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Research

Rachunia cymbiformis, a new genus and species of Gesneriaceae from Thailand

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Rachunia, a new genus of Gesneriaceae from Thailand, is described with a single species, *Rachunia cymbiformis*. Its relationship to the rest of subtribe Didymocarpinae is investigated through a phylogenetic study based on Bayesian Inference and Parsimony analyses of nuclear ITS and plastid *trn*L-*trn*F (intron-spacer) sequences. Morphologically, *Rachunia* differs from the related genera *Codonoboea* in the large boat-shaped bracts and orthocarpic vs plagiocarpic fruit; from *Microchirita* in the bracts, wiry vs fleshy stem, the campanulate vs tubular corolla and the clavate vs chiritoid stigma, and from *Henckelia* in the clavate vs chiritoid stigma, large boat-shaped bracts in the inflorescence, free and imbricate sepals, short and campanulate corolla, clavate stigma, and relatively robust orthocarpic fruit.

Keywords: phylogeny, *Henckelia*, Didymocarpinae

Introduction

In November 2015 a plant in the Gesneriaceae was collected in Thong Pha Phum district in Kanchanaburi province that neither the collectors nor researchers on the family were able to place to genus. Amongst genera already known from Thailand it shows superficial similarities to *Henckelia* Spreng. in general appearance, the caulescent habit (in the Thai *Henckelia* species), opposite leaves (in most *Henckelia* species) and two fertile stamens, but differs in the large boat-shaped bracts in the inflorescence, free and imbricate sepals, short and campanulate corolla, clavate stigma, and relatively robust orthocarpic fruit. Although under the genus concept adopted by Weber et al. (2011), most species of *Henckelia* have a long corolla tube, there are species with a campanulate corolla, including the type species *H. incana* Spreng., but no known species has as short and wide corolla tube as the unknown plant. Also, several species of *Henckelia*, including the widespread *H. anachoreta* (Hance) D.J.Middleton & Mich.Möller and *H. pumila* (D.Don) A.Dietr., have orthocarpic or almost orthocarpic fruits but in these species they are much longer and narrower resulting in a delicate appearance. These differences from *Henckelia*, and the lack of any other genus occurring in Thailand, or indeed Asia as a whole, to which it could be readily compared, led to the need

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for a more detailed study of its generic relationships before what was quite clearly a new species could be described. Fortunately, the collectors also collected material in silica gel and this material was included in a molecular phylogenetic study to determine the affinities of this plant.

Material and methods

Our phylogenetic study is based on Bayesian Inference (BI) and Maximum Parsimony (MP) analyses of nuclear ITS and plastid *trn*L-*trn*F (intron-spacer) sequences. The ingroup encompassed 32 of the 33 known genera in subtribe Didymocarpinae (sensu Weber et al. 2013), excluding only *Sepikea* Schltr., a genus that has tentatively been included in *Cyrtandra* J.R.Forst. & G.Forst. (Burtt 2001) and for which no suitable material for DNA analysis is available. We aimed to incorporate two or more taxa per genus, including the type species of each, and excluding ambiguous data whenever possible. Following the outcome of preliminary analyses, we sampled more densely from the genera *Codonoboea* Ridl., *Henckelia* and *Microchirita* (C.B.Clarke) Yin Z.Wang. The outgroup comprised a selection of genera belonging to the sister subtribes Loxocarpinae and Didissandrinae (Möller et al. 2011a, Middleton and Möller 2012), plus two species of subtribe Streptocarpinae.

Many of the sequences used were published in our previous studies (Möller et al. 2011a, 2016, Middleton and Möller 2012, Middleton et al. 2015, Puglisi et al. 2016), with the addition of newly generated data for 12 taxa, including the unknown plant from Thong Pha Phum, Poopath, Sae Wai, Kheiwbang and Jirakon 1370. A few remaining sequences were downloaded from GenBank. The final matrices included 82 ingroup and 17 outgroup samples (Appendix 1).

For the newly generated data, we extracted the DNA with CTAB (Doyle and Doyle 1987) or with the innuPREP PlantDNA Kit. The PCR protocols follow Middleton et al. (2017). The sequencing reactions were based on BigDye Terminator technology, and were run by AIT biotech (Singapore) on an ABI3730 DNA Analyser. The sequences were edited in Sequencher ver. 4.7, preliminarily aligned in Muscle (Edgar 2004) and then manually adjusted in Mesquite ver. 2.75 (Maddison and Maddison 2011).

The Bayesian inference (BI) phylogenetic analyses were run in MrBayes ver. 3.2.6 (Ronquist and Huelsenbeck 2003, Ronquist et al. 2012). We used a likelihood model of DNA substitution (GTR) with rate variation across sites following a gamma distribution, run over 10 000 000 generations and sampling every 10 000 generations. The heat was set at 0.01 to increase chain swaps. The final standard deviation of the split sequences was 0.006967 in the combined analysis ($\leq$ 0.01 in the preliminary single-marker analyses) and the other output parameters all indicated sufficient sampling, swaps between chains and convergence. After discarding the first 25% of the trees, the retained ones were summarised in the majority-rule consensus tree presented here (Fig. 1), which was edited in FigTree ver. 1.3.1 (Rambaut and Drummond 2009). The Parsimony analyses were run in PAUP v. 4.0a (build 161) (Swofford 2002). The heuristic analyses were run over 100 000 replicates from random stepwise addition, with MulTrees and TBR with steepest descent on. Statistical support was inferred by a Parsimony-based bootstrap analysis, sampled 10 000 times, with random stepwise addition, TBR on, MulTrees on and steepest descent off.

We analysed the ITS and *trn*L-*trn*F independently (not shown) to assess the combinability of the data. As there were no hard incongruences (Nishii et al. 2015) between the tree topologies, we proceeded to analyse the data in a combined matrix.

Results

The Bayesian analysis of the combined dataset resulted in a consensus tree with the sample of Poopath et al. 1370 on the earliest diverging ingroup branch (PP = 0.95), followed by *Codonoboea* (low support, PP = 0.69), *Microchirita* (low support, PP = 0.55), *Henckelia* (PP = 0.99), and a polytomy including all the remaining Didymocarpinae (PP = 1) (Fig. 1). The 36 trees derived from Parsimony analysis did not have a robust structure (CI = 0.3827, RI = 0.5809, RC = 0.2223) and the bootstrap analysis provided high support values only for the terminal nodes. The resulting polytomy had most of the genera of Didymocarpinae placed along individual branches, and this was the case also for Poopath et al. 1370, *Codonoboea*, *Henckelia* and *Microchirita*. Among the remaining Didymocarpinae, the few supported relationships between genera matched those obtained by Bayesian Inference (Fig. 1).

Discussion

The molecular phylogenetic analysis places Poopath et al. 1370 firmly in the Gesneriaceae, subfamily Didymocarpoideae, tribe Trichosporeae, subtribe Didymocarpinae, by far the largest subtribe in Asian Gesneriaceae. The outcome of the analysis presented is similar to those of previous phylogenetic studies which extensively covered the Didymocarpinae (Möller et al. 2009, 2011b, 2016, Middleton et al. 2015). Phylogenetic relationships among the members of the subtribe remain mostly uncertain, though several clear clades can be identified among the early diverging branches in the phylogeny of the Didymocarpinae. In the present study, at the base of the subtribe, the collection Poopath et al. 1370 and the genera *Codonoboea*, *Microchirita*, and *Henckelia* form four distinct units, followed by the remaining Didymocarpinae genera on a large polytomy. *Codonoboea*, *Microchirita* and *Henckelia* have been established as monophyletic and well-supported (Möller et al. 2011b, Middleton and Möller 2012, Middleton et al. 2015). Poopath et al. 1370 occupies a separate branch from the other genera in all the analyses, with

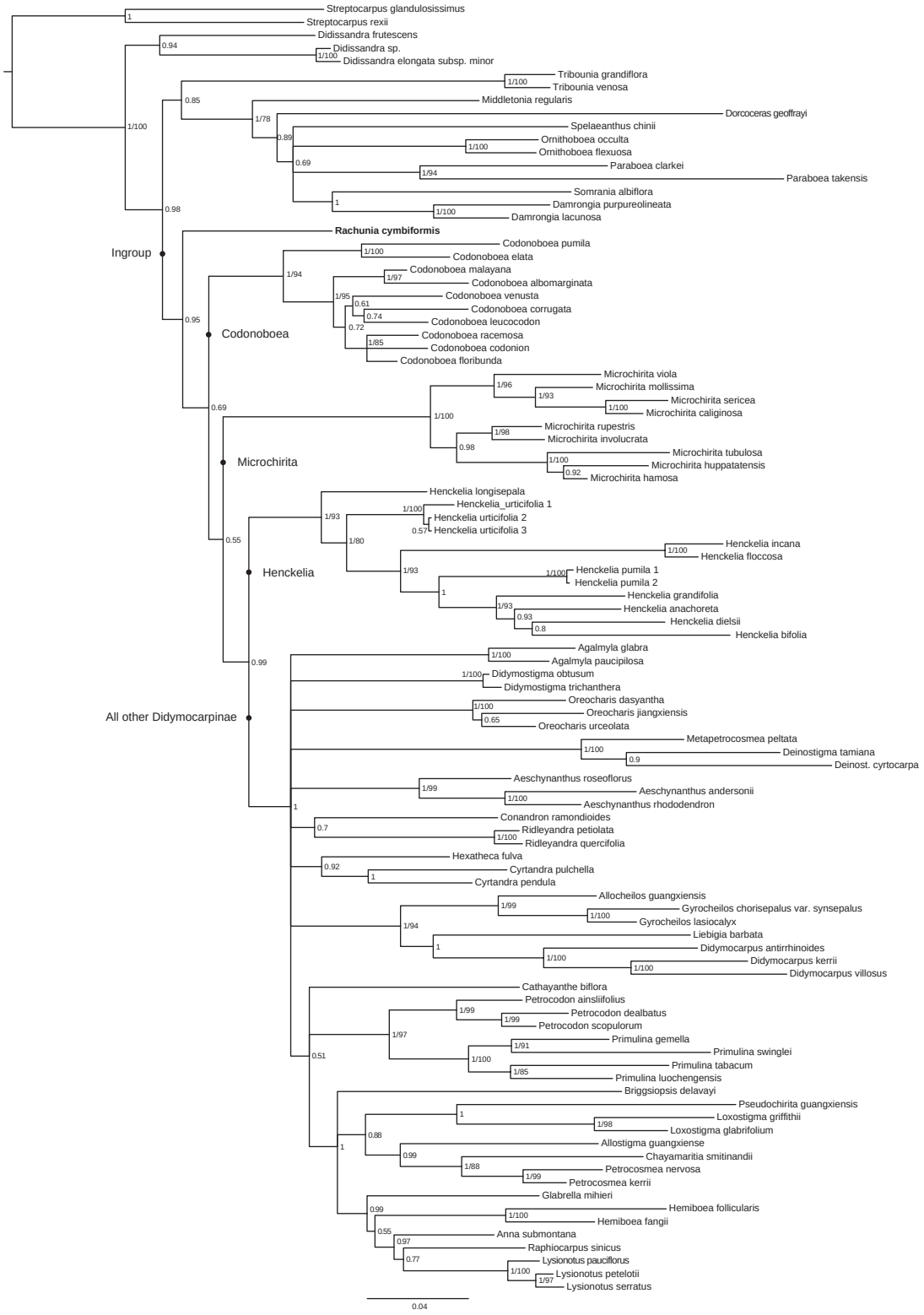

Figure 1. Bayesian Inference 50% majority rule consensus tree from the analysis of ITS and *trn*L-*trn*F. All the posterior probabilities and only the bootstrap values ≥ 75% are added at the nodes.

no immediate sister taxon. Morphologically, it differs from *Codonoboea* in the large boat-shaped bracts and orthocarpic vs plagiocarpic fruit; from *Microchirita* in the bracts, wiry vs fleshy stem, the campanulate vs tubular corolla and the clavate vs chiritoid stigma (a stigma in which the upper lip of the stigma is absent and the lower lip is expanded and often bilobed), and from *Henckelia* in the characters noted in the introduction and particularly in the clavate vs chiritoid stigma.

The other genera in this subtribe are in a clade well-supported by the Baysian Inference (PP = 1), and are more distantly related to Poopath et al. 1370. Morphologically, the genera which share a caulescent habit and two fertile stamens with Poopath et al. 1370 are *Allostigma* W.T.Wang, *Cyrtandra* J.R.Forst. & G.Forst., *Deinostigma* W.T.Wang, most *Didymocarpus* Wall., *Didymostigma* W.T.Wang, *Hemiboea* C.B.Clarke, *Liebigia* Endl. and *Pseudochirita* W.T.Wang. Poopath et al. 1370 differs from all of them in the presence of large boat-shaped bracts, and additionally from *Allostigma* in the parietal (Poopath et al. 1370) vs axile (*Allostigma*) placentation, from *Cyrtandra* in dehiscent vs indehiscent fruit, from *Deinostigma* in opposite vs alternate leaves and clavate vs chiritoid stigma, from *Didymocarpus* in the imbricate vs tubular or widely separated sepals and having a shorter corolla tube, from *Didymostigma* in the insertion of stamens being basal vs distal in the corolla tube and the clavate vs bi-lobed stigma, from *Hemiboea* in the unilocular ovary with 2 equal parietal placentae vs bi-locular ovary with only 1 axile placenta developing, from *Liebigia* in the campanulate vs tubular corolla and clavate vs chiritoid stigma, and from *Pseudochirita* in the free and imbricate vs tubular calyx and in the clavate vs bi-lobed and unequal-lobed stigma shape.

Based on the results of the molecular phylogenetic study and the morphological distinctness of the plant, we hereby describe a new species in a new genus which we name *Rachunia cymbiformis*.

Rachunia D.J.Middleton & C.Puglisi gen. nov.

Type species: *Rachunia cymbiformis* D.J.Middleton.

Etymology

The genus is named in honour of the Thai botanist Dr Rachun Pooma of the Forest Herbarium Bangkok (BKF) to recognise his great contribution to our understanding of plant diversity in Thailand and the wider region.

Description

Caulescent herb; stems not fleshy. Leaves opposite, petiolate; blades entire to serrulate at margin, with pinnate venation. Inflorescences axillary, dichasial, 6–14 pair-flowers, similar in length to subtending leaves, with large boat-shaped bracts. Calyx 5-lobed; lobes free to base, strongly imbricate; margins entire. Corolla tube short, campanulate; limb 2-lipped, with upper lip 2-lobed and lower lip 3-lobed, all lobes orbicular. Fertile stamens 2, inserted near base of corolla tube; filaments narrow at base, thickened above, strongly curved with the anther apex pointing towards dorsal side of corolla; anthers coherent at apices, held face to face; staminodes 3, medial one smaller than 2 lateral ones. Disc annular. Ovary uni-locular with 2 equal-sized parietal placentae; style narrow; stigma clavate. Fruit a long and narrow capsule, orthocarpic (only seen immature but likely to be loculicidal as in most other Didymocarpinae with dehiscent fruit); seeds tiny, globose.

Distribution

Thailand, Kanchanaburi. The genus currently only has one species which is only known from the type collection. Given the collection locality on the Thai-Myanmar border it almost certainly also occurs in Myanmar.

Rachunia cymbiformis D.J.Middleton sp. nov. (Fig. 2)

Type: Kanchanaburi, Thong Pha Phum, Ban E Tong, near Thai-Myanmar border, 900 m a.s.l., 3 Nov 2015, M. Poopath, J. Sae Wai, W. Kheiwbang and S. Jirakon 1370 (holotype: BKF, isotype: SING).

Etymology

The specific epithet '*cymbiformis*' refers to the boat-shaped bracts in the inflorescence.

Figure 2. *Rachunia cymbiformis* D.J.Middleton. (A) upper leaves, inflorescence and infructescence, (B) close-up of bracts and flower. Photos by Manop Poopath.

Description

Terrestrial herb, 20–40 cm tall, decumbent at base and rooting along stems; stems densely covered in long dark brown hairs to 3 mm long. Leaves opposite; petioles 13–19 mm long, densely covered in long golden brown hairs; blade elliptic to obovate, symmetrical or slightly falcate, 6.0–13.5 × 2.7–5.3 cm, 2.1–3.6 times as long as wide, narrowed at base but then ultimately rounded, sometimes inserted on pedicel at slightly different levels on each side, acuminate at apex; margin entire at base to serrulate in upper half; secondary veins 7–9 on each side; tertiary venation reticulate, sparsely covered with golden brown hairs above and beneath, beneath more densely so on venation. Inflorescence 5–15 cm long, as a simple dichasium with 6 pair-flowers or branched one more time and then with up to 14 flowers; peduncle 3.0–10.1 cm long, sparsely to densely covered in long golden brown hairs which are 1.2–2.5 mm long; bracts boat-shaped, green tinged purplish, ovate to elliptic, 9–11 × 4.5–8.0 mm, acuminate at apex, sparsely to densely covered in long golden brown hairs; pedicels 8.0–13.5 mm long, glabrous. Calyx purplish, of 5 lobes which are free to base; lobes elliptic, 4.8–5.5 × 1.4–2.2 mm, acute at apex, with entire margin, glabrous. Corolla with a short campanulate tube and spreading limb; limb 2-lipped, with all lobes orbicular; tube whitish, its lobes pale purple, whitish at base of upper lip, glabrous outside, with sparse long hairs in lower half of tube inside; tube 4.5 mm long; upper lip 5 mm long, its lobes 4 × 5 mm; lower lip 6 mm long, its lateral lobes 4.5 × 5.0 mm and medial lobe 4.5 × 4.5 mm. Fertile stamens 2, inserted at 1.2 mm from base; filaments white, narrow at base, thickened above, strongly curved with the anther apex pointing towards dorsal side of corolla, glabrous; anthers coherent at apices, held face to face, ca 1.1 × 2.7 mm, glabrous; lateral staminodes 0.6 mm long, medial 0.3 mm long. Disc annular, 0.2 mm long. Pistil white; ovary 3 mm long, glabrous, unilocular with 2 equal-sized parietal placentae; style 7 mm long, glabrous; stigma clavate. Fruit orthocarpic, 4–6 cm long, 1.2–1.5 mm wide; seeds globose, 0.2 mm in diameter.

Distribution and habitat

Rachunia cymbiformis is currently only known from the type collection from Thailand, Kanchanaburi province, Thong Pha Phum district, Ban E Tong, near the Thai-Myanmar border at 900 m a.s.l. Given the collection locality on the Thai-Myanmar border it almost certainly also occurs in Myanmar. The habitat is in moist evergreen forest on a slope in shade. Although not recorded on the specimen label, the underlying bedrock is granite and the soil is loam.

Conservation status

As *Rachunia cymbiformis* is only known from the type collection and its distribution and population size are unknown, it must currently be categorised as 'Data Deficient' (DD). On the Thai side of the border the forest is rather degraded but on the Myanmar side the forest is currently rather extensive.

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Appendix 1. The following is a list of the accessions used, ordered alphabetically by taxon, with the GenBank codes for the ITS and *trn*L-*trn*F sequences respectively. The taxa whose sequences are newly published are listed with the complete voucher information.

Aeschynanthhus andersonii, Myanmar, Shan State, 19 Sep 2015, Y. Baba et al. FOMIC103456 (SING); *Aeschynanthus rhododendron* HQ632993, HQ632895; *Aeschynanthus roseoflorus* FJ501333, HQ632896; *Agalmyla glabra* HQ632989, HQ632892; *Agalmyla paucipilosa* HQ632990, HQ632893; *Allocheilos guangxiensis* HQ632994, HQ632897; *Allostigma guangxiense* HQ632977, HQ632880; *Anna submontana* FJ501362, FJ501542; *Briggsiopsis delavayi* HQ632976, HQ632879; *Cathayanthe biflora* HQ632996, HQ632899; *Chayamaritia smitinandii* KP325425, KP325432; *Codonoboea albomarginata* HQ632961, AJ492297; *Codonoboea codonion* JF912565, JF912538; *Codonoboea corrugata* HQ632962, FJ501484; *Codonoboea elata* JF912550, JF912523; *Codonoboea floribunda* JF912566, JF912539; *Codonoboea leucocodon* JF912567, JF912540; *Codonoboea malayana* JF912568, JF912541; *Codonoboea pumila* JF912570, JF912543; *Codonoboea racemosa* JF912571, JF912544; *Codonoboea venusta* JF912572, JF912545; *Conandron ramondioides* FJ501340, FJ501515; *Cyrtandra pendula* FJ501354, FJ501530; *Cyrtandra pulchella* EU919941, HQ632906; *Damrongia lacunosa* KU203801, KU203896; *Damrongia purpureo*lineata KU203798, KU203893; *Deinostigma cyrtocarpa* JX506885, JX506777; *Deinostigma tamiana*, Vietnam, Quang Ngai, 2 Apr 2017, Lý Ngọc Sâm Lý 882 (VNM); *Didissandra elongata* ssp. *minor* KP325420, KP325427; *Didissandra frutescens* JN934793, FJ501522; *Didissandra* sp. KP325422, KP325429; *Didymocarpus antirrhinoides* DQ912671, FJ501513; *Didymocarpus kerrii*, Thailand, Mae Hong Son, 21 Oct 2014, D.J. Middleton, C. Hemrat, P. Karaket, C. Puglisi and S. Suddee 5812 (SING); *Didymocarpus villosus* HQ633001, HQ63290; *Didymostigma obtusum* HQ632971, HQ632875; *Didymostigma trichanthera* HQ632972, HQ632876; *Dorcoceras geoffrayi*, Thailand, Sukhothai, 24 Oct 2014, D.J. Middleton, C. Hemrat, P. Karaket, C. Puglisi and S. Suddee 5833 (SING); *Glabrella mihieri* FJ501363, FJ501544; *Gyrocheilos chorisepalus* var. *synsepalus* HQ632997, HQ632900; *Gyrocheilos lasiocalyx* HQ632998, HQ632901; *Hemiboea fangii* HQ632979, HQ632882; *Hemiboea follicularis* HQ632982, HQ632885; *Henckelia anachoreta* 1 HQ632966, HQ632870; *Henckelia bifolia* JF912549, JF912522; *Henckelia dielsii* HQ632967, HQ632871; *Henckelia floccosa* HQ632964, FJ501486; *Henckelia grandifolia* JF912554, JF912527; *Henckelia incana* HQ632965, HQ632869; *Henckelia longisepala* HQ632963, HQ632868; *Henckelia pumila* 1 JF912556, JF912529; *Henckelia pumila* 2 FJ501327, FJ501491; *Henckelia urticifolia* 1 DQ872835, DQ872821; *Henckelia urticifolia* 2 JF912559, JF912532; *Henckelia urticifolia* 3 FJ501328, FJ501492; *Hexatheca fulva* HQ632969, HQ632873; *Liebigia barbata* DQ912668, FJ501538; *Loxostigma glabrifolium* HQ633006, HQ632910; *Loxostigma griffithii* FJ501338, FJ501508; *Lysionotus pauciflorus* FJ501331, FJ501497; *Lysionotus petelotii* HQ632974, FJ501496; *Lysionotus serratus*, Myanmar, Shan State, 13 Sep 2015, Y. Baba et al. FOMIC103132 (SING); *Metapetrocosmea peltata* HQ632968, HQ632872; *Microchirita caliginosa* FJ501325, FJ501488; *Microchirita hamosa*, Thailand, Tak, 17 Oct 2014, D.J. Middleton, C. Hemrat, P. Karaket, C. Puglisi and S. Suddee 5762 (BKF); *Microchirita huppatatensis*, Thailand, Uthai Thani, 14 Oct 2014, D.J. Middleton, C. Hemrat, P. Karaket, C. Puglisi and S. Suddee 5689 (BKF); *Microchirita involucrata* 2 JF912553, JF912526; *Microchirita mollissima* JF912555, JF912528; *Microchirita rupestris*, Thailand, Kanchanaburi, 28 Oct 2009, D.J. Middleton and P. Triboun 5204 (E); *Microchirita sericea* JF912548, JF912521; *Microchirita tubulosa* JF912558, JF912531; *Microchirita viola* JF912560, JF912533; *Middletonia regularis* KU203789, KU203884; *Oreocharis dasyantha* HQ633014, HQ632918; *Oreocharis jiangxiensis* HQ633029, HQ632933; *Oreocharis urceolata* HQ633018, HQ632922; *Ornithoboea flexuosa* KU203836, KU203931; *Ornithoboea occulta*, Thailand, Tak, 15 Oct 2014, D.J. Middleton, C. Hemrat, P. Karaket, C. Puglisi and S. Suddee 5702 (SING); *Paraboea clarkei* JN934757, JN934715; *Paraboea takensis*, Thailand, Tak, 16 Oct 2014, D.J. Middleton, C. Hemrat, P. Karaket, C. Puglisi and S. Suddee 5706 (SING); *Petrocodon ainsliifolius* HQ633038, HQ632941; *Petrocodon dealbatus* FJ501358, FJ501537; *Petrocodon scopulorum* HQ633044, HQ632947; *Petrocosmea kerrii* FJ501334, FJ501502; *Petrocosmea nervosa* FJ501335, AJ492299; *Primulina gemella* FJ501345, FJ501523; *Primulina luochengensis* HQ633046, HQ632949; *Primulina swinglei*, Vietnam, cultivated at Singapore Botanic Gardens, 27 Feb 2017, J. Leong-Škorničková JLS 3188 (SING); *Primulina tabacum* FJ501352, AJ492300; *Pseudochirita guangxiensis* HQ633003, HQ632908; *Rachunia cymbiformis*, Thailand, Kanchanaburi, 3 Nov 2015, M. Poopath, J. Sae Wai, W. Kheiwbang and S. Jirakon 1370 (BKF); *Raphiocarpus sinicus* HQ632973, HQ632877; *Ridleyandra petiolata* HQ633032, HQ632935; *Ridleyandra quercifolia* HQ633033, HQ632936; *Somrania albiflora* KU203792, KU203887; *Spelaeanthus chinii* FJ501307, FJ501457; *Streptocarpus glandulosissimus* AF316918, KR703972; *Streptocarpus rexii* AF316979; AJ492305; *Tribounia grandiflora* JX839280, JX839281; *Tribounia venosa* JX839283, JX839282.