

The *Isoëtes flaccida* (Isoëtaceae) complex in the southeastern United States: unrecognized species diversity in southern Georgia and northern Florida

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Abstract

Isoëtes flaccida, the first species of *Isoëtes* described from the Southeast, is easily distinguished from other species in eastern North America by its boldly tuberculate—ridged megaspores (Fig. 1A-D). Until relatively recently, *Isoëtes* featuring this megaspore ornamentation found in Florida and Georgia (Fig. 1E) were considered members of *I. flaccida* (including some occasionally recognized infraspecific taxa). Since the late 20th century, cytologic and DNA sequence data have uncovered evolutionarily distinct individuals of *I. flaccida* in southern Georgia and northern Florida. Phylogenetic analysis of low-copy nuclear DNA sequences and whole chloroplast genomes shows non-monophyly of several lineages, suggesting they could be considered distinct species under a phylogenetic species concept. These include the diploid *I. chapmanii* (Jackson Co., FL), tetraploid *I. junciformis* (Tift Co., GA), and the proposed diploids *I. “snowii”* (Coffee Co., GA) and *I. “learyensis”* (Calhoun Co., GA).

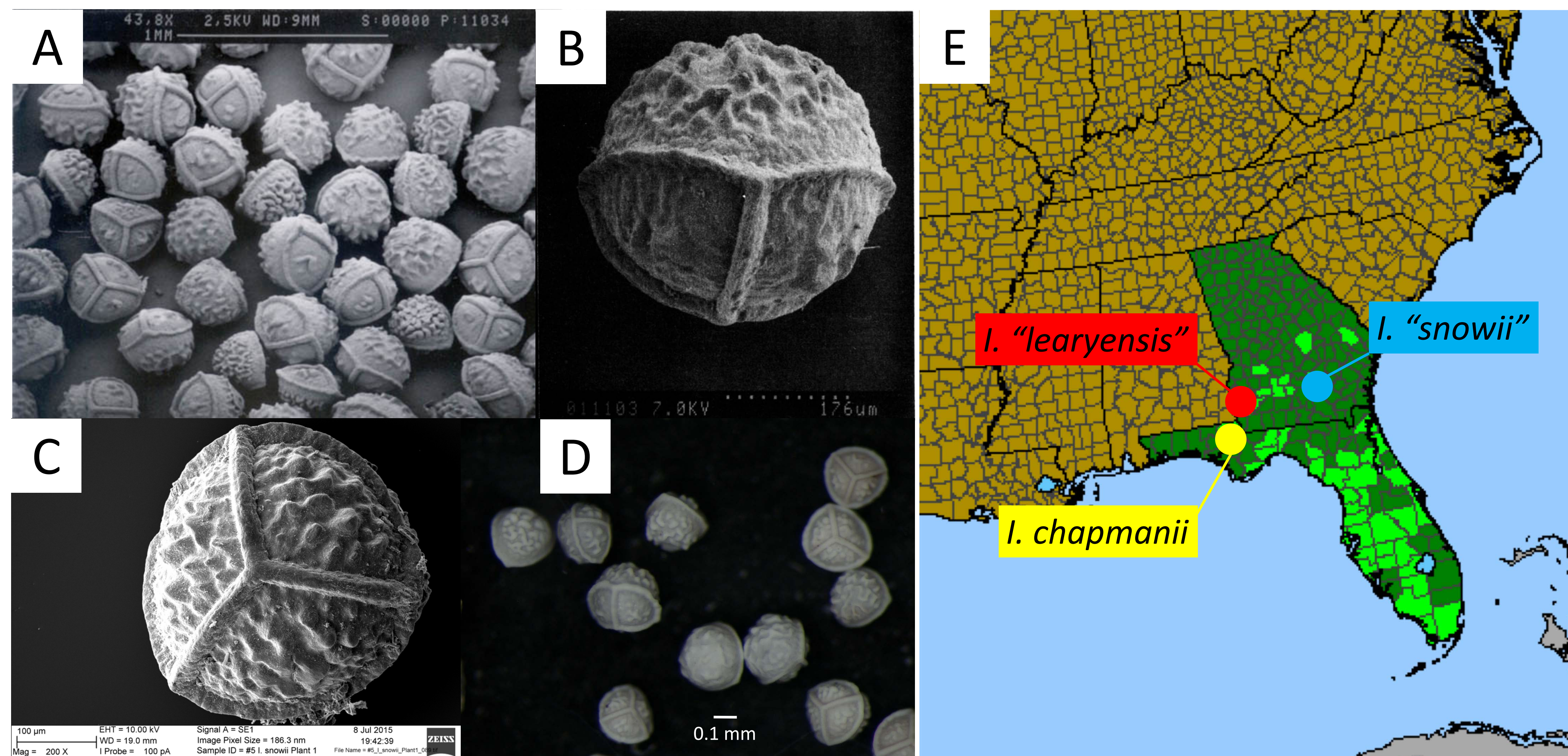


Figure 1. Megaspores of A) *I. flaccida* s.s. B) *I. junciformis* C) *I. “snowii”* D) *I. “learyensis”*. E) Distribution of *I. flaccida* s.l. with locations of other diploid taxa. Fig. 1A from ODU Plant Site, Fig. 1B from Brunton and Britton 1999, Fig. 1E from Biota of North America Program.

Methods

Leaf tissue from several individuals per taxon was collected from the field and DNA extracted by the Qiagen DNeasy Plant Mini Kit. Whole chloroplast genomes (plastomes) were assembled from Illumina MiSeq reads generated by genome skimming. DNA sequences of a *LEAFY* intron were obtained by PCR and sequencing with the PacBio RS II platform. Phylogenies were inferred with RAXML and MrBayes.

Conclusion

DNA sequence data indicate that taxa included in *I. flaccida* s.l. represent several independent evolutionary lineages. Most of these are supported by informal assessments of their habitats:

- *I. flaccida* s.s. occurs primarily in shallow freshwater rivers, lakes, and wetlands along Florida’s Gulf coast
- *I. chapmanii* is known only from swamps adjoining a spring-fed river in Jackson Co., FL
- *I. “snowii”* grows on Altamaha Grit sandstone outcrops in the Georgia coastal plain
- *I. “learyensis”* occurs in karst sinkhole ponds in southwestern Georgia
- *I. junciformis* is found in seasonally flooded swamp forest of southwestern Georgia and Altamaha Grit outcrops with *I. “snowii”*

Further work is necessary to evaluate the hypothesis that some of these taxa are long-distance dispersals from South America, where other morphologically similar *Isoëtes* exist.

Results

Phylogenetic inference reveals polyphyly among members of the *I. flaccida* complex. Though diploid species relationships differ between plastid (Fig. 2) and nuclear (Fig. 3) data, neither dataset places any putative species of the *I. flaccida* complex sister to another. Alleles from *I. junciformis* and other unidentified tetraploids from Broxton Rocks, GA, mostly fall within the *I. “snowii”* clade.

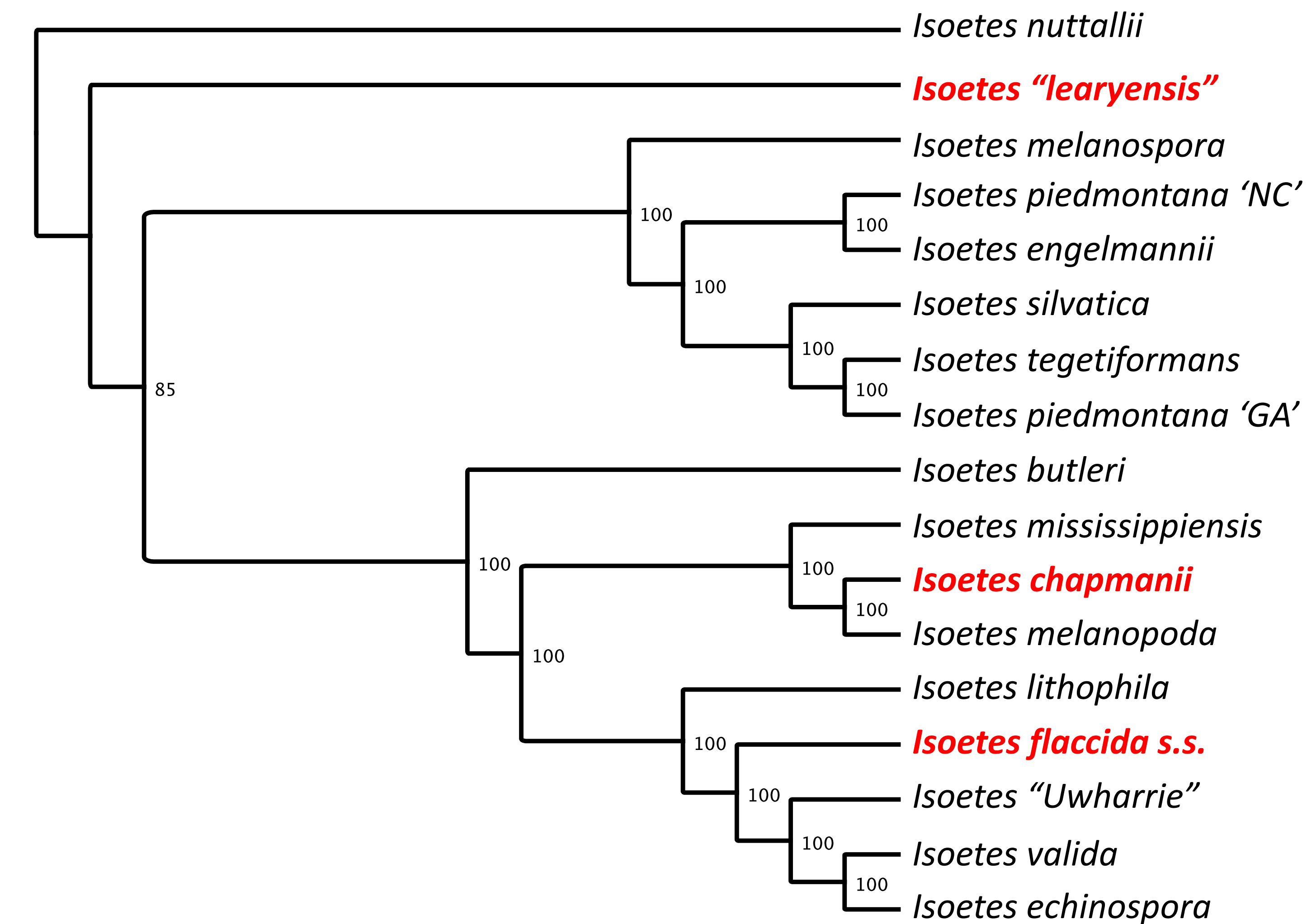


Figure 2. Plastome ML scaled phylogram. Members of *I. flaccida* complex indicated in red.

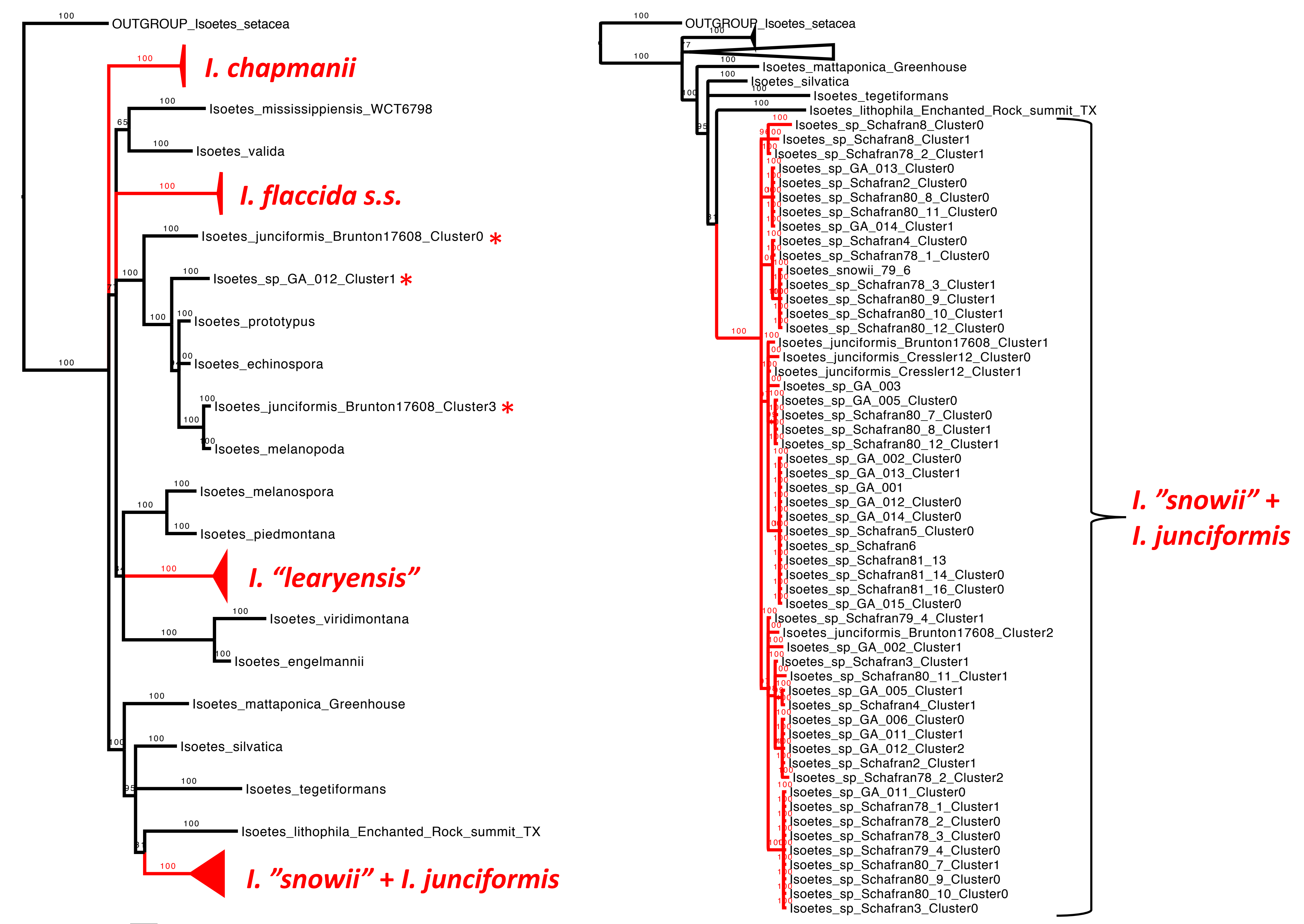


Figure 3. *LEAFY* intron Bayesian phylogram. Members of *I. flaccida* complex indicated in red. Sequences marked with asterisks may represent PCR contamination.

Acknowledgements

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