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PHYLOGENY OF ANTHOSPERMEAE OF THE COFFEE FAMILY INFERRED USING CLOCK AND NONCLOCK MODELS

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Premise of research. With wind-pollinated flowers and partly temperate distribution, the tribe Anthospermeae stands out in the otherwise mostly animal-pollinated and tropical coffee family (Rubiaceae). Nevertheless, few attempts to resolve the phylogeny of the group have been made, and inter- and infrageneric relationships have been only partly addressed. Here we investigate evolutionary relationships and generic and subtribal delimitations of Anthospermeae. We assess the influence of alternative evolutionary rate models on topology and node support.

Methodology. Using sequence data from the nuclear (nrITS and nrETS) and plastid (*atpB-rbcL*, *ndhF*, *rbcL*, *rps16*, and *trnT-trnF*) genomes collected for a broad sample of taxa, we conducted Bayesian analyses using nonclock, strict clock, and relaxed clock models. The resulting topologies and support values were compared, and the relative fit of evolutionary models to our data was evaluated. Marginal likelihood estimates were used to discriminate between the competing rate models.

Pivotal results. The monophyly of Anthospermeae was confirmed with *Carpacoce* resolved as sister to the remaining species. We found several cases of supported topological conflict between results based on nuclear and plastid data, but the deepest splits of the tribe were congruent among all analyses and incompatible with traditional subtribal delimitations of Anthospermeae. Monophyly of the genera *Anthospermum*, *Nenax*, and *Coprosma* was not supported. While the relaxed clock model was consistently favored over the nonclock and strict clock models for all data sets, the use of the different models had little impact on phylogenetic results.

Conclusions. We propose a revised subtribal classification of Anthospermeae, including a new subtribe, the monogeneric Carpacocinae. Introgression/hybridization and incomplete lineage sorting are the most likely causes for the plastid-nuclear incongruences detected for Anthospermeae, but their relative contribution could not be concluded.

Keywords: Anthospermeae, incongruence, model choice, nonclock, relaxed molecular clock, Rubiaceae.

Online enhancements: appendix figures and tables.

Introduction

The species-rich and mostly tropical coffee family (Rubiaceae) is one of the largest flowering plant families. Most species are animal-pollinated, but the tribe Anthospermeae stands out as comprising only wind-pollinated genera (Puff 1982). In general, Anthospermeae comprise small trees or shrubs with inconspicuously colored, actinomorphic, nectarless, and odorless flowers distributed in the tropics, subtropics, and temperate regions of the Southern Hemisphere (Puff 1982). Even though Anthospermeae thus exhibit some features that are atypical of Rubiaceae (wind-pollinated flowers and partly temperate distribution), attempts to resolve the phylogenetic relationships among and within genera are few. Puff (1982) redelimited Anthospermeae using flower type (anemophilous and nonheterostylous flowers) and other floral characters (e.g., filament insertion) and included 12 genera in the tribe. On the basis of floral and fruit characters and consideration of geography, he grouped these genera into three subtribes. His treatment has since been slightly modified (Fosberg 1982; Puff and Robbrecht 1988; Robbrecht 1988, 1993; Andersson 2000); for a summary, see Anderson et al. (2001).

Anthospermeae are thus currently subdivided into (1) subtribe Anthosperminae (*Anthospermum* L., *Carpacoce* Sond., *Galopina* Thunb., *Nenax* Gaertn., and *Phyllis* L.), (2) subtribe Coprosminae (*Coprosma* J.R.Forst. & G.Forst., *Durringtonia* R.J.F.Hend & Guymer, *Leptostigma* Arn., *Nertera* Banks & Sol. ex Gaertn., and *Normandia* Hook.f.), and (3) subtribe Opercullariinae (*Oper-*

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cularia Gaertn. and *Pomax* Sol.). The monotypic genus *Eleuthranthes* F.Muell. ex Benth. and its single species *Eleuthranthes opercularina* F.Muell. ex Benth. are illegitimate names and taxonomic synonyms of *Opercularia* Gaertn. and *Opercularia liberiflora* F.Muell., respectively (Australian Plant Census: CHAH 2019; IPNI 2019). Anthosperminae occur in mainland Africa, Madagascar, Macaronesia, and the southwestern part of the Arabian Peninsula and are typically characterized by unisexual or protandrous bisexual flowers and dry dehiscent fruits. Some *Nenax* species constitute exceptions in having dry indehiscent fruits (Puff 1982). Coprosminae have a broad transpacific distribution (*Nertera* expands the range to also include the Caribbean and Tristan da Cunha). The subtribe is characterized by fleshy to semifleshy fruits (as opposed to dry fruits in the other subtribes) and unisexual to protogynous bisexual flowers (Puff 1982). Opercullariinae are restricted to Australia and characterized by umbel- or headlike inflorescences, unisexual or protogynous bisexual flowers, and dry fruits, which open by means of an operculum (Puff 1982). This subtribal delimitation is also supported by pollen morphology (Robbrecht 1982).

The first study to address the phylogeny of Anthospermeae using molecular data was based on parsimony analyses of nrITS and *rps16* sequence data of 28 taxa from 12 genera (Anderson et al. 2001). While offering new insights into the intergeneric relationships, many nodes of the phylogeny were not statistically supported, and the monophyly of the tribe was unsupported because the South African genus *Carpacoce* was sister to the tribe Knoxieae. Subsequent studies (Bremer and Eriksson 2009; Rydin et al. 2009) focusing on deeper relationships in Rubiaceae supported the monophyly of Anthospermeae, with *Carpacoce* resolved as sister to the rest of the tribe in the latter study. However, these early studies used a limited sample of taxa from each tribe, and the delimitation and phylogenetic relationships within Anthospermeae have remained unclear. Phylogeny and historical biogeography of the large Pacific genus *Coprosma* have recently been studied (Cantley et al. 2014, 2016; Heads 2017). Cantley et al. (2016) found *Coprosma* to be nonmonophyletic, since *Coprosma moorei* and *Coprosma talbrockiei* were resolved as more closely related to *Durringtonia paludosa* than to the other species of *Coprosma*. These clades will hereafter be referred to as the *Durringtonia-Coprosma* clade and as *Coprosma* sensu stricto (s.s.), respectively. Apart from those studies, no phylogenetic work on specific genera or subtribes of Anthospermeae has been conducted.

The main objective of this study was to produce a robust phylogeny of Anthospermeae. We used plastid and nuclear data from a comprehensive sample of representative taxa. Through the course of the work, we also wanted to assess relative fit of evolutionary models to our data. We were particularly interested in assessing the potential impact of different clock models on phylogenetic results, and in addition to a nonclock model, we explored two clock models (relaxed and strict) when analyzing the relationships of Anthospermeae. In comparison with, for example, choice of nucleotide substitution model and data partitioning (e.g., Nylander et al. 2004; Pagel and Meade 2004; Kainer and Lanfear 2015), the impact of clock models (which result in ultrametric trees) on topology and node support has been less well studied, but increasing evidence indicates that the assumption of a relaxed molecular clock may influence topological results (e.g., Miller and Bergsten 2012; Lambert et al. 2015; Rydin et al. 2017). We therefore estimated the marginal likelihood of selected models and used them for subsequent Bayes factor tests to determine the competing models' relative fit to data. The phylogenetic results were used as a basis for discussions on evolutionary relationships and generic and subtribal delimitations of Anthospermeae.

Material and Methods

Data

A total of 120 specimens of Anthospermeae, representing 87 species from all 12 genera of the tribe, were included. The phylogenetic relationships within the genus *Coprosma* have been dealt with previously (Cantley et al. 2014, 2016) and will not be discussed further here. We therefore restricted the sample of *Coprosma* to include a set of species that, although limited, represents the major lineages of the genus (including *Coprosma moorei* and *Coprosma talbrockiei*). For outgroup rooting, 39 specimens from 33 different genera were chosen to represent the other 11 recognized tribes of the Spermacoceae alliance (Rydin et al. 2009; Wen and Wang 2012; Ginter et al. 2015)—Argostemmateae, Cyanoneuroneae, Danaideae, Dunnieae, Foonchewieae, Knoxieae, Paederieae, Putorieae, Rubieae, Spermacoceae, and Theligoneae—as well as one species from its sister group, the Psychotrieae alliance.

We used five molecular markers from the plastid genome (*atpB-rbcL* intergenic spacer [IGS], *ndhF*, *rbcL*, *rps16* intron, and the *trnT-trnL-trnF* region) and two from the nuclear genome (nrETS and nrITS). These markers have proven useful for constructing phylogenies on similar taxonomic depths as this study within Rubiaceae (e.g., Backlund et al. 2007; Kårehed et al. 2008; Ginter et al. 2015). For the outgroup taxa, only the plastid markers *ndhF*, *rbcL*, and *atpB-rbcL* IGS were used. Newly produced sequences (88 nrETS, 89 nrITS, 91 *atpB-rbcL*, 94 *ndhF*, 92 *rbcL*, 86 *rps16*, and 86 *trnT-F*) were complemented with relevant sequence data from GenBank. For information regarding investigated specimens and GenBank accession numbers, see appendix A.

Extraction of DNA was performed using the cetyl trimethylammonium bromide method (Doyle and Doyle 1987) with the modification that instead of manual grinding of leaf tissue, a TissueLyser LT (Qiagen) with two stainless steel beads at 5000 rpm for 2 min was used. The extracted DNA was purified with a QIAquick polymerase chain reaction (PCR) kit (Qiagen, Hilden) according to the instructions provided by the manufacturer and subsequently used as PCR templates. The PCR mixtures included the following: 5 μL reaction buffer, 5 μL TMACl, 0.2 mM of each dNTP, 0.5 μL Paq5000 DNA polymerase (5 U/μL; Agilent Technologies, Santa Clara, CA), 0.5 μL of each primer (20 μM), 0.5 μL BSA 1%, 1 μL of DNA template, and sterilized water up to 50 μL. Primers and profiles used for PCR amplification and sequencing of the included regions are given in tables B1 and B2 (tables B1–B4 are available online). PCR products were cleaned on Multiscreen PCR plates (Millipore, Billerica, MA) according to the instructions provided by the manufacturer. Sequencing was performed by the Macrogen Sequencing Service (Amsterdam).

Obtained raw reads were assembled in Geneious version 9.1.8 (Kearse et al. 2012). Bases of primer sequences were identified by aligning the new sequences with the respective amplification/sequencing primers and subsequently removed.

Plastid and nuclear markers were annotated in Geneious with the complete chloroplast sequence of *Coffea arabica* (GenBank accession no. NC_008535; Samson et al. 2007) and the complete ribosomal cistron sequence of *Asclepias syriaca* (GenBank accession no. JF312046; Straub et al. 2011) as references. Separate alignments of the following gene regions were performed: *ndhF*, *rbcL*, *rps16* intron, *atpB-rbcL* IGS, *trnT-trnL* IGS, *trnL*, *trnL-trnF* IGS, nrETS, nrITS1, nr5.8S, and nrITS2. The nr5.8S region was manually aligned. All other regions were aligned using MAFFT version 7 (Katoh and Standley 2013) with the algorithm G-INS-i with a variable scoring matrix. This alignment method was chosen because it reduces the risk of overalignment (Katoh and Standley 2016). Protein coding genes were aligned as amino acids and back translated to nucleotides. The alignments were visually inspected in AliView (Larsson 2014), and some minor adjustments were occasionally made.

Before phylogenetic analyses were performed, we checked for putative nrITS pseudogenes and putative recombinant sequences. For the detection of putative pseudogenic regions, the nrITS sequences were aligned with the Viridiplantae conserved 5.8S motifs: GAATTGCAGAAwyC, TTTGAAyGCA, CGATGAAGA ACGyAGC. The absence of one or more of these conserved motifs indicates that the sequence is a putative pseudogene (Harpke and Peterson 2008). No such putative pseudogenes were found. For detection of putative recombinants and their major and minor parents, we conducted automated exploratory screens for recombination of the nrETS and nrITS alignments using seven different automated detection methods implemented in RDP4 v.4.95 (Martin et al. 2015): 3SEQ (Boni et al. 2006), BOOTSCAN (Salminen et al. 1995), CHIMAERA (Posada and Crandall 2001), GENECONV (Padidam et al. 1999), MAXCHI (Smith 1992), RDP (Martin and Rybicki 2000), and SISCAN (Gibbs et al. 2000). All seven methods were used as primary detection methods. Sequences were treated as linear, and window size for the BOOTSCAN and SISCAN methods were set to 80 base pairs (bp); otherwise, default settings were used. Following the default setting in RDP4 and the arbitrarily set criteria defined by Tsaousis et al. (2005), we considered recombination signals identified by at least two methods as good evidence for a sequence to be a putative recombinant. No such putative recombinants were found.

Incongruence between individual and combined gene regions was evaluated by manually comparing their corresponding tree topologies. We considered nodes to be in conflict if alternative resolutions had Bayesian posterior probability (BPP) values equal to or above 0.95. The separate analyses of the individual markers (not shown) did not show any supported topological conflicts when compared with markers from the same genome. Because of supported incongruence between plastid versus nuclear tree topologies, we did not combine plastid and nuclear data. Three separate data matrices were constructed using SequenceMatrix v1.8 (Vaidya et al. 2011): one plastid data set including both ingroup and outgroup taxa (7805 bp in length/1862 variable characters), one plastid data set including ingroup (Anthospermeae) taxa only (7605/1006 bp), and one nuclear data set containing only ingroup taxa (1022/483 bp; see also table B3).

Phylogenetic Analyses

PartitionFinder 2 (Lanfear et al. 2016) was used to find algorithmically optimal partitioning schemes (table B4). As input for the plastid data sets, a total of 11 data blocks were specified (*atpB-rbcL* IGS, *rps16* intron, *trnT-trnL* IGS, *trnL*, *trnL-trnF* IGS, and first, second, and third codon positions of the protein coding genes *ndhF* and *rbcL*). For the nuclear data set, four data blocks were specified (nrETS, nrITS1, nr5.8S, and nrITS2). Using PartitionFinder 2, a search for the best partitioning scheme was conducted with the greedy algorithm under the Bayesian information criterion (Schwarz 1978) using all models. The reversible jump Markov chain Monte Carlo (RJ-MCMC) procedure for model selection implemented in MrBayes 3.2 (Ronquist et al. 2012*b*) was used for each partition. The method, described by Huelsenbeck et al. (2004), allows for sampling across all the 203 possible 4×4 nucleotide substitution models in proportion to each model's marginal probability. Among-site rate variation was modeled using a discrete gamma distribution with four categories and a proportion of invariant sites. All parameters except those for topology and branch lengths were unlinked across partitions, and rates were allowed to vary across partitions.

For the plastid data set with outgroups, two MrBayes analyses were performed, one using a nonclock model and rooted on *Schizocolea linderi* and one using a relaxed clock model where the Spermacoceae alliance (i.e., all taxa except *Schizocolea linderi*) was constrained to be monophyletic. For the data sets without outgroups, three MrBayes analyses were performed, one using a nonclock model, one using a relaxed clock model, and one using a strict clock model. All other model settings were identical between analyses. The trees resulting from the clock analyses were rooted by the model, and the trees resulting from the nonclock analyses were rooted on *Carpacoce* (based on results of the analyses including outgroups).

For the nonclock analyses, the default settings were used. For the relaxed clock analyses, we used the independent gamma rates (IGR) model (Lepage et al. 2007), with the prior on the variance of the branch rate parameter (igrvarpr) set to the default exponential (10) value (expected mean 0.1). The tree prior for the clock analyses was set to fossilized birth-death (FBD; Heath et al. 2014), with the fossilization rate set to zero. The reason for using the FBD model with the fossilization rate set to zero instead of the birth-death model was that they have different priors on root age. The birth-death model has a uniform (zero to infinity) distribution, and the FBD model has a gamma (1, 1) distribution. Because choosing proper priors is of great importance when inferring marginal likelihoods (Baele et al. 2013), the improper tree age prior (i.e., does not integrate to 1) of the birth-death model was not used.

Metropolis-coupled MCMC for all analyses included four runs with four chains each (one cold and three heated), sampling trees, and parameter estimates every 1000th generation. The total number of generations for the nuclear, plastid without outgroup, and plastid with outgroup data sets was 40 million, 50 million, and 60 million, respectively. To achieve better mixing and faster convergence, the temperature setting varied between 0.02 and 0.1 among analyses. Convergence was diagnosed by monitoring the average standard deviation of split frequencies (ASDSF) to be below 0.01, the potential scale reduction factor values (Gelman and Rubin 1992) to be close to 1.0 for all parameters, and the effective sample size values to be above 200 for each parameter and by evaluating whether apparent stationarity of the log likelihood estimates had been reached. The first two statistics were monitored in the MrBayes output, and the remaining

were visualized in Tracer v1.6 (Rambaut et al. 2014). On the basis of the convergence assessment, the default burn-in (the first 25% of the samples) was used in the majority of analyses, but for the relaxed clock analysis of the plastid with outgroups data set, a burn-in of 50% of samples was used. The post-burn-in trees from each analysis were summarized as 50% majority rule trees. To identify possible conflicts between individual markers from the same genome, nonclock analyses (including two runs) for each marker (one partition) were conducted using RJ-MCMC with the number of generations set to 10 million.

Model Selection

To compare the nonclock and clock models' relative fit to the data, the stepping-stone sampling procedure (Xie et al. 2011) implemented in MrBayes 3.2 was used for log marginal likelihood estimations. The same settings for number of runs, number of chains, temperature, and sampling frequency for the corresponding regular analysis were used. The number of steps and the alpha value were set to their default values (50 and 0.4, respectively), and the steps were sampled from the posterior to prior. Each analysis was rerun with increasing number of generations to assess that the marginal likelihood estimates were stable (the analyses of the plastid data set with outgroups demanded much longer computational times than the analyses without outgroups, and the decision to abandon them was taken). The highest number of total generations for the plastid nonclock, plastid clock (relaxed and strict), nuclear nonclock, and nuclear clock (relaxed and strict) analyses was 100 million, 150 million, 160 million, and 240 million, respectively. The first 10 million generations in each analysis were discarded as burn-in. The first 25% of the total number of generations within each step was also discarded.

Convergence was assessed by inspecting differences of marginal likelihood estimates among runs as well as those between analyses. The ASDSF for each step was examined. The log marginal likelihoods estimated from the longest stepping-stone analyses were deemed stable as estimates obtained from previous shorter runs were almost unchanged, the log marginal likelihoods obtained were stable also among runs in each analysis, and the ASDSF was at least below 0.035 for each step in all analyses. The log marginal likelihoods obtained from the final analyses were used for subsequent Bayes factor tests. Models were compared with the 2 × log Bayes factor (2lnBF) statistic, which is calculated by taking twice the difference of the log marginal likelihoods between two models. For calculation of the 2lnBF statistics, the mean from four independent stepping-stone estimations of the marginal likelihood of each model was used. The 2lnBF scores were interpreted following Kass and Raftery (1995), where 2lnBF in the range 0–2 is not worth more than mentioning, 2lnBF in the range 2–6 means positive support, 2lnBF in the range 6–10 means strong support, and 2lnBF >10 means very strong support. All Bayesian analyses were run using the software MrBayes 3.2.6 (Ronquist et al. 2012*b*) on the CIPRES computing cluster (Miller et al. 2010).

Results

Model Selection

Log Bayes factor tests showed with very strong support that the relaxed clock model fits both the plastid data set and the nuclear data set better than both the nonclock model and the strict clock model (table 1). The mean and the 95% highest posterior density (HPD) interval of the amount of rate variation among branches (as defined by the IGRvar parameter from the relaxed clock analyses of the plastid data set with outgroups, plastid data set without outgroups, and nuclear data set) were 1.46×10^{-3} (95% HPD interval: 1.05×10^{-3}, 1.90×10^{-3}), 2.04×10^{-4} (95% HPD interval: 1.12×10^{-4}, 3.02×10^{-4}), and 8.12×10^{-4} (95% HPD interval: 2.48×10^{-4}, 1.39×10^{-3}), respectively. The HPD intervals indicate that the data sets are overall quite clock-like but do not include zero, thus providing additional support for rejection of a strict clock model.

Phylogenetic Reconstruction

Because the relaxed clock model provided a better fit to both the plastid data set and the nuclear data set, we will (unless otherwise stated) focus on the results obtained from the relaxed clock analyses without outgroups (figs. 1, 2). The nonclock and relaxed clock analyses of the plastid data set including outgroups

Table 1

Marginal Likelihood Estimation and Bayes Factor Values (2lnBF) for Models under Consideration

	Marginal likelihood					
Data set and model	SS run 1	SS run 2	SS run 3	SS run 4	Mean	2lnBF
Plastid:						
Relaxed clock	−19,174.35	−19,175.15	−19,178.64	−19,172.24	−19,173.46	na
Strict clock	−19,192.65	−19,195.84	−19,194.77	−19,193.61	−19,193.60	40.28
Nonclock	−19,234.64	−19,229.16	−19,235.59	−19,234.07	−19,230.54	114.16
Nuclear:						
Relaxed clock	−8704.87	−8705.31	−8703.55	−8714.37	−8704.57	na
Strict clock	−8708.97	−8714.77	−8712.13	−8713.22	−8710.29	11.44
Nonclock	−8835.28	−8830.68	−8836.02	−8831.81	−8831.77	254.40

Note. Marginal log likelihood estimates from the four independent stepping-stone sampling (SS) runs under the nonclock, relaxed clock, and strict clock models for the nuclear and plastid data sets. The Bayes factor test statistic (2lnBF) was calculated by taking twice the difference of the log marginal likelihoods between the two competing models. The 2lnBF values given are from comparing the relaxed clock model against the other models. na = not applicable.

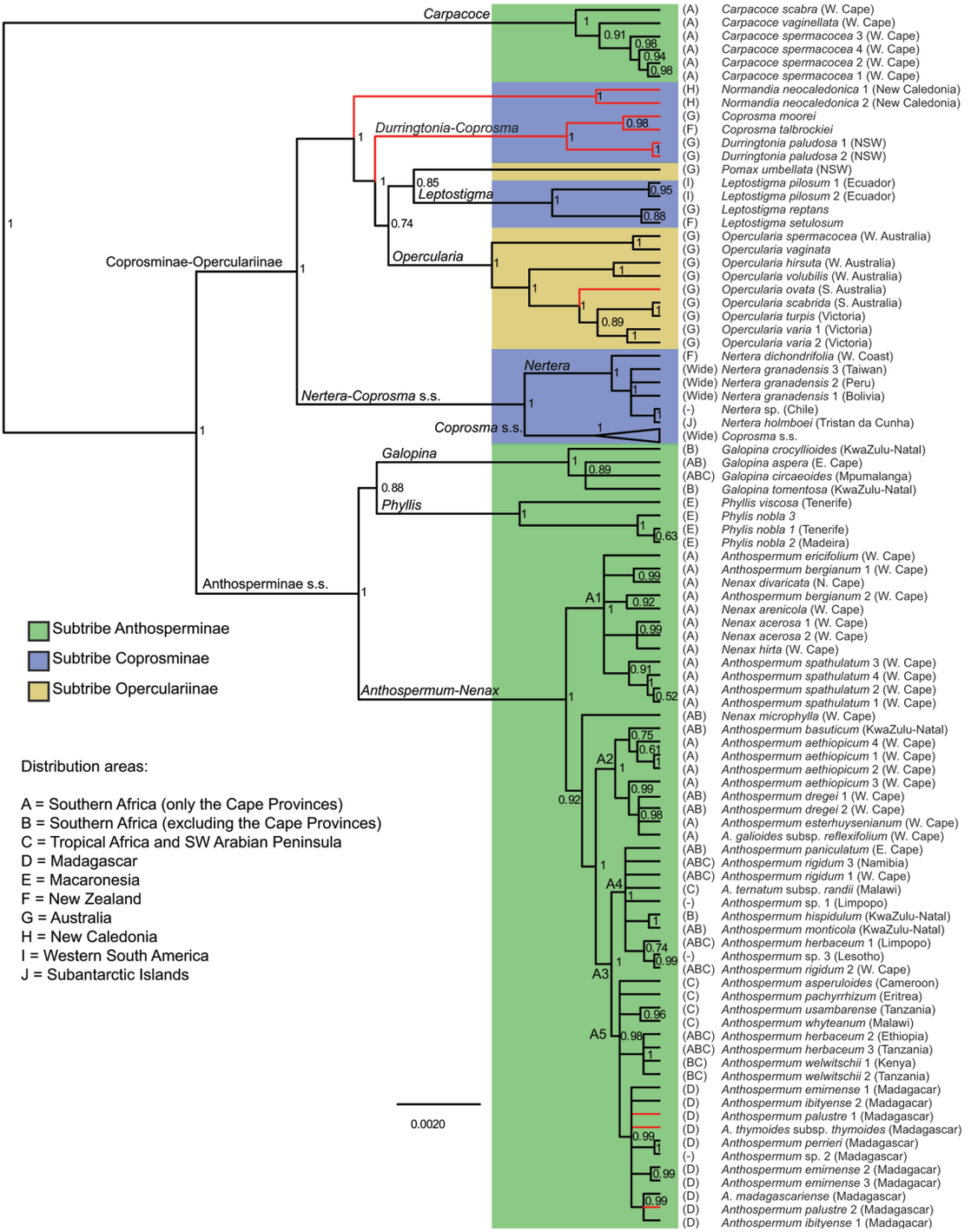

Fig. 1 Fifty percent majority rule consensus tree showing phylogenetic relationships among Anthospermeae based on the relaxed clock analysis of the plastid data set excluding outgroups. Support values next to the nodes are Bayesian posterior probabilities (BPPs). Scale bar represents the expected number of substitutions per site. Text in the tree corresponds to clades mentioned in the text. Red branch color indicates selected supported (BPP ≥ 0.95) incongruence with the nuclear-derived trees discussed in the text. The *Coprosma* s.s. clade is collapsed into a triangle. Capital letter(s) within parentheses before taxon names corresponds to distribution area code(s). Text within parentheses after taxon names corresponds to sampling locality for species/specimens with newly produced sequences. Distribution areas were based on information from the cited literature and the World Checklist of Selected Plant Families (WCSP 2019), with geographical areas based on Brummitt (2001).

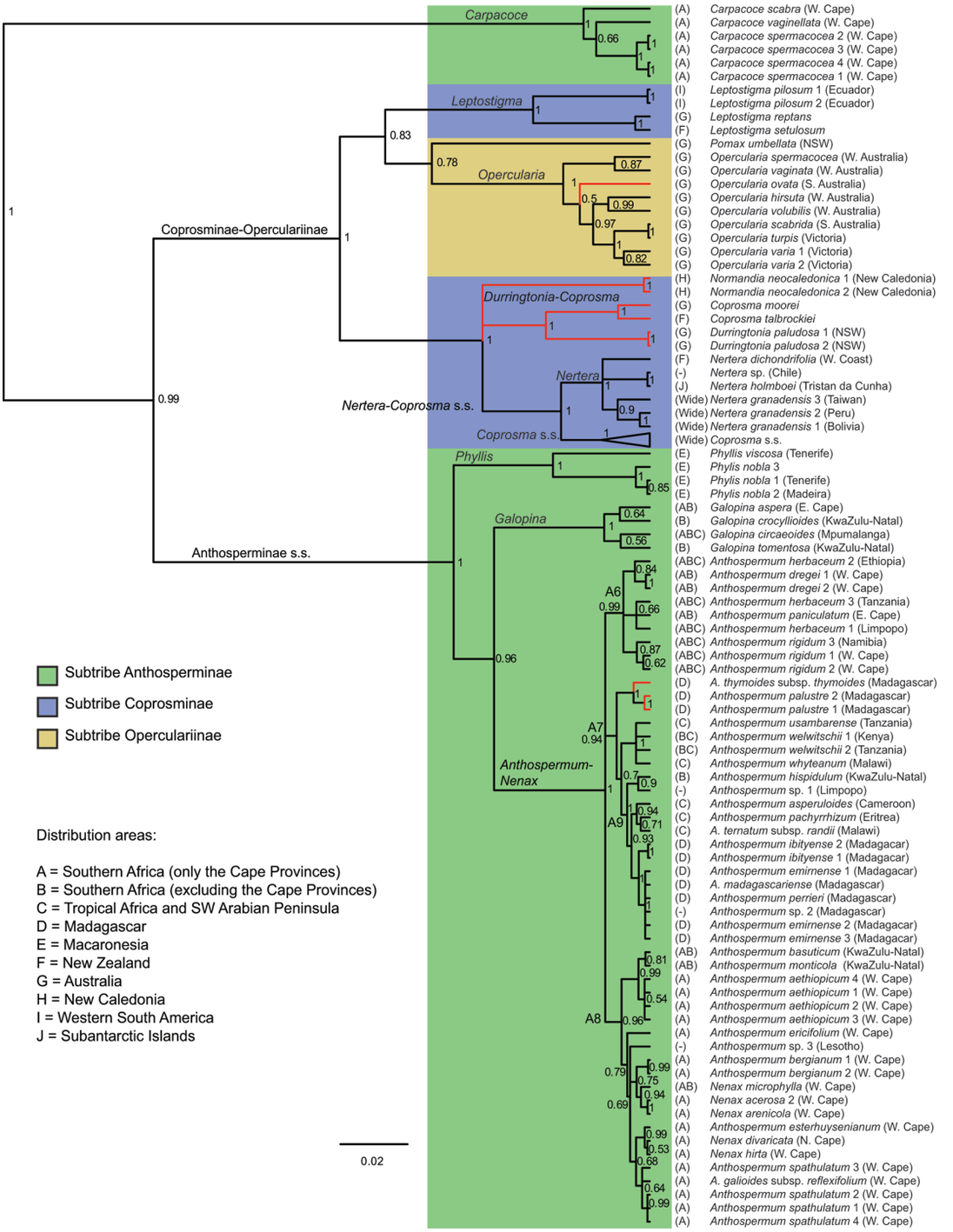

Fig. 2 Fifty percent majority rule consensus tree showing phylogenetic relationships among Anthospermeae based on the relaxed clock analysis of the nuclear data set. Support values next to the nodes are Bayesian posterior probabilities (BPPs). Scale bar represents the expected number of substitutions per site. Text in the tree corresponds to clades mentioned in the text. Red branch color indicates selected supported (BPP ≥ 0.95) incongruence with the plastid-derived trees discussed in the text. The *Coprosma* s.s clade is collapsed into a triangle. Capital letter(s) within parentheses before taxon names corresponds to distribution area code(s). Text within parentheses after taxon names corresponds to sampling locality for species/specimens with newly produced sequences. Distribution areas were based on information from the cited literature and the World Checklist of Selected Plant Families (WCSP 2019), with geographical areas based on Brummitt (2001).

(figs. B1, B2; figs. B1–B6 are available online) recovered a monophyletic Anthospermeae (BPP = 1) with *Carpacoce* (BPP = 1) resolved sister to the remaining species (BPP = 1). In general, the results were consistent with those from analyses of the plastid data set without outgroups. Results from the nonclock and strict clock analyses without outgroups (figs. B3–B6) were also mostly consistent with those retrieved from the relaxed clock analyses of respective data set.

Consistency of Topological Results

Several taxa occupied conflicting positions in trees on the basis of plastid versus nuclear data (figs. 1, 2), but no supported topological conflicts were identified when comparing the tree topologies resulting from the analyses using the nonclock model, relaxed clock model, and strict clock model of the same data set.

Phylogenetic Reconstruction Based on the Plastid Data Set without Outgroups

The rooting obtained with nonclock and relaxed clock analyses of the plastid data set with outgroups (figs. B1, B2) was also obtained by the relaxed clock analyses of the plastid data set without outgroups (fig. 1), which are presented here. The six included *Carpacoce* specimens formed a monophyletic group (BPP = 1), which was resolved as sister to a large supported (BPP = 1) clade formed by the remaining species of Anthospermeae (fig. 1). This latter lineage was split into two supported sister clades; one clade (BPP = 1) comprised the genera corresponding to the members of subtribes Coprosminae and Opercullariinae, and a second clade (BPP = 1) included all genera except *Carpacoce* of subtribe Anthosperminae sensu Puff (1982). These clades will hereafter be referred to as the Coprosminae-Opercullariinae clade and *Anthosperminae* s.s., respectively. The Coprosminae-Opercullariinae clade was split into two supported sister clades, and neither subtribe was resolved as monophyletic (fig. 1). One of these clades (BPP = 1) comprised the monotypic genus *Normandia*, the *Durringtonia-Coprosma* clade (BPP = 1), *Leptostigma* (BPP = 1) of the subtribe Coprosminae, and the genera *Opercularia* (BPP = 1) and *Pomax* of the Australian subtribe Opercullariinae. *Normandia* was sister to the remaining members of the clade (BPP = 1). Opercullariinae were not resolved monophyletic as currently circumscribed, since *Pomax* was sister to *Leptostigma* (although with low support; BPP = 0.85). The other clade (BPP = 1) comprised the sister groups *Nertera* (BPP = 1) and *Coprosma* s.s. (BPP = 1).

Within the Anthosperminae s.s. clade (BPP = 1), *Galopina* (BPP = 1) and *Phyllis* (BPP = 1) formed an unsupported monophyletic group (BPP = 0.88), which was resolved as sister to a clade comprising *Anthospermum* and *Nenax* (BPP = 1). In the *Anthospermum-Nenax* clade, neither *Nenax* nor *Anthospermum* was resolved as monophyletic (fig. 1). The phylogeny of the *Anthospermum-Nenax* group was geographically structured with a few larger supported clades. Clade A1 (BPP = 1; fig. 1) comprised species of *Anthospermum* and *Nenax* mainly restricted to the western parts of the Western and Northern Cape Provinces. Of the remaining species (BPP = 0.92), *Nenax microphylla* was sister to all remaining *Anthospermum* species (BPP = 1), which were split into two supported clades, labeled A2 and A3 (fig. 1). Clade A2 (BPP = 1) included the generic type of *Anthospermum* (*Anthospermum aethiopicum*) along with other species occurring only in southern Africa. Clade A3 (BPP = 1) was split into two supported sister clades, one comprising species occurring in southern and tropical Africa (clade A4; BPP = 1) and the other (clade A5; BPP = 0.98; fig. 1) comprising all Malagasy species resolved in one clade (BPP = 0.99), a clade including the widespread species *Anthospermum herbaceum* and *Anthospermum welwitschii* (BPP = 1) as well as a polytomy of species from tropical Africa. Relationships among the Malagasy group, the *A. herbaceum* + *A. welwitschii* group, and remaining taxa of clade A5 were unresolved.

Phylogenetic Reconstruction Based on the Nuclear Data Set

The relaxed clock analysis of nuclear data (fig. 2) inferred the root to be between the monophyletic *Carpacoce* (BPP = 1) and the remaining species of Anthospermeae (BPP = 0.99). The remaining species were split into two sister clades, the Coprosminae-Opercullariinae clade (BPP = 1) and *Anthosperminae* s.s. (BPP = 1).

The Coprosminae-Opercullariinae clade consisted of two sister clades. One group (BPP = 0.83) comprised *Leptostigma* (BPP = 1) of subtribe Coprosminae and *Opercularia* (BPP = 1) and *Pomax* of subtribe Opercullariinae (BPP = 0.78). The other clade (BPP = 1) comprised the remaining members of subtribe Coprosminae, that is, the monotypic genus *Normandia*, the *Durringtonia-Coprosma* clade (BPP = 1), and the *Nertera-Coprosma* s.s. clade (BPP = 1). Relationships among the *Durringtonia-Coprosma* clade, the *Nertera-Coprosma* s.s. clade, and *Normandia* were unresolved.

Within the Anthosperminae s.s. clade, the Macaronesian genus *Phyllis* (BPP = 1) was sister to a clade containing the remaining species (BPP = 0.96). The southeastern Africa-centered genus *Galopina* (BPP = 1) was the next lineage to split off, sister to a group (BPP = 1) comprising *Anthospermum* and *Nenax*. The *Anthospermum-Nenax* clade constitutes an unresolved trichotomy of three clades that are partly different from any clade of the plastid tree. These clades are therefore given unique names: clades A6, A7, and A8, respectively (fig. 2). Clade A6 (BPP = 0.99) comprised southern African and more widespread species. Clade A7 (BPP = 0.94) included *Anthospermum* species occurring in southern and tropical Africa as well as all the investigated Malagasy species. These Malagasy species did not group together but formed two monophyletic groups. Clade A8 (BPP = 0.96) comprised species restricted to southern Africa and included the generic types of *Anthospermum* (*A. aethiopicum*) and *Nenax* (*Nenax acerosa*) as well as several other species of *Anthospermum* and all investigated species of *Nenax*.

Discussion

Although this study reveals several cases of supported topological conflict, the deepest splits in Anthospermeae are consistently resolved. All analyses resolved the genus *Carpacoce* as sister to the remaining Anthospermeae. All analyses supported a Coprosminae-Opercullariinae clade (including all genera traditionally referred to the subtribes Coprosminae and Opercullariinae) and an Anthospermineae s.s. clade (comprising the genera corresponding to subtribe Anthosperminae except *Carpacoce*). These deep splits in Anthospermeae are retrieved and strongly

supported regardless of data sets used and are unaffected by the choice of evolutionary model and rooting strategy. They are, in addition, incompatible with the traditional subtribal delimitation of Anthospermeae (Anthosperminae, Coprosminae, and Opercullariinae; Puff 1982) primarily on the basis of floral and fruit characters. Using a denser sample of species/specimens than in previous work, our results also reveal that the two genera *Anthospermum* and *Nenax* are not monophyletic, and the nonmonophyly of *Coprosma* shown by Cantley et al. (2016) is further confirmed. Also, these results appear robust. They are consistently returned, present in analyses of plastid data as well as of nuclear data, and are apparently not linked to certain analytical approaches (i.e., nonclock vs. relaxed clock vs. strict clock).

However, there are also noteworthy and previously undetected cases of statistically supported conflicts between results based on plastid and nuclear data. Conflicts include the positions of the monotypic genus *Normandia*, the *Durringtonia-Coprosma* clade, one species of *Opercularia* (*Opercularia ovata*), and several taxa in the *Anthospermum-Nenax* clade. The former two are successive sisters to *Pomax* + *Leptostigma* + *Opercularia* on the basis of plastid data but included in a clade together with *Nertera* and *Coprosma* s.s. on the basis of nuclear data. Finally, it is worth noting that the position of the species-poor Australian genus *Pomax* is inconsistent between analyses, although with low statistical support. *Pomax umbellata* is sister to *Leptostigma* on the basis of plastid data but to *Opercularia* on the basis of nuclear data.

Potential Reasons for Plastid-Nuclear Incongruence

Analytical factors (such as taxon sampling, model misspecification, and sampling error [low signal-to-noise ratio]) as well as biological factors (such as hidden paralogs, incomplete lineage sorting, and hybridization/introgression) are often considered likely reasons for incongruent patterns between plastid and nuclear phylogenetic trees (Rieseberg and Soltis 1991; Soltis and Kuzoff 1995; Wendel and Doyle 1998; Dávalos et al. 2012; Som 2015). Our sample of taxa is dense within Anthospermeae, with an almost complete taxonomical overlap between nuclear and plastid data sets, and results are generally strongly statistically supported. It is therefore unlikely that insufficient taxon sampling and/or sampling error are major causes for the observed incongruence. We have described conflicts on the basis of results from the statistically favored relaxed clock model, but most of the incongruences are supported in results on the basis of all models considered in this study. Model misspecification therefore does not appear a likely explanation for the observed phylogenetic incongruence. Paralogous and recombinant sequences are additional unlikely explanations for the detected conflicting topologies between the plastid and nuclear trees because several studies have shown that variation of functional paralogs from the same species/specimen form monophyletic groups (Razafimandimbison et al. 2004; Won and Renner 2005; Pelser et al. 2010) and because of lack of evidence for the presence of nrITS pseudogenes and putative recombinants in our data sets.

Instead, the incongruent patterns between the nuclear and plastid trees detected in this study most probably stem from biological factors, such as incomplete lineage sorting and hybridization/introgression. However, the persistence of ancestral polymorphisms due to incomplete lineage sorting can give rise to the same pattern as those generated by hybridization/introgression (Doyle 1992; Joly et al. 2009), and on the basis of the information in this study, it is not possible to distinguish between the two processes.

Comparison of Clock and Nonclock Models

Stepping-stone sampling (Xie et al. 2011) analyses and subsequent Bayes factor tests provided very strong support in favor of the relaxed clock model over a strict clock and the nonclock model as best fit to all data sets we analyzed. However, using these different models had little impact on phylogenetic results; the nonclock, strict clock, and relaxed clock models result in similar tree topologies, although there may be differences in level of support. Support for many intergeneric relationships is, for example, higher in the nonclock analyses than in the relaxed clock analyses, in particular in results based on plastid data. Digging deeper into this is beyond the scope of this study, but it is possible that the higher support values in analyses using the nonclock model are due to higher precision at the expense of accuracy, as suggested by Wertheim et al. (2010). Drummond et al. (2006) analyzed five real data sets and argued that employing a relaxed clock model outperforms a nonclock model in terms of both accuracy and precision. In contrast, Wertheim et al. (2010) found a trade-off effect between accuracy and precision. They argued that while relaxed clock models were more accurate than the nonclock model, the relaxed clock models were not as precise. The lower support values of our analyses using a relaxed clock model may thus indicate lower precision but higher accuracy, which should be preferred over the opposite since having high precision on the wrong tree is more detrimental than low support values (on the correct tree).

Another possible reason for differences in support values in analyses employing nonclock models compared with those using relaxed clock models is rate-smoothing effects of the clock models. Topological artifacts have been demonstrated from strict clock analyses as well as from relaxed clock analyses (Ronquist et al. 2012*a*) possibly because of the presence of large rate shifts that the utilized clock models cannot accommodate for (Smith et al. 2010; Dornburg et al. 2012; Ronquist et al. 2012*a*). In this study, *Pomax* has a long branch in both the nuclear and plastid tree and may be an outlier in terms of molecular evolutionary rate within Anthospermeae. Although differences are minor, it is interesting to note that support for the placement of *Pomax* is higher in the nonclock trees than in the clock trees, possibly because the nonclock model is less sensitive to problems with strong rate heterogeneity among lineages.

More Details of the Subgroups of Anthospermeae

Carpacoce. The genus *Carpacoce* is here represented by six specimens representing three species, and both molecular clock rooting and outgroup rooting resolve a monophyletic *Carpacoce* as sister to the remaining Anthospermeae. This finding is consistent with the study by Rydin et al. (2009) but inconsistent with that by Anderson et al. (2001), in which *Carpacoce* was sister to the tribe Knoxieae. There are seven species of *Carpacoce*, all confined to South Africa, endemic to the southwest Cape Floristic Region, with the exception of *Carpacoce vaginellata*, which extends further east (Puff 1986). They generally grow as dwarf shrubs or rarely as perennial herbs (Puff 1982, 1986). They can be found in both damp/moist and dry areas from the sea coast

to the highest mountains of the Cape Provinces (Puff 1982, 1986).

Puff (1982) included *Carpacoce* in Anthosperminae but considered it not as closely allied to the other genera within Anthosperminae as the other genera are to each other. A putatively distant relationship to the other members of Anthosperminae was again pointed out by Puff (1986), where he mentions differences in leaf, flower (e.g., calyx, corolla, carpel), and fruit morphologies and in the sizes of chromosomes and pollen. Also, other pollen characters indicate that the genus is distantly related to the rest of Anthosperminae (Robbrecht 1982), but Puff (1986) considered the morphological evidence insufficient to motivate a placement of *Carpacoce* in its own subtribe. The strongly supported sister relationship between *Carpacoce* and the remaining Anthospermeae found in this study clearly supports the exclusion of *Carpacoce* from Anthosperminae and motivates a placement of the genus in its own subtribe to render Anthosperminae monophyletic.

The Coprosminae-Opercullariinae clade. The mostly Pacific Coprosminae-Opercullariinae clade has previously been identified by Anderson et al. (2001) and Rydin et al. (2009). Anderson et al. (2001) included seven genera from Coprosminae and Opercullariinae (*Coprosma*, *Durringtonia*, *Leptostigma*, *Nertera*, *Normandia*, *Opercularia*, *Pomax*) in their analyses and indicated a monophyletic Coprosminae but found no support for Opercullariinae. Rydin et al. (2009) found support for both subtribes, but their analyses did not include *Durringtonia* and *Leptostigma*.

In this study, the Coprosminae-Opercullariinae clade is strongly supported across all data sets and analyses. However, strongly supported phylogenetic incongruences between the nuclear-based and the plastid-based trees are demonstrated within the group. These incongruences were undetected in previous studies where members of this group have been included (e.g., Anderson et al. 2001; Rydin et al. 2009; Cantley et al. 2016). In addition, phylogenetic estimates based on nonclock and relaxed clock approaches are partly different. Although there is no support for each of the subtribes Opercullariinae and Coprosminae, we prefer not to make taxonomic changes regarding these subtribes, pending further study. The traditional subtribal classification (Puff 1982) is neither rejected nor supported in the relaxed clock nuclear tree, and the plastid phylogeny neither rejects nor supports the monophyly of Opercullariinae.

All genera of Coprosminae and Opercullariinae are monophyletic in both trees, with the exception of *Coprosma*. The *Coprosma* species *Coprosma talbrockiei* and *Coprosma moorei* and the monospecific genus *Durringtonia* (here represented by two specimens) form a highly supported group clearly separated from other species of *Coprosma*. However, the position of this group is incongruent between results based on plastid data (where it belongs to the same clade as *Normandia*, *Leptostigma*, and the sampled members of Opercullariinae) and those based on nuclear data (where it belongs to the same clade as the other Coprosminae members, except *Leptostigma*). A close relationship between *C. talbrockiei*, *C. moorei*, and *Durringtonia* was first shown by Cantley et al. (2016), but their main focus was biogeography and diversification of *Coprosma* s.s., and the *Durringtonia-Coprosma* clade was not discussed. The support for the *Durringtonia-Coprosma* clade in our analyses is strong (BPP = 1.0), and although both are rhizomatous glabrous perennial herbs with drupaceous fruits, there are no obvious morphological synapomorphies shared by *Durringtonia* and these two *Coprosma* species. *Durringtonia* is found in swamps along the Australian east coast and was, when described, placed in its own tribe (Henderson and Guymer 1985) distinct from other genera of Anthospermeae by the combination of the characters unilocular ovary, consistently single-seeded drupaceous fruits, and a subsessile single stigma (Henderson and Guymer 1985). *Coprosma moorei* and *C. talbrockiei* stand out in having strictly bisexual flowers (other *Coprosma* are dioceous) and resemble in many aspects (e.g., bisexual flowers and creeping habit) the genera *Leptostigma* and *Nertera* more than they do *Durringtonia paludosa*, which is dioceous and taller. Like *Durringtonia*, *C. moorei* is found in Australia (Victoria and Tasmania) and in Victoria restricted to bogs and peaty heaths at high altitude (Jeanes 1999). *Coprosma talbrockiei* is endemic to New Zealand (northwestern part of the South Island; Moore and Mason 1974). A close relationship between these two *Coprosma* species was noticed by Moore and Mason (1974), who placed them in the same section together with *Coprosma atropurpurea*, but *C. atropurpurea* has been found deeply nested in *Coprosma* s.s. (Cantley et al. 2016), and it is thus not closely related to *C. moorei* and *C. talbrockiei*.

Taxonomically, *C. moorei* and *C. talbrockiei* should be removed from the genus *Coprosma*. A transfer to *Durringtonia* would be possible but appears unsuitable, as it would create a very heterogeneous group. Another alternative is to raise a new genus encompassing only *C. moorei* and *C. talbrockiei*. However, since our results are based on only one individual per species and no additional molecular sequence data of *C. moorei* and *C. talbrockiei* have been added to those used by Cantley et al. (2016), we prefer to await information from additional specimens of those species before any taxonomical changes are made.

The genus *Nertera* has in previous studies (Cantley et al. 2014, 2016) been shown to form a sister relationship with the large (ca. 110 species) and widely distributed *Coprosma* s.s., and this result is confirmed in this study. The nine species of *Nertera* are morphologically homogeneous and distinct from *Coprosma* s.s. by their bisexual flowers and herbaceous habit. The distribution of *Nertera* is similar to that of *Coprosma*, scattered in and around the Pacific Ocean, but *Nertera* also includes one species that has been found only on Tristan da Cunha Islands as well as the very widespread species *Nertera granadensis*, which occurs in southern China, the Indian subcontinent, Southeast Asia, Australia, Pacific Islands, Caribbean, Mexico to southern South America, and subantarctic Islands (Fosberg 1982; Puff 1982; Andersson 2000). Improved taxon sampling would be needed to investigate the evolutionary history and biogeography of this genus, for example, if *Nertera* also originated in New Zealand, as is suggested for its sister group *Coprosma* s.s (Cantley et al. 2016).

Normandia neocaledonica, a shrub or small tree with leathery leaves and bisexual flowers, is the only species of its genus. It is endemic to New Caledonia, where it is considered to be the only representative of Anthospermeae (Puff 1982; Heads 2017). *Normandia* takes up strongly incongruent positions in the nuclear and plastid trees (figs. 1, 2). In the study by Anderson et al. (2001), it was nested among species of *Coprosma*, and the authors argued for the inclusion of *Normandia* in *Coprosma*. Since then, DNA sequences obtained from alternative specimens of *Normandia neocaledonica* have been produced, none of which have provided support for *Normandia* being nested within *Coprosma* (Cantley et al. 2014, 2016; this study).

Opercullariinae of Australia (including Tasmania), comprising *Opercularia* and the species-poor genus *Pomax*, are primarily distinguished by their head-like inflorescences (in *Pomax*, the heads are arranged in an umbel-like inflorescence), in which fusion of ovaries results in a dry infructescence made up of fused capsules that open by the means of a deciduous apical operculum in most species (either one per capsule or several united opercula shed as one single unit; Puff 1982; Jeanes 1999; Markey 2018). Relationships within *Opercularia* were in general well resolved and highly supported in both the plastid and the nuclear trees. The plastid tree shows a geographic structure with species distributed in south and southeastern Australia (*Opercularia ovata*, *Opercularia scabrida*, *Opercularia turpis*, and *Opercularia varia*) more closely related to each other than to species endemic to Western Australia (*Opercularia hirsuta*, *Opercularia spermacocea*, *Opercularia vaginellata*, and *Opercularia volubilis*). In the nuclear tree, this pattern is somewhat blurred because of the incongruent position of *O. ovata*.

The genus *Leptostigma* comprises seven species with a trans–South Pacific distribution with species occurring in Australia, New Zealand, and western parts of South America (Fosberg 1982). With the prostrate creeping habit and hermaphroditic flowers, *Leptostigma* is most similar to *Nertera* but are distinct from the latter by having well-developed persistent calyx lobes, long tubular corollas, far-exerted anthers, and semifleshy rather than fleshy fruits (Fosberg 1982). The position of *Leptostigma* is uncertain and mostly poorly supported. Plastid data indicate a sister relationship between *Pomax* and *Leptostigma*, whereas nuclear data place *Leptostigma* as sister to Opercullariinae. Monophyly of each of the subtribes Coprosminae and Opercullariinae thus cannot be confirmed or rejected by our results.

The Anthosperminae sensu stricto clade. The genera originally included in Anthosperminae (Puff 1982) except *Carpacoce* (the African genera *Anthospermum*, *Galopina*, *Nenax*, and *Phyllis*), here referred to as Anthosperminae s.s., are highly supported as a clade in our study as well as in previous studies (Anderson et al. 2001; Bremer and Eriksson 2009; Rydin et al. 2009). Anderson et al. (2001) included one representative each of *Nenax* and *Anthospermum* in their analyses, and they grouped together with strong support, but the relationships between *Galopina* and *Phyllis* were ambiguous and not well supported. In this study, the monophyly of *Phyllis*, *Galopina*, and the *Anthospermum-Nenax* clade is consistently highly supported, *Phyllis* and *Galopina* are always included in Anthosperminae s.s. and always placed outside of the *Anthospermum-Nenax* clade, but the relative placement of *Phyllis* and *Galopina* is uncertain. On the basis of morphology, Sunding (1979) stated that the southeastern Africa-centered genus *Galopina* is *Phyllis*'s closest relative. Also, Puff (1986) found the two genera to be similar with respect to inflorescence and leaf characters but believed those similarities to be the result of convergent evolution and rejected the claims of Sunding (1979).

The Macaronesian genus *Phyllis* consists of two species that are large, often single-stemmed shrubs (Mendoza-Heuer 1972; Mendoza-Heuer 1977). *Phyllis nobla* occurs on both Canary and Madeira Islands generally in the Macaronesian laurel forest, whereas *Phyllis viscosa* inhabits drier habitats and is known only from the Canary Islands (Mendoza-Heuer 1977). Previous studies based on molecular data have included only *P. nobla*; *P. viscosa* is here sequenced for the first time. The two species differ in many aspects, and authors of previous studies have considered *P. viscosa* more derived because of its dioecious habit, hairy fruits, congested inflorescence, and habitat preference of drier places (Mendoza-Heuer 1977; Puff 1986). The node height of the *Phyllis* clade, as estimated in this study (figs. 1, 2), indicates that the two species diverged from each other relatively long ago.

The genus *Galopina* comprises four species of perennial herbs, all of which were represented in this study, with *Galopina aspera* and *Galopina tomentosum* sequenced for the first time. However, infrageneric relationships are poorly resolved and unsupported in both the plastid and the nuclear trees. Species of *Galopina* can be recognized by their relatively broad and decussately arranged leaves and terminal paniculate to thyrso-paniculate inflorecences (Puff 1986). They are most frequently found in Swaziland and eastern parts of South Africa, although the distribution of *Galopina circaeioides* extend northward to Zimbabwe, Mozambique, and southern Malawi and southwest to the Cape Floristic Region (Puff 1986). Habitat preference within the genus seems to vary between moist/wet and shady places to more sun-exposed places (Puff 1986).

The *Anthospermum-Nenax* clade was highly supported by both the nuclear and plastid data, and a close relationship between the two genera has also been indicated in previous work (Anderson et al. 2001; Rydin et al. 2009). With the current sampling of 25 species of *Anthospermum* species and five species of *Nenax*, neither genus is resolved as monophyletic, a result that is consistent among all our analyses. Deeper understanding of the relationships within the *Anthospermum-Nenax* clade is hampered by topological conflicts between results based on nuclear data and plastid data, but there is support for geographical groupings in both trees (figs. 1, 2). Interpreting the distribution areas of the sampled species, the *Anthospermum-Nenax* clade may have originated in southern Africa with subsequent dispersal and colonization of tropical Africa and Madagascar. *Anthospermum* and *Nenax* are morphologically similar to each other—for example, in flower structure, fruit structure, growth form, and phyllotaxis (Puff 1986)—but *Nenax* has nevertheless been considered distinct from *Anthospermum* by being dioecious woody dwarf shrubs and having needle-like leaves and reduced inflorescences with few flowers (Puff 1986). Considering this and the (still) incomplete sampling of species and uncertain phylogenetic results, we find it premature to make taxonomic changes in the *Anthospermum-Nenax* clade.

The genus *Nenax* comprises (as currently circumscribed) 10 species distributed in southern Africa, with most species occurring in the Western Cape and western parts of the Northern Cape. The genus *Anthospermum* comprises dioecious and non-dioecious shrubs, dwarf shrubs, subshrubs, and perennial herbs with condensed leafy short shoots and is the largest (39 species) and most widely distributed genus of the African Anthospermeae. The species of *Anthospermum* occur in mainland Africa and Madagascar, with one species extending to the southwestern part of the Arabian Peninsula, but most species are found in southern Africa (Puff 1986). *Anthospermum* endemics of Madagascar display incongruent geographical patterns based on nuclear versus plastid data. Six out of eight species endemic to Madagascar were included in our analyses, and in the plastid tree, they all form a supported clade. In the nuclear tree, however, the species pair *Anthospermum thymoides* subsp. *thymoides* and *Anthospermum palustre* and the remaining Malagasy species are intervened by poorly to highly supported groups of tropical

African taxa, indicating additional colonization events to the island.

Taxonomic Implications

On the basis of our results, the tribe Anthospermeae comprises three main clades: *Carpacoce* (South Africa), Anthosperminae s.s. (Anthosperminae as delimited by Puff [1982] but excluding *Carpacoce*; mainly Africa), and Opercullariinae-Coprosminae (mainly Oceania, Hawaii, and South America). Taxonomic adjustments are made accordingly. Our results do not support the monophyly of subtribe Anthosperminae as currently circumscribed, and we here propose a revised subtribal classification of Anthospermeae. We keep for now the current delimitation for the subtribes Coprosminae and Opercullariinae, pending further study. Number of currently recognized species follows that of the Australian Plant Census (CHAH 2019) for those genera that are endemic to Australia (*Durringtonia*, *Opercularia*, and *Pomax*) and the World Checklist of Selected Plant Families (WCSP 2019) for the remaining genera.

Tribe Anthospermeae Cham. & Schlecht. ex DC.—Prodr. 4: 343, 578. 1830.

Type genus. *Anthospermum* L.

Descriptions. See Puff (1982), Robbrecht (1988), and Bremer and Manen (2000).

Distribution. Anthospermeae contains 212 species predominantly distributed in tropical, subtropical, and temperate regions of the Southern Hemisphere.

Subtribe Carpacocinae Thureborn, Rydin & Razafim., subtrib. nov.

Type genus. *Carpacoce* Sond.

Diagnosis. The new monogeneric subtribe Carpacocinae is characterized by a combination of the following characters: leaves without tannin-containing cells, leaf-like calyx lobes, corolla lobes with hood-like structures or apical appendages, and dry fruits dehiscing into three to six exocarp valves and endocarps together with seed(s).

Description. Dwarf shrubs or rarely perennial herbs. Leaves decussate or rarely in whorls of three; blades frequently ericoid. Stipular sheaths with minute setae or more rarely longer bristles. Flowers unisexual or bisexual, protandrous; calyx lobes leaf-like; corolla lobes with hood-like structures or apical appendages, linear to more or less lanceolate, spreading to spreading-recurved; stamens four to seven; ovary with one (rarely two) fertile ovule(s), stigmas one (rarely two). Fruits with one (rarely two) seed(s), crowned with persistent calyx lobes, dry, exocarp splits into several valves, releasing seed-bearing endocarp.

Distribution. This monogeneric subtribe contains seven species confined to South Africa (endemic to the Cape Provinces).

Included genus. *Carpacoce* (seven species).

Subtribe Anthosperminae Benth. (as Anthospermeae Benth.)—Fl. Austral. 3: 401, 429. 1866, emend. Thureborn, Rydin & Razafim.

Type genus. *Anthospermum* L.

Diagnosis. The recircumscribed Anthosperminae is characterized by a combination of the following characters: leaves with tannin-containing cells, calyx strongly reduced/absent (*Galopina*, *Phyllis*, some *Anthospermum* and *Nenax* species) or present with lobes rarely as large as those of *Carpacoce*, corolla lobes without hood-like structures or apical appendages, and dry fruits dehiscing into indehiscent mericarps (except a few *Nenax* species with dry indehiscent fruits).

Description. Large shrubs, dwarf shrubs, short-lived shrubs, subshrubs, or perennial herbs. Leaves decussate or sometimes in whorls of three to four; blades relatively broad and large to small and more or less ericoid. Stipular sheaths with or without one to many setae or fimbriae. Flowers unisexual or bisexual, protandrous; calyx absent or present, with lobes being indistinct to large; corolla lobes linear to lanceolate, recurved, spreading, or erect; stamens four to five; stigmas almost always two (only one species has one), ovary bicarpellate and biovulate (only two species have one carpel reduced). Fruits with or without persistent calyx lobes, dry, mostly dehiscing into two mericarps or sometimes indehiscent.

Distribution. This subtribe contains 55 species confined to mainland Africa, Macaronesia, Madagascar, and the southwestern part of the Arabian Peninsula.

Included genera. *Anthospermum* (39 species), *Galopina* (four species), *Nenax* (10 species), and *Phyllis* (two species).

Excluded genus. *Carpacoce*.

Subtribe Coprosminae Fosberg—Acta Phytotax. Geobot. 33: 75. 1982.

Syn. Coprosminae Puff in Bot. J. Linn. Soc. 84(4): 370. 1982.

Syn. Durringtonieae R.J.F.Hend. & Guymer in Kew Bull. 40(1): 99. 1985.

Type genus. *Coprosma* J.R.Forst. & G.Forst.

Descriptions. See Fosberg (1982) and Puff (1982).

Distribution. This subtribe contains 130 species and has a mainly trans-Pacific distribution (*Nertera* expands the distribution area to also include the Caribbean and Tristan da Cunha).

Included genera. *Coprosma* (111 species), *Durringtonia* (one species), *Leptostigma* (seven species), *Nertera* (10 species), and *Normandia* (one species).

Subtribe Opercullariinae Benth. (as Opercularieae Benth.)—Fl. Austral. 3: 401, 429. 1866.

Type genus. *Opercularia* Gaertn.

Description. See Puff (1982).

Distribution. This subtribe contains 20 species confined to Australia.

Included genera. *Opercularia* (18 species) and *Pomax* (two species). Some treatments have recognized a third genus, *Eleuthranthes* F.Muell. ex Benth, but the name is illegitimate and a taxonomic synonym of *Opercularia* Gaertn.

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Appendix A

Information on Included Taxa and GenBank Accession Numbers

Voucher information is given for new sequences generated for this study. For sequences obtained from other studies, GenBank accessions are given. ***Taxon***, voucher, locality, lab identification, GenBank accessions: *atpB-rbcL*, *ndhF*, *rbcL*, *rps16*, *trnT-trnF*, nrETS, nrITS.

Anthospermeae: Anthospermum aethiopicum L. 1, Bremer et al. 4363 (UPS), South Africa: Western Cape, aL78, MK141093*, MK141363*, MK141457*, MK141546*, MK141633*, MK141184*, MK141274*; ***Anthospermum aethiopicum*** L. 2, Bremer et al. 4367 (UPS), South Africa: Western Cape, aL79, MK141094*, MK141364*, MK141458*, MK141547*, MK141634*, MK141185*, MK141275*; ***Anthospermum aethiopicum*** L. 3, Bremer et al. 4377 (UPS), South Africa: Western Cape, aL81, MK141096*, MK141366*, MK141460*, MK141549*, MK141636*, MK141187*, MK141277*; ***Anthospermum aethiopicum*** L. 4, Hafström & Lindeberg (S), South Africa: Western Cape, cX47, MK141063*, MK141331*, MK141425*, MK141517*, MK141603*, MK141154*, MK141243*; ***Anthospermum asperuloides*** Hook.f., Breteler et al. 108 (UPS), Cameroon: South-West, cX89, MK141065*, MK141333*, MK141427*, MK141519*, MK141605*, MK141156*, MK141245*; ***Anthospermum basuticum*** Puff, Hilliard & Burtt 7121 (S), South Africa: KwaZulu-Natal, cX48, MK141066*, MK141334*, MK141428*, MK141520*, -, MK141157*, MK141246*; ***Anthospermum bergianum*** Cruse 1, Bremer et al. 4413 (UPS), South Africa: Western Cape, aL49, MK141067*, MK141335*, MK141429*, MK141521*, MK141606*, MK141158*, MK141247*; ***Anthospermum bergianum*** Cruse 2, Hafström & Acock 1418 (S), South Africa: Western Cape, cX49, MK141068*, MK141336*, MK141430*, MK141522*, MK141607*, MK141159*, MK141248*; ***Anthospermum dregei*** Sond. 1, Bremer et al. 4410 (UPS), South Africa: Western Cape, aL83, MK141069*, MK141337*, MK141431*, MK141523*, MK141608*, MK141160*, MK141249*; ***Anthospermum dregei*** Sond. 2, Acocks 15194 (S), South Africa: Western Cape, cX51, MK141070*, MK141338*, MK141432*, MK141524*, MK141609*, MK141161*, MK141250*; ***Anthospermum emirnense*** Baker 1, Afzelius s.n. (S), Madagascar: Toasmina, cX52, MK141071*, MK141339*, MK141433*, -, MK141610*, MK141162*, MK141251*; ***Anthospermum emirnense*** Baker 2, Razafimandimbison & Krüger 863 (S), Madagascar: Fianarantsoa, cX68, MK141087*, MK141356*, MK141450*, MK141539*, MK141626*, MK141177*, MK141267*; ***Anthospermum emirnense*** Baker 3, Razafimandimbison & Krüger 862 (S), Madagascar: Fianarantsoa, cX69, MK141088*, MK141357*, MK141451*, MK141540*, MK141627*, MK141178*, MK141268*; ***Anthospermum ericifolium*** (Licht. ex Roem. & Schult.) Kuntze, Esterhuysen 35553 (S), South Africa: Western Cape, cX53, MK141072*, MK141340*, MK141434*, MK141525*, MK141611*, MK141163*, MK141252*; ***Anthospermum esterhuysenianum*** Puff, Esterhuysen 34159a (S), South Africa: Western Cape, cX54, MK141073*, MK141341*, MK141435*, MK141526*, MK141612*, -, MK141253*; ***Anthospermum galioides*** **subsp.** ***reflexifolium*** (Kuntze) Puff, Bremer et al. 4379 (UPS), South Africa: Western Cape, aL82, MK141097*, MK141367*, MK141461*, MK141550*, MK141637*, -, MK141278*; ***Anthospermum herbaceum*** L.f. 1, Bremer et al. 4340 (UPS), South Africa: Limpopo, aL63, MK141091*, MK141361*, MK141455*, MK141544*, MK141631*, MK141182*, MK141272*; ***Anthospermum herbaceum*** L.f. 2, Friis & Demissew 10140 (UPS), Ethiopia: Tigray, cX90, MK141074*, MK141342*, MK141436*, -, MK141613*, MK141164*, MK141254*; ***Anthospermum herbaceum*** L.f. 3, Bremer 3093 (UPS), Tanzania: Morogoro, h72, MK141075*, MK141343*, MK141437*, MK141527*, EU145544[1], -, EU145355[1]; ***Anthospermum hispidulum*** E.Mey. ex Sond., Stray 7505 (S), South Africa: KwaZulu-Natal, cX57, MK141076*, MK141344*, MK141438*, MK141528*, MK141614*, MK141165*, MK141255*; ***Anthospermum ibityense*** Puff 1, Eriksson & Lundberg T975 (S), Madagascar: Antananarivo, cX65, MK141085*, MK141354*, MK141448*, MK141537*, MK141624*, MK141175*, MK141265*; ***Anthospermum ibityense*** Puff 2, Schatz et al. 4094 (P), Madagascar: Antananarivo, cY70, MK141077*, MK141345*, MK141439*, MK141529*, MK141615*, MK141166*, MK141256*; ***Anthospermum madagascariense*** Homolle ex Puff, Razafimandimbison 559 (S), Madagascar: Antananarivo, cX59, MK141078*, MK141346*, MK141440*, MK141530*, MK141616*, MK141167*, MK141257*; ***Anthospermum monticola*** Puff, Hilliard & Burtt 17995 (S), South Africa: KwaZulu-Natal, cX60, MK141079*, MK141347*, MK141441*, MK141531*, MK141617*, MK141168*, MK141258*; ***Anthospermum pachyrrhizum*** Hiern, Ryding 2045 (UPS), Eritrea: Anseba, cX93, MK141080*, MK141348*, MK141442*, MK141532*, MK141618*, MK141169*, MK141259*; ***Anthospermum palustre*** Homolle ex Puff 1, Eriksson et al. T973 (S), Madagascar: Fianarantsoa, cX66, MK141086*, MK141355*, MK141449*, MK141538*, MK141625*, MK141176*, MK141266*; ***Anthospermum palustre*** Homolle ex Puff 2, De Block et al. 1922 (P), Madagascar: Fianarantsoa, cY73, MK141081*, MK141349*, MK141443*, MK141533*, MK141619*, MK141170*, MK141260*; ***Anthospermum paniculatum*** Cruse, Wall s.n. (S), South Africa: Eastern Cape, cX61, -, MK141350*, MK141444*, MK141534*, MK141620*, MK141171*, MK141261*; ***Anthospermum perrieri*** Homolle ex Puff, Rasoarivelo s.n (P), Madagascar: Antananarivo, cY74, MK141082*, MK141351*, MK141445*, -, MK141621*, MK141172*, MK141262*; ***Anthospermum rigidum*** Eckl. & Zeyh. 1, Bremer et al. 4527 (UPS), South Africa: Western Cape, aL47, MK141089*, MK141359*, MK141453*, MK141542*, MK141629*, MK141180*, MK141270*; ***Anthospermum rigidum*** Eckl. & Zeyh. 2, Bremer et al. 4267 (UPS), South Africa: Western Cape, aL48, MK141090*, MK141360*, MK141454*, MK141543*, MK141630*, MK141181*, MK141271*; ***Anthospermum rigidum*** Eckl. & Zeyh. 3, Wanntorp & Wanntorp 196 (S), Namibia: Khomas, cX63, MK141083*, MK141352*, MK141446*, MK141535*, MK141622*, MK141173*, MK141263*; ***Anthospermum*** **sp.**

L. 1, Bremer et al. 4351 (UPS), South Africa: Limpopo, aL84, MK141102*, MK141372*, MK141466*, MK141555*, MK141641*, MK141192*, MK141283*; ***Anthospermum* sp.** L. 2, Bremer et al. 5336 (S), Madagascar. Antananarivo, cX67, MK141084*, MK141353*, MK141447*, MK141536*, MK141623*, MK141174*, MK141264*; ***Anthospermum* sp.** L. 3, Phillipson 4487 (P), Lesotho, cY69, MK141064*, MK141332*, MK141426*, MK141518*, MK141604*, MK141155*, MK141244; ***Anthospermum spathulatum*** Spreng. 1, Bremer et al. 4366 (UPS), South Africa: Western Cape, aL64, MK141092*, MK141362*, MK141456*, MK141545*, MK141632*, MK141183*, MK141273*; ***Anthospermum spathulatum*** Spreng. 2, Bremer et al. 4405 (UPS), South Africa: Western Cape, aL69, KY378687[2], KY378687[2], KY378687[2], KY378687[2], KY378687[2], -, MK141242*; ***Anthospermum spathulatum*** Spreng. 3, Bremer et al. 4372 (UPS), South Africa: Western Cape, aL80, MK141095*, MK141365*, MK141459*, MK141548*, MK141635*, MK141186*, MK141276*; ***Anthospermum spathulatum*** Spreng. 4, Wall s.n. (S), South Africa: Western Cape, cX71, MK141098*, MK141368*, MK141462*, MK141551*, MK141638*, MK141188*, MK141279*; ***Anthospermum ternatum* subsp. *randii*** (S.Moore) Puff, Iversen & Martinsson 89147 (UPS), Malawi: Central, cX94, MK141099*, MK141369*, MK141463*, MK141552*, MK141639*, MK141189*, MK141280*; ***Anthospermum thymoides* subsp. *thymoides*** Baker, J., Krüger & Razafimandimbison 70 (S), Madagascar, Fianarantsoa, cX70, -, MK141358*, MK141452*, MK141541*, MK141628*, MK141179*, MK141269*; ***Anthospermum usambarense*** K.Schum., Mwangoka et al. 1180 (S), Tanzania: Kilimanjaro, cX73, MK141100*, MK141370*, MK141464*, MK141553*, MK141640*, MK141190*, MK141281*; ***Anthospermum welwitschii*** Hiern 1, Luke et al. 8928 (UPS), Kenya: Rift Valley, ai76, MK141101*, MK141371*, MK141465*, MK141554*, DQ662220[3], MK141191*, MK141282*; ***Anthospermum welwitschii*** Hiern 2, Thulin & Mhoro 3244 (UPS), Tanzania: Iringa, cX95, MK141103*, MK141373*, MK141467*, MK141556*, MK141642*, MK141193*, MK141284*; ***Anthospermum whyteanum*** Hiern, Brummitt 10056 (UPS), Malawi: Central, cX96, MK141104*, MK141374*, MK141468*, MK141557*, MK141643*, MK141194*, MK141285*; ***Carpacoce scabra*** (Thunb.) Sond., Acocks 23743 (S), South Africa: Western Cape, cY60, MK141105*, MK141375*, MK141469*, MK141558*, MK141644*, MK141195*, MK141286*; ***Carpacoce spermacocea*** (Rchb. ex Spreng.) Sond. 1, 9225 (S), South Africa: Western Cape, cX75, MK141109*, MK141378*, MK141472*, MK141561*, MK141647*, MK141199*, MK141289*; ***Carpacoce spermacocea*** (Rchb. ex Spreng.) Sond. 2, Bremer et al. 4365 (UPS), South Africa: Western Cape, aL85, MK141106*, FJ695293[4], MK141470*, MK141559*, MK141645*, MK141196*, FJ695438[4]; ***Carpacoce spermacocea*** (Rchb. ex Spreng.) Sond. 3, Bremer et al. 4385 (UPS), South Africa: Western Cape, aL86, MK141107*, MK141376*, FJ695231[4], FJ695261[4], FJ695404, MK141197*, MK141287*; ***Carpacoce spermacocea*** (Rchb. ex Spreng.) Sond. 4, Bremer & Bremer 3708 (UPS), South Africa: Western Cape, s44, MK141108*, MK141377*, MK141471*, MK141560*, MK141646*, MK141198*, MK141288*; ***Carpacoce vaginellata*** Salter, Acocks 22841 (S), South Africa: Western Cape, cX76, MK141110*, MK141379*, MK141473*, MK141562*, MK141648*, MK141200*, MK141290*; ***Coprosma acutifolia*** Hook.f., -, -, -, -, -, -, KF688224[5], -, KF688306[5], KF688389[5]; ***Coprosma baueri*** Endl., Bremer & Rydin 5009b (S), Cult. Botanical Garden Aarhus, bp88, MK141111*, MK141380*, MK141474*, MK141563*, MK141649*, MK141201*, MK141291*; ***Coprosma brunnea*** (Kirk) Cockayne ex Cheeseman, -, -, -, -, -, -, KF688226[5], -, KF688308[5], KF688391[5]; ***Coprosma chathamica*** Cockayne, -, -, -, -, -, -, KF688227[5], -, KF688309 [5], KF688392[5]; ***Coprosma cheesemanii*** W.R.B.Oliv., -, -, -, -, -, -, KF688228[5], -, KF688310[5], KF688393[5]; ***Coprosma crenulata*** W.R.B.Oliv., -, -, -, -, -, -, KF688230[5], -, KF688313[5], KF688396[5]; ***Coprosma cuneata*** Hook.f., -, -, -, -, -, -, JF950826[6], -, KF688356 [5], JF950726[6]; ***Coprosma elatirioides*** de Lange & A.S.Markey, -, -, -, -, -, -, KF688233[5], -, KF688317[5], KF688400[5]; ***Coprosma esulcata*** (F.Br.) Fosberg, -, -, -, -, -, -, KF688290[5], -, KF688373[5], KF688458[5]; ***Coprosma fatuhivaensis*** W.L.Wagner & Lorence, -, -, -, -, -, -, KF688289[5], -, KF688372[5], KF688457[5]; ***Coprosma foetidissima*** J.R.Forst. & G.Forst., Skottsberg s.n. (S), New Zealand: South Island, cY8, MK141112*, MK141381*, MK141475*, MK141564*, MK141650*, MK141202*, MK141292*; ***Coprosma foweraker*i** D.A.Norton & de Lange, -, -, -, -, -, -, KF688234[5], -, KF688318[5], KF688401[5]; ***Coprosma grandifolia*** Hook.f., Skottsberg s.n. (S), New Zealand: South Island, cY95, MK141116*, MK141385*, MK141479*, MK141568*, MK141654*, MK141206*, MK141296*; ***Coprosma hirtella*** Labill., Nordenstam & Anderberg s.n. (S), Australia: Victoria, cY96, MK141117*, MK141386*, MK141480*, MK141569*, MK141655*, MK141207*, MK141297*; ***Coprosma huttoniana*** P.S.Green, -, -, -, -, -, -, JF950835[6], -, KT861902, JF950734[6]; ***Coprosma intertexta*** G.Simpson, -, -, -, -, -, -, KF688236[5], -, KF688320[5], KF688403[5]; ***Coprosma linariifolia*** (Hook.f.) Hook.f., Tibell NZ70 (UPS), New Zealand: South Island, cY97, MK141118*, MK141387*, MK141481*, MK141570*, MK141656*, MK141208*, MK141298*; ***Coprosma lucida*** J.R.Forst. & G.Forst., Tibell NZ210 (UPS), New Zealand: South Island, cY9, -, MK141388*, MK141482*, MK141571*, MK141657*, MK141209*, MK141299*; ***Coprosma montana*** Hillebr., Degener & Degener 34421 (UPS), United States: Hawaii, cY98, MK141119*, MK141389*, MK141483*, MK141572*, MK141658*, MK141210*, MK141300*; ***Coprosma moorei*** F.Muell. ex Rodway, -, -, -, -, -, -, KT861911[7], -, -, KT861893[7]; ***Coprosma oliveri*** Fosberg, Swenson & Tepe 469 (UPS), Chile: Juan Fernandez Islands, cX97, MK141120*, MK141390*, MK141484*, MK141573*, MK141659*, MK141211*, MK141301*; ***Coprosma propinqua* var. *propinqua*** A.Cunn., -, -, -, -, -, -, KF688272[5], -, KF688355[5], KF688440[5]; ***Coprosma pumila*** Hook.f., -, -, -, -, FJ695294[4], X87146[8], FJ695262[4], FJ695405[4], -, FJ695439 [4]; ***Coprosma rhamnoides*** A.Cunn., Tibell NZ46 (UPS), New Zealand: South Island, cY12, MK141121*, MK141391*, MK141485*, MK141574*, MK141660*, MK141212*, MK141302*; ***Coprosma rhynchocarpa*** A.Gray, -, -, -, -, -, -, KF688281[5], -, KF688364[5], KF688449[5]; ***Coprosma rotundifolia*** A.Cunn., -, -, -, -, -, -, KF688250[5], -, KF688334[5], KF688417[5]; ***Coprosma rubra*** Petrie, -, -, -, -, -, -, KF688251[5], -, KF688335[5], KF688418[5]; ***Coprosma serrulata*** Hook.f. ex Buchanan, -, -, -, -, -, -, KF688253[5], -, KF688337[5], KF688420[5]; ***Coprosma spathulata*** A.Cunn., -, -, -, -, -, -, KF688265[5], -, KF688348[5], KF688433[5]; ***Coprosma tahitensis*** A.Gray, Swenson et al. 1073 (S), Tahiti, cY16, MK141122*, MK141392*, MK141486*, MK141575*, MK141661*, MK141213*, MK141303*; ***Coprosma talbrockiei*** L.B.Moore & R.Mason, -, -, -, -, -, -, KT861918[7], -, -, KT861896[7]; ***Durringtonia paludosa*** R.J.F.Hend. & Guymer 1, Henderson et al H 3044 (NSW), Australia: New South Wales,

bv75, MK141123*, MK141393*, MK141487*, MK141576*, MK141662*, MK141214*, MK141304*; ***Durringtonia paludosa*** R.J.F.Hend. & Guymer 2, Henderson et al H3048 (P), Australia: New South Wales, cY80, MK141124*, MK141394*, MK141488*, MK141577*, MK141663*, MK141215*, MK141305*; ***Galopina aspera*** (Eckl. & Zeyh.) Walp., Phillipson 1461 (UPS), South Africa: Eastern Cape, cX98, MK141125*, MK141395*, MK141489*, MK141578*, MK141664*, MK141216*, MK141306*; ***Galopina circaeoides*** Thunb., Bremer & Bremer 3797 (UPS), South Africa: Mpumalanga, cY6, MK141126*, MK141396*, MK141490*, MK141579*, MK141665*, MK141217*, -; ***Galopina crocyllioides*** Bär, Hilliard & Burtt 10184 (P), South Africa: KwaZulu-Natal, cY81, MK141127*, MK141397*, MK141491*, MK141580*, MK141666*, MK141218*, MK141307*; ***Galopina tomentosa*** Hochst., Stray 9561 (S), South Africa: KwaZulu-Natal, cX80, MK141128*, MK141398*, MK141492*, MK141581*, MK141667*, MK141219*, MK141308*; ***Leptostigma pilosum*** (Benth.) Fosberg 1, Erik Asplund 7171 (UPS), Ecuador: Imbabura, cX99, MK141129*, MK141399*, MK141493*, -, -, MK141220*, MK141309*; ***Leptostigma pilosum*** (Benth.) Fosberg 2, Benkt Sparre 13451 (S), Ecuador: Imbabura, cY92, MK141130*, MK141400*, MK141494*, -, -, -, MK141310*; ***Leptostigma reptans*** (F.Muell.) Fosberg, -, -, -, -, -, -, AF257921[9], -, -, AF257920[9]; ***Leptostigma setulosum*** (Hook.f.) Fosberg, -, -, -, -, -, KT626730[10], KT861919[7], -, -, KT861897 [7]; ***Nenax acerosa*** Gaertn. 1, Esterhuysen 33327 (S), South Africa: Western Cape, cY61, MK141131*, MK141401*, MK141495*, MK141582*, MK141668*, -, -; ***Nenax acerosa*** Gaertn. 2, Hafström & Acock 1432 (S), South Africa: Western Cape, cY62, MK141132*, MK141402*, MK141496*, MK141583*, MK141669*, MK141221*, MK141311*; ***Nenax arenicola*** Puff, Acocks 19635 (UPS), South Africa: Western Cape, cX100, MK141133*, MK141403*, MK141497*, MK141584*, MK141670*, MK141222*, MK141312*; ***Nenax divaricata*** Salter, Acocks 17458 (UPS), South Africa: Northern Cape, cY1, MK141134*, MK141404*, MK141498*, MK141585*, MK141671*, MK141223*, MK141313*; ***Nenax hirta*** (Cruse) Salter, Hafström & Acock 1433 (S), South Africa: Western Cape, cX82, MK141135*, MK141405*, MK141499*, MK141586*, MK141672*, MK141224*, MK141314*; ***Nenax microphylla*** (Sond.) Salter, Hafström & Acock 1441 (S), South Africa: Western Cape, cX83, MK141136*, MK141406*, MK141500*, MK141587*, MK141673*, MK141225*, MK141315*; ***Nertera dichondrifolia*** (A.Cunn.) Hook.f., Tibell NZ119 (UPS), New Zealand: West Coast, cY3, MK141137*, MK141407*, MK141501*, MK141588*, MK141674*, MK141226*, MK141316*; ***Nertera granadensis*** (Mutis ex L.f.) Druce 1, Persson & Gustafsson 368 (S), Bolivia, cX77, MK141115*, MK141384*, MK141478*, MK141567*, MK141653*, MK141205*, MK141295*; ***Nertera granadensis*** (Mutis ex L.f.) Druce 2, Asplund 12752 (S), Peru, cX78, MK141114*, MK141383*, MK141477*, MK141566*, MK141652*, MK141204*, MK141294*; ***Nertera granadensis*** (Mutis ex L.f.) Druce 3, Chung & Anderberg 1348 (S), Taiwan, cX79, MK141113*, MK141382*, MK141476*, MK141565*, MK141651*, MK141203*, MK141293*; ***Nertera holmboei*** Christoph., Christophersen 2021 (P), Tristan da Cunha, cY82, MK141138*, MK141408*, MK141502*, MK141589*, MK141675*, MK141227*, MK141317*; ***Nertera*** **sp**. Banks ex Gaertn., Sparre 19514 (S), Chile: Bío-Bío, cX81, MK141139*, MK141409*, MK141503*, MK141590*, MK141676*, -, MK141318*; ***Normandia neocaledonica*** Hook.f. 1, Munzinger 532 (MO), New Caledonia, af77, MK141140*, MK141410*, MK141504*, MK141591*, EU145543[1], MK141228*, MK141319*; ***Normandia neocaledonica*** Hook.f. 2, Selling 125.b (S), New Caledonia, cY63, MK141141*, MK141411*, MK141505*, MK141592*, MK141677*, MK141229*, MK141320*; ***Opercularia hirsuta*** F.Muell. ex Benth., Nordenstam & Anderberg 1989 (S), Western Australia, cX84, MK141142*, MK141412*, MK141506*, MK141593*, MK141678*, MK141230*, MK141321*; ***Opercularia ovata*** Hook.f., Ising s.n. (S), South Australia, cX85, MK141143*, MK141413*, MK141507*, MK141594*, MK141679*, MK141231*, -; ***Opercularia scabrida*** Schltdl., Blaylock 2072 (S), South Australia, cX86, MK141144*, MK141414*, MK141508*, MK141595*, MK141680*, MK141232*, MK141322*; ***Opercularia spermacocea*** Juss., Morat 8340 (P), Western Australia, cY88, MK141145*, MK141415*, MK141509*, MK141596*, MK141681*, MK141233*, MK141323*; ***Opercularia turpis*** F.Muell., Jeanes & Lay 2485 (S), Australia: Victoria, cX87, MK141146*, MK141416*, -, MK141597*, MK141682*, MK141234*, MK141324*; ***Opercularia vaginata*** Juss., -, -, -, FJ695376[4], FJ695316[4], Z68809[8], AF257936[9], FJ695418[4], -, AF257935[9]; ***Opercularia varia*** Hook.f. 1, Karunajeewa 832 (S), Australia: Victoria, cY4, MK141147*, MK141417*, MK141510*, MK141598*, MK141683*, MK141235*, MK141325*; ***Opercularia varia*** Hook.f. 2, Muir 1818 (UPS), Australia: Victoria, cY5, -, MK141418*, MK141511*, MK141599*, MK141684*, MK141236*, MK141326*; ***Opercularia volubilis*** R.Br. ex Benth., Lepschi & Fuhrer BJL 3671 (P), Western Australia, cY89, MK141148*, MK141419*, MK141512*, -, MK141685*, MK141237*, MK141327*; ***Phyllis nobla*** L. 1, Wikström et al. 76 (S), Tenerife, cY51, MK141150*, MK141421*, -, -, MK141686*, MK141238*, -; ***Phyllis nobla*** L. 2, Anderberg et al. (S), Madeira, cY59, MK141151*, MK141422*, MK141514*, MK141600*, MK141687*, MK141239*, MK141328*; ***Phyllis nobla*** L. 3, -, -, -, AJ234031[11], FJ695324[4], Z68814[8], AF003613[12], AY538468[13], -, AF2579399; ***Phyllis viscosa*** Webb ex Christ, Santesson 26911 (S), Tenerife, cX88, MK141152*, MK141423*, MK141515*, MK141601*, MK141688*, MK141240*, MK141329* ***Pomax umbellata*** (Gaertn.) Sol. ex A.Rich., Bremer & Bremer 3918 (UPS), Australia: New South Wales, v7, MK141153*, MK141424*, MK141516*, MK141602*, FJ695420[4], MK141241*, MK141330*.

Outgroups: Argostemma yappii King, -, -, -, KY378693[2], KY378693[2], KY378693 [2], -, -, -, -; ***Argostemma hookeri*** King, -, -, -, FJ695349 [4], FJ695287[4], FJ695225[4], -, -, -, -; ***Batopedina pulvinellata*** Robbr., -, -, -, FJ695355[4], FJ695291[4], AJ288596[11], -, -, -, -; ***Cyanoneuron cyaneum*** (Hallier f.) Tange, -, -, -, KP212708[14], KP212786[14], KP212812[14], -, -, -, -; ***Danais xanthorrhoea*** (K.Schum.) Bremek., -, -, -, KY378686[2], KY378686[2], KY378686[2], -, -, -, -; ***Danais*** **sp**. Comm. ex Vent., -, -, -, FJ695361[4], FJ695297[4], FJ695235[4], -, -, -, -; ***Didymaea alsinoides*** (Cham. & Schltdl.) Standl., -, -, -, AJ234036[11], FJ695298[4], Z68795[8], -, -, -, -; ***Dunnia sinensis*** Tutch. 1, -, -, -, KY378692[2], KY378692[2], KY378692[2], -, -, -, -; ***Dunnia sinensis*** Tutch. 2, -, -, -, EU1453401, EU1454431, EU1454681, -, -, -, -; ***Fooncheuia guangdongensis*** R.J.Wang & H.Z.Wen, -, -, -, JQ002649[15], JQ002645[15], JQ002641[15], -, -, -, -; ***Galium album*** Mill., -, -, -, X76459[16], FJ695299[4], X81090[17], -, -, -, -; ***Houstonia caerulea***

L., -, -, -, FJ695362[4], FJ695300[4], AJ288604[11], -, -, -, - ***Kelloggia galioides*** Torr. in C.Wilkes, -, -, -, AY570768[18], FJ695301[4], DQ662179[3], -, -, -, -; ***Knoxia platycarpa*** Arn., -, -, -, FJ695363[4], FJ695302[4], AJ288631[11], -, -, -, -; ***Kohautia caespitosa*** Schnizl., -, -, -, KY378684[2], KY378684[2], KY378684[2], -, -, -, -; ***Leptodermis potaninii*** Batalin, -, -, -, FJ695365[4], FJ695304[4], AM117241[19], -, -, -, -; ***Manostachya ternifolia*** E.S.Martins, -, -, -, FJ695366[4], FJ695305[4], AJ616213[20], -, -, -, -; ***Mouretia larsenii*** Tange, -, -, -, FJ695367[4], FJ695306[4], FJ695236[4], -, -, -, -; ***Myrioneuron faberi*** Hemsl., -, -, -, KP212726[14], KP212805[14], KP212831[14], -, -, -, -; ***Neohymenopogon parasiticus*** (Wall.) Bennet, -, -, -, FJ695373[4], FJ695313[4], FJ695242[4], -, -, -, -; ***Paederia foetida*** L., -, -, -, KY378691[2], KY378691[2], KY378691[2], -, -, -, -; ***Paederia majungensis*** Homolle ex Puff, -, -, -, FJ695378[4], FJ695319[4], DQ662184[3], -, -, -, -; ***Parapentas silvatica*** (K.Schum.) Bremek., -, -, -, AJ234021[11], FJ695320[4], X83657[21], -, -, -, -; ***Payera coriacea*** (Humbert) Buchner & Puff, Malcomber 2775 (MO), Madagascar, bo65, MK141149*, MK141420*, MK141513*, -, -, -, -, -; ***Pentas lanceolata*** (Forssk.) Deflers, -, -, -, KY378685[2], KY378685[2], KY378685[2], -, -, -, -; ***Pentodon pentandrus*** Vatke, -, -, -, -, FJ695323[4], X83660[21], -, -, -, -; ***Plocama pendula*** Aiton, -, -, -, KY378690[2], KY378690[2], KY378690[2], -, -, -, -; ***Plocama hymenostephana*** (Jaub. & Spach) M.Backlund & Thulin, -, -, -, FJ695385[4], FJ695328[4], DQ662190[3], -, -, -, -; ***Rubia horrida*** (Thunb.) Puff, -, -, -, KY378689[2], KY378689[2], KY378689[2], -, -, -, -; ***Saprosma fruticosum*** Blume, -, -, -, FJ695387[4], FJ695330[4], DQ662194[3], -, -, -, -; ***Schismatoclada* sp.**, -, -, -, FJ695389[4], FJ695332[4], FJ695246[4], -, -, -, -; ***Schizocolea linderi*** (Hutch. & Dalziel) Bremek., -, -, -, EU145323[1], FJ695334[4], AM945286[19], -, -, -, -; ***Serissa japonica*** (Thunb.) Thunb., -, -, -, AJ234034[11], FJ695336[4], Z68822[22], -, -, -, -; ***Sherardia arvensis*** L., -, -, -, X76458[16], FJ695337[4], X81106[17], -, -, -, -; ***Spermacoce remota*** Lam., -, -, -, -, AJ236309[23], Z68823[22], -, -, -, -; ***Spermadictyon suaveolens***, -, -, -, FJ695391[4], FJ695338[4], Z68824[22], -, -, -, -; ***Theligonum cynocrambe*** L. 1, -, -, -, KY378688[2], KY378688[2], KY378688[2], -, -, -, -; ***Theligonum cynocrambe*** L. 2, -, -, -, FJ695393[4], FJ695339[4], FJ695248[4], -, -, -, -; ***Triainolepis mandrarensis*** Homolle ex Bremek., -, -, -, FJ695394[4], FJ695341[4], FJ695250[4], -, -, -, -.

*New sequence generated for this study. Sequences obtained from other studies: 1: Rydin et al. (2008). 2: N. Wikström, B. Bremer, and C. Rydin, unpublished data 2018 (GenBank unpublished). 3: Backlund et al. (2007). 4: Rydin et al. (2009). 5: Cantley et al. (2014). 6: Papadopulos et al. (2011). 7: Cantley et al. (2016). 8: Bremer (1996a). 9: Anderson et al. (2001). 10: Smissen et al. (GenBank unpublished). 11: Bremer and Manen (2000). 12: Andersson and Rova (1999). 13: Andersson and Antonelli (2005). 14: Ginter et al. (2015). 15: Wen and Wang (2012). 16: Manen et al. (1994). 17: Manen and Natali (1995). 18: Nie et al. (2005). 19: Bremer and Eriksson (2009). 20: Thulin and Bremer (2004). 21: Bremer et al. (1995). 22: Bremer (1996b). 23; Bremer et al. (1999).

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