Evolutionary Insights from DNA Sequences from *Chaetanthera* Ruiz & Pav. and *Oriastrum* Poepp. & Endl. (Asteraceae; Mutisieae). I. Of Molecules and Systematics

Mark A. Hershkovitz
Santiago, Chile
cistanthe@gmail.com

ABSTRACT

Phylogenetic analysis of combined ribosomal DNA internal transcribed spacer (ITS) and chloroplast DNA *rpl32-trnL* intergenic spacer sequences greatly improves phylogenetic resolution within the central/southern Andean genera *Chaetanthera* Ruiz & Pav. and *Oriastrum* Poepp. & Endl. (Asteraceae; Mutisieae) over a previously published phylogeny based on ITS alone. The results support segregation of *Chaetanthera* subg. *Liniphyllum* Less. from *C.* subg. *Chaetanthera*. One sample with peculiar ITS and *rpl32-trnL* sequences may be of extraterrestrial origin. Fifteen of 16 nominal species sampled more than once for both loci were polymorphic for at least one of them, and only half of the polymorphic samples were demonstrably monophyletic in the combined data analysis. An additional five species sampled only for ITS all were polymorphic. These results underscore the ontological difference between gene trees and species trees and further discredit the notion of "species barcodes." The gene trees for both loci manifest departures from all evolutionary models implemented for phylogenetic reconstruction. This result is explained as a consequence of evolutionary idiosyncraticity, in turn a function of the determinacy of biological organisms and processes consequent to autopoiesis. This determinacy implicates a chaotic evolutionary function that theoretically cannot be reconstructed or predicted by stochastic models. However, because phylogenetic history and clades are materially tangible entities, their reconstruction is within the realm of scientific inquiry. I discuss the phylogeny of *Chaetanthera/Oriastrum* in this epistemological framework.

Key words: *Chaetanthera*, *Oriastrum*, Mutisieae, Asteraceae, rDNA-ITS, cpDNA *rpl32-trnL*, gene trees, species trees, molecular evolutionary models, phylogenetic methods, evolutionary idiosyncraticity, alien species

CITATION: Hershkovitz, M. [A.]. 2021. Evolutionary insights from DNA sequences from *Chaetanthera* Ruiz & Pav. and *Oriastrum* Poepp. & Endl. (Asteraceae; Mutisieae). I. Of molecules and systematics. EcoEvoRxiv, 4 January 2021. https://doi.org/10.32942/osf.io/ak68m/ [Verify most recent version].

INTRODUCTION

Hershkovitz et al. (2006a) published a phylogeny of the central/southern Andean genus *Chaetanthera* Ruiz & Pav. (sensu lato, including the later segregated *Oriastrum* Poepp. & Endl.; Davies, 2010; Asteraceae; Mutisieae) based on ribosomal DNA (rDNA) internal transcribed spacer region (ITS) sequences. Per a revised species taxonomy (Davies, 2010; see also Davies, 2013), Hershkovitz et al. (2006a) sampled, 24/30 and 13/18 (total, 37/48) nominal species of *Chaetanthera* and *Oriastrum*, respectively. A few taxonomic adjustments were proposed by Nicola et al. (2015), as elaborated later in the text.

The principal conclusion of Hershkovitz et al. (2006a) was that the exclusively alpine genus *Oriastrum* originated millions of years before the development of their modern habitat, whereas alpine species of *Chaetanthera* s. str. are of relatively recent origin. Subsequent analysis of the same ITS data (Guerrero et al., 2013) did not challenge this conclusion. Likewise, a more limited analysis of some of the same and some additional ITS sequences of *Chaetanthera* s. str. (Cabezas Álvarez, 2015) did not alter significantly the phylogenetic conclusions of Hershkovitz et al. (2006a), except as discussed later.

The present work adds to Hershkovitz et al. (2006a) analysis of chloroplast DNA (cpDNA) intergenic spacer sequences between the Large Ribosomal Protein 32 and tRNA-Leucine (UAG) genes (*rpl32-trnL*; Shaw et al., 2007). The sequences are analyzed separately and in combination with the ITS sequences using maximum parsimony (MP) and maximum likelihood (ML) criteria. Addition of the *rpl32-trnL* sequences greatly enhances phylogenetic resolution among the samples, as evidenced by bootstrap analysis.

The data support segregation of *Chaetanthera* subg. *Liniphyllum* Less. from *C.* subg. *Chaetanthera* sensu Davies (2010). However, the position of a sample identified in Hershkovitz et al. (2006a) as *C. flabelleta* D.Don is incongruent in the ITS and *rpl32-trnL* trees, and, consequently, this sample cannot be assigned to subgenus. It may have an extraterrestrial origin. While the data are consistent with the segregation of the monophyletic and presumed sister-groups *Chaetanthera* s. str. and *Oriastrum*, they also are axiomatically consistent with retention of the latter in the former. In fact, their remerging may be favored on morphological evidence and also in the interest of taxonomic stability. In particular, the 150 year-old concept of *Chaetanthera* s. l. readily distinguishes from other genera, whereas *Chaetanthera* s. str. and *Oriastrum* are distinguished only by less or not macroscopic traits that appear to intergrade more than proposed by Davies (2010).

But the present work emphasizes more the epistemological basis of the relation between molecular evolution and systematics (Hershkovitz, 2018a, 2019a, b), here using *Chaetanthera/Oriastrum* as the "model." For example, the *ontological* distinction between "gene trees" and "species trees" predicts incongruencies (as opposed to "conflicts") between these, and this is what is observed. The explicit or subliminal effort to force congruence between these, as in the "species barcodes" paradigm, thus is misguided at best. The present data demonstrate strong incongruence between genetic haplotype diversity and nominal species taxonomy. This incongruence is theoretically predicted, hence its evidence should be sought, appreciated, and scientifically exploited rather than avoided or marginalized.

The present work also emphasizes the theoretically well-articulated but usually ignored differences between molecular evolution and simplistic, erroneous, and mindlessly-applied reductionist phylogenetic reconstruction models/methods. But while the consequences of *technically* misspecified stochastic evolutionary models have been extensively studied, practically no attention has been paid to their *epistemological* misspecification. In particular, evolution is not stochastic, as commonly presumed or asserted, but, rather, *idiosyncratic* (Hershkovitz, 2018a, 2019a, b).

This evolutionary idiosyncraticity is a function of the determinacy of biological organisms and processes, which is a function of the autopoietic character of life (Maturana and Mpodozis, 2000). This determinacy implicates a chaotic evolutionary function that theoretically cannot be reconstructed or predicted by stochastic models. However, because phylogenetic history and clades are materially tangible entities, their reconstruction is within the realm of scientific inquiry. Thus, I argue that methods based on unrealistic models/assumptions can have heuristic value in phylogenetic reconstruction, but only in the framework of reason and logic. I discuss the phylogeny of *Chaetanthera/Oriastrum* in this epistemological framework.

MATERIALS AND METHODS

1. Samples and sequences

Molecular and sequence analysis methods for *rpl32-trnL* were the same as described in Hershkovitz et al. (2006a) for ITS, but using the corresponding primers. The DNA samples are the same as in Hershkovitz et al. (2006a), but the most of the vouchers here are identified according to Davies (2010; Table 1). Table 1 also notes taxonomic adjustments proposed by Nicola et al. (2015), and consequences on the present results are addressed in the Results and Discussion. Nicola et al. (2015) did not examine the collections sampled here. The present analysis includes 62/63 of the samples in the phylogeny of Hershkovitz et al. (2006a), these representing 24/30 and 13/18 of the nominal species of, respectively, *Chaetanthera* s. str. and *Oriastrum* as recognized by Davies (2010), plus one unidentified *Chaetanthera* sample.

The vouchers of 12 samples analyzed here were not mentioned and presumably not seen by Davies (2010). Their species identification here is based on circumstantial evidence (morphology, geography, and DNA sequence), but one or more of the identifications might be "incorrect" per Davies' (2010) taxonomy. The inclusion in the present analysis is, in any case, "informative," just as the analysis of Hershkovitz et al. (2006a) was informative, even though many of the vouchers have been since identified differently by Davies (2010). "Ambiguous" identification in no way signifies no identity at all. Moreover, technically, the only absolutely unambiguously identified individuals are holotypes. All other identifications are relative.

One sample identified in Hershkovitz et al. (2006a) as *Chaetanthera flabellata* is listed here as *sp_indet_25161*. This is because the ITS sequence apparently is highly divergent from that of a specimen of *C. flabellata* identified by Davies (2010) and analyzed by Cabezas Álvarez (2015). The latter unpublished sequence evidently is very similar to that from the same specimen of *C. euphrasioides* used in both analyses. In fact, Davies (2010) noted that *C. flabellata* and *C. euphrasioides* are very similar species. But the ITS and *rpl32-trnL* sequences of *sp_indet_25161* (not listed in Davies, 2010) both are highly divergent and, moreover, their phylogenetic position is incongruent in the respective gene trees (see results). This sample is considered further in the discussion.

Table 1 also includes reference to the outgroup sequences used here. Otherwise, the *Chaetanthera* and *Oriastrum* ITS sequences are the same as in Hershkovitz et al. (2006a), and the *rpl32-trnL* sequences are reported here. The amplification and sequencing primers used for *rpl32-trnL* differed from those developed independently by Shaw et al. (2007). Functionality of the latter obviates the need to report here the sequences of the former. Alignments were constructed manually.

The aligned *Chaetanthera*/*Oriastrum* ITS and partial *rpl32-trnL* sequences (with outgroups, without indel characters) and the combined alignment (without outgroups, with indel characters) are provided in NEXUS format at https://osf.io/r764b/. The outgroup sequences are "modified." The modification is deletion of "insertions" in the outgroup relative to the ingroup sequences. This is because the alignment of the former was prepared several years ago, and the insertions in the outgroup sequences were uninformative. All three alignments include various superfluous gaps (in all taxa) owing to earlier alignment manipulations. These gaps were not closed, because they had no affect on the data analysis.

2. *Tree construction methods*

Phylogenetic tree construction of the sequence data applying both MP and ML criteria were undertaken using PAUP Version 4.0a168 (Swofford, 2003). As in Hershkovitz et al. (2006a), all analyses applied the heuristic search (HS) protocol with the tree bisection-reconnection algorithm, with other parameters (replications, maxtrees, starting tree, number of trees held at each step when using the stepwise addition [SA] algorithm, number of rearrangements per tree) varying depending upon the objective of the particular analysis and in order to achieve a balance between rigor and computational speed.

Both data sets were analyzed using MP, both with and without indel characters. As in Hershkovitz et al. (2006a), apparently discrete insertions/deletions (indels) were scored as additional 4-state characters coded using DNA base symbols. Ambiguously aligned positions were scored as missing. In general, four seemed to be approximately the limit of states that could be scored "unambiguously," i.e., indels with more than four states were deemed unalignable. For MP, 10 trees were held at each SA step, with no limit on maxtrees or rearrangements. Trees were rooted along the branch between *Chaetanthera* s. str. and *Oriastrum*, as suggested in Hershkovitz et al. (2006a; see also below).

For (frequentist, not Bayesian) ML, only substitution data were analyzed. Identical samples were represented by a single one. Statistically optimized linear substitution models were deduced using the automated model selection tool in PAUP. However, the models so-estimated are known or at least presumed in practice to not be the "true" models (Yang and Xhu, 2018; Abadi et al., 2019), hence will be termed hereafter "fake models" (FM). Hershkovitz (2019b) proposed that conventional DNA substitution models are false not (or just) because they are misspecified statistically, but rather misspecified epistemologically (see Discussion).

FM selection, however, was somewhat subjective owing to Abadi et al. (2019). These workers noted that that there is no consensus basis for selecting among different FMs selected by different FM selection procedures. At the same time, they found that, at least with respect to tree topology and ancestral state reconstruction, different FMs selected by different procedures performed the same. In fact, vastly suboptimal FMs performed nearly as well. They recommended skipping model selection and using the most generalized DNA substitution model, "GTR+I+G," the general time-reversible model (with specified base frequencies) coupled with the invariant sites and gamma rate corrections for among-site heterogeneity. The FM models and parameters were estimated using a neighbor-joining (NJ) tree and then re-estimated using ML trees optimized with the initial parameters. The HS procedure was the same as for MP.

In addition to the above, distance-based trees were constructed using the Balanced Minimum Evolution (BME) criterion (Desper and Gascuel, 2004, 2005) implemented in PAUP. This procedure was undertaken only to compare BME with the MP and ML tree scores. The same HS procedure was applied as for MP, specifying the GTR model with among site rate heterogeneity parameters/values as applied in the ML procedures and empirical base frequencies.

3. Outgroups

For ML tree construction only, each data set was analyzed both with and without sequences from additional outgroups. This was essentially a "formality," in order to demonstrate that *Chaetanthera* s. str. and *Oriastrum* were mutual outgroups of each other, hence no additional outgroups were necessary. This result was reported in Hershkovitz et al. (2006a) and is confirmed here. However, outgroup comparison still was useful to examine whether either the *Chaetanthera* s. str. or *Oriastrum* sequences were more pleisiomorphic, viz., whether the inferred root was biased towards one or the other end of the branch between the genera. This, in turn, might affect molecular dating analysis.

The precise sister lineage of the *Chaetanthera*/*Oriastrum* clade evidently has not been determined. Katinas and Funk (2020) classified these taxa along with four other genera in their Subtribe Mutisiinae. Among the four genera are the evidently sister taxa *Mutisia* Lf. and *Pachylaena* D.Don ex Hook. & Arn. But for phylogenetic reconstruction purposes, sister relation precision may not be as important as sequence divergence, as demonstrated for *Tropaeolum* by Hershkovitz et al. (2006b).

Hershkovitz et al. (2006a) reported poor alignability of *Mutisia*, *Pachylaena*, and *Trichocline* (Mutisieae; Katinas and Funk 2020) ITS sequences to the *Chaetanthera*/*Oriastrum* sequences. Here, adequate alignment was obtained using sequences of *M. decurrens* and *M. hamata*. These appear to represent the basalmost diverging species of each of the two major clades of *Mutisia* (cf. Moreira-Muñoz et al., 2020). Alignability was fairly good except in the two hypervariable regions of ITS1 (Hershkovitz et al., 1999). Especially in the 3' hypervariable region, the alignment to the *Chaetanthera*/*Oriastrum* sequences was essentially random. This might be problematic, because random sequence has no phylogenetic signal and is apt to insert anywhere in a sequence phylogenetic reconstruction. But this hypervariability is mitigated, because 70–80% of the *Mutisia* sequences are more conserved and readily aligned.

For the *rpl32-trnL* ML analysis, outgroup sequences were from *Mutisia spinosa*, *Proustia ilicifolia* (Mutisioideae, Nassauvieae; Katinas and Funk, 2020), and *Richterago discoidea* (Gochnatioideae, Gochnatieae; Katinas and Funk, 2020). The latter two were selected on the basis of sequence similarity with an *Oriastrum acerosum* sequence, as determined by a BLAST search (Altschul et al., 1990).

4. Data congruence analysis

Phylogenetic congruence of the ITS and *rpl32-trnL* data and of infralocus substitution and indel data were examined using the MP-based partition homogeneity or incongruence length difference (ILD) test, as implemented in PAUP. Despite its known faults, among these a high Type I (but presumably not Type II) error rate (Barker and Lutzoni, 2002), this approximation was applied as a complement to interpret combined data analysis (see below). Homogeneity of substitution characters of the two genes was tested using the HS as above, with 1000 replicates, maxtrees limited to 100, 10 trees held at each step of SA, and unlimited rearrangements. The substitution and indel characters of each gene were tested similarly, but with 100 trees held at each SA step and rearrangements increased to 10,000.

5. Data support for constructed trees

Data support for clades in optimal trees was analyzed using the bootstrap (Felsenstein, 1985a) as implemented in PAUP. The ITS and *rpl32-trnL* data each were analyzed using both MP and ML. The MP bootstrap analysis was applied to each data set both with and without indel characters. In addition, the MP bootstrap was applied to combined ITS and *rpl32-trnL* data with indel data, with only ITS indel data (because the ILD test rejected homogeneity between the *rpl32-trnL* substitution and indel data; see Results), and without indel data. For the MP bootstrap analyses, 1000 replicates were performed, maxtrees was set to 1000, 10 trees held at each SA step, and rearrangements limited to 1000. The ML bootstrap was performed similarly except limited to 500 replicates, beginning with a NJ starting tree and no limit on maxtrees (none was necessary).

Substitutions from the two data sets were weighted equally in the combined data analysis. This might be problematic, because the ITS data are far more variable and the locus evidently evolves overall 2–3 times faster than *rpl32-trnL*. Thus, the combined analysis should tend to reinforce signal when the two loci are congruent, but the ITS probably overrules *rpl32-trnL* when they are not. This problem might be alleviated by weighting the *rpl32-trnL* data, but this is not as simple a matter as it appears. For one, the relationship between evolutionary rates in ITS and *rpl32-trnL* evidently is not strictly linear (see Results). For another, not all informative sites evolve at the same rate (Hershkovitz and Zimmer, 1996; Hershkovitz et al., 1999). Thus, the difference in overall rates between loci might be a red herring.

The sequence data sets also had been subjected to “Bayesian analysis” using Mr. Bayes 3.2 (Ronquist et al., 2012) and following protocols typical in contemporaneously published analyses. Perhaps the greatest advantage of this approach was the facility with which heterogeneous data (different loci and/or substitution and indel data) can be analyzed. However, owing to both theoretical and practical shortcomings of this approach (see Discussion), these results are not presented.

6. NeighborNet visualization

To complement tree construction and bootstrap analysis, phylogenetic networks were constructed using the NeighborNet (Bryant and Moulton, 2004) feature implemented in Splitstree 4.14.8 (Huson and Bryant, 2006; 32-bit Windows version). NeighborNet adapts the neighbor-joining (NJ) algorithm to construct reticulating networks that permit visualization of conflict/incongruence in the data, whether owing to homoplasy, incongruent evolutionary history, or both. Thus, alternative topologies are superimposed, such that alternative branch paths intersect and form parallelograms whose area correlates with the degree of character support for the alternative branching.

NeighborNet was use to constructs networks for the separate ITS and *rpl32-trnL* substitution data and for their combination, all without outgroups. The procedure was applied primarily for visualization/interpretation rather than analytical purposes (as suggested by Bryant and Moulton, 2004: 262). A full split decomposition analysis was not undertaken at this time, though the results of the present implementation suggest that this would be worthwhile.

For ITS, alternative branching may be consequent to multiple factors. It is presumed here that it owes mainly to homoplasy, given the relatively rapid rate of sequence evolution. But it may owe to either or both of infragenomic and intergenomic recombination. The former would be consequent to recombination among the hundreds to thousands of rDNA copies in the genome. The latter would be consequent to hybridization. The extent to which recombination might have occurred among these ITS sequences was not analyzed. Likewise, for *rpl32-trnL*, homoplasy is presumed to be the source of alternative branchings. Intergenomic recombination is rare to absent for cpDNA, and infragenomic recombination affects mainly the inverted repeat and immediately adjacent regions. In the combined analysis, alternative branching owe to both homoplasy and incongruent histories of the loci.

For the separate data sets, distances were calculated using the implemented HKY85+G model, which corrects for transition/transversion (Ti/Tv) rate, base compositional bias, and among-site rate heterogeneity. The program does not accommodate the full GTR model, though the HKY85+G model is perfectly adequate for the present purposes. The model parameter values were those calculated for the HKY85+G model in the PAUP model selection protocol applied above. In addition, the "Fitch1" option for branch length fitting was selected. For the combined data NeighborNet tree, the Jukes-Cantor model plus gamma was applied. This is because the HKY85 parameter values for the separate data sets were extremely different. I presume here that most of the error in branch length estimates owes to uncorrected simple substitution distance and among-site rate heterogeneity. As for the combined data bootstrap analyses, substitutions in the two data set were weighted equally (see above). Likewise, the ITS signal might be expected to dominate the combined data networks,

RESULTS

1. Basic sequence characteristics

The ITS sequences range in length between 615–655 base pairs (bp). The alignment length was 691. Nine alignable informative indels having 2–4 states were scored for the ITS sequences. The *rpl32-trnL* sequences range in length between 501–807 bp, but typically are 750–760 bp. The alignment length was 959. Especially short sequences include *peruviana_25254* (501 bp), *pusillum_25120* (516 bp), *chilense_25180* (526 bp), and *euphrasioides_25176* (555 bp). This owes to especially large deletions in these samples, though the position of these deletions is not shared among the taxa and otherwise manifests no phylogenetic relation to sequence length of other taxa. Twenty-three informative indels were scored with 2–4 states for the *rpl32-trnL*data. The sequences include two notable hypervariable microsatellites. A poly-A microsatellite towards the 5' end of the sequence varies in length between 3–14 bases, and could not be scored unambiguously. A poly-T microsatellite near the 3' end varies in length between 6-11 bases. It could not be scored between genera, but manifested only four states within genera. Thus, this microsatellite was scored as a 4-state indel within each genus, with the other genus scored as missing.

2. Polymorphism within species

Sixteen of the 38 nominal species (including the unidentified one) sampled in this analysis were sampled twice or, less commonly, 3–5 times (Table 2). Considering additional previously reported ITS sequences with vouchers identified by Davies (2010), 22 nominal species were sampled more than once for ITS. Sixteen of the 22 species sampled twice for ITS manifested base polymorphisms (substitutions), and 18/22 were polymorphic considering both base and length polymorphisms.

The figures above and in Table 2 do not consider possible polymorphisms within samples. Hershkovitz et al. (2006a) reported that some samples appeared to be polymorphic at one or more base positions, evidenced by superimposed dual peaks in sequence chromatograms. These positions were scored as ambiguous in the sequence reports in GenBank. Considering sample polymorphism as species polymorphism, the ITS summaries change as follows: (1) 21/22 nominal species sampled more than once are polymorphic for ITS substitutions and substitutions plus length variation; (2) for the relevant samples analyzed in this work, 36/39 are distinct considering only base substitutions, and 37/39 are distinct considering both base substitutions and length variation; and (3) for all relevant samples, 48/53 are distinct considering only base substitutions, and 51/53 are distinct considering both base substitutions and length variation. The only nominally infraspecific sequences that are completely identical are two sequence pairs pertaining to *C. flabellifolia* and *O. acerosum*. But other samples of the latter species are polymorphic for ITS.

For *rpl32-trnL* sequences, 15/16 multiply-sampled species manifested both base and length polymorphisms. Including length polymorphisms, no two sequences among these 15 species were identical, even in the case of the two species sampled 4–5 times. Oddly enough, therefore, the *rpl32-trnL* sequences were *more* variable "infraspecifically" than ITS, even though, among all species of *Chaetanthera* s. str. and *Oriastrum*, they are about one third *less* variable, and the MP trees (total patristic distances) somewhat less than half as long. Distance-based ITS trees, which correct for superimposed substitutions, were three times as long as the *rpl32-trnL* trees (see below). Only the two samples of *C. flabellifolia* were identical for both ITS and *rpl32-trnL* sequences. In fact, the collecting localities and elevations of the specimens were rather proximal.

The substitution data include three cases of identical interspecific ITS sequences, although in all three cases, one of the nominal species is itself polymorphic. In fact, all of the apparently identical sequences are distinct considering both sequence ambiguities and length variation. The *rpl32-trnL* data include two cases of identical interspecific sequences, in both cases where one of the species is itself polymorphic. The sequences of one pair (*chilensis_25042* and *elegans_26000*) are identical, while the other (*pubescens_25076* and *glabrata_25130*) differ by a one-base indel.

3. Phylogenetic signal heterogeneity

The ILD test rejected homogeneity between the ITS and *rpl32-trnL* sequence data (p = 0.001) and between the *rpl32-trnL* substitution and indel data (p = 0.003). Homogeneity was not rejected between the ITS substitution and indel data (p = 0.95). Significant incongruence between the *rpl32-trnL* indel and substitution characters was eliminated by downweighting the indel characters to 0.3 relative to the substitution characters (p = ca. 0.3). Nonetheless, MP bootstrap analyses of the *rpl32-trnL* and combined ITS + *rpl32-trnL* assigned equal weighting to indel and substitution characters. Comparison of the trees with those produced by downweighting or excluding indel data did not seem to produce significantly different results (see below).

4. *MP tree construction*

MP summary statistics for both sequences are provided in Table 3. The MP trees are not shown, but well-supported MP tree construction results can be inferred from the bootstrap analysis (see below). The longer *rpl32-trnL* sequences yielded ca. 2/3 (69%; 63% excluding indel characters) as many informative sites as ITS. However, homoplasy of the *rpl32-trnL* data is rather lower, as indicated by all of the homoplasy-related indices. For ITS, the homoplasy-related indices are essentially the same with or without indel characters. For the *rpl32-trnL* data, inclusion of the indel data consistently increased homoplasy for all indices. This presumably reflects the phylogenetic signal heterogeneity between the substitution and indel data, reported above.

5. *ML substitution model*

The Figs. 1 and 3 captions include the ML-estimated FM parameters applied for the ITS and *rpl32-trnL* sequences, respectively. These parameters also were applied in the analyses illustrated in Figs. 2 and 4.

For ML analysis of the ITS data, FM parameter optimization was performed first with an NJ tree and refined with the consequently optimized ML tree. However, the FMs selected were the same regardless of the test tree, although the estimated parameters different somewhat. Both the standard and "corrected" Akaike Information Criterion (AIC[c]) methods selected the "SYM+I+G" model, which is the GTR+I+G model with equal base frequencies, hence a "symmetrical" matrix with all six substitution rates differing ("abcdef"). However, the Bayesian Information Criterion (BIC) and decision-theory criterion (DT) selected the "simpler" TIM3ef+I+G model, in which base frequencies are equal, r(AC) = r(CG), and r(AT) = r(GT) ("abcaec").

The likelihood of the GTR+I+G model was only -*ln*L 1 better than the SYM+I+G model (on the order of *e* times better), but the latter was -*ln*L 5 better than TIM3ef+I+G (ca. e^5 times better). The GTR+I+G model "ranked" second (out of 88 models) by AIC and third by AICc (after TIM3ef+I+G). But it ranked 11th and 15th under, respectively, BIC and DT. In the latter case, it ranked behind a model that ranked 23 out of 88 in likelihood score. The -*ln*L of the various models with gamma rates only (without invariants) increased by 4 or more, effectively $> e^4$ times worse. The -*ln*L of the various models with invariant rates only (without gamma) increased by about 50, which sucks.

Estimates of relative transversion rates different among the FMs. The substitution rates estimated under GTR+I+G were rather different from those of one of the BIC/DT selected model, TIM3ef+I+G. In the former, not only was r(AC) ca. twice (rather than equal to) r(AG), but r(AC) was very close to r(AT) and r(GT) (all close or equal to one). It is not clear, then, why the model was not reduced to two transversion rates and two transition rates. It must be kept in mind, however, that each estimated rate has a variance, and the FM test procedure did not reveal these. Evidently, in these data, r(AC) has a high variance, as its estimated value varied two-fold across the different models. All of the FMs estimated high r(CT), these ca. 5-10X the variously estimated rates of the various transversions.

FM selection preferred equal base frequencies and, indeed, base frequencies estimated using GTR+I+G were approximately equal: p(A,C,G,T) = (0.232, 0.247, 0.257, 0.264). But empirically calculated base frequency averages (excluding "redundant" sequences) are G/C-rich: (0.213, 0.285, 0.283, 0.219). Variable site base frequency averages are somewhat differently proportioned, but still G/C-rich: (0.181, 0.320, 0.250, 0.249), and these proportions are not stationary, as described below. A few FMs rejected by FM selection all estimated base frequencies as C/T-rich (ca. 58%) rather than G/C-rich. The difference between the ML estimates and the empirical averages presumably owes to the frequencies with which bases actually are "selected" as a function of substitutions along branches. As CT transitions dominate, these bases appear to form larger "pools" than suggested by empirical frequencies.

However, base frequency evidently had negligible impact on tree construction: identical trees were obtained whether using SYM, GTR with ML-estimated base frequencies, or GTR with empirical base frequencies. Naturally, the trees differed somewhat in likelihood score depending upon the model specified, and also showed miniscule differences in branch length.

For the ITS data, based on the results of FM selection together with the recommendation of Abadi et al. (2019), I used the GTR+I+G model for tree construction, though I also compared the results with the SYM+I+G model. These FMs yielded the two best likelihood scores for the data, and the SYM+I+G model ranked best for AIC and AICc and third best for BIC and DT. However, the funky behavior of the parameter estimates under different FMs, as well as the discord between FM selection criteria, affirms what is known but not adequately appreciated about DNA sequence evolution: standard linear models, however estimated, are not the true models (Yang and Zhu, 2018; Abadi et al., 2019), but, rather, FMs.

For the *rpl32-trnL* data, FM selection was more straightforward: all selection criteria preferred the TVM+G model [r(AG) = r(CT)], with GTR+G always second and not terribly worse. Moreover, there was no funkiness in parameter estimates across FMs. The GTR+G model estimated r(AG) and r(AC) as, respectively, 0.460 and 0.345. Thus, the increase in information content from combining these rates may well overshadow the -*ln*L decrease. The base frequency estimates were very close to the empirical averages, both A/T-rich (see below). Adding invariant sites to the models improved the -*ln*L < 1 (less than twice as good). However, likelihoods of FMs with invariant sites only were substantially worse. For tree construction, following Abadi et al. (2019), I preferred the GTR+G model.

Regardless of FM, molecular evolutionary patterns for the two sequences are strikingly different. For the ITS sequences, the estimated r(CT) is the highest and is about 12 times greater than for the lowest rate, r(CG). The second highest rate is for r(AG), and clearly the overall transition rate is much higher than transversion. For the *rpl32-trnL*, r(GT) is the highest but only slightly higher than r(AC); these are about eight times higher than the lowest transversion rate, r(AT). Here, the overall transversion rate is higher than the transition.

The base frequencies in the two genes also is strikingly different, ITS being more G/C-rich than *rpl32-trnL* (Table 4). The high G/C content of the former is typical of ITS (Hershkovitz and Zimmer, 1997), and the high A/T content of the latter is typical of noncoding plastome sequences. The data also indicate some differences in base composition among total versus variable sites, as well as among clades. Both sequences manifest a degree of evolutionary nonstationarity in substitution patterns. In the present analyses, base composition was estimated over all sites, although it would have been possible to apply values estimated from variable sites only.

The total and variable site ITS base A/T% within clades are approximately the same, but the range among variable sites mostly is broader. Constant sites are ca. 43% A/T, which also is within the range for total and variable sites. In contrast, variable sites of the *rpl32-trnL* sequences are far less A/T-rich than total sites. Constant sites are ca. 80% AT. This is a remarkable figure and itself is related to the observed high transversion/transition rate in this locus (see Discussion).

6. *ML tree construction*

ML phylograms for the ITS and *rpl32-trnL* data sets are shown in Figs. 1–4. Figures 1 and 3 show phylograms generated using divergent outgroup sequences and Figs. 2 and 4 show phylograms generated using only *Chaetanthera*/*Oriastrum* data, designating the latter as the monophyletic outgroup of the former.

The topologies generated with and without outgroups are very similar, at least with respect to species-level topology. However, the ITS topology with outgroups (Fig. 1) resolves *C*. subg. *Chaetanthera* and *C*. subg. *Tylloma* as sister clades, whereas the topology without outgroups (Fig. 2) resolves *C*. subg. *Chaetanthera* and *C*. subg. *Liniphyllum* as sister clades. The contrary incongruence emerges in the *rpl32-trnL* trees (Figs. 3–4). The relationships of the subgenera are not resolved in the ITS or combined bootstrap analyses (see below). All of the *rpl32-trnL* bootstrap trees show weak support for the sister relation between *C*. subg. *Chaetanthera* and *C*. subg. *Tylloma*.

The topologies generated with and without outgroups also resolve differently branch lengths within the ingroup. This is more common and pronounced in the ITS topologies, perhaps because branches are generally longer than in the *rpl32-trnL* tree. Comparing the ITS topologies with and without outgroups, 16 topologically congruent ingroup internal branches differ in length at the third decimal place (data not shown). The differences occur in all subgenera. The incongruent branch lengths involve branches 0.004–0.051 substitutions/site (s/s) in length. The numerous shorter branches and, oddly enough, the very longest branches are unchanged.

A pronounced allometry is evident comparing the two divergent branches in between the *Oriastrum* and *O*. subg. *Oriastrum* and *O*. subg. *Egania* crown nodes. In the outgroup-rooted ITS topology, these branches are, respectively, 0.029 and 0.043 s/s, whereas in the ingroup-rooted topology, they are, respectively, 0.023 and 0.049 s/s. Thus, the sum of the branch lengths is the same, but the distribution from the *Oriastrum* crown is considerably different. Correspondingly, the *O*. subg. *Oriastrum* crown appears “older” in the outgroup-rooted topology, although the topologies are not time-calibrated. Whether the dating of these and other clades would differ using time-calibration methods is not clear, but not within the scope of the present work.

Ten branch lengths differ between the *rpl32-trnL* outgroup-rooted and ingroup-rooted topologies, occurring in all subgenera except *C*. subg. *Chaetanthera*. The number is about half that for ITS, perhaps reflecting the overall shorter tree length. As for the ITS topologies, allometry is evident comparing the two divergent branches in between the *Oriastrum* and *O*. subg. *Oriastrum* and *O*. subg. *Egania* crown nodes. The allometry is less extreme, each branch differing by only 0.001 s/s, but it is parallel to that for ITS, viz., the *O*. subg. *Oriastrum* crown appears slightly “older” in the outgroup-rooted topology.

The outgroup-rooted ITS and *rpl32-trnL* topologies (Figs. 1, 3) also contrast in the apparent divergence between the outgroups and ingroup. In the ITS topology, the length of the outgroup-ingroup branch is 0.225 s/s (not shown), about four times the divergence between *Chaetanthera* s. str. and *Oriastrum*. In the *rpl32-trnL* tree, the outgroup-rooted branch is 0.036, actually about the same as the *Chaetanthera*/*Oriastrum* divergence. This, however, ignores numerous and considerable sequence length differences between the ingroup and outgroups, Still, a midpoint-rooted *rpl32-trnL* topology that includes the outgroups (not shown) does not resolve monophyly of *Chaetanthera*/*Oriastrum*. But a midpoint rooted ITS topology clearly does. Additional sampling would not seem to alter these observations, at least based on eyeball alignment of additional Mutisieae ITS and *rpl32-trnL* sequences available in GenBank at this writing.

Another feature of both the outgroup-rooted ITS and *rpl32-trnL* topologies is lower divergence of *Chaetanthera* s. str. than *Oriastrum* from the root node. This per se has no bearing on the absolute stem age of each genus. It merely means that the ML ancestral sequences of *Chaetanthera* s. str. are more similar to the ancestral sequences of *Chaetanthera*/*Oriastrum* than are the ancestral sequences of *Oriastrum*. It might and probably does mean that *extant Chaetanthera* s. str. diverged from an ancestor older than *extant Oriastrum*. This is essentially the conclusion of Hershkovitz et al. (2006a): the ancestral lower elevation lineages of *Oriastrum* are extinct.

Both the ITS and *rpl32-trnL* topologies manifest evolutionary rate heterogeneity (nonstationarity), though this is much more pronounced in the ITS topologies. This is especially evident in the contrast between branch lengths of *Oriastrum* subg. *Oriastrum* and *O*. subg. *Egania*, as well as between the two clades within *C*. subg. *Liniphyllum*. Additional examples are apparent in the trees. Rate changes seem fewer in the *rpl32-trnL* topology, but are nonetheless notable. Here, the contrast in *Oriastrum* is not between subgenera but within *O*. subg. *Oriastrum*, where divergence has been notably slower in the *O. gnaphalioides* samples, even slower than that of *O*. subg. *Egania*. Additional contrasts appear elsewhere in the topology, conspicuously in the *euphrasioides_25176* branch.

The question of whether the inferred changes in molecular evolutionary rate relate to life form will be deferred to the follow-up paper. But, in a word, the answer is no, though this conclusion bases partially on the rejection of current correlative phylogenetic comparative methods in favor of the simple criterion of falsification (cf. Hershkovitz, 2018a, 2019a, b). The "obvious" evidence for a causal relation between life form and molecular evolutionary rate is the stark contrast in ITS topology branch lengths between the annual *O*. subg. *Oriastrum* and the caudicose perennial *O*. subg. *Egania*. But, as noted, in the *rpl32-trnL* topology, this contrast occurs within *O*. subg. *Oriastrum*. Likewise, a rate difference is apparent within the annual clade *C*. subg. *Liniphyllum*.

Branch lengths in the ITS and *rpl32-trnL* topologies superficially seem approximately proportional, which would suggest that, whatever might be supposed to drive molecular evolution, it is shared among the nucleosome and plastome. But closer inspection reveals that the proportionality is, at best, loose. One difference, noted above, is the contrast between the two topologies in branch lengths within *O*. subg. *Oriastrum*. A more generalized observation: the ITS topology includes a total of nine nearest neighbor divergences from a common node and/or terminal that exceed the length of the basal divergence between *Chaetanthera* s. str. and *Oriastrum*. In two cases, the divergences are more than double the latter. These are the two divergences in the clade (*lycopodioides_25169*, (*chilense_25180*, *pusillum_25120*)). The *rpl32-trnL* topology includes only a single nearest neighbor divergence that exceeds the length of the *Chaetanthera*–*Oriastrum* divergence. This involves the exceptionally long *euphrasioides_25176* branch. Another divergence, separating *C*. subgenus *Chaetanthera* and its sister (different in the outgroup- and ingroup-rooted topologies), is about the same length as the intergeneric divergence, but all remaining divergences are considerably less.

7. *Scores of MP, ML, and BME trees under alternative criteria*

For the 90 ITS MP trees for the ML data set (without outgroups), the -*ln*L scores per the GTR+I+G model ranged from 5606.947–5613.482, i.e., some were only slightly worse than the ML tree (5609.267) under this model. The MP score of the ML tree was 903, versus 899 for the MP tree. The BME tree scored 903 by MP and its -*ln*L was 5613.875, i.e., e^7 times worse than the ML tree and slightly worse than the worst ML score among MP trees.

For the 1478 *rpl32-trnL* MP trees for the ML data set (without outgroups), the -*ln*L scores per the GTR+G model ranged from 3650.418–3664.799. The lower value is equal to the optimal ML tree, and 48 of the MP trees (3%) had this score. Correspondingly, the MP score of the ML tree was 397, the same as the MP trees, i.e., the Fig. 3 ML topology is also an MP topology. The BME tree scored 402 by MP and its -*ln*L was 3670.309, ca e^{20} times worse than the ML tree (see also below).

8. Bootstrap analyses

The bootstrap results are summarized in Figs. 5–7 and in Table 5. Table 5 lists 71 clades supported in the majority rule tree of at least one of the three bootstrap tests for each of the separate ITS and *rpl32-trnL* data sets and their combination, nine tests altogether. The “true” maximum number of partitions between 62 taxa is 60, the extra 11 observed corresponding to clades incompatible among the different bootstrap tests.

The combined data bootstraps (all MP) support monophyly of each genus and the subgenera recognized by Davies (2010) plus *C.* subg. *Liniphyllum*. Support from ITS for *C.* subg. *Liniphyllum* appears reduced (discussed below), and support for *O.* subg. *Oriastrum* from *rpl32-trnL* is weak to nil. However, the combined data bootstraps indicate that the individual data sets at least are not incongruent with respect to the generic/subgeneric clades. The bootstraps support also additional clades within each of the subgenera. These results are considered in the discussion. However, a few cases of apparent incongruency between ITS and *rpl32-trnL* are highlighted in the Results below.

In interpreting the bootstrap trees, it must be emphasized that while “highly” supported clades are, likewise, “highly” informative, contrapositively, “weakly” (or not) supported clades are likewise, “weakly” informative. This is because highly supported nodes are supported against all possible conflicting clades. The principle caveat is sample adequacy, hence whether or not all possible conflicting clades are adequately tested. By contrast, weak support for a clade, *by itself*, says little about the truth of that clade. It simply indicates inadequacy of evidence one way or another. Depending on the divergence of related samples, support might be weak even for a clade comprising identical sequences, e.g., Table 5, Clade II, tests 5 and 6, and Clade XVII, tests 2 and 3. The complete bootstrap partition table permits evaluation of support for all competing clades, hence to determine whether apparently weakly (or not) supported clades are *relatively* well-supported in the context of the data.

The above caveat kept in mind, Table 5 indicates that the number of resolved clades for the majority rule consensus of the ITS data is ca. 10% better than for the *rpl32-trnL* data. But for clades supported by bootstrap proportions (BP) $\geq$ 70%; (~ 70% confidence level; see Discussion), ITS resolves 15-25% more clades. The better-resolved ITS majority rule bootstrap trees resolve 70–82% of the possible 60 clades, and at the 70% confidence level, 55–62%. For the *rpl32-trnL* data, the figures are, respectively, only 68–78% and 42–50%. The lower bootstrap resolution of the *rpl32-trnL* data evidently owes to lower phylogenetic quantity rather than quality. This follows from the superior homoplasy indices (Table 3). Although not analyzed here, compared to the ITS bootstrap, support for partitions conflicting with the best supported one likely is relatively low and more dispersed across the *rpl32-trnL* tree.

But for the combined data bootstraps, the figures increase: support at the 50% and 70% level are, respectively, 82–85% and 70–72%. Overall, the ITS and *rpl32-trnL* data are complementary or at least mutually compatible at 35–36 nodes of the total 42–43 nodes supported at the 70% (mostly higher) level in the combined analysis. This is despite the IDL test results. Complementarity/compatibility is evident when the combined data BP is equal or greater than either separate data BP.

The Table 5 data demonstrate that the ITS contains most of the phylogenetic signal. About 17 clades (varying slightly with the test conditions) are supported at the 70% (mostly higher) confidence interval in both data sets. About another 14 achieve this level in the ITS and an equal or *usually greater* level in the combined data analyses, but not in the *rpl32-trnL* data alone. This indicates that, at *these* nodes, signal in the *rpl32-trnL* data is not incompatible/incongruent with that in the ITS data, but is by itself insufficient to resolve the node with high confidence.

The reverse case, strong support in the *rpl32-trnL* and combined data but not in the ITS data, occurs for only three clades plus one additional "problematic" one (see below). In these cases, it is the ITS data that are not incompatible/incongruent with the *rpl32-trnL* data, but are themselves insufficient to resolve the clade with high confidence. There seems to be only one clade, Clade IV, that is marginally well-supported *only* in the *rpl32-trnL* and combined (hence ITS) data bootstraps that *exclude* the *rpl32-trnL* indel data. This possibly underlies the ILD rejection of homogeneity between *rpl32-trnL* substitution and indel data. But there are three counterexamples of clades (XXII, XXVI, XXXXV) strongly supported by the *rpl32-trnL* substitution and indel data and also in the combined (hence ITS) data bootstrap. As noted by Barker and Lutzoni (2002), congruence/compatibility of heterologous data thus is clade-specific. Only one clade (XV) is supported at < 70% in each data set but > 80% in the combined data bootstrap.

The bootstrap data for both ITS and *rpl32-trnL* also reveal that ML analysis (Table 5, tests 3 and 6) disproportionately increases the confidence interval over MP analysis (tests 2 and 4) of the same data, viz. substitution data without indel data. For the ITS data, the confidence interval is increased at 27 nodes, compared to 11 nodes where it is decreased. For *rpl32-trnL*, the proportion is nearly the same, ML increasing the confidence level at 29 nodes and decreasing it at 12. Although these figures base on as little as a single percentage point difference, a bias one way or the other is not expected.

Given the HS protocols applied here, it would seem that the ML bootstrap would be no better or *less* well resolved than the MP bootstraps. The ML bootstrap was less rigorous, 500 rather than 1000 replicates. While both bootstraps limited the number of permitted rearrangements to 1000, MP generally searches more of tree space than the default ML procedure. This is because MP usually saves (often very much) larger numbers of (sometimes quite different) trees with identical scores, and branch-swaps on each of these in search of a better one. The default ML procedure discriminates more between similar trees, hence saves and swaps on one to a few at a time, hence explores less of tree space. Also, the MP bootstrap used SA starting trees, whereas the ML bootstrap used NJ starting trees (because SA with ML was exceedingly slow on my antique notebook). In general, and confirmed in the present case, SA trees are a priori much better optimized and therefore require fewer rearrangements to optimize further. The ML bootstrap performed here was monitored to examine the course of likelihood improvement during branch-swapping. Indeed, likelihoods improved notably during the first 500 rearrangements and much less, if ever, during the second 500 rearrangements and very rarely at all in the final 100 rearrangements. Nonetheless, the ML bootstraps procedures were overall less rigorous than the MP bootstraps, which ought to yield poorer resolution.

I suggest that the improved bootstrap resolution of the ML tree owes to two factors. One, as noted, MP saves more trees, hence axiomatically more topological incongruencies. But this effect might be offset to the degree that individual ML bootstrap replicates are more likely to converge on collectively more incongruent topologies. But this, in turn, may be offset by the second factor, effective "weighting" by ML. In particular, ML substitution models have an effect comparable to (but distinct from) successive homoplasy-based character weighting in MP. The latter indeed can improve apparent tree resolution and reduce the number of MP trees, because less homoplasious characters are weighted relative to "noisy" ones that support alternative topologies. But this method itself can converge on a spurious topology, and, similarly, so can ML when model assumptions are not met.

Nonetheless, in the present case, I presume that the underlying data themselves did not discriminate strongly between the (false) ML and MP assumptions. This is demonstrated especially by the *rpl32-trnL* ML tree, which is also an MP tree. Consequently, ML bootstrap resolution appears improved simply because the procedure saves fewer (usually only single) trees. This, in turn, is a function of ML scores, which discriminate trees to the scale of on the order of $e^{0.001}$ (i.e., the third decimal place in the -*ln*L). In practice, ML saves multiple trees not in cases involving "hard" topological differences (as in MP), but involving multiple resolutions of branches very close to zero in length. Thus, the ML bootstrap trees were more or less a subset of the MP bootstrap trees. But because both the ML and MP assumptions are inherently "unrealistic" (see Discussion), greater resolution of the ML bootstrap does not mean greater reliability.

Detailed examination of Table 5 reveals that, in the case of ITS, the "improved" performance of ML occurs at nodes fairly well supported in the combined data analyses. But this is not always the case. For example, at node XXXXVI, the ITS MP bootstrap yields 91% confidence and ML only 67%. The combined data analyses support this node at the 98–99% interval. But whether node XXXXVI is in the "true" tree is another question. Clearly there are incongruencies between the ITS and *rpl32-trnL* data, so it cannot be discounted that the ML result for the ITS data is a fluke.

The bootstrap data reveal few incongruencies in phylogenetic signal between the two data sets. A notably lower bootstrap proportion in the combined data bootstrap means signifies incongruence in the separate data sets. Table 5 manifests very slight to considerable incongruence at perhaps 12–14 of the 71 identified nodes. But the incongruence is less than this since, in most cases, one incongruent node is complementary to another. As expected, most of the apparent incongruence manifests in the comparison of *rpl32-trnL* and combined data bootstraps. This is because, as noted above, the ITS data have more informative characters and resolve more nodes.

There are only two nodes in the ITS bootstrap majority rule trees absent in the combined data bootstraps, and only one of these is supported at >70% confidence in the former. There are six nodes present in all three *rpl32-trnL* bootstrap trees, and another six that occur in at least one of the three trees, that are absent or have substantially reduced support in the combined data bootstraps. Two of these nodes, XXXIV and LXIV, involve cases where the *rpl32-trnL* ML bootstrap increased the confidence interval to ≥ 80%.

A subtle example of incongruence not evident in the bootstrap analysis involves the sample *glabrata_25130*. The ITS ML topology (Figs. 1–2) indicates that the sequence is identical to *schroederi_25150*. Actually, the sequences differ by a single base indel. But the *rpl32-trnL* topology indicates that *glabrata_25130* is identical to *pubescens_25076*, although here both of these differ by only a single base from *schroederi_25150*. In the ITS and combined data bootstraps, *pubescens_25076* is separated from *glabrata_25130/schroederi_25150* by two strongly supported nodes. Because *rpl32-trnL* divergence in this complex is very low, neither the *rpl32-trnL* nor combined data bootstraps reveal the underling incongruency.

Clade XXXXII represents the strongest incongruence between the two data sets. This involves the sample *sp_indet_25161*, which is supported at 91–97% BP as sister to *C.* subg. *Liniphyllum* (Clade XXXXI) only the *rpl32-trnL* data. In Hershkovitz et al. (2006a), this sample was identified as *C. flabellata*. The position of this sample in the ITS data is ambiguous and varies especially because of the relatively poor resolution at adjacent nodes. However, the partition data from the ITS bootstrap provide no evidence at all (to the 5% level) of a sister relation between *sp_indet_25161* and *C.* subg. *Liniphyllum*. This is to say that the relation of *sp_indet_25161* supported with 91-97% confidence by the *rpl32-trnL* data is *rejected* at ≥ 95% confidence by the ITS data.

Ambiguity of the ITS data with respect to the position of *sp_indet_25161* should be highlighted. The ITS ML bootstrap indicates with weakest possible support (51%) a sister relation to *C.* subg. *Tylloma.* Monophyly of the latter in this bootstrap is reasonably well supported. However, monophyly of *C.* subg. *Tylloma* is much less well supported in the ITS MP bootstraps (62% with indel data, 68% without). But the partition table indicates that this reduced support for monophyly of *C.* subg. *Tylloma* owes to support for a sister relation between *sp_indet_25161* and *euphrasioides_25176* (23% with indel data, 11% without). This presumably is branch attraction, as this relation is absent in the ML partition data. MP bootstrap analysis of the ITS data without *sp_indet_25161* (not shown) yields 95% support for monophyly of *C.* subg. *Tylloma*. The results demonstrate the underlying influence of sampling, optimization criteria, and branch lengths in bootstrap results (see also Discussion).

However, the most significant aspect of the results for *sp_indet_25161* relates to the erstwhile identification of the voucher as *C. flabelleta*. The ITS tree of Cabezas Álvarez (2015) indicates a very different result for a sample of *C. flabellata* identified by A. Davies. The ITS sequence evidently was very similar to *euphrasioides_25176*, which is why I now consider the species identity of the sample analyzed as not determined. It possible identity is considered in the Discussion.

9. NeighborNet results

Figures 9–11 show the NeighborNet trees for, respectively, the ITS, *rpl32-trnL*, and combined substitution data. The ITS network shows good separation of all strongly-supported clades in the ITS bootstrap analyses. The central axes ("backbone") are relatively thick and "busy," most including many superimposed parallelograms of mostly small size. A single parallelogram denotes alternative partitions of the separated nodes, and the size of the parallelogram reflects character support for the alternative splits. The length of edges of the parallelogram is the branch length between the indicated nodes. Thus, the network indicates multiple possible branch lengths between terminals according to alternative trees, which represent sets of alternative splits of the terminals. The number of superimposed parallelograms in an axis reflects the number of alternative partitions (splits) of the terminals across the axis. The more terminals, the more possible splits, hence the greater number of superimposed parallelograms

In alternative splits, one or more samples on one side partition with those of the other, albeit with different character support. For example the axis between *Chaetanthera* s. str. and *Oriastrum* samples suggests alternative splits in which one or more samples of one genus partitions with those of the other. Since the genera appear to be well separated, these splits likely are consequent to accumulated random similarities between the terminals on either side, i.e., a form of long branch attraction. Note that while the distance correction itself should mitigate branch attraction along the principal paths, the random similarities between divergent terminals still may cause branch attractions that underlie the secondary paths.

The complexity of the backbone owes to otherwise counteracting parameters. To the degree that sequence divergence is both sufficiently high and uniform among the terminals, closely related but nonetheless divergent samples may form separate splits, resulting in more parallelograms along the more central axes. Alternatively, to the degree that sequences of the terminals are less divergent from each other, the NeighborNet algorithm will tend to consolidate them at a single, more distal node. It is from this single node that a single alternative split, rather than multiple splits, might form.

The geometric complexity of axes also relates to the number of terminals. Note, for example, that the *O.* subg. *Egania* axis is much more finely divided than that of *O.* subg. *Oriastrum*. The former has 16 terminals and the latter six. Evidently, the sequences of *O.* subg. *Egania* are similar enough to cluster but divergent enough to form splits independent of most closely related sequences. Note also that parallelograms of the *O.* subg. *Oriastrum* axis are rather larger than those of *O.* subg. *Egania*. Quite likely this owes to the especially divergent sequences of the former. The alternative splits likely reflect long branch attractions.

The ITS network appears to show evidence of two "clades" not supported the bootstrap analyses. One includes samples of three species of *C.* subg. *Tylloma*, *C. renifolia*, *C. spathulifolia*, and *C. villosa*. The clade is present in the ITS ML trees (Figs. 1–2), but not in the *rpl32-trnL* ML trees, and it is not apparent in the *rpl32-trnL* (Fig. 10) or combined data (Fig. 11) NeighborNet networks. Within this grouping, the ITS NeighborNet clusters *C. spathulifolia* and *C. villosa*, whereas the ITS ML tree clusters *C. renifolia* and *C. spathulifolia*.

The other "clade," barely perceptible in Fig. 9, comprises samples of three species of *O.* subg. *Egania*, *O. acerosum*, *O. apiculatum*, and *O. dioicum*, the first two further clustering. This "clade" is absent in the *rpl32-trnL* network, in which *O. acerosum* appears as "polyphyletic." In the combined data network, only the *O. acerosum* and *O. apiculatum* samples cluster. None of these interspecific relations are well-supported in the bootstrap analyses or even in the ML trees. The *rpl32-trnL* bootstraps (Fig. 7) partition the *O. apiculatum* and one of the *O. acerosum* samples with 53–57%. BPs. The ITS sequences of all three *O. acerosum* samples are identical, and the three group strongly in the combined data analysis.

The *rpl32-trnL* network (Fig. 10) appears less well-resolved than the ITS network, especially near the terminals, but also "cleaner," i.e., with fewer (and larger) parallelograms along the backbone. The appearance reflects the underlying data, which is overall less variable (and less homoplasious), even though there are more haplotypes (see above). Four of the five subgenera include clusters of samples (representing species complexes) whose divergence is mostly less than ITS divergence for the same samples. Thus, as suggested above, the NeighborNet algorithm likely coalesces these samples closer to the terminals. Their splits thus are consolidated.

In the *rpl32-trnL* network, only *C.* subg. *Chaetanthera* is notably well-separated from the backbone, and *O.* subg. *Oriastrum* appears as "paraphyletic" with respect to *O.* subg. *Egania*. Likewise, resolution of samples at the interspecific level in each subgenus is much less than in the ITS network. A notable peculiarity is the geometry within *C.* subg. *Chaetanthera*. The *rpl32-trnL* bootstrap analysis (Fig. 6) strongly supports a clade comprising samples of all of the species except *C. glandulosa* and *C. peruviana*. This "clade" is essentially absent in the NeighborNet network, owing to the split complex of the *C. peruviana* samples. The four large parallelograms indicate that sites within *each* of the two *C. peruviana* samples independently (or mutually) support closer relations with the larger clade. This is to say that there are splits in which one or the other but not both *C. peruviana* partition with the larger clade, rendering *C. peruviana* "paraphyletic."

The combined data network (Fig. 12) appears more or less intermediate between the separated data networks, with one notable exception. Samples of *C.* subg. *Tylloma* form a distinct "clade" in both the ITS and *rpl32-trnL* networks. Expectedly it is somewhat less distinct in the latter network, but so are the *C.* subg. *Liniphyllum* and other "clades." The separated networks and the bootstrap results (see above) suggest that *C.* subg. *Tylloma* also should be very distinct in the combined data network, but it is not. Especially with the highly divergent *C. euphrasioides* sequence, the subgenus appears practically "paraphyletic," with alternative splits with the other two *Chaetanthera* subgenera and with *Oriastrum*. Even ignoring the *C. euphrasioides* sequence, the subgenus is, at best, barely distinct, and much less so than expected.

The mechanical explanation for the above is that both the ITS and *rpl32-trnL* sequences in at least some samples of this group have strong signal supporting relationships with those of other *C.* subg. *Tylloma*, but also weak signal supporting relationships with either or all of the other clades. The signal is too weak to be detected in networks of the separate data sets, but appears when these are combined.

Another notable feature of the NeighborNet networks is the disposition of the sample *sp_indet_25161*. In the ITS network, this sample appears to split between *C.* subg. *Chaetanthera* and *C.*

subg. *Tylloma*, slightly more with the latter. From the network, it appears that the attraction to the former is via the long *C. euphrasioides* branch, and thus represents branch attraction.

The *rpl32-trnL* network places the sample decidedly within *C.* subg. *Liniphyllum* close to *C. perpusilla* and *C. taltalensis*. Recall that bootstrap support for the inclusion of *C. perpusilla* and *C. taltalensis* in *C.* subg. *Liniphyllum* is low in the *rpl32-trnL* analysis with *sp_indet_25161* included (Fig. 6), but close to 100% with *sp_indet_25161* excluded (not shown). This indicates that the association of *sp_indet_25161* with *C.* subg. *Liniphyllum* owes to its similarity to, specifically, *C. perpusilla* and *C. taltalensis*. The *rpl32-trnL* NeighborNet network suggests that the similarity attracts these three samples to *C.* subg. *Chaetanthera*, specifically to the *C. peruviana* samples.

While the separated data networks agree exactly with the separated data bootstrap analysis, the combined data network adds a new dimension (literally). One might expect a prominent split associating *sp_indet_25161* with *C.* subg. *Liniphyllum*, but this is not realized. Rather, *sp_indet_25161* most strongly (yet weakly) appears as part of a partition including *both C.* subg. *Chaetanthera* and *C.* subg. *Liniphyllum*. Careful examination reveals the relatively larger splits with the *C.* (subg. *Chaetanthera*) *peruviana* and *C.* (subg. *Liniphyllum*) *perpusilla* and *taltalensis* samples, and a lesser (branch attraction) split with *C. euphrasioides*. Meanwhile the samples of *sp_indet_25161* and *C. euphrasioides* appear to be the principal cause of the ambiguous distinction of *C.* subg. *Tylloma*. These and other possibilities could be explored in a full split decomposition analysis. In the meantime, the graphics presented here demonstrate a dimensionality of phylogenetic history not appreciated in conventional tree construction.

DISCUSSION: OF MOLECULES AND SYSTEMATICS

It should be clear that the present work is conceptually very different from Hershkovitz et al. (2006a). While its major conclusion is robust, Hershkovitz et al. (2006a) was advanced more for political than scientific reasons. It was conceived and undertaken ostensibly to support the study of "evolutionary patterns in floral morphology and breeding systems in a phylogenetic context using the South American genus *Chaetanthera* as a model" (https://plants.jstor.org/stable/10.5555/al.ap.person.bm000000261, https://chile.unt.edu/faculty/dr-mary-kalin-arroyo). But the promised floral and breeding data never materialized, not then, not even 15 years and more than a million US dollars later. Fortunately and fortuitously, I noticed that the data revealed an unusual biogeographic phenomenon. Focusing on that "sound byte," I was able to fashion a paper short enough to be accepted by an indexed "high impact" molecular systematics journal. But the paper considered only cursorily the molecular systematics of *Chaetanthera*/*Oriastrum*, and only dogmatically at best. Perhaps it was just as well, given the evidently "rudimentary" taxonomic treatment available at that time (i.e., Cabrera, 1937; cf. Davies, 2010).

The publication by Davies (2010) changed the landscape dramatically, finally motivating me to resurrect and my unprocessed *rpl32-trnL* data. Davies' (2010) work gave "life" to the molecular phylogenetic data, permitting analysis of not just one, but a great many phenomena, at not just one, but many organizational levels (molecular to organismal to ecological/geographic), and in not just empirical, but also theoretical and epistemological frameworks. Naturally, and in the venerable tradition of *systematics*, the present work is an order of magnitude longer than Hershkovitz et al. (2006a). For these reasons, the present synthesis should be considered an appendage not to Hershkovitz et al. (2006a), but to Davies (2010).

Sections 1–5 of this discussion emphasize the relationship between the molecular phylogenetic results and the systematics of *Chaetanthera*/*Oriastrum*. Sections 6–7 discuss nominally infraspecific ITS and *rpl32-trnL* sequence polymorphism and nominally interspecific sequence identity in the context of taxonomic species delimitation. Section 8 discusses relevant issues of methodology issues in molecular phylogenetic reconstruction. Sections 9–12 challenge the notion of stochasticity of DNA sequence evolution that underlies molecular phylogenetic methods. Section 13 thus argues that the basis for the present phylogenetic conclusions is not inherent in the data or methodology, but in the epistemology.

1. Higher level phylogeny and taxonomy of **Chaetanthera** *s. str. and* **Oriastrum**

The present analysis confirms Hershkovitz et al. (2006a) in demonstrating monophyly of both *Chaetanthera* s. str. and *Oriastrum* sensu Davies (2010). The sister-relation between these two genera does not seem to have been proven in prior studies, nor was it rigorously analyzed here. The sister relation of *Chaetanthera* s. str. and *Oriastrum* seems most likely on the basis of inspection of other available Mutiseae DNA sequences.

Based on ITS only and an evolutionary rate smoothing procedure, Hershkovitz et al. (2006a; cf. Guerrero et al., 2013) concluded that the split between *Chaetanthera* s. str. and *Oriastrum* occurred on the order of 16 mya. The calibration point in that analysis was an estimate of the crown age of Asteraceae at 128 mya. The take home was that, even considering variance, *Oriastrum* must have originated long before the development of their current high alpine habitat, believed to be on the order of 5 mya. Thus, it was proposed that the genus existed at lower elevations in what is currently northern Chile and migrated upwards, becoming extinct at lower elevations during relatively recent development of the hyperarid Atacama Desert. Meanwhile the ancestor of *Chaetanthera* s. str. was distributed somewhat further south and adapted to less severe seasonal lowland aridity, with a few lineages also finding their way into the alpine zone more recently.

Nothing in the present data seems to challenge the earlier interpretation. In the meantime, using ITS, rDNA ETS (external transcribed spacer), the cpDNA *trnL-trnF* region, and an estimated crown age of Asteraceae of ca. 70 mya, Muñoz et al. (2020) estimated the crown age of *Mutisia* to be on the order of 20 mya. Given the close relationship between *Chaetanthera/Oriastrum* and *Mutisia*, both estimates are in reasonable agreement, since crown ages are younger than stem ages and not the same in different radiations.

In the meantime, the *rpl32-trnL* data presented here suggest somewhat different relative ages for the Mutisieae genera. As noted above, ITS substitution data suggested a divergence between *Chaetanthera* s. str. and *Oriastrum* of about one fourth that between the *Chaetanthera/Oriastrum* crown and the *Mutisia* crown. Meanwhile, the *rpl32-trnL* divergence between *Chaetanthera* s. str. and *Oriastrum* is about the same as that between the *Chaetanthera* crown and the *Mutisia-Proustia* split and less than twice that between the *Chaetanthera* crown and the terminal of a rather recently evolved *Mutisia* species. The allometry suggests that an *rpl32-trnL* dating (alone) would result in either an older *Chaetanthera/Oriastrum* crown or a younger *Mutisia*. However, molecular dating still retains a strong component of sorcery (cf. Hershkovitz, 2019a).

Assuming that *Chaetanthera* s. str. and *Oriastrum* are indeed sister taxa, then *Chaetanthera* s. l. (and sensu Bentham and Hooker, 1873, Cabrera, 1937, and Hershkovitz et al., 2006a) indeed is monophyletic. Monophyly always has been suggested by morphology, in particular the intergradation of leaf and capitulum bract morphology, in contrast to the sharp distinction between these in related genera (Davies, 2010).

Curiously, Davies (2010: 18; cf. 16) characterized Cabrera's (1937) circumscription as an "unnatural paraphyletic entity." This would suggest that Cabrera (1937) segregated from *Chaetanthera* taxa phylogenetically nested within this clade. This does not seem to be the case, and Davies (2010) provided no examples. Certainly Cabrera´s (1937) subgeneric classification appears to be phylogenetically unnatural, but not the generic circumscription itself. Nevertheless, Davies' (2010) assertion of paraphyly seems to underlie partially her argument for segregating *Oriastrum* (including *Egania* J.Rémy), which Cabrera included in *Chaetanthera*.

Davies' (2010) circumscriptions of *Chaetanthera* s. str. and *Oriastrum* are consistent with the molecular evidence, but their erstwhile lumping is also. For phylogenetic purposes, their segregation seems to be a matter of taxonomic "taste." Nonetheless, Davies' (2010) segregation of *Oriastrum* seems to have been accepted "politically" (e.g., Rodriguez et al., 2018; POWO, 2019; Flora Cono Sur, without year).

While I am deferential to the segregation of *Chaetanthera* s. str. and *Oriastrum*, I do not believe it was necessary or especially well-justified based on the evidence presented by Davies (2010). Objective phylogenetic evidence is neutral in this case, so the criteria for lumping versus splitting only can be subjective and based on similarity versus distinctiveness in easily observed characteristics, including geographic range. An accessory factor is the size of the genus. Ultimately, the question is whether splitting or lumping serves science and society by making generic recognition easier and more intuitive and/or to associate the genera with characteristics of broader scientific interest.

In many ways, *Oriastrum* fails to meet the desiderata for generic segregation. Davies (2010) referred to generic size as one justification for segregation of *Oriastrum*. But while the sum of species in both segregates, 48, is a relatively large for a plant genus in Chile, by global standards, the number is relatively modest (cf. Humphreys and Linder, 2009). The split genera, especially *Oriastrum*, are pretty puny. It seems that such small genera are justified in cases where divergence/distinctiveness is exceptional, where merging is impractical/impossible on the basis of phylogenetic evidence, or and/or where circumscriptions are well established. None of these apply in the case of *Chaetanthera*/*Oriastrum*.

The geographic ranges substantially overlap; most of the range of *Oriastrum* is contained within the range of *Chaetanthera* s. stricto. This means that knowing *where* it is does not mean knowing *what* it is. While lowland annual *C*. subg. *Chaetanthera* and alpine perennial *O*. subg. *Egania* species are easily discriminated by life form, morphology, and geography, the characteristics that supposedly discriminate the split genera evidently overlap/intergrade more than Davies (2010) suggests, especially via *C*. subg. *Tylloma* (see below). Just as importantly, most of the discriminating characteristics are microscopic to submicroscopic (including DNA characteristics). Thus, segregation of the genera is somewhat inconvenient in that, in many cases, it requires diagnosing first the species in order to diagnose the genus (see below), and the *explanation* for the segregation is not obvious except to knowledgeable specialists.

Davies (2010: 112–113, Table 8) tabulated about 25 qualitative/quantitative traits that discriminate between *Chaetanthera* s. str. and *Oriastrum* and the two subgenera she recognized within each (with *C*. subg. *Liniphyllum* included in *C*. subg, *Chaetanthera*). Inspection reveals overlap/intergradation especially between *C*. subg. *Tylloma* and *O*. subg. *Oriastrum*, e.g., in pappus setae dehiscence, stigma lobe hairs, carpopodium presence, achene hair shape, testa epidermal cell shape, and pollen ectosexine structure.

However, comparison of the table data with the actual species descriptions reveals that additional traits intergrade, as well. As an initial example, Davies (2010: 112–113, Table 8) described the habit of *C*. subg. *Tylloma* as "stem rosettes," in contrast to that of both *Oriastrum* subgenera, viz., "compact to laxly spreading dwarf cushions." However, Davies (2010: 211) described the stems of *C*. (subg. *Tylloma*) *philippii* as "densely clustered to form loose cushion[s]." Indeed, from the many photos/illustrations, the habit of *C. philippii* and species of *O*. subg. *Oriastrum* appears very similar. Perhaps this is not surprising, given that *C. philippii* is a high elevation species that shares its range with several *Oriastrum* species.

From Davies' (2010: 211) detailed description, *C. philippii* appears to have additional similarities to species of *O.* subg. *Oriastrum*. Davies (2010: 112–113, Table 8) discriminated *C.* subg. *Tylloma* from *O.* subg. *Oriastrum* on the basis of stigma lobe length, ca. 0.5 mm in the former vs. ca. 0.1–0.25 mm in the latter. But she described the stigma lobe length in *C. philippii* as 0.3 mm, and the length reported for several other species of *Oriastrum* range from 0.3–0.6 mm, the last value reported for *O. lycopodioides* (Davies, 2010: 242). Also, for three traits reported in Davies (2010: Table 8) as polymorphic in *C.* subg. *Tylloma*, *C. philippii* has the traits characteristic of *O.* subg. *Oriastrum*: pappus setae sometimes dehiscent, carpopodium poorly formed or absent, and spherical achene hairs. The length of the last, 20 µm, is on the high end reported for *C.* subg. *Tylloma* and the low end reported for *O.* subg. *Oriastrum* (Davies, 2010: 112–113, Table 8), i.e., it is intermediate, hence the achene hair lengths intergrade.

The DNA data and other morphological traits leave no doubt that *C. philippii* pertains to *C.* subg. *Tylloma* and, despite morphological similarities, is highly divergent from *Oriastrum*. Nonetheless, its similarities to *Oriastrum* beg the question as how someone other than a taxonomic specialist would be able to distinguish the genera without memorizing beforehand the species and their generic classification. And the ultimate explanation for the different classification, given the failures of morphology, also leaves something to be desired. Yes, the species pertain to different clades, but the clades are morphologically intergrading sister-clades. At the same time, since the time of Cabrera (1937; cf. Bentham and Hooker, 1873), notwithstanding Federico Philippi's (1881; hence Reiche, 1905) vandalistic intervention (see Davies, 2010: 123–125), there has not been a problem distinguishing *Chaetanthera* s. l. from other genera of Mutisieae.

To put the above another way, segregating *Oriastrum* from *Chaetanthera* s. str., notwithstanding molecular phylogenetic coincidence, replaces an easily distinguished genus with two morphologically/ecologically poorly distinguishable ones. Perhaps more problematic, their segregation leaves *Chaetanthera* s. l. as taxonomically unrecognized *and nomenclaturally unrecognizable*. This is because the current nomenclatural code for plants (Turland et al., 2018) does not offer a rank in between subtribe and genus. This is unfortunate, because the clade *Chaetanthera* s. l. is both well-known and *easily* distinguished from other Mutisieae-Mutisiinae by the intergrading leaves and capitulum bracts, as well as other traits (Davies, 2010).

In practice, I suspect that hereafter, any substantial work on these taxa will necessarily refer to the "twin genera" *Chaetanthera* s. str. and *Oriastrum* (¿"*Chaetantiastrum*"?) and that, in any case, any work dealing with one of the taxa will not be able to avoid reference to the other. In contrast, I cannot see where segregation of *Chaetanthera* s. str. and *Oriastrum* at the subgeneric level would introduce any confusion or difficulty. Thus, while I adopt here Davies' (2010) taxonomy, there are good arguments for *reinstating* Cabrera's (1937) circumscription of *Chaetanthera*, recognize therein two subgenera (*Chaetanthera* and *Oriastrum*), and recognize the subgenera delimited here as sections.

I hasten to add that this opinion in no way diminishes the importance of the work of Davies (2010), especially because the present discussion would have been impossible without it. It probably represents the most thorough and up-to-date monograph of any sizeable genus of the Chilean Floristic Region. And it represents the first largely "accurate" (i.e., largely in agreement with molecular phylogenetic evidence) classification of *Chaetanthera* s. l. based on morphology. The only disagreements pertain to evidence for segregating *C.* subg. *Liniphyllum* from *C.* subg. *Chaetanthera*, and the suggested relations of *C. taltalensis* (see below). But it appears that Davies' (2010) segregation of *Oriastrum* as a genus (as opposed to a subgenus with two sections) was motivated by faulty 19th Century generic splitting, as well as Cabrera's (1937) problematic subgeneric classification. In other words, had all prior taxonomies consistently classified all of the species in *Chaetanthera*, there would have been less justification and motivation for splitting the genus in two. Davies' (2010) also may have been influenced by her impression that *Chaetanthera* sensu Cabrera. is paraphyletic. It is not.

This also is not to say that segregation of *Oriastrum* has no merit. While the traits of species like *C. philippii* challenge the phenetic distinctiveness of *Chaetanthera* s. str. and *Oriastrum*, segregation of the latter renders clear that these similarities are convergences. With both classified in *Chaetanthera* s. l., notwithstanding in different subgenera, "nonexperts" (familiar only with binomial classification) might be inclined to believe that *C. philippii* actually is most closely related to species of *O.* subg. *Oriastrum*. But this is somewhat of a red herring. Morphological/ecological convergence is a constant process during diversification, viz., not contingent on taxonomic rank. It is as least as apt (if not more so) to characterize more closely related as more distantly related species. Evidently, for example, *C.* (subg. *Liniphyllum*) *taltalensis* is convergent in form and ecology upon species of *C.* subg. *Chaetanthera* (see below). Should *C.* subg. *Liniphyllum* be segregated from *Chaetanthera* in order to illuminate this fact?

But perhaps a final consideration is simply that the "deed is done," i.e., the segregation proposed by Davies (2010) was adopted in major references, evidently on authority and without consideration of the points discussed here. Consequently, the taxonomy of *Chaetanthera* and *Oriastrum* cannot win for losing. It will be referred to as "*Chaetanthera*, including *Oriastrum*" or "*Chaetanthera* excluding *Oriastrum*" and *Oriastrum*, formerly *Chaetanthera*."

2. *Subgeneric classification of* **Chaetanthera** *s. str.: recognition of* **C.** *subg.* **Liniphyllum**

Davies (2010: 70) referred informally to a "linear-leaved group" comprising five annual species. With the addition of *C. taltalensis*, this group forms a clade corresponding to *C.* subg. *Liniphyllum*, recognized here as distinct from *C.* subg. *Chaetanthera*. A peculiarity is that Davies (2010: 70) described the "linear-leaved group" as having entire leaves. But most (all?) of the species have dentate leaves, as she noted in the individual species descriptions. The clade is strongly supported by the ITS (here and in Hershkovitz et al., 2006a) and combined bootstrap analyses, but not by the *rpl32-trnL* bootstrap. As explained in the results, this is consequent to the position of *sp_indet_25161* in the *rpl32-trnL* analysis (see below). With this sample removed, the *rpl32-trnL* data also strongly support monophyly of *C.* subg. *Liniphyllum*.

Recognition here of *C.* subg. *Liniphyllum* bases not merely on its apparent monophyly, but also the lack of evidence for monophyly of *C.* subg. *Chaetanthera* with *C.* subg. *Liniphyllum* included. Thus, unlike the case of *Chaetanthera* s. str. and *Oriastrum*, it is not merely a question of lumping versus splitting. Figures 1–6 and 9–11 demonstrate that neither or both of the ITS and *rpl32-trnL* data resolve the relationships among the three major clades within *Chaetanthera* s. stricto. I doubt, therefore, that the relationships are resolvable. Each clade must be recognized as an equivalent taxon.

3. *Relationships within the subgenera of* **Chaetanthera** *s. str.*

a. Relationships of the **C.** *(subg.* **Liniphyllum***)* **albiflora** *complex*

Davies (2010: 54) referred to the molecular phylogenetic results of Hershkovitz et al. (2006a) for three species of the "linear-leaved group" (*C. albiflora*, *C. linearis*, *C. microphylla*), but only with respect to nominal infraspecific ITS polymorphism and nominal interspecific sequence identity. She did not mention the strongly supported clade comprising the "linear-leaved group" plus *C. taltalensis*, which was (and is) strongly supported as sister to *C. perpusilla*.

Davies (2010: 179) considered *C. perpusilla* to be "close to" *C. depauperata* and otherwise not notably distinct from the rest of the "linear-leaved group." She did not mention the ITS results (Hershkovitz et al., 2006a) showing *C. perpusilla* as relatively highly diverged from *C. depauperata*, the latter pertaining to the genetically similar and intertwined species complex that includes *C. albiflora*, *C. linearis*, and *C. microphylla*. The *rpl32-trnL* results fully corroborate the ITS.

Davies (2010, 2013) discussed at length hybridization and intergradation between *C. albiflora* and *C. linearis* Poepp. ex Less. The latter was not sampled here. However, both the *ITS* and *rpl32-trnL* haplotypes of *C. albiflora* are polymorphic and appear in the tree as paraphyletic with respect to *C. microphylla*. The *rpl32-trnL* gene tree (Figs. 3–4; cf. Fig. 10) indicates possible paraphyly with respect to *C. depauperata* as well. Davies' (2010, 2013) discussion tacitly presumes that *C. albiflora* and *C. linearis* are sister species. This may well be the case, but their history may entwine also the other two species. However, at such low level of divergence, it also is possible that the gene trees are incongruent with the species trees, reflecting lineage sorting and/or homoplasy.

[The collection here identified as *C. depauperata* was identified in Hershkovitz et al. (2006a) as *C. leptocephala* Cabrera, which Davies includes in *C. depauperata*. Davies (2010) did not see this specimen, but I collected it east of Copiapó, Chile at ca. 2100 m. All other species of this complex occur at much lower elevations (Davies, 2010: 65–66).]

***b. Relationships of* C. *(subg.* Liniphyllum*)* taltalensis**

Davies (2010: 73) referred *C. taltalensis* to the "dentate-ciliate group," to which she referred the six species comprising all of the annual taxa of *C.* subg. *Chaetanthera* (as recognized here). Within this group, Davies (2010: 74; cf. 173, 186, 192) remarked that "*C. ramosissima*, *C. taltalensis* and *C. moenchioides* form a triad of morphologically close species." Davies (2010: 173, Table 13) thus tabulated a diagnostic key to these three species. Davies (2010) did not mention the contradictory molecular results of Hershkovitz et al. (2006a). Guerrero et al. (2013) reanalyzed the data of Hershkovitz et al. (2006a), specifically highlighting the evolution of *C. taltalensis*, but did not refer to the contrary opinion of Davies (2010). Likewise, Cabezas Álvarez (2015) partially reproduced the results of Hershkovitz et al. (2006a), but did not refer to the disagreement with Davies (2010).

Scrutiny of the species descriptions in Davies (2010), however, reveals similarities between *C. taltalensis* and *C. perpusilla* not shared with *C. moenchioides* or *C. ramosissima*. These include leaves with a single pair of teeth near the apex, shape of the capitulum (campanulate rather than cylindrical), and, more esoterically, almost double the density of barbs along the pappus setae. Thus, the molecular data are not incongruent with morphology. There seems to be no characteristics of *C. taltalensis* shared exclusively with *C. moenchioides* and *C. ramosissima*. Meanwhile, both the ITS and *rpl32-trnL* data show the samples of *C. moenchioides* and *C. ramosissima* as diverging at adjacent nodes, viz., the data do not indicate that these species are phylogenetically closest relatives or even especially similar genetically.

c. Relationships of the* C. *(subg.* Chaetanthera*)* chilensis *complex

Based on Hershkovitz et al. (2006a), Davies (2010: 73) recognized that the "dentate-ciliate group" is paraphyletic with respect to the perennial and evidently interbreeding species complex (Davies, 2010, 2013) comprising *C. chilensis*, *C. elegans*, and *C.* x *serrata*. The subgenus includes another perennial, *C. glandulosa*, but both the ITS and *rpl32-trnL* data indicate that its relations with the other perennials is remote.

But the DNA data indicate that the relationship between the annual "dentate-cilate" species and the perennial *C. chilensis* complex it itself complex. Both the ITS and *rpl32-trnL* data include a strongly supported clade comprising the perennial species plus the annual species *C. ciliata* and *C. incana*. The ITS analysis of Cabezas Álvarez (2015) indicates that *C. multicaulis* DC (not sampled here) also pertains to this clade. Davies (2010) considered *C. ciliata* and *C. multicaulis* to be closely related. Davies (2010: 73,

163) indicated that *C. incana* was morphologically similar and closely related to the *C. chilensis* species complex, but made no reference to *C. ciliata* or *C. multicaulis* in this regard. The ITS and *rpl32-trnL* data indicate that the relations of the remaining annual species of the subgenus to the *C. chilensis* complex is more remote.

The genetic divergence of samples of both sequences of the annual and perennial species of the *C. chilensis* complex is low, and their relations otherwise are not resolved here. But the combined data bootstrap, evidently reflecting mainly resolution of the *rpl32-trnL* data, shows the *C. incana* samples as sister to the remaining *C. chilensis* complex, with the *C. ciliata* sample nested therein. Thus, there is evidence that, within this annual/perennial clade, the annual and perennial species are not, respectively, closest relatives. However, especially given the low degree of genetic divergence, the aphorism that "gene trees are not species trees" (see below) should be invoked. At this divergence level, lineage sorting and/or gene flow must be considered.

Meanwhile, Davies (2010, 2013) discussed at length evidence for evolutionary relationships within the perennial *C. chilensis* complex, which presumes that these form a monophyletic or at least evolutionarily autonomous entity. This may well be the case, but it does not follow from the genetic data. Many possible evolutionary scenarios could explain the sequence data for this clade of three annual and three perennial species.

d. Relationships of the **C.** *(subg.* **Tylloma***)* **glabrata** *complex*

Davies (2010: 67) circumscribed a complex of six annual species that includes *C. frayjorgensis*, *C. glabrata*, *C. kalinae*, *C. limbata*, *C. pubescens* and *C. schroederi*. In a discussion of the relation between climate and leaf form (Davies, 2010: 85), she also referred to a "*C. glabrata – C. limbata* complex." It is not clear whether this refers only to this pair or to all six species. The climate/leaf discussion was summarized in Davies (2013), but here she referred only to *C. glabrata* and not *C. limbata*. All but *C. kalinae* and *C. limbata* were sampled in the present work, but *C. kalinae* was sampled for ITS by Cabezas Álvarez (2015).

The present data strongly support a clade that includes the four sampled species of this complex. From Cabezas Álvarez' (2015) ITS tree, it can be inferred that *C. kalinae* also belongs to this clade. Davies (2010: 67) asserted that these species represent a "very recent radiation event, based on a founder event that occurred at higher elevations, with rapid expansion at lower elevations as a result of environmental adaptation to semi-arid conditions." It is not clear whether this hypothesis was influenced by the ITS trees in Hershkovitz et al. (2006a; cf. Figs. 4, 5). These show this radiation as recent, and the high elevation *C. pubescens* sample (there identified as *C. kalinae*) as sister to the remaining species. The latter is not corroborated by the less resolved *rpl32-trnL* data (Fig. 6), and the combined data bootstrap support for this relationship is decreased relative to the ITS bootstrap (Fig. 7).

Within this clade, the relationships of the *C. glabrata* samples evidently are complex given polymorphism of samples of this species (see Results and below). The ITS sequence of the *schroederi_25150* sample is identical to *glabrata_25130* but not *glabrata_25163* (Figs. 1–2). The combined data bootstrap (Fig. 7) shows fairly strong support for a sister relation between the first two samples, but also polyphyly of the two *C. glabrata* samples. As in the case of the *C. chilensis* complex, sequence divergences in the *C. glabrata* complex are very low, hence lineage sorting and/or gene flow might occlude correspondence with the species trees.

Davies (2010, 2013) noted that leaf form and size are highly variable in *C. glabrata*. This species also has the broadest latitudinal range. While *C. glabrata* is (like the other five species) distributed mainly between 25°–30°S, disjunct ranges occur at 24°N and 33°–34°S (Davies, 2010: 69, Fig. 24). She described three leaf forms in the species. Two are similar: leaves well differentiated into blade and somewhat broad petiole, but some are larger with more orbicular blades and others are smaller with more ovate blades. A third form has longer/larger and oblanceolate with decurrent leaf bases (i.e., hardly or not petiolate) and also undulating margins (Davies, 2013: 76, Fig. 6). The overall size and shape of this form is suggestive of *C. frayjorgensis*, whose range at ca. 29.5°–31°S is essentially parapatric with the principal continuous range *C. glabrata* (cf. Davies, 2010: 69, Fig. 24). Significantly (see below), this form occurs only in the northern range and is absent in the disjunct southern range.

Davies (2010, 2013) astutely noted that leaf size/form in herbarium collections of *C. glabrata* varied according to the year of collection, with the larger leaves being more common among plants collected during El Niño years, when rainfall in central and northern Chile is much higher than in "normal" years. She favored the notion that leaf morphological variability in *C. glabrata* reflected not incipient speciation (i.e., genetic differentiation), but phenotypic plasticity, and that this plasticity accounted for the difference in the frequencies of different forms in wet versus dry years.

The current sequence data do not permit adequate evaluation of Davies (2010, 2013) plasticity conclusion. As it happens, the two *C. glabrata* collections sampled here represent the southern and the northern disjunct ranges, with no samples from the principal continuous range. The fact that the sequences for both loci appear polyphyletic thus may be meaningful, because the southern disjunct range lacks the large oblanceolate leaf form of the northern range. Its absence in the southern range seems peculiar, because Davies (2010, 2013) reported it to be more common in the northern range during wet years. But the southern range *always* is wetter than the northern, more so in dry years than wet.

But even given the inadequacy of the genetic data, I regard Davies' (2010, 2013) argument to be plausible but far from proven. Low elevation annual species distributions at 25°–30°S are extremely different in rare wet and more common dry years. In dry years, annuals occur only very near the coast, where they are irrigated by a combination of fog humidity/precipitation and a relatively high water table owing to runoff from the high Andes. But as little as 1–2 km inland, annuals are then absent, though they emerge (usually different species) further inland in the Andes at higher elevations.

In contrast, in wet years, often the same operational annual species may distribute continuously at low elevations from the coast to the Andes. This phenomena manifests as Chile's famed "desierto florido." Likewise, I have found nominally alpine annual species growing in the precordillera at 1000–2000 m elevation (see also later discussion of *Oriastrum gnaphalioides*). The effect is less conspicuous but not insignificant even in the mediterranean climate in the southernmost range of *C. glabrata*. During drier years, annuals broadly abundant during wet years are restricted to sites more mesic owing to topographic effects.

With this in mind, it is possible that more mesophytic and xerophytic forms of the same operational species indeed are differentiated genetically. The more drought-tolerant forms may be more uniformly abundant in all years, while the less drought-tolerant forms are rare in dry years but abundant only in wet years. Elsewhere (Hershkovitz, 2019a: 55; 2020: 9), I pointed out that the extreme moisture periodicity (coupled with topographic mitigation) results in localized annual plant genetic differentiation ("speciation," if you like) in the *temporal* as well as spatial dimension. This is because seed banks in drier locations germinate an order of magnitude less frequently than those of moist locations. The phenomenon might explain the biogeographic origin of phylogenetic disjunctions between alpine and coastal annuals in arid Chile, viz., via seed bank-mitigated vicariance rather than dispersal. In any case, this phenomenon might explain the pattern Davies (2010, 2013) deduced from herbarium specimens.

Davies (2010) greatly clarified diversity of forms of the *C. glabrata* complex. But the group demands additional study from a genetic perspective in order to understand its morphological and ecological evolution. I suspect that further research will demonstrate that the southernmost range of *C. glabrata* is well differentiated genetically from the northern range, and that the latter has had and perhaps continues to have gene flow with other northern species, especially *C. frayjorgensis*. The northernmost population, near Antofagasta, Chile, is less problematic. Numerous species characteristic of more southerly coastal deserts are disjunct in this localized fog oasis. Temporal as much as spatial genetic differentiation likely will be found in the lowland species of the Atacama and Coquimbo Regions.

e. Other relationships among **C.** ***subg.*** **Tylloma** ***species***

Within *C.* subg. *Tylloma*, Davies (2010: 194, 198, 229) referred to three other pairs of species as being closely related: *C. euphrasioides*/*C. flabellata*, *C. flabellifolia*/*C. splendens*, and *C. spathulifolia*/*C. villosa*. The present data partially support the last conjecture, but do not address the first two given current specimen identifications (cf. Hershkovitz et al., 2006a). However, Cabezas Álvarez (2015) found a close relationship of ITS samples of Davies-identified specimens of *C. euphrasioides* and *C. flabellata* (but see discussion of the sample *sp_indet_25161* below).

The ITS ML trees (Figs. 1–2) show a clade comprising the samples of *C. renifolia*, *C. spathulifolia*, and *C. villosa*. The grouping also is apparent in the ITS NeighborNet network (Fig. 9). These three species are high elevation perennials that share nearly sessile capitulate and similar pappus setae morphology (Davies, 2010). The last two further share similar rosette morphology, achene size/shape, and achene trichome morphology (Davies, 2010). Davies (2010: 229) remarked that *C. villosa* was “close” to *C. spathulifolia*, but did not comment on the possible relations of *C. renifolia*. But the clade is absent in the ML bootstrap majority rule tree (Fig. 5) and is strongly refuted in the *rpl32-trnL* bootstrap tree (Fig. 6). However, samples of the three species emerge at least as adjacent branches in both the ITS and *rpl32-trnL* trees. Their similarities might owe to symplesiomorphy mitigated also by their similar ecology.

4. Species relationships within **Oriastrum**

Among *Oriastrum* subg. *Oriastrum*, Davies (2010: 238–239) merged the species *Chaetanthera minuta* (Phil.) Cabrera with *Oriastrum gnaphalioides*, but she did not cite the type of the former. Here, she cited the unpublished opinion of M. T. K. Arroyo, who reported that *C. minuta* is a smaller and *lower* elevational form of *O. gnaphalioides*. But she also reported that the type of the former is from 4000 m elevation, which is at the high end of the reported range for *O. gnaphalioides* (2000–4300 m). Davies (2010) did not refer to this discrepancy, which makes me wonder if she or Arroyo “reversed” the supposed distinction between these taxa. Nicola et al. (2015) argued that *C. minuta* is distinct from *O. gnaphalioides*. They cited but otherwise did not address the supposed (and erroneous?) elevational distinction reported by Davies (2010).

Hershkovitz et al. (2006a) recognized both [*O.*] *gnaphalioides* and *C. minuta* according to identifications by Arroyo. One sample used here, *gnaphalioides_02_154*, not seen by Davies (2010) was listed there as [*O.*] *gnaphalioides* and not *C. minuta*. But it was collected at ca. 1000 m near Combarbalá, Chile, which is not only low elevation, it is well below the elevational range reported by Davies. I do not regard this as problematic. As reported earlier, I have found that in especially rainy (El Niño) years in semiarid/arid Chile, it is not uncommon for a few nominally alpine annuals to appear at much lower elevations in the precordillera. The propagules are bound to disperse here, and the seeds persist in the seed bank.

In any case, the present data are problematic with respect to Nicola et al.’s (2015) proposal, because both sequences of the samples identified in Hershkovitz et al. (2006) as *C. minuta* are polyphyletic, each clustering with different specimens previously distinguished as [*O.*] *gnaphalioides*. If the identifications in Hershkovitz et al. (2006) are "correct," then this would be an additional example of nominally infraspecific polyphylesis in the gene trees. However, Nicola et al. (2015) did not examine these specimens. They cited Hershkovitz et al. (2006), but not the polyphyly of the *C. minuta* specimens reported therein.

Davies (2010: 244, 247) remarked that "*O. lycopodioides* is closely related to *O. pusillum* and *O. chilense*" and that "*O. pusillum* is similar to *O. chilense*. These remarks suggest the relations indicated by both DNA loci, viz. (*O. gnaphalioides*, (*O. lycopodioides*, (*O. pusillum*, *O. chilense*))). At the same time, it must be noted that the genetic divergence between the samples is considerable and much greater than for other "closely related species."

Among the *O*. subg. *Egania* taxa sampled here, Davies (2010: 252, 255, 259, 274, 276) noted four interspecific phenetic similarities: (1) *O. abbreviatum*/*O. famatinae*; (2) *O. acerosum*/*O. apiculatum*/*O. dioicum*; (3) *O. polymallum*/*O. pulvinatum*; and (4) *O. revolutum*/*O. stuebelii*. Corroboration by ITS and *rpl32-trnL* is hampered by infraspecific polymorphism and overall low sequence divergence. But only conjecture 3 is *partially* supported by bootstrap analyses; conjecture 2 is evidenced only barely in the ITS NeighborNet network, while conjectures 1 and 4 are refuted.

Davies (2010: 81) referred to three species of *O*. subg. *Egania*, *O. acerosum*, *O. apiculatum*, and *O. dioicum* as a "species radiation" she called the "Andino group." This language is suggestive of a clade. She indicated that *O. acerosum* and *O. apiculatum* were geographically and morphologically more similar. None of these relationships are supported by the sequence data, although the *rpl32-trnL* bootstrap shows weak support for a clade comprising *O. apiculatum* and *one* of the *O. acerosum* samples (cf. Fig. 10). The ITS NeighborNet network (Fig. 9) shows faint evidence of the "Andino group," but does cluster the *O. acerosum* and *O. apiculatum* samples. The combined data network (Fig. 11) shows a "clade" comprising *O. apiculatum* and all three *O. acerosum* samples, but the *O. dioicum* samples diverge apart.

Nicola et al. (2015) reduced *O. abbreviatum* to varietal status within *O. stuebelii*. The ITS (Fig. 5) and combined data (Fig. 7) bootstraps strongly support the *O. abbreviatum* samples as sisters nested within a paraphyletic *O. stuebelii*. Under Nicola et al.'s (2015) taxonomy, in these trees, the consequent *O. stuebelii* var. *stuebelii* then becomes the paraphyletic specioid. Same difference. However, neither bootstrap strongly supports monophyly of all of the samples of this complex. The *rpl32-trnL* bootstrap (Fig. 6) is insufficiently resolved to pronounce on this matter. Here, the two *O. abbreviatum* samples intermix among multiple species.

Nicola et al. (2015) also reduced *O. famatinae* to *O. stuebelii* var. *argentinum* (Cabrera) Nicola, S.E. Freire & Ariza, which Davies (2010) recognized as *O. abbreviatum*, which, as noted, Nicola et al. (2015) recognized as *O. stuebelii* var. *abbreviatum* (Cabrera) Nicola, S.E. Freire & Ariza. But in the ITS and combined data bootstraps (Figs. 5, 7), the single specimen sampled here, not seen by Nicola et al. (2015) but identified by Davies (2010) as *O. famatinae*, groups strongly with the sample of *O. revolutum*. Nicola et al. (2015) did not mention *O. revolutum*, but Davies believed it to be closely related to *O. abbreviatum*. Thus, Davies (2010) and Nicola et al. (2015) clearly disagree not only on the taxonomy of *O. famatinae*, but also its relations to the types of *O. stuebelii* vars. *abbreviatum* and *argentinum*. Confusing? This is worse than trying to sort out who are Diego Maradona's children. In any case, taken at face value, the genetic data disagree with both Davies (2010) and Nicola et al. (2015).

Nicola et al. (2015) also reduced *O. polymallum* to a variety of *O. pulvinatum*. But in the gene trees, the polymorphic *O. pulvinatum* samples are "all over the place." The combined data bootstrap strongly supports their dispersal in three different clades (cf. Fig. 11). The single *O. polymallum* sample does cluster within two of them. But, again, Nicola et al. (2015) did not examine the specimens sampled here.

5. *Relationships of sp_indet_25161 – an unidentified phylogenetic object*

As noted in the results, relations of the sample *sp_indet_25161* differed in the ITS and *rpl32-trnL* trees. In the latter, the sample is strongly supported as sister to *C.* subg. *Liniphyllum* (Figs. 2, 3, 6, 10). In the former, it appears as an isolated lineage of *Chaetanthera*, but seems most closely related to *C.* subg. *Tylloma* (Figs. 1, 2, 5, 9) and, in any case, not at all related to *C.* subg. *Liniphyllum*.

This sample was identified in Hershkovitz et al. (2006a) by its collector, M. T. K. Arroyo, as *C.* (subg. *Tylloma*) *flabellata*. The voucher, *Arroyo et al. 25161* (CHILE: Región Metropolitana, 2200 m elev.) is not listed in Davies (2010). I have not studied it, either. Cabezas Álvarez (2015) constructed an ITS phylogeny using a different sample, *Arroyo et al. 25162*, from a nearby locality at 2310 m elevation, and identified in Davies (2010) as *C. flabellata*. Both localities are the along the road between metropolitan Santiago and the high elevation ski resorts near the small town of Farellones.

From Cabezas Álvarez' (2015: 29, Fig. 4) phylogram, the ITS sequence of the *Arroyo et al. 25162* sample is very similar to that of *euphrasioides_25176*. This result is not surprising. The two species are similar, *C. flabellata* (endemic to the precordillera near Santiago) being a bit larger, with more dentate leaves, and generally occurring at somewhat lower elevation that the more widespread and variable *C. euphrasioides* (Davies, 2010). In fact, data from Davies (2010) indicates that the elevational ranges of the two species overlap in the Santiago vicinity.

Setting aside for a moment the *rpl32-trnL* sequence, several explanations for the peculiar ITS sequence can be discounted. Examination of its highly conserved 5.8S and conserved ITS regions (Hershkovitz and Zimmer, 1996; Hershkovitz et al., 1999) discard the possibility that the sequence is a pseudogene. It also is possible that it represents a highly diverged but functional ITS paralog that descended from an ancestral paralogous sequence different from the ancestor of all of the other ITS sequences in *Chaetanthera*. But the persistence of this paralog in only one of all of the sampled taxa is not only implausible, its veracity would universally discredit rDNA ITS as a phylogenetic marker. Mixing of the samples during lab procedure is another possibility, but it would beg the question of where is the *other* mixed sample, the "true" *C. flabellata* sequence that is highly similar to *C. euphrasioides*.

But the *rpl32-trnL* sequence of *sp_indet_25161* render moot such explanations for the ITS sequence. While strongly supported as sister to *C.* subg. *Liniphyllum*, its *rpl32-trnL* sequence clearly is highly divergent. The data are suggestive of an ancient hybrid, but the high divergence of *both* sequences suggest a plant that ought not to be confused morphologically with any other. Furthermore, how is it possible that a species so divergent could have been overlooked in the work of Davies (2010) or, for that matter, by Cabrera (1937) and dozens of Chilean plant collectors ever since? So, the DNA sequences of the sample *sp_indet_25161* are highly divergent, but *what is it*?

Several remaining possibilities might explain the sequences of the sample *sp_indet_25161*, though none of them are entirely satisfactory. One possibility is that *Arroyo et al. 25162* analyzed by Cabezas Álvarez (2015) and identified by Davies (2010) as *C. flabellata* is actually *C. euphrasioides*, and that *Arroyo et al. 25161* is "true" *C. flabellata*. *Arroyo et al. 25162* was collected at 2310 m elevation, 110 m higher elevation than *Arroyo et al. 25161*. Although lower in elevation than other *C. euphrasioides* collections from this particular road, Davies (2010) listed other Región Metropolitana specimens from as low as 1600 m elevation. The apparently small ITS divergence between the *C. flabellata* of Cabezas Álvarez (2015) and *C. euphrasioides* is not problematic, because such polymorphisms are the rule rather than the exception in this genus (see Results and below).

The preceding explanation is appealing, but if *Arroyo et al. 25161* actually is *C. flabellata*, the remarkable nuclear and chloroplast genetic divergence remains unexplained, especially given the strong phenotypic similarity and intergradation of *C. flabellata* and *C. euphrasioides*. But it may be worth noting that *C. euphrasioides* is a "basal" lineage of *C.* subg. *Tylloma*, thus that it may retain ancestral phenotypic features. This raises the possibility that, whether or not *Arroyo et al. 25161* is *C. flabellata*, it descended from a relictual hybrid between the ancestor of *C.* subg. *Liniphyllum* and *C.* subg. *Tylloma* that later evolved to resemble *C. euphrasioides*. If *Arroyo et al. 25161* exemplifies this species and this species is true *C. flabellata*, then both the morphological and geographic proximity of *C. flabellata* to *C. euphrasioides* owes to convergence and coincidence rather than close relationship. And forms of the polymorphic *C. euphrasioides* might be confused with *C. flabellata*. Alternatively, if *Arroyo et al. 25162* exemplifies true *C. flabellata*, then *Arroyo et al. 25161* represents an undescribed species of ancient hybrid origin that is, nonetheless, so similar to *C. flabellata* as to be confused with it.

The other possibility is that the plant material of *Arroyo et al. 25161* itself (rather than the DNA sample) was mixed or swapped with that of one of the five species recognized by Davies (2010) that were not sampled in the present analysis. None of these species occurs in the range of *C. flabellata*. However, three of the five species (*C. kalinae* A.M.R.Davies, *C. linearis* Poepp. ex Less., and *C. multicaulis* DC) were sampled for ITS by Cabezas Álvarez (2015: 29: Fig. 4), and evidently none have a sequence corresponding to *sp_indet_25161*.

The remaining two species are *C.* (*Tylloma*) *limbata* (D.Don) Less. and *C.* (*Tylloma*) *splendens* (J.Rémy) B.L.Rob. The latter is very similar to *C. flabellifolia*, but smaller and parapatric at lower elevations (Davies, 2010: 198, 225). But *C. flabellifolia*, which *was* sampled, actually is the better candidate for swapping with *C. flabellata*, because of similarity in its name and sharing of its flabellate leaves and higher elevation niche. Meanwhile, *C. limbata* belongs to the "*C. glabrata – C. limbata* complex" discussed above. Because of presumed taxonomic affinities, it would be surprising if *sp_indet_25161* corresponded to either *C. limbata* or *C. splendens*. Then again, no less peculiar is its possible correspondence to *C. flabellata*, as identified originally.

A final possibility is that the collection *Arroyo et al. 25161* does not pertain to *Chaetanthera* or any other natural taxon. Its peculiar sequences suggest that it is an extraterrestrial invader. Indeed, Chile is well established as a principal destination for extraterrestrials (Dobson, 2018; Ortega, 2020), and the Chilean government closely monitors extraterrestrial activity. Likewise, it is well established that the mediterranean zone of Chile, where *Arroyo et al. 25161* was collected, is especially vulnerable to invasion by alien plants (Fuentes et al., 2008). Extraterrestrial civilizations, far superior to ours, hardly could have overlooked this datum. They easily could have Googled it and downloaded the paper from Sci-Hub.

Not coincidently, it has been established also that one method of extraterrestrial colonization of the Earth is via plantlike propagules that germinate and grow into plantlike organisms that produce fruitlike pods whose contents assimilate the form of terrestrial organisms (Finney, 1955; Fig. 12). These have been known to assimilate the form of individual humans who happen to fall asleep in their proximity. However, they can assimilate any life form and, being themselves plantlike, most certainly plants.

It is also established that the extraterrestrial forms are very superficially similar but not identical to the terrestrial organisms that they assimilate. This manifests in behavior and, to the degree behavior reflects genetics, probably also in the genome. It should be expected, then, that the DNA sequences of the extraterrestrial forms are very similar to yet obliquely distinct from the assimilated forms. This prediction is borne out in *Arroyo et al. 25161*. The sequences of this *Chaetanthera*-like imposter *seem* to pertain to *Chaetanthera*, but nonetheless are very different, and the nuclear and chloroplast sequences are not concordant.

But another possibility emerges as well. Possibly extraterrestrial assimilation already is advanced far more than appreciated. Peculiar natural and political phenomena across the globe in the past few years make this all the more likely. Thus, *Arroyo et al. 25161* may have escaped assimilation in a remote pre-Andean canyon and was one of the few remaining organisms that are *not* extraterrestrial. This means that the entire molecular "Tree of Life" (D. Soltis and P. Soltis, 2018; perhaps not coincidentally also addressed as "the Soltoids") is already assimilated. Oddly enough, the Tree of Life was constructed in a remarkably short period of time, seemingly appearing out of nowhere, and now it is all over the Worldwide Web. Accordingly, DNA sequences of the unworldly order Caryophyllales, which are clearly angiosperm imposters, placed them among the recently evolved Asteridae. Not coincidentally, many Caryophyllales species are invasive (Pyšek, 1998; the strange accent on the "s" in Pyšek also intriguing).

Of course, despite there being "more evidence than ever before" (cf. Nespolo, 2003) for extraterrestrial visits, least of all *Arroyo et al. 25161*, an extraterrestrial origin of some or as much as nearly all earthly biota cannot be considered proven. Like all theories, it must be subjected to the most rigorous scientific analysis. Otherwise, it is merely dogma, or "*an abstract phenomenon that obtains in all possible*…[cases, and that]…*holds in any history in which the terms of the theory can be jointly interpreted in a way that accords with the abstract requirements of the theory*" (Matthen and Ariew, 2009: 222). Like, for example, the Theory of Natural Selection (Matthen and Ariew, 2009; cf. Maturana and Mpodozis, 2000; Hershkovitz, 2019b).

In the meantime, perhaps it is better to keep quiet about *Arroyo et al. 25161*, and not call its attention to the authorities. They might well be pod people already. But it may be prudent, as a precaution, when travelling on the road between Santiago and Farellones, Chile, to avoid falling asleep near 2200 m.

6. Infraspecific DNA sequence polymorphism (and interspecific sequence identity)

Infraspecific DNA sequence polymorphism was the rule rather than the exception, found in 15/16 of the multiply-sampled nominal species of *Chaetanthera* s. str. and *Oriastrum*. Only a single species, *C. flabellifolia*, sampled only twice, had identical sequences for both ITS and *rpl32-trnL*. At the same time, both sequences yielded examples of nominal interspecific distances greater than infraspecific. Just as notable, in half of the cases, the combined sequence data did not support or refuted monophyly of the nominally infraspecific sequences. The results are all the more notable given the recency and thoroughness of the species taxonomic monograph, viz., Davies (2010). This means that the polymorphism is less likely to reflect taxonomic error. But polymorphism also was common when the same samples were identified according to the previous monograph, viz., Cabrera (1937; cf. Hershkovitz et al., 2006a).

The above observations are not unusual. Molecular phylogenetic analyses that focus on the infraspecific level usually tell a story different from that superficially evident from interspecific and higher-level analyses. Interspecific sequence divergence characterized multiply-sampled nominal species of *Tropaeolum* L. (Hershkovitz et al., 2006b) and genera of Montiaceae (Hershkovitz, 2006), the latter also manifesting considerable interspecific sequence identity.

The present work also chanced upon the considerable DNA sequence polymorphism found in a phylogeographic study of *Richterago discoidea* (Barres et al., 2019). Here, 51 ITS sequences from 17 populations (3 samples/population) yielded seven haplotypes differing by 1–3 mutations (substitutions and/or indels). Five defined geographic regions were each characterized by multiple haplotypes, with considerable interregional haplotype sharing.

Even greater variability was found in four noncoding cpDNA loci, including *rpl32-trnL*. Here, 88 concatenated sequences (3378 bp total) from 19 populations (4–5 samples/population) yielded 25 haplotypes. But sharing of haplotypes among the five different geographic regions was considerably less. This is not surprising given both the larger number of haplotypes and their virtually absolute linkage, their uniparental inheritance, and the lack of recombination. Nonetheless, infraregional polymorphism in the cpDNA was found.

In any case, the infraregional polymorphism in both nuclear and plastid loci anticipates its propagation in the case of eventual geographic speciation, and also demonstrates how lineage sorting might obfuscate phylogenetic analysis. [Barres et al. (2019) reported monophyly of *R. discoidea*. This may well be the case, but outgroup sampling was limited to three individuals of only two of the reported total of 16 *Richterago* species.]

I call attention here to the finding that there were more *rpl32-trnL* than ITS haplotypes found among the *Chaetanthera/Oriastrum* samples (see Results and Table 2). This seems unexpected. *Rpl32-trnL* is about 40% longer, but ITS apparently evolved about three times faster. The *Richterago* data described above also suggest excess cpDNA compared to ITS haplotypes when corrected for length and overall evolutionary rate. I would be tempted to offer a possible explanation, such as the well-known “concerted evolution” (infragenomic homogenization) of ITS copies, which would tend to reduce haplotype diversity.

But the pattern in the preceding taxa apparently is not universal. Data from Poaceae (Peterson et al., 2014; see below) revealed slightly fewer *rpl32-trnL* than ITS haplotypes. Among sampled Montiaceae (Hershkovitz, 2006), three polytypic genera summed 58 haplotypes for ITS and 45 (i.e., fewer) for cpDNA *ycf3-trnS* (a spacer comparable to *rpl32-trnL*). But sampled *Montiopsis* Kuntze summed 16 ITS and 20 *ycf3-trnS*. So the relationship between ITS and cpDNA haplotypes is not constant, hence has no single explanation.

Perhaps a more “consummate” example is Stoughton et al. (2018), who used two different methods of whole genome sampling, each analyzed two different ways, to evaluate correspondence with species/subspecies of the *Claytonia* sect. *Claytonia* (Montiaceae, Montieae, Montiinae; cf. Hershkovitz, 2019a, b). One of the two genome sampling methods (“genome skimming”) performed “better” than the other in yielding higher coalescent tree bootstrap support (both ≥ 70% and ≥ 95%) and splits-network “branches” having greater correspondence to independent taxonomic identifications. But the samples identified as *Claytonia umbelleta* S. Watson were “all over the place,” their genomes originating 4–5 times. Bootstrap support for monophyly was lacking for an additional three of the multiply-sampled taxa (including two subspecies).

[Hershkovitz (2019a, b) misinterpreted the split networks of Stoughton et al. (2018: 541, Fig. 1), reporting that eight individuals not therein classified into one of the eight shaded boxes were therefore unidentified. In fact, the individuals were indeed identified, four corresponding to unclassifiable samples of *C. umbellata* and the other four corresponding to taxa sampled only once. Still, the result is not unproblematic, because three of the species sampled only once are rather widespread and sympatric with other species. It seems that additional sampling of these taxa most likely would yield infraspecific divergences similar to the more intensively sampled taxa. Considering the results for *Claytonia umbellata*, it is not clear a priori whether this would clarify or muddle the correspondence between genome and taxa based on the current data.]

More importantly in the present context, the results of whole genome sampling represent the asymptotic expectation for “infinite” targeted gene sampling. No two samples (individuals) in Stoughton et al. (2018) were genetically identical, and total nominal infraspecific sequence divergence often was considerable and greater than nominal interspecific divergence. The splits networks also manifest (by design) a considerable degree of “incongruence” in the gene sequence data, although they do not identify its cause. It would include gene tree incongruencies because of lineage sorting or hybridization, but also simple homoplasy and sharing of more conserved loci among some but not all sampled species.

Noting evidence of nominal infraspecific polymorphism, about six years ago, I further examined about 120 of the then most recently published interspecific-level angiosperm phylogenetic analyses, these representing perhaps 3000 nominal species. Only about 300 of the nominal species had been sampled more than once. But it could be gleaned from the phylograms that about 80% of these were polymorphic for the analyzed sequence (usually ITS). Likewise, the phylograms suggested polyphyly of a major proportion of the nominal species. Even so, the Discussion sections of the articles generally did not even mention the observed infraspecific polymorphism. Thus, it seems that an expectation of infraspecific sequence uniformity is simply a consequence of rare infraspecific sampling in *phylogenetic* analyses and then not even noting polymorphism when it is found.

In any case, the present results for *Chaetanthera*/*Oriastrum* represent an infinitesimal sampling of the genome (two loci, ca. 1300 bp) and also very little sampling within nominal species (37 of 48 nominal species sampled for both loci, 16 species sampled more than once, but 10/16 only twice). Nonetheless, both loci were identical in only one twice-sampled species. Likewise, nominal infraspecific divergence often is considerable and greater than interspecific divergence, gene trees of some nominal species are not monophyletic, and the gene trees manifest incongruencies. But whole genome studies demonstrate that these are not anomalies.

7. ***Barcodes, anyone?***

The present results and those of cited and countless uncited references demonstrate the absurdity of the concept of "species barcoding," at least with respect to species delimitation and definition. This is not at all to say that loci such as ITS do not provide powerful tools for essentially "automated" approximation of identification of individuals in many cases to the level of species or even populations. Nor does it suggest that such loci are not useful for cryptic lineage recognition and refining species taxonomy. But in these cases, the loci are an appendage to conventional taxonomic research. Both theory and empirical data refute any notion that sequence differences from one to a few DNA loci can be proxies for taxa at the (sub)specific level, i.e., *by themselves* identify, diagnose, distinguish, or delimit species.

"Despite efforts by many scientists, the standardization of DNA barcodes for all land plants has not yet been achieved" (Peterson et al., 2014). Indeed it has not and it cannot, and the effort itself is at best "ill-informed" and, at worst, hardly "scientific" at all (see also below). Peterson et al.'s (2014) work underscores this. In search of species-discriminating markers, they tested four loci, plastome *rbcL* and *matK*, and the two loci used here, ITS and *rpl32-trnL*. Not unexpectedly, they demonstrated that the first two loci did not adequately discriminate between species, which might have been news 20 years earlier.

Peterson et al. (2014) based their conclusions on a phylogenetic analysis of 50 taxa (46 species plus 4 subspecies) spanning Poaceae subfamily Chloridoideae, which Wikipedia tells me comprises ca. 1600 species. However, the study focused on the genus *Leptochloa* P.Beauv. s. l. and its segregates, comprising 32 species, of which Peterson et al. (2014) sampled 23 plus some subspecies for a total of 27 taxa ("specioids;" Hershkovitz, 2019a: 2). They referred to the tested loci repeatedly (ontologically) as "barcode regions." While the combination of the ITS and *rpl32-trnL* "barcode regions" did discriminate among these relatively few species, they only sampled three taxa more than once, one nominal species three times and two of the nominal subspecies twice. This does not qualify as a rigorous test of the interspecific discriminatory power of the loci. The *Chaetanthera*/*Oriastrum* sampling also was not or intended to be a test of discriminatory abilities of these same loci. Yet, the data clearly demonstrate that both loci would fail this test.

But it is theory rather than data that undermines the species barcode concept. The data merely corroborate theory. Firstly, purely from a molecular genetics viewpoint, DNA sequences do not evolve uniformly across taxa nor in lockstep with other sequences (Ogilvie et al., 2016; but see also below). There is no theoretical reason why they should. This has been corroborated empirically for decades. The data presented here add nothing new to this notion, nevertheless provide suitable examples. For example, molecular evolutionary rates for both loci evidently have been faster in *Oriastrum* subg. *Oriastrum* than in *O.* subg. *Egania*. And within *O.* subg. *Oriastrum*, the rates between loci in samples of *O. gnaphalioides* are strikingly different.

More philosophically, the aphorism that "gene trees are not species trees" refers not so much to empirical incongruence as it does to ontological distinction, such that gene trees never can be species trees, even when they appear to be perfectly congruent (Hershkovitz, 2019a, b). Put another way, DNA sequences do not specify or cause or impose upon the organization level perceived as species (see also below), and perceived species do not specify or cause or impose upon DNA sequences. The degree to which gene trees and species trees appear to agree is an artifact of their hierarchical physical relationship (Hershkovitz, 2019b). The genome and the individual organisms represent hierarchically separated organizational levels. Each can evolve (diverge/diversify) semi-autonomously as long as their respective operating conditions are maintained at their respective (and dynamic) upper and lower hierarchical bounds. Species represent an organizational level hierarchically higher than individuals, but, in practice, *most* biological species are not organized, hence persist only via inertia and evolve without bounds at all (see also below). [I use the term "semi-autonomously" rather than "autonomously" or "independently" (e.g., Ogilvie et al., 2016) in order to emphasize that components in a hierarchical system are mutually constrained and/or canalized.]

Some evolutionary histories will tend to render nominal species relatively genetically homogeneous and distinct from other species, e.g., in a highly diverged and geographically highly localized lineage (itself likely consequent not to the absence of diversity and intergradation, but rather its extinction). But both of these conditions are unrealistic for the overwhelming majority of taxonomic species, and also trivial, because such species will tend to be easily identified without a DNA barcode. In practice, molecular genetics approaches are not applied to easy cases, but difficult ones, such as recently evolved species complexes, where lineage sorting and/or hybridization are rampant. Four of the five *Chaetanthera*/*Oriastrum* subgenera include such species complexes. These are the taxa that challenge not only taxonomic, but also macroevolutionary, ecological, and conservation analysis. Here, the barcode approach is not only useless, it is likely to be misinformative and disinformative.

But the feeblest aspect of the species barcode notion owes not to ontological distinction, but ontological ambiguity. A "species barcode" is supposed to be a discrete aligned DNA sequence fragment – a reasonably tangible and quantifiable entity – whose identity is supposed to approximate that of a species.

But…*¿What is a "species?"*

To quote the back cover material of Wheeler and Meier (2000), "no question in theoretical biology has been more perennially controversial or perplexing than 'What is a species?'" In the realm of ecology and macroevolutionary biology, Pennell and Harmon (2013) remarked that "...species delimitation is a thorny issue…estimates of species-level diversity are notoriously unreliable and subject to myriad sources of bias." Brooks and McClennan (1999) remarked that "...at the end of the 19th century…species were whatever good taxonomists said they were…" (cf. Hey, 2001, 2006).

Unless I have missed something, none of the preceding has changed. A "good taxonomist," Davies (2010), published a detailed revision of the species of *Chaetanthera*/*Oriastrum*. Preliminary to this, she published separately a few new species descriptions. It appears that current research [with the exception of Nicola et al. (2015) with respect to a few species] and databases accept that the species are whatever Davies (2010) said they were. There is little basis for dissent, because nobody *ever* has conducted such a thorough study of the entire group, and probably nobody will ever again.

If Davies (2010) had not monographed the group, then species of *Chaetanthera*/*Oriastrum* would be today whatever the "good taxonomist" Cabrera (1937) said they were. This classification was used in Hershkovitz et al. (2006a). And if not for Cabrera (1937), the species would be today whatever the "good taxonomist" Reiche (1905) said they were for Chile and who-knows-which "good taxonomist" for non-Chilean plants. Yet Davies (2010), Nicola et al. (2015), and the present work make clear that species delimitation in *Chaetanthera*/*Oriastrum* remains problematic. In the meantime, what was and is the status of the "species barcodes," viz., the highly vaunted ITS and *rpl32-trnL* sequences? The species taxonomy has changed, but the sequences have not.

The crux of the problem is that species are broadly conceived to be tangible (and often implicitly equivalent) entities, to one or another of which all organisms pertain. But none of the 30-some objective species definitions and/or delimitation criteria have ever "worked" (Hey, 2001, 2006; cf. Schlick-Steiner et al., 2010). The "barcode species concept" does not work, either.

The various parochial species concepts/definitions of the 20th Century seem to have been largely abandoned in favor of a so-called "unified species concept" (De Queiroz, 2007) in the context of "integrative taxonomy" (Schlick-Steiner et al., 2010). Both appear to be 21st Century terms for 18th Century taxonomy. In the unified concept, species delimitation remains as it always was: subjective and specialist-specific. The only apparent difference is the approach, which includes modern methods, data, and analytical technology. But if Linnaeus and his powdered wig pals had had these, they would have done the same thing. More problematic, the extraordinary complexity (and bureaucracy) recommended for "integrative taxonomy" (Schlick-Steiner et al., 2010) seems to presume that there are 10 government-funded researchers studying every species on earth. I do not know what is the true ratio, but I suggest it is somewhat less.

It is not possible to articulate here fully on the "species problem." But elsewhere (Hershkovitz, 2019a, b), I have emphasized that species ontology must be resolved in the perceptual dimension, because species have no established ontology in any material dimension. This is why throughout the present work, I refer to "nominal species," i.e., taxonomic units that are *called* species, whatever are species ontologically. I also have drawn analogy between perception of species and perception of water waves on a dynamic wavescape, i.e., deciding where each wave (and wavelet) begins and ends and to which wave each water molecular pertains – as the wavescape continues to evolve. Likely the classification of waves would be difficult, and different observers would classify them differently. In many cases, some water may not appear to pertain to or associate with any wave at all.

Classification of individuals into operational species is the essentially the same, and taxonomic literature often refers to unclassified or unclassifiable or otherwise oddball individuals whose classification seems tenuous. To address this dilemma, I coined the terms "apospecies" and "synspecies." Operational species are the latter, analogous to n-dimensional waves [or, alternatively, "property clusters" (Wilson et al., 2007; though dynamic/evolving rather than homeostatic)]. Synspecies are not fixed; they are both perceptually (operationally) and, incidentally, biologically dynamic.

Seemingly paradoxically, a synspecies *excludes* its nomenclatural type, which, by definition, proxies for all individuals of whatever synspecies might be circumscribed around them. But these nontype individuals have no nomenclaturally fixed identity. Hence neither does the synspecies. A synspecies merely takes its name from and refers to a type. But it is not the same thing.

In the meantime, a nomenclatural type refers to a specimen that, *also* by definition, is purportedly distinct from all other types. It is used as a proxy for a perceived and never fixed synspecies, but it specifies no synspecies. In other words, ontologically, it is *not synspecific*. Rather, it is *apospecific*, hence nomenclatural types represent *apospecies*. Apospecies are nondimensional. Nomenclatural types represent arbitrary points in the wavescape that have been formally recognized and named not because of what they are, but rather, what they are not, i.e., not pertinent to other species. Think about it.

I stress that the notion of apospecies and synspecies pertains to operational species and imply/impose no *particular* biological quality. This is critical, because, in fact, all taxonomic species are operational, and evidently owe to no *particular* biological quality (cf. Schlick-Steiner et al., 2014). Likewise, the apospecies/synspecies association implies no particular genetic or phylogenetic relation. Again, this is standard taxonomic practice. At the same time, the biological qualities of organisms and their genetics and phylogenetics are not denied. Any of them may well be used by "good taxonomists" to recognize apospecies and/or delimit synspecies.

In the case of *Chaetanthera/Oriastrum*, Davies (2010) perceived 48 species. But formally, she perceived 48 apospecies, that is, nomenclatural types that are distinct from all other types (or not synspecific with any of them), hence belong to no other species. Within the 48 associated synspecies, she included an additional 79 nomenclatural types whose presumed apospecificity she therefore rejected. She also cited additional conceptually heterotypic invalid names, but not their "types." In other words, in Davies' (2010) perception, these specimens are not distinct from other types, hence indeed are synspecific with other types, and therefore pertain to synspecies and not apospecies. Cabrera (1937) had perceived a different set of apospecies and, accordingly, a different assemblage of synspecies. Nicola et al. (2015) disagreed with some of Davies (2015) apospecies designations and, accordingly, synspecies waves. These workers have different perceptions of waves, hence a distinct operational wavescape. I won't attend the International Botanical Congress in Rio de Janeiro in 2023, but if I did, I would give a lecture on the wave model on the beach, using just the surf as my Power Point.

Notably, much of the difference between the taxonomy of Cabrera (1937), Davies (2010), and Nicola et al. (2015) involves taxa considered in one or another way problematic or difficult by Davies (2010, 2013), e.g., the *C.* (subg. *Chaetanthera*) *chilensis* complex, the *C.* (subg. *Tylloma*) *glabrata* complex, and several taxa of *O.* subg. *Egania*. (cf. Nicola et al., 2015). These are, metaphorically, taxonomically rough waters, a churning foam in which waves are not so readily distinct. The DNA sequence data likewise did not resolve into distinct synspecies.

8. *Perspectives on methodology applied in the present work*

a. Where is the Mr. Bayes tree?

As noted in the Materials and Methods section, I did subject the *Chaetanthera/Oriastrum* data to BE phylogenetic reconstruction. For a variety of reasons explained below, I opted to not include these results here. I can report, however, that the results were "as expected," with clades supported by ≥ 70 % BP in the bootstrap consensus generally supported by ≥ 95% Bayesian posterior probabilities (PP; see below).

BE methods have become standard practice in phylogenetics and macroevolutionary analysis. This presumably owes to several factors: (1) their reasonably user-friendly implementation in freely-available software; (2) the ease with which BE can analyze heterogeneous data and data types; (3) the ease with which models far more complex than conventional ones can be specified; and (4) their speed relative to full frequentist ML analysis (and more so, full ML bootstrap analysis). Other factors are more sociological/political than scientific, i.e., the bandwagon effect. Many, if not most, practitioners would never have conceived of its application otherwise.

b. The epistemology of Bayesian phylogenetic estimation: "induction on steroids"

The popularity of BE in phylogenetics also probably owes something to misconceptions of the theoretical equivalency of PPs and BPs. This favors use of the former, since they often are much higher than the latter (Alfaro and Holder, 2006). This seems to have evoked a popular but mistaken sentiment that nodes supported by PPs ≥ 0.95 are therefore "true." These misconceptions must be corrected. BE is interpreted better as an ML optimization algorithm, whereby the PPs represent – at best – neither the "probability" nor of the "truth" of the tree, but merely an overestimated probability that the ML tree is the optimal one given the FM ("fake model," remember?; cf. Yang and Xhu, 2018; Abadi et al., 2019).

It is important, in the first place, to understand that, despite using Bayes' theorem, BE methods in phylogenetics are distinct from classical empirical applications of Bayesian statistics. An example of the latter is the classical case of the woman whose brother is hemophiliac. Thus, it can be deduced from classical genetics and classical statistics that the fixed probability that the woman carries the hemophilia allele is 0.5. Of course, she is not a 50% carrier – she is a carrier or she is not. Bayesian statistics establishes the 50% figure as a *prior* probability, which it seeks to refine using subsequent observations. As the woman gives birth to non-hemophiliac male children, Bayes' formula calculates a reduced (but nonzero) PP that the woman is a carrier. But if her 101st male child is hemophiliac, the formula yields a PP of 1.

BE implemented in phylogenetics and macroevolutionary analysis is another "beast" altogether (the pun purely accidental). Setting aside the controversial theme of the basis for prior probability abduction in the absence of empirical knowledge of the ancestral conditions, the more critical difference is that the PPs are derived *without* additional empirical observations. And they are derived not analytically (owing to computational expense), but heuristically and algorithmically. The "metropolis-coupled Markov chain Monte Carlo" (MCMCMC) procedure tweaks the FM parameters (including the tree topology) and algorithmically accepts or rejects the changes. The acceptance criterion and other parameters themselves can be adjusted seat-of-the-pants by the user. But the procedure simply evaluates the *same* data millions of times from slightly different angles. It is difficult to propose an analogy with the hemophilia example. It is more as though the woman´s genetic history was unknown and she had only one male child and the child was lacerated millions of times to see if eventually he would bleed to death. Then the woman would be pronounced a hemophilia carrier.

Contrary to widespread, if not popular belief, BE PPs, unlike bootstrap BPs, do not provide *corroboration*, more commonly referred to as "character support" for the tree (García-Sandoval, 2014). Alfaro and Holder (2006) pointed out that the statistical meaning of PPs in BE phylogenetics may be misunderstood by non-experts. They are not equivalent to p-values in frequentist statistics, e.g., the probability that the mean of the test distribution is different from the mean of the null distribiution. This means that the underlying distributions, hence causes, also are different. P-values are the probability of Type I error. BE does not formally (statistically) test branches in the standing tree, i.e., the standing tree against a null distribution. Such tests can be performed "manually" using likelihood ratio tests. But, theoretically, statistical rejection of tree branches generated with different parameters is not a simple matter, because the tree itself is a parameter (Yang et al., 1995). But more importantly and philosophically speaking, BE does not constitute a rigorous test of a tree

The BE procedure increasingly biases in favor of the standing tree as it searches algorithmically for a better one. PPs are calculated as the proportion of node incidence in the trees accepted in the MCMCMC process. But, these mostly *are not* trees constructed independently, but rather the exact *same* tree that survives against millions of proposed alternatives. Partially for these reasons, BE PPs are considered to be "overconfident" (see also below). García-Sandoval (2014) provides another clue as to the nature of BE, noting that a clade supported by a single character with no conflicting characters will appear in the ML tree and also have a high PP, whereas the BP might be rather low, because its incidence among the bootstrap trees depends upon the resampling of that single character.

c. Performance issues of BE

Several theoretical and performance problems of BE were discussed by, among others, Alfaro and Holder (2006), Grünwald and van Omen (2017), Autzen (2018), and Yang and Xhu (2018). And these are apart from epistemological problems. The problems range from PP overconfidence, statistical inconsistency when the FM is misspecified (which I consider to be "always;" see below), to the underlying theoretical basis of what I call BE's "Boeing 737 MAX" behavior, the known tendency of BE to sometimes and unpredictably indicate extremely high PP support for nonexistent branches. The cited and other references have suggested "fixes" to the various problems, generally involving intensive "manual" inspection of the data to detect these problems in the first place. Grünwald and van Ommen (2017) devised an algorithmic method to correct performance problems. But this does not change the epistemological nature of the beast. Moreover, the fixes do not retroactively inspect and correct countless of BE studies already published.

The reported inconsistency of BE under misspecified models (Grünwald and van Ommen, 2017) especially caught my attention. It seems somewhat at odds with the work of Abadi et al. (2019; see above), whose frequentist ML simulations found that model selection criteria had little effect on tree selection per se: different FMs selected by different FM selection criteria selected the true tree with similar frequency. Also, they found that even a very poor FM did not perform much worse than an optimized one. These results, in turn, were at odds with most prior work that tended to conclude the opposite, viz., that "accurate" models were essential. Abadi et al. (2019) explained this discrepancy as a consequence of prior research basing on more hypothetical and cherry-picked data, whereas they based their simulations on a large sample of qualitatively diverse empirical data sets.

The results of Abadi et al. (2019) suggest that BE ML performance likewise ought to be somewhat insensitive to model and/or parameter estimates. After all, the tweaks realized by MCMCMC are certainly no more and generally much less severe than the tweaks performed by Abadi et al. (2019) and by FM selection undertaken with suboptimal trees. Likewise, they are far less severe than the tweaks realized by conventional bootstrap analysis, in which the data are resampled with replacement. BE "resamples" the *same* data. Moreover, the conventional bootstrap (e.g., in PAUP) does not reoptimize FM parameters according to each bootstrapped data set.

But possibly the discrepancy owes also to another factor. While Abadi et al. (2019) concluded that frequentist ML was relatively "indifferent" towards different models, they also indicated that *all* of the different models selected the "true" simulated tree at best only about half of the time. This they attributed to the inadequacy of the models in general (hence FMs). The reported inconsistency of BE under misspecified models might owe to either or both circumstances, viz., simulations designed to exacerbate differences between models and/or differences between idealized and unknown "true" models.

In summary, BE is more informative than the frequentist ML analysis alone in suggesting possible alternative topologies statistically equivalent to the optimal one (given the FM). This and other advantages of BE (e.g., model flexibility) may seem to render the procedure worthwhile. But the downside is that the approach may be as misinformative as it is informative, especially when applied by researchers not expert in its underlying theory. BE seems to be as much or more sensitive to axiomatic model assumption violations than frequentist approaches and MP, but detection of such artifacts may be more difficult. The approach seems to yield generally overconfidence in results. And the meaning of BE PPs does not seem to be well-understood in practice. Indeed, the PPs derive from prior beliefs, but not so much about the data or the phylogeny as the prior superstitious belief that BE yields the true tree.

d. BE as a biased ML optimization algorithm

The preceding observations suggest that BE is an ML optimization algorithm. It is a much more powerful and flexible algorithm than conventional frequentist ML optimization programs, such as that in PAUP. Its superiority lies in the greater number and diversity of models that can be specified, and the speed with which it can simultaneously optimize both model parameters and the tree. "Full heuristic" (not "rapid") ML for more than a very few taxa is computationally feasible *only* with a priori specified and fixed model parameter estimates. Nonetheless, I believe that in the case of the present data, the number of taxa and length of sequences were not prohibitive for a reasonably rigorous frequentist ML analysis and bootstrap. Especially when the results can be compared with MP, ME, and NeighborNet results. In this case, the shortcomings of BE outweigh its benefits.

But even ignoring the theoretical and empirically demonstrated shortcomings of BE, it must be appreciated that even with 100 million BE MCMCMC generations, the number of trees examined is close to 0% of the number possible for even a modest dataset. The efficiency with which BE finds an apparently optimal tree (given the FM) is purely algorithmic (but see below). This, in turn, betrays its true operational nature. It demonstrates that phylogenetic BE is indeed an ML optimization procedure that is not better than and more likely worse than frequentist ML.

Also, as noted previously, unlike MP, ML (whether derived conventionally or via BE) tends to yield a single or very few trees having the same optimized -*ln*L. Multiple optimal trees result not from truly conflicting resolutions, whose -*ln*Ls usually are different, but when the optimal tree includes one or more true or virtual polytomies, i.e., because of essentially zero-length branches. Near-optimal trees probably include truly conflicting resolutions, but the default option in PAUP is to save only optimal trees. It is possible to save suboptimal trees, but this generally is not done in practice. In the case of BE, during its exploration of "tree space," the MCMCMC algorithm generally "accepts" some truly conflicting near-optimal trees and saves these to the treefile. PPs are calculated directly from the partition table, which is similar to a BP partition table.

But it must be appreciated that this treefile contains mostly redundant copies of the optimized tree, which MCMCMC rarely rejects, plus some number of conflicting near-optimal trees, which MCMCMC occasionally accepts. Thus, the PPs are essentially a weighted consensus of mostly many "copies" of the same optimized tree plus some near-optimal trees that were accepted during the MCMCMC. This is quite different from bootstrap replicates, in which no two trees are constructed from the same data, not even from the *original* data.

e. "Mr. Bayes" meets "Mr. Bootstrap"

The present work emphasized bootstrap support. It has been suggested, correctly, that the bootstrap itself is conceptually Bayesian (Efron, 2012; Bååth, 2015), whereby the original optimized tree is a prior belief conditioned by the original data. The procedure provides a posterior estimate of the reliability of each branch based on a conceptually different *data* sample, viz., a pseudo-independent sample. But pseudo-independent is better than not independent, which is the case for BE. The logic of the bootstrap is that it presumes that the empirical data possibly represents a biased sample of the data universe, hence that resampling with replacement compensates for that bias and that BPs indicate bias severity. High BPs mean that the bias is low to nil.

More generally, Bayesian estimation is a formalization of default human reasoning, which favors naivety. "Knowledge" is constantly updated as a function of how new observations affect conditioned beliefs. This theme is far beyond the scope of the present work, except that it helps put methodological results in a philosophical perspective. Science is supposed to *challenge* naivety. It is not supposed to determine how *reliable* are beliefs (or methodological results), but how *unreliable*. Phylogenetic BE does not do this, at least not in popular practice. The bootstrap, especially when applied to different data sets analyzed with conceptually different methods, is a more scientific approximation.

f. Interpretation of BPs – again

The exact meaning of BPs in the phylogenetic context is not established, but typically they are referred to as confidence intervals (Felsenstein, 1985a; also confidence "regions" and "limits;" see also Efron, 2012; Holmes, 2003; P. Soltis and D. Soltis, 2003). In any case, clearly BPs, like BE PPs, do not correspond to p-values. As noted above, p-values represent the probability that two or more sets of observations are outcomes of the same (null) or different processes, *where the processes are presumed to be stochastic (indeterminate).* But a tree and all of its branches are *determinate*: they are true or false independent of the process presumed to have generated them (see also below). Tree space is *not* a statistical distribution. Trees and their branches may be quantified by one or another criteria as being closer to or further from each other. But the distribution of these values is inherent in the criterion and not in the trees themselves.

The present work emphasized 70% BP as a cut-off for "strong support" for a branch. Many workers have criticized the "popularity" of this value as arbitrary (e.g., Alfaro et al., 2003; P. Soltis and D. Soltis, 2003; cf. Alfaro and Holder, 2006). By itself, indeed it is, even in the present work, but I argue that it is justifiable intuitively and, in any case, its reliability can be evaluated subjectively on a branch-by-branch basis.

To begin with, a branch with BP of 50.1% (as in a bootstrap majority rule tree) obviously is contradicted in 49.9% of bootstrap trees. This does not inspire much confidence in the branch, and generally it should not. But it is not as simple as that. Note that the simplest case of a fully resolved four-terminal tree with an internal branch has not two, but three possible solutions: $((x_1x_2)(x_3x_4))$, $((x_1x_3)(x_2x_4))$, and $((x_1x_4)(x_2x_3))$. The 49.9% is apportioned among these and might be as little as half of 50.1% (i.e., 49.9/2). Now, if we up the BP ante to 70%, each of the two alternatives in the four-taxon case converge on 15% BP. In this case, each alternative is contradicted in a total of 85% of the bootstrap trees. So why are we fretting about the significance of 70% while *not* fretting about the *insignificance* of 15%?

Of course, the BPs of the alternative branches may be greatly unequal, such that one of them is much closer to 50% in the first case and 30% in the second. But with increasing numbers of taxa and length of DNA data, the likelihood of such high BPs for alternative branching greatly decreases. And the 70% value becomes all the more imposing. This is because the number of possible contradicting partitions greatly increases, hence their BPs greatly reduced. All resolved internal quartets can be reduced to a four-subtree rather than four-taxon statement, $((x_{T1}x_{T2})(x_{T3}x_{T4}))$, etc., where T_n denotes a subtree that includes any possible combination of one or more taxa up to x_{n-3} (i.e., leaving one taxon for each of the other three branches). Intuitively, the number of possible tree quartets increases linearly with the number of all possible trees, but this number itself increases astronomically with the number of taxa. Hence so does the number of possible BPs

Dramatic reduction of BPs for increasing numbers of contradictory partitions is exactly what we observe in real data bootstrap partition tables. *Most* of the BPs are single-digit and decimal-scale. The default option in PAUP lists only BPs $\geq$ 5%, which is reasonable, because the branch is contradicted in 95% of bootstrap replicates. In that 95% replicate set, there might be, but usually is not, a *single* complementary BP of 5%. Usually, this 5% support itself is spread among many, possibly hundreds of different contradictory quartet subtree resolutions.

It should be clear from the preceding that the "significance" of 70% is not a constant, but varies contextually with the number of taxa, size of data, and distribution of contradictory BPs. In this sense, indeed the value is arbitrary. At the same time, it appears to be fairly robust with typical real data, in which maximum BPs of *contradictory* partitions usually are on the order of 10% or less. Given that one partition must be true and all of the others false, the partitions contradicting 70% usually can be considered reasonably well falsified, attributable to oversampling of homoplasy in some proportion of bootstrap replicates.

Nonetheless, depending on the degree of importance associated with the "truth" of any or all branches in a tree, scrutiny of the BP table is prudent. Some analyses might focus on the reliability of one or a few tree branches. But it is becoming increasingly common to subject *entire* "fully-resolved" trees to "automated" downstream evolutionary analysis, e.g., Moreira-Muñoz et al. (2020; see below). In such cases, branch-by-branch scrutiny should be requisite. In some cases, resolution of contradictory partitions should defer not only to BP, but also scrutiny of possible branch length artifacts and also the actual character data supporting the various contradictory partitions.

The present analysis of *Chaetanthera*/*Oriastrum* was generalized and emphasized relatively few of the possible internal branches, especially those supporting the five subgenera recognized here (see also below). One relationship was scrutinized in greater detail, that of the sample *sp_indet_25161*. Bootstrap analysis of the *rpl32-trnL* data strongly supported a sister relation with *C.* subg. *Liniphyllum*, whereas as much as one half of the ITS BPs supported a close relation with *C.* subg. *Tylloma*, with on the order of 10% actually supporting a sister relation with the sample of *C.* (*Tylloma*) *euphrasioides*. As discussed previously, the last may represent a long branch attraction.

In any case the ITS bootstrap majority rule consensus alone does not quantify support for the relationship between *sp_indet_25161* and *C.* subg. *Liniphyllum*. If the BP were on the order of 40+%, it would be absent in the majority rule tree, yet be numerically similar to support for the relation to *C.* subg. *Tylloma*. But here scrutiny of the BP table rules out this possibility, its support < 5%. The NeighborNet tree likewise illustrates that the ITS of *sp_indet_25161* shares weak similarity with both *C.* subg. *Chaetanthera* and *C.* subg. *Tylloma* exclusive of *C.* subg. *Liniphyllum*. These observations illustrate the importance of scrutiny of data beyond those highlighted in methodological results.

g. Other empirical examples of "data support"

In other contexts above, I have cited two phylogenetic analyses that reported "data support," one citing both BPs and PPs (Moreira-Muñoz et al., 2020) and the other only PPs (Peterson et al., 2014). Moreira-Muñoz et al. (2020) analyzed molecular phylogenetic relationships among 43 samples of *Mutisia* (the ingroup) along with samples of single species of 33 outgroup genera. The 43 ingroup samples included 42 nominal species; only one nominal species was sampled twice. The data comprised combined rDNA ITS and external transcribed spacer (ETS) and cpDNA *trnL-trnF* sequences. The aligned length was 2236, thus ca. 35% longer than in the *Chaetanthera*/*Oriastrum* alignment with 62 ingroup samples representing 37 nominal species. "Support for clades" [sic; see above] was derived via BE PPs and "rapid [ML] bootstrapping" using the program RAxML.

Moreira-Muñoz et al. (2020) considered that "clades were…well-supported" when their PPs were ≥ 95% *and* BPs ≥ 90% [italics mine]. They then asserted that, based on their analysis, "relationships within…[*Mutisia*]…are *mostly* well-resolved [italics mine]." Scrutiny of their tree (Moreira-Muñoz et al., 2020: Fig. 4) indicates otherwise. Only 9/41 clades (22%) within *Mutisia* are "well-supported" by their *own* standard. This figure represents the intersection of the 16/41 clades (39%) with ≥ 0.95 PP and 13/41 clades (32%) with ≥ 90% BP. Relaxing the standard of BP support to 70% (18/41 clades; 44%) improves this intersecting support to 13/41 clades (32%). Notably, while 9–13/41 nodes are mutually corroborated by BP and PP, I count 9/41 nodes whose support "conflicts" between BP and PP, i.e., ≥ 70% BP but < 0.95 PP or ≥ 0.95 PP but < 70% BP. The *Mutisia* tree is not "well-resolved."

Peterson et al. (2014) analyzed several "barcode regions" (see above). Unlike Peterson et al. (2012) for a similar data set, they performed only BE with "all parameters…left at default settings." Thus, it is not clear whether the analysis partitioned the ITS and *rpl32-trnL* data. In any case, the trees included only PPs, whereas Peterson et al.´s (2012) analyses revealed some "discrepancies" (as described above) between PPs and frequentist ML BPs. From the discussion above, it should be clear that PPs based on default-setting BE are inadequate.

More to point, Peterson et al. (2014) and Moreira-Muñoz et al. (2020) represent the common superstitious approach to molecular phylogenetics. It probably is true that, owing to their first order simplicity, DNA sequences can be analyzed more automatically than other forms of data, and the output, a gene tree, generally is highly informative. But computer programmers and theorists themselves advertise the theoretical assumptions and limitations of programs and advise due caution in their use. In practice, this often is ignored. The chemistry and biology of DNA sequences a propos phylogenetic analysis are not appreciated. Typically the raw data are blindly force-fit into a prefabricated evolutionary model, and then discarded completely, not referred to again, replaced with trees, BPs, and PPs. As though the computer "knows" what to do with the data. "Reason" – the singular distinction of scientific inquiry – is abandoned in favor of faith in calculations that the researchers did not perform and often do not even understand. To paraphrase Dragicevic (2016: 2), computers in science are intended to enhance human cognition and not, as is too often the case, to replace it.

The accuracy of phylogenetic trees per se is not especially problematic if the only objective is to estimate phylogeny. The problem emerges only when inadequate phylogenetic estimates then are used in downstream statistical (and often also BE) analyses. For example, Moreira-Muñoz et al.'s (2020) *Mutisia* data *did not* yield a well-resolved tree, and the BPs and PPs manifested incongruencies. Nonetheless, applying the dicey criterion of "maximum clade credibility," their poorly resolved tree magically resolved itself completely for downstream purposes of biogeographic and macroevolutionary analysis, with an eye also on conservation applications. Likewise, the broader objective of Peterson et al. (2014) is in the realm of species conservation. Theory and practice of species conservation is beyond the scope of the present work. The question here is the utility and/or even relevance of phylogenetic analysis of sparse DNA sequence data towards conservation objectives. But even if we patronizingly assume high relevance of the DNA data, then their utility is contingent upon their due diligent, rigorous, and accurate interpretation. Misinformation may be relevant, but it is hardly useful.

9. DNA sequence evolution: stochastic or idiosyncratic?

The accuracy of molecular (organismal) phylogenetics rests not only upon both biological and methodological assumptions of how DNA sequences and organisms evolve, but also the relation between these. In any empirical analysis, these assumptions must be scrutinized. Likely it is not adequately appreciated that current assembly line methods assume a stochastic (indeterminate) evolutionary processes. To my knowledge, this assumption, which can be traced back to R. A. Fisher's work nearly a century ago, has no proof. But it is incorrect. Especially in Hershkovitz (2018b), I argued that DNA sequence evolution is not stochastic (see also Hershkovitz, 2018a, 2019a, b, and below).

Just reviewing literature on hand for the present paper, it seems that this assumption is at best tacit. Generally, it is not even mentioned. As noted, Desper and Gascuel (2005: 10) wrote in passing,

"…the distance-based approach involves estimating the evolutionary distance from the differences we observe today between taxa, *assuming a stochastic model of evolution* [italics mine]." Otherwise, their lengthy work did not use the word "stochastic." Other papers apply methods that assume a stochastic evolutionary model (e.g., Hershkovitz et al., 2006a; Moreira-Muñoz et al., 2020), but do not refer to this assumption. Abadi et al.'s (2019) study of ML, hence stochastic, substitution models does not use the word "stochastic." They emphasize that available models inadequately describe sequence evolution, but do not consider whether more "adequate" models might be nonstochastic (determinate).

Notably, Kryazhimskiy et al. (2014) considered that evolution might be idiosyncratic at the phenotypic level (via epistasis), while remaining stochastic at the DNA sequence level (i.e., mutations). In their analysis of the effect of epistasis on adaptation and fitness, they found that most of the (statistical!) variance owed to the fitness of the Founder individual and much less to the "inherent stochasticity" of evolution (at the DNA sequence level). But they did not provide proof that the latter is indeed stochastic. I will not here provide an exhaustive consideration of evidence for stochasticity in evolution, DNA or otherwise. But it should be emphasized that fitting data points to a stochastic distribution does not qualify for proof of stochasticity.

10. Idiosyncrasy in the **Chaetanthera/Oriastrum** *data*

Some aspects of the *Chaetanthera*/*Oriastrum* data normally not noted in conventional molecular systematics papers caught my attention, because they represent departures from tacit and sometimes explicit analytical assumptions. One was the polymorphism aspect, discussed above. Another aspect is the relatively large variation in ITS substitution parameter estimates across different models, nonstationarity in base frequencies and overall evolutionary rate, and the peculiar estimate of equal base frequencies given that the empirical frequencies at all and just variable sites are decidedly G/C-rich.

For the *rpl32-trnL* sequences, the opposite occurred: estimated base frequencies were equal to empirical frequencies over all sites. But, peculiarly, the frequencies at variable sites were very different from this estimate and also somewhat nonstationary. And, as noted above, despite evolving evidently less than half as fast as ITS, the *rpl32-trnL* sequences, nevertheless manifest greater "infraspecific" polymorphism and number of haplotypes.

These observations are not predicted by the linear substitution models used to reconstruct the phylogeny. Thus, evolution of both sequences seems to be evolving according to rules besides and/or different from those assumed. This does not mean necessarily that the reconstructed phylogeny is "wrong," although some parts may be less reliable than they appear. But it does mean that some aspects of sequence evolution remain unexplained and perhaps undiscovered. It is incumbent upon science to seek such explanations.

I highlight here only one other among the various molecular evolutionary idiosyncrasies that may have figured into the evolution of the *rpl32-trnL* sequences analyzed here. This is the "Morton effect" (Morton, 1997, 2003). Based on analysis of the few then-available grass chloroplast genomes, Morton found that in A/T-rich noncoding cpDNA regions, transversions are more common than transitions. This runs contrary to the transition bias more common in the "universe" of genomes. The present *rpl32-trnL* sequences are A/T-rich (Table 3) and, indeed, Tv > Ti. In contrast, for the ITS sequences, Ti > Tv.

More importantly, Morton (1997, 2003) found that substitution biases were not site-specific and constant, but context-dependent and dynamic. In particular, substitutions were biased depending upon the bases at flanking sites, in some cases whether they were A/T, in others whether they were purines (R) or pyramidines (Y). Of course, substitutions at these sites sometimes themselves alter the context of the flanking bases, thereby biasing subsequent substitutions. Note that the biases are not "site-specific," because the position of biased sites changes with substitution. I am not aware of a model that corrects for such biases.

I have not analyzed context-specificity of substitution biases in the present *rpl32-trnL* data. Analysis of this and other data sets is a work in progress. But I recall, in this context, that the extreme A/T bias of the *rpl32-trnL* sequences is at constant sites (ca. 80% A/T), whereas the variable sites are ca. 60% A/T. Thus, the three-base sliding-window A/T "context" is especially dynamic at variable sites. Also, perhaps notably, r(AT) is the lowest estimated substitution rate, less than one-fifth that of both r(AC) and r(GT). Note that the latter two substitutions alter the three-base sliding-window A/T "context," whereas the first does not. Note also that, because Tv > Ti, most substitutions also alter the three-base sliding-window Y/R "context."

11. ML and distance-based methods cannot deal with idiosyncrasy

The preceding is merely an example of the problem inherent in statistical approaches to…ANYTHING. Statistical power is achieved only when data are pooled, i.e., when different observations are presumed to be phenomenologically identical and independent outcomes of trials sharing exactly the same underlying cause. Matthen and Ariew (2009) and Walsh (2010) provide two of many available criticisms of this approach and demonstrate that, quite commonly, different outcomes having different causes converge on common means. Yet convergence on a common mean is considered to be evidence for uniformity of the process. Thus, standard statistical molecular phylogenetic methods would lump "Morton effect" substitutions within a different substitution rate class, such that the estimated mean substitution rate in that class owes to nonuniform processes.

Applied molecular phylogenetic analyses (like this one) generally exploit only popular/accessible methods that only measure DNA substitutions at aligned positions (and sometimes also indels) across a sequence alignment. While popular/accessible methods can accommodate evolutionary heterogeneity among sites, they generally cannot accommodate heterogeneity among lineages, or nonstationarity, and other idiosyncratic phenomena. Such phenomena have not been overlooked in theoretical circles, but methodological implementation and computational practicality are another matter.

For example, while standard ML and ML-based distance methods restrict to stationary evolutionary rates, the problem of rate nonstationarity, and their solution, covarion models, have been acknowledged for decades (Desper and Gascuel, 2004). In BE methods, only the simplest form of this model, site-specific rate oscillation, is implemented. But in practice, most applied phylogenetic analyses do not even explore/acknowledge evidence for nonstationarity in the data. Also generally ignored are other molecular evolutionary phenomena for which there are not protocols both widely accepted and methodologically implemented. For example, in the case rDNA, including ITS, substitutions are thought to be constrained by secondary structure and recombination among loci. Adjustments for coevolving sites can be introduced manually in data analysis, but conventional phylogenetic methods themselves do not automatically detect them. Likewise, no methods detect extraterrestrial assimilation.

Indeed, it seems obvious that substitutions in a DNA sequence alignment typically are not "independent and identically distributed." The solution to this problem for phylogenetics has been parameterization, viz., divide the observations into smaller, distinct classes that collectively improve the statistical fit between the data and the tree. This, of course, trades off with statistical power, because each parameter class includes fewer observations, hence increases variance. Parameterization and model optimization were foci of theoretical phylogenetics research in the two decades flanking the millennium. The legacy is, e.g., PAUP now with 88 distinct DNA site-static sequence evolution models.

But 88-plus models evidently are not nearly enough to capture all aspects of DNA sequence evolution, not even in the realm of stationary models (i.e., where parameters are assumed to be stationary throughout phylogeny; cf. Abadi et al., 2019). Paradoxically, parameterization of stationary models asymptotically leads to the "no common mechanism" model, where there are as many "rate classes" as there are sites. This is otherwise known as "maximum (unweighted) parsimony" (cf. Huelsenbeck et al., 2008). MP is known to be statistically inconsistent (Type 1 error increasing with increasing data) in the case of DNA sequence evolution, especially because MP equates divergence with the smallest number of observed base differences along tree branches. It does not correct for multiple superimposed substitutions along long branches, nor concern itself with the inevitability of random similarity between diverging sequences. [In the context of (inductive) statistics, (abductive) parsimony is perhaps better termed "irrational" rather than "inconsistent," because statistical inconsistency has no meaning under MP, and the inconsistency of MP in statistical analysis perhaps owes to the difference between induction and abduction rather than "good" versus "bad" statistical modeling.]

This discussion would not be complete without reference to DNA distance methods (Swofford et al., 1996; Nei and Kumar, 2000; Desper and Gascuel, 2005), which were applied albeit cursorily to the *Chaetanthera/Oriastrum* data. The methods originated and were popularized more in the statistical paradigm of "molecular evolution" rather than character-oriented "molecular systematics." Distance methods, especially NJ and "Minimum Evolution" (ME), were hawked especially because of their supposed accuracy, statistical consistency, and computational speed relative to MP and ML. Popularity of the methods (in some circles) probably owes mainly to computational speed, suggesting that "ME" refers better to "Minimum Effort."

Carefully read and understood, Desper and Gascuel (2005) burst the ME bubble. They point out that *analytical* solutions to ME are indeed computationally intensive and impractical. They explain how the apparent speed in ME programs owes to shortcuts, assumption-based approximations, noting that "it is well known in statistics that approximate values are sufficient to obtain reliable estimators." This reliability, in turn, presumes that molecular evolution is stochastic. This presumption, which the authors passively acknowledge but do not otherwise question, is the fatal flaw of all statistical evolutionary reconstruction (see below).

Violation of the stochasticity assumption is more critical in the context of ME than ML. This is because "approximations" are applied at not one, but two different procedural levels. The pairwise distances are calculated using the same estimators applied in ML analysis, hence subject to the same errors and consequent inconsistency. Likewise, implemented models cannot accommodate lineage-specific or site-dynamic evolutionary phenomena.

But then, unlike ML, another level of approximation is introduced when the distances are used to estimate the tree topology and fit the branch lengths. Several methods exist, but all base fundamentally on least squares estimates of branch length error. Here, Desper and Gascuel (2005) render clear that the much-touted statistical consistency of ME, as in the case of ML, owes to data cherry-picked to conform with the assumptions of the particular method.

Desper and Gascuel (2004, 2005) indeed highlighted a major theoretical advance that apparently renders ME trees more reliable without significantly compromising on speed. This is the BME method, which applies Weighted Least Squares (WLS) in branch length error calculations. The qualification as "balanced" itself is not intended to denote superior performance. The term substitutes the "weighted" of WLS with "balanced," in order not confuse BME with the established nomenclature of WLS, which refers to a different distance method. Conventional ME (Nei and Kumar, 2000) is now called ME/OLS, because it applies an "Ordinary Least Squares" branch length correction. Then there are "Generalized Least

Squares," whose performance theoretically is supposed to be superior to WLS, but evidently is not (Desper and Gascuel, 2005).

Indeed, BME seems to perform better than ME/OLS with the *Chaetanthera/Oriastrum* data. Despite its aggressive marketing, in 25 years of experimentation, I had never found ME/OLS to perform satisfactorily. MP scores of ML/OLS trees always were substantially less "P" than MP trees. Likewise, their ML scores were less "L," often $e^{\text{double-digits}}$ times worse than ML-optimized trees. This was true also for ME/OLS constructed using the *Chaetanthera/Oriastrum* data. But the MP and ML scores of the BME trees were much improved. Unfortunately, the literature is replete with trees constructed with ME/OLS. Perhaps "OLS" would be better defined as "Old and Lousy Squares."

12. Evolutionary idiosyncraticity: the rule, not the exception

The classical narrative approach in systematics and evolutionary biology – natural history – tended to highlight idiosyncrasy. Indeed, it aspired to discover the unique. In contrast, the prevalent cookie-cutter statistical approach, especially with emphasis on BE, occludes the uniqueness in nature. This is a natural consequence of ever larger data mass, which is why in the first place it must be analyzed by ever more streamlined and reductionist and less stringent computation. Idiosyncrasy is relegated to the residual and, in any case, not proactively sought (but see Kryazhimskiy et al., 2014; also Gerhold et al., 2015).

Consequently, current practice tends to reinforce rather than challenge the dogmas inherent in computational protocols. Yet scientific discovery tends to emerge not from data that fit the dogma, but data that do not. Data that do not conform to expectations suggest flaws in expectations. This philosophical point was highlighted in Hershkovitz (2019b) and references cited therein and elaborated especially in Hershkovitz (2018a). This is not to say that current popular practices are incapable of "discovery." Indeed, countless heavenly bodies were discovered in the background of now obsolete notions of the universe and astrophysics.

But the critical shortcoming of comparative methods, including tree construction methods, is that all base on the unproven assumption that organic evolution (whether DNA or organisms) is a stochastic (indeterminate) process. This not only justifies, it practically obligates discarding of idiosyncratic observations. As noted above, the notion traces to R. A. Fisher's innovations in the field of population genetics nearly a century ago, and, Sewell Wright's criticisms notwithstanding, its subsequent extension to the discipline of microevolutionary genetics. About 50–60 years ago, these notions later extended to the discipline of molecular genetics and evolution, and then to phylogenetics generally.

The modern dominance of statistical approaches to macroevolution owes especially to three classic works by Joe Felsenstein: development of the ML approach to tree construction (Felsenstein, 1981), the bootstrap (Felsenstein, 1985a), and the phylogenetic "comparative method" in macroevolutionary analysis (Felsenstein, 1985b). But more than anyone else, modern evolutionary methods were popularized especially by…Steve Jobs. His efforts established the low bar of broad accessibility of scientific computation to the masses of otherwise untrained and inexperienced researchers. The consequent trend towards computational literacy, in turn, increased the sophistication of the methodological "pipeline." Current research thus can be appreciated as line-assembled mass production. Likely, the epistemological consequences of the stochasticity assumption are not appreciated. As noted above, this is partially because the assumption itself is "hidden" in the methodology, certainly not highlighted, and in practice often not mentioned at all

There is, however, considerable theoretical and empirical evidence that life processes themselves, at all of hierarchical levels, are *determinate*, hence not stochastic (Maturana and Mpodozis, 2000; cf.

Hershkovitz, 2018a, 2019a, b). This determinacy should extend to the evolutionary process. Elsewhere (Hershkovitz, 2018a, 2019a, b), I have reviewed evidence of the similarity of life processes to chaotic processes, which are determinate. I thus described evolution (hence evolutionary history) as an *idiosyncratic* process, in which the evolving intrinsically determinate chaos-like character of life processes is mitigated by presumably indeterminate (stochastic) extrinsic processes. This I stated as ***f***(chaos)***f***(stochasticity), which is the "God function."

The "principle of evolutionary idiosyncraticity" (PEI) has several implications. One is that, because life is determinate, statistical means of organisms and their traits have no biological meaning. Likewise, no organism or trait thereof can be considered as statistical "noise" or an "outlier." It must be stressed here that PEI does not qualify *only* "outliers" as idiosyncratic. *All* evolutionary change is idiosyncratic (and *never* stochastic), as are *all* organisms and traits. This is because all evolutionary changes are historically unique events.

This uniqueness is true even in the case of DNA substitutions, because, even when superficially identical, the historical context (or milieu) of each is spatiotemporally and materially unique. The frequencies of evolutionary changes and means of trait values are ad hoc epiphenomena. This should be intuitively obvious. *All* organisms and traits were "unusual" at their origin and all have or had the potential to become "usual." Indeed, evolution is a process in which the absent becomes present and eventually again absent; the "unusual" *sometimes* becoming "usual" and then again "unusual." Nonstationarity described above for *Chaetanthera*/*Oriastrum* DNA sequence evolution thus is predicted to be the rule, not the exception.

13. Reconciling phylogenetic reconstruction with idiosyncraticity

Perhaps a more inconvenient implication of PEI is that, to the degree life processes indeed are chaotic, evolutionary history cannot be reliably recovered. This is because chaotic processes, while determinate, are mathematically unrecoverable and unpredictable, unless *both* the mathematical chaotic function and the starting conditions are known exactly. The starting conditions cannot be "approximated," e.g., ancestral states reconstructed statistically. This, in turn, implies that phylogeny, more generally "evolutionary history," *cannot* be reconstructed *statistically/mathematically*. Obviously, this conclusion runs counter to current mainstream superstition. Yet, technically, it is true.

…So, wait a minute. If I am asserting that evolutionary history cannot be reconstructed, then why am I even writing this paper? Just as important, why are you reading it? (Or are you just looking at the pictures?)…

Because I qualify the assertion: there is no *statistical/mathematical* solution to phylogenetic reconstruction. The assertion does not mean that there is no solution at all. The assertion does not deny either that statistical/mathematical tools cannot be used heuristically to reconstruct evolutionary history, as long as the nature of the statistical/mathematical procedures and the difference between evolutionary history and its mathematical estimation are understood explicitly.

Reconstruction of phylogeny and evolutionary history roots philosophically *only* in the simplest assumption that these *have* occurred, and not *how* they have occurred. Axiomatically, evolutionary history therefore is materially tangible and can be *materially* recovered/discovered. Historical hypotheses remain within the realm of scientific inquiry. As long as the inquiry itself is scientific, viz., with explicit reference to the notions of corroboration and falsification. Unfortunately, it seems that the culture of phylogenetic investigation has taken the opposite trajectory, tending to believe superstitiously that trees output by computer programs are "true" rather than consequent to methods and assumptions (cf. Swofford et al., 1996), in turn necessarily rooted in theory and epistemology.

The assumption of phylogeny axiomatically assumes also the tangibility of clades, agglomerations of organisms that descended from a common ancestor. This is the basis for phylogenetic taxonomy, which posits that clades comprise the only biologically/historically natural taxa. However, as emphasized by Hershkovitz (2019a, b), in nature, clades are not nearly as discrete as cladists have idealized. This is a theoretical prediction now amply demonstrated empirically with phylogenomic data. Thus, in its idealized extreme, cladistic taxonomy is no more biologically/historically natural than the ad hoc taxonomy it replaced.

The underlying evidence for phylogeny owes to the pattern of presumed evolutionary conservation in the wake of evolutionary (idiosyncratic) change. Indeed, if the conservation principle were not presumed, phylogenetic reconstruction would be futile. In this light, it can be appreciated that the fashionable notion of "phylogenetic conservatism" is tautological to phylogeny itself (Losos, 2008). The notion of phylogeny itself would collapse if conservation (similarity) were excessive or, alternatively, erased by change (differences). Paradoxically, therefore, similarity and difference are evidence both for and against evolutionary history. Too much of either is precisely what obfuscates phylogenetic reconstruction. The obvious example of too little change is different taxa with identical DNA sequences. And of too much change is the superimposition of substitutions during molecular sequence divergence, such that DNA sequences are predicted to become randomized relative to one another.

Phylogenetic reconstruction is a process of comparative analysis of many traits in which conservation is maximized and change minimized per the principle of parsimony. It is always subjective and never objective. Unfortunately, current mathematical/statistical approaches render the impression that change is an objective function. This is illusory. The approaches simply replace the subjectivity of the phylogenetic researcher with that of the programmer. The researcher might be completely unaware of the nature of the comparative analysis that is being undertaken. The researcher might simply report that the tree is well-resolved, even when it is not. And evidence/proof that the method is statistically consistent or otherwise "reliable" is a red herring.

As noted, all evolutionary methods base on unrealistic assumptions and are therefore bound to inconsistency. But this is not at all to say that current methods generally do not, much less *cannot*, yield accurate results and/or have no heuristic value. The key is to recognize when the results are meaningful and, more importantly, why. This is a tall order. As Abadi et al. (2019: 8) wrote, "…when topological uncertainties exist, reconstruction with the true model can result in an inaccurate topology while the reconstruction with a wrong model results in the accurate one." This implies that subjective evaluation is not merely desirable, but essential. Without it, computational results have no meaning at all.

The present work analyzed relationships among taxa of *Chaetanthera*/*Oriastrum*. The evidence for the relations does not manifest in the reconstructed trees per se or any associated quantities. It manifests in the *nature* of the comparisons, each line of evidence being a mutual attempt to falsify the other. Including the use of two loci rather than one, methods that base on different assumptions, and the bootstraps, which not only falsify inherently, they do so even more when using different data sets and methods. Given idiosyncraticity, none of the individual approaches or data sources can be considered "correct." It is the juxtaposition of different data analyzed by different methods that yields discovery. As conjectured by Abadi et al. (2019), each data source and method will err, but not in the same direction.

The analysis presumed, but did not demonstrate definitively, that *Chaetanthera*/*Oriastrum* is monophyletic. It is or it is not, independent of phylogenetic analysis. The presumption of monophyly is subjective. All of the included taxa share a distinctive inflorescence morphology, in which the stem leaves intergrade with the capitulum bracts. But this alone cannot demonstrate monophyly of *Chaetanthera*/*Oriastrum*. This rests on comparative analysis of many traits (discussed in Davies, 2010), which, at least intuitively, suggest that conservation is maximized and change minimized if this trait is allowed to originate only once (maybe twice, in the case of extraterrestrial assimilation). Note that the trait itself has no inherent or intrinsic probability of change. Any mathematical evaluation of its "retention index" or likelihood is contingent upon joint comparative analysis of many traits.

The ITS data suggest that *Chaetanthera*/*Oriastrum* is monophyletic, especially because the computationally inferred ancestral sequences of *Chaetanthera* and *Oriastrum* are far more similar to each other than they are to sequences of sampled outgroups. But not all possible outgroups were examined. The *rpl32-trnL* sequences are inconclusive. Estimated divergence between *Chaetanthera* and *Oriastrum* is about the same as that between these taxa and the sampled outgroups. But this was measured only for aligned substitutions and not for indel traits. It is possible that intergeneric divergence in alignable substitutions is effectively maxed out for this sequence. My subjective expectation is that analysis of additional DNA loci will support monophyly of *Chaetanthera*/*Oriastrum*. But the caveat is that phylogenomic analyses of other taxa already demonstrate empirically the theoretically expected decoupling between gene and organismal phylogeny. Quite likely, some DNA loci will indeed suggest polyphyly of *Chaetanthera*/*Oriastrum*.

Within *Chaetanthera*/*Oriastrum*, I recognized five subgeneric taxa arbitrarily at the formal subgeneric rank, with one sample (*sp_indet_25161*) not classified. The five subgenera are one more than recognized by Davies (2010) based on morphological evidence and with reference to Hershkovitz et al. (2006a). The additional subgenus results from splitting of *C.* subg. *Chaetanthera*. The evidence for these taxa bases on corroboration, including high bootstrap support for each in the combined analysis of ITS and *rpl32-trnL* using MP both with and without indel data and ML (Fig. 7). Careful analysis of Davies (2010) reveals morphological corroboration, as well. The relationship between the three *Chaetanthera* s. str. remains unresolved, which is precisely why all three should be recognized as distinct at the same rank.

As noted, the combined data ML analyses of the two loci yielded BPs ≥ 70% at 72% of the 60 possible nodes. This is considerably better than the 44% obtained in the bootstrap analysis of Moreira-Muñoz et al. (2020) for 41 possible *Mutisia* nodes using *six* loci (see above). Although not directly comparable (because of greater sampling within nominal species), BP resolution also is dramatically better than that obtained collectively using MP and two loci (ITS and cpDNA *ycf3-trnS*, which behaves similar to *rpl32-trnL*) for several genera of primarily Chilean Montiaceae (Hershkovitz, 2006).

But the estimated reliability of the combined bootstrap tree did not rest on the face value of the BPs. I noted where the combined data increased or decreased the BP, irrespective of the value of the BP. I attempted to explain differences in the BPs derived using different methods and data sets. And I applied, however cursorily, the NeighborNet approach to help convey the degree of incongruency in the data. The purpose of these protocols was not to determine, but to challenge the conclusions and, moreover, the theories on which they are based.

I mostly did not examined in detail corroborative morphological evidence for the nodes in the trees. I highlighted both corroborative and incongruent morphological evidence only in certain cases, e.g., the relations of *C. taltalensis* and other specific relations suggested by Davies (2010). Here, I also stressed the ontological distinction between gene trees and species tress as a possible explanation for disparate conclusions. Davies (2010: 56) remarked that the molecular phylogeny of Hershkovitz et al. (2006a) rendered "morphological cladistic analysis …largely redundant." This is not the case at all. Because of the ontological distinction between gene trees and species trees, phenotypic analysis never is obviated, but especially in the case where DNA divergence is low. Thus, high bootstrap support for DNA sequence data from two loci does not necessarily correspond perfectly to the phylogenetic history of the organisms.

In summary, the conclusions presented here do not defer to any quantitative result, not any probability, not any index of reliability. Nor, for that matter, do they even defer to the sequences analyzed. Idiosyncraticity predicates that there does not and cannot exist any such yardsticks of evolutionary truth. Conclusions of contemporary works that do defer to such measures may be correct or incorrect. But they are not vetted scientifically. The conclusions presented here base entirely on reason and logic. It does not matter so much their accuracy as their purpose. They raise the bar. They are merely new placeholders, new targets to challenge with ever more rigorous and detailed investigation and analysis.

I defer to a subsequent work evaluation of the relation between DNA divergence and phenotypic, geographic, and ecological evolution in *Chaetanthera*/*Oriastrum*. Such analysis was undertaken by Cabezas Álvarez (2015) for several species of *Chaetanthera* s. str. based on ITS data. But elsewhere, I have criticized, indeed rejected, the current prevalent protocol-driven statistical approach to these phenomena. As in the case of phylogenetic reconstruction, the methods have heuristic value, provided the distinction is made between methodological results and history.

However, even to the degree that statistical approaches yield useful statistical descriptions, I generally reject attempts to interpret statistical correlation with historical cause. I have given examples in Hershkovitz (2018a), but I offer another example more relevant in the present context.

In Kryazhimskiy et al.'s (2014) theoretical/computational study of the effect of the "idiosyncratic" effect of epistasis on adaptive evolution, the authors concluded that, after 500 generations, most of the observed variance owed not to the "inherent stochasticity of evolution," but to the fitness of the Founder. They concluded that fitness of the Founder thus rendered predictable adaptive evolution. They did not seem to appreciate that the Founder itself is an idiosyncrasy, such that a different Founder would have *enacted* (Varela et al., 1992; cf. Virgo, 2019) a different history, and that each of its descendents likewise would have enacted a different history, even under the same historical circumstances.

To make the preceding clear, consider the genus *Pachylaena*, which is believed to be the sister taxon of *Mutisia* (Moreira-Muñoz et al., 2020). Certainly, therefore, it must be considered among the closest relatives of *Chaetanthera*/*Oriastrum*. Thus, the common ancestor of each of these taxa must share a relatively contemporary common ancestor – a Founder – probably in the central or southern Andes (Moreira-Muñoz et al., 2020). Accordingly, the descendents of the Founder, including the Founders of the now segregated genera, share a similar ecological history, even up to the present day (given their degree of sympatry).

But *Pachylaena* is a not especially common monotypic genus restricted to the central-southern Andes. Unlike *Mutisia* and *Chaetanthera*/*Oriastrum*, it does not appear to have undergone an "adaptive radiation." Thus, whatever was the "fitness" of the Founder, it did not seem to render "predictable" subsequent adaptive evolution of *Pachylaena*. Nor, for that matter, did the ecological history. From this, we can deduce, logically, that neither did ancestry nor ecology cause diversification of *Mutisia* or *Chaetanthera*/*Oriastrum*. All evolutionary history owes to idiosyncraticity.

ACKNOWLEDGEMENTS

Elaboration of this and all of my works posted since 2018 would not have been possible except for the generous support and dedicated efforts of Sandra Steuermann and Gabriela Feldman of Fundación Reshet in Chile (www.reshet.cl). Thanks to their help in renting a ROOM, I was able to do the present work sitting on a CHAIR (I found in the trash) at a TABLE. All of my 2018–2019 works were done with my notebook between my crossed legs, sitting on a wet, dirty floor, where I also slept. Martin Gardner (E) arranged two years ago a gift of a used HP 530 notebook, which I used for all analyses. Slow, but it did the job. A propos, Daniel Huson kindly reposted a Windows 32-bit version of Splitstree 4 per my request.

Once again, I acknowledge the support of several central Santiago businesses for regularly throwing out their garbage containing a wide variety of damaged or expired but still edible food: McDonald's (Avda. República), OK Market (Avda. República and Avda. Toesca), La Sureñita produce market (Avda. Club Hípico), El Mercadito produce market(Avda. Almirante Latorre), and an unnamed produce market (Avda. Sazie). Also some undocumented immigrant Haitian illegal banana vendors near the Vega Central. Laboratory and fieldwork was funded partially by Chilean government FONDECYT Grant 1000909 to MAH (2000–2002), partially with ca. 10% of FONDECYT Grant 1020956 to Mary Kalin Arroyo and MAH (2002–2005), an A. W. Mellon Foundation Grant, and nontrivially out-of-pocket. Finally, all but the more sterile aspects of this work would not have been possible without the exceptional and exceptionally detailed and scholarly monograph by Alison Davies. The present work takes issue with some of Davies' conclusions, but, as General George Patton once said, if everybody is thinking alike, then somebody isn't thinking. Davies' monograph gives plant evolutionary biology a lot to think about,

LITERATURE CITED

Abadi, S., D. Azouri,T. Pupko and I. Mayrose. 2019. Model selection may not be a mandatory step for phylogeny reconstruction. Nature Communications 10: 934. https://doi.org/10.1038/s41467-019-08822-w

Alfaro, M.E. and M.T. Holder. 2006. The posterior and the prior in Bayesian phylogenetics. Ann. Rev. Ecol. Evol. Syst. 37: 19–42. https://doi.org/10.1146/annurev.ecolsys.37.091305.110021

Alfaro, M.E., S. Zoller and F. Lutzoni. 2003. Bayes or bootstrap? a simulation study comparing the performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol. Biol. Evol. 20: 255–266. https://doi.org/10.1093/molbev/msg028

Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410. http://dx.doi.org/10.1016/S0022-2836(05)80360-2

Autzen, B. 2018. Bayesian convergence and the fair-balance paradox. Erkenntnis 83: 23–263. https://doi.org/10.1007/s10670-017-9888-0

Bååth, R. 2015. The non-parametric bootstrap as a Bayesian model. http://www.sumsar.net/blog/2015/04/the-non-parametric-bootstrap-as-a-bayesian-model/

Barker, F.K. and F.M. Lutzoni. 2002. The utility of the incongruence length difference test. Syst. Biol. 51: 625–637. https://doi.org/10.1080/10635150290102302

Barres, L., H. Batalha-Filho, A.S. Schnadelbach and N. Roque. 2019. Pleistocene climatic changes drove dispersal and isolation of *Richterago discoidea* (Asteraceae), an endemic plant of campos rupestres in the central and eastern Brazilian sky islands. Bot. J. Linn. Soc. 189: 132–152. https://doi.org/10.1093/botlinnean/boy080

Bentham, G. and J.D. Hooker. 1873. Genera Plantarum, vol. 2, pt. 1. A. Black, London. https://www.biodiversitylibrary.org/item/14685

Brooks, D.R. and D.A. McClennan. 1999. Species: turning a conundrum into a research program. J. Nematol. 31: 117–133. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2620355/pdf/117.pdf

Bryant, D. and V. Moulton. 2004. NeighborNet: an agglomerative algorithm for the construction of planar phylogenetic networks. Mol. Biol. Evol. 21: 255–265. https://doi.org/10.1093/molbev/msh018

Cabezas Álvarez, J.L. 2015. Relación entre la forma de vida, sistema de reproducción y rasgos florales en el género *Chaetanthera* (Asteraceae): un acercamiento filogenético. Masters Thesis, University of Chile. http://repositorio.conicyt.cl/bitstream/handle/10533/182424/CABEZAS_JOSEFINA_2812M.pdf?sequence=1

Cabrera, A.L. 1937. Revisión del genero *Chaetanthera* (Compositae). Revista Mus. La Plata, Secc. Bot. 1(3): 8–210., Lam. 1–4. https://publicaciones.fcnym.unlp.edu.ar/rmlp/article/view/1514/683

Davies, A.M.R. 2010. A systematic revision of *Chaetanthera* Ruiz & Pav., and the reinstatement of *Oriastrum* Poepp. & Endl. (Asteraceae: Mutisieae). PhD Dissertation, University of Munich. https://edoc.ub.uni-muenchen.de/11538/1/Davies_Alison.pdf

Davies, A.M.R. 2013. Hairy anthers and mountain stars: the speciation of Andean Asteraceae in response to geological and climate change. Arch. Sci. 66: 69–84. https://www.e-periodica.ch/cntmng?pid=ads-004%3A2013%3A66%3A%3A232

De Queiroz, K. 2007. Species concepts and species delimitation. Syst. Biol. 56: 879–886. https://doi.org/10.1080/10635150701701083

Desper, R. and O. Gascuel. 2004. Theoretical foundation of the balanced minimum evolution method of phylogenetic inference and its relationship to weighted least-squares tree fitting. Mol. Biol. Evol. 21: 587–598. https://doi.org/10.1093/molbev/msh049

Desper, R. and O. Gascuel. 2005. The Minimum Evolution distance-based approach to phylogenetic inference. Pp. 1–32 in O. Gascuel, Mathematics of Evolution and Phylogeny. Oxford Univ. Press, Oxford, UK. https://books.google.cl/books?id=Ao_MNOaFRHEC&pg=PA1&dq=THE+MINIMUM+EVOLUTION+DISTANCE-BASED+APPROACH+TO+PHYLOGENETIC+INFERENCE+Richard+Desper+and+Olivier+Gascuel&lr=&source=gbs_toc_r&cad=4#v=onepage&q=THE%20MINIMUM%20EVOLUTION%20DISTANCE-BASED%20APPROACH%20TO%20PHYLOGENETIC%20INFERENCE%20Richard%20Desper%20and%20Olivier%20Gascuel&f=false

Dobson, J. 2018. The world's 8 best places to hunt for extraterrestrials and search for UFOs. Forbes Magazine, 28 January 2018. https://www.forbes.com/sites/jimdobson/2018/01/28/the-worlds-8-best-places-to-hunt-for-extraterrestrials-and-search-for-ufos/?sh=133497232ddb

Dragicevic P. 2016. Fair statistical communication in HCI. Pp. 291–330 in J. Robertson and M. Kaptein (editors), Modern Statistical Methods for HCI. Human–Computer Interaction Series. Springer, Cham. https://doi.org/10.1007/978-3-319-26633-6_13

Efron, B. 2012. Bayesian inference and the parametric bootstrap. Ann. Appl. Stat. 6: 1971–1997. https://doi.org/10.1214/12-AOAS571

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17: 368–376. https://doi.org/10.1007/BF01734359

Felsenstein, J. 1985a. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Felsenstein J. 1985b. Phylogenies and the comparative method. Amer. Nat. 125: 1–15. https://doi.org/10.1086/284325

Finney, J. 1955. The Body Snatchers. Dell Publishing, New York.

Flora Cono Sur. Without year. Catálogo de las Plantas Vasculares, Instituto de Botánica Darwinion. http://www.darwin.edu.ar/proyectos/floraargentina/fa.htm (accessed December 2020)

Fuentes, N., E. Ugarte, I. Kühn and S. Klotz. 2008. Alien plants in Chile: inferring invasion periods from herbarium records. Biological Invasions 10: 649–657. https://doi.org/10.1007/s10530-007-9159-0

García-Sandoval, R. 2014. Why some clades have low bootstrap frequencies and high Bayesian posterior probabilities. J. Israel Ecol. Evol. 60: 41–44. https://doi.org/10.1080/15659801.2014.937900

Gerhold, P., J.F. Cahill Jr., M. Winter, I.V. Bartish and A. Prinzing. 2015. Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). Functional Ecol. 29: 600–614. https://doi.org/10.1111/1365-2435.12425

Grünwald, P. and T. van Ommen. 2017. Inconsistency of Bayesian inference for misspecified linear models, and a proposal for repairing it. Bayesian Analysis 12: 1069–1103. https://doi.org/10.1214/17-BA1085

Guerrero, P.C., M. Rosas, M.T.K. Arroyo and J.J. Wiens. 2013. Evolutionary lag times and recent origin of the biota of an ancient desert (Atacama–Sechura). Proc. Nat. Acad. Sci. (USA) 110: 11469–11474. https://doi.org/10.1073/pnas.1308721110

Hershkovitz, M.A. 2006. Evolution of western American Portulacaceae in the Andean region. Gayana 63: 13–74. http://dx.doi.org/10.4067/S0717-66432006000100002

Hershkovitz, M. [A.]. 2018a. Perspectives from Montiaceae (Portulacineae) evolution. II. Ecological evolution, phylogenetic comparative analysis, and the principle of evolutionary idiosyncraticity. Preprints 2018, 2018090566. https://doi.org/10.20944/preprints201809.0566.v2

Hershkovitz, M. [A.] 2018b. Perspectives from Montiaceae (Portulacineae) evolution. I. Phylogeny and phylogeography. Preprints 2018, 2018090096. https://doi.org/10.20944/preprints201809.0096.v2

Hershkovitz, M. A. 2019a. Systematics, evolution, and phylogeography of Montiaceae (Portulacineae). Phytoneuron 2019-27: 1–77. http://www.phytoneuron.net/2019Phytoneuron/27PhytoN-Montiaceae.pdf

Hershkovitz, M. [A.]. 2019b. The 'Holy Grail' in Phylogenetic Reconstruction: Seeing the Forest for the Trees? EcoEvoRxiv. June 2019. https://doi.org/10.32942/osf.io/b9mtn.

Hershkovitz, M. [A.]. 2020. *Calandrinia jompomae* (MONTIACEAE), another overlooked species in the Chilean flora. EcoEvoRxiv. https://doi.org/10.32942/osf.io/gv5wr

Hershkovitz, M.A. and E.A. Zimmer. 1996. Conservation patterns in angiosperm rDNA-ITS2 sequences. Nucleic Acids Res. 24: 2857–2867. https://doi.org/10.1007/s10670-017-9888-0

Hershkovitz, M.A., E.A. Zimmer and W.J. Hahn. 1999. Ribosomal DNA and angiosperm evolution. Pp. 268–326 in P.M. Hollingsworth, R.M. Bateman and R.J. Gornall (editors), Molecular Systematics and Plant Evolution. Taylor & Francis, London. [No digital version available. I do not even have a print copy, since my copy of the book was among the first items stolen from me in Chile back in 1998.]

Hershkovitz, M.A., M.T.K. Arroyo, C. Bell and L.F. Hinojosa. 2006a. Phylogeny of *Chaetanthera* (Asteraceae: Mutisieae) reveals both ancient and recent origins of the high elevation lineages. Mol. Phylogen. Evol. 41: 594–605. https://doi.org/10.1016/j.ympev.2006.05.003

Hershkovitz, M.A., C. Hernandez Pellicer and M.T.K. Arroyo. 2006b. Ribosomal DNA evidence for diversification of *Tropaeolum* sect. *Chilensia*. Plant Systematics and Evolution 260: 1–24. https://doi.org/10.1007/s00606-006-0428-7

Hey, J. 2001. The mind of the species problem. Trends Ecol. Evol. 16: 326–329. https://doi.org/10.1016/S0169-5347(01)02145-0

Hey, J. 2006. On the failure of modern species concepts. Trends Ecol. Evol. 21: 447–450. https://doi.org/10.1016/j.tree.2006.05.011

Holmes, S. 2003. Bootstrapping phylogenetic trees: theory and methods. Statistical Science 18: 241–255. https://doi.org/10.3417/3182854

Humphreys, A.M. and H. P. Linder. 2009. Concept versus data in delimitation of plant genera. Taxon, 58: 1054–1074. https://doi.org/10.1002/tax.584002

Huson, D.H. and D. Bryant. 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23: 254–267. https://doi.org/10.1093/molbev/msj030

Katinas, L. and V.A. Funk. 2020. An updated classification of the basal grade of Asteraceae (= Compositae): from Cabrera's 1977 tribe Mutisieae to the present. New Zeal. J. Bot. 58: 67–93. https://doi.org/10.1080/0028825X.2020.1718168

Kryazhimskiy, S., D.P. Rice, E.R. Jerison and M.M. Desai. 2014. Global epistasis makes adaptation predictable despite sequence-level stochasticity. Science 344: 1519–1522. https://doi.org/10.1126/science.1250939

Losos, J.B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol. Lett. 11: 995–1003. https://doi.org/10.1111/j.1461-0248.2008.01229.x

Maturana, H.R. and J. Mpodozis. 2000. The origin of species by means of natural drift. Rev. Chil. Hist. Nat. 73: 261–310. https://www.researchgate.net/profile/Jorge_Mpodozis/publication/262497422_El_origen_de_la_especies_por_medio_de_la_deriva_natural/links/0c96053bd4f4696eb5000000.pdf

Moreira-Muñoz, A., R.A. Scherson, F. Leubert, M.J. Román, M.Monge, M Diazgranados, and H. Silva. 2020. Biogeography, phylogenetic relationships and morphological analyses of the South American genus *Mutisia* L.f. (Asteraceae) shows early connections of two disjunct biodiversity hotspots. Org. Diver. Evol. 20: 639–656. https://doi.org/10.1007/s13127-020-00454-z

Morton, B.R. 1997. The influence of neighboring base composition on substitutions in plant chloroplast coding sequences. Mol. Biol. Evol. 14: 189–194. https://doi.org/10.1093/oxfordjournals.molbev.a025752

Morton, B.R. 2003. The role of context-dependent mutations in generating compositional and codon usage bias in grass chloroplast DNA. J. Mol. Evol. 56: 616–629. https://doi.org/10.1007/s00239-002-2430-1

Nei, M. and S. Kumar. 2000. Molecular evolution and phylogenetics. Oxford Univ. Press, Oxford, UK. https://books.google.cl/books?id=hcPSag2pn9IC&printsec=copyright#v=onepage&q&f=false

Nicola, M.V., S.E. Freire and L.A. Espinar. 2015. Nuevas combinaciones, nuevos sinónimos y lectotipificaciones en *Chaetanthera* y *Oriastrum* (Asteraceae, Mutisieae). Darwiniana 3: 65–72. https://doi.org/10.14522/darwiniana.2015.31.658

Nespolo, R.F. 2003. Evolution by natural selection: more evidence than ever before. Rev. Chil. Hist. Nat. 76: 699–716. https://www.uv.mx/personal/tcarmona/files/2010/08/Nespolo-2003.pdf

Ogilvie, H.A., J. Heled, D. Xie and A.J. Drummond. 2016. Computational performance and statistical accuracy of *BEAST and comparisons with other methods. Syst. Biol. 65:381–396. https://doi.org/10.1093/sysbio/syv118

Ortega, F. 2020. Alienígenas Chilenos. Planeta Chilena, Santiago, Chile.

Peterson, P.M, K. Romaschenko, N. Snow and G. Johnson. 2012. A molecular phylogeny and classification of *Leptochloa* (Poaceae: Chloridoideae: Chlorideae) sensu lato and related genera. Annals Bot. 109: 1317–1329. https://doi.org/10.1093/aob/mcs077

Peterson, P.M, K. Romaschenko and R.J. Soreng. 2014. A laboratory guide for generating DNA barcodes in grasses: a case study of *Leptochloa* s. l. (Poaceae: Chloridoideae). Webbia 69: 1–12. http://dx.doi.org/10.1080/00837792.2014.927555

Philippi, F. 1881. Catalogus Plantarum Vascularium Chilensium. Imprenta Nacional, Santiago, Chile. https://books.google.cl/books/download/Catalogue_of_Publications_Relating_to_Bo.pdf?id=G_ciAQAAMAAJ&output=pdf&sig=ACfU3U0w_myTCZ0l15VIlQvGhyhpb0KgO

POWO. 2019. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org (accessed December 2020)

Pyšek, P. 1998. Is there a taxonomic pattern to plant invasions? Oikos 82: 282–294. https://doi.org/10.2307/3546968

Reiche, K. 1905. Flora de Chile, vol. 4. Cervantes, Santiago, Chile. https://www.biodiversitylibrary.org/item/10734

Rodríguez, R., C. Marticorena, D. Alarcón, C. Baeza, L. Cavieres, V.L. Finot, N. Fuentes, A. Kiessling, M. Mihoc, A. Pauchard, E. Ruiz, P. Sanchez, and A. Marticorena. 2018. Catálogo de las plantas vasculares de Chile. Gayana 75: 1–430. https://scielo.conicyt.cl/pdf/gbot/v75n1/0717-6643-gbot-75-01-1.pdf

Ronquist, F., M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard, and J.P. Huelsenbeck. 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Syst. Biol. 61:539-542. https://doi.org/10.1093/sysbio/sys029

Schlick-Steiner, B.C., F.M. Steiner, B. Selfert, E.C. Stauffer and R.H. Crozier. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. Annu. Rev. Entemol. 55: 421–438. https://doi.org/10.1146/annurev-ento-112408-085432

Shaw, J., E.B. Lickey, E.E. Schilling and R.L. Small. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Amer. J. Bot. 94: 275–288. https://doi.org/10.3732/ajb.94.3.275

Soltis, D.[E.] and P.[S.] Soltis. 2018. The Great Tree of Life. Academic Press, London, UK.

Soltis, P.S. and D.E. Soltis. 2003. Applying the bootstrap in phylogeny reconstruction. Statistical Science 18: 256–267. https://doi.org/10.3417/3182855

Stoughton, T.R., D.D. Jolles, and R.L. O'Quinn. 2018. Next-generation lineage discovery: A case study of tuberous *Claytonia* L. Amer. J. Bot.105: 536–548. https://doi.org/10.1002/ajb2.1061

Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts. https://paup.phylosolutions.com/

Swofford, D.L., G.J. Olsen, P.J. Waddell and D.M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 in D.M. Hillis, C. Moritz and B.K. Mable (editors), Molecular Systematics, 2nd ed. Sinauer, Sunderland, Massachusetts. [no digital version available]

Turland, N.J., J.H. Wiersema, F.R. Barrie, W. Greuter, D.L. Hawksworth, P.S. Herendeen, S. Knapp, W.-H. Kusber, D.-Z. Li, K. Marhold, T.W. May, J. McNeill, A.M. Monro, J. Prado, M.J. Price and G.F. Smith. 2018. International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. https://doi.org/10.12705/Code.2018; http://www.iapt-taxon.org/nomen/main.php

Varela, F.J., E. Thompson, and E. Rosch. 1992. The Embodied Mind: Cognitive Science and Human Experience. MIT Press, Cambridge, Massachusetts.

Virgo, N. 2019. The necessity of extended autopoiesis. Adaptive Behavior. 617. https://doi.org/10.1177/1059712319841557

Walsh, D. 2010. Not a sure thing: fitness, probability, and causation*. Philos. Sci. 77: 147–171. https://doi.org/10.1086/651320

Wheeler, Q.D. and R. Meier (editors). 2000. Back cover material. Species Concepts and Phylogenetic Theory: A Debate. Columbia University, New York. [No digital version available; see http://cup.columbia.edu/book/species-concepts-and-phylogenetic-theory/9780231101424]

Wilson, R.A., M.J. Barker and I. Brigandt. 2007. When traditional essentialism fails: biological natural kinds. Phil. Topics 35: 189–215. https://philpapers.org/archive/BRIWTE.pdf

Yang, Z., N. Goldman and A. Friday. 1995. Maximum likelihood trees from DNA sequences: a peculiar statistical estimation problem. Syst. Bot. 44: 384–399. https://doi.org/10.1093/sysbio/44.3.384

Yang, Z. and T. Zhu. 2018. Bayesian selection of misspecified models is overconfident and may cause spurious posterior probabilities for phylogenetic trees. Proc. Nat. Acad. Sci. USA 115: 1854-1859. https://doi.org/10.1073/pnas.1712673115

Table 1. Taxa, samples acronyms, and ITS sequence accessions. Names of taxa as accepted by Davies (2010) are in the left column, followed by the acronym corresponding to the vouchers and DNA samples referred to in the present work. This is a combination of the species epithet plus the voucher collection number, which are the same as those used in Hershkovitz et al. (2006a) and provided in GenBank. An asterisk denotes vouchers not identified in Davies (2010). Samples lacking an acronym were used only in analysis of nominal species polymorphism. Their voucher identity was verified in Davies (2010). The acronyms are followed by the GenBank accession of the ITS sequence. The corresponding and aligned *rpl32-trnL* sequences are provided in Fasta format in the supporting data files. The right column indicates the ID or name of the sample, when different from the current ID/name, according to Hershkovitz et al. (2006a) and/or the current ID in GenBank. A hyphen indicates that both IDs/names are the same.

Taxon	**Sample acronym**	**GenBank sequence accession**	**Voucher ID or name used in Hershkovitz et al. (2006a) if different**
Chaetanthera* subg. *Chaetanthera			
C. chilensis (Willd.) DC	*chilensis_25042*	DQ355841	*C. chilensis* var. *tenuifolia* (D.Don) Cabrera
"	**chilensis_25229*	DQ355840	*C. chilensis* (Willd.) DC var. *chilensis*
C. ciliata Ruiz & Pav.	*ciliata_25157*	DQ355888	-
"		DQ355887	*Arroyo 25002*
C. elegans Phil.	*elegans_25069*	DQ355889	*C. elegans* Phil. var. *elegans*
"	*elegans_26000*	DQ355839	*C. elegans* var. *pratensis* (Phil.) Cabrera
C. euphrasioides F.Meigen	*euphrasioides_25176*	DQ355868	-
"		DQ355866	*Arroyo 25119*
C. glandulosa J.Rémy	**glandulosa_25181*	DQ355881	-
C. incana Poepp. ex Less.	*incana_25013*	DQ355885	-
C. moenchioides Less.	*moenchioides_25122*	DQ355847	-
"	*moenchioides_25177*	DQ355883	*C. australis* Cabrera
"		DQ355884	*Arroyo 25003*
"		DQ355846	*Arroyo 25018*
C. peruviana Gray	**peruviana_25252*	DQ355850	*C. chiquianensis* Ferreyra
"	**peruviana_25254*	DQ355850	-
C. x serrata Ruiz & Pav.	*x_serrata_25131*	DQ355886	*C. serrata* Ruiz & Pav.
"	*x_serrata_25250*	DQ355848	*C. brachylepis* Phil.
***C.* subg. *Liniphyllum* Less.**			
. *C. albiflora* (Phil.) A.M.R. Davies	*albiflora_25012*	DQ355909	*C. linearis* Poepp. ex Less var. *albiflora* Phil.
"	*albiflora_25033*	DQ355869	*C. linearis* var. *linearis*
"	*albiflora_25129*	DQ355870	*C. linearis* var *taltalensis* I.M.Johnst.
"		DQ355909	*C. linearis* var *taltalensis*
C. depauperata (Hook. & Arn.) A.M.R. Davies	**depauperata_02-109*	DQ355873	*C. leptocephala* Cabrera
C. microphylla (Cass.) Hook. & Arn.	*microphylla_25007*	DQ355871	-
C. multicaulis DC		DQ355882	*C. tenella* var. *Tenella*

Table 1, continued.

Taxon	Sample acronym	GenBank sequence accession	Voucher ID or name used in Hershkovitz et al. (2006a) if different
C. perpusilla (Wedd.) Anderb. & S.E.Freire	**perpusilla_25202*	DQ355880	-
C. taltalensis (Cabrera) A.M.R. Davies	*taltalensis_25055*	DQ355878	*C. tenella* Less. var. *taltalensis* Cabrera
"	*taltalensis_25128*	DQ355879	*C. tenella* var. *taltalensis*
***C.* subg. *Tylloma* (D.Don) Less.**			
C. flabellifolia Cabrera	*flabellifolia_25078*	DQ355852	-
"	*flabellifolia_25084*	DQ355853	*C. splendens* (J.Rémy) B.L.Rob.
C. glabrata (DC.) F.Meigen	*glabrata_25065*	DQ355854	-
"	*glabrata_25130*	DQ355858	-
"	*glabrata_25163*	DQ355856	-
C. philippii B. L. Rob.	**philippii_02-96*	DQ355857	*C.* sp. '02-96'; [1]cf. Davies (2010: 55, Fig. 22)
"	*philippii_25075*	DQ355863	[2]*C. lanata* (Phil.) I.M.Johnst.
C. pubescens A.M.R. Davies	*pubescens_25076*	DQ355861	*C. kalinae* A.M.R. Davies
C. renifolia (J.Rémy) Cabrera	*renifolia_25175*	DQ355860	-
C. schroederi G. F. Grandjot & K. Grandjot	*schroederi_25150*	DQ355843	*C. limbata* (D.Don) Less.
C. spathulifolia Cabrera	*spathulifolia_25098*	DQ355864	-
C. villosa D.Don	*villosa_20646*	DQ355845	-
"	*villosa_210671*	DQ355865	-
Incertae sedis			
C. sp. indet.	**sp_indet_25161*	DQ355867	*C. flabellata* D.Don
Oriastrum* subg. *Oriastrum			
O. chilense (J.Rémy) Wedd.	*chilense_25121*	DQ355891	*C. pusilla* (D.Don) Hook. & Arn.
"	*chilense_25180*	DQ355916	*C. pusilla*
O. gnaphalioides (J.Rémy) Wedd.	**gnaphalioides_02-154*	DQ355906	*C.* aff. *gnaphalioides* (J.Rémy) I.M.Johnst
"	*gnaphalioides_25079*	DQ355890	*C. minuta* (Phil.) Cabrera
"	*gnaphalioides_25086*	DQ355908	*C.* aff. *gnaphalioides*
"	*gnaphalioides_25127*	DQ355907	*C. minuta*
O. lycopodioides (J.Rémy) Wedd.	*lycopodioides _25169*	DQ355920	*C. lycopodioides* (J.Rémy) Cabrera
"		DQ355919	voucher *Arroyo 210637* (CONC)
O. pusillum Poepp. & Endl.	*pusillum_25120*	DQ355892	*C. planiseta* Cabrera
***O. subg. Egania* (J.Rémy) A.M.R. Davies**			
O. abbreviatum (Cabrera) A.M.R. Davies[3]	*abbreviatum _25109*	DQ355896	*C. stuebelii* var. *argentina* Cabrera

Table 1, continued.

Taxon	**Sample acronym**	**GenBank sequence accession**	**Voucher ID or name used in Hershkovitz et al. (2006a) if different**
"	*abbreviatum_25110*	DQ355900	*C. stuebelii* Hieron var. *abbreviata* Cabrera
O. acerosum (J.Rémy) Phil.	*acerosum_25077*	DQ355905	*C. acerosa* (J.Rémy) Benth. & Hook. f. var. indet.
"	*acerosum_25087A*	DQ355909	*C. acerosa* var. *acerosa*
"	*acerosum_25087B*	DQ355914	*C. acerosa* var. *dasycarpa* Cabrera
O. apiculatum (J.Rémy) A.M.R. Davies	**apiculatum_25244*	DQ355910	*C. apiculata* (J.Rémy) F.Meigen
"		DQ355902	*C. apiculata*
O. dioicum (J.Rémy) Phil.	*dioicum_25099*	DQ355893	*C. pentacaenoides* (Phil.) Hauman
"	*diocum_25168*	DQ355904	*C. pentacaenoides*
O. famatinae A.M.R. Davies[4]	*famatinae_25102*	DQ355898	*C. dioica* (J.Rémy) B. L. Robinson
O. polymallum Phil.[5]	*polymallum_25082*	DQ355897	*C. sphaeroidalis* (Reiche) Hicken
O. pulvinatum Phil.	*pulvinatum_25083*	DQ355903	*C. pulvinata* (Phil.) Hauman var. *pulvinata*
"	*pulvinatum_25100*	DQ355909	*C. pulvinata* var. *pulvinata*
"	*pulvinatum_25104*	DQ355913	*C.* aff. *sphaeroidalis*
"	*pulvinatum_25111*	DQ355895	*C. cochlearifolia* (Gray) B. L. Robinson
O. revolutum (Phil.) A.M.R. Davies	*revolutum_25126*	DQ355899	*C. revoluta* (Phil.) Cabrera
O. stuebelii (Hieron.) A.M.R. Davies "sensu stricto" sensu Davies (2010)	*stuebelii_25200*	DQ355911	*C. stuebelii* var. *stuebelii*
"	**stuebelii_25201*	DQ355912	*C. stuebelii* var. indet.
"	**stuebelii_25203*	DQ355901	*C.* sp. '25203'
"	*stuebelii_25204*	DQ355913	*C.* sp. '25204'
Outgroup taxa			
Mutisia decurrens Cav.	*Mutisia decurrens*	EU841169	
Mutisia hamata Reiche	*Mutisia hamata*	EF530242	
Mutisia spinosa Ruiz & Pav.	*Mutisia spinosa*	MG553881	
Proustia ilicifolia Hook. & Arn.	*Proustia ilicifolia*	KY223784	
Richterago discoidea (Less.) Kuntze	*Richterago discoidea*	MH886333	

Table 1, continued.

Taxon	Sample acronym	GenBank sequence accession	Voucher ID or name used in Hershkovitz et al. (2006a) if different

NOTES:

[1] Davies (2010: 55, Fig. 22) reproduced the maximum likelihood phylogram of Hershkovitz et al. (2006a) with a modified taxonomy. There, the name "*C. lanata*" is superimposed as the "accepted name" over a clade of two taxa that includes the sample *Hershkovitz 02-96*, which she had not seen. The specimen is from the road to the Pascua Lama mine, Huasco Province, Atacama Region, altitude 2264 m. The ID can be inferred from locality and DNA sequence. However, in the taxonomic treatment, Davies listed *C. lanata* as a synonym of *C. philippii* (see below).

[2] Davies (2010: 211) listed *Chaetanthera. lanata* as a synonym *C. philippii* B.L.Rob., Proc. Amer. Acad. Arts 49: 514 (1913), ≡ *Chondrochilus involucratus* Phil., Fl. Atacam. pp. 27, t. 3. (1860), non *Chaetanthera involucrata* Phil., Anal. Univ. Chile 47: 6 (1894), = *C. chilensis* (Davies, 2010: 140). The heterotypic *C. lanata* (Phil.) I. M. Johnst., Physis (Buenos Aires) 9: 325 (1929), ≡ *Tylloma lanatum* Phil., Linnaea 33: 112. (1864-65), thus is a later synonym when the two types are considered conspecific. At this writing, GBIF (GBIF Secretariat, 2017), Plants of the World Online (POWO, 2019), and World Flora Online (WFO, 2020) all list *C. philippii* as a synonym of *C. lanata*. This is an error.

[3] Classified by Nicola et al. (2015) as *O. stuebelii* var. *abbreviatum* Nicola, S.E. Freire & Ariza.

[4] Classified by Nicola et al. (2015) as *O. stuebelii* var. *argentinum* (Cabrera) Nicola, S.E. Freire & Ariza.

[5] Classified by Nicola et al. (2015) as *O. pulvinatum* var. *polymallum* (Phil.) Hicken.

Table 2. DNA sequence polymorphism in nominal species of *Chaetanthera* s. str. and *Oriastrum*. In the left-hand column are the names of species recognized by Davies (2010), followed by the number of samples sequenced and analyzed phylogenetically for ITS and *rpl32-trnL* in the´present work. In parentheses is the total number of samples for ITS including additional sequences in GenBank of samples with vouchers identified by Davies (2010).

Species sampled > 1X (by epithet)	**# samples**	**ITS**			***rpl32-trnL***			**ITS + *rpl32-trnL***		
		genotypes w/o indels	**genotypes w/indels**	**phylo status***	**genotypes w/o indels**	**genotypes w/indels**	**phylo status**	**genotypes w/o indels**	**genotypes w/indels**	**phylo status**
***Chaetanthera* s. str.**										
C. albiflora	3	3	3	poly ≥ 70	3	3	poly ≥ 70	3	3	poly ≥ 50
C. chilensis	2	1	2	-**	2	2	poly ≥ 70	2	2	-
C. ciliata	1 (2)	1 (1)	1 (2)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)
C. elegans	2	2	2	poly ≥ 70	2	2	poly ≥ 70	2	2	poly ≥ 50
C. euphrasioides	1 (2)	1 (1)	1 (1)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)
C. flabellifolia	2	1	1	-**	1	1	-**	1	1	-**
C. glabrata	2	2	2	poly ≥ 70	2	2	poly ≥ 50	2	2	poly ≥ 70
C. moenchioides	2 (4)	2 (3)	2 (4)	mono (NA)	2	2	mono	2	2	mono
C. peruviana	2	2	2	mono	2	2	mono	2	2	mono
C. philippii	2	2	2	mono	2	2	mono	2	2	mono
C. taltalensis	1 (2)	1 (2)	1 (2)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)
C. villosa	2	2	2	mono	2	2	mono	2	2	mono
C. x serrata	2	1	1	-**	2	2	poly ≥ 50	2	2	poly ≥ 50
Oriastrum										
O. abbreviatum	2	2	2	mono	2	2	poly ≥ 50	2	2	mono
O. acerosum	3	1	1	mono	3	3	poly ≥ 50	3	3	mono
O. apiculatum	1 (2)	1 (2)	1 (2)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)
O. chilense	1 (2)	1 (2)	1 (2)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)
O. dioicum	2	2	2	mono	2	2	mono	2	2	mono
O. gnaphalioides	4	3	3	mono	3	4	mono	4	4	mono
O. lycopodioides	1 (2)	1 (2)	1 (2)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)	(NA)
O. pulvinatum	4	4	4	poly ≥ 70	4	4	poly ≥ 70	4	4	poly ≥ 70
O. stuebelii	4	3	3	poly ≥ 70	4	4	poly ≥ 50	4	4	poly ≥ 70
Totals:										
15/16 (20/22) of 2+ sampled taxa polymorphic	**39 (53)**	**32/39 (43/53)**	**33/39 (46/53)**	**8 mono 5 poly 3 ambig**	**37/39**	**38/39**	**6 mono 9 poly 1 ambig**	**38/39**	**38/39**	**8 mono 6 poly 2 ambig**

Notes:

Figures in parentheses are totals for all samples in GenBank whose vouchers are identified in Davies (2010). Otherwise the figures include totals only for nominal species sampled more than once in the present phylogenetic analysis, i.e., excluding species sampled once in the present analysis.

* Phylogenetic status of samples in the corresponding trees: mono = monophyletic with ≥ 70% bootstrap support in at least one of the three corresponding bootstrap analyses; poly = para- or polyphyletic; ≥ 50 or ≥ 70 = bootstrap proportion; - = clade unresolved.

** Even though the two samples share the same genotype, the genotypes are shared between taxa, hence monophyly of each taxon is not tested.

Table 3. Summary statistics for MP analysis of the ITS and *rpl32-trnL* data.

	ITS		***rpl32-trnL***	
Length				
	with indel characters	w/o indel characters	with indel characters	w/o indel characters
Variable sites	338	327	266	243
Informative sites	260	249	180	157
MP tree length	922	899	451	397
Consistency index (CI)	0.539	0.540	0.712	0.728
Homoplasy index (HI)	0.461	0.466	0.288	0.272
CI w/o uninformative sites	0.489	0.483	0.637	0.645
HI w/o uninformative sites	0.511	0.517	0.363	0.355
Retention index	0.831	0.827	0.918	0.925
Rescaled consistency index	0.450	0.441	0.654	0.674

Table 4. %A/T content extremes in the five subgenera of *Chaetanthera*/*Oriastrum* and the content in *sp_indet_25161*.

Clade	**ITS %AT**				***rpl32-trnL* %AT**			
	total sites		**variable sites**		**total sites**		**variable sites**	
	lowest %	**highest %**	**lowest %**	**highest %**	**lowest %**	**highest %**	**lowest %**	**highest %**
C. subg. Chaetanthera	41.4	43.1	39.0	42.9	72.4	73.6	55.2	59.3
C. subg. Liniphyllum	41.4	42.7	38.9	41.7	74.3	74.7	61.2	62.6
C. subg. Tylloma	42.9	46.9	42.3	50.8	72.6	75.0	59.4	63.5
sp_indet_25161	43.2		43.0		74.2		61.3	
O. subg. Oriastrum	42.1	48.4	40.9	54.0	72.8	74.8	57.8	63.9
O. subg. Egania	42.0	43.8	40.8	44.4	72.4	73.7	56.7	60.4

Table 5. Comparison of bootstrap proportions for the different datasets and optimization criteria.

The bootstrap treatments are as follows: **1)** ITS substitution and indel data, MP; **2)** ITS substitution data only, MP; **3)** ITS substitution data only, ML; **4)** *rpl32-trnL* substitution and indel data, MP; **5)** *rpl32-trnL* substitution data only, MP; **6)** *rpl32-trnL* substitution data only, ML; **7)** combined substitution and indel data, MP; **8)** combined substitution and ITS indel data; and **9)** combined substitution data only. The sample numbers are according to Fig. 8. The clade numbers (Roman numerals) are arbitrary and were assigned more or less from terminal to basal clades of *C.* subg. *Chaetanthera*, thereafter repeating the procedure for each of the five major clades corresponding to the subgenera recognized here. The five subgenera are shaded as follows: pink, *Chaetanthera* subg. *Chaetanthera*; green, *C.* subg. *Tylloma*; yellow, *C.* subg. *Liniphyllum*; blue, *Oriastrum* subg. *Oriastrum*; lavender, *O.* subg. *Egania*. The final rows sum the number of clades supported at ≥ 50% and ≥ 70% in each bootstrap analysis.

CLADE[1]	**TERMINAL, CLADE NUMBERS[2]**	**BOOTSTRAP DATA & METHOD[3]**								
		ITS			***rpl32-trnL***			**ITS + *rpl32-trnL***		
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
I	2, 36	-	-	68	-	-	-	-	-	-
II	35, 36	-	-	**-**	**73**	55	*	68	52	-
III	2,3,7,33, 34, 35, 36	**73**	**74**	**72**	-	**-**	-	-	-	-
IV	II, 7	-	-	-	62	**75**	**82**	59	**76**	**74**
V	IV, 2	-	-	59	-	-	54	-	-	-
VI	IV, 1, 2, 3 **-or-** V, 2, 3	-	-	-	54	61	52	59	50	-
VII	VI, 34	-	**-**	**-**	**89**	**96**	**98**	**93**	**93**	**91**
VIII	III, 1	**92**	**93**	**96**	61	60	58	**99**	**100**	**99**
IX	VIII, 31	-	-	59	-	-	-	-	-	-
X	6, 32	**100**	**100**	**100**	**95**	**97**	**98**	**100**	**100**	**100**
XI	VIII, X	-	**-**	**-**	56	55	-	-	-	-
XII	IX, X-**or**- XI, 31	**86**	**82**	**98**	**96**	**93**	**87**	**99**	**99**	**99**
XIII	8, 9	**100**	**100**	**100**	**100**	**99**	**100**	**100**	**100**	**100**
XIV	XIII, 30	-	-	-	-	-	50	-	-	-
XV	XII, XIV	64	59	**70**	-	-	**-**	61	**71**	**73**
XVI	XV, 30 or XI, XIV	**96**	**93**	**98**	**100**	**100**	**100**	**100**	**100**	**100**
XVII	4, 14	60	57	*	-	-	*	**82**	**86**	**86**
XVIII	XVII, 12	**99**	**98**	**100**	-	-	-	**99**	**99**	**98**
XIX	4, 14, 16	-	-	-	66	62	**60	-	-	-
X	XIX, 12	-	-	-	61	-	-	-	-	-
XXI	XVIII, 13	**91**	**86**	**97**	-	-	-	**88**	**92**	**86**
XXII	XXI, 16 **-or-** XIX, XX	**99**	**100**	**100**	**81**	58	69	**100**	**99**	**100**
XXIII	10, 11	**100**	**100**	*	**100**	**100**	*	**100**	**100**	**100**
XXIV	17,18	**89**	**78**	**80**	**99**	**99**	**100**	**99**	**100**	**99**
XXV	XXIII, XXIV	**74**	**74**	66	-	-	**-**	66	65	69
XXVI	XXII, XXV	**99**	**97**	**98**	**76**	51	58	**98**	**98**	**97**
XXVII	XXVI, 19	60	55	-	-	-	-	-	-	-
XXVIII	XXVI, 15	-	-	-	**96**	**92**	**95**	**75**	69	66
XXIX	5, 20	**91**	**89**	**84**	**72**	57	**70**	**96**	**95**	**91**
XXX	XXVIII, XXIX, 19	-	-	**-**	55	55	52	56	53	55
XXXI	XXVII, XXIX, 15, 22 **-or-** XXX, 22	62	68	**88**	**81**	**83**	**97**	**88**	**93**	**94**
XXXII	XVI, XXXI	-	-	-	55	64	54	-	-	-
XXXIII	XVI, XXXI, 21	56	-	-	-	-	-	-	-	-
XXXIV	XXXI, 21	-	-	51	-	-	-	-	-	-

Table 5, continued.

CLADE[1]	**TERMINAL, CLADE NUMBERS[2]**	**BOOTSTRAP DATA & METHOD[3]**								
		ITS			***rpl32-trnL***			**ITS + *rpl32-trnL***		
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
XXXV	23, 25	**100**	**100**	*	**83**	**80**	**81**	**100**	**100**	**100**
XXXVI	XXXV, 27	**90**	**86**	**85**	-	-	-	60	59	59
XXXVII	XXXV, 24	-	-	-	-	62	65	-	55	-
XXXVIII	XXXV, 24 **-or-** XXXVII, 27	69	67	66	**-**	-	-	-	-	55
XXXIX	XXXVIII, 26 **-or-** XXXV, 24, 26, 27	**100**	**100**	**100**	**98**	**99**	**100**	**100**	**100**	**100**
XXXX	28, 29	**100**	**100**	**100**	**77**	**80**	**70**	**100**	**100**	**100**
XXXXI	XXXIX, XXXX	**95**	**96**	**100**	-	-	60	**99**	**99**	**100**
XXXXII	XXXXI, 21	-	-	-	**91**	**93**	**97**	-	-	-
XXXXIII	XXXVIII, XXXXII **-or-** XXXV, XXXXI	**99**	**99**	**100**	**100**	**100**	**100**	**100**	**100**	**99**
XXXXIV	37, 53	**100**	**100**	*	**100**	**100**	*	**100**	**100**	**100**
XXXXV	51, 52	**98**	**99**	**95**	**89**	69	68	**100**	**99**	**100**
XXXXVI	XXXXIV,XXXXV	**96**	**97**	**99**	**84**	**75**	**71**	**98**	**98**	**98**
XXXXVII	38, 60	**92**	**92**	**99**	**82**	**92**	**97**	**98**	**98**	**98**
XXXXVIII	XXXXVII, 62	**87**	**91**	**71**	**91**	**90**	**100**	**99**	**99**	**99**
XXXXIX	XXXXVII, XXXXVIII	**87**	**85**	**98**	-	-	60	**89**	**88**	**88**
L	39, 49	**83**	**84**	68	**91**	**84**	**90**	**98**	**98**	**98**
LI	40, 43	50	-	53	-	-	-	-	-	-
LII	40, 48	-	-	-	**91**	**95**	**98**	**77**	**75**	**77**
LIII	LII, 43 **-or-** LI, 48	64	65	64	-	-	-	**71**	**70**	**71**
LIV	44, 45	**99**	**98**	**100**	-	-	**-**	**96**	**94**	**96**
LV	LIV, 61	**87**	**86**	**93**	-	-	**-**	**96**	**96**	**96**
LVI	44, 61	-	-	**-**	64	62	60	-	-	-
LVII	LIII, LV	**88**	**83**	**99**	-	-	-	**96**	**96**	**97**
LVIII	42, 46	**99**	**98**	**98**	-	-	-	**93**	**93**	**93**
LIX	47, 58, [**56]	**87**	**86**	**52	-	-	*	**89**	**88**	**86**
LX	LIX, 56	-	-	-	**100**	**99**	*	**99**	**96**	**97**
LXI	LVIII, LX	**87**	**89**	**96**	-	-	-	**91**	**91**	**92**
LXII	LX, 46, 57	-	-	-	60	64	**53	-	-	-
LXIII	LXI, 57	-	-	56	-	-	-	60	60	61
LXIV	LVI, LXIII, 45	-	-	-	-	-	64	-	-	-
LXV	L, LXIII, 42,43	-	-	-	66	64	**81**	-	-	-
LXVI	LX, 41, 57 **-or-** LXII, 41	**94**	**93**	**100**	-	-	-	**95**	**94**	**100**
LXVII	LVII, LXVI or LXV, 41	-	-	55	68	68	**75**	**71**	69	**71**
LXVIII	50, 55	-	-	-	56	53	57	-	-	-
LXIX	54, 59	-	-	*	**90**	**87**	**92**	**96**	**96**	**95**
LXX	LXIX, 50	**100**	**100**	*	-	-	-	**99**	**99**	**99**
LXXI	L, LVII, LXVI, LXX, 55 **-or-** L, LXVII, LXVIII, LXIX **-or-** L, LXVII, LXX, 55	**98**	**98**	**100**	**93**	**95**	**99**	**100**	**100**	**99**
# Clades ≥ 50% bootstrap support		*44*	*42*	****49*	*41*	*41*	****47*	*50*	*51*	*49*
# Clades ≥ 70% bootstrap support		*33*	*33*	****37*	*28*	*25*	****30*	*42*	*42*	*43*

Table 5, continued.

CLADE[1]	**TERMINAL, CLADE NUMBERS**[2]	**BOOTSTRAP DATA & METHOD**[3]								
		ITS			***rpl32-trnL***			**ITS +** ***rpl32-trnL***		
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

Notes:

* Denotes clades in the MP bootstraps absent in an ML bootstrap because the indicated samples were identical and therefore merged.

** Denotes bootstrap support for clades implicit in ML bootstraps because of sample merging, whereas in MP bootstraps the samples were not merged because of indel differences. Thus, an MP bootstrap might indicate different relations for the samples here shown as identical.

*** The totals for the ML bootstraps include clades absent because of merging of identical samples. Had they been included, presumably the bootstrap recovery of these clades would approach 100% depending upon the divergence of the identical sequences from nonidentical sequences. However, bootstrap support for monophyly of identical sequences would be less as divergence from nonidentical sequences decreases towards a single substitution, because some bootstrap replicates would not sample the diverging site.

Figure captions

Figs. 1–4. ML phylograms. The Figs. 1 and 3 captions include the ML-estimated FM parameters applied for the ITS and *rpl32-trnL* sequences, respectively. These parameters also were applied in the analyses illustrated in Figs. 2 and 4. Sample acronyms are defined in Table 1. The five subgenera are shaded as follows: pink, *Chaetanthera* subg. *Chaetanthera*; green, *C*. subg. *Tylloma*; yellow, *C*. subg. *Liniphyllum*; blue, *Oriastrum* subg. *Oriastrum*; lavender, *O*. subg. *Egania*. Samples with identical sequences are indicated on the right hand side. Samples denoted with an asterisk refer to vouchers not cited by Davies (2010).

Fig. 1. ML phylogram for the *Chaetanthera* and *Oriastrum* ITS data with outgroups. The GTR+I+G parameters are: r(AC) 0.911, r(AG) 2.131, r(AT) 1.050, r(CG) 0.438, r(CT) 5.676, r(GT) 1, p(invariant sites) 0.332, λ 1.213, p(A) 0.231, p(C) 0.247, p(G) 0.253, p(T) 0.268. -*ln*L 6192.867.

Fig. 2. ML phylogram for the *Chaetanthera* and *Oriastrum* ITS data as in Fig. 1 but without outgroups. -*ln*L 5618.606.

Fig. 3. ML phylogram for the *Chaetanthera* and *Oriastrum rpl32-trnL* data with outgroups. The GTR+ G parameters are: r(AC) 0.925, r(AG) 0.461, r(AT) 0.122, r(CG) 0.699, r(CT) 0.343, r(GT) 1, λ 0.616, p(A) 0.370, p(C) 0.142, p(G) 0.122, p(T) 0.365. -*ln*L 4032.403.

Fig. 4. ML phylogram for the *Chaetanthera* and *Oriastrum rpl32-trnL* data as in Fig. 1 but without outgroups. -*ln*L 3650.763.

Figs. 5–7. Bootstrap majority rule trees. Sample acronyms are defined in Table 1. The five subgenera are shaded as follows: pink, *Chaetanthera* subg. *Chaetanthera*; green, *C*. subg. *Tylloma*; yellow, *C*. subg. *Liniphyllum*; blue, *Oriastrum* subg. *Oriastrum*; lavender, *O*. subg. *Egania*. Samples with identical sequences are indicated on the right hand side. Samples denoted with an asterisk refer to vouchers not cited by Davies (2010).

Fig. 5. ML majority bootstrap consensus for the *Chaetanthera* and *Oriastrum* ITS substitution data. Indicated on each branch are three BPs for the different analysis as follows: MP, substitutions and indel characters/MP, substitutions only/ML. One clade present in the MP substitution plus indel bootstrap but absent in the ML bootstrap is not shown (see Table 5). The BPs supporting *C*. subg. *Tylloma* in the two MP bootstraps without *sp_indet_25161* were both 95%.

Fig. 6. ML majority bootstrap consensus for the *Chaetanthera* and *Oriastrum rpl32-trnL* substitution data. Indicated on each branch are three BPs for the different analysis as follows: MP, substitutions and indel characters/MP, substitutions only/ML. Two clades present in the MP bootstraps but absent in the ML bootstrap are not shown (see Table 5). The BPs supporting *C*. subg. *Liniphyllum* in the two MP bootstraps without *sp_indet_25161* were 98% and 97%.

Fig. 7. ML majority bootstrap consensus for the *Chaetanthera* and *Oriastrum* combined substitution data plus ITS indel data. Indicated on each branch are three BPs for the different analysis as follows: MP, substitutions and indel characters/MP, substitutions and ITS indel characters/MP, substitutions only. There were no clades present in one of the other bootstraps not present in this one (see Table 5).

Fig. 8. Sample bootstrap tree showing taxon numbers used in Table 5. The tree is a replicate of the Fig. 7 tree but with a different starting seed. The BPs are slightly different, with two very weakly supported branches in Fig. 7 here collapsed.

Figs. 9–11. NeighborNet networks. Branch angles were adjusted manually to improve clarity. Samples with identical sequences are indicated with an arrow. Samples names in lower case refer to vouchers not cited by Davies (2010). The five subgenera are encircled as follows: pink, *Chaetanthera* subg. *Chaetanthera*; green, *C*. subg. *Tylloma*; yellow, *C*. subg. *Liniphyllum*; blue, *Oriastrum* subg. *Oriastrum*; purple, *O*. subg. *Egania*.

Fig. 9. NeighborNet network for the ITS data.

Fig. 10. NeighborNet network for the *rpl32-trnL* data.

Fig. 11. NeighborNet network for the combined data.

Fig. 12. Scientists examining fruitlike pod of extraterrestrial plantlike entity. Within the pod develops an embrioid of the organism that it will assimilate. When that organism is resting, the pod opens and the embrioid matures and assumes its being.

Fig. 1

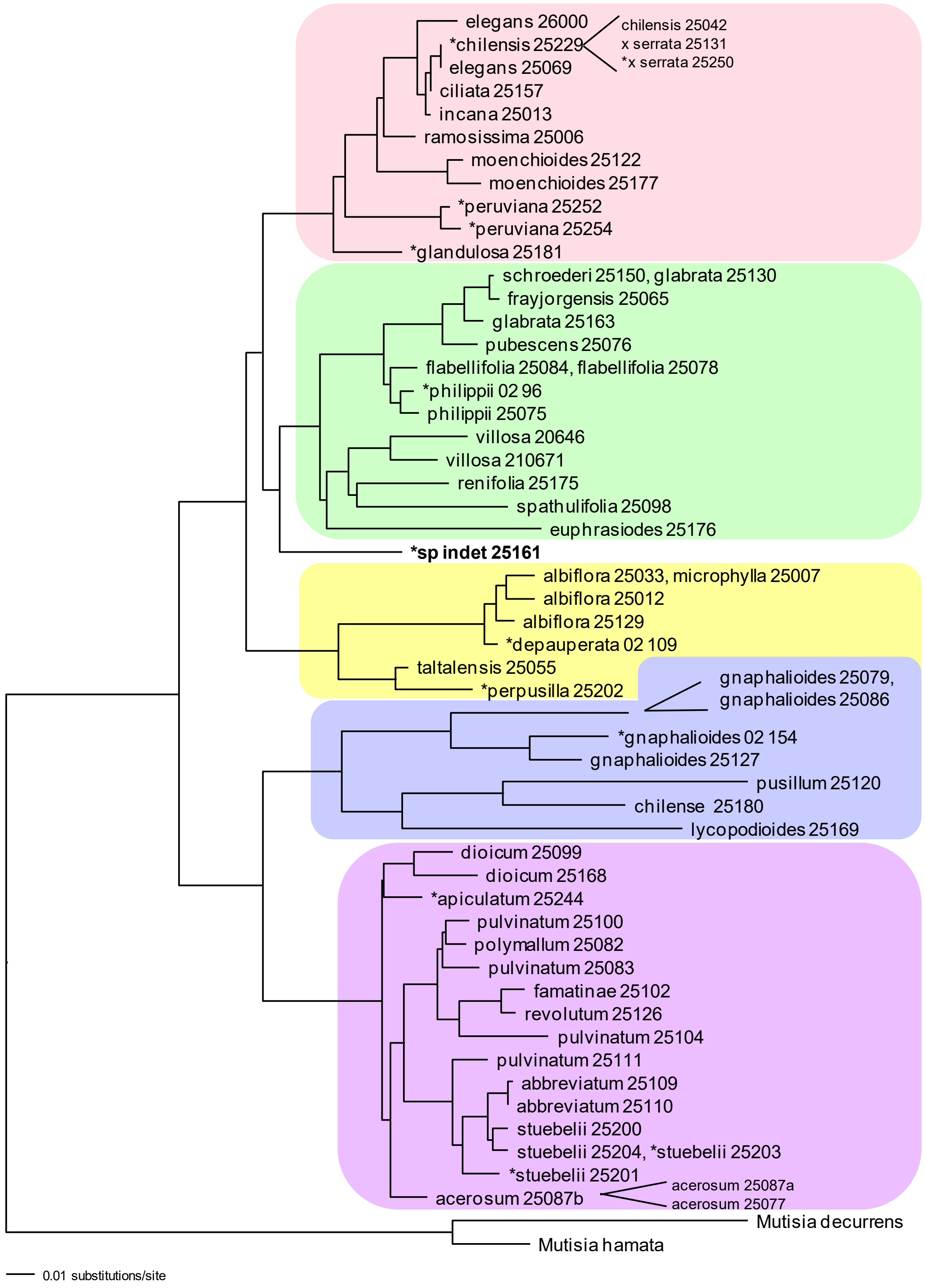

elegans 26000
*chilensis 25229
chilensis 25042
x serrata 25131
*x serrata 25250
elegans 25069
ciliata 25157
incana 25013
ramosissima 25006
moenchioides 25122
moenchioides 25177
*peruviana 25252
*peruviana 25254
*glandulosa 25181
schroederi 25150, glabrata 25130
frayjorgensis 25065
glabrata 25163
pubescens 25076
flabellifolia 25084, flabellifolia 25078
*philippii 02 96
philippii 25075
villosa 20646
villosa 210671
renifolia 25175
spathulifolia 25098
euphrasiodes 25176
*sp indet 25161
albiflora 25033, microphylla 25007
albiflora 25012
albiflora 25129
*depauperata 02 109
taltalensis 25055
*perpusilla 25202
gnaphalioides 25079,
gnaphalioides 25086
*gnaphalioides 02 154
gnaphalioides 25127
pusillum 25120
chilense 25180
lycopodioides 25169
dioicum 25099
dioicum 25168
*apiculatum 25244
pulvinatum 25100
polymallum 25082
pulvinatum 25083
famatinae 25102
revolutum 25126
pulvinatum 25104
pulvinatum 25111
abbreviatum 25109
abbreviatum 25110
stuebelii 25200
stuebelii 25204, *stuebelii 25203
*stuebelii 25201
acerosum 25087b
acerosum 25087a
acerosum 25077
Mutisia decurrens
Mutisia hamata
0.01 substitutions/site

Fig. 2

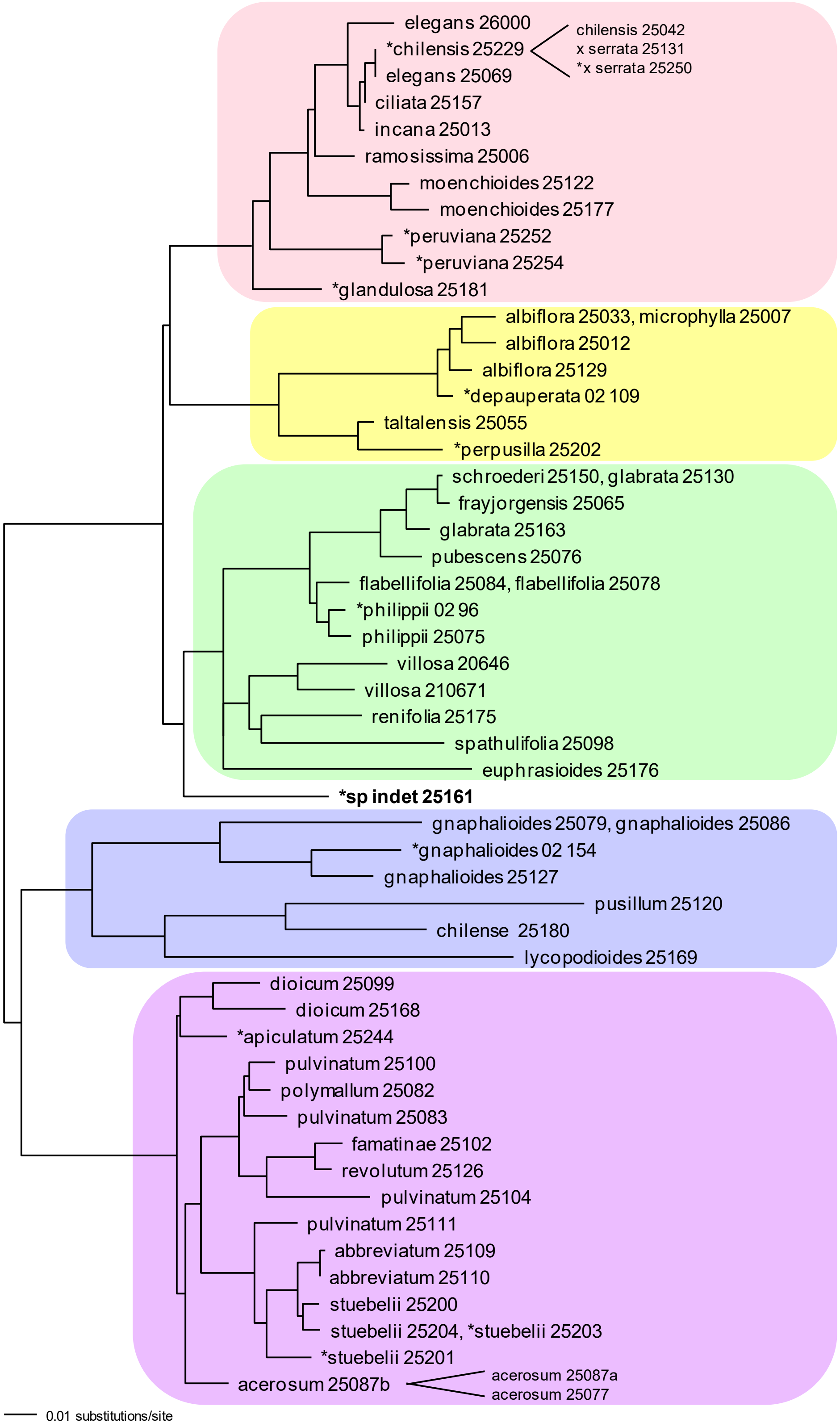

elegans 26000
*chilensis 25229
chilensis 25042
x serrata 25131
*x serrata 25250
elegans 25069
ciliata 25157
incana 25013
ramosissima 25006
moenchioides 25122
moenchioides 25177
*peruviana 25252
*peruviana 25254
*glandulosa 25181
albiflora 25033, microphylla 25007
albiflora 25012
albiflora 25129
*depauperata 02 109
taltalensis 25055
*perpusilla 25202
schroederi 25150, glabrata 25130
frayjorgensis 25065
glabrata 25163
pubescens 25076
flabellifolia 25084, flabellifolia 25078
*philippii 02 96
philippii 25075
villosa 20646
villosa 210671
renifolia 25175
spathulifolia 25098
euphrasioides 25176
*sp indet 25161
gnaphalioides 25079, gnaphalioides 25086
*gnaphalioides 02 154
gnaphalioides 25127
pusillum 25120
chilense 25180
lycopodioides 25169
dioicum 25099
dioicum 25168
*apiculatum 25244
pulvinatum 25100
polymallum 25082
pulvinatum 25083
famatinae 25102
revolutum 25126
pulvinatum 25104
pulvinatum 25111
abbreviatum 25109
abbreviatum 25110
stuebelii 25200
stuebelii 25204, *stuebelii 25203
*stuebelii 25201
acerosum 25087b
acerosum 25087a
acerosum 25077
0.01 substitutions/site

Fig. 3

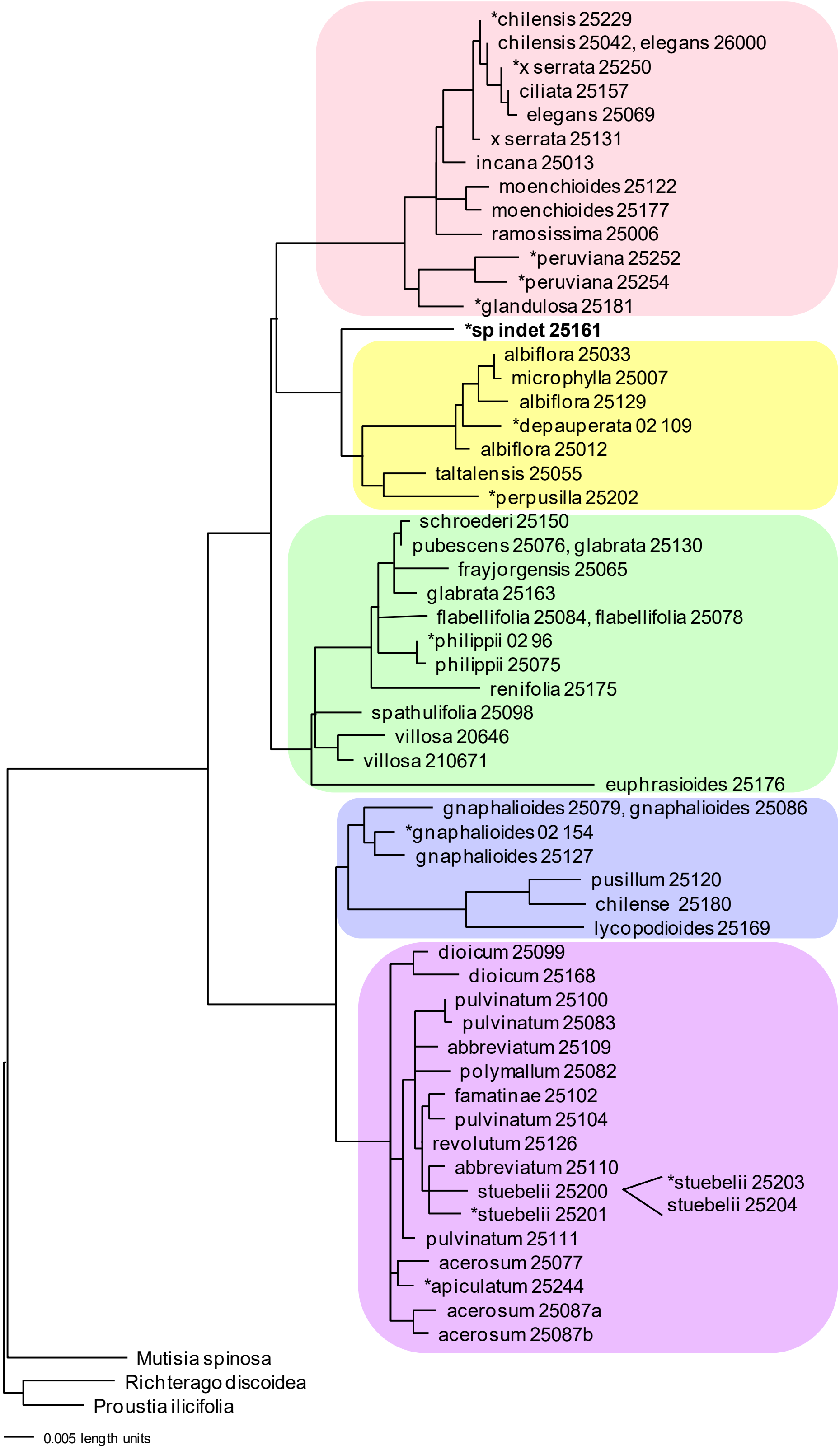

*chilensis 25229
chilensis 25042, elegans 26000
*x serrata 25250
ciliata 25157
elegans 25069
x serrata 25131
incana 25013
moenchioides 25122
moenchioides 25177
ramosissima 25006
*peruviana 25252
*peruviana 25254
*glandulosa 25181
*sp indet 25161
albiflora 25033
microphylla 25007
albiflora 25129
*depauperata 02 109
albiflora 25012
taltalensis 25055
*perpusilla 25202
schroederi 25150
pubescens 25076, glabrata 25130
frayjorgensis 25065
glabrata 25163
flabellifolia 25084, flabellifolia 25078
*philippii 02 96
philippii 25075
renifolia 25175
spathulifolia 25098
villosa 20646
villosa 210671
euphrasioides 25176
gnaphalioides 25079, gnaphalioides 25086
*gnaphalioides 02 154
gnaphalioides 25127
pusillum 25120
chilense 25180
lycopodioides 25169
dioicum 25099
dioicum 25168
pulvinatum 25100
pulvinatum 25083
abbreviatum 25109
polymallum 25082
famatinae 25102
pulvinatum 25104
revolutum 25126
abbreviatum 25110
stuebelii 25200
*stuebelii 25201
*stuebelii 25203
stuebelii 25204
pulvinatum 25111
acerosum 25077
*apiculatum 25244
acerosum 25087a
acerosum 25087b
Mutisia spinosa
Richterago discoidea
Proustia ilicifolia
0.005 length units

Fig. 4

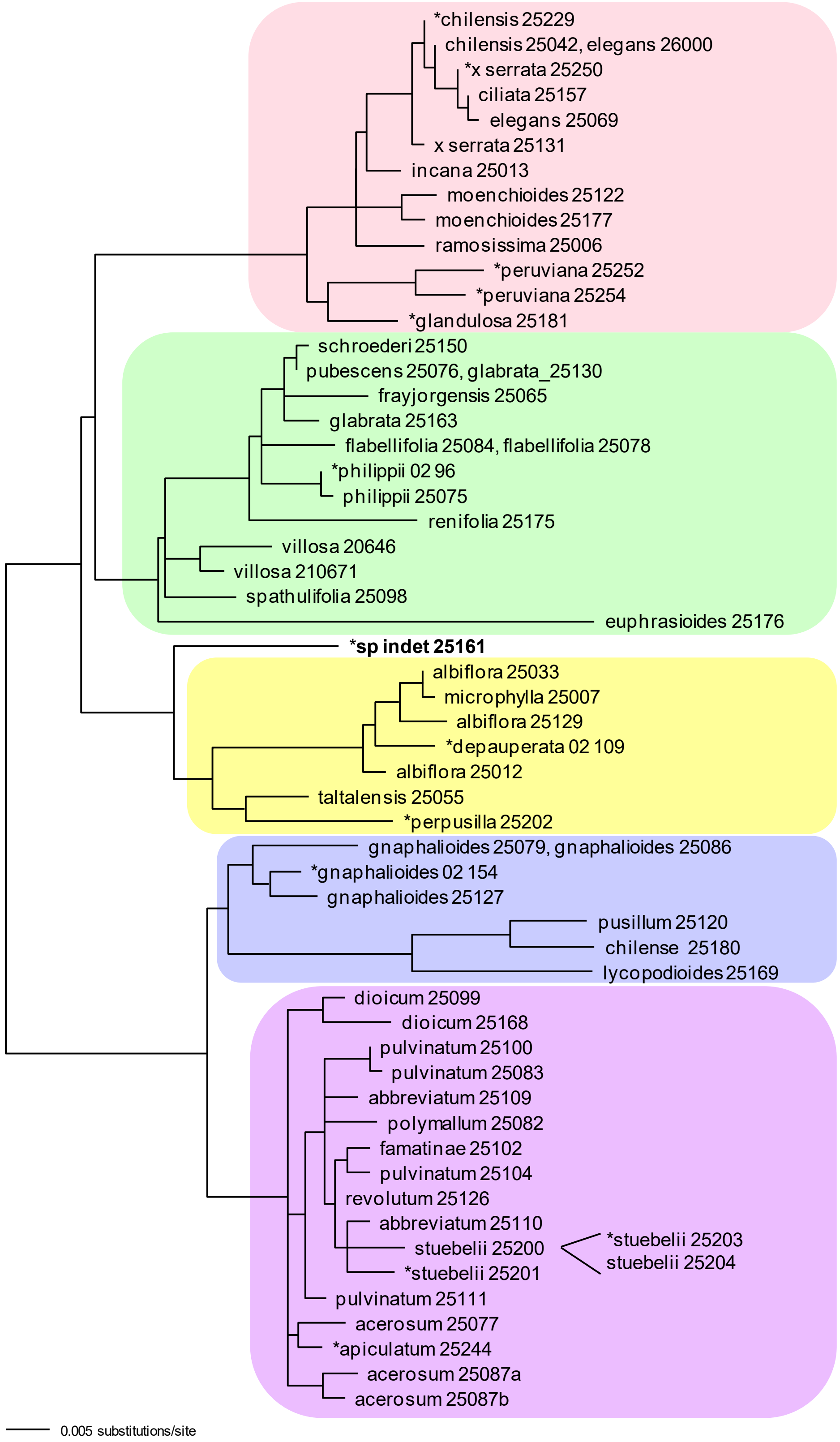
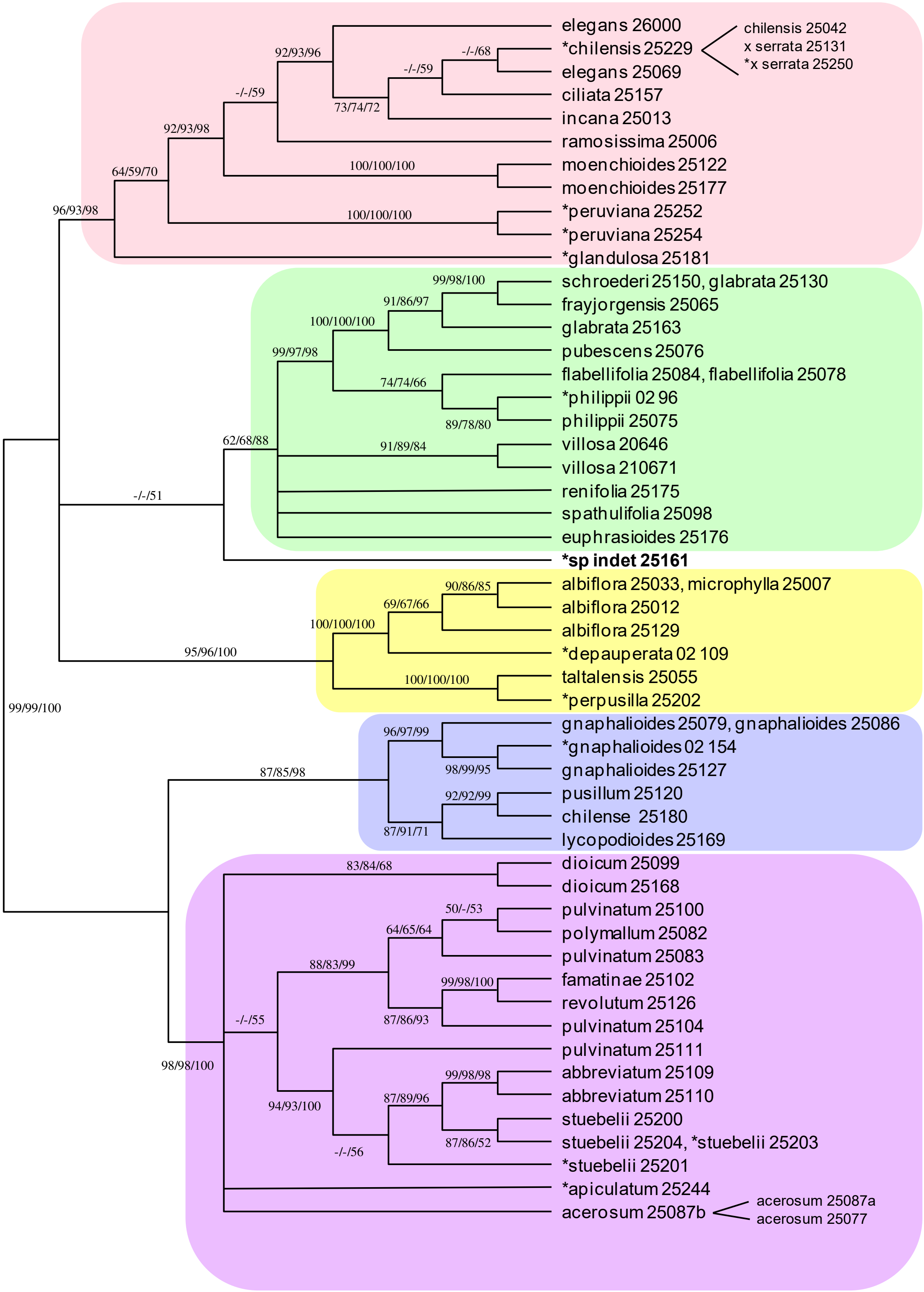

*chilensis 25229
chilensis 25042, elegans 26000
*x serrata 25250
ciliata 25157
elegans 25069
x serrata 25131
incana 25013
moenchioides 25122
moenchioides 25177
ramosissima 25006
*peruviana 25252
*peruviana 25254
*glandulosa 25181
schroederi 25150
pubescens 25076, glabrata_25130
frayjorgensis 25065
glabrata 25163
flabellifolia 25084, flabellifolia 25078
*philippii 02 96
philippii 25075
renifolia 25175
villosa 20646
villosa 210671
spathulifolia 25098
euphrasioides 25176
*sp indet 25161
albiflora 25033
microphylla 25007
albiflora 25129
*depauperata 02 109
albiflora 25012
taltalensis 25055
*perpusilla 25202
gnaphalioides 25079, gnaphalioides 25086
*gnaphalioides 02 154
gnaphalioides 25127
pusillum 25120
chilense 25180
lycopodioides 25169
dioicum 25099
dioicum 25168
pulvinatum 25100
pulvinatum 25083
abbreviatum 25109
polymallum 25082
famatinae 25102
pulvinatum 25104
revolutum 25126
abbreviatum 25110
stuebelii 25200
*stuebelii 25201
*stuebelii 25203
stuebelii 25204
pulvinatum 25111
acerosum 25077
*apiculatum 25244
acerosum 25087a
acerosum 25087b
0.005 substitutions/site

Fig. 5


elegans 26000
*chilensis 25229
chilensis 25042
x serrata 25131
*x serrata 25250
elegans 25069
ciliata 25157
incana 25013
ramosissima 25006
moenchioides 25122
moenchioides 25177
*peruviana 25252
*peruviana 25254
*glandulosa 25181
92/93/96
-/-/68
-/-/59
73/74/72
-/-/59
92/93/98
100/100/100
64/59/70
100/100/100
96/93/98
schroederi 25150, glabrata 25130
frayjorgensis 25065
glabrata 25163
pubescens 25076
flabellifolia 25084, flabellifolia 25078
*philippii 02 96
philippii 25075
villosa 20646
villosa 210671
renifolia 25175
spathulifolia 25098
euphrasioides 25176
***sp indet 25161**
99/98/100
91/86/97
100/100/100
99/97/98
74/74/66
89/78/80
62/68/88
91/89/84
-/-/51
albiflora 25033, microphylla 25007
albiflora 25012
albiflora 25129
*depauperata 02 109
taltalensis 25055
*perpusilla 25202
90/86/85
69/67/66
100/100/100
95/96/100
100/100/100
99/99/100
gnaphalioides 25079, gnaphalioides 25086
*gnaphalioides 02 154
gnaphalioides 25127
pusillum 25120
chilense 25180
lycopodioides 25169
96/97/99
98/99/95
87/85/98
92/92/99
87/91/71
dioicum 25099
dioicum 25168
pulvinatum 25100
polymallum 25082
pulvinatum 25083
famatinae 25102
revolutum 25126
pulvinatum 25104
pulvinatum 25111
abbreviatum 25109
abbreviatum 25110
stuebelii 25200
stuebelii 25204, *stuebelii 25203
*stuebelii 25201
*apiculatum 25244
acerosum 25087b
acerosum 25087a
acerosum 25077
83/84/68
50/-/53
64/65/64
88/83/99
99/98/100
-/-/55
87/86/93
98/98/100
99/98/98
94/93/100
87/89/96
87/86/52
-/-/56

Fig, 6

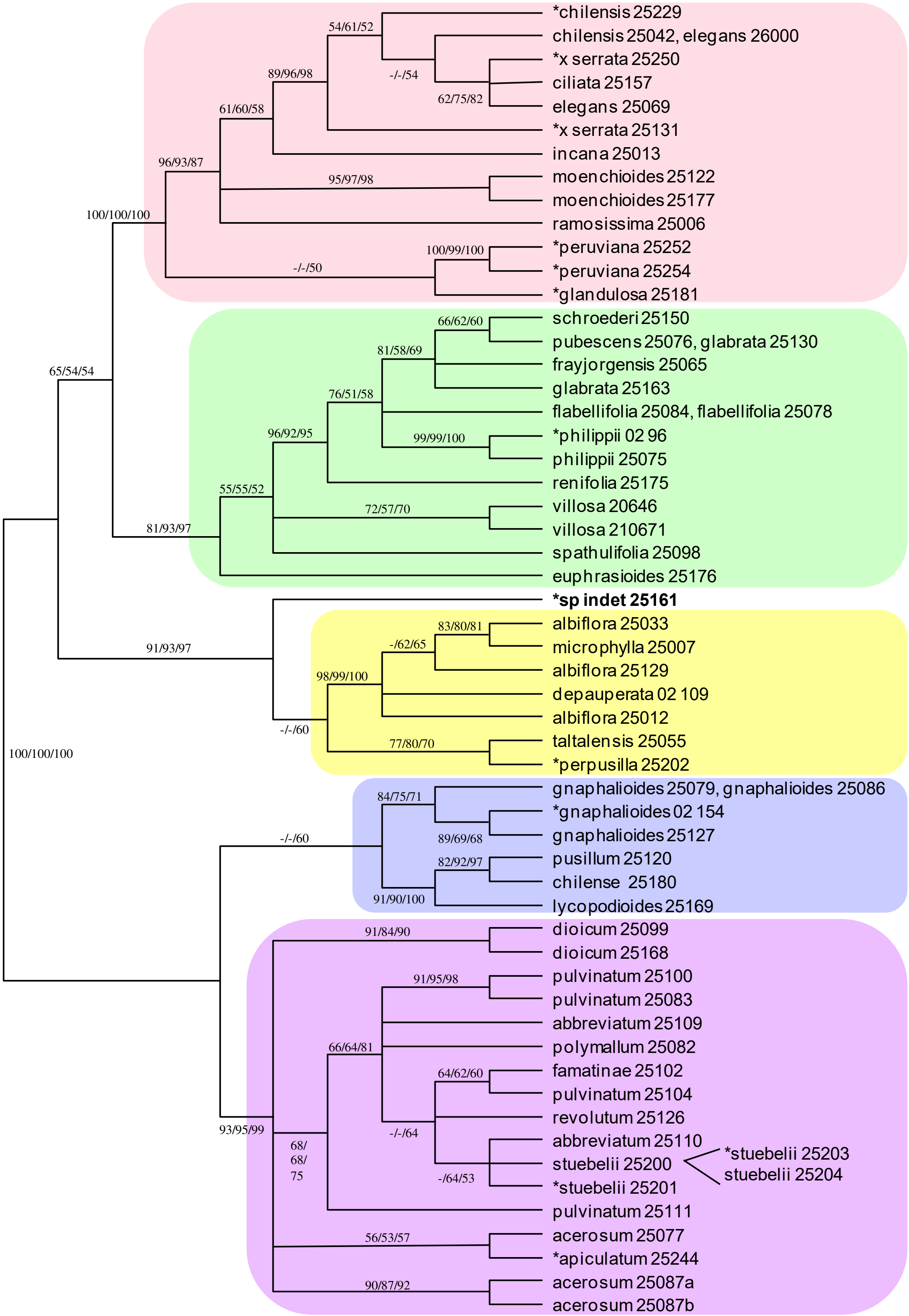

*chilensis 25229
chilensis 25042, elegans 26000
*x serrata 25250
ciliata 25157
elegans 25069
*x serrata 25131
incana 25013
moenchioides 25122
moenchioides 25177
ramosissima 25006
*peruviana 25252
*peruviana 25254
*glandulosa 25181
schroederi 25150
pubescens 25076, glabrata 25130
frayjorgensis 25065
glabrata 25163
flabellifolia 25084, flabellifolia 25078
*philippii 02 96
philippii 25075
renifolia 25175
villosa 20646
villosa 210671
spathulifolia 25098
euphrasioides 25176
*sp indet 25161
albiflora 25033
microphylla 25007
albiflora 25129
depauperata 02 109
albiflora 25012
taltalensis 25055
*perpusilla 25202
gnaphalioides 25079, gnaphalioides 25086
*gnaphalioides 02 154
gnaphalioides 25127
pusillum 25120
chilense 25180
lycopodioides 25169
dioicum 25099
dioicum 25168
pulvinatum 25100
pulvinatum 25083
abbreviatum 25109
polymallum 25082
famatinae 25102
pulvinatum 25104
revolutum 25126
abbreviatum 25110
stuebelii 25200
*stuebelii 25201
*stuebelii 25203
stuebelii 25204
pulvinatum 25111
acerosum 25077
*apiculatum 25244
acerosum 25087a
acerosum 25087b
54/61/52
89/96/98
-/-/54
62/75/82
61/60/58
96/93/87
95/97/98
100/100/100
100/99/100
-/-/50
66/62/60
81/58/69
76/51/58
96/92/95
99/99/100
65/54/54
55/55/52
72/57/70
81/93/97
83/80/81
-/62/65
98/99/100
91/93/97
-/-/60
77/80/70
100/100/100
84/75/71
-/-/60
89/69/68
82/92/97
91/90/100
91/84/90
91/95/98
66/64/81
64/62/60
-/-/64
93/95/99
68/
68/
75
-/64/53
56/53/57
90/87/92

Fig. 7

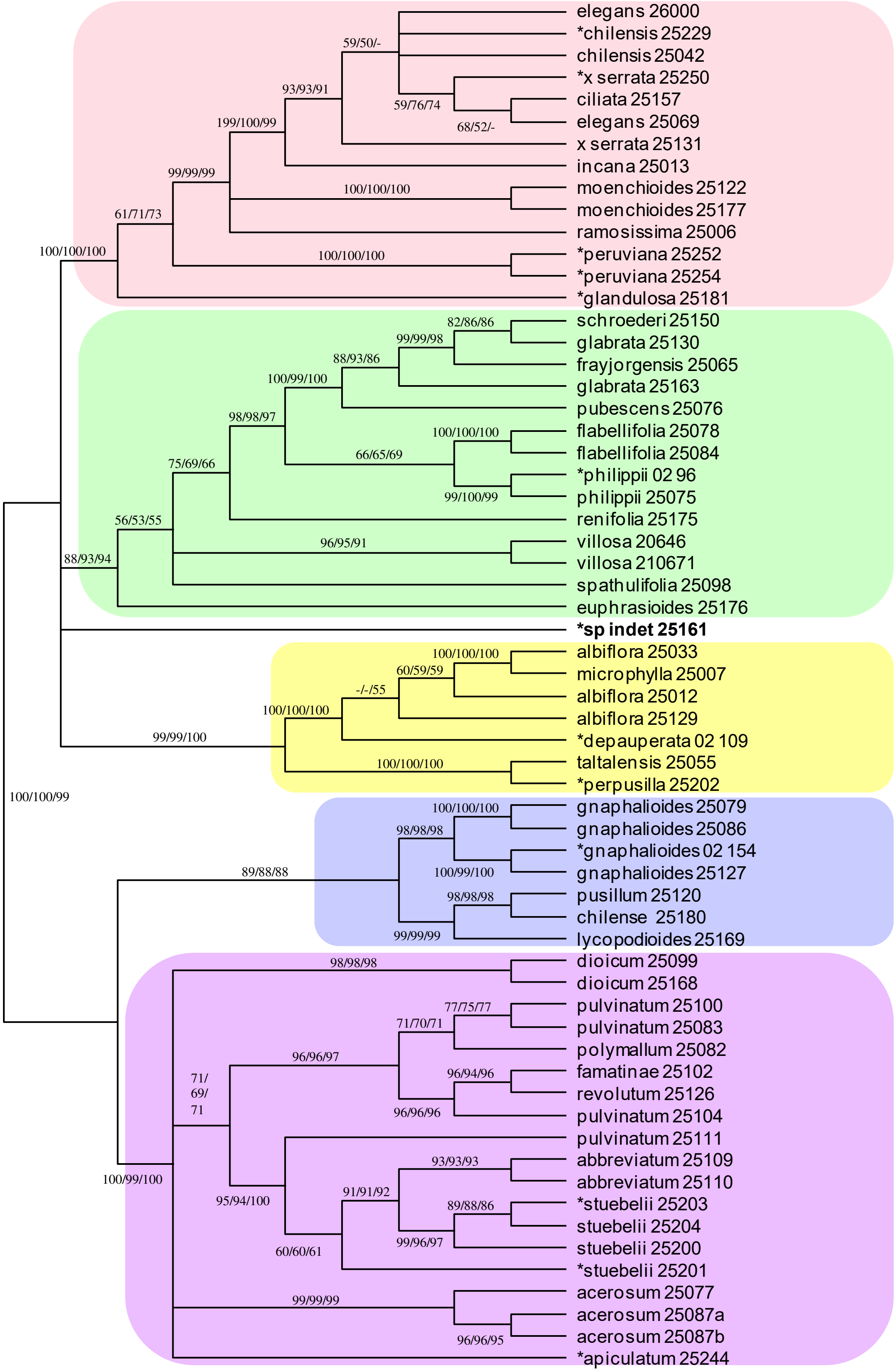

elegans 26000
*chilensis 25229
chilensis 25042
*x serrata 25250
ciliata 25157
elegans 25069
x serrata 25131
incana 25013
moenchioides 25122
moenchioides 25177
ramosissima 25006
*peruviana 25252
*peruviana 25254
*glandulosa 25181
schroederi 25150
glabrata 25130
frayjorgensis 25065
glabrata 25163
pubescens 25076
flabellifolia 25078
flabellifolia 25084
*philippii 02 96
philippii 25075
renifolia 25175
villosa 20646
villosa 210671
spathulifolia 25098
euphrasioides 25176
*sp indet 25161
albiflora 25033
microphylla 25007
albiflora 25012
albiflora 25129
*depauperata 02 109
taltalensis 25055
*perpusilla 25202
gnaphalioides 25079
gnaphalioides 25086
*gnaphalioides 02 154
gnaphalioides 25127
pusillum 25120
chilense 25180
lycopodioides 25169
dioicum 25099
dioicum 25168
pulvinatum 25100
pulvinatum 25083
polymallum 25082
famatinae 25102
revolutum 25126
pulvinatum 25104
pulvinatum 25111
abbreviatum 25109
abbreviatum 25110
*stuebelii 25203
stuebelii 25204
stuebelii 25200
*stuebelii 25201
acerosum 25077
acerosum 25087a
acerosum 25087b
*apiculatum 25244
59/50/-
93/93/91
59/76/74
199/100/99
68/52/-
99/99/99
100/100/100
61/71/73
100/100/100
100/100/100
82/86/86
99/99/98
88/93/86
100/99/100
98/98/97
100/100/100
75/69/66
66/65/69
99/100/99
56/53/55
96/95/91
88/93/94
100/100/100
60/59/59
-/-/55
100/100/100
99/99/100
100/100/100
100/100/99
100/100/100
98/98/98
89/88/88
100/99/100
98/98/98
99/99/99
98/98/98
77/75/77
71/70/71
96/96/97
96/94/96
71/
69/
71
96/96/96
93/93/93
100/99/100
95/94/100
91/91/92
89/88/86
99/96/97
60/60/61
99/99/99
96/96/95

Fig. 8

```
                                                                                           /.......................... elegans 26000          [1]
                                                                                           +.......................... chilensis 25229        [2]
                                                                                           +.......................... chilensis 25042        [3]
                                                                                /....93....+           /.............. x serrata 25250        [7]
                                                                                |          +.....76.....+............. ciliata 25157          [35]
                                                                   /.....99.....+          |            \............. elegans 25069          [36]
                                                                   |            |          \.......................... x serrata 25131        [34]
                                                                   |            \..................................... incana 25013           [33]
                                                       /....99.....+                                  /............... moenchioides 25122     [6]
                                                       |           +...............100...............+................ moenchioides 25177     [32]
                                            /....74....+           \.................................................. ramosissima 25006      [31]
            /..............100..............+          |                                         /.................... peruviana 25252        [8]
            |                               |          \...................100....................+................... peruviana 25254        [9]
            |                               \......................................................................... glandulosa 25181       [30]
                                                                                                             /........ schroederi 25150       [4]
            |                                                                                  /.....84.....+......... glabrata 25130         [14]
            |                                                                        /....99....+..................... frayjorgensis 25065    [12]
            |                                                           /.....92.....+................................ glabrata 25163         [13]
            |                                               /....100....+............................................. pubescens 25076        [16]
            |                                   /....98.....+                                           /............. flabellifolia 25078    [10]
            |                                   |           |                              /....100.....+............. flabellifolia 25084    [11]
            |                       /....69.....+           \..............68...............+           /............. philippii 02 96        [17]
/..........+                        |           |                                          \....100.....+............. philippii 25075        [18]
|           |          /.....53.....+           \..................................................................... renifolia 25175        [15]
|           |          |            |                                                            /.................... villosa 20646          [5]
|          +....92.....+            +............................95..............................+.................... villosa 210671         [20]
|           |          |            \................................................................................. spathulifolia 25098    [19]
|           |          \.............................................................................................. euphrasiodes 25176     [22]
|          +..........................................................................................................sp indet 25161          [21]
|           |                                                                                          /.............. albiflora 25033        [23]
|           |                                                                             /....100.....+.............. microphylla 25007      [25]
|           |                                                                 /....60.....+........................... albiflora 25012        [27]
|           |                                                    /.....57.....+....................................... albiflora 25129        [24]
|           |                                        /....100....+.................................................... depauperata 02 109     [26]
100         \...................99...................+                                          /.................... taltalensis 25055      [28]
|                                                    \...................100....................+..................... perpusilla 25202       [29]
|                                                                                                /.................... gnaphalioides 25079    [37]
|                                                                                  /....100......+.................... gnaphalioides 25086    [51]
|                                                                       /....98....+            /..................... gnaphalioides 02 154   [51]
|                   /........................88.........................+          \.....99.....+..................... gnaphalioides 25127    [52]
|                   |                                                   |                       /..................... pusillum 25120         [38]
|                   |                                                   |          /.....98.....+..................... chilense  25180        [60]
|                   |                                                   \....98....+.................................. lycopodioides 25169    [62]
|                   |                                                                                     /........... dioicum 25099          [39]
|                   |            /..............................98.............................+...................... dioicum 25168          [49]
|                   |            |                                                                  /................. pulvinatum 25100       [40]
\...................+            |                                                      /....79.....+................. pulvinatum 25083       [48]
                    |            |                                          /....72.....+............................. polymallum 25082       [43]
                    |            |           /..............97..............+                        /................ famatinae 25102        [44]
                    |            |           |                              |            /.....96....+................ revolutum 25126        [45]
                    |            +....70.....+                              \....95.....+............................. pulvinatum 25104       [61]
                    |            |           |           /............................................................ pulvinatum 25111       [41]
                    \.....100....+           |           |                                                /........... abbreviatum 25109      [42]
                                 |           \.....94....+                          /..........92.........+........... abbreviatum 25110      [46]
                                 |                        |            /.....91.....+                         /....... stuebelii 25203        [47]
                                 |                        \.....60.....+            |            /.....88.....+....... stuebelii 25204        [58]
                                 |                                     |            \.....97.....+.................... stuebelii 25200        [56]
                                 |                                     \.............................................. stuebelii 25201        [57]
                                 |                                                  /................................. acerosum 25077         [50]
                                 +........................98........................+           /..................... acerosum 25087a        [54]
                                 |                                                  \.....96....+..................... acerosum 25087b        [59]
                                 \................................................................................... apiculatum 25244       [55]
```

Fig. 9

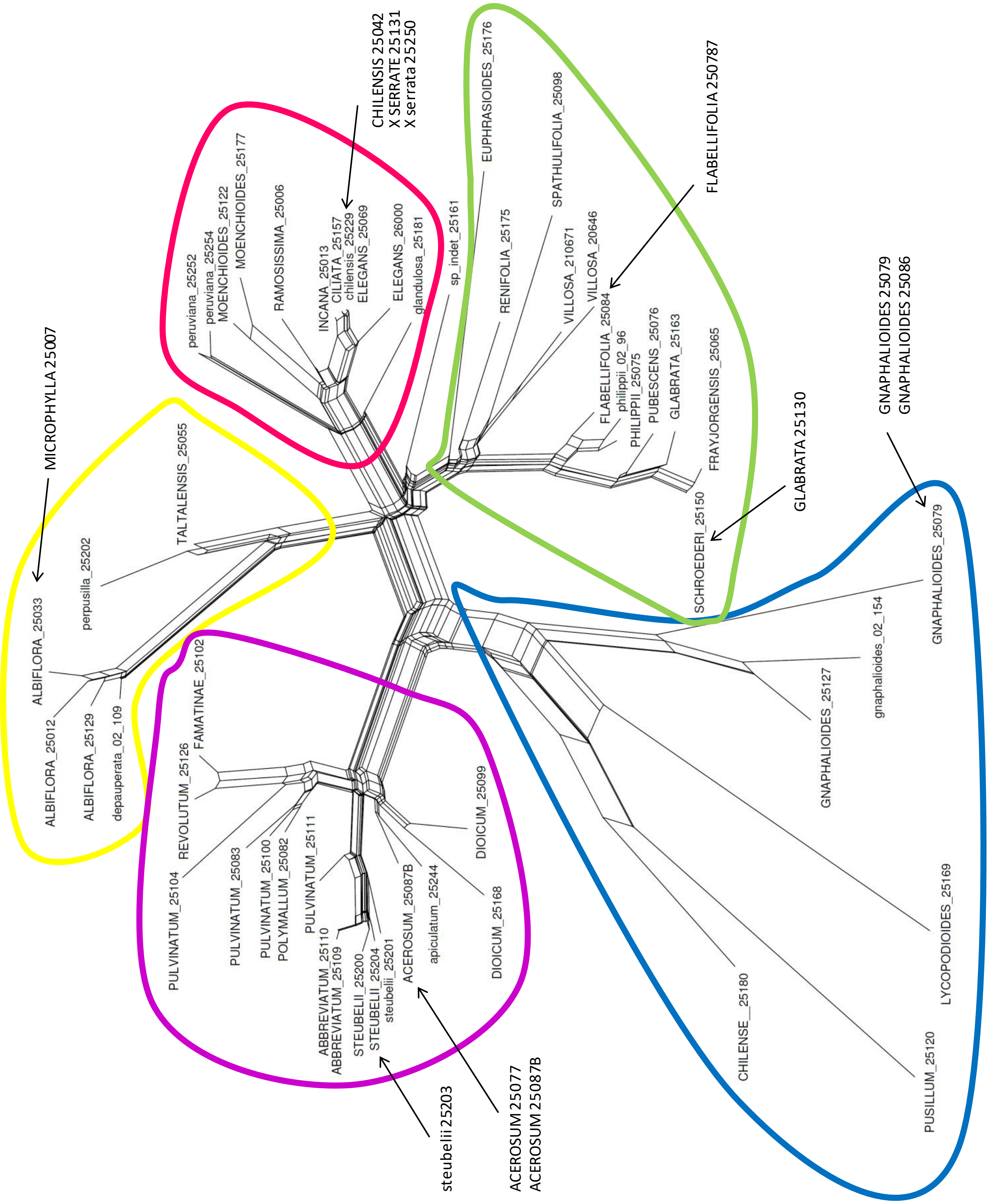

CHILENSIS 25042
X SERRATE 25131
X serrata 25250
peruviana_25252
peruviana_25254
MOENCHIOIDES_25122
MOENCHIOIDES_25177
RAMOSISSIMA_25006
INCANA_25013
CILIATA_25157
chilensis_25229
ELEGANS_25069
ELEGANS_26000
glandulosa_25181
sp_indet_25161
EUPHRASIOIDES_25176
SPATHULIFOLIA_25098
RENIFOLIA_25175
VILLOSA_210671
VILLOSA_20646
FLABELLIFOLIA 250787
FLABELLIFOLIA_25084
philippii_02_96
PHILIPPII_25075
PUBESCENS_25076
GLABRATA_25163
FRAYJORGENSIS_25065
SCHROEDERI_25150
GLABRATA 25130
GNAPHALIOIDES 25079
GNAPHALIOIDES 25086
GNAPHALIOIDES_25079
gnaphalioides_02_154
GNAPHALIOIDES_25127
LYCOPODIOIDES_25169
CHILENSE__25180
PUSILLUM_25120
MICROPHYLLA 25007
TALTALENSIS_25055
perpusilla_25202
ALBIFLORA_25033
ALBIFLORA_25012
ALBIFLORA_25129
depauperata_02_109
FAMATINAE_25102
REVOLUTUM_25126
PULVINATUM_25104
PULVINATUM_25083
PULVINATUM_25100
POLYMALLUM_25082
PULVINATUM_25111
ABBREVIATUM_25110
ABBREVIATUM_25109
STEUBELII_25200
STEUBELII_25204
steubelii_25201
ACEROSUM_25087B
apiculatum_25244
DIOICUM_25099
DIOICUM_25168
steubelii 25203
ACEROSUM 25077
ACEROSUM 25087B

Fig. 10

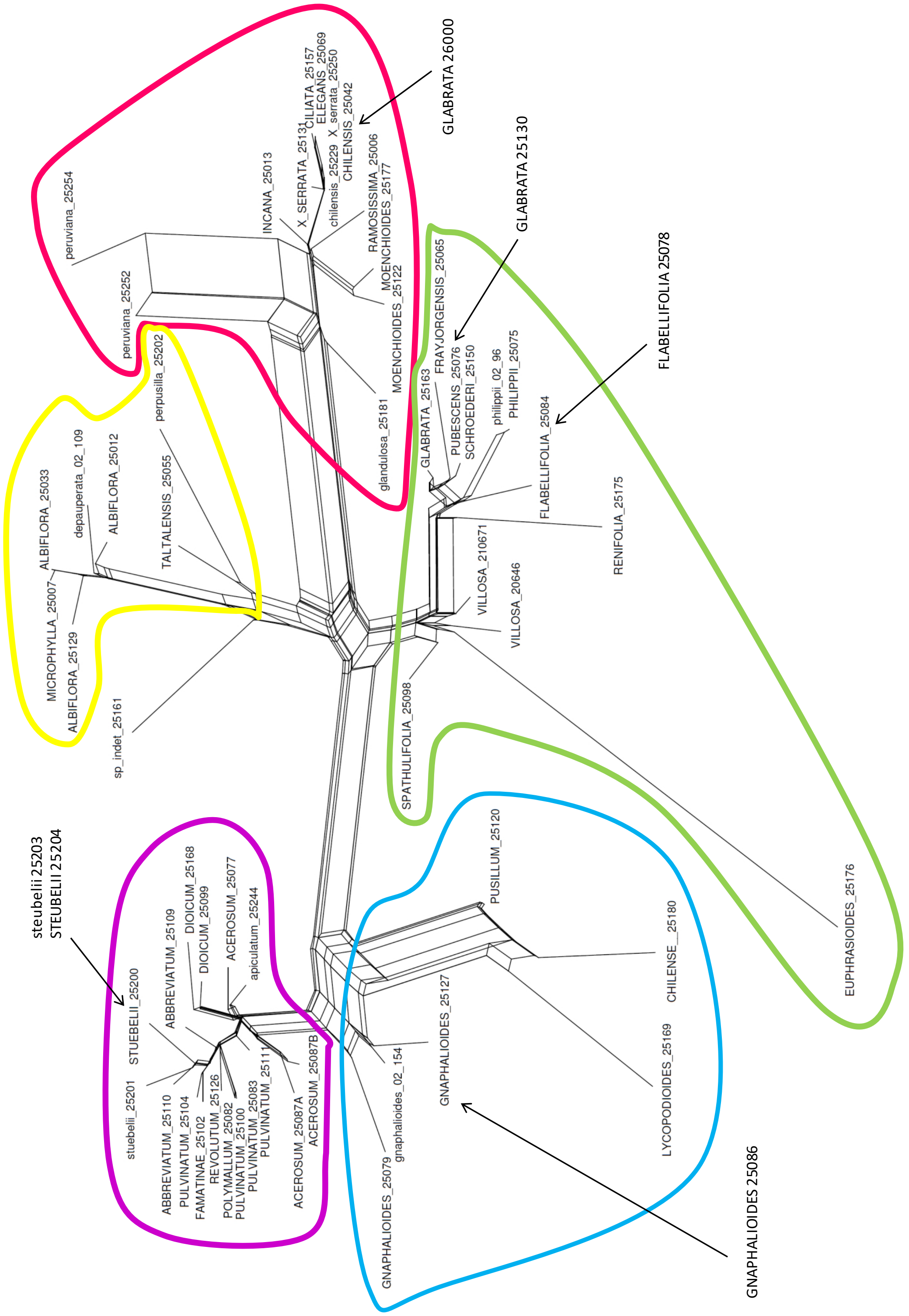

GLABRATA 26000
GLABRATA 25130
FLABELLIFOLIA 25078
peruviana_25254
peruviana_25252
INCANA_25013
X_SERRATA_25131
CILIATA_25157
ELEGANS_25069
X_serrata_25250
chilensis_25229
CHILENSIS_25042
RAMOSISSIMA_25006
MOENCHIOIDES_25177
MOENCHIOIDES_25122
MOENCHIOIDES_25181
glandulosa_25181
perpusilla_25202
ALBIFLORA_25033
depauperata_02_109
ALBIFLORA_25012
TALTALENSIS_25055
MICROPHYLLA_25007
ALBIFLORA_25129
sp_indet_25161
GLABRATA_25163
FRAYJORGENSIS_25065
PUBESCENS_25076
SCHROEDERI_25150
philippii_02_96
PHILIPPII_25075
FLABELLIFOLIA_25084
RENIFOLIA_25175
VILLOSA_210671
VILLOSA_20646
SPATHULIFOLIA_25098
EUPHRASIOIDES_25176
steubelii 25203
STEUBELII 25204
STUEBELII_25200
stuebelii_25201
ABBREVIATUM_25109
DIOICUM_25168
DIOICUM_25099
ACEROSUM_25077
apiculatum_25244
ABBREVIATUM_25110
PULVINATUM_25104
FAMATINAE_25102
REVOLUTUM_25126
POLYMALLUM_25082
PULVINATUM_25100
PULVINATUM_25083
PULVINATUM_25111
ACEROSUM_25087A
ACEROSUM_25087B
PUSILLUM_25120
CHILENSE__25180
GNAPHALIOIDES_25127
gnaphalioides_02_154
GNAPHALIOIDES_25079
LYCOPODIOIDES_25169
GNAPHALIOIDES 25086

Fig. 11

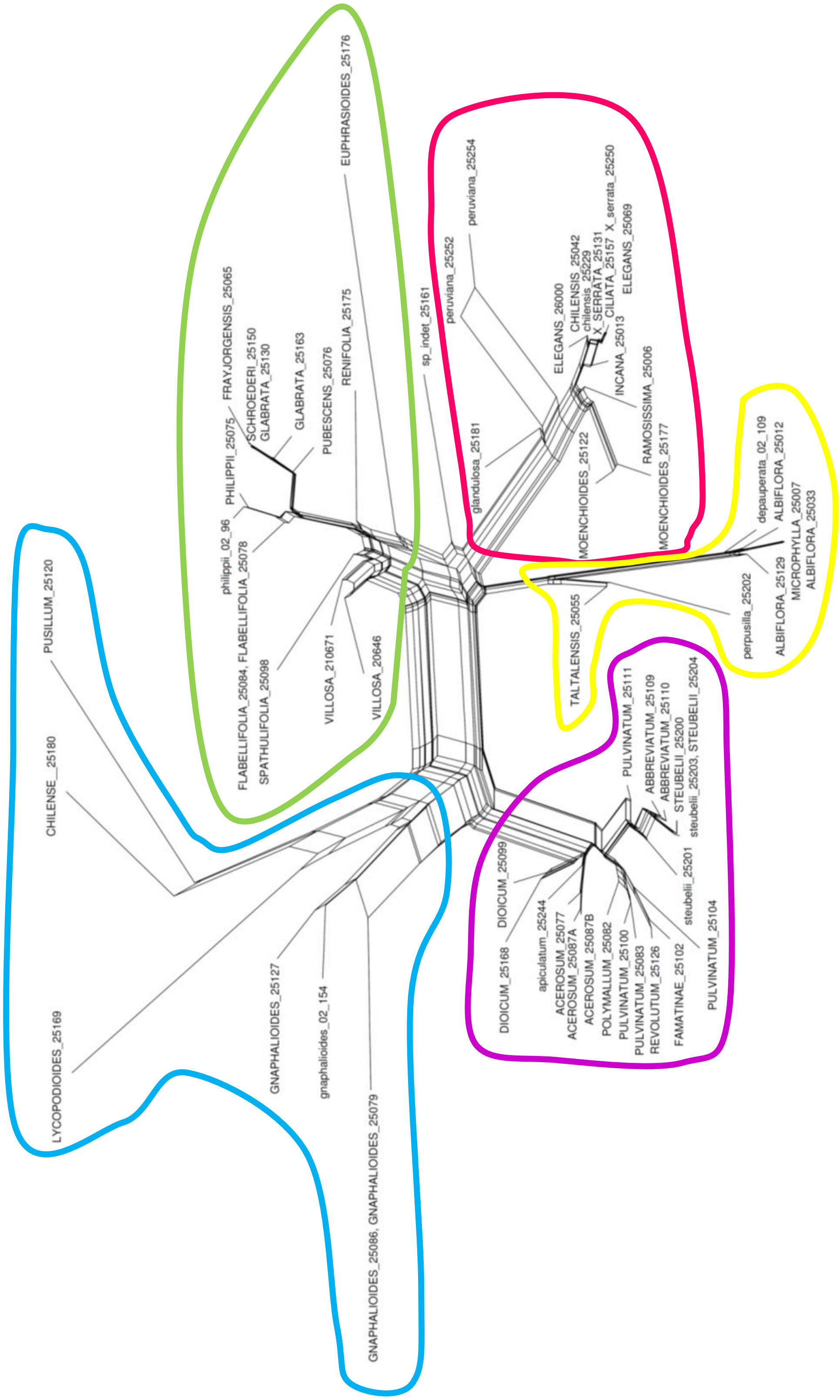

EUPHRASIOIDES_25176
FRAYJORGENSIS_25065
SCHROEDERI_25150
GLABRATA_25130
GLABRATA_25163
PUBESCENS_25076
RENIFOLIA_25175
PHILIPPII_25075
philippii_02_96
FLABELLIFOLIA_25084, FLABELLIFOLIA_25078
SPATHULIFOLIA_25098
VILLOSA_210671
VILLOSA_20646
sp_indet_25161
peruviana_25252
peruviana_25254
glandulosa_25181
ELEGANS_26000
CHILENSIS_25042
chilensis_25229
X_SERRATA_25131
CILIATA_25157
X_serrata_25250
ELEGANS_25069
INCANA_25013
RAMOSISSIMA_25006
MOENCHIOIDES_25122
MOENCHIOIDES_25177
depauperata_02_109
ALBIFLORA_25012
MICROPHYLLA_25007
ALBIFLORA_25033
ALBIFLORA_25129
perpusilla_25202
TALTALENSIS_25055
PULVINATUM_25111
ABBREVIATUM_25109
ABBREVIATUM_25110
STEUBELII_25200
steubelii_25203, STEUBELII_25204
steubelii_25201
PULVINATUM_25104
DIOICUM_25099
DIOICUM_25168
apiculatum_25244
ACEROSUM_25077
ACEROSUM_25087A
ACEROSUM_25087B
POLYMALLUM_25082
PULVINATUM_25100
PULVINATUM_25083
REVOLUTUM_25126
FAMATINAE_25102
PUSILLUM_25120
CHILENSE_25180
LYCOPODIOIDES_25169
GNAPHALIOIDES_25127
gnaphalioides_02_154
GNAPHALIOIDES_25086, GNAPHALIOIDES_25079

Fig. 12