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New nomenclature combinations in the green alder species complex (Betulaceae)

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Abstract

The name *Alnus viridis* (Chaix) DC., based on *Betula viridis* Chaix (1785), has traditionally been attributed to green alders although it is based on a later basionym. *Alnus alnobetula* (Ehrh.) K. Koch based on *Betula alnobetula* Ehrh. (1783) is the correct name for green alders. In light of the increasing use and recognition of the name *Alnus alnobetula* (Ehrh.) K. Koch in the literature. I herein propose new nomenclatural combinations to account for the Japanese and Chinese subspecies respectively: *Alnus alnobetula* subsp. *maximowiczii* (Callier ex C.K. Schneid.) J. Chery and *Alnus alnobetula* subsp. *mandschurica* (Callier ex C.K. Schneid.) J. Chery. Recent phylogenetic analyses place these two taxa in the green alder species complex, suggesting that they should be treated as infraspecific taxa under the polymorphic *Alnus alnobetula*.

Keywords

Green alders, *Alnus viridis*, *Alnus alnobetula*, Betulaceae

Introduction

Characteristic to the genus, *Alnus alnobetula* (Ehrh.) K. Koch is an anemophilous shrub with carpellate catkins that develop into woody strobili. It has a circumpolar distribution with subspecies in Europe (Greuter and Raab-Straube 2011, Flora Europea [http://rbg-web2.rbge.org.uk/FE/fe.html – accessed 22.07.2015], North America (Furlow 1979, Furlow 1990, Fl. North. Amer. North of Mexico Editorial Committee 1997), and Asia (Löve 1968, Li and Skvortsov 1999, Ohba 2006). A phylogeny using nuclear ribosomal DNA ITS sequences generated a polytomy containing five taxa within the green alder species complex due to low sequence divergence among the individuals (Chen and Li 2004). Ren et al. (2010) found the green alder species complex to be a monophyletic clade with the unique character state of a thymine at position 192 of the ITS region. Banaev and Adel'shin (2009) also found close affinity of green alder species using molecular data.

The name *Alnus viridis* (Chaix) DC. has long been attributed to green alders; however a closer look at the literature reveals the name *Alnus alnobetula* (Ehrh.) K. Koch has priority (Pouzar 1982, Holub 1986). Appropriate nomenclatural combinations have recently been published for *A. alnobetula* subsp. *crispa* (Aiton) Raus, *A. alnobetula* subsp. *sinuata* (Aiton) Raus (Greuter and Raab-Straube 2011), and *A. alnobetula* subsp. *suaveolens* (Req.) Lambinon & Kerguélen (Lambinon and Kerguélen 1988). Subspecies names for the Japanese green alder and Chinese green alder are assigned here.

Nomenclature history

The confusion lies in the appropriate basionym of this taxon. The name *Betula viridis* Chaix dates from 1785 (unable to access original text; revisited in Perret and Burdet 1981). No type specimen was designated. Two years earlier, *Betula alnobetula* Ehrh. was published by Ehrhart (in Gartenkalender 1783) describing a shrub in which "the homeland is unknown to me" (translated from German). In Ehrhart (1788), he republished his work where the name *Betula alnobetula* Ehrh. reappeared.

As *Betula* species were transferred to *Alnus*, authors were evidently unaware of the original 1783 publication of the name *B. alnobetula* Ehrh., so *B. viridis* Chaix was thought to be the older name and was taken to be the basionym for green alders. *Alnus alnobetula* Ehrh. has consistently been associated with the 1788 reproduced work and thus listed as a later synonym of *A. viridis* (Chaix) DC.

Major databases such as plantlist.org [accessed 22.07.2015], list the name *Alnus viridis* (Chaix) DC. as a synonym of *A. alnobetula* (Ehrh.) K. Koch. Other databases seem to be waiting for formal action to account for all subspecies names. For example, USDA, Germplasm Resources Information Network (GRIN 2015) [http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?2483], states: "the name *A. alnobetula* (Ehrh.) K. Koch, based on *Betula alnobetula* Ehrh. (1783) has priority over *A. viridis* (Chaix) DC., based on *B. viridis* Chaix (1786); nevertheless, *A. viridis* is retained here until all infraspecific taxa are accounted for under *A. alnobetula*". Other major databases have incomplete citation list for synonyms such as Fl. North Amer. North of Mexico Editorial Committee [http://www.efloras.org/flora_page.aspx?flora_id=1 – accessed 22.07.2015]. Flora Europea [http://rbg-web2.rbge.org.uk/FE/fe.html – accessed 22.07.2015] omits citations for green alder names.

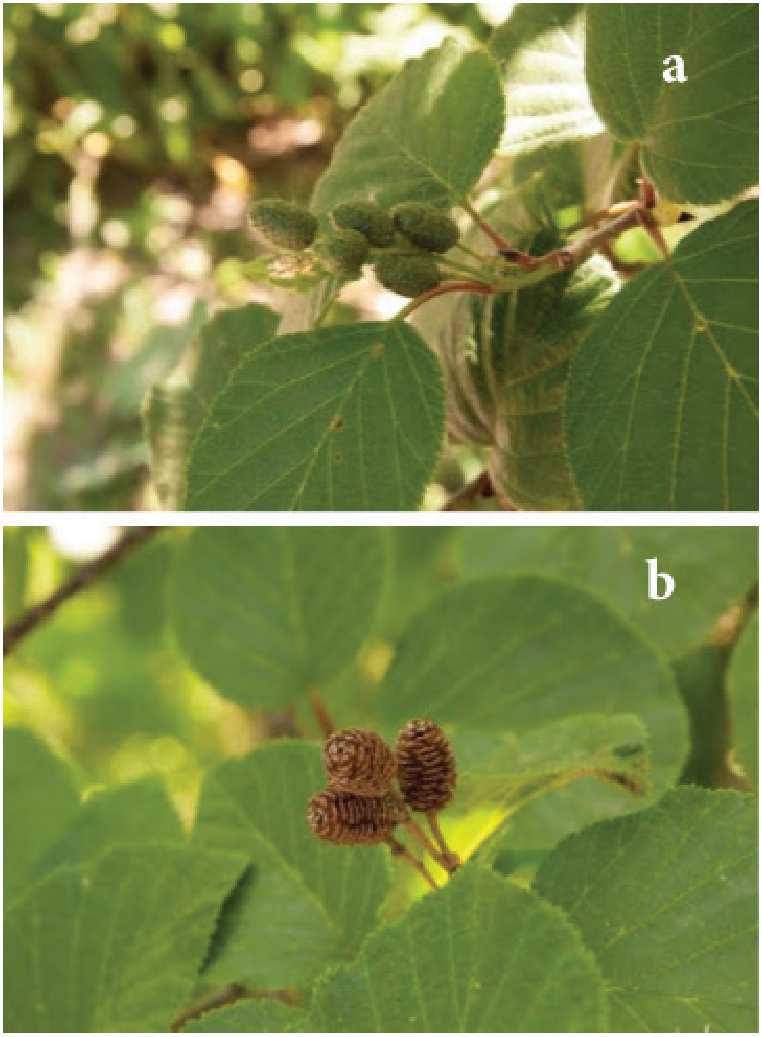

Figure 1. *Alnus alnobetula* subsp. *maximowiczii* – images (taken by Jordan Wood) from Arnold Arboretum 1462-77*E **a**) developing infructescenes; **b**) old infructescences.

Conclusions

The close relatedness of the green alder species complex members is supported by recent phylogenetic anaylses. The use of a single nrDNA marker, ITS, generated a weakly supported clade of *A. mandshurica*, *A. firma*, *A. pendula* and *A. sieboldiana* embedded within a greater polytomy that includes all other green alders (see strict consensus parsimony tree by Chen and Li 2004). In more recent phylogenetic analysis, *A. maximowiczii* and *A. mandshurica* always form a monophyletic clade with the rest of the green alders (Ren et al. 2010, Banaev and Adel'shin 2009). Given this evidence, it is appropriate to change the rank of these taxa to subspecies of the green alders. The proposed nomenclature changes utilize the correct species epithet and recognize their phylogenetic placement as lineages of a polymorphic *Alnus alnobetula*.

Infraspecific rankings of plants, specifically subspecies and variety, have been used rather interchangeably (Hamilton and Reichard 1992). The green alder species complex has historically been separated into subspecies due to geographic and morphological distinctiveness. I here agree with this subspecies concept and propose two new nomenclatural combinations to account for the Japanese and Chinese green alder subspecies. This change provides the proper nomenclature for future taxonomic and phylogenetic studies in the green alder species complex.

***Alnus alnobetula* subsp. *maximowiczii* (Callier ex C.K. Schneid.) Chery, comb. n.**

urn:lsid:ipni.org:names:77149153-1

Alnus maximowiczii Callier ex C.K. Schneid., Illustr. Handb. Laubholzk. 1: 122. 1904: typified by the plate accompanying the protologue (Basionym).

Alnus crispa subsp. *maximowiczii* (Callier ex C.K. Schneid.) Hultén, Acta Univ. Lund. Avd. 2. 40(1): 590. 1944.

Alnaster maximowiczii (Callier) Czerep., Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk. S.S.S.R. 17: 97. 1955.

Alnaster crispus subsp. *maximowiczii* (Callier ex C.K. Schneid.) Murai, Bull. Gov. Forest Exp.Sta.154: 62. 1963.

Duschekia maximowiczii (Callier ex C.K. Schneid.) Pouzar, Preslia 36: 339. 1964.

Alnaster maximowiczii (Callier) Czerep., Fl. Arct. URSS Fasc. 5, 133 in obs. 1966.

Alnus viridis subsp. *maximoviczii* (Callier ex C.K. Schneid.) D. Löve, Taxon 17: 89. 1968.

Alnus viridis subsp. *maximowiczii* (Callier ex C.K. Schneid.) H. Ohba, Fl. Japan 2a: 27. 2006.

Distribution. Temperate Asia: Russian Federation - Khabarovsk, Kurile Islands, Primorye, Sakhalin; Japan - Hokkaido, Honshu; Korea

***Alnus alnobetula* subsp. *mandschurica* (Callier ex C.K. Schneid.) Chery, comb. n.**

urn:lsid:ipni.org:names:77149155-1

Alnus fruticosa var. *mandschurica* Callier ex C.K. Schneid., Illustr. Handb. Laubholzk. 1:121. 1904: Lectotype: Nadelholzzone des Tschangpei-schan, immer vereinzelt, 1600–1800 m (Fenze 262); designated by Hand.-Mazz., not seen) (Basionym).

Alnus fruticosa var. *mandschurica* Callier ex Kom., Acta Hort. Petr. 22: 59. 1903.

Alnus fruticosa var. *mandschurica* f. *normalis* Callier, Fedde, Rep. Spec. Nov. 10: 227. 1911.

Alnus fruticosa var. *mandschurica* f. *grandifolia* Callier, Fedde, Rep. Spec. Nov. 10: 227.1911.

Alnus mandschurica (Callier ex C.K. Schneid.) Hand.-Mazz., Oesterr. Bot. Z. 81: 306–307.1932.

Alnus crispa (Aiton) Pursh subsp. *mandshurica* (Callier) Hara, J. Fac. Sci. Univ. Tokyo III, -6, (2): 32. 1952.

Alnus mandschurica var. *pubescens* Baranov, in T. N. Liou, Illustrated Flora of Ligneous plants of N. E. China 206, t. 75, fig. 112, t. 76, figs 1–4. 1955.

Duschekia mandschurica (Callier ex C.K. Schneid.) Pouzar, Preslia 36(4): 339. 1964.

Alnaster crispa (Aiton) ssp. *mandshurica* (Callier) Murai, Bull. Gov. For. Expt. Sta. Jap. 171: 34. 1964.

Distribution. Russian Federation: Khabarovsk, Primorye; China: Heilongjiang, Jilin, Liaoning, Nei Monggol; Korea

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References

Banaev EV, Adel'shin RV (2009) Structure of *Alnus fruticosa* Rupr. s.l. and its relationships with other taxa of subgenus Alnobetula (Ehrhart) Peterman. Contemporary Problems of Ecology 2(6): 601–610. doi: 10.1134/S1995425509060186

Chen Z, Li J (2004) Phylogenetics and biogeography of *Alnus* (Betulaceae) inferred from sequences of nuclear ribosomal DNA ITS region. International Journal of Plant Sciences 165(2): 325–335. doi: 1086/382795

Ehrhart JF (1783) Bestimmung einiger Bäume und Sträucher aus unseren Lustgebüschen.

Gartenkalender 2: 193. http://www.ub.uni-bielefeld.de/cgi-bin/neubutton.cgi?pfad=/diglib/aufkl/gartenkal/095831&seite=00000248.TIF

Ehrhart JF (1788) Beiträge zur Naturkunde, und den damit verwandten wissenschaften, besonders der botanik, chemie, haus- und landwirthschaft, arzneigelahrtheit und apothekerkunst vol (Bd 2). Hannover & Osnabrück, 67–72. doi: 10.5962/bhl.title.44806

Hamilton CW, Reichard SH (1992) Current practice in the use of subspecies, variety, and forma in the classification of wild plants. Taxon 41(3): 485–498. doi: 10.2307/1222819

Furlow JJ (1979) The Systematics of the American Species of *Alnus* (Betulaceae). Rhodora 81(825): 1–121. http://biostor.org/reference/137992

Furlow JJ (1990) The genera of Betulaceae in the southeastern United States. Journal of the Arnold Arboretum 71: 1–67. http://biostor.org/reference/61852

GRIN (2015) Germplasm Resources Information Network. http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl [accessed 22.07.2015]

Greuter W, Von Raav-Straube E (2011) Euro+Med Notulae, 5. Willdenowia 41(1): 129–138. doi: 10.3372/wi.41.41117

Holub J (1986) Comments on the “Med-Checklist 1”. Preslia 58: 289–306.

Ohba H (2006) *Alnus*. Flora of Japan Vol. 2a, Angiospermae, Dicotyledoneae, Archichlamydeae(a). Kodansha, Tokyo, 26–31.

Koch K (1872) Dendrologie. Bäume, Sträucher und Halbsträucher, welche in Mittel- und Nord Europa im Freien kultivirt werden (Vol 2). F. Enke, Erlangen, 623–638. doi: 10.5962/bhl.title.20459

de Lamarck J, Candolle A (1805) Flore française: ou descriptions succinctes de toutes les plantes qui croissent naturellement en France, disposées selon une novelle méthode d’analyse, et précédées par un exposé des principes élémentaires de la botanique (Vol 3). H. Agasse, Paris, 304 pp. http://hdl.handle.net/2027/hvd.32044107276479?urlappend=%3Bseq=354

Lambinon J, Kerguélen L (1988) Trois combinaisons nomenclatures nouvelles relatives à la flore corse. Candollea 43: 405–406.

Li P, Skvortsov AK (1999) Betulaceae. Flora of China Vol 4: Cycadaceae through Fagaceae. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 286–313. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=10101

Löve D (1968) Nomenclatural Notes on Mt. Washington Plants. Taxon 17(1): 89. doi: 10.2307/1216168

Pandora Taxonomic Database System (digital version of the Flora Europaea). Royal Botanic Garden Edinburgh. http://rbg-web2.rbge.org.uk/FE/fe.html [accessed 22.07.2015]

Perret P, Burdet HM (1981) 2. Les “Plantae Vapincenses” de Dominique Chaix et les travaux floristiques de Dominique Villars en Dauphiné. In: Burdet HM (Ed.) Med-Checklist Notulae Bibliographicae, 1 et 2. Candollea 36: 400–408.

Pouzar Z (1982) Otázka správného jména pro olši zelenou. Journal of the National Museum (Prague), Natural History, 151: 20.

Ren B, Xiag X, Chen Z (2010) Species identification of *Alnus* (Betulaceae) using nrDNA and cpDNA genetic markers. Molecular Ecology Resources 10(4): 594–605. doi: 10.1111/j.1755-0998.2009.02815.x

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Problematic specimens turn out to be two undescribed species of *Bignonia* (Bignoniaceae)

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Abstract

Bignonia comprises 29 species of lianas characterized by eight phloem wedges, leaves usually 2-foliolate, mostly simple tendrils and opaque seed wings. The analysis of herbarium specimens in preparation for a taxonomic revision of the genus led to the recognition of two new species: (i) *Bignonia cararensis* from Costa Rica, characterized by a thyrse with lateral compound dichasia and lack of interpetiolar ridge, and (ii) *Bignonia sanctae-crucis* from Bolivia and Brazil, distinguishable by its membranous leaflets, membranous calyx and small fruits. We provide detailed descriptions, illustrations, distribution maps, initial conservation status assessments, and comparisons of the newly described taxa with closely related species.

Keywords

Amazonia, Costa Rica, Bignoniaceae, Lianas, Neotropical Flora

Introduction

Bignonia L. is the fifth largest genus in the Neotropical tribe Bignonieae (Bignoniaceae), with 29 species distributed from Argentina to USA (Zuntini et al. 2015). The genus comprises lianas with eight phloem wedges, leaves usually 2-foliolate, prophylls of the axillary buds foliaceous and bromeliad-like (small, decussate, triangular prophylls resembling a bromeliad), mostly simple tendrils and opaque seed wings (Lohmann and Taylor 2014). Additionally, these plants have showy rather large flowers, pink corollas, and septicidal capsules that contain numerous seeds, usually winged. Molecular studies have found that *Bignonia* is a highly supported clade that combines previously recognized genera, such as *Clytostoma* Miers ex Bureau and *Cydista* Miers (Lohmann 2006). The species of these two former genera share a variety of morphological features, such as variously cylindrical or quadrangular stems, a cupular calyx, dorso-ventrally flattened corollas, and a reduced nectariferous disk, which made their generic identification sometimes difficult. While preparing a monograph of *Bignonia* (Zuntini, Taylor and Lohmann, in prep.), more than 4,000 collections were analyzed and several problematic specimens that had been previously identified in a variety of Bignonieae genera were found to belong to *Bignonia*. However before these materials were finally identified to genus, the identity of these specimens was so unclear that they were confused with four different genera, and the flowering and fruiting specimens of each of these new species were considered to belong to different genera. Once identified as *Bignonia*, it became clear that these specimens represent two undescribed species.

These two new species are *Bignonia cararensis* Zuntini from Costa Rica and *Bignonia sanctae-crucis* Zuntini from Bolivia and western Brazil. Within *Bignonia*, these new species are not very similar and are not closely related to each other, however, these species are each similar to previously described species.

With these two new species, *Bignonia* is now composed of 31 species, with no morphological or geographical changes in the circumscription of the genus. Our results highlight the importance of large diverse herbarium collections for understanding the systematics of tropical plants, and also of broadly surveying all the specimens of a group before finalizing monographic studies rather than studying only a selected set of specimens of a given genus.

Methods

Specimens from the following herbaria were examined: CR, F, INB, MO, NY, SPF, USJ (acronyms following Thiers 2015). The morphology descriptions follow mainly Lohmann and Taylor (2014), with additional terminology from Leaf Architecture Working Group (1999), Radford et al. (1974) and Weberling (1989). For indumentum, we follow Nogueira et al. (2013) with each trichome type described separately; peltate glandular trichomes are described according to their density as sparsely, moderately or densely lepidote, and patelliform glandular trichomes are presented here as "glands." In the descriptions, terms inside parentheses denote rare conditions. The conservation status assessments follow IUCN guidelines (IUCN 2012), with the evaluation of geographic range based on the extent of occurrence (EOO). Distribution maps were prepared using the specimen database that was compiled as part of an ongoing monographic study of the whole genus (Zuntini, Taylor and Lohmann, in prep).

Taxonomic treatment

Bignonia cararensis Zuntini, sp. nov.

urn:lsid:ipni.org:names:77149637-1

Type. Costa Rica. Puntarenas: Reserva Biológica [Parque Nacional] Carara, Sector Quebrada Bonita. Sitio Area administrativa, 09°45.6'N, 084°36.0'W, 20 m, 9 February 1990, *R. Zúñiga 90* (holotype: CR-145925, mounted in two sheets!; isotypes: F!, INB!, MO!). Figure 1.

Diagnosis. This new species is closely related to *Bignonia uleana* (Kraenzl.) L.G.Lohmann, but differs by the absence of interpetiolar ridges, inflorescences with compound dichasia (vs. simple dichasia in *B. uleana*) and fruits up to 14 cm long with cylindrical and delicate spines (vs. longer than 16 cm with triangular and rough spines in *B. uleana*). Table 1.

Description. Lianas. **Stems** solid, cylindrical, not winged, with lenticels, without interpetiolar gland fields, without interpetiolar ridge, puberulous at least at nodes, sparsely lepidote; foliaceous prophylls caducous, cymbiform, ascending, sessile, symmetrical, 1.7–2.0 mm × ca. 1.4 mm, ciliate, sparsely lepidote, without glands; bromeliad-like prophylls present. **Leaves** 2-foliolate; petiole semi-cylindrical, 35.9–46.5 mm, without simple trichomes or puberulous, sparsely lepidote; petiolules semi-cylindrical, 25.0–45.3 mm, without simple trichomes or puberulous, sparsely lepidote; blades concolorous to slightly discolorous, chartaceous, matte, symmetrical, elliptic to widely elliptic, shortly acuminate apically, rounded basally, 17.1–23.4 × 11.0–12.8 cm, on adaxial surface puberulous at base, sparsely lepidote, with glands clustered at apex and few scattered, on abaxial surface without simple trichomes or puberulous on mid and secondary veins, sparsely lepidote, with a few scattered glands; venation pinnate, with tertiary venations mixed opposite-alternate percurrent; tendrils rarely present, simple, without simple trichomes, sparsely lepidote, with simple apex. **Inflorescences** thyrses, terminal, multi-flowered, with lateral dichasia compound and pedunculate, without simple trichomes, sparsely to moderately lepidote, primary axis ca. 255.0 mm long; bracts caducous, narrowly triangular, 1.8–2.1 × 0.5–0.6 mm, without simple trichomes, sparsely lepidote, without glands; pedicels 6.7–14.4 mm, without simple trichomes, sparsely lepidote, without glands. **Flowers** with calyx cupular, 5-toothed, subchartaceous, 3.7–5.9 × 4.7–6.4 mm wide at apex, ciliate, moderately lepidote, with a few scattered glands, teeth 0.6–1.4 mm; corolla purple outside, inside color unknown, infundibuliform, dorso-ventrally flattened, membranous, 40.8–75.0 mm, externally sericeous, sparsely lepidote, without glands, internally sericeous at lobes, not lepidote, with stipitate glandular trichomes at base, tube 28.4–52.5 × 2.7–3.3 mm wide at base and 10.9–14.1 mm wide at apex, lobes sub-circular, 9.7–22.0 × 10.7–15.5 mm; androecium didynamous, with stamens included, the largest 16.4–18.6 mm, the shortest 11.2–11.3 mm, without simple trichomes, not lepidote, with stipitate glandular trichomes at base, thecae 3.3–3.5 mm, staminode ca. 4.2 mm; gynoecium 25.5–27.9 mm, ovary cylindrical, verrucose, without simple trichomes, not lepidote, ovules in 2

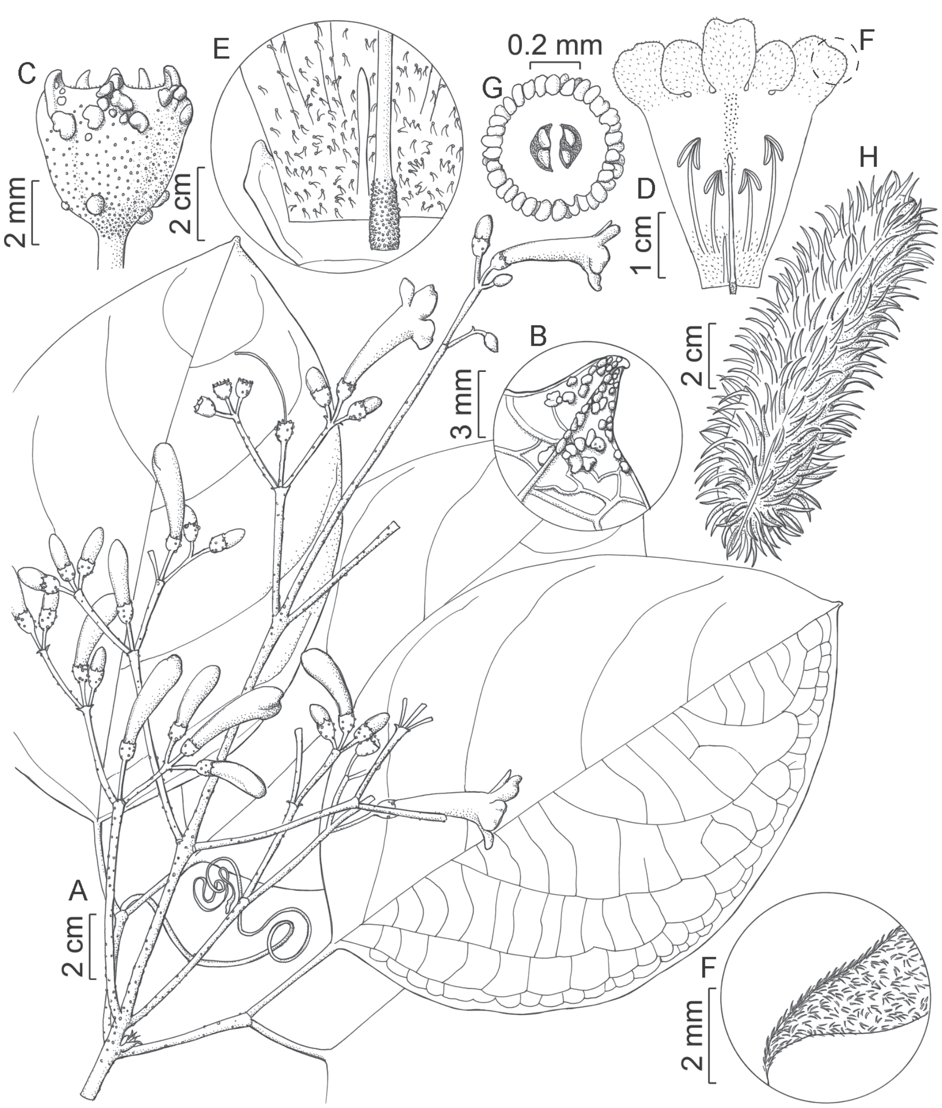

Figure 1. *Bignonia cararensis* Zuntini **A** Flowering branch **B** Gland cluster at leaflet apex on adaxial surface **C** Calyx **D** Opened flower **E** Detail of internal flower base, showing the glandular stipitate trichomes at corolla **F** Detail of the internal sericeous indument of the corolla lobes **G** Ovary cross-section **H** Fruit. Illustrated from *Zuñiga 90* (MO) [**A–G**] and *Weinberg s.n.* (MO-3842040) [**H**].

series per locule, style not lepidote; nectariferous disk reduced. **Fruits** inflated, narrowly elliptic, 11.2–14.5 × 3.1–4.0 wide × ca. 1.4 cm thick, valves woody, without ridges, moderately echinate, without simple trichomes, not lepidote, without glands; spines cylindrical, 8.2–13.4 mm. **Seeds** unknown.

Distribution. This species is known only from Parque Nacional Carara, in Puntarenas, Costa Rica, between 20 and 100 m elevation (Fig. 2).

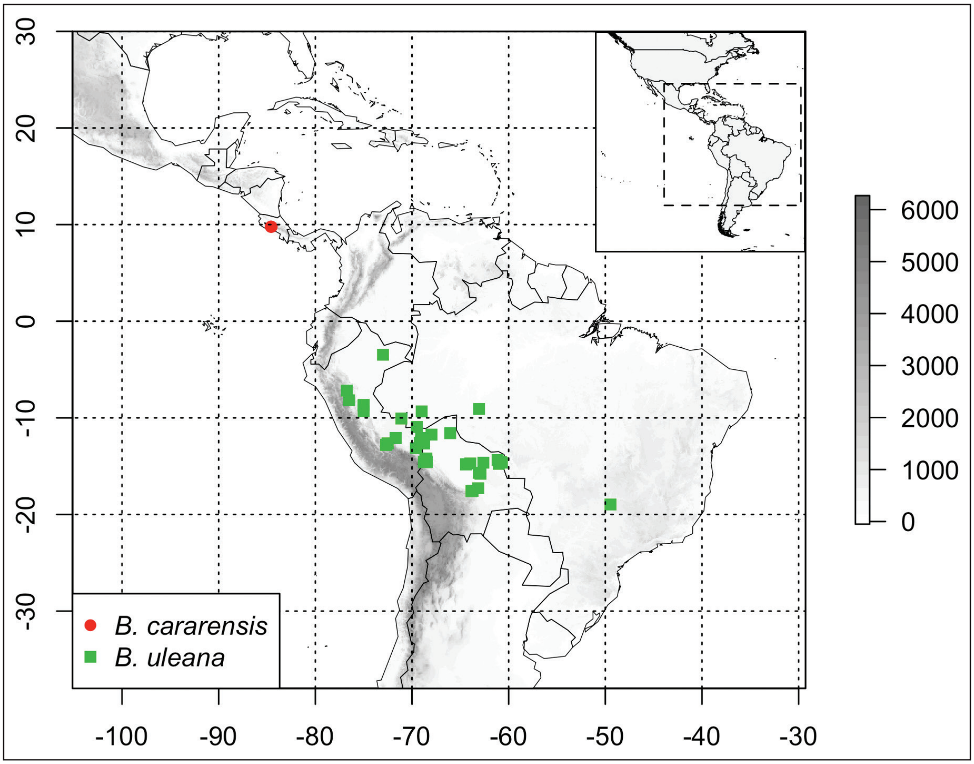

Figure 2. Distribution of *Bignonia cararensis* (red circles) and *B. uleana* (green squares). Elevation in meters, following the scale on the right.

Phenology. Three fertile collections are documented for *Bignonia cararensis*: a single flowering specimen was collected in February and two fruiting specimens were collected in February and October.

Etymology. The name is a reference to the type locality.

Conservation status. The collections from the main herbaria of Costa Rica (CR, INB and USJ) were consulted, but so far this species is only documented from Parque Nacional Carara. Since *B. cararensis* is known exclusively from the type locality, its full distribution cannot be accurately assessed and is here listed as Data Deficient (DD). Additional fieldwork is necessary to estimate the number of mature individuals and to assess the full extend of the species' distribution.

Discussion. This species is similar to *B. uleana*, a species from Bolivia, central western Brazil and Peru. *Bignonia cararensis* can be recognized by the absence of interpetiolar ridges (vs. present in *B. uleana*), the inflorescences in lateral compound dichasia (vs. lateral simple dichasia in *B. uleana*), and the fruit up to 14 cm and with cylindrical delicate spines (vs. longer than 16 cm with triangular rough spines in *B. uleana*) (Table 1).

The flowering collection *Zuñiga 90* was previously identified as *Cydista lilacina* A.H.Gentry [≡ *B. lilacina* (A.H.Gentry) L.G.Lohmann] (Burger and Gentry 2000,

Table 1. Contrasting characters of *Bignonia cararensis* and *B. uleana*.

Character	*B. cararensis*	*B. uleana*
Interpetiolar ridge	Absent	Present
Inflorescence lateral structure	Compound dichasia	Simple dichasia
Fruit length (cm)	11.2–14.5	16.8–26.0
Fruit spines	Cylindrical, moderately distributed	Triangular, sparsely distributed
Distribution	Costa Rica	Bolivia, Brazil and Peru

Hauk 1997, *in sched.* at CR, INB and MO), and so was the sterile specimen *Acosta Vargas 826* (*in sched.* at INB and MO). *Bignonia lilacina* is similar to *Bignonia cararensis*, with which it shares cylindrical stems, large leaflets, and inflorescences in thyrses with compound lateral dichasia. However, *Bignonia cararensis* differs from *B. lilacina*, an Amazonian species, by its glabrous and verrucose ovary (vs. densely lepidote and smooth in *B. lilacina*), sparsely lepidote stems, inflorescences and flowers (all of these structures are densely lepidote in *B. lilacina*), gland clusters borne on the adaxial surface of leaflet apices (vs. basal gland clusters on the abaxial leaflet surface in *B. lilacina*) and buds with straight apices (vs. curved apices in *B. lilacina*).

In contrast, the fruiting collection *Jiménez 2042* was previously identified as *Clytostoma pterocalyx* Sprague ex Urb. [≡ *B. pterocalyx* (Sprague ex Urb.) L.G.Lohmann] (*in sched.* at INB), and as *Clytostoma sciuripabulum* Bureau & K.Schum. [≡ *B. sciuripabulum* (Bureau & K.Schum.) L.G.Lohmann] (*in sched.* at CR and INB). The other fruiting material of this new species (*Weinberg s.n.*) was also identified as *C. sciuripabulum* (*in sched.* at MO). However, *Bignonia cararensis* differs from *B. pterocalyx* by its puberulous stems and inflorescences (vs. pilose in *B. pterocalyx*), 2-foliolate leaves (vs. 1-foliolate in *B. pterocalyx*) and moderately echinate fruit (vs. densely echinate in B. pterocalyx). *Bignonia cararensis* differs from *B. sciuripabulum* by the cylindrical stems (vs. quadrangular in *B. sciuripabulum*) and apical gland clusters borne on the adaxial leaflet surface (vs. no apical clusters in *B. sciuripabulum*).

Clytostoma pterocalyx and *Cydista lilacina* were reported as new records for Costa Rica (Burger and Gentry 2000, Hauk 1997, Jiménez and Grayum 2002) based on the specimens studied here, but with the re-identification of these specimens both of these species are now known only from South America. *Bignonia pterocalyx* is found in Venezuela and Colombia, and *B. lilacina* is distributed throughout Amazonia.

The Carara National Park is located in the northern portion of the Tárcoles-Térraba floristic region, which extends through the central portion of Pacific coastal Costa Rica (Hammel et al. 2004). This region has a combination of dry and moist forests, and includes elements from Nicoya and Osa Peninsulas, where *B. cararensis* might also be found.

Additional examined specimens. COSTA RICA. Puntarenas: Carara Biological Reserve, 2.6 km del portón de la entrada del sendero Laguna Meandrica. Primer desviación a mano izquierda entrando, 9°48.0'N, 84°35.16'W, 100 m, 6 Apr 2000, *L.G. Acosta Vargas 826* (INB, MO); Camino a Coopecarara, 9°47.16'N, 84°36.16'W, 100 m, 11 Oct 1995, *Q. Jiménez 2042* (CR, INB); Carara Biological Reserve. 15 minute walk from entrance of Carara taking trail winding right (counter-clockwise), 9°46'N, 84°31'W, 18 Feb 1991, *R. Weinberg s.n.* (MO-3842040).

Bignonia sanctae-crucis **Zuntini, sp. nov.**

urn:lsid:ipni.org:names:77149638-1

Type. Bolivia. Santa Cruz: Prov. Ichilo. El Carmen (8 km al SSW de Buena Vista), tramo de 2km al W de la comunidad por el camino al Campamento del Río Saguayo, 17°31.98'S, 63°41.85'W, 400 m, 5 October 1996, *I.G. Vargas C. 5382 & S. Hurtado P.* (holotype: MO-5878679!; isotypes: K, NY!). Figure 3.

Diagnosis. This new species is similar to *Bignonia potosina* (K.Schum. & Loes.) L.G.Lohmann, but is distinguished by its membranous leaflets with mixed opposite-alternate percurrent tertiary venation (vs. chartaceous with alternate percurrent tertiary venation in *B. potosina*), membranous calyx (vs. chartaceous calyx in *B. potosina*) and fruits shorter than 6.8 cm long (vs. fruits longer than 15 cm in *B. potosina*). Table 2.

Description. Lianas. **Stems** solid, tetragonal, winged or ribbed, with lenticels, without interpetiolar gland field, with interpetiolar ridge, puberulous to pilose at least at nodes, sparsely lepidote; foliaceous prophylls caducous, falcate (subulate), ascending, stipitate, asymmetrical, 0.9–2.3 mm × 0.5–1.5 mm, without simple trichomes, sparsely lepidote, with a few glands on abaxial surface (no glands); bromeliad-like prophylls present. **Leaves** 2-foliolate; petiole semi-cylindrical, (6.1–)15.1–38.9 mm, pubescent, puberulous or pilose, sparsely lepidote; petiolules semi-cylindrical, 9.7–29.8 mm, pilose, sparsely lepidote; blades slightly discolorous, membranous, matte, slightly asymmetrical to asymmetrical, elliptic to widely ovate, acuminate to long acuminate apically, rounded basally (short attenuate), 8.3–13.3(–18.9) × 5.1–8.5(–12.3) cm, on adaxial surface without simple trichomes, densely lepidote, without glands, on abaxial surface pilose along midvein and secondary veins, sparsely lepidote, with a few scattered glands; venation pinnate, with tertiary venations mixed opposite-alternate percurrent; tendrils rarely present, simple, without simple trichomes, sparsely lepidote, with simple apex. **Inflorescences** racemes, terminal, 2–4-flowered, without simple trichomes or puberulent, sparsely lepidote, primary axis 8.3–13.8 mm long; bracts caducous, not observed; pedicels 5.5–10.0 mm, without simple trichomes, moderately lepidote. **Flowers** with calyx cupular, 5-toothed, membranous, 4.5–6.0 × 4.3–5.1 mm wide at apex, ciliate, moderately lepidote, with glands clustered in columns, teeth 0.6–1.5 mm; corolla creamish outside, yellowish inside, infundibuliform, dorso-ventrally flattened, membranous, 30.6–52.8 mm, externally pubescent at lobes, moderately lepidote, without glands, internally with pubescent lobes, not lepidote, with shortly stipitate glandular trichomes at base, tube 19.8–40.0 × 2.1–5.0 mm wide at base and 9.2–16.3 mm wide at apex, lobes rounded or oblong, 9.7–15.4 × 8.8–15.2 mm; androecium didynamous, with stamens included, the largest 10.7–18.0 mm, the shortest 6.4–11.7 mm, without simple trichomes, not lepidote, with shortly stipitate glandular

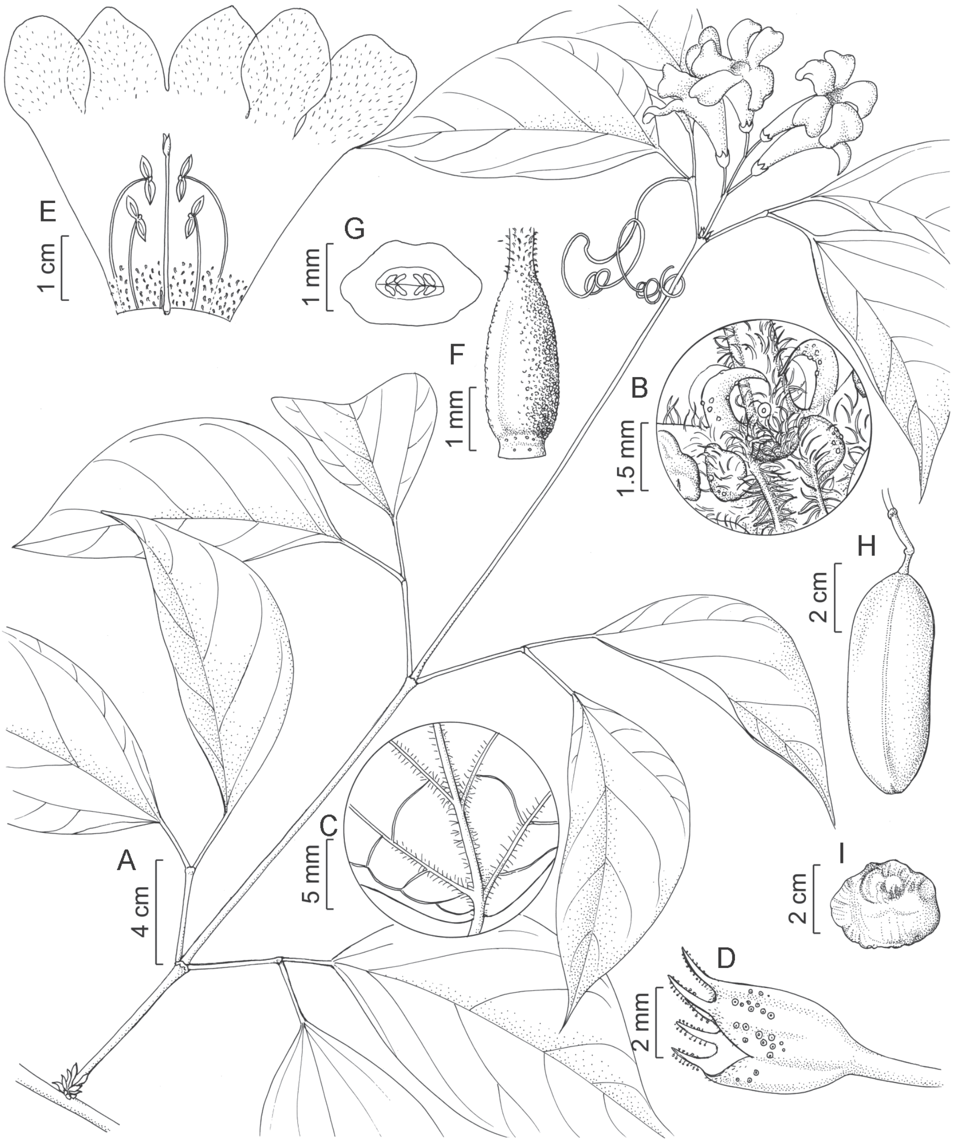

Figure 3. *Bignonia sanctae-crucis* Zuntini. **A** Flowering branch **B** Stem node with prophylls of the axillary buds **C** Detail of the abaxial leaflet surface **D** Calyx **E** Opened flower **F** Ovary side-view **G** Ovary cross-section **H** Fruit **I** Seed. Illustrated from *Vargas 5382* (NY) [**A,D**], *Saldias 4775* (NY) [**E–G**] and *Nee 52361* (NY) [**B, C, H, I**].

trichomes at base, thecae 1.7–3.6 mm, staminode 0.9–2.5 mm; gynoecium 21.5–29.2 mm, ovary ovoid to cylindrical, smooth, without simple trichomes (pilose at apex), densely lepidote, ovules in 4 series per locule, style sparsely lepidote at base; nectariferous disk reduced. **Fruits** inflated, oblong, ca. 6.8 × 2.8 wide × 0.8 cm thick, valves

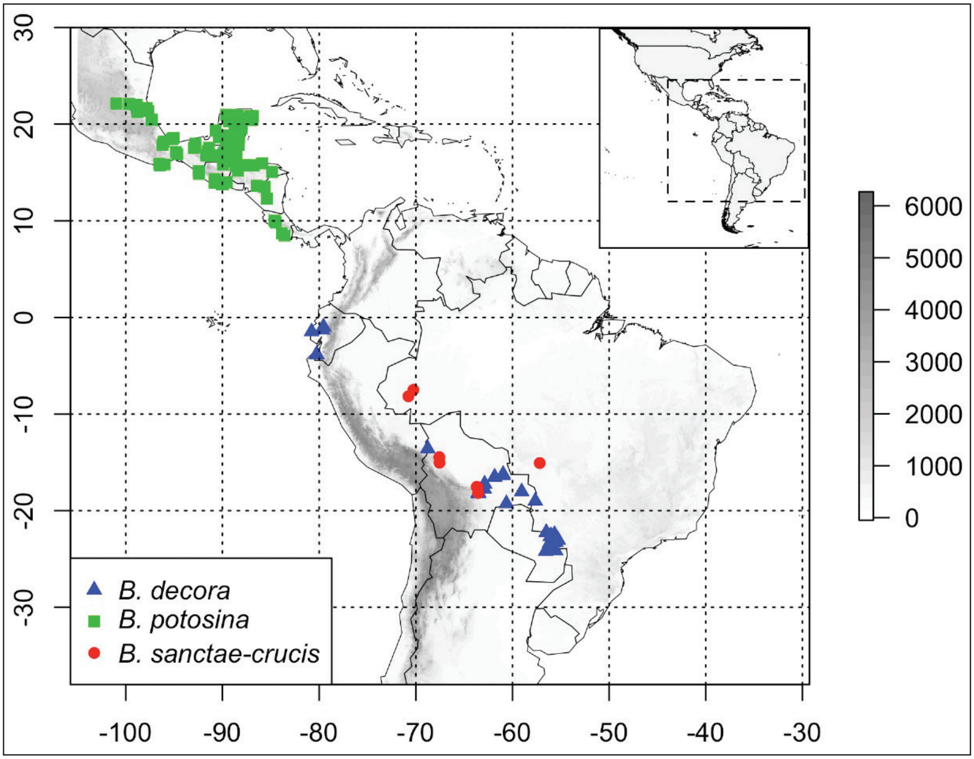

Figure 4. Distribution of *Bignonia sanctae-crucis* (red circles), *B. decora* (blue triangles), and *B. potosina* (green squares). Elevation in meters, following the scale on the right.

woody, without ridges, smooth, without simple trichomes, sparsely lepidote, without glands. **Seeds** beige, thin, transversally elliptic to narrowly transversally oblong, symmetrical, 13.7–24.3 × 28.5–39.4 mm, with two opaque wings; seed body flattened, 0.8–1.2 mm thick.

Distribution. This species is found in evergreen or semideciduous forests in Western Amazonia, occurring in Bolivia (Beni, La Paz and Santa Cruz) and Brazil (Acre, Amazonas and Mato Grosso), between 160 to 700 m alt. (Fig. 4).

Phenology. This species was collected with flowers in June, September, October and November. A single fruiting specimen was collected in July.

Conservation status. *Bignonia sanctae-crucis* is known from only seven locations but is considered Least Concern (LC) given its wide extent of occurrence (over 600.000 km^2) and the different physiognomies where it occurs, including secondary formations. The number of locations where this species is known to occur is likely underestimated because *Bignonia* species are usually not densely distributed and because this entire region is not well documented floristically. Additional fieldwork is needed in order to fully document the extent of distribution of this species.

Etymology. The epithet refers to the type locality, the Department of Santa Cruz (Bolivia), where most specimens were collected.

Table 2. Contrasting characters of *Bignonia decora*, *B. potosina* and *B. sanctae-crucis*.

Character	*B. decora*	*B. potosina*	*B. sanctae-crucis*
Prophylls of axillary buds	Foliaceous, persistent and spreading	Falcate, caducous and ascending	Falcate, caducous and ascending
Leaf texture	Sub-chartaceous to chartaceous	Chartaceous to sub-coriaceous	Membranous
Leaf tertiary venation	Alternate percurrent	Alternate percurrent	Mixed opposite-alternate percurrent
Inflorescence	Thyrse	Raceme	Raceme
Calyx texture	Chartaceous	Chartaceous	Membranous
Fruit length (cm)	14.7–37.7	12.0–24.0	Ca. 6.8
Distribution	Bolivia, Brazil, Ecuador and Peru	Central America	Bolivia and Brazil

Discussion. *Bignonia sanctae-crucis* and *B. potosina* share quadrangular and ribbed (winged) stems, prominent interpetiolar ridges, falcate and caducous prophylls, and few-flowered racemes. Apart from being morphologically similar, these species are also closely related and can be confused. However, *Bignonia sanctae-crucis* can be distinguished from *B. potosina* by the membranous calyx (vs. chartaceous in *B. potosina*) and fruits shorter than 6.8 cm long (vs. fruits longer than 15 cm in *B. potosina*) (Table 2). These two species are also geographically widely separated, with *B. sanctae-crucis* found in Bolivia and central to western Brazil while *B. potosina* is widely found in Mexico and Central America but not in South America. *Bignonia sanctae-crucis* can also be confused with the sympatric species *B. decora* (S.Moore) L.G.Lohmann due to the quadrangular stems shared by both species. However, *B. sanctae-crucis* can be recognized by its falcate and caducous prophylls (vs. foliaceous and persistent in *B. decora*), few-flowered racemes (vs. multi-flowered thyrses in *B. decora*) and fruit without ridges (vs. three longitudinal ridges in *B. decora*) (Table 2). Quadrangular stems are also characteristic of *B. sciuripabulum* (Bureau & K.Schum.) L.G.Lohmann, a distantly related species (Zuntini and Lohmann, in prep.) that has a verrucose and glabrous ovary (vs. smooth and lepidote in *B. sanctae-crucis*) and echinate fruits (vs. smooth in *B. sanctae-crucis*); *B. sciuripabulum* is found in Amazonia and the Atlantic forest of Brazil.

The only fruiting material of this new species (*Nee 52361*) was previously identified as *Cydista cf. decora* (S.Moore) A.H.Gentry [≡ *B. decora*] (*in sched.* at NY), a closely related species. The flowering specimens of *B. sanctae-crucis*, however, were identified as *Clytostoma sciuripabulum* Bureau & K.Schum. [≡ *B. sciuripabulum*], *Clytostoma uleanum* Kraenzl. [≡ *Bignonia uleana*], and some other *Clytostoma* species (*in sched.* at MO and NY). The thin-textured corolla probably confused the generic identification, given that most *Cydista*, as previously circumscribed, were characterized by thicker corollas whereas such thin corollas were characteristic of the previously recognized *Clytostoma*. Despite its corolla texture, *B. sanctae-crucis* is not closely related to the species that were included in *Clytostoma*, and does not have the verrucose glabrous ovary that is characteristic of that group.

Additional examined specimens. BOLIVIA. Beni: Rurrenabaque, Rurrenabaque, 14°28'S, 67°34'W, 333 m, 8 Oct 1921, *White 874* (NY). La Paz: Alto Beni, Concesión de San Jose de Papay, 15°02'S, 67°33'W, 500 m, 23 Oct 1987, *E. Vargas 2022* (LPB, MO); San Buena Ventura, 500 m, 29 Nov 1901, *R.S. Williams 363* (NY). Santa Cruz: Cercado, Lomas del Rio Cúcha, 450 m, 28 Oct 1925, *J. Steinbach G. 7307* (F, MO); Ibáñez, Gorge of Río Bermejo, 6.5km (by road) W of the checkpoint at Angostura, 18°10'S, 63°33'W, 690 m, 25 Jul 2003, *M.H. Nee 52361* (LPB, NY, USZ). Ichilo, 2 km W of Center of San Carlos, older secondary growth along highway from Buena Vista to Villa Tunari, 17°24.5'S, 63°45'W, 310 m, 31 Oct 1999, *M.H. Nee 50398* (NY); Ichilo, Estáncia San Rafaél (propiedad de la Unversidad NUR), 16 km SW de Buena Vista, 17°36'S, 63°36'W, 432 m, 1 Oct 1996, *M. Saldias P. 4775* (NY, USZ). BRAZIL. Acre: Tarauacá, 1–3 km east of Rio Tarauacá, 24 Sep 1968, *G.T. Prance 7513* (K, INPA, MG, MO, NY). Amazonas: Envira, Rio Juruá, Basin of Rio Jurua, near mouth of Rio Embira, 7°30'S, 70°15'W, 160 m, 28 Jun 1933, *B.A. Krukoff 5046* (MICH, MO, NY, US). Mato Grosso: Barra do Bugres, Fazenda Ochsenfeld, 23 Oct 1995, *G. Hatschbach 63777* (MBM, SPF).

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References

Burger W, Gentry AH (2000) Bignoniaceae. In: Burger W (Ed.) Flora costaricensis. Fieldiana, Bot, n.s., 41: 77–162.

Hammel BE, Grayum MH, Herrera C, Zamora N (2004) 1 Manual de plantas de Costa Rica. Missouri Botanical Garden Press, St. Louis, 324 pp.

Hauk WD (1997) A review of the genus *Cydista* (Bignoniaceae). Annals of the Missouri Botanical Garden 84: 815–840. doi: 10.2307/2992028

IUCN (2012) IUCN red list categories and criteria: Version 3.1. Second edition. IUCN Red List Unit, Cambridge, 32 pp.

Jiménez MQ, Grayum MH (2002) Vegetación del Parque Nacional Carara, Costa Rica. Brenesia 57–58: 25–66.

Leaf Architecture Working Group (1999) Manual of leaf architecture: morphological description and categorization of dicotyledons and net-veined monocotyledonous angiosperms. Smithsonian Institution, Washington, 65 pp.

Lohmann LG (2006) Untangling the phylogeny of neotropical lianas (Bignonieae, Bignoniaceae). American Journal of Botany 93: 304–318. doi: 10.3732/ajb.93.2.304

Lohmann LG, Taylor CM (2014) A new generic classification of Tribe Bignonieae (Bignoniaceae). Annals of the Missouri Botanical Garden 99: 348–489. doi: 10.3417/2003187

Nogueira A, Ottra JHL El, Guimarães E, Machado SR, Lohmann LG (2013) Trichome structure and evolution in Neotropical lianas. Annals of Botany 112: 1331–1350. doi: 10.1093/aob/mct201

Radford AE, Dickison WC, Massey JR, Bell CR (1974) 1 Vascular Plant Systematics. Harper-Collins, New York, EUA, 891 pp.

Thiers B (2015) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Weberling F (1989) 1 Morphology of flowers and inflorescences. Cambridge University Press, Cambridge, UK, 415 pp.

Zuntini AR, Taylor CM, Lohmann LG (2015) Deciphering the Neotropical *Bignonia binata* species complex (Bignoniaceae). Phytotaxa 219: 69–77. doi: 10.11646/phytotaxa.219.1.5

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RESEARCH ARTICLE

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A new record of the rare alga *Pachycladella* P. C. Silva (Chlorophyceae) in New England

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Abstract

A rarely reported taxon, the microscopic green alga *Pachycladella*, was found in a pond in Connecticut. Due to an unresolved taxonomic debate within the genus, the species-level identity of the newly discovered population cannot be determined with absolute certainty. However, according to the currently accepted classification the Connecticut specimens best match *Pachycladella zatoriensis*, heretofore only known from Europe. The find represents not only the first record of *Pachycladella* in Connecticut, but also in the entire New England region. This study highlights the need for continuing floristic surveys even in regions previously well explored.

Keywords

Connecticut, floristics, microalgae, plankton

Introduction

USA's New England region, which encompasses the states of Connecticut (CT), Maine, Massachusetts, New Hampshire, Rhode Island and Vermont, has certainly received plenty of floristic attention over the past century. Freshwater algal floristic works in this region, however, have dwindled in recent years. In 1984, a 1020 pages long list of New England algal records and the related literature was compiled by Colt (1984) from previously published works, but relatively few studies have been added since. Yet, even those few have demonstrated the need for continuing floristic work on New England freshwater algae: for example, Vaccarino et al. (2011) added 140 generic records to the flora of Acadia National Park and Fučíková et al. (2015) reported 65 new species records for Maine. Clearly, much of the freshwater algal diversity still remains to be documented even in this relatively well-studied region.

The genus *Pachycladella* was originally described from Palisades Interstate Park in New York by Smith (1924, as *Pachycladon* G.M. Smith), and remained monotypic for nearly four decades. The type species, *P. umbrina* (G.M. Smith) P.C. Silva, is represented in public culture collections by a single strain (SAG 10.85 and its duplicates in other collections), which has been studied extensively in terms of its morphology, life cycle, and ultrastructure (e.g., Reymond and Hegewald 1990, Friedl and Reymond 1997). This strain was also used in molecular phylogenetic reconstructions. Both molecular data and ultrastructural features indicate that the strain (and presumably the genus) belongs to Chlorophyceae, and molecular phylogenies, e.g., in Škaloud et al. (2013), show it as member of the volvocalean clade Stephanosphaerinia.

Pachycladella and the species within it have a somewhat convoluted taxonomic history (Smith 1924, Silva 1970, Reymond 1980, Reymond et al. 1992). Of the five currently recognized species, three are not well understood in terms of morphological variation, occurrence, and ultimately taxonomic status (Reymond et al. 1992, Guiry and Guiry 2015). Through careful morphological examinations and literature review Reymond et al. (1992) clarified the blurry distinction between *P. umbrina* and *P. zatoriensis* (Bednarz & Mrozinska-Webb) Komárek, but the distinctness of *P. chodatii* (Bern.) Hegewald*, P. minor* (Chudybowa & Chudyba) P.C. Silva, and *P. komarekii* (Fott & Kovácik) Reymond still needs to be confirmed (Komárek and Fott 1983).

The present study contributes a new record of *Pachycladella* cf. *zatoriensis* from the USA, and is the first report of the genus *Pachycladella* for CT and for New England. Given the past taxonomic confusion it is difficult to interpret all historical records with certainty, but it is possible that this study also represents the first record of *P. zatoriensis* in the United States. Continuing survey studies will be necessary to understand the occurrence patterns of *Pachycladella* and its species.

Methods

In 2014 and 2015 I conducted a survey of selected lakes, streams, and wetlands in northeastern CT and recorded the algal diversity in these habitats. Samples were collected using a 10 µm mesh plankton net, stored in small Ziploc bags, and examined microscopically using an Olympus BX60 microscope with Nomarski DIC optics equipped with an Olympus DP25 digital camera (Olympus Imaging America, Center Valley, PA, USA). The Olympus CellSens software was used to capture images and measure cell dimensions. Georeferenced records of observed algae, accompanied by micrographs where possible, have been deposited in iNaturalist (https://www.inaturalist.org/projects/freshwater-algae-of-new-england). Most algal species were identified using North American and New England taxonomic literature (Conn and Webster 1908, Hylander 1928, Prescott 1964, Whitford and Schumacher 1984).

Results

On August 4th 2015, I collected a plankton sample from a pond on Bonemill Rd., Storrs, CT (41°48'12", -72°16'48") and in it found a population of a species I had not seen in any previous collection, including a 2014 sample from the same pond. I identified the alga as *Pachycladella umbrina* based on the unmistakable reddish-brown cell wall processes (Fig. 1A–F) that were either bluntly or bifurcately terminated (Fig. 1C). Upon later review of the literature it became apparent that the tetrahedral arrangement of the processes (also referred to as appendages in past literature) is characteristic of *P. zatoriensis* rather than *P. umbrina*, the latter of which has predominantly a cruciate arrangement of processes, i.e., all four processes are in the same plane (Reymond et al. 1992).

I collected an additional sample on August 12th. In both samples, *Pachycladella* occurred infrequently but consistently: I usually saw 5-10 specimens on every slide. Other algae abundant in the planktonic community included nonmotile colonial chlorophytes resembling *Chlamydocapsa planktonica* (West & G.S. West) Fott, members of the chlorophycean family Hydrodictyaceae, the large dinophyte *Ceratium hirundinella* (O.F. Müller) Dujardin, alongside diatoms (e.g., *Cymbella* C. Agardh, *Eunotia* Ehrenberg, and *Melosira* C. Agardh), synurophytes (*Mallomonas* Perty and *Synura* Ehrenberg), and euglenoids (*Euglena* Ehrenberg, *Lepocinclis* Perty, *Phacus* Dujardin, and *Trachelomonas* Ehrenberg).

The observed *Pachycladella* cells were spherical and 8–11.5 µm in diameter, and possessed one chloroplast with a single pyrenoid (not always visible). Cell wall was protracted into four (rarely five, Fig. 1D) long, hollow processes arranged in a tetrahedral, exceptionally somewhat irregular manner. Of the ca. 30 specimens observed, none exhibited a cruciate arrangement of processes. The processes were light to dark reddish-brown in color, (26-) 30–42 µm long, 3.5–4.1 µm thick at the base and 1.6–2 µm thick ca. at half their length, and had a rough surface. Process apices were either bluntly pointed or bifurcated (Fig. 1C). Additional micrographs showing the observed morphological variation are available online as Fig. S1.

Discussion

Globally, *Pachycladella* has been reported broadly but infrequently. For some time, the genus and its sole species were only known from North America (e.g., as noted by Bourrelly 1966). The geographically scattered records of the genus now also include several European countries, Argentina, Bangladesh, Brazil, India, Singapore, Taiwan, and possibly Australia (Reymond et al. 1992 and references within, Keppeler et al. 1999, Islam and Alfasane 2005, Pham et al. 2011, Hentschke and Prado 2012). *Pachycladella umbrina* is by far the most commonly reported species, although many reports do not contain enough detail in morphological descriptions or figures to critically evaluate the species-level identifications. Thus, some of these reports may in fact represent the occurrence of another species of *Pachycladella*.

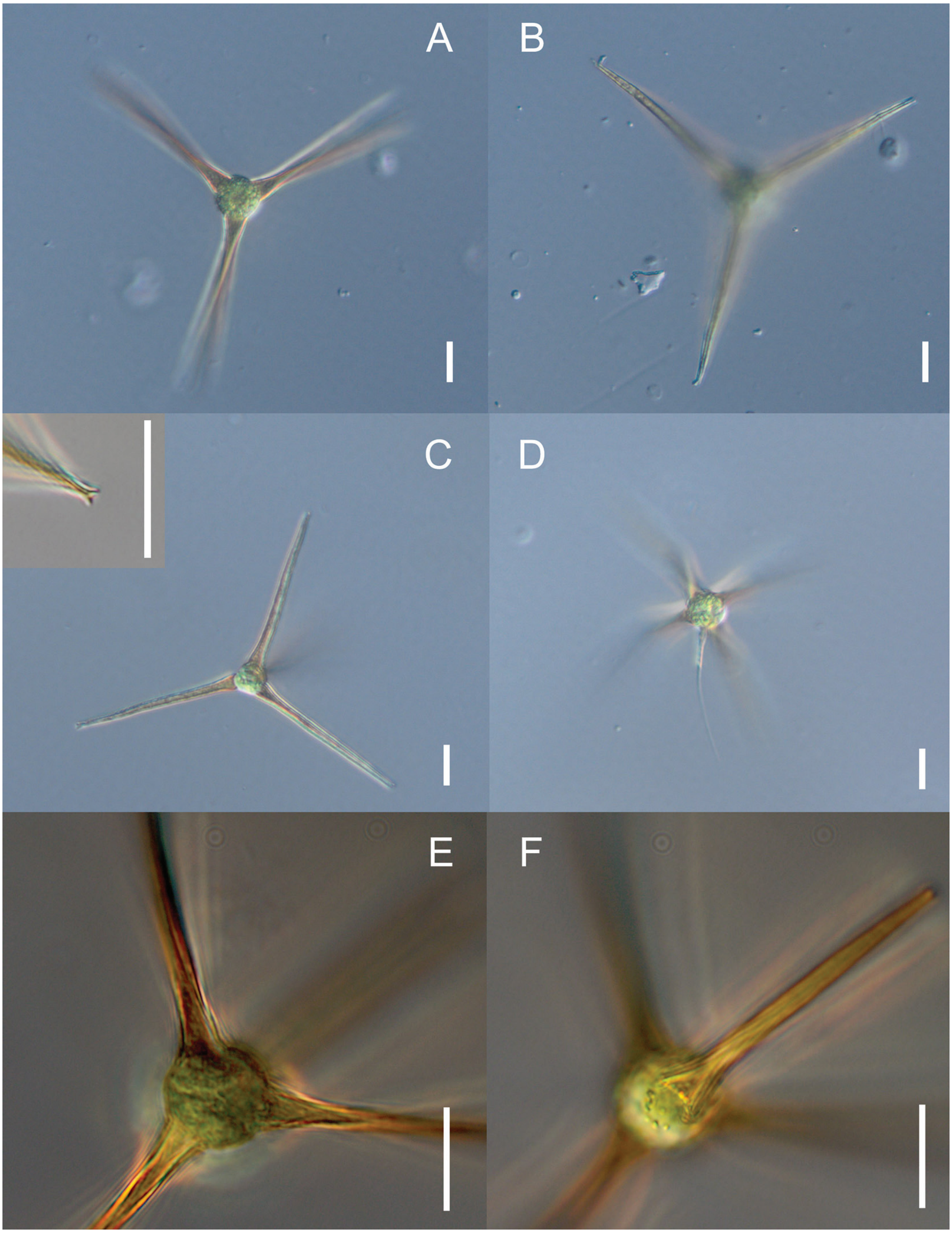

Figure 1. Light micrographs of *Pachycladella* cf. *zatoriensis* found in Connecticut. **A** gross morphology of a vegetative cell **B** same cell as in **A**, with focus on process apices **C** small vegetative cell with a clearly bifurcated process apex (enlarged in the inset) **D** an unusual cell with a fifth, irregularly placed and colorless appendage **E** high-magnification micrograph showing the hollow bases of cell wall processes as well as their dark coloration **F** same cell as in E with focus on the cell surface and attachment of the fourth process. Scale bars represent 10 µm in all images.

Based on past records and newly collected data, Reymond et al. (1992) argued that the tetrahedral vs. cruciate arrangement of processes is a stable trait within populations and can be used to distinguish *P. umbrina* (cruciate) from *P. zatoriensis* (tetrahedral). Following this distinction they assigned several previous records of *Pachycladella* to either of the two species, but also left many as uncertain due to lack of information in the reports or because the reported morphological variation spanned both types of appendage arrangement. *Pachycladella zatoriensis* is so far only confirmed to occur in Europe (Reymond et al. 1992). However, Reymond et al. (1992) also suggested that Playfair's (1918) report of *Bernardia tetraedrica* from Australia is in fact the first report of *Pachycladella zatoriensis*. Notably, Playfair (1918) does not mention two of the generic characters assigned to *Pachycladella* by Smith (1924): a common bifurcation of the process apices and the brown tint of the cell wall. Both traits were also extensively documented in *P. zatoriensis* (Bednarz and Mrozinska-Webb 1971, Reymond et al. 1992, 1993). While these characters may be variable and facultative (Wawrik 1977, Reymond et al. 1993), their complete absence in Playfair's description and figure makes the synonymy with *Pachycladella* far less certain.

In the USA, *Pachycladella* has been heretofore reported from Alabama, Kentucky, New York, North Carolina, Tennessee, and from the Great Lakes region, and has been noted to be rare or uncommon (e.g., Prescott 1964, Shubert 2003). The genus was not found during the extensive National Lakes Assessment conducted by the United States Environmental Protection Agency in 2007. Only *P. umbrina* has been reported thus far, but many US records of *Pachycladella* cannot be assigned to species with confidence (Reymond et al. 1992 and references within).

The population of *Pachycladella* newly found in CT morphologically matched the original description of *P. umbrina* except for having appendages consistently in a tetrahedral orientation, rather than being mostly cruciate (Smith 1924). The tetrahedral disposition classifies the CT specimens as *P. zatoriensis sensu* Reymond et al. (1992), which has heretofore not been reported from the USA. The original description of *P. zatoriensis* by Bednarz and Mrozinska-Webb (1971) however reports slightly smaller cells and considerably shorter processes, only up to 20 µm long. The size difference between the two species appears to be consistent across the reports reviewed and assigned to species by Reymond et al. (1992). With their larger size and tetrahedral appendage orientation, the CT specimens fit neither species perfectly, nor do they fit other *Pachycladella* species morphologically or ecologically. However, several reports marked as uncertain by Reymond et al. (1992) suggest a similar trait combination as the CT specimens (e.g., Wawrik 1977, Dillard 1989). Thus, the distinction between the two species may not be as clear-cut as implied by Reymond et al. (1992).

The rarity of *Pachycladella* combined with difficult-to-interpret past records makes species-level taxonomy in this genus quite tricky. DNA barcode data are available only from SAG 10.85, and therefore genetic comparisons of morphologically and geographically distinct populations are not possible at present. A morphological study aided by a molecular phylogeny would help deciding which traits should be considered taxonomically informative. Because even morphologically distinct taxa can be non-monophyletic (e.g., McManus and Lewis 2011), such a study on *Pachycladella* would also elucidate whether the *Pachycladella*-like morphology has a common origin or represents multiple taxa. For the purposes of this study, I followed the taxonomic scheme of Reymond et al. (1992) and assigned the CT specimens to *P. zatoriensis*. However, it is possible that they represent an unusual population of *P. umbrina*, or possibly even a novel species of *Pachycladella*. Given this uncertainty I am hesitant to proclaim this find the first US record of *P. zatoriensis*, but based on the available literature it is the first report of the genus and species in New England.

It is possible that this interesting alga had been overlooked in past floristic studies or misidentified as another taxon, despite its distinct morphology. *Pachycladella* could conceivably be mistaken for *Treubaria triappendiculata* Bernard, a species reported from Massachusetts by Gustafson (1942). It could also possibly be confused with other species of *Treubaria* (which have been reported from Maine and New Hampshire, Colt 1984 and references within). However, the two genera, despite both bearing tetrahedrally or cruciately arranged processes, are quite morphologically different. When present, the brown cell wall and bifurcate processes give *Pachycladella* away immediately. Furthermore, e.g., Smith (1950) summarizes the differences in cell shape (spherical in *Pachycladella* vs. pyramidal in *Treubaria*), appendage morphology (slender, brown and blunt/bifurcate in *Pachycladella* vs. stout, hyaline, and sharp in *Treubaria*), and chloroplast features (single plastid with one pyrenoid in *Pachycladella* vs. four pyrenoids or even four distinct plastids in older cells of *Treubaria*). Other than the unlikely misidentification for *Treubaria*, no records of *Pachycladella* or its synonyms are listed in Colt's New England checklist (Colt 1984) or subsequent works (e.g., Sheath and Harlin 1988, Vaccarino et al. 2011).

It may be somewhat surprising to see a new genus record in a region so well studied – and particularly in CT, which of all the New England states likely has the best documented freshwater algal microflora, perhaps only rivaled by Rhode Island (Conn and Webster 1908, Hylander 1928, Colt 1984, Sheath and Harlin 1988). However, many of the comprehensive studies are several to many decades old, and given the drastic environmental and habitat changes this region has undergone in the past century, one cannot simply assume that the same biodiversity still occurs here. As shown in the present study, a previously unreported taxon may have expanded its range into New England from more southern states. In other cases, species may have been lost from New England due to habitat deterioration or destruction without this biodiversity loss ever being noticed. With this small study I hope to underscore the importance of continuing survey studies on algae, as they are key players in aquatic ecosystems.

Conclusion

Despite past taxonomic confusion and uncertainty about the classification and specific diversity of *Pachycladella*, this study presents a new record of an alga that matches the original generic description perfectly, best matching the species *P. zatoriensis*. As such, it is the first record of *Pachycladella* in New England and possibly the first record of *P. zatoriensis* in North America.

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References

Bednarz T, Mrozinska-Webb T (1971) A new species of the genus *Pachycladon* G.M. Smith found on the terrain of Zator. Fragmenta Floristica et Geobotanica 17(1): 171–173.

Bourrelly P (1966) Les algues d'eau douce. Initiation à la systématique. I. Les algues vertes. Boubée, Paris, 511 pp.

Colt LC (1984) A guide to the algae of New England 1829–1984: A comprehensive guide to the algae of the New England region as reported in the literature. Parts I and II. University of Massachusetts Dartmouth, North Dartmouth, 1020 pp.

Conn HW, Webster LWH (1908) A preliminary report on the algae of the fresh waters of Connecticut. State Geological and Natural History Survey, Hartford, 78 pp.

Dillard GE (1989) Freshwater algae of the southeastern United States. Part 1. Chlorophyceae: Volvocales, Tetrasporales and Chlorococcales. Bibliotheca Phycologica 81: 1–202.

Friedl T, Reymond OL (1997) Zoospore ultrastructure of the rare coccoid green alga *Pachycladella umbrina* (Chlorophyta, Chlorophyceae). Nova Hedwigia 65: 377–384.

Fučíková K, Johansen JR, Hall JD (2015) A contribution to the desmid flora of Southeastern Maine. Journal of the Torrey Botanical Society 142(2): 166–178. doi: 10.3159/TORREY-D-14-00046.1

Guiry MD in Guiry MD and Guiry GM (2015) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org [searched on 11 August 2015]

Gustafson AH (1942) Notes on some fresh-water algae from New England. Rhodora 44: 64–69.

Hentschke GS, Prado JF (2012) Chlorococcales *s. l.* (Chlorophyceae) e Zygnematales (Zygnematophyceae) em um açude do Balneário do Lérmen, Rio Grande do Sul, Brasil. Iheringia 67: 59–74.

Hylander CJ (1928) The Algae of Connecticut. State Geological and Natural History Survey, Hartford, CT.

Islam AKMN, Alfasane MA (2005) Notes on two green plankton found in Bangladesh. Bangladesh Journal of Plant Taxonomy 12(2): 97–99. doi: 10.3329/bjpt.v12i2.606

Keppeler EC, Lopes MRM, Lima CS (1999) Ficoflórula do Lago Amapá em Rio Branco-Acre, II: Chlorophyta. Revista Brasileira de Biologia 59: 687–691. doi: 10.1590/S0034-71081999000400017

Komárek J, Fott B (1983) Chlorophyceae (Grünalgen), Ordnung: Chlorococcales. In: Huber-Pestalozzi G (Ed.) Das Phytoplankton des Süsswassers. Die Binnengewässers 16(7/1): 1–1044.

McManus HA, Lewis LA (2011) Molecular phylogenetic relationships in the freshwater family Hydrodictyaceae (Sphaeropleales, Chlorophyceae), with an emphasis on *Pediastrum duplex*. Journal of Phycology 47(1): 152–163. doi: 10.1111/j.1529-8817.2010.00940.x

Pham MN, Tan HTW, Mitrovic S, Yeo HHT (2011) A checklist of the algae of Singapore. Raffles Museum of Biodiversity Research, Singapore, 100 pp.

Playfair GI (1918) New and rare freshwater algae. Proceedings of the Linnean Society of New South Wales 43: 497–543.

Prescott GW (1964) How to know the fresh-water algae. In: Jaques HE (Ed.) Pictured key nature serie. WMC Brown Comp. Publisher, Dubuque, 272 pp.

Reymond OL (1980) Contribution à l'étude de *Treubaria* Bernard (Chlorococcales, Chlorophyceae). Candollea 35: 37–70.

Reymond OL, Hegewald E (1990) Morphology, life cycle and taxonomy in *Pachycladella umbrina* (Chlorophyceae, Chlorococcales). Algological Studies 58: 15–28.

Reymond OL, Yamagashi T, Druart JC (1992) Morphological and taxonomical assessment of former and present records of *Pachycladella umbrina* and *P. zatoriensis* (green alga, Chlorococcales). Cryptogamie, Algologie 13(2): 93–107.

Reymond OL, Birchem R, Rouelle-Rossier VB (1993) Cell morphology and localization of Fe and Mn in field-collected algae *Pachycladella umbrina* and *P. zatoriensis* (Chlorococcales, Chlorophyceae). Algological Studies 68: 51–63.

Sheath RG, Harlin MM (1988) Freshwater and marine plants of Rhode Island. Kendall/Hunt Publishing Company, Dubuque, 149 pp.

Shubert E (2003) Nonmotile coccoid and colonial green algae. In: Wehr JD, Sheath RG (Eds) Freshwater algae of North America: Ecology and classification. Academic Press, San Diego, 253–309. doi: 10.1016/b978-012741550-5/50008-8

Silva PC (1970) Remarks on algal nomenclature IV. Taxon 19: 941–945. doi: 10.2307/1218317

Škaloud P, Kalina T, Nemjová K, De Clerck O, Leliaert F (2013) Morphology and phylogenetic position of the freshwater green microalgae *Chlorochytrium* (Chlorophyceae) and *Scotinosphaera* (Scotinosphaerales, ord. nov., Ulvophyceae). Journal of Phycology 49: 115–129. doi: 10.1111/jpy.12021

Smith GM (1924) Ecology of the plankton algae in the Palisades Interstate Park: including the relation of control methods to fish culture. Roosevelt Wild Life Forest Experiment Station, 108 pp.

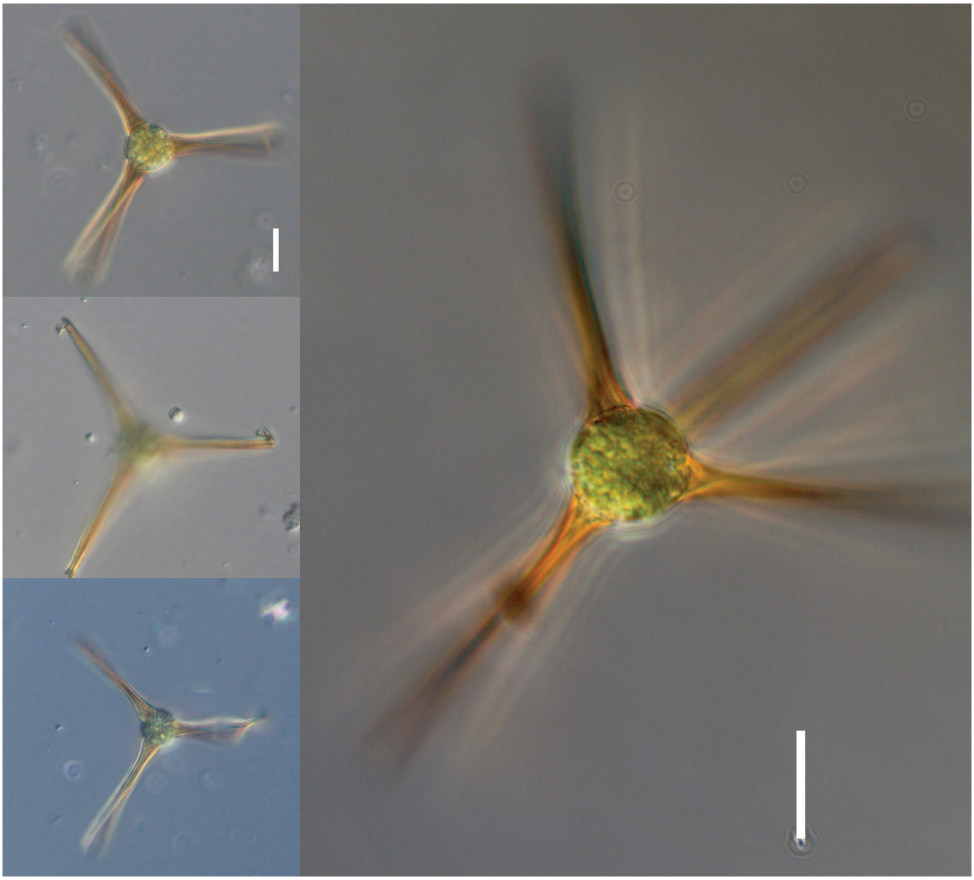
Smith GM (1950) The fresh-water algae of the United States. MacGraw-Hill, New York, 719 pp.

Vaccarino MA, Veselá J, Johansen JR (2011) The algal flora of Acadia National Park, Maine. Northeastern Naturalist 18(4): 457–474. doi: 10.1656/045.018.0404

Wawrik F (1977) Beobachtungen an *Pachycladon umbrinus*, *Micractinium bornhemiense*, *Paradoxia multiseta* und der Infektionen einer *Mallomonas*-Population. Archiv für Protistenkunde 119: 60–67.

Whitford LA, Schumacher GJ (1984) A manual of freshwater algae (revised). Sparks Press, 338 pp.

Appendix



Figures S1. Additional light micrographs showing the morphology of *Pachycladella* cf. *zatoriensis* found in Connecticut. Scale bars represent 10 µm.

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RESEARCH ARTICLE

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Study of phylogenetic relationship of Turkish species of *Klasea* (Asteraceae) based on ISSR amplification

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Abstract

Klasea is a taxonomically complex genus in which there are many problems, mostly with *Klasea kotschyi* and *K. hakkiarica*. It is challenging to differentiate the genera based on morphological characters alone. Revision studies performed on the basis of molecular data obtained from studies conducted in recent years have made the phylogenetic relationships and systematic positions of the taxa more apparent and reliable. In this study, *Klasea*, *Serratula*, *Jurinea* and *Centaurea* species native to Turkey, were collected from different localities of Anatolia and DNA was isolated from the collected samples. The data were analyzed ordination analyses including UPGMA and PCA using NTSYSpc 2.1. The infrageneric and intergeneric phylogenetic relationships between *Klasea* and other related genera were also characterized. The *Klasea* species were grouped into three clusters. It was determined that taxa *Klasea kotschyi* and *K. hakkiarica* are separate but closely related. Moreover, it was observed that the *Klasea lasiocephala* a separate group within the genera. Clearly the genera *Klasea*, *Serratula*, *Jurinea* and *Centaurea* are phylogenetically differentiated on the dendogram.

Keywords

Asteraceae, ISSR, *Klasea*, *Serratula*, Molecular systematics, Phylogeny

Introduction

The tribe Cardueae (Asteraceae) is generally accepted to be classified into five subtribes named Echinopinae, Carlininae, Carduinae, Centaureinae and Cardopatiinae (Susanna et al. 2006). Cardueae include perennial, biennial, or monocarpic herbs and shrubs and, less often, annual herbs or small trees (Barres et al. 2013). However, delineation of these taxonomic entities is highly problematic. Beyond the limits of the tribes, the boundaries between these units are also very difficult to establish. Also, some large genera of the tribes have generic delimitation problems: *Carduus* L. (90 species), *Cirsium* Mill. (250 species), *Centaurea* L. (400 species), *Cousinia* Cass. (800 species), *Serratula* L. (70 species), and *Saussurea* DC. (more than 300 species) (Garcia-Jacas et al. 2002). Extensive work conducted recently by Garcia-Jacas et al. (2000, 2001) and Font et al. (2002) have clarified the delineation of *Centaurea*. Limited studies also exist on *Cirsium* and *Carduus* (Haffner and Hellwig 1999), but most of the taxonomic problems persist. The genus *Klasea* Cass. constitutes a taxonomically complex group of plants with generic boundaries are unclear, especially at the generic level surrounding genus *Serratula* (Martins and Hellwig 2005). *Klasea* Cass., traditionally treated as a section within *Serratula* L., is widely accepted at the generic level (Martins 2006). *Klasea* is naturally distributed in Central Asia, Iran, Turkey, China, Himalayas, south east Europe and south Russia. *Klasea* is located within the monophyletic tribe Cardueae, in the subtribe Centaureinae (Susanna et al. 2006).

16 species were reported for the genus *Serratula* in Turkey (Davis and Kupicha 1975; Davis et al. 1988). Then all Turkish *Serratula* species were transferred to *Klasea* except *Serratula tinctoria* (Greuter 2003; Martins 2006). Thus, *Klasea* is represented by 15 species and *Serratula* is represented by one species within the Mediterranean and Irano-Turanian phytogeographic regions of Turkey (Dogan et al. 2012). Five of these species are endemic to Turkey, resulting in an endemism ratio of 33.3% (Dogan et al. 2012).

Currently, morphological revisions of various plant taxa are often supported by molecular data (APG 2003). As compared with morphological data, DNA sequences are not influenced by the environmental conditions in which the plants have grown; hence they serve as a powerful tool in resolving taxonomical and systematical problems. When compared with the phenotypic characters, by using different molecular marker systems, more reliable results were also obtained by a number of researchers that used different plant groups (Yang et al. 1996; Joel et al. 1998; Soranzo et al. 1999; Bremer et al. 2001; Mengitsu et al. 2002; Ash et al. 2003; Jump et al. 2003; Pharmawati et al. 2004; Dogan et al. 2007; Ali et al. 2013).

The RAPD (Randomly Amplified Polymorphic DNA) fingerprinting method is widely used and has a wide range of applications (Williams et al. 1990). However, because RAPD is a highly sensitive method, it should be used with great care. The ISSR (Inter Simple Sequence Repeat) has much higher levels of reproducibility than RAPD, for which reason it is preferable (Zietkiewicz et al. 1994, Prevost and Wilkinson 1999; Dogan et al. 2007; Hakki et al. 2010). The ISSR method is very widely used for the analysis of genetic diversity (Prevost and Wilkinson 1999).

Simple sequence repeats (SSRs), also known as microsatellites, are tandemly repeated di-, tri, tetra- or penta-nuclotide sequences (mainly within the range of 10–80 repeats of the core unit) that are abundant within eukaryotic genomes. A high level of genomic variation is generated by the more or less evenly distributed microsatellite sequences present within the plant and animal genomes. The high levels of genomic variation are widely used for genetic variation analysis of both wild plants (Wolfe et al. 1998; Dogan et al. 2010; Laosatit et al. 2013; Khalik et al. 2014) and crop plants (Vosman and Arens 1997; Hakki et al. 2001; Mohammadzadeh et al. 2011). Microsatellites can be used in inter- as well as intra-species analyses (Soranzo et al. 1999). However, the technique requires prior sequence information for the locus-specific primers, a feature that renders it difficult to be applied to plants for which no adequate genomic sequencing studies exist. Without considering their difficulty or cost (Hakki and Akkaya 2000), numerous microsatellite loci have been identified for economically important crops such as wheat, rice or maize. In *Klasea*, however, they have not been utilized.

In this study, *Klasea* species, which are difficult to delineate using morphological traits, were collected from their natural habitats in Turkey. DNA was isolated and fingerprinting was performed using a highly reliable and reproducible technique that mimics the application ease of RAPDs. The method employed to assess the genetic diversity and to resolve the genetic relationships among the species is a technique derived from SSR characterization based on PCR amplification of ISSR regions primed by a single oligonucleotide corresponding to the targeted repeat motif. The SSR-containing primers are usually 16-25 base pair long oligonucleotides anchored at the 3'- or 5'-end by two to four arbitrary, and often degenerate, nucleotides (Fang et al. 1997). The primer can be based on any of the motifs found at SSR loci. In these conditions, only sequence regions flanked by the two adjacent identical and inversely oriented microsatellites are amplified. Overall, the technique does not require prior sequence information (an advantage against microsatellites) and its reliability is higher than RAPD's.

The aim of this study was to determine the genetic relationships among selected Anatolian-originated *Klasea*, *Serratula*, *Jurinea* and *Centaurea* species collected from diverse regions of Turkey and to use a DNA-based molecular marker system to resolve the unclear and controversial status of these species based on conventional morphological characters.

Material and methods

Specimen collection

Silica gel dried plant leaf samples belonging to 15 *Klasea* taxa and *Serratula tinctoria*, and 2 out-group taxa (*Jurinea* and *Centaurea*) were collected from the natural flora of Turkey. The species and provinces of their localities are as follows: *Klasea quinquefolia* (Artvin), *K. oligocephala* (Kahramanmaraş), *K. kotschyi* (Bitlis), *K. serratuloides* (Van), *K. erucifolia* (Erzurum), *K. lasiocephala* (Antalya), *K. cerinthifolia* (Kahramanmaraş), *K. grandifolia*

Table 1. List of sampled taxa. Including location data, collectors, and herbarium in which the voucher specimens are accessioned.

Species	Voucher
Klasea serratuloides	Turkey, Van: Van to Gurpinar, 2125 m, 38024.434’N, 0430 23.079’E, 19.07.2009, B.Doğan 2117 & A.Duran (KNYA).
K. lasiocephala	Turkey, Antalya, Gazipasa, Çayıryaka mountain pasture, 1730 m, 36°.30.027'N, 032°.32.181'E, 30.06.2009, B.Doğan 2105 & A.Duran (KNYA), Endemic.
K. bornmuelleri	Turkey, Malatya, Darende, near Akçatoprak, 1010 m, 38°30.064'N, 037°33.907'E, 17.07.2009, B.Doğan 2110 & A.Duran (KNYA), Endemic.
K. kurdica	Turkey, Osmaniye, Yarpuz, 1465 m, 37°00.774'N, 036°26.683'E, 15.07.2009, B.Doğan 2106 & A.Duran (KNYA).
K. coriaceae	Turkey, Kars, Tuzluca to Kağızman, 1055 m, 40°06.399'N, 043°29.567'E, 20.07.2009, B.Doğan 2122 & A.Duran (KNYA)
K. cerinthifolia	Turkey, Kahramanmaraş, Ahir mountain, 990 m, 37°36.470'N, 036°52.917'E, 16. 07.2009, B.Doğan 2107 & A.Duran (KNYA)
K. grandifolia	Turkey, Antalya, Akseki, Süleymanlı village, 1425 m, 37°17.980'N, 031°46.520'E, 31.07.2009, B.Doğan 2130 & A.Duran (KNYA).
K. haussknechtii	Turkey, Muş, Malazgirt, Karıncalı village, 1840 m, 39°21.219'N, 042°20.010'E, 18.07.2009, B.Doğan 2113 (KNYA).
K. radiata subsp. *biebersteiniana*	Turkey, Kars, Kağızman, Akçay to Cumaçay, 1830 m, 20.07.2009, B.Doğan 2124 & A.Duran (KNYA).
K. radiata subsp. *radiata*	Turkey, Kars, Arpaçay, Kardeşköy to Dağköy, 2190 m, 06.08.2010, 40°55.087'N, 043°11.209'E, B.Doğan 2283 & A.Duran
K. hakkiarica	Turkey, Hakkari, Cilo mountain, Kırıkdağ, near dez stream, 2210 m, 37 °32.974'N, 043°57.615'E, 07.08.2009, B.Doğan 2132 & A.Duran (KNYA), Endemic.
K. kotschyi	Turkey, Bitlis, Tatvan, Sapur village, 1965 m, 38°26.154'N, 042°24.413'E, 06.08.2009, B.Doğan 2131 & A.Duran (KNYA).
K. quinquefolia	Turkey, Artvin, Ardanuç, Boyalı village, 1210 m, 41°06.967'N, 042°07.283'E, 11.08.2009, B.Doğan 2139 & A.Duran (KNYA).
K. erucifolia	Turkey, Erzurum, Köprüköy, Eğirmez village, 1635 m, 39°57.056'N, 041°51.530'E, 09.08.2009, B.Doğan 2137 & A.Duran (KNYA).
K. oligocephala	Turkey, Kahramanmaraş, Ahir mountain, 995 m, 37°36.475'N, 036°52.947'E, 16. 07.2009, B.Doğan 2108 & A.Duran (KNYA).
Serratula tinctoria	Turkey, Bolu, Gerede to Bolu, 28. km, 1105 m, 09.08.2010, 40°45.340'N, 031°54.888'E, B.Doğan 2290 & A.Duran (KNYA).
Jurinea cataonica	Turkey, Erzincan, Old Çayırlı road, 10. km, 1750 m, 39047.954'N, 039030.343'E, 07.08.2005, B.Doğan 1029 (KNYA). Endemic.
Centaurea ptosimopappoides	Turkey, Adana,Aladağ to Kızıldag, 890 m, 19. 06. 2010, A.Duran 9042 & M.Öztürk (KNYA).
C. straminicephala	Turkey, Erzurum,Uzundere to Artvin, 1100 m, 26.07.2002, A.Duran 6048 & M.Sağıroğlu (KNYA).

(Antalya), *K. radiata* subsp. *radiata* (Kars), *K. hakkiarica* (Hakkari), *K. haussknechtii* (Muş), *K. coriaceae* (Kars), *K. radiata* subsp. *biebersteiniana* (Kars), *K. kurdica* (Osmaniye), *K. bornmuelleri* (Malatya), *Serratula tinctoria* (Bolu), *Centaurea ptosimopappoides* (Adana), *C. straminicephala* (Erzurum), and *Jurinea cataonica*. For details see Table 1.

DNA extraction

Nuclear DNA of silica gel dried leaf samples were extracted according to the instructions of the Nucleon phytopure plant DNA extraction kit (RPN 8510, Amersham Life Science, England). For each sample, DNA was extracted from 100 mg of leaf. After concentrations were determined using an Eppendorf BioPhotometer, DNA samples were diluted to the working concentration of 25 ng/µL. To better quantify the DNA and to assess the quality of the DNA, samples were run on an agarose gel (0.9%), stained with ethidium bromide, against a DNA standard with known concentrations. Stock DNA was kept at -86 °C.

ISSR Amplifications

Of the 20 primers investigated during our initial screening, the primers that gave the most informative patterns (in terms of repeatability, scorability, and the ability to distinguish between varieties) were selected for fingerprinting. The characteristics of the primers used are given in Table 2.

Each reaction contained 2.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.8), 50 mM KCl; 0.8% Nonidet P40, 200 mM of each of dNTP, 0.5 mM primer, 25 ng DNA template and 0.4 units of Taq DNA Polymerase (Bioron, Germany) in a final reaction volume of 25 µl. After a pre-denaturation step of 3 minutes at 94 °C, amplification reactions were cycled 40 times at 94 °C for 1 minute, at annealing temperature (Table 1) for 50 seconds and 72 °C for one minute followed by a final 10 minutes 72 °C extension in an Eppendorf Mastercycler gradient thermocycler. Upon completion of the reaction, aliquots of PCR products (15 µL) were mixed with 3 µL of loading buffer (50% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanol), loaded onto a 2.0% agarose/1x Tris-Borate EDTA gel and electrophoresed at 4 V/cm.

Amplifications were repeated at least twice at different time periods for each primer using the same reagents and procedures.

Table 2. List of the ISSR primers used in this study and their specifications.

Primer	Primer sequence	T_m (°C)	Size (bp)	GC (%)	Number of polymorphic bands
ISSR F1	GAG CAA CAA CAA CAA CAA	49.1	18	38.9	13
ISSR F2	CTC GTG TGT GTG TGT GTG T	56.7	19	52.6	11
ISSR F3	AGA GAG AGA GAG AGA GCG	56	18	55.6	14
ISSR F4	AGA GAG AGA GAG AGA GTG	53.7	18	50	12
ISSR F5	AGA GAG AGA GAG AGA G	49.2	16	50	10
ISSR F6	CCA CCA CCA CCA CCA	53.3	15	66.7	13
ISSR F7	ACA CAC ACA CAC ACA C	49.2	16	50	12

Data collection and cluster analysis

Amplified fragments were visualized under a UV transiluminator and photographed using a gel documentation system (Vilbert Lourmat, Infinity model). All of the amplified fragments were treated as dominant genetic markers. Each DNA band generated was visually scored as an independent character or locus (1 for presence and 0 for absence). Qualitative differences in band intensities were not considered. Every gel was scored in triplicate (independent scorings) and only the fragments consistently scored were considered for analysis. A rectangular binary data matrix was prepared and all the data analysis was performed using the Numerical Taxonomy System, NTSYS-pc version 2.1 (Applied Biostatistic, Exeter Software, Setauket, New York, USA).

In cluster analysis of the samples, the unweighted pair-group method with the arithmetic mean (UPGMA) procedure was followed (Rohlf 1992). The genetic distances were calculated with the SM coefficient. In order to determine the ability of ISSR data to display the inter-relationships among the samples, principle co-ordinate analysis (PCA) of pair-wise genetic distances (Nei 1972) was also conducted using the NTSYS-pc package.

Results and discussion

Silica gel dried plants collected from 19 different natural habitats were taken to the laboratory. The total number of species collected and used in the phylogenetic analysis was 19. DNA extractions were first attempted using a standard 2X CTAB method. Due to the poor DNA quality produced by the CTAB procedure, a commercial kit (Nucleon phytopure) was used in all isolations and repeated extractions were conducted whenever necessary.

From an initial screening of 20 ISSR primers, seven primers revealed high levels of polymorphisms. These primers generated 85 highly polymorphic fragments that were consistently amplified in repeated experiments conducted on separate dates. The GC percentages of the selected primers were within the range of 38.8–66.7%. The characteristics as well as the sequences of the primers revealing a polymorphism are shown in Table 2. The primer ISSR F3 amplified the highest number of polymorphic fragments (14 bands) and primer ISSR F5 yielded the lowest number of fragments (10 bands). In total, the average number of polymorphic fragments per primer used was roughly 12. A representative figure containing ISSR F3 and ISSR F5 banding patterns is given in Figure 1.

A total of 15 *Klasea*, 1 *Serratula*, 1 *Jurinea* and 2 *Centaurea* taxa were used in the scoring analysis. The *Jurinea* and *Centaurea* taxa, which were used as the out-group, formed a cluster that was distinct from the *Klasea* and *Serratula* cluster in the constructed dendogram. Furthermore the *Klasea* and the *Serratula* taxa form clearly separate clusters among themselves (Figure 2).

The *Klasea radiata* subsp. *radiata* and *Klasea radiata* subsp. *biebersteiniana* taxa were observed to be very closely positioned in the dendogram. The *K. kotschyi*, *K. hak-*

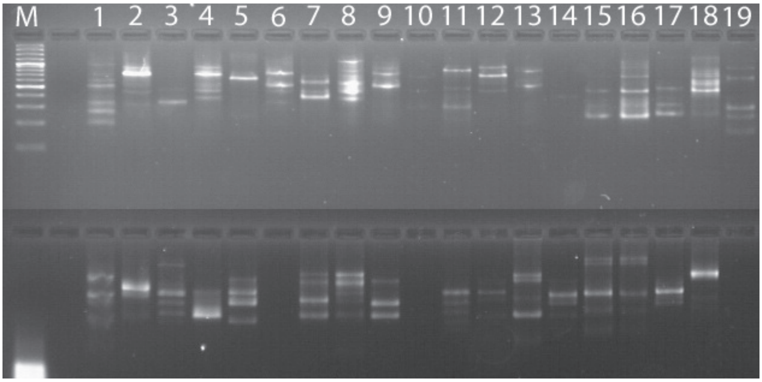

Figure 1. Representative agarose gels where PCR products were amplified with the primers ISSR F5 (highest number of polymorphic bands, top) and ISSR F5 (lowest level of polymorphic bands, down). **1** *Serratula tinctoria* **2** *Klasea quinquefolia* **3** *K. oligocephala* **4** *K. kotschyi* **5** *K. serratuloides* **6** *K. erucifolia* **7** *K. lasiocephala* **8** *K. cerinthifolia* **9** *K. grandifolia* **10** *K. radiata* subsp. *radiata* **11** *K. hakkiarica* **12** *K. haussknechtii* **13** *K. coriaceae* **14** *K. radiata* subsp. *radiata* **15** *K. kurdica* **16** *K. bornmuelleri* **17** *Centaurea ptosimopappoides* **18** *C. straminicephala* **19** *Jurinea cataonica*, M: marker.

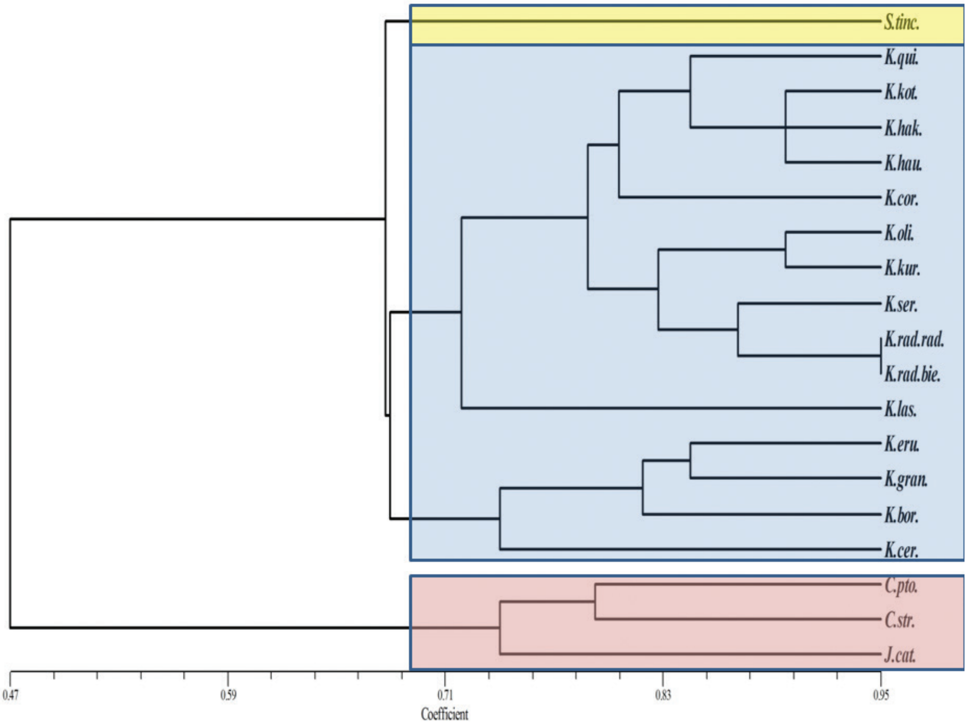

Figure 2. Dendrogram showing genetic relationship of *Klasea*, *Serratula*, *Centaurea* and *Jurinea* species as shown using inter simple sequence repeats. (*Serratula tinctoria*, *Klasea quinquefolia*, *K. oligocephala*, *K. kotschyi*, *K. serratuloides*, *K. erucifolia*, *K. lasiocephala*, *K. cerinthifolia*, *K. grandifolia*, *K. radiata* subsp. *radiata*, *K. hakkiarica*, *K. haussknechtii*, *K. coriaceae*, *K. radiata* subsp. *radiata*, *K. kurdica*, *K. bornmuelleri*, *Centaurea ptosimopappoides*, *C. straminicephala*, *Jurinea cataonica*)

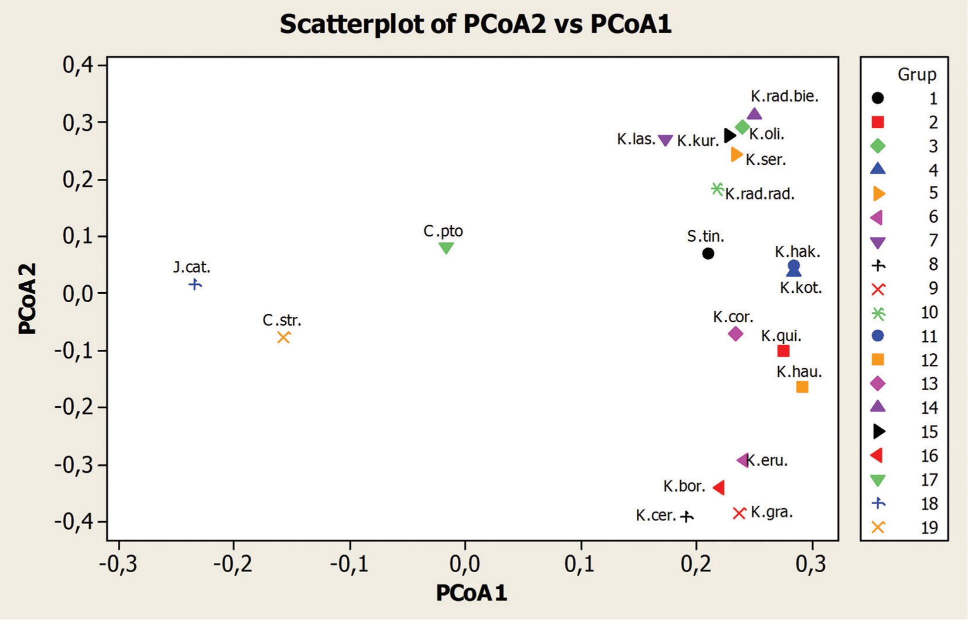

Figure 3. Principal co-ordinate analysis (PCA) of *Klasea*, *Serratula*, *Centaurea* and *Jurinea* species. **1** *Serratula tinctoria* **2** *Klasea quinquefolia* **3** *K. oligocephala* **4** *K. kotschyi* **5** *K. serratuloides* **6** *K. erucifolia* **7** *K. lasiocephala* **8** *K. cerinthifolia* **9** *K. grandifolia* **10** *K. radiata* subsp. *radiata* **11** *K. hakkiarica* **12** *K. haussknechtii* **13** *K. coriaceae* **14** *K. radiata* subsp. *radiata* **15** *K. kurdica* **16** *K. bornmuelleri* **17** *Centaurea ptosimopappoides* **18** *C. straminicephala* **19** *Jurinea cataonica*

kiarica and *K. haussknechtii* taxa, which have similar leaf characteristics, were also correlated in terms of their molecular data and were located in the same sub-cluster. *K. coriaceae* is a taxon, which spreads over distinct areas in the Eastern Anatolia and is distinguished owing to its distinctive height. The *Klasea oligocephala* and *K. kurdica* clustered together. The two taxa are very similar also in terms of morphological properties such as leaf, capitulum and pollen characteristics. *K. serratuloides* taxon has the largest capitulum among the genus. It has a similar profile to the *K. radiata* subspecies with respect to the leaf characteristics and the similarity of overspreading areas. *Klasea serratuloides* and *K. radiata* were also positioned close to one another on the dendogram.

K. lasiocephala is distinguished within the genus by its very short stems or the absent stems.. *K. lasiocephala* differs morphologically from other *Klasea* taxa in having absent or reduced stems and that it is also somewhat genetically distinct from other *Klasea* taxa, as the sole taxon in the cluster in which it is placed. The *K. erucifolia* and *K. grandifolia* taxa have similar leaf characteristics and were also located in the same sub-cluster owing to their molecular characteristics. *K. bornmuelleri* taxon does not have a morphologically close relative in the genus. Its position on the dendogram confirmed this classification. *K. cerinthifolia* is distinguished by its yellow flowers and semiamplexicaul leaf structure and was also molecularly identified to be distinct. All these findings were consistent with the morphological classifications made in the Flora of Turkey (Davis and Kupicha 1975; Dogan et al. 2012). Martins and Hellwig (2005) showed that *Klasea* and *Serratula* taxa to belong to separate clusters in a molecular study conducted using the ITS and ETS sequences. The same study reported shorter distances on the dendogram constructed based on molecular similarities for the taxa, which showed morphological similarities.

The inspection of the dendogram indicated that molecularly similar taxa were also morphologically similar. This separation was also shown in the PCA plot (Figure 3).

Our study has demonstrated that ISSR is a powerful tool in resolving the genetic relationships within problematic taxonomical entities. In conclusion, the morphologically close taxa were, in the molecular aspect, also located in the same clade. The genera used as out-groups (*Serratula*, *Jurinea*, and *Centaurea*) were clearly separate from the genus *Klasea*. According to our knowledge, this is the first report on the use of ISSR in *Klasea*.

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References

Ali MA, Al-Hemaid FM, Choudhary RK, Lee J, Kim SY, Rub MA (2013) Status of *Reseda pentagyna* Abdallah & A.G. Miller (Resedaceae) inferred from combined nuclear ribosomal and chloroplast sequence data. Bangladesh Journal Plant Taxonomy 20(2): 233–238. doi: 10.3329/bjpt.v20i2.17397

APG (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society 141: 399–436. doi: 10.1046/j.1095-8339.2003.t01-1-00158.x

Ash GJ, Raman R, Crump NS (2003) An investigation of genetic variation in *Carthamus lanatus* in New South Wales, Australia, using intersimple sequence repeats (ISSR) analysis. Weed Research 43: 208–213. doi: 10.1046/j.1365-3180.2003.00335.x

Barres L, Sanmartin I, Anderson CL, Susanna A, Buerki S, Galbany-Casals M, Vilatersana R (2013) Reconstructing the evolution and biogeographic history of tribe Cardueae (Compositae). American Journal of Botany 100(5): 867–882. doi: 10.3732/ajb.1200058

Bremer K, Backlund A, Sennblad B, Swenson U, Andreasen K, Hjertson M, Lundberg J, Backlund M, Bremer B (2001) A phylogenetic analysis of 100+ genera and 50+ families of euasterids based on morphological and molecular data with notes on possible higher level morphological synapomorphies. Plant Systematics and Evolution 229: 137–169. doi: 10.1007/s006060170009

Davis PH, Kupicha FK (1975) *Serratula* L. In: Davis PH (Ed.) Flora of Turkey and the East Aegean Islands, vol 5. Edinburgh Univ. Press, Edinburgh, 452–460.

Davis P, Tan K, Mill RR (Eds) (1988) Flora of Turkey and the East Aegean Islands, vol. 10. Edinburgh Univ. Press, Edinburgh.

Dogan B, Duran A, Hakki EE (2007) Phylogenetic analysis of *Jurinea* (Asteraceae) species from Turkey based on ISSR amplification. Annales Botanici Fennici 44: 353–358.

Dogan B, Duran B, Bagcı Y, Dinc M, Martin E, Cetin Ö, Oztürk M (2010) Phylogenetic relationships among the taxa of the genus *Johrenia* DC. (Apiaceae) from Turkey based on molecular method. Bangladesh Journal of Plant Taxonomy 17(2): 113–120. doi: 10.3329/bjpt.v17i2.6693

Dogan B, Duran A, Martin E, Coşkun F (2012) Türkiye *Serratula* L. cinsinin revizyonu. Proje no: TÜBİTAK-TBAG-109T243. [In Turkish]

Fang DQ, Roose ML, Krueger RR, Federici CT (1997) Fingerprinting of trifoliate orange germplasm accessions with isozymes, RFLPs, and inter-simple sequence repeat markers. Theoretical and Applied Genetics 95: 211–219. doi: 10.1007/s001220050550

Font M, Garnatje T, Garcia-Jacas N, Susanna A (2002) Delineation and phylogeny of Centaurea sect. Acrocentron based on DNA sequences: a restoration of the genus *Crocodylium* and indirect evidence of introgression. Plant Systematics and Evolution 243: 15–26. doi: 10.1007/s00606-002-0203-3

Garcia-Jacas N, Garnatje T, Susanna A, Vilatersana R (2002) Tribal and subtribal delimitation and phylogeny of the Cardueae (Asteraceae): A combined nuclear and chloroplast DNA analysis. Molecular Phylogenetics and Evolution 22(1): 51–64. doi: 10.1006/mpev.2001.1038

Garcia-Jacas N, Susanna A, Garnatje T, Vilatersana R (2001) Generic delimitation and phylogeny of the subtribe Centaureinae (Asteraceae): A combined nuclear and chloroplast DNA analysis. Annals of Botany 87: 503–515. doi: 10.1006/anbo.2000.1364

Garcia-Jacas N, Susanna A, Mozaffarian V, Ilarslan R (2000) The natural delimitation of *Centaurea* (Asteraceae : Cardueae): ITS sequence analysis of the *Centaurea jacea* group. Plant Systematics and Evolution 223: 185–199. doi: 10.1007/BF00985278

Greuter W (2003) The Euro+Med treatment of Cardueae (Compositae) generic concepts and required new names. Willdenowia 33: 49–61. doi: 10.3372/wi.33.33104

Haffner E, Hellwig FH (1999) Phylogeny of the Cardueae (Compositae) with emphasis on the subtribe Carduinae: an analysis based on ITS sequence data. Willdenowia 29: 27–39. doi: 10.3372/wi.29.2902

Hakki EE, Savaskan C, Akkaya MS (2001) Genotyping of Anatolian doubled-haploid durum lines with SSR markers. Euphytica 122: 257–262. doi: 10.1023/A:1012955516198

Hakki EE, Akkaya MS (2000) Microsatellite isolation using amplified fragment length polymorphism markers: no cloning, no screening. Molecular Ecology 9: 2149–2154. doi: 10.1046/j.1365-294X.2000.11143.x

Joel D, Portnoy V, Tzuri G, Greenberg R, Katzir N (1998) Molecular markers of the identification of *Orobanche* species. Advance in Parasitic Plant Research Proceedings Sixth International Symposium on Parasitic Weeds, Cordoba, 152–160.

Jump AS, Woodward FL, Burke T (2003) *Cirsium* species show disparity in patterns of genetic variations at their range-edge, despite similar patterns of reproduction and isolation. New Phytologist 160: 359–370. doi: 10.1046/j.1469-8137.2003.00874.x

Khalik KA, El-Twab MA, Gala R (2014) Genetic diversity and relationships among Egyptian *Galium* (Rubiaceae) and related species using ISSR and RAPD markers. Biologia 69(3): 300–310.

Laosatit K, Tanta P, Saensuk C, Srinives P (2013) Development and characterization of EST-SSR markers from *Jatropha curcas* EST database and their transferability across jatropha-related species/genus. Biologia 68(1): 41–47. doi: 10.2478/s11756-012-0143-5

Martins L (2006) Systematics and Biogeography of *Klasea* (Asteraceae-Cardueae) and a synopsis of the genus. Botanical Journal of the Linnean Society 152: 435–465. doi: 10.1111/j.1095-8339.2006.00583.x

Martins L, Hellwig FH (2005) Systematic position of the genere *Serratula* and *Klasea* within Centaureinae (Cardueae, Asteraceae) and new combinations in *Klasea*. Taxon 54: 632–638. doi: 10.2307/25065420

Mengitsu LW, Messersmith CG (2002) Genetic diversity of kochia. Weed Science 50: 498–503. doi: 10.1614/0043-1745(2002)050[0498:GDOK]2.0.CO;2

Mohammadzadeh F, Monirifar H, Saba J, Valizadeh M, Haghighi AR, Zanjani BM, Barghi M, Tarhriz V (2011) Genetic variation among Iranian Alfalfa (Medicago sativa L.) populations based on RAPD markers. Bangladesh Journal of Plant Taxonomy 18(2): 93–104. doi: 10.3329/bjpt.v18i2.9296

Nei M (1972) Genetic distance between populations. The American Naturalist 106: 283–292. doi: 10.1086/282771

Pharmawati M, Yan G, McFarlane IJ (2004) Application of RAPD and ISSR markers to analyse molecular relationships in *Grevillea* (Proteaceae). Australian Systematic Botany 17: 49–61. doi: 10.1071/SB03016

Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato accessions. Theoretical and Applied Genetics 98: 107–112. doi: 10.1007/s001220051046

Rohlf FJ (1992) NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.0. State Univ. of New York, Stony Brook, NY.

Soranzo N, Provan J, Powel W (1999) An example of microsatellite length variation in the mitochondrial genome of conifers. Genome 42: 158–161. doi: 10.1139/g98-111

Susanna A, Garcia-Jacas N, Hidalgo O, Vilatersana R, Garnatje T (2006) The Cardueae (Compositae) Revisited: Insights from ITS, Trnl-Trnf, and Matk Nuclear and Chloroplast DNA Analysis. Annals of the Missouri Botanical Garden 93: 150–171. doi: 10.3417/0026-6493(2006)93[150:TCCRIF]2.0.CO;2

Vosman B, Arens P (1997) Molecular characterization of GATA/GACA microsatellite repeat in tomato. Genome 40: 25–33. doi: 10.1139/g97-004

Williams JGK, Kubelik AR, Livak KJ (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531–6535. doi: 10.1093/nar/18.22.6531

Wolfe AD, Qiu-Yun X, Kepkart SR (1998) Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. Molecular Ecology 7: 1107–1125. doi: 10.1046/j.1365-294x.1998.00425.x

Yang W, De Oliveria AC, Godwin I, Schertz K, Bennetzen JL (1996) Comparison of DNA marker technologies in characterizing plant genome diversity: variability of Chinese sorghums. Crop Science 36: 1669–1676. doi: 10.2135/cropsci1996.0011183X003600060042x

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20: 176–183. doi: 10.1006/geno.1994.1151

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RESEARCH ARTICLE

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A new species of *Symplocos* (Symplocaceae) from southern Ecuador

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Abstract

A new species from Ecuador, *Symplocos limonensis,* is here described and illustrated. It resembles *S. clethrifolia* but differs by having larger leaves with evident (i.e., not concealed) areoles on lower surface, sessile inflorescences, smaller white corollas, and fewer stamens. The species is only known from three collections in the Andean forests of Morona-Santiago Province in southern Ecuador.

Resumen

Se describe e ilustra una nueva especie, *Symplocos limonensis*, de Ecuador. Se parece a *S. clethrifolia* pero se diferencia por tener las hojas más grandes, con las aréolas evidentes (no ocultas) en el envés, las inflorescencias sésiles y las flores más pequeñas, blancas y con menos estambres. La especie solo se conoce de tres ejemplares de los bosques andinos de la provincia de Morona-Santiago en el sur del país.

Keywords

Symplocos, Ecuador, Andes

Introduction

The genus *Symplocos* Jacq. is represented in Ecuador by ca. 33 species (Ståhl 2010a), the majority of which occur in Andean forests at 2500–3500 m elevation (Ståhl 1995). Thirteen of the species are country endemics (Barriga 2011) and have been described in the last 25 years, significantly increasing the knowledge of this still poorly known genus. In his treatment of the Symplocaceae for the Flora of Ecuador series, Ståhl (1991) described several new species of *Symplocos*, especially from southern Ecuador. One of these, *S. clethrifolia* Ståhl, was based on five collections, one of which (*Ulloa 487*) was indicated to deviate from the remaining four in floral and leaf features. This collection was provisionally treated under *S. clethrifolia* until more material could help elucidate its taxonomic status.

Materials and methods

Recent examination of the *Symplocos* collections in Ecuadorian herbaria revealed additional gatherings from the same general area that have led us to conclude that the Ulloa collection, along with some new ones, belong to a previously undescribed species, which is described here. Acronyms of the herbaria follow Thiers (2015).

Description of the new species

Symplocos limonensis Ståhl, C. Ulloa & Minga, sp. nov.

urn:lsid:ipni.org:names:77150152-1
Fig. 1

Diagnosis. *Symplocos limonensis* differs from *S. clethrifolia* Ståhl by having larger leaves (up to 13.5 vs. 9.5 cm) with evident (i.e., not concealed) venation areoles abaxially (vs. densely ferrugineous-velutinous with matted hairs concealing areoles), sessile inflorescences (vs. pedunculate), somewhat smaller, white (vs. red to pink) corollas, and fewer stamens (40–50 vs. 60–70).

Type: ECUADOR. Morona-Santiago: road Cuenca-Macas (road Gualaceo-Limón East of the pass), 2700–3400 m, 19 Aug 1987, *C. Ulloa 487* (Holotype: QCA-209874!; isotypes AAU!, GB!, MO-6500819!).

Tree or treelet to 12 m tall; branchlets reddish brown, sparsely villous to glabrescent, apical buds densely long-villous. Leaves alternate, petiolate; blade oblong to widely elliptic, 8–13.5 × 4–9 cm, the blades exceptionally almost circular and ca. 3 × 3 cm, coriaceous, light green and long-villous beneath, olive green and glabrous above, base rounded or truncate, slightly decurrent on petiole, apex rounded to widely obtuse, mucronulate at the tip, margins denticulate, teeth 3–4 mm apart, lateral veins 8–10 per side, midvein, lateral veins, and veinlets on lower side prominently raised and

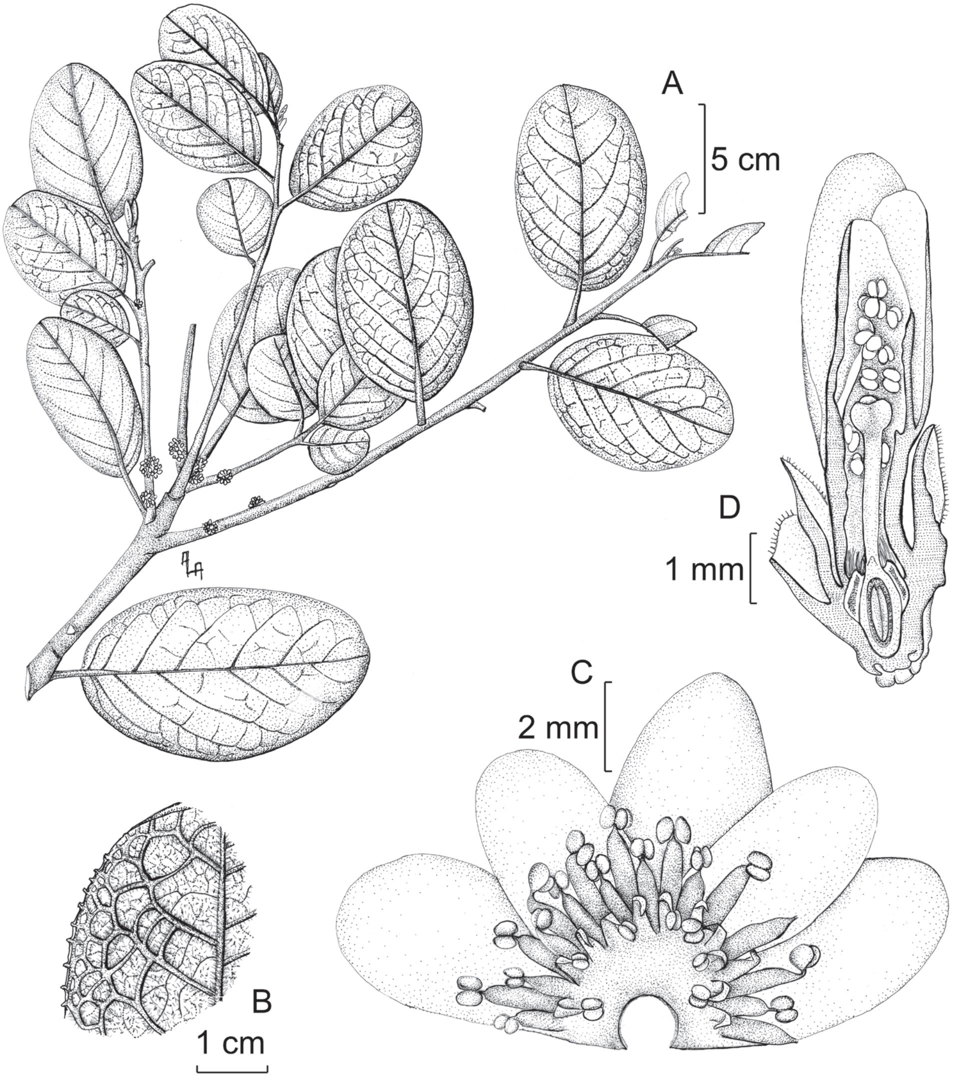

Figure 1. *Symplocos limonensis*. **A** Flowering branch **B** Detail of venation on leaf lower surface **C** Corolla and stamens whorls **D** Young flower in longitudinal section. (**A, B** from *Jørgensen 92876*; **C, D** from *Ulloa 487*). Illustration by A.L. Arbeláez.

verrucose, impressed above; petiole 1–1.5 cm long, rounded and long-villous beneath, flattened and glabrous above. Inflorescences fasciculate, sessile, borne in axils of extant leaves and along branchlets beneath the foliage; flowers 8–10 per inflorescence; bracts 4, very broadly ovate, 2–3 × 2–2.5 mm, strigulose on midvein toward apex, otherwise glabrous, margins ciliolate. Flowers with the calyx synsepalous, tube ca. 1.5 mm long, lobes 5, very broadly ovate, 1.5–2.5 × 2.2– 2.8 mm, margins ciliolate; corolla sympetalous, glabrous, white, 5–6 mm long, lobes 5 to 7, broadly oblong, 1.8–4 mm wide, margins entire; stamens 40–45 in 3(4) rows, filaments smooth, fused for ca. 2 mm to the corolla tube, those of the inner whorl fused most of their length leaving ca. 0.5 mm of the filaments free, stamens of the outer whorls with free filaments 1–2.8 mm long, more or less flattened and constricted at apex, anthers ca. 0.5 mm long; disc intrastaminal, dome-shaped, densely villous; style ca. 2.5 mm long, glabrous; stigma capitate and irregularly 3-lobed; ovary inferior, 3-locular with 3 or 4 ovules per locule. Mature fruits not seen.

Speciemens examined (Paratypes). ECUADOR. Morona-Santiago: road Gualaceo-Limón, km 33.3, 03°02'S, 78°35'W, 3010 m, 27 Dec 1990 (fl), *P.M. Jørgensen, C. Ulloa & B. Øllgaard 92876* (AAU!, QCA!, QCNE!); Collay, Maylas, on road to Limón 03°00.36'S, 78°38.00'W, 3150 m, 14 Jun 2000 (bud), *F. Serrano, D. Minga & A. Verdugo 1459* (HA!).

Etymology. The name of the species refers to the town of Limón, officially known as General Leonidas Plaza Gutiérrez. The road leading from the Andean town of Gualaceo to Limón, situated at 1400 m altitude on the east Andean slopes, has long been considered an important locality for botanical exploration where many new species have been found.

Distribution and conservation status. The species is known solely from three collections made in disturbed upper Andean forests and scrub páramo on the highest point of the Gualaceo-Limón-Macas road in Morona-Santiago Province of southern Ecuador. The area of occupancy (AOO) of the species is less than 20 km^2 and falls within the "Área de Conservación Municipal Tinajillas-Río Gualaceño," a locally managed reserve; however, the category of protection is of lesser status than that of a National Park. Since 2000, we have searched for additional plants but could not locate any, finding only one population of *Symplocos quitensis* Brand (1901: 76), a very different species with a wide Andean distribution. The road was poorly maintained and treacherous until recently, but has now been considerably widened in the process of being paved, and consequently the natural vegetation alongside is heavily destroyed. Ongoing wood extraction for charcoal production, expansion of areas under cultivation, and mining activities for clay, gravel, and metals are threats to the natural habitats in this region. Given current knowledge, we assign a provisional IUCN conservation status of Endangered (IUCN 2014) to this species.

Discussion. *Symplocos limonensis* resembles *S. clethrifolia* Ståhl and *S. golondrinae* Ståhl by having leaves with conspicuous venation, the veins of the lower side being prominent and verrucose and those on the upper side impressed. It differs from *S. clethrifolia,* which also is restricted to southern Ecuador (but from other localities), by having larger leaves (up to 13.5 vs. 9.5 cm) with evident (i.e., not concealed) venation areoles abaxially (vs. densely ferrugineous-velutinous with matted hairs concealing areoles), sessile inflorescences (vs. pedunculate), smaller (5–6 vs. c. 8 mm long), white (vs. red to pink) corollas, and fewer stamens (40–50 vs. 60–70). From *S. golondrinae* it differs by the larger size of leaves and flowers, with the leaves being coriaceous (vs. cartilaginous), long-villous abaxially (vs. sparsely strigose) and having longer (1–1.5 vs.

< 1 cm) petioles, and by having a densely villous flower disk (vs. glabrous). Moreover, *S. golondrinae* occurs in northwestern Ecuador, on the opposite side of the Andes.

Symplocos quitensis has been collected in the same area of the new species, but it is readily distinguished from both *S. limonensis* and *S. clethrifolia* by the densely hispid branchlets, smaller (up to 6.5 × 4 cm), membranaceous leaves, and pinkish flowers borne in short racemes.

In the most recent key to Andean *Symplocos* (Ståhl 2010a), *S. limonensis* keys to *S. clethrifolia* (albeit with the inflorescence pedunculate) or to *S. robusta* Ståhl. The latter species is known only from Bolivia and differs from *S. limonensis* in, e.g., its larger (to 14.5 × 8.5 cm), longer-petiolate (to 2 cm) leaves, more numerous flowers (up to 20) per inflorescence, and strigose corollas (vs. glabrous).

Symplocos limonensis has flowers with 5 to 7 petals, but being notoriously instable in many species of *Symplocos*, and often not studied, the number of petals may show to be of little taxonomic significance.

In the key to infrageneric taxa of the genus (Fritsch et al. 2008), *Symplocos limonensis* falls into the tropical American clade *Symplocos* subg. *Symplocos* sect. *Symplocos* having exerted, monodelphous stamens adnate to the corolla beyond its base, and the filaments flattened and constricted apically.

Acknowledgments

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References

Barriga P (2011) Symplocaceae. In: León-Yánez S, Valencia R, Pitman N, Endara L, Ulloa Ulloa C, Navarrete H (Eds) Libro Rojo de las plantas endémicas del Ecuador, 2da. Edición. Publicaciones del Herbario QCA, Pontificia Universidad Católica del Ecuador, Quito, 795–797.

Brand A (1901) IV. 242. Symplocaceae. In: Engler A von (Ed.) Das Pflanzenreich regni vegetablilis conspectus. Heft 6. W. Engelmann, Leipzig, 100 pp.

Fritsch P, Kelly LM, Wang Y, Almeda F, Kriebel R (2008) Revised infrafamilial classificaton of Symplocaceae based on phylogenetic data from DNA sequences and morphology. Taxon 57: 823–852.

Jacquin NJ von (1760) *Symplocos*. Enumeratio Systematica Plantarum. Theodorum Haak, Lugduni Batavorum [Leiden]. 5 pp.

IUCN (2014) Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. http://jr.iucnredlist.org/documents/RedListGuidelines.pdf [accessed: 27 Feb 2015]

Ståhl B (1991) Symplocaceae. In: Harling G, Andersson L (Eds) Flora of Ecuador 43. University of Göteborg, 44 pp.

Ståhl B (1994) The genus *Symplocos* (Symplocaceae) in Bolivia. Candollea 49: 369–388.

Ståhl B (1995) New or noteworthy Andean species of the genus *Symplocos* (Symplocaceae). Candollea 50: 445–452.

Ståhl B (2010a) Four new species and records of *Symplocos* (Symplocaceae) from Peru and Bolivia, and a key to all species of *Symplocos* known to occur in Ecuador, Peru and Bolivia. Nordic Journal of Botany 28: 79–87. doi: 10.1111/j.1756-1051.2009.00445.x

Ståhl B (2010b) Additions to the knowledge of the genus *Symplocos* (Symplocaceae) in Ecuador and Peru. Novon 20: 84–94. doi: 10.3417/2008079

Thiers B (2015) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. http://sweetgum.nybg.org/ih/ [accessed: 27 Feb 2015]

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DATA PAPER

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SIFlore, a dataset of geographical distribution of vascular plants covering five centuries of knowledge in France: Results of a collaborative project coordinated by the Federation of the National Botanical Conservatories

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Abstract

More than 20 years ago, the French Muséum National d'Histoire Naturelle[1] (MNHN, Secretariat of the Fauna and Flora) published the first part of an atlas of the flora of France at a 20km spatial resolution, accounting for 645 taxa (Dupont 1990). Since then, at the national level, there has not been any work on this scale relating to flora distribution, despite the obvious need for a better understanding. In 2011, in response to this need, the Federation des Conservatoires Botaniques Nationaux[2] (FCBN, http://www.fcbn.fr) launched an ambitious collaborative project involving eleven national botanical conservatories of France. The project aims to establish a formal procedure and standardized system for data hosting, aggregation and publication for four areas: flora, fungi, vegetation and habitats. In 2014, the first phase of the project led to the development of the national flora dataset: SIFlore. As it includes about 21 million records of flora occurrences, this is currently the most comprehensive dataset on the distribution of vascular plants (Tracheophyta) in the French territory. SIFlore contains information for about 15'454 plant taxa occurrences (indigenous and alien taxa) in metropolitan France and Reunion Island, from 1545 until 2014. The data records were originally collated from inventories, checklists, literature and herbarium records. SIFlore was developed by assembling flora datasets from the regional to the national level. At the regional level, source records are managed by the national botanical conservatories that are responsible for flora data collection and validation.

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[1] The National Museum of Natural History

[2] The Federation of The National Botanical Conservatories

In order to present our results, a geoportal was developed by the Fédération des conservatoires botaniques nationaux that allows the SIFlore dataset to be publically viewed. This portal is available at: http://siflore.fcbn.fr. As the FCBN belongs to the Information System for Nature and Landscapes' (SINP), a governmental program, the dataset is also accessible through the websites of the National Inventory of Natural Heritage (http://www.inpn.fr) and the Global Biodiversity Information Facility (http://www.gbif.fr). SIFlore is regularly updated with additional data records. It is also planned to expand the scope of the dataset to include information about taxon biology, phenology, ecology, chorology, frequency, conservation status and seed banks.

A map showing an estimation of the dataset completeness (based on Jackknife 1 estimator) is presented and included as a numerical appendix.

Purpose:
SIFlore aims to make the data of the flora of France available at the national level for conservation, policy management and scientific research. Such a dataset will provide enough information to allow for macroecological reviews of species distribution patterns and, coupled with climatic or topographic datasets, the identification of determinants of these patterns. This dataset can be considered as the primary indicator of the current state of knowledge of flora distribution across France. At a policy level, and in the context of global warming, this should promote the adoption of new measures aiming to improve and intensify flora conservation and surveys.

Keywords
Metropolitan France, La Reunion Island, Plantae, Tracheophyta, FCBN, CBN

Data published through

FCBN: http://www.siflore.fcbn.fr

Introduction

Taxonomic coverage

Note: The taxonomic and nomenclatural reference for the first edition of the SIFlore dataset is the fifth edition of the taxonomic repository for the fauna, flora and fungi of metropolitan France and overseas territories, TAXREF, which was developed within the framework of a convention between the French ministry of ecology, the MNHN, the FCBN and Tela Botanica. The overall methodological framework at the basis of the TAXREF repository is presented in Gargominy et al. (2014).

The version originally used for data aggregation is TAXREF v5.0, posted online on July 20th, 2012.

At the time of writing, the current version of TAXREF was v8.0, posted online on December 1st, 2014. Data is available in the taxonomic and nomenclatural reference TAXREF v5.0 on the http://siflore.fcbn.fr web atlas. For practical reasons, data has

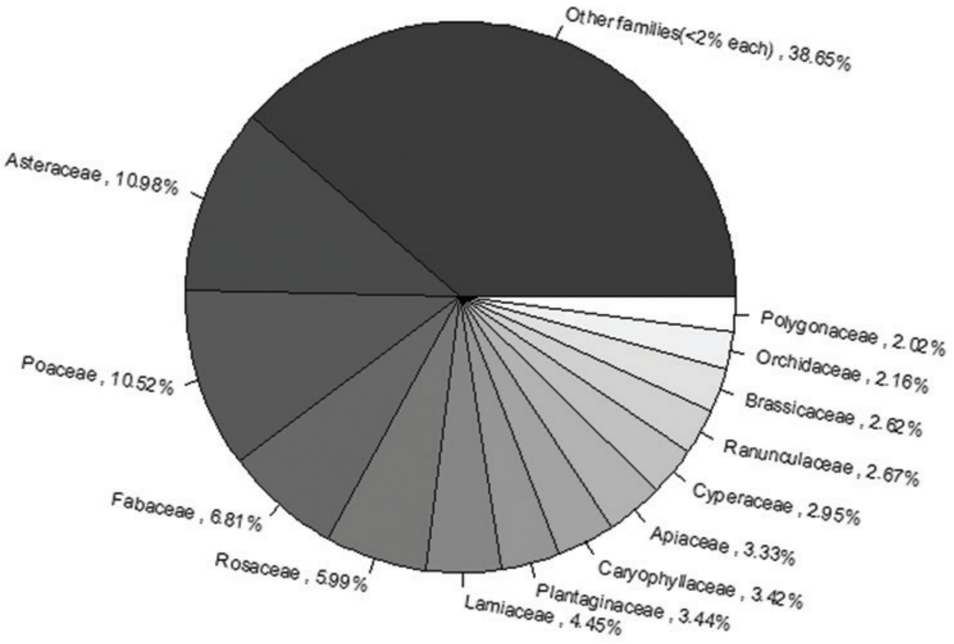

Figure 1. Distribution of the dataset by family.

been automatically linked to TAXREF v8.0 on the GBIF website, which may generate some taxonomic errors. This work was carried out under the responsibility of the Service du patrimoine naturel (SPN/MNHN). In order to prevent any error of interpretation, amended taxa were tagged in the Darwin core archive within the field datageneralization as follows: "taxon initially attached to the Taxonomic repository TAXREF v5. This taxon has undergone changes since".

It has to be noted that the taxonomic coverage of La Reunion Island was incomplete in this 1st version of SIFlore dataset as TAXREF v5.0 did not include most of La Reunion Island taxa. This issue was adressed in TAXREF v7, and subsequent versions, with the integration of the " Index des Trachéophytes de La Réunion (ITR, Boullet & al, 2012) ". Consequently, SIFlore completeness will be improved in the near future by including more taxa originating from La Réunion.

General taxonomic coverage description: The taxonomic coverage of this dataset spans the phylum Tracheophyta (vascular plants) present in metropolitan France (excluding the departments of Alsace and Lorraine) and La Reunion Island. Taxa are first identified at the species level and, if appropriate, subspecies level. The largest number of data records belong to the Asteraceae family (2,317,247 records), followed by Poaceae (2,220,479 records), Fabaceae (1,436,758 records) and Rosaceae (1,264,321 records). The families with the least number of records are Escalloniaceae, Malpighiaceae and Schizaeaceae with one data record each (Figure 1).

Taxonomic ranks

Kingdom: Plantae
Phylum: Tracheophyta
Class: Equisetopsida
Order: Acorales, Alismatales, Apiales, Aquifoliales, Araucariales, Arecales, Asparagales, Asterales, Boraginales, Brassicales, Buxales, Caryophyllales, Celastrales, Ceratophyllales, Commelinales, Cornales, Crossosomatales, Cucurbitales, Cupressales, Dioscoreales, Dipsacales, Ephedrales, Equisetales, Ericales, Escalloniales, Fabales, Fagales, Garryales, Gentianales, Geraniales, Ginkgoales, Gunnerales, Hymenophyllales, Isoetales, Lamiales, Laurales, Liliales, Lycopodiales, Magnoliales, Malpighiales, Malvales, Myrtales, Nymphaeales, Ophioglossales, Osmundales, Oxalidales, Pandanales, Pinales, Piperales, Poales, Polypodiales, Proteales, Psilotales, Ranunculales, Rosales, Salviniales, Santalales, Sapindales, Saxifragales, Schizaeales, Selaginellales, Solanales, Vitales, Zingiberales, Zygophyllales
Family: Acanthaceae, Acoraceae, Actinidiaceae, Adoxaceae, Aizoaceae, Alismataceae, Altingiaceae, Amaranthaceae, Amaryllidaceae, Anacardiaceae, Annonaceae, Apiaceae, Apocynaceae, Aponogetonaceae, Aquifoliaceae, Araceae, Araliaceae, Araucariaceae, Arecaceae, Aristolochiaceae, Asparagaceae, Aspleniaceae, Asteraceae, Balsaminaceae, Basellaceae, Begoniaceae, Berberidaceae, Betulaceae, Bignoniaceae, Blechnaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Butomaceae, Buxaceae, Cabombaceae, Cactaceae, Campanulaceae, Cannabaceae, Cannaceae, Capparaceae, Caprifoliaceae, Caricaceae, Caryophyllaceae, Casuarinaceae, Celastraceae, Ceratophyllaceae, Cistaceae, Cleomaceae, Clusiaceae, Colchicaceae, Combretaceae, Commelinaceae, Convolvulaceae, Coriariaceae, Cornaceae, Crassulaceae, Cucurbitaceae, Cunoniaceae, Cupressaceae, Cymodoceaceae, Cyperaceae, Cytinaceae, Dennstaedtiaceae, Dioscoreaceae, Droseraceae, Dryopteridaceae, Ebenaceae, Elaeagnaceae, Elatinaceae, Ephedraceae, Equisetaceae, Ericaceae, Escalloniaceae, Euphorbiaceae, Fabaceae, Fagaceae, Frankeniaceae, Garryaceae, Gentianaceae, Geraniaceae, Gesneriaceae, Ginkgoaceae, Goodeniaceae, Grossulariaceae, Gunneraceae, Haloragaceae, Heliconiaceae, Hydrangeaceae, Hydrocharitaceae, Hymenophyllaceae, Hypericaceae, Hypoxidaceae, Iridaceae, Isoetaceae, Juglandaceae, Juncaceae, Juncaginaceae, Lamiaceae, Lardizabalaceae, Lauraceae, Lecythidaceae, Lentibulariaceae, Liliaceae, Linaceae, Linderniaceae, Lycopodiaceae, Lythraceae, Magnoliaceae, Malpighiaceae, Malvaceae, Marantaceae, Marsileaceae, Martyniaceae, Melanthiaceae, Melastomataceae, Meliaceae, Menispermaceae, Menyanthaceae, Molluginaceae, Moraceae, Moringaceae, Musaceae, Myricaceae, Myrtaceae, Nartheciaceae, Nelumbonaceae, Nephrolepidaceae, Nitrariaceae, Nyctaginaceae, Nymphaeaceae, Oleaceae, Onagraceae, Ophioglossaceae, Orchidaceae, Orobanchaceae, Osmundaceae, Oxalidaceae, Paeoniaceae, Pandanaceae, Papaveraceae, Passifloraceae, Paulowniaceae, Phrymaceae, Phyllanthaceae, Phytolaccaceae, Pinaceae, Piperaceae, Pittosporaceae, Plantaginaceae, Platanaceae, Plumbaginaceae, Poaceae, Polemoniaceae, Polygalaceae, Polygonaceae, Polypodiaceae, Pontederiaceae, Portulacaceae, Posidoniaceae, Pota-

mogetonaceae, Primulaceae, Proteaceae, Psilotaceae, Pteridaceae, Ranunculaceae, Resedaceae, Rhamnaceae, Rosaceae, Rubiaceae, Ruppiaceae, Rutaceae, Salicaceae, Salviniaceae, Santalaceae, Sapindaceae, Sapotaceae, Sarraceniaceae, Saxifragaceae, Scheuchzeriaceae, Schizaeaceae, Scrophulariaceae, Selaginellaceae, Simaroubaceae, Smilacaceae, Solanaceae, Staphyleaceae, Strelitziaceae, Styracaceae, Tamaricaceae, Taxaceae, Theaceae, Thelypteridaceae, Thymelaeaceae, Tofieldiaceae, Tropaeolaceae, Typhaceae, Ulmaceae, Urticaceae, Verbenaceae, Violaceae, Vitaceae, Woodsiaceae, Xanthorrhoeaceae, Zingiberaceae, Zosteraceae, Zygophyllaceae

Spatial coverage

General geographic description

This national dataset collates species occurrences from metropolitan France and Reunion Island. Four different climates are covered in the metropolitan area: oceanic, continental, mediterranean and alpine. Reunion Island is an overseas department and region of France, located in the southwest Indian Ocean, in the Mascareignes archipelago, to the east of Madagascar. The island has a tropical climate.

The records documented in the dataset are distributed across 21 (out of a total of 27) administrative regions of France: Aquitaine, Auvergne, Bourgogne, Bretagne, Centre, Champagne-Ardenne, Corse, Franche-Comté, Ile-de-France, Languedoc-Roussillon, Limousin, Midi-Pyrénées, Nord-Pas-de-Calais, Basse-Normandie, Haute-Normandie, Pays de la Loire, Picardie, Poitou-Charentes, Provence-Alpes-Côte d'Azur, La Réunion, Rhône-Alpes.

This geographical zone is characterized by a large range in altitude, from 0 to 4,810 m above sea level, and extends over an area of 516,499 km^2 representing 74% of Metropolitan France, its overseas departments and other territories.

Geographical method

Reference grids

In France, the Muséum National d'Histoire Naturelle (MNHN) recommends the use of standardized grids for species distribution maps. The grid reference of MNHN is defined according to the French official map projection systems: Lambert 93 in Metropolitan France (grid name "L93_10X10") and UTM 40 S in La Reunion Island (grid name "grille_10km_ZEE_974").

Lambert-93 (EPSG 2154) is a Lambert conic projection using RGF93 geodetic system (compatible with WGS84) and defined by two reference parallels: 44°N and 49°N. The central meridian, 3°E, is the Greenwich meridian and the latitude of origin is 46°30'N. Prime coordinates are 700,000 meters and 6,600,000 meters.

The Universal Transverse Mercator Projection 40 South (UTM 40S) is an adaptation of the standard Mercator projection. This is a cylindrical and conformal projection using RGR92 geodetic system (compatible with WGS84). The central meridian is 57°E and the latitude of origin is 0. Prime coordinates are 500,000 meters and 10,000,000 meters.

Grids are composed of cells of 10 km by 10 km. Continental and maritime metropolitan France (954, 500 sq.km) is divided into 9546 cells and La Reunion Island terrestrial area (2, 512 sq.km) is divided into 34 cells (information on maritime area is not given as it is disproportionate in relation to terrestrial one).

All SIFlore records are georeferenced through the code of the corresponding grid square.

French municipalities repository: The official geographic boundaries of the municipalities and associated data (BD CARTO®) were provided by the National Geographic Institute (IGN) based on the National Institute of Statistics and Economic Studies (Insee) database. Each municipality is referenced by an official geographic code (code Insee) and its name. The records in SIFlore are georeferenced through the code of the corresponding municipality.

The BD CARTO® version originally used for data aggregation was published in 2011.

The current version is BD CARTO® 3.1 which was revised in 2013.

Coordinates

Metropolitan France : 40°0'0"N – 52°0'0"N Latitude; 07°0'0"W – 12°0'0"W Longitude.
La Reunion Island : 20°52'0"S – 21°24'0"S Latitude; 55°10'0"E – 55°50'0"E Longitude.

Temporal coverage

The oldest record in the dataset is from the year 1545 and the most recent records are from 2014. Most records (69.6%) were obtained after 2000 (Figure 2).

Records for which the date of collection is unknown are registered with the year 1500.

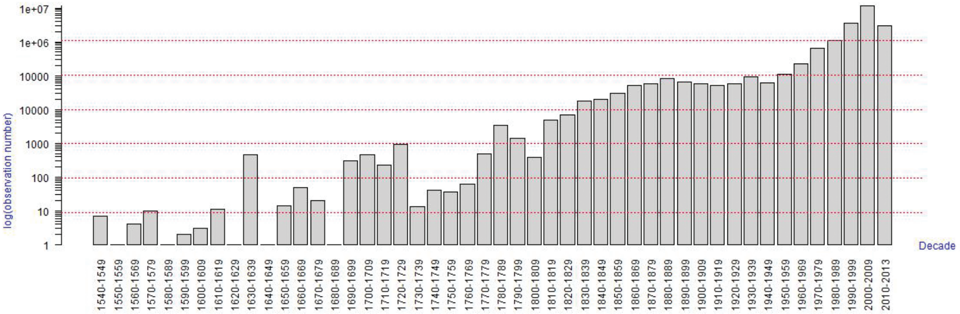

Figure 2. Temporal distribution of records by decade (shown on logarithmic scale).

Project description

Title: «*A dataset on vascular plant distribution covering five centuries of knowledge in France:Results of a collaborative project coordinated by the Federation of the National Botanical Conservatories*» is the outcome of a collaboration between 11 Conservatoires botaniques nationaux (CBN) and their Federation (FCBN): (a) Conservatoire Botanique National Alpin , Domaine de Charance 05000 Gap, France (b) Conservatoire Botanique National de Bailleul , Hameau des Haendries 59270 Bailleul, France (c) Conservatoire Botanique National du Bassin Parisien, Muséum national d'Histoire naturelle, 61, rue Buffon 75005 Paris, France (d) Conservatoire National Botanique de Brest , 52, allée du Bot 29200 Brest, France (e) Conservatoire National Botanique de Corse, Office de l'environnement de la Corse, 14, avenue Jean Nicoli 20250 Corte, France (f) Conservatoire Botanique National de Franche-Comté, Maison de l'environnement de Franche-Comté, 7, rue Voirin

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Les colimaçons 97436 Saint-Leu, Ile-de-La-Réunion, France (h) Conservatoire Botanique National du Massif Central, Le Bourg 43230 Chavaniac-Lafayette, France (i) Conservatoire Botanique National méditerranéen de Porquerolles, 34, avenue Gambetta 83400 Hyères, France (j) Conservatoire Botanique National des Pyrénées et de Midi Pyrénées, Vallon de Salut - BP 315- 65203 Bagnères-de-Bigorre Cedex, France (k) Conservatoire Botanique National Sud-Atlantique, Domaine de Certes Graveyron 33980 Audenge, France

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Methods

Data collection

Primary data are collected by both professional (from CBN and other organisations) and volunteer. As data originate from various sources (field inventories, scientific literature and herbaria), this task involves different trades such as botanists and archivists. Data are entered into CBN databases and then follow a validation process. To insure dataset homogeneity, data records are extracted from CBN databases and provided to the national system in a simplified format that is compatible with the SINP format (edited by MNHN). This format is also based on the Data Specification on Species distribution, produced by the INSPIRE Thematic Working Group *Species distribution* (http://inspire.ec.europa.eu/).

For each observation, key variables are recorded, such as a unique code for the record, valid scientific name of the taxon and its identifier in the French national taxonomic repository (TAXREF). Additional details including observation and data transmission dates, the collector name, the source basis of record name and geospatial information, including municipality name and code, and the square code of the national grid, are also provided. The grid system used for species inventories in France is defined by the Muséum National d'Histoire Naturelle: this is a grid composed of cells of 10km by 10km. Information about municipalities are collected from the Insee national repository. When available, additional information is collected such as bibliographic and herbarium references or primary source of the data record.

The evolution of the dataset is described in the Database history section.

Once standardized, data are checked for consistency before being incorporated into the Postgresl/Postgis national database by using Talend Open Studio for Data Integration. At the end of the process, data are posted on the FCBN geoportal.

Sampling description: Most records originate from field inventories (88.7%), with other records identified in scientific literature (11%) and in herbaria (0.2%). The protocols for collection vary over time and between collection sites, but also in response to other projects launched. However, in this first version, SIFlore does not include information on the data collection procedures. It is expected that a simplified description of the different protocols used by each CBN will be provided in the near future.

Nevertheless, according to Vallet et al. (2012), it is possible to assess survey completeness of regional floristic inventories despite heterogeneous sources and protocols, through the use of the Jackknife 1, a non-parametric estimator. This estimator

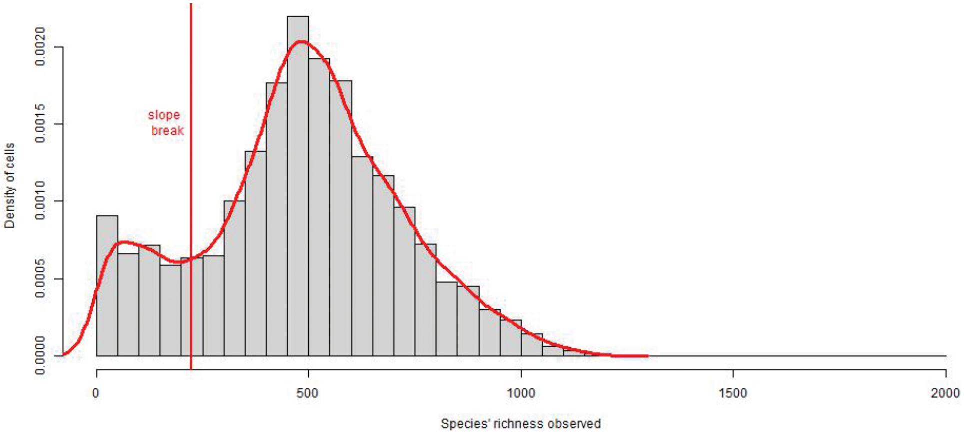


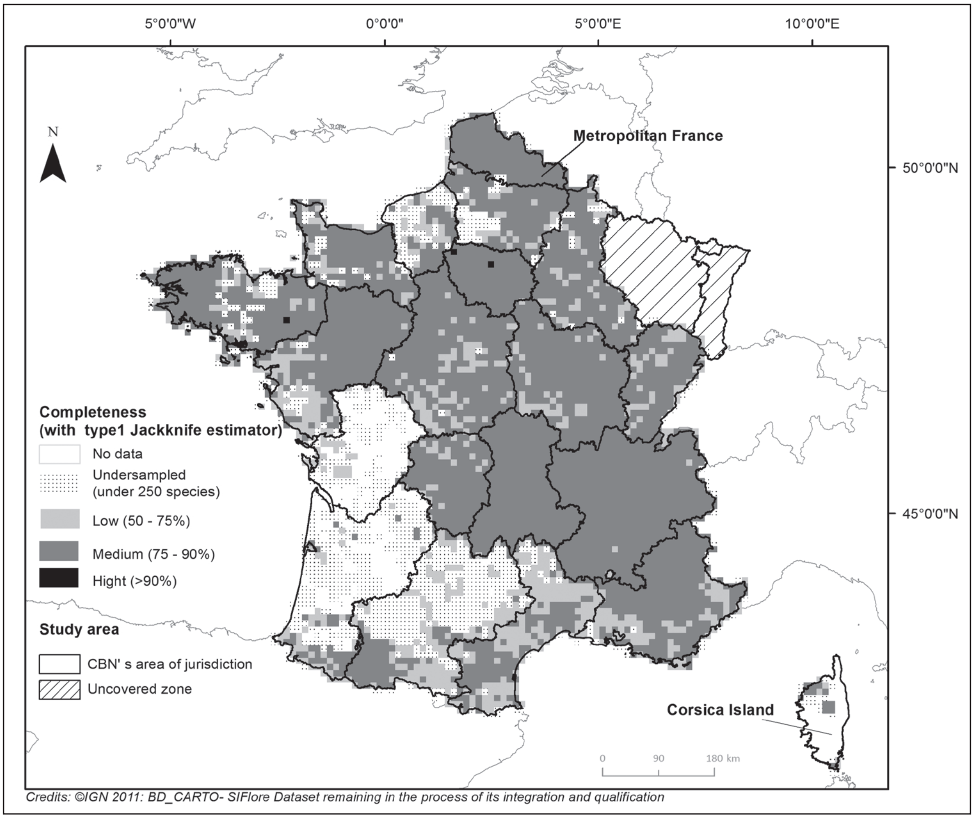
Figure 3. Density of cells by richness of observed species: looking at the distribution within the dataset, it appears that cells with less than 250 distinct species recorded are over-represented.

was calculated for each 10km by 10km cell of the French map, aiming to mitigate sampling bias as effectively as possible. Thus, occurrences were all generalized to the rank of species (including all infraspecies into this one rank) and only recent data were taken in account (from 1990). Cells with less than 250 species were excluded from the analyses, as they were considered as undersampled and therefore over represented (Figure 3). The resulting map (Figure 4) should allow users to interpret their results appropriately.

It has to be noted that the completeness of La Reunion Island survey could not be assured in this work. Indeed, as mentioned above, in 2012, at the time of working on the 1st version of SIFlore, there had been a lack of La Reunion taxa in the French national taxonomic referential for fauna, flora and fungi, TAXREF V5. As the analysis only takes into account the species which correspond across the two taxonomic guidelines (ITR and TAXREF v5), all of the La Reunion cells had been undersampled, according to Jackknife calculation requirements.

Quality control description: Quality control is implemented at different levels, under different responsibilities, throughout the data collection and validation process. Following digitalization, the dataset is first checked by regional data administrators, in order to ensure compliance with the survey protocols. The records are individually reviewed according to specified criteria, including the accuracy of the scientific name and the correctness of the geographical position entered, according to known chorology.

At the national level, a second step is carried out to ensure conformity of the data to the national standards, before compilation. Non-compliant data are rejected and an audit report is sent to the data provider. FCBN is currently working on an additional quality control step in order to ensure global coherence and to provide a relevance score for the distribution map of each species.



Credits: ©IGN 2011: BD_CARTO- SIFlore Dataset remaining in the process of its integration and qualification

Figure 4. Dataset completeness for Metropolitan France according to the Jackknife 1 estimator (data from 1990 to 2013). The number of records in each cell was used as an estimator of the sampling effort. The ratio between the observed and estimated richness of species measures the completeness of inventory in each surveyed cell (Vallet et al. 2012).

Dataset

Dataset history

In 1975, the Botanical Conservatory of Brest was created, with the support of the Ministry of Environment. This was the first establishment in the world entirely devoted to flora conservation. In 1988, the label "conservatoire botanique national" was legally recognized in France. There are currently eleven national botanical conservatories (CBN) in France.

As they are mandated to share their expertise to the national and local authorities, CBN have built knowledge databases in which information is structured to allow data sharing.

CBN have operational and managerial autonomy. Consequently, their databases differ in terms of structural design and the information contained. Nevertheless, there is an urgent need to provide information on flora distribution at the national level and, in particular, to define more clearly the flora conservation priorities. Furthermore, FCBN is involved in establishing the IUCN Red List of Threatened Flora at the national level, and in evaluating the conservation status of natural habitats and wild flora, according to the *Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora*. For these reasons, it was decided to pool all CBN flora records and also to apply a subsidiarity relationship between the FCBN and CBN. This means that data must only be handled by national botanical conservatories, and that the federation may only facilitate networking and ensure data aggregation and management, without altering the records in any way.

In 2010, a working group was created to implement this project. It includes botanists and data managers from across the CBN, and project facilitators. Due to the heterogeneity of the CBN databases, data cannot easily and quickly be aggregated unless determining a database exchange standard. As such, a standardized format was proposed and, in 2011, 10 taxa were selected for a first trial run. Based on this pilot, the data standard was refined. In January 2013, the flora regional dataset aggregation was officially launched. Data were transmitted through various channels under the scenarios prepared by the working group. Data were then compiled and integrated into a Postgresql/Postgis database using an extract, transform and load system (ETL). In May 2013, 18 million data records were aggregated. In January 2014, an additional 3 million records were integrated. Meanwhile, a geoportal was developed to respond to the needs of the CBN and their partners for improving their understanding of flora distribution, as well as allowing the CBN to share its expertise with the public. The portal was finally published on the FCBN's website in February 2014.

Dataset description

The SIFlore dataset is a custom-made SQL view of the global database hosted by the FCBN. Only tracheophyta data are shown. In the current version of the database, the key information provided for each record includes: unique identifier of the data record in SIFlore, identifier that points to the data in the original database file, source institution and database identifier, scientific name, valid identifier in the French national taxonomic referential (TAXREF), taxon rank, location of sighting (grid cell and municipality code), date that the flora was sighted and name of the data collector.

Dataset preview

Object name: SIFlore, a dataset of geographical distribution of vascular plants covering five centuries of knowledge in France: Results of a collaborative project coordinated by the Federation of the National Botanical Conservatories
Character encoding: UTF-8
Format name: Darwin Core Archive Format

Format version: 1.0
Distribution: http://www.gbif.org/dataset/75956ee6-1a2b-4fa3-b3e8-ccda64ce6c2d
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References

Antonetti P, Brugel E, Kessler F, Barbe J-P, Tort M (2006) Atlas de la Flore d'Auvergne. CBN du Massif Central, 984 pp. http://www.calameo.com/books/0005641497d3f9521789f

Armand M, Gourgues F, Marciau R, Villaret J-C, Portal R (2008) Atlas des plantes protégées de l'Isère. Editions biotope- publications scientifiques du Muséum - Collection Parthénope, 320 pp. http://www.leclub-biotope.com/fr/ouvrages-soldés/87-atlas-des-plantes-protegees-de-l-isere-9782914817318.html

Arnal G, Guittet J (2004) Atlas de la flore sauvage du département de l'Essonne Editions biotope- publications scientifiques du Muséum - Collection Parthénope, 608 pp. http://www.leclub-biotope.com/fr/ouvrages-soldés/17-atlas-de-la-flore-sauvage-du-département-de-l-essonne-9782914817042.html

Bardet O, Fédoroff E, Gaël C, Moret J (2008) Atlas de la flore sauvage de Bourgogne. Editions biotope- publications scientifiques du Muséum - Collection Parthénope, 752 pp.

Bédé B, Bédé N, Martegoute J-C (2011) Les Plantes de Dordogne et des départements limitrophes avec indications des propriétés apicoles. (Bacofin., p. 912). Société botanique du Périgord.

Boudin L, Cordier J, Moret J (2007) Atlas de la flore remarquable du Val de Loire entre le bec d'Allier et le bec de Vienne. Editions biotope- publications scientifiques du Muséum - Collection Parthénope.

Boullet V (2012) Index de la flore vasculaire de la Réunion (Trachéophytes): statuts, menaces et protections. http://flore.cbnm.org/

Braud S, Charrier M, Mourgaud G (1999) Les Ptéridophytes du Maine-et-Loire. Inventaire et cartographie. E.R.I.C.A, (Bull. de botanique armoricaine , n° 12), 62 pp.

Chas E (1994) Atlas de la flore des Hautes-Alpes (Éd). CBNA G., 816 pp.

Chas E, Le Driant F, Dentant, Cédric GLG, VanEs J, Gillot P, Rémy C, Gattus J-C, Salomez P, Quelin L (2007) Atlas des plantes rares ou protégées des Hautes-Alpes. Naturalia publications.

Collectif (2002) Plantes et végétation en Limousin. Atlas de la flore vasculaire. Espaces naturels du Limousin, 863 pp.

Collectif (2007) Atlas floristique Alpes-de-Haute-Provence. Conservatoire Botanique National Alpin, 418 pp.

Collectif (2011) Inventaire de la Flore vasculaire du Nord-Pas de Calais (Ptéridophytes et Spermatophytes): raretés, protections, menaces et statuts. Version n° 4b / décembre 2011. Conservatoire botanique de Bailleul et Collectif des botanistes du Nord-Pas de Calais, 85 pp.

Collectif (2014) Plantes sauvages de la Loire et du Rhône. Conservatoire botanique national du Massif Central, 760 pp.

Collectif Floraine (2013) Atlas de la Flore Lorraine. Editions vent d'Est, 1296 pp.

Cruon R, Aboucaya A, Astier J-M, Blais P-M, Lanfranchi H (2008) Le Var et sa flore, plantes rares ou protégées. Naturalia Publications, 544 pp.

David C, Gérard M, Hubert H, Jarri B, DeLabarre Y, Ravet M (2009) Atlas floristique des Pays de la Loire. La Flore de la Mayenne: flore vasculaire (p. 679). Editions Siloë.

Diard L (2005) Atlas floristique de Bretagne. La Flore d'Ille-et-Vilaine. Editions Siloë, Ed., 670 pp.

Dupont P (1990) Atlas partiel de la flore de France. Museum d'Histoire Naturelle, 442 pp.

Dupont P (2001) Atlas floristique de la Loire-Atlantique et de la Vendée (2 volumes). Tome 1: État et avenir d'un patrimoine - Tome 2: Cartes et commentaires. Editions Siloë, 734 pp.

Dupré R, Boudier P, Delahaye P, Joly M, Cordier J, Moret J (2009) Atlas de la Flore sauvage du département d'Eure-et-Loir. Editions biotope- publications scientifiques du Muséum- Collection Parthénope, 488 pp.

Filoche S, Perriat F, Moret J, Hendoux F (2010) Atlas de la flore sauvage de Seine-et-Marne. Illustria Librairie des Musées, 690 pp.

Gargominy O, Tercerie S, Daszkiewicz P, Régnier C, Ramage T, Dupont P, Poncet L (2012) TAXREF v5.0, référentiel taxonomique pour la France: mise en œuvre et diffusion. Rapport SPN 2012, 75 pp. Retrieved from http://inpn.mnhn.fr/downloads/taxref-docs/TAXREFv5.0.pdf

Garraud L, Leprince J-H, Lieutaghi P, Danton P (2003) Flore de la Drôme: Atlas écologique et floristique. Conservatoire botanique national Alpin de Gap-Charance, 930 pp.

Girerd B, Roux J-P (2011) Flore du Vaucluse. Editions biotope- publications scientifiques du Muséum- Collection Parthénope, 1024 pp.

Hunault G, Moret J (2009) Atlas de la flore sauvage du département de la Sarthe. Editions biotope- publications scientifiques du Muséum- Collection Parthénope, 640 pp.

Jordan D (2015) La flore rare ou menacée de Haute-Savoie. Naturalia Publications, 496 pp.

Noble V, Diadema K (2011) Flore des Alpes-Maritimes et de la Principauté de Monaco, originalité et diversité. Naturalia Publications, 500 pp.

Philippon D, Prelli R, Poux L (2006) Atlas floristique de Bretagne. La flore des Côtes-d'Armor. Editions Siloë, 566 pp.

Pierrat F, Filoche S, Moret J (2010) Atlas de la flore sauvage du département du Val de Marne. Editions biotope- publications scientifiques du Muséum- Collection Parthénope, 480 pp.

Pierrat F, Filoche S, Hendoux F (2015) Atlas de la flore patrimoniale du Val d'Oise. Editions biotope- publications scientifiques du Muséum- Collection Parthénope, 368 pp.

Provost M (1993) Atlas de répartition des plantes vasculaires de Basse-Normandie. Presses universitaires de Caen, 331 pp.

Pujol D,Cordier J, Moret J (2008) Atlas de la flore sauvage du Loiret. Editions biotope- publications scientifiques du Muséum- Collection Parthénope, 472 pp.

Quéré E, Gager L, Hardy F, Magnanon S, Ragot R (2008) Atlas floristique de Bretagne. La Flore du Finistère. Editions Siloë, 693 pp.

Rivière G (2007) L'atlas Floristique de Bretagne. La flore du Morbihan. Editions Siloë, 500 pp.

Vallet J, Rambaud M, Coquel L, Poncet L, Hendoux F (2012) Sampling effort and floristic atlases: Survey completeness of localities and description of knowledge gaps. Biologies 335(12): 753–763. doi: 10.1016/j.crvi.2012.11.005

Supplementary material 1

Numerical appendix

Authors: Anaïs Just, Johan Gourvil, Jérôme Millet, Vincent Boullet, Thomas Milon, Isabelle Mandon, Bruno Dutrève

Data type: dataset

Explanation note: A shapefile representing the dataset completeness (based on the Jackknife 1, a non-parametric estimator) on a grid of 10 km by 10 km cells. The number of records in each cell was used as an estimator of the sampling effort. The ratio between the observed and estimated richness of species measures the completeness of the inventory in each surveyed cell (Vallet et al. 2012).

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DATA PAPER

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Dataset of MIGRAME Project (Global Change, Altitudinal Range Shift and Colonization of Degraded Habitats in Mediterranean Mountains)

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Abstract

In this data paper, we describe the dataset of the Global Change, Altitudinal Range Shift and Colonization of Degraded Habitats in Mediterranean Mountains (MIGRAME) project, which aims to assess the capacity of altitudinal migration and colonization of marginal habitats by *Quercus pyrenaica* Willd. forests in Sierra Nevada (southern Spain) considering two global-change drivers: temperature increase and land-use changes. The dataset includes information of the forest structure (diameter size, tree height, and abundance) of the *Quercus pyrenaica* ecosystem in Sierra Nevada obtained from 199 transects sampled at the treeline ecotone, mature forest, and marginal habitats (abandoned cropland and pine plantations). A total of 3839 occurrence records were collected and 5751 measurements recorded. The dataset is included in the Sierra Nevada Global-Change Observatory (OBSNEV), a long-term research project designed to compile socio-ecological information on the major ecosystem types in order to identify the impacts of global change in this mountain range.

Keywords

Quercus pyrenaica forests, altitudinal migration, colonization of abandoned croplands, global change, Sierra Nevada (Spain), occurrence data, measurement data

Project details

Project title

Global Change, altitudinal range shift and colonization of degraded habitats in Mediterranean mountains (MIGRAME)

Personnel

Regino Jesús Zamora Rodríguez (Principal Investigator, University of Granada)

Funding

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Rationale

Currently, there is strong scientific evidence of the effects of global change on natural systems (Parmesan 2006, Rosenzweig et al. 2008, García et al. 2014, O'Connor et al. 2015). Some ecological processes are being altered due to the changing climate, such as species distribution (Thuiller et al. 2005, Lenoir et al. 2008), phenology (Parmesan and Yohe 2003, Gordo and Sanz 2010, Wolkovich et al. 2014), ecological interactions (Hughes 2000, Suttle et al. 2007); among others. Land-use changes and climate change are the most important drivers of biodiversity shifts (Sala et al. 2000).

One of the most obvious biotic responses from global warming are the latitudinal and altitudinal shifts of species and communities (Allen and Breshears 1998, Jump and Peñuelas 2005, Lenoir et al. 2008). Species tend to expand into new areas that are becoming favourable, and retract from those that turn hostile. In consideration of two main drivers of global change (climatic warming and land abandonment), an understanding of the dynamics of altitudinal migration and colonization of marginal habitats is critical in order to develop effective forest-management strategies.

The project Global Change, altitudinal range shift, and colonization of degraded habitats in Mediterranean mountains (MIGRAME) was designed to assess the capacity of altitudinal migration and colonization of marginal habitats by a Mediterranean forest ecosystem (Zamora et al. 2013, Benito et al. 2013). This assessment considers two global change drivers: temperature increase and land-use changes. In so doing, this project analyzes the pattern of altitudinal migration and colonization of marginal habitats by a vulnerable ecosystem in a Mediterranean mountain region, which represents the rear edge of their distribution: forests of *Quercus pyrenaica* Willd.

The Mediterranean region has shown broad climate shifts in the past (Luterbacher et al. 2006) and is potentially vulnerable to forthcoming climatic changes (Pacifici et al. 2015), being considered a key region in future climate-change projections (Giorgi 2006, Giorgi and Lionello 2008). Concomitantly, land-use changes are considered a major driver of vegetation change (McGill 2015). This is especially relevant in Mediterranean region, which has undergone intense antrophic activities for millennia (Padilla et al. 2010) shaping the current landscape (Valbuena-Carabaña et al. 2010).

In this context, Mediterranean ecosystems are considered natural laboratories in which to study global change, due to their high sensitivity to global-change drivers (Matesanz and Valladares 2014, Doblas-Miranda et al. 2015).

Study area descriptions/descriptor

The target ecosystem of the project encompasses the Pyrenean oak forests (*Quercus pyrenaica* Willd.) of Sierra Nevada.

Sierra Nevada is a high-mountain range located in southern Spain (37°N, 3°W) with altitudes of between 860 m and 3482 m a.s.l. The climate is Mediterranean, characterized by cold winters and hot summers, with pronounced summer drought (July-August). The Sierra Nevada mountain range hosts a high number of endemic plant species (c. 80) (Lorite et al. 2007) for a total of 2,100 species of vascular plants (25% and 20% of Spanish and European flora, respectively), and thus it is considered one of the most important biodiversity hotspots in the Mediterranean region (Blanca et al. 1998). This mountain area has 27 habitat types (listed in the European Union Habitat Directive) harbouring 31 animal species (20 birds, 5 mammals, 4 invertebrates, 2 amphibians and reptiles) and 20 plant species listed in the Annex I and II of EU Habitat and Bird Directives. Sierra Nevada has several types of legal protection:

Biosphere Reserve MAB Committee UNESCO; Special Protection Area and Site of Community Importance (Natura 2000 network); and National Park. There are 61 municipalities with more than 90,000 inhabitants. The main economic activities are agriculture, tourism, beekeeping, mining, and skiing (Bonet et al. 2010).

For a description of the Pyrenean oak forests in Sierra Nevada, see *Study extent description* section.

Design description

The specific aims of the MIGRAME project are:

- To analyse the relevance of altitudinal migration at the leading edge (high elevation) of the range distribution of Pyrenean oak formation.
- To analyse the importance of the recolonization process of marginal habitats (abandoned croplands and pine plantations) close to Pyrenean oak formation.

Derived from the two global-change drivers, we have considered two main hypothesis (Figure 1):

Altitudinal migration hypothesis

Several studies have pointed out a trend towards higher temperatures and lower precipitation for the Mediterranean area (Giorgi and Lionello 2008, García-Ruiz et al. 2011). Climate projections forecast an increase of +4.8 °C at the end of the 21st century (Benito et al. 2011) for Sierra Nevada. In this context, shifts in the altitudinal (and latitudinal) distribution of species and communities are expected (Thuiller et al. 2008, Gottfried et al. 2012).

We hypothesised that the range shift of *Q. pyrenaica* in Sierra Nevada is changing as a consequence of recent changes to temperature, and we would expect an upward expansion (Figure 1a).

Marginal habitat colonization hypothesis

In Mediterranean area, cropland abandonment has been widespread during the second half of the last century (Valbuena-Carabaña et al. 2010, Pías et al. 2014). Land-use change models predict an increase in this trend in the future (Rounsevell et al. 2006). In fact, land abandonment is considered one of the most powerful global-change drivers in developed countries (Escribano-Avila et al. 2012).

We hypothesised that the land-use changes in high mountain (e.g. abandonment of croplands, management of pine plantations) should facilitate the native forest regeneration, and a process of colonization of marginal habitat (abandoned cropland, pine plantations) will occur (Figure 1b).

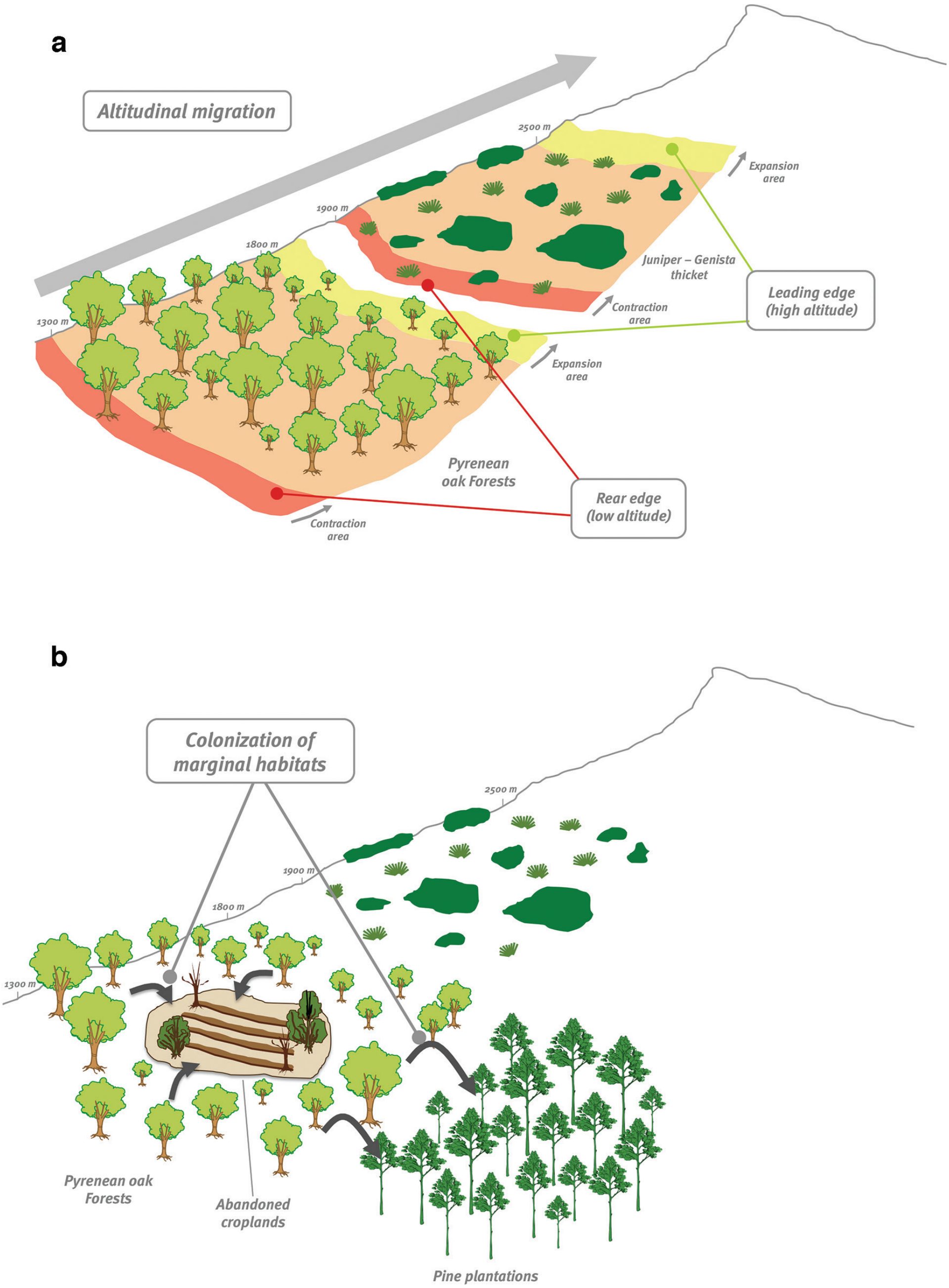


Figure 1. Schematic representation of the two main hypothesis of the project: altitudinal migration (**a**) and colonization of marginal areas (**b**) of *Q. pyrenaica* forests.

Overall, focusing on changes will occur in altitudinal migration and/or colonization of marginal habitats, we examine the following questions: Are altitudinal changes in Pyrenean oak forests associated with recent climate changes? Are they more consistent with changes in land use, or are they consistent with both global-change drivers?

Data published through GBIF

http://www.gbif.es/ipt/resource.do?r=migrame

Taxonomic coverage

This dataset includes records of the phylum Magnoliophyta (3823 records, 99.58%) and marginally Pinophyta (16 records, below 1% of total records). Most of the records included in this dataset belong to the class Magnoliopsida (99.58%). There are 5 orders represented in the dataset, with Fagales (98.98%) being the most important order. The other 4 orders (Rosales, Cupressales, Sapindales and Pinales) represent only 1.02% of the records. In this collection, 5 families are represented: Fagaceae, Rosaceae, Cupressaceae, Pinaceae, and Sapindaceae. The most represented taxa are *Quercus pyrenaica* Willd. and *Quercus ilex* L. (81.74 and 17.24%, respectively). Of the six taxa included on the dataset, three are considered threatened (Table 1).

Taxonomic ranks

Kingdom: Plantae
Phylum: Magnoliophyta, Pinophyta
Class: Magnoliopsida (Dicotyledones), Pinopsida

Table 1. Conservation status and threats of the species included in the dataset.

Scientific Name	Andalusian Red List[1]	IUCN[2]	Threat[3]
Acer opalus subsp. *granatense* (Boiss.) Font Quer & Rothm.	NT	VU	1,2,3
Quercus pyrenaica Willd.	NT	LR-cd	1,2,4,5,6
Sorbus aria Wimm.	NT	VU	1,2,3,7

[1] 2005 Red List of vascular flora of Andalusia (Cabezudo et al. 2005). [2] IUCN category in Sierra Nevada (Blanca et al. 1998, Blanca et al. 2001, IUCN 2001, Lorite et al. 2007). [3] Threats against the species (Herrera et al. 2000, Prados et al. 2000, Vivero et al. 2000, Marañón et al. 2004, Cabezudo et al. 2005, Gómez-Aparicio et al. 2005, Gómez-Aparicio et al. 2008). *1*: regeneration; *2*: fire; *3*: overgrazing; *4*: inappropriate forestry practices; *5*: changes in agriculture and agricultural practices; *6*: erosion; *7*: demography. *VU*: Vulnerable; *NT*: Near threatened; LR-nt: *LR-cd*: Lower Risk-Conservation Dependent.

Order: Fagales, Pinales, Cupressales, Sapindales, Rosales
Family: Fagaceae, Pinaceae, Cupressaceae, Sapindaceae, Rosaceae
Genus: *Quercus*, *Pinus*, *Juniperus*, *Acer*, *Sorbus*
Species: *Quercus pyrenaica*, *Pinus sylvestris*, *Juniperus communis*, *Acer opalus* subsp. *granatense*, *Sorbus aria*, *Quercus ilex*

Spatial coverage

General spatial coverage

Quercus pyrenaica forests
The Pyrenean oak (*Quercus pyrenaica* Willd.) forests extend through south-western France and the Iberian Peninsula (Franco 1990) (Figure 2a) reaching its southern limit in north of Morocco. In the Iberian Peninsula these forests live under meso-supramediterranean and mesotemperate areas and subhumid, humid and hyperhumid ombroclimate (Rivas-Martínez et al. 2002) living on siliceous soils, or soils poor in basic ions (Vilches de la Serna 2014). *Q. pyrenaica* requires between 650 and 1200 mm of annual precipitation and a summer minimal precipitation between 100 and 200 mm (Martínez-Parras and Molero-Mesa 1982, García and Jiménez 2009), summer rainfall being a key factor in the distribution of the species (Gavilán et al. 2007, Río et al. 2007).

The forests dominated by *Q. pyrenaica* constitute an ecosystem included in the Annex I of the Habitat Directive (habitat code 9230: *Quercus pyrenaica* oak woods and *Quercus robur* and *Quercus pyrenaica* oak woods from Iberian northwestern). The conservation status of this habitat is not well known (EIONET 2014), partly due to lack of detailed ecological studies (García and Jiménez 2009).

This species reaches its southernmost European limit at Sierra Nevada mountains, where eight oak patches (2400 Has) have been identified (Figure 2b), ranging between 1100 and 2000 m a.s.l. and generally associated to major river valleys. Sierra Nevada is considered a glacial refugia for deciduous *Quercus* species during glaciation (Brewer et al. 2002, Olalde et al. 2002, Rodríguez-Sánchez et al. 2010) and these populations are considered as a rear edge of the habitat distribution, which is important in determining habitat responses to expected climate change (Hampe and Petit 2005).

These forests, like other vegetation types, have undergone intense human pressure (wood cutting, grazing, etc.) which has reduced their distribution area and in some cases has altered their floristic pattern (Gavilán et al. 2000, Gavilán et al. 2007).

Q. pyrenaica is considered as vulnerable in southern Spain (Vivero et al. 2000). The populations of Pyrenean oak forests at Sierra Nevada are considered relict forests (Melendo and Valle 2000, Vivero et al. 2000), undergoing intensive anthropic use in the last few decades (Camacho-Olmedo et al. 2002, Valbuena-Carabaña et al. 2010). The relict presence of this species in Sierra Nevada is related both to its genetic resil-

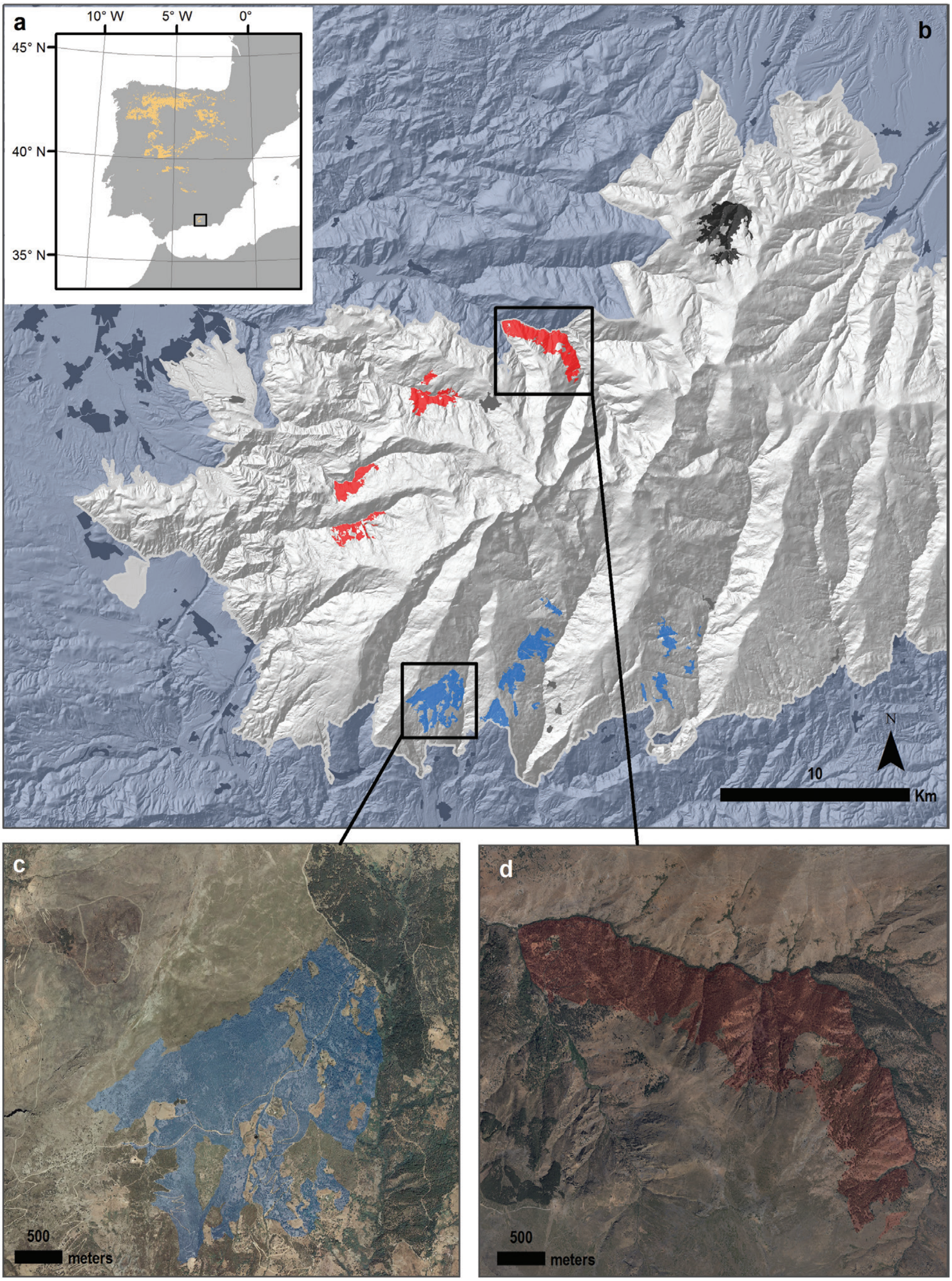


Figure 2. Distribution of *Quercus pyrenaica* forests in Iberian Peninsula (**a**). Sierra Nevada harbours eight populations of *Q. pyrenaica* clustered into three groups (different colours). We selected two study sites: Robledal de Cañar (**c**) and Robledal San Juan (**d**). Colour Orthophotography of 2009 from Regional Ministry of the Environment, Regional Government of Andalusia.

ience as well as to its high intraspecific genetic diversity (Valbuena-Carabaña and Gil 2013). However, they are also expected to suffer the impact of climate change, due to their climate requirements (wet summers). Thus, simulations of the climate change effects on this habitat forecast a reduction in suitable habitats for Sierra Nevada (Benito et al. 2011).

Coordinates

36°56'13.2"N and 37°8'9.6"N Latitude; 3°26'16.8"W and 3°20'16.8"W Longitude

Temporal coverage

2012–2014

Collection name

Dataset of MIGRAME Project (Global Change, Altitudinal Range Shift and Colonization of Degraded Habitats in Mediterranean Mountains)

Collection identifier

http://www.gbif.es/ipt/resource.do?r=migrame

Methods

Study extent description

The MIGRAME dataset covers the Pyrenean oak forests (see *Spatial coverage* section) in Sierra Nevada mountain range (see *Study area descriptions* section).

Sampling description

We sampled two localities of the Pyrenean oak forests in Sierra Nevada: Robledal de Cañar and Robledal de San Juan. We selected those two sites based on previous works (Pérez-Luque 2011, Pérez-Luque et al. 2013) that clustered the populations of *Q. pyrenaica* forests based on their plant species composition and environmental features. The Robledal de Cañar site (Figure 2c) (1366-1935 m a.s.l., 37°57'28.04"N, 3°25'57.1"W; Cáñar, Granada, SE Spain) was located in the Alpujarras Region on the southern slopes of Sierra Nevada. The Robledal de San Juan (Figure 2d) (1189-1899 m a.s.l., 37°7'29.63"N, 3°21'54.60"W; Güejar-Sierra, Granada, SE Spain) site was located in the northern slopes of Sierra Nevada.

The sampling design was determined by the hypothesis of the project (see Project Design description section).

Altitudinal migration design

To test our hypothesis of altitudinal migration, we sampled a total of 104 transects (Table 2) distributed along an altitudinal gradient at the two sites. We sampled two transects (at least 10 m apart) every 25 m of elevation from forest limit to treeline ecotone at both study sites. At each locality, we performed three replicates of this design (Figure 3a).

Habitat colonization design

To test the hypothesis of *colonization of marginal habitats*, we laid out transects in two types of marginal habitats: *abandoned agricultural areas* and *pine plantations* (Figure 3b). A total of 64 transects were located within the marginal habitat and on the edge between marginal habitat and Pyrenean oak forest. The number of transects inside the marginal habitat was determined by the size of the marginal habitat (Table 3).

Forest samplings

In addition to the above surveys, we conducted a survey inside *Q. pyrenaica* forests. A total of 31 transects were distributed at the two sites.

Table 2. Transect number of the *Altitudinal migration* design.

Locality	Altitudinal gradient	Transects[1]		
		R1	*R2*	*R3*
Robledal de Cañar	1900–2150	12	20	20
Robledal de San Juan	1775–2000	18	18	16

[1] For each replicate (R1 to R3) the number of transects is shown.

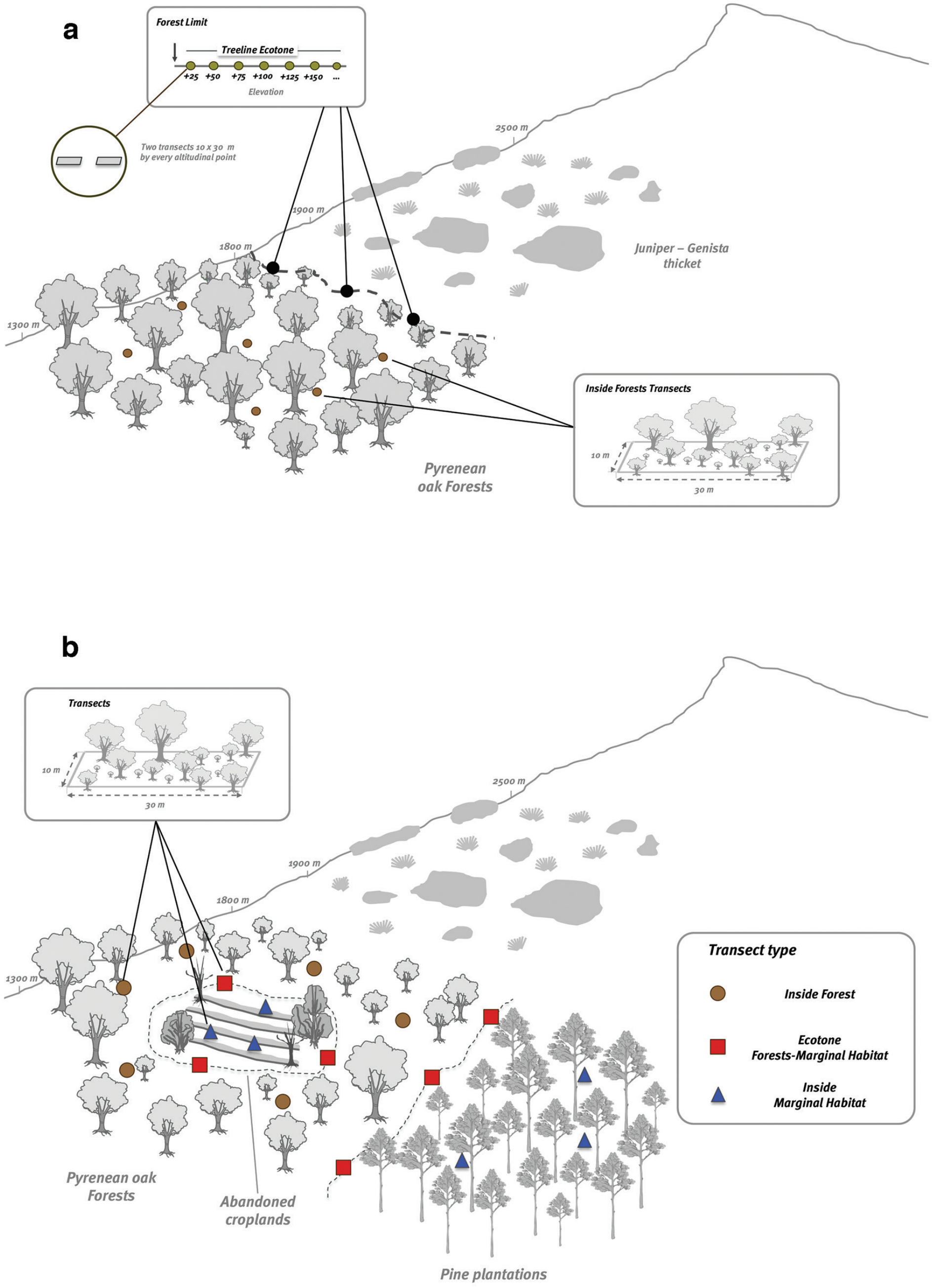


Figure 3. Sampling Design. **a** Altitudinal migration hypothesis. At each study site, from the forest edge to treeline ecotone, we sampled each 25 m of elevation **b** Colonization of marginal habitat hypothesis. Transects were located on three habitat types: Forests (brown circles), Forest Edges (red squares) and Inside Marginal Habitats (blue triangles).

Table 3. Transects number of the *Colonization of marginal habitat* design.

				Transects	
Locality	**Marginal habitat**	**Replicate**	**Surface (ha)**	**Inside**	**Edge**
Robledal de Cañar	Abandoned Cropland	R1	3.29	6	3
		R2	5.80	9	3
		R3	1.55	3	3
	Pine plantation		80.70	6	6
Robledal de San Juan	Abandoned Cropland	R1	3.46	6	3
		R2	10.36	13	3

Data collection

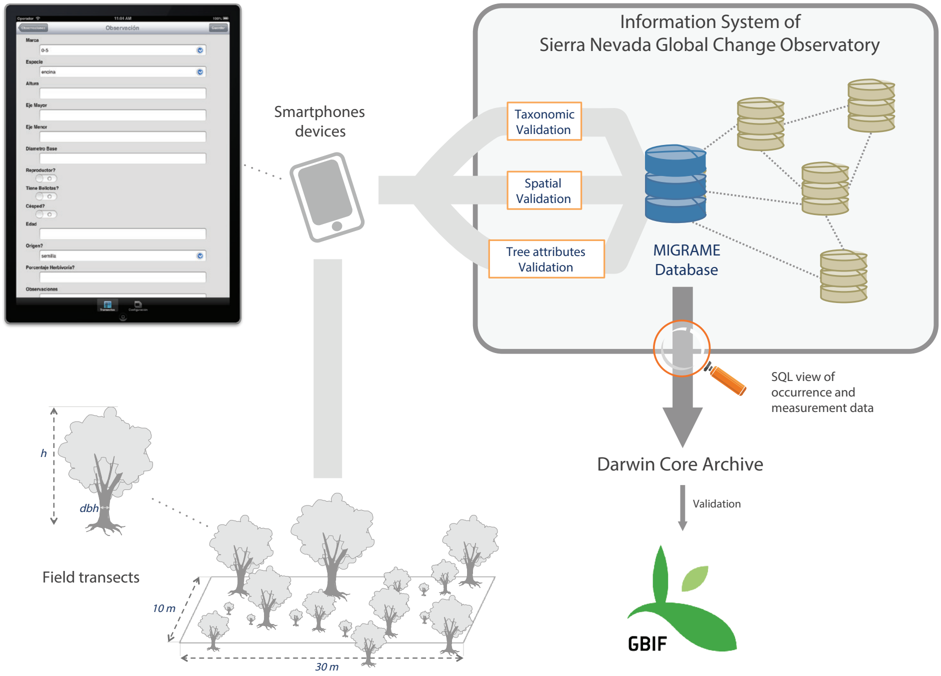
We sampled a total of 199 linear transects of 30 m × 10 m (Suppl. material 1). Within each transect, all tree species were recorded and the species identity was recorded. Diameter size and tree height were measured for all individuals. Field data were recorded using handheld PDAs. A customized application (app) (Figure 4) was built to facilitate both data collection and storage (Pérez-Pérez et al. 2013 – http://obsnev.es/noticia.html?id=4513). The data were automatically integrated into an information system using this application.

Method step description

All data were stored in a relational database (PostgreSQL) and added to the Information System of Sierra Nevada Global-Change Observatory (Figure 4) (http://obsnev.es/linaria.html – Pérez-Pérez et al. 2012; Free access upon registration). Taxonomic and spatial validations were made on this database (see *Quality-control description*). A custom-made SQL view of the database was performed to gather occurrence data and other variables associated with some occurrence data (diameter size and tree height of each individual).

The occurrence and measurement data were accommodated to fulfil the Darwin Core Standard (Wieczorek et al. 2009, Wieczorek et al. 2012). We used Darwin Core Archive Validator tool (http://tools.gbif.org/dwca-validator/) to check whether the dataset met Darwin Core specifications. The Integrated Publishing Toolkit (IPT v2.0.5) (Robertson et al. 2014) of the Spanish node of the Global Biodiversity Information Facility (GBIF) (http://www.gbif.es/ipt) was used both to upload the Darwin Core Archive and to fill out the metadata.

The Darwin Core elements for the occurrence data included in the dataset were: occurrenceId, modified, language, institutionCode, collectionCode, basisOfRecord, catalogNumber, recordedBy, eventDate, day, month, year, continent, country, countryCode, stateProvince, county, locality, minimumElevationInMeters, maximumEle-



Observación

Marca
0-5

Especie
encina

Altura

Eje Mayor

Eje Menor

Diametro Base

Reproductor?

Tiene Bellotas?

Césped?

Edad

Origen?
semilla

Porcentaje Herbivoría?

Observaciones

Figure 4. Diagram of integration of the dataset within Information System of Sierra Nevada Global Change Observatory (http://obsnev.es/linaria.html). Field data were recorded with Smartphone devices (see Pérez-Pérez et al. 2013). After a validation process (see Quality Control section) the occurrence and measurement data were accommodated to Darwin Core Archive and integrated into GBIF.

vationInMeters, decimalLongitude, decimalLatitude, coordinateUncertaintyinMeters, geodeticDatum, scientificName, kingdom, phylum, class, order, family, genus, specificEpithet, infraspecificEpithet, scientificNameAuthorship.

For the measurement data, the Darwin Core elements included were: occurrenceId, measurementID, measurementType, measurementValue, measurementAccuracy, measurementUnit, measurementDeterminedDate, measurementDeterminedBy, measurementMethod.

Quality control description

Transects coordinates were recorded with a handheld Garmin eTrex Vista Global Positioning System (GPS, ±5 m accuracy, Garmin (2007)) (WGS84 Datum). We also used colour digital orthophotographs provided by the Andalusian Cartography Institute and GIS (ArcGIS 9.2; ESRI, Redlands, California, USA) to verify the geographical coordinates of each sampling plot (Chapman and Wieczorek 2006).

The specimens were taxonomically identified using Flora iberica (Castroviejo 1986–2005). The scientific names were checked with databases of International Plant Names Index (IPNI 2013) and Catalogue of Life/Species 2000 (Roskov et al. 2015). We also used the R package taxize (Chamberlain and Szöcs 2013, Chamberlain et al. 2014) to verify the taxonomical classification.

We also performed validation procedures (Chapman 2005a, 2005b) (geographic coordinate format, coordinates within country/provincial boundaries, absence of ASCII anomalous characters in the dataset) with DARWIN_TEST (v3.2) software (Ortega-Maqueda and Pando 2008).

Dataset description

Object name: Darwin Core Archive Dataset of MIGRAME Project (Global Change, Altitudinal Range Shift and Colonization of Degraded Habitats in Mediterranean Mountains)
Character encoding: UTF-8
Format name: Darwin Core Archive format
Format version: 1.0
Distribution: http://www.gbif.es/ipt/resource.do?r=migrame
Publication date of data: 2015-05-13
Language: English
Licenses of use: This "Dataset of MIGRAME Project (Global Change, Altitudinal Range Shift and Colonization of Degraded Habitats in Mediterranean Mountains)" is licensed under a made available under the Creative Commons Attribution Non Commercial (CC-BY-NC) 4.0 License http://creativecommons.org/licenses/by-nc/4.0/legalcode
Metadata language: English
Date of metadata creation: 2015-05-13
Hierarchy level: DataSet

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References

Allen CD, Breshears DD (1998) Drought-induced shift of a forest–woodland ecotone: Rapid landscape response to climate variation. Proceedings of the National Academy of Sciences 95: 14839–14842. doi: 10.1073/pnas.95.25.14839

Benito B, Lorite J, Peñas J (2011) Simulating potential effects of climatic warming on altitudinal patterns of key species in Mediterranean-alpine ecosystems. Climatic Change 108: 471–483. doi: 10.1007/s10584-010-0015-3

Benito B, Pérez-Pérez R, Zamora R, Pérez-Luque AJ (2013) Colonización de hábitats marginales y migración altitudinal del roble mediada por el arrendajo: Simulación dinámica mediante sistemas multi-agente. In: XI Congreso Nacional de la Asociación Española de Ecología Terrestre. Invitación a la ecología. Pamplona, Spain. doi: 10.7818/AEET.XICongress.2013

Blanca G, Cueto M, Martínez-Lirola M, Molero-Mesa J (1998) Threatened vascular flora of Sierra Nevada (Southern Spain). Biological Conservation 85: 269–285. doi: 10.1016/S0006-3207(97)00169-9

Blanca G, López Onieva M, Lorite J, Martínez Lirola MJ, Molero Mesa J, Quintas S, Ruíz Girela M, Varo MA, Vidal S (2001) Flora amenazada y endémica de Sierra Nevada. Editorial Universidad de Granada, Granada, 410 pp.

Bonet FJ, Pérez-Luque AJ, Moreno R, Zamora R (2010) Sierra Nevada Global Change Observatory. Structure and Basic Data. Environment Department (Andalusian Regional Government). University of Granada, 1–48. http://refbase.iecolab.es/files/bonet/2010/2905_Bonet_etal2010.pdf

Brewer S, Cheddadi R, Beaulieu J de, Reille M (2002) The spread of deciduous *Quercus* throughout Europe since the last glacial period. Forest Ecology and Management 156: 27–48. doi: 10.1016/S0378-1127(01)00646-6

Cabezudo B, Talavera S, Blanca G, Salazar C, Cueto M, Valdés B, Hernández-Bermejo J, Herrera C, Rodríguez-Hiraldo C, Navas D (2005) Lista roja de la flora vascular de Andalucía. Consejería de Medio Ambiente. Junta de Andalucía, Sevilla, 1–126.

Camacho-Olmedo M, García-Martínez P, Jiménez-Olivencia Y, Menor-Toribio J, Paniza-Cabrera A (2002) Dinámica evolutiva del paisaje vegetal de la Alta Alpujarra granadina en la segunda mitad del s. XX. Cuadernos Geográficos 32: 25–42.

Castroviejo S (Ed.) (1986–2005) Flora iberica. Real Jardín Botánico CSIC, Madrid.

Chamberlain S, Szöcs E (2013) taxize: Taxonomic search and retrieval in R. F1000Research 2: 191. doi: 10.12688/f1000research.2-191.v2

Chamberlain S, Szöcs E, Boettiger C, Ram K, Bartomeus I, Baumgartner J (2014) taxize: Taxonomic information from around the web. R package version 0.3.0. https://github.com/ropensci/taxize

Chapman AD (2005a) Principles and methods of Data Cleaning: Primary species and species-occurrence data, version 1.0. Global Biodiversity Information Facility, Copenhagen, 75 pp. http://www.gbif.org/orc/?doc_id=1262

Chapman AD (2005b) Principles of Data Quality, version 1.0. Global Biodiversity Information Facility, Copenhagen, 61 pp. http://www.gbif.org/orc/?doc_id=1229

Chapman A, Wieczorek J (2006) Guide to Best Practices for Georeferencing. Global Biodiversity Information Facility, Copenhagen. http://www.gbif.org/orc/?doc_id=1288

Doblas-Miranda E, Martínez-Vilalta J, Lloret F, Álvarez A, Ávila A, Bonet FJ, Brotons L, Castro J, Curiel Yuste J, Díaz M, Ferrandis P, García-Hurtado E, Iriondo JM, Keenan TF, Latron J, Llusia J, Loepfe L, Mayol M, Moré G, Moya D, Peñuelas J, Pons X, Poyatos R, Sardans J, Sus O, Vallejo VR, Vayreda J, Retana J (2015) Reassessing global change research priorities in Mediterranean terrestrial ecosystems: How far have we come and where do we go from here? Global Ecology and Biogeography 24: 25–43. doi: 10.1111/geb.12224

EIONET (2014) Online report on article 17 of the Habitats Directive: Conservation status of habitats and species of community interest (2001–2006). http://bd.eionet.europa.eu/activities/Reporting/Article_17/Reports_2007/index_html [accessed 24.07.2015]

Escribano-Avila G, Sanz-Pérez V, Pías B, Virgós E, Escudero A, Valladares F (2012) Colonization of abandoned land by *Juniperus thurifera* is mediated by the interaction of a diverse dispersal assemblage and environmental heterogeneity. PLoS ONE 7: e46993. doi: 10.1371/journal.pone.0046993

Franco J (1990) *Quercus* L. In: Castroviejo A, Laínz M, López-González G, Montserrat P, Muñoz-Garmendia F, Paiva J, Villar L (Eds) Flora iberica 2. Real Jardín Botánico CSIC, Madrid, 15–36.

García I, Jiménez P (2009) 9230 Robledales de *Quercus pyrenaica* y robledales de *Quercus robur* y *Quercus pyrenaica* del noroeste ibérico. In: Ministerio de Medio Ambiente, y Medio Rural y Marino (Ed.) Bases Ecológicas Preliminares para la Conservación de los Tipos de Hábitat de Interés Comunitario En España, Ministerio de Medio Ambiente, y Medio Rural y Marino, Madrid, 1–66.

García RA, Cabeza M, Rahbek C, Araújo MB (2014) Multiple dimensions of climate change and their implications for biodiversity. Science 344. doi: 10.1126/science.1247579

García-Ruiz JM, López-Moreno JI, Vicente-Serrano SM, Lasanta–Martínez T, Beguería S (2011) Mediterranean water resources in a global change scenario. Earth-Science Reviews 105: 121–139. doi: 10.1016/j.earscirev.2011.01.006

Garmin (2007) Garmin eTrex vista personal navigator. Owner's manual and reference guide. https://buy.garmin.com/shop/store/manual.jsp?product=010-00630-00 [accessed 10.05.2012]

Gavilán R, Sánchez-Mata D, Vilchez B (2000) Effects of disturbance on floristic patterns of *Quercus pyrenaica* forests in central Spain. In: White P, Mucina L, Leps J (Eds) Vegetation science in retrospect and perspective – Proceedings 41st IAVS symposium. Opulus Press, Uppsala, 227–230.

Gavilán R, Sánchez-Mata D, Vilchez B (2007) Modeling current distribution of spanish *Quercus pyrenaica* forests using climatic parameters. Phytocoenologia 37: 561–581. doi: 10.1127/0340-269X/2007/0037-0561

Giorgi F (2006) Climate change hot-spots. Geophysical Research Letters 33: L08707. doi: 10.1029/2006GL025734

Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Global and Planetary Change 63: 90–104. doi: 10.1016/j.gloplacha.2007.09.005

Gordo O, Sanz JJ (2010) Impact of climate change on plant phenology in Mediterranean ecosystems. Global Change Biology 16: 1082–1106. doi: 10.1111/j.1365-2486.2009.02084.x

Gottfried M, Pauli H, Futschik A, Akhalkatsi M, Barancok P, Benito Alonso JL, Coldea G, Dick J, Erschbamer B, Fernández Calzado MR, Kazakis G, Krajci J, Larsson P, Mallaun M, Michelsen O, Moiseev D, Moiseev P, Molau U, Merzouki A, Nagy L, Nakhutsrishvili G, Pedersen B, Pelino G, Puscas M, Rossi G, Stanisci A, Theurillat J-P, Tomaselli M, Villar L, Vittoz P, Vogiatzakis I, Grabherr G (2012) Continent-wide response of mountain vegetation to climate change. Nature Climate Change 2: 111–115. doi: 10.1038/nclimate1329

Gómez-Aparicio L, Pérez-Ramos IM, Mendoza I, Matías L, Quero JL, Castro J, Zamora R, Marañón T (2008) Oak seedling survival and growth along resource gradients in Mediterranean forests: Implications for regeneration in current and future environmental scenarios. Oikos 117: 1683–1699. doi: 10.1111/j.1600-0706.2008.16814.x

Gómez-Aparicio L, Zamora R, Gómez JM (2005) The regeneration status of the endangered *Acer opalus* subsp. *granatense* throughout its geographical distribution in the Iberian Peninsula. Biological Conservation 121: 195–206. doi: 10.1016/j.biocon.2004.04.019

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: The rear edge matters. Ecology Letters 8: 461–467. doi: 10.1111/j.1461-0248.2005.00739.x

Herrera C, Manzaneda A, Benavente A, Luque P, Jordano P (2000) *Sorbus aria* (L.) Crantz. In: Blanca G, Cabezudo B, Hernández-Bermejo J, Herrera C, Muñoz J, Valdés B (Eds) Libro rojo de la flora silvestre amenzada de Andalucía. II. Especies vulnerables. Consejería de Medio Ambiente. Junta de Andalucía, Sevilla, 337–339.

Hughes L (2000) Biological consequences of global warming: Is the signal already apparent? Trends in Ecology & Evolution 15: 56–61. doi: 10.1016/S0169-5347(99)01764-4

IPNI (2013) The international plant names index. http://www.ipni.org [accessed 12.12.2014]

IUCN (2001) IUCN Red List Categories. Prepared by the IUCN Species Survival Commission. As approved by the 51st Meeting of the IUCN Council Gland, Switzerland. IUCN, Gland, Switzerland.

Jump AS, Peñuelas J (2005) Running to stand still: Adaptation and the response of plants to rapid climate change. Ecology Letters 8: 1010–1020. doi: 10.1111/j.1461-0248.2005.00796.x

Lenoir J, Gégout JC, Marquet PA, Ruffray P de, Brisse H (2008) A significant upward shift in plant species optimum elevation during the 20th century 320: 1768–1771. doi: 10.1126/science.1156831

Lorite J, Navarro FB, Valle F (2007) Estimation of threatened orophytic flora and priority of its conservation in the Baetic range (S. Spain). Plant Biosystems 141: 1–14. doi: 10.1080/11263500601153560

Luterbacher J, Xoplaki E, Casty C, Wanner H, Pauling A, Küttel M, Rutishauser T, Brönnimann S, Fischer E, Fleitmann D, Gonzalez-Rouco FJ, García-Herrera R, Barriendos M, Rodrigo F, Gonzalez-Hidalgo JC, Saz MA, Gimeno L, Ribera P, Brunet M, Paeth H, Rimbu N, Felis T, Jacobeit J, Dünkeloh A, Zorita E, Guiot J, Türkes M, Alcoforado MJ, Trigo R, Wheeler D, Tett S, Mann ME, Touchan R, Shindell DT, Silenzi S, Montagna P, Camuffo D, Mariotti A, Nanni T, Brunetti M, Maugeri M, Zerefos C, Zolt SD, Lionello P, Nunes MF, Rath V, Beltrami H, Garnier E, Ladurie ELR (2006) Mediterranean climate variability over the last centuries: A review. In: P. Lionello PM-R, Boscolo R (Eds) Mediterranean climate variability. Elsevier, 27–148.

Marañón T, Zamora R, Villar R, Zavala M, Quero J, Pérez-Ramos I, Mendoza I, Castro J (2004) Regeneration of tree species and restoration under constrasted Mediterranean habitats: Field and glasshouse experiments. International Journal of Ecology and Environmental Sciences 30: 187–196.

Martínez-Parras J, Molero-Mesa J (1982) Ecología y fitosociología de *Quercus pyrenaica* Willd. en la provincia Bética. Los melojares béticos y sus etapas de sustitución. Lazaroa 4: 91–104.

Matesanz S, Valladares F (2014) Ecological and evolutionary responses of Mediterranean plants to global change. Environmental and Experimental Botany 103: 53–67. doi: 10.1016/j.envexpbot.2013.09.004

McGill B (2015) Biodiversity: Land use matters. Nature 520: 38–39. doi: 10.1038/520038a

Melendo M, Valle F (2000) Estudio comparativo de los melojares nevadenses. In: Chacón J, Rosúa J (Eds) 1ª Conferencia internacional Sierra Nevada. Universidad de Granada, Granada, 463–479.

Olalde M, Herrán A, Espinel S, Goicoechea PG (2002) White oaks phylogeography in the Iberian Peninsula. Forest Ecology and Management 156: 89–102. doi: 10.1016/S0378-1127(01)00636-3

Ortega-Maqueda I, Pando F (2008) DARWIN_TEST v3.2: Una aplicación para la validación y el chequeo de los datos en formato Darwin Core 1.2 or Darwin Core 1.4. Unidad de Coordinación de GBIF, CSIC. Ministerio de Educación y Ciencia, Madrid, Spain. http://www.gbif.es/Darwin_test/Darwin_test.php [accessed 12.12.2014]

O'Connor MI, Holding JM, Kappel CV, Duarte CM, Brander K, Brown CJ, Bruno JF, Buckley L, Burrows MT, Halpern BS, Kiessling W, Moore P, Pandolfi JM, Parmesan C, Poloczanska ES, Schoeman DS, Sydeman WJ, Richardson AJ (2015) Strengthening confidence in climate change impact science. Global Ecology and Biogeography 24: 64–76. doi: 10.1111/geb.12218

Pacifici M, Foden WB, Visconti P, Watson JEM, Butchart SHM, Kovacs KM, Scheffers BR, Hole DG, Martin TG, Akçakaya HR, Corlett RT, Huntley B, Bickford D, Carr JA, Hoffmann AA, Midgley GF, Pearce-Kelly P, Pearson RG, Williams SE, Willis SG, Young B, Rondinini C (2015) Assessing species vulnerability to climate change. Nature Climate change 5(3): 215–224. doi: 10.1038/nclimate2448

Padilla FM, Vidal B, Sánchez J, Pugnaire FI (2010) Land-use changes and carbon sequestration through the twentieth century in a Mediterranean mountain ecosystem: Implications for land management. Journal of Environmental Management 91: 2688–2695. doi: 10.1016/j.jenvman.2010.07.031

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics 37: 637–669. doi: 10.1146/annurev.ecolsys.37.091305.110100

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems 421: 37–42. doi: 10.1038/nature01286

Pérez-Luque AJ (2011) Análisis multivariante ambiental de los melojares de *Quercus pyrenaica* Willd. de Sierra Nevada. Master's thesis. University of Granada, Granada, 63 pp.

Pérez-Luque AJ, Bonet FJ, Benito B, Zamora R (2013) Caracterización ambiental de los robledales de *Quercus pyrenaica* Willd. de Sierra Nevada. In: XI Congreso Nacional de la Asociación Española de Ecología Terrestre. Invitación a la ecología. Pamplona, Spain. doi: 10.7818/AEET.XICongress.2013

Pérez-Pérez R, Bonet FJ, Pérez-Luque AJ, Zamora R (2012) Linaria: a set of information management tools to aid environmental decision making in Sierra Nevada (Spain) LTER site. In: Long Term Ecological Research (LTER) (Ed.) Proceedings of the 2013 LTER All Scientist Meeting: The Unique Role of the LTER Network in the Antropocene: Collaborative Science Across Scales. LTER, Estes Park - Colorado, USA.

Pérez-Pérez R, Pérez-Luque AJ, Navarro I, Bonet FJ, Zamora R (2013) Seguimiento y divulgación de procesos ecológicos mediante dispositivos móviles: Un caso práctico. In: XI Congreso Nacional de la Asociación Española de Ecología Terrestre. Invitación a la ecología. Pamplona, Spain. doi: 10.7818/AEET.XICongress.2013

Pías B, Escribano-Avila G, Virgós E, Sanz-Pérez V, Escudero A, Valladares F (2014) The colonization of abandoned land by spanish juniper: Linking biotic and abiotic factors at different spatial scales. Forest Ecology and Management 329: 186–194. doi: 10.1016/j.foreco.2014.06.021

Prados J, Vivero J, Hernández-Bermejo J (2000) *Acer opalus* Mill. subsp. *granatense* (Boiss.) Font Quer & Rothm. In: Blanca G, Cabezudo B, Hernández-Bermejo J, Herrera C, Muñoz J, Valdés B (Eds) Libro rojo de la flora silvestre amenzada de Andalucía. II. Especies vulnerables. Consejería de Medio Ambiente. Junta de Andalucía, Sevilla, 21–23.

Rivas-Martínez S, Díaz T, Fernández-González F, Izco J, Loidi J, Lousa M, Penas A (2002) Vascular plant communities of Spain and Portugal. Addenda to the syntaxonomical checklist of 2001. Itinera Geobotanica 15: 5–922.

Río S del, Herrero L, Penas A (2007) Bioclimatic analysis of the *Quercus pyrenaica* forests in Spain. Phytocoenologia 37: 541–560. doi: 10.1127/0340-269X/2007/0037-0541

Robertson T, Doring M, Guralnick R, Bloom D, Wieczorek J, Braak K, Otegui J, Russell L, Desmet P (2014) The GBIF Integrated Publishing Toolkit: Facilitating the efficient publishing of biodiversity data on the internet. PLoS ONE 9: e102623. doi: 10.1371/journal.pone.0102623

Rodríguez-Sánchez F, Hampe A, Jordano P, Arroyo J (2010) Past tree range dynamics in the Iberian Peninsula inferred through phylogeography and palaeodistribution modelling: A review. Review of Palaeobotany and Palynology 162: 507–521. doi: 10.1016/j.revpalbo.2010.03.008

Rosenzweig C, Karoly D, Vicarelli M, Neofotis P, Wu Q, Casassa G, Menzel A, Root T, Estrella N, Seguin B, Tryjanowski P, Liu C, Rawlins S, Imeson A (2008) Attributing physical and biological impacts to anthropogenic climate change. Nature 453: 353–357. doi: 10.1038/nature06937

Roskov Y, Abucay L, Orrell T, Nicolson D, Kunze T, Culham A, Bailly N, Kirk P, Bourgoin T, DeWalt R, Decock W, De Wever A (Eds) (2015) Species 2000 & ITIS Catalogue of Life. Species 2000: Naturalis, Leiden, the Netherlands. http://www.catalogueoflife.org/col [accessed 05.02.2015]

Rounsevell M, Reginster I, Araújo M, Carter T, Dendoncker N, Ewert F, House J, Kankaanpää S, Leemans R, Metzger M, Schmit C, Smith P, Tuck G (2006) A coherent set of future land use change scenarios for Europe. Agriculture, Ecosystems & Environment 114: 57–68. doi: 10.1016/j.agee.2005.11.027

Sala OE, Stuart Chapin F, III, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287: 1770–1774. doi: 10.1126/science.287.5459.1770

Suttle KB, Thomsen MA, Power ME (2007) Species interactions reverse grassland responses to changing climate. Science 315: 640–642. doi: 10.1126/science.1136401

Thuiller W, Albert C, Araújo MB, Berry PM, Cabeza M, Guisan A, Hickler T, Midgley GF, Paterson J, Schurr FM, Sykes MT, Zimmermann NE (2008) Predicting global change impacts on plant species' distributions: Future challenges. Perspectives in Plant Ecology, Evolution and Systematics 9: 137–152. doi: 10.1016/j.ppees.2007.09.004

Thuiller W, Lavorel S, Araújo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. Proceedings of the National Academy of Sciences 102: 8245–8250. doi: 10.1073/pnas.0409902102

Valbuena-Carabaña M, Heredia UL de, Fuentes-Utrilla P, González-Doncel I, Gil L (2010) Historical and recent changes in the spanish forests: A socio-economic process. Review of Palaeobotany and Palynology 162: 492–506. doi: 10.1016/j.revpalbo.2009.11.003

Valbuena-Carabaña M, Gil L (2013) Genetic resilience in a historically profited root sprouting oak (*Quercus pyrenaica* Willd.) at its southern boundary. Tree Genetics & Genomes 9: 1129–1142. doi: 10.1007/s11295-013-0614-z

Vilches de la Serna B (2014) Comprehensive study of *Quercus pyrenaica* Willd. forests at Iberian Peninsula: Indicator species, bioclimatic, and syntaxonomical characteristics. PhD thesis. Complutense University of Madrid, 194 pp.

Vivero J, Prados J, Hernández-Bermejo J (2000) *Quercus pyrenaica* Willd. In: Blanca G, Cabezudo B, Hernández-Bermejo J, Herrera C, Muñoz J, Valdés B (Eds) Libro rojo de la flora silvestre amenzada de Andalucía. II. Especies vulnerables. Consejería de Medio Ambiente. Junta de Andalucía, Sevilla, 303–306.

Wieczorek J, Döring M, De Giovanni R, Robertson T, Vieglais D (2009) Darwin core terms: A quick reference guide. http://rs.tdwg.org/dwc/terms/ [accessed 12.12.2014]

Wieczorek J, Bloom D, Guralnick R, Blum S, Doring M, Giovanni R, Robertson T, Vieglais D (2012) Darwin core: An evolving community-developed biodiversity data standard. PLoS ONE 7: e29715. doi: 10.1371/journal.pone.0029715

Wolkovich EM, Cook BI, Davies TJ (2014) Progress towards an interdisciplinary science of plant phenology: Building predictions across space, time and species diversity. New Phytologist 201: 1156–1162. doi: 10.1111/nph.12599

Zamora R, Pérez-Luque AJ, Benito B, Bonet FJ, Navarro I, Pérez-Pérez R, Hódar J, Matías L (2013) Cambio global, migración altitudinal y colonización de hábitats degradados en montañas mediterráneas (MIGRAME). In: XI Congreso Nacional de la Asociación Española de Ecología Terrestre. Invitación a la ecología. Pamplona, Spain. doi: 10.7818/AEET.XICongress.2013

Supplementary material 1

Table S1

Authors: Antonio Jesús Pérez-Luque, Regino Zamora, Francisco Javier Bonet, Ramón Pérez-Pérez

Data type: Table

Explanation note: Information about transects of the project. Elevation in m a.s.l.. Type: *AM* = Altitudinal migration; *FO* = Forest; *MH* = Marginal Habitat. Subtype: *AC-e*: Abandoned Cropland: edge; *AC-i*: Abandoned Cropland: inside; *Pp-e*: Pine plantations: edge; *Pp-i*: Pine plantations: inside; *TE*: Treeline Ecotone. Locality: *CA* = Robledal de Cáñar; *SJ* = Robledal de San Juan.

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Dendrochilum hampelii (Coelogyninae, Epidendroideae, Orchidaceae) traded as 'Big Pink' is a new species, not a hybrid: evidence from nrITS, *mat*K and *ycf*1 sequence data

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Abstract

In 2013, an unidentified species of *Dendrochilum* appeared in cultivation under the commercial trade name 'Big Pink'. Using sequences of the nuclear ribosomal ITS1-5.8S-ITS2 region and of the plastid *mat*K and *ycf*1 genes, we examined the phylogenetic relationships between 'Big Pink' and six other species of the phenetically defined *Dendrochilum* subgen. *Platyclinis* sect. *Eurybrachium*. Separate and combined analyses (using Bayesian, Maximum Likelihood and Parsimony inference) showed consistent placement of the unidentified species within a statistically well supported clade. Furthermore, the multi-copy nrITS marker showed clear distinct peaks. Thus, we found no evidence that 'Big Pink' could be a hybrid. Against this background, and further supported by species-specific mutations in (at least) nrITS and *ycf*1, we formally describe 'Big Pink' as a new species under the name *Dendrochilum hampelii*. Morphologically, it is most similar to *D. propinquum*, but it differs in a number of characters. Of the two cultivated individuals available for our study, one was of unrecorded provenance. The other allegedly originated from the Philippines. Observations of the species occurring in the wild in the Philippines in the northern provinces of Bukidnon and Misamis Oriental on the island of Mindanao confirmed this.

Keywords

Dendrochilum hampelii, Molecular Phylogenetics, Orchids, the Philippines

Introduction

The largely Malesian genus *Dendrochilum* Blume (Blume 1825–1827: 398–399; published 1825) accommodates ca. 275 species—most of them described based on field inventories in the periods ca. 1900–1940 and ca. 1985–2000 (Pedersen 2007a). However, since the turn of the millenium, most new species of *Dendrochilum* have been described based on cultivated material of unrecorded provenance (cf. Pedersen 2011). Following the publication of protologues based on cultivated material, four of these species have been located in the wild (Cootes 2011). This is the case for *D. coccineum* H.A.Pedersen & Gravend. (Pedersen et al. 2004), *D. croceum* H.A.Pedersen (Pedersen 2005), *D. quinquecallosum* H.A.Pedersen (Pedersen 2007b) and *D. undulatum* H.A.Pedersen (Pedersen 2007b). The discovery of *D. coccineum* in the Philippines confirmed a phylogeny-based hypothesis put forward by Pedersen et al. (2004). Similar phylogenetic inference of *D. warrenii* H.A.Pedersen & Gravend. (Pedersen et al. 2004) as probably originating from the Philippines and/or Sulawesi (Pedersen et al. 2004) is still awaiting confirmation.

The formal description of new species of unknown natural distribution has undoubtedly served to stimulate the (partly successful) search for these species in the wild, thus demonstrating the relevancy of this practice—not least in a conservation context. Nevertheless, describing new species based on material in commercial trade also involves a few problems. Thus, Vermeulen et al. (2014) raised some issues of moral concern, and Pedersen (2011) emphasized that care should be taken not to accidentally describe artificial hybrids as new species. Until recently, this risk was negligible in connection with *Dendrochilum* since not a single *Dendrochilum* hybrid had been registered (cf. Pedersen 2011). However, now there are two *Dendrochilum* artificial hybrids registered in *The International Orchid Register* (http://www.apps.rhs.org.uk/horticulturaldatabase/orchidregister/orchidregister.asp; accessed on 13 February 2015), originating from Bornean parental species. This demonstrates that human assisted hybridization between different species of *Dendrochilum* is indeed possible—for which reason we must consider the possibility that seemingly undescribed species suddenly appearing in cultivation could in reality be artificial hybrids.

Since chloroplast DNA is usually maternally inherited and nuclear DNA is biparentally inherited in orchids, incongruences between nuclear and plastid gene trees might indicate past events of hybridization. For example, by comparing phylogenies based on cpDNA and nrDNA, Barkman and Simpson (2002) inferred a natural hybrid origin of *D. acuiferum* Carr whereas Gravendeel et al. (2004) detected natural hybrids within the more distantly related genus *Pleione* D.Don. Further evidence for hybridization could potentially come from significant within-individual variation in multi-copy DNA markers.

This paper reports our study of an unidentified *Dendrochilum* (trade name: 'Big Pink') that appeared in cultivation in 2013. A live plant presented to the Hortus botanicus in Leiden carried a tag indicating a Philippinese provenance. However, as the plant came from a commercial nursery that trades much material of unknown geographic origin, we felt this provenance was in need of verification.

Synanthous inflorescences in combination with an entire rostellum, presence of stelidia and an apical wing on the column place the study plant in the phenetically defined subgenus *Platyclinis* Engl. as circumscribed by Pedersen et al. (1997). Within this subgenus, a firmly attached entire labellum and a stout and straight column with stelidia but without a foot place the plant in the phenetically defined section *Eurybrachium* Carr ex J.J.Wood, H.A.Pedersen & J.B.Comber (Pedersen et al. 1997). However, the morphology of 'Big Pink' does not match any previously described species in section *Eurybrachium*—implying that it should be formally described as a new species, provided it is not either an artificial or natural hybrid.

Altogether, we decided to examine 'Big Pink' in a molecular phylogenetic framework—and to describe it as a new species, if the results of the phylogenetic study could reject the possibility of 'Big Pink' being a hybrid.

Methods

Plant sampling and DNA extraction

The possible hybrid status of 'Big Pink' was tested using a molecular phylogenetic approach based on three markers, namely the biparentally inherited multi-copy nuclear ribosomal internal transcribed spacer (nrITS), and the maternally inherited plastid *mat*K and *ycf*1 genes. The ingroup consisted of 'Big Pink' and six other species belonging to *Dendrochilum* subgen. *Platyclinis* sect. *Eurybrachium* (cf. Pedersen et al. 1997), see Table 1.

A live plant of 'Big Pink' was available from the Hortus botanicus in Leiden, whereas the remaining *Dendrochilum* plants sampled for this study were reared in the Botanical Garden, Natural History Museum of Denmark. For information on vouchers, see Table 1. *Thunia alba* Rchb.f. was chosen as outgroup, based on the placement of the genus *Thunia* as sister to *Dendrochilum* using sequences from nrITS, *mat*K, *trn*L-F, and *rbc*L (Goldman et al. 2001, Gravendeel et al. 2001, van den Berg et al. 2005).

Total genomic DNA was obtained from 50 mg of silica dried or fresh leaf tissue. In the case of 'Big Pink', the tissue was mechanically reduced to dry powder using liquid nitrogen; for all other taxa, it was ground in Lysing Matrix A tubes (MP Biomedicals) and extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol.

Amplification and Sanger sequencing

The ITS1-5.8S-ITS2 region of the nuclear ribosomal internal transcribed spacer (nrITS) was amplified using primers 17SE (ACGAATTCATGGTCCGGTGAAGTGTTC) and 26SE (TAGAATTCCCCGGTTCGCTCGCCGTTAC), as described by Sun et al. (1994). Subsequently, a M13 universal sequencing primer was added to the

Table 1. List of species sampled for our DNA-based phylogenetic analysis with voucher data. All species in the table belong to the phenetically defined *Dendrochilum* subgen. *Platyclinis* sect. *Eurybrachium*. Abbrevations of herbaria: C=University of Copenhagen, Copenhagen, Denmark; K=Royal Botanic Gardens Kew, United Kingdom; L=Naturalis Biodiversity Center, Leiden, The Netherlands; N=Nanjing University, Nanjing, China.

Species **Voucher**	**NCBI GenBank accession numbers**		
	nrITS	*mat*K	***ycf*1**
Dendrochilum apoense T.Hashim. *cult. Hort. Bot. Hafn. s.n.* (C!)	KT334200	KT334206	KT334213
Dendrochilum auriculare Ames *cult. Hort. Bot. Hafn. P2012.5172* (C!)	KT334201	KT334207	KT334214
Dendrochilum coccineum H.A.Pedersen & Gravend. *cult. Richard C. Warren, Warren EQ 3060* (C!)	AY534923	KT334208	KT334215
Dendrochilum convallariiforme Schauer *cult. Hort. Bot. Hafn. P2012.5177* (C!)	KT334202	KT334209	KT334216
**Dendrochilum hampelii* Sulistyo et al. *cult. Hort. bot. Leiden 20130654* (L! [WAG0116920]])	KT334203	KT334210	KT334217
Dendrochilum septemnervium H.A.Pedersen *cult. Hort. Bot. Hafn. P2012.5195* (C!)	KT334204	KT334211	KT334218
Dendrochilum tortile H.A.Pedersen *cult. Hort. Bot. Hafn. P2012.5200* (C!)	KT334205	KT334212	KT334219
Thunia alba (Lindl.) Rchb.f. Nepal, *Chase 589* (K!)	AY008466	AY121731	-
Thunia alba (Lindl.) Rchb.f. China, *B. Hou EThuA* (N!)	-	-	KF361675

*Trade name: 'Big Pink'

5' end of the forward (TGTAAAACGACGGCCAGT) and reverse (CAGGAAACAGCTATGAC) primers to improve Sanger sequencing efficiency. Each PCR reaction consisted of 25 μl, containing the template DNA, CoralLoad PCR buffer (Qiagen), dNTPs, Taq DNA Polymerase (Qiagen), and both primers. The PCR reactions were carried out using a MyCycler Thermal Cycler (Bio-Rad) or a C1000 Touch Thermal Cycler (Bio-Rad). The thermal cycling protocol began with 5 min initial denaturation at 95 °C followed by 35 amplification cycles, each with 30 sec denaturation at 95 °C, 30 sec annealing at 50 °C, and 1 min extension at 72 °C, which were concluded by a 7 min final extension at 72 °C.

The primers for the amplification of the chloroplast *mat*K region were also used by Gravendeel et al. (2001), and specified as follows: 19F (CGTTCTGACCATATTGCACTATG) and 881R (TMTTCATCAGAATAAGAGT), 731F (TCTGGAGTCTTTCTTGAGCGA) and 2R (AACTAGTCGGATGGAGTAG). The PCR mix for the amplification of *mat*K followed that of nrITS, but with additional BSA. The thermal cycling protocol for *mat*K PCR began with 5 min initial denaturation at 94 °C followed by 28 amplification cycles, each with 30 sec denaturation at 94 °C, 30 sec annealing at 49 °C, 1 min extension at 72 °C, and ended with a 7 min final extension at 72 °C.

The 3' end portion of the chloroplast *ycf*1 region was amplified using primers newly designed in this study. The design was based on the *ycf*1 sequences data set of Neubig et al. (2008) available on NCBI GenBank (http://www.ncbi.nlm.nih.gov/GenBank), specifically from the species of subfamily Epidendroideae. The sequences were aligned using Geneious 5.6.7 (Kearse et al. 2012), and conserved regions were identified to be used as annealing sites. The *ycf*1 sequences produced in this study had a complete marker size of approximately 1.5 kb. The *ycf*1 region was amplified using a Hot start PCR protocol with primers d147F (TGCAGCRAATTYATTTATGAGTC) and intR2 (GATTTGATTGGGATGATCCAAGG), d557F (TCAAGAGATCAAACCATKCAATCA) and 1560R (CTCTACGACGTCTGGGAGATAG). Each PCR reaction consisted of 25 µl, containing DNA template, Phire Hot Start II DNA Polymerase and Phire buffer (ThermoScientific), dNTPs, BSA, and both primers. The cycling condition started with 30 sec initial denaturation at 98 °C and followed by 30 cycles of 5 sec denaturation at 98 °C, 5 sec annealing at 65.5 °C, and 10 sec extension at 72 °C, concluded with a 1 min final extension at 72 °C.

Sanger sequencing of the amplification products was performed at Baseclear (http://www.baseclear.com/), using an ABI 3730xl sequencer (Applied Biosystems). All new sequences are deposited in NCBI GenBank (Table 1). All sequences of *T. alba* were obtained from NCBI GenBank.

Phylogenetic analyses

Raw Sanger sequencing results in the form of AB1 files were edited and contigged using Sequencher 5.3 sequence analysis software (http://www.genecodes.com). The ends of all data sets were trimmed to avoid character misinterpretation. Ambiguous bases were replaced with "N" in the data matrix. The sequences were aligned using Geneious multiple sequence alignment in Geneious 5.6.7 (Kearse et al. 2012) with subsequent manual adjustments. Missing data were replaced with "?".

Phylogenetic analyses were carried out by means of Maximum Parsimony and Maximum Likelihood using PAUP* and Bayesian methods using the software Bayesian Evolutionary Analysis and Sampling of Trees (BEAST ver. 1.8.0; Drummond et al. 2012). Both PAUP* and the BEAST program were used to analyze the nrITS, the combined plastid *mat*K + *ycf*1, and the combined nrITS + *mat*K + *ycf*1 data matrices. For the Bayesian analysis, the substitution and clock model was set as unlinked, and the chosen nucleotide substitution model was General Time Reversible (GTR), plus Gamma with 10 categories. The best fit substitution model for each partition was determined using the Find Model web tool (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). A lognormal relaxed clock model was used for each partition, and the chosen tree prior was the Yule speciation process (Gernhard 2008). Tree samplings were generated through Markov Chain Monte Carlo (MCMC), with the number of generations set to 20,000,000 and a tree sampling for every 1000 generations. Three consecutive replicates were done to assess the consistency of the method. The three consistent replicates were then combined into a single matrix using LogCombiner c1.8.0 (http://beast.bio.ed.ac.uk/logcombiner) and used to search for the best probable tree using the program TreeAnnotator ver. 1.8.0 (http://beast.bio.ed.ac.uk/TreeAnnotator) with a 20% burnin value to avoid the reduction of posterior probability (PP) values, and visualized using FigTree ver. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The nrITS sequence alignment contained 854 positions with a mean ungapped length of 834 bp. Included in the alignment were the nrITS1 (236 positions), 5.8S RNA (166 positions), and mrITS2 (253 positions) regions. In total, the number of variable sites for the included positions was 164 (19.2%), of which 39 were potentially phylogenetically informative. Mean pairwise distances within the ingroup varied from 0.2–6.9%. There were six synapomorphic indels, with a size ranging from 1–5 bp. All the sequences included in the nrITS matrix were complete except for *T. alba*, which lacked 110 characters.

The *mat*K matrix was characterized by a fairly high number of missing data, mostly due to amplification failures. Samples lacking approximately half (800 bp) of the entire *mat*K sequence included *D. apoense* T.Hashim., *D. septemnervium* H.A.Pedersen and *D. tortile* H.A.Pedersen. The *mat*K alignment consisted of 1,783 positions, with a mean ungapped length of approximately 1,769 bp. The alignment contained the least variable sites out of the three alignments, 91 sites (5.1%) with 5 potentially phylogenetically informative sites. Mean pairwise distances within the ingroup ranged from 0.8–71.1%. There was a synapomorphic indel of 1 bp.

The *ycf*1 alignment consisted of 1,297 positions with mean ungapped length of 1065 bp. The number of variable sites was similar to that of nrITS, 244 (18.8%). Out of these positions, 30 were phylogenetically informative. There was one synapomorphic indel with a size of 9 bp. Mean pairwise distances within the ingroup varied from 10–33.5%. Amplification failures for *D. septemnervium* resulted in almost half of the desired *ycf*1 marker missing for this species.

The phylogenetic trees based on the combined *matK* + *ycf*1 matrix obtained by all three methods yielded overall stronger branch support relative to that of nrITS (Fig. 1A). The internal nodes gained high (PP = 0.97–1.0) support values, and the preceding replicates showed consistent topologies as well as well-supported clades. Clades presented by the *mat*K + *ycf*1 tree were highly congruent with the nrITS tree, and with higher support value.

No hard incongruence was present between the nrITS and the plastid phylogenetic trees obtained by all three methods. The combined nrITS + *mat*K + *ycf*1 matrix yielded a single tree with highly consistent topology and strong support values; the same clades as those in the separate nrITS and *mat*K + *ycf*1 analyses were identified (Fig. 1B). In all our trees, 'Big Pink' was positioned as a sister to *D. tortile* and *D. septemnervium*, which were very closely related, in terms of relative branch length (Fig. 1).

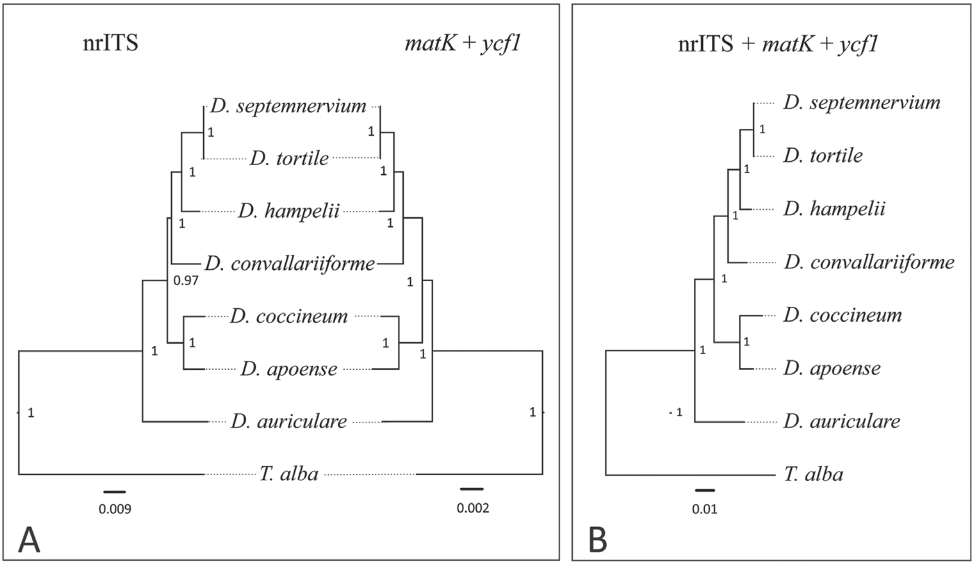
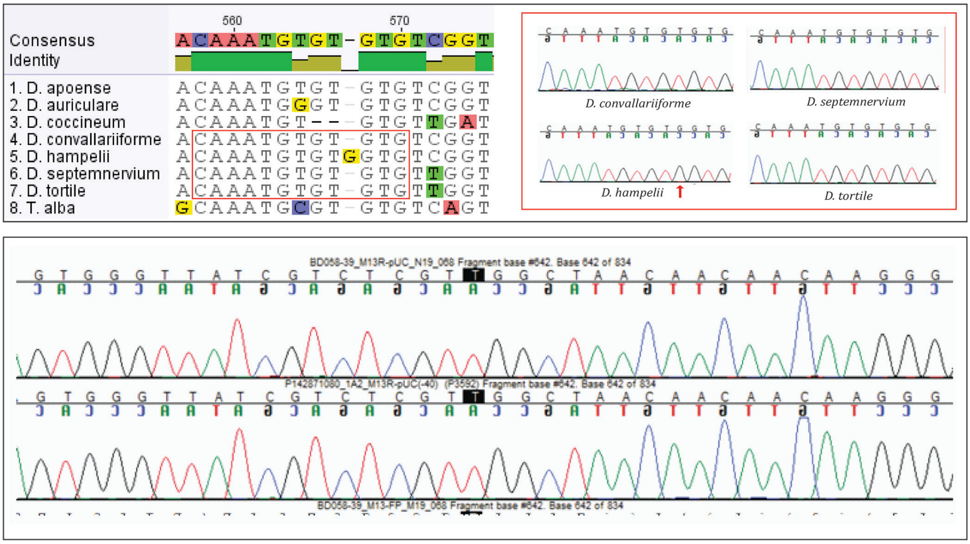


Figure 1. Phylogenetic relationships amongst the sampled species of *Dendrochilum*, created using BEAST and PAUP*. The values on the nodes represent posterior probabilities, whereas branch lengths indicate to the relative number of changes: **A** comparison between topologies based on nrITS and *mat*K + *ycf*1 matrices **B** topology resulting from the combined nrITS + *mat*K + *ycf*1 data matrices.



```
                       560        570
Consensus            ACAAATGTGT-GTGTCGGT
Identity
1. D. apoense        ACAAATGTGT-GTGTCGGT
2. D. auriculare     ACAAATGGGT-GTGTCGGT
3. D. coccineum      ACAAATGT---GTGTTGAT
4. D. convallariiforme ACAAATGTGT-GTGTCGGT
5. D. hampelii       ACAAATGTGTGGTGTCGGT
6. D. septemnervium  ACAAATGTGT-GTGTTGGT
7. D. tortile        ACAAATGTGT-GTGTTGGT
8. T. alba           GCAAATGCGT-GTGTCAGT
```

Figure 2. Above: alignment of nrITS sequences of the ingroup species from our phylogenetic analyses. For 'Big Pink' (*Dendrochilum hampelii*) and its apparently closest relatives among our study species, electropherograms are shown in red boxes. The electropherograms show clear distinct peaks; the species-specific mutation of 'Big Pink' is indicated by a red arrow. Below: electropherogram of 'Big Pink' (*Dendrochilum hampelii*) covering a larger region of nrITS; the distinct single peaks in both the forward and reverse sequences suggest this is a wild species rather than an artificial hybrid (see text for details).

Analysis of the nrITS sequence alignment revealed a species-specific mutation of 'Big Pink' at position 567 (Fig. 2). Electropherograms of the ITS sequences of 'Big Pink' and its three closest relatives among the study species showed clear, distinct signals for positions included in the alignment, including the 'Big Pink' species-specific mutation site (Fig. 2).

Discussion

Nuclear sequence variation found was largely in agreement with previous studies in Coelogyninae (Gravendeel et al. 2001). In general, sequence divergence of nrITS between different species of *Dendrochilum* is known to vary between 0.2–20% (Pedersen et al. 2004; Sulistyo, unpublished data). Additionally, the nrITS sequence obtained from *D. hampelii* is not likely to be a paralogous locus since branch lengths are similar to the sequences of other *bona fide* species analyzed. The position of 'Big Pink' was in agreement with preliminary analyses based on larger sampling (Sulistyo, unpublished data), as well as with the analyses based on different sequences. We found no evidence suggesting that the morphologically distinctive 'Big Pink' could be an artificial hybrid rather than a new wild species. Firstly, the separate phylogenetic analyses of nuclear and plastid markers proved highly congruent. Secondly, the electropherogram of the nrITS sequence of 'Big Pink' showed distinct single peaks with no indication of any heterozygosity of the specimen examined.

Genetically, 'Big Pink' possessed a number of automorphic mutations compared to the other species included in our small phylogenetic study. These unique mutations were found in nrITS and *ycf*1, whereas one in *mat*K is in need of verification due to the alignment being characterized by a fairly high number of missing data. Morphologically, 'Big Pink' is most similar to *D. propinquum* Ames. Unfortunately, no material was available of *D. propinquum* for DNA sequencing for this study. However, 'Big Pink' has much larger flowers (approximately twice the size of those of *D. propinquum*), its flowers have petals that are 1.4–1.5 times as broad as the sepals (0.8–1.1 times in *D. propinquum*), its labellum is broadly cordate (broadly elliptic to ovate in *D. propinquum*), and the stelidia of its column are acute (obtuse in *D. propinquum*). Against this background, we describe 'Big Pink' as a new species below.

Contrary to the studies of Wanntorp et al. (2002) and Pedersen et al. (2004), the taxon sampling underlying the phylogenetic analyses in this study was too small to allow for any inference of a probable geographic origin of 'Big Pink'. However, in a preliminary study based on systematically and geographically much broader sampling of *Dendrochilum* s.l., individual phylogenetic analyses using sequences from nrITS, plastid *mat*K, and *ycf*1 have all indicated 'Big Pink' to be nested within a clade nearly exclusively consisting of Philippine endemics (Sulistyo, unpublished data). In subsequent field surveys by the second author, a total of 12 plants were observed in February 2014 in the northern provinces of Bukidnon and Misamis Oriental on the island of Mindanao at high elevation, a few of which were flowering. In March 2015, only 4 individuals were observed *in situ* in the wild by the second and third author, and none were flowering. These observations confirm that *D. hampelii* is indeed a Philippinese, almost certainly endemic species. It is conjectured to have entered cultivation in Europe through the many domestic markets in Southeast Asia that sell orchid species. For these markets, wild plants are harvested but traded as 'cultivated' to circumvent CITES legislation. All Coelogyninae are listed on Appendix 2. Despite this legal protection, illegal trade is continuing at international orchid shows and by web based orders from buyers of specific species or nursery owners hoping to incorporate desirable wild traits into new hybrids (Hinsley et al. 2015).

Taxonomic treatment

Based on these results, it is determined that "Big Pink" is a new species in need of recognition. Formally naming the species is relevant for horticulture and *ex situ* conservation, because the name provides an unambiguous way to refer to the species.The morphology of 'Big Pink' was described using terminology of the vocabulary and list of individual absolute terms in Stearn (1983), if relevant standardized according to the Orchidaceae glossary in Pedersen et al. (2011).

Dendrochilum hampelii **Sulistyo, Gravend., R.Boos & Cootes, sp. nov.**

urn:lsid:ipni.org:names:77150223-1
Figs 3, 4

Type. Sine loco et anno, *Perry 490* (holotype L!).

Diagnosis. This new species is similar to *D. propinquum* Ames, but is distinguished by its larger flowers with petals proportionally broader (1.4–1.5×) than the sepals, a broadly cordate labellum (6.8–8.0 × 7.2–7.6 m) and acute stelidia.

Medium-sized, tufted epiphytic herb. *Roots* appearing from the rhizome, ca. 2.7 mm in diameter. *Pseudobulbs* tightly clustered on a short rhizome, fusiform, 3.5–5.0 cm long, 0.5–1.4 cm in diameter, longitudinally striated when dry, 1-leaved, initially covered by ca. 3 imperfectly to nearly perfectly tubular, rounded to acute cataphylls that soon disintegrate into persistent fibers. *Leaves* convolute, dorsiventrally flattened, petiolate; petiole channeled, 3.0–4.5 cm long; lamina (ob)lanceolate, obtuse, 13.0–20.0 × 3.7–5 cm, subcoriaceous, with 7–8 distinct (and many indistinct) nerves. *Inflorescence* synanthous, racemose; peduncle suberect, arched, slender, somewhat flattened, 18.0–21.2 cm long, sparsely and finely setose; rachis pendent with distichously alternating flowers (but the rachis axis twisted so as to produce a cylindrical inflorescence), many-flowered with internodes of 3–7 mm, somewhat furrowed, 20.0–27.5 cm long, sparsely and finely setose, basally with 1 appressed non-floriferous bract; flowering starting from the proximal part of the rachis. *Floral bracts* glumaceous, broadly lanceolate to (ovate-)oblong when flattened, obtuse to acute, 4.0–9.5 × 2.2–4.3 mm, entire,

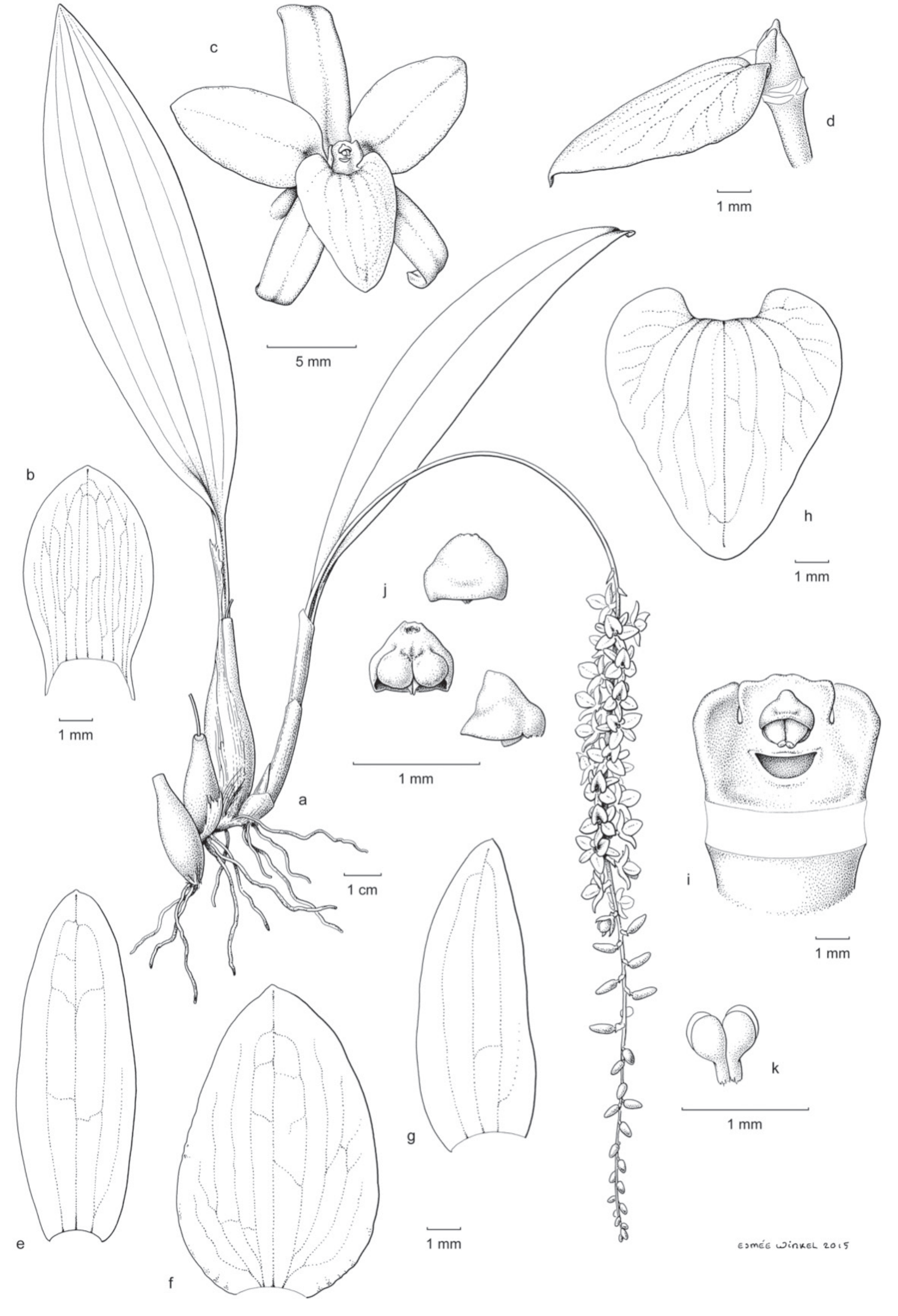


Figure 3. *Dendrochilum hampelii*: **a** habit **b** floral bract **c** flower **d** flower (sepals and petals removed) **e** dorsal sepal **f** petal **g** lateral sepal **h** labellum **i** column, front view **j** anther **k** pollinia. Drawing by Esmee Winkel based on *Hort. bot. Leiden 20130654* (L! [spirit no. WAG0116920]).

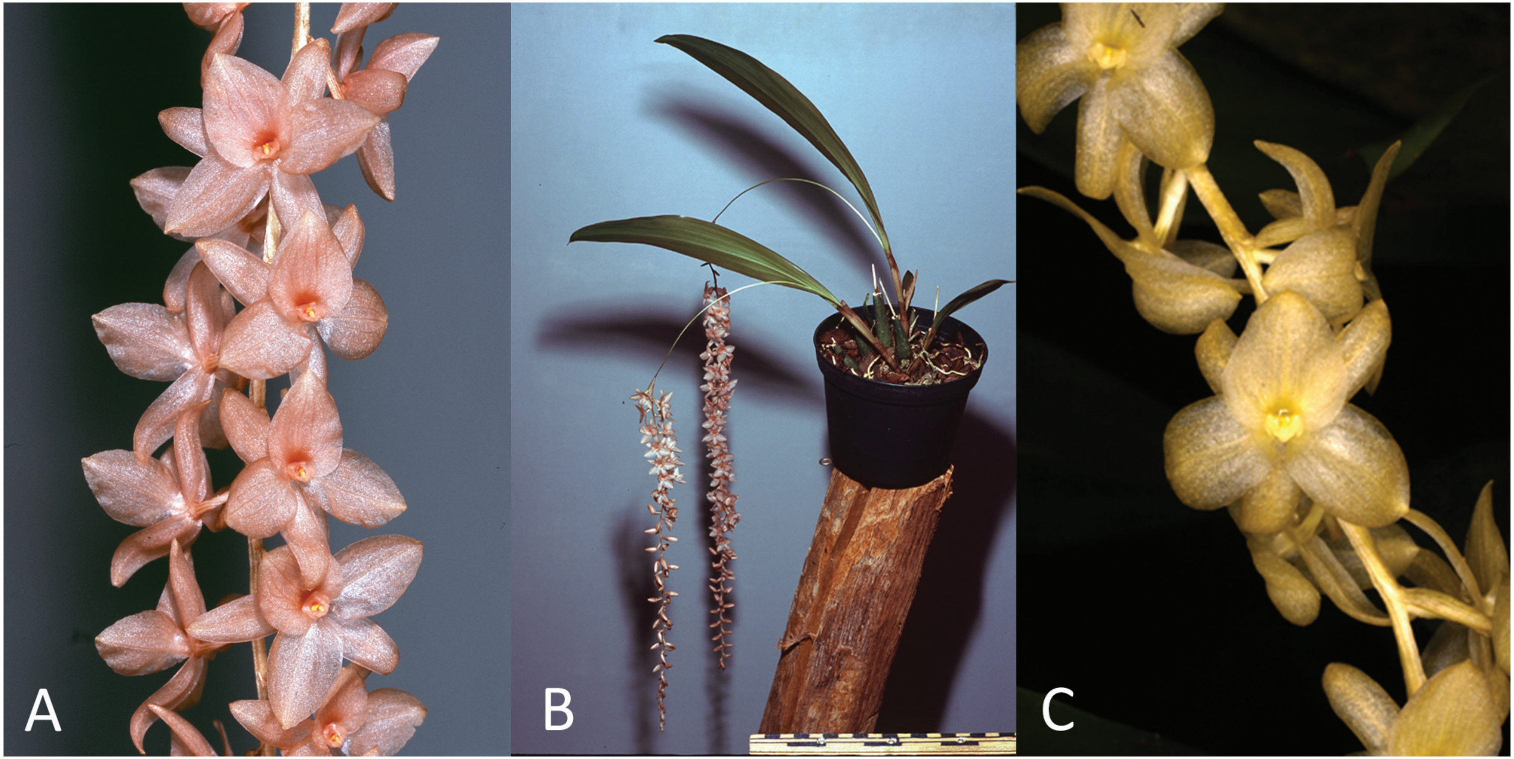


Figure 4. *Dendrochilum hampelii*: **A** portion of inflorescence of cultivated pinkish salmon-coloured form **B** habit. Photographs by Lubbert Westra of *Hort. bot. Leiden 20130654* (L! [spirit no. WAG0116920]) **C** portion of inflorescence of pale yellow-coloured form of a plant growing in the wild in the Philippines in the Misamis Oriental province of the island of Mindanao. Photograph by James Cootes.

9- to 19-nerved from the base, finely setose on the dorsal side. *Flowers* non-resupinate, pinkish salmon-coloured (Fig. 4A–B) or pale yellow (Fig. 4C) with yellow anther. *Sepals* recurved with revolute margins, entire, obtuse to rounded, minutely mucronate, glabrous, 3- to 5-veined from the base; dorsal sepal lanceolate-oblong, 8.7–11.0 × 3.5–3.7 mm when flattened; lateral sepals ovate-oblong, slightly oblique, 8.1–10.0 × 3.7–4.0 mm when flattened. *Petals* recurved with flat margins, broadly (ovate-)elliptic, often with a subbasal fold in either side, rounded to acute, 8.4–11.1 × 5.4–5.7 mm, 1.4–1.5 times as wide as the sepals, entire, glabrous, 3- to 5-veined from the base. *Labellum* firmly attached, sessile, describing a right to obtuse angle to the column, flat, broadly cordate with entire margins, acute to short-acuminate, 6.8–8.0 × 7.2–7.6 mm, without ornaments, glabrous and smooth, 5- to 7-veined from the base. *Column* suberect, straight, semiterete, 1.7–2.1 mm long, smooth, distally prolonged into a truncate to obscurely 3-lobed wing that distinctly exceeds the anther; stelidia appearing from the distal part of column proper, erect, falcately triangular-oblong, acute, subequal to the apical wing; anther circular to transversely elliptic in upper view, rounded in front, lobed at the back, with a small wart on top; pollinia 4, ellipsoid, devoid of caudicles; rostellum slightly protruding, flat, semicircular; fertile stigma part crescent-shaped, concave. *Ovary* (including pedicel) subterete, slightly longitudinally furrowed, twisted through 180°, distally incurved, 3.8–4.5 mm long, glabrous. *Fruit* not seen.

Additional material examined. PHILIPPINES? Sine loco et anno, *sine coll./cult. Hort. bot. Leiden 20130654* (L! [spirit no. WAG0116920]).

Etymology. The specific epiphet honours Georg Hampel, who was one of the first to provide us with study material of the newly described species.

Distribution and ecology. The species occurs in the wild in the Philippines in the northern provinces of Bukidnon and Misamis Oriental on the island of Mindanao (Fig. 4C). It grows as an epiphyte at elevations approximately 1,200 m above sea level among mosses on the trunks and branches of trees. Fresh flowers of plants observed in the wild were pale yellow whereas fresh flowers of the cultivated plants studied were pinkish salmon-coloured. We do not consider this reason to describe them as a different variety or forma as color dimorphism is known to occur in other Coelogyninae as well (Gravendeel 2000).

Reproductive biology. The live plant in Leiden flowered in mid-December. Attempts to pollinate flowers of *D. hampelii* were made using pollinia from the same flower and pollinia from a different flower in the same inflorescence. None of these efforts led to fruit formation. This indicates that *D. hampelii* is probably self-incompatible, as previously demonstrated for *D. longibracteatum* Pfitzer (Pedersen 1995), although it should be noted that experimental pollination was severely challenged by the small size of the stigmatic cavity.

Conservation status. Although the species occurs in cultivation we as yet know very little about the distribution and abundance of *D. hampelii* in the wild. As such, we recommend the species to be considered for the Data Deficient category of the IUCN Red List of Threatened Species (IUCN 2012).

Acknowledgements

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References

Ames O (1920) Orchidaceæ. Illustrations and studies of the family Orchidaceæ 6. The Merrymount Press, Boston, 1–335.

Barkman TJ, Simpson BB (2002) Hybrid origin and parentage of *Dendrochilum acuiferum* (Orchidaceae) inferred in a phylogenetic context using nuclear and plastid sequence data. Systematic Botany 27: 209–220.

Bentham G (1881) Notes on Orchideae. Journal of the Linnean Society, Botany 13: 281–360. doi: 10.1111/j.1095-8339.1881.tb01258.x

van den Berg C, Goldman DH, Freudenstein JV, Pridgeon AM, Cameron KM, Chase MW (2005) An overview of the phylogenetic relationships within Epidendroideae inferred from multiple DNA regions and recircumscription of Epidendreae and Arethuseae (Orchidaceae). American Journal of Botany 92: 613–624. doi: 10.3732/ajb.92.4.613

Blume CL (1825–1827) Bijdragen tot de flora van Nederlandsch Indië. Jakarta, 1–1169.

Carr CE (1935) Two collections of orchids from British North Borneo, part I. Gardens' Bulletin Straits Settlements 8: 165–240.

Cootes J (2011) Philippine native orchid species. Katha Publishing Co., Quezon City, 1–289.

Don D (1825) Prodromus floræ nepalensis, sive enumeratio vegetabilium, quæ in itinere per Nepaliam proprie dictam et regiones conterminas, ann. 1802–1803. J. Gale, London, 1–256.

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST. Molecular Biology and Evolution 29: 1969–1973. doi: 10.1093/molbev/mss075

Engler A (1907) Das Pflanzenreich. Regni vegetabilis conspectus, IV 50 II B 7. Verlag von Wilhelm Engelmann, Leipzig, 1–169.

Gernhard T (2008) The conditioned reconstructed process. Journal of Theoretical Biology 253: 769–778. doi: 10.1016/j.jtbi.2008.04.005

Goldman DH, Freudenstein JV, Kores PJ, Molvray M, Jarrell DC, Whitten MW, Cameron KM, Jansen RK, Chase MW (2001) Phylogenetics of Arethuseae (Orchidaceae) based on plastid *matK* and *rbcL* sequences. Systematic Botany 26: 670–695.

Gravendeel B (2000) Reorganising the orchid genus *Coelogyne* – a phylogenetic classification based on molecules and morphology. Grafische Vormgeving Kanters, Sliedrecht, 1–208.

Gravendeel B, Chase MW, de Vogel EF, Roos MC, Mes THM, Bachmann K (2001) Molecular phylogeny of *Coelogyne* (Epidendroideae; Orchidaceae) based on plastid RFLPs, *mat*K, and nuclear ribosomal ITS sequences: evidence for polyphyly. American Journal of Botany 88: 1915–1927. doi: 10.2307/3558367

Gravendeel B, Eurlings MCM, van den Berg C, Cribb PJ (2004) Phylogeny of *Pleione* (Orchidaceae) and parentage of its wild hybrids based on plastid and nuclear ribosomal ITS sequences and morphological data. Systematic Botany 29: 50–63. doi: 10.1600/036364404772973988

Hashimoto T (1981) A new species of the genus *Dendrochilum* Bl. (Orchidaceae) from the Philippines. Journal of Japanese Botany 56: 335–338.

Hinsley A, Verissimo D, Roberts DL (2015) Heterogeneity in consumer preferences for orchids in international trade and the potential for the use of market research methods to study demand for wildlife. Biological Conservation 190: 80–86. doi: 10.1016/j.biocon.2015.05.010

IUCN (2012) IUCN red list of threatened species categories and criteria, vers. 3.1. Second edition. IUCN, Gland, Switzerland and Cambridge, UK, iv + 32 pp.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Pedersen HÆ (1995) Anthecological observations on *Dendrochilum longibracteatum*—a species pollinated by facultatively anthophilous insects. Lindleyana 10: 19–28.

Markowitz S, Duran C, Tierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. doi: 10.1093/bioinformatics/bts199

Neubig KM, Whitten MW, Carlsward BS, Blanco MA, Endara L, Williams NH, Moore M (2008) Phylogenetic utility of *ycf*1 in orchids: a plastid gene more variable than *mat*K. Plant Systematics and Evolution 277: 75–84. doi: 10.1007/s00606-008-0105-0

Pedersen HÆ (1995) Anthecological observations on *Dendrochilum longibracteatum*—a species pollinated by facultatively anthophilous insects. Lindleyana 10: 19–28.

Pedersen HÆ (2005) A new miniature *Dendrochilum*. Orchid Review 113: 286–287.

Pedersen HÆ (2007a) Hotspots of narrow endemism: adequate focal points for conservation in *Dendrochilum* (Orchidaceae). Lankesteriana 7: 83–92.

Pedersen HÆ (2007b) Changes to *Dendrochilum*. Orchid Review 115: 220–226.

Pedersen HÆ (2011) Three new species of *Dendrochilum*, with notes on the practice of formally describing cultivated species of unknown provenance. Malesian Orchid Journal 7: 117–124.

Pedersen HÆ, Gravendeel B, Mudiana D (2004) Three new species of *Dendrochilum* (Orchidaceae) and their phylogenetic positions according to plastid and nuclear ribosomal ITS sequences. Blumea 49: 351–360. doi: 10.3767/000651904X484315

Pedersen HÆ, Kurzweil H, Suddee S, Cribb PJ (2011) Orchidaceae 1 (Cypripedioideae, Orchidoideae, Vanilloideae). In: Santisuk T, Larsen K (Eds) Flora of Thailand 12(2). The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, 1–302, Pls. I–XXIV.

Pedersen HÆ, Wood JJ, Comber JB (1997) A revised subdivision and bibliographical survey of *Dendrochilum* (Orchidaceae). Opera Botanica 130: 1–85.

Reichenbach HG (1852) Gartenorchideen. III. Botanische Zeitung 10: 761–772.

Stearn WT (1983) Botanical Latin: history, grammar, syntax, terminology and vocabulary. 3rd ed. David & Charles, Newto Abbot, xiv, 1–566.

Sun Y, Skinner DZ, Liang GH, Hubert SH (1994) Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theoretical and Applied Genetics 89: 26–32. doi: 10.1007/BF00226978

Vermeulen JJ, Phelps J, Thavipoke P (2014) Notes on *Bulbophyllum* (Dendrobiinae; Epidendroideae; Orchidaceae): two new species and the dilemmas of species discovery via illegal trade. Phytotaxa 184: 12–22. doi: 10.11646/phytotaxa.184.1.2

Wallich N (1830–1831) Plantae asiaticae rariores; or, descriptions and figures of a select number of unpublished East Indian plants 2. Treuttel and Würtz, London, 1–86.

Wanntorp L, Wanntorp HE, Källersjö M (2002) The identity of *Gunnera manicata* Linden ex Andre – resolving a Brazilian–Colombian enigma. Taxon 51: 493–497. doi: 10.2307/1554861

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RESEARCH ARTICLE

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A new subspecies of *Seseli gummiferum* (Apiaceae) from Ilgaz Mountain National Park, northern Turkey

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Abstract

A new subspecies *Seseli gummiferum* Pall. ex Sm. subsp. *ilgazense* A.Duran, Ö.Çetin & M.Öztürk, **subsp. nov.** (Apiaceae) is described from Kastamonu province, Turkey. It was collected from the open *Pinus sylvestris* L. and *Abies nordmanniana* (Steven) É.Spach. mixed forest in the northern Anatolian region. An endemic apparently confined to the Ilgaz Mountain National Park, the new taxon is closely related to *Seseli gummiferum* subsp. *gummiferum*. Diagnostic morphological characters for closely similar taxa are discussed, and a key to the subspecies of *Seseli gummiferum* is presented. ITS (Internal Transcribed Spacer) region of the nuclear ribozomal DNA of closely related *Seseli* L. taxa and *Pimpinella* is used to constract phylogenetic tree by using BioEdit and Seaview Programme.

Keywords

New taxon, *Seseli gummiferum*, Turkey, Umbelliferae

Introduction

The Apiaceae comprise approximately 450 genera and 3700 species worldwide (Pimenov and Leonov 1993). However, the distribution of species among the genera vary, with almost half of the genera monotypic and 26% consisting of only two or three species. Sixty percent of the species in the family assign to just a few genera, which genera encompass over 20 species have been noted as polyphyletic (Spalik et al. 2004). Asian countries with the greatest biodiversity for the Apiaceae include China, Asian Turkey, Iran, Asian Russia, and Kazakhstan, with the most species given for the Chinese flora (677 species in 108 genera) (Pimenov and Leonov 2004). Turkey, with a considerably smaller geographic area, is second only to China in its diversity for the Apiaceae, with 450 species in 109 genera. There are four endemic genera in Turkey, with 140 species among 42 genera. This suggests that the Asiatic region in Turkey has the highest known species-level diversity for the Apiaceae in Asia, if not in the world (Pimenov and Leonov 2004).

Seseli L. is one of the largest genera in the Apiaceae with 125 to 140 taxa ascribed to the genus worldwide. Represented by both intraspecific and interspecific diversity, *Seseli* is distributed in Europe, Asia, Africa, North America and Australia (Pimenov and Leonov 2004). The first revision of *Seseli* in Turkey was made by Hedge and Lamond (1972), who recognized 10 infrageneric taxa. *Seseli gummiferum* subsp. *gummiferum* was reported as a new record from Turkey (Duman 2000). After that *Seseli ramosissimum* replaced with *Seseli hartvigiii* by Parolly (Parolly and Nordt 2001). Latest revision of *Seseli* in Turkey was made by Doğan Güner and Duman (2013). So the total number of these taxa is 13 now.

Material and methods

In 2008, during a field trip in the Black Sea region of Turkey, an unusual specimen of *Seseli* was collected by the authors. Study of the descriptions in Hedge and Lamond (1972), Davis et al. (1988), Duman (2000), Ball (1968), Rechinger (1987), Parolly and Nordt (2001), Shishkin (1950), Özhatay et al. (2009), Doğan Güner and Duman (2013) as well as comparison with herbarium material in GAZI, HUB, KNYA and ANK revealed that the specimens indeed represented a new taxon. In particular, the new taxon was compared with the closely similar taxa *Seseli gummiferum* Pall. ex Sm. subsp. *gummiferum* and *S. corymbosyum* Boiss. & Heldr. In the morphological description below, each numerical value is the average of ten measurements from different specimens. The abbreviations of the authors of plant names were checked from Brummitt and Powell (1992).

DNA isolation: Total DNA was obtained from 50–75 mg leaf and fruit from six different individuals. DNAs are isolated with CTAB method and after concentrations were determined by Nanodrop. Sample DNAs were diluted 25 ng/µl. Stok DNAs were kept at -86 °C.

ITS amplifications: ITS region of studied taxa were amplified using ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') and ITS5 (5' GGA AGG AGA AGT CGT AAC AAG 3') primers. PCR condition is 95 °C for 5 min initial denaturation, 35 cycles of 94 °C for 30 s denaturation, 50 °C for 30 s anneling, and 72 °C for 1 min extension, 72 °C for 10 min final extension.

Data collection and cluster of phylogenetic analysis: PCR products were visualised by agarose jel. The amplified fragments were sequenced using the same primers used for amplification. ITS sequences of the taxa were aligned via Bioedit and were used to construct phylogenetic trees by using Seaview.

Taxonomic treatment

Seseli gummiferum gummiferum Pall. ex Sm., Exot. Bot. [Smith] ii, 121 (1807).

Replaced synonym: *Bubon rigidus* Spreng., Syst. Veg. (ed. 16) [Sprengel] 1: 900 1825 [1824], nom. illeg., non Bubon rigidus (Waldst. & Kit.) Spreng. Pl. Min. Cogn. Pug. 2: 53. 1815. Type: [London] Cult. in Oxford Bot. Garden and Hort. Lady Hume.

Seseli gummiferum crithmifolium (Boiss.) P.H.Davis, Notes Roy Bot. Gard. Edinb. 21: 120 (1953).

Basionym: *Seseli crithmifolium* Boiss., Fl. Orient. [Boissier] 2: 962 (1872). Type: Greece, Insulae maris Aegei, *Tournefort* 324 (holotype P, photo!, E!).

Seseli gummiferum ilgazense A.Duran, Ö.Çetin & M.Öztürk, subsp. nov.

urn:lsid:ipni.org:names:77150275-1
Figs 1–6

Affinis S. gummiferum Pall. ex Sm. subsp. gummiferum sed umbellis centralibus radiis 13–21 (nec 25–30), bracteis 2–7 (nec plerumque 8–15), umbellis lateralibus radiis 7–13 (nec 15–20), ovariis glabris (nec cum pilis), fructibus glabris (nec cum pilis) differt.

Type. TURKEY. A4 Kastamonu: Ilgaz Mountain Natural Park, Kastamonu road, from Çatören village to Büyük Hacet Hill, 6 km, in open *Pinus sylvestris* L. and *Abies nordmanniana* (Stev.) Spach. mixed forest, serpentine stony slopes, 41°06'344"N, 33°48'628"E, 1465 m, 22 August 2008, *A.Duran* 8135, *Ö.Çetin & M.Öztürk* (holotype KNYA! isotypes ANK!, GAZI!, HUB!).

Description. Plants monocarpic, 15–30 cm tall; rootstock thickened, cylindrical, oblong, ± vertical, 8–15 mm diameter; stems 3 to 5, terete, finely ridged, mostly glabrous, sparsely puberulent above, with a developed fibrous collar 2.5–7 cm; stems mostly branching from the base, below and rarely at the middle part, green to purplish green. Basal leaves ovate to oblong-ovate, 2-pinnate, 6–20 × 3–10 cm, glabrous; ultimate segments lanceolate, linear-oblong, 5–15 × 0.5–1.5 mm, acute to acuminate; sheaths developed, distinctly widened at base, upper sheath surface sulcate, margin membranous; cauline leaves similar to basal leaves, partly reduced, amplexicaule, middle and upper portions of the stem leafless. Flowers hermaphroditic; the central umbel stout, 7–11 cm diam., equal to or longer than lateral umbels, rarely shorter, with 13 to 21 rays, 1.8–5.5 cm long, puberulent above, unequal, each central umbel with by 2 to 7 bracts; bracts lanceolate, rarely widened at base, 10–17 mm long, margin ± membranous, sometimes bifid to trifid, glabrous or puberulent; umbellules 9–15 mm diam, each comprising

Figure 1. *Seseli gummiferum* subsp. *ilgazense* A.Duran, Ö.Çetin & M.Öztürk, subsp. nov.

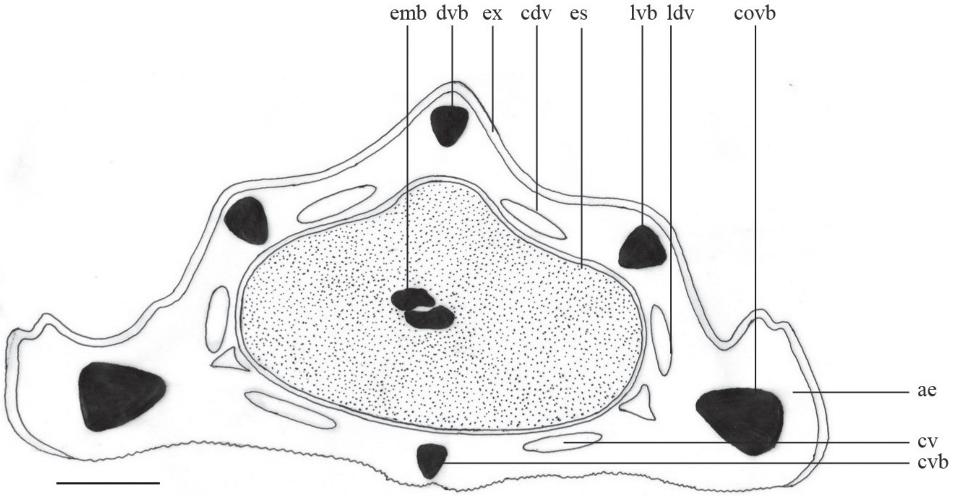

Figure 2. Cross section of fruit: emb = embriyo; dvb = dorsal vascular bundle; ex = exocarp; cdv = central dorsal vittae; es = endosperm; lvb = lateral vascular bundle; ldv = lateral dorsal vittae; ae = aerenchyma; cv = commissural vittae; cvb = carpophore vascular bundle; covb = commissural vascular bundle. Scale bar = 0.2 mm.

55 to 70 flowers; bracteoles 15–20, connate at base, 5–8 mm long, linear- lanceolate, acuminate, entire or bifid to trifid, green to partly purple, margin distinctly membranous, especially ciliate at apex, out side glabrous to puberulent; lateral umbels 3.5–6 cm diam. with 7 to 13 rays, 1–3.5 cm long, with bracts or without. Flowers subsesile, glabrous, sepals ±purplish, broadly lanceolate, glabrous, persistent, ca. 1 mm; petals white, glabrous with deflexed apex; filament white, anther clearly purple; ovary glabrous. Fruit oblong-elliptic, 3.5–4 × 1.5–2 mm, glabrous, with 5 ribs prominent, obtuse; stylopodium short-conical; styles deflexed in fruit, distinctly purple.

Distribution and IUCN red list category. The new subspecies is known only from the type locality, with specimens collected only from Ilgaz Mountain National Park (Kastamonu province) in Turkey, where the species seems to be very rare. This area is ca. 1.5 km^2, and mature individuals of the type population number approximately 125. The location is very close to the road side and near forest management. The population is going to be negatively affected from cars, trucks and people in the future. The habitat of this subspecies is clearly under threat of destruction, and therefore, the taxon should be considered Critically Endangered (CR), according to IUCN Red List Criteria (IUCN 2001).

Habitat and ecology. This new subspecies grows at 1450–1470 m with *Lapsana communis* L., *Abies nordmanniana* (Stev.) Spaach, *Centaurea drabifolia* Sm., *Erysimum thyrsoideum* Boiss., *Pteridium aquilinum* (L.) Kuhn, *Sorbus umbellata* (Desf.) Fritsch, *Valeriana alliariifolia* Adams, *Eryngium giganteum* M.Bieb., *Bupleurum falcatum* L. subsp. *persicum* (Boiss.) Koso-Pol., *Salvia verticillata* L., *Teucrium chamaedrys* L., *Dactylis glomerata* L., *Asyneuma rigidum* (Willd.) Grossh. subsp. *rigidum*.

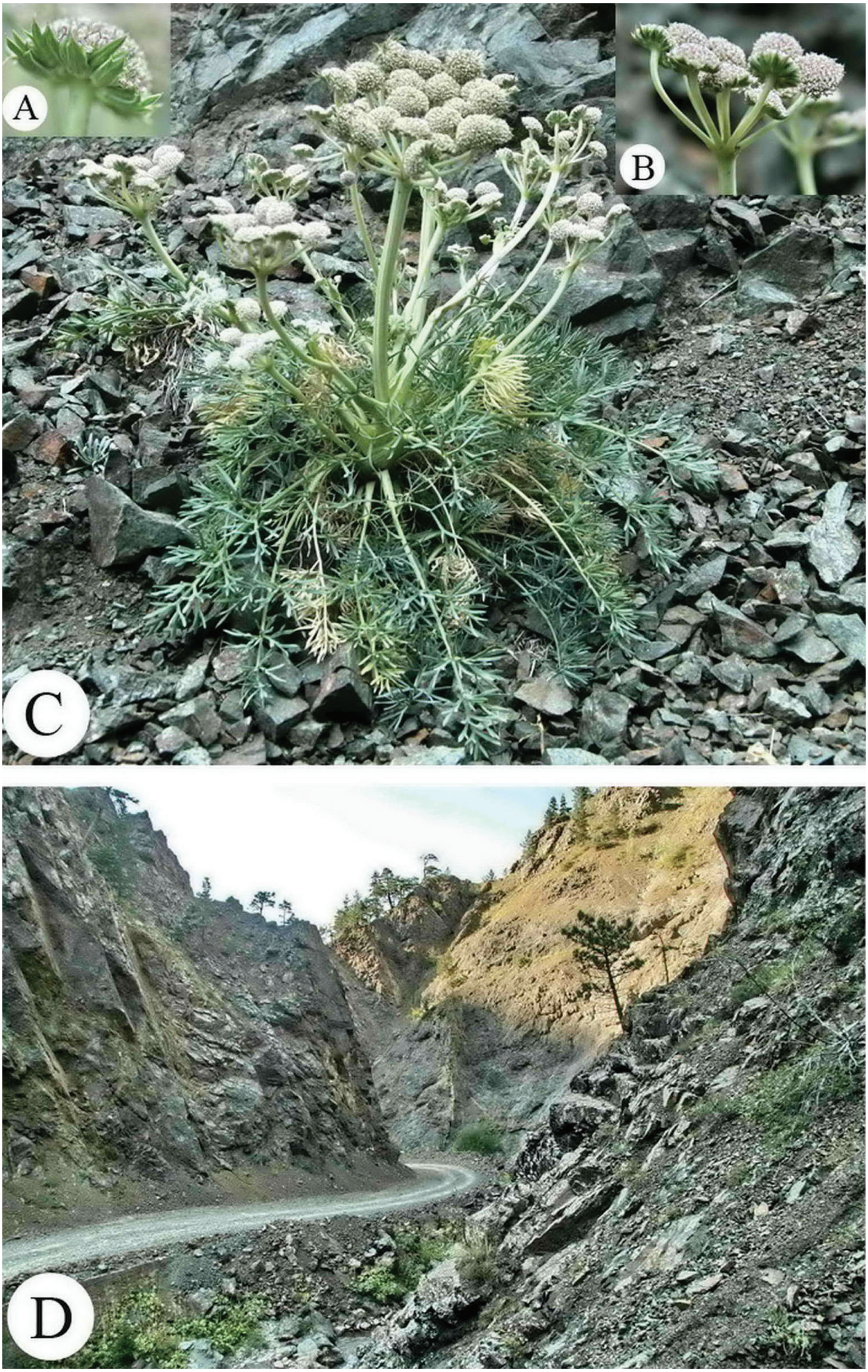

Figure 3. *Seseli gummiferum* subsp. *ilgazense*. **A** trifid and entire bracteoles **B** lateral umbel **C** general aspects **D** habitat and general view of type locality. Photo by A.Duran.

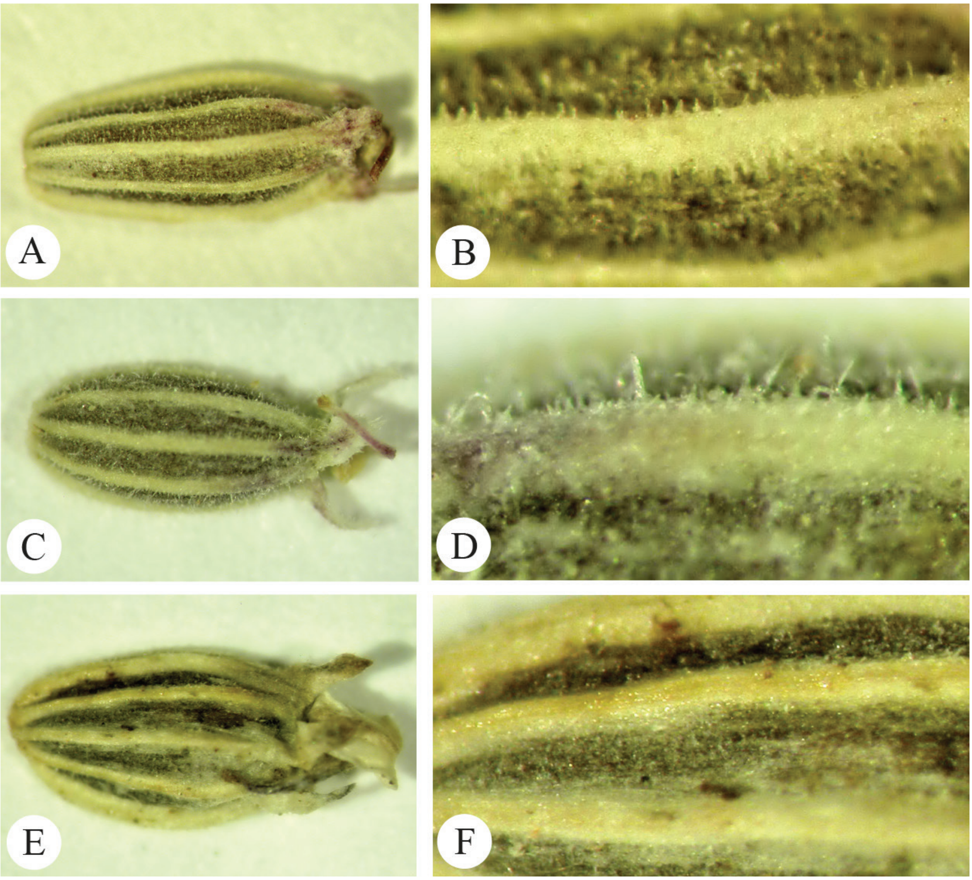

Figure 4. Comparison of *Seseli gummiferum* fruits. *S. corymbosum* fruits. **A** general view **B** surface details. *S. corymbosum*: **C** general view **D** surface details. *S. gummiferum* subsp. *ilgazense*: **E** general view **F** surface details.

Discussion and conclusion. Turkey is the most complex country in the Middle East with regard to geographic structure and landforms. It's comprised of comparatively narrow and long, variously oriented mountain chains, separated by deep valleys and also high- and medium-elevational plateaus. The geological composition and physical direction, exposure and altitude of these mountains are here largely influential not only upon the diversity of vegetation, but also on the richness of the flora (Zohary 1973). Ilgaz Mountain, which is situated in a transitional zone in central and North of Anatolia and is generally composed of serpentine, schist and volcanic rocks. The mountain is orogenically interesting, with the quite active north Anatolian fault found along the southern slopes of Ilgaz Mountain (Kuter 2008). One of the more important reasons for protecting the Ilgaz Mountain is the richness and endemism of its flora. Approximately 100 endemic plants occur within the boundaries of the National Park and the type localities of 19 endemic taxa are found on Ilgaz Mountain. *Delphinium ilgazense*

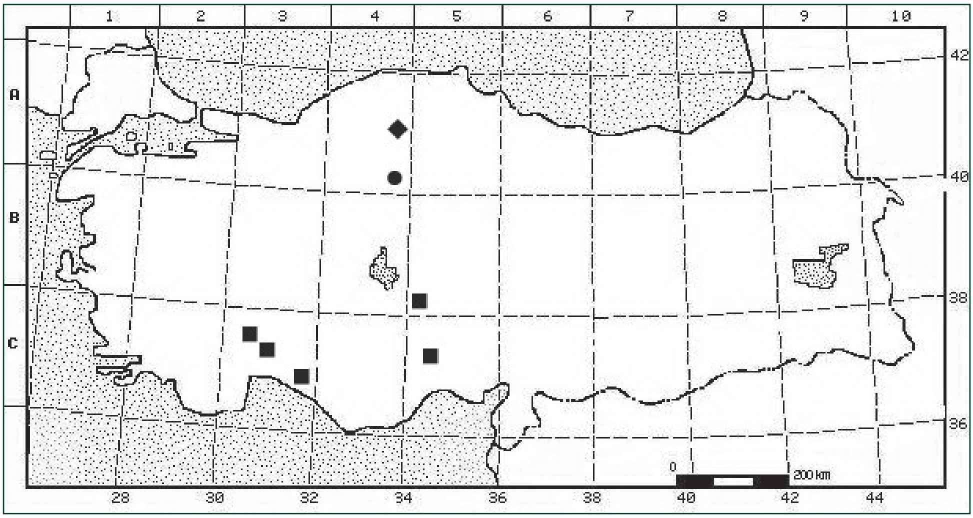

Figure 5. Distribution of *Seseli gummiferum* subsp. *ilgazense* (♦), *S. gummiferum* subsp. *gummiferum* (●) and *S. corymbosum* (■) in Turkey.

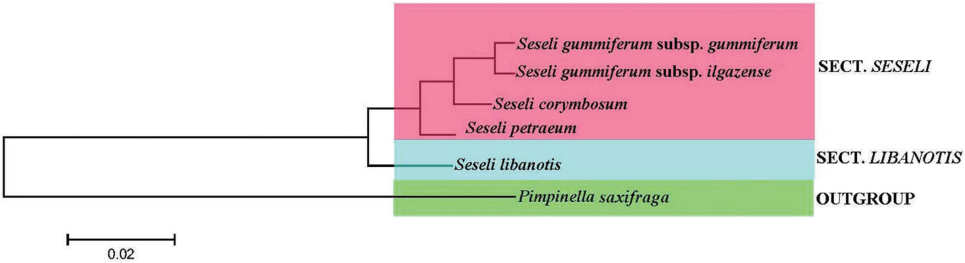

Figure 6. The neighbour joining tree generated using nrITS DNA sequences of some *Seseli* taxa and *Pimpinella saxifraga*.

P.H.Davis, *Arabis abietina* Bornmüller, *Draba anatolica* A.Duran & Dinç, *Astragalus nabelekii* Czeczott, *Heracleum paphlagonicum* Czeczott, *Hieracium macrogonum* (Zahn) P.D.Sell & C.West and *H. tuberculatum* Freyn, *Festuca ilgazensis* Markgr.-Dann. are all noteworthy endemic taxa confined to the national park (Davis 1965–1985, Davis et al. 1988, Duman 2000, Duran et al. 2008).

Seseli gummiferum subsp. *ilgazense* is closely related to two other subspecies found in Turkey. *S. gummiferum* subsp. *gummiferum* is distributed in Crimea, Central Anatolia (Shishkin 1950, Ball 1968, Duman 2000). The new subspecies differs from *S. gummiferum* subsp. *gummiferum*, based on its glabrous fruits (not with indument), fewer central umbels, with 13 to 21 rays (not 25 to 30), fewer lateral umbels, with seven to 13 rays (not 15 to 20), as well as the bracteoles 15 to 20 (not 11 to 16) (Hedge and Lamond 1972, Duman 2000).

Seseli gummiferum subsp. *ilgazense* also differs from *S. gummiferum* subsp. *crithmifolium* (DC.) P.H.Davis, which is distributed in west and east Crete, Folegandros, Sikinos, Amargos, Karpathos, Saria and neigbouring islands in Aegean Sea. The new taxon is not so broadly distributed, endemic and found only in northern Anatolia (Ilgaz Mountain). *Seseli gummiferum* subsp. *crithmifolium* principally differs from subsp. *ilgazense* by its puberulent fruits, the central umbels with 20–45 rays, and the oblong leaf segments (6–) 10–30 × 2–5 mm (Ball 1968, Hedge and Lamond 1972).

Key to closely related *Seseli gummiferum* subspecies

1 Fruit glabrous; the central umbel 13 to 21 rays; lateral umbels with 7 to 13 rays .... **subsp. *ilgazense***

– Fruit with hairs; the central umbel 22 to 70 rays; lateral umbels with 13 to 47 rays ....

2 Basal leaves glabrous .... **subsp. *crithmifolium***

– Basal leaves puberulent or finely pubescent .... **subsp. *gummiferum***

Since Morison's (1672), *Plantarum umbelliferarum*, fruit morphology and anatomy have been regarded as essential to the taxonomy of Apiaceae (Drude 1898, Constance 1971, Spalik et al. 2001). Details of the fruits have been traditionally viewed as rich sources of taxonomic characters, exhibiting some, but not excessive variation in features such as fruit shape, the degree and direction of mericarp compression, modifications of the pericarp ribs (e.g. wings or spines), and the shape of mericarp commissural faces. Thus, most traditional classifications of Apiaceae have relied almost exclusively on fruit characters (Plunkett and Downie 1999, Pimenov et al. 2004). *Seseli gummiferum* subsp. *ilgazense* has some distinctive characteristics in terms of carpological features. Mericarps of *Seseli gummiferum* subsp. *ilgazense* have five large vascular bundles situated beneath the each rib. Large vittae present in mesocarp layer adjacent to the endocarp. There are also two large vittae in the commissure. Differing from *Seseli gummiferum* subsp. *gummiferum*, the new subspecies does not have short secretory ducts around vascular bundles. In addition, the vittae around the endocarp are always large and elliptical. The endosperm is round shaped. Commissure width is approximately equal to mericarp width.

Diagnostic characters of *Seseli gummiferum* subsp. *ilgazense* with the three related taxa are provided in Table 1.

Five *Seseli* taxa and *Pimpinella* were evaluated in the phylogenetic analysis. PCR amplification with ITS 4/ITS 5 primers generated bands ranging from 595 to 665 bp. Alignment of the ITS sequences was done using Bioedit. Neighbour-Joining (NJ) tree were constructed using Seaview programme (Figure 6). According to NJ analyses, the taxa of *Pimpinella* placed in first clade. This species was used as outgroup. The second clade includes *Seseli libanotis,* which is found in Section *Libanotis*. *Seseli libanotis* is separated from *S. gummiferum* and *S. petraeum* by having highly wide leaf segment. The second clade incudes *Seseli petraeum*, *S. corymbosum*, *S. gummiferum* in sect. *Seseli*. *Seseli petraeum* is close to *Seseli gummiferum*. It mainly differs from *S. gummiferum*

Table 1. Diagnostic characters of *Seseli gummiferum* subsp. *ilgazense*, *S. gummiferum* subsp. *gummiferum*, *S. gummiferum* subsp. *crithmifolium* and *S. corymbosum*.

Characters	subsp. *ilgazense*	subsp. *gummiferum*	subsp. *crithmifolium*	*S. corymbosum*
Stems per plant	3 to 5	1 to 4	solitary	solitary
Stem branching	mostly below, rarely in middle portion	below the middle	above the middle	above the middle
Central umbel rays	13 to 21	25 to 30	22 to 45	30 to 70
Lateral umbel rays	7 to 13	15 to 20	17 to 35	13 to 47
Bracts	2 to 7	mostly 8 to 15	mostly absent, rarely up to 2	0 to 1
Bracteoles	15 to 20	11 to 16	19 to 26	19 to 23
Petals	glabrous	glabrous	pubescent	pubescent
Ovary	glabrous	pubescent	puberulent	pubescent
Fruit	glabrous	pubescent	pubescent	pubescent

because it has free bracteol from base and main umbel shorter than lateral umbels. *Seseli corymbosum* (Boiss. & Heldr.) P.H.Davis which only occurs in South Anatolia, is readily distinguished from *S. gummiferum*, by its solitary stem branching above, bracts either lacking or one below the central umbel, more numerous bracteoles, 19 to 23, larger central umbels with 30 to 70 rays and the lateral umbels with 13 to 47 rays, and finally by the pubescent petals and fruits (Hedge and Lamond 1972). Although *Seseli gummiferum* subsp. *ilgazense* is closely related to *S. gummiferum* subsp. *gummiferum*, this taxa is treated as different subspecies.

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References

Ball PW (1968) *Seseli* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (Eds) Flora Europaea. Vol 2, Cambridge University Press, Cambridge, 334–338.

Brummitt RK, Powell CE (1992) Authors of Plant Names. Royal Botanic Gardens, Kew, 736 pp.

Constance L (1971) History of the classification of Umbelliferae (Apiaceae). In: Heywood VH (Ed.) The Biology and Chemistry of the Umbelliferae. Academic Press, New York, 1–8.

Davis PH (1965–1985) Flora of Turkey and the East Aegean Islands. Vols 1–9. Edinburgh University Press, Edinburgh, 567 pp.

Davis PH, Mill RR, Tan T (1988) *Seseli* L. In: Davis PH, Mill RR, Tan T (Eds) Flora of Turkey and the East Aegean Islands – 11. Edinburgh University Press, Edinburgh, 150 pp.

Doğan Güner E, Duman H (2013) The revision of genus *Seseli* (Umbelliferae) in Turkey. Turkish Journal of Botany 37: 1018–1037. doi: 10.3906/bot-1302-41

Drude CGO (1898) Umbelliferae. In: Engler A, Prantl K (Eds) Die Natürlichen Pflanzenfamilien – Volume 3. Leipzig, 63–250.

Duman H (2000) *Seseli* L. In: Güner A, Özhatay N, Ekim T, Başer KHC (Eds) Flora of Turkey and the East Aegean Islands (Suppl. 2). Edinburgh University Press, Edinburgh, 141 pp.

Duran A, Dinç M, Martin E (2008) A new species of *Draba* (Brassicaceae) from North Anatolia, Turkey. Novon 18: 464–468. doi: 10.3417/2007023

Hedge IC, Lamond JM (1972) *Seseli* L. In: Davis PH (Ed.) Flora of Turkey and the East Aegean Islands – Volume 4. Edinburgh University Press, Edinburgh, 367–372.

IUCN (2001) IUCN red list categories, ver. 3.1. IUCN Species Survival Commission.

Kuter N (2008) Ilgaz Dağı milli Parkının orman peyzajı ve estetiği açısından değerlendirilmesi. Süleyman Demirel Üniversitesi Orman Fakültesi Dergisi 1: 36–47.

Morison R (1672) Plantarum umbelliferarum distributio nova. Oxford.

Özhatay N, Kültür Ş, Aslan S (2009) Check-list of Additional Taxa to the Supplement Flora of Turkey, IV. Turkish Journal of Botany 33: 191–226.

Parolly G, Nordt B (2001) *Seseli hartvigii* (Apiaceae), a new name for *S. ramosissimum* Hartvig & Strid., with carpological and ecological notes on this species. Willdenowia 31: 87–93. doi: 10.3372/wi.31.31107

Pimenov MG, Valiejo-Roman CM, Terentieva EI, Samigullin TH, Mozaffarian V (2004) Enigmatic genus *Haussknechtia* (Umbelliferae): systematic relationships based on molecular and carpological data. Nordic Journal of Botany 24(5): 555–565. doi: 10.1111/j.1756-1051.2004.tb01640.x

Pimenov MG, Leonov MV (2004) The Asian Umbelliferae biodiversity database (ASIUM) with particular reference to South-West Asian taxa. Turkish Journal of Botany 28: 139–145.

Pimenov MG, Leonov MV (1993) *Seseli* L. The genera of the Umbelliferae: a nomenclature. Kew Royal Botanic Gardens, Kew.

Plunkett GM, Downie SR (1999) Major lineages within Apiaceae subfamily Apioideae: A comparison of chloroplast restriction site and DNA sequence data. American Journal of Botany 86: 1014–1026. doi: 10.2307/2656619

Rechinger KH (1987) Umbelliferae. Flora Iranica. Akademische Druck-u., Verlagsanstalt, Graz-Austria, 347–349.

Shishkin BK (1950) *Seseli* L. In: Shishkin BK (Ed.) Flora of the USSR, Vol. 16, Izdatel'stvo Akademii Nauk SSSR, Moskva-Leningrad, 349–378. [translated from Russian, Israel Prog. Sci. Transl., 1973, Jerusalem]

Spalik K, Wojewódzka A, Downie SR (2001) The evolution of fruit in Scandiceae subtribe Scandicinae (Apiaceae). Canadian Journal of Botany 79: 1358–1374. doi: 10.1139/cjb-79-11-1358

Spalik K, Reduron J-P, Downie SR (2004) The phylogenetic position of *Peucedanum* sensu lato and allied genera and their placement in tribe Selineae (Apiaceae, subfamily Apioideae). Plant Systematics and Evolution 243: 189–210. doi: 10.1007/s00606-003-0066-2

Zohary M (1973) Geobotanical foundations of the Middle East, Vol. 1. Gustav Fischer, City.

Appendix

Representative specimens examined (*which used for DNA samples)**: – *Seseli corymbosum*:** Turkey, C3 Antalya: Akseki, Pınarbası village, S of Gidefi Mountain, *A.Duran* 2970 (GAZI); Akseki, Gidefi Mountain, *A.Duran* 1847 (GAZI); C5 Niğde; Ulukışla, between Alihoca-Maden villages, *A.Duran* 6078* (KNYA). **– *S. gummiferum* subsp. *gummiferum*:** Turkey, A4 Ankara: Hasanoğlan, İdris Mountain, above Adilahmet village, *M.Koyuncu* 16348 (GAZI); Hasanoğlan, İdris Mountain, 1500–1600 m., 14.09.2002, *E.Doğan* 1650* (GAZI). **– *S. gummiferum* subsp. *crithmifolium*:** Greece, Dodecanese, Karpathos, side of Kali Limni, 10 m, 21.07.1950, *Davis* 18010 (E). **– *Seseli petraeum:*** Turkey, Trabzon, between Araklı-Bayburt, 27.07.2002, *A.Duran* 6059* (KNYA). **– *Seseli libanotis:*** Turkey, Erzurum, between İspir-Ovit, 1700 m, 18.08.2013, *A.Duran* 9778* (KNYA).

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Reinstatement of the Loyalty Islands Sandalwood, *Santalum austrocaledonicum* var. *glabrum* (Santalaceae), in New Caledonia

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Abstract

Sandalwoods encompass 19 species restricted to southeast Asia and the Pacific. The species *Santalum austrocaledonicum* Vieill. (Santalaceae) is endemic to New Caledonia (Grande-Terre, Isle of Pines, Loyalty Islands) and Vanuatu, where several varieties are recognized. The Loyalty Islands sandalwood variety is here reinstated as *Santalum austrocaledonicum* var. *glabrum* Hürl. emend. Butaud & P.Firmenich, mut. char. It was previously considered a synonym of the type variety; however, new morphological and genetic studies confirmed its distinctiveness. The key for New Caledonian varieties of *Santalum austrocaledonicum* has been updated and a short description of its essential oil composition and organoleptic quality is given.

Keywords

Santalum austrocaledonicum, Santalaceae, Loyalty Islands, New Caledonia, essential oil

Introduction

Sandalwoods are shrubs or small trees well known for the essential oil extracted from their fragrant heartwood and used in perfumery. They belong to the genus *Santalum* (Santalaceae) comprising 19 species restricted to southeast Asia and the Pacific (Harbaugh and Baldwin 2007, Harbaugh 2007, Harbaugh et al. 2010). The most sought-after species is the Indian sandalwood, *Santalum album* L.

Santalum austrocaledonicum Vieill. is the only native sandalwood in New Caledonia and Vanuatu (Guillaumin 1925, Hallé 1988, Harbaugh and Baldwin 2007), and is endemic to these archipelagoes. It has been heavily harvested for the past 150 years (Shineberg 1967) and its wood is still exploited for the fragrance industry as its essential oil can be considered as a substitute for Indian sandalwood oil (Braun et al. 2005).

The most recent taxonomical work on *S. austrocaledonicum* was carried out by Hallé (1988) who recognized three botanical varieties based on specimens from New Caledonia in Paris (P) herbarium:

- *S. austrocaledonicum* Vieill. var. *austrocaledonicum* from Grande-Terre (main island of New Caledonia), Isle of Pines, Loyalty Islands and Vanuatu;
- *S. austrocaledonicum* var. *pilosulum* N.Hallé in the vicinity of Nouméa on the southwest coast of Grande-Terre;
- *S. austrocaledonicum* var. *minutum* N.Hallé from the northwest coast of Grande-Terre.

Since that revision, several studies have shown the great morphological variations of var. *austrocaledonicum* in New Caledonia and Vanuatu which are linked to geographical distribution (Quemin 1988, Nasi 1995, Chauvin and Ehrhart 1998, Bottin 2006, Bouvet et al. 2005, Bottin et al. 2007). The main differences in New Caledonia can be summarized as follows:

- Loyalty Islands: large seeds (L = 8–11 mm, D = 7–10 mm), short and wide juvenile leaves (L = 30–52 mm, W = 8–15 mm);
- Isle of Pines: medium-sized seeds (L = 8–10 mm, D = 6–9 mm), long and narrow juvenile leaves (L = 25–76 mm, W = 2–10 mm);
- Grande-Terre: small seeds (L = 6–9 mm, D = 5–7 mm), long and very narrow juvenile leaves (L = 52–70 mm, W = 2–4 mm).

No comprehensive study of the variation of these characters is available in Vanuatu due to lack of herbarium specimens from most of sandalwood populations there.

For this reason, Nasi (1994) intended to describe the Loyalty endemic sandalwood under the variety *loyaltensis* but his manuscript was never published.

More recently, molecular studies of New Caledonian sandalwood showed strong genetic differentiation between islands and led to the recognition of two evolutionarily significant units, i.e. Grande-Terre and Isle of Pines for the first, and Loyalty Islands for the second (Bottin et al. 2005, 2007).

The New Caledonian sandalwood is subject to exploitation through harvesting and is grown in plantations to satisfy the increasing international demand of sandalwood essential oil. However, its taxonomy appears to be in need of revision to more precisely describe the variability of this New Caledonian biodiversity hotspot species (Myers et al. 2000) and to contribute to the sustainable management of this natural resource.

A first step of this revision is presented here, with the recognition of an endemic sandalwood variety from the Loyalty Islands based on morphological and molecular studies as well as examination of living plants and herbarium specimens.

Nomenclature

The New Caledonian sandalwood, *Santalum austrocaledonicum*, was described by Vieillard (1861) from samples collected on the hills of Arama, in the Northern extremity of Grande-Terre (*E. Vieillard 1090*, holotype P00645808). Later, it was confirmed that this species was also present on the Isle of Pines, Loyalty Islands and Vanuatu (Guillaumin 1925, 1970, Virot 1950).

In 1964, Hürlimann described a new variety based on a sample collected on the island of Maré in the Loyalty Islands (Stauffer and Hürlimann 1964), *Santalum austrocaledonicum* var. *glabrum* Hürl. characterized by glabrous flowers, differing from the type variety (*Santalum austrocaledonicum* Vieill. var. *austrocaledonicum*) represented erroneously by samples gathered around Nouméa (Ouen Toro, Anse Vata, Baie de l'Orphelinat) which have villous flowers.

In 1988, the revision of the New Caledonian *Santalum* by Hallé recognized the three varieties mentioned in the introduction. Hallé reduced var. *glabrum* of Hürlimann to synonymy under the type (autonym) variety, considering it superfluous due to confusion by Hürlimann on what was really the type of the species. Indeed, the latter described var. *glabrum* in comparison with what is now recognized as var. *pilosulum*, this one differing morphologically from var. *austrocaledonicum*. The true var. *austrocaledonicum* and var. *glabrum* are in fact identical relative to the morphological differences pointed out by Hürlimann in his diagnosis.

As the native Loyalty Islands sandalwoods are quite homogeneous, only one variety is to be recognized for the entire Loyalty archipelago, which is the one of Hürlimann. Thus, to reinstate the varietal name *glabrum* for the endemic Loyalty sandalwood, its description must be amended to distinguish it from the true type specimen.

Systematics

***Santalum austrocaledonicum* var. *glabrum* Hürl. emend. Butaud & P.Firmenich, mut. char.**

S. austrocaledonicum var. *glabrum* Hürl., Mém. Mus. Hist. Nat., ser. B, Bot. 15(1): 15 (1964).

Type. New Caledonia, Loyalty Islands, Maré, près de Rawa, arbre, 8 m, en fleurs et en fruits, forêt mésophile, 17 July 1951, *M.G. Baumann-Bodenheim 14762* (holotype: P scan!; isotype: Z scan!).

Diagnosis. *Santalum austrocaledonicum* var. *glabrum* is most similar to var. *austrocaledonicum* in its glabrous inflorescence and leaves wider than 1.5 cm, which differentiates them from the other New Caledonian *S. austrocaledonicum* varieties. *Santalum austrocaledonicum* var. *glabrum* differs from *S. austrocaledonicum* var. *austrocaledonicum* by the seed size, which is more than 7.5 mm wide for the former and less than 7.5 mm for the latter.

Description. Shrub to small tree 2–10 m tall, trunk up to 30 cm dbh; bark rough, grey to reddish-brown, longitudinally fissured; heartwood fragrant, yellowish to brownish. ***Leaves*** glabrous; petiole canaliculate, 7–13 mm long; blades of the mature leaves 3.5–6.0 (–6.6) × (1.6–) 2.0–3.5 (–4.1) cm, usually elliptic or rarely obovate, apex obtuse to acute or apiculate, base acute, secondary veins mostly 7–9 pairs. ***Inflorescences*** glabrous, in axillary or terminal panicles, usually trichotomous and several times branched, with 10–40 flowers; peduncles 10–38 mm long. ***Flowers*** bisexual with outer surface of petal greenish and glabrous; pedicels 1.5–2 mm long. ***Petals*** 4, narrowly triangular, 2.5–3.0 × 1.5–2.0 mm, inner surface white when opening turning brownish later; petal internal margin glabrous. ***Stamens*** 4, surrounded by long hairs at the base; the outer ones reaching the anther apex, the inner ones reflexed in the cup-shaped disk; anthers 1.5–2.7 × 0.8–1.0 mm. ***Disk*** concave, more than 2 mm deep; disk lobes fleshy and erected between petals, 1.3–1.4 × 0.8–0.9 mm. ***Ovary*** unilocular, conic, acute, 1.1–1.7 × 0.6 mm; style free, 4 mm long; stigma 3 or 4 lobed. ***Fruit*** a globose fleshy drupe, 15–21 × 12–15 mm when fresh, topped by the petal scars 3–5 mm diameter, green turning red to deep purple and black at maturity. ***Seed*** globose, with a hard endocarp (8.5–) 9.0–11.5 × 7.5–10.0 (–10.5) mm.

Phenology. Flowering and fruiting probably occurring throughout the year but with some peaks; herbarium samples provide the following data: flowers from December to August, fruits from January to August.

Distribution. New Caledonia, endemic to Loyalty Islands; known only from Ouvéa, Lifou and Maré islands (Figure 1). Not recorded on the smaller islands of Beautemps-Beaupré, Tiga and Walpole.

Habitat and biology. This variety is restricted to the calcareous soils of uplifted atolls between 5 and 80 m elevation and is closely linked with traditional agriculture which consists of shifting cultivation. Regeneration by seeds occurs mainly in the open cultivated areas and young fallow lands. Loyalty sandalwood is therefore characteristic of fallows, shrublands and secondary forests, and rarely occurs in mature forests. It is also commonly found along roads and close to villages in open areas where it is generally protected and managed by inhabitants. The surrounding vegetation is often composed of trees and shrubs, such as *Acacia spirorbis* Labill., *Acalypha* spp., *Acronychia laevis* J.R.Forst. & G.Forst., *Dodonaea viscosa* (L.) Jacq., *Elattostachys apetala* (Labill.) Radlk., *Glochidion billardierei* Baill., *Melochia odorata* L.f., *Morinda citrifolia* L., *Pandanus macrocarpus* (Brongn.) Solms, *Podonephelium homei* (Seem.) Radlk., *Polyscias bracteata* (R.Vig.) Lowry subsp. *bracteata*., *Psidium guajava* L., and *Schinus terebenthifolius* Raddi. Loyalty sandalwood is a hemiparasitic tree like all *Santalum* species; its pollination is insect-mediated whereas its fleshy fruits are dispersed mainly by doves and pigeons (Bottin et al. 2005).

Conservation status. Using the categories and criteria of IUCN (2001), we propose for *S. austrocaledonicum* var. *glabrum* the IUCN Red List Category Vulnerable (VU): B (1+2) ab (iii,v). Its population size is estimated at more than 10,000 mature individuals (excluding criteria C and D) with an extent of occurrence around 8,000 km^2 and an area of occupancy around 1,000 km^2. Criteria A can not be used due to

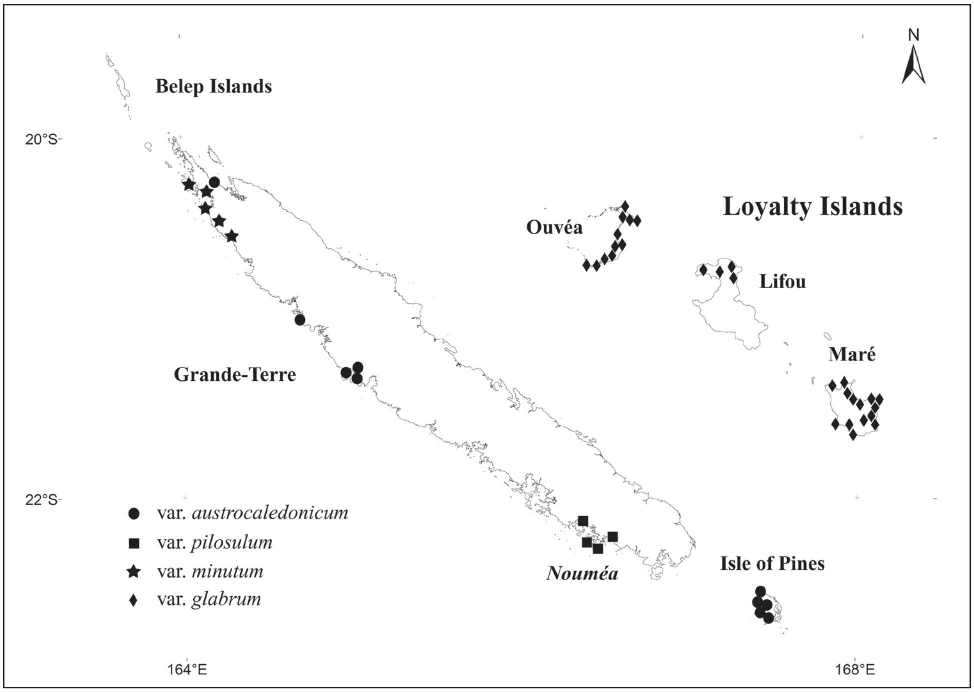

Figure 1. Distribution map of New Caledonian varieties of *Santalum austrocaledonicum* based on the specimens examined.

lack of knowledge of generation length and magnitude of population size reduction. Three locations (one per island) can be distinguished without any fragmentation. A continuing decline is observed and projected in terms of habitat quality and number of mature individuals due to harvest, competition with invasive plant species (*Schinus terebenthifolius* Raddi, *Pluchea odorata* (L.) Cass., *Lantana camara* L.), hybridization with other *S. austrocaledonicum* varieties used in plantations, and changes in the traditional agricultural system (less cultivated fields, short fallows...). Indeed, despite provincial regulations establishing quota and exploitability criteria, illegal logging is still occurring (Butaud et al. 2013) whereas hybridization is suspected due to sandalwood interspecific crossability (Tamla et al. 2012).

Common names. The common names recorded for *S. austrocaledonicum* var. *glabrum* are “tapakae” (pers. obs. 2014) or “tapakai” (Lenormand 1968) on Lifou, “wekesi” (Lormée et al. 2011) on Maré and “wahata” (Ozanne-Rivierre 1984) on Ouvéa.

Discussion. *Santalum austrocaledonicum* var. *glabrum* is the sole native sandalwood in the Loyalty Islands. Nevertheless, two other varieties have been introduced for plantation purposes, mainly in Maré and Lifou: var. *pilosulum* from Ouen Toro in Nouméa, and var. *austrocaledonicum* from Isle of Pines. These plantations can be considered a risk for the Loyalty variety because of hybridization and subsequent introgression. Plantations with exotic varieties should be discouraged to preserve the

Figure 2. Flowers of *Santalum austrocaledonicum* var. *glabrum* on Ouvéa atoll in January 2015 (specimen *Butaud 3414*).

Figure 3. Fruit of *Santalum austrocaledonicum* var. *glabrum* on Ouvéa atoll in January 2015 (specimen *Butaud 3414*).

Loyalty sandalwood's morphological, genetic, sylvicultural and chemical specificities (Bottin 2006, Bottin et al. 2007, Butaud et al. 2013, Ehrhart 1998). On the other hand, the sustainable exploitation of natural stands of Loyalty Islands sandalwood is becoming increasingly difficult with the lack of regeneration and the increasing international demand for sandalwood essential oil. Well-managed plantations with variety *glabrum* could be promoted to preserve the natural stands, to develop the Loyalty sandalwood sector, and to increase the production of heartwood and essential oil (Butaud 2011, Butaud et al. 2013).

The taxonomy of *S. austrocaledonicum* still needs to be further investigated. Indeed, the study of herbarium samples of var. *austrocaledonicum* and previous molecular and morphometric studies (Bottin 2006, Bouvet et al. 2005) showed significant variability. It is expected that future work supported by increased surveys and sampling of northern Grande-Terre sandalwood could reveal one or two new varieties, including one on Isle of Pines (taxon previously described as *Santalum homei* Seem.). Moreover, two new endemic varieties of *S. austrocaledonicum* are expected based on the recent study of Millet et al. (2012) on the genetic structure of Vanuatu sandalwood, one for the northern islands and one for the southern islands.

Key to New Caledonian varieties of *Santalum austrocaledonicum*

(adapted from Hallé 1988)

1 Inflorescence peduncles, rachis segments and outer surface of petals sparsely to densely hairy .... **var. *pilosulum***
– Inflorescence peduncles, rachis segments, outer surface of petals and petal internal margins glabrous **2**
2 Leaf blade usually less than 3.5 × 1.5 cm; petiole less than 7 mm; blade adaxial surface glaucous and bluish in color; interstaminal disk lobes very narrow (W/L = 1/3–1/4) .... **var. *minutum***
– Leaf blade usually more than 3.5 × 1.5 cm; petiole more than 7 mm; blade adaxial surface not glaucous and bluish in color; interstaminal disk lobes wide (W/L = 1/2) **3**
3 Seed usually more than 9 × 7.5 mm; globose (L/D < 1.2) .... **var. *glabrum***
– Seed usually less than 9.5 × 7.5 mm; ovoid (L/D > 1.2) .... **var. *austrocaledonicum***

Essential oil quality

Essential oil of *Santalum austrocaledonicum* was previously assessed for its composition and organoleptic properties, and was considered as a possible substitute for Indian sandalwood oil (*Santalum album*) but also as a promising new raw material for the fra-

Table 1. Main constituents of Loyalty Islands sandalwood (var. *glabrum*) essential oil compared to ISO standards for *S. album* oil.

Essential oil	(Z)-α-santalol (%)	(Z)-β-santalol (%)	(E)-lanceol (%)
ISO Standard 3518 : 2002 (*S. album*)	41 to 55	16 to 24	-
New Caledonia Braun et al. (2005)	38.2	18.2	9.1
Loyalty Oil 1	44.3	19.4	14.1
Loyalty Oil 2	47.4	21.3	8.5

grance industry (Braun et al. 2005). These evaluations were performed on essential oils resulting from mixtures of sandalwood varieties *glabrum* (Loyalty islands), *pilosulum* (surroundings of Nouméa) and *austrocaledonicum* (Isle of Pines), leaving unknown the quality of each individual variety.

To investigate the quality of Loyalty Islands sandalwood essential oil (var. *glabrum*), two samples were analyzed for their main sesquiterpenoids but also for their organoleptic properties:

– Loyalty Oil 1 (EI1) obtained in 2015 from Distillerie de Boulouparis (New Caledonia) with Ouvéa sandalwood,
– Loyalty Oil 2 (EI2) obtained in 2002 from Michel Point and processed by Koop Cuada distillery with Maré and Lifou sandalwoods.

The gas-chromatography was performed on an apolar column (HP-1, 10 m x 0.1 mm, film 0.1m, 50° (1') to 280° (2') at 50°/min., vector gas: hydrogen). Its results are given in Table 1 (C. Vial and S.A. Firmenich, pers. comm. 2015).

The organoleptic evaluation showed that the Loyalty essential oil is lighter and less milky than the Indian sandalwood; the former has also a character less woody but more animal and masculine (P.-A. Blanc and S.A. Firmenich, pers. comm. 2015).

These findings are in accordance with Braun et al. (2005), especially for the organoleptic evaluation. Moreover, the composition of both Loyalty essential oils meets the ISO Standard for Indian sandalwood, which was not the case for the samples of the previous study. Thus, *Santalum austrocaledonicum* var. *glabrum* or Loyalty sandalwood constitutes a true substitute for Indian sandalwood in the perfume industry.

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References

Bottin L (2006) Déterminants de la variation moléculaire et phénotypique d'une espèce forestière en milieu insulaire : cas de *Santalum austrocaledonicum* en Nouvelle-Calédonie. PhD Thesis, ENSAM – CIRAD, Montpellier, France.

Bottin L, Tassin J, Nasi R, Bouvet JM (2007) Molecular, quantitative and abiotic variables for the delineation of evolutionary significant units: case of sandalwood (*Santalum austrocaledonicum* Vieillard) in New Caledonia. Conservation genetics 8(1): 99–109. doi: 10.1007/s10592-006-9152-7

Bottin L, Verhaegen D, Tassin J, Olivieri I, Vaillant A, Bouvet JM (2005) Genetic diversity and population structure of an insular tree *Santalum austrocaledonicum* in New Caledonia archipelago. Molecular ecology 14: 1979–1989. doi: 10.1111/j.1365-294X.2005.02576.x

Bouvet JM, Cardi C, Verhaegen D, Butaud JF, Nasi R, Tassin J (2005) Molecular and morphometric diversity in *Santalum insulare* and *Santalum austrocaledonicum*. In: Thomson L, Bulai S, Sovea L (Eds) Proceedings of the regional workshop on sandalwood research, development and extension in the Pacific islands and Asia. 7–11 October 2002, Noumea, New Caledonia, 124–134.

Braun NA, Meier M, Hammerschmidt FJ (2005) New Caledonian Sandalwood Oil – a Substitute for East Indian Sandalwood Oil? Journal of Essential Oil Research 17: 477–480. doi: 10.1080/10412905.2005.9698969

Butaud JF (2011) Analyse bibliographique et technique des références existantes sur le santal en vue de sa valorisation économique en plantations à l'échelle de la Nouvelle-Calédonie. Rapport n°15/2011. IAC – Programme Forêt Sèche, Nouvelle-Calédonie, 1–238.

Butaud JF, Guy L, Lefeuvre JC (2013) Etude sur l'état de la ressource naturelle en santal aux Iles Loyauté pour le développement de filières aromatiques certifiées. Conservation International & Province des Iles Loyauté, 1–370.

Chauvin JP, Ehrhart Y (1998) Germination of two provenances of *Santalum austrocaledonicum* var. *austrocaledonicum*. In: Radomiljac AM, Ananthapadmanabho HS, Welbourn RM, Satyanarayana Rao K (Eds) Sandal and its products. Proceedings of an international seminar held on 18-19 December 1997 organised by the Institute of Wood Science and Technology (ICFRE) and Karnataka State Forest Department, Bangalore, India. ACIAR, 113–116.

Ehrhart Y (1998) Description of some sandal tree populations in the South West Pacific: consequences for the silviculture of these species and provenances. In: Radomiljac AM, Ananthapadmanabho HS, Welbourn RM, Satyanarayana Rao K (Eds) Sandal and its products. Proceedings of an international seminar held on 18-19 December 1997 organised by the Institute of Wood Science and Technology (ICFRE) and Karnataka State Forest Department, Bangalore, India. ACIAR, 113–116.

Guillaumin A (1925) Matériaux pour la flore de Nouvelle-Calédonie, XVIII : Révision des Santalacées. Bulletin de la Société Botanique de France 72(1): 89–91. doi: 10.1080/00378941.1925.10832717

Guillaumin A (1970) Le santal en Nouvelle-Calédonie. Journal d'agriculture tropicale et de botanique appliquée 17(7-8-9): 340–341. doi: 10.3406/jatba.1970.3073

Hallé N (1988) Santalaceae. In: Flore de la Nouvelle-Calédonie et Dépendances. Tome 15. MNHN, Paris.

Harbaugh DT (2007) A taxonomic revision of Australian northern sandalwood (*Santalum lanceolatum*, Santalaceae). Australian Systematic Botany 20: 409–416. doi: 10.1071/SB07009

Harbaugh DT, Baldwin BG (2007) Phylogeny and biogeography of the sandalwoods (*Santalum*, Santalaceae): repeated dispersal throughout the Pacific. American Journal of Botany 94: 1028–1040. doi: 10.3732/ajb.94.6.1028

Harbaugh DT, Oppenheimer HL, Wood KR, Wagner WL (2010) Taxonomic Revision of the Endangered Hawaiian Red-flowered Sandalwoods (*Santalum*) and Discovery of an Ancient Hybrid Species. Systematic Botany 35(4): 827–838. doi: 10.1600/036364410X539899

ISO (2002) Standard ISO 3518:2002: Oil of sandalwood (*Santalum album* L.). International Organization for Standardization, Geneva, Switzerland.

IUCN (2001) IUCN Red List categories (version 3.1). IUCN Species Survival Commission, Gland, Switzerland. http://www.iucnredlist.org/info/categories_criteria2001

Lenormand MH (1968) Vocabulaire des noms de plantes médicinales et alimentaires utilisées par les Mélanésiens de Lifou (Iles Loyalty). Journal d'Agriculture Tropicale et de Botanique Appliquée 15(4-5-6): 173–194. doi: 10.3406/jatba.1968.2979

Lormée N, Cabalion P, Hnawia ES (2011) Hommes et plantes de Maré, îles Loyauté, Nouvelle-Calédonie. IRD Editions, 1–358.

Millet L, Malapa R, Maggia L (2012) Diversité génétique des populations de Santalum austrocaledonicum (Santalaceae) au Vanuatu. Rapport Final Appel à projet 2008 - Ministère chargé de l'Outre-Mer. Institut Agronomique néo-Calédonien & Centre Technique et de Recherche en Agronomie du Vanuatu, 1–41.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. doi: 10.1038/35002501

Nasi RP (1994) Le genre *Santalum* L. en Nouvelle-Calédonie. CIRAD-forêt, Nouvelle-Calédonie. Unpublished manuscript.

Nasi RP (1995) Germination and seed dormancy in *Santalum austrocaledonicum*: a synopsis. In: Gjerum L, Fox JED, Ehrhart Y (Eds) Sandalwood Seed Nursery and Plantation Technology. Proceedings of a Regional Workshop for Pacific Island Countries held at Nouméa, New Caledonia, 1–11 August 1994, FAO, Suva, Fiji. RAS/92/361. Field Document 8: 59–74.

Ozanne-Rivierre F (1984) Dictionnaire Iaai - Français (Ouvéa Nouvelle-Calédonie) suivi d'un lexique français-iaai. Laboratoire de langues et civilisations à tradition orale, CNRS, Ivry-sur-Seine, France, 1–181.

Quemin C (1988) Etudes sur le santal (*Santalum austrocaledonicum*). Mémoire de 3$^{\text{ème}}$ année ENGREF. Centre Technique Forestier Tropical, France.

Shineberg D (1967) They came for sandalwood. Melbourne University Press, 1–299.

Stauffer H, Hürlimann H (1964) Santalacées. In: Guillaumin A (Ed.) Résultats scientifiques de la Mission franco-suisse de botanique en Nouvelle-Calédonie (1950-1952) n°3. Mémoires du Muséum National d'Histoire Naturelle, série B, Botanique 15(1): 14–15.

Tamla HT, Cornelius JP, Page T (2012) Reproductive biology of three commercially valuable *Santalum* species: development of flowers and inflorescences, breeding systems, and interspecific crossability. Euphytica 184: 323–333. doi: 10.1007/s10681-011-0530-y

Vieillard E (1861) Plantes utiles de la Nouvelle-Calédonie. Annales des Sciences Naturelles – Botanique, série 4 – 16: 28–76.

Virot R (1950) Le santal néo-calédonien. Revue internationale de botanique appliquée et d'agriculture tropicale 30: 79–86.

Appendix

Specimens examined

Santalum austrocaledonicum var. *glabrum* Hürl.: New Caledonia, Loyalty Islands.

Ouvéa. Lékine, 15 May 1976, *D. Bourret 815* (NOU); Lekiny, bord de route goudronnée, 24 January 2015, *J.-F. Butaud 3414* (NOU); Lekiny, bord de route goudronnée, 24 January 2015, *J.-F. Butaud 3415* (NOU); Lekiny, bord de route goudronnée, 24 January 2015, *J.-F. Butaud 3416* (NOU); Mouli, bord de route goudronnée, 24 January 2015, *J.-F. Butaud 3417* (NOU); Hnyimehe, route menant à la salle omnisport, 24 January 2015, *J.-F. Butaud 3421* (NOU); Hwaadrila, piste carrossable menant au Cap St Hilaire, 25 January 2015, *J.-F. Butaud 3422* (NOU); St Joseph, 14 August 1925, *A.U. Däniker 2546* (P scan, Z n.v.); Saint-Paul, 10 m, forêt, February 1980, *H.S. MacKee 37848* leg. *Douheret* (NOU, P scan); Nimaha, 29 April 1987, *H.S. MacKee 43516* leg. *Cherrier* (NOU, P scan, Z n.v.); Nimaha, 29 April 1987, *H.S. MacKee 43517* leg. *Cherrier* (P scan); Hanawa, forêt dense littorale, 29 April 1987, *H.S. MacKee 43520* leg. *Cherrier* (P scan); Hanawa, forêt dense littorale, 30 April 1987, *H.S. MacKee 43526* leg. *Cherrier* (NOU, P scan); Pointe de Mouli, 30 April 1987, *H.S. MacKee 43527* leg. *Cherrier* (NOU, P scan); Wakat, 30 April 1987, *H.S. MacKee 43530* leg. Cherrier (NOU, P scan); Wakat, 30 April 1987, *H.S. MacKee 43532* leg. *Cherrier* (NOU, P scan); Gossanat, forêt, 11 May 1987, *H.S. MacKee 43545* leg. *Ongat* (NOU, P scan); Gossanat, forêt, 11 May 1987, *H.S. MacKee 43546* leg. *Ongat* (NOU, P scan););

Teouta, 11 May 1987, *H.S. MacKee 43547* leg. *Ongat* (NOU, P scan); Teouta, 11 May 1987, *H.S. MacKee 43548* leg. *Ongat* (P scan); Ogniat, en lisière d'une cocoteraie, 11 May 1987, *H.S. MacKee 43549* leg. *Ongat* (P scan); Ogniat, en lisière d'une cocoteraie, 11 May 1987, *H.S. MacKee 43550* leg. *Ongat* (P scan); Takedji, 11 May 1987, *H.S. MacKee 43551* leg. *Ongat* (NOU, P scan); Takedji, 11 May 1987, *H.S. MacKee 43552* leg. *Ongat* (NOU, P scan); 19 July 1984, *P. Morat 7782* (NOU).

Lifou. July 1869, *B. Balansa 1692b* (P scan); 1928, *C. Bergeret 106* (P scan); Mutschaweng, 30 m, lisière de forêt, 18 February 1974, *H.S. MacKee 28169* (P scan, Z n.v.); Natchaom, 5 June 1987, *H.S. MacKee 43577* leg. *Wapae* (P scan); Natchaom, 5 June 1987, *H.S. MacKee 43578* leg. *Wapae* (P scan); Natchaom, 5 June 1987, *H.S. MacKee 43579* leg. *Wapae* (P scan); Ouanaham, 3 August 1987, *H.S. MacKee 43638* leg. Case (NOU, P scan); Hunete, 4 August 1987, *H.S. MacKee 43639* leg. *Case* (P scan); Hunete, dans un jardin, 4 August 1987, *H.S. MacKee 43640* leg. *Case* (NOU, P scan); Hunete, 4 August 1987, *H.S. MacKee 43641* leg. *Case* (NOU, P scan); March 1979, *J.-M. Veillon 3920* leg. *Lespès* (NOU); March 1979, *J.-M. Veillon 3921* leg. *Lespès* (NOU, P scan).

Maré. Medu, dans les fourrés, 22 December 1925, *A.U. Däniker 2546a* (P scan, Z n.v.); *E.I. Franc 1286* (P scan); La Roche, fourré secondaire, 24 April 1987, *H.S. MacKee 43602* leg. *Cornaille* (NOU, P scan); La Roche, fourré secondaire, 24 April 1987, *H.S. MacKee 43603* leg. *Cornaille* (NOU, P scan); Hnadid, 3 August 1987, *H.S. MacKee 43620* leg. *Cornaille* (NOU, P scan); Hnadid, fourré secondaire, 3 August 1987, *H.S. MacKee 43621* leg. *Cornaille* (NOU, P scan); Wakone, fourré secondaire, 3 August 1987, *H.S. MacKee 43622* leg. *Cornaille* (NOU, P scan); Tawainedre, Pewaete, fourré secondaire, 3 August 1987, *H.S. MacKee 43623* leg. *Cornaille* (NOU, P scan); Tawainedre, Pewaete, fourré secondaire, 3 August 1987, *H.S. MacKee 43624* leg. *Cornaille* (NOU, P scan); Cuadene, lisière de forêt, 3 August 1987, *H.S. MacKee 43625* leg. *Cornaille* (NOU, P scan); Penelo, fourré, 3 August 1987, *H.S. MacKee 43626* leg. *Cornaille* (NOU, P scan); Penelo, fourré secondaire, 3 August 1987, *H.S. MacKee 43627* leg. *Cornaille* (NOU, P scan); La Roche, jardin abandonné, 3 August 1987, *H.S. MacKee 43628* leg. *Cornaille* (NOU, P scan); Penelo, Dadac, fourré secondaire, 3 August 1987, *H.S. MacKee 43629* leg. *Cornaille* (NOU, P scan); Peyece, fourré secondaire, 3 August 1987, *H.S. MacKee 43630* leg. *Cornaille* (NOU); Kaewatine, fourré secondaire, 3 August 1987, *H.S. MacKee 43631* leg. *Cornaille* (NOU, P scan); Thogone, fourré secondaire, 3 August 1987, *H.S. MacKee 43632* leg. *Cornaille* (NOU, P scan); Ro, fourré secondaire, 3 August 1987, *H.S. MacKee 43633* leg. *Cornaille* (NOU, P scan); Pede, 3 August 1987, *H.S. MacKee 43634* leg. *Cornaille* (NOU, P scan); Pede, lisière de forêt, 3 August 1987, *H.S. MacKee 43635* leg. *Cornaille* (NOU, P scan); Eni, dans un jardin, 3 August 1987, *H.S. MacKee* 43636 leg. *Cornaille* (NOU, P scan, Z n.v.); Eni, cocoteraie littorale, 3 August 1987, *H.S. MacKee 43637* leg. *Cornaille* (NOU, P scan); 31 August 1987, *H.S. MacKee 43679* (NOU, P scan).

Santalum austrocaledonicum Vieill. var. *austrocaledonicum*:

New Caledonia, Grande-Terre. Arama, collines près de la mer, 1855-60, *E. Vieillard 1090* (holotype: P scan; isotypes: P [2] scan, K p.p. scan); Arama, collines près de la mer, 1855-60, *E. Vieillard 1090bis* (P scan; K p.p. scan); Vavouto, 26 September 2007, *J. Munzinger 4518* (NOU); Presqu'île de Pindai, propriété Kuhn, 2 July 1987, *J.M. Veillon 6422* (NOU, P scan); Presqu'île de Népoui, Pindai, 100 m, 18 November 1987, *J.M. Veillon 6574* (NOU, P scan); Presqu'île de Pindai, 100 m, 1 April 1988, *J.M. Veillon 6794* (NOU, P scan); Poya, forêt de Nekoro, 5 m, 28 September 1988, *J.M. Veillon 6917* (NOU); Poya, station de Mueo, propriété Johnston entre 10-20 m, 6 May 1998, *J.M. Veillon 8106* (NOU); Poya, station de Mueo, propriété Johnston entre 10-20 m, 6 May 1998, *J.M. Veillon 8107* (NOU, P scan).

New Caledonia, Isle of Pines. 1852, *Home s.n.* (BM scan); 1853, *MacGillivray 818b* (K scan);); Kuto, 1 February 1980, *H.S. MacKee 37852* leg. *Douheret* (NOU, P scan); Kuto, 9 February 1980, *H.S. MacKee 37853* leg. *Douheret* (NOU, P scan); Kuto, 1 February 1980, *H.S. MacKee 37872* leg. *Sevenet* (NOU, P scan); Kuto, 1 February 1980, *H.S. MacKee 37873* leg. *Sevenet* (NOU, P scan); Plateau, 25 February 1980, *H.S. MacKee 37874* leg. *Sevenet* (NOU, P scan); Ouro, 7 October 1987, *H.S. MacKee 43738* leg. *Quemin* (NOU, P scan); Ouro, 9 October 1987, *H.S. MacKee 43739* leg. *Quemin* (NOU, P scan); Kuto, 9 October 1987, *H.S. MacKee* 43740 leg. *Quemin* (NOU, P scan); Ouameo, 8 October 1987, *H.S. MacKee 43741* leg. *Quemin* (NOU, P scan); Oupotoue, Gadji, 8 October 1987, *H.S. MacKee 43742* leg. *Quemin* (NOU, P scan).

Santalum austrocaledonicum var. *pilosulum* N.Hallé: New Caledonia, Grande-Terre. Nouméa, Ouen Toro, 50 m, forêt côtière sur pente caillouteuse schisteuse 29 December 1971, *H.S. MacKee 24766* (holotype: P scan); Mont Dore, Ilot Pêcheur, 27 January 2015, *G. Gâteblé 708* (IAC); Paita, Stand de Tir, 27 January 2015, *G. Gâteblé 709* (IAC); Ouen Toro, March-April 1983, *Herbier Convention 10* (NOU); Ouen Toro, 12 February 1979, *M. Hoff 184* (NOU); Nouméa, île Nou, 5 February 1978, *H.S. MacKee 34688* (NOU, P scan); Plantule provenance Ouen Toro, Port-Laguerre, Paita, 1 October 1982, *H.S. MacKee 40837* (NOU, P scan); Nouméa, Ouen Toro, 50 m, 26 June 1987, *H.S. MacKee 43600* (NOU, P scan); Plantule provenance Ouen Toro, Port-Laguerre, Paita, 17 August 1987, *H.S. MacKee 43657* (NOU, P scan); Ouen Toro hill at S End of Noumea, 50 m, 31 January 1982, *G. MacPherson 4558* (NOU, P scan); Nouméa, Anse Vata, SW-Hang des Mt Ouen Toro, 100 m, 22 February 1964, *H.U. Stauffer 5701* (NOU, P scan, Z n.v.); Nouméa, Anse Vata, SW-Hang des Mt Ouen Toro, 100 m, 22 February 1964, *H.U. Stauffer 5702* (NOU, P scan, Z n.v.); Areal der IFO, Anse Vata, Nouméa, 11 March 1964, *H.U. Stauffer 5792* (NOU, P scan, Z n.v.); IFO, Anse Vata, 6 January 1965, *J.M. Veillon 18* (NOU, P scan); Nouméa, Colline du Ouen Toro, 1 April 1969, *J.M. Veillon 1934* (NOU, P scan).

Santalum austrocaledonicum var. *minutum* N.Hallé: New Caledonia, Grande-Terre. Montagne de Poum, pic 272, versant Est, 50 m, arbuste 3m, 25 March 1982, *J.M. Veillon 4852* (holotype: P n.v.; isotype NOU scan); Arama, collines près de la mer, 1855-60, *E. Vieillard 1090ter* (P scan); Koumac, Camp militaire, SMA, 8 August 1994, *A. Dessert 2* (NOU); Koumac, Babouillat, 14 June 2014, *G. Gâteblé 518* (IAC); Entrée de Koumac, terrain du SMA, 16 December 1994, *T. Jaffré 3257* (NOU, P scan); Au pied de la Tiebaghi 18 October 1970, *M. Schmid 3465* (NOU); Montagne de Poum, 25 March 1982, *B. Suprin 1744* (NOU); Montagne de Poum, 9 June 1982, *B. Suprin 1948* (NOU); Koumac, Camp militaire, SMA, 9 August 1994, *B. Suprin 2435* (NOU).

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Corrigenda: A revision of infrageneric classification in *Astelia* Banks & Sol. ex R.Br. (Asteliaceae)

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In Birch (2015), *Astelia banksii* A.Cunn. was incorrectly stated as present in New Caledonia within the "Included species and distribution" section for *Astelia* sect. *Isoneuron* Skottsb. (page 112). The correct distribution of *Astelia banksii* A.Cunn. is New Zealand. The *Astelia* sect. *Isoneuron* Skottsb. "Included species and distribution" section should read: Included species and distribution. New Caledonia: *A. neocaledonica* Schltr. New Zealand: *A. banksii* A.Cunn.

Additionally, the designation of the *A. microsperma* Colenso *pro parte* type was incorrectly stated (page 113) as being made by Skottsberg on page 81 (1934). This type designation was made by Skottsberg on page 87 (1934). The designation of the *A. microsperma* type should correctly read:

Astelia microsperma Colenso *pro parte*, Trans. & Proc. New Zealand Inst. 17: 251. 1885 (description of fruit only). Type: NEW ZEALAND. North Island. Seventy-mile Bush, between Norsewood and Dannevirke, County of Waipawa. 1884, *W. Colenso s.n.* (Lectotype: K [000524879, digital image!], fruiting material in packet, designated by Skottsberg, 1934, 87).

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References

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Skottsberg C (1934) Studies in the genus *Astelia* Banks et Solander. Kungliga Svenska Vetenskapsakademiens Handlingar 14(2): 1–128.