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Adding time-calibrated branch lengths to the Asteraceae supertree

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Abstract New inference techniques, such as supertrees, have improved the construction of large phylogenies, helping to reveal the tree of life. In addition, these large phylogenies have enhanced the study of other evolutionary questions, such as whether traits have evolved in a neutral or adaptive way, or what factors have influenced diversification. However, supertrees usually lack branch lengths, which are necessary for all these issues to be investigated. Here, divergence times within the largest family of flowering plants, namely the Asteraceae, are reviewed to estimate time-calibrated branch lengths in the supertree of this lineage. An inconsistency between estimated dates of basal branching events and the earliest asteraceous fossil pollen record was detected. In addition, the impact of different methods of branch length assignment on the total number of transitions between states in the reconstruction of sexual system evolution in Asteraceae was investigated. At least for this dataset, different branch length assignation approaches influenced maximum likelihood (ML) reconstructions only and not Bayesian ones. Therefore, the selection of different branch length information is not arbitrary and should be carefully assessed, at least when ML approaches are being used. The reviewed divergence times and the estimated time-calibrated branch lengths provide a useful tool for future phylogenetic comparative and macroevolutionary studies of Asteraceae.

Key words Asteraceae, character mapping, divergence times, sexual systems, trait evolution.

The development of phylogenetic supertree inference techniques has promoted the construction of new large phylogenetic hypotheses (e.g. Salamin et al., 2002; Davies et al., 2004; Baker et al., 2009). These methodological advances, as well as supermatrix (de Queiroz & Gatesy, 2007) and megatrees (Smith et al., 2009) approaches, will help shed light on the tree of life. In addition, they allow us to gain access to comprehensive and large phylogenies (Gittleman et al., 2004), which are necessary for answering decisive questions about trait evolution (e.g. Gittleman et al., 2004; Bolmgren & Cowan, 2008; Torices & Anderberg, 2009), rates of speciation, extinction, and diversification (e.g. Purvis, 1995; Bininda-Emonds et al., 1999; Davies et al., 2004; Moore et al., 2004), phylogenetic community ecology (e.g. Webb et al., 2002), or establishing conservation priorities (e.g. Mooers et al., 2005).

Supertree construction is a phylogenetic approach in which many overlapping source trees are combined to produce a single, larger supertree (Bininda-Emonds et al., 2002). Source trees need only be overlapping, and not identical, with respect to the terminal taxa they contain (Bininda-Emonds et al., 2002). Thus, the resulting supertree is usually larger than any of the source trees. However, the supertrees usually lack branch length information (e.g. Jones et al., 2002; Kennedy & Page, 2002; Funk et al., 2005; Beck et al., 2006). However, the study of most evolutionary issues requires branch length information (Pagel, 1999; Cunningham, 1999; Oakley, 2003; Felsenstein, 2004). To avoid this problem, one commonly used alternative is to make all branch lengths equal (i.e. an implicitly 'punctuated' model of evolution). However, assuming equal branch length may lead to an increase in Type I error rates (Purvis et al., 1994). Other possibilities of incorporating branch lengths are to assign them based on the number of terminal taxa (Purvis, 1995; Bininda-Emonds et al., 1999), but some traits can be correlated with diversification rates (Heilbuth, 2000; Vamosi & Vamosi, 2004). This makes it inappropriate to assign branch lengths in this way, particularly if the tree is then going to be used to investigate the evolution of traits correlated with diversification rates. Other simple method for estimating time-calibrated branch lengths of large phylogenies is to fix those nodes for which some estimate of their ages is available and to distribute the rest of the nodes evenly between the dated nodes (e.g. Moles et al., 2005; Milla & Reich, 2007; Cahill et al., 2008; Willis et al., 2008).

Newly available methods for studying trait evolution, such as maximum likelihood (ML) and Bayesian analyses (BA), assume that branch lengths carry information on the probability of phenotypic change (Oakley, 2003; Ronquist, 2004). The length of a branch will influence the probability of estimating some change between states in a given trait, character transitions

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being much more likely on long branches (Cunningham, 1999; Oakley, 2003). Therefore, branch length information should be assessed carefully before any analysis of trait evolution. Nevertheless, the effect of different methods of assigning branch length information to phylogenies on character reconstructions has not been examined in great detail using empirical data (Xiang & Thomas, 2008).

The main objective of the present study was to estimate time-calibrated branch lengths based on published studies dating branching events for one supertree of the largest family of flowering plants, namely Asteraceae (Funk et al., 2005). First, data from distinct analyses were reviewed, testing whether different dating analyses show major inconsistencies between dates of branching events. Second, the impact of different methods of branch length assignment on the reconstruction of trait evolution was evaluated. In particular, whether different common approaches of estimating branch lengths had any effect on the total number of changes between different sexual systems in the evolution of Asteraceae was assessed.

1 Material and methods

1.1 Branch length estimation

Branch lengths were estimated for the Asteraceae supertree (Funk et al., 2005) using the BLADJ function of Phylocom v. 4.0.1b software (Webb et al., 2008a), which fixes the age of internal nodes based on clade age estimates, whereas undated internal nodes in the phylogeny are spaced evenly between dated nodes to minimize tree-wide variance in branch length (Webb et al., 2008b). BLADJ is a simple tool that fixes the root node of a phylogeny at a specified age and fixes other nodes or which age estimates are available. It sets all other branch lengths by placing the nodes evenly between dated nodes, as well as between dated nodes and terminals (of Age 0).

First, the Asteraceae supertree from Funk et al. (2005, figs. 6–9) was translated into a Newick tree file format. Second, nodes for which there were age estimates were fixed. Phylocom's authors (Webb et al., 2008b) suggest using the age estimates of Wikström et al. (2001), however this analysis is not suitable for estimated branch lengths within families. Therefore, published articles were searched for clade age estimates and age estimates were selected mainly on the basis of molecular dating in which some fossil calibration had been used, although other dating methods were also considered (i.e. geological dating). Fossil dates alone were not used in the first approach because the first appearance in the fossil record does not necessarily correspond with the origin of a taxon. Clade age estimates are usually given as time intervals. However, for the BLADJ algorithm, only one date for each node must be provided. Thus, the mean value of the minimum and maximum time estimates was used in the analysis. Moreover, when more than one analysis had been performed within the same article, the minimum and maximum dates were used to calculate the mean, irrespective of the method used in the study. The same rationale was followed when more than one date was obtained from different articles for the same branching event, such as for the origin of Asteraceae (Table 1) or the origin of Asteroid tribes (Table 1). Unfortunately, not all estimated ages were useful for this analysis because some of the branching events were not included in the supertree.

In addition, fossil data, mainly pollen, were used as a posterior calibration test to improve the robustness of branch length estimation; specifically, the estimated ages for nodes for which age data were not available were compared for consistency with dates from the fossil record (Table 2). Thus, available fossil data were only used when, after the first analysis with BLADJ, the estimated age of the origin of the lineages was earlier than the fossil age, as in the case of *Chuquiraga*. In this case, fossil pollen grains have been recovered from marine Miocene deposits from southern South America (Table 2) that have been attributed to *Dasyphyllum* and *Chuquiraga* (23–20 mya) and *Schlechtendalia* (11–9 mya; Palazzesi et al., 2009). In the first analysis, the origin of *Chuquiraga* was estimated to be earlier than 20 mya. Therefore, it was considered more appropriate to use the fossil age to fix this branching event, although it corresponded to a minimum age.

1.2 Effects of different branch lengths on sexual system reconstruction

Sexual system data for Asteraceae were used to explore how different branch lengths may influence the total number of transitions between seven sexual systems that are present in Asteraceae (hermaphroditism, gynomonoecy, monoecy, andromonoecy, trimonoecy, dioecy, and gynodioecy; Torices, 2009). Three common approaches used to assign branch lengths were assessed: (i) an implicitly 'punctuated' model of evolution, where all branch lengths are equal; (ii) simulated branch lengths under a birth and death model of diversification; and (iii) the time-calibrated estimation described above. The birth and death simulation of branch lengths was implemented in SIMMAP 1.0 Beta 2.3.2 software (Bollback, 2006), in which branch length values are assigned by sampling from the birth–death method described by Rannala & Yang (1996). In this

Table 1 Estimated age of branching events

Branching event	Estimated time (mya)	Reference	Node label[A]
Origin of Goodeniaceae	64.5 (49–80)	Kim et al. (2005)	1
Origin of Asteraceae[B]	45.5 (42–49)	Kim et al. (2005)	2 (47.0)
Origin of Asteraceae	48 (43–52)	Devore & Stuessy (1995)	
Barnadesioideae/rest of Asteraceae[C]	39.0 (36–42)	Kim et al. (2005)	3
Barnadesioideae genera diversification	28.5 (22–35)	Kim et al. (2005)	4
Onoseris/rest of Asteraceae[D]	38.0	Kim et al. (2005)	5
Cardueae + Mutisieae/LALV + Asteroid tribes	35.5 (33–38)	Kim et al. (2005)	
LALV tribes/Asteroid tribes	35 (32–38)	Kim et al. (2005)	7
Origin of LALV tribes	27.5 (24–31)	Kim et al. (2005)	8
Origin of Liabeae	10 (5–15)	Funk et al. (2005)	9
Origin of Asteroid tribes	32.5 (26–39)	Kim et al. (2005)	10 (33.2)
Origin of Asteroid tribes	43.0 (56.6–29.6)	Bergh & Linder (2009)[E]	
Origin of Helianthoid tribes	19 (17–21)	Kim et al. (2005)	13 (26.0)[F]
Origin of Hecastocleidoideae	35 (32–38)	Kim et al. (2005)	6
Origin of Gnaphalieae	34.5 (52.3–20.6)	Bergh & Linder (2009)	11
Origin of Australasian Gnaphalieae	14.6 (20.6–8.3)	Bergh & Linder (2009)	
Diversification of Anthemideae	23.1 (19.0–27.2)	Oberprieler (2005)	12
Hesperomannia/rest of African Vernonieae	21.5 (17–26)	Kim et al. (1998)	
Origin of *Coreocarpus*	1	Kimball et al. (2003)	
Leontodon/*Hypochaeris*	6.58	Tremetsberger et al. (2005)	
Helianthus/*Tagetes*	17.4 (15.1–22.3)	Tremetsberger et al. (2005)	14
Origin of *Abrotanella*	19.41(17.1–21.9)	Wagstaff et al. (2006)	
Diversification of Subtribe Chrysantheminae	8	Oberprieler (2005)	
Diversification of Subtribe Chrysantheminae	2.75 (2.5–3.0)	Francisco-Ortega et al. (1995)	
Origin of *Argyranthemum*	0.26–2.1	Francisco-Ortega et al. (1997)	
Origin of *Argyranthemum*	5	Oberprieler (2005)	
Origin of Hawaiian silverword alliance	5.2 (4.4–6.0)	Baldwin & Sanderson (1998)	

[A]Branching events selected to estimate branch lengths on the supertree. The mean value of the minimum and maximum time estimates was used when more than one dating was obtained from different articles for the same branching event.
[B]Kim et al. (2005) estimated divergence times using two methods: (i) the average synonymous substitutions of *ndhF* gene in conventional distance-based molecular clock method; and (ii) the combined whole sequence data set of *ndhF* and *rbcL* in the non-parametric rate smoothing (NPRS)-based molecular clock method. Using these two methods, the estimated age for the origin of Asteraceae was 49–45 and 48–42 mya, respectively. Thus, in the present study, interval limits of 49 and 42 mya were used.
[C]Origin of two chloroplast DNA inversions.
[D]This age was obtained from fig. 6 in Kim et al. (2005) and not from tables, and therefore does not have maximum and minimum limits.
[E]The "d" analysis from Bergh & Linder (2009) was chosen, which was performed with a narrower root prior corresponding closely to the range of estimates in Kim et al. (2005). Thus, inconsistencies between both dating analyses were reduced.
[F]The maximum limit to calculate the mean value was set by *Ambrosia* type pollen (35 mya; Graham, 1996).
LALV, Lactuceae, Arctoteae, Liabeae, and Vernonieae.

way, ultrametric trees were obtained that varied in their branch lengths.

Several methods exist to reconstruct the evolutionary history of a character on a given phylogenetic hypothesis (Felsenstein, 2004; Ronquist, 2004). Recently, some authors have reported different results using different methodological approaches for character reconstruction, such us maximum parsimony (MP), ML and Bayesian BA (Ekman et al., 2008; Xiang & Thomas, 2008). In particular, Xiang & Thomas (2008) suggested that the discrepancies between ML and BA when branch lengths are considered equal disappear in chronogram-based analyses. Therefore, in addition to using different sets of branch lengths, different approaches of trait reconstruction should be assessed.

Branch lengths are irrelevant for reconstructing the evolutionary history of a character using MP, but not for reconstructions using ML (Felsenstein, 2004). Thus, the transitions between sexual systems determined by ML and BA reconstructions were explored on a set of

Table 2 List of first fossils for different lineages of Asteraceae

Lineage	Period	Type of fossil	Reference
Barnadesioideae	Early Miocene	*Dasyphyllum* and *Chuquiraga* pollen grains	Palazzesi et al. (2009)
Subtribe Nassauviinae	Miocene	Pollen grains	Barreda et al. (2008)
Dicomeae	Mid-Eocene	*Dicoma*-type pollen grains	Scott et al. (2006)
Asteroid tribes	Late Eocene–Late Oligocene	*Ambrosia*-type pollen grains	Graham (1996)
Anthemideae	Late Oligocene	*Artemisia* pollen grains	Graham (1996)
Lactuceae	Early Miocene	*Taraxacum* fruits/seeds	Graham (1996)
Cardueae	Mid-Miocene	*Cirsium* fruits/seeds	Graham (1996)
Heliantheae	Late Miocene	*Xanthium* fruits/seeds	Graham (1996)

500 trees (R. Torices et al., unpubl. data, 2010). The ML reconstructions were implemented in MESQUITE 2.6 software (Maddison & Maddison, 2009). One mapping per tree was sampled in ML analysis because this analysis does not allow for multiple mappings of character reconstructions (Maddison & Maddison, 2006). MESQUITE provides the mean, minimum and maximum of each transition over all inferred mappings. The BA character mapping technique (Huelsenbeck et al., 2003) was implemented in the SIMMAP 1.0 Beta 2.3.2 program (Bollback, 2006; for recent use of this methodology in the study of sexual system evolution, see Renner et al., 2007; Torices & Anderberg, 2009). The BA method requires the use of priors. A prior is a probability distribution specifying knowledge about the model and its parameters before a BA is run (Ronquist, 2004). In some instances the particular form of the prior can dominate the posterior results (Schultz & Churchill, 1999; Pagel et al., 2004). To determine the importance of this issue, three different priors were explored to obtain the posterior distribution. Twenty maps or realizations were sampled from each tree and 10 realizations were sampled from the prior distribution over three sets of morphological priors to test the influence on the results. SIMMAP uses a gamma prior on the overall rate of change of tree length. Thus, the gamma distributions for the priors on the rate parameter were set from low to high rates as follows, where T is the rate of change of tree length, E(T) is the expected value and SD(T) is the standard deviation: low rates E(T) = 1.00, SD(T) = 1.00; medium rates E(T) = 1.50, SD(T) = 0.87; and high rates E(T) = 5.00, SD(T) = 2.24. The posterior expectation of the total number of transitions between sexual systems was investigated (Bollback, 2006).

2 Results and discussion

2.1 Branch length estimation

The present analysis provided, for the first time, a supertree of the Asteraceae with time-calibrated branch lengths (Fig. 1; for a complete view of the whole supertree, see Supplementary Fig. S1). Although it is not a proper dating analysis, this tree is valuable in: (i) providing relative branch lengths for comparative methods; and (ii) verifying divergence times from distinct analyses, testing whether the ages of younger or older lineages are consistent between different dating analyses.

Distinct analyses have suggested different dates for some branching events within Asteraceae diversification (Table 1). However, only one major inconsistency was found between dates of branching events. The origin for the Dicomeae lineage (32 mya; Fig. 1) was estimated more recently than the oldest fossil of this group (Table 2). This fossil is considered the earliest unequivocal asteraceous fossil pollen record; however, there still are some uncertainties about its taxonomic affinity and its date. It was initially attributed to Mutisieae-type pollen from the Paleocene–Eocene (Zavada & de Villiers, 2000), but the same samples have recently been dated to the mid-Eocene, approximately to 40 mya, instead of the Paleocene–Eocene, and reattributed to the *Dicoma*-like taxon (Scott et al., 2006). The *Dicoma*-type fossil age (Scott et al., 2006) was not used for the main analysis because it disagreed with some of the estimated dates of basal branching events (Nodes 3, 5, and 6; Fig. 1; Table 1). Nevertheless, in a secondary analysis in which the *Dicoma*-type fossil age was used as calibration point (Supplementary Fig. S2) instead of the estimated ages obtained by Kim et al. (2005), Nodes 3, 5, and 6 were dated at earlier ages (46, 45, and 42 mya respectively; Supplementary Fig. S2). Hence, the first branching events of Asteraceae are likely to be dated to earlier ages when this fossil would be used as a calibration age in a molecular age estimation study. Despite this inconsistency, other pollen fossil grains supported the estimation provided in this analysis. For instance, pollen fossil grains of Nassauviinae (Barreda et al., 2008) support the age estimated here (Fig. 1).

Purvis (1995) and Bininda-Emonds et al. (1999, 2007) dated their supertrees following a similar procedure; however, they estimated the divergence age of the nodes without published ages using a pure birth model, under which a clade's age is proportional to the logarithm of the number of species it contains. It is doubtful whether it is appropriate to assign branch lengths to a clade considering only the number of taxa it contains. To avoid this potential problem, the BLADJ approach was followed (Webb et al., 2008a), in which the undated nodes are placed evenly between dated nodes, as well as between dated nodes and terminals. Another option is the method proposed by Vos & Mooers (2004), which combines the estimates from different genes for the same node to reconstruct divergence times for supertrees. This method is limited by the availability of gene sequences for the group being investigated and would require more effort than the other methods. Although the estimates of Vos & Mooers (2004) were strongly correlated with those of Purvis (1995), it would be necessary to test the effectiveness of the method of Vos & Mooers (2004) using more data sets.

One potential weakness of the present analysis was the low number of dated nodes included for the large size of this lineage (Table 1). Although some events in the evolution of Asteraceae have been well characterized, such as the origin of two chloroplast DNA

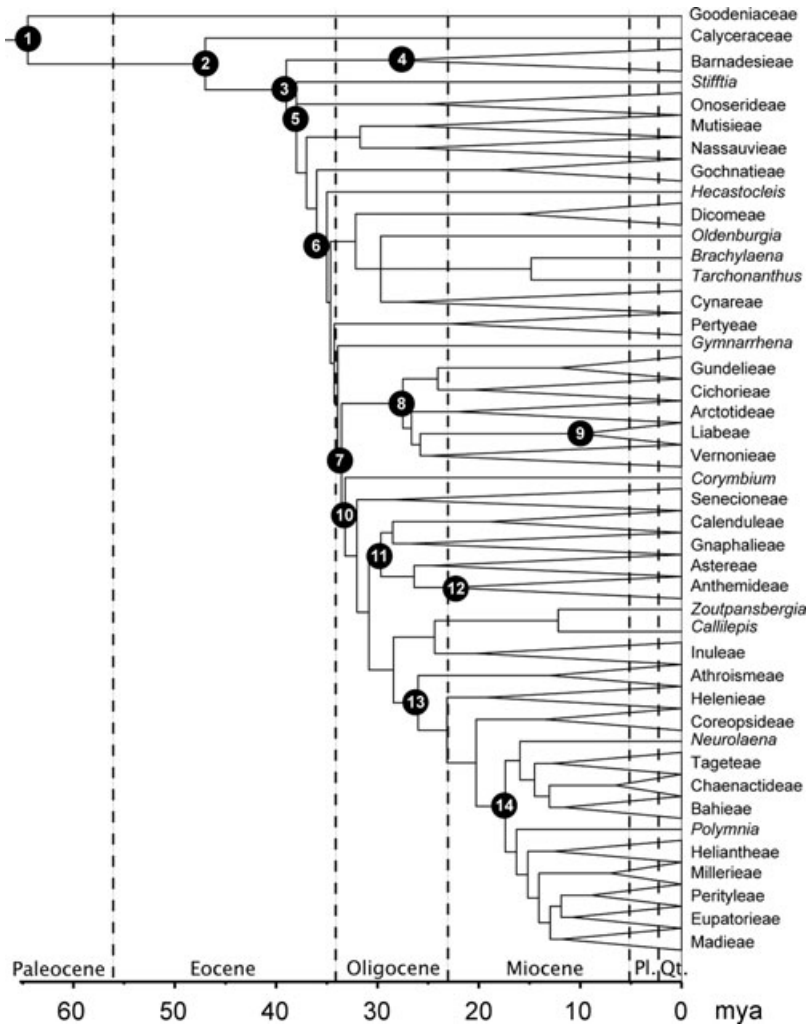

Fig. 1. Time-calibrated Asteraceae supertree. Node labels are given in Table 1. Pl, Pliocene; Qt, Quaternary.

inversions (Kim et al., 2005), we know very little about the age of most branching events, which could have been very important in the diversification of this evolutionary successful lineage (Panero & Funk, 2008). Some precautions should also be considered in this analysis because the estimated branch lengths proposed in could be undoubtedly wrong in many details. However, this is the first attempt to combine the evidence of the ages of branching events within this family and it points out where broad agreement does and does not exist and therefore constitutes a useful baseline for further research. These branch lengths must be treated as approximations and it could be better to use them as relative age estimates rather than absolute ages. Although it is not a substitute for dating analyses of primary molecular and fossil data, the interpolations are intended more to accommodate those comparative methods requiring a complete set of branch lengths than as precise estimates of divergence times. Therefore, these time-calibrated branch lengths provide a useful tool for future phylogenetic comparative and macroevolutionary studies of the largest family of flowering plants (e.g. R. Torices et al., unpubl. data, 2010).

2.2 Effect of different branch lengths on sexual system reconstruction

In the analysis of sexual system evolution in Asteraceae, the three sets of branch lengths yielded almost the same total number of transitions between sexual systems in BA reconstructions (Table 3). In contrast, the use of different branch lengths using an ML reconstruction approach provided very different total numbers of transitions between sexual systems (Table 3). Therefore, at least for this dataset, the different branch length assignation approaches only influenced ML reconstruction and not BA reconstructions.

Table 3 Total number of changes between sexual systems for different branch lengths and using different methods of character reconstruction

Branch length[A]	Method[B]	No. changes[C]		
		n	Mean ± SD	Range
Simulated	Bayesian 1	100 000	161.75 ± 2.50	152–173
	Bayesian 2	100 000	161.65 ± 2.47	152–172
	Bayesian 3	100 000	163.16 ± 2.78	153–176
	Maximum likelihood	500	109.17	
Equal	Bayesian 1	100 000	161.93 ± 2.51	152–173
	Bayesian 2	100 000	161.88 ± 2.50	152–173
	Bayesian 3	100 000	162.84 ± 2.69	150–175
	Maximum likelihood	500	75.63	
Time calibrated	Bayesian 1	100 000	161.33 ± 2.36	152–172
	Bayesian 2	100 000	161.25 ± 2.34	152–171
	Bayesian 3	100 000	162.42 ± 2.59	152–174
	Maximum likelihood	500	96.53	

[A]Simulated: branch lengths were assigned to each tree from the topology according to the model of diversification from birth and death option in SIMMAP 1.0 Beta 2.3.2 software; Equal: branch lengths were set to 1; Time calibrated: branch lengths were estimated using BLADJ function of Phylocom version 4.0.1b software. Branch lengths were always rescaled to 1 before every analysis.
[B]Rate parameter prior distributions were set in E(T) = 0.50, SD(T) = 0.50 for Bayesian 1; in E(T) = 1.50, SD(T) = 0.87 for Bayesian 2; and in E(T) = 5.00, SD(T) = 2.24 for Bayesian 3, where E(T) is the expected value and SD(T) is the standard deviation.
[C]Maximum likelihood analyses were performed with MESQUITE 2.6, which does not provide error measurement of the number of changes, and hence only the mean value is given for the 500 mappings.

The total number of changes under an ML approach was always lower than under a BA approach, irrespective of the branch length assigned (Table 3). This result agrees with the findings of Ekman et al. (2008), who reported that ML reconstruction with the decision threshold set to 2 ln likelihood units (which was also used in the present analysis) was much less certain. With this decision threshold, there are many cases of several almost equally good state assignments, reducing the number of estimated transitions on the tree. The same analysis, but using an MP criterion that does not make use of branch lengths, provided results more similar to those obtained using a BA approach than an ML approach (Torices, 2009). Pedersen et al. (2007) also found congruent results between an MP model and a BA reconstruction of trait evolution in the moss family Bryaceae.

The selection of different branch length information is not arbitrary and it should be assessed carefully, at least when ML approaches are being used (Cunningham et al., 1998; Cunningham, 1999; Pagel, 1999; Oakley, 2003). Xiang & Thomas (2008) suggest that time-calibrated branch lengths are better in that they provide temporal information on the events of character state transitions. However, Cunningham (1999) proposed that even if the branch lengths are estimated perfectly, at the very least a model with equal branch lengths should be used as a point of comparison. The impact of branch lengths on trait reconstruction seems method specific (Xiang & Thomas, 2008). Thus, trait evolution should be assessed with more than one method of reconstruction (Pedersen et al., 2007; Ekman et al., 2008; Xiang & Thomas, 2008), and with more than one type of branch length information, while awaiting clarification of the statistical properties of each method under different sets of branch lengths.

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Supporting Information

The following supporting information is available for this article:

Supplementary Figure S1. Time-calibrated Asteraceae supertree. This tree is available in NEWICK format from the author's web site (http://sites.google.com/site/rubentoricesblanco/).

Supplementary Figure S2. Time-calibrated Asteraceae supertree using *Dicoma*-type fossil age. This tree is available in NEWICK format from the author's web site (http://sites.google.com/site/rubentoricesblanco/).

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