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Phylogenetic relationships of Santalales with insights into the origins of holoparasitic Balanophoraceae

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■ APPENDIX S1. JUSTIFICATION FOR WHY *CYNOMORIUM* WAS EXCLUDED FROM THIS STUDY

Historically, *Cynomorium* L. has either been included in Balanophoraceae or not. For example, Eichler (1867) removed *Cynomorium* from Balanophoraceae as did Van Tieghem (1896). Interestingly, Eichler (1873) reversed his position on this in the *Prodromus*. Workers in the 20th century usually included Cynomoriaceae in Balanophoraceae (Cronquist, 1981) or at least placed it proximally in the classification system (Takhtajan, 1997, 2009).

Nickrent & al. (2005) were the first to use molecular data to show that *Cynomorium* and Balanophoraceae were not closely related. Support was strong for Balanophoraceae in or near Santalales and *Cynomorium* in Saxifragales. Curiously, APG III (2009) stated that the evidence for placing Cynomoriaceae in Saxifragales "is not strong". The MP plus BI tree shown in Nickrent & al. (2005) utilized nuclear SSU rDNA, chloroplast *rbcL* and *atpB*, and mitochondrial *matR*. That tree (their fig. 2) shows *Cynomorium* as sister to *Peridiscus* (MPBS 98, BIPP 0.78) and that clade sister to *Hamamelis* (MPBS 98, BIPP 100), both members of Saxifragales. The tree that resulted from an attempt to more precisely place *Cynomorium* within Saxifragales (their fig. 3) did not have strong support for any particular relationship.

Barkman & al. (2007) recovered a relationship with Saxifragales using *matR* but a combined *atp1* and *coxI* analyses supported a placement with Sapindales. This conflicting relationship is likely the result of horizontal gene transfer, known to occur with both *atp1* and *coxI*.

Cynomorium was excluded from the study of Saxifragales by Jian & al. (2008) because the authors report that it was placed in Santalales in an unpublished analysis of 561 angiosperms. Given that phylogenetic position, it is likely that the authors accidentally used a sample of Balanophoraceae; however, no voucher was cited to verify the identity of the plant sampled.

Zhang & al. (2009) conducted a phylogenetic study using the inverted repeat of the plastid genome. Their data indicated that *Cynomorium songaricum* was sister to Rosaceae (*Prunus* and *Fragaria*) with 99% BS, a result also seen by Moore & al. (2011) using the same molecular marker. As was shown by García & al. (2004), *Cynomorium* has extensive intraindividual variation in plastid rDNA, likely deriving from heteroplasmy. For this reason, one must use extreme caution before using phylogenetic data from the plastid genome of *Cynomorium* or other extreme haustorial parasites (Naumann & al., 2013).

In their study of angiosperm phylogeny using mitochondrial genes, Qiu & al. (2010) discuss but did not include *Cynomorium* in their trees because it was placed in Saxifragales with *matR* and *nad5* but with Sapindales with *atp1* and *rps3*. Again, it is well documented that holoparasite mitochondrial genes can derive from horizontal transfer, particularly with *atp1* and ribosomal protein genes (Molina & al., 2014). But interestingly, the study by Naumann & al. (2013) also used mitochondrial *atpA* (= *atp1*?), *coxI* and *matR*. Using a relaxed molecular clock approach to estimate the timing of origins for all angiosperm parasite lineages, they found that Cynomoriaceae diverged ca. 100 Ma and that it was most closely related to Saxifragales.

In summary, the genes that support placing *Cynomorium* in (or sister to) Saxifragales include nuclear SSU rDNA and mitochondrial *atp1*, *coxI*, *matR* and *nad5*. Because the mitochondrial genes *atp1* and *coxI* are known to be subjected to horizontal transfer, particularly in holoparasitic angiosperms, it is not clear how different results were obtained in Qiu & al. (2010) vs. Naumann & al. (2013). Finally, one should be skeptical about phylogenetic results derived from plastid-encoded genes from *Cynomorium* given the heteroplasmic nature of this organelle and the extreme reduction and modification its plastome experienced as the plant evolved the holoparasitic habit.

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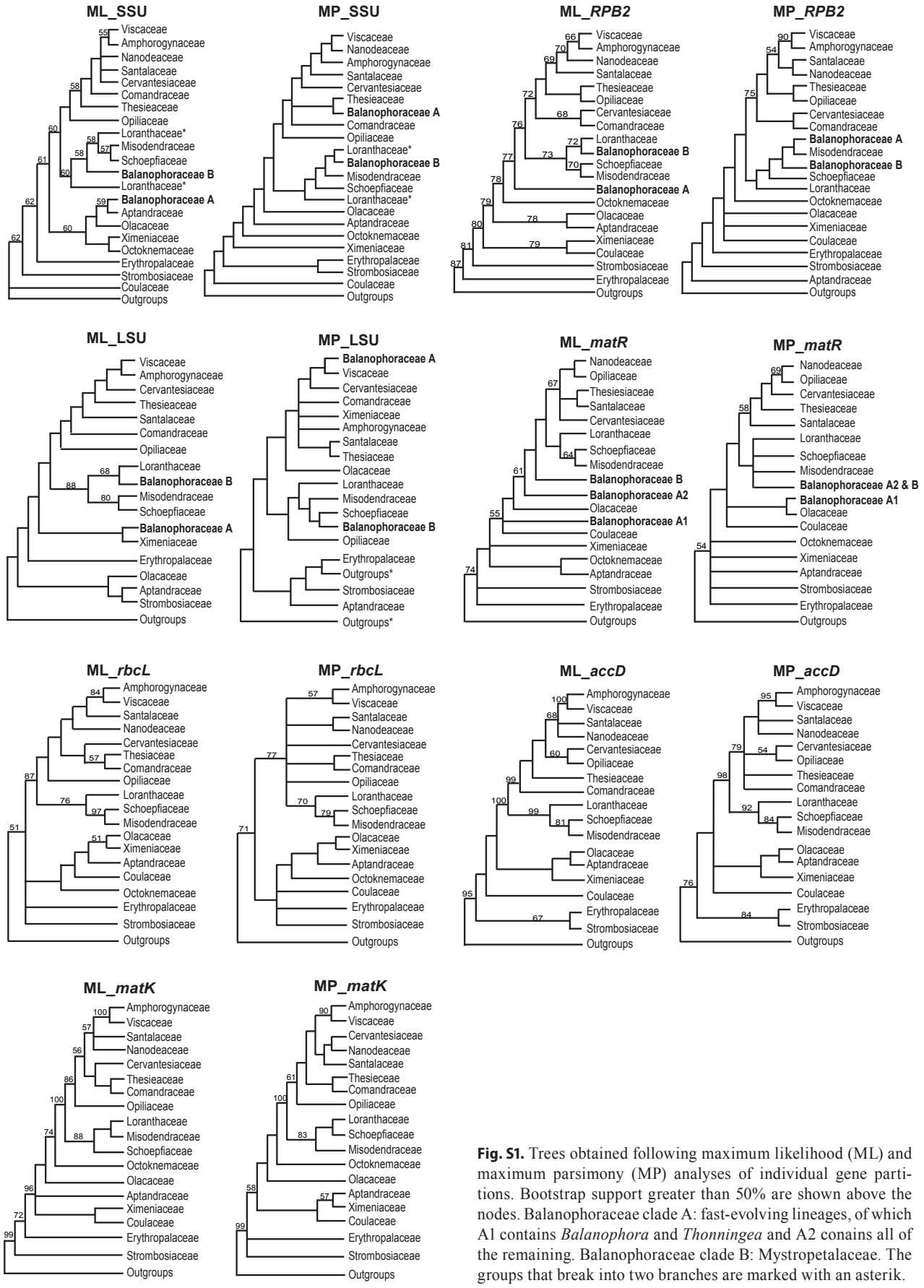

Fig. S1. Trees obtained following maximum likelihood (ML) and maximum parsimony (MP) analyses of individual gene partitions. Bootstrap support greater than 50% are shown above the nodes. Balanophoraceae clade A: fast-evolving lineages, of which A1 contains *Balanophora* and *Thonningea* and A2 conains all of the remaining. Balanophoraceae clade B: Mystropetalaceae. The groups that break into two branches are marked with an asterik.

Fig. S2. The strict consensus of 6912 MP trees inferred from the concatenated 7-gene dataset. Bootstrap support values greater than 50% are shown above the nodes. Tree length = 28,736, consistency index = 0.380, retention index = 0.641.

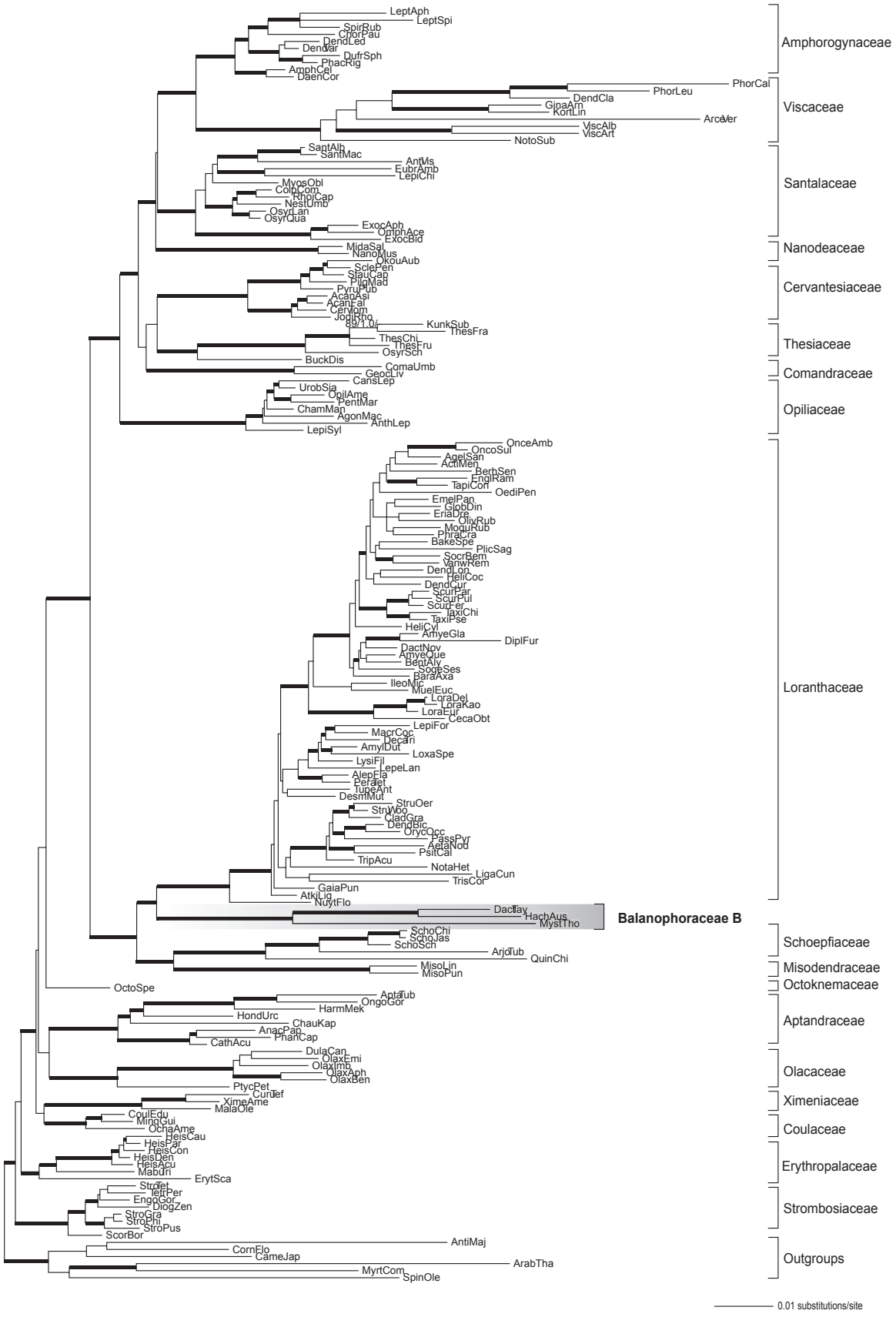

Fig. S3. The ML tree inferred from the concatenated 7-gene dataset that excluded Balanophoraceae clade A (fast-evolving). Bootstrap support greater than 80% and Bayesian posterior probabilities greater than 0.95 are shown with bold lines.

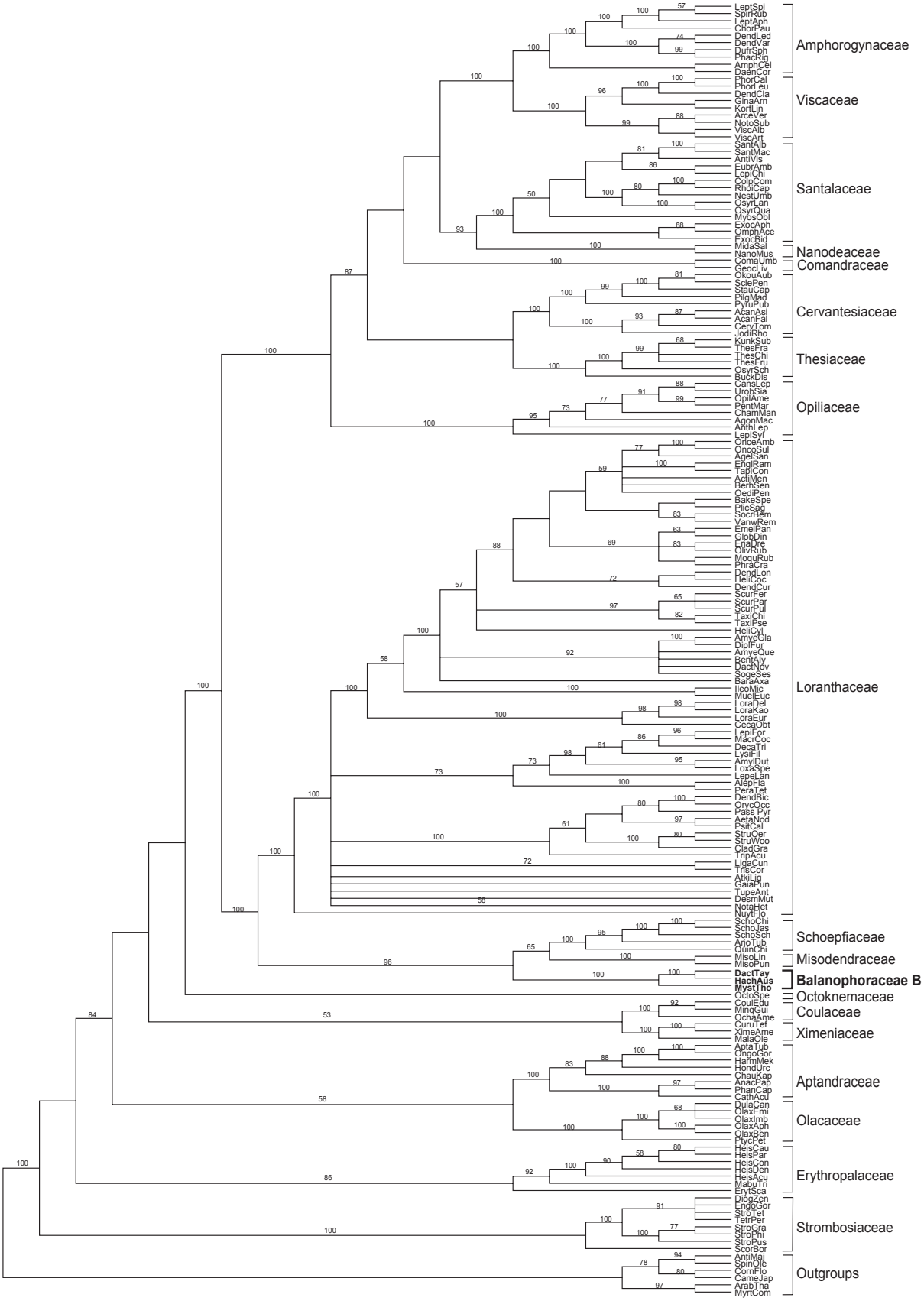

Fig. S4. The strict consensus of 15,552 MP trees inferred from the concatenated 7-gene dataset that excluded Balanophoraceae clade A (fast-evolving). Bootstrap support greater than 50% are shown above the nodes. Tree length = 25,219, consistency index = 0.384, retention index = 0.657.

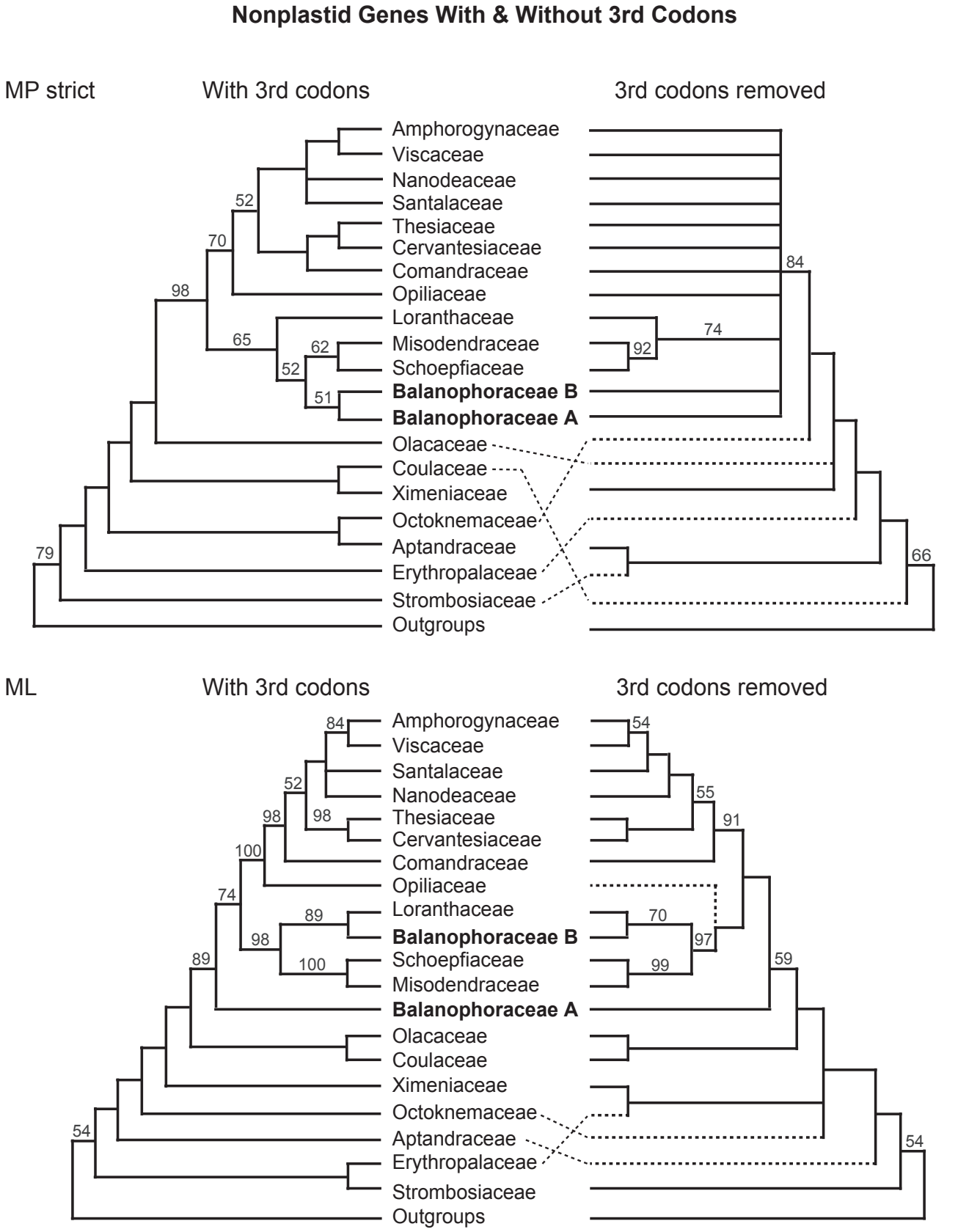

Fig. S5. Santalales tree topologies using the four-non-plastid gene partition: SSU and LSU rDNA, *RPB2*, and *matR*. Trees on the left included third codons whereas trees on the right excluded third codons from *RPB2* and *matR*. Strict consensus maximum parsimony trees (MP) shown on the top, maximum likelihood trees (ML) on the bottom. Support values greater than 50% are shown above the nodes.

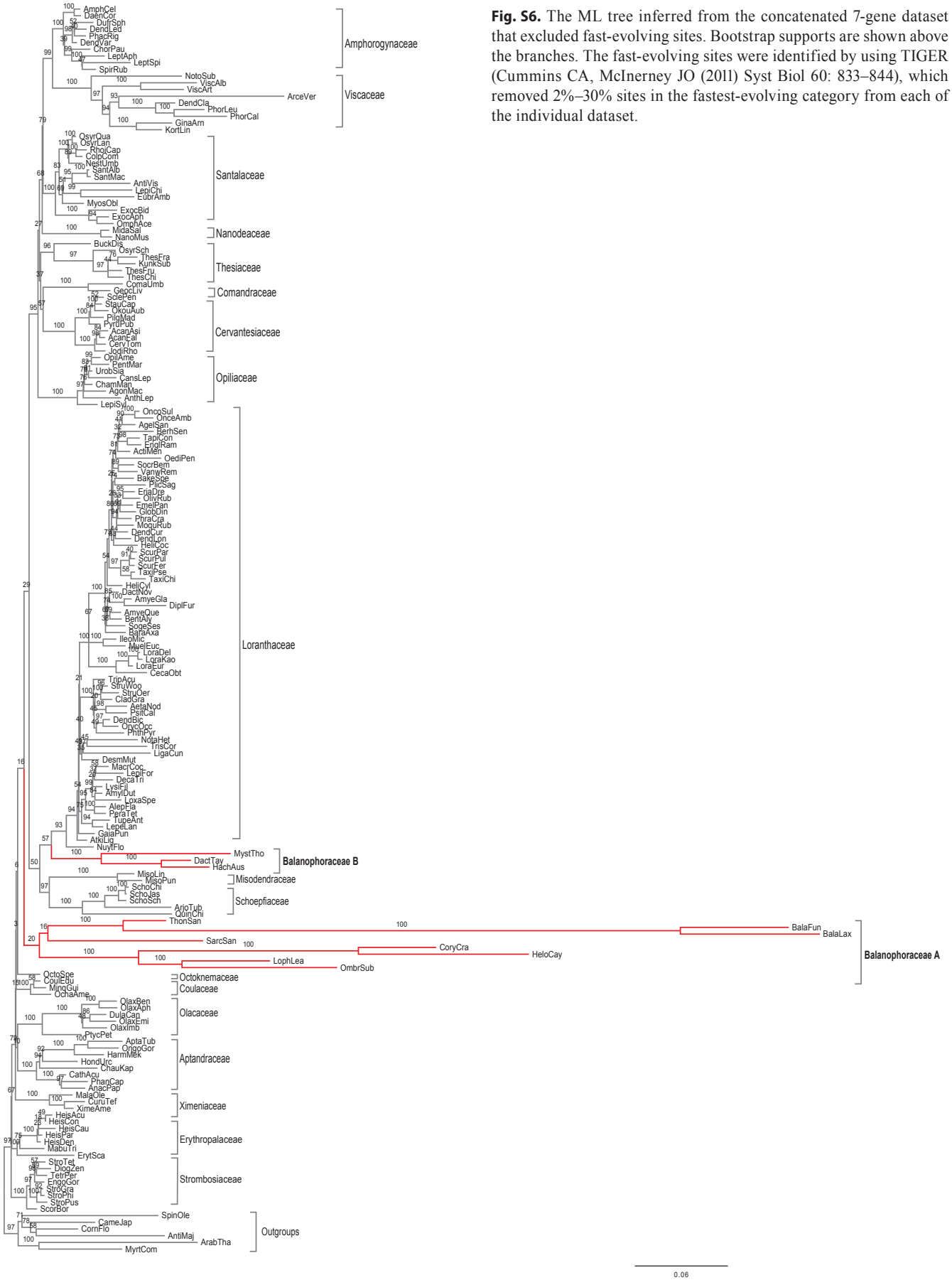

Fig. S6. The ML tree inferred from the concatenated 7-gene dataset that excluded fast-evolving sites. Bootstrap supports are shown above the branches. The fast-evolving sites were identified by using TIGER (Cummins CA, McInerney JO (2011) Syst Biol 60: 833–844), which removed 2%–30% sites in the fastest-evolving category from each of the individual dataset.

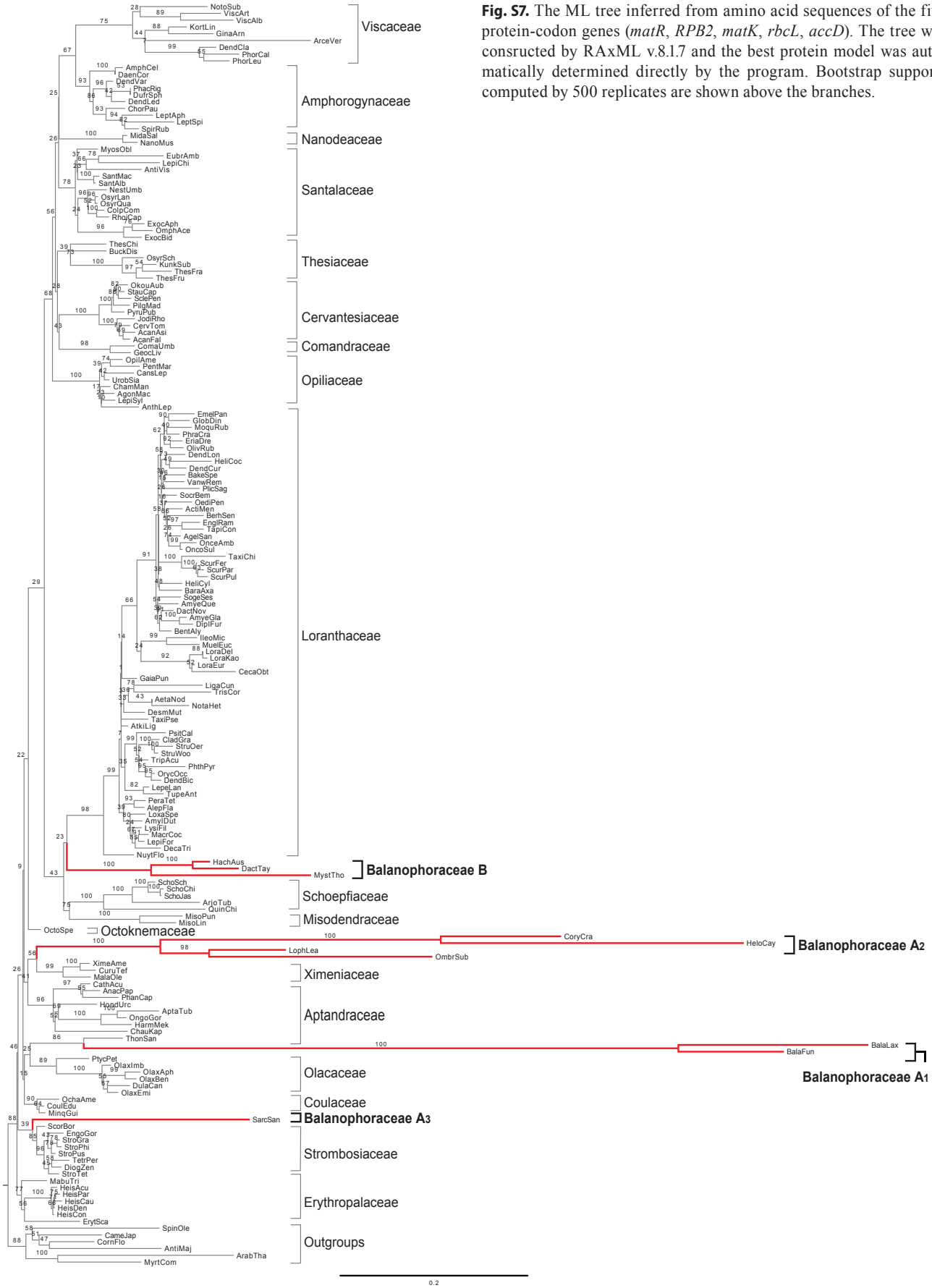

Fig. S7. The ML tree inferred from amino acid sequences of the five protein-codon genes (*matR*, *RPB2*, *matK*, *rbcL*, *accD*). The tree was consructed by RAxML v.8.1.7 and the best protein model was automatically determined directly by the program. Bootstrap supports computed by 500 replicates are shown above the branches.