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A new black nightshade (Morelloid clade, *Solanum*, Solanaceae) from the caatinga biome of north-eastern Brazil with a key to Brazilian morelloids

Sandra Knapp[1], Tiina Särkinen[2]

1 *Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK* **2** *Royal Botanic Garden Edinburgh, 20A Inverleith Row, EH3 5LR Edinburgh, UK*

Corresponding author: *Sandra Knapp* (s.knapp@nhm.ac.uk)

Abstract

Solanum caatingae **sp. nov.** is described from the arid caatinga biome of north-eastern Brazil. It is known from only a few specimens, but these were found amongst the many sheets of the widespread circumtropical weed *S. americanum* Mill.; it is possible that more will be found once its distinct nature has been recognised. It differs from *S. americanum* and all other herbaceous black nightshades known in Brazil, in its combination of glandular pubescence and shiny black fruit with small spreading sepals. The description of *S. caatingae* brings the number of morelloid solanums in Brazil to seven and a key is provided for their identification.

Keywords

Brazil, dry forests, endemism, identification key, new species, *Solanum*, Solanaceae, weeds

Introduction

Solanum L. is one of the most species-rich vascular plant genera in South America (Jørgensen et al. 2011; Ulloa Ulloa et al. 2017) and, within the continent, the tropical Andes represent one of the main centres of species diversity for *Solanum* for both spiny

(Leptostemonum clade, see Stern et al. 2011) and non-spiny clades (Weese and Bohs 2007; Särkinen et al. 2013b). Significant diversity is also found in dry regions such as the Atacama desert (Regmandra clade, Bennett 2008) and seasonally dry tropical forests (Cyphomandropsis clade, Bohs 2001; Dulcamaroid clade, Knapp 2013; Geminata clade, Knapp 2002a, 2008; section *Gonatotrichum* Stern et al., 2013; section *Erythrotrichum*, Agra 2008; Elaeagnifolium clade, Knapp et al. 2017). These dry habitats, however, are less species-rich, but also less well-explored (Sobral and Stehmann 2009; Sousa-Baena et al. 2014), than the more humid forests of the Andes and south-eastern Brazil, which have been considered the foci of diversity in the genus (Knapp 2002b).

Within *Solanum*, the Morelloid clade is a group of ca. 75 species most of which are endemic to the tropical Andes (Bohs 2005; Särkinen et al. 2015c). The clade includes five major groups traditionally recognised at the sectional level (sections *Solanum*, *Campanulisolanum* Bitter, *Parasolanum* A.Child pro parte, *Chamasarachidium* Bitter and *Episarcophyllum* Bitter), which are in the process of re-circumscription based on molecular results (Särkinen et al. 2015c). The black nightshade group (section *Solanum* sensu D'Arcy 1972; see Särkinen et al. 2018) is the largest of these with ca. 52 species and ca. 580 published names and is the only group to occur outside of the Americas. Black nightshades are distinguished by their herbaceous to subshrubby habit, stems that sometimes bear spinescent processes (not true prickles, see Edmonds 1972; Särkinen et al. 2015c), inflorescences usually positioned along the internodes, small flowers and fruits and the usual possession of stone cells in the fruits (Bitter 1911), which appear as small, seed-like structures that are usually white and spherical rather than flattened and brown or yellowish-brown like the seeds. These stone cells are derived from accretions of sclerenchyma in the mesocarp (Bitter 1911, 1914; Danert 1969). Although some studies have been undertaken to clarify the taxonomy of the Old World and North American species of the Morelloid group (Edmonds 1977, 1978; Schilling 1981; Särkinen et al. 2018), a monographic study is needed to aid species identification and to clarify synonymy, especially in Andean South America where most of the species diversity is found (Edmonds 1972; Barboza et al. 2013) and where the Morelloid clade is amongst the most diverse groups of *Solanum*. The weedy nature of many of the common species of the group means that distinct taxa are often identified as common species and collections are often not made because botanists consider these plants uninteresting weeds (see Särkinen et al. 2013a for a typical case).

Recent taxonomic work, focusing on delivering a global monographic treatment of the Morelloid clade, has resulted in the description of various new species from the tropical Andes (Särkinen et al. 2013a, 2015a, 2015b; Särkinen and Knapp 2016). Unlike for many groups of solanums (e.g. Giacomin and Stehmann 2014; Knapp et al. 2015; Gouvêa and Stehmann 2016), Brazil is not a centre of diversity for the Morelloid clade, but in work undertaken revising these species we have encountered specimens from the poorly explored caatingas of north-eastern Brazil that do not correspond to any of the currently recognised species from the region. We describe this taxon here and provide a key to all morelloid species occurring in Brazil and current documentation of our knowledge of specimens of these taxa in Brazil.

Materials and methods

The description of *S. caatingae* is based on examination of herbarium specimens from CE-PEC, HUEFS, RB and W (acronyms follow Index Herbariorum; http://sweetgum.nybg.org/science/ih/). Specimens of this species may be found identified as *S. americanum* in other herbaria, but in our extensive work on the morelloid solanums in European, American and Latin American herbaria (see Särkinen et al. 2018), we have failed to find collections other than those cited here. Specimens (1,241 in total) included in the Suppl. material 1 are those morelloids occurring in Brazil for which we have examined material from 68 herbaria worldwide; many of these are not yet georeferenced but will be as we complete the monographic treatment of this group for South America (e.g. see Särkinen et al. 2018). The specimen data are included here to assist herbarium curators with species identification.

Specimens with coordinates were mapped directly and those lacking coordinates were located using Google Earth and gazetteers. The Extent of Occurrence (EOO) and Area of Occupancy (AOO) were calculated using GeoCat (www.geocat.kew.org) with a 2 km cell width for AOO calculation. The preliminary conservation status was assessed using the IUCN (2017) criteria based on the GeoCat analyses (Bachman et al. 2011) combined with field knowledge. All specimens are cited in the text and full data is provided in the supplemental file and on the NHM Data Portal (https://doi.org/10.5519/0034287).

Taxonomic treatment

Solanum caatingae S.Knapp & Särkinen, sp. nov.
urn:lsid:ipni.org:names:60476868-2
Figure 1

Diagnosis. Like *Solanum americanum* Mill., but differing in its glandular pubescence on all vegetative parts, larger flowers with longer anthers, glabrous adaxial calyx lobe surfaces and spreading to appressed calyx lobes in fruit.

Type. Brazil. Bahia: Mun. Maracajú, Lagoa Itaparica 10 km W of São Inacio-Xique-Xique road at the turning 13.1 km N of São Inacio, 300–400 m alt., 26 Feb 1977, *R.M. Harley [with S.J. Mayo, R.M. Storr & T.S. Santos] 19125* (holotype: RB [RB00464327, acc. # 271981]; isotype: CEPEC [acc. # 19367]).

Description. Perennial herb, 0.4–1 m tall, perhaps occasionally annual or only persisting for a few years. Stems terete or slightly angled, lacking spinescent processes; young stems densely to sparsely pubescent with spreading glandular, simple uniseriate trichomes 0.5–1 mm long, the trichomes 4–15 celled, drying translucent; new growth densely glandular pubescent; bark of older stems greenish-brown or pale tan. Sympodial units unifoliate or difoliate, the leaves not geminate. Leaves simple, shallowly toothed, 2.5–10 cm long, 1–4.5 cm wide, ovate to broadly elliptic, widest in the lower half, membranous; adaxial and abaxial surfaces evenly glandular-pubescent with simple uniseriate trichomes to 2 mm long, these denser abaxially and along the veins, densely pubescent with minute glandular papillae on both leaf surfaces especially in young leaves; principal veins 4–6

Figure 1. Photograph of the holotype of *Solanum caatingae* (*Harley et al. 19125*, RB). Image courtesy of the Jardim Botânico do Rio de Janeiro (JBRJ).

pairs, drying paler than the lamina; base truncate and then abruptly attenuate on to the distal part of the petiole; margins shallowly and irregularly toothed, the teeth ca. 0.5 mm long, rounded at the tips and broadly deltate to semi-circular in outline; apex acuminate, the tip blunt; petiole (0.5) 1–2 cm, only winged from the attenuate leaf base in the distal half to third. Inflorescences internodal, 2–3.5 cm long, subumbelliform with most flowers in the distal portion or spaced ca. 0.5 mm apart, unbranched or furcate, with 5–8 flowers, densely and finely glandular-pubescent like the stems and leaves; peduncle 1.8–3 cm long; pedicels 0.7–0.8 cm long at anthesis, ca. 0.5 mm in diameter at the base, ca. 0.7 mm in diameter at the apex, slender and tapering, densely glandular-pubescent with short uniseriate trichomes and glandular papillae, spreading at anthesis, articulated at the base but the articulation point somewhat swollen and a minute stump that is darker in colour left on the rhachis, this especially visible in fruiting material; pedicels scars closely packed in the distal part of the inflorescence to 0.5 mm apart, with the lowermost ca. 1 mm distant from the rest. Buds globose to broadly ellipsoid, the corolla strongly exserted from the calyx tube before anthesis. Flowers 5-merous, all perfect. Calyx tube 1–1.5 mm long, conical to broadly conical, the lobes 1–1.5 mm long, ca. 1 mm wide, deltate and spathulate, densely glandular-pubescent like the pedicels with uniseriate trichomes and papillae, the tips rounded. Corolla 0.6–0.9 cm in diameter, white with a darker (green?) central star, stellate, lobed 2/3–3/4 of the way to the base, the lobes 2.5–3.5 mm long, 1.5–3 mm wide, triangular, reflexed to spreading at anthesis, the abaxial surfaces glabrous to sparsely papillate with a few glandular trichomes ca. 0.2 mm long. Stamens equal; filament tube minute; free portion of the filaments 0.5–1 mm long, glabrous or sparsely pubescent with a few weak tangled simple uniseriate trichomes adaxially at the very base; anthers 1.8–2.2 mm long, 0.7–1 mm wide, ellipsoid, bright yellow, smooth, poricidal at the tips, the pores elongating to slits with age. Ovary conical, glabrous; style 3.5–4 mm long, sparsely glandular pubescent with weak tangled trichomes and papillae in the basal half where included in the anther cone; stigma minutely capitate, densely papillate, not markedly different from the style. Fruit a globose berry, 0.7–1 cm in diameter, green when young, maturing shiny black; the pericarp thin but not translucent when dry (drying black); fruiting pedicels 0.9–1.2 mm long, tapering from a base ca. 1 mm in diameter to an apex 1–1.2 mm in diameter, not distinctly woody, spreading and becoming deflexed at fruit maturity, remaining on inflorescence; fruiting calyx not accrescent, the tube 1–1.5 mm long, the lobes 2–2.5 mm long, spreading and later reflexed, covering the lower ca. 1/4 of the berry, the abaxial surfaces not densely papillate (different to *S. americanum* where the surfaces are densely papillate). Seeds (30)50–80 per berry, 1–1.5 mm long, 1–1.2 mm wide, tear-drop shaped with a subapical hilum, reddish-gold, the surfaces minutely pitted, the testal cells pentagonal. Stone cells absent. Chromosome number: Not known.

Distribution (Figure 2). *Solanum caatingae* is endemic to Brazil; widely scattered collections are known from the states of Bahia, Ceará, Paraiba and Goiás.

Ecology and habitat. *Solanum caatingae* grows in dry formations known as "caatinga" or "savana estépica" (Eiten 1983; Prado 2003), between 300 and 400 m elevation. The caatinga (from the Tupi language words "caa" forest and "tinga" white) formation is a xerophytic thorn scrub/forest with marked differences in rainfall between wet and dry seasons. The biome occurs within the Caatinga Biogeographic Domain (IBGE 2004) in
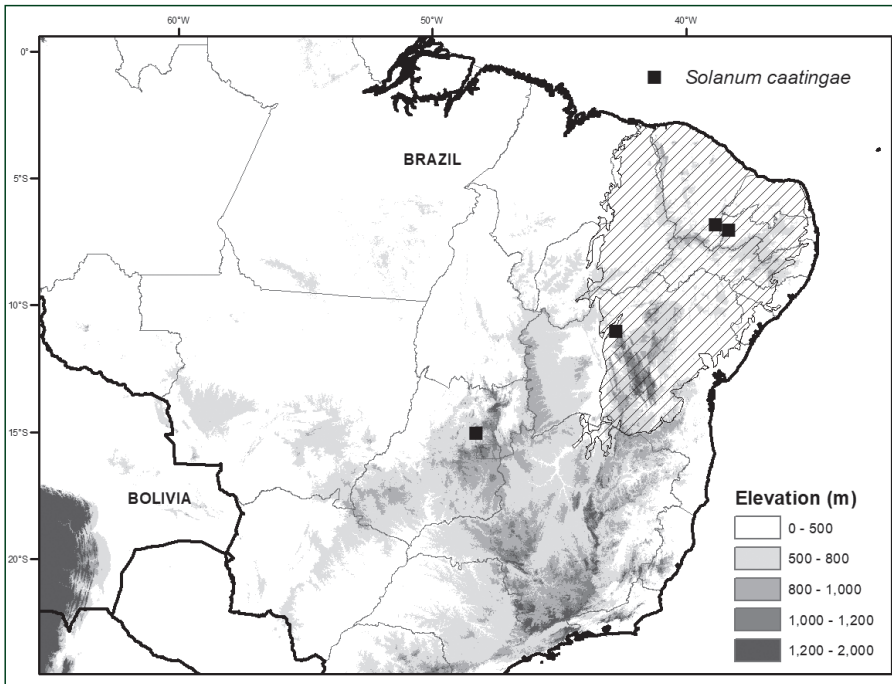
Figure 2. Distribution of *Solanum caatingae*. Hatched area indicates the Caatinga Biogeographic Domain (sensu IBGE 2004).

north-eastern Brazil. The Caatinga Domain is a complex mosaic of many biomes, ranging from the thorn forests of the caatinga proper (see Andrade-Lima 1981) to gallery forest, to humid forests on higher elevations ("brejos de altitude") and cerrado savannas (Andrade-Lima 1981; Lleras 1997). Like many other morelloid species, *S. caatingae* apparently grows in somewhat disturbed and moist areas within the broader more xerophytic habitat and details of its ecological preferences will remain somewhat unclear until more field observations and collections can be made. All specimens seen were in both flower and fruit, so it is likely to flower and fruit all year round or at least when water is available.

Etymology. The species epithet is a genitive noun and comes from the caatinga vegetation formation (e.g. Olson et al. 2000; IBGE 2004) from where most of the collections of this species are known.

Preliminary conservation status (IUCN 2017). DD (Data Deficient; EOO=55,971 km² [LC]; AOO=16 km² [EN]). The paucity of collections of *S. caatingae* means we cannot assign a preliminary conservation status with any certainty. The widely scattered nature of collections, coupled with the extreme threats to caatinga vegetation, mean that the species is possibly of conservation concern, despite its relatively large EOO. More collections may be hiding in the many sheets of the widespread and common *S. americanum* held in Brazilian herbaria and we hope that the description of this taxon will stimulate its future discovery both in the field and in herbaria.

Discussion. *Solanum caatingae* is morphologically most similar to the widespread circumtropical weed *S. americanum*. It differs from it most strikingly in its spread-

ing glandular pubescence of translucent trichomes (versus appressed eglandular pubescence of white trichomes) and longer anthers (ca. 2 mm long versus ca. 1.5 mm long). Several other glandular pubescent species of herbaceous solanums occur in the dry forests of South America, but these are mostly from the Chaco biome and do not overlap in distribution with *S. caatingae* (see Särkinen and Knapp 2016). *Solanum caatingae* can, however, be distinguished from these species (e.g. *S. michaelis* Särkinen & S.Knapp, *S. nitidibaccatum* Bitter, *S. physalifolium* Rusby, *S. sarrachoides* Sendtn., *S. tweedianum* Hook. and *S. woodii* Särkinen & S.Knapp) by its calyx that is not accrescent in fruit with the lobes spreading or slightly reflexed and its shiny black berries with no stone cells. *Solanum arenicola* Särkinen & P.Gonzáles is another glandular pubescent species with which *S. caatingae* could potentially be confused, but that taxon is Amazonian and occurs along rivers in the tropical rainforest; it has not yet been recorded for Brazil, but we expect it to occur in the western part of the country along the border with Bolivia and Peru. Morphologically, *S. arenicola* differs from *S. caatingae* in its larger flowers (8–12 mm in diameter versus 6–9 mm in diameter in *S. caatingae*), longer anthers (3–4 x 0.8–0.9 mm versus 1.8–2.2 x 0.7–1 mm), smaller berry (3.5–7 mm versus 7–10 mm in diameter) and presence of stone cells in the berries.

Several species of European and African polyploid morelloids (e.g. *S. nigrum* L., *S. retroflexum* Dunal, *S. villosum* Mill.) are polymorphic for presence or absence of glandular trichomes and their occurrence does not correlate with relationships based on phenetic studies with molecular markers (Manoko 2007; see Särkinen et al. 2018 for a discussion). In the Americas, however, glandular pubescence is correlated with other characters such as anther length and stone cell presence or absence, suggesting it can be of taxonomic significance.

The type collection *(Harley et al. 19125)* comes from near the edge of the Caatinga Biogeographic Domain as defined by the Instituto Brasileiro de Geografia e Estatística (IBGE 2004) in a highly heterogeneous mosaic of caatinga and cerrado around a seasonal lake (Lagoa Itaparica) with stands of carnaúba palm (*Copernicia prunifera* [Mill.] H.Moore, Arecaceae). The plant itself was found near abandoned houses in a weedy area with *Waltheria rotundifolia* Schrank (Malvaceae), *Sida spinosa* L. (Malvaceae) and *Calotropis procera* (Aiton) W.T.Aiton (Apocynaceae) (R. Harley, in litt., 31 May 2018, extract from field diary dated 26 Feb 1977), suggesting that, like many other morelloid species, *S. caatingae* grows in disturbed sites with at least some moisture, perhaps accounting for its sparse distribution across its range. A duplicate of Harley's collection was not found in the herbarium at Kew. The specimen collected by Johann Pohl at "Rio Maranhao" was collected between 1817 and 1821 and is from a small tributary of the Rio Tocantins slightly to the north of the Distrito Federal. This is at the very southern edge of the Caatinga Domain and is one of the priority areas for both conservation and study (Tabarelli and Cardoso da Silva 2003).

Specimens examined (paratypes). **BRAZIL**. Ceará: Mun. Lavras de Mangabeira, area a ca. 12 km a N do Distrito de Felixardo, 299 m, 24 Jul 2014, *A. Costa-Lima et al. 1406* (HUEFS, RB). Goiás: Rio Maranhão, sin.dat., *J.B.E. Pohl 2393* (W). Paraiba: Mun. Carrapateira, Sitio Volta, nos arredores do Açude Volta, 404 m, 24 Sep 2014, *A. Costa-Lima et al. 1862* (HUEFS, RB).

Key to the Brazilian species of black nightshades (Morelloid clade)

Solanum arenicola is included here although it has not yet been recorded for Brazil; the species is known from adjacent Peru and Bolivia in lowland Amazonian rainforest.

1	Plants with simple glandular pubescence on stems and leaves **2**
–	Plants with simple eglandular trichomes (without glandular tips) on stems and leaves..**4**
2	Calyx markedly accrescent in fruit, covering more than half of the berry; flower buds completely enclosed within the calyx lobes; rare annuals of coastal habitats in southern Brazil (mostly known from Argentina) ....*Solanum sarrachoides* **Sendtn.**
–	Calyx not markedly accrescent in fruit, the lobes spreading and only covering ca. 1/4 of the berry; flower buds strongly exerted from the calyx lobes; perennials (annuals?) of dry areas within the Caatinga Domain in north-eastern Brazil or in moist Amazonian lowland rain forest in western Brazil 3
3	Anthers 1.8–2.2 mm long; calyx lobes deltate-rounded with rounded apices; mature berry shiny black or purplish-black, without stone cells; in dry areas within the Caatinga Domain in north-eastern Brazil.................................. ....*Solanum caatingae* **S.Knapp & Särkinen**
–	Anthers 3–4 mm long; calyx lobes long-triangular with acuminate apices; mature berry matte purple-black, with stone cells; in moist Amazonian lowland rain forest probably in western Brazil (not yet recorded)....................... .... *Solanum arenicola* **Särkinen & P.Gonzáles**
4	Anthers 0.8–2.5 mm long..**5**
–	Anthers (2.5-) 3.5–5.5 mm long...**6**
5	Anthers 0.8–1.5 mm long; fruiting pedicels spreading, not recurved or reflexed; calyx lobes in fruit ca. 1 mm long, strongly reflexed; fruits drop off without pedicels and calyx, leaving behind peduncles with pedicels and calyces still attached; berries shiny black, with 0–4 stone cells; widespread weed . ...*Solanum americanum* **Mill.**
–	Anthers 1.5–2.5 mm long; fruiting pedicels recurved; calyx lobes in fruit 1.5–3.0 mm long, appressed to the berry, not reflexed; pedicels drop off with fruit, leaving peduncles behind; berries matte black, with no stone cells; in littoral from Santa Catarina to Ceará, less commonly inland.................................. ....*Solanum chenopodioides* **Lam.**
6	Leaf bases truncate; leaves broadly delate to ovate; inflorescence unbranched (rarely furcate); calyx lobes in fruit ca. 1 mm long; lowlands in southern Brazil, from inland or along Amazonian rivers .... *Solanum pilcomayense* **Morong**
–	Leaf bases attenuate, often decurrent on the petiole; leaves lanceolate, elliptic or more rarely ovate; inflorescence branched or less commonly unbranched; calyx lobes in fruit >1 mm long; Amazonian or southern Brazil **7**

7	Calyx lobes long triangular with acuminate apices; corolla lobes long and narrow; buds more than 2 times longer than wide, narrowly ellipsoid; plants of lowland Amazonia probably occurring in western Brazil (not yet recorded) .. .. ***Solanum arenicola* Särkinen & P.Gonzáles**
–	Calyx lobes deltate or triangular with acute apices; corolla lobes deltate; buds ca. 2 times longer than wide, ellipsoid; plants of south-eastern Brazil..........**8**
8	Anthers (2.5-) 3.5–4.5 mm long; pedicels tightly to loosely spaced, recurving/reflexed in most specimens and fruiting inflorescences appearing secund; calyx lobes in fruit 1.5–2 mm long, appressed to the berry; corolla with a dark purple eye, lobed nearly to the base, the lobes strongly reflexed at anthesis; berries with 2 small stone cells; 0–1,700 (-2,300) m elevation in southern Brazil ....***Solanum paucidens* Bitter**
–	Anthers 4.4–5.5 mm long; pedicels arising closely together yet regularly spaced 1 mm apart, spreading in fruit; calyx lobes in fruit 1.5–2.5 mm long, slightly spreading; corolla with a yellow eye, lobed to 1/2–2/3 to the base, the lobes spreading at anthesis; berries without stone cells; above 2,000 m elevation in coastal mountains of Minas Gerais, Rio de Janeiro and São Paulo ....***Solanum enantiophyllanthum* Bitter**

Acknowledgements

References

Agra M (2008) Four new species of *Solanum* section *Erythrotrichum* (Solanaceae) from Brazil and Peru, and a key to the species of the section. Systematic Botany 33(3): 556–565. https://doi.org/10.1600/036364408785679897

Andrade-Lima D (1981) The caatingas dominium. Revista Brasileira de Botanica. Brazilian Journal of Botany 4: 149–163.

Bachman S, Moat J, Hill A, de la Torre J, Scott B (2011) Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

Barboza GE, Knapp S, Särkinen T (2013) *Solanum* Grupo VII. Moreloide. In: Barboza GE (Ed.) Flora Argentina: Flora Vascular de la República Argentina, Dicotyledoneae, Solanaceae Vol. 13. Instituto de Botanica Darwinion, San Isidro, Argentina, 231–264.

Bennett JR (2008) Revision of *Solanum* section *Regmandra* (Solanaceae). Edinburgh Journal of Botany 65(01): 69–112. https://doi.org/10.1017/S0960428608004903

Bitter G (1911) Steinzellkonkretionen im Fruchtfleisch beerentragender Solanaceen und deren systematische Bedeutung. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 45: 483–507.

Bitter G (1914) Weitere Untersuchungen über das Vorkommen von Steinzellkonkretionen in Fruchtfleisch beerentragender Solanaceen. Abhandlungen Naturwissenschaften Vereine Bremen 23: 114–163.

Bohs L (2001) Revision of *Solanum* section *Cyphomandropsis* (Solanaceae). Systematic Botany Monographs 61: 1–85. https://doi.org/10.2307/25027891

Bohs L (2005) Major clades in *Solanum* based on *ndhF* sequence data. In: Keating R, Hollowell VC, Croat TB (Eds) A festschrift for William G. D'Arcy – The legacy of a taxonomist. Missouri Botanical Garden Press, St. Louis, 27–49.

Danert S (1969) Über die Entwicklung der Steinzellkonkretionen in der Gattung *Solanum.* Die Kulturpflanze 17(1): 299–311. https://doi.org/10.1007/BF02097952

D'Arcy WG (1972) Solanaceae studies II: Typification of subdivisions of *Solanum.* Annals of the Missouri Botanical Garden 59(2): 262–278. https://doi.org/10.2307/2394758

Edmonds JM (1972) A synopsis of the taxonomy of *Solanum* sect. *Solanum* (Maurella) in South America. Kew Bulletin 27(1): 95–114. https://doi.org/10.2307/4117874

Edmonds JM (1977) Taxonomic studies on *Solanum* section *Solanum* (Maurella). Botanical Journal of the Linnean Society 75(2): 141–178. https://doi.org/10.1111/j.1095-8339.1977.tb01482.x

Edmonds JM (1978) Numerical taxonomic studies on *Solanum* L. section *Solanum* (Maurella). Botanical Journal of the Linnean Society 76(1): 27–51. https://doi.org/10.1111/j.1095-8339.1978.tb01497.x

Eiten G (1983) Classificação da vegetação do Brasil. Editorial CNPq, Brasilia.

Giacomin LL, Stehmann JR (2014) Three new species of *Solanum* (Brevantherum clade) endemic to the Brazilian Atlantic forest. PhytoKeys 38: 69–87. https://doi.org/10.3897/phytokeys.38.7055

Gouvêa YF, Stehmann JR (2016) Two new species of the *Solanum asterophorum* species group (*Solanum* subg. *Leptostemonum*, Solanaceae) from the Brazilian Atlantic Forest. Phytotaxa 288(2): 120–130. https://doi.org/10.11646/phytotaxa.288.2.2

IBGE (2004) Mapa de Biomas do Brasil. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro. http://www.terrabrasilis.org.br/ecotecadigital/images/Mapa%20de%20Biomas%20do%20Brasil%202%20-%20IBGE.pdf

IUCN (2017) Guidelines for using the IUCN Red List Categories and Criteria. Version 13. Prepared by the Standards and Petitions Subcommittee. http://www.iucnredlist.org/documents/RedListGuidelines.pdf [accessed: 1 May 2018]

Jørgensen PM, Ulloa Ulloa C, León B, León-Yánez S, Beck SG, Nee M, Zarucchi JL, Celis M, Bernal R, Gradstein R (2011) Regional patterns of vascular plant diversity and endemism.

In: Herzog SK, Martínez R, Jørgensen PM, Tiessen H (Eds) Climate Change and Biodiversity in the Tropical Andes. Inter-American Institute for Global Change Research (IAI) and Scientific Committee on Problems of the Environment (SCOPE), 192–203.

Knapp S (2002a) *Solanum* section *Geminata* (G. Don) Walpers (Solanaceae). Flora Neotropica 84: 1–405.

Knapp S (2002b) Assessing patterns of plant endemism in Neotropical uplands. Botanical Review 68(1): 22–37. https://doi.org/10.1663/0006-8101(2002)068[0022:APOPEI]2.0.CO;2

Knapp S (2008) A revision of the *Solanum havanense* species group (section *Geminata* (G. Don) Walp. pro parte) and new taxonomic additions to the Geminata clade (*Solanum*: Solanaceae). Annals of the Missouri Botanical Garden 95: 405–458. https://doi.org/10.3417/2006159

Knapp S (2013) A revision of the Dulcamaroid Clade of *Solanum* L. (Solanaceae). PhytoKeys 22(0): 1–432. https://doi.org/10.3897/phytokeys.22.4041

Knapp S, Giacomin LL, Stehmann JR (2015) New species, additions and a key to the Brazilian species of the Geminata clade of *Solanum* L. (Solanaceae) in Brazil. PhytoKeys 47: 1–48. https://doi.org/10.3897/phytokeys.47.9076

Knapp S, Sagona E, Carbonell AKZ, Chiarini F (2017) A revision of the *Solanum elaeagnifolium* clade (Elaeagnifolium clade; subgenus *Leptostemonum*, Solanaceae). PhytoKeys 84: 1–104. https://doi.org/10.3897/phytokeys.84.12695

Lleras E (1997) Interior dry and mesic forest: CPD site SA19. Caatinga of north-eastern Brazil. In: Davis SD, Heywood VH, Herrera-MacBryde O, Villa-Lobos J, Hamilton AC (Eds) Centres of plant diversity. Volume 3: The Americas. WWF and IUCN, Gland, 393–396.

Manoko MLK (2007) A systematic study of African *Solanum* L. section *Solanum* (Solanaceae). PhD Thesis, Radboud University, Nijmegen, The Netherlands. http://dare.ubn.kun.nl/bitstream/2066/30032/1/30032.pdf

Olson D, Dinerstein E, Hedao P, Walters S, Allnutt T, Loucks C, Kura Y, Kassem K, Webster A, Bookbinder M (2000) Terrestrial Ecoregions of the Neotropical Realm (map). Conservation Science Program, WWF-US, Washington DC.

Prado DE (2003) As caatingas da América do Sul. In: Leal IR, Tabarelli M, Cardoso da Silva JM (Eds) Ecologia e conservação da caatinga. Universidade Federal de Pernambuco, Recife, 3–74.

Särkinen TS, Gonzáles P, Knapp S (2013a) Distribution models and species discovery: The story of a new *Solanum* species from the Peruvian Andes. PhytoKeys 16: 1–20. https://doi.org/10.3897/phytokeys.31.6312

Särkinen TS, Bohs L, Olmstead RG, Knapp S (2013b) A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree. BMC Evolutionary Biology 13(1): 214. https://doi.org/10.1186/1471-2148-13-214

Särkinen T, Gonzáles P, Knapp S (2015a) Four new non-spiny *Solanum* (Solanaceae) species from South America. PhytoKeys 44: 39–64. https://doi.org/10.3897/phytokeys.44.8693

Särkinen T, Knapp S, Nee M (2015b) Two new non-spiny *Solanum* species from the Bolivian Andes (Morelloid Clade). PhytoKeys 47: 97–109. https://doi.org/10.3897/phytokeys.47.4423

Särkinen TS, Barboza GE, Knapp S (2015c) True Black nightshades: Phylogeny and delimitation of the Morelloid clade of *Solanum.* Taxon 64(5): 945–958. https://doi.org/10.12705/645.5

Särkinen T, Knapp S (2016) Two new non-spiny *Solanum* (Solanaceae) from the Gran Chao Americano and a key for the herbaceous glandular-pubescent solanums from the region. PhytoKeys 74: 19–33. https://doi.org/10.3897/phytokeys.74.10159

Särkinen T, Poczai P, Barboza GE, van der Weerden GM, Baden M, Knapp S (2018) A revision of the Old World Black Nightshades (Morelloid clade of *Solanum* L., Solanaceae). PhytoKeys.

Schilling EE (1981) Systematics of *Solanum* sect. *Solanum* (Solanaceae) in North America. Systematic Botany 6(2): 172–185. https://doi.org/10.2307/2418547

Sobral M, Stehmann JR (2009) An analysis of new angiosperm species discoveries in Brazil (1990–2006). Taxon 58: 227–232.

Sousa-Baena MS, Garcia LC, Peterson AT (2014) Completeness of digital accessible knowledge of the plants of Brazil and priorities for survey and inventory. Diversity & Distributions 20(4): 369–381. https://doi.org/10.1111/ddi.12136

Stern S, Agra F, Bohs L (2011) Molecular delimitation of clades within New World species of the "spiny solanums" (*Solanum* sugb. *Leptostemonum*). Taxon 60: 1429–1441.

Tabarelli M, Cardoso da Silva JM (2003) Áreas e ações prioritárias par a conservação da biodiversidade da caatinga. In: Leal IR, Tabarelli M, Cardoso da Silva JM (Eds) Ecologia e conservação da caatinga. Universidade Federal de Pernambuco, Recife, 777–196.

Ulloa Ulloa C, Acevedo-Rodriguez P, Beck S, Belgrano MJ, Bernal R, Berry PE, Brako L, Celis M, Davidse G, Forzza RC, Gradstein SR, Hokche O, León B, León-Yanez S, Magill RE, Neill DA, Nee M, Raven PH, Stimmel H, Strong MT, Villaseñor JL, Zarucchi JL, Zuloaga FO, Jørgensen PM (2017) An integrated assessment of the vascular plant species of the Americas. Science 358: 1614–1617. https://doi.org/10.1126/science.aao0398

Weese TL, Bohs L (2007) A three-gene phylogeny of the genus *Solanum* (Solanaceae). Systematic Botany 32(2): 445–463. https://doi.org/10.1600/036364407781179671

Supplementary material 1

Specimen data for species of the Morelloid Clade occurring in Brazil
Authors: Sandra Knapp, Tiina Särkinen
Data type: occurrence
Explanation note: CSV file of specimen and locality data for morelloid species (black nightshades) occurring in Brazil. These data are also deposited as a dataset on the Natural History Museum Data Portal (https://doi.org/10.5519/0034287).

Link: https://doi.org/10.3897/phytokeys.108.27454.suppl1

RESEARCH ARTICLE

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Evidence from checklists for a Holarctic (circumboreal) kingdom of diatoms

Loren Bahls[1]

1 *Montana Diatom Collection, 1032 12th Avenue, Helena, MT 59601 USA*

Corresponding author: *Loren Bahls* (eemahtuskie@gmail.com)

Abstract

Published checklists of freshwater diatoms that represent the American Northwest, Laurentian Great Lakes, Germany and the South Polar Region were compared systematically and the numbers of taxa shared by two or more of these regions were noted. There is a higher level of floristic correspondence between the American Northwest and Germany (71%) and between the American Northwest and the Laurentian Great Lakes (64%) than between the American Northwest and the South Polar Region (45%). These findings support a Holarctic Kingdom of diatoms that is parallel to the Holarctic Kingdom of flowering plants. Mountains and coastal areas and/or inland waters of high salinity may explain why the American Northwest and Germany have more taxa in common than the American Northwest and the Laurentian Great Lakes. Common riverine diatom taxa in the American Northwest are similar to those reported from nationwide monitoring stations. The number of truly cosmopolitan species – those found on all continents – is probably less than 300. The terms "cosmopolitan", "endemic" and "native" are often misused when applied to diatoms and the first two terms always need to be qualified.

Keywords

Introduction

Until the end of the last century, the prevailing view of freshwater diatom biogeography had been one of cosmopolitan distribution, where the majority of species occur worldwide in suitable habitats and endemism is exceptional. This view assumed that

diatoms and other eukaryotic microbes were dispersed globally with few restrictions (Finlay 2002, Finlay et al. 2002). During this era, light microscopy was the primary taxonomic tool and similar taxa were often "lumped" into a single species (e.g., Patrick and Reimer 1966, 1975, Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b).

This era ended with the widespread availability of electron microscopy, which accelerated the description of new diatom species, most of which appear to be endemic. Several lines of evidence have shown that dispersal of viable diatoms is problematic, that endemism is commonplace, and that geologic history and evolution are just as important as environmental factors in explaining the distribution of diatom species (Edlund and Jahn 2001, Kociolek and Spaulding 2000, Potapova and Charles 2002).

Evolutionary studies of diatom lineages with restricted distributions demonstrate that many regions have unique floristic elements (Kociolek and Spaulding 2000). This suggests that diatom distributions follow the generalized tracks of Croizat (Cox and Moore 2005), which result from tectonic history (vicariance) rather than random dispersal. For example, Ehrenberg (1849, 1850) noted that the flora of western North America was more like that of eastern Asia than the flora of eastern North America. On the Pacific Rim of western North America, the Cascade Mountains are rich in endemic diatom species compared to other regions (Sovereign 1958, 1963), especially in the genera *Gomphoneis* Cleve (Kociolek and Stoermer 1986, 1988) and *Navicula* Bory de Saint-Vincent (Bahls 2011, 2014, Bahls and Potapova 2015, Schmidt 1874–1959).

European floras (e.g., Krammer 2002, 2003) are widely used in the United States and contain many taxa that are common to both Europe and North America. They often refer to "Holarctic", "northern-alpine", "circumboreal", or "Northern Hemisphere" distributions. The monograph by Lange-Bertalot (2001) on *Navicula sensu stricto* is an example of diatom taxa shared by the two continents and the usefulness of European floras in North America: of the 128 taxa addressed by Lange-Bertalot, 110 have been identified from the northwestern United States (Bahls 2009). The occurrence of many diatom species in both North America and Europe suggests a biogeographic model for diatoms that conforms to the Holarctic Kingdom of flowering plants (Takhtajan 1986).

Other taxa that were thought to be endemic to North America or Europe are showing up on both continents. For example, *Amphora calumetica* (Thomas *ex* Wolle) Peragallo and *Distrionella incognita* (Reichardt) Williams were assumed to be endemic to the Laurentian Great Lakes and to northern Europe, respectively (Edlund and Stoermer 1999, Reichardt 1988, Krammer and Lange-Bertalot 1991a). However, *A. calumetica* was subsequently found in lakes of northern Europe (Reichardt 2000) and *D. incognita* was reported from the Rocky Mountains of North America (Morales et al. 2005).

Systematic comparisons of large diatom checklists are rarely undertaken because they are tedious, especially when the checklists do not exist in electronic format. In addition, identifications of taxa within checklists may be questionable because they often don't rely on type material or original descriptions. Comparisons are further compli-

cated when different checklists use different names (synonyms) for the same taxon. Nevertheless, diatom checklists contain many hundreds of morphologically distinct taxa that are easily identified and as such they provide a wealth of useful biogeographic information. Four regional diatom checklists from North America, Europe, and the southern hemisphere are compared here to test the Holarctic Kingdom model of diatom biogeography.

Methods

Four published diatom checklists were systematically compared in this study, each representing a distinct geographic region (Table 1): American Northwest (ANW, combined Bahls 2009 + Prescott and Dillard 1979), Laurentian Great Lakes (LGL, Stoermer et al. 1999), Germany (GER, Lange-Bertalot and Steindorf 1996) and south Polar regions (SPR, Kellogg and Kellogg 2002). These checklists are roughly contemporary, contain about the same number of taxa and represent land areas of similar size. The three regions in the Northern Hemisphere span approximately the same temperate latitudes.

The combined American Northwest checklist was also compared to a diatom dataset produced for rivers of the contiguous United States by the US Geological Survey's National Water Quality Assessment (NAWQA) Program (Potapova and Charles 2002), which allowed for a comparison of the most frequently occurring taxa in the two datasets.

Because the combined ANW checklist is newer than the others and contains taxa that were described after the other checklists were published, it had to be adjusted to reflect the taxonomy in place at the time the other checklists were prepared. For comparison with the LGL and GER checklists, diatom names with a publication date of 1997 or later were removed from the ANW checklist (Table 1). For comparison with the SPR checklist, names with a publication date of 2001 or later were removed from the ANW checklist.

Synonyms were counted as a match. For example, if one list contained *Achnanthidium minutissimum* (Kützing) Czarnecki and the other list had *Achnanthes minutissima* Kützing (but not *Achnanthidium minutissimum*), this was counted as one taxon in common to both lists. For genera, old names were brought up-to-date with combinations used in the ANW list. For example, the GER list contained *Navicula minima* Grunow and this same taxon is listed as *Eolimna minima* (Grunow) Lange-Bertalot in the ANW list, hence *Eolimna* was counted as a genus that is common to both lists.

The SPR checklist (Kellogg and Kellogg 2002) contains a number of taxa from marine littoral areas. Strictly marine genera (e.g., *Hyalodiscus*, *Licomorpha*, *Paralia*) and their included taxa were removed from the SPR list prior to comparisons. Marine species in other genera (e.g., *Melosira*, *Tabularia*, *Tropidoneis* [*Plagiotropis*]) that include both marine and freshwater representatives were also removed.

Table 1. Characteristics of diatom floras compared in this paper (NAWQA=National Water Quality Assessment Program; ANSP=Academy of Natural Sciences of Philadelphia).

Flora	References	Latitude range	Total names	Synonyms	Total taxa	Unknowns	Named taxa[1]	Genera[2]
American Northwest (12/31/1996)	Bahls 2009, Prescott and Dillard 1979	38–49°N	1989	549	1440		1440	
American Northwest (12/31/2000)	Bahls 2009, Prescott and Dillard 1979	38–49°N	2125	622	1503		1503	
American Northwest (08/31/2008)	Bahls 2009, Prescott and Dillard 1979	38–49°N	2552	637	1915	334	1581	120
Laurentian Great Lakes (LGL)	Stoermer et al. 1999	40–50°N	2188	716	1472		1472	124
Germany (GER)	Lange-Bertalot and Steindorf 1996	47–55°N			1632		1632	128
South Polar Region (SPR)	Kellogg and Kellogg 2002	35–90°S			1526		1526	105
NAWQA (ANSP)	Potapova and Charles 2002	25–49°N			1548	381	1167	

[1]At the species and infra-species level.

[2]Non-marine genera.

Results

Regional checklists compared

At the sub-genus level, the ANW shares from 676 (SPR) to 1017 (GER) taxa with the three other checklists that were compared in this study (Table 2). Notably, the 1017 taxa shared with Germany represent 71% of the taxa reported from the ANW. Curiously, the ANW shares about 100 more taxa with Germany than it does with the much closer Laurentian Great Lakes region (916 taxa shared).

Although the ANW, LGL, and SPR checklists are nearly equal in size (the SPR list is slightly larger), there were many more matches between ANW and LGL (916) than between ANW and SPR (676). Matches between ANW and LGL represent 64% of the ANW flora, whereas matches between ANW and SPR represent only 45% of the ANW flora. This 45% agreement might seem large given that the ANW and South Polar regions are on nearly opposite ends of the Earth, however the SPR checklist extends to 35°south latitude and includes temperate as well as sub-Antarctic and Antarctic habitats (Kellogg and Kellogg 2002). Also, researchers working on samples from the Southern Hemisphere rely mostly on floras prepared for the Northern Hemisphere and may be inclined to "force" southern specimens into northern taxa.

At the genus level there is much more agreement among checklists (Table 2). When the GER and ANW checklists are compared, the 114 genera they have in common represent 95% of the 120 genera reported from the American Northwest. The 109 genera in common between LGL and ANW represent 91% of the 120 genera reported from ANW and the 106 genera shared by SPR and ANW represent 88% of the ANW genera. Even when all four checklists are compared, the 96 shared genera

represent 80% of the genera present in the ANW checklist. This suggests a lower rate of endemism at the genus level than at the sub-genus level.

When all three checklists from the Northern Hemisphere are compared, there is a considerable decline in the number of shared taxa to 472 or 33% of the taxa in the ANW checklist (Table 2). These may be core or "signature" taxa that define the Holarctic Kingdom of diatoms. When all four checklists are compared, the number of shared taxa declines to 309, which is only 21% of the sub-generic taxa in the ANW checklist. These taxa are widespread in temperate regions of both the Northern and Southern Hemispheres. If comparable checklists of taxa from temperate regions of Africa, Asia and South America were also included, the list of truly cosmopolitan taxa (found on all continents) would be shorter, perhaps much shorter.

ANW flora compared to NAWQA

The NAWQA dataset includes 1167 named taxa and 381 unknowns based on 2735 samples collected at sites scattered across the contiguous United States but concentrated in the eastern part of the country (Potapova and Charles 2002). The combined ANW checklist contains 1581 named taxa plus 334 unknowns based on more than 8,500 samples collected across the ANW, but mostly in Montana (Bahls 2009). All of the NAWQA sites are flowing-water sites, whereas the ANW checklist includes taxa from a wide variety of habitats, including springs, seeps, ephemeral pools, rivers, streams, lakes and wetlands.

The most frequently occurring diatom taxa in the ANW and their corresponding frequency and rank in NAWQA samples are presented in Table 3. Most of these taxa are widespread in temperate regions worldwide and all of them are addressed in the *Freshwater Flora of Middle Europe* (Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b). As would be expected of taxa with wide geographic ranges, the taxa in Table 3 have broad ecological amplitudes. Twenty-four of these same taxa are among the 30 most frequently occurring taxa at NAWQA sampling sites. Differences between the two lists may be expected given the different geographic scopes of the two datasets and additional habitats represented by the ANW list.

Table 2. Comparing the American Northwest flora with other diatom floras in the Northern and Southern Hemispheres. (ANW=American Northwest; LGL=Laurentian Great Lakes; GER=Germany; SPR=South Polar Region).

Floras compared	Number of taxa in common		Shared taxa as a % of ANW taxa	
	Species/varieties/forms	Genera	Species/varieties/forms	Genera
ANW * LGL	916	109	63.6%	90.8%
ANW * GER	1017	114	70.6%	95.0%
ANW * SPR	676	106	45.0%	88.3%
ANW * LGL * GER	472	103	32.8%	85.8%
ANW * LGL * GER * SPR	309	96	20.6%	80.0%

Table 3. The most frequently occurring diatom taxa in the American Northwest (ANW) (Bahls 2009) and corresponding frequency and rank in National Water Quality Assessment (NAWQA) Program samples (Potapova and Charles 2002). NAWQA and ANW ranks are based on percent of samples in which the taxon was encountered.

Rank	Taxon name	ANW % samples	NAWQA % samples	NAWQA rank
1	*Achnanthidium minutissimum* (Kützing) Czarnecki	88.1	59.7	1
2	*Cocconeis placentula* Ehrenberg	79.7	37.8, 26.8[1]	7, 16[1]
3	*Nitzschia dissipata* (Kützing) Grunow	65.2	22.6	21
4	*Navicula cryptotenella* Lange-Bertalot	64.1	39.8	5
5	*Synedra ulna* (Nitzsch) Ehrenberg	60.9	16.7	30
6	*Achnanthes lanceolata* (Brébisson) Grunow	58.7	26.4	17
7	*Fragilaria vaucheriae* (Kützing) Petersen	56.1	20.8	25
8	*Nitzschia palea* (Kützing) W. Smith	53.7	32.2, 17.0[2]	10, 28[2]
9	*Gomphonema parvulum* (Kützing) Kützing	47.4	41.1	3
10	*Amphora pediculus* (Kützing) Grunow	47.3	39.4	6
11	*Encyonema silesiacum* (Bleisch *ex* Rabenhorst) Mann	44.4		
12	*Navicula minima* Grunow	42.8	41.0	4
13	*Nitzschia inconspicua* Grunow	42.5	36.3	8
14	*Reimeria sinuata* (Gregory) Kociolek & Stoermer	42.1	31.0	12
15	*Nitzschia linearis* (Agardh) W. Smith	42.0		
16	*Navicula tripunctata* (O.F. Müller) Bory	39.7	22.5	22
17	*Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot	39.4	29.1	14
18	*Gomphonema minutum* (Agardh) Agardh	38.6		
19	*Fragilaria capucina* Desmazières	38.1		
20	*Navicula reichardtiana* Lange-Bertalot	37.9		
21	*Navicula capitatoradiata* Germain	36.5	27.5	15
22	*Nitzschia fonticola* Grunow	35.8		
24	*Staurosira construens* (Ehrenberg) Williams & Round	35.5		
25	*Cymbella excisa* Kützing (=*C. affinis* sensu Patrick & Reimer)	35.0	16.8	29
26	*Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot	34.7	42.6	2
27	*Cyclotella meneghiniana* Kützing	34.6		
28	*Cocconeis pediculus* Ehrenberg	34.1	18.1	27
29	*Navicula gregaria* Donkin	33.5	29.4	15
30	*Nitzschia paleacea* (Grunow) Grunow	33.5		
31	*Gomphonema olivaceum* (Hornemann) Brébisson	31.7		
32	*Diatoma mesodon* (Ehrenberg) Kützing	29.0		
33	*Epithemia sorex* Kützing	28.9		
34	*Caloneis bacillum* (Grunow) Cleve	28.2		
35	*Melosira varians* Agardh	27.3	24.3	18
36	*Meridion circulare* (Greville) Agardh	26.7		
37	*Navicula veneta* Kützing	25.7		
38	*Hannaea arcus* (Ehrenberg) Patrick	24.4		
39	*Epithemia turgida* (Ehrenberg) Kützing	24.4		
40	*Rhopalodia gibba* (Ehrenberg) O. Müller	23.8		
41	*Nitzschia amphibia* Grunow	23.7	33.2	9

[1]*Cocconeis placentula* var. *euglypta* (37.8%) and *C. placentula* var. *lineata* (26.8%) were counted separately in NAWQA samples.

[2]*Nitzschia palea* var. *palea* (32.2%) and *Nitzschia palea* var. *debilis* (17.0%) were counted separately in NAWQA samples.

Many of the unknown taxa from both the NAWQA and ANW datasets are probably undescribed and new to science. Most of these will likely have limited distributions and be endemic at one scale or another (local, regional, continental or hemispheric).

Discussion

Evidence for a Holarctic Kingdom of diatoms

Two lines of evidence presented here support a Holarctic distribution model for freshwater diatoms: (1) a high level of floristic affinity between the ANW and Germany, with shared sub-generic taxa accounting for 71% of the ANW flora; and (2) a much lower level of floristic affinity between the ANW and the South Polar Region (45% of sub-generic taxa) than between the ANW and the Laurentian Great Lakes (64%) or between the ANW and Germany. The percentages of genera shared by the regions are larger, ranging from a high of 95% (ANW vs. GER) to a low of 88% (ANW vs. SPR).

There are three possible explanations why the ANW shares more taxa with Germany than it does with the Great Lakes region: (1) The German checklist includes 160 more taxa than the LGL checklist and therefore provides more opportunities for matches; (2) Germany and the ANW both have mountains higher than 2,000 m elevation, which allow for more southerly range extensions of northern-alpine taxa (the Laurentian Great Lakes region lacks high mountains); and (3) Germany and the ANW both have marine coasts and many brackish to hyper-saline inland waters, features that are either not present or not as common in the Great Lakes region. Many salt-tolerant taxa that are common to both Germany and the ANW are addressed in a monograph on diatoms of marine coasts (Witkowski et al. 2000).

Potapova and Charles (2002) note that 1016 (70%) of the 1461 non-planktonic diatom taxa in the NAWQA dataset have been recorded on continents other than North America, mainly Europe. In addition, 49 taxa known only from North America and 15 taxa known only from the New World are included in the NAWQA dataset (Potapova and Charles 2002). A similar but undetermined number of North American and New World endemics also occur in the ANW checklist, including many of those described by Sovereign (1958, 1963) and Kociolek and Stoermer (1986, 1988).

Reinforcing the relative dissimilarity of floras from the Northern and Southern Hemispheres are the findings of Kociolek and Spaulding (2000). They compared lists of diatoms from the Arctic and Subarctic Region of North America to lists from the Antarctic that were presented at a workshop on Arctic-Antarctic diatoms (e.g., Håkansson and Jones 1994, Hamilton et al. 1994). The Arctic and Antarctic regions have aquatic habitats with similar physical conditions at comparable latitudes and may be expected to have similar diatom floras under a cosmopolitan model for diatom distributions. However, only 80 (8.9%) of 897 taxa reported from the two regions are common to both regions (Kociolek and Spaulding 2000).

A Holarctic model for the distribution of diatoms is further supported by established Holarctic distributions for flowering plants (Takhtajan 1986). Historically,

dispersal of flowering plants, and probably diatoms, was aided by land bridges that connected Europe with North America (through Greenland) until the late Eocene, and Asia with North America (Bering Land Bridge) off and on until the end of the Pleistocene (Cox and Moore 2005). Finally, all areas in the Holarctic region share a common history of glaciation. Since the end of the Pliocene, several advancing continental glaciers essentially stripped the Holarctic of its preexisting flora and resulted in a "drastic modernization" of the flora across the region (Cox and Moore 2005, Pielou 1991). The Holarctic region thereby became common ground for the establishment of a modern, cold-tolerant flora.

Rappaport's Rule holds that high-latitude organisms have broader geographic ranges than those from the low latitudes (Cox and Moore 2005). The occurrence of broad-range species at higher latitudes could be a consequence of (1) a common tectonic history (Laurasia giving rise to Laurentia and Eurasia); (2) a common history of glaciation in the Northern Hemisphere, leaving only the most adaptable species behind; or (3) greater seasonal fluctuations at higher latitudes, which select for species with broad ecological amplitudes (Cox and Moore 2005), or a combination of these. Whatever the underlying cause or causes, the broad ranges of many diatom taxa in the northern Hemisphere conform to Rappaport's Rule.

Cosmopolitan, endemic, and native diatom species

The term "cosmopolitan" is often used offhandedly to describe freshwater diatom taxa that are widely distributed. Webster's New World Dictionary defines cosmopolitan as "belonging to the whole world, not national or local; at home in all countries or places". The term therefore implies global distribution, that is, the taxon is found on all continents. But the term is often used without evidence that the taxon is present on all continents.

Lange-Bertalot, as reported by Kociolek and Spaulding (2000), estimates that there are 150–200 taxa that "occur across great ecological and geographic space that has been influenced by urbanization". It is true that taxa with broad ecological amplitudes tend to have widespread distributions, but we often don't know if these taxa occur on all continents. Unless we do know this, diatomists should refrain from using the term cosmopolitan and use more precise terminology, e.g., "widespread in the Northern Hemisphere" or "widespread in temperate regions", assuming the writer has the distribution records to support these statements.

Similarly, for clarity, the term endemic should not be used without qualification. Appropriate modifiers for endemic might include local, regional, and continental, e.g., North American endemic. For example, the diatom species *Cymbella janischii* (A. Schmidt) De Toni is endemic to the American Northwest region (Bahls 2007). Endemics can occur at all taxonomic levels, but diatom endemics at the genus level or higher are uncommon. An example is the diatom genus *Gomphocymbella* O. Müller, which is endemic to Africa (Kociolek and Spaulding 2000).

There is also some confusion with and misuse of the term "native" as it applies to diatoms. For example, Potapova and Charles (2004) define native diatom species as

those "generally confined to the Americas; known only from America, or common in America but found extremely rarely elsewhere". What they describe here are actually species that are endemic to the Americas, as well as being native to the Americas. Native means occurring before European settlement or before intercontinental travel and commerce became common and widespread. Documented cases of freshwater diatoms introduced from other continents since European settlement are not common (e.g., Edlund et al. 2000, Spaulding et al. 2010, Reid et al. 2012, Kilroy and Bothwell 2011). If one accepts evolution and vicariance or "historical biogeography" as the prevailing mechanisms that explain diatom distributions (as opposed to unrestricted dispersal and "ecological biogeography"), then one must assume that all taxa are native, unless there is evidence to the contrary of their recent introduction.

Conclusions

1. Freshwater diatoms in the Northern Hemisphere display a Holarctic distribution that conforms to the Holarctic Kingdom of flowering plants; temperate and polar floras in the Northern Hemisphere are distinct from those of the Southern Hemisphere at the sub-generic level.
2. European diatom floras and monographs are useful for identifying at least 70% of the named sub-generic diatom taxa in the northwestern United States.
3. Shared geographic features (mountains, coastal areas, inland brackish waters) may explain why some regions at temperate latitudes in the Northern Hemisphere have higher levels of floristic similarity (ANW and GER) than regions that are much closer together (ANW and LGL).
4. The terms "cosmopolitan", "endemic", and "native" are often misused when applied to diatoms; the first two terms always need to be qualified.
5. The number of truly cosmopolitan diatom taxa (native to all continents) is probably less than 300.

Acknowledgements

References

Bahls LL (2007) *Cymbella janischii* – giant endemic diatom of the Pacific Northwest: Morphology, ecology and distribution compared to *Cymbella mexicana*. Northwest Science 81(4): 284–292. https://doi.org/10.3955/0029-344X-81.4.284

Bahls LL (2009) A checklist of diatoms from inland waters of the northwestern United States. Proceedings of the Academy of Natural Sciences of Philadelphia 158(1): 1–35. https://doi.org/10.1635/053.158.0101

Bahls LL (2011) Three new species of *Navicula* (Bacillariophyta) from Oregon and Montana and a review of diatom endemism in the Northwest. Northwest Science 85(4): 517–526. https://doi.org/10.3955/046.085.0402

Bahls L (2014) New diatoms from the American West – A tribute to citizen science. Proceedings of the Academy of Natural Sciences of Philadelphia 163(1): 61–84. https://doi.org/10.1635/053.163.0109

Bahls LL, Potapova M (2015) Two new species of *Navicula* (Bacillariophyta, Naviculales) from the Cascade Mountains of the American Northwest. Phytotaxa 218(3): 253–267. https://doi.org/10.11646/phytotaxa.218.3.4

Cox CB, Moore PD (2005) Biogeography: an ecological and evolutionary approach (7th edn). Blackwell Publishing, 428 pp.

Edlund MB, Jahn R (2001) Report of a workshop on biogeography and endemism of diatoms. In: Economou-Amilli A (Ed.) Proceedings of the 16th international diatom symposium. AMVROSIOU Press, Athens, 575–587.

Edlund MB, Stoermer EF (1999) Taxonomy and morphology of *Amphora calumetica* (BW Thomas ex Wolle) Peragallo, an epipsammic diatom from post-pleistocene large lakes. In: Mayama S, Idei M, Koizumi I (Eds) Proceedings of the 14th international diatom symposium. Koeltz Scientific Books, 63–74.

Edlund MB, Taylor CM, Schelske CL, Stoermer EF (2000) *Thalassiosira baltica* (Grunow) Ostenfeld (Bacillariophyta), a new exotic species in the Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 57(3): 610–615. https://doi.org/10.1139/f99-284

Ehrenberg CG (1849) Über das mächtigste bis jetzt bekannt gewordene (angeblich 500 Fufs mächtige) Lager von mikroskopischen reinen kieselalgen Süßwasser-Formen am Wasserfall-Flusse in Oregon. Bericht über die zur Bekanntmachung geeingneten Verhandlungen der Königlichen Pruessischen Akademie der Wissenschaften zu Berlin, 76–87.

Ehrenberg CG (1850) On infusorial deposits on the River Chutes in Oregon. American Journal of Science 9: 140.

Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. Science 296(5570): 1061–1063. https://doi.org/10.1126/science.1070710 PubMed

Finlay BJ, Monaghan EB, Maberly SC (2002) Hypothesis: The rate and scale of dispersal of freshwater diatom species is a function of their global abundance. Protist 153(3): 261–273. https://doi.org/10.1078/1434-4610-00103 PubMed

Håkansson H, Jones VJ (1994) The compiled freshwater diatom taxa list for the maritime Antarctic region of the South Shetland and South Orkney Islands. In: Hamilton PB (Ed.) Proceedings of the Fourth Arctic-Antarctic Diatom Symposium. Canadian Museum of Nature Technical Report of Fisheries and Aquatic Sciences 1957, 77–83.

Hamilton PB, Douglas MSV, Fritz SC, Pienitz R, Smol JP, Wolfe AP (1994) A compiled freshwater diatom taxa list for the arctic and subarctic regions of North America. In: Hamilton PB (Ed.) Proceedings of the Fourth Arctic-Antarctic Diatom Symposium. Canadian Museum of Nature Technical Report of Fisheries and Aquatic Sciences 1957: 85–102.

Kellogg TB, Kellogg DE (2002) Non-marine and littoral diatoms from Antarctic and subarctic regions. In: Witkowski A (Ed.) Diatom monographs, Volume 1. ARG Gantner, 1–795.

Kilroy C, Bothwell, M (2011) Environmental control of stalk length in the bloom-forming, freshwater benthic diatom *Didymosphenia geminata* (Bacillariophyceae). Journal of Phycology 47: 981–989.

Kociolek JP, Spaulding SA (2000) Freshwater diatom biogeography. Nova Hedwigia 71: 223–241.

Kociolek JP, Stoermer EF (1986) Observations on North American *Gomphoneis* (Bacillariophyceae). II. Descriptions and ultrastructure of two new species. Transactions of the American Microscopical Society 105(2): 141–151. https://doi.org/10.2307/3226386

Kociolek JP, Stoermer EF (1988) Taxonomy, ultrastructure and distribution of *Gomphoneis herculeana*, *G. eriense* and closely related species (Naviculales: Gomphonemataceae). Proceedings of the Academy of Natural Sciences of Philadelphia 140(2): 24–97.

Krammer K (2002) *Cymbella*. In: Lange-Bertalot H (Ed.) Diatoms of Europe, Volume 3. ARG Gantner, 1–584.

Krammer K (2003) *Cymbopleura*, *Delicata*, *Navicymbula*, *Gomphocymbellopsis*, *Afrocymbella*. In: Lange-Bertalot H (Ed.) Diatoms of Europe, Volume 4. ARG Gantner, 1–530.

Krammer K, Lange-Bertalot H (1986) Bacillariophyceae. 1. Teil: Naviculaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (Eds) Süßwasserflora von Mitteleuropa 2/1. Gustav Fischer, 1–876.

Krammer K, Lange-Bertalot H (1988) Bacillariophyceae. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (Eds) Süßwasserflora von Mitteleuropa 2/2. Gustav Fischer, 1–596.

Krammer K, Lange-Bertalot H (1991a) Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D (Eds) Süßwasserflora von Mitteleuropa 2/3. Gustav Fischer, 1–576.

Krammer K, Lange-Bertalot H (1991b) Bacillariophyceae. 4. Teil: Achnanthaceae. In: Ettl H, Gärtner G, Gerloff J, Heynig H, Mollenhauer D (Eds) Süßwasserflora von Mitteleuropa 2/4. Gustav Fischer, 1–437.

Lange-Bertalot H (2001) *Navicula* sensu stricto, 10 genera separated from *Navicula* sensu lato, *Frustulia*. In: Lange-Bertalot H (Ed.) Diatoms of Europe, Volume 2. ARG Gantner, 1–526.

Lange-Bertalot H, Steindorf A (1996) Rote liste der limnischen kieselalgen (Bacillariophyceae) Deutschlands. Schrittenreihe fur Vegetationskunde 28: 633–677.

Morales EA, Bahls LL, Cody WR (2005) Morphological studies of *Distrionella incognita* (Reichardt) Williams (Bacillariophyceae) from North America with comments on the taxonomy of *Distrionella* Williams. Diatom Research 20(1): 115–135. https://doi.org/10.1080/0269249X.2005.9705622

Patrick R, Reimer CW (1966) The diatoms of the United States, Volume 1. Monograph 13, The Academy of Natural Sciences of Philadelphia, 1–688.

Patrick R, Reimer CW (1975) The diatoms of the United States. Volume 2, Part 1. Monograph 13, The Academy of Natural Sciences of Philadelphia, 213 pp.

Pielou EC (1991) After the ice age: the return of life to glaciated North America. The University of Chicago Press, 366 pp.

Potapova MG, Charles DF (2002) Benthic diatoms in USA rivers: Distributions along spatial and environmental gradients. Journal of Biogeography 29(2): 167–187. https://doi.org/10.1046/j.1365-2699.2002.00668.x

Potapova MG, Charles DF (2004) Potential use of rare diatoms as environmental indicators in USA rivers. In: Poulin M (Ed.) Proceedings of the 17th international diatom symposium. Biopress Limited, 281–295.

Prescott GW, Dillard GE (1979) A checklist of algal species reported from Montana, 1891–1977. Montana Academy of Sciences, Monograph 1, 102 pp.

Reichardt E (1988) Neue Diatomeen aus Bayerischen und Nordtiroler Alpenseen. Diatom Research 3(2): 237–244. https://doi.org/10.1080/0269249X.1988.9705036

Reichardt E (2000) Das vorkommen von *Amphora calumetica* (BW Thomas *ex* Wolle) Peragallo in Europa. Diatom Research 15(1): 191–193. https://doi.org/10.1080/026924 9X.2000.9705492

Reid BL, Hernández KL, Frangópulos M, Bauer G, Lorca M, Kilroy C, Spaulding SA (2012) The invasion of the freshwater diatom *Didymosphenia geminata* in Patagonia: prospects, strategies, and implications for biosecurity of invasive microorganisms in continental waters. Conservation Letters 5(6): 432–440.

Schmidt A (1874–1959) Atlas der Diatomaceen-Kunde, von Adolf Schmidt, continued by Martin Schmidt, Friedrich Fricke, Heinrich Heiden, Otto Muller, Friedrich Hustedt. Koeltz Scientific Books, Königstein.

Sovereign HE (1958) The diatoms of Crater Lake, Oregon. Transactions of the American Microscopical Society 77(2): 96–134. https://doi.org/10.2307/3224112

Sovereign HE (1963) New and rare diatoms from Oregon and Washington. Proceedings of the California Academy of Sciences 31: 349–368. [fourth series]

Spaulding SA, Kilroy C, Edlund MB (2010) Diatoms as non-native species. In: Smol JP, Stoermer EF (Eds) The diatoms: applications for the environmental and earth sciences, Second Edition. Cambridge University Press, 560–569.

Stoermer EF, Kreis Jr RG, Andresen NA (1999) Checklist of diatoms from the Laurentian Great Lakes. II. Journal of Great Lakes Research 25(3): 515–566. https://doi.org/10.1016/S0380-1330(99)70759-8

Takhtajan A (1986) Floristic Regions of the World. University of California Press, 522 pp.

Witkowski A, Lange-Bertalot H, Metzeltin D (2000) Diatom flora of marine coasts I. In: Lange-Bertalot H (Ed.) Iconographia Diatomologica, Volume 7. ARG. Gantner, 1–925.

RESEARCH ARTICLE

Total evidence phylogeny of Pontederiaceae (Commelinales) sheds light on the necessity of its recircumscription and synopsis of *Pontedera* L.

Marco O. O. Pellegrini[1], Charles N. Horn[2], Rafael F. Almeida[3]

1 *Universidade de São Paulo, Departamento de Botânica, Rua do Matão 277, CEP 05508-900, São Paulo, SP, Brazil* **2** *Newberry College, Department of Sciences and Mathematics, 2100 College Street, Newberry, SC 29108, USA* **3** *Universidade Federal de Minas Gerais, Programa de Pós-Graduação em Biologia Vegetal, Avenida Antonio Carlos 6627, CEP 31270-901, Belo Horizonte, MG, Brazil*

Corresponding author: *Marco O. O. Pellegrini* (marcooctavio.pellegrini@gmail.com)

Abstract

A total evidence phylogeny for Pontederiaceae is herein presented based on new morphological and previously published molecular data. Our results led us to re-circumscribe *Pontedera* to include *Monochoria*, *Pontedera s.s.* and the polyphyletic *Eichhornia*. We provide the needed ten new combinations and 16 typifications, arrange a total of 25 accepted species (six representing re-established names) in 5 new subgenera. Furthermore, we provide an identification key for the two genera accepted by us in Pontederiaceae, an identification key to the subgenera, identification keys to the species of each subgenus and commentaries on *Pontedera s.l.*, as well as for each subgenus and each species.

Keywords

Introduction

Pontederiaceae is a small aquatic monocot family, placed in Commelinales as sister to Haemodoraceae, with both families being sister to Philydraceae (Saarela et al. 2008). This clade can be morphologically characterised by its: distichously-alternate and unifacial leaves, with xylem and phloem alternate (or rarely phloem circular with central

xylem); the presence of styloid crystals; perianth whorls partially to completely connate forming a hypanthium, perianth petaloid, flowers bisexual, zygomorphic and enantiostylous; pollen shed with raphides; the presence of placental sclereid idioblasts; and seeds longer than wide with longitudinal wings or striations (Simpson 1990; Prychid et al. 2003; Simpson and Burton 2006; Pellegrini, unpublished data). Furthermore, the relationship between Pontederiaceae and Haemodoraceae is morphologically supported by their endothecium with a basal thickening, non-columellate-tectate exine and the presence of septal nectaries (Simpson 1987, 1990). Pontederiaceae can be easily distinguished from the remaining families of Commelinales by its roots not sandbinding; dimorphic, late bifacial and ligulate leaves, ptyxis involute enclosing the petiole of the preceding leaf; xylem and phloem alternate near the centre of the blades, plus xylem abaxial and phloem adaxial near the margins; bisulcate pollen grains; and the presence of an anthocarp (Arber 1925; Simpson 1987, 1990; this study). The family is currently arranged in four genera (i.e. *Eichhornia* Kunth, *Heteranthera* Ruiz & Pavón, *Monochoria* C.Presl and *Pontederia* L.) and possesses ca. 45 species (Lowden 1973; Horn 1985; Cook 1989; Pellegrini 2017a; Pellegrini and Horn 2017). Pontederiaceae has a pantropical distribution, with the Neotropical region as its diversity centre, where ca. 70% of its species can be found (Barrett 2004; Pellegrini and Horn 2017). Furthermore, Brazil retains most of the diversity for the group, with 24 species known to occur in all kinds of aquatic and damp environments (BFG 2015; Pellegrini and Horn 2017). Despite being unquestionably monophyletic (Eckenwalder and Barrett 1986; Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011), generic boundaries in Pontederiaceae are still in great need of revision (Ness et al. 2011; Pellegrini 2017a). A total of 30 genera have been described and assigned to Pontederiaceae throughout the years (eMonocot 2010; Govaerts 2018; Tropicos.org 2018) and some authors have accepted up to nine genera in the family (e.g. Cook 1998). All phylogenetic studies invariably recover most genera as non-monophyletic, with *Eichhornia* and *Heteranthera* being the most problematic groups (Eckenwalder and Barrett 1986; Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011). Based on these published phylogenies, it is clear that these genera have been circumscribed based either on autapomorphic or homoplastic characters. Thus, traditionally proposed generic boundaries need to be urgently revisited.

Recently, *Heteranthera* was recircumscribed to include *Hydrothrix* Hook.f. and *Scholleropsis* H.Perrier, thus being finally rendered monophyletic (Pellegrini 2017a). Nonetheless, the *Pontederia* clade (i.e. *Eichhornia s.l.*, *Monochoria* and *Pontederia*) remains neglected (Pellegrini 2017a), with the hopelessly polyphyletic *Eichhornia* being recovered as three distinct lineages within it (Eckenwalder and Barrett 1986; Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011). The first *Eichhornia* lineage is composed by the erect-emergent, non-clonal species, with perianth spirally-coiled at post-anthesis. The second lineage is composed exclusively by *E. crassipes* (Mart.) Solms, which is characterised by its free-floating and stoloniferous rosette, flabellate ligules and its peculiarly inflated

petioles. The last *Eichhornia* lineage is composed by procumbent-emergent species, with distichously-alternate leaves evenly distributed along the stems, infundibuliform perianth and glabrous styles (Pellegrini and Horn, pers. observ.).

According to Pellegrini (2017a), there are two approaches for solving the generic limits in the *Pontederia* clade: (1) sink *Eichhornia* and *Monochoria* into a broader, but morphologically cohesive *Pontederia*; or (2) split *Eichhornia* into three ill-defined genera, in order to maintain *Pontederia* and *Monochoria* as independent genera. The first option is considerably more taxonomically stable and would greatly facilitate the identification of Pontederiaceae specimens, especially for the non-specialists, ecologists, plant growers, farmers etc.

Here, we present a total evidence phylogeny for Pontederiaceae, based on plastid and morphological data, in order to recircumscribe *Pontederia* to include *Eichhornia* and *Monochoria* and provide an identification key to the genera in Pontederiaceae. We also present a synopsis for *Pontederia s.l.*, with an updated description for the genus, propose five new subgenera, provide an identification key to the accepted subgenera of *Pontederia* and provide identification keys to the species of each subgenus. Finally, we propose the needed 10 new combinations, present six new synonyms and accept a total of 25 species, five of these representing reestablished names. The present study concludes the bi-generic classification of Pontederiaceae initiated by Pellegrini (2017a) and is a result of the first author's ongoing systematic studies on Commelinales.

Methods

Taxonomy

Specimens from the following herbaria were analysed: AAU, ALCB, B, BA, BAF, BHCB, BHZB, BLH, BM, BOL, BOTU, BR, BRIT, C, CAS, CEPEC, CESJ, COL, CORD, CTES, CVRD, DS, E, EA, ESA, F, FCAB, FLOR, FURB, G, GH, GMUF, GOET, GUA, HAL, HAMAB, HAS, HB, HBR, HERBAM, HNMN, HRB, HRCB, HSTM, HUEFS, HUFSJ, HURB, IAC, IBE, ICN, INPA, IPA, K, KANU, L, LE, LG, LIL, LL, LP, M, MA, MBM, MBML, MG, MO, MVM, MY, NBYC, NY, OS, P, PH, PMSP, PR, PRC, PRE, R, RB, RFA, RFFP, S, SMU, SP, SPF, SRGH, TEX, UEC, UMO, UNA, UPCB, US, USF, VDB, VIC, W and WAG (herbaria acronyms according to Thiers, cont. updated). Fresh specimens, field notes, photographs and specimens for cultivation were gathered by the authors during several field trips across North, Central and South America, between 1980 and 2017. The indumentum and shape terminology follow Radford et al. (1974); the inflorescence terminology and morphology follow Weberling (1965, 1989) and Panigo et al. (2011), as implemented by Pellegrini and Horn (2017); fruit terminology follows Spjut (1994); and seed terminology follows Faden (1991). Species distribution is based on literature, herbarium specimens and fieldwork data.

Morphological character selection, coding, mapping and morphological analysis

Characters were scored mainly from living specimens in the field and specimens in cultivation and later complemented by spirit and herbarium samples from the aforementioned herbaria. When no living or herborised specimens were available for examination, information was taken from published literature. We have studied at least five specimens for each taxon, with the most representative specimen chosen as the voucher for the morphological matrix (Table 1). Some characters were chosen based on previous studies (i.e. Eckenwalder and Barrett 1986; Simpson 1987; Barrett and Graham 1997; Simpson and Burton 2006), with most characters being scored for the present study. Character coding followed the recommendations of Sereno (2007) for morphological phylogenies. Primary homology hypotheses (De Pinna 1991) were proposed for root, stem, leaf, inflorescence architecture, floral, fruit, seed, palynological and anatomical characters. A total of 96 discrete micro- and macromorphological characters were scored, being treated as unordered and equally weighted (Suppl. material 1).

Data were entered into a matrix of characters per taxa using the software Mesquite 3.20 (Maddison and Maddison 2017; Suppl. material 2). All characters were treated as unweighted and unordered. Maximum Parsimony (MP) analysis was performed using PAUP* 4 (Swofford 2003), with a heuristic search with 1000 random taxon additions and tree bisection-reconnection (TBR) branch swapping. Consistency index (CI) and retention index (RI) were used to assess the degree of homoplasy in the dataset and ACCTRAN (accelerated transformation optimisation; Swofford and Maddison 1987) was used for character optimisation. Statistical support for each branch of the cladogram was evaluated with Bootstrap Support (BS) analyses with 1000 random addition replication. The search parameters used to estimate the bootstrap values were the same as the initial heuristic search. The Bremer Index (BI) was also used to evaluate clade reliability based on the presence of secondary homologies (Bremer 1994). The Bremer Index was calculated by increasing the number of the optimal tree steps until all clades collapsed. Mesquite 3.20 was used to reconstruct the ancestral character states, while WinClada ver. 1.0000 (Nixon 2002) was used to trace the synapomorphic characters on the strict consensus tree.

Taxon sampling, alignment and phylogenetic analysis

Sequences of the genes *ndhF* and *rbcL* were retrieved from GenBank for 26 taxa representing all currently accepted genera in Pontederiaceae, including outgroups *Anigozanthos* Labill. and *Xiphidium* Aubl. (Haemodoraceae) and the tree was rooted with Philydraceae. All sequences were aligned using Muscle (Edgar 2004) implemented on Geneious software (Kearse et al. 2012), with subsequent adjustments in the preliminary matrices made by eye.

Combined analyses of the plastid regions and plastid+morphology datasets were performed. Prior to combining our data, we performed the incongruence length difference

Table 1. Voucher specimens used in the morphological and combined phylogenetic analyses, and Genbank accession numbers for all DNA regions sampled in this study. *Type species of the genus.

Family	Species	Collector & no.	Herbarium acronym	ndhF	rbcL
Philydraceae	*Helmholtzia acorifolia F.Muell.	Mueller 1876	K	EF422989.1	AF206774.1
Philydraceae	*Philydrum lanuginosum Banks & Sol. ex Gaertn.	Banks & Solander s.n.	BM barcode BM000990702	U41622.2	U41596.2
Haemodoraceae	Anigozanthos flavidus DC.	Brown s.n.	K barcode K000846259	EF422987.1	EF422992.1
Haemodoraceae	*Xiphidium caeruleum Aubl.	Perdiz 2376	RB	AF547013.1	AY149359.1
Pontederiaceae	Monochoria cyanea (F.Muell.) F.Muell.	Leichhardt s.n.	K barcode K000873493	U41613.1	U41588.1
Pontederiaceae	Monochoria korsakovii Regel & Maack	Maack s.n.	K barcode K000873544	U41615.2	U41590.1
Pontederiaceae	*Monochoria hastata (L.) Solms	Hermann s.n.	BM barcode BM000621681	U41614.1	U41589.1
Pontederiaceae	Monochoria vaginalis Burm.f.	Boea 8471	US	U41616.1	KX527476.1
Pontederiaceae	Eichhornia crassipes (Mart.) Solms	Martius 60	M	FJ861142.1/U41599.2	FJ861142.1/EF422991.1
Pontederiaceae	Eichhornia diversifolia (Vahl) Urb.	Harley 10248	RB	U41600.1	U41575.1
Pontederiaceae	*Eichhornia azurea (Sw.) Kunth	Marrinelli 18669	RB	U41598.1	U41573.1
Pontederiaceae	Eichhornia heterosperma Alexander	Smith 2290	NY	U41601.1	U41576.1
Pontederiaceae	Eichhornia paniculata (Spreng.) Solms	Machado 574	RB	U41603.1	U41578.1
Pontederiaceae	Eichhornia paradoxa (Mart.) Solms	Harley 21401	K	U41607.1	U41579.1
Pontederiaceae	*Pontederia cordata L.	Barton s.n.	PH barcode PH00038346	U41617.1	U41592.1
Pontederiaceae	Pontederia lancifolia Muhl.	Muhlenberg 242	PH	U41618.1	U41593.1
Pontederiaceae	Pontederia rotundifolia L.f.	Alvarenga 952	RB	U41620.1	U41595.1
Pontederiaceae	Pontederia ovalis Mart.	Pellegrini 474	RB	U41619.1	U41594.1
Pontederiaceae	Pontederia sagittata C.Presl	Catharino 342	RB	U41621.1	U41597.1
Pontederiaceae	Heteranthera gardneri (Hook.f.) M.Pell.	Gardner 1863	K	U41606.2	U41582.1
Pontederiaceae	Heteranthera rotundifolia (Kunth) Griseb.	Walter 6644	RB	U41610.1	U41585.1
Pontederiaceae	Heteranthera limosa (Sw.) Willd.	Assunção 721	RB	U41608.2	U41583.1
Pontederiaceae	Heteranthera zosterifolia Mart.	Fontana 8316	RB	U41612.1	U41587.1
Pontederiaceae	Heteranthera seubertiana Solms	Gardner 1864	BM	U41611.1	U41586.1
Pontederiaceae	Heteranthera oblongifolia Mart. ex Schult. & Schult.f.	Araújo 38	RB	U41609.1	U41584.1

(ILD) test (Farris et al. 1994) to investigate the incongruence between DNA data sets. Analyses, using maximum parsimony (MP) on both matrices, were conducted with PAUP* 4 (Swofford 2003). A heuristic search was performed using TBR swapping (tree-bisection reconnection) and 1000 random taxon-addition sequence replicates with TBR swapping limited to 15 trees per replicate in order to prevent extensive searches (swapping) in suboptimal islands, followed by TBR in the resulting trees with a limit of 1000 trees. In all analyses, the characters were equally weighted and unordered (Fitch 1971). Relative support for individual nodes was assessed using non-parametric bootstrapping (Felsenstein 1985), with 1000 bootstrap pseudo-replicates, TBR swapping, simple taxon addition and a limit of 15 trees per replicate.

For the DNA partitions of the model-based approach, we selected the model using hierarchical likelihood ratio tests (HLRT) on J Modeltest 2 (Darriba et al. 2012. For the morphological partition, the standard discrete Markov model (Mkv) was used, following Lewis (2001) with rates set to equal. A Bayesian analysis (BA) was conducted with mixed models and unlinked parameters, using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The Markov Chain Monte Carlo (MCMC) was performed using two simultaneous independent runs with four chains each (one cold and three heated), saving one tree every 1000 generations, for a total of ten million generations. We excluded as 'burn in' trees from the first two million generations and tree distributions were checked for a stationary phase of likelihood. The posterior probabilities (PP) of clades were based on the majority-rule consensus, using the remaining trees, calculated with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003).

Results

Morphological analysis

The cladistic analysis retrieved 228 equally parsimonious trees with 209 steps, Consistency Index (CI) of 0.5913, Homoplasy Index (HI) of 0.4087, Retention Index (RI) of 0.8618 and Rescaled Consistency Index (RC) of 0.5096. All 96 coded characters were parsimony-informative. The strict consensus (Fig. 1) and the majority-rule trees (Fig. 2) are presented and discussed below.

The Haemodoraceae+Pontederiaceae clade is supported by seven characters: the presence of septal nectaries (Character 44), perianth 6-lobed (Character 58, plesiomorphic), perianth with 3+3 arrangement (Character 59, plesiomorphic), epipetalous stamens (Character 66, homoplastic), stamens dimorphic (Character 69), endothecium with a basal thickening (Character 72) and non-tectate-columellate exine (Character 76). Pontederiaceae is recovered as monophyletic with high statistical support (BS=100; BI=7; Fig 2), being supported by: dimorphic leaves (Character 12), leaf-blades late bifacial (Character 13), involute ptyxis where the blade of the new leaf encloses the petiole of the preceding leaf (Character 14), leaf-blades with xylem and phloem alternate in the central portion of the blade and xylem abaxial and phloem

Figure 1. Strict consensus tree (length=209 steps; CI=0.5913; RI=0.8618) recovered by the morphological dataset, showing the character state optimisations at each node of the cladogram, represented by circles. In each circle, the numbers above and below represent the character and character state numbers, respectively (as presented in Suppl. material 1).

adaxial at the margins (Character 15), the presence of a ligule (Character 16), non-equitant leaves (Character 18, reversion), sessile leaves early-deciduous (Character 18), inflorescence deflexed at post-anthesis and in fruit (Character 37), sessile flowers (Character 39), absence of fibrillar tannin cells in the perianth (Character 47), presence of aerenchymatous tissue in the receptacle (Character 48) and in the perianth (Character 49), perianth connate producing a conspicuous tube (Character 56, homoplastic), perianth ranging from lilac to purple or blue (Character 57, homoplastic), posterior lobe(s) with a nectar guide (Character 63, homoplastic), pollen grains bisulcate (Character 75), presence of aerenchymatous tissue in the ovary walls (Character 79) and the presence of an anthocarp (Character 91).

Heranthera sensu Pellegrini (2017a), is recovered as monophyletic with high statistical support (BS=99; BI=3; Fig. 2). It is supported by: plants mostly to completely submersed (Character 3, homoplastic), indefinite base (Character 4), water-binding/
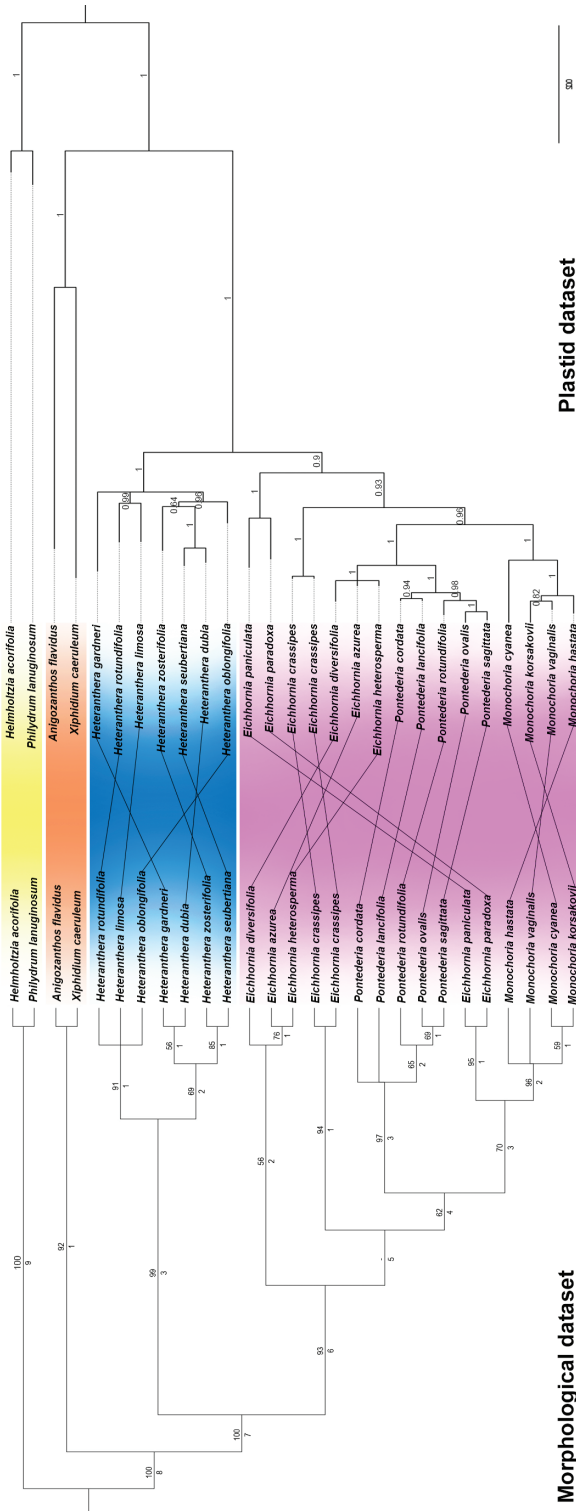
Figure 2. Majority-rule tree recovered for the morphological and plastid datasets. Morphology: bootstrap support values are depicted over the branches, while Bremer Index support values are depicted under the branches. Plastid: posterior probability values are depicted over the branches. Yellow: Philydraceae. Orange: Haemodoraceae. Blue: *Heteranthera s.l.* Pink: *Pontederia s.l.*

mucilaginous roots (Character 6), rhizome absent (Character 7), stems freely branching and elongated (Character 9 and 10, homoplastic), ligules 2–several parted (Character 17), spirally-alternate sessile leaves (Character 18), sessile leaves evenly distributed along the stem (Character 20, homoplastic), basal bract conduplicate (Character 30), main florescence reduced to a solitary cincinnus (Character 32), sparse aerenchymatous tissue in the perianth (Character 49), perianth tubular (Character 50), filaments obliquely inserted (Character 65) and unevenly trilobate stigma (Character 87). Within *Heteranthera s.l.*, we recover two main clades in the majority rule (Fig. 2), with only one of these being also recovered in the strict consensus (Fig. 1). The *H. limosa* group is composed by *H. limosa* (Sw.) Willd., *H. oblongifolia* Mart. *ex* Schult. & Schult.f. and *H. rotundifolia* (Kunth) Griseb., being characterised by: the absence of clonal reproduction (Character 2, homoplastic), sessile leaves late-deciduous (Character 19, homoplastic), petiolate leaves with elliptic to ovate blades (Character 27, homoplastic), the posterior perianth lobe with flanged base (Character 62) and a nectar guide consisting of a sole spot or dark band (Character 63, homoplastic), sigmoid filaments (Character 67), ovary hemiseptalous (Character 80, homoplastic), axile-parietal placentation (Character 83) and placentation 2-flanged (Character 84, homoplastic). The second clade, named by us as the *H. dubia* group, is composed of *H. dubia* (Jacq.) MacMill., *H. gardneri* (Hook.f.) M.Pell., *H. seubertiana* Solms and *H. zosterifolia* Mart. This group is characterised by: the presence of cleistogamous flowers (Character 43), inflated filaments (Character 68), gynoecium 1-locular (Character 77, homoplastic), ovary aposeptalous (Character 80, homoplastic), intrusive-parietal placentation (Character 83, homoplastic) and placentation slightly 2-flanged (Character 84).

Pontederia s.l. is also recovered as monophyletic with high statistical support (BS=93; BI=6; Fig. 2), being supported by: distichously-alternate sessile leaves (Character 18), petiolate leaves pulvinate (Character 25), tristylous flowers (Character 42), dense aerenchymatous tissue in the perianth (Character 49), perianth campanulate or infundibuliform or hypocrateriform (Character 50, homoplastic), perianth coiled and tightly enclosing the fruit at post-anthesis (Characters 53 and 55), perianth lobes equal in shape in the same whorl (Character 60) and with obtuse apex (Character 61, homoplastic), stamens 6 (Character 64, reversion), filaments J-shaped or recurved-decurved (Character 67), anthers dorsifixed (Character 71), style J-shaped (Character 85), stigmas evenly trilobate to trifid or capitate (Characters 87), stigma wet (Characters 88), anthocarp tightly enveloping the fruit (Character 92) and anthocarp hardened and ornamented (Characters 93 and 94). *Pontederia s.l.* is recovered by us arranged in five clades in the strict consensus (Fig. 1) and in the majority rule (Fig. 2). The *E. paniculata* group is highly supported (BS=95; BI=1; Fig. 2), being composed by *E. paniculata* (Spreng.) Solms and *E. paradoxa* (Mart. *ex* Schult. & Schult.f.) Solms. It is characterised by: its annual life cycle (Character 1, homoplastic), the lack of clonal reproduction (Character 2, homoplastic), inflated sheath of the leaf subtending the inflorescence (Character 29, homoplastic), flat basal bract (Character 30, homoplastic) with a caudate apex (Character 31, homoplastic), main florescence with a fistulose main axis (Character 34, homoplastic), inflorescence erect at post-anthesis and in fruit

(Character 37, reversion), floral organs lacking tannin cells of the homogeneous type (Character 45), perianth with a moderate amount of granular tannin cells (Characters 51 and 52), perianth spirally-coiled at post-anthesis (Character 54, homoplastic), ovary walls lacking tannin cells (Character 78, homoplastic), ovary hemiseptalous (Character 80, homoplastic) and septae lacking tannin cells (Character 82, homoplastic). Based on morphology, *E. meyeri* A.G.Schulz should also be placed in the *E. paniculata* group. *Monochoria* is recovered as monophyletic with high statistical support (BS=96; BI=2; Fig. 2), being characterised by eight non-homoplastic synapomorphies: pedicellate flowers (Character 39, reversion), perianth only basally connate (Character 56, reversion), absence of a nectar guide (Character 63, reversion), presence of a petalostaminal tube (Character 66), stamens unequal (Character 69), presence of a filament appendage (Character 70), enantiostylous flowers (Character 71, reversion) and poricidal anthers (Character 72). *Eichhornia crassipes* is recovered as a sole species with high statistical support (BS=94; BI=1; Fig. 2), being characterised by: its free-floating habit (Character 5), the production of new rosette through stolons (Character 8), flabellate ligules (Character 17), spirally-alternate petiolate leaves (Character 22, homoplastic), perianth loosely enveloping the fruit (Character 55, homoplastic) and nectar guide consisting of a sole spot (Character 63, homoplastic). *Eichhornia s.s.* was recovered with low statistical support (BS=56; BI=2; Fig. 2), being composed by *E. azurea* (Sw.) Kunth, *E. diversifolia* (Vahl) Urb. and *E. heterosperma* Alexander. It is characterised by: growing as mostly submerged plants (Character 3, homoplastic), stems freely branching and elongated (Character 9 and 10, homoplastic), sessile leaves late-deciduous (Character 19, homoplastic), petiolate leaves evenly distributed along the stem (Character 23, homoplastic), flowers self-compatible (Character 38, homoplastic), floral tissues lacking granular tannin cells (Character 46, homoplastic) and presenting fibrillar tannin cells (Character 47, reversion), nectar guide consisting of a sole spot or dark band (Character 63, homoplastic) and ovary walls lacking aerenchymatous tissue (Character 79, reversion). Finally, *Pontederia sensu* Lowden (1973) was recovered by us as monophyletic with high statistical support (BS=97; BI=3; Fig. 2). It is characterised by: flowers self-compatible (Character 38, homoplastic), nectar guide consisting of two spots (Character 63, homoplastic), pseudomonomerous ovary (Character 77), the presence of epithelial cells in the septae (Character 81, homoplastic), pendulous and unflanged placentation (Characters 83 and 84), fruit an achene (Character 89), seeds one per locule (Character 90) and smooth testa (Character 95). Nonetheless, the subgenera proposed by Lowden (1973) cannot be maintained, due to *P. rotundifolia* L.f. (i.e. *P.* subg. *Reussia*) being nested within *P.* subg. *Pontederia* (*sensu* Lowden 1973).

Plastid and combined analyses

The *ndhF* characters represented 503 characters of the plastid dataset, with GTR+G as the nucleotide model selected. The *rbcL* characters represented 1355 characters of the plastid dataset, with HKY+G+I as the nucleotide model selected. The plastid dataset

represented 1858 characters, of which 241 characters were variable and 119 characters were parsimony-informative. The plastid Bayesian analysis recovered a mostly resolved tree with 23 well-supported clades (>PP 95%) (Fig. 2). The congruence between the plastid and morphological datasets is illustrated in Figure 2. In both analyses, *Pontederia s.l.* and *Heteranthera sensu* Pellegrini (2017a) are strongly supported, but the relationship between the species is greatly different. In *Heteranthera,* the morphologically based topology is better resolved and recovers two clades, while the plastid dataset recovers two clades plus *H. gardneri* in a polytomy (Fig. 2). In *Pontederia s.l.*, both datasets recover the genus arranged in five clades, but the relationship between them is different. In the morphological dataset, *Eichhornia s.s.* is the first lineage to diverge, followed by *E. crassipes*, *Pontederia s.s.* and *Monochoria*, sister to the *E. paniculata* group. Alternatively, in the plastid dataset, the *E. paniculata* group is undoubtedly recovered as the first lineage, followed by *E. crassipes*, *Monochoria* and *Pontederia s.s.*, sister to *Eichhornia s.s.*

Topologies produced by MP and BI analyses, based on the combined plastid + morphology datasets, were highly congruent and provided higher support for more clades than the results based on independent datasets (Fig. 3). Thus, based on the combined plastid + morphological datasets (1858 analysed characters, of which 353 were variable and 140 parsimony-informative), the maximum parsimony analysis found 24 trees (CI=0.6471, RI=0.7858) whose MRC presented 23 highly supported clades (BSP 75%). The combined Bayesian analysis recovered a fully resolved tree with 25 mostly well-supported clades (>PP 95%) (Fig. 3). The topology recovered for the Bayesian combined analysis (Fig. 3) is almost identical to the one recovered for the plastic dataset (Fig. 2), differing in only very small details. On the other hand, the Parsimony combined analysis recovers *E. crassipes*, *Pontederia s.s.* and *Eichhornia s.s.* in a well-supported clade, with this clade being recovered in a polytomy together with the *E. paniculata* group and *Monochoria*.

Discussion

Phylogenetics of Pontederiaceae

The topologies recovered from the combined plastid and the total evidence datasets strongly corroborate the bi-generic circumscription of Pontederiaceae suggested by Pellegrini (2017a). They are also congruent with previous phylogenetic studies using molecular and/or combined datasets (Graham and Barrett 1995; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011) and partially congruent with the morphologically based phylogenetic tree of Eckenwalder and Barrett (1986). The phylogenetic tree recovered by Kohn et al. (1996) differs greatly from our results and all previous studies due to part of the polyphyletic *Eichhornia* being recovered as sister to *Heteranthera s.l.* Most molecular studies in the family (Graham and Barrett 1995; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011) recover a well-supported Pontede-
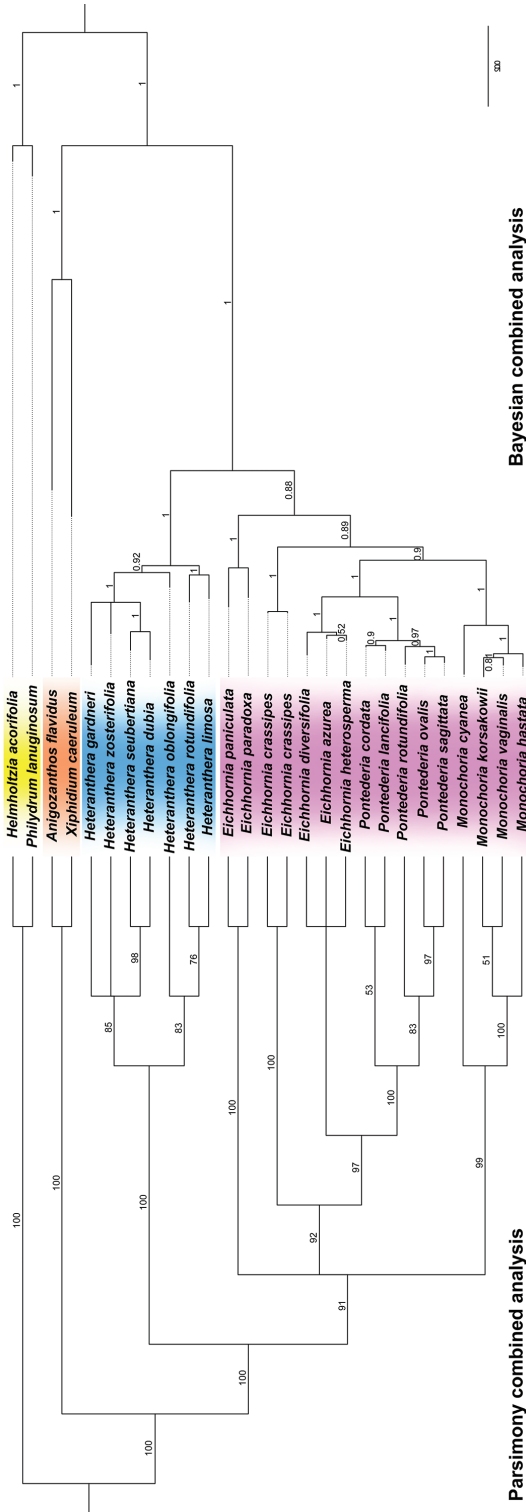
Figure 3. Majority-rule tree recovered for the parsimony and Bayesian analysis of the combined morphological + plastid dataset. Yellow: Philydraceae. Orange: Haemodoraceae. Blue: *Heteranthera s.l.* Pink: *Pontederia s.l.*

riaceae, divided into two main lineages, corresponding to a well-supported *Heteranthera s.l.* (*sensu* Pellegrini 2017a) and poorly-supported *Pontederia s.l.*; using *ndhF*, *rbcL*, plus a restriction-site in the chloroplast genome in Graham et al. (1998, 2002) and five nuclear gene families recovered employing an expressed sequence tag (EST) study by Ness et al. (2011). As in previous studies (Graham and Barrett 1995; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011), we recover *Pontederia s.l.* arranged in five main lineages, each representing a well-supported morphological group (i.e. *Eichhornia paniculata* group, *Monochoria*, *E. crassipes* group, *Eichhornia s.s.* and *Pontederia s.s.*). The monophyly of *Heteranthera sensu* Pellegrini (2017a) is indisputable and the inclusion of *Hydrothrix* and *Scholleropsis* in *Heteranthera* was strongly corroborated.

Morphology and systematics of Pontederiaceae

The monophyly of Pontederiaceae was rarely, if ever, questioned by previous authors. Perhaps for this reason, little attention was ever given to the family's putative morphological synapomorphies. Amongst the 18 morphological synapomorphies recovered for Pontederiaceae, one was previously suggested by Arber (1925; i.e. with xylem and phloem alternate near the centre of the blades, plus xylem abaxial and phloem adaxial near the margins), three were suggested by Simpson (1987, 1990; i.e. late bifacial and ligulate leaves and bisulcate pollen grains) and four were suggested by Simpson and Burton (2006; absence of fibrillar tannin cells in the perianth and presence of aerenchymatous tissue in the receptacle, perianth and ovary walls). Nonetheless, the peculiar involute ptyxis where the blade of the new leaf encloses the petiole of the preceding leaf, non-equitant leaves, sessile leaves early-deciduous, inflorescence deflexed at post-anthesis and in fruit, sessile flowers, perianth connate producing a conspicuous tube and the presence of an anthocarp, are suggested here for the first time as synapomorphies for Pontederiaceae.

Almost, if not all, leaf synapomorphies recovered for Pontederiaceae seem to be directly correlated. These characters seem to be related to the adaptive shift to a completely aquatic lifestyle in the family and an adaptation to changes in water level. The leaves of Pontederiaceae are characteristically dimorphic, being morphologically divided into sessile and petiolate leaves (Horn 1988). Leaf dimorphism is widely distributed across the Embryopsida, being generally related to changes in function (e.g. reproductive leaves in ferns), growth form (e.g. juvenile and mature leaves of *Monstera* spp.) or environmental changes (Allsopp 1965). The dimorphic leaves of Pontederiaceae seem to fit the latter situation, since the petiolate leaves are always floating or aerial, while the ribbon-like or acicular sessile leaves are the first type produced by the germinating plantlet and seen to be an adaptation to the aquatic environment. Furthermore, the presence of a petiole greatly helps to keep the leaves at or above the water level, through cell elongation in the petiolar region. This strategy can be easily observed in several distantly related aquatic plant families (e.g. Alismataceae, Asteraceae, Cabombaceae, Haloragaceae, Nymphaeaceae, Onagraceae, Ranunculaceae etc.; Allsopp 1965;

Sculthorpe 1967; Cook 1996). The peculiar vascular bundle arrangement observed in Pontederiaceae is exclusive to the family and few other monocots (Arber 1925). This feature seems to be a result of the reversion from abaxialised unifacial leaves to bifacial leaves, which, according to Simpson (1990), might be related to the adaptive shift and radiation to an aquatic lifestyle in the family. The remaining closely related families (i.e. Haemodoraceae and Philydraceae) possess consistently abaxialised unifacial leaves, with blades ranging from cylindrical, terete, laterally compressed to more rarely plicate (Simpson 1990, 1998; Hamann 1998). Nonetheless, the evolutionary relevance of bifacial leaves is significantly harder to infer, since unifacial leaves are noticeably common in several aquatic plants. The reversal from equitant to alternate leaves seems to be a by-product from the reversion from unifacial to bifacial leaves. As aforementioned, the involute ptyxis in Pontederiaceae is extremely unusual, since the blade of the new leaf encloses the petiole of the preceding leaf. This feature is also unique in the Angiosperms and is easily observed in most species in the family but is especially obvious in *E. crassipes* (Fig. 7C). This feature might also be related to the adaptive shift and radiation to a completely aquatic lifestyle in Pontederiaceae, being most likely a result of the reversion to bifacial leaves. Developmental studies focusing on the ontogeny of the leaves in Pontederiaceae, in comparison to some members of Haemodoraceae and Philydraceae, might help us better understand the mechanics of the reversal from unifacial to bifacial leaves in the family and how this shift might have affected general leaf morphology and the appearance of novel structures such as the ligule.

As aforementioned, the leaves of Pontederiaceae are dimorphic, with both sessile and petiolate leaves being produced at different times in the plants' life. Sessile leaves represent the plesiomorphic state and are the first ones produced after seed germination. They vary in number from 5–many per plant and allow plants to become established in a submersed habitat (Horn 1988). The sessile leaves can range from early-deciduous to persistent in mature plants, while in some species of *Heteranthera s.l.*, petiolate leaves are never or very rarely produced (Horn 1985, 1988; Eckenwalder and Barrett 1986). The petiolate leaves are produced at posteriori and are considered the mature leaf type in the family. The initial petiolate leaves are morphologically plastic, allowing for a transition from a submersed to an immersed environment. This plasticity, coupled with the elongation of the stem, allows Pontederiaceae plants to successfully develop to and at the water surface (Horn 1988). In *Heteranthera s.l.*, the sessile leaves suffer a reversion from distichously to spirally arranged, producing the characteristic basal rosettes in the juvenile phase of many *Heteranthera* species (Horn 1988). Thus, early-deciduous sessile leaves and early production of petiolate leaves give a clear adaptive advantage to the Pontederiaceae, enabling them to tolerate a wide variation in water depth during their development, also allowing juvenile plants to successfully reach mature emergent or floating growth-forms (Horn 1988). This might have ultimately allowed the diversification of Pontederiaceae and their complete invasion of the aquatic environment.

The presence of a leaf sheath projection is striking in Pontederiaceae, with its morphology being relevant to the systematics of the family. Ligules and ligule-like

structures are recorded for several members of Embryopsida, being especially common in some lycophytes (i.e. Selaginellales and Isoëtales) and several monocots (i.e. Alismatales, Arecales, Asparagales, Commelinales, Dioscoriales, Poales and Zingiberales) (Kubitzki 1998; Rudall and Buzgo 2002; Kellogg 2015). Despite possessing the same name, there is no evidence supporting the homology of these structures between lycophytes and monocots and not even between different groups within the monocots (Rudall and Buzgo 2002). The definition and characterisation of ligules in monocots has varied greatly depending on the author, having Poaceae as their main focus. These authors have proposed three distinct definitions for ligules: (1) a subtype of stipule (Bischoff 1834; Regel 1843; Lubbock 1891, 1895; Arber 1925); (2) a structure of mixed origin between stipules and petioles (Glück 1901; Majumbdar 1956); and (3) an avascular projection of the leaf-sheath, situated between the leaf-sheath and the blade (Colomb 1887; Philipson 1935; Dahlgren et al. 1985; Chaffey 1994; Rudall and Buzgo 2002). In Commelinid monocots, ligules and ligule-like structures are recorded for Arecales (i.e. the hastulae present is some Arecaceae leaves), several families of Poales (e.g. Cyperaceae, Joinvilleaceae, Juncaceae, Poaceae, Restionaceae), Commelinales (exclusively in Pontederiaceae) and Zingiberaceae (i.e. Costaceae and Zingiberaceae) (Kubitzki 1998; Rudall and Buzgo 2002; Kellogg 2015). As aforementioned, ligules and ligule-like structures in Commelinales seem to be restricted to Pontederiaceae and are unknown to any of the other four families of the order (Kubitzki 1998; Rudall and Buzgo 2002; Pellegrini pers. obs.). These structures might also be a result of the reversion from unifacial leaves to bifacial leaves or even an independent adaptation to the aquatic lifeform in the family. In the unifacial-leaved clade, composed by Philydraceae (Haemodoraceae+Pontederiaceae), Pontederiaceae is the only exclusively aquatic family and also the only one to possess ligule-like structures (Figs 4F, 6C, 7C, 9E), dimorphic leaves, petiolate leaves and bifacial leaves. Nonetheless, ontogenetic studies are necessary to understand the origin of these structures in the family. In Pontederiaceae, these ligule-like structures have been treated under different names according to the authors, having been named stipules (Schwartz 1926), ligules (Castellanos 1958; Pellegrini and Horn 2017), ochreas (Rutishauser 1999) or simply as leaf-sheath projections (Pellegrini 2017a). Different names have also been applied by the same author, depending on the development and shape of these structures (i.e. Cook 1998). Regardless of the name adopted for these ligule-like structures in Pontederiaceae, their systematic and taxonomic relevance is undeniable. As aforementioned, this structure is recovered as synapomorphic for the family. Alternatively, within Pontederiaceae, the morphology of this structure can be easily used to define the two clades recovered in phylogenetic studies. *Pontederia s.l.* can be easily characterised by it mainly truncate ligules, being rarely flabellate (i.e. *E. crassipes*); while *Heteranthera s.l.* can be characterised by its 2–several-parted ligules.

Out of the reproductive synapomorphies recovered by us for Pontederiaceae, some of them seem to be related to pollination, while the others seem to be related to fruit dispersal. Sessile flowers are recovered by us as a synapomorphy of Pontederiaceae, with the sole reversion occurring in *Monochoria*. This character seems to be directly

related to another reproductive synapomorphy for the family (i.e. perianth connate to part of the receptacle and the filaments producing a conspicuous tube). Pedicel and floral tube length seem to be inversely correlated, with tube elongation helping with the floral display by elevating the perianth lobes. Added to that, the contraction of the pedicel might also provide extra stability for heavier floral visitors that require landing platforms in order to properly visit flowers (e.g. butterflies). Alternatively, the reversion from sessile to pedicellate flowers in *Monochoria* might have played a key role, by giving flowers the needed mobility in order to avoid floral damage during buzz pollination (Wang et al. 1995). Bisulcate pollen grains are rather rare in the monocots, being recorded for only a handful of families, such as: Araceae (Grayum 1992), Arecaceae (Harley and Baker 2001), Dioscoreaceae (Caddick et al. 1998), Iridaceae (Rudall and Wheeler 1988) and Velloziaceae (Halbritter and Hesse 1993). Of the aforementioned families, only Arecaceae (Arecales) is a member of the Commelinid monocots and it is but distantly related to Pontederiaceae (Saarela et al. 2008; Hertweck et al. 2015; APG IV 2016). In Haemodoraceae, Simpson (1983) recorded the occurrence of biporate pollen grains in some genera from subfamily Conostylidoideae. Nonetheless, Simpson (1987, 1990) considers the biporate pollen grains in Haemodoraceae not homologous to the bisulcate pollen grains in Pontederiaceae. This view is also shared by us in the present study.

The first synapomorphy related to diaspore dispersal is the deflexed position of the inflorescence at post-anthesis and in fruit. This shift in the inflorescence position during fruit development will almost certainly allow the mature fruits to reach the water after their maturity. The deflexed inflorescences also elongate in length, which ultimately places the maturing fruits at or under the water surface. This seems to be the first step in diaspore dispersion in most species of Pontederiaceae. The following adaptations are related to increasing the floatation period of the diaspores. The first and most obvious seems to be the presence of an anthocarp. According to Spjut (1994), an anthocarp is a type of fruit which possesses attached and developed floral parts that aid in its dispersal. It is more commonly recorded for plants with inferior ovaries, but it is not exclusive to them (Spjut 1994). In Commelinales, all fruits have persistent perianth parts, but only in Pontederiaceae does an enlarged perianth actively aid in the dispersal of the diaspores (Pellegrini, pers. observ.), with *Tradescantia zanonia* (L.) Sw. (Commelinaceae) being an exception (Pellegrini 2017b; Pellegrini and Faden 2017). In Pontederiaceae, the anthocarp seems to be related to hydrochoric dispersion, which is also supported by the remaining synapomorphies for the family (i.e. presence of aerenchymatous tissue in the receptacle, perianth and ovary walls). The anthocarp is especially developed with thick aerenchymatous tissue in *Monochoria*, *Pontederia s.s.* and in the *E. paniculata* group (Lowden 1973; Cook 1989, 1998; Simpson and Burton 2006; Pellegrini, pers. observ.; Figs 5F, 6K & 9K), that provides long flotation periods for the diaspores (i.e. around 15 days; Barrett 1988). In the remaining lineages of Pontederiaceae (i.e. *Heteranthera s.l.*, *E. crassipes* group and *Eichhornia s.s.*), the anthocarp is thin, probably resulting in a much shorter flotation period (i.e. probably around 24h), with seeds being secondarily dispersed by other biotic and/or abiotic means (Bar-

rett 1978; Pellegrini and Horn, pers. observ.). In the closely-related Haemodoraceae and Philydraceae, the perianth is also connate, producing a characteristic hypanthium and partially to completely persistent in fruit (Hamann 1998; Simpson 1998). Nonetheless, they do not aid in the dispersal of diaspores, since in all species, the persistent perianth is only marcescent and does not develop during fruit development, being ultimately torn open by the mature fruit (Pellegrini, pers. observ.). These observations are also supported by the complete lack of aerenchymatous tissues in floral organs of both families, with aerenchyma being recorded only in the septae of the hydrochoric Philydraceae (Simpson and Burton 2006). In Commelinaceae and Hanguanaceae, the persistent perianth also does not develop during fruit maturation; with the exception of *Buforrestia* C.B.Clarke (Commelinaceae), where the persistent sepals are as long as, or longer than, the mature capsule (Bayer et al. 1998; Faden 1998). Nonetheless, the perianth of *Buforrestia* does not seem to aid in the dispersion of the diaspores, since the perianth only loosely involves the capsules, which remain attached to the pedicel and dehisce at maturity (Pellegrini, pers. observ.). In Hanguanaceae, the fruits consist of variously coloured berries that detach from the persistent sepaloid perianth and are most probably zoochoric (Bayer et al. 1998). On the other hand, in Commelinaceae, the fruits are primarily dehiscent capsules (rarely indehiscent capsules or berries), that do not rely on the persistent sepals for dispersal, with fruits or seeds being autochoric or more rarely zoochoric (Pellegrini and Faden 2017).

Systematics and characterisation of *Pontederia* s.l.

All 18 synapomorphies recovered by us for *Pontederia s.l.* are suggested here for the first time. Sand-binding roots were recovered by Smith et al. (2011) as plesiomorphic for Haemodoraceae and probably for all Commelinales, despite the authors' not sampling Hanguanaceae in their analysis. These sand-binding roots produce specialised hairs that bind soil, especially larger sand crystals, creating a protective layer that envelops the roots (Smith et al. 2011). These authors also state that all studied specimens of Philydraceae and Pontederiaceae had non-sand-binding roots, in contrast to Haemodoraceae. On the other hand, sand-binding roots are commonly observed in several lineages of Commelinaceae, but especially in species growing in dry environments (Smith et al. 2011; Pellegrini, pers. observ.). After several field studies and cultivation of several species of Pontederiaceae, we have observed that all species of *Heteranthera s.l.* possess water-binding (i.e. mucilaginous) roots, while the absence of an external mucilage layer on the roots was characteristic of *Pontederia s.l.* The water-binding roots of *Heteranthera s.l.* are most probably not homologous to the sand-binding roots in the order, since they do not seem to have specialised hairs, like those described for Haemodoraceae (Smith et al. 2011). The mucilage layer seems to be produced by the secretion of chemical compounds near the root apex which polymerises in contact with water (Pellegrini, pers. observ.). Nonetheless, further anatomical and histochemical studies are needed to better understand this feature.

The presence of leaves with pulvinate petioles in *Pontederia s.l.* is easily observed in the field, since most pulvini are lighter or darker than the rest of the petiole. On the other hand, in dried specimens, this difference in colouration is only sometimes maintained, making this character not always obvious to untrained eyes. Added to that, the pulvini in *Pontederia s.l.* are seldom swollen, as would be expected in most eudicot plants with articulated leaves. Nonetheless, this feature seems to be key for the emergent and floating species, especially the perennial ones, since they are subjected to the greatest amount of environmental variation. Floating species like *E. crassipes* are easily dragged by water currents, forcing all leaf-blades to change their position in order to better absorb sunlight. Perianth-coiling at post-anthesis seems to be poorly documented in the literature for most Angiosperm families and more so in the monocots. It is known to occur in the monocots only in the distantly related Bromeliaceae (Poales), being characteristic to some genera of subfamilies Pitcairnioideae and Puyoideae (Smith et al. 1998; Hornung-Leoni and Sosa 2008). In Commelinales, the persistent perianth is marcescent in Philydraceae, Haemodoraceae and Hanguanaceae, while in Commelinaceae, the sepals are marcescent and the petals are deliquescent (Pellegrini, pers. observ.). In Pontederiaceae, the perianth in *Heteranthera s.l.* is also marcescent at post-anthesis, only loosely enclosing the developing capsule. In *Pontederia s.l.*, the perianth is either spirally-coiled or revolute at post-anthesis, tightly enclosing the developing fruit, with two independent shifts to deliquescent perianths loosely enclosing the developing fruit (i.e. *E. crassipes* and *Eichhornia s.s.*). This might be related with increasing long-distance diaspore dispersal in the rooted species, with the anthocarp ridges possessing aerenchymatous tissue in most species. This character seems to greatly increase the dispersion range of most *Pontederia s.l.* lineages that, unlike *E. crassipes* and *Eichhornia s.s.*, are not easily vegetatively dispersed by the fragmentation of floating stems. In *E. crassipes*, the plants are free-floating and can easily disperse in waterbodies with moving waters, while in *Eichhornia s.s.,* the plants have elongated stems, which possibly help diaspores to disperse further away from the mother plant's base, thus decreasing parental/offspring competition.

Tristyly is an extremely rare type of heterostyly, recorded for a handful of families, only two being monocots (i.e. Amaryllidaceae and Pontederiaceae; Barrett 1993). According to Kohn et al. (1996), tristyly evolved only once in Pontederiaceae. As aforementioned, in Kohn et al. (1996), they recover part of the polyphyletic *Eichhornia* as sister to *Heteranthera s.l.* and tristyly as a synapomorphy for Pontederiaceae as a whole, with four reversions to homostyly. However, we recover tristyly as a synapomorphy of *Pontederia s.l.* alone, with only two reversions to homostyly. In *E. diversifolia* (Vahl) Urb. and *E. natans* (P.Beauv.) Solms, the flowers seem to be consistently pseudo-homostylous, which could be related to miniaturisation connected with these species' floating growth-form (Barrett 1988). In *Monochoria,* there is a shift from tristyly to enantiostyly (i.e. two different types of heterostyly; Barrett 1993), that could be easily explained by the shift in the group's pollination syndrome. *Monochoria* species are enantiostylous, lack septal nectaries and exclusively offer pollen as a floral reward (Wang

et al. 1995) and this, most likely, is connected with the buzz pollination syndrome of their flowers. Furthermore, poricidal, basifixed, polymorphic anthers are typical to buzz-pollinated flowers (Cook 1989; Wang et al. 1995). This shift from nectar-flowers to pollen-flowers seems to be the main cause of the peculiar floral morphology and loss of tristyly in *Monochoria*.

In Pontederiaceae, three different patterns in perianth-lobe shape can be observed: (1) perianth lobes all equal, thus producing an actinomorphic perianth (e.g. *H. dubia*); (2) equal to subequal in the same whorl, producing either actinomorphic or zygomorphic perianths, depending on the presence of a nectar guide [e.g. actinomorphic in *M. hastata* (L.) Solms and zygomorphic in *E. crassipes*]; and (3) unequal perianth lobes, with more than one morph in the same whorl, producing strongly zygomorphic perianths (e.g. *H. gardneri*). In Commelinales, the perianth lobes pattern seems to be extremely variable, being equal in the same whorl in Hanguanaceae, unequal in Philydraceae (due to the fusion of three posterior lobes) and variable in Commelinaceae and Haemodoraceae (Pellegrini, pers. observ.). In Commelinaceae, sepals are almost invariably different from the petals, except in *Palisota* Rchb. *ex* Endl. in which the sepals are characteristically petaloid (Faden 1998). Furthermore, both sepals and petals can range from equal to unequal, producing strongly zygomorphic flowers (e.g. *Aneilema* R.Br., *Commelina* L., *Polyspatha* Benth.; Faden 1998). In Haemodoraceae, there is much variation in the shape of the perianth lobes (Simpson 1990, 1998). Nonetheless, equal perianth lobes seem to be plesiomorphic in the monocots (Sauquet et al. 2017; Stevens 2001–onwards) and dominant in the family, being recorded for 11 out of 14 genera (Pellegrini, pers. observ.). Thus, equal to subequal lobes in one perianth whorl (the apices are obtuse to round) is recovered by us as a homoplastic synapomorphy for *Pontederia s.l.* (Fig. 1). The perianth in *Pontederia s.l.* ranges from campanulate to infundibuliform to hypocrateriform, while in *Heteranthera s.l.,* it is almost exclusively tubular, a distinctive synapomorphy for the latter genus. The only exception is *H. gardneri*, which possesses an infundibuliform perianth and which might be explained by miniaturisation. In Philydraceae, the perianth is consistently infundibuliform, while the perianth in Haemodoraceae shows great plasticity, depending on the genus, ranging from flat to hypocrateriform to tubular to the peculiar split and falcate perianth of *Anigozanthos* (Simpson 1990, 1998).

Systematics and characterisation of the five main lineages of *Pontederia* s.l.

Out of the four synapomorphies recovered for the *E. paniculata* group, two had been previously proposed by Eckenwalder and Barrett (1986; annual life cycle) and Barrett and Graham (1997; annual life cycle and the absence of clonal reproduction). All currently accepted species in this group are known to inhabit seasonal and, generally, short-lived waterbodies. Thus, the annual life cycle and the absence of clonal reproduction are more than expected. However, all previous studies in the family failed to notice

the peculiarly inflated sheath of the leaf subtending the inflorescence and the flat basal bract (Fig. 5B). These characters are easily observed in *E. paniculata* and *E. meyeri*, due to their elongated inflorescences, while in *E. paradoxa,* the inflorescence has its internodes greatly contracted, thus making the flat basal bract extremely hard to observe, especially in dried specimens.

Monochoria comprises species with extremely autapomorphic morphology, being traditionally grouped based on their: pedicellate, actinomorphic and enantiostylous flowers, basally connate perianth and its basifixed and poricidal anthers (Cook 1989, 1998). Due to its enantiostylous flowers and basifixed anthers, *Monochoria* has traditionally been considered closely related to *Heteranthera* (Eckenwalder and Barrett 1986; Cook 1998). Nonetheless, molecular data provide strong support that *Monochoria* is instead sister to the clade composed of *E. crassipes*, *Eichhornia s.s.* and *Pontederia s.s.* (Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011; this study). Aside from the six aforementioned synapomorphies, *Monochoria* is also supported in our present analysis by other six characters. Out of these characters, only the basal bract with a caudate apex was previously described as characteristic of *Monochoria* by Cook (1989). The presence of an inflated sheath in the leaf subtending the inflorescence, flat basal bract and fistulose main axis are shared between the *E. paniculata* group and *Monochoria* and are most likely plesiomorphic for *Pontederia s.l.* The caudate apex in the basal bract is observed in all species of *Monochoria*. Nonetheless, *M. korsakowii* can also present a leaf-like basal bract (Cook 1989). The actinomorphic perianth is a result of the loss of the nectar guide in this lineage which, as aforementioned, is directly related to the shift in pollination syndrome in the group. Additionally, other four floral modifications in *Monochoria* seem to be associated with this shift in the group's pollination syndrome: (1) pedicellate, actinomorphic and enantiostylous flowers; (2) basally connate perianth (which helps to expose the stamens and allows the bees to properly visit the flowers); (3) unequal, basifixed and poricidal anthers; and (4) the loss of septal nectaries. The presence of a petalo-staminal tube is also unique in the family and most probably is the result of the reduction of the length of the hypanthium. Finally, the thickened and ridged anthocarps are also observed in the *E. paniculata* group and *Pontederia s.s.*, being directly related to the fruits primary hydrochoric dispersal syndrome (see comment above).

Despite being well-known, *E. crassipes* possesses the most peculiar vegetative morphology in the polyphyletic *Eichhornia* and one of the most peculiar in the family as a whole. It is so peculiar that specimens are easily identified, even when lacking any reproductive structures (Pellegrini and Horn, pers. observ.). It is the only species in the family to possess a free-floating growth form, the only one to produce stolons and the only one to possess inflated petioles. Nonetheless, one of the most peculiar characters in *E. crassipes* has been greatly disregarded by most specialists in the family. Castellanos (1958) was one of the first to properly describe and illustrate the flabellate ligules of *E. crassipes*. All synapomorphies recovered for *E. crassipes* seem to be directly related to its peculiar free-floating growth form, which also enabled it to become the most trouble-

some weed of the world (Gopal and Sharma 1981). The morphology of *Eichhornia s.s.* is clearly a result of its floating growth form and the tendency of these plants to grow in deeper water bodies. The late-deciduous sessile leaves (sometimes persistent for most of the plant's adult life) are characteristic of this group, but especially striking in *E. diversifolia*, hence its name. This protraction of the submerged phase seems to give the species in this clade a clear developmental advantage by helping them to reach the water surface and produce enough petiolate leaves to allow them to float properly. Furthermore, the even arrangement of the petiolate leaves along the mature stem might help provide the needed stability to the elongated floating stem.

From all the recovered clades in *Pontederia s.l.*, *Pontederia s.s.* goes hand-in-hand with *Monochoria* in the number of reproductive synapomorphies. Out of the eight recovered synapomorphies for this clade, six are reproductive, with only the presence of epithelial cells in the septae, which are shared with *Monochoria*, being homoplastic. All the remaining five reproductive synapomorphies are directly correlated, but their evolutionary chronology is much harder to infer. The most parsimonious view is probably that all characters were triggered concomitantly by the appearance of the pseudomonomerous ovary, which caused the change in placentation morphology and ovule number. The abortion of most of the gynoecium might have caused a key shift in the reproductive strategy in this lineage from investing in a great number of small seeds with little chance of reaching maturity, to investing into a single big seed with a good amount of provision and guaranteeing that it has a greater chance of reaching maturity. The smooth testa seems to be a simple byproduct of negative selection of ornamentation, since the seeds stopped being individually dispersed with the change of reproductive strategy. Finally, the achene gives this lineage a great evolutionary advantage since it is easily dispersed by water, with a long floatation period due to its thick parenchymatous walls. Furthermore, many species also possess complex ornate achenes, with teeth and spikes that efficiently stick to fur, feathers, fabric etc., most likely having animals as their primary dispersers (Pellegrini, pers. observ.).

Taxonomy

With the present recircumscription of *Pontederia*, Pontederiaceae now is organised in two monophyletic genera (i.e. *Heteranthera* and *Pontederia*). As stated by Pellegrini (2017a) and corroborated by nine phylogenetic studies (Eckenwalder and Barrett 1986; Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011; this study), the recognition of two genera seems to be the best and most taxonomically conservative option available, since it avoids the description of new genera and the reestablishment of names that were rarely, if ever, used in any relevant taxonomic or floristic study. Finally, this option makes the differentiation of the two accepted genera easy, using either fresh, liquid or herbarium samples. Thus, the genera of Pontederiaceae can be differentiated using the key below:

Key to the genera of Pontederiaceae

1   Sessile leaves spirally-alternate, petiolate leaves sometimes present in mature specimens, when present non-pulvinate, blade membranous; inflorescence reduced to a solitary cincinnus; stamens (1–)3, staminodes sometimes present, septal nectaries absent, stigma unevenly trilobate .... **Heteranthera Ruiz & Pavón** (Fig. 4)
–   Sessile leaves distichously-alternate, petiolate leaves always present in mature specimens, pulvinate, blade chartaceous to coriaceous; inflorescence a 2– many branched thyrsi (rarely reduced to a solitary flower); stamens 6, staminodes absent, septal nectaries present (if absent, then flowers pedicellate and anthers poricidal), stigma capitate or trilobate, rarely trifid .... ***Pontederia*** **L.** (Figs 5–9)

Pontederia L., Sp. Pl. 1: 288. 1753.
Figs 5–9

Type species (designated by Lowden 1973). *Pontederia cordata* L.

   Description. *Herbs* perennial or annual, aquatic to amphibious, erect-emergent, procumbent-emergent or free-floating. *Roots* thin, fibrous or spongy. *Rhizome* short and generally inconspicuous. *Stems* trailing to erect, delicate to spongy, branching at the base, rarely branching at the upper half, rooting at the basal nodes or along the whole stem; internodes reduced to elongate, producing stolons or not. *Sessile leaves* distichously-alternate, congested at the apex of the stem, submerged, deciduous or persistent in mature plants, blades linear to linear-obovate, membranous, rarely chartaceous. *Petiolate leaves* distichously or spirally-alternate, congested at the apex of the stem or evenly distributed along the stem, floating or emergent, ligule truncate or with a flabellate projection; petioles conspicuous, rarely indistinct, inflated or not; blades elliptic to lanceolate or ovate to cordate to reniform or obovate to rounded, chartaceous to coriaceous. *Synflorescence* composed by a solitary main florescence subtended by a vegetative, petiolate leaf. *Main florescences (inflorescences)* axillary or apparently terminal, consisting of a pedunculate, many-branched thyrse, rarely a reduced thyrse; inflorescence leaf with or without an inflated leaf-sheath; basal bract flat or tubular; cincinnus' bract absent; cincinni (1–3–)4 – many per thyrse, alternate or fascicle-like, 1 – many-flowered, sessile or pedunculate, internodes contracted, rarely elongate; bracteoles absent, rarely present. *Flowers* bisexual, sessile or pedicellate, chasmogamous, pseudo-homostylous or tristylous, enantiostylous, zygomorphic, perianth connate usually forming a tube (hypanthium), rarely only basally fused, campanulate or infundibuliform or hypocrateriform, white to light pink to pink to mauve to pale lilac to lilac to bluish-lilac to purple, lobes 6 (3 outer and 3 inner), elliptic to oblong to obovate, 3 superior and 3 inferior, rarely 5 superior and 1 inferior, the central superior lobe generally with a nectar guide, consisting of 1–2 yellow to green spots, generally surrounded by a dark purple to bluish-purple, rarely white blur, spirally-coiling or
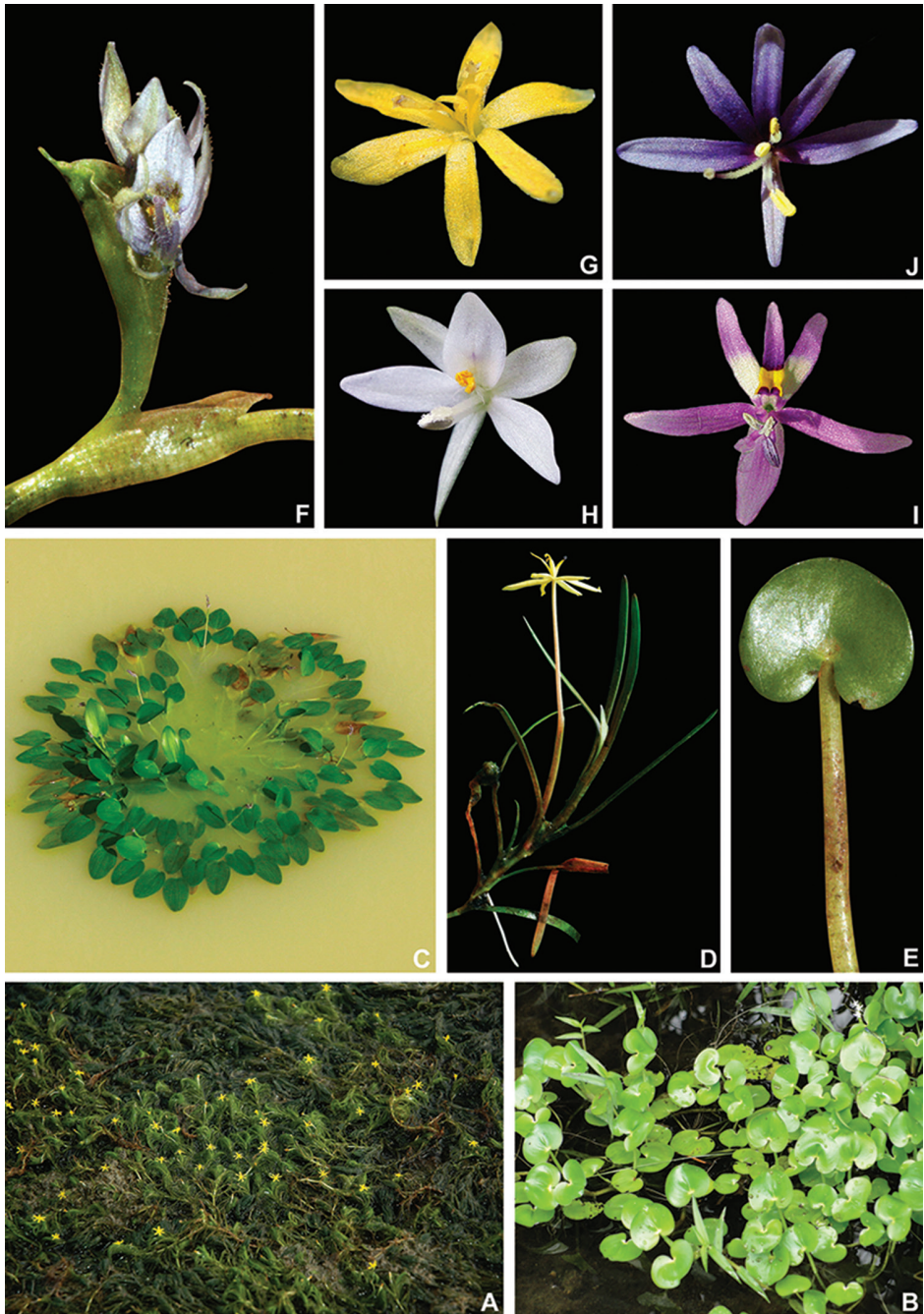
Figure 4. *Heteranthera* Ruiz & Pav. **A–D** habit: **A** emerged and flowering population of *H. gardneri* (Hook.f.) M.Pell. during the dry season **B** floating specimen of *H. reniformis* Ruiz & Pav. **C** emergent habit with floating and emerged leaves of *H. rotundifolia* (Kunth) Griseb. **D** habit of *H. dubia* (Jacq.) MacMill., showing the persistent sessile leaves E petiolate leaf of *H. pumila* M.Pell. & C.N.Horn, showing the lack of a pulvinus **F** Ligule and inflorescence of *H. pumila* **G–J** flowers: **G** pseudanthium of *H. gardneri* **H** *H. reniformis* **I** *H. rotundifolia* **J** *H. zosterifolia* Mart. **A** by A.P. Fontana **B, H** by C.N. Horn **C, I** by A. Popovkin **D** by S.R. Turner **E, F** by M.O.O. Pellegrini **G** by C.P. Bove and **J** by S.S. Oliveira.
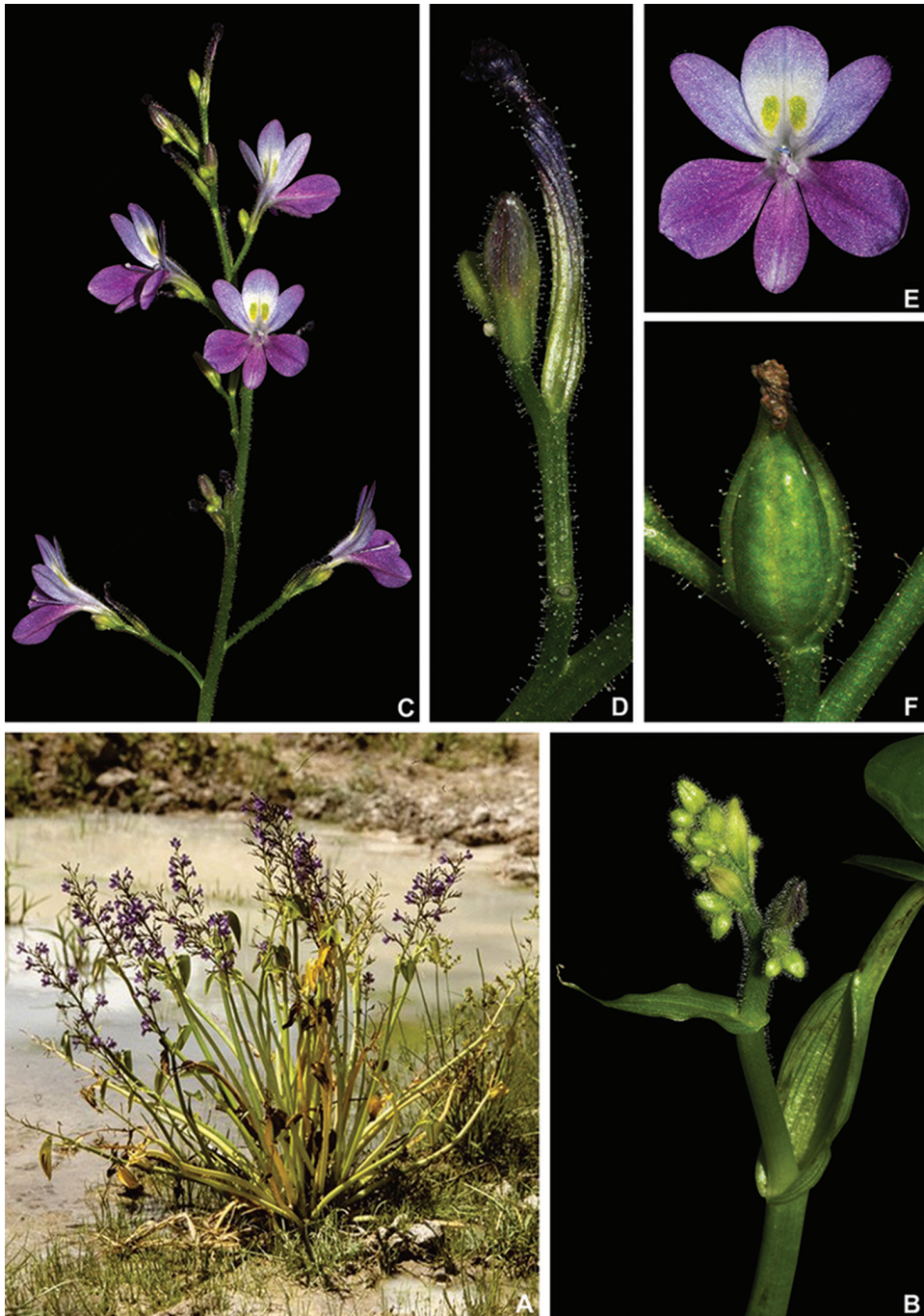
Figure 5. *Pontederia* subg. *Cabanisia* (Klotzsch *ex* Schltdl.) M.Pell. & C.N.Horn. **A** habit **B–C** inflorescence: B young inflorescence, showing the inflated leaf-sheath and flat basal bract with caudate apex **C** mature inflorescence showing the pedunculate cincinni with elongate internodes **D** detail of a cincinni, showing (from left to right) an immature floral bud, a pre-anthesis floral bud and a post-anthesis flower **E** front view of a flower F detail of an immature capsule, showing the ridged anthocarp. All photos of *P. paniculata* Spreng.; **A** by C. Willig & L. Nusbaumer, remaining photos by M.O.O. Pellegrini.

revolute at post-anthesis, deliquescent or not; stamens 6, epipetalous, dimorphic (the superior 3 shorter than the inferior 3) or unequal (1 inferior longer with a differently coloured anther), filaments J-shaped or recurved-decurved, terete, glabrous to glandular-pubescent, anthers dorsifixed, sometimes basifixed, rimose or poricidal, oblong to elliptic or sagittate; ovary ellipsoid to oblongoid, glabrous, locules 3, (1–)3 fertile, (1–) multi-ovulate, placentation axial or pendulous, septal nectaries generally present, rarely absent, style J-shaped, glabrous to glandular-pubescent, stigma capitate to trilobate, rarely trifid. *Fruit* a capsule with loculicidal or irregular dehiscence, rarely an achene, ellipsoid to oblongoid to subglobose or ovoid, rarely pyriform, light to medium brown, apiculate due to persistent style base; anthocarp thin or hardened, smooth or ridged, ridges ornamented or not. *Seeds* oblongoid or ellipsoid or subglobose to broadly oblongoid or ovoid or curved narrowly ovoid, brown to light-brown, testa longitudinally conspicuously to inconspicuously winged, rarely smooth, when present, wings membranous and testa also transversally striated between each wing; funiculi generally persistent, hilum punctate; embryotega dorsal, not prominently apiculate, darker than the rest of the seed.

Distribution and habitat. *Pontederia* currently comprises 26 mainly Neotropical species. Almost all Paleotropical species belong to *P.* subg. *Monochoria* (C.Presl) M.Pell. & C.N.Horn *comb. et stat. nov.*; except for *P. natans* P.Beauv., which is restricted to Africa and is a member of *P.* subg. *Eichhornia* (Kunth) M.Pell. & C.N.Horn *comb. et stat. nov.* Species in *Pontederia* can range from paludal to free-floating plants, thus occurring in a wide range of water bodies, from perennial to temporary, but most commonly in slow or stagnated water.

Generic circumscription and infrageneric classification. The circumscription adopted by us is almost equivalent to the original one proposed by Linnaeus (1753). It differs only by the exclusion of *P. ovata* L., which is currently placed in Marantaceae as a synonym for *Phrynium pubinerve* Blume (Horn and Haynes 1987; eMonocot 2010). Thus, no amendments are necessary for the herein adopted circumscription. We propose the subdivision of *Pontederia* in five monophyletic subgenera, based on the previously published molecular and morphological phylogenies (Eckenwalder and Barrett 1986; Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011), added to the new morphological and molecular analyses presented by us and data gathered by us while working on the family. Despite being monophyletic, these subgenera are not easily morphologically differentiated, since many of the characters supporting each clade are not always easy to observe, especially in dried specimens. Thus, it is our opinion that a broader sense of *Pontederia* should be accepted, instead of elevating each *Eichhornia* lineage (i.e. the herein proposed subgenera) to the generic rank.

Key to the subgenera of Pontederia

1   Basal bract commonly with a caudate apex, rarely leaf-like; flowers pedicellate, enantiostylous, perianth only basally connate, campanulate; stamens

with filaments connate forming a petalo-staminal tube, anthers basifixed, poricidal; septal nectaries absent ....
..... ***Pontederia*** **subg.** ***Monochoria*** **(C.Presl.) M.Pell. & C.N.Horn** (Fig. 6)

– Basal bract with an acute to acuminate to aristate apex, rarely caudate; flowers sessile, non-enantiostylous, perianth connate forming a conspicuous tube, infundibuliform or hypocrateriform; stamens with free filaments, anthers dorsifixed, rimose; septal nectaries present **2**

2 Ovary 1-locular by abortion, fertile locule 1-ovulate, placentation pendulous; fruit an achene, anthocarp hardened, ridges sinuate, toothed or echinate; seeds smooth...................................***Pontederia*** **L. subg.** ***Pontederia*** (Fig. 9)

– Ovary 3-locular, locules many-ovulate, placentation axial; fruit a capsule, anthocarp thin to thickened, if thickened ridges smooth; seeds longitudinally winged...**3**

3 Herbs procumbent-emergent, stems elongate; sessile leaves late deciduous, rarely persistent in mature plants, petiolate leaves distichously-alternate, evenly distributed along the stem; perianth infundibuliform, style glabrous...........
...........***Pontederia*** **subg.** ***Eichhornia*** **(Kunth) M.Pell. & C.N.Horn** (Fig. 8)

– Herbs erect emergent or free-floating, stems inconspicuous; sessile leaves early deciduous, petiolate leaves spirally-alternate, congested at the apex of the stem; perianth hypocrateriform, style glandular-pubescent...........................**4**

4 Herbs stoloniferous; ligule flabellate, petioles generally inflated; inflorescences deflexed post-anthesis and in fruit, emerging from a non-inflated leaf-sheath, basal bract tubular; flowers ca. 4–6 cm diam., perianth loosely enclosing the developing fruit; seeds oblongoid..
...........................***Pontederia*** **subg.** ***Oshunae*** **M.Pell. & C.N.Horn** (Fig. 7)

– Herbs never producing stolons; ligule truncate, petioles never inflated; inflorescences erect at post-anthesis, emerging from an inflated leaf-sheath, basal bract flat; flowers ca. 2–3 cm diam., perianth tightly enclosing the developing fruit; seeds subglobose to broadly oblongoid....................................***Pontederia*** **subg.** ***Cabanisia*** **(Klotzsch ex Schltdl.) M.Pell. & C.N.Horn** (Fig. 5)

1. *Pontederia* subg. *Cabanisia* (Klotzsch *ex* Schltdl.) M.Pell. & C.N.Horn, comb. et stat. nov.

urn:lsid:ipni.org:names:77188076-1
Fig. 5

Cabanisia Klotzsch *ex* Schltdl., Abh. Naturf. Ges. Halle 6: 176. 1862. Type species (designated here). *Cabanisia caracasana* Klotzsch *ex* Schltdl., nom. illeg. (≡ *P. paniculata* Spreng.).

Description. *Herbs* perennial or annual, aquatic to amphibious, erect-emergent. *Rhizome* short and generally inconspicuous. *Stems* erect, spongy, branching at the base. *Ses-*

sile leaves early deciduous. *Petiolate leaves* spirally-alternate, congested at the apex of the stem, emergent, ligule truncate, petioles not-inflated, blades cordate to broadly cordate, rarely elliptic to lanceolate or narrowly ovate. *Main florescences (inflorescences)* terminal, sessile or pedunculate; inflorescence leaf with an inflated leaf-sheath; basal bract flat; cincinni alternate or fascicle-like, 1–3-flowered, pedunculate, rarely sessile, internodes elongate, rarely contracted. *Flowers* sessile, chasmogamous, tristylous, zygomorphic, non-enantiostylous, perianth connate forming a tube, hypocrateriform, spirally-coiled at post-anthesis, non-deliquescent and tightly enclosing the developing fruit, lobes 3 superior and 3 inferior, rarely 5 superior and 1 inferior, the central superior lobe with a nectar guide, consisting of 2 yellowish-green to green spots, generally surrounded by a dark purple to bluish-purple, rarely white blur; stamens dimorphic, filaments free from each other, J-shaped, glandular-pubescent, anthers dorsifixed, rimose; ovary with 3 fertile locules, multi-ovulate, septal nectaries present, style glandular-pubescent, stigma capitate to trilobate. *Capsules* loculicidal, ellipsoid to oblongoid; anthocarp thickened, ridged. *Seeds* subglobose to broadly oblongoid, testa longitudinally winged.

Circumscription. *Pontederia* subg. *Cabanisia* is composed by *P. meyeri* (A.G.Schulz) M.Pell. & C.N.Horn *comb. nov.*, *P. paniculata* Spreng. and *P. paradoxa* Mart. All three species occur in moist environments or shallow waters, being similar in habit to well-known species of *P.* subg. *Pontederia*, such as *P. cordata* L. Nonetheless, both subgenera can be differentiated based on gynoecium, fruit and seed morphology.

Distribution. Mainly Central-West and Northeastern Brazil (reaching Argentina and Paraguay), growing in temporary water bodies in the Caatinga, Cerrado and Chaco domains. However, two species have very peculiar disjunctions in their distributions, also occurring in north-western South America (Colombia, Ecuador, Guyana and Venezuela), Central America (Costa Rica, Guatemala and Nicaragua), Antilles (Jamaica) and North America (Mexico).

Key to the species of *Pontederia* subg. *Cabanisia*

1   Petiolate blades without posterior divisions, elliptic to lanceolate or narrowly ovate in outline; inflorescences 2–5-flowered, sessile, cincinni sessile, fascicle-like; perianth arranged in a 5+1 pattern, tube 2–2.5 cm long ....***P. paradoxa* Mart.**
–   Petiolate blades with posterior divisions, cordate to broadly ovate in outline; inflorescences 10–many-flowered, pedunculate, cincinni pedunculate, alternate; perianth arranged in a 3+3 pattern, tube 0.8–1.6 cm long..................**2**
2   Main axis with a mixture of glandular and eglandular hairs, basal bract with cordate base and caudate apex, basal cincinni 1–2(–3)-flowered, bracteoles present; central superior perianth lobe with one green spot, surrounded by purple striations, all stamens exserted from the floral tube, anthers yellow ....
       ....***P. meyeri* (A.G.Schulz) M.Pell. & C.N.Horn**
–   Main axis glandular-pubescent, basal bract with round base and acute to acuminate apex, basal cincinni 4–9-flowered, bracteoles absent; central su-

perior perianth lobe with two green spots, surrounded by a white blur, 3 stamens included and 3 stamens exserted from the floral tube, anthers bluish-lilac to lilac .... ***P. paniculata* Spreng.**

1.1. *Pontederia meyeri* (A.G.Schulz) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:60476935-2

Eichhornia meyeri A.G.Schulz, Darwiniana 6: 56. 1942. Lectotype (designated here). ARGENTINA. Chaco, Cote Lai, 25 June 1939, fl., fr., *T. Meyer 2640* (SI barcode SI000621!; isolectotypes: GH barcode GH00057534!, LIL barcode LIL000196!).

Distribution. Restricted to Argentina, Paraguay and Brazil (states of Ceará, Mato Grosso and Mato Grosso do Sul).

Nomenclatural notes. Schulz (1942), when describing his new *E. meyeri,* cites two specimens from the same collection, one housed at SI and another at GH. Furthermore, a third specimen, housed at LIL was found by us. After carefully analysing the syntypes, we noticed that the specimen at SI perfectly matches the original illustration. Furthermore, it is widely known that Schulz worked at the Instituto de Botánica Darwinion, thus, making the specimen at SI the obvious choice of a lectotype.

Taxonomical notes. Current databases (eMonocot 2010; The Plant List 2013; Govaerts 2018; Tropicos.org 2018) have treated *E. meyeri* (≡ *P. meyeri*) as a synonym of *E. paniculata* (≡ *P. paniculata*). Nonetheless, as indicated in our identification key and by Horn (1998), both species are distinct, being easily differentiated in the field and herbaria. Thus, *E. meyeri* is here re-established and transferred to *Pontederia s.l.*

1.2. Pontederia paniculata Spreng., Neue Entdeck. Pflanzenk. 3: 18. 1822.

Piaropus paniculatus (Spreng.) Small, Fl. S.E. U.S. (ed. 2): 1328. 1913.
Eichhornia paniculata (Spreng.) Solms, Monogr. Phan. 4: 530. 1883.
Cabanisia caracasana Klotzsch *ex* Schltdl., Abh. Naturf. Ges. Halle 6: 176. 1862, nom. superfluous. Neotype (designated here). BRAZIL. S.loc., fl., Mar 1817, M. Wied s.n. (BR barcode BR0000005188734!).

Distribution. *Pontederia paniculata* possesses a peculiarly disjunctive distribution between North-eastern Brazil (states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Rio Grande do Norte and Sergipe), north-western South America (Colombia, Ecuador, Guyana and Venezuela), Central America (Nicaragua), Antilles (Cuba and Jamaica) and North America (Mexico).

Nomenclatural notes. When describing *P. paniculata*, Sprengel (1822) makes no mention of any specimen, just mentioning that his newly described species is native to

Brazil. According to Stafleu and Cowan (1985), Sprengel's herbarium was acquired by B, but later entirely lost during the WWII. The specimen *Wied s.n.* (BR0000005188734) is an excellent match to the diagnosis provided by Sprengel, was collected prior to the publication of *P. paniculata* and was originally part of the Martius Herbarium. Despite having no proof that this specimen might have been examined by Sprengel, this specimen was surely available at the time of the publication, being originally identified as *P. paniculata* and later examined by Seubert (1847) and identified as *Eichhornia tricolor* Seub, thus making it a good choice for a neotype for *P. paniculata* and being here designated as such.

Taxonomical notes. The very evident disjunctions in the distribution of *P. paniculata* might indicate a species complex, instead of a sole species. Nonetheless, we believe that without proper studies, it would be precocious to re-establish any names or recognise any new taxa at this time.

1.3. *Pontederia paradoxa* Mart. in Schultes & Schultes f., Syst. Veg. (ed. 15 bis) 7(2): 1144. 1830.

Eichhornia paradoxa (Mart.) Solms, Monogr. Phan. 4: 531 1883.
Eichhornia schultesiana Seub., Fl. Bras. 3(1): 94. 1847, nom. superfluous. Lectotype (designated here). BRAZIL. Maranhão: Alcântara oppidium at ad Porto de Carvalho, fl., fr., 1817, C.F.P. Martius 2575 (M barcode M0242209!).

Distribution. *Pontederia paradoxa* has a disjunctive distribution between Northern and North-eastern Brazil (states of Pará, Bahia, Ceará, and Rio Grande do Norte), north-western South America (Venezuela) and Central America (Costa Rica and Guatemala).

Nomenclatural notes. In the original description of *P. paradoxa* (Schultes and Schultes f. 1830), it is mentioned that the description was based on a Martius collection, from the state of Maranhão, Brazil. After consulting M, we came across the specimen *Martius 2575* (M0242209) that matches the protologue in great detail. Thus, it is the obvious choice for a lectotype. Later, Seubert (1847) noticed that *P. paradoxa* did not fit in the circumscription of *Pontederia* at the time. When describing *E. schultesiana*, Seubert clearly mentions *P. paradoxa*, even citing the *Martius 2575* specimen. According to the Code (McNeill et al. 2012, Art. 52.1.), Seubert provided a superfluous replacement name, thus rendering *E. schultesiana* illegitimate.

Taxonomical notes. Similarly as *P. paniculata*, *P. paradoxa* possesses a highly mind-boggling distribution, which makes us believe that it might actually represent a species complex. Two names are available for the putative disjunctive taxa, but since *P. paradoxa* in its current circumscription is known for only a handful of specimens, we discourage any taxonomic changes before the species is properly studied.

2. *Pontederia* subg. *Monochoria* (C.Presl) M.Pell. & C.N.Horn, comb. et stat. nov.

urn:lsid:ipni.org:names:77188078-1

Fig. 6

Monochoria C.Presl, Reliq. Haenk. 1(2): 127. 1827. Type species. *Monochoria hastifolia* C.Presl., nom. illeg. (≡ *P. hastata* L.).

Calcarunia Raf., Med. Fl. 2: 106. 1830. Type species. *Calcarunia hastata* (L.) Raf., nom. inval. (≡ *P. hastata* L.).

Carigola Raf., Fl. Tellur. 2: 10. 1837. Type species. *Carigola hastata* (L.) Raf. (≡ *P. hastata* L.).

Gomphima Raf., Fl. Tellur. 2: 10. 1837. Type species. *Gomphima vaginalis* (Burm.f.) Raf. (≡ *P. vaginalis* Burm.f.).

Kadakia Raf. Fl. Tellur. 2: 9. 1837. Type species. *Kadakia dilatata* (Buch.-Ham.) Raf. (= *P. hastata* L.). **Syn. nov.**

Limnostachys F.Muell., Fragm. 1: 24. 1858. Type species. *Limnostachys cyanea* F.Muell. [≡ *P. cyanea* (F.Muell.) M.Pell. & C.N.Horn].

Description. *Herbs* perennial or annual, aquatic to amphibious, erect-emergent or procumbent-emergent. *Rhizome* short to elongated. *Stems* erect, spongy, branching at the base. *Sessile leaves* early deciduous. *Petiolate leaves* distichously to spirally-alternate, congested at the apex of the stem, sometimes evenly distributed along the stem, floating to emergent, ligule truncate, petioles not-inflated, blades cordate to broadly cordate, rarely elliptic to narrowly ovate. *Main florescences (inflorescences)* terminal, sessile or pedunculate; inflorescence leaf with an inflated leaf-sheath; basal bract tubular, apex caudate, sometimes acute to acuminate, rarely leaf-like; cincinni alternate or fascicle-like, 1–3-flowered, sessile or pedunculate, internodes elongate, rarely contracted. *Flowers* pedicellate, chasmogamous, monostylous, actinomorphic or zygomorphic, enantiostylous, perianth connate only at base, campanulate, spirally-coiled at post-anthesis, non-deliquescent and tightly enclosing the developing fruit, lobes 3 superior and 3 inferior, the central superior lobe lacking a nectar guide; stamens unequal, filaments connate forming a petalo-staminal tube, J-shaped or recurved-decurved, glabrous, anthers basifixed, poricidal, dehiscent through two apical pores; ovary with 3 fertile locules, multi-ovulate, septal nectaries absent, style glabrous, stigma capitulate to capitate or trilobate to trifid. *Capsules* loculicidal, ellipsoid to oblongoid to subglobose; anthocarp thickened, ridged. *Seeds* cylindrical or ellipsoid to narrowly oblongoid to broadly oblongoid to subglobose or ovoid, testa longitudinally winged.

Circumscription. *Pontederia* subg. *Monochoria* is composed of ten exclusively Paleotropical species. All species occur in permanently moist environments or shallow waters, growing either as erect or procumbent-emergent, resembling in habit smaller members of *P.* subg. *Pontederia* and even some species of *Heteranthera*. The members of this subgenus are quite unique within *Pontederia s.l.* due to their pedicellate flowers, perianth only basally connate, unequal stamens, basifixed and poricidal anthers and due to the secondary loss of the septal nectaries.
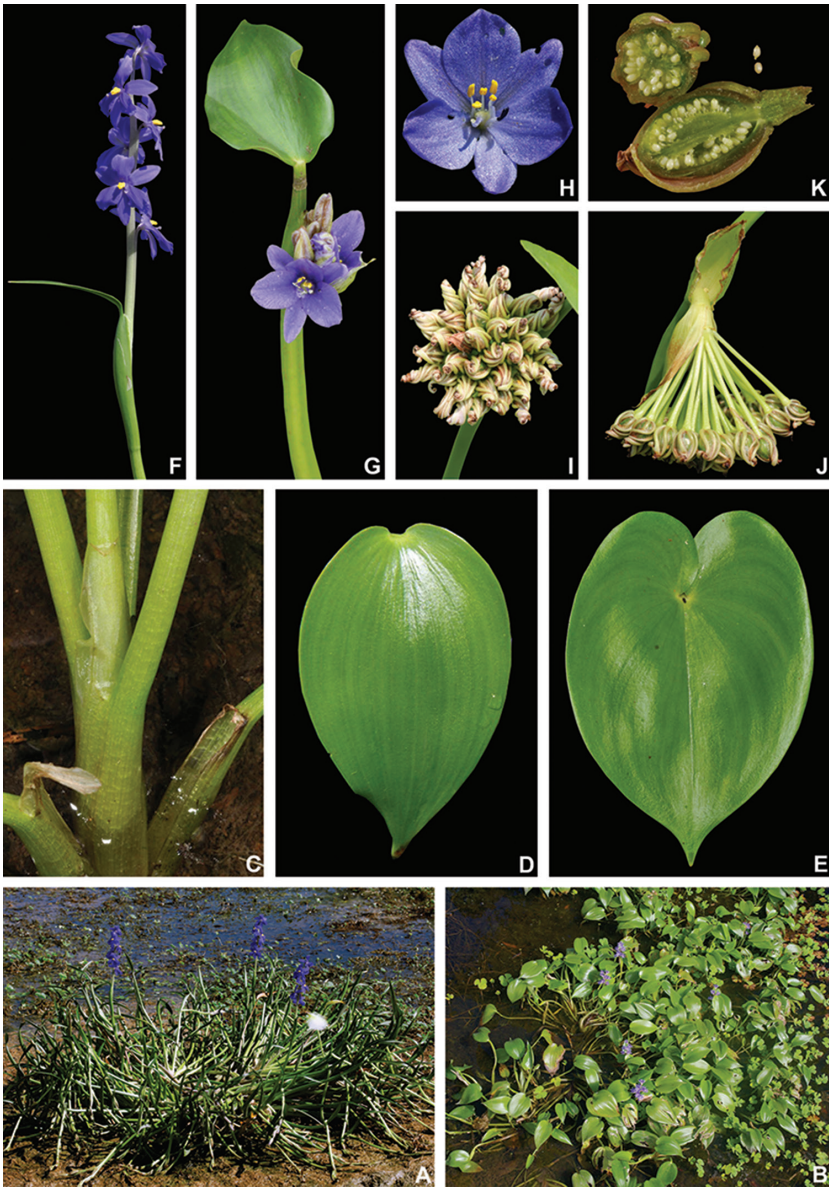
Figure 6. *Pontederia* subg. *Monochoria* (C.Presl) M.Pell. & C.N.Horn. **A–B** habit: **A** paludal habit of *P. australasica* (Ridl.) M.Pell. & C.N.Horn **B** paludal habit of *P. cyanea* (F.Muell.) M.Pell. & C.N.Horn **C** ligule of *P. vaginalis* Burm.f., showing the truncate apex **D–E** petiolate leaf-blades: **D** blade of *P. cyanea*, showing the lack of a posterior division **E** blade of *P. vaginalis*, showing the presence of a posterior division **F–G** inflorescences: **F** inflorescence of *P. australasica*, showing the developed main axis **G** inflorescence of *P. plantaginea* Roxb., showing the contracted main axis **H** front view of a flower of *P. korsakowii* (Regel & Maack) M.Pell. & C.N.Horn **I–J** inflorescences at post-anthesis: **I** erect inflorescence of *P. hastata* L. bearing flowers at post-anthesis J infructescence of *P. hastata*, showing the deflexed posture and the elongated pedicels K sections of immature capsules of *P. vaginalis*, showing developing seeds. **A, F** by M. Barritt **B** by R. Cumming **C, E, K** by P.B. Pelser & J.F. Barcelona **D** by A. & S. Pearson **G** by D. Valke **H** by Ashitaka-f Studio and **I** & **J** by Cerlin Ng.

Distribution. Exclusively Paleotropical (Cook 1989), with two species native to Africa (Verdcourt 1961), four to Australia (two endemic, Aston 1985) and six to Asia (Wang et al. 2004).

Key to the species of *Pontederia* subg. *Monochoria*

1   Filaments without a tooth-like appendage, anthers yellow; stigma trilobate to trifid, with glandular hairs...**2**
–   Central inferior filament with 1(–2) tooth-like appendage, anthers greyish-blue to purple, remaining stamens with unappendaged filaments and yellow anthers; stigma capitulate to capitate, with eglandular hairs **3**
2   Petiole of the leaves bearing inflorescences shorter than or ca. equal to the length of its leaf-sheath; anthers equal or longer than the filaments..............
        ....*P. australasica* (Ridl.) M.Pell. & C.N.Horn
–   Petiole of the leaves bearing inflorescences 2/5 to 5 times longer than its leaf-sheath; anthers smaller than the filaments ....
        .... *P. cyanea* (F.Muell.) M.Pell. & C.N.Horn
3   Basal bract leaf-like, rarely reduced to a bladeless sheath, lower cincinni 3–several-flowered; capsules ovoid; seeds cylindrical..
        ....*P. korsakowii* (Regel & Maack) M.Pell. & C.N.Horn
–   Basal bract always reduced to a bladeless sheath, lower cincinni 1(–2)-flowered; capsules ellipsoid to broadly ellipsoid; seeds oblongoid to ellipsoid or ovoid or subglobose **4**
4   Rhizome robust; petiolate leaves with posterior divisions with acuminate apex; flowers opening from apex to base of the inflorescence; perianth strongly spirally-coiled at post-anthesis **5**
–   Rhizome delicate to inconspicuous; petiolate leaves with posterior divisions generally absent, if present posterior divisions with round apex; flowers opening from base to apex of the inflorescence; perianth strongly patent to slightly spirally-coiled at post-anthesis...**7**
5   Petioles longitudinally sulcate, leaves narrowly hastate or narrowly sagittate to linear sagittate, narrower than 3 cm wide; inflorescences surpassing the leaves; inner tepals obovate ....*P. elata* (Ridl.) M.Pell. & C.N.Horn
–   Petioles smooth, leaves hastate to broadly hastate or sagittate to broadly sagittate, equal or broader than 8 cm wide; inflorescences shorter than the leaves; inner tepals elliptic to oblong..**6**
6   Petiolate leaf-blades patent, posterior division 2–5 cm long; inflorescences sessile to subsessile, cincinni fascicle-like ....*P. hastata* L.
–   Petiolate leaf-blades upright, posterior division 7–11 cm long; inflorescences pedunculate, cincinni alternate ....
        ....*P. valida* (G.X.Wang & Nagam.) M.Pell. & C.N.Horn
7   Leaf blades patent; thyrsi lax, raceme- or fascicle-like, deflexed post-anthesis and in fruit; pedicels ca. as long as the floral buds **8**

–   Leaf blades pendulous; thyrsi dense, spike-like, erect post-anthesis and in fruit; pedicels equal to shorter than ½ the length of the floral buds.............**9**

8   Petiolate leaf-blades without posterior divisions, base round to obtuse, sometimes auriculate; inflorescence 2–7-flowered; seeds oblongoid, longitudinally conspicuously winged .... *P. plantaginea* **Roxb.**

–   Petiolate leaf-blades with conspicuous posterior divisions, base characteristically cordate; inflorescence 9–25-flowered; seeds ovoid, longitudinally inconspicuously winged...*P. vaginalis* **Burm.f.**

9   Petiolate leaves cordate to ovate, leaves bearing inflorescences with petioles (5–)10–12(–16) cm long; seeds ellipsoid to narrowly oblongoid, with 8–10 longitudinal wings .... *P. africana* **(Solms) M.Pell. & C.N.Horn**

–   Petiolate leaves narrowly ovate to elliptic to linear, leaves bearing inflorescences with petioles (0.7–)1–2(–4) cm long; seeds subglobose to broadly oblongoid, with 12–14 longitudinal wings ....*P. brevipetiolata* **(Verdc.) M.Pell. & C.N.Horn**

2.1. *Pontederia africana* (Solms) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:77188080-1

Monochoria africana (Solms) N.E.Br., Fl. Trop. Afr. 8: 5. 1901.
Monochoria vaginalis var. *africana* Solms, Monogr. Phan. 4: 525. 1883. Holotype. B†; Lectotype (designated here). CENTRAL AFRICAN REPUBLIC: Djur Region, Seriba Ghattas, fl., 27 Aug 1869, G.A. Schweinfurth 2296 (PRE barcode PRE0792113-0!; isolectotypes: K barcodes K000321232!, K000321233!).

Distribution. Angola, Kenya, Malawi, Mozambique, South Africa and Sudan.
  Nomenclatural notes. Solms-Laubach (1883) clearly designates the specimen at B as the holotype for his new taxon *Monochoria vaginalis* var. *africana*. However, since the holotype was destroyed during WWII (Cook 1989), a lectotype is needed. The specimen at PRE is in great condition and possesses a complete preserved individual, thus being selected by us as the lectotype.

2.2. *Pontederia australasica* (Ridl.) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:60476936-2

Monochoria australasica Ridl., J. Straits Branch Roy. Asiat. Soc. 79: 100. 1918. Lectotype (designated by Aston 1985). AUSTRALIA. Northern Territory near Darwin, fl., fr., 4 Feb 1914, C.E.F. Allen 81 (K barcode K000873495!; isolectotype: NSW barcode NSW686319!).

Distribution. Restricted to northern Australia.

2.3. *Pontederia brevipetiolata* (Verdc.) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:60476937-2

Monochoria brevipetiolata Verdc., Kirkia 1: 81 1961. Type. GUINEA-BISSAU. Gabú, depressóes alagadas de savana entre Pitche e Canquelifá, fl., fr., 18 Sep 1950, J.V.G. Espírito Santo 2777 (holotype: K barcode K000321231!).

Distribution. Gabón, Gambia, Guinea-Bissau, Ivory Coast, Mali, Níger, Senegal and Sierra Leone.

2.4. *Pontederia cyanea* (F.Muell.) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:60476938-2

Monochoria cyanea (F.Muell.) F.Muell., Fragm. 8: 44. 1872.
Limnostachys cyanea F.Muell., Fragm. 1: 24. 1858. Lectotype (designated by Aston 1985). AUSTRALIA. Northern Territory, Depot Creek, upper Victoria River, fl., fr., 1 Apr 1856, F.W.L. Leichhardt s.n. (K barcode K000873493!: isolectotypes: G barcode G00164431!, K barcode K000873494!, MEL barcodes MEL665251! MEL665252!).

Distribution. Restricted to northern and western Australia.

2.5. *Pontederia elata* (Ridl.) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:77188081-1

Monochoria hastata var. *elata* (Ridl.) Backer, Fl. Males. 4: 258. 1951.
Monochoria elata Ridl., J. Straits Branch Roy. Asiat. Soc. 79: 99. 1918. Lectotype (designated by Cook 1989). MALAYSIA. Kedah: Jenun, fl., fr., 19 Nov 1915, M. Haniff 1208 (K barcode K000291970!; isolectotypes: BM barcode BM000958428!, K barcode K000291971!).

Distribution. From Myanmar to Malaysia, Thailand and China.

Taxonomical notes. *Monochoria elata* (≡ *P. elata*) was treated by Cook (1989) as well as Guofang and Horn (2000) as an accepted name, but subsequent floras (e.g. Wang et al. 2004) and online databases (eMonocot 2010; The Plant List 2013; Govaerts 2018; Tropicos.org 2018) have either considered *M. elata* a synonym of *M. hastata* (≡ *P. hastata*) or as a variety of the latter. Nonetheless, both species can be easily differentiated based on the petiolate ornamentation, the width of the petiolate leaf-blades, length of their inflorescences and number of flowers per inflorescence. Thus, *M. elata* is here re-established and transferred to *Pontederia s.l.*

2.6. *Pontederia hastata* L., Sp. Pl. 1: 288. 1753.

Monochoria hastata (L.) Solms, Monogr. Phan. 4: 523. 1883.
Carigola hastata (L.) Raf., Fl. Tellur. 2: 10. 1837.
Calcarunia hastata (L.) Raf., Med. Fl. 2: 106. 1830. Lectotype (designated by Horn
    and Haynes 1987). SRI LANKA. Herb. P. Hermann 2: 52, No. 129 (BM barcode
    BM000621681!).

Distribution. Bangladesh, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal,
New Guinea, Philippines, Sri Lanka, Thailand and Vietnam.

2.7. *Pontederia korsakowii* (Regel & Maack) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:77188082-1

Monochoria vaginalis var. *korsakowii* (Regel & Maack) Solms, Monogr. Phan. 4: 525.
    1883.
Monochoria korsakowii Regel & Maack, Mém. Acad. Imp. Sci. Saint Pétersbourg, Sér.
    7, 4(4): 155. 1861. Lectotype (designated here). RUSSIA. Ussuri, Keugxa Laa, fl.,
    fr., 1859, R.K. Maack s.n. (LE barcode LE01007092!; isolectotypes: K barcode
    K000873544!; LE barcodes LE01007090!, LE01007091!, LE01007093!, P bar-
    code P00730337!).

Distribution. China, India, Indonesia, Japan, Korea, Malaysia, Pakistan, Russia, Sri
Lanka and Vietnam.
    Nomenclatural notes. Cook (1989), in his revision for *Monochoria*, cites one of
the specimens at LE as a holotype. Nonetheless, Regel and Maack (1861) make no
direct mention of which herbaria the type specimens were deposited and which speci-
men was to be considered the type. Thus, we designate the specimen LE01007092 as
the lectotype, since it possesses well-preserved flowers and seems to have been a model
for the original illustration.

2.8. *Pontederia plantaginea* Roxb., Fl. Ind. (ed. 1832) 2: 123. 1832.

Monochoria vaginalis var. *plantaginea* (Roxb.) Solms, Monogr. Phan. 4: 524. 1883.
Monochoria plantaginea (Roxb.) Kunth, Enum. Pl. 4: 135. 1843. Lectotype (des-
    ignated here). NEPAL: Nathpur, fl., Aug. 1821, N. Wallich 5096 (K bar-
    code K001104737!; isolectotypes: K barcodes K001104733!, K001104734!,
    K001104735!, K001104736!, K001104738!, K001104739!, K001104740!).
Monochoria vaginalis var. *angustifolia* G.X.Wang, Acta Phytotax. Sin. 41: 569. 2003.
    Type. THAILAND. Koksung: in a marshy place, fl., 18 Sep 1984, N. Fukuoka
    T-36166 (holotype: KYO!; isotypes: A n.v., BKF n.v., L n.v.). **Syn. nov.**

Boottia mairei H.Lév., Cat. Pl. Yun-Nan 131. 1916. Type. CHINA. Yunnan: Dongchuan [Tangdan], fl., Aug 1912, *E.E. Maire s.n.* (holotype: E barcode E00386692!). **Syn. nov.**

Monochoria junghuhniana Hassk., Flora 35: 115. 1852. Lectotype (designated here). INDONESIA. Java, Yogyakarta, Djokjakarta, prope Samas ad affim Opar, fl., s.dat., *Junghuhn s.n.* (L barcode L0041652!). **Syn. nov.**

Monochoria linearis (Hassk.) Miq., Fl. Ned. Ind. 3: 549. 1859.

Pontederia linearis Hassk., Flora 25(2, Beibl.): 4. 1842. Type (not found). INDONE-SIA. Java (L?). **Syn. nov.**

Monochoria ovata Kunth, Enum. Pl. 4: 665. 1843.

Pontederia ovata Hook. & Arn., Bot. Beechey Voy. 218 1837, nom. illeg. non *P. ovata* L. Lectotype (designated here). SRI LANKA. Canton, fl., s.dat., *Millet s.n.* (G barcode G00164757!; isolectotype: E n.v.).

Pontederia cernua L. *ex* B.D.Jacks., Index Linn. Herb.: 129. 1912, nom. nud.

Pontederia alba Buch.-Ham. *ex* Wall., Numer. List: 5095 D. 1831, nom. nud.

Pontederia racemosa Buch.-Ham. *ex* Wall., Numer. List: 5095C. 1831, nom. nud.

Pontederia lanceolata Wall. *ex* Kunth, Enum. Pl. 4: 135. 1843, pro. syn.

Distribution. Australia, Bangladesh, Cambodia, China, India, Indonesia, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam.

Nomenclatural notes. Cook (1989) cites that no suitable specimens, collected by Roxburgh, were found. Nonetheless, according to Stafleu and Cowan (1983) and Forman (1997), many of Roxburgh's new species described after 1831 were based on specimens at the Wallich Herbarium (currently housed at K). After visiting Kew, we came across a series of specimens at Wallich Herbarium (*Wallich 5096*), collected in the Bengal region (Bangladesh, Myanmar, Nepal and India), that perfectly matched the protologue of *P. plantaginea*. One of the herbarium sheets contained several complete flowering specimens in perfect condition. Thus, we designate the gathering under the barcode K001104737, as the lectotype for *P. plantaginea*.

Hasskarl (1852), when describing *M. junghuhniana*, makes no reference to any specimens. However, the author does mention that his new species is native to Sumatra, near Samas and Opar. After analysing the collection at L, we came across a specimen from exactly the same locality as indicated in the protologue and most likely collected by Junghuhn. Thus, it is designated by us as the lectotype for *M. junghuhniana*.

Taxonomical notes. *Monochoria vaginalis,* in its current circumscription (Cook 1989), is widely morphologically variable and distributed. However, recent studies (Wang et al. 2003; Tungmunnithum et al. 2016) have highlighted the need to revisit the species boundaries in this taxon. Recently, Tungmunnithum et al. (2016) published a thorough morphometric study on *M. vaginalis s.l.* from Thailand and showed that two taxa are easily recognisable. The authors informally recognised *M. vaginalis* Burm.f. var. *vaginalis* and *M. vaginalis* var. *angustifolia* G.X.Wang as representing each of the recovered morphotypes. Nonetheless, after studying all the names treated as synonyms of *M. vaginalis s.l.* by Cook (1989), we concluded that *M. vaginalis* var. *angustifolia* and *M. junghuhniana* are conspecific to *P. plantaginea* Roxb. Thus, *P. plan-*

taginea is here re-established and *M. vaginalis* var. *angustifolia* and *M. junghuhniana* are treated as synonyms of the latter.

2.9. *Pontederia vaginalis* Burm.f., Fl. Indica: 80. 1768.

Monochoria vaginalis (Burm.f.) C.Presl *ex* Kunth, Enum. Pl. 4: 134. 1843.
Gomphima vaginalis (Burm.f.) Raf., Fl. Tellur. 2: 10. 1837.
Monochoria hastifolia C.Presl., Reliq. Haenk. 1(2): 127. 1827, nom. illeg. Lectotype
    (designated by Cook 1989). INDIA. Ind. Orien., fl., s.dat., W. Roxburgh s.n. (G
    barcode G00164756!).

Distribution. Widespread throughout Asia (Afghanistan, Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Iran, Japan, Korea, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Russia, Sri Lanka, Thailand and Vietnam) and Oceania (Australia, Fiji, Papua New Guinea and Pacific Islands).

2.10. *Pontederia valida* (G.X.Wang & Nagam.) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:60476939-2

Monochoria valida G.X.Wang & Nagam., Acta Phytotax. Geobot. 45(1): 41. 1994.
    Type. CHINA. Hainan: Sanya, Yanglan, fl., 21 Sep 1990, G.X. Wong 901001
    (holotype: WH; isotype: KYO!).

Distribution. Southern China and Thailand.
    Taxonomical notes. *Monochoria valida* (≡ *P. valida*) was described by Wang and Nagamasu (1994), being compared to *M. elata* (≡ *P. elata*) and *M. hastata* (≡ *P. hastata*). These species are morphologically similar, due to their robust rhizomes, petiolate blades hastate to sagittate and posterior division with acuminate apex. However, they can be easily differentiated by inflorescence morphology (inflorescence sessile, many-flowered, not surpassing the leaves and cincinni fascicle-like in *P. hastata*; inflorescence pedunculate, many-flowered, surpassing the leaves and alternate cincinni in *P. valida*; inflorescence pedunculate, few-flowered, surpassing the leaves and alternate cincinni in *P. elata*). Aside from that, leaf morphology is also helpful in species delimitation in this group. Thus, *M. valida* is here re-established and transferred to *Pontederia s.l.*

3. *Pontederia* subg. *Oshunae* M.Pell. & C.N.Horn, subg. nov.
urn:lsid:ipni.org:names:77188083-1
Fig. 7

Piaropus Raf., Fl. Tellur. 2: 81. 1837, nom. rej. Type species. *Piaropus mesomelas* Raf.,
    nom. illeg. (≡ *Pontederia crassipes* Mart.). **Syn. nov.**
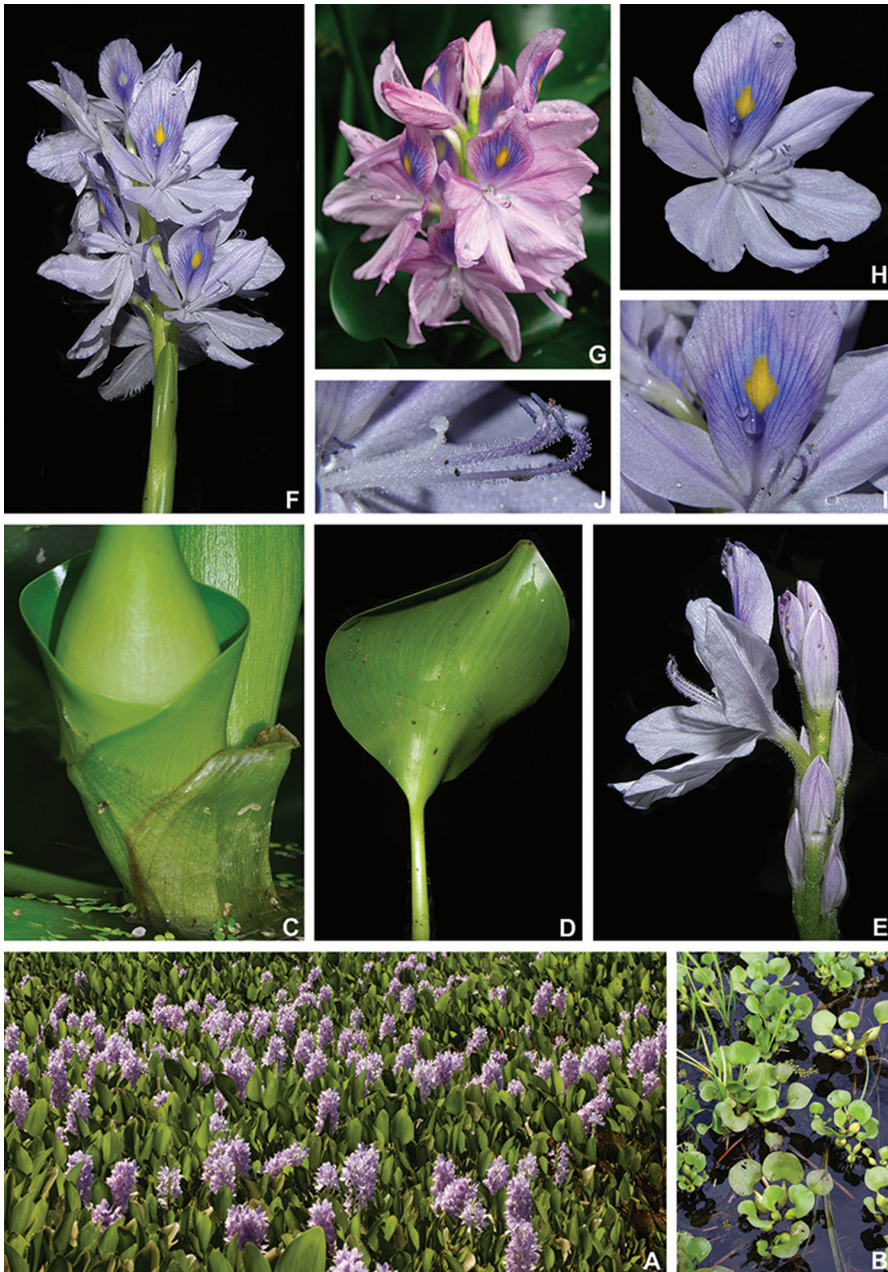
Figure 7. *Pontederia* subg. *Oshunae* M.Pell. & C.N.Horn. **A–B** habit: **A** dense population of the pink-flowered form **B** detail of a population, showing the free-floating rosettes, stolons and inflated petioles **C–D** petiolate leaves: **C** blade **D** detail of a young leaf showing its blade enclosing the inflated petiole of the presiding leaf **E–G** inflorescence: **E** young inflorescence of a lilac-flowered form **F** inflorescence of a lilac-flowered form at anthesis G inflorescence of a pink-flowered form at anthesis **H–J** flowers: **H** oblique view of a lilac flower **I** detail of the nectar guide J detail of the androecium and gynoecium showing the glandular hairs. All photos of *P. crassipes* Mart.; **A** by C. Willig & L. Nusbaumer **B** by O. Gaubert **C** by K. Pritchard & S.A. Harris, **D–F, H–I** by R. Aguilar and **G** by M.O.O. Pellegrini.

Type species. *Pontederia crassipes* Mart. [≡ *Eichhornia crassipes* (Mart.) Solms].

Description. *Herbs* perennial, aquatic, free-floating. *Rhizome* short and inconspicuous. *Stems* inconspicuous, unbranched, producing stolons. *Sessile leaves* early deciduous. *Petiolate leaves* spirally-alternate, congested at the apex of the stem, emergent, ligule flabellate, petioles inflated, blades broadly ovate to cordate to reniform. *Main florescences (inflorescences)* terminal; inflorescence leaf without an inflated leaf-sheath; basal bract tubular; cincinni alternate, 1(–2)-flowered, sessile, internodes contracted. *Flowers* sessile, tristylous, zygomorphic, non-enantiostylous, perianth connate forming a tube, hypocrateriform, spirally-coiled at post-anthesis, deliquescent and loosely enclosing the developing fruit, 3 superior and 3 inferior, the central superior lobe with a nectar guide, consisting of 1 yellow spot, surrounded by a dark purple to bluish-purple blur; stamens dimorphic, filaments free from each other, J-shaped, glandular-pubescent, anthers dorsifixed, rimose; ovary with 3 fertile locules, multi-ovulate, septal nectaries present, style glandular-pubescent, stigma capitate to trilobate. *Capsules* loculicidal, oblongoid; anthocarp thin, smooth. *Seeds* oblongoid, testa longitudinally winged.

Circumscription. *Pontederia* subg. *Oshunae* is monospecific, being composed solely by *P. crassipes*.

Distribution. Widespread throughout South America.

Etymology. The name of this new subgenus derives from the Yoruba words "*Oxum*", "*Oshun*" and "*Osun*". These are the names given in the Candomblé religion to the orisha (i.e. a deity that reflects one of the manifestations of God) mother and guardian of freshwater bodies. Oshun is known for her beauty and vanity, being also known as the deity of luxury, pleasure, sexuality, fertility, beauty and love. The sole species accepted in *Pontederia* subg. *Oshunae* is commonly named "*mãe d'água*" (i.e. mother of the freshwaters) in Brazil, also one of the popular names for Oshun. This popular name in Brazil makes reference to the water-hyacinth's ability to dominate freshwater environments, as well as its ability to produce beautiful flowers.

3.1. *Pontederia crassipes* Mart., Nov. Gen. Sp. Pl. 1: 9. 1823.

Eichhornia crassipes (Mart.) Solms, Monogr. Phan. 4: 527. 1883.
Piaropus mesomelas Raf., Fl. Tellur. 2: 81. 1837, nom. illeg. Lectotype (designated by Horn 1994). BRAZIL. Bahia. Provinciae Minas Gerais, in stagnis ad fl. St. Francisci prope Malhada, s.dat., C.F.P. Martius 60 (M barcode M0242217!).

Distribution. Widespread throughout South America and naturalised worldwide.

4. *Pontederia* subg. *Eichhornia* (Kunth) M.Pell. & C.N.Horn, comb. et stat. nov.
urn:lsid:ipni.org:names:77188084-1
Fig. 8

Eichhornia Kunth, Enum. Pl. 4: 129. 1843. Type species. *Eichhornia azurea* (Sw.)
    Kunth. (≡ *P. azurea* Sw.).
Leptosomus Schltdl., Abh. Naturf. Ges. Halle 6: 174. 1862. Type species. *Leptosomus
    natans* (P.Beauv.) Schltdl. (≡ *P. natans* P.Beauv.).

Description. *Herbs* perennial, aquatic, procumbent-emergent. *Rhizome* short and gen-
erally inconspicuous. *Stems* trailing, spongy, branched to unbranched. *Sessile leaves* late
deciduous, sometimes persistent in mature plants. *Petiolate leaves* distichously-alter-
nate, evenly distributed along stem, emergent, ligule truncate, petioles not-inflated,
blades cordate to ovate or obovate to broadly obovate to rounded. *Main florescences
(inflorescences)* axillary or terminal, pedunculate; inflorescence leaf without an inflated
leaf-sheath; basal bract tubular; cincinni alternate, 1–3-flowered, sessile to subsessile,
internodes contracted. *Flowers* sessile, chasmogamous, tristylous or pseudo-homosty-
lous, zygomorphic, non-enantiostylous, perianth connate forming a tube, infundibu-
liform, revolute at post-anthesis, deliquescent and loosely enclosing the developing
fruit, lobes 3 superior and 3 inferior, rarely 5 superior and 1 inferior, the central supe-
rior lobe with a nectar guide, consisting of 2 yellowish-green to green spots, generally
surrounded by a dark purple to bluish-purple, rarely white blur, coiling or post-anthe-
sis; stamens dimorphic, filaments free from each other, J-shaped, glandular-pubescent,
anthers dorsifixed, rimose; ovary with 3 fertile locules, multi-ovulate, septal nectaries
present, style glabrous, stigma capitate to trilobate. *Capsules* loculicidal or with irregu-
lar dehiscence, ellipsoid to oblongoid; anthocarp thin, smooth. *Seeds* subglobose to
broadly oblongoid, testa longitudinally winged.

Circumscription. *Pontederia* subg. *Eichhornia* is composed of four species. All spe-
cies occur in permanently or seasonal water bodies, growing as procumbent-emergent
and resembling in habit some members of *P.* subg. *Monochoria* and *P.* subg. *Pontede-
ria.* The members of this subgenus are peculiar within *Pontederia s.l.* due to their late
deciduous sessile leaves (sometimes persistent throughout the plant's entire lifespan),
perianth infundibuliform, revolute at post-anthesis, deliquescent and loosely enclosing
the developing fruit, glandular-pubescent filaments, glabrous styles and anthocarp thin
and smooth.

Distribution. Mainly Neotropical, except for *P. natans*, which is restricted to con-
tinental Africa and Madagascar.

Key to the species of *Pontederia* subg. *Eichhornia*

1   Petiolate leaves floating, blades cordate to ovate, base auriculate to cordate;
        inflorescences 1–4-flowered; flowers pseudo-homostylous; margins if the in-
        ternal lobes of the perianth entire...**2**
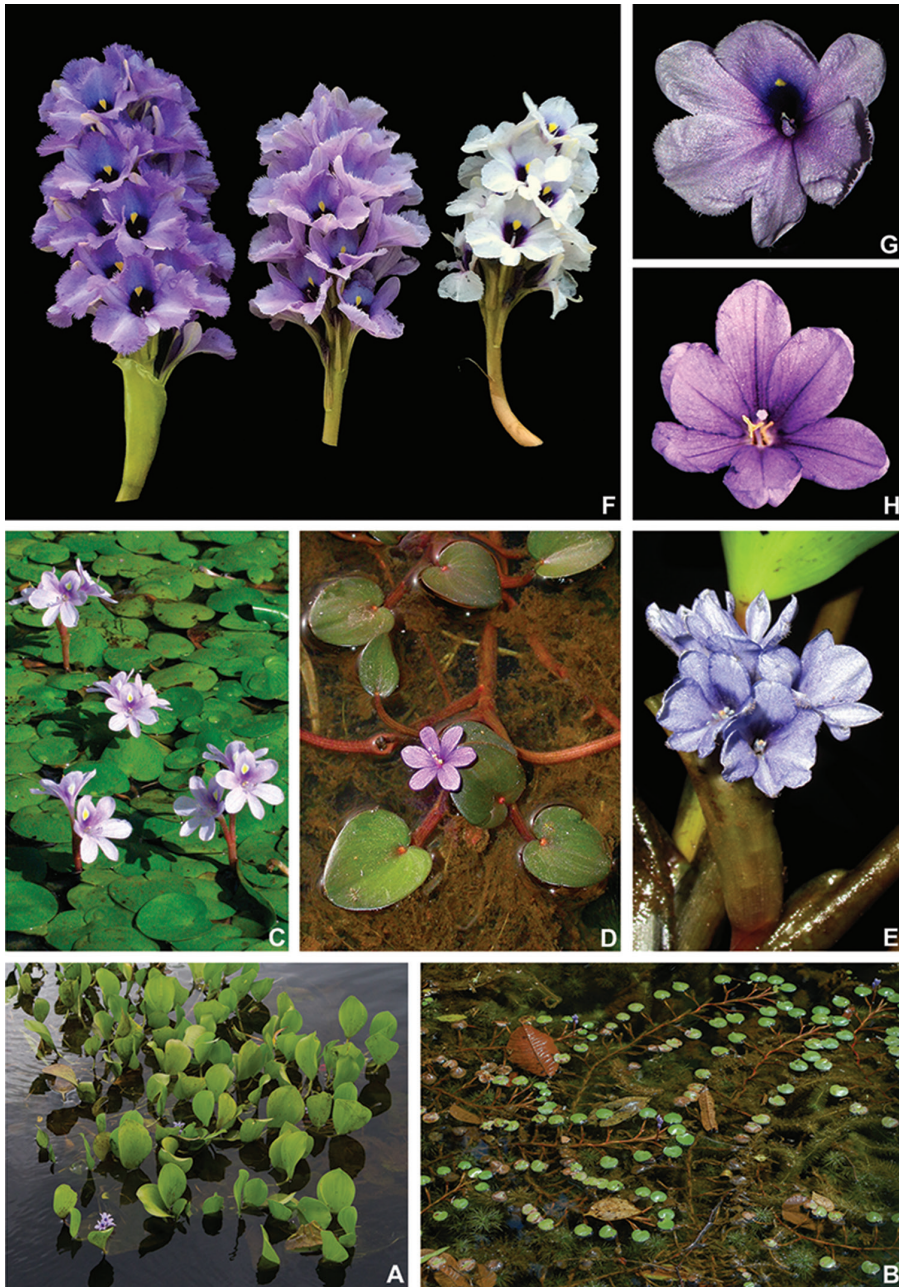
Figure 8. *Pontederia* subg. *Eichhornia* (Kunth) M.Pell. & C.N.Horn. **A–B** habit: **A** habit of *P. heterosperma* (Alexander) M.Pell. & C.N.Horn, showing the emerged petiolate leaves **B** habit of *P. diversifolia* (Vahl) M.Pell. & C.N.Horn, showing the floating petiolate leaves **C–F** inflorescence: **C** 2–3-flowered inflorescences of *P. diversifolia*, showing the flowers with a yellow nectar guide in the posterior perianth lobes **D** 1-flowered inflorescence of *P. natans* P.Beauv., showing the lack of a nectar guide **E** inflorescence of *P. heterosperma*, showing the lack of nectar guides in the posterior perianth lobes **F** morphological variation of inflorescences and perianth colour of *P. azurea* Sw **G** front view of a flower of *P. azurea* H front view of a flower of *P. natans*. **A, B** by O. Gaubert **C** by A.S. Castro **D** by P. Birnbaum **E** by H. Medeiros **F** by L.O.A. Teixeira **G** by M.O.O. Pellegrini and I by T.C. Buruwate.

–   Petiolate leaves emergent, blades obovate to broadly obovate to rounded, base cuneate; inflorescences 5–many-flowered; flowers heterostylous; margins of the internal lobes of the perianth erose to fimbriate, rarely entire **3**

2   Inflorescences (1–)2–4-flowered; flowers 2–3.2 cm diam., perianth lilac to bluish-lilac, central superior lobe with a yellow spot, surrounded by a purple to bluish-purple blur, filaments glandular-pubescent; capsules 3-valved ....*P. diversifolia* **(Vahl) M.Pell. & C.N.Horn**

–   Inflorescences 1(–2)-flowered; flowers 0.7–1 cm diam., perianth purple to mauve, central superior lobe concolorous with the remaining lobes or with a dark purple blur, filaments glabrous; capsules with irregular dehiscence ....*P. natans* **P.Beauv.**

3   Inflorescences axillary, much exceeding the basal bract, main axis glandular-pubescent; perianth with central superior lobe with a yellow spot, filaments glandular-pubescent; seeds monomorphic................................*P. azurea* **Sw.**

–   Inflorescences terminal, enclosed or approximately the same size as the basal bract, main axis glabrous; perianth with central superior lobe with a dark purple to bluish-purple blur, filaments glabrous; seeds dimorphic................. ....*P. heterosperma* **(Alexander) M.Pell. & C.N.Horn**

4.1. *Pontederia azurea* Sw., Prodr. 57. 1788.

Eichhornia azurea (Sw.) Kunth, Enum. Pl. 4: 129. 1843.
Piaropus azureus (Sw.) Raf., Fl. Tellur. 2: 81. 1837. Type. JAMAICA. s.loc., s.dat., *Brown s.n.* (holotype: S No. S-R-5196!).

Distribution. Widespread in the American continent from Mexico to Uruguay.

4.2. *Pontederia diversifolia* (Vahl) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:77188085-1

Eichhornia diversifolia (Vahl) Urb., Symb. Antill. 4: 147. 1903.
Heteranthera diversifolia Vahl, Enum. Pl. 2: 44. 1805. Lectotype (designated here). GUIANA. s.loc., fl., s.dat., L.C. Richard s.n. (C barcode C10017422!).

Distribution. Antilles (Cuba, Dominican Republic, and Puerto Rico), Central America (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama) and South America (Bolivia, Colombia, Ecuador, French Guiana, Guyana, Suriname, Venezuela and Brazil – states of Acre, Amazonas, Amapá, Pará, Rondônia, Roraima, Tocantins, Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe, Goiás, Mato Grosso do Sul, Mato Grosso, Minas Gerais and Rio de Janeiro).

Nomenclatural notes. When describing *Heteranthera diversifolia*, Vahl (1805) makes no direct mention of any analysed specimens in which he might have based the description of his new species. The author only mentions that his new species is native to Guiana and was sent to him by "Richard". After analysing the collection at C, we came across a specimen part of Herb. Vahlian., collected by *Richard s.n.* and identified in Vahl's handwriting as *H. diversifolia*. Thus, it is chosen by us as the lectotype.

4.3. *Pontederia heterosperma* (Alexander) M.Pell. & C.N.Horn, comb. nov.
urn:lsid:ipni.org:names:77188086-1

Eichhornia heterosperma Alexander, Lloydia 2: 170. 1939. Lectotype (designated here). GUIANA. Basin of Rupununi River, Wichabai, fl., fr., 25–26 Oct 1937, A.C. Smith 2290 (NY barcode NY00247522!; isolectotypes: F barcode F0047046F!, G barcode G00168031!, GH barcode GH00255059!, K barcode K000644009!, MO barcode MO-1936311!, NY barcode NY00247521!, P barcode P00730322!, S No. S05-5985!, U barcode U0005719!, US barcode US00091644!).

Distribution. Antilles (Cuba), Central America (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama) and South America (Bolivia, Colombia, Ecuador, French Guiana, Guyana and Suriname, Venezuela and Brazil – states of Acre, Amazonas, Amapá, Pará, Rondônia, Tocantins, Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe, Goiás, Mato Grosso do Sul, Mato Grosso and Minas Gerais).

4.4. *Pontederia natans* P.Beauv., Fl. Oware 2: 18. 1807.

Eichhornia natans (P.Beauv.) Solms, Abh. Naturwiss. Vereins Bremen 7: 254. 1882.
Leptosomus natans (P.Beauv.) Schltdl., Abh. Naturf. Ges. Halle 6: 174. 1862. Lectotype (designated here). NIGERIA. Benin, fleuve Formosa, fl., fr., s.dat., A.M.F. Palisot de Beauvois s.n. (G on 3ex barcode G00418251!; isolectotype: G-DC on 4ex GDC048496!).

Distribution. Angola, Benin, Botswana, Burkina Faso, Cameroon, Central African Republic, Chad, Congo, Egypt, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Liberia, Madagascar, Mali, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Sudan, Sudan, Tanzania, Togo, Uganda, Zaire, Zambia and Zimbabwe.

Nomenclatural notes. When describing *P. natans*, Palisot de Beauvois (1807) comments that his new species is common at the margins of the Formosa River (currently called Benin River). After analysing specimens from G and G-DC herbarium, we came across two specimens, mounted on seven sheets. The specimen GDC048496

is mounted on four sheets, composed of several flowering and fruiting specimens, with an extremely detailed annotation in the handwriting of Palisot de Beauvois. Nonetheless, the specimen G00418251 is mounted on three sheets, with the second sheet possessing a detached petiolate leaf and a copy of the original illustration and the third possessing the specimen on which the illustration was based. Thus, the G00418251 specimen is the obvious choice for a lectotype.

Taxonomical notes. The African *E. natans* (≡ *P. natans*) is currently treated as a synonym of the Neotropical *Eichhornia diversifolia* (≡ *P. diversifolia*) by all online databases (i.e. eMonocot 2010; The Plant List 2013; Govaerts 2018; Tropicos.org 2018). Nonetheless, as indicated in our identification key (see above), both species can be easily differentiated based on the number of flowers per inflorescence, floral diameter, presence or absence of a nectar guide, pubescence of the filaments and capsule dehiscence. Thus, *P. natans* is here re-established.

5. *Pontederia* L. subg. *Pontederia*
Fig. 9

Michelia Adans., Fam. Pl. 2: 201. 1763, nom. illeg. Type species (designated here). *Pontederia cordata* L.
Narukila Adans., Fam. Pl. 2: 54. 1763, nom. illeg. Type species (designated here). *Narukila cordata* (L.) Nieuwl. (≡ *P. cordata* L.).
Pontederaea Kuntze, Revis. Gen. Pl. 2: 718. 1891, orth. var.
Pontederas Hoffmanns., Verz. Pfl.: 137. 1824, orth. var.
Reussia Endl., Gen. Pl.: 139. 1836. Type species (designated by Lowden 1973). *Reussia triflora* Endl. *ex* Seub. [≡ *P. triflora* (Endl. *ex* Seub.) G.Agostini et al.].
Unisema Raf. Med. Repos. 5: 352. 1808, nom. illeg. Type species. *Unisema obtusifolia* (Raf.) Raf. (≡ *P. cordata* L.).
Umsema Raf. Med. Repos. 5: 352 1808, orth. var.
Unisemma D.A.Godron, in Orbigny CVD, Dict. Univ. Hist. Nat.: 761. 1848, orth. var.

Description. *Herbs* perennial or annual, aquatic to amphibious, erect-emergent or procumbent-emergent. *Rhizome* short and generally inconspicuous. *Stems* erect or trailing, spongy, unbranched to branching only at the base to branched. *Sessile leaves* early deciduous. *Petiolate leaves* distichously-alternate, evenly distributed along the stem or congested at the apex of the stem, emergent, ligule truncate, petioles not-inflated, blades cordate to broadly cordate, rarely elliptic to lanceolate or narrowly ovate. *Main florescences (inflorescences)* terminal, sessile or pedunculate; inflorescence leaf without an inflated leaf-sheath; basal bract flat; cincinni alternate, 1–3-flowered, sessile to shortly-pedunculate, internodes contracted. *Flowers* sessile, tristylous, zygomorphic, non-enantiostylous, perianth connate forming a tube, infundibuliform, revolute at post-anthesis, non-deliquescent and loosely enclosing the developing fruit, lobes 3 superior and 3 inferior, rarely 5 superior and 1 inferior, the central superior
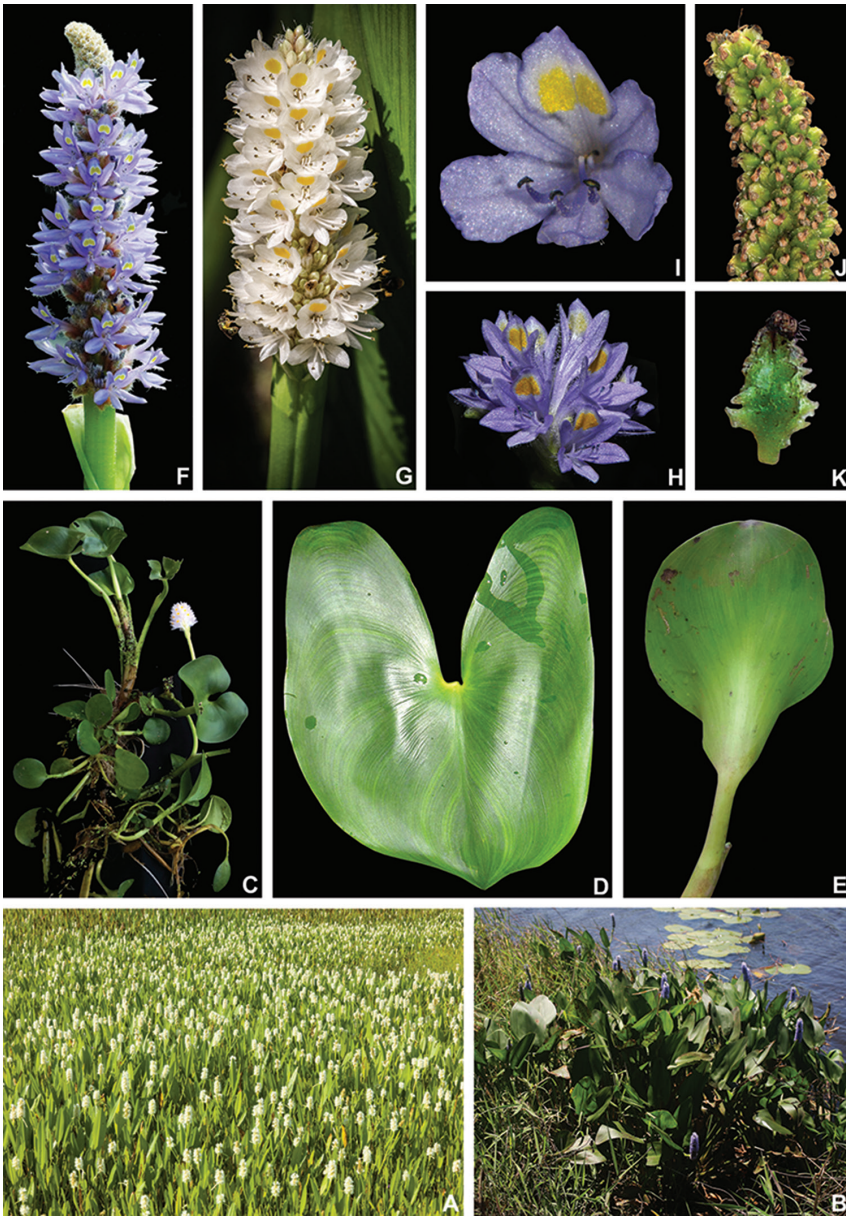
Figure 9. *Pontederia* L. subg. *Pontederia*. **A–C** habit: **A** dense population of *P. parviflora* Alexander **B** population of *P. ovalis* Mart. *ex* Schult. & Schult.f. **C** habit of *P. rotundifolia* L.f. **D–E** petiolate leaves: **D** blade of *P. rotundifolia* **E** blade of *P. parviflora* **F–H** inflorescences: **F** inflorescence of *P. cordata* L., showing flowers with two yellow nectar guides in the posterior perianth lobes **G** inflorescence of *P. parviflora*, showing flowers with a sole yellow nectar guide in the posterior perianth lobes **H** inflorescence of *P. rotudifolia*, showing a lilac-flowered form **I** oblique view of a flower of *P. ovalis* **J–K** fruits: **J** detail of the apex of the infructescence of *P. ovalis*, showing the anthocarp with sinuate ridges **K** detail of an achene of *P. cordata*, showing the toothed ridges. **A** by C. Willig & L. Nusbaumer **B, I, J** by M.O.O. Pellegrini **C** by L.O.A. Teixeira, **D, H** by R. Aguilar **E** by M.R. Engels **F** by Ashitaka-f Studio **G** by M.V. Lameiras and **K** by A. Haines.

lobe with a nectar guide, consisting of 2 yellowish-green to green spots, generally surrounded by a dark purple to bluish-purple, rarely white blur, coiling or post-anthesis; stamens dimorphic, filaments free from each other, J-shaped, glandular-pubescent, anthers dorsifixed, rimose; ovary with 1 fertile locule, 1-ovulate, septal nectaries present, style glandular-pubescent or glabrous, stigma truncate or capitate or trilobate. *Achene* ovoid or pyriform; anthocarp hardened, ridged, ridges sinuate, toothed or echinate. *Seeds* curved narrowly ovoid or ovoid, testa smooth.

Circumscription. *Pontederia* subg. *Pontederia* is circumscribed by us to comprise eight species. Our concept of *P.* subg. *Pontederia* is equivalent to the concept of *Pontederia* adopted by Lowden (1973). Nonetheless, we accept *P. triflora* as distinct from *P. subovata* and increase the number of species in the *P. cordata* complex by the re-establishment of *P. ovalis*. The members of this subgenus are peculiar within *Pontederia s.l.* due to their spike-like main florescences, ovaries 1-locular by abortion, fertile locule 1-ovulate, pendulous placentation, fruit an achene, hardened and ornate anthocarps and smooth seeds.

Distribution. Exclusively Neotropical.

Key to the species of *Pontederia* subg. *Pontederia*

1	Rhizomes absent; stems elongated, trailing; leaves evenly distributed along the stem; anthocarp echinate; seeds straight, ovoid......................................**2**
–	Rhizomes present, short; stems short, erect; leaves congested at the apex of the stem; anthocarp toothed or with sinuate ridges; seeds curved, narrowly ovoid **4**
2	Petiolate leaf-blades with cordate to sagittate base; inflorescences 30–80-flowered, cincinni 2–3-flowered; flowers lilac or light to medium pink, rarely white, perianth lobes with a 3+3 arrangement..................***P. rotundifolia* L.f.**
–	Petiolate leaf-blades with obtuse to cuneate base; inflorescences 2–15-flowered, cincinni 1-flowered; flowers light to medium blue, rarely white, perianth lobes with a 5+1 arrangement **3**
3	Petiolate leaf-blades emergent, elliptic to narrowly ovate to ovate to rhomboid; inflorescences (6–)8–20-flowered............***P. subovata* (Seub.) Lowden**
–	Petiolate leaf-blades floating, linear-lanceolate to linear-elliptic to linear rhomboid; inflorescences 2–4(–5)-flowered ....***P. triflora* (Endl. ex Seub.) G.Agostini et al.**
4	Petioles green, blades with a thickened midvein; inflorescences and flowers covered with light yellow hairs, flowers homostylous, central superior lobe with 1 spot, anthers dark brown to black, style equal in length with the inferior stamens .... ***P. parviflora* Alexander**
–	Petioles red to vinaceous to purple, rarely green, blades lacking a thickened midvein; inflorescences and flower covered with hyaline hairs, flowers tri-

stylous, central superior lobe with 2 spots, anthers yellow or greyish-blue to purple, style either shorter or longer than the inferior stamens....................**5**

5   Basal bract deflexed, main axis glabrous; central superior lobe with 2 green spots, style glandular-pubescent, stigma trilobate; anthocarp with toothed ridges ....***P. cordata* L.**

–   Basal bract upright, main axis velutine or sparsely to densely villose; central superior lobe with 2 yellow spots, style glabrous, stigma truncate; anthocarp with sinuate ridges **6**

6   Petiolate leaf-blades elliptic to narrowly ovate to ovate to broadly ovate; cincinni 2–3-flowered..***P. ovalis* Mart.**

–   Petiolate leaf-blades sagittate to broadly sagittate or hastate to broadly hastate; cincinni 4–6-flowered .... ***P. sagittata* C.Presl**

5.1. *Pontederia cordata* L., Sp. Pl. 1: 288. 1753.

Unisema cordata (L.) Farw., Pap. Michigan Acad. Sci. 3: 91. 1924.

Narukila cordata (L.) Nieuwl., Amer. Midl. Naturalist 3: 101. 1913. Lectotype (designated by Reveal et al. 1987). UNITED STATES. Virginia and Maryland, fl., fr., s.dat., P. Kalm s.n. (LINN barcode LINN-HL407-4).

Pontederia lancifolia Muhl., Cat. Pl. Amer. Sept.: 34. 1813.

Unisema cordata fo. *lancifolia* (Muhl.) Farw., Pap. Michigan Acad. Sci. 3: 92. 1924.

Narukila cordata var. *lancifolia* (Muhl.) Nieuwl., Repert. Spec. Nov. Regni Veg. 12: 101. 1913.

Pontederia cordata var. *lancifolia* (Muhl.) Torr., Fl. N. Middle United States: 343. 1824. Lectotype (designated by Lowden 1973). UNITED STATES. Carolina, fl., fr., s.dat., G.H.E. Muhlenberg 242 (PH barcode PH00033652!).

Distribution. Widely distributed in North, Central and South America from Canada to Uruguay and the West Indies.

Taxonomical notes. *Pontederia cordata* has always been the origin of much debate and taxonomical confusion in the genus. Most of the species currently accepted by us in *Pontederia s.l.* have either been confused or compared with *P. cordata*, at some point. This can be demonstrated by how many of them have been treated either as synonyms or infraspecific taxa by different authors (Fernald 1950; Lowden 1973; Godfrey & Wooten 1979; Novelo & Lot 1994). *Pontederia cordata* is morphologically and phylogenetically related to *P. lancifolia*, with only weak differences related to leaf morphology, thus should not be recognised taxonomically. Otherwise, we believe that, based on the current phylogenetic and morphological data, *P. cordata*, *P. ovalis*, *P. parviflora* and *P. sagittata* should be treated at the species level, until further studies can properly deal with the problem.

5.2. *Pontederia ovalis* Mart. *ex* Schult. & Schult.f., Syst. Veg. (ed. 15 bis) 7(2): 1140. 1830.

Pontederia lanceolata f. *ovalis* (Mart. *ex* Schult. & Schult.f.) A.Cast., Arch. Jard. Bot. Rio de Janeiro 15: 62. 1957.

Pontederia cordata var. *ovalis* (Mart. *ex* Schult. & Schult.f.) Solms, Monogr. Phan. 4: 533. 1883. Lectotype (designated here). BRAZIL. s.loc., fl., s.dat., C.F.P. Martius 14 (M barcode M0242238!).

Distribution. Costa Rica, Guatemala, Honduras, Bolivia, Brazil (states of Bahia, Maranhão, Paraíba, Distrito Federal, Goiás, Mato Grosso, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul), Colombia, Paraguay and Uruguay.

Nomenclatural notes. When describing *P. ovalis*, Schultes and Schultes (1830) mention that their new species is based in Martius specimens from Brazil. However, the author makes no mention in which herbarium the specimens are housed or their collectors' numbers. While consulting the specimens at M, we came across two Martius' specimens (i.e. *Martius 14* M0242238; *Martius 16* M0242244) that matched the protologue of *P. ovalis*. Both specimens were annotated in Martius handwriting and were probably analysed by Schultes. Since the specimen *Martius 14* (M0242238) is a more complete collection, when compared with *Martius 16* (M0242244), which is composed of two detached leaves and two inflorescences, it is selected by us as the lectotype for *P. ovalis*.

Taxonomical notes. *Pontederia ovalis* has been considered by most authors and online databases as either a variety (Dubs 1998, Tropicos.org 2018) or a synonym (Schulz 1942, Tropicos.org 2018) of *P. cordata*. Nonetheless, both morphologically and phylogenetically, *P. ovalis* is much more similar to *P. sagittata*, due to its pubescent inflorescence main axis and fruits with sinuate ridges. Thus, *P. ovalis* is here re-established, being also part of the *P. cordata* species complex.

5.3. *Pontederia parviflora* Alexander, N. Amer. Fl. 19: 59. 1937.

Pontederia cordata var. *parviflora* (Alexander) Schery, Ann. Missouri Bot. Gard. 31: 156. 1944. Lectotype (designated here). PANAMA. Camino del Boticario, near Chapo, fl., Oct 1911, H. Pittier 4556 (NY barcode NY00260019!: isolectotypes: NY barcode NY00260020!, US barcode US00091647!).

Distribution. Panama, Venezuela, Colombia and Brazil (states of Tocantins, Alagoas, Ceará, Maranhão, Paraíba, Pernambuco, Piauí, Distrito Federal, Goiás, Mato Grosso do Sul, Mato Grosso, Minas Gerais and São Paulo).

5.4. *Pontederia rotundifolia* **L.f., Suppl. Pl. 192 1782.**

Reussia rotundifolia (L.f.) A.Cast., Lilloa 25: 593. 1952. Lectotype (designated by Lowden 1973). SURINAM. s.loc., fl., s.dat., C.G. Dahlberg 137 (LINN barcode LINN-HL407-2!; isolectotype: S No. S09-33701!).

Distribution. Mexico, Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama, French Guiana, Guyana, Suriname, Venezuela, Colombia, Ecuador, Peru, Bolivia, Argentina, Paraguay, Uruguay and Brazil (states of Amazonas, Pará, Rondônia, Roraima, Tocantins, Alagoas, Bahia, Maranhão, Paraíba, Pernambuco, Distrito Federal, Goiás, Mato Grosso do Sul, Mato Grosso, Minas Gerais, Rio de Janeiro, Paraná, Rio Grande do Sul and Santa Catarina).

5.5. *Pontederia sagittata* **C.Presl, Reliq. Haenk. 1(2): 116. 1827.**

Pontederia cordata f. *sagittata* (C.Presl) Solms, Monogr. Phan. 4: 533. 1883.
Pontederia cordata var. *sagittata* (C.Presl) Schery, Ann. Missouri Bot. Gard. 31: 157. 1944. Holotype. MEXICO. s.loc., fl., fr., s.dat., T.P.X. Haenke s.n. (PRC barcode PRC450416!).

Distribution. Mexico, Costa Rica, Guatemala, Honduras, Panama and Brazil (states of Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul and Santa Catarina).

    Taxonomical notes. *Pontederia sagittata* is a poorly circumscribed taxon that is morphologically similar to *P. cordata*, due to the shape of the blade of their petiolate leaves. However, it is molecularly more closely related to *P. ovalis*, having in common the anthocarp with sinuate ridges. The disjunctive distribution of *P. sagittata* is probably related to misidentified specimens and/or the presence of cryptic species in what we currently accept as *P. sagittata s.l.* Great variation in petiolate leaf shape can be observed throughout its distribution, especially in Brazil. We believe that *P. sagittata* should be properly studied, using different approaches than traditional taxonomy, in order to solve this issue.

5.6. *Pontederia subovata* **(Seub.) Lowden, Rhodora 75: 478. 1973.**

Reussia subovata (Seub.) Solms, Monogr. Phan. 4: 534. 1883.
Eichhornia subovata Seub., Fl. Bras. 3(1): 91. 1847. Lectotype (designated by Lowden 1973). BRAZIL. Goiás: Provincia de Goyaz, fl., 1836–1841, G. Gardner 4022 (NY barcode NY00247524!; isolectotypes: BM, G barcodes G00168015!, G00168018!, G00168019!, K barcode K000644012!, P barcodes P00730329!, P00730589!, US barcode US00091645!).

Distribution. Venezuela, Guyana, Bolivia, Argentina, Paraguay and Brazil (states of Acre, Amazonas, Amapá, Pará, Tocantins, Bahia, Piauí, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, São Paulo, Paraná, Rio Grande do Sul and Santa Catarina).

5.7. *Pontederia triflora* (Endl. *ex* Seub.) G.Agostini et al., Ernstia 27: 9. 1984.

Reussia triflora Endl. *ex* Seub., Fl. Bras. 3(1): 96. 1847. Type (not found). BRAZIL. Pohl; Sellow (B?).

Distribution. Colombia, Venezuela, Guyana, Bolivia, Argentina and Brazil (states of Roraima, Mato Grosso, Mato Grosso do Sul and Minas Gerais).

    Nomenclatural notes. Due to the impossibility of finding the type specimen(s?) of *Reussia triflora* in any of the visited herbaria, we do not designate any types for this name at this point.

    Taxonomical notes. *Pontederia triflora* has been greatly confused with *P. subovata*, since its original description as *R. triflora* by Seubert (1847). Both species share similar habit, leaf and floral morphology. Nonetheless, in *P. triflora,* the petiolate leaf-blades are linear-lanceolate to linear-elliptic or linear rhomboid (vs. emergent and elliptic to narrowly ovate to ovate or subrhomboid in *P. subovata*) and the inflorescences are 2–4(–5)-flowered [vs. (6–)8–20-flowered]. Thus, we reaffirm *P. triflora* as an accepted name, distinct from *P. subovata*.

Conclusions

Pontederiaceae was one of the first families of flowering plants to be the focus of studies dealing with its phylogenetic history, based on morphological, molecular and combined data (Eckenwalder and Barrett 1986; Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1997; Graham et al. 1998, 2002; Ness et al. 2011). Nonetheless, until very recently (Pellegrini 2017a), the taxonomy of the family remained dogmatic and outdated, with the recognition of several non-monophyletic taxa. The arguments used as the basis for maintaining such assemblages are based especially on misunderstandings of the principles of phylogenetic systematics (Schmidt-Lebuhn 2012). According to Simpson (2006), one of the main paradigms of modern phylogenetic systematics is the proposal of classification systems that accurately reflect the evolutionary history of the studied group, being simultaneously easy to use. In order to achieve that, novel classification systems should be based on molecular phylogenetic studies, together with morphological and, whenever possible, also including less common characters (e.g. anatomy, ecology, geography, palynology, micromorphology, phytochemistry etc.; Pellegrini 2017b). Furthermore, without the inclusion of morphological characters in a phylogenetic analysis, there is no way to obtain morphological synapomorphies to support the recovered relationships and any proposed new classification (Lipscomb et al. 2003; Wiens 2004; Assis and Rieppel 2011). The implementation of these ideals

on the systematics of Pontederiaceae has generated not only monophyletic genera but has considerably facilitated the taxonomy of the group. With the classification implemented here, species of Pontederiaceae are easily and unambiguously placed under two genera supported by morphological and molecular data. An infrafamilial classification for Pontederiaceae has always been of little taxonomic and systematic relevance, due to the families' reduced size. With Pontederiaceae consisting now of only two genera, the recognition of subfamilies and tribes seems rather pointless, since each genus would be placed in its own subfamily/tribe. Thus, we do not accept any taxonomic ranks between family and genus in Pontederiaceae.

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References

Allsopp A (1965) Heteroblastic development in cormophytes. In: Lang A (Ed.) Encyclopedia of Plant Physiology (Vol. 15). Springer-Verlag, Berlin, 1172–1221. https://doi.org/10.1007/978-3-642-50088-6_33

APG IV [The Angiosperm Phylogeny Group] (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society 181(1): 1–20. https://doi.org/10.1111/boj.12385

Arber A (1925) Monocotyledons: A Morphological Study. Cambridge University Press, Cambridge, 282 pp.

Assis LCS, Rieppel O (2011) Are monophyly and synapomorphy the same or different? Re-
visiting the role of morphology in phylogenetics. Cladistics 27: 94–102. https://doi.
org/10.1111/j.1096-0031.2010.00317.x

Aston HI (1985) *Monochoria cyanea* and *M. australasica* (Pontederiaceae) in Australia. Muel-
leria 6(1): 51–57.

Balslev H (1998) Juncaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants
(vol. 4). Springer Verlag, Berlin, 252–260. https://doi.org/10.1007/978-3-662-03531-3_26

Barret SCH (1978) The floral biology of *Eichhornia azurea* (Swartz) Kunth (Pontederiaceae).
Aquatic Bot. 5: 217–228. https://doi.org/10.1016/0304-3770(78)90064-5

Barrett SCH (1988) Evolution of breeding systems in Eichhornia (Pontederiaceae): a review.
Annals of the Missouri Botanical Garden 75(3): 741–760

Barrett SCH (1993) The evolutionary biology of tristyly. In: Futuyma D, Antonovics J (Eds) Ox-
ford Surveys in Evolutionary Biology (vol. 9). Oxford University Press, Oxford, 283–326.

Barrett SCH (2004) Pontederiaceae (water hyacinth family). In: Smith N, et al. (Eds) Flower-
ing Plants of the Neotropics. Princeton University Press, Princeton, 474–476.

Barrett SCH, Graham SW (1997) Adaptive radiation in the aquatic plant family Pontede-
riaceae: insights from phylogenetic analysis. In: Givnish TJ, Sytsma K (Eds) Molecular
Evolution and Adaptive Radiation. Cambridge University Press, Cambridge, 225–258.

Bayer C, Appel O, Rudall PJ (1998) Hanguanaceae. In: Kubitzki K (Ed.) The Families and
Genera of Vascular Plants. Flowering plants: Monocotyledons. Alismatanae and Com-
melinanae (except Gramineae), vol. IV. Springer Verlag, Berlin, 223–225. https://doi.
org/10.2307/2399363

Bischoff GW (1834) Lehrbuoh der Botanik. E Schweizerbart's Verlagsbuchhandlung, Stuttgart,
524 pp.

BFG [The Brazilian Flora Group] (2015) Growing knowledge: An overview of Seed Plant diver-
sity in Brazil. Rodriguésia 66: 1085–1113. https://doi.org/10.1590/2175-7860201566411

Bremer K (1994) Brach support and tree stability. Cladistics 10: 295–304. https://doi.
org/10.1111/j.1096-0031.1994.tb00179.x

Caddick LR, Furness CA, Stobart KL, Rudall PJ (1998) Microsporogenesis and pol-
len morphology in Dioscoreales and allied taxa. Grana 37(6): 321–336. https://doi.
org/10.1080/00173139809362687

Castellanos A (1959) Las Pontederiaceae de Brasil. Arquivo Jardim Botânico do Rio de Janeiro
16: 147–236.

Chaffey NJ (1994) Structure and function of the membranous grass ligule: a com-
parative study. Botanical Journal of the Linnean Society 116: 53–69. https://doi.
org/10.1111/j.1095-8339.1994.tb00421.x

Colomb M (1886) Note sur l'ochrea des Polygonées. Bulletin de la Société botanique de France
33: 506–507. https://doi.org/10.1080/00378941.1886.10828484

Cook CDK (1989) Taxonomic revision of *Monochoria* (Pontederiaceae). In: Tan K, et al. (Eds)
The Davis & Hedge Festschrift: Plant Taxonomy, Phytogeography, and Related Subjects.
Edinburgh University Press, Edinburgh, 149–184.

Cook CDK (1996) Aquatic Plant Book (2nd and revised edn). Kugler Publications, Amsterdam,
228 pp.

Cook CDK (1998) Pontederiaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants. Flowering plants: Monocotyledons. Alismatanae and Commelinanae (except Gramineae), vol. IV. Springer Verlag, Berlin, 395–403. https://doi.org/10.1007/978-3-662-03531-3_37

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Dahlgren RMT, Clifford HT, Yeo PF (1985) The Families of the Monocotyledons: Structure, Evolution, and Taxonomy. Springer Verlag, Berlin, Heidelberg, New York & Tokyo, 520 pp. https://doi.org/10.1007/978-3-642-61663-1

De Pinna MCC (1991) Concepts and tests of homology in the cladistic paradigm. Cladistics 7(4): 367–394. https://doi.org/10.1111/j.1096-0031.1991.tb00045.x

Dubs B (1998) Prodromus Florae Matogrossensis, Series B, No. 3. Betrona-Verlag.

Eckenwalder JE, Barrett SCH (1986) Phylogenetic systematics of Pontederiaceae. Systematic Botany 11(3): 373–391. https://doi.org/10.2307/2419074

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Informatics 5:113. https://doi.org/10.1186/1471-2105-5-113

eMonocot (2010) eMonocot (Version 1.0.2). http://e-monocot.org [accessed: 1.6.2018]

Faden RB (1991) The morphology and taxonomy of *Aneilema* R.Brown (Commelinaceae). Smithsonian Contributions to Botany (Washington) 76: 1–166.

Faden RB (1998) Commelinaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants. Flowering plants: Monocotyledons. Alismatanae and Commelinanae (except Gramineae), vol. IV. Springer Verlag, Berlin, 109–128. https://doi.org/10.1007/978-3-662- 03531-3_12

Farris JS, Kallersjo M, Kluge AG, Bult C (1994) Testing significance of congruence. Cladistics 10: 315–319. https://doi.org/10.1111/j.1096-0031.1994.tb00181.x

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fernald ML (1950) Gray's Manual of Botany (8th edn). Dioscorides Press. https://doi.org/10.2307/4110285

Fitch WM (1971) Towards defining the course of evolution: minimum change for a specific tree topology. Systematic Zoology 20: 406–416. https://doi.org/10.2307/2412116

Forman LL (1997) Notes concerning the typification of names of William Roxburgh's species of Phanerogams. Kew Bulletin 52(3): 513–534.

Glück H (1901) Stipulargebilde der Monokotyledonen. Verh. Naturh.-med. Ver. Heildenberg 7: 1–96.

Godfrey RK, Wooten JW (1979) Aquatic and wetland plants of the Southeastern United States, Monocotyledons. The University of Georgia Press.

Gopal B, Sharma KP (1981) Water Hyacinth (*Eichhornia crassipes*): The Most Troublesome Weed of the World. Hindasia Publishers, Delhi, 227 pp.

Govaerts R (2018) World Checklist of Pontederiaceae. Facilitated by the Royal Botanic Gardens, Kew. http://wcsp.science.kew.org/ [accessed: 2.5.2018]

Graham SW, Barrett SCH (1995) Phylogenetic systematics of Pontederiales: implications for breeding-system evolution. In: Rudall P, et al. (Eds) Monocotyledons: Systematics and Evolution. Condon, Royal Botanic Gardens, Kew, 415–441.

Graham SW, Kohn JR, Morton BR, Eckenwalder JE, Barrett SCH (1998) Phylogenetic congruence and discordance among one morphological and three molecular data sets from Pontederiaceae. Systematic Biology 47: 545–567. https://doi.org/10.1080/106351598260572

Graham SW, Olmstead RG, Barrett SCH (2002) Rooting phylogenetic trees with distant outgroups: A case from the Commelinoid Monocots. Molecular Biology and Evolution 19(10): 1769–1781. https://doi.org/10.1093/oxfordjournals.molbev.a003999

Grayum MH (1992) Comparative external pollen ultrastructure of the Araceae and putatively related taxa. Monographs in Systematic Botany from Missouri Botanical Garden 43: 1–167.

Guofang W, Horn CN (2000) Pontederiaceae. In: Wu ZY, Raven PH (Eds) Flora of China (vol. 24). Science Press, Beijing, 40–42.

Halbritter H, Hesse M (1993) Sulcus morphology in some monocot families. Grana 32(2): 87–99. https://doi.org/10.1080/00173139309429459

Hamann U (1998) Philydraceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants. Flowering plants: Monocotyledons. Alismatanae and Commelinanae (except Gramineae), vol. IV. Springer Verlag, Berlin, 389–394. https://doi.org/10.1007/978-3-662-03531-3_36

Harley MM, Baker WJ (2001) Pollen aperture morphology in Arecaceae: Application within phylogenetic analyses, and a summary of record of palm-like pollen the fossil. Grana 40(1/2): 45–77. https://doi.org/10.1080/00173130152591877

Hasskarl JC (1852) Flora oder Botanische Zeitung, vol. 35. Regensburg, Die Gesellschaft, 784 pp.

Hertweck K, Kinney M, Stuart S, Maurin O, Mathews S, Chase MW, Gandolfo M, Pires JC (2015) Phylogenetics, divergence times, and diversification from three genomics partitions in monocots. Botanical Journal of the Linnean Society 178: 375–393. https://doi.org/10.1111/boj.12260

Hornung-Leoni C, Sosa V (2008) Morphological phylogenetics of *Puya* subgenus *Puya* (Bromeliaceae). Botanical Journal of the Linnean Society 156: 93–110. https://doi.org/10.1111/j.1095-8339.2007.00740.x

Horn CN (1985) A systematic revision of the genus *Heteranthera* (*sensu lato*; Pontederiaceae). PhD Dissertation, University of Alabama, Tuscaloosa.

Horn CN (1988) Developmental heterophylly in the genus *Heteranthera* (Pontederiaceae). Aquatic Botany 31: 197–209. https://doi.org/10.1016/0304-3770(88)90014-9

Horn CN (1994) 197. Pontederiaceae. In: Görts-Van Rljn ARA (Ed.) Flora of the Guianas, Series A: Phanerogams. Koeltz Scientific Books, Koenigstein, 91–102.

Horn CN (1998) Notulae ad Floram Paraguaiensem 66: A note on *Eichhornia paniculata* (Spreng.) Solms and *Eichhornia meyeri* A.G.Schulz (Pontederiaceae) in Paraguay. Candollea 53: 127–130.

Horn CN, Haynes RR (1987) Typification of *Pontederia* and its Linnaean species names (Pontederiaceae). Taxon 36(3): 621–625. https://doi.org/10.2307/1221857

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, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kellogg EA (2015) The Families and Genera of Vascular Plants (vol. XII: Poaceae). Springer Verlag, Cham, Heidelberg, New York, Dordrecht & London, 443 pp.

Kohn JR, Graham SW, Morton BR, Doyle JJ, Barrett SCH (1996) Reconstruction of the evolution of reproductive characters in Pontederiaceae using phylogenetic evidence from chloroplast DNA restriction-site variation. Evolution; International Journal of Organic Evolution 50(4): 1454–1469. https://doi.org/10.1111/j.1558-5646.1996.tb03919.x

Kubitzki K [Ed.] (1998) The Families and Genera of Vascular Plants. Flowering plants: Mono-cotyledons. Alismatanae and Commelinanae (except Gramineae), vol. IV. Springer Verlag, Berlin, 511 pp.

Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. Systematic Biology 50: 913–925. https://doi.org/10.1080/106351501753462876

Lipscomb D, Platnick N, Wheeler QD (2003) The intellectual content of taxonomy: A comment on DNA taxonomy. Trends in Ecology & Evolution 18: 65–66. https://doi.org/10.1016/S0169-5347(02)00060-5

Linnaeus C (1753) Species Plantarum, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, mominibus trivialibus, synonymis selectis, locis natalibus, secundum Systema Sexuale digestas, Tomus I. Imprensis Laurentii Salvii, Stockholm, Sweden, 560 pp.

Lowden RM (1973) Revision of the genus *Pontederia* L. Rhodora 75: 426–487.

Lubbock J (1891) On stipules, their form and function. Botanical Journal of the Linnean Society 28: 217–243. https://doi.org/10.1111/j.1095-8339.1891.tb01458.x

Lubbock J (1895) On stipules, their form and function – part II. Botanical Journal of the Linnean Society 30: 436–532.

Maddison WP, Maddison DR (2017) Mesquite: a modular system for evolutionary analysis. Version 3.20. http://mesquiteproject.org

Majumdar GP (1956) Stipules, stipels, ligules and leaf-sheath. Proceedings of the Indian Academy of Sciences 43B: 9–22.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'Homme Van Reine WF, Smith GF, Wiersema JH, Turland NJ (2012) International Code of Botanical Nomenclature (Melbourne Code). Adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Regnum Vegetabile 154. A.R.G. Gantner Verlag KG, 240 pp.

Ness RW, Graham SW, Barrett SCH (2011) Reconciling gene and genome duplication events: Using multiple nuclear gene families to infer the phylogeny of the aquatic plant family Pontederiaceae. Molecular Biology and Evolution 28(11): 3009–3018. https://doi.org/10.1093/molbev/msr119

Nixon KC (2002) WinClada ver. 1.0000 Published by the author, Ithaca. http:// www.cladistics.com/about_winc.htm

Novelo A, Lot A (1994) 252. Pontederiaceae. In: Davidse G, et al. (Eds) Flora Mesoamerica, vol. 6, Alismataceae a Cyperaceae. Universidad Nacional Autonoma de Mexico, Mexico, 65–71.

Palisot de Beauvois AMFJ (1807) Flore d'Oware et de Benin, en Afrique, Tome 2. De L'Imprimerie de Fain et Compagnie, Paris, 95 pp.

Panigo E, Ramos J, Lucero L, Perreta M, Vegetti A (2011) The inflorescence in Commelinaceae. Flora 206(4): 294–299. https://doi.org/10.1016/j.flora.2010.07.003

Pellegrini MOO (2017a) Two new synonyms for *Heteranthera* (Pontederiaceae, Commelinales). Nordic Journal of Botany 35(1): 124–128. https://doi.org/10.1111/njb.01152

Pellegrini MOO (2017b) Morphological phylogeny of *Tradescantia* L. (Commelinaceae) sheds light on a new infrageneric classification for the genus and novelties on the systematics of subtribe Tradescantiinae. PhytoKeys 89: 11–72. https://doi.org/10.3897/phytokeys.89.20388

Pellegrini MOO, Faden RB (2017) Recircumscription and taxonomic revision of *Siderasis*, with comments on the systematics of subtribe Dichorisandrinae (Commelinaceae). PhytoKeys 83: 1–41. https://doi.org/10.3897/phytokeys.83.13490

Pellegrini MOO, Horn CN (2017) Two peculiar new species of *Heteranthera* Ruiz & Pavón (Pontederiaceae) from Brazil, with notes on inflorescence architecture in the family. PhytoKeys 82: 35–56. https://doi.org/10.3897/phytokeys.82.13752

Philipson WR (1935) The development and morphology of the ligule in grasses. New Phytologist 34: 310–325. https://doi.org/10.1111/j.1469-8137.1935.tb06849.x

Prychid CJ, Furness CA, Rudall PJ (2003) Systematic significance of cell inclusions in Haemodoraceae and allied families: silica bodies and tapetal raphides. Annals of Botany 92: 571–580. https://doi.org/10.1093/aob/mcg172

Radford AE, Dickison WC, Massey JR, Bell CR (1974) Vascular Plant Systematics. Harper & Row Publishers, New York, 891 pp.

Regel EA (1843) Beobachtung über den Ursprung und Zweck der Stipeln. Linnea 17: 193–234.

Regel EA, Maack RK (1861) Tentamen florae Ussuriensis oder Versuch einer Flora des Ussuri-Genietes. Mémoires de l'Académie Impériale des Sciences de Saint Pétersbourg, Septième Série (Sér. 7) 4(4): 1–228.

Reveal JL, Frick GF, Broome CR, Brown ML (1987) Botanical explorations and discoveries in colonial Maryland, 1688 to 1753. Huntia 7: 5–60.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Rudall PJ, Buzgo M (2002) Evolutionary history of the monocot leaf. In: Cronk CBQ, et al. (Eds) Developmental genetics and plant evolution. Taylor and Francis, London, 431–458. https://doi.org/10.1201/9781420024982.ch23

Rutishauser R (1999) Polymerous leaf whorls in vascular plants: developmental morphology and fuzziness of organ identities. International Journal of Plant Sciences 160: 81–103. https://doi.org/10.1086/314221

Rudall PJ, Wheeler A (1988) Pollen morphology in Tigridieae (Iridaceae). Kew Bulletin 43(4): 693–701. https://doi.org/10.2307/4129967

Saarela JM, Prentis PJ, Rai HS, Graham SW (2008) Phylogenetic relationships in the monocot order Commelinales, with a focus on Philydraceae. Botany 86: 719–731. https://doi.org/10.1139/B08-063

Sauquet H, von Balthazar M, Magallón S, Doyle JA, Endress PK, Bailes EJ, Morais EB, Bull-Hereñu K, Carrive L, Chartier M, Chomicki G, Coiro M, Cornette R, El Ottra JHL, Epicoco C, Foster CSP, Jabbour F, Haevermans A, Haevermans T, Hernández R, Little

SA, Löfstrand S, Luna JA, Massoni J, Nadot S, Pamperl S, Prieu C, Reyes E, Santos P, Schoonderwoerd KM, Sontag S, Soulebeau A, Staedler Y, Tschan GF, Wing-Sze Leung A, Schönenberger J (2017) The ancestral flower of angiosperms and its early diversification. Nature Communications 8: 16047. https://doi.org/10.1038/ncomms16047

Seubert MA (1847) Pontederiaceae. In: Martius CFP (Ed.) Flora Brasiliensis (Vol. III, part 1). Leipzig aput Frid. Fleischer, Munich, 85–96.

Schmidt-Lebuhn AN (2012) Fallacies and false premises – a critical assessment of the arguments for the recognition of paraphyletic taxa in botany. Cladistics 28: 174–187.

Schwartz O (1926) Anatomische, morphologische und systematische Untersuchungen tiber die Pontederiaceen. Beih. Bot. Centralbl. 42 (l. Abt.): 263–320.

Schultes JA, Schultes JH (1830) Caroli a Linné equitis Systema vegetabilium secundum classes, ordines, genera, species; cum characteribus, differentiis et synonymiis, vol. 7, part 2. Stuttgardtiae, Sumtibus J.G. Cottae, 1816 pp.

Schulz AG (1942) Las Pontederiaceas de la Argentina. Darwiniana 6(1): 45–87.

Sculthorpe CD (1967) The Biology of Aquatic Vascular Plants. Edward Arnold Ltd., London, 610 pp.

Sereno PC (2007) Logical basis for morphological characters in phylogenetics. Cladistics 23: 565–587. https://doi.org/10.1111/j.1096-0031.2007.00161.x

Simpson MG (1983) Pollen ultrastructure of the Haemodoraceae and its taxonomic significance. Grana 22: 79–103. https://doi.org/10.1080/00173138309431969

Simpson MG (1987) Pollen ultrastructure of the Pontederiaceae. Grana 26(2): 113–126. https://doi.org/10.1080/00173138709429941

Simpson MG (1990) Phylogeny and classification of the Haemodoraceae. Annals of the Missouri Botanical Garden 77(4): 722–784. https://doi.org/10.2307/2399670

Simpson MG (1998) Haemodoraceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants. Flowering plants: Monocotyledons. Alismatanae and Commelinanae (except Gramineae), vol. IV. Springer Verlag, Berlin, Germany, 212–128. https://doi.org/10.1007/978-3-662-03531-3_20

Simpson MG (2006) Plant Systematics. Elsevier Academic Press, Burlington, Massachusetts, 590 pp. https://doi.org/10.5642/aliso.20062201.39

Simpson MG, Burton DH (2006) Systematic floral anatomy of Pontederiaceae. Aliso 22: 499–519.

Smith LB, Till W (1998) Bromeliaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants. Flowering plants: Monocotyledons. Alismatanae and Commelinanae (except Gramineae), vol. IV. Springer Verlag, Berlin, 74–99. https://doi.org/10.1007/978-3-662-03531-3_8

Smith RJ, Hopper SD, Shane MW (2011) Sand-binding roots in Haemodoraceae: Global survey and morphology in a phylogenetic context. Plant and Soil 348: 453–470. https://doi.org/10.1007/s11104-011-0874-z

Solms-Laubach HM (1883) Pontederiaceae. In: De Candolle ALPP, De Candolle ACP (Eds) Monographiae Phanerogamarum, vol. IV. Sumptibus G. Masson, Paris, 501–535.

Spjut RW (1994) A systematic treatment of fruit types. Memoirs of the New York Botanical Garden 70: 1–181.

Sprengel CPJ (1822) Species plantarum minus cognitae. Neue Entdeckungen im Ganzen Umfang der Pflanzenkunde 3: 3–65.

Stafleu FA, Cowan RS (1983) Taxonomic literature. A selective guide to botanical publications and collections with dates, commentaries and types (ed. 2, Vol. IV). Regnum Vegetabile 110, Rugell: A.R.G. Gantner Verlag K.G., 1214 pp.

Stafleu FA, Cowan RS (1985) Taxonomic literature. A selective guide to botanical publications and collections with dates, commentaries and types, ed. 2, Vol. V. Regnum Vegetabile 112, Rugell: A.R.G. Gantner Verlag K.G., 1066 pp.

Stevens PF (2001–onwards) Angiosperm Phylogeny Website. Version 14, July 2017 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/ [accessed: 6.10.2018]

Swofford DL (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*And Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Swofford DL, Maddison WP (1987) Reconstructing ancestral character states under Wagner parsimony. Mathematical Biosciences 87: 199–229. https://doi.org/10.1016/0025-5564(87)90074-5

The Plant List (2013) The Plant List (Version 1.1). http://www.theplantlist.org [accessed: 3.10.2018]

Thiers B (cont. updated) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Gardens' Virtual Herbarium. Available from: http://sweetgun.nybg.org/ih/ [accessed: 11.20.2017]

Tropicos.org (2018) Tropicos. Missouri Botanical Garden. http://www.tropicos.org/ [accessed: 2.26.2018]

Tungmunnithum D, Boonkerd T, Zungsontiporn S, Tanaka N (2016) Morphological variations among populations of *Monochoria vaginalis s.l.* (Pontederiaceae) in Thailand. Phytotaxa 268(1): 057–068.

Vahl MH (1805) Enumeratio Plantarum, vel ab aliis, vel ab ipso observatarum, cum differentiis specificis, synonymis selectis et descriptionibus succinctis, vol. 2. Hauniae, Impenis auctoris & prostat apud J.H. Schubothe, 423 pp.

Verdcourt B (1961) The genus *Monochoria* C.Presl (Pontederiaceae) in Africa. Kirkia 1: 80–83.

Wang G-X, Miura R, Kusanagi T (1995) The enantiostyly and the pollination biology of *Monochoria korsakowii* (Pontederiaceae). Acta Phytotaxonomica et Geobotanica 46(1): 55–65.

Wang G-X, Nagamasu H (1994) A new species of *Monochoria* (Pontederiaceae) from Hainan, China. Acta phytotaxonomica et geobotanica 45: 41–44.

Wang G-X, Li W, Wan X, Ito M (2003) *Monochoria vaginalis* var. *angustifolia*, a new variety of the Pontederiaceae from Thailand. Phytotaxonomica Sinica 41: 569–572.

Wang G-X, Wei L, Xiao-Chun W, Itoh K (2004) Taxonomy of the genus *Monochoria* (Pontederiaceae) in Asia. Current Topics in Plant Biology 5: 39–52.

Weberling F (1965) Typology of inflorescences. Botanical Journal of the Linnean Society 59: 15–221.

Weberling F (1989) Morphology of flowers and inflorescences. Cambridge University Press, Cambridge, 348 pp. https://doi.org/10.1111/j.1095-8339.1965.tb00058.x

Wiens JJ (2004) The role of morphological data in phylogeny reconstruction. Systematic Biology 53: 653–661. https://doi.org/10.1080/10635150490472959

Supplementary material I

List of morphological characters and coding

Authors: Marco O. O. Pellegrini, Charles N. Horn, Rafael F. Almeida

Data type: measurement

Explanation note: List of morphological characters and their respective coding, used in the morphological and total evidence analyses.

Link: https://doi.org/10.3897/phytokeys.108.27652.suppl1

Supplementary material 2

Morphological matrix

Authors: Marco O. O. Pellegrini, Charles N. Horn, Rafael F. Almeida

Data type: matrix

Explanation note: Matrix with the 27 terminals and the first 96 characters. The characters that were not coded due to lack of data of the analyzed specimens and/or from literatures sources are coded as "?"; characters that did not apply were coded as "-"; and the polymorphic characters were coded with a "/" between each state it presented.

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RESEARCH ARTICLE

Nomenclatural review of *Acalypha* (Euphorbiaceae) of the Western Indian Ocean Region (Madagascar, the Comoros Archipelago, the Mascarene Islands and the Seychelles Archipelago)

Iris Montero Muñoz[1], José María Cardiel[1], Geoffrey A. Levin[2,3]

1 *Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Ciudad Universitaria de Cantoblanco, 28049, Madrid, Spain* **2** *Illinois Natural History Survey, Prairie Research Institute, University of Illinois, 1816 S Oak St., Champaign, IL 61820, USA* **3** *Canadian Museum of Nature, P.O. Box 3443, Station D, Ottawa, ON K1P 6P4, Canada*

Corresponding author: *Iris Montero Muñoz* (iris.montero@uam.es)

Abstract

This work presents a critical nomenclatural review of the *Acalypha* species of the Western Indian Ocean Region (Madagascar, the Comoros Archipelago, the Mascarene Islands and the Seychelles Archipelago). This is the first treatment of *Acalypha* of Madagascar since Leandri's monograph in 1942. A total of 151 scientific names related to *Acalypha* from this region are treated. We recognise 35 species (28 native and seven introduced), treat 93 names as synonyms (28 of them for the first time) and identify three as doubtful or excluded names. We designate lectotypes for 41 names, make two new combinations and propose one new name.

Keywords

Introduction

This work is part of the taxonomic and biogeographic revision of *Acalypha* (Euphorbiaceae) in the Western Indian Ocean Region (WIOR). According to the Taxonomic Databases Working Group standards (Brummit 2001), this region includes Madagascar, the Mascarene Islands (Mauritius and the French Department of Réunion), the Comoros Islands (Union of the Comoros and the French Department of Mayotte) and the Seychelles Archipelago. There are no species of *Acalypha* known from any of the smaller Scattered Islands of the Western Indian Ocean.

The first two species of Madagascan *Acalypha* (*A. filiformis* and *A. venosa*) were described by Poiret (1804) in Lamarck's encyclopaedia. More than half a century later, Baillon (1861) published *Euphorbiacées Africaines, Afrique Orientale* (*Bourbon, Maurice, Madagascar, Zanguebar* etc.), where 21 species of *Acalypha* are cited, of which 19 are from the study area. Baker (1883, 1884), 20 years later, described four new species. In 1891, Baillon, in his contribution to Grandidier`s *Histoire Physique, Naturelle et Politique de Madagascar,* provided 10 excellent plates of *Acalypha* species from Madagascar, five of which are cited for the first time (Baillon 1891). These plates are not accompanied by descriptions or any additional information. Soon thereafter, Baillon (1892, 1895a, 1895b) published in several chapters his *Liste des Plantes de Madagascar* citing 32 *Acalypha* species, six of which were newly described.

In the treatment of *Acalypha* for Engler´s Das Pfanzenreich, Pax and Hoffmann (1924) included 31 species from WIOR. In this work, *Acalypha* was formally divided into three subgenera, "*Euacalypha*", *Androcephala* and *Linostachys*; the first two occur in WIOR. The most recent work treating Madagascan *Acalypha* (Leandri 1942) is now almost 80 years old. Leandri's work included complete information about 22 species and 14 varieties of *Acalypha*, of which eight species and six varieties were described for the first time.

Only three floristic works included *Acalypha* species from the other islands of the Western Indian Ocean region. Baker (1877) cited five species from Mauritius and the Seychelles and de Cordemoy (1895) treated five species from Réunion. Most recently, Coode (1982) included five species, three subspecies and seven varieties from the Mascarene Islands.

While undertaking this nomenclatural review, we found 144 names related to *Acalypha* from the study area, many of which involved serious problems of taxonomic identity. All of these names have been evaluated in this work and our interpretation of their current taxonomic status and distribution is presented in Tables 1 and 2. Our aim is to lay the foundation for a complete taxonomic and biogeographical revision of *Acalypha* in the WIOR, a region of particular relevance to the origin and evolution of this genus. Preliminary results of *Acalypha* molecular phylogeny (Levin et al. 2005) suggest that the genus first appeared in Africa, where the highest morphological diversity within the genus is found. We share the same purpose as the recently published WIOR nomenclator of *Croton* (Berry et al. 2017), the second largest genus of the Euphorbiaceae, preceded by *Euphorbia* (Horn et al. 2012) and followed by *Acalypha*.

Materials and methods

We conducted a thorough bibliographical review and consulted the following herbaria for the Western Indian Ocean *Acalypha* types: B, BM, BREM, BRNU, C, CAS, G, GB, GDC, GH, JE, K, M, MA, MAU, MO, MPU, NY, P, S, US, TAN, TUB and W (acronyms according to Thiers 2018). Scanned images of types from these and other herbaria, available on JSTOR Global Plants (http://plants.jstor.org/), were also consulted.

The accepted species are cited in alphabetical order and all known synonyms are included, except for the introduced species. We include the notation "**syn. nov.**" whenever we newly treat a taxon as a synonym. We provide details of the type collection(s) for each name. Lectotypes are designated after a careful review of the relevant original literature and examination of the nomenclatural types.

Five of the *Acalypha* treated names were illustrated first, without description, in Baillon`s *Histoire Naturelle des Plantes* (Baillon 1891). These are *A. diminuta*, *A. humblotiana*, *A. leptomyura*, *A. madreporica* and *A. polynema*. Except *A. humblotiana,* we consider each of the plates provided, which include extensive vegetative and reproductive morphological details, as the holotypes of the associated names, according to art. 38.8 of the ICN (McNeill et al. 2012).

When two or more syntypes were cited in the protologue, a lectotype is designated, using the best preserved specimen or the one most consistent with the protologue. The remaining syntypes are also cited.

The type locality information is taken mainly from the type specimen labels. We add additional information such as the province name of Madagascar or a modern or accepted spelling of a place name. If the locality is ambiguous or imprecise, we keep the literal citation (in quotation marks) as it appears in the protologue. Barcode numbers of type specimens are cited when available.

Under the "Distribution" section, we list the general distribution of each species in Africa (following Brummit 2001), if they occur there and in the WIOR region. We include the Madagascar provinces or island names within the studied archipelagos where they occur. This information should be taken as preliminary until a more complete study of the genus, in progress, is completed.

In the references section, we only include references which cite *Acalypha* in the WIOR region.

All information gathered as part of this work, including the complete listing of studied specimens, is available online at the regularly updated *Acalypha Taxonomic Information System* website, www.acalypha.es (Cardiel et al. 2018).

Results and discussion

In the present work, we record 151 published scientific names related to *Acalypha* of the WIOR (Table 1). Of these, 35 are accepted names, 93 are considered synonyms and

Table 1. Taxa included in *Acalypha* of the Western Indian Ocean Region. Accepted names in bold.

Published names	Accepted names in this paper
A. acuminata Vahl ex Baill. *nom. illeg.*	**A. burmanii I.Montero & Cardiel nom. nov.**
A. aldabrica Pax & K.Hoffm.	**A. claoxyloides Hutch.**
A. amentacea Roxb. subsp. *wilkesiana* (Müll.Arg.) Fosberg	**A. wilkesiana Müll.Arg.**
A. andringitrensis Leandri	**A. radula Baker**
A. arborea Comm. in Poir. *nom. nud.*	**A. filiformis Poir.**
A. aspretorum Leandri *nom. nud.*	**A. medibracteata Radcl.-Sm. & Govaerts**
A. bailloniana Müll.Arg.	
A. bakeriana Baill.	**A. emirnensis Baill.**
A. baronii Baker	**A. emirnensis Baill.**
A. boinensis Leandri	
A. buchenavii Müll.Arg.	**A. spachiana Baill.**
A. burmanii I.Montero & Cardiel *nom. nov.*	
A. chibomboa Baill.	
A. claoxyloides Hutch.	
A. cloiselana M. Denis in Leandri *nom. nud.*	**A. gracilipes Baill.**
A. codonocalyx Baill.	**A. chibomboa Baill.**
A. colorata (Poir.) Spreng.	**A. integrifolia Willd.**
A. commersoniana Baill. *nom. nud.*	**A. integrifolia Willd.**
A. commersoniana Baill. ex Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *acutifolia* Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *acutifolia* f. *concolor* Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *acutifolia* f. *purpurea* Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *acutifolia* f. *purpureo-marginata* Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *brevifolia* Baill. ex Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *concolor* Baill. *nom. nud.*	**A. integrifolia Willd.**
A. commersoniana var. *discolor* Baill. *nom. nud.*	**A. integrifolia Willd.**
A. commersoniana var. *gracilipes* (Baill.) Müll.Arg.	**A. gracilipes Baill.**
A. commersoniana var. *longifolia* Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *obtusifolia* Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *obtusifolia* f. *colorata* (Poir.) Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *obtusifolia* f. *discolor* Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *obtusifolia* f. *unicolor* Müll.Arg.	**A. integrifolia Willd.**
A. commersoniana var. *parvifolia* Baill. *nom. nud.*	**A. integrifolia Willd.**
A. commersoniana var. *parvifolia* Baill. ex Müll.Arg.	**A. integrifolia Willd.**
A. commersonii Baill. *nom. nud.*	**A. gracilipes Baill.**
A. comorensis Pax	**A. chibomboa Baill.**
A. crenata var. *glandulosa* Müll.Arg.	**A. lanceolata var. glandulosa (Müll.Arg.) Radcl.-Sm.**
A. decaryana Leandri	
A. diminuta Baill.	
A. discolor Bojer *nom. nud.*	**A. integrifolia Willd.**
A. emirnensis Baill.	
A. emirnensis var. *bara* Leandri	**A. emirnensis Baill.**

Published names	Accepted names in this paper
A. emirnensis var. *jabohaziana* Leandri	***A. emirnensis* Baill.**
***A. fasciculata* Müll.Arg.**	
A. fasciculata var. *humbertiana* Leandri	***A. fasciculata* Müll.Arg.**
A. fasciculata var. *lyallii* (Baker) Leandri	***A. fasciculata* Müll.Arg.**
***A. filiformis* Poir.**	
A. filiformis var. *arborea* Poir.	***A. filiformis* Poir.**
A. filiformis var. *goudotiana* (Baill.) Govaerts	***A. urophylla* Boivin ex Baill.**
A. filiformis var. *ovalifolia* (Baill.) Govaerts	***A. ovalifolia* Baill.**
A. filiformis var. *pervilleana* (Baill.) Govaerts	***A. paxii* Aug.DC.**
A. filiformis var. *urophylla* (Boivin ex Baill.) Govaerts	***A. urophylla* Boivin ex Baill.**
A. filiformis var. *urophylloides* (Pax & K.Hoffm.) Govaerts	***A. paxii* Aug.DC.**
***A. fimbriata* Schumach. & Thonn.**	
A. fryeri Hutch.	***A. claoxyloides* Hutch.**
A. gagnepainii Leandri *nom. illeg.*	***A. medibracteata* Radcl.-Sm. & Govaerts**
A. gagnepainii var. *calcicola* Leandri	***A. medibracteata* Radcl.-Sm. & Govaerts**
A. goudotiana Baill.	***A. urophylla* Boivin ex Baill.**
***A. gracilipes* Baill.**	
A. hildebrandtii Baill.	***A. radula* Baker**
***A. hispida* Burm.f.**	
A. hologyna Baker	***A. emirnensis* Baill.**
***A. humbertii* Leandri**	
A. humblotiana Baill.	***A. paxii* Aug.DC.**
***A. indica* L.**	
A. indica var. *bailloniana* (Müll.Arg.) Hutch.	***A. bailloniana* Müll.Arg.**
***A. integrifolia* Willd.**	
A. integrifolia subsp. *marginata* (Poir.) Coode	***A. marginata* (Poir.) Spreng.**
A. integrifolia subsp. *marginata* var. *crateriana* Coode	***A. marginata* (Poir.) Spreng.**
A. integrifolia subsp. *marginata* var. *saltuum* Coode	***A. marginata* (Poir.) Spreng.**
A. integrifolia subsp. *panduriformis* Coode	***A. marginata* (Poir.) Spreng.**
A. integrifolia var. *colorata* (Poir.) Pax & K.Hoffm.	***A. integrifolia* Willd.**
A. integrifolia var. *concolor* (Müll.Arg.) Pax & K.Hoffm.	***A. integrifolia* Willd.**
A. integrifolia var. *gracilipes* (Baill.) Pax & K.Hoffm.	***A. gracilipes* Baill.**
A. integrifolia var. *longifolia* (Müll.Arg.) Coode	***A. integrifolia* Willd.**
A. integrifolia var. *parvifolia* (Baill. ex Müll.Arg.) Pax & K.Hoffm.	***A. integrifolia* Willd.**
A. juliflora Pax	***A. rottleroides* Baill.**
***A. lamiana* (Leandri) I.Montero & Cardiel** *comb. nov.*	
***A. lanceolata* var. *glandulosa* (Müll.Arg.) Radcl.-Sm.**	
A. lantanaefolia Bojer *nom. nud.*	***A. filiformis* Poir.**
***A. leonii* Baill.**	
A. leonii var. *perrierana* Leandri	***A. leonii* Baill.**
***A. lepidopagensis* Leandri**	
***A. leptomyura* Baill.**	
***A. linearifolia* Leandri**	

Published names	Accepted names in this paper
A. lyallii Baker	**A. fasciculata** Müll.Arg.
A. madagascariensis Pax & K.Hoffm.	**A. fasciculata** Müll.Arg.
A. madreporica Baill.	**A. fasciculata** Müll.Arg.
A. marginata (Poir.) Spreng.	
A. medibracteata Radcl.-Sm. & Govaerts	
A. medibracteata var. *calcicola* (Leandri) Radcl.-Sm. & Govaerts	**A. medibracteata** Radcl.-Sm. & Govaerts
A. meiodonta Baill.	**A. paxii** Aug.DC.
A. menabeana Leandri *nom. nud.*	**A. medibracteata** Radcl.-Sm. & Govaerts
A. menavody (Leandri) I.Montero & Cardiel *comb. nov.*	
A. neptunica Müll.Arg.	
A. ovalifolia Baill.	**A. richardiana** Baill.
A. paxii Aug.DC.	
A. perrieri Leandri	
A. pervilleana Baill.	**A. paxii** Aug.DC.
A. poiretii Spreng.	
A. polynema Baill.	**A. rottleroides** Baill.
A. radula Baker	**A. radula** Baker
A. reticulata (Poir.) Müll.Arg.	**A. filiformis** Poir.
A. reticulata var. *arborea* (Poir.) Müll.Arg.	**A. filiformis** Poir.
A. reticulata var. *cloiselana* Leandri	**A. gracilipes** Baill.
A. reticulata var. *goudotiana* (Baill.) Müll.Arg.	**A. urophylla** Boivin ex Baill.
A. reticulata var. *longifolia* Müll.Arg.	**A. integrifolia** Willd.
A. reticulata var. *longifolia* f. *aberrans* Müll.Arg.	**A. integrifolia** Willd.
A. reticulata var. *meiodonta* (Baill.) Leandri	**A. paxii** Aug.DC.
A. reticulata var. *meiodonta* f. *andronea* Leandri	**A. paxii** Aug.DC.
A. reticulata var. *ovalifolia* (Baill.) Müll.Arg.	**A. ovalifolia** Baill.
A. reticulata var. *pervilleana* (Baill.) Müll.Arg.	**A. paxii** Aug.DC.
A. reticulata var. *urophylla* (Boivin ex Baill.) Müll.Arg.	**A. urophylla** Boivin ex Baill.
A. reticulata var. *urophylla* f. *glabrescens* Leandri	**A. urophylla** Boivin ex Baill.
A. reticulata var. *urophylla* f. *humblotiana* (Baill.) Leandri	**A. paxii** Aug.DC.
A. reticulata var. *urophylla* f. *lamiana* Leandri	**A. lamiana (Leandri) I.Montero & Cardiel comb. nov.**
A. reticulata var. *urophylla* f. *longa* Leandri	**A. urophylla** Boivin ex Baill.
A. reticulata var. *urophylla* f. *meeusei* Leandri	**A. urophylla** Boivin ex Baill.
A. reticulata var. *urophylla* f. *typique* Leandri *nom. inval.*	**A. urophylla** Boivin ex Baill.
A. reticulata var. *urophylla* f. *vohitrae* Leandri	**A. urophylla** Boivin ex Baill.
A. reticulata var. *urophylloides* Pax & K.Hoffm.	**A. paxii** Aug.DC.
A. richardiana Baill.	
A. rottleroides Baill.	
A. salviifolia Baill. *nom. nud.*	**A. radula** Baker
A. spachiana Baill.	
A. spachiana var. *acutifolia* Baill.	**A. spachiana** Baill.
A. spachiana var. *latifolia* Baill.	**A. spachiana** Baill.
A. spachiana var. *minor* Baill.	**A. spachiana** Baill.
A. spiciflora Burm.f.	***Claoxylon spiciflorum*** **(Burm.f.) A.Juss.**
A. spiciflora var. *menavody* Leandri	**A. menavody (Leandri) I.Montero & Cardiel comb. nov.**

Published names	Accepted names in this paper
A. squarrosa Pax	**A. spachiana Baill.**
A. urophylla Boivin ex Baill.	
A. urophylla Pax *nom. illeg.*	**A. paxii Aug.DC.**
A. venosa Poir.	**Leptonema venosum (Poir.) A.Juss.**
A. vulneraria Baill.	
A. wilkesiana Müll.Arg.	
Caturus sessilis Pet.Thou. in Baill. *nom. nud.*	**A. integrifolia Willd.**
Ricinocarpus baillonianus (Müll.Arg) Kuntze	**A. bailloniana Müll.Arg.**
Ricinocarpus hispidus (Brum.f.) Kuntze	**A. hispida Brum.f.**
Ricinocarpus poiretii (Spreng.) Kuntze	**A. poiretii Spreng.**
Ricinocarpus wilkesianus (Müll.Arg.) Fosberg	**A. wilkesiana Müll.Arg.**
Tragia colorata Poir.	**A. integrifolia Willd.**
Tragia filiformis Poir.	**A. burmanii I.Monero & Cardiel nom. nov.**
Tragia fruticosa Commers. in Baill. *nom. nud.*	**A. integrifolia Willd.**
Tragia lobata Wall. *nom. nud.*	**A. integrifolia Willd.**
Tragia macrophylla Wall. *nom. nud.*	**A. integrifolia Willd.**
Tragia marginata Poir.	**A. integrifolia Willd.**
Tragia obtusata Vahl. in Baill. *nom. nud.*	**A. integrifolia Willd.**
Tragia reticulata Poir.	**A. filiformis Poir.**
Tragia salviifolia Bojer in Baill. *nom. nud.*	**A. radula Baker**
Tragia saxatilis Bojer in Pax & K.Hoffm. *nom. nud.*	**A. spachiana Baill.**

three are doubtful or excluded (*A. neptunica* Müll.Arg., *A. spiciflora* Burm.f. and *A. venosa* Poir.). We also include 19 *nomina nuda* found in literature and one *nomen invalidum*. Type specimens are indicated for all valid names and we have designated 41 lectotypes.

In Madagascar, we accept 28 species, 23 native (of which 20 are endemic) and five introduced (Table 2). Leandri (1942) recognised 22 species of *Acalypha* from Madagascar, of which we accept 19 (including *A. gagnepainii* under its correct name, *A. medibracteata*). We consider *A. andringitrensis* to be a synonym of *A. radula*. Our concept of what Leandri called *A. reticulata* is narrower than his; we consider *A. reticulata s.s.* (under its correct name, *A. filiformis*) to be endemic to the Mascarines and treat the Madagascan material Leandri assigned to this species as four distinct species (*A. gracilipes*, *A. lamiana*, *A. paxii* and *A. urophylla*). We exclude the name *Acalypha spiciflora* (accepted by Leandri), because it is not an *Acalypha*. We report two species, *A. fimbriata* and *A. lanceolata*, not previously cited for Madagascar. We also treat the name *A. madagascariensis*, previously excluded by Leandri, as a synonym of *A. fasciculata*. We anticipate the description of at least 2 more new species from Madagascar based on the material we now have on hand.

In the Comoros Archipelago, there are seven species of *Acalypha*, four native (one endemic) and three introduced, one of which, *A. bailloniana*, is first reported here. There are seven species on the Mascarene Islands, three native (all endemic) and four introduced. There are four species on the Seychelles, one endemic and three introduced.

Table 2. Synoptic table of the species distribution of *Acalypha* of the Western Indian Ocean Region (E: endemic, N: native, I: introduced).

Species	Madagascar	Comoros	Mascarenes	Seychelles
A. bailloniana		I		
A. boinensis	E			
A. burmanii	E			
A. chibomboa	N	N		
A. claoxyloides				E
A. decaryana	E			
A. diminuta	E			
A. emirnensis	E			
A. fasciculata	E			
A. filiformis			E	
A. fimbriata	I			
A. gracilipes	E			
A. humbertii	E			
A. indica	I	I	I	I
A. integrifolia			E	
A. lamiana	E			
A. lanceolata var. *glandulosa*	I		I	
A. leonii	E			
A. lepidopagensis	E			
A. leptomyura	E			
A. linearifolia	E			
A. marginata			E	
A. medibracteata	E			
A. menavody	E			
A. paxii	N	N		
A. perrieri	E			
A. radula	E			
A. richardiana		E		
A. rottleroides	E			
A. spachiana	E			
A. urophylla	N	N		
A. vulneraria	E			
A. hispida	I	I	I	I
A. poiretii			I	
A. wilkesiana	I			I
Native	23	4	3	1
Endemic	20	1	3	1
Introduced	5	3	4	3
Total	28	7	7	4

Nomenclatural synopsis of Malagasy, Comoros, Mascarene and Seychelles *Acalypha*

1. *Acalypha bailloniana* Müll.Arg., Linnaea 34: 44. 1865.

Ricinocarpus baillonianus (Müll.Arg.) Kuntze, Revis. Gen. Pl. 2: 617. 1891.
Acalypha indica var. *bailloniana* (Müll.Arg.) Hutch., Fl. Trop. Afr. 6(1): 904. 1912.

Type. Tanzania: Zanzibar: s.l., 1848, *L. H. Boivin s.n.* (holotype: P [P04809900]!).
　　Distribution. East Tropical Africa. Comoros Archipelago (Anjouan).
　　Notes. *Acalypha bailloniana* was considered as a subspecies of *A. indica* L. by Hutchinson (1913) and this treatment was followed in the subsequent floristic works. Radcliffe-Smith (1987, 1996) treated *A. bailloniana* as a synonym of *A. indica*. We consider that *A. bailloniana* is a distinct species which can be distinguished from *A. indica* by its dentante bracts with a prominent central tooth and with glandular hairs (vs. subentire bracts without prominent central tooth and without glandular hairs, in *A. indica*). *A. bailloniana* is distributed in the east coast of tropical Africa and cited for the WIOR region for the first time, where it is probably introduced.

2. *Acalypha boinensis* Leandri, Notul. Syst. (Paris) 10: 268. 1942.

Type. Madagascar. Prov. Mahajanga: Ambongo et Boïna, bassin moyen du Bemarivo, 200 m, Nov 1919, *H. Perrier de la Bâthie 9823* (lectotype, designated here: P [P00508582]!).
　　Syntypes. Madagascar. Prov. Mahajanga: Maintirano, s.d., *R. Decary 8216* P [P00508588]!; Madagascar. Prov. Mahajanga: Maromandia (Andranosamontana), s.d., *R. Decary 1045* P [P00508585]!; Madagascar. Prov. Mahajanga: Morovoay, s.d., *H. Humbert & H. Perrier de la Bâthie 2350* P [P00508570!, P00508571!, P00508572!, P00508573!, P00508574!]; Madagascar. Prov. Mahajanga: Ambongo et Boïna, 300 m, s.d., *H. Perrier de la Bâthie 9546* P [P00508576]!, *9551* P [P00508577]!; Madagascar. Prov. Toliara: Forêt de Besomaty, entre le Fiherena et l'Isahaina (Mangoky), 750–800 m, s.d., *H. Humbert 11234* P [P00508575]!; Madagascar: s.l., s.d., *Baron 5393* P [P00508586]!, *5450* [n.v.].
　　Distribution. Madagascar (Antsiranana, Fianarantsoa, Mahajanga, Toliara)
　　References. Govaerts et al. (2000: 51).

3. *Acalypha burmanii* I.Montero & Cardiel. nom. nov.
urn:lsid:ipni.org:names:60477001-2

Tragia filiformis Poir., Encycl. 7: 727. 1806. *Acalypha acuminata* Vahl ex. Baill. Adansonia 1: 267. 1861 *nom. illeg.*, non. *A. acuminata* Benth. (1854).

Type. Madagascar?: s.l., s.d., *Herb. de Lamarck. s.n.* (holotype: P-LAM [P00367371]!).

Distribution. Madagascar (Antsiranana).

References. Müller Argoviensis (1865: 40) as *A. spiciflora* Burm.f.; Müller Argoviensis (1866: 867) as *A. spiciflora*; Baillon (1891: tab. 188) as *A. acuminata* Vahl. ex Baill.; Baillon (1892: 1004) as *A. acuminata*; Heckel (1903: 198) as *A. acuminata*; Palacký (1907: 24) as *A. acuminata*; Pax and Hoffmann (1924: 137) as *A. spiciflora*; Leandri (1942: 269) as *A. spiciflora*.

Notes. *Acalypha burmanii* is proposed as a new name for *Tragia filiformis* Poir. We cannot combine *T. filiformis* under *Acalypha* because it is blocked by *A. filiformis* Poir., nor can we use the illegitimate name *A. acuminata* Baill. See also comments under the excluded species *A. spiciflora*.

4. *Acalypha chibomboa* Baill., Adansonia 1: 269. 1861.

Type. Comoros, Anjouan, 1850, *L. H. Boivin s.n.* (holotype: P [P00196274]!).

    Acalypha codonocalyx Baill., Adansonia 1: 271. 1861. Type: Comoros: Mohéli, "Ile Mohilla", s.d., *M. Richard 286* (lectotype, designated here: P [P00196282]!; isolectotype: P [P00196283]!).

    Acalypha comorensis Pax, Bot. Jahrb. Syst. 19(1): 95. 1894. Type: Comoros, Anjouan, "Johanna", Jun–Aug 1875, *J. M. Hildebrandt 1662* (holotype B or WRSL, presumably destroyed; lectotype, designated here: BREM [BREM0001792]!; isolectotypes: K [K000186524!, K000186525!], L [L0241274]!, P [P00196280]!, W [W0004243]!) Syntype: Comoros, Grande Comore, 1886. *C. W. Schmidt 192* [n.v.].

Distribution. Madagascar (Antsiranana, Antananarivo), Comoros Archipelago (Grande Comore, Anjouan, Mohéli, Mayotte).

References. Baillon (1891: Pl. 192 as *A. codonocalyx*); Palacký (1907: 24); Voeltzkow (1917: 447); Pax and Hoffmann (1924: 165); Leandri (1942: 280); Govaerts et al. (2000: 55).

5. *Acalypha claoxyloides* Hutch., Bull. Misc. Inform. Kew 1918: 205. 1918.

Type. Seychelles, Astove, Cosmoledo and Aldabra, Apr 1907, *H. P. Thomasset 243* (lectotype, designated here: K [K000186504]!).

Syntypes. Seychelles, Aldabra, s.d., *W. L. Abbott s.n.* P [P00887488]!, [P00887489]!; Seychelles, Aldabra, Oct-Dec 1892, *J. Fryer 18* K [K000186505]!.

    Acalypha fryeri Hutch. Bull. Misc. Inform. Kew 1918: 206. 1918. Type. Seychelles, Aldabra, s.d., *J. Fryer 92* (holotype: K [K000186506]!).

    Acalypha aldabrica Pax & K.Hoffm., Pflanzenr. 147,16(Heft 85): 136. 1924. Type. Seychelles, Aldabra, s.d., *W. L. Abbott s.n.* (holotype B?, presumably destroyed; lectotype, designated here: P [P00887488]!; isolectotype: P [P00887489]!, **syn. nov.**).

Distribution. Seychelles Archipelago.

References. Hemsley (1919: 130); Hemsley (1919: 131) *A. fryeri*; Pax and Hoffmann (1924: 136) *A. aldabrica*; Fosberg (1974: 263); Renvoize (1975: 152); Robertson (1989: 199); Govaerts et al. (2000: 56).

Notes. *Acalypha claoxyloides* is widespread in the Seychelles archipelago. It is very close to *A. pubiflora* (Klotzsch) Baill., known from south-eastern Africa (Botswana, Malawi, Mozambique, South Africa and Zimbabwe), of which it may be a synonym. More studies of the African material, as well as the Australian material treated as *A. pubiflora* var. *australica* Radcl.-Sm. (Radcliffe-Smith 1990, 1996), is needed to unravel the *A. pubiflora* complex.

6. *Acalypha decaryana* Leandri, Notul. Syst. (Paris) 10: 284. 1942.

Type. Madagascar. Prov. Toliara: Ambovombe, 20 Aug 1924, *R. Decary 2985* (holotype: P [P00508553]!; isotype W [W1962-0013399]!).

Distribution. Madagascar (Antananarivo, Antsiranana, Fianarantsoa, Toliara).

References. Govaerts et al. (2000: 59); Seebaluck et al. (2015: 150).

Notes. We consider as holotype of *Acalypha decaryana,* the only specimen with the word "type" hand-writen by Leandri.

7. *Acalypha diminuta* Baill., Hist. Pl. Madag., Atlas, t. 194. 1891.

Type. Plate 194 in Baillon ibid. loc., holotype.

Distribution. Madagascar (Antananarivo, Mahajanga, Toamasina, Toliara).

References. Baillon (1895b: 1197); Palacký (1907: 25); Pax and Hoffmann (1924: 21); Leandri (1935: 42); Leandri (1942: 253); Govaerts et al. (2000: 60).

Notes. *Acalypha diminuta* was first illustrated, without description, in Baillon`s *Histoire Naturelle des Plantes* (Baillon 1891). The first description of this species, based only on Baillon`s illustration, appears in Engler`s Pflanzenreich (Pax and Hoffmann 1924). This is the only species included in *Acalypha* subgen. *Androcephala* Pax & K.Hoffm.

8. *Acalypha emirnensis* Baill., Adansonia 1: 270. 1861.

Type. Madagascar. Prov. Antananarivo: Antananarivo "in prov. Emirna, prope Tananarivou", 1833, *M. Bojer s.n.* (holotype: P [P00536723]!; isotypes GD-C [GDC005713]!, P [P00536725]!).

    Acalypha baronii Baker, J. Linn. Soc., Bot. 20: 254. 1883. Type: Madagascar: "Central Madagascar", 1882, *R. Baron 1725* (holotype: K [K000186523]!; isotype P [P00324467]!).

Acalypha hologyna Baker, J. Linn. Soc., Bot. 21: 441. 1885. Type: Madagascar: s.l., s.d., *R. Baron 2889* (holotype: K [K000186526]!; isotype P [P00536724]!).

Acalypha bakeriana Baill., Bull. Mens. Soc. Linn. Paris 2. 1180. 1895. Type: Madagascar: "Centr. Madag." *R. Baron 4425* (holotype: P [P00324466]!).

Acalypha emirnensis var. *bara* Leandri, Notul. Syst. (Paris) 10: 282. 1942. Type: Madagascar. Prov. Toliara: Massif of l'Ivakoany, 1928, *H. Humbert 6986* (lectotype, designated here: P [P00508509]!; isolectotype: P [P00508508]! **syn. nov**.).

Syntypes. Madagascar. Prov. Toliara: bassin supérieur du Mandrare: col et sommet de Marosohy, 1000–1400 m, 14–15 Nov 1928, *H. Humbert 6623* P [P00536758]!; Madagascar. Prov. Toliara: Massif de l'Ivakoany, *H. Humbert 12185* P [P00508506!, P00508507]!; Madagascar. Prov. Toliara: entre l'Andohahela et l'Elakelaka, *H. Humbert 13941* [P00324472].

Acalypha emirnensis var. *jabohaziana* Leandri, Notul. Syst. (Paris) 10: 283. 1942. Type: Madagascar. Prov. Mahajanga: "Boina, Jabohazo, près du mont Tsitondroina" Dec 1900, *H. Perrier de la Bâthie 9793* (holotype: P [P00536722]!. **syn. nov**).

Distribution. Madagascar (Antananarivo, Antsiranana, Fianarantsoa, Mahajanga, Toamasina, Toliara).

References. Müller Argoviensis (1866: 804); Baillon (1892: 1003); Baillon (1895a: 1180) as *A. baronii*; Baillon (1895b: 1196) as *A. hologyna*; Palacký (1907: 24, 25); Pax and Hoffmann (1924: 94, 171); Leandri (1942: 281); Govaerts et al. (2000: 50, 61); Govaerts et al. (2000: 67) sub. *A. hologyna*.

9. *Acalypha fasciculata* Müll.Arg., Linnaea 34: 31. 1865.

Type. Madagascar: s.l., s.d., *L. M. A. Du-Petit Thouars s.n.* (lectotype, designated here: P [P00324476]!; isolectotypes: P [P00324495!, P00508505!]).

Acalypha lyallii Baker, J. Linn. Soc., Bot. 20: 255. 1883. *Acalypha fasciculata* var. *lyallii* (Baker) Leandri, Notul. Syst. (Paris) 10: 284. 1942. Type: Madagascar: "Central Madagascar", s.d., *R. Lyall s.n.* (holotype: K [K000186529]! **syn. nov**.).

Acalypha madreporica Baill., Hist. Pl. Madag., Atlas t. 186 (1891). Type: Plate 186 in Baillon ibid. loc., holotype.

Acalypha madagascariensis Pax & K.Hoffm., Pflanzenr. 147, 16 (Heft 85): 162. 1924. Type: Madagascar. Prov. Toliara: Forêt d'Antsianaka, 19 Jan 1882, *H. Humblot 447* ["*449*"] (holotype B, presumably destroyed; lectotype, designated here: P [P00324501]!, **syn. nov.**).

Acalypha fasciculata var. *humbertiana* Leandri, Notul. Syst. (Paris) 10: 284. 1942. Type: Madagascar. Prov. Toliara: haute vallée de Mandrare, 8 Nov 1928, *H. Humbert 6514.* Lectotype, designated here: P [P00508503]!; isolectotypes: P [P00324487]!, US [US00096332]! **syn. nov**.). Syntypes. Madagascar. Prov.

Toliara: Bassin de la Manampanihy (Sud-Est), col de Fitana, *H. Humbert 6044* P [P00324486]!]; Madagascar. Prov. Toliara: Massif du Beampingaratra, du col de Bevava au sommet de Bekoho, *H. Humbert 6478* P [P00508504]!.

Distribution. Madagascar (Antsiranana, Fianarantsoa, Mahajanga, Toamasina, Toliara).

References. Müller Argoviensis (1866: 851); Baillon (1895a: 1181) *A. madreporica*. and *A. lyallii*; Palacký (1907: 25); Pax and Hoffmann (1924: 94) *A. madreporica*; Pax and Hoffmann (1924: 171); Leandri (1942: 283); Leandri (1948: 186) *A. lyallii*; Leandri (1952) *A. fasciculata* var. *humbertiana*; Govaerts et al. (2000: 61); Govaerts et al. (2000: 73) *A. lyallii*; Govaerts et al. (2000: 74) *A. madreporica*; Schatz (2001: 142) as *A. fasciculata* var. *humbertiana*; Seebaluck et al. (2015: 152) as *A. lyallii*.

Notes. The correct number of the type specimen of *A. madagascariensis* is *Humblot 447*. In the protologue of this name, it is wrongly transcribed as "*Humblot 449,* which corresponds to a specimen of *Psorospermum* (Clusiaceae).

10. *Acalypha filiformis* Poir., Encycl. 6(1): 205. 1804.

Type. Mauritius, "Île de France", s.d., *P. Commerson s.n.* (lectotype, designated here: P [P05604464]!; isolectotypes: MPU [MPU014933]!, P [P05604471]!).

Acalypha filiformis var. *arborea* Poir., Encycl. 6(1): 205. 1804. *Acalypha reticulata* var. *arborea* (Poir.) Müll.Arg. Linnaea 34: 32. 1865. Type: Reunion, "Elle croît a l'île Bourbon", 1774, *P. Commerson s.n.* (holotype: P [P05604473]!; isotype: MPU [MPU014949]!).

Tragia reticulata Poir., Encycl. 7: 725. 1806. *Acalypha reticulata* (Poir.) Müll.Arg., Prodr. 15(2): 851. 1866. Type: Reunion: "l'Ile -de-Bourbon", s.d., *P. Commerson s.n.* (holotype: P-LAM [P00382118]!; isotype P [P05604477]!).

Acalypha arborea Commers. in Poir., Encycl. 6: 205. 1804 *nom. nud.*

Acalypha lantanaefolia Bojer, Hortus Maurit. 286. 1837 *nom. nud.*

Distribution. Mascarene Islands (Mauritius, Réunion).

References. Bojer (1837: 286); Baillon (1858: 443) as *Tragia reticulata*; Baillon (1861: 266) as *A. arborea*; Müller Argoviensis (1866: 851) as *A. reticulata*; Baker (1877: 316) as *A. reticulata*; Müller Argoviensis (1882: 26) as *A. reticulata*; Pax (1890: 61) as *A. reticulata*; Pax (1894: 96) as *A. reticulata*; de Cordemoy (1895: 342) as *A. reticulata*; Voeltzkow (1917: 447) as *A. arborea*; Pax and Hoffmann (1924: 102) as *A. reticulata*; Leandri (1942: 258) as *A. reticulata*; Coode (1979: 45) as *A. reticulata*; Coode (1982: 69, 76); Govaerts et al. (2000: 62, 99, 105); Seebaluck et al. (2015: 150).

Notes. *Acalypha reticulata* has been usually considered as the accepted name of this species. Leandri (1942) noticed that *A. filiformis* and *A. reticulata* are conspecific, but he kept *A. reticulata* as the accepted name. Applying the rule of priority, the accepted name must be *A. filiformis* and *A. reticulata* should be placed as a synonym.

11. *Acalypha fimbriata* Schumach. & Thonn., Beskr. Guin. Pl. 409. 1827.

Type. Ghana: s.l., s.d., *P. Thonning s.n.* (holotype: C [C10003279]!; isotypes: C [C10003278!, C10003280]!, S [S14-42539]!).

Distribution. West Tropical Africa, West Central Tropical Africa, East Tropical Africa, Northeast Tropical Africa and Southern Africa. Madagascar (Antananarivo, Fianarantsoa, Toliara).

Notes. This is the first time that this species is cited for the WIOR region, where it is almost certainly introduced.

12. *Acalypha gracilipes* Baill., Adansonia 1: 273. 1861.

Acalypha commersoniana var. *gracilipes* (Baill.) Müll.Arg., Prodr. 15(2): 850. 1866. *Acalypha integrifolia* var. *gracilipes* (Baill.) Pax & K.Hoffm., Pflanzenr. 147,16 (Heft 85): 106. 1924.

Type. Madagascar: s.l., s.d., *P. Commerson s.n.* (holotype: P [P04022747]!).
Acalypha reticulata var. *cloiselana* Leandri, Notul. Syst. (Paris) 10: 266. 1942. Type: Madagascar: s.l., s.d., *P. Commerson s.n.* (lectotype, designated here: P [P00513166]!, **syn. nov.**).
Syntypes. Madagascar. Prov. Toamasina: Fénérive, *H. Perrier de la Bâthie 9707* [P00513169]!; Madagascar. Prov. Toliara: Fort Dauphin, *J. Cloisel 156* P [P00513165]!; Madagascar. Prov. Toliara: Forêt de Manantantely, *H. Humbert 5835* P [P00513167]!, P [P00513168]!; Madagascar. Prov. Toliara: Fort Dauphin, *G. F. Scott-Elliot 2493* P [P00513171]!; Madagascar: s.l., s.d., *R. Baron 5980* P [P00513164]!; *R. Baron 6420* [P00324562]!.
Acalypha cloiselana Denis ex Leandri, Notul. Syst. (Paris) 10: 266. 1942 *nom. nud.* as synonym of *A. reticulata* var. *cloiselana* Leandri.
Acalypha commersonii Baill. ex Leandri, Notul. Syst. (Paris) 10: 266. 1942 *nom. nud.* as synonym of *A. reticulata* var. *cloiselana* Leandri.

Distribution. Madagascar (Antsiranana, Fianarantsoa, Toamasina, Toliara).

References. Müller Argoviensis (1866: 850) as *A. commersoniana* var. *gracilipes*; Baillon (1892: 1004); Palacký (1907: 25); Pax and Hoffmann (1924: 106) as *A. integrifolia* var. *gracilipes*; Govaerts et al. (2000: 69, 100).

Notes. *Acalypha gracilipes* has been usually treated as a variety of *A. integrifolia* Willd., which is endemic of Mascarene Islands, but *A. gracilipes* can be clearly distinguished by its elliptic-lanceolate leaves, denticulate female bracts and glabrous and glandular ovaries, vs. linear-lanceolate leaves, entire female bracts and hispidulous and echinate ovary in *A. integrifolia*.

A. gracilipes can be distinguished from both *A. urophylla* Boivin ex Baill. and *A. paxii* Aug.D.C. mainly by its glabrous leaves with crenate to subdentate margins and obtuse to subacute apices, glabrous female bracts and glabrous ovaries with minute sessile glands, vs. pubescent leaves with serrate margins and acuminate (*A. urophylla*) or

usually caudate (*A. paxii*) apices, pubescent female bracts and hispidulous ovaries with long papillae. In addition, the female bracts of *A. paxii* have a prominent central tooth, which is absent in both *A. gracilipes* and *A. urophylla*.

Leandri (1942) included the collection *H. Perrier de la Bâthie 9746* as *Acalypha reticulata* var. *cloiselana* Leandri, however the specimen of this collection in P (P00513170) corresponds to *A. urophylla*.

13. *Acalypha hispida* Burm.f., Fl. Ind. 303, pl. 61, f. 1. 1768.

Ricinocarpus hispidus (Burm. f.) Kuntze, Revis. Gen. Pl. 2: 618. 1891.

Type. Habitat in India, Tab. 61 in Burm. f., loc. cit. 302. 1768.

Distribution. Introduced in Tropical Africa and the WIOR. Madagascar (Toliara).

References. Bojer (1837: 25); Baillon (1861: 274); Palacký (1907: 25); Robertson (1989: 199).

Notes. This shrub, native to Melanesia or Malesia, is frequent in gardens throughout the tropics and rarely appears naturalised. As all plants are pistillate, it can only reproduce clonally. We found collections from Madagascar and Seychelles where it is cultivated. It has been reported from Madagascar (Palacký 1907), Mauritius (Bojer 1837; Baillon 1861), Réunion (Baillon 1861: 274) and the Seychelles (Robertson 1989).

14. *Acalypha humbertii* Leandri, Notul. Syst. (Paris) 10: 274. 1942.

Type. Madagascar. Prov. Toliara: vallées du Mangoky et de l'Isahaina, aux environs de Beroroha, 200 m, Oct 1933, *H. Humbert 11289* (lectotype, designated here: P [P00508400]!; isolectotype: P [P00508399]!).

Syntypes. Madagascar. Prov. Toliara: Bassin supérieur de Mandrare du Sud-Est, entre le col de Vavara et la vallée de la Manambolo, 700–1200 m, 20–22 Dec. 1928, *H. Humbert 6758* P [P00508401!, P00508402!, P00508403!, P00508404!]).

Distribution. Madagascar (Toliara).

References. Govaerts et al. (2000: 67).

15. *Acalypha indica* L., Sp. Pl. 2: 1003. 1753.

Type. India: s.l., s.d., *Herb. Hermann 3: 2, #34.* (lectotype, designated by Radcliffe-Smith (1986: 65): BM; isolectotype: BM).

Distribution. Widely distributed in the Paleotropics and introduced in the Americas. In the WIOR, it is found only in disturbed areas and almost certainly is introduced there. Madagascar (Antananarivo, Antsiranana, Fianarantsoa). Comoros Archipelago (Anjouan, Mohéli, Mayotte). Mascarene Islands (Mauritius, Réunion). Seychelles Archipelago.

References. Bojer (1837: 285); Baillon (1861: 274); Müller Argoviensis (1865: 42; 1866: 868); Baker (1877: 314); Müller Argoviensis (1882: 27); Baillon (1895b: 1197); de Cordemoy (1895: 342); Palacký (1907: 25); Voeltzkow (1917: 447); Hemsley (1919: 148); Pax and Hoffmann (1924: 33); Leandri (1935: 43); Leandri (1942: 256); Renvoize (1975: 152); Coode (1982: 69, 78); Robertson (1989: 200); Govaerts et al. (2000: 68).

16. *Acalypha integrifolia* Willd., Sp. Pl. 4(1): 530. 1805.

Type. Mauritius, s.l., s.d., *Anonymous, s.n.* (lectotype designated by Coode (1978: 39): B [B-W17834-020]).

Tragia colorata Poir., Encycl. 7: 725. 1806. *Acalypha colorata* (Poir.) Spreng., Syst. Veg. 3: 879. 1826. *Acalypha commersoniana* var. *obtusifolia* f. *colorata* (Poir). Müll.Arg., Prodr. 15(2): 850. 1866. *Acalypha integrifolia* var. *colorata* (Poir.) Pax & K.Hoffm. Pflanzenr. 147,16 (Heft 85): 106. 1924. Type: Mauritius: "Cete plante croit dans les indes orientales, & à l'Ile de France,", s.l. s.d., *P. Commerson s.n.* (holotype: P-LAM [P00382140]!).

Acalypha reticulata var. *longifolia* Müll.Arg., Linnaea 34: 32. 1865. *Acalypha integrifolia* var. *longifolia* (Müll.Arg.) Coode, Kew Bull. 34: 41. 1979. Type: Mauritius: s.l., s.d., *L. Bouton s.n.* (holotype: GDC [G00324522]!, **syn. nov.**).

Acalypha reticulata var. *longifolia* f. *aberrans* Müll.Arg., Linnaea 34: 32. 1865. Type: Mauritius: "In sylvis Mauritii", 1833, *M. Bojer s.n.* (holotype: GDC [G00324521]!).

Acalypha commersoniana Baill. ex Müll.Arg., Prodr. 15(2): 849. 1866. *Acalypha commersoniana* var. *brevifolia* Baill. ex Müll.Arg., Prodr. 15(2): 850. 1866. Type: Mauritius: "Cum var. praecedentibus", s.d., *Anonymous, s.n.* (*Hb. Willd. fol. 17834 pag. 1*) (lectotype, designated here: B [B-W17834-010]!). Syntypes. Mauritius: s.l., s.d., *L. Bouton s.n.* GDC [G00324543]!.

Acalypha commersoniana var. *acutifolia* Müll.Arg., Prodr. 15(2): 849. 1866. *Acalypha commersoniana* var. *acutifolia* f. *purpurea* Müll.Arg., Prodr. 15(2): 849. 1866. Type: Mauritius: s.l., s.d., *F. W. Sieber 181 pr. p.* (lectotype, designated here: GDC [G00324550]!). Syntype: Mauritius: s.l., s.d., *L. Bouton s.n.* GDC [G00324559]!.

Acalypha commersoniana var. *acutifolia* f. *purpureo-marginata* Müll.Arg., Prodr. 15(2): 849. 1866. Type: Mauritius: s.l., s.d., *Hb. Boiss. s.n.* (holotype: GDC [G00324557]!; isolectotype: K [K000431097]!, **syn. nov.**).

Acalypha commersoniana var. *acutifolia* f. *concolor* Müll.Arg., Prodr. 15(2): 849. 1866. *Acalypha integrifolia* var. *concolor* (Müll.Arg.) Pax & K.Hoffm, Pflanzenr. 147,16 (Heft 85): 106. 1924. Type: Mauritius: "Cum form. Praecentibus", s.d., *J. B. G. M. Bory s.n.* (lectotype, designated here: GDC [G00324554]!). Syntype: Mauritius: s.l., s.d., *F. W. Sieber 18*2 GDC [G00324556]!.

Acalypha commersoniana var. *longifolia* Müll.Arg., Prodr. 15(2): 850. 1866. Type: Mauritius: s.l., s.d., *Bouton s.n.* (lectotype, designated here: GDC [G00324553]!; isolectotypes: GDC [G00324552!, G00324551!], K [K000431101!], **syn. nov.**).

Acalypha commersoniana var. *parvifolia* Baill. ex Müll.Arg., Prodr. 15(2): 850. 1866. *Acalypha integrifolia* var. *parvifolia* (Baill. ex Müll.Arg.) Pax & K.Hoffm., Pflanzenr. 147,16(Heft 85): 106. 1924. Type: Mauritius: "Cum var. praecedentibus", s.d., *F. W. Sieber 369* p.p. (holotype: GDC [G00324538]!, **syn. nov.**).

Acalypha commersoniana var. *obtusifolia* Müll.Arg., Prodr. 15(2): 850. 1866. *Acalypha commersoniana* var. *obtusifolia* f. *discolor* Müll.Arg., Prodr. 15(2): 850. 1866. Type: Mauritius: "Cum praecedentibus", s.d., *F. W. Sieber 181 pr. p.* (holotype: GDC [G00324558]!, **syn. nov.**).

Acalypha commersoniana var. *obtusifolia* f. *unicolor* Müll.Arg., Prodr. 15(2): 850. 1866. Type: Mauritius: s.l., s.d., *F. W. Sieber 178* (holotype: GDC [G00324539]!; isotypes P [P04779345!, P04780015!].

Acalypha discolor Bojer, Hortus Maurit. 286. 1837 *nom. nud.*

Tragia macrophylla Wall., Numer. List n. 7796. 1847 *nom. nud.*

Tragia lobata Wall., Numer. List n. 7796. 1847 *nom. nud.*

Acalypha commersoniana Baill., Adansonia 1: 267. 1861 *nom. nud.*

Acalypha commersoniana var. *concolor* Baill., Adansonia 1: 267. 1861 *nom. nud.*

Acalypha commersoniana var. *discolor* Baill., Adansonia 1: 267. 186 *nom. nud.*

Acalypha commersoniana var. parvifolia Baill., Adansonia 1: 267. 1861 *nom. nud.*

Caturus sessilis Pet. Thou. ex Baill., Adansonia 1: 267. 1861 *nom. nud.*

Tragia fruticosa Commers. ex Baill., Adansonia 1: 267. 1861 *nom. nud.*

Tragia obtusata Vahl. ex Baill., Adansonia 1: 267. 1861 *nom. nud.*

Distribution. Mascarene Islands (Mauritius, Réunion).

References. Bojer (1837: 286); Baillon (1858: 443) *A. colorata*; Müller Argoviensis (1866: 850); Baker (1877: 315) *A. colorata*; de Cordemoy (1895: 342) *A. colorata*; Palacký (1907: 24) *A. commersoniana*; Palacký (1907: 25); Pax and Hoffmann (1924: 105); Coode (1982: 69); Robertson (1989: 200); Govaerts et al. (2000: 69).

Notes. Coode (1982) accepted three subspecies with six varieties within *Acalypha integrifolia*, but we find the varieties he placed within subsp. *integrifolia* to overlap too much to accept as distinct taxa. See *A. marginata* (Poir.) Spreng. for our treatment of what Coode treated within *A. integrifolia* subsp. *marginata* (Poir.) Coode and subsp. *panduriformis* Coode.

17. *Acalypha lamiana* (Leandri) I.Montero & Cardiel., comb. nov.

urn:lsid:ipni.org:names:60476986-2

Acalypha reticulata var. *urophylla* f. *lamiana* Leandri, Notul. Syst. (Paris) 10: 263. 1942.

Type. Madagascar, Prov. Mahajanga, Reserve de Marohogo, 28 Dec 1938, *H. J. Lam & A. D. J. Meeuse, 6127* (lectotype, designated here: P [P05604417]!; isolectotype: L [L0242109]!).

Syntypes. Madagascar, Prov. Antananarivo, Tsarasaotra, Feb 1898, *H. Perrier de la Bâthie 457* P [P05604408!, P05604409!, P05604410!, P05604413!]; Madagascar, Prov. Fianarantsoa, Ankirihitra près du mont Tsilondroina, Mar 1902 , *H. Perrier de la Bâthie 9817* P [P05604403]!, *9817 bis* P [P05604404!, P05604405!]; Madagascar, Prov. Mahajanga, NW of Ankazobe,Vallée de l'Ikopa, 14 Mar 1930, *R. Decary 7535* P [P05604421]!; Massif de l'Ankarafantsika, 11 Jan 1938, *R. Decary 12876* P [P05604420]!; Bekodoka, 17 Sept 1930, *R. Decary 8109* P [P05604422]!; Region d'Antsalova, 1932-1933, *J. Leandri 998* P [P05604411]!; Tsingy du Bemaraha, 3-6 Oct 1932, *J. Leandri 176* P [P05604415]!; Dokolahy, Feb-Apr 1933, J. Leandri 602 P [P05604414]!; Madagascar, Prov. Toliara, Soahazo Forest, 100 m, 22 Oct 1932, *J. Leandri 414* P [P05604416]!.

Distribution. Madagascar (Antananarivo, Fianarantsoa, Mahajanga and Toliara).

Notes. *Acalypha lamiana* was treated by Leandri (1942) as a form of *A. reticulata* var. *urophylla* (Boivin ex Baill.) Müll.Arg. (treated here as *A. urophylla*). After studying the numerous type collections, it seems clear to us that *A. lamiana* must be considered as a distinct species. *A. lamiana* differs from *A. urophylla* mainly by the leaves that are rounded at the base and reddish at the margins and its subentire, eglandular female bracts vs. leaves that are usually acute at the base and not reddish at the margins and dentate female bracts with small sessile glands at margins, in *A. urophylla*.

One of the mentioned syntypes, *Perrier de la Bâthie 9817,* was wrongly transcribed by Leandri (1942: 263) as "*8917*".

18. *Acalypha lanceolata* var. *glandulosa* (Müll.Arg.) Radcl.-Sm., Kew Bull. 44(3): 444. 1989.

Acalypha crenata var. *glandulosa* Müll.Arg., Linnaea 34: 43. 1865.

Type. Tanzania: Zanzibar, 1847-1852, *L. H. Boivin s.n.* (lectotype, designated here: P [P05511211]!; isolectotypes: P [P05511212!, P05511225!, P05510174!]).

Distribution. *Acalypha lanceolata* Willd. is widely distributed in the Paleotropics; the var. *glandulosa* occurs in East Tropical Africa and South Tropical Africa. Madagascar (Antsiranana). Mascarene Islands (Réunion).

References. Müller Argoviensis (1866: 872); Coode (1982: 69, 79); Govaerts et al. (2000: 71); Seebaluck et al. (2015: 152).

Notes. *Acalypha lanceolata* var. *lanceolata* occurs in Asia. The main diference between the African var. *glandulosa* and the Asian var. *lanceolata* is the presence or absence of stipitate glands. Additional studies are needed to clarify the taxonomic status of these taxa.

19. *Acalypha leonii* Baill., Bull. Mens. Soc. Linn. Paris ii. (1895) 1197.

Type. Madagascar. Prov. Toamasina: Forêt d'Antsianaka, 14 Dec 1882, *L. Humblot 514* (lectotype, designated here: P [P00513056]!; isolectotypes: K [K000186528]!, P [P00513057!, P00513058!, P00513055!]).

> *Acalypha leonii* var. *perrierana* Leandri, Notul. Syst. (Paris) 10: 271. 1942. Type: Madagascar. Prov. Mahajanga: bassin du Bemarivo, versant NE, 100 m, 1912, *H. Perrier de la Bâthie 9719* (lectotype, designated here: P [P00513061]!; isolectotypes: P [P00513059!, P00513060!], **syn. nov.**).
>
> **Distribution.** Madagascar (Antananarivo, Antsiranana, Toamasina, Toliara).
>
> **References.** Palacký (1907: 25); Pax and Hoffmann (1924: 112); Leandri (1942: 271); Leandri (1952); Govaerts et al. (2000: 72); Schatz (2001: 142).

20. *Acalypha lepidopagensis* Leandri, Notul. Syst. (Paris) 10: 280. 1942.

Type. Madagascar. Prov. Antsiranana: Massif du Tsaratanana, 1000 m, Dec 1912, *H. Perrier de la Bâthie 9726* (lectotype, designated here: P [P00513062]!; isolectotype: P [P00513063]!).

> **Distribution.** Madagascar (Antsiranana).
>
> **References.** Leandri (1952); Govaerts et al. (2000: 72); Schatz (2001: 142).

21. *Acalypha leptomyura* Baill., Hist. Pl. Madag., Atlas (1891) t. 191.

Type. Madagascar. Plate 191 in Baillon ibid. loc., holotype.

> **Distribution.** Madagascar (Antananarivo, Antsiranana, Fianarantsoa, Mahajanga, Toamasina and Toliara).
>
> **References.** Baillon (1892: 1004); Koehne (1892: 131); Palacký (1907: 25); Pax and Hoffmann (1924: 112); Leandri (1942: 271); Govaerts et al. (2000: 72).

22. *Acalypha linearifolia* Leandri, Notul. Syst. (Paris) 10: 275. 1942.

Type. Madagascar. Prov. Toliara: Ambovombe, Kotoala, 21 Jan 1931, *R. Decary 8423* (lectotype, designated here: P [P00513090]!; isolectotypes: S [S07-14664]!, TAN [TAN000510]!, US [US01014148]!).

> **Syntypes.** Madagascar. Prov. Toliara: delta de la Linta, 17–24 Aug 1928, *H. Humbert & C. F. Swingle 5385* P [P00513086!, P00513087!, P00513088!, P00513089!]; US [US00096361]!.
>
> **Distribution.** Madagascar (Toamasina, Toliara).
>
> **References.** Govaerts et al. (2000: 72).

23. *Acalypha marginata* (Poir.) Spreng., Syst. Veg. 3: 879. 1826.

Tragia marginata Poir., Encycl. 7: 725. 1806. *Acalypha integrifolia* subsp. *marginata*
    (Poir.) Coode, Kew Bull. 34: 42. 1979.

Type. Mauritius: "Les Indes Orientales", s.d. (holotype P-LAM [P00382145]!).
    Acalypha integrifolia subsp. *panduriformis* Coode, Kew Bull. 34: 42. 1979. Type:
        Réunion: Cliff between St Philippe and St Joseph near Basse vallée, ca. 100 m,
        26 Feb 1975. *M. J. E. Coode & T. H. Cadet 4968* (holotype K [K000431108]!;
        isotype K [K000431107]!, **syn. nov.**).
    Acalypha integrifolia subsp. *marginata* var. *saltuum* Coode, Kew Bull. 34: 43. 1979.
        Type: Mauritius: Macabé, 650 m, 15 Feb 1975, *M. J. E. Coode et al. 4874* (holo-
        type: K [K000431106]!; isotypes P [P04779351]!, MAU [n.v.], **syn. nov.**).
    Acalypha integrifolia subsp. *marginata* var. *crateriana* Coode, Kew Bull. 34: 44.
        1979. Type: Mauritius: Tamarin Falls, 2 Mar1975, *D. Lorence 1138* (holotype:
        K [K000431104]!; isotype K [K000431105]!, MAU [n.v.], **syn. nov.**).
    Distribution. Mascarene Islands (Mauritius, Réunion).
    References. Bojer (1837: 286); Baillon (1858: 443); Baillon (1861: 267); Baker
(1877: 315); de Cordemoy (1895: 343); Palacký (1907: 25); Pax and Hoffmann (1924:
106); Coode (1982: 73) *A. integrifolia* subsp. *panduriformis*, *A. integrifolia* subsp. *mar-
ginata*; Coode (1982: 74) *A. integrifolia* var. *saltuum*, *A. integrifolia* var. *crateriana*;
Govaerts et al. (2000: 69) *A. integrifolia* subsp. *marginata*; Seebaluck et al. (2015: 152).
    Notes. *Acalypha marginata* was treated by Coode (1979) as *A. integrifolia* subsp.
marginata. We consider *A. marginata* to be a distinct species, differentiated mainly
by its variegated leaf blades and vestigial female bracts that are not accrescent in fruit
vs. non variegated leaf blades and conspicuous female bracts that are accrescent in
fruit in *A. integrifolia*. We include as synonyms *A. integrifolia* subsp. *panduriformis*, *A.
integrifolia* var. *crateriana* and *A. integrifolia* var. *saltuum* because they have the same
characters as *A. marginata*.

24. *Acalypha medibracteata* Radcl.–Sm. & Govaerts, Kew Bull. 52(2): 477. 1997.

Acalypha gagnepainii Leandri Notul. Syst. (Paris) 10: 274. 1942 *nom. illeg.* non *A.
    gagnepainii* Merr. (1938).

Type. Madagascar. Prov. Toliara: Massif du Vohitsiombe (Fort-Dauphin), 31 Jul
1926, *R. Decary 4664* (lectotype, designated here: P [P00508417]!; isolectotype: S
[S07-14667]!).
    Syntypes. Madagascar. Prov. Toliara: Vallée du Mandrare, s.d., *R. Decary 2620* P
[P00508418]!; Madagascar. Prov. Toliara: Vallée de l'Ikonda, au N. d'Ambovombe,
R. Decary 8913, P [P00887487]!; Madagascar. Prov. Toliara: Imangory, s.d., *R. De-
cary 8948* P [P00508416]!; Madagascar. Prov. Toliara: Beteny, (limite Nord-Est de

l'Androy), 22 Nov 1931, *R. Decary 9355* P [P00508415]!, G [G00034242]!, GB [GB0047682]!.

> *Acalypha medibracteata* var. *calcicola* (Leandri) Radcl.–Sm. & Govaerts, Kew Bull. 52(2): 477. 1997. *Acalypha gagnepainii* var. *calcicola* Leandri Notul. Syst. (Paris) 10: 275. 1942. Type: MADAGASCAR. Prov. Toliara: vallée moyenne du Mandrare près d'Anadabolava, Dec 1933, *H. Humbert 12422* (lectotype, designated here: P [P00508409]!; isolectotypes: P [P00508405!, P00508406!, P00508407!, P00508408!], **syn. nov.**).

> Syntypes. MADAGASCAR. Prov. Toliara: Fort-Dauphin, *J. Cloisel 18* P [P00508410]!, P [P00508411]!; MADAGASCAR. Prov. Toliara: Basse vallée du Fiherenana, s.d., *H. Humbert 11573* P [P00508412!, P00508413!].

> *Acalypha menabeana* Leandri Notul. Syst. (Paris) 10: 275. 1942 *nom. nud.* as *A. gagnepainii.*

> *Acalypha aspretorum* Leandri Notul. Syst. (Paris) 10: 275. 1942 *nom. nud.* as *A. gagnepainii.*

> **Distribution.** MADAGASCAR (Antananarivo, Toamasina, Toliara).

> **References.** Govaerts et al. (2000: 75, 100).

25. *Acalypha menavody* (Leandri) I.Montero & Cardiel, comb. nov.
urn:lsid:ipni.org:names:60476988-2

Acalypha spiciflora var. *menavody* Leandri, Notul. Syst. (Paris) 10: 270. 1942.

Type. MADAGASCAR. Prov. Antsiranana: Collines et plateaux calcaires de l'Analamera, Jan 1938, *H. Humbert 19149* (lectotype, designated here: P [P00536737]!; isolectotypes: P [P00536736!, P00536738!]).

> **Distribution.** MADAGASCAR (Antsiranana).

> **Notes.** *Acalypha spiciflora* has been excluded because it does not belong to *Acalypha* (see notes under this name). We only recognise *A. spiciflora* var. *menavody* as *A. menavody.*

26. *Acalypha paxii* Aug.D.C., Bull. Herb. Boissier, sér. 2, 1: 567. 1901.

Acalypha urophylla Pax, Bot. Jahrb. Syst. 19: 96. 1894. *nom. illeg.* non *A. urophylla* Boivin ex Baill. (1861). *Acalypha reticulata* var. *urophylloides* Pax & K.Hoffm., Pflanzenr. 147, 16 (Heft 85): 105. 1924. *Acalypha filiformis* var. *urophylloides* (Pax & K.Hoffm.) Govaerts, World Checkl. Bibliogr. Euphorbiaceae 63. 2000.

Type. MADAGASCAR. Prov. Antsiranana: Nossibé, nordwest, Feb 1880, *J. M. Hildebrandt 3356* (holotype B or WRSL, presumably destroyed; lectotype, designated here: BREM [BREM0001784]!; isolectotypes: JE [JE00004294!, JE00004293!], K [K000186531]!, M [M0110600]!, P [P00536741!, P00536742!, P00536743!]).

Acalypha pervilleana Baill., Adansonia 1: 273. 1861. *Acalypha reticulata* var. *pervilleana* (Baill.) Müll.Arg., Linnaea 34: 32. 1865. *Acalypha filiformis* var. *pervilleana* (Baill.) Govaerts, World Checkl. Bibliogr. Euphorbiaceae 62. 2000. Type. Madagascar. Prov. Antsiranana: Nossibé, 1840, *M. Richard 384* (lectotype, designated here: P [P00536745]!; isolectotype: P [P05604474]!, **syn. nov.**).

Syntypes. Madagascar, Prov. Antsiranana: Nossibé, *M. Pervillé 368* P [P00536746!, P00536747!].

Acalypha humblotiana Baill., Hist. Pl. Madag., Atlas (1891) t. 1891. *Acalypha reticulata* var. *urophylla* f. *humblotiana* (Baill.) Leandri, Notul. Syst. (Paris) 10: 262. 1942. Type: Comoros: Grande comore, 14 Nov 1885, *L. Humblot 1461* (lectotype, designated here: P [P00196295]!; isolectotypes: P [P02712292!, P00196296]!, **syn. nov.**).

Acalypha meiodonta Baill., Bull. Mens. Soc. Linn. Paris 2: 1197. 1895. *Acalypha reticulata* var. *meiodonta* (Baill.) Leandri. Notul. Syst. (Paris) 10: 267. 1942. Type: Madagascar: Centr. Madag., Dec. 1883, *R. Baron 2826* (lectotype, designated here: P [P05604378]!; isolectotype: K [K000186508]!, **syn. nov.**). Syntype: Madagascar: "Centr. Madag.", s.d., *R. Baron 6581* K [n.v.].

Acalypha reticulata var. *meiodonta* f. *andronea* Leandri, Notul. Syst. (Paris) 10: 268. 1942. Type: Madagascar, Prov. Mahajanga, Bemarivo (Boïna), Dec 1906, *H. Perrier de la Bâthie 9561* (lectotype, designated here: P [P00513143]!; isolectotype: P [P00513144]!, **syn. nov.**).

Former syntypes: Madagascar, Prov. Mahajanga, bord du massif du Manongarivo, versant du Sambirano, 1909, *H. Perrier de la Bâthie 9934* P [P00513149]!; Massif du Manongarivo, versant du Sambirano, Sep 1909, *H. Perrier de la Bâthie 9939* P [P00513150]!; haut Bemarivo (Andranofosy), Boïna, Jan 1907, *H. Perrier de la Bâthie 9635* P [P00513145]!; Massif du Manongarivo, Sambirano, Apr 1909, *H. Perrier de la Bâthie 9928* P [P00513142]!; Manongarivo (Ambongo), Oct 1904, *H. Perrier de la Bâthie 1677*, P [P00513146!, P00513147!].

Distribution. Madagascar (Antsiranana, Mahajanga, Toamasina). Comoros Archipelago (Grande Comore, Anjouan, Mohéli and Mayotte).

References. Müller Argoviensis (1866: 852) as *A. reticulata* var. *pervilleana*; Müller Argoviensis (1882: 26) as *A. reticulata* var. *urophylla*; Müller Argoviensis (1866: 852) as *A. reticulata* var. *pervilleana*; Baillon (1892: 1004) as *A. pervilleana*; Baillon (1895b: 1197) as *A. humblotiana*; Palacký (1907: 25, 26); Pax and Hoffmann (1924: 105, 112) as *Acalypha reticulata* var. *urophylloides* and *A. reticulata* var. *pervilleana*; Leandri (1942: 258, 260) as *A. reticulata* var. *pervilleana*; Govaerts et al. (2000: 62, 63, 75, 104 105, 107).

Notes. *Acalypha paxii* was proposed by August De Candolle as a replacement name for the illegitimate *A. urophylla* Pax. Leandri (1942) treated *A. paxii* as a synonym of *A. reticulata* var. *pervilleana*. We consider that *A. paxii* is a well-differentiated species.

Acalypha paxii, A. pervilleana and *A. meiodonta* have been considered synonyms of *A. reticulata*, which we consider to be a synonym of *A. filiformis*. *A. paxii* can be differentiated from *A. filiformis* by its sessile, dentate female bracts with a prominent

central tooth vs. pedicellate, crenate to subentire female bracts in *A. filiformis* (see notes in *A. filiformis*).

Acalypha paxii can be distinguished from *A. urophylla*, which has also been placed within *A. reticulata*, mainly by its leaves with usually caudate apices, its dentate female bracts with a prominent central tooth and eglandular margins vs. leaves with usually acuminate apices, dentate female bracts without a prominent central tooth and with small sessile glands at the margins. See notes under *A. gracilipes* Baill. for the differences between *A. paxii* and that species.

Acalypha humblotiana was first illustrated, without description, in Baillon`s (1891) *Histoire Naturelle des Plantes*, but this illustration is not consistent with Baillon`s (1895b) later description nor with the specimen on which the description presumably is based (*L. Humblot 1461*).

Although *Richard 384* [P00536745]! and *385* [P04779454]! have labels giving the locality as "Bourbon", now Réunion, these are not Richard's original labels and the species is otherwise unknown from the Mascarene Islands. We do not have evidence that it occurs there.

27. *Acalypha perrieri* Leandri, Notul. Syst. (Paris) 10: 273. 1942.

Type. Madagascar. Prov. Mahajanga: Belambo, près de Maevatanana, Aug 1901, *H. Perrier de la Bâthie 981* (lectotype, designated here: P [P00513095]!).

Syntypes. Madagascar. Prov. Mahajanga: Menabé, Tsiampihy, *J. Leandri 275* P [P00513092]!, P [P00513093]!, TAN [TAN000511]!; Madagascar. Prov. Mahajanga: ibid. loc., s.d., *J. Leandri 294*, P [P00513091]!; Madagascar. Prov. Mahajanga: Forêt de Tsimembo, s.d., *J. Leandri* 420, P [P00513094]!.

Distribution. Madagascar (Antsiranana, Mahajanga, Toliara).

References. Govaerts et al. (2000: 81).

28. *Acalypha poiretii* Spreng., Syst. Veg. 3: 879. 1826.

Ricinocarpus poiretii (Spreng.) Kuntze, Gen. Pl. 2: 618. 1891.

Type. "Amer. trop." s. loc., s.d., *Anonymous s.n.* (holotype: P-LAM [P00382110]!).

Distribution. Introduced in Tropical Africa and the WIOR region. Mascarene Islands (Mauritius, Réunion, Rodríguez).

References. Baker (1877: 315); de Cordemoy (1895: 312); Coode (1982: 79).

Notes. Herb native to the Americas. It has been reported from continental Africa (Cardiel and Montero Muñoz 2018) and from the Mascarene Islands (Baker 1877; de Cordemoy 1895; Coode 1982). We found specimens from the Mascarene Islands (Mauritius, Réunion and Rodríguez).

29. *Acalypha radula* Baker, J. Linn. Soc., Bot. 20: 254. 1883.

Type. Madagascar: "Central Madagascar", 1882, *R. Baron 1818* (holotype: K [K000186509]!; isotypes: P [P00513119!, P00513120!]).

  Acalypha hildebrandtii Baill., Bull. Mens. Soc. Linn. Paris 2: 1005, 1180. 1892. Type: Madagascar. Prov. Fianarantsoa: "Betsileo, Nandahizana,". *J. M. Hildebrandt 3900* (holotype: P [P00513121]; isotypes: BREM [BREM0001783]!, G [G00190630!, G00074184!], JE [JE00000289!, JE00000288!], K [K000186507]!, M [M0110604!], P [P00513122]!).

  Acalypha andringitrensis Leandri, Notul. Syst. (Paris) 10: 277. 1942. Type. Madagascar. Prov. Fianarantsoa: Massif of Andringitra, Apr 1921, *H. Perrier de la Bâthie 13640* (lectotype, designated here: P [P00508596]!; isolectotypes: P [P00508594!, P00508595]!, **syn. nov.**).

    Syntypes. Madagascar, ibid. loc., 1924, *H. Humbert 3709* P [P00224706]!, P [P00508591]!, P [P00508592]!, P [P00508593]!; Madagascar, ibid. loc., Apr 1921, *H. Perrier de la Bâthie 9671* P [P00508589!, P00508590!].

  Acalypha salviifolia Baill., Étude Euphorb. 443. 1858 *nom. nud.*

  Tragia salviaefolia Boj. ex Baill., Étude Euphorb. 443. 1858 *nom. nud.*

  Distribution. Madagascar (Antananarivo, Fianarantsoa, Toamasina and Toliara).

  References. Baillon (1861: 268) as *A. salviifolia*; Müller Argoviensis (1866: 889) *A. salviifolia*; Baillon (1891: 193); Baillon (1892: 1004) *A. salviifolia*; Baillon (1895a: 1180) *A. hildebrandtii*; Palacký (1907: 25) *A. hildebrandtii*; Palacký (1907: 26); Palacký (1907: 26) *A. salviifolia*; Pax and Hoffmann (1924: 102) as *A. salviifolia*; Pax and Hoffmann (1924: 156) *A. hildebrandtii*; Leandri (1942: 278); Leandri (1952); Jenkins (1987:347); Jenkins (1990: 408, 433); Goodman (1996: 61) *A. andringitrensis*; Govaerts et al. (2000: 49) *A. andringitrensis*; Govaerts et al. (2000: 67) *A. hildebrandtii*; Govaerts et al. (2000: 85, 86, 105) *A. salviifolia*; Schatz (2001: 142); Seebaluck et al. (2015: 149, 153).

  Notes. Although Leandri (1942) distinguished *Acalypha andringitrensis* and *A. radula* based on leaf shape and bract incision, more recent collections show continuous variation between the extremes recognised by Leandri, and DNA sequences do not differentiate these forms (G. A. Levin, pers. obs.).

30. *Acalypha richardiana* Baill., Adansonia 1: 268. 1861.

Type. Comoros: Mohéli, "Ile Mohilla", s.d., *M. Richard 287* (lectotype, designated here: P [P04779566]!; isolectotypes: P [P04779562!, P04779564!, P04779565!]).

  Syntypes. Madagascar: s.l., s.d., *M. Richard 544* [P04779563]!; Mayotte, s.l., s.d., *L. H. Boivin 3373* GDC [G00324505]!, P [P00196299!, P00196300!], W [W-Rchb. 1889-0166704]!.

  Acalypha ovalifolia Baill., Adansonia 1: 269. 1861. *Acalypha reticulata* var. *ovalifolia* (Baill.) Müll.Arg., Linnaea 34: 32. 1865. *Acalypha filiformis* var. *ovalifo-*

lia (Baill.) Govaerts, World Checkl. Bibliogr. Euphorbiaceae 62. 2000. Type: Mayotte: Nov 1848. *L. H. Boivin 3372* (holotype: P [P00196298]!).

Distribution. Comoros Archipelago (Anjouan, Mohéli, Mayotte).

References. Müller Argoviensis (1866: 852 as *A. reticulata* var. *ovalifolia*, 855); Baillon (1892: 1004); Palacký (1907: 25 as *A. ovalifolia*, 26); Voeltzkow (1917: 447); Pax and Hoffmann (1924: 127); Leandri (1942: 272); Govaerts et al. (2000: 62, 85, 105).

Notes. The specimens, indicated as isolectotypes, do not have Richard's original label. They instead have labels with Baillon's handwriting giving the location as "Madagascar". We believe that the correct location is the one indicated on the selected lectotype, "Ile Mohilla", whose current name is Mohéli, in the Comoros Archipelago. A search of specimens at P showed that Richard's collections numbered *284–286, 288, 290, 291* and *293* are also from Mohéli (*289* has no locality and *292* is not listed). *Richard 544* has an apparently original label showing the locality only as "Madagascar." Collections at P with nearby numbers are labelled as being from either "Nord de Madagascar" or "Ile Nos-bé" (now Nossi-bé), so this collection could be from northern Madagascar, although we have seen no other specimens from outside the Comoros Archipelago.

31. *Acalypha rottleroides* Baill., Adansonia 1: 270. 1861.

Type. Madagascar. Prov. Antsiranana: Nossibé, 1837, *M. Richard 215* (holotype P [P00536728]!).

Acalypha polynema Baill., Hist. Pl. Madag., Atlas (1891) t. 187. Type: Madagascar: Plate 187 in Baillon ibid. loc., holotype.

Acalypha juliflora Pax, Bot. Jahrb. Syst. 19: 95. 1894. Type: Madagascar. Prov. Antsiranana: Nossibé, "Urwald von Loko-bé", Dec 1879, *J. M. Hildebrandt 3279* (holotype: W [W1889-0089773]!; isotypes: JE [JE00004291]!, K [K000186527]!, P [P00536729!, P00536730!, P00536731!]).

Distribution. Madagascar (Antsiranana).

References. Müller Argoviensis (1866: 854); Baillon (1891: 182); Baillon (1895b: 1197); Baillon (1895b: 1197) *A. polynema*; Palacký (1907: 25) *A. juliflora*; Palacký (1907: 26) *A. polynema*; Palacký (1907: 26); Nitschke (1923: 280) *A. juliflora*; Pax and Hoffmann (1924: 127); Leandri (1942: 277); Govaerts et al. (2000: 70, 105) *A. juliflora*; Govaerts et al. (2000: 86).

32. *Acalypha spachiana* Baill., Adansonia 1: 272. 1861.

Acalypha spachiana var. *latifolia* Baill., Adansonia 1: 272. 1861.

Type. Madagascar. Prov. Antsiranana: Baies de Rigny et de Diego-Suarès, 1848, *L. H. Boivin 2654* (lectotype, designated here: P [P00536733]!; isolectotype: G [G00034251]!, GDC [G00324359]!, P [P00536734]!).

Acalypha spachiana var. *acutifolia* Baill., Adansonia 1: 272. 1861. Type: Madagascar. Prov. Antananarivo: Antananarivo, 12 Feb 1840, *J. P. Goudot s.n.* (holotype: G [G00383582]!, **syn. nov.**).

Acalypha spachiana var. *minor* Baill., Adansonia 1: 272. 1861. Type: Madagascar: s.l., s.d., *M. Bojer s.n.* (holotype: P [P00536735]!; isotypes: TUB [TUB002081!, TUB002082]!, **syn. nov.**).

Acalypha buchenavii Müll.Arg., Abh. Naturwiss. Verein Bremen 7: 27. 1880. Type: Madagascar. Prov. Antananarivo: Antananarivo, 18 Dec. 1877, *D.C. Rutenberg s.n.* (lectotype (probably holotype), designated here: BRNU [BRNU347926]!).

Acalypha squarrosa Pax, Bot. Jahrb. Syst. 19: 97. 1894. Type. Madagascar. Prov. Antananarivo: Antsirabe, "Sírabé", Aug 1880, *J. M. Hildebrandt 3560* (holotype B or WRSL, presumably destroyed; lectotype, designated here: JE [JE00004308]!; isolectotype: JE [JE00004309]!, K [K000186510]!, P [P00536732]!).

Tragia saxatilis Bojer ex Pax & K.Hoffm., Pflanzenr. 147, 16 (Heft 85): 33. 1924 nom. nud.

Distribution. Madagascar (Antananarivo, Antsiranana, Mahajanga, Toamasina, Toliara).

References. Müller Argoviensis (1866: 827); Müller Argoviensis (1882: 27) *A. buchenavii*; Baillon (1892: 1003); Baillon (1895b: 1199) *A. buchenavii*; Palacký (1907: 26); Pax and Hoffmann (1924: 33); Pax and Hoffmann (1924: 33) *A. squarrosa*; Leandri (1942: 255, 257); Jenkins (1987: 347); Jenkins (1990: 408, 433); Govaerts et al. (2000: 89, 96); Govaerts et al. (2000: 89) *A. squarrosa*; Seebaluck et al. (2015: 153).

Notes. The holotype of *Acalypha buchenavii* should be at BREM but Rutenberg's specimens arrived at BRNU after World War II. Originally in the Überseemuseum in Bremen (BREM), they were transferred to northern Moravia (Czechia was at that time a Protectorate of Nazi Germany) to save them from potential destruction by bombardment. After the collapse of the Nazi regime, these collections were confiscated as "German property" and sent to BRNU.

33. *Acalypha urophylla* Boivin ex Baill., Adansonia 1: 273. 1861.

Acalypha reticulata var. *urophylla* (Boivin ex Baill.) Müll.Arg. Linnaea 34: 32. 1865.
    Acalypha filiformis var. *urophylla* (Boivin ex Baill.) Govaerts, World Checkl. Bibliogr. Euphorbiaceae 63. 2000.

Type. Madagascar. Prov. Antsiranana: Nossibé, plateau de Hell-Ville, Jun 1847, *L. H. Boivin 2178* (lectotype, designated here: P [P00536752]!; isolectotypes: G [G00034246]!, GDC [G00324519]!, P [P00536751!, P00536753!]).

Syntypes. Madagascar: s.l., s.d., *L. M. A. Du Petit-Thouars s.n.* P [P00536748]!; Madagascar. Prov. Antsiranana: Nossibé, Dec 1840, *A. Pervillé 364* P

[P00536749]!; Madagascar. Prov. Antsiranana: Nossibé, *M. Richard 385* P [P00536750]!.

Acalypha goudotiana Baill., Adansonia 1: 268. 1861. *Acalypha reticulata* var. *goudotiana* (Baill.) Müll.Arg., Linnaea 34: 32. 1865. *Acalypha filiformis* var. *goudotiana* (Baill.) Govaerts, World Checkl. Bibliogr. Euphorbiaceae 62. 2000. Type: Madagascar: s.l., 1830, *J. P. Goudot s.n.* (holotype: G!; isotype: P [P00536727]! fragment), **syn. nov.**

Acalypha reticulata var. *urophylla* f. *meeusei* Leandri, Notul. Syst. (Paris) 10: 264. 1942. Type: Madagascar, Prov. Fianarantsoa, base Est du Pic d'Ivohibe, 19 Sep 1926, R. Decary 5352 P [P00224690]!; Prov. Toamasina, Analamazaotra forest, 1912, *H. Perrier de la Bâthier 9741* (lectotype, designated here: P [P05604377]!, **syn. nov.**). Syntypes. Madagascar, Prov. Toamasina, Soanierana, Antasibé, 9 Dec 1938, *H. J. Lam & A. D. J. Meeuse 5814* WAG [WAG0133229]!, L [L0242110]!; Moramanga, 900 m, 11 Nov 1938, H. J. Lam & A. D. J. Meeuse 5363 L [L0242105]!; Analamazaotra, 1000 m, 10 Nov. 1938, *H. J. Lam & A. D. J. Meeuse 5290* L [L0242106]!.

Acalypha reticulata var. *urophylla* f. *longa* Leandri, Notul. Syst. (Paris) 10: 265. 1942. Type: Madagascar, Prov. Toliara, bassin de la Manampanihy, Col de Fitana, 700 m, 15 Oct 1928, H. Humbert 6015 (lectotype, designated here: P [P05604383]!, **syn. nov.**). Syntypes. Madagascar, Prov. Toliara, Col d'Ivolo (District de Fort-Dauphin), 500 m, 5 Sept 1932, *R. Decary 10558* P [P05604384]!; Fort-Dauphin, col de Tsitongabarika, 600 m, 9 Sept 1932, *R. Decary 10595* P [P05604399]!.

Acalypha reticulata var. *urophylla* f. *vohitrae* Leandri, Notul. Syst. (Paris) 10: 265. 1942. Type: Madagascar, Prov. Toamasina, Andevorante, rive droite de la Vohitra près de Lohariandava, 200-250 m, 10 Oct 1912, *R. Viguier & H. Humbert 661* (lectotype, designated here: P [P05604437]!; isolectotypes: P [P05604440!, P05604441!], **syn. nov.**).

Acalypha reticulata var. *urophylla* f. *glabrescens* Leandri, Notul. Syst. (Paris) 10: 266. 1942. Type: Madagascar, Prov. Antsiranana, Massif du Tsaratanana, 2200 m, H. Perrier de la Bâthie 16180 (lectotype, designated here: P [P05604386]! **syn. nov.**). Syntypes. Madagascar, Prov. Antsiranana, Sambirano, Nov-Dec 1937, *H. Humbert 18659* P [P05604397!, P05604400!]; Massif du Tsaratanana, Sep 1912, *H. Perrier de la Bâthie 18614* P [P05604382]!; Massif du Tsaratanana, 1600 m, H. Perrier de la Bâthie 15371 P [P05604385]!; Madagascar, Prov. Fianarantsoa, Ranohira, Isalo, 30 Jul 1928, *H. Humbert 5014* P [P05604401!, P05604402!]; Madagascar, Prov. Mahajanga, Beritsoka, *H. Perrier de la Bâthie 413* P [P05604392!, P05604390!]; Beritsoka, Dec 1897, *H. Perrier de la Bâthie 422* P [P05604387!, P05604388!, P05604391!]; Beritsoka, H. Perrier de la Bâthie 9822 P [P05604389]!; Madagascar, s. l., *R. Baron 5987* P [P05604407]!; s. l., *L. Humblot 335* P [P05604393!, P05604394!, P05604395!, P05604396!].

Acalypha reticulata var. *urophylla* f. *typique* Leandri, Notul. Syst. (Paris) 10: 262. 1942 *nom. inval.*

Distribution. Madagascar (Antananarivo, Antsiranana, Fianarantsoa, Mahajanga, Toamasina and Toliara). Comoros Archipelago (Grande Comore, Anjouan, Mohéli and Mayotte).

References. Müller Argoviensis (1866: 260, 852) as *A. reticulata* var. *goudotiana* and *A. reticulata* var. *urophylla*; Baron (1889: 262); Baillon (1891: 189); Baillon (1892: 1004); De Candolle (1901: 567); Palacký (1907: 25 as *A. goudotiana*, 26); Pax and Hoffmann (1924: 105, 260) as as *A. reticulata* var. *goudotiana* and *A. reticulata* var. *urophylla*; Leandri (1942: 258, 260, 262) as *A. reticulata* var. *goudotiana* and *A. reticulata* var. *urophylla*; Leandri (1942: 281) as *A. emirnensis*; Leandri (1948: 186) as *A. reticulata* var. *goudotiana*; Govaerts et al. (2000: 62, 63, 100, 105, 108).

Notes. *Acalypha urophylla* has been considered to be a variety of *A. reticulata*, but *A. urophylla* can be distinguished by its sessile female bracts with dentate margins vs. pedicellate female bracts with crenate to subentire margins in *A. filiformis*. (see notes in *A. filiformis*). See notes under *A. gracilipes*, *A. lamiana* and *A. paxii* for the differences between those species and *A. urophylla*.

34. *Acalypha vulneraria* Baill., Bull. Mens. Soc. Linn. Paris 2: 1180-1181. 1895.

Type. Madagascar. Prov. Toliara: Fort-Dauphin, s.d., *G. F. Scott-Elliot 3010* (lectotype, designated here: P [P00536740]!; isolectotypes: K [K000186511]!, P [P00536739]!).

Syntypes. Madagascar. Prov. Toliara: Fort-Dauphin, *M. Cloisel 51* (wrongly transcribed as "*Cloisel 50*" in the protologue) P [P04779526]!.

Distribution. Madagascar (Fianarantsoa, Mahajanga, Toliara).

References. Palacký (1907: 26); Nitschke (1923: 281); Pax and Hoffmann (1924: 128); Leandri (1935: 46); Leandri (1942: 279); Govaerts et al. (2000: 93).

35. *Acalypha wilkesiana* Müll.Arg., Prodr. 15(2): 817. 1866.

Ricinocarpus wilkesianus (Müll.Arg.) Kuntze, Revis. Gen. Pl. 2: 618. 1891. *Acalypha amentacea* Roxb. subsp. *wilkesiana* (Müll.Arg.) Fosberg, Smithsonian Contr. Bot. 45: 10. 1980.

Type. Fiji, s.d., (U.S. Expl. Exped. Under. Capt. Wilkes), *B. C. Seeman s.n.* (holotype: G-DC [G00324022]!; isotypes: GH [00045512]!, K [K000959008]!, US [00096423!, 00096424!]).

Distribution. Tropical Africa. Madagascar (Antananarivo).

References. Robertson (1989: 200).

Notes. Shrub native to Fiji (Melanesia), used as an ornamental plant throughout the tropics. It has been reported from Seychelles (Robertson 1989). We found some specimens from Madagascar

Doubtful or excluded species

Acalypha neptunica Müll.Arg., Abh. Naturwiss. Vereins Bremen 7: 26. 1880. Type. Tanzania, Zanzibar: Kidosi, Oct 1873, *J. M. Hildebrandt 1146.* (lectotype, designated by Cardiel and Montero Muñoz (2018): K [K000431078]!; isolectotype: G [G00007675]!).

This species occurs in West Tropical Africa, West Central Tropical Africa, Northeast Tropical Africa and East Tropical Africa. There are only two references of *Acalypha neptunica* from Madagascar. They appear in Baillon (1895b) and in the checklist of Palacký (1907). We have not yet confirmed the presence of this species in the study area.

Acalypha spiciflora Burm.f., Fl. Ind. 203, pl. 61-2. 1768. *Claoxylon spiciflorum* (Burm.f.) A.Juss., Euphorb. Gen. 43. 1824. *Cleidion spiciflorum* (Burm.f.) Merr., Interpr. Herb. Amboin. 322. 1917.

Acalypha spiciflora Burm.f. was described and illustrated in Burman´s *Flora Indica,* but the plate and the description are very imprecise. Subsequently, Poiret (1804) cited under this name a specimen in the Lamarck herbarium, P00382113, from Réunion. That specimen is morphologically close to Burman´s plate, but it is not an *Acalypha* species. Later, Jussieu combined *A. spiciflora* under the genus *Claoxylon,* as *Cla. spiciflorum.* Merrill combined it under *Cleidion* as *Cle. spiciflorum,* the name that is accepted today. Müller Argoviensis (1866) wrongly placed *A. spiciflora* as a synonym of *A. acuminata* Vahl ex Baill. Pax and Hoffmann (1924) and Leandri (1942) followed the treatment of Müller Argoviensis, but, applying the rule of priority, chose *A. spiciflora* as the accepted name. This is the origin of the confusion in the use of this name.

Acalypha venosa Poir., Encycl. 6: 204. 1804.

The type specimen of *Acalypha venosa* (*Commerson s.n.,* from Madagascar) corresponds to *Leptonema venosum* (Poir.) A.Juss. (Phyllanthaceae), as was pointed out by Steudel (1841: 31).

References

Baillon HE (1858) Etude générale du groupe des Euphorbiacées. Librarie de Victor Masson, Paris, 1–700. https:// doi.org/10.5962/bhl.title.50439

Baillon HE (1861) Euphorbiacées Africaines. Deixiéme partie, Afrique Orientale (Bourbon, Maurice, Madagascar, Zanguebar, etc.). Adansonia 1: 251–286.

Baillon HE (1891) Histoire Naturelle des Plantes Vol. 34, Tome 4 (Atlas 2), 3ª partie, 27 fascicule. In: Grandidier A. (ed.) Histoire Physique, Naturelle et Politique de Madagascar. M. le Grade des Sceaux, à l'Imprimerie Nationale, Paris, 161–204.

Baillon HE (1892) Liste des plantes de Madagascar (suite de la page 996). Bulletin Mensuel de la Société Linnéenne de Paris 2(126): 1003–1005.

Baillon HE (1895a) Liste des plantes de Madagascar (suite de la page 1005). Bulletin Mensuel de la Société Linnéenne de Paris 2(149): 1177–1184.

Baillon HE (1895b) Liste des plantes de Madagascar (suite de la page 1181). Bulletin Mensuel de la Société Linnéenne de Paris 2(151): 1193–1200.

Baker JG (1883) Contributions to the Flora of Madagascar. Part III. Incompletae, Monocotyledons, and Filices. Journal of the Linnean Society. Botany 20: 237–304.

Baker JG (1884) Further Contributions to the Flora of Madagascar. Second and Final Part. Journal of the Linnean Society. Botany 21: 407–455.

Baker JG (1877) Flora of Mauritius and the Seychelles: a description of the flowering plants and ferns of those islands. L Reeve, London, 1–557.

Baron R (1889) The Flora of Madagascar. Journal of the Linnean Society. Botany 25: 246–294.

Berry PE, Kainulainen K, van Ee BW (2017) A Nomenclator of *Croton* (Euphorbiaceae) in Madagascar, the Comoros Archipelago, and the Mascarene Islands. PhytoKeys 90: 1–87. https://doi.org/10.3897/phytokeys.90.20586

Bojer W (1837) Hortus Mauritianus. Aimé Mararot & Co., Mauritius, 1–456.

Brummitt RK (2001) World geographical scheme for recording plant distributions, 2nd ed. Plant Taxonomic Database Standards, No. 2. International Working Group on Taxonomic Databases for Plant Sciences (TDWG). Hunt Institute for Botanical Documentation Carnegie Mellon University, Pittsburgh, 1–137.

Cardiel JM, Montero Muñoz I (2018) Synopsis of *Acalypha* (Euphorbiaceae) of West Tropical Africa, including Cameroon, Chad, Equatorial Guinea, Gabon, and São Tomé and Príncipe Plant Systematics and Evolution 304: 93–110. https://doi.org/10.1007/s0060

Cardiel JM, Muñoz-Rodríguez P, Dorda E, Pardo de Santayana M (2018) *Acalypha* Taxonomic Information System. http://www.acalypha.es [accessed 03.05.2018]

Coode MJE (1979) Notes on Euphorbiaceae in the Mascarene Islands: II. Kew Bulletin 34(1): 39–48. https://doi.org/10.2307/4117969

Coode MJE (1982) *Acalypha* L. In: Bosser J, Cadet T, Guého J, Marais W (Eds) Flore des Mascareignes: La Réunion, Maurice, Rodrigues. 153. Lauracées à 160. Euphorbiacées. The Sugar Industry Research Institute, Mauritius, L'Office de la Recherche Scientifique et Technique Outre-Mer, Paris, and The Royal Botanic Gardens, Kew, 68–79.

de Cordemoy EJ (1895) Flore de l'île de la Réunion (phanérogames, cryptogames, vasculaires, muscinées) avec l'indication des propriétés économiques & industrielles des plantes. Jacques Lechevalier, Librairie Médicale & Scientifique, Paris, 1–574.

De Candolle A (1901) Plantae Madagascarienses ab Alberto Mocquerysio lectae. Bulletin de l'Herbier Boissier, sér. 2 1(6): 564–567.

Fosberg FR (1974) Miscellaneous notes on the flora of Aldabra and neighbouring islands: III*. Kew Bulletin 29(2): 253–266. https://doi.org/10.2307/4108539

Goodman SM (1996) A Floral and faunal inventory of the eastern slopes of the Réserve naturelle intégrale d'Andringitra, Madagascar: with reference to elevational variation. Fieldiana, Zoology, new. ser. 85: 1–319.

Govaerts R, Frodin DG, Radcliffe-Smith A (2000) World Checklist and Bibliography of Euphorbiaceae (and Pandaceae). Royal Botanic Gardens, Kew, 1–1621.

Heckel E (1903) Catalogue alphabetique raisonne des plantes medicales et toxiques de Madagascar avec leur emploi indigene. Annales du Museé colonial de Marseille v. 11 (s. 2, v. 1), fasc. 2: 59–204.

Hemsley WB (1919) Flora of Aldabra: With Notes on the Flora of the Neighbouring Islands. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) 3: 108–153.

Horn JW, van Ee BW, Morawetz JJ, Riina R, Steinmann VW, Berry PE, Wurdack KJ (2012) Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). Molecular Phylogenetics and Evolution 63(2): 305–326. https://doi.org/10.1016/j.ympev.2011.12.022

Hutchinson J (1913) *Acalypha*. In: Thiselton-Dyer WT (Ed.) Flora of tropical Africa 6, 1: nyctagineae-euphorbiaceae. Lovell Reeve & Co., London, 880–910.

Jenkins MD (1987) Madagascar, an environmental profile. IUCN, Gland, Switzerland and Cambridge, U.K., 1–396.

Jenkins MD (1990) Madagascar: profil de l'environnement. IUCN, Gland, Switzerland and Cambridge, U.K., 1–464.

Koehne E (1892) Just's botanischer jahresbericht. Systematisch geordnetes repertorium der botanischen literatur aller länder, Jahrg. 20, Abt. 1. Gebr. Borntraeger, Leipzig, Berlin, 1– 636.

Leandri J (1935) Catalogue des Plantes de Madagascar, Euphorbiaceae. Pitot G, Tananarive, 1–51.

Leandri J (1942) *Acalypha*. Contribution a l'etude des Euphorbiacées de Madagascar (VI). Notululae Systematicae 10: 252–290.

Leandri J (1948) Euphorbiacées récoltées a Madagascar et aux Mascareignes par MM. Lam et Meeuse en 1938. Notulae Systematicae 13: 182–187.

Leandri J (1952) Les arbres et grands arbustes malgaches de la familie Euphorbiacées. Naturaliste Malgache 4(1): 47–82.

Levin GA, Steinmann VW, Sagun VG (2005) Phylogeny and biogeography of *Acalypha* (Euphorbiaceae). Abstracts of the XVII International Botanical Congress: 68. Vienna, Austria.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme Van Reine WF, Smith GF, Wiersema JH, Turland NJ (Eds) (2012) International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Regnum Vegetabile 154. Koelz Scientific Books.

Müller-Argoviensis J (1865) Euphorbiaceae. Vorläufige Mitthielungen aus den für De Candolle Prodromus bestimmtem Manuscript über diese Familie. Linnaea 34: 1–54.

Müller-Argoviensis J (1866) *Acalypha*. In: De Candolle AP (Ed.) Prodromus Systematis Naturalis Regni Vegetabilis. Vol 15(2). Paris, Treuttel et Würtz, 799–889.

Müller-Argoviensis J (1882) Euphorbiaceen. In: Buchenau, Fr (Ed.) Reliquiae Rutenbergianae I (Botanik, Taf. I, II). Abhandlungen herausgegeben vom Naturwissenschaftlichen Verein zu Bremen 7: 24–30.

Nitschke R (1923) Die geographische Verbreitung der Gatung Acalypha. In: Mez K (Ed.) Botanisches Archiv: Zeitschrift für die gesamte Botanik. Verlag des Repertoriums, Berlin, 277–317.

Palacký J (1907) Catalogus plantarum Madagascariensium II. Pragae, 1–89.

Pax FA (1890) Euphorbiaceae. In: Engler A, Prantl K (Eds) Die natürlichen Pflanzenfamilien 3(5): 1–123.

Pax FA (1894) Enphorbiaceae africanae. II. In: Engler A (Ed.) Beiträge zur Flora von Afrika. VIII. Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie 19: 76–127.

Pax FA, Hoffmann K (1924) *Acalypha*. In: Engler A (Ed.) Das Pflanzenreich. IV, 147-16 (heft 85), 1–231.

Poiret JLM (1804) *Acalypha*. In: Lamarck L, Poiret JLM (Eds) Encyclopédie Métodique. Botanique. Paris, 6: 202–209.

Renvoize SA (1975) A Floristic Analysis of the Western Indian Ocean Coral Islands. Kew Bulletin 30(1): 133–152. https://doi.org/10.2307/4102881

Radcliffe-Smith A (1987) Euphorbiaceae (Part 1). In: Polhill RM (Ed.) Flora of tropical East Africa. Balkema, Rotterdam, 185–213.

Radcliffe-Smith A (1990) New record of *Acalypha* in Australia. Kew Bulletin 45(4): 677–679. https://doi.org/10.2307/4113874

Radcliffe-Smith A (1996) Euphorbiaceae. In: Pope GV (Ed.) Flora Zambesiaca, vol 9. Part 4. Royal Botanic Gardens, Kew, 1–138.

Robertson SA (1989) Flowering Plants of Seychelles. Royal Botanic Gardens, Kew, 1–343.

Schatz GE (2001) Generic tree flora of Madagascar. Royal Botanic Gardens, Kew, Richmond, United Kingdom, 1–477.

Seebaluck R, Gurib-Fakim A, Mahomoodally F (2015) Medicinal plants from the genus *Acalypha* (Euphorbiaceae)–A review of their ethnopharmacology and phytochemistry. Journal of Ethnopharmacology 159: 137–157. https://doi.org/10.1016/j.jep.2014.10.040

Steudel ET (1841) Nomenclator Botanicus seu: synonymia plantarum universalis, enumerans ordine alphabetico nomina atque synonyma, tum generic tum specifica et A Linnaeo et A recentioribus de re botanica scriptoribus plantis phanerogamis imposita. Pars II. Stuttgartiae et Tubingae, Typis et Sumptibus, Cottae, 1–810.

Thiers B (2018) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/ [accessed 03.05.2018]

Voeltzkow A (1917) Flora und Fauna der Comoren. In: Voetzkow A (Ed.) Reise in Ostafrika in den Jahren 1903–1905. Wissenschaftliche Ergebnisse, Stuttgart, Schweizerbart 3: 429–480.

RESEARCH ARTICLE

Taxonomic status of *Populus wulianensis* and *P. ningshanica* (Salicaceae)

Lei Zhang[1], Mingcheng Wang[1], Tao Ma[1], Jianquan Liu[1]

I *Key Laboratory of Bio-Resource and Eco-Environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610065, Sichuan, P. R. China*

Corresponding author: *Jianquan Liu* (liujq@nwipb.cas.cn)

Abstract

Species delimitation in the genus *Populus* is particularly challenging due to high levels of intraspecific polymorphism as well as frequent interspecific hybridisation and introgression. In this study, we aimed to examine the taxonomic status of *Populus ningshanica* and *P. wulianensis* using an integrative taxonomy that considers multiple operational criteria. We carried out morphometric analyses of leaf traits and genetic examinations (including sequence variations at five barcoding DNAs and polymorphisms at 14 nuclear microsatellite SSR primers) at the population level between them and two closely related species *P. adenopoda* and *P. davidiana*. Results suggest that *P. wulianensis* belongs to the polymorphic species, *P. adenopoda* and should be considered as a synonym of the latter. *P. ningshanica* may have arisen as a result on the hybridisation between *P. adenopoda* and *P. davidiana* and therefore should be treated as *P. × ningshanica*. This study highlights the importance of the integrated evidence in taxonomic decisions of the disputed species.

Keywords

Geometric morphometrics, microsatellites, DNA barcodes, integrated species delimitation

Introduction

Species delimitation is essential to conserve and assess biodiversity (Agapow et al. 2004). Any incorrect species recognition may result in serious after-effects in related studies, for example, by an increase in species conservation (Wiens 2007) and un-

der- or over-estimation of biodiversity (Douady 2007). Therefore, in addition to morphological traits, significant efforts have been made to delimit species based on DNA sequence variation (Wiens and Penkrot 2002; Sites and Marshall 2003; Kress et al. 2005; Bond and Stockman 2008; Fujita et al. 2012; Hendrixson et al. 2013) or other genetic polymorphisms that can assess gene flow and identify interspecific hybrids according to the biological species concept (Pérez-Losada et al. 2005). These molecular markers have been used to differentiate species, hybrids and even clones in the genus *Populus* (Salicaceae) (Hamzeh and Dayanandan 2004; Cervera et al. 2005; Hamzeh et al. 2006; Fladung and Buschbom 2009; Schroeder et al. 2012; Feng et al. 2013; Wan et al. 2013). Poplars are widely distributed in the Northern Hemisphere with an important ecological role in natural and artificial forests in both boreal and temperate regions (Dickmann et al. 2001). However, due to high levels of morphological variation and extensive inter-specific hybridisation, species delimitation within the genus is highly contentious (Eckenwalder 1996; Dickmann and Kuzovkina 2008). The number of the proposed species ranges from 22 to 85, plus hundreds of hybrids, varieties and cultivars (Dickmann and Stuart 1983; Fang et al. 1999). Numerous described species were doubted as being hybrids of the other independently evolving lineages (good species) or intra-specific variations of the polymorphic species. However, these ambiguous species have not been well examined.

In this study, we aimed to determine the taxonomic status of two species described from China: *P. wulianensis* S.B.Liang & X.W.Li and *P. ningshanica* C. Wang & Tung (Fang et al. 1999) based on morphometric analyses and genetic examinations at the population level as recently suggested for an integrated species delimitation (Liu 2016). *P. wulianensis* is restricted to eastern Shandong while *P. ningshanica* is distributed in southern Shaanxi and Northwest Hubei. Both are morphologically similar to *P. davidiana* Dode and *P. adenopoda* Maxim. of sect. *Populus* with widespread distributions in northern or middle to southern China. The key traits for their diagnosis are mainly based on leaf characters: blade and apex shape and margin incision (Fang et al. 1999). We firstly conducted morphometric analyses of leaf traits for representative populations of all four species. Then we examined genetic delimitations between them based on evidence from sequence variation of internal transcribed spacer (ITS) and four chloroplast DNA (cpDNA) and genetic polymorphisms from nuclear microsatellite loci (nSSR).

Materials and methods

Sample collection

We sampled 163 individuals from 17 populations of four species (Table 1), including all recorded natural populations of both *P. ningshanica* and *P. wulianensis*. All individual trees were chosen with typical morphological leaf traits (Fang et al. 1999). Each tree was set apart by at least 50m in each population. Except for collecting specimens (SZ, herbarium of Sichuan University, Chengdu, China) for geometric morphometric

Table 1. Detailed information for the 17 sampled populations of the Sect. *Populus* species that were adopted for Data analysis using SSR and Geometric morphology.

Species	Pop	Individuals	Lon (N)	Lat (E)	Alt (m)	CS	Vouchers
P. davidiana	1	21	111.2848	38.21627	1467	Lvliang, SX	LiuJQ-MZL-2013-117
	2	8	111.3395	38.14662	1587	Lvliang, SX	LiuJQ-MZL-2013-121
	3	6	112.3880	38.92512	1402	Qizhou, SX	LiuJQ-MZL-2013-109
	4	10	112.0744	38.8556	1855	Qizhou, SX	LiuJQ-MZL-2013-115
	5	8	111.4328	37.8976	1961	Lvliang, SX	LiuJQ-MZL-2013-124
	6	9	111.2637	37.203483	1459	Lvliang, SX	LiuJQ-MZL-2013-136
P. ningshanica	7	8	105.249	32.74979	657	Longnan, GS	LiuJQ-SHX-2015-20
	8	1	107.1394	32.60744	865	Hanzhong, SaX	LiuJQ-SHX-2015-14
	9	3	106.0741	33.55506	768	Hanzhong, SaX	LiuJQ-SHX-2015-10
P. wulianensis	10	10	121.7556	37.2983	188	Yantai, SD	LiuJQ-ZL-2016-300
P. adenopoda	11	5	108.8565	28.1423	798	Tongren, GZ	MaoKS-CX-2014-326
	12	5	109.1866	28.2958	643	Tongren, GZ	MaoKS-CX-2014-327
	13	5	108.7551	28.3148	707	Tongren, GZ	MaoKS-CX-2014-328
	14	18	105.3035	32.5254	598	Guangyuan, SC	LiuJQ-ZF-2016-01
	15	10	117.8054	30.4742	677	Liuan, AH	LiuJQ-ZF-2016-02
	16	17	117.9531	30.5850	26	Chizhou, AH	LiuJQ-ZF-2016-03
	17	19	110.3215	32.6738	683	Shiyan, HB	LiuJQ-ZF-2016-04

Abbreviations: Pop, Population; Lon (N), Longitude; Lat (E), Latitude; Alt (m), Altitude; CS, Collection site. SX, Shanxi; GS, Gansu; SaX, Shaanxi; SD, Shandong; GZ, Guizhou; SC, Sichuan; AH, Anhui; HB, Hubei.

analyses, we further selected healthy and fresh leaves from each tree and dried them immediately in silica gel for DNA extraction. We also used an Etrex GIS monitor (Garmin, Taiwan) to record latitude, longitude and altitude of each sampled population (Table 1; Fig. 1A).

Geometric morphometrics

Although we failed to find type specimens of *P. ningshanica* and *P. wulianensis*, we included the newly collected specimens from their type localities. A Canon 60D digital camera was used to photograph typical leaves of all specimens. We transformed every image into a vector diagram using TpsUtil version 1.64 (Rohlf 2013). Thirty-two homologous landmarks were assigned in order to quantify leaf blades shape in all specimens. Landmark positions of leaves included base, tip and margin. All landmarks were digitised for each individual using the software TpsDig version 2.22 (Rohlf 2015). We created a combined data file including all specimens. We implemented morphometrics analyses in MorphoJ version 1.01b (Klingenberg 2011), within which a principal component analysis of morphological variations was conducted and plotted.
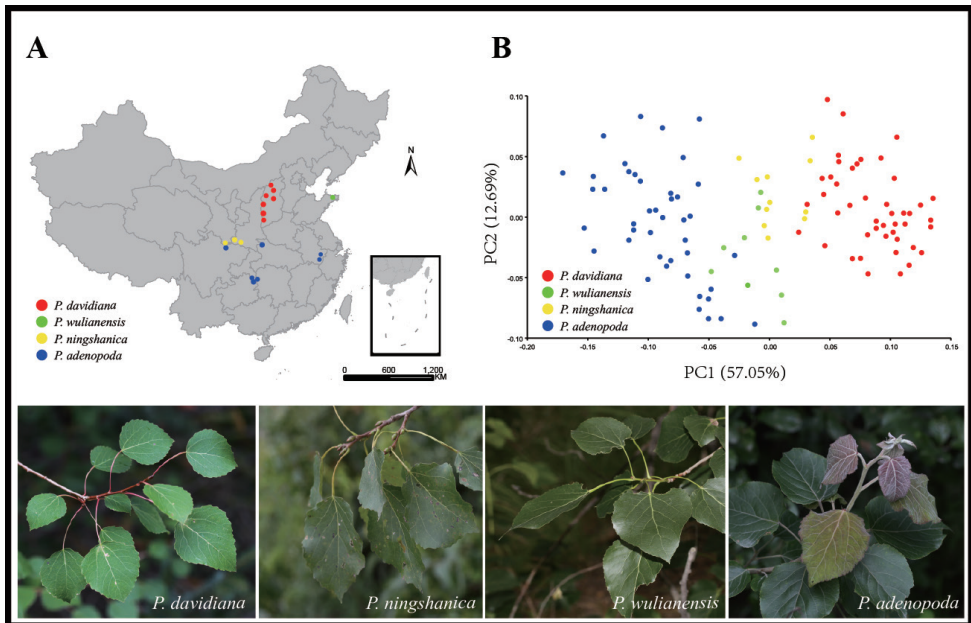
Figure 1. A Geographical distribution of 17 populations of the four species (*Populus adenopoda*, *Populus davidiana*, *Populus ningshanica*, *Populus wulianensis*) **B** The Principal Component Analysis (PCA) plot for the morphological variations of 17 populations of 4 species.

Genetic analyses

We isolated the total genomic DNA from leaves of each individual, based on the hexa-decyltrimethyl ammonium bromide (CTAB) method (Doyle 1987). We used a total of 14 SSRs primers (Suppl. material 1: Table S1) developed previously, based on the genome sequences of *Populus euphratica* and *P. trichocarpa* (Ma et al. 2013; Jiang et al. 2016) to genotype our samples. The PCRs were performed in a volume of 25 ml, which contained: 50–100 ng diluted genomic DNA, 0.5 mM of each dNTP, 0.5 µl of each primer, 2.5 µl 10 × Taq buffer and 0.5 units of Taq polymerase (Vazyme Biotech, Nanjing, China). The PCR programme used was: initially a single cycle at 95 °C for 5 min, followed by 36 cycles at 95 °C for 45s, 55 °C for 40 s and 72 °C for 80 s, with a final extension at 72 °C for 10 min. The PCR products at each locus were analysed on an ABI 3830xl DNA analyser (Applied Biosystems, Inc., Foster City CA) at Tsingke Biological Technology (Beijing, China). We used STRUCTURE version 2.3.4 (Falush et al. 2003) that allows a Bayesian hybrid mixture computation to identify genetic compositions of all sampled trees. We pre-assigned a number of genetic clusters (K) ranging from 1 to 10. All runs involved 1,000,000 Markov chain Monte Carlo repetitions after a burn-in period of 500,000 iterations. We used the long burn-in and run lengths as well as 10 replicates to ensure the reproducibility of STRUCTURE results (Gilbert et al. 2012). We estimated the posterior probability of K and Delta K (ΔK),

the rate of change of Ln P (K) between successive K values (Evanno et al. 2005). We determined the most likely number of clusters.

We also sequenced internal transcribed spacer (ITS) and four chloroplast DNA (cpDNA) fragments: *matK*, *trnH-psbA*, *trnG-psbK* and *psbK-psbI* for three to five individuals from each sampled population of four species used for nSSR genotyping. In addition, one individual of *P. euphratica* was sequenced as the outgroup. Primers, PCRs and sequencing followed Feng et al. (2013) (Suppl. material 1: Table S2). Sequences for each fragment were aligned and sequences from four cpDNAs were connected using MEGA 7.0 (Kumar et al. 2016). We constructed unrooted neighbour-joining (NJ) trees for both ITS and cpDNAs datasets by MEGA 7.0 (Kumar et al. 2016) respectively, using pairwise deletion and the *P*-distance model. Bootstrap values were estimated with 1000 random addition sequence replicates.

Results

PCA analyses of geometric morphometric data

Geometric morphometric analyses of leaf traits yielded 30 principal components (PC), which accounted for all leaf variations. PC1 to PC3 were the only PCs that individually represented >5% of the variance (PC1=57.05%; PC2=12.69%; PC3=7.68%) and they together represented 77.43% of the variance. All other PCs accounted for <5% of the variance individually. The greatest amount of shape variance is observed across PC1 and PC2 (Fig. 1B). Across these two axes, individuals of *P. davidiana* and *P. adenopoda* were treated as a clear division, whereas individuals of *P. wulianensis* and *P. ningshanica* are clustered into one subgroup of the *P. adenopoda* group. All other PCs showed similar relationships.

Clustering analyses based on the SSR polymorphisms

We genotyped 14 nuclear SSR loci for 163 sampled individuals of four species. Using the method originally described by Pritchard et al. (Pritchard et al. 2000) and also the ΔK approach described by Evanno et al. (Evanno et al. 2005), we found the most likely number of Bayesian clusters was two (*K* = 2) (Fig. 2A). When *K* = 2, individuals from *P. davidiana* clustered into one group and those from *P. adenopoda* into the other. Within each group, some samples indicated the weak genetic introgression from the other. All sampled individuals of both *P. wulianensis* and *P. ningshanica* were assigned to the group represented by *P. adenopoda* (Fig. 2B). However, approximately 10% of the genetic composition of *P. ningshanica* derived from the cluster represented by *P. davidiana*, while more than 90% was from *P. adenopoda*. Similar results were obtained based on PCA analyses of genetic polymorphisms and that two groups were identified to be, respectively, represented by *P. davidiana* and the other three (Fig. 2C).
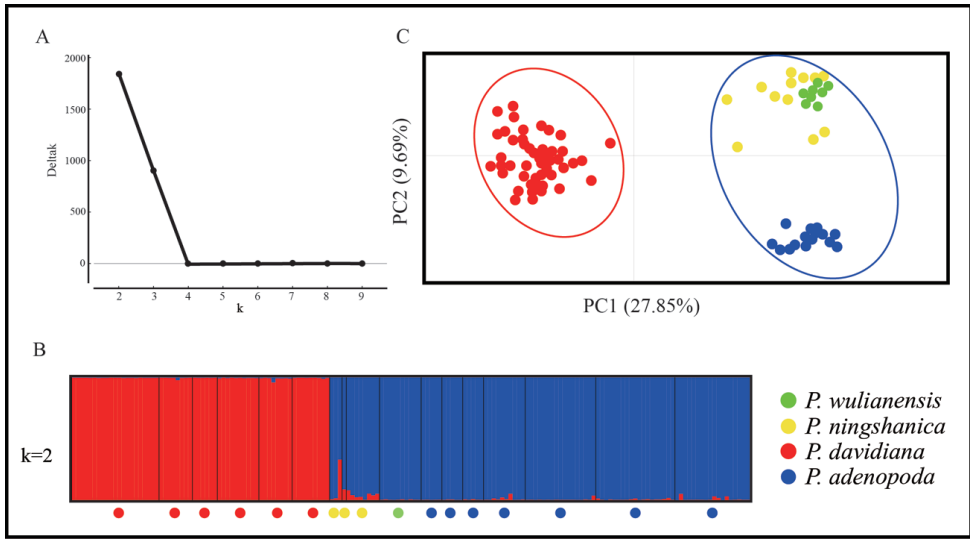
Figure 2. Principal Coordinates Analysis (PCA) of the 17 populations of 4 species based on genetic distance using SSR data (**A**); the optimal K value was estimated using (**B**) the distribution of delta K (*K=2*) and Bayesian clustering plots for 17 populations of 4 species based on variation at 14 nSSR loci (**C**).
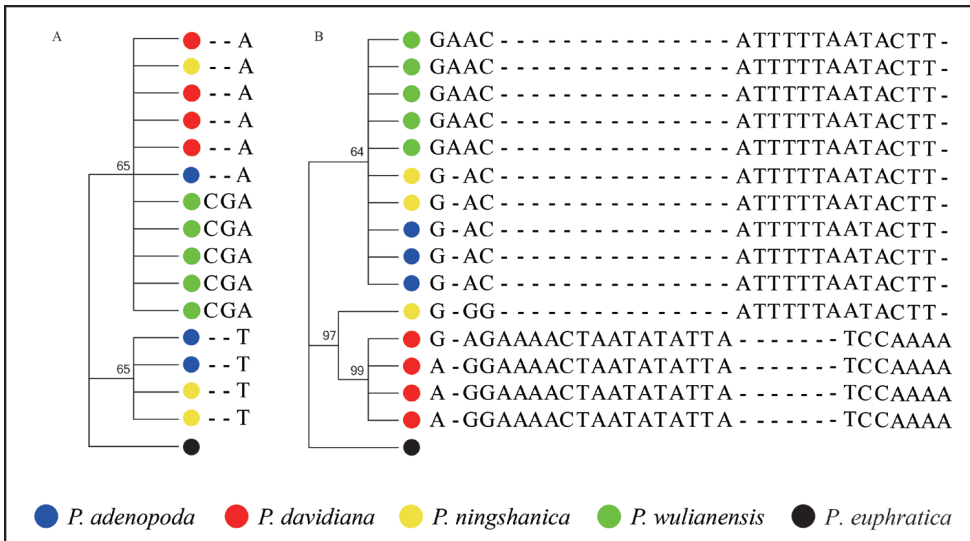

Figure 3. The neighbour-joining (NJ) tree of ITS variable sites (**A**); The neighbour-joining (NJ) tree of four cpDNA variable sites (**B**).

We have combined sequences of four cpDNAs for each individual into one cpDNA sequence. We aligned the cpDNA sequences of all individuals and identified 2, 1, 1 and 2 sequences for *P. davidiana*, *P. adenopoda*, *P. wulianensis* and *P. ningshanica*, respectively. The total length of the aligned cpDNA sequence was 1866 bp with 9 vari-

able sites amongst different sequences from four species (Fig. 3B). NJ clustering of all different cpDNA sequences from four species similarly identified two tentative groups: one comprised of *P. davidiana* and *P. ningshanica,* while the other included those from *P. adenopoda*, *P. wulianensis* and *P. ningshanica*. We identified 1, 2, 1 and 2 different ITS sequences for the sampled individuals for *P. davidiana*, *P. adenopoda*, *P. wulianensis* and *P. ningshanica*. We aligned these ITS sequences from four species, which were 552 bps long with 1 variable site amongst all the different sequences from four species (Suppl. material 1: Table S3; Fig. 3A). NJ analyses of the ITS dataset identified two tentative groups: one comprised 4 sequences from *P. adenopoda* and *P. ningshanica* while the other, all four species.

Discussion

Statistical analyses based on geometric morphometric measurements are highly successful at separating similar species (Villemant et al. 2007; Francuski et al. 2009), even when the individual character shows the overlapped variations between them (Lumley and Sperling 2010; Buck et al. 2012). Especially, geometric morphometrics could differentiate the overall changes in the gross morphology (Rohlf and Marcus 1993). Poplar leaves are ideal for geometric morphometric analyses, as they are two-dimensional, easily imaged and the venation provides many points that are clearly homologous and straightforward to landmark accurately. In addition, flower traits are highly static across the genus without variations and leaf characters are therefore used to classify different species (Dickmann and Stuart 1983; Eckenwalder 1996; Fang et al. 1999; Dickmann and Kuzovkina 2008). We tried to classify four popular species based on geometric morphometric analyses of leaf traits. Our results obviously suggested that *P. davidiana* and *P. adenopoda* differed distinctly from each other. *P. wulianensis* and *P. ningshanica* could not be distinguished from each other and they together clustered into one subgroup, which obviously belonged to the *P. adenopoda* group (Fig. 1B). Therefore, this statistical clustering indicated that both *P. wulianensis* and *P. ningshanica* may belong to the polymorphic *P. adenopoda.*

Genetic evidence, based on nuclear SSR loci, similarly recognised the distinct species boundary between *P. davidiana* and *P. adenopoda* (Fig. 2A, B). However, all sampled individuals of *P. wulianensis* belong to the *P. adenopoda* group without distinct introgression from *P. davidiana*. All sampled individuals of *P. ningshanica* shared similar genetic compositions, together belonging to the *P. adenopoda* group but with obvious genetic introgressions from the *P. davidiana* group. These individuals comprise the obvious backcrosses from *P. adenopoda*. Similarly, sequence variations from five DNAs (ITS, *matK*, *trnH-psbA*, *trnG-psbK* and *psbK-psbI*) seem to support these inferences. The connected sequences of four cpDNAs distinguished *P. davidiana* and *P. adenopoda* while all *P. wulianensis* individuals shared the same cpDNA sequences with *P. adenopoda*. We found two types of cpDNA sequences in *P. ningshanica* (Fig. 3B), clustering respectively with those from *P. davidiana* and *P. adenopoda*, which further suggested the hybrid origin of *P. ningshanica*. However, the initial hybrids must have

repeatedly backcrossed with *P. adenopoda*, which resulted in the high genetic similarity of the sampled individuals of *P. ningshanica* to *P. adenopoda* but with introgression with *P. davidiana* (Fig. 3B). The interspecific hybrids in the genus *Populus* could be F1, F2 to multiple generation backcrossing hybrids (Braatne et al. 1992; Bradshaw et al. 2000; Feng et al. 2013; Jiang et al. 2016). We failed to find stable ITS differences between *P. davidiana* and *P. adenopoda*. It is highly probably that the gene flow, mediated by interspecific hybrids, had caused the concerted evolutions and indistinct differences in the ITS sequence variations (Feng et al. 2013; Jiang et al. 2016).

Overall, multiple lines (Figs 1B, 2B, C;) of evidence suggested that *P. wulianensis* was described based on the intraspecific variations of the polymorphic *P. adenopoda* and individuals ascribed to *P. ningshanica* are, in fact, hybrids between *P. adenopoda* and *P. davidiana* with the repeated backcrosses to the former. Both taxa should be treated accordingly in the taxonomic revision of the genus *Populus*.

Additional specimens examined. China. Anhui: Jiuhuashan mountain, on slope, 500 m elev., 18 Aug 1934 *C. S. Fan & Y. Y. Li 262* (NAS!). Huoshan county, on slope, 17 Apr 1959, *M. B. Deng & J. Q. Pan 0208* (NAS!). She county, in woods, 300 m elev., 04 May 1959, *S. She 1218* (NAS!). Jinzhai county, on slope, 12 Jul 1959, *Z. Jin 6044* (PE!). Jin county, in woods, 300 m elev., 10 Oct 1959, *Anonymous 793* (NAS!). Xiuning county, in roadside, 450 m elev., 29 Jun 1959, *R. H. Shan et al. 2661* (NAS!). Xuancheng city, in woods, 130 m elev., 02 Nov 1959 *Anonymous 262* (NAS!). Chong-Qing: Fengjie county, 860 m elev., 29 Apr 1959, *J. C. Zhang, 174* (SM!) Nanchuan county, 970 m elev., 13 Apr 1957, *G. F. Li 60474* (PE!). Nanchuan county, jin fo mountain, in forest edge, 1070 m elev., 20 Apr 1957, *J. H. Xiong & Z. L. Zhou 90383* (PE!). Qianjiang county, on slope, 980 m elev., 14 Aug 1988, *Z. C. Zhao, 88-1502* (PE!). Pengshui county, on slope, 800 m elev., 26 May 1959, *J. Z. Chuan, 03125* (PE!). Wushan county, 1080 m elev., 31 Mar 1958, *G. H. Yang, 57592* (PE!). Wushan county, huangniba mountain, 1100 m elev., 14 Apr 1958, *G. H. Yang 57715* (PE!). Wushan county, on slope, 1500 m elev., 17 May 1939, *T.P.Wang 10653* (PE!). Gansu: Wen county, 16 Oct 1958, *Z. P. Wei, 3047* (HIMC!). Wen county, 04 Apr 1964, *Z. B. Wang, 18862* (HNWP!). Guangxi: Longlin county, in woods, 1600 m elev., 09 Apr 1991, *H. Q. Wen 00375* (IBK!). Rongshui county, on slope, 1280 m elev., 20 Aug 1958, *S. Q. Chen 16359* (PE!). Tianyang county, 29 Nov 1978, *Z. Y. Chen 54101* (IBK!). Yangshuo county, 19 Apr 1956, *H. F Qin 700139* (IBK!). Guizhou: Dushan county, in grassland, 900 m elev., 24 Jul 1959, *Team of Libo 1198* (PE!). Guiding county, 400 m elev., 29 Jun 1930, *Y. Tsiang 5435* (IBSC!). Guiding county, in woods, 16 Jun 2014, *K. S. Mao & L. Zhang 2014-313* (SZ!). Guiyang city, Baiyun county, on slope, 1320 m elev., 22 Mar 2003, *M. T. An 5014* (PE!). Huangping county, in bushwoods, 1505 m elev., 04 May 1987, *J. M. Li 14* (GZTM!). Huishui county, on slope, 20 Jun 2014 *K. S. Mao & L. Zhang 2014-306* (SZ!). Luodian county, 300 m elev., 20 Mar 1960, *Z. S. Zhang & Y. T. Zhang 634* (IBSC!). Luodian county, in woods, 400 m elev., 22 Mar 1960, *Z. S. Zhang & Y. T. Zhang 133* (PE!). Pingtang county, in woods, 15 Jun 2014, *K. S. Mao & L. Zhang 2014-310* (SZ!). Qinglong county, in woods, 1600 m elev., 25 May 1987, *F. J. Li 403* (GZTM!). Suiyang county, in woods, 17 Jun 2014, *K. S. Mao & L.*

Zhang 2014-315 (SZ!). Wangmo county, in woods, 850 m elev., 01 Apr 2005, *G. F. Wang 1-1048* (PE!). Tongzi county, 23 May 1987, *K. M. Lan 870314* (GFS!). Yuqing county, in woods, 16 Jun 2014, *K. S. Mao & L. Zhang 2014-315* (SZ!). Zunyi county, in woods, 17 Jun 2014, *K. S. Mao & L. Zhang 2014-320* (SZ!). Henan: Luanchuan county, in woods, 28 Jun 2013, *K. S. Mao & L. Zhang 2013-078A* (SZ!). Nanyang city, funiu mountain, in woods, 1000 m elev., Jun 1959, *Anonymous 063* (HENU!). Tongbai county, tongbai mountain, on slope, 1000 m elev., 01 Apr 1960, *S. S. Kuang 468* (HENU!). Tongbai county, in woods, 27 Jun 2013, *K. S. Mao & L. Zhang 2013-063* (SZ!). Xixia county, 04 Agu 1956, *forestry department of Henan 27* (PE!). Hubei: Enshi city, in woods, 18 Jun 2013, *K. S. Mao & L. Zhang 2013-050* (SZ!). Hefeng county, 1250 m elev., 27 Aug 1958, *H. J. Li 5862* (PE!). Jianshi county, in woods, 23 Jun 2013, *K. S. Mao & L. Zhang 2013-048* (SZ!). Luotian county, in woods, 700 m elev., 10 Jul 1979, *Q. G. He 75-3* (PE!). Shennongjia, in woods, 06 Apr 1977, *Team of shennongjia 20635* (PE!). Xianfeng county, in woods, 25 Sep 1958, *H. J. Li 9252* (PE!). Xianfeng county, in woods, 22 Jun 2013, *K. S. Mao & L. Zhang 2013-053* (SZ!). Xinshan county, in woodlands, 1993 m elev., 27 March 2012, *D. G. Zhang 4383* (JIU!). Xinshan county, on slope, 1300 m elev., 14 May 1975, *Z. F. Fang et al 2005* (NAS!). Xinshan county, in woods, 20 Jun 2013, *K. S. Mao & L. Zhang 2013-046* (SZ!). Xuanen county, in woods, 22 Jun 2013, *K. S. Mao & L. Zhang 2013-052* (SZ!). Yun county, in woods, 20 Jun 2013, *K. S. Mao & L. Zhang 2013-038A* (SZ!). Hunan: Cili county, on slope, 840 m elev., 07 May 1986, *C. L. Peng 86040* (CSFI!). Dao county, on slope, 550 m elev., 04 May 1978, *Q. Z. Lin 0262* (CSFI!). Longshan county, 31 May 1958, *L. H. Liu 1885* (IBK!). Longshan county, in woods, 25 Jun 2013, *K. S. Mao & L. Zhang 2013-055* (SZ!). Luxi county, on slope, 400 m elev., 09 Apr 1982, *K. W. Liu 30045* (CSFI!). Sangzhi county, in woods, 25 Jun 2013, *K. S. Mao & L. Zhang 2013-061* (SZ!). Shimen county, 09 Jul 1979, *P. C. Cai 20198* (CSFI!). Shimen county, in woods, 420 m elev., 01 May 1980, *D. C. Xiao 80311* (CSFI!). Zhangjiajie city, zhangjiajie mountain, in woods, 870 m elev., 15 Apr 2015, *H. Zhou & D. S. Zhou 15041503* (CSFI!). Yizhang county, in woods, 09 Aug 1942, *S. Q. Chen 2107* (PE!). Yuanling county, in woods, 600 m elev., 22 Apr 1976, *Z. H. Shen 058* (CSFI!). Yuanling county, 600 m elev., 22 Apr 1976, *Anonymous 58* (IBSC!). Jiangxi: Lushan mountain, 15 May 1977, *C. F. Liang 34455* (IBK!). Tonggu county, 400 m elev., 06 Jun 1959, *J. Xiong 04268* (LBG!). Yushan county, 500 m elev., 14 Sep 1977, *S. K. Lai & H. R. Shan & D. F. Huang 039* (LBG!). Zhejiang: Chunan county, in broad-leaved forest, 700 m elev., 31 May 1959, *M. L. She 26991* (NAS!). Linan city, tianmu mountains in woods, 1 Oct 1934, J. *Shen 264* (NAS!). Linan city, tianmu mountain, on roadside, 430 m elev., 20 Jun 1983, *Q. X. Zheng S815-16* (PE!). Linan city, tianmu mountains in woods, 400 m elev., 22 Aug 1959, *Anonymous 28877* (NAS!). Taishun county, 25 May 2007, *Anonymous 24100* (HHBG!). Shaanxi: Foping county, in woods, 15 Jun 2013 *K. S. Mao & L. Zhang 2013-027A* (SZ!). Lueyang county, in valley, 600 m elev., 11 Nov 1989 *T. Y. Ding 2159* (IFP!). Mian county, 23 May 1942, *K.T.Fu 3508* (PE!). Nanzhen county, in woods, 15 Jun 2015 *L. Zhang 2015-19* (SZ!). Pingli county, on slope, 550 m elev., Apr 1959,*Y. L. Qiao, 1114* (PE!). Shiquan county, in woods, 15 Jun 2013, *K. S. Mao &*

L. Zhang 2013-033B (SZ!). Xixiang county, on slope, 650 m elev., 08 Apr 1958, *J. Q. Xing 18* (NAS!). Xixiang county, in woods, 16 Jun 2013, *K. S. Mao & L. Zhang 2013-032* (SZ!). Shandong: Kunyushan mountain, 12 Jul 1957, *Anonymous 3095* (IBSC!, PE!). Kunyushan mountain, in woods, 188 m elev., 12 May 2016, *L. Zhang 2016300* (SZ!). Sichuan: Cangxi county, 1070m elev., 08 May 1959, *Z. S. Qin 02663* (CDBI!). Da county, 800 m elev., 23 Feb 1979, *Team of Bazhong 830* (SM!). Da county, 1000 m elev., 20 Aug 1978, *Team of Kaijiang 706* (SM!). Dujiangyan city, in valley, 1200 m elev., 11 May 1930, *F. T. Wang 20749* (PE!). Emeishan mountain, on slope, 400 m elev., 03 Apr 1940, *W. P. Fang 13968* (WUK!). Jiangjin county, 1100 m elev., 26 Jul 1978, *Team of Dazu 584* (SM!). Jiulong county, on slope, 1000 m elev., 03 May 1959, *M. X. Wang 7680* (PE!). Leibo county, 1100 m elev., Jun 1963, *Z. T. Guan 373* (IBSC!). Mabian county, 1000 m elev., 31 May 1962, *Q. L. Zhang 10123* (IBSC!). Qingchuan county, in woods, 10 May 2015, *L. Zhang 201501* (SZ!). Tianquan county, on slope, 950 m elev., 14 Sep 1963, *K. J. Guan & W. C. Wang 3470* (PE!). Tongjiang county, on roadside, 1900 m elev., 19 Sep 1978, *Team of Tongjiang 1385* (SM!). Guangyuan city, on roadside, 1720 m elev., 08 Jul 1978, *Team of Guangyuan 0880* (SM!).

Populus × ningshanica C. Wang & Tung in Journal of Beijing Forestry University 4: 19. 1979. TYPE: China (holotype, WUK not seen).

Additional specimens examined. China. Gansu: Wen county, 660 m elev., 15 Jun 2015 *L. Zhang & Z. Q. Wang, 01014868* (SZ!). Shaanxi: Lueyang county, 770 m elev., 10 Jun 2015, *L. Zhang & Z. Q. Wang, 01014866* (SZ!).

Acknowledgments

References

Agapow PM, Binindaemonds OR, Crandall KA, Gittleman JL, Mace GM, Marshall JC, Purvis A (2004) The impact of species concept on biodiversity studies. The Quarterly Review of Biology 79(2): 161–179. https://doi.org/10.1086/383542

Bond JE, Stockman AK (2008) An integrative method for delimiting cohesion species: Finding the population-species interface in a group of Californian trapdoor spiders with extreme genetic divergence and geographic structuring. Systematic Biology 57(4): 628–646. https://doi.org/10.1080/10635150802302443

Braatne JH, Hinckley TM, Stettler RF (1992) Influence of soil water on the physiological and morphological components of plant water balance in *Populus trichocarpa*, *Populus*

deltoides and their F1 hybrids. Tree Physiology 11(4): 325–339. https://doi.org/10.1093/treephys/11.4.325

Bradshaw HD, Ceulemans R, Davis J, Stettler R (2000) Emerging model systems in plant biology: Poplar (*Populus*) as a model forest tree. Journal of Plant Growth Regulation 19(3): 306–313. https://doi.org/10.1007/s003440000030

Buck M, Cobb TP, Stahlhut JK, Hanner RH (2012) Unravelling cryptic species diversity in eastern Nearctic paper wasps, *Polistes* (*Fuscopolistes*), using male genitalia, morphometrics and DNA barcoding, with descriptions of two new species (Hymenoptera: Vespidae). Zootaxa 3502(3502): 1–48.

Cervera MT, Storme V, Soto A, Ivens B, Van MM, Rajora OP, Boerjan W (2005) Intraspecific and interspecific genetic and phylogenetic relationships in the genus *Populus* based on AFLP markers. Theoretical and Applied Genetics 111(7): 1440–1456.

Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J (Eds) (2001) Poplar Culture in North America. NRC Research Press, Ottawa, Canada.

Dickmann DI, Kuzovkina YA (2008) Poplars and willows of the world. Forest Management, Rome, Italy.

Dickmann DI, Stuart KW (1983) The culture of Poplars in Eastern North America. Department of Forestry, Michigan State University.

Doyle J (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19(1): 11–15.

Douady CJ (2007) Contribution of molecular techniques to the species delimitation problem. Bulletin de la Société Zoologique de France 132(4): 281–291.

Eckenwalder JE (1996) Taxonomic signal and noise in multivariate interpopulational relationships in *Populus mexicana* (Salicaceae). Systematic Botany 21(3): 261–271. https://doi.org/10.2307/2419658

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14(8): 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164(4): 1567–1587.

Fang Z, Zhao S, Skvortsov A (1999) Salicaceae In: Wu Z, Raven P (Eds) Flora of China. Volume 4. Science Press, Beijing, China.

Feng J, Jiang DC, Shang HY, Dong M, Wang GN, He XY, Zhao CM, Mao KS (2013) Barcoding Poplars (*Populus* L.) from Western China. PLoS One 8(8): e71710. https://doi.org/10.1371/journal.pone.0071710

Fladung M, Buschbom J (2009) Identification of single nucleotide polymorphisms in different *Populus* species. Trees (Berlin) 23(6): 1199–1212. https://doi.org/10.1007/s00468-009-0359-3

Francuski L, Ludoski J, Vujic A, Milankov V (2009) Wing geometric morphometric inferences on species delimitation and intraspecific divergent units in the *Merodon ruficornis* group (Diptera, Syrphidae) from the Balkan Peninsula. Zoological Science 26(4): 301–308. https://doi.org/10.2108/zsj.26.301

Fujita MK, Leaché AD, Burbrink FT, McGuire JA, Moritz C (2012) Coalescent-based species delimitation in an integrative taxonomy. Trends in Ecology & Evolution 27(9): 480–488. https://doi.org/10.1016/j.tree.2012.04.012

Gilbert KJ, Andrew RL, Bock DG, Franklin MT, Kane NC, Moore JS, Moyers BT, Renaut B, Rennison DJ, Veen T, Vines TH (2012) Recommendations for utilizing and reporting population genetic analyses: The reproducibility of genetic clustering using the program structure. Molecular Ecology 21(20): 4925–4930. https://doi.org/10.1111/j.1365-294X.2012.05754.x

Hamzeh M, Périnet P, Dayanandan S (2006) Genetic relationships among species of *Populus* (Salicaceae) based on nuclear genomic data. The Journal of the Torrey Botanical Society 133(4): 519–527. https://doi.org/10.3159/1095-5674(2006)133[519:GRASOP]2.0.CO;2

Hamzeh M, Dayanandan S (2004) Phylogeny of *Populus* (Salicaceae) based on nucleotide sequences of chloroplast *trnt-trnf* region and nuclear rDNA. American Journal of Botany 91(9): 1398–1408. https://doi.org/10.3732/ajb.91.9.1398

Hendrixson BE, Derussy BM, Hamilton CA, Bond JE (2013) An exploration of species boundaries in turret-building tarantulas of the Mojave Desert (Araneae, Mygalomorphae, Theraphosidae, Aphonopelma). Molecular Phylogenetics and Evolution 66(1): 327–340. https://doi.org/10.1016/j.ympev.2012.10.004

Jiang D, Feng JJ, Dong M, Wu GL, Mao KS, Liu JJ (2016) Genetic origin and composition of a natural hybrid poplar *Populus × jrtyschensis* from two distantly related species. BMC Plant Biology 16(1): 89. https://doi.org/10.1186/s12870-016-0776-6

Klingenberg CP (2011) MorphoJ: An integrated software package for geometric morphometrics. Molecular Ecology Resources 11(2): 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences of the United States of America 102(23): 8369–8374. https://doi.org/10.1073/pnas.0503123102

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Liu J (2016) The integrative species concept and species on the speciation way. Shengwu Duoyangxing 24(9): 1004–1008. https://doi.org/10.17520/biods.2016222

Lumley LM, Sperling FAH (2010) Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). Systematic Entomology 35(3): 416–428. https://doi.org/10.1111/j.1365-3113.2009.00514.x

Ma T, Junyi Wang JY, Zhou GK, Yue Z, Hu QJ, Chen Y, Liu BB, Qiu Q, Wang Z, Zhang J, Wang K, Jiang D, Gou CY, Yu LL, Zhan DL, Zhou R, Luo WC, Ma H, Yang YZ, Pan SK, Fang DM, Luo YD, Wang X, Wang GN, Wang J, Wang Q, Lu X, Chen Z, Liu JC, Lu Y, Yin Y, Yang HM, Abbott RJ, Wu YX, Wan DS, Li J, Yin TM, Lascoux M, DiFazio SP, Tuskan GA, Wang J, Liu JQ (2013) Genomic insights into salt adaptation in a desert poplar. Nature Communications 4(1): 2797. https://doi.org/10.1038/ncomms3797

Pérez-Losada M, Eiroa J, Mato S, Domínguez J (2005) Phylogenetic species delimitation of the earthworms *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 (Oligochaeta,

Lumbricidae) based on mitochondrial and nuclear DNA sequences. Pedobiologia 49(4): 317–324. https://doi.org/10.1016/j.pedobi.2005.02.004

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Rohlf FJ (2015) TpsDig. Version 2.22. Ecology and Evolution, SUNY at Stony Brook.

Rohlf FJ (2013) TpsUtility. Version 1.64. Ecology and Evolution, SUNY at Stony Brook.

Rohlf FJ, Marcus LF (1993) A revolution in morphometrics. Trends in Ecology & Evolution 8(4): 129–132. https://doi.org/10.1016/0169-5347(93)90024-J

Sites Jr JW, Marshall JC (2003) Delimiting species: A Renaissance issue in systematic biology. Trends in Ecology & Evolution 18(9): 462–470. https://doi.org/10.1016/S0169-5347(03)00184-8

Schroeder H, Hoeltken AM, Fladung M (2012) Differentiation of *Populus* species using chloroplast single nucleotide polymorphism (SNP) markers-essential for comprehensible and reliable poplar breeding. Plant Biology 14(2): 374–381. https://doi.org/10.1111/j.1438-8677.2011.00502.x

Villemant C, Simbolotti G, Kenis M (2007) Discrimination of *Eubazus* (Hymenoptera, Braconidae) sibling species using geometric morphometrics analysis of wing venation. Systematic Entomology 32(4): 625–634. https://doi.org/10.1111/j.1365-3113.2007.00389.x

Wan XQ, Zhang F, Zhong Y, Ding YH, Wang CL, Hu TX (2013) Study of genetic relationships and phylogeny of the native *Populus* in Southwest China based on nucleotide sequences of chloroplast *trnt-trnf* and nuclear DNA. Plant Systematics and Evolution 299(1): 57–65. https://doi.org/10.1007/s00606-012-0702-9

Wiens JJ (2007) Species delimitation: New approaches for discovering diversity. Systematic Biology 56(6): 875–878. https://doi.org/10.1080/10635150701748506

Wiens JJ, Penkrot TA (2002) Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). Systematic Biology 51(1): 69–91. https://doi.org/10.1080/106351502753475880

Supplementary material I

Tables S1–S3

Authors: Lei Zhang, Mingcheng Wang, Tao Ma, Jianquan Liu

Data type: molecular data

Explanation note: **Table S1.** Details for the 14 microsatellite loci adopted in genetic survey. **Table S2.** Details for the four chloroplast DNA fragments adopted in genetic survey. **Table S3.** Length and variations for each DNA region and for the combination of the four plastid regions

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RESEARCH ARTICLE

Eutrema nanum (Brassicaceae), a new species from Chola Shan, Southwest China

Guoqian Hao[1,2], Ihsan A. Al-Shehbaz[3], Lei Zhang[2],
Xinyi Guo[2], Hao Bi[2], Songbai Xu[1], Jianquan Liu[2]

1 *Biodiversity Institute of Mount Emei, Mount Emei Scenic Area Management Committee, Leshan 614000, Sichuan, P. R. China* **2** *Key Laboratory for Bio-Resources and Eco-Environment of Ministry of Education, College of Life Science, Sichuan University, Chengdu 610065, Sichuan, P. R. China* **3** *Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299, USA*

Corresponding author: *Jianquan Liu* (liujq@nwipb.ac.cn)

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Abstract

Eutrema nanum, a new high-elevation (4500–4600 m) species from Chola Shan, Sichuan (Southwest China), is described and illustrated. It is similar morphologically to *E. nepalense* but is readily distinguished by having oblong to elliptic or obovate to spatulate (vs. suborbicular to broadly ovate) leaves, glabrous (vs. puberulent) sepals and ovate to oblong fruit 4–7 × 2–3 mm with flattened valves (vs. ovoid to subglobose fruit 2–3 × 1.8–2 mm with rounded valves). The genetic differences amongst *E. nanum*, *E. nepalense* and other close relatives are further confirmed by phylogenetic analyses using ITS and cpDNA sequence variations. The new combination *E. sinense* is proposed.

Keywords

Cruciferae, *Eutrema nanum*, molecular phylogeny, Sichuan, *Eutrema sinense*

Introduction

The boundaries of *Eutrema* R.Br. (Brassicaceae or Cruciferae) have recently been expanded to include 38 species, several of which were previously placed in the six genera *Taphrospermum* C.A.Mey., *Thellungiella* O.E.Schulz, *Neomartinella* Pilg., *Platycraspedum* O.E.Schulz, *Chalcanthus* Boiss. and *Pegaeophyton* Hayek & Hand.-Mazz. (Al-Shehbaz and Warwick 2005; Hao et al. 2017a). The taxonomic knowledge

of this genus is still incomplete because numerous collections from the high-elevation regions in Southwest China were often overlooked and many areas remain poorly explored. We reported two new species during recent field investigations and molecular analyses (Hao et al. 2016, 2017b). Here we report the third one, *Eutrema nanum*, found in Chola Shan at a high elevation of 4500–4600 m in Sichuan Province, Southwest China. This new species is morphlogically similar to *E. nepalense* (Al-Shehbaz, Kats Arai & H.Ohba) Al-Shehbaz, G.Q.Hao & J.Quan Liu but, as shown below, it is readily distinguished by several aspects of leaves and fruit. The phylogenetic studies on both species and their other relatives were also conducted herein and the results support the recognition of this novelty. In addition, one of six species which were used to determine the systematic position of *E. nanum* was found to need a taxonomic combination and a new name *Eutrema sinense* (Hemsl.) G.Q.Hao, J.Quan Liu & Al-Shehbaz is therefore proposed herein.

Material and methods

We examined morphological traits of *Eutrema nanum* and several relative species. We followed Hu et al. (2015) and Hao et al. (2017a) in examining the genetic differences between this novelty (two accessions) and the morphologically similar *E. nepalense* (one accession). In order to determine the systematic position of *E. nanum*, we futher included six species (*E. scapiflorum* (Hook.f. & Thomson) Al-Shehbaz, G.Q.Hao & J.Quan Liu, *E. sinense* (Hemsl.) G.Q.Hao, J.Quan Liu & Al-Shehbaz, *E. hookeri* Al-Shehbaz & Warwick, *E. fontanum* (Maxim.) Al-Shehbaz & Warwick, *E. verticillatum* (Jeffrey & W.W.Sm.) Al-Shehbaz & Warwick and *E. deltoideum* (Hook.f. & Thomson) O.E.Schulz) in our analyses. All six species were shown to be close relatives to *E. nepalense* in our previous study (Hao et al. 2017a) and two (*E. scapiflorum* and *E. sinense*) were previously placed in the genus *Pegaeophyton*. The related *E. integrifolium* Bunge (see Hao et al. 2017a) was selected as the outgroup. The collection information of the sampled species is listed in Table 1 and Figure 3 and the voucher specimens were deposited in the Sichuan University Herbarium (SZ).

We extracted the total DNA and amplified and sequenced four DNA markers, the nuclear internal transcribed spacer (ITS) and three chloroplast DNA (cpDNA) regions (*psb*A-*trn*H, *rbc*L, *mat*K), following Hu et al. (2015) and Hao et al. (2017a). The sequences firstly reported here were placed in GenBank under the accession numbers (MH702367, MH793597, MH793598, MH793599). We aligned all sequences using Clustal X (Thompson et al. 1997) and refined them manually. We concatenated three cpDNA sequences into a single matrix for Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses. We coded indels using the simple code method by Gap-Coder (Young and Healy 2003). We constructed phylogenetic relationships based on two datasets (ITS and cpDNAs) using MP analyses by PAUP* 4.10b (Swofford 2003) and ML analyses using RAxML 7.2.6 (Stamatakis 2006). MP analyses employed a heuristic search with 10,000 replicates and TBR branch swapping and bootstrap values (Felsenstein 1985) were estimated with 1000 replicates and 100 random-addition-

Table 1. The sources of materials used for molecular analyses of Himalayan *Eutrema* (all vouchers at SZ).

| Taxon	Voucher	Source	Coordinate
E. nanum	Liu & Hao 14091	Chola Shan, Sichuan, China	31°55'N, 98°54'E
E. nanum	Liu 17124	Chola Shan, Sichuan, China	31°55'N, 98°54E
E. nepalense	Long et al. 605	Sikkim, India	27°36'N, 88°12'E
E. sinense	Liu 13114	Biluo Snow Mountain, Yunnan, China	27°59'N, 98°47'E
E. scapiflorum	Liu & Hao 13074	Yarla Shampo Mountain, Tibet, China	28°51'N, 91°59E
E. fontanum	Liu & Hao 13144	Zhuodala Mountain, Sichuan, China	31°24'N, 99°56'E
E. hookeri	Liu 17108a	Mila Mountain, Tibet, China	29°49'N, 92°90'E
E. verticillatum	Liu & Hao 14094	Maila Mountain, Sichuan, China	30°58'N, 98°58'E
E. deltoideum	Liu 13024	Lasa, Tibet, China	29°42'N, 91°09'E
E. integrifolium	Liu & Hao 13049	Tianshan Mountain, Xinjiang, China	43°12'N, 84°49'E
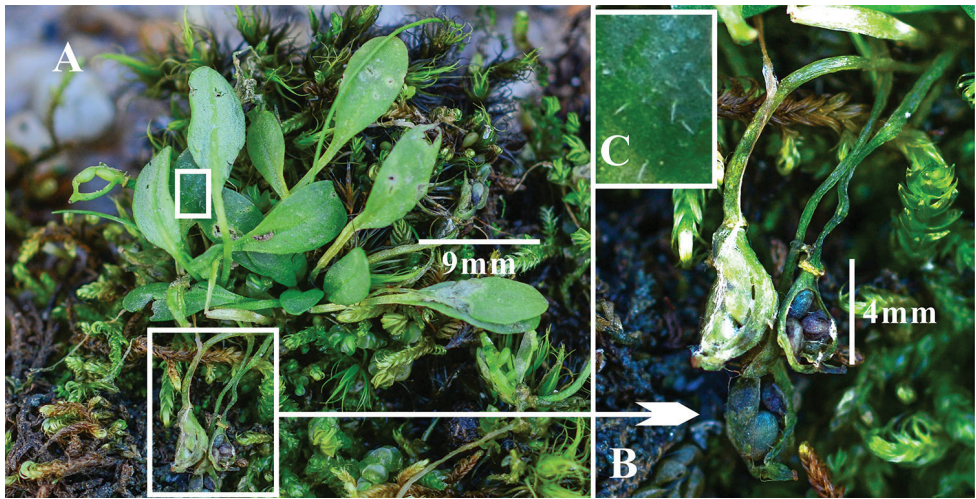
Figure 1. *Eutrema nanum.* G.Q. Hao, J.Quan. Liu & Al-Shehbaz. **A** Plant **B** Fruit **C** Leaf trichomes.

sequence replicates per bootstrap replicate. ML analyses were performed with raxml-HPC -f a -s sequence. phy -n boot2 -m GTRGAMMA -x 1234 -# 1000 -n outname. The most suitable GTRGAMMA models were used and bootstrap analyses were estimated with 1000 replicates.

Taxonomy

Eutrema nanum G.Q.Hao, J.Quan Liu & Al-Shehbaz, sp. nov.
urn:lsid:ipni.org:names:60477015-2
小山嵛菜

Type. China. Sichuan: Chola Shan, 31°55'32"N, 98°54'35"E, 4500 m elev., 16 August 2014, *Liu & Hao 14091* (Holotype, SZ). Figures 1, 2.

Figure 2. Holotype of *Eutrema nanum*.

Description. Herbs perennial, 3–6 cm tall, glabrous or puberulent; caudex slender, ca. 3–5 mm long. Leaves basal, rosulate, 20–25 per caudex; petiole 13–20 mm long, slender at base, glabrous or with few trichomes; blade oblong, elliptic,

Figure 3. Geographical distribution of the sampled populations of *Eutrema nanum* and related species.

obovate, spatulate, 6–10 × 3–4 mm, fleshy, glabrous or abaxially pubescent with trichomes, 0.3–0.6 mm long, base subattenuate, to cuneate, margin entire, apex obtuse to subrounded. Pedicels slender, 18–23 mm long at anthesis, not elongated in fruit, not persistent. Flowers 5–8 per plant; sepals ovate to oblong, 1–1.5 mm long; petals white, broadly obovate to spatulate, blade 2–3 ×1–2 mm, persistent to fruit maturity, claw-like base 0.5–1 mm long. Ovules 2–4 per ovary. Fruit latiseptate, dehiscent, ovate to oblong, somewhat curved, 4–7 × 2–3 mm; valves nearly flat, extending along part of fruit length; gynophore 0.1–0.3 mm long; replum 0.3–0.4 mm wide; style 0.6–1 mm long. Seeds broadly ovate, brown, plump, 2–4 per fruit, 1.4–2 × 0.6–1 mm.

Eutrema nanum is morpholgically most similar to *E. nepalense*, from which it is readily distinguished by having oblong, elliptic, obovate to spatulate leaves, glabrous sepals and ovate to oblong larger fruit 4–7 × 2–3 mm with flattened, glabrous valves. In contrast, *E. nepalense* (https://www.gbif.org) has suborbicular to broadly ovate leaves, puberulent sepals and ovoid to subglobose smaller fruit 2–3 × 1.8–2 mm with rounded, puberulent valves. *Eutrema nanum* was only found with around 100 individuals along a stream in a valley about 2 kilometres from the Chola Shan peak, whereas *E. nepalense* occurs across Himalyas Mountains in Bhtan, China, Nepal and India.

Phenology. Flowering: June–August. Fruiting: August–September.

Distribution and habitat. *Eutrema nanum* is currently known only from Chola Shan, part of Hengduan Mountains in West Sichuan, China (Fig. 3). It grows under rocks by streams close to glaciers, damp or gravelly scree, wet sand at a very high elevation of 4500–4600 m.

Paratype. China. Sichuan: Chola Shan, 31°55'32"N, 98°54'35"E, 4500 m elev., *Liu 17124* (SZ).

Table 2. Diagnosing sites of the aligned ITS and three cpDNA sequences between *Eutrema nanum* and *E. nepalense*.

Species	ITS	rbcL		matK											
	508	82	337	165	276	333	342	391	449	483	495	497	549		
Eutrema nanum	C	C	T	C	T	T	T	T	T	T	C	T	T		
Eutrema nepalense	T	A	C	T	C	G	C	A	G	C	T	C	A		

Species	matK					psbA-trnH									
	601	603	633	638	657	28	40	48	92	114	115	138-	212	228	235
Eutrema nanum	C	C	T	T	G	T	C	G	-	G	C	-	2 nt	C	C
Eutrema nepalense	T	T	C	A	A	A	G	A	6 nt	T	A	74 nt	-	T	A

nr=nucleotide.

Eutrema sinense **(Hemsl.) G.Q.Hao, J.Quan Liu & Al-Shehbaz, comb. nov. Based on *Braya sinensis* Hemsl., J. Linn. Soc., Bot. 30: 303. 1892.**
urn:lsid:ipni.org:names:77190124-1

Syn.: *Eutrema robustum (O.E.Schulz) Al-Shehbaz, G.Q.Hao & J.Quan Liu, Bot.* J. Linn. Soc. 184: 219. 2017. Basionym: *Pegaeophyton sinense* var. *robustum* O.E.Schulz, Notizbl. Bot. Gart. Gerlin-Dahlem 9: 477. 1926.

The earliest available epithet of this taxon at the species rank is "*sinensis*" and it should have been been transferred to *Eutrema* by Hao et al. (2017a) instead of using the varietal epithet "*robustum*."

Genetic differences between *Eutrema nanum*, *E. nepalense* and other relatives

Sequence data from *Eutrema nanum* and *E. nepalense* reveals that one nucleotide substitution in ITS, two in *rbc*L, 18 in *mat*K and eight substitutions and three indels in *psb*A-*trn*H distinguish them very well (Table 2).

Based on sequence variations of ITS and cpDNAs (Table 3), phylogenetic analyses suggested that *Eutrema nanum* is mostly related to *E. nepalense*, *E. sinense* and *E. scapiflorum*. However, phylogenetic relationships of these four species are incongurent between ITS and plastid DNA tree (Fig. 4). In the ML analyses of ITS sequence data, *E. nanum* and *E. nepalense* formed a single cluster sister to *E. sinense* and together are sister to *E. scapiflorum* with high support values (>80%) (Fig. 4A). By contrast, in the ML analyses of cpDNAs sequences, the phylogenetic relationships were maintained between *E. nanum* and sister *E. sinense* and together as sister to *E. scapiflorum*, but *E. nepalense* fell outside that relationship and was separated from them by *E. hookeri* with medium support (>50%) (Fig. 4B). MP analyses produced almost the same tree topologies with similar bootstrap support values.

Table 3. Tree statistics for analyses of the datasets.

| Data set	ITS*	*psb*A-*trn*H	*rbc*L	*mat*K	Combined cpDNA*
No. of sequences	30	30	30	30	30
Aligned length used in analyses	698	455	506	779	1786
No. of variable characters	141	58	17	66	169
No. of parsimony-informative characters	56	15	7	22	47
Tree length (steps)	78	70	10	71	195
Consistency (CI)	0.833333	0.900000	1.000000	0.873239	0.892308
Retention index (RI)	0.803030	0.708333	1.000000	0.790698	0.764045
Rescaled consistency index (RC)	0.669192	0.637500	1.000000	0.690468	0.681763

*gaps were coded and included.
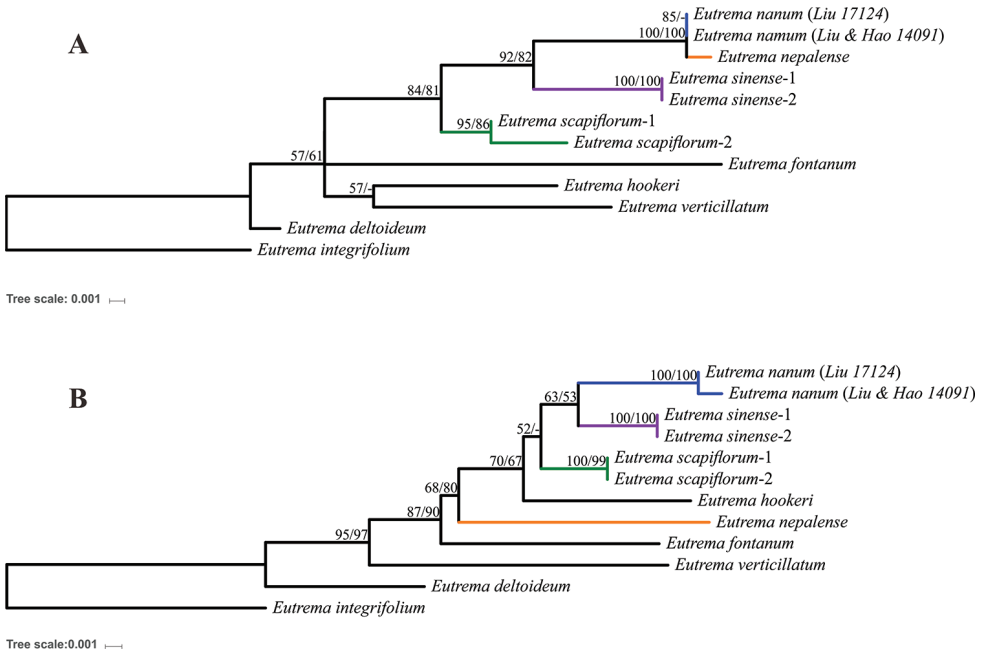
Figure 4. The Maximum Likelihood tree based on analysis of ITS (**A**) and Three cpDNA regions (**B**). Numbers above branches are maximum likelihood bootstrap support values and maximum parsimony bootstrap support values. '-' represents <50%.

Discussion

Both *Eutrema nanum* and *E. nepalense* are small plants similar in flower traits and seed size. However, as discussed above, they are quite different morphologically. In addtion, phylogenetic analyses of cpDNAs variations suggested these two species did not comprise a monophyletic clade. Furthermore, the Himalayan *E. nepalense* is disjunctly

separated by a distance of at least 1200 air kilometres from the Chola Shan (Sichuan, SW China), where *E. nanum* is endemic (Fig. 3). *Eutrema nanum* is also closely related to *E. sinense* in the phylogenetic analyses of the cpDNA sequence variations, but both are easily distinguished from each other. *Eutrema nanum* is a small and weak herb with entire leaves (0.5–1.5 cm long) and small flowers (petals 2–3 mm long), whereas *E. sinense* is obviously stout with entire or toothed leaves (1.5–8 cm long) and distinctly larger flowers (petals 8–15 mm long) and fruit (10–20 mm long).

Pylogenetic relationships amongst *E. nanum*, *E. nepalense* and *E. sinense* are incongruent between ITS and cpDNA trees. This incongruence may suggest possible hybridisations or incomplete lineage sorting during the rapid and recent species diversifications (Soltis and Soltis 2000, 2009). However, it is not possible at present to determine which of these two factors had caused the incongruent phylogenies observed here. More analyses and molecular data, especially based on more individuals and genomic evidence, are needed to solve these phylogenetic inconsistences.

Acknowledgements

References

Al-Shehbaz IA, Warwick SI (2005) A synopsis of *Eutrema* (Brassicaceae). Harvard Papers in Botany 10(2): 129–135. https://doi.org/10.3100/1043-4534(2005)10[129:ASOEB]2.0.CO;2

Felsenstein J (1985) Confidence limits on phylogenies, an approach using the bootstrap. Evolution; International Journal of Organic Evolution 39(4): 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Hao GQ, Al-Shehbaz IA, Liang QL, Liu JQ (2016) *Eutrema tianshanense* (Brassicaceae), a new species from Tian Shan Mountains of central Asia. Phytotaxa 286(1): 23–31. https://doi.org/10.11646/phytotaxa.286.1.3

Hao GQ, Al-Shehbaz IA, Ahani H, Liang QL, Mao KS, Wang Q, Liu JQ (2017a) An integrative study of evolutionary diversification of *Eutrema* (Eutremeae, Brassicaceae). Botanical Journal of the Linnean Society 184(2): 204–223. https://doi.org/10.1093/botlinnean/box024

Hao GQ, Zhang CB, Al-Shehbaz IA, Guo XY, Bi H, Wang JY, Liu JQ (2017b) *Eutrema giganteum* (Brassicaceae), a new species from Sichuan, southwest China. PhytoKeys 82: 15–26. https://doi: 10.3897/phytokeys.82.12329

Hu H, Al-Shehbaz IA, Sun YS, Hao GQ, Wang Q, Liu JQ (2015) Species delimitation in Orychophragmus (Brassicaceae) based on chloroplast and nuclear DNA barcodes. Taxon 64(4): 714–726. https://doi.org/10.12705/644.4

Soltis PS, Soltis DE (2000) The role of genetic and genomic attributes in the success of polyploids. Proceedings of the National Academy of Sciences of the United States of America 97(13): 7051–7057. https://doi.org/10.1073/pnas.97.13.7051

Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. Annual Review of Plant Biology 60(1): 561–588. https://doi.org/10.1146/annurev.arplant.043008.092039

Stamatakis A (2006) RAxML-VI-HPC, maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics (Oxford, England) 22(21): 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Swofford DL (2003) PAUP*, phylogenetic analyses using parsimony (*and other methods), Version 4. Sunderland, MA, USA, Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface, flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25(24): 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Young ND, Healy J (2003) GapCoder automates the use of indel characters in phylogenetic analysis. BMC Bioinformatics 4(1): 6. https://doi.org/10.1186/1471-2105-4-6