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The taxonomic status of *Hypsela sessiliflora* E.Wimm. (Campanulaceae: Lobelioideae)

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Abstract

The taxonomic status of *Hypsela sessiliflora*, a threatened NSW endemic, was examined using morphological and molecular data. Although the Type specimen appears to be missing and the protologue is of limited value, available evidence suggests that it is best included within a slightly broadened circumscription of the highly variable *Isotoma fluviatilis* subsp. *fluviatilis*.

Introduction

Hypsela sessiliflora E.Wimm. was described in 1943 from undated material collected by Ferdinand Bauer in the vicinity of Sydney between 1801 and 1805. A new nomenclatural combination, *Isotoma sessiliflora* (E.Wimm.) Lammers was made for the species (Lammers 1999), which has been tentatively accepted by Australian herbaria pending comprehensive revision of generic limits in the family Campanulaceae. For consistency the name *Hypsela sessiliflora,* rather than *Isotoma sessiliflora* is used throughout the paper.

For many years *Hypsela sessiliflora* was presumed extinct (Briggs and Leigh 1996). A specimen collected at Homebush in 1951 and another collected at Agnes Banks in 1992 were provisionally identified as *H. sessiliflora* but were later re-determined as *Isotoma fluviatilis* (R.Br.) F.Muell. ex Benth. In 1999 another specimen possibly conforming to *H. sessiliflora* was collected from Erskine Park by ecologist Teresa James. This specimen was referred, somewhat tentatively, to *H. sessiliflora* by staff at the National Herbarium of New South Wales (NSW). Several factors contributed to the lack of certainty with the determination of the specimen. These included a general lack of information on the Type specimen of *H. sessiliflora*, the lack of detail in the protologue and the apparent close morphological similarity of *H. sessiliflora* to *I. fluviatilis*. *Isotoma fluviatilis* has three currently recognised described subspecies and there are additional related taxa from mound spring sites in Queensland that await description.

A repercussion of the putative rediscovery of an extant population of *H. sessiliflora* was the changing of the species in NSW threatened species legislation from Extinct to Endangered (NSWSC 2003). It remains listed as an extinct species (as *Hypsela sessiliflora*) under the Federal (EPBC) act (DOE 2015). An imperative to clarify the conservation status of *H. sessiliflora* prompted a re-evaluation of the taxonomic status of the species, the results of which are reported below.

Methods

Morphological study: Several lines of investigation were pursued. Attempts were made to relocate the Type of *Hypsela sessiliflora* at the Vienna Herbarium (W). The protologue of *H. sessiliflora* and all available taxonomic literature relating to *H. sessiliflora* and the currently recognised subspecies of *Isotoma fluviatilis* were consulted, and potential character differences tabulated. Unpublished data for the subspecies of *I. fluviatilis* collected by DEA and NGW were also included in the comparative tables. This data was based on herbarium specimens housed at NSW, MEL, BRI, CANB and HO. Floral measurements were based on fresh or rehydrated material. Herbarium specimens referred at some time to *H. sessiliflora* at NSW were examined and characters coded for individual specimens. Due to the limited available material present on some herbarium sheets it was not possible to assess all characters for all specimens. Where sufficient flowers were available a representative flower was detached, rehydrated and the component parts examined and measured.

Five live accessions collected at Erskine Park (NSW) by Teresa James were sent to DEA in Alice Springs in July 2002. According to the collector this material included one accession tentatively referred to *Hypsela sessiliflora* (H7), one accession of *Isotoma fluviatilis* subsp. *fluviatilis* (H2) and three accessions (H3, H4, H5) of uncertain identity or possible hybrids between *H. sessiliflora* and *I. fluviatilis* subsp. *fluviatilis*. Accessions mostly consisted of a single plant fragment though one accession comprised two fragments. Data on pedicel length, floral sex and fruit type was collected when the material arrived in Alice Springs. Corresponding voucher specimens for most of the accessions were lodged at NSW. These specimens were examined at NSW and data corroborated with the data from the live material. Live accessions were potted separately and grown on in glasshouse conditions at the Alice Springs Desert Park until December 2002 to assess changes in morphological features. Care was taken to ensure plants from adjacent pots did not intermingle. Hand pollinations were undertaken in attempt to improve fruit set and seed development. Further data on pedicel length, floral sex and fruit type were collected in September and December 2002.

Molecular study: Initial investigations focused on obtaining DNA sequence data from a specimen with morphological features most closely conforming to the protologue of *Hypsela sessiliflora*. A cultivated specimen grown on from Teresa James' plant H7, originally collected at Erskine Park was selected for this purpose. Total DNA was extracted from silica-dried samples of leaf tissue using a modified CTAB method (Doyle and Doyle 1987). DNA was also extracted from representative samples of the three named subspecies of *Isotoma fluviatilis* (Appendix 1). The adjacent plastid genes *atpB* and *rbcL* and their intergenic region were amplified and sequenced as previously described (Knox et al. 2008). The closely related New Zealand *Lobelia ionantha* was used to polarize the variation found in *Isotoma fluviatilis* (Heenan et al. 2008). The aligned data matrix (comprising 3683 nucleotides and two coded alignment gaps) was analysed using PAUP* (Swofford 2002).

Further DNA sampling was undertaken on the morphologically variable Erskine Park population to assess sequence variability. DNA was extracted from five herbarium specimens (H2, H3, H5, H6 and H7) housed at NSW, that, along with live material sent to DEA in Alice Springs (see above) constitute the original population sample from the Erskine Park site. Three additional herbarium specimens from across the *Isotoma fluviatilis subsp. fluviatilis* distribution were also sampled (Appendix 1). DNA was extracted using the Qiagen DNeasy Plant micro-prep protocol described in Struwe *et al.* (1998). The plastid *atpB-rbcL* intergenic spacer region was amplified and sequenced as previously described (Knox et al. 2008). Sequences were deposited in Genbank (refer to Appendix 1).

Results and discussion

Status of the Type of *Hypsela sessiliflora*: Wimmer's (1943) protologue indicates that the Type specimen was extant at Wien (Vienna) herbarium (W) in 1943. Attempts to correspond with W between 2001-2004 regarding the presence and potential loan of the Type were unsuccessful. During a visit to Vienna in 2005, Peter Wilson (NSW) kindly searched W and the Vienna University Herbarium (WU) for the Type specimen. He spent several hours over two days examining all likely sets of specimens, without result. Thus, it would appear that the Type specimen is missing.

Protologue and accompanying illustration: In the absence of the Type specimen the protologue becomes the most significant source of information to fix the identity of the name. The original description of *Hypsela sessiliflora* appeared in *Pflanzenreich* IV.276b: 121 and was accompanied by a line drawing (fig. 31b). There are a number of issues with the protologue and illustration that reduce their value in fixing the application of the name. These include:

1. The description is based on a single specimen and therefore encompasses little morphological variation. The size ranges cited for leaf and corolla length, for instance, are much narrower than would be expected for Australian Lobelioideae taxa.

2. It is uncertain whether Wimmer's floral measurements were based on dried or rehydrated material. Some shrinkage occurs during the drying process and therefore measurement made on rehydrated flowers will be slightly larger than for dried specimens.
3. Some terms used in the original description are imprecise, and there is uncertainty about how to interpret some information due to the lack of detail in how the character was assessed. According to the protologue, the flowers are sessile or subsessile and fruit subsessile, although without measurements the dimensions included in the definition of subsessile is uncertain. The corolla is described as glabrous, however it is unclear whether this relates to the entire corolla or the adaxial or abaxial surface only. Importantly, the fruit type is described as "apparently a berry" (i.e. indehiscent) though there is evidence to suggest that this was not based on observations of ripe fruit. Had Wimmer had access to ripe fruit, one would have expected the description to include fruit dimensions and information on seeds, which are presented in the descriptions of the three other species of *Hypsela* in his monograph. The illustration also lacks fruit, though fruit are shown on the two other species of *Hypsela* illustrated.
4. Floral sex is not specifically stated in the description and the illustration does not provide enough detail to determine sex. This is significant as floral sex has a bearing on the size of floral organs and potential to develop fruit. The only measurement in the protologue potentially indicating the gender of the Type is hypanthium length.

Literature-based comparison of *Hypsela sessiliflora* and *Isotoma fluviatilis*: Wimmer does not directly compare *Hypsela sessiliflora* and *Isotoma fluviatilis* in the protologue and his publications do not provide a key including both species. To assist comparison of the two species, data for a selection of characters were extracted from the protologue of *H. sessiliflora* and from published and our own data on the three described subspecies of *I. fluviatilis* (table 1).

Table 1. Morphological comparison between *Hypsela sessiliflora* and *Isotoma fluviatilis* based on selected characters from published and unpublished sources.

Character	*Hypsela sessiliflora*[A]	*Isotoma fluviatilis subsp. fluviatilis*	*Isotoma fluviatilis subsp. borealis*	*Isotoma fluviatilis subsp. australis*
Stem apex indumentum	hairy	glabrous or minutely pubescent[CF]	glabrous or minutely pubescent[CF]	glabrous or minutely pubescent[CEF]
Leaf length (mm)	2–4	5–10(–15)[B]; 5–15[D]; 3–15(–19)[F]	(4–)5–12[BD]; 4–18.5[F]	5–13[BD]; 2–15[EF]
Leaf width (mm)	1–2.5	3–5[B]; 1–7(–9)[F]	2–5[BD]; 1.5–8[F]	2–7[BD]; 1–8[EF]
Leaf indumentum	glabrous	glabrous or minutely pubescent[CF]	glabrous or minutely pubescent[CF]	glabrous or minutely pubescent[CEF]
Pedicel length in flower (mm)	sessile or subsessile	1–15(–30)[B]; 0.5–35[F]	5–40(–50)[BD]; 2.5–53[F]	(2.5–)5–60(–85)[BEF]
Floral sex	not stated	unisexual[BDF]	bisexual[BD]; rarely female flowers present within a population[BF]	bisexual[BDF]; rarely female flowers present within population[EF]
Hypanthium length (mm) (flowering)	c. 0.5	(2–)4–5 in females[BD]; 1.8–3 in females[F], 1–2 in males[BDF]	1.5–4.5[F]	1–4[F]
Hypanthium indumentum	inconspicuously hairy	with short sparse or long dense hairs[BD]; glabrous to sparsely or densely hairy[F]	glabrous or minutely pubescent[BDF]	mostly glabrous[B]; glabrous to sparsely hairy[F]
Calyx lobe length (mm)	barely 0.5	c. 2[C]; 0.7–1.8[F]	c. 2[C]; 0.8–2[F]	c. 2[C]; 0.9–2.5[EF]
Corolla length (mm)	4–5	4–6(–8) in females[BDF]; (3–)4–7(–11) in males[BDF]	6–10(–13)[BD]; 6–10.2[F]	7–12(–15)[BD]; 6.5–16[EF]
Corolla tube length (mm)	1.5*	1.2–2 in females[F]; 1.7–3 in males[F **]	2.2–4[F **]	2–5[F **]
Corolla tube indumentum internally	glabrous	glabrous[BD]; glabrous or rarely with few hairs[F]	hairy[BDF]	hairy[BD]; sparsely hairy or rarely glabrous[F]

Corolla lobe indumentum internally	glabrous	glabrous[CF]	hairy[BD]; hairy, rarely glabrous[F]	hairy[BD]; hairy, rarely glabrous[F]
Corolla colour	apparently white (dried material)	sky blue to almost white[C]; white to pale blue, often with darker blue band at base of 3 adaxial lobes[F]	sky blue to almost white[C]; white to blue or mauvish-blue, with darker blue band at base of 3 adaxial lobes[F]	sky blue, rarely pinkish[B]; white to blue or mauvish-blue[EF,] with darker blue band at base of 3 adaxial lobes[F]
Staminal filaments	inserted midway along corolla tube	adnate to corolla for 1–1.7 mm in females, 0.9–2.2 mm in males[F]	adnate to corolla for 1.5–3 mm[F]	adnate to corolla for 2–4 mm[F]
Anther tube indumentum (upper exterior surface) and length	glabrous, 0.8 mm long	usually minutely pubescent[C]; glabrous or minutely pubescent, c. 0.7–0.8 mm long in females, c. 1–1.2 mm long in males[F]	usually minutely pubescent[C]; glabrous or minutely pubescent, 1.5–2 mm[F]	usually minutely pubescent[C]; glabrous or minutely pubescent, 1.3–2.5 mm[F]
Pedicel length in fruit (mm)	subsessile	1–15(–30)[B]; 1.5–35[F]	5–40(–60)[BD]; 3.5–53[F]	(3–)5–60(–85)[BEF]
Fruit type	apparently a berry	capsule[CF]; rarely somewhat fleshy and tardily dehiscent[C],	capsule[CF], rarely somewhat fleshy and tardily dehiscent[C]	capsule[CEF]; rarely somewhat fleshy and tardily dehiscent[C],
Chromosome No. (2n)	not stated	14[B]	14[B]	28[B]
Mean pollen diameter	not stated	n/a	<33.5μ[B]	>34.5μ[B]

Source: A - Wimmer 1943; B - McComb 1970 (data specific to subspecies); C - McComb 1970 (species level data but assumed applies to subspecies); D - Wiecek 1992, note outer extreme measurements (often glasshouse grown) not included; E - Albrecht 1999; F - Albrecht and Walsh unpublished data. * = method of measurement uncertain; ** = measured from base of corolla to lowest point of dorsal slit.

On the basis of corolla length, corolla indumentum, anther tube length and pedicel length, *H. sessiliflora* appears to be most similar to *I. fluviatilis* subsp. *fluviatilis*. Many character states and dimensions in the protologue of *H. sessiliflora* fall within the range of *I. fluviatilis* subsp. *fluviatilis*. Our unpublished data for *I. fluviatilis* subsp. *fluviatilis* supports the assertions of McComb (1968) that *I. fluviatilis* (in the broad sense and with respect to its infraspecific taxa) is highly variable with respect to leaf shape, presence or absence of a petiole, presence or absence of leaf margin teeth and vegetative indumentum, even on the same plant.

Based on available literature there may be differences between *H. sessiliflora* and *I. fluviatilis* subsp. *fluviatilis* in the following characters: leaf length, pedicel length, floral sex, hypanthium length, calyx lobe length, and fruit type. However any number of these may be unreliable distinctions due to factors discussed in the previous section. Interestingly, hypanthium length is stated as c. 0.5 mm long in the protologue of *H. sessiliflora*, which is considerably smaller than our *I. fluviatilis* subsp. *fluviatilis* measurements for female hypanthium length but closer to our measurements for male hypanthium length. If the Type of *H. sessiliflora* was indeed a male then the assertion that the fruits are a berry could be discounted as conjecture or a mistaken interpretation of the hypanthium post-anthesis.

Evaluation of specimens provisionally determined as *Hypsela sessiliflora*: Three specimens housed at NSW have been identified at some time, at least provisionally, as *Hypsela sessiliflora*. Selected characters with potential to distinguish *H. sessiliflora* and *I. fluviatilis* subsp. *fluviatilis* (based on table 1) were assessed for these specimens (Table 2). Comparable data from the protologue of *H. sessiliflora* and for *I. fluviatilis* subsp. *fluviatilis* are also included in Table 2.

Table 2. Morphological comparison between *Hypsela sessiliflora*, *Isotoma fluviatilis* subsp. *fluviatilis* and three specimens provisionally determined as *Hypsela sessiliflora* based on selected critical characters.

Character	*Hypsela sessiliflora*	R.G.Coveny 16286 (NSW 260567)	H.S.McKee s.n. (NSW 591425)	T.A.James s.n. (NSW 436612)	*Isotoma fluviatilis* subsp. *fluviatilis*
Leaf length (mm)	2–4	c. 4–8	c. 2.5–8	4–8	5–10(–15)[B]; 5–15[D]; 3–15(–19)[F]
Pedicel length in flower	sessile or subsessile	0.5–2	0.5–1.3	1.3–7	1–15(–30)[B]; 0.5–35[F]
Floral sex	not stated	unisexual, female	unisexual, male	unisexual,mostly female specimens on sheet, few specimens male	unisexual[BDF]
Hypanthium length (mm) (flowering)	c. 0.5	1.3–1.5	c. 1	n/a	(2–)4–5 in females[BD]; 1.8–3 in females[F], 1–2 in males[BDF]
Calyx lobe length (mm)	barely 0.5	1.1	1–1.5	n/a	c. 2[C]; 0.7–1.8[F]
Pedicel length in fruit (mm)	subsessile	n/a	n/a	1–7	1–15(–30)[B]; 1.5–35[F]
Fruit type	apparently a berry	n/a	n/a	capsule	capsule, rarely somewhat fleshy and tardily dehiscent[C]; capsule[F]

Data sources for *Hypsela sessiliflora* and *Isotoma fluviatilis* subsp. *fluviatilis* as for Table 1.

Based on the characters scored, all three specimens align more closely with *I. fluviatilis* subsp. *fluviatilis* than with *H. sessiliflora*. If these three specimens are included with *I. fluviatilis* subsp. *fluviatilis* then the current circumscription of *I. fluviatilis* subsp. *fluviatilis* would require minor amendment to include a lower value for leaf length of 2.5 mm and female hypanthium length to 1.3 mm long.

Cultivation study: Data on pedicel length, floral sex and fruit type collected at the time the live accessions arrived in Alice Springs was combined with data collected from the corresponding voucher specimens lodged at NSW. The combined live and herbarium data collected is included under 'Field collection July 2002' in Table 3. Data on pedicel length, floral sex and fruit type collected from the cultivated plants in September and December 2002 is included in Table 3 under 'Glasshouse Sept–Dec 2002'. The results for plant H4 were similar to H3, however the former is not included in table 3 as no voucher was lodged at NSW.

Table 3. Temporal variation in four morphological characters for four cultivated specimens originating from Erskine Park.

Voucher	H2 (NSW732388)	H3 (NSW732391)	H5 (NSW732390)	H7 (NSW732387)
Field collection July 2002				
Floral sex	female	female	male	female
Pedicel length in flower (mm)	2.5–3	to 3	1.2–2	0.5–1
Pedicel length in fruit (mm)	to 13	7–10	n/a	n/a
Fruit	dehiscent	n/a	n/a	n/a
Glasshouse Sept-Dec 2002				
Floral sex	male	female	male and female	female and male
Pedicel length in flower (mm)	3–15	to 20	3–8.5	1.5–15
Pedicel length in fruit (mm)	n/a	to 20	n/a	1.5–15
Fruit	n/a	dehiscent	n/a	dehiscent

An important result across all accessions included in the cultivation study was the presence of longer pedicels in September and December relative to those present at the start of the study in July. Plant H7 was of particular interest as it had been tentatively identified as *H. sessiliflora* and had very short (0.5–1 mm long) flowering pedicels at the start of the study. At the conclusion of the study both male and female flowers present in the pot had flowering and fruiting pedicels ranging from 1.5–15 mm long.

Based on these results it is apparent that pedicel length is highly variable in *I. fluviatilis* subsp. *fluviatilis*. The results allude to the potential influence of day length on pedicel length, though more detailed study would be required to be definitive. Experiments undertaken by McComb (1968) showed that when grown under continuous light (long day length) plants of *I. fluviatilis* subsp. *fluviatilis* produce elongated pedicels (mean length 12.7 mm in males and 12.9 mm in females). By contrast, plants grown under short day length (and induced to flower under long day length) produced nearly sessile flowers (mean pedicel length 1.1 mm in male plants and 1.0 mm in female plants). Another important result of the cultivation study was that plant H7 (tentatively identified as *H. sessiliflora*) produced dehiscent (capsular) fruits.

Molecular results: Maximum parsimony analysis of the data matrix for one sample each of ?*Hypsela sessiliflora* (H7), *Isotoma fluviatilis* subsp. *fluviatilis*, *I. fluviatilis* subsp. *borealis*, *I. fluviatilis* subsp. *australis* and *Lobelia ionantha* yields a single tree of 45 steps with a consistency index of 1.00 (Fig. 1). Subspecies *borealis* and *australis* are sister to each other and are collectively sister to subsp. *fluviatilis* (including ?*Hypsela sessiliflora*), which is diagnosed by four point mutations and a 4-bp deletion in the intergenic region. The gap in *I. fluviatilis* subsp. *australis* results from deletion of one base-pair in a 7-bp homonucleotide run located elsewhere in the intergenic region.

For sequencing of the within-population accessions (Erskine Park) and two additional specimens, tentatively identified as *H. sessiliflora*, we were able to successfully sequence James s.n. (H2, H3, H5, H6) and McKee s.n., but not Coveny 16286 (Appendix 1). The aligned matrix containing these five sequences showed zero character variation when compared against sequences representing the H7 plant from Erskine Park and P.C. Jobson 7458 (data not shown).

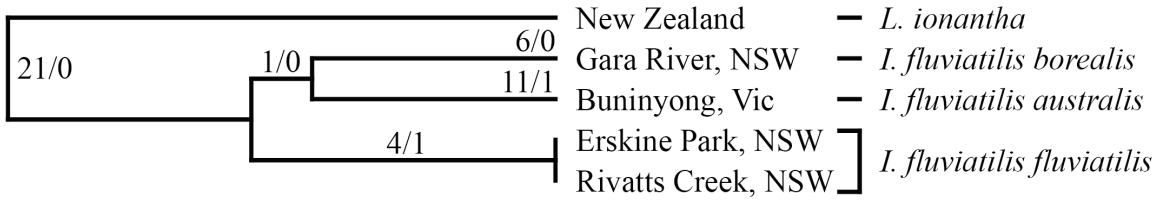

Fig. 1. Phylogenetic estimate from plastid DNA sequence data showing relationships among the three subspecies of *Isotoma fluviatilis* (Erskine Park, NSW = ?*H. sessiliflora* plant H7), as polarized using *Lobelia ionantha*. The numbers above each node and for each terminal segment indicate the number of point mutations (before the slash) and deletions (after the slash).

Conclusions

Although in the absence of the Type specimen there is still a slight margin of doubt, morphological evidence points to there being only a remote chance that *Hypsela sessiliflora* is distinct from *Isotoma fluviatilis* subsp. *fluviatilis*. It is likely that the missing Type specimen was collected from a plant similar to plant H7 from the Erskine Park site and at the time of collection the pedicels were very short. This study has confirmed the work of McComb (1968, 1970) indicating that *I. fluviatilis* subsp. *fluviatilis* is a highly variable taxon, particularly with respect to habit, indumentum, leaf size and shape, presence of marginal teeth, presence of petiole and pedicle length.

Most features included in the original description of *H. sessiliflora* fall within the recorded range for *I. fluviatilis* subsp. *fluviatilis*. Those characters that are outside the range recorded for *I. fluviatilis* subsp. *fluviatilis* (i.e. leaf length, hypanthium length and calyx lobe length) are only slightly so and are considered of negligible taxonomic significance. That they do not fall within the range for *I. fluviatilis* subsp. *fluviatilis* may merely be a function of the way in which they were measured. Fruit type and floral sex remain the most significant uncertainties. The absence of floral gender in the protologue adds to the uncertainty of the assertion that the fruit are indehiscent. Although the fruit of *H. sessiliflora* are recorded as indehiscent in the protologue, the description may not have been based on ripe fruit, as it lacks fruit dimensions and information on seeds, and the accompanying illustration also lacks fruit. Should the Type specimen surface at some time in future it would be possible to resolve or partly resolve the main uncertainties around floral sex and fruit type.

The results from the molecular study corroborate those of the morphological study. Sequence data for the Erskine Park population showed that plants matching the protologue of *H. sessiliflora* with respect to the short pedicels were identical to plants with elongated pedicels from the same site, and that both were identical to *I. fluviatilis* subsp. *fluviatilis* from elsewhere.

We consider the information presented here is sufficiently compelling to warrant placing *H. sessiliflora* in synonymy under *I. fluviatilis* subsp. *fluviatilis*, an action formally effected below.

Taxonomy

Isotoma fluviatilis (R.Br.) F.Muell. ex Benth. subsp. ***fluviatilis***

Hypsela sessiliflora E.Wimm., *Pflanzenreich* IV.276b:121 + fig. 31b

Type citation: 'Ost-Australien: N.S. Wales, Port Jackson (Ferd. Bauer – Hb. Wien).

Lectotype (here designated): Figure 31b accompanying the description.

We have been unable to find Bauer's specimen and it is presumed missing. In the absence of a specimen that has both subsessile flowers and subsessile indehiscent fruits, figure 31b is here designated as the lectotype, despite its poor quality.

Amended description of *I. fluviatilis* subsp. *fluviatilis*

Mat-forming, glabrous to sparsely puberulous perennial, rooting at the nodes. *Leaves* lanceolate, oblanceolate, elliptic or obovate, 2–15(–19) mm long, 1–7(–9) mm wide, obtuse to acute, margins entire to toothed, petiole 0–2 mm long. *Flowers* solitary in the axils, unisexual; bracteoles at base of pedicels minute; pedicels 0.5–35 mm long, glabrous or puberulous. *Flowering hypanthium* 1.3–3 mm long in females, 1–2 mm long in males, glabrous to minutely pubescent. *Calyx lobes* 0.5–2 mm long. *Corolla* (3–)4–8(–11) mm long, white to pale blue, often with deeper blue markings at base of lower 3 lobes, lobes subequal or the upper 2 slightly more deeply cleft, lanceolate to oblanceolate or elliptic, 1.6–4.5 mm long, 0.5–1.5 mm wide, glabrous; tube split to within 1.7–3 mm of base on the dorsal side, glabrous internally or rarely with few hairs. *Filaments* 1.8–3.2 mm long, distally connate for 0.2–0.7 mm long, adnate to the corolla tube for 0.9–2.2 mm; anther tube 1–1.2 mm long in males, 0.6–0.8 mm long in females, glabrous or very sparsely puberulous dorsally, seta of lower anthers 0.2–0.4 mm long, with a tuft of associated microhairs. *Capsule* ovoid, ellipsoid or obovoid, not compressed to slightly compressed, 2.5–5 mm long, 1.5–3 mm wide; seeds (based on a small sample) broadly ellipsoid, slightly compressed, c. 0.35–0.5 mm long, c. 0.3–0.35 mm wide, light brown to yellowish-brown, weakly reticulate.

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Appendix Table 1. Samples of *Lobelia* and *Isotoma* used in the DNA sequencing study.

Taxon	Collection site	Herbarium voucher	Tissue accession	GenBank accession
L. ionantha	Macraes Flat, Otago, New Zealand	CHR573373	E.B. Knox 5039	EF999984*
I. fluviatilis subsp. *borealis*	Gara River, NSW	NE 79301	I.R. Telford 12520	KR011114*
I. fluviatilis subsp. *australis*	Buninyong, Victoria	MEL2385053	N.G. Walsh 5135a	KR011115*
I. fluviatilis subsp. *fluviatilis*	Erskine Park, NSW	NSW732387	T. James H7	KR011116*#
I. fluviatilis subsp. *fluviatilis*	Rivatts Creek, NSW	IND143865	P.C. Jobson 7458	EF999977*
I. fluviatilis subsp. *fluviatilis*	Howells Swamp, NSW	NSW862366	R. Coveny 19427	KR028358
I. fluviatilis subsp. *fluviatilis*	Homebush, NSW	NSW591425	McKee s.n	KR028359
I. fluviatilis subsp. *fluviatilis*	Agnes Banks, NSW	NSW734728	W.S. Cuddy 114	KR028360
I. fluviatilis subsp. *fluviatilis*	Erskine Park, NSW	NSW732390	T. James H5	KR028361#
I. fluviatilis subsp. *fluviatilis*	Erskine Park, NSW	NSW732388	T. James H2	KR028362#
I. fluviatilis subsp. *fluviatilis*	Erskine Park, NSW	NSW732391	T. James H3	KR028363#
I. fluviatilis subsp. *fluviatilis*	Erskine Park, NSW	NSW732396	T. James H6	KR028364#

* Sequences (*atpB* & *rbcL* + intergenic region) used in phylogeny (Fig. 2)

Sequences (*atpB-rbcL* intergenic region only) used for Erskine Park population study.