Glass sponges (Hexactinellida) are a group of deep-water benthic animals that have a unique syncytial organization and possess a characteristic siliceous skeleton. Although hexactinellids are traditionally grouped with calcareous and demosponges in the phylum Porifera, the monophyly of sponges and the phylogenetic position of the Hexactinellida remain contentious. We determined and analyzed the nearly complete mitochondrial genome sequences of the hexactinellid sponges *Iphiteon panicea* and *Sympagella nux*. Unexpectedly, our analysis revealed several mitochondrial genomic features shared between glass sponges and bilaterian animals, including an Arg → Ser change in the genetic code, a characteristic secondary structure of one of the serine tRNAs, highly derived tRNA and rRNA genes, and the presence of a single large noncoding region. At the same time, glass sponge mtDNA contains *atp9*, a gene previously found only in the mtDNA of demosponges (among animals), and encodes a tRNA^Pro_{157}^ with an atypical A11–U24 pair that is also found in demosponges and placozoans. Most of our sequence-based phylogenetic analyses place Hexactinellida as the sister group to the Bilateria; however, these results are suspect given accelerated rates of mitochondrial sequence evolution in these groups. Thus, it remains an open question whether shared mitochondrial genomic features in glass sponges and bilaterian animals reflect their close phylogenetic affinity or provide a remarkable example of parallel evolution.

**Introduction**

Glass sponges (class Hexactinellida) are an exclusively marine and predominantly deep-water group of animals that contains about 500 described extant species and plays an important role in benthic communities. The group is defined by the presence of siliceous spicules of triaxonic (cubic) symmetry or their derivatives that are often fused to form a rigid framework (Reiswig 2002) and sometimes display remarkable optical characteristics (Sundar et al. 2003; Aizenberg et al. 2004). In addition to their characteristic spicules, hexactinellids differ from other sponges in their unique syncytial organization (reviewed in Leys [2003]) and their unusual capability of impulse conduction (Lawn, Mackie, and Silver 1981; Mackie, Lawn, and Pavans de Ceccatty 1983; Leys, Mackie, and Meech 1999).

Although glass sponges have been traditionally placed with calcareous and demosponges in the phylum Porifera (Thomson 1869; Schmidt 1870), this association is based on the overall morphological similarity of sponges rather than any specific synapomorphies (Hooper, Van Soest, and Debrenne 2002). Furthermore, most sequence-based phylogenetic studies fail to recover Porifera as a monophyletic group (Collins 1998; Kruse et al. 1998; Adams, McInerney, and Kelly 1999; Borchelli et al. 2001; Medina et al. 2001; Rokas, Kruger, and Carroll 2005; Müller, Müller, and Schröder 2006). Some of these studies place the Hexactinellida as a sister group to the rest of the Metazoa (Kruse et al. 1998; Borchelli et al. 2001; Müller, Müller, and Schröder 2006), whereas others support their affinity with the Demospongiae, but exclude Calcarea from the group (Adams, McInerney, and Kelly 1999; Medina et al. 2001). However, statistical support for these alternative hypotheses of poriferan relationships is generally low, and a recent attempt to use multiple genes to elucidate animal phylogeny found no resolution for the relationships among the 3 classes of sponges and other nonbilaterian animals (Rokas, Kruger, and Carroll 2005).

Comparisons of mitochondrial genomes have been useful for investigating ancient animal relationships (Boore, Lavrov, and Brown 1998; Reyes et al. 2004; Lavrov and Lang 2005). In addition to the large amount of sequence data, which minimizes sampling error in sequence-based phylogenetic analysis, mtDNA harbors additional rare genomic characters that are valuable for phylogenetic inference, including indels in the coding sequences, variations in the genetic code, changes in the secondary structures of encoded transfer and ribosomal RNAs, and gene rearrangements (Rokas and Holland 2000). To gain a better understanding of the phylogenetic position of the Hexactinellida and to fill a gap in our sampling of animal mitochondrial genomes, we determined the nearly complete mitochondrial genome sequences of 2 glass sponges, *Iphiteon panicea* and *Sympagella nux*. Here, we describe these genomes and analyze their features in relationship to the phylogenetic position of glass sponges and animal mitochondrial evolution.

**Materials and Methods**

**Specimen Collection, DNA Extraction, Amplification, Cloning, and Sequencing**

Specimens of *I. panicea* (Bowerbank, 1869) (Class Hexactinellida: Order Hexactinosida: Family Dactylocalyceidae) and *S. nux* (Schmidt 1870) (Class Hexactinellida: Order Lyssacinosida: Family Rossellidae) were collected by the “Johnson-Sea-Link” manned submersible near Turks & Caicos and processed as described in Adams et al. (1999). Small fragments of *cox1*, *cox2*, *cox3*, and *rnl* were amplified from total DNA using sponge-specific primers, checked against the GenBank database to minimize the possibility of contamination, and used to design specific primers for these regions (table 1). Complete mtDNA
of I. panicea and partial mtDNA of S. nux mtDNA were amplified in overlapping fragments using the Takara LA-PCR kit. Regions of the S. nux mtDNA upstream of coxl and downstream of cox3 were amplified using a modified step-out polymerase chain reaction (PCR) approach (Wesley UV and Wesley CS 1997).

Random clone libraries were constructed from purified PCR products by nebulating them into fragments 1–3 kbp in size and cloning them into the pCR 4Blunt-TOPO vector (Invitrogen, Carlsbad, CA) as described previously (Wang and Lavrov 2007), sequenced at the Iowa State University DNA Facility on an ABI 3730 DNA Analyzer, and assembled in local databases using the FASTA program (Pearson by comparisons between the 2 genomes, similarity searches in both mtDNAs. The arrangements of protein and rRNA genes within the mitochondrial genome.

Results

Mitochondrial Genome Organization Is Similar in Glass Sponges and Bilaterian Animals

The sequenced portions of the mitochondrial genomes of I. panicea and S. nux are 19.0 and 16.3 kb in size and contain 37 and 35 genes, including 13 and 12 protein-coding genes, 2 rRNA genes, and 22 and 20 tRNA genes, respectively (fig. 1). Identified protein-coding genes include all those typical for bilaterian animals with the exception of atp8 (and nad6 in S. nux), as well as the gene for subunit 9 of adenosine triphosphate (ATP) synthase (atp9), previously found only in the mtDNA of demosponges among animals. Identified mt-tRNA genes include only those typical for bilaterian animals; none of the additional mt-tRNAs identified for I. panicea and S. nux are present in demosponges and the placozoan T. adhaerens.

A concatenated alignment of protein-coding genes was prepared as described previously (Lavrov et al. 2005). Searches for the maximum likelihood (ML) topologies were performed with the TreeFinder program (Jobb, von Haeseler, and Strimmer 2004) using the mtArt + G (Abascal, Posada, and Zardoya 2006), cpREV + G (Adachi et al. 2000), mtREV + G (Adachi and Hasegawa 1996), and JTT + G (Jones, Taylor, and Thornton 1992) models of amino acid substitutions and with the RAxML program (Stamatakis 2006) using the cpREV + F + G model. Bayesian inferences were conducted with the MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and PhyloBayes 2.1c programs. For the MrBayes analysis, we used the cpREV model of amino acid substitutions with Γ4 + I distributed rates and conducted 2 simultaneous runs, each with 4 Markov chain Monte Carlo chains, for 1,100,000 generations. Trees were sampled every 1,000th cycle after the 1st 100,000 burn-in cycles. The results of the 2 runs were compared with the AWTY program (Wilgenbusch, Warren, and Swoford 2004). For the PhyloBayes analysis, we used the CAT + Γ model and ran 3 chains for 35,000 cycles. Trees were sampled every 10th cycle after the 1st 5000 burn-in cycles (the maximum difference in the frequency of bipartitions between the 2 runs was 0.03). Likelihood-based tests of alternative topologies were conducted as follows: ML branch lengths of alternative topologies were calculated with Tree-Puzzle (Schmidt et al. 2002) using the mtREV24 + F + Γ model of amino acid substitutions; log-likelihood values for individual sites in the alignment were computed with Codeml (Yang 1997) using the cpREV + F + Γ substitution model; and the probability values for different likelihood-based tests were determined with Consel (Shimodaira and Hasegawa 2001).
coding genes in the 2 genomes are identical with the exception of the position of \textit{cox2} and the absence of the \textit{nad6} gene in \textit{S. nux} (fig. 1). In contrast, only 6 of the tRNA genes share the same relative position in the 2 genomes. A maximum of 5 gene boundaries are shared with the demosponge \textit{G. neptuni}: 3 of these gene boundaries (+\textit{nad2}+\textit{nad5}, +\textit{atp6}+\textit{cox3}, and +\textit{nad3}+\textit{trnR}) are well conserved in the mitochondrial DNA of demosponges and cnidarians, and, except for the +\textit{nad2}+\textit{nad5} boundary, have also been reconstructed in ancestral bilaterian mtDNA (Lavrov and Lang 2005).

The A + T content of \textit{I. panicea} and \textit{S. nux} is 65.3\% and 70.4\%, respectively, which is within the range of other animals and similar to that of demosponges. The coding strand of \textit{I. panicea} mtDNA has an AT skew of 0.36 and a GC skew of −0.36. In \textit{S. nux}, the AT skew is 0.19 and the GC skew is −0.28. The polarity of each skew holds true regardless of the gene type analyzed: protein, tRNA, and rRNA (table 2). However, the AT-skew is reversed at the 2nd codon position in coding sequences, consistent with the fact that most codons specifying nonpolar amino acids, which are prevalent in mitochondrial (and other transmembrane) proteins, have thymidine (uridine) at the 2nd position (table 2). The compositional biases observed in the coding strands of glass sponge mtDNA are similar to those of many bilaterian animals rather than demosponges, cnidarians, and the choanoflagellate \textit{M. brevicolis}, in which coding strands have an overall positive GC skew and negative AT skew (Beagley, Okimoto, and Wolstenholme 1998; Burger et al. 2003; Lavrov et al. 2005; Dellaporta et al. 2006).

Although most genes in hexactinellid DNA are compactly arrayed, a single large noncoding region is present in both genomes downstream of \textit{nad4}. This region has been only partially sequenced and contains at least 645 bp (41.3\%) and 348 bp (33.3\%) of the noncoding nucleotides in \textit{I. panicea} and \textit{S. nux} mtDNA, respectively. Like in many bilaterian animals, this large noncoding region includes multiple repeated sequences and was problematic for PCR amplification, cloning, and sequencing. Other noncoding regions are substantially smaller, and several genes in \textit{S. nux} mtDNA appear to overlap. Thus, with the exception of \textit{atp9}, hexactinellid mtDNA resembles the mtDNA of bilaterian animals in gene content, nucleotide composition, and general organization of mtDNA. A more detailed analysis revealed several specific mitochondrial features that are potentially informative for understanding the phylogenetic position of glass sponges.

### Table 2

<table>
<thead>
<tr>
<th>Coding Sequences</th>
<th>Total (bp)</th>
<th>1st Positions</th>
<th>2nd Positions</th>
<th>3rd Positions</th>
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<th>tRNA Genes</th>
<th>Intergenic Regions</th>
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<td>11,337</td>
<td>11,087</td>
<td>11,337</td>
<td>11,087</td>
<td>11,087</td>
<td>11,087</td>
</tr>
<tr>
<td>\textit{S. nux} (SN)</td>
<td>11,337</td>
<td>11,087</td>
<td>11,087</td>
<td>11,087</td>
<td>11,087</td>
<td>11,087</td>
<td>11,087</td>
</tr>
</tbody>
</table>

**Fig. 1.**—Gene maps of hexactinellid mtDNAs. Protein and ribosomal RNA genes (lighter gray) are \textit{atp6} and 9, subunits 6 and 9 of F\textsubscript{0} ATP synthase; \textit{cob}, apocytochrome b; \textit{cox1–3}, cytochrome c oxidase subunits 1–3; \textit{nad1–6} and \textit{nad4L}, NADH dehydrogenase subunits 1–6 and 4L; \textit{rns} and \textit{rnl}, small and large subunit rRNAs. The tRNA genes (black) are identified by the 1-letter code for their corresponding amino acid. Large ORFs (darker gray) are named according to their lengths. All genes are transcribed clockwise. Curly lines indicate the lack of sequence data from the region.
Glass Sponges and Bilaterian Animals Share an Arg → Ser Reassignment of Mitochondrial AGR Codons

The analysis of mitochondrial coding sequences in hexactinellids both manually and using genetic code prediction software (Abascal, Zardoya, and Posada 2006) showed that glass sponges use a modified genetic code for mitochondrial protein synthesis with UGA coding for tryptophan and AGR coding for serine. Specifically, nearly all tryptophan residues in encoded proteins are specified by the UGA codon (the UGG codon is extremely rare in both genomes and is potentially involved in translational frame-shifting [Haen KM, Lavrov DV, unpublished data]), whereas ~42% of AGR codons in evolutionarily conserved positions in each genome code for serine, and none of them code for arginine. Furthermore, the inference that AGR codons specify serine is supported by the presence of only one tRNA for AGN codons (tRNA$^{AGN}_{GC}$. Whereas the reassigned UGA codon is found in the mtDNA of all animals, the only other known reassignments of AGR codons occurred in the mtDNA of bilaterian animals, first in the lineage leading to this group (Arg → Ser), then in the lineage leading to chordates (Ser → ?), and finally in 2 chordate lineages: Urochordata (? → Gly) and Vertebrata (? → stop) (Knight, Freeland, and Landweber 2001).

Interestingly, although the AUA codon appears to have a standard meaning (isoleucine) in glass sponges, the sequenced portions of glass sponge mtDNA do not code for the tRNA$^{AGN}_{AU}$, which usually translates this codon in eubacteria and eubacteria-derived organelles (Muramatsu et al. 1988; Weber et al. 1990; Soma et al. 2003). This lack of AUA appears to be disadvantageous in translation, at least in vitro (Hanada et al. 2001), it is conserved throughout the evolution of the Bilateria (Garey and Wolstenholme 1989; Wolstenholme 1992; Ruiz-Trillo et al. 2004).

Second, an atypical R11–Y24 pair is present in tRNA$^{Pro}_{GGG/GCG}$ of glass sponges as well as all demosponges and the placozoan Trichoplax but is not found in the outgroup species M. brevicollis and A. parastictum or in bilaterian animals (Wang and Lavrov 2007). Because the R11–Y24 base pair is an important recognition element for initiator tRNA, it is usually strongly countersel ected in elongator tRNAs (Marck and Grosjean 2002), and its presence in the proline tRNA of Sponges and T. adhaerens may indicate the monophyly of nonbilaterian animals or at least the monophyly of the Porifera and Placozoa (this character is not available for Cnidaria because they lack this and most other tRNA genes in their mtDNA).

Mitochondrial Genomes of Glass Sponges Encode Highly Derived tRNA and tRNA Structures

The analysis of tRNA and tRNA genes in glass sponge mtDNA revealed that both the primary and secondary structures of encoded RNAs are highly derived and resemble those encoded by bilaterian rather than nonbilaterian animals. The changes in tRNA structures are particularly striking (fig. 2). Canonical tRNAs in nuclear, bacterial, and most organellar genomes have several highly conserved features: a 7-bp aminoacyl arm, a dihydrouridine (DHU) stem varying in size from 3–4 bp, a DHU loop of 8–10 nt, a 5-bp anticodon stem with a 7-nt anticodon loop, and a 5-bp TΨC stem with a 7-nt loop (Marck and Grosjean 2002). Furthermore, several nucleotides are nearly universally conserved in standard tRNAs, including 2 guanines within the DHU-loop that bind to well-conserved nucleotides in the TΨC loop, stabilizing the 3-dimensional L-shaped structure of tRNAs. Conventional tRNAs are found in demosponge, cnidarian, and Trichoplax mtDNA, but in most bilaterian animals there are multiple deviations from this pattern of conservation (Wolstenholme 1992). Similar to bilaterian animals, glass sponge mt-tRNAs display a great degree of variation in both the size and nucleotide sequence of the DHU and TΨC arms, including a highly variable sequence of the TΨC loop and a typical absence of guanine residues in the DHU loops (fig. 2). The authors are not aware of any other group of organisms that encode tRNAs with similar characteristics.

Phylogenetic Analyses Based on Sequence Data Give a Mixed Message about the Phylogenetic Position of the Hexactinellida

Phylogenetic analyses based on deduced amino acid sequences using traditional empirical substitution models provide strong support for the sister group relationship between glass sponges and bilaterian animals (fig. 4A). These analyses separate animals into 2 groups: the Diploblastica (excluding the Hexactinellida) and the Bilateria, with glass sponges grouping with the latter group. The inferred phylogeny is stable with respect to the substitution matrix—identical ML topologies were found by the TreeFinder program when using the JTT + Γ, mtREV + Γ, cpREV + Γ, or mtART + Γ models of sequence evolution—and with respect to the phylogenetic program used—both TreeFinder and RAxML (using the best available cpREV + F + Γ model) found the same ML topology with similar bootstrap support values. Alternative positions of
<table>
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<th>Alanine (A)</th>
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<th>Asparagine (N)</th>
<th>Aspartate (D)</th>
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<th>Glutamine (Q)</th>
<th>Glycine (G)</th>
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<th>Isoleucine (I)</th>
<th>Leucine (L1)</th>
<th>Leucine (L2)</th>
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<td><img src="image12" alt="Leucine L2" /></td>
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<table>
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<th>Methionine (M)</th>
<th>Phenylalanine (F)</th>
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<table>
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<th>Threonine (T)</th>
<th>Tryptophan (W)</th>
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<tr>
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<tr>
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<td><img src="image22" alt="Valine" /></td>
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</table>
the Hexactinellida (e.g., as the sister group to other Metazoa and as the sister group to the Demospongiae) were rejected by likelihood tests (fig. 4C). In addition, the ML topology received 98% posterior probability in the MrBayes analysis.

We have previously proposed (Lavrov et al. 2005) that the clustering of nonbilaterian animals in phylogenetic analyses based on mtDNA data might be explained by elevated rates of mitochondrial sequence evolution in Bilateria, which would pull the latter group toward the base of the metazoan tree due to a long-branch attraction (LBA) artifact (Felsenstein 1978; Hendy and Penny 1989). The unusual position of the Hexactinellida can be rationalized in the same way. In fact, the LBA artifact in the present analysis should be exacerbated by the similar nucleotide compositions of coding strands in the Hexactinellida and Bilateria, which is a feature that may reflect phylogenetic relationships but is also prone to convergence (Steel, Lockhart, and Penny 1993). Several strategies have been suggested to alleviate the effect of the LBA artifact on phylogenetic inference, which involve either better detection of multiple substitutions or minimizing their number (Baurain, Brinkmann, and Philippe 2007). To minimize the number of multiple substitutions, we redid our analyses without bilaterian animals (one of the long branches on the tree) and also conducted a Bayesian analysis on the amino acid data recoded into 6 Dayhoff groups (Hrdy et al. 2004). Neither of these modifications changed the relative phylogenetic position of the Hexactinellida on the tree (not shown).

We also used a potentially better model of sequence evolution—the category (CAT) + I model (Lartillot and Philippe 2004) that explicitly handles the heterogeneity of the substitution process across amino acid positions. The results of this analysis placed glass sponges as an unresolved polyphyly with the demosponges A. corrugata, G. neptuni, and T. actinia (fig 4B). Although this position of glass sponges is more reasonable from a traditional perspective, it appears to be sensitive to the choice of taxa: glass sponges are placed as a sister group to G. neptuni when bilaterian animals are excluded from the analysis, but as a sister group to the jellyfish A. aurita when additional unpublished demosponge sequences are added to the data set (not shown).

Discussion

Genomic analyses of hexactinellid mtDNA revealed several derived mitochondrial features shared with bilaterian animals, including changes in the genetic code, gene content, nucleotide composition, tRNA and rRNA structures, and the presence of a single large noncoding region. These unexpected results can be explained either by a sister group relationship between glass sponges and bilaterian animals or by parallel mitochondrial evolution in these two groups.

In our view, the strongest support for the sister group relationship between glass sponges and bilaterian animals...
Fig. 4.—Phylogenetic position of the glass sponges based on the analysis of mitochondrial sequence data. (A) ML tree obtained from the analysis of 2,678 aligned amino acid positions using the mtART substitution matrix plus 4-category gamma rate correction \((\ln L = -75759.61)\) in the TreeFinder program. Identical ML topologies and very similar bootstrap values were recovered using the JTT + \(C\) \((\ln L = -77651.98)\), mtREV + \(C\) \((\ln L = -77071.95)\), and cpREV + \(C\) +\(I\) models in TreeFinder and using the cpREV + \(C\) +\(I\) model in RaXml. An identical topology received \(98\%\) posterior probability in a MrBayes analysis using the cpREV + \(C\) +\(I\) model of sequence evolution. The 1st number at each node indicates the percentage of bootstrap support in ML analysis; the 2nd number is the posterior probability in Bayesian analysis. An asterisk indicates \(100\%\) bootstrap support and Bayesian posterior probability equal to 1.0. (B) Posterior consensus tree under the CAT +\(F\) +\(C\) model. Only the part of the tree that differs from A is shown. (C) Results of likelihood-based tests of alternative positions of the Hexactinellida. Five alternative positions of the group (including the one from fig 4B) have been tested using approximately unbiased (AU), weighted Shimodaira–Hasegawa (WSH) tests, and resampling of estimated log-likelihood bootstrap percentage (RELL BP). B, bilaterians; G, glass sponges; D, demosponges; C, cnidarians; P, placozoan.
comes from the reassignment of the mitochondrial AGR codons from serine to arginine and from the changes in the secondary structure of the tRNA\textsubscript{Ser} GCU/UCU in both of these groups (fig. 3). Changes in the genetic code are complex and rare events; therefore, they are usually regarded as reliable indicators of phylogenetic relationships (Telford et al. 2000). Similarly, changes in the secondary structure of mitochondrial tRNAs and, in particular, tRNA\textsubscript{Ser} GCU/UCU also appear to be rare and complex events that, so far, are known to occur only in the Bilateria and Hexactinellida. It should be noted, however, that because tRNA\textsubscript{Ser} GCU/UCU translates AGR codons that changed their meaning in both groups, the modifications in the genetic code and tRNA\textsubscript{Ser} GCU/UCU structure may be not independent. Other features shared between these 2 groups are known to be prone to convergence.

Not all mitochondrial genomic features support the affinity of glass sponges and bilaterian animals. MtDNA of glass sponges and demosponges share the presence of \textit{atp9}, a gene for subunit 9 of ATP synthase that is absent in the mtDNA of all other animals. Although the presence of \textit{atp9} is clearly a plesiomorphic feature for these taxa that says nothing about sponge monophyly, the sister group relationship between hexactinellids and bilaterians would imply multiple independent mitochondria-to-nucleus transfers of \textit{atp9} within the Metazoa. It should be noted, however, that such independent transfers of organellar genes are quite common (Martin et al. 1998), and a specific example of an independent \textit{atp9} transfer to the nucleus has been reported recently in the demosponge \textit{Amphimedon queenslandica} (Erpenbeck et al. 2007). The 2nd mitochondrial genomic feature that contradicts the grouping of Hexactinellida and Bilateria is the unusual R11-Y24 pair in the proline tRNA. This unusual base pair is an apomorphy for sponges and placozoa and to our knowledge has not been found in the mt-tRNA\textsubscript{Pro} of either bilaterian animals or any outgroups. Because the R11-Y24 base pair is an important recognition element for initiator tRNA, it is usually strongly counterselected in elongator tRNAs, and its presence in tRNA\textsubscript{Pro} may be phylogenetically informative.

Interestingly, phylogenetic analyses of deduced amino acid sequences also produced ambiguous results for the position of the Hexactinellida. The use of traditional empirical models of amino acid evolution resulted in strong support (with high bootstrap and posterior probability numbers) for the sister group relationship between glass sponges and bilaterian animals. By contrast, phylogenetic inference based on the newly developed CAT model placed glass sponges within the Demospongia. However, the alternative positioning proposed by CAT is not stable upon taxonomic resampling, suggesting the influence of other artifacts, such as compositional biases, that are not accounted for by the CAT model (Lartillot N, personal communication).

To conclude, most of the mitochondrial genomic characters support the sister group relationship between glass sponges and bilaterian animals. If confirmed, this position of glass sponges would prompt the reassessment of mainstream ideas about early animal evolution in general and the evolution of the sponge body plan in particular. If, conversely, this relationship is refuted, then all the mitochondrial features shared between glass sponges and bilaterian animals—a change in the genetic code, changes in secondary structures of the encoded tRNAs, and the loss of the D-arm in tRNAs, among others—will serve as remarkable examples of parallel evolution in mitochondrial genomes and may provide insights into the origin of unusual features in animal mtDNA. Further studies are needed to distinguish between these 2 possibilities.

**Supplementary Materials**

Mitochondrial genome sequences of \textit{I. panicea} and \textit{S. nux} have been deposited in the GenBank database under the accession numbers EF537576 and EF537577, respectively.

**Acknowledgments**

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Martin Embley, Associate Editor

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