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Authors: Rodda, Michele, Juhonewe, Nadhanielles Simonsson, and Ercole, Enrico

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Hoya corymbosa (Apocynaceae, Asclepiadoideae), a New Unusual Species from Sabah, Borneo, and its Systematic Position based on Phylogenetic Analysis

Michele Rodda,^{1,4} Nadhanielle Simonsson Juhonewe,² and Enrico Ercole³

¹Herbarium, Singapore Botanic Gardens, 1 Cluny Road, Singapore 259569.

²National Research Institute of Papua New Guinea, Post Office Box 1 – 524, Ukarumpa, Eastern Highlands Province 444, Papua New Guinea.

³Dipartimento di Biologia Vegetale, Viale PA Mattioli 25, 10125 Torino, Italy.

⁴Author for correspondence (rodda.michele@gmail.com)

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Abstract—A new species, *Hoya corymbosa*, from Sabah (Borneo), is described and illustrated. It is a horizontally growing epiphytic shrub with diminutive flowers. Its minute staminal corona lobes lacking revolute outer margins and broad, flat caudicles of the pollinarium (resembling those of *Dischidia*) are quite peculiar within the genus, which is otherwise characterized by corona lobes with revolute margins and generally round to oval caudicles. Its position in *Hoya* has been confirmed by phylogenetic analysis based on nuclear ribosomal ITS region, the *matK* gene and *psbA-trnH* intergenic spacer.

Keywords—critically endangered, ITS, lower montane forest, *matK*, *psbA-trnH*.

Hoya R. Br. is a large genus with more than 500 published species names (The International Plant Name Index, 2013), and an estimated 200–300 species (Kleijn and van Donkelaar 2001). It includes mostly climbing epiphytes found in inaccessible places high in the canopy in tropical and subtropical forests of mainland Asia, Malesia, Melanesia, and Australia (Forster et al. 1998). Only a few species are pendulous, such as *Hoya linearis* Wall. ex D. Don, or form epiphytic shrubs, such as *Hoya medinillifolia* Rodda & Simonsson (Rodda and Simonsson 2011).

The flowers of *Hoya* are held by slender terete pedicels borne on peduncles that can bear flowers multiple times and often last several years (Rintz 1978). They form concave, flat, or convex inflorescences and are characterized by a corolla with five lobes, ranging from almost entirely fused, forming a campanulate flower, to nearly free, spreading, reflexed or revolute, and by a staminal corona (Cs sensu Liede and Kunze 1993) with lobes often horizontally spreading, including an inner process appressed to the anthers, and a variable outer process with revolute lateral margins (see figures in Kunze and Wanntorp 2008). The seeds are comose, spindle-shaped, and lack differentiated margins. The pollinaria are formed by two pollinia with a conspicuous, pellucid, germination zone (Omlor 1996) connected to a pigmented retinaculum by caudicles, generally round or oval, rarely ribbon-shaped, as observed in species of *Hoya* section *Eriostemma* Schltr.

Recent molecular studies based on *atpB-rbcL* spacer, ITS and *trnL* regions (Wanntorp et al. 2006a, b), the *matK* gene and *psbA-trnH* intergenic spacer (Wanntorp et al. 2011) have led to a recircumscription of the genus and the merger of genera previously considered as belonging elsewhere in *Marsdenieae* (Wanntorp and Forster 2007; Wanntorp et al. 2011; Wanntorp and Meve 2011) reducing the genera belonging to *Marsdenieae* from 29 (Endress and Bruyns 2000) to 25. The inclusion of these taxa in *Hoya* introduced novel characters to the genus, such as small urceolate corollas (in *Hoya manipurensis* Deb), revolute basal skirt in the corona lobe and broadly urceolate corolla (*Hoya inflata* (P. I. Forst., Liddle & I. M. Liddle) L. Wanntorp & P. I. Forst. (Forster et al. 1997; Wanntorp and Forster 2007)), fleshy coronas with valvate lobes and clavate pollinia without pellucid margins, small and narrow retinacula and long ribbon-shaped caudicles

(*Hoya mariae* (Schltr.) L. Wanntorp & Meve and *Hoya omlorii* (Livsh. & Meve) L. Wanntorp & Meve (Wanntorp and Meve 2011)). New potential synapomorphies for *Hoya* have also been identified: pollinia with pellucid margins, anther skirts, modified guide rails, and secondary nectaries (Wanntorp and Kunze 2009).

As part of ongoing work on the systematics of *Hoya*, a new species was identified among living collections cultivated at Kipandi Butterfly Farm, Sabah, Malaysia. Based on morphological characters, the new species described below is atypical within the genus because it exhibits (1) horizontal or pendulous shrubby habit, (2) anvil-shaped corona lobes lacking revolute outer margins and (3) broad, flat caudicles of the pollinarium. In light of the atypical morphology, the objective of this study was to verify the generic placement of the new species in the genus *Hoya* by a molecular phylogenetic analysis and subsequently to describe it formally.

MATERIALS AND METHODS

Morphological Observation—This study is based on the comparison of specimens of *Marsdenieae* from BM, BRUN, FI, K, KEP, L, LAE, P, SAN, SAR, SNP, and SING herbaria and from living specimens cultivated in Kipandi Butterfly Farm, Sabah, Malaysia. Observation of the specimens was carried out with the aid of a light microscope. Pollinaria were extracted and mounted on glass slides prior to microscopic observation.

Phylogenetic Analysis—To confirm the generic position of the new species, we performed a phylogenetic analysis of *Hoya* based on nuclear ribosomal internal transcribed spacer (ITS), *matK* gene, and *psbA-trnH* intergenic spacer DNA sequences. The choice of markers was dictated by the availability of reference sequences in GenBank published as part of recent investigations on *Hoya* (Wanntorp et al. 2011). ITS, *matK*, and *psbA-trnH* sequences are available for most *Hoya* taxa, while sequences of *atpB-rbcL* spacer and *trnL* regions are generally incomplete or not available.

DNA extraction and sequencing was performed as described in Wanntorp et al. (2011) from one accession of *Hoya corymbosa* Rodda & Simonsson (Voucher: Rodda MR10–003, SING) and of two further shrubby species, *Hoya lockii* V. T. Pham & Aver. (Voucher: Rodda M. Hort2010.X, TO) and *Hoya ignorata* T. B. Tran et al. (Tran et al. 2011) (Voucher: Simonsson & Somadee NS10–007, SING). The PCR products were purified using Wizard® PCR and gel clean-up system (Promega Corporation, Madison, Wisconsin), according to the manufacturer's recommendations. Sequencing was performed by AITBiotech Pte Ltd, Singapore, and all newly obtained sequences were deposited in GenBank (Appendix 1). For the phylogenetic analysis, our sequences were aligned with 67 reference taxa, including 62 species of *Hoya*, two species of *Dischidia* R. Br. (ingroup), and three species of *Marsdenia* R.Br. (outgroup taxa) selected

among those published in recent phylogenetic studies on *Hoya* (Wanntorp et al. 2011) for which ITS, *matK*, and *psbA-trnH* sequences were available. These may not fully represent the morphological and geographical complexity of the genus, which still requires further investigation. The specimen of *Hoya wallichii* (Wight) C. M. Burton included in Wanntorp et al. (2011) was re-identified as *Hoya danumensis* Rodda & Nyhuus. All sequences used are listed in Appendix 1.

Single alignments for each data set of ITS, *matK*, and *psbA-trnH* sequences were generated using CLUSTALX 2.0 (Larkin et al. 2007) with default conditions for gap openings and gap extension penalties. Best-fit model estimation was performed by jModeltest (Posada 2008). The best-fit substitution models adopted were GTR + G, HKY + G, GTR + G for the ITS, *matK* and *psbA-trnH* analysis, respectively. A combined molecular phylogenetic analysis was performed using the Bayesian Inference (BI) approach. Congruence of the three datasets was verified by computation of Congruency Index I_{cong} (de Vienne et al. 2007). Trees based on the single regions are available upon request. Bayesian Inference of phylogeny using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Four incrementally heated simultaneous MCMC were run over 10,000,000 generations, under model assumptions. Trees were sampled every 1,000 generations resulting in an overall sampling of 10,001 trees, with the first 2,500 trees discarded as "burn-in" (25%). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. Only BPP values over 0.8 are reported in the resulting tree. Bayesian analysis was repeated three times, always using random starting trees and values for model parameters to test independence of the results from revisiting prior topologies during chain growth (Huelsenbeck et al. 2002).

RESULTS

The examination of herbarium materials allowed us to exclude that the new taxon was already described either in *Hoya* or in any other genus within Marsdenieae. Similarities were recognized only with *H. ignorata* (see Notes below), which as a result was included in the phylogenetic analysis.

Trees reconstructed from single gene analyses (ITS, *matK*, and *psbA-trnH*) were poorly resolved (data not shown, available on request). The topology of the combined tree (Fig. 1) is in accordance with recent phylogenetic investigations of the genus (Wanntorp et al. 2011) and all previously recognized groups (clades 1–7 of Fig. 3 in Wanntorp et al. 2011) are maintained.

Three further clades can be observed. Clade 5 includes species from New Guinea and the Pacific Islands, which was already observed in Wanntorp et al. (2006a), but not in Wanntorp et al. (2011) because in the latter case two species, *Hoya erythrostemma* Kerr from Thailand and Peninsular Malaysia, and *Hoya globulosa* Hook. f. from the Himalayan region and Indochina were nested within the clade. Clade 9 includes two taxa from the Himalayan/Indian region. *Hoya ignorata* and *H. corymbosa* form clade 8 that belongs to a polytomy containing clade 6, 'Acanthostemma clade', clade 7, containing a further three species from the Himalayan/Indian region, clade 9, and the poorly supported clade 10, 'core *Hoya*'. Another shrubby species, *H. lockii*, instead, is part of clade 1, '*Hoya multiflora* Blume clade', which includes other shrubby species.

DISCUSSION

Although initial examination of the new species did not allow its straightforward assignment to *Hoya*, the molecular phylogeny allowed us to verify that it belongs to the genus, and confirm its sister relationship to *H. ignorata*. The following description is based on living materials in cultivation at

Kipandi Butterfly Farm, Sabah, Malaysia, and on the examined specimens from SAN and SING herbaria.

TAXONOMIC TREATMENT

Hoya corymbosa Rodda & Simonsson, sp. nov.—TYPE: MALAYSIA. Sabah: Cultivated in Kipandi Butterfly Farm, originally collected in Malaysia, Sabah, Sipitang District, Mt. Rimau area, lower montane forest being logged at the moment of collection, ca. 1,600 m, 18 June 2009, Gokusing et al. *Al1803/2009* (holotype: SAN!).

Similar to *H. ignorata* because both taxa are epiphytic shrubs bearing positively geotropic inflorescences of small flowers (< 2.8 mm diam.) with reflexed corollas. The two species can be distinguished because *H. ignorata* flowers lack corona lobes and the pollinia are pendulous, connected to the retinaculum by long, narrow, ribbon-shaped caudicles, while *H. corymbosa* has anvil-shaped corona lobes and erect pollinia, connected to the retinaculum by broader caudicles (15 μ m vs. 150 μ m).

Horizontal or pendulous epiphytic shrub with white latex in vegetative parts. All trichomes unicellular eglandular. Leafy stems cylindrical, 2–2.5 mm in diam., dark green, with sparse erect or spreading trichomes; older stems leafless, up to 5 mm in diam., brown, glabrous with peeling bark; internodes 1.5–3 cm long. Leaves held horizontally; petiole terete, 1.5–2 \times ca. 1.2 mm in diam., green, with sparse spreading trichomes; lamina ovate-elliptic, 0.7–2 \times 2–4 cm, thick, fleshy, dark green on adaxial surface with purple margin, light green on abaxial surface, sparsely pubescent on the abaxial surface of young leaves only, adaxial side and old leaves glabrous, apex cuspidate (caudate), base round to attenuate, margin entire, slightly revolute with a purple edge; venation pinnate, midrib depressed on adaxial surface, secondary veins 2–4 on each side, not evident in living and dry material grown in high light, but clearly visible on living material cultivated in low light, anastomosing near the leaf margin, branching from the midrib at 30–60°; colleters 2–4 at the base of the lamina, triangular, brown. Inflorescences one per node, extra-axillary, positively geotropic, corymbiform, flat to convex, consisting of 7–10 flowers; peduncle terete, 1.5–3 mm \times ca. 1.2 mm, old ones with scars from previous flowerings, new peduncles sparsely covered by antrorse trichomes (old peduncles glabrous), presenting ca. 10 apical bracts, round, ca. 0.5 \times 0.4 mm, light green or purple, with sparse antrorse unicellular trichomes, margin ciliate; pedicels terete, 3–4 \times ca. 0.6 mm in diam., producing a bulbous expansion beneath the calyx lobes ca. 1 mm in diam., light green-yellow, papillate. Flower buds globular, light yellow to green. Flowers unscented (lasting about one week in cultivation). Calyx ca. 2 mm in diam., calyx lobes ovate-orbicular, ca. 0.7 \times 0.7 mm, apex rounded, glabrous adaxially, papillose abaxially, margin ciliate; colleters 1–2 in each sepal sinus, triangular, ca. 50 μ m long. Corolla reflexed, 2.3–2.7 mm diam. (ca. 7 mm when flattened), yellow flushed pink; lobes ovate-lanceolate, 2.3–2.7 \times 1.3–1.5 mm, basally fused for ca. 1.5 mm, adaxially thickly covered with unicellular retrorse trichomes, abaxially glabrous, margins entire, apex acuminate, revolute. Staminal corona (Cs), ca. 2 mm high, 2.2 mm in diam., fleshy, yellow; corona lobes anvil-shaped, free portion 0.3 \times 0.5 mm, inner process slightly erect, outer process apically enlarged, truncate when observed from above, deeply grooved between

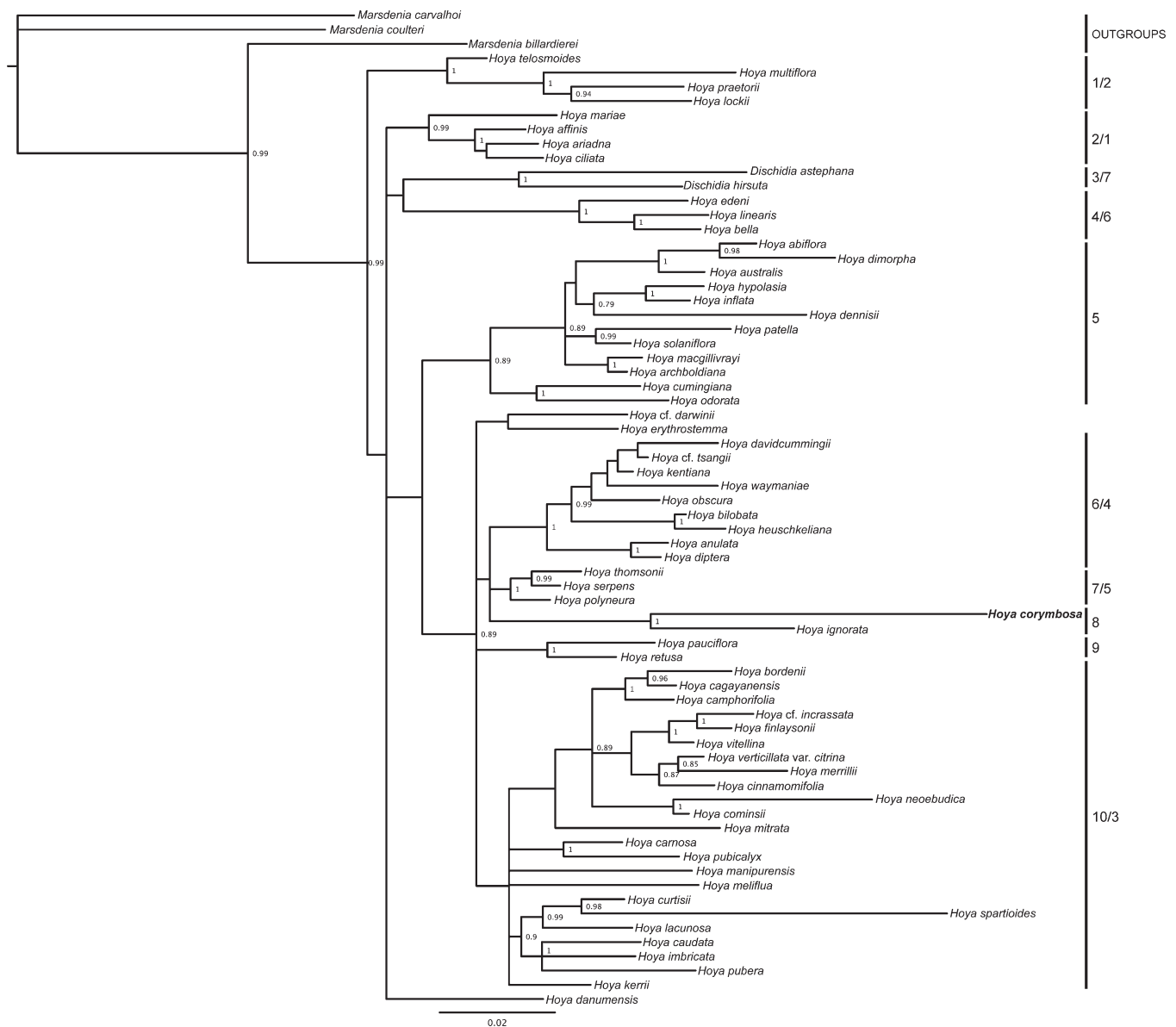


FIG. 1. Bayesian phylogram obtained from the combined *psbA-trnH* intergenic spacer, *matK* gene and ITS ribosomal region data set. Voucher information and GenBank accession numbers can be found in Appendix 1. Support values (> 0.80) for major clades resulting from Bayesian BPP analyses are indicated above the branches. Clade numbers identified in Wanntorp et al. (2011) are indicated after the slash: '1/2' *Hoya multiflora* clade; '2/1' *Hoya* sect. *Eriostemma* clade; '3/7' *Dischidia* clade; '4/6', '7/5' and '9' Himalayan/Indian clades; '5' Australia/New Guinea clade (clade 2 in Wanntorp et al. 2006a); '6/4' sect. *Acanthostemma* clade; '8' *Hoya corymbosa* clade; '10/3' Core *Hoya*.

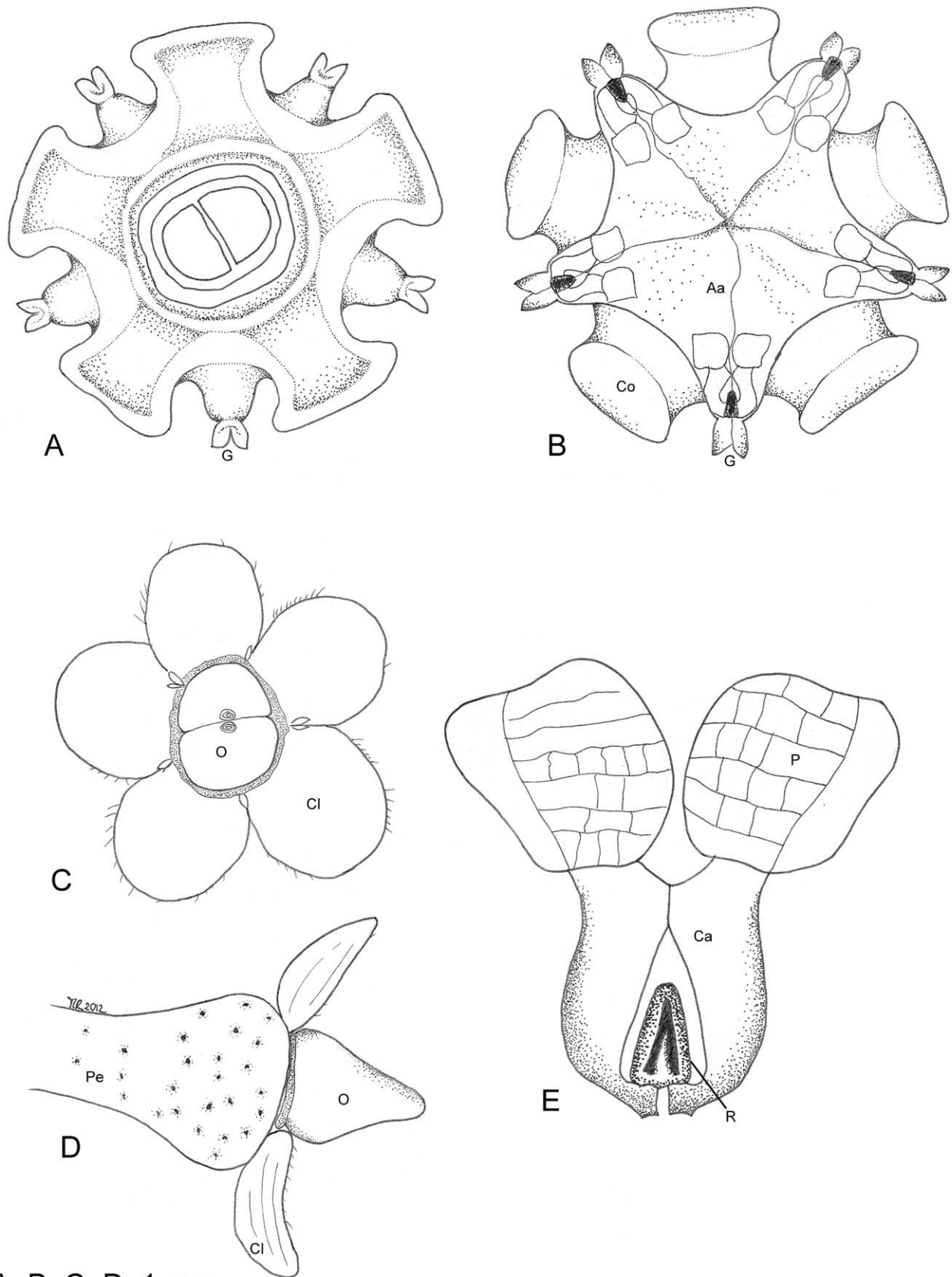
inner and outer lobe, basally fused with the filament tube, lacking basal revolute margins. Style-head convex, covered by membranaceous, triangular anther appendages. Guide rails abruptly terminating ca. 350 μm below retinaculum forming a prominent membranaceous appendage. Pollinarium ca. 750 \times 750 μm , with two nearly round erect pollinia with evident pellucid margin 300 μm across, retinaculum small (compared to the size of the pollinarium), ca. 170 \times 100 μm , caudicles attached at the base of retinaculum, ca. 400 \times 150 μm , flat. Ovary bi-carpellate, triangular, apically constricted, rounded, ca. 0.8 mm long; each carpel ca. 0.5 mm wide at base, light green, glabrous. Fruits and seeds unknown. Figures 2, 3.

Representative Specimens Examined—ITALY. Turin, cultivated in Turin Botanic Gardens, ex hort., 7 March 2011, Rodda MR10-003 (SING).

MALAYSIA. Sabah: cultivated in Mr Ben Chai Garden, 3 Mar 2009, Lamb AL1726/2009 (SAN).

Etymology—The species name refers to the corymbiform inflorescence.

Distribution and Ecology—The only documented locality of *H. corymbosa* is in Sabah, Sipitang district, near Mt. Rimau (Gokusing et al. A11803/2009). The plant was also collected in a second, undocumented locality in Sabah (Lamb A11726/2009). The only information on the habitat of *H. corymbosa* is derived from the type locality. The plant is epiphytic and roots in holes in the bark of the host trees. It was collected at 1,600 m altitude, in primary lower montane forest, which was being logged at the time of collection. It is successfully cultivated at the Kipandi Butterfly Farm, Kampung Kipandi, Moyog, Sabah, where we observed that most flowers were lacking pollinaria (Fig. 2D). This suggests that potential pollinators may be present also in this locality (although they



A, B, C, D, 1 mm

E, 500 μ m

FIG. 2. *Hoya corymbosa*: A. Corona from below. B. Corona from above. C, D. Calyx. E. Pollinarium. Aa = anther appendage, Ca = caudicle, Cl = calyx lobe, Co = corona lobe, G = guide rail, O = ovary, P = pollinium, Pe = pedicel, R = retinaculum (all by M. Rodda from Gokusing et al. A11803/2009 (SAN)).

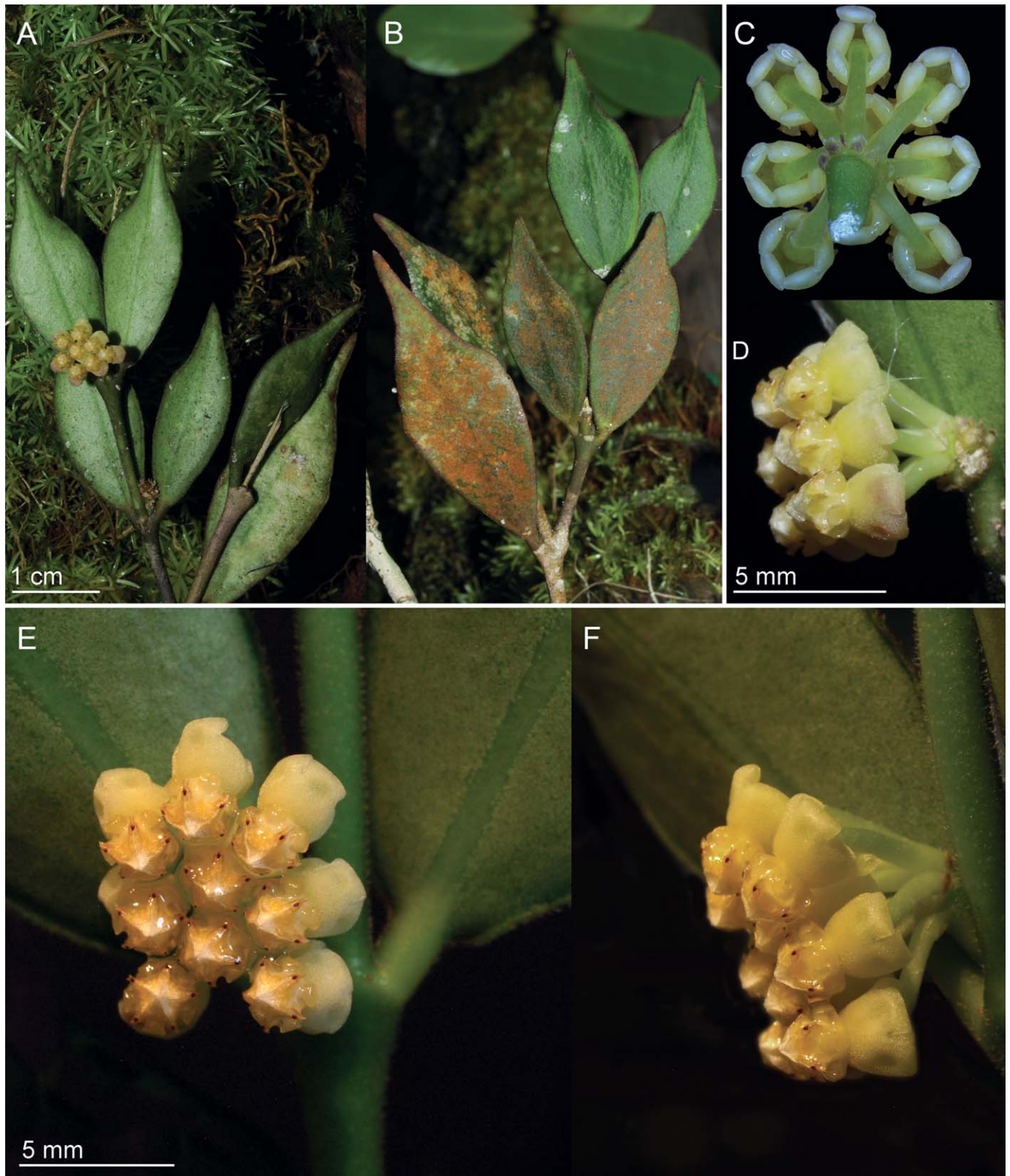


FIG. 3. *Hoya corymbosa*: A, B. Flowering specimen. C. Inflorescence from below: peduncle with apical bracts, pedicels and revolute tips of the corolla lobes. D. Inflorescence from the side. Most anthers lack pollinaria on this specimen cultivated in Kipandi Butterfly Farm, Sabah. E, F. Inflorescences on specimens cultivated in Turin. Here the pollinaria have not been dislodged (all pictures M. Rodda from *Rodda MR10-003* prior to pressing (C, E, F), or from living plant at Kipandi Butterfly Farm, Sabah (A, B, D)).

have not been observed), and, so far, no fruits have been produced.

Conservation Status—No extant wild population of *H. corymbosa* is known. The type locality was being logged when the plant was collected, and the second locality in Sabah was not documented. It is currently conserved *ex situ* at the Kipandi Butterfly Farm. For these reasons the conservation status of this species is critically endangered (IUCN Standards and Petitions Subcommittee 2011).

Notes—*Hoya corymbosa* is peculiar within the genus in being an epiphytic shrub while most other species of *Hoya*

are climbers. Shrubby species of *Hoya* have flowers broader than 0.5 cm across, the only exception is its sister taxon *H. ignorata*, a species distributed from Vietnam to Borneo (including Sabah). *Hoya corymbosa* and *H. ignorata* can be superficially confused because they are both epiphytic shrubs and bear diminutive yellowish flowers. They can be distinguished as *H. corymbosa* has anvil-shaped corona lobes (lacking revolute outer margins), erect pollinia with flat, broad caudicles (resembling those of *Dischidia* spp.), while *H. ignorata* flowers lack corona lobes, and the pollinaria are pendulous with long, thin caudicles.

KEY TO BORNEAN HOYA SPECIES WITH A SHRUBBY HABIT

1. Flower diameter > 0.5 cm 2
 2. Corona lobe processes elongated with acuminate tips *Hoya multiflora*
 2. Corona lobe processes otherwise 4
 4. Corona lobes heart-shaped *Hoya medinillifolia*
 4. Corona lobes otherwise 5
 5. Corona lobes laterally compressed, corolla with long sericeous trichomes *Hoya lastiantha*
 5. Corona lobes ovate, corolla finely puberulous *Hoya cumingiana*
1. Flower diameter < 0.5 cm 3
 3. Corona lobes absent *Hoya ignorata*
 3. Corona lobes anvil-shaped *Hoya corymbosa*

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LITERATURE CITED

- Endress, M. E. and P. V. Bruyns. 2000. A Revised Classification of the Apocynaceae s.l. *Botanical Review* 66: 1–56.
- Forster, P. I., D. J. Liddle, and I. M. Liddle. 1997. *Madangia inflata* (Asclepiadaceae: Marsdenieae), a new genus and species from Papua New Guinea. *Austrobaileya* 5: 53–57.
- Forster, P. I., D. J. Liddle, and I. M. Liddle. 1998. Diversity in the genus *Hoya* (Asclepiadaceae-Marsdenieae). *Aloe* 35: 44–48.
- Huelsenbeck, J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
- Huelsenbeck, J. P., B. Larget, R. E. Miller, and F. Ronquist. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology* 51: 673–688.
- The International Plant Name Index. 2013. Published on the Internet <http://www.ipni.org> [accessed 21 January 2013].
- IUCN Standards and Petitions Subcommittee. 2011. Guidelines for using the IUCN red list categories and criteria. Version 9.0. Prepared by the Standards and Petitions Subcommittee. Downloadable from <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>.
- Kleijn, D. and R. van Donkelaar. 2001. Notes on the taxonomy and ecology of the genus *Hoya* (Asclepiadaceae) in Central Sulawesi. *Blumea* 46: 457–483.
- Kunze, H. and L. Wanntorp. 2008. The gynostegium of *Hoya spartioides* (Apocynaceae Asclepiadoideae): a striking case of incongruence between molecular and phenotypic evolution. *Organisms, Diversity & Evolution* 8: 346–357.
- Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.

- Liede, S. and H. Kunze. 1993. A descriptive system for corona analysis in the Asclepiadaceae. *Plant Systematics and Evolution* 185: 99–284.
- Omlor, R. 1996. Notes on Marsdenieae (Asclepiadaceae): A new, unusual species of *Hoya* from Northern Borneo. *Novon* 6: 288–294.
- Posada, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.
- Rintz, R. E. 1978. The Peninsular Malaysian species of *Hoya* (Asclepiadaceae). *Malayan Nature Journal* 30: 467–522.
- Rodda, M. and N. Simonsson. 2011. *Hoya medinillifolia* (Apocynaceae, Asclepiadoideae), a new species from lowland forests of Sarawak, Borneo. *Webbia* 66: 149–154.
- Tran, T. B., J. H. Kim, D. K. Kim, J. Lee, B. T. Ha, N. Simonsson Juhonewe, and M. Rodda. 2011. *Hoya ignorata* (Apocynaceae, Asclepiadoideae) an overlooked species widely distributed across southeast Asia. *Novon* 21: 508–514.
- de Vienne, D. M., T. Giraud, and O. C. Martin. 2007. A congruence index for testing topological similarity between trees. *Bioinformatics* 23: 3119–3124.
- Wanntorp, L., A. Kocyan, and S. S. Renner. 2006a. Wax plants disentangled: a phylogeny of *Hoya* (Marsdenieae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* 39: 722–733.
- Wanntorp, L., A. Kocyan, R. van Donkelaar, and S. S. Renner. 2006b. Towards a monophyletic *Hoya* (Marsdenieae, Apocynaceae): inferences from the chloroplast *trnL* region and the *rbcL-atpB* spacer. *Systematic Botany* 31: 586–596.
- Wanntorp, L. and P. I. Forster. 2007. Phylogenetic relationships between *Hoya* and the monotypic genera *Madangia*, *Absolmsia* and *Micholitzia* (Apocynaceae, Marsdenieae): insights from flower morphology. *Annals of the Missouri Botanical Garden* 94: 36–55.
- Wanntorp, L. and H. Kunze. 2009. Identifying synapomorphies in the flowers of *Hoya* and *Dischidia* – towards phylogenetic understanding. *International Journal of Plant Sciences* 170: 331–342.
- Wanntorp, L., K. Gotthardt, and A. N. Muellner. 2011. Revisiting the wax plants (*Hoya*, Marsdenieae, Apocynaceae): phylogenetic tree using the *matK* gene and *psbA-trnH* intergenic spacer. *Taxon* 60: 4–14.
- Wanntorp, L. and U. Meve. 2011. New combinations in *Hoya* for the species of *Clemensiella* (Marsdenieae, Apocynaceae). *Willdenowia* 41: 97–99.

APPENDIX 1. Taxa included in the present study are presented in the following order: species: GenBank accession numbers for sequences of the *psbA-trnH* region, *matK*, and ITS, respectively; collection locality, voucher (herbarium acronyms follow Index Herbariorum).

Dischidia astephana Scort. ex King & Gamble. HQ327682, HQ327603, DQ334459; MALAYSIA. Pahang: Cameron Highlands, Wanntorp 562 (S). *Dischidia hirsuta* Decne. HQ327669, HQ327590, DQ334452; ex hort., Wanntorp 563 (S). *Hoya affinis* Hemsl. HQ327615, HQ327536, DQ334481; RBG-Kew,

- Liv. Coll. 1983-4478, *Chase 17128* (K). *Hoya albiflora* Zipp. ex Blume. HQ327647, HQ327567, DQ334493; *Wanntorp 584* (S). *Hoya anulata* Schltr. HQ327663, HQ327584, DQ334485; ex hort., IPPS 8603, L990438, *Wanntorp 585* (S). *Hoya archboldiana* C. Norman. HQ327646, HQ327566, HQ327513; ex hort., *Wanntorp 613* (S); *Hoya ariadna* Decne. HQ327614, HQ327535, DQ334502; RBG-Kew, Liv. Coll. 1983-4474, *Chase 17125* (K). *Hoya australis* R. Br. ex J. Traill. HQ327631, HQ327551, DQ334428; ex hort., *Wanntorp 564* (S). *Hoya bella* Hook. HQ327660, HQ327581, HQ327518; ex hort., *Wanntorp 616* (S). *Hoya bilobata* Schltr. HQ327678, HQ327599, DQ334489; RBG-Kew, Liv. Coll. 1983-4481, *Chase 17129* (K). *Hoya bordenii* Schltr. HQ327686, HQ327607, HQ327527; 1998-3111, Uppsala Bot. Gard., *Wanntorp 595* (S). *Hoya cagayanensis* C. M. Burton. HQ327681, HQ327602, HQ327525; ex hort., *Wanntorp 618* (S). *Hoya camphorifolia* Warburg. HQ327644, HQ327564, DQ334474; PHILIPPINES. Quezon National Park, *Wanntorp 590* (S). *Hoya carnosia* (L. f.) R. Br. HQ327665, HQ327586, DQ334460; ex hort., *Wanntorp 566* (S). *Hoya caudata* Hook. f. HQ327685, HQ327606, DQ334483; ex hort., *Wanntorp 587* (S). *Hoya ciliata* Elmer ex C. M. Burton. HQ327616, HQ327537, DQ334512; ex hort., IPPS 3071, L920785, *Wanntorp 586* (S). *Hoya cinnamomifolia* Hook. HQ327642, HQ327562, HQ327510; ex hort., *Wanntorp 596* (S). *Hoya cominsii* Hemsl. HQ327656, HQ327577, HQ327515; Liv. Coll. 1983-4485, *Chase 17133* (K). *Hoya corymbosa* Rodda & Simonsson. JQ950747; JQ950746; JQ950745; ex hort., M. Rodda MR10-003 (SING). *Hoya cumingiana* Decne. HQ327673, HQ327594, HQ327521; ex hort., *Wanntorp 611* (S). *Hoya curtisii* King & Gamble. HQ327687, HQ327608, DQ334479; 1998-3180, Uppsala Bot. Gard., *Wanntorp 578* (S). *Hoya* cf. *darwinii* Loher. HQ327670, HQ327591, DQ334477; RBG-Kew, Liv. Coll. 1984-2899, *Chase 17135* (K). *Hoya davidcummingii* Schltr. HQ327653, HQ327574; ex hort., *Wanntorp 567* (S). *Hoya danumensis* Rodda & Nyhuus. HQ327649, HQ327569, HQ327514; ex hort., *Wanntorp 605* (S). *Hoya dennisii* P. I. Forst. & Liddle. HQ327622, HQ327543, HQ327524; ex hort., *Wanntorp 608* (S). *Hoya dimorpha* F. M. Bailey. HQ327645, HQ327565, HQ327512; RBG-Kew, Liv. Coll. 1983-4477, *Chase 17127* (K). *Hoya diptera* Seem. HQ327662, HQ327583, HQ327519; ex hort., *Wanntorp 602* (S). *Hoya edeni* King ex Hook. f. HQ327661, HQ327582, DQ334476; ex hort., IPPS 8292, *Wanntorp 579* (S). *Hoya erythrostemma* Kerr. HQ327643, HQ327563, HQ327511; ex hort., *Wanntorp 615* (S). *Hoya finlaysonii* Wight. HQ327637, HQ327557, HQ327506; RBG-Kew, Liv. Coll. 1984-3827, *Chase 17138* (K). *Hoya heuschkeliana* Kloppenb. HQ327621, HQ327542, DQ334416; ex hort., *Wanntorp 568* (S). *Hoya hypolasia* Schltr. HQ327627, HQ327548, DQ334470; ex hort. IPPS 7006, L901824, *Wanntorp 588* (S). *Hoya ignorata* T. B. Tran, Rodda, Simonsson & Joongku Lee. JX627339, JX627338, JX627337; THAILAND. Nakhon Si Thammarat: Khao Luang, 810 m, 6 May 2010, Simonsson & Somadee NS10-007, (SING). *Hoya imbricata* Callery ex Decne. HQ327684, HQ327605, DQ334480; ex hort., *Wanntorp 569* (S). *Hoya* cf. *incrassata* Elmer ex Merr. HQ327636, HQ327556; PHILIPPINES. Palawan: RBG-Kew, Liv. Coll. 1984-3340, *Chase 17136* (K). *Hoya inflata* (P. I. Forst., Liddle & I. M. Liddle) L. Wanntorp & P. I. Forst. HQ327618, HQ327539, DQ334508; NEW GUINEA. Madang Province: I.M. Liddle IML1076 (BRI). *Hoya kentiana* C. M. Burton. HQ327677, HQ327598, DQ334424; ex hort., *Wanntorp 570* (S). *Hoya kerrii* Craib. HQ327624, HQ327545, DQ334458; RBG-Kew, Liv. Coll. 1982-2786, *Chase 17123* (K). *Hoya lacunosa* Blume. HQ327680, HQ327601, DQ334499; ex hort., *Wanntorp 571* (S). *Hoya linearis* Wall. ex D. Don. HQ327659, HQ327580, HQ327517; ex hort., *Wanntorp 598* (S). *Hoya lockii* V. T. Pham & Aver. JQ823007, JQ823006, JQ823005; ex hort Turin, Italy, originally from VIETNAM. Thua Thien Hue: A Roang, *Rodda Hort2010.X* (TO); *Rodda Hort2010.X* (TO). *Hoya macgillivrayi* F. M. Bailey. HQ327648, HQ327568, DQ334488; ex hort., *Wanntorp 572* (S). *Hoya manipurensis* Deb. HQ327628, HQ327549, DQ334501; *Seidenfaden s.n.* (K). *Hoya mariae* (Schltr.) L. Wanntorp & Meve. HQ327613, HQ327534, HQ327500; PHILIPPINES. Manila, *Wanntorp 617* (S). *Hoya meliflua* Merr. HQ327651, HQ327571, DQ334434; PHILIPPINES. Mindoro Occidental: Puerto Galera, *Wanntorp 591* (S). *Hoya merrillii* Schltr. HQ327635, HQ327555, HQ327505; 1998-3113, Uppsala Bot. Gard., *Wanntorp 614* (S). *Hoya mitrata* Kerr. HQ327683, HQ327604, DQ334500; IPPS 7684, L914643, *Wanntorp 589* (S). *Hoya multiflora* Blume. HQ327629, HQ327550, DQ334487; ex hort., *Wanntorp 573* (S). *Hoya neobudica* Guillaumin. HQ327657, HQ327578, HQ327516; RBG-Kew, Liv. Coll. 1983-4475, *Chase 17126* (K). *Hoya obscura* Elmer ex C. M. Burton. HQ327676, HQ327597, HQ327523; PHILIPPINES. Luzon, Nueva Vizcaya: Salton Pass, Royal Botanic Gardens Sydney, 883625, NSW 4103384, *Wanntorp 597* (S). *Hoya odorata* Schltr. HQ327634, HQ327554, HQ327504; PHILIPPINES. Luzon, Nueva Vizcaya: Salton Pass, Royal Botanic Gardens Sydney, 860083, NSW 4025383, *Wanntorp 600* (S). *Hoya patella* Schltr. HQ327633, HQ327553, DQ334498; ex hort., *Wanntorp 575* (S). *Hoya pauciflora* Wight. HQ327620, HQ327541, DQ334465; ex hort., *Wanntorp 574* (S). *Hoya polyneura* Hook. f. HQ327640, HQ327560, HQ327509; ex hort., *Wanntorp 610* (S). *Hoya praetorii* Miq. HQ327664, HQ327585, HQ327520; ex hort., *Wanntorp 593* (S). *Hoya pubera* Blume. HQ327623, HQ327544, HQ327526; ex hort. RBG-Kew, Liv. Coll. 1983-4489, *Chase 17134* (K). *Hoya pubicalyx* Merr. HQ327666, HQ327588, DQ334447; ex hort., *Wanntorp 576* (S). *Hoya retusa* Dalzell. HQ327632, HQ327552, DQ334457; 1998-3127, SWEDEN. Rosendal Uppsala, Uppsala Bot. Gard., *Wanntorp 580* (S). *Hoya serpens* Hook. f. HQ327654, HQ327575, DQ334482; RBG-Kew, Liv. Coll. 1988-3338, *Chase 17118* (K). *Hoya solaniflora* Schltr. HQ327617, HQ327538, DQ334507; ex hort., IPPS 3773, *Wanntorp 583* (S). *Hoya spartioides* (Benth.) Kloppenb. HQ327619, HQ327540, DQ334484; Borneo, MALAYSIA. Sipitang: *Wanntorp 592* (S). *Hoya telosmoides* Omlor. HQ327671, HQ327592, DQ334486; MALAYSIA. Sabah: Mount Kinabalu, *Wanntorp 577* (S). *Hoya thomsonii* Hook. f. HQ327625, HQ327546, HQ327502; ex hort., Zurich Botanical Garden, Nr. 19981401, *Wanntorp 606* (S). *Hoya* cf. *tsangii* C. M. Burton. HQ327679, HQ327600, DQ334425; ex hort., Uppsala Bot. Gard., *Wanntorp 581* (S). *Hoya verticillata* var. *citrina* (Ridl.) Veldkamp. HQ327638, HQ327558, HQ327507; Royal Botanic Gardens Sydney, 852229, NSW 4103363, *Wanntorp 604* (S). *Hoya vitellina* Blume. HQ327639, HQ327559, HQ327508; IPPS 4072, L990489, *Wanntorp 603* (S). *Hoya waymaniae* Kloppenb. HQ327675, HQ327596, HQ327522; IPPS 3424, L892662/28120, *Wanntorp 612* (S). *Marsdenia billardieri* Decne. HQ327612, HQ327533, HQ327499; NEW CALEDONIA. Nouméa: *Selling s.n.* (S). *Marsdenia carvalhoi* Morillo & Carnevali. HQ327610, HQ327531, DQ334419; BRAZIL. Bahia: RBG-Kew, Liv. Coll. 1982-1949, *Chase 17115* (K). *Marsdenia coulteri* Hemsl. HQ327611, HQ327532, HQ327498; MEXICO. Yucatan: Bradburn & Darwin 1267 (S).