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SHORT COMMUNICATION

# Phylogeny of *Isolepis* (Cyperaceae) revisited: non-monophyletic nature of *I. fluitans* sensu lato and resurrection of *I. lenticularis*

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**Abstract** The aquatic and wetland ephemeral genus *Isolepis* (Cyperaceae) comprises 76 species mostly in the southern hemisphere, and especially Africa and Australasia. The latest taxonomic revision recognizes three subgenera (*Fluitantes*, *Isolepis* and *Micranthae*) and three sections in subgen. *Isolepis*. Subgen. *Fluitantes*, mat-forming perennial herbs typically bearing a single terminal spikelet, comprises nine species with a nearly cosmopolitan distribution except in Americas and Antarctica. Of these, *I. fluitans* includes infraspecific taxa from Africa–Europe and Asia–Australasia that are distinguished by the length of the involucral bract relative to the spikelet. This morphological character is also used in the key to subgen. *Fluitantes* that separates African–European and Asian–Australasian species. The overall morphological evidence conflicts with the species recognition of *I. fluitans* sensu lato and rather indicates the non-monophyly of *I. fluitans*, which we tested in a phylogenetic framework. Sequence data from three plastid DNA regions and nuclear ITS were analyzed using maximum parsimony, maximum likelihood, and Bayesian inference. We obtained moderately resolved phylogenies with the plastid DNA and ITS data sets. Although partially conflicting, both phylogenies rejected the monophyly of *I. fluitans* and instead revealed intercontinental pattern with infraspecific taxa showing close relationships with species in the subgenus within their geographic area. A revised key to species of subgenus *Fluitantes* is provided with the Asian–Australasian *I. fluitans* var. *lenticularis* resurrected to species rank as *I. lenticularis*. The phylogeny reveals a single dispersal event from Africa to Australasia, or vice versa in subgen. *Fluitantes*.

**Keywords** Aquatic plants · Cyperaceae · *Isolepis* · ITS · Molecular phylogeny · Plastid DNA

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## Introduction

*Isolepis* R.Br. (Cyperaceae) is an aquatic and wetland or ephemeral plant genus that comprises 76 species mostly in the southern hemisphere, and especially Africa and Australasia (Muasya 1998; WCSP 2015). This sedge genus, defined as having "bisexual flowers with glumes spirally (or occasionally distichously) arranged" (Muasya and Simpson 2002; Muasya et al. 2006, 2007), has been phylogenetically studied based on morphological (Muasya and Simpson 2002) and molecular data (Muasya et al. 2001, 2009; Muasya and de Lange 2010). Although the *Ficinia-*

*Isolepis* clade was strongly supported (>90 % Bootstrap support; Muasya et al. 2009), none of these studies recovered *Isolepis* as monophyletic and instead, for example, dispersed the genus at least into three weakly supported clades (Muasya et al. 2009).

Muasya and Simpson (2002) revised the taxonomy of the genus *Isolepis*, recognizing three subgenera (*Isolepis, Fluitantes* (C.B.Clarke) Muasya and *Micranthae* (C.B.Clarke) Muasya) and three sections in subgenus *Isolepis* (*Isolepis*, *Cernuae* (C.B.Clarke) Muasya and *Proliferae* Muasya). Subgen. *Fluitantes* is defined as being "plants mat-forming, with above ground rhizomes; spikelet single and not proliferating" and has been previously classified as the segregate genus *Eleogiton* Link (Muasya and Simpson 2002). *Isolepis fluitans* is among the nine species of subgen. *Fluitantes* and includes three varieties that are in part distinguished by the length of involucral bract relative to the spikelet: Australasian var. *lenticularis* ("longer") and African–European var. *fluitans* and Ethiopian endemic var. *nervosa* ("shorter") (Muasya and Simpson 2002). This morphological character is also found in the key to species of subgen. *Fluitans* provided by Muasya and Simpson (2002; Table 1), where "longer" is given for Australasian *I. crassiuscula* and *I. producta*, while "shorter" is for South African Cape endemic *I. rubicunda* and *I. striata*. Besides, the other African members of subgen. *Fluitantes* had involucral bracts "shorter" than spikelets: *I. graminoides*; *I. inyangensis*; *I. ludwigii* (Muasya and Simpson 2002). Meanwhile, the length of glumes and the length of anthers divide *I. fluitans*, *I. graminoides*, *I. inyangensis*, plus *I. ludwigii* and *I. crassiuscula*, *I. producta*, *I. rubicunda*, plus *I. striata* (Muasya and Simpson 2002; Table 1). These characters, however, need to be reevaluated because, for example, in the Flora of Victoria, Australia, *I. crassiuscula*, *I. fluitans*, and *I. producta* are largely overlapped (Wilson 1994; Table 1). The overall morphological evidence conflicts with the species recognition of *I. fluitans* sensu Muasya and Simpson (2002) and rather indicates that the Australasian and African plus African–European varieties of *I. fluitans* have close relationships with the respective geographical relatives in subgen. *Fluitantes*.

The primary aim of this study was to test the monophyly of *Isolepis fluitans* sensu Muasya and Simpson (2002). To do so, we performed simultaneous molecular phylogenetic analyses based on plastid DNA (ptDNA) and nuclear DNA (nDNA) data sets. For ptDNA markers, two regions (*rbc*L, *trn*L) of Muasya et al (2001) and one region (*rps*16) of Muasya and de Lange's (2010) were chosen and combined altogether. The nuclear ITS data set that Muasya and de Lange (2010) used was expanded and then analyzed separately.

## Materials and methods

### Taxon sampling

With a primal focus on *Isolepis* subgen. *Fluitantes*, we obtained a total of 43 samples (41 unique OTUs), which were equivalent to 32 species from all subgenera and sections of *Isolepis* sensu Muasya and Simpson (2002) including five samples of each of the three varieties of *I. fluitans* (Online Resource 1). The sample set contained those used in the previous molecular phylogenetic studies (Muasya et al. 2001; Hirahara et al. 2007a, 2007b; Muasya et al. 2009; Muasya and de Lange 2010; Yano et al. 2012) and newly added 15 samples, including *I. pottsii* and *I. reticularis*, species new to molecular phylogenetic studies.

The outgroup included *Dracoscirpoides falsa*, *Ficinia pinguior*, and *Hellmuthia membranacea*, following Muasya et al. (2001, 2009) and Muasya and de Lange (2010).

### DNA extraction, amplification and sequencing

Total genomic DNA was extracted from either silica gel-dried leaf tissues or herbarium specimens using the CTAB method described in Ito et al. (2010). The targeted DNA regions, three from plastid DNA (ptDNA; *rbc*L, *rps*16, and *trn*L) and one from nuclear DNA (ITS), were amplified using the following primers: *rbc*L-1F (Fay et al. 1997) and *rbc*L-729R (5′-CTTCGCATGTACCTGCAGTAGC-3′; modified from Fay et al. 1997) plus *rbc*L-636F (Asmussen and Chase 2001) and *rbc*L-1379R (Little and Barrington 2003) for *rbc*L, "c" and "d" for the *trn*L intron (Taberlet et al. 1991), *rps*F and *rps*R2 for *rps*16 (Oxelman et al. 1997), and ITS-4 and ITS-5 for ITS (Baldwin 1992). PCR amplification was performed following the procedure of Ito et al. (2010).

Sequences of the *rbc*L, *rps*16, and *trn*L, and ITS were aligned using the software Mafft (ver. 7.058; Katoh and Standley 2013) and then edited manually. Gaps associated with mononucleotide repeats were not included in the phylogenetic analyses, because homology assessment can be difficult for these repeated nucleotides (Kelchner 2000) and they might be technical artifacts of the PCR amplification (Clarke et al. 2001). Ambiguously aligned regions, found in *trn*L, were excluded from the analyses.

Phylogenetic inference was determined using maximum parsimony (MP) in PAUP* (ver. 4.0b10; Swofford 2002), maximum likelihood (ML) in the RAxML web-server program (Stamatakis et al. 2008), and Bayesian inference (BI) in MrBayes (ver. 3.2.2; Ronquist et al. 2012). The incongruence length difference test (Farris et al. 1994) was conducted to test congruence between the four DNA

**Table 1** Distribution and some morphological characters of *Isolepis* subgen. *Fluitantes*

| | Distribution | Involucral bract[a] (mm) | Spikelet[a] | Length of involucral bract over spikelet[a,b] | Glumes[a,c] | Glumes[d] | Anthers[a,c] | Anthers[d] |
|---|---|---|---|---|---|---|---|---|
| *Isolepis beccarii* | Indonesia | 4–18 × 0.3–0.7 | 3.1–7.3 × 1.5–2.7 mm | Longer | 1.9–3 × 0.9-1.2 mm | N/A | 0.4–0.7 mm long | N/A |
| *I. crassiuscula* | Australasia, Asia | 3–10 × 0.6–1.6 | 3.7–11.2 × 1.9–3.6 mm | Equal[a] | 2.7–4.5 × 0.8-1.6 mm[a] | 3–4 mm long | 0.7–1.5 mm long[a] | 0.8–1.5 mm long |
| *I. fluitans* var. *fluitans* | Africa, Europe | 2–12 × 0.3–1.3 | 2.4–9.4 × 0.7–2.7 mm | Shorter[a] | 1.5–3.4 × 0.5-1.3 mm[b] | N/A | 0.4–1.5 mm long[b] | N/A |
| *I. fluitans* var. *lenticularis* | Australasia | 2–12 × 0.3–1.3 | 2.4–9.4 × 0.7–2.7 mm | Longer[a] | 1.5–3.4 × 0.5-1.3 mm[b] | 1.7–2.8 mm long | 0.4–1.5 mm long[b] | 0.5–1.0 mm long |
| *I. fluitans* var. *nervosa* | Africa | 2–12 × 0.3–1.3 | 2.4–9.4 × 0.7–2.7 mm | Shorter[a] | 1.5–3.4 × 0.5-1.3 mm[b] | N/A | 0.4–1.5 mm long[b] | N/A |
| *I. graminoides* | Africa | 3–4 × 0.8–1 | 3–7.1 × 0.9–2 mm | Shorter | 2–3.1 × 0.6-1 mm[b] | N/A | 0.7–0.9 mm long[b] | N/A |
| *I. inyangensis* | Africa | 2–4 × 0.7–1.2 | 4.7–9.3 × 1.5–3 mm | Shorter | 1.6–3.4 × 0.7-1.2 mm[b] | N/A | 0.6–1 mm long[b] | N/A |
| *I. ludwigii* | Africa | 2–3 × 0.6–1 | 2.6–8 × 1–1.8 mm | Shorter | 1.5–2.8 × 0.6-1 mm[b] | N/A | 0.7–1.3 mm long[b] | N/A |
| *I. producta* | Australasia | 3–9 × 0.7–1 | 2.7–5.5 × 1.2–2.2 mm | Longer[a] | 2.4–4.1 × 0.5-2 mm[a] | 2.5–3.3 mm long | 1.3–2.2 mm long[a] | 1.5–2.7 mm long |
| *I. rubicunda* | Africa | 3–4 × 0.6–1.5 | 3.2–5.4 × 1.1-3.3 mm | Shorter[a] | 2.4–4.2 × 0.6-1.5 mm[a] | N/A | 1.6–2.3 mm long[a] | N/A |
| *I. striata* | Africa | 3–4 × 0.9–1.5 | 3.5–8.2 × 1.3-2.5 mm | Shorter[a] | 2.6–4.5 × 0.7-1.6 mm[a] | N/A | 1.2–2.5 mm long[a] | N/A |

[a] Muasya and Simpson (2002)

[b] This study

[c] Two groups distinguished by length of glume and length of anther are shown (Muasya and Simpson 2002)

[d] Wilson (1994)

regions using a partition homogeneity test with 1000 replicates in the program PAUP* (ver. 4.0b10; Swofford 2002).

In the MP analysis, a heuristic search was performed with 100 random addition replicates involving tree-bisection–reconnection (TBR) branch swapping, with the MulTrees option in effect. The MaxTrees option was set at no limits for the analysis. Bootstrap analyses (Felsenstein 1985) were performed using 1000 replicates with TBR branch swapping and the simple addition sequences.

For the maximum likelihood (ML) analysis, the RAxML BlackBox online server (http://phylobench.vital-it.ch/raxml-bb/) was used, which supports GTR-based models of nucleotide substitution (Stamatakis 2006). The maximum likelihood search option was used to find the best-scoring tree after bootstrapping. Statistical support for branches was calculated by rapid bootstrap analyses of 100 replicates (Stamatakis et al. 2008).

Bayesian analyses were conducted using MrBayes, after evaluating the best model in MrModeltest (ver. 3.7; Nylander 2002), which were GTR+I+G for ptDNA and HKY+G for ITS. Gap characters were coded as standard datatype. Analyses were run for three million generations, sampling every 100th generation and discarding the first 25 % as burn-in. Convergence and effective sampling sizes (ESS) of all parameters were checked in Tracer (ver. 1.6; Rambaut et al. 2014). The data matrices and the MP, RAxML, and MrBayes trees are available at Treebase (S17774).

## Results

### Molecular phylogeny of combined plastid DNA sequences

The ptDNA dataset of three regions (*rbc*L, *rps*16, and *trn*L) includes 2843 aligned characters excluding ambiguous sites, of which 134 characters were parsimony informative. Analysis of this data set resulted in 1945 MP trees (tree length = 496, CI = 0.78, RI = 0.81). The 50 % majority MP topology showed no significantly supported incongruences [≥70% MP bootstrap support (BS), ≥70% ML BS, ≥0.95 Bayesian posterior probabilities (PP)] with those resulting from the MrBayes and RAxML analyses, and thus the phylogenetic tree reconstructed with MrBayes was shown in which MP and ML BS and PP calculated with PAUP, RAxML, and MrBayes, respectively, are given (Fig. 1a).

The combined ptDNA sequences showed sufficient variation among the 44 ingroups (42 OTUs), and the phylogeny was moderately resolved (Fig. 1a). The tree is mostly consistent with previous ones, such as Muasya et al. (2001) and Muasya et al. (2009), i.e., the three subgenera and the three sections of *Isolepis* sensu Muasya and Simpson (2002) were moderately supported. Some, however, were resolved differently compared to previous phylogenetic studies: (i) *Isolepis digitata* first branched off in subgen. *Isolepis* (Fig. 1) but was clustered with sect. *Cernua* and sect. *Isolepis* of subgen. *Isolepis* (Muasya et al. 2001); (ii) *Isolepis levynsiana* was placed sister to a clade comprising sect. *Cernua* and sect. *Isolepis* (Fig. 1), while phylogenetic position was less resolved among the species of subgen. *Isolepis* in Muasya et al. (2009). Two newly included species, *I. pottsii* and *I. reticularis*, were clustered in sect. *Prolifera* of subgen. *Isolepis* as inferred morphologically by Muasya and Simpson (2002).

Three varieties of *Isolepis fluitans* were resolved as non-monophyletic: (i) var. *fluitans* and var. *nervosa* were strongly clustered with African *I. graminoides* and *I. inyangensis* (92 % MP BS, 91 % ML BS, and 1.0 PP); (ii) var. *lenticularis* was placed in a moderately supported clade with Australasian-Asian *I. crassiuscula* and *I. producta* (91 % MP BS, 68 % ML BS, and 1.0 PP).

### Molecular phylogeny of nuclear ITS sequences

The nuclear ITS data set includes 578 aligned characters, of which 129 are parsimony informative. The analysis of this data set resulted in 1188 MP trees (tree length = 434, CI = 0.64, RI = 0.77). The 50 % majority MP topology showed no significantly supported incongruences [≥70% MP BS, ≥70% ML BS, ≥0.95 PP] with those resulting from the MrBayes and RAxML analyses, and thus the phylogenetic tree reconstructed with MrBayes was shown in which MP and ML BS and PP calculated with PAUP, RAxML, and MrBayes, respectively, are given (Fig. 1b).

The ITS sequences showed sufficient variation among the 43 out of 44 ingroup (*I. cernua* var. *cernua* from UK was missing) (41 OTUs), including the heterogeneous sequences from *I. fluitans* var. *lenticularis* (Fig. 1b). The phylogenetic resolution was lower than that of ptDNA. The topology is consistent with the Muasya and de Lange's (2010) combined tree of 14 species of *Isolepis* sensu stricto based on nuclear ITS and plastid *rps*16, yet none of the Muasya and Simpson's (2002) taxonomic groups of *Isolepis* was supported, except subgen. *Micranthae* of two OTUs (100 % MP BS, 100 % ML BS, and 1.0 PP). Subgen. *Fluitantes* was divided into three lineages: (i) African–European clade of seven OTUs (96 % MP BS, 96 % ML BS, and 1.0 PP); (ii) *Isolepis fluitans* var. *lenticularis* clade of two OTUs (100 % MP BS, 100 % ML BS, and 1.0 PP); and (iii) *Isolepis crassiuscula-I. producta* clade of three OTUs (96 % MP BS, 96 % ML BS, and 1.0 PP). *Isolepis levynsiana* was placed sister to sect. *Cernua* of subgen. *Isolepis* (80 % MP BS, 83 % ML BS, and 0.98 PP). Subgen. *Isolepis* was also resolved as non-monophyletic: (i) Sect. *Cernua* (100 % MP BS, 100 % ML BS, and 1.0

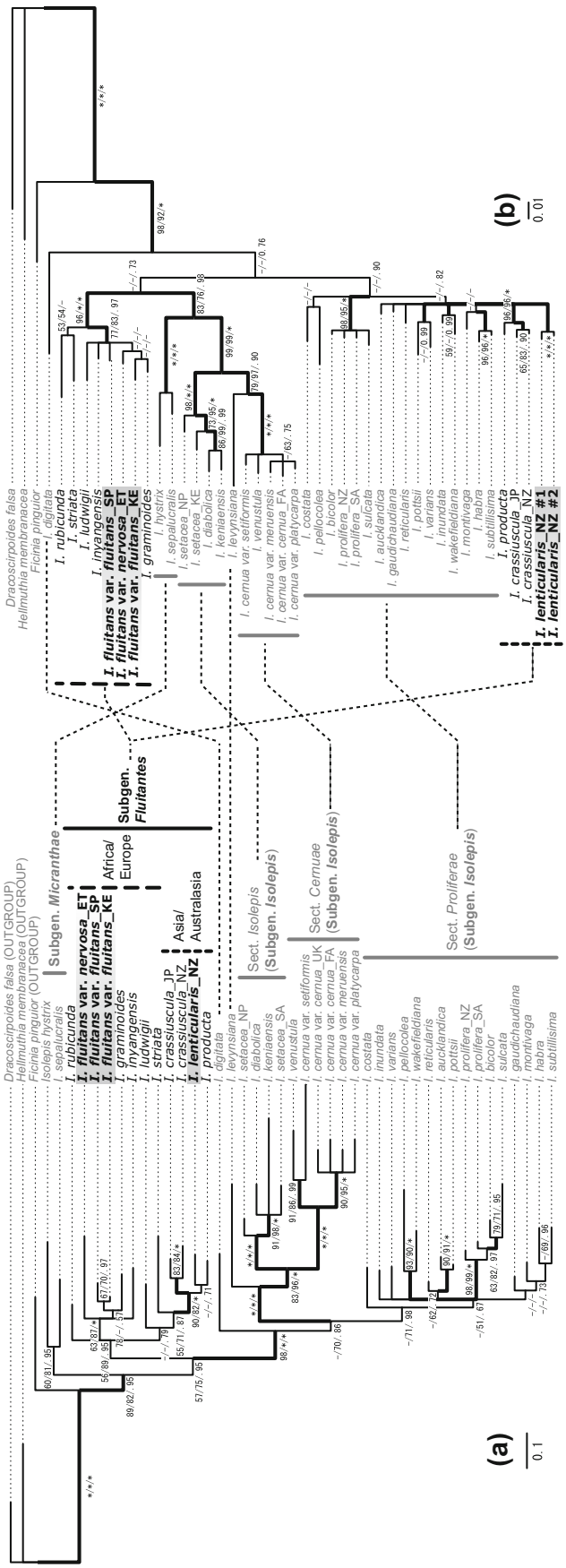


**Fig. 1** RAxML trees of *Isolepis* sensu stricto based on **a** combined chloroplast DNA dataset (*rbc*L, *rps*16, and *trn*L) and **b** nuclear ITS. *Dracoscirpoides falsa*, *Ficinia pinguior*, and *Hellmuthia membranacea* are used as outgroup. Muasya and Simpson's (2002) subgenera and sections are indicated. Subgenus *Fluitantes* are colored in *black*; *I. fluitans* sensu Muasya and Simpson (2002) are further emphasized in bold in background in gray. Maximum parsimony (PAUP) and maximum likelihood (RAxML) bootstrap supports (BS) and Bayesian posterior probabilities (PP; MrBayes) are indicated above and below the branches (100 % BS and 1.0 PP are shown with *asterisks*, and <50 % BS and <0.7 PP are shown with *dashes*). Nodes with significant supports (≥70 % BS and ≥0.95 PP) are shown with *thick lines*. Heterogeneous ITS sequences derived from single accessions are shown with #1 and #2, respectively

PP); (ii) Sect. *Isolepis* (100 % MP BS, 100 % ML BS, and 1.0 PP); (iii) Sect. *Proliferae* (non-monophyletic).

The varieties of *Isolepis fluitans* did not cluster with each other and instead were placed sister to or near their geographic relatives: (i) var. *fluitans* and var. *nervosa* with *I. graminoides*, *I. inyangensis*, *I. ludwigii*, *I. rubicunda*, and *I. striata* (96 % MP BS, 96 % ML BS, and 1.0 PP); (ii) var. *lenticularis* with *I. crassiuscula* and *I producta* plus sect. *Proliferae* of subgen. *Isolepis* (<50 % MP BS, <50 % ML BS, and 0.89 PP).

### Incongruence between nuclear and chloroplast DNA data

There was no significant heterogeneity indicated by ILD test among the three ptDNA regions ($P$ value >0.05 for all three pairs). In contrast, the test yielded $P$ values of 0.001 between *trn*L vs ITS and ptDNA (combined) vs ITS. Hence, the phylogenetic analyses were performed simultaneously and compared the results between the datasets (ptDNA vs ITS).

## Discussion

### Topological incongruence between ptDNA and ITS trees

Topological conflicts between two or more data sets are commonly observed phenomena in phylogenetic studies (Wendel and Doyle 1998). As reported in other Cyperaceae groups (Escudero et al. 2010; Gehrke et al. 2010), the present study found some significant incongruence between ptDNA and ITS trees in *Isolepis*: (i) subgen. *Micranthae*, which was first branched off in *Isolepis* in the ptDNA tree but clustered with sect. *Cernua* and sect. *Isolepis* of subgen. *Isolepis* in the ITS tree; (ii) Subgen. *Fluitantes*, which was monophyletic in the ptDNA tree but separated into three lineages in the ITS tree; (iii) *Isolepis digitata*, which was in the basal position of subgen. *Isolepis* in the ptDNA tree, but was first branched off in *Isolepis* in the ITS tree; (iv) *Isolepis levynsiana*, which was sister to a clade of sect. *Cernua* and sect. *Isolepis* of subgen. *Isolepis* but a sister to sect. *Cernua* of subgen. *Isolepis* in the ITS tree (Fig. 1).

Gene choice is among the "technical causes" of phylogenetic incongruence (Wendel and Doyle 1998). It is known that "if the rate of (gene) evolution is too high relative to the scale of taxon divergence, phylogenetic signal may be obscured by homoplasy" (Wendel and Doyle 1998). In the case of *Isolepis*, while taxonomic groups were highly to moderately supported, e.g., sect. *Cernua* of subgen. *Isolepis*, the deeper-scale phylogenetic resolution was significantly low, especially on ITS tree (Fig. 1), which at least partly attributed to the topological conflicts. A better supported inter-sectional phylogeny of *Isolepis* may thus be obtained from analyses using additional slowly evolving markers, such as *mat*K and *ndh*F (ptDNA) or nuclear DNA genes, such as phytochrome genes (Mathews et al. 1995). This will ultimately discard topological incongruence and recover a more accurate phylogeny of *Isolepis*.

Hybridization is among well-recognized evolutionary events that causes phylogenetic incongruences. Of these, chloroplast capture is known to occur most frequently (Wendel and Doyle 1998). However, this seems not to be the case in the present study, where the ptDNA tree showed: (i) higher resolution and (ii) better matching with morphology when compared to the ITS tree (Fig. 1). Hybridization affects nuclear DNA regions, which might be a case for ITS tree of *Isolepis*, yet its detection is more difficult (Wendel and Doyle 1998).

Incomplete lineage sorting is another cause for topological conflicts (Wendel and Doyle 1998). This phenomenon occurs due to genetic polymorphisms and subsequent "random gene extinction of all but one of these ancestral gene lineages" (Simpson 2010) and so that is "generally of importance to population and species-level studies" (Wendel and Doyle 1998). It seems, therefore, not to be the case for *Isolepis*.

The last possibility is "intragenic recombination" (Wendel and Doyle 1998) in relation to ancient hybridization. This could especially apply to the above-mentioned case of (iii), where Asia–Australasian members of subgen. *Fluitantes* clustered with sect. *Proliferae* of subgen. *Isolepis* (Fig. 1) with assumption of ancient hybridization between the taxonomic lineages. Although no hybridization has been reported between the lineages, it is reported that "ancient hybridization occurs among species that no longer are able to form fertile hybrid" (Wendel and Doyle 1998).

### Non-monophyletic nature of *Isolepis fluitans* sensu Muasya and Simpson (2002) and resurrection of *I. lenticularis*

The present study includes all three varieties of *Isolepis fluitans* sensu Muasya and Simpson (2002): var. *fluitans* from Africa and Europe, var. *lenticularis* from New Zealand, and var. *nervosa* from Ethiopia (Online Resource 1). Neither ptDNA nor ITS trees supported the monophyly of the species. Instead, in the trees, the varieties showed close relationships with the respective geographical relatives in subgen. *Fluitantes* (Fig. 1). To avoid the non-monophyly of *I. fluitans* sensu Muasya and Simpson (2002), we resurrect the taxonomic status of *I. lenticularis* (see Taxonomic treatment).

### Implications for biogeography of subgen. *Fluitantes* of *Isolepis*

Subgen. *Fluitantes* includes African–European and Asian–Australasian species (Muasya and Simpson 2002). Given the divergence time estimates between *Ficinia* and *Isolepis* (8.9 Mya; Besnard et al. 2009), in which major continents were at their current positions, inter-continental oceanic dispersal may explain the disjunct distribution.

Muasya et al.'s (2001) ptDNA phylogeny weakly implied multiple dispersal events between the areas, where neither six from Africa nor two species from Australia were resolved as monophyletic. In contrast, our ptDNA phylogeny supported the monophyly of Asia–Australasia lineage while the ITS tree clustered the African–European species with each other (Fig. 1). *Isolepis beccarii* (Boeck.) Goetgh. & D.A.Simpson from Sumatra, Indonesia is the only member of subgen. *Fluitantes* that has not yet been subjected to phylogenetic analysis. Morphological evidence, especially the length of involucral bract over spikelet (Table 1), the character that divides the African–European and Asian–Australasian species of subgen. *Fluitantes*, indicates the close relationship between *I. beccarii* and other Asian–Australasian relatives. It is, therefore, most parsimonious to infer a single dispersal event from Africa to Australasia, or vice versa in subgen. *Fluitantes* by means of, for instance, "wind highways" (Muñoz et al. 2004).

## Taxonomic treatment

***Isolepis lenticularis*** R.Br., Prodr.: 222. 1810. ≡*Isolepis fluitans* var. *lenticularis* (R.Br.) Muasya, Kew Bulletin. 57: 281. 2002. ≡*Scirpus lenticularis* (R.Br.) Poir., Encycl., Suppl. 5: 103. 1817. —TYPE: Australia, *Brown 5984* (holotype: BM; isotype: K).

= *Scirpus carsei* Kuik., Trans. & Proc. New Zealand Inst. 48: 240. 1915. —TYPE: New Zealand, *Mathews AK59177* (lectotype: AK).

### Key to species of *Isolepis* subgen. *Fluitantes*

1a. Involucral bract equal to or longer than spikelet ..... 2
1b. Involucral bract shorter than spikelet ..... 5
2a. Spikelet pseudolateral, bract nearly twice as long as spikelet; style bifid or trifid ..... ***I. beccarii***
2b. Spikelet always terminal, bract shorter or as long as spikelet; style always bifid ..... 3
3a. Spikelets ovate to broad-elliptic in outline, only slightly flattened, 12-40-flowered, 5–9 mm long ..... ***I. crassiuscula***
3b. Spikelets more or less elliptic in outline; slender; strongly flattened; 5-10(-15)-flowered; 3–5 mm long ..... 4
4a. Glumes 2.5–3.3 mm long, usually dark red-brown to blackish; stamens 3; anthers 1.5–2.7 mm long; nutlet obovoid with angles slightly ribbed ..... ***I. producta***
4b. Glumes 1.7–2.8 mm long, occasionally with red-brown patches; stamens 2 (rarely 3); anther 5–10 mm long; nutlet broad-obovoid to ellipsoid, angles not ribbed ..... ***I. lenticularis***
5a. Glumes (2.4-)3.5-4.5 mm long; anthers (0.7-)1.5-2.5 mm long ..... 6
5b. Glumes 1.5-3(-3.5) mm long; anthers 0.4-1(-1.5) mm long ..... 7
6a. Nutlet orbicular, reticulate ..... ***I. rubicunda***
6b. Nutlet oblong, striate ..... ***I. striata***
7a. Peduncles clustered at internodes (>10) ..... ***I. ludwigii***
7b. Peduncles not clustered at internodes (<5) ..... 8
8a. Peduncle <0.5 cm long, partially covered by leaf sheath ..... ***I. graminoides***
8b. Peduncle >1 cm long, not covered by leaf sheath ..... 9
9a. Plants base woody; glume 19–59 per spikelet ..... ***I. inyangensis***
9b. Plants base not woody; glume 4-12(-28) per spikelet ..... ***I. fluitans***

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Research involving human participants and/or animals** Not applicable for this study.

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