The earliest diverging extant scleractinian corals recovered by mitochondrial genomes

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Evolutionary reconstructions of scleractinian corals have a discrepant proportion of zooxanthellate reef-building species in relation to their azooxanthellate deep-sea counterparts. In particular, the earliest diverging “Basal” lineage remains poorly studied compared to “Robust” and “Complex” corals. The lack of data from corals other than reef-building species impairs a broader understanding of scleractinian evolution. Here, based on complete mitogenomes, the early onset of azooxanthellate corals is explored focusing on one of the most morphologically distinct families, Micrabaciidae. Sequenced on both Illumina and Sanger platforms, mitogenomes of four micrabaciids range from 19,048 to 19,542 bp and have gene content and order similar to the majority of scleractinians. Phylogenies containing all mitochondrial genes confirm the monophyly of Micrabaciidae as a sister group to the rest of Scleractinia. This topology not only corroborates the hypothesis of a solitary and azooxanthellate ancestor for the order, but also agrees with the unique skeletal microstructure previously found in the family. Moreover, the early-diverging position of micrabaciids followed by gardineriids reinforces the previously observed macromorphological similarities between micrabaciids and Corallimorpharia as well as its microstructural differences with Gardineriidae. The fact that both families share features with family Kilbuchophylliidae ultimately points towards a Middle Ordovician origin for Scleractinia.

Scleractinian corals are renowned for their capacity to create spectacular shallow-water calcium carbonate reef structures. Azooxanthellate scleractinians—corals that do not establish a symbiotic relationship with dinoflagellates of the family Symbiodiniaceae1,2—total approximately the same number of extant zooxanthellate species of the order, but are not restricted to tropical, shallow-waters as the latter. The early evolutionary history of the order Scleractinia has been the subject of intense scientific debate. For example, the monophyly of this order has been challenged since the order Corallimorpharia was previously recovered as a clade nested within the main scleractinian lineage (“naked coral” hypothesis). Nevertheless, it has been shown that such a corallimorpharian position was an artefact of the use of amino acid sequences in phylogenetic analyses4. Furthermore, the evolutionary history of the main reef builders has also attracted great attention as the “molecular revolution” challenged the long-established morphological systematics especially for the higher taxonomic ranks5–11. Originally, based on gross morphology, the order Scleractinia was divided into five12, or thirteen13 suborders. However, more recently, molecular data pointed to three main clades: “Basal”; “Complex”; and “Robust” corals11,14–16.

In contrast to widely accepted Triassic emergence of Scleractinia, divergence of the earliest scleractinian clade with extant representatives (families Micrabaciidae and Gardineriidae) was suggested to have occurred between the Ordovician and Silurian, around 425 million years ago (mya)14, or in the Silurian, 407 mya16. Although recovering a slightly later onset, a recent study by Quattrini and collaborators17 has also pointed to a Paleozoic origin for the order. Composed of exclusively azooxanthellate taxa18, representatives of Micrabaciidae share some morphological skeletal characters (septal bifurcations) with the Ordovician Kilbuchophylliidae (~ 460 mya), but otherwise represent a morphologically unique coral group19. On the other hand, gardineriids develop usually a thick, exclusively epithelial wall20 typical of some of the oldest known solitary Mesozoic (Middle Triassic) corals14 (~ 230 mya; Fig. 1a–c,f,g). Gardineriidae is also composed exclusively of azooxanthellate solitary corals, occurring

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from 2 to 1200 m depth\textsuperscript{31}. Due to the unique micrabaciid microstructure\textsuperscript{14,19,22,23}, which is not comparable to that of gardineriids (Fig. 1d,e,h,i) nor any other modern or fossil scleractinian coral, the phylogenetic position of micrabaciids within the “Basal” clade is intriguing. Known to occur from 15 m to as deep as 5000 m\textsuperscript{21,24}, that of gardineriids (G. hawaiiensis) nor any other modern or fossil scleractinian coral, the phylogenetic position of micrabaciids (here L. formosissima) Thickening Deposits (TDs) are composed of an irregular meshwork of fiber bundles oriented sub-parallel to the skeleton surface (d,e), whereas in gardineriids (G. hawaiiensis) TDs are arranged in small bundles of fibers oriented approximately perpendicular to the skeleton surface. Consequently, micrabaciid TDs show variable crystallographic orientation (d, seen as lack of larger areas of similar vivid interference colors in polarized light), whereas in gardineriids TDs are crystallographically ordered and larger areas of similar vivid interference colors are visible in polarized light (h). Thin-sections in polarized microscope views (d,h), and polished and lightly etched sections in Scanning Electron Microscopy views (e,i).

Overall, scleractinian mitogenomes have unique features, such as few transfer RNA genes (trnW, which is duplicated in Seriatopora and Stylophora, and trnM\textsuperscript{4,13,34}), as well as the occurrence of introns in two protein-coding genes: nad5 and cox1. In nad5, this feature is present in all scleractinian mitogenomes determined to date\textsuperscript{3,6,26}, while the cox1 intron is absent in some species and appears to have been regained at least five times in the “Robust” coral clade\textsuperscript{37,38}. Regarding gene order, the same pattern seems to be shared among the majority of species sequenced to date\textsuperscript{35}, except for Madrepora spp\textsuperscript{35,39}, Desmophyllum dianthus and D. pertusum\textsuperscript{35,36,40,41}, and Solenosmilia variabilis (Fig. 2).

To date, the majority of data used to investigate the evolutionary history of scleractinian corals is derived from shallow-water zooxanthellate species, limiting a broader understanding of several aspects of the evolution of the order\textsuperscript{14,15}. Such a pattern has been reproduced for genomic and transcriptomic data. A search for scleractinian mitogenomes on Nucleotide database\textsuperscript{42} and genomic or transcriptomic data on Sequence Read Archive\textsuperscript{43} (excluding metagenomic data and both accessed on May, 2020) turned up 81 mitogenomes and 107 nuclear genomic/nuclear genomic/transcriptomic-level datasets from colonial and zooxanthellate species compared to 11 and 24, respectively, from solitary or azooxanthellate/facultative species. Among azooxanthellate species, only two mitogenomes (Fungia cyathus stephanus [JF825138] and Gardineria hawaiiensis [MT376619]) and seven nuclear (Balanophyllia elegans, Balanophyllia Europaea, Caryophyllia Arnoldi, Flabellum Alabastrum, Paraconotrochus Antarticus, Rhizotrochus spp., Thecosomamia sp.) datasets were from solitary species. Also, from the mitogenomes presented herein, the only available data (mitochondrial and nuclear genomes or transcriptomes) from “Basal” representatives is resumed to the mitogenome of Gardineria hawaiiensis (Gardineriidae). Therefore, in this study, we refined the understanding of the evolutionary history of these early diverging lineages, more specifically by determining the mitogenomes of four micrabaciids (Letepsammia franki, L. superstes, L. formosissima and Rhombopammina rhiphade). Together, the results presented here shed light on the Early Paleozoic origin of the order but also raise further questions on the discrepancy between mitochondrial and nuclear-based phylogenies within Scleractinia, a phenomenon detected for Cnidaria\textsuperscript{44} and other animal groups\textsuperscript{45,46}.
Results

Generated raw reads ranged from 3,037,202 to 5,605,634 (MiSeq run) and corresponded to 26,584,520 and 29,910,418 (NextSeq run). Trimmed reads ranged from 2,767,107 to 4,840,710 and 14,566,182 to 15,824,076, respectively (Supplementary Table S1 online). Mitogenomes determined herein (Table 1) were each assembled in one contig, all above 19 kbp (Rhombopsammia niphada: 19,542 bp; Letepsammia formosissima: 19,048 bp; Letepsammia superstes: 19,073 bp; see number of reads mapped in each assembly on Supplementary Table S1 online), although that from L. formosissima lacks a part of the 16S ribosomal gene (probably around ~30 bp; Table 2). Sanger data from Letepsammia franki was assembled into five contigs comprising 18,206 bp in total and the only absent gene was trnW. Micrabaciidae mitogenomes have 37.8–37.9% GC levels, values slightly lower than that observed for G. hawaiiensis and Corallimorpharia (both with 39.7%). Nevertheless, “Complex” corals include a wide range of values, from 36.2% in Porites lobata to 40.5% in Pavona claus (see Table 1).

Gene number and order mirrors that observed in the majority of scleractinian corals (13 protein coding genes, two ribosomal RNA genes, and two transfer RNA genes), comprising two genes coding for ATP synthase subunits (atp6 and atp8), seven for NADH dehydrogenase subunits (nad4L and nad1–6), three for cytochrome c oxidase subunits (cox1–3), and one for cytochrome b oxidase (cob). Cox1 has a 1027 bp long intron in all three Illumina sequenced species and the intron in nad5 includes 11 genes and is 11,627 (R. niphada), 11,155 (L. formosissima) and 11,158 (L. superstes) bp long (Fig. 2). Genes and intergenic regions (IGRs) are similar in length among R. niphada, L. formosissima and L. superstes in almost all cases, except for two genes (nad1 and 12S) and six IGRs (igr1, igr7, igr8, igr13, igr14, igr17; Table 2). Micrabacid mitochondrial genes start codons are ATG (N = 9) and GTG (N = 4) (Table 2), while stop codons are more equitatively shared between TAA (N = 7) and TAG (N = 6). The evolutionary reconstruction using mitogenome data (Fig. 3) recovered the family Micrabaciidae as monophyletic with high statistical support (ML: 100%; BI: 1), and placed it as a sister group to all other scleractinians (i.e. Gardineriidae + “Robust” + “Complex”) with moderate to high support (ML: 83%; BI: 0.99). The family Gardineriidae was recovered as a lineage sister to “Complex” and “Robust” with moderate to high support (ML: 81%; BI: 0.99). In “Complex”, all nodes except one (Euphylliidae + Acroporidae; ML: 81%; BI: 0.99) displayed maximum values of support, while in “Robust” corals, six had non-full support values (inside family Merulinidae), being three of them with low (clade comprising Platygryra cariosa, Favites abdita and Dipsastrea rotiamenta; ML: 69% and 68%; BI: 0.99 for both) and moderate to high (Orbicella annularis + Cyphastrea serailia; ML: 87%; BI: 1) support values.

Discussion

The phylogenetic analysis presented here indicates a paraphyly of the previously thought earliest diverging scleractinian “Basal” group11,14–16, represented by the two families Micrabaciidae and Gardineriidae. Based on all mitochondrial genes, the recovered phylogeny suggests that the micrabacids were the first to diverge, being a sister group to all other scleractinians, including gardineriids. These results add further evidence for the hypothesis of solitary azooxanthellate corals as origin for the group.
| Species          | Accession      | Length (bp) | GC content (%) |
|------------------|----------------|-------------|----------------|
| **Actiniaria**   |                |             |                |
| Metridium senile | AF000023       | 17,443      | 38.1           |
| Nematostella sp. | DQ643835       | 16,389      | 39.1           |
| **Corallimorpharia** |            |             |                |
| Corallimorphus profundus | KP938440        | 20,488      | 39.7           |
| Discosoma nummiforme | KP938434       | 20,925      | 39.0           |
| Pseudocorynactis sp. | KP938437       | 21,239      | 39.1           |
| **Micrabcidae**  |                |             |                |
| Letepsammia formosissima | MT705247      | 19,048      | 37.9           |
| Letepsammia franki | MT706036-MT706040 | 18,206      | 37.8           |
| Letepsammia superstes | MT706035      | 19,073      | 37.9           |
| Rhombopsammia niphada | MT706034      | 19,542      | 37.8           |
| **Gardineriidae** |                |             |                |
| Gardineria hawaiiensis | MT376619      | 19,429      | 39.7           |
| **“Complex”**    |                |             |                |
| Acropora tenuis  | AF338425       | 18,338      | 38.0           |
| Agaricia humilis | DQ643833       | 18,735      | 40.4           |
| Alveopora sp.    | KJ634271       | 18,146      | 37.9           |
| Anacropora matthai | AY903295      | 17,888      | 38.4           |
| Dendrophyllia arbuscula | KR824937  | 19,069      | 37.3           |
| Fimbriaphyllia ancora | IF825139      | 18,875      | 37.8           |
| Fungucathus stephanus | IF825138    | 19,381      | 37.8           |
| Galaxea fascicularis | KU159433    | 18,751      | 38.3           |
| Goniopora columna | IF825141       | 18,766      | 37.1           |
| Pavona clavus    | DQ643836       | 18,315      | 40.5           |
| Porites lobata   | KU572435       | 18,647      | 36.2           |
| Porites porites  | DQ643837       | 18,648      | 36.3           |
| Pseudodiodactylus tayamai | KF260633 | 19,475      | 36.3           |
| Tubastraea coccinea | KX024566       | 19,094      | 37.2           |
| Turbinaria peltata | KJ725201       | 18,966      | 37.0           |
| **“Robust”**     |                |             |                |
| Astrangia sp.    | DQ643832       | 14,853      | 31.9           |
| Colphophyllia natans | DQ643833      | 16,906      | 33.6           |
| Cyphastrea serailia | KY094484     | 17,138      | 33.5           |
| Desmophyllum dianthus | KX000893      | 16,310      | 35.1           |
| Desmophyllum pertusum | KC875348      | 16,149      | 34.9           |
| Dipsastra rotutmana | KY094481      | 16,466      | 33.2           |
| Echinophyllia aspera | MG792550      | 17,697      | 34.1           |
| Favites abdita   | KY094479       | 17,825      | 33.8           |
| Hydnophora exesa | KY094486       | 17,790      | 33.4           |
| Madracis decactis | KX982259       | 16,970      | 31.7           |
| Madracis mirabilis | EU400212      | 16,951      | 31.7           |
| Madrepora oculata | JX236041       | 15,841      | 30.3           |
| Mussa angulosa   | DQ643834       | 17,245      | 33.7           |
| Orbicella annularia | AP008974      | 16,138      | 33.6           |
| Platygryra carnea | JX011333       | 16,463      | 33.0           |
| Plesiastrea versipora | MH025639    | 15,320      | 32.0           |
| Pocillopora damicornis | EU400213    | 17,425      | 30.2           |
| Pocillopora eydouxi | EFS26303      | 17,422      | 30.1           |
| Polycyathus sp.  | IF825140       | 15,357      | 29.1           |
| Sclerophyllia maxima | FO904931      | 18,168      | 33.7           |
| Solenosmilia variabilis | KM609293 | 15,968      | 34.7           |

Table 1. Lengths and GC contents of mitogenomes included in the phylogeny. *Samples sequenced in this study. **Mitogenomes that were not circularized successfully or were stated as linear or incomplete on NCBI.
| Gene/IGR/Intron | Letepsammia formosissima | Letepsammia superstes | Rhombopsammia niphada | Letepsammia franki |
|----------------|--------------------------|----------------------|----------------------|-------------------|
| Position | Codonsb | Length | Position | Codonsb | Length | Position | Codonsb | Length | Positionc | Codonsb | Length |
| nad5′-5′ | 342–1061 | GTG/GGT | 720 | 1–720 | GTG/GGT | 720 | (A) | 1510–2029 | GTG/GGT | 720 |
| Igr1 | – | 247 | – | – | 247 | – | – | 247 | – | – |
| nad1 | 1309–2292 | ATG/TAA | 984 | 968–1951 | ATG/TAA | 984 | (A) | 2277–3065 | ATG/TAA | 789 |
| Igr2 | – | 57 | – | – | 57 | – | – | 57 | – | – |
| Coxb | 2350–3522 | ATG/TAA | 1173 | 2009–3181 | ATG/TAA | 1173 | (B) | 1–540 | TCC/TAA | 540 |
| Igr3 | – | 336 | – | – | 336 | – | – | 336 | – | – |
| Nad2 | 3859–4956 | ATG/TAA | 1098 | 3518–4615 | ATG/TAA | 1098 | (B) | 877–1974 | ATG/TAA | 1098 |
| Igr4 | – | 88 | – | – | 88 | – | – | 89 | – | – |
| Nad6 | 5045–5605 | ATG/TAA | 561 | 4704–5264 | ATG/TAA | 561 | (B) | 2063–2623 | ATG/TAA | 561 |
| Igr5 | – | 16 | – | – | 16 | – | – | 16 | – | – |
| Atp6 | 5622–6320 | ATG/TAG | 699 | 5281–5979 | ATG/TAG | 699 | (C) | 15–485 | TCT/TAG | 471 |
| Igr6 | – | 45 | – | – | 45 | – | – | 45 | – | – |
| Nad4 | 6366–7841 | ATG/TAG | 1476 | 6025–7500 | ATG/TAG | 1476 | (C) | 531–2006 | ATG/TAG | 1476 |
| Igr7 | – | 188 | – | – | 188 | – | – | 176 | – | – |
| 12S | 8030–8977 | – | 950 | 7678–8639 | – | 962 | (C) | 2183–3145 | – | 963 |
| Igr8 | – | 869 | – | – | 870 | – | – | 1338 | – | – |
| Cox3 | 9847–10,635 | ATG/TAG | 789 | 9509–10,766 | ATG/TAG | 789 | (C) | 4016–4804 | ATG/TAG | 789 |
| Igr9 | – | 8 | – | – | 8 | – | – | 8 | – | – |
| Cox2 | 10,644–11,387 | ATG/TAG | 744 | 10,306–11,049 | ATG/TAG | 744 | (C) | 4813–5556 | ATG/TAG | 744 |
| Igr10 | – | 17 | – | – | 17 | – | – | 17 | – | – |
| Nad4L | 11,405–11,704 | ATG/TAG | 300 | 11,067–11,366 | ATG/TAG | 300 | (C) | 5574–5873 | ATG/TAA | 300 |
| Igr11 | – | 15 | – | – | 15 | – | – | 15 | – | – |
| Nad3 | 11,720–12,076 | ATG/TAG | 357 | 11,382–11,738 | ATG/TAG | 357 | (C) | 5689–6245 | ATG/TAG | 357 |
| Igr12 | – | 140 | – | – | 140 | – | – | 140 | – | – |
| Nad5-3′ | 12,217–13,246 | ATG/TAG | 1080 | 11,879–12,958 | ATG/TAG | 1080 | (C) | 6,386–7,465 | ATG/TAG | 1080 |
| Igr13 | – | 38 | – | – | 26 | – | – | 26 | – | – |
| TrnW | 13,335–13,404 | TCA | 70 | 12,985–13,054 | TCA | 70 | (C) | 513–5523 | TCA | N.A. |
| Igr14 | – | 32 | – | – | 34 | – | – | 34 | – | – |
| Atp8 | 13,437–13,661 | ATG/TAA | 225 | 13,089–13,313 | ATG/TAA | 225 | (D) | 49–273 | ATG/TAA | 225 |
| Igr15 | – | 765 | – | – | 765 | – | – | 765 | – | – |
| Cox1 | 14,427–16,953 | ATG/TAA | 2527 | 14,079–16,605 | ATG/TAA A | 2527 | (D) | 1039–1849; (E) 1–657 | ATG/TAA | 811; 657 (total 1,468) |
| Igr16 | – | 97 | – | – | 97 | – | – | 97 | – | – |
| TrnM | 17,051–17,121 | CAT | 71 | 16,703–16,773 | CAT | 71 | (E) | 755–825 | CAT | 71 |
| Igr17 | – | 260 | – | – | 260 | – | – | 260 | – | – |
| 16S | 1–188; 17,382–19,048 | – | 188; 1667 | 17,034–18,920 | – | 1887 | (E) | 1056–1773; (A) 1–1156 | – | 718; 1156 (total 1,874) |
| Igr18 | – | 153 | – | – | 153 | – | – | 153 | – | – |
| Cox1 intron | 15,231–16,257 | – | 1027 | 14,883–15,969 | – | 1027 | – | 1027 | – | N.A. |
| Nad5 intron | 1062–12,216 | 11,155 | 721–11,878 | – | 11,158 | 721–12,347 | – | 11,627 | – | N.A. |

**Table 2.** Micrabaciidae mitochondrial gene content. aLetters in parentheses indicate different *L. franki* Sanger sequencing contigs to which each gene belongs. bFor tRNA genes, anticodons. c“Start codon” annotated by MITOS2 for an incompletely assembled gene.
Despite its crucial relevance for improving our understanding of the deep evolutionary patterns in Scleractinia, phylogenetic analyses have been based on limited data from a few partial genes (majoritarily cox1, 16S rDNA, 18S rDNA and 28S rDNA). Only recently, using 933 loci (278,819 bp) captured with a targeted-enrichment approach, Quattrini et al. recovered the family Micrabaciidae as the first diverging lineage within “Robust” corals. Nevertheless, they lack representatives of Gardineriidae or other exclusively deep-water azooxanthellate families, such as Deltocyathidae and Anthemiphylliidae. On one hand, Anthozoa mitochondrial genes tend to evolve at slower rates in comparison with other metazoans, thus favouring their application to investigate Scleractinia relationships through deep time or among taxonomic ranks above genus. On the other hand, mitogenomes may be genetically saturated, making them problematic for studying deeper levels of relationship inside Cnidaria (e.g. class level). Therefore, sequencing of more mitogenomes and tests for saturation will elucidate the utility of mitochondrial genes in clarifying deep phylogenies within Scleractinia.

In general, micrabacid mitogenomes have similar sizes to those of *G. hawaiiensis* and some “Complex” corals (e.g. *Tubastrea coccinea*, *Dendrophyllia arbuscula*, *Fungiacyathus stephanus*, and *Pseudosiderastrea tayamai*; Table 1). Among the mitogenomes sequenced herein, differences in length were mainly due to IGR sizes rather than among genes. A similar length variation pattern has previously been observed for the entire order and supports our findings for this family. At 19,542 bp (Fig. 2), *R. niphada* holds the longest Scleractinia mitogenome known to date, followed by two *Pseudosiderastrea* species with 19,475 bp. Micrabacids were expected to bear long mitogenomes (> 19 kbp) based on their phylogenetic placement since the mitogenome of *Gardineria hawaiiensis* is 19,429 bp long and the overall size of scleractinian mitogenomes appears to be shrinking in relation to Corallimorpharia (between 1 and 6 kbp) and also within the order (i.e. “Robust” corals have 2–3 kbp shorter mitogenomes than “Complex” corals). Nevertheless, a clear pattern is still not evident in the class Anthozoa as a whole. While octocorals seem to present a narrower range of variation regarding mitogenome size (18 to 19.8 kbp), hexacorals show a broader range. Actiniarian mitogenomes vary between 16 and 20 kbp, which is close to that observed in scleractinians. Antipatharia ranges from 18 to 20 kbp and Zoantharia includes 20-kbp long mitogenomes, similar to Corallimorpharia.
Different from size correlations, the recovered topology does not match previous reconstructions that included “Basal” lineages\(^\text{11,14,15,59}\) (Fig. 3), with a few exceptions such as reconstructions based on 28S rDNA and mitochondrial 16S rDNA that recovered Micrabaciidae and Gardineriidae as a polytomy and a paraphyly, respectively\(^\text{64}\). Here, micrabaciids and gardineriids were recovered in a paraphyly, the former being the earliest to diverge. Notably, Micrabaciidae share anatomical features in common with corallimorpharians, including the presence of a thick mesoglea\(^\text{66}\) and acrospheres positioned around and above the polyps\(^\text{66}\). The molecular discrepancy between Gardineriidae and Micrabaciidae is strongly supported by differences in microstructural organization of their skeleton. For example, although the presence of an epithelial wall is common in many fossil and extant scleractinians, its occurrence as the only wall of the corallum, like in gardineriids, is exclusive in modern corals, but seems to have been a more common feature in early Mesozoic corals\(^\text{66,62}\). In contrast to the Gardineriidae skeletal thickening deposits (TD) (i.e. bundles of fibers arranged perpendicularly to the growing surfaces), micrabacid TD are shaped in form of chip-like fiber bundles, sub-parallel to the skeletal surfaces, creating an irregular meshwork within the skeleton, which is not comparable with any microstructural organization from other modern or fossil scleractinian\(^\text{14,19}\). Because distinct patterns of TD organization are highly conservative traits in the evolution of scleractinian corals\(^\text{63}\), a unique micrabacid fine-scale skeletal organization clearly suggests a long-period of independent evolutionary history in relation to gardineriids. On the other hand, although microstructural organization of Ordovician kilbuchophyllids is unknown (these fossils occur as moulds), these Paleozoic corals with scleractinian pattern of septal insertion had an epithelial wall (somewhat similar to gardineriids), and a pattern of bifurcations of higher septal cycles similar to micrabaciids. Together, these morphological characteristics allied to the molecular based phylogeny point towards a common and deep Paleozoic root for the order Scleractinia.

The early divergence of the azooxanthellate, solitary, deep-water micrabaciids and gardineriids (also supported by Stolarski et al.\(^\text{11}\) and Kitahara et al.\(^\text{15}\)) contrasts with some hypotheses for whether first scleractinians were symbiotic and if they inhabited shallow or deep water environments\(^\text{64}\). In fact, Campoy and colleagues\(^\text{16}\) used four markers (18S rDNA, 28S rDNA, 16S rDNA and cox1) and 513 scleractinian coral species from almost all extant families and hypothesized that the first scleractinian would have been azooxanthellate and solitary. Nonetheless, symbiosis with zooxanthellae was widespread in Triassic corals\(^\text{65}\) and there is some degree of disagreement about it being lost and reappearing a few times\(^\text{63,66}\) or being gained only once during scleractinian evolution\(^\text{66}\). In contrast, it appears that coloniality was the first one to be gained and there is an agreement that it was lost and gained more than once\(^\text{16,64}\), and even the presence of multiple mouths in one polyp seems to be a labile trait in some families of this order as well (e.g. Dendrophylliidae\(^\text{66}\) and Fungiidae\(^\text{77,68}\)).

Interestingly, all Paleozoic purported scleractiniamorph corals (i.e. Kilbucophyllia, Houchangocythus, and possibly Numidiaphyllum) were solitary polyps or had a loosely constructed phaceloid growth form (see Scrutton\(^\text{69}\); Ezaki\(^\text{70,71}\)). Due to the several similarities with living solitary azooxanthellate scleractinians\(^\text{69,72}\), these aforementioned corals were purported to be azooxanthellate. Also, coral-zooxanthellae symbiosis has been assessed on a Bioanalyzer (Agilent). Samples were pooled with other libraries and sequenced on two different MiSeq v3 2 × 300 bp runs (L. formosissima in a run with other seven libraries and L. superstes and R. niphada in a different run with other eight). The same libraries from species L. superstes and R. niphada were also included on a NextSeq v2 High Output 2 × 75 bp pooled run with 16 samples in total. Illumina sequencing was performed at the Genome Investigation and Analysis Laboratory of the Centro de Facilidades para a Pesquisa, University of São Paulo. Raw sequences were trimmed using Trimmmomatic\(^\text{77}\) under default settings, and the trimmed reads

**Methods**

Specimens belonging to four species of the family Micrabaciidae (Letepsammnia formosissima (Moseley, 1876): IK-2012-3802; L. franki Owens, 1984: IK-2012-3748; L. superstes (Ortmann, 1888): IK-2012-3754; and Rhombopsammia niphada Owens, 1986: IK-2012-3832) were sourced from the Muséum national d’Histoire naturelle (Paris, France) Cnidaria collection. Total genomic DNA extraction was performed using the DNaeasy Blood and Tissue kit (Qiagen) and libraries for Next Generation Sequencing (NGS) were prepared using TruSeq DNA Nano library preparation kit (Illumina; one library per species, based on one sample each). Since DNA from R. niphada was particularly degraded, Covaris shearing parameters were changed for this sample according to manufacturer suggestions (duty cycle: 5%; duration: 70 s). Moreover, in order to avoid adapter-dimer formation, adapters were diluted (3×) and the number of cycles at the PCR step was set to 12 cycles, following Illumina recommendations. Library concentrations were quantified on a Qubit 2.0 fluorometer and size distributions were assessed on a Bioanalyzer (Agilent). Samples were pooled with other libraries and sequenced on two different MiSeq v3 2 × 300 bp runs (L. formosissima in a run with other seven libraries and L. superstes and R. niphada in a different run with other eight). The same libraries from species L. superstes and R. niphada were also included on a NextSeq v2 High Output 2 × 75 bp pooled run with 16 samples in total. Illumina sequencing was performed at the Genome Investigation and Analysis Laboratory of the Centro de Facilidades para a Pesquisa, University of São Paulo. Raw sequences were trimmed using Trimmmomatic\(^\text{77}\) under default settings, and the trimmed reads
were used to assemble mitogenomes using MITObim78. Complete assembly of mitogenomes was ascertained by a circular mitogenome recovered, as determined by circules.py79. Assembled mitogenomes were then annotated by MITOS280. Protein coding genes with start or stop codons that did not match the mold/coelenterate mitochondrial genetic code were inspected for the presence of suitable codons before the beginning or after the end of their annotations and re-annotated accordingly. Data from previous attempts to sequence Micrabaciidae mitogenomes by primer walking (using the same samples; primer sequences and PCR settings from Lin et al.74) were used to refine *R. niphada* and *L. formosissima* assemblies. Data for *L. franki* were generated by Sanger sequencing followed by editing and assembling in Sequencher80.

A selection of published mitogenomes (Table 1) was downloaded from GenBank and re-annotated on MITOS2. Nucleotide sequences were aligned by gene (11 PCGss, 2 rRNAs and 2 tRNAs) or exon for multi-exon genes (i.e. *nad5* and *cox1*) in MAFFT v7 using L-INS-i algorithm81. Alignments were tested for substitution saturation on DAMBE v7.0.1282. In saturated alignments, the third codon position nucleotide was removed and tested for saturation again. Only non-saturated alignments were concatenated using catsequences (https://github.com/ChrisCreevey/catsequences) and the final matrix is available at Zenodo (https://doi.org/10.5281/zenodo.4133805). For phylogenetic reconstructions, both maximum likelihood (ML) and Bayesian inference (BI) methods were used. The matrix was partitioned by either gene or exon in multi-exon genes. The former was carried out in RAxML v8.2.1283 with 1000 bootstrap replicates and 100 random starting trees (GTR + G model). The latter was performed in MrBayes v3.2.784 on CIPRES portal85, after searching for the best substitution model for each alignment using Bayesian Information Criterion on jModelTest286 (run on CIPRES portal). Two Markov chain Monte Carlo runs with four chains each were run for 20 million generations, sampling once every 1000 trees, and Monte Carlo runs with four chains each were run for 20 million generations, sampling once every 1000 trees, and discarding the first 30% of them as burn-in, following run convergence check in Tracer v1.7.187.

**Data availability**

The data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/nuccore/ and can be accessed with accessions MT705247, MT706034, MT706035 and MT706036–MT706040. The alignment used for phylogenetic reconstructions is available in Zenodo at https://doi.org/10.5281/zenodo.4133805.

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### Author contributions

I.G.L.S. and M.V.K. conceived the experiment; I.G.L.S. and K.C.C.C. extracted/sequenced DNA, and analysed data; Z.B.R.Q. and D.H. contributed to data analysis; J.S. provided data on skeletal microstructures. All authors interpreted results and wrote the manuscript.

### Competing interests

The authors declare no competing interests.

### Additional information

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