 Discussion

3.4.1 Phylogeny of Hypericaceae

Although we found multiple ITS copies within an individual, no obvious chimeric sequences could be identified that would introduce reticulating or divergently branching phylogenetic signals between larger clades in the dataset (McDade 1990; 1992). The multiple ITS copies found by cloning did consistently group within the big clades, *i. e.* we did not detect reticulate evolution between these clades. Within the large clades hybridization and/or polyploidization might be frequent and responsible for low resolution or reticulate phylogenetic signals (*e. g.,* within *H.* sect. *Ascyreia*), as indicated by the occurrence of multiple ITS copies. Extended taxon sampling and statistic support of clades is necessary to aim at identifying potentially parental lineages. Further studies, which focus on reticulate evolution within the genus, demand an experimental setup that explicitly aims at detecting all possible ITS loci copies, like *e. g.,* pooling of several PCR reactions, usage of a proof reading polymerase, sequencing of more clones per accession, and a comprehensive species sampling for the clade of interest.

More important for the purpose of this study is the possibility of paralogous ITS sequences or pseudogenes that would also confound phylogenetic reconstruction (Álvarez & Wendel 2003). No evidence, however, was detected in our dataset that would hint towards the existence of paralogous loci, which might be due to technical reasons (PCR drift or consideration of too few clones) or genomic reasons (does not exist at all or almost complete concerted evolution of rDNA in *Hypericum* s. l.). Preliminary analyses of the *pet*D region from the plastid genome (Borsch & Nürk *et al.*, unpubl. data) revealed the same major grouping as found by ITS sequence analysis, which supports the phylogenetic accuracy of our rDNA sequence analysis.

The relationships of genera, with *Cratoxylum* (Cratoxyleae) defined as outgroup, followed by *Vismia* and *Harungana* in a clade (Vismieae), followed by *Thornea* as sister to *Hypericum* s. l. (incl. *Triadenum*; Hypericeae) in our ITS analysis is generally in accordance with the recent classification of Hypericaceae (Stevens 2007), and with data of Wurdack & Davis (2009) based on multi-gene analysis of sequences from all three plant genomes. *Triadenum*, that in our analysis groups as sister to the New World clade ("Brathys s. l." + *Myriandra*) within *Hypericum* s. l., was resolved as sister to *Hypericum* in studies of Gustafsson *et al.* (2002) and Wurdack & Davis (2009). However, both analyses included single species representatives of the two genera, thus providing no conflict with our data.

Within Hypericeae, the analysis of the ITS region revealed a grade, for practical reasons called **A,** and a clade, here called **B**. The grade **A** is made out of *Thornea* as sister to the remainder, followed by a grade (or a clade) here called "Mediterranean I" as sister to *Hypericum* s.l., followed by the mainly New World clade containing the genus *Triadenum* as sister to the *Hypericum* "Brathys s.l." + *Myriandra* clade. Although each of these clades obtained maximum support values in all three analyses (BI, ML, MP), the relationships between the clades were highly supported only in BI (≥0.99 pp), less in ML (75 and 92 bs, respectively; Fig. 3.2-A), but received no support ≥ 50 % in MP.

A morphological description of the described clades/grades is possible, but it should be kept in mind that exceptions exist for several characters, especially within the large sections from the New World, *H.* sect. *Myriandra*, *Brathys* and *Trigynobrathys*.

The two *Thornea* species are shrubs possessing pale glands only (*i. e.* dark glands are absent). Their petals are deciduous, campanulate, and pink or white. Stamens are persistent, with the filaments basally united in three fascicles. Between the fascicles three staminodes are present. The gynoecium is trimerous and placentation axile.

"Mediterranean I" (section 25 & 28) contains shrubs (*H.* sect. *Adenotrias*) and herbs (*H.* sect. *Elodes*). Dark glands are present only at the connective of the anthers and the sepals of *H. elodes* (described as red glands). The petals are persistent, (pseudo-) tubular, and yellow. Stamens are persistent and in three fascicles. The filaments are united to above the middle and they are heterostylus in *H.* sect. *Adenotrias*. Three staminodes are present between the fascicles. The gynoecium is trimerous, with placentation parietal in *H.* sect. *Elodes* and axial in *H.* sect. *Adenotrias.*

Triadenum species are herbs that occur in ± aquatic habitats like swamps and lake margins. No dark glands are present. The petals are deciduous, campanulate, and pink to purple or white. Stamens are deciduous, and in three fascicles. Filaments are united, and three staminodes between the fascicles are present. The gynoecium is trimerous, and placentation axial.

"Brathys s.l." + *Myriandra* (section 20, 29 & 30) contains shrubs and herbs, but few annuals. Dark glands are generally absent. The petals are stellate, yellow and persistent in *H.* sect. *Brathys* and *Trigynobrathys*, but deciduous in *H.* sect. *Myriandra*. Stamens are mostly persistent. They are in a broad ring in *H.* sect. *Myriandra* and in a narrow ring or modifications/reductions thereof in *H.* sect. *Brathys* and sect. *Trigynobrathys*. The gynoecium is trimerous, and placentation is parietal, or loosely axile in some *Myriandra* species.

Group **B** contains all remainder of *Hypericum* s.l., and consists of a grade and two larger clades.

(1) The grade called "Mediterranean II" + *Arthrophyllum* (section 5, 6, 8, 9c, 21 & 22) consists of *H.* sect. *Arthrophyllum*, followed by a clade containing *H.* sect. *Inodora* and *Webbia*, *Bupleuroides* and *Androsaemum*, and *Sampsonia* from China. Support values are again high to moderate for the two clades themselves, but low for the relationships between the two clades and to the remainder of group **B** (Fig. 3.2-B). It is characterized by shrubby habits, but some herbs do occur. Dark glands are present in *H.* sect. *Arthrophyllum* and sect. *Sampsonia*, and in *H.* sect. *Inodora* and sect. *Bupleuroides* in reproductive parts only. The petals are stellate, yellow and persistent, but deciduous in *H.* sect. *Androsaemum*. Stamens are persistent, but deciduous in *H.* sect. *Androsaemum*. They are in three fascicles, but in five in *H.* sect. *Androsaemum*. The gynoecium is trimerous, and placentation is loosely axile to axile in *H.* sect. *Bupleuroides*.

(2) The mainly Indo-Malayan "Ascyreia s.l." + *Campylosporus* clade (section 1, 2, 3, 4 & 7), grouping together Afrotropic *H.* sect. *Campylosporus* as sister to a clade containing *H.* sect. *Psorophytum*, *Ascyreia*, *Takasagoya* and *Roscyna*. It contains mostly shrubs, but tree-like habits are described in *H.* sect. *Campylosporus*. Dark glands are present only in *H.* sect. *Campylosporus* and in reproductive parts only in *H. ascyron* (*H.* sect. *Roscyna*). The petals are deciduous, but persistent in *H.* sect. *Roscyna* and in some species of *H.* sect. *Campylosporus*, where they are described as tardily deciduous. They are stellate and yellow, sometimes tinged red. Stamens are generally deciduous, but persistent in *H.* sect. *Roscyna* and in some species of *H.* sect. *Campylosporus*, and in five fascicles. The gynoecium is pentamerous, and placentation is loosely axile.

(3) The mainly Palaearctic crown clade "core Hypericum" (section 9, 9b, 9d, 9e, 10–19, 23, 24, 26 & 27) is well supported as a clade but without sound bipartitions within. This clade contains most of the sections described in *Hypericum* and has the highest species-richness (Fig. 3.2-C). It consists of herbs, and (secondarily) some dwarf shrubs. Dark glands are present, except in three species (*H. heterophyllum*, *H. taygeteum,* and sometimes in *H. saxifragum*) and only in reproductive parts in *H.* sect. *Coridium* and in some species of *H.* sect. *Hirtella* and *Taeniocarpium*. The petals are persistent (except in some species of *H.* sect. *Coridium*), stellate and yellow. Stamens are persistent, in three fascicles, or in a narrow ring in *H.* sect. *Humifusoideum*. The gynoecium is trimerous, with axile placentation, or loosely axile placentation in *H.* sect. *Adenosepalum*, *Humifusoideum*, *Triadenioides* and in the unclassified species *H. haplophylloides* Halácsy & Bald.

A recently published five-marker analysis of the clusioid clade of the Malpighiales (Ruhfel *et al.* 2011) that included twenty-one representatives of the Hypericeae (representing four genera: *Hypericum*, *Santomasia*, *Triadenum* and *Thornea*) revealed identical groupings, however, our (grade) **A** was a clade in their analysis (*i. e.* as sister to clade **B**) that also included the monotypic genera *Santomasia* from Oaxaca, Mexico. The ITS-based topology described here is in part congruent with the analysis of morphological characters (Chapter 2), with the main difference that ITS groups together the three large New World *H.* sect. *Myriandra*, *Brathys* and *Trigynobrathys*. Other differences in morphological inference in comparison to the results from ITS are the position of *Triadenum* (and/or *Lianthus*) as sister to *Hypericum*, and positions of the species distributed in the Mediterranean, which could not be clearly assigned to larger clades due to low statistic support of the groups (Chapter 2). The Mediterranean clades are almost identical to the ones found by analysis of morphology. Few exceptions exist, like *H.* sect. *Elodes* and *H.* sect. *Bupleuroides*, being grouped within "Euhypericum" (Chapter 2), or *H.* sect. *Triadenioides* and *H.* sect. *Heterophylla* suggested by ITS to belong to "core Hypericum". However, the main groupings revealed in *Hypericum* by the cladistic analysis of morphological characters were evident also in the ITS sequence analysis presented here (Fig. 3.1).

Compared to previous phylogenetic studies using molecular markers in *Hypericum*, the phylogenetic tree (Fig. 3.1) is highly congruent with the results of Crocket *et al.* (2004), but differs in several parts from Park & Kim (2004). Crocket *et al.* (2004) analyzing ITS sequences with MP and using *Clusia rosea* as outgroup recovered the same major clades as presented here, *i. e.* "Brathys s.l." + *Myriandra*, "Ascyreia s.l." + *Campylosporus* and "core Hypericum", called C, B and A in Crocket *et al.* (2004), respectively. The split of *Hypericum* into two main clades in Crocket *et al.* (2004) as (C(AB)), is reflected in our grade **A** and clade **B**. Clade C of Crocket *et al.* (2004), however, consisted of taxa belonging to *H.* sect. *Myriandra* only. The MP tree presented in Park & Kim (2004) uses ITS and the two *Thornea* species as outgroup, but is also topologically different

regarding the placement of accessions classified into *H.* sect. *Hypericum* (grouping together with species from *H.* sect. *Trigynobrathys*) and *H.* sect. *Adenotrias* (grouping together with species from *H.* sect. *Roscyna*). To test whether incorrect species determination could have contributed to these differences, we sequenced ITS for additional individuals of these species, each determined by Norman Robson (Nat. Hist. Museum, London). We found that the newly sequenced individuals of such 'misplaced' taxa grouped according to their sectional affiliation. For this reason, we excluded several sequences of Park & Kim (2004) and other similarly obvious problematic sequences available from GenBank from our analysis.

3.4.2 Character evolution

Morphological support for major groupings within *Hypericum* s.l. is limited although some apomorphic characters can be identified, which may have played a major role in evolution. The occurrence of three alternipetalous staminodes (*i.* e., vestigial fascicles opposite the sepals, also called fasciclodes) between the (three) stamen fascicles is a characteristic of *Thornea* and *Triadenum* (and *Lianthus* and, with five fascicles, in *Santomasia*; both genera are not included in our analysis), as well as of the *H.* sect. *Elodes* and *Adenotrias* ("Mediterranean I"), but is absent in all other *Hypericum* species. The topology revealed by the ITS analyses (Fig. 3.1) suggests the loss of these staminodes at least two times, once in the ancestors of the "Brathys s.l." + *Myriandra* clade and once in the ancestors of group **B**. The presence of such staminodes goes along with a 'pseudo-tubular' corolla, which has been hypothesized as modifications towards specialized insect pollination (Robson, 1981: 302). With respect to the ITS phylogeny and regarded from this functional viewpoint, the stellate flowers with 'unspecialized' pollination are typical for the clades of *Hypericum* s.l. with much higher species-richness (in Hypericeae: 14 species with staminodes *versus* > 460 without).

The reconstruction of the evolution of growth form revealed a shrubby ancestor for *Hypericum* s.l. from which herbs evolved several times independently within *Hypericum* s.l. (Fig. 3.1), the latter being the characteristic habit of the "core Hypericum" clade. In two sections only, *H.* sect. *Brathys* and *Trigynobrathys*, annuals evolved, most probably twice independently. Trees that are growing with a single trunk attaining over 10 m in height are reported for some Afrotropical species of *Hypericum*, namely *H. bequaertii* endemic to the Rwenzori Mountains and *H. revolutum* from the Ethiopian highlands. Such trees have been observed, however, in disturbed and open habitats. In natural habitats they are usually tall, but bushy or slender shrubs (Robson 1993). The evolution of such tall erect shrubs, which have been called tree-like by some authors, is evidently connected to tropical montane habitats.

Within *Hypericum* s.l., sclerophylous arborescent shrubs have also evolved in the páramos of Andean South America (*H.* sect. *Brathys*) and in the mountains of New Guinea and Sumatra (*H.* sect. *Humifusoideum*). The altitudinal range, *c.* 1600–4500 m, of these arborescent species is similar in South America, Africa and New Guinea, and so are the habitats, reaching from the montane forest belt to shrublands and alpine grasslands. This phenomenon has been hypothesized to result from 'parallel evolution' (homoplasy) as a response to the conditions in tropical montane

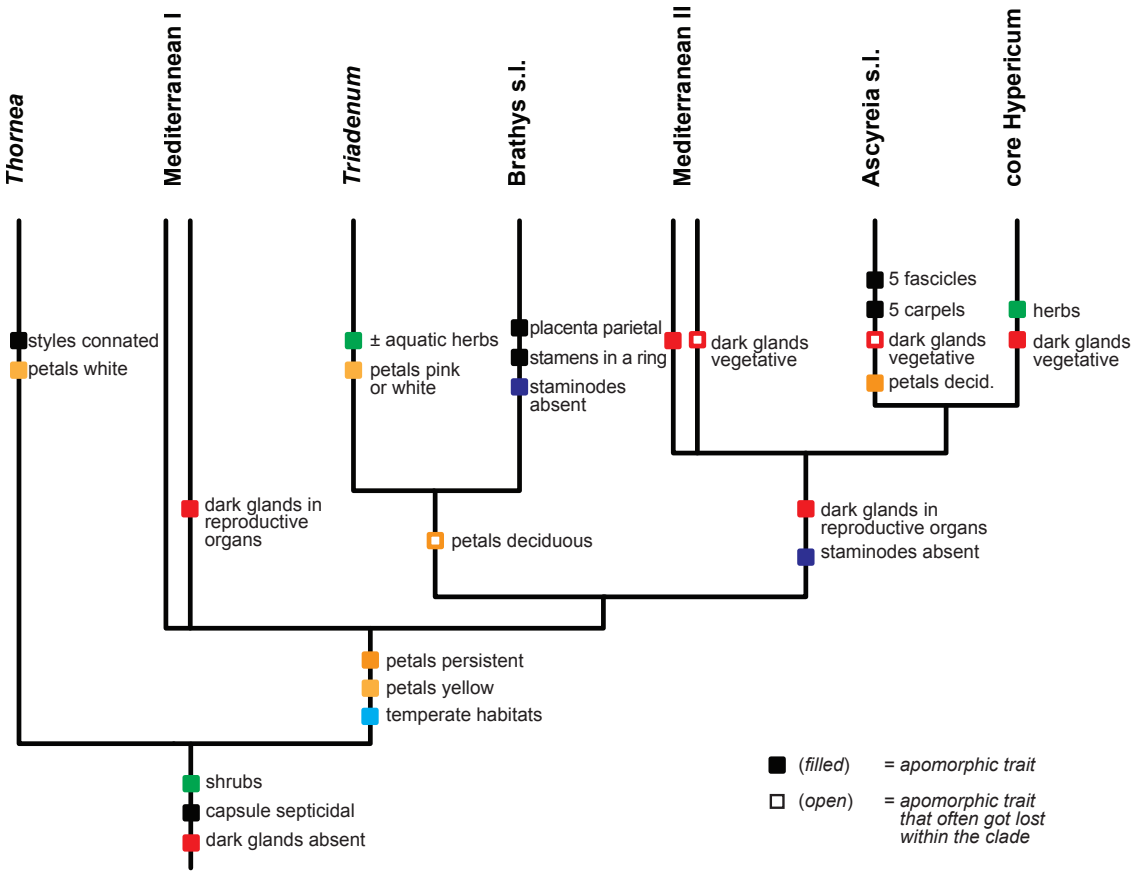


Fig. 3.3 Phylogeny of Hypericeae – Scenario of character evolution optimized on the phylogenetic tree derived from Bayesian inference of ITS sequences (of Hypericaceae). Apomorphic character states belonging to a class of characters are highlighted by the same color. Stem node apomorphies of Hypericeae are derived by the comparison to the outgroups (Vismieae and Cratoxyleae, not displayed). According to that, plesiomorphic character states of Hypericeae are the presence of pale glands, deciduous petals, stamens in three fascicles, three staminodes between the stamen fascicles, and a trimerous gynoecium.

habitats (Robson 1993). This hypothesis is supported by results of the present study. At least in the species belonging to *H.* sect. *Brathys* (South America) and *Campylosporus* (Africa), such habits have evolved in parallel. It is worthwhile pointing out the unique radiation that occurred in the páramos of Andean South America (*c.* 65 species and 16 subspecies are native to the páramo, belonging mainly to *H.* sect. *Brathys*). In contrast, only 17 species are reported from tropical montane regions of Africa (belonging to *H.* sect. *Campylosporus* [8 sp.] *Adenosepalum* [4 sp.] and *Trigynobrathys* [5 sp.]). For taxa from New Guinea (*c.* 9 species belonging to *H.* sect. *Humifusoideum*) and for taxa from Africa that belong to *H.* sect. *Adenosepalum* (*c.* 6 sp.), *Humifusoideum* (*c.* 3 sp.), and *Trigynobrathys* (*c.* 5 sp.), increased sampling is necessary to reveal phylogenetic evidence to interpret evolution of growth forms. Within the remaining genera of Hypericaceae trees are the predominant growth form, but shrubs are reported from *Vismia* and *Harungana* and from some *Cratoxylum* species (Stevens 2007).

In *Hypericum* two types of glandular tissue occurs: (*i*) multicellular black or red nodules containing hypericin, generally called 'dark glands' (Curtis & Lersten 1990; Karppinen *et al.* 2008), and (*ii*) translucent cavities occurring in all species of *Hypericum* s.l., containing hyperforin and essential oils, called 'pale glands'. Both, pale and dark glands are biochemically and to a certain extent also anatomically different and, thus, may not be homologous structures. However, Curtis and Lersten (1990) report the observation of 'chimerical' canals (elongated glands), changing color abruptly at some point from black to translucent. They conclude that the dark nodules are a modification of common (translucent) secretory reservoirs. Dark glands have also been reported from *Cratoxylum*, *Vismia* and *Harungana*. Presence of hypericin, however, has up to now only been detected in *Hypericum* (Crockett & Robson 2011). Comparative studies focusing on ontogeny and biochemical constitution (also in other genera of Hypericaceae), which also implement phylogenetic results, are needed to clarify homology and evolution of these secretory structures. Within Hypericeae, dark glands do not occur in species belonging to **A**, with the exception of *H. elodes* (*H.* sect. *Elodes*), having red glands at the sepals and black glands at the connective of the anthers. According to the phylogenetic hypothesis presented here, the occurrence of dark glands and therefore presence of hypericin in reproductive parts of the plant is also apomorphic for *Hypericum* s.l. species of clade **B** (but was lost again in *H.* sect. *Androsaemum*, *Webbia*, *Ascyreia*, *Takasagoya* and *Heterophylla*). The presence of dark glands in vegetative organs did evolve even later, and is apomorphic for *H.* sect. *Campylosporus*, *Arthrophyllum* and "core Hypericum" (except *H.* sect. *Coridium* and *Taeniocarpium* p.p.).

In "core Hypericum", the occurrence of dark glands in vegetative parts of the plant correlates with a rapid radiation (indicated by very short branch lengths in ITS). It has been suggested that hypericin plays a major role in plant response to herbivore attack (Sirvent *et al.* 2003) and it might be a key innovation for "core Hypericum", triggering fast speciation. The puzzling pattern of occurrence of pale and dark glands, however, exemplifies the problem of homology assessment in species belonging to such a large taxa as *Hypericum* s.l.

The evolution of fruit types does not allow far reaching conclusions, as the septicidal capsule is the sole form present in *Hypericum* s.l. Only in *H. androsaemum* (*H.* sect. *Androsaemum*, placed by ITS in "Mediterranean II") and in *H. peplidifolium* (*H.* sect. *Humifusoideum*, not included in the analysis) an indehiscent and crimson berry-like capsule is described. At least for *H. androsaemum* the crimson fruit offered new dispersal possibilities, as the plant has become a popular component in flower bunches, and thus were distributed almost all over the World during the last years (Groenteman *et al.* 2011).

Apomixis is a trait of interest for plant breeding (Schallau *et al.* 2010). In *Hypericum*, apomixis is reported from at least 16 species (Matzk *et al.* 2003), which are classified into the *H.* sect. *Ascyreia*, *Hirtella* and *Hypericum*. Myers (1964) also reported an apomictic embryo development in *Triadenum*. According to the phylogenetic hypothesis presented here (Fig. 3.1), apomixis has evolved several times independently within *Hypericum* s.l.: once in *Triadenum*, once in the "Ascyreia s.l." clade, and independently thereof probably twice in "core Hypericum". Whether the genetic basis is similar in all species to that which is reported for *H. perforatum* (Schallau *et al.* 2010) needs to be investigated.

3.4.3 Phylogeny and classification

One outcome of phylogenetic research is to provide a basis for establishing new classifications and for revising existing ones. The sectional classification in *Hypericum* (Robson 1977, 1981, 1987, 1990, 2001, 2010x) is based on the "recognition and correlation of morpho-geographical trends" (Robson 2010a) to identify sister-taxon relationships. It is part of a monograph of *Hypericum* that presents a remarkable resource for research on the genus (for a numerical list of all studies, see Carine & Christenhusz 2010). The classification in principle is an evolutionary one as it incorporates the concept of plesiospecies and character polarity (Robson 2006).

Of the 36 sections recognized in Robson's scheme (Table 3.1), ten are monotypic. The inclusion of multiple species for 20 of the remaining 26 sections allowed us to test their monophyly. The monophyly of nine sections (1, 5, 10, 12, 14, 16, 19, 20, 22) was supported by the analysis (Table 3.1). Of the remainder, five sections (9, 9b, 13, 17, 27, 29) were resolved as non-monophyletic but without support. The non-monophyly of section 30 is strongly supported but low levels of variation and support mean that the relationships of sections 30 and 29 remain unclear with the paraphyly of section 30 a possibility. Four sections (3, 7, 9d and 18) are strongly supported as polyphyletic.

The sectional classification developed by Robson (1977 onwards) works at a practical level and we believe that researchers will use it also in future. Nevertheless, a re-evaluation of the classification in light of these results and, in particular the status of polyphyletic sections identified in this analysis may be appropriate.

Whilst incongruence between the sectional circumscriptions of Robson and the results presented in this study is limited, relationships between sections differ markedly from those proposed by Robson (2003: fig. 1.16). This revised hypothesis of phylogenetic relationships between larger parts within the genus (*i. e.* the clades/grades described above) bears on the interpretation of biogeographical patterns and ancestral character state reconstructions. In the ITS tree presented in this study, a split between the New and the Old World species of *Hypericum* s. l. is evident that has not been hypothesized earlier. Within the Old World taxa, sections from Africa and Southeast Asia ("Ascyreia s. l." + *Campylosporus*) are placed as sister to a clade containing mainly species from the Palaearctic ("core Hypericum"). The sections belonging to our "Ascyreia s. l." + *Capylosporus* clade have been hypothesized to contain 'basal' species (Robson 1985). However, according to character state reconstructions based on ITS sequence phylogenetic inference, all of the "Ascyreia s. l." + *Campylosporus* species have character states that are apomorphic within the genus, *e. g.,* a 5-numerous gynoecium (Fig. 3.3).

The split of *Hypericum* s. l. into a New World and an Old World clade ("Brathys s. l." + *Myriandra* + *Triadenum vs.* clade **B**), as well as the position of species almost exclusively distributed in the Mediterranean on initial splits within the ITS tree has implications on the biogeography of *Hypericum* s. l. For the Old World taxa of the genus, the West Asian-pan Mediterranean region seems to be an important area of diversification and a center for dispersal (which might be referred to as "the Tethys hypothesis" as biogeographic scenario for Old World *Hypericum* s. l.). The New World taxa belonging to **A** seem to have a biogeographic history independent thereof. More detailed conclusions, however, demand model based biogeographic reconstructions that are placed in a historical background (Chapter 4).

Table 3.1 Classification of the genus *Hypericum* L. detailing sections (*sensu* Robson, 1977 onwards), results of Nürk & Blattner (2010; Chapter 2) and results found in this study: number of included species (and individuals [acces.] for ITS) and statistic support values of both, morphology and sequence based analyses (m = monophyletic, mt = monotypic, p = not monophyletic).

Classification		Morphology		ITS sequence data		Phylogenetic status
№	sections	included spec	support [BI | MP]	included spec./acces.	support [BI | ML | MP]	(both analyses)
1.	*Campylosporus*	10	0.58 | –	3/9	1 | 98 | 85	m
2.	*Psorophytum*	1		1/3	1 | 100 | 99	mt
3.	*Ascyreia*	43	–	29/58	–	p
4.	*Takasagoya*	5	–	1/1		p
5.	*Androsaemum*	4	0.99 | 51	5/12	1 | 90 | 87	m
6.	*Inodora*	1		1/3	1 | 100 | 100	mt
6a.	*Umbraculoides*	1		0		mt
7.	*Roscyna*	2	1 | 57	2/4	–	m
8.	*Bupleuroides*	1		1/1		mt
9.	*Hypericum*	42	–	23/50	–	p
9a.	*Concinna*	1		0		mt
9b.	*Graveolentia*	9	–	6/8	–	p
9c.	*Sampsonia*	2	1 | 57	1/2	1 | 100 | 100	m
9d.	*Elodeoida*	5	–	3/6	–	p
9e.	*Monanthema*	7	–	1/2	–	p
10.	*Olympia*	4	0.71 | –	2/9	1 | 96 | 96	m
11.	*Campylopus*	1		1/2	1 | 100 | 99	mt
12.	*Origanifolia*	13	1 | –	4/4	1 | 90 | 75	m
13.	*Drosocarpium*	11	0.69 | –	7/12	–	m?
14.	*Oligostema*	6	–	2/6	0.77 | 54 | 58	p?
15.	*Thasia*	1		0		mt
16.	*Crossophyllum*	3	0.99 | –	2/4	1 | 91 | 90	m
17.	*Hirtella*	30	–	7/10	–	p
18.	*Taeniocarpium*	28	–	10/15	–	p
19.	*Coridium*	6	1 | –	3/7	0.63 | 46 | –	m
20.	*Myriandra*	29	1 | 56	27/28	1 | 61 | 62	m
21.	*Webbia*	1		1/4	1 | 100 | 99	mt
22.	*Arthrophyllum*	5	–	3/4	1 | 100 | 100	m
23.	*Triadenioides*	5	–	1/3	1 | 100 | 100	p?
24.	*Heterophylla*	1		1/1		mt
25.	*Adenotrias*	3	1 | 96	1/4	1 | 100 | 100	m
26.	*Humifusoideum*	12	–	1/1	–	p?
27.	*Adenosepalum*	25	–	11/22	–	p
28.	*Elodes*	1		1/3	1 | 100 | 100	mt
29.	*Brathys*	87	–	16/28	–	p
30.	*Trigynobrathys*	52	–	11/15	–	p

A summary of the results of the cladistic analysis of morphological characters (Chapter 2) and ITS sequence analysis is given in Table 3.1. This can be used in future studies as a base to select the groups of interest, *i. e.* sections where species sampling is still too low to infer insights into their evolutionary history.

3.5 Conclusions

According to the results of the discussed ITS based phylogeny, 1) *Thornea* is sister to *Hypericum* s. l. and 2) the small genus *Triadenum* (6 species) is included within *Hypericum* s. l. 3) The position of the taxa of the basal grade, *Thornea*, *H.* sect. *Elodes* and *Adenotrias* (and *Triadenum*) on initial splits within Hypericeae is supported by the occurrence of three staminodes in these taxa, when assuming possession of such staminodes as a plesiomorphic character state for Hypericeae (five staminodes are present in Vismieae and three in Cratoxyleae). 4) Dark glands evolved probably several times in the Old World, first in reproductive parts only, later also in vegetative parts. 5) All *Hypericum* s. l. species occurring in the New World and not possessing dark glands are monophyletic. 6) *Hypericum* s. l. had a shrubby ancestor, and herbs evolved later in the genus. 7) The evolution of sclerophylous arborescent shrubs is related to shifts into tropical montane habitats. In the New World it has resulted in a burst of species in Andean páramos, a habitat that is not older than ~5 million years. 8) The crown clade "core Hypericum" is characterized by a perennial life style, and the occurrence of dark glands in vegetative parts of the plants.

4 Out of the tropics? Historical biogeography of the temperate genus *Hypericum*

This chapter is a manuscript in preparation for publication by Nicolai M. Nürk. Simon Uribe-Converse, David C. Tank, Santiago Madriñán and Frank R. Blattner will be coauthors.

4.1 Introduction

Since Darwin and Wallace it is known that "…species of the same genus have usually […] some similarity in habits and constitution…" (Darwin 1859, p 76). That is, variation in traits across species has non-random components, for example, traits are a legacy from their ancestors (Prinzing 2001), which is known as phylogenetic conservatism.

Systematics has a major influence on our understanding of biodiversity, as it provides knowledge about the relationship of lineages, and evidence for the interpretation of observed patterns. With the growing availability of dated phylogenies, it becomes feasible to access deeper insights into both general biogeographic and ecological models that explain biodiversity (Donoghue *et al.* 2001; Morley 2003; Wiens & Donoghue 2004; Crisp *et al.* 2009; Sanmartín *et al.* 2010). Knowledge about 'what limits the distribution of species' essentially bears on conservation biology as well as on speciation research (Wiens 2004).

One main problem for plants confronted with changing environments is the ecological shift that goes along with the transition from tropical to temperate climate conditions (Donoghue 2008). Adaptation to cold conditions might demand complex reorganizations of the genome (Sakai & Larcher 1987), implicating that it might take time to evolve tolerances to temperate climates with highly seasonal conditions (Fine & Ree 2006). That is, species also tend to retain ancestral ecological characteristics, *i. e.* descents diverge ecologically from their ancestors less than would be expected under an unconstrained evolution (Ricklefs & Latham 1992; Peterson *et al.* 1999), which has been termed 'niche conservatism' or 'phylogenetic niche conservatism' (PNC), if one emphasizes that related species have a tendency to occupy similar environments (Harvey & Pagel 1991; Wiens & Graham 2005; Donoghue 2008; Losos 2008).

Here, the question is not whether niches are precisely conserved or not, which is quite dependent on the level of relationship and/or demanded similarity (*e. g.,* Wiens & Graham 2005; Jakob *et al.* 2010). The question rather is about the implications of PNC in explaining phenomena like the latitudinal diversity gradient (Hildebrand & Jensen 1991; Mittelbach *et al.* 2007), and other general biodiversity patterns (Sanmartín *et al.* 2001; Sanmartín & Ronquist 2004; Hoorn *et al.* 2010; Antonelli & Sanmartín 2011).

Only few lineages of flowering plants have managed the transition from tropical to temperate climates (Judd *et al.* 1994), despite presumably having an ample of opportunities to do so with the expansion of temperate climates (Ricklefs & Renner 1994; Donoghue 2008). These findings suggest that it may be easier, under changing climates, for species to migrate into an area to which they are adapted (at least to a certain degree), than to evolve the relevant adaptations in place ("it's easier to move than to evolve"; Donoghue 2008, p 11551). Contrariwise, in the absence of plants with relevant adaptations in an area that undergoes environmental changes and that lacks migration routes, resident lineages will presumably evolve the relevant traits (Engler 1879; Baldwin & Sanderson 1998).

The effect of both mechanisms on evolution may cause reticulating biogeographic patterns, which result from different events at different time periods (Donoghue & Moore 2003). Thus, revealing insights into biogeographic history of biological organisms demands implication of dated phylogenetical, ecological and palaeogeological evidence (Ricklefs & Renner 1994; Manos & Donoghue 2001; Smith & Donoghue 2010; Crisp *et al.* 2011; Wertheim & Sanderson 2011).

St. John's wort (*Hypericum* L., Hypericaceae) is a medically useful genus distributed worldwide with a main center of species richness in the temperate regions of the Northern Hemisphere (*e. g.,* Stevens 2007). In cold temperate climates *Hypericum* is native mainly to lowland and upland areas, while in the tropic and warm temperates it is almost always confined to high elevation mountain habitats (*e. g.,* the Andes or East African mountain ranges). Beside the enormous morphological variation visible in the genus, its members share characteristic traits, like *e. g.,* yellow petals with many stamens in fascicles. *Hypericum* is native to habitats that range from dry rocky places, *e. g.,* in the Mediterranean, to moist woodland-meadow borders, *e. g.,* in Central Europe, to grasslands, *e. g.,* in the Páramos of South America, or fens and swamps in, *e. g.,* North America.

Robson (1977; 1993) assumed that the equatorial species/sections of *Hypericum* were of early descent within the genus and, therefore, hypothesized a Gondwanan origin for *Hypericum* (specifically: Central Africa). As closely related genera (*Thornea*, *Vismia*, *Harungana*, *Cratoxylum* and *Eliea*) are pantropical flora elements, this assumption seemed plausible. The time frame for the

break-up of South Gondwana (*i. e.* South America and Africa) more than 105 million years (Ma) ago (McLoughlin 2001), however, contradicts this vicariance hypothesis for *Hypericum*, as the family Hypericaceae has been estimated to be around 60–70 Ma old (Davis *et al.* 2005). Thus, Robson's biogeographic 'out of Africa' scenario would imply several long distance dispersal events at an early stage during the evolution of the genus.

Recent phylogenetic studies of *Hypericum* and representatives of other genera of the Hypericaceae render the most current taxonomic description of the genus (Robson 1977 onwards) as too restricted, suggesting to merge the genera *Santomasia* (Chapter 2; Ruhfel *et al.* 2011), as well as *Triadenum* and *Lianthus* (Chapter 3; Ruhfel *et al.* 2011), and even *Thornea* (Ruhfel *et al.* 2011) into *Hypericum*, all of them belong to the Hypericeae. On the other hand, the phylogeny of Cratoxyleae + Vismieae + Hypericeae within the family Hypericaceae has been revealed as congruent to classification (Stevens 2007) in morphological (Chapter 2), and molecular studies (Chapter 3; Gustafsson *et al.* 2002; Wurdack & Davis 2009; Ruhfel *et al.* 2011).

More than 80 % of the species within the Hypericaceae belong to the temperate *Hypericum* s. l. group. The remaining 20 % consist of tropical plants, classified into Vismieae and Cratoxyleae. Thus, the group offers attractive opportunities to investigate PNC by means of historical biogeography and diversification rate analysis. Apomorphic key novelties that would explain the species richness of *Hypericum* could not convincingly be identified (Chapter 3). Although growth form and secondary metabolites might play a role in evolution of certain clades within *Hypericum*, investigating divergence times of lineages, diversification rates, and biogeography can offer further insights into the evolutionary history of the group (Donoghue & Moore 2003), *i. e.* can reveal possible processes and mechanisms underlying the divergence of the temperate *Hypericum* s. l. lineage from its tropical relatives.

Here, we give the results of analyses of historical biogeography and diversification rates based on nrDNA sequence analysis of a representative subsample of the dataset that will be published in Nürk *et al.* (submitted; Chapter 3). We ask for the scenario of area colonization explaining the distribution of *Hypericum* species (i) in temperate and tropical regions, (ii) between Northern and Southern Hemisphere, (iii) between the New and the Old World, and (iv) within Eurasia and America. We use a recently developed maximum likelihood method (Ree *et al.* 2005; Ree & Smith 2008) and place the ancestral area reconstructions in a paleogeological background to distinguish events of long-distance dispersal (LDD) from events of vicariance, which might rather be caused by plate tectonics and climate changes (*e. g.,* Bartish *et al.* 2011). We analyze diversification rate shifts between lineages to identify time points in the evolutionary history of the group that might have had a major influence of the species richness of *Hypericum* nowadays. In general, we ask for reasons, which explain the species richness of temperate *Hypericum* compared to its tropical relatives, and if PNC can contribute to the answers.

4.2 Methods

All analyses were conducted on the nrDNA ITS sequence dataset of Nürk *et al.* (submitted, Chapter 3) that was reduced to 173 accessions representing the clades described in the original publication as well as their geographic ranges (Table A4.1).

4.2.1 Divergence time estimations

We choose the Bayesian tree (Chapter 3) to test for rate constancy among lineages. The likelihood score associated with branch length were calculated on this tree in PAUP* (Swofford 2002) under the optimal model of sequence evolution and associated parameters with and without a strict molecular clock enforced. We followed the approach described in Huelsenbeck & Rannala (1997) to assess significance. A global molecular clock was rejected ($P< 0.05$) for the nrDNA ITS sequence data. Therefore, divergence times were estimated under a relaxed molecular clock employing the uncorrelated lognormal (UCLN) model (Drummond *et al.* 2006) that assumes branch specific substitution rates to be drawn from a single lognormal distribution estimated from the data. Implementation of the UCLN model in BEAST v1.5.4 (Drummond & Rambaut 2007) together with the use of Markov chain Monte Carlo (MCMC) sampling methods takes into account uncertainty and inconsistency in both the topology and substitution rates.

Due to the lack of fossils clearly assigned to a nearest living relative or to a certain clade within the clusioid taxa (Reid 1923; Friis 1985; Crepet & Nixon 1998; Zhao *et al.* 2004; Velichkevich & Zastawniak 2007; Graham 2010), we used age estimations as reported by Davis *et al.* (2005) for the stem node of the family Hypericaceae ([66–] 72–76 [–82]) as secondary calibration point, and the time frame of the Andean uplift (reviewed in: Gregory-Wodzicki 2000) to calibrate an internal node containing species only native to the páramos of South America as biogeological calibration point (~2–7 Ma). Both calibrations were constrained with a lognormal distribution, that had for the root of the tree an offset of 50 million years (Ma), a mean of 2.4, and a standard deviation of 0.5, and for the Andean clade an offset of 1.7 Ma, a mean of 0.2, and a standard deviation of 0.6; incorporating uncertainty in the calibration of the nodes (Ho & Phillips 2009).

To ensure convergence in divergence times, analyses were performed in five independent runs in Beast, each consisted of 10^7 generations sampling a tree every 1 000 generation. The GTR model of nucleotide substitution was applied with the Γ model of site heterogeneity and the tree priors were kept as default under the birth-death process of speciation. Each run started from the tree obtained by a maximum likelihood search in RAxML (Stamatakis 2006), after performing a semi-parametric method based on penalized likelihood (Sanderson 2002) in R (R Development Core Team 2011) with the chronopl command as implemented in the package APE (Paradis *et al.* 2004).

Convergence of the parameters were monitored using Tracer (Rambaut & Drummond 2007) and the resulting trees (represented the maximum clade credibility trees and had 95 % of the highest posterior density [HPD]) of the five runs were combined in LogCombiner with a burnin of 25 %.

Means and confidence intervals were calculated in TreeAnnotator (Drummond & Rambaut 2007) to obtain a final consensus tree for visualization in FigTree v1.3.1 (Rambaut 2006–2009).

4.2.2 Diversification rates

Diversification rate shifts were analyzed in MEDUSA (modeling stepwise diversification using stepwise AIC [Akaike Information Criterion; Akaike 1974]; Alfaro *et al.* 2009; Santini *et al.* 2009) on 1 000 trees randomly chosen (using the program Phyutility; Smith & Dunn 2008) from the posterior distribution of dated trees generated by the BEAST analyses to avoid conditioning results on any particular tree topology and branch length (Smith 2009; Smith & Donoghue 2010).

MEDUSA, as implemented in the R package GEIGER (Harmon *et al.* 2009), is an extension of the approach described by Rabosky *et al.* (2007) which uses phylogenetic (topology and branch length) and taxonomic (species richness) information to estimate birth and death rates for an incompletely resolved phylogenetic tree. Additionally, MEDUSA uses a stepwise procedure to look for shifts in diversification rates comparing AIC scores of increasingly complex birth-death models until there is no substantial improve of the AIC scores, *i. e.* $\Delta AIC \geq 4$ (Burnham & Anderson 2002). The detected shifts are then confirmed by a backward elimination process, whereat individual shifts are removed and the model reevaluated. In other words, MEDUSA takes a phylogeny, in which the tips of the phylogeny may represent stems of unresolved clades, and a list of taxonomic richness (species numbers) for each tip clade and fits a series of birth-death models to each branch in the tree to detect diversification rate shifts.

To conduct our analysis, we obtained species richness for twelve clades representing all genera and major clades in our study (Fig. 4.1, Appendix S4.1) as reported in the monograph of *Hypericum* by Robson (1977–2010; cited in Nürk & Blattner 2010) and for the outgroups as reported by Stevens (2007). We pruned our 1 000 dated trees in a way that the remaining tips represent the twelve clades. The resulting rates found in the analysis were summarized and tested for significance using Fisher's Sign test ($p < 0.001$) in the R package BSDA (Alan 2010).

4.2.3 Biogeographic analyses

The biogeographic history of *Hypericum* and allied genera was reconstructed employing an improved version of the software package Lagrange (likelihood analysis of geographic range evolution; Ree & Smith 2008). And over the same posterior distribution of 1 000 trees used for the diversification rate analysis (see above). Lagrange implements a model for geographic range evolution by dispersal, extinction and cladogenesis (the DEC model; Ree *et al.* 2005) to estimate ancestral ranges (areas) and biogeographic parameters (dispersal and extinction rates) based on current geographic distribution of species by maximum likelihood. The DEC model defines a matrix of

probabilities of lineage dispersal between areas and extinction within an area based on the assumption that only a single dispersal/extinction event can occur in an instant of time (Ree & Sanmartín 2009).

We considered species to be distributed within seven broad areas: Africa (A), the Mediterranean (M), western Palaearctic (EU), eastern Palaearctic (EA), Indo-Pacific (*i. e.* Asia tropical + Australasia + Pacific; IP), North America (NA), and South America (SA) following in general Brummit *et al.* (2001) for area subdivision (Fig. 4.1). We assigned species nowadays distributed in the Mediterranean to a separate area (M) to be able to distinguish ancestral distribution patterns not only between Europe and temperate Asia, but also between West and East temperate Asia and North and South Europe, respectively. As the advent of the Mediterranean (summer dry) climate was around (2–) 3–5 Ma ago (Thompson 2005; Donoghue 2008), one should keep in mind, that the high diversity of these floras results mainly from its heterogeneous origin, notably by immigration from the ancestral floras of the tropical and temperate regions (Thompson 2005). We assume similar patterns being mainly responsible for the species richness of *Hypericum* in this area, *i. e.* that species with adaptive traits characteristic for the Mediterranean climate moved in from outside the region, perhaps from dry areas further to the east.

To take the impact of dispersal probabilities between areas into account necessary to run Lagrange, we designed three models differing in their dispersal/extinction probability matrix (Fig. 4.1). Model I is the most complex. It incorporates varying dispersal probabilities over time and is based on previous geographic knowledge on connectivity of the areas, *e. g.,* the North Atlantic land bridge (NALB) and the Beringian land bridge (BLB) as summarized by Tiffney and Manchester (2001). Model II is similar to model I by incorporating different dispersal probabilities between the areas, but differs from the latter by being constant over time (*i. e.* only one dispersal probability matrix defines different dispersal probabilities between different areas over the entire time period). The most conservative is model III being completely unconstrained, *i. e.* assuming equal dispersal probabilities between areas over the entire time period. The results of the three analyses (each with one of the three dispersal probability models) were summarized in R (R Development Core Team 2011) by histograms showing the frequency of ancestral areas, which were reconstructed for a certain node over a posterior distribution of 1 000 trees.

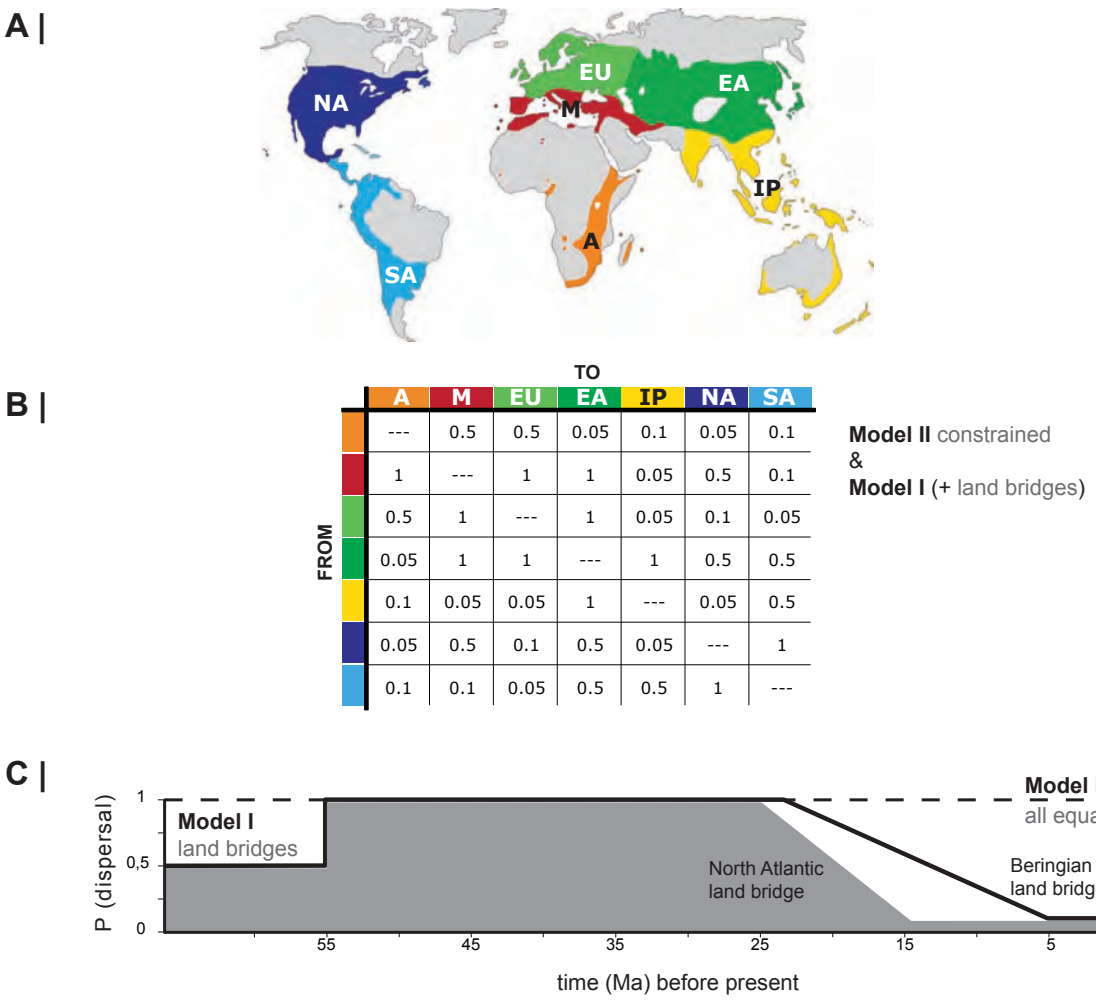


Fig. 4.1 Biogeographical models used by the dispersal-extinction-cladogenesis (DEC) model for the parametric reconstruction of ancestral areas. **A**) Distribution of *Hypericum* s.l. (colored areas) and biogeographical regions (different colors) for the three models (I–III) discussed in the study (Africa [A], the Mediterranean [M], western Palaearctic [EU], eastern Palaearctic [EA], Indo-Pacific [*i. e.* Asia tropical + Australasia + Pacific; IP], North America [NA], and South America [SA]). **B**) Constraints-matrix defining dispersal/extinction probabilities (*P*) across areas (in Model I and II) by specifying the probability that a given lineage will disperse from one area into another by conditioning the intrinsic rates of lineage dispersal and extinction. **C**) Illustration of varying dispersal probabilities (*P*) over time to incorporate land bridges (in model I) by modifying the probability matrix. The probability of dispersal success across each connection is plotted through time and is symmetrical with respect to direction. The dashed line illustrates the maximal dispersal probability ($P = 1$) across areas, as used in the 'unconstrained' model III.

4.3 Results & discussion

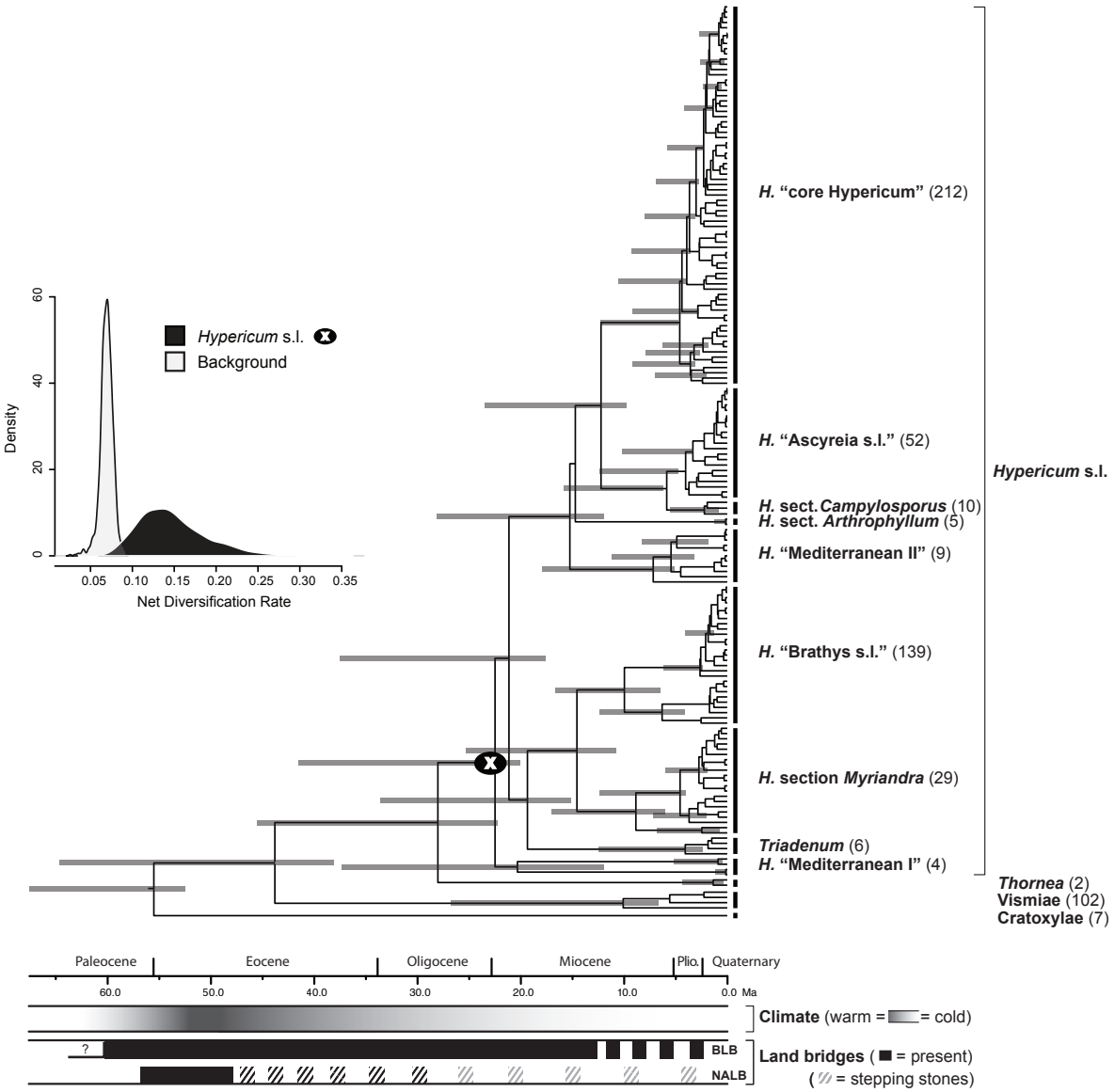
4.3.1 Age estimations

Our divergence estimates indicate that the temperate *Hypericum* s.l. lineage (*i.e. Hypericum* including *Triadenum*) obviously originated after the Eocene thermal maximum (52 Ma; Tiffney & Manchester 2001) within a 20-million year time window at a mean age of 22 Ma (20–41 Ma, 95 % HPD; Fig 4.2, Table 4.1). Shortly before, around the Oligocene, the two-species genus *Thornea* from Central America diverged from this lineage with a mean estimation for this split of 28 Ma (22–45) ago. Thus, origin of the 'cold' adapted *Hypericum* lineage correlates with the cooling of the world climate during the Late Eocene-Oligocene-Early Miocene (Zachos *et al.* 2001; Zachos *et al.* 2008).

Within *Hypericum* s.l. the divergence between a New World lineage with *Triadenum*, *Myriandra*, and "Brathys s.l.", and an Old World lineage consisting of the remaining clades ("Mediterranean II", *Arthrophyllum*, "Ascyreia s.l." + *Campylosporus* and "core Hypericum"; Fig. 4.2) was estimated to have occurred in the Oligocene to Miocene 21.13 Ma (11.98–37.54) ago, with subsequent divergence of the New World lineages at 19.34 Ma (15.13–33.57) ago and of the Old World lineages at 15.23 Ma (11.92–28.07) ago, respectively (Table 4.1). The divergence time estimation for the Old World lineage correlates with the fossil record, as the oldest seed fossils clearly belonging to the genus *Hypericum* are reported from Middle Miocene of Jutland, Denmark (Friis 1985), and from Yunnan in southwestern China (Zhao *et al.* 2004). These estimations are much too young to explain the current distribution of *Hypericum* by vicariance resulting from plate tectonics during the Gondwanan break-up sequence, which is congruent to findings by other studies (see introduction).

Oceanic barriers separating the New and the Old World impede plant dispersal since the break-up of South Gondwana (in the Cretaceous, at least more than 105 Ma ago; McLoughlin 2001), and the opening of the North Atlantic Ocean (Upper Cretaceous, *c.* 90 Ma ago; reviewed in: Sanmartín *et al.* 2001). On the other hand, physical connections in the Northern Hemisphere were present during the Tertiary (Paleocene to Neocene), particular the North Atlantic land bridge(s) (NALB) and the Beringian land bridge (BLB), which have facilitated biotic exchange between the continents (Tiffney 1985a, b; Tiffney & Manchester 2001; Donoghue 2008). On both connections, however, prevailing climatic and floristic conditions changed considerably over time, enabling different flora elements at different times to migrate (Sanmartín *et al.* 2001).

During the estimated time frame for the divergence of the temperate *Hypericum* lineage from its tropical relative 20–40 Ma ago (Mid Eocene to Early Miocene), the NALB, especially the De Geer Bridge, a trans-Atlantic connection relatively far in the North, allowed cold-adapted organisms to migrate between North America and Europe until the Greenland Sea broke this route (*c.* 39 Ma ago; Tiffney 1985b). Even later, until the Miocene, islands in the North Atlantic likely functioning as 'stepping stones' (the Greenland-Faeroes Bridge) could have facilitated biotic dispersal



(Tiffney 1985b), although this connection is not considered to have been an important migration

Fig. 4.2 Maximum clade credibility tree of *Hypericum* s.l. and outgroups produced from Bayesian divergence time analysis, and density plot produced from diversification rates analysis (above). The X marks the crown height estimate for the most recent common ancestor of *Hypericum* s.l. and corresponds to a significant shift in diversification rates at this node, resulting in two times increased speciation (net diversification) rates within this clade compared to the background. Below the tree the time scale and the corresponding paleontological events are depicted (i) the climate cooling after the Eocene thermal maximum (later, smaller fluctuations not considered) and (ii) Land bridges connecting the continents of the Northern Hemisphere (BLB = Beringian land bridge, NALB = North Atlantic land bridge; adopted from Tiffney & Manchaster 2001).

route (Sanmartín *et al.* 2001; but see also Denk *et al.* 2010). The opening of the oldest and probably most important dispersal route, the Thulean Bridge, that connected southern Europe to Greenland via the British Isles until *c.* 50 Ma ago (Tiffney 1985b), predates with at least 5 Ma the node age of *Hypericum* s.l. + *Thornea* (45 Ma, upper 95 % HPD, node 3; Table 4.1).

4.3.2 Ancestral area reconstructions: model comparison

Independent of the dispersal model given to the analysis, the reconstructions of the ancestral area for the stem node of *Hypericum* s.l. excluding "Mediterranean I" (node 5, Table 4.1), suggest a distribution of the populations between North America and southern Europe (or which is today the Mediterranean region; Fig. 4.3). One divergence event before, at the stem node of *Hypericum* s.l. (node 4, Table 4.1), ancestral area reconstructions differ between the models. While model I (counting for land bridges in the dispersal constraints) suggests a distribution of the ancestors of *Hypericum* s.l. already between North America and southern Europe, model II (same constrained dispersal probabilities over the entire period) and model III (unconstrained, *i. e.* dispersal is equally probable between all areas) suggest an occurrence of *Hypericum* s.l. ancestors restricted to which is today the Mediterranean region (Fig. 4.3). The reconstruction of both latter models, however, seems less plausible, as the restriction at node 4 to the Old World implies dispersal to North America 20–41 Ma ago (node 5), after a dispersal event from Central America into the Old World 22–45 Ma ago (node 3, reconstructed under model I & III, Table 4.1). Thus, a distribution of the ancestors of *Hypericum* s.l. around the western part of the Tethys Ocean and North America, is suggested as the origin for diversification of this temperate lineage of the Hypericaceae, independent of the model used.

In comparison, differences between ancestral areas reconstructed among the three models are almost absent towards higher nodes (node 5–17). Towards the deeper nodes (node 1–4), however, reconstructions differ considerably (Fig. 4.3; Table 4.1), a phenomenon resulting from the parametric approach of the DEC model (Ree *et al.* 2005) that has been reported also from other studies (*e. g.,* Buerki *et al.* 2011). Therefore, the biogeographic scenario for the most basal nodes in the tree (Fig. 4.3) that represent the phylogeny of Hypericaceae (*i. e. Cratoxylum*, *Vismia* + *Harungana*, *Thornea* + *Hypericum* s.l.) cannot be inferred with confidence. Studies that incorporate more distantly related taxa (*i. e.* dense sampling of representatives of the clusioid clade; see Ruhfel *et al.* 2011) are needed to reveal insights into the biogeographic history of all genera belonging to the Hypericaceae. For the purpose of this study, however, it is adequate to recognize that the taxa belonging to the Cratoxyleae and Vismieae share pantropical distribution, which is expressed in the ancestral area reconstructed for the root of the tree (node 1) that suggests a Southeast Asian to South American occurrence of the ancestors of Hypericaceae.

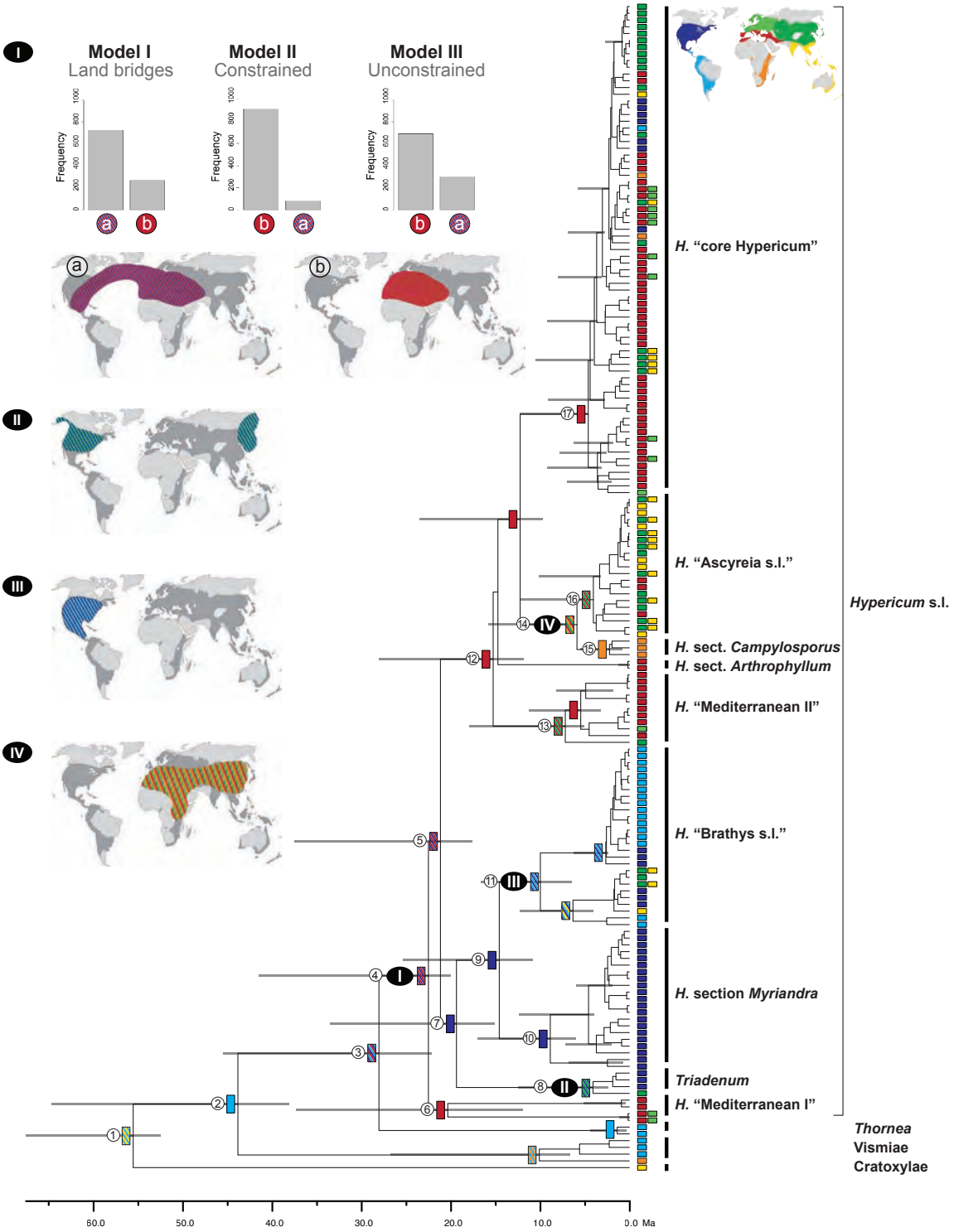


Fig. 4.3 Biogeographical optimization produced from a posterior probability of 1 000 trees using maximum likelihood (ML) analysis and the DEC model. Colored squares depict recent areas (right to tips) and ancestral areas (at stem nodes) as reconstructed under model I (Fig. 4.1). Numbers 1–17 mark nodes as referred to in the text and Table 4.1. Numbers I–IV mark nodes for which the potential ancestral population is illustrated (to the left). For Node I only, frequencies of reconstructions are given for the three models in comparison, and the two potential ancestral populations are illustrated in (a) and (b).

4.3.3 Historical biogeography

The biogeographic scenario for the temperate lineage of the Hypericaceae might be inferred as illustrated in Figure 4.4, assuming for *Hypericum* s.l. an origin of diversification around the Mid Eocene to Early Miocene (20–40 Ma ago) in an area ranging from North America to the western part of the Tethys Ocean. Ancestors of *Hypericum* s.l. + *Thornea* (probably native to southern North America during the Eocene) diversified into a *Thornea* and a temperate *Hypericum* s.l. lineage. The latter evolved into (i) a New World population probably native to (southeastern) North America, and (ii) an Old World population native around the western part of the Tethys Ocean, probably at the outset connected via the NALB (Thulean or rather De Geer Bridge). Subsequent to the opening of the Greenland Sea *c.* 39 Ma ago, both ancestral New World and Old World populations got separated and diversified (i) in North America (*H.* sect. *Myriandra*, *c.* 6–17 Ma ago, and "Brathys s.l.", *c.* 6–16 Ma ago), into South America (within the "Brathys s.l." clade probably two times independently), and via the BLB into East Asia (within *Triadenum c.* 2–12 Ma ago, and some "Brathys s.l." representatives even later).

In (ii) the Old World, diversification and dispersal patterns are more complex, suggesting rather recent migration from the Tethys region into Africa at least two times independently. Once for *H.* sect. *Campylosporus c.* 2–5 Ma ago, and once within "core Hypericum" in *H.* sect. *Adenosepalum*. And diversification of at least three lineages in Asia, one "Ascyreia s.l." lineage that also dispersed into southern Asia *c.* 5–12 Ma ago, and probably several lineages within "core Hypericum" that dispersed into temperate Asia, *i.e.* species belonging to *H.* sect. *Hypericum*, *Elodea*, and *Monanthemum*. Furthermore, one or two dispersal events into North America within "core Hypericum" species belonging to *H.* sect. *Hypericum*, *Concinna* and *Graveolentia*, either via migration through Beringia or long-distance dispersal across the Atlantic Ocean (Fig. 4.4).

The importance of long-distance dispersal in colonization of oceanic islands has been recognized since Darwin (1859). Recent studies indicate that long-distance dispersal has played a significant role in shaping modern distributions of plants (Renner 2004; Blattner 2006; Bartish *et al.* 2011; Emadzade *et al.* 2011). Robson (1981) mentions the tiny seeds of *Hypericum* (0.3–1.5 mm long) and their sculptured testa, and conclude that they might easily be attached to dispersal vectors as migrating birds (Robson 1981). We suggest long-distance dispersal likely explaining migration into South America in one of the two inferred colonization events (Figs. 4.3, 4.4). As relatively few samples from South America are included in our data set (*c.* 18 % of the species richness described for this continent; *cf.* Robson 1987, 1990), denser sampling of species is needed to infer more detailed hypotheses about biogeographic patterns within this group.

Especially the migration into Africa might include long-distance dispersal. Five species native to Central Africa and Madagascar (of which non is included in our data set) are classified into a group with species native mainly to South America (*H.* sect. *Trigynobrathys*; Robson 1990). The five African species of this group had been found to be nested within South American species in an analysis of morphological data that included almost all species described in *Hypericum* (Chapter 2). If the nested position of the five African species is confirmed by further studies, long distance dispersal by birds or even wind drift during (at least) the last 16 Ma is likely to explain this transoceanic distribution (Fig. 4.4, dashed grey arrows). A further migration into South Africa

(three species classified into a group mainly native to New Guinea, *H.* sect. *Humifusoideum*; Robson 1996) that might also be explained by long-distance dispersal (Robson 1993; Fig. 4.4) cannot be concluded without knowledge about their phylogenetic relationship. Further studies with a representative sampling of these African species and there relatives might clarify whether (i) migration via the East African mountain ranges (hypothesized for species belonging to *H.* sect. *Campylosporus* and *H.* sect. *Adenosepalum* which is placed by ITS sequence phylogenetic inference within "core Hypericum"; Figs. 4.3, 4.4) or (ii) long-distance dispersal as mentioned above (for species belonging to *H.* sect. *Trigynobrathys* and *Humifusoideum*) is the more probable explanation.

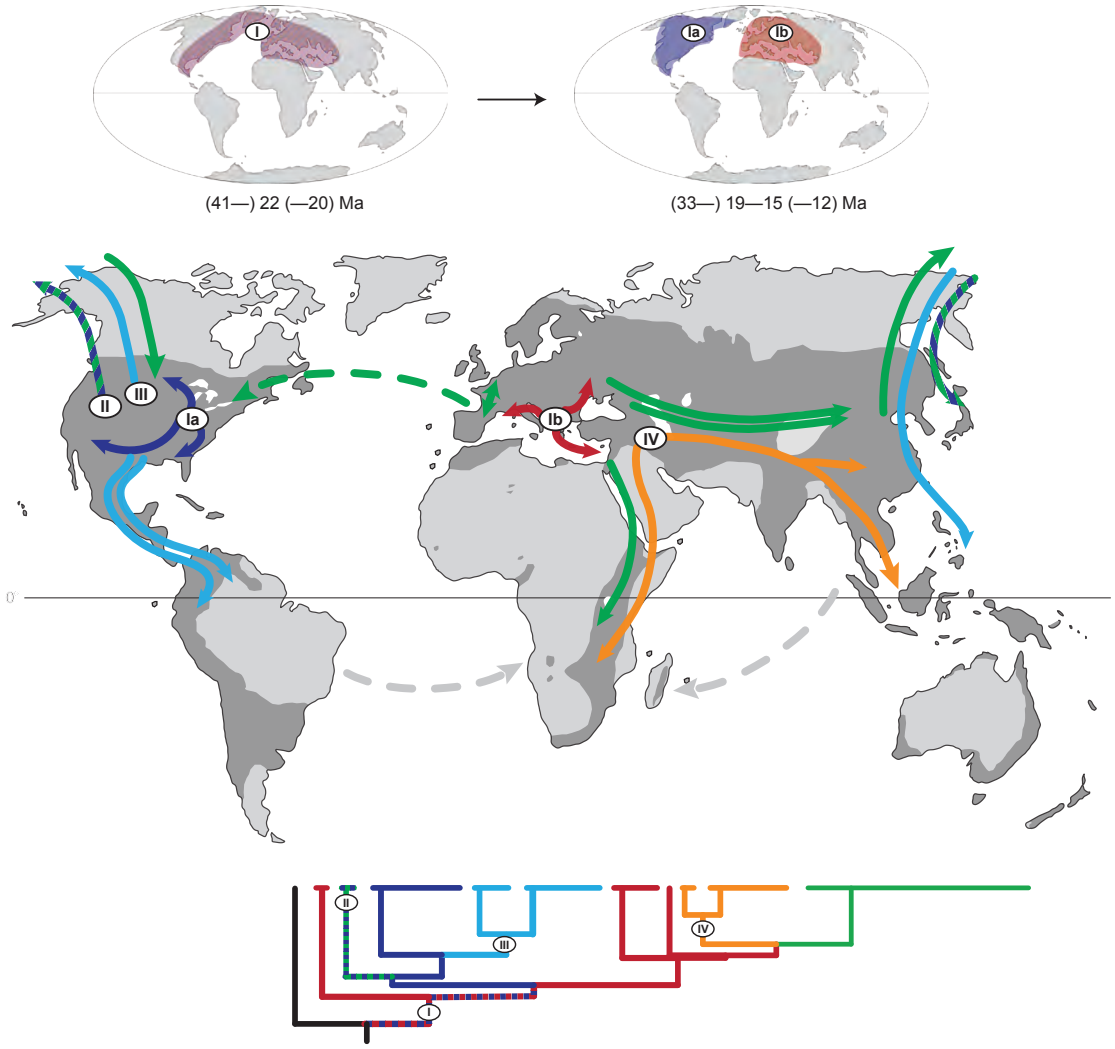


Fig. 4.4 Scenario of the historical biogeography of *Hypericum* s. l. The colors in the tree depict major clades. In the tree taxa of Hypericeae are only shown, with *Thornea* as outgroup. Arrows on the map indicate the hypothesized migration routs (solid lines) or dispersal events (dashed lines) of the clades. Numbers I–IV corresponds to nodes in the tree (Fig. 4.3). The ancestral area reconstructed for the stem node of *Hypericum* s. l. (I) suggests an ancestral population distributed across North America and the western Tethys region, with a subsequent split into a New World (Ia) and an Old World (Ib) population. The two grey-dashed lines depict long-distance dispersal events predicted by Nürk and Blattner (2010; Chapter 2) and by the classification of Robson (1977 onwards) for species not included in our data set.

A similar migration scenario as described for *Hypericum* s.l. via the NALB has been found for *e.g.,* Malpighiaceae (Davis *et al.* 2002). Malpighiaceae, however, are tropical flora elements, and divergence time estimations for the migration of ancestors of Malpighiaceae via the NALB certainly predates the NALB migration of the temperate *Hypericum* s.l. lineage of about 20 Ma. During the Eocene a boreotropical flora was native to the Thulean Bridge that became extinct in these northern latitudes during the cooling of the world climate after the thermal maximum early in this period (Donoghue & Smith 2004; Donoghue 2008). The expansion of temperate climates around the Northern Hemisphere, however, favored dispersal of temperate adapted lineages, and *Hypericum* s.l. could have survived colder conditions prevailing at the NALB (De Geer Bridge) during the Late Eocene or later.

Denk and colleagues (2010) hypothesize migration of oaks via the NALB even later as here suggested for *Hypericum* s.l. Based on pollen records of *Quercus* from Iceland, they conclude that dispersal and establishment of populations could have taken place twice, 9 and 5 Ma ago in the Late Miocene. Their finding that the NALB have been a corridor for plant migration during the Miocene is supported by fossil records from Iceland, which suggests a warm temperate to temperate flora until 9 Ma ago, followed by cool temperate forests until the early Middle Miocene, *c.* 3.6 Ma ago (Denk *et al.* 2010, and citations within). Possible gene flow across the North Atlantic via Iceland in the Late Miocene, was also indicated by a molecular phylogenetic study of *Rhododendron* subsection *Ponticum*, suggesting divergence time between Eurasian and North American members of *c.* 5 Ma (Milne 2004). Thus, the biogeographic scenario for *Hypericum* s.l. presented here provides further support for a functioning NALB in the Middle to Late Cenozoic, and that that the NALB was not open for plant dispersal only until the Early Eocene, as it was inferred for terrestrial animal migrations (McKenna 1983a, b).

The question whether some LDD events maybe via stepping stones of the NALB or vicariance caused the distribution range from the New World to the Old World cannot be answered with certainty. Beside this, vicariance is the more parsimonious explanation, taking into account the position of "Mediterranean I" at the initial split of *Hypericum* s.l. (node 4) before the divergence of the two large New and Old world lineages (node 5) occurred. This position of "Mediterranean I" as sister to remaining *Hypericum* s.l. (Nürk *et al.* submitted; Fig 4.3), however, needs phylogenetic validation based on further data from, *e.g.,* the plastid genome. Preliminary data from the *pet*D region of the chloroplast genome (Borsch & Nürk *et al.*, unpubl. data) hints towards the position of "Mediterranean I" as sister to the Old World lineage, and Ruhfel *et al.* (2011) revealed these species to be within our New World clade. The first topology described will not bear on the biogeographic scenario of *Hypericum* s.l., as the reconstructed distribution area between North America and the western part of the Tethys region will stay the same (*i. e.* it will be reconstructed just for less nodes, *cf.* Fig. 4.3). The latter topology described will certainly bear much more on ancestral area reconstruction, and might demand a revised biogeographic scenario.

4.3.4 Diversification rate shifts

Only one significant shift in diversification rates could be detected given the topology and the species numbers of the twelve well-supported clades of the Hypericaceae as presented in Figure 4.1. For the temperate *Hypericum* s. l. lineage (node 5) a significant shift in net diversification (speciation) rates was inferred to be two times higher compared to the background ($p<0.005$). Extinction rates within *Hypericum* s. l. are even faster. Compared to the background around 20 times higher. Such increased diversification rates prove much higher turnover within *Hypericum* s. l. compared to its tropical relatives. This is reflected in the species richness of this temperate lineage, in which 80 % of the described species of the family belong to. Increased evolutionary turnover is a well-known phenomenon in temperate floras triggered by seasonal and long term climate fluctuations in comparison to long-term stable tropical habitats (*e. g.,* Fine & Ree 2006; Field *et al.* 2009).

4.4 Conclusions

Hypericum s. l. has adapted to colder conditions, which is evident in its current habitat preferences. With the ecological shift from tropical to temperate conditions within ancestors of *Hypericum* s. l., a significant increase in diversification rates is correlated. Moreover, the divergence of this temperate lineage, which is estimated to have occurred around the Mid Eocene (*c.* 40 Ma ago) to Early Miocene (*c.* 20 Ma ago), correlates with the enlargement of temperate habitats in the Northern Hemisphere.

With the cooling of the world climate during the Tertiary, temperate habitats expanded around the globe, and *Hypericum* s. l. dispersed and diversified within these enlarging habitats. The decline of tropical habitats during the Tertiary certainly restricted the potential habitats of the tropical lineages of the family (*Cratoxylum*, *Eliea*, *Vismia*, and *Harungana*). Thus, the high turnover in diversification rates in this lineage seems to be triggered by climate fluctuations and expanded habitats in the Northern Hemisphere, likely explaining the high diversity within *Hypericum* s. l.

While in all tropical lineages of the Hypericaceae ecological preferences stayed the same, *i. e.* strong phylogenetic nice conservatism (PNC) can be inferred, an ecological shift occurred during evolution of the ancestors of *Hypericum* s. l., allowing these species to establish in habitats with temperate conditions. In the Old World, probably relatively shortly after the evolution of cold tolerance (Table 4.1) *Hypericum* s. l. diversified in the temperate habitats enlarging around the Tethys Ocean in the Miocene, and subsequently migrated into Africa and Asia. The species never really left habitats with temperate climate conditions. Similar patterns were inferred for the New World lineage, where species diversified in North America, as well as in South America during or after the uplift of the Andean mountain range. Thus, again PNC is obvious within the *Hypericum* s. l. lineage of the Hypericaceae, but subsequent to an ecological shift from tropical to temperate conditions.

This effect might not be exclusively explaining species richness found in *Hypericum* s. l. Physiological novelties, like *e. g.,* hypericin synthesis involved in plant protection against herbivores (Sirvent *et al.* 2003), or morphological key innovations, might also contribute to the species richness of *Hypericum*. However, we hypothesize that a high turnover in evolution of *Hypericum* might be triggered by temperate climate conditions prevailing in the Northern Hemisphere, and that the adaptation to freezing conditions is a physiological key innovation contributing to the evolutionary success of *Hypericum*.

Table 4.1 Summary statistics of the ancestral area reconstruction using maximum likelihood (ML), the DEC model, and molecular dating analyses for key nodes in Hypericaceae and *Hypericum* s. l. phylogeny (A = Africa, M = Mediterranean, EU = Europe, EA = East Asia, IP = Indo-Pacific, NA = North America, SA = South America).

			Ancestral area reconstructions [%]						**Age estimations** [Ma]		
			Model I land bridges considered		Model II constrained dispersal		Model III not constrained (*i.e.* all equal)				
№	**Clade**	**pp**	1.	2.	1.	2.	1.	2.	Mean	Lower	Upper
1	Hypericaeae (root)	–	PA-G: 97	M-PA-G: 2	M-PA: 76	PA-G: 23	PA-G: 98	M-PA-G: 1	55.51	52.48	67.53
2	Hypericaeae excl. *Cratoxylum*	1.00	G: 94	all: 3	M: 73	G: 25	G: 99	M-G: 0.5	43.79	38.09	67.53
3	*Hypericum* s.l.+*Thornea*	1.00	M-NA-G: 83	M-G: 8	M: 71	M-G: 21	M-G: 75	M-NA-G: 23	28.01	22.20	45.51
4	*Hypericum* s.l.	1.00	M-NA: 73	M: 27	M: 91	M-NA: 8	M: 69	M-NA: 30	22.47	20.07	41.54
5	*Hypericum* s.l. excl. "Mediterranean I"	1.00	M-NA: 97	M: 3	M-NA: 84	M: 15	M-NA: 91	M: 8	21.13	17.63	37.54
6	"Mediterranean I"	–	M: 96	M-NA: 4	M: 100	–	–	M: 100	20.31	11.98	37.34
7	*Triadenum*+*Myriandra*+ "Brathys s.l."	1.00	NA: 100	–	NA: 99	M-NA: 0.5	NA: 100	–	19.34	15.13	33.57
8	*Triadenum*	1.00	EA-NA: 100	EA-NA: 100	EA-NA: 100	–	–	–	04.07	02.37	12.48
9	*Myriandra*+"Brathys s.l."	1.00	NA: 100	–	NA: 100	–	NA: 100	–	14.55	10.81	25.30
10	*Myriandra*	1.00	NA: 100	–	NA: 100	–	NA: 100	–	08.84	06.02	17.03
11	"Brathys s.l."	1.00	NA-G: 77	NA: 21	NA: 73	NA-G: 27	NA: 56	NA-G: 43	09.95	06.47	16.66
12	"Mediterranean II"+*Arthrophyllum* +*Camphylosporus*+"Ascyreia s.l." +"core Hypericum"	1.00	M: 100	–	M: 100	–	M: 100	–	15.23	11.92	28.07
13	"Mediterranean II"	1.00	M-EA: 85	M: 15	M-EA: 86	M: 14	M-EA: 86	M: 14	07.16	05.09	17.84
14	*Camphylosporus*+"Ascyreia s.l."	1.00	A-M-EA: 68	A-M: 24	A-M-EA: 52	A-M: 29	A-M-EA: 50	A-M-EA-PA: 29	05.85*	06.17	15.80
15	*Camphylosporus*	1.00	A: 66	M: 18	A: 66	M: 20	A: 66	M: 19	02.21	00.81	05.48
16	"Ascyreia s.l."	1.00	M-EA: 56	EA: 29	M-EA: 47	M-EA-PA: 27	M-EA-PA: 43	M-EA: 27	04.02*	04.72	12.30
17	"core Hypericum"	1.00	M: 93	M-EA: 9	M: 94	M-EA: 6	M: 95	M-EA: 5	04.59*	04.73	12.25

5 Synthesis

The cosmopolitan genus *Hypericum* (St. John's wort, Hypericaceae) is one of the few large plant genera for which a comprehensive (alpha-) taxonomy is available (Robson 1977 onwards). During the course of the DFG-funded project within which this thesis is written, the main objective has been to infer phylogenetic relationships within this large flowering plant group. In two datasets (morphological and nrDNA ITS sequence data) representatives of almost all genera of the Hypericaceae are included. Revealed phylogenetic relationships of the three tribes (Cratoxyleae, Hypericeae, and Vismieae) are congruent to the classification (Stevens 2007). The genera belonging to the tribe Hypericeae (*Hypericum*, *Lianthus*, *Santomasia*, *Thornea*, and *Triadenum*) form a monophyletic group in all analyses.

Within the genus *Hypericum*, the two datasets allow me (1) to consider all described species (morphological dataset; Chapter 2), and (2) to use a standard molecular marker (nrDNA ITS sequences) to analyze phylogenetic relationships for a representative sampling of *Hypericum* species (Chapter 3). Comparing the results obtained by cladistic and model-based analyses allows me to validate the main phylogenetic findings of both datasets. Following Blattner (2004) and Jakob and Blattner (2006), the results of this thesis exemplify the accuracy of a deep sampling approach. A comprehensive sampling of taxonomic groups within *Hypericum* s.l. offered insights into phylogenetic relationships, which had not been reported by other studies using less representative sampling designs (Crockett *et al.* 2004; Park & Kim 2004; Pilepic *et al.* 2010, 2011). In the following I will summarize the main findings of the study concerning phylogeny, character evolution and biogeography of *Hypericum* s.l.

5.1 Phylogeny and biogeography

Of the 36 sections recognized by Robson (1977 onwards; Fig. 1.1; Table 2.1, 3.1), ten are monotypic. For the remaining 26 sections the broad approach using several individuals per section allowed me to test their monophyly. Despite low incongruence between the sectional circumscriptions of Robson (1977, onwards) and the results presented in this thesis, the groupings of the sections in larger clades differ markedly from those proposed by Robson (1977, 2003). Moreover, the molecular phylogeny suggests that the small genus *Triadenum* is included in a New World lineage within *Hypericum* s.l., which is in accordance with the findings of Ruhfel *et al.* (2011).

It has been suggested that *Hypericum* originated in Africa and that its worldwide occurrence results from a Gondwanan distribution (Robson 1977: 308, 1981: Fig. 73a, b). This hypothesis was based on the assumption that the African species represent the most basal lineages within *Hypericum*. Thus, these species are assumed to possess plesiomorphic character states within *Hypericum*, such as a tree-like habit and large 'tulipa-like' flowers with stamens in five free fascicles (Robson 1981). Character state optimizations on the phylogeny obtained by both molecular and morphological datasets, however, revealed that a shrub is the ancestral state and that three fascicles are plesiomorphic within *Hypericum* s.l. Furthermore, age estimations for the split of Hypericaceae from its sister group Podostemaceae (Davis *et al.* 2005) do not support the 'out of Africa' scenario, as the break-up sequence of South Gondwana predates the origin of the Hypericaceae by about 30–40 Ma (McLoughlin 2001).

As described in Chapter 4, ancestral area reconstructions for the most basal divergence event within *Hypericum* s.l. revealed a distribution of the ancestral population in the area stretching from North America to the western part of the Tethys Ocean (Fig. 4.3). Divergence time estimation for this node suggests that this ancestral population existed within a time frame of 20 Ma from the Late Eocene to Middle Miocene, *c.* 20–40 Ma ago (Fig. 4.2). At that time the Atlantic Ocean separated North America from Eurasia, and both vicariance and dispersal via stepping stones of the North Atlantic land bridge are likely explanations for the existence of the ancestral population. Limited gene flow across this widespread population may have contributed to speciation at early stages of the evolution of *Hypericum* s.l. Unfortunately, ITS sequence information could not resolve these ancestral diversifications with sound support (Fig. 2.1, 3.1). The difficulty in resolving these relationships within *Hypericum* s.l., seems to be mirrored in early-diverging angiosperms (Moore *et al.* 2007), Saxifragales (Jian *et al.* 2008), campanulids (Tank & Donoghue 2010), rosids (Wang *et al.* 2009), and Malpighiales (Wurdack & Davis 2009). Further information that can be obtained by sequencing of multiple protein coding genes from the nuclear genome (Sang 2002) is needed to infer a sound topology of these basal splits within *Hypericum* s.l.

In summary, phylogenetic relationships within *Hypericum* s.l. inferred by ITS sequence analysis and by the cladistic analysis of morphological characters, suggest the existence of a basal grade ("Mediterranean I and II" and *H.* sect. *Arthrophyllum*) and three large clades. The three clades are *Triadenum* + "Brathys s.l." + *H.* sect. *Myriandra* in the New World, "Ascyreia s.l." + *H.* sect. *Campylosporus* from Southeast Asia and Africa, and "core Hypericum" that is distributed mainly in the Palaearctic region. Within these large clades more recent radiations, apparently less than 5–15 Ma ago, resulted in the species richness described for *Hypericum* s.l. today. When counted according to the groupings revealed by ITS and by morphological characters (for the species that are missing

in ITS) respectively, 36 % of the species belong to the New World lineage of *Hypericum* s. l. The majority of species, over 62 %, are native to the Old World and mainly belong to "core Hypericum" (45 %) and to "Ascyreia s. l." (13 %).

5.2 Character evolution

The evolution of the herbaceous habit and the dark glands in vegetative tissues are identified as apomorphic characters for "core Hypericum" (Chapter 3). Life history is suggested to trigger rates of molecular evolution (Smith & Donoghue 2008). Thus, the herbaceous life form with its shorter generation times might trigger higher species turnover in "core Hypericum", as mutations might become fixed more quickly in herbaceous populations. However, the occurrence of dark glands and, therefore, hypericin in vegetative organs of the plants might also have an effect on evolution rates.

Hypericin is suggested to be involved in plant defense against herbivores (Sirvent *et al.* 2003). An effective defense system in vegetative parts of the plant is certainly of selective advantage. Comparative approaches may allow one to disentangle the influence of both characters on evolution in "core Hypericum". The influence of traits becomes testable (Harvey & Pagel 1991; Wiens 2004) when the evolutionary patterns of different groups possessing the relevant traits are compared. For example, in the South America-centered "Brathys s. l." clade the herbaceous habit is described for several species but not the occurrence of dark glands. For such a comparative approach, however, a comprehensive phylogeny of the South American species would be needed. Today, only 18 % of the species described for South America are included in the ITS dataset, which is not sufficient to infer the evolutionary history of this lineage of *Hypericum* s. l. It will be the task of my DFG-funded follow-up project to acquire further insights into the evolutionary history of South American *Hypericum* species.

5.3 Nuclear rDNA internal transcribed spacer, hybridization and concerted evolution

Due to hybridization, paralogy and concerted evolution, molecular markers derived from nuclear rDNA have certain limitations for phylogenetic inference (Álvarez & Wendel 2003, and citations within). By considering several accessions per species, as well as by cloning selected ITS amplicons, it is possible to test for the inherent molecular evolutionary problems in using rDNA spacers (Hershkovitz & Zimmer 1996; Blattner 2004; Soltis *et al.* 2008). Nuclear ribosomal genes are constituents of individual 18S–5.8S–26S cistron repeats, which are tandemly reiterated at one or more chromosomal loci per haploid genome (Baldwin *et al.* 1995; Álvarez & Wendel 2003). Concerted evolution, resulting from inter-genic sequence homogenization (Zimmer *et al.* 1980), may cause the loss of parental ITS types. Hence, ancient hybridization events may not be detected in ITS sequence analyses (Blattner 2004). First results of chloroplast *pet*D sequence analysis (Borsch & Nürk *et al.*, unpubl. data) revealed similar clades and a topology congruent to that produced from

ITS. This supports the deductions discussed in this thesis, according to which reticulate evolution across the major clades did not take place during early evolution within *Hypericum* s. l.

Cloning of ITS amplicons revealed frequent hybridization between more closely related species (*i. e.* species belonging to one of the large clades; Chapter 3), especially in "core Hypericum" and in "Ascyreia s. l.". Thus, further evidence was found that polyploidy or hybridization are common phenomena within *Hypericum*, although they seem to occur only among close relatives.

Robson taxonomically describes 40 hybrids in his monographic treatment of the genus (Robson 1977 onwards). Scheriau & Koch (in prep.) show ongoing introgression and massive gene flow between *H. perforatum* and *H. maculatum*. Schallau *et al.* (2010) highlight that apomixis is often associated with extensive heterozygosity and polyploidy, although some diploid apomicts have been described in literature (Schallau *et al.* 2010, and citations within). The results of this thesis provide the explicit and comprehensive phylogenetic context necessary to put studies on polyploidy and apomixis in *Hypericum* s. l. into an evolutionary framework. Population genetic approaches are required to investigate the influence of such events on the evolution of certain lineages of *Hypericum* s. l.

5.4 Evolutionary scenario of *Hypericum* s. l.

In summary I would like to develop a hypothesis for the phylogeny and biogeography of *Hypericum* s. l. based on current knowledge derived from the results presented in this thesis.

After the thermal maximum in the Early Eocene *c.* 52 Ma ago (Tiffney & Manchester 2001; Zachos *et al.* 2001), with the slow cooling of the earth climate, *Hypericum* s. l. adapted to temperate climates and diverged from its tropical relatives. During the estimated time frame for the evolution of the ancestor of *Hypericum* s. l. (20–40 Ma ago) temperate zones enlarged worldwide and tropical floras, once connected through the continents of the Northern Hemisphere, become separated. While warm adapted populations were receded towards tropical zones, the adaptation to colder climate conditions offered new colonization possibilities to this temperate lineage of the Hypericaceae. That is, *Hypericum* s. l. remained and diversified in the Northern Hemisphere, while the population expansion of its tropical relatives was reduced. The niche shift into temperate habitats correlates with a significant increase in diversification rates within *Hypericum* s. l.

At that time, the Tethys Ocean still separated large parts of the Old World until the Middle Miocene. Large estuaries and gulfs expanded deep into the Eurasian continent and Africa was separated from the rest of Eurasia. This is the area in which Old World *Hypericum* s. l. diversified. To date the highest species density of *Hypericum* s. l. is still native to the Mediterranean Basin and the Caucasus region, two areas counted under the 25 hotspots of biodiversity (Myers *et al.* 2000).

Migration into Africa took place several times, not during an early stage in the evolution of *Hypericum* s. l. but instead more recently, *e. g., H.* sect. *Campylosporus* is estimated to be less than 5 Ma old. Migration into East Asia happened earlier, estimated to have occurred *c.* 15 Ma ago, which correlates with the fossil record (Zhao *et al.* 2004). As detailed above, the lack of a comprehensive sampling in the ITS phylogeny impedes sound conclusions about the dispersal history for South America.

The detected shift in diversification rates for *Hypericum* s. l. correlates with the evolution of cold tolerance (Chapter 4). The questions whether the twice as high speciation rates are caused by this niche shift, or whether the niche shift offered new colonization possibilities (Stephens & Wiens 2003; Moore & Donoghue 2007), which allowed *Hypericum* s. l. to diversify twice as fast as its tropical relatives, cannot be answered yet. Similarly, the central question subsuming the findings of this thesis is about cohesive causal motives (Pagel 1999; Crisp *et al.* 2011; Vamosi & Vamosi 2011), which explain the diversity that evolved in *Hypericum* s. l. within the large clades after adaptation to temperate climates.

Whether and to which amount dispersal abilities, physiological novelties (hypericin synthesis) or novel morphological traits contribute to the evolution within the major clades of *Hypericum* s. l. awaits investigations. The study presented here provides for the first time a comprehensive phylogeny and deduces explicit hypotheses, which may form the baseline for comparative studies within *Hypericum* s. l.

Abstract

St. John's wort (*Hypericum*, Hypericaceae) is a cosmopolitan genus with almost 500 species, including the medically used, facultative apomictic species *H. perforatum*. It is one of the few large plant genera for which an almost complete alpha taxonomy and classification is available. *Hypericum* is a temperate plant genus belonging to the clusioid clade of the Malpighiales that otherwise consists of tropical flora elements. In this dissertation, new insights into the evolutionary history of the genus *Hypericum* are provided. I investigate mechanisms that might have contributed to the observed species richness within this flowering plant group.

To infer phylogenetic relationships within *Hypericum* and related taxa, I used morphological data and nuclear ribosomal DNA internal transcribed spacer (ITS) sequence information. A phylogenetic hypothesis had first to be formulated in order to position the analysis of evolution of the morphological and ecological characteristics, the reconstruction of historical biogeography, as well as the identification of diversification events into a testable framework, that is, into an explicit phylogenetic context.

I coded 89 morphological characters for all (598) described taxa to conduct a formal cladistic analysis of the genus. For molecular phylogenetic inference, I used 366 sequences of the ITS region for 206 species representing *Hypericum* and five other genera of Hypericaceae. I analyzed the data with parsimony and model based methods to generate an explicit phylogenetic hypothesis of the genus. The results indicate that the small genera *Lianthus*, *Triadenum*, and *Santomasia* are nested within *Hypericum,* included in a clade containing most of the New World species. Sister to *Hypericum* is the small genus *Thornea* from Central America. Within *Hypericum*, three large clades and two smaller grades were found. Ancestral character state reconstructions yielded the recognition of characters, which support major clades within the genus. Shrubs represent the ancestral growth form from which herbs evolved several times. Sclerophylous treelets have radiated convergent in high elevation tropical habitats in Africa and the páramos of South America.

To investigate the historical biogeography I conducted maximum likelihood analyses and compared the influence of different parametric models incorporating geological information. These analyses resulted in a revised biogeographic scenario for *Hypericum* and relatives. A cold adapted *Hypericum* s. l. lineage evolved with the emergence of temperate habitats on the Northern Hemisphere. This ancestral population was distributed across North America and West Eurasia (at this time the western Tethys region), and subsequently became disjunct and diversified independently in the New and the Old World. Since adaptation to cold climate conditions, species of this lineage stayed within temperate habitats (*e. g.*, high mountains in the tropics). Together with this physiological shift, a significant increase in net diversification is correlated, resulting in eight times higher species richness within this temperate lineage of the Hypericaceae. Thus, the adaptation to cold climate conditions is a physiological key innovation triggering the increased evolutionary turnover in this temperate lineage. These results add to the growing evidence that phylogenetic niche conservatism is an important principle influencing biodiversity, especially during global climate changes.

Zusammenfassung

Johanniskraut (*Hypericum*, Hypericaceae) ist eine kosmopolitische Gattung, die annähernd 500 Arten beinhaltet, darunter die medizinisch verwendete, fakultativ apomiktische *H. perforatum*. *Hypericum* ist eine der wenigen großen Gattungen, für welche eine umfassende Taxonomie vorhanden ist. Die Arten kommen vor allem in gemäßigten Zonen vor. Die Gattung gehört phylogenetisch in den Clusioid Klade der Malpighiales, welche ansonsten Arten der tropischen Flora enthält. In dieser Arbeit gebe ich neue Einblicke in die Phylogenese der Gattung *Hypericum*. Weiterhin untersuche ich Gründe, welche zu dem beschriebenen Artenreichtum in dieser Gattung beigetragen haben können.

Morphologische Daten und DNA Sequenzen der *internal transcribed spacer* (ITS) Region wurden verwendet, um die Verwandtschaftsbeziehungen innerhalb von *Hypericum* zu untersuchen. Eine phylogenetische Hypothese muss formuliert werden, um die Evolution von morphologischen und ökologischen Besonderheiten, die Rekonstruktion der historischen Biogeographie, sowie die Identifizierung von Diversifizierungs-Ereignissen in eine überprüfbare Rahmenbedingung stellen zu können, das heißt in einen klaren phylogenetischen Zusammenhang.

Ich habe für alle (598) beschriebenen Taxa 89 morphologische Merkmale codiert und eine kladistische Analyse für die Gattung durchgeführt. Die molekular-phylogenetischen Analysen basieren auf 366 Sequenzen der ITS Region für 206 *Hypericum* Arten und Vertreter von fünf weiteren Gattungen der Hypericaceae. Die Daten wurden mithilfe von Parsimonie und modellbasierten Methoden analysiert, um eine Phylogenie der Gattung zu generieren. Die Ergebnisse zeigen, dass die Gattungen *Lianthus*, *Triadenum* und *Santomasia* phylogenetisch *Hypericum* zugeordnet sind. Die Schwestergruppe zu diesem Klade ist die kleine Gattung *Thornea* aus Mittelamerika. Innerhalb von *Hypericum* s. l. wurden drei große monophyletische Gruppen und mehrere kleinere, sukzessiv abzweigende Linien identifiziert.

Durch die Rekonstruktion von Merkmalszuständen innerhalb der Gattung lassen sich über morphologische Merkmale die großen Gruppen unterstützen. Die Stammart von *Hypericum* s. l. waren Sträucher. Kräuter entwickelten sich mehrmals konvergent innerhalb der Gattung. Ein baumartiger Habitus hat sich unabhängig in tropischalpinen Lebensräumen in Afrika und in Südamerika entwickelt.

Die historische Biogeographie wurde über ML basierte Analysen rekonstruiert. Dabei wurde der Einfluss verschiedener parametrischer Modelle verglichen. Die Ergebnisse führten zu einem revidierten biogeographischen Szenario für *Hypericum* und seine Verwandten. Eine kalt-adaptierte *Hypericum* s. l. Linie entwickelte sich mit der Entstehung gemäßigter Lebensräume in der Nordhemisphäre. Aufgrund der Adaptation an kalte Klimate konnten sich die Arten/Individuen dieser Linie in gemäßigten Lebensräumen über die gesamte Erde ausbreiten. Ein signifikanter Anstieg in Artbildungsraten korreliert mit dieser physiologischen Veränderung, was zu einen achtmal größerem Artenreichtum in dieser gemäßigten Linie der Hypericaceae führt. Das heißt, die Anpassung an gemäßigte Klimabedingungen stellt eine wichtige physiologische Neuerung in der Evolution von *Hypericum* s. l. dar. Diese Resultate verdeutlichen die Bedeutung von Nischenstabilität als ein wichtiges Prinzip, das Biodiversität vor allem in Zeiten globaler Klimaveränderung beeinflusst.

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Abbreviations

AIC	=	Akaike information criterion
APG	=	Angiosperm phylogeny group
BC	=	before Christ
BI	=	Bayesian inference
bp	=	base pair (two nucleotides)
bs	=	bootstrap support
c.	=	*circa*
cp	=	chloroplast
DEC	=	dispersal, extinction and cladogenesis (model)
DNA	=	Deoxyribonucleic acid
e. g.	=	*exempli gratia* (for example)
et al.	=	*et alia* (and others)
GTR	=	general time reversible (DNA substitution) model
HPD	=	highest posterior density (comprising normally 95 % of highest clade credibility trees)
i. e.	=	*it est*
ITS	=	internal transcribed spacer
kp	=	kilo bp
Lagrange	=	likelihood analysis of geographic range evolution
LBA	=	long branch attraction
LRT	=	likelihood ratio test
Ma	=	million years (any)
MCMC	=	Markov chain Monte Carlo
MC^3	=	Metropolis coupled MCMC
MEDUSA	=	modeling stepwise diversification using stepwise AIC
ML	=	maximum likelihood
MP	=	maximum parsimony
nrDNA	=	nuclear ribosomal DNA (formulating the nuclear organizing [NOR] region), multi copy genes.
OTU	=	operational taxonomic unit
PCR	=	Polymerase chain reaction
*pet*D	=	chloroplast gene, encoding for the subunit IV of the cytochrome b6/*f* complex (Sakamoto et al. 1993)
pp	=	posterior probability
s. l.	=	*sensu lato* (in the wider sense)
spec.	=	species
TBR	=	tree bisection recognition

Figures & Tables

Appendix

Appendix S1 Active compounds

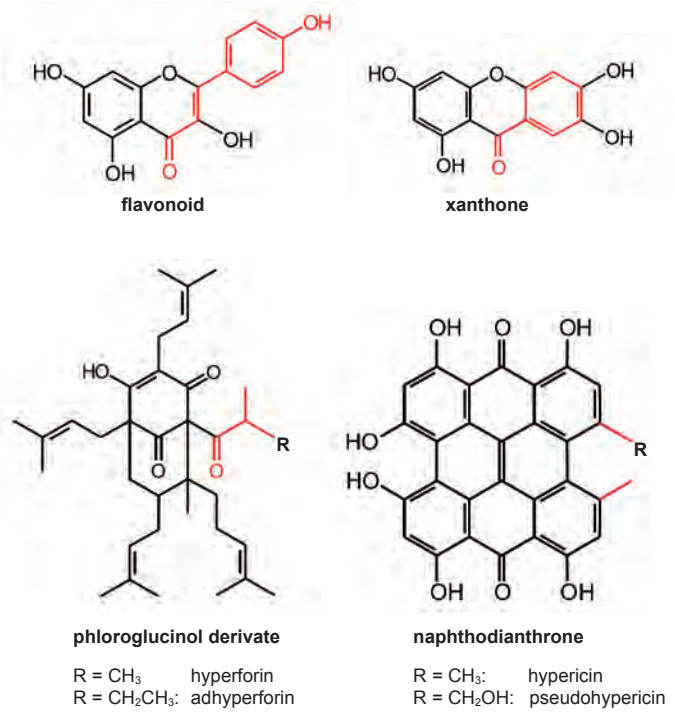

Fig. S1 Classes of active compounds in *Hypericum*. The biosynthetic starter unit is highlighted in red.
Adopted from Beerhues (2011); Hölzel & Peterson (2003).

Table S1 Biological active compounds found in *H. perforatum* and their medical properties (modified from: Czok & Lang 1961; Roth 1990; Hölzl & Petersen 2003; Linde 2009).

Component group	Example	Characteristic	Effect
Naphthodianthrones (lipophilic)	hypericin [2,2′-dimethyl-4,4′,5,5′,7,7′-hexahydroxy-mesonaphtodianthrone] pseudohypericin and related compounds, *e.g.,* their photo-derivates and their precursors protohypericin and protopseudohypericin	red pigments, present in aerial parts (stem, leaves, flowers and fruits), *i.e.* produced in dark nodules	antidepressant, antiviral, photosensitizing
Phloroglucinol derivates (polyketide derivates; lipophilic)	hyperforin adhyperforin	light-sensitive and unstable, accumulated in pale glands	antidepressant, antimicrobial
Xanthones (lipophilic)	kielcorin mangiferin norathyriol [1,3,6,7-tetrahydroxyxanthen- 9-one]	enriched in roots and flowers, but found in all parts of the plant	antidepressant (concentrations too low in the plant to be responsible for AD activity in extracts)
Flavonoids (lipophilic/hydrophilic)	rutin quercetin quercitin isoquercitin and its 3-O-galactoside: hyperoside	UV absorbent	antidepressant, antiphlogistic, antiviral
Biflavonoids (lipophilic)	I3,II8-biapigenin I3´,II8-biapigenin	produced in flowers	sedating, antiphlogistic
Tannins and **procyanidins** (*i.e.* condensed tannins; lipophilic)	procyanidin catechin epicatechin	produced in aerial parts	antiphlogistic, astringent, antioxidant
Phenylpropanes (*i.e.* phenolic acids; hydrophilic)	chlorogenic acid caffeic acid		spasmolytic activity
Amino acids (hydrophilic)	GABA (*gamma*-Aminobutyric acid)	in flowers and leaves	antidepressant (amount considered too low in the plant to be responsible for AD activity in extracts)
Essential oils (lipophilic)	terpenes (*e.g.*, the monoterpenes a- and b-pinene, myrcene, limonene, and sesquiterpenes) hydrocarbons long chain alcohols	in flowers and leaves	might contribute to the sedative effect of the crude plant extract (but ≤ 1% of extract)

Appendix S1.1 Description of *Hypericum*

The following diagnosis of the genus is based on species descriptions from the *Hypericum* monograph (Robson, 1977, 1981, 1985, 1987, 1990, 1996, 2001, 2002, 2006, 2010a, 2010b) and additional information summarized from Judd *et al.* (2008) and Stevens (2007). Description of architectural features of leaves follows Hickey (1973).

Hypericum L., *Sp. Pl.*: 783 (1753); *Gen. pl.* 5th ed.: 341 (1754).
Type species: *H. perforatum* L.
Shrubs or perennial to (few) annual herbs and some trees (*i. e.* arborescent shrubs) up to *c.* 12 m tall, evergreen or deciduous; glabrous or with simple uniseriate hairs; with canals ('linear') or cavities/nodules ('punctiform') containing resins (amber in color), essential oils and hyperforin (translucent cavities or 'pale glands') and often hypericin and derivates (red to blackish nodules or 'dark glands'). *Stems* green to yellow-brown or red, holly terete in some herbs, or with 2–4 (–6) raised lines along each internode when young (those lines decurrent from the midrib of the leaf above usually most prominent and sometimes expanded to form narrow wings), eventually terete; glabrous or with indumentum; eglandular or with pale glands and/or dark nodules; bark smooth, red-brown to purple-brown or silvery, thin (or rarely corky), persistent or exfoliating in sheets, flakes or irregular stripes or scales; with clear sap (!), *i. e.* not exuding colored resiniferous sap or latex. *Leaves* opposite, decussate or distichous, sometimes in alternating whorls of 3–4, simple, exstipulate, sessile to shortly and gradually petiolate (petiole usually <2 mm long), sometimes with basal articulation, persistent or deciduous at or above the articulation; lamina entire, rarely glandular denticulate or -fimbriate, sometimes with gland-fringed auricles or base, venation normally pinnate (camptodromous [open or closed] to hydrodromus), sometimes parallelodromous, the tertiary absent to densely reticulate; pale glands linear to punctiform, sometimes dark nodules, linear to punctiform, marginal to laminar; indumentum present or absent. *Inflorescence* terminal, 1–∞ -flowered, cymose (sympodial, *i. e.* determinated), acropedal (terminal flower is usually the first to mature), elaborated acrotonally by dichasial and/or monochasial cymes (resulting in a dichasium or a bostryx), or by a pseudo-dichotomy (*i. e.* a dichasium showing bracts that are not bearing a flowering branch between the regular branching; see (Robson, 1981): Fig. 13n), or a mixture of both patterns, basitonally by single flowers or flowering branches, resembling the described patterns, sometimes separated from the terminal flower by leaves, and sometimes become racemose by suppression of the terminal bud; often bracteose, sometimes frondose (*i. e.* bracts look like leaves), or frondobracteose (*i. e.* gradual transition from leaves to sepals) persistent as long as leaves or occasionally 'caducous' (deciduous). *Flowers* bisexual, actinomorph (radial symmetric), mostly stellate, sometimes campanulate or pseudo-tubular, homostylous or rarely heterostylous. *Sepals* (3–) 4–5 (–6), green and sometimes tinged red, equal to dimorphic, sometimes foliaceous, free or up to ⅔ united, persistent or occasionally deciduous, margin entire or glandular-denticulate to -fimbriate or eglandular-fimbriate; vains 1–*c.* 11, parallelodromous to pinnate; glands marginal to laminar, linear to punctiform, pale and/or dark; indumentum absent or on dorsal surface only. *Petals* (3–) 4–5 (–6), lemon to golden yellow or orange or rarely cream to white, often tinged or veined red dorsally where visible in bud, very really spotted or wholly carmine-red, contorted, equal, free, persistent or deciduous, normally asymmetrical and usually with ± evident projection

at apical point of margin in bud ('apiculus'), entire or with sessile marginal glands or glandular-ciliate margin, rarely with entire and cucullate or trifid and flat ligule; veins few to numerous; glands laminar and often marginal, linear to punctiform, pale and/or dark; indumentum absent. *Stamens* arranged in 4–5 fascicles (bundles), antipetalous, free or variously united (2 + 1 + 1 + 1 [resulting in 4 visible fascicles], 2 + 2 + 1 [resulting in 3 visible fascicles], (5), (4)) and then with combined fascicles antisepalous, each with 1–60 (–80) stamens, persistent or deciduous, glabrous; staminodes (staminodal fascicles: 'fasciclodes') absent or rarely 3, alternating with the '3' fascicles, scale like, entire or bilobed, functioning like grass lodicules; filaments yellow to orange or rarely cream to white or crimson, slender, united towards the base only or apparently free or united to above the middle in a few '3'-fascicled species; anthers yellow to orange to reddish, oblong to elliptic, bithecal, with amber or red to blackish gland on connective, normally dorsifixed, dehiscing introrsely by longitudinal slits; pollen tricolporate, speroidal to prolate, with exine microreticulate to reticulate or a tectum perforatum. *Ovary* superior, (2–) 3–5-numerous, yellow to greenish, placentation ± complete axile to parietal; styles (2–) 3–5, elongate, free or partially to completely united, with stigmas distinct, minute (to capitate); ovules (2–) ∞ on each placenta, erect to horizontal or pendulous. *Fruit* capsular, generally septicidal, dehiscing from the apex, silvery to light to dark brown to blackish, with valves lignite (somewhat woody or coriaceous to papyraceous), persistent rarely deciduous, rarely tardily dehiscent or indehiscent with valves ± fleshy (drupe like) and colored red to blackish, styles partially to wholly persistent; vittae (*i. e.* a tube like cavity in the pericarp containing oil and/or resins) often ± prominent, linear to punctiform ('vesicles'), amber or rarely blackish. *Seeds* small, 0.3–1.5 mm long, light to dark brown to black, mat to agleam, linear to ovoid-cylindrical to ellipsoid, slightly curved to straight, sometimes with unilateral carina or thin and papyraceous wingrarely with an apical whitish caruncle; testa with sculpturing reticulate to scalariform to papillose; endosperm absent (or tiny); embryo slender, straight, with cotyledons equal, free, plano-convex, shorter than hypocotyls. *Chromosomes c*. 1.3–3.5 µm long, basic numbers (n): 6, 7, 8, 9, 10, 11, 12; ploidy 2–6.

Appendix S1.2 Species taxonomy

Table S1.2 Taxonomic names used in this thesis. References and illustrations are cited. Section (first) and species numbers (second) are given before the names and follow Robson (1977 onwards).

Cratoxyleae Benth.
Cratoxylum Blume, Stevens (2007): 194 ff.
Cr. arborescens Blume.
Cr. celebicum Blume.
Cr. formosum (Jack) Benth. & Hook.f. ex Dyer.
Cr. pruniflorum Dyer.

Eliea Cambess., Stevens (2007): 194 ff.
E. articulata Cambess.

Hypericeae Choisy
Hypericum L.,
27. 2. ***H. abilianum*** N.Robson, Robson (1996): 175 Fig. 25.
29. 48. ***H. aciculare*** Kunth, Robson (1987): 66 (No. 35) Fig. 11.
25. 3. ***H. aciferum*** (Greuter) N.Robson, Robson (1996): 153 „[…] very restricted relict distributin. […]" (Robson 1996: 153).
3. 23. ***H. acmosepalum*** N.Robson, Robson (1985): 245.
29. 9. ***H. acostanum*** N.Robson, Robson (1987): 27 (No. 5).
3. 27. ***H. addingtonii*** N.Robson, Robson (1985): 251 Fig. 17.
—x. ***Hypericum x cyathiflorum*** N.Robson, Robson (1985): 253, 27. *H. addingtonii* x *H. hookeriano,* to be knowen as *H.* x *cyatiflorum* 'Gold Cup'.
—xx. ***Hypericum x ,Hidcote'*** _, Robson (1985): 254, 27x. *H.* x *cyathiflorum* ,Gold Cup' x 14. *H. calycinum*.
15/16 (16). 3. ***H. adenotrichum*** Spach, Robson (2010b): 115 Fig. 24.
20. 21. ***H. adpressum*** W.P.C.Barton, Robson (1996): 119.
25. 1. ***H. aegypticum*** L., Robson (1996): 148.
—a. subsp. ***maroccanum*** (Pau) N.Robson, Robson (1996): 150 Fig. 22.
—b. subsp. ***webbii*** (Spach) N.Robson, Robson (1996): 150.
—c. subsp. ***aegypticum,*** Robson (1996): 152.
27. 6. ***H. aethiopicum*** Thunb., Robson (1996): 179.
—a. subsp. ***sonderi*** (Bredell) N.Robson, Robson (1996): 180.
27. —b. subsp. ***aethiopicum,*** Robson (1996): 181.
27. 5. ***H. afrum*** Lam., Robson (1996): 178 Fig. 25.
12. 5. ***H. albiflorum*** (Hub.-Mor.) N.Robson, Robson (2010b): 45 Fig. 17.
19. 5. ***H. amblycalyx*** Coustur. & Grandid., Robson (2010a): 251 Fig. 20.
17. 21. ***H. amblysepalum*** Hochst., Robson (2010a): 176 Fig. 11.
30. 47. ***H. anagalloides*** Cham. & Schltdl., Robson (1990): 136.
30. 24. ***H. anceps*** Larrañaga, Robson (1990): 87.
29. 72. ***H. andinum*** Gleason, Robson (1987): 97 (No. 58).
14. 3. ***H. andjerinum*** Font Quer & Pau, Robson (2010b): 98.
5. 3. ***H. androsaemum*** L., Robson (1985): 301.
—x. ***Hypericum x inodorum*** Mill. „elatum", Robson (1985): 304, *H. androsaemum x H. hircinum subsp. majus / cambessedesii (or hircinum), „H. inodorum appears to have originated spontaneously, both in cultivation […] and in natural habits, […]" (Robson 1985).*
—a. ***Hypericum x inodorum ,Elstead',*** Robson (1985): 307, named cultivars also include ‚Summergold', ‚Ysella', Goudelsje', ‚Hysan', and ‚Betty´s Variety' (Robson 1985: 307).
27. 18. ***H. annulatum*** Moris, Robson (1996): 199.
—a. subsp. ***intermedium*** (Steud. ex A.Rich.) N.Robson, Robson (1996): 199.
—b. subsp. ***annulatum,*** Robson (1996): 201.
—c. subsp. ***afromontanum*** (Bullock) N.Robson, Robson (1996): 202 Fig. 29.
30. 35. ***H. aphyllum*** Lundell, Robson (1990): 107 Fig. 19.
17. 11. ***H. apiculatum*** (N.Robson) Sennikov, Robson (2010b): 159 Fig. 9.
20. 16. ***H. apocynifolium*** Small, Robson (1996): 113.
17. 10. ***H. apricum*** Kar. & Kir., Robson (2010b): 156.
29. 77. ***H. arbuscula*** Stanley & Steyerm., Robson (1990): 30.
30. 40. ***H. arenarioides*** A.Rich., Robson (1990): 121.
18. 11. ***H. armenum*** Jaub. & Spach, Robson (2010a): 219 Fig. 16.
—a. subsp. ***armenum,*** Robson (2010a): 222.
—b. subsp. ***iranicum*** N.Robson, Robson (2010a): 222.
9. 34. ***H. asahinae*** Makino, Robson (2006): 63 Fig. 16.
7. 1. ***H. ascyron*** L., Robson (2001): 52, *„H. ascyron* is a highly polymorphic species or species complex with a very wide distribution. […] the variation appears to be olmost continuous." (Robson 2001: 53).
—a. subsp. ***ascyron,*** Robson (2001): 54 Fig. 9.
—b. subsp. ***gebleri*** (Ledeb.) N.Robson, Robson (2001): 57 Fig. 9.
—c. subsp. ***pyramidatum*** (Aiton) N.Robson, Robson (2001): 58 Fig. 9.
19. 1. ***H. asperuloides*** Czern. ex Turcz., Robson (2010a): 240 Fig. 19.
17. 23. ***H. asperulum*** Jaub. & Spach, Robson (2010a): 180.
29. 20. ***H. asplundii*** N.Robson, Robson (1990): 24 Fig. 7.
9c. 2. ***H. assamicum*** S.N.Biswas, Robson (2001): 66 Fig. 11.
27. 20. ***H. athoum*** Boiss. & Orph., Robson (1996): 203.
27. 21. ***H. atomarium*** Boiss., Robson (1996): 204.
9. 6. ***H. attenuatum*** Fisch. ex Choisy, Robson (2002): 108 Fig. 10.

15/16 (16). 2. ***H. aucheri*** Jaub. & Spach, Robson (2010b): 113 Fig. 24.
3. 7. ***H. augustinii*** N.Robson, Robson (1985): 219 Fig. 14.
10. 2. ***H. auriculatum*** (N.Robson & Hub.-Mor.) N.Robson, Robson (2010b): 20 Fig. 13.
14. 2. ***H. australe*** Ten., Robson (2010b): 96 Fig. 23.
12. 6. ***H. aviculariifolium*** Jaub. & Spach, Robson (2010b): 46 Fig. 17.
29. 39. ***H. baccharoides*** Cuatrec., Robson (1990): 27.
2. 1. ***H. balearicum*** L., Robson (1985): 203 Fig. 11.
1. 5. ***H. balfourii*** N.Robson, Robson (1985): 191.
13. 8. ***H. barbatum*** Jacq., Robson (2010b): 76 Fig. 20.
29. 85. ***H. beamanii*** N.Robson, Robson (1990): 40.
3. 40. ***H. beanii*** N.Robson, Robson (1985): 282 27.
26. 7. ***H. beccarii*** N.Robson, Robson (1996): 164.
—a. ***H. beccarii*** N.Robson subsp. ***beccarii,*** Robson (1996): 165.
—b. ***H. beccarii*** N.Robson subsp. ***steenisii*** N.Robson, Robson (1996): 165.
3. 35. ***H. bellum*** H.L.Li, Robson (1985): 273.
1. 1. ***H. bequaertii*** De Wild., Robson (1985): 180 Fig. 9.
26. 4. ***H. bifurcatum*** N.Robson, Robson (1996): 159.
13. 5. ***H. bithynicum*** Boiss., Robson (2010b): 66.
29. 53. ***H. bolivaricum*** N.Robson, Robson (1987): 72 (No. 40).
12. 3. ***H. bourgaei*** (Boiss.) N.Robson, Robson (2010b): 41 Fig. 16.
20. 11. ***H. brachyphyllum*** (Spach) Steud., Robson (1996): 109 Fig. 16.
30. 16. ***H. brasiliense*** Choisy, Robson (1990): 73 Fig. 15.
30. 31. ***H. brevistylum*** Choisy, Robson (1990): 100.
29. 52. ***H. bryoides*** Gleason, Robson (1987): 71 (No. 39).
20. 15. ***H. buckleyi*** M.A.Curtis, Robson (1996): 112.
8. 1. ***H. bupleuroides*** Griseb., Robson (2001): 50 Fig. 8.
30. 26. ***H. caespitosum*** Cham. & Schltdl., Robson (1990): 91 Fig. 17.
29. 13. ***H. callacallum*** N.Robson, Robson (1990):18 Fig. 7.
17. 3. ***H. callithyrsum*** Coss., Robson (2010b): 147 Fig. 7.
3. 14. ***H. calycinum*** L., Robson (1985): 228.
30. 18. ***H. campestre*** Cham. & Schltdl., Robson (1990): 75.
—a. subsp. ***pauciflorum*** N.Robson, Robson (1990): 76 Fig. 15.
—b. subsp. ***campestre,*** Robson (1990): 76 Fig. 15.
—c. subsp. ***tenue*** N.Robson, Robson (1990): 78 Fig. 15.
30. 37. ***H. canadense*** L., Robson (1990): 110.
—x. ***Hypericum x dissimulatum*** E.P.Bicknell, Robson (1990): 113, *H. canadense* x *mutilum* or *H. canadense* x *boreale* „The more northern hybrids are more *H. canadense* x *mutilum* subsp. *boreale* and the more southern *H. canadense* x *mutilum* subsp. *mutilum* […]" (Robson 1990): 113, 115 ff.).
21. 1. ***H. canariense*** L., Robson (1996): 134 Fig. 19.
17. 27. ***H. capitatum*** Choisy, Robson (2010a): 186.
—a. var. ***luteum*** N.Robson, Robson (2010a): 187.
—b. var. ***capitatum,*** Robson (2010a): 187.
30. 4. ***H. caprifoliatum*** Cham. & Schltdl., Robson (1990): 56.
27. 15. ***H. caprifolium*** Boiss., Robson (1996): 192 Fig. 27.
29. 64. ***H. caracasanum*** Willd., Robson (1987): 89 (No. 51).
—a. subsp. ***caracasanum,*** Robson (1987): 89 (No. 51a) Fig. 13.
—b. subsp. ***turumiquirense*** (Steyerm.) N.Robson, Robson (1987): 90 (No. 51b).
22. 3. ***H. cardiophyllum*** Boiss., Robson (1996): 138 Fig. 20.
29. 63. ***H. cardonae*** Cuatrec., Robson (1987): 87 (No. 50).
30. 23. ***H. carinatum*** Griseb., Robson (1990): 84.
29. 6. ***H. carinosum*** R.Keller, Robson (1987): 33 (No. 9).
29. 42. ***H. cassiopiforme*** N.Robson, Robson (1987): 59 (No. 29).
29. 19. ***H. castellanoi*** N.Robson, Robson (1990): 23.
30. 7. ***H. cavernicola*** L.B.Sm., Robson (1990): 62 Fig. 13.
11. 1. ***H. cerastioides*** (Spach) N.Robson, Robson (2010b): 31 Fig. 15.
29. 76. ***H. chamaemyrtus*** Triana & Planch., Robson (1990): 29.
—a. subsp. ***chamaemyrtus,*** Robson (1990): 30 Fig. 9.
—b. subsp. ***pseudocaracasanum*** (Steyerm.) N.Robson, Robson (1990): 30 Fig. 9.
20. 14. ***H. chapmanii*** W.P.Adams, Robson (1996): 112.
3. 34. ***H. choisianum*** Wall. ex N.Robson, Robson (1985): 271.
20. 18. ***H. cistifolium*** Lam., Robson (1996): 116 Fig. 17.
27. 13. ***H. coadunatum*** C.Sm., Robson (1996): 189 Fig. 27.
3. 17. ***H. cohaerens*** N.Robson, Robson (1985): 235.
27. 11. ***H. collenettiae*** N.Robson, Robson (1996): 188 Fig. 26.
9b. 3. ***H. collinum*** Schltdl. & Cham., Robson (2006): 84 Fig. 20.
9a. 1. ***H. concinnum*** Benth., Robson (2001): 61 Fig. 9.
18. 5. ***H. confertum*** Choisy, Robson (2010a): 207.
—a. subsp. ***stenobotrys*** (Boiss.) Holmboe, Robson (2010a): 209 Fig. 14.
—b. subsp. ***confertum,*** Robson (2010a): 210 Fig. 14.
27. 3. ***H. conjungens*** N.Robson, Robson (1996): 177 Fig. 25.
30. 5. ***H. connatum*** Lam., Robson (1990): 57.
30. 6. ***H. cordatum*** (Vell.Conc.) N.Robson, Robson (1990): 59.
—a. subsp. ***cordatum,*** Robson (1990): 60 Fig. 13.
—b. subsp. ***kleinii*** N.Robson, Robson (1990): 60 Fig. 13.
3. 2. ***H. cordifolium*** Choisy, Robson (1985): 213.
19. 2. ***H. coris*** L., Robson (2010a): 241 Fig. 19.
29. 51. ***H. costaricense*** N.Robson, Robson (1987): 70 (No. 38).
18. 13. ***H. crenulatum*** Boiss., Robson (2010a): 222 Fig. 16.
20. 25. ***H. crux-andreae*** (L.) Crantz, Robson (1996): 124 Fig. 18.
29. 23. ***H. cuatrecasii*** Gleason, Robson (1987): 41 (No. 16).
27. 22. ***H. cuisinii*** Barbey, Robson (1996): 205 Fig. 29.
30. 13. ***H. cumulicola*** (Small) W.P.Adams, Robson (1990): 70 Fig. 14.

3. 39. ***H. curvisepalum*** N.Robson, Robson (1985): 281 Fig. 19.
12. 10. ***H. cymbiferum*** Boiss. & Balansa, Robson (2010b): 50 Fig. 18.
29. 75. ***H. cymobrathys*** N.Robson, Robson (1987): 103 (No. 61).
9e. 6. ***H. daliense*** N.Robson, Robson (2001): 83.
17. 12. ***H. davisii*** N.Robson, Robson (2010b): 160 Fig. 9.
27. 24. ***H. decaisneanum*** Coss. & Daveau, Robson (1996): 208.
29. 43. ***H. decandrum*** Turcz., Robson (1987): 59 (No. 30) Fig. 10.
27. 19. ***H. delphicum*** Boiss. & Heldr., Robson (1996): 202.
20. 5. ***H. densiflorum*** Pursh, Robson (1996): 100.
—x. ***H. densiflorum x galioides ?,*** Robson (1996): 101, *H.* x *arnoldianum,* parents are unsure, *H. lopocarpum* x? *densiflorum* (Robson 1996: 101).
—x. ***H. densiflorum x prolificum,*** Robson (1996): 101.
30. 10. ***H. denticulatum*** Walter, Robson (1990): 64.
—a. subsp. ***denticulatum,*** Robson (1990): 65.
—b. subsp. ***acutifolium*** (Elliott) N.Robson, Robson (1990): 66.
30. 14. ***H. denudatum*** A.St.-Hil., Robson (1990): 71.
29. 80. ***H. dichotomum*** Lam., Robson (1990): 35.
29. 81. ***H. diosmoides*** Griseb., Robson (1990): 36.
20. 24. ***H. dolabriforme*** Vent., Robson (1996): 123.
29. 87. ***H. drummondii*** (Grev. & Hook.) Torr. & A.Gray, Robson (1990): 41 Fig. 11.
3. 26. ***H. dyeri*** Rehder, Robson (1985): 249.
29. 83. ***H. eastwoodianum*** I.M.Johnst., Robson (1990): 39.
20. 27. ***H. edisonianum*** (Small) W.P.Adams, Robson (1996): 128 Fig. 18.
29. 65. ***H. ekmanii*** Alain, Robson (1987): 90 (No. 52) Fig. 13.
3. —a. ***H. elatoides*** R.Keller, Robson (2001): 49 Fig. 8.
18. 12. ***H. eleanorae*** Jelenc, Robson (2010a): 222.
9. 7. ***H. elegans*** Stephan ex Willd., Robson (2002): 111 Fig. 10.
—x. ***H. elegans Stephan ex Willd. x H. perforatum L.?,*** Robson (2002): 112, „[…] seems to be this hybrid, which has not hitherto been recorded. It is morphologically intermediate between the two suggested parents." (Robson 2002: 112).
20. 22. ***H. ellipticum*** Hook., Robson (1996): 120.
9d. 4. ***H. elodeoides*** Choisy, Robson (2001): 72.
—a. ***eledeoides,*** Robson (2001): 74 Fig. 13.
—b. ***wardii*** N.Robson, Robson (2001): 74 Fig. 13.
28. 1. ***H. elodes*** L., Robson (1996): 209 Fig. 27.
17. 1. ***H. elongatum*** Ledeb., Robson (2010b): 139.
—a. var. ***elongatum,*** Robson (2010b): 142 Fig. 7.
—b. var. ***lythrifolium*** (Boiss.) N.Robson, Robson (2010b): 144 Fig. 7.
—c. var. ***racemosum*** (Kuntze) N.Robson, Robson (2010b): 145.
—d. var. ***antasiaticum*** (Grossh.) N.Robson, Robson (2010b): 145 Fig. 9.
19. 4. ***H. empetrifolium*** Willd., Robson (2010a): 246.
—a. subsp. ***empetrifolium,*** Robson (2010a): 247 Fig. 20.
—b. subsp. ***oliganthum*** (Rech.f.) Hagemann, Robson (2010a): 249.
—c. subsp. ***tortuosum*** (Rech.f.) Hagemann, Robson (2010a): 250 Fig. 20.
9b. 4. ***H. epigeium*** R.Keller, Robson (2006): 86 Fig. 20.
9. 31. ***H. erectum*** Thunb., Robson (2006): 53.
—a. var. ***erectum,*** Robson (2006): 57.
—aa. f. ***vaniotii*** (H.Lév.) Y.Kimura, Robson (2006): 57 Fig. 15.
—ab. f. ***erectum,*** Robson (2006): 58.
—ac. f.***debile*** R.Keller, Robson (2006): 59.
—ad. f. ***angustifolium*** (Y.Kimura) Y.Kimura, Robson (2006): 59 Fig. 15.
—ae. f. ***papillosum*** (Y.Kimura) Y.Kimura, Robson (2006): 59.
—af. f. ***tateukianum*** (Koidz.) Y.Kimura, Robson (2006): 59.
—ag. f. ***perforatum*** Y.Kimura, Robson (2006): 60.
—ah. f. ***lutchuense*** (Koidz.) Y.Kimura, Robson (2006): 60.
—b. var. ***caespitosum*** Mokino, Robson (2006): 60.
—c. var. ***deviatum*** Y.Kimura, Robson (2006): 61.
19. 3. ***H. ericoides*** L., Robson (2010a): 244 Fig. 19.
29. 18. ***H. espinalii*** N.Robson, Robson (1990): 22.
9. 17. ***H. faberi*** R.Keller, Robson (2006): 38 Fig. 11.
20. 13. ***H. fasciculatum*** Lam., Robson (1996): 110 Fig. 16.
23. 1. ***H. fieriense*** N.Robson, Robson (1996): 142 Fig. 21.
18. 14. ***H. fissurale*** Woron., Robson (2010a): 223 Fig. 16.
5. 2. ***H. foliosum*** Aiton, Robson (1985): 300.
4. 1. ***H. formosanum*** Maxim., Robson (1985): 289 Fig. 21.
9b. 6. ***H. formosum*** Kunth, Robson (2006): 87 Fig. 21.
3. 42. ***H. forrestii*** (Chitt.) N.Robson, Robson (1985): 286.
—x. ***Hypericum x dummeri*** N.Robson, Robson (1985): 287, 42. *H. forrestii* (female) x 14. *H. calycinum* (male).
18. 26. ***H. fragile*** Heldr. & Sart., Robson (2010a): 236.
20. 1. ***H. frondosum*** Michx., Robson (1996): 94 Fig. 14.
—x. ***H. frondosum x prolificum,*** Robson (1996): 96, „[…] species appear remain distinkt in the field, they hybridize in cultivation; and artificial hybrids between them have been made (Myers, 1963). There is a series of garden forms, intermediate in size of parts between *H. frondosum* and *H. prolificum* […]" (Robson 1996).
29. 79. ***H. fuertesii*** Urb., Robson (1990): 33 Fig. 10.
18. 3. ***H. fursei*** N.Robson, Robson (2010a): 202 Fig. 13.
9. 29. ***H. furusei*** N.Robson, Robson (2006): 52 Fig. 14.
3. 12. ***H. gaitii*** Haines, Robson (1985): 225.
29. 86. ***H. galinum*** S.F.Blake, Robson (1990): 41 Fig. 11.
20. 6. ***H. galioides*** Lam., Robson (1996): 102 Fig. 15.
29. 8. ***H. garciae*** Pierce, Robson (1987): 26 (No. 4).
4. 4. ***H. geminiflorum*** Hemsl., Robson (1985): 292.
—a. subsp. ***geminiflorum,*** Robson (1985): 294 30.
—b. subsp. ***simplicistylum*** (Hayata) N.Robson, Robson (1985): 295.
29. 88. ***H. gentianoides*** (L.) Britton, Sterns & Poggenb., Robson (1990): 44.

29. 15. ***H. gladiatum*** N.Robson, Robson (1990): 21.
27. 1. ***H. glandulosum*** Aiton, Robson (1996): 172 Fig. 25.
—x. ***Hypericum x joerstadii*** Lid, Robson (1996): 175, „shrub intermediate in characters between 1. *H. glandulosum* and 16. *H. reflexum*. […]" (Robson 1996: 175).
29. 37. ***H. gleasonii*** N.Robson, Robson (1987): 55 (No. 25).
30. 49. ***H. globuliferum*** R.Keller, Robson (1990): 141.
1. 10. ***H. gnidiifolium*** A.Rich., Robson (1985): 201.
29. 82. ***H. gnidioides*** Seem., Robson (1990): 37.
29. 24. ***H. goyanesii*** Cuatrec., Robson (1987): 38 (No. 14).
3. 11. ***H. gracilipes*** Stapf ex C.E.C.Fischer, Robson (1985): 224.
9. 35. ***H. gracillimum*** Koidz., Robson (2006): 65.
30. 27. ***H. gramineum*** G.Forst., Robson (1990): 92 Fig. 17.
5. 1. ***H. grandifolium*** Choisy, Robson (1985): 298.
9b. 1. ***H. graveolens*** Buckley, Robson (2006): 80 Fig. 19.
—x. ***Hypericum x mitchellianum*** Rydb., Robson (2006): 80, 1. *H. graveolens* x 9. *H. punctatum (see* Robson 2006: 82 for discussion).
3. 15. ***H. griffithii*** Hook.f. & Thomson ex Dyer, Robson (1985): 230.
30. 38. ***H. gymnanthum*** Engelm., Robson (1990): 113 Fig. 20.
— ***H. gymnanthum x canadense,*** Robson (1990): 115.
— ***H. gymnanthum x mutilum,*** Robson (1990): 115.
9. 37. ***H. hachijyoense*** Nakai, Robson (2006): 68.
9. 38. ***H. hakonense*** Franch. & Sav., Robson (2006): 69 Fig. 17.
29. 69. ***H. harlingii*** N.Robson, Robson (1990): 28.
30. 11. ***H. harperi*** R.Keller, Robson (1990): 67.
29. 34. ***H. hartwegii*** Benth., Robson (1987): 51 (No. 22).
18. 20. ***H. havvae*** Güner, Robson (2010a): 231.
17. 13. ***H. hedgei*** N.Robson, Robson (2010a): 162 Fig. 10.
17. 29. ***H. helianthemoides*** (Spach) Boiss., Robson (2010a): 190 Fig. 12.
9d. 3. ***H. hengshanense*** W.T.Wang, Robson (2001): 72.
3. 30. ***H. henryi*** H.Lév. & Vaniot, Robson (1985): 260.
—a. subsp. ***hancockii*** N.Robson, Robson (1985): 261.
—b. subsp. ***henryi,*** Robson (1985): 263.
3—c. subsp. ***uraloides*** (Rehder) N.Robson, Robson (1985): 263.
24. 1. ***H. heterophyllum*** Vent., Robson (1996): 147 Fig. 22.
9e. 4. ***H. himalaicum*** N.Robson, Robson (2001): 81 Fig. 15.
5. 4. ***H. hircinum*** L., Robson (1985): 307.
—a. subsp. ***majus*** (Aiton) N.Robson, Robson (1985): 310 Fig. 22.
—b. subsp. ***cambessedesii*** (Nyman) Sauvage, Robson (1985): 311 Fig. 22.
—c. subsp. ***metroi*** (Maire & Sauvage) Sauvage, Robson (1985): 312.
—d. subsp. ***hircinum,*** Robson (1985): 312 Fig. 22.
—e. subsp. ***albimontanum*** (Greuter) N.Robson, Robson (1985): 313 Fig. 22.
18. 4. ***H. hirsutum*** L., Robson (2010a): 203.
17. 22. ***H. hirtellum*** (Spach) Boiss., Robson (2010a): 179.
—a. var. ***hirtellum,*** Robson (2010a): 179.
—b. var. ***var._assyriacum*** (Boiss.) N.Robson, Robson (2010a): 180.
3. 28. ***H. hookerianum*** Wight & Arn., Robson (1985): 255.
—a. ***H. hookerianum ‚Charles Rogers',*** Robson (1985): 258.
29. 59. ***H. horizontale*** N.Robson, Robson (1987): 81 (No. 46).
30. 50. ***H. humbertii*** Staner, Robson (1990): 143 Fig. 22.
29. 14. ***H. humboldtianum*** Steud., Robson (1990): 20.
14. 6. ***H. humifusum*** L., Robson (2010b): 102.
—x. ***Hypericum x caesariense*** Druce ex N.Robson, Robson (2010b): 108, 6. *H. humifusum* x 1. *H. linariifolium*.
20. 29. ***H. hypericoides*** (L.) Crantz, Robson (1996): 129.
—a. subsp. ***hypericoides,*** Robson (1996): 131.
—b. subsp. ***multicaule*** (Michx. ex Willd.) N.Robson, Robson (1996): 132.
—c. subsp. ***prostratum*** N.Robson, Robson (1996): 133.
17. 7. ***H. hyssopifolium*** Vill., Robson (2010b): 152.
—a. subsp. ***hyssopifolium,*** Robson (2010b): 154.
—b. subsp. ***acutum*** N.Robson, Robson (2010b): 154.
12. 8. ***H. ichelense*** N.Robson, Robson (2010b): 48.
12. 9. ***H. imbricatum*** Poulter, Robson (2010b): 49.
29. 4. ***H. irazuense*** Kunze ex N.Robson, Robson (1987): 30 (No. 7) Fig. 5.
9. 9. ***H. iwatelittorale*** H.Koidz., Robson (2002): 114.
30. 42. ***H. japonicum*** Thunb. ex Murray, Robson (1990): 122, „[…] very variable species […]": (1)–(3) *‚japonicum'* pro parte & (4) *‚laxum',* (4a) , *maximowiczii',* (4b) *‚laxum',* (4c) *‚calyculatum',* (4d) *javanicum',* (5) *‚humifusum'.* „[…] but the continuous variation prevents their being given formal taxonomic ranks." (Robson 1990: 127 ff.).
29. 62. ***H. jaramilloi*** N.Robson, Robson (1987): 86 (No. 49).
19. 6. ***H. jovis*** Greuter, Robson (2010a): 252.
9. 11. ***H. jozoënse*** Maxim., Robson (2002): 115 Fig. 11.
29. 54. ***H. juniperinum*** Kunth, Robson (1987): 72 (No. 41).
20. 3. ***H. kalmianum*** L., Robson (1996): 98 Fig. 14.
—x. ***H. kalmianum x prolificum,*** Robson (1996): 99, „Utech & Iltis (1970: 335) indicate that intermediates between *H. prolificum* and *H. kalmianum* may occur in Wisconsin […]. In gardens they seem to remain distinct; but it may be impossibile to distinguish depauperate *H. prolificum* plants from true *H. kalmianum*. (For *H. kalmianum* x *densiflorum* see p. 102.)" (Robson 1996: 99).
9. 14. ***H. kamtschaticum*** Ledeb., Robson (2006): 29 Fig. 10.
17. 4. ***H. karjaginii*** Rzazade, Robson (2010b): 148 Fig. 8.

9. 20. ***H. kawaranum*** N.Robson, Robson (2006): 44 Fig. 12.
14. 5. ***H. kelleri*** Bald., Robson (2010b): 101 Fig. 23.
27. 4. ***H. kiboënse*** Oliv., Robson (1996): 177 Fig. 25.
30. 29. ***H. killipii*** N.Robson, Robson (1990): 96 Fig. 18.
9. 22. ***H. kimurae*** N.Robson, Robson (2006): 46 Fig. 12.
9. 36. ***H. kinashianum*** Koidz., Robson (2006): 66 Fig. 17.
9d. 5. ***H. kingdonii*** N.Robson, Robson (2001): 74.
9. 27. ***H. kitamense*** (Y.Kimura) N. Robson, Robson (2006): 51.
9. 40. ***H. kiusianum*** Koidz., Robson (2006): 71.
—a. subsp. ***var._kiusianum,*** Robson (2006): 72 Fig. 18.
—b. subsp. ***var._yakusimense*** (Koidz.) T.Kato, Robson (2006): 72 Fig. 18.
18. 7. ***H. kotschyanum*** Boiss., Robson (2010a): 212.
3. 36. ***H. kouytchense*** H.Lév., Robson (1985): 275.
—x. ***H. kouytchense x calycinum,*** Robson (1985): 276, without formal diagnosis (Robson 1985: 276 f).
—xx. ***Hypericum x ‚Eastleigh Gold'*** Robson (1985): 277, „hybrid (?) seedling in cultivation" (Robson 1985: 278).
9. 28. ***H. kurodakeanum*** N.Robson, Robson (2006): 51.
3. 29. ***H. lacei*** N.Robson, Robson (1985): 258 Fig. 18.
3. 24. ***H. lagarocladum*** N.Robson, Robson (1985): 247.
30. 48. ***H. lalandii*** Choisy, Robson (1990): 138.
3. 38. ***H. lancasteri*** N.Robson, Robson (1985): 279 Fig. 19.
1. 3. ***H. lanceolatum*** Lam., Robson (1985): 188.
—a. subsp. ***angustifolium*** (Lam.) N.Robson, Robson (1985): 190.
—b. subsp. ***lanceolatum,*** Robson (1985): 190.
29. 58. ***H. lancifolium*** Gleason, Robson (1987): 80 (No. 45).
29. 73. ***H. lancioides*** Cuatrec., Robson (1987): 98 (No. 59).
—a. subsp. ***lancioides,*** Robson (1987): 99 (No. 59a) Fig. 14.
—b. subsp. ***congestiflorum*** (Triana & Planch.) N.Robson, Robson (1987): 101 (No. 59b) Fig. 14.
27. 23. ***H. lanuginosum*** Lam., Robson (1996): 206 Fig. 29.
29. 32. ***H. laricifolium*** Juss., Robson (1987): 47 (No. 20) Fig. 8.
3. s.n. ***H. latisepalum*** N.Robson, Robson (2005): 276.
12. 2. ***H. laxiflorum*** N.Robson, Robson (2010b): 39 Fig. 16.
30. 9. ***H. legrandii*** L.B.Sm., Robson (1990): 64.
12. 7. ***H. leprosum*** Boiss., Robson (2010b): 47.
3. 22. ***H. leschenaultii*** Choisy, Robson (1985): 242.
—x. ***Hypericum x ‚Rowallane' (Armytage Moore),*** Robson (1985): 244, 22. *H. leschenaultii* x 33a. *H. hookerianum* ‚Charles Rogers' (= *H. leschenaultii* x "rogersii").
17. 14. ***H. libanoticum*** N.Robson, Robson (2010a): 164.
20. 10. ***H. limosum*** Griseb., Robson (1996): 108.
14. 1. ***H. linariifolium*** Vahl, Robson (2010b): 91 Fig. 23.
18. 10. ***H. linarioides*** Bosse, Robson (2010a): 214.
—a. subsp. ***linarioides,*** Robson (2010a): 217 Fig. 15.
—b. subsp. ***alpestre*** (Steven) N.Robson, Robson (2010a): 218 Fig. 15.
30. 19. ***H. linoides*** A.St.-Hil., Robson (1990): 79.
20. 12. ***H. lissophloeus*** W.P.Adams, Robson (1996): 110 Fig. 16.
29. 70. ***H. llanganaticum*** N.Robson, Robson (1987): 95 (No. 56).
20. 8. ***H. lloydii*** (Svenson) W.P.Adams, Robson (1996): 105 Fig. 15.
3. 10. ***H. lobbii*** N.Robson, Robson (1985): 223.
20. 4. ***H. lobocarpum*** Gatt., Robson (1996): 99.
—x. ***H. lobocarpum x prolificum,*** Robson (1996): 100, „[…]. Adams (1972) agreed that these plants were likely to be hybrids with H. lobocarpum and suggested that they arose spomtaneously in garden; and […], I agree with their suggested parentage. […]" (Robson 1996: 100).
3. 19. ***H. longistylum*** Oliv., Robson (1985): 238.
—a. subsp. ***longistylum,*** Robson (1985): 238.
—b. subsp. ***giraldii*** (R.Keller) N.Robson, Robson (1985): 239.
30. 21. ***H. lorentzianum*** Gilg ex R.Keller, Robson (1990): 80 Fig. 16.
29. 27. ***H. loxense*** Benth., Robson (1987): 43 (No. 18).
—a. subsp. ***aequatoriale*** (R.Keller) N.Robson, Robson (1987): 43 (No. 18a).
—b. subsp. ***loxense,*** Robson (1987): 45 (No. 18b).
9e. 5. ***H. ludlowii*** N.Robson, Robson (2001): 82 Fig. 15.
10. 1. ***H. lycium*** (N.Robson & Hub.-Mor.) N.Robson, Robson (2010b): 19 Fig. 13.
29. 28. ***H. lycopodioides*** Triana & Planch., Robson (1987): 36 (No. 12).
17. 16. ***H. lydium*** Boiss., Robson (2010a): 166 Fig. 10.
17. 25. ***H. lysimachioides*** Boiss. & Noë, Robson (2010a): 182.
—a. var. ***lysimachioides,*** Robson (2010a): 183.
—b. var. ***spathulatum*** N.Robson, Robson (2010a): 184.
26. 3. ***H. macgregorii*** F.Mull., Robson (1996): 158.
3. 33. ***H. maclarenii*** N.Robson, Robson (1985): 270.
9. 1. ***H. maculatum*** Crantz, Robson (2002): 68.
—a. subsp. ***immaculatum*** (Murb.) A.Fröhl., Robson (2002): 68 Fig. 6, the link to *perforatum* in the Balkan region (Robson 2008 pers com.; see Robson 2002: 63, Fig 2, 4).
—b. subsp. ***maculatum,*** Robson (2002): 71 Fig. 6.
—c. subsp. ***obtusiusculum*** (Tourlet) Hayek, Robson (2002): 73 Fig. 6.
—x. ***Hypericum x laschii*** A.Fröhl., Robson (2002): 76, 1. *H. maculatum* x 3. *H. tetrapterum* (see Robson 2002: 76 for discussion.
—xa. ***Hypericum x laschii*** nf. ***laschii,*** Robson (2002): 76, 1c. *H. maculatum* subsp. *maculatum* x 3. *H. tetrapterum*.
—xb ***Hypericum x laschii*** nf. ***froelichii*** N.Robson, Robson (2002): 76, 1c. *H. maculatum* subsp. *obtusiusculum* x 3. *H. tetrapterum*.
— (= 5x.) ***H. maculatum x H. perforatum,*** Robson (2002): 76, „see 5x, p. 102" (Robson 2002).
9b. 8. ***H. macvaughii*** N.Robson, Robson (2006): 91 Fig. 21.
1. 4. ***H. madagascariense*** (Spach) Steud., Robson (1985): 191.
29. 45. ***H. magdalenicum*** N.Robson, Robson (1987): 63 (No. 32).

29. 36. ***H. magniflorum*** Cuatrec., Robson (1987): 53 (No. 24) Fig. 9.
29. 35. ***H. maguirei*** N.Robson, Robson (1987): 52 (No. 23).
30. 36. ***H. majus*** (A.Gray) Britton, Robson (1990): 107 Fig. 20.
—x. ***H. majus x canadense,*** Robson (1990): 110, hybrids occur in mixed populations (Robson 1990: 110).
—xx. ***H. majus x mutilum,*** Robson (1990): 110, hybrids are formed with both subspecies and are intermediate in form between the parents (Robson 1990: 110).
—xxa. subsp. ***mutilum,*** Robson (1990): 110.
—xxb. subsp. ***boreale,*** Robson (1990): 110.
18. 21. ***H. malatyanum*** Peş men, Robson (2010a): 232.
29. 57. ***H. marahuacanum*** N.Robson, Robson (1987): 77 (No. 44).
—a. subsp. ***marahuacanum,*** Robson (1987): 79 (No. 44a) Fig. 12.
—b. subsp. ***strictissimum*** N.Robson, Robson (1987): 79 (No. 44b) Fig. 12.
—c. subsp. ***chimantaicum*** N.Robson, Robson (1987): 80 (No. 44c) Fig. 12, correction of type: Col. No. 758, not 7581 (part 7: 80, part 8: 28).
18. 25. ***H. marginatum*** Woron., Robson (2010a): 236 Fig. 18.
29. 33. ***H. martense*** N.Robson, Robson (1987): 51 (No. 21).
29. 7. ***H. matangense*** N.Robson, Robson (1990): 17.
29. 38. ***H. mexicanum*** L., Robson (1987): 56 (No. 26).
17. 8. ***H. microcalycinum*** Boiss. & Heldr., Robson (2010b): 155 Fig. 7.
30. 2. ***H. microlicioides*** L.B.Sm., Robson (1990): 55.
20. 19. ***H. microsepalum*** (Torr. & A.Gray) A.Gray ex S.Watson, Robson (1996): 117 Fig. 17.
29. 67. ***H. millefolium*** Urb. & Ekman, Robson (1987): 93 (No. 54) Fig. 13.
9. 10. ***H. momoseanum*** Makino, Robson (2002): 115.
18. 22. ***H. monadenum*** N.Robson, Robson (2010a): 233 Fig. 18.
9e. 1. ***H. monanthemum*** Hook.f. & Thomson ex Dyer, Robson (2001): 75.
—a. ***monanthemum,*** Robson (2001): 77 Fig. 14.
—b. ***filicaule*** (Dyer) N.Robson, Robson (2001): 78 Fig. 14.
3. 16. ***H. monogynum*** L., Robson (1985): 231 Fig. 15, 4 forms: (i) 'salicifolium', (ii) 'obtusifolium', (iii) 'latisepalum', (iv) 'ovatum' (Robson (2085: 235).
27. 17. ***H. montanum*** L., Robson (1996): 194 Fig. 28.
13. 6. ***H. montbretii*** Spach, Robson (2010b): 68 Fig. 19.
30. 45. ***H. moranense*** Kunth, Robson (1990): 134 Fig. 21.
30. 39. ***H. mutilum*** L., Robson (1990): 115.
—a. subsp. ***mutilum,*** Robson (1990): 116.
—b. subsp. ***latisepalum*** (Fernald) N.Robson, Robson (1990): 119.
—c. subsp. ***boreale*** (Britton) J.M.Gill&t, Robson (1990): 120.
30. 22. ***H. myrianthum*** Cham. & Schltdl., Robson (1990): 82.
—a. subsp. ***myrianthum,*** Robson (1990): 83 Fig. 16.
—b. subsp. ***tamariscinum*** (Cham. & Schltdl.) N.Robson, Robson (1990): 83 Fig. 16.
29. 25. ***H. myricariifolium*** Hieron., Robson (1987): 40 (No. 15).
20. 23. ***H. myrtifolium*** Lam., Robson (1996): 122.
3. 1. ***H. mysurense*** Wall. ex Wight & Arn., Robson (1985): 210 Fig. 12.
26. 6(i). ***H. nagasawai*** Hayata, Robson (1996): 162.
9. 15. ***H. nakaii*** H.Koidz., Robson (2006): 34.
—a. subsp. ***nakaii,*** Robson (2006): 35 Fig. 10, „description excludes the depauperate ‚var. *debile*'." (Robson 2006: 35).
—b. subsp. ***miyabei*** (Y.Kimura) N.Robson, Robson (2006): 35.
—c. subsp. ***tatewakii*** (S.Watan.) N.Robson, Robson (2006): 35.
4. 2. ***H. nakamurai*** (Masam.) N.Robson, Robson (1985): 289.
22. 4. ***H. nanum*** Poir., Robson (1996): 140 Fig. 20.
—a. subsp. ***nanum,*** Robson (1996): 140.
—b. subsp. ***prostratum*** Boiss., Robson (1996): 141.
26. 8. ***H. natalense*** J.M.Wood & M.S.Evans, Robson (1996): 165 Fig. 24.
27. 14. ***H. naudinianum*** Coss. & Durieu, Robson (1996): 190 Fig. 27.
18. 6. ***H. neurocalycinum*** Boiss. & Heldr., Robson (2010a): 211 Fig. 14.
9. 39. ***H. nikkoense*** Makino, Robson (2006): 70 Fig. 17.
20. 9. ***H. nitidum*** Lam., Robson (1996): 106.
—a. subsp. ***cubense*** (Turcz.) N.Robson, Robson (1996): 106 Fig. 16.
—b. subsp. ***nitidum,*** Robson (1996): 108.
—c. subsp. ***exile*** (W.P.Adams) N.Robson, Robson (1996): 108.
26. 6(ii). ***H. nokoense*** Ohwi, Robson (1996): 163.
20. 17. ***H. nudiflorum*** Michx., Robson (1996): 114 Fig. 17.
18. 18. ***H. nummularioides*** Trautv., Robson (2010a): 228 Fig. 17.
18. 19. ***H. nummularium*** L., Robson (2010a): 229 Fig. 17.
9. 30. ***H. nuporoense*** N.Robson, Robson (2006): 52 Fig. 14.
9b. 2. ***H. oaxacanum*** R.Keller, Robson (2006): 82.
—a. subsp. ***veracrucense*** N.Robson, Robson (2006): 83 Fig. 19.
—b. subsp. ***oaxacanum,*** Robson (2006): 84 Fig. 19.
3. 13. ***H. oblongifolium*** Choisy, Robson (1985): 226.
30. 52. ***H. oligandrum*** Milne-Redh., Robson (1990): 144 Fig. 22.
9. 18. ***H. oliganthum*** Franch. & Sav., Robson (2006): 40.
17. 26. ***H. olivieri*** (Spach) Boiss., Robson (2010a): 184 Fig. 11.
10. 4. ***H. olympicum*** L., Robson (2010b): 23.
—a. f. ***olympicum,*** Robson (2010b): 26 Fig. 14.
—b. f. ***uniflorum*** D.Jord. & Kozuharov, Robson (2010b): 28 Fig. 14.
—c. f. ***tenuifolium*** (D.Jord. & Ko˘zuharov) N.Robson, Robson (2010b): 28 Fig. 14.
—d. f. ***minus*** Hausskn., Robson (2010b): 29 Fig. 14, in culture often called *polyphyllum* (Robson pers com. 19.11.2008).
15/16 (16). 4. ***H. orientale*** L., Robson (2010b): 116 Fig. 24.
12. 1. ***H. origanifolium*** Willd., Robson (2010b): 36 Fig. 15.

—a. var. ***origanifolium,*** Robson (2010b): 37.
—b. var. ***depilatum*** (Freyn & Bornm.) N.Robson, Robson (2010b): 38.
9. 33. ***H. ovalifolium*** Koidz., Robson (2006): 62 Fig. 16.
—a. subsp. ***hisauchii*** (Y.Kimura) N.Robson, Robson (2006): 63 Fig. 16.
—b. subsp. ***ovalifolium*** N.Robson, Robson (2006): 63.
3. 6. ***H. pachyphyllum*** Coll&t & Hemsl., Robson (1985): 219.
23. 5. ***H. pallens*** Banks & Sol., Robson (1996): 145 Fig. 21.
22. 2. ***H. pamphylicum*** N.Robson & P.H.Davis, Robson (1996): 138 Fig. 20.
12. 11. ***H. papillare*** Boiss. & Heldr., Robson (2010b): 52 Fig. 18.
29. 12. ***H. papillosum*** N.Robson, Robson (1987): 34 (No 11) Fig. 6.
26. 5. ***H. papuanum*** Ridl., Robson (1996): 159 Fig. 23.
29. 56. ***H. parallelum*** N.Robson, Robson (1987): 76 (No. 43).
29. 22. ***H. paramitanum*** N.Robson, Robson (1990): 25 Fig. 8.
30. 46. ***H. parvulum*** Greene, Robson (1990): 135.
3. 31. ***H. patulum*** Thunb. ex Murray, Robson (1985): 265.
—x. ***Hypericum x moserianum*** Luquet & ex André, Robson (1985): 266, 31. *H. patulum* x 14. *H. calycinum*; „known only in cultivation" (Robson 1985).
—xa. ***Hypericum x moserianum ‚Tricolor'*** Robson (1985): 268.
30. 32. ***H. pauciflorum*** Kunth, Robson (1990): 101.
30. 44. ***H. paucifolium*** S.Watson, Robson (1990): 132 Fig. 21.
30. 15. ***H. pedersenii*** N.Robson, Robson (1990): 72.
29. 84. ***H. peninsulare*** Eastw., Robson (1990): 40.
26. 10. ***H. peplidifolium*** A.Rich., Robson (1996): 169 Fig. 24.
13. 1. ***H. perfoliatum*** L., Robson (2010b): 56 Fig. 19.
9. 5. ***H. perforatum*** L., Robson (2002): 87, „[…] is apparently an allotetraploid (2n=32), wich, for reasons of morphology and geography, would appear to have arisen from a cross between two diploid taxa (2n=16), viz. 1a. *H. maculatum* subsp. *immaculatum* (Balkans) and 6. *H. attenuatum* (western Siberia to China). […]. The morphological variation in *H. perforatum*, though great, appears to be continuous and therefore theroretically indivisible. For reasons of practicalyty, however, it is convenient to recognize some previously described variants as subspecies, a rank that reflects their geographical basia better than the hitherto more usual varieties. […]" (Robson 2002: 88).
—a. subsp. ***perforatum,*** Robson (2002): 89 Fig. 8.
—b. subsp. ***songaricum*** (Ledeb. ex Rchb.) N. Robson, Robson (2002): 95 Fig. 8.
—c. subsp. ***veronense*** (Schrank) Ces., Robson (2002): 96 Fig. 8.
—d. subsp. ***chinense*** N.Robson, Robson (2002): 101 Fig. 8.
—x. ***Hypericum x desetangsii*** Lamotte, Robson (2002): 102, 1. *H. maculatum* x 5. *H. perforatum* L.
—xa. nsubsp. ***desetangsii*** N.Robson, Robson (2002): 104 Fig. 9, 5. *H. perforatum* x 1c. *H. maculatum* subsp. *obtusiusculum*.
—xb. nsubsp. ***carinthiacum*** (A.Fröhl) N.Robson, Robson (2002): 105, 5. *H. perforatum* L. x 1b. *H. maculatum* subsp. *maculatum*.
—xba. nf. ***maculatiforme*** (A.Fröhl) N.Robson, Robson (2002): 106 Fig. 9.
—xbb. nf. ***perforatiforme*** (A.Fröhl) N.Robson, Robson (2002): 106 Fig. 9.
—xc. nsubsp. ***balcanicum*** N.Robson, Robson (2002): 107 Fig. 9, 1a. *H. maculatum* subsp. *immaculatum* x *perforatum*.
—xx. ***Hypericum x medium*** Peterm., Robson (2002): 108 5. *H. perforatum* x 4. *H. tetrapterum*.
—xxx. ***H. perforatum x H. maculatum x H. tetrapterum,*** Robson (2002): 108, 5x. *H.* x *desetangsii* x 3. *H. tetrapterum*.
18. 23. ***H. peshmenii*** Yild., Robson (2010a): 233.
9d. 2. ***H. petiolulatum*** Hook.f. & Thomson ex Dyer, Robson (2001): 69.
—a. subsp. ***yunnanense*** (Franch.) N.Robson, Robson (2001): 70 Fig. 12.
—b. subsp. ***petiolulatum,*** Robson (2001): 71 Fig. 12.
29. 3. ***H. phellos*** Gleason, Robson (1987): 23 (No. 3).
—a. subsp. ***marcescens*** N.Robson, Robson (1990): 16.
—b. subsp. ***phellos,*** Robson (1987): 23 (No. 3a, variants ii—vi).
—c. subsp. ***oroqueanum*** N.Robson, Robson (1987): 25 (No. 3b).
—d. subsp. ***platyphyllum*** (Gleason) N.Robson, Robson (1987): 25 (No. 3c).
30. 34. ***H. philonotis*** Cham. & Schltdl., Robson (1990): 105 Fig. 19.
9. 13. ***H. pibairense*** (Miyabe & Y.Kimura) N.Robson, Robson (2006): 29 Fig. 10.
29. 44. ***H. pimeleoides*** Planch. & Linden ex Triana & Planch., Robson (1987): 60 (No. 31).
29. 10. ***H. piriai*** Arechav., Robson (1987): 28 (No. 6).
30. 41. ***H. pleiostylum*** C.Rodr.Jim., Robson (1990): 122.
3. 3. ***H. podocarpoides*** N.Robson, Robson (1985): 214 Fig. 13.
30. 17. ***H. polyanthemum*** Klotzsch ex Reichardt, Robson (1990): 75.
10. 3. ***H. polyphyllum*** Boiss. & Balansa, Robson (2010b): 22 Fig. 13.
30. 33. ***H. pratense*** Cham. & Schltdl., Robson (1990): 102 Fig. 19.
3. 18. ***H. prattii*** Hemsl., Robson (1985): 236.
29. 41. ***H. prietoi*** N.Robson, Robson (1987): 58 (No. 28).
9b. 5. ***H. pringlei*** S.Watson, Robson (2006): 87 Fig. 20.
20. 2. ***H. prolificum*** L., Robson (1996): 96 Fig. 14.
29. 55. ***H. prostratum*** Cuatrec., Robson (1987): 75 (No. 42).
18. 8. ***H. pruinatum*** Boiss. & Balansa, Robson (2010a): 213 Fig. 14.
7. 2. ***H. przewalskii*** Maxim., Robson (2001): 59 Fig. 10.
9. 24. ***H. pseudoerectum*** N.Robson, Robson (2006): 47.

3. 41. ***H. pseudohenryi*** N.Robson, Robson (1985): 283 Fig. 20.
17. 28. ***H. pseudolaeve*** N.Robson, Robson (2010a): 187 Fig. 12.
9b. 7. ***H. pseudomaculatum*** Bush, Robson (2006): 90.
9. 42. ***H. pseudopetiolatum*** R.Keller, Robson (2006): 75 Fig. 18.
—x. ***Hypericum x hyugamontanum*** Y.Kimura, Robson (2006): 78, 42. *H. pseudopetiolatum* x 40a. *H. kiusianum* subsp. *kiusianum*, „[…] the parents of this hybrid grow in different habits and thus rarely hybridise. In Kyushu, however, habitat disturbance has often resulted in their growing in close proximity and hybridising. The hybrids […] are partially fertile, wich results in back-crossing and introgression." (Robson 1006: 78 f).
18. 9. ***H. pseudorepens*** N.Robson, Robson (2010a): 214 Fig. 15.
27. 8. ***H. psilophytum*** (Diels) Maire, Robson (1996): 184.
27. 7. ***H. pubescens*** Boiss., Robson (1996): 181 Fig. 26.
—x. ***H. pubescens x tomentosum,*** Robson (1996): 184, 7. *H. pubescens* x 9. *H. tomentosum*, „[…] intermediate in form between the parents. […]" (Robson 1996: 184).
18. 2. ***H. pulchrum*** L., Robson (2010a): 199 Fig. 13.
26. 6. ***H. pulogense*** Merr., Robson (1996): 162.
18. 24. ***H. pumilio*** Bornm., Robson (2010a): 234 Fig. 18.
30. 43. ***H. pumillum*** Sessé & Moc., Robson (1990): 130.
—a. subsp. ***diffusum*** (Rose) N.Robson, Robson (1990): 131.
—b. subsp. ***pumillum,*** Robson (1990): 131.
9b. 9. ***H. punctatum*** Lam., Robson (2006): 92 Fig. 21.
29. 66. ***H. pycnophyllum*** Urb., Robson (1987): 92 (No. 53) Fig. 13.
1. 7. ***H. quartinianum*** A.Rich., Robson (1985): 194 Fig. 10.
29. 26. ***H. quitense*** R.Keller, Robson (1987): 41 (No. 17) Fig. 7.
29. 21. ***H. radicans*** N.Robson, Robson (1990): 24.
29. 49. ***H. recurvum*** N.Robson, Robson (1987): 68 (No. 36).
27. 16. ***H. reflexum*** L., Robson (1996): 193 Fig. 28.
30. 28. ***H. relictum*** N.Robson, Robson (1990): 95 Fig. 18.
14. 4. ***H. repens*** L., Robson (2010b): 99 Fig. 23.
3. 5. ***H. reptans*** Hook.f. & Thomson ex Dyer, Robson (1985): 218.
17. 15. ***H. retusum*** Aucher, Robson (2010a): 165 Fig. 10.
1. 2. ***H. revolutum*** Vahl, Robson (1985): 182.
a. subsp. ***keniense*** (Schweinf.) N.Robson, Robson (1985): 184.
b. subsp. ***revolutum,*** Robson (1985): 185.
13. 11. ***H. richeri*** Vill., Robson (2010b): 83.
—a. subsp. ***grisebachii*** (Boiss.) Nyman, Robson (2010b): 86 Fig. 22.
—b. subsp. ***richeri,*** Robson (2010b): 88 Fig. 22.
—c. subsp. ***burseri*** (DC) Myman, Robson (2010b): 89 Fig. 22.
—x. ***Hypericum x reinosae*** A.Ramos, Robson (2010b): 90, 10c. *H. richeri* subsp. *burseri* x 5. *H. perforatum*.
30. 1. ***H. rigidum*** A.St.-Hil., Robson (1990): 51.
—a. subsp. ***rigidum,*** Robson (1990): 52 Fig. 12.
—b. subsp. ***meridionale*** (L.B.Sm.) N.Robson, Robson (1990): 52 Fig. 13.
—c. subsp. ***sellowianum*** (R.Keller) N.Robson, Robson (1990): 54 Fig. 14.
—d. subsp. ***brecteatum*** N.Robson, Robson (1990): 55.
13. 9. ***H. rochelii*** Griseb. & Schenk, Robson (2010b): 79 Fig. 21.
1. 9. ***H. roeperianum*** G.W.Schimp. ex A.Rich., Robson (1985): 198.
29. 16. ***H. roraimense*** Gleason, Robson (1990): 22.
29. 78. ***H. rubritinctum*** N.Robson, Robson (1990): 33 Fig. 10.
13. 7. ***H. rumeliacum*** Boiss., Robson (2010b): 71.
—a. subsp. ***rumeliacum,*** Robson (2010b): 74 Fig. 20.
—b. subsp. ***apollinis*** (Boiss. & Heldr.) N.Robson & Strid, Robson (2010b): 75 Fig. 20.
22. 1. ***H. rupestre*** Jaub. & Spach, Robson (1996): 137 Fig. 20.
29. 68. ***H. ruscoides*** Cuatrec., Robson (1987): 93 (No. 55).
25. 2. ***H. russeggeri*** (Fenzl) R.Keller, Robson (1996): 152.
29. 31. ***H. sabiniforme*** Trevir., Robson (1987): 45 (No. 19).
17. 6. ***H. salsolifolium*** Hand.-Mazz., Robson (2010b): 151 Fig. 8.
12. 13. ***H. salsugineum*** N.Robson & Hub.-Mor., Robson (2010b): 54.
30. 20. ***H. salvadorense*** N.Robson, Robson (1990): 79.
9c. 1. ***H. sampsonii*** Hance, Robson (2001): 63 Fig. 11.
26. 2. ***H. saruwagedicum*** Diels, Robson (1996): 157 Fig. 23.
18. 28. ***H. saxifragum*** N.Robson & Hub.-Mor., Robson (2010a): 238 Fig. 18.
17. 18. ***H. scabroides*** N.Robson & Poulter, Robson (2010a): 170.
17. 19. ***H. scabrum*** L., Robson (2010a): 171.
30. 51. ***H. scioanum*** Chiov., Robson (1990): 143 Fig. 22.
23. 2. ***H. scopulorum*** Balf., Robson (1996): 143 Fig. 21.
9. 12. ***H. scouleri*** Hook., Robson (2002): 117 Fig. 11.
29. 74. ***H. selaginella*** N.Robson, Robson (1987): 102 (No. 60).
9. 16. ***H. senanense*** Maxim., Robson (2006): 35.
—a. subsp. ***senanense,*** Robson (2006): 37 Fig. 11.
—b. subsp. ***mutiloides*** (R.Keller) N.Robson, Robson (2006): 37 Fig. 11.
9d. 1. ***H. seniawinii*** Maxim., Robson (2001): 67 Fig. 12.
4. 3. ***H. senkakuinsulare*** Hatus., Robson (1985): 292.
30. 12. ***H. setosum*** L., Robson (1990): 68 Fig. 14.
26. 1. ***H. sewense*** N.Robson, Robson (1996): 154 Fig. 23.
3. 4. ***H. sherriffii*** N.Robson, Robson (1985): 217.
3. 21. ***H. siamense*** N.Robson, Robson (1985): 240 Fig. 16.
9. 19. ***H. sikokumontanum*** Makino, Robson (2006): 42.

30. 25. ***H. silenoides*** Juss., Robson (1990): 87.
—a. subsp. ***silenoides,*** Robson (1990): 88 Fig. 17.
—b. subsp. ***minus*** N.Robson, Robson (1990): 90 Fig. 17, corection of type citatiopn: Robson (1996): 76, first paragraph.
29. 11. ***H. simonsii*** N.Robson, Robson (1987): 34 (No 10) Fig. 6.
27. 12. ***H. sinaicum*** Hochst. ex Boiss, Robson (1996): 188 Fig. 26.
1. 6. ***H. socotranum*** Good, Robson (1985): 192.
—a. subsp. ***socotranum,*** Robson (1985): 193.
—b. subsp. ***smithii*** N.Robson, Robson (1985): 194.
27. 10. ***H. somaliense*** N.Robson, Robson (1996): 187.
17. 9. ***H. sorgerae*** N.Robson, Robson (2010b): 155 Fig. 9.
17. 24. ***H. spectabile*** Jaub. & Spach, Robson (2010a): 182 Fig. 11.
20. 20. ***H. sphaerocarpum*** Michx., Robson (1996): 118.
29. 47. ***H. sprucei*** N.Robson, Robson (1987): 65 (No. 34).
13. 10. ***H. spruneri*** Boiss., Robson (2010b): 82 Fig. 21.
3. 37. ***H. stellatum*** N.Robson, Robson (1985): 278 Fig. 19.
29. 5. ***H. stenopetalum*** Turcz., Robson (1987): 31 (No. 8).
29. 61. ***H. strictum*** Kunth, Robson (1987): 83 (No. 48).
—a. subsp. ***strictum,*** Robson (1987): 84 (No. 48a).
—b. subsp. ***compactum*** (Triana & Planch.) N.Robson, Robson (1987): 85 (No. 48b).
29. 71. ***H. struthiolifolium*** Juss., Robson (1987): 96 (No. 57).
29. 40. ***H. stuebelii*** Hieron., Robson (1987): 58 (No. 27).
29. 2. ***H. styphelioides*** A.Rich., Robson (1987): 20 (No. 2).
—a. subsp. ***clarense*** Lippold, Robson (1987): 21 (No. 2a).
—b. subsp. ***styphelioides,*** Robson (1987): 21 (No. 2b).
—c. subsp. ***moaense*** Lippold, Robson (1987): 22 (No. 2c).
4. 5. ***H. subalatum*** Hayata, Robson (1985): 296.
9e. 2. ***H. subcordatum*** (R.Keller) N.Robson, Robson (2001): 78 Fig. 14.
3. 20. ***H. subsessile*** N.Robson, Robson (1985): 239.
20. 28. ***H. suffruticosum*** W.P.Adams, Robson (1996): 128 Fig. 18.
1. 8. ***H. synstylum*** N.Robson, Robson (1985): 197.
9. 41. ***H. taihezanense*** Sasaki ex Sad.Suzuki, Robson (2006): 74 Fig. 18.
18. 27. ***H. taygeteum*** Quézel & Contandr., Robson (2010a): 237.
3. 9. ***H. tenuicaule*** Hook.f. & Thomson ex Dyer, Robson (1985): 222.
20. 7. ***H. tenuifolium*** Pursh, Robson (1996): 104 Fig. 15, 16.
30. 3. ***H. teretiusculum*** A.St.-Hil., Robson (1990): 56.
23. 4. ***H. ternatum*** Poulter, Robson (1996): 145 Fig. 21.
30. 8. ***H. ternum*** A.St.-Hil., Robson (1990): 62.
29. 1. ***H. terrae firmae*** Sprague & Riley, Robson (1987): 18 (No. 1) Fig. 4.
20. 26. ***H. tetrapetalum*** Lam., Robson (1996): 127 Fig. 18.
9. 3. ***H. tetrapterum*** Fr., Robson (2002): 80.
—a. var. ***tetrapterum,*** Robson (2002): 82 Fig. 7.
—b. [var.] *gamma* ***corsicum*** (Steud.) Boiss., Robson (2002): 84.
—c. var. ***anagallidifolium*** Boiss., Robson (2002): 84.
29. 60. ***H. tetrastichum*** Cuatrec., Robson (1987): 82 (No. 47).
15/16 (15). 1. ***H. thasium*** Griseb., Robson (2010b): 110 Fig. 24.
18. 16. ***H. theodori*** Woron., Robson (2010a): 227 Fig. 16.
30. 30. ***H. thesiifolium*** Kunth, Robson (1990): 98.
29. 30. ***H. thuyoides*** Kunth, Robson (1987): 37 (No. 13).
17. 17. ***H. thymbrifolium*** Boiss. & Noë, Robson (2010a): 169.
18. 15. ***H. thymifolium*** Banks & Sol., Robson (2010a): 224 Fig. 17.
17. 20. ***H. thymopsis*** Boiss., Robson (2010a): 175.
27. 9. ***H. tomentosum*** L., Robson (1996): 185 Fig. 26.
23. 3. ***H. tortuosum*** Balf., Robson (1996): 143 Fig. 21.
9. 8. ***H. tosaense*** Makino, Robson (2002): 112.
12. 4. ***H. trachyphyllum*** Griseb., Robson (2010b): 42 Fig. 17.
13. 2. ***H. trichocaulon*** Boiss. & Heldr., Robson (2010b): 61 Fig. 19.
9e. 3. ***H. trigonum*** Hand.-Mazz., Robson (2001): 79 Fig. 15.
9. 4. ***H. triquetrifolium*** Turra, Robson (2002): 84 Fig. 7, „*H. triquetrifolium* is the easterm Mediterranean counterpart of the Atlantic 2. *H. undulatum*; but it has a qiute different habitat. […]" (Robson 2003: 87).
17. 2. ***H. tymphresteum*** Boiss. & Spruner, Robson (2010b): 146 Fig. 7.
13. 4. ***H. umbellatum*** A.Kern., Robson (2010b): 64.
6a. 1. ***H. umbraculoides*** N.Robson, Robson (1985): 318 Fig. 24.
9. 2. ***H. undulatum*** Schousb. ex Willd., Robson (2002): 76.
—a. var. ***undulatum,*** Robson (2002): 78 Fig. 7.
—b. var. ***boeticum*** (Boiss.) Lange, Robson (2002): 79.
2. ***H. undulatum x H. tetrapterum*** Schousb. ex Willd., Robson (2002): 80.
12. 12. ***H. uniflorum*** Boiss. & Heldr., Robson (2010b): 53.
17. 5. ***H. uniglandulosum*** Hausskn. ex Bornm., Robson (2010b): 151 Fig. 8.
3. 32. ***H. uralum*** Buch.-Ham. ex D.Don, Robson (1985): 268.
22. 5. ***H. vacciniifolium*** Hayek & Siehe, Robson (1996): 141 Fig. 20.
18. 17. ***H. vaccinioides*** N.Robson, Robson (2010a): 227 Fig. 16.
29. 46. ***H. valleanum*** N.Robson, Robson (1987): 64 (No. 33) Fig. 11.
18. 1. ***H. venustum*** Fenzl, Robson (2010a): 196 Fig. 13.
17. 30. ***H. vermiculare*** Boiss. & Hausskn., Robson (2010a): 192 Fig. 12.
13. 3. ***H. vesiculosum*** Griseb., Robson (2010b): 63.
9. 25. ***H. vulcanicum*** Koidz., Robson (2006): 48 Fig. 13.

9. 21. ***H. watanabei*** N.Robson, Robson (2006): 44 Fig. 12.
9e. 7. ***H. wightianum*** Wall. ex Wight & Arn., Robson (2001): 83 Fig. 13.
3. 8. ***H. williamsii*** N.Robson, Robson (1985): 221.
26. 9. ***H. wilmsii*** R.Keller, Robson (1996): 168 Fig. 24.
3. 25. ***H. wilsonii*** N.Robson, Robson (1985): 248.
29. 29. ***H. woodianum*** N.Robson, Robson (1990): 25.
29. 50. ***H. wurdackii*** N.Robson, Robson (1987): 69 (No. 37).
6. 1. ***H. xylosteifolium*** (Spach) N.Robson, Robson (1985): 314 Fig. 23.
9. 26. ***H. yamamotoanum*** H.Koidz., Robson (2006): 50 Fig. 13.
9. 23. ***H. yamamotoi*** Miyabe & Y.Kimura, Robson (2006): 46.
9. 32. ***H. yojiroanum*** Tate. & Koji Ito, Robson (2006): 62 Fig. 14.

Lianthus N.Robson, Robson (2001): 38,
L. ellipticifolius (H.L.Li) N.Robson, Robson (2001): 38; Xiwen & Robson (2007) Fig. 1, type as for *Hypericum ellipticifolium* H.L.Li.

Santomasia N.Robson, Robson (1981): 62,
S. steyermarkii (Standley) N.Robson, Robson (1981): 62 Fig. 1.

Thornea Breedlove & E.M.McClint., Stevens (2007):194 ff.,
Th. matudae (Lundell) Breedlove & E.M.McClint., Breedlove 40408.
Th. calcicola (Standl. & Steyerm.) Breedlove & E.M.McClint., Madroño 23: 370. 1976.

Triadenum Raf., Stevens (2007): 194 ff.,
Tr. japonicum (Blume) Makino, Naito s.n. (1973); Robson (2006); Xiwen & Robson (2007).
Tr. breviflorum (Wallich ex Dyer) Y.Kimura, Xiwen & Robson (2007).
Tr. fraseri (Spach) Gleason, Phytologia 2: 289. 1947.
Tr. viginicum Raf., Fl. Tellur. 3: 79. 1837 [1836 publ. Nov-Dec 1837].
Tr. viginicum Raf. subsp. ***fraseri*** (Spach) Á.Löve & D.Löve, Taxon 31: 344. 1982.
Tr. walteri (J.F.Gmel.) Gleason, Phytologia 2: 289. 1947.
Tr. tubulosum (Walter) Gleason, Phytologia 2: 289. 1947.

Vismieae Choisy

Harungana Lam., Stevens (2007): 194 ff.,
Har. madagascariensis Poir.

Vismia Vandt., Stevens (2007): 194 ff.,
V. cayennensis (Jacq.) Pers.
V. guianensis (Aubl.) Choisy, Prodr. (DC.) 1: 542. 1824 [mid Jan 1824].
V. macrophylla Kunth, Nov. Gen. Sp. [H.B.K.] v. 184.

Appendix S2.1 Morphological phylogeny

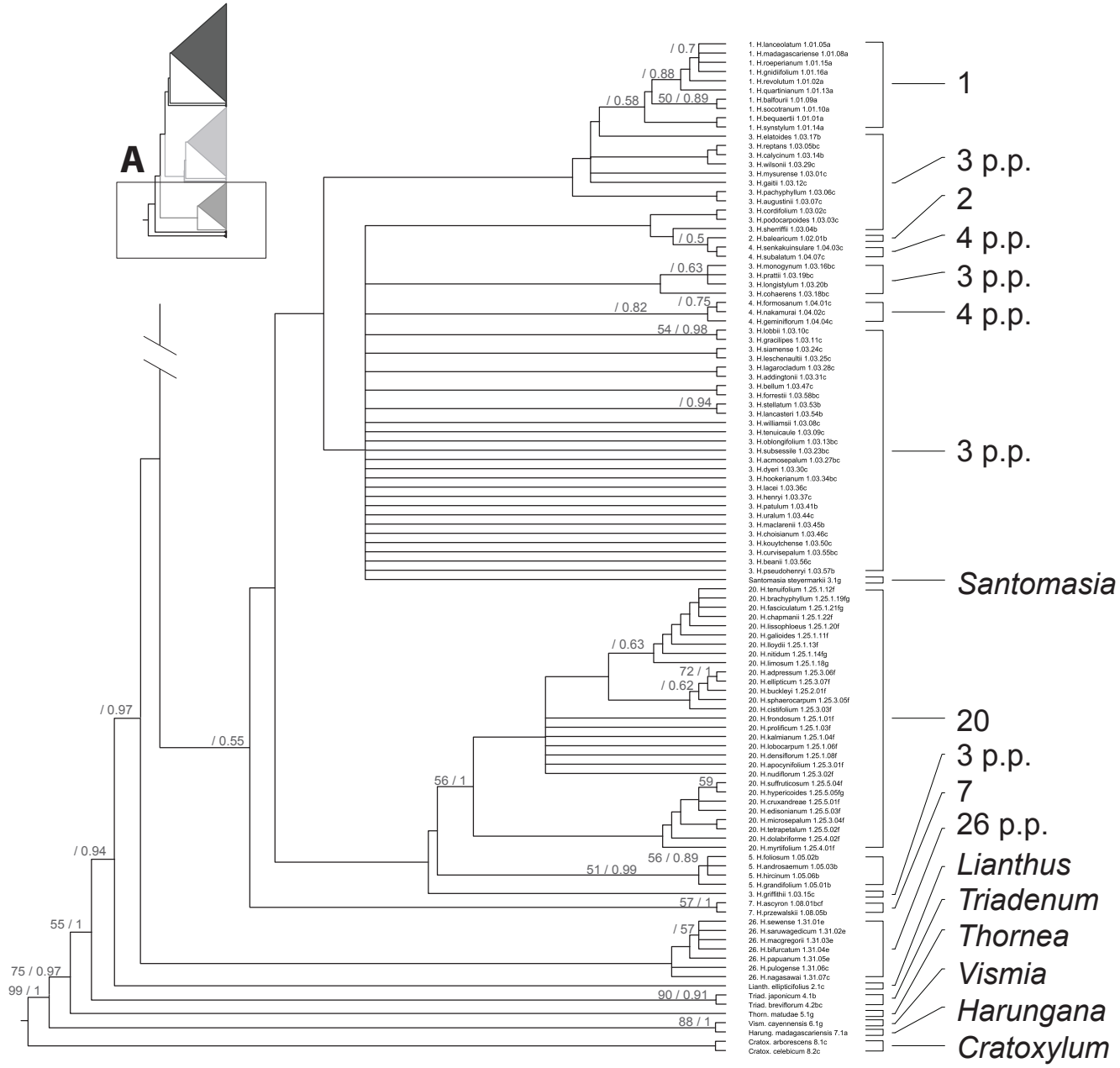

Fig. S2.1-A–C (part A), at right page. Strict consensus MP tree based on 89 morphological characters, showing the relationship of 457 *Hypericum* species and nine outgroup representatives. Square brackets and section numbers (numbers refer to Table 2.1) mark the position of sections within *Hypericum*. Numbers of subsections and series are given for section 9. *Hypericum* only. Bootstrap values (bs in %) and posterior probabilities (pp) are given above the branches [bs/pp]. Section numbers before, and an artificial code behind the species name (giving the species an exact position (in upward order) in Robson's classification), as well as distribution of species are given (a = Afrotropic, b = Palaearctic, c = Indo-Malaysia, d = Oceania, e = Australasia, f = Nearctic, g = Neotropic).

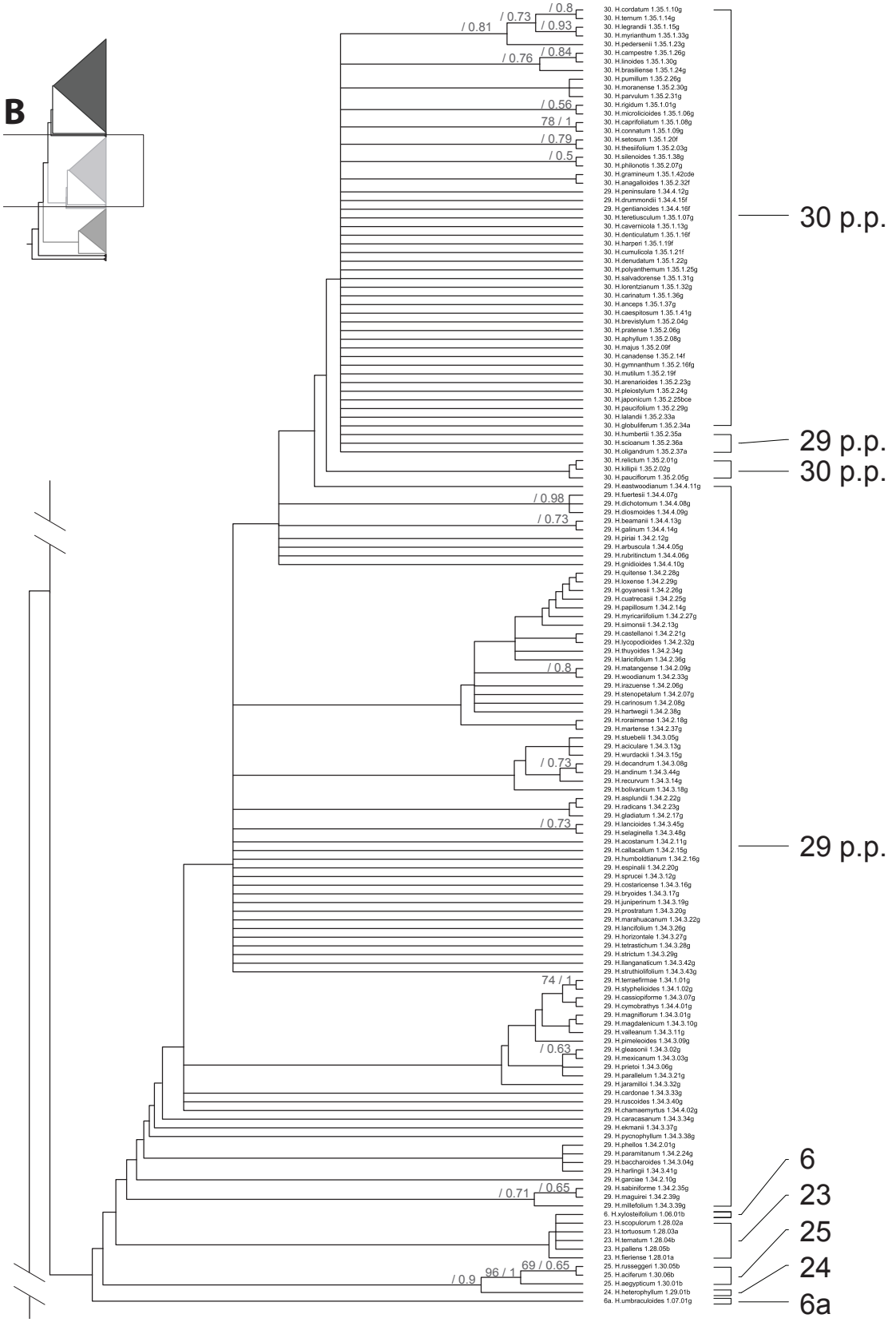

Fig. S2.1-A–C (part B), continued. a = Afrotropic, b = Palaearctic, c = Indo-Malaysia, d = Oceania, e = Australasia, f = Nearctic, g = Neotropic

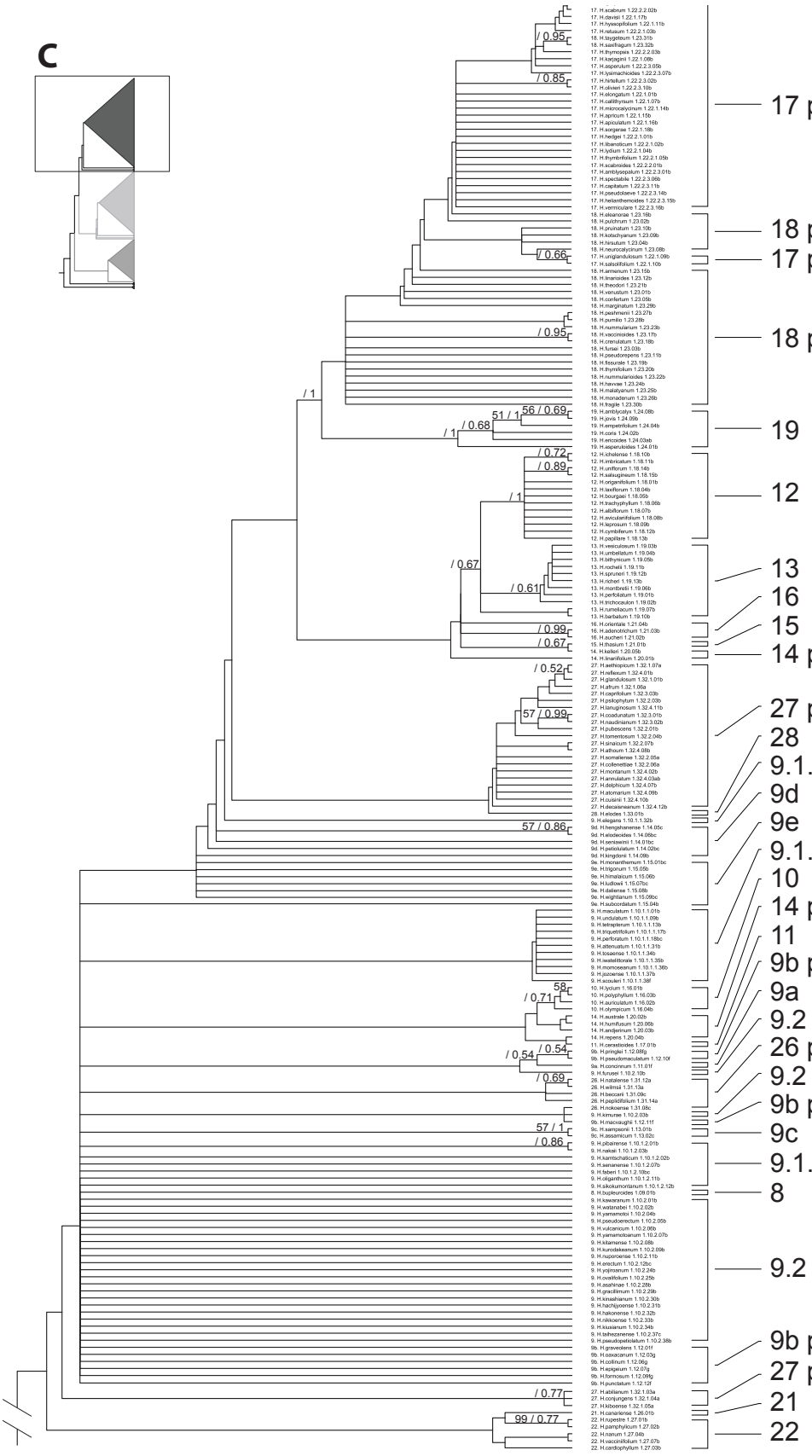

Fig. S2.1-A–C (part C), continued.

Appendix S2.2 Morphological character coding

Habitat: **1)** Elevation: lowland (0 to ~1000 m a.s.l.) (n), montane (>1000 m a.s.l. and not <500 m a.s.l.) (y). **2)** Humidity-preference: wet (plants standing in water) (a), humid (b), dry (c). Habit: **3)** Live form: tree (a), shrub (b), perennial herb (c), annual herb (d). **4)** Runners: absent (n), present (y). **5)** Taproot: absent (n), present (y). **6)** Vegetative layer (aerial bulbils etc.): absent (n), present (y). **7)** Wood parenchyma: absent (n), present (y). Stem: **8)** Number of stem lines: absent (a), two (b), three (c), four (d), six (e). **9)** Stem ancipitous (*i. e.*, complanate, two-edged): not ancipitous (n), ancipitous (y). **10)** Stem terete (rounded at node): not terete (n), terete (y). **11)** Glands on stem – type: pale (a), red (b), black (c), amber (d), absent (eglandular) (e). **12)** Indumentum: stem glabrous (n), stem hairy (y). **13)** Indumentum stellate (multicellular): unicellular trichomes (n), stellate trichomes (y). **14)** Cortex exfoliating: persistent (cortex not exfoliating) (a), exfoliating in flakes (b), sheets or plates (c), strips (d), scales (e), irregularly (f). **15)** Internodes: shorter than leaves (n), longer than leaves (y). Leaves: **16)** Phyllotaxis: opposite (a), three-whorled (b), four-whorled (c). **17)** Leaf type: foliage (a), linear to "ericoid" (b), scale (<2 mm long) (c). **18)** Insertion: sessile (a), sub-sessile (or sub-petiolate: ≤0.5 long) (b), petiolate (>0.5 mm long) (c). **19)** Adnation of opposite leaf bases: not clasping the stem (free) (a), amplexicaul (stem clasping) (b), perfoliate (connate pairs) (c). **20)** Indumentum: leaves glabrous (n), leaves hairy (y). **21)** Margin: not entire (denticulate to ciliate) (n), entire (y). **22)** Leaf persistence (deciduousness): persistent (evergreen plant) (n), deciduous (y). **23)** Stomatal type: paracytic (n), anomocytic or cyclocytic (y). **24)** Leaf venation I – type: parallelodromus (a), pinnate (b), one nerved (midrib only) (c). **25)** Leaf venation II – adnation of pinnate veins: not all lateral veins adnate among themselves (n), all lateral veins adnate among themselves (y). **26)** Leaf venation III – tertiary reticulum: absent (n), present (y). Glands on leaves: **27)** Laminar glands I – type: pale (a), red (b), dark (c), absent (d). **28)** Laminar glands II – shape: linear (not interrupted) (a), streaks and short lines (interrupted or striiform) (b), small dots (punctiform) (c). **29)** Marginal glands – type: pale (a), red (b), dark (c), absent (d). **30)** Ventral glands: absent (n), present (y). Inflorescence: (Inflorescences in *Hypericum* are generally cymose (Robson, 1981: **83)**, but several modifications make it difficult to describe inflorescences within *Hypericum* by single terms without loosing information. Therefore we coded inflorescences in a key-like way, following the descriptions given in Robson (1981: 83 ff.).) **31)** Ramification on terminal node: alternate (a), decussate (b), pleiochasial (c), not branched (uniflor) (d). **32)** Position of flowering branches: on terminal node only (n), flowering branches also from lower nodes (y). **33)** Ramification of inflorescence branches above the terminal node: alternate (a), decussate (b), monochasial (c), dichasial (d), pseudo-dichotomous (e), "sympodial" (f), not branched (uniflor) (g). **34)** Subsidiary inflorescence branches: not divided by only-leaf-bearing nodes from terminal inflorescence (n), divided by only-leaf-bearing nodes from terminal inflorescence (y). **35)** Bracts margin: entire (n), not entire (denticulate, ciliate or fimbriate) (y). **36)** Glands on bracts margin: absent (n), present (y). Flowers: **37)** Corolla type: stellate (or infundibuliform = obconic) (a), cyathiform (b), campanulate (c), tubular (d). **38)** Ligulate outgrowth of petals: absent (a), ligula entire (b), ligula trifid (c), ligula fringed (d). **39)** Style: homostylous (n), heterostyous (y). **40)** Merosity: pentamerous (a), tetramerous (b), trimerous (c), hexamerous (d). Sepals: **41)** Connation of sepals: free (a), united at base (b), ≥2/3 united (c). **42)** Sepals persistence (deciduousness): persistent (n), deciduous (y). **43)** Position of sepals in fruit: erect (a), spreading (b), reflexed or recurved (c). **44)** Margin of sepals: entire (n), not entire (fringed) (y). **45)** Sepal veins branched: not branched (n), branched (y). **46)** Dimorphism in sepals: equal (to subequal) (a), unequal (to subequal) (b), dimorphic ('markedly unequal') (c) [also coded: "subequal or equal to unequal" (ab)]. Glands on Sepals: **47)** Laminar glands I – type: pale (a), red (b), dark (c), absent (d). **48)** Laminar glands II – shape: linear (not interrupted) (a), streaks and short lines (interrupted or striiform) (b), small dots (punctiform) (c). **49)** Marginal glands I – type: pale (a), red (b), dark (c), absent (d). **50)** Marginal glands II – position: marginal (a), inframarginal (b), submarginal (c). **51)** Marginal glands: sessile (n), stipitate (raised) (y). Petals: **52)** Color: yellow (a), pink (b), white (c), greenish (d). **53)** Corolla aestivation: imbricate (n), contorted (y). **54)** Petals shape: symmetrical (n), asymmetrical (y). **55)** Petals pubescent on adaxial surface: not pubescent (glabrous) (n), pubescent (y). **56)** Petals persistence (deciduousness): persistent (n), deciduous (y). **57)** Apiculus on petals: absent (a), apical (b), lateral or subapical (c). **58)** Margin of petals: entire (n), not entire (fringed, ciliate or denticulate) (y). Glands on petals: **59)** Laminar glands I – type: pale (a), red (b), dark (c), absent (d). **60)** Laminar glands II – shape: linear (not interrupted) (a), streaks and short lines (interrupted or striiform) (b), small dots (punctiform) (c). **61)** Marginal glands – type: pale (a), red (b), dark

(c), absent (d). **62)** Marginal glands raised: not raised (sessile) (n), raised (stipitate) (y). Androecium: **63)** Configuration (arrangement of stamens): 5 free fascicles (a), 2 + 1 + 1 + 1 fascicles (b), 2 + 2 + 1 fascicles (c), 2 + 2 + 1 fascicles + 3 sterile fascicles (d), narrow continuous ring (e), broad continuous ring (f), tetramer ring by elimination (g), 5 obscure fascicles (h), 3 obscure fascicles (i), 5 single stamens (j). **64)** Stamens persistence (deciduousness): persistent (n), deciduous (y). **65)** Proportion of stamen length to petal length (stamen/ petal): 0.20–0.59 (a), 0.60–0.90 (b), 0.91–1.50(–2.00) (c). **66)** Interstaminal glands: absent (n), present (y). **67)** Connation of stamina: free (a), shortly united (b), united above middle (c). **68)** Gland on anthers: absent (a), amber (b), black (c). **69)** Staminodes (vestigial fascicles): absent (a), three staminodes (b), five staminodes (c). **70)** Pollen grains type: I (a), II (b), III (c), IV (d), V (e), VI (f), VII (g), VIII (h), IX (i), X (j), XI (k). Gynoecium: **71)** Placentation: parietal (a), loosely axile (b), axile (c). **72)** Number of seeds per ovary: few (n), many (∞) (y). **73)** Number of styles: five (a), four (b), three (c), two (d), six (e), seven (f), eight (g) [f, g: only in species *H. pleiostylum* C. Rodr.Jim.]. **74)** Proportion of style length to ovary length (style/ovary): 0.01–0.59 (a), 0.60–0.99 (b), 1.00–1.59 (c), 1.60–1.99 (d), 2.00–2.99 (e), 3.00–3.99 (f), 4.00–4.99 (–7.00) (g). **75)** Union of styles: free (a), partly united (in flower) (b), complete union (also united in fruit) (c). **76)** Stigma shape: (sub-)globose (a), (sub-)capitate (also 'rounded', 'truncate', 'peltate') (b), narrow or small (at least not capitate) (c), ellipsoid (d), cylindrical (e), clavate (f), infundibuliform (g). **77)** Persistence of style: breaks off in fruit (n), persists on fruit (y). Fruit: **78)** Type of fruit: capsule (n), berry (y). **79)** Capsule aperture mechanism: loculicidal (n), septicidal (y). **80)** Proportion of fruit length to sepals length: shorter than sepals (a), equaling sepals (b), exceeding sepals (c). **81)** Fruit enclosed by twisted petals: not enclosed (n), enclosed (y). **82)** Surface structure of capsules (Vittae & Vesicles): not vittate (without stripes) (a), vertical raised vittae (b), vertical vittae with glands (c), lateral (towards the margin of the single carpel) vittae diagonal and dorsal vittae vertical (d), swollen vittae (e), pale vesicles ('vesiculate', bubble-shaped) (f), black vesicles (g), only 1–2 pale vesicles (h). Seeds: **83)** Seed shape: cylindrical (a), fusiform (spindle-shaped) (b), pyriform (pear-shaped) (c), ellipsoid (d), ovoid (e), clavate (club-shaped) (f), elongate and flattened (g). **84)** Seed appendages I – laterally carinate: not carinate (n), carinate (y). **85)** Seed appendages II – terminally winged: not winged (n), winged (y). **86)** Seed appendages III – with a distal expansion: absent (n), present (y). **87)** Seed appendages IV – with an elaiosome ('carunculate'): absent (n), present (y). **88)** Testa sculpturing: reticulate (also 'linear-reticulate', 'irregularly reticulate', 'linear-foveolate' or 'foveolate') (a), scalariform (also 'linear-scalariform' or 'ribbed-scalariform') (b), papillose (also 'rugulose' or 'smooth') (c). Cytology: **89)** Chromosome numbers ($n = \ldots$): 6 (a), 7 (b), 8 (c), 9 (d), 10 (e), 12 (f), 14 (g), 16 (h), 18 (i), 19 (j).

For BI, letters were changed to numbers (0–9). Character no. 70 (pollen grains type) had more than ten states, therefore I excluded the pollen type XI in the BI analysis (originally described only for *H.* sect. *Hirtella*).

Appendix S3.1 Voucher: ITS sequences, direct and cloned (below)

Direct sequenced: Cratoxyleae, *Cratoxylum*: *Cr. formosum* (Jack) Benth. & Hook.f. ex Dyer subsp. *formosum,* 377nmn, Larson & Larson 33255 (B); *Cr. pruniflorum* Dyer, 379nmn, Larson, Larson, Nielsen & Santisuk 32141 (B). **Hypericeae**, ***Hypericum***: **3**, *H. acmosepalum* N.Robson, 338nmn, N.M. Nürk 401 (GAT); **3**, *H. acmosepalum* N.Robson, 339nmn, N.M. Nürk 402 (GAT); **3**, *H. acmosepalum* N.Robson, 40nmn, Sino-British Expedition to Cangshan (SBEC) K052 (BM); **3**, *H. addingtonii* N.Robson, 6nmn, N.M. Nürk 348 (GAT); **16**, *H. adenotrichum* Spach, T15.1, cultivated: 1981-35, Kew-Wakehurst; **20**, *H. adpressum* W.P.C.Barton, AY555865.2; **25**, *H. aegypticum* L. subsp. *maroccanum* (Pau) N.Robson, 43nmn, S.l. Jury and B. Tahiri & T.M. Upson 14264 (BM); **25**, *H. aegypticum* L. subsp. *marrocanum* (Pau) N.Robson, Sara.new, KEW 1978-4468 (K); **25**, *H. aegypticum* L. subsp. *webbii* (Spach) N.Robson, 44nmn, E. Stamatiadou 12008 (BM); **25**, *H. aegypticum* L. subsp. *webbii* (Spach) N.Robson, 41nmn, Turland 111 (BM); **27**, *H. aethiopicum* Thunb. subsp. *sonderi* (Bredell) N.Robson, 45nmn, D. & S. Pigott s.n. 8.11.98 (BM); **5**, *H. androsaemum* L., 11nmn, Ch. Scheriau HEID-808382 (HEID); **5**, *H. androsaemum* L., T06.3, cultivated: Bed 256G, Kew-Wakehurst; **5**, *H. androsaemum* L., T12.7, cultivated: 1969-31240, Kew-Wakehurst; **20**, *H. apocynifolium* Small, AY555883.2; **9**, *H. asahinae* Makino, AY572997; **7**, *H. ascyron* L., 318nmn, S. Fujii 11937 (KYO); **7**, *H. ascyron* L., 51nmn, Wan & Chow 81093 (BM); **7**, *H. ascyron* L. subsp. *ascyron,* 52nmn, Hort. Bot. Acad. Sci. s.n. St 1580/63/64 (GAT); **7**, *H. ascyron* L. subsp. *longistylum* Maxim., AY573015; **27**, *H. athoum* Boiss. & Orph., 53nmn, Bot. Gard. Berlin-Dahlem s.n. EPG 6/2001 (GAT); **27**, *H. athoum* Boiss. & Orph., 12nmn, Ch. Scheriau HEID-808390 (HEID); **27**, *H. athoum* Boiss. & Orph., 13nmn, Ch.

Scheriau HEID-801636 (HEID); **27**, *H. atomarium* Boiss., 201nmn, Bot. Gard. Potsdam s.n. 385/57/60 (GAT); **27**, *H. atomarium* Boiss., 55nmn, E. Stamatiadou 9106 (BM); **9**, *H. attenuatum* Fisch. ex Choisy, AY572993; **9**, *H. attenuatum* Fisch. ex Choisy var. *confertissimum* (Nakai) T.B.Lee, AY572995; **12**, *H. aviculariifolium* Jaub. & Spach, T25.4, Ulrich s.n. (BM); **2**, *H. balearicum* L., 59nmn, J.F.M. & M.J. Cannon 3780 (BM); **2**, *H. balearicum* L., JE4.8, s.n.; **2**, *H. balearicum* L., AY555862; **13**, *H. barbatum* Jacq., 362nmn, N.M. Nürk 410 (GAT); **3**, *H. beanii* N.Robson, 206nmn, Alpine Garden Society Expedition to China 1994 (ACE) ACE 32 (BM); **3**, *H. beanii* N.Robson, T11.6, cultivated: 452-81/05839, Kew-Wakehurst; **3**, *H. beanii* N.Robson, T06.2, cultivated: 1996-744, Kew-Wakehurst; **3**, *H. bellum* H.L.Li, 60nmn, D.E. Boufford, S.L Kelly, R.H. Ree & S.K. Wu 29827 (BM); **3**, *H. bellum* H.L.Li subsp. *latisepalum* N.Robson, G9, N.K.B. Robson s.n. 15.8.1995 (BM); **3**, *H. bellum* H.L.Li subsp. *latisepalum* N.Robson, 61nmn, Sino-British Expedition to Cangshan (SBEC) 0424 (BM); **13**, *H. bithynicum* Boiss., 343nmn, N.M. Nürk 398 (GAT); **13**, *H. bithynicum* Boiss., E04.4, s.n.; **20**, *H. brachyphyllum* (Spach) Steud., AY555870.2; **30**, *H. brevistylum* Choisy, AY573019; **20**, *H. buckleyi* M.A.Curtis, AY555880.2; **8**, *H. bupleuroides* Griseb. haplotype 1, 376nmn, A. Gröger & W. Lobin 113-3 (M, cultivated: at the Botanical Garden Munich, No. GE-0-M-2000/3927, DNA extracted from seeds); **8**, *H. bupleuroides* Griseb. haplotype 2, 376nmn, A. Gröger & W. Lobin 113-3 (M, cultivated: at the Botanical Garden Munich, No. GE-0-M-2000/3927, DNA extracted from seeds); **3**, *H. calycinum* L., 63nmn, Bot. Gard. Frankfurt/Main s.n. EPG 7/2001 (GAT); **3**, *H. calycinum* L., T14.1, cultivated: 1969-16045, Kew-Wakehurst; **3**, *H. calycinum* L., 207nmn, D. McClintock s.n. 1993 (BM); **3**, *H. calycinum* L., FJ694194.1; **30**, *H. canadense* L., Sara.new, S. Crockett 19 (UGA); **21**, *H. canariense* L., T23.1, cultivated: 2000-0075, Jardín Botánico Barcelona; **21**, *H. canariense* L., FJ694195.1; **21**, *H. canariense* L. var. *canariense*, 003nmn, R. Davis 10261 (BM); **21**, *H. canariense* L. var. *flori* (Aiton) Bornm., 344nmn, N.M. Nürk 386 (GAT); **11**, *H. cerastioides* (Spach) N.Robson, T25.2, voucher data not available; **11**, *H. cerastioides* (Spach) N.Robson, AY555884; **6**, *H. cf xylosteifolium* (Spach) N.Robson, 365nmn, N.M. Nürk 411 (GAT); **3**, *H.* cf. *kouytchense* x *calycinum*, 350nmn, N.M. Nürk 406 (GAT); **16**, *H. cf. orientale* L., 347nmn, N.M. Nürk 404 (GAT); **20**, *H. chapmanii* W.P.Adams, AY555869.2; *H. chejuense* S.-J. Park & K.-J. Kim, AY572996; **3**, *H. choisianum* Wall. ex N.Robson, 372nmn, Ikeda *et al.* 20913019 (TI); **3**, *H. choisianum* Wall. ex N.Robson, AY555856; **20**, *H. cistifolium* Lam., AY555881.2; **18**, *H. confertum* Choisy, G6, Himmetõglu H22 (BM); **27**, *H. conjungens* N.Robson, 68nmn, L.B. Mwasumbi 16191A (BM); **19**, *H. coris* L., 71nmn, R.E. Longton 4436 (BM); **19**, *H. coris* L., 70nmn, S.l. Jury, M.F. Watson, D.A. Webb & M. B. Wyse Jackson 6415 (BM); **19**, *H. coris* L., FJ694196.1; **20**, *H. crux-andreae* (L.) Crantz, AY555874.2; **3**, *H. curvisepalum* N.Robson, T12.5, cultivated: 1993-3327, Kew-Wakehurst; **17**, *H. davisii* N.Robson, 214nmn, A. Çubukçu s.n. (1978) (BM); **29**, *H. decandrum* Turcz., 335nmn, M. Weigend & G. Brokamp 9102 (B); **27**, *H. delphicum* Boiss. & Heldr., 14nmn, Ch. Scheriau HEID-808391 (HEID); **27**, *H. delphicum* Boiss. & Heldr., 15nmn, Ch. Scheriau HEID-808395 (HEID); **27**, *H. delphicum* Boiss. & Heldr., T15.2, cultivated: 000-69.19158, Kew-Wakehurst; **20**, *H. densiflorum* Pursh, AY555886; **30**, *H. denticulatum* Walter subsp. *acutifolium* (Elliott) N.Robson, C7, Kral 48272 (BM); **1**, *H. dogobadanicum* Assadi, 73nmn, Assadi & Aboohamzeh 38585 (BM); **20**, *H. dolabriforme* Vent., AY555889; **3**, *H. dyeri* Rehder, 74nmn, Toshiyuki Nakaike 1797 (BM); **3**, *H. elatoides* R.Keller, 7F, Boufford *et al.* 26156 (BM); **9d**, *H. elodeoides* Choisy, 371nmn, Ikeda *et al.* 20911111 (TI); **9d**, *H. elodeoides* Choisy, 076nmn, L.W. Beer, C.R. Lancaster & D. Morris 9492 (BM); **9d**, *H. elodeoides* Choisy, H5, Miyamoto *et al.* 96062 (BM); **28**, *H. elodes* L., 16nmn, Ch. Scheriau HEID-808396 (HEID); **28**, *H. elodes* L., 17nmn, Ch. Scheriau HEID-808399 (HEID); **28**, *H. elodes* L., T20.3, Michael F. Fay 374 (K); **17**, *H. elongatum* Ledeb. var. *lythrifolium*, 79nmn, K. Sutory 112 (BM); **19**, *H. empetrifolium* Willd., 80nmn, R.C. Lancaster 1118 (BM); **9b**, *H. epigeium* R. Keller, D5, M. Véliz, Gallardo & Vásquez MV 2m.9542 (BM); **9**, *H. erectum* Thunb. var. *erectum*, 304nmn, N.M. Nürk 365 (BM); **9**, *H. erectum* Thunb. var. *erectum* f. *papillosum* (Y.Kimura) Y.Kimura, 332nmn, N.M. Nürk 383 (GAT); **9**, *H. erectum* Thunb. var. *erectum* f. *tateutianum* (Koidz.) Y.Kimura, 303nmn, N.M. Nürk 354 (GAT); **9**, *H. erectum* Thunb. var. *erectum* f. *vaniottii* (H.Lév.) Y.Kimura, 305nmn, N.M. Nürk 372 (KYO); **19**, *H. ericoides* L., T13.8, cultivated: 1985-922, Kew-Wakehurst; **19**, *H. ericoides* L., 83nmn, P.F. Cannon, P.R. Crane, S.R. Jury & D.M. Moore (R.U. Botany Dept. Exped.) 475 (BM); **19**, *H. ericoides* L., 82nmn, Stübing 25 (BM); **20**, *H. fasciculatum* Lam., AY555868.2; **5**, *H. foliosum* Aiton, 20nmn, H. Schaefer HS 208 (K); **3**, *H. forrestii* (Chitt.) N.Robson, 85nmn, C.R. Lancaster L2032 (BM); **3**, *H. forrestii* (Chitt.) N.Robson, 86nmn, D. & S. Pigott s.n. 15.11.98 (BM); **3**, *H. forrestii* (Chitt.) N.Robson, 84nmn, Sino-British Expedition to Cangshan (SBEC) 0472 (BM); **3**, *H. fosteri* N.Robson, F9, N.K.B. Robson s.n. 4.8.2004 (BM); **20**, *H. frondosum* Michx., AY555887; **20**, *H. galioides* Lam., AY555864.2; **29**, *H. garciae* Pierce, T05.2, Santiago Madriñán 2063 (ANDES); **4**, *H. geminiflorum* Hemsl., C12andrea, HM162838, Kuo-Fang Chung 1266 (NRM); **29**, *H. gentianoides* (L.) Britton, Sterns & Poggenb., T14.2, cultivated: 2000-3136, Kew-Wakehurst; **29**, *H. gentianoides* (L.) Britton, Sterns & Poggenb., T14.2.1, cultivated: 2000-3136, Kew-Wakehurst; **29**, *H. gentianoides* (L.) Britton, Sterns & Poggenb., 337nmn, N.M. Nürk 384 (GAT); **29**, *H. gleasonii* N.Robson, T03.5, Santiago Madriñán 2285 (ANDES); **29**, *H. gleasonii* N.Robson, T01.2, Santiago Madriñán 2011 (ANDES); **29**, *H. goyanesii* Cuatrec., T01.4, Carlos García 143 (ANDES); **9**, *H. gracillimum* Koidz., 302nmn, N.M. Nürk 371 (BM); **9**, *H. gracillimum* Koidz., 306nmn, N.M. Nürk 373 (BM); **30**, *H. gramineum* G.Forst., EU352256; **30**, *H. gramineum* G.Forst., EU352257; **5**, *H. grandifolium* Choisy, 226nmn, C.E. Jarvis, Gibby & Humphries 411 (BM); **5**, *H. grandifolium* Choisy, 346nmn, N.M. Nürk 403 (GAT); **9b**, *H. graveolens* Buckley, AY555843; **9**, *H. hakonense* Franch. & Sav., 307nmn, N.M. Nürk 375 (KYO); **9**, *H. hakonense* Franch. & Sav., 319nmn, Tomitarô Makino 33650 (KYO);, *H. haplophylloides* Halácsy & Bald., 227nmn, F.K. Meyer 5973 (BM); **3**, *H. henryi* H.Lév. & Vaniot subsp. *henryi*, B9, N.K.B. Robson s.n. 28.8.1983 (BM); **3**, *H. henryi* H.Lév. & Vaniot subsp. *uraloides* (Rehder) N. Robson, AY555859; **24**, *H. heterophyllum* Vent., 66nmn, A.A. Dönmez 3812 (BM); **5**, *H. hircinum* L. subsp. *albimonta-*

num (Greuter) N.Robson, 92nmn, C. Whitefoord 185 (BM); **5**, *H. hircinum* L. subsp. *hircinum*, 93nmn, G. Bocquet, Itinera Galica 15507 (BM); **5**, *H. hircinum* L. subsp. *majus* (Aiton) N.Robson, 95nmn, J.R. Akeroyd, S.l. Jury, C.J. Miles & F.J. Rumsey 3788 (BM); **5**, *H. hircinum* L. subsp. *majus* (Aiton) N.Robson, 94nmn, Turland 769 (BM); **5**, *H. hircinum* L. subsp. *metroi* (Maire & Sauvage) Sauvage, 233nmn, S.l. Jury, J.B. Peris & G. Stübing 64 (BM); **18**, *H. hirsutum* L., 96nmn, F. Dvorák 1648 (HEL); **18**, *H. hirsutum* L., T20.2, Michael F. Fay 350 (K); **18**, *H. hirsutum* L., 364nmn, N.M. Nürk 390 (GAT); **3**, *H. hookerianum* Wight & Arn., 234nmn, L.W. Beer, C.R. Lancaster & D. Morris 12316 (BM); **3**, *H. hookerianum* Wight & Arn., 369nmn, N.M. Nürk 413 (GAT); **3**, *H. hookerianum* Wight & Arn., 100nmn, Sino-British Expedition to Cangshan, 1981 (SBE) 469 (BM); **29**, *H. humboldtianum* Steud., T02.8, María Angélica Bello 21 (ANDES); **14**, *H. humifusum* L., 236nmn, A. Strid 22275 (BM); **14**, *H. humifusum* L., 101nmn, Carine, Ait Lafkih, Rumsey & Rutherford 262 (BM); **14**, *H. humifusum* L., 103nmn, J.F. Veldkamp 8837 (BM); **14**, *H. humifusum* L., 102nmn, K. Harris s.n. 1988 (BM); **14**, *H. humifusum* L., 328nmn, N.M. Nürk 381 (GAT); **20**, *H. hypericoides* (L.) Crantz subsp. *hypericoides*, AY555879.2; **17**, *H. hyssopifolium* Vill., 104nmn, B. de Retz 67577 (BM); **17**, *H. hyssopifolium* Vill., 238nmn, P. Bamps 9004 (BM); **30**, *H. japonicum* Thunb. ex Murray, 374nmn, Ikeda *et al.* 20913073 (TI); **30**, *H. japonicum* Thunb. ex Murray, 322nmn, Kazuaki Masuda 3360 (KYO); **30**, *H. japonicum* Thunb. ex Murray, GQ396674; *H. jeongjocksanense* S.-J. Park & K.-J. Kim, AY573023; **9**, *H. jozoeënse* Maxim., AY573004; **9**, *H. jozoeënse* Maxim., FJ793046.1; **29**, *H. juniperinum* Kunth, T12.1, Santiago Madriñán 2123 (ANDES); **29**, *H. juniperinum* Kunth, T05.11, Santiago Madriñán 2062 (ANDES); **29**, *H. juniperinum* Kunth, T05.12, Santiago Madriñán 2062 (ANDES); **20**, *H. kalmianum* L., FJ694209.1; **9**, *H. kamtschaticum* Ledeb., 308nmn, N.M. Nürk 366 (GAT); **9**, *H. kamtschaticum* Ledeb., AY572992; **9**, *H. kamtschaticum* Ledeb. haplotype 1, 321nmn, K. Yonekura 12937 (KYO); **9**, *H. kamtschaticum* Ledeb. haplotype 2, 321nmn, K. Yonekura 12937 (KYO); **9**, *H. kinashianum* Koidz., AY573001; **9**, *H. kiusianum* Koidz. var. *yakusimense* (Koidz.) T.Kato, A10.13, s.n.; **3**, *H. kouytchense* H.Lév., 107nmn, J.R. Hosking & P.T. Gorham 2007 (BM); **3**, *H. kouytchense* H.Lév., 349nmn, N.M. Nürk 405 (GAT); **3**, *H. kouytchense* H.Lév., AY555853; **3**, *H. lagarocladum* N.Robson, T10.6, cultivated: 1988-3144, Kew-Wakehurst; **3**, *H. lagarocladum* N.Robson, 244nmn, Sino-British Expedition to Cangshan (SBEC) K149 (BM); **3**, *H. lancasteri* N.Robson, T11.8.2, cultivated: 1990-2357, Kew-Wakehurst; **3**, *H. lancasteri* N.Robson, 246nmn, Sino-British Expedition to Cangshan (SBEC) K039 (BM); **3**, *H. lancasteri* N.Robson, AY555854; **27**, *H. lanuginosum* Lam., 248nmn, R. Ulrich s.n. 1998 (BM); **29**, *H. laricifolium* Juss., T02.3, Carlos García 24 (ANDES); **29**, *H. laricifolium* Juss., 334nmn, M. Weigend & G. Brokamp 9101 (B); **29**, *H. laricifolium* Juss., T03.6, Santiago Madriñán 2284 (ANDES); **29**, *H. laricifolium* Juss., T05.6, Santiago Madriñán 2125 (ANDES); **29**, *H. laricifolium* Juss. "CoordCetrl", T09.8, Santiago Madriñán 2113 (ANDES); **12**, *H. laxiflorum* N.Robson, 11D, Ulrich s.n. (15.6.2000) (BM); **3**, *H. leschenaultii* Choisy, AY555857; **14**, *H. linariifolium* Vahl, 329nmn, N.M. Nürk 379 (GAT); **18**, *H. linarioides* Bosse, 249nmn, P. Hein 64 (BM); **20**, *H. lissophloeus* W.P.Adams, AY555885; **20**, *H. lloydii* (Svenson) W.P.Adams, AY555867.2; **20**, *H. lobocarpum* Gatt., AY555876.2; **3**, *H. maclarenii* N.Robson, 112nmn, C.R. Lancaster L2016 (BM); **3**, *H. maclarenii* N.Robson, E8, N.K.B. Robson s.n. 13.8.2000 (BM); **9**, *H. maculatum* Crantz, 22nmn, Ch. Scheriau HEID-811874 (HEID); **9**, *H. maculatum* Crantz, 24nmn, Ch. Scheriau HEID-808359 (HEID); **9**, *H. maculatum* Crantz, 28nmn, Ch. Scheriau HEID-704351 (HEID); **9**, *H. maculatum* Crantz subsp. *obtusiusculum* (Tourlet) Hayek, FJ694199.1; **18**, *H. marginatum* Woron., D6, Davis & Hedge D.32436 (BM); **29**, *H. mexicanum* L., T04.4, Santiago Madriñán 2051 (ANDES); **20**, *H. microsepalum* (Torr. & A.Gray) A.Gray ex S.Watson, AY555877.2; **9e**, *H. monanthemum* Hook.f. & Thomson ex Dyer, 116nmn, Sino-American Botanical Expedition to Yunnan (SABEY) 1166 (BM); **3**, *H. monogynum* L., 118nmn, C.R. Lancaster 1848B (BM); **3**, *H. monogynum* L., F8, N.K.B. Robson s.n. (BM); **27**, *H. montanum* L., 30nmn, Ch. Scheriau HEID-808415 (HEID); **27**, *H. montanum* L., 12A, Hein 7541 (BM); **27**, *H. montanum* L., 119nmn, P. Hein 7504 (BM); **13**, *H. montbretii* Spach, 121nmn, D. McClintock s.n. 1993 (BM); **13**, *H. montbretii* Spach, 257nmn, E. Stamatiadou 14999 (BM); **13**, *H. montbretii* Spach, 11B, Stearn A.3. (BM); **30**, *H. mutilum* L., DQ006013; **30**, *H. mutilum* L. subsp. *boreale*, AY573026; **20**, *H. myrtifolium* Lam., AY555875.2; **9**, *H. nakaii* H.Koidz. subsp. *nakaii*, 309nmn, N.M. Nürk 360 (GAT); **9**, *H. nakaii* H.Koidz. subsp. *nakaii*, 310nmn, N.M. Nürk 363 (KYO); **22**, *H. nanum* Poir., T13.2, cultivated: 1945-31204, Kew-Wakehurst; **22**, *H. nanum* Poir., 123nmn, P.H. Davis 10149 (BM); **20**, *H. nitidum* Lam. subsp. *nitidum*, AY555871.2; **20**, *H. nudiflorum* Michx., AY555888; **18**, *H. nummularioides* Trautv. haplotype 1, 262nmn, C.R. Lancaster s.n. 1.viii.1979 (BM); **18**, *H. nummularioides* Trautv. haplotype 2, 262nmn, C.R. Lancaster s.n. 1.viii.1979 (BM); **18**, *H. nummularium* L., 125nmn, C.-A. Hæggström 7063 (BM); **9b**, *H. oaxacanum* R.Keller, AY573003; **3**, *H. oblongifolium* Choisy, FJ694226.1; **9**, *H. oliganthum* Franch. & Sav., 317nmn, T. Kawahara, H. Im & T. Yahara 55 (TI); **9**, *H. oliganthum* Franch. & Sav., 323nmn, Turu Sawada 236 (KYO); **9**, *H. oliganthum* Franch. & Sav., AY573005; **10**, *H. olympicum* L. forma *minus* Hausskn., T11.3, cultivated: 1973-21185, Kew-Wakehurst; **10**, *H. olympicum* L. forma *olympicum*, 127nmn, D. McClintock s.n. 1983 (BM); **10**, *H. olympicum* L. forma *olympicum*, 128nmn, W. Greuter 16146 (BM); **10**, *H. olympicum* L. forma *uniflorum* D.Jord. & Kozuharov, 130nmn, E. Stamatiadou 10094 (BM); **10**, *H. olympicum* L. forma *uniflorum* D.Jord. & Kozuharov, 351nmn, N.M. Nürk 387 (GAT); **16**, *H. orientale* L., 352nmn, N.M. Nürk 396 (GAT); **16**, *H. orientale* L., FJ694213.1; **12**, *H. origanifolium* Willd. var. *origanifolium*, 131nmn, A. Çubukçu, E. Yesilada, A. Basaran & H. Koçak 1412 (BM); **9**, *H. ovalifolium* Koidz., AY572998; **23**, *H. pallens* Banks & Sol., 31nmn, Ch. Scheriau HEID-801626 (HEID); **23**, *H. pallens* Banks & Sol., T13.4, cultivated: 1945-31202, Kew-Wakehurst; **23**, *H. pallens* Banks & Sol., AY555848; **22**, *H. pamphylicum* N.Robson & P.H.Davis, 132nmn, R. Ulrich s.n. 1998 (BM); **12**, *H. papillare* Boiss. & Heldr., 265nmn, A. Çubukçu & A. Basaran A-12 (BM); **26**, *H. papuanum* Ridl., T21.1, Marsden 91 (K); **3**, *H. patulum* Thunb. ex Murray, 133nmn, C.R. Lancaster L.623 (BM); **3**, *H. patulum* Thunb. ex Murray, 134nmn, J.R. Hosking & M.J.

Williams 1845 (BM); **3**, *H. patulum* Thunb. ex Murray, 268nmn, O.M. Hilliard & B.L. Burtt 16088 (BM); **13**, *H. perfoliatum* L., 354nmn, N.M. Nürk 385 (GAT); **13**, *H. perfoliatum* L., 139nmn, S.l. Jury and M. Ait Lafkih, M. El Haila & R.G. Wilson 16522 (BM); **9**, *H. perforatum* L. subsp. *chinense* N.Robson, 311nmn, N.M. Nürk 353 (GAT); **9**, *H. perforatum* L. subsp. *chinense* N.Robson, 312nmn, N.M. Nürk 370 (GAT); **9**, *H. perforatum* L. subsp. *perforatum,* 146nmn, M. Wayda s.n. 2006 (BM); **9**, *H. perforatum* L. subsp. *veronense* (Schrank) Ces., 142nmn, C.R. Lancaster 232 (BM); **9**, *H. perforatum* L. subsp. *veronense* (Schrank) Ces., 144nmn, S. Collenette 6079 (BM); **9d**, *H. petiolulatum* Hook.f. & Thomson ex Dyer, 148nmn, A.J.C. Grierson & D.G. Long 2549 (BM); **9d**, *H. petiolulatum* Hook.f. & Thomson ex Dyer subsp. *yunnanense* (Franch.) N.Robson, 274nmn, B. Bartholomew, D.E. Boufford, Q.H. Chen *et al.* (1986 Sino-American Guizhou Botanical Expedition) 2163 (BM); **9**, *H. pibairense* (Miyabe & Y.Kimura) N.Robson, 316nmn, Tatsumi Kato 3132-3 (TI); **9**, *H. pibairense* (Miyabe & Y.Kimura) N.Robson haplotype 1, 313nmn, N.M. Nürk 367 (GAT); **9**, *H. pibairense* (Miyabe & Y.Kimura) N.Robson haplotype 2, 313nmn, N.M. Nürk 367 (GAT); **3**, *H. podocarpoides* N.Robson, 373nmn, Ikeda *et al.* 20913062 (TI); **3**, *H. podocarpoides* N.Robson, 149nmn, J.R.I. Wood 6110 (BM); **10**, *H. polyphyllum* Boiss. & Balansa, 355nmn, N.M. Nürk 407 (GAT); **10**, *H. polyphyllum* Boiss. & Balansa, 357nmn, N.M. Nürk 391 (GAT); **10**, *H. polyphyllum* Boiss. & Balansa, 358nmn, N.M. Nürk 392 (GAT); **10**, *H. polyphyllum* Boiss. & Balansa, 367nmn, N.M. Nürk 388 (GAT); **20**, *H. prolificum* L., AY555873.2; **20**, *H. prolificum* L., FJ694217.1; **29**, *H. prostratum* Cuatrec., T01.3, Carlos García 108 (ANDES); **7**, *H. przewalskii* Maxim., 150nmn, G. & S. Miehe 9215/04 (BM); **3**, *H. pseudohenryi* N. Robson, AY555850; **3**, *H. pseudohenryi* N.Robson, FJ694218.1; **17**, *H. pseudolaeve* N.Robson, 151nmn, J. Roper 68 (BM); **9b**, *H. pseudomaculatum* Bush, E5, Culwell & Tucker s.n. 12.5.1968 (BM); **9**, *H. pseudopetiolatum* R.Keller, AY573002; **27**, *H. pubescens* Boiss., 153nmn, Davis 52932 (BM); **27**, *H. pubescens* Boiss., 152nmn, S.l. Jury with M.A. Carine, M. Rejdali, F.J. Rumsey & R.W. Rutherford 19630 (BM); **18**, *H. pulchrum* L., 156nmn, Cubr 39891 (B); **18**, *H. pulchrum* L., T20.1, Michael F. Fay 298 (K); **18**, *H. pulchrum* L., 155nmn, S.l. Jury & M.F. Watson 6219 (BM); **9b**, *H. punctatum* Lam., 158nmn, D.E. Boufford & E.W. Wood 23250 (BM); **9b**, *H. punctatum* Lam., AY555844; **9b**, *H. punctatum* Lam. haplotype 1, 278nmn, K.G. Sikes & J. Stone 24 (BM); **9b**, *H. punctatum* Lam. haplotype 2, 278nmn, K.G. Sikes & J. Stone 24 (BM); **1**, *H. quartinianum* A.Rich., 159nmn, J.C. Lovett & C.J. Kayombo 4922 (BM); **1**, *H. quartinianum* A.Rich., E1, Nkhoma & Changwe 2032 (BM); **1**, *H. quartinianum* A.Rich., 160nmn, T.R.I. Wood 2817 (BM); **29**, *H. quitense* R.Keller, 333nmn, M. Weigend & G. Brokamp 9100 (B); **27**, *H. reflexum* L., T23.2, cultivated: 1999-00370, Jardín Botánico Barcelona; **27**, *H. reflexum* L., 002nmn, F. Blattner FRB-2008-004 (GAT); **27**, *H. reflexum* L., FJ694221.1; **3**, *H. reptans* Hook.f. & Thomson ex Dyer, T10.2, cultivated: 1972-6301, Kew-Wakehurst; **1**, *H. revolutum* Vahl, T10.8, cultivated: 1972-3163, Kew-Wakehurst; **1**, *H. revolutum* Vahl, 280nmn, I.F. LaCroix 3098 (BM); **1**, *H. revolutum* Vahl, 279nmn, S. Chaudhary 3901 (BM); **13**, *H. richeri* Vill., T15.6, cultivated: 1993-1024, Kew-Wakehurst; **30**, *H. rigidum* A.St.-Hil., AY573021; **1**, *H. roeperianum* G.W.Schimp. ex A.Rich., T15.7, cultivated: 1982-2124, Kew-Wakehurst; **1**, *H. roeperianum* G.W.Schimp. ex A.Rich., 284nmn, W.T. Stearn s.n. 1977 (BM); **1**, *H. roeperianum* G.W.Schimp. ex A.Rich., AY555863; **13**, *H. rumeliacum* Boiss., 12H, s.n.; **13**, *H. rumeliacum* Boiss. subsp. *rumeliacum,* 164nmn, A.O. Chater 21 (BM); **29**, *H. ruscoides* Cuatrec., T02.4, Amalia Díaz 13 (ANDES); **29**, *H. sabiniforme* Trevir., T09.1, Favio González 3838 (ANDES); **9c**, *H. sampsonii* Hance, 324nmn, unknown (name writen in Japanese) 16917 (KYO); **9c**, *H. sampsonii* Hance, AY573011; **18**, *H. saxifragum* N.Robson & Hub.-Mor., 165nmn, R. Ulrich s.n. 6.10.1997 (BM); **17**, *H. scabroides* N.Robson & Poulter, 36nmn, Ch. Scheriau HEID-808410 (HEID); **17**, *H. scabroides* N.Robson & Poulter, 37nmn, Ch. Scheriau HEID-808412 (HEID); **17**, *H. scabrum* L., T15.3, cultivated: 1995-3560, Kew-Wakehurst; **17**, *H. scabrum* L., 169nmn, K. Sutory 110 (BM); **9**, *H. scouleri* Hook., 171nmn, R. Halse 5427 (BM); **9**, *H. senanense* Maxim., 315nmn, N.M. Nürk 362 (GAT); **9**, *H. senanense* Maxim. *mutiloides* (R.Keller) N.Robson, 314nmn, N.M. Nürk 361 (GAT); **9d**, *H. seniawinii* Maxim., 287nmn, Xiao Bai-Zhong 3778 (BM); **30**, *H. setosum* L., AY573020; **9**, *H. sikokumontanum* Makino, AY572999; **27**, *H. sinaicum* Hochst. ex Boiss, 173nmn, A. Danin 962609 (BM); **20**, *H.* spec., AY555866.2; **20**, *H. sphaerocarpum* Michx., AY555878.2; **13**, *H. spruneri* Boiss., 359nmn, N.M. Nürk 408 (GAT); **29**, *H. strictum* Kunth, T02.6, María Angélica Bello 97 (ANDES); **29**, *H. strictum* Kunth, T04.1.1, Santiago Madriñán 2048 (ANDES); **29**, *H. strictum* Kunth, T04.1.2, Santiago Madriñán 2048 (ANDES); **3**, *H. subsessile* N.Robson, 67nmn, Alpine Garden Society Expedition to China 1994 (ACE) 2526 (BM); **3**, *H. subsessile* N.Robson, T10.5, cultivated: 1981-5841, Kew-Wakehurst; **20**, *H. suffruticosum* W.P.Adams, Sara.new, S. Crockett 156 (UGA); **18**, *H. taygeteum* Quézel & Contandr., 005nmn, W. Greuter & H. Merxmüller 17233 (BM); **3**, *H. tenuicaule* Hook.f. & Thomson ex Dyer, 289nmn, F. Miyamoto, M. Amano, H. Iked, C.M. Joshi, K. Arai & T. Komatsu 9596032 (BM); **20**, *H. tenuifolium* Pursh, AY555872.2; **30**, *H. ternum* A.St.-Hil., AY573022; **29**, *H. terrae firmae* Sprague & Riley, T18.1, Rees 221 (BM); **20**, *H. tetrapetalum* Lam., AY555882.2; **9**, *H. tetrapterum* Fr., T13.1, cultivated: 1983-533, Kew-Wakehurst; **9**, *H. tetrapterum* Fr., FJ694224.1; **29**, *H. tetrastichum* Cuatrec., T11.1, Santiago Madriñán 2039 (ANDES); **29**, *H. tetrastichum* Cuatrec., T14.6, Santiago Madriñán 2010 (ANDES); **30**, *H. thesiifolium* Kunth, 336nmn, M. Weigend & G. Brokamp 9119 (B); **17**, *H. thymbrifolium* Boiss. & Noë, 178nmn, A. Çubukçu 3 (BM); **18**, *H. thymifolium* Banks & Sol., 179nmn, R. Ulrich 0/52 (BM); **18**, *H. thymifolium* Banks & Sol., E6, Ulrich 0/52 (BM); **27**, *H. tomentosum* L., 368nmn, N.M. Nürk 412 (GAT); **27**, *H. tomentosum* L., 291nmn, S.l. Jury and L.S. Springate & M. Ait Lafkih 11271 (BM); **9**, *H. tosaense* Makino, 325nmn, Tamiki Kobayashi 41978 (KYO); **9**, *H. triquetrifolium* Turra, T14.4, cultivated: 1990-100, Kew-Wakehurst; **9**, *H. triquetrifolium* Turra, 182nmn, J,R. Akeroyd, S.l. Jury & F.J.Rumsey 3572 (BM); **9**, *H. triquetrifolium* Turra, 185nmn, Tomkinson 29 (BM); **9**, *H. undulatum* Schousb. ex Willd., 35nmn, Ch. Scheriau HEID-808335 (HEID); **9**, *H. undulatum* Schousb. ex Willd. subsp. *undulatum,* 293nmn, D.J. Goyer & S.l. Jury 545 (BM); **22**, *H. vacciniifolium* Hayek & Siehe, 188nmn, R.

Ulrich s.n. 2000 (BM); **3**, *H. wardianum* N. Robson, E9, N.K.B. Robson s.n. (BM); **3**, *H. wilsonii* N.Robson, G8, N.K.B. Robson s.n. (BM); **5**, *H.* x *inodorum* Mill., FJ694208.1; **9b**, *H.* x *mitchellianum* Rydb., 331nmn, N.M. Nürk 382 (GAT); **3**, *H. x moserianum* Luquet & ex André, 360nmn, N.M. Nürk 409 (GAT); **3**, *H.* x *moserianum* Luquet & ex André, AY555855; **6**, *H. xylosteifolium* (Spach) N.Robson, 38nmn, Ch. Scheriau HEID-808337 (HEID); **6**, *H. xylosteifolium* (Spach) N.Robson, T06.8, cultivated: 1979-6434, Kew-Wakehurst. ***Thornea***: *Th. calcicola* (Standl. & Steyerm.) Breedlove & E.M.McClint., AY573028; *Th. matudae* (Lundell) Breedlove & E.M.McClint., 195nmn, D.E. Breedlove 40408 (B); *Th. matudae* (Lundell) Breedlove & E.M.McClint., AY573027; *Tr. fraseri* (Spach) Gleason, 400nmn, S.R. Hill 17290 (GH); ***Triadenum***: *Tr. fraseri* (Spach) Gleason, 326nmn, W. Hess & N. Stoynoff 7351 (KYO); *Tr. japonicum* (Blume) Makino, 301nmn, N.M. Nürk 376 (GAT); *Tr. japonicum* (Blume) Makino, 370nmn, N.M. Nürk 414 (GAT); *Tr. tubulosum* (Walter) Gleason, 402nmn, J.D. Ray 5412 (GH); *Tr. virginicum* Raf., 403nmn, R.S. Mitchel & J. Focht 8507 (GH); *Tr. virginicum* Raf. subsp. *fraseri* (Spach) Á.Löve & D.Löve, 401nmn, B. Boivin & A. Champagne 14188 (GH); *Tr. walteri* (J.F.Gmel.) Gleason, 405nmn, R. Kral & R.K. Godfrey 5921 (GH); **Vismieae**, ***Harungana***: *Har. madagascariensis* Poir., 193nmn, A.J.M. Leeuwenberg 8143 (B); ***Vismia***: *V. cayennensis* (Jacq.) Pers., 381nmn, Mori, Lokova & Keeley 25662 (B); *V. guianensis* (Aubl.) Choisy, 382nmn, Jansen-Jacobs, Lilwah, Raghoenandan, Scheplitz & Vermeer 5501 (B); *V. macrophylla* Kunth, 383nmn, Jansen-Jacobs, Welle, James & Andrew 4920 (B).

Cloned sequences (***Hypericum***): **3**, *H. choisianum* Wall. ex N.Robson 372-1 nmn, 372-2 nmn, 372-3 nmn, 372-4 nmn, 372-5 nmn, Ikeda *et al.* 20913019 (TI); **3**, *H. lagarocladum* N.Robson 244-1 nmn, 244-2 nmn, 244-3 nmn, 244-4 nmn, 244-5 nmn, 244-6 nmn, 244-7 nmn, 244-8 nmn, Sino-British Expedition to Cangshan (SBEC) K149 (BM); **3**, *H. lancasteri* N.Robson 246-1 nmn, 246-2 nmn, 246-3 nmn, 246-4 nmn, 246-5 nmn, 246-6 nmn, 246-7 nmn, Sino-British Expedition to Cangshan (SBEC) K039 (BM); **9**, *H. nakaii* H.Koidz. subsp. *nakaii* 309-1 nmn, 309-2 nmn, 309-3 nmn, 309-4 nmn, 309-5 nmn, 309-6 nmn, N.M. Nürk 360 (GAT); **9**, *H. scouleri* Hook. 171-1 nmn, 171-2 nmn, 171-3 nmn, 171-4 nmn, 171-5 nmn, 171-6 nmn, 171-7 nmn, R. Halse 5427 (BM); **9**, *H. undulatum* Schousb. ex Willd. subsp. *undulatum* 293-1 nmn, 293-2 nmn, 293-3 nmn, 293-4 nmn, 293-5 nmn, 293-6 nmn, 293-7 nmn, D.J. Goyer & S.l. Jury 545 (BM); **10**, *H. polyphyllum* Boiss. & Balansa 355-1 nmn, 355-2 nmn, 355-3 nmn, 355-4 nmn, 355-5 nmn, 355-6 nmn, 355-7 nmn, 355-8 nmn, N.M. Nürk 407 (GAT); **17**, *H. davisii* N.Robson 214-1 nmn, 214-2 nmn, 214-3 nmn, 214-4 nmn, 214-5 nmn, 214-6 nmn, 214-7 nmn, 214-8 nmn, A. Çubukçu s.n. (1978) (BM); **17**, *H. scabroides* N.Robson & Poulter 37-1 nmn, 37-2 nmn, 37-3 nmn, 37-4 nmn, 37-5 nmn, 37-6 nmn, Ch. Scheriau HEID-808412 HEID); **27**, *H. reflexum* L. 33-1 nmn, 33-2 nmn, 33-3 nmn, 33-4 nmn, 33-5 nmn, 33-6 nmn, 33-7 nmn, F. Blattner FRB-2008-002 (GAT).

Appendix S3.2 Character coding for ancestral character reconstructions by Bayesian MCMC optimization on the ITS phylogeny

Live form: tree (0), shrub (1), herb (2). Dark glands in vegetative parts: absent (0), present (1). Dark glands in reproductive parts: absent (0), present (1). Petals: persistent (0), deciduous (1). Stamens: persistent (0), deciduous (1). Arrangement of stamens: 5 free fascicles (0), 2 + 2 + 1 free (3 visible) fascicles (1), narrow continuous ring (2), broad continuous ring (3), 5 obscure fascicles (4), 3 obscure fascicles (5), 5 single stamens (6). Staminodes (sterile fascicles, 'fasciclodes'): absent (0), present (1). Placentation: parietal (0), loosely axile (1), axile (2).

Appendix S4 Historical biogeography and diversification rate shift analyses

Operational taxonomic units used in the analyses of divergence times and biogeography, listed under the clade names (defined in Figs. 4.2, 4.3), detailing accession numbers and distribution areas (A = Africa, M = the Mediterranean, EU = western Palaearctic, EA = eastern Palaearctic, IP = Indo-Pacific [*i. e.* Asia tropical + Australasia + Pacific], NA = North America, and SA = South America).

— ***Cratoxylum***: *Cr. formosum* (Jack) Benth. & Hook.f. ex Dyer subsp. *formosum* 377nmn, **IP**.
— ***Harungana***: *Har. madagascariensis* Poir. 193nmn, **A**.
— ***Hypericum* section *Arthrophyllum***: *H. pamphylicum* N.Robson & P.H.Davis 132nmn, **M**; *H. vacciniifolium* Hayek & Siehe 188nmn, **M**.

— *Hypericum* "Ascyreia s.l.": *H. acmosepalum* N.Robson 338nmn, **EA**-**IP**; *H. acmosepalum* N.Robson 339nmn, **EA**-**IP**; *H. acmosepalum* N.Robson 40nmn, **EA**-**IP**; *H. ascyron* L. subsp. *ascyron* 52nmn, **EA**-**IP**; *H. balearicum* L. 59nmn, **M**; *H. beanii* N.Robson 206nmn, **IP**; *H. beanii* N.Robson T06.2, **IP**; *H. bellum* H.L.Li subsp. *latisepalum* N.Robson 61nmn, **IP**; *H. calycinum* L. 207nmn, **M**; *H. choisianum* Wall. ex N.Robson AY555856, **IP**; *H. dogobadanicum* Assadi 73nmn, **M**; *H. dyeri* Rehder 74nmn, **IP**; *H. elatoides* R.Keller 7F, **EA**; *H. geminiflorum* Hemsl. C12andrea, **IP**; *H. hookerianum* Wight & Arn. 100nmn, **EA**-**IP**; *H. hookerianum* Wight & Arn. 369nmn, **EA**-**IP**; *H. monogynum* L. 118nmn, **EA**-**IP**; *H. monogynum* L. F8, **EA**-**IP**; *H. przewalskii* Maxim. 150nmn, **EA**; *H. reptans* Hook.f. & Thomson ex Dyer T10.2, **EA**-**IP**; *H. wardianum* N. Robson E9, **EA**.
— *Hypericum* "Brathys s.l.": *H. brevistylum* Choisy AY573019, **SA**; *H. canadense* L. Sara.2, **NA**; *H. decandrum* Turcz. 335nmn, **SA**; *H. denticulatum* Walter subsp. *acutifolium* (Elliott) N.Robson C7, **NA**; *H. garciae* Pierce T05.2, **SA**; *H. gentianoides* (L.) Britton, Sterns & Poggenb. 337nmn, **NA**; *H. gleasonii* N.Robson T01.2, **SA**; *H. gramineum* G.Forst. EU352257, **IP**; *H. japonicum* Thunb. ex Murray 322nmn, **EA**-**IP**; *H. japonicum* Thunb. ex Murray GQ396674, **EA**-**IP**; *H. jeongjocksanense* S.-J. Park & K.-J. Kim AY573023, **EA**; *H. juniperinum* Kunth T05.11, **SA**; *H. juniperinum* Kunth T05.12, **SA**; *H. laricifolium* Juss. T03.6, **SA**; *H. mexicanum* L. T04.4, **SA**; *H. mutilum* L. DQ006013, **NA**; *H. mutilum* L. subsp. *boreale* AY573026, **NA**; *H. prostratum* Cuatrec. T01.3, **SA**; *H. quitense* R.Keller 333nmn, **SA**; *H. rigidum* **A**.St.-Hil. AY573021, **SA**; *H. ruscoides* Cuatrec. T02.4, **SA**; *H. setosum* L. AY573020, **NA**; *H. strictum* Kunth T02.6, **SA**; *H. ternum* **A**.St.-Hil. AY573022, **SA**; *H. tetrastichum* Cuatrec. T11.1, **SA**; *H. tetrastichum* Cuatrec. T14.6, **SA**; *H. thesiifolium* Kunth 336nmn, **SA**.
— *Hypericum* section *Campylosporus*: *H. quartinianum* **A**.Rich. 160nmn, **A**; *H. revolutum* Vahl 279nmn, **A**; *H. roeperianum* G.W.Schimp. ex **A**.Rich. 284nmn, **A**.
— *Hypericum* "core Hypericum": *H. adenotrichum* Spach T15.1, **M**; *H. aethiopicum* Thunb. subsp. *sonderi* (Bredell) N.Robson 45nmn, **A**; *H. athoum* Boiss. & Orph. 13nmn, **M**; *H. atomarium* Boiss. 55nmn, **M**; *H. attenuatum* Fisch. ex Choisy AY572993, **EA**; *H. aviculariifolium* Jaub. & Spach T25.4, **M**; *H. bithynicum* Boiss. 343nmn, **M**; *H. cerastioides* (Spach) N.Robson T25.2, **M**; *H. confertum* Choisy G6, **M**; *H. conjungens* N.Robson 68nmn, **A**; *H. coris* L. 70nmn, **EU**; *H. delphicum* Boiss. & Heldr. 14nmn, **M**; *H. elodeoides* Choisy H5, **EA**-**IP**; *H. elodeoides* Choisy 371nmn, **EA**-**IP**; *H. empetrifolium* Willd. 80nmn, **M**; *H. epigeium* R.Keller D5, **NA**-**SA**; *H. erectum* Thunb. var. *erectum* 304nmn, **EA**-**IP**; *H. ericoides* L. 82nmn, **M**; *H. gracillimum* Koidz. 302nmn, **EA**; *H. graveolens* Buckley AY555843, **NA**; *H. hakonense* Franch. & Sav. 307nmn, **EA**; *H. haplophylloides* Halácsy & Bald. 227nmn, **M**; *H. heterophyllum* Vent. 66nmn, **M**; *H. hirsutum* L. 364nmn, **M**-**EU**; *H. humifusum* L. 101nmn, **M**-**EU**; *H. hyssopifolium* Vill. 104nmn, **M**; *H. jozoeënse* Maxim. AY573004, **EA**; *H. kamtschaticum* Ledeb. AY572992, **EA**; *H. kamtschaticum Ledeb. (haplotype 1)* 321nmn, **EA**; *H. kiusianum Koidz. var. yakusimense (Koidz.) T.Kato* A10.13, **EA**; *H. lanuginosum* Lam. 248nmn, **M**; *H. maculatum* Crantz 24nmn, **M**-**EU**; *H. maculatum* Crantz subsp. *obtusiusculum* (Tourlet) Hayek FJ694199.1, **M**-**EU**; *H. monanthemum* Hook.f. & Thomson ex Dyer 116nmn, **EA**-**IP**; *H. montbretii* Spach 121nmn, **M**; *H. nakaii* H.Koidz. subsp. *nakaii* 310nmn, **EA**; *H. nummularium* L. 125nmn, **M**; *H. oaxacanum* R.Keller AY573003, **NA**; *H. oliganthum* Franch. & Sav. 317nmn, **EA**; *H. oliganthum* Franch. & Sav. 323nmn, **EA**; *H. olympicum* L. forma *uniflorum* D.Jord. & Kozuharov 130nmn, **M**; *H. orientale* L. 352nmn, **M**-**EU**; *H. origanifolium* Willd. var. *origanifolium* 131nmn, **M**; *H. pallens* Banks & Sol. 31nmn, **M**; *H. papillare* Boiss. & Heldr. 265nmn, **M**; *H. papuanum* Ridl. T21.1, **IP**; *H. perfoliatum* L. 139nmn, **M**; *H. perforatum* L. subsp. *chinense* N.Robson 312nmn, **EA**-**IP**; *H. perforatum* L. subsp. *veronense* (Schrank) Ces. 142nmn, **M**-**EU**; *H. petiolulatum* Hook.f. & Thomson ex Dyer subsp. *yunnanense* (Franch.) N.Robson 274nmn, **EA**-**IP**; *H. pibairense* (Miyabe & Y.Kimura) N.Robson 316nmn, **EA**; *H. polyphyllum* Boiss. & Balansa 355nmn, **M**; *H. pseudolaeve* N.Robson 151nmn, **M**; *H. pseudomaculatum* Bush E5, **NA**; *H. pseudopetiolatum* R.Keller AY573002, **EA**; *H. pubescens* Boiss. 152nmn, **M**; *H. pulchrum* L. 155nmn, **M**-**EU**; *H. punctatum* Lam. 158nmn, **NA**; *H. punctatum Lam. haplotype 1* 278nmn, **NA**; *H. reflexum* L. 002nmn, **M**; *H. reflexum* L. T23.2, **M**; *H. rumeliacum* Boiss. subsp. *rumeliacum* 164nmn, **M**; *H. scabroides* N.Robson & Poulter 36nmn, **M**; *H. scouleri* Hook. 171nmn, **NA**; *H. sinaicum* Hochst. ex Boiss 173nmn, **M**; *H. taygeteum* Quézel & Contandr. 005nmn, **M**; *H. tetrapterum* Fr. FJ694224.1, **M**-**EU**; *H. thymifolium* Banks & Sol. 179nmn, **M**; *H. tomentosum* L. 368nmn, **M**; *H. tosaense* Makino 325nmn, **EA**; *H. triquetrifolium* Turra 182nmn, **M**; *H. triquetrifolium* Turra T14.4, **M**; *H. undulatum* Schousb. ex Willd. subsp. *undulatum* 293nmn, **M**-**EU**.
— *Hypericum* "Mediterranean I": *H. aegypticum* L. subsp. *maroccanum* (Pau) N.Robson 43nmn, **M**; *H. aegypticum* L. subsp. *webbii* (Spach) N.Robson 44nmn, **M**; *H. elodes* L. 16nmn, **M**-**EU**; *H. elodes* L. 17nmn, **M**-**EU**.

— *Hypericum* "Mediterranean II": *H. androsaemum* L. T12.7, **M**-**EU**; *H. bupleuroides Griseb. haplotype 1* 376nmn, **M**; *H. canariense* L. var. *canariense* 003nmn, **M**; *H. canariense* L. var. *flori* (Aiton) Bornm. 344nmn, **M**; *H. canariense* L. T23.1, **M**; *H. foliosum* Aiton 20nmn, **M**; *H. grandifolium* Choisy 226nmn, **M**; *H. hircinum* L. subsp. *metroi* (Maire & Sauvage) Sauvage 233nmn, **M**; *H. sampsonii* Hance 324nmn, **EA**; *H. xylosteifolium* (Spach) N.Robson 38nmn, **M**; *H. xylosteifolium* (Spach) N.Robson T06.8, **M**.
— *Hypericum* section *Myriandra*: *H. adpressum* W.P.C.Barton AY555865.2, **NA**; *H. apocynifolium* Small AY555883.2, **NA**; *H. brachyphyllum* (Spach) Steud. AY555870.2, **NA**; *H. buckleyi* **M**.**A**.Curtis AY555880.2, **NA**; *H. chapmanii* W.P.Adams AY555869.2, **NA**; *H. cistifolium* Lam. AY555881.2, **NA**; *H. crux-andreae* (L.) Crantz AY555874.2, **NA**; *H. dolabriforme* Vent. AY555889, **NA**; *H. fasciculatum* Lam. AY555868.2, **NA**; *H. frondosum* Michx. AY555887, **NA**; *H. galioides* Lam. AY555864.2, **NA**; *H. lissophloeus* W.P.Adams AY555885, **NA**; *H. lloydii* (Svenson) W.P.Adams AY555867.2, **NA**; *H. lobocarpum* Gatt. AY555876.2, **NA**; *H. microsepalum* (Torr. & **A**.Gray) **A**.Gray ex S.Watson AY555877.2, **NA**; *H. myrtifolium* Lam. AY555875.2, **NA**; *H. nudiflorum* Michx. AY555888, **NA**; *H. sphaerocarpum* Michx. AY555878.2, **NA**; *H. suffruticosum* W.P.Adams Sara.2, **NA**; *H. tenuifolium* Pursh AY555872.2, **NA**; *H. tetrapetalum* Lam. AY555882.2, **NA**.
— *Triadenum*: *Tr. japonicum* (Blume) Makino 370nmn, **EA**; *Tr. tubulosum* (Walter) Gleason 402nmn, **NA**; *Tr. walteri* (J.F.Gmel.) Gleason 405nmn, **NA**; *Tr. fraseri* (Spach) Gleason 400nmn, **NA**.
— *Thornea*: *Th. matudae* (Lundell) Breedlove & E.**M**.McClint. 195nmn, **SA**; *Th. calcicola* (Standl. & Steyerm.) Breedlove & E.**M**.McClint. AY573028, **SA**. – ***Vismia***: *V. cayennensis* (Jacq.) Pers. 381nmn, **SA**; *V. guianensis* (Aubl.) Choisy 382nmn, **SA**; *V. macrophylla* Kunth 383nmn, **SA**.

Nicolai M. Nürk

Curriculum vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

Publications

Nürk NM, Uribe-Convers S, Tank DC, Madriñán S & Blattner FR (in prep.) Out of the Tropics? Historical biogeography of the temperate genus *Hypericum*.

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Other publications

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Posters (selected)

Nürk NM, Madriñán S, Carine M, Chase M & Blattner FR. Molecular phylogeny and historical biogeography *of Hypericum. Botany 2010*, Providence, Rhode Island, US, 31.07.–04. August 2010.

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Stays of NMN at other institutes

02.11.–20.11.2010	Tank Lab – molecular plant systematics, College of Natural Resources & Stillinger Herbarium, University of Idaho, Moscow, USA.
31.10–19.11.2009	Natural History Museum, London, UK.
03.08.–31.08.2009	Department of Botany, University Museum, University of Tokio & Kyoto University Museum, Kyoto University, Japan.
15.06–23.06.2008	Natural History Museum, London, UK.