"*Keep your Nassauvieae close…and your Mutisieae CLOSER*"…Phylogenetic relations of *Macrachaenium* Hook.f. (Asteraceae; Mutisieae)

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ABSTRACT

Reanalysis of Mutisioideae nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) and chloroplast DNA (cpDNA) *rpl32-trnL* spacer sequences demonstrates that the monotypic genus *Macrachaenium* Hook.f. pertains to Mutisioideae (Asteraceae) tribe Mutisieae, in which it was classified formerly, and not Nassauvieae, in which it is classified currently. The analysis also highlights persistent uncertainty regarding the relations of the genus *Spinoliva* G.Sancho, Luebert & Katinas.

Key words: *Macrachaenium*, *Spinoliva*, Asteraceae, Mutisioideae, Mutisieae, Nassauvieae,

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Introduction

Hershkovitz (2024a, b) published results of phylogenetic analyses of *Leucheria* Lag. and selected Nassauvieae (Asteraceae; Mutisioideae) outgroups. That work demonstrated that *Polyachyrus* Lag. is nested within *Leucheria*, and that *Oxyphyllum* Phil. relates to the *Leucheria* crown as either its sister or as simply an additional lineage equivalent to the other 2–4 basal *Leucheria* lineages described in Jara-Arancio et al. (2017). Hershkovitz (2024a, b; cf. Jara-Arancio et al., 2017 [without reference to *Polyachyrus* or *Oxyphyllum*], Sancho et al. 2018) confirmed that the sister group of the *Leucheria* sensu lato crown is a clade comprising *Marticorenia* Crisci and *Moscharia* Ruiz & Pav.

Hershkovitz (2024a) concluded that the sister to this assemblage was the monotypic *Spinoliva* G.Sancho, Luebert & Katinas. This was not based on my own broader phylogenetic analysis of Nassauvieae, but partially on Jara-Arancio et al. (2017)'s analysis of sequences of the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) and two chloroplast gene (cpDNA) loci. However, this relationship was not evident in analyses of the three loci separately. I also considered Sancho et al.'s (2018) analyses of cpDNA (four loci), which showed *Spinoliva* as sister to the *Leucheria* assemblage. But Sancho et al.'s (2018) analysis of nrDNA ITS and external transcribed spacer (ETS) sequences showed the *Leucheria* assemblage as sister to remaining Nassauvieae, with *Spinoliva* segregating at the next split. But close study of these results show that neither *Spinoliva* relation is well-supported. The data strongly support monophyly of Nassauvieae, including *Spinoliva*, but the relations among the *Leucheria* assemblage, *Spinoliva*, and remaining Nassauvieae are not resolved with significant support.

In this context I overlooked the results of Moreira-Muñoz et al. (2020), which are more in line with Sancho et al.'s (2018) nrDNA tree, except that the relations (*Leucheria* assemblage [±], (*Spinoliva*, Nassauvieae)) are strongly supported. But Moreira-Muñoz et al.'s (2020) results also are somewhat unsatisfactory, because it bases ITS and ETS, both *highly* variable (and difficult to align; see below), and only *one* cpDNA locus, the *trnL*(UAA) intron plus *trnL*(UAA)-*trnF*(GAA) intergenic spacer (*trnL-trnF*). The last locus contains relatively little phylogenetic information (Hershkovitz, 2024a, b). Hence, the similarity to Sancho et al. (2018) is not surprising.

Hershkovitz (2024b) pointed out that Jara-Arancio et al. (2017) showed *Spinoliva* not as sister per se to the *Leucheria* assemblage, but as sister to *Macrachaenium* Hook.f. This relation was supported by 1.0 posterior probability (PP) in their Bayesian (BE) maximum likelihood (ML) phylogenetic analysis of combined ITS and cpDNA data and also in their analysis of ITS alone. *Macrachaenium* comprises a single perennial herbaceous species endemic to forests of southern Patagonia. It had been classified in what is now Mutisieae (e.g., Hoffmann, 1893), but Crisci (1974) argued that it fit better in what is now Nassauvieae. It seems that all subsequent classifications have followed Crisci (1974; e.g., Katinas et al., 2009; Katinas & Funk, 2020).

In any case, in Jara-Arancio et al. (2017), the clade comprising *Spinoliva* and *Macrachaenium* was sister to the *Leucheria* assemblage, supported by 0.91 PP, a value I consider tantalizing but still "insignificant" Hershkovitz (2024b). But this relation had no support in their separate ITS and cpDNA *rpl32-trnL*(UAA) intergenic spacer (*rpl32-trnL*) tree, and no *trnL-trnF* sequence was reported. Since Hershkovitz (2024b) demonstrated that the Jara-Arancio et al. (2017) analysis was based on an unedited alignment of untrimmed, unedited, and low quality sequence chromatograph data, and that some high PP branches were false, I ought to have been more cautious about accepting *Spinoliva* as the de facto sister of the *Leucheria* assemblage. Still, its sequences performed adequately as outgroup for the purpose of Hershkovitz (2024a, b).

In Hershkovitz (2024b), I reported difficulty in aligning the *Macrachaenium* ITS sequence with the rest of the alignment. For this reason, and because this question was not critical to the conclusions of that paper, I punted on the question of its phylogenetic relations, referring the matter to a future publication. Two weeks later, and not exactly expectedly, *this* is that manuscript. Sorry for the delay. But once again, while sweeping the porch in Isla Negra, Chile (cf. Hershkovitz, 2024a), I accidently confirmed that *Macrachaenium* pertains to Mutisieae. I now consider the position of *Spinoliva* among Nassauvieae to be unresolved except that it *definitely* is *not* sister to the *Leucheria* assemblage (contra Hershkovitz, 2024a, b). These results emerged from additional analysis of ITS and *rpl32-trnL* sequences described here.

Materials and Methods

For this work, I downloaded from GenBank sequences of additional taxa and incorporated them into my existing alignments of Nassauvieae ITS and *rpl32-trnL*[1] sequences (Hershkovitz, 2024a, b). I reduced the representation of *Leucheria* samples to include only adequate sampling of the major clades plus *Polyachyrus*, *Oxyphyllum*, and *Marticorenia*. No *rpl32-trnL* sequence is available for *Moscharia*, but its sister relation to *Marticorenia* was confirmed in Hershkovitz (2024a).

I then added to the alignment taxa from additional Nassauvieae from Jara-Arancio et al. (2017), along with representatives from Asteraceae subfamily Barnadesioideae (*Chuquiraga* Juss. and *Schlechtendalia* Less.) and Mutisioideae tribes Mutisieae (*Adenocaulon* Hook. and *Mutisia* L.f.) and Onoserideae (*Plazia* Ruiz & Pav. and *Gypothamnium* Phil.). Table 1 provides the GenBank accession numbers for all samples not listed in Jara-Arancio et al. (2017). Excess 5' and 3' sequences were trimmed to the length of my existing alignment. Alignments were adjusted manually using BioEdit (Hall, 2004) as in Hershkovitz (2024b), aligning first sequences of closely related taxa and then aligning these grouped sequences to each other.

[1] Although I had not mentioned this before, my *rpl32-trnL* alignment begins in the *rpl32-trnL* gene 89 bases upstream from the canonical beginning of the spacer region and ends 14 bases upstream of the beginning of the *trnL* gene. This is because this was the range of sequence available for most taxa. Some available sequences were shorter on one and/or both ends.

Initially, I added to each alignment all of the available sequences for Nassauvieae genera from Jara-Arancio et al. (2017) and additional outgroups and conducted preliminary analyses for reference. I then reduced the sampling to include only taxa for which both sequences are available. Then I deleted the sequence for *Calorezia* Panero because its *rpl32-trnL* sequence includes two large deletions totaling 200-250 bases relative to the ca. 900 bases of other taxa. This amount of missing information destabilized its position in the tree and reduced bootstrap support for many branches.

As in Hershkovitz (2024a), I performed MP, ML, and MP bootstrap (1000 replicates) analyses for the separate and combined data sets using PAUP version 4 (Swofford, 2003). The ML analysis used the general time-reversible substitution model with gamma among-site rate variation correction and all parameters estimated using an MP tree. I performed all analyses with and without the *Spinoliva* sequence, because its relations in the ITS and *rpl32-trnL* trees were significantly incongruent and, otherwise, its inclusion reduced bootstrap support at multiple branches in both trees.

Both MP and ML analysis of the ITS data yielded significant phylogenetic incongruencies with respect to the *rpl32-trnL* data. I suspected that this might owe at least partially to highly variable base composition of the ITS sequences (see Results). For this reason, I also bootstrapped (500 replicates) the ITS and combined data using the Balanced Minimum Evolution distance method (Desper & Gascuel, 2004) with the LogDet distance correction (see Swofford et al., 1996) and otherwise the PAUP distance method default settings. The LogDet method calculates pairwise sequence "p" distances by constructing a 4x4 matrix of sitewise base differences and applies the log of the matrix determinant as a correction for the raw distance. LogDet cannot accommodate substitution parameters.

Both data sets manifested inconsiderable length heterogeneity owing to insertion/deletion (indel) events. ML and related analyses ignore this potentially useful source of additional phylogenetic information, but it can be exploited in MP analysis. Hershkovitz (2024a) scored length variable regions with up to four states (plus "ambiguous"). This was an expedited version of a much more tedious protocol I have used in previous analyses (Hershkovitz & Zimmer, 2000: Fig. 1). In the case of Hershkovitz (2024a), MP trees generated without this indel data were superior, because the indel data included that for highly complex and homoplasious length variability. Normally, I partition these regions phylogenetically in order to recover the less homoplasious indel information *within* each phylogenetic sector, while scoring the remaining taxa as ambiguous "N" for that indel.

In the *present* analysis, I scored as *two*-state characters only *fixed*-length informative indels embedded in otherwise highly conserved flanking sequences. This is because this work emphasizes deeper relations between a few of the analyzed taxa and not the fine-level resolution of the entire tree. This allows, for the purpose of MP analysis, addition of character data I consider a priori reliable, while excluding those likely to introduce artifacts (as in Hershkovitz, 2024a). And I analyzed the data both with and without the indel characters. Of course, the indel data have no bearing on the ML and distance analyses.

Results

1. Sequence and alignment characteristics

Several characteristics of the sequences and alignments analyzed in this work are presented in Table 2. ITS and *rpl32-trnL* are exploited in finer-level angiosperm systematics because of their relatively high variability. But their biology and characteristics are completely different, and understanding these differences aids in phylogenetic interpretation.

a. ITS

As summarized in Hershkovitz et al. (1999), ITS is in the "middle" of the 18S-26S nrDNA (in plants) gene, which, along with their 5' and 3' ETS, is transcribed as a unit. ITS includes two untranslated spacers, ITS1 and ITS2, separated by the 5.8S gene, which pertains to the ribosomal large subunit comprising 5.8S and 26S. ITS2 originated as an "expansion sequence" within the prokaryotic large subunit gene. ITS1, in contrast, is homologous to the prokaryotic small subunit and large subunit *intergenic* spacer.

The 18S-26S nrDNA gene exists in hundreds to thousands of tandem copies at one to a few chromosomal loci. For this reason, the paralogs can recombine, which can yield phylogenetic artifacts. The nrDNA paralogs (obviously) can be substantially polymorphic within an organism, and lineage-sorting of these alleles also can mislead estimation species phylogeny. Nonetheless, the paralogs tend to be remarkably uniform owing to a phenomenon known as "concerted evolution." Consequently, PCR amplifications of ITS show very little or no polymorphism, especially because polymorphism is further reduced by the PCR process itself

Variation in ITS sequences is limited mainly to ITS1 and ITS2. The 5.8S is highly conserved in length (161-162 bases) and sequence across angiosperms (Hershkovitz & Lewis, 1996). In the present analysis, the 5.8S gene included only 9/282 (3%) of the total ITS parsimony-informative sites. Sequence and length variation is not distributed evenly across ITS1 and ITS2. Hershkovitz & Zimmer (1996) found six regions of ITS2 that were conserved across angiosperms, and Hershkovitz et al. (1999) reported two such regions in ITS1. Actually, few "sites" are conserved within these regions. The conservation is more "structural," viz. the regions share similarly highly *biased* patterns of nucleotide diversity, presumably associated with function. Among the spacers, ITS1 is more variable in length and sequence. For the present sequences, 65% of the total ITS length variation – and 60% of the parsimony-informative sites – occurred in ITS 1.

Besides length and sequence, ITS also varies substantially in base composition, even at low divergence levels. The conserved angiosperm ITS regions are GC-biased, while the variable regions tend to be GC-biased but can vary considerably (Hershkovitz & Zimmer, 1996). The present data are no exception. This extreme variation affects both MP and ML analysis, though in different ways. In the former, sequences with similar base composition tend to attract. In the latter, the variation reflects nonstationarity of the substitution dynamics, which are presumed to be constant across the phylogeny.

The GC-richness of the ITS sequences is somewhat evident in the estimated substitution matrix. The rate parameters are similar to those for ITS for angiosperms in general. In particular, the GC substitution rate is exceptionally low, which is expected for an inherently GC-rich sequence, since these bases attract be three rather than two hydrogen bonds. Consequently, Gs and Cs in a DNA sequence replicate with greater *fidelity*. The GC-richness itself owes to the fact that the nrDNA gene translates to *functional ("enzymatic") RNA* rather than an mRNA template for protein synthesis. Functionality of ribosomal and related RNAs owes to their secondary structure, viz. *base pairing*. This base pairing is stabilized by high GC. Also typical of ITS is the exceptionally high CT substitution rate. This owes to "wobble" pairing of G with T rather than C and, in RNA, relatively stable wobble pairing of G with U (uracil). Thus, in the DNA sequence, C/T mutations are much more tolerable than other mutations.

Because of the length variability, concentrated in variable ITS regions, ITS alignment can be extremely difficult at raw sequence divergences much higher than 10%. Except within the *Leucheria* assemblage, *total* pairwise raw divergences in the present alignment were mostly in the range of 15-30%,

the divergences much higher in the variable regions. Worse, variable ITS sequence regions differ in *both* length and sequence, rendering almost futile the homologization of variable-length fragments across a phylogenetically deep alignment.

For example, consider the fragments AATGGTTC and CGTGTCGT from ITS sequences of, respectively, a *Leucheria* and *Mutisia* species. In my alignment, these fragments superpose, implying four substitutions. But one or the other fragment can be extended such that the difference can be explained by two insertions. In such a length-variable region, two insertions cannot be considered especially improbable. In the context of all the phylogenetically intervening sequences, however, my alignment becomes more parsimonious.

This example underscores the problem not so much of alignment, but of the underlying assumption that, by virtue of their alignment, aligned positions are *presumed* (viz. a priori) to be *homologous*. This presumption may be ill-founded in the case of highly divergent ITS sequences. At this level, phylogenetic analysis becomes essentially phenetic analysis, viz., based on overall sequence *similarity* rather than phylogeny. Still, this does not *necessarily* mislead phylogenetic reconstruction in part of the tree, because identical sequences embedded even among *random* sequences will naturally cluster. But here, the misalignment will create artifacts related to rooting of the similar sequences (e.g., Hershkovitz et al., 2006).

In terms of phylogenetic utility at the level of the present analysis, ITS exhibits high variability, which is desirable, but also very high homoplasy, as evidenced by its indices in Table 1. About half of the sequence data in the present alignment have no phylogenetic signal. This can create branch attraction artifacts in MP analysis that are partially corrected in ML analysis. But even ML cannot correct for convergence, because, in the end, a base is a base is a base, etc. The problem is most critical for the popular BE method, which is merely an algorithm that aids in finding the optimal ML tree (Hershkovitz, 2021). The PPs are misinterpreted as data support, which they are not. Convergence thus yields simply high PPs for *wrong* branches (e.g., Jara-Arancio et al., 2017; cf. Hershkovitz, 2024b).

b. *rpl32-trnL*

Characteristics of the *rpl32-trnL* locus are very different from those of ITS. While the length variability is proportionally much greater than for ITS, the sequences are highly alignable at this and (not shown) even deeper levels. This is because, unlike ITS, sequence adjacent to many indels at this locus and in noncoding cpDNA in general tends to be highly conserved. However, noncoding cpDNA regions usually contain also another type of length variable sequence consisting of mononucleotide (less often di- or trinucleotide) repeats, viz. “microsatellites.” Repeat number is highly homoplasious and best ignored at deeper phylogenetic levels (Hershkovitz, 2024a).

Another characteristic of this locus and noncoding cpDNA is extremely *long* indels. The present alignment includes deletions ca. 200 bases long. In *Chaetanthera* Ruiz &n Pav. (Mutisieae), I reported deletions of ca. 300 bases. This seems paradoxical. When sequences are highly conserved, one suspects that the conservation reflects critical functionality, even if the sequences are noncoding. Yet, in the case noncoding cpDNA, large fragments whose sequence otherwise is highly conserved, evidently are functionally *dispensable*. This suggests that sequence conservation owes to higher *replicative fidelity* in cpDNA than in nuclear DNA rather than function per se. In fact, this might be expected given that variation in nuclear DNA owes partially allelic interaction in *cis* and in *trans*.

In contrast to nrDNA, noncoding cpDNA tends to have very low GC (hence high AT) content. But as in the case of ITS, this can be inferred from the estimate substitution rates. In particular, here it is the

AT rate that is exceptionally low, which is expected if high AT sequences are highly conserved. *Rpl32-trnL* is perhaps the most variable of cpDNA loci. Yet, it remains *both* too conserved and too variable at the finest phylogenetic levels. This is because variation at this level often involves not so much substitution as variability in mononucleotide repeat numbers (viz. indels), and because closely related species often are polymorphic and overlapping in their haplotype variability (Hershkovitz, 2021). But at "medium" levels studied here, where there is more substitution and where haplotypes are strictly divergent, *rpl32-trnL* is very useful. Indeed, in the present analysis, the homoplasy indices for this locus are about 40% better than those for ITS, suggesting that convergence is not a serious problem.

2. Phylogenetic results

Results of the phylogenetic analyses obtained here are illustrated in Figures 1–7. Figure 1 shows the ITS MP bootstrap consensus, Fig. 2 the ITS ML tree, Fig. 3 the ITS LogDet bootstrap consensus, Fig. 4 the *rpl32-trnL* MP bootstrap consensus, Fig. 5 the *rpl32-trnL* ML tree, Fig. 6 the combined data MP bootstrap consensus, and Fig. 7 the combined data LogDet bootstrap consensus. As in Hershkovitz (2024a), this work applies the *Leucheria* taxonomy used in Jara-Arancio et al. (2017). Some taxa were reduced to synonymy by Katinas et al. (2022).

The ITS and *rpl32-trnL* trees share many similarities at various phylogenetic levels, but also some significant differences. Most important for the present problem, all of the trees associate *Macrachaenium* with Mutisieae and *most* strongly *separate* these from Nassauvieae. This result is summarized in Table 3, which shows the number and magnitude of bootstrap partitions supporting the separation, viz. counterevidencing the inclusion of *Macrachaenium* in Nassauvieae. Only one analysis failed to place a bootstrap partition ≥ 70% between *Macrachaenium* and "core Nassauvieae"[2] This was the *rpl32-trnL* data with indels excluded and *Spinoliva* included. But adding the indel data, with or without *Spinoliva*, created *two* partitions, each with 79–87% bootstrap support.

In total, 11 of the 14 analyses place 2–3 partitions ≥ 70% between *Macrachaenium* and core Nassauvieae. Separation was greatest (and quite convincing) using the combined data *with* indels and without *Spinoliva*. Just as importantly, *none* of the analyses showed even *weak* support for a close relation between *Macrachaenium* and core Nassauvieae or *Spinoliva*.

Inclusion of *Macrachaenium* in Mutisieae is a separate question. Bootstrap support for this relation is the first number in each entry in Table 3. This support is < 70% in 4/14 analyses but > 80% in 11/14 analyses. Notably, for MP analysis of both loci, the *Macrachaenium*/Mutisieae association is *strengthened* by inclusion of the indel data. Again, the indel characters used here were highly conservative and unambiguous.

It may seem problematic that the ITS and combined data MP bootstrap and ITS ML trees including *Spinoliva* show Mutisieae as paraphyletic, although with < 70% bootstrap support. These trees root Mutisioideae along the *Adenocaulon* branch. But I believe that this owes to a combination of low taxon sampling and GC content variation, both of which might yield spurious branch attraction. Also, the paraphyly in the combined data bootstrap owes to the disproportionate weight of the ITS data. It is notable that this paraphyly is not supported in the analyses with *Spinoliva* excluded. In the LogDet ITS and combined data bootstrap consensuses, the Mutisieae taxa are monophyletic, with a very strong attraction between *Macrachaenium* and *Mutisia*. In any case, paraphyly of the Mutisieae taxa in some trees does not affect the conclusions regarding *Macrachaenium*.

[2] Because of the uncertain position of *Spinoliva*, I use the designation "core Nassauvieae" for all Nassauvieae sensu Katinas & Funk (2020) except for *Macrachaenium* and *Spinoliva*.

The ITS and *rpl32-trnL* bootstrap trees show incongruent relations for *Spinoliva*. The former show *Spinoliva* as sister to core Nassauvieae, while the latter show it as sister to Mutisieae + core Nassauvieae. The combined data bootstrap agrees, not surprisingly, with the ITS bootstrap. But it is notable that the support in the combined data bootstrap is not reduced relative to the ITS bootstrap. This suggests that the *rpl32-trnL* data contain *latent* support for this relation. Meanwhile, the combined data LogDet bootstrap strongly supports a sister relation between *Spinoliva* and Onosorideae.

LogDet might seem inappropriate for combined analysis of data sets having very different base compositional bias, as it the case for the present ITS and *rpl32-trnL* data. Base bias in different data might variously cancel out or, alternatively, become more extreme, but in a *taxon-specific* manner that would distort phylogenetic results. But this is not a problem for the present application, because the *rpl32-trnL* sequences analyzed here vary negligibly in base composition. Thus, the base compositional variation in the combined data owes only to that of the ITS data, while *rpl32-trnL* simply adds *phylogenetic* signal. This explains the higher bootstrap values in the combined data versus ITS trees. But the inability of LogDet to accommodate substitution bias and among-site rate heterogeneity remains its weakness. In any case, in the present analysis, I am mainly concerned with the relations of *Macrachaenium*. In this case, it is reassuring that LogDet corrected the obvious misrooting of the ITS MP and ML trees and yielded the otherwise generally accepted Mutisieae monophyly derived from the *rpl32-trnL* data.

Discussion

The present analyses demonstrate that there is no evidence supporting the inclusion of *Macrachaenium* in Nassauvieae, as suggested by Crisci (1974) and apparently universally accepted currently. *Most* of the analyses strongly support its inclusion in Mutisieae, as suggested in 19th Century taxonomy (Hoffmann, 1893). One or two of the present 14 analyses fail to robustly resolve this question, but those analyses were based on the least data, viz. the less variable *rpl32-trnL* data excluding unambiguous and informative indels. Support for inclusion in Mutisieae increased with *all* combinations of data addition and ambiguity source exclusion (i.e., *Spinoliva* and base compositional bias).

The present results in no way resolve the question of the relations of *Macrachaenium among* Mutisieae. This question must be addressed with additional taxon and sequence sampling. Likewise, I consider the relations of *Spinoliva* to remain unresolved. I suggest that most likely it will prove to be sister to core Nassauvieae. Meanwhile, I find Moreira-Muñoz et al.'s (2020) results unconvincing at this point, partially because they conflict with the present results, and partially because of the ITS (and certainly ETS) alignment question. As I discussed above, alignment of variable regions of these loci at this divergence level is not only exceedingly difficult, but possibly inherently inaccurate because of the high probability of convergence. After all, Jara-Arancio et al. (2017) *proved* that it is possible to obtain high BE PPs for spurious relationships using a low quality alignment of low quality sequences (Hershkovitz, 2024b).

These results call for, besides additional molecular sampling and analysis, a reevaluation of morphological evidence regarding *Macrachaenium*. Crisci (1974) cited only three morphological traits that linked *Macrachaenium* with Nassauvieae rather than Mutisieae. One was pollen exine structure, which I have not studied. The other two were tailed anthers and bilabiate corollas, both of which characterize also Mutisieae (Katinas, 2000). The cited trait linking *Macrachaenium* with *Mutiseae* was its rounded rather than truncate style branches. Katinas et al. (2009) also noted that the style papillae of *Macrachaenium* agreed with Mutisieae and not core Nassauvieae. Thus, on the balance, perhaps the evidence for transfer of *Macrachaenium* to Nassauvieae was not so robust in the first place.

The return of *Macrachaenium* to Mutisieae raises interesting biogeographic questions. Mutisieae includes (now) ca. 19 genera distributed ± worldwide but concentrated at warm-temperate to tropical American latitudes (Katinas et al., 2009; Moreira-Muñoz et al., 2012). The cold-adapted species that occur at higher latitudes/altitudes seem to be secondarily derived. The ca. 25 genera of Nassauvieae, in contrast, concentrate in Patagonia. Possibly this is one of the reasons that the inclusion of the Patagonian *Macrachaenium* was not questioned.

Only two Mutiseae genera seem to be fundamentally Patagonian, *Adenocaulon* and the monotypic *Eriachaenium* Sch.Bip. *Adenocaulon* has one Patagonian species and several presumably derived species in cool temperate Eurasia. *Macrachaenium* represents a third Patagonian genus. My examination of available *Eriachaenium* DNA sequences suggests that this genus is not closely related to *Macrachaenium*. A "close" relation with *Adenocaulon* cannot be ruled out at this point, but it remains possible that all three genera represent independent occupations of southern Patagonia.

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Supplemental file

The data set in NEXUS format for this work is available at:

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Table 1. GenBank accessions for taxa sampled here. Accessions for taxa not in the list below are listed in Jara-Arancio et al. (2017: Table 1).

Taxon	**ITS**	***rpl32-trnL***
Adenocaulon chilense Less.	KX349359.1	FJ979775.1
Chuquiraga jussieui J.F.Gmel.	KU182733.1	MG553885.1
Gypothamnium pinifolium Phil.	EU729342.1	MG553853.1
Mutisia hamata Reiche	EF530242.1	-
Mutisia speciosa Aiton ex Hook	-	KX349405.1
Oxyphyllum ulicinum Phil.	EU729344.1	MG553854.1
Plazia daphnoides Wedd.	EF530226.1	MG553867.1
Polyachyrus fuscus (Meyen) Walp.	EF530263.1	MG553855.1
Schlechtendalia luzulifolia Less.	AF412836.1	NC_051501.1
Spinoliva ilicifolia subsp. *baccharoides* (D.Don ex Hook. & Arn.) G.Sancho	MG553793.1	MG553872.1

Table 2. Characteristics of the ITS and *rpl32-trnL* sequences. Notes: [1]As noted in the text, the alignment here begins within the *rpl32* gene and terminates upstream of the *trnL* gene. [2]Parsimony-informative sites excluding indel characters. [3]Range of GC content in parsimony-informative sites. [4]The number of informative indels used in the analysis, which is not a measure of the number of indels that exist. [5]The ensemble *Rescaled Consistency Index*, which is the average difference between the observed and minimum number of parsimony steps for each site but ignoring variable uninformative sites (Farris, 1989). [6]The ranges are for the four data sets, with /without indel data and with/without *Spinoliva*, with the *highest* values for with indel data and without *Spinoliva*. [7]The ensemble *Homoplasy Index*, which is 1 – (the *Homoplasy Excess Ratio*[Archie (1989]). This is the average proportion of the informative data that lack phylogenetic signal, viz. the empirically observed homoplasy of the characters is equal to that expected in a random tree. [8]The ranges are for the four data sets, with/without indel data and with/without *Spinoliva*, with the *lowest* values for with indel data and without *Spinoliva*. [9]ML substitution rates estimated using an MP tree. [10]Alpha parameter (curve shape) of the among-site rate heterogeneity modeled as a gamma distribution and estimated using an MP tree.

	ITS	***rpl32-trnL***
Length	568–647	672 – 890[1]
Informative sites[2]	282	166
GC %[3]	40–74	26–29
Informative indels[4]	15	16
RC[5]	0.36–0.39[6]	0.65–0.67[6]
HI[7]	0.50–0.52[8]	0.30–0.32[8]
AC, AG, AT, CG, CT, GT[9]	0.8, 1.8, 1.0, 0.4, 5.4, 1	1.1, 0.9, 0.2, 1.1, 0.9, 1
Alpha[10]	0.5	0.7

Table 3. Bootstrap support for the partitions *counterevidencing* a relation between *Macrachaenium* and core Nassauvieae and/or *Spinoliva*. Below are the bootstrap %'s for these partitions for each of the 14 bootstrap analyses. For ITS and the combined (comb) MP data analyses, the *Spinoliva* branch (if included) intervenes between *Macrachaenium* and core Nassauvieae. The middle of the three numbers corresponds to the *Spinoliva* branch when it is included. When it is excluded, the middle number is given as NA. For the *rpl32-trnL* data (*rpl32*), the *Macrachaenium* branch intervenes between core Nassauvieae and *Spinoliva*. The numbers in the first column are the partitions counterevidencing a relation between the former taxa and in the second column between the latter. For the LogDet analysis, the two numbers in the left column in parentheses are for the partitions of, respectively, *Macrachaenium* + *Mutisia* and Mutisieae. The numbers in the second column are for *Macrachaenium* + *Mutisia*, Mutisieae, and *Spinoliva*. Bootstrap values < 50 are indicated with "-."

Data set; method	***(Macrachaenium*+Mutisieae), ± *Spinoliva*, Nassauvieae**	***(Macrachaenium*+Mutisieae), *Spinoliva***
ITS, indels, *Spinoliva*; MP	82, 93, 78	
ITS, no indels, *Spinoliva*; MP	76, 88, 76	
ITS, indels, no *Spinoliva*; MP	71, NA, 97	
ITS, no indels, no *Spinoliva*; MP	64, NA, 95	
rpl32, indels, *Spinoliva*; MP	84, NA, 79	84, 71
rpl32, no indels, *Spinoliva*; MP	-, NA, 56	-, 57
rpl32, indels, no *Spinoliva*; MP	85, NA, 87	NA
rpl32, no indels, no *Spinoliva*; MP	-, NA, 70	NA
comb, indels, *Spinoliva*; MP	87, 95, 81	
comb, no indels, *Spinoliva*; MP	82, 87, 78	
comb, indels, no *Spinoliva*; MP	88, NA, 99	
comb, no indels, no *Spinoliva*; MP	75, NA, 99	
ITS, *Spinoliva*; LogDet	(89, 65), -, 88	(89, 65), -
comb, *Spinoliva*; LogDet	(82, 95), NA, 99	(82, 95), 80

Figure 1. MP bootstrap consensus for the ITS data. Numbers and symbols above the branches are bootstrap % for analysis with/without indel data. Numbers and symbols below the branches are for the same but excluding *Spinoliva*. "!" = 100%; "-" = < 50%; "*" = not applicable because of exclusion of the *Spinoliva* branch. The *Leucheria* species taxonomy follows Jara-Arancio et al. (2017).

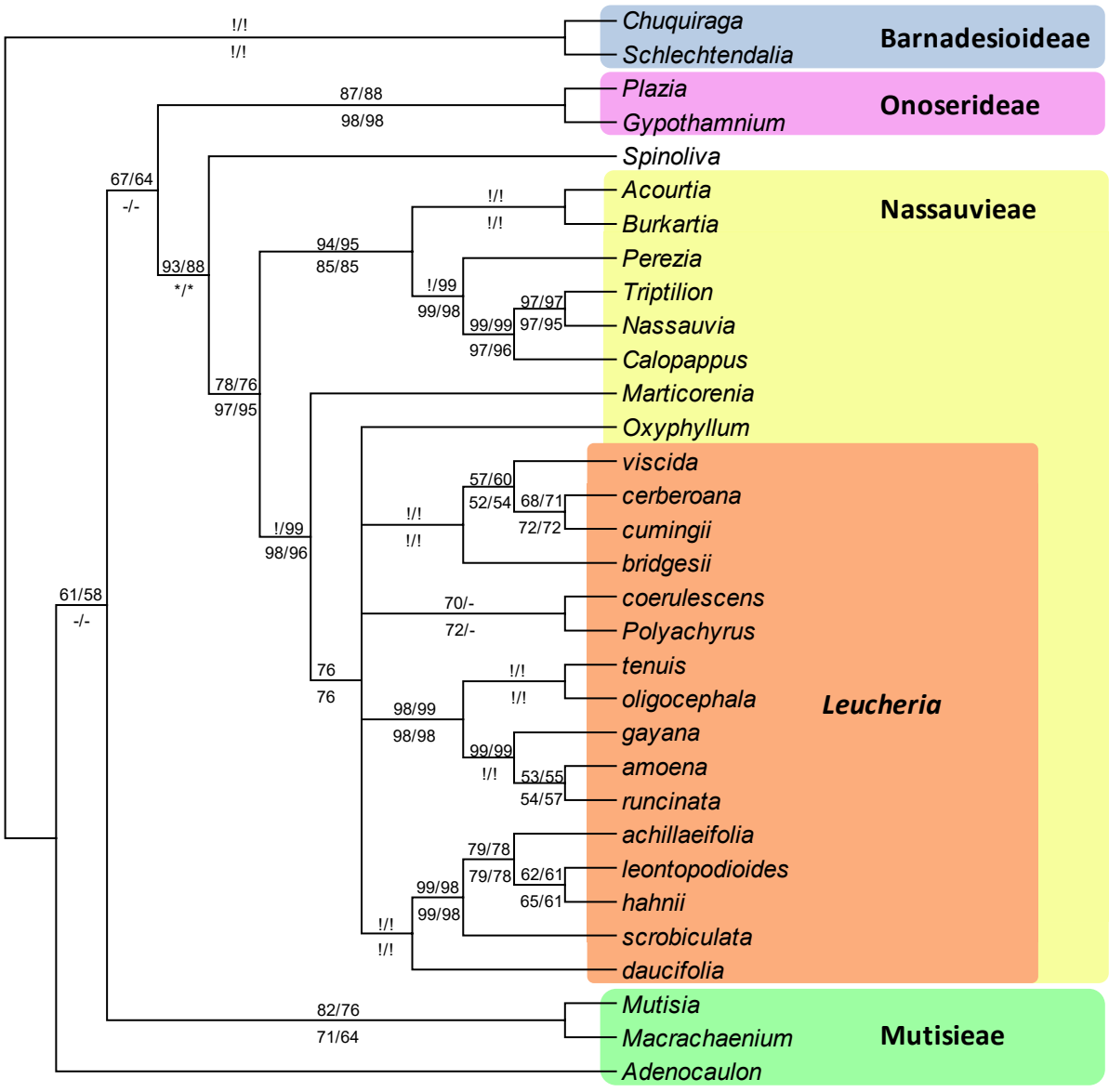

Figure 2. ML phylogram for the ITS data. The *Leucheria* species taxonomy follows Jara-Arancio et al. (2017).

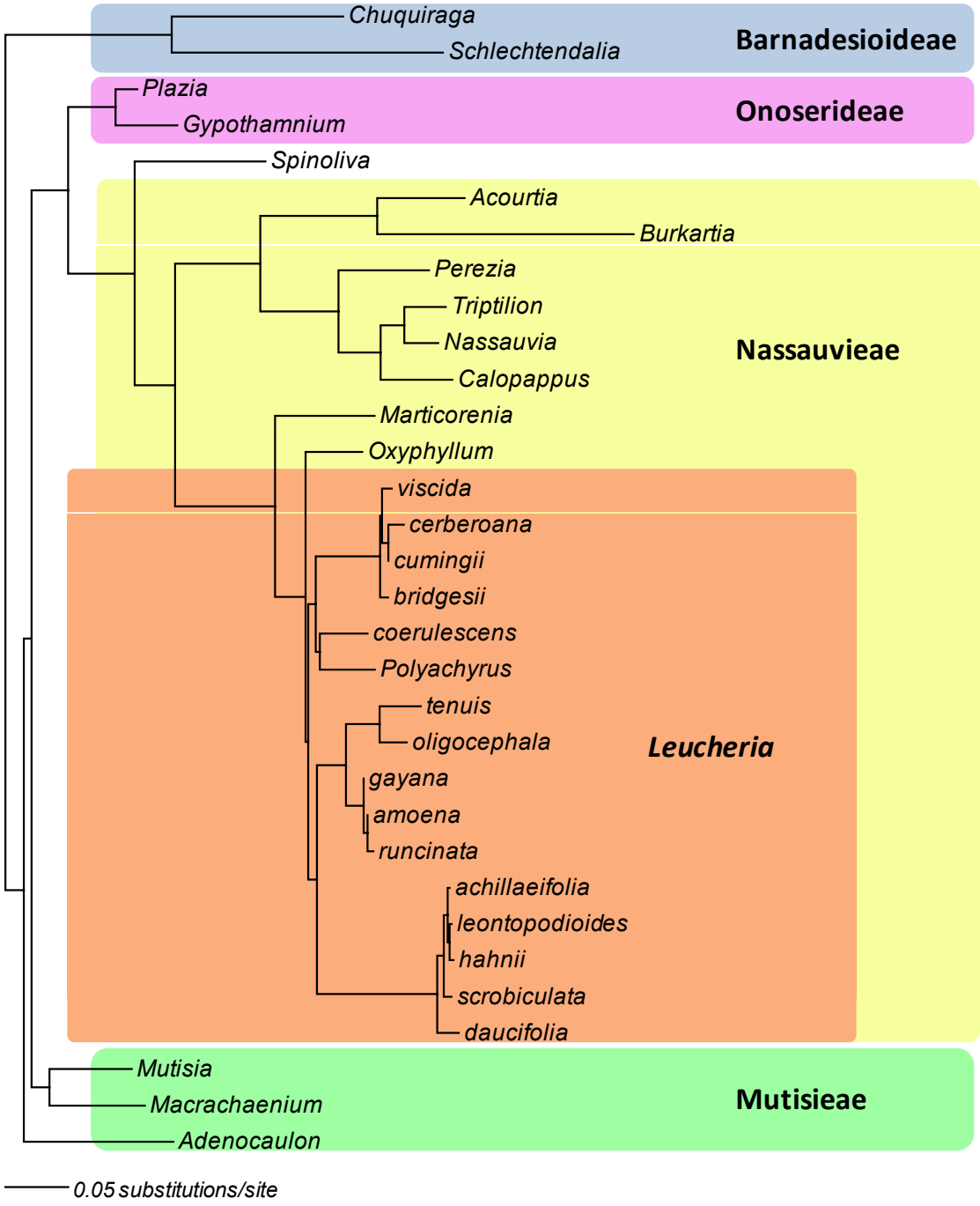

Figure 3. LogDet distance bootstrap consensus for the ITS data. Numbers above the branches are bootstrap %. The *Leucheria* species taxonomy follows Jara-Arancio et al. (2017).

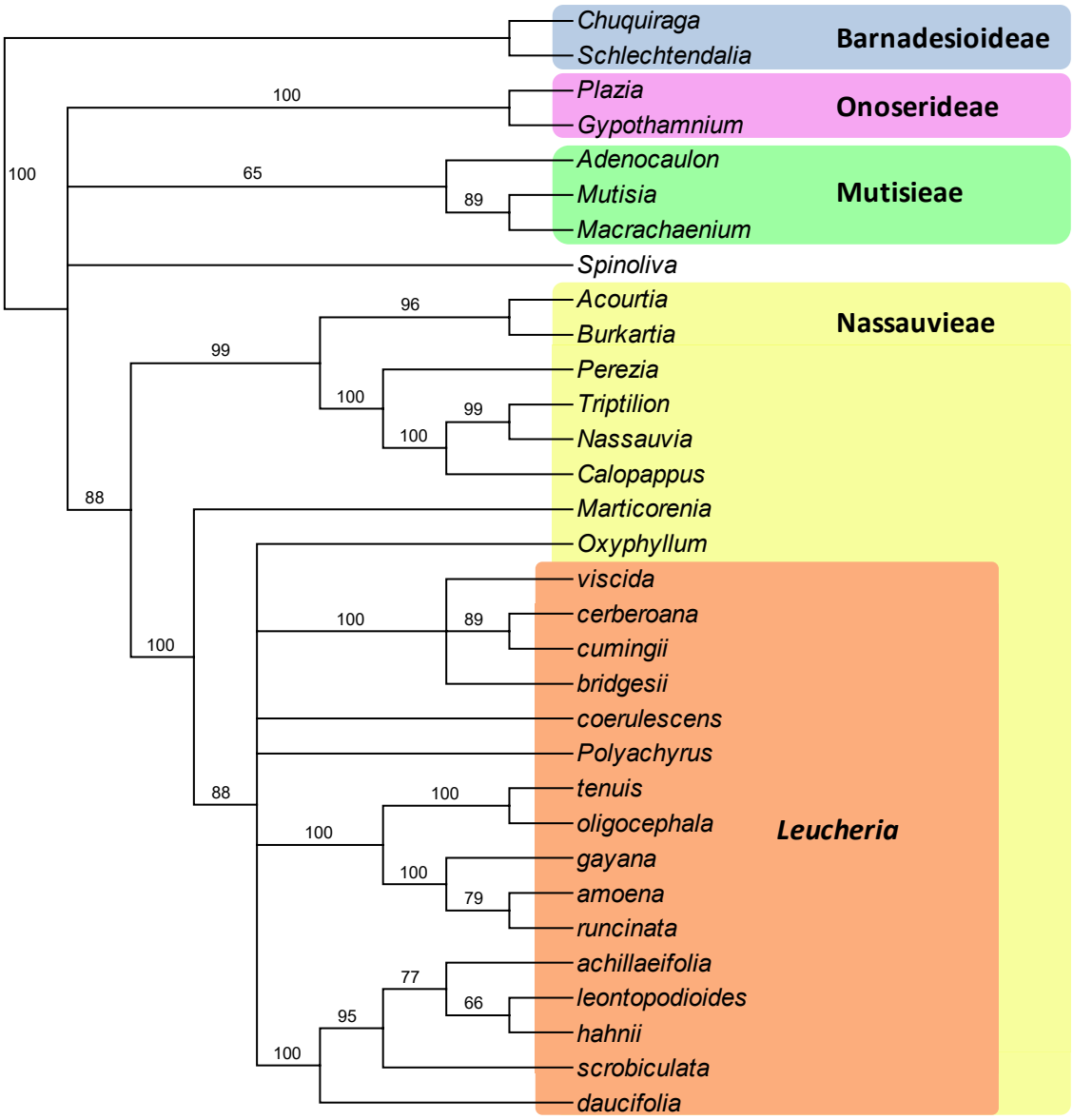

Figure 4. MP bootstrap consensus for the *rpl32-trnL* data. Numbers and symbols above the branches are bootstrap % for analysis with/without indel data. Numbers and symbols below the branches are for the same but excluding *Spinoliva*. "!" = 100%; "-" = < 50%; "*" = not applicable because of exclusion of the *Spinoliva* branch. The *Leucheria* species taxonomy follows Jara-Arancio et al. (2017).

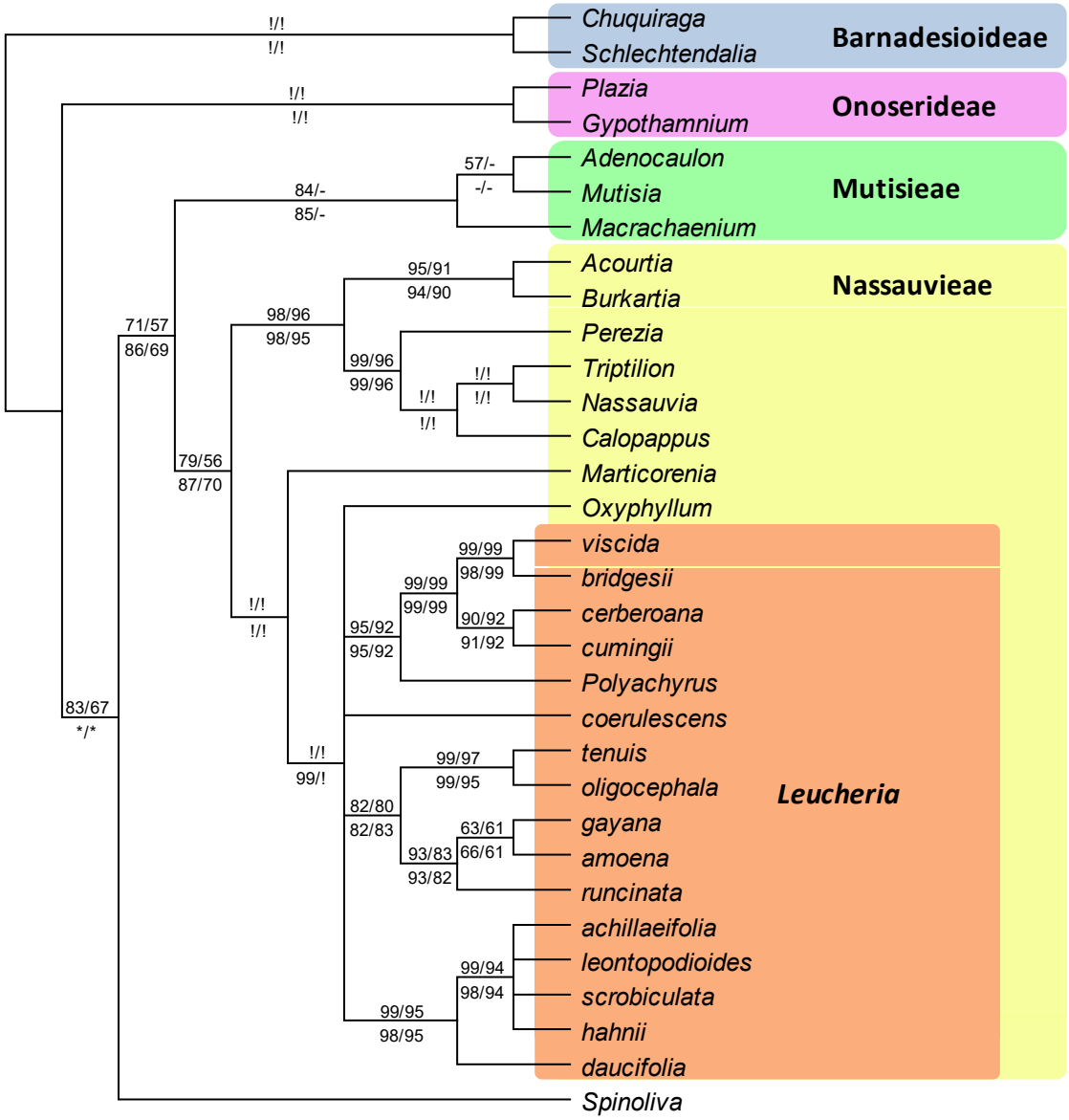

Figure 5. ML phylogram for the *rpl32-trnL* data. The *Leucheria* species taxonomy follows Jara-Arancio et al. (2017).

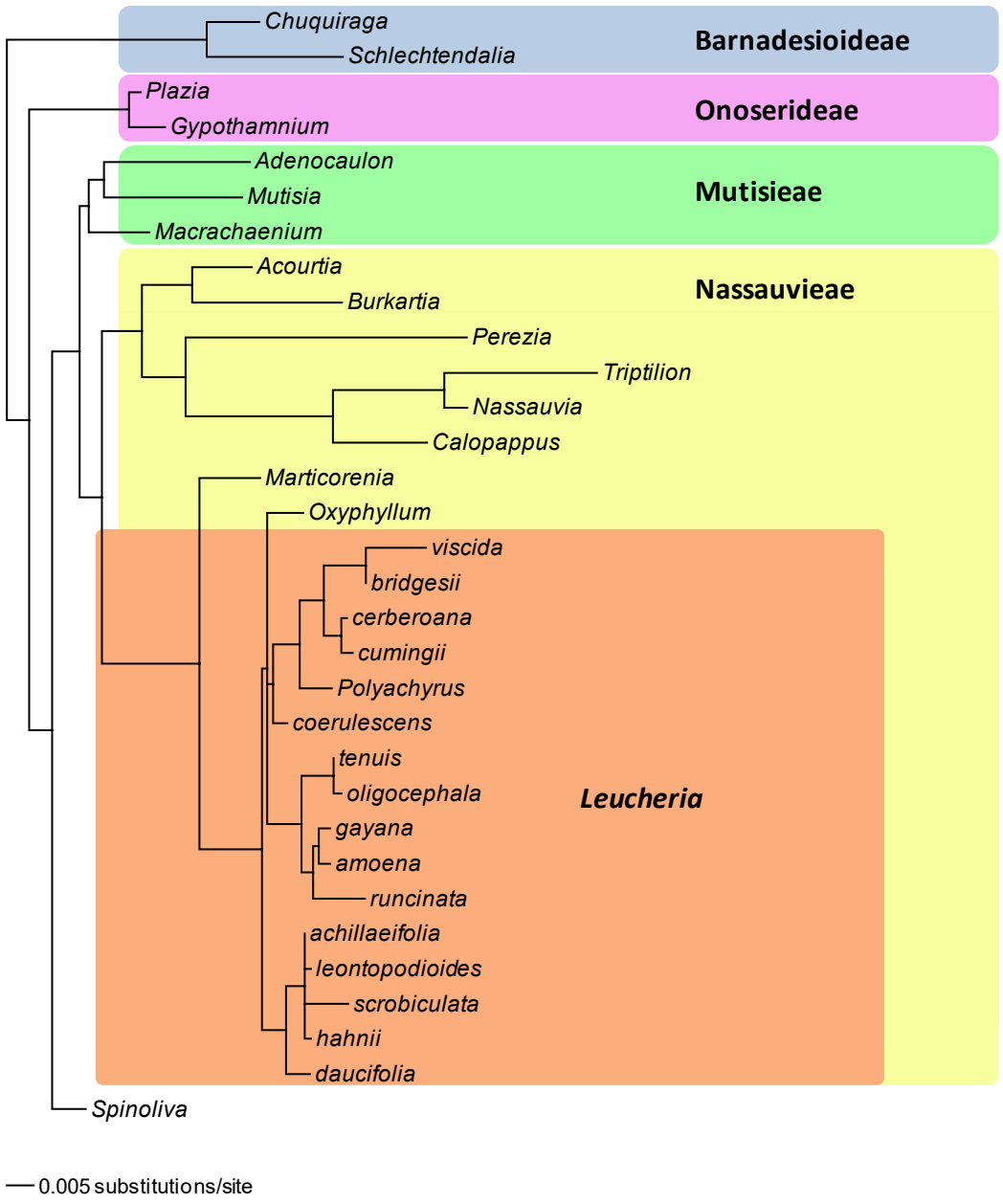

Figure 6. MP bootstrap consensus for the combined ITS and *rpl32-trnL* data. Numbers and symbols above the branches are bootstrap % for analysis with/without indel data. Numbers and symbols below the branches are for the same but excluding *Spinoliva*. "!" = 100%; "-" = < 50%; "*" = not applicable because of exclusion of the *Spinoliva* branch. The *Leucheria* species taxonomy follows Jara-Arancio et al. (2017).

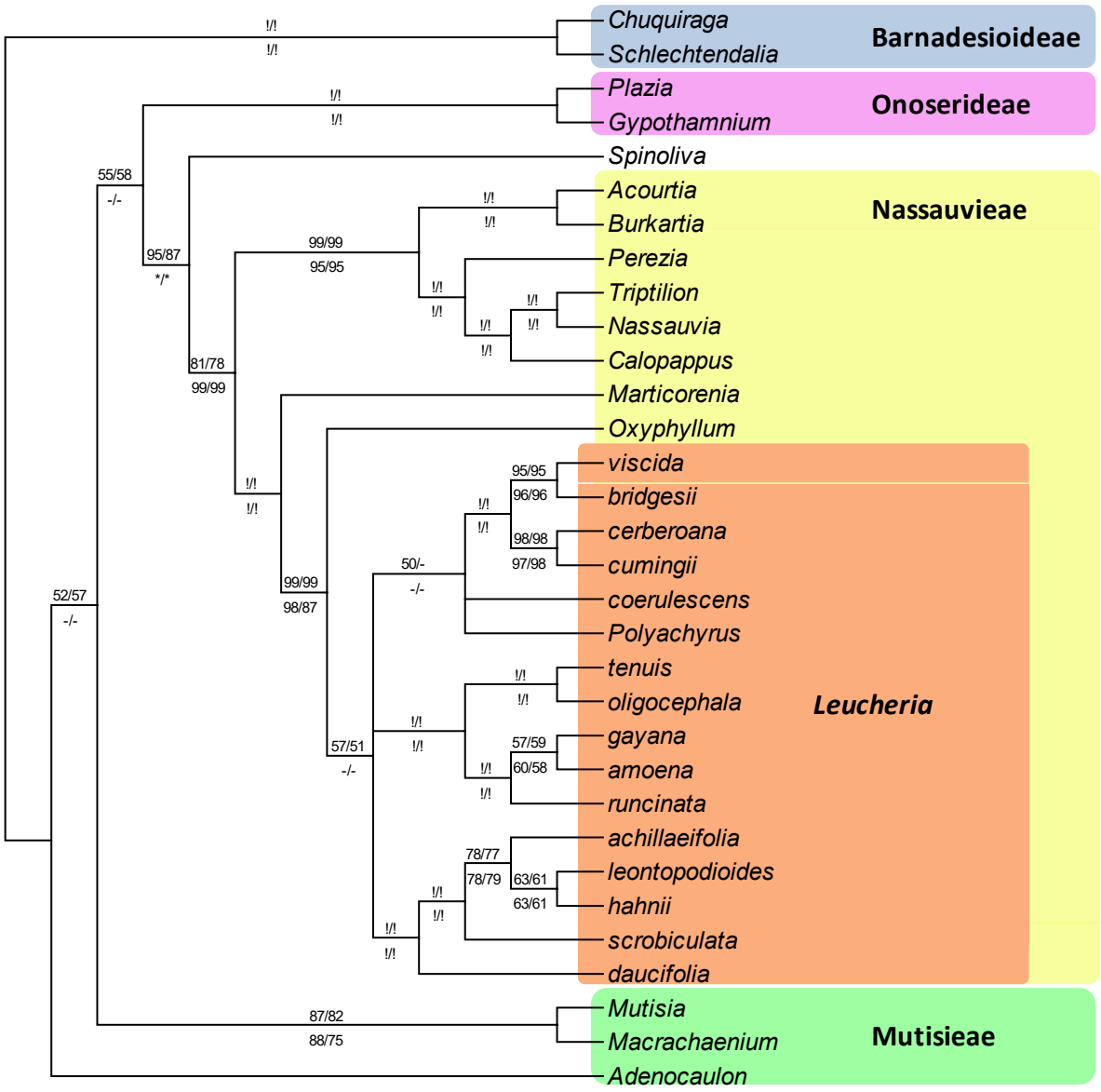

Figure 7. LogDet distance bootstrap consensus for the combined ITS and *rpl32-trnL* data. Numbers above the branches are bootstrap %. The *Leucheria* species taxonomy follows Jara-Arancio et al. (2017).