

Supporting Information

This document comprises the following items:

- APPENDIX S3: Additional discussion relative to the phylogenetic position of the genus *Tetrapus* (Agaonidae).
- APPENDIX S4: Review of the literature on fig fossils (leaves, woods, seeds and fruits).
- SUPPORTING FIGURES S1-S14.
- SUPPORTING TABLES S1-S6.

APPENDIX S3. Additional discussion relative to the phylogenetic position of the genus *Tetrapus* (Agaonidae).

By not placing *Tetrapus* as sister to all other agaonids, our topology challenges all previous molecular studies on fig pollinators. However, for clarity and brevity of the main manuscript, we prefer to address the issues raised by this result in the following supplementary section (written by Astrid Cruaud and Jean-Yves Rasplus).

INTRODUCTION: POSITION OF *TETRAPUS*, A BRIEF OVERVIEW

Molecular phylogenies — *Tetrapus* has consistently been recovered as sister to all other agaonids by previous molecular studies (Herre et al., 1996; Machado et al., 1996; Machado et al., 2001; Lopez-Vaamonde et al., 2009; Cruaud et al., 2010, see Table S3 for a review of taxonomic and gene sampling used as well as phylogenetic reconstruction methods). Furthermore, two independent datasets, both analysed using Bayesian methods, have provided strong support for this placement (Lopez-Vaamonde et al., 2009; Cruaud et al., 2010). As a consequence, *Tetrapus* has been often used to root agaonid phylogenies (Jiang et al., 2006; Cruaud et al., 2011).

Structural variation in the mtDNA of the Agaonidae— Herre et al. (1996) proposed that *Tetrapus* placement as sister to all other pollinators was supported by a long AT rich intergenic spacer between COI and t-RNA_{Leu}, which was present in all agaonids but was lacking in both *Tetrapus* and the non pollinating fig wasps they used as outgroups (*Idarnes* sp. and *Critogaster* sp.).

Morphology—While *Tetrapus* is consistently recovered as sister to the other agaonids based on molecular data, the two available morphological studies by Ramirez (1978) and Wiebes (1982b) do not support this view (Table S3). In Ramirez' hypothesis, *Tetrapus* is sister to a group clustering *Dolichoris*, *Pleistodontes*, the pollinators of the section *Galoglychia* and the subsection *Conosycea*, which is in agreement with the topology we propose here. In Wiebes' hypothesis, *Tetrapus* is part of the subfamily Agaoninae and sister to *Pleistodontes* and the pollinators of section *Galoglychia*. Specifically, two characters were proposed as evidence for a sister group relationships between *Tetrapus* and the other agaonids: the structure of the maxillary palpi (Wiebes, 1982a) and the presence of sensilla on flagellomere 2 (Wiebes, 1995). The character states present in *Tetrapus* were proposed to be plesiomorphic (i.e. shared with all other Chalcidoidea).

ADDITIONAL RESULTS AND DISCUSSION: WHAT DO THE MOLECULES TELL US?

Trying to understand why we observe a shift in *Tetrapus* position on our phylogenetics trees, we conducted several additional analyses.

Exploration of bias in the proposed phylogeny

Missing data—Analyses conducted to assess the impact of missing data on the accuracy of our phylogeny, resulted in topologies that were congruent with the topology estimated from the global dataset (Fig. S2 B&C, Table S2). In both cases (removal of incomplete taxa sequenced on less than five genes or removal of gene fragments for which less than 60% of the taxa were available), *Tetrapus* is recovered nested within Agaonidae and AU and SH tests

indicate that the combined ML tree, pruned from incomplete taxa, explains the data from a given reduced dataset as well as the ML tree for that reduced dataset. Therefore, missing data are not responsible for the new placement of the genus *Tetrapus*.

Long Branch attraction of the Kradibiinae toward the outgroups— Previous analyses of mtDNA sequences (*COI*) have shown that *Ceratosolen* and *Kradibia* have significantly higher rates of substitution (Machado et al., 2001). Hence, there is a suspicion that Kradibiinae may tend to be artificially attracted towards the outgroups. Kradibiinae is sister to all other agaonids in the mtDNA topology (Fig. S3a). The nuDNA dataset places Kradibiinae as the sister group of the remaining Agaonidae after Group 4 (most *Wiebesia* species and pollinators of subsection *Frustescentiae*, see Main Text) (Fig. S3b). In both cases, there is no support for these sister taxa relationships.

Examination of branch lengths on all trees (mtDNA, nuDNA and combined dataset) indicates considerable variation in rates of molecular evolution among agaonid lineages, which is confirmed by the Bayesian relative rate (BRR) tests (Figs. S4 & S5). For each partition of the combined analysis, BEAST returns a 95% credible interval for the coefficient of variation of rates that is not abutting against zero, suggesting among branch rate heterogeneity. Furthermore, the covariance statistics show no strong evidence of autocorrelation of rates in the combined phylogeny (covariance values spanning zero). Mapping of the average rate for each branch on the BEAST chronogram indicates that fast rates of nucleotide substitution are not specific to *Ceratosolen* and *Kradibia* (Fig. S4).

Among *Ceratosolen* species, those associated with *Ficus* section *Sycocarpus* show higher mitochondrial substitution rates than most other pollinator species (FigS5a). This could explain the Machado et al. (2001) observation. Indeed, Machado et al.'s (2001) sampling includes ten *Ceratosolen* species, 60% of them being associated with *Ficus* section

Sycocarpus. They also sampled *C. arabicus* (and one closely related species), which appears here as one of the fastest evolving *Ceratosolen* species associated with *Ficus* section *Sycomorus*. *Kradibia* species do not exhibit significant higher mitochondrial substitution rate.

Finally, when we conducted analyses without *Ceratosolen* and *Kradibia* species or with a reduced number of species from both genera, Group 4 is recovered as sister to the rest of the Agaonidae with strong (BP=76) or low (BP=31) support, respectively (Fig. S2 K&L).

Based on these results, the question of which group is sister to the rest of agaonids should be regarded as unsettled. Statistical supports for the deeper nodes of the phylogeny are low and preclude us from drawing any definite conclusion. While either Kradibiinae or Group 4 are good candidates for a sister group to the remainder of Agaonidae, *Tetrapus* is probably best placed nested within the Agaonidae.

Were previous molecular studies misled by a LBA artifact?

Additional analyses on our dataset— Interestingly, on both the nuDNA tree (Fig S3b) and the ML and Bayesian combined trees (Fig S1), the branch leading to *Tetrapus* is long. BRR tests and branch-specific rates inferred by BEAST also reveal a lineage-specific increase in nucleotide substitution rates on this branch (Fig. S4).

To test whether our analyses (or previous studies) could be confounded by an LBA artifact, we first RY-coded 1) third mtDNA codon positions and 2) first plus third mtDNA codon positions. ML analysis of these datasets generated topologies that were not significantly different from the original one (Fig. S2 D&E, Table S2). Using RY-coding increases support for *Tetrapus* nested within the Agaonidae (BP=76 when nt1 is RY-coded; BP=75 when nt1 and nt3 are RY-coded). It is also noteworthy that, while not supported

(BP=31), Group 4 is recovered as sister to all other agaonids when the first and third mtDNA codon positions are RY-coded.

We also ran analyses without the outgroups included. Unrooted and rooted topologies appeared congruent (Fig. S2 J) showing that rooting does not alter the ingroup topology by pulling any agaonid group to a different position. However, rooting with the most distantly related outgroup only (*Trichogramma evanescens*, (Munro et al., 2011) places *Tetrapus* as sister to all other agaonids with strong support (BP=77, Fig. S2 I). Either Kradibiinae or Group 4 (or an unsupported clade clustering both groups) were recovered as sister to the remaining agaonids when the more closely related *Ficomila* sp., *Megastigmus* sp. or *Sycophaga cyclostigma* were used as outgroup (Fig. S2 F-H). In these last cases, support for *Tetrapus* nested within Agaonidae were strong (BP=85, BP=77, BP=70 respectively).

Finally, parsimony analysis of our combined dataset recovers *Tetrapus* as sister to all other agaonids with moderate support (BP=64) (Fig. S6).

Based on all these results we suspect that *Tetrapus* past position as sister to all other agaonid might have resulted from a LBA to the outgroups.

Re-analysis of previous datasets— As mentioned in the introduction, two independent datasets, both analysed using Bayesian methods, have provided strong support for the placement of *Tetrapus* as sister to the remaining Agaonidae (Lopez-Vaamonde et al., 2009; Cruaud et al., 2010). When we reanalyze these two datasets using ML methods (with same partition schemes), support for *Tetrapus* placement as sister to all other agaonids remains strong for the Lopez-Vaamonde et al. (2009) dataset (BP=92), but it decreases for the Cruaud et al. (2010) dataset (BP=40) (not shown). This confirms that PP may overestimate clade support in some cases (e.g. Suzuki et al., 2002).

For the Cruaud et al. (2010) dataset, both AU and SH tests fail to reject alternative topologies in which Kradibiinae or Group 4 were constrained to be sister to all other agaonids (Table S2). For the Lopez-Vaamonde et al. (2009) dataset, both AU and SH tests fail to reject alternative topologies in which Group 4 was constrained to be sister to all other agaonids, whereas placement of Kradibiinae as sister to all other agaonids is rejected at $P \leq 0.05$ but not at $P \leq 0.03$ (Table S2). Consequently, there is no genuine evidence to support *Tetratus* as sister to all other Agaonidae based on molecular studies.

To test whether *Tetratus* past position as sister to all other agaonids could have resulted from an LBA to the outgroups, we first reanalysed the Cruaud et al. (2010) dataset without *Trichogramma evanescens*. In the recovered topology, *Tetratus* is nested within the Agaonidae (without support, Fig. S7 a), and its long branch becomes more evident. Then, we reanalysed the Lopez-Vaamonde et al. (2009) and the Cruaud et al. (2010) datasets without the outgroups included and found that none of the recovered topologies show conflict with the topology we propose here (Fig. S7 b-c). It has been shown that ML and Bayesian methods can be subject to LBA (e.g. Lockhart et al., 1996; Brinkmann et al., 2005; Kolaczkowski and Thornton, 2009). Considering only one distant outgroup (*Anaphes nitens*, Mymaridae, see Munro et al., 2011) could exacerbate the LBA artifact for the Lopez-Vaamonde et al. (2009) dataset (e.g. Philippe et al., 2005b; Holton and Pisani, 2010)

These results provide additional support to the hypothesis that *Tetratus* previous position could have been due to an LBA artifact to the outgroups.

Differences between this dataset and previous ones?

One notable difference between ours and previous studies concerns taxon sampling, which can be critical in phylogenetic analyses (e.g. Philippe et al., 2005a). By including a

larger number of species and using a sampling that reflects the known diversity of each group, we may counteract potential problems with long branches (e.g. Bergsten, 2005). Increasing taxonomic sampling to break up long branches has been applied repeatedly often with the conclusion that earlier studies were misled (e.g. Soltis and Soltis, 2004; Stefanović et al., 2004; Leebens-Mack et al., 2005; Phillips et al., 2010; Philippe et al., 2011).

Reassessment of the evidence from the structural variation in the mtDNA of the Agaonidae proposed by Herre et al. (1996)

Herre et al. (1996) and Machado et al. (2001) proposed that *Tetrapus* position as sister to all other pollinators was supported by a long AT rich intergenic spacer between COI and t-RNA_{Leu}, which was present in all agaonids but was lacking in both *Tetrapus* and the non pollinating fig wasps they used as outgroups (*Idarnes* sp. and *Critogaster* sp.). They argue that this observation is significant “given that the mitochondrial genomes of the majority of metazoans lack such non-coding sequences [with a reference to Crozier and Crozier, 1993]”.

It appears from a review of the literature that with increasing sequencing of mtDNA, the presence of non-coding intergenic spacer regions between coding genes is now considered relatively common in insects. Intergenic spacers have been found in several groups of Hymenoptera [*Apis* (Crozier et al., 1989; Cornuet et al., 1991; Garnery et al., 1998a; Rortais et al., 2011), *Atta* (Wetterer et al., 1998; Martins Jr. et al., 2007), Evaniidae (Wei et al., 2010b), Pteromalidae (Oliveira et al., 2008), Agaonidae (Herre et al., 1996)], and in most insect orders [Coleoptera (Bae et al., 2004; Sheffield et al., 2009), Diptera (da Silva et al., 2009; Yang et al., 2011), Hemiptera (Dotson and Beard, 2001), Lepidoptera (Sperling et al., 1999; Kim et al., 2009; Feng et al., 2010; Zhao et al., 2011), Mecoptera (Beckenbach, 2011), Neuroptera (Negrisolo et al., 2011), Odonata (Lin et al., 2010), Orthoptera (Kim et al., 2005), Strepsiptera (McMahon et al., 2009) and Thysanoptera (Shao and Barker, 2003)]. Their exact

origin and function are often unclear but their number and size and the length of main non-coding regions are responsible for the length variations of mitogenomes in species.

Furthermore, recent studies on Hymenoptera and Chalcidoidea mitogenomes have shown that a series of rearrangements in gene order affect the relative position of tRNAs and several protein-coding genes (Castro et al., 2006; Oliveira et al., 2008; Wei et al., 2010a; Wei et al., 2010b; Xiao et al., 2011). Indeed, a large inversion (COIII, A6, A8, D, K, COI, L2, COII) appeared specific to the Chalcidoidea (Oliveira et al., 2008; Xiao et al., 2011), and a translocation of ND2 is found in Pteromalidae (Xiao et al., 2011). Consequently, the hypothesis that mtDNA structure is fixed in insects and more specifically in Hymenoptera is not valid (Dowton et al., 2003; Shao and Barker, 2003; Cameron et al., 2006; Castro et al., 2006; Cameron et al., 2008; Gotzek et al., 2010). On average, intergenic spacers are less than 50 bp but the spacer between COI and t-RNA^{Leu} can for example range in length from 144 to 152 bp in the genus *Atta* (Formicidae) (Wetterer et al., 1998; Martins Jr. et al., 2007). Some longer non-coding spacers are also found in *Apis* (193 bp between t-RNA^{Leu} and COII, thought to be another origin of replication, (Tan et al., 2011)), *Triatoma* (314 bp between t-RNA^{Ser} TCN and ND1 (Dotson and Beard, 2001)), *Diadegma* (1515 bp between COI and COII, (Wei et al., 2009)), and *Pyrocoelia* (1724 bp of tandem repeats unit that is possibly an intergenic spacer, (Bae et al., 2004)). These spacers are AT-rich, sometimes composed of tandem repeats, and may exhibit infraspecific variability (i.e. the COI-COII intergenic region mtDNA enable to characterize diversity between and within honeybee lineages (Franck et al., 1998; Garnery et al., 1998a, b)).

To examine Herre et al.s (1996) hypothesis, we downloaded and aligned all the chalcidoid sequences covering the zone between between COI and t-RNA^{Leu} available in GenBank. Unfortunately, the sequences of Herre et al. (1996) were not available, so three

sequences of Chalcidoidea belonging to the same clades as their outgroups (Pteromalidae s.s. and Sycophaginae) were used instead: *Nasonia*, *Philotrypesis* and *Sycophaga* (Weiblen, 2001; Oliveira et al., 2008; Munro et al., 2011; Xiao et al., 2011). Fourteen sequences belonging to agaonid species and representing twelve of the twenty genera, namely *Tetrapus*, *Dolichoris* (2), *Wiebesia*, *Ceratosolen*, *Kradibia* (3), *Blastophaga*, *Valisia*, *Platyscapa*, *Eupristina*, *Alfonsiella*, *Pleistodontes* were used as ingroup (Yokohama, 1994; Weiblen, 2001; Lopez-Vaamonde et al., 2009; Table S4).

When aligning all these sequences, we identified a portion of COI that did not align well. The length of this region was highly variable (from 9 bp to 153 bp; Fig. S8, Table S4). While this region was at most 21 bp long in the outgroups, it reached 43 bp in *Tetrapus* species. The same region was shorter in two *Kradibia* species (19 bp), which made it shorter than the one from *Philotrypesis* (outgroup). Consequently, *Tetrapus* was neither as short as the outgroups nor shorter than any other Agaonidae.

The zone considered by Herre et al (1996) as an « AT rich intergenic sequence » corresponds to the C-terminal region of COI (Lunt et al., 1996), which is not always an intergenic non-coding spacer. Indeed, this region may exhibit either terminal or intermediate stop codons. In the first condition, the coding COI ends with an incomplete termination codon (T) that abuts the t-RNA^{Leu} and the completion of the stop codon (TAA) is probably realised by post-transcriptional polyadenylation (Ojala et al., 1981). This condition is observed in the outgroups and also in three Agaonidae species (*Kradibia wassae*, *Wiebesia frustratae* and *W. punctatae*). However, this is not the case for *Tetrapus*. In the second condition, which is encountered in most studied Agaonidae including *Tetrapus* but not in the outgroups, a stop codon is present in the terminal part of COI in variable position. This stop codon (mostly TAA but TAG in *Ceratosolen*) delimits a coding COI gene upstream and a non-coding intergenic spacer downstream. The spacer length varies from 1 pb (*Kradibia copiosae*) to 56

pb (*Eupristina verticillata*). In *Tetrapus* the spacer is short (11 pb) but longer than spacers observed in *Kradibia* species, *Dolichoris umbilicata* or *Valisia malayana* (Table S4). Consequently, *Tetrapus* does not exhibit a structure of the ends of COI similar to the outgroups (Pteromalidae and Sycophaginae). In fact *Kradibia copiosae* exhibit a much similar structure to the outgroups and the structure observed in *Tetrapus* closely match those observed in some *Kradibia* and in *Dolichoris boschmai*.

Conclusion

Following all these results, we argue that there is no genuine evidence to support *Tetrapus* as sister to all other Agaonidae based on molecular studies. To the contrary we suggest that *Tetrapus* previous position was probably due to an LBA artifact to the outgroups and that the C-terminal region of COI of *Tetrapus* is not closer to the outgroups than any other Agaonidae.

ADDITIONAL RESULTS AND DISCUSSION: WHAT DOES THE MORPHOLOGY TELL US?

Although a detailed discussion on the characters used by Ramirez (1978) and Wiebes (1982) in the light of molecular results could have been useful, it has never been conducted so far. Conducting a morphological analysis of the Agaonidae is also beyond the scope of the present study. However, to further investigate *Tetrapus* phylogenetic placement, we mapped three morphological character-state transformations [1) male mesosoma (external and internal structures), 2) maximum number of teeth on the protibia of males and 3) female first flagellomere (fl1)] onto the two alternative phylogenetic hypotheses (*Tetrapus* nested within the Agaonidae *versus* sister of all other Agaonidae). Indeed, mapping morphological characters with discrete states onto phylogenetic hypotheses may help to reveal the polarity of

the character state transitions and determine whether the state observed in *Tetrapus* disrupts the transformation series. Illustrations of the character states and results of the mappings are shown in Figure S14. Character state coding is based on observations of more than 200 species of Agaonidae.

All three characters show strong evidence of evolutionary directionality. There is a trend toward reduction of either the number of segments (male mesosoma and female fl1) or the number of teeth (protibia of males). In all cases *Tetrapus* does not exhibit the ancestral character state. In *Tetrapus*, the propodeum and the metanotum are fused externally, and there is no sulcus between these sclerites. Consequently, the male mesosoma is subdivided into three parts (Fig. S14 A&C). The ancestral condition is observed in several species of *Ceratosolen*, *Kradibia* and *Wiebesia*, which have a separated propodeum and metanotum. Consequently, the male mesosoma is subdivided into four parts (Fig. S14 A&C). The internal mesosomal structure also suggests that *Tetrapus* is best placed within our phylogenetic hypothesis rather than within previous reconstructions. Indeed, the mesosomal structure of males is similar to that observed in several *Dolichoris* species. Both genera exhibit a metasomal notum that bridges the metasomal pleurae (Fig. S14 B&D), an apomorphy linking these two genera. The maximum number of teeth on the protibia of male *Tetrapus* is five, the same number as in *Dolichoris* and *Valisia* (Fig. S14 E). This number places *Tetrapus* within Agaonidae but not sister to all other Agaonidae. Indeed the maximum number of teeth is respectively nine and eight in *Ceratosolen* and *Kradibia* (Kradibiinae). Finally, the female first flagellomere structure is uninformative, as the character state observed in *Tetrapus* is a reduction in the number of segments, a character state that has independently evolved four times in the family (Fig. S14 F). We also analysed two characters that were supposed to be shared exclusively by *Tetrapus* and the other Chalcidoidea (maxillary palpus and sensillae placodea on fl2). Again, character states observed in *Tetrapus* are shared with *Dolichoris*

species (Fig. S14 G&H) and do not support a sister-group relationship with other Agaonidae.

In conclusion, there is no morphological character that unambiguously place *Tetrapus* sister to all other Agaonidae. Instead, there is growing morphological evidence suggesting that *Tetrapus* is more closely related to *Dolichoris* than to any other Agaonidae genera.

GENERAL CONCLUSION: GO BACK TO WORK!

As mentioned in the main text, the question of which group is sister to the rest of the Agaonidae should be regarded as unsettled and either Kradibiinae or Group 4 (most *Wiebesia* species and pollinators of subsection *Frustescentiae*) are good candidates. Further studies using more genes and increased taxonomic sampling (ingroup and outgroup species) are needed.

We argue that there is no genuine evidence to support *Tetrapus* as sister to all other Agaonidae based on molecular and morphological data. Instead, *Tetrapus* is probably nested within Agaonidae. Its phylogenetic position is still to be resolved but several independent morphological characters suggest strong affinities with *Dolichoris*. However, more data (both morphological and molecular characters) are needed to provide a robust phylogenetic position of genus *Tetrapus*.

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APPENDIX S4: Review of the literature on fig fossils (leaves, woods, seeds and fruits).

(written by Jean-Yves Rasplus).

We conducted a thorough examination of the literature dealing with fig fossils, not only including leaves but also woods, seeds and fruits. We also re-examined the descriptions and illustrations of several fossils to ascertain or not their assignation to *Ficus*. Most recent discussions on fig fossils are based on rather old reviews and mostly did not consider fossils of fig woods (i.e. Compton et al, 2010). They consequently diffuse a partial understanding of the paleo-distribution of figs.

Although numerous fossils have been referred to *Ficus* (including *Ficoxylon* and *Ficophyllum*), a high proportion of them was misidentified and did not belong to figs (Collinson, 1989). Recent re-assessment of putative *Ficus* fossils showed that they instead belong to other families of plants (Lauraceae, Rhamnaceae, Malvaceae) (Givilescu, 1994; Mai and Walther, 2000; Jungwirth, 2004; Kvacek and Wilde, 2010; Srivastava et al, 2010).

Nowadays, most if not all of the reports of *Ficus* leaves from North America are now considered not to belong to Moraceae. To give an example, re-examination of the photos of the type of *Ficus affinis* (shown on page 993 in Taylor et al, 2009) undoubtly suggests that it is not a *Ficus* species but instead an Ulmaceae.

In Asia, *Ficus* is the genus best represented in the fossil record of Moraceae and has been collected from Late Cretaceous to Late Pliocene sediments (Li and Zheng, 1995; Sun et al., 1995). The oldest fig fossil known from Asia (India) has been discovered in the Deccan intertrappean flora. This formation dated back to 61.7-70.6 Ma and hosted some putative

Ficus leaves (*Ficus ramentacea*) (Khare *et al.*, 2000) but was not formally described and is controversial (Srivastava and Guleria, 2006). For most of the Asiatic *Ficus* fossils, the affinities are considered very uncertain and some of these fossils that we re-examined clearly does not belong to *Ficus* (*F. longipedia*, *F. parasemicordata*, *F. shanwangensis*). Among the fossils of fig leaves described in China and India, only a few are still considered as true *Ficus* (Srivastava *et al.*, 2010). Until all available material of described fig fossils from Asia will be thoroughly re-examined (which is clearly not the purpose of this paper), there will be doubt about their correct assignation to the genus.

Several Eurasian fig fossils are still believed to belong to the genus *Ficus* (Srivastava *et al.*, 2010) and some clearly belong there. This is the case for two fossils of fig leaves from the Paleocene-Eocene boundary. One species has been discovered in the Kutch of Gujarat, Upper Paleocene (55.8 – 58.7 Ma)(Lakhanpal *et al.*, 1984; Mehrotra, 2003) and another fig (*Ficus cherrapunjiensis*) has been found in Late Eocene – Paleocene (48.6 – 58.7 Ma) coal deposits in Cherrapunji, West Khasi Hills District, Meghalaya (Ambwani, 1991). These specimens could present the oldest fig fossils known to date and deserve further examination to ascertain the recent re-examination (Srivastava *et al.*, 2010).

In the Neotropics, most of the identification of fig fossils by Berry (1921) have been repeatedly questioned over the past 50 years (Burham and Graham, 1999). Indeed, most of the species described by Berry clearly does not belong to the genus whereas some other like *F. tressensis* from the Palo Pintado formation (Miocene) appears to be good species of figs (sect. *Americana*) (Anzótegui, 1998). Nice fossils of fig leaves have been discovered from the Miocene-Pliocene formation of Bolivia (www-personal.umich.edu/~rburnham/). However, most of the Eocene citations from South-America remains to be confirmed and examination

of the fig drawings in Berry's papers clearly show that most of the species he described are not *Ficus* species. It is noteworthy that no *Pharmacosycea* fossils have been reported so far.

Ficus leaves (putative or not) have never been collected from ancient formations of South-America and the oldest fossil is probably an *Americana* species dating back to the Oligocene-Miocene boundary (*F. talamanca*) (Berry, 1921). Indeed, it appears that fossil *Ficus* were neither discovered in Laguna del Hunco and Río Pichileufú floras from Patagonia, Argentina (Wilf et al., 2003) nor in the Cerrejon formation of Colombia (Wing et al, 2009). Although studies of fossil floras in Central and South America are somewhat limited, no fig fossils have been discovered to date from these well known deposits. Despite that Laguna del Hunco flora is not considered a tropical flora, it could nevertheless include fig trees. Indeed, this Patagonian fig flora hosts one *Papuacedrus* species very closely related to extent tropical Papuan species (Wilf et al., 2009). In Papua New Guinea and in Papua Barat (Indonesia), this conifer is found in the same mountainous habitats as *Malvanthera* fig species at altitudes above 2000m (for example in the Arfak mountains, Kebar Valley, Bulolo-Wau, Mt Kerewa). Consequently, we argue that figs could indeed be found in these deposits and the observed absence of figs in these deposits is therefore relevant to the question of when figs arrived in the Neotropics.

Ficus wood (*Ficoxylon*) anatomy is remarkably homogeneous and *Ficus* wood can be distinguished from wood of other Moraceae by several structural features (Koek-Noorman et al., 1984). Several fossil woods have been assigned to *Ficoxylon* (Kaiser, 1880). Since the review by Collison (1989), anatomical characteristics of *Ficoxylon* have been redescribed accurately by Jolly-Saad et al. (2010) and several Old world species probably belong to *Ficus*. Wood considered as *Ficus* was first cited (*Ficoxylon cretaceum*) from sediments dating from

the Cretaceous to Lower Oligocene-Lower Miocene of northern Africa (Schenk et al., 1883, Kamal-El-Din, 2003) and from Danian (65,5 et 61,7 Ma) of Mali (Boureau and Monod, 1949). However, all these fossils need to be re-examined to confirm their assignment to the genus *Ficus* following the standards given by Koek-Noorman et al., (1984), Martínez-Cabrera, (2006) and Jolly-Saad et al. (2010). Several other *Fycoxylon* species are known from the Oligocene of Libya (Dupéron-Laudoueneix and Dupéron, 1995).

To the possible exception of a *Ficoxylon* fossil from the middle Eocene reported by Manchester (1981), the oldest *Ficoxylon* woods from the American continent appears to belong to the El Cien Formation in Mexico from the Miocene (Martínez-Cabrera, 2006). This wood has been shown to be both quantitatively and qualitatively very similar to the extant species of *Ficus* (Martínez-Cabrera, 2006). In South America, only one species of *Ficoxylon* has been buried out in Colombia (Boureau and Salard, 1962) but was stated to be the same as *Crudioxylon* (Pons, 1980).

In Asia, the oldest fossil wood of *Ficoxylon* was reported from the Eocene of China (Prakash, 1965) and *F. saurinii* from Cambodia (Boureau, 1950) is possibly from late Cretaceous, but the exact age of this fossil is still unclear.

In Europe, a *Ficus* wood was recently reported in Italy from Middle Eocene (Bartonian, ca 40 Ma) deposits, a report considered as the oldest known European wood of the genus (Bernabei et al., 2010).

Recently, seeds of *Ficus* have been discovered from Miocene Coal in Yunnan (Zhao et al., 2004). However, *Ficus* are cleistogamous and wasp pollinated. Therefore, the genus is severely under-represented in modern dispersed pollen assemblages (Bush & Riviera 2001).

In summary, our review shows that:

- 1) Assignment of fossil leaves to the genus *Ficus* results mostly from misidentification. Most of these fossil leaves belong to other plant families.
- 2) Oldest fig fossils (leaves and woods) are known only from Eurasia and northern Africa. Fig fossils appear to be ubiquitous by the Miocene and are found on all continents at that time.
- 3) Only recent fossils have been discovered from South America and figs appear to be absent from the Eocene well prospected formation of the Laguna del Hunco. Specifically no *Pharmacosycea* fossils have been reported so far.

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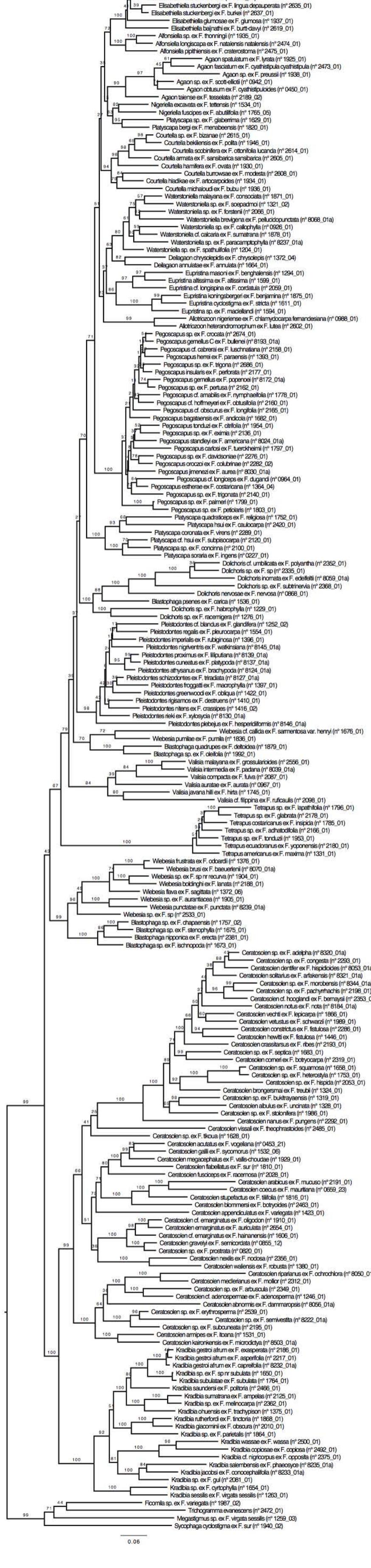
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FIGURE S1. Phylogram of relationships among agaonoids

a- based on ML analyses. Bootstrap percentages are indicated above branches.

b- based on Bayesian analyses. Posterior probabilities are indicated above branches.

a)



b)

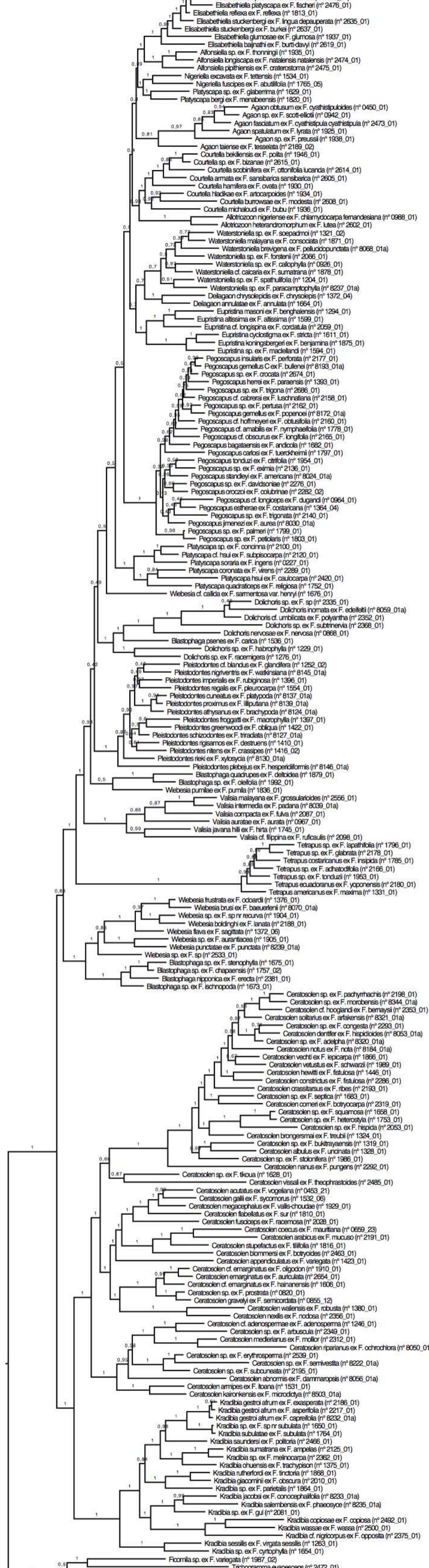


FIGURE S2: Summary results of the additional analyses conducted on the agaonid dataset.

A: original dataset, B: using only taxa for which more than five genes were sequenced, C: without gene fragment for which less than 60% of the taxa were available, D: with mtDNA third codon position RY-coded, E: with mtDNA first and third codon positions RY-coded, F: with *Ficomila* sp. used as outgroup, G with *Megastigmus* sp. used as outgroup, H with *Sycophaga cyclostigma* used as outgroup I: with *Trichogramma evanescens* used as outgroup, J: without outgroup, K: without *Ceratosolen* and *Kradibia* species, L: with a reduced sampling of *Ceratosolen* and *Kradibia* species (see Table S2 for the detailed list). Bootstrap percentages (1000 replicates) are indicated at nodes. Nodes are collapsed for BP<70, to the exception of the backbone of the tree.

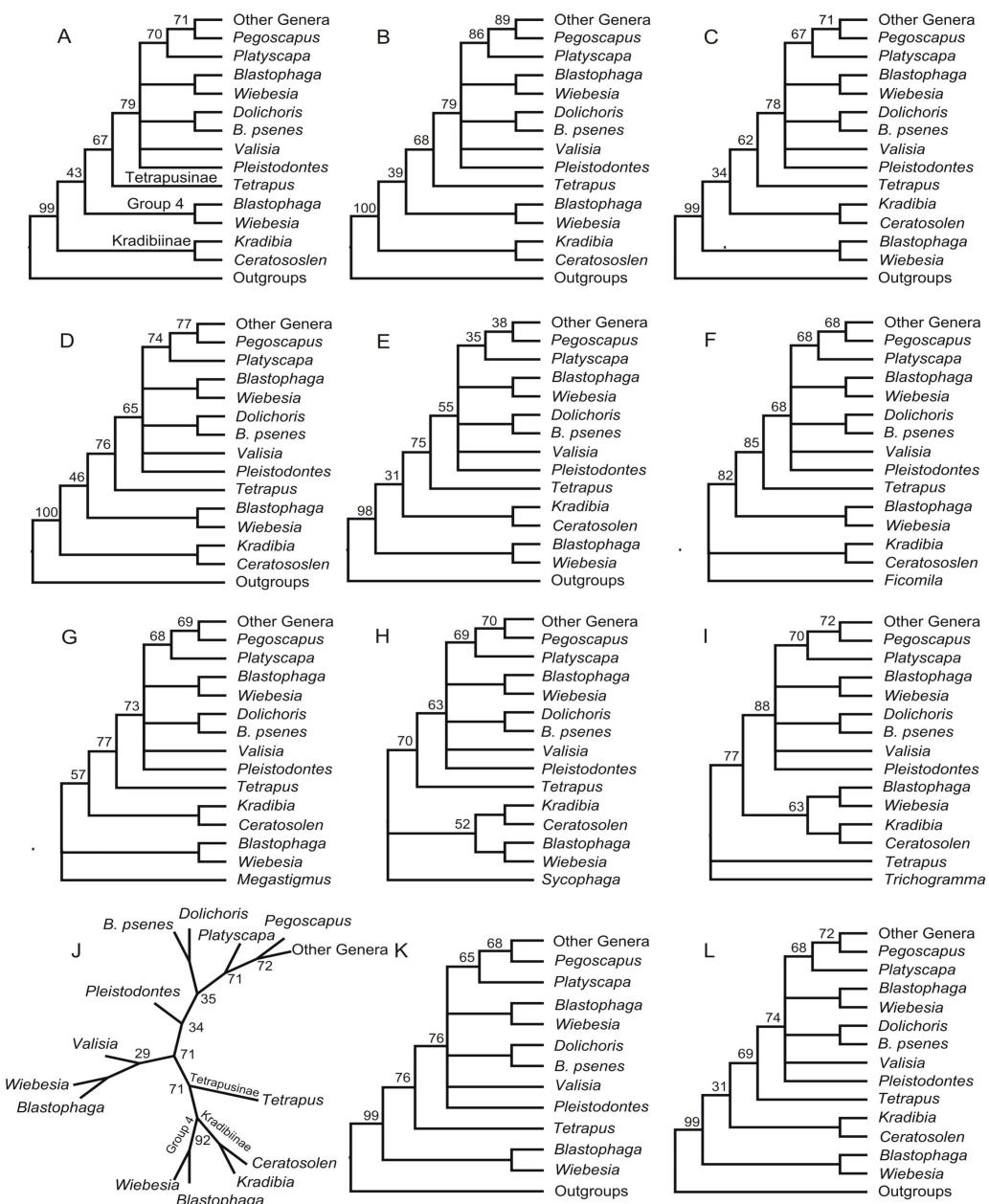
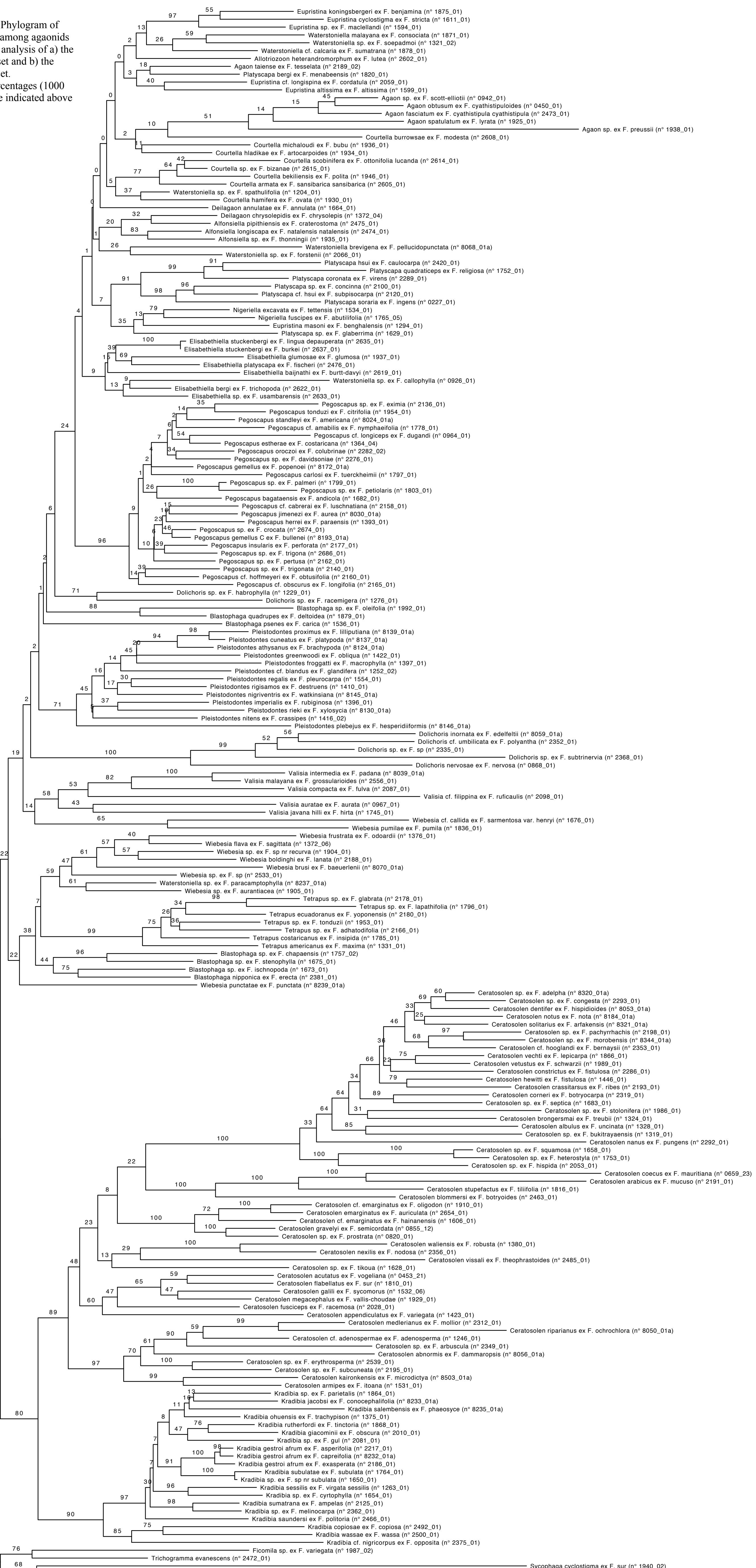


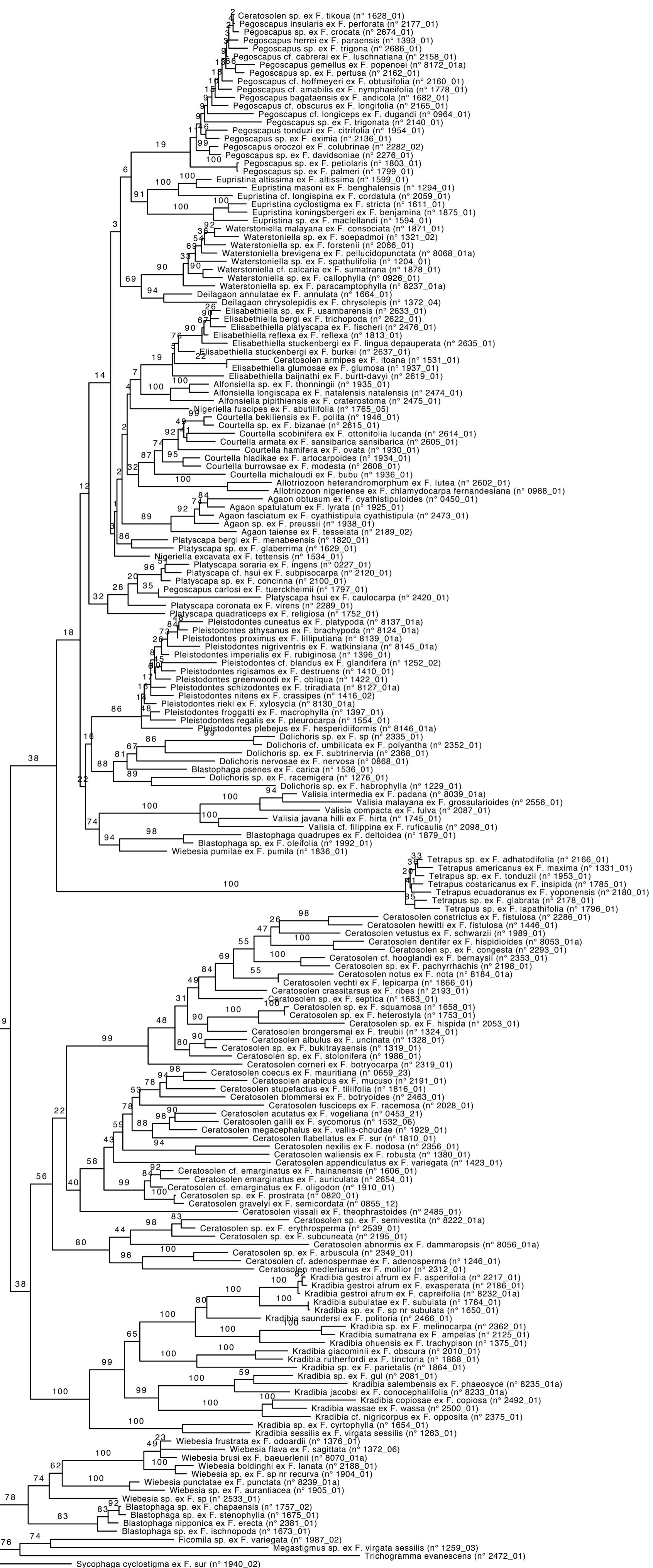
FIGURE S3. Phylogram of relationships among agaonids based on ML analysis of a) the mtDNA dataset and b) the nuDNA dataset. Bootstrap percentages (1000 replicates) are indicated above branches.

a)



0.09

b)



0.07

FIGURE S4. Comparison of rates of molecular evolution among agaonid lineages.

a- BEAST chronogram from the combined dataset (cf Fig S13) with branches colored to indicate substitution rates. Colour gradient is as follows: from green (slow rates) to black branches (fast rates) through red. Median rates are indicated above branches. Units are in substitutions/site/Myr.

b- Distribution of branch lengths from the most recent common ancestor of the agaonids to all tips. Branch lengths were compiled with v1.08b based on 1000 randomly chosen post burn-in trees from the bayesian analyses of the combined dataset. The tick marks indicate the mean branch length and the 95% confidence intervals are highlighted using pale grey bars. Units are in substitutions/site.

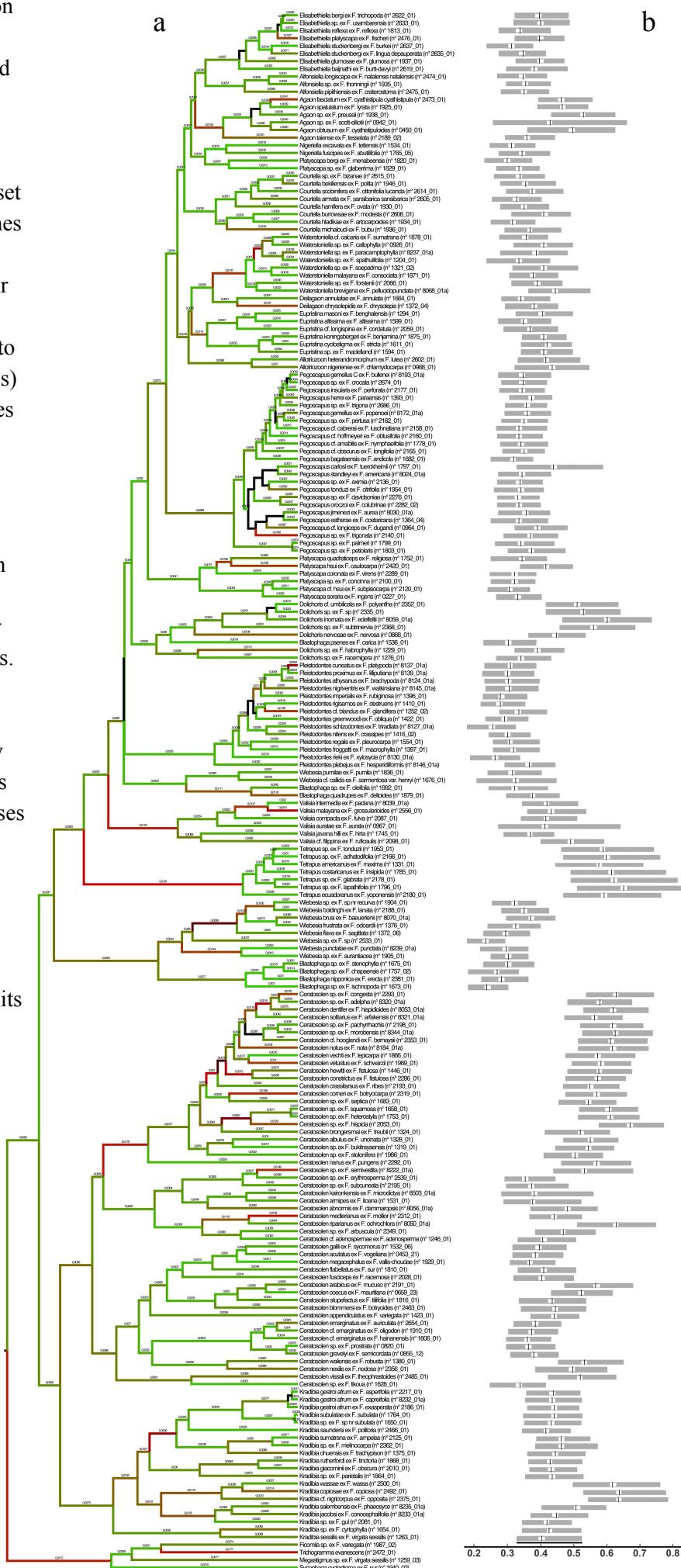
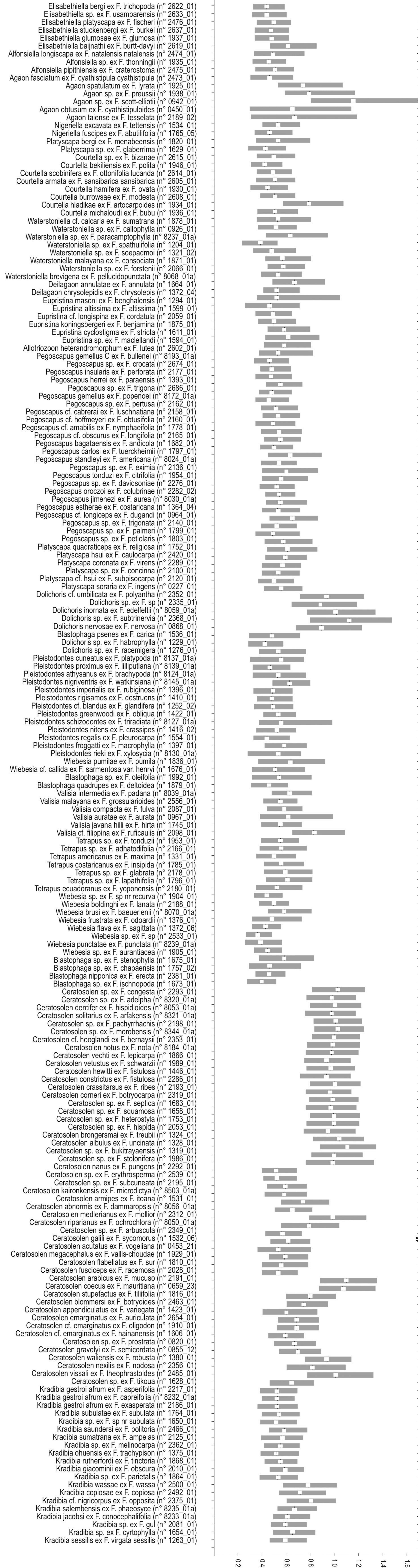


FIGURE S5. Distribution of branch lengths from the most recent common ancestor of the agaonids to all tips for:

- a) the mtDNA dataset.
b) the nuDNA dataset.

Branch lengths were compiled with v1.08b based on 1000 randomly chosen post burn-in trees from the bayesian analyses of either the mtDNA or the nuDNA dataset. Species are arranged following the same branching order as in the combined tree. Units are in substitutions/site.



b)

Elisabethiella bergii ex *F. trichopoda* (n° 2622_01)
Elisabethiella sp. ex *F. usambarensis* (n° 2633_01)
Elisabethiella reflexa ex *F. reflexa* (n° 1813_01)
Elisabethiella platyscapa ex *F. fischeri* (n° 2476_01)
Elisabethiella stuckenbergi ex *F. burkei* (n° 2637_01)
Elisabethiella stuckenbergi ex *F. lingua depauperata* (n° 2635_01)
Elisabethiella glumosa ex *F. glumosa* (n° 1937_01)
Elisabethiella bajinathi ex *F. burtt-davyi* (n° 2619_01)
Alfonsiella longiscapa ex *F. natalensis natalensis* (n° 2474_01)
Alfonsiella sp. ex *F. thonningii* (n° 1935_01)
Alfonsiella pipithiensis ex *F. craterostoma* (n° 2475_01)
Agaon fasciatum ex *F. cyathistipula cyathistipula* (n° 2473_01)
Agaon spatulatum ex *F. lyrata* (n° 1925_01)
Agaon sp. ex *F. preussii* (n° 1938_01)
Agaon obtusum ex *F. cyathistipuloides* (n° 0450_01)
Agaon tainense ex *F. tessellata* (n° 2189_02)
Nigeriella excavata ex *F. tettensis* (n° 1534_01)
Nigeriella fuscipes ex *F. abutilifolia* (n° 1765_05)
Platyscapa bergi ex *F. menabeensis* (n° 1820_01)
Platyscapa sp. ex *F. glaberrima* (n° 1629_01)
Courtella sp. ex *F. bizanae* (n° 2615_01)
Courtella bekiliensis ex *F. polita* (n° 1946_01)
Courtella scobinifera ex *F. ottonifolia lucanda* (n° 2614_01)
Courtella armata ex *F. sansibarica sansibarica* (n° 2605_01)
Courtella hamifera ex *F. ovata* (n° 1930_01)
Courtella burrowsae ex *F. modesta* (n° 2608_01)
Courtella hladikae ex *F. artocarpoides* (n° 1934_01)
Courtella michaloudi ex *F. bubu* (n° 1936_01)
Waterstoniella cf. *calcaria* ex *F. sumatrana* (n° 1878_01)
Waterstoniella sp. ex *F. callophylla* (n° 0926_01)
Waterstoniella sp. ex *F. paracamptophylla* (n° 8237_01)
Waterstoniella sp. ex *F. spathulifolia* (n° 1204_01)
Waterstoniella sp. ex *F. soepadmoi* (n° 1321_02)
Waterstoniella malayana ex *F. consociata* (n° 1871_01)
Waterstoniella sp. ex *F. forsternii* (n° 2066_01)
Waterstoniella brevigena ex *F. pellucidopunctata* (n° 8068_01a)
Delagoaon annulatae ex *F. annulata* (n° 1664_01)
Deilagaon chrysolepidis ex *F. chrysolepis* (n° 1372_04)
Eupristina masoni ex *F. benghalensis* (n° 1294_01)
Eupristina altissima ex *F. altissima* (n° 1599_01)
Eupristina cf. *longispina* ex *F. cordatula* (n° 2059_01)
Eupristina koningsbergeri ex *F. benjamina* (n° 1875_01)
Eupristina cyclostigma ex *F. stricta* (n° 1611_01)
Eupristina sp. ex *F. maclellandii* (n° 1594_01)
Allotriozoon heterandromorphum ex *F. lutea* (n° 2602_01)
Allotriozoon nigeriense ex *F. chlamydocarpa* (n° 0988_01)
Pegoscapus sp. ex *F. crocata* (n° 2674_01)
Pegoscapus insularis ex *F. perforata* (n° 2177_01)
Pegoscapus herrei ex *F. paraensis* (n° 1393_01)
Pegoscapus sp. ex *F. trigona* (n° 2686_01)
Pegoscapus gemellus ex *F. poponei* (n° 8172_01a)
Pegoscapus sp. ex *F. pertusa* (n° 2162_01)
Pegoscapus cf. *cabrerai* ex *F. luschnatiana* (n° 2158_01)
Pegoscapus cf. *hoffmeyeri* ex *F. obtusifolia* (n° 2160_01)
Pegoscapus cf. *amabilis* ex *F. nymphaeifolia* (n° 1778_01)
Pegoscapus cf. *obscurus* ex *F. longifolia* (n° 2165_01)
Pegoscapus bagataensis ex *F. andicola* (n° 1682_01)
Pegoscapus carlosi ex *F. tuerckheimii* (n° 1797_01)
Pegoscapus sp. ex *F. exigua* (n° 2136_01)
Pegoscapus tonduzi ex *F. citrifolia* (n° 1954_01)
Pegoscapus sp. ex *F. davidsoniae* (n° 2276_01)
Pegoscapus orozcoi ex *F. colubrinae* (n° 2282_02)
Pegoscapus cf. *longiceps* ex *F. dugandi* (n° 0964_01)
Pegoscapus sp. ex *F. trigonata* (n° 2140_01)
Pegoscapus sp. ex *F. palmeri* (n° 1799_01)
Pegoscapus sp. ex *F. petiolaris* (n° 1803_01)
Platyscapa quadraticeps ex *F. religiosa* (n° 1752_01)
Platyscapa hsui ex *F. caulocarpa* (n° 2420_01)
Platyscapa coronata ex *F. virens* (n° 2289_01)
Platyscapa sp. ex *F. concinna* (n° 2100_01)
Platyscapa cf. *hsui* ex *F. subpisocarpa* (n° 2120_01)
Platyscapa soraria ex *F. ingens* (n° 0227_01)
Dolichoris cf. *umbilicata* ex *F. polyantha* (n° 2352_01)
Dolichoris sp. ex *F. sp* (n° 2335_01)
Dolichoris sp. ex *F. subtrinervia* (n° 2368_01)
Dolichoris nervosae ex *F. nervosa* (n° 0868_01)
Blastophaga psenes ex *F. carica* (n° 1536_01)
Dolichoris sp. ex *F. habrophylla* (n° 1229_01)
Dolichoris sp. ex *F. racemigera* (n° 1276_01)
Pleistodontes cuneatus ex *F. platypoda* (n° 8137_01a)
Pleistodontes proximus ex *F. illipitupiana* (n° 8139_01a)
Pleistodontes athysanus ex *F. brachypoda* (n° 8124_01a)
Pleistodontes nigritentrus ex *F. watkinsiana* (n° 8145_01a)
Pleistodontes imperialis ex *F. rubiginosa* (n° 1396_01)
Pleistodontes rigisamos ex *F. destruens* (n° 1410_01)
Pleistodontes cf. *blandus* ex *F. glandifera* (n° 1252_02)
Pleistodontes greenwoodi ex *F. obliqua* (n° 1422_01)
Pleistodontes schizodontes ex *F. triradiata* (n° 8127_01a)
Pleistodontes nitens ex *F. crassipes* (n° 1416_02)
Pleistodontes regalis ex *F. pleurocarpa* (n° 1554_01)
Pleistodontes froggatti ex *F. macrophylla* (n° 1397_01)
Pleistodontes rieki ex *F. xyloscia* (n° 8130_01a)
Pleistodontes plebejus ex *F. hesperiiformis* (n° 8146_01a)
Wiebesia pumila ex *F. pumila* (n° 1836_01)
Blastophaga sp. ex *F. oleifolia* (n° 1992_01)
Blastophaga quadrupes ex *F. deltoidea* (n° 1879_01)
Valisia intermedia ex *F. padana* (n° 8039_01a)
Valisia malayana ex *F. grossularioides* (n° 2556_01)
Valisia compacta ex *F. fulva* (n° 2087_01)
Valisia auratae ex *F. aurata* (n° 0967_01)
Valisia javana hilli ex *F. hirta* (n° 1745_01)
Valisia cf. *filippina* ex *F. ruficaulis* (n° 2098_01)
Tetrapus sp. ex *F. tonduzii* (n° 1953_01)
Tetrapus sp. ex *F. adhatodifolia* (n° 2166_01)
Tetrapus americanus ex *F. maxima* (n° 1331_01)
Tetrapus costaricanus ex *F. insipida* (n° 1785_01)
Tetrapus sp. ex *F. glabrata* (n° 2178_01)
Tetrapus sp. ex *F. lapathifolia* (n° 1796_01)
Tetrapus ecuadoranus ex *F. yoponensis* (n° 2180_01)
Wiebesia sp. ex *F. sp* (n° 1904_01)
Wiebesia boldinghi ex *F. lanata* (n° 2188_01)
Wiebesia brusi ex *F. baueuerlenii* (n° 8070_01a)
Wiebesia frustula ex *F. odoardi* (n° 1376_01)
Wiebesia flava ex *F. sagittata* (n° 1372_06)
Wiebesia sp. ex *F. sp* (n° 2533_01)
Wiebesia punctata ex *F. punctata* (n° 8239_01a)
Wiebesia sp. ex *F. aurantiacea* (n° 1905_01)
Blastophaga sp. ex *F. stenophylla* (n° 1675_01)
Blastophaga sp. ex *F. chapaensis* (n° 1757_02)
Blastophaga nipponica ex *F. erecta* (n° 2381_01)
Blastophaga sp. ex *F. ischnopoda* (n° 1673_01)
Ceratosolen dentifer ex *F. hispidioides* (n° 8053_01a)
Ceratosolen sp. ex *F. pachyrhachis* (n° 2198_01)
Ceratosolen cf. *hooglandii* ex *F. bernaysii* (n° 2353_01)
Ceratosolen notus ex *F. nota* (n° 8184_01a)
Ceratosolen vechi ex *F. lepicarpa* (n° 1866_01)
Ceratosolen vetustus ex *F. schwartzii* (n° 1989_01)
Ceratosolen hewitti ex *F. fistulosa* (n° 1446_01)
Ceratosolen constrictus ex *F. fistulosa* (n° 2286_01)
Ceratosolen crassitarsus ex *F. ribes* (n° 2193_01)
Ceratosolen corneri ex *F. botryocarpa* (n° 2319_01)
Ceratosolen sp. ex *F. septica* (n° 1683_01)
Ceratosolen sp. ex *F. squamosa* (n° 1658_01)
Ceratosolen sp. ex *F. heterostyla* (n° 1753_01)
Ceratosolen sp. ex *F. treubii* (n° 1324_01)
Ceratosolen albus ex *F. uncinata* (n° 1328_01)
Ceratosolen sp. ex *F. bukitrayaensis* (n° 1319_01)
Ceratosolen sp. ex *F. stolonifera* (n° 1986_01)
Ceratosolen sp. ex *F. semivestita* (n° 8222_01a)
Ceratosolen sp. ex *F. erythrosperma* (n° 2539_01)
Ceratosolen sp. ex *F. subcuneata* (n° 2195_01)
Ceratosolen armipes ex *F. itoana* (n° 1531_01)
Ceratosolen abnormis ex *F. dammaropsis* (n° 8056_01a)
Ceratosolen medlerianus ex *F. mollior* (n° 2312_01)
Ceratosolen sp. ex *F. arbuscula* (n° 2349_01)
Ceratosolen galili ex *F. sycomorus* (n° 1532_06)
Ceratosolen coecus ex *F. mauritiana* (n° 0659_23)
Ceratosolen acutatus ex *F. vogeliana* (n° 0453_21)
Ceratosolen megacephalus ex *F. vallis-choudae* (n° 1929_01)
Ceratosolen flabellatus ex *F. sp* (n° 1810_01)
Ceratosolen fusciceps ex *F. racemosa* (n° 2028_01)
Ceratosolen arabicus ex *F. mucoso* (n° 2191_01)
Ceratosolen coecus ex *F. mauritiana* (n° 0659_23)
Ceratosolen stupefactus ex *F. tilifolia* (n° 1816_01)
Ceratosolen blommersi ex *F. botryoides* (n° 2463_01)
Ceratosolen appendiculatus ex *F. variegata* (n° 1423_01)
Ceratosolen emarginatus ex *F. auriculata* (n° 2654_01)
Ceratosolen cf. *emarginatus* ex *F. oligodon* (n° 1910_01)
Ceratosolen cf. *emarginatus* ex *F. hainanensis* (n° 1606_01)
Ceratosolen sp. ex *F. prostrata* (n° 0820_01)
Ceratosolen graveyi ex *F. semicordata* (n° 0855_12)
Ceratosolen waliensis ex *F. robusta* (n° 1380_01)
Ceratosolen nexilis ex *F. nodosa* (n° 2356_01)
Ceratosolen vissali ex *F. theophrastoides* (n° 2485_01)
Ceratosolen sp. ex *F. tikoua* (n° 1628_01)
Kradibia gestroi afrum ex *F. asperifolia* (n° 2217_01)
Kradibia gestroi afrum ex *F. capreifolia* (n° 8232_01a)
Kradibia gestroi afrum ex *F. exasperata* (n° 2186_01)
Kradibia subulatae ex *F. subulata* (n° 1764_01)
Kradibia sp. ex *F. sp* (n° 1650_01)
Kradibia saundersi ex *F. politoria* (n° 2466_01)
Kradibia sumatrana ex *F. ampelas* (n° 2125_01)
Kradibia sp. ex *F. melinocarpa* (n° 2362_01)
Kradibia ohuensis ex *F. trachypison* (n° 1375_01)
Kradibia rutherfordi ex *F. tinctoria* (n° 1868_01)
Kradibia giacomini ex *F. obscura* (n° 2010_01)
Kradibia sp. ex *F. parietalis* (n° 1864_01)
Kradibia wassa ex *F. wassa* (n° 2500_01)
Kradibia copiosae ex *F. copiosa* (n° 2492_01)
Kradibia cf. *nigriceps* ex *F. opposita* (n° 2375_01)
Kradibia salemensis ex *F. phaeosyce* (n° 8235_01a)
Kradibia jacobi ex *F. conocephalifolia* (n° 8233_01a)
Kradibia sp. ex *F. gul* (n° 2081_01)
Kradibia sp. ex *F. cyrtophylla* (n° 1854_01)
Kradibia sessilis ex *F. virgata sessilis* (n° 1263_01)

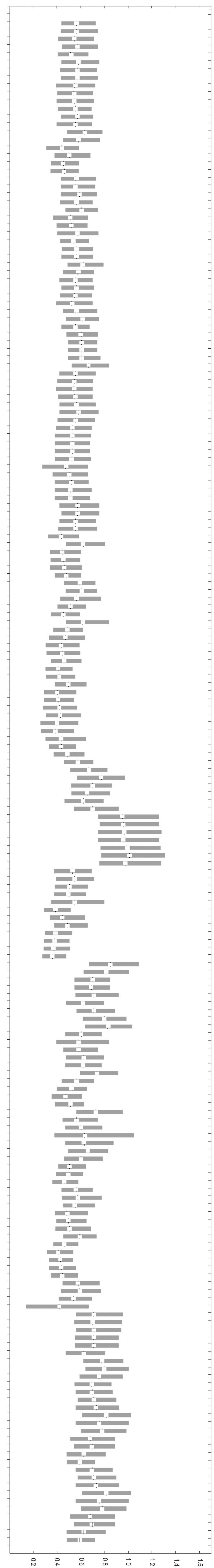


FIGURE S6. Fifty percent majority rule consensus of the 34 most parsimonious trees from the TNT analysis.

Bootstrap percentages ($> 60\%$) are indicated above branches.

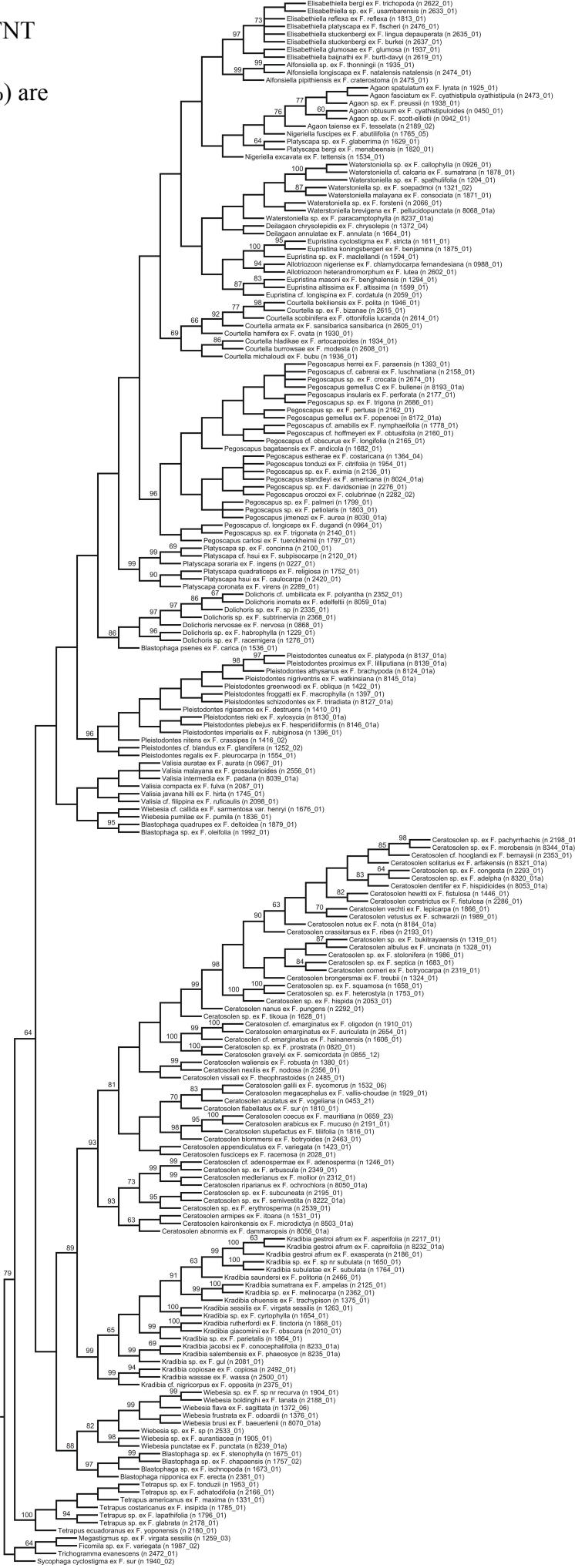
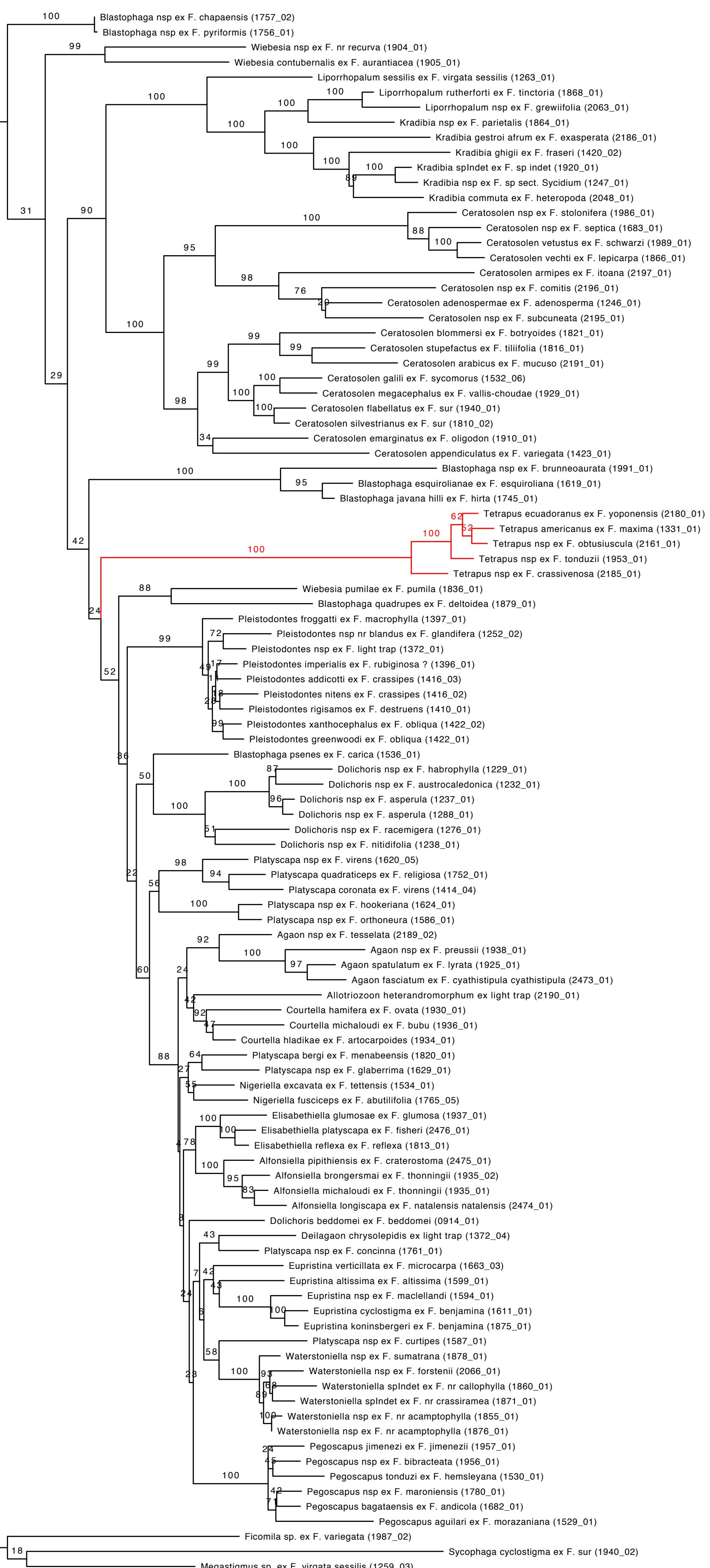


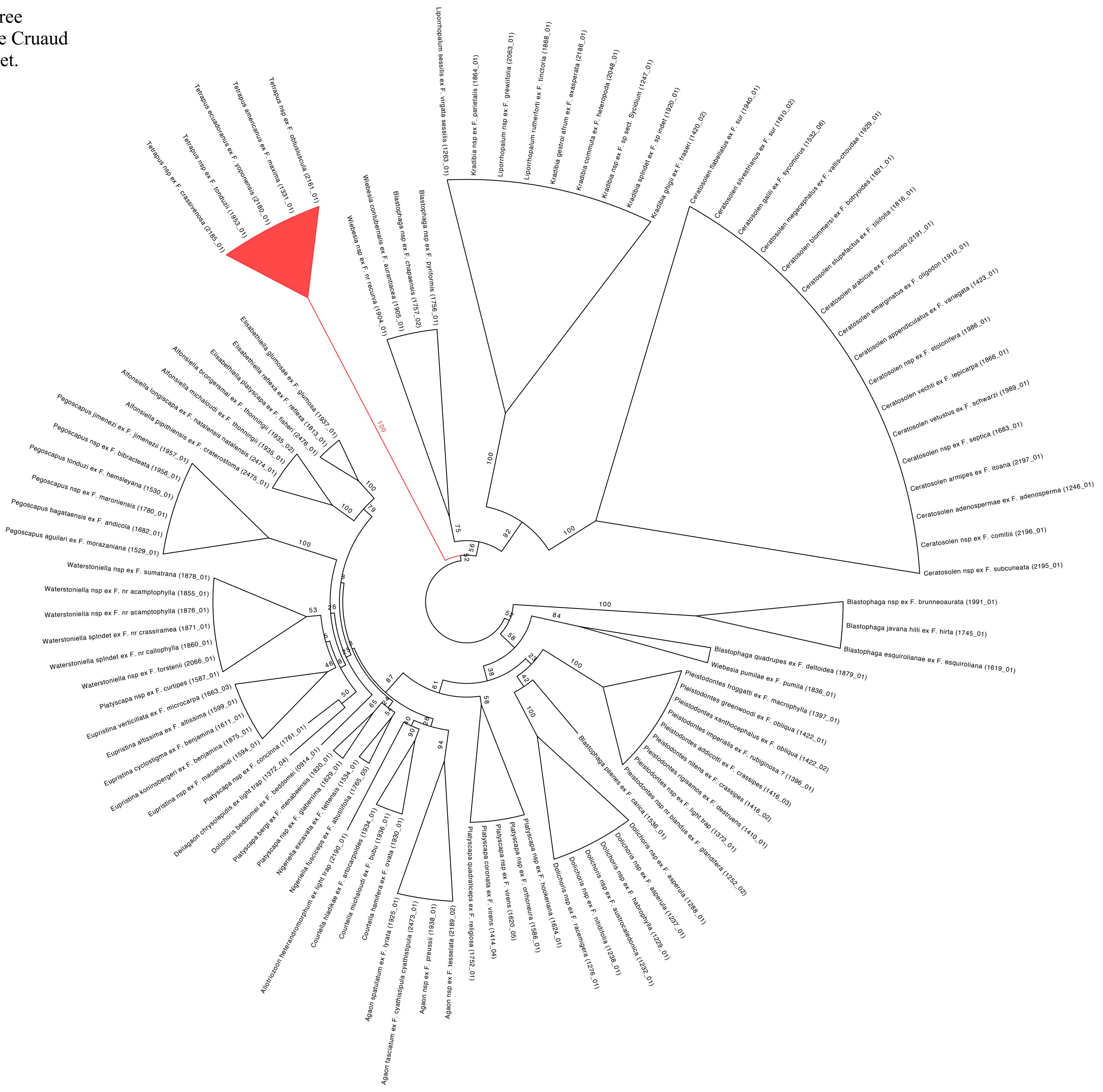
FIGURE S7.

Impact of
outgroup
choice on
previous
datasets.

a- ML analysis
of the Cruaud
et al. (2010)
dataset without
Trichogramma
evanescens.



b- Unrooted ML tree
estimated from the Cruaud
et al. (2010) dataset.



c- Unrooted ML tree estimated from the Lopez-Vaamonde et al. (2009) dataset.
 Bootstrap percentages are indicated above branches.

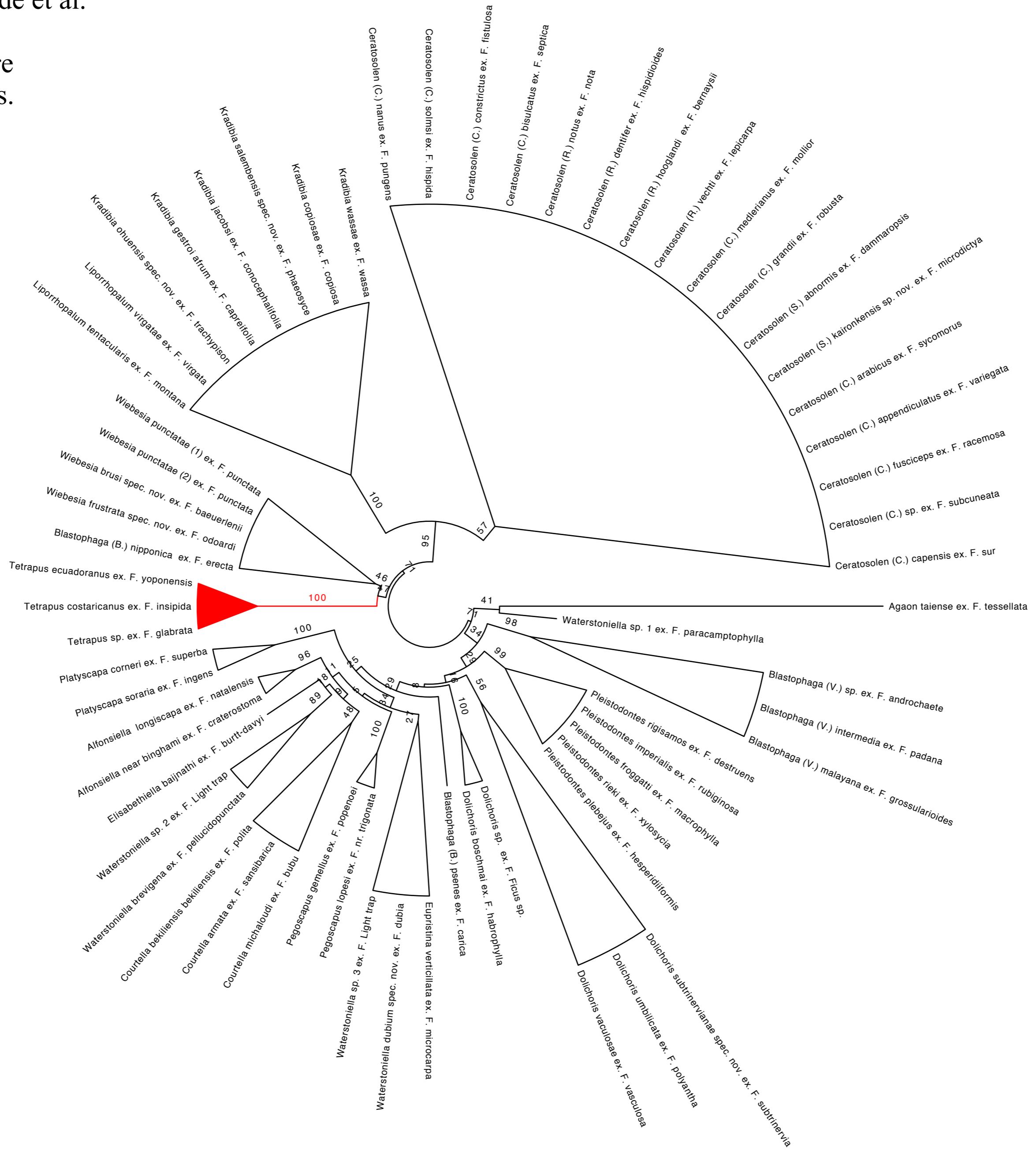
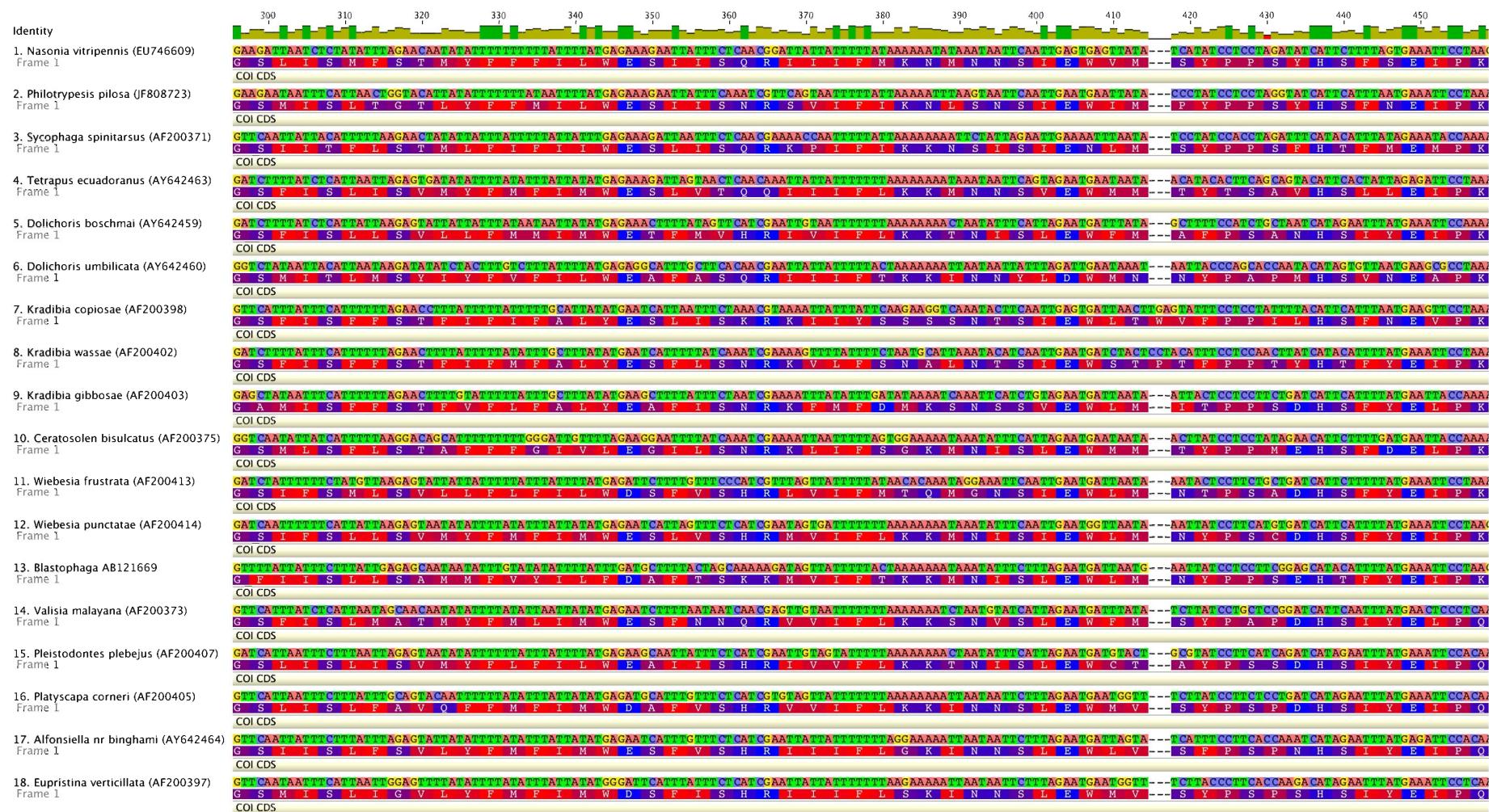
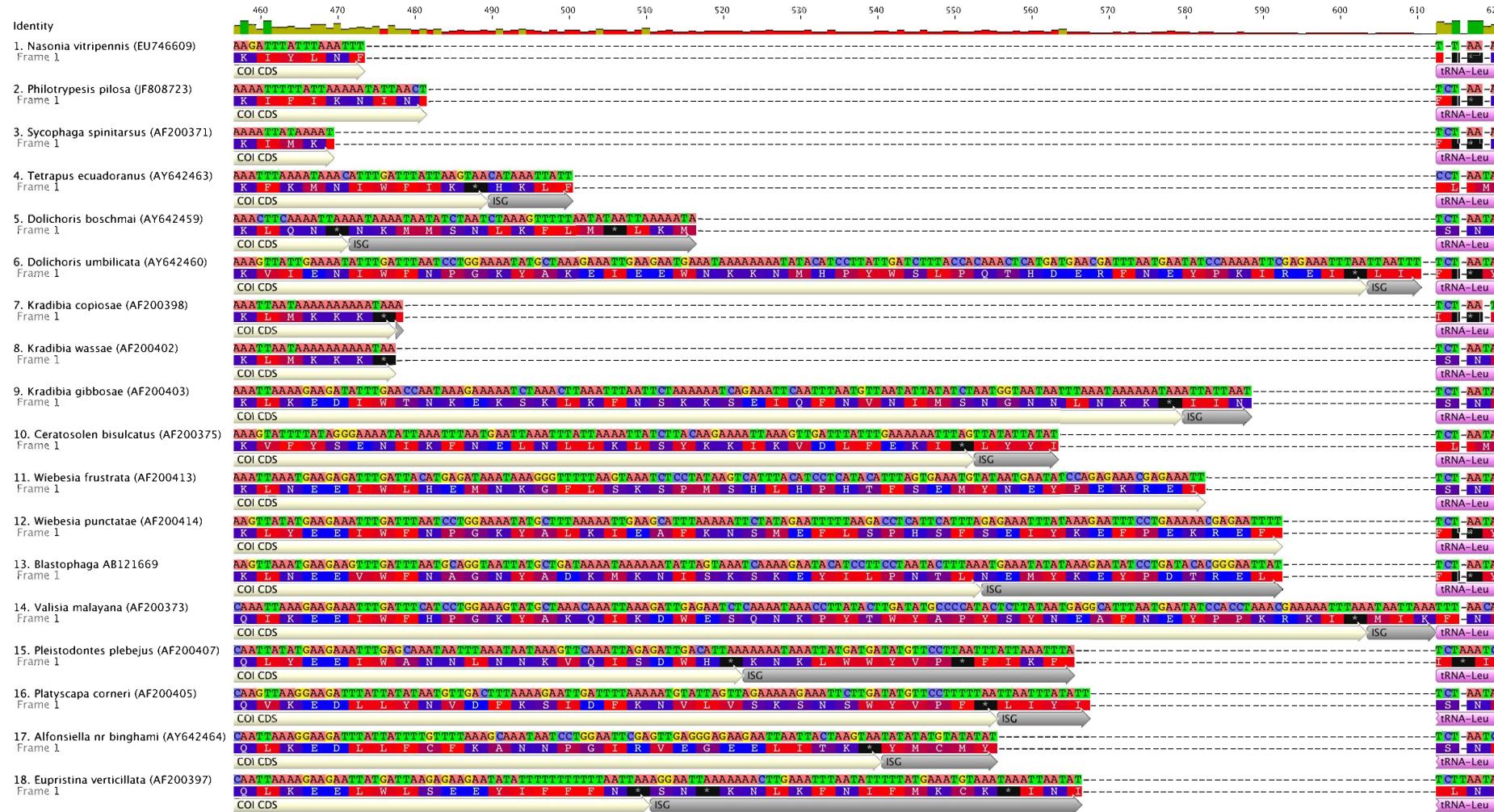
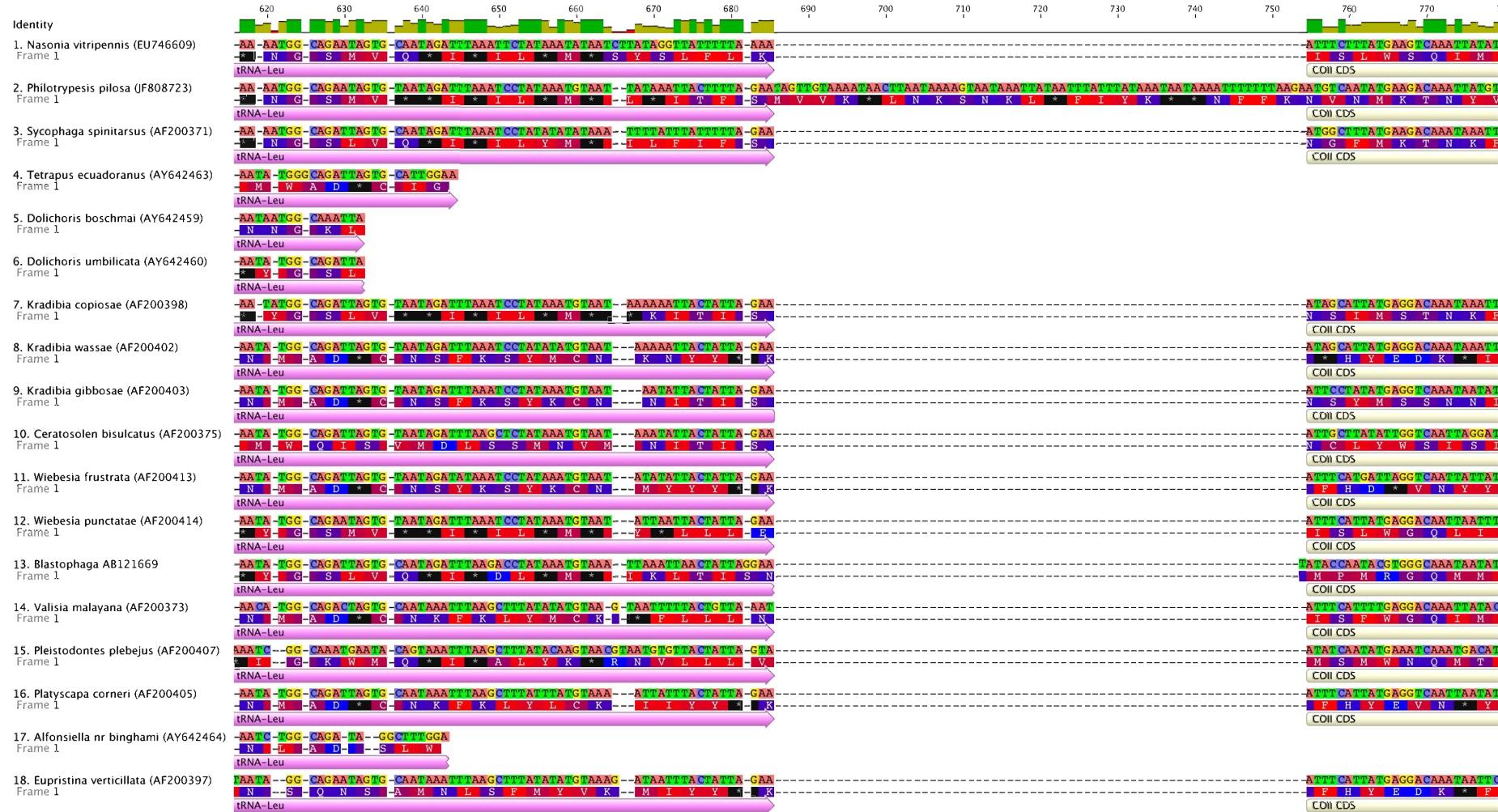


FIGURE S8. Analysis of the structural variation of the end of COI.

a- Alignment of all the chalcidoid sequences covering the zone between COI and t-RNAleu available in GenBank. Screenshots from Geneious v5.4.2. For clarity, the alignment has been split into 3 sub-alignments, with a short overlap between ends and beginnings of consecutive sub-alignments. Due to program features, we could not remove the amino-acid translation of the t-RNAleu fragment, though this has no sense. The intergenic segment (ISG) is left-aligned.







b- Focus on the alignment of the intergenic segment.

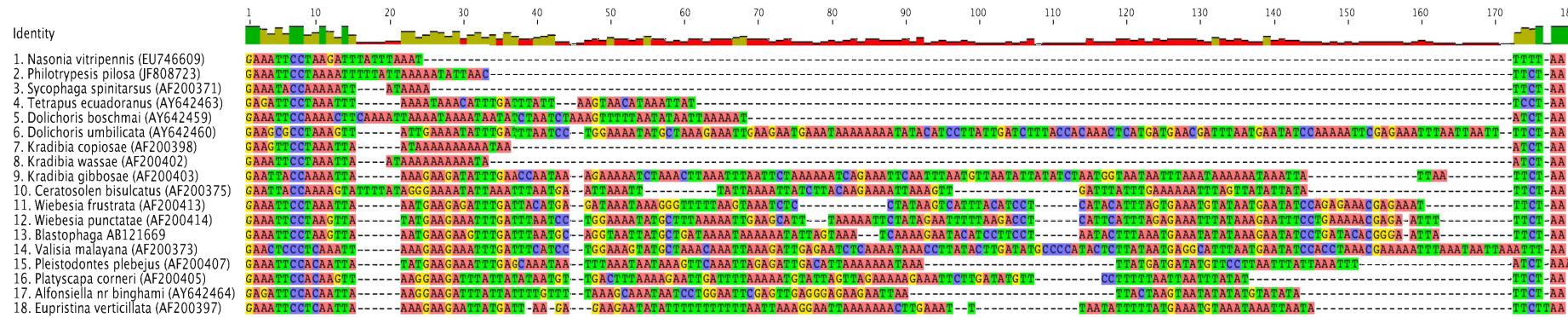
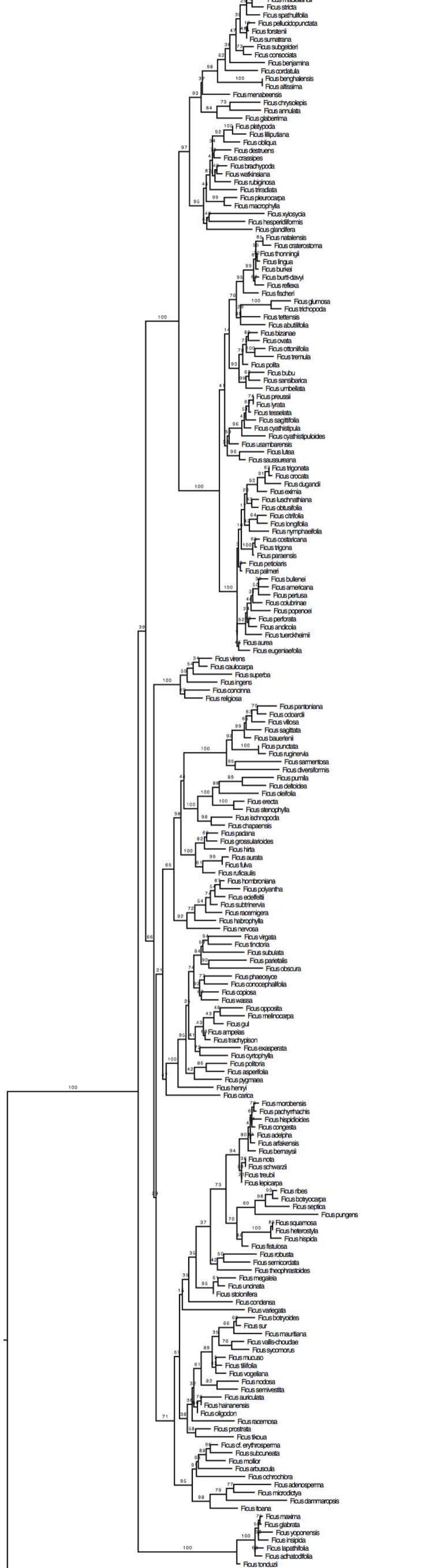


FIGURE S9. Phylogenograms of relationships among *Ficus*

a- based on ML analyses. Bootstrap percentages are indicated above branches.

b- based on Bayesian analyses. Posterior probabilities are indicated above branches.

a)



b)

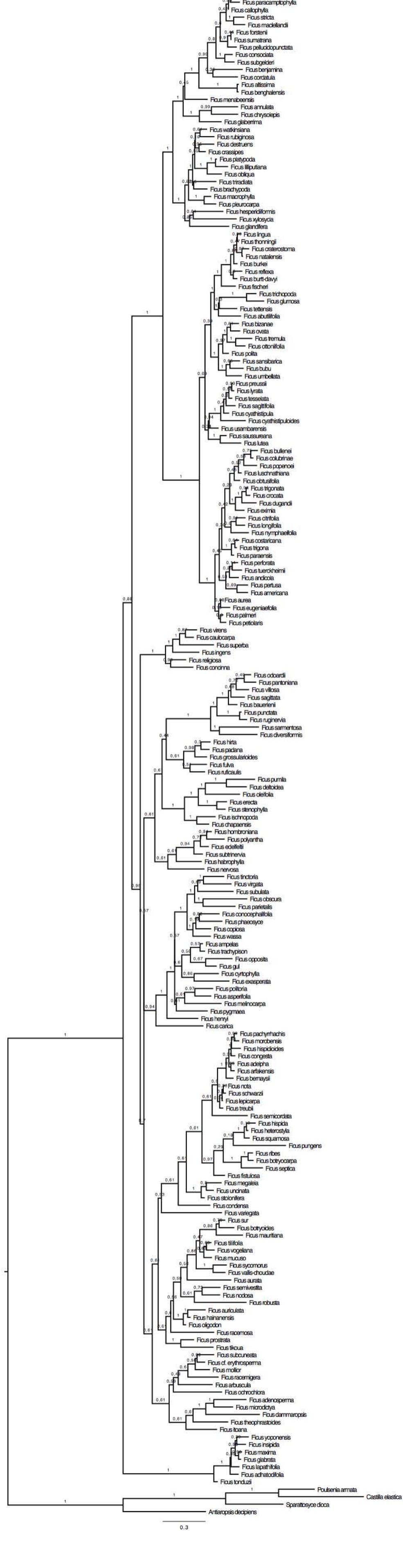
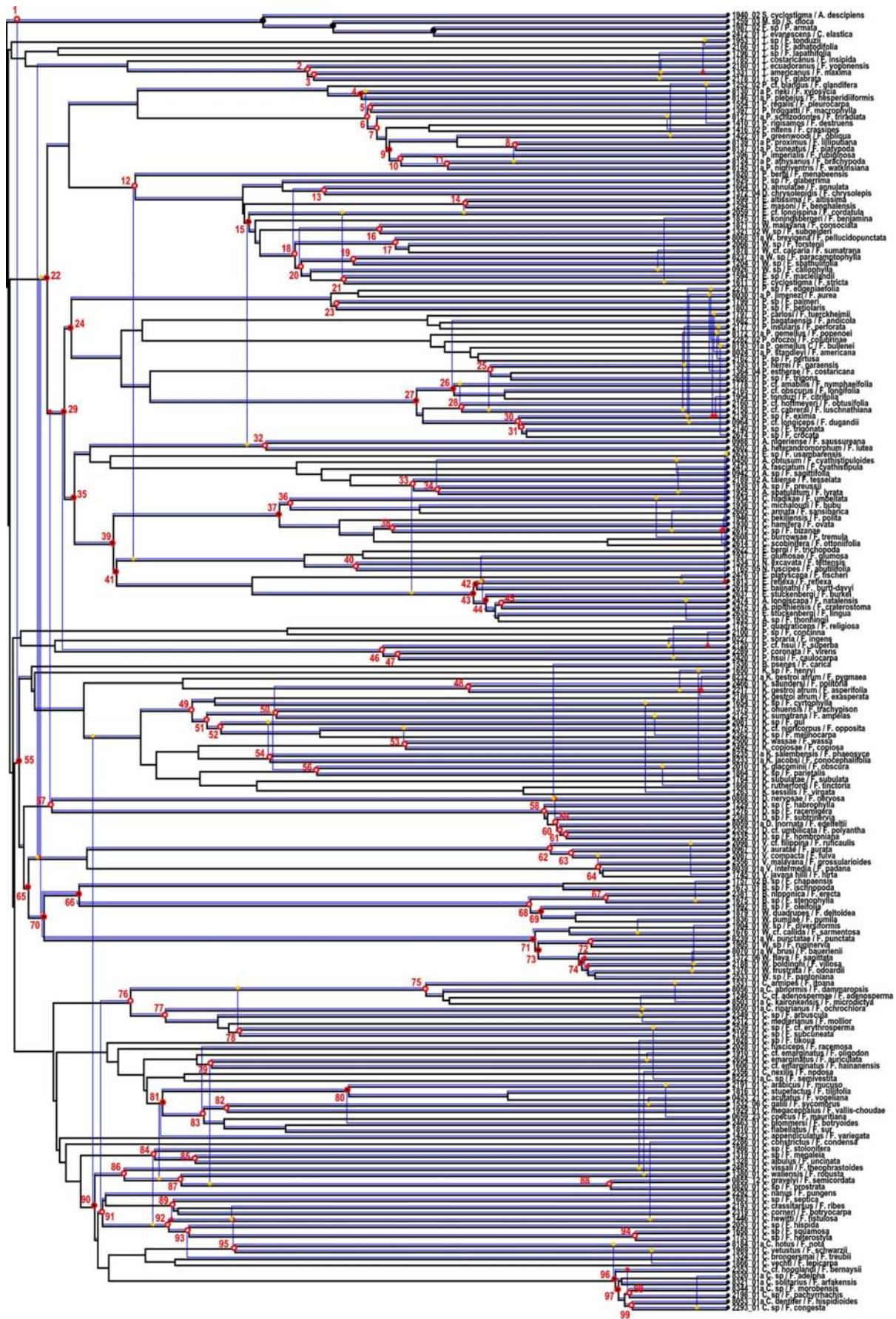


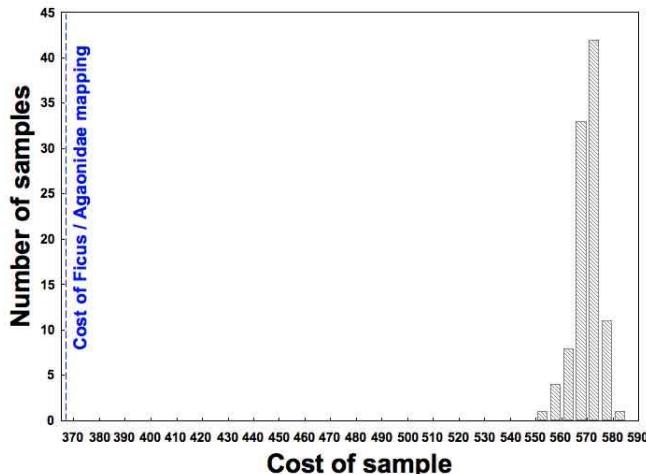
FIGURE S10. Results of cophylogenetic analyses conducted with Jane 2. a-Hypothetical codivergence scenario inferred by Jane 2. The Ficus tree is drawn in black and the pollinator tree is drawn in blue. The cophylogeny mapping was conducted using the following cost model: 0 for cospeciation and 1 for the other evolutionary events (under the Treemap cost model). The 198 (= 99 x 2) putative cospeciation events are numbered and marked by hollow red circles. Jane 2 inferred 198 cospeciation events, 204 duplications (solid red circles), 102 host shifts (horizontal and vertical lines coming out of the node) and 61 losses (dashed lines) between figs and wasp BEAST-topologies, accounting for an optimal cost of 367. It is noteworthy that numerous host shifts occurred between Americana figs.



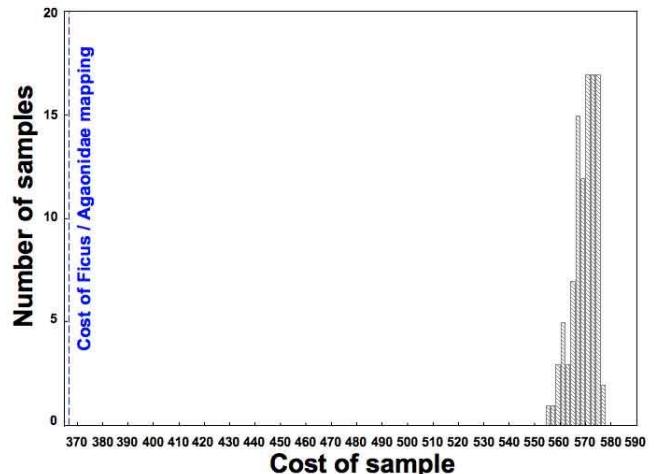
b- Results of the randomization tests of significance. Three different cost models were used (A, B and C). Under each cost model, two randomisations were conducted (1&2). In all cases the observed cost was compared to the null distribution of costs derived from each randomization procedure to determine whether the number of cospeciation events recovered from the reconciliation analysis was significant (see SI methods for details).

Cost model A: 0 for cospeciation, 1 for the other events (using the TreeMap cost model). Optimal cost found by Jane 2: 367

1) Tip randomization (x100)

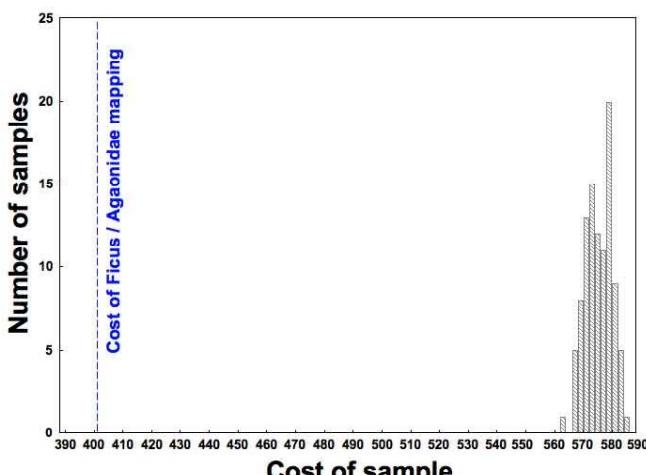


2) Parasite tree and tip randomization (x100)

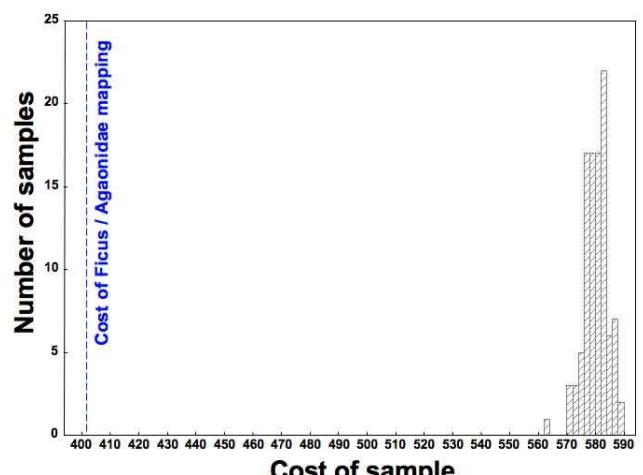


Cost model B: 0 for cospeciation, 1 for switch and duplication (using the TreeMap cost model) and 2 for losses (Jane 2 default parameters). Optimal cost found by Jane 2: 401

1) Tip randomization (x100)

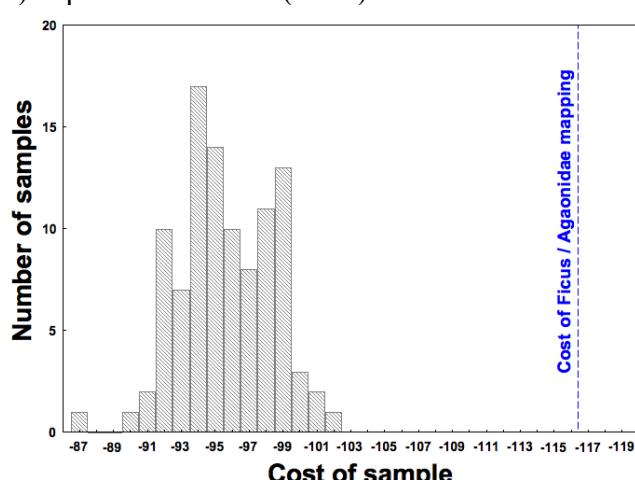


2) Parasite tree and tip randomization (x100)

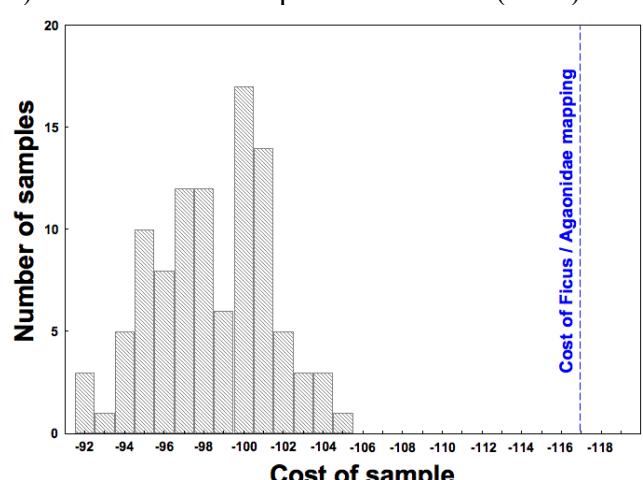


Cost model C: -1 for cospeciation, 0 for the other events. Optimal cost found by Jane =-117

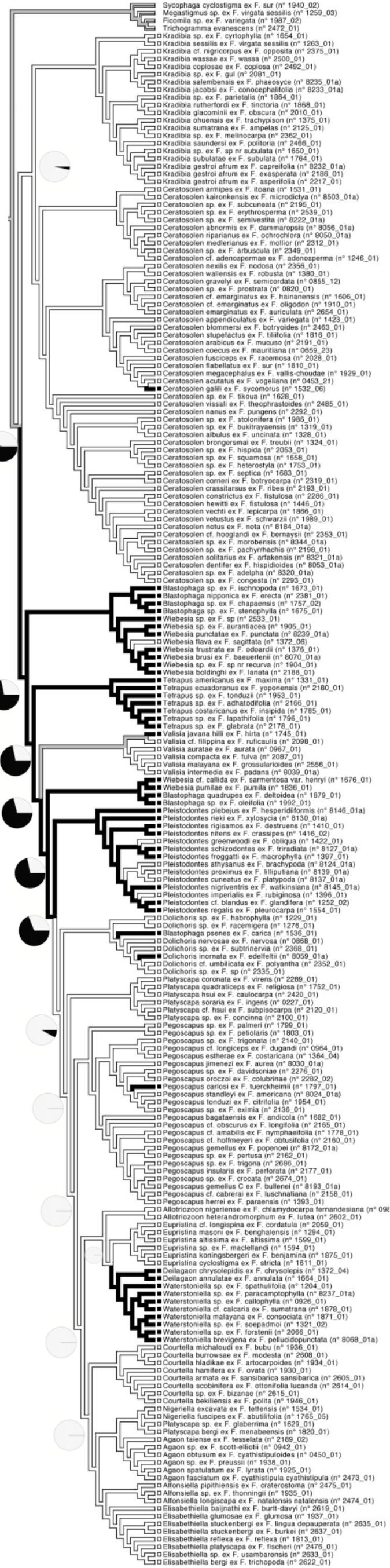
1) Tip randomization (x100)



2) Parasite tree and tip randomization (x100)



□ Active
■ Passive



□ Active
■ Passive

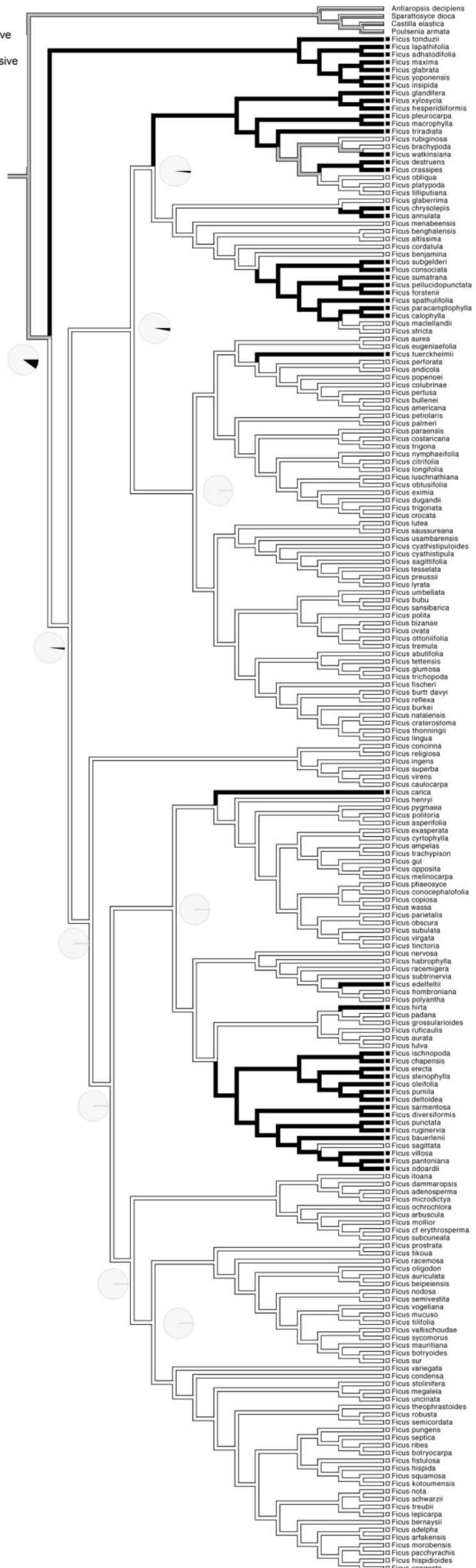
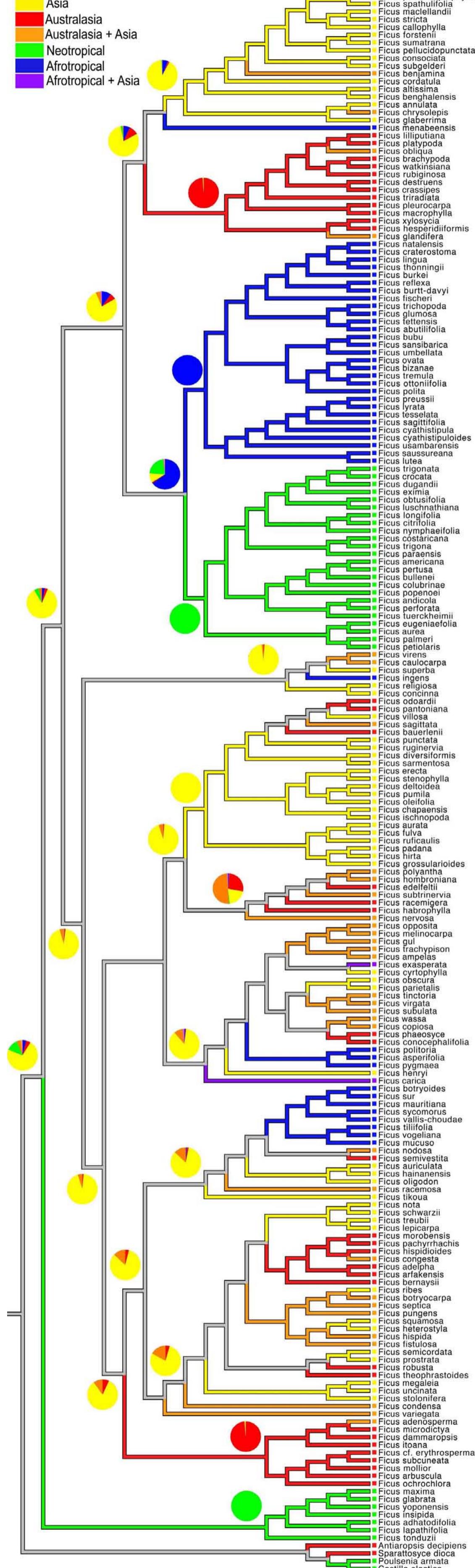


FIGURE S12. Reconstruction of ancestral area of major clades of *Ficus* and pollinators using Mesquite. a- *Ficus* and b-pollinators. Branch color reflects the most parsimonious ancestral area for that branch while pie charts at nodes represent the proportion of the total likelihood received by each biogeographical region as the ancestral area. Reconstruction was performed using the ML-topology. Mesquite requiring fully resolved topologies, we arbitrarily fixed the topology depicted in this figure. This topology is one of the fully resolved topology among others and consequently shows small topological differences with the topology depicted in Figure 2. These minor changes affect only the shallower nodes that are not strongly supported by any of the phylogenetic method used. Moreover, they involved species that occur in the same biogeographical area and have consequently no effect on the biogeographical interpretations.



b)

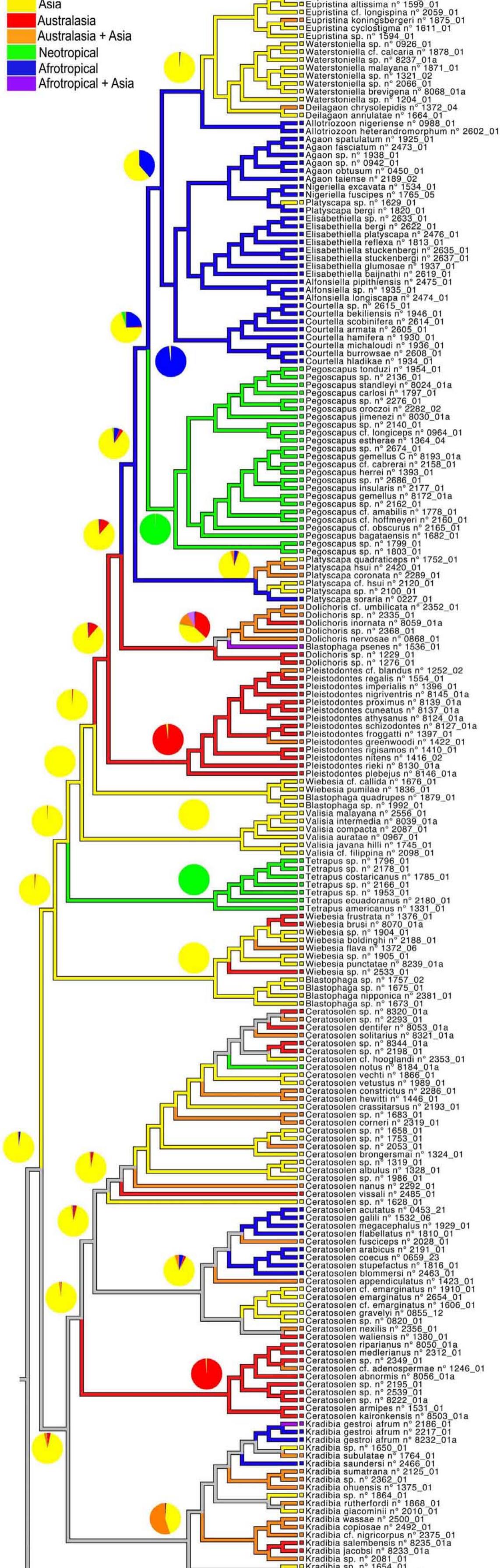
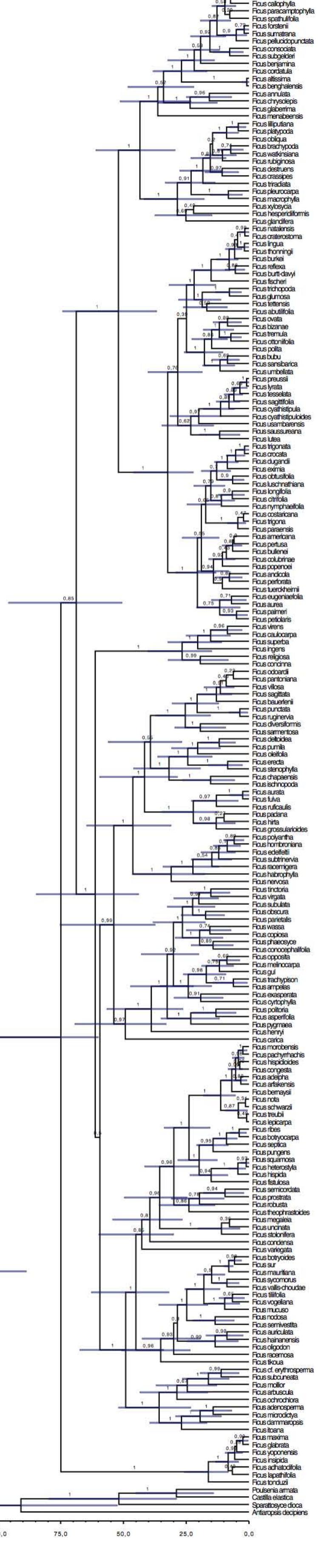


FIGURE S13. BEAST chronograms (maximum clade credibility trees) showing the timing of evolution of the partners.

a- Ficus and b- pollinators. Grey bars around node ages (Ma) indicate the 95% highest posterior density (HPD) intervals.

a)



b)

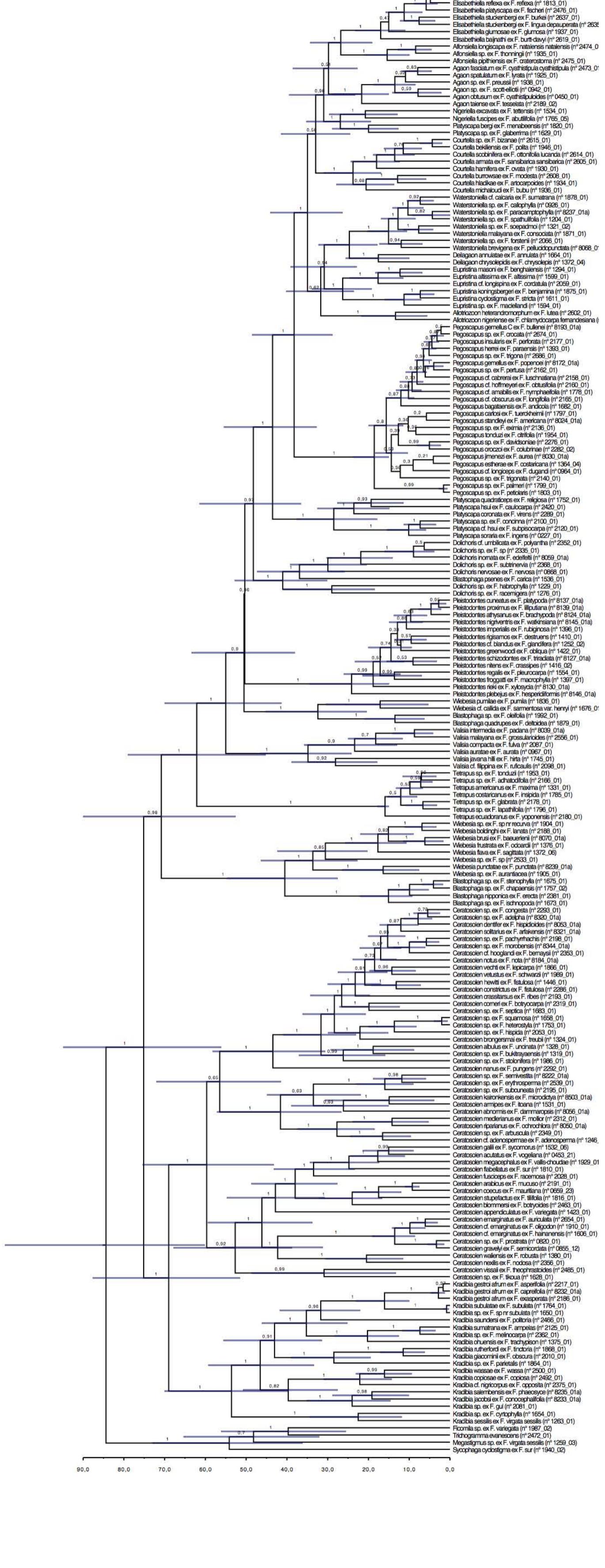


FIGURE S14. Analysis of key morphological character-state transformations.

Morphological characters were mapped onto the two alternative phylogenetic hypotheses (i.e., *Tetrapus* nested within the Agaonidae *versus* sister of all other Agaonidae). Character state coding is based on observations of more than 200 species of Agaonidae.

A-External male mesosoma structure. Illustration of the characters states are provided in C.

B-Internal male mesosoma structure. Illustration of the characters states are provided in D.

E-Maximum number of teeth on the male protibia

F-Structure of the flagellomere 1 in female

G-Presence of sensilla on flagellomere 2 in female

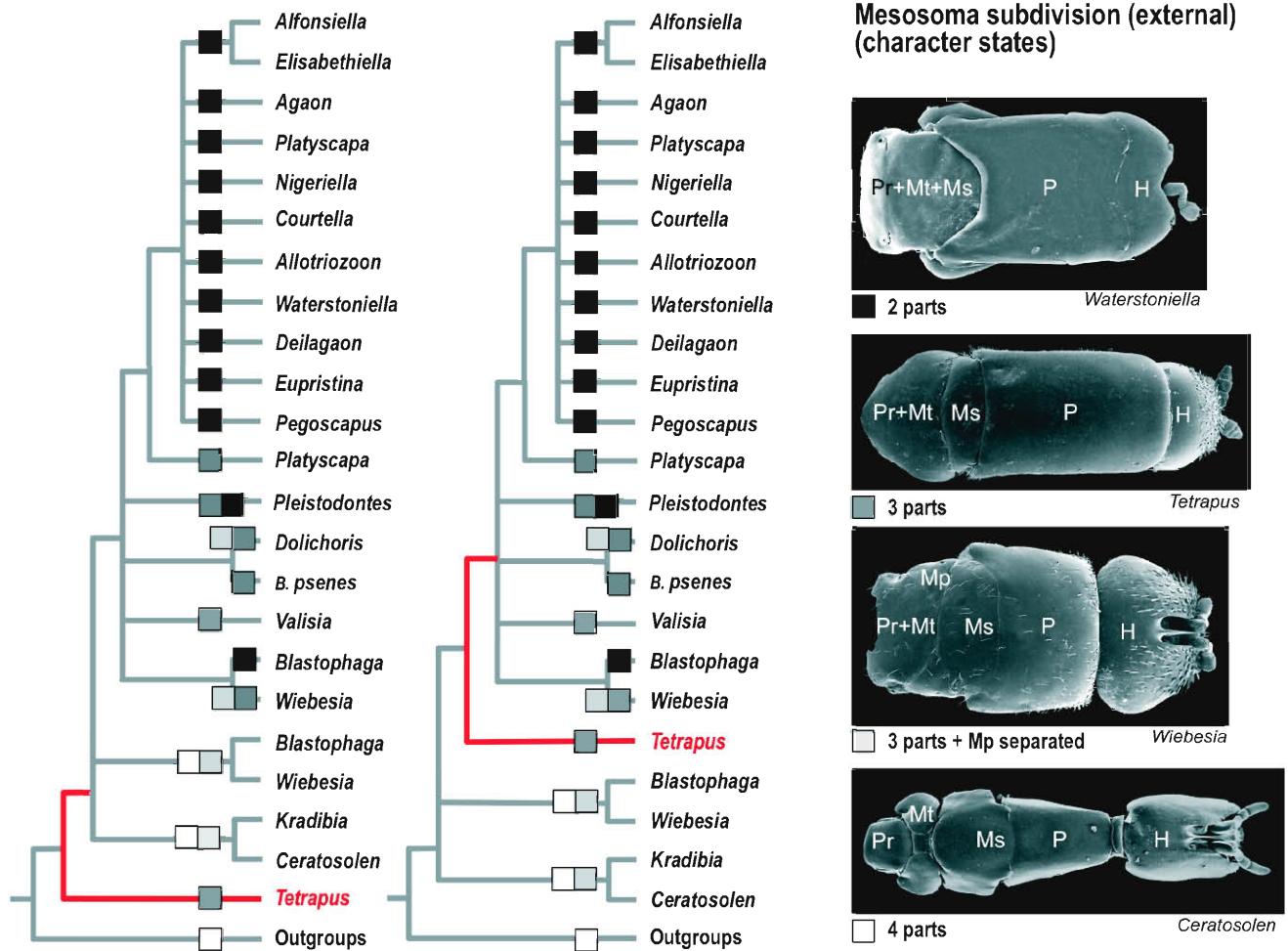
H- Structure of the maxillary palpi

I-Presence of pollen pocket

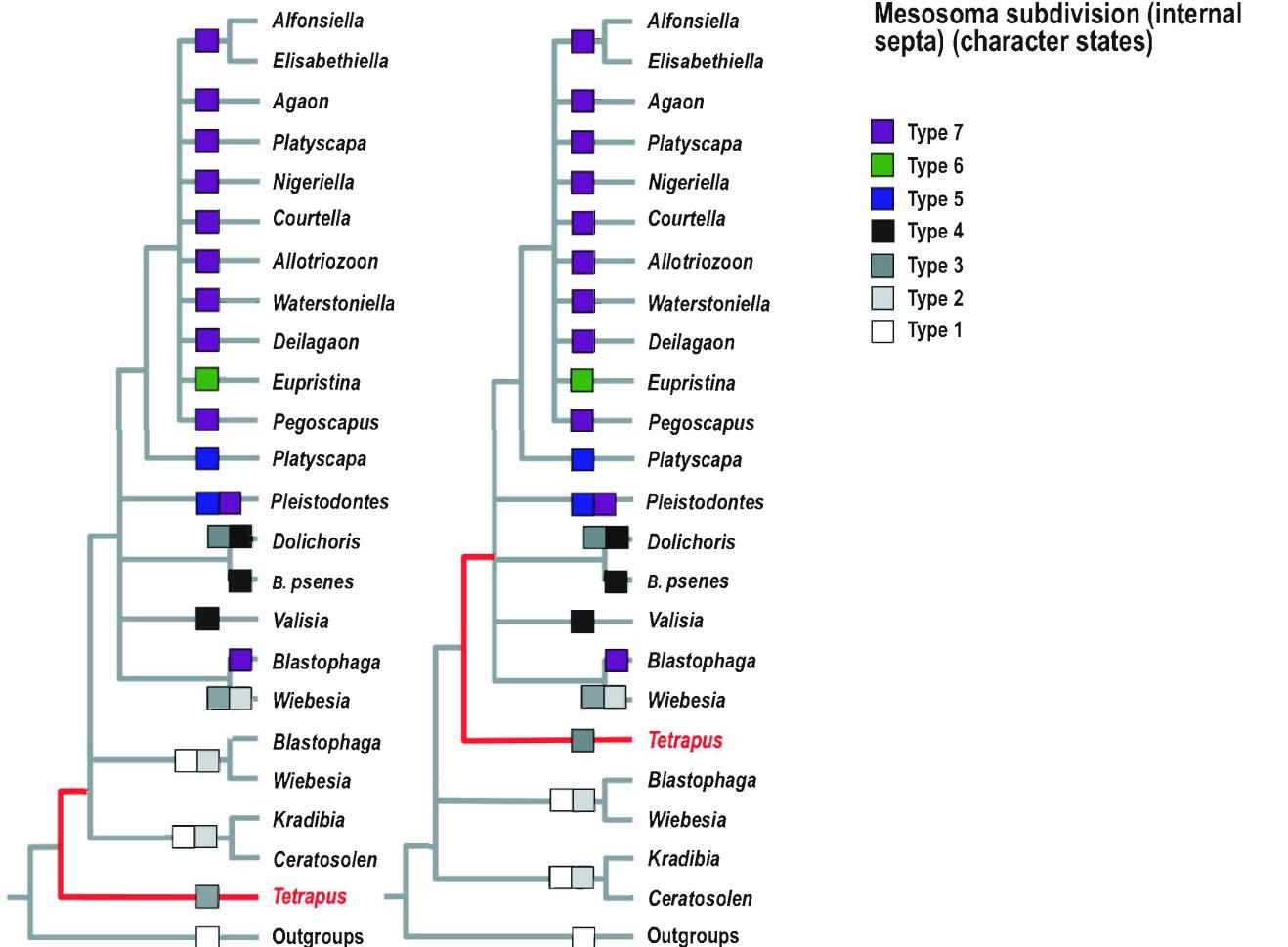
J-Pollination syndrome

Fig. S14

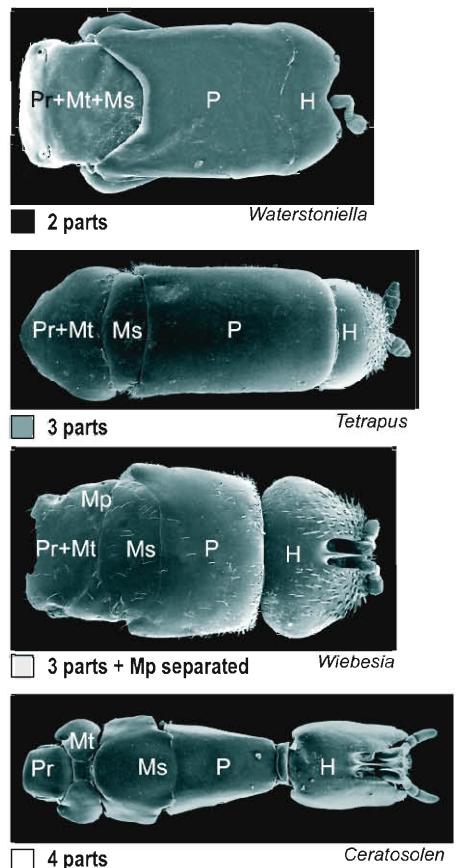
A



B

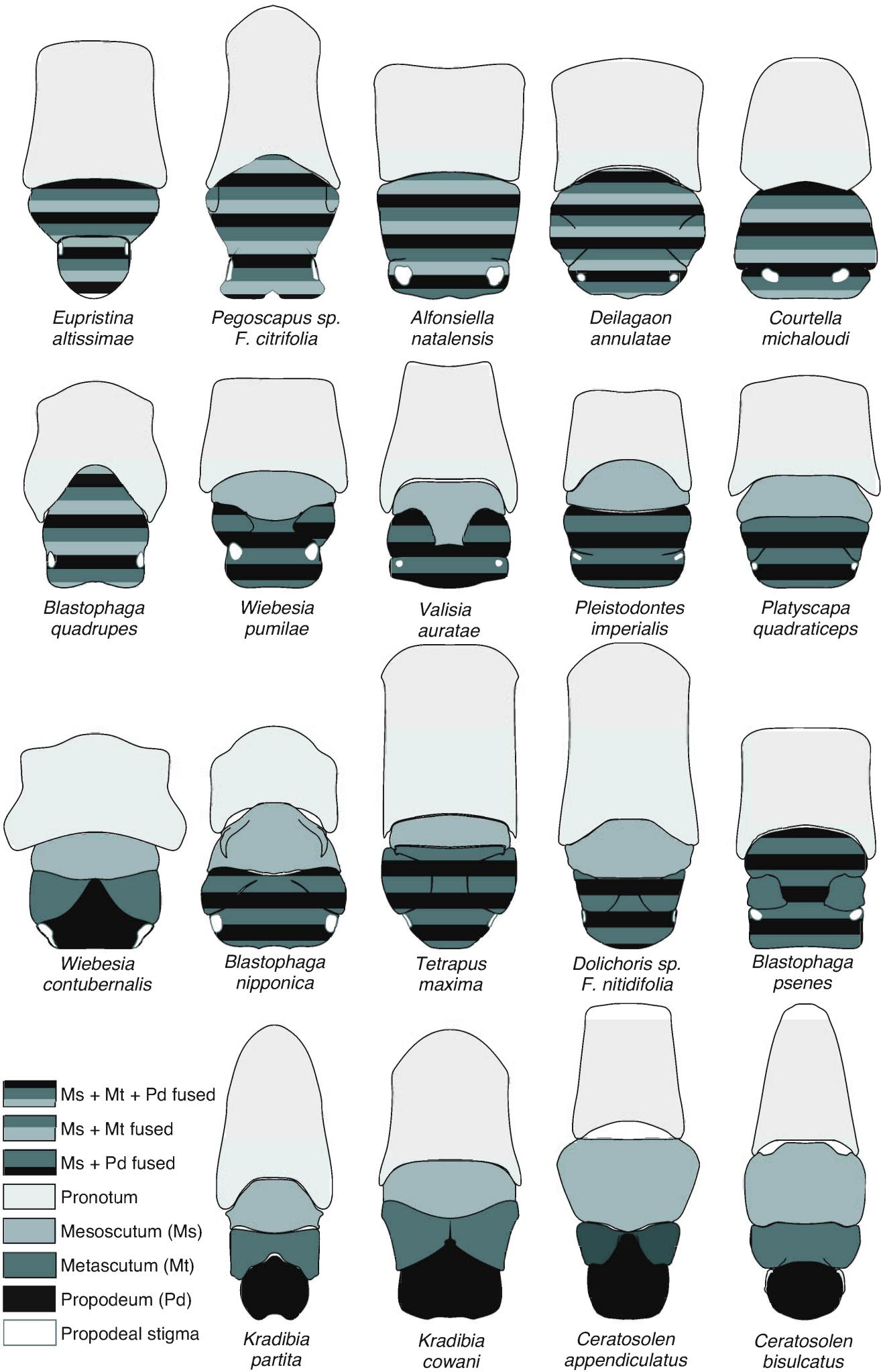


Mesosoma subdivision (external) (character states)

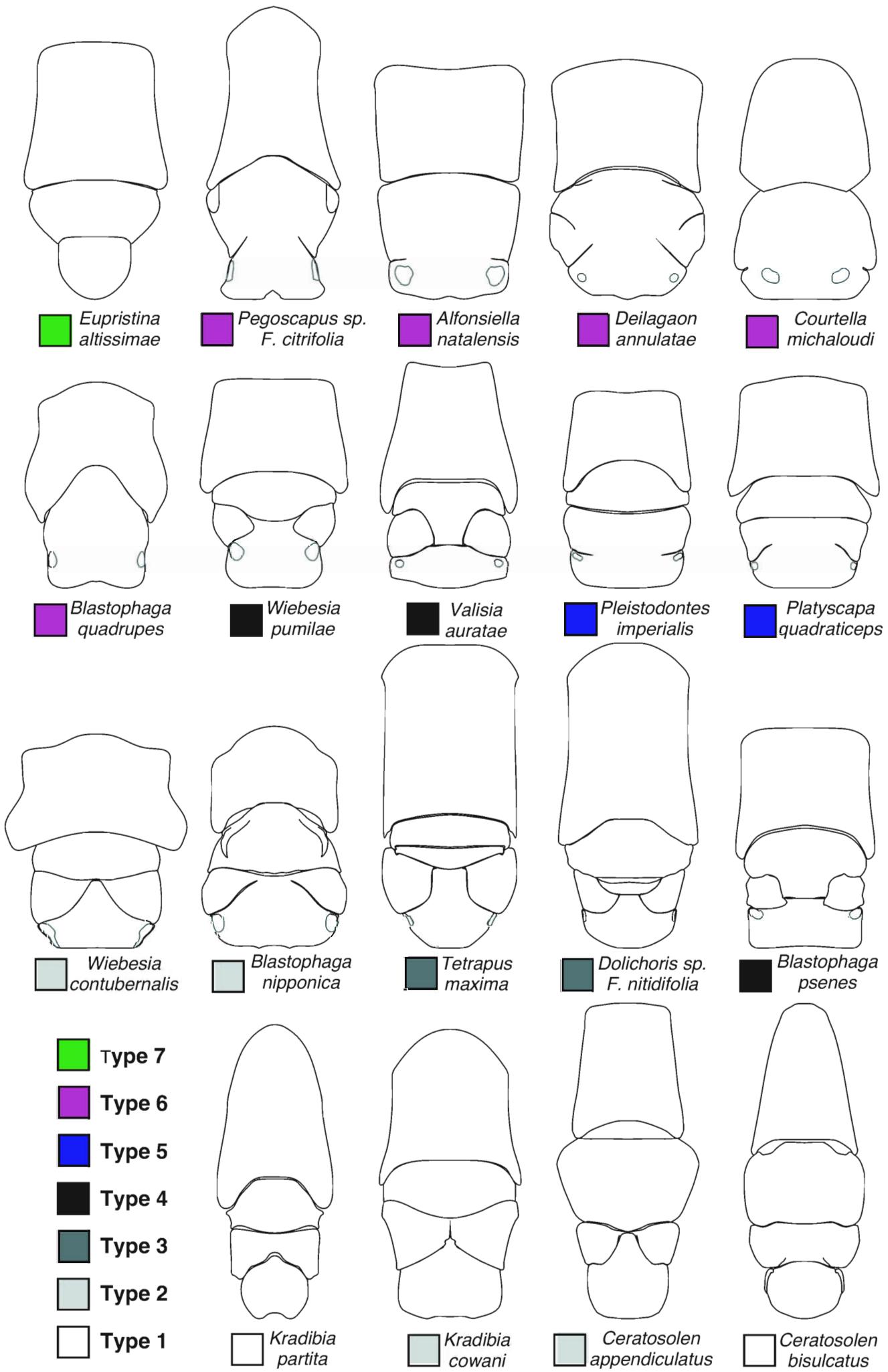


Mesosoma subdivision (internal septa) (character states)

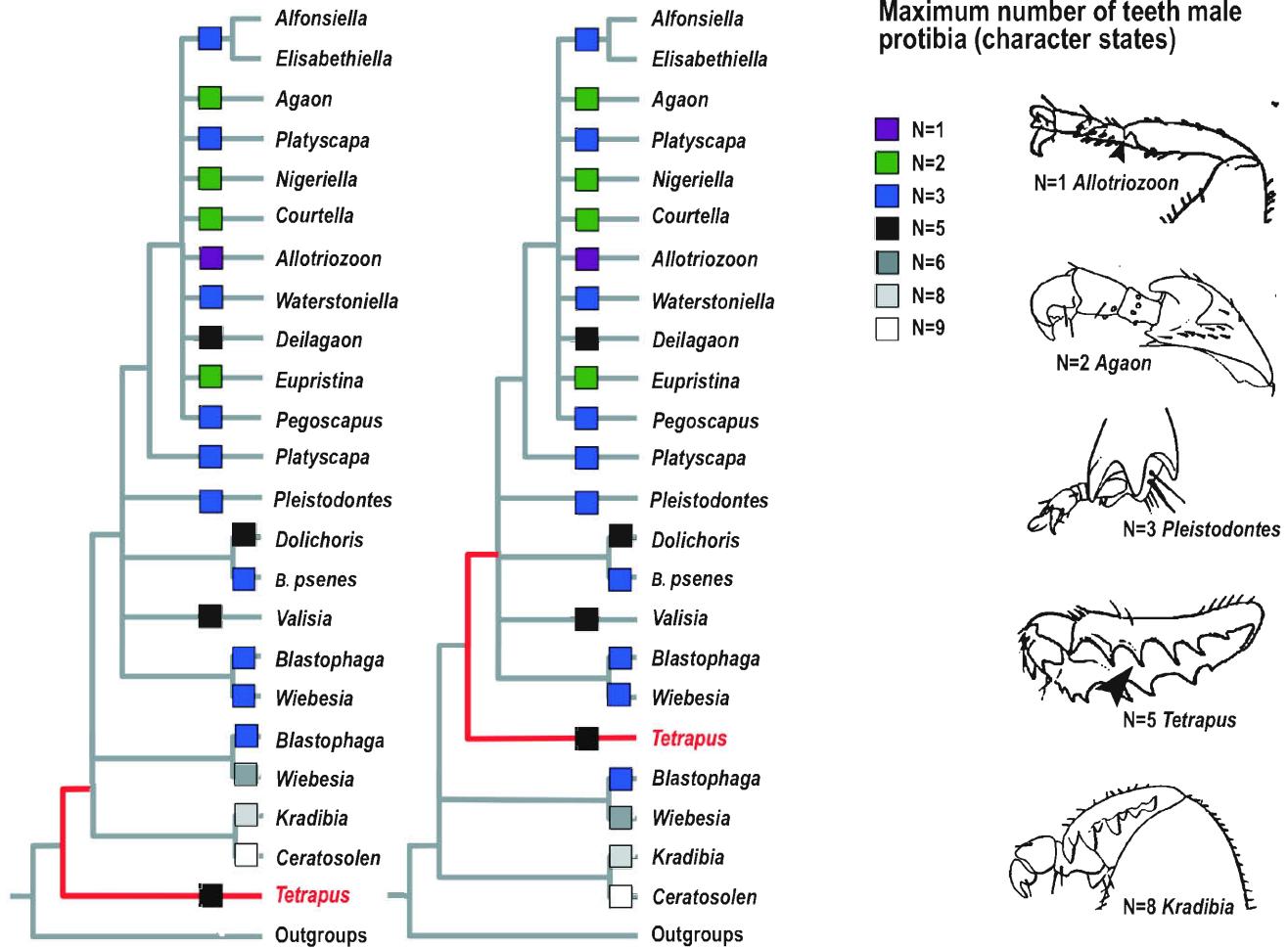
C



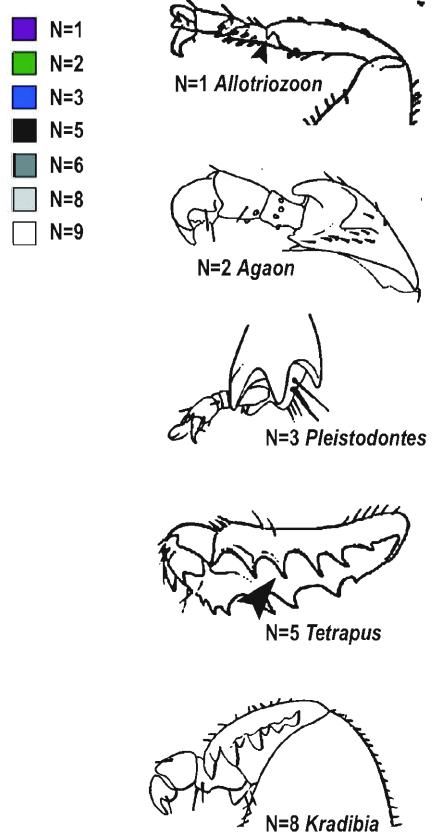
D



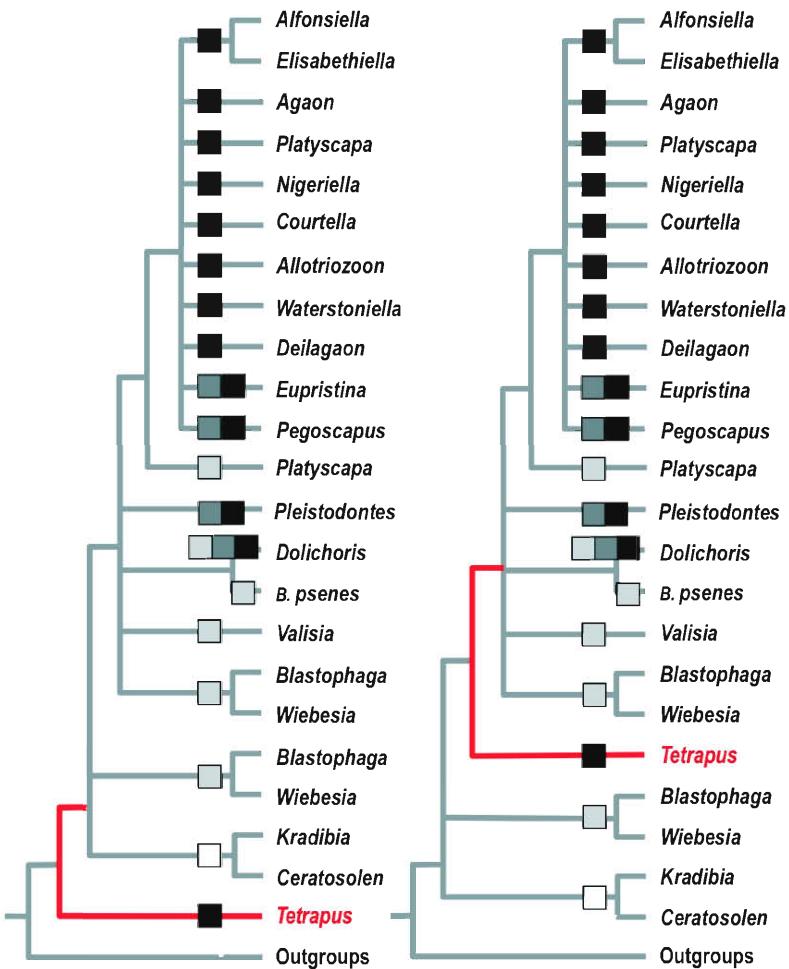
E



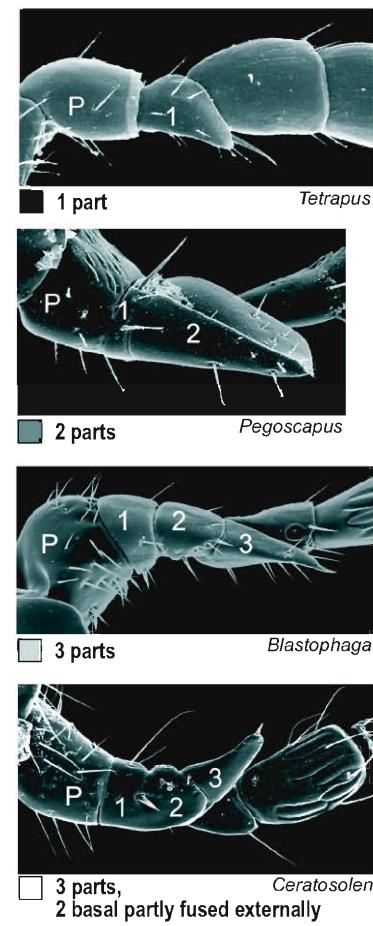
Maximum number of teeth male protibia (character states)



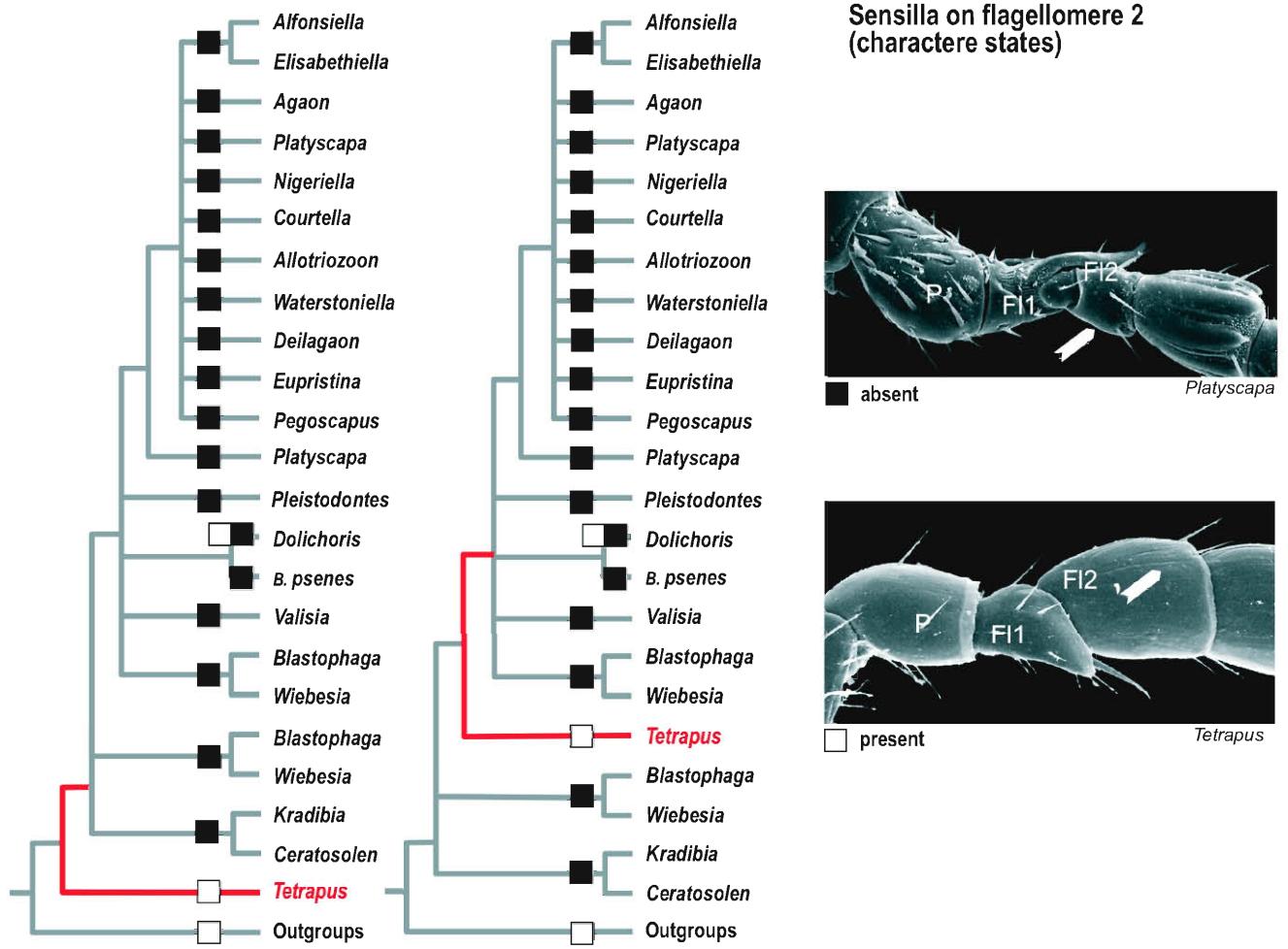
F



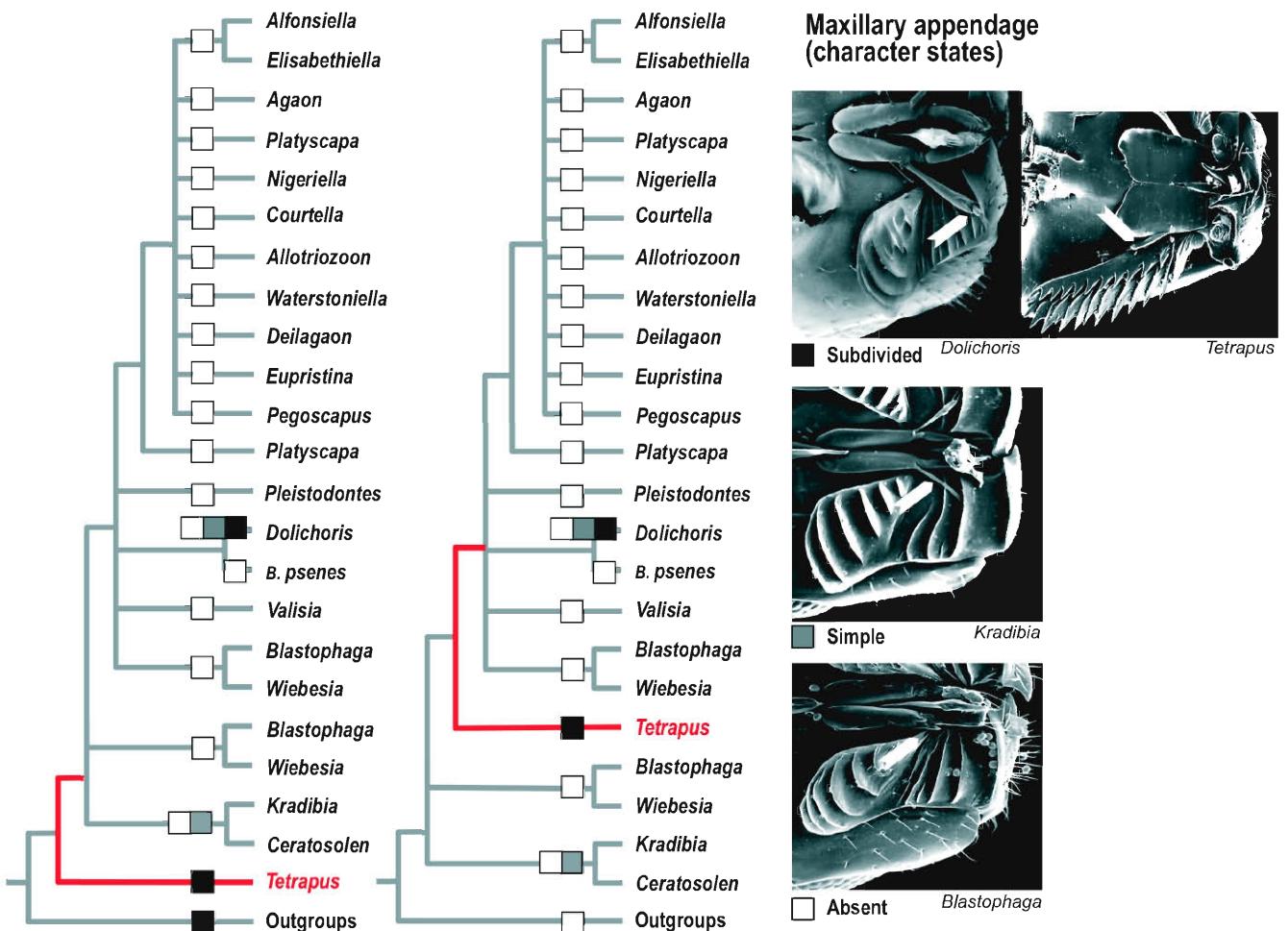
Structure of the first flagellomere (character states)



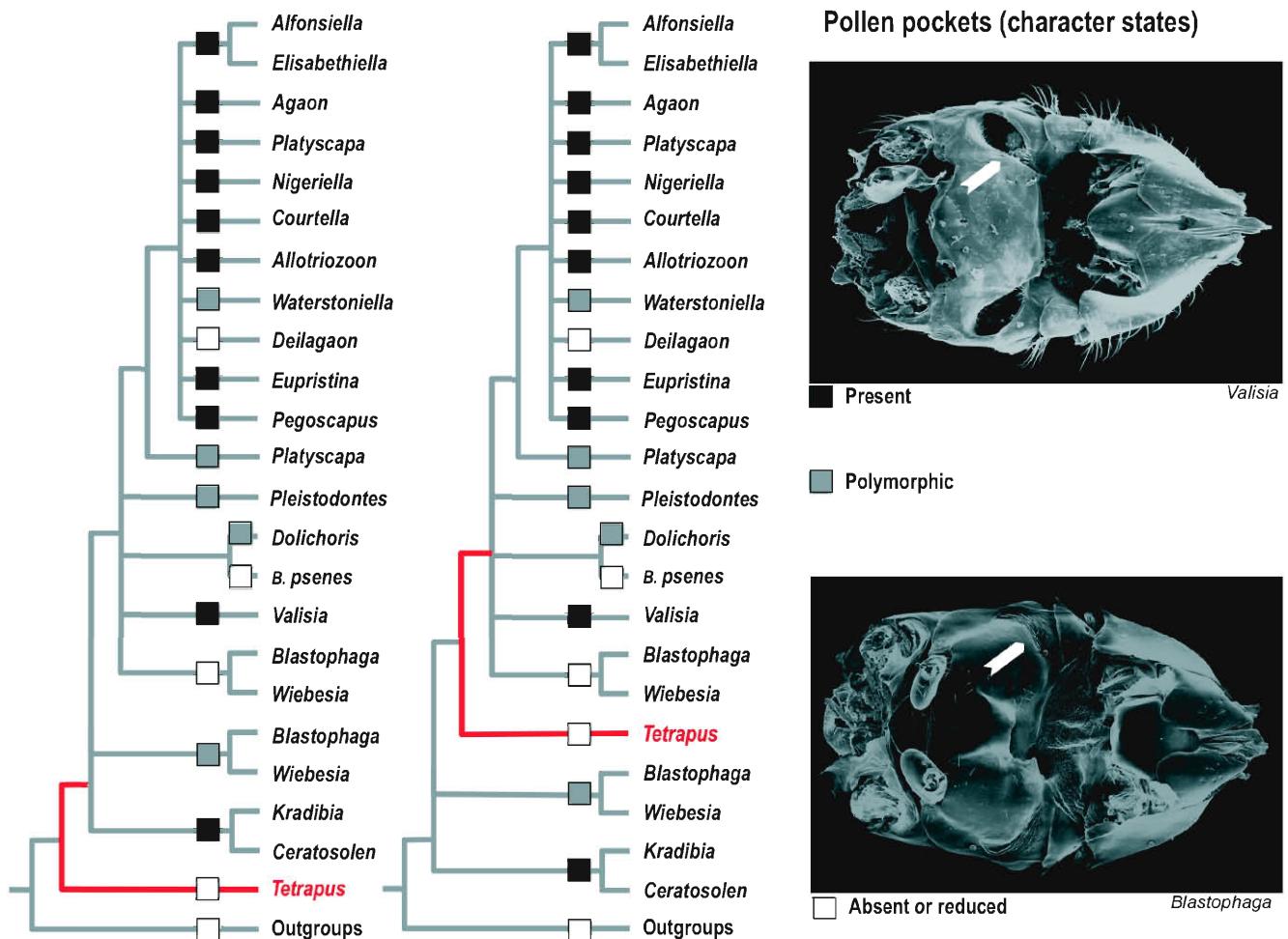
G



H



I



J

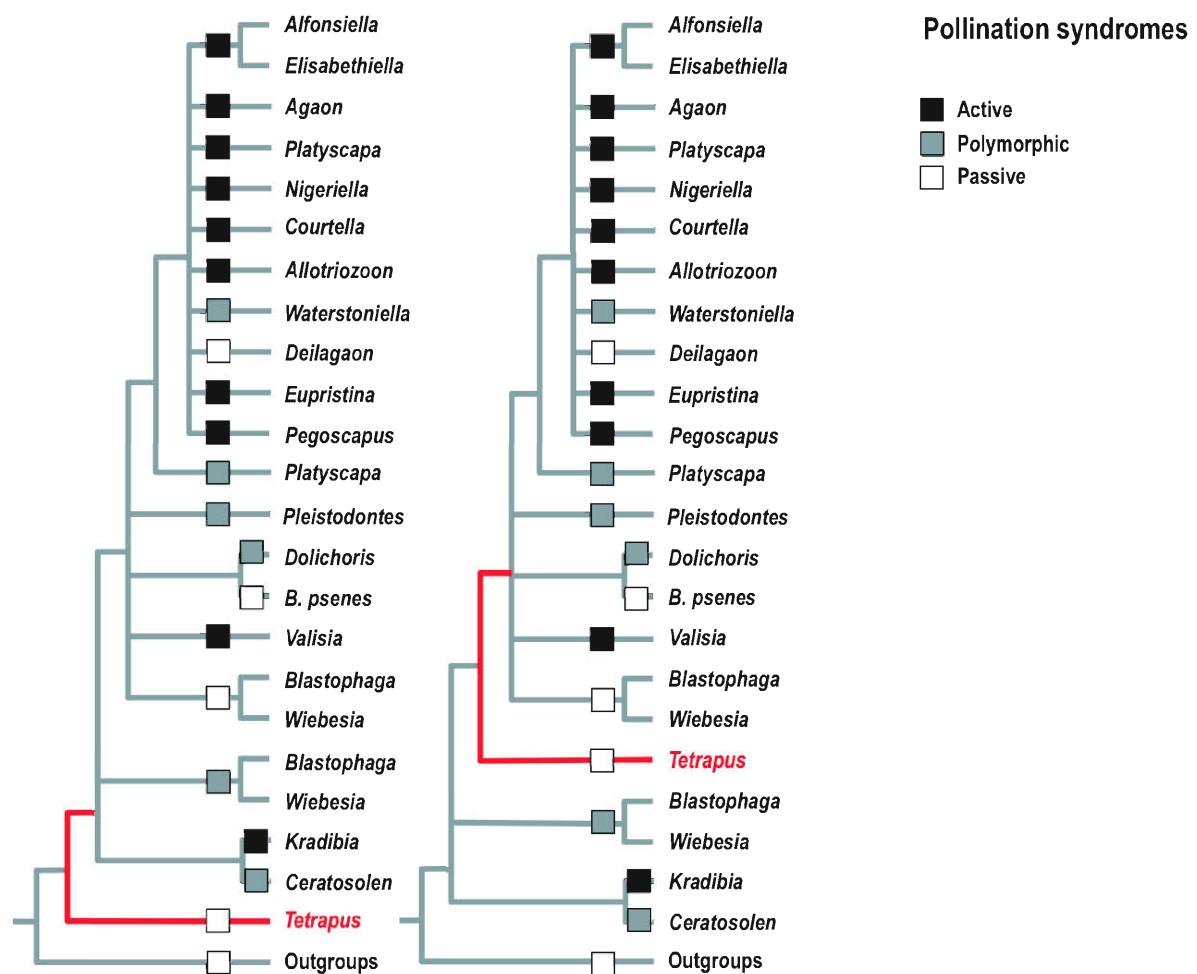


TABLE S1. Statistics regarding *Ficus* (a) and fig wasp (b) matrix completeness.

a) *Ficus*

Average percent of genes per species	59.9%
Average number and percent of nucleotides per species	2251 bp (40.5%)
Gene matrix completeness	
ITS (891 aligned basepairs)	100%
ETS (527 aligned basepairs)	78.4%
G3pdh (768 aligned basepairs)	74.5%
ncpGS (1629 aligned basepairs)	24.0%
Waxy (1733 aligned basepairs)	22.5%

b) Fig wasps

Average percent of genes per species	77.3%
Gene matrix completeness	
COI	84.8% (barcode fragment 79.9%; 2183-3014 fragment 46.1 %)
Cytb	84.8%
EF	72.1%
Wg	61.8%
28S	84.3% (D2D3 fragment 78.4%; D4D5 fragment 65.2 %)
18S	76.0%

TABLE S2. Results of comparative tests conducted on agaonid and *Ficus* datasets, AU and SH tests *P*-values.

Newick files for all topologies tested are available upon request. *Group 4 = most pollinators associated with subgenus *Synoecia* and subsection *Frustescentiae* (see main Text). **species included correspond to *Ceratosolen cf. adenispermae* ex *F. adenisperma* (n° 1246_01) ; *C. sp.* ex *F. subcuneata* (n° 2195_01) ; *C. stupefactus* ex *F. tiliifolia* (n° 1816_01) ; *C. arabicus* ex *F. mucoso* (n° 2191_01) ; *C. galili* ex *F. sycomorus* (n° 1532_06) ; *C. megacephalus* ex *F. vallis-choudae* (n° 1929_01) ; *C. appendiculatus* ex *F. variegata* (n° 1423_01) ; *C. cf. emarginatus* ex *F. oligodon* (n° 1910_01) ; *C. sp.* ex *F. stolonifera* (n° 1986_01) ; *C. sp.* ex *F. septica* (n° 1683_01) ; *C. vechti* ex *F. lepicarpa* (n° 1866_01) ; *C. vetustus* ex *F. schwarzii* (n° 1989_01) ; *Kradibia gestroi afrum* ex *F. exasperata* (n° 2186_01) ; *K. sp.* ex *F. parietalis* (n° 1864_01) ; *K. rutherfordi* ex *F. tinctoria* (n° 1868_01) ; *K. sessilis* ex *F. virgata sessilis* (n° 1263_01), which were all included in the Cruaud et al. (2010) dataset.

Dataset	Best topology	Alternative topology	Difference (-ln <i>L</i>)	AU test <i>P</i> value	SH test <i>P</i> value
Agaonidae					
combined dataset	best ML tree	Tetrapusinae sister to the remaining Agaonidae	12.7	0.261	0.416
combined dataset	best ML tree	Group 4* sister to the remaining Agaonidae	3.7	0.488	0.567
combined dataset	best ML tree	best ML tree estimated from the combined dataset with nt3 of the mitochondrial partition RY-coded	46.4	0.177	0.193
combined dataset	best ML tree	best ML tree estimated from the combined dataset with nt1 and nt3 of the mitochondrial partition RY-coded	61.4	0.124	0.121
combined dataset including only species for which more than 5 genes were sequenced	best ML tree estimated from the combined dataset including only species for which more than 5 genes were sequenced	best ML tree estimated from the combined dataset with pruning of taxa for which less than 5 genes were sequenced	45.5	0.061	0.064
combined dataset excluding	best ML tree estimated from the	best ML tree estimated from the	26.3	0.155	0.154

2183-3014 fragment of the COI gene	combined dataset excluding 2183-3014 fragment of the COI gene	combined dataset with pruning of taxa for which 2183-3014 fragment was missing			
combined dataset without <i>Ceratosolen</i> and <i>Kradibia</i> species	Best ML tree estimated from the combined dataset without <i>Ceratosolen</i> and <i>Kradibia</i> species	best ML tree estimated from the combined dataset with pruning of <i>Ceratosolen</i> and <i>Kradibia</i> species	18.1	0.265	0.266
combined dataset with a reduced sampling of <i>Ceratosolen</i> and <i>Kradibia</i> species**	Best ML tree estimated from the combined dataset with a reduced sampling of <i>Ceratosolen</i> and <i>Kradibia</i> species	best ML tree estimated from the combined dataset with pruning of <i>Ceratosolen</i> and <i>Kradibia</i> species not included in the reduced dataset	8.2	0.338	0.339
Cruaud et al., 2010	Cruaud et al., 2010	Kradibiinae sister to the remaining Agaonidae	11.6	0.231	0.338
Cruaud et al., 2010	Cruaud et al., 2010	Group 4 sister to the remaining Agaonidae	4.2	0.457	0.566
Lopez-vaamonde et al., 2009	Lopez-vaamonde et al., 2009	Kradibiinae sister to the remaining Agaonidae	32.1	0.032	0.098
Lopez-vaamonde et al., 2009	Lopez-vaamonde et al., 2009	Group 4 sister to the remaining Agaonidae	14.3	0.257	0.352
<i>Ficus</i>					
combined dataset	best ML tree	Subgenus <i>Sycomorus</i> sister to the remaining <i>Ficus</i>	6.9	0.418	0.556
combined dataset	best ML tree	Subgenera. <i>Sycomorus</i> , <i>Sycidium</i> , <i>Ficus</i> and <i>Synoecia</i> sister to the remaining <i>Ficus</i>	7.8	0.398	0.527
combined dataset including only species for which more than 3 genes were sequenced	best ML tree estimated from the combined dataset including only species for which more than 3 genes were sequenced	best ML tree estimated from the combined dataset with pruning of taxa for which less than 3 genes were sequenced	13.5	0.213	0.217

TABLE S3 Review of previous studies that attempted to reconstruct the evolutionary history of the Agaonidae.

Studies	Ramirez (1978)	Wiebes (1982)	Machado et al. (1996)	Herre et al. (1996)	Machado et al. (2001)	Weiblen (2001)	Jiang et al. (2006)	Lopez-Vaamonde et al. (2009)	Cruaud et al. (2010)	Cruaud et al. (2011)	
Taxonomic sampling	Phylogeny of Agaonidae 39 species, 18 genera <i>Blastophaga</i> (1) <i>Dolichoris</i> (2) <i>Wiebesia</i> (2) <i>Kradibia</i> (5) <i>Ceratosolen</i> (8) <i>Tetrapus</i> (1) <i>Pegoscapus</i> (1) <i>Platyscapa</i> (3) <i>Galaglychia</i> poll. (5) <i>Pleistodontes</i> (2) <i>Conosycea</i> poll. (8) <i>Valisia</i> (1)	Phylogeny of Agaonidae 23 species, 17 genera <i>Blastophaga</i> (1) <i>Dolichoris</i> (1) <i>Kradibia</i> (4) <i>Ceratosolen</i> (1) <i>Tetrapus</i> (1) <i>Pegoscapus</i> (1) <i>Platyscapa</i> (3) <i>Galaglychia</i> poll. (6) <i>Pleistodontes</i> (1) <i>Conosycea</i> poll. (4)	Phylogeny of Agaonidae and non pollinating fig wasps 3 Agaonidae + 7 species non pollinating fig wasps <i>Tetrapus</i> (1) <i>Pegoscapus</i> (1) <i>Platyscapa</i> (1) <i>Galaglychia</i> poll. (1)	Phylogeny of Agaonidae 13 species, 6 genera <i>Blastophaga</i> (1) <i>Dolichoris</i> (0) <i>Wiebesia</i> (0) <i>Kradibia</i> (0) <i>Ceratosolen</i> (1) <i>Tetrapus</i> (3) <i>Pegoscapus</i> (5) <i>Platyscapa</i> (0) <i>Galaglychia</i> poll. (2) <i>Pleistodontes</i> (1) <i>Conosycea</i> poll. (0)	Phylogeny of Agaonidae 32 species, 15 genera <i>Blastophaga</i> (1) <i>Dolichoris</i> (1) <i>Wiebesia</i> (2) <i>Kradibia</i> (2) <i>Ceratosolen</i> (10) <i>Tetrapus</i> (3) <i>Pegoscapus</i> (3) <i>Platyscapa</i> (1) <i>Galaglychia</i> poll. (5) <i>Pleistodontes</i> (2) <i>Conosycea</i> poll. (2)	Phylogeny of Agaonidae 43 species, 10 genera <i>Dolichoris</i> (3) <i>Wiebesia</i> (3) <i>Kradibia</i> (7) <i>Ceratosolen</i> (20) <i>Tetrapus</i> (0) <i>Pegoscapus</i> (0) <i>Platyscapa</i> (2) <i>Galaglychia</i> poll. (0) <i>Pleistodontes</i> (3) <i>Conosycea</i> poll. (3) <i>Valisia</i> (2)	Phylogeny of Agaonidae 101 species, 16 genera (Genbank + 20 species) <i>Blastophaga</i> (5) <i>Dolichoris</i> (5) <i>Wiebesia</i> (4) <i>Kradibia</i> (8) <i>Ceratosolen</i> (17) <i>Tetrapus</i> (3) <i>Pegoscapus</i> (2) <i>Platyscapa</i> (2) <i>Galaglychia</i> poll. (7) <i>Pleistodontes</i> (5) <i>Conosycea</i> poll. (6) <i>Valisia</i> (3)	Phylogeny of Agaonidae 64 species, 16 genera <i>Blastophaga</i> (2) <i>Dolichoris</i> (5) <i>Wiebesia</i> (3) <i>Kradibia</i> (8) <i>Ceratosolen</i> (17) <i>Tetrapus</i> (3) <i>Pegoscapus</i> (2) <i>Platyscapa</i> (9) <i>Galaglychia</i> poll. (17) <i>Pleistodontes</i> (9) <i>Conosycea</i> poll. (12) <i>Valisia</i> (3)	Phylogeny of Agaonidae 101 species, 19 genera <i>Blastophaga</i> (4) <i>Dolichoris</i> (7) <i>Wiebesia</i> (3) <i>Kradibia</i> (12) <i>Ceratosolen</i> (15) <i>Tetrapus</i> (5) <i>Pegoscapus</i> (12) <i>Platyscapa</i> (5) <i>Galaglychia</i> poll. (12) <i>Pleistodontes</i> (6) <i>Conosycea</i> poll. (5) <i>Valisia</i> (4)	Phylogeny of Agaonidae 89 species, 18 genera <i>Blastophaga</i> (4) <i>Dolichoris</i> (4) <i>Wiebesia</i> (6) <i>Kradibia</i> (12) <i>Ceratosolen</i> (15) <i>Tetrapus</i> (4) <i>Pegoscapus</i> (12) <i>Platyscapa</i> (5) <i>Galaglychia</i> poll. (12) <i>Pleistodontes</i> (6) <i>Conosycea</i> poll. (5) <i>Valisia</i> (4)	
(356 species described in 20 genera											
Estimated biodiversity > 1000 species*											
Outgroups	None	None	1 species Braconidae	2 species <i>Idarnes</i> sp. (Sycophaginae) <i>Critogaster</i> sp. (Sycoryctinae)	4 species <i>Philocaenus warei</i> (Sycocinae) <i>Seres solweziensis</i> (Sycocinae) <i>Critogaster</i> sp. 9A (Sycoryctinae) <i>Critogaster</i> sp. 10A (Sycoryctinae)	1 species <i>Apocryphopagrus spinitarsus</i> (Sycophaginae)	None	1 species <i>Anaphes nitens</i> (Mymaridae)	4 species <i>Sycophaga cyclostigma</i> (Sycophaginae) <i>Ficimila</i> sp (Eurytomidae) <i>Megastigmus</i> sp (Torymidae) <i>Trichogramma evanescens</i> (Trichogrammatidae)	None	
N nucleotids	N/A	N/A	238	626	816	1724	816	2137	4182	4763	
Molecular markers	N/A	N/A	12S (238 bp)	12S (350 bp) COII (276 bp)	COI (816 bp)	COI-tRNA(Leu)-COII (1724 bp)	COI (816 bp)	COI (824 bp) 28S (1313 bp)	COI (696 bp) Cytb (739 bp) Wg (481 bp) 28S (1480 bp) 18S (786 bp)	COI (1511 bp) Cytb (739 bp) EF (516 bp) Wg (403 bp) 28S (1594 bp)	
Morphological data	yes organs implied in pollen transfer	Yes (21 characters)	No	No	No	Yes (57 characters)	no	no	no	no	
Phylogenetic reconstruction methods	Intuitive reconstruction	Intuitive reconstruction	Parsimony Distance (NJ) Maximum likelihood	Parsimony	Maximum likelihood Distance (NJ) (data not shown) Parsimony (data not shown)	Maximum likelihood Parsimony	Bayesian inferences Parsimony	Bayesian inferences	Parsimony Bayesian inferences	Maximum likelihood Bayesian inferences	
Tetrapus position	2 groups proposed - Group A = <i>Ceratosolen</i> + <i>Kradibia</i> + <i>Blastophaga</i> (associated with subg. <i>Synoezia</i> and sect. <i>Ficus</i> subg. <i>Ficus</i>) Group B = <i>Tetrapus</i> sister to the remaining genera	2 subfamilies proposed Agaoninae: <i>Tetrapus</i> sister to <i>Pleistodontes</i> + pollinators of sect. <i>Galaglychia</i> <i>Blastophaginiae</i> : all the remaining genera	Sister to the 2 other agaonid species BP (parsimony?)=99	Sister to the remaining agaonids with low support BP = 45 for 12S and BP = 51 for COII	Sister to the remaining agaonids with low support BP < 50 "pollinators of the subgenera <i>Sycidium</i> , <i>Ficus</i> and <i>Sycomorus</i> appear in a basal position but with weak support (not shown) in the maximum parsimony tree"	N/A (no <i>Tetrapus</i> species included)	N/A (<i>Tetrapus</i> used to root the trees)	Sister to the remaining agaonids with high support PP=1.00	Sister to the remaining agaonids with high support in the bayesian analysis PP = 0.99 with low support in the parsimony analyses BP = 59, 55	N/A (<i>Tetrapus</i> used to root the trees)	

Studies	Ramirez (1978)	Wiebes (1982)	Machado et al. (1996)	Herre et al. (1996)	Machado et al. (2001)	Weiben (2001)	Jiang et al. (2006)	Lopez-Vaamonde et al. (2009)	Craaud et al. (2010)	Craaud et al. (2011)
Dating analyses	N/A	N/A	No	No	Strict molecular clock after removing the 19 lineages evolving at significantly different rates from the rest	No	No	Relaxed molecular clock r8s (NPRs and PL), BEAST, Multidivtime several calibration schemes	No	No
Agaonid age (crown group) Ma	N/A	N/A	N/A	N/A	87.5 Ma (100.3 - 74.7)	N/A	N/A	98 Ma (130 - 84) youngest mean estimate 149 Ma (216 - 130) oldest mean estimate global range 54-216	N/A	N/A
Biogeographic analyses	N/A	N/A	No	No	No	No	No	Mesquite (ML approach)	No	No
Agaonid ancestral area	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Depend on the topology used : From [Asia 0.54 ± 0.08; Australasia 0.28 ± 0.09; Neotropics 0.14 ± 0.02 for the highest probability for Asia] to [Asia 0.38 ± 0.09; Australasia 0.38 ± 0.08; Neotropics 0.13 ± 0.02 for the lowest probability for Asia]	N/A	N/A

*Craaud et al., 2010; **including probable contamination with *Ceratosolen* species

N/A non applicable

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TABLE S4 Length of intergenic regions for the chalcidoid sequences covering the zone between COI and t-RNA_{Leu} available in GenBank.

Species	N° Genbank	Length between the EIPK conserved amino acids and the t-RNA _{Leu}	Length of the coding part ⁽¹⁾	Length of the intergenic non coding spacer ⁽²⁾
<i>Nasonia vitripennis</i>	EU746609	13	13	0
<i>Philotrypesis pilosa</i>	JF808723	21	21	0
<i>Sycophaga spinitarsus</i>	AF200371	9	9	0
<i>Tetrapus ecuadoranus</i>	AY642463	41	27	11
<i>Dolichoris boschmai</i>	AY642459	57	9	45
<i>Dolichoris umbilicata</i>	AY642460	151	141	7
<i>Kradibia copiosae</i>	AY642460	19	18	1
<i>Kradibia wassae</i>	AF200402	19	18	0
<i>Kradibia gibbosae</i>	AF200403	129	117	9
<i>Ceratosolen bisulcatus</i>	AF200375	104	90 (TAG)	11
<i>Wiebesia frustratae</i>	AF200413	123	123	0
<i>Wiebesia punctatae</i>	AF200414	133	133	0
<i>Blastophaga sp</i>	AB121669	133	91	39
<i>Valisia malayana</i>	AF200373	153	141	9
<i>Pleistodontes plebejus</i>	AF200407	106	60	43
<i>Platyscapa corneri</i>	AF200405	108	93	12
<i>Alfonsiella nr binghami</i>	AY642464	96	78	15
<i>Eupristina verticillata</i>	AF200397	107	48	56

(1) not including the TAA, TAG or T stop codons ; (2) from stop codon to t-RNA_{Leu}.

Nota : COI from *Waterstoniella* sp. (Genbank ID AY642462) has been removed from the alignment as this sequence is probably a contamination from a *Wiebesia* species.

TABLE S5 Review of previous studies that attempted to reconstruct the evolutionary history of the *Ficus*.

Studies	Herre et al. (1996)	Herre et al. (1996)	Weiblen (2000)	Jousselin et al. (2003)	Datwyler and Weiblen (2004)	Zerega et al. (2005)	Rønsted et al. (2005)	Rønsted et al. (2008)	Craaud et al. (2011)	Xu et al. (2011)
Taxonomic sampling (subgenera) 735 to 750 species recognized in 6 subgenera and about 19-20 sections	Phylogeny of Moraceae 3 <i>Ficus</i> species and 7 other Moraceae <i>Pharmacosycea</i> (1) <i>Ficus</i> (1) <i>Americana</i> (1)	Phylogeny of <i>Ficus</i> 10 species, 6 subgenera, 9 sections <i>Pharmacosycea</i> (2) <i>Ficus</i> (2) <i>Synoezia</i> (1) <i>Sycidium</i> (0) <i>Sycomorus</i> (1) <i>Urostigma</i> (4)	Phylogeny of functionally dioecious <i>Ficus</i> 46 species, 6 subgenera, 14 sections <i>Pharmacosycea</i> (5) <i>Ficus</i> (2) <i>Synoezia</i> (4) <i>Sycidium</i> (8) <i>Sycomorus</i> (18) <i>Urostigma</i> (18)	Phylogeny of <i>Ficus</i> 41 species, 6 subgenera, 16 sections <i>Pharmacosycea</i> (6) <i>Ficus</i> (4) <i>Synoezia</i> (2) <i>Sycidium</i> (3) <i>Sycomorus</i> (3) <i>Urostigma</i> (2)	Phylogeny and origin of Moraceae 33 Moraceae genera incl. 11 <i>Ficus</i> species <i>Pharmacosycea</i> (4) <i>Sycidium</i> (2) <i>Sycomorus</i> (2) <i>Urostigma</i> (1)	Historical biogeography of Moraceae 32 Moraceae genera incl. 9 <i>Ficus</i> species <i>Pharmacosycea</i> (4) <i>Sycidium</i> (2) <i>Sycomorus</i> (2) <i>Urostigma</i> (1)	Phylogeny and dating of <i>Ficus</i> 146 species, 6 subgenera, 16 sections <i>Pharmacosycea</i> (10) <i>Ficus</i> (13) <i>Synoezia</i> (7) <i>Sycidium</i> (13) <i>Sycomorus</i> (21) <i>Urostigma</i> (82)	Phylogeny of <i>Ficus</i> 100 species, 6 subgenera, 16 sections <i>Pharmacosycea</i> (10) <i>Ficus</i> (13) <i>Synoezia</i> (7) <i>Sycidium</i> (13) <i>Sycomorus</i> (21) <i>Urostigma</i> (82)	Co-speciation testing Based on Rønsted (2008) matrix	Phylogeny of <i>Ficus</i> About 206 species (Genbank+34 Chinese species) 6 subgenera, 19 sections <i>Pharmacosycea</i> (13) <i>Ficus</i> (14) <i>Synoezia</i> (6) <i>Sycidium</i> (15) <i>Sycomorus</i> (44) <i>Urostigma</i> (114)
Outgroups	1 species <i>Magnolia magnophylla</i>	none	none	none	4 families	17 families	<i>Antiaropsis decipiens</i> <i>Castilla elastica</i> <i>Poulsonia armata</i> <i>Sparattosyne dioca</i>	2 species <i>Antiaropsis decipiens</i> <i>Castilla elastica</i>	none	2 species <i>Antiaropsis decipiens</i> <i>Castilla elastica</i>
N nucleotids	800	1800	643	1220	2089	4006	1354	2097	2097	2097
Molecular markers	RbcL (800 bp)	RbcL (800 bp) TrnLF (1000 bp)	ITS (643 bp) morphology	ITS (722 bp) ETS (498 bp)	NdhF (2089 bp)	NdhF (2092 bp) 26S (1014 bp)	ITS ETS (1354 bp in total)	ITS (820 bp) ETS (515 bp) G3pdH (762 bp)	ITS (820 bp) ETS (515 bp) G3pdH (762 bp)	ITS (820 bp) ETS (515 bp) G3pdH (762 bp)
Phylogenetic reconstruction methods	Parsimony	Parsimony	Parsimony	Parsimony	Parsimony Likelihood	Parsimony Likelihood	Parsimony Bayesian	Parsimony	Likelihood Bayesian	Parsimony Bayesian (MrBayes, BEAST)
Position of the section <i>Pharmacosycea</i>	<i>F. maxima</i> sister to the 2 other <i>Ficus</i> species BP=69	N/A (used as outgroup based on the results from the Moraceae phylogeny)	N/A (sect. <i>Pharmacosycea</i> used to root the trees)	N/A (sect. <i>Pharmacosycea</i> used to root the trees)	<i>F. insipida</i> sister to the remaining <i>Ficus</i> BP (parsimony only) < 50	<i>F. insipida</i> sister to the remaining <i>Ficus</i> BP (parsimony only)=84	Sister to the remaining <i>Ficus</i> species BP=71, PP=0.41	Sister to the remaining <i>Ficus</i> species BP < 50	N/A (sect. <i>Pharmacosycea</i> used to root the trees)	Sister to the remaining <i>Ficus</i> species PPMrBayes=0.91, PPBEAST=1.00, BP=87
Dating analyses	No	No	No	No	Penalized Likelihood (r8s)	MultiDivTime	NPRS Penalized Likelihood	No	No	BEAST
<i>Ficus</i> age (crown group)	N/A	N/A	N/A	N/A	60 Ma (minimum age constrained by fossil) stem group: at least 83 Ma	43.3 Ma (40.1–51.0)	60 Ma (minimum age constrained by fossil) stem group: 101.2 Ma (104.9 - 97.5)	N/A	N/A	86.67 (60.00 - 136.83)
Biogeographic analyses	No	No	No	No	No	No	No	No	No	DIVA
<i>Ficus</i> ancestral area	N/A	N/A	N/A	N/A	N/A	Eurasia as plausible as Gondwana (based on interpretation)	N/A	N/A	N/A	Neotropical America + India-Southeast Asian region

*Berg and Corner (2005): 735 species in 19 sections. Most authors estimate over 750 -800 species.

N/A non applicable

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TABLE S6 Results of tests of individual *Ficus*-pollinator links using ParaFit.

The global test of cospeciation resulted in rejection of random association between host and pollinator taxa (ParaFitGlobal = 1.37866, P ≤ 0.01). 176 of the 200 tests of individual host-associate pairs (highlighted in pale grey) resulted in significant associations between figs and their agaonid pollinators (P ≤ 0.01).

Significance	Agaonid	host <i>Ficus</i> species	P-value_1	P-value_2	ParaFitLink1 statistic (F1)	ParaFitLink2 statistic (F2)
+	1372_04	<i>Ficus chrysolepis</i>	0.00220	0.00220	0.00876	0.00007
+	2059_01	<i>Ficus cordatula</i>	0.00200	0.00200	0.00938	0.00008
+	1294_01	<i>Ficus benghalensis</i>	0.00300	0.00300	0.01104	0.00009
+	1599_01	<i>Ficus altissima</i>	0.00190	0.00190	0.00956	0.00008
+	1594_01	<i>Ficus maclellandii</i>	0.00200	0.00200	0.01138	0.00009
+	1875_01	<i>Ficus benjamina</i>	0.00240	0.00240	0.01144	0.00010
+	1611_01	<i>Ficus stricta</i>	0.00210	0.00210	0.01200	0.00010
+	1935_01	<i>Ficus thonningii</i>	0.00020	0.00020	0.01124	0.00009
+	2474_01	<i>Ficus natalensis</i>	0.00040	0.00040	0.01139	0.00009
+	2475_01	<i>Ficus craterostoma</i>	0.00090	0.00090	0.01262	0.00010
+	2619_01	<i>Ficus burtt-davyi</i>	0.00080	0.00080	0.01310	0.00011
+	2635_01	<i>Ficus lingua</i>	0.00080	0.00080	0.01179	0.00010
+	2637_01	<i>Ficus burkei</i>	0.00020	0.00020	0.01079	0.00009
+	1813_01	<i>Ficus reflexa</i>	0.00030	0.00030	0.01062	0.00009
+	2633_01	<i>Ficus usambarensis</i>	0.00100	0.00100	0.01028	0.00009
+	2622_01	<i>Ficus trichopoda</i>	0.00050	0.00050	0.01407	0.00012
+	2476_01	<i>Ficus fischeri</i>	0.00060	0.00060	0.01250	0.00010
+	1937_01	<i>Ficus glumosa</i>	0.00020	0.00020	0.01639	0.00014
+	2189_02	<i>Ficus tesselata</i>	0.00110	0.00110	0.01109	0.00009
+	1938_01	<i>Ficus preussii</i>	0.00240	0.00240	0.01860	0.00015
+	1925_01	<i>Ficus lyrata</i>	0.00150	0.00150	0.01660	0.00014
+	2473_01	<i>Ficus cyathistipula</i>	0.00120	0.00120	0.01545	0.00013
+	0942_01	<i>Ficus sagittifolia</i>	0.00120	0.00120	0.01360	0.00011
+	0450_01	<i>Ficus cyathistipuloides</i>	0.00140	0.00140	0.01586	0.00013
+	1534_01	<i>Ficus tettensis</i>	0.00080	0.00080	0.01047	0.00009
+	1765_05	<i>Ficus abutilifolia</i>	0.00060	0.00060	0.01176	0.00010
+	1629_01	<i>Ficus glaberrima</i>	0.00410	0.00410	0.00721	0.00006
+	1820_01	<i>Ficus menabeensis</i>	0.00360	0.00360	0.00572	0.00005
+	1936_01	<i>Ficus bubu</i>	0.00040	0.00040	0.01234	0.00010
+	2608_01	<i>Ficus tremula</i>	0.00060	0.00060	0.01562	0.00013
+	1934_01	<i>Ficus umbellata</i>	0.00030	0.00030	0.00999	0.00008
+	1930_01	<i>Ficus ovata</i>	0.00040	0.00040	0.01151	0.00010
+	2605_01	<i>Ficus sansibarica</i>	0.00060	0.00060	0.01081	0.00009
+	2615_01	<i>Ficus bizanae</i>	0.00080	0.00080	0.01087	0.00009
+	1946_01	<i>Ficus polita</i>	0.00120	0.00120	0.01094	0.00009

+	2614_01	<i>Ficus ottoniifolia</i>	0.00020	0.00020	0.01343	0.00011
+	1799_01	<i>Ficus palmeri</i>	0.00060	0.00060	0.01045	0.00009
+	1803_01	<i>Ficus petiolaris</i>	0.00100	0.00100	0.01236	0.00010
+	2140_01	<i>Ficus trigonata</i>	0.00030	0.00030	0.01441	0.00012
+	0964_01	<i>Ficus dugandii</i>	0.00020	0.00020	0.01737	0.00014
+	1364_04	<i>Ficus costaricana</i>	0.00030	0.00030	0.01146	0.00010
+	8030_01	<i>Ficus aurea</i>	0.00090	0.00090	0.01050	0.00009
+	2276_01	<i>Ficus eugeniaefolia</i>	0.00090	0.00090	0.01122	0.00009
+	2282_02	<i>Ficus colubrinae</i>	0.00050	0.00050	0.01230	0.00010
+	8024_01	<i>Ficus americana</i>	0.00040	0.00040	0.01347	0.00011
+	1954_01	<i>Ficus citrifolia</i>	0.00020	0.00020	0.01366	0.00011
+	2136_01	<i>Ficus eximia</i>	0.00040	0.00040	0.01380	0.00011
+	1797_01	<i>Ficus tuerckheimii</i>	0.00060	0.00060	0.01470	0.00012
+	1682_01	<i>Ficus andicola</i>	0.00050	0.00050	0.01149	0.00010
+	2165_01	<i>Ficus longifolia</i>	0.00030	0.00030	0.01395	0.00012
+	1778_01	<i>Ficus nymphaefolia</i>	0.00030	0.00030	0.01406	0.00012
+	2160_01	<i>Ficus obtusifolia</i>	0.00070	0.00070	0.01220	0.00010
+	2686_01	<i>Ficus trigona</i>	0.00100	0.00100	0.01296	0.00011
+	2177_01	<i>Ficus perforata</i>	0.00020	0.00020	0.01174	0.00010
+	2674_01	<i>Ficus crocata</i>	0.00030	0.00030	0.01340	0.00011
+	8193_01	<i>Ficus bullenei</i>	0.00040	0.00040	0.01311	0.00011
+	2158_01	<i>Ficus luschnathiana</i>	0.00030	0.00030	0.01278	0.00011
+	1393_01	<i>Ficus paraensis</i>	0.00060	0.00060	0.01303	0.00011
+	8172_01	<i>Ficus popenoei</i>	0.00020	0.00020	0.01433	0.00012
+	2162_01	<i>Ficus pertusa</i>	0.00030	0.00030	0.01356	0.00011
-	2289_01	<i>Ficus virens</i>	0.98770	0.98770	-0.00163	-0.00001
-	1752_01	<i>Ficus religiosa</i>	0.99320	0.99320	-0.00213	-0.00002
-	2420_01	<i>Ficus caulocarpa</i>	0.99490	0.99490	-0.00241	-0.00002
-	0227_01	<i>Ficus ingens</i>	0.99220	0.99220	-0.00189	-0.00002
-	2120_01	<i>Ficus superba</i>	0.98910	0.98910	-0.00161	-0.00001
-	2100_01	<i>Ficus concinna</i>	0.99030	0.99030	-0.00163	-0.00001
-	1536_01	<i>Ficus carica</i>	0.99290	0.99290	-0.00165	-0.00001
-	0868_01	<i>Ficus nervosa</i>	0.98070	0.98070	-0.00170	-0.00001
-	8059_01	<i>Ficus edelfeltii</i>	0.94979	0.94979	-0.00158	-0.00001
-	2352_01	<i>Ficus polyantha</i>	0.90429	0.90429	-0.00102	-0.00001
-	2335_01	<i>Ficus hombroniana</i>	0.93699	0.93699	-0.00131	-0.00001
-	2368_01	<i>Ficus subtrinervia</i>	0.97460	0.97460	-0.00196	-0.00002
-	1229_01	<i>Ficus habrophylla</i>	0.98470	0.98470	-0.00145	-0.00001
-	1276_01	<i>Ficus racemigera</i>	0.97010	0.97010	-0.00111	-0.00001
-	1676_01	<i>Ficus sarmentosa</i>	0.96300	0.96300	-0.00101	-0.00001
-	1836_01	<i>Ficus pumila</i>	0.72107	0.72107	-0.00013	-0.00000
-	1879_01	<i>Ficus deltoidea</i>	0.80798	0.80798	-0.00027	-0.00000
-	1992_01	<i>Ficus oleifolia</i>	0.74737	0.74737	-0.00016	-0.00000
+	1331_01	<i>Ficus maxima</i>	0.00200	0.00200	0.01540	0.00013
+	2180_01	<i>Ficus yoponensis</i>	0.00230	0.00230	0.01657	0.00014

+	1785_01	<i>Ficus insipida</i>	0.00260	0.00260	0.01665	0.00014
+	1796_01	<i>Ficus lapathifolia</i>	0.00300	0.00300	0.01774	0.00015
+	2178_01	<i>Ficus glabrata</i>	0.00330	0.00330	0.01629	0.00014
+	2166_01	<i>Ficus adhatodifolia</i>	0.00230	0.00230	0.01568	0.00013
+	1953_01	<i>Ficus tonduzii</i>	0.00260	0.00260	0.01319	0.00011
+	2533_01	<i>Ficus pantoniana</i>	0.00350	0.00350	0.00466	0.00004
+	1905_01	<i>Ficus ruginervia</i>	0.00330	0.00330	0.00502	0.00004
+	8239_01	<i>Ficus punctata</i>	0.00620	0.00620	0.00503	0.00004
+	1372_06	<i>Ficus sagittata</i>	0.00380	0.00380	0.00551	0.00005
+	1376_01	<i>Ficus odoardii</i>	0.00450	0.00450	0.00601	0.00005
+	8070_01	<i>Ficus bauerlenii</i>	0.00660	0.00660	0.00584	0.00005
+	1904_01	<i>Ficus diversiformis</i>	0.00440	0.00440	0.00601	0.00005
+	2188_01	<i>Ficus villosa</i>	0.00460	0.00460	0.00609	0.00005
+	1673_01	<i>Ficus ischnopoda</i>	0.00540	0.00540	0.00328	0.00003
+	2381_01	<i>Ficus erecta</i>	0.00600	0.00600	0.00347	0.00003
+	1757_02	<i>Ficus chapaensis</i>	0.00720	0.00720	0.00329	0.00003
+	1675_01	<i>Ficus stenophylla</i>	0.00670	0.00670	0.00348	0.00003
+	1531_01	<i>Ficus itoana</i>	0.00290	0.00290	0.00940	0.00008
+	8503_01	<i>Ficus microdictya</i>	0.00250	0.00250	0.01054	0.00009
+	2195_01	<i>Ficus subcuneata</i>	0.00360	0.00360	0.00931	0.00008
+	2539_01	<i>Ficus cf. erythrosperma</i>	0.00390	0.00390	0.00840	0.00007
+	8222_01	<i>Ficus semivestita</i>	0.00750	0.00750	0.01083	0.00009
+	8050_01	<i>Ficus ochrochlora</i>	0.00890	0.00890	0.01211	0.00010
+	2312_01	<i>Ficus mollior</i>	0.00560	0.00560	0.00966	0.00008
+	2349_01	<i>Ficus arbuscula</i>	0.00460	0.00460	0.01002	0.00008
+	1246_01	<i>Ficus adenosperma</i>	0.00220	0.00220	0.01244	0.00010
+	8056_01	<i>Ficus dammaropsis</i>	0.00350	0.00350	0.01405	0.00012
+	2463_01	<i>Ficus botryoides</i>	0.00230	0.00230	0.01481	0.00012
+	2191_01	<i>Ficus mucoso</i>	0.00470	0.00470	0.01326	0.00011
+	0659_23	<i>Ficus mauritiana</i>	0.00240	0.00240	0.01703	0.00014
+	1816_01	<i>Ficus tiliifolia</i>	0.00350	0.00350	0.01170	0.00010
+	1929_01	<i>Ficus vallis-choudae</i>	0.00130	0.00130	0.01244	0.00010
+	0453_21	<i>Ficus vogeliana</i>	0.00250	0.00250	0.01127	0.00009
+	1532_06	<i>Ficus sycomorus</i>	0.00170	0.00170	0.01318	0.00011
+	1810_01	<i>Ficus sur</i>	0.00230	0.00230	0.01274	0.00011
+	2028_01	<i>Ficus racemosa</i>	0.00250	0.00250	0.01120	0.00009
+	1423_01	<i>Ficus variegata</i>	0.00430	0.00430	0.01176	0.00010
+	2356_01	<i>Ficus nodosa</i>	0.00290	0.00290	0.01213	0.00010
+	1380_01	<i>Ficus robusta</i>	0.00190	0.00190	0.01557	0.00013
+	1606_01	<i>Ficus hainanensis</i>	0.00420	0.00420	0.00885	0.00007
+	1910_01	<i>Ficus oligodon</i>	0.00380	0.00380	0.00870	0.00007
+	2654_01	<i>Ficus auriculata</i>	0.00270	0.00270	0.01029	0.00009
+	0855_12	<i>Ficus semicordata</i>	0.00160	0.00160	0.01395	0.00012
+	0820_01	<i>Ficus prostrata</i>	0.00300	0.00300	0.00927	0.00008
+	1628_01	<i>Ficus tikoua</i>	0.00220	0.00220	0.01062	0.00009

+	2292_01	<i>Ficus pungens</i>	0.00030	0.00030	0.04351	0.00036
+	1986_01	<i>Ficus stolonifera</i>	0.00270	0.00270	0.01893	0.00016
+	1319_01	<i>Ficus megaleia</i>	0.00250	0.00250	0.02246	0.00019
+	1328_01	<i>Ficus uncinata</i>	0.00240	0.00240	0.02118	0.00018
+	2286_01	<i>Ficus condensa</i>	0.00290	0.00290	0.02210	0.00018
+	1446_01	<i>Ficus fistulosa</i>	0.00100	0.00100	0.03300	0.00027
+	1866_01	<i>Ficus lepicarpa</i>	0.00150	0.00150	0.03139	0.00026
+	1989_01	<i>Ficus schwarzii</i>	0.00080	0.00080	0.03259	0.00027
+	8321_01	<i>Ficus arfakensis</i>	0.00120	0.00120	0.03541	0.00029
+	8053_01	<i>Ficus hispidioides</i>	0.00100	0.00100	0.03854	0.00032
+	8320_01	<i>Ficus adelpha</i>	0.00130	0.00130	0.03546	0.00029
+	2293_01	<i>Ficus congesta</i>	0.00100	0.00100	0.03746	0.00031
+	2353_01	<i>Ficus bernaysii</i>	0.00090	0.00090	0.03683	0.00031
+	8344_01	<i>Ficus morobensis</i>	0.00110	0.00110	0.03886	0.00032
+	2198_01	<i>Ficus pachyrrhachis</i>	0.00140	0.00140	0.03777	0.00031
+	8184_01	<i>Ficus nota</i>	0.00200	0.00200	0.03425	0.00028
+	2193_01	<i>Ficus ribes</i>	0.00080	0.00080	0.03916	0.00033
+	1683_01	<i>Ficus septica</i>	0.00040	0.00040	0.04061	0.00034
+	2319_01	<i>Ficus botryocarpa</i>	0.00060	0.00060	0.03945	0.00033
+	1324_01	<i>Ficus treubii</i>	0.00060	0.00060	0.02902	0.00024
+	2053_01	<i>Ficus hispida</i>	0.00080	0.00080	0.04387	0.00036
+	1658_01	<i>Ficus squamosa</i>	0.00050	0.00050	0.03965	0.00033
+	1753_01	<i>Ficus heterostyla</i>	0.00070	0.00070	0.03998	0.00033
+	2485_01	<i>Ficus theophrastoides</i>	0.00160	0.00160	0.01755	0.00015
+	1654_01	<i>Ficus cyrtophylla</i>	0.00660	0.00660	0.00760	0.00006
+	1263_01	<i>Ficus virgata</i>	0.00580	0.00580	0.00845	0.00007
+	2375_01	<i>Ficus opposita</i>	0.00580	0.00580	0.01147	0.00010
+	2500_01	<i>Ficus wassa</i>	0.00850	0.00850	0.00942	0.00008
+	2492_01	<i>Ficus copiosa</i>	0.01020	0.01020	0.00966	0.00008
+	2081_01	<i>Ficus gul</i>	0.00450	0.00450	0.00851	0.00007
+	8235_01	<i>Ficus phaeosyce</i>	0.00580	0.00580	0.01001	0.00008
+	8233_01	<i>Ficus conocephalifolia</i>	0.00530	0.00530	0.00899	0.00007
+	2466_01	<i>Ficus politoria</i>	0.00440	0.00440	0.00848	0.00007
+	8232_01	<i>Ficus pygmaea</i>	0.00530	0.00530	0.00825	0.00007
+	2186_01	<i>Ficus exasperata</i>	0.00310	0.00310	0.00986	0.00008
+	2217_01	<i>Ficus asperifolia</i>	0.00560	0.00560	0.00850	0.00007
+	1650_01	<i>Ficus henryi</i>	0.00560	0.00560	0.00702	0.00006
+	1764_01	<i>Ficus subulata</i>	0.00400	0.00400	0.01038	0.00009
+	1375_01	<i>Ficus trachypison</i>	0.00540	0.00540	0.00796	0.00007
+	2125_01	<i>Ficus ampelas</i>	0.00660	0.00660	0.00775	0.00006
+	2362_01	<i>Ficus melinocarpa</i>	0.00520	0.00520	0.01055	0.00009
+	1864_01	<i>Ficus parietalis</i>	0.00460	0.00460	0.01032	0.00009
+	1868_01	<i>Ficus tinctoria</i>	0.00530	0.00530	0.00917	0.00008
+	2010_01	<i>Ficus obscura</i>	0.00340	0.00340	0.01124	0.00009
-	1745_01	<i>Ficus hirta</i>	0.39434	0.39434	0.00041	0.00000

-	2098_01	<i>Ficus ruficaulis</i>	0.83178	0.83178	-0.00030	-0.00000
-	0967_01	<i>Ficus aurata</i>	0.47685	0.47685	0.00033	0.00000
-	2556_01	<i>Ficus grossularioides</i>	0.51285	0.51285	0.00029	0.00000
-	8039_01	<i>Ficus padana</i>	0.50185	0.50185	0.00031	0.00000
-	2087_01	<i>Ficus fulva</i>	0.51675	0.51675	0.00029	0.00000
+	8146_01	<i>Ficus hesperidiiformis</i>	0.00710	0.00710	0.00511	0.00004
+	8130_01	<i>Ficus xylosycia</i>	0.00290	0.00290	0.00367	0.00003
+	1410_01	<i>Ficus destruens</i>	0.00480	0.00480	0.00330	0.00003
+	1416_02	<i>Ficus crassipes</i>	0.00690	0.00690	0.00336	0.00003
+	8124_01	<i>Ficus brachypoda</i>	0.00600	0.00600	0.00338	0.00003
+	8139_01	<i>Ficus hilliputiana</i>	0.00360	0.00360	0.00406	0.00003
+	8137_01	<i>Ficus platypoda</i>	0.00500	0.00500	0.00380	0.00003
+	8145_01	<i>Ficus watkinsiana</i>	0.00570	0.00570	0.00360	0.00003
+	1396_01	<i>Ficus rubiginosa</i>	0.00530	0.00530	0.00350	0.00003
+	1252_02	<i>Ficus glandifera</i>	0.00540	0.00540	0.00524	0.00004
+	1554_01	<i>Ficus pleurocarpa</i>	0.00660	0.00660	0.00420	0.00003
+	8127_01	<i>Ficus triradiata</i>	0.00440	0.00440	0.00298	0.00002
+	1397_01	<i>Ficus macrophylla</i>	0.00670	0.00670	0.00404	0.00003
+	1422_01	<i>Ficus obliqua</i>	0.00510	0.00510	0.00397	0.00003
+	0988_01	<i>Ficus saussureana</i>	0.00190	0.00190	0.01270	0.00011
+	2602_01	<i>Ficus lutea</i>	0.00080	0.00080	0.01350	0.00011
+	1204_01	<i>Ficus spathulifolia</i>	0.00120	0.00120	0.00787	0.00007
+	8237_01	<i>Ficus paracamptophylla</i>	0.00200	0.00200	0.01006	0.00008
+	0926_01	<i>Ficus callophylla</i>	0.00270	0.00270	0.01030	0.00009
+	1878_01	<i>Ficus sumatrana</i>	0.00260	0.00260	0.00833	0.00007
+	1871_01	<i>Ficus consociata</i>	0.00150	0.00150	0.00905	0.00008
+	1321_02	<i>Ficus subgelderri</i>	0.00200	0.00200	0.01017	0.00008
+	2066_01	<i>Ficus forstenii</i>	0.00220	0.00220	0.00917	0.00008
+	8068_01	<i>Ficus pellucidopunctata</i>	0.00260	0.00260	0.01121	0.00009
+	1664_01	<i>Ficus annulata</i>	0.00120	0.00120	0.00764	0.00006