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Molecular paleobiology of early-branching animals: integrating DNA and fossils elucidates the evolutionary history of hexactinellid sponges

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Abstract.—Reconciliation of paleontological and molecular phylogenetic evidence holds great promise for a better understanding of the temporal succession of cladogenesis and character evolution, especially for taxa with a fragmentary fossil record and uncertain classification. In zoology, studies of this kind have largely been restricted to Bilateria. Hexactinellids (glass sponges) readily lend themselves to test such an approach for early-branching (non-bilaterian) animals: they have a long and rich fossil record, but for certain taxa paleontological evidence is still scarce or ambiguous. Furthermore, there is a lack of consensus for taxonomic interpretations, and discrepancies exist between neontological and paleontological classification systems. Using conservative fossil calibration constraints and the largest molecular phylogenetic data set assembled for this group, we infer divergence times of crown-group Hexactinellida in a Bayesian relaxed molecular clock framework. With some notable exceptions, our results are largely congruent with interpretations of the hexactinellid fossil record, but also indicate long periods of undocumented evolution for several groups. This study illustrates the potential of an integrated molecular/paleobiological approach to reconstructing the evolution of challenging groups of organisms.

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Introduction

The fossil record provides the only tangible evidence of the temporal distribution of taxa, but it is well appreciated that these data are often incomplete. Furthermore, taxonomic interpretation of fossils can be difficult when key characters are not preserved, leaving much room for speculation. Molecular sequences provide an independent source of data that can be brought to bear on evolutionary questions. Using these data to elucidate the timing of evolutionary events by estimating dates of clade divergence, however, requires external information, typically taken from the fossil record. Therefore, synthesizing paleontological and molecular data for a more holistic understanding of evolutionary history is one of the most promising—and most challenging—lines of modern paleobiological research (Brochu et al. 2004; Magallón 2004; Donoghue and Benton 2007; Peterson et al. 2007).

Taxa vary greatly in their suitability for such integrated analyses. For diverse groups with still no appreciable fossil record, e.g., Platyhelminthes or Placozoa, analyses integrating paleontological data will probably never be possible. The evolution of other important groups for which there are only scattered fossil occurrences, usually as partly isolated Lagerstätten, e.g., Nematoda or Medusozoa, may also be prohibitively difficult to address with synthetic analyses combining fossil and molecular data because first fossil occurrences of subclades are not likely to correspond closely to their evolutionary origin (Cartwright and Collins 2007). A further difficulty is posed for groups that have a relatively rich fossil record, but whose phylogenetic histories are difficult to reconstruct with morphological data owing to the paucity of informative

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characters and high levels of homoplasy. Sponges (Porifera) are perhaps the most notorious example of such a taxon (see Hooper and van Soest 2002; Cárdenas et al. 2012). Although molecular systematics has greatly helped resolve relationships of extant sponges (Erpenbeck and Wörheide 2007; Wörheide et al. 2012), it has also generally indicated that traditional sponge classification and taxonomy is based on characters that do not accurately reflect evolutionary history (Cárdenas et al. 2012). Morphology-based classification, which is already difficult for extant taxa, is thus even more problematic for fossil sponges. Paleontologists naturally have to rely on morphological characters for taxonomic assignment, and the task for sponges is complicated by a fossil record biased toward groups with fused or articulated skeletons, leaving substantial gaps for taxa that more readily disintegrate after death (Pisera 2006). This difficult situation, however, is less severe in the Hexactinellida (glass sponges), which have recently been shown to have an evolutionary history, as elucidated by molecular sequence data, that is largely consistent with the distribution of morphological features across its traditional taxa (Dohrmann et al. 2008, 2009). However, the fossil record presently provides incomplete or ambiguous evidence regarding the origin and evolution of extant hexactinellid subtaxa (see next section). Furthermore, the poor concordance between paleontological and neontological systematics greatly complicates matters (Krautter 2002; Reiswig 2006).

Here we use glass sponges to illustrate how a molecular paleobiological approach (Peterson et al. 2007) can enhance our understanding of the evolution of such "problematic" animal groups. We estimate divergence times of crown-group Hexactinellida from the largest molecular phylogenetic data set assembled to date for this class of sponges (Dohrmann et al. 2012a), using a relaxed molecular clock approach (see, e.g., Welch and Bromham 2005; Yang 2006 for reviews) and fossil-based age constraints for calibration. We then compare the dated phylogeny with the fossil record and discuss implications of congruencies and discrepancies between the two sources of evidence, thereby coming to an enhanced appreciation of hexactinellid evolution.

Fossil Record and Systematics of Glass Sponges.—Hexactinellids were important components of deep- and at times also shallower-water benthic ecosystems throughout the Phanerozoic, often associated with reef communities (e.g., Finks 1960; Mehl 1992; Brunton and Dixon 1994; Leinfelder et al. 1994; Krautter et al. 2001; Carrera and Botting 2008). Their rich fossil record (see Krautter 2002; Pisera 2006) dates back to the late Neoproterozoic (Steiner et al. 1993; Gehling and Rigby 1996; Brasier et al. 1997). The two extant subclasses, Hexasterophora and Amphidiscophora, whose monophyly is strongly supported by both morphological and molecular data (Mehl 1992; Dohrmann et al. 2008), appear in the early Paleozoic, as indicated by isolated microscleres (Mostler 1986). Because the oldest hexasters (the defining autapomorphy of Hexasterophora) are known from the lowermost Ordovician, the hexasterophoran and amphidiscophoran stem-lineages must have already evolved during the Cambrian (Mostler 1986; Mehl 1996). However, given that the earliest (late Ediacaran and early Cambrian) bodily preserved hexactinellid fossils (e.g., Steiner et al. 1993; Gehling and Rigby 1996; Brasier et al. 1997; Wu et al. 2005; Xiao et al. 2005) bear no resemblance to any specific extant subtaxon, it is likely that they represent stem-group members, implying that the origin of the crown group (i.e., the split between the two subclasses) does not predate the Ediacaran/Cambrian boundary. Thus, although the origin of Hexactinellida from a common ancestor with demosponges certainly occurred in Precambrian times, their crown group likely evolved rapidly as part of the Cambrian radiation (see Zhang and Pratt 1994; Reitner and Mehl 1995; Xiao et al. 2005; see also Erwin 2011).

Following a high Paleozoic diversity, most groups dominant in that era had disappeared by the end of the Permian (Mostler 1990; Mehl 1996; Mehl-Janussen 1999; Krautter 2002). Because the taxonomically important microscleres are rarely preserved in situ (but see, e.g., Kling and Reif 1969 and Rigby et al. 2007 for notable exceptions), and Paleozoic skeletal

architectures differ greatly from Mesozoic and modern forms (Mehl and Mostler 1993; Mehl 1996), relationships of these taxa to extant hexactinellids remain largely elusive. Thus, fossils assigned to modern families are mostly confined to the Mesozoic–Cenozoic. The fossil record of Amphidiscophora is poor (Mehl 1992, 1996), and the earliest crown-group member (family Hyalonematidae) was described from the Late Cretaceous (Mehl and Hauschke 1995). Likewise, there is no conclusive evidence for Paleozoic Lyssacinosida, a hexasterophoran order characterized by largely unfused (lyssacine) skeletons (Mehl 1992, 1996). Although most Paleozoic glass sponges are lyssacine, assignment of early Paleozoic or even late Neoproterozoic fossils, solely based on this single character, to the Lyssacinosida (e.g., Krautter 2002) is highly questionable because this type of skeletal organization also characterizes Amphidiscophora and is therefore likely plesiomorphic (Mehl 1992). The modern families of Lyssacinosida (Rossellidae, Euplectellidae, Leucopsacidae) are definitely present by the Late Cretaceous (Salomon 1990; Brückner and Janussen 2005; Brückner 2006), but because of their limited fossilization potential the earlier history of Lyssacinosida remains obscure.

The earliest unambiguous evidence for crown-group Hexasterophora is the occurrence of dictyonal frameworks—rigid skeletons produced by fusion of hexactine megascleres—in the Late Devonian (e.g., Rigby et al. 1981, 2001; Rigby 1986; Mehl and Mostler 1993; Mehl 1996). These structures are diagnostic for the "Hexactinosida," a hexasterophoran order that underwent major radiations during the Mesozoic (Mehl 1992; Mehl and Mostler 1993; Pisera 1999) and is still abundant and diverse today (see Hooper and van Soest 2002; Leys et al. 2007). Curiously, dictyonal skeletons are not documented from the Carboniferous, Permian, or Early Triassic (Pisera and Bodzioch 1991; Mehl and Mostler 1993; Mehl 1996), an absence that is hypothesized to be a preservational artifact (Mehl 1996; Rigby et al. 2001). Although molecular data (Dohrmann et al. 2008, 2009) suggest that the "Hexactinosida" are paraphyletic with respect to Lyssacinosida, the majority of hexactinosidans form a highly supported clade, the Sceptrulophora (Mehl 1992; Dohrmann et al. 2011), which is the sister group of the remaining hexasterophorans (Dohrmann et al. 2008, 2009). This taxon is characterized by the possession of sceptrules, a scepter-like spicule type that occurs in various forms, mostly scopules or clavules (see Dohrmann et al. 2011). In contrast, sceptrules are lacking in the Dactylocalycidae, which were resolved as the sister group of Lyssacinosida in molecular studies (Dohrmann et al. 2009, 2012a). Spicule fragments interpreted as sceptrules have been reported from late Cambrian and Ordovician strata (e.g., Bengtson 1986; Webby and Trotter 1993; Dong and Knoll 1996; Kozur et al. 1996; Zhang and Pratt 2000). However, their poor preservation and the next appearance of sceptrules in the Triassic (Donofrio 1991; Krainer and Mostler 1991) raise doubts about the homology of the Paleozoic and Mesozoic–Recent forms.

Another important hexasterophoran taxon is the Lychniscosida, species-poor in today's oceans (Reiswig 2002a) but once highly diverse and reef-building. Lychniscosidans appeared in the Middle Jurassic (Pisera and Bodzioch 1991; Mehl 1992; Mehl and Mostler 1993; Pisera 1999) and also have dictyonal skeletons. However, their skeletons probably evolved convergently, because they are built from lantern-like hexactins rather than simple hexactins (Mehl 1992). Lychniscosidans have not been sampled yet for molecular systematics, so the hypothesis that this taxon is nested within Lyssacinosida (Mehl 1992) remains to be tested.

In general, after a Late Cretaceous peak hexactinellid diversity underwent a gradual decline, which might be related to restrictions of shelf habitats (Mehl 1992) and/or changes in ocean chemistry (Maldonado et al. 1999).

Methods

We based our study on a DNA-sequence data set (~4600 bp) consisting of concatenated nuclear 18S and partial 28S ribosomal DNA (rDNA), partial mitochondrial 16S rDNA, and partial mitochondrial cytochrome oxidase subunit I (COI) from 50 hexactinellid species.

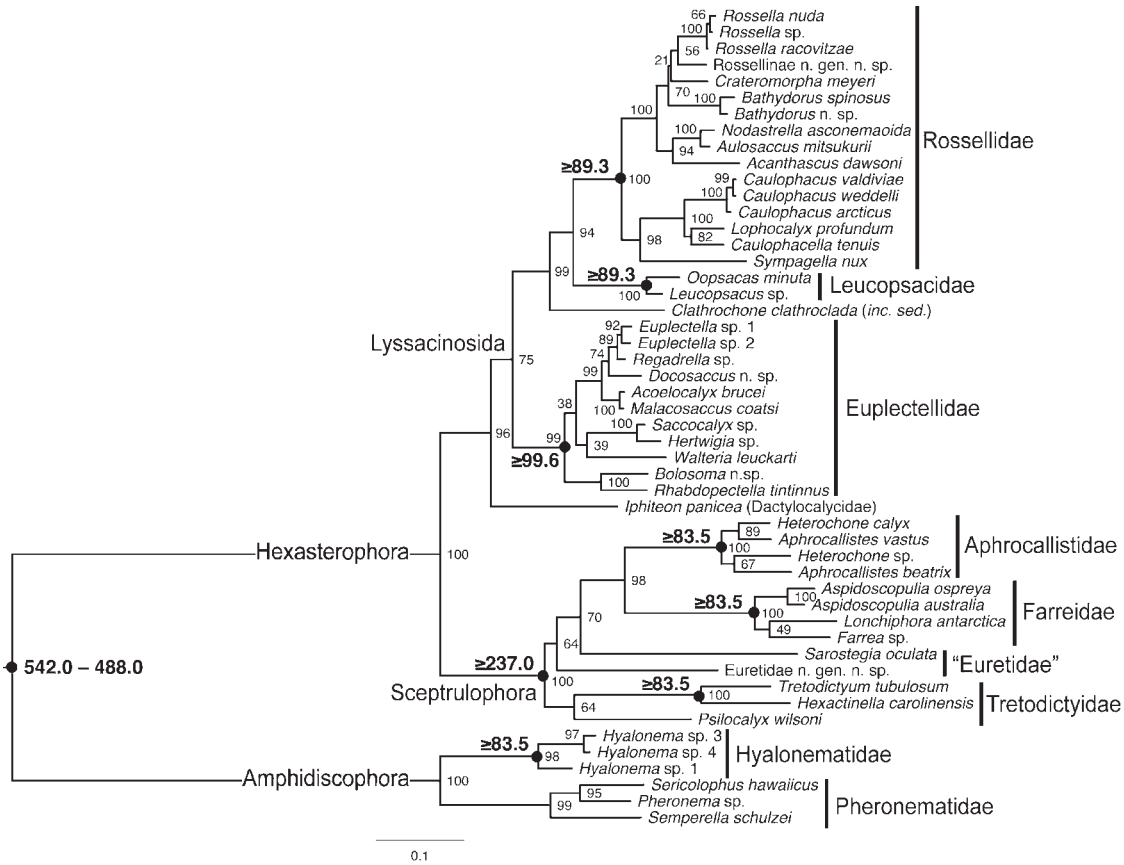

FIGURE 1. Maximum likelihood molecular phylogeny of Hexactinellida, based on combined rRNA and COI genes (Dohrmann et al. 2012a). Clade support values are bootstrap proportions; scale bar indicates expected number of substitutions per site. Black dots indicate calibration nodes; ages in million years ago (Ma). See text for explanation and references. Note: the species *Nodastrella asconemaoida* corresponds to *Rossella nodastrella* in Dohrmann et al. (2012a); see Dohrman et al. (2012b).

For details on the molecular methods and phylogenetic analysis of this data set see Dohrmann et al. (2012a). In brief, we applied independent substitution models to COI, 16S, 18S single-stranded regions (loops), 28S loops, and 18S+28S double-stranded regions (stems), including an RNA model to account for coevolution of paired sites for the latter (see Savill et al. 2001). This analysis was conducted in a maximum likelihood (ML) framework using the software RAxML (Stamatakis 2006).

For calibration, we constrained the ages of eight internal nodes and the root node, using one maximum and nine minimum constraints in total, as detailed below. Analyses without data, i.e., sampling only from the prior distribution, showed that the prior mean divergence times of the internal nodes significantly differed from the posterior estimates (results not shown), confirming that this calibration set was suitable, allowing the data to dominate the results. The calibrations used are as follows (see also Fig. 1; stratigraphy follows Gradstein et al. 2004 throughout this paper):

1. For the age of the root (= origin of crown-group Hexactinellida, or the Amphidiscophora/Hexasterophora-split) we used a minimum of 488 Ma (million years ago) (early Tremadocian: first hexasters [Mostler 1986]). Although the fossil record generally only provides minimum ages for taxa, at least one maximum age constraint is required to produce meaningful results from relaxed molecular clock analyses

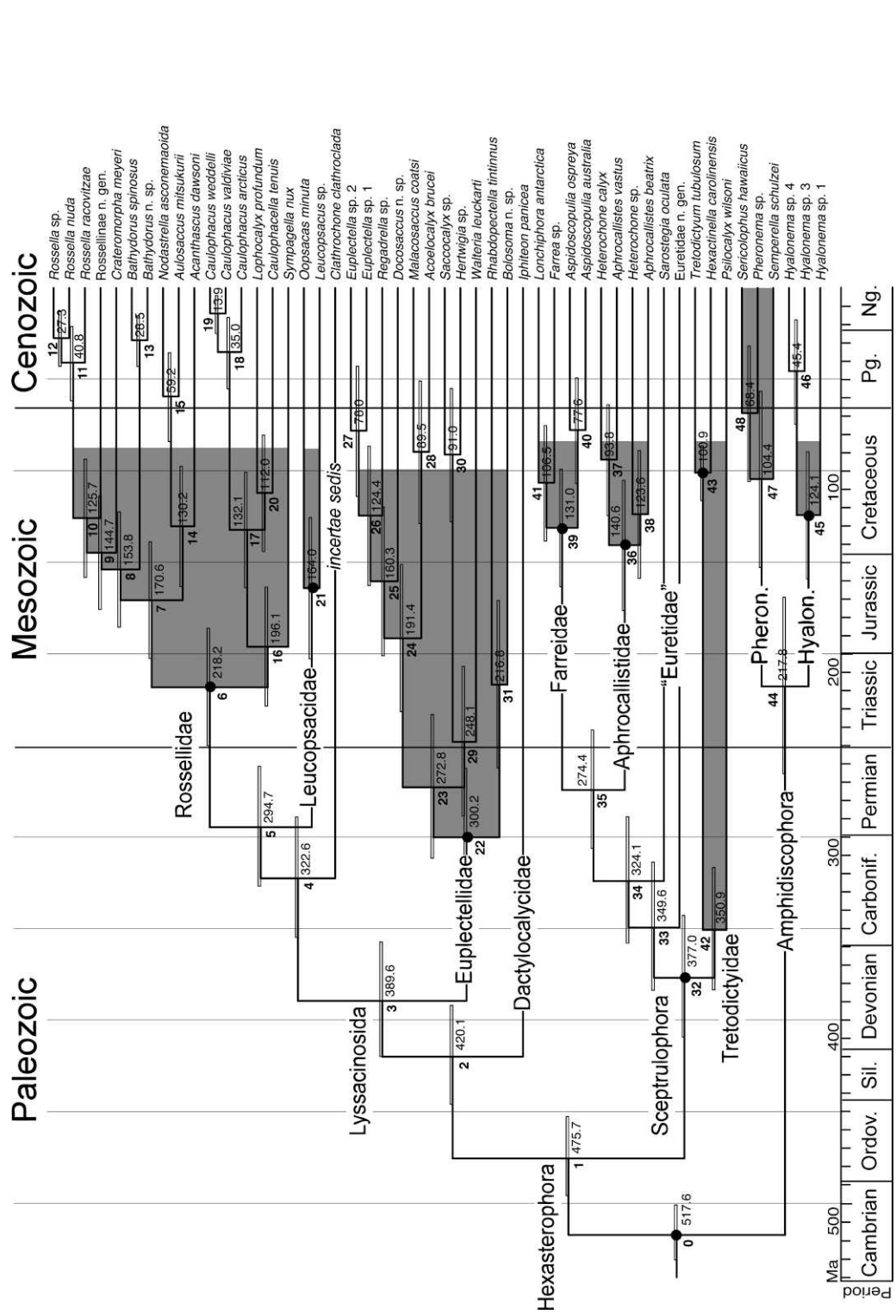

FIGURE 2. Time-calibrated molecular phylogeny of Hexactinellida plotted on stratigraphic chart. Calibration nodes indicated by black dots (see Fig. 1). Numbers on the right side of nodes are mean age estimates in million years ago (Ma); bold numbers on the left correspond to the node numbers in Supplementary Table 1 (first column). Gray areas illustrate the implied temporal extent of missing fossil records for the crown groups of the hexactinellid families (where "missing" refers to the absence of undisputed fossils; see text). Ordov., Ordovician; Sil., Silurian; Pg., Paleogene; Ng., Neogene.

(see Warnock et al. 2012). Therefore, we also used a maximum of 542 Ma (Ediacaran/Cambrian boundary) for the root age (see *Introduction* for justification). In preliminary analyses (results not shown) we also applied soft bounds (Yang and Rannala 2006) in order to relax this assumption. However, this still resulted in a Cambrian estimate for the root age while producing younger ages for many internal nodes. The reason for this is probably that all minimum constraints were also relaxed since the software we used for dating (PhyloBayes; see below) currently does not support application of soft bounds only to specific nodes or only to maximum constraints. As we have very little doubt about the validity of our minimum constraints, we therefore considered it more appropriate to use hard bounds for the final analysis.

2. The age of crown-group Sceptrulophora was constrained to be at least 237 Ma, according to the earliest unambiguous finds of sceptrules in the Middle Triassic (lower Ladinian) (Krainer and Mostler 1991). Although the Late Devonian dictyonal frameworks (see *Introduction*) were assigned to extant sceptrulophoran families by Rigby et al. (2001), this interpretation has to be viewed with caution (see *Results and Discussion*). These fossils might instead represent stem-group Sceptrulophora, so we did not use them to calibrate this node.
3. Aphrocallistidae, Farreidae, and Tretodictyidae (all Sceptrulophora), as well as Hyalonematidae (Amphidiscophora) are known with certainty from fossils that clearly exhibit crown-group morphology from the Late Cretaceous Campanian Stage (see, e.g., Schrammen 1912 for the sceptrulophoran families, and Mehl and Hauschke 1995 for Hyalonematidae). Thus, we assigned minimum ages of 83.5 Ma (base of the Campanian) to the crown nodes of these clades. However, in the case of the Tretodictyidae we assigned the constraint to the node that separates *Hexactinella carolinensis* and *Tretodictyum tubulosum*, because the third included species, *Psilocalyx wilsoni*, exhibits a rather peculiar morphology (Reiswig 2002b; Dohrmann et al. 2011; Reiswig and Kelly 2011) that differs from the Late Cretaceous fossils, which more closely resemble the other two genera (in fact, some of these fossils were actually assigned to *Hexactinella* or *Tretodictyum*, although non-preservation of microscleres makes this assignment rather speculative).
4. Crown-group members of the lyssacinosidan families Rossellidae and Leucopsacidae are known from bodily preserved fossils since the Late Cretaceous Coniacian Stage (Brückner and Janussen 2005; Brückner 2006), and the earliest unambiguous crown-euplectellid was described from the Cenomanian (Salomon 1990). Although several of these fossils were assigned to extant genera that are included in our molecular data set, the lack of microscleres renders these interpretations somewhat speculative (Brückner 2006). Thus, we assigned minimum ages of 89.3 Ma (Rossellidae, Leucopsacidae) and 99.6 Ma (Euplectellidae) to the crown nodes of these families in order to test if those generic assignments were consistent with the molecular age estimates.

Using these constraints, we re-estimated branch lengths of the ML tree topology (Fig. 1) from the sequence data in units of absolute time, employing the Bayesian Markov chain Monte Carlo (BMCMC) framework provided by the PhyloBayes (version 3.2f) package (Lartillot et al. 2009). Because the models used to infer the tree topology are not implemented in PhyloBayes, we used the CAT model (Lartillot and Philippe 2004) with GTR exchange rates (CAT-GTR), as the manual recommends for nucleotide data. Among-site rate variation was modeled with the Dirichlet process of Huelsenbeck and Suchard (2007), and among-lineage rate variation was accounted for by employing a log-normal autocorrelated relaxed molecular clock model (see Lepage et al. 2007 for details and justification). We ran two chains simultaneously (sampling every 1000th point) and checked for convergence using the *tracecomp* application of the PhyloBayes package. Chains

were stopped when minimum effective sizes/maximum discrepancies of model parameters had achieved values >100/<0.1, as recommended in the manual. Node age summary statistics were then extracted with *readdiv*, with the first 25% of sampled points discarded as burn-in. Additionally, node ages were extracted from each tree in the post burn-in sample using ETE, version 2.1 (Huerta-Cepas et al. 2010; script available in Supplementary Material); the posterior distribution of the node ages was then plotted in R (http://www.r-project.org/) and used to evaluate alternative hypotheses concerning the systematic position of a number of hexactinellid fossils of ambiguous taxonomic affinities. In brief, the assignment of a fossil to a clade on the chronogram constrains the age that fossil can have in order to be congruent with the chronogram. Thus, for every fossil assigned to a clade on the chronogram it is possible to evaluate whether the fossil's age lies within the 95% credibility interval (CrI) of the node's estimated age and to reject the assignment when it does not.

Results and Discussion

The BMCMC analyses took several weeks to reach convergence, and the two runs produced very similar results. We base our discussion on the chronogram shown in Figure 2, which is derived from the arbitrarily chosen "chain 1." The means, standard errors, and 95% CrIs of age estimates derived from both chains are summarized in Supplementary Table 1. For selected nodes discussed below, we also show histograms of the posterior distributions of estimated ages (Figs. 3–7).

The root age estimate of ca. 518 Ma (Cambrian Series 2) is consistent with the notion of a strong mid-Cambrian radiation of Hexactinellida (Mehl 1996; Krautter 2002). However, one has to bear in mind that we constricted the root age a priori to the Cambrian (see *Methods*), so the influence of the prior on this estimate might be strong (sampling only from the prior distribution gave a mean of ca. 516 Ma [results not shown]). Therefore, a more precise estimate of the age of crown-group Hexactinellida might require further study.

The overall distribution of nodes (i.e., cladogenetic events or splits) through time in our chronogram is broadly consistent with paleontological views on Phanerozoic hexactinellid diversification (see *Introduction*): a "deep" Paleozoic radiation (13 splits) followed by a peak Mesozoic diversification (29 splits) and a marked Cenozoic decline (only seven splits, which are confined to two families). Age estimates for the origin of most families' crown groups are considerably older than unambiguous fossil evidence suggests (gray areas in Fig. 2); however, the estimated age ranges in many cases are consistent with more contentious assignment of older fossils to the respective families, as discussed in more detail below.

One striking result of our analysis is the huge difference in the estimated ages of the crown groups of the two subclasses: whereas the Hexasterophora had already radiated some 40 Myr after the origin of their stem lineage, the Amphidiscophora crown radiation was delayed until the Late Triassic, suggesting the extinction of deeply branching amphidiscophoran lineages. In contrast, our results suggest an Early Ordovician origin of crown-group Hexasterophora, in good congruence with the first appearance of hexasters in the fossil record (Mostler 1986; Mehl 1996). Intriguingly, paleontological data indicate that major transitions in siliceous sponge morphology and ecology occurred during that time, including colonization of nearshore siliciclastic settings by hexactinellids (Carrera and Botting 2008). Thus, our estimate suggests that the early crown-group diversification of Hexasterophora might have been connected to these events.

Because Sceptrulophora is the sister group to all other Hexasterophora, the crown-group origin of this subclass coincides with the origin of the Sceptrulophora stem-group. Although the mean age estimate of ca. 476 Ma (Floian; late Early Ordovician) post-dates Paibian (late Cambrian) and Tremadocian (earliest Ordovician) fragmentary microfossils interpreted as scopules (Dong and Knoll 1996; Kozur et al. 1996), these stages lie within the 95% CrI (Fig. 3, Supplementary Table 1). Thus, we cannot reject the hypothesis that these

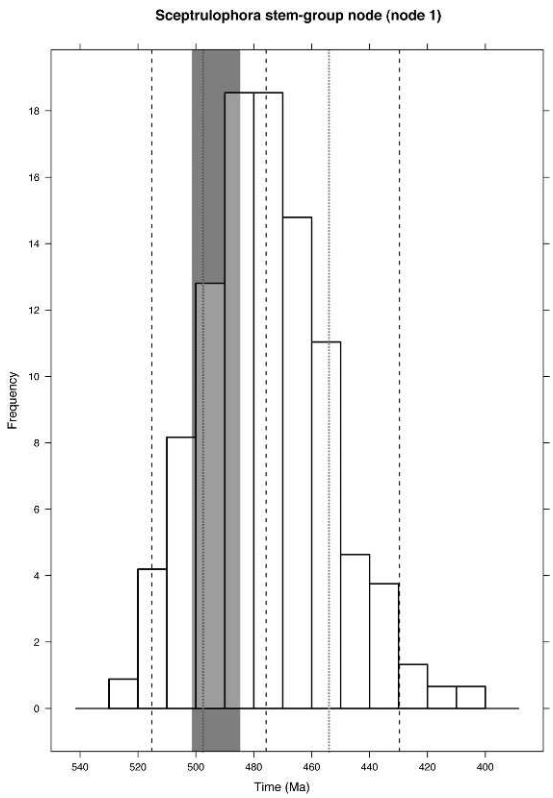

FIGURE 3. Frequency distribution for the posterior age estimate of the stem-group node of Sceptrulophora (= crown-group node of Hexasterophora). Dashed lines, mean and 95% Bayesian credibility interval (CrI); dotted lines, standard error (SE). The shaded area indicates the time window from which putative Paleozoic scopules have been reported. See text for further explanation.

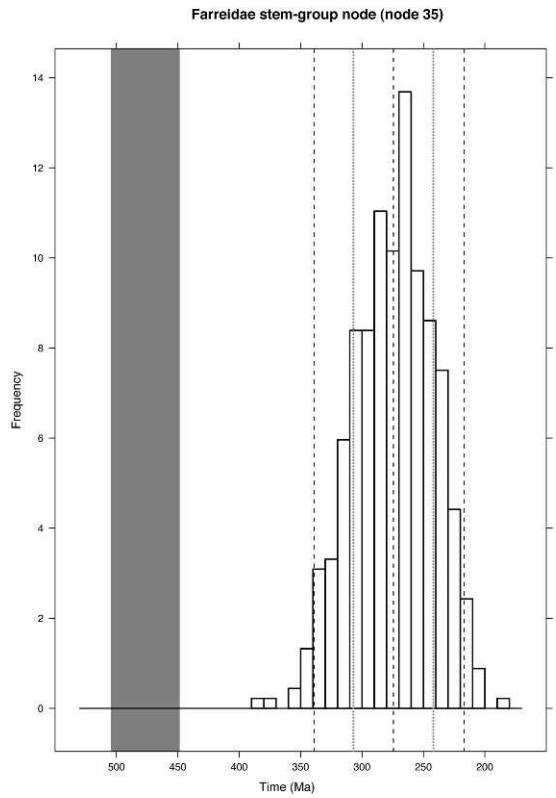

FIGURE 4. Frequency distribution for the posterior age estimate of the stem-group node of Farreidae. Dashed lines, mean and 95% CrI; dotted lines, SE. The shaded area indicates the time window from which putative Paleozoic clavules have been reported. See text for further explanation.

spicules really came from early stem-group sceptrulophorans. In contrast, our results clearly reject an alleged early Paleozoic occurrence of clavules (e.g., Bengtson 1986; Webby and Trotter 1993; Dong and Knoll 1996; Kozur et al. 1996; Zhang and Pratt 2000): these spicules are restricted to the Farreidae (see Dohrmann et al. 2011), and our results suggest a late Paleozoic origin of this clade (ca. 343 Ma at most; Fig. 4, Supplementary Table 1). We therefore suggest that at least the Paleozoic "clavules," which are in fact morphologically rather different from those of extant Farreidae, either represent different spicule types, such as anchorate basalia, or are convergently evolved spicules unrelated to the modern forms ("paraclavules").

Our mean estimate of the age of crown-group Sceptrulophora (377 Ma; Frasnian) almost perfectly matches the age of the oldest known dictyonal frameworks (ca. 380 Ma [Rigby et al. 2001]), suggesting that their worldwide appearance in Late Devonian strata was the result of extensive radiations that gave rise to the modern sceptrulophoran lineages. Although assignment of some of these fossils to the Euretidae (Rigby et al. 2001) is incompatible with our mean estimate of ca. 350 Ma (Early Mississippian) for the origin of the lineage leading to Euretidae n. gen., their age lies within the standard error bounds for that node (Supplementary Table 1). However, because monophyly of Euretidae is questionable from a morphological point of view and currently not resolved by molecular data (see Dohrmann et al. 2011), one should be cautious

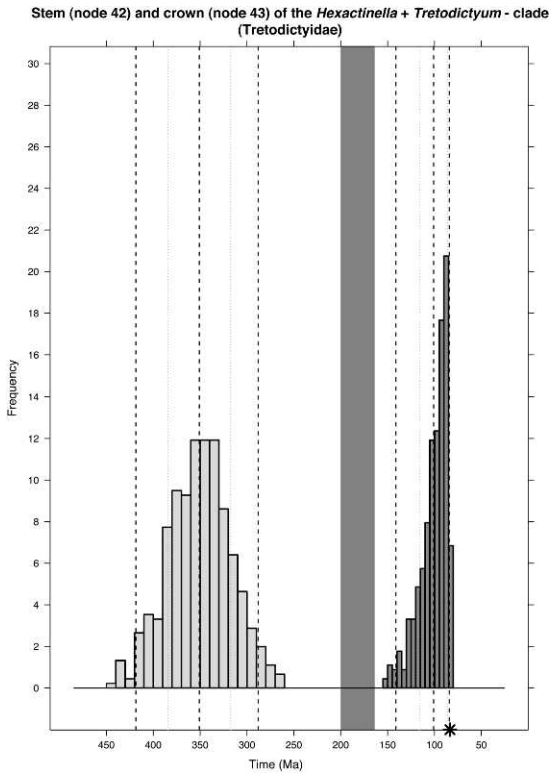

FIGURE 5. Frequency distribution for the stem (light gray histogram) and crown (dark gray histogram) posterior node age estimates of the *Hexactinella* + *Tretodictyum* – clade (Tretodictyidae). Dashed lines, mean and 95% CrI; dotted lines, SE. The shaded area indicates the time window from which putative Jurassic members of this clade have been reported; the star on the time line indicates the calibration constraint (≥83.5 Ma). See text for further explanation.

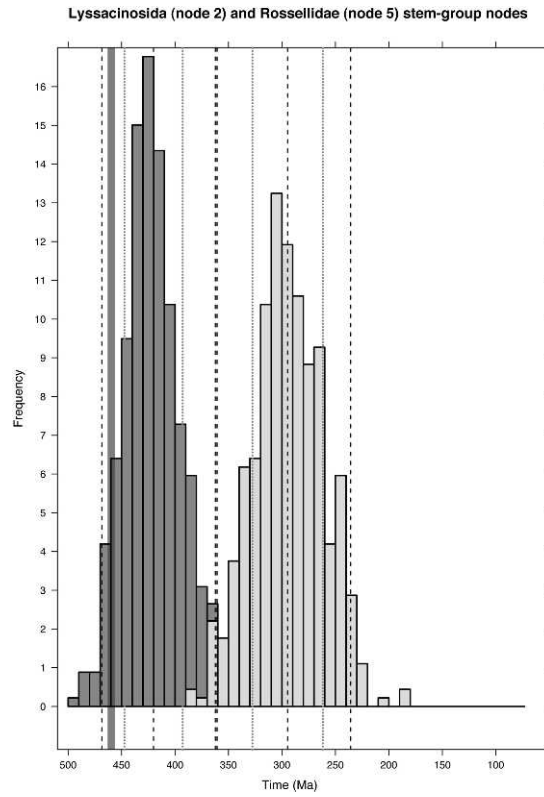

FIGURE 6. Frequency distribution for the posterior age estimates of the stem-group nodes of Lyssacinosida (dark gray histogram) and Rossellidae (light gray histogram), respectively. Dashed lines, mean and 95% CrI; dotted lines, SE. The shaded area indicates the approximate age of putative Ordovician stem-group rossellids. See text for further explanation.

to "shoe-horn" fossils of simple dictyonal skeletons into that family. According to our results, extensive cladogenesis within Sceptrulophora also occurred during the Carboniferous and Permian, which is intriguing because neither dictyonal skeletons nor sceptrules are documented from these periods (Mehl 1996). Thus, our results suggest that the early radiation of crown-Sceptrulophora continued in habitats that either were cryptic (Mehl 1996) or are simply not preserved due to a bias toward terrestrial sedimentary outcrops from the late Paleozoic (Smith and McGowan 2007).

Crown-Tretodictyidae are estimated to date back to the Early Mississippian (ca. 351 Ma), which is considerably older than the first unambiguous records of this family from the Late Cretaceous. This result might suggest that the monospecific genus *Psilocalyx* belongs to a very ancient lineage of Tretodictyidae. However, no fossil record of this genus is known, and inclusion of additional tretodictyid genera, which might break up the long branch leading to *Psilocalyx* in the molecular phylogeny, will be required to further test this hypothesis. In contrast, the split between *Hexactinella* and *Tretodictyum*, which we used for calibration (see *Methods*), is estimated to be only ~17 Myr older (Albian; late Early Cretaceous) than those fossils (e.g., Schrammen 1912). This is inconsistent with assignment of putative tretodictyids from the Early (Mostler 1990) and Middle (Mehl and Fürsich 1997) Jurassic to these genera, rather suggesting a stem-lineage membership of the *Hex-*

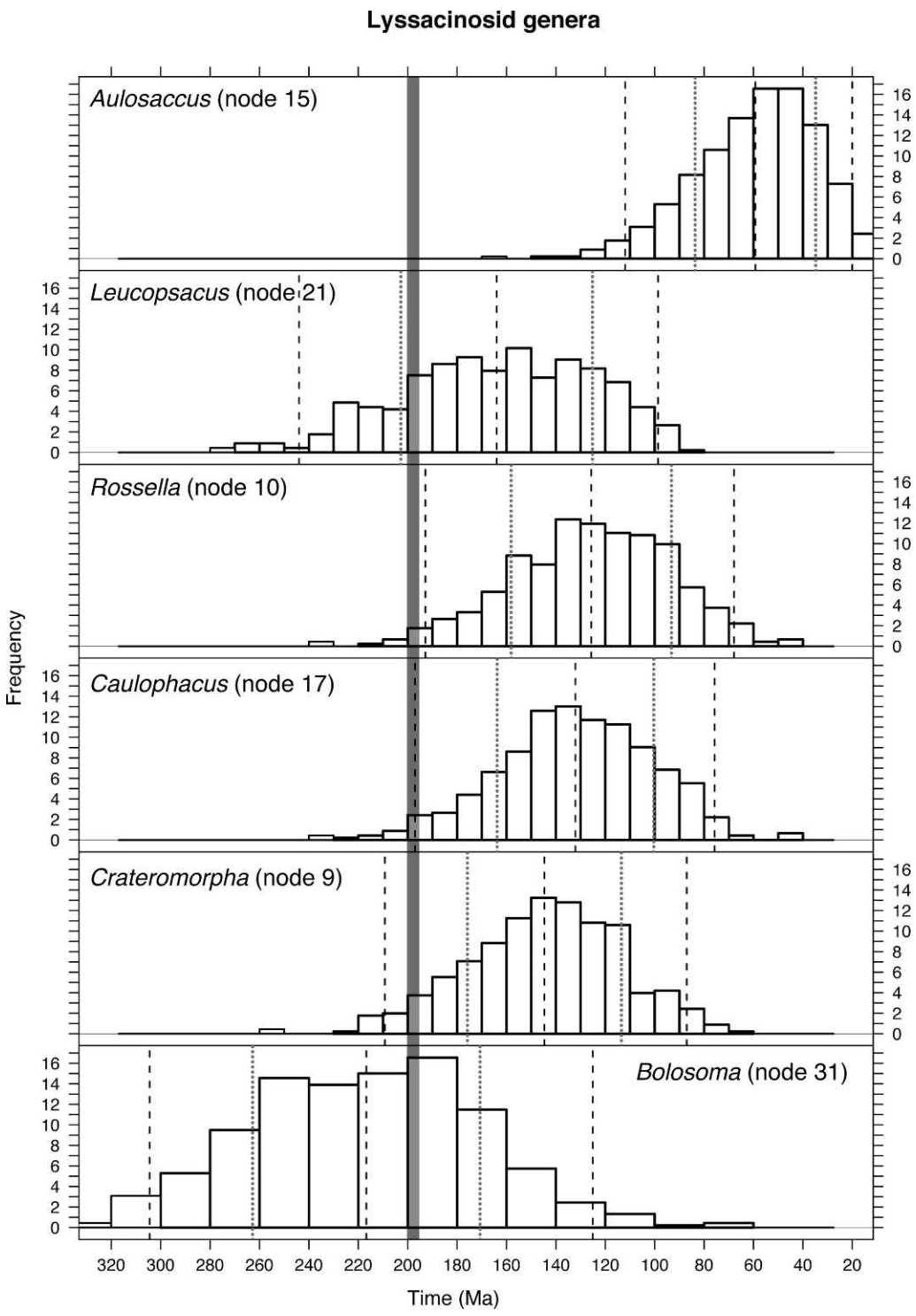

FIGURE 7. Frequency distribution for the posterior node age estimates of several lyssacinosid genera. Dashed lines, mean and 95% CrI; dotted lines, SE. The shaded area indicates the approximate age of Early Jurassic isolated microscleres attributed to these taxa. See text for further explanation.

actinella + *Tretodictyum* clade (Fig. 5). In contrast to the Tretodictyidae, the crown groups of Farreidae and Aphrocallistidae are estimated to have originated much later, in the Early Cretaceous, although their stem lineages already separated in the mid-Permian. Farreoid skeletons and clavules have been reported as early as the Middle Triassic (Donofrio 1991; Krainer and Mostler 1991; Mehl and Mostler 1993), so our results suggest that these autapomorphies (see Dohrmann et al. 2011) evolved very early in the farreid stem lineage. Other than that, it is unclear at present which pre-Cretaceous fossil taxa might be good candidates for stem-group Farreidae and Aphrocallistidae.

Speculations about Ordovician stem-group Rossellidae (Botting 2004) are clearly incompatible with our chronogram, because we estimated an Early Permian origin for this lineage (Late Devonian at most; Fig. 6, Supplementary Table 1). Instead, according to our results the sponges described by Botting (2004) fall on the lineage leading to a late Silurian (ca. 420 Ma) Dactylocalycidae/Lyssacinosida split (assuming they are indeed hexasterophorans). Because these fossils show a lyssacine skeletal organization, this supports the hypothesis that dictyonal frameworks of Dactylocalycidae are not homologous to those of Sceptrulophora but evolved independently from a lyssacine condition. However, the CrIs for this node include the age of Botting's (2004) fossils (Fig. 6, Supplementary Table 1), so we cannot completely rule out that they were stem-group Lyssacinosida. The inferred Silurian age of Lyssacinosida also further discourages classification of older lyssacine hexactinellids in this order (see *Introduction*).

According to our estimate, Euplectellidae diverged from its sister lineage by the Middle Devonian (ca. 390 Ma), followed by crown-group radiation around the Carboniferous/Permian boundary (ca. 300 Ma). This is much older than, and therefore consistent with, assignment of Early Triassic (Rigby and Gosney 1983) and Early Jurassic (du Dresnay et al. 1978) fossils to the Euplectellidae, although these interpretations have been questioned (Pisera and Bodzioch 1991).

Our results are also consistent with assignment of Early Jurassic isolated spicules to the Recent families of Lyssacinosida (Mostler 1989, 1990), although the claim that extant genera were already present at that time should be viewed with caution. Mostler (1989, 1990) lists the following: *Aulosaccus*, *Caulophacus*, *Crateromorpha*, *Rossella* (Rossellidae), *Leucopsacus* (Leucopsacidae), and *Bolosoma* (Euplectellidae). Among these, only *Bolosoma* and *Leucopsacus* could have been present in the Early Jurassic with reasonable certainty if our results are accurate (Fig. 7, Supplementary Table 1). In contrast, although the upper bounds of the CrIs for the stem nodes of *Caulophacus*, *Crateromorpha*, and *Rossella* reach back to ca. 200 Ma, the rossellid genera are estimated to be younger (Fig. 7, Supplementary Table 1). However, even in the case of *Bolosoma*, increased taxon sampling might break up the long branch leading to the single species sampled here and therefore lead to much younger estimates. Nonetheless, the hypothesis that the spicules reported by Mostler came from crown-group members of the extant lyssacinosidan families is corroborated by our study.

Finally, although the classification of several Coniacian (Late Cretaceous) fossils from Denmark (Brückner and Janussen 2005; Brückner 2006) in extant genera is somewhat tentative due to non-preservation of microscleres (Brückner 2006), our results are consistent with assignments to *Rossella* (Rossellidae), *Regadrella*, *Docosaccus*, and *Acoelocalyx* (Euplectellidae). Interestingly, Rossellidae is the only hexasterophoran family that shows post-Cretaceous cladogenesis in our chronogram. This might indicate that large parts of the diversity of this most speciose hexactinellid family are the product of relatively recent radiations, in contrast to other taxa. However, increased taxon sampling among the remaining families might reveal a more homogeneous pattern of diversification across the Hexactinellida.

Conclusions

By integrating molecular and fossil data, we have further illuminated the evolutionary history of Hexactinellida, a group of non-

bilaterian animals that contributed significantly to benthic communities, including reefs, throughout the Phanerozoic. Although our results corroborate some attempts by paleontologists to classify hexactinellid fossils, especially from the Mesozoic record, within Recent taxa, in other cases we could reject some rather speculative hypotheses of systematic affinities by using probability distributions of molecular node age estimates for the respective clades. This demonstrates how molecular chronograms can help narrow down the possibilities in the face of ambiguously interpretable fossils, which is particularly relevant in studies of taxonomically difficult groups (see also Waggoner and Collins 2004; Peterson et al. 2008). On the other hand, our dated phylogeny revealed extensive periods of missing fossil records for many clades, thereby providing a framework for more targeted efforts by paleontologists to recover older fossils of the respective taxa. We hope this work will stimulate future paleobiological research and reevaluation of the hexactinellid fossil record, and also encourage researchers working on other non-bilaterian animal groups to apply molecular paleobiological approaches.

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