

Towards a Monophyletic *Hoya* (Marsdenieae, Apocynaceae): Inferences from the Chloroplast *trnL* Region and the *rbcL-atpB* Spacer

LIVIA WANNTORP,^{1,2,4} ALEXANDER KOCYAN,¹ RUURD VAN DONKELAAR,³ and
SUSANNE S. RENNER¹

¹Systematic Botany, Ludwig Maximilians University Munich, Menzinger Strasse 67,
D-80638 Munich, Germany;

²Molecular Systematics Laboratory, Swedish Museum of Natural History, Box 50007,
SE-104 05 Stockholm, Sweden;

³Laantje 1, 4251 EL Werkendam, The Netherlands

⁴Author for correspondence, present address: Department of Botany, Stockholm University, Lilla Frescativägen
5, SE-10691, Stockholm, Sweden (livia.wanntorp@botan.su.se)

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ABSTRACT. The delimitation of the genus *Hoya*, with at least 200 species distributed from India and China to Australia, from its closest relatives in the Marsdenieae has long been problematic, precluding an understanding of the evolution and biogeography of the genus. Traditional circumscriptions of genera in the *Hoya* alliance have relied on features of the flower, but these overlap extensively between clades and may be evolutionarily labile. We obtained chloroplast DNA sequences to infer the phylogenetic relationships among a sample of 35 taxa of *Hoya* and 11 other genera in the tribe Marsdenieae, namely *Absolmsia*, *Cionura*, *Dischidia*, *Dregea*, *Gongronema*, *Gunnessia*, *Madangia*, *Marsdenia*, *Micholitzia*, *Rhyssolobium*, and *Telosma*. Trees were rooted with representatives of Asclepiadeae, Ceropegieae, Fockeeae, Periplocoideae, and Secamonoideae. *Hoya* and *Dischidia* form a monophyletic group, but the phylogenetic signal in the chloroplast data analyzed here was insufficient to statistically support the mutual monophyly of the two genera. A monophyletic *Hoya*, however, must include the monotypic *Absolmsia*, *Madangia*, and *Micholitzia*, a result congruent with their flower morphology. The data also identified several well-supported groups within *Hoya*. The morphologically unusual *Gunnessia* belongs firmly in the Marsdenieae, but it is not close to *Hoya* and *Dischidia*.

KEYWORDS: chloroplast, flowers, *Hoya*, Marsdenieae, phylogeny, *rbcL-atpB* spacer, *trnL* region.

Species of *Hoya* R. Br. (Figs. 1A, 2A, B), the wax flower genus, are among the most commonly cultivated indoor plants in Europe, the United States, and Australia. Named in 1810 by Robert Brown in honor of his friend, the gardener Thomas Hoy, the popularity of *Hoya* as ornamentals has grown continuously, and according to The Plant Names Project (1999), *Hoya* now includes over 500 species names. However, problems with species delimitation abound, and a number of 200 species may be more realistic (Kleijn and van Donkelaar 2001). The genus mainly occurs in tropical rain forests of Southeast Asia with outposts in Sri Lanka, China, Japan, Australia, and the Pacific.

Most species of *Hoya* are epiphytic herbaceous climbers, basally woody, and with twining or pendulous stems that develop adventitious roots from the internodes. Like many other Apocynaceae, *Hoya* is laticiferous, with succulent leaves that vary greatly in shape, degree of hairiness, thickness, venation, and color of the latex. The flowers are grouped in umbelliform extra-axillary inflorescences (Forster et al. 1998; Omlor 1998) that can be concave and positively geotropic, convex and positively geotropic, or convex and negatively geotropic (Rintz 1978). New flowers are generally produced on persistent peduncles (Omlor 1998). The corolla varies in shape, from globose with revolute lobes to salver-shaped with erect, spreading or reflexed lobes, to campanulate or even tubular. *Hoya* has

staminal coronas (Fig. 2A), consisting of five lobes arising from the backs of the stamens (Forster et al. 1998). Each corona lobe generally has an erect inner projection and a spreading outer projection with revolute margins (Fig. 2A). As in all Asclepiadoideae, the stamens in *Hoya* are fused to each other and to the style head forming a rigid pentangular conical gynostegium. Each anther produces two pollinia that, in most species are provided with a lateral germination area, called the pellucid margin, a potential apomorphy of *Hoya* s.l. (Fig. 2B). The pollinia from adjacent anthers are united by a translator apparatus formed from hardened stigma secretions. The resulting pollinaria are highly variable in shape and size. The fruits of *Hoya* are narrow follicles with a thin pericarp and contain comose fusiform seeds without prominent wings but with keel-shaped thickenings on the lateral walls of cells in the seed coats (Omlor 1998; Kleijn and van Donkelaar 2001).

Ten genera are currently considered synonymous to *Hoya*: *Acanthostemma* (Blume) Blume, *Centrostemma* Decne., *Cyrtoceras* Benn., *Cystidianthus* Hassk., *Otostemma* Blume, *Physostelma* Wight, *Plocostemma* Blume, *Pterostelma* Wight, *Schollia* Jacq., and *Sperlingia* Vahl (Omlor 1998). Several, such as *Acanthostemma*, *Centrostemma*, *Otostemma*, *Physostelma*, *Plocostemma*, and *Pterostelma*, were included in *Hoya* as sections once it became clear that morphological characters of the corolla and coro-

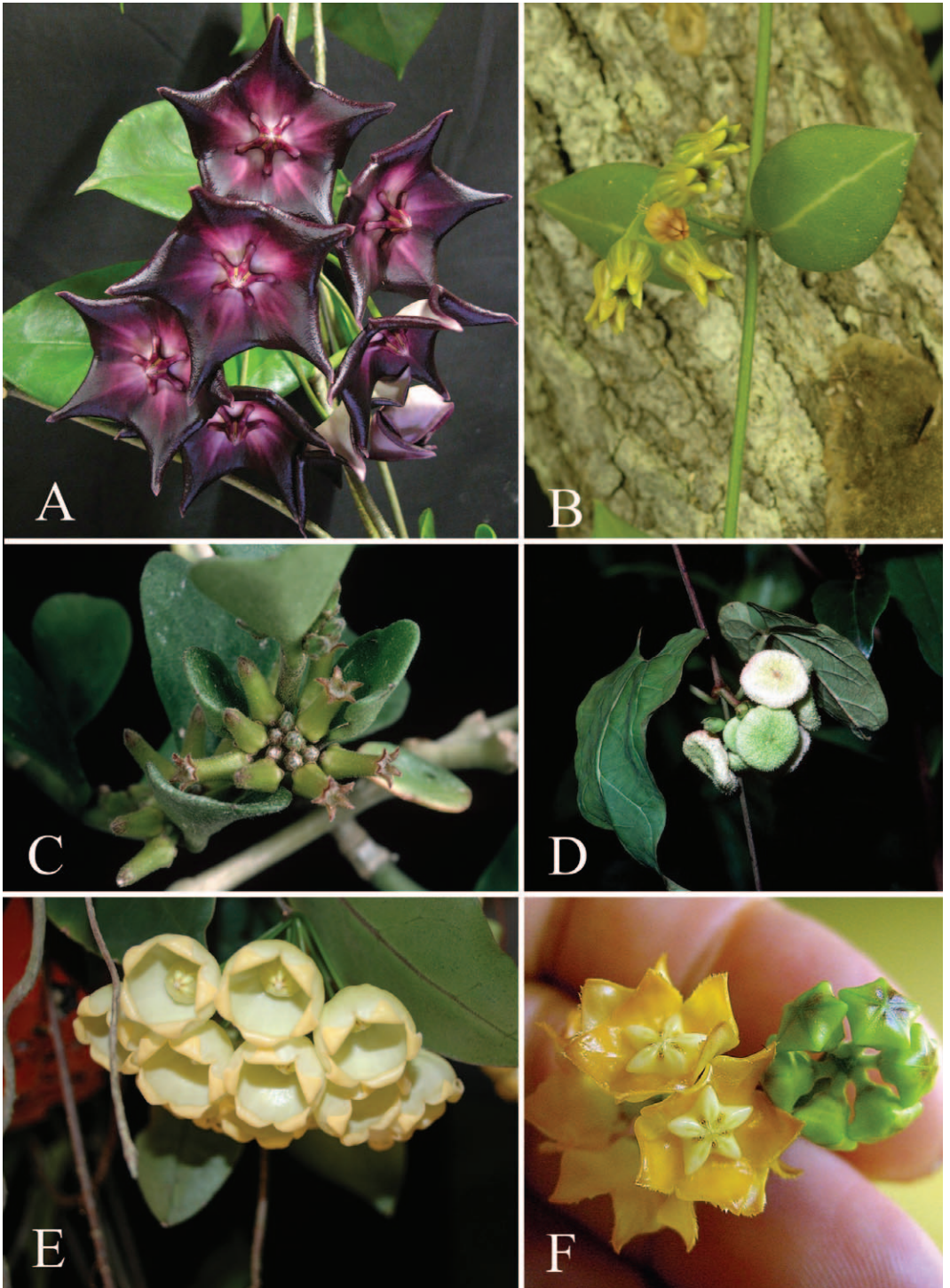


FIG. 1. Representative genera of Marsdenieae. A. *Hoya macgillivrayi* (Australia). B. *Dischidia ovata* (New Guinea). C. *Micholitzia obcordata* (Thailand, India, and southern China). D. *Gunnessia pepo* (Australia). E. *Madangia inflata* (New Guinea). F. *Absolmsia spartioides* (Borneo). Photographs: A, B, E by D. Liddle, C by D. Goyder, D by G. Sankowsky and F by T. Nyhuus.

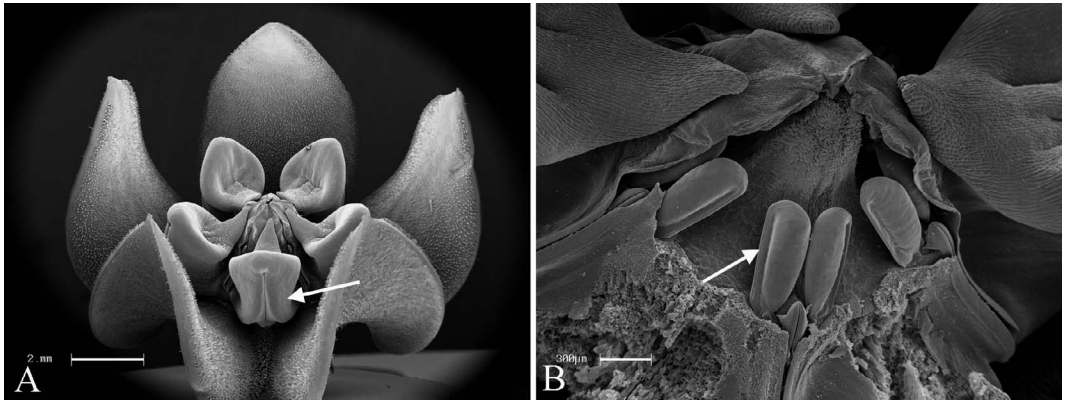


FIG. 2. *Hoya australis*. A. Mature flower showing outer coronal lobes with revolute margins (arrow). B. Detail of flower showing pollinia with pellucid margins (arrow). Scanning electronic microscopic (SEM)-micrographs: Livia Wanntorp.

na, previously considered distinctive, were also exhibited by more typical species of *Hoya*. (Schumann 1895; Schlechter 1913; Forster and Liddle 1990).

The first more complete infrageneric classification of the genus was provided by Hooker (1885) and consisted of the four sections *Ancistrostemma* Hook. f., *Cyrtoceras* Hook. f. (= *Cyrtoceras* (Benn.) Schum.), *Euhoja* Miq. (*Hoya* according to today's nomenclatural rules), and *Pterostelma* Wight. Schlechter (1913, 1916) added six sections to this classification: *Eriostemma* Schltr., *Otostemma* (Blume) Schltr., *Oreostemma* Schltr., *Peltostemma* Schltr., *Physostelma* (Wight) Schltr., and *Plocostemma* (Blume) Schltr. Recent attempts to subdivide *Hoya* into infrageneric entities have been made by horticulturalists (14 sections, Burton 1985, 1995, 1996; 16 sections, Kloppenburg 2001b). Besides these efforts, no comprehensive taxonomic revision of *Hoya* has yet been attempted, and many species of *Hoya* have never been placed into sections, pointing to the need for phylogenetic infrageneric studies.

While the position of *Hoya* in Asclepiadaceae (Schumann 1895), now considered part of the Apocynaceae (Judd et al. 1994; Sennblad and Bremer 1996; Endress and Bruyns 2000; Potgieter and Albert 2001), has never been contested, the circumscription of the genus is still unclear. Recent classifications (Liede and Albers 1994; Omlor 1998; Endress and Bruyns 2000) and molecular phylogenies (Sennblad and Bremer 2002) agree in placing *Hoya* in the Marsdenieae, a tribe of about 29 genera and 570 species (Liede and Albers 1994; Omlor 1998). Molecular sampling shows that a monophyletic Marsdenieae includes *Cionura* Griseb., *Dischidia* R.Br. (Fig. 1B), *Dregea* E. Mey., *Gongronema* (Endl.) Decne., *Gymnema* R. Br., *Hoya*, *Marsdenia* R. Br., *Micholitzia* N.E. Br. (Fig. 1C), *Rhyssolobium* (Meve and Liede 2004), *Stephanotis* Thouars (Sennblad and Bremer 2002), and *Telosma* Coville (Potgieter and Albert 2001). The remaining genera of Marsdenieae have not yet been sequenced.

Among them is the Australian monotypic genus *Gunnessia* (Fig. 1D). Flowers of *G. pepo* have previously been compared to those of Ceropogeeae (Bruyns 1981, 1988), Asclepiadeae, and Marsdenieae (Forster 1990), and the genus was placed in the latter tribe by Omlor (1998).

Molecular studies have found a clade comprising *Dischidia*, *Hoya*, and *Micholitzia* (Livshultz 2000; Potgieter and Albert 2001; Sennblad and Bremer 2002). These three genera had also been grouped based on morphology, together with the monotypic New Guinean *Madangia* (Fig. 1E) and the likewise monotypic Bornean *Absolmsia* (Fig. 1F; Omlor 1998). All five taxa share an epiphytic habit, succulent leaves (only in very young plants of *Absolmsia*), long lasting flowers on extra-axillary inflorescences departing from persistent peduncles, valvate corolla lobes, and seeds without prominent wings (Omlor 1996, 1998; Livshultz 2003). However, while flowers of *Absolmsia*, *Hoya*, *Madangia*, and *Micholitzia* have staminal corona lobes with revolute outer margins (Fig. 2A), pollinia with pellucid margins (Fig. 2B), and narrow fusiform seeds with thickenings on the lateral walls of cells in the seed coats (Omlor 1998), none of these diagnostic characters occur in *Dischidia*. Here, pollinia have no pellucid margins, staminal corona lobes, when present, have no revolute margins, and the radial cell walls of the seed coats do not have any thickenings (Omlor 1998; Livshultz 2003). A feature common to *Absolmsia*, *Dischidia*, and *Hoya* are interactions with ants, involving leaf modifications and plant architecture. *Hoya* and *Dischidia*, in addition, include species whose seeds are dispersed by ants and planted in ant-gardens (Kaufmann et al. 2001; Kaufmann 2002). Resolving the relationships among these three genera contributes towards a better understanding of the evolution of ant-symbiosis in Marsdenieae.

Other genera potentially closely related to *Hoya* are

Oreosparte Schltr. from Sulawesi, *Heynella* Backer from Java, and *Anatropanthus* Schltr. from Borneo, each with a single species (Omlor 1998). *Oreosparte celebica* Schltr., only collected a few times, has flowers with a stalked gynostegium and a corona reminiscent of that of *H. multiflora*. The close relationship between *Hoya* and *Heynella lactea* Backer hypothesized by Omlor (1998) was only based on their similar habit and leaves. Unfortunately, neither the pellucid margins of the pollinia nor the seed morphology of *Oreosparte* and *Heynella* have been examined yet. *Anatropanthus borneensis* Schltr. has pollinia with pellucid margins similar to those of *Hoya* (Schlechter 1908) but its holotype was lost in the fire that destroyed the Berlin herbarium in 1943 (Omlor 1998), and we were unable to obtain new material for this study. The positions of *Heynella*, *Oreosparte*, and *Anatropanthus* in tribe Marsdenieae therefore remain uncertain (Omlor 1998).

As the first step in an ongoing project on the evolution of *Hoya* and close relatives by the first author, we here analyze the phylogenetic relationships between *Hoya* and the three monotypic genera *Absolmsia*, *Madangia*, and *Micholitzia*. We mainly rely on the chloroplast gene region used in previous studies of asclepiad generic relationships, namely the *trnL* intron and adjacent spacer (Potgieter and Albert 2001; Rapini et al. 2003; Verhoeven et al. 2003; Meve and Liede 2004), but have added a second chloroplast spacer to augment resolution. Taxa sampled here for the first time are *Absolmsia spartioides* (Benth.) Kunze (*Hoya spartioides* (Benth.) Kloppenb.), *Gunnessia pepo* (Forster 1990), and *Madangia inflata* (Forster et al. 1997), together with *Micholitzia obcordata* and 38 species of *Hoya* and *Dischidia*.

MATERIALS AND METHODS

Taxon Sampling. Eighty-five sequences were newly generated for this study and the remaining 59 were downloaded from GenBank (Appendix 1). To represent *Hoya* and its closest relatives in the Marsdenieae, we sampled 35 species of *Hoya*, three species of *Dischidia*, two of *Marsdenia*, and the monotypic genera *Absolmsia*, *Gunnessia*, *Madangia*, and *Micholitzia*, plus another five species representing the remaining Marsdenieae. Representatives of Asclepiadoideae, Ceropogonioideae, Fockeae, and Secomonoideae (sensu Endress and Stevens 2001; Verhoeven et al. 2003) from among those already sequenced and available in GenBank, were added to the ingroup. Trees were either rooted with three species of Periplocoideae (Appendix 1) or with *Marsdenia carvalhoi* (Marsdenieae).

There are currently major problems with the taxonomy of *Hoya*, due both to the large number of species in the genus and to the continuing description of new species, likely synonymous to species already described. The ingroup of this study included species chosen to represent both the range of geographic and floral variation in the genus. We also tried to include at least one taxon from each of the ten sections recognized by Hooker and Schlechter. The identity of the taxa was verified with the help of literature and *Hoya* experts. Two data sets were constructed; to assess the relationships between *Absolmsia spartioides*, *Madangia inflata*, *Micholitzia obcordata*, and *Hoya* in the Marsdenieae, data set 1 included these three genera, six species of *Hoya*, three species of *Dischidia*, and representatives of the main tribes of Asclepiadoideae. Data set 2 in-

cluded a denser sampling of *Hoya* to identify major groups in the genus. Appendix 1 lists all species sequenced, their sources, GenBank accession numbers, with their tribal assignment, and status as generic type. The data sets are available from TreeBASE (study accession number S1421).

DNA Isolation, Amplification, and Sequence Alignment. Total genomic DNA was isolated from fresh or silica-dried leaves using DNeasy kits (QIAGEN, Valencia, California, USA), and polymerase chain reactions (PCR) were performed with 10 μ M of primers per 25- μ L reaction volume, using PCR beads from Amersham-Pharmacia Biotech (Uppsala, Sweden). To amplify the *trnL* intron and the adjacent spacer before the *trnF* gene, we used the universal primers of Taberlet et al. (1991). The *atpB-rbcL* spacer was amplified using the forward primer 'oligo 2' of Manen et al. (1994) and a primer complementary to the *rbcL* forward primer 1F for the reverse reaction (Renner 1999). Reactions were run in a thermal cycler, using standard settings: one cycle of 95°C for 5 min, followed by 30 cycles (*trnL* region) or 35 cycles (*rbcL-atpB* region) of 95°C for 30 s, 55°C (trnL region)/ 48°C (*rbcL-atpB* region) for 30 s (*trnL* region) or 1 min (*rbcL-atpB* region), 72°C for 1 min and 30 sec (*trnL* region) or 1 min and 40 sec (*rbcL-atpB* region), and 72°C for 8 min (*trnL* region) or for 7 min (*rbcL-atpB* region). PCR products were purified with the QIAquick Gel Extraction Kits (QIAGEN) and the Wizard SV Gel and PCR Clean-Up System (Promega, Wisconsin, USA). Cycle sequencing of the purified PCR products used the BigDye Terminator Cycle Sequencing kit v3.1 (Applied Biosystems [ABI], Norwalk, Connecticut, USA) according to the manufacturer's suggested protocol. The dye was removed by 2 μ L of 3 mol/L NaOAc (pH 4.6) and 50 μ L ethanol precipitation. Samples were run on an ABI 377 automated sequencer at the Molecular Systematics Laboratory of the Swedish Museum of Natural History or on an ABI capillary sequencer (3100 Avant) at the Institute of Systematic Botany at Ludwig Maximilians University, Munich. Both strands were sequenced and consensus sequences were generated, using the Staden Package (Staden 1998) and the software Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Alignment and Phylogenetic Analyses. Alignments were performed manually. In data set 1, 20 insertions or deletions (indels) were detected in the *trnL* region and four in the *rbcL-atpB* spacer. Data set 2 included 25 indels in the *trnL* region and four in the *rbcL-atpB* spacer. Indels were coded following the simple coding indel method of Simmons and Ochoterena (2000), and in all parsimony analyses, substitutions and indels were weighted equally. The two DNA regions were first analyzed separately and later in combination. Sequences of the *rbcL-atpB* spacer were not available for 30 species (see Appendix 1). The effects of missing data on phylogenetic accuracy have been extensively addressed (e.g., Wiens and Reeder 1995; Wiens 2003), and it has been shown that missing data do not necessarily reduce accuracy. We therefore chose to add question marks to the data sets in the combined analyses instead of deleting taxa from the data set. As a result, 33.6% of the data were scored as missing in data set 1. In data set 2, 2.13% of the data cells were scored as missing.

Data set 1 included 12 new *trnL* sequences and 13 new *rbcL-atpB* spacer sequences plus 31 sequences retrieved from GenBank. Data set 2 included 41 new *trnL* and 43 new *rbcL-atpB* spacer sequences plus two sequences from GenBank. All parsimony analyses were conducted in version 4.0b.10 of PAUP* (Swofford 2002). Nonparametric bootstrap analyses based on 1,000 replicates were performed to explore the robustness of the trees. The following options were used in all parsimony analyses: heuristic search with 1,000 random taxon addition replicates, keeping 10 trees in memory, tree bisection-reconnection (TBR) swapping, and collapse zero-length branches. Clade support was also assessed by calculating the Bremer support indices (1994), using the program Autodecay version 4.0.2' (Eriksson 1998).

Bayesian analyses were conducted on data sets 1 and 2 using MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003 on-line manual), after excluding indels. Evolutionary models were selected using MrModeltest version 1.1b (Nylander 2003) in combination with PAUP. MrModeltest uses

hierarchical likelihood ratio testing to choose a best-fitting model from among 24 substitution models. The model best fitting our data was the General Time Reversible model (GTR; Lanave et al. 1984; Tavaré 1986; Rodríguez et al. 1990) with a gamma distribution of substitution rates, and this model was used in all Bayesian analyses. One cold and three incrementally heated Markov chain Monte Carlo (MCMC) chains were run for 2 million generations, saving every 100th tree using random trees as starting points and a temperature parameter value of 0.2 (the default in MrBayes). For each data set, MCMC runs were repeated three times as a safeguard against spurious results. The first 15,000 trees were discarded as burn-in, and a majority rule consensus tree was constructed based on all remaining trees. Examination of the log-likelihoods and the observed consistency between runs suggested that this burn-in was sufficiently long for chains to have become stationary.

RESULTS

The Position of *Absolmsia*, *Madangia*, and *Micholitzia* in the *Marsdenieae*. In data set 1, the *trnL* region comprised 1046 aligned positions and contributed 118 informative characters (including 20 indels). The data set combining sequences of the *trnL* region and of the *rbcl-atpB* spacer comprised 1915 aligned positions of which 128 (including 24 indels) were informative. Parsimony analysis swapped to completion and identified the same well-supported clades as found in the Bayesian analyses. A majority rule consensus of the 6067 most parsimonious trees obtained for the 43 taxa in the combined analysis is shown in Fig. 3 (tree length = 419; consistency index [CI] = 0.86; retention index [RI] = 0.88).

Determination of phylogenetic relationships between and within the tribes Periplocoideae, Secamonoideae, Asclepiadeae, Ceropegieae, and Fockeeae was not the goal of this study. Nevertheless, the tribal relationships recovered, namely Periplocoideae, Secamonoideae (((Marsdenieae, Ceropegieae) Asclepiadeae) Fockeeae), were generally in concordance with those found by Sennblad and Bremer (2002) with *rbcl* and *ndhF* data, and by Potgieter and Albert (2001) and Rapini et al. (2003) with many of the same *trnL* sequences also used here. The sister group to the Marsdenieae are the Ceropegieae (Meve and Liede 2004).

Of the taxa newly sequenced for this study, (a) *Gunnessia pepo* belonged to a well-supported clade with *Cionura erecta*, *Telosma accedens*, *Marsdenia gillespieae*, *M. carvalhoi*, and *Dregea sinensis*. Relationships within this clade were weakly supported (Fig. 3). (b) *Absolmsia*, *Madangia*, and *Micholitzia* clearly grouped with species of *Hoya*. A close relationship between *Dischidia* and *Hoya* s.l. was supported, but the data contained insufficient signal to clearly assess the monophyly of either genus.

Major Clades Within *Hoya*. In data set 2, the *trnL* region provided 61 informative characters (including 25 indels) out of 938 aligned positions, and the *rbcl-atpB* spacer contributed 30 informative characters (including four indels) out of 849 positions. Of the 1787

aligned positions, this matrix contained 85 parsimony informative characters including 29 indels. Parsimony analysis did not swap to completion and we saved the first 100 most parsimonious trees. The *trnL* region and the *rbcl-atpB* spacer did not contribute enough phylogenetic information within *Hoya* and only a few supported clades were identified in the consensus trees of the parsimony and Bayesian analyses. A majority rule consensus from the Bayesian analysis is shown in Fig. 4 (tree length = 346; CI = 0.73; RI = 0.67), including 35 representatives of *Hoya* and closest relatives. The main groupings are (1) *H. bilobata*, *H. gracilis*, *Hoya kentiana*, *H. heuschkeliana*, and *H. tsangii* form a well-supported clade. (2) The two accessions of *H. australis* (Fig. 2) and *H. albiflora* group together and are part of a larger clade with *H. hypolasia*, *H. macgillivrayi*, *H. patella*, *H. venusta*, and *Madangia inflata*, the latter two being sister species. (3) *Hoya carnosa*, *H. kerrii*, *H. meliflua*, *H. pubicalyx*, *H. serpens*, and *Micholitzia obcordata* form a clade with two subclades; one with *H. camphorifolia*, *H. cf. darwinii*, *H. cf. incrassata*, and *H. mitrata*; the other with *Absolmsia spartioides*, *H. caudata*, *H. curtisii*, *H. imbricata*, and *H. lacunosa*. (4) Lastly, *H. affinis*, *H. ariadna*, and *H. ciliata* form a clade.

DISCUSSION

The Marsdenieae in the circumscription of Omlor (1998) comprise 29 genera, of which 12 were included here. This study is the first to include the Australian *Gunnessia*, with the single species *G. pepo* from Northern Queensland, a taxon found to clearly belong to Marsdenieae (Fig. 3). Earlier studies have compared the flowers of *Gunnessia* to those of several genera of the Ceropegieae (*Echinodopsis*, *Heterostemma*, and *Stapeliopsis*), pointing out similarities in their corollas and staminal coronal tubes with fused lobes (Bruyns 1981, 1988; Swarupanandan et al. 1989). However, other characters, such as pollinia without pellucid margins, white latex, and apical anthers appendages present in *Gunnessia* but not in Ceropegieae, do not support a close relationship between *Gunnessia* and this tribe (Forster 1990). Pollinia are globose in shape in both *Gunnessia* and *Tylophora* of the Asclepiadeae but this seems to be their only shared character (Forster 1990). The molecular results show *Gunnessia* to be part of the Marsdenieae (Fig. 3) and *Sarcolobus* probably also belongs in this tribe. An affinity of *Gunnessia* to *Sarcolobus* has also been hypothesized, based on their similar geniculate pollinarium caudicles (Forster 1990), the compact shape of their gynostegia, papillose style heads, and corona lobes (Omlor 1998).

The second newly placed genus of Marsdenieae is *Madangia inflata*, discovered in the Madang Province of northeastern Papua New Guinea in 1997. Forster et al. (1997) thought it closely allied to *Hoya* because of similarities in the pollinaria and overall habit. The results

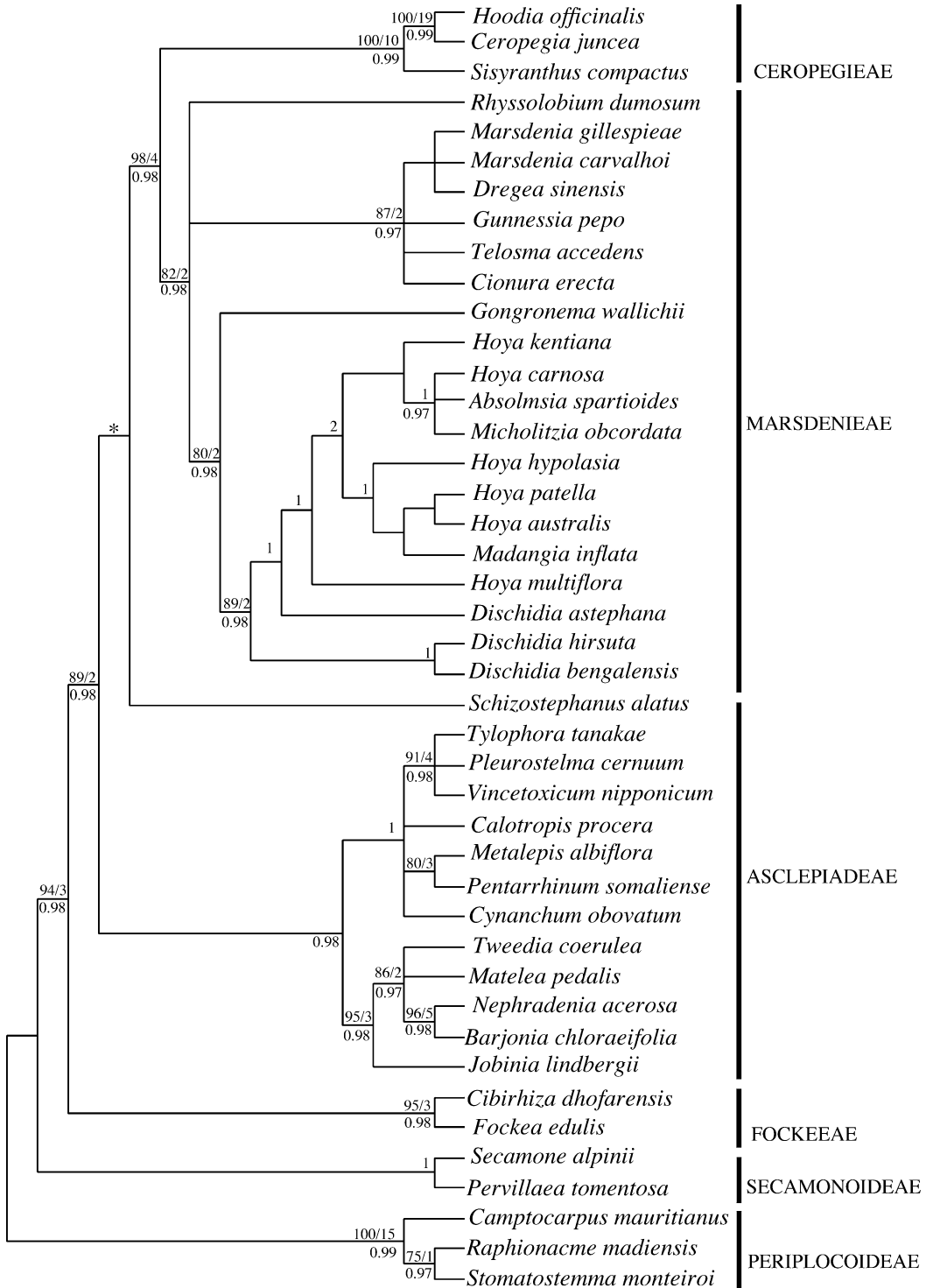


FIG. 3. Position of *Absolmsia*, *Madangia*, and *Micholitzia* in the Marsdenieae. Majority rule consensus tree obtained from the Bayesian analysis, based on the *trnL* region and the *rbcL-atpB* spacer with Bremer support, and bootstrap support greater than 75% shown above branches, and Bayesian posterior probabilities greater than 0.97 shown below branches. The branch marked with an asterisk collapses in the strict consensus tree.

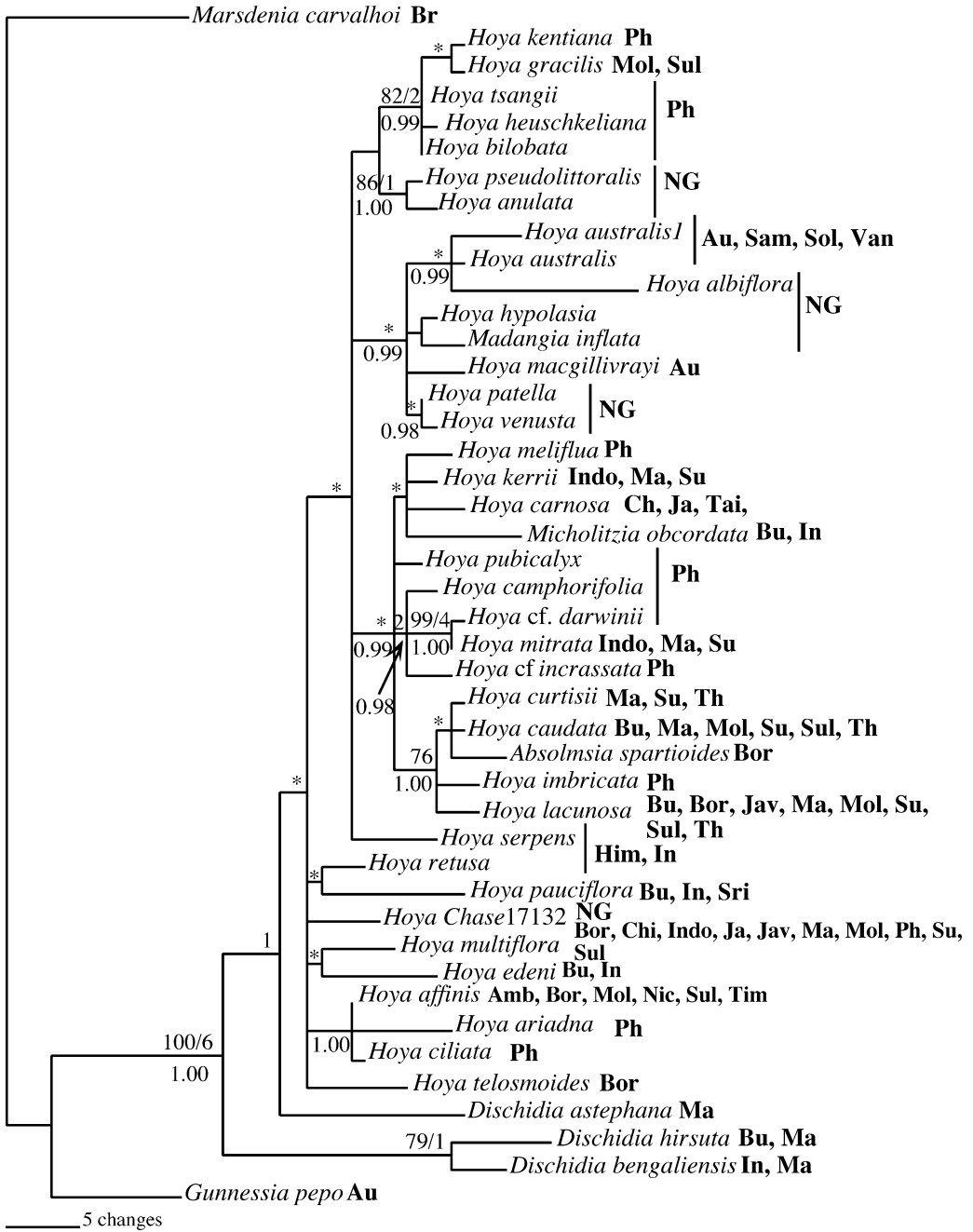


FIG. 4. *Hoya* phylogeny. Majority rule consensus tree obtained from the Bayesian analysis, based on the *trnL* region and the *rbcl-atpB* spacer with Bremer support, bootstrap support greater than 75% shown above branches, and Bayesian posterior probabilities greater than 0.97 below branches. An asterisk shows branches collapsing in the strict consensus tree. Geographic occurrences are abbreviated as follows: Amb=Amboin, Au=Australia, Bor=Borneo, Br=Brazil, Bu=Burma, Ch=China, Him=Himalaya, In=India, Indo=Indochina, Ja=Japan, Jav=Java, Ma=Malaysia, Mol=Moluccas, NG=New Guinea, Nic=Nicobar, Ph=Philippines, Sam=Samoa, Sol=Solomon, Sri=Sri Lanka, Su=Sumatra, Sul=Sulawesi, Tai=Taiwan, Th=Thailand, Tim=Timor, Van=Vanuatu. Taxon abbreviations: *H.* = *Hoya*.

of the present study are in agreement with this morphological evidence and *Madangia* clearly is a part of *Hoya* (Fig. 3). Forster compared the globose corollas of *Madangia* to the urceolate corollas of *H. heuschkeliana*. However, the corolla lobes of that species are not as fused as those of *Madangia*, and Omlor (1998) therefore thought *Madangia* closer to *H. telosmoides* and *H. pusilla*, also with urceolate corollas. Although its exact sister species was not identified, *Madangia* is not close to either *H. heuschkeliana* or *H. telosmoides*, but instead nested in a well-supported clade including the New Guinean and Australian species *H. australis* (Fig. 2), *H. albiflora*, *H. hypolasia*, *H. macgillivrayi*, *H. patella*, and *H. venusta* (Fig. 4).

Absolmsia is a sparsely-branched epiphytic dwarf shrub endemic to Borneo. Leaves are only found on young plants, while older plants only exhibit long, green, photosynthetic peduncles that hang out from under tree crowns. These peduncles bear large clusters of orange-yellow flowers (Fig. 1F). Schlechter (1916) noted the similarity between *Absolmsia* and *Hoya*, and Omlor (1998) stressed characters of the corona, pollinia, and seeds common to *Absolmsia* and *Hoya* (and to *Madangia* and *Micholitzia*). Based on these similarities, Kloppenburg (2001a) transferred *Absolmsia* to *Hoya*. With the present sampling of species of *Hoya*, *Absolmsia* is part of a well-supported clade including *H. caudata*, *H. curtisii*, *H. imbricata*, and *H. lacunosa* (Fig. 4). Although being ecologically indistinguishable (T. Livshultz, pers. comm., 2005), ant gardens in *Absolmsia* should, according to the results of this study, be compared to ant gardens of *Hoya*, rather than to those found in *Dischidia*.

Micholitzia is a slightly succulent epiphyte from India, Thailand, and southern China. It has narrowly urceolate flowers with staminal coronas, reminiscent of those of some species of *Dischidia* (Goyder and Kent 1994). Following its description by N. Brown (1909), *Micholitzia* was repeatedly synonymized either with *Dischidia* (Craib and Kerr 1951; Maxwell 1991) or with *Hoya* (Deb 1955). Further confusion was added by the description of *Hoya lantsangense* Tsiang and Ping-Tao (1974) from China, a species subsequently raised to generic rank as *Antiostelma lantsangense* (Ping-Tao 1992), but later discovered to be a synonym of *Micholitzia obcordata*. According to Goyder and Kent (1994) *Micholitzia* is similar to *Hoya* and *Dischidia* in its habit, the leaves containing white latex, and several floral traits. Previous molecular studies also identified the clade of *Micholitzia*, *Hoya*, and *Dischidia* without resolving their precise relationships (Livshultz 2000; Potgieter and Albert 2001; Sennblad and Bremer 2002). Our results show that *Micholitzia* is nested within *Hoya* but do not resolve its exact position (Fig. 4). Studies on the flower ontogeny and morphology of *Madangia*, *Mich-*

olitzia, *Absolmsia*, and *Hoya* will contribute to the understanding of the relationships among these genera.

Dischidia includes about 80 species of epiphytes from Southeast Asia and Australia often associated with ants that sometimes live in pouched leaves (Omlor 1998; Livshultz 2000; Kaufmann et al. 2001; Kaufmann 2002). Previous *trnL*-based studies (Potgieter and Albert 2001; Rapini et al. 2003) only sampled one species each of *Dischidia* and *Hoya* (plus the monotypic *Micholitzia*) and could therefore not address the mutual monophyly of *Dischidia* and *Hoya*. The present study supports a close relationship between the two genera (Fig. 3), but species sampling in *Dischidia* is again insufficient to resolve whether these genera are both monophyletic.

Problems with classifications of *Hoya* that are based on morphology are well known (Burton 1985, 1995, 1996; Kloppenburg 2001b). An example is the clade formed by the species *H. caudata*, *H. curtisii*, *H. imbricata*, and *H. lacunosa* from Thailand, Malaysia, and the Philippines, as well as *Absolmsia spartioides* from Borneo (Fig. 4). Of these species, *Hoya curtisii* was placed in section *Plocostemma* by Burton (1996), but included in a monotypic section (*Kloiphora* King) by Kloppenburg (2001b). Both authors classified *H. lacunosa* in section *Otostemma*, and *H. caudata* and *H. imbricata* in section *Peltostemma*. Clearly, a denser taxon sampling in combination with additional molecular information is needed before current classifications can be revised to reflect evolutionary lineages. Other clades identified here are in closer agreement with earlier morphological studies of *Hoya*. For example, species from the Philippines, Moluccas, and Sulawesi (*H. bilobata*, *H. gracilis*, *H. kentiana*, *H. heuschkeliana*, and *H. tsangii*) group together (Fig. 4), in keeping with their traditional placement in section *Acanthostemma* (Blume) Blume, and *H. affinis*, *H. ariadna*, and *H. ciliata* group together fitting with their traditional placement in section *Eriostemma* (Schlechter 1913).

The Southeast Asian/Australian geographic range of *Hoya*, with outposts in India and Sri Lanka to the West, in China to the North, and in the Fiji Islands to the East, makes this genus an interesting group for studying floristic relationships between Southeast Asia, Australia, New Guinea, and India. The species included here cover almost the entire distribution of *Hoya*, and the results suggest complex biogeographical patterns in *Hoya*, which, for example, reached the Philippines several times from different source areas; some Philippines species group with species from Sulawesi, the Moluccas, and New Guinea (Fig. 4), others with species from China, Taiwan, and Japan, and yet others with species from the Malaysian archipelago or the Southeast Asian mainland. In addition, most species from New Guinea and Australia (*H. albiflora*, *H. australis*, *H. hypolasia*, *H. macgillivrayi*, *H. patella*, *H. venusta*,

and *Madangia inflata* group together (Fig. 4), which fits with New Guinea and Australia lying on the same continental plate and having been connected at times of low sea levels during the Pliocene and Pleistocene (McLoughlin 2001). The two regions are often found as sister areas in biogeographical studies (e.g., Linder and Crisp 1995; Wanntorp and Wanntorp 2003).

This study has shown that a monophyletic *Hoya* must include the monotypic genera *Absolmsia*, *Madangia*, and *Micholitzia*. A comprehensive phylogeny of *Hoya*, based on a larger taxon sample and amount of sequence data from faster-evolving gene regions from the nuclear genome, as well as morphological data will provide the basis for a taxonomic revision of the genus, and for the understanding the evolution of its complex flowers.

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APPENDIX 1. Names of the specimens, voucher details, and GenBank accession numbers (in the following sequence: *trnL-F* spacer, *trnL* intron, *trnL-F* spacer & *trnL* intron, *atpB-rbcL* spacer). Herbarium abbreviations follow *Index Herbariorum* (Holmgren et al. 1990). Italicized accession numbers indicate sequences downloaded from GenBank.

ASCLEPIADOIDEAE R. Br. Ex Burnett/ASCLEPIADEAE (R. Br.)

Duby: *Barjonia chloraeifolia* Decne., *Rapini* 495 (SPF), Brazil, –, –, AY163667, –, *Calotropis procera* (Aiton) W. T. Aiton. (Genus type), *Huber s.n.* (UBT), Gambia, AJ428797, AJ428796, –, –, *Cynanchum obovatum* R. Holm, *Mangelsdorff* 14 (UBT), Madagascar, AJ428803, AJ428802, –, –, *Jobinia lindbergii* Fourn., *Farinaccio* 194 (SPF), Brazil, –, –, AY163694, –, *Matelea pedalis* Fontella & E.A.Schwarz, *Rapini* 714 (SPF), Brazil, –, –, AY163699, –, *Metalepis albiflora* Urb., *Burnham* 1611 (MO), Ecuador, AJ428776, AJ428775, –, –, *Nephradenia acerosa* Decne. (Genus type), *Philcox* 3303 (K), Brazil, AY163705, AY163704, –, –, *Pentarrhinum somaliense* (N.E. Br.) *Liede, Mesfin & Vollesen* 4238 (UPS), Ethiopia, AJ428824, AJ428823, –, –, *Pleurostelma cernuum* (Decne.) *Bullock, Liede & Meve* 3377 (UBT), Tanzania, AJ410243, AJ410242, –, –, *Schizostephanus alatus* Hochst. ex K.Schum. (Genus type), *Noltee s.n. sub IPPS* 8111 (UBT), Kenya, AJ410249, AJ410248, –, –, *Tylophora tanakae* Maxim., T. Y. 4003 (URO), Japan, AB109951, AB109919, –, –, *Tweedia coerulescens* D. Don ex Sweet, unknown, Brazil and Uruguay, AF214289, AF214443, –, –, *Vincetoxicum nipponicum* (Matsumura) *Kitag., T. Y. & A. Y.* 7571 (TUS), Japan, AB109963, AB109932, –, –

CEROPEGIEAE Orb.: *Ceropegia juncea* Roxb., *Rikaneek & Hanacek* 92 (UBT), India, AJ428800, AJ428799, –, –, *Hoodia officinalis* (N.E. Br.) D.C.H. Plowes, *Meve* 176 (MSUN, UBT), Namibia, AJ488395, AJ488394, –, –, *Sisyranchus compactus* N.E. Br., *Nicholas* 2825 (UDW), South Africa, AJ410069, AJ410068, –, –

FOCKEEAE H. Kunze, Meve & Liede: *Cibirhiza dhofarensis* P. Bruyns (Genus type), *Butler s.n.* (UBT), Oman, AJ431739, AJ431738, –, –, *Fockea edulis* K. Schum., ex hort. (UBT), South Africa, AJ431742, AJ431741, –, –

MARSDENIEAE Benth.: *Hoya spartioides* (Benth.) *Kloppenb.* = *Absolmsia spartioides* (Benth.) *Kunze* (Genus type), *Wanntorp* L. 592 (S), Borneo, –, –, DQ334549, DQ334591; *Cionura erecta* (R. Br.) *Grieseb.* (Genus type), *Heyne* 120 (UBT), Turkey, AJ410174, AJ410173, –, –, *Dischidia astephana* *Scort. ex King & Gamble*, *Wanntorp* L. 562 (S), Malaysia, –, –, DQ334534, DQ334576; *Dischidia bengalensis* *Col-ber*, 920392 (CONN), Southeast Asia, India, and Burma, AF214189, AF214343, –, –, *Dischidia hirsuta* *Decne.*, *Wanntorp* L. 563 (S), ex hort. *Departm. Bot., Stockholm University, Burma and Malaysia*, –, –, DQ334531, DQ334573; *Dregea sinensis* *Hemsl.*, unknown, China, AF214191, AF214345, –, –, *Gongronema wallichii* *Decne.*, *Schneidtf* 95–130 (ABD), Malaysia, AJ431748, AJ431747, –, –, *Gunnessia pepo* *P. I. Forster* (Genus type), *Pf. Forster* PIF6465 (BRI), Australia, –, –, DQ334528, DQ334570; *Hoya affinis* *Hemsl.*, *Chase* 17128 (K), (RBG-Kew), *Liv. Coll.* 1983–4478, Solomon Islands, –, –, DQ334546, DQ334588; *Hoya albiflora* *Zipp. ex Blume*, *Wanntorp* L. 584 (S), L20006646, New Guinea, –, –, DQ334555, DQ334597; *Hoya anulata* *Schltr.*, *Wanntorp* L. 585 (S), IPPS 8603, L990438, New Guinea, –, –, DQ334550, DQ334592; *Hoya ariadna* *Decne.*, *Chase* 17125 (K), (RBG-Kew), *Liv. Coll.* 1983–4474, Malayan Archipelago, –, –, DQ334559, DQ334602; *Hoya australis* *R.Br. ex Traill*, *Wanntorp* L. 564 (S), ex hort. *Departm. Bot., Stockholm University, Pacific Islands and Australia*, –, –, DQ334527, DQ334569; *Hoya australis* *1 R.Br. ex Traill*, *Wanntorp* L. 565 (S), ex hort. *Departm. Bot., Stockholm University, Pacific Islands and Australia*, –, –, DQ334524, DQ334566; *Hoya bilobata* *Schltr.*, *Chase* 17129 (K), (RBG-Kew), *Liv. Coll.* 1983–4481, Philippines, –, –, DQ334554, DQ334596; *Hoya cam-*

- phorifolia* Warburg, *Wanntorp* L. 590 (S), Philippines, -, -, DQ334539, DQ334581; *Hoya carnosia* R.Br. (Genus type), *Wanntorp* L. 566 (S), ex hort. Departm. Bot., Stockholm University, China, Japan, and Taiwan, -, -, DQ334535, DQ334577; *Hoya caudata* Hook. f., *Wanntorp* L. 587 (S), ex hort. Departm. Bot., Stockholm University, Southeast Asia, -, -, DQ334548, DQ334590; *Hoya ciliata* Elmer ex C.M.Burton, *Wanntorp* L. 586 (S), IPPS 3071, L920785, Philippines, -, -, DQ334562, DQ334605; *Hoya curtisii* King & Gamble, *Wanntorp* L. 578 (S), 1998–3180, Uppsala Bot. Gar., Thailand, Malaysia, and Indonesia, -, -, DQ334544, DQ334586; *Hoya* aff. *darwinii* Loher, *Chase* 17135 (K), RBG-Kew, Liv. Coll. 1984–2899, Philippines, -, -, DQ334542, DQ334584; *Hoya edeni* King ex Hook.f., *Wanntorp* L. 579 (S), IPPS 8292, India and Burma, -, -, DQ334540, DQ334582; *Hoya gracilis* Schltr., *Wanntorp* L. 567 (S), ex hort. Departm. Bot., Stockholm University, Sulawesi and Moluccas, -, -, DQ334426, DQ334568; *Hoya heuschkeliana* Kloppenb., *Wanntorp* L. 568 (S), ex hort. Departm. Bot., Stockholm University, Philippines, -, -, DQ334529, DQ334571; *Hoya hypolasia* Schltr., *Wanntorp* L. 588 (S), IPPS 7006, L901824, New Guinea, -, -, DQ334538, DQ334580; *Hoya imbricata* Decne., *Wanntorp* L. 569 (S), ex hort. Departm. Bot., Stockholm University, Philippines, -, -, DQ334545, DQ334587; *Hoya* cf. *incrassata* Elmer ex Merr., *Chase* 17136 (K), RBG-Kew, Liv. Coll. 1984–3340, Philippines, -, -, DQ334561, DQ334604; *Hoya kentiana* C. M. Burton, *Wanntorp* L. 570 (S), ex hort. Departm. Bot., Stockholm University, Philippines, -, -, DQ334522, DQ334564; *Hoya kerrii* Craib, *Chase* 17123 (K), RBG-Kew, Liv. Coll. 1982–2786, Southeast Asia, -, -, DQ334533, DQ334575; *Hoya lacunosa* Blume, *Wanntorp* L. 571 (S), ex hort. Departm. Bot., Stockholm University, Southeast Asia, -, -, DQ334557, DQ334599; *Hoya macgillivrayi* F. M. Bailey, *Wanntorp* L. 572 (S), ex hort. Departm. Bot., Stockholm University, Australia, -, -, DQ334553, DQ334595; *Hoya meliflua* Merr., *Wanntorp* L. 591 (S), Philippines, Mindoro Occidental, Puerto Galera, Philippines, -, -, DQ334525, DQ334567; *Hoya mitrata* Kerr, *Wanntorp* L. 589 (S), IPPS 7684, L914643, Southeast Asia, -, -, DQ334558, DQ334600; *Hoya multiflora* Blume, *Wanntorp* L. 573 (S), ex hort. Departm. Bot., Stockholm University, China and Southeast Asia, -, -, DQ334552, DQ334594; *Hoya patella* Schltr., *Wanntorp* L. 575 (S), ex hort. Departm. Bot., Stockholm University, New Guinea, -, -, DQ334556, DQ334598; *Hoya pauciflora* Wight, *Wanntorp* L. 574 (S), ex hort. Departm. Bot., Stockholm University, India, Burma, and Sri Lanka, -, -, DQ334536, DQ334578; *Hoya pseudolittoralis* C. Norman, *Wanntorp* L. 582 (S), IPPS 4551, New Guinea, -, -, DQ334543, DQ334585; *Hoya pubicalyx* Merr., *Wanntorp* L. 576 (S), ex hort. Departm. Bot., Stockholm University, Philippines, -, -, DQ334530, DQ334572; *Hoya retusa* Dalz., *Wanntorp* L. 580 (S), ex hort. 1998–3127, Rosendal Uppsala, Uppsala Bot. Gar., Himalaya and India, -, -, DQ334532, DQ334574; *Hoya serpens* Hook. f., *Chase* 17118 (K), RBG-Kew, Himalaya and India, -, -, DQ334547, DQ334589; *Hoya telosmoides* R. Omlor, *Wanntorp* L. 577 (S), Malaya, -, -, DQ334551, DQ334593; *Hoya tsangii* C. M. Burton, *Wanntorp* L. 581 (S), 1998–3136, Uppsala Bot. Gar., Philippines, -, -, DQ334523, DQ334565; *Hoya venusta* Schltr., *Wanntorp* L. 583 (S), IPPS 3773, New Guinea, -, -, DQ334560, DQ334603; *Hoya*—*Chase* 17132, *Chase* 17132 (K), RBG-Kew, Liv. Coll. 1983–4484, New Guinea, -, -, DQ334537, DQ334579; *Madangia inflata* P.I. Forst., D.J. Liddle & I.M. Liddle (Genus type), I.M. Liddle IML1076 (BRI), New Guinea, -, -, DQ334541, DQ334583; *Marsdenia gillespieae* Morillo, Ollerton et al. 212 (UBT), Guyana, AJ431757, AJ431756, -, -, *Marsdenia carvalhoi* Morillo & Carnevali, *Chase* 17115 (K), RBG-Kew, Liv. Coll. 1982–1949, Brazil, -, -, DQ334521, DQ334563; *Micholitzia obcordata* N.E. Br. (Genus type), *Seidenfaden* s.n. (K) (MWC 733); *Wanntorp* L. 596 (S), ex hort. Departm. Bot., Stockholm University, Thailand, India, and China, AJ431766, AJ431765, -, -, DQ334601; *Rhyssolobium dumosum* E. Mey (Genus type), *Bruyns* 3948 (BOL; MWC 3857), South Africa, AJ431775, AJ431774, -, -, *Telosma accedens* (Blume) Backer, *Schneid* 96–101 (UBT), Philippines, AJ431784, AJ431783, -, -
- PERIPLOCOIDEAE** Bartl.: *Camptocarpus mauritianus* Decne. (Genus type), *Petignat* s. n. (UBT), Madagascar, AJ581796, AJ581795, -, -; *Stomatostenma monteiroi* N. R. Br. (Genus type), IPPS 1427 (UBT), -, AJ431781, AJ431780, -, -; *Raphionacme madiensis* S. Moore, *Specks* 682 (UBT), Tanzania, AJ581826, AJ581827, -, -
- SECAMONOIDEAE** G. Don: *Pervillaea tomentosa* Decne. (Genus type), 187498, ex hort. Palmengarten, Madagascar, AJ431769, AJ431768, -, -; *Secamone alpinii* Schult., *Liede* 2931 (UBT), South Africa, AJ428830, AJ428829, -, -