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Early divergence dates of demosponges based on mitogenomics and evaluated fossil calibrations

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Abstract

Demosponges are among the most primitive biomineralized metazoans to appear first in the fossil record with hard skeletons; their confirmed earliest fossils are from the lower Cambrian rocks about 520 Ma, with putative demosponge biomarkers reported from 713 to 635 Ma sediments. In this study, we use mitogenomic data to approach the early divergence timescale of demosponges using relaxed molecular clock techniques and likelihood-evaluated fossil calibration strategies. We found that among various molecular dating models, the correlated rate model yielded time estimates of demosponges in this analysis which is most congruent with the fossil appearance dates of demosponges. Our dating analyses show that crown groups of Demospongiae appeared at about 704 (674–741) Ma, and the silicification in demosponges (divergence of spicular sponges) began about 633 (616–648) Ma indicating a gap of over 100 million years between the origin of silicification and their first unequivocal appearance of siliceous spicules in the fossil record (520–525 Ma); demosponges with tetraxon-type spicules (Tetractinellida) are dated here at about 514 (498–530) Ma, an estimate comparable with the earliest tetraxial megasclere fossil records (510–520 Ma, Ordian Age, middle Cambrian).

Keywords: Demospongiae; Mitochondrial genes; Relaxed clock; Silicification origination

1. Introduction

Recent molecular studies have contributed substantially to the understanding of phylogenetic relationships among sponges; they also raised additional questions; e.g., Are the sponges monophyletic (Dohrmann et al., 2008; Philippe et al., 2009; Pick et al., 2010; Nosenko et al., 2013) or paraphyletic with Calcarea closer to Eumetazoan than other sponges (Borchiellini et al., 2001; Sperling et al., 2007, 2009; Erwin et al., 2011; Mallatt et al., 2012)? Is the subclass Homoscleromorpha more closely related with Calcarea (Dohrmann et al., 2008; Philippe et al., 2009; Gazave et al., 2010; Erwin et al., 2011) or Eumetazoa (Sperling et al., 2007; Hejnol et al., 2009), forming the fourth class of Phylum Porifera (Gazave et al., 2012)? The phylogeny of sponges is critical to understanding the origin of metazoans.

Demospongiae is the most diverse group among Porifera; the earliest demosponge fossils with siliceous skeletons have been documented in the lower Cambrian (Rigby and Collins, 2004; Xiao et al., 2005), although questionable demosponge-related fossils were reported from the Neoproterozoic Doushantuo Formation (ca. 580 Ma) in South China (Li et al., 1998) and demosponge biomarkers were reported in Cryogenian strata in Oman (713–635 Ma and later sediments) (Bowring et al., 2007; Love et al., 2009), which was considered to be accordant with molecular dating (Sperling et al., 2010). Considering the huge gap of about 150–200 million years between the earliest biomarkers and unquestionable fossil sponges (mainly archaeocyaths) found in the lower Cambrian Tommotian Age (540–535 Ma; Riding and Zhuravlev, 1995), Sperling et al. (2010) provided the following possible scenarios: (1) the first appearance of spicules signifies the origination of the demosponges during the early Cambrian, and the biomarkers found in Precambrian, while the molecular divergence dates are inaccurate and supposedly demosponge-related were likely related

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with non-demosponge organisms; (2) the Silicea, including the hexactinellids and the spicule-bearing demosponges, is a monophyletic group, both derived from the aspiculate Keratosa possibly rooted deep in the Precambrian as molecular divergence estimates and biomarkers indicate; (3) due to taphonomic bias, siliceous sponges are not preserved in Precambrian rocks.

Mitochondrial genomes of Porifera, like Cnidaria, are characterized by exceptionally slow evolution, especially for Demospongiae and Homoscleromorpha (Lavrov, 2007; Wang and Lavrov, 2008), suitable for phylogenetic analyses and divergence dating (Lavrov et al., 2008; Erpenbeck et al., 2009), although some lineages experienced relatively faster rate such as Hexactinellida and Calcarea (Haen et al., 2007, 2014; Rosengarten et al., 2008; Lavrov et al., 2013). In this study, we designed an approach to estimate the origination of biomineralization in demosponges in a phylochronological framework on the basis of mitogenomics and carefully evaluated fossil calibration.

Because the fossil record is generally incomplete and biased among different organism groups, the early divergence time of an organism group can be inferred by applying molecular clock analysis in the context of the geological timescale and the early fossil record (Ayala et al., 1998; Peterson et al., 2004, 2008; Peterson and Butterfield, 2005; Yang et al., 2007). By phylochronological analysis, we trace the evolutionary history of major demosponge lineages on the basis of their spicules fossil record and molecular-based phylogenetic timescale. In this study, we adopted the molecular phylogeny of spiculate and aspiculate demosponges to identify the important evolutionary event in the history of early silicification in Demospongiae on the basis of the mitochondrial gene dataset.

2. Materials and method

2.1. Sequence

Mitochondrial genomes of 24 demosponges, 2 homoscleromorphs, 11 eumetazoans (including 9 cnidarians and 2 arthopods), 1 hexactinellid, and 1 outgroup (choanoflagellate *Monosiga brevicollis*) were downloaded from Genbank for the phylogenetic analyses of this study (Table 1). Nucleotide sequences of the 12 coding genes are translated into protein sequences and each is individually aligned using the MEGA v4.0 (Tamura et al., 2007). Nucleotide sequences are subsequently aligned matching the amino acid (AA) sequence alignment. The 12 alignments are concatenated into a single matrix with 11526 nucleotide sites (Supplementary datafile).

2.2. Fossil calibration dates

Based on their morphological characters and previous published data, nine fossil/geological dates are used for calibration in our divergence time estimation (Fig. 1, Table 2). The root age prior, representing the divergence of Choanoflagellida-Metazoa, is set to 1000 Ma, which is a quite conservative maximum constraint as no true metazoan fossils (body, trace or biomarkers) have been confirmed to occur in older sediments; this date is also broadly compatible with previous phylochronological estimates, such as those in Peterson et al. (2008) and Sperling et al. (2010). In order to test the effects of root age prior, 1200 and 1500 Ma were also used in the dating analysis.

The earliest monaxial demosponge spicules are found in the lower Cambrian Atdabanian strata worldwide (Bengtson et al., 1990; Gruber and Reitner, 1991; Rozanov and Zhuravlev, 1992; Zhang and Pratt, 1994; Xiao et al., 2005; Carrera and Botting, 2008). According to Xiao et al. (2005), the lower Cambrian monaxial sponges found in Anhui and Yunnan, South China, may be dated to 520–535 Ma. For calibrating the demosponge tree in this study, we use the minimum age of 520 Ma as the first appearance of monaxial demosponges. Because biomineralized animals have not been found before the Ediacaran Period

Table 1
Species and genomes used in this study.

Species	Classification	GenBank Accession#
Agelas schmidti	Agelasida	EU237475
Ampphimedon compressa	Haplosclerida/Haplosclerina	EU237474
Amphimendon queenslandica	Haplosclerida/Haplosclerina	NC_008944
Aplysina fulva	Verongida	EU237476
Axinella corrugate	Halichondrida	NC_006894
Callyspongia plicifera	Haplosclerida/Haplosclerina	EU237477
Chondrilla aff. *nucula*	Chondrosida	EU237478
Cinachyrella kuekenthali	Spirophorida	EU237479
Ectyopla siaferox	Poecilosclerida/Microcionina	EU237480
Ephydatia muelleri	Haplosclerida/Spongillina	EU237481
Geodia neptuni	Astrophorida	NC_006990
Halisarca dujardini	Halisarcida	EU237483
Hippospongia lachne	Dictyoceratida	EU237484
Hyattella sinuosa	Dictyoceratida	JX535019
Ircinia sp.	Dictyoceratida	KC510273
Ircinia sp.	Dictyoceratida	KC510274
Ircinia strobilina	Dictyoceratida	GQ337013
Igernella notabillis	Dendroceratida	EU237485
Iotrochota birotulata	Poecilosclerida/Myxillina	EU237486
Ptilocaulis walpersi	Halichondrosida	EU237488
Tethya actiraphidites	Hadromerida	NC_006991
Topsentia ophiraphidites	Halichondrida	EU237482
Oscarella carmela	Homoscleromorpha	NC_009090
Plakortis angulospiculatus	Homoscleromorpha	EU237487
Vaceletia sp.	Verticillitida	EU237489
Xestospongia muta	Haplosclerida/Petrosian	EU237490
Aphrocallistes vastus	Hexactinellida; Hexactinosida	NC_010769
Astrangia sp.	Cnidaria; Sclleractinia	NC_008161
Montastraeaan nularis	Cnidaria; Sclleractinia	NC_007224
Acropora tenuis	Cnidaria; Sclleractinia	NC_003522
Agaricia humilis	Cnidaria; Sclleractinia	NC_008160
Pavona clavus	Cnidaria; Sclleractinia	NC_008165
Savalia savaglia	Cnidaria; Zoanthidea	NC_008827
Calicogorgia granulosa	Cnidaria; Holaxonia	GU047880
Hydra oligactis	Cnidaria; Hydroida	NC_010214
Aurelia aurita	Cnidaria; Semaeostomeae	NC_008446
Anopheles darlingi	Arthopoda; Diptera; Nematocera	NC_014275
Drosophila littoralis	Arthopoda; Diptera; Brachycera	NC_011596
Monosiga brevicollis	Choanoflagellida; Codonosigidae	AF538053