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Henckelia wijesundarae (Gesneriaceae), a new endemic species from Sri Lanka, and lectotypification of *Chirita walkerae* and *C. walkerae* var. *parviflora*

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Abstract: A new species, *Henckelia wijesundarae*, endemic to Hiniduma, Galle, Sri Lanka, is described and illustrated. Diagnostic features of the new species are discussed and compared to the closely allied species *H. walkerae*. Based on fieldwork studies, we propose an IUCN conservation status of Critically Endangered (CR) for *H. wijesundarae*. At the same time we lectotypify the names *Chirita walkerae* (the basionym of *H. walkerae*) and *C. walkerae* var. *parviflora* (treated here in the synonymy of *H. wijesundarae*).

Key words: Gesneriaceae, *Henckelia*, *Chirita*, *Henckelia walkerae*, *Chirita walkerae*, *Chirita walkerae* subsp. *parviflora*, *Chirita walkerae* var. *parviflora*, new species, lectotype, Sri Lanka, Hiniduma

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Introduction

After its recent restructuring, *Henckelia* Spreng. has approximately 56 species with a distribution in NE and S India and Sri Lanka, the Himalayas and continental SE Asia, excluding Peninsular Thailand and Malaysia (Möller & al. 2011; Weber & al. 2011). The Sri Lankan species had previously been assigned to two genera: *Chirita* Buch.-Ham. ex D. Don (Wood 1974) and *Didymocarpus* Wall. (Wallich 1828–1849). When the genus *Henckelia* was re-established by Weber & Burt (1998), the three species of *Didymocarpus* occurring in Sri Lanka were moved into *Henckelia*. Later, based on molecular phylogenetic data (Möller & al. 2011; Weber & al. 2011), the four species of *Chirita* were also moved into

Henckelia. Six out of these seven species are endemic to Sri Lanka: *H. angusta* (C. B. Clarke) D. J. Middleton & Mich. Möller (Weber & al. 2011), *H. communis* (Gardner) D. J. Middleton & Mich. Möller (Weber & al. 2011), *H. moonii* (Gardner) D. J. Middleton & Mich. Möller (Weber & al. 2011), *H. walkerae* (Gardner) D. J. Middleton & Mich. Möller (Weber & al. 2011), *H. floccosa* (Thwaites) A. Weber & B. L. Burt (1998) and *H. zeylanica* (R. Br.) A. Weber & B. L. Burt (1998). The only species not endemic is *H. humboldtiana* (Gardner) A. Weber & B. L. Burt (1998), which occurs in both Sri Lanka and S India. All species have a rather restricted distribution in Sri Lanka, with their localities in undisturbed moist montane and lowland forests of the C and SW parts of the island.

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Thwaites (1864) distinguished two varieties in what he called *Chirita* “*walkeri*” (correctly *walkerae* and hereafter this spelling is used even if misspelt by earlier authors), and referred to them as “var. α ” and “var. β ”. Clarke (1883) named var. β *C. walkerae* var. *parviflora* C. B. Clarke while Theobald & Grupe (1972) raised it to *C. walkerae* subsp. *parviflora* (C. B. Clarke) Theobald & Grupe. In the restructuring of *Henckelia*, Weber & al. (2011) placed it into synonymy with *H. walkerae*, but without comment.

During fieldwork in Hiniduma, Galle, Sri Lanka, in May 2008, a population of *Henckelia* plants was discovered that appeared distinct in leaf-morphological characters compared to its morphologically closest ally *H. walkerae*. The population was not flowering at that time and only seed capsules were present. Hence, seeds were collected and germinated in the floriculture nursery at the Peradeniya gardens. The resultant plants flowered during June 2009. In August 2013, a second population of the same taxon was observed in the forest behind the Nugalala Buddhist monastery, Hiniduma, Galle. From both populations, herbarium specimens and flowers preserved in FAA for detailed morphological work were collected to test whether the newly collected material corresponds to the infraspecific groupings recognized previously in *H. walkerae*. We also collected silica-dried leaf samples for molecular work, along with materials of *H. walkerae* sensu stricto to test whether the two taxa represent separate evolutionary lineages and can be taxonomically delineated.

Material and methods

Studies of micro and macro morphological characters were carried out on field-collected and cultivated material of the newly collected specimens, by eye and with a Zeiss Stemi 2000c dissection microscope equipped with a Zeiss Axiocam MRc 5 digital camera and Zeiss Axio-Vision software (Carl Zeiss, Inc., Thornwood, NY 10594 U.S.A.). Preliminary observations suggested that the newly collected specimens were most closely affiliated to *Henckelia walkerae*. Thus, the newly collected material was compared specifically with herbarium specimens of this taxon.

Molecular work followed Mayer & al. (2003), Möller & al. (2009, 2011), Weber & al. (2011) and Samarakoon & al. (2013) to obtain sequence data for the nuclear ribosomal internal transcribed spacer (ITS) region constituting ITS1, the 5.8S gene and ITS2 (Möller & Cronk 1997). PCR amplification for the ITS region was performed using primers 5P and 8P (Möller & Cronk 1997) for three individuals from each of two populations of the new taxon, and two *Henckelia walkerae* populations, totalling 12 samples. The closest relative to these taxa is *H. moonii* (Ranasinghe, unpublished data), and therefore six samples from three populations of *H. moonii* were included as outgroups. Presenting the

relationships among all Sri Lankan *Henckelia* taxa is beyond the scope of the present paper, and will be the subject of a forthcoming publication.

All sequence electropherograms were edited using Sequencher version 5.3 (Gene Codes Corporation, MI, U.S.A.). The sequences were aligned using ClustalW in BioEdit version 7.1.11 (Hall 1999) and the alignments manually adjusted in Mesquite version 2.75 (Maddison & Maddison 2011). The ITS sequences were analysed by maximum parsimony (MP) implemented in PAUP* beta Version 4.0a146 for Microsoft Windows (Swofford 2002). All characters were used unordered, with equal weight. Parsimony trees were obtained via Branch-and-Bound searches with MulTrees in effect. Statistical branch support was performed as bootstraps (BS) (Felsenstein 1985) of 10000 heuristic bootstrap replicates of random step-wise addition, with TBR branch-swapping algorithm and MulTrees option in effect in PAUP.

Results and Discussion

Morphological observations — These confirmed that the newly collected material is very similar to *Henckelia walkerae*, but differs from it in several features, most prominently in the shorter calyx with a sharp ridge along the middle axis of the calyx lobes, white corolla lobes, fertile stamens with toothed filaments, and spatulate stigma (Table 1, Fig. 3, 4 & 5). The collection localities for the new taxon and *H. walkerae* are also disjunct, in the SW lowland wet zone forests at 200–300 m for the former, and above 1000 m in submontane and montane forests for the latter (Fig. 1).

The basis for Thwaites’s (1864) *Chirita walkerae* “var. β ”, Clarke’s (1883) *C. walkerae* var. *parviflora* and Theobald & Grupe’s *C. walkerae* subsp. *parviflora* was the gathering CP 542 [CP = Ceylon Plants] (see also lectotypification of *C. walkerae* var. *parviflora*), which was studied in different herbaria (one herbarium sheet at K, two at PDA, two at P and two at W; codes after Thiers 2015+). Several characters of the specimens on these sheets match the newly collected material (less dense pubescence, small flowers and distribution data). Our morphological studies therefore established that the newly collected specimens are very similar to the specimens collected under *C. walkerae* var./subsp. *parviflora*. The only discernible difference appears to concern the corolla colour. In *Enumeratio plantarum Zeylaniae* (Thwaites 1864) and *A hand-book to the flora of Ceylon* (Trimen 1895), the colour of the corolla of *C. walkerae* var. *parviflora* was described as pale purple, whereas in our collected material it is pure white with a pale-pink-tinged tube. In the former, the corolla colour was given in rather general terms, and may perhaps represent an imprecise description, in fact corresponding to the newly collected material, or variation within a spe-

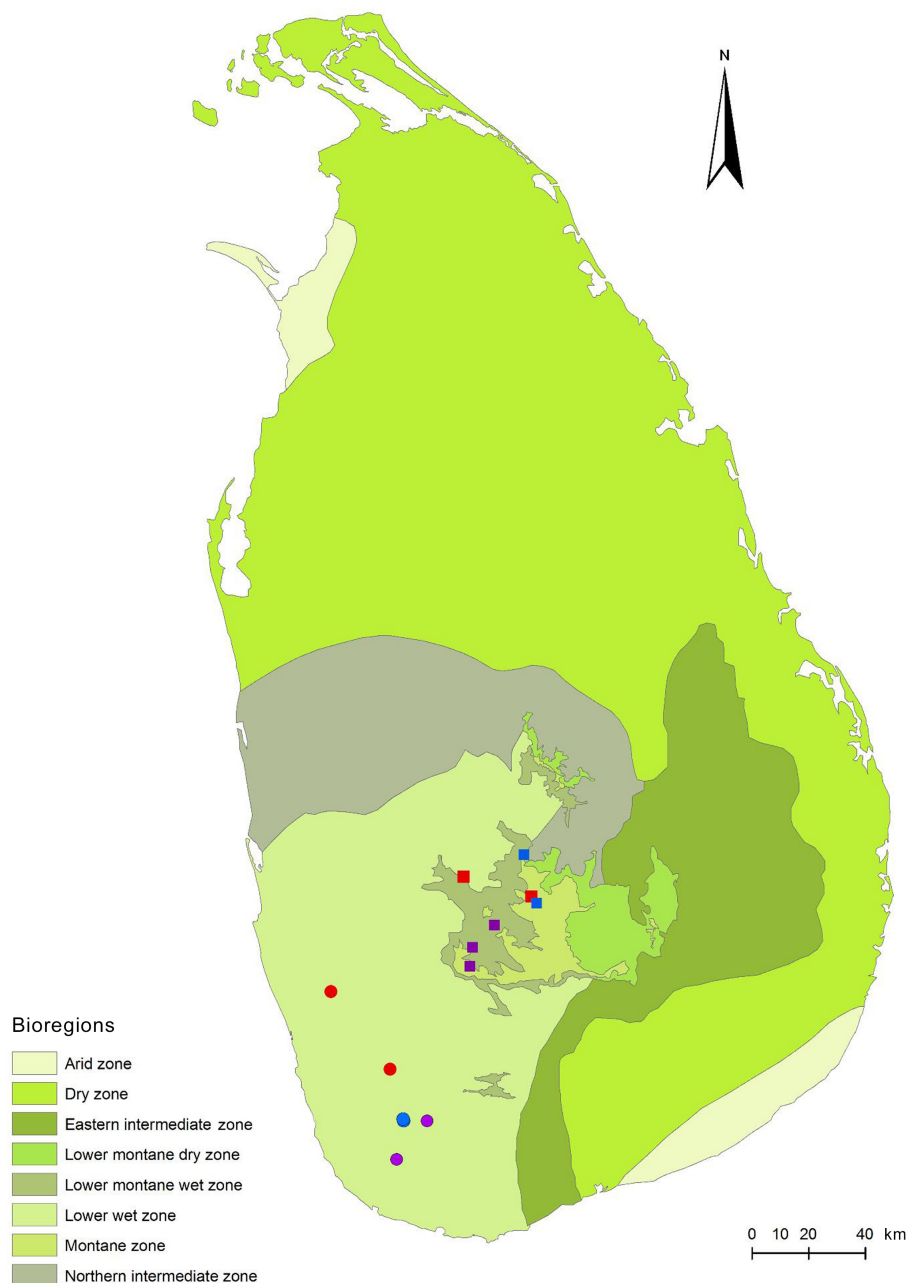


Fig. 1. Distribution points of *Henckelia wijesundarae* (circles) and *H. walkerae* (squares) based on herbarium specimen information projected on a bioclimatic map of Sri Lanka. Collections before 1850s (red), after 1850 (purple) and in 2013–2014 (blue).

cies. On herbarium sheets of CP 542, the colour of the corollas has been lost. Overall, because of their similar morphologies, we regard *C. walkerae* var./subsp. *parviflora* as conspecific with the newly collected material.

Phylogenetic analysis — We found several morphological character differences between our new material (including *Chirita walkerae* var./subsp. *parviflora*) and *Henckelia walkerae*. To test the independence of the two taxa we performed the molecular phylogenetic study utilizing our newly collected material.

In our molecular work, the matrix of the aligned ITS1, 5.8S and ITS2 sequences had 660 characters of

which 600 characters were constant, three were variable but parsimony-uninformative, and 57 were parsimony-informative. The MP analysis in PAUP* generated a single most parsimonious tree (length = 63 steps, consistency index, CI = 0.9524, retention index, RI = 0.9880) (Fig. 2). The tree shows a highly supported (BS = 100%) single clade for the new taxon described here. The analysis also revealed that the newly collected specimens are genetically distant from *Henckelia walkerae* (Fig. 2), with 23 nucleotide substitutions separating the two taxa. Based on average substitution rates for ITS for herbaceous plants of Kay & al. (2006), as applied to *Gesneriaceae* in Puglisi & al. (2011), this represents an approximate divergence time of 4.6 million years ago ($\pm 0.734SE$). This is a considerable length of time and supports a species status for this taxon.

Our careful observations suggested that our newly collected material is likely conspecific with *Chirita walkerae* var./subsp. *parviflora*. However, because of the sparse, imprecise and incomplete description of this taxon (Clarke 1883; Thwaites 1864; Theobald & Grupe 1972), it seems better to describe our material as a

new *Henckelia* species from Sri Lanka.

Henckelia wijesundarae Ranasinghe & Mich. Möller, **sp. nov.** – Fig. 3 & 4.

Holotype: Sri Lanka, Southern Province, Galle District, Hiniduma, forest behind Nugagala Monastery, 208 m, 26 Jul 2013, *Subhani Ranasinghe & Tharanga Wijewickrama* 31 (PDA; isotypes: E, K).

= *Chirita walkerae* [“walkeri”] var. *parviflora* C. B. Clarke in Candolle & Candolle, *Monogr. Phan.* 5(1): 112. 1883 = *Chirita walkerae* [“walkeri”] subsp. *parviflora* (C. B. Clarke) W. L. Theob. & Grupe in Ceylon J.

Table 1. Diagnostic features differentiating *Henckelia walkerae* and *H. wijesundarae*; see also Fig. 3, 4 & 5.

Character	<i>Henckelia walkerae</i>	<i>Henckelia wijesundarae</i>
Altitude and habitat	above 1000 m in submontane and montane forests	200–300 m in wet lowland rain forests
Branching pattern	irregularly or dichotomously branched	dichotomously branched
Hairs on adaxial leaf surface	sericeous with densely appressed short straight hairs	puberulous with less dense short straight hairs giving a velvet-like appearance
Leaf margin	serrate, with prominent hydathodes	shallowly crenulate, with inconspicuous hydathodes
Number of lateral veins	12(–14) pairs	8–10 pairs
Number of flowers per inflorescence	2 or 3	mostly 3 or 4
Calyx length	> 2 cm	< 2 cm
Calyx lobes arrangement	free to c. ½ length of calyx	free to c. ½ length of calyx, calyx lobes in 2+2+1 arrangement
Outer calyx lobe surface	keel inconspicuous, hairs scattered throughout calyx lobe	keeled, with a sharp ridge along middle axis of calyx lobe, hairs aggregated along this ridge
Corolla length	4–6 cm	3–4 cm
Corolla tube colour	diffuse purple	diffuse pink or white
Corolla lobes colour	deep purple	white
Filament	tooth absent	tooth present
Stigma	cuneate-truncate	spatulate
Fruit length	8–13 cm	6–7 cm

Sci., Biol. Sci. 10: 70. 1972. – Lectotype: see p. 220.
– *Chirita walkerae* [“*walkeri*”] var. “β” in Thwaites, Enum. Pl. Zeyl.: 207. 1864.

Diagnosis — *Henckelia wijesundarae* Ranasinghe & Mich. Möller differs from *H. walkerae* (Gardner) D. J. Middleton & Mich. Möller by its less dense short straight hairs on the adaxial and abaxial leaf surfaces, inconspicuous hydathodes (vs prominent ones in *H. walkerae*) on shallowly crenulate (vs serrate) leaf margins, 8–10 pairs (vs 12–(14) pairs) of lateral veins, calyx less than 2 cm long (vs more than 2 cm long), calyx lobes keeled, with a sharp ridge along the middle axis and hairs aggregated along this ridge (vs keel inconspicuous, hairs scattered throughout calyx lobe), shorter corolla, 3–4 cm long (vs 4–6 cm long), white (vs deep purple) corolla lobes, toothed (vs untoothed) filaments, distinctly spatulate (vs cuneate-truncate) stigma, and shorter fruit, 6–7 cm long (vs 8–13 cm long).

Description — *Herbs* caulescent, suffruticose, 30–90 cm tall, dichotomously branched. *Mature stem* cylindrical, 0.7–0.8 cm in diam., with brownish pink hairs throughout; *leaf scars* present on mature stems; *young stem* cylindrical, c. 0.5 cm in diam., fleshy, pubescent, with fine silvery hairs scattered throughout. *Leaves* predominantly 3-whorled at nodes, rarely decussate; *petiole* light green, cylindrical, 2–4 cm long, fleshy, pubescent with fine silvery and purple hairs throughout, fleshy leaf texture, *leaf blade* greenish white on abaxial

surface, bright light green on adaxial surface, ovate to lanceolate, 6–8 cm long, 3.5–4.5 cm wide, pinnately veined, silky to pubescent on abaxial surface, more velvety on adaxial surface, base obtuse, slightly unequal, margin shallowly crenulate, with inconspicuous hydathodes, apex acuminate; *lateral veins* in 8–10 pairs, sometimes oblique, conspicuous on abaxial surface. *Inflorescence* predominantly a compound dichasium (pair-flowered cymes), mostly with 3 or 4 flowers open simultaneously; *peduncle* 3.5–4.5 cm long, pubescent; *bracts* oblong-linear, 2–3 mm long, 0.5–1 mm wide, caducous; *pedicels* 0.5–1 cm long. *Calyx* 5-lobed; *calyx tube* 0.5–1 cm long; *calyx lobes* free to c. ½ length of calyx, in 2+2+1 arrangement (with single calyx lobe on upper side), lanceolate, 0.5–1 cm long, c. 0.2 cm wide, keeled, glandular pubescent, hairs on abaxial surface aggregated to sharp ridge along middle axis, apex acuminate. *Corolla* 3–4 cm long, with 2 longitudinal ridges along floor of tube and lower lip, outside with glandular hairs especially in tube, inside glabrous; *corolla tube* white with a pink tinge, sometimes with a broad yellow line in throat, c. 3 cm long, c. 1 cm wide at throat, throat somewhat angular in outline; *corolla lobes* 5, white, suborbicular, with c. 3 veins per lobe, lower lip with 3 lobes 0.7–1 cm long, c. 1 cm wide, upper lip with 2 lobes slightly smaller than those of lower lip. *Ovary* glandular hairy; *style* green-white, 2.5–3.5 cm long, glandular pubescent; *stigma* chiritoid, abaxial lip greenish white, spatulate, 2–3 mm long, 1.5–2 mm wide, adaxial lip inconspicuous. *Stamens* 2, anterior; *filaments* white, geniculate, swollen at bend, twisted in lower half,

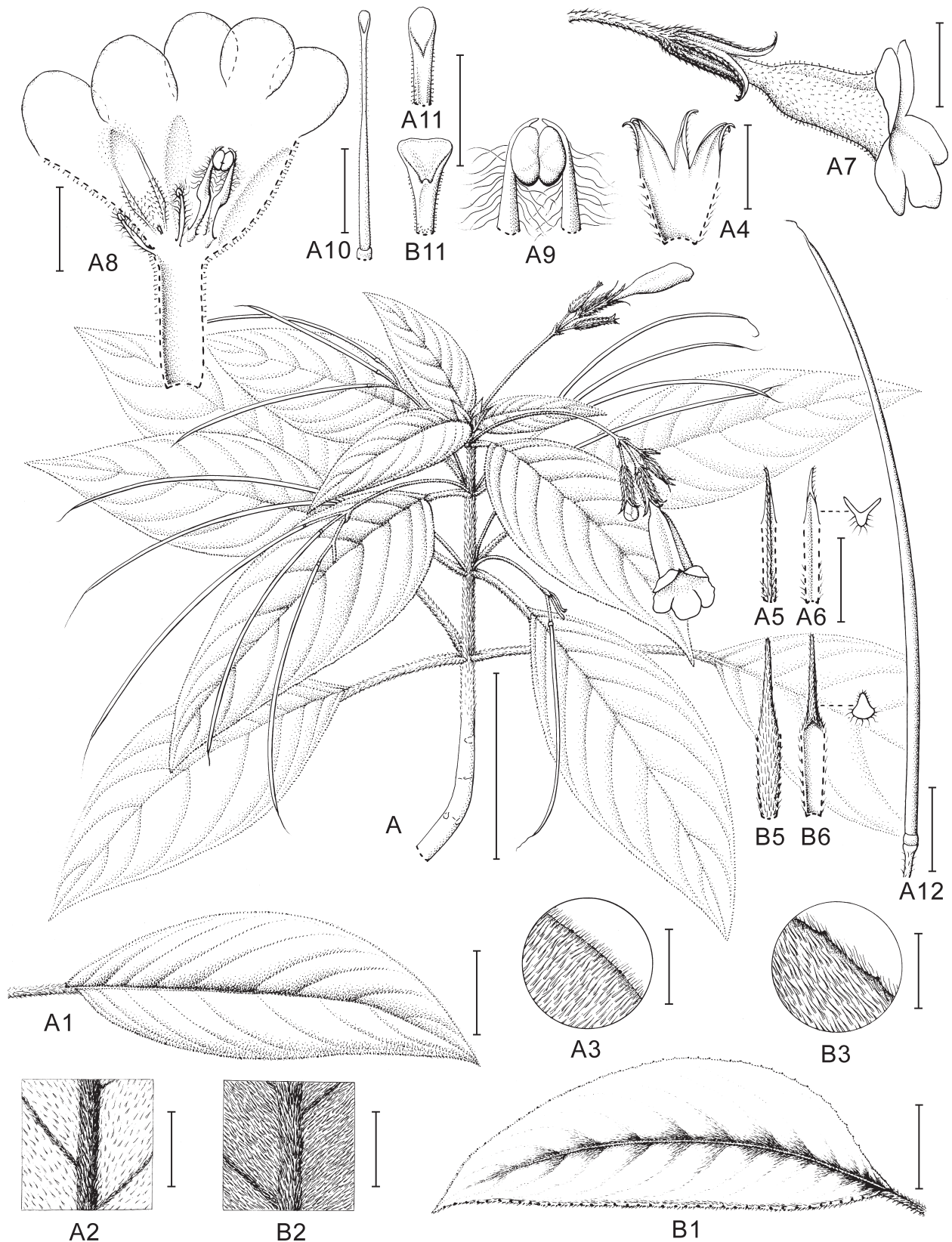


Fig. 3. *Henckelia wijesundarae* (A) and *H. walkerae* (B). – A: habit; A1 & B1: adaxial leaf surface; A2 & B2: abaxial leaf surface; A3 & B3: leaf margin; A4: calyx cut open showing 2+2+1 arrangement of lobes; A5 & B5: calyx lobe abaxial surface; A6 & B6: calyx lobe adaxial surface and cross-section; A7: flower lateral view; A8: flower longitudinal section; A9: coherent anther pair showing toothed filaments; A10: pistil; A11 & B11: stigma; A12: fruit. – Scale bars: A = 4 cm; A1, B1, A4, A5, B5, A6, B6, A7, A8, A10, A12 = 1 cm; A2, B2, A3, B3, A9, A11, B11 = 5 mm. – Drawn from Ranasinghe & Wijewickrama 31 by Claire Banks.

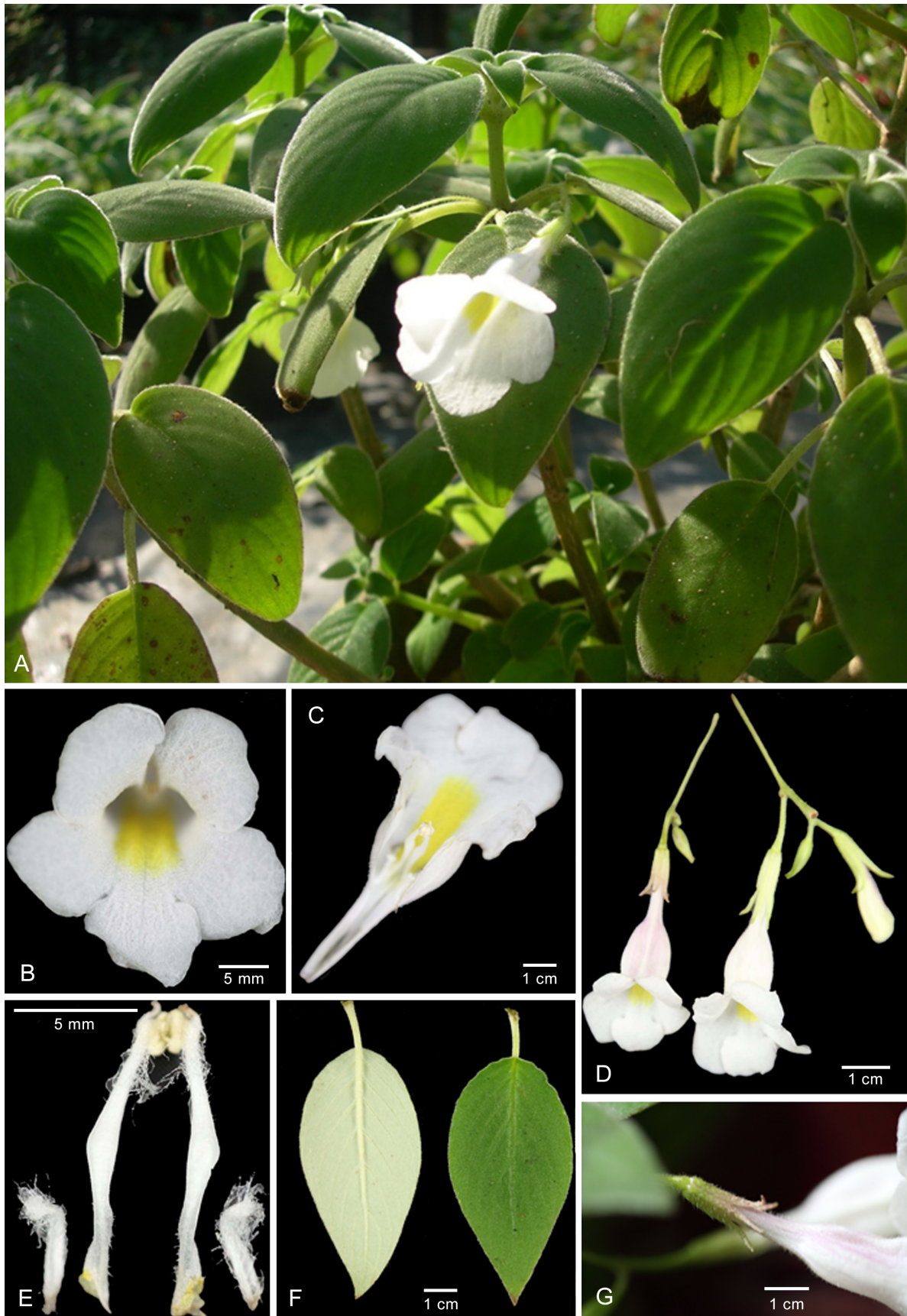


Fig. 4. *Henckelia wijesundarae* – A: habit; B: corolla front view; C: corolla longitudinal section; D: two inflorescences; E: two stamens with toothed filaments and coherent anthers and two lateral staminodes; F: leaf abaxial (left) and adaxial (right) surfaces; G: calyx showing lobes divided less than $\frac{1}{2}$ length of calyx.

15479 (MO, PDA). Kalutara district, Rayigam Korale, *Thwaites s.n.* part of CP 542 (PDA); Pasdun Korale, *Thwaites s.n.*, part of CP 542 (PDA). Locality unknown, *Thwaites s.n.*, CP 542 (K, P [2 sheets], W [2 sheets]); *Mrs Walker s.n.* (P).

Lectotypification of *Chirita walkerae*

Chirita walkerae Gardner in Calcutta J. Nat. Hist. 6: 480. 1846 ["*walkeri*"] = *Roettlera walkerae* (Gardner) Kuntze, Revis. Gen. Pl. 2: 477. 1891 ["*walkeri*"] = *Henckelia walkerae* (Gardner) D. J. Middleton & Mich. Möller in Taxon 60: 777. 2011. – **Lectotype (designated here)**: Sri Lanka ["Ceylon"], *Mrs General Walker 191* (K top left specimen on sheet K000858368) – Fig. 6.

Henckelia walkerae was first described from Ceylon (= Sri Lanka) as *Chirita "walkeri"* by Gardner (1846), correctly *C. walkerae* under Art. 60.12, Note 4 and Rec. 60C.1 of the ICN (McNeill & al. 2012), based on Mrs Walker's collections. However, Gardner (1846) did not cite any specimen number or any specific location of Mrs Walker's collections in Sri Lanka in his description. Until now, no lectotype has been designated. Gardner (1846) stated that he described the species based on part of Mrs Walker's collections, which he received from Prof. William Hooker then at Glasgow University. Gardner took them to Ceylon when he accepted his appointment as Director of the Botanic Gardens at Peradeniya. However, none of the specimens of *H. walkerae* from Mrs Walker's collections is present at the herbarium at Peradeniya (PDA). The collections of Mrs Walker annotated as *C. walkerae* are available at the herbaria in Kew (K), Edinburgh (E) and Paris (P). Study of these specimens confirmed that there is no specific location cited on those herbarium sheets, except for the then country name "Ceylon". There are three sheets at K: *Walker 191* (K000858368), *Walker 1722* (K000858369) and *Walker 177* (not barcoded). On sheet *Walker 191* there are three specimens mounted, and only those on the top left and bottom left are *H. walkerae*, whereas the one on the right belongs to *H. communis*. The other sheets contain only *H. walkerae* specimens. Another three specimens of collection number *Walker 1722* are at E, mounted on two sheets (E00155265 and E00155266); these were confirmed as *H. walkerae*. Only one specimen from Mrs Walker's collection is present at P (P03884698) but it does not have a collection number. The two specimens mounted on this sheet match in morphology *H. wijesundarae*. Therefore, only specimens at K and E match the description of *C. walkerae* made by Gardner (1846). The top left and bottom left specimens on the sheet of *Walker 191* at K (K000858368) are complete for the description of *C. walkerae*. However, careful observations have suggested that it is very unlikely that these specimens are from a single gathering. Therefore, the top left specimen on sheet *Walker 191* at K, which also

has capsules (Fig. 6), is selected as the lectotype of the name *C. walkerae*.

Lectotypification of *Chirita walkerae* var. *parviflora*

Chirita walkerae var. *parviflora* C. B. Clarke in Candolle & Candolle, Monogr. Phan. 5: 112. 1883 (for full synonymy, see account of *H. wijesundarae* above). – **Lectotype (designated here)**: Sri Lanka ["Ceylon"], *Thwaites s.n.*, CP 542 (K [not barcoded]) – Fig. 7.

Thwaites (1864) recognized two varieties under *Chirita walkerae*, "var. α " and "var. β ". Later, Clarke (1883) described "var. β " as *C. walkerae* var. *parviflora*, citing as its basis "Thwaites C. P. n. 542 in hh. Kew, DC, Mus. Brit., Berol.", a treatment that was followed by Trimen (1895). During revisionary work on the *Flora of Ceylon* for the family Gesneriaceae, Theobald & Grupe (1972) elevated *C. walkerae* var. *parviflora* to subspecific rank as *C. walkerae* subsp. *parviflora*. This was based on the same material examined by Thwaites (1864) and Clarke (1883), because no other material determined as this taxon had been collected since. However, none of these accounts cited a particular specimen of Thwaites's CP 542 as the type specimen of *C. walkerae* var. *parviflora*. As noted by Stafleu & Cowan (1986: 342) the CP numbers of material distributed by Thwaites were not true collection numbers, the material stemming from various locations. We have located seven herbarium sheets in different herbaria bearing the number CP 542. Two sheets at W with the herbarium accession numbers 162356 and 65460, originally annotated as *C. walkerae* var. β under CP 542, had in 2014 been annotated as isotypes of *C. walkerae* by J. Walter (annotation numbers W-1889-0162356 and W-1889-0065460, respectively). Although these two specimens do not bear flowers, there are other important characters, such as leaf pubescence, lateral vein number, fruit length and leaf margin characteristics that are comparable with other specimens of CP 542. Hence, these specimens are better referred to *C. walkerae* var. *parviflora*, i.e. *H. wijesundarae*, rather than to typical *C. walkerae*, i.e. *H. walkerae*, and therefore this re-annotation as isotype is not in accord with the present lectotypification of *C. walkerae*. There were two specimens of CP 542 observed from P: one sheet with no. 542 (P03884094) annotated as *C. walkerae* var. β and a second sheet CP 542 (P03884093) annotated as *C. walkerae* var. *parviflora*. There are two further herbarium sheets of CP 542 at PDA with the same annotation. There is only one sheet at K, mounted with two specimens annotated as *C. walkerae* var. β . Our present investigation suggests that all specimens numbered CP 542 represent *C. walkerae* var. *parviflora*. The herbarium sheet of CP 542 at K was noted by Clarke (1883) in his original description of *C. walkerae* var. *parviflora*. The two specimens mounted on this sheet appear to be from a single gathering. Therefore, we have chosen *Thwaites s.n.*, CP 542 at K as the lectotype of *C. walkerae* var. *parviflora* (Fig. 7).



Fig. 5. *Henckelia walkerae* at Kikilyamana Forest Reserve in August 2013. – A: habitat and habit; B: flower front view; C: flower top view.

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Fig. 6. Herbarium sheet of *Mrs Walker 191* (K000858368). – Top left specimen: lectotype of *Chirita walkerae* Gardner (= *Henckelia walkerae* (Gardner) D. J. Middleton & Mich. Möller); bottom left specimen: *H. walkerae*, but probably from a different gathering; right-hand specimen: *H. communis* (Gardner) D. J. Middleton & Mich. Möller. – Reproduced with kind permission of the Board of Trustees of the Royal Botanic Gardens, Kew.

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References

- Bachman S., Moat J., Hill A. W., de la Torre J. & Scott B. 2011 [online version 2015]: GeoCAT (geospatial conservation assessment tool). – Published at <http://geocat.kew.org/> [accessed 27 Jul 2015].
- Clarke C. B. 1883: *Cyrtandreae*. – Pp. 111–112 in: Can-



Fig. 7. Lectotype of *Chirita walkerae* var. *parviflora* C. B. Clarke: Thwaites s.n., CP 542 (K). – Reproduced with kind permission of the Board of Trustees of the Royal Botanic Gardens, Kew.

- dolle A. & Candolle C., Monographiae phanerogamarum **5(1)**. – Paris: G. Masson.
- Felsenstein J. 1985: Confidence-limits on phylogenies – An approach using the bootstrap. – *Evolution* **39**: 783–791.
- Gardner G. 1846: Contributions towards a flora of Ceylon. – *Calcutta J. Nat. Hist.* **6**: 480–481.
- Hall T. A. 1999: BioEdit: a user friendly biological sequence alignment editor and analysis program for windows 95/98/NT. – *Nucl. Acids Symp. Ser.* **41**: 95–98.
- IUCN 2012: IUCN Red List categories and criteria. Version 3.1, ed. 2. – Gland and Cambridge: IUCN.
- Kay K. M., Whittall J. B. & Hodges S. A. 2006: A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. – *B. M. C. Evol. Biol.* **6**: 36.
- Kuntze O. 1891: Pp. 473–477 in: *Revisio generum plantarum* **2**. – Paris: Charles Klincksieck.
- Maddison W. P. & Maddison D. R. 2011: Mesquite: A modular system for evolutionary analysis. Version 2.75. – Published at <http://mesquiteproject.org>
- Mayer V., Möller M., Perret M. & Weber A. 2003: Phylogenetic position and generic differentiation of *Epithemateae* (*Gesneriaceae*) inferred from plastid DNA sequence data. – *Amer. J. Bot.* **90**: 321–329.
- McNeill J., Barrie F. R., Buck W. R., Demoulin V., Greuter W., Hawksworth D. L., Herendeen P. S., Knapp S., Marhold K., Prado J., Prud'homme van Reine W. F., Smith G. F. & Wiersema J. H. 2012: International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. – Königstein: Koeltz Scientific Books. – *Regnum Veg.* **154**.
- Möller M. & Cronk Q. C. B. 1997: Origin and relationships of *Saintpaulia* (*Gesneriaceae*) based on ribosomal DNA internal transcribed spacer (ITS) sequences. – *Amer. J. Bot.* **84**: 956–965.
- Möller M., Forrest A., Wei Y. G. & Weber A. 2011: A molecular phylogenetic assessment of the advanced Asiatic and Malesian didymocarpoid *Gesneriaceae* with focus on non-monophyletic and monotypic genera. – *Pl. Syst. Evol.* **292**: 223–248.
- Möller M., Pfosser M., Jang C. G., Mayer V., Clark A., Hollingsworth M. L., Barfuss M. H. J., Wang Y. Z., Kiehn M. & Weber A. 2009: A preliminary phylogeny of the 'didymocarpoid *Gesneriaceae*' based on three molecular data sets: incongruence with available tribal classifications. – *Amer. J. Bot.* **96**: 989–1010.
- Puglisi C., Wei Y. G., Nishii K. & Möller M. 2011: *Oreocharis* × *heterandra* (*Gesneriaceae*): a natural hybrid from the Shengtangshan Mountains, Guangxi, China. – *Phytotaxa* **38**: 1–18.
- Samarakoon T., Wang S. Y. & Alford M. H. 2013: Enhancing PCR amplification of DNA from recalcitrant plant specimens using a trehalose-based additive. – *Appl. Plant Sci.* **1(1)**: 1200236.
- Stafleu F. A. & Cowan R. S. 1986: Taxonomic literature, ed. 2, **6**. – Utrecht & Antwerpen: Bohn, Scheltema & Holkema; The Hague & Boston: dr. W. Junk b.v. – *Regnum Veg.* **115**.
- Swofford D. L. 2002: PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. – Sunderland: Sinauer Associates.
- Theobald W. L. & Grupe D. A. 1972: Critical notes on Ceylon plants: A new name and combinations in the *Gesneriaceae*. – *Ceylon J. Sci., Biol. Sci.* **10**: 70–71.
- Thiers B. 2015+ [continuously updated]: Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. – Published at <http://sweetgum.nybg.org/science/ih/> [last accessed 13 Jul 2016].
- Thwaites G. H. K. 1864: *Cyrtandraceae*. – Pp. 206–208 in: *Enumeratio plantarum Zeylaniae*. – London: Dulau & Co.
- Trimen H. 1895: *Gesneraceae* (*Cyrtandreae*). – Pp. 271–280 in: *Hand-book to the flora of Ceylon. Part III*. – London: Dulau & Co.
- Wallich N. 1828–1849: A numerical list of dried specimens of plants in the East India Company's Museum, collected under the superintendence of Dr. Wallich of the Company's Botanic Garden at Calcutta. – London and Hereford.
- Weber A. & Burt B. L. 1998: Remodeling of *Didymocarpus* and associated genera (*Gesneriaceae*). *Beitr. Biol. Pflanzen* **70**: 293–363.
- Weber A., Middleton D., Forrest A., Kiew R., Lim C. L., Rafidah A. R., Sontag S., Triboun P., Wei Y. G., Yao T. L. & Möller M. 2011: Molecular systematics and remodelling of *Chirita* and associated genera (*Gesneriaceae*). – *Taxon* **60**: 767–790.
- Wood D. 1974: A revision of *Chirita* (*Gesneriaceae*). *Notes Roy. Bot. Gard. Edinburgh* **33**: 123–205.

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