Morphology and molecules support the new monotypic genus *Parainvolucrella* (Rubiaceae) from Asia

Yi-Da Xu¹,², Ming-Deng Yuan¹,², Rui-Jiang Wang¹

¹ Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, Guangdong 510650, China ² University of Chinese Academy of Sciences, Beijing 100049, China

Corresponding author: Rui-Jiang Wang (wangrj@scbg.ac.cn)

Abstract

*Parainvolucrella* R.J. Wang, a new monotypic genus for *P. scabra* (Wall. ex Kurz) M.D.Yuan & R.J.Wang, new combination, is segregated from the *Hedyotis-Oldenlandia* complex, based on morphological and molecular evidence. Phylogenetically, the new genus is sister to *Scleromitrion*, from which it differs by a combination of morphological characters: herbaceous habit, terminal inflorescence with subtended leaves, heterostylous flowers, indehiscent fruits and pollen with double microreticulate tectum. A key to the genera of the *Hedyotis-Oldenlandia* complex in China is provided for further identification.

Keywords

new combination, palynology, *Parainvolucrella*, *Scleromitrion*, taxonomy

Introduction

As one of the largest species groups of the family Rubiaceae, the *Hedyotis-Oldenlandia* complex contains hundreds of species distributed in the tropical and subtropical regions worldwide. Due to morphological intermediacy and homoplasy, systematic studies in herbaceous Rubiaceae are very difficult (Gibbons 2020). The generic delimitation within this complex is complicated and controversial (Neupane et al. 2015) and historically disputed. The commonly shared morphological characters, such as four petals and calyx lobes, 2-celled ovaries with numerous ovules on axile placenta and...
capsular fruits made some studies treat this complex as one genus, *Hedyotis* L., in a broad sense (Lamarck 1792; Fosberg and Sachet 1991; Dutta and Deb 2004; Chen and Taylor 2011). Whereas, morphological differences in habit, inflorescence position, homo- or heterostylous flowers, dehiscent or indehiscent fruits, as well as the shape and ornamentation of seeds and pollen, provide unquestionable evidence to separate this complex into several small genera (Bremerkamp 1952; Terrell et al. 1986; Terrell and Robinson 2003). Recent phylogenetic analyses, based on multiple nuclear and chloroplast DNA markers, revealed that this complex was polyphyletic and supported its subdivision into small genera (Groeninckx et al. 2009; Neupane et al. 2009; Guo et al. 2013; Wikström et al. 2013; Neupane et al. 2015; Gibbons 2020). Then the *Hedyotis* species in China fall into the following genera of *Debia* Neupane & N.Wikstr., *Dimetia* (Wight & Arn.) Meisn., *Edrastima* Raf., *Hedyotis*, *Involucrella* (Benth. & Hook.f.) Neupane & N.Wikstr., *Leptopetalum* Hook. & Arn., *Oldenlandia* L. and *Scleromitrion* (Wight & Arn.) Meisn. (Neupane et al. 2015; Wang 2018).

During our field investigation in Guangxi Zhuang Autonomous Region, we came across the species *Hedyotis scabra* Wall. ex Kurz, not recorded previously in China (Wei 2018), in bamboo forest nearby the Nonggang National Nature Reserve. This species has arbitrarily been treated as *Scleromitrion scabrum* (Wall. ex Kurz) Neupane & N.Wikstr. with insufficient morphological and molecular evidence (Neupane et al. 2015). Morphologically, it is similar to *Involucrella coronaria* (Kurz) Neupane & N.Wikstr. for its terminal inflorescence subtended by four involucral leaves. Our subsequent morphological comparison and phylogenetic analysis, based on multiple DNA markers, support that this species represents a new genus.

**Materials and methods**

Morphological characters of *Hedyotis scabra* were scored from living materials and dried specimens. All vouchers which we collected were deposited at the herbarium of South China Botanical Garden, Chinese Academy of Sciences (IBSC). Pollen and seeds were observed using scanning electron microscopy (JSM-6360LV) under 15.00 kV accelerating voltage. Pollen terminology for description followed Punt et al. (2007).

Methods of DNA extraction and PCRs followed Guo et al. (2011). Sequences of all taxa were downloaded from GenBank for molecular phylogenetic analysis, except for the newly added *Hedyotis hainanensis*, *H. ovata*, and three samples of *Hedyotis scabra* (Table 1). Geneious v.11.0.3 (Kearse et al. 2012) was used for sequence alignment and MrModeltest 2.0 was applied for selecting the best-fit nucleotide substitution model (GTR+G+I) on the basis of the AIC criterion (Nylander 2004). Bayesian Inference (BI) was performed using MrBayes v.3.2.7 (Ronquist et al. 2012), with a calculation of posterior probabilities (PP) to each clade. The bootstrap (BS) values were obtained by IQ-TREE v. 2.0 (Nguyen et al. 2015) for Maximum Likelihood analyses based on the best-fit nucleotide substitution model (GTR+F+R3) selected by ModelFinder (Kalyaanamoorthy et al. 2017).
Table 1. Taxa, vouchers, localities and GenBank accession numbers of ITS, petD, rps16, trnH-pobA and trnL-F sequences for phylogenetic analysis.

| Taxon | Voucher (herbarium) | ITS | petD | rps16 | trnH-pobA | trnL-F |
|-------|---------------------|-----|------|-------|-----------|--------|
| Debia ovatifolia (Cass.) Neupane & N. Wikstr. | China: Xing Guo & Ping Yang 20-1 (IBSC) | JF699940 | JF700090 | JX111109 | JF699795 | JX111382 |
| Deinandra repens J.R. Forst. & G. Forst. | Australia: Andersson 2262 (GB) | AM939440 | EU557693 | AF333701 | / | EU543091 |
| Dimetia ampliflora (Hance) Neupane & N. Wikstr. | China: Ruijiang Wang et al. 1147 (IBSC) | JX111198 | JX111086 | JX111242 | JX111161 | JX113317 |
| Dimetia auriculata (L.) R.J. Wang | China: Ruijiang Wang & Yiding Gao 1185 (IBSC) | JF699904 | JF700053 | JX111298 | JF699765 | JX113372 |
| Dimetia capitellata (Wall. ex G. Don) Neupane & N. Wikstr. var. capitellata | China: Xingguo Huang et al. GBOWS1278 (IBSC) | JX11201 | JX111089 | JX111250 | JX111164 | JX113327 |
| Dimetia scandens (Benth.) R.J. Wang | China: Guo Xing & Ping Yang 10 (IBSC) | JF699949 | JF700099 | / | JF699804 | / |
| Eduatima trineriata (Retz.) Neupane & N. Wikstr. | Sri Lanka: F. Fagerlind 4338 (S) | HE657769 | HE657652 | HE649907 | / | / |
| Hedysotis acutangula Champ. ex Benth. | China: Ruijiang Wang HA-02 (IBSC) | JX111197 | JX111085 | JX111241 | JX111160 | JX113316 |
| Hedysotis cantoniensis F.C. How ex W.C. Ko | China: Ruijiang Wang et al. 1250 (IBSC) | JP764844 | JF700601 | JX111247 | JF699773 | JX113322 |
| Hedysotis cuneifolia Merc. & EP. Meratelf | China: Ruijiang Wang et al. 1269 (IBSC) | JF699916 | JF700065 | JX111256 | JF699777 | JX113329 |
| Hedysotis effusa Hance | China: Ruijiang Wang et al. 1268_1 (IBSC) | JF699933 | JF700083 | JX111262 | JF699790 | JX113335 |
| Hedysotis hainanensis (Chun) W.C. Ko | China: Guobing Jiang & Xinxin Zhou 1121 (IBSC) | MZ326000* | MZ403798* | MZ343047* | MZ403808* | MZ403794* |
| Hedysotis oxasa Thunb. ex Maxim. | China: Guobin Jiang et al. 1508 (IBSC) | MZ326003* | MZ403799* | MZ343053* | MZ403807* | MZ403793* |
| Hedysotis shenzhenensis Tao Chen | China: Ruijiang Wang et al. 1262-1 (IBSC) | JF765092 | JF700101 | JX111276 | JF699805 | JX113350 |
| Hedysotis uncinella Hook. & Arn. | China: Ruijiang Wang 1217 (IBSC) | JF699963 | JF700113 | JX111282 | JF699814 | JX113356 |
| Involucrella cherevenensis (Pierre ex Pil.) Neupane & N. Wikstr. | Thailand: Suanthane 799 (ODU) | K994258 | KR005743 | KR005803 | / | / |
| Involucrella coronaria (Kurz) Neupane & N. Wikstr. | China: Xing Guo & Ping Yang 22-1 (IBSC) | JX112118 | JX111104 | JX111270 | JX111177 | JX113344 |
| Leptopetalum beflower (L.) Neupane & N. Wikstr. | Singapore: Ruijiang Wang SIN02 (IBSC) | JX11238 | JX111120 | JX11302 | JX111192 | JX113376 |
| Leptopetalum pteritum (Blume) Neupane & N. Wikstr. | China: Ruijiang Wang 1478 (IBSC) | JF699944 | JF700094 | / | JF699799 | / |
| Oldenlandia capensis L. var. capensis | Zambia: Dessein et al. 843 (BR) | AM939436 | EU557577 | EU543048 | / | EU543133 |
| Oldenlandia corymbosa L. var. corymbosa | Singapore: Ruijiang Wang SIN02 (IBSC) | JX11239 | JX111121 | JX11306 | JX111194 | JX113380 |
| Oldenlandia duemneri S. Moore | Uganda: W. H. Lewis 6018 (GH) | HE657744 | HE657169 | HE649881 | / | / |
| Oldenlandia umbellata L. | Sri Lanka: F. Fagerlind 3320 (S) | HE657674 | HE657569 | HE649806 | / | / |
| Oldenlandia wiedemannii K.Schum. | Kenya: Luke & Luke 8362 (UPS) | AM939525 | EU557576 | EU543063 | / | EU543151 |
| Panainvolucrella scabra (Wall. ex Kurz) M.D. Yuan & R.J. Wang | China: Mingdeng Yuan & Yida Xu YS398_1 (IBSC) | MZ326006* | MZ403801* | MZ343079* | MZ403806* | MZ403796* |
| Panainvolucrella scabra (Wall. ex Kurz) M.D. Yuan & R.J. Wang | China: Mingdeng Yuan & Yida Xu YS398_2 (IBSC) | MZ326007* | MZ403802* | MZ343070* | MZ403805* | MZ403797* |
| Panainvolucrella scabra (Wall. ex Kurz) M.D. Yuan & R.J. Wang | China: Mingdeng Yuan & Yida Xu YS399 (IBSC) | MZ326008* | MZ403803* | MZ343071* | MZ403804* | MZ403795* |
| Panainvolucrella scabra (Wall. ex Kurz) M.D. Yuan & R.J. Wang | Thailand: Neupane 183 (ODU) | K994264 | KR005751 | KR005812 | / | / |
| Pentodon pentandrus Vatke | Zambia: Dessein et al. 598 (BR) | AM939528 | EU557759 | EU543066 | / | EU543154 |
| Scleromitrion angustifolium (Cham. & Schdl.) Benth. | China: Xing Guo & Ping Yang 12 (IBSC) | JF765066 | JF700108 | JX111297 | JF699810 | JX113370 |
| Scleromitrion davidi (Wild.) R.J. Wang | China: Xing Guo 51 (IBSC) | JF699932 | JF700081 | JX111308 | JF699789 | JX113381 |
| Scleromitrion koanum (R.J.Wang) R.J. Wang | China: Ruijiang Wang et al. 978 (IBSC) | JX111215 | JX111101 | JX111267 | JX111174 | JX113341 |
| Scleromitrion pinifolium (Wall. ex G.Don) R.J. Wang | China: Ruijiang Wang 1231 (IBSC) | JX111240 | JF700094 | JX111311 | JX111196 | JX113384 |

Notes: "*" indicates the newly-seqenced fragments, "/" indicates the missing data.
Results

Phylogenetic analysis

The phylogenetic analysis, based on nuclear ITS and four chloroplast DNA regions (petD, rps16, trnH-psbA and trnL-F), generated an almost identical tree to that of Neupane et al. (2015). It showed that all the samples of *Hedyotis scabra* cluster into an independent clade which is sister to *Scleromitrion* with robust support (PP = 1, BS = 100, Fig. 1). In addition, the morphological similar species, *Involucrella coronaria*, nested in the *Involucrella* clade (PP = 1, BS = 93, Fig. 1) and is sister to the lineage of (*Debia* clade + (*Leptopetalum* clade + (*Dimetia* clade + (*Scleromitrion* clade + *H. scabra* clade)))) with robust support (PP = 1, BS = 100, Fig. 1).

![Phylogenetic tree](image)

**Figure 1.** Phylogenetic relationships of the *Hedyotis-Oldenlandia* complex derived from a combined analysis of ITS and plastid petD, rps16, trnH-psbA and trnL-F. Bayesian Posterior Probability (PP ≥ 0.5) and Bootstrap values (BS ≥ 50%) are indicated above and below the branches, respectively.
**Parainvolucrella, a new monotypic genus of Rubiaceae**

**Taxonomic treatment**

Based on the morphological and palynological differences between *Hedyotis scabra* and *Scleromitrion*, as well as the molecular evidence, a new genus is proposed here.

*Parainvolucrella* R.J. Wang, gen. nov.
urn:lsid:ipni.org:names:77218849-1
拟合叶耳草属 (Nǐ Hé Yè ěr Cǎo Shǔ)

**Note.** Annual or perennial herbs. Stem decumbent. Inflorescences terminal, congested-cymose, involucrated. Flowers heterostylyous; petals 4; ovary 2-loculed, ovules many. Pollen 3-colporate; tectum double microreticulate. Fruits indehiscent. Seeds trigonous; testa reticulate.

**Type.** *Parainvolucrella scabra* (Wall. ex Kurz) M.D. Yuan & R.J. Wang (*Hedyotis scabra* Wall. ex Kurz)

*Parainvolucrella scabra* (Wall. ex Kurz) M.D. Yuan & R.J. Wang, comb. nov.
urn:lsid:ipni.org:names: 77218850-1
Figs 2, 3

Basionym: *Hedyotis scabra* Wall. ex Kurz, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46(2): 133, 136 (1877). Type: MYANMAR. from Martaban down to Upper Tenasserim, *Wall. Cat. 880* (holotype: CAL; isotypes: G [G00436284!; G00436285!]; K [K001110148!; K001110149! K000031881!]).

Synonym: *Scleromitrion scabrum* (Wall. ex Kurz) Neupane & N.Wikstr., Taxon 64(2): 317 (2015)

**Description.** Annual or perennial herbs. Stems decumbent, ca. 1 m long, roughly angular, usually rooted at nodes; branches ascending to 30 cm high. Leaves opposite, subsessile to petiolate, petiole to 3 mm long; blades 2.0–7.0 × 1.0–3.0 cm, narrowly ovate to ovate, apex acute, base cuneate; leaf scabrid adaxially and along the veins abaxially; mid-rib depressed adaxially and prominent abaxially; secondary veins 5–6 on each side. Stipules ca. 3.0 × 2.0 mm, triangular, fimbriate with tipped colleters, excurved, pubescent abaxially. Inflorescence terminal, (2–)3–8(–12)-flowered, congested-cymose, usually subtended by 4 involucral leaves; peduncle subsessile; bracts 2–3 mm long, narrowly ovate, scabrid; bracteoles ca. 1 mm long, truncate to broadly ovate-triangular, fimbriate with tipped colleters, glabrous. Flowers heterostylyous, pedicels to 0.8 mm long. Hypanthium ca. 0.8 mm long, obconic, 4 longitudinal projections against the lobes; lobes 4, ca. 1.5 × 0.4 mm long, narrowly triangular to narrowly oblong, scabrid. Corolla white, tube 1.5–2.0 mm long, glabrous abaxially and pubescent adaxially; lobes 4, 2.3–2.8 × 0.7–0.8
Figure 2. Parainvolucrella scabra (Wall. ex Kurz) M.D. Yuan & R.J. Wang. A habit B leaf adaxial (left) and abaxial (right) surface C stem and stipule D infructescence with four involucral leaves E infructescence with bracts F calyx with bracteole at base G–I longistylos flower J–L brevistylos flower M, N fruits O seeds.
Parainvolucrella, a new monotypic genus of Rubiaceae

mm, oblong. Stamens 4, anthers 0.6–0.7 mm long. Stigma bilobed, 0.5–0.6 mm long, papillate. Longistylous flowers: stamens included, filaments adnate to the base of corolla tube, filaments ca. 2 mm long; styles ca. 4.3 mm long, exserted, included part pubescent, stigma ellipsoid. Brevistyloous flowers: stamens included; filaments adnate to the base of corolla tube, filaments ca. 5.6 mm long; styles ca. 2 mm long, exserted, pubescent, stigma clavate. Fruits ca. 2.1 × 2.3 mm, subglobose, with 4 longitudinal projections when young, scabrid, indehiscent. Seeds trigonous, 0.4–0.5 mm, numerous, black; testa reticulate.

**Phenology.** Flowering from July to September; fruiting from October to December.

**Etymology.** The generic name *Parainvolucrella* alludes to similarity to *Involucrella coronaria* in possessing terminal inflorescence subtended by four involucral leaves.

**Distribution and habitat.** Bangladesh, India, Myanmar, Thailand and Vietnam (Fukuoka 1970; Dutta and Deb 2004), and China (new record). Only one subpopulation including about 200 individuals was found in dense bamboo forest and at the edge of the forest nearby the Nonggang National Nature Reserve. The habitat there belongs to a tropical monsoon climate, main associated species are *Dendrocalamus latiflorus* Munro (Poaceae) and *Centotheca lappacea* (L.) Desv. (Poaceae).

**Palynology.** Monads, isopolar and prolate-spheroidal, with 3-colporate apertures; the tectum is double microreticulate, with a psilate suprareticulum and a microechinate infrareticulum. The pollen size is 22.2 (20.9–23.7) × 20.2 (18.3–21.8) μm with P/E value 1.10 in brevistyloous flowers (Fig. 4A–C); and 20.2 (18.5–21.2) × 19.0 (16.6–20.6) μm with P/E value 1.06 in longistylous flowers (Fig. 4D–F).

**Additional specimens examined.** China. Guangxi Zhuang Autonomous Region: Chongzuo City, Longzhou County, Zhubu Town, Nonggang Village, 1 Nov 1978, Nonggang Investigation Team 11263 (IBK!); same locality, 22°29′16″N, 106°56′13″E, elev. 287 m, 29 Oct 2020, Ming-Deng Yuan & Yi-Da Xu YS398, YS399 (IBSC!); same locality, 22°29′22″N, 106°56′11″E, elev. 290 m, 2 Feb 2021, Ming-Deng Yuan YS407 (IBSC!); Zhubu Town, Lenglei Village, 9 Oct 1979, Nonggang Investigation Team 20457 (GXM!). India. India orientalis: in Bengalia circa Calcuttam, J.W. Helfer 40 (P03904580). Thailand. Kampeng: A.F.G. Kerr 6161 (SING!); Tak: Ban Musoe, 22 Jul 1973, Gen Murata et al. 16719 (P03904581).
Key to the genera of the *Hedyotis-Oldenlandia* complex in China

1 Decumbent or prostrate herbs or climbers ................................................. 2
   – Erect or ascending herbs, subshrubs or shrubs ....................................... 5
2 Herbs; venation triplinerved inconspicuously above base; flowers homosty- 
   lous ............................................................................................................ *Edrastima*
   – Herbs or climbers; pinnated venation; flowers heterostylos ..................... 3
3 Climbers ...................................................................................................... *Dimetia*
   – Decumbent or prostrate herbs ................................................................ 4
4 Stipules triangular, fimbriate with tipped colleters; inflorescence terminal, 
   subtended by four leaves ............................................................................ *Parainvolucrella*
   – Stipules broadly triangular, apex spinous; inflorescence terminal or axillary, 
     without subtended leaves ....................................................................... *Dimetia*
5 Shrubs or subshrubs .................................................................................... *Hedyotis*
   – Herbs ..................................................................................................... 6
6 Inflorescence terminal, subtended by two or four leaves ............................ 7
   – Inflorescence terminal or axillary, without subtended leaves ................. 8
7 Inflorescence large and loose, peduncles and pedicels long .................... *Debia*
   – Inflorescence small and congested, peduncles and pedicels subsessile ....
     ........................................................................................................... *Involucrella*

Figure 4. Pollen morphology of *Parainvolucrella scabra* (A–C from Mingdeng Yuan & Yida Xu YS398, 
brevistylous flower D–F from Mingdeng Yuan & Yida Xu YS399, longistylous flower) A, D  
equatorial view B, E polar view C, F double microreticulate ornamentation of mesocolpium.
Parainvolucrella, a new monotypic genus of Rubiaceae

8  Fruits winged conspicuously or inconspicuously ............................................Leptopetalum
 – Fruits wingless .............................................................................................9
9  Herbs gracile; growing in limestone area ......................................................Involucrella
 – Herbs robust; growing in non-limestone area .................................................10
10 Stipules papery, hard, entire or fimbriate; flower homo- or heterostylos; fruits dehisce diplophragmously ..........................................................Hedyotis
 – Stipules membrane, fimbriate; flower homostylos; fruits dehisce loculicidally ..........................................................11
11 Stamens and stigma included in corolla tube .................................................Oldenlandia
 – Stamens and styles exserted from corolla tube ...........................................Scleromitrion

Discussion

The plant habit, stipule shape, inflorescence position, flower distyly and the dehiscence pattern of the fruits are of diagnostic significance in the different genera of the Hedyotis-Oldenlandia complex (Dutta and Deb 2004). Several successive field collections observed that the fruits of Hedyotis scabra are completely indehiscent, which was obscurely diagnosed by Hooker (1880) and incorrectly described by Dutta and Deb (2004). Hedyotis scabra differs from Scleromitrion by the terminal inflorescences with involucral leaves (vs. axillary or terminal and axillary in the uppermost leaf axils in Scleromitrion), the heterostylos flowers (vs. homostylos in Scleromitrion), pollen grains tectum double microreticulate, with psilate suprareticulum and microechinate infrareticulum (vs. rugulate tectum with microechinate muri in Scleromitrion) and indehiscent fruits (vs. loculicidally dehiscent in Scleromitrion). On the other hand, Parainvolucrella scabra is similar to Involucrella coronaria with respect to their terminal inflorescence subtended by involucral leaves, heterostylos flowers and indehiscent fruits, but Parainvolucrella has decumbent habit (vs. erect or ascending in Involucrella coronaria), young fruits with 4 longitudinal projections (vs. smooth surfaces in Involucrella coronaria) and trigonous seeds with no pits on the surface (vs. ellipsoidal and 3–5 pitted seeds in Involucrella coronaria) (Table 2).

Based on the combined nuclear (ITS, ETS) and plastid (petD, rps16) data, Neupane et al. (2015) did not provide a well-resolved phylogenetic tree to support the placement of Hedyotis scabra as sister to the remainder of Scleromitrion in the Hedyotis-Oldenlandia complex, neither did Gibbons (2020). In addition, it seemed that the morphological confliction between the H. scabra and Scleromitrion and the phylogenetic exclusion of H. scabra from Scleromitrion clade were overlooked before making the new combination by Neupane et al. (2015). Our further integrated analysis, based on the morphological incongruence and the robust phylogenetic support (BS = 100, PP = 1), based on nrITS and plastid petD, rps16, trnH-psbA and trnL-F, elucidated the taxonomic and phylogenetic confusions and thus the new monotypic genus Parainvolucrella is proposed here.
| Taxon                      | Habit                        | Stipules                                  | Flowers                                      | Fruits                                      | Seeds                                         | Pollen                                      |
|---------------------------|------------------------------|-------------------------------------------|----------------------------------------------|---------------------------------------------|-----------------------------------------------|---------------------------------------------|
| *Debia* Neupane & N. Wikstr. | Annual small herbs, erect    | Papery, broadly triangular, fimbriate with tipped colleters | Homostylos with exerted stigma and stamens | Compressed globose, loculicidally dehiscint | Conoidal with deeply depressed exotesta, anticlinal boundaries nearly straight or rounded | 3-colporate, perforate tectum with psilate muri |
| *Dimetia* (Wight & Arn.) Meisn. | Perennial herbs or subshrubs, prostrate, decumbent or climber | Papery, truncate, broadly rounded or broadly triangular, spinous | Heterostylos                                 | Subglobose to ellipsoidal, dehiscent diplopaphragmously or indehiscent | Dorsiventrally flattened or trigonous, reticulate, anticlinal boundaries nearly straight | 3- or 4-colporate, double microreticulate tectum with psilate suprareticulum and microechinate infrareticulum |
| *Edrastima* Raf.           | Annual small herbs, decumbent | Membranous, truncate, fimbriate with tipped colleters | Homostylos with exerted stigma and stamens | Subglobose, loculicidally dehiscint           | Trigonous to ellipsoidal, reticulate, anticlinal boundaries nearly straight | 3-colporate, microreticulate tectum with psilate muri |
| *Hedyotis* L.             | Perennial herbs to shrubs, erect or ascending | Papery, triangular or truncate, margin fimbriate or acicular spinous with tipped colleters | Homostylos or rarely homostylos with exerted stigma and stamens | Ellipsoidal, dehiscent diplopaphragmously or rarely indehiscent | Ellipsoidal, 3–5 pitted, anticlinal boundaries nearly straight or undulate | 3- or 4-colporate, double microreticulate tectum with psilate suprareticulum and microechinate infrareticulum |
| *Involucella* (Hook. f.) Neupane & N. Wikstr. | Annual herbs, erect or ascending | Papery, triangular or truncate, margin fimbriate with tipped colleters | Homostylos or rarely homostylos with included stigma and stamens | Hemispherical to ellipsoidal, loculicidally dehiscint or indehiscent | Ellipsoidal, 3–5 pitted, anticlinal boundaries nearly straight or undulate | 3- or 4-colporate, double microreticulate tectum with psilate suprareticulum and microechinate infrareticulum |
| *Oldenlandia* L.          | Annual small herbs, erect or ascending | Membranous, flabellate or broadly rounded, fimbriate with tipped colleters | Homostylos with included stigma and stamens | Globose to ellipsoidal, loculicidally dehiscint | Trigonous, reticulate, anticlinal boundaries nearly straight | 3- or 4-colporate, microreticulate tectum with psilate muri |
| *Parainvolucella* R.J. Wang | Annual or perennial herbs, decumbent | Papery, triangular, fimbriate with tipped colleters | Heterostylos                                 | Subglobose, 4 longitudinal projections when young, indehiscent | Trigonous, reticulate, anticlinal boundaries nearly straight | 3-colporate, double microreticulate tectum with psilate suprareticulum and microechinate infrareticulum |
| *Scleromitrion* (Wight & Arn.) Meisn. | Annual small herbs, erect or ascending | Membranous, triangular to rounded, fimbriate with tipped colleters | Homostylos with exerted stigma and stamens | Subglobose, loculicidally dehiscint           | Trigonous to conoidal, reticulate, anticlinal boundaries nearly straight | 3- or 4-colporate, rugulate tectum with microechinate muri |
| *Leptopetalum* Hook. & Arn. | Annual small herbs, erect    | Papery, triangular or broadly triangular, fimbriate with tipped colleters | Homostylos with included stigma and stamens | Obconical, winged, loculicidally dehiscint | Ellipsoidal with deeply depressed exotesta, anticlinal boundaries undulate | 3-colporate, microreticulate tectum with psilate muri |
Acknowledgements

This work was supported by the General Program of National Natural Science Foundation of China (Grant no. 31770217). We are grateful to Mr. Xin-Xin Zhou for field assistance and Ms. Xiao-Ying Hu for SEM observation.

References

Bremekamp CEB (1952) The African species of *Oldenlandia* L. sensu Hiern & K. Schumann. Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen, Afd. Natuurkunde 48(2): 1–297.

Chen T, Taylor CM (2011) *Hedyotis*. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China (Vol. 19). Science Press and Missouri Botanical Garden Press, Beijing and St. Louis, 147–174.

Dutta R, Deb DB (2004) Taxonomic Revision of *Hedyotis* L. (Rubiaceae) in Indian Sub-continent. Botanical Survey of India, Kolkata, 211 pp.

Fosberg FR, Sachet MH (1991) Studies in Indo-Pacific Rubiaceae. Allertonia 6(3): 191–278. https://www.jstor.org/stable/23185880

Fukuoka N (1970) Contributions to the flora of Southeast Asia (III). *Hedyotis* (Rubiaceae) of Thailand. Tonan Ajia Kenkyu 8(3): 305–336. [The Southeast Asian Studies]

Gibbons KL (2020) *Hedyotis, Oldenlandia* and related genera (Rubiaceae: Spermacoceae) in Australia: New genera and new combinations in an Asian-Australian-Pacific lineage. Taxon 69(3): 515–542. https://doi.org/10.1002/tax.12236

Groeninckx I, Dessein S, Ochoterena H, Persson C, Motley TJ, Kdrehed J, Bremer B, Huysmans S, Smets E (2009) Phylogeny of the Herbaceous tribe Spermacoceae (Rubiaceae) based on plastid DNA data. Annals of the Missouri Botanical Garden 96(1): 109–132. https://doi.org/10.3417/2006201

Guo X, Simmons MP, But PPH, Shaw PC, Wang RJ (2011) Application of DNA barcodes in *Hedyotis* L. (Spermacoceae, Rubiaceae). Journal of Systematics and Evolution 49(3): 203–212. https://doi.org/10.1111/j.1759-6831.2011.00130.x

Guo X, Wang RJ, Simmons MP, But PPH, Yu J (2013) Phylogeny of the Asian *Hedyotis-Oldenlandia* complex (Spermacoceae, Rubiaceae): Evidence for high levels of polyphyly and the parallel evolution of diplophragmous capsules. Molecular Phylogenetics and Evolution 67(1): 110–122. https://doi.org/10.1016/j.ympev.2013.01.006

Hooker JD (1880) The Flora of British India (Vol. 3). Lovell Reeve & Co., London, 712 pp. https://www.biodiversitylibrary.org/page/353862

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and
analysis of sequence data. Bioinformatics (Oxford, England) 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Lamarck JBAPM (1792) Hedyotis. In: Lamarck JBAPM, Poiret JLM (Eds) Tableau encyclopédique et méthodique des trois règnes de la nature. Botanique (Vol. 1(2)). Chez Panckouvek, Paris, 269–272. https://www.biodiversitylibrary.org/page/794169

Neupane S, Dessein S, Motley TJ (2009) The Hedyotis-Oldenlandia-Kohautia complex (Rubiaceae) in Nepal: A study of fruit, seed and pollen characters and their taxonomic significance. Edinburgh Journal of Botany 66(3): 371–390. https://doi.org/10.1017/S0960428609990035

Neupane S, Dessein S, Wikström N, Lewis P, Long CL, Bremer B, Motley T (2015) The Hedyotis-Oldenlandia complex (Rubiaceae: Spermacoceae) in Asia and the Pacific: Phylogeny revisited with new generic delimitations. Taxon 64(4): 299–322. https://doi.org/10.12705/64.2

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Nylander JAA (2004) MrModeltest v.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology 143(1–2): 1–81. https://doi.org/10.1016/j.revpalbo.2006.06.008

Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Terrell EE, Robinson H (2003) Survey of Asian and pacific species of Hedyotis and Exallage (Rubiaceae) with nomenclatural notes on Hedyotis types. Taxon 52(4): 775–782. https://doi.org/10.2307/3647351

Terrell EE, Lewis WH, Robinson H, Nowicke JW (1986) Phylogenetic implications of diverse seed types, chromosome numbers, and pollen morphology in Houstonia (Rubiaceae). American Journal of Botany 73(1): 103–115. https://doi.org/10.1002/j.1537-2197.1986.tb09686.x

Wang RJ (2018) Rubiaceae. In: Li DZ (Ed.) A Dictionary of the Families and Genera of Chinese Vascular Plants. Science Press, Beijing, 685 pp.

Wei YG (2018) The Distribution and Conservation Status of Native Plants in Guangxi, China. China Forestry Publishing House Beijing, 876 pp.

Wikström N, Neupane S, Kårehed J, Motley TJ, Bremer B (2013) Phylogeny of Hedyotis L. (Rubiaceae: Spermacoceae): Redefining a complex Asian-Pacific assemblage. Taxon 62(2): 357–374. https://doi.org/10.12705/622.2