

Two new genera in the *Omphalodes* group (Cynoglosseae, Boraginaceae)

Dous novos xéneros no grupo *Omphalodes* (Cynoglosseae, Boraginaceae)

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Abstract

Omphalodes (Boraginaceae, Cynoglosseae) molecular phylogenetic relationships are surveyed in the context of the tribe Cynoglosseae, being confirmed that genus *Omphalodes* is paraphyletic. Our work is focused both in the internal relationships among representatives of *Omphalodes* main subgroups (and including *Omphalodes verna*, the type species), and their relationships with other Cynoglosseae genera that have been related to the *Omphalodes* group. Our phylogenetic analysis of ITS and *trnL-trnF* molecular markers establish close relationships of the American *Omphalodes* with the genus *Mimophytum*, and also with *Cynoglossum paniculatum* and *Myosotidium hortensia*. The southwestern European annual *Omphalodes* species form a discrete group deserving taxonomic recognition. We describe two new genera to reduce the paraphyly in the genus *Omphalodes*, accommodating the European annual species in *Iberodes* and *Cynoglossum paniculatum* in *Mapucheia*. The pollen of the former taxon is described in detail for the first time.

Keywords: Madrean-Tethyan, phylogeny, pollen, systematics, taxonomy

Resumo

Neste estudo analisamos as relacións filoxenéticas de *Omphalodes* (Boraginaceae, Cynoglosseae) no contexto da tribo Cynoglosseae, confirmándose como parafilético o xénero *Omphalodes*. Neste traballo colocamos o foco tanto nas relacións internas entre diversos representantes dos principais subgrupos dentro de *Omphalodes* (e incluíndo *Omphalodes verna*, a especie tipo), como nas relacións destes con outros xéneros das Cynoglosseae que teñen sido relacionados co grupo *Omphalodes*. A nosa análise filoxenética cos marcadores moleculares ITS e *trnL-trnF* establece a proximidade entre as *Omphalodes* americanas e o xénero *Mimophytum*, así como con *Cynoglossum paniculatum* e *Myosotidium hortensia*. As *Omphalodes* anuais do suroeste europeo forman un grupo característico merecedor de recoñecemento taxonómico. Describimos dous novos xéneros para reducir a parafilia do xénero *Omphalodes*, acomodando as especies europeas anuais en *Iberodes* e *Cynoglossum paniculatum* en *Mapucheia*. O pólen deste último taxón é descrito en pormenor por vez primeira.

Palabras chave: Madreano-Tetiano, filoxenia, pólen, sistemática, taxonomía

INTRODUCTION

The genus *Omphalodes* Moench (1794: 419) (Boraginaceae) comprises ca. 25 annual, biennial and perennial herbaceous species mostly from temperate habitats of the northern hemisphere. The distribution of the genus showed a striking disjunct range, with three separate groups of species occurring in North America (Mexico and Texas), Western Eurasia and the Japanese archipelago, but the Japanese species were recently accommodated in the new genus *Nihon* (OTERO *et al.*, 2014). As a result, *Omphalodes* as is now recognized appears to fit relatively well to the Madrean-Tethyan Flora biogeographical pattern (RAVEN & AXELROD, 1974; WEN & ICKERT-BOND, 2009), a disjunct pattern of species distribution between the Mediterranean areas in Eurasia and North America.

The internal systematics of Boraginaceae have paid particular attention to fruit morphological characters to establish distinctions among and within tribes, being *Omphalodes* traditionally recognized as a member of the tribe Cynoglosseae (DE CANDOLLE, 1846; JOHNSTON, 1924; POPOV, 1953; RIEDL, 1997). The Cynoglosseae show great variation in external fruit morphology, and therefore the traditional systematics of the tribe heavily relied on few relatively constant traits, mainly gynobase shape and apical attachment scar position in the nutlet (JOHNSTON, 1924; RIEDL, 1997). However LÅNGSTRÖM & CHASE (2002) molecular phylogenies suggested that the Cynoglosseae should be merged with other tribes (LÅNGSTRÖM & OXELMAN, 2003; WEIGEND *et al.*, 2010; NAZAIRE & HUFFORD, 2012; WEIGEND *et al.*, 2013; OTERO *et al.*, 2014), among them the large tribe Eritrichieae, morphologically defined by having a nutlet attachment scar in a basal position (JOHNSTON, 1924; AL-SHEHBAZ, 1991; RIEDL, 1997). These results gave final support to previous considerations that Cynoglosseae systematics based on fruit morphology could be rather artificial (AL-SHEHBAZ, 1991). In this context, the genus *Omphalodes* never accommodated well in terms of the attachment scar to the Cynoglosseae or the Eritrichieae, having big scars covering a great part of the inner side of the nutlet (POPOV, 1953) that hardly can be considered apical. This feature can be

related to a generic trend to nutlets comparatively bigger than gynobasis (BRAND, 1921). Beside that, the most defining trait of *Omphalodes* is dorsally compressed nutlets with a wing circling the margins of the upper side. Published molecular phylogenies (WEIGEND *et al.* 2013) and our own previous results (see COUTINHO *et al.*, 2012) have revealed unsuspected close relationships of *Omphalodes* with two taxa, *Myosotidium hortensia* Baillon (1890: 383) and *Cynoglossum paniculatum* Hook. & Arn. (1830: 37). The former is a shrub species occurring in the Chatham archipelago in New Zealand, very different to *Omphalodes* both in terms of habit and inflorescence but in fruit features resembles *Omphalodes*, being somewhat winged nutlets and an expanded scar. The latter is a Chilean endemic that was considered a member of the core *Cynoglossum* group by BRAND (1921). This species has unwinged nutlet covered by glochidiate hooks, a typical feature of the genus *Cynoglossum* (JOHNSTON, 1924, 1927), but that is also present in a number of taxa of the Cynoglosseae (i.e. *Trichodesma*, *Lappula*, ...).

Since *Myosotidium hortensia* and *Cynoglossum paniculatum* are recovered among the *Omphalodes* in the phylogenetic works cited above, the genus as currently circumscribed is paraphyletic. In this work we want firstly to explore the phylogenetic relationships, based both on plastid (*trnL-trnF* spacer) and nuclear (ITS1+5.8S+ITS2 region) molecular markers of *Omphalodes* and *Cynoglossum paniculatum* with other possibly close genera in family-wide phylogenetic analysis.

Secondly, we want to clarify the identity of two conspicuous groups of *Omphalodes*, the Mexican-Texan group and the south-western European annual taxa group. Regarding the former, possible relationships with genus *Mimophytum* Greenm. (1905: 242) have to be surveyed. This genus is a member of the Cynoglosseae of which habit similarities with Mexican *Omphalodes* have been highlighted, in spite of its glochidiate fruit (GREENMAN, 1905; JOHNSTON, 1924). In this sense, recently, *Omphalodes richardsonii* Nesom 1988:27, the only previously *Omphalodes* described species with slightly glochid-like appendages in fruit has been transferred to the hitherto monotypic genus *Mimophytum* (*Mimophytum richardsonii* (Nesom 1988: 27) NESOM,

2013: 9). Regarding the European annuals, it must be noted that the annual species of south western Europe form a well identified group of taxa (POPOV, 1953; GRAU, 1967). , being *Omphalodes aliena* A. Gray ex Hemsley (1882: 377) and *Omphalodes alienoides* G.L. Nesom (2013: 10), two Texan-Mexican species closely related between them, the only two other annual (sometimes biennial) taxa in the genus. Identity and possible relationships between these groups are also surveyed. And thirdly, in order to have a more natural classification, we will propose new genera, supported by the molecular phylogeny and giving morphological and palynological information of the characteristic features of the genera, to accommodate the nomenclature to the evolutionary relationships.

MATERIAL AND METHODS

Phylogenetic analysis

Plant material used in the phylogenetic analysis derives from field collections or herbarium sheets. The ingroup dataset included *Myosotidium hortensia*, *Mimophytum omphalodoides* and *Cynoglossum paniculatum*, together with some representatives of the three main *Omphalodes* groups, 10 of 25 species, but including *Omphalodes verna* Moench (1794: 420), the type species of the genus. The main morphological-geographical groups (POPOV, 1953) of *Omphalodes* are the Texan-Mexican species, the southwestern European annual species and the western Eurasian perennial species, the last represented by *Omphalodes verna* and *Omphalodes nitida* Hoffmanns. & Link (1811: 194).

The taxonomic coverage is completed with a wide representation of the Cynoglosseae (Table I), including *Cynoglossum officinale* L. (1753: 134), type species of both the genus and the tribe.

DNA isolation followed the CTAB method (DOYLE & DOYLE, 1987). The *trnL-trnF* spacer of chloroplast DNA was amplified using primers *e* and *f* from TABERLET *et al.* (1991); regions ITS1, 5.8S and ITS2 used the ITS2 and ITS5 (modified) primers of WHITE *et al.* (1990). The sequence of the modified ITS5 primer is

5'-GGAAGGAGAAGTCGTAACAAGG-3'.

PCR reactions were performed using Ready-To-Go PCR beads (GE Healthcare) in a Thermo Electron PXE 0,2 thermal cycler. PCR conditions were an initial denaturation of 94 °C for 3-4 min, followed by 35 cycles of 94 °C denaturation for 1-2 min, 46-53 °C annealing for 1 min and 71 °C extension for 1-5 min sec. Sequencing was performed in an automated DNA sequencer (Model 377, Applied Biosystems).

Automatic alignments were conducted by Geneious (Geneious 7.1.4, created by Biomatters. Available from <http://www.geneious.com/>) and followed by manual analysis in BioEdit (HALL, 1999). The complete data set had 1277 aligned positions, of which 763 belonged to the ITS1+5.8S+ITS2 region and 514 to the *trnL-trnF*. PartitionFinder (LANFEAR *et al.*, 2012) was used for selecting best-fit partitioning schemes and models of molecular evolution using both the “greedy” and “all” algorithms. Four representatives of the order Boraginales belonging to the Cordiaceae, Hydrophyllaceae and Heliotropiaceae families were used as outgroup (Table I). Maximum Likelihood phylogenetic analyses were run in RAxML (STAMATAKIS *et al.*, 2008). Three subset partitions were identified with two different selected models, GTR+G for ITS1+ITS2 and for *trnL-F* and GTR+I+G for 5.8S gene. RAxML partitioned analyses used these evolutionary substitution models. Statistical support was calculated by 1000 bootstrap replicates.

Parsimony analyses were performed using PAUP 4.0b10 (SWOFFORD, 2002) for each region separately and in combination. Heuristic searches were conducted with 1000 random stepwise addition replicates, with TBR branch swapping, with Multrees on, all characters treated as equally weighted and unordered and branches collapsed if the minimum length was zero and gaps considered as missing data. Relative clade support was assessed using bootstrap analyses (Felsenstein, 1985), which were performed running 100 bootstrap replicates, each with 1000 random stepwise addition replicates, equal weighting and TBR branch swapping.

Bayesian phylogenetic analyses were performed using MrBayes v3.1.2 (RONQUIST & HUELSENBECK, 2003). The selected model

Taxon	ITS	trnL-trnF
Outgroups		
<i>Cordia decandra</i> Hook. & Arn. (outgroup)	EF688903	EF688851
<i>Heliotropium curassavicum</i> Hook. (outgroup)	EF688843	EF688896
<i>Heliotropium paronychioides</i> A.DC. (outgroup)	*KP027100	*KP027131
<i>Hydrophyllum capitatum</i> Douglas ex Benth. (outgroup)	AF091169	HQ412982
Tribes of Boraginoideae (not Cynoglosseae s.l.)		
<i>Antiphytum heliotropioides</i> A.DC.	AJ555898	
<i>Borago officinalis</i> L.	FJ763248	FJ763302
<i>Echium rosulatum</i> Lange	*KP027097	*KP027129
<i>Glandora difussa</i> (Lag.) D.C.Thomas	FJ789863	FJ789881
<i>Lithospermum officinale</i> L.	FJ763189	FJ763254
<i>Lithospermum viride</i> Greene	FJ763209	FJ763271
<i>Moritzia lindenii</i> Benth.	GQ285231	GQ285255
<i>Ogastemma pusillum</i> (Coss. & Durieu ex Bonnet & Barratte) Brummitt	AB808586	FJ763265
<i>Pentaglottis sempervirens</i> (L.) Tausch	*KP027112	*KP027143
<i>Pulmonaria longifolia</i> Bast. ex. Boreau	*KP027114	*KP027145
Cynoglosseae s.l. clade		
<i>Actinocarya tibetica</i> Benth.	JX976803	KC542502
<i>Amsinckia vernicosa</i> Hook. & Arn.	*KP027090	*KP027122
<i>Asperugo procumbens</i> L.	*KP027091	*KP027123
<i>Bothriospermum tenellum</i> (Hornem.) Fisch. & C.A.Mey.	AB808585	KC542507
<i>Brachybotrys paridiformis</i> Maxim. ex Oliv.	JQ388498	
<i>Caccinia macranthera</i> BRAND	*KP027092	*KP027124
<i>Cryptantha affinis</i> Greene	JQ513395	JQ582297
<i>Cryptantha virginensis</i> Payson	*KP027093	*KP027125
<i>Cynoglossum amabile</i> Stapf & J.R.Drumm.	*KP027094	*KP027126
<i>Cynoglossum bottae</i> Deflers	*KP027120	
<i>Cynoglossum clandestinum</i> Desf.	FR715300	
<i>Cynoglossum creticum</i> Mill.	DQ320749 / DQ320759	*KP027151
<i>Cynoglossum officinale</i> L.	*KP027095	*KP027127
<i>Eritrichium nanum</i> Schrad. ex Gaudin	JQ388499	JQ388581
<i>Eritrichium splendens</i> Kearney	JQ388501	JQ388582
<i>Hackelia deflexa</i> Opiz	*KP027098	*KP027130
<i>Harpagonella palmeri</i> A.Gray	*KP027099	KC542590
<i>Lappula redowskii</i> (Hornem.) Greene	*KP027121	
<i>Lappula squarrosa</i> Dumort.	*KP027101	*KP027132
<i>Lindelofia longiflora</i> Baill.	*KP027115	*KP027146
<i>Memoremea scorpioides</i> (Haenke) A.OTERO, Jim.Mejías, Valcárcel & P.Vargas	*KP027102	*KP027133
<i>Mertensia franciscana</i> A.Heller	*KP027103	*KP027134
<i>Microula tibetica</i> Maxim.	*KP027104	*KP027135
<i>Myosotis alpestris</i> F.W.Schmidt	*KP027106	*KP027137
<i>Nihon japonicum</i> (Thunb.) A.OTERO, Jim.Mejías, Valcárcel & P.Vargas	DQ320747/ DQ320757	*KP027152
<i>Paracaryum rugulosum</i> Boiss.	*KP027110	*KP027141
<i>Pardoglossum watieri</i> (Batt. & Maire) Barbier & Mathez	FR715321	
<i>Pectocarya setosa</i> A.Gray	*KP027111	*KP027142
<i>Plagiobothrys figuratus</i> (Piper) I.M.Johnst. var. <i>corallicarpus</i> (Piper) K.L.Chambers	*KP027113	*KP027144
<i>Rochelia cardiosepala</i> Bunge	AB564701	
<i>Rochelia disperma</i> (L.) Wettst.	*KP027116	*KP027147
<i>Solenanthus circinatus</i> Ledeb.	FR715324	
<i>Thyrocarpus sampsonii</i> Hance	*KP027119	*KP027150
<i>Trichodesma boissieri</i> Post	*KP027117	*KP027148
<i>Trigonotis peduncularis</i> (Trevis.) Benth. ex Baker & S.Moore	*KP027118	*KP027149

Taxon	ITS	trnL-trnF
Omphalodes clade		
<i>Iberodes brassicifolia</i> (Lag.) M. Serrano, R. Carbajal and S. Ortiz	AY837606	AY837607
<i>Iberodes commutata</i> (G. López) M. Serrano, R. Carbajal and S. Ortiz	AY837604	AY837610
<i>Iberodes kuzinskyana</i> (Willk.) M. Serrano, R. Carbajal and S. Ortiz	AY837595	AY837609
<i>Iberodes linifolia</i> (L.) M. Serrano, R. Carbajal and S. Ortiz	AY837605	AY837612
<i>Iberodes littoralis</i> (Lehm.) M. Serrano, R. Carbajal and S. Ortiz subsp. <i>littoralis</i>	AY837603	AY837611
<i>Iberodes littoralis</i> (Lehm.) M. Serrano, R. Carbajal and S. Ortiz subsp. <i>gallaecica</i> (M. Lainz) M. Serrano, R. Carbajal and S. Ortiz	AY837600	AY837611
<i>Mapucheia paniculata</i> (Hook. & Arn.) M. Serrano, R. Carbajal & S. Ortiz	*KP027096	*KP027128
<i>Mimophytum omphalodoides</i> Greenm.	*KP027105	*KP027136
<i>Myosotidium hortensia</i> (Decne.) Baill.	GQ281764	KC542488
<i>Omphalodes alienoides</i> Nesom	*KP027107	*KP027138
<i>Omphalodes cardiophylla</i> A.Gray ex Hemsl.	*KP027108	*KP027139
<i>Omphalodes nitida</i> Hoffmanns. & Link	AY837615	AY837613
<i>Omphalodes verna</i> Moench	*KP027109	*KP027140

Table I. List of species included in this research ordered by main clades in Figure 1. The Genbank accession numbers of the nucleotide sequences are presented. Asterisks indicate new Genbank accession numbers produced for this work.

Taboa I. Listaxe de especies incluídas neste traballo ordenadas segundo os cladros principais da Figura 1. Preséntanse tamén os números de orde de secuencias nucleotídicas do GenBank. Os asteriscos indican aquelas novas secuencias en GenBank producidas para este traballo.

was General Time Reversible for the two datasets. Four simultaneous runs with one cold and three heated chains each were run for 1,000,000 generations, and every 100th tree was sampled. The first 2000 trees (20%) of each run were discarded as burn-in, and a 50% majority rule consensus tree was constructed, with clade support reported as posterior probabilities.

Palynological study of *Cynoglossum paniculatum* and morphological information for description of new taxa

Pollen samples were collected from herbarium specimens of *Cynoglossum paniculatum* in order to compare with *Omphalodes* pollen types described by COUTINHO *et al.* (2012). All pollen samples were subjected to acetolysis according to the method proposed by ERDTMAN (1960). The terminology used for pollen descriptions follows PUNT *et al.* (2007) and HESSE *et al.* (2009). For Light Microscopy, pollen grains were pre-treated with butanol, mounted in silicone oil (ANDERSEN, 1960) and observed using a Motic BA 310 light microscope with an oil immersion objective lens (magnification=1000×). The exine thickness was measured in 15 pollen grains from each taxon using a micrometer. For SEM, the acetolysed pollen grains were air dried (AHN & LEE,

1986), mounted on aluminium stubs and coated with a 30 nm layer of gold/palladium for eight minutes at high vacuum in a sputtering chamber (Jeol JFC-1100 Ion Sputter). Pollen grains were then observed with a Zeiss FESEMULTRA plus scanning electron microscope (operating at 15 kV) and micrographs of at least 15 pollen grains were taken using ImageTool (v.3.0 for Windows, University of Texas Health Science Centre, San Antonio, TX, USA). Due to the small pollen size, micrographs were then used for morphometric analysis. The following characters were measured in 15 pollen grains: polar axis (P), equatorial axis (E), maximum width of the grain, pseudocolpi length, colpori ectoaperture length and endoaperture width. The P:E ratio was then calculated. The general pollen morphology and particularly the type of sculpture of the margins of the pseudocolpi and colpori and the presence/absence of a ring-like aperture were studied also from the SEM micrographs. The ring-like aperture is an outer circumferential aperture at the equator of the pollen grain (following HESSE *et al.*, 2009). Descriptive statistics (mean and standard deviation of the mean) of the quantitative variables were calculated. Scanning Electron Micrographs were also used to observe and to show some morphological features of the fruit, like surface and shape of glochidiate appendages of the fruit. Observation

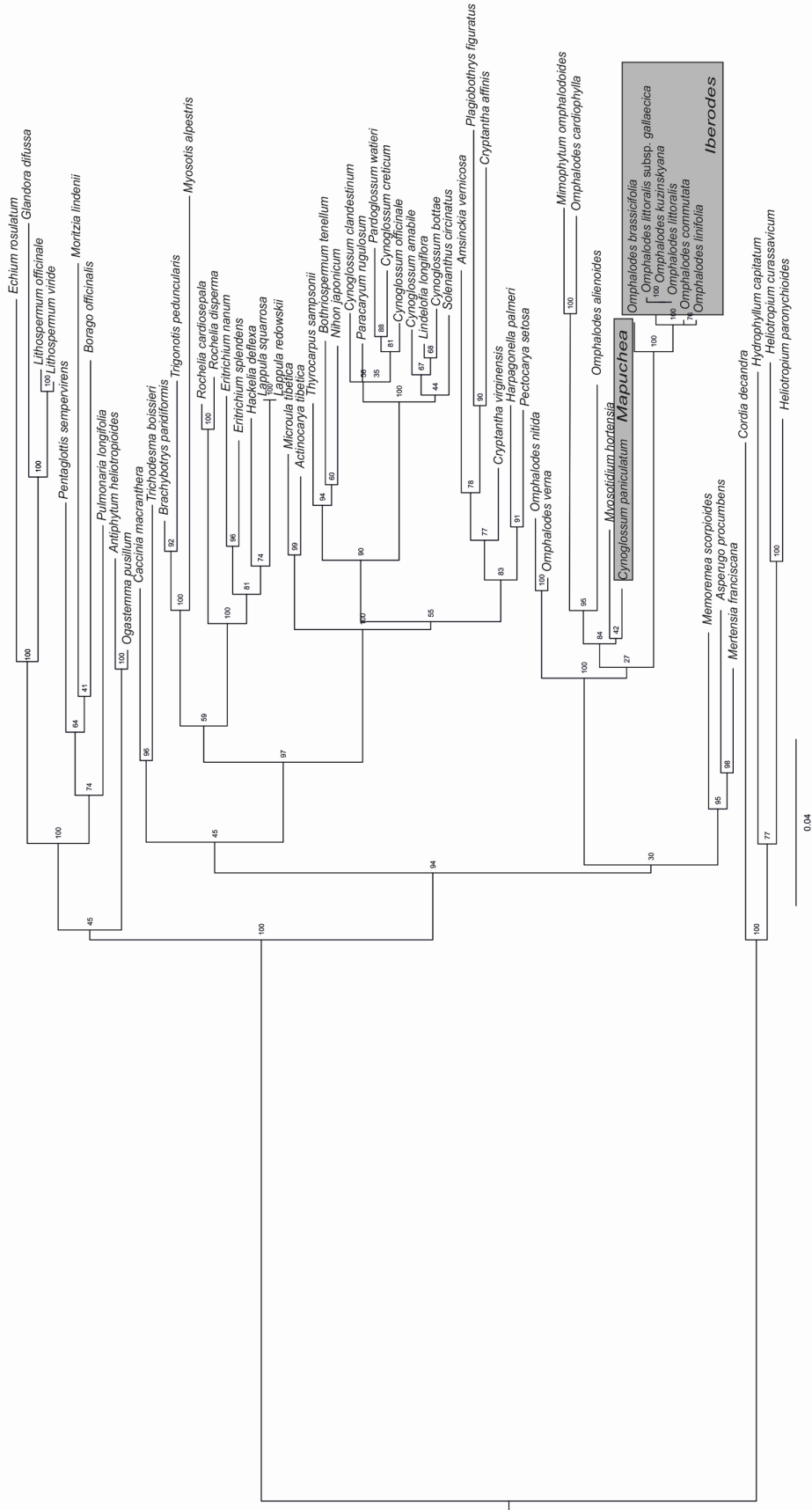


Figure 1. Maximum likelihood tree based on ITS and *trnL-trnF* markers with bootstrap support on nodes. *Mapucheae* and *Iberodes* clades are indicated in gray. **Figura 1.** Árbore de máxima verosimilitud baseada en marcadores ITS e *trnL-trnF* con apoio bootstrap sobre os nodos. Clados *Mapucheae* e *Iberodes* indicados en grisallo.

of the general features of the plant were conducted and incorporated in the taxonomic description of the new taxa.

Flower and nutlet samples were collected from 17 *Omphalodes* species, two *Mimophytum* species, *Myosotidium hortensia* and five *Cynoglossum* species, including the type species of the genus *Cynoglossum officinale*, and *Cynoglossum paniculatum* from the following Herbaria: COI, CONC, K, SALAF and SANT.

RESULTS

Phylogenetic analysis

Only results from the Maximum Likelihood (ML) analysis are described and shown in Figure 1. Maximum Parsimony (MP) and Bayesian analyses revealed no conflict between plastid and nuclear regions and recovered topologies highly congruent with the more conservative tree from the ML analysis. The Bayesian tree with Posterior Probabilities, and bootstrap values from MP on the branches can be seen as supplementary material (Fig. S1), or on request to the authors.

The Cynoglosseae s.l. clade is recovered with high bootstrap support (94 BS) (Fig. 1). Four well supported clades, although with not resolved relationships, are recovered in the Cynoglosseae s.l., in agreement with previous phylogenetic analysis (WEIGEND *et al.* 2013, OTERO & *et al.* 2014). The *Trichodesma* R. Brown (1810: 496) clade (BS 96), including *Caccinia* Savi (1832: 1), the core Cynoglosseae clade (BS 97) with the type species *Cynoglossum officinale* and allied taxa, and including the old tribe Eritrichieae among others, the *Mertensia* Roth (1797: 34) clade (95 BS), including *Memoremea* Otero, Jim.-Mejías, Valcárcel & P. Vargas (2014: 266) and *Asperugo* L. (1753: 138) and the *Omphalodes* clade (100 BS), including *Myosotidium hortensia*, *Mimophytum omphalodoides* and one *Cynoglossum* species, the South-American *Cynoglossum paniculatum*.

Within the *Omphalodes* clade, three main clades are recovered, although with the relationships among them unresolved. Two of these clades are recovered with high BS support, the European annual species clade (100 BS), a clade grouping the

Eurasian perennial representatives, with the type species *Omphalodes verna* and *Omphalodes nitida* (100 BS), meanwhile the third clade is recovered only with relatively good support (84 BS), the last including *Myosotidium hortensia*, *Cynoglossum paniculatum*, *Mimophytum omphalodoides* and two representatives of the American *Omphalodes*: *Omphalodes cardiophylla* A. Gray ex Hemsl. (1882: 377) and the annual *Omphalodes alienoides*. *Mimophytum omphalodoides* forms a well supported clade (95 BS), with *Omphalodes alienoides* and *Omphalodes cardiophylla*. Interestingly, *Mimophytum omphalodoides* and *Omphalodes cardiophylla* group in a well supported subclade (100 BS) with *Omphalodes alienoides* as sister.

Pollen morphology of *Cynoglossum paniculatum*

Pollen grains are radially symmetrical, isopolar, rectangular-elliptic and slightly constricted at the equator, and rounded at the poles, sub-circular to subhexagonal in polar view; prolate to perprolate; P/E = 1.85–2.08; size very small; P = 7.00–9.30 μm ; E = 3.60–5.00 μm ; maximum width of the grains = 4.20–5.30 μm . Apertural system is 6-zono-heterocolpate: three pseudocolpi 5.50–7.60 μm long; three colpi elongated-rhomboidal, operculate with ectoaperture 6.00–7.00 μm long; margins of the pseudocolpi and of the colpi thin, conspicuously granulate; equatorial ring-like aperture absent; polar pori absent. Exine is 0.5–1.0 μm thick, costae slightly developed. Sculpture finely granulate to scabrate (Fig. 2). Pollen is *Omphalodes verna* type (COUTINHO *et al.*, 2012).

DISCUSSION

Our phylogenetic results show that the *Omphalodes* group is one of the four main clades of the Cynoglosseae s.l.. Albeit the relationships among these clades are not well resolved in our phylogeny, the clades reflect the initial processes of divergence experimented by the ancestors of the extant members of the tribe. These results agree with other published phylogenies (WEIGEND *et al.* 2013; OTERO *et al.*, 2014). The internal topo-

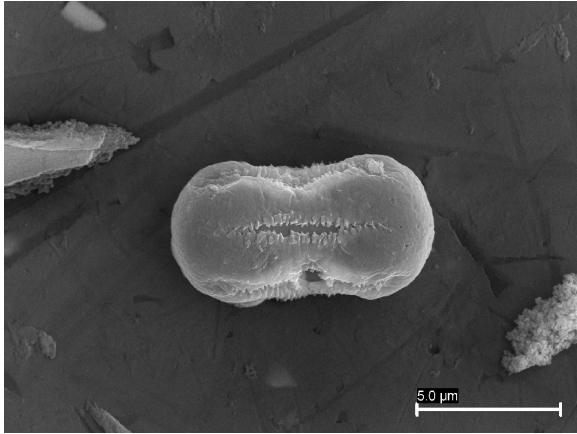


Figure 2. SEM micrograph of pollen grain of *Mapucheana paniculata* in equatorial view (CONC herbarium).

Figura 2. Fotografía MEV (Microscopio Electrónico de Varrimento) dun gran de pólen de *Mapucheana paniculata* en vista ecuatorial (herbarium CONC).

logy of the *Omphalodes* clade makes the genus paraphyletic, since within one of three main subclades are grouped *Myosotidium hortensia*, *Mimophytum omphalodoides* and *Cynoglossum paniculatum* together with two American *Omphalodes* species. In order to reduce the paraphyly of *Omphalodes* the genera *Mimophytum* and *Myosotidium*, and the species *Cynoglossum paniculatum* could be transferred to *Omphalodes*. However, *Myosotidium hortensia* is a very characteristic species, a robust perennial herb with huge leaves (petiole and lamina of the basal leaves up to 90 cm) with dense corymbose cymes, endemic of the Chatham islands (New Zealand). In our view, it is preferable to maintain the New Zealand taxon in the genus *Myosotidium* and to consider the creation of a new genus to accommodate the endemic Chilean *Cynoglossum paniculatum*, since this species is phylogenetically nested in the *Omphalodes* clade far from the type species of the genus *Cynoglossum* (*C. officinale*) and allied taxa, and therefore can no longer be considered a member of this genus. *Cynoglossum paniculatum* is clearly divergent in morphology from the remaining taxa of the clade, with distinctive features as the unwinged, ovoid, glochidiate nutlet or the glochid fruit appendages with unicuspidate excrecences.

Within the subclade including *Mimophytum* and two North American *Omphalodes*, *Omphalodes cardiophylla* is more related to *Mimophytum*

omphalodoides than to *Omphalodes alienoides*. To completely eliminate the paraphyly of *Omphalodes* it could be proposed the transference of these *Omphalodes* species to the genus *Mimophytum*, as was recently done for another Mexican *Omphalodes* species (NESOM, 2013). This clade is both geographically and morphologically consistent, grouping taxa with a mostly Mexican distribution range and sharing some synapomorphies, as the distinctive cordate leaves. However, we prefer not to propose any nomenclatural changes in this North American clade because of the relatively important morphological differences and nucleotide divergences among the species considered in our analyses. More North American *Omphalodes* taxa should be analysed to deal with the internal systematics of this subclade.

Finally, we also propose the splitting of *Omphalodes* with the creation of a new genus for the annual western European group of species, of which a possible immediate relationship with the annual American group is discarded by our analysis. This clade is morphologically well defined with respect to the core *Omphalodes*, a perennial group of *c.* seven mostly trailing and rhizomatous species represented in our analyses by the clade of *Omphalodes nitida* and *Omphalodes verna*, the type species of the genus. Currently ongoing work of biogeographical basis (unpublished data) including more markers and taxa indicates that the remaining western Eurasian perennial *Omphalodes* cluster with *Omphalodes verna*. The main diverging characters of the western European annual group with respect to the typical perennial *Omphalodes* group are, besides the annual habit, the distinctly accrescent glabrous calyx with big trichomes, awn-bearing nutlet and highly pyramidal gynobasis. Distinctive morphological features of the proposed new genera are described in detail below, in the diagnoses of the Taxonomic Treatment section.

Taxonomic treatment

1. *Iberodes* M. Serrano, R. Carbajal & S. Ortiz, *gen. nov.*

Diagnosis: Genus of annual herb species occurring in South-western Europe, mainly in the Iberian peninsula. *Iberodes* is strictly annual

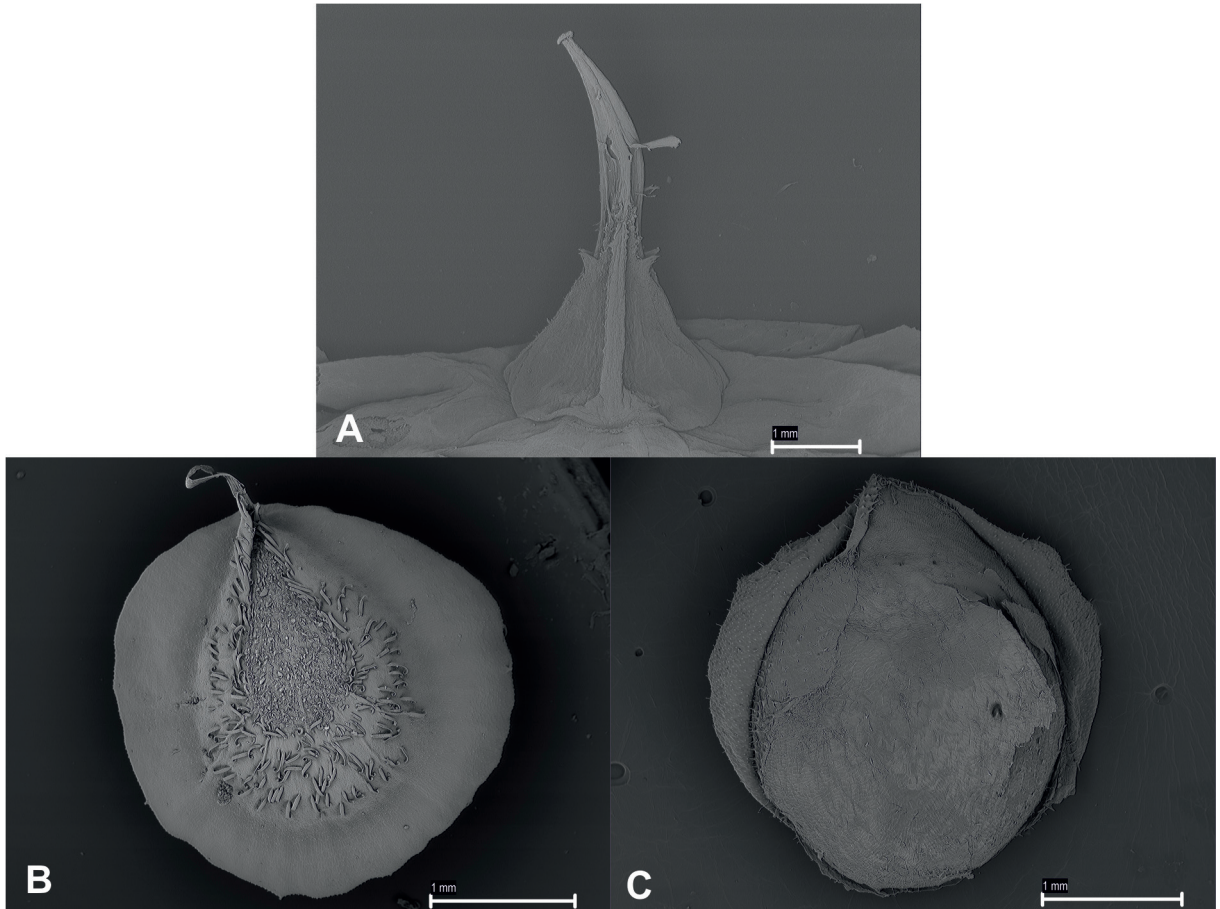


Figure 3. SEM micrographs of *Iberodes* and *Omphalodes* fruit characters (SANT herbarium). A. *Iberodes brassicifolia* gynobase after nutlet freeing to show the highly pyramidal gynobase of genus *Iberodes*. B. Under side of *Iberodes linifolia* nutlet showing the apical awn of *Iberodes*. C. Under side of *Omphalodes nitida* nutlet without awn.

Figura 3. Fotografías MEV de caracteres do froito de *Iberodes* e *Omphalodes* (herbario SANT). A. Xinobase de *Iberodes brassicifolia* despois da liberación das núculas mostrando a elevada xinobase piramidal do xénero *Iberodes*. B. Face inferior de núcula de *Iberodes linifolia* mostrando a aresta apical de *Iberodes*. C. Face inferior de núcula de *Omphalodes nitida* sen aresta.

whereas *Omphalodes* is perennial, frequently rhizomatous. The habit is erect or somewhat divaricated, particularly when withering in fruiting, never trailing as in many *Omphalodes*. The stems branch in the upper part is always subglabrous, slightly succulent, and the leaves are unpetiolate, in contrast with *Omphalodes*. The corolla is mostly white, rarely bluish, as opposite to *Omphalodes*. Pedicel is glabrous and horizontally disposed, short, slightly drooping in fruit, not reflexed and long as is common in *Omphalodes*. The calyx is very accrescent, glabrous with a few thick trichomes in the margins, not with small, thin and abundant hairs as is common in *Omphalodes*. The gynobasis is highly pyramidal, so nutlets form a pyramid over the calyx. In *Omphalodes* the gynobasis is commonly smaller and flatter, with

nutlets forming low elevated cross over the calyx. Nutlets wing can be from dentate to entire. Nutlet apex bears an awn, that can seldom be found in *Omphalodes*. Chromosome number of this group would be $2n=28$, with basal number $X=14$ whereas *Omphalodes* would have $X=12$ as basal number ($2n=24, 48$).

Pollen very small, isopolar, rectangular-elliptic, more or less constricted at the equator, prolate to perprolate, 6-zono-heterocolpate, with three pseudocolpi and three elongated-rhomboidal colpi, operculate. Colpal and pseudocolpal membranes psilate; equatorial ring-like aperture absent; Pseudocolpi and colpi margins conspicuously granulate. Polar pori absent. Sculpture finely granulate to scabrate (Fig. 3).

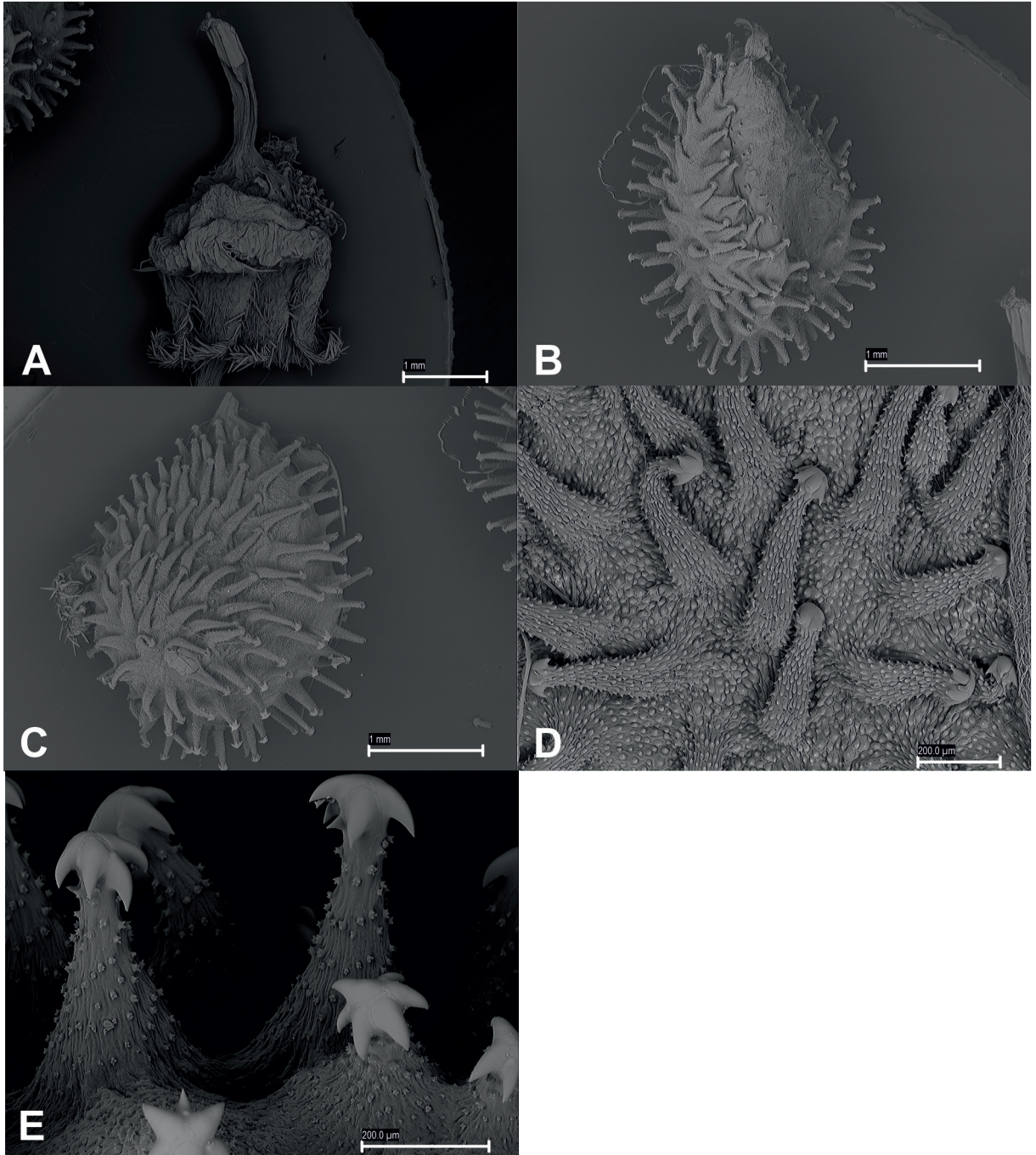


Figure 4. SEM micrographs of *Mapucheia paniculata* (CONC herbarium) and *Cynoglossum officinale* (SANT herbarium) nutlets. A. Gynobase and calyx with reflexed sepals of *Mapucheia paniculata*. B. Side view of *Mapucheia paniculata* nutlet. C. Upper view of *Mapucheia paniculata* nutlet. D. Detail of *Mapucheia paniculata* glochids, densely arranged stem excrecences with a single tip. E. Detail of *Cynoglossum officinale* glochids, scattered stem excrecences with several tips or cusps.

Figura 4. Fotografias MEV de núculas de *Mapucheia paniculata* (herbario CONC) e *Cynoglossum officinale* (herbario SANT). A. Xinobase e cálice com sépalas reflexas de *Mapucheia paniculata*. B. Vista lateral de núcula de *Mapucheia paniculata*. C. Vista superior de núcula de *Mapucheia paniculata*. D. Pormenor dos gloquídios de *Mapucheia paniculata*, coas excrecencias do fuste dispostas densamente e rematadas nunha única punta. E. Pormenor dos gloquídios de *Cynoglossum officinale*, con excrecencias do fuste dispostas laxamente e rematadas en varias puntas ou cúspides.

Type species: *Iberodes linifolia* (L.) M. Serrano, R. Carbajal and S. Ortiz

Etiology: The name *Iberodes* refers to the Iberian Peninsula as center of diversification of the genus.

1.1. *Iberodes linifolia* (L.) M. Serrano, R. Carbajal and S. Ortiz *comb. nov.*

Basionym: *Cynoglossum linifolium* Linnaeus (1753: 134)

≡ *Omphalodes linifolia* (L.) Moench (1794: 419)

≡ *Omphalodes lusitanica* (L.) Schrank (1812: 221)

= *Cynoglossum lusitanicum* Linnaeus (1762: 193)

1.2. *Iberodes brassicifolia* (Lag.) M. Serrano, R. Carbajal and S. Ortiz *comb. nov.*

Basionym: *Cynoglossum brassicifolium* Lagasca (1816: 10)

≡ *Omphalodes brassicifolia* (Lag.) Sweet (1826: 293)

= *Omphalodes amplexicaulis* Lehmann (1818: 98)

= *Omphalodes pavoniana* Boissier (1849: 128)

1.3. *Iberodes commutata* (G. López) M. Serrano, R. Carbajal and S. Ortiz *comb. nov.*

Basionym: *Omphalodes commutata* (López 1980: 83)

1.4. *Iberodes kuzinskyana* (Willk.) M. Serrano, R. Carbajal and S. Ortiz *comb. nov.*

Basionym: *Omphalodes kuzinskyanae* Willkomm (1889: 318)

≡ *Omphalodes linifolia* (L.) Moench subsp. *kuzinskyana* (Willk.) BRAND (1921: 78)

1.5. *Iberodes littoralis* (Lehm.) M. Serrano, R. Carbajal and S. Ortiz *comb. nov.*

Basionym: *Omphalodes littoralis* Lehmann (1818: 98)

1.6. *Iberodes littoralis* (Lehm.) M. Serrano, R. Carbajal and S. Ortiz subsp. *gallaecica* (M. Laínz) M. Serrano, R. Carbajal and S. Ortiz *comb. nov.*

Basionym: *Omphalodes littoralis* Lehm. subsp. *gallaecica* Laínz (1971: 25)

2. *Mapuchea* M. Serrano, R. Carbajal & S. Ortiz, *gen. nov.*

Diagnosis: Weak perennial herb, in age becoming somewhat fruticulose, with few erect subsimple branches 20–80 cm long, strigose or merely appressed hispid-villous. Leaves oblanceolate, acute, slightly mucronate, 5–12 cm long, 1–2 cm wide, veinless, margins sparsely ciliate. Lower surface pale and strigose, upper sparsely short villous-strigose. Lower leaves with a short petiole, upper leaves sessile. Inflorescence in loose, open panicle, 1–30 cm long with leafy bracts towards the base. Pedicels horizontally disposed, 8–30 mm long. Calyx 1.5–2 mm, densely strigose, with deltoid-lanceolate lobes, scarcely accrescent in age, and strongly reflexed in fruit. Corolla blue, subrotate. Gynobase pyramidal. Nutlets unwinged, orbicular-ovate, ascending, 2–2.6 mm long, unmarginated, covered by glochidiate appendages, the stem of the glochid completely covered by prickly excrescences with a single tip. In *Iberodes*, *Mimophytum*, *Myosotidium* and *Omphalodes* the nutlet is winged. In typical *Cynoglossum* species the stem of the glochid has scattered papillae-like excrescences, smaller than in *Mapuchea* and with several tips or cuspid at the top. In *Mimophytum* the glochids (or glochid-like appendages) have smooth stems. The attachment scar cover most of the nutlet ventral face. Pollen very small, isopolar, rectangular-elliptic, slightly constricted at equator, prolate to perprolate, 6-zono-heterocolpate, with three pseudocolpi and three elongated-rhomboidal colpi, operculate. Pseudocolpi margins conspicuously granulate, equatorial ring-like aperture absent (present in typical *Cynoglossum* species). Sculpture finely granulate to scabrate (Fig. 4).

Monospecific genus endemic from Central Chile.

Etymology: Dedicated to the Mapuche, aboriginal people from Chile.

2.1 *Mapuchea paniculata* (Hook. & Arn.) M. Serrano, R. Carbajal & S. Ortiz, *comb. nov.*

Basionym: *Cynoglossum paniculatum* Hooker & Arnott (1830: 37)

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