Sinularia leptoclados (Ehrenberg, 1834) (Cnidaria, Octocorallia) re-examined

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Abstract

Sinularia leptoclados (Ehrenberg, 1834) is re-described. Sinularia leptoclados var. gonatodes Kolonko, 1926 is synonymized with S. maxima Verseveldt, 1977. Two new species of Sinularia with digitiform lobules, leptoclados-type surface clubs and unbranched interior spindles, are described. An updated maximum likelihood tree of Sinularia species with leptoclados-type clubs (clade 5C) based on two mitochondrial genes (mtMutS, COI) and a nuclear gene (28S rDNA) is presented.

Keywords

Alcyonacea, re-description, new species, Indo-Pacific, Red Sea, taxonomy, phylogeny

Introduction

In his revision of the soft coral genus Sinularia, Verseveldt (1980) mentioned three stalked Sinularia species with digitiform lobules, leptoclados-type surface clubs and unbranched interior spindles. These are Sinularia firma Tixier-Durivault, 1970, S. leptoclados (Ehrenberg, 1834), and S. maxima Verseveldt, 1971. Subsequently, comparison
of material collected from New Caledonia (RMNH Coel. 10447-10449) to type material proved *S. firma* to be an encrusting species. The original description of *S. firma* was based on a tiny fragment that obscured the colony growth form. In the key of Verseveldt (1980: 12) *S. leptoclados* and *S. maxima* were separated from each other by colony growth form, *S. maxima* with robust lobes, up to 120 mm high, and *S. leptoclados* with shorter ones. Verseveldt based his description of *Sinularia leptoclados* on a RMNH specimen from the Red Sea, without mentioning its catalogue number. He certainly did not have the type specimen, as he states that he failed to find that in museum collections (Verseveldt: 9). Additionally, in his revision Verseveldt synonymized *Sinularia leptoclados* var. *gonatodes* Kolonko, 1926, with *S. leptoclados* and stated the species exhibited an Indo-Pacific distribution.

The first two authors have based their identifications of *S. leptoclados* on the microscope slides of Verseveldt at their disposal, and following the *Sinularia* revision of Verseveldt (1980), have considered *S. leptoclados* specimens to be stalked with finger-like lobules and variable *leptoclados*-type clubs in the surface layer of the colony. The results have been published in a series of studies (see below) that have further supported Verseveldt’s (1980) statement that the species is widespread in the Red Sea and in the Indo-West Pacific area.

Alderslade and Shirwaiker (1991) were the first after Verseveldt’s (1980) revision to describe another species with characters similar to *S. leptoclados*, their *S. kavarattiensis* from the Laccadive Archipelago, India. They compared *S. kavarattiensis* with the holotype of *S. leptoclados* var. *gonatodes* and considered the many small spindles present in the surface layer of the lobes of the latter as a major difference between the two species. Later on, Manuputty and Ofwegen (2007) described three species from Ambon (Indonesia) which resembled *S. leptoclados*: *S. acuta*, *S. corpulentissima* and *S. longula*. In that study they used for comparison a specimen from Ambon (RMNH Coel. 38426), considered by them to be *S. leptoclados*.

McFadden et al. (2009), the first molecular study of the genus *Sinularia* ever conducted, discovered that specimens from Australia identified as *S. leptoclados* by P. Alderslade (NTM C5421) and the first author (NTM C14492, 14519-21) differed genetically from Red Sea specimens identified as *S. leptoclados* by the second author (ZMTAU CO 34095). This unexpected finding prompted us to re-examine the *leptoclados* collections of the RMNH and ZMTAU and to search for the type material of this widespread species (e.g., Verseveldt). Fortunately, we discovered the type specimen of *S. leptoclados* still exists in the ZMB, probably overlooked by Verseveldt, while revising the genus, most likely because it was labelled as *Lobularia leptoclados* Ehrenberg, 1834. After examination of its sclerites and comparison to RMNH and ZMTAU material identified as *S. leptoclados* it became obvious that this species does not exhibit an Indo-West Pacific distribution as stated by Verseveldt (1980), but is rather limited to the Red Sea and eastern Indian Ocean. Material wrongly assigned to *S. leptoclados* from other parts of the Indo-Pacific by the two first authors proved to be a mixture of misidentifications and as yet undescribed species. Interestingly, the specimens from Australia that were erroneously identified as *S. leptoclados* have sclerites and a colony morphology that closely resemble that
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31 species. However, certain small morphological differences, its unique genetic haplotype, and the now disjunct distribution (Red Sea and western Indian Ocean vs. Pacific Ocean, Australia), convinced us to describe this material as a new species.

While collecting new material of *S. leptoclados* at Eilat, northern Gulf of Aqaba, Red Sea, we unexpectedly found two other species with *leptoclados*-type clubs and *leptoclados*-like colony shape: *S. verseveldti* Ofwegen, 1996 (Fig. 5f), so far only known from the Pacific, and a yet undescribed species which is described here.

**Material and methods**

**Morphological examination**

In order to identify the material, sclerites from different parts of the colony were obtained by dissolving the tissues in 10% sodium hypochlorite, followed by rinsing in fresh water. When appropriate, they were prepared for scanning electron microscopy as follows: the sclerites were carefully rinsed with double-distilled water, dried at room temperature, coated with gold and examined with a Jeol 6480LV electron microscope, operated at 10 kV.

Material studied is deposited in the Naturalis Biodiversity Center (formerly Rijksmuseum van Natuurlijke Historie, Leiden, the Netherlands (RMNH)), Zoological Museum, Department of Zoology, Tel Aviv University, Israel (ZMTAU), Museum für Naturkunde der Humboldt-Universität, Berlin, Germany (ZMB), Zoological Reference Collection (ZRC) of the Raffles Museum of Biodiversity Research, Singapore, and the Museum and Art Gallery of the Northern Territory, Darwin, Australia (NTM).

**Molecular phylogenetic analysis**

Extraction of DNA from ethanol-preserved tissue samples, PCR amplification, and sequencing of the *mtMutS* (*msh1*), *COI* and 28S rDNA genes followed the protocols published in McFadden et al. (2011) and McFadden and Ofwegen (2012). Sequence data were proofread using LaserGene software, and aligned using the L-INS-i method in MAFFT (Katoh et al. 2005). Pairwise measures of genetic distance (uncorrected p) among sequences were computed using MEGA v.5 (Tamura et al. 2011). Modeltest 3.0 (Posada and Crandall 1998) was used to select appropriate models of evolution for maximum likelihood analyses that were run using GARLI 2.0 (Zwickl 2006). Trees for *mtMutS* and *COI* were generally congruent with those for 28S rDNA, so in addition to separate analyses of the mitochondrial and nuclear genes we also ran a combined analysis with different models of evolution applied to each data partition (*mtMutS + COI*: TrN+I; 28S: GTR+I+G). Bayesian analyses of the same separate and combined data sets were run using MrBayes v. 3.2.1 (Ronquist et al. 2012) and a GTR+I+G model of evolution applied to both partitions; analyses were run for 2 million genera-
tions (until standard deviation of split partitions < 0.01) with a burn-in of 25% and default Metropolis coupling parameters. We included in our analyses all other species from *Sinularia* clade 5C for which sequence data were available for at least two of the three genes (Table 1); three species belonging to clades 5A (club sclerites with a distinct central wart, polyps with collaret, points and tentacle scales; *S. gardineri*) and 5B (club sclerites with a distinct central wart, polyps with collaret, points and tentacle rods; *S. hirta, S. terspilli*) were used as outgroup taxa.

**Taxonomy**

*Sinularia australiensis* sp. n.
urn:lsid:zoobank.org:act:C0EC77D7-A9DF-49A6-8BC4-C93AC3AFE8AF
http://species-id.net/wiki/Sinularia_australiensis

Figs 1–4

*Sinularia leptoclados*; Lüttschwager, 1915: 3 (West Australia); Macfadyen: 37 (Great Barrier Reef Australia).

*Sinularia leptoclados*; Ofwegen, 2008a: 131; McFadden et al.: 320 (Gulf of Carpentaria, West Australia).

**Material examined.** Holotype: NTM C14519, Australia, Northern Territory, Gulf of Carpentaria, West of Bremer island, 12°05.660’S, 136°47.754’E, depth 1–3 m, coll. P. Alderslade & party, 17 December 2003. Paratypes: NTM C14492, C14520, C14521, same data as holotype.

**Description.** The holotype is 6 cm high and 9.5 cm wide, attached to a piece of rock (Fig. 1A). The middle part of the colony is devoid of lobes, possibly a colony in the process of colony fission. The primary lobes branch off once or twice, lobules knob-to finger-shaped, up to 4 mm wide and 1 cm long.

The polyps have a collaret and eight points. Points with poorly developed clubs, up to 0.15 mm long (Fig. 2A). Collaret has bent spindles, up to 0.20 mm long (Fig. 2B). Tentacle sclerites were not present.

The surface layer of the lobules has *leptoclados*-type clubs, the smallest are 0.07 mm long, most are around 0.10 mm, but some even reach a length of 0.15 mm (Fig. 2C); in addition, longer wart clubs are present, up to 0.25 mm long (Fig. 2D). Furthermore, the surface layer of the lobules has spindles, up to 0.40 mm long, with simple tubercles (Fig. 2E).

The sclerites of the surface layer of the base of the colony resemble those of the surface layer of the lobules but the clubs have wider handles and the spindles are wider (Fig. 3).

The interior of the colony has mostly unbranched spindles; a few have one or two side branches. In the lobules the spindles are up to 2.5 mm long (Fig. 4A), almost all having simple tubercles (Fig. 4B). In the base of the colony they are up to 3 mm long (Fig. 4C), with more complex tubercles (Fig. 4D).
Sinularia leptoclados (Ehrenberg, 1834) (Cnidaria: Octocorallia) re-examined

Colour. The preserved specimen is brown.

Etymology. Named after Australia, where the type was collected.

Intraspecific variation. NTM C14492 (Fig. 1B) and NTM C14521 (Fig. 1D) have stouter lobules, up to 1 cm wide.

Remarks. The species resembles Sinularia leptoclados regarding clubs and colony shape. It differs in having small surface lobule spindles with uniformly placed tubercles and many internal lobule spindles with simple tubercles. Other species resembling S. australiensis are S. acuta Manuputty & Ofwegen, 2007, S. corpulentissima Manuputty & Ofwegen, 2007 and S. longula Manuputty & Ofwegen, 2007, all three described from Ambon. S. acuta and S. longula have more slender spindles and wart clubs in the surface layer of the lobules (Manuputty and Ofwegen 2007: Figs 3, 19). S. corpulentis-
Figure 2. Sinularia australiensis sp. n., holotype NTM C14519. A point clubs B collaret spindles C lep- toclados-type clubs of surface layer of lobule D wart clubs of surface layer of lobule E spindles of surface layer of lobule. Scale of 0.10 mm at E only applies to E.
Figure 3. Sinularia australiensis sp. n., holotype NTM C14519. Sclerites of the surface layer of the base of the colony A leptoclados-type clubs B wart clubs C–D spindles. Scale of 0.10 mm at D only applies to D.
Figure 4. *Sinularia australiensis* sp. n., holotype NTM C14519. sclerites of the interior A spindles from the lobules B tuberculation of one of the lobule spindles C spindles from the base D tuberculation of one of the base spindles. Scale of 1 mm at C also applies to A.
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*Simia*, like *S. leptoclados*, differs in having many internal spindles with complex tubercles (Manuputty and Ofwegen: Fig. 7c). Moreover, in the current molecular study *S. corpulentissima* is assigned to a distinct subclade together with *S. maxima*, while *S. acuta* and *S. longula* fall into a separate well-supported subclade (Figs 16, 17). *S. australiensis* sp. n. does not belong to either of those subclades, but is close genetically to *S. leptoclados* and *S. abrupta*. The latter species has clubs resembling those of *S. leptoclados* and *S. australiensis*, but a totally different colony shape, with ridges instead of lobes with lobules.

Lüttschwager (1915) and Macfadyen (1936) had *Sinularia* material from Australia that could belong to *S. australiensis*, but re-examination of sclerites of these specimens is necessary to confirm this possibility.

**Sinularia eilatensis** sp. n.

urn:lsid:zoobank.org:act:2DE6BD04-F415-48CB-AFB5-ABF3EF9BA63D
http://species-id.net/wiki/Sinularia_eilatensis
Figs 5A–E, 6–9

**Type material examined.** holotype ZMTAU Co 35260, Israel, Red Sea, northern Gulf of Aqaba, Eilat, IUI (the Interuniversity Institute for Marine Sciences in Eilat) reef, depth 6 m, coll. Y. Benayahu, 10 January 2011; paratypes: ZMTAU Co 35261, same data as holotype; ZMTAU Co 35305, same data as holotype, 30 May 2011.

**Other material examined:** ZMTAU Co 35303-04, Israel, Red Sea, northern Gulf of Aqaba, Eilat, IUI reef, depth 5 m, coll. Y. Benayahu, 30 May 2011.

**Description.** The holotype is 3.4 cm high and wide (Fig. 5A). The primary lobes branch off once or twice, lobules finger-shaped, up to 2 mm wide and 1 cm long.

The polyps have a collaret and eight points. Points with poorly developed clubs, up to 0.25 mm long (Fig. 6A), collaret with bent spindles, up to 0.25 mm long (Fig. 6B) Tentacles with rods, about 0.05 mm long (Fig. 6C).

The surface layer of the lobules has *leptoclados*-type clubs, the smallest are 0.07 mm long, most are around 0.10 mm, but some reach a length of 0.15 mm (Fig. 6D); in addition longer wart clubs are present, up to 0.25 mm long (Fig. 6E). Furthermore, the surface layer of the lobules has spindles, up to 0.35 mm long, with simple tubercles (Fig. 6F).

The sclerites of the surface layer of the base of the colony resemble those of the surface layer of the lobules but they are wider (Fig. 7).

The interior of the colony has mostly unbranched spindles, a few have one or two side branches. In the lobules they are up to 2.5 mm long (Fig. 8A), with simple or complex tubercles (Fig. 8B). In the base of the colony the spindles are up to 2 mm long (Fig. 8C–D), with more complex tubercles (Fig. 8E).

**Colour.** The preserved holotype is dark brown.

**Etymology.** Named after Eilat, the type locality.
**Intraspecific variation.** ZMTAU Co 35305 (Fig. 5C) has distinctly longer lobules, up to 2 cm long.

**Remarks.** The species is unique among *Sinularia* species with *leptoclados*-type clubs by its very long point and collaret sclerites.

We excluded ZMTAU Co 35303-04 (Fig. 5D–E) from the type series. Morphologically we could not find a difference between these two specimens and the types, but their mitochondrial gene haplotypes differ by 0.5%. For comparison, we also present sclerites of ZMTAU Co 35304 (Fig. 9).
Figure 6. Sinularia eilatensis sp. n., holotype ZMTAU Co 35260. A point clubs B collaret spindles C tentacle rods D leptoclados-type clubs of surface layer of lobule E wart clubs of surface layer of lobule F spindles of surface layer of lobule. Scale of 0.10 mm at F only applies to F.
Figure 7. *Sinularia eilatensis* sp. n., holotype ZMTAU Co 35260. Sclerites of the surface layer of the base of the colony. **A** *leptoclados*-type clubs **B** wart clubs **C** spindles.
Figure 8. *Sinularia eilatensis* sp. n., holotype ZMTAU Co 35260. Sclerites of the interior A spindles from the lobules B tuberculation of one of the lobule spindles C–D spindles from the base E tuberculation of one of the base spindles. Scale at D only applies to D.
Figure 9. *Sinularia eilatensis* sp. n., ZMTAU Co 35304. A point clubs B collaret spindles C leptoclados-type clubs of surface layer of lobule D wart clubs of surface layer of lobule E spindles of surface layer of lobule F interior spindles of lobule G tuberculation of one of the lobule spindles. Scale of 0.10 mm at E only applies to E, 1 mm scale at F only to F.
**Sinularia leptoclados (Ehrenberg, 1834)**

(Ehrenberg, 1834) (Cnidaria: Octocorallia) re-examined

Figs 10A–E, 11–14

*Lobularia leptoclados* Ehrenberg, 1834: 58 (Red Sea).

*Alcyonium leptoclados*; Klunzinger 1877: 26, pl. 1 fig. 7a-d (Red Sea).

*Sinularia leptoclados*; Tixier-Durivault 1951: 124, figs 173-175 (Red Sea); 1966: 218, 222, figs 212–214 (Madagascar); Verseveldt 1965: 29 (Red Sea); 1971: 4 (Madagascar); Ofwegen and Benayahu 1992: 140 (Tanzania); Benayahu and Schleyer 1996: 6 (Mozambique); Benayahu et al. 2002: 278 (Southern Red Sea).

**NOT** *Alcyonium leptoclados*; Burchardt 1903: 661, pl. 54 fig. 6, pl. 56 fig. 4 (Torres Strait, Ambon).

**NOT** *Sinularia leptoclados*; Thomson and Dean 1931: 45, pl. 11 fig. 5, pl. 21 figs 6, 9 (Indonesia); Roxas 1933: 350, pl. 2 fig. 8 (Philippines); Verseveldt 1974: 96 (New Caledonia); 1977: 3 (Gambier Island, Fanning Atoll, Enewetak); 1978: 50 (Guam); Ofwegen and Vennam 1994: 138 (Ambon, Indonesia); Benayahu 1993: 6 (South Africa); 1995: 107 (Ryukyu Archipelago, Japan); Ofwegen 1996: 208 (Bismarck Sea); Benayahu 1997: 210 (Guam); Benayahu 2002: 14 (Ryukyu Archipelago, Japan); Benayahu et al. 2004: 551 (Taiwan); Manuputty and Ofwegen 2007: 192, figs 2b, 5 (Ambon, Indonesia; = *S. verseveldti*); Ofwegen 2008a: 131 (Gulf of Carpentaria, Australia; = *S. australiensis* sp. n.).

**NOT** *Sinularia aff. leptoclados* Ofwegen, 2008b: 671 (Palau; = *S. verseveldti*).

**NOT** *Sinularia leptoclados var. gonatodes* Kolonko, 1926: 309, pl. 2 fig. 1 (Philippines); Roxas 1933: 351 (same data as Kolonko) (= *S. maxima* Verseveldt, 1971)

? *Sclerophytum herdmanni* Pratt, 1905: 235, pl. 2 figs 8–9 (Sri Lanka; needs re-examination).

**Material examined.** ZMB 304, holotype of *Lobularia leptoclados* Ehrenberg; 1834, Rotes Meer, leg. Hemprich. Additional material: **Red Sea**; ZMTAU Co 25763, Egypt, Sinai, Tiran Strait, Thomas W., depth 3 m, coll. Y. Benayahu, 25 June 1985; ZMTAU Co 25940, Egypt, Gulf of Suez, Jubal Island, Bluf Point, depth 16 m, coll. Y. Benayahu, 24 March 1988; ZMTAU Co 34093-95, Israel, Gulf of Aqaba, Eilat, Nature Reserve, 29°30.6’N, 34°55.35’E, depth 2.4–5.5 m, coll. Y. Benayahu, 24 July 2007; ZMTAU Co 35308, Israel, Gulf of Aqaba, Eilat, Nature Reserve, depth 3 m, coll. Y. Benayahu, 31 May 2011; **Kenya**; ZMTAU Co 30354, off Mombasa, Shelly Reef, 04°07’S, 39°40’E, depth 12–13 m, coll. Y. Benayahu & S. Perkol, 20 January 2000; ZMTAU Co 32549, Shimoni, Wasini Is., opposite the building, depth 5 m, coll. Y. Benayahu, 2 February 2003; **Tanzania**; RMNH Coel. 18953, off Dar es Salaam, Pangavinne Island, seaward slope (P02), 6°50’S, 39°17’E, depth 6 m, coll. J.N. Nyanda; RMNH Coel. 18954, off Dar es Salaam, Pangavinne Island, seaward slope (P18), 6°50’S, 39°17’E, depth 8 m, coll. J.N. Nyanda; RMNH Coel. 18955, off Dar es Salaam, Mbudya Island, seaward slope (P35), 6°50’S, 39°17’E, depth 5 m, coll. J.N.
Figure 10. *Sinularia leptoclados* colonies. **A** ZMB 304 holotype **B** ZMTAU Co 34093 **C** ZMTAU Co 34094 **D** ZMTAU Co 34095 **E** ZMTAU Co 35308 **F** *Sinularia maxima*, ZRC1999.1066. Scale of 2 cm only applies to **A** and **F**.
Sinularia leptoclados (Ehrenberg, 1834) (Cnidaria: Octocorallia) re-examined

Figure 11. *Sinularia leptoclados* holotype ZMB 304. A point clubs B *leptoclados*-type clubs of surface layer of lobule C wart clubs of surface layer of lobule D spindles of surface layer of lobule.
Figure 12. *Sinularia leptoclados* holotype ZMB 304. A spindles of the surface layer of lobule B–D sclerites of the interior B spindles from the lobules C tuberculation of one of the lobule spindles D spindles from the base E tuberculation of two of the base spindles. Scale of 0.10 mm at A only applies to A.
Figure 13. *Sinularia leptoclados* holotype ZMB 304. Sclerites of the surface layer of the base of the colony. A *leptoclados*-type clubs B wart clubs C–D spindles. Scale of 0.10 mm at C only applies to C.
Figure 14. *Sinularia leptoclados* colonies. A ZMTAU Co 25763 B ZMTAU Co 25940.

Nyanda; ZMTAU Co 26314, Pangavinne Is., depth 6 m, coll. J.N. Nyanda, 1991; ZMTAU Co 26316, Mbudya Is., depth 5 m, coll. J.N. Nyanda, 1991; **Mozambique**; ZMTAU Co 28796, Bazaruto Is., Manta Reef, depth 15 m, coll. M. Schleyer, 7 Oc-
Sinularia leptoclados (Ehrenberg, 1834) (Cnidaria: Octocorallia) re-examined

tober 1994; Madagascar; RMNH Coel. 6653, Ankify, on mainland of Madagascar, opposite Nosy Komba, depth 1 m, 22 July 1967, coll. A.G. Humes (1183); RMNH Coel. 6654, Ankify, on mainland of Madagascar, opposite Nosy Komba, depth 1 m, 11 August 1967, coll. A.G. Humes (1250); RMNH Coel. 6655, Ankify, on mainland of Madagascar, opposite Nosy Komba, depth 1 m, 23 August 1967, coll. A.G. Humes (1320); RMNH Coel. 6659, Nosy Iranja, SW Nosy Bé, depth 15 m, 9 August 1967, coll. A.G. Humes (1239); RMNH Coel. 6660, W of Andilana, 13°18’S, 48°07’E, 20 m deep, 24 August 1967, coll. A.G. Humes (1331); RMNH Coel. 6661, Pass at Pte Lokobe, Nosy Bé, Madagascar, depth 15 m, 19 June 1967, coll. A.G. Humes (A28).

Description. The holotype is 18 cm high and 13 cm wide (Fig. 10A). The primary lobes branch off once or twice, lobules finger-shaped, up to 1 cm wide and 3 cm long. The polyps have a collaret and eight points. Points with poorly developed clubs, up to 0.13 mm long (Fig. 11A), collaret with bent spindles. Tentacle sclerites not observed.

The surface layer of the lobules has leptoclados-type clubs, the smallest are 0.05 mm long, most are around 0.10 mm, but some reach a length of 0.15 mm (Fig. 11B); in addition longer wart clubs are present, up to 0.20 mm long (Fig. 11C). Furthermore, the surface layer of the lobules has spindles, up to 0.45 mm long, with simple tubercles (Fig. 11D, 12A); the smaller ones with a distinct median waist.

The interior of the colony has unbranched spindles. In the lobules they are up to 2.5 mm long (Fig. 12B), with simple or complex tubercles (Fig. 12C). In the base of the colony the spindles are also up to 2 mm long (Fig. 12D), with more complex tubercles (Fig. 12E).

The sclerites of the surface layer of the base of the colony resemble those of the surface layer of the lobules but they are wider (Fig. 13).

Colour. The holotype is brown.

Intraspecific variation. Most of the colonies of S. leptoclados are stalked and rarely feature an encrusting colony shape (Fig. 14).

Remarks. Verseveldt (1980) re-examined ZMB 6495, the type specimen of Kolonko’s S. leptoclados var. gonatodes from the Bata islands (East coast of Palawan, Philippines), and considered it nothing else than S. leptoclados (Ehrenberg, 1834). Alderslade and Shirwaiker (1991) also re-examined ZMB 6495, assuming it was S. leptoclados, to differentiate their S. kavarattiensis. They noticed many small rods in the surface layer of the lobes. Unfortunately, neither Verseveldt nor Alderslade and Shirwaiker presented any figures of sclerites of Sinularia leptoclados var. gonatodes. Here we present such sclerites of the lobe surface (Fig. 15), which are more like those of S. maxima Verseveldt, 1971, and therefore we consider S. leptoclados var. gonatodes to be S. maxima. The main difference between S. leptoclados and S. maxima is not in the sclerites but concerns the much wider lobes of the latter (Fig. 10E). As a consequence,
Figure 15. *Sinularia leptoclados* var. *gonatodes* ZMB 6495 A point clubs B *leptoclados*-type clubs of surface layer of lobule C wart clubs of surface layer of lobule D spindles of surface layer of lobule.
Alderslade and Shirwaiker (1991) compared their S. kavarattiensis with S. maxima instead of with S. leptoclados. Their new species differs from both in having leptoclados-type clubs with an angle between the head and handle of about 90 degrees, thus considered to be valid.

One other species that can be confused with S. leptoclados is S. verseveldti Ofwegen, 1996. Its colony shape was described as being cup-shaped, but examination of many specimens from Indonesia showed that colony shape to be exceptional. Mostly the colonies resemble S. leptoclados very closely. Manuputty and Ofwegen (2007, fig. 2b, fig. 5) showed such a colony and its sclerites. The species differs in club shape, with the angle between the head and handle larger than 90 degrees in S. leptoclados and about 90 degrees in S. verseveldti. S. aff. leptoclados in Ofwegen (2009: 671) we now consider also to be S. verseveldti.

**Molecular Results**

Sequences for mtMutS and COI (including igr1) were available or newly obtained for 31 specimens representing 19 morphospecies of Sinularia belonging to clade 5C; 28S rDNA sequences were obtained for all but four specimens (Table 1). mtMutS (735 nt) and COI (888 nt) sequences were concatenated for a total mitochondrial gene alignment of 1623 nt. 28S sequences ranged from 797–799 nt in length for a total alignment length of 801 nt. Maximum likelihood and Bayesian analyses resulted in identical tree topologies for all three data sets (mt genes only, 28S only, all three genes combined). Support values were generally somewhat stronger for Bayesian analyses, however, and several nodes that were not supported by maximum likelihood (bootstrap values <50%) nonetheless had Bayesian posterior probabilities >0.9 (Figs 16, 17). All alignments and trees have been submitted to TreeBASE (www.treebase.org).

Within Sinularia species with leptoclados-type clubs (clade 5C), genetic distances (uncorrected p) among recognized morphospecies range from only 0–1.7% for mtMutS, 0–0.8% for COI and 0–1.4% for 28S rDNA. Despite these relatively low levels of genetic differentiation among taxa, several moderately- to well-supported clades appear in both the mitochondrial and 28S gene trees (Fig. 16). S. maxima and S. corpulentissima share identical mt and 28S haplotypes with one another, but are well differentiated from all other species in clade 5C. S. acuta, S. longula and S. molesta are also very similar to one another genetically (S. molesta and S. acuta share identical mt and 28S haplotypes), and form a well-supported clade in both trees. Finally, S. erecta is genetically distinct, separated from all other species by genetic distances of >0.8% at mtMutS (28S was not available for S. erecta).

Two additional clades are moderately supported by the combined analysis of the mt and 28S genes (Fig. 17); the species in these clades also group together in the separate analyses, but with low bootstrap support (<50%) (Fig. 16). S. penghuensis, S. bisulca, S. robusta, S. digitata and S. slievingi comprise one of these moderately-supported clades (Fig. 17); these species share identical or nearly identical 28S sequences.
Table 1. Specimens of *Sinularia* included in the molecular phylogenetic analyses. NTM = Museum and Art Gallery of the Northern Territory; RMNH = Naturalis Biodiversity Center; ZMTAU = Zoological Museum, Tel Aviv University. Bold = new GenBank accessions; NA = no sequence obtained.

| Species                     | Museum Acc. No. | ICO  | mtMutS   | 28S rDNA |
|-----------------------------|-----------------|------|----------|----------|
| *S. abrupta*                | NTM C14012      | KC542862 | KC542849 | NA       |
| *S. abrupta*                | ZMTAU Co 33623  | JX991256 | JX991168 | KC542822 |
| *S. acuta*                  | RMNH Coel. 38721| KC542863 | FJ621376 | NA       |
| *S. australiensis sp. n.*   | NTM C14492      | KC542864 | FJ621437 | KC542824 |
| *S. australiensis sp. n.*   | NTM C14519      | KC542865 | FJ621438 | KC542825 |
| *S. bisulca*                | RMNH Coel. 38724| KC542866 | FJ621378 | KC542826 |
| *S. corpulentissima*        | RMNH Coel. 40839| KC542867 | KC542850 | KC542827 |
| *S. daiti*                  | ZMTAU Co 34665  | JX991258 | JX991170 | KC542828 |
| *S. densa*                  | RMNH Coel. 40840| KC542868 | KC542851 | KC542829 |
| *S. digitata*               | RMNH Coel. 40841| KC542869 | KC542852 | KC542830 |
| *S. eilatensis sp. n.*      | ZMTAU Co 35260  | KC542870 | KC542853 | KC542831 |
| *S. eilatensis sp. n.*      | ZMTAU Co 35305  | KC542873 | KC542856 | KC542834 |
| *S. ?eilatensis sp. n.*     | ZMTAU Co 35303  | KC542871 | KC542854 | KC542832 |
| *S. ?eilatensis sp. n.*     | ZMTAU Co 35304  | KC542872 | KC542855 | KC542833 |
| *S. eilatensis sp. n.*      | ZMTAU Co 35304  | KC542872 | KC542855 | KC542833 |
| *S. erecta*                 | ZMTAU Co 34144  | GU355981 | FJ621404 | KC542835 |
| *S. gardineri (5A)*        | ZMTAU Co 34097  | GU355982 | FJ621414 | KC542819 |
| *S. hirta (5B)*            | ZMTAU Co 34100  | GU355983 | FJ621428 | KC542820 |
| *S. leptoclados*            | ZMTAU Co 35308  | KC542874 | KC542857 | KC542836 |
| *S. leptoclados*            | ZMTAU Co 34095  | GU355980 | FJ621439 | KC542837 |
| *S. longula*                | RMNH Coel. 38439| KC542875 | FJ621441 | KC542838 |
| *S. maxima*                 | NTM C14512      | KC542876 | FJ621448 | KC542839 |
| *S. molesta*                | RMNH Coel. 38440| KC542877 | FJ621449 | NA       |
| *S. penghuensis*            | ZMTAU Co 34659  | JX991273 | JX991183 | KC542840 |
| *S. penghuensis*            | ZMTAU Co 34681  | JX991274 | JX991184 | KC542841 |
| *S. penghuensis*            | ZMTAU Co 34739  | JX991276 | JX991186 | KC542842 |
| *S. robusta*                | NTM C14518      | KC542878 | FJ621473 | KC542843 |
| *S. slieringsi*             | ZMTAU Co 34654  | JX991277 | JX991187 | NA       |
| *S. terspilli (5B)*         | ZMTAU Co 34156  | GU355984 | FJ621481 | KC542821 |
| *S. verseveldti (5B)*       | ZMTAU Co 35309  | KC542879 | KC542858 | KC542844 |
| *S. verseveldti*            | RMNH Coel. 40842| KC542880 | KC542859 | KC542845 |
| *S. verseveldti*            | RMNH Coel. 40843| KC542881 | KC542860 | KC542846 |
| *S. verseveldti*            | RMNH Coel. 40844| KC542882 | KC542861 | KC542847 |
| *S. wanannensis*            | ZMTAU Co 34704  | JX991281 | JX991190 | KC542848 |

(28S was not available for *S. slieringsi* (Fig. 16b). Within the mt gene tree (Fig. 16a) they constitute two distinct clades, one comprised by *S. robusta*, *S. digitata* and *S. slieringsi* and the other by *S. penghuensis*, *S. bisulca* and *S. daiti*. The latter is, however, distinct from all other species at 28S, and falls outside of this clade in the combined analysis (Fig. 17). *S. leptoclados*, *S. abrupta*, *S. australiensis* sp. n. and *S. densa* also
Figure 16. Maximum likelihood trees of *Sinularia* clade 5C (McFadden et al. 2009) based on (a) combined analysis of two mitochondrial genes (*mtMutS, COI*), and (b) nuclear 28S rDNA. Specimens described in this publication in bold. Numbers above branches are bootstrap values from maximum likelihood analysis (only values >50% shown; ns = value <50%); numbers below branches are Bayesian posterior probabilities (only values > 0.85 shown).

form a moderately-supported clade in the 28S tree (Fig. 16b) and in the combined tree (supported by Bayesian but not maximum likelihood analyses; Fig. 17), but their relationship is unresolved in the mt tree (Fig. 16a). *S. australiensis* sp. n. and *S. abrupta* share identical 28S haplotypes, but differ from *S. leptoclados* by 0.3%. *S. australiensis* sp. n. differs from both *S. leptoclados* and *S. abrupta* by 0.1% and 0.1-0.2% at *mtMutS* and *COI* respectively.

The relationships among the remaining species in the clade — *S. verseveldti*, *S. wanannensis* and *S. eilatensis* sp. n. — were poorly resolved and exhibited some incongruence between the mitochondrial and 28S gene trees. *S. wanannensis*, all four specimens of *S. verseveldti*, and two specimens (ZMTAU Co 35303, ZMTAU Co 35304) that were tentatively assigned to *S. eilatensis* sp. n. share identical or nearly identical *mt-
MutS and COI haplotypes, and cluster together within the mt tree (but with bootstrap values <50%). Two specimens of S. eilatensis sp. n. (ZMTAU Co 35305, ZMTAU Co 35260) fall outside of that group, and differ from it by >0.5% at mtMutS (Fig. 16a). At 28S, however, ZMTAU Co 35303 and ZMTAU Co 35304 are genetically identical to both individuals of S. eilatensis sp. n., and those four specimens form a moderately-supported clade together with S. verseveldti ZMTAU Co 35309 (Fig. 16b). Two additional specimens of S. verseveldti share identical 28S haplotypes with S. wanannensis.

The combined tree reflects the topology of the mt gene tree, and shows the separation of S. eilatensis sp. n. (ZMTAU Co 35305, ZMTAU Co 35260) from ZMTAU Co 35303, ZMTAU Co 35304 and all other species (Fig. 17).

Our findings indicate that specimens of the same species generally shared identical or nearly identical sequences at all three loci. The only exceptions were the two distinct mitochondrial haplotypes of S. eilatensis sp. n. discussed above, and the four specimens of S. verseveldti. All S. verseveldti shared identical or nearly identical mtMutS and COI
sequences, but differed at 28S. Most of these differences, however, reflected polymorphic nucleotide positions at which one or more specimens exhibited heterozygosity. For example, at position 533 of the 28S alignment, ZMTAU Co 35309 and Coel. 40842 had C, Coel. 40843 had T, and Coel. 40844 had both C and T. A total of 8 such heterozygous nucleotide sites among the four *S. verseveldti* specimens contribute to their disjunct distribution within the 28S and combined trees.

**Discussion**

The two new species described here are supported both by morphological characters and by the molecular analysis. Although *S. australiensis* sp. n. is similar genetically to *S. leptoclados* and both belong to the same sub-clade within *Sinularia* clade 5C, they differ at all three of the loci sequenced here. Furthermore, the 28S and combined analyses suggest that *S. leptoclados* and *S. australiensis* sp. n. are not sister taxa, but that *S. australiensis* sp. n. is closer to *S. abrupta*, a species with which it shares a 28S haplotype. The disjunct geographical distribution between *S. leptoclados*, which occurs in the Red Sea and western Indian Ocean, and *S. australiensis* sp. n. from Australia, further supports their distinction.

Although sympatric with *S. leptoclados* in the Red Sea, *S. eilatensis* sp. n. is clearly distinct from that species, both morphologically and genetically. Within clade 5C, *S. eilatensis* sp. n. is most similar genetically to the geographically widespread *S. verseveldti* and to *S. wanannensis*, a species recently described from Taiwan (Ofwegen and Benayahu 2012). Morphologically, however, *S. eilatensis* clearly differs from *S. verseveldti* and *S. wanannensis* by its long polyp sclerites, up to 0.25 mm long in *S. eilatensis* vs up to 0.15 mm long in the other two species. Undoubtedly, the phylogenetic relationships among these three species need further investigation. In particular, the conflicting phylogenetic signals obtained from the mitochondrial and nuclear genes suggest the possibility of past hybridization events between *S. verseveldti* and *S. eilatensis* sp. n. Two specimens from the Red Sea (ZMTAU Co 35030 and ZMTAU Co 35304) appear morphologically to belong to *S. eilatensis* sp. n. and have the same 28S sequence as that species but share a distinct mitochondrial haplotype with *S. verseveldti*. This observed mito-nuclear discord could reflect a hybrid origin of these specimens, as has been suggested for some other octocorals (reviewed in McFadden et al. 2010). In addition, the polymorphism observed at the 28S locus in *S. verseveldti* could be indicative of recent hybridization events involving this species, although it could also be the result of incomplete lineage sorting following recent speciation. The possible hybrid origin of ZMTAU Co 35303 and ZMTAU Co 35304 should be investigated further using single-copy nuclear gene markers.

Previous molecular systematic work on *Sinularia* and other octocoral genera has highlighted the inadequacies of mitochondrial gene markers for species discrimination and species-level phylogenetic analyses in the group (McFadden et al. 2009, 2011). Although both *mtMutS* and *COI* effectively distinguish genera and distinct clades within genera, neither gene is variable enough to distinguish all congeneric
species pairs unequivocally. The region of the nuclear 28S rDNA gene we sequenced exhibits somewhat greater variability than mtMutS in some genera of the family Alcyoniidae (Benayahu et al. in press), but did not distinguish among all of the morphospecies of Sinularia examined in the current study. Despite the relatively small genetic distances separating morphospecies and the low resolution of the resulting phylogenies, we believe the analysis presented here adequately supports the distinctions of the new species that are the focus of this study. Development of additional, more variable molecular markers, will be necessary in order to fully resolve the relationships among morphospecies in Sinularia clade 5C and to address the possibility of hybridization among them.

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