

Two new species of *Athenaea* Sendtn. (Solanaceae) from the Atlantic forests of south-eastern Brazil

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Abstract

Two new species of *Athenaea* Sendtn. (Solanaceae) from the Brazilian Atlantic Forest are described and illustrated. *Athenaea altoserranae* I.M.C.Rodrigues & Stehmann, **sp. nov.** from the Serra do Mar range, in São Paulo State and *Athenaea hunzikeriana* I.M.C.Rodrigues & Stehmann, **sp. nov.** from a restricted area in the north-eastern region of Minas Gerais State and the southern part of Bahia State. Both species have brown to purple-brown or purple mature fruits, a character not found in other species of *Athenaea*. Descriptions, illustrations, complete specimen citations and maps of both species are provided. A dichotomous key to all species of *Athenaea* is also presented.

Resumo

Duas espécies novas de *Athenaea* Sendtn. (Solanaceae) da floresta atlântica brasileira são descritas e ilustradas. *Athenaea altoserranae* I.M.C.Rodrigues & Stehmann, **sp.nov.** conhecida apenas da floresta atlântica da Serra do Mar, no estado de São Paulo, e *Athenaea hunzikeriana* I.M.C.Rodrigues & Stehmann, **sp.nov.**, conhecida somente de uma área restrita na região nordeste do estado de Minas Gerais e sul da Bahia, divisa entre os estados. Ambas as espécies apresentam frutos amarronzados a arroxeados, um caractere até então não observado em outras espécies de *Athenaea*. São disponibilizadas descrições, ilustrações, citações completas de espécimes e um mapa de distribuição. Uma chave dicotômica de identificação para todas as espécies de *Athenaea* também é apresentada.

Keywords

Alto da Serra, *Aureliana*, Brazilian Atlantic Forest, Mata Atlântica, Physalideae, taxonomy, Withaninae

Palavras-chave

Alto da Serra, *Aureliana*, floresta atlântica, Mata Atlântica, Physalideae, taxonomia, Withaninae

Introduction

Athenaea Sendtn. (Solanaceae) is a small, exclusively Neotropical genus with highest species diversity in south-eastern Brazil (Rodrigues et al. 2019). It comprises 12 species of shrubs or small trees mostly growing in forest understorey or openings in highly fragmented Atlantic forests (Rodrigues et al. 2019). Recent re-circumscription of the genus (Rodrigues 2013; Zamberlan et al. 2015) meant that all species of the previously recognised *Aureliana* Sendtn. were transferred to *Athenaea* (Rodrigues et al. 2019).

The genus is characterised by axillary inflorescences, stellate, actinomorphic flowers with five stamens and fruits that are fleshy berries, borne on erect or spreading pedicels. The fruiting calyx is variously accrescent (Rodrigues 2013), ranging from not enlarging at all in fruit to completely enclosing the berry (see Zamberlan et al. 2015 for illustrations of the states). The genus was previously considered a member of the subtribe Withaninae of the tribe Physalideae, based on molecular phylogenetic studies (Olmstead et al. 2008). Recent work, however, has shown that Withaninae is not monophyletic (Deanna et al. 2019); *Athenaea* and two European species of *Withania* Pauq. (*W. frutescens* (L.) Pauq. and *W. aristata* Pauq. from the Mediterranean region and Canary Islands, respectively) are sister to the Andean genera *Deprea* Raf. and *Cuatresia* Hunz., while the rest of *Withania* species resolve as more closely related to *Physalis* L. and relatives (see Deanna et al. 2019 for clade composition).

As part of on-going work producing a monographic treatment of *Athenaea* in this new circumscription, new taxa have been discovered and are described here. *Athenaea altoserranae* I.M.C.Rodrigues & Stehmann (described here) was included in the molecular phylogenetic studies of Zamberlan et al. (2015) and resolved as distinct (as *Aureliana* sp. nov.), but was not given a name at that time (Zamberlan et al. 2015; GenBank numbers in Suppl. material 1). These new taxa can be included in future studies of both morphology and biogeography of the family Solanaceae.

Materials and methods

Species circumscriptions are based on a combination of morphological (Rodrigues 2013) and molecular (Zamberlan et al. 2015) information. Descriptions are based on observations and data taken from specimens collected in the field between 2003 and 2014. Examination of herbarium specimens from 15 herbaria (acronyms according to Thiers 2019+) <http://sweetgum.nybg.org/science/ih/>): BHCB, BM, CEPEC, CEN,

CORD, ESA, IAC, MBM, MBML, MO, NY, R, RB, SP and SPF; and specimens accessed as digital images via INCT Herbário Virtual (<http://inct.splink.org.br>) and Re-flora – Herbário Virtual (<http://reflora.jbrj.gov.br/reflora/herbarioVirtual/>) databases were conducted to confirm the novelty of the species described here. All barcode and accession numbers, as well as label details for the new species, are presented in Suppl. material 2 and on the Natural History Museum Data Portal (<https://doi.org/10.5519/zc80n093>). In the field, fresh flowers were fixed in alcohol to permit detailed descriptions and illustrations using dissection and compound microscopy.

We calculated the Extent of Occurrence (EOO) and Area of Occupancy (AOO) using GeoCat (<http://geocat.kew.org>) calibrated with the standard 2 km² cell width for the AOO measurement. A preliminary conservation status was assigned using the IUCN (2019) criteria implemented in GeoCat analyses (Bachman et al. 2011) combined with our field knowledge of habitats and threats to the Brazilian Atlantic forests.

Taxonomic treatment

Athenaea altoserranae I.M.C.Rodrigues & Stehmann, sp. nov.

urn:lsid:ipni.org:names:77217158-1

Figs 1–3

Diagnosis. Similar to *Athenaea fasciculata* from which it can be distinguished in leaves arranged in clusters at the top of the stems (versus evenly distributed), warty somewhat lenticellate floral pedicels (versus without lenticels) and dark purplish-brown mature fruits (versus green).

Type. BRAZIL. São Paulo: Santo André, Distrito de Paranapiacaba, mais ou menos no km 3 da rodovia Estação de Campo Grande – Paranapiacaba, 18 Oct 1967 (fl, fr), J. Mattos & N. Mattos 15078 (holotype: SP [acc. # 106084]).

Description. Small trees, 3–8 m tall; branching dichotomous, especially distally. Stems quadrangular in cross-section, longitudinally striate, glabrous, brownish. Sympodial units difoliate, the leaves usually geminate, occasionally solitary. Leaves simple, alternate, spreading, arranged in clusters (short internodes) near branch apices, lanceolate, narrowly lanceolate, sometimes elliptic-lanceolate; blades (3.5–) 8.9–28.5 (50) cm long, (1)1.8–8.3 (15) cm wide, 2–6 times longer than wide, coriaceous, slightly discoloured; abaxial and adaxial surfaces glabrous, shiny; venation brochidodromous and prominent abaxially, with 6–12 pairs of principal veins; base attenuate, somewhat decurrent and asymmetric, occasionally somewhat cordate; margins entire and slightly revolute; apex acute or acuminate, sometimes notched (retuse); petioles (0.65–) 1–4(5.3) cm long, glabrous. Inflorescences axillary, sessile fascicles, with 4–8 flowers; pedicels 1.2–2.5 cm long, spreading or deflexed, glabrous and lenticellate, distally annular (constricted below a swollen pedicel apex). Flower buds globose, greenish-cream. Flowers 5-merous, heteromorphic with short- and long-styled forms. Calyx 1.8–3.5 mm long, campanulate-rotate, green, glabrous, the tube 0.4–1 mm long, the lobes 1–2.5 mm long, 2–3 mm wide,



Figure 1. *Athenea altoserranae* I.M.C.Rodrigues & Stehmann **a** flower bud **b** flower **c** branches with buds, flowers and fruits **d** fruit. Illustrated from I.M.C. Rodrigues et al. 625 (BHCB). Illustration by I.M.C. Rodrigues. Scale bars 1 cm.

triangular, the apex acute or obtuse, glabrous on both surfaces. Corolla 7–13 mm long, stellate, white abaxially, cream with green to brownish spots adaxially, the tube 3–4 mm long, the lobes 3.5–9 mm long, 3–5.6 mm wide, triangular-lanceolate, glabrous abaxially, with glandular trichomes (stalk 2-celled, the head unicellular) at the base adaxially, the margins densely papillate. Stamens equal, glabrous; filaments 1.8–3 mm long, the staped (basal extension of the filaments) ca. 1.5 mm long; anthers 1.8–3 mm long, 1–1.5 mm wide, oblong, yellowish-green, dehiscent by longitudinal slits. Ovary ca. 2.5 mm long, subglobose, greenish-cream, glabrous, surrounded by a yellowish green nectary; styles heteromorphic, long styles 4.2–5 mm long, short styles 2.3–2.8 mm long, greenish-white; stigma dilated, discoidal, greenish-white. Fruit a subglobose berry, 8–16 mm long, 8–19 mm in diameter, green when immature, dark brown to almost purplish-black at maturity, the surface glabrous; fruiting calyx not accrescent, up to 1.8 times longer in fruit than in flower, the apex of lobes up to 1/4 of fruit length; fruiting pedicels 2.1–4.1 cm long, enlarged at the apex, glabrous, pendent. Seeds up to 22 per berry, 2.4–2.8 mm long, 2.2–2.5 mm wide, semicircular to ovoid-reniform, yellow to brown, the testa reticulate, foveolate, the embryo curved. Chromosome number: not known.

Etymology. The specific epithet is a reference to “Alto da Serra”, located in the District of Paranapiacaba, Municipality of Santo André, São Paulo State, where



Figure 2. *Athenaea altoserranae* I.M.C.Rodrigues & Stehmann in the field **a** I.M.C. Rodrigues *et al.* 80 **b, c, e** Stehmann *et al.* 4800 (BHCB) **d** I.M.C. Rodrigues *et al.* 83 **f** I.M.C. Rodrigues *et al.* 625. Photographs **a–e** by J.R. Stehmann **f** by S.N. Moreira.

the species, including the type gathering, has been collected since 1917. This area was the first biological station in South America, established in 1909 by Hermann Friedrich Albrecht von Ihering. In 1938, under the administration of the Instituto de Botânica de São Paulo, the name was changed to Reserva Biológica do Alto da Serra de Paranapiacaba (Marcolin 2009).

Phenology. *Athenaea altoserranae* flowers and fruits from August to March.

Distribution and habitat. *Athenaea altoserranae* is endemic to Brazil and restricted to the edges of the plateau of the Serra do Mar mountain range in São Paulo State.

It grows in the wet Atlantic rain forest (Mata Atlântica), often in clearings and along trails or other open places from 714 to 1,100 m elevation.

Preliminary conservation assessment (IUCN 2019). Endangered (EN). EOO 1,240.9 km² (EN); AOO 48 km² (EN). This species occurs in the plateau of Serra do Mar and near the coast in the Municipality of Cananéia, São Paulo State, an area originally totally covered by Atlantic rain forest. The restricted range, coupled with the fragmented habitat, suggests a preliminary assessment of Endangered B1a and B2a, b (ii and iii).

Discussion. Specimens of *Athenaea altoserranae* have been variously identified in herbaria as *Aureliana fasciculata* (Vell.) Sendtn., *Aureliana fasciculata* var. *longifolia* (Sendtn.) Hunz. and Barboza or *Aureliana glomuliflora* Sendtn. – all currently recognised as synonyms of the widespread glabrous species *Athenaea fasciculata* (Vell.) I.M.C.Rodrigues & Stehmann. *Athenaea fasciculata* is the most widespread species of the genus and is distributed along the Atlantic rain forest from southern to north-eastern Brazil, but also occurs south to Paraguay and Argentina and west to eastern Peru and Bolivia (see Rodrigues et al. 2019). *Athenaea altoserranae* is sympatric with *A. fasciculata*, *A. cuspidata*, *A. pogogena*, *A. picta*, *A. sellowiana* and *A. wettsteiniana*.

Hunziker and Barboza (1990) recognised three infraspecific taxa within their circumscription of *Aureliana fasciculata* (*A. fasciculata* var. *fasciculata*, *A. fasciculata* var. *longifolia* Sendtn. and *A. fasciculata* var. *tomentella* Hunz. & Barboza). They included material of *Athenaea altoserranae* in their circumscription of var. *longifolia* along with other specimens collected in Bahia and the border of São Paulo with Paraná (SP/PR); the only original material we have seen for var. *longifolia* was destroyed in the bombing of the Berlin herbarium and no duplicates have been found, but a photograph (F neg. 2880, of an un-numbered F. Sellow collection from “Brasilia australiore” annotated “*Bassovia glomuliflora* Dun. β *longifolium*”) corresponds to *A. fasciculata* s.l. and not to *A. altoserranae* (Rodrigues et al. 2019). Although all of the specimens included in var. *longifolia* by Hunziker and Barboza (1990) share completely glabrous leaves, the plants from Bahia and from the São Paulo/Paraná border have small flowers, long-obovate leaves with a cuspidate apex and longer petioles, thus distinguishing them from the plants here recognised as *A. altoserranae*. We currently include these plants from Bahia and from the São Paulo/Paraná border in our circumscription of *A. fasciculata* s.l.

Athenaea altoserranae can be distinguished from *A. fasciculata* s.l. by its erect and long-lanceolate leaves clustered at the tips of shoots versus broader leaves evenly spaced along the stem, warty versus smooth fruiting pedicels and dark brown versus green mature fruits. Phylogenetic studies of the group, using molecular data (plastid and nuclear markers), provide additional support for the distinction of these populations. Zamberlan et al. (2015) found that *Athenaea altoserranae* (as *Aureliana* sp. nov.) does not belong to the same clade as *Aureliana fasciculata*, but is, instead, a member of a clade with *A. sellowiana* (Sendtn.) I.M.C.Rodrigues & Stehmann and *A. velutina* (Sendtn.) D’Arcy that is sister to *Athenaea pogogena* (Moric.) Sendtn. + *Athenaea cuspidata* Witasek + *Athenaea picta* (Mart.) Sendtn.

Athenaea sellowiana is also morphologically similar to *A. altoserranae* in its glabrous stems and leaves and is broadly sympatric with it in São Paulo State. The two species

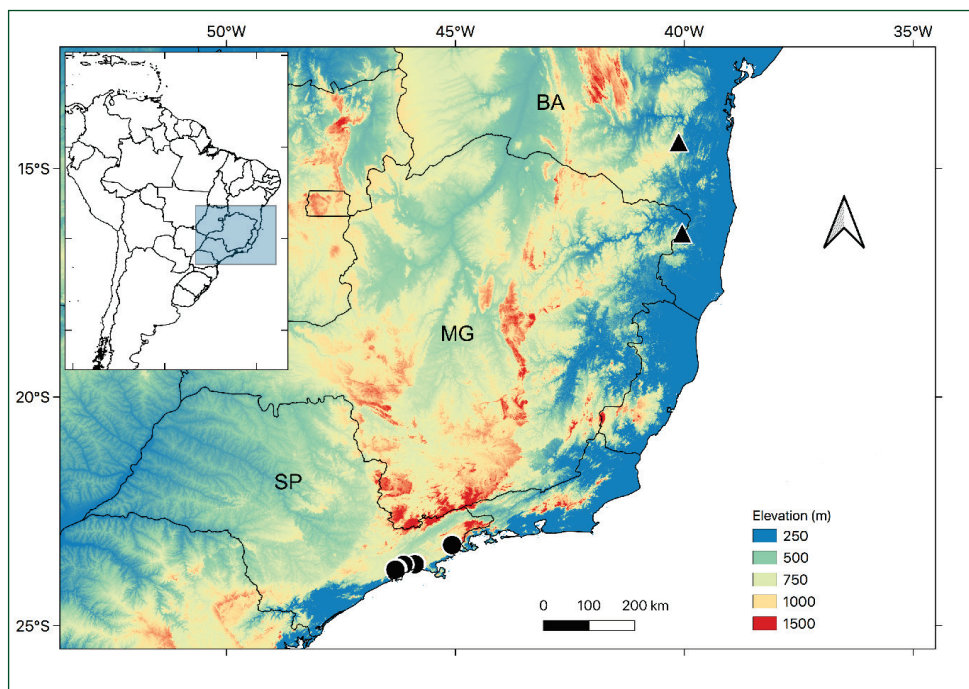


Figure 3. Distribution map for *Athenaea altoserranae* I.M.C.Rodrigues & Stehmann (circles) and *Athenaea hunzikeriana* I.M.C.Rodrigues & Stehmann (triangles). Bahia = BA; Minas Gerais = MG; São Paulo = SP.

can be distinguished by flower texture (calyx and corolla fleshy in *A. sellowiana* and foliaceous in *A. altoserranae*) and the more strictly lanceolate leaves of *A. altoserranae* (versus narrowly oblanceolate, elliptic or oblong leaves in *A. sellowiana*).

Additional specimens examined. BRAZIL. São Paulo: Cunha, Parque Estadual da Serra do Mar, 12 Dec 1989 (fl), *O.T. Aguiar* 338 (SPSF); Cunha, Parque Estadual da Serra do Mar, Estrada de acesso ao Núcleo, margens do Rio Paraibuna, 28 Jan 2004 (fl, fr), *F.A.R.D.P. Arzolla* 435 (ESA); Biritiba Mirim, Estação Biológica de Boracéia, 29 Sep 1983 (fl), *A. Custódio Filho* 1570 (BHCB); Biritiba Mirim, Estação Biológica de Boracéia, 29 Sep 1983 (fl), *A. Custódio Filho* 1592 (SP); Biritiba Mirim, Estação Biológica de Boracéia, 21 Oct 1983 (fl), *A. Custódio Filho* 1723 (SP); Biritiba Mirim, Estação Biológica de Boracéia, 26 Oct 1983 (fl, fr), *A. Custódio Filho* 1735 (SP); Alto da Serra, 20 Nov 1984 (fl), *T.P. Guerra & M. Kirizawa* 90 (BHCB); Alto da Serra, Caixa d'água, 6 Oct 1922 (fl), *F.C. Hoehne* s.n. (SP); Natividade da Serra, Parque Estadual da Serra do Mar, Núcleo Santa Virgínia, 14 Nov 2005 (fl, fr), *N.M. Ivanauskas et al.* 5196 (MBM); Bertioiga, Rodovia BR 101, Km 216, 21 Aug 1995 (fl, fr), *M. Kirizawa et al.* 3177 (BHCB); Cunha, Parque Estadual da Serra do Mar, trilha para a casa do Silvestre, 20 Mar 1996 (fr), *M. Kirizawa et al.* 3271 (SPF); Alto da Serra, Estação Biológica, 17 Mar 1944 (fr), *M. Kuhlmann* s.n. (SP); Salesópolis, Boracéia, 22 Nov 1957 (fl), *M. Kuhlmann* 4308 (SP); Estação Biológica do Alto da Serra de Paranapiacaba, 27 Nov 1980 (fl), *E.A. Lopes et al.* 92 (SP); Cunha, Parque Estadual da Serra do Mar, trilha do Rio Bonito, 16 Nov 2006 (fl, fr), *E.J. Lucas et al.* 369

(BHCB); Santo André, A. da Serra, Est. Biológica, 19 Oct 1918 (fl), *A. Pedroso 13* (SP); Salesópolis, Estação Biológica de Boracéia, 20 Oct 2001 (fl, fr), *J.R. Pirani et al. 4906* (SP); Distrito de Paranapiacaba, Estrada para a parte alta do distrito, em frente à guarita da Estação Biológica do Instituto de Botânica, 12 Oct 2009 (fl, fr), *I.M.C. Rodrigues et al. 71* (BHCB); Caminho da Bela Vista, Parque Ecológico Municipal das Nascentes de Paranapiacaba, 13 Oct 2009 (fl, fr), *I.M.C. Rodrigues et al. 75* (BHCB); Caminho da Bela Vista, 13 Oct 2009 (fl, fr), *I.M.C. Rodrigues et al. 76* (BHCB); Caminho da Bela Vista, Parque Ecológico Municipal das Nascentes de Paranapiacaba, 13 Oct 2009 (fl), *I.M.C. Rodrigues et al. 77* (BHCB); Caminho da Bela Vista, Parque Ecológico Municipal das Nascentes de Paranapiacaba, 13 Oct 2009 (fl, fr), *I.M.C. Rodrigues et al. 80* (BHCB); Caminho da Bela Vista, Parque Ecológico Municipal das Nascentes de Paranapiacaba, 13 Oct 2009 (fl, fr), *I.M.C. Rodrigues et al. 81* (BHCB); Caminho da Bela Vista, Parque Ecológico Municipal das Nascentes de Paranapiacaba; 13 Oct 2009 (fl, fr), *I.M.C. Rodrigues et al. 82* (BHCB); Caminho da Bela Vista, Parque Ecológico Municipal das Nascentes de Paranapiacaba; 13 Oct 2009 (fl, fr), *I.M.C. Rodrigues et al. 83* (BHCB); Santo André, Distrito de Paranapiacaba, em frente à guarita da Reserva Biológica do Instituto de Botânica de São Paulo, 23 Jan 2014 (fl, fr), *I.M.C. Rodrigues et al. 625* (BHCB); Cananéia, Ilha do Cardoso, Reserva Biológica, Margem do Rio Perequê, 14 Sep 1983 (fl), *S. Romaniuc Neto 97* (SP); Paranapiacaba, 3 Oct 1998 (fl), *L.C.Q.M.P. Sampaio & D. Vedovello 119* (BHCB); Santo André, Alto da Serra, Dec 1917 (fl), *E. Schwebel 132* (SP); Mogi das Cruzes, Sítio do Mauro Peixoto, 24 Oct 2007 (fl, fr), *J.R. Stehmann et al. 4800* (BHCB); Biritiba Mirim, Estação Biológica de Boracéia, 24 Oct 2007 (fl), *J.R. Stehmann et al. 4818* (BHCB); Biritiba Mirim, Estação Biológica de Boracéia, 24 Oct 2007 (fl, fr), *J.R. Stehmann et al. 4819* (BHCB); Biritiba Mirim, Estação Biológica de Boracéia, 24 Oct 2007 (fl, fr), *J.R. Stehmann et al. 4820* (BHCB); Biritiba Mirim, Estação Biológica de Boracéia, 24 Oct 2007 (fl, fr), *J.R. Stehmann et al. 4823* (BHCB); Biritiba Mirim, Estação Biológica de Boracéia, 24 Oct 2007 (fl), *J.R. Stehmann et al. 4824* (BHCB); Biritiba Mirim, Estação Biológica de Boracéia, 24 Oct 2007 (fl), *J.R. Stehmann et al. 4825* (BHCB); Biritiba Mirim, Estação Biológica de Boracéia, Estrada para Salesópolis, 24 Oct 2007 (fl, fr), *J.R. Stehmann et al. 4839* (BHCB); Cunha, Parque Estadual da Serra do Mar, 24 Oct 2007 (fl), *J.R. Stehmann et al. 4846* (BHCB); Cunha, Parque Estadual da Serra do Mar, 24 Oct 2007 (fl), *J.R. Stehmann et al. 4848* (BHCB); Santo André, Estrada Paranapiacaba – Taquarussú, 14 Oct 2009 (fl, fr), *V.A. Thode et al. 251* (BHCB); Santo André, Estrada Paranapiacaba – Taquarussú, 14 Oct 2009 (fl, fr), *V.A. Thode et al. 253* (BHCB); Santo André, Estrada Paranapiacaba – Taquarussú; 14 Oct 2009 (fl, fr), *V.A. Thode et al. 254* (BHCB).

***Athenaea hunzikeriana* I.M.C. Rodrigues & Stehmann, sp. nov.**

urn:lsid:ipni.org:names:77217159-1

Figs 3–5

Diagnosis. Similar to *A. pogogena*, but differing in the campanulate rather than inflated calyx and a subglobose (versus ovoid) fruit that is purplish-red when mature

and pubescent with eglandular trichomes (versus green to yellow fruits with glandular trichomes).

Type. BRAZIL **Minas Gerais:** Santa Maria do Salto, Fazenda Duas Barras, 16°24'45"S, 40°02'51"W, 817 m elev., 1 Nov 2013 (fl, fr), *J.R. Stehmann, L.L. Giacomini, S. Knapp & L. Bohs* 6328 (holotype: BHCB [acc. # 182684; duplicates to be distributed to BM, RB).

Description. Shrubs to small trees, up to 3 m tall; branching virgate and distally dichotomous. Stems rounded in cross-section, dark brown, tomentose with yellowish-brown, simple uniseriate trichomes, up to 2 mm long. Sympodial units difoliate, the leaves geminate, members of a pair markedly unequal. Leaves simple, alternate, elliptic to elliptic lanceolate, subcoriaceous, discolorous; blades of major leaves elliptic lanceolate, 8.7–16.4 cm long, 2.8–5 cm, 2.3–4.5 times longer than wide, the minor leaves elliptic to obelliptic 2.7–6.8 cm long, 1.3–3.2 cm wide, ca. 2.5 times longer than wide; abaxial and adaxial surfaces densely pubescent with 8–15-celled eglandular trichomes 0.5–2 mm long and scarce 1-celled glandular trichomes with usually 3-celled multicellular head; venation camptodromous and prominent abaxially, with 3–6 pairs of principal veins; base decurrent, the leaves appearing sessile or sub-sessile; margins entire and slightly revolute; apex acute to somewhat acuminate; petioles 0–0.8 cm long, densely pubescent with trichomes like those of the leaves. Inflorescences axillary, with 10–15 flowers along a distinct axis; rhachis 1.5–3.4 mm long; pedicels 0.6–2 cm long, erect to spreading, densely pubescent with simple uniseriate trichomes. Flower buds ovoid, white, densely glandular-pubescent. Flowers 5-merous, heteromorphic with long- and short-styled forms. Calyx 3.5–4.5 mm long, green, campanulate, densely pubescent, the tube ca. 2 mm long, the lobes 1.6–2.7 mm long, 0.8–1.4 mm wide, triangular, densely pubescent with 5–8-celled eglandular trichomes and longer 5–10-celled glandular trichomes with multicellular heads abaxially, sparsely pubescent with 4–8-celled glandular trichomes with multicellular heads adaxially, the lobe apices acute. Corolla 4.1–7.3 mm long, stellate, white with green and purple spots adaxially near the base, the tube 1.1–1.6 mm long, the lobes 3.4–5.6 mm long, 1.3–2.7 mm wide, lanceolate, covered with eglandular trichomes (3–4-celled) abaxially at the apex and 4–6-celled glandular trichomes with multicellular heads abaxially over the entire surface, adaxially with 3–5-celled glandular trichomes with unicellular heads. Stamens 5, equal, glabrous; filaments ca. 0.8 mm long, the staminal tube (basal extension of the filaments) ca. 0.6 mm long; anthers 1.4–1.9 mm long, 0.8–1 mm wide, oblong, yellowish-cream, slightly cordate at the base. Ovary ca. 0.6 mm long, subglobose, yellowish-cream, densely pubescent with glandular trichomes, surrounded by a yellowish-green nectary; styles heteromorphic, greenish-white, long styles 2.8–3 mm long, short styles ca. 2.2 mm long; stigma dilated, capitate, yellowish-white. Fruit a subglobose berry, ca. 12.6 mm long, 13.7 mm in diameter, green when immature, wine-red to purplish-red at maturity, the pericarp densely pubescent with simple, uniseriate 4–8-celled eglandular trichomes; fruiting calyx not accrescent, up to 1.8 times longer than in flower; fruiting pedicels 1.5–2 cm long, erect, enlarged at the apex, densely glandular-pubescent. Seeds ca. 6 per berry, 5.5–5.7 mm long, 3.8–4.0 mm wide, flattened-reniform, yellowish-brown, the testa minutely reticulate, foveolate, the embryo curved. Chromosome number: not known.



Figure 4. *Athenaea hunzikeriana* I.M.C.Rodrigues & Stehmann **a** fruit **b** stem showing reduced peduncle **c** branch with buds, flowers and fruits; illustrated from *J.A. Lombardi et al. 5447* (BHCB) **d** flower **e** branch with buds and flowers; illustrated from *J.R. Stehmann et al. 6328* (BHCB). Illustration by I.M.C. Rodrigues. Scale bars 1 cm.

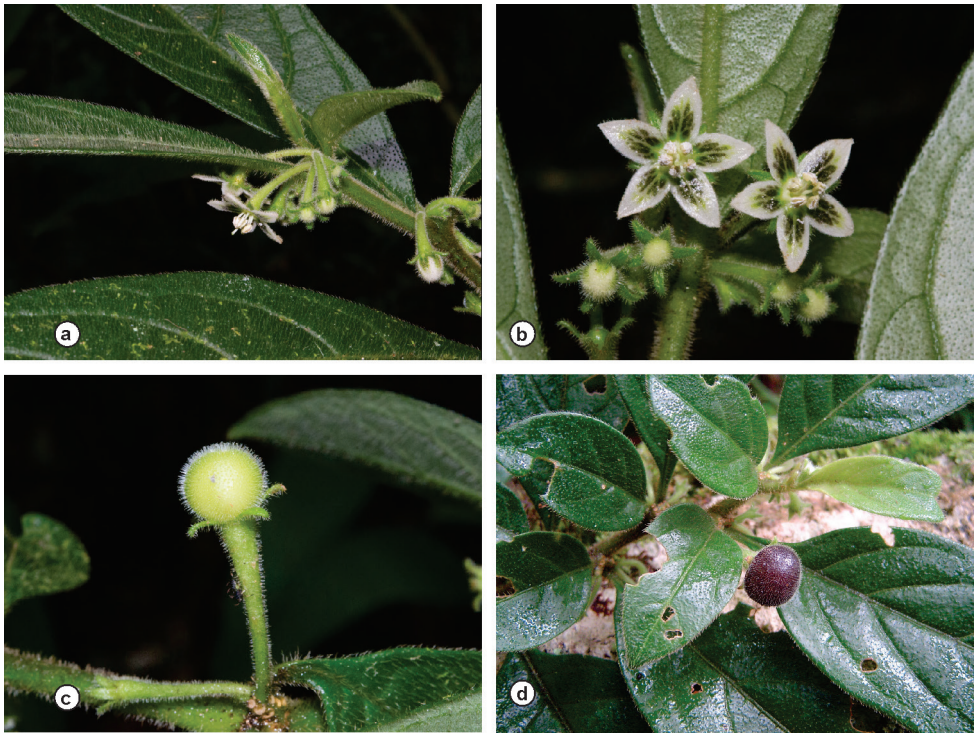


Figure 5. *Athenaea hunzikeriana* I.M.C.Rodrigues & Stehmann in the field **a–c** J.R. Stehmann et al. 6328 **d** J.A. Lombardi et al. 5447 – All photographs by J.R. Stehmann.

Etymology. The specific epithet honours Ing. Armando Teodoro Hunziker (1919–2001), who dedicated his life to the study of the family Solanaceae in the Neotropics and whose mentorship formed a whole generation of Solanaceae workers.

Phenology. *Athenaea hunzikeriana* flowers and fruits from August to November.

Distribution and habitat. *Athenaea hunzikeriana* is endemic to Brazil and only known from the type locality, in north-eastern Minas Gerais State and in adjacent Bahia State. *Athenaea hunzikeriana* grows in the understorey of well-preserved remnants of wet Atlantic forests (Floresta Ombrófila Densa, Mata Atlântica; IBGE 2012), from 700 to 1000 m elevation.

Preliminary conservation assessment (IUCN 2019). Data Deficient (DD). Just two populations of *A. hunzikeriana* are known, one growing in a private reserve (RPPN Fazenda Duas Barras), in the Municipality of Santa Maria do Salto, Minas Gerais and another in Fazenda Farofa in the Municipality of Boa Nova, Bahia. Although the locality in Bahia is well-protected, the extremely restricted range indicates the species is of some conservation concern.

Discussion. *Athenaea hunzikeriana* is easily recognised by its purple, eglandular-pubescent mature fruits on erect or spreading pedicels, its large seeds and the distinctive

inflorescences with a short but persistent axis. Other species of *Athenaea* have axillary fascicles, with no rachis along which pedicel scars can be observed. This species is morphologically similar to and sympatric with *A. pogogena*, with which it shares almost all flower characteristics, but can be distinguished by its subglobose (versus conic or ovoid) berry and leaf pubescence of mostly eglandular trichomes with sparse minute glandular trichomes, rather than densely glandular pubescent leaves. *Athenaea hunzikeriana* is also vegetatively similar to *A. anonacea* Sendtn. and *A. martiana* Sendtn. in having pubescent leaves but is differentiated from both those species by its pubescent fruit and non-acrescent fruiting calyx. It is also sympatric with *A. fasciculata* but differs from that species in its conspicuous pubescence (*A. fasciculata* is glabrous, see discussion of *A. altoserranae* above).

Additional specimens examined. BRAZIL. Bahia: Boa Nova, Faz. Farofa (Dr. Mauro), estrada entre Boa Nova e Dário Meira, 24 Oct 2007 (fr), *F.M. Ferreira 1641* (CEPEC). **Minas Gerais:** Santa Maria do Salto, Fazenda Duas Barras, 23 Aug 2003 (fl, fr), *J.A. Lombardi, J.R. Stehmann, R.C. Mota & L.G. Temponi 5447* (BHCB).

Dichotomous key to the species of *Athenaea*

All of the species in this key occur in Brazil, their distribution can be found in Rodrigues et al. (2019); *A. fasciculata* is the only species that occurs outside of Brazil.

- 1 Leaves glabrous or with eglandular trichomes only.....2
- Leaves with glandular and eglandular trichomes10
- 2 Leaves geminate, anisophyllous, usually sessile, with both surfaces hispid; fruiting calyx accrescent, the tip extending beyond the fruit length.....
.....*Athenaea martiana* Sendtn.
- Leaves solitary or geminate, not anisophyllous, subsessile or petiolate, glabrous to variously pubescent on both surfaces; fruiting calyx not or sometimes only partially accrescent, with the tip reaching up to 1/3 of fruit length.....3
- 3 Stems, leaves, pedicels and calyx glabrous; leaves in clusters at branch tips4
- Stems, leaves, pedicels and calyx variously pubescent; leaves evenly spaced along branches, not clustered at branch tips5
- 4 Leaves solitary, obovate-lanceolate; calyx more than 4 mm long; corolla thick and fleshy, with purple spots; fruits yellowish-green when mature.....
.....*Athenaea sellowiana* (Sendtn.) I.M.C.Rodrigues & Stehmann
- Leaves geminate, rarely solitary, elliptic to lanceolate; calyx less than 3.5 mm long; corolla not fleshy, with brownish spots; fruits dark brown when mature*A. altoserranae* I.M.C.Rodrigues & Stehmann
- 5 Leaves variously pubescent on both surfaces; calyx lobes triangular and of equal size and shape; fruits globose or subglobose6
- Leaves pubescent on the adaxial surface, hirsute on the abaxial surface; calyx lobes of unequal size and variously subulate; fruits ovoid
.....*Athenaea wettsteiniana* (Witasek) I.M.C.Rodrigues & Stehmann

- 6 Leaves adaxially glabrescent, abaxially sparsely pubescent to tomentose; inflorescences with up to 12 flowers; fruiting calyx lobes equally divided 7
- Leaves with both surfaces velvety pubescent; inflorescences generally with (6-) 13 to 26 flowers; fruiting calyx lobes unequally divided
..... *Athenaea velutina* (Sendtn.) D’Arcy
- 7 Stems, abaxial leaf surfaces, pedicels and calyx tomentose; leaves ovate-elliptic, drying black; calyx tube narrowly urceolate in floral buds; calyx (tube+lobes) more than 3.5 mm long at anthesis
..... *Athenaea tomentosa* (Sendtn.) I.M.C.Rodrigues & Stehmann
- Stems, abaxial leaf surfaces, pedicels and calyx glabrescent to sparsely pubescent; leaves elliptic to lanceolate, drying pale green; calyx tube campanulate in floral buds; calyx (tube+lobes) up to 2.5 (-3) mm long at anthesis 8
- 8 Trichomes appressed, with broad bases and curved apices; calyx lobes with acute apices *Athenaea brasiliana* Hunz.
- Trichomes patent, straight or curly; calyx lobes with obtuse to acute apices or with short caudate projections 9
- 9 Leaves narrowly lanceolate (length/width ratio > 4.8); corolla pubescent abaxially; seeds ca. 10 per fruit
..... *Athenaea angustifolia* (Alm.-Lafetá) I.M.C.Rodrigues & Stehmann
- Leaves elliptic, ovate to lanceolate (length/width ratio < 4.5); corolla glabrescent or glabrous abaxially; seeds more than 31 per fruit
..... *Athenaea fasciculata* (Vell.) I.M.C.Rodrigues & Stehmann
- 10 Leaves sessile or subsessile; leaf apices acute; leaf bases decurrent; fruiting calyx not accrescent 11
- Leaves long-petiolate, the petioles up to 9 cm long; leaf apices cuspidate, leaf bases attenuate; fruiting calyx accrescent 12
- 11 Stems dichotomously branching throughout, sometimes trichotomously branching; calyx lobes strongly unequal, with one or two longer than the others; fruits ovoid, glabrous, yellowish-green when mature
..... *Athenaea anonacea* Sendtn.
- Stems dichotomously branching only distally; calyx lobes equal; fruits subglobose, pubescent, vinaceous purple when mature
..... *Athenaea hunzikeriana* I.M.C.Rodrigues & Stehmann
- 12 Calyx lobes with bases auriculate in flower and fruit; pedicels sparsely pubescent *Athenaea cuspidata* Witasek
- Calyx lobes with bases not auriculate or only slightly cordate in fruit; pedicels densely pubescent 13
- 13 Trichomes of unequal size, arranged in two levels over the entire plant surface, translucent white; fruits glabrous; seeds more than 60 per fruit
..... *Athenaea picta* (Mart.) Sendtn.
- Trichomes of equal size, arranged in a single level over the entire plant surface, red-ferruginous; fruits pubescent; seeds up to 50 per fruit
..... *Athenaea pogogena* (Moric.) Sendtn.

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Supplementary material 1

GenBank accession numbers for *Athenaea altoserranae* I.M.C.Rodrigues & Stehmann

Authors: Izabella Martins da Costa Rodrigues, Sandra Knapp, João Renato Stehmann

Data type: Genbank accession numbers

Explanation note: Complete Genbank accession number data for *Athenaea altoserranae*, published as *Aureliana* sp. nov. in Zamberlan et al. (2015).

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Link: <https://doi.org/10.3897/phytokeys.178.64609.suppl1>

Supplementary material 2

Complete specimen details for *Athenaea altoserranae* and *Athenaea hunzikeriana*

Authors: Izabella Martins da Costa Rodrigues, Sandra Knapp, João Renato Stehmann

Data type: specimens data

Explanation note: Table with habitat, loc notes, coordinates, altitude and specimen description notes of all examined specimens (also available on the NHM Data Portal as a dataset, <https://doi.org/10.5519/zc80n093>).

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Link: <https://doi.org/10.3897/phytokeys.178.64609.suppl2>

Hybridization of *Oxalis corniculata* and *O. dillenii* in their non-native range

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Abstract

Many species have been introduced beyond their native ranges and many have become global weeds. Human mediated dispersal has removed the geographic isolation of these species, reversing millions of years of independent evolution. Examples are the *Oxalis* species in section *Corniculatae* where several species have become invasive. Here we characterize and formally describe a hybrid between *O. dillenii* and *O. corniculata*, which occurs spontaneously in Belgium and Japan. *Oxalis corniculata* is probably native to Japan, but both species are alien to Belgium and *O. dillenii* is native to North America. We formally name this hybrid as *Oxalis* × *vanaelstii*. Although this hybrid is sterile, it is nevertheless vigorous and perennial. Both parent species grow as weeds in gardens; therefore, it is likely to be more common than currently appreciated in countries where these species co-occur.

Keywords

DNA content, *Oxalis* × *vanaelstii*, pollen, stomata

Introduction

The human mediated redistribution of plant species, whether by accident or design, facilitates the hybridization of species that were once separated by millennia of independent evolution (Largiadèr 2008). Hybridization is a major force in the evolution of plants and the fingerprint of this breakdown in geographic isolation is likely to be

marked on the future evolution of plants (Vallejo-Marín and Hiscock 2016). Hybrids can occur in a geographic range where one parent is native and the other is alien (e.g. Ayres and Strong 2001), but also between two species brought together in a range where they are both alien (e.g. Vallejo-Marín 2012).

The genus *Oxalis* is one of the weediest genera and many species have become naturalized beyond their native ranges (Pyšek et al. 2017). Therefore, *Oxalis* species from all over the world are being brought together with sibling species where they may have the opportunity to cross pollinate.

Molecular genetic techniques have shown that hybridization has been an important force in the evolution of *Oxalis* (Emshwiller and Doyle 2002; Vaio et al. 2013; Vaio et al. 2016). However, few hybrids from the genus have been formally described and, as Salter (1944) and du Preez et al. (2018) point out, field observations of hybrids in the genus are rare.

Studies have indicated that hybridization can occur between species in the section *Corniculatae* (Mulcahy 1965; Doust et al. 1981). For example, artificial crosses between *O. corniculata* L. and *O. dillenii* Jacq. were reported to be fertile irrespective of the direction of the crossing (Mulcahy 1965). However, only recently have sterile wild hybrids of *O. dillenii* and *O. corniculata* been characterized from Japan (Fukatsu et al. 2018). *Oxalis dillenii* is a North American species, which has naturalized in other parts of the world, including Japan and Europe (Mito and Uesugi 2004; Groom et al. 2017). *Oxalis corniculata* is now a cosmopolitan species, probably native to Japan (Groom et al. 2019).

Fukatsu et al. (2018) characterized hybrid plants from four sites in mixed populations of *O. corniculata* and *O. dillenii*. These plants are sterile and have intermediate characters of their stipules, stem hairs and floral morphology. They also have an intermediate chromosome count and DNA content. In the summer of 2016 a putative hybrid plant was found in a mixed population of *O. dillenii* and *O. corniculata* var. *atropurpurea* Planch. from Belgium. Here we characterize that plant and formally describe the hybrid. Following article H.3.1 of the International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) we propose to formally name this hybrid so that it can be unambiguously referred to (Turland et al. 2018).

Material and methods

Hybrid origin and habitat

For some years, one of the authors (IH) has maintained a small living collection of *Oxalis* species in his home garden in Aalter (Belgium, prov. of East Flanders). Among these plants are *Oxalis corniculata* var. *atropurpurea* and *O. dillenii*, both collected as weeds in plant containers offered for sale in garden centers and nurseries (although the former taxon was already present in the garden as a weed years before additional plants were deliberately introduced for cultivation). The precise origin of the cultivated

plants is not known. Over the years, plants have escaped from the pots in which they are cultivated, as ripe seeds are catapulted away over distances that frequently exceed 1 m. It is among these escapees that, in 2016, putative hybrids were detected (voucher BR0000025668254V, see the taxonomic description). A few more plants of the possible hybrid were also found in 2017 and 2018. They grew next to plants of the two parents and flowered abundantly, yet produced no fruits. They were observed in a neglected part of the garden where selective manual weeding created opportunities for several different *Oxalis* to maintain themselves among competitors such as *Epilobium* sp., *Poa annua* and other common weeds. The hybrid grew on moderately damp, sandy, humus-rich soil. We note that all populations are homostylous.

Additional material examined

Oxalis dillenii

BELGIUM. • Museumstraat 93, Bellem, Aalter; 51°05'44.46"N, 3°29'47.21"E; 8 Aug. 2013; Ivan Hoste 13023 (BR (BR0000013236892)).

Oxalis corniculata var. *atropurpurea*

BELGIUM. • Museumstraat 93, Bellem, Aalter; 51°05'44.46"N, 3°29'47.21"E; 2 Jul. 2019; Ivan Hoste (BR (BR0000025959222V)).

Molecular protocols and sequence analysis

We applied a modified CTAB protocol for total genomic DNA isolation (Tel-Zur et al. 1999). Secondary metabolites were removed by washing ground, silica-dried leaf material with 1000 µL extraction buffer (100 mM Tris-HCl pH 8, 5 mM EDTA pH 8, 0.35 M sorbitol). Samples were incubated at 60 °C (1 h) with a CTAB lysis buffer (incl. 1% PVP-40 and 0.3% 2-mercaptoethanol). Extraction was done twice with SEVAG (chloroform-isoamylalcohol (24/1 v/v)) and was followed by an isopropanol precipitation (0.8 volumes). After centrifugation, the pellet was washed in 70% ethanol, air-dried, and dissolved in 50 µL buffer (10 mM Tris-HCl pH 8, 1 mM EDTA pH 8). Amplification and sequencing of ITS and *trnL-F* was performed using the primers of White et al. (1990) and Taberlet et al. (1991), respectively. PCR reactions of ITS and *trnL-F* were carried out using a touchdown PCR protocol (25 µL). PCR reactions were initiated with a 3-min heating at 95 °C followed by 20 cycles consisting of a denaturation step of 30 s at 95 °C, a 30 s annealing step starting at 58 °C decreasing each cycle with 0.5 °C, and an extension step of 72 °C for 60 s, and ending with 10 cycles (similar in timing as with the first 20 cycles) at an annealing temperature of 48 °C. PCR products were enzymatically purified using the ExoSap protocol and sequenced by the Macrogen sequencing facilities (Macrogen Europe, Amsterdam, Netherlands). Contiguous sequences were assembled using Geneious v7.0.6 (Biomatters, New Zealand). Automatic alignments were carried out with MAFFT (Katoh et al. 2002) under an E-INS-i algorithm, a 100 PAM/k = 2 scoring matrix, a gap open penalty of 1.3 and

an offset value of 0.123. Subsequent manual fine-tuning of the aligned dataset was done in Geneious v7.0.6. Gaps were treated as missing data. All sequences and sample information were uploaded to European Nucleotide Archive and can be found under the project number PRJEB41412.

Flow cytometry

Genome sizes were measured with a Partec PAS III flow cytometer equipped with an 20 mW 488 nm solid state laser. Samples were prepared using the commercial kit Cystain PI absolute P (Partec, Germany). Each of the three plants of interest, *O. corniculata*, *O. dillenii* and the new hybrid were analyzed separately and chopped with a sharp razor blade at room temperature together with an internal standard (*Zea mays*, 2C = 5.43 pg, Lysák and Doležel (1998)). The nuclei suspension was filtered through a 50 µm mesh CellTrics disposable filter (Partec GmbH, Münster, Germany), stained with propidium iodide following the specifications of the kit. At least five replicates per sample were incubated for at least 30 min in the dark before measuring with the flow cytometer on two (*O. corniculata*) to three (*O. dillenii*, hybrid) different days using the FloMax software (Quantum Analysis, Germany). Average genome size was calculated from the relative fluorescence intensities of the sample of interest and the internal standard with known genome size.

Guard cell length

Epidermal leaf impressions were made from the abaxial side of the leaves in the middle of the leaf, between the midvein and edge. Transparent nail polish (Bourjois Crystal ball) was used to make the impressions which, once dried, were mounted pointing upward with double-sided tape (Scotch) on a microscope slide.

Stacked photomicrographs were taken per leaf print (view fields = 0.09 mm²) using a digital microscope (VH-5000 Ver 1.5.1.1, Keyence Corporation) with full coaxial lighting and default factory settings for shutter speed at ×1000 lens magnification (VH-Z250R).

Pollen electron microscopy and viability count

Anthers were collected from mature buds and no distinction was made between the two whorls of anthers. Material for scanning electron microscopy was washed in 70% ethanol for 20 minutes and washed twice with 100% DMM (dimethoxymethane) for 20 minutes while being sonicated each time for a couple of seconds. Then it was washed with 100% acetone. The material was critical point dried using liquid CO₂ with a Leica EMCPD3000 critical point dryer. The dried samples were mounted on aluminum stubs using carbon adhesive tape and coated with a platinum palladium mix with a Cressington JFC-2300/208HR sputter coater. SEM images were obtained with a JEOL JSM7100F field emission scanning electron microscope. Pollen size was measured on

200 grains for each parent and for the hybrid. The diameter of the roughly spherical pollen was measured horizontally on the photograph regardless of orientation of the pollen grain. Pollen viability was measured using the staining protocol of Peterson et al. (2010). Viability was evaluated by counting the proportion of stained versus unstained pollen grains. More than 100 grains were counted per flower for a total of three flowers.

Results

Morphology

Oxalis corniculata var. *atropurpurea* has purple-brown leaves and *O. dillenii* bright green leaves (Figs 1, 2). The hybrid is intermediate with pale purple-brown leaves and sometimes green leaves with a purple underside. Guard cells of *O. corniculata* are about 30% larger than those of *O. dillenii* (Table 1). However, the guard cells of the hybrid are almost exactly the same size as those of *O. dillenii*. The hybrid also has an intermediate growth form. *Oxalis dillenii* is generally erect, though it becomes decumbent with age because the main stem tends to topple over once the crown becomes too heavy. *Oxalis corniculata* var. *atropurpurea* is largely prostrate and roots at the nodes. The hybrid also roots at the nodes, but is not as strongly creeping as *Oxalis corniculata* var. *atropurpurea* (Fig. 1).

The stipules and stem of the putative hybrid are illustrated in Figure 2. *Oxalis corniculata* has short adnate stipules with an angular tip. *Oxalis dillenii* has narrow stipules with a more rounded tip. The hybrid is intermediate, though perhaps closer to *O. corniculata*. The stipule is distinct, longer than wide, but with more of a rounded tip than *O. corniculata*.

Stem and petiole hairs are also visible on Figure 2. In *Oxalis corniculata* var. *atropurpurea* they are patent and sometimes glandular, whereas in *O. dillenii* they are antrorse, eglandular and appressed to the stem or petiole. The hybrid is again intermediate in character with eglandular, antrorsely directed hairs, but they are not as strongly appressed as in *O. dillenii*.

Cellular DNA content

Total DNA content (2C) is 0.88 pg (SD = 0.05, n = 7) for the *O. dillenii* parent, 2.13 pg (SD = 0.03, n = 5) for the *O. corniculata* parent and an intermediate DNA content of 1.50 pg (SD = 0.06, n = 8) for the putative hybrid.

Pollen

Representative pollen grains of the parents and the hybrid are illustrated in Figure 3. All taxa have significantly different pollen diameters ($P < 0.01$) based upon a pairwise comparison using Wilcoxon rank sum test. The diameter of *O. corniculata*

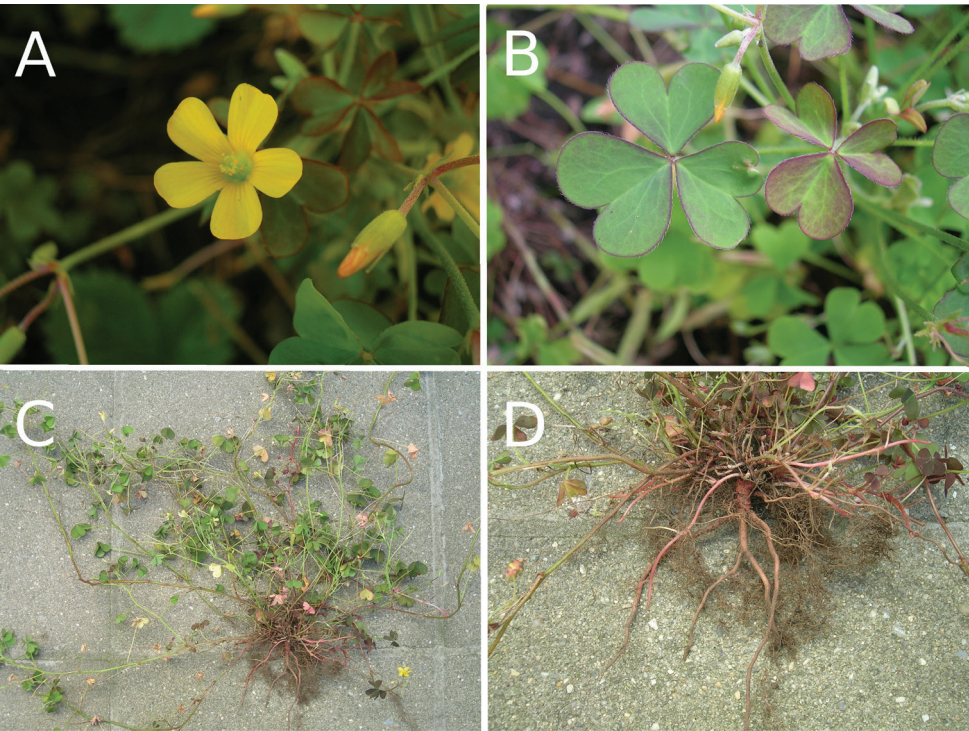


Figure 1. Photographs of the putative hybrid **A** flower and bud **B** leaves showing their intermediate coloration between the green *O. dillenii* and purple *O. corniculata* var. *atropurpurea* **C** whole plant showing its straggly habit **D** root system and stoloniferous shoots.

Table 1. Guard cell length (μm), pollen diameter (μm) and total (2C) DNA content (pg) for *O. corniculata*, *O. × vanaelstii* and *O. dillenii*.

	<i>O. corniculata</i>	<i>O. × vanaelstii</i>	<i>O. dillenii</i>
Guard cell length (μm)	23.9 (2.0, n = 45)	18.0 (1.9, n = 119)	18.4 (2.2, n = 39)
Pollen diameter (μm)	31.0 (3.1, n = 200)	20.2 (6.2, n = 200)	26.5 (2.6, n = 200)
Total DNA content 2C (pg)	2.13 (0.03, n = 5)	1.50 (0.06, n = 8)	0.88 (0.05, n = 7)

pollen is larger (mean 31 μm, SD = 3.1 μm, n = 200) than *O. dillenii* pollen (mean 26 μm, SD = 2.6 μm, n = 200) (Fig. 3). On average, both parents have larger pollen grains than the putative hybrid (mean 20 μm, SD = 6.2 μm, n = 200). However, note the large standard deviation and broad spread of hybrid pollen in Figure 3. This is both because some grains are exceedingly small and because about 4% of hybrid pollen grains exceed 40 μm. In pollen viability staining pollen of the parents are strongly stained and all appear to be viable. Viability staining of the pollen of the putative hybrid is 45.6% (SD = 3.0%, n = 3 flowers). Those pollen of the hybrid that were exceptionally large were seen to be stained strongly whereas small grains remained unstained.



Figure 2. The leaf nodes of (a) *Oxalis corniculata* (b) *O. × vanaelstii* and (c) *O. dillenii* showing the stipules and stem hairs and for the latter two the peduncle.

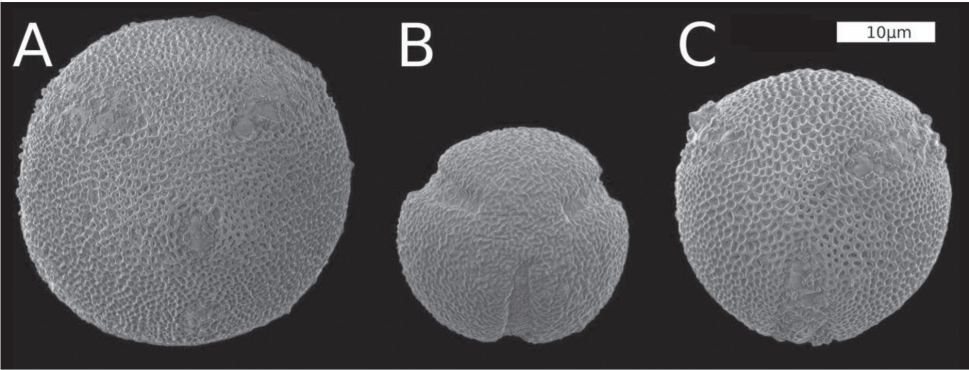


Figure 3. Scanning electron micrographs of pollen adjusted to the same scale **A** *Oxalis corniculata* **B** *O. x vanaelstii* **C** *O. dillenii*.

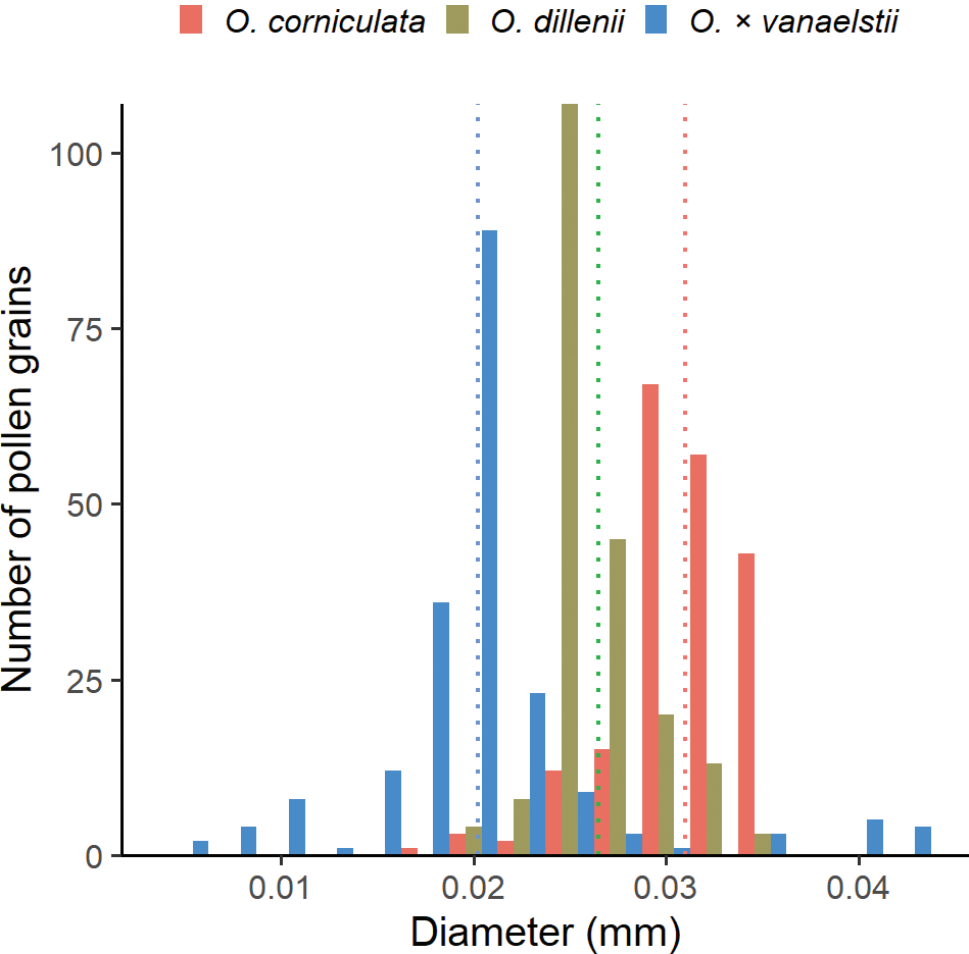


Figure 4. A histogram of pollen size measurements made from scanning micrographs. The dotted line indicates the mean diameter of the pollen grains.

Sequence analysis results

ITS polymorphisms between *O. corniculata* and *O. dillenii* occurred at nucleotide positions 51, 52, 102, 103, 115, 122, 130, 138, 152, 165, 178, 187, 224, 232, 235, 237, 379, 423 (Fig. 5). ITS sequencing of the putative hybrid shows a clear ambiguity at each of the aforementioned polymorphic loci consistent with both parental alleles (Fig. 5). We also note a frameshift at position 423 where there are four cytosine base pairs in *O. dillenii*, versus five cytosine base pairs in *O. corniculata*. This frameshift blurs the sequencing read in the hybrid beyond this point. The chloroplastic marker *trnL-F* has a single polymorphism at position 279 (Suppl. material 1: Fig. S1). At this position, *Oxalis corniculata* has a guanine as character state whereas both *O. dillenii* and the putative hybrid are characterized by a cytosine.

Discussion

Fukatsu et al. (2018) characterized a hybrid between *Oxalis dillenii* and *O. corniculata* var. *corniculata*. It has intermediate morphological characters as does the one we describe here. Their specimens are of intermediate habit and the stipules are winged as are ours. Similarly, they found that the amount of nuclear DNA of *O. corniculata* was about twice that of *O. dillenii* with the hybrid being intermediate. Our result of 2.13 pg is in line with the genome size of between 2.11 and 2.20 pg in the octaploid *O. corniculata* as analysed by Kubešová et al. (2010) and Vaio et al. (2013). For tetraploid *O. dillenii* Kubešová et al. (2010) found a genome size of 1.01 pg, also comparable to the 0.88 pg measured in our study.

Fukatsu et al. (2018) also counted chromosomes numbers and concluded that their parental *O. corniculata* is an octoploid ($2n = 48$), their *O. dillenii* is a tetraploid ($2n = 24$) and the hybrid is a hexaploid. This is also consistent with our hybrid and parental plants. The larger size of the *O. corniculata* pollen is consistent with a larger genome. The small size of the majority of hybrid pollen is consistent with its sterility as is the poor pollen viability. Similarly, the larger guard cell size agrees with a larger genome size for *O. corniculata*. Guard cell size is generally positively correlated with genome size in vascular plants and *Oxalis* (Beaulieu et al. 2008; Jooste 2015).

The DNA sequencing results are consistent with our sterile plant being a hybrid of *O. corniculata* and *O. dillenii*. The nuclear ITS sequence has ambiguity at each of the polymorphic loci between the proposed parent species, which suggests the hybrid genome contains alleles of both parents. The chloroplastic marker, *trnL-F*, differs in only one nucleotide in *O. corniculata* and *O. dillenii*. This single polymorphism indicates that *O. dillenii* is the likely maternal parent. This also accords with the results of Fukatsu et al. (2018) who found that *O. dillenii* was the maternal parent in 85% of Japanese specimens.

Our results agree with, and add to those of, Fukatsu et al. (2018), giving further support to the parentage of these sterile putative hybrids. The *Corniculatae* are a group of similar-looking taxa with few distinctive features. The plants described here are distinctive in their sterility, however, fertile hybrids would be considerably harder to recognize, particularly as they are likely to be more closely related and perhaps more similar morphologically. In North America and Japan, where *O. dillenii* and *O. corniculata* are respectively native, there is likely to be more genetic variation and perhaps greater chance of fertile hybrids being produced. Much more work is needed to understand the extent of hybridization in this group.

Taxonomic description

Oxalis × *vanaelstii* Hoste, Meeus & Groom, sp. nov.

urn:lsid:ipni.org:names:77217160-1

Type material. Holotype. BELGIUM. • Bellem, Aalter; 51.09°N, 3.49°E; 31 Oct. 2016; Ivan Hoste 16054 (holotype: BR (BR0000025668254V); isotype: K, isotype: MO).

Paratypes. BELGIUM. • Cultivated at Meise Botanic Garden; Quentin Groom 19001 (BR (BR0000025668247V)). • Cultivated at Meise Botanic Garden; Ivan Hoste & Quentin Groom S.N. (BR (BR0000025668209V)).

Diagnosis. Intermediate in characters between its parents *O. corniculata* and *O. dillenii*. Prostrate to ascending, mid-sized stipules, stem hairs antrorse, not tightly appressed to the stem. Flowers with at most weak orange marks in the throat, marks sometimes absent.

Description. A short-lived perennial, prostrate to ascending, herb with a thin taproot. Leaves trifoliolate with three similarly sized, heart-shaped leaflets. A narrow stipule is fused to the base of the petiole and is intermediate in width between *O. corniculata* and *O. dillenii*. The stem hair density is moderate with simple, arcuate, antrorse, pointed hairs. Not strongly appressed to the stem as in *O. dillenii*. The leaves are green or purple-brown, though if purple, not as darkly colored as *Oxalis corniculata* var. *atropurpurea*. The flowers are yellow, sometimes with weak orange streaks in the throat. Fruits are unknown.

Etymology. *Oxalis* × *vanaelstii* is named to commemorate the Belgian naturalist, conservationist and mycologist Etienne Vanaelst (1948–2017) who, as a volunteer collaborating with mycologists at Ghent University, contributed to a better understanding of the diversity of mushrooms, especially those growing in and around his hometown, Knesselare (prov. of East Flanders).

Habitat. Gardens.

Distribution area. Europe and Japan where parental distribution overlaps. The hybrid is also highly likely to occur in North America where the parents also co-occur (Eiten 1963).

Acknowledgements

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Supplementary material 1

Figure S1

Authors: Quentin Groom, Sofie Meeus, Steven B. Janssens

Data type: image

Explanation note: Part of the aligned electropherogram of the plastid gene marker trnL-F for the hybrid taxon *O. corniculata* × *dillenii* and its putative parental accessions *Oxalis corniculata* (QG351) and *O. dillenii* (QG320).

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Link: <https://doi.org/10.3897/phytokeys.178.61031.suppl1>

Supplementary material 2

Taxonomic details of *Oxalis* × *vanaelstii* and its parents

Author: Quentin Groom

Data type: COL

Explanation note: A Darwin Core file containing the details of *Oxalis* × *vanaelstii*, *O. dillenii* and *O. corniculata*.

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Link: <https://doi.org/10.3897/phytokeys.178.61031.suppl2>

Centric diatom diversity in the lower part of the Southern Bug river (Ukraine): the transitional zone at Mykolaiv city

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Abstract

The diversity of centric diatoms is documented for the transitional zone of the lower part of the Southern Bug River (Ukraine) just before entering the Dnipro-Bug Estuary and compared to earlier results from the upstream sampling sites of the same river system. Benthic samples of the following sites were investigated: north of Mykolaiv City (approximately 5 km), in Mykolaiv City (near Varvarivskyi Bridge), and 5 km south of the city. Twenty-four centric diatom taxa belonging to 11 genera were identified, analysed, and documented by scanning electron microscopy (SEM) and light microscopy (LM). Among them, *Aulacoseira nivalis* is the first report for Ukraine, *A. islandica* and is the first confirmed record for the studied area since the 1930s. The maximum number of centric diatom taxa found in one station was 21, the minimum 10. *Melosira subglobosa* was the most common (documented in 57–80% of sites with centric diatoms) and abundant species 7.3–15.7% in relative abundance to all diatom taxa. The discovered diversity of taxa and its comparison with previous results is discussed with regard to the relevance of estuary zones in the research of diatoms.

Keywords

Centric diatoms, diversity, Dnipro-Bug Estuary, ecology, phytobenthos, Southern Bug River, transitional zone

Introduction

Transitional waters are the continuum between freshwaters and coastal marine waters which according to the EU Water Framework Directive (The Directive 2000/60/EC) are defined as “bodies of surface water in the vicinity of river mouths which are partially saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows”. All over the world, such waters attract scientific attention owing to the tremendous biodiversity that they sustain. The salinity is a major, if not the most important natural factor structuring the algal communities and explaining their variability within these ecosystems (Cebrián and Valiela 1999; Bode et al. 2005; Muylaert et al. 2006, 2009; Gameiro and Brotas 2010; Garmendia et al. 2011; Hartnett et al. 2011; Seoane et al. 2011). Furthermore, transitional waters have intrinsically higher productivity in comparison to open oceanic waters (Basset et al. 2013; Facca 2020). This may be attributed to the fact that such zones are deemed to be naturally stressed systems as they work as basins for runoff from their catchments and impact of saline waters from the sea (Zaldivar et al. 2008).

With shifts in the ecological ranges of organisms apparent in response to changes in freshwater flow, the ecological model of a transitional zone was studied in the Southern Bug River. A continuum of assemblages exists along the salinity gradient from the freshwater part of the River to the Dnipro-Bug estuary and within the estuary and the Black Sea. The Dnipro-Bug estuary consists of two parts: the wide Dnipro estuary (55 km long, up to 17 km wide), and the narrower Bug estuary (47 km long, from 5 to 11 km wide) with average depths of 6–7 metres and a maximum of 12 metres (Marynych 1993). The salinity gradient in the Bug estuary itself varies in a wide range of 0.3–9.5 g/dm³, and the mean salinity equals 3.6‰ (Mykolaiv regional state administration 2019). These waters move upstream to Mykolaiv City forming a buffer zone, which may be defined as having two overlapping gradients formed by major saline stressors: freshwater species from the river and marine species from the estuary. Therefore, this area could be termed a transitional zone because it represents a transition community consisting of freshwater and marine species being at the edge of their ecological range.

In addition, the studied sites could also be impacted by severe stress from anthropogenic pressures. Precisely for this reason transitional waters are considered to be among the most impacted and ‘at risk’ ecosystems. Considering that, it is difficult to exclude these impacts on the species diversity of spatial and ecological gradual boundaries between these systems; nevertheless, salinity is considered as a prevailing stressor (van der Maarel 1990; Attrill and Rundle 2002).

The concept of this paper was inspired by the lack of knowledge in algal ecological variability, fluctuations and changes within transition zones, as well as by the importance of improving our understanding on the variability of different spatio-temporal scales and biological interactions (Smayda 1998). Of immense interest are the diatoms (Chromista, Bacillariophyta), which demonstrate a wide array of morphological, physiological, and behavioural traits and are a major component of

marine and freshwater ecosystems (Kociolek et al. 2015). Many centric species are known from marine waters, but the group is present, with a considerably lower diversity, in freshwater habitats as well (Harwood and Nikolaev 1995). Based on available evidence, it appears that the majority of centric diatom genera occurring in freshwaters are ultimately derived from multiple immigration events from the marine realm (e.g., Alverson et al. 2007, 2011). Additionally, the transition zones greatly constitute ecosystems for rare and potentially neophytic centric diatom species. Hence, the investigation of diversity in this group of algae concerning salinity is of high interest and pertinence.

The centric diatom flora near the Mykolaiv region is not particularly well explored, however, some studies have been done (Genkal and Bilous 2015; Bilous et al. 2019). Our previous investigations revealed that these sites at the Southern Bug River have a higher diversity of algae in comparison to the sites over the entire water body explored in former studies (Bilous et al. 2012; Bilous et al. 2014; Belous 2016). Thus, the highlighted facts served as an incentive for continuation of the work and a more detailed analysis in order to better understand the development of different biotic components in response to changed salinity conditions. The aim of this work was the refined evaluation of the centric diatom species composition and its diversity in the transitional zone of the Southern Bug River. The results in this manuscript as well as the follow-up paper with a detailed pennate diatom species list could set the baseline and serve as a contribution to the discussions of high biodiversity in transitional zones.

Materials and methods

Benthic samples were collected in autumn 2017. The analysis of the species was divided into two parts: centric diatoms for this study and pennate diatoms for a follow-up study. The investigation was carried out at three sites assumed to have saline and freshwater impact on the lower portion of the Southern Bug River bed (Fig. 1): north of the Mykolaiv City (approximately 5 km) – 47°03'05"N, 31°52'35"E, in Mykolaiv City (near Varvarivskyi Bridge) – 46°59'07"N, 31°57'40"E, and 5 km south of the city – 46°48'59"N, 31°57'02"E. For the full picture of the studied area, our previous results (list of species at the Mykolaiv city site) from the sampling research in 2013, but with a different focus, were also considered.

Samples were taken by scraping diatoms from stones with a brush at a depth of 10–30 cm, homogenized and fixed with 70% ethanol. For LM and SEM investigations samples were cleaned from organic matter by standard procedures involving treatment with concentrated hydrogen peroxide and washing with deionized water. We focussed on benthic samples since they contain all diatoms, which occurred in the river at some time either as true benthos, as settled plankton or in between. Benthic samples treated by standard methods provide the best comparison with current and historic diatom studies.

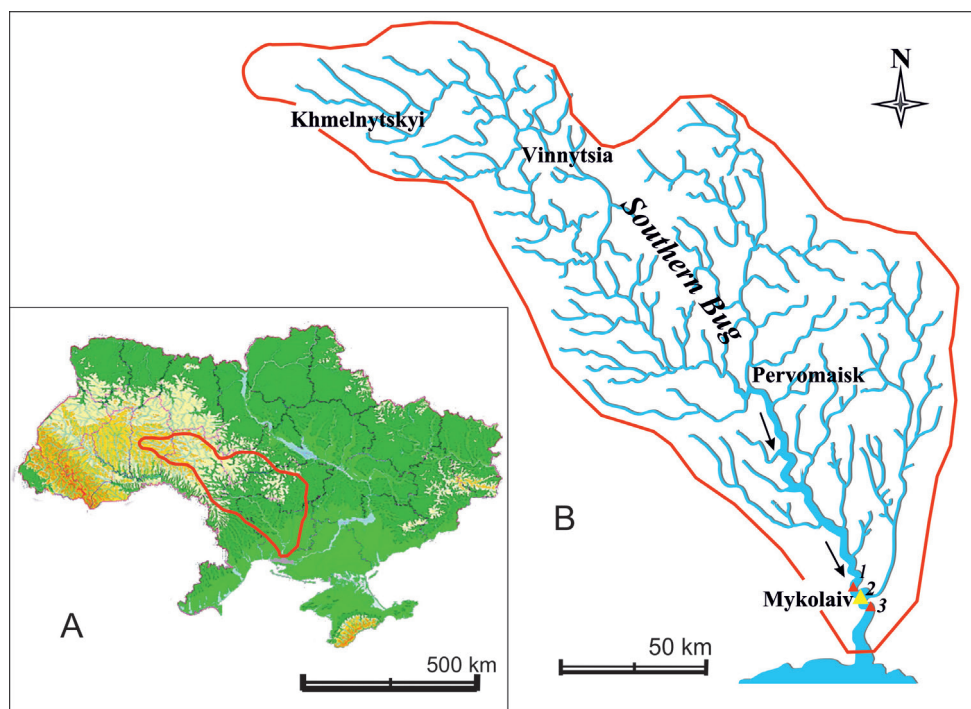


Figure 1. **A** Outline of Ukraine with the catchment area of the Southern Bug River in red **B** schematic drawing of the Southern Bug River with the sampling stations of the research in autumn 2017; the sampling site 2 – Mykolaiv City – was sampled also in the year 2013.

Permanent diatom preparations were done by drying the cleaned samples on cover slips and mounting them with Naphrax (Kelly et al. 1998; European Committee for Standardization 2003, 2004; CEN 2014b). Light microscopic (LM) observations were performed by means of Axio.Imager2 (Carl Zeiss, Germany) equipped with oil immersion objective ($\times 1000$, DIC). Valve ultrastructure was examined on cleaned unsputtered material with a field scanning electron microscope FE-SEM (Hitachi SU8010) with 1.0 kV and 7.8–8.2 mm distance. LM slides and SEM stubs were studied at and are stored in the Algae Herbarium (B) Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin.

Diatom identification was based on the books by Lange-Bertalot et al. (2017), Houk et al. (2010, 2014, 2017), Krammer and Lange-Bertalot (2000), Witkowski et al. (2000), and some selected works by Tanimura et al. (2004) and Aké-Castillo et al. (2012). Nine slides were investigated, at least 400 valves on each slide were counted following European Standard EN 14407 (CEN 2004b). The measurements of the taxa were made for at least 5 valves; most of the taxa were measured for 10–30 valves. In this paper, the focus is set on the centric diatoms, which include all diatoms from the modern systematic groups *Coscinodiscophyceae* and *Mediophyceae* (Medlin and Kaczmarska 2004).

Results

The species diversity of centric diatoms from the three sites of the Southern Bug River bed (see Fig. 1) accounted for 24 taxa constituted by the following 11 genera: *Aulacoseira* Thwaites, *Actinocyclus* Ehrenb., *Conticribra* Stachura-Suchoples & D.M. Williams, *Cyclotellaphanos* Round, *Cyclotella* (Kütz.) Bréb., *Melosira* C. Agardh, *Minidiscus* Hasle, *Pleurosira* (Menegh.) Trevisan, *Skeletonema* Greville, *Stephanodiscus* Ehrenb., *Thalassiosira* Cleve.

The following 15 species were found in our previous investigation for this region of the river in 2013: *Aulacoseira subarctica* (O. Müll.) E.Y. Haworth, *Conticribra weissflogii* (Grunow) Stachura-Suchoples & D.M. Williams, *Cyclotellaphanos dubius* (Hust.) Round, *Cyclotellaphanos invisitatus* (M.H. Hohn & Hellerman) E.C. Theriot, Stoermer & Håk., *Cyclotella atomus* Hust., *Cyclotella choctawhatcheeana* A.K.S. Prasad, *Cyclotella meduanae* H. Germ., *Cyclotella meneghiniana* Kütz., *Melosira subglobosa* (Grunow) Houk, Klee & Tanaka, *Melosira varians* C. Agardh, *Skeletonema subsalsum* (Cleve-Euler) Bethge, *Stephanodiscus hantzschii* Grunow, *Stephanodiscus minutulus* (Kütz.) Cleve & J.D. Möller, *Thalassiosira incerta* I.V. Makarova and *Thalassiosira faurii* (Gasse) Hasle (Genkal and Bilous 2015). The survey in 2017 revealed 9 more centric diatoms for the investigated stations as specified in detail below (Table 1).

Table 1. Species composition of centric diatoms at the investigated sites near Mykolaiv city in the Southern Bug River in the year 2017 (2013 added for station 2).

List of species	Stations			
	1: Upstream Mykolaiv	2: Mykolaiv (2017)	2: Mykolaiv (2013)	3: Downstream Mykolaiv
<i>Actinocyclus normanii</i>	+	+		+
<i>Aulacoseira islandica</i>	+	+		+
<i>Aulacoseira nivalis</i>	+			+
<i>Aulacoseira subarctica</i>		+	+	
<i>Conticribra weissflogii</i>		+	+	
<i>Cyclotellaphanos dubius</i>	+	+	+	
<i>Cyclotellaphanos invisitatus</i>		+	+	+
<i>Cyclotella atomus</i> var. <i>atomus</i>	+	+	+	+
<i>Cyclotella atomus</i> var. <i>gracilis</i>	+	+		+
<i>Cyclotella choctawhatcheeana</i>		+	+	+
<i>Cyclotella cryptica</i>				+
<i>Cyclotella marina</i>	+	+		+
<i>Cyclotella meduanae</i>		+	+	+
<i>Cyclotella meneghiniana</i>	+	+	+	+
<i>Melosira subglobosa</i>		+	+	
<i>Melosira varians</i>			+	
<i>Minidiscus proschkinae</i>		+		+
<i>Pleurosira laevis</i>		+		+
<i>Skeletonema subsalsum</i>		+	+	
<i>Stephanodiscus hantzschii</i>	+	+	+	+
<i>Stephanodiscus makarovae</i>				+
<i>Stephanodiscus minutulus</i>		+	+	+
<i>Thalassiosira faurii</i>		+	+	
<i>Thalassiosira incerta</i>	+	+	+	
Taxa (number)	10	21	15	16

* in the publication of sampling research from 2013 (Genkal and Bilous 2015), this species was identified as *Melosira nummuloides* C. Agardh, however, Houk et al. (2017) provided evidence that the correct species name should be *Melosira subglobosa*.

However, the absence of *Melosira varians* in comparison to the previous investigation has to be noted. In summary, for the explored transitional zone the taxa belonged to the following systematic groups: class *Coscinodiscophyceae* (6 taxa) and class *Mediophyceae* (18 taxa), as orders these are *Melosirales* (2 taxa), *Aulacoseirales* (3 taxa), *Coscinodiscales* (1 taxon), *Triceratiales* (1 taxon) and *Thalassiosirales* (17 taxa), and as families *Melosiraceae* (2 taxa), *Aulacoseiraceae* (3 taxa), *Triceratiaceae* (1 taxon), *Hemidiscaceae* (1 taxon), *Thalassiosiraceae* (4 taxa), *Skeletonemataceae* (1 taxa) and *Stephanodiscaceae* (12 taxa).

With regard to the relative abundancies of the above counted taxa, *Melosira subglobosa* was the most abundant centric diatom with 7.3% – 15.7% relative abundance of all diatoms found in the benthic samples in 2017. Within centric diatoms only, its relative abundance varied from 57.1% to 80.4% and had its maximum values at the station 2 (80.4%) and 3 (80.3%). In contrast, *Actinocyclus normanii* had a relative abundance of 18.2% in station 3 (7.1% for station 2) and *Cyclotella atomus* var. *atomus* prevailed in station 1 with 14.3%. The other centric taxa made a much lower contribution to the diatom composition having less than 8% abundancies of all centric diatoms at each station.

List of taxa with comments on their nomenclature, taxonomy, ecology and worldwide distribution

Class Coscinodiscophyceae

Order Melosirales Glezer in Glezer, Moiss. and I.V. Makarova 1990

Family Melosiraceae Kütz. emend. Round, R.M. Crawford & Mann, 1990

Genus *Melosira* C. Agardh, 1824

***Melosira subglobosa* (Grunow) Houk, Klee & Tanaka in Fottea 17 (Supplement):17, pl. 17, figs 1–10; Pl. 19, figs 1–15; pl. 19, figs 1–11. 2017.**

Synonyms. *Melosira borreri* var. *subglobosa* Grunow, *Melosira moniliformis* var. *subglobosa* (Grunow) Hustedt.

Morphological description. Frustule shape is cylindrical to approximately octagonal (Fig. 2A, F). Valve 12.5–26 µm in diameter, mantle height 3.1–8.5 µm. Valve face nearly octagonal with flat tops (Fig. 2B–E, G). Girdle with puncta forming straight and transverse rows 32–56 in 10 µm.

Ecology. Marine and brackish, benthic-planktonic (tychoplanktonic) species, halophilic, widely distributed. Indicates significant organic pollution (eutrophication), α -mesosaprobiont (Kolpacov et al. 2014). This species prefers water enriched with dissolved organic matter, and is capable to switch from autotrophic to heterotrophic or mixed type of nutrition (Andreeva et al. 2008). Additionally, these diatoms are ubiquitous, and widely distributed in seas and estuaries of temperate zones in habitats with a moderate level with increased level of human impact (Kuzminova and Rudneva 2005; Ryabushko 2009).

Distribution. *Melosira subglobosa* is one of the most common species at the sampling sites near the Mykolaiv area in the Southern Bug River (Table 1). For Ukrainian territory, it is known from the coastal zone in the northwestern part of the Black Sea, Crimean seaboard, and some estuaries.

For a worldwide distribution, it was recorded from coastal zones of Europe, and Asia; specifically, from Lake Aral, Baltic, Bering, Black, Caspian, Mediterranean, North and Norway Seas (Tsarenko et al. 2009).

Melosira varians C. Agardh, Syst. Alg.: 64. 1816.

Synonym. *Aulacoseira varians* (C. Agardh) Simonsen.

Morphological description. Frustule is cylindrical, valve flat, 15.7–46.6 µm in diameter with numerous small-scale granules, 5.7–15.5 µm high (Fig. 2H).

Ecology. Common species for freshwaters in streams and lakes, as well as in slightly brackish waters, oligotrophic, eutrophic to dystrophic or polluted environments (Nardelli et al. 2016; Hofmann et al. 2018). Taxon has preferences of alkaline conditions (pH 7–8.5), with moderate oxygen, regularly found in humid environments, requiring periodically high levels of nitrogen (Soltanpour-Gargari et al. 2011).

Distribution. Valves were found near Mykolaiv city in the Southern Bug River in our previous sampling study (Table 1; Genkal and Bilous 2015). In Ukraine, it is known from the Southern Bug and lakes in the basins of the rivers Danube, Dnister, Siverskyi Donets, Desna, Prypiat, Dnipro and its reservoirs and estuary.

On the global level, it is a widely distributed taxon, known from Europe (i.e. Berlin, Germany, see Geissler and Kies 2003), Russia (Genkal et al. 2020), Asia (Iran), North America (Canada, USA), Africa (Egypt, RSA); Adriatic, Aral, Azov, Baltic, Barents, Black, Caspian, Kara, Mediterranean, North and Red Seas, and throughout North America (Stoermer and Julius 2003). It is also very common in Brazilian waters (Stoermer and Julius 2003; Tremarin et al. 2009; Nardelli et al. 2016).

Order Aulacoseirales V.A. Nikolajev ex Moiss. & I.V. Makarova, 1990

Family Aulacoseiraceae Moiss., 1990

Genus *Aulacoseira* Thwaites, 1848

Aulacoseira islandica (O. Müll.) Simonsen, Bacillaria 2: 60, pl. 1, figs 1–10. 1979.

Basionym. *Melosira islandica* O. Müll., J. Wiss. Bot. 43 (1): 56, pl. 1, figs 3–6. 1906.

Synonyms. *Melosira islandica* subsp. *helvetica* O. Müll., *M. islandica* subsp. *vaenensis* A. Cleve.

Morphological description. Frustule cylindrical, valve face flat with randomly located areolae, diameter is 13.2–14.4 µm (Fig. 3A). Curve of the valve with longitudinal

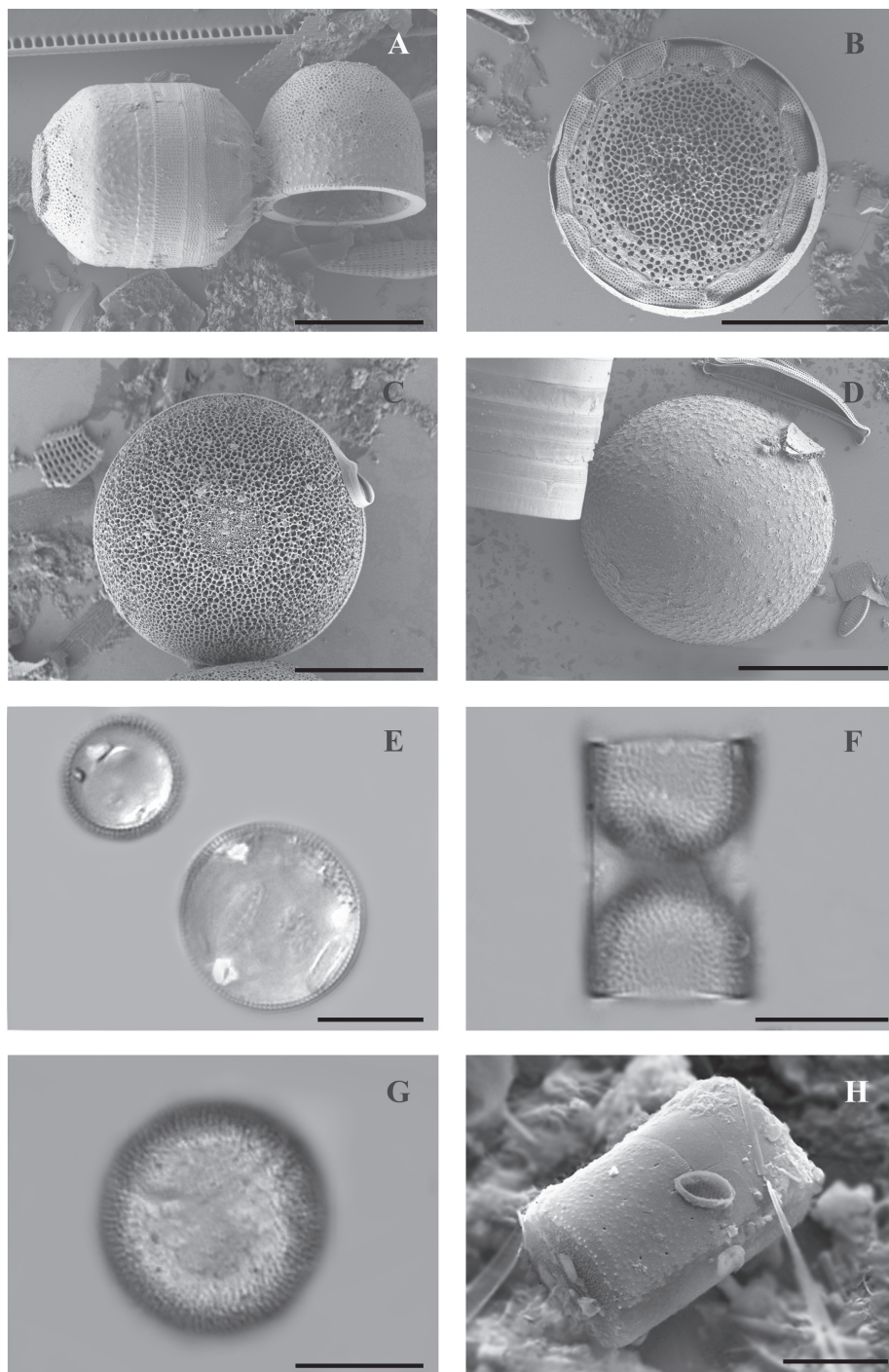


Figure 2. SEM (A–D, H) and LM (E–G) images of species found in the transitional zone at Mykolaiv city: **A–G** *Melosira subglobosa* (A, F girdle views, B–E, G, valve views) **H** *Melosira varians*, partial valve view and girdle view of complete frustule. Scale bars: 10 µm (A–C, E–H); 20 µm (D).

areolae (12–14 in 10 μm) and transverse curly rows of areolae. The ringleiste is wide, connective spines are small-sized, sharp-ended, tear-drop-shaped or branched.

Ecology. *Aulacoseira islandica* is most often occurring as planktonic or tycho-planktonic taxon in water bodies of different types in high latitude or high altitude oligotrophic to mesotrophic large waters (Houk et al. 2010; Stoermer and Julius 2013; Genkal et al. 2020). Sometimes this species may even cause a spring bloom (Stoermer and Julius 2013).

Distribution. This species was observed in all sampling sites and is the first confirmed record for the whole basin of the Southern Bug River (Table 1). In the mid-1930s Swirenko (1941) found this species as *Melosira islandica* var. *helvetica* O. Müller in the lower part of the River starting from Mygea rapids and some other lower stations along the River bed to the mouth (Swirenko 1941). *Aulacoseira islandica* occurs in the Dnipro River basin and its reservoirs (Tsarenko et al. 2009).

It is also a common species for Europe (Bulgaria, Iran, Finland, Germany, Lithuania, Romania, Russia, Sweden, Ukraine), Asia (Georgia, Russia, Turkmenistan), North America (Canada, Greenland, USA); Barents, Bering and White Seas (Tsarenko et al. 2009).

***Aulacoseira nivalis* (W. Sm.) J. English & Potapova, Proc. Acad. Nat. Sci. Philadelphia 158 (1): 39. 2009.**

Basionym. *Melosira nivalis* W. Sm. Syn. Brit. Diat. 2, p. 58, pl. LIII, fig. 336. 1856.

Synonyms. *Melosira distans* var. *nivalis* (W. Sm.) O. Kirchner, *Aulacoseira distans* var. *nivalis* (W. Sm.) E.Y. Haworth.

Morphological description. Cells cylindrical, valve face flat, 7.5–12.8 μm in diameter, valve mantle 3.8–4.7 μm high. Valve face with rough areolae, located in intersecting diagonal rows (Fig. 3B).

Ecology. This is a freshwater species, found growing in or near snow and in alpine pools (Houk et al. 2007; English and Potapova 2009) but was also reported for alkaline and acid waters (Kulikovskiy et al. 2016).

Distribution. At the localities of upstream Mykolaiv, in Mykolaiv City and downstream Mykolaiv *Aulacoseira nivalis* is the first record for Ukraine (Table 1).

As for other countries, it is known from alpine and northern regions (Krammer and Lange-Bertalot 2000); frequently occurring in western North America (English and Potapova 2009) and as being abundant in lake sediments in Brazil.

***Aulacoseira subarctica* (O. Müll.) Haworth in Round. Alg. aquat. environm.: 143–144. 1988.**

Basionym. *Melosira italica* subsp. *subarctica* O. Müll., Jahrb. Wiss. Bot. 43: 78, pl. 2, figs 10–11. 1906.

Synonyms. *A. italica* subsp. *subarctica* (O. Müll.) Simonsen.

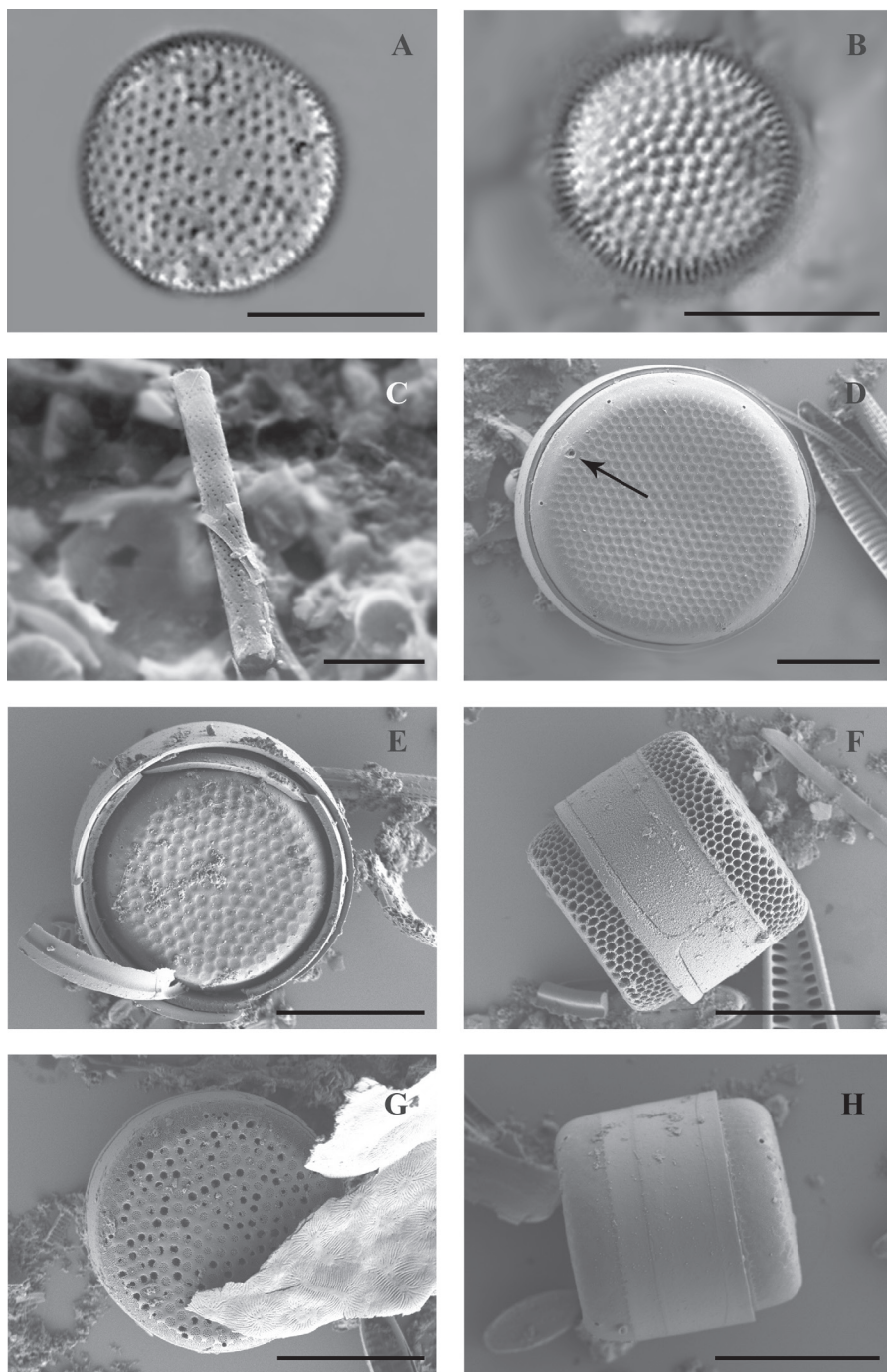


Figure 3. LM (A,B) and SEM (C–H) images of species found in the transitional zone at Mykolaiv city: **A** *Aulacoseira islandica*, valve view **B** *Aulacoseira nivalis*, valve view **C** *Aulacoseira subarctica*, girdle view of complete frustule **D–H** *Actinocyclus normanii* valve view (**D** pseudonodulus shown with arrow, **E**, **G**) and girdle view (**H**, **F**). Scale bars: 10 µm.

Morphological description. Frustules are usually low to high-cylindrical, valve face flat. Valve is 16.6 μm in diameter, 4.4 μm high, number of areolae lines on valve bend 15 in 10 μm , in the line 18 areolae in 10 μm . Ringleiste is wide, linking spines are large, sharpened (Fig. 3C).

Ecology. *Aulacoseira subarctica* is a planktonic alga in lakes, rivers, reservoirs and temporary water bodies and is confined to higher latitudes. It usually appears in response to moderate increases in nutrients, supposedly to phosphorus concentrations controlling its presence and is disadvantaged by further enrichment (Chris et al. 2003).

Distribution. Occurred in the Southern Bug River in Mykolaiv city (near Varvarivskyi Bridge) (Table 1). This species is known from the Dnipro River basin (Tsarenko et al. 2009).

Aulacoseira subarctica is widely distributed across Europe (Germany, Great Britain, Holland, Norway, Russia, Ukraine, Scandinavia), Asia (Russia, Japan, China), North America, Australia and New Zealand. The species is rare in the tropics, and positive identifications are lacking for Africa (Chris et al. 2003).

Order Coscinodiscales Round & R.M. Crawford in Round, Crawford and D.G. Mann 1990

Family Hemidiscaceae Hendey ex G.R. Hasle, 1996

Genus *Actinocyclus* Ehrenb., 1837

Actinocyclus normanii (W. Greg.) Hust. in Abh. Naturwiss. Vereine Bremen 34 (3): 218, pl. 1, figs 5, 6. 1957.

Basionym. *Coscinodiscus normanii* W. Greg. in Grev., Quart. J. Microsc. Sci. 7: 80, pl. 6, fig. 3. 1859.

Synonyms. *Coscinodiscus curvatulus* Grunow, *C. fasciculatus* A.W.F. Schmidt, *C. normannicus* W. Greg., *A. normanii* f. *subsalsus* (Juhlin-Dannfelt) Hustedt

Morphological description. The frustule is drum-shaped, the valve is flat or slightly concave or convex (Fig. 3D–H). Valve diameter 13.6–26.2 μm , height 3.4–5.2 μm . Areolae are arranged into sectors. The external openings of the process (4–6) are clearly visible on the curved outer surface the mantle (Fig. 3F, H). The pseudonodulus is located above the openings of the rimoportulae, it has a slight depression.

According to Hasle (1977), there are no significant taxonomic differences between *A. normanii* f. *subsalsus* and the nominate form (the ranges of valve diameters coincide), but there may be ecological preferences. Krammer and Lange-Bertalot (2000) did not identify forms, but gave so-called morphotypes that do not have a rank in nomenclature and, according to their data, in the *A. normanii* population from the Rhine region, a continuous range of forms was observed during the life cycle. Kozyrenko et al. (2008) synonymized *A. normanii* f. *subsalsus* with the nominate form and we adhere to their point of view.

Ecology. Cosmopolitan, planktonic and phytobenthic, alkalibiontic and halophytic species, occurring in brackish inland waters influenced by anthropogenic nutrients

and salts, waters with moderate to high conductivity (222–918 $\mu\text{S}/\text{cm}$), pH ranges from 7.8–8.6, at a water temperature between 8.0–25.7 °C and may serve as indicator of nutrient-rich habitats and polluted waters (Christie 2014; Vidaković et al. 2016).

Distribution. *Actinocyclus normanii* is found sporadically in epilithic benthic samples from the the Southern Bug River at the three investigated stations (Table 1). It has previously been recorded for Ukrainian water bodies, especially for the Steppe zone noted in the monograph (Tsarenko et al. 2009) and for the Dnipro-Bug Estuary in particular (Vladimirova 1971; Zhukinskiy et al. 1989). It may travel upstream with highly mineralized waters from the estuary and appear near Mykolaiv City.

Upstream occurrences have been documented for *Actinocyclus normanii* f. *subsalsus* for Germany. According to diatom core analyses, this taxon reached the River Havel around 1900 (Schönfelder 1997). It was missing in Berlin (which is situated more than 200 km inland from the North and Baltic Seas) in the 1830s–1850s (Jahn and Kusber, unpubl. data from the Ehrenberg collection at BHUPM) but occurred in recent samples at the beginning of the 20th century (Kolbe 1925; Geissler and Kies 2003) where it became an established part of the flora (Geissler and Kies 2003; Geissler et al. 2006). It was discussed by Schönfelder (1997) that a prerequisite for naturalisation might be the anthropogenically induced increase of salinity over the minimum value of salt tolerance. In other inland waters, e.g in the Czech Republic, the taxon occurred but did not establish (Fránková-Kozáková et al. 2007).

Additionally, it is a widely distributed species occurring in Europe, North and South America, the islands of the Atlantic Ocean, Africa, Asia, Australia, and New Zealand (Guiry and Guiry 2021). However, the species was considered invasive for Russia (see Kaštovský 2010; Korneva 2014) but was not included in the Handbook of alien species in Europe, outcome of the DAISIE (Delivering Alien Invasive Species Inventories for Europe) project (Handbook of alien species in Europe DAISIE 2009).

Class Mediophyceae

Order Triceratales Round & R.M. Crawford in Round, Crawford and Mann 1990

Family Triceratiaceae (F. Schütt) Lemmerm., 1899

Genus *Pleurosira* (Meneghini) Trevisan, 1848

***Pleurosira laevis* (Ehrenb.) Compère in Bacillaria 5: 177, figs 1–17, 20, 39. 1982.**

Basionym. *Biddulphia laevis* Ehrenb. in Ber. Bekanntm. Verh. Königl. Preuss. Akad. Wiss. Berlin: 122. 1843.

Synonyms. *Cerataulus laevis* Ralfs in A. Pritch., *C. polymorpha* Van Heurck, *Odontella polymorpha* Kütz.

Morphological description. The frustule is cylindrical, valves are elliptical-round-oval, with diameter 39–61 μm , 15–17 areolae in 10 μm (Fig. 4A–D).

Ecology. This taxon occurs in brackish and fresh water habitats, commonly found in estuaries of large rivers, also surviving in inland waters with high conductivity, this is a halophilic species. It has considerable abundance in epiphytic, benthic hard waters

with relatively high electrical conductivity. Distributed and more abundant in warm-temperate and tropical waters, being mesohalobic, from alkalibiontic to alkaliphilic indicator, β -mesosaprobic, and eutraphentic (El-Awamri 2008).

Distribution. Valves were found in benthic samples in the Mykolaiv city of the Southern Bug River and downriver (Table 1). For Ukrainian territory it was recorded for estuaries of the Black Sea, also reported for the Southern Bug River (Tsarenko et al. 2009).

Pleurosira laevis is quite cosmopolitan, distributed in the Boreal, near coasts of Europe (Czech Republic, Finland, Germany, Romania, Russia, Sweden, Ukraine), Asia (Korea, Turkey), South America (Brazil), Africa (Egypt), Hawaiian Islands; Azov, Black and Marmora Seas (Tsarenko et al. 2009; Park et al. 2017b). For Europe this taxon is considered an invasive species (Handbook of alien species in Europe DAISIE 2009). An upstream colonisation of *Pleurosira laevis* with help of different vectors for the River Labe was discussed by Fránková-Kozáková et al. (2007).

Order Thalassiosirales Glezer & I.V. Makarova, 1986

Family Thalassiosiraceae M. Lebour, 1930

Genus *Conticribra* Stachura-Suchoples & D.M. Williams, 2009

***Conticribra weissflogii* (Grunow) Stachura-Suchoples & D.M. Williams, Eur. J. Phycol. 44: 482. 2009.**

Basionym. *Micropodiscus weissflogii* Grunow in Van Heurck., 1885.

Synonyms. *Eupodiscus weissflogii* Grunow, nom. inval., *Eupodiscus weissflogii* (Grunow) De Toni, *Thalassiosira weissflogii* (Grunow) G.A. Fryxell & Hasle

Morphological description. The frustule has the form of a drum, valves are almost flat, diameter 24.4–26.6 μm , 8–10 marginal processes in 10 μm , 2–5 central processes (Fig. 4E).

Ecology. *Conticribra weissflogii* is a planktonic diatom, from marine and brackish-water environments that also may occur in lacustric and riverine waters. It is reported to occur in a wide range of salinity 2–26‰ (representing oligohalobes to polyhalobes), especially at salinities above 5‰ (Stachura-Suchoples and Kulikovskiy 2014). This taxon tends to increase in population density with rising temperature (Lomas and Glibert 1999) as well as with eutrophication (Zheng et al. 2016). It is also known to grow in waters with relatively high pH, around 8–9.4 (Sala 1997).

Distribution. This centric taxon appeared at the Mykolaiv site in the Southern Bug River; for Ukraine it was mentioned for the first time in our previous investigation (Table 1; Genkal and Bilous 2015), afterwards it was found in the tributaries of Dnipro in eastern and central parts of the country (Berezovskaya 2019; Kryvosheia and Kapustin 2019).

This is a widely distributed species: Europe, Asia, America (North and South), Africa, Australia and New Zealand; it was even found in Lake Baikal, also in the oceans over the world (Stachura-Suchoples and Kulikovskiy 2014; Genkal et al. 2020).

Order Thalassiosirales Glezer & I.V. Makarova, 1986**Family Thalassiosiraceae M. Lebour, 1930****Genus *Thalassiosira* Cleve, 1873**

***Thalassiosira incerta* I.V. Makarova in Bot. mater. otd. spor. rast. Bot. inst. AN SSSR 14: 50, pl. 1, figs 9–16. 1961.**

Synonym. *Coscinodiscus bulla* M.H. Hohn & Hellerman.

Morphological description. The frustule is cylindrical, valves approximately flat, 21.4–27.8 µm in diameter, in the middle of a valve 5 processes are located, 4–5 marginal fuloportulae in 10 µm, situated on the valve margin (Fig. 4F). The rimoportula is short-necked with an elongated, compressed narrow lip usually perpendicular to the margin.

Ecology. The species was recorded as planktonic in water bodies of different types, typical for eutrophic/hypertrophic and highly saprobic marine, brackish, and fresh waters. It is euryhaline and eurythermal, in addition to being known as an alkaliphilic taxon (Okhapkin et al. 2016). *Thalassiosira incerta* was named an invasive taxon for Russia (Kaštovský 2010; Korneva 2014).

Distribution. *Thalassiosira incerta* was recorded near Mykolaiv city in the Southern Bug River (Table 1). For Ukrainian territory, there are few records from reservoirs of the Dnipro River and some estuaries of the Black Sea and coastal waters near Crimea (Tsarenko et al. 2009).

This taxon is quite cosmopolitan, and distributed in the Boreal of Europe (Great Britain, Russia, Ukraine), Asia (Azerbaijan), North America (Canada, USA), Africa (Egypt); Aral, Black and Caspian Seas (Sims 1996; Kuo and Guo 2003; Okhapkin et al. 2016; Genkal et al. 2020).

***Thalassiosira faurii* (Gasse) Hasle in Phycologia 17(3): 282, figs 61–65. 1978.**

Basionym. *Coscinodiscus faurii* Gasse, 1975. PhD dis. Univ. Paris VI, Vol. II: 24, pl. 32, figs 1, 2. 1975.

Morphological description. The frustule is low-cylindrical, valves are flat, 21.4–25.5 µm in diameter, central processes located on the valve ingroups, 6 marginal fuloportulae in 10 µm (Fig. 4G).

Ecology. Planktonic and benthic in freshwater reservoirs, lakes, rivers, and fossil. Lack of information for this taxon must be noted, but it is known that this species is very sensitive to salinity (Roubeix et al. 2014).

Distribution. *Thalassiosira faurii* (Gasse) Hasle occurred near Mykolaiv city in the Southern Bug River (Table 1). This taxon was registered for the Dnipro River and its reservoirs, mouth of the Danube in the Black Sea (Maystrova et al. 2007; Roubeix et al. 2014).

Worldwide distribution shows this species to occur in some European countries (Hungary, Russia), Asia (Russia), Africa (Ethiopia, Tanzania, Kenia, Kongo) (Tsarenko et al. 2009; Roubeix et al. 2014; Genkal et al. 2020).

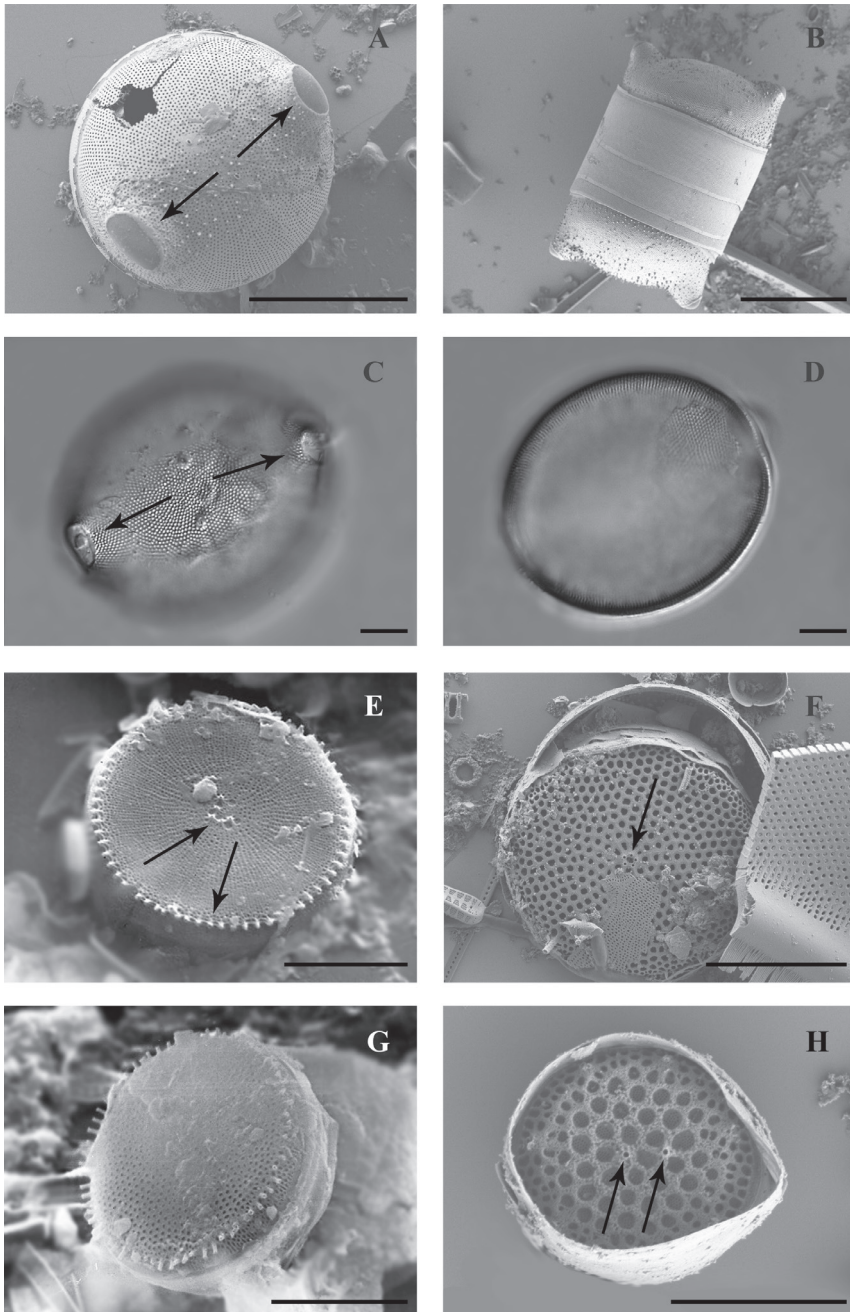


Figure 4. SEM (**A, B** and **E–H**) and LM (**C, D**) images of species found in the transitional zone at Mykolaiv city: **A–D** *Pleurosira laevis* **A, C** valve views with arrows shown the ocelli **B** girdle view **E** *Conticribra weissflogii*, valve view with arrows shown central and marginal fultoportulae **F** *Thalassiosira incerta*, valve view with arrow shown central fultoportulae **G** *Thalassiosira faurii*, valve view **H** *Minidiscus proschkinae*, valve view with arrow in the centre shown central fultoportula, and closer to the edge – rimoportula. Scale bars: 10 μm (**C–G**); 30 μm (**A**); 50 μm (**B**); 3 μm (**H**).

Order Thalassiosirales Glezer & I.V. Makarova, 1986**Family Thalassiosiraceae M. Lebour, 1930****Genus *Minidiscus* Hasle, 1973**

Minidiscus proschkinae (I.V. Makarova) J.S. Park & J.H. Lee in J.S. Park et al., PLoS ONE 2(9): 18. 2018.

Basionym. *Thalassiosira proschkinae* I.V. Makarova in Makarova, Genkal and Kuzmin, Bot. Zhurn. 64(7): 922, pl. 1, figs 1–7. 1979.

Morphological description. The frustule is cylindrical, valve flat, diameter 3.8–4.9 μm , areolae polygonal, in quantities 25 in 10 μm , near centre of a valve, the central process and rimoportula are located (Figs 4H, 5A).

Ecology. This planktonic taxon has been mainly found in estuaries characterized by low salinity and high turbulence, and in seas, but also in freshwaters and may be a halophile indicator (Makarova 1988; Park et al. 2017a; Barinova et al. 2019).

Distribution. Valves were found in benthic samples in Mykolaiv city of the Southern Bug River and downriver (Table 1). For Ukrainian territory it was recorded for estuaries of the Black Sea and nearshore regions (Tsarenko et al. 2009; Genkal and Terenko 2014).

Minidiscus proschkinae is widely distributed across estuaries and seacoasts of Europe (Germany, Great Britain, Netherlands, Russia, Ukraine), Asia (Azerbaijan, China), Argentina; Azov Sea, Baltic Sea, Caspian Sea (Park et al. 2017a).

Order Thalassiosirales Glezer & I.V. Makarova, 1986**Family Skeletonemataceae M. Lebour, 1930****Genus *Skeletonema* Grev., 1865**

Skeletonema subsalsum (A. Cleve) Bethge in Ber. Deutsch. Bot. Ges. 46(5): 343, pl. 2, figs 1–11. 1928.

Basionym. *Melosira subsalsa* A. Cleve in. Arch. Hydrobiol. 7: 509, fig. 1. 1912.

Morphological description. The frustule is cylindrical, valves are flat or slightly convex, diameter 7.8–12.7 μm (Fig. 5B). Frustules are connected with marginal flat-spoon fuloportulae providing a very close connection between valve mantles (Fig. 5C, D).

Ecology. Planktonic taxon, preferring low salinities, usually occurring in salinities up to 15‰, recorded mainly for the brackish waters, however, is known from rivers, lakes, inland seas, coastal waters, and marshes, and often associated with eutraphentic conditions, temperate taxon, alkaliphilic (Hasle and Evensen 1975; Sarno et al. 2005; Hofmann et al. 2018). In addition, in the DAISIE database *Skeletonema subsalsum* is considered to be an invasive species for Russia's and Ireland's water bodies (Kaštovský 2010; Korneva 2014) but is not listed in the *Handbook of alien species in Europe* (2009).

Distribution. This taxon was found at the site of Mykolaiv city in the Southern Bug River (Table 1). *Skeletonema subsalsum* is widely distributed in Ukrainian waters,

known from the Dnipro River in its freshwater reservoirs, in estuaries connected to the Black Sea and from coastal waters of different river basins as well as Crimea coast.

It is a cosmopolitan species, known from the Boreal, Europe (Finland, Germany, Ireland, Italy, the Netherlands, Romania, Russia, Sweden, Ukraine), North America (Canada, USA); Baltic, Black and Caspian Seas. (Gibson et al. 1993; Krammer and Lange-Bertalot 2000; Tsarenko et al. 2009; Genkal et al. 2020).

Order Thalassiosirales Glezer & I.V. Makarova, 1986

Family Stephanodiscaceae I.V. Makarova in Glezer and Makarova 1986

Genus *Stephanodiscus* Ehrenberg, 1845

***Stephanodiscus hantzschii* Grunow in Cleve and Grunow in Bih. Kongl. Sven. Vet. Akad. handl. 17(2): 115, pl. 7, fig. 131. 1880.**

Synonyms. *Stephanodiscus hantzschianus* Grunow, *S. hantzschii* var. *delicatula* A. Cleve, *S. hantzschii* var. *zachariasii* (Brun) Fricke, *S. zachariasii* Brun.

Concept synonym. *Cyclotella operculata* sensu Hantzsch in Rabenhorst, Fl. Alg. Eur.: N 1104. 1861.

Morphological description. The frustule is low-cylindrical, valves flat 13.6–21.4 µm in diameter, striae multiseriate with 6–7 in 10 µm, central processes are absent, spines large and pointy, growing from each rib (Fig. 5E).

Ecology. Planktonic in lakes and rivers, indifferent, alkaliphilic, α-mesosaprobic, eutraphentic serving as an indicator of eutrophication in rivers, reservoirs, lakes worldwide mostly because of phosphorus loads (Håkansson and Stoermer 1984; Van Dam et al. 1994; Burge and Edlund 2016; Hofmann et al. 2018).

Distribution. *Stephanodiscus hantzschii* was identified for the Southern Bug River in Mykolaiv city and downstream (Table 1). It is widespread taxon in Ukrainian water bodies: the rivers Danube, Dniester, Southern Bug, Siverskyi Donets, Dnipro and its reservoirs, coastal waters.

It is a cosmopolitan species, known from Europe (Belarus, Bulgaria, Finland, France, Germany, Moldova, Norway, Romania, Russia, Ukraine), Asia (Armenia, China, Georgia, Japan, Korea, Mongolia, Russia, Tadjikistan, Uzbekistan), North America (Canada, USA); Aral, Azov, Baltic, Black and Caspian Seas (Krammer and Lange-Bertalot 2000; Tsarenko et al. 2009; Genkal et al. 2020).

***Stephanodiscus makarovae* Genkal in Nov. Syst. Nizsh. Rast. 15: 13, pl. 2, fig. 1. 1978.**

Morphological description. Frustule disciform, valve with slightly convex or concave centre, frequently flat, diameter 5.9–8.3 µm, striae are double, rarely triple, in numbers of 14–16 in 10 µm. One central process is present. Spines pointy, small-scale, growing from each costa (Fig. 5F).

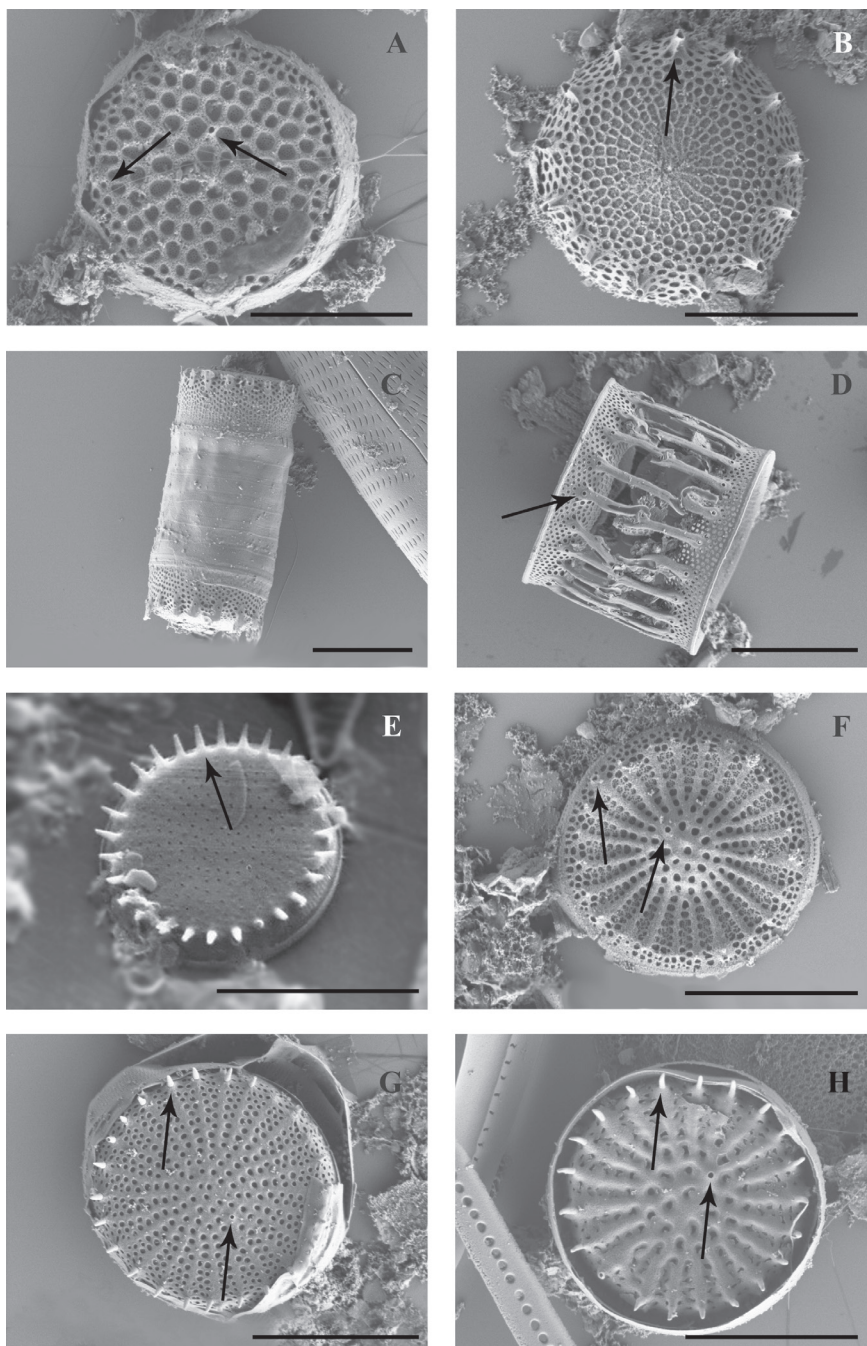


Figure 5. SEM images of species found in the transitional zone at Mykolaiv city (**A, B, E–H** valve views, **C, D** girdle views): **A** *Minidiscus proschkinae* with arrows showing rimoportula and marginal fultoportula **B–D** *Skeletonema subsalsum* with arrows showing marginal fultoportulae **E** *Stephanodiscus hantzschii* with arrow shown spines **F** *Stephanodiscus makarovae* with arrows showing marginal and central fultoportulae **G, H** *Stephanodiscus minutulus* with arrows showing spines and central fultoportula. Scale bars: 3 μm (**A**); 4 μm (**B, F, H**); 5 μm (**C, D, G**); 10 μm (**E**).

Ecology. Planktonic in rivers, lakes and reservoirs, freshwater, but mainly in mesotrophic-eutrophic water bodies. In addition, reported occurrence of this species in high numbers in highly mineralized waters (Genkal 2007; Genkal et al. 2009; Tsarenko et al. 2009).

Distribution. *Stephanodiscus makarovae* was observed downstream from Mykolaiv city in the Southern Bug River (Table 1). It was observed in the Dnipro River and its reservoirs, the Danube River and in the coastal area of the Black Sea (Genkal et al. 2009; Tsarenko et al. 2009; Genkal and Terenko 2014).

As for general distribution, this taxon has only a few records around the world – Europe (Russia, Ukraine), Asia (Armenia, Russia), Africa (Egypt) (Kulikovskiy et al. 2016; Genkal et al. 2020).

Comments. According to Houk et al. (2014), *S. makarovae* (Genkal 2007) was erroneously included in the synonym *C. delicatus* (Genkal) Casper & Scheffler, since, according to the diagnosis, the marginal fulcra of *S. makarove* have 2 satellite pores, and for *C. delicatus* 3. The difference in the number of satellite pores at the marginal fulcra in centric diatoms is a good diagnostic feature. For *S. makarovae*, the valve relief also varies from flat to slightly convex or concave, and there are also other morphological differences (see same publication Genkal 2007). As for the transfer of *S. makarovae* to the genus *Cyclotellus*, this is a debatable issue and molecular genetic studies are needed.

***Stephanodiscus minutulus* (Kütz.) Cleve & J.D. Möller, Collect. Diat.: 300. 1882.**

Basionym. *Cyclotella minutula* Kütz. Kieselschal. Bacill.: 50, pl. 2, fig. 3. 1844.

Synonyms. *S. astraea* var. *minutula* (Kütz.) Grunow, *S. minutulus* (Kütz.) Round, *S. parvus* Stoermer & Håk., *S. perforatus* Genkal & Kuzmin, *S. rotula* var. *minutulus* (Kütz.) R. Ross & P.A. Sims.

Morphological description. The frustule is disciform, valves flat or with slightly convex or concave centre, 8.8–9.1 µm in diameter, striae double to triple, numbering 10 in 10 µm (Fig. 5G, H). A central process is present. The spines are short, growing from each rib (Figs 5G, H, 6A).

Ecology. It is a planktonic taxon, described as eutraphentic (Hofmann et al. 2018). *Stephanodiscus minutulus* reaches greatest abundance in productive nearshore regions, in the mouths of large rivers and coastal embayments. This taxon is an indicator of increased TP concentrations, alkaliphilic, mesosaprobic indicator (Stoermer and Yang 1969; Bradbury et al. 2002; Reavie and Kireta 2015).

Distribution. *Stephanodiscus minutulus* occurred at the Mykolaiv city and downstream the Southern Bug river sites (Table 1). This taxon is distributed over the entire territory of Ukraine, common for such rivers as the Dnipro with its reservoirs and tributaries, the rivers Dnister, Danube, Siverskyi Donets, estuaries of main rivers (Tsarenko et al. 2009).

Concerning worldwide distribution, *Stephanodiscus minutulus* is a widespread taxon, recorded for Europe (i.e. Estonia, Finland, France, Germany, Great Britain, Hungary, Ireland, Moldova, Norway, Russia, Ukraine), Asia (Armenia, Georgia, Iran,

Israel, Russia, Japan), North America (Canada, Greenland, USA) (Tsarenko et al. 2009; Kiss et al. 2012; Kulikovskiy et al. 2013; Genkal et al. 2020).

Comments. Houk et al. (2014) considered *Stephanodiscus minutulus* to be different from *Stephanodiscus parvus* (and Stoermer and Håkansson 1984) and noted the main difference between *S. minutulus* and *S. parvus* the convex-concave valve relief in contrast to flat valves, respectively. However, many authors have shown that in *S. minutulus* the valve relief varies from convex-concave to flat, and therefore *S. parvus* was treated as a synonym (see Genkal 2010), and we adhere to this point of view.

Order Thalassiosirales Glezer & I.V. Makarova, 1986

Family Stephanodiscaceae I.V. Makarova in Glezer and Makarova 1986

Genus *Cyclotella* (Kütz.) Bréb., 1838

Cyclotella atomus Hust. var. *atomus* in Arch. Hydrobiol. 15: 143, pl. 9, figs 1–4. 1938.

Morphological description. Frustule low-cylindrical, central part of the valve is slightly tangentially undulated, 3.6–5.6 µm in diameter, clear boundary between regional and central zones absent, 10–15 striae in 10 µm, and a central process (Fig. 6B, C).

Ecology. Euplanktonic species, that may exist in marine, brackish or nearshore areas and freshwaters, indicating eutraphentic, α -mesosaprobous conditions and often associated with polluted, warm nutrient-rich water, however particularly tolerating high total phosphorus loads (Denys 1991; Van Dam et al. 1994; Yang et al. 2005; Lowe 2015), halophilic, alkaliphilic, tolerates higher ion concentrations and frequent osmotic stress as well as high temperature conditions and turbulence (Krammer and Lange-Bertalot 2000).

Distribution. Valves were found at all investigated sites of the Southern Bug during this research (Table 1). For Ukrainian territory, it has been reported for the Dnipro River (Maystrova et al. 2007).

In general, *Cyclotella atomus* is a cosmopolitan species (Krammer and Lange-Bertalot 2000), widespread in freshwater and marine environments in North America, Europe, and Asia, and has also been recorded from Argentina and South Africa (Pouličková 1993; Medioli and Brooks 2003; Tanimura et al. 2004; Yang et al. 2005; Wojtal and Kwadrans 2006; Genkal et al. 2020).

Cyclotella atomus var. *gracilis* Genkal & K.T. Kiss in Hydrobiologia 269: 43, figs 10–16. 1993.

Morphological description. The frustule is low-cylindrical, central part of valve is slightly tangentially undulated, valves 4.6–7 µm in diameter, and a clear boundary between regional and central zones is present, 15–20 wedge-shaped striae in 10 µm, with central process (Fig. 6D, E).

Ecology. Planktonic in rivers, lakes, freshwater, eutraphentic (Hofmann et al. 2018). It is regarded as an euryhaline species (Kiss et al. 2012).

Distribution. *Cyclotella atomus* var. *gracilis* is here first reported for the studied area, and was found at all investigated sites during this study (Table 1). In turn, its existence was reported in Dnipro waters (Maystrova et al. 2007), as well as for the Danube River (Genkal and Ivanov 1990).

This species is cosmopolitan, i.e. it was recorded for European waters (Kiss et al. 2012; Genkal et al. 2020).

***Cyclotella choctawhatcheeana* A.K.S. Prasad., 1990; emend. Genkal. Biol. vnutr. vod. 2: 1–10. 2012.**

Synonym. *Cyclotella hakanssoniae* Wendker, Nova Hedwigia 52: 360. 1991.

Morphological description. Frustule low-cylindrical, central part of valve tangentially undulated, valves 9.1–12.3 μm in diameter, 12–14 striae in 10 μm , 1–4 central fultoportulae, 6 marginal fultoportulae in 10 μm (Fig. 6G).

Ecology. *Cyclotella choctawhatcheeana* is a small centric diatom from the plankton of water bodies tolerating a wide temperature range. Originally this species was described as a marine species in the northern Gulf Coast of Florida; it is also recorded from several localities in Florida Bay and its global distribution is discussed (Prasad et al. 1990). For Germany, it was described from the River Schlei close to the Baltic Sea (Wendker 1991). Nowadays, it may be classified as an invasive species in brackish waters (Kiss et al. 2012). This species may grow in different seasons and with high and low nutrient availability (Oliva et al. 2008). In turn, some authors note that the existence of this species has a positive linear relationship with nutrient concentration (Jaanus et al. 2009).

Distribution. It was recorded for the first time in Ukraine in our previous investigation near Mykolaiv city of the Southern Bug River and this study confirms its existence in Mykovaiv city and at the downriver sites (Table 1).

Cyclotella choctawhatcheeana was recorded as a cosmopolitan species. Its presence has been confirmed in different localities around the world in brackish waters and rivers connected with saline lakes (Prasad et al. 1990). It was found as a component of the phytoplankton in the saline Mexican lake Alchichica (Oliva et al. 2008), in the Baltic Sea, with salinity between 3 and 11‰ (Wendker 1991; Håkansson et al. 1993), and the Salton Sea, with a salinity in excess of 40‰ (Lange and Tiffany 2002). Additionally, it is known from saline lakes in North America and Africa (Carvalho et al. 1995), reservoirs in Russia (Genkal et al. 2020).

***Cyclotella cryptica* Reimann, J.C. Lewin & Guillard in Phycologia 3: 82, figs 4–11. 1963.**

Morphological description. Frustule cylindrical, the medium part of a valve slightly tangentially undulated, or flat, valve diameter is 6.4–6.7 μm , a clear boundary between edge and central zone is absent, striae wedge-shaped, 8 in 10 μm , single central fultoportula (Figs 6H, 7A).

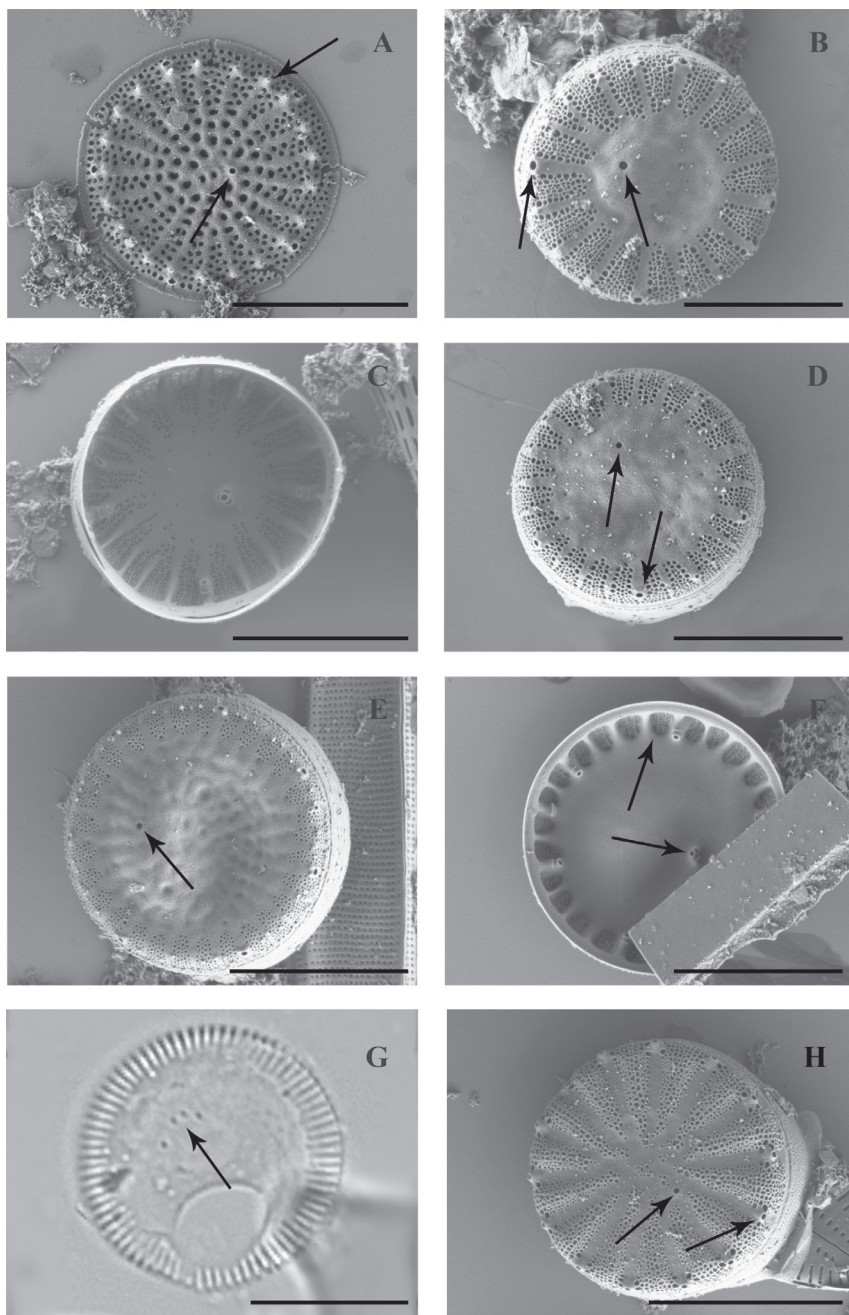


Figure 6. SEM (A–F, H) and LM images (G) of species found in the transitional zone at Mykolaiv city, valve views: **A** *Stephanodiscus minutulus* with arrows shown marginal and central fuloportulae **B, C** *Cyclotella atomus* var. *atomus* with arrows shown marginal and central fuloportulae **D–F** *Cyclotella atomus* var. *gracilis* with arrows shown marginal and central fuloportulae on **D** picture, only central fuloportula on **E** and central fuloportula and alveolae on **(F)** **G** *Cyclotella choctawhatcheeana* with arrow shown central fuloportulae **H** *Cyclotella cryptica* with arrows shown marginal and central fuloportulae. Scale bars: 3 μ m (**B, C**); 4 μ m (**D, F**); 5 μ m (**A, E, H**); 10 μ m (**G**).

Ecology. *Cyclotella cryptica* is a planktonic species, known from marine and brackish environments, may be found in high chloride concentrations. It occurs at maximum abundance around 20 °C (Liu and Hellebust 1976; Makarewicz 1987; Mills et al. 1993). *Cyclotella cryptica* is a saprophilic species (Barinova et al. 2019), requires NO₃ as its source of nitrogen and Ni ions in order to grow autotrophically, however is capable of heterotrophic growth in bottom water or mud enriched in glucose and known to grow mesotrophically (Oliveira and Antia 1984; Saros and Fritz 2000).

Distribution. Valves were identified at the site in the south of Mykolaiv city in the Southern Bug River (Table 1).

It is a widespread species, recorded for Europe, North America, and Asia (Mills et al. 1993; Guiry and Guiry 2021).

***Cyclotella marina* (Tanimura, Nagumo & M. Kato) Aké-Castillo, Okolodk. & Ector, in Nova Hedwigia, Beih. 141: 267, figs 2–9. 2012.**

Basionym. *Cyclotella atomus* var. *marina* Tanimura, Nagumo & Kato in, Bull. Natn. Sci. Mus., Tokyo, Ser. C. 30: 6–7, figs 3–15. 2004.

Morphological description. Frustule low-cylindrical, valve diameter is 3.2–3.9 µm, a clear boundary between edge and central zone is absent, striae wedge-shaped, 18–20 in 10 µm, central process is absent (Fig. 7B).

Ecology. According to literature data, *C. marina* has a high ecological relevance, with a preference in brackish waters, also inhabiting marine environments even with salinity ranges around 30‰; smaller numbers were recorded for freshwaters under 10‰ (Tanimura et al. 2004; Chung et al. 2010; Aké-Castillo et al. 2012). There are known cases where *C. marina* exists in shallow waters with freshwater discharges. At the same time, the appearance of this taxon is connected to high nutrient concentrations. In addition, cases of blooming of this species are known in the south-eastern Gulf of Mexico (Aké-Castillo et al. 2012).

Distribution. *C. marina* is found in epilithic benthic samples at all investigated sites during this research for the Southern Bug River (Table 1). For Ukrainian territory it is also reported from Khmelnytsky NPS, (Genkal et al. 2012) and in phytoplankton in Odessa Bay of the Black Sea (Genkal and Terenko 2014).

It is also a common species for Europe, North America, and Asia (Aké-Castillo et al. 2012; Genkal et al. 2012; Guiry and Guiry 2021).

***Cyclotella meduanae* H. Germ., Fl. Diat. Mass. Armor. 36, pl. 8, fig. 28, pl. 154, figs 4, 4 a. 1981.**

Morphological description. Frustule low-cylindrical, central part of valve is slightly tangentially undulated or flat, valves 5.8–9.4 µm in diameter, striae wedge-shaped, 6–9 in 10 µm, central process is absent (Fig. 7C, D).

Ecology. *Cyclotella meduanae* was recorded ecologically as a planktonic taxon from different types of water bodies (lakes, reservoirs, rivers), freshwater and brackish, of different halobity, eutrapphentic (Kiss et al. 2012; Genkal 2014; Hofmann et al. 2018).

Distribution. This taxon is sporadically found in epilithic benthic samples from the Southern Bug River at two investigated stations (in Mykolaiv City and downstream Mykolaiv) (Table 1). For other Ukrainian waters it has been reported for the Dnipro River (Maystrova et al. 2007), the Danube River (Genkal and Ivanov 1990).

Globally, this taxon is distributed in the Boreal zone, Europe, Asia and North America (Genkal 2014; Genkal et al. 2020).

Cyclotella meneghiniana Kütz., Kieselchal. Bacill.: 50, pl. 30, fig. 68. 1844.

Synonyms. *C. kuetzingiana* Thwaites, *C. rectangula* Bréb. ex Rabenh.

Morphological description. Frustule cylindrical, valves with a tangentially undulated central part, 10.4–33.3 µm in diameter, striae wedge-shaped 5–8 in 10 µm. Central processes (usually from 1 to 9) and spines are present at the mantle of the valve (Fig. 7E, F).

Ecology. *Cyclotella meneghiniana* was recorded as tycho planktonic, in coastal and estuarine locations with water of varied chemistry (Trigueros and Orive 2000). Its optimal development occurred at temperatures in the range of 20.1–20.6 °C (Stoermer and Ladewski 1976) but it was eurythermal (Gasse 1986). This is a mesopolysaprobic, and eutrapphentic taxon, particularly common for shallow, nutrient rich waters, favoured by moderately alkaline conditions (Håkansson 1993; Van Dam et al. 1994).

Distribution. Valves were found at all investigated sites of Southern Bug during this research (Table 1). For Ukrainian territory it has been reported for the Dnipro Estuary, the Southern Bug as well, but near Vinnitsya (300 km upriver from Mykolayiv), the rivers Siverskyi Donets, Dnister, Danube, Dnipro, Desna, Prypiat, Teteriv, Oskol, small rivers in Odessa region and other rivers (Tsarenko et al. 2009).

Concerning global distribution, *Cyclotella meneghiniana* is considered a widespread taxon (Houk et al. 2010); it was also recorded for Berlin, Germany (Geissler and Kies 2003).

Order Thalassiosirales Glezer & I.V. Makarova, 1986

Family Stephanodiscaceae I.V. Makarova in Glezer and Makarova 1986

Genus *Cyclostephanos* Round in Theriot et al. 1987

Cyclostephanos dubius (Hust.) Round in Theriot et al., Brit. Phycol. J. 22 (4): 346. 1987.

Basionym. *Stephanodiscus dubius* Hust., Krypt.-Fl. Deutschl., 2.Aufl., 7 (1): 367, fig. 192. 1928.

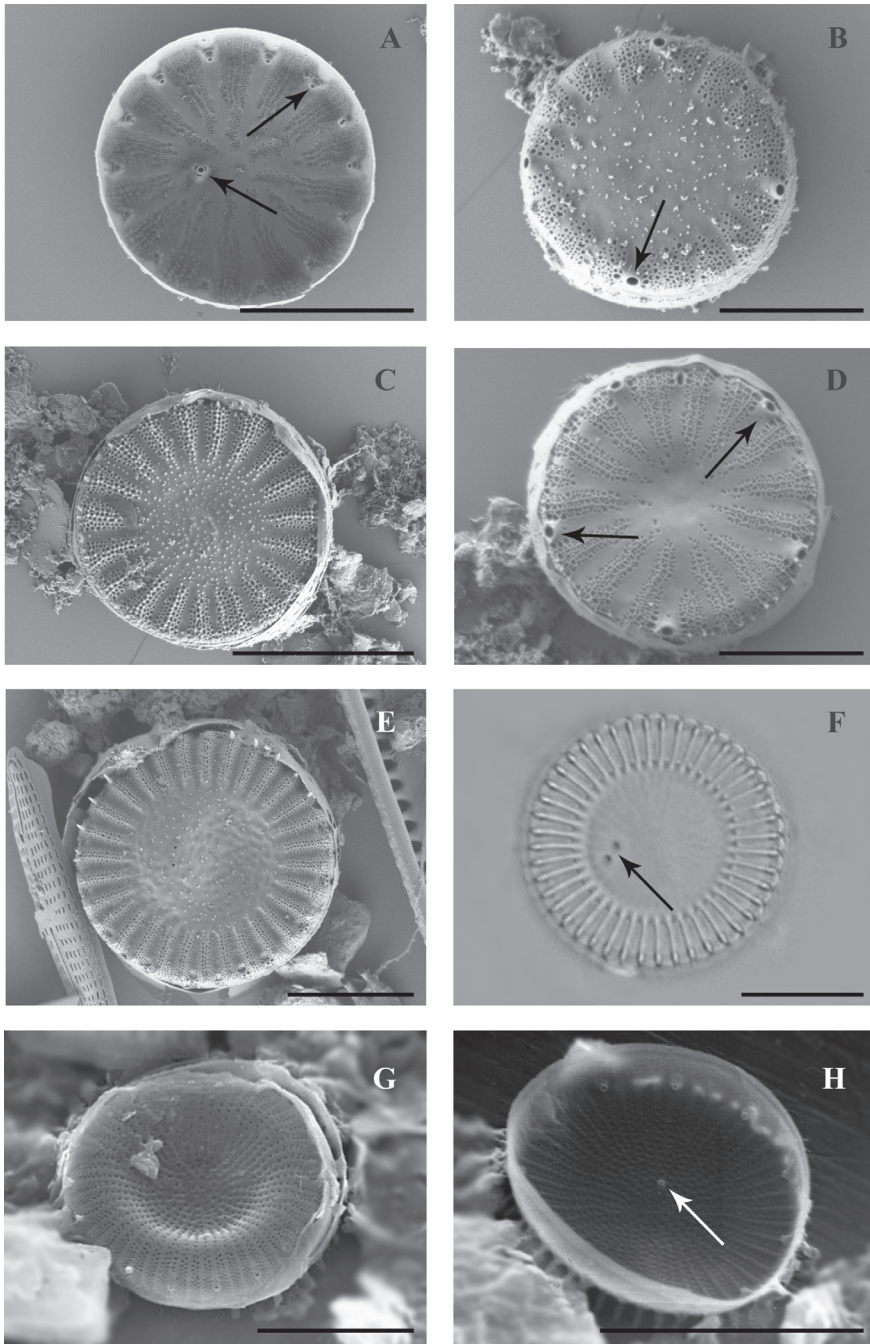


Figure 7. SEM (A–E, G, H) and LM images (F) of species found in the transitional zone at Mykolaiv city, valve views: **A** *Cyclotella cryptica* **B** *Cyclotella marina* **C, D** *Cyclotella meduanae* **E, F** *Cyclotella meneghiniana* **G** *Cyclostephanos dubius* **H** *Cyclostephanos invisitatus*, with arrows shown on **A, D, H** marginal and central fultoportulae, **B** marginal fultoportulae, **F** central fultoportulae. Scale bars: 2 µm (B, D); 4 µm (A); 5 µm (C, E); 10 µm (F–H).

Synonyms. *Cyclotella dubia* Fricke in A.W.F. Schmidt

Morphological description. The frustule is disciform, valve face concentrically undulate, 13.6–21 µm in diameter, striae multiseriate with 9–12 areolae in 10 µm, ribs continue on the curve of a valve, spines grow from each rib (Fig. 7G).

Ecology. *Cyclostephanos dubius* is considered to be a pelagic taxon, planktonic component of both fresh and brackish lakes (Cholnoky 1968). Often indicating meso- to eutrophic conditions, sometimes recorded for hypertrophic lakes (Van Dam et al. 1994; Kirilova et al. 2010). The species is common in flowing and stagnant water in coastal area, oligosaprobic, alkalibiontic, halophilous “0.0–5 g/dm³” (Hustedt 1957); pH value above 7.0 (van der Werff and Huls 1957–1974; Foged 1973).

Distribution. This species was found above Mykolaiv city in the Southern Bug River bed (Table 1). From Ukrainian territory, it is known from the Dnipro River, the Dnipro-Bug Estuary (Topachevsky and Oksiyuk 1960; Vladimirova 1971) and the Danube River (Tsarenko et al. 2009).

Cyclostephanos dubius is a cosmopolitan species, recorded for Berlin, Germany (Geissler and Kies 2003) and further sites in Europe (Hungary, Dania, Estonia, Moldova, Russia, Ukraine), Asia (Georgia, Russia, Uzbekistan), North America (Canada, USA), Africa (Egypt); Baltic and White Seas (Tsarenko et al. 2009; Kiss et al. 2012; Genkal et al. 2020).

***Cyclostephanos invisitatus* (M.H. Hohn & Hellerman) Stoermer, E.C. Ther. & Håk. in Theriot, Stoermer and Håkansson, Diatom Res. 2: 256, figs 10 d–f. 1988.**

Basionym. *Stephanodiscus invisitatus* M.H. Hohn & Hellerman, Trans. Am. Microscop. Soc. 82 (3): 325. 1963.

Morphological description. Frustule disciform, valve face flat, 9.4–14.5 µm in diameter, multiseriate striae 10–14 in 10 µm, ribs are continuing on curve of the valve, spines grow from every rib (Fig. 7H).

Ecology. *Cyclostephanos invisitatus* was recorded as planktonic species from rivers, ponds, lakes, reservoirs and seas, freshwater, brackish and marine waters. Also known from waters of eutraphentic conditions, moderate and higher trophic and moderate alkalinity (Krammer and Lange-Bertalot 2000; Siver et al. 2005; Kirilova et al. 2010; Hofmann et al. 2018).

Distribution. *C. invisitatus* was found 5 km downstream Mykolaiv city (Table 1), and was recorded for this River earlier (Bilous et al. 2012; Bilous et al. 2014; Belous 2016). In turn, it is common for the Dnipro River and there are some findings in the Danube River (Tsarenko et al. 2009).

Probably a cosmopolitan species, known from Europe (Germany, Hungary, Poland, Russia), Asia (Armenia, Azerbaijan, Russia), Northern America (USA), Africa (Egypt); Caspian Sea (Wojtal and Kwadrans 2006; Tsarenko et al. 2009; Kiss et al. 2012; Genkal et al. 2020).

Discussion

Based on the results above, indicator species for eutrophication in the coastal area of Black Sea waters, which are entering the estuary and then move upstream into the Southern Bug River, were identified. From the investigated taxa the following have been reported as tolerant to nutrient pollution: *Aulacoseira subarctica*, *Cyclotella atomus*, *C. choctawhatcheeana*, *Cyclostephanos dubius*, *Melosira varians*, *Skeletonema subsalsum*, *Stephanodiscus hantzschii*, *S. minutulus* (Hasle and Evensen 1975; Van Dam et al. 1994; Bradbury et al. 2002; Chris et al. 2003; Sarno et al. 2005; Yang et al. 2005; Jaanus et al. 2009; Kirilova et al. 2010; Högländer et al. 2013; Lowe 2015; Reavie and Kireta 2015; Burge and Edlund 2016; Nardelli et al. 2016; Hofmann et al. 2018). Therefore, it is not surprising, that all of them were recorded at Mykolaiv city during different sampling studies and only some of them sporadically at each site.

The conducted research was supplemented by the information concerning the basic biology of centric diatoms, their distribution and occurrence in the transition zone of a freshwater-saline environment. Comparison of the two investigations of 2013 and 2017, revealed the absence of *Melosira varians* in 2017. This might be initialised by changes of ecological conditions near the investigated territory of the Mykolaiv area in the Southern Bug River and eventually by the displacement of this taxon by other representatives of the genus that are better adapted to increasing salinity. The evident displacement representative is *Melosira subglobosa*, which prevailed in the studied area with relatively high abundancies from 57.1 to 80.4% for all centric diatoms over all investigated sites of the transitional zone of the Southern Bug River.

During the sampling research in 2017 a new taxon for Ukraine, *Aulacoseira nivalis*, was found. Since this species is rare, up to now poorly studied and not enough data concerning its ecology and distribution are available, our finding could serve to supplement the existing information. In Hofmann et al. (2018) the taxon is characterised as oligotraphentic and living in dystrophic waters; but this does not seem to correspond to the characteristics of the Ukrainian waters in this area (Mykolaiv regional state administration 2019). Different information for *A. nivalis* is presented in Kulikovskiy et al. (2016), where it is found in alkaline as well as in acidic waters. Ten more taxa found by us in this area are alkalibionts and probably reveal the appropriate conditions for *A. nivalis* in the investigated water body, if all publications are talking about the same taxon. Further focused investigations to establish the appropriate ecology, distribution and identity of *A. nivalis* should be conducted to validate the presented information.

One more interesting finding is *Cyclotella atomus* var. *gracilis* that was reported for the first time for the studied area (Tsarenko et al. 2009). For other water bodies in Ukraine, this species is rare and recorded only once for the Dnipro (Maystrova et al. 2007), however, it is probably more widespread in Ukraine, since its varieties *Cyclotella atomus* var. *gracilis* and *Cyclotella atomus* var. *atomus* are difficult to differentiate using light microscopy, and the nominate one is widespread in Ukraine (Tsarenko et al. 2009). As we have done detailed morphological investigations using LM and SEM

microscopy, it gave us the possibility to distinguish *C. atomus* var. *gracilis*. This taxon is marked as common for freshwaters, however some authors mention it as an euryhaline species (Kiss et al. 2012). It is supposed that the ecology of this taxon as well as the previous one need further focused investigations.

Aulacoseira islandica is also the first confirmation from Swirenko's investigations of the benthic flora of the lower part of the River in 1925–1926 that has reappeared during our study (Swirenko 1941). As it was mentioned above, this taxon is common for the Dnipro river basin, moreover it is also known for swamps from the eastern part of Ukraine as well as from estuaries in the southern part of the country (Ivanov and Karpeso 1999). Moreover, this is a common species for neighbouring countries in Europe as well as worldwide. *A. islandica* is reported from waters of different trophy levels. Thus, it seems surprising that *A. islandica* was not reported for the Southern Bug river until now, especially for the well explored lower part of the river.

Previously, our investigations revealed ten centric diatom species as new for this territory: seven species (*Aulacoseira subarctica*, *Conticribra weissflogii*, *Cyclostephanos invisitatus*, *Cyclotella atomus*, *C. choctawhatcheeana*, *C. meduanae*, *Thalassiosira faurii*) were found in our study of the sampled material from 2013 (Genkal and Bilous 2015) and three species were discovered in 2017 but considered as rare (*Cyclotella cryptica*, *C. marina*, *Stephanodiscus makarovae*) in our publication from 2019 (Bilous et al. 2019). In this paper, four further species (*Aulacoseira nivalis*, *A. islandica*, *Cyclotella atomus* var. *gracilis*, *Melosira subglobosa*, *Skeletonema subsalsum*) were revealed as new to this river. Altogether, for the examined zone of the Southern Bug River 14 centric diatom species were published for the first time for this river due to detailed examination of the species of our 2013 and 2017 sampling studies. The total number of centric diatoms in the studied area shows the existence of 24 centric diatom taxa representing 11 genera (Table 1).

Among the centric diatoms we also found three alien or potentially neophytic species (*Actinocyclus normanii*, *Skeletonema subsalsum*, *Thalassiosira incerta*) that might be considered as an immigration of marine species to freshwaters (Kaštovský et al. 2010; Korneva 2014; Vidaković et al. 2016), consequently their monitoring is important for biodiversity conservation. For *Actinocyclus normanii* f. *subsalsus*, here treated as a synonym of the nominate forma, the Ukrainian findings might be comparable to Germany where this taxon migrated from coastal habitats to inland waters (Geissler and Kies 2003; Geissler et al. 2006).

When comparing the composition of species, we found about 5–6 species for each investigated site along the river (Bilous et al. 2012; Bilous et al. 2014; Belous 2016). Our current findings show that the studied sites of the Lower Southern Bug River near Mykolaiv city have centric diatom species numbers a few times higher than the sampling sites analysed in our former studies (Genkal and Bilous 2015). Therefore, we are classifying this area as a transitional zone.

For more specific definitions for transitional zones two ecological terms are currently used (among others), the ecotone and the ecocline. An ecotone, being defined as highly dynamic and usually unstable, results in an environmentally stochastic stress zone. For diatoms an ecotone would mean that each species can be assigned to clear-cut

specific zones such as freshwater, brackish, and marine (Attrill and Rundle 2002). The term ecocline in diatoms would refer to an area that due to e.g. physicochemical variation represents a boundary of more gradual, progressive change, meaning that species could more easily migrate from freshwaters through brackish to marine waters and vice versa with less distinct/clear cut zones (Attrill and Rundle 2002). Although this study was not designed to answer this question, the combined data of all sampling researches could indicate that the here studied transitional zone fits the definition of an ecocline.

The occurrence of marine taxa in estuaries and freshwaters below the physiological salt barrier of about 5‰ was discussed in Geissler and Kies (2003) for Hamburg, Germany. The presence of single valves of marine diatoms in eutrophic fresh waters in cities far away from marine habitats was interpreted as air borne valves or part of anthropogenic wastewater impacts (Geissler and Kies 2003). Only taxa with high euryhalinity tolerance are able to move upstream and adapt to eutrophic freshwaters, which have also an increased conductivity.

Although the majority of centric genera are discovered in strictly marine waters, and relatively few of them are present in strictly freshwaters (Kociolek et al. 2015), a growing number of them seem to be able to live in the wide salinity spectrum of brackish waters. This might also be attributed to the increased studies of these large transitional zones. Indeed, in this paper the distribution of centric diatoms near Mykolaiv city according to their ecological characteristics showed the prevailing number of species as estuarine. Mykolaiv city at the lower part of the Southern Bug River represents a river region with salt water inflow into the freshwater river. The salinity here varies in a wide range from 2.39 up to 6.36 g/dm³, indicating the effect of marine and freshwater discharge (Governmental portal 2020). Nevertheless, not only salinity but also anthropogenic influences from Mykolaiv city might also add to the higher centric diatom species number at this site. These questions need to be elaborated in detail and in future investigations of this area.

Conclusion

Due to the presence of many centric diatoms in all types of water bodies, they have often been considered as a cosmopolitan if not ubiquitous group. This makes centric diatoms a very good organism group to be used for bioindication purposes owing to the widescale presence of the environmental conditions suitable for their development within the studied area.

Our study shows that salinity is one of the most influential factors for diatom species composition. The transitional zone of the Mykolaiv area with its changing salinities is offering conditions for the existence of 24 centric diatom taxa representing 11 genera. The occurrence of three marine taxa may be considered as an immigration of marine species into this area due to changing salinities. These trends should be thoroughly monitored for the inland waters of Ukraine in the future. The presented results are a documented contribution to the regional flora of Ukraine.

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Artabotrys pachypetalus (Annonaceae), a new species from China

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Abstract

Artabotrys pachypetalus **sp. nov.** is described from Guangdong, Guangxi, Guizhou, Hunan and Jiangxi in China. A detailed description, distribution data, along with a color plate and a line drawing are provided. In China, specimens representing this species were formerly misidentified as *A. multiflorus* or *A. hongkongensis* (= *A. blumei*). *Artabotrys blumei* typically has a single flower per inflorescence, whereas both *Artabotrys pachypetalus* and *A. multiflorus* have multiple flowers per inflorescence. In addition, *A. pachypetalus* is readily distinguished from *A. multiflorus* in having thicker and shorter petals, and connivent and somewhat trigonal or terete inner petal blades. *Artabotrys pachypetalus* is most similar to *A. punctulatus* because both have multi-flowered inflorescences and similar petal length, but *A. pachypetalus* differs in having cream petals *in vivo*, connivent inner petal blades, and a short, raised rim above the inner petal claw. *Artabotrys multiflorus* should be excluded from the flora of China because none of the Chinese specimens of *Artabotrys* collected so far fall within the variation of *A. multiflorus*.

Keywords

Annonaceae, *Artabotrys*, morphology, South China, taxonomy

Introduction

Artabotrys R.Br. is one of the largest genera in the Annonaceae, with over 100 species of woody climbers distributed in Africa (including Madagascar) and Asia (Chen et al. 2019; Rainer and Chatrou 2021). Among the lianescent genera of the Annonaceae, *Artabotrys* is distinctive in possessing hooked inflorescence axes that facilitate climbing (Posluszny and Fisher 2000). Although the persistent inflorescence hook is a synapomorphy for the genus, identification at the species level is often complex. In general, the flowers of *Artabotrys* possess a uniform structure characterised by a tightly enclosed floral chamber (Chen et al. 2019, 2020). Both the outer and inner petals are concave at the base, with an expanded, generally flattened (sometimes terete or triquetrous) blade above the concave base. In addition, the inner petals have a projecting rim between its blade and the concave base (see fig. 9d in Chen et al. 2020); the elaborate rims of the three inner petals are tightly connivent, forming a dome around the reproductive organs. The concave base of the petals has been traditionally referred to as the ‘claw’ by Sinclair (1955) and widely followed by recent workers (e.g., Turner 2012; Prabhu et al. 2015; Turner and Utteridge 2015; Chen et al. 2018; Chen and Eiadthong 2020), although the term ‘claw’ is also used (in a strict sense) to refer to the narrowed, stalk-like, basal portion of the inner petals of other genera, e.g. *Mitrephora* (Blume) Hook.f. & Thomson, *Monodora* Dunal and *Pseuduvaria* Miq. (Sinclair 1955; Su and Saunders 2006; Couvreur 2009; Weerasooriya and Saunders 2010). The terms ‘claw’ and ‘blade’ have been explained in detail by Chen et al. (2020) in the context of the pollination biology of *Artabotrys*. To date, eight *Artabotrys* species have been recorded in China, viz. *A. fragrans* Jovet-Ast, *A. hainanensis* R.E.Fr., *A. hexapetalus* (L.f.) Bhandari, *A. blumei* Hook.f. & Thomson (recorded as *A. hongkongensis* Hance), *A. multiflorus* C.E.C. Fisch., *A. pilosus* Merr. & Chun, *A. punctulatus* C.Y.Wu ex S.H.Yuan, *A. rhynchocarpus* C.Y.Wu ex S.H.Yuan (Li and Gilbert 2011).

During our field exploration in Yangchun City in Guangdong Province, we collected an *Artabotrys* species with multi-flowered inflorescences. Morphological comparison with herbarium specimens from China revealed that the newly collected specimen is conspecific with many specimens that were previously identified as *A. multiflorus* or *A. hongkongensis*. However, our Yangchun collections (and the above-mentioned specimens) do not match type specimens or descriptions of *A. multiflorus* and *A. hongkongensis*. Originally described from Myanmar (Fischer 1937; Kress et al. 2003; Turner 2015), *Artabotrys multiflorus* also occurs in adjacent forests in Thailand (Chen et al. 2018; Chen and Eiadthong 2020) and is distinct in having multiple flowers per inflorescence and lanceolate petals (Fischer 1937; Chen and Eiadthong 2020). In China, *A. multiflorus* was first incorrectly recorded by Xu and Li (1985) based on three collections from Guizhou Province (*Z.R. Xu. L1224, S827, L437, SYS*). Subsequently, more specimens in China were misidentified as *A. multiflorus*. Thus, Li and Gilbert (2011) recorded this species in the *Flora of China* with a quite wide distribution in Guangdong, Guangxi, Guizhou and Yunnan. Originally described from Hong

Kong, *A. hongkongensis* is also reportedly widespread in China, where it is known from Hunan, Guangdong, Guangxi, Yunnan, Guizhou and Hainan provinces (Li and Gilbert 2011); it is also recorded in Vietnam (Bân 2000). This species usually bears a single-flowered inflorescence (rarely two-flowered). However, this name has now been synonymized with *A. blumei* Hook.f. & Thomson (Turner 2018).

Apart from Yangchun, flowering individuals are also found in Ruyuan in Guangdong Province, as well as Mulun National Nature Reserve in Guangxi. Based on detailed comparisons of available living material as well as herbarium specimens, we confirm that our collections and many specimens misidentified as *A. multiflorus* and *A. hongkongensis* in China represent a new species described here as *Artabotrys pachypetalus*. In addition, *A. multiflorus* is not present in China, as none of the Chinese specimens of *Artabotrys* collected so far fall within the variation of *A. multiflorus*.

Materials and methods

Physical and scanned herbarium specimens of *Artabotrys* from 15 Chinese herbaria (BNU, CCNU, GE, GFS, GZAC, GXMG, GXMI, GZTM, HGAS, HITBC, IBK, IBSC, KUN, PE and SYS; acronyms according to Thiers 2021) were studied in detail and compared with the type specimens and descriptions of *A. multiflorus*, *A. blumei* and other similar species from China and Indochina. Fresh material was collected from Chun-wan Town, Yangchun City of Guangdong, China. The morphological study was based on fresh material and herbarium specimens, but the measurements in the description below are based on dried material. The distribution, habitat and phenology of the new species are based on field observations and specimen records.

Taxonomy

***Artabotrys pachypetalus* B.Xue & Junhao Chen, sp. nov.**

urn:lsid:ipni.org:names:77217250-1

Figs 1, 2

Chinese name. Hou ban ying zhua hua (厚瓣鹰爪花)

Diagnosis. Similar to *Artabotrys punctulatus* C.Y.Wu ex S.H.Yuan in having multi-flowered inflorescences and similar outer and inner petal length (9–20 mm long), but differs in having non-punctate (vs. densely punctate) abaxial leaf lamina, cream (vs. beige, maroon-tinged) petals *in vivo*, connivent (vs. spreading) inner petal blades *in vivo*, a short, ca. 1 mm long (vs. an elongate, ca. 5 mm long) raised rim above the inner petal claw, and thick pericarp (2–3 mm thick vs. less than 1 mm thick).

Type. CHINA. Guangdong Province, Yangchun, Chun-wan Town, Zi-you Village, Ma-tang, alt. 110 m, limestone, 08 Apr. 2019, X.X. Zhou, G.T. Wang & Y.N. Guo 0028 (holotype: IBSC [barcode no. IBSC0861926]; isotypes: KUN, SING).

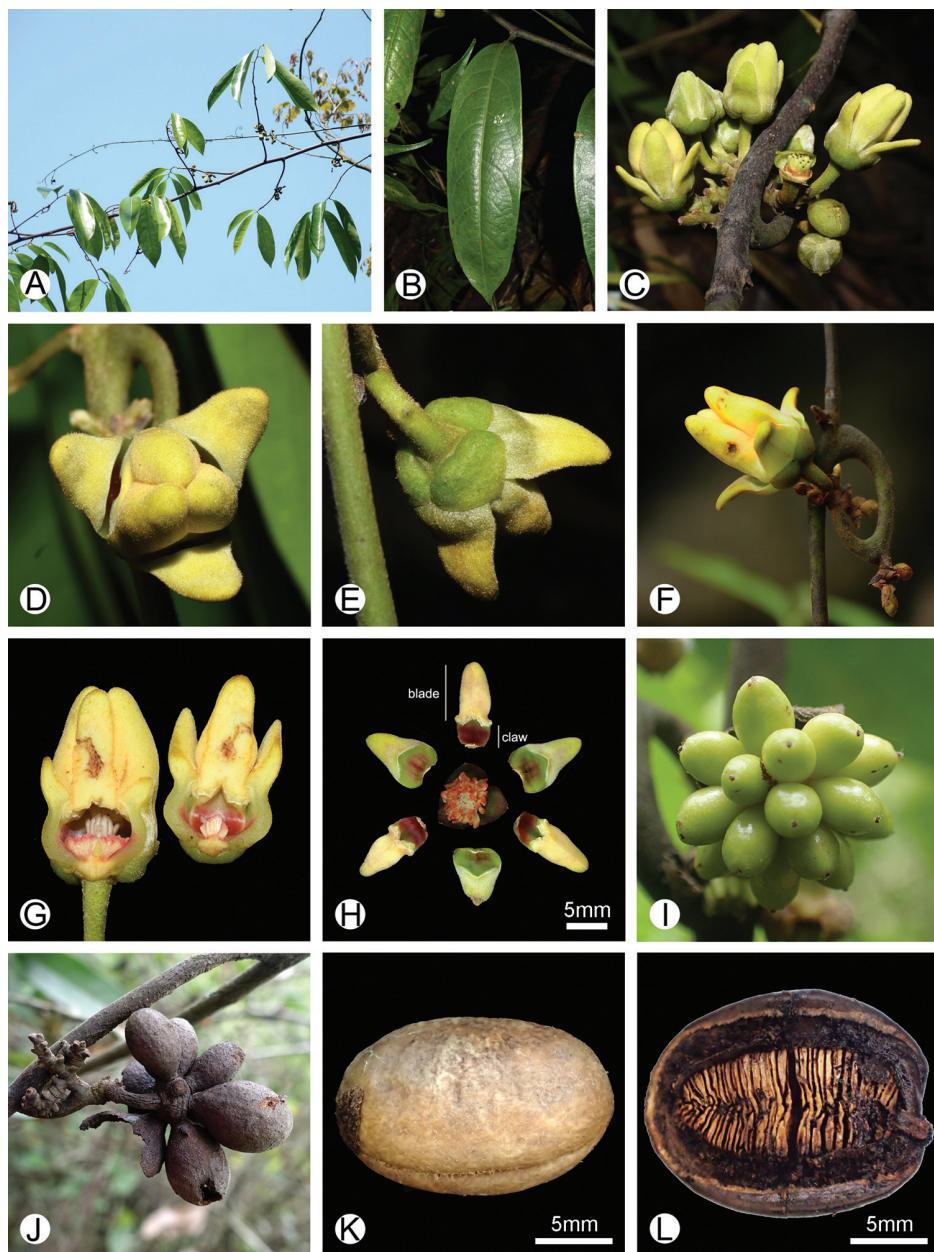


Figure 1. Photographs of *Artabotrys pachypetalus* sp. nov. **A** habit **B** adaxial leaf surface, showing the raised midrib **C** hooked inflorescence with many loosely clustered flowers **D** apical view of the flower **E** basal view of the flower **F** lateral view of the flower **G** longitudinal section of the flower, showing the clavate stigma and orange-red stamen connective apex **H** dissected flower, showing three sepals, three outer petals and three inner petals, and many carpels and stamens (Y. Huang 341, IBSC) **I** developing young fruit with many monocarps (B. Xue XB342, IBSC) **J** dried fruit on old branch (B. Xue XB342, IBSC) **K** seed (B. Xue XB342, IBSC) **L** longitudinal section of the seed, showing lamelliform endosperm ruminations (B. Xue XB342, IBSC). Photos: Yi Huang (A, H); Gang-Tao Wang (B–E, G); Yi Tong (F); Bine Xue (I–L).

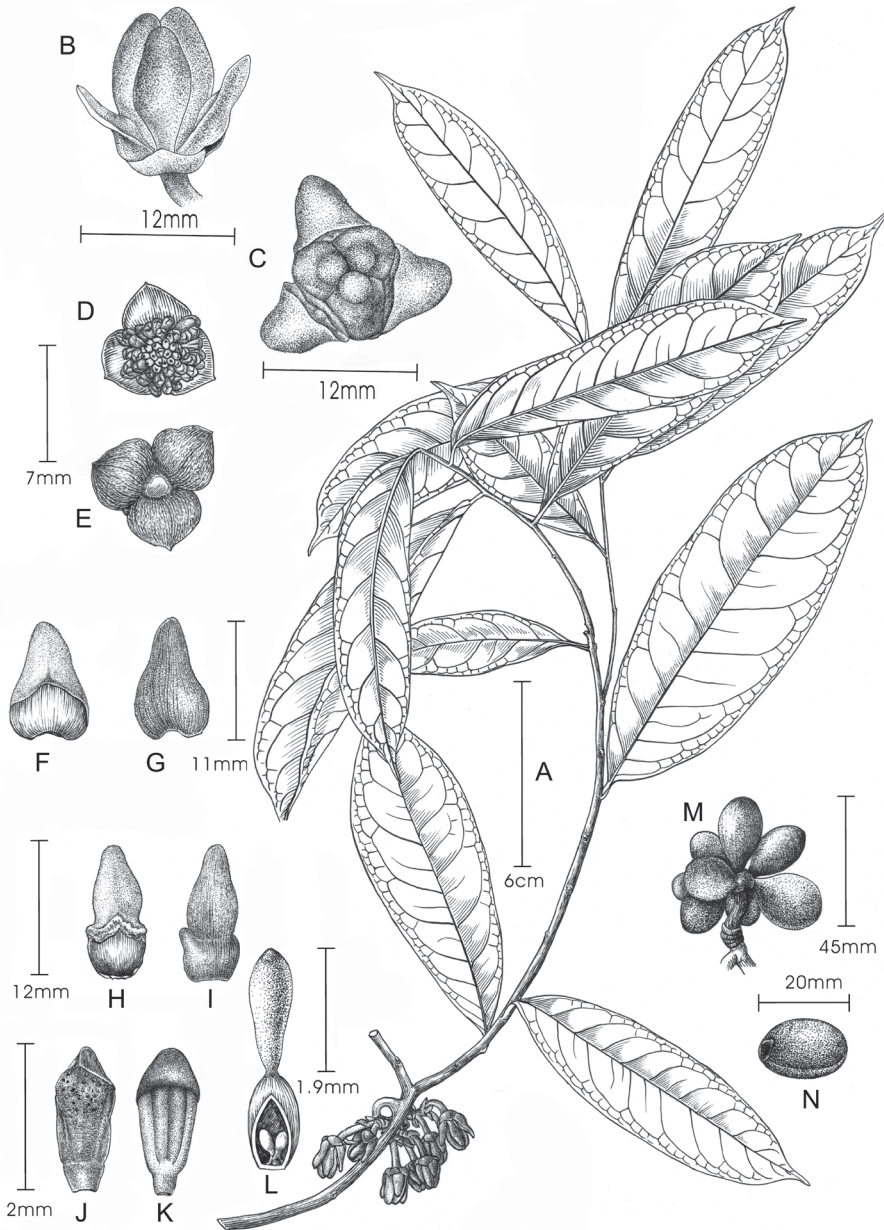


Figure 2. Illustration of *Artabotrys pachypetalus* sp. nov. **A** flowering branch, showing the hooked inflorescence with multiple flowers **B** lateral view of the flower **C** adaxial view of the flower, showing three outer petals and three inner petals forming a closed floral chamber **D** adaxial view of the sepals and torus with stamens and carpels **E** abaxial view of the sepals **F** adaxial view of the outer petal **G** abaxial view of the outer petal **H** adaxial view of the inner petal **I** abaxial view of the inner petal **J** adaxial view of the stamen, showing the rounded connective apex with a sharp edge **K** abaxial view of the stamen, showing four thecae **L** carpel, showing the clavate stigma and two basal ovules **M** immature fruit **N** seed. Drawn by Yun-Xiao Liu, X.X. Zhou, G.T. Wang & Y.N. Guo 0028 (**A–L**); B. Xue XB342 (**M, N**).

Description. Climbers ca. 7 m tall. Twigs drying brown, glabrous to sparsely appressed-pubescent when young. Leaf laminas chartaceous, 10–16 cm long, 3.5–5.0 cm wide, length:width ratio 3–4(–5), narrowly oblong, apex acute or acuminate to cuspidate, base cuneate, glabrous both ab- and adaxially; midrib appressed-pubescent abaxially, glabrous adaxially, raised on both surfaces; secondary veins 8–13 pairs per leaf, impressed adaxially, raised abaxially; tertiary venation reticulate, visible on both surfaces; petioles 3–5 mm long, ca. 1 mm in diameter, glabrous to sparsely appressed-pubescent, drying with transverse striations. Inflorescence axis recurved, laterally compressed, hook-like, with 6–10 flowers; flowering pedicels 6–15 mm long, ca. 1 mm in diameter, densely spreading-pubescent. Sepals 3, free, coriaceous, valvate at base, ca. 5 mm long, ca. 5 mm wide, triangular, glabrous adaxially, sparsely appressed-pubescent abaxially. Petals 6, in two whorls of 3 inner and 3 outer petals, free, valvate, coriaceous when dry, cream *in vivo*, with distinct upper blade and concave base. Outer petals 3, 9–14 mm long; blades spreading, ca. 5–9 mm long, 3–7 mm wide, ca. 1 mm thick, ovate, densely appressed-pubescent both ab- and adaxially, apex rounded; claws 4–5 mm long, 4–8 mm wide, densely appressed-pubescent abaxially, glabrous adaxially. Inner petals 3, 11–17 mm long; with a short, ca. 1 mm long raised rim above the claw; blades connivent, 7–11 mm long, 4–6 mm wide, ca. 2 mm thick, somewhat trigonal or terete *in vivo*, densely appressed-pubescent both ab- and adaxially, apex rounded; claws ca. 4–6 mm long, 3–5 mm wide, densely appressed-pubescent abaxially, glabrous adaxially, tinged orange-red at the very base *in vivo*. Stamens extrorse, ca. 50–60 per flower, ca. 2 mm long, ca. 1 mm wide; apex of connectives rounded, with a sharp edge adaxially, orange red *in vivo*. Carpels 8–20 per flower, ca. 3.5 mm long, glabrous; ovaries ellipsoid, ca. 1.5 mm long, ca. 0.6 mm wide, glabrous; ovules 2, basal; stigmas 1.5–2 mm long, cylindrical to slightly clavate, glabrous. Fruiting pedicels 10–20 mm long, ca. 3 mm in diameter, subglabrous. Monocarps 8–20 per fruit, unripe monocarps *in vivo* light green, at maturity unknown, drying black, 25–30 mm long, 15–22 mm wide, ellipsoid, glabrous, subsessile or with stipes up to ca. 2 mm long, apex rounded, pericarp 2–3 mm thick. Seeds 2 per monocarp, light yellow, smooth, plano-convex, 16–20 mm long, 12–14 mm wide, 10–12 mm thick, raphe depressed, endosperm ruminations lamelliform.

Etymology. The specific epithet '*pachypetalus*' alludes to the thick inner petals.

Phenology. Flowering in April; fruiting in August to December.

Distribution and habitat. Guangdong, Guangxi, Guizhou, Hunan and Jiangxi (Fig. 3), at 100–1400 m elev, mainly growing on limestone.

Additional specimens examined (Paratypes). CHINA. **Guangdong:** Yangchun City, Chun-wan Town, Zi-you Village, Ma-tang, alt. 110 m, limestone, 13 Apr. 2020, Y. Huang 341 (IBSC, SING); *ibid.*, 19 Apr. 2020, B. Xue XB342, XB343 (IBSC, SING); Ru-yuan Hsien, 21 Oct. 1939, S.K. Lau 29060 (IBSC, PE); Ru-yuan Hsien, Da-qiao Town, Da-fu village, forest valley, alt. 550 m, 13 Apr. 2013, L. Wu & Y. Tong 3234 (BNU). **Guangxi:** Feng-shan Hsien, Qiao-yin Town, Ba-la-hou Mountain, limestone, forest edge, alt. 940 m, 3 Mar. 2013, B.Y. Huang, X.Y. Hu & J.J. Yao 451223130331025LY (GXMG); Qi-cheng Hisen, Cui-ping Mountain, alt. 270 m, limestone, 5 May 1983, C.Z. Gao & A.Y. Lan 61263 (GXMI); Na-po Hisen, Nong-bu, alt. 1200 m, limestone, 02 May 1981, Ding Fang et al. 25102 (GXMI). **Guizhou:** Li-bo Hsien, Mao-lan, 21

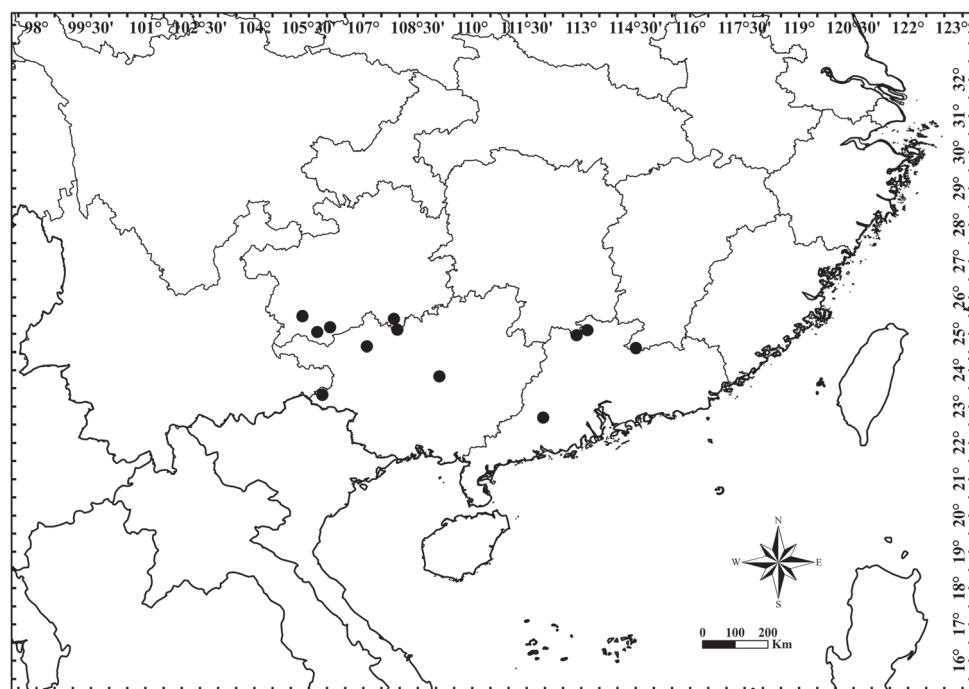


Figure 3. Distribution of *Artabotrys pachypetalus* sp. nov.

Apr. 1981, J.Q. Zhang 1144 (CCNU, GF, GZAC); *ibid.*, alt. 730 m, 8 May 1981, R.B. Jiang 81-0080 (GF, IBSC); *ibid.*, 8 May 1981, R.B. Jiang 80 (CCNU); *ibid.*, 8 May 1981, M.Z. Yang 810247 (HGAS); *ibid.*, alt. 750 m, 7 Mar. 1982, Y.K. Li 10039 (IBSC); *ibid.*, alt. 710 m, 6 Sep. 1982, Y.K. Li 10462 (HGAS); *ibid.*, alt. 800–1000 m, limestone, 03 Apr. 1984, Z.R. Xu L1224 (IBSC); *ibid.*, 26 Apr. 1984, K.M. Lan 840097 (GFS, GZAC); *ibid.*, 02 May 1984, K.M. Lan 841268 (GFS, GZAC); *ibid.*, 2 May 2005, Q.W. Sun 0505019 (GZTM); Xing-ren Hsien, alt. 1300 m, 28 Jun. 1986, C.Z. Dang & P. Dang 156 (PE); Ce-heng Hsien, 21 May 1977, *anonymous* 071, 77-1635 (HGAS); *ibid.*, alt. 1400 m, 5 Sep. 1958, Z.Y. Cao 0575 (PE); Wang-mo Hsien, Cheng-guan, forest valley, 15 May 1977, *anonymous* 77-1594 (HGAS); *ibid.*, 15 May 1977, C.Z. Dang 030 (HGAS). **Hunan:** Yi-zhang Hsien, Mang Mountain, forest valley, alt. 430 m, 16 Oct. 1942, S.H. Chun 2490 (IBSC, IBK). **Jiangxi:** Ganzhou City, Long-nan Hsien, Jiu-lian Mountain, limestone, alt. 596 m, 05 Apr. 2021, J.Y. Xu, L.X. Yuan, Y.R. Wang, J.R. Chen JLS-304 (SYS); *ibid.*, 09 Jun. 1970, Group-236 0852 (PE).

Discussion

Artabotrys pachypetalus is similar to *A. multiflorus* in having multiple flowers per inflorescence (Figs 1, 2), but the two species can readily be distinguished by their disparate petal morphology. The petals of *A. multiflorus* are 18–30 mm long, and 2–5 mm wide (Fischer 1937; Chen and Eiadthong 2020) whereas the petals of *A. pachypetalus*

are much shorter (9–17 mm long, 3–8 mm wide). The petals of *A. pachypetalus* are also considerably thicker (1–2 mm thick when dry) than those of *A. multiflorus* (less than 1 mm thick when dry). In addition, the inner petal blades of *A. pachypetalus* are connivent and somewhat trigonal or terete whereas the inner petal blades of *A. multiflorus* are spreading and planar. Xu and Li (1985) recorded *A. multiflorus* in China based on a few collections from Li-bo Hsien, Guizhou Province (Z.R. Xu L1224, S827, L437, SYS). We failed to locate these specimens in SYS, but found a duplicate of Z.R. Xu L1224 in IBSC as well as many other collections from the same locality, i.e., Mao-lan National Nature Reserve, Guizhou Province, and hence were able to confirm that they are conspecific with the new species described that we collected in Guangdong and Guangxi. The misidentification probably arose in the absence of careful comparison against the type specimens of *A. multiflorus*.

Some specimens representing *A. pachypetalus* are also misidentified with the name *A. hongkongensis* in China, which is now a synonym of *A. blumei* (Turner 2018). The distinction between these two species is quite straightforward: the inflorescence of *A. blumei* usually consists of a single flower or rarely two (Li and Gilbert 2011), whereas that of *A. pachypetalus* has 6–10 co-occurring flowers (Figs 1C, F, 2A). Additionally, *A. blumei* can be distinguished by having planar (vs. somewhat trigonal or terete), spreading (vs. connivent) inner petal blades, subglabrous (vs. sparsely to densely pubescent) pedicels and abaxial surface of sepals, and thin pericarp (< 1 mm thick vs. 2–3 mm thick).

Although *A. pachypetalus* is most similar to *A. punctulatus*, it has not been confused with the latter. This is probably because *A. punctulatus* is restricted to the montane forests of Yunnan in China and is characterized by densely punctate abaxial leaf lamina. The similarities and differences between the two species are stated in the diagnosis.

Besides the above-mentioned species, *A. pachypetalus* is also similar to *Artabotrys hienianus* Bân from Vietnam, both with multiple small hairy flowers per inflorescence (Bân 2000). The two species differ, however, in the number of the flowers per inflorescence, petiole length, leaf shape, and thickness of the pericarp. The inflorescence of *A. hienianus* has more flowers borne on shorter pedicels (5–6 mm), leading to densely clustered flowers on the inflorescence (Bân 2000), whereas that of *A. pachypetalus* has fewer flowers borne on longer pedicels (6–15 mm, Figs 1C–F, 2A), leading to loosely clustered flowers on the inflorescence (Figs 1C, 2A). *Artabotrys hienianus* has longer and broader leaves (12–17 × 5–6 cm), whereas *A. pachypetalus* has smaller and narrower leaves (10–16 × 3.5–5 cm). In addition, *A. hienianus* has a thin pericarp (Bân 2000), whereas that of *A. pachypetalus* is quite thick (2–3 mm thick) (Fig. 1L).

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Finding missing diversity from synonyms of *Haplopteris* (Pteridaceae)

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Abstract

Although taxonomists target the remote wild regions to discover new species, taxa lacking a comprehensive and modern systematic treatment may be the new hotspot for biodiversity discovery. The development of molecular systematics integrated with microscopic observation techniques has greatly improved the ability of taxonomists to identify species correctly. *Vittaria centrochinensis* Ching ex J.F. Cheng, regarded as a synonym of *Haplopteris fudzinoi* (Makino) E.H. Crane, remained hidden from the eyes of fern taxonomists for more than 20 years. Herein, we collected several population samples of *V. centrochinensis* by performing molecular phylogenetic analysis of five cpDNA regions (*rbcL*, *atpA*, *matK*, *ndhF*, and *trnL-trnF*) and through micromorphological observation of specimens which differs from *H. fudzinoi* by lamina width and exospores. Considering the differences in morphology, geographical range, and genetic distance between these two species, we formally recognized *V. centrochinensis* as an authentic species and proposed a new combination *Haplopteris centrochinensis* (Ching ex J.F. Cheng) Y.H. Yan, Z.Y. Wei & X.C. Zhang, **comb. nov.** Our findings demonstrate that several taxa in synonyms are missing, and nowadays taxonomy should also include re-evaluation of the past taxonomy.

Keywords

Haplopteris, molecular phylogeny, new combination, nomenclature, Pteridaceae, taxonomy

Introduction

The question “How many species are there on earth?” is one of the top 125 questions in science, and exploring it is considered equivalent to imagining the number of stars in the sky (Kennedy and Norman 2005). To understand the biodiversity of species, taxonomists should not only explore new species but also re-evaluate the published species’ names that are considered synonymous with older species’ names. According to the stasis of the web of TPL (The Plant List 2013), more than one-third of species names are unclear and approximately one-third of species names are considered synonymous. Unfortunately, once a species name is treated as a synonym, it remains in the pile of synonyms forever. With the development in molecular phylogeny research, DNA barcoding, and detailed taxonomic observations, an increasing number of species names have been re-established from the checklist of synonyms, which ranges from ferns (Shu et al. 2017; Shu et al. 2018; Wei et al. 2018; Wang et al. 2020) to spermatophyte (Luo et al. 2016; Jin et al. 2017; Wang et al. 2018). Consequently, we found that the synonym database could be a new hotspot for biodiversity discovery.

Accurate specimen identification through sequencing of the type specimens or samples from type locality is the key to solving questions regarding taxonomic synonyms. In addition, a clear understanding of the taxonomic status and barcoding database of the species suspected of being independent is required. *Haplopteris* C.Presl is a genus of vittarioid ferns, long treated as a synonym of *Vittaria* Sm. (Kramer 1990; Wu and Ching 1991; Chen et al. 2013). Recently, it has been widely adopted and followed that the Old World *Vittaria* species were transferred to *Haplopteris* (Chen et al. 2013). Because of limited morphology exhibited by members of the *Haplopteris* as well as convergent and parallel evolution of morphological characteristics, the circumscription of species in the genus has been ambiguous. *Vittaria centrochinensis* Ching ex J.F.Cheng, just one of a long sleeping suspicious species in the synonym list of *Haplopteris*, was initially published in “Flora of Jiangxi” (Xu 1993) as a new species and subsequently considered as a synonym of *V. fudzinoides* (Zhang 1999). To date, it has been regarded as a synonym of *Haplopteris fudzinoides* (Makino) E.H.Crane (Zhang and Gilbert 2013; Yan et al. 2016). *Haplopteris fudzinoides* is a species originally described in Japan, and then used to refer to a Chinese fern (Zhang 1999). During our field investigation in Wuyi Mountain (Jiangxi, China), we collected some population samples of *V. centrochinensis* and found some obvious differences between *V. centrochinensis* and *H. fudzinoides*.

In this study, we analyzed morphological characteristics and geographic distribution along with the molecular phylogeny to confirm the identity of *V. centrochinensis* and phylogenetic affinities of this species with *H. fudzinoi*. We hope that this study can provide a paramount example of re-evaluating of synonyms for new insights into biodiversity discovery.

Materials and methods

Morphological analyses and geographical distribution

For morphology, the *H. centrochinensis* was compared with similar species by analyzing photographs of type specimens and field photos. The features of rhizome scales were obtained using Nikon SMZ-1500 (Japan). The morphology of spores was observed with a Quanta 250 scanning electron microscope (FEI, USA), and spore size was measured using ImageJ software (Collins 2007). The descriptions of spore ornamentation abided by Wang and Dai (2010) and Ranker et al. (1993). The map of the geographical distribution of two species, namely *H. centrochinensis* and *H. fudzinoi*, was obtained through field investigation and National Specimen Information Infrastructure (NSII). The specimens of *H. centrochinensis* in this study were deposited in Shanghai Chenshan Herbarium (CSH).

Phylogenetic analyses

The total genomic DNA was extracted from silica-dried leaves by using a plant total genomic DNA kit (Tiangen, Beijing, China), according to the manufacturer's instructions. The primers used for amplification and sequencing were shown in Table 1. Sequencing was performed with an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). The cpDNA sequences of the three samples of *H. centrochinensis* were submitted to GenBank under accession numbers: MW810047–MW810061 (Table 2). In addition, five cpDNA regions of nine species were downloaded from GenBank (Table 3). Of these, the unavailable data (Table 3) were treated as missing data when they were concatenated. The newly generated sequences were assembled and edited using SeqMan (Burland 1999). Subsequently, all sequences were aligned and manually adjusted on MEGA software (v7.0) (Kumar et al. 2016), with default alignment parameters. Alignments of five cpDNA regions were concatenated using PhyloSuite (Zhang et al. 2018). Then the matrix was used to construct phylogenetic trees with maximum likelihood (ML) and MrBayes. Maximum likelihood analysis was conducted using IQ-TREE (Nguyen et al. 2015) integrated in PhyloSuite with standard bootstrap and TVM+F+G4 model. Bayesian analysis was performed using MrBayes (v3.2.6) (Ronquist et al. 2012) with the GTR+F+G4 model. Four Markov chains were

Table 1. List of PCR amplification and sequencing primers used in the study.

Regions	Primer name	Primer sequence (5'-3')	Reference
<i>rbcL</i>	AF	ATGTCACCACAAACGGAGACTAAAGC	Hasebe et al. (1994)
	ESRBCL1361R	TCAGGACTCCACTTACTAGCTTCACG	Schuettpelz and Pryer (2007)
<i>atpA</i>	ESATPF412F	GARCARGTTTCGACAGCAAGT	Schuettpelz et al. (2006)
	ESTRNR46F	GTATAGGTTTCRARTCCTATTGGACG	Schuettpelz et al. (2006)
<i>matK</i>	Vt matK1610F*	GCARTCAARCGTTTAATTRGTA	Chen et al. (2013)
	Vt matK rRFQ	TTATTACTGAATTTGGRATCT	Chen et al. (2013)
<i>ndhF</i>	Vt ndhF fAYS	GCTTATTCTACHATGTCTCAGYTRGGATATATGG	Kuo et al. (2016)
	Vt trnN 2210R	TCTGTGARACGAAAATAGCAGTTTATGG	Kuo et al. (2016)
<i>trnL-F</i>	F	ATTTGAACCTGGTGACACGAG	Taberlet et al. (1991)
	FernL 11r1	GGYAATCCTGAGCCAAATC	Li et al. (2010)

Table 2. GenBank accession number of sequences newly generated in this study.

Species	Location	Voucher	GenBank accession number				
			<i>rbcL</i>	<i>atpA</i>	<i>matK</i>	<i>ndhF</i>	<i>trnL-trnF</i>
<i>Haplopteris centrochinensis</i> comb. nov.	Jiangxi, China	YYH15442-1	MW810047	MW810050	MW810053	MW810056	MW810059
<i>Haplopteris centrochinensis</i> comb. nov.	Jiangxi, China	YYH15442-2	MW810048	MW810051	MW810054	MW810057	MW810060
<i>Haplopteris centrochinensis</i> comb. nov.	Jiangxi, China	YYH15442-3	MW810049	MW810052	MW810055	MW810058	MW810061

run 1,000,000 generations, with the sampling frequency of 100. The standard deviation of split frequencies was set to less than 0.01 to achieve the convergence of the independent runs. A majority-rule consensus tree was constructed to estimate the posterior probabilities (PP); the first 25% of samples were discarded as the burn-in phase.

Results

Morphological comparisons and geographical distribution

The morphological and micromorphological characters of *H. centrochinensis* and *H. fudzinoi* are presented in Figure 1 and Table 4. The lamina of *H. centrochinensis* was shorter and wider than that of *H. fudzinoi* (Fig. 1A, B, F); in *H. fudzinoi* costa it was raised adaxially with two prominent long grooves beside the costa on adaxial surface (Fig. 1D). The rhizome scales were yellow-brown, margin denticulate, linear-lanceolate, and clathrate (Fig. 1E left); scale margins of *H. centrochinensis* were toothed and those of *H. fudzinoi* were subentire to minutely denticulate at lower margin and upper part, respectively. The scales length of *H. centrochinensis* were visibly longer than that of *H. fudzinoi* (Fig. 1E). Spores were monoletate for both the species. Spore ornamentation observed in *H. centrochinensis* was scabrate and rugate (Fig. 1G, I), whereas it was laevigate or inconspicuous-granulate in *H. fudzinoi* (Fig. 1H, J). Additionally, sori position was distinct between the two species; the soral line in *H. fudzinoi* was located close to the edge of lamina and immersed in groove (Fig. 1D), whereas it was immersed between the frond

Table 3. Information on species and GenBank accession numbers used in the study. Dash (-) indicates unavailable data.

Species	Location	Voucher	GenBank accession number				
			<i>rbcL</i>	<i>atpA</i>	<i>matK</i>	<i>ndbF</i>	<i>trnL-trnF</i>
<i>Haplopteris taeniophylla</i> (Copel.) E.H. Crane	Luzon, Philippines	FWL974	–	–	KC812901	KC812935	KC812969
	Nantou, Taiwan, China	Chen1493	–	–	KC812874	KC812908	KC812942
<i>Haplopteris doniana</i> (Mett. ex Hieron.) E.H. Crane	Yunnan, China	Kuo1418	–	–	KC812880	KC812914	KC812948
	Tamdao, Vietnam	Kuo1801	–	–	KC812905	KC812939	KC812973
<i>Haplopteris fudzinoi</i> (Makino) E.H. Crane	Sichuan, China	Kuo2225	KX165003	KX165201	KC812895	KC812929	KC812963
<i>Haplopteris linearifolia</i> (Ching) X.C. Zhang	Yunnan, China	Liu9457	KX165012	KX165209	KC812899	KC812933	KC812967
<i>Haplopteris mediosora</i> (Hayata) X.C. Zhang	Nantou, Taiwan	Chen1492	KX165015	KX165211	KC812875	KC812909	KC812943
<i>Haplopteris amboinensis</i> (Fée) X.C. Zhang	Hainan, China	Kuo1715	–	–	KC82879	KC812913	KC812947
<i>Haplopteris flexuosa</i> (Fée) E. H. Crane	Yunnan, China	Kuo1142	–	–	KC812881	KC812915	KC812949
<i>Antrophyum parvulum</i> Blume	Nantou, Taiwan, China	Chen1495	–	–	KC812877	KC812911	KC812945
<i>Antrophyum sessilifolium</i> (Cav.) Spreng	Taitung, Taiwan, China	Chen1502	KX164974	KX165181	KC812876	KC812910	KC812944

Table 4. Morphological comparisons between *H. centrochinensis* and *H. fudzinoi*.

Features	<i>H. centrochinensis</i>	<i>H. fudzinoi</i>
Lamina width	10–15 mm	8–10 mm
Lamina margin	Flat	Reflexed
Adaxial costa	Slightly raised	Greatly raised
Abaxial costa	Carinated	Sharp carinate
Rhizome scale	Long, margin toothed	Short, lower margin subentire, upper part minutely denticulate
Exospores	Scabrate	Psilate
Sorus position	Between the frond costa and margin	Close to the lamina edge

costa and margin in *H. centrochinensis* (Fig. 1C). The geographical distribution for these two species was obtained on the basis of the information of the specimens. The result indicated that most distributions are shared by *H. centrochinensis* and *H. fudzinoi* (Fig. 3).

Haplopteris centrochinensis is an independent species according to molecular data obtained using phylogenetic analysis

The two phylogenetic analyses (BI, ML) recovered congruent topologies, with *Antrophyum parvulum* and *Antrophyum sessilifolium* as outgroups (Fig. 2). The results revealed strong support for the monophyly of *H. centrochinensis* (PP = 1.0, BS = 100) (Fig. 2), and it was strongly supported as a sister to another four species (i.e., *H. fudzinoi*, *H. doniana*, *H. taeniophylla*, and *H. linearifolia*) of *Haplopteris* (PP = 1.0, BS = 100) (Fig. 2). The genetic distance between the *H. centrochinensis* and these four *Haplopteris* species ranged from 0.073 to 0.120, and the intraspecific distances of these four species were 0 and 0.001 (Table 5).

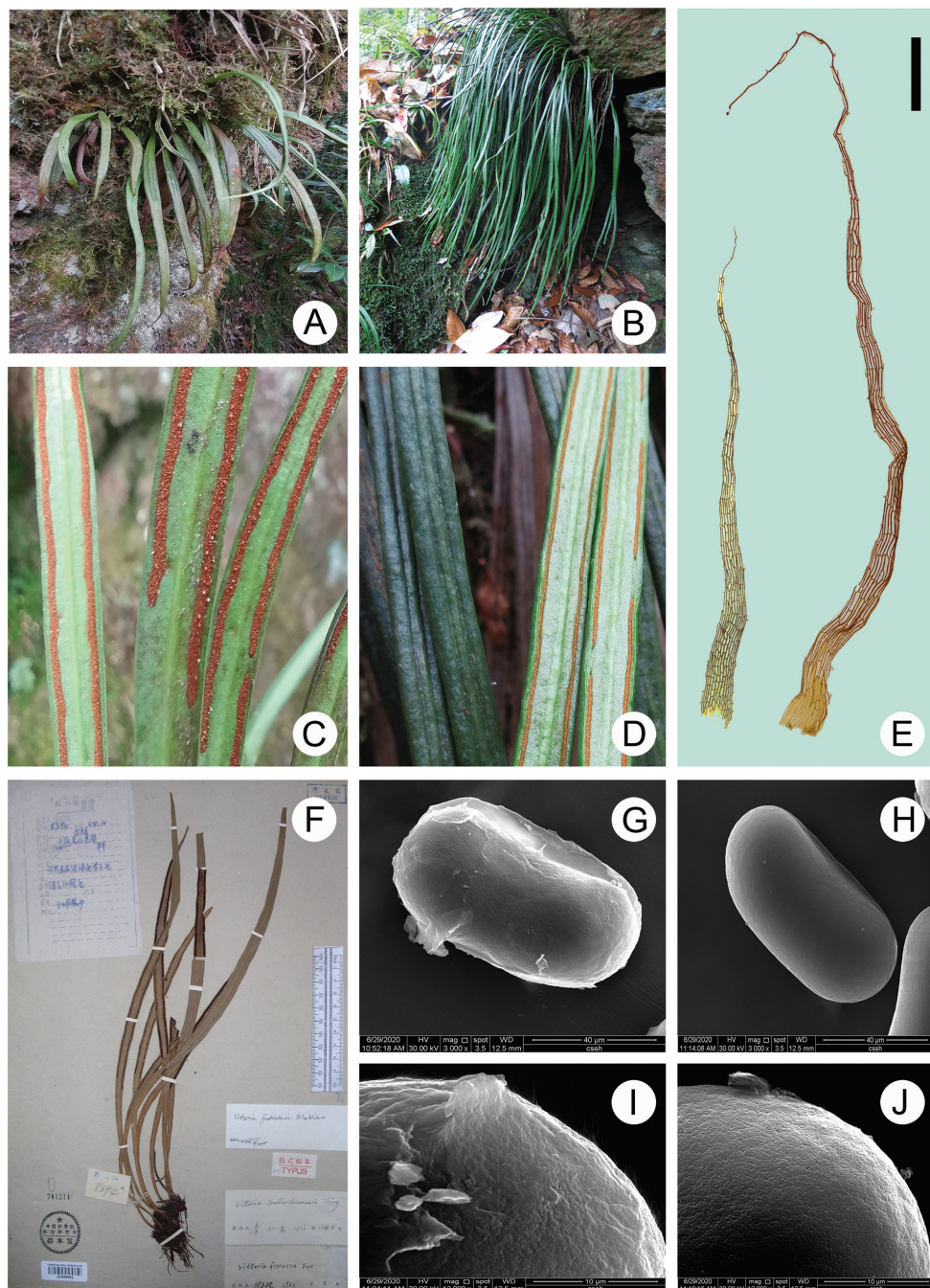


Figure 1. Morphological observations in *H. centrochinensis* YYH15442 (**A,C,F,G,I**) and *H. fudzinoi* SG1654 (**B,D,H,J**) **A** habitat **C** sorus position and flat lamina **F** type specimen (provided by National Plant Specimen Resource Center, <http://www.cvh.ac.cn>); and **G,I** spore and ornamentation in *H. centrochinensis* YYH15442 **B** habitat (taken by Hong-Jin Wei) **D** sorus position and flat lamina (taken by Hong-Jin Wei) **H,J** spore and ornamentation in *H. fudzinoi* SG1654 **E** rhizome scale, left: *H. fudzinoi*, right: *H. centrochinensis*.

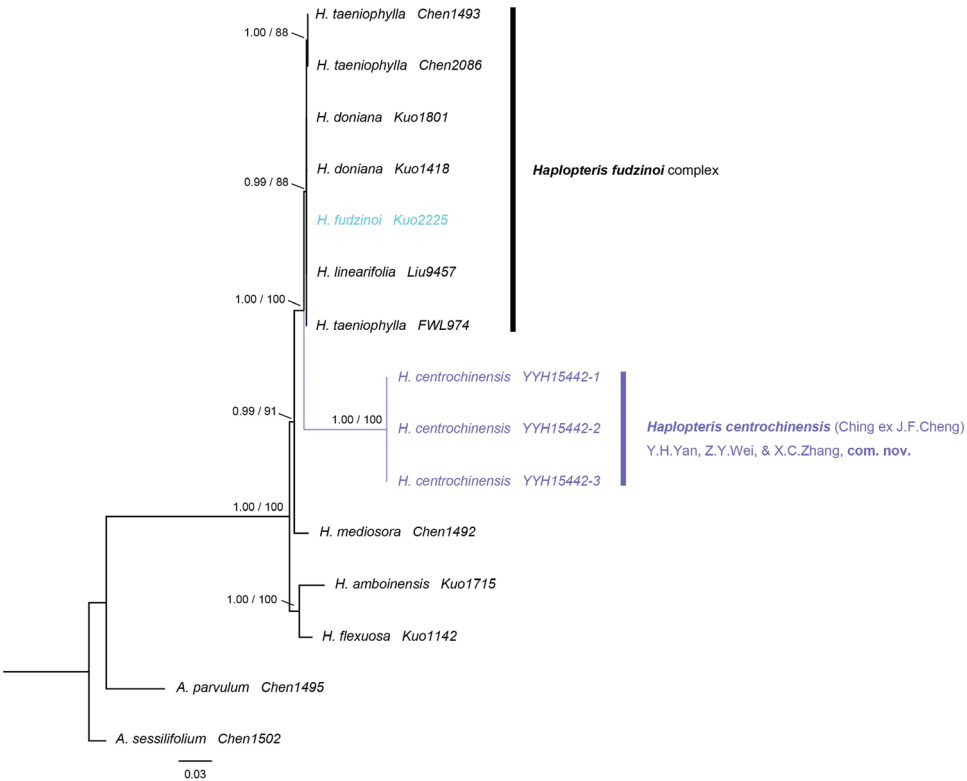


Figure 2. Majority consensus tree derived from Bayesian tree based on 5 cpDNA loci (*rbcL*, *atpA*, *matK*, *ndhF*, and *trnL-F*). Numbers above the branches are support values in the order of PP_{BI}/BS_{ML}.

Table 5. Genetic distance between eight individuals of five *Haplopteris* species.

	1	2	3	4	5	6	7
2	0.073*						
3	0.120*	0.001					
4	0.120*	0	0				
5	0.120*	0.001	0.001	0.001			
6	0.120*	0.001	0.001	0.001	0		
7	0.120*	0	0	0	0.001	0.001	
8	0.073*	0	0	0.000	0.001	0.001	0

Note: 1 = *H. centrochinensis* (YYH15442); 2 = *H. fudzinoi* (Kuo2225); 3 = *H. donoana* (Kuo 1418); 4 = *H. doniana* (Kuo1801); 5 = *H. taeniophylla* (Chen 2086); 6 = *H. taeniophylla* (Chen 1493); 7 = *H. taeniophylla* (FWL 974); 8 = *H. linearifolia* (Liu 9457); Genetic distances of *H. centrochinensis* from others species are shown with *.

Discussion

Re-evaluation of synonyms for new insights into biodiversity discovery

Synonym is the first concern in the estimation of the total number of species in one taxon, and only after its resolution can one ask the next question regarding how many additional species there are in the taxon (Joppa and Pimm 2011). Surprisingly, near-

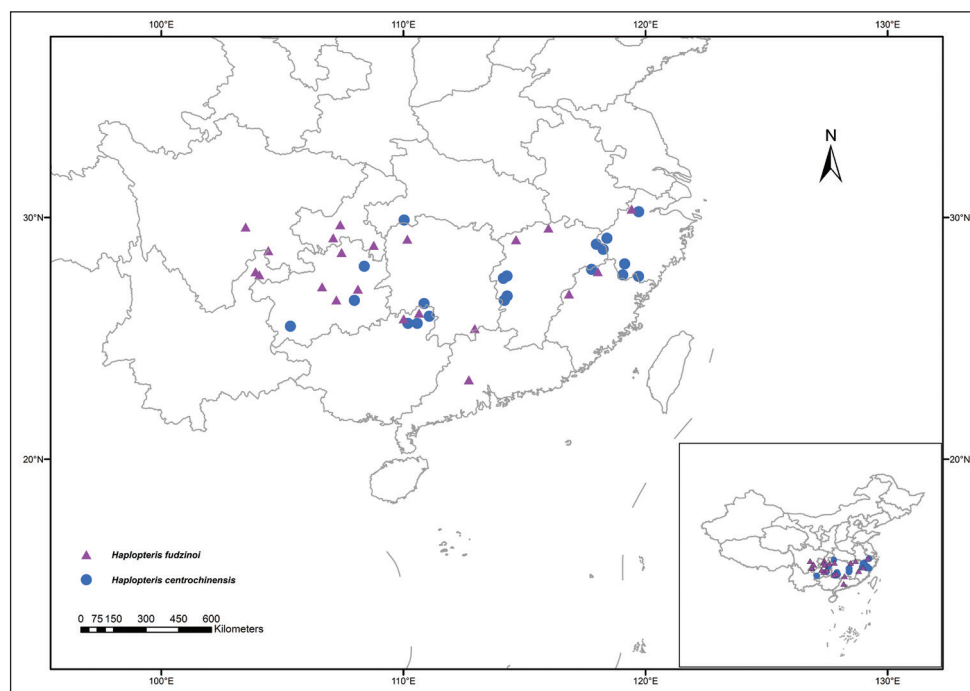


Figure 3. Geographic distribution of *H. centrochinensis* and *H. fudzinoi* in China. The dataset is provided by the National Specimen Information Infrastructure (<http://www.nsii.org.cn>).

ly two-thirds of the plant names are synonymous or recorded as unresolved in *TPL* (2013), which consists of 26,000 additional synonyms that were not listed in its earlier version (v.1.0). The increase in the number of newly discovered species has been consistent in line with the use of molecular evidence; however, information on synonyms is meager. For instance, International Plant Names Index (IPNI 2020) provides information on nomen novum, combination nova, and taxa nova, but it provides no information on new species resurrected from the established synonyms. Although many species of ferns (Liu et al. 2013; Morigengaowa et al. 2018; Shu et al. 2017; Shu et al. 2018; Sigel et al. 2014; Wang et al. 2020; Wei et al. 2018) and seed plants (Tkach et al. 2015; Hu et al. 2015) have been reinstated as independent species, this type of study has been rarely reported. The extent of biodiversity hidden in taxonomic literature is an interesting question to explore. To understand the biodiversity of our planet and for efficient conservation of valuable natural resources, the prime objective of taxonomists should be to correctly identify our planet biodiversity by using modern taxonomic facilities.

Various taxa, especially widely distributed ones, still require a comprehensive systematic treatment that also involves evaluating their nomenclature. Then, if cryptic taxa or misunderstood species have to be segregated, naming these taxa needs first to be evaluated against synonymy as potential sources of the needed name, otherwise a new name needs to be proposed. However, the number of taxonomists has significantly declined (Haas and Hauser 2005), and young taxonomists do not pay enough atten-

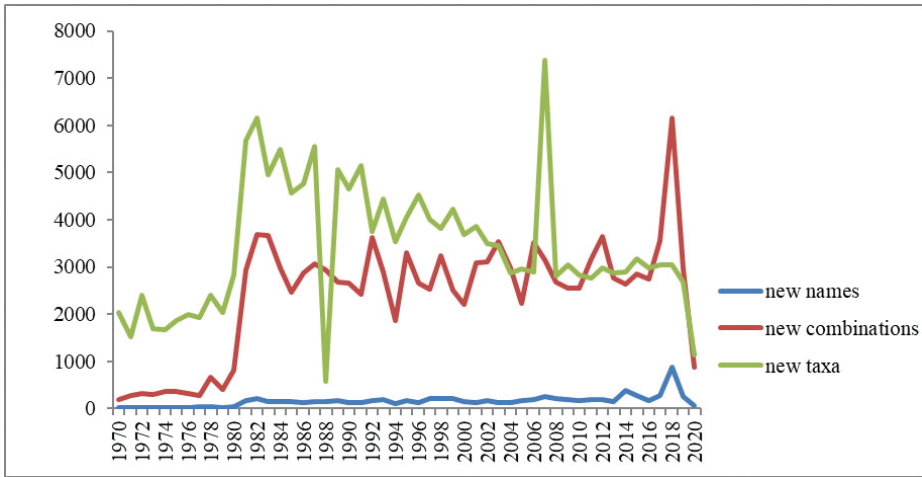


Figure 4. Trends in the number of new names, new combinations, and new taxa published over 50 years (1970–2020).

tion either to the evaluation of synonymous names already listed in the taxonomy or to the assessment of thousands of different species names and their type specimens. Therefore, the number of species on earth remains uncertain. Fortunately, technological advancement has considerably affected taxonomy. According to the IPNI data, two obvious turning points in taxonomy have affected the trends in new combinations and new taxa (Fig. 4), and those are related to the development of electron microscopy and molecular phylogeny in the 1970s and the 1990s, respectively (Endess et al. 2000). Although the new taxa have been displaying a steady or even a downward trend, the new combination is expected to display an upward trend in future, with the application of molecular biology in taxonomy (Fig. 4). Unsurprisingly, new combinations will continue to occur for a long time because of the abundance of listed synonyms and suspected species names, which are equivalent to the new species in the wild. Thus, synonyms and suspected species will serve as the new biological diversity hotspot for the exploration of new unknown species.

Integrative taxonomy contributes to clarifying species delimitation

The reason for numerous synonyms existing only in books may be the lack of sufficient morphological judgments made in the past. In the present study, the phylogeny (Fig. 2) based on the 5-locus dataset revealed strong support for the monophyly of *H. centrochinensis* (PP = 1.0, BS = 100) (Fig. 2) and strong support for *H. centrochinensis* as sister to *H. fudzinoi* (PP = 1.0, BS = 100) (Fig. 2). Although our result was different from that of a study by Zhang and Gilbert (2013) that indicated distinction between the two species, no further research was performed merely because of limited conditions at that time. Moreover, genetic distance in line with the K2P model showed an obvious hereditary difference between the *H. centrochinensis* and another three *Haplopteris* species (Table 5). For morphological comparisons, several traits were observed. Of these, the most

unambiguous differences between the two species (*H. centrochinensis* and *H. fudzinoi*) were the wider lamina, longer rhizome scale, and shorter stipe in *H. centrochinensis*. In addition, *H. fudzinoi* costa was raised adaxially with two prominent long grooves besides the costa on adaxial surface. Furthermore, the scabrate and rugate ornamentation of spores observed in *H. centrochinensis* was found to be laevigate or inconspicuous-granulate in *H. fudzinoi*. To sum up, monophyletic clade, long genetic distance, stable morphological differentiation, and independent geographical distribution form the basis of establishing *H. centrochinensis* as a divergent species or an independent species, and therefore, it should not be considered synonymous with *H. fudzinoi*.

Here, we proposed a new combination *H. centrochinensis* (Ching ex J.F.Cheng) Y.H.Yan, Z.Y.Wei & X.C.Zhang, comb. nov. The taxonomic treatment of *H. centrochinensis* is as follows.

Taxonomic treatment

***Haplopteris centrochinensis* (Ching ex J.F. Cheng) Y.H.Yan, Z.Y.Wei & X.C.Zhang, comb. nov.**

urn:lsid:ipni.org:names:77217251-1

Vittaria centrochinensis Ching ex J.F.Cheng: Fl. Jiangxi 1: 365. 1993. Basionym.

Vittaria taeniophylla sensu F.Zhang, non Copel.: Fl. Zhejiang 1: 111. 1993. p.p.

Vittaria fudzinoi sensu X.C.Zhang, non Makino: Fl. Rep. Poup. Sin. 3(2):20.1999. p.p.

Haplopteris fudzinoi sensu Zhang & Gilbert, non (Makino) E. H. Crane: Fl. China 2(3): 254.2013. p.p.

Type. China. Hubei Province, Enshi Tujia and Miao Autonomous Prefecture, Hefeng District, elev. 1200 m, October 1958, Hong-Jun Li, 8394 (holotype, PE!; isotypes, IBSC!, NAS!).

Additional specimens examined. **Guangxi Province:** Damiaoshan District, 26 July 1958, Shao-Qing Chen, 15853 (IBSC); Quanzhou District, April 27, 2013, Quanzhou census team, 450324130427042LY (GXMG). **Guizhou Province:** Kaili City, census team, 3592 (CNBG); Xingren District, 9 August 1960, census team, 7872 (CNBG); Yinjiang, December 26, 1930, Y. Tsiang, 7867 (CNBG). **Hunan Province:** Shaoyang City, Dongkou District, 24 May 1983, Ze-Yong Yang, 166 (IBSC); Xinning District, September 9, 1984, Anonymous, 394 (PE). **Jiangxi Province:** Shangrao City, Yanshan District, Wuyi Mountain National Nature Reserve, 1729 m, October 7, 2019, Yue-Hong Yan, Zuo-Ying. Wei, Quan Yuan, YYH15442 (NOCC); Shangrao City, Yushan District, Sanqingshan, July 27, 1991, Sheng-Xiu Xu, 91018 (JXU); Jinggangshan City, Jinggangshan, April 13, 1983, Sheng-Xiu Xu, 83422 (JXU); Jinggangshan City, Jinggangshan, February 1982, 8210118 (JXU); Jinggangshan City, Jinggangshan, November 4, 1982, 8220349 (JXU); Jinggangshan City, Jinggangshan, July 2, 1973, Jing -Fu Cheng, 730433 (JXU); Shangrao City, Yushan District, Huaiyushan, July 1970, 0028466 (PEY); Pingxiang City, Luxi District, March 24, 2014, Gong-Xi, Chen and Dai-Gui Zhang, LXP-06-

1246, LXP-06-1251, LXP-06-1201 (SYS). **Zhejiang Province:** Quzhou City, Kaihua District, September 1, 2019, She-Lang Jin, Hong-Yu Wei, Jiao Zhang, JSL5850 (CSH); Longquan City, September 27, 1963, Shao-Guang Zhang, 4453 (CNBG); Linan City, May 25, 1958, Anonymous, 28714; Qingyuan District, Pei-Xi Qiu, 3935 (PE); Taishun District, July 17, 1960, Anonymous, 8576 (CNBG).

Note. *Vittaria centrochinensis* Ching ex J.F.Cheng was initially published in “Flora of Jiangxi” as a new species found in two distributed provinces (i.e., Jiangxi and Hubei). The type locality is situated in the Hefeng District from which a single specimen was cited. Additional specimens were cited from Jiangxi Province.

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Typifications of some species names in *Anthurium* section *Pachyneurium* (Araceae)

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Abstract

During a taxonomic study of *Anthurium* sect. *Pachyneurium*, it was found that the names of four species required typification. Verification of the protologues and cited collections is discussed and typifications are proposed as follows: the illustration *Schott Icones Aroideae* No. 465 is designated as the neotype of *A. affine* Schott. A lectotype is designated for *A. bonplandii* G.S. Bunting since the holotype, cited in the protologue at MY, was not found there. An epitype is selected for *A. solitarium* Schott because the lectotype illustration of J.M.C. Vellozo (*Flora Fluminensis* t. 123) lacks sufficient detail to determine it unambiguously to species in *A.* sect. *Pachyneurium*. A lectotype is selected for *A. glaziovii* Hook.f., a synonym of *A. solitarium*.

Keywords

Anthurium, Araceae, epitype, lectotype, Pothoideae, section *Pachyneurium*

Introduction

Anthurium Schott (Schott 1829) is a neotropical genus, the largest and possibly the most complex of the *Araceae* family, belonging to the subfamily Pothoideae (Cusimano et al. 2011), with 950 accepted species and many more which remain to be described

(Boyce and Croat 2021 onwards; Cole et al. 2020). It is particularly diverse in perhumid cloud forests of Central and South America (Croat 1992; Mora et al. 2006).

In Brazil, the genus is represented by 152 species, of which 122 are endemic (Coelho et al. 2020). Classical field studies of the 19th century in the country were carried out by many botanists, such as H.W. Schott, F. Sellow, L. Riedel, A. F. Regnell, A.F.M. Glaziou and C.A.M. Lindman, who made extensive living and dried collections later deposited in European herbaria and botanical gardens, from which many *Anthurium* taxa were described and named (Coelho and Mayo 2007).

Schott (1860), the pioneer in taxonomic studies of *Anthurium*, presented a classification of 28 infrageneric taxa named “*grex*” (plural “*grexes*”). Many of these were later classified as sections and subsections by Engler (1878, 1898), who consolidated his revision in his final treatment, published in *Das Pflanzenreich* (Engler 1905), the last monograph of the genus at species level which was considered complete in its time.

Currently, the genus is subdivided into 20 sections (Carlsen and Croat 2019; Croat and Carlsen 2020). Only five of these have representatives in Brazil and the sections *Urospadix* Engl. and *Pachyneurium* (Schott) Engl. are those that circumscribe the majority of endemic species (e.g. Croat 1991; Coelho and Croat 2005; Gonçalves 2005; Temponi 2006; Coelho et al. 2009; Camelo et al. 2018a, b; Coelho and Valadares 2019; Hammes et al. 2020; Camelo et al. 2020; Coelho et al. 2020).

The first description of a species of section *Pachyneurium* was published by Linnaeus (1763: 75) as *Pothos crenata* L. and later transferred to *Anthurium* by Kunth (1841: 75). Schott (1860) recognised 20 species in his *grex Pachyneurium* and Engler (1905) included 56 species in his *A. sect. Pachyneurium*. In the most recent revision, Croat (1991) included 114 species and 126 taxa and divided the section into two series, *Multinervia* Croat and *Pachyneurium* (Schott) Croat (Carlsen and Croat 2013). Subsequently, Carlsen and Croat (2019), in their recent study of sectional circumscriptions in *Anthurium*, proposed that series *Multinervia* should be recognised as a section because it was shown to be a monophyletic clade, all other species belonging to section *Pachyneurium*.

Anthurium sect. *Pachyneurium* is distributed throughout the Neotropics from Mexico to Argentina and distinguished by the dark brown colour of the leaves in dried specimens and the usually wide spacing of the rib-like secondary veins (more than 3 cm apart), which are more conspicuous than the inter-secondary veins (Croat 1991). The leaf blade has involute venation and eucamptodromous secondary venation (Croat and Sheffer 1983, 2002; Croat 1991). Probable synapomorphies of the section are the stem with very short internodes, the entire leaf blade and one ovule per locule (Temponi 2006).

Croat (1991) described three new species for Brazil from the Amazon Region in his taxonomic revision of *A. section Pachyneurium*. Taking into consideration taxonomic modifications and subsequent descriptions of new species, there are currently 23 taxa native to Brazil, occurring mainly in the Amazon and Atlantic Forest biomes (e.g. Croat 1991; Coelho and Croat 2005; Gonçalves 2005; Coelho et al. 2020). These species exhibit a wide range of morphological variability, partly due to a lack of de-

tailed field observations and, consequently, incomplete published descriptions (Temponi 2005). However, the taxonomic difficulties of this group are also linked to the history of species diversification and delimitation in the genus (Carlsen and Croat 2013, 2019) and the generally very plastic vegetative characters used in morphological species delimitations (Mayo et al. 1997). These issues can lead to nomenclatural and taxonomic confusion and a lack of clarity in species limits.

Currently, Camelo and collaborators (in preparation) are carrying out revisionary studies of *Anthurium* sect. *Pachyneurium* in Brazil. During these investigations, some names have been found to lack type specimens, requiring typification to stabilise the nomenclature of their respective species. Therefore, we propose here one neotypification, two lectotypifications and one epitype.

Materials and methods

In order to discover the types of H.W. Schott's Brazilian species of *Anthurium*, it is, above all, necessary to make a detailed consultation of his published works, particularly his monograph *Prodromus Systematis Aroidearum* (Schott 1860). We also studied his magnificent collection of drawings, available in microfiche (Schott 1984) with the accompanying index (Nicolson 1984), the originals of which are deposited in the Archive Department of the Natural History Museum of Vienna, as well as black-and-white photographs made by the New York Botanical Garden. The coloured drawings represent plants cultivated by Schott himself in the Imperial Gardens of Schönbrunn Palace, Vienna, whereas the black and white drawings depict specimens from many European herbaria. Other publications and sources relevant to researching Schott types are Fenzl (1865), Wawra (1879), Riedl (1965a, b), Riedl and Riedl-Dorn (1988), Riedl-Dorn (1992), Grayum (1996), Coelho and Mayo (2007) and Mayo (2020a, b). We also studied the original black and white drawings of the "*Pothos*" (= *Anthurium*) species illustrated in the *Florae fluminensis* (Vellozo 1831), which are deposited in the National Library of Brazil in Rio de Janeiro.

Protologues of the treated species were studied using the databases of the Biodiversity Heritage Library (2020) (<http://www.biodiversitylibrary.org/>) and publications in scientific journals. Images of the type specimens were accessed through the database JSTOR Global Plants (2020) (<http://plants.jstor.org/>), GBIF.org (2020) (Global Biodiversity Information Facility; <https://www.gbif.org/>), REFLOA (2020) (<http://reflora.jbrj.gov.br/>) and the virtual herbaria of K, MO and NY (acronyms following Thiers 2020 continuously updated).

Typifications followed the nomenclature standards of the International Code of Nomenclature (ICN) criteria (Turland et al. 2018). The primary taxonomic literature sources consulted were the monographs of Schott (1860), Engler (1898, 1905) and Croat (1991). Complementary information on external morphology and phenology was made through observations of populations in the field and analysis of herbarium collections.

Typification

Anthurium affine Schott, Oesterr. Bot. Wochenbl. 5: 82. 1855

Type. (neotype, designated here), [icon] the original illustration H.W. Schott's *Icones Aroideae et Reliquiae* No. 465, deposited in the Archive Department of the Natural History Museum of Vienna and available in the microfiche edition (Nicolson 1984; Schott 1984).

Note. The name *A. affine* was first cited as a *nomen nudum* by Schott (1829) in a short work in which he separated *Anthurium* from the broader Linnaean genus *Pothos* L. and mentioned this species as one of the plants flowering in the glasshouses of the Schönbrunn Gardens: “*Anthurium affine*. (Schott.) *Verwandter Blüthenschweif. Aus Brasilien. Aroideae*”.

The protologue given by Schott (1855: 82) to *A. affine* consists only of a passing remark during a discussion of the taxonomic problems then surrounding *A. hookeri* Kunth, written in German in an article entitled “Vermischtes”, which discusses various nomenclatural confusions in *Anthurium*. Schott observes that the name *A. hookeri* has been used for various cultivated plants, some with an elongated, long-pedunculate spadix and a basally cuneate leaf and others with basal lobes that are rounded or cordate at the petiole junction. However, *A. hookeri* is strikingly recognisable by its short spadix and short peduncle and is somewhat similar only to a Brazilian species, Schott's *A. affine*, which, while certainly having a very short spadix, develops a long peduncle and is generally closer to *A. crassinervium* [“...*Anthurien mit langgestrecktem und langgestieltem Blüthenkolben und an der Basis keilig verlaufendem Blatte, andere mit unten rund oder herzförmig in den Blattstiel vereinigten Blattausbreitungen, sollen die echte, durch kurzstieligen, kurzen Blütenstand so auffallend kenntliche Species kund geben, **die nur einigermassen mit einer brasilischen Art, unserem Anthurium affine, Aehnlichkeit hat (das zwar sehr kurzen Spadix, aber langen pedunculus entwickelt, überhaupt dem A. crassinervium näher steht)**...*”].

The validating phrase of the *A. affine* name (marked in bold italics above), although short and in German, contrasts the inflorescence characters which distinguish it from *A. hookeri* and indicates a similarity to *A. crassinervium*. According to ICN (Art. 38.2), this descriptive phrase is sufficient to validate a name and therefore can be accepted as a valid original description. Later, Schott published a full description of *A. affine* in his monograph *Prodromus Systematis Aroidearum* (Schott 1860: 473–474). This description was clearly made from a living plant, since there are such details as leaf, spadix and berry colour and petiole cross-sectional shape, which are lost in dried specimens. In addition, Schott cites the locality and specimens he saw as follows “– *Brasilia* (S.) – *v.u.spont. et cult.*”, [“vidi vivam spontaneam et cultam”], an abbreviated Latin phrase meaning “I have seen living plants both wild and cultivated” (Stearn 1983: 292). Here, Schott makes it explicit that he had seen living plants in the field and associates his name (“S.”) with the locality “Brasilia”.



Figure 1. Neotype of *Anthurium affine*, the reproduction of the original coloured gouache Schott *Icones Aroideae* n° 465, deposited in herbarium NYBG (NYBG Neg. N53869).

Croat (1991) in his revision of *A.* sect. *Pachyneurium* designated the drawing Schott *Icones Aroideae* no. 465 as the lectotype of *A. affine*, but without explaining the type designation. This is one of the five drawings of the species (nos. 463–467) preserved in Schott's *Icones Aroideae et reliquiae* archive of illustrations (Nicolson 1984). They are coloured gouache *icones*, drawn and painted by the artists Oberer and Liepoldt (Riedl-Dorn 1992: 92) and no. 465 shows a single flowering plant as a whole, i.e. the complete habit of the plant with leaves and an inflorescence (Fig. 1). However, this lectotypification by Croat (1991) is vulnerable since Grayum (1996) and Coelho and Mayo (2007) argued that lectotypes should be chosen from materials known to have been in the author's hands at the time of description. The drawing cited by Croat bears no date and so it cannot be established with certainty that Schott had it in his hands in 1855 at the time of the original valid description of *A. affine*. Consequently, this drawing must be considered a neotype rather than a lectotype, according to ICN (Art. 9.3 and 9.4).

Coelho and Mayo (2007) highlight that, in the case of binomials published by Schott himself, the coloured drawings (i.e. those depicting plants he cultivated in Vienna) are regarded as a source of neotypes rather than lectotypes, because they normally bear no date, making it impossible to determine whether they had been drawn before or after the publication of the names. They also point out that, in the absence of a type specimen, Schott's drawings may be the only remaining evidence of the type. There is no evidence that the drawings of *A. affine* were available to Schott in 1855 when this species was validly published. Consequently, Icon no. 465 cannot be considered as the lectotype. Since no type specimen has been found, we designate here the illustration *Icones Aroideae et Reliquiae* No. 465 as the neotype of *A. affine* because it agrees well with the description of the spadix and peduncle length presented in the protologue and later complete description (Schott 1855, 1860).

***Anthurium bonplandii* G.S.Bunting, Acta Bot. Venez. 10: 267 (1975)**

Type. VENEZUELA. [Amazonas], Dept. Atures, Rio Orinoco near Siquita, between Isla Castillito and San Fernando de Atabapo, 1969, 100–140 m alt., *G.S. Bunting, L. Akkermans et J. van Rooden* 3676 (holotype, MY!: not located); (lectotype, designated here: K barcode K000434112!; (isolectotypes: K barcode K000434111!, NY barcodes NY00133732!, NY00133733!, NY00133734!, NY000133735!).

Note. *Anthurium bonplandii* was described by Bunting (1975) as a new species, based on a single collection, in honour of Aimé Bonpland, who travelled with Alexander von Humboldt in regions of Venezuela where this species occurs. The author cited the specimen *G.S. Bunting, L. Akkermans et J. van Rooden* #3676 (MY) as the holotype, without mentioning any isotype. According to the curator of Maracay Herbarium (MY), all type material of *A. bonplandii* was sent to K and NY sometime after the species' publication. No type material was found at MY herbarium, but there are four sheets of this collection at NY and two at K. These specimens are all marked on their original labels as "Typus", but none with the designation holotype. In 1987, the NY sheets were labelled by J. Burgess as isotypes, probably following Bunting's "holotype" designation for MY in the original description.



Figure 2. Lectotype of *Anthurium bonplandii* subsp. *bonplandii* in K herbarium (K000434112).

We cannot be sure if one of them is the original holotype designated by Bunting, but it is clear from their original labels that they are isotypes, according to ICN (Art. 9.5). Since the holotype has not been found, we have designated *G.S. Bunting*, *L. Akkermans*

et *J. van Rooden* #3676 (K000434112) (Fig. 2) as the lectotype, chosen from amongst the isotypes, in accordance with ICN (Art. 9.3). This specimen was selected because it is the single most complete preserved specimen, including the stem with connected vegetative and reproductive structures and agrees with the protologue description.

***Anthurium solitarium* Schott, Prodr. Syst. Aroid. p. 478. 1860.**

- ≡ *Pothos solitarius* Vell., Fl. flum. text. p. 390, tab. 123 (“122”). 1881, as “*solitaria*”, *nom. illeg.*, [*Pothos solitarius* (“*solitaria*”) Vell., Fl. flum. t. 123 (“122”). 1831 [“1827”], *nom. non valid. publ.*].
- = *Anthurium glaziovii* Hook. f., Bot. Mag. t. 6833. 1885. Type: BRAZIL. Rio de Janeiro, *Glaziou* 188. (lectotype, designated here: 3 sheets, part 1 K barcode K000434142!, part 2 K barcode K000434143!, part 3 K [barcode K000434144!]).
- = *Anthurium nobile* Engl., Bot. Jahrb. Syst. 25: 366. 1898. Type: BRAZIL. Rio de Janeiro, *Glaziou* 9039. (holotype: B barcode B100242945!; isotype: P barcode P00748724!).

Type. BRAZIL. Rio de Janeiro, Santa Cruz (“*Regii Praedii S. Crucis*”), illustration of “*Pothos solitaria*” in J.M.C. Vellozo, *Flora Fluminensis*, t. 123 (1831 [“1827”]) (lectotype, designated here): original parchment illustration of *Flora fluminensis* t. 123 in the Manuscript Section, Biblioteca Nacional, Rio de Janeiro, indexed under digital object code mss1198658_127! (epitype, designated here: BRAZIL. Rio de Janeiro, Nova Friburgo, PE dos Três Picos, picada para os Três Picos, 22°20'9"S, 42°43'0"W, Floresta Ombrófila Densa Altomontana, 05 April 2016, *M. Nadruz et al.* 3064 (RB barcode RB01111541!)).

Note. The plates of Araceae were published in volume 9 of the *Flora Fluminensis* of José Mariano da Conceição Veloso (Vellozo 1831), long before the text of the species descriptions (Vellozo 1881). Plate 123 shows a species of *Anthurium* sect. *Pachyneurium* and bears the name “*Pothos solitaria*”, but this was a *nomen nudum* at this date, according to ICN (Art. 38, 38.8 and 38.9), since the plate lacks both an accompanying description and any analytical figure.

Schott (1860) then validly published the name *Anthurium solitarium*, citing only “*Brasilia. (Flor. Flum.)*”. This is an indirect, but unambiguous reference to Vellozo’s plate 123 of “*P. solitaria*” in the *Flora Fluminensis*, since the original 1831 volume 9 of the plates was the only published form of the Araceae then available. Schott’s short description seems clearly to be based on the leaves and inflorescence shown in plate 123, which can thus be regarded as the type of Schott’s binomial name. According to ICN (Art. 6.9 and 6.10), the name *Anthurium solitarium* therefore cannot be regarded as a new combination, with *Pothos solitarius* (as “*Pothos solitaria*”) as its basionym, but is rather to be treated as the name of a newly-published species.

The name *Pothos solitarius* (as “*Pothos solitaria*”) was eventually validly published in Vellozo (1881: 390), with a description of the plant’s leaves, inflorescence, locality, habitat and rupicolous habit (“*Habitat fruticetis Regii Proedii S. Crucis supra lapides*”),

besides the original illustration. However, *P. solitarius* is an illegitimate name, being superfluous according to ICN (Art. 52.1 and 52.2), due to its later publication and inclusion of the eligible type of *A. solitarium* Schott (1860), the original plate of "*P. solitaria*" (Vellozo 1831: tab. 123).

The locality, cited by Vellozo, corresponds to one of those listed by Lima (1995) in his study of Vellozo's Leguminosae. The site of the "*Regium Praedium Sanctae Crucis*" – literally "Royal Estate of Santa Cruz" – lies in the modern Santa Cruz District of Rio de Janeiro, where, during the colonial period, there was an estate owned by the Crown and used for the cultivation of *Camellia sinensis* (L.) Kuntze, popularly known as Indian tea (*chá-da-india*). In addition, Vellozo cites "*Habitat fruticetis ... supra lapides*", meaning "Occurring in shrubby vegetation ... on rocks". In the Municipalities (Mangaratiba, Angra dos Reis and Paraty) adjoining Santa Cruz Municipality, *A. solitarium* is very common as a rupicolous plant growing on rocks by the sea.

Vellozo's plate 123 (Fig. 3) shows only very few characteristics in common with *A. solitarium*, such as the presence of numerous roots and the slightly wavy leaf blade margins. However, the most diagnostic characteristic of this species – the pendent inflorescence – is not shown; instead the inflorescence is shown on the contrary as an erect structure. From the modern taxonomic standpoint, the plate is ambiguous and inconsistent, because it shows the leaves cut in half, does not include the complete shape of the leaf blade and details of the spathe are lacking. According to current understanding of *Anthurium solitarium*, the spathe and spadix are almost the same size and the inflorescence is pendent, both characteristics being important for the circumscription of the species. Consequently, this illustration could be confused with certain other species of *A.* sect. *Pachyneurium* from southeastern Brazil, i.e. *A. leonii* E.G. Gonçalves and *A. santaritense* Nadrusz & Croat (Gonçalves 2005; Coelho and Croat 2005).

Engler (1898: 352) confused *A. solitarium* with *A. affine*. In the synonymy of the latter, he included the names *P. solitaria* and *A. solitarium* with question marks, indicating his doubt. This can also be seen in his incorrect determinations, as *A. affine*, of specimens of *A. solitarium* deposited in various herbaria. Two examples are: a drawing and specimen of: (*A. Engler Araceae exsiccatae et illustratae* no. 295 [K!]) from a plant cultivated in the Breslau (today Wrocław) University botanic garden and the specimen *Glaziou #9040* (F0BN011838), collected in Rio de Janeiro and deposited at the Field Museum of Natural History.

Despite this earlier confusion, *A. solitarium* differs markedly from *A. affine* in its morphology, ecology and geographical distribution. Current revisionary studies of *Anthurium* sect. *Pachyneurium* (Camelo et al., in prep.), have revealed that four species occur in the Southeast Major Region of Brazil: *A. affine*, *A. leonii*, *A. santaritense* and *A. solitarium*, of which the taxon most closely related to *A. solitarium* is *A. leonii*. *Anthurium solitarium* differs 1) from *A. leonii* by the vinaceous to brownish, cylindrical to 1-ribbed peduncle, the spathe purple or brown on both surfaces and the whitish pollen grains (vs. peduncle greenish, cylindrical, never ribbed, spathe lilac on adaxial surface, greenish on abaxial surface and yellowish pollen grains) (Camelo et al., in prep.); 2) from *A. santaritense* by the obovate leaf wider than 21 cm and the pale



Gynand. Polyand
POTHOS SOLITARIA
(Tab.123.)

Figure 3. Lectotype of *Anthurium solitarium* (Garamond *Pothos solitarius*), the illustration “*Pothos solitaria*” in Vell., Fl. Flum. t. 123. 1831.



Figure 4. Epitype of *Anthurium solitarium* (\equiv *Pothos solitarius*) in RB herbarium (RB01111541).

purple or brown, sessile spadix > 11 cm long (vs. narrowly lanceolate leaf blade up to 20 cm wide, spadix < 10 cm long., lilac, with 0.3–2.5 cm stipe); and 3) from *A. affine* by an obovate leaf blade with non-wavy to slightly wavy margins (vs. elliptic leaf blade with strongly wavy margins), pendent inflorescence (vs. erect inflorescence), elongated purple or brown spathe (vs. spathe elliptic to ovate, green or green with vinaceous striations) and a slender, tapered, pale purple or brown flowering spadix (vs. cylindrical to clavate, yellowish-green to yellow flowering spadix) (Camelo et al., in prep.).

Anthurium leonii is a common rupicole in humid areas of the Atlantic Forest. *Anthurium santaritense* and *A. solitarium* are common epiphytes or rupicoles in humid areas, montane forest or in restingas of the Atlantic Forest Region in south-eastern Brazil. In contrast, *A. affine* is a rupicole in *campos rupestres* and *cerrado* or occurs as a terrestrial plant in *campos* in *restingas* of the Atlantic Forest Region (Coelho et al. 2020; Camelo et al., in prep.).

Following the recommendation of ICN (Art. 9.9) to correct the taxonomic ambiguity of Vellozo's plate, we designated here as epitype of *A. solitarium* Schott, the specimen *M. Nadruz et al.* 3064 (RB01111541) (Fig. 4). This specimen is complete and consistent with the original description. Since there is no material collected in the type location (the City of Rio de Janeiro), we have selected a specimen collected in the Atlantic Forest Region of Nova Friburgo Municipality, in Rio de Janeiro State.

The original description of *A. glaziovii* Hook.f. was based on a living plant sent by A.F.M. Glaziou in 1880, from Rio de Janeiro to the Royal Botanic Gardens, Kew, where it flowered in June 1881. Hooker (1885) noted in the plant's description that its exact origin was unknown, but was presumably Rio de Janeiro. Three sheets of this cultivated plant are preserved at K, prepared by N.E. Brown and labelled in his handwriting as "Anthurium Glaziovii Hook. f.! Type specimen of Bot. Mag. t. 6833! Rio de Janeiro, Glaziou Hort. Kew June 8. 1881 labelled "Glaziou No. 3 188/1880" N.E. Brown". Therefore, we designated this specimen as the lectotype, referring to it as *Glaziou #188* (K) and consisting of the following three elements: sheet 1: (K000434142), sheet 2: (K000434143) and sheet 3: (K000434144).

The holotype of *A. nobile* was located at B herbarium (B100242945) and an isotype at P herbarium (P00748724). Based on the study of these type materials, the application of the names *A. glaziovii* and *A. nobile* is confirmed as heterotypic synonyms of *A. solitarium*, as previously accepted by Croat (1991).

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An unknown hotspot of plant diversity in the heart of the Central Apennine: flora and vegetation outline of Mt. Pozzoni-St. Rufo valley (Cittareale, Rieti)

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Abstract

Surprisingly enough, Italy still has some botanically unexplored areas; among these there are some territories between Lazio, Umbria and Abruzzo not included in any protected area. The study area, ranging for 340 ha, includes the mountainous area of Mt. Pozzoni-Mt. Prato-St. Rufo valley, which forms the upper part of the river Velino basin, located in the territory of the municipality of Cittareale (Rieti, Lazio), at an elevation from 1150 to 1903 m a.s.l. The substrate is mainly made of marly limestone of the Meso-Cenozoic Umbria-Marche sedimentary succession. The climate is Temperate and comprises vegetation belts from the montane to sub-alpine. Land cover is dominated by pastures and deciduous forests, with only a few hay meadows. 794 entities have been detected: 16% are considered rare or very rare for the regional territory with several floristic novelties for the regional flora, 6% of the total was found to be endemic to Italy and only eight *taxa* were aliens. Four *taxa* are new for the regional flora of Lazio: *Arum cylindraceum*, *Alopecurus pratensis* subsp. *pratensis*, *Hieracium bupleuroides* and *Trinia glauca* subsp. *glauca*. Forest vegetation is represented by beech forests, while dry grasslands are the most widespread vegetation type. The greatest phytocoenotic diversity was found within the secondary pastures. Particularly interesting is the plant community with *Iris marsica*, which suggests that limestone mountain ledges can represent a primary habitat for this endemic species of the Central Apennine. The presence of several habitats listed

in the EU Habitat Directive indicates how the lack of detailed territorial knowledge can lead to the non-designation of conservation sites in areas of high naturalistic value. These findings showed that botanical explorations in territories which are still not known could contribute significantly to the identification of areas of high interest in conserving plant diversity.

Keywords

endemic species, floristic records, Italy, phytosociology, Red lists

Introduction

The study of Central Apennine attracted the attention of several botanists in the past (e.g. Gravina 1812; Tenore 1830; Paolucci 1891; Crugnola 1900; Grande 1904; Zodda 1931, 1954; Anzalone 1951; Montelucci 1952, 1953) for the presence of the highest peaks of the Italian peninsula and of a rich flora, characterized by the presence of numerous endemics (Conti 2004). The floristic knowledge of this territory has since been progressively increased by numerous contributions (e.g. Conti 1998, 2004; Ballesi 2003; Tondi et al. 2003; Di Pietro et al. 2008; Iocchi et al. 2010; Gubellini et al. 2014; Falcinelli et al. 2016; Conti et al. 2018; Rosati et al. 2020) and some synopses have recently involved the National Parks of the Central Apennine (Conti and Bartolucci 2015, 2016; Conti et al. 2019). On a regional scale, the state of floristic knowledge of this territory has been synthesized in the recent checklist of Italian vascular flora by Conti et al. (2005) and Bartolucci et al. (2018) whilst, for Lazio, a detailed flora was published by Anzalone et al. (2010). Despite this, as already highlighted in previous publications (Scoppola and Blasi 2005; Bartolucci et al. 2012, 2019), floristic exploration of several areas of the Central Apennine cannot be considered exhaustive and homogeneous throughout the territory; consequently, particularly interesting species, of high phytogeographic interest, are still being discovered (e.g. Cancellieri et al. 2017; Filibeck et al. 2020).

As for interior areas of the Central Apennine, the attention of botanists has always been directed towards the main mountain ranges (e.g. Terminillo, Sibillini, Laga), thus large portions of the surrounding territory have been neglected, both by floristic and vegetational studies. This is the case with the area constituting the upland drainage basin of Velino River, located between Lazio, Umbria and Abruzzo administrative regions where only wet meadows were studied (Venanzoni 1992). Some studies on a national scale had already highlighted that this area lacked specific floristic knowledge; in particular the Map of the Important Plant Areas in Italy (Blasi et al. 2009, 2011) indicated how this part of the Central Apennine is surrounded by areas of high interest for plant conservation, suggesting that covering such a gap of knowledge (Hortal et al. 2015) could lead to interesting results for its flora and vegetation.

In this study we present the results of the flora and vegetation surveys conducted by the authors during the period 2008–2010 in the mountainous area of Mt. Pozzoni-Mt. Prato-St. Rufo valley, which represents the upland drainage basin of the Velino River.

Data and methods

Study area

The study area is included within the municipality of Cittareale (province of Rieti), belonging to the Lazio administrative region. It extends for 343 ha, at altitudes ranging between 1150 and 1903 m a.s.l. (Fig. 1). The study area includes the peak of Mt. Pozzoni (1903 m), extends to southeast including the mountain ridge of Mt. Laghetto-Mt. Prato (1834 m), ending in the south almost in correspondence of the provincial road Cittareale-Norcia. To the west the limit runs along the watershed that delimits the valley of St. Rufo-Pozzoni (Fig. 1).

The substrate is mainly made up of Meso-Cenozoic marly limestone ("Scaglia" formation) belonging to the Umbria-Marche succession; these sedimentary layers are heavily fractured and faulted, due to the proximity of a regional overthrust overlapping the Sibillini unit on the Gran Sasso-Cittareale unit (Calamita et al. 1995). The head of the valley is modeled by an evident glacial cirque, with a threshold placed at an altitude of 1660 m; the valley bottom is largely covered with slope debris, mixed with sediments of fluvio-glacial origin partly terraced and dissected by the upper course of Velino River. Along St. Rufo valley, an important karst cavity, 3 km long and over 400 m deep, opens at about 1440 m set in the marly limestones of Scaglia rossa formation (Gatti and Uffreduzzi 1989); the cavity has returned interesting fossil remains of bats, testifying to a cold period fauna dated to the end of the Pleistocene (Argenti et al. 2008).

Following the bioclimatic classification of Rivas-Martínez et al. (2011), the climate is Temperate oceanic/semi-continental, with the presence of two phytoclimatic belts along the altitudinal gradient, from the lower supratemperate to the lower orotemperate of the cacuminal areas; ombrotypes are comprised between humid and hyperhumid (Pesaresi et al. 2017).

The land cover is dominated by secondary grasslands, deciduous woodlands and small patches of artificial coniferous forest. Meadows and fallows are very limited and arable lands are nowadays completely missing in the study area. In the past they must have been quite common in the lower part of the valley, as evidenced by the presence of several still visible terraced parcels. Forests are used regularly as coppices and summer grazing of cattle and sheep is still widespread in this sector, together with horse grazing, which is conversely continuously present almost all year round.

The study area does not comprise any protected area, even if some Natura 2000 sites, defined according to European Union Habitat Directive (European Union 1992), are present in the neighboring territory of the Umbria region.

Flora and vegetation survey

The flora of the studied area was investigated in depth by carrying out numerous herborizations and field excursions, both in spring, summer and autumn, during three consecutive years from 2008 to 2010. Identification of vascular plants was mostly based

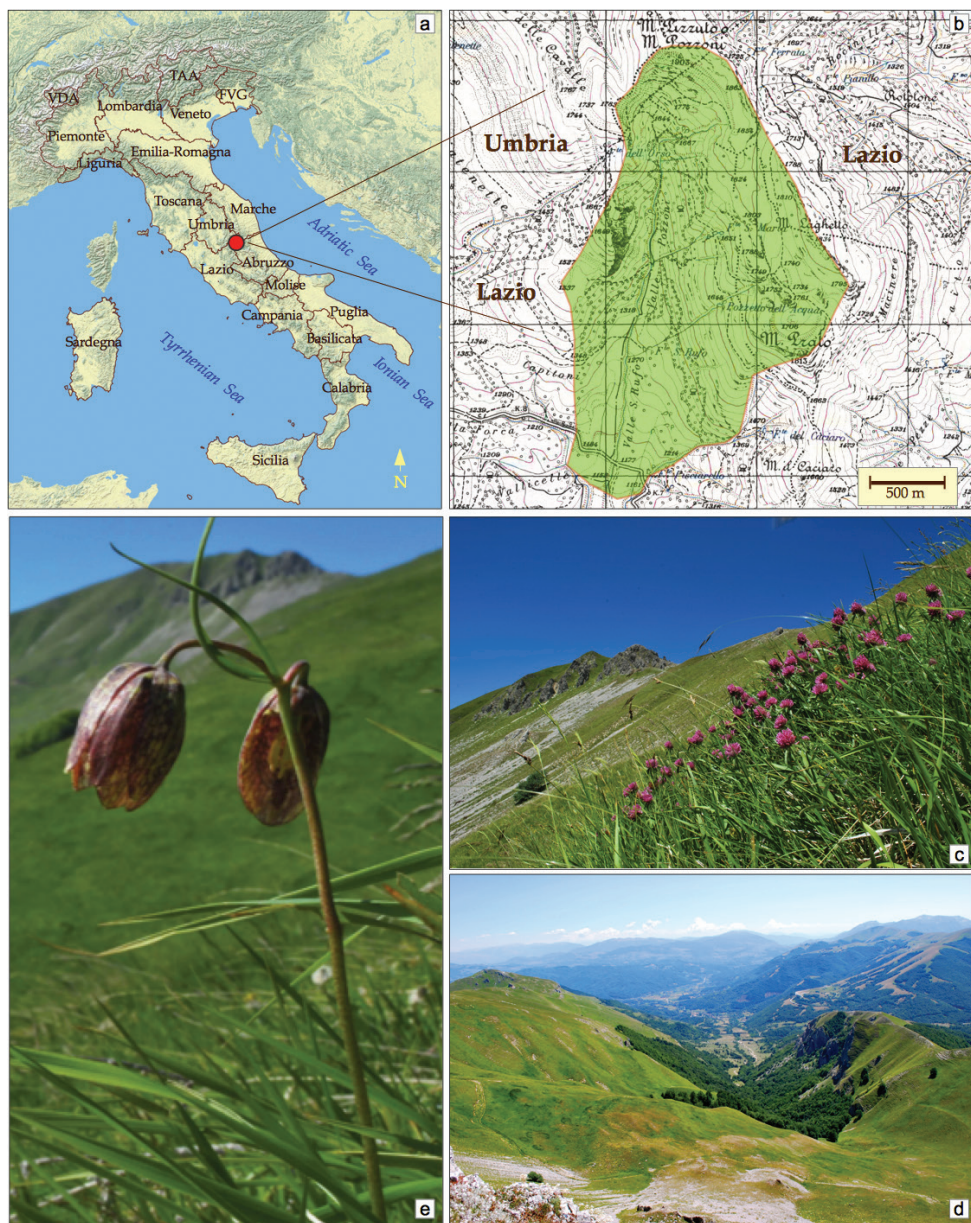


Figure 1. Study area location (a) and its landscape (c–e) b the limits of study area drawn on I.G.M.I-1:25,000 topographic map c high mountain pastures of Mt. Pozzoni dominated by *Brachypodium genuese* (DC.) Roem. et Schult. with *Trifolium alpestre* L. e *Fritillaria montana* Hoppe ex W.D.J.Koch, a rare species considered “Near Threatened” for extinction risk. Photos: R. Tranquilli and E. Del Vico.

on Pignatti (1982) and Flora Europaea (Tutin et al. 1968, 1972, 1976, 1980, 1993). *Taxa* delimitation was based on Anzalone et al. (2010) and nomenclature accords to Bartolucci et al. (2018), Galasso et al. (2018) and the subsequent updates summarized

in the “Portal to the Flora of Italy” (<http://dryades.units.it/floritaly/>). Families of vascular plants correspond to APG IV (2016), whereas life forms and chorotypes were retrieved from Pignatti (1982). Exsiccata are preserved in *Herbarium Lucanum* (HLUC), *Herbarium Del Vico* (Roma) and *Herbarium Lattanzi* (Roma), the latter is now moving to *Herbarium Sapienza* (RO). In the floristic list we reported the rarity level in the regional flora of Lazio for each *taxon* according to Anzalone et al. (2010), adopting three levels: rare, medium rare and very rare (coded as R, MR, RR). New *taxa* for the regional flora were marked with an asterisk. Italian endemics were retrieved from Bartolucci et al. (2018). As for alien *taxa*, we also reported the status of naturalization in the study area following the same codes used by Galasso et al. (2018). For each *taxon* the status of threatened species was derived by the published Italian Red lists (Conti et al. 1992, 1997) and updated, when new assessments were available, according to the most recent ones (Rossi et al. 2013; Orsenigo et al. 2020). Vegetation was analyzed using the phytosociological method (Braun-Blanquet 1965) by carrying out 30 surveys of the main vegetation types in the territory located in the field with a GPS unit with ± 5 m accuracy. The syntaxonomic nomenclature, at the level of alliance, order and class, follows the ‘Prodrome of the Italian Vegetation’ (Biondi et al. 2014).

Main vegetation types were identified through multivariate methods, Hierarchical Cluster Analysis and NMDS ordination. For each vegetation type a floristic-ecological description and the syntaxonomic framework were provided.

Results

Flora

794 *taxa* of vascular plants were identified, belonging to 331 genera and 69 families (Appendix 1). The families with more than 40 *taxa* (Fig. 2a) were: Asteraceae (107), Poaceae (71), Fabaceae (67), Caryophyllaceae (46) and Rosaceae (42). The most diverse genera were *Trifolium* (19), *Carex* (16), *Ranunculus* (14), *Hieracium* (13) and *Silene* (12) (Fig. 2b). Hemicryptophytes were the dominant life form (52%), followed by therophytes (21%) and geophytes (13%) (Fig. 2c). As for chorology, (Fig. 2d) species with Eurasian-Paleotemperate distribution prevailed (39%), slightly exceeding the Mediterranean element (29%). The Mediterranean group was mainly composed of Eurimediterranean (15%) and Mediterranean-Mountain species (6%). A significant contingent of Circumboreal species was also present (6%) while only a few species displayed eastern chorotypes (e.g. SE-European and Pontic).

Forty-seven *taxa*, approximately 6% of the total, were found to be endemic to Italy (Table 1). Only eight *taxa* were found to be aliens, among these *Malus domestica* (Suckow) Borkh., *Matricaria discoidea* DC., *Medicago sativa* L. and *Veronica persica* Poir. can be considered naturalized, whereas *Abies alba* Mill., *Abies cephalonica* Loudon, *Picea abies* (L.) H. Karst. and *Pinus nigra* J.F. Arnold subsp. *nigra* have been observed only as planted in reforested areas.

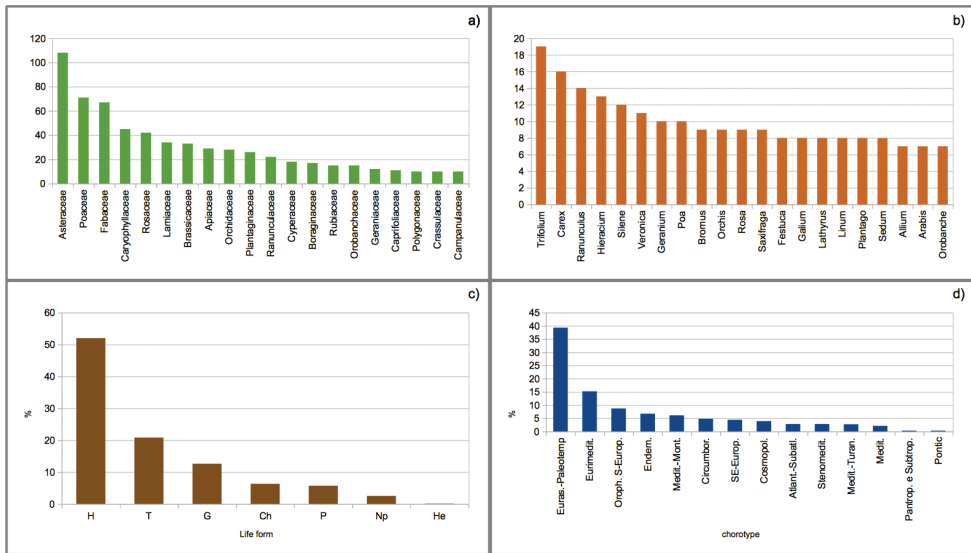


Figure 2. **a** number of *taxa* per family; only the families with more than 10 *taxa* are shown **b** number of *taxa* per genus; genera containing more than 7 *taxa* are shown **c** life form percentages **d** chorological spectrum of the detected flora in the study area.

Two *taxa*, *Arum cylindraceum* Gasp. and *Corydalis densiflora* C.Presl subsp. *apennina* F.Conti, Bartolucci & Uzunov have been recorded for the first time for Lazio during this research, whereas four *taxa* whose presence was considered doubtful for Lazio have been confirmed (*Alopecurus pratensis* L. subsp. *pratensis*, *Hieracium bupleuroides* C.C.Gmel., *Scandix macrorhyncha* C.A.Mey and *Trisetaria flavescens* (L.) Baumg. subsp. *flavescens*). These floristic records at regional level have been anticipated by Del Vico et al. (2014). However, probably due to oversight, *Alopecurus pratensis* subsp. *pratensis* is still considered to be confirmed for Lazio in the continuously updated database of the Portal to the Flora of Italy (Available at <http://dryades.units.it/floritaly>, accessed: 16/11/2020). Additionally, we herein confirm the presence of *Trinia glauca* (L.) Dumort. subsp. *glauca*, previously considered recorded erroneously for Lazio (Bartolucci et al. 2018).

Thirty-four *taxa* (Table 2) are considered very rare at the regional level (2010). Considering all the rare species (R, MR and RR), these *taxa* represent altogether approximately 20% of the studied flora.

The following 15 species have been identified as being at risk of extinction in the first published Italian red lists (Conti et al. 1992, 1997): *Iris marsica* I. Ricci et Colas., *Achillea tomentosa* L., *Carex panicea* L., *Fritillaria montana* Hoppe ex Koch, *Gentiana lutea* L. subsp. *lutea*, *Gentiana utriculosa* L., *Klasea nudicaulis* (L.) Fourr., *Lathyrus nissolia* L., *Lilium bulbiferum* L. subsp. *croceum* (Chaix) Jan, *Narcissus poeticus* L., *Onobrychis arenaria* (Kit.) DC. subsp. *arenaria*, *Ornithogalum comosum* L., *Scorzonera purpurea* L., *Trifolium phleoides* Willd. and *Trisetaria villosa* (Bertol.) Banfi et Soldano.

Table 1. List of endemic *taxa* recorded in the study area.

Taxon
<i>Armeria canescens</i> (Host) Ebel
<i>Astragalus sirinicus</i> Ten.
<i>Betonica alopecuroides</i> L. subsp. <i>divulsa</i> (Ten.) Bartolucci & Peruzzi
<i>Brachypodium genuense</i> (DC.) Roem. et Schult.
<i>Campanula tanfanii</i> Podlech
<i>Carduus nutans</i> L. subsp. <i>perspinosus</i> (Fiori) Arènes
<i>Centaurea ambigua</i> Guss. subsp. <i>ambigua</i>
<i>Centaurea ambigua</i> Guss. subsp. <i>nigra</i> (Fiori) Pignatti
<i>Cerastium tomentosum</i> L.
<i>Corydalis densiflora</i> C.Presl subsp. <i>apennina</i> F.Conti, Bartolucci & Uzunov
<i>Crepis lacina</i> Ten.
<i>Cynoglossum apenninus</i> L.
<i>Cynoglossum magellense</i> Ten.
<i>Digitalis micrantha</i> Roth ex Schweigg.
<i>Epipactis meridionalis</i> H. Baumann et R. Lorenz
<i>Erysimum majellense</i> Polatschek
<i>Erysimum pseudorhaeticum</i> Polatschek
<i>Galium magellense</i> Ten.
<i>Gentianella columbae</i> (Ten.) Holub
<i>Helictotrichon praetutiana</i> (Parl. ex Arcang.) Bartolucci, F.Conti, Peruzzi & Banfi subsp. <i>praetutiana</i>
<i>Iris marsica</i> I. Ricci et Colas.
<i>Klasea flavescens</i> (L.) Holub subsp. <i>cichoracea</i> (L.) Greuter et Wagenitz
<i>Koeleria splendens</i> C. Presl
<i>Linaria purpurea</i> (L.) Mill.
<i>Myosotis decumbens</i> Host subsp. <i>florentina</i> Grau
<i>Myosotis graui</i> Selvi
<i>Ornithogalum etruscum</i> Parl.
<i>Oxytropis pilosa</i> (L.) DC. subsp. <i>caputoi</i> (Moraldo et La Valva) Brilli-Catt., Di Massimo et Gubellini
<i>Pedicularis elegans</i> Ten.
<i>Potentilla rigoana</i> Th.Wolf
<i>Pulmonaria vallisae</i> A.Kern. subsp. <i>apennina</i> (Cristof. & Puppi) L.Cecchi & Selvi
<i>Ranunculus apenninus</i> (Chiov.) Pignatti
<i>Ranunculus thomasi</i> Ten.
<i>Rhinanthus wettsteinii</i> (Sterneck) Soó
<i>Saxifraga exarata</i> Vill. subsp. <i>ampullacea</i> (Ten.) D.A.Webb
<i>Saxifraga porophylla</i> Bertol. subsp. <i>porophylla</i>
<i>Sempervivum riccii</i> Iberite et Anzal.
<i>Senecio apenninus</i> Tausch
<i>Sesleria nitida</i> Ten.
<i>Silene notariisii</i> Ces.
<i>Siler montanum</i> Crantz subsp. <i>siculum</i> (Spreng.) Iamónico, Bartolucci & F.Conti
<i>Stipa dasyvaginata</i> Martinovský subsp. <i>apenninica</i> Martinovský et Moraldo
<i>Trifolium pratense</i> L. subsp. <i>semipurpureum</i> (Strobl) Pignatti
<i>Trisetaria villosa</i> (Bertol.) Banfi et Soldano
<i>Viola eugeniae</i> Parl. subsp. <i>eugeniae</i>

Meanwhile, in the most recent ones (Rossi et al. 2013; Orsenigo et al. 2020), excluding the species classified as “Least Concern”, *Viola kitaibeliana* Schult. was listed as “Endangered”, *Epipactis meridionalis* H. Baumann et R. Lorenz as “Vulnerable” and *Fritillaria montana*, *Gentiana lutea*, *Iris marsica* and *Senecio scopolii* Hoppe et Hornsch. ex Bluff et Fingerh. as “Near Threatened”.

Table 2. List of *taxa* found in the study area considered very rare (RR) at regional level including those that are new records for Lazio with respect to Anzalone et al. (2010).

Taxon
<i>Achillea tomentosa</i> L.
<i>Alchemilla cinerea</i> Buser
<i>Arabis auriculata</i> Lam.
<i>Arum cylindraceum</i> Gasp.
<i>Avenella flexuosa</i> (L.) Drejer subsp. <i>flexuosa</i>
<i>Carex liparocarpos</i> Gaudin subsp. <i>liparocarpos</i>
<i>Carex panicea</i> L.
<i>Centaurea arachnoidea</i> subsp. <i>adonidifolia</i> (Rchb.) F. Conti, Moraldo & Ricceri
<i>Conringia austriaca</i> (Jacq.) Sweet
<i>Corydalis densiflora</i> C.Presl subsp. <i>apennina</i> F.Conti, Bartolucci & Uzunov
<i>Epipactis meridionalis</i> H. Baumann et R. Lorenz
<i>Erysimum majellense</i> Polatschek
<i>Gagea minima</i> (L.) Ker Gawl.
<i>Genista sagittalis</i> L.
<i>Herniaria glabra</i> L. subsp. <i>nebrodensis</i> Nyman
<i>Hienacium tomentosum</i> L.
<i>Hypericum hyssopifolium</i> Chaix
<i>Iris marsica</i> I. Ricci et Colas.
<i>Juncus striatus</i> Schousb. ex E. Mey.
<i>Mcneillia graminifolia</i> (Ard.) Dillenb. & Kadereit subsp. <i>clandestina</i> (Port.) Dillenb. & Kadereit
<i>Medicago prostrata</i> Jacq. subsp. <i>prostrata</i>
<i>Onobrychis arenaria</i> (Kit.) DC. subsp. <i>arenaria</i>
<i>Oxytropis pilosa</i> (L.) DC. subsp. <i>caputoi</i> (Moraldo et La Valva) Brilli-Catt., Di Massimo et Gubellini
<i>Parnassia palustris</i> L. subsp. <i>palustris</i>
<i>Pilosella cymosa</i> (L.) F.W.Schultz & Sch.Bip.
<i>Pilosella hoppeana</i> (Schult.) F.W.Schultz & Sch.Bip.
<i>Pilosella piloselloides</i> (Vill.) Soják subsp. <i>praealta</i> (Vill. ex Gochnat) S.Bräut. & Greuter
<i>Scorzonera laciniata</i> L.
<i>Scorzonera purpurea</i> L. subsp. <i>purpurea</i>
<i>Thymus oenipontanus</i> Heinr.Braun ex Borbás
<i>Trifolium phleoides</i> Willd.
<i>Trinia glauca</i> (L.) Dumort. subsp. <i>glauca</i>
<i>Trisetaria villosa</i> (Bertol.) Banfi et Soldano
<i>Tulipa pumila</i> Moench

Plant community descriptions

Hierarchical cluster analysis and NMDS ordination (the latter not shown) enabled the detection of 10 clusters that were clearly interpretable, floristically and ecologically, as shown in Fig. 3. A further inspection of the ordered table led to the identification of 12 plant communities (some of these represented by only one relevè); the greatest phyto-coenotic diversity was found within the secondary pastures. The relevès of these communities are presented in Suppl. material 1: Table S1 and described in detail below.

Forests and shrublands

Forest vegetation in the Mt. Pozzoni valley is represented almost exclusively by beech forests (cluster 1). *Quercus cerris* woodlands are present only on the edges of the investigated area on southern exposures, but they are widespread at lower altitudes where flyschoid substrates emerge. Small patches of coniferous plantation, planted for reforestation pur-

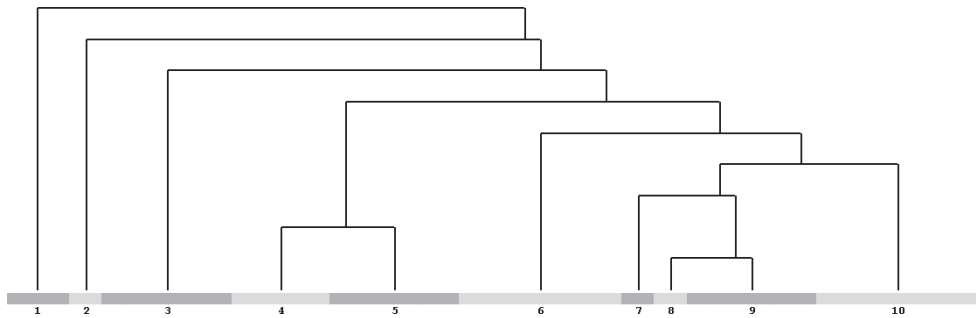


Figure 3. Hierarchical clustering resulting from Modified TWINSpan analysis. Cluster 1) *Fagus sylvatica* woodlands; 2) *Amelanchier ovalis* forest edges; 3) *Saxifraga callosa* chasmophytic vegetation; 4) *Cynosurus cristatus* hay meadows; 5) *Bromopsis erecta* montane grasslands; 6) *Iris marsica* community; 7) *Rumex scutatus* screes vegetation; 8) *Paronychia kapela* rocky outcrops vegetation; 9) upper-montane grasslands, including also the *Brachypodium genuense* community and the *Astragalus sempervirens* community; 10) chamaephytes-rich dry grasslands.

poses (e.g. *Pinus nigra*, *Abies spp.*) are also present. *Fagus sylvatica* forests cover approx. 60 ha, forming a more or less continuous belt between 1350 and 1700 m a.s.l.; they are managed as coppices with stands, meanwhile mixed aged and old-growth formations are absent. Tree layer is always dominated by *Fagus sylvatica*, frequently accompanied by *Acer pseudoplatanus* and *Acer opalus* subsp. *obtusatum*. In relevè no. 1, surveyed at 1500 m, in the tree layer we also found *Tilia plathyphyllos* and *Prunus avium*, while in rel. no. 2, placed at 1360 m, the presence of *Quercus cerris* and *Acer campestre* can be noted. In the herb layer, nemoral species commonly found in mesic and beech forests are present, such as *Cardamine bulbifera*, *Pulmonaria vallisarsae* subsp. *apennina*, *Moehringia trinervia*, *Rabiera holostea* (= *Stellaria holostea*); from a phytosociological point of view most of these species are typical of *Fagetalia sylvaticae* order. Related to the altitude, the second relevè in table S1 hosts a larger contingent of species having a relatively thermophilic character, such as *Lathyrus venetus*, *Cyclamen hederifolium*, *Cephalanthera damasonium*, *Sesleria autumnalis*, *Viola odorata*, which also characterize the lower altitudinal vegetation belt dominated by oak forests. These relevès can be classified in the alliance *Aremonio agrimonoidis-Fagion sylvaticae* (*Quercus roboris-Fagetalia sylvaticae*, *Fagetalia sylvaticae*) describing the beech forests of E-Europe, Balkans and extending to Central-Northern Apennine (Biondi et al. 2014). The beech woods at lower altitudes, as evidenced by the relevè no. 2, show a floristic composition referable to *Lathyro veneti-Fagetum sylvaticae*, a widespread association which frames beech forests of lower supratemperate belt of the Central Apennines mainly on limestones (Blasi et al. 2010). At higher altitudes, generally above 1500 m, it is possible to observe a clear decrease in the number of species of *Quecetalia pubescenti-petraeae*, indicating a shift towards the association *Cardamino kitaibelii-Fagetum sylvaticae* Ubaldi ex Ubaldi 1995. The latter represents the potential vegetation of the upper supratemperate belt, which in the St. Rufo valley is almost completely substituted by the secondary grasslands belonging to *Festuco-Brometea* class.

The edge of the beech forest (cluster 2), in some cases, presents characteristic pre-forest shrub formations, as evidenced by relevè no. 3, physiognomically dominated by

Amelanchier ovalis, *Atadinus fallax* (= *Rhamnus alpina* subsp. *fallax*) and *Sorbus aria*, accompanied by smaller shrubs of *Rosa montana*, *Crataegus laevigata* and *Cytisophyllum sessilifolium*. These formations can be attributed to the association *Rhamno alpinae-Amelanchieretum ovalis* (*Berberidion vulgaris*, *Prunetalia spinosae*, *Rhamno-Prunetea*), described for Sibillini mountains by Pedrotti (1994) and revised by Cutini et al. (2002), even if the herb layer appeared strongly impoverished.

Along the river incisions, especially in the middle and lower part of the investigated area, there are other shrub and pre-forest formations of difficult syntaxonomic classification at association level, dominated by *Corylus avellana* and *Acer spp.*, which are not represented in our surveys.

Sparse vegetation of outcrops

Limestone rocky walls, from 1400 m up to the highest peak of Mt. Pozzoni, are colonized by a characteristic chasmophytic vegetation dominated by *Saxifraga callosa* (rel. 4–6) with the presence of *Saxifraga paniculata*, *Campanula tanfanii* and *Saxifraga exarata* subsp. *ampullacea* (cluster 3). Even if *Trisetum villosum* was not found within the relevés, this species is present in such habitats of the study area (see floristic list). The overall floristic composition of these coenoses allows us to refer them to *Saxifraga australis-Trisetetum bertolonii* (*Saxifragion lingulatae*, *Potentilletalia caulescentis*, *Asplenietea trichomanis*), a widespread association in the Umbria-Marche Apennine and Central Italy (Giancola and Stanisci 2002).

On sub-vertical calcareous rocky outcrops, large boulders and ledges, we found some peculiar communities characterized by the abundance and dominance of *Sedum album*. At higher altitudes, above 1650 m (rel. no. 7–9) these coenoses are characterized by *Paronychia kapela*, *Anthemis cretica* subsp. *columnae*, *Sempervivum arachnoideum* and *Poa molinerii* (cluster 8). The floristic composition is dominated by species belonging to *Sedo albi-Scleranthetea biennis* such as *Sedum album*, *Sempervivum arachnoideum* and *Petrosedum rupestre* together with several transgressive species from dry grasslands of *Festuco-Brometea*. These coenoses developed on a calcareous substrate have to be attributed to the alliance *Alysso alyssoidis-Sedion albi*. At the alliance level, the same attribution is valid for the relevés no. 10–12 (cluster 6), carried out on large rocky ledges at lower altitudes (ca 1500 m) which are physiognomically characterized by the endemic *Iris marsica* and by *Petrosedum rupestre*, with the presence of several thermophilous species such as *Crupina vulgaris*, *Lactuca perennis* subsp. *perennis* and *Centaurea deusta*. The survey no. 13, characterized also by the presence of *Iris marsica* but with the dominance of *Bromopsis erecta*, represents the ecotone between the vegetation of the *Alysso-Sedion* on ledges and the contiguous dry grasslands of *Festuco-Brometea*, which are developed where the soils are more evolved and deeper. Our current state of knowledge about *Alysso-Sedion* vegetation in the studied area does not make it possible to find a clear reference to any already described association; therefore these two coenoses are provisionally indicated as *Sedum album-Paronychia kapela* community and *Sedum album-Iris marsica* community.

Calcareous screes and mobile debris have only small surfaces in the study area; they are represented by the relevè no. 14 (cluster 7), carried out at medium altitude (1480 m), on the scree fed by the characteristic rocky spike present on the right side of the St. Rufo valley, known as Peschio dell'Aquila. This plant community has sparse cover and is characterized by *Rumex scutatus*, *Galium magellense* and *Scrophularia canina*. The scree vegetation of the alpine and subalpine belt in the Central Apennine is quite well known, while at lower altitudes it has rarely been investigated (see Ciaschetti et al. 2020 and references therein). Also in this case, it was not possible to identify a reference at the association level for the *Petrosedum rupestre*-*Rumex scutatus* community of the study area. The presence of *Galium magellense* suggests a probable framework within the alliance *Linario-Festucion dimorphae*, describing Apennine glaucochromous communities of calcareous screes, from the supratemperate to orotemperate thermotypes, even if most of the diagnostic species are lacking. The floristic impoverishment could be justified by the fact that in the study area the most important screes are located at rather low altitudes, at the lower ecological limit of *Linario-Festucion dimorphae*. In fact, at higher altitude we observed in this habitat also the presence of *Drypis spinosa*. Although the abundance of *Petrosedum rupestre* would seem to indicate an affinity with the association *Linario purpureae*-*Petrosedum rupestre* recently described for several localities of the Abruzzo territory (Ciaschetti et al. 2020), the overall floristic composition of the community herein reported is rather different from the one described for Abruzzo and the diagnostic species are absent. In addition, this thermophilic association was referred to a different order and alliance (*Scrophulario bicoloris*-*Helicrhyselia italici* Brullo, 1984, *Linarion purpureae* Brullo, 1984) within the class *Thlaspietea rotundifolii*.

Meadows and grasslands

In the lower part of the study area, at an altitude below 1200 m, small patches of regularly mowed grasslands are still present (cluster 4; rel. no. 15–17), developed on deep, partially terraced, well drained alluvial soil. They are species rich, with 54 species per plot on average. Floristically, the dominant species (*Trifolium incarnatum*, *T. repens*, *T. pratense*, *Poa trivialis*, *Dactylis glomerata*, *Phleum nodosum*, *Cynosurus cristatus*) are indicators of the class *Molinio-Arrhenatheretea*, accompanied by several nitrophilous and ruderal species such as *Bromus hordeaceus* and *Dasyphyrum villosum*. The perennial meso and supratemperate Apennine grasslands, usually grazed and mowed once a year, which develop on mesotrophic soils with good water availability and rich in nutrients, have been commonly attributed to the *Cynosurion cristati* alliance, (see the Italian review of the alliance in Blasi et al. 2012 and references therein). The classification of *Cynosurion* grasslands has often been considered to be somewhat problematic (Blasi et al. 2012) due to the fact that exclusive species are lacking and most of the diagnostic species range widely from both a geographical and ecological point of view. Although the surveys carried out in the study area can be referred to *Cerastio arvensis*-*Cynosurion cristati* sub-alliance, the attribution to an already described association remains unresolved, also be-

cause the Apennine's associations have not yet been the subject of a critical review which clarifies delimitation and differential species. Probably the closest association could be identified in the *Colchico lusitani-Cynosuretum*, repeatedly reported on the Umbria-Marche Apennines (e.g. Allegrezza 2003; Biondi et al. 2004; Catorci et al. 2007).

The most widespread vegetation in the study area is represented by the dry grasslands of the *Festuco-Brometea* class (cluster 5; rel. no. 18–30), mainly used for horse and cattle grazing, which replaced the beech forest vegetation on the mountain slopes. In particular, they can be classified in *Phleo ambigu-Brometalia* order including xerophilous and semi-mesophilous secondary grasslands of the Central-southern Apennines, that occur from the supramediterranean to the upper supratemperate thermotype. These plant communities have a high physiognomic and floristic diversification in relation to local characteristics of the site (exposure, rockiness, soil depth and pH) and to the degree of grazing. The relevés no. 18–22 (cluster 10) are particularly species rich (mean 52 species per releve) and were found at lower altitude (max 1300 m), frequently on moraine deposits. They are characterized by a high incidence of chamaephytes (e.g. *Helianthemum apenninum*, *H. oelandicum* subsp. *incanum*, *H. nummularium* subsp. *obscurum*, *Thymus longicaulis*, *T. oenipontanus*) and by the presence of several therophytes (e.g. *Trifolium campestre*, *T. scabrum*, *Euphrasia liburnica*). However, they are physiognomically dominated by hemicryptophytes such as *Bromopsis erecta*, *Festuca circummediterranea*, *Phleum hirsutum* subsp. *ambiguum*. From a phytosociological point of view, the ecology and floristic analysis led to the inclusion of this community in the association *Asperulo purpureae-Brometum erecti*, frequently reported for the Umbria-Marche Apennine (e.g. Catorci et al. 2007) and described with several variants and subassociations. The releve no. 23 represents a particular stand on strongly inclined slopes at higher altitude (1500 m) dominated by *Bromopsis erecta* but with a high cover of *Securigera varia* and *Petrosedum rupestre*, which cannot be easily classified at the level of association.

At higher altitude (cluster 9) and on more inclined slopes, in the dry grasslands can be noted the presence of *Sesleria nitida* and a floristic composition close to the association *Seslerio nitidae-Brometum erecti*. Near the summit area of the mountain slopes or in eroding areas, the floristic composition becomes impoverished and the spiny chamaephyte *Astragalus sempervirens* was found as co-dominant with *Sesleria nitida*. The reference for this community is the *Astragalo sempervirenti-Seslerietum nitidae*, an association described for the grasslands of the summit sectors of Coscerno and Civitella Mountains in Umbria (Biondi and Ballelli 1995). The parts at higher altitudes of the mountain slopes, relying on calcareous-marly substrata, are occupied by grasslands dominated by *Brachypodium genuense*. The presence of some acidophilic species (e.g. *Luzula campestris*, *Genista sagittalis*, *Campanula micrantha*) which are frequently found in the vegetation of *Nardetea strictae* (see Di Pietro et al. 2017 for a review of these communities in the Italian Peninsula), indicates the presence of decarbonated soils with a lower pH. Despite this, the floristic composition is clearly dominated by *Festuco-Brometea* species; therefore, the reference for these coenoses, is the order *Phleo ambigu-Brometalia erecti* and the alliance *Phleo-Bromion erecti*, but unlike the previous ones, in this case the arid high-montane grasslands are to be referred to the sub-alliance *Brachypodienion genuensis*. At the association level, the floristic analysis led to refer-

ring this community to the *Potentillo rigoanae-Brachypodium genuensis*, an association quite widespread in the Central Apennine, in particular in Lazio and Abruzzo regions (Lucchese et al. 1995).

We did not survey other relevant coenoses that are present with significant extensions but only outside the surveyed area: the mountain acidophilic grasslands of the *Nardetea strictae* class, present on the northern slopes of Mt. Pozzoni and in the concave morphologies in the high-mountain orotemperate belt and the discontinuous prairies of the steep slopes dominated by *Sesleria juncifolia*; the latter can be observed on the steep and inaccessible mountain slope surrounding the cliff of Peschio dell'Aquila.

Discussions and conclusions

The floristic composition of the study area, at the family level, does not significantly differ from the neighboring regional floras (Anzalone et al. 2010; Conti 1998). The percentage of endemics (6%) was quite similar to the flora of the nearby Terminillo massif (5.1%) studied by Montelucci (1952, 1953), slightly lower than the flora of Gran Sasso National Park (8.7%) (Conti and Bartolucci 2016), but perfectly aligned, for example, with the value of Abruzzo National Park (6.5%) (Conti and Bartolucci 2015). This difference can be due to the fact that the higher elevation of Gran Sasso National Park allows the presence of a large alpine vegetation belt, known to host several endemic *taxa*. On the contrary, in the study area, Terminillo massif and Abruzzo National Park, the alpine belt is completely absent. In fact, only considering the ipsophilous flora (above 1900 m a.s.l.), Conti (2004) calculated a rate of 13.2% of endemics for Central Apennine.

Similarly, from the chorological point of view, no particular differences were observed with the flora of Terminillo, except for the contingent of Illirian, SE-European and Pontic species, which are slightly more represented in the flora of the Terminillo, probably because the flora of the latter also includes the lower vegetation belts of thermophilous oak forests and mixed deciduous woods, known to be characterized by the presence of numerous eastern species (Blasi et al. 2004).

Based on overall floristic results, the area of the St. Rufo Valley-Mt. Pozzoni can be considered of particular floristic interest, due to the high number of endemic and rare species detected, as recognized also by Lucchese (2018) who incorporated our preliminary data (Del Vico et al. 2014) in his recent, but not yet completed, atlas of the flora of Lazio.

The studied area is also undoubtedly characterized by a high floristic diversity if we consider that the 794 *taxa* recorded in this study have been found in an area of only 3,4 km². The relevance of these data can be easily understood by comparing the number of *taxa* detected in local floras that have a comparable extension. For example, considering the data reported in Pierini et al. (2009), regarding numerous floras from Tuscany, and limiting to those having extension between 2 and 8 km², only in the flora of Mt. Ferrato a similar number of *taxa* (800) was found, but within a study area almost double the size (6 km²) (Biagioli et al. 2002).

Moreover, we have to keep in mind that the studied area has a modest altitude range, relatively few types of lithologies and thus, a limited number of habitats. Despite this, the vegetation analysis revealed the presence of varied and species rich plant communities, with 311 *taxa* detected in only 30 relevés. As expected, the higher number of vegetation types was found within the secondary habitats of mountain pastures and dry grasslands. Particularly interesting is the plant community with *Iris marsica* (referred to the *Alyso-Sedion* alliance), which indicates, probably for the first time, as limestone mountain ledges can represent a primary habitat for this species endemic of the Central Apennine.

Almost all the plant communities identified here are referable to habitats listed by the Habitat Directive (habitat codes: 6110, 6210, 6520, 8120, 8210, 9210), some of them with priority status (6110, 6210, 9210), thus their presence would have required the proposal of a Natura 2000 site according to the Habitat Directive (European Union 1992).

The intriguing aspects of this territory combined with, until recently, the complete lack of detailed botanical knowledge, led, in 2016, the Italian Botanical Society to carry out, in the Cittareale municipality, an annual field trip of the working group for Floristics, Systematics and Evolution. During the field trip, with the participation of some of the authors, several other additional localities (e.g. Mt. Boragine 1824 m a.s.l.), surrounding those herein investigated, were explored floristically (Bartolucci et al. 2019).

The very limited number of alien species identified, none of which is considered invasive, can be considered an indicator of the fairly good state of conservation of the territory in which a completely traditional land use still persists. However, reforested areas, planted with several non- native conifers, are now composed of mature trees able to produce seeds. Recruitment from these could involve a process of spontaneization of non-native coniferous as frequently observed in other territories of Central Apennine, involving, for example, the spread of *Pinus nigra*. Moreover, the presence of *Abies cephalonica* and *Picea abies* in reforested areas could also lead to spontaneization of these species in Lazio, as already observed in the neighboring Abruzzo region (Galasso et al. 2018).

Taking into account how important updating distribution data is, for example for the Red List assessment (Orsenigo et al. 2020), it is certainly possible to affirm that this study constitutes a valid contribution towards filling the gap in our botanical knowledge of a sector of the Central Apennines of high conservation interest.

Syntaxonomic scheme

ASPLENIETEA TRICHOMANIS (Br.-Bl. in Meier & Br.-Bl., 1934) Oberdorfer, 1977

POTENTILLETALIA CAULESCENTIS Br.-Bl. in Br.-Bl. & Jenny, 1926

Saxifragion australis Biondi & Ballelli ex Brullo 1984

Saxifrago-Trisetetum villosi Biondi & Ballelli, 1982

THLASPIETEA ROTUNDIFOLII Br.-Bl., 1948

THLASPIETALIA STYLOSI Avena & Bruno, 1975

Linarion purpureae Brullo, 1984

Petrosedum rupestre-Rumex scutatus community**SEDO ALBI-SCLERANTHETEA BIENNIS Br.-Bl., 1955**

ALYSSO ALYSSOIDIS-SEDETALIA ALBI Moravec, 1967

Alyso alyssoidis-Sedion albi Oberdorfer & Müller in Müller 1961

Sedum album-Paronychia kapela community*Sedum album-Iris marsica* community**FESTUCO VALESIIACAE-BROMETEA ERECTI Br.-Bl. & Tüxen ex Br.-Bl. 1949**PHLEO AMBIGUI-BROMETALIA ERECTI Biondi, Allegrezza, Blasi & Galdenzi in Biondi, Allegrezza, Casavecchia, Galdenzi, Gasparri, Pesaresi, Vagge and Blasi 2014
Phleo ambigu-Bromion erecti Biondi, Ballelli, Allegrezza & Zuccarello ex Biondi and Galdenzi 2012

Phleo ambigu-Bromenion erecti Biondi, Allegrezza & Zuccarello ex Di Pietro 2011

Asperulo purpureae-Brometum erecti Biondi & Ballelli ex Di Pietro 2011*Seslerio nitidae-Brometum erecti* Bruno & Covarelli, 1968*Astragalo sempervirentis-Seslerietum nitidae* Biondi & Ballelli, 1995

Brachypodienion genuensis Biondi, Ballelli, Allegrezza & Zuccarello ex Biondi and Galdenzi 2012

Potentillo rigoanae-Brachypodietum genuensis Lucchese et al., 1995**MOLINIO-ARRHENATHERETEA Tüxen, 1937**

TRIFOLIO REPENTIS-PHLEETALIA PRATENSIS Passarge, 1969

Cynosurion cristati Tüxen, 1947

Cerastio arvensis-Cynosurenion cristati Blasi et al., 2012

Trifolium incarnatum-Cynosurus cristatus community**RHAMNO CATHARTICAE-PRUNETEA SPINOSAE Rivas Goday & Borja ex Tüxen 1962**

PRUNETALIA SPINOSAE Tüxen, 1952

Berberidion vulgaris Br.-Bl., 1950

Rhamno alpinae-Amelanchieretum ovalis Pedrotti, 1994**QUERCO ROBORIS-FAGETEA SYLVATICAE Br.-Bl. & Vlieger in Vlieger 1937**

FAGETALIA SYLVATICAE Pawłowski in Pawłowski, Sokołowski and Wallisch 1928

Aremonio agrimonoidis-Fagion sylvaticae (Horvat) Borhidi in Török, Podani and Borhidi 1989

Lathyro veneti-Fagetum sylvaticae Biondi et al. ex Biondi, Casavecchia, Pinzi, Allegrezza and Baldoni in Biondi, Allegrezza, Casavecchia, Galdenzi, Gigante and Pesaresi 2013

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Appendix I

Floristic list of detected *taxa* in the study area. E = endemic *taxon* of Italian territory; R, MR, RR = increasing level of rarity, from rare to very rare in the regional flora of Lazio coded as in Anzalone et al. (2010). Floristic novelties for the regional flora are marked with asterisk. CULT = *taxon* detected only as cultivated. NAT = alien *taxon* naturalized in the study area.

		Taxon
FERNS AND FERNS ALLIES		
	Selaginellaceae	
		<i>Selaginella denticulata</i> (L.) Spring
	Equisetaceae	
		<i>Equisetum palustre</i> L.
		<i>Equisetum ramosissimum</i> Desf.
		<i>Equisetum telmateja</i> Ehrh.
	Ophioglossaceae	
R		<i>Botrychium lunaria</i> (L.) Sw.
	Cystopteridaceae	
		<i>Cystopteris fragilis</i> (L.) Bernh.
	Aspleniaceae	
		<i>Asplenium ceterach</i> L. subsp. <i>bivalens</i> (D.E.Mey.) Greuter & Burdet
		<i>Asplenium onopteris</i> L.
		<i>Asplenium ruta-muraria</i> L. subsp. <i>ruta-muraria</i>
		<i>Asplenium trichomanes</i> L. subsp. <i>quadrivalens</i> D.E. Mey.
GYMNOSPERMS		
	Pinaceae	
CULT		<i>Abies alba</i> Mill.
CULT		<i>Abies cephalonica</i> Loudon
CULT		<i>Picea abies</i> (L.) H. Karst.
CULT		<i>Pinus nigra</i> J.F. Arnold subsp. <i>nigra</i>
	Cupressaceae	
		<i>Juniperus communis</i> L. subsp. <i>alpina</i> (Suter) Celak
		– <i>taxon</i> delimitation according to Anzalone et al. (2010)
		<i>Juniperus communis</i> L. subsp. <i>communis</i>
		– <i>taxon</i> delimitation according to Anzalone et al. (2010)
		<i>Juniperus oxycedrus</i> L. subsp. <i>oxycedrus</i>
		– <i>taxon</i> delimitation according to Anzalone et al. (2010)
ANGIOSPERMS		
	Araceae	
*RR		<i>Arum cylindraceum</i> Gasp. ex Guss.
		<i>Arum italicum</i> Mill. subsp. <i>italicum</i> var. <i>italicum</i>
		<i>Arum maculatum</i> L.
	Colchicaceae	
		<i>Colchicum lusitanum</i> Brot.
	Liliaceae	
R		<i>Fritillaria montana</i> Hoppe ex W.D.J.Koch
		<i>Gagea lutea</i> (L.) Ker Gawl.
RR		<i>Gagea minima</i> (L.) Ker Gawl.
		<i>Gagea villosa</i> (M. Bieb.) Sweet
		<i>Lilium bulbiferum</i> L. subsp. <i>croceum</i> (Chaix) Jan
RR		<i>Tulipa pumila</i> Moench
	Orchidaceae	
		<i>Anacamptis morio</i> (L.) R.M.Bateman, Pridgeon & M.W.Chase
		<i>Anacamptis pyramidalis</i> (L.) Rich.
		<i>Cephalanthera damasonium</i> (Mill.) Druce
		<i>Cephalanthera longifolia</i> (L.) Fritsch
		<i>Cephalanthera rubra</i> (L.) Rich.
		<i>Coeloglossum viride</i> (L.) Hartm.

Taxon	
	<i>Dactylorhiza maculata</i> (L.) Soó subsp. <i>saccifera</i> (Brongn.) Diklić
	<i>Dactylorhiza sambucina</i> (L.) Soó
E – RR	<i>Epipactis atrorubens</i> (Hoffm.) Besser
	<i>Epipactis meridionalis</i> H. Baumann & R. Lorenz
	<i>Epipactis microphylla</i> (Ehrh.) Sw.
	<i>Epipactis muelleri</i> Godfery
	<i>Gymnadenia conopsea</i> (L.) R. Br.
	<i>Himantoglossum adriaticum</i> H. Baumann
	<i>Neotinea tridentata</i> (Scop.) R.M.Bateman, Pridgeon & M.W.Chase
	<i>Neotinea ustulata</i> (L.) R.M.Bateman, Pridgeon & M.W.Chase
	<i>Neottia nidus-avis</i> (L.) Rich.
	<i>Neottia ovata</i> (L.) Bluff & Fingerh.
	<i>Ophrys apifera</i> Huds.
	<i>Ophrys holosericea</i> (Burnm.f.) Greuter subsp. <i>holosericea</i>
	<i>Ophrys sphegodes</i> Mill. subsp. <i>sphgodes</i>
	<i>Orchis anthropophora</i> (L.) All.
MR	<i>Orchis mascula</i> (L.) L. subsp. <i>mascula</i>
	<i>Orchis pallens</i> L.
	<i>Orchis pauciflora</i> Ten.
	<i>Orchis purpurea</i> Huds.
	<i>Orchis simia</i> Lam.
	<i>Platanthera bifolia</i> (L.) Rich.
Iridaceae	
E – RR	<i>Crocus vernus</i> (L.) Hill
	<i>Iris marsica</i> I. Ricci & Colas.
Asphodelaceae	
	<i>Asphodeline lutea</i> (L.) Rchb.
Amaryllidaceae	
	<i>Allium dentiferum</i> Webb et Berthel.
R	<i>Allium flavum</i> L. subsp. <i>flavum</i>
R	<i>Allium horvatii</i> Lovrić
R	<i>Allium lusitanicum</i> Lam.
	<i>Allium sphaerocephalon</i> L.
	<i>Allium tenuiflorum</i> Ten.
	<i>Allium vineale</i> L.
	<i>Galanthus nivalis</i> L.
	<i>Narcissus poeticus</i> L.
Asparagaceae	
	<i>Loncomelos brevistylus</i> (Wolfner) Dostál
	<i>Loncomelos pyrenaicum</i> (L.) Hrouda ex J. Holub subsp. <i>pyrenaicum</i>
	<i>Muscari neglectum</i> Guss. ex Ten.
MR	<i>Ornithogalum comosum</i> L.
E	<i>Ornithogalum etruscum</i> Parl.
	<i>Ornithogalum umbellatum</i> L.
	<i>Polygonatum multiflorum</i> (L.) All.
	<i>Scilla bifolia</i> L.
Juncaceae	
	<i>Juncus articulatus</i> L. subsp. <i>articulatus</i>
	<i>Juncus bufonius</i> L.
	<i>Juncus inflexus</i> L. subsp. <i>inflexus</i>
RR	<i>Juncus striatus</i> Schousb. ex E. Mey.
	<i>Luzula campestris</i> (L.) DC. subsp. <i>campestris</i>
	<i>Luzula sylvatica</i> (Huds.) Gaudin subsp. <i>sylvatica</i>
Cyperaceae	
	<i>Carex caryophyllea</i> Latourr.
	<i>Carex distans</i> L.
	<i>Carex divulsa</i> Stokes

Taxon	
	<i>Carex flacca</i> Schreb. subsp. <i>flacca</i>
	<i>Carex halleriana</i> Asso
	<i>Carex hirta</i> L.
	<i>Carex kitaibeliana</i> Degen ex Bech.
R	<i>Carex leporina</i> L.
RR	<i>Carex liparocarpos</i> Gaudin subsp. <i>liparocarpos</i>
	<i>Carex macrolepis</i> DC.
	<i>Carex otrubae</i> Podp.
R	<i>Carex pinnata</i> F.W. Schultz
RR	<i>Carex panicea</i> L.
	<i>Carex pendula</i> Huds.
	<i>Carex sylvatica</i> Huds.
R	<i>Carex viridula</i> Michx.
	<i>Schoenoplectus lacustris</i> (L.) Palla
	<i>Scirpoides holoschoenus</i> (L.) Soják
Poaceae	
R	<i>Agrostis canina</i> L.
	<i>Agrostis stolonifera</i> L.
	<i>Aira caryophyllaea</i> L. subsp. <i>caryophyllaea</i>
	<i>Aira elegantissima</i> Schur subsp. <i>elegantissima</i>
	<i>Alopecurus aequalis</i> Sobol.
*	<i>Alopecurus pratensis</i> L. subsp. <i>pratensis</i>
	<i>Anisantha sterilis</i> (L.) Nevski
	<i>Anisantha tectorum</i> (L.) Nevski
	<i>Anthoxanthum odoratum</i> L. subsp. <i>odoratum</i>
	<i>Arrhenatherum elatius</i> (L.) P. Beauv. ex J. Presl & C. Presl subsp. <i>elatius</i>
RR	<i>Avenella flexuosa</i> (L.) Drejer subsp. <i>flexuosa</i>
R	<i>Bellardiachloa variegata</i> (Lam.) Kerguelen subsp. <i>variegata</i>
E – R	<i>Brachypodium genuense</i> (DC.) Roem. et Schult.
	<i>Brachypodium rupestre</i> (Host) Roem. et Schult.
	<i>Brachypodium sylvaticum</i> (Huds.) P.Beauv. subsp. <i>sylvaticum</i>
	<i>Briza media</i> L.
	<i>Bromopsis erecta</i> (Huds.) Fourr.
	<i>Bromopsis ramosa</i> (Huds.) Holub subsp. <i>ramosa</i>
	<i>Bromus commutatus</i> Schrad.
	<i>Bromus hordeaceus</i> L. subsp. <i>hordeaceus</i>
R	<i>Bromus lanceolatus</i> Roth
	<i>Bromus racemosus</i> L. subsp. <i>racemosus</i>
	<i>Bromus squarrosus</i> L. subsp. <i>squarrosus</i>
	<i>Cynodon dactylon</i> (L.) Pers.
	<i>Cynosurus cristatus</i> L.
	<i>Cynosurus echinatus</i> L.
	<i>Dactylis glomerata</i> L. subsp. <i>glomerata</i>
	<i>Dasyphyrum villosum</i> (L.) P. Candargy
	<i>Elymus caninus</i> (L.) L.
	<i>Elymus repens</i> (L.) Gould. subsp. <i>repens</i>
	<i>Festuca circummediterranea</i> Patzke
	<i>Festuca heterophylla</i> Lam.
R	<i>Festuca inops</i> De Not.
	<i>Festuca laevigata</i> Gaudin
	<i>Festuca stricta</i> Host subsp. <i>trachyphylla</i> (Hack.) Patzke ex Pils
	<i>Glyceria notata</i> Chevall.
E	<i>Helictichloa praetutiana</i> (Parl. ex Arcang.) Bartolucci, F.Conti, Peruzzi & Banfi subsp. <i>praetutiana</i>
	<i>Holcus lanatus</i> L. subsp. <i>lanatus</i>
R	<i>Hordelymus europaeus</i> (L.) Harz
	<i>Koeleria australis</i> A. Kern.
	<i>Koeleria callierii</i> (Domin) Ujhelyi

Taxon	
E	<i>Koeleria splendens</i> C. Presl <i>Koeleria subcaudata</i> (Asch. & Graebn.) Ujhelyi <i>Leucopoa dimorpha</i> (Guss.) H.Scholz & Foggi <i>Lolium arundinaceum</i> (Schreb.) Darbysh. subsp. <i>arundinaceum</i> <i>Lolium multiflorum</i> Lam. <i>Lolium perenne</i> L. <i>Lolium pratense</i> (Huds.) Darbysh. <i>Melica ciliata</i> L. subsp. <i>ciliata</i> <i>Melica uniflora</i> Retz. <i>Nardus stricta</i> L. <i>Phleum alpinum</i> L. <i>Phleum nodosum</i> L. <i>Phleum hirsutum</i> Honck. subsp. <i>ambiguum</i> (Ten.) Cif. & Giacom. <i>Phleum pratense</i> L. subsp. <i>pratense</i> <i>Poa alpina</i> L. subsp. <i>alpina</i> <i>Poa annua</i> L. <i>Poa bulbosa</i> L. <i>Poa compressa</i> L. <i>Poa infirma</i> Kunth MR <i>Poa molinerii</i> Balb. <i>Poa nemoralis</i> L. subsp. <i>nemoralis</i> <i>Poa pratensis</i> L. subsp. <i>pratensis</i> <i>Poa sylvicola</i> Guss. <i>Poa trivialis</i> L. <i>Sesleria autumnalis</i> (Scop.) F.W. Schultz <i>Sesleria juncifolia</i> Suffren subsp. <i>juncifolia</i> E <i>Sesleria nitida</i> Ten. subsp. <i>nitida</i> E <i>Stipa dasyvaginata</i> Martinovský subsp. <i>apenninicola</i> Martinovský et Moraldo <i>Trisetaria flavescens</i> (L.) Baumg. subsp. <i>flavescens</i> E – RR <i>Trisetaria villosa</i> (Bertol.) Banfi et Soldano
Ranunculaceae	
	<i>Actaea spicata</i> L. <i>Anemone ranunculoides</i> (L.) Holub <i>Clematis vitalba</i> L. <i>Delphinium ajacis</i> L. <i>Delphinium consolida</i> L. subsp. <i>consolida</i> <i>Delphinium fissum</i> Waldst. et Kit. subsp. <i>fissum</i> <i>Eranthis hyemalis</i> (L.) Salisb. <i>Ficaria verna</i> Huds. subsp. <i>verna</i> <i>Hepatica nobilis</i> Schreb. R <i>Ranunculus acris</i> L. subsp. <i>acris</i> E – R <i>Ranunculus apenninus</i> (Chiov.) Pignatti MR <i>Ranunculus bulbosus</i> L. subsp. <i>bulbosus</i> R <i>Ranunculus gramineus</i> L. <i>Ranunculus illyricus</i> L. <i>Ranunculus lanuginosus</i> L. <i>Ranunculus millefoliatus</i> Vahl <i>Ranunculus monspeliacus</i> L. subsp. <i>monspeliacus</i> <i>Ranunculus repens</i> L. <i>Ranunculus sardous</i> Crantz <i>Ranunculus sceleratus</i> L. E – MR <i>Ranunculus thomasi</i> Ten. MR <i>Ranunculus tuberosus</i> Lapeyr.
Papaveraceae	
	<i>Corydalis cava</i> (L.) Schweigg. et Körte subsp. <i>cava</i> <i>Corydalis pumila</i> (Host) Rchb. E – RR <i>Corydalis densiflora</i> C.Presl subsp. <i>apennina</i> F.Conti, Bartolucci & Uzunov

Taxon	
	<i>Papaver dubium</i> L. subsp. <i>dubium</i>
	<i>Papaver rhoeas</i> L. subsp. <i>rhoeas</i>
Crassulaceae	
	<i>Petrosedum rupestre</i> (L.) P.V.Heath
	<i>Sedum acre</i> L.
	<i>Sedum album</i> L.
R	<i>Sedum atratum</i> L. subsp. <i>atratum</i>
	<i>Sedum dasyphyllum</i> L.
	<i>Sedum hispanicum</i> L.
	<i>Sedum rubens</i> L.
	<i>Sedum sexangulare</i> L.
R	<i>Sempervivum arachnoideum</i> L.
E	<i>Sempervivum riccii</i> Iberite et Anzal.
Grossulariaceae	
R	<i>Ribes alpinum</i> L.
MR	<i>Ribes multiflorum</i> Kit. ex Roem. et Schult. subsp. <i>multiflorum</i>
R	<i>Ribes uva-crispa</i> L. subsp. <i>uva-crispa</i>
Saxifragaceae	
	<i>Saxifraga adscendens</i> L. subsp. <i>adscendens</i>
	<i>Saxifraga bulbifera</i> L.
R	<i>Saxifraga callosa</i> Sm. subsp. <i>callosa</i>
E – R	<i>Saxifraga exarata</i> Vill. subsp. <i>ampullacea</i> (Ten.) D.A.Webb
	<i>Saxifraga granulata</i> L. subsp. <i>granulata</i>
	<i>Saxifraga paniculata</i> Mill.
E	<i>Saxifraga porophylla</i> Bertol. subsp. <i>porophylla</i>
	<i>Saxifraga rotundifolia</i> L. subsp. <i>rotundifolia</i>
	<i>Saxifraga tridactylites</i> L.
Fabaceae	
	<i>Anthyllis montana</i> L. subsp. <i>jacquinii</i> (Rchb.f.) Rohlena
R	<i>Anthyllis vulneraria</i> L. subsp. <i>pulchella</i> (Vis.) Bornm.
	<i>Anthyllis vulneraria</i> L. subsp. <i>rubriflora</i> (DC.) Arcang.
	<i>Astragalus depressus</i> L. subsp. <i>depressus</i>
	<i>Astragalus glycyphyllos</i> L.
R	<i>Astragalus sempervirens</i> Lam.
E – MR	<i>Astragalus sirinicus</i> Ten.
	<i>Colutea arborescens</i> L.
	<i>Coronilla minima</i> L. subsp. <i>minima</i>
	<i>Coronilla scorpioides</i> (L.) W.D.J. Koch
	<i>Cytisophyllum sessilifolium</i> (L.) O. Lang
	<i>Cytisus hirsutus</i> L.
	<i>Lotus herbaceus</i> (Vill.) Jauzein
RR	<i>Genista sagittalis</i> L.
	<i>Genista tinctoria</i> L.
	<i>Hippocrepis comosa</i> L. subsp. <i>comosa</i>
MR	<i>Hippocrepis glauca</i> Ten.
	<i>Lathyrus cicera</i> L.
	<i>Lathyrus latifolius</i> L.
	<i>Lathyrus nissolia</i> L.
	<i>Lathyrus pratensis</i> L. subsp. <i>pratensis</i>
	<i>Lathyrus sphaericus</i> Retz.
	<i>Lathyrus sylvestris</i> L. subsp. <i>sylvestris</i>
	<i>Lathyrus venetus</i> (Mill.) Wohlff.
	<i>Lathyrus vernus</i> (L.) Bernh.
MR	<i>Lotus corniculatus</i> L. subsp. <i>alpinus</i> (DC.) Rothm.
	<i>Lotus corniculatus</i> L. subsp. <i>corniculatus</i>
	<i>Medicago falcata</i> L. subsp. <i>falcata</i>
	<i>Medicago lupulina</i> L.

		Taxon
		<i>Medicago minima</i> (L.) L.
RR		<i>Medicago prostrata</i> Jacq. subsp. <i>prostrata</i>
NAT		<i>Medicago sativa</i> L.
RR		<i>Onobrychis arenaria</i> (Kit.) DC. subsp. <i>arenaria</i>
		<i>Onobrychis viciifolia</i> Scop.
		<i>Ononis pusilla</i> L. subsp. <i>pusilla</i>
		<i>Ononis reclinata</i> L.
		<i>Ononis spinosa</i> L. subsp. <i>spinosa</i>
E – RR		<i>Oxytropis pilosa</i> (L.) DC. subsp. <i>caputoi</i> (Moraldo et La Valva) Brilli-Catt., Di Massimo et Gubellini
		<i>Securigera varia</i> (L.) Lassen
		<i>Spartium junceum</i> L.
		<i>Trifolium alpestre</i> L.
		<i>Trifolium angustifolium</i> L. subsp. <i>angustifolium</i>
		<i>Trifolium arvense</i> L.
		<i>Trifolium campestre</i> Schreb.
		<i>Trifolium dubium</i> Sibth.
		<i>Trifolium fragiferum</i> L. subsp. <i>fragiferum</i>
		<i>Trifolium incarnatum</i> L. subsp. <i>molinerii</i> (Balb. ex Hornem.) Ces.
		<i>Trifolium micranthum</i> Viv.
R		<i>Trifolium montanum</i> L. subsp. <i>rupestre</i> (Ten.) Nyman
		<i>Trifolium nigrescens</i> Viv. subsp. <i>nigrescens</i>
		<i>Trifolium ochroleucum</i> Huds.
		<i>Trifolium pallidum</i> Waldst. et Kit.
RR		<i>Trifolium phleoides</i> Willd.
		<i>Trifolium pratense</i> L. subsp. <i>pratense</i>
E		<i>Trifolium pratense</i> L. subsp. <i>semipurpureum</i> (Strobl) Pignatti
		<i>Trifolium repens</i> L.
		<i>Trifolium scabrum</i> L.
		<i>Trifolium squarrosum</i> L.
R		<i>Trifolium thalii</i> Vill.
		<i>Trigonella gladiata</i> M. Bieb.
		<i>Trigonella officinalis</i> (L.) Coulot & Rabaut
		<i>Vicia angustifolia</i> L.
		<i>Vicia cracca</i> L.
		<i>Vicia dasycarpa</i> Ten.
		<i>Vicia incana</i> Gouan
MR		<i>Vicia onobrychioides</i> L.
		<i>Vicia sepium</i> L.
	Polygalaceae	
R		<i>Polygala alpestris</i> Rchb. subsp. <i>alpestris</i>
		<i>Polygala major</i> Jacq.
MR		<i>Polygala nicaeensis</i> W.D.J. Koch subsp. <i>mediterranea</i> Chodat
	Rosaceae	
		<i>Agrimonia eupatoria</i> L. subsp. <i>eupatoria</i>
RR		<i>Alchemilla cinerea</i> Buser
MR		<i>Alchemilla glaucescens</i> Wallr.
R		<i>Alchemilla monticola</i> Opiz
MR		<i>Alchemilla strigosula</i> Buser
		<i>Amelanchier ovalis</i> Medik. subsp. <i>ovalis</i>
		<i>Aremonia agrimonoides</i> (L.) DC. subsp. <i>agrimonoides</i>
MR		<i>Cotoneaster integerrimus</i> Medik.
		<i>Crataegus laevigata</i> (Poir.) DC.
		<i>Crataegus monogyna</i> Jacq.
		<i>Filipendula vulgaris</i> Moench
		<i>Fragaria vesca</i> L. subsp. <i>Vesca</i>
MR		<i>Geum molle</i> Vis. et Pancic
		<i>Geum urbanum</i> L.
NAT		<i>Malus domestica</i> (Suckow.) Borkh.

	Taxon
	<i>Malus sylvestris</i> (L.) Mill.
R	<i>Potentilla detommasii</i> Ten.
	<i>Potentilla micrantha</i> Ramond ex DC.
R	<i>Potentilla pedata</i> Willd ex Hornem.
	<i>Potentilla recta</i> L. subsp. <i>recta</i>
	<i>Potentilla reptans</i> L.
E	<i>Potentilla rigoana</i> Th. Wolf
	<i>Poterium sanguisorba</i> subsp. <i>balearicum</i> (Bourg. ex Nyman) Stace
	<i>Prunus avium</i> (L.) L.
	<i>Prunus mahaleb</i> L.
	<i>Prunus spinosa</i> L. subsp. <i>spinosa</i>
	<i>Pyrus communis</i> L. subsp. <i>pyraster</i> (L.) Ehrh.
	<i>Rosa arvensis</i> Huds.
	<i>Rosa balsamica</i> Besser
	<i>Rosa canina</i> L. s.s.
	<i>Rosa corymbifera</i> Borkh.
MR	<i>Rosa montana</i> Chaix
	<i>Rosa pouzinii</i> Tratt.
	<i>Rosa spinosissima</i> L.
	<i>Rosa squarrosa</i> (A. Rau) Boreau
MR	<i>Rosa villosa</i> L.
	<i>Rubus caesius</i> L.
	<i>Rubus canescens</i> DC.
	<i>Rubus hirtus</i> Waldst. et Kit.
	<i>Rubus idaeus</i> L. subsp. <i>idaeus</i>
	<i>Rubus ulmifolius</i> Schott
	<i>Sorbus aria</i> (L.) Crantz
	Rhamnaceae
R	<i>Atadinus alpinus</i> (L.) Raf.
R	<i>Atadinus fallax</i> (Boiss.) Hauenschild
R	<i>Atadinus pumilus</i> (Turra) Hauenschild subsp. <i>pumilus</i>
R	<i>Rhamnus saxatilis</i> Jacq.
	Urticaceae
	<i>Urtica dioica</i> L. subsp. <i>dioica</i>
	Fagaceae
	<i>Fagus sylvatica</i> L. subsp. <i>sylvatica</i>
	<i>Quercus cerris</i> L.
	<i>Quercus pubescens</i> Willd. subsp. <i>pubescens</i>
	Betulaceae
	<i>Corylus avellana</i> L.
	<i>Ostrya carpinifolia</i> Scop.
	Cucurbitaceae
	<i>Bryonia dioica</i> Jacq.
	Celastraceae
	<i>Euonymus europaeus</i> L.
	<i>Euonymus latifolius</i> (L.) Mill.
RR	<i>Parnassia palustris</i> L. subsp. <i>palustris</i>
	Violaceae
	<i>Viola alba</i> Besser subsp. <i>dehnhardtii</i> (Ten.) W. Becker
	<i>Viola arvensis</i> Murray
E	<i>Viola eugeniae</i> Parl. subsp. <i>eugeniae</i>
MR	<i>Viola kitaibeliana</i> Schult.
	<i>Viola odorata</i> L.
	<i>Viola reichenbachiana</i> Jord. ex Boreau
	Salicaceae
	<i>Populus tremula</i> L.
	<i>Salix apennina</i> A.K. Skortsov
	<i>Salix caprea</i> L.

		Taxon
MR		<i>Salix eleagnos</i> Scop.
		<i>Salix purpurea</i> L. subsp. <i>purpurea</i>
	Linaceae	
		<i>Linum catharticum</i> L. subsp. <i>catharticum</i>
		<i>Linum corymbulosum</i> Rchb.
		<i>Linum strictum</i> L.
		<i>Linum tenuifolium</i> L.
MR		<i>Linum tommasinii</i> (Rchb.) Nyman
		<i>Linum trigynum</i> L.
		<i>Linum usitatissimum</i> L. subsp. <i>angustifolium</i> (Huds.) Thell.
R		<i>Linum viscosum</i> L.
	Hypericaceae	
RR		<i>Hypericum byssopifolium</i> Chaix
		<i>Hypericum perforatum</i> L. subsp. <i>veronense</i> (Schränk.) Ces.
		<i>Hypericum tetrapterum</i> Fr.
	Euphorbiaceae	
		<i>Euphorbia amygdaloides</i> L.
		<i>Euphorbia cyparissias</i> L.
		<i>Euphorbia falcata</i> L. subsp. <i>falcata</i>
R		<i>Euphorbia myrsinites</i> L. subsp. <i>myrsinites</i>
	Geraniaceae	
		<i>Erodium ciconium</i> (L.) L'Hér.
		<i>Erodium cicutarium</i> (L.) L'Hér. subsp. <i>cicutarium</i>
		<i>Geranium columbinum</i> L.
		<i>Geranium dissectum</i> L.
		<i>Geranium lucidum</i> L.
		<i>Geranium molle</i> L.
MR		<i>Geranium nodosum</i> L.
		<i>Geranium purpureum</i> Vill.
		<i>Geranium pyrenaicum</i> Burm. fil. subsp. <i>pyrenaicum</i>
		<i>Geranium robertianum</i> L.
		<i>Geranium rotundifolium</i> L.
		<i>Geranium sanguineum</i> L.
	Onagraceae	
		<i>Chamaenerion angustifolium</i> (L.) Scop.
		<i>Chamaenerion dodonaei</i> (Vill.) Schur ex Fuss
		<i>Epilobium montanum</i> L.
		<i>Epilobium parviflorum</i> Schreb.
	Lythraceae	
		<i>Lythrum byssopifolia</i> L.
	Sapindaceae	
		<i>Acer campestre</i> L.
		<i>Acer opalus</i> Mill. subsp. <i>obtusatum</i> (Waldst. & Kit. ex Willd.) Gams
		<i>Acer pseudoplatanus</i> L.
	Thymelaeaceae	
		<i>Daphne laureola</i> L.
		<i>Daphne oleoides</i> Schreb. subsp. <i>oleoides</i>
	Cistaceae	
		<i>Helianthemum apenninum</i> (L.) Mill. subsp. <i>apenninum</i>
MR		<i>Helianthemum nummularium</i> (L.) Mill. subsp. <i>grandiflorum</i> (Scop.) Schinz et Thell.
		<i>Helianthemum nummularium</i> (L.) Mill. subsp. <i>obscurum</i> (Celak.) Holub
		<i>Helianthemum oelandicum</i> (L.) Dum. Cours. subsp. <i>incanum</i> (Willk.) G. López
		<i>Helianthemum salicifolium</i> (L.) Mill.
	Malvaceae	
		<i>Malva alcea</i> L.
		<i>Malva sylvestris</i> L.
		<i>Tilia platyphyllos</i> Scop. subsp. <i>platyphyllos</i>
	Resedaceae	

	Taxon
	<i>Reseda luteola</i> L.
Brassicaceae	
	<i>Aethionema saxatile</i> (L.) R.Br.
	<i>Alliaria petiolata</i> (M. Bieb.) Cavara et Grande
	<i>Alyssum alyssoides</i> (L.) L.
	<i>Alyssum simplex</i> Rudolphi
	<i>Arabidopsis thaliana</i> (L.) Heynh.
RR	<i>Arabis alpina</i> L. subsp. <i>caucasica</i> (Willd.) Briq.
	<i>Arabis auriculata</i> Lam.
	<i>Arabis collina</i> Ten. subsp. <i>collina</i>
	<i>Arabis hirsuta</i> (L.) Scop.
	<i>Arabis sagittata</i> (Bertol.) DC.
	<i>Barbarea bracteosa</i> Guss.
	<i>Barbarea vulgaris</i> R. Br.
	<i>Biscutella laevigata</i> L. subsp. <i>laevigata</i> var. <i>laevigata</i>
	<i>Bunias erucago</i> L.
	<i>Capsella bursa-pastoris</i> (L.) Medik. subsp. <i>bursa-pastoris</i>
	<i>Capsella rubella</i> Reut.
	<i>Cardamine bulbifera</i> (L.) Crantz
	<i>Cardamine impatiens</i> L. subsp. <i>impatiens</i>
RR	<i>Conringia austriaca</i> (Jacq.) Sweet
	<i>Draba aizoides</i> L. subsp. <i>aizoides</i>
	<i>Draba verna</i> L. subsp. <i>verna</i>
E – RR	<i>Erysimum majellense</i> Polatschek – after Iocchi et al. (2010) this is the second record for Lazio, thus representing a confirmation of its presence in the region.
E	<i>Erysimum pseudorhaeticum</i> Polatschek
R	<i>Hesperis laciniata</i> All. subsp. <i>laciniata</i>
	<i>Hesperis matronalis</i> L. subsp. <i>matronalis</i>
	<i>Hornungia petraea</i> (L.) Rchb. subsp. <i>petraea</i>
	<i>Isatis tinctoria</i> L. subsp. <i>tinctoria</i>
	<i>Microthlaspi perfoliatum</i> (L.) F.K.Mey.
	<i>Mummenhoffia alliacea</i> (L.) Esmailbegi & Al-Shehbaz
	<i>Pseudoturritis turrita</i> (L.) Al-Shehbaz
	<i>Rapistrum rugosum</i> (L.) All.
	<i>Sinapis arvensis</i> L. subsp. <i>arvensis</i>
MR	<i>Turritis glabra</i> L.
Loranthaceae	
	<i>Loranthus europaeus</i> Jacq.
Santalaceae	
	<i>Thesium humifusum</i> DC.
R	<i>Thesium linophyllum</i> L.
R	<i>Viscum album</i> L.
Plumbaginaceae	
E	<i>Armeria canescens</i> (Host) Ebel
Polygonaceae	
R	<i>Bistorta officinalis</i> Delarbre
	<i>Fallopia convolvulus</i> (L.) A. Löve
	<i>Polygonum arenastrum</i> Boreau subsp. <i>arenastrum</i>
	<i>Polygonum aviculare</i> L. subsp. <i>aviculare</i>
	<i>Rumex acetosa</i> L. subsp. <i>acetosa</i>
	<i>Rumex acetosella</i> L. subsp. <i>pyrenaicus</i> (Pourr. ex Lapeyr.) Akeroyd
R	<i>Rumex alpinus</i> L.
R	<i>Rumex arifolius</i> All.
	<i>Rumex crispus</i> L.
	<i>Rumex scutatus</i> L. subsp. <i>scutatus</i>
Caryophyllaceae	
	<i>Agrostemma githago</i> L. subsp. <i>githago</i>
	<i>Arenaria leptoclados</i> (Rchb.) Guss. subsp. <i>leptoclados</i>

	Taxon
	<i>Arenaria serpyllifolia</i> L. subsp. <i>serpyllifolia</i>
	<i>Cerastium arvense</i> L. subsp. <i>arvense</i>
	<i>Cerastium brachypetalum</i> Desp. ex Pers. subsp. <i>brachypetalum</i>
	<i>Cerastium brachypetalum</i> Desp. ex Pers. subsp. <i>roeseri</i> (Boiss. et Heldr.) Nyman
	<i>Cerastium brachypetalum</i> Desp. ex Pers. subsp. <i>tenoreanum</i> (Ser.) Soó
	<i>Cerastium ligusticum</i> Viv.
E	<i>Cerastium tomentosum</i> L.
	<i>Dianthus carthusianorum</i> L. subsp. <i>carthusianorum</i>
	<i>Dianthus deltoides</i> L. subsp. <i>deltoides</i>
R	<i>Dianthus hyssopifolius</i> L.
	<i>Dianthus longicaulis</i> Ten.
R	<i>Drypis spinosa</i> L. subsp. <i>spinosa</i>
RR	<i>Herniaria glabra</i> L. subsp. <i>nebrodensis</i> Nyman
	<i>Herniaria hirsuta</i> L. subsp. <i>hirsuta</i>
	<i>Herniaria incana</i> Lam.
RR	<i>Mcneillia graminifolia</i> (Ard.) Dillenb. & Kadereit subsp. <i>clandestina</i> (Port.) Dillenb. & Kadereit
	<i>Moehringia trinervia</i> (L.) Clairv.
	<i>Paronychia kapela</i> (Hacq.) A. Kern. subsp. <i>kapela</i>
	<i>Petrorhagia dubia</i> (Raf.) G. López et Romo
	<i>Petrorhagia prolifera</i> (L.) P. W. Ball. et Heywood
	<i>Petrorhagia saxifraga</i> (L.) Link subsp. <i>saxifraga</i>
	<i>Rabclera holostea</i> (L.) M.T.Sharple & E.A.Tripp
	<i>Sabulina glauca</i> (Dvořáková) Dillenb. & Kadereit
	<i>Sabulina tenuifolia</i> (L.) Rchb. subsp. <i>tenuifolia</i>
R	<i>Sabulina verna</i> (L.) Rchb. subsp. <i>verna</i>
	<i>Sagina alexandrae</i> Iamónico
	<i>Saponaria ocymoides</i> L.
	<i>Scleranthus annuus</i> L.
	<i>Scleranthus polycarpus</i> L.
MR	<i>Silene ciliata</i> Pourr. subsp. <i>graefferi</i> (Guss.) Nyman
	<i>Silene conica</i> L.
	<i>Silene dioica</i> (L.) Clairv.
	<i>Silene italica</i> (L.) Pers. subsp. <i>italica</i>
	<i>Silene latifolia</i> Poir.
R	<i>Silene multicaulis</i> Guss. subsp. <i>multicaulis</i>
	<i>Silene nemoralis</i> Waldst. et Kit.
E – MR	<i>Silene notarisii</i> Ces.
MR	<i>Silene nutans</i> L. subsp. <i>nutans</i>
	<i>Silene otites</i> (L.) Wibel
MR	<i>Silene saxifraga</i> L.
	<i>Silene vulgaris</i> (Moench) Garcke subsp. <i>vulgaris</i>
	<i>Stellaria media</i> (L.) Vill. subsp. <i>media</i>
	<i>Stellaria nemorum</i> L. subsp. <i>montana</i> (Pierrat) Berher
	<i>Stellaria pallida</i> (Dumort.) Crép.
	Chenopodiaceae
	<i>Blitum bonus-henricus</i> (L.) Rchb.
	<i>Chenopodium album</i> L.
	<i>Chenopodium opulifolium</i> Schrad. ex W.D.J. Koch et Ziz
	<i>Chenopodium vulvaria</i> L.
	Primulaceae
	<i>Cyclamen hederifolium</i> Aiton subsp. <i>hederifolium</i>
	<i>Lysimachia arvensis</i> (L.) U.Manns & Anderb. subsp. <i>arvensis</i>
R	<i>Primula veris</i> L. subsp. <i>columnae</i> (Ten.) Maire & Petitm.
	<i>Primula vulgaris</i> Huds. subsp. <i>vulgaris</i>
	Ericaceae
	<i>Monotropa hypopitys</i> L.
	<i>Orthilia secunda</i> (L.) House

Taxon	
R	<i>Vaccinium myrtillus</i> L.
Rubiaceae	
R	<i>Asperula aristata</i> L.f. subsp. <i>scabra</i> Nyman
	<i>Asperula cynanchica</i> L.
	<i>Asperula purpurea</i> (L.) Ehrend.
	<i>Cruciata glabra</i> (L.) C.Bauhin ex Opiz
	<i>Cruciata laevipes</i> Opiz
MR	<i>Cruciata pedemontana</i> (Bellardi) Ehrend.
	<i>Galium album</i> Mill. subsp. <i>album</i>
	<i>Galium aparine</i> L.
	<i>Galium corrudifolium</i> Vill.
	<i>Galium lucidum</i> All.
E – MR	<i>Galium magellense</i> Ten.
	<i>Galium mollugo</i> L.
	<i>Galium odoratum</i> (L.) Scop.
	<i>Galium verum</i> L. subsp. <i>verum</i>
	<i>Sherardia arvensis</i> L.
Gentianaceae	
	<i>Centaurium pulchellum</i> (Sw.) Druce subsp. <i>pulchellum</i>
	<i>Gentiana cruciata</i> L. subsp. <i>cruciata</i>
	<i>Gentiana lutea</i> L. subsp. <i>lutea</i>
MR	<i>Gentiana utriculosa</i> L.
	<i>Gentiana verna</i> L. subsp. <i>verna</i>
E	<i>Gentianella columnae</i> (Ten.) Holub
Apocynaceae	
	<i>Vincetoxicum hirundinaria</i> Medik. subsp. <i>hirundinaria</i>
Boraginaceae	
	<i>Aegonychon purpureoeruleum</i> (L.) Holub
	<i>Anchusa azurea</i> Mill.
	<i>Buglossoides arvensis</i> (L.) I.M. Johnst.
MR	<i>Buglossoides incrassata</i> (Guss.) I.M. Johnst. subsp. <i>incrassata</i>
E	<i>Cynoglossum apenninum</i> L.
E – R	<i>Cynoglossum magellense</i> Ten.
	<i>Cynoglossum montanum</i> L.
	<i>Cynoglossum officinale</i> L.
	<i>Cynoglottis barrelieri</i> (All.) Vural et Kit Tan subsp. <i>barrelieri</i>
	<i>Echium italicum</i> L. subsp. <i>italicum</i>
	<i>Echium vulgare</i> L.
R	<i>Myosotis alpestris</i> F.W.Schmidt
	<i>Myosotis arvensis</i> (L.) Hill subsp. <i>arvensis</i>
E	<i>Myosotis decumbens</i> Host subsp. <i>florentina</i> Grau
MR	<i>Myosotis graui</i> Selvi
	<i>Onosma echioides</i> (L.) L.
E	<i>Pulmonaria vallisarvae</i> A.Kern. subsp. <i>apennina</i> (Cristof. & Puppi) L.Cecchi & Selvi
Convolvulaceae	
	<i>Convolvulus arvensis</i> L.
	<i>Cuscuta epithymum</i> (L.) L. subsp. <i>epithymum</i>
	<i>Cuscuta planiflora</i> Ten.
Plantaginaceae	
	<i>Chaenorhinum minus</i> (L.) Lange subsp. <i>minus</i>
	<i>Digitalis ferruginea</i> L.
E	<i>Digitalis micrantha</i> Roth ex Schweigg.
	<i>Globularia bisnagarica</i> L.
	<i>Globularia meridionalis</i> (Podp.) O.Schwarz
E	<i>Linaria purpurea</i> (L.) Mill.
	<i>Plantago argentea</i> Chaix
	<i>Plantago atrata</i> Hoppe subsp. <i>atrata</i>

Taxon	
	<i>Plantago lanceolata</i> L.
	<i>Plantago lanceolata</i> L. var. <i>sphaerostachya</i> Mert. et W.D.J. Koch
	<i>Plantago major</i> L. subsp. <i>major</i>
	<i>Plantago media</i> L. subsp. <i>media</i>
	<i>Plantago sempervirens</i> Crantz
	<i>Plantago subulata</i> L.
	<i>Veronica anagallis-aquatica</i> L. subsp. <i>anagallis-aquatica</i>
	<i>Veronica arvensis</i> L.
MR	<i>Veronica barrelieri</i> H.Schott ex Roem. & Schult. subsp. <i>barrelieri</i>
	<i>Veronica beccabunga</i> L. subsp. <i>beccabunga</i>
	<i>Veronica chamaedrys</i> L. subsp. <i>chamaedrys</i>
	<i>Veronica cymbalaria</i> Bodard subsp. <i>cymbalaria</i>
	<i>Veronica hederifolia</i> L. subsp. <i>hederifolia</i>
R	<i>Veronica orsiniana</i> Ten. subsp. <i>orsiniana</i>
NAT	<i>Veronica persica</i> Poir.
	<i>Veronica polita</i> Fr.
MR	<i>Veronica prostrata</i> L.
	<i>Veronica serpyllifolia</i> L.
Scrophulariaceae	
	<i>Scrophularia auriculata</i> L. subsp. <i>auriculata</i>
	<i>Scrophularia canina</i> L.
R	<i>Scrophularia juratensis</i> Schleich.
	<i>Scrophularia nodosa</i> L.
	<i>Scrophularia scopolii</i> Hoppe ex Pers.
	<i>Scrophularia vernalis</i> L.
	<i>Verbascum longifolium</i> Ten.
	<i>Verbascum mallophorum</i> Boiss. et Heldr.
	<i>Verbascum pulverulentum</i> Vill.
Lamiaceae	
	<i>Ajuga chamaepitys</i> (L.) Schreb. subsp. <i>chamaepitys</i>
	<i>Ajuga reptans</i> L.
E – MR	<i>Betonica alopecuroides</i> L. subsp. <i>divulsa</i> (Ten.) Bartolucci & Peruzzi
	<i>Clinopodium menthifolium</i> (Host) Merino subsp. <i>menthifolium</i>
	<i>Clinopodium vulgare</i> L. subsp. <i>vulgare</i>
	<i>Galeopsis angustifolia</i> Hoffm. subsp. <i>angustifolia</i>
	<i>Lamium bifidum</i> Cirillo subsp. <i>bifidum</i>
	<i>Lamium garganicum</i> L. subsp. <i>laevigatum</i> Arcang.
	<i>Lamium maculatum</i> L.
	<i>Lamium purpureum</i> L.
	<i>Marrubium incanum</i> Desr.
	<i>Melittis melissophyllum</i> L. subsp. <i>melissophyllum</i>
	<i>Mentha longifolia</i> (L.) L.
MR	<i>Mentha microphylla</i> C. Koch
	<i>Origanum vulgare</i> L. subsp. <i>vulgare</i>
	<i>Prunella laciniata</i> (L.) L.
	<i>Prunella vulgaris</i> L. subsp. <i>vulgaris</i>
	<i>Salvia glutinosa</i> L.
	<i>Salvia pratensis</i> L.
	<i>Salvia verbenaca</i> L.
	<i>Salvia virgata</i> Jacq.
	<i>Stachys heraclea</i> All.
	<i>Stachys recta</i> L. subsp. <i>recta</i>
R	<i>Stachys recta</i> L. subsp. <i>subcrenata</i> (Vis.) Briq.
	<i>Stachys tymphaea</i> Hausskn.
	<i>Teucrium chamaedrys</i> L. subsp. <i>chamaedrys</i>
	<i>Teucrium montanum</i> L.
	<i>Thymus longicaulis</i> C.Presl subsp. <i>longicaulis</i>

Taxon	
RR	<i>Thymus oenipontanus</i> Heinr.Braun ex Borbás
R	<i>Thymus praecox</i> Opiz subsp. <i>polytrichus</i> (Borbás) Jalas <i>Thymus striatus</i> Vahl <i>Ziziphora acinos</i> (L.) Melnikov <i>Ziziphora granatensis</i> (Boiss. & Reut.) Melnikov subsp. <i>alpina</i> (L.) Bräuchler & Gutermann
Orobanchaceae	
MR	<i>Euphrasia liburnica</i> Wettst. <i>Euphrasia stricta</i> D. Wolff ex J.F. Lehm. <i>Orobanche artemisiae-campestris</i> Gaudin <i>Orobanche caryophyllacea</i> Sm. <i>Orobanche gracilis</i> Sm. <i>Orobanche minor</i> Sm.
MR	<i>Orobanche reticulata</i> Wallr. subsp. <i>reticulata</i>
R	<i>Orobanche teucarii</i> Holandre
R	<i>Orobanche variegata</i> Wallr. <i>Parentucellia latifolia</i> (L.) Caruel <i>Pedicularis comosa</i> L. subsp. <i>comosa</i>
E	<i>Pedicularis elegans</i> Ten.
MR	<i>Pedicularis tuberosa</i> L. <i>Rhinanthus alectorolophus</i> (Scop.) Pollich <i>Rhinanthus minor</i> L.
E	<i>Rhinanthus wettsteinii</i> (Sterneck) Soó
Campanulaceae	
E – MR	<i>Campanula glomerata</i> L. subsp. <i>glomerata</i> <i>Campanula micrantha</i> Bertol. <i>Campanula rapunculus</i> L.
E	<i>Campanula scheuchzeri</i> Vill. subsp. <i>scheuchzeri</i> <i>Campanula tanfanii</i> Podlech <i>Campanula trachelium</i> L. subsp. <i>trachelium</i> <i>Edraianthus graminifolius</i> (L.) A. DC. subsp. <i>graminifolius</i> <i>Legousia hybrida</i> (L.) Delarbre <i>Legousia speculum-veneris</i> (L.) Chaix subsp. <i>speculum-veneris</i>
R	<i>Phyteuma orbiculare</i> L.
Asteraceae	
RR	<i>Achillea setacea</i> Waldst. et Kit. subsp. <i>setacea</i> <i>Achillea tomentosa</i> L. <i>Adenostyles alpina</i> (L.) Bluff & Fingerh. subsp. <i>alpina</i> <i>Anthemis arvensis</i> L. subsp. <i>arvensis</i>
R	<i>Anthemis cretica</i> L. subsp. <i>columnae</i> (Ten.) Franzén <i>Arctium lappa</i> L. <i>Arctium minus</i> (Hill) Bernh. <i>Bellis perennis</i> L. <i>Bellis sylvestris</i> Cirillo <i>Bombycilaena erecta</i> (L.) Smoljan <i>Carduus defloratus</i> L. subsp. <i>carlinifolius</i> (Lam.) Ces. <i>Carduus nutans</i> L. subsp. <i>nutans</i>
E	<i>Carduus nutans</i> L. subsp. <i>perspinosus</i> (Fiori) Arènes <i>Carlina acanthifolia</i> All. subsp. <i>acanthifolia</i> <i>Carlina acaulis</i> L. subsp. <i>caulescens</i> (Lam.) Schübl. et G. Martens <i>Carlina corymbosa</i> L. <i>Carlina vulgaris</i> L. subsp. <i>vulgaris</i> <i>Carthamus lanatus</i> L. subsp. <i>lanatus</i>
E – MR	<i>Centaurea ambigua</i> Guss. subsp. <i>ambigua</i>
E	<i>Centaurea ambigua</i> Guss. subsp. <i>nigra</i> (Fiori) Pignatti
RR	<i>Centaurea arachnoidea</i> subsp. <i>adonidifolia</i> (Rchb.) F. Conti, Moraldo & Ricceri <i>Centaurea deusta</i> Ten. <i>Centaurea jacea</i> L. subsp. <i>gaudini</i> (Boiss. et Reut.) Gremlí

	Taxon
R	<i>Centaurea scabiosa</i> L. <i>Centaurea triumfetti</i> All. <i>Chondrilla juncea</i> L. <i>Cichorium intybus</i> L. subsp. <i>intybus</i> <i>Cirsium arvense</i> (L.) Scop. <i>Cirsium creticum</i> (Lam.) d'Urv. subsp. <i>triumfetti</i> (Lacaita) Werner <i>Cirsium eriophorum</i> (L.) Scop.
R	<i>Cirsium palustre</i> (L.) Scop. <i>Cota segetalis</i> (Ten.) Holub <i>Cota tinctoria</i> (L.) J. Gay subsp. <i>australis</i> (R. Fern.) Oberprieler et Greuter <i>Cota tinctoria</i> (L.) J. Gay subsp. <i>tinctoria</i>
MR	<i>Crepis biennis</i> L. <i>Crepis foetida</i> L.
E	<i>Crepis lacera</i> Ten. <i>Crepis neglecta</i> L. <i>Crepis vesicaria</i> L. subsp. <i>vesicaria</i> <i>Crupina vulgaris</i> Cass. <i>Doronicum columnae</i> Ten. <i>Echinops sphaerocephalus</i> L. subsp. <i>sphaerocephalus</i> <i>Erigeron acris</i> L. subsp. <i>acris</i> <i>Erigeron epiroticus</i> (Vierh.) Halácsy <i>Eupatorium cannabinum</i> L. subsp. <i>cannabinum</i> <i>Helminthotheca echinoides</i> (L.) Holub
R	<i>Hieracium bifidum</i> Kit. ex Hornem.
*	<i>Hieracium bupleuroides</i> C.C. Gmel.
RR	<i>Hieracium cymosum</i> L. subsp. <i>cymosum</i>
E – RR	<i>Hieracium hoppeanum</i> Schult.
R	<i>Hieracium lachenalii</i> C.C.Gmel. <i>Hieracium murorum</i> L. <i>Hieracium prenanthoides</i> Vill. <i>Hieracium racemosum</i> Waldst. et Kit. ex Willd.
RR	<i>Hieracium tomentosum</i> (L.) L.
MR	<i>Hieracium villosum</i> Jacq. <i>Jacobaea erucifolia</i> (L.) G.Gaertn., B.Mey. & Scherb. subsp. <i>erucifolia</i>
E – R	<i>Klasea flavescens</i> (L.) Holub subsp. <i>cichoracea</i> (L.) Greuter et Wagenitz <i>Klasea nudicaulis</i> (L.) Fourr. <i>Lactuca perennis</i> L. subsp. <i>perennis</i> <i>Lactuca sativa</i> L. subsp. <i>serriola</i> (L.) Galasso, Banfi, Bartolucci & Ardenghi <i>Lactuca viminea</i> (L.) J. Presl et C. Presl subsp. <i>chondrilliflora</i> (Boreau) St.-Lag. <i>Lapsana communis</i> L. subsp. <i>communis</i> <i>Leontodon crispus</i> Vill. <i>Leontodon hispidus</i> L. <i>Leontodon rosanoi</i> (Ten.) DC. <i>Leontodon tuberosus</i> L. <i>Leucanthemum vulgare</i> Lam. subsp. <i>vulgare</i>
NAT	<i>Matricaria discoidea</i> DC. <i>Mycelis muralis</i> (L.) Dumort. subsp. <i>muralis</i> <i>Onopordum acanthium</i> L. subsp. <i>acanthium</i> <i>Onopordum illyricum</i> L. subsp. <i>illyricum</i> <i>Pentanema montanum</i> (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort. <i>Pentanema squarrosum</i> (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort. <i>Petasites hybridus</i> (L.) P. Gaertn., B. Mey. et Scherb. subsp. <i>hybridus</i> <i>Picris hieracioides</i> L. subsp. <i>hieracioides</i> <i>Pilosella officinarum</i> Vaill. <i>Pilosella piloselloides</i> (Vill.) Soják
RR	<i>Pilosella piloselloides</i> (Vill.) Soják subsp. <i>praealta</i> (Vill. ex Gochnat) S.Bräut. & Greuter

	Taxon
	<i>Prenanthes purpurea</i> L.
	<i>Pseudopodospermum hispanicum</i> (L.) Zaika, Sukhor. & N.Kilian
	<i>Prilostemon strictus</i> (Ten.) Greuter
	<i>Pulicaria dysenterica</i> (L.) Bernh.
	<i>Scorzonera cana</i> (C.A.Mey.) Griseb.
RR	<i>Scorzonera laciniata</i> L.
RR	<i>Scorzonera purpurea</i> L. subsp. <i>purpurea</i>
	<i>Scorzoneroides cichoriacea</i> (Ten.) Greuter
MR	<i>Senecio apenninus</i> Tausch
	<i>Senecio scopolii</i> Hoppe et Hornsch. ex Bluff et Fingerh.
R	<i>Serratula tinctoria</i> L. subsp. <i>tinctoria</i> var. <i>tinctoria</i>
	<i>Solidago virgaurea</i> L. subsp. <i>virgaurea</i>
	<i>Sonchus asper</i> (L.) Hill subsp. <i>asper</i>
R	<i>Sonchus asper</i> (L.) Hill subsp. <i>glaucescens</i> (Jord.) Ball
R	<i>Tanacetum corymbosum</i> (L.) Sch. Bip. var. <i>corymbosum</i>
	<i>Tanacetum corymbosum</i> (L.) Sch. Bip. var. <i>tenuifolium</i> (Willd.) Briq. et Cavill.
	<i>Tanacetum parthenium</i> (L.) Sch. Bip.
	<i>Taraxacum fulvum</i> gr.
	<i>Taraxacum minimum</i> (V.Br.) N.Terracc.
	<i>Taraxacum officinale</i> Weber
R	<i>Tragopogon crocifolius</i> L.
R	<i>Tragopogon dubius</i> Scop.
	<i>Tragopogon porrifolius</i> L.
	<i>Tragopogon pratensis</i> L.
	<i>Tragopogon samaritani</i> Heldr. et Sartori ex Boiss.
	<i>Tussilago farfara</i> L.
	<i>Xeranthemum cylindraceum</i> Sm.
	<i>Xeranthemum inapertum</i> (L.) Mill.
Viburnaceae	
	<i>Adoxa moschatellina</i> L. subsp. <i>moschatellina</i>
	<i>Sambucus ebulus</i> L.
	<i>Sambucus nigra</i> L.
Dipsacaceae	
	<i>Dipsacus fullonum</i> L.
	<i>Knautia purpurea</i> (Vill.) Borbás
	<i>Scabiosa columbaria</i> L.
Caprifoliaceae	
	<i>Lonicera alpigena</i> L. subsp. <i>alpigena</i>
	<i>Lonicera etrusca</i> Santi
Valerianaceae	
	<i>Centranthus ruber</i> (L.) DC. subsp. <i>ruber</i>
	<i>Valeriana officinalis</i> L.
	<i>Valeriana tuberosa</i> L.
	<i>Valerianella carinata</i> Loisel.
	<i>Valerianella eriocarpa</i> Desv.
	<i>Valerianella locusta</i> (L.) Laterr.
Araliaceae	
	<i>Hedera helix</i> L. subsp. <i>helix</i>
Apiaceae	
	<i>Aegopodium podagraria</i> L.
	<i>Angelica sylvestris</i> L. subsp. <i>sylvestris</i>
	<i>Anthriscus sylvestris</i> (L.) Hoffm. subsp. <i>sylvestris</i>
	<i>Bunium bulbocastanum</i> L.
	<i>Bupleurum baldense</i> Turra
	<i>Bupleurum falcatum</i> L. subsp. <i>cernuum</i> (Ten.) Arcang.
	<i>Chaerophyllum aureum</i> L.

Taxon	
MR	<i>Chaerophyllum hirsutum</i> L. <i>Chaerophyllum temulum</i> L. <i>Daucus carota</i> L. subsp. <i>carota</i> <i>Eryngium amethystinum</i> L.
R	<i>Heracleum sibiricum</i> L. subsp. <i>sibiricum</i>
R	<i>Heracleum sibiricum</i> L. subsp. <i>ternatum</i> (Velen.) Briq. <i>Katapsuxis silaifolia</i> (Jacq.) Reduron, Charpin & Pimenov <i>Oenanthe pimpinelloides</i> L. <i>Opopanax chironium</i> (L.) W.D.J. Koch <i>Orlaya grandiflora</i> (L.) Hoffm.
R	<i>Pimpinella major</i> (L.) Huds. <i>Pimpinella saxifraga</i> L. <i>Pimpinella tragi</i> Vill. <i>Sanicula europaea</i> L. <i>Scandix macrorhyncha</i> C.A.Mey. <i>Scandix pecten-veneris</i> L. subsp. <i>pecten-veneris</i> <i>Seseli montanum</i> L. subsp. <i>montanum</i>
E	<i>Siler montanum</i> Crantz subsp. <i>siculum</i> (Spreng.) Iamonico, Bartolucci & F.Conti <i>Tordylium maximum</i> L. <i>Torilis arvensis</i> (Huds.) Link subsp. <i>arvensis</i> <i>Trinia dalechampii</i> (Ten.) Janch.
*RR	<i>Trinia glauca</i> (L.) Dumort. subsp. <i>glauca</i>

Supplementary material I

Phytosociological releves surveyed in the study area and their syntaxonomical classification

Authors: Edda Lattanzi, Eva Del Vico, Roberto Tranquilli, Emmanuele Farris, Michela Marignani, Leonardo Rosati

Data type: Table

Explanation note: Table with the phytosociological releves surveyed in the study area and their syntaxonomical classification.

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Two new species of *Otoba* (Myristicaceae) from Colombia

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Abstract

Otoba is the third largest genus of Myristicaceae in the Neotropics with 12 species, nine of them native to Colombia. Two new species from the department of Antioquia, *O. scottmorii* **sp. nov.** and *O. squamosa* **sp. nov.**, are described and illustrated. *Otoba scottmorii* occurs in humid, lowland forests, while *O. squamosa* occurs in premontane forest. Previously, *Otoba scottmorii* was confused with *O. acuminata* (which here is considered restricted to Costa Rica and Panama), while *O. squamosa* was confused with *O. gordoniiifolia*. The similarities and differences between these and other species are discussed.

Keywords

Antioquia, Chocó, Magnoliales, Neotropics, Parque Nacional Natural Las Orquídeas, taxonomy

Introduction

Otoba (A. DC.) H. Karst. is one of six genera of Myristicaceae native to the Neotropics. Morphologically, the genus is relatively easy to recognize. Its species have sessile or short-stalked (e.g. *O. gordoniiifolia* (A. DC.) A.H. Gentry; *W. Devia et al.* 2291, MO) malpighiaceous foliar trichomes; conduplicate vernation; staminate flowers with filaments fused in an elongated column (except *O. novogranatensis* Moldenke in South America) with fused or free anthers; globose to ellipsoid green fruits; and seeds usually

covered by a white aril and marked by the presence of a lateral or apical gibba (Kühn and Kubitzki 1993; Jaramillo-Vivanco and Balslev 2020). As currently delimited, the majority of *Otoba* species occur in the Andes of Colombia and Ecuador and the Chocó biogeographic region of Colombia and Ecuador, with eight and six species respectively. Outside these regions in South America, Peru, Bolivia, Venezuela, and Brazil are each home to two species; in Venezuela, these are *O. novogranatensis* and the widespread *O. glycyarpa* (Ducke) W.A. Rodrigues & T.S. Jaram., while in the remaining countries, *O. glycyarpa* and *O. parviflora* (Markgr.) A.H. Gentry can be found.

Otoba has been comprehensively treated twice in the last century. In the first treatment (Smith and Wodehouse 1938), in which *Otoba* was treated as *Dialyanthera* Warb., six species were recognized. Eight decades later, this was followed by a comprehensive revision of the genus now recognized as *Otoba* (Jaramillo-Vivanco and Balslev 2020). This revision recognizes 10 species distributed from Nicaragua to Brazil (Fig. 1), with six found in Central America[†] and nine in South America. Based on morphology, species of *Otoba* can be placed into two informal groups that can be differentiated according to the anther size (0.2–0.6 vs. 0.7–1.5 mm long), vestiture on the ovary (glabrous vs. pubescent), fruits size, and pericarp thickness (see Table 1). These groups do not correspond to clades (Frost et al. 2021).

Two new species of *Otoba* are here described. Both occur in the Antioquia department of Colombia in premontane (1330–1450 m elevation) and humid (20–80 and 410–730 m) forests. Currently, there are seven species registered in this department: *O. acuminata*, *O. gordoniiifolia*, *O. gracilipes*, *O. latialata*, *O. lehmannii*, *O. novogranatensis*, and *O. parvifolia* (Cogollo 2011). However, according to our interpretation, the name *O. acuminata* attributed in herbaria and literature (e.g., Cogollo et al. 2007; Jaramillo-Vivanco and Balslev 2020) has been misapplied to Colombian specimens. Instead, this name should be applied to populations from Costa Rica and Panama (see description of *O. scottmorii* below). Thus, with the inclusion of *Otoba scottmorii* and *O. squamosa*, there are now eight known species of the genus for this department, and nine for the country. This makes Colombia the country with the highest number of native *Otoba* species (Table 1).

Natural history

Otoba often is abundant within their habitats (Hartshorn 1983; Gentry 1993; Macía and Svenning 2005; Guevara Andino et al. 2017; Honorio Coronado et al. 2019). Illustrating this abundance, it is often one of the most collected genera in Gentry plots (i.e., those in which all plants with stem diameters equal to or exceeding 2.5 cm diameter at breast height along ten 2 × 50 m transects are documented) (Phillips and Miller 2002).

Despite its abundance, our knowledge of floral biology of *Otoba* is very limited. The small flowers, which usually have a greenish-yellow or yellow perianth, are reported to have unpleasant odors (e.g., H. van der Werff 10764, MO), sometimes reported to smell of human semen (M.H. Grayum et al. 5502, MO). Small coleop-



Figure 1. Geographic distribution of *Otoba*.

terans are known pollen vectors for *O. novogranatensis* (Flores 2010), while flowers of *O. gordoniiifolia* are visited by Coleoptera (Curculionidae, Nitidulidae, Tenebrionidae), Diptera (Ceratopogonidae, Drosophilidae, Sciaridae), and Hymenoptera (Kanstrup and Olesen 2000). It is likely that all members of the genus are generalist insect pollinated, with a potentially important role for beetles, as observed in other Myristicaceae, including *Gymnacranthera* (A. DC.) Warb., *Knema* Lour., *Myristica fragrans* Houtt., and *M. insipida* R. Br. (Armstrong and Drummond 1986; Armstrong and Irvine 1989; Armstrong 1997; Momose et al. 1998).

More is known about dispersal in *Otoba*. Fruits are generally aromatic and globose, with a green, often shiny pericarp and a seed covered by a white or, occasionally, reddish aril. These arils can be very sweet (van Roosmalen et al. 1996; R. Aguilar, I. Chacón pers. comm.). Species with smaller fruits ($\sim 1.5\text{--}2.8 \times 1.5\text{--}2.4$ cm) and thin pericarps ($\sim 0.68\text{--}2.3$ mm) (e.g., *O. cyclobasis*, *O. scottmorii*, Costa Rican *O. novogranatensis*, and *O. parvifolia*) are generally found at low elevations, where they are frequently reported to be dispersed by bats (Swamy 2008; Melo et al. 2009). On the other end of the fruit size spectrum, species that have large fruits ($\sim 3.4\text{--}6 \times 2.8\text{--}4.5$ cm) and thick pericarp (2.6–5 mm), such as *O. gordoniiifolia*, *O. lehmannii*, *O. novogranatensis*, and *O. squamosa*, are likely dispersed by larger animals (birds or primates). In some of these higher elevation species, fruits are eaten by toucans (*Aulacorhynchus prasinus* in Costa Rica, *Pteroglossus erythropygius* in Ecuador) (Berg 2001; Chaves-Campos 2004) and monkeys (e.g. *Ateles belzebuth*, *Logothrix lagotricha*

Table 1. Species of *Otoba* showing anther long, vestiture on the ovary, fruit size, pericarp thickness, and distributions.

Taxon	Anther long (mm)	Ovary vestiture	Fruit size (cm)	Pericarp thickness (mm)	Distribution
<i>O. acuminata</i> (Standl.) A.H. Gentry (1979: 417)	0.45–0.8	Pubescent	1.9–2.5 (–2.8) × 1.7–2.2 (–2.4)	1–2.1	Costa Rica, Panama
<i>O. cyclobasis</i> T.S. Jaram. & Balslev (2001: 563) [‡]	ca. 0.3	Glabrous	1.5–2.5 × 1.5–2	1–2	Ecuador
<i>O. glycyarpa</i> (Ducke) W.A. Rodrigues & T.S. Jaram. (2001: 446) [‡]	0.5–0.6	Pubescent	3.4 × 3.4	5–8	Colombia, Ecuador, Peru, Bolivia, Brazil
<i>O. gordoniiifolia</i> (DC.) A.H. Gentry (1979: 417) [‡]	0.7–1.5	Pubescent	(3–) 5 × 4	3–5	Colombia, Ecuador
<i>O. gracilipes</i> (A.C. Sm.) A.H. Gentry (1979: 417) [‡]	0.6–0.8	Glabrous	3–3.5 × 2.5	3–4	Colombia, Ecuador
<i>O. latialata</i> (Pittier) A.H. Gentry (1979: 417)	0.2–0.3 (in Panama; pers. obs.)	Glabrous	2.2–2.8 × 1.8–2.3 (in Panama pers. obs.)	2 [‡] (ca. 1 in Panama pers. obs.)	Panama, Colombia
<i>O. lehmannii</i> (A.C. Sm.) A.H. Gentry (1979: 417) [‡]	0.7–1.3	Glabrous	4–6 × 4–4.5	3–5	Colombia
<i>O. novogranatensis</i> Moldenke (1932: 156) s.l.	1–1.5 [‡] (0.6–1.1 in Central America; pers. obs.)	Pubescent in South America [‡] ; (always glabrous in Central America; pers. obs.)	3–5.5 × 2.5–4 [‡] (2.3–3.5 × 2–2.5 in Central America; pers. obs.)	2–5 [‡] ([1.4–] 2.1–2.3 in Central America; pers. obs.)	Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Venezuela
<i>O. parvifolia</i> (Markgr.) A.H. Gentry (1979: 417) [‡]	0.3–0.7	Glabrous	2.3–3 × 1.5–2	1–2	Colombia, Ecuador, Peru, Bolivia, Brazil, Venezuela
<i>O. squamosa</i>	0.5–0.7	Glabrous	(2.5–) 3.4–3.6 × (1.9–) 2.8–3	2.6–3	Colombia
<i>O. scottmorii</i>	0.2–0.4	Unknown (speculated is glabrous)	1.8–2.1 × 1.5–1.7	0.68–0.89	Colombia
<i>O. vespertilio</i> D. Santam. & J.E. Jiménez (2019: 371)	ca. 0.2–0.3	Glabrous	2.2–2.7 × 1.6–1.7	1.3–1.8	Costa Rica, Panama

†*Otoba gracilipes* is attributed to Costa Rica and Panama, and *O. latialata* to Costa Rica by Jaramillo-Vivanco and Balslev 2020. Part of the material that is cited as *O. gracilipes* by Jaramillo-Vivanco and Balslev (2020) corresponds to our concept of *O. acuminata* (i.e., [L.] Poveda et al. 3793, CR, MO; [P.H.] Allen 2004, MO; [T.B.] Croat 25225, MO, PMA; as well M.H. Grayum et al. 7675 CR, F [n.v.], MO, cited under *O. novogranatensis*). Other collections referred to as *O. gracilipes* in that work (i.e., [R.] Lent 2526, F digital image; [G.] Hartshorn 1310, F-2 sheets, digital imagen), as well as those referred to *O. latialata* from Costa Rica ([P.H.] Allen 5591, F digital image, CR, US digital image), and [M.H.] Grayum & [J.E.] Morales 20786 [the name of the collector correctly given to B. Hammel et al. 20786, CR-2 sheets, MO]) we consider to belong to a very broad sense of *O. novogranatensis* (D. Santam. In prep.).
‡From Jaramillo-Vivanco and Balslev 2020.

in Yasuní National Park, Ecuador; *Alouatta seniculus* in Andean forests of Colombia (Dew 2005; Giraldo et al. 2007). Various small, terrestrial animals also consume fruits of *Otoba*. This has been noted when large numbers of fallen fruit (i.e., intact pericarps and seeds; Fig. 2A, B) accumulate around the base of a tree. In Costa Rica, the red-orange arils of *O. novogranatensis* are eaten by agoutis (*Dasyprocta punctata*), pacas (*Agouti paca*), and squirrels (pers. comm. by R. Aguilar, J.M. Chaves, F. Oviedo, J.A. Rosales). Similarly, in Panama, the white-transparent aril of *O. acuminata* is consumed by the brown four-eyed opossum, *Metachirus nudicaudatus* (Fig. 2C, D) (Sosa-Bartuano 2016). It is not known if these animals are dispersers, seed predators, or both. Germination of the seeds of *O. novogranatensis* in natural conditions is very

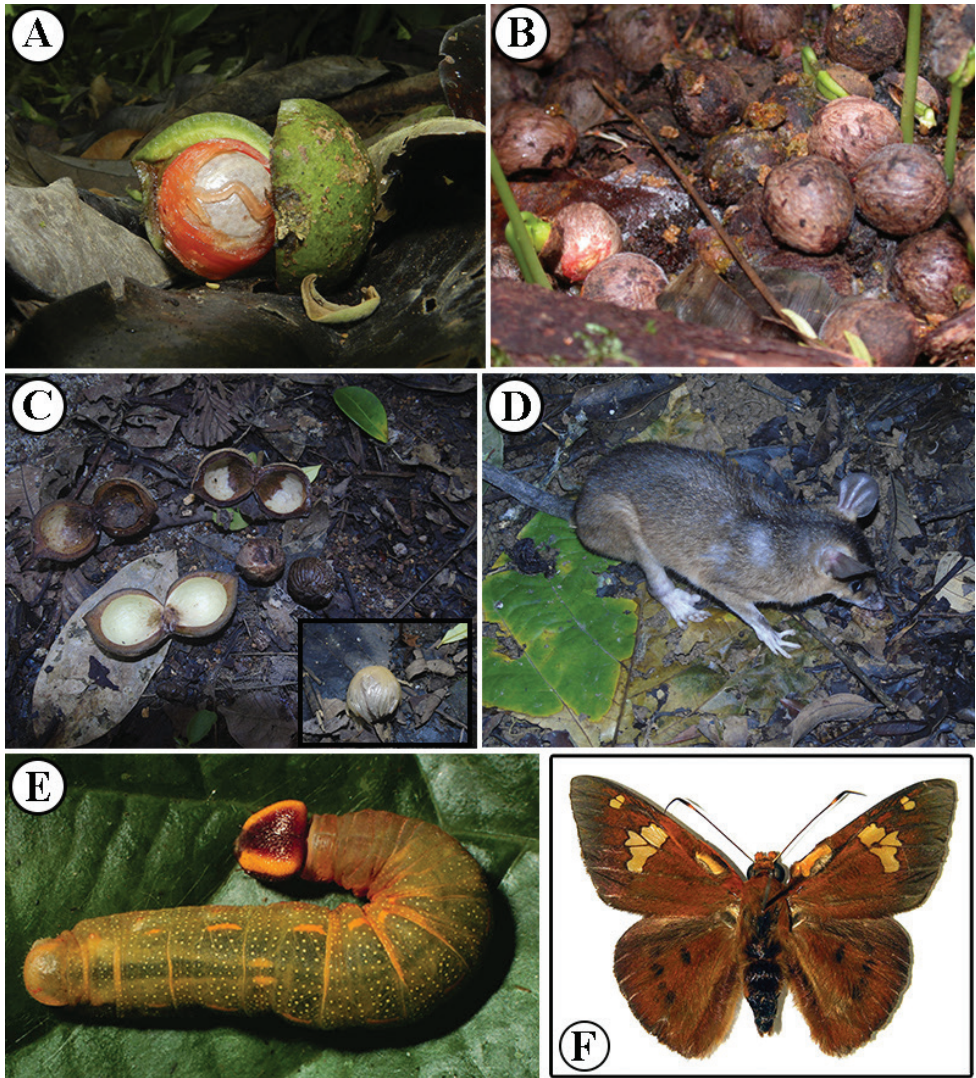


Figure 2. Examples of natural history of *Otoba* in Central America **A, B** *Otoba novogranatensis*, fruits (**A**) and seeds (**B**) accumulating on the ground in Costa Rica **C, D** Fruits of *O. acuminata*, inset seed covered by aril (**C**), which are eaten by *Metachirus nudicaudatus* (**D**) in Panama **E, F** *Dyscophellus phraxanor* (Hesperiidae), a herbivore of *O. novogranatensis*, as a larvae (**E**) and adult (**F**). Photos by Reinaldo Aguilar (**A**), Orlando Vargas (**B**) from <https://sura.ots.ac.cr/florula4/index.php>, Ángel Sosa-Bartuano (**C, D**), Daniel H. Janzen (**E, F**) from <http://janzen.sas.upenn.edu/caterpillars/database.lasso>.

low due to granivory by insects including a weevil (*Conotrachelus* sp., Curculionidae), although seeds collected shortly after falling germinate at high rates in nursery conditions (pers. comm. by J.A. Rosales).

Leaves of *Otoba* are also an important food source for animals. This includes mammals, such as the Caquetá titi (*Plecturocebus caquetensis*), which eats the young leaves

of *Otoba parvifolia* (Acero-Murcia et al. 2018), and herbivorous insects. In Área de Conservación Guanacaste in Costa Rica, the leaves of *O. novogranatensis* are eaten by the larvae of nine families of Lepidoptera, including *Dyscophellus* (Hesperiidae) (Fig. 2E, F) (Janzen and Hallwachs 2009).

Materials and methods

In the course of herbarium investigations, we identified two new taxonomic species that we describe here. Descriptions are based on herbarium specimens observed at the following herbaria: CR (including ex INB), LSCR, LSU, MO, NO and USJ, as well as loaned material from: GH, NY, and US (acronyms follow Thiers 2020 [continuously updated]), though specimens from MO represent the majority of the material studied. All specimens hosted by virtual herbaria, including types specimens, were consulted, including those maintained by: the Field Museum (F; <http://emuweb.fieldmuseum.org/botany/taxonomic.php>), Instituto Nacional de Pesquisas da Amazônia (INPA; <http://inct.florabrasil.net/en/>), JSTOR Global Plants (<http://plants.jstor.org>), Museum of Natural History, Paris (P; <http://www.mnhn.fr>), and Universidad Nacional Autónoma de México (MEXU; <https://datosabiertos.unam.mx/biodiversidad/>). Images of specimens not available online were provided by F and JAUM. If necessary and material permitted, flowers from specimens were rehydrated before measurement. A ruler was used to measure leaves and inflorescences; a digital Neiko caliper was used to measure fruits, seeds, and the thickness of the twigs, petioles and peduncles; and, finally, flowers, trichomes and thickness of the pericarp were measured with a micrometer calibration tool (1div = 1mm) under a dissecting stereoscope (Bausch & Lomb).

In the nomenclatural section for each new species, we cite both accession numbers and barcodes when present. Barcodes are included in square brackets and follow the format of a series of numbers preceded by the herbarium acronym (e.g., [04206141]); all other numbers correspond to accession numbers.

When the coordinates and/or elevation were not included on the herbarium label but were present in the TROPICOS database (Tropicos.org. 2020), the values from TROPICOS are included in brackets. Dot-distribution maps were compiled from studied specimens and generated with SimpleMappr (Shorthouse 2010).

The preliminary conservation status of each new species was assessed using quantitative criteria recommended by the IUCN Red List (IUCN Standards and Petitions Subcommittee 2014). Georeferenced specimen data were used to determine the area of occupancy (AOO) and the extent of occurrence (EOO), which in turn were used to determine threat status. All analyses were performed in the R package conR (Dauby et al. 2017). When the recommendation differed between AOO and EOO assessments for a given species, we opted to conservatively recommend the more vulnerable status, following Knapp (2013).

Taxonomy

Otoba scottmorii D. Santam., sp. nov.

urn:lsid:ipni.org:names:77217289-1

Figs 3, 5B, 6C

Type. COLOMBIA. Antioquia: [Municipio] Mpio. Segovia, 24.5 km N of Remedios (17 km N of La Cruzada) on road to Zaragoza, hills side forest above río Poconé, 07°12'N, 074°48'W, [not elev.], 20 Jul. 1987 (♂ fl), W.W. Thomas & C.J. Castaño 5501 (holotype: NY! [04206141]; isotypes: INPA-159329 [digital image!], UPCB-35245 [n.v.]).

Diagnosis. *Otoba scottmorii* is similar to *O. cyclobasis* from Ecuador. However, it differs in having smaller leaves (5.5–8.5 [–11.2] vs. 9–14 [–18] cm long) with an attenuate to acuminate apex (vs. cuspidate), inconspicuous marginal and secondary veins (vs. conspicuous), fewer lateral veins (4–7 vs. 14–17), straight axes of staminate inflorescences (vs. zig zag), a longer filament column ([1.3–] 1.5–1.6 vs. 1 mm long), larger fruits (1.8–2.1 vs. 1.5–2.5 mm long) with thinner pericarp (0.68–0.89 vs. 1–2 mm thick), and seeds that are gibbose at the apex (vs. near-basal and lateral gibba).

Description. **Tree** (2–) 15–20 m tall × 45 cm diam., external and internal bark not described. **Exudate** once described as red in the bark, transparent and without specifying from where, or without exudate. **Twigs** 0.88–0.1 cm thick, terete to slightly flattened laterally, the external bark brown to grayish, with malpighiaceae trichomes that are 0.2–0.3 mm long, brown to ferruginous or sometimes glabrescent. **Young foliar bud** 0.6–1.2 cm long, densely pubescent. **Leaves:** petiole 0.5–1.1 × 0.055–0.1 cm, canaliculate, not winged; lamina 5.5–8.5 (–11.2) × 1.7–2.5 (–3.7) cm, elliptic; adaxial side glabrous, drying blackish, sometimes brown in young leaves, the surface muricate; abaxial side drying brown to whitish grayish, the surface wrinkled to muricate, sparsely pubescent to glabrescent, with malpighiaceae trichomes that are 0.2–0.4 mm long, sessile, and ferruginous, and squamate trichomes that are ca. 0.08 mm diam. with the central portion dark and sides lighter (sometimes these sides appearing absent), with whitish crystals; vernation line absent; midvein flat to very slightly ribbed on adaxial side, the same color as the surface, abaxially 0.2–0.4 mm wide, slightly raised, darker than the surface; secondary veins brochidodromous, the loops 0.1–0.3 cm from the margin (sometimes the loops not visible), lateral veins 4–7 per side, 3–5 veins per 3 cm, on adaxial side flat and visible, on abaxial side flat and not very conspicuous, arcuate distally, the marginal vein not visible on adaxial side, slightly visible on abaxial side; tertiary veins indistinct; base attenuate, not revolute; margin entire, not revolute; apex attenuate to acuminate, the acumen 0.6–1 (–2) cm long. **Staminate inflorescence:** axillary (only very young twigs) or supraaxillary, with 1–2 (–3) main axes, spiciform, these axes 1.5–4.3 cm × 0.41–0.1 mm, pubescent, the trichomes ferruginous to coppery, each axis compound with 2–3 (–6) fascicles of flowers, each fascicle with 1–5 (–8) flowers, alternate; bracts not seen; pedicel 3–5 mm long, pubescent; bracteoles absent. **Staminate flowers:** flower bud 1–2.5 × 0.6–0.1 mm, elliptic to lanceolate; perianth 2–2.5 mm long,



Figure 3. *Oroba scottmorii* **A** staminate flowering branch, with detail of leaf surface (detail below left) **B** leaf base, petiole and basal portion of inflorescence **C** detail of staminate inflorescence **D** flower bud **E** staminate flowers, entire (above right) and with one perianth lobe removed, showing the androecium (below center) and perianth lobe (above left) **F** androecium **G** stem with infructescence, including an immature, opening fruit **H** view of dehiscent fruit (left, below G) showing the seed and aril (right) *Oroba cyclobasis* **I** staminate flowering branch showing leaves with brochidodromous venation, including detail of marginal vein **J** staminate flower with one perianth lobe removed, showing the androecium and perianth lobe (above left) **K** androecium. Illustration by Bobbi Angell, W.W. Thomas & C.J. Castaño 5501 (NY) (**A–F**), J. Brand & M. Narváez 682 (MO) (**G, H**), E. Narváez et al. 1072 (MO-2468293) (**I–K**).

yellow or orange (in fresh material), sub-membranaceous, connate by 0.5–0.7 (–1) mm; lobes 3 (4), 1.5–1.8 × 0.8–1.1 mm, without resinous punctuations or lines, pubescent outside, the trichomes ferruginous to coppery, inside glabrous, the apex acute to obtuse, without inflexed-apicula, the edges flat or slightly turned inwards distally, without a swollen lobed ring; filament column (1.3–) 1.5–1.6 mm long, bowling pin-shaped, fleshy, connate, glabrous; anthers 3, 0.2–0.4 mm long, free, lanceolate to oblong, apex slightly incurved. **Pistillate inflorescence** and **flowers**: not seen. **Infructescence** 2–4 cm long, with a solitary fruit; pedicel 1.2–1.6 cm long. **Fruits** 1.8–2.1 × 1.5–1.7 cm, green when fresh, globose, surface glabrous, colliculate-rugose, sometimes with whitish lenticels (J. Brand 682), the line of dehiscence smooth, the base obtuse, apex apiculate, the acumen 0.1–0.3 cm long; pericarp 0.68–0.89 mm thick; seed ca. 1.6 × 1.5 cm, similar in shape to the fruit, brownish (in dry material), gibbose at the apex (ca. 0.3 mm wide), the testa ca. 0.4 mm thick; aril described once as red (E. Rentería 4680), brownish to yellowish translucent in dry material, dry and membranous in texture.

Distinctive characters. *Otoba scottmorii* is recognized by a combination of leaf, inflorescence, and fruit traits. Its small leaf blades have long apices and thin petioles, and lack vernation lines on the abaxial surface. The staminate inflorescences are delicate with flowers on relatively thin, long pedicels; these flowers have sub-membranaceous perianth and lack a swollen-lobed ring, a bowling pin-shaped filament column, and anthers that are lanceolate to oblong. Finally, fruits are small with thin pericarp and membranous aril.

Etymology. Is a pleasure to name this species after Dr. Scott A. Mori (1941–2020), a wonderful person and skilled botanist; a dedicated explorer of Central and South America humid forests (where this species occurs), especially in the Guianas and the Amazon basin; and an authority on Neotropical Lecythidaceae. His taxonomic and ecological publications gave great inspiration to the first author, as did Dr. Mori's personal support. For an account of Dr. Mori's legacy, see Boom (2020), Forget (2020), and Prance et al. (2021).

Common name. Cuángare otobo (Cogollo et al. 2007).

Distribution. *Otoba scottmorii* is known only from the humid forests in the Department of Antioquia in northwestern Colombia in the Municipios of Mutatá, Segovia, Tarazá, Turbo, and Valdivia (Fig. 4). Three collections were collected under 100 m elevation (20–80 m), and three others above 400 m elevation (410–730 m).

Phenology. Herbarium specimens of *Otoba scottmorii* have been collected with staminate flowers in May, July, and November, and with fruits in November. Pistillate flowers have yet to be observed.

Preliminary conservation status. *Otoba scottmorii* is Endangered following IUCN criteria B2a. Justifying this status, it is known from five localities, has an EOO of 5,341 km², and an AOO of 20 km². The regions where it occurs are threatened by high levels of deforestation (González-Caro and Vásquez 2017). Of the relatively few specimens of this species that we were able to verify, the most recent specimen was collected in 1993.

Discussion. *Otoba scottmorii* is similar to *O. acuminata* and *O. vespertilio* D. Santam. & J.E. Jiménez, from Costa Rica and Panama, and *O. cyclobasis* T.S. Jaram. &



Figure 4. Geographic distribution of *Otoba scottmorii* (black circle) and *O. squamosa* (black triangle). The shaded map area corresponds to the department of Antioquia.

Balslev from Ecuador. They all have relatively small leaf blades of similar shape and thin petioles, and usually lack vernation lines (Figs 5, 6); staminate flowers with short perianth and small anthers; and similarly sized fruit with thin pericarp (Fig. 5). The four species can be distinguished by the characteristics in Table 2.

As mentioned in the introduction, *Otoba scottmorii* was confused with the Mesoamerican species *O. acuminata*, which we now consider to be endemic to the Caribbean slope of Costa Rica and Panama. The specimens referred to as *O. acuminata* from Colombia in recent floras or checklists (e.g., Cogollo et al. 2007; Cogollo 2011; Ulloa Ulloa et al. 2017; Jaramillo-Vivanco and Balslev 2020) correspond to *O. scottmorii*. The confusion between these two species is likely driven by the similar morphology of the leaf blades (i.e., size and shape, usually without vernation lines, and thin petioles). However, *O. scottmorii* differs from *O. acuminata* in having narrower leaf blades (Fig. 5B, A respectively), staminate flowers with short perianth and narrow perianth lobes, a longer column of filaments, and fruits with a smooth surface and thin pericarp

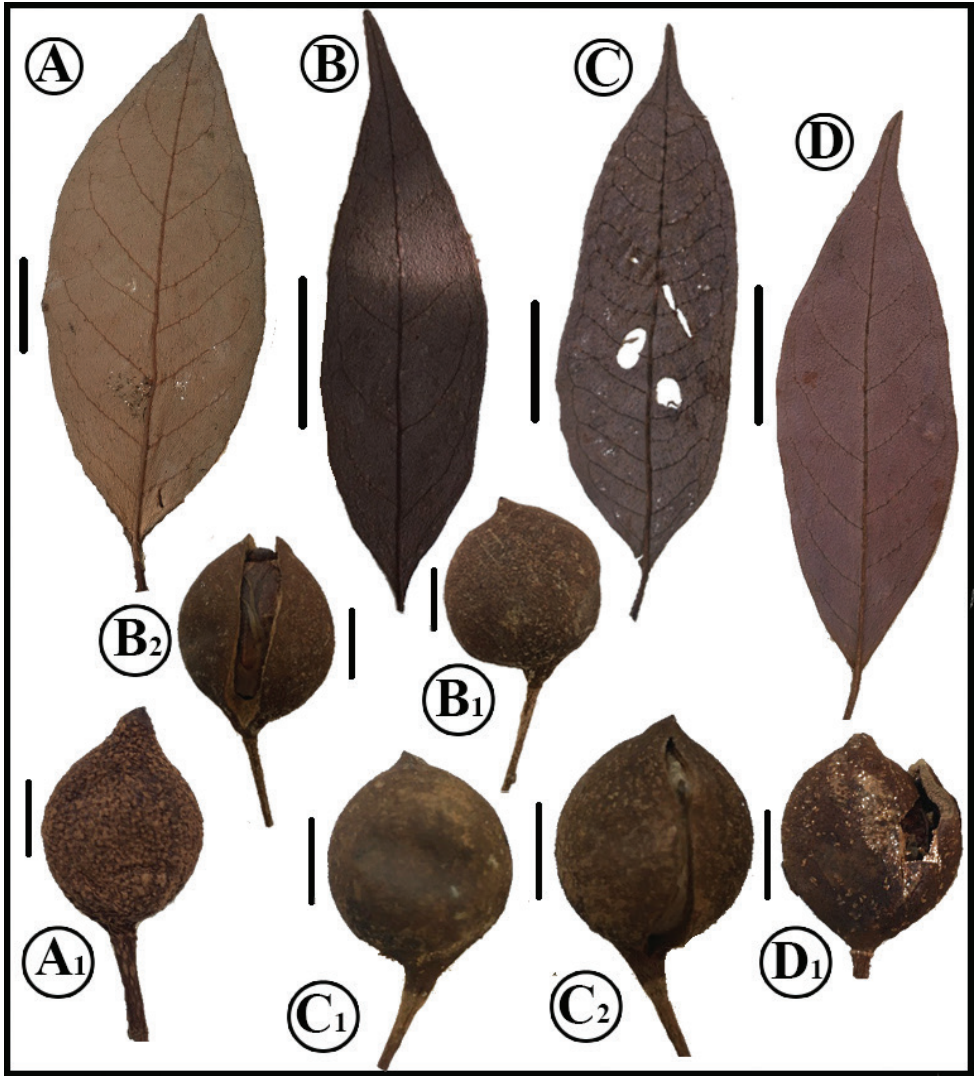


Figure 5. Comparison of leaf blades and fruits of *O. acuminata* (**A** L. Poveda et al. 3793, MO **A**₁ from H.W. Churchill & G.C. de Nevers 4976 MO; immature fruit) *O. scottmorii* (**B** R. Callejas et al. 3440 MO **B**₁–**B**₂ from R. Callejas et al. 5789, MO) *O. cyclobasis* (**C** E. Narvaéz et al. 1072, MO **C**₁–**C**₂ from W.A. Palacios & M. Tirado 11318 MO) and *O. vespertilio* (**D** G. McPherson 13597, MO, juvenile leaf, **D**₁ G. McPherson 12543, MO). Scale bars: leaves: 2 cm; fruits: 0.75 cm.

(Table 2). Although we have not observed the pistillate flowers of *O. scottmorii*, it is likely that the ovary is glabrous as the fruits do not have traces of trichomes, while in *O. acuminata* the ovary is pubescent; see for example: A. Rodríguez et al. 1559 (CR-2 sheets, MO); A. Estrada et al. 4829 (CR); B. Hammel & M. Grayum 14288 (CR, INPA [digital image], MEXU [digital image], MO). For a list of specimens that correspond to *O. acuminata*, see Santamaría-Aguilar et al. (2019).

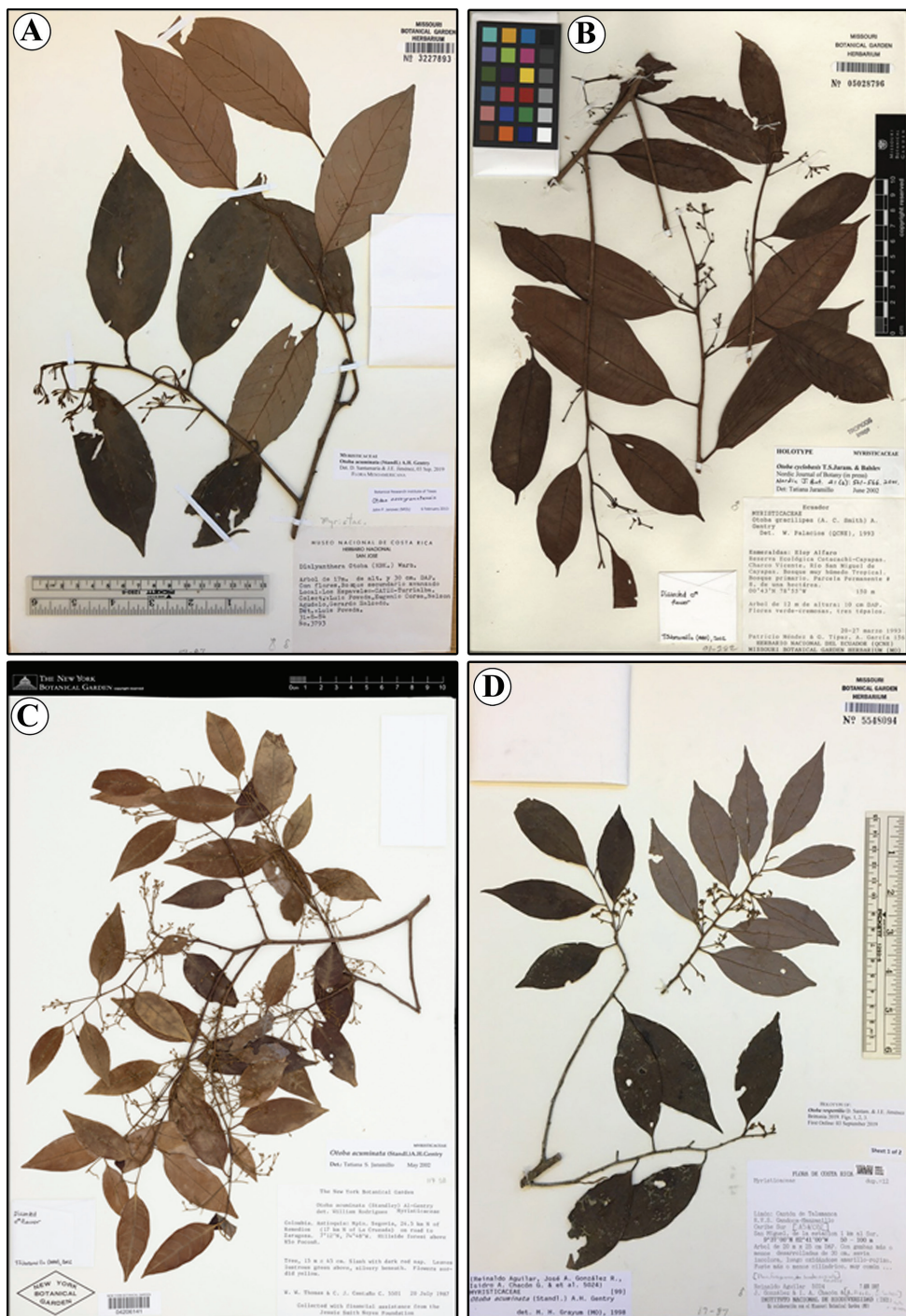


Figure 6. Comparisons of herbarium specimens of *Otoba acuminata* (A L. Poveda et al. 3793, MO) *O. cyclobasis* (B P. Méndez et al. 156, MO) *O. scottmorii* (C W.W. Thomas & C.J. Castaño 5501, NY), and *O. vesperilio* (D R. Aguilar et al. 5024, MO).

Table 2. Comparison of *Otoba scottmorii* with similar species (Fig. 6).

Taxon	<i>O. scottmorii</i>	<i>O. acuminata</i>	<i>O. cyclobasis</i>	<i>O. vespertilio</i>
Petiole length (cm)	0.5–1.1	(1.1–) 1.8–2.6	1–1.5 [‡]	0.8–2
Leaf size (cm)	5.5–8.5 (–11.2) × 1.7–2.5 (–3.7) (Fig. 5B)	(6.5–) 8.5–15.5 × (2.7–) 4.4–6.1 (Fig. 5A)	9–14 (–18) × 3–5 [‡] (Fig. 5C)	5.3–12.5 × 2–3.5 (–4.8) (Fig. 5D)
Lateral vein number	4–7	8–12	14–17 [‡]	6–8
Staminate flower pedicel length (mm)	3–5	5–8	4–5 [‡]	2–3.6
Staminate perianth length (mm)	2–2.5	4.5–5.5	ca. 2.5 [‡]	2–3
Perianth lobe width (mm)	0.8–1.1	1.5–2	ca. 1 [§]	0.5–1
Ring	Absent	Absent	Present [‡]	Absent
Filament column length (mm) and shape	(1.3–) 1.5–1.6, bowling pin	2–2.7, cylindrical, ovoid, to pyriform	1, cylindrical	0.6–1.5, cylindrical
Anther length (mm) and shape	0.2–0.4 lanceolate to oblong	0.45–0.8, oblong	ca. 0.3, globose [‡]	ca. 0.2–0.3, globose to subglobose
Ovary vestiture	Unknown	Pubescent	Glabrous [‡]	Glabrous
Fruit length and width (cm)	1.8–2.1 × 1.5–1.7 (Fig. 5 _{1,2})	1.9–2.5 (–2.8) × 1.7–2.2 (–2.4) (Fig. 5A ₁)	1.5–2.5 × 1.5–2 [‡] (Fig. 5C _{1,2})	2.2–2.7 × 1.6–1.7 (Fig. 5D ₁)
Pericarp thickness (mm)	0.68–0.89	1–2.1	1–2 [‡]	1.3–1.8
Seed length and width (cm)	1.6 × 1.5	ca. 2.2 × 1.9	1–2 × 1–1.5 [‡]	1.8–1.9 × ca. 1.7 cm
Distribution and elevation	Colombia, 20–80 m and 410–730 m	Costa Rica, 100–900 m; Panama, 500–1000	Ecuador, 150–300 m [‡]	Costa Rica 0–200 m, Panama 100–800 m

[‡]Information from Jaramillo-Vivanco and Balslev 2020; [§]from E. Narvaéz et al. 1072, MO-2 sheets.

Notes. The type specimen of *Otoba scottmorii* and other collections here mentioned have been previously identified in herbaria and cited in literature as *O. acuminata* and *O. gracilipes*, and duplicates may have been distributed under these names.

Specimens examined. COLOMBIA. Antioquia: Mutatá, Sitio Rio Surambay, 12 km N de Mutata, 07°20'N, 076°30'W, 30–80 m, 21 Nov. 1987 (♂ fl), R. Callejas et al. 5752 (INPA digital image, MO, NY); ibid, 21 Nov. 1987 (fr), R. Callejas et al. 5789 (INPA digital image, MO, NY); Turbo, Carretera Tapón del Darién, Sector Río León-Lomas Aisladas, km 37, [07°39'11"N, 076°58'02"W], 20 m, 29 Nov. 1993 (fr), J. Brand & M. Narvaéz 682 (COL [n.v.], JAUM digital image, MO); río Cianurá, Paso de la Reina, 730 m, 13 Mar 1986 (fr), E. Renteria et al. 4680 (JAUM digital image); Tarazá, Corregimiento El 12, 210 kms, NE de Medellín, vía El 12-Barroblanco, km 3, 07°30'N, 075°20'W, [450 m], 09 Nov. 1987 (♂ fl), R. Callejas et al. 5509 (INPA digital image, NY); Valdivia, corregimiento Puerto Valdivia, km 5 de Puerto Valdivia hacia “El 12”, colecciones a lo largo del Río Pescado, 07°20'N, 075°20'W, 410 m, 14 May. 1987 (♂ fl), R. Callejas et al. 3440 (MO-2 sheets, NY).

***Otoba squamosa* D. Santam., sp. nov.**

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Fig. 7

Type. COLOMBIA. Antioquia: Urrao, Vereda Calles, Parque Nacional Natural “Las Orquídeas”, margen derecha Quebrada La Honda, 06°32'N, 076°19'W, 1330–1400 m,

08 May 1993 (fr), Á. Cogollo, R. Carmona, E. Álvarez 6197 (holotype: MO-05011088! [1500541]; isotypes: n.v.).

Diagnosis. *Otoba squamosa* is similar to *O. gordoniiifolia* from Colombia and Ecuador, and both species grow in montane forest in the Andes. However, it differs in its leaves with shorter petioles (1.7–2.7 [–3.8] vs. [3–] 5–7 cm long) and smaller lamina (6.7–14.5 vs. [13–] 24–34 cm long), staminate flowers with a perianth with a swollen ring (vs. without) and smaller anthers (0.5–0.7 vs. 0.7–1.5 mm long), pistillate flowers with a glabrous ovary (vs. pubescent), and fruits with thin pericarp (2.6–3 vs. 3–5 mm thick).

Description. *Tree* 8–18 (–24) m tall × 11.9–34.7 cm diam., external and internal bark not described. *Exudate* hyaline, oxidizing reddish, only reported from in flowers and fruits. *Twigs* 0.13–0.27 cm thick, terete to slightly flattened laterally, the external bark brown to blackish, with malpighiaceae trichomes 0.2–0.6 mm long, brown to ferruginous, the indument denser in young parts. *Young foliar bud* 1.6–4.3 (–5.8) cm long, densely pubescent. *Leaves:* petiole 1.7–2.7 (–3.8) × 0.1–0.2 (–0.25) cm, canaliculate, very short-winged; lamina 6.7–14.5 × 3.4–6 (–8) cm, elliptic, rarely widely elliptic; adaxial side glabrous, usually drying dark brown to blackish, the surface muricate-reticulate; abaxial side usually drying pale to dark brown, the surface muricate, sparsely pubescent, with malpighiaceae trichomes 0.3–0.6 mm long, sessile, ferruginous, and scale-like trichomes ca. 0.1 diam. with the central part dark, contrasting with the lighter sides, crystals generally absent and if present very few; vernation line imprints 2 parallel lines, 0.7–1.5 (–1.8) cm from the margin, the panel area 1.8–2.5 (–3.4) cm wide (in the central portion), the same color as the surface; midvein flat on adaxial side, the same color as the surface or blackish, abaxially 0.5–0.9 mm wide, raised, a little darker than the surface; secondary veins brochidodromous, the loops 0.2–0.3 cm from the margin, lateral veins 13–17 per side, (3–) 4–6 veins per 3 cm, on adaxial side slightly caniculate, on abaxial side flat to slightly raised, not very conspicuous, arcuate distally, the marginal vein not visible on adaxial side, slightly visible on abaxial side; tertiary veins indistinct; base acute to cuneate, not revolute; margin entire, not revolute; apex acute, the acumen 0.3–0.9 cm long. *Staminate inflorescence:* axillary and/or ramiflorous, with 1–2 main axes, spiciform, these axes 2–7 cm × 0.6–1.2 mm, pubescent, the trichomes ferruginous, each axis compound with 2–5 fascicles of flowers, each fascicle with 3–6 flowers, alternate; bracts ca. 1.5–1.6 × 1 mm (observed in very young inflorescences), densely pubescent outside, the trichomes ferruginous; pedicel 1.3–4 mm long, pubescent; bracteoles absent. *Staminate flowers:* flower bud 2–3 × 1–1.5 mm; perianth 3.5–4.7 mm long, yellowish to yellowish-green (in fresh material), fleshy (hardening near the base by the ring), connate by 1–1.7 mm; lobes 3 (4), (2–) 2.5–3.4 × (0.9–) 1.4–2 mm, without resinous punctuations or lines, pubescent outside, the trichomes ferruginous, inside glabrous, smooth to lightly spongy, the apex in some flowers with a minutely inflexed-apiculate, the margin edges slightly turned inwards, slightly wavy; ring present, 0.1–0.4 mm wide, lobed, smooth, or sometimes spongy; filament column 1.5–2.1 mm long, usually cylindrical, slightly narrow towards the apex, fleshy, glabrous; anthers 3 (4), 0.5–0.7 mm long, free, lanceolate to oblong, apex slightly incurved. *Pistillate inflorescence:* axillary, 1.4–3.2 cm long, pubescent, the

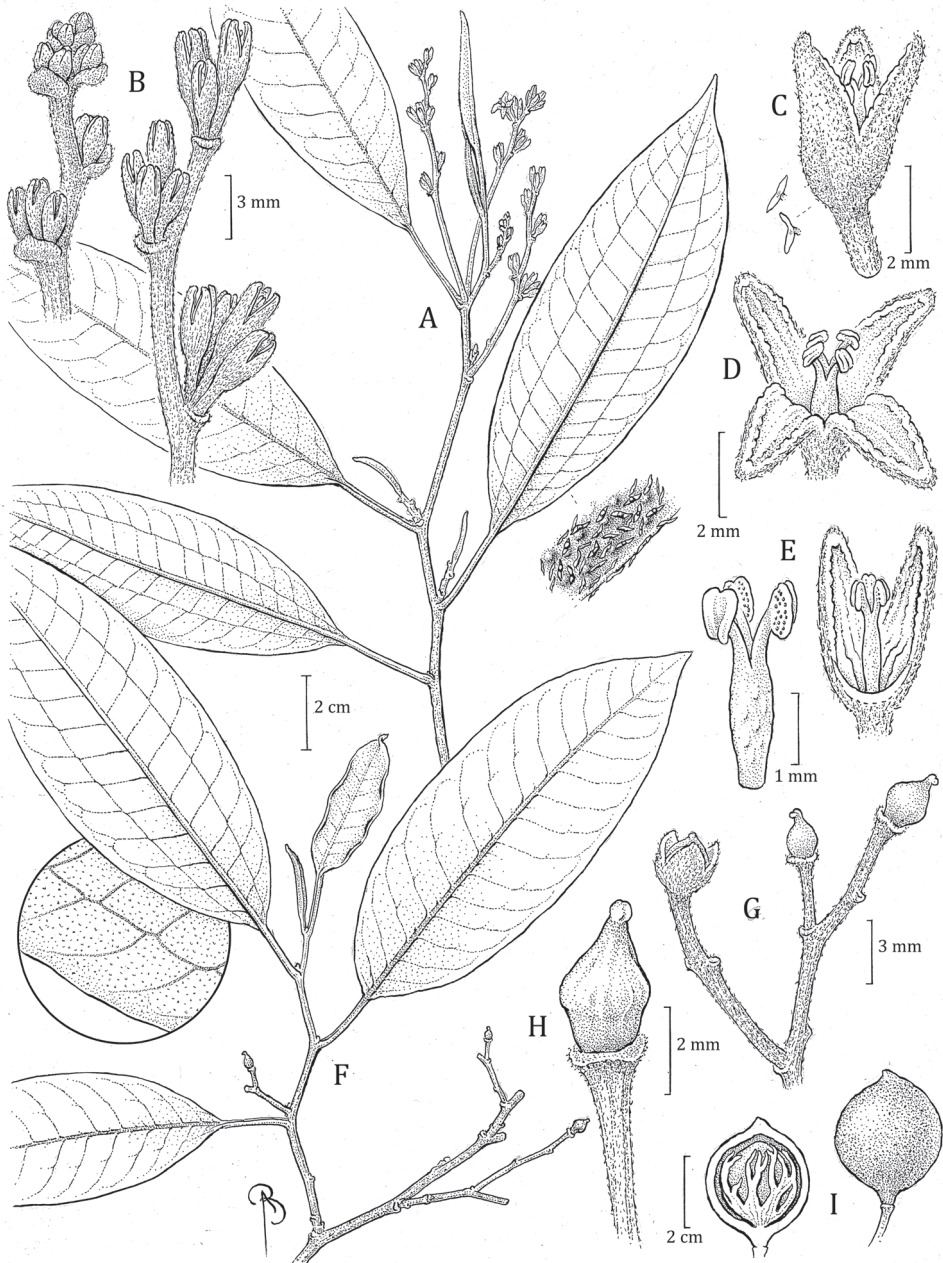


Figure 7. *Otoba squamosa* **A** staminate flowering branch, with detail of malpighiate trichomes on abaxial leaf surface (detail below right) **B** detail of staminate inflorescence **C** staminate flower, with detail of trichomes **D** open staminate flower **E** longitudinal section of staminate flower and androecium **F** pistillate flowering branch **G** portion of pistillate inflorescence, showing the perianth (left) and ovary after the perianth has fallen **H** ovary **I** closed and open fruit (left), showing the pericarp and aril. Illustration by Bobbi Angell. Á. Cogollo et al. 6279 (MO) (**A–D**), J. Pipoly et al. 17019 (MO) (**E**), J. Pipoly et al. 16798 (**G**), Á. Cogollo et al. 6197 (MO) (**F, H, E**).

trichomes ferruginous, each axis compound with 1–2 fascicles of flowers, each fascicle with 2–3 flowers, alternate; bracts not seen; pedicel 3–4 mm long, pubescent; bracteoles absent. **Pistillate flowers:** flower bud 3–4 × ca. 2 mm; perianth 3–5 mm long, the color and texture as in the staminate flower, connate by 1–1.5 mm; lobes 3 (4), 2–3.5 × 2–2.5 mm, without resinous punctuations or lines, pubescent outside, the trichomes ferruginous, inside glabrous; ring present; gynoecium 2–3 × 1.5–2.3 mm, glabrous, ovary sessile to short-stalked, ca. 0.6 mm long; stigma 2-lipped, subsessile; stigmatic lips ca. 0.6 mm long. **Infructescence** probably with one fruit (fruits separated from the axis in all specimens observed); pedicel ca. 1–1.1 cm long. **Fruits** (2.5–) 3.4–3.6 × (1.9–) 2.8–3 cm, green, globose, the surface glabrous, rugose, sometimes with whitish to brownish lenticels, the line of dehiscence smooth, the base obtuse, and sometimes getting narrower towards the pedicel, apex obtuse or acute, the acumen 0.8–1 cm long; pericarp 2.6–3 mm thick; seed (2.1–) 2.5–2.9 × (1.7–) 2.3–2.4 cm, similar in shape to the fruit, whitish or brown (in dry material), gibbose at the apex or nearly so, the testa 0.4–0.6 mm thick; aril described once as white (D. Sánchez et al. 1529), brownish to white-yellowish in dry material, waxy to dry in texture.

Distinctive characters. *Otoba squamosa* is recognized by a variety of leaf traits, including: squamate or scale-like indument mixed with malpighiaceae trichomes on the abaxial surface, lateral veins that are more or less conspicuous, forming a marginal vein, and vernation lines that parallel the midvein. Additionally, the staminate flowers have a perianth with swollen-lobed ring in the inner surface, a typically cylindrical filament column with lanceolate to oblong anthers and pistillate flowers that have a glabrous gynoecium. Finally, the fruits are relatively large, with thick pericarp.

Etymology. The specific epithet refers to the squamate or scale-like indument that is present with more typical malpighiaceae trichomes on the abaxial surface of the leaf blades. The squamate or scale-like indument is not unique to this species; it is also present in most specimens of *O. acuminata*, *O. glycyarpa*, *O. scottmorii*, and *O. vespertilio*; also Smith and Wodehouse (1938), they described for *O. lehmannii* (as *Dialyanthera Lehmannii*).

Common name. None recorded.

Distribution. *Otoba squamosa* is known from the Cordillera Occidental of Colombia, specifically in the municipalities of Frontino and Urrao in the Department of Antioquia (Fig. 4). It grows in premontane forest between 1330–1450 m.

Phenology. Fertile herbarium specimens of *Otoba squamosa* have been collected with staminate flowers in May and December, with pistillate flowers in May, September, and December, and with fruits in March, May, September, October, and December.

Preliminary conservation status. *Otoba squamosa* is Endangered following IUCN criteria B2a. Justifying this status, it is known from only two localities and has an AOO of 4 km²; there are too few verified localities to reasonably estimate the EOO. The Andean forests of the Antioquia Department of Colombia where it occurs are particularly at risk for deforestation (González-Caro and Vásquez 2017).

Discussion. The collections now identified as *Otoba squamosa* were previously included under the concept of *O. gordoniiifolia* (Fig. 8), and identified as such in

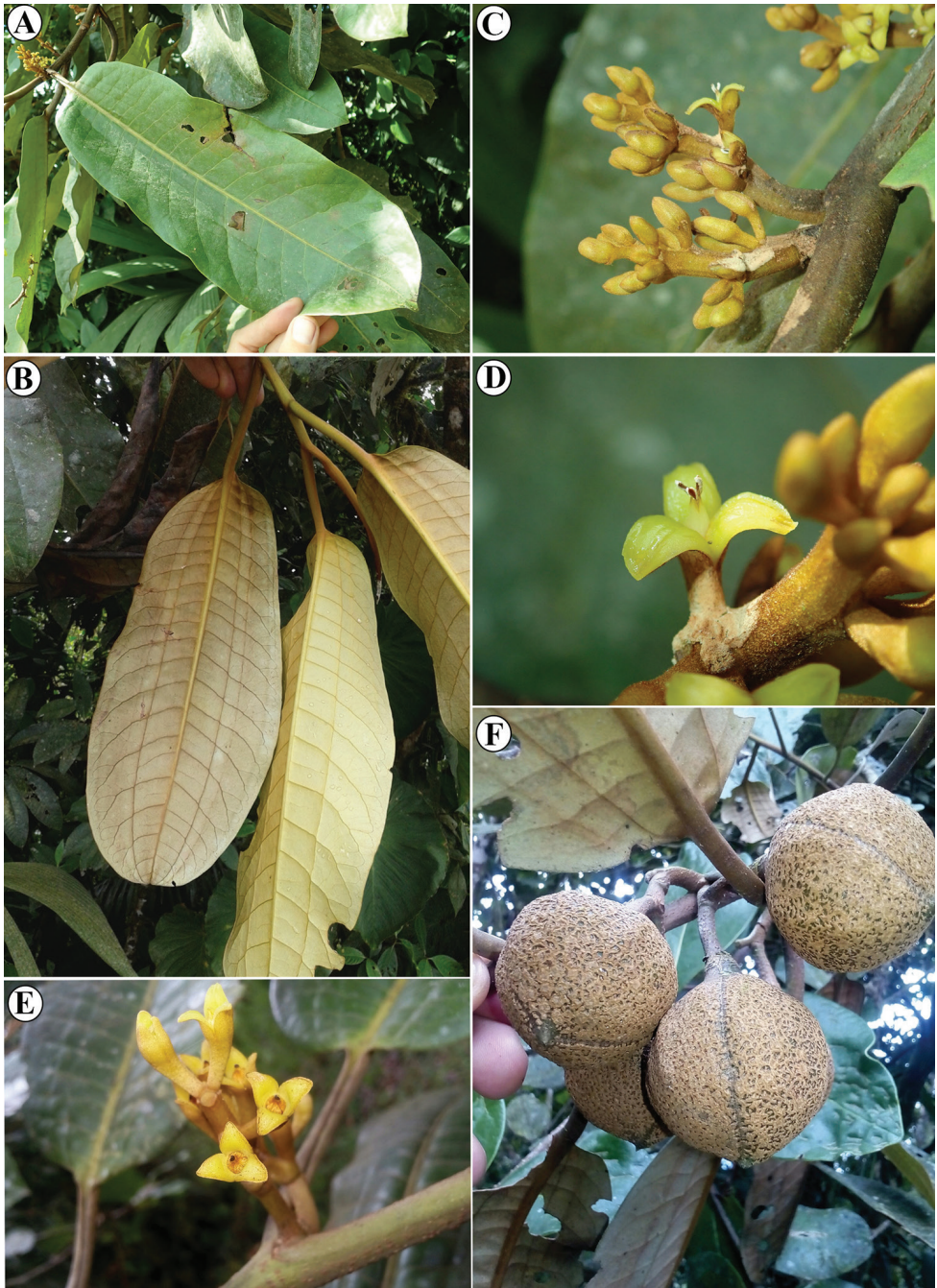


Figure 8. *Oroba gordoniiifolia* **A** leaf on adaxial side **B** leaf on abaxial side, illustrating long petioles and venation lines **C** staminate inflorescence **D** staminate flower **E** pistillate flowers, note the pubescent ovary **F** immature fruits. Photos by Rudy Gelis taken in Ecuador (unvouchered).

Table 3. Comparison of *Otoba gordoniiifolia*, and *O. squamosa*.

	<i>Otoba squamosa</i>	<i>O. gordoniiifolia</i> [‡]
Petiole length (cm)	1.7–2.7 (–3.8) × 0.1–0.2 (–0.25)	(3–) 5–7 × 0.3–0.4
Leaf size (cm)	6.7–14.5 × 3.4–6 (–8)	(13–) 24–34 × (6–) 8–12
Staminate perianth length (mm)	3.5–4.7	(2–) 4–6
Ring	Present	Absent
Anthers length (mm)	0.5–0.7	0.7–1.5
Ovary vestiture	Glabrous	Pubescent
Fruit size (cm)	(2.5–) 3.4–3.6 × (1.9–) 2.8–3	(3–) 5 × 4
Pericarp thickness (mm)	2.6–3	3–5

[‡]From Jaramillo-Vivanco and Balslev 2020

herbarium specimens and in the literature (e.g., Cogollo 2011). These species share similarities: both grow in montane forests and have the leaf blades with conspicuous veneration lines (Figs 7A, F, 8B), long petioles (shorter in the new species), lanceolate to oblong anthers, and fruit with thick pericarp. However, *Otoba squamosa* differs in its smaller leaf blades with thinner petioles, staminate flowers with a shorter perianth with a swollen-lobed ring in the inner surface and smaller anthers, and pistillate flowers with glabrous ovaries. See Table 3 for a comparison of these distinguishing characteristics. In addition, *O. squamosa*’s leaf blades are less pubescent abaxially, the terminal young foliar bud is shorter, and petioles are shorterwinged.

Within *Otoba*, the swollen-lobed ring in the staminate perianth is shared between *O. cyclobasis* (and even gives this species its specific epithet; Jaramillo and Balslev 2001) and *O. squamosa*; in addition to this feature, both species have glabrous ovaries. However, the new species has longer petioles (1.7–2.7 [–3.8] vs. 1–1.5 cm) and leaves (6.7–14.5 vs. 3.4–6 [–8] cm), lanceolate to oblong anthers (vs. globose), and larger fruits ([2.5–] 3.4–3.6 vs. 1.5–2.5 cm long); further, *O. squamosa* grows at higher elevations (1330–1450 vs. 150–300 m).

In the “Key to the species (pistillate or fruiting plants)” from Jaramillo-Vivanco and Balslev (2020), *Otoba squamosa* matches the recently described *O. vespertilio* from Costa Rica and Panama, though it is clearly not conspecific. *Otoba squamosa* differs from *O. vespertilio* in its longer petioles (1.7–2.7 [–3.8] vs. 0.8–2 cm long), abaxially conspicuous veneration lines (vs. absent), staminate perianth with a swollen-lobed ring (vs. without), and larger fruits ([2.5–] 3.4–3.6 vs. 2.2–2.7 × 1.6–1.7 cm) with thicker pericarp (2.6–3 vs. 1.3–1.8 mm).

Notes. The specimen [J.] Pipoly 17003 (MO nv), included in Jaramillo-Vivanco and Balslev (2020), was collected in the same area of the type and other material of *O. squamosa* cited here, and we suspect that it corresponds to this new species, though this specimen was not located in our study.

Specimens of *O. squamosa*, including the type, have been previously identified in herbaria as *O. gordoniiifolia*, and duplicates may have been distributed under these names.

The following sterile specimens from MO were collected in the same locality as fertile material or nearby (Parque Nacional Natural “Las Orquídeas”, Sector Calles); because they were not fertile, they were not used in the description presented above,

though we believe that they correspond to this new species: Á. Cogollo et al. 6069, 6132, 6169, 6298, 6344, 6642, 6644, 6657, 6683, 6684, 6869, 6926, 6929, 7109, 7180, 7221, 7237, 7238, 7259, 7262, 7268, 7269; and J. Pipoly et al. 16663, 17022, 17033, 17042, and 17076.

Specimens examined. COLOMBIA. Antioquia: Frontino, Corregimiento Nutibara, cuenca alta del Río Cuevas, bosque al lado carretera a La Blanquita, 1100 m, 21 Sep. 1987 (♀ fl & fr), D. Sánchez et al. 1529 (MO); Urrao, Parque Nacional Natural “Las Orquídeas”, Sector Calles, margen derecha del Río Calles, 06°32'N, 076°19'W, 1420 m, 25 Mar. 1988 (fr), Á. Cogollo et al. 2573 (MO); Vereda Calles, Parque Nacional Natural “Las Orquídeas”, margen derecha Quebrada La Honda, 06°32'N, 076°19'W, 1330–1400 m, 03 May 1993 (♂ fl), Á. Cogollo et al. 6074 (MO); *ibid*, 08 May 1993 (♂ fl), Á. Cogollo et al. 6190 (K-2 sheets [n.v.], MO); *ibid*, 08 May 1993 (♀ fl), Á. Cogollo et al. 6198 (MO); *ibid*, 11 May 1993 (♂ fl), Á. Cogollo et al. 6279 (MO, NY); *ibid*, 11 May 1993 (♂ fl), Á. Cogollo et al. 6300 (K n.v., MO); Vereda Calles, Parque Nacional Natural “Las Orquídeas”, margen derecha del Río Calles, en el filo NW de la Cabaña de Calles, 06°32'N, 076°19'W, 1450 m, 15 Oct. 1993 (fr), Á. Cogollo et al. 6918 (MO); *ibid*, 18 Oct. 1993 (fr), Á. Cogollo et al. 7107 (MO); *ibid*, 08 Dec. 1993 (fr), Á. Cogollo et al. 7959 (GH digital image, K [n.v.], MO, NY); Las Orquídeas, Vereda Calles, Parque Nacional Natural Las Orquídeas, Quebrada Honda, 06°29'N, 076°14'W, 1300 m, 08 Dec. 1992 (fr), J. Pipoly et al. 16745 (MO); *ibid*, 1330 m, 08 Dec. 1992 (♀ fl), J. Pipoly et al. 16798 (MO); *ibid*, 10 Dec. 1992 (♂ fl), J. Pipoly et al. 16902 (MO); *ibid*, 1300 m, 11 Dec. 1992 (♂ fl), J. Pipoly et al. 17019 (K [n.v.], MO); Parque Nacional Natural Las Orquídeas, Vereda Calles, margen derecha del Río Calles, 06°32'N, 076°19'W, 1350–1450 m, 05 Dec. 1993 (fl bud), J. Pipoly et al. 17721 (MO).

The identification key below is modified from Jaramillo-Vivanco and Balslev 2020. Except where specified otherwise, the information comes from Smith 1950¹, measurements of the digital image of the holotype², or measurements of herbarium specimens[#]. The key was difficult to build, in part because of current limited access to physical specimens during the COVID-19 pandemic and in part because we were not able to identify obvious characters to separate species in some cases (e.g., between *O. gordoniifolia* and *O. lehmannii*). This may mean that the key is difficult to use, but we have decided to include it in case it is of use to some.

Key to the species of *Otoba* in Colombia

- 1 Abaxial leaf blades with vernation lines (e.g. 7A, 8B).
- 2 Leaf blades 6.7–14.5 cm long, abaxial surface with sparse, scale-like and trichomes; perianth of staminate flowers with swollen-lobed ring; staminate flowers with anthers 0.5–0.7 mm long; fruits with pericarp 2.6–3 mm thick.....*O. squamosa*
- 2' Leaf blades (13–) 24–43 cm long, abaxial surface with dense, scale-like and trichomes; perianth of staminate flowers without swollen-lobed ring; staminate flowers with anthers 0.7–1.5 mm; fruits with pericarp 3–5 mm thick.

- 3 Pistillate flowers with pubescent ovaries *O. gordonii*folia
- 3' Pistillate flowers with glabrous ovaries..... *O. lehmannii*
- 1' Abaxial leaf blades without vernation lines.
- 4 Leaf blades with petioles less than 0.2 cm thick.
- 5 Staminate inflorescence 6–7 cm long, flower bud 1.2–1.6 mm wide^l, perianth ca. 3 mm long; filament column divided distally; anthers ca. 0.5 mm long...
.....*O. gracilipes*'
- 5' Staminate inflorescence 1.5–4.3 cm long, flower bud 0.6–0.1 mm wide, perianth 2–2.5 mm long; filament column completely connate; anthers 0.2–0.4 mm long.....*O. scottmorii*
- 4' Leaf blades with petioles up to (0.1–) 0.2 cm thick.
- 6 Staminate flowers with filament column distinct almost to the base.....
..... *O. novogranatensis*
- 6' Staminate flowers with filament column connate, if distinct never to the base
- 7 Leaf blades with petioles conspicuously winged; staminate inflorescence with the first fascicles of flowers 2.2–4 (–8) cm[#] from the base, fascicles of flowers arranged more or less in a zig-zag, fascicles well separated (1–1.5 cm apart[#]), pedicel filiform, flowers with membranaceous perianth..... *O. latialata*
- 7 Leaf blades with petioles usually not winged, if winged, the wing very narrow; staminate inflorescence with the first fascicles of flowers 0.5–2 cm[#] from the base, fascicles of flowers arranged in a straight line, each fascicle in close proximity (0.5–1 cm apart[#]), pedicel stout, flowers with fleshy perianth.
- 8 Abaxial leaf surface densely covered with scale-like trichomes; pistillate flowers with pubescent gynoeceum; fruits with pericarp 5–8 mm thick *O. glycyarpa*
- 8' Abaxial leaf surface sparsely covered with scale-like trichomes; pistillate flowers with glabrous gynoeceum; fruits with pericarp 1–2 mm thick..... *O. parvifolia*

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Begonia shenzhenensis, a new species of Begoniaceae from Guangdong, China

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Abstract

Begonia shenzhenensis D.K.Tian & X.Yun Wang, **sp. nov.**, a new species in *Begonia* sect. *Platycentrum* of Begoniaceae from Shenzhen of Guangdong province, China, is described and illustrated. Morphologically, it is primarily similar to *B. coelocentroides* in the same section but differs by its denser hairs on leaf, petiole, and pedicel, obtuse anther apex, hairy ovary, and narrower adaxial fruit wing. Based on only one small population found to date, its conservation status is assigned to Critical Endangered according to the IUCN Red List Categories and Criteria.

Keywords

Conservation status, deciduous, morphology, rhizomatous begonia, southern China, taxonomy

Introduction

Guangdong province is located in southern China, and it borders Macau and Hong Kong, two Chinese special administrative regions. Numerous surveys on plant diversity of this province have been widely conducted by a number of botanists, mainly from several research institutions, including South China Botanical Garden of Chinese Academy of Sciences, Sun Yat-sen University, Guangdong Academy of Forestry, South China Agricultural University, and others. Nonetheless, some new species and

new records have been recently discovered (Huang et al. 2020; Zhou et al. 2020), including two begonia species (Ding et al. 2018; Tu et al. 2020). Here we describe and illustrate a new species of *Begonia*, *Begonia shenzhenensis* D.K.Tian & X.Yun Wang, which increases the number of begonias species to 14 in Guangdong province. The other 13 species are *B. circumlobata* Hance, *B. coptidifolia* H.G.Ye, F.G.Wang, Y.S.Ye & C.-I Peng, *B. cucullata* Willd. (naturalized), *B. edulis* H.Lév., *B. ehuanzhangensis* Q.L.Ding, W.Y.Zhao & W.B.Liao, *B. fimbriatipula* Hance, *B. fordei* Irmsch., *B. grandis* Dryand., *B. guangdongensis* Wen-Hui Tu, Bing-Mou Wang & Yu-Ling Li, *B. handelii* Irmsch., *B. leprosa* Hance, *B. longifolia* Blume, and *B. palmata* D.Don.

Taxonomic treatment

Begonia shenzhenensis D.K.Tian & X.Yun Wang, sp. nov.

urn:lsid:ipni.org:names:77217426-1

Chinese name: 深圳秋海棠

Figs 1, 2

Type. CHINA. Guangdong: Shenzhen (深圳), Pingshan (坪山) District, Tiantou Mountain (田头山) Natural Reserve, on shallow humus soil above the rocky surface under the forest along streams, 22°43'29"N, 114°24'2"E, elev. 70 m, 6 June 2020, Dai-Ke Tian, Xiao-Yun Wang, Bin Chen & Shi-Ping Zhong, TDK4160 (holotype, CSH! CSH0182483).

Specimen examined. Same locality as type, 21 September 2019, Xiao-Yun Wang, TDK3981 (paratype, CSH); Shanghai Chenshan Plant Science Research Center, cultivated plants under 25 °C room temperature, 7 November 2020, Dai-Ke Tian, TDK4832 (paratype, CSH).

Diagnosis. The new species is most similar to *B. coelocentroides* Y.M.Shui & Z.D.Wei in the same section, *Begonia* Section *Platycentrum* (J.F.Klotzseh) A.DC. with rhizomatous habit, four tepals of staminate flowers, five tepals of pistillate flower and two-located ovary, but clearly differs by its densely (vs. sparsely) hairy petioles and blades, hairy (vs. glabrous) ovaries, obtuse (vs. concave) anther apex, and narrower (vs. wider) adaxial fruit wing. It is also close to the small-sized individuals (before mature) of *B. edulis* in appearance but differs by its short and small (vs. tall and large) and non-stemmed (vs. stemmed) plants, and hairy (vs. glabrous) pedicels, flowers, and fruits (Table 1, Fig. 2).

Herb perennial, rhizomatous, 7–15 cm tall, monoecious, epiphytic on rock with moss or humus soil. **Rhizome** creeping, 5–10 cm long, internodes unclear to 10 mm long, 5–13 mm thick, unbranched to rarely branched, greenish-brown. Aerial stem absent, or occasionally one very short internoded stem at anthesis. **Leaves** simple, basal, alternate, 4–8 each plant, deciduous in winter. **Stipules** persistent, pale pink, long triangular, 5–8 × 4–6 mm, abaxially hairy, apex with short arista. **Petioles** light-green to pink, 4–18 cm long, 1.5–5 mm thick, grooved in full-length, short greyish pubescent, less than 1.5 mm long, hair tips often curly. **Leaf-blades** obliquely oval-cordate, 5–22 ×

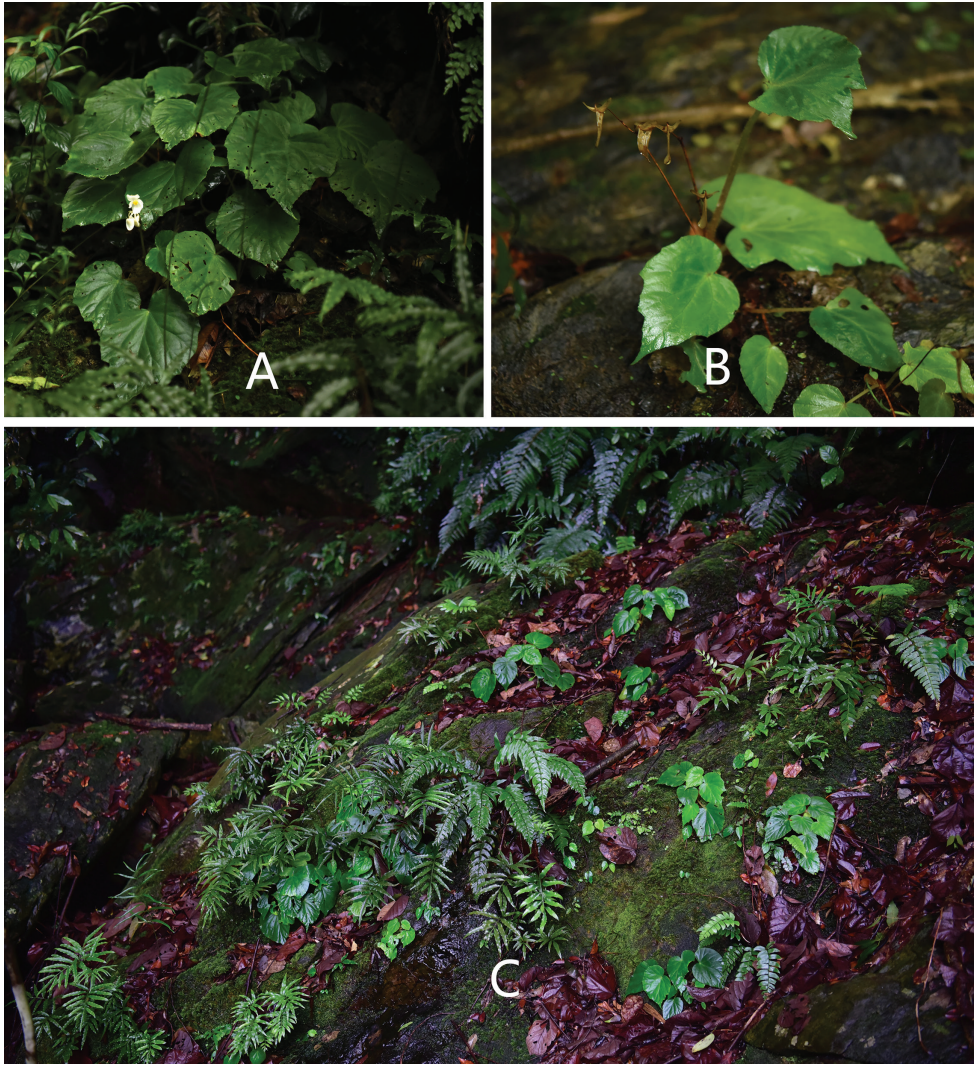


Figure 1. Plants and habitat of *B. shenzhenensis* D.K.Tian & X.Yun Wang **A** plant at the earliest flowering time (6 June 2020) **B** plants with the dried fruits matured in the previous year **C** habitat showing mature plants and seedlings of the new species growing together with mosses and ferns above the rocks. (Photo by Dai-Ke Tian).

3.5–16 cm; adaxially green, rarely dark green, rough, short greyish strigose, less than 1 mm long; adaxial veins slightly convex at the base, concave upper part; abaxially greyish-green or lightly red-purple, veins convex, with short greyish strigose, 1–1.5 mm long, leaf veins 7 to 6; **Leaf base** shallowly cordate, obtuse to nearly overlapping, decurrent part 1–4 cm long; leaf margins serrate, rarely double serrate, occasionally cleft-teethed, with arista; apex acuminate or short-caudate. **Inflorescences** 2–4, successively growing from leaf axil of the stem near the shoot, 11–17 cm long; peduncle light green, 8–15 cm

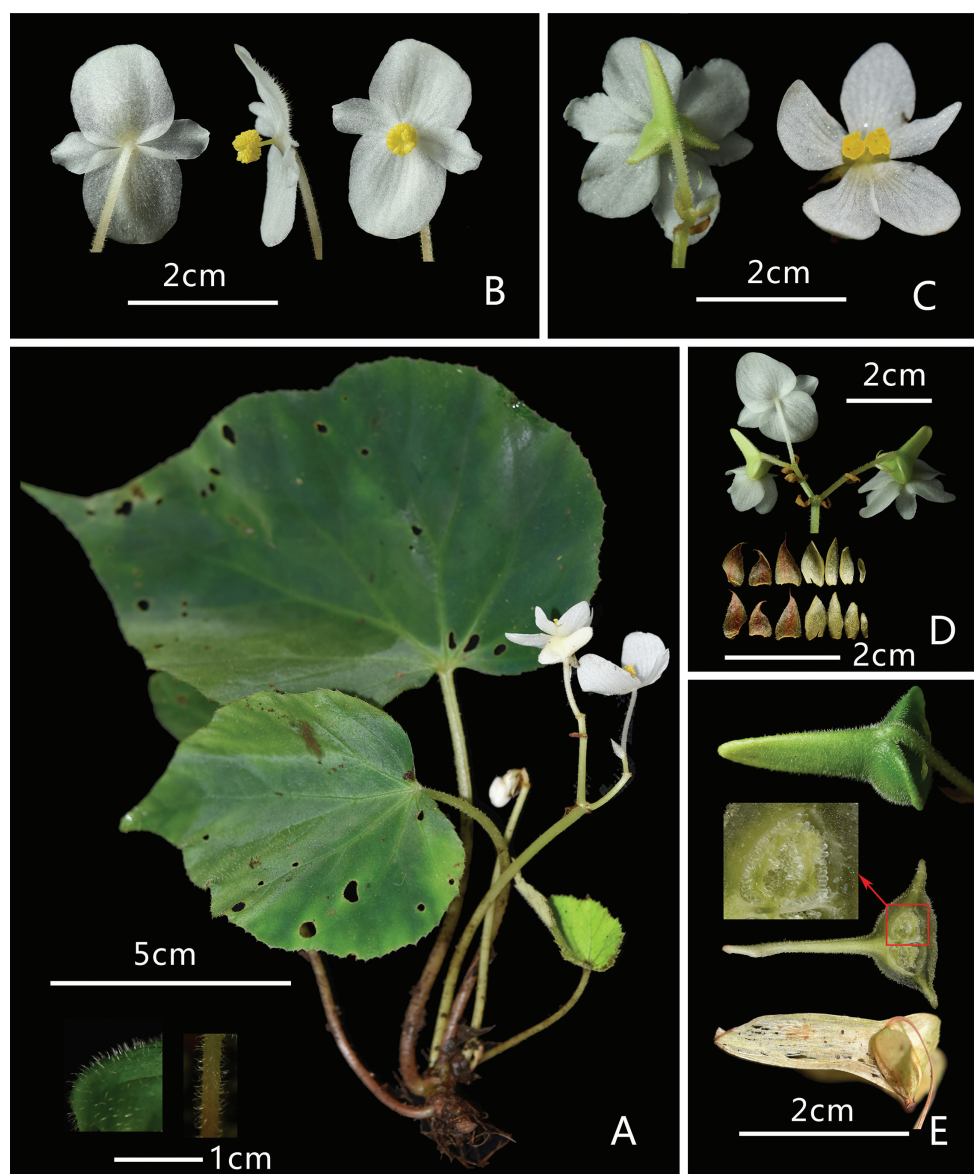


Figure 2. Morphology of *Begonia shenzhenensis* D.K.Tian & X.Yun Wang **A** one of the largest plants with flowers (left corner: sections of leaf and petiole showing hairs) **B** staminate flowers showing in different directions **C** pistillate flowers, bracts (left: back view of 6-tepalled flower; right: front view of 5-tepalled flower) **D** inflorescence, stipules (left) and bracts (right) **E** fruits (top: fresh, bottom: dried) and ovary dissection (Photo by Dai-Ke Tian).

long, 2–3.5 mm thick, greyish-white villose, pedicel less hairy, each inflorescence 3–7 flowers, 0–2 pistillate. **Bracts** and bracteoles greenish-white, long triangular, bracts 5–12 × 3–7 mm; bracteoles 4–11 × 2–6 mm, both abaxially sparsely white pubescent, ca. 1 mm long, apex acuminate, with sparse cilia. **Staminate flower**: pedicel white or light-



Figure 3. Distribution of *Begonia shenzhenensis* (red dot) from Shenzhen, Guangdong of China.

green at low part, 1.8–3.4 (4.5) cm long, 1–2 mm thick, short greyish white-pubescent (less than 1 mm long); flower white, 2.5–4.5 × 2.4–3.7 cm; outer 2, ovate, entire, 1.2–2.2 × 1.2–2.1 cm, central part thick and abaxial with short grayish white or pink pubescent, indumentum same as pedicel; inner 2, long obovate–lanceolate, glabrous, 10–19 × 5–9 mm. androecium, 3–4 × 4–5 mm, stamens 50–92, filaments basally connate into stamen column, 2–3 mm × ca. 1 mm, free part 1–2 mm long, anthers yellow, 0.8–1.2 mm long. **Pistillate flower:** a pair of pale green bracteoles persistent near ovary, 3–10 × 1–4 mm; pedicel 9–18 mm long, 1.2–2 mm thick, with extremely short greyish white pubescent, less than 0.5 mm long; flower 30–37 × 25–37 mm, tepals 5 (rarely 6), outer 4 (5), slightly large, 13–18 × 7–15 mm, sparsely greyish white short pubescent, inner 1, small, glabrous, oblanceolate, 11–15 × 5–9 mm wide; gynoecium 3–4 × 3–7 mm, styles 2, stigmas 2, nearly U-shape branched, both sides spirally twisted nearly one circle; ovary greenish-white, short hairy, placentation axile, 2-loculed, each placenta 2-branched; **Peduncles** light-green, hairy, 15–32 cm long, ca 2 mm thick, hairy, a pair of green bracteoles often persistent near ovary; **Fruits** capsule, green, densely short grayish-white pubescent, 6–10 × 5–8 mm, 3-winged, unequal, abaxial wing long rectangular or subtriangular, 18–28 × 8–10 mm; lateral wings narrow, sickle-shaped or cornice or auriculate triangular, ca. 4–8 × 8–10 mm. Flowering June–July. Fruiting July–October.

Table 1. Comparison of *Begonia shenzhenensis* and *B. coelocentroides*.

	<i>B. shenzhenensis</i>	<i>B. coelocentroides</i>
Plant height	7–15 cm	5–10 cm
Leaf-blade	densely hairy	sparsely pilose to nearly glabrous
Petiole's hair	dense	sparse
Peduncle and pedicel	hairy	glabrous
Outer staminate tepal	hairy	sparsely hairy
Anther apex	obtuse	concave
Fruit	densely hairy	glabrous
Adaxial wing	narrower, 8–10 mm wide	wider, 9–14 mm wide

Etymology. The specific epithet refers to the name of Shenzhen, which includes the type locality of the new species.

Distribution and habitat. The new species is only known from its type locality in Tiantou Mountain Natural Reserve of Pingshan District of Shenzhen, Guangdong Province, China (Fig. 3). It grows together mainly with mosses, ferns, and other herbs on shallow humus soil above the rocks along a small stream under forest canopy.

Conservation status. *Begonia shenzhenensis* is only observed in its type locality with a very small population containing fewer than 50 individuals. Due to the plant's relatively low ornamental value, it is possibly unlikely to be collected by plant hunters. However, there are no suitable places nearby for spreading its population. Therefore, this species should be considered as Critical Endangered (CE) according to the IUCN Red List Categories and Criteria (IUCN 2019) based on the current data.

Note. The new species was first discovered by Mr. Xiao-Yun Wang, a plant enthusiast working at Hechang Weilai Science and Technology (Shenzhen) Co. Ltd. in Guangdong province of China. On 21 September 2019, Xiao-Yun Wang posted photos of a wild begonia from Shenzhen of Guangdong province, China. At the request of Dr. Dai-Ke Tian, the living plants were collected and introduced to Shanghai Chenshan Botanical Garden for further study. The introduced plants grow well in two plastic pots placed in a squared polymethyl box at 25 °C room temperature and bloom from November to June. On 6 June of 2020, with the help of Xiao-Yun Wang, Dr. Dai-Ke Tian and Dr. Bin Chen conducted a field survey on this species and further confirmed that it is new to science.

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Smelowskia sunhangii (Brassicaceae), a new species from China, with a re-evaluation of the *S. tibetica* complex

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Abstract

Smelowskia sunhangii, from Qinghai and Tibet (China), is described and illustrated. Morphological and molecular data indicate that *S. sunhangii* is closely related to *Smelowskia tibetica*, from which it is easily distinguished by the densely hirsute (vs. glabrous or sparsely pubescent), elliptic to ovate-lanceolate (vs. suborbicular, oblong, or lanceolate) fruits with undulate (vs. straight) margins. A re-evaluation of the widely distributed *S. tibetica* and related taxa is also provided.

Keywords

Brassicaceae, China, Cruciferae, new species, phylogeny, taxonomy

* The authors contributed equally to this study.

Introduction

The genus *Smelowskia* C.A.Mey. (Brassicaceae; Cruciferae) comprises 25 species distributed mainly in central and northeastern Asia, with fewer species in North America (Al-Shehbaz and Warwick 2006). The North American-Beringian taxa are believed to have originated from Asian ancestors (Carlsen et al. 2010). However, as in many other genera of the family, *Smelowskia* lacks unique synapomorphies (Warwick et al. 2004), though it is currently placed in the monogeneric tribe Smelowskieae (Al-Shehbaz et al. 2006; Al-Shehbaz 2012). Schulz (1936) and Appel and Al-Shehbaz (2003) considered the genera *Ermania* Cham. ex Botsch., *Gorodkovia* Botsch. & Karav., *Hedinia* Ostenf., *Redowskia* Cham. & Schltdl., *Sinosophiopsis* Al-Shehbaz, and *Sophiopsis* O.E. Schulz to be morphologically closely related to *Smelowskia*. Warwick et al. (2004) and Al-Shehbaz and Warwick (2006) incorporated all these genera into *Smelowskia* based on molecular phylogenetic and morphological evidences.

Nine species of *Smelowskia* are native to China (Al-Shehbaz and Warwick 2006; German and Chen 2009), and they are distributed mainly in the western parts of the country. In their most recent account for the *Flora of China*, these species were placed by Zhou et al. (2001) in the genera *Hedinia*, *Sinosophiopsis*, *Smelowskia*, and *Sophiopsis*. Of these, *H. tibetica* (Thomson) Lipsky is the most widely distributed (Bhutan, W and Himalayan China, India, Kyrgyzstan, Nepal, Tajikistan) and was recognized by these authors as most highly variable in fruit shape. Four new taxa were described by Zhou and An (1990) and He and An (1996) from Xinjiang province, including *H. lata* Xue L.He & C.H.An, *H. rotundata* C.H.An, *H. taxkargannica* G.L.Zhou & C.H.An, *H. taxkargannica* var. *hejigensis* G.L.Zhou & C.H.An, but these were reduced by Zhou et al. (2001) and Al-Shehbaz and Warwick (2006) to synonymy of *Smelowskia tibetica* (Thomson) Lipsky based on the examination of all type collections of *Smelowskia*. The above *Smelowskia tibetica* complex is re-examined here in light of additional field and molecular studies.

During a recent field survey, we collected an unidentified specimen of *Smelowskia* in Qinghai province, China (Fig. 1), and it appeared initially to be allied morphologically to the *S. tibetica* complex. The current study is devoted to resolve the mystery of the Chinese components of this complex.

Materials and methods

Morphological observation

Morphological data were recorded from field collections and herbarium specimens covering the full spectrum of geographical, plant type, and habitat variation in the *S. tibetica* complex. The voucher specimens of our collections were deposited in the herbarium of Kunming Institute of Botany (**KUN**), Kunming, China. Herbarium specimens of the *S. tibetica* complex and related taxa were examined from BNU, KUN, PE,

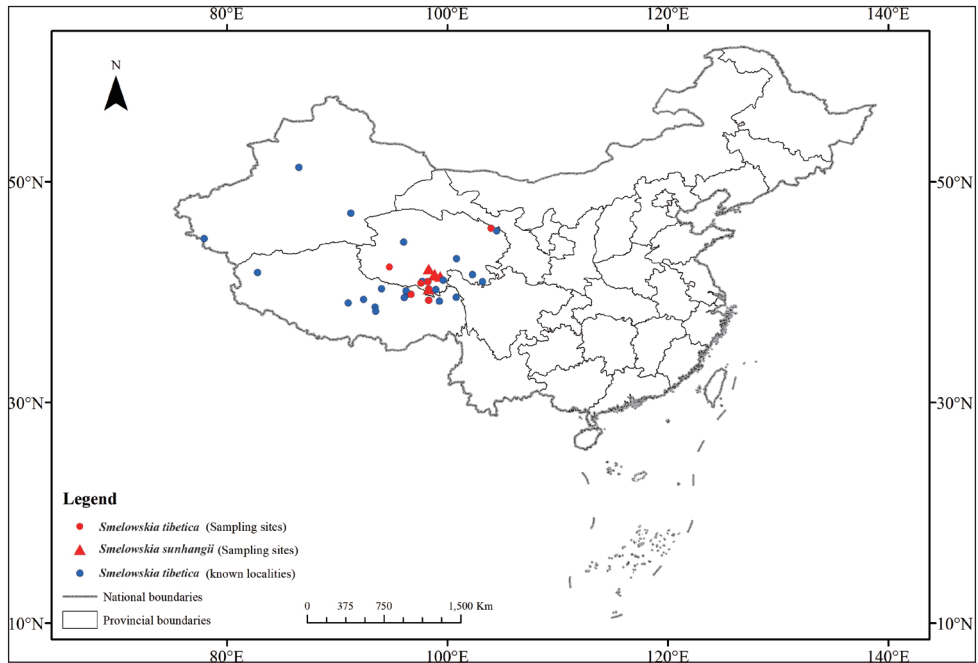


Figure 1. Distributions of sampling sites (red triangles) of *Smelowskia sunhangii*, known localities (blue dots) and sampling sites (red dots) of *Smelowskia tibetica* in China.

WUK and XJA (acronyms follow Thiers 2018), either by examining the specimens directly, or electronically through the National Plant Specimen Resource Center (<http://www.cvh.ac.cn/index.php>), and JSTOR Global Plants web portal (<https://plants.jstor.org/>). The voucher specimens for morphological observation were cited in the section of “Chinese specimens examined” of taxonomic treatment and Table 1.

Molecular analyses

We sampled 12 collections representing the *Smelowskia tibetica* complex, including the presumed new species. Leaf materials were collected from field works and dried herbarium specimens. *Descurainia pinnata* from tribe Descurainieae (sister to tribe Smelowskieae) and *Shehbazia tibetica* from tribe Chorisporeae were selected as out-groups based on previous molecular phylogenetic relationships (Warwick et al. 2004; Al-Shehbaz et al. 2006; Al-Shehbaz 2012; Liu et al. 2020). Sequences for other taxa were obtained from GenBank (Table 1). Voucher information and GenBank accession numbers are also provided in Table 1.

Total genomic DNA extracted from leaf materials using DP305 Plant Genomic DNA kits (Tiangen, Beijing, China) following the manufacturer’s protocol. The entire ITS region (including internal transcribed spacers ITS1 and ITS2 of nuclear ribosomal DNA and the 5.8S rRNA gene) were amplified using the primers ITS1-18S described

Table 1. Voucher information and GenBank accessions for phylogenetic analysis.

Taxon	Voucher	GenBank accession number
<i>Smelowskia tibetica</i> (1) (as <i>Hedinia rotundata</i>)	Yang Jingsheng 402 (KUN)	MZ089467
<i>S. tibetica</i> (2) (as <i>Hedinia lata</i>)	HuangXH018-9 (KUN)	MZ089476
<i>S. tibetica</i> (4) (as <i>H. lata</i>)	HuangXH015-20 (KUN)	MZ089475
<i>S. tibetica</i> (5) (as <i>H. lata</i>)	Deng7359 (KUN)	MZ089474
<i>S. tibetica</i> (6) (as <i>H. tibetica</i>)	ZDG23-7 (KUN)	MZ089468
<i>S. tibetica</i> (7) (as <i>H. tibetica</i>)	Deng7261 (KUN)	MZ089473
<i>S. sunhangii</i> (1)	Deng7262 (KUN)	MZ089472
<i>S. sunhangii</i> (3)	DengT128-9 (KUN)	MZ089471
<i>S. sunhangii</i> (4)	HuangXH025-13 (KUN)	MZ089470
<i>S. sunhangii</i> (5)	DengT105-23 (KUN)	MZ089469
<i>Shebbazia tibetica</i>	HuangXH028-4 (KUN)	MZ089466
Sequences downloaded from NCBI		
<i>Smelowskia sunhangii</i> (2) (as <i>S. tibetica</i>)	Zh641 (KUN)	KX244397
<i>S. tibetica</i> (3) (as <i>H. lata</i>)	LJQ-QLS-2008-0115 (KUN)	JF941772
<i>S. czukotica</i>	TC03_60	EU489520
<i>S. altaica</i>	TC03_61	EU489519
<i>S. bartholomewii</i>	Ho et al. 3000 (MO)	AY230609
<i>S. sophiifolia</i>	Geonova 148 (LE)	AY230608
<i>S. calycina</i>	Velychnin N495 (LE)	AY230604
<i>S. borealis</i>	Murray 8582 (DAO)	AY230571
<i>S. jacutica</i>	Elias & Murray 11462 (ALA)	AY230646
<i>S. johnsonii</i>	Johnson, Viereck, & Melchior 688 (ALA)	AY230631
<i>S. ovalis</i>	CCDB-23367-G06	MG234816
<i>S. sisymbrioides</i>	Egorova 2349 (LE)	AY230612
<i>S. annua</i>	Anonymous 870473 (HNWP)	AY230610
<i>Descunainia pinnata</i>	–	AF183122
<i>Shebbazia tibetica</i> (as <i>Dontostemon tibeticus</i>)	GH:33576	LN713849

in O’Kane et al. (1997) and ITS4 described in White et al. (1990). Parallel chromatograms derived from bi-directional sequencing were checked for accuracy by visual inspection with Chromas v. 2.6.6 (<http://www.technelysium.com.au>) and integrated into a single sequence. Sequences were then aligned with MEGA version 7.0 and gaps were treated as missing data (Sudhir et al. 2016).

Phylogenetic reconstruction was performed using Bayesian inference (BI) and maximum likelihood (ML). The phylogenetic tree based on Bayesian inference was generated using MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001). The phylogenetic analysis based on maximum likelihood was conducted with PhyML version 3.0 (Guindon et al. 2010). Detected by the jModeltest 2.1.7, the GTR+G model selected by Akaike information criterion (AIC) was used in BI and ML analyses (Posada 2008).

Results

Morphology and taxonomy

Morphological studies of strictly Chinese material revealed a wide variation in fruit indumentum and shape and leaf divisions in the *Smelowskia tibetica* complex, and such

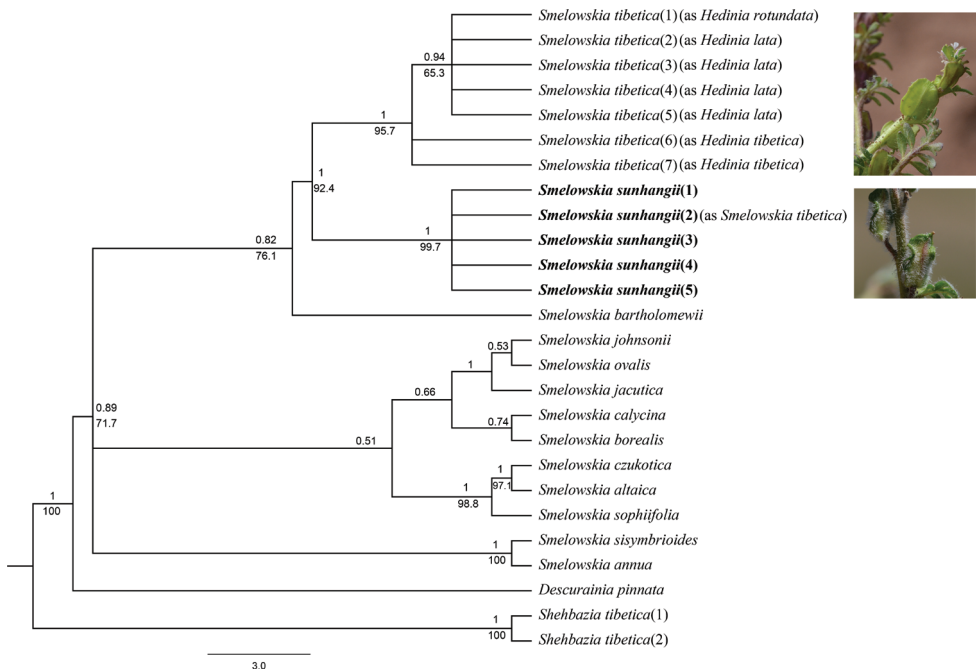


Figure 2. Bayesian consensus tree of 13 species of *Smelowskia* based on their ITS sequences, with *Descurainia pinnata* and *Shehbazia tibetica* as the outgroup. Numbers above branches indicate Bayesian posterior probability, numbers below branches are ML bootstraps.

differences lead Zhou and An (1990) and He and An (1996) to recognize several novelities under the synonymized *Hedinia*. For example, plants with 1- or 2-pinnatifid leaves and oblong glabrous fruits characterize the type collection of *Smelowskia tibetica*, those with 2-pinnatifid leaves and glabrous oblong fruits are found in the type of *H. lata*, those with lanceolate glabrous fruits are in plants of the type of *H. taxkargannica*, and those with pubescent suborbicular fruits are seen in the type of *H. rotundata*. All of the above taxa do not resemble the densely fruited plants we collected in Qinghai (Table 2).

These densely hirsute plants with densely hirsute, elliptic to ovate-lanceolate fruits undulate along the margin, do not match any of the other 25 species we carefully studied throughout the range of *Smelowskia*. Therefore, these Qinghai plants are described as the new species *S. sunhangii* and recognized hereafter as such.

Phylogenetic analyses

A total of 15 taxa were included in this analysis (Fig. 2). The resulting multiple alignment of the ITS region, including 5.8S gene, was 651 bp. The 50% majority-rule consensus tree based on Bayesian posterior probability (PP) and maximum likelihood bootstraps (ML) of the ITS sequences both showed that four accessions of *Smelowskia sunhangii* grouped together (PP=1, ML=99.7), however, an accession of previously

Table 2. Comparison of selected distinguishing features of *Smelowskia sunhangii* and related taxa.

Taxon	Cauline leaf shape	Fruit shape	Indumentum in silicle	Distribution
<i>Smelowskia tibetica</i>	1- or 2-pinnatifid	oblong	Glabrous	Qinghai, Tibet, Xinjiang
<i>S. tibetica</i> (as <i>Hedinia lata</i>)	2-pinnatifid	oblong	Glabrous	Qinghai, Tibet, Xinjiang
<i>S. tibetica</i> (as <i>H. taxkargannica</i>)	1-or 2-pinnatifid	lanceolate	Glabrous	Xinjiang
<i>S. tibetica</i> (as <i>H. rotundata</i>)	1-pinnatifid	suborbicular	sparsely pubescent	Xinjiang
<i>S. sunhangii</i>	1-pinnatifid	elliptic to ovate-lanceolate	densely hirsute	Qinghai, Tibet

determined *Smelowskia tibetica* (KX244397) was nested in it. After a critical examination of the voucher of this accession, *Zh641* (KUN), we immediately concluded that it is *S. sunhangii*.

All of the seven accessions of the *Smelowskia tibetica* complex formed a monophyletic clade, sister to *S. sunhangii* with a strong support (PP = 1, ML = 95.7). In addition, the clade of *Smelowskia tibetica* complex formed a polytomy of three subclades, of which two belong to *Smelowskia tibetica* and the third is a polytomy of samples identifiable as members of the *Hedinia lata* and *H. rotundata* as characterized morphologically above.

Discussion

Our molecular and morphological analyses indicate that *Smelowskia tibetica sensu lato* contains two, easily distinguished species, of which one is the new *S. sunhangii* and the other is *S. tibetica* including the taxa described by Zhou and An (1990) and He and An (1996) as three species and one variety of *Hedinia*. Although our data show some differentiation within *S. tibetica*, namely a polytomy of the *Hedinia* taxa forming another polytomy with the remaining samples of *S. tibetica* proper, such a current slight differentiation does not justify the recognition of more than two species. The principal reasons for not recognizing the *Hedinia* taxa are as follows.

First and foremost, as discussed above in the section of “Morphology and taxonomy” of the results, the variation in leaf and fruit morphology in the entire *Smelowskia tibetica* complex was critically studied by one of us (Al-Shehbaz) in plants from the entire range of this complex in Bhutan, India, China, Nepal, and Tajikistan both in herbaria worldwide and in the field in Xinjiang (China), Nepal, and Tajikistan. The conclusion of such observations was the acceptance of a single polymorphic species in the Himalayan region (Al-Shehbaz 2015), including Nepal and its neighbors (Al-Shehbaz and Watson 2011). That conclusion was also reflected in BrassiBase (Kiefer et al. 2014), the comprehensive and continuously updated database on the entire Brassicaceae.

Then similar variation in fruit and leaf morphology of the *Smelowskia tibetica* complex, especially that of the *Hedinia* taxa discussed above, was observed sporadically elsewhere in the species range and, therefore, the alleged distinction of *Hedinia* taxa has no merit.

Additionally, although our molecular data show a slight differentiation in the *Hedinia* taxa (Fig. 2), our sampling cannot be considered as the final word without

doing similar analyses from the other countries where *Smelowskia tibetica* grows. We only used just two samples of this highly variable and widespread species. Therefore, the most reasonable conclusion is to avoid the recognition of any of the *Hedinia* taxa and create additional synonymies without more convincing data.

Finally, our morphological studies strongly support the novelty of *Smelowskia sunhangii* because its fruit morphology is unique in the genus and has not yet been observed in Asian and North American taxa. Furthermore, our molecular data also strongly support the above recognition of the novelty and its sister relationship to *S. tibetica* including the *Hedinia* taxa of Zhou and An (1990) and He and An (1996).

Taxonomic treatment

***Smelowskia sunhangii* T. Deng, X.J. Zhang & J.T. Chen, sp. nov.**

urn:lsid:ipni.org:names:77217427-1

Figs 3, 4

Type. CHINA. Qinghai. Yushu Tibetan Autonomous Prefecture, Yushu City, Longbao Town, 33°30'13.54"N, 96°24'38.79"E, 4,602 m, 26 July 2019, *J.T. Chen, X. Zhang & T.H. Kuang HuangXH025-13* (holotype: KUN1498313!; isotype: KUN1498314!).

Diagnosis. *Smelowskia sunhangii* is easily distinguished from its closest relative *S. tibetica* by having densely hirsute (vs. glabrous or sparsely pubescent), elliptic to ovate-lanceolate (vs. oblong, suborbicular, or lanceolate) fruits undulate (vs. straight) along margin.

Description. Herbs 5–15 cm tall, covered with simple trichomes, canescent. Stems procumbent or ascending, densely white hirsute. Basal leaves densely hirsute; petiole 0.5–1.5 cm long, often ciliate basally; leaf blade ovate or narrowly oblong in outline, 1-pinnatifid, 1–4 × 1–2.5 cm; cauline leaves similar to basal, reduced in size upwards. Racemes bracteate throughout, distal bracts subsessile. Sepals oblong, 1–2 × 0.5–0.8 mm, hirsute. Petals obovate, 2–3.2 × 0.9–1.4 mm, claw ca. 1.5 mm long. Fruit elliptic to ovate-lanceolate, densely white hirsute, 5–10 × 3–5 mm, undulate along margin, appressed to rachis. Seeds light to dark brown, oblong, 0.9–1.2 × 0.4–0.6 mm. Fl. Jul–Sep, fr. Aug–Oct.

Etymology. *Smelowskia sunhangii* is named after Prof. Sun Hang (1963–), director of the Kunming Institute of Botany (China) who conducted extensive research on plant taxonomy, biogeography, and evolutionary biology and made outstanding contributions towards understanding the plant diversity of China. Vernacular name: The Chinese name is given as “毛果藏芥” (máo guǒ zàng jì), referring to the densely hirsute fruit of the new species.

Distribution. China (Qinghai, Tibet [Xizang], Fig. 1).

Paratypes. CHINA. **Tibet:** Lhasa, Damxung County, Gangla Mountain, 30°41'6.77"N, 91°6'16.88"E, 4,802 m, 27 July 2018, *D.G. Zhang DengT051-14* (KUN). **Qinghai:** Yushu Tibetan Autonomous Prefecture, Yushu City, 32°17'20.62"N, 95°50'15.29"E, 4,848 m, 23 July 2019, *P.J. Liu & H.H. Shi deng7262* (KUN); Yushu

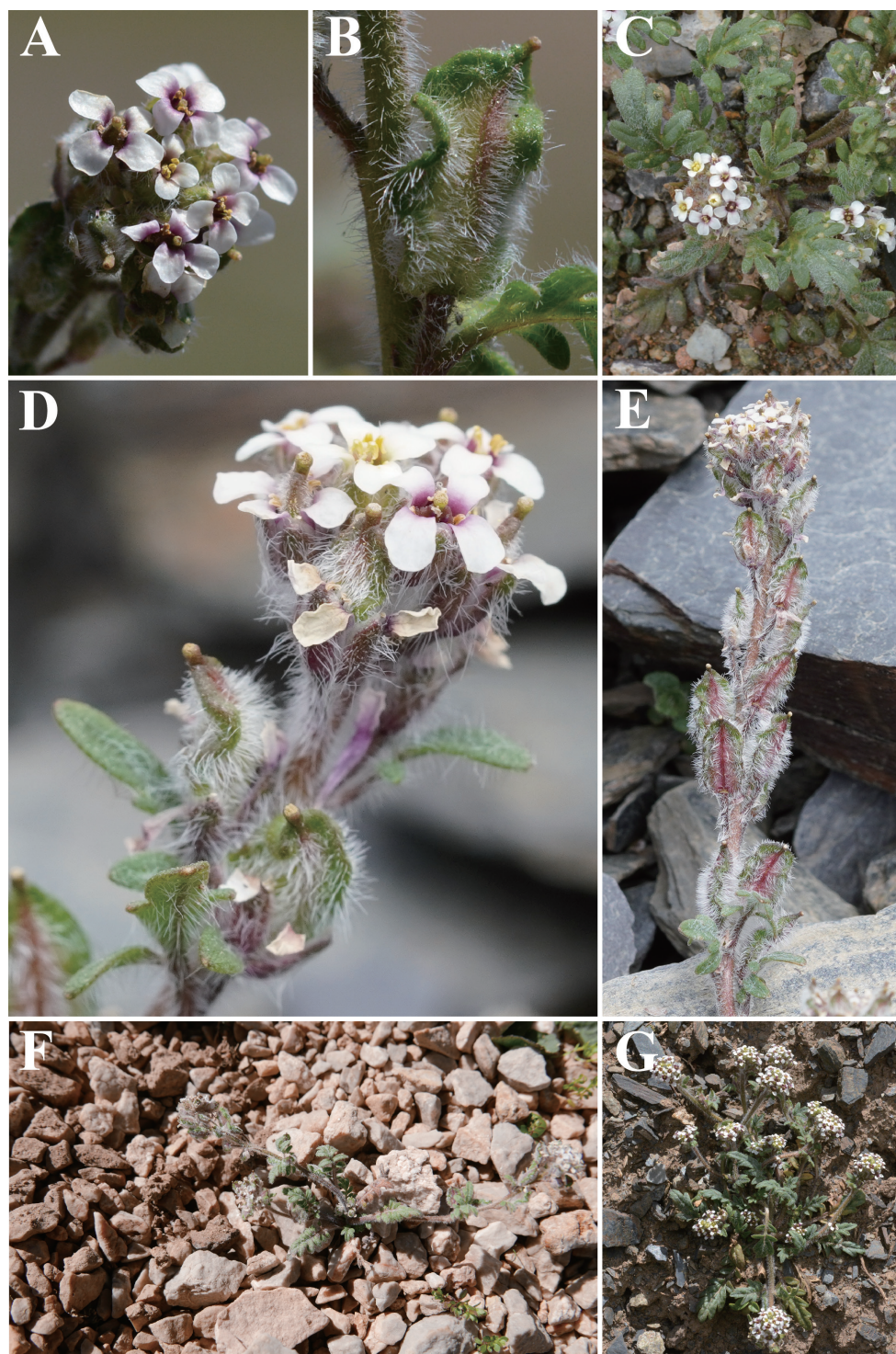


Figure 3. *Smelowskia sunhangii* T. Deng, X.J. Zhang & J.T. Chen **A** flower **B** fruit **C** leaves and flowers **D** inflorescence **E** infructescence **F, G** plants and habitat.



Figure 4. Photograph of the holotype of *Smelowskia sunhangii* T. Deng, X.J. Zhang & J.T. Chen (KUN1498313).

Tibetan Autonomous Prefecture, Chindu City, 31 July 2019, *T. Deng, X.H. Huang, Z.Y. Lv & L.J. Li DengT128-9* (KUN); Yushu Tibetan Autonomous Prefecture, *T. Deng, X.H. Huang, Z.Y. Lv & L.J. Li DengT105-23* (KUN); Yushu Tibetan Autonomous Prefecture, Qumarlêb County, 2 September 2013, *J.W. Zhang, B. Yang & H.L. Chen Zh641* (KUN).

***Smelowskia tibetica* (Thomson) Lipsky**

Basionym: *Hutchinsia tibetica* Thomson, Hooker's Icon. Pl. 9: t. 900. 1852. TYPE: WESTERN TIBET. Lanak Pass, 18–19,000 ft [ca. 5,480–5,790 m], *Thomas Thomson s.n.* (holotype: K!).

Synonyms: *Hedinia tibetica* (Thomson) Ostenf. in Hedin, S. Tibet 6: fig. 2. 1922.

Hedinia lata Xue L.He & C.H.An, Acta Phytotax. Sin. 34(2): 205. 1996. TYPE: TIBET. Ando, alt. 4,750 m, on stony mountain slopes, 14 August 1963, *J.X. Yang 2220* (holotype: WUK, n.v.).

Hedinia rotundata C.H.An, Acta Bot. Boreali-Occid. Sinica 10: 325. 1990. TYPE: XINJIANG. The west of Altum Mountains, Qimantag, 2,700 m, 25 July 1984, *Zhang Li-Yun 84-A-411* (holotype: XJA!).

Hedinia taxkargannica G.L.Zhou & C.H.An, Acta Bot. Boreal.-Occid. Sin. 10: 323. 1990. TYPE: XINJIANG. Tajik Autonomous County of Taxkargan Vaka, in alpina desert steppe zone, July 1986, *An Zheng-xi N. 268* (holotype: XJA!).

Description. Detailed descriptions of the species are found in the Flora of China (Zhou et al. 2001), Nepal (Al-Shehbaz and Watson 2011), and the entire Himalayan Region (Al-Shehbaz 2015). Therefore, there is no need to repeat it here.

Distribution. China (Gansu, Qinghai, Sichuan, Tibet, Xinjiang, Fig. 1), Bhutan, India, Kyrgyzstan, Nepal, Tajikistan.

Chinese specimens examined. CHINA. **Tibet:** Baingoin County, 15 August 1988, *S.G. Wu, H. Ohba, Y.H. Wu & Y. Fei No. 4095* (KUN); Lhasa, Damxung County, Namtso, 25 July 2018, *D.G. Zhang, Y. Wu & H. Ye ZDG18-9* (KUN); Nagqu, Sog County, 28 July 2018, *D.G. Zhang, Y. Wu & H. Ye ZDG23-7* (KUN); Nagqu, Sog County, 28 July 2018, *D.G. Zhang, Y. Wu & H. Ye ZDG24-24* (KUN); Nagqu, Shenza County, 3 August 1987, *B.S. Li & D. Zheng 10888* (PE); Nagqu, Biru County, 3 September 1976, *D.D. Tao 11181a* (KUN); Nagqu, Anduo County, 10 September 2008, *J.H. Chen, H.F. Zhuang & D.T. Liu Yangyp-Q-0258* (KUN); Nagqu, Baqing County, 26 June 2016, *J.P. Yue, Z. Zhou & H.L. Chen YZC226* (KUN); Nagqu, Anduo County, 21 August 2009, *J.H. Chen, H.F. Zhuang & P. Tashi YangYP-Q-2166* (KUN); Ngari, Rutog County, 29 July 1987, *B.S. Li, D. Zheng 10848* (PE); Changdu, Jomda County, 1 August 2004, *D.E. Boufford, J.H. Chen, S.L. Kelley, J. Li, R. H. Ree, H. Sun, J.P. Yue & Y.H. Zhang 31531* (KUN); Changdu, Riwoqê County, 11 August 2004, *D.E. Boufford, J.H. Chen, S.L. Kelley, J. Li, R.H. Ree, H. Sun, J.P. Yue & Y.H. Zhang 32037* (KUN). **Qinghai:** Yushu Tibetan Autonomous Prefecture, Nangqên County, 24 July 1965, *Y.C. Yang 1261* (KUN); Yushu Tibetan Autonomous Prefecture, Nangqên County, 24 July 2019,

X. Zhang, J.T. Chen & T.H. Kuang HuangXH018-9 (KUN); Yushu Tibetan Autonomous Prefecture, Nangqên County, 23 July 2019, *X. Zhang, J.T. Chen & T.H. Kuang HuangXH015-20* (KUN); Yushu Tibetan Autonomous Prefecture, Yushu City, 23 July 2019, *P.J. Liu & H.H. Shi deng7261* (KUN); Yushu Tibetan Autonomous Prefecture, Zadoi County, 25 July 2019, *P.J. Liu & H.H. Shi deng7359* (KUN); Haibei Tibetan Autonomous Prefecture, Menyuan Hui Autonomous County, 26 July 2008, *Y.H. Wu LJQ-QLS-2008-0115* (KUN); Haibei Tibetan Autonomous Prefecture, Menyuan Hui Autonomous County, 18 June 2018, *XX. Zhu & Y.M. An ZXX18140* (KUN); Golog Tibetan Autonomous Prefecture, Madoi County, 31 July 2011, *S.L. Chen, Q.B. Gao & F.Q. Zhang ChenSL1379* (KUN); Golog Tibetan Autonomous Prefecture, Baima County, 29 August 2013, *J.W. Zhang, B. Yang & H.L. Cheng Zh537* (KUN); Golog Tibetan Autonomous Prefecture, Darlag County, 10 August 1993, *H.B.G. 1033* (PE); Haixi Mongol and Tibetan Autonomous Prefecture, Golmud City, 11 September 2008, *H.Y. Feng LiuJQ-08KLS-139* (KUN); Haixi Mongol and Tibetan Autonomous Prefecture, Golmud City, 19 August 2010, *X.M. Tian, Z.Q. Wang & J.B. Zou LiuJQ-Txm10-097* (KUN); Haixi Mongol and Tibetan Autonomous Prefecture, Golmud City, Fenghuo mountain, 18 August 2010, *X.M. Tian, Z.Q. Wang & J.B. Zou LiuJq-Txm10-074* (KUN); Haixi Mongol and Tibetan Autonomous Prefecture, Golmud City, Ulan Moron, 22 August 1990, *Yang Jingsheng 402* (KUN). **Xinjiang:** Bayin'gholin Mongol Autonomous Prefecture, Ruoqiang County, 10 August 1988, *S.G. Wu, H. Ohba, Y.H. Wu & Y. Fei 2678* (KUN); Bayin'gholin Mongol Autonomous Prefecture, Hejing County, 16 August 1958, *J.N. Zhu & A.R. Li 6543* (KUN); Kashgar Prefecture, Taxkorgan Tajik Autonomous County, 11 August 2008, *J. Qiu & J.J. Feng LiuJQ0171* (KUN).

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