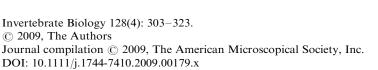# Invertebrate Biology

Molecular systematics of the speciose Indo-Pacific soft coral genus, *Sinularia* (Anthozoa: Octocorallia)

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Abstract. The speciose tropical soft coral genus *Sinularia* traditionally has been divided into five intrageneric taxonomic groups based on variation in a single morphological character: the shape of the club sclerites (calcite skeletal elements) embedded in the surface tissues of the colony. To test the phylogenetic utility of this system of classification, we used a 735-bp fragment of the octocoral-specific mitochondrial *msh1* gene to construct a molecular phylogeny that included 80 of the ~150 recognized morphospecies of *Sinularia*. The *msh1* phylogeny recovered five well-supported clades, but they were not congruent with the traditional intrageneric taxonomic groups. Mapping of characters onto the tree suggested that the five major clades plus several additional sub-clades of *Sinularia* can be distinguished based on a suite of four morphological characters; these include the presence of sclerites in the tentacle, collaret, and point regions of the polyps, in addition to the shape of the club sclerites in the surface tissues. The overall growth form of the colony also distinguishes some clades. Polyp sclerites have for the most part been overlooked taxonomically in *Sinularia,* and as a result information on these characters is lacking or is incorrect in many species descriptions. As has been the case in other recent studies of lower metazoan groups, construction of a molecular phylogeny has led us to recognize the phylogenetic and taxonomic importance of previously overlooked morphological characters. A revised taxonomic key that includes these characters is already improving our ability to discriminate species boundaries, and facilitating description of new *Sinularia* species.

Additional key words: Alcyoniidae, molecular phylogenetics, taxonomy, *msh1*

Use of molecular systematic approaches continues to revolutionize our understanding of evolutionary relationships within and among higher taxa in the lower metazoan phyla, in particular, the cnidarians and sponges. Demosponges, scleractinian corals, and octocorals are three major groups in which molecular data are necessitating a radical reappraisal of ordinal- and family-level taxonomy (Romano & Palumbi 1996; Fukami et al. 2004, 2008; Boury-Esnault 2006; McFadden et al. 2006b). Historically, taxonomic classification and phylogenetic inference in these groups has been challenging because of their relative morphological simplicity and a consequent paucity

of characters suitable for analysis (e.g., Bayer 1956). Many of the morphological characters traditionally used for higher level classification in these taxa have now been shown to be discordant with molecular phylogenetic evidence, and likely represent homoplasies.

In many cases in which there is a disagreement between classification based on traditional morphology-based and molecular phylogenetic approaches, careful re-examination of morphology has revealed new sets of characters that are indeed congruent with the molecular data. For example, new sets of phylogenetically informative microskeletal characters have been discovered for the scleractinian families Faviidae (Fukami et al. 2004) and Mussidae (Budd & Stolarski 2009) after molecular studies showed the traditional taxonomy of these groups to be incorrect
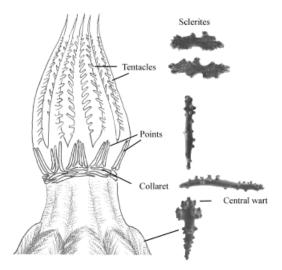
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(Fukami et al. 2004, 2008). In Octocorallia, molecular studies clearly indicate that the traditional morphology-based sub-ordinal and family-level taxonomy does not reflect phylogenetic relationships (McFadden et al. 2006b). Re-examination of some unexpected relationships has, however, facilitated identification of new morphological characters that are congruent with molecular data. For example, by mapping morphological characters onto a molecular phylogeny, Sánchez et al. (2003) found that the characters that have traditionally been considered most important for classifying octocorals belonging to the holaxonian group of sea fans (axial structure and the size and shape of surface sclerites) were not congruent with the phylogeny, whereas several characters heretofore considered of only minor taxonomic importance (presence of polyp sclerites and sclerite ornamentation) were diagnostic for the major molecular clades. Similarly, in their analysis of a cryptic genus of soft corals revealed by molecular data, McFadden et al. (2006a) showed that several previously overlooked morphological characters (polyp sclerites and subtle aspects of colony growth form) clearly separate this genus from two other genera with which its members had been confounded. In the present study, we use a similar character-mapping approach to identify morphological characters congruent with sub-generic phylogenetic relationships in the speciose soft coral genus *Sinularia* MAY 1898.

Members of the genus *Sinularia* are among the most widespread and commonly encountered octocorals on Indo-Pacific coral reefs (Benayahu & Loya 1977; Tursch & Tursch 1982; Dinesen 1983; Riegl et al. 1995; Fabricius 1997). Their fleshy colonies often attain a large size and monopolize extensive areas of shallow reef habitat (Benayahu & Loya 1981; Fabricius 1995, 1997; Fabricius & Dommisse 2000; Bastidas et al. 2004), particularly in disturbed, near-shore areas where they are sometimes considered invasive (Fabricius 1998). Despite their ecological dominance in some habitats, species of *Sinularia* have also been impacted severely by recent coral bleaching events, suffering mortality rates >90% in some locations (Fabricius 1999; Bruno et al. 2001; Loya et al. 2001). Work on the ecology of this important group has, however, been hampered by taxonomic uncertainties and difficulty in distinguishing species in the field, limiting most studies to the generic level (e.g., Fabricius 1995). Colony growth morphology is often highly variable (Benayahu et al. 1998), even among parts of the same colony (Alderslade & Shirwaiker 1991; Benayahu 1998), and examination of microscopic sclerites (calcite skeletal elements) in the tissues is required to distinguish species reliably. Many

species remain undescribed, while the validity of others is uncertain (van Ofwegen 2002).

Until now, most taxonomic work on *Sinularia* has followed the protocol of Verseveldt (1980), who published the last comprehensive taxonomic revision of the genus. The two characters that he considered the most important for taxonomy and that are the exclusive focus of his and most subsequent studies are: (1) the morphology of the club-shaped sclerites found in the surface layer of the colony and (2) the overall colony growth form as assessed primarily from preserved material. In his revision, Verseveldt (1980) presented a key in which he subdivided *Sinularia* into five groups based on characteristics of the club sclerites, a system of classification that taxonomists have continued to use (e.g., Verseveldt & Benayahu 1983; Alderslade 1987; Alderslade & Baxter 1987; van Ofwegen & Vennam 1991; Benayahu 1993; van Ofwegen 2001). Verseveldt's Group I is the most distinctive of these five, having club sclerites with a characteristic shape referred to as "*leptoclados*-type" clubs after the nominate species in the group, *Sinularia leptoclados* (EHRENBERG 1834). Group II includes species in which the sclerites are not of the *leptoclados*-type, but instead have a central wart (Fig. 1). Groups III and IV have club sclerites that lack a central wart and are distinguished from one another by size (Group III: clubs <0.12 mm long; Group IV: clubs >0.12 mm long). Species in Group V have few or no club sclerites on the colony surface.

van Ofwegen (2002) summarized the current taxonomic status of *Sinularia*, and recognized 128 valid species. Since then, an additional 26 species of this

Fig. 1. Illustration of an octocoral polyp indicating the locations and types of sclerites typically found in genus *Sinularia*. Modified from Bayer et al. (1983).

genus have been described (Manuputty & van Of-wegen 2007; van Ofwegen 2008a,b), bringing the total to ~150. This recent taxonomic explosion has been fueled in part by the recognition that preserved specimens may not retain important aspects of colony growth form that can be observed easily *in situ*. Species whose colony form differs markedly when alive are often transformed by preservation such that their differences are less apparent (van Ofwegen 2008b), and as a result many distinct species have gone unrecognized. Careful documentation of colony growth form *in situ* is now improving taxonomic resolution as well as facilitating identification of species in the field (van Ofwegen 2008b).

A recent molecular phylogeny of the related alcyoniid genera *Sarcophyton* LESSON 1834 and *Lobophytum* VON MARENZELLER 1886 revealed unexpected clades (including a previously unsuspected third genus), and subsequently led to the identification of a new set of morphological characters for taxonomy (McFadden et al. 2006a). In particular, the presence, distribution, and form of sclerites in the polyps, characters that were rarely mentioned by Verseveldt (1980, 1982, 1983) in his revisions, were discovered to be phylogenetically and taxonomically informative characters for these genera. In the present study, we construct a molecular phylogeny for the genus *Sinularia* using the same genetic marker (the octocoral-specific mitochondrial protein-coding gene *msh1*) used effectively in our work on *Sarcophyton* and *Lobophytum* (McFadden et al. 2006a). We test for congruence between the molecular phylogeny and Verseveldt's (1980) taxonomic groups, and subsequently use the phylogeny to identify a new suite of morphological characters that distinguish five major clades and several distinct sub-clades within the genus *Sinularia*.

Methods

Specimens of *Sinularia* were collected and identified by the authors from Ambon, Moluccas, Indonesia (November 1996); Gulf of Carpentaria, NT, Australia (December 2003); Republic of Palau (May 2005); and Eilat, Israel (July 2007). Taxonomic accounts and descriptions of new species have been published for the Gulf of Carpentaria, Moluccas, and Palau collections (Manuputty & van Ofwegen 2007; van Ofwegen 2008a,b). Specimens have been deposited in the Museum and Art Gallery of the Northern Territory, Australia (NTM); the Nationaal Natuurhistorish Museum, Leiden (NNM, formerly RMNH); and the Tel Aviv University Zoological Museum, Israel (ZMTAU) (Appendix 1). Additional species of *Sinularia* were obtained from collections in the NTM and the Florida Natural History Museum (UF), a majority of them collected throughout the Indo-Pacific from 1995 to 2002 by the Coral Reef Research Foundation, Republic of Palau, and identified by PA.

Extraction of DNA from ethanol-preserved tissue samples, PCR amplification, and sequencing of the *msh1* gene followed the protocols published in McFadden et al. (2006a). Sequence data were proofread using LaserGene software (DNASTAR, Inc., Madison, WI, USA), and aligned using Muscle v. 3.6 (Edgar 2004). PhyML (Guindon & Gascuel 2003) was used to construct maximum likelihood trees using a $GTR+I+\Gamma$ model of substitution with 100 bootstrap replicates. Maximum parsimony analyses were run using TNT (Goloboff et al. 2000) with default parameters and 100 bootstrap replicates. Bayesian analysis was conducted using MRBAYES v 3.1 (Huelsenbeck & Ronquist 2001) with a $GTR+I+\Gamma$ model run for 5×10^6 generations (burnin = 10^6 generations). PAUP* (Swofford 2002) was used to compute pairwise genetic distances (Kimura 2-parameter) among taxa, and to generate maximum likelihood scores for trees constrained to reflect monophyly of Verseveldt's (1980) five taxonomic groups. Constrained trees were compared with the best-fit maximum likelihood tree using the Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa 1999) with 10,000 RELL bootstrap replicates. Species of *Sarcophyton, Lobophytum,* and *Dampia* ALDERSLADE 1983 were included as outgroup taxa in all analyses; molecular analyses of relationships within Octocorallia support these three genera as the clades most closely related to *Sinularia* (McFadden et al. 2006b).

Following phylogenetic reconstruction, morphological character states were mapped onto the tree manually. Specific characters examined included the presence, form, and size of the sclerites in the tentacles, collaret and point regions of the polyp (Fig. 1; see Bayer et al. 1983 for further definitions of anatomical terms); presence, shape, and size of the club sclerites in the colony surface tissues; and overall colony growth form. When information on particular characters (especially polyp sclerites) could not be found in published taxonomic descriptions or conflicted with the observed phylogenetic distribution of traits, we re-examined original material if it was available, including type specimens and the permanent sclerite slides prepared by Verseveldt, now kept at NNM.

Results

A total of 735 nucleotides of the *msh1* sequence were obtained for 119 specimens representing 80

morphospecies of *Sinularia* (Appendix 1). No indels were present, and nucleotide alignments were unambiguous. All phylogenetic methods supported the same tree topology (Fig. 2). We identified five major clades of *Sinularia,* each supported by bootstrap values >90% and Bayesian posterior probabilities >0.95. The two largest clades (4, 5) each could be further subdivided into three or four sub-clades; the three sub-clades within clade 5 were supported strongly by all analyses, whereas only one of the four sub-clades identified within clade 4 was well supported by all phylogenetic methods (Fig. 2). All analyses recovered clade 1 as the sister clade to all other *Sinularia* species. The phylogenetic relationships among the other four major clades, however, were unresolved (Fig. 2).

Clade 1 includes four specimens: three identified as *Sinularia brassica* MAY 1898, and one as *Sinularia dura* (PRATT 1903). These two species, which differ only in colony growth form, have been synonymized previously (Benayahu et al. 1998), although the two distinct haplotypes we found are suggestive of two species (Fig. 2). The shape of the club sclerites (very wide heads with no central wart) is unique within the genus (Fig. 3E, b), as is the enormous variation in colony growth form that these species exhibit (Benayahu et al. 1998). Clade 1 is further distinguished from the other four major clades by the presence of scales in the tentacles (Fig. 3E, a). Point and collaret sclerites are absent (Table 1).
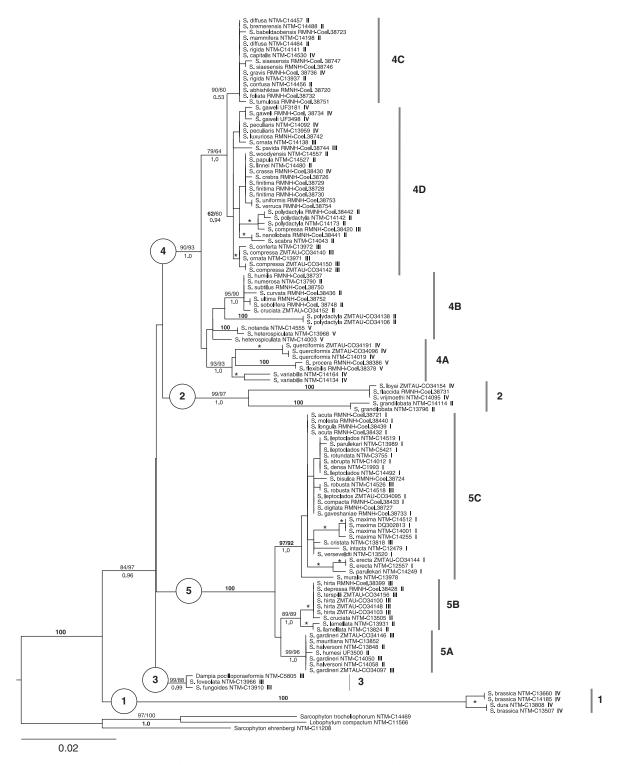
Clade 2 is formed by *Sinularia vrijmoethi* VERSEVELDT 1971, *Sinularia flaccida* VAN OFWEGEN 2008b, *Sinularia loyai* VERSEVELDT & BENAYAHU 1983, and *Sinularia grandilobata* VERSEVELDT 1980. All four species have in common a well-developed collaret and points (collaret spindles to 0.25–0.4 mm length [Fig. 3D, b]; point clubs to 0.17–0.28 mm length [Fig. 3D, a]) as well as rods in the tentacles (Table 1). On the colony surface, they all have club sclerites with a central wart (see Fig. 1) that is somewhat obscured by three lateral warts (Fig. 3D, c). *Sinularia vrijmoethi, S. flaccida,* and *S. loyai* are very similar in having long clubs (averaging 0.26, 0.29, and 0.30 mm, respectively); *S. grandilobata,* which forms a sister clade to the other three species, has much shorter clubs (0.10 mm). *Sinularia grandilobata* also has an encrusting colony growth form, while the other three species are stalked.

Clade 3 is formed by *Sinularia foveolata* VERSEVELDT 1974, *Sinularia fungoides* THOMSON & HENDERSON 1906, and the monotypic species *Dampia pocilloporaeformis* ALDERSLADE 1983, which falls within genus *Sinularia* rather than within the other outgroup taxa (Fig. 2). Verseveldt (1980) classified

the two former species in his Group III: "most clubs 0.06–0.12 mm long, not of the *leptoclados*-type, and without central wart." However, all three species clearly have clubs with a central wart, although it is mostly nestled within the three lateral warts below it, which tend to obscure it and give the club head a triangular shape (Fig. 3C). This unique club shape is diagnostic for clade 3. In addition, species in this clade have indistinct points formed by rod-like sclerites (see Alderslade 1983: fig. 4J–M; Alderslade 1987: fig. 9).
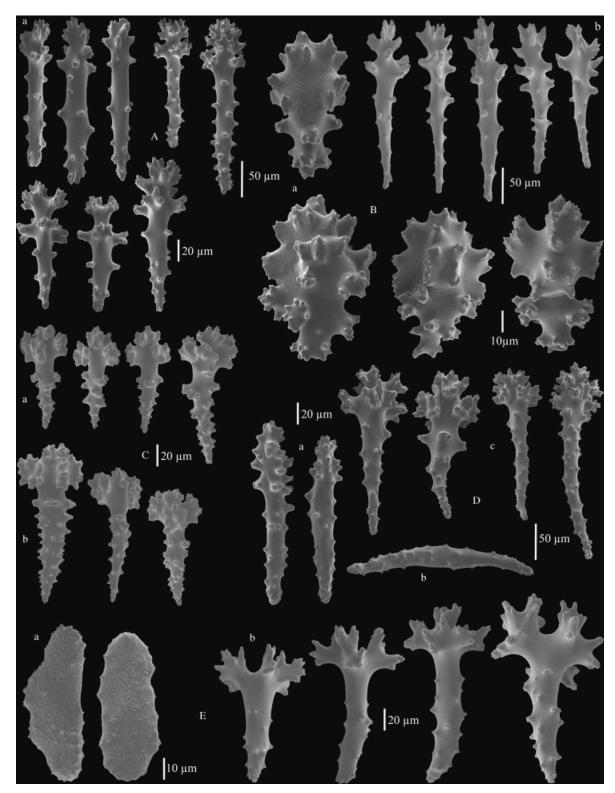
Clade 4 is the largest clade in the genus (Fig. 2). Species belonging to this group have clubs with a central wart, and lack collaret and tentacle sclerites (but see "Discussion"); the majority also lack point sclerites. Clade 4 can be divided into four sub-clades (4A–D) that vary in support values but can be distinguished morphologically (Table 1). Sub-clade 4A is the most distinct of these groups genetically, and is supported by bootstrap values >90%. It includes *Sinularia querciformis* (PRATT 1903) and *Sinularia variabilis* TIXIER-DURIVAULT 1945, both of which Verseveldt (1980) classified as Group IV ("clubs with no central wart"), as well as *Sinularia flexibilis* (QUOY & GAIMARD 1833) and *Sinularia procera* VERSEVELDT 1977, placed by Verseveldt in Group V ("No [or hardly any] clubs in the surface layer of the lobes"). *Sinularia querciformis* and *S. variabilis* do have clubs with a central wart, but this central wart is often leaf-like, obscuring the arrangement (Fig. 3B, b). *Sinularia procera* and *S. flexibilis* normally have no clubs on the surface layer of the lobes (Verseveldt 1977, 1980). van Ofwegen & Vennam (1994), however, found a specimen of *S. flexibilis* from Ambon that had surface clubs, mostly with a central wart and not unlike those of *S. querciformis* and *S. variabilis*. All four species in sub-clade 4A have a similar colony growth form with a stalk.

Sub-clade 4B is formed by *Sinularia heterospiculata* VERSEVELDT 1970, *Sinularia notanda* TIXIER-DURIVAULT 1966, *Sinularia humilis* VAN OFWEGEN 2008b, *Sinularia numerosa* TIXIER-DURIVAULT 1970, *Sinularia sublimis* VAN OFWEGEN 2008b, *Sinularia curvata* MANUPUTTY & OFWEGEN 2007, *Sinularia ultima* VAN OFWEGEN 2008b, *Sinularia sobolifera* VERSEVELDT & TURSCH 1979, *Sinularia cruciata* TIXIER-DURIVAULT 1970, and *Sinularia polydactyla* (EHRENBERG 1834). Although support for monophyly was lacking for this group, all of these species have in common the presence of point sclerites (Fig. 3A, a), a character that distinguishes them from other members of clade 4.

Sub-clades 4C and 4D include a large number of species (Table 1) that all lack sclerites in the polyps

Fig. 2. Maximum likelihood tree of genus *Sinularia* constructed using 735 bp of the mitochondrial gene *msh1*. Major clades discussed in the text are indicated by circled numbers, and sub-clades by vertical bars to the right of node labels. Numbers above branches indicate bootstrap values from maximum likelihood (left) and maximum parsimony (right) analyses; numbers below branches are Bayesian posterior probabilities. 100, both bootstrap values 100 and Bayesian credibility 1.0; Asterisk, bootstrap values > 70 and posterior probabilities > 0.90. Roman numerals following specimen names indicate the sub-generic taxonomic group to which a species has been assigned traditionally based on the classification of Verseveldt (1980).

Fig. 3. Typical morphology of sclerites from the colony surface and polyps of *Sinularia,* clades 1 to 4B. **A.** Clade 4B: *Sinularia humilis* RMNH Coel. 38737; a, point clubs. **B.** Clade 4A: a, *Sinularia flexibilis,* clubs from the base of the colony (uncatalogued colony from Raja Ampat, Indonesia); b, *Sinularia querciformis* RMNH Coel. 34308. **C.** Clade 3: a, *Sinularia fungoides* RMNH Coel. 34321; b, *Dampia pocilloporaeformis* RMNH Coel. 19843. **D.** Clade 2: *Sinularia flaccida* RMNH Coel. 38731; a, point clubs; b, collaret spindle; c, surface clubs. **E.** Clade 1: *Sinularia brassica* RMNH Coel. 34304; a, tentacle scales; b, surface clubs.

Table 1. Summary of diagnostic morphological characteristics of clades of *Sinularia* identified in the molecular phylogeny (Fig. 2). Confirmed clade members = species included in the phylogeny. Additional species not included in the phylogeny, but predicted to belong to each clade on the basis of morphology: 2: *S. dactyloclados, S. elongata, S. licroclados, S. macrodactyla, S. manaarensis, S. parva, S. schumacheri.* 3: *S. molokaiensis. S. megasclera,* 4A: *S. arborea, S. asterolobata, S. flabelliclavata, S. inexplicita, S. portieri.* 4B: *?S. barcaformis, S. capillosa, S. macropodia, S. mira, S. mollis, ?S. muqeblae, S. platylobata, S. sandensis.* 4C/4D: *S. acetabulata, S. agilis, S. deformis, S. discrepens, S. flexuosa, S. gibberosa, S. higae, S. incompleta, S. inflata, S. microclavata, S. ovispiculata, S. paulae, S. tanakai, S. venusta, S. yamazatoi.* 5A/B: *S. crustaformis, S. granosa, S. lochmodes, S. ramosa, S. recurvata, S. slieringsi.* 5C: *S. corpulentissima, S. dissecta, S. exilis, S. facile, S. firma, S. fishelsoni, S. inelegans, S. jasminae, S. kavarattiensis, S. microspiculata, S. vanderlandi.*

Clade	Confirmed clade members	Points	Collaret	Tentacle sclerites	Surface clubs	Other
1	*S. brassica* MAY 1898 *S. dura* (PRATT 1903)	No	No	Yes, scales	Wide heads	Variable colony shape
2	*S. flaccida* VAN OFWEGEN 2008b *S. grandilobata* VERSEVELDT 1980 *S. loyai* VERSEVELDT & BENAYAHU 1983 *S. vrijmoethi* VERSEVELDT 1971	Yes, strong	Yes, strong	Yes, rods	Central wart obscured by three lateral warts	
3	*D. pocilloporaeformis* ALDERSLADE 1983 *S. foveolata* VERSEVELDT 1974 *S. fungoides* THOMSON & HENDERSON 1906	Yes, indistinct	No	No	Central wart obscured by three lateral warts	
4A	*S. flexibilis* (QUOY & GAIMARD 1833) *S. procera* VERSEVELDT 1977 *S. querciformis* (PRATT 1903) *S. variabilis* TIXIER-DURIVAULT 1945	No	No	No	Central wart often leaf-like; or no surface clubs	Colony has a stalk
4B	*S. cruciata* TIXIER-DURIVAULT 1970* *S. curvata* MANUPUTTY & OFWEGEN 2007 *S. heterospiculata* VERSEVELDT 1970 *S. humilis* VAN OFWEGEN 2008b *S. notanda* TIXIER-DURIVAULT 1966 *S. numerosa* TIXIER-DURIVAULT 1970 *S. polydactyla* (EHRENBERG 1834)* *S. sobolifera* VERSEVELDT & TURSCH 1979 *S. sublimis* VAN OFWEGEN 2008b *S. ultima* VAN OFWEGEN 2008b	Yes	No	No	Central wart distinct	
4C	*S. abhishiktae* OFWEGEN & VENNAM 1991 *S. babeldaobensis* VAN OFWEGEN 2008b *S. bremerensis* VAN OFWEGEN 2008a *S. capitalis* (PRATT 1903)	No	No	No	Central wart distinct, warts often leaf-like	

Table 1.   (cont'd).

Clade	Confirmed clade members	Points	Collaret	Tentacle sclerites	Surface clubs	Other
	S. confusa VAN OFWEGEN 2008a					
	S. diffusa VAN OFWEGEN 2008a					
	S. foliata VAN OFWEGEN 2008b					
	S. gravis TIXIER-DURIVAULT 1970					
	S. mammifera MALYUTIN 1990					
	S. rigida (DANA 1846)					
	S. siaesensis VAN OFWEGEN 2008b					
	S. tumulosa VAN OFWEGEN 2008b					
4D	*S. compressa* TIXIER-DURIVAULT 1945	No	No	No	Central wart distinct	
	S. conferta (DANA 1846)					
	S. crassa TIXIER-DURIVAULT 1945					
	S. crebra VAN OFWEGEN 2008b					
	S. finitima VAN OFWEGEN 2008b					
	S. gaweli VERSEVELDT 1978					
	S. linnei VAN OFWEGEN 2008a					
	S. luxuriosa VAN OFWEGEN 2008b					
	S. nanolobata VERSEVELDT 1977					
	S. ornata TIXIER-DURIVAULT 1970					
	S. papula VAN OFWEGEN 2008a					
	S. pavida TIXIER-DURIVAULT 1970					
	S. polydactyla (EHRENBERG 1834)*					
	S. scabra TIXIER-DURIVAULT 1970					
	S. uniformis VAN OFWEGEN 2008b					
	S. verruca VAN OFWEGEN 2008b					
	S. woodyensis VAN OFWEGEN 2008a					
5A	*S. gardineri* (PRATT 1903)	Yes	Yes	Yes, scales	Central wart distinct	
	S. halversoni VERSEVELDT 1974					
	S. humesi VERSEVELDT 1968					
	S. mauritiana VENNAM & PARULEKAR 1994					
5B	*S. cruciata* TIXIER-DURIVAULT 1970*	Yes	Yes	Yes, rods	Central wart distinct	
	S. depressa TIXIER-DURIVAULT 1970					
	S. hirta (PRATT 1903)					
	S. lamellata VERSEVELDT & TURSCH 1979					
	S. terspilli VERSEVELDT 1971					

Table 1. (cont'd).

Clade	Confirmed clade members	Points	Collaret	Tentacle sclerites	Surface clubs	Other
5C	*S. abrupta* TIXIER-DURIVAULT 1970	Yes	Yes	?rods	*Leptoclados-* type clubs	
	S. acuta MANUPUTTY & OFWEGEN 2007					
	S. bisulca VAN OFWEGEN 2008b					
	S. compacta TIXIER-DURIVAULT 1970					
	S. cristata TIXIER-DURIVAULT 1969					
	S. densa (WHITELEGGE 1897)					
	S. digitata VAN OFWEGEN 2008b					
	S. erecta TIXIER-DURIVAULT 1945					
	S. gaveshaniae ALDERSLADE & SHIRWAIKER 1991					
	S. intacta TIXIER-DURIVAULT 1970					
	S. leptoclados (EHRENBERG 1834)					
	S. longula MANUPUTTY & OFWEGEN 2007					
	S. maxima VERSEVELDT 1971					
	S. molesta TIXIER-DURIVAULT 1970					
	S. muralis MAY 1899					
	S. parulekari ALDERSLADE & SHIRWAIKER 1991					
	S. robusta MACFADYEN 1936					
	S. rotundata TIXIER-DURIVAULT 1970					
	S. verseveldti OFWEGEN 1996					

*Different specimens of *S. polydactyla* and *S. cruciata* exhibit *msh1* haplotypes characteristic of two different clades or sub-clades.

and have clubs with a distinct central wart. Maximum likelihood analysis supported the monophyly of sub-clade 4C (bootstrap value = 90%), but other phylogenetic methods did not support the distinction of sub-clades 4C and 4D (Fig. 2). Nonetheless, there is often a detectable, albeit subtle, difference in the typical shape of the club sclerites between these two groups, with those in sub-clade 4C having more leaf-like warts (Fig. 4D,E).

Two very different *msh1* haplotypes were found in specimens identified as *S. polydactyla:* one belonging to sub-clade 4B and the other to 4D (Fig. 2). Specimens collected from the Red Sea (type locality of the species) that were present in the NNM and identified as *S. polydactyla* show the normal point clubs of sub-clade 4B, while a re-examination of RMNH-Coel. 38442 found no polyp sclerites whatsoever, consistent with its placement in sub-clade 4D.

Clade 5 is another large clade that can be divided into three sub-clades, each with high support values (Fig. 2). All of the species in clade 5 have a collaret and point sclerites present in the polyps. Sub-clade 5A includes *Sinularia gardineri*, *Sinularia halversoni* VERSEVELDT 1974, *Sinularia mauritiana* VENNAM & PARULEKAR 1994, and *Sinularia humesi* VERSEVELDT 1968, all of which have club sclerites with a distinct central wart (Fig. 4C, c). *Sinularia gardineri*, *S. halversoni*, and *S. humesi* also have many scales in

Fig. 4. Typical morphology of sclerites from the colony surface and polyps of *Sinularia,* clades 4C to 5C. **A.** Clade 5C; a–c, *Sinularia digitata* VAN OFWEGEN 2008b RMNH Coel.38727; a, point clubs; b, collaret spindle; c, surface clubs; d, *Sinularia acuta* RMNH Coel. 38432. **B.** Clade 5B: *Sinularia depressa* RMNH Coel. 38399; a, point clubs; b, collaret spindle; c, surface clubs. **C.** Clade 5A: *Sinularia humesi* RMNH Coel. 38418; a, point clubs; b, collaret spindle; c, surface clubs; d, tentacle scales. **D.** Clade 4D: *Sinularia uniformis* VAN OFWEGEN 2008b RMNH Coel. 38753. **E.** Clade 4C: *Sinularia foliata* VAN OFWEGEN 2008b RMNH Coel. 38732.

the tentacles (Fig. 4C, d), the latter two species even sharing a particular type of tentacle scale (see Manuputty & van Ofwegen 2007: fig. 13A). The tentacle scales of *S. gardineri* are less pronounced. *Sinularia mauritiana* has been described as lacking polyp sclerites (Vennam & Parulekar 1994), but we were unable to verify that character state for the specimen included here.

Sub-clade 5B includes *Sinularia lamellata* VERSE-VELDT & TURSCH 1979, *S. cruciata* NTM-C13505, *Sinularia hirta* (PRATT 1903), *Sinularia depressa* TIX-IER-DURIVAULT 1970, and *Sinularia terspilli* VERSE-VELDT 1971. Like sub-clade 5A, these species all have club sclerites with a distinct central wart, and the polyps have a collaret and points (Fig. 4B). Notably, however, the tentacular sclerites are rods rather than scales, a character that distinguishes this sub-clade from 5A (Table 1). Two distinct *msh1* haplotypes were found for *S. cruciata*: one in sub-clade 5B and the other in sub-clade 4B. Microscope slides of the type show characters consistent with placement in sub-clade 4B, and suggest that the specimen identified as *S. cruciata* in sub-clade 5B (NTM-C13505) is a different species.

Sub-clade 5C makes up the majority of clade 5 and includes species with *leptoclados*-type clubs in the colony surface (Fig. 4A), as well as collaret and point sclerites in the polyps. A majority of the species in this sub-clade (*Sinularia abrupta* TIXIER-DURIVAULT 1970, *Sinularia parelukari* ALDERSLADE & SHIRWAIKER 1991, *Sinularia densa* (WHITELEGGE 1897), *Sinularia leptoclados*, *Sinularia rotundata* TIXIER-DURIVAULT 1970, *Sinularia intacta* TIXIER-DURIVAULT 1970, *Sinularia verseveldti* OFWEGEN 1996, *Sinularia compacta* TIXIER-DURIVAULT 1970, *Sinularia acuta* MANUPUTTY & OFWEGEN 2007, *Sinularia longula* MANUPUTTY & OFWEGEN 2007, *Sinularia molesta* TIXIER-DURIVAULT 1970, *Sinularia maxima* VERSEVELDT 1971, *Sinularia muralis* MAY 1899, and *Sinularia erecta* TIXIER-DURI-VAULT 1945) have previously been placed in Group I of Verseveldt (1980) (*leptoclados*-type clubs). The sub-clade also includes *Sinularia robusta* MACFADYEN

1936 and *Sinularia cristata* TIXIER-DURIVAULT 1969, neither of which has been assigned to this group previously (Group III; Verseveldt 1980). In these two species, however, some *leptoclados*-type clubs are present although they are not well developed. Insufficient information is available to determine whether or not all of the species in sub-clade 5C share the presence of tentacle rods with sub-clade 5B.

The tree shown in Fig. 2 had a significantly greater log likelihood ($-\ln L = 2999.22$) than an alternative tree in which the five taxonomic groups of Verseveldt (1980) were constrained to be monophyletic ($-\ln L = 3528.84$) (SH test, $p < 0.001$). Among our five major clades, clade 1 included species placed in Verseveldt's Group IV, and clade 3 only consisted of Group III species, but each of the other three clades comprised a mix of species from two or more different taxonomic groups (Fig. 2).

Within clades, the mean pairwise genetic distances (Kimura 2-parameter) between species ranged 0.002–0.029 (Table 2), and some morphospecies shared the same *msh1* sequence, reflecting the typical lack of variability in anthozoan mitochondrial genes (Shearer et al. 2002). The mean genetic distances between clades 2, 3, 4, and 5 ranged 0.028–0.072, comparable to the mean distance between the genera *Sarcophyton* and *Lobophytum* at *msh1* (McFadden et al. 2006a). Clade 1, however, differed from the other four clades by 0.085–0.112, distances that were slightly greater than those between each of the other *Sinularia* clades and the outgroup taxa, *Sarcophyton* and *Lobophytum* (Table 2).

Discussion

The five major clades and sub-clades of *Sinularia* distinguished by *msh1* can all also be recognized and separated from one another based on a suite of just four primary morphological characters: presence of sclerites in the (a) tentacle, (b) collaret, and (c) point regions of the polyp, and (d) shape of the club sclerites in the colony surface tissues (Table 1, Fig. 1).

Table 2. Average pairwise genetic distances (Kimura 2-parameter) between species within (bold) and among the five major clades of *Sinularia*. Clades are defined as in Fig. 2. Values are means (SD).

	Outgroup	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5
1	0.121 (0.027)	**0.005 (0.004)**				
2	0.113 (0.007)	0.112 (0.003)	**0.029 (0.023)**			
3	0.080 (0.006)	0.085 (0.001)	0.052 (0.002)	**0.002 (0.001)**		
4	0.091 (0.008)	0.100 (0.003)	0.059 (0.005)	0.028 (0.004)	**0.015 (0.010)**	
5	0.105 (0.008)	0.112 (0.003)	0.072 (0.004)	0.042 (0.003)	0.048 (0.005)	**0.011 (0.007)**

Colony growth form and type of sclerites in the tentacles further distinguish several clades. Identification of morphological characters diagnostic for each of the clades and sub-clades allows us to predict the phylogenetic placement of a majority of the *Sinularia* species that were not included in our molecular phylogeny (Table 1), thereby providing a hypothetical framework for further tests of the phylogenetic utility of these characters. The only species whose phylogenetic placement we were unable to predict because of unclear species descriptions and lack of access to type material were *Sinularia andamanensis* (THOMSON & SIMPSON 1909), *Sinularia anomala* VERSEVELDT & BENAYAHU 1983, *Sinularia pedunculata* TIXTER-DURIVAULT 1945, *Sinularia schleyeri* BENAYAHU 1993, and *Sinularia whiteleggei* LÜTTSCHWAGER 1914, and the species described by LI (1982) (*Sinularia corpulenta* LI 1982, *Sinularia fibrillosa* LI 1982, *Sinularia monstrosa* LI 1982, *Sinularia papillosa* LI 1982, and *Sinularia tenella* LI 1982).

There was strong support for the monophyly of genus *Sinularia,* provided that *Dampia pocilloporaeformis* is regarded as a species of *Sinularia.* In his original description of *Dampia,* Alderslade (1983) discussed the similarities between it, *Sinularia foveolata,* and *Sinularia fungoides,* the two other species with which it groups in clade 3. All three of these species have calyx-like structures surrounding the polyps, although they are developed to a much greater degree in *Dampia.* Fabricius & Alderslade (2001) also expressed the opinion that *D. pocilloporaeformis* may be simply an aberrant species of *Sinularia;* it bears a close resemblance to *Sinularia triangula* TIXIER-DURIVAULT 1970, and may in fact be that species.

Although the phylogeny presented here supports monophyly of *Sinularia,* it should be noted that in some molecular analyses based on different genes and including a wider range of outgroup taxa, the species in clade 1 (*Sinularia brassica* and *Sinularia dura*) fall outside of *Sinularia* (C.S. McFadden, unpubl. data). Clade 1 was recovered here as sister to all remaining *Sinularia* species, but the genetic distances separating it from the other *Sinularia* clades are as great or greater than those among the genera *Sinularia, Sarcophyton,* and *Lobophytum* (Table 2). If future molecular phylogenetic analyses confirm a paraphyletic relationship between clade 1 and the other four *Sinularia* clades, clade 1 might merit a generic status. Because *S. brassica* is the type species of *Sinularia,* however, such a revision would make *Sinularia* a monotypic genus and require that a new genus be established for the other ∼150 species.

The only one of Verseveldt's (1980) five taxonomic groups that was congruent with the molecular phylogeny and formed a monophyletic group was his Group I (our sub-clade 5C). All of the species in this sub-clade have the *leptoclados*-type club sclerites that distinguish them clearly from other clades. Only *Sinularia robusta* and *Sinularia cristata,* in both of which the *leptoclados*-type clubs appear to be rare, were not included in Group I by Verseveldt (1980). Species belonging to his other four taxonomic groups are distributed throughout the other nine sub-clades in the *msh1* phylogeny, with some sub-clades (e.g., 4D) comprising species from three different groups (Fig. 2). In particular, species from Verseveldt's Group II (club sclerites with a central wart) and Groups III/IV (club sclerites without a central wart) often fall into the same clade, and our re-examination of type material suggests that his classification of club sclerites as having a central wart or not was often incorrect. For instance, species in clade 3 were classified by Verseveldt as lacking a central wart and were therefore placed in his Group III, but careful examination of these clubs reveals the presence of a small central wart that is often obscured (Fig. 3C). In fact, the only *Sinularia* species whose clubs truly lack a central wart are those in clade 1 (*S. brassica/dura*) and those with *leptoclados*-type clubs (5C).

Three of the four characters that we found to be the most informative phylogenetically for *Sinularia* were the presence/absence of sclerites in three distinct regions of the polyp (tentacles, collaret, and points; Fig. 1). Characters associated with the polyps, however, have not traditionally been considered important in taxonomic work on this genus. As discussed previously (McFadden et al. 2006a), the methods used by Verseveldt (1980) to prepare microscope slides of sclerites may inadvertently have resulted in the loss of many of the smallest sclerites, particularly those from the tentacles. His and many subsequent descriptions of *Sinularia* species do not include information on tentacle sclerites, as a consequence of which we are unable to verify their presence/absence in all members of some of the clades identified here (Table 1). In addition to omissions, in a number of cases, our subsequent re-examination of type material has revealed errors in original taxonomic descriptions. For instance, *Sinularia curvata* (sub-clade 4B) was described recently by Manuputty & van Ofwegen (2007) as having no sclerites in the polyps, but a careful re-examination of type material revealed some point sclerites. No polyp sclerites have been described previously for *Sinularia sobolifera* (4B) either, but the specimen identified here (RMNH-Coel. 38748) clearly showed them. Re-examination of permanent microscope slides of the type material for *Sinularia cruciata* and *Sinularia numerosa* also showed point

sclerites to be present in those species. In contrast, Vennam & van Ofwegen (1996) erroneously reported the presence of polyp sclerites in *Sinularia gravis* Tixier-Durivault 1970, mistaking immature surface clubs for those typical of the points. Further examination of type material for species whose reported morphological character states appear to be incongruent with the molecular phylogeny is likely to reveal additional omissions or errors in the primary taxonomic literature. For example, both *Sinularia mauritiana* and *Sinularia jasminae* Alderslade & Shirwaiker 1991 have been reported to lack polyp sclerites (Alderslade & Shirwaiker 1991; Vennam & Parulekar 1994), and yet both species belong to a clade (5) whose other members have them, a discrepancy that should motivate a future re-evaluation of the type material.

Assessment of species boundaries

Although *msh1* effectively distinguishes the major clades and sub-clades within *Sinularia*, it should be noted that this gene is not variable enough to distinguish all species of this genus from one another. Within each clade, there are numerous examples of distinct morphospecies that nonetheless share the same *msh1* sequence. The traditional morphological differences that distinguish some of these genetically similar species from one another are discussed further in van Ofwegen (2008b). Analysis of a more rapidly evolving gene region will be necessary to detect molecular differences among these closely related *Sinularia* species. Indeed, preliminary results from the more variable nuclear ribosomal *ITS* genes show clear genetic differences among species such as *Sinularia diffusa* van Ofwegen 2008a, *Sinularia bremerensis* van Ofwegen 2008a, *Sinularia mammifera* Malyutin 1990, and *Sinularia confusa* van Ofwegen 2008a, four species in sub-clade 4C that share identical *msh1* sequences (C.S. McFadden, unpubl. data).

Because *msh1* sequences often do not differ among closely related species, cases where individuals identified to the same species do have quite different *msh1* sequences may suggest either cryptic species or misidentification of specimens. The most obvious examples of this in the *msh1* tree are *Sinularia polydactyla* and *S. cruciata*, for which specimens identified to the same species using traditional morphological characters fell into entirely different phylogenetic clades. Other examples of genetic differences among specimens of a magnitude that suggests the possibility of cryptic species can be seen for *Sinularia grandilobata* (clade 2), *Sinularia variabilis* (sub-clade 4A), *Sinularia heterospiculata* (sub-clade 4B), *Sinularia compressa* Tixier-Durivault 1945 (sub-clade 4D), and *Sinularia leptoclados* (sub-clade 5C). Indeed, a subsequent taxonomic re-evaluation of *S. leptoclados* based on morphological characters has revealed this taxon to be a complex of several different species (L.P. van Ofwegen & Y. Benayahu, unpubl. data). In addition, *Sinularia rotundata, Sinularia intacta,* and *Sinularia molesta,* three species in the *leptoclados* group that had been synonymized (van Ofwegen 2001), have *msh1* sequences that are sufficiently different to suggest that they do represent distinct species, as is also the case for the previously synonymized *S. brassica* and *S. dura* (clade 1). Clearly, much work still needs to be carried out to verify species boundaries in *Sinularia* in cases where a significant genetic variation is present within a recognized taxon, or, conversely, where morphospecies are indistinguishable genetically.

Although the morphological characters identified here (Table 1) distinguish clades rather than species, consideration of these characters in future taxonomic studies will also improve our ability to discern species boundaries (see van Ofwegen 2008b). This point is best illustrated by the cases we have identified in which specimens that were assigned to the same species on the basis of traditional taxonomic characters actually belong to very different clades. For example, by examining the polyp sclerites, it was possible to distinguish morphologically between genetically disparate specimens of *S. polydactyla* and *S. cruciata,* and in retrospect assign them to the correct clades. Examination of polyp sclerites *a priori* (coupled with recognition of the phylogenetic importance of those characters) would have prevented these specimens from ever having been assigned to the same species.

Recent evidence for hybridization among species of *Sinularia* may, however, complicate our ability to assess species boundaries and to reconcile morphological with molecular data. Slattery et al. (2008) document the ability of *Sinularia maxima* and *S. polydactyla* to hybridize successfully in the laboratory, and identify a putative naturally occurring hybrid zone between these species in Guam. *Sinularia maxima* belongs to the distinctive *leptoclados*-club sub-clade (5C) while *S. polydactyla* belongs to clade 4, characterized by club sclerites with a distinct central wart. Hybrid offspring contain a mixture of both parental types of club sclerites (Slattery et al. 2008). This case is particularly instructive because it demonstrates (1) that successful hybridization can occur between *Sinularia* species from very different genetic groups and (2) that hybrid individuals can exhibit morphological traits diagnostic for more than one clade. *ITS* polymorphisms that are shared among species also suggest

the possibility of ongoing or past hybridization between some members of sub-clades 4C and 4D (C.S. McFadden, unpubl. data). Much additional work needs to be carried out, however, before any conclusions can be drawn about the prevalence or evolutionary ramifications of hybridization in *Sinularia*.

In summary, as has also been shown recently for a variety of other cnidarian groups (Sánchez et al. 2003; Fukami et al. 2004, 2008; McFadden et al. 2006b), we have demonstrated that the morphological characters traditionally considered most important for inferring taxonomic relationships within the genus *Sinularia* (i.e., the form and size of club sclerites) are incongruent with molecular phylogenetic data. However, other characters whose phylogenetic importance has until now been overlooked (i.e., polyp sclerites) recover relationships that are congruent with the phylogeny. Recognition of the utility of these new characters will necessitate a re-working of the established taxonomy and taxonomic protocols in *Sinularia,* an effort that is already underway (van Ofwegen 2008b). In octocorals, the character-mapping approach used here continues to be useful for reconciling genus- and family-level taxonomy with molecular phylogenetics (Sánchez et al. 2003; McFadden et al. 2006a). In future, we hope to be able to use a similar method to identify morphological characters that are congruent with and diagnostic for even higher level (ordinal and sub-ordinal) clades within Octocorallia, a problem that historically has and continues to bedevil the taxonomy of this group (McFadden et al. 2006b).

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Appendix 1. Specimens of *Sinularia* and outgroup taxa included in molecular phylogenetic analysis. NTM, Museum and Art Gallery of the Northern Territory, Darwin, Australia; RMNH, Nationaal Natuurhistorisch Museum, Leiden, ZMTAU, Zoological Museum, Tel Aviv University; UF, Florida Natural History Museum.

Species	Museum Cat. No.	Collection location	Latitude	Longitude	Date	GenBank no.
S. abhishiktae	RMNH Coel. 38720	Palau, Koror, Mutremdiu	7°16.25.2″N	134°31′26.8″E	2005	FJ621373
S. abrupta	NTM C13799	Palau, Neco Channel	7°12.34′N	134°22.32′E	1995	FJ621374
S. acuta	RMNH Coel. 38432	Indonesia, Ambon, near Morela	3°33′S	128°12′E	1996	FJ621375
S. acuta	RMNH Coel. 38721	Palau, Koror, Wonder Channel	7°10′53.3″N	134°21′38.6″E	2005	FJ621376
S. babeldaobensis	RMNH Coel. 38723	Palau, Babeldaob, Toagel Mlungi Channel	7°32′33″N	134°28′06.6″E	2005	FJ621377
S. bisulca	RMNH Coel. 38724	Palau, Koror, Wonder Channel	7°10′53.3″N	134°21′38.6″E	2005	FJ621378
S. brassica	NTM C13507	Malaysia, Sabah, Semporna I.	4°39.48′N	118°46.94′E	1999	FJ621379
S. brassica	NTM C14185	Papua New Guinea, Cape Nelson, Tufi	9°04.89′S	149°19.10′E	2002	FJ621380
S. brassica	NTM C13660	Australia, WA, Ashmore Reef	12°14.29′S	123°00.77′E	2002	FJ621381
S. bremerensis	NTM C14488	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621382
S. capitalis	NTM C14530	Australia, Gulf of Carpentaria, Bremer Is.	12°05.66′S	136°47.75′E	2003	FJ621383
S. compacta	RMNH Coel. 38433	Indonesia, Ambon, Latuhalat	03°46′S	128°06′E	1996	FJ621384
S. compressa	RMNH Coel. 38420	Indonesia, Ambon, Ambon Bay	03°43′S	128°04′E	1996	FJ621385
S. compressa	ZMTAU CO 34140	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621386
S. compressa	ZMTAU CO 34142	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621387
S. compressa	ZMTAU CO 34150	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621388
S. conferta	NTM C13972	Mauritius	20°17.60′S	57°21.06′E	1999	FJ621389
S. confusa	NTM C14456	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621390
S. crassa	RMNH Coel. 38430	Indonesia, Ambon, Ambon Bay	03°40′S	128°10′E	1996	FJ621391
S. crebra	RMNH Coel. 38726	Palau, Koror, Uchelbeluu Reef	7°16′25.2″N	134°31′27″E	2005	FJ621392
S. cristata	NTM C13818	Palau, Ngiwal	07°31.43′N	134°37.66′E	1996	FJ621393
S. cruciata	NTM C13505	Malaysia, Sabah, Semporna I.	04°38.06′N	118°42.58′E	1999	FJ621394
S. cruciata	ZMTAU CO 34152	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621395
S. curvata	RMNH Coel. 38436	Indonesia, Ambon, W of Sahuru	03°40′S	128°09′E	1996	FJ621396
S. densa	NTM C1993	Australia, GBR, Qld, Rib Reef			1980	FJ621397
S. depressa	RMNH Coel. 38428	Indonesia, Ambon, Latuhalat	03°46′S	128°06′E	1996	FJ621398

Appendix 1. (cont'd).

Species	Museum Cat. No.	Collection location	Latitude	Longitude	Date	GenBank no.
S. diffusa	NTM C14457	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621399
S. diffusa	NTM C14464	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621400
S. digitata	RMNH Coel. 38727	Palau, Babeldaob, Toagel Mlungi Channel	7°32′33″N	134°28′06.6″E	2005	FJ621401
S. dura	NTM C13808	Philippines, Davao, N of Talikud I.	7°57.72′N	125°40.94′E	1996	FJ621402
S. erecta	NTM C12557	Persian Gulf, Iarak I.	26°52′N	56°25′E	1997	FJ621403
S. erecta	ZMTAU CO 34144	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621404
S. finitima	RMNH Coel. 38729	Palau, Koror, Toachel Mid	7°20′21.9″N	134°31′03.8″E	2005	FJ621405
S. finitima	RMNH Coel. 38730	Palau, Koror, Wonder Channel	7°10′53.3″N	134°21′38.6″E	2005	FJ621406
S. finitima	RMNH Coel. 38728	Palau, Babeldaob, Ngaregabal Reef	7°24′50.4″N	134°26′38.2″E	2005	FJ621407
S. flaccida	RMNH Coel. 38731	Palau, Koror, Big Drop Off	7°06′48.8″N	134°15′36.4″E	2005	FJ621408
S. flexibilis	RMNH Coel. 38378	Indonesia, Ambon, Latuhalat	3°46S	128°06′E	1996	FJ621409
S. foliata	RMNH Coel. 38732	Palau, Koror, Mutremdiu	7°16″25.2′N	134°31′26.8″E	2005	FJ621410
S. foveolata	NTM C13966	Mauritius	20°14.38′S	57°22.80E	1999	FJ621411
S. fungoides	NTM C13910	Palau, Ngerm 1	7°33.47′N	134°0.03E	1998	FJ621412
S. gardineri	NTM C14050	Vanuatu, Santo, Tutuba	15°33.07′S	167°16.65E	2000	FJ621413
S. gardineri	ZMTAU CO 34097	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621414
S. gardineri	ZMTAU CO 34146	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621415
S. gaveshaniae	RMNH Coel. 38733	Palau, Koror, Ngederrak Reef	7°17.05′N	134°29′20″E	2005	FJ621416
S. gaweli	UF3498	Guam, Agat Bay			1998	FJ621417
S. gaweli	UF3181	Guam, Double Reef			1998	FJ621418
S. gaweli	RMNH Coel. 38734	Palau, Koror, Big Drop Off	7°06′48.8″N	134°15′36.4″E	2005	FJ621419
S. grandilobata	NTM C13796	Palau, Lighthouse Channel marker 3			1995	FJ621420
S. grandilobata	NTM C14114	American Samoa, Pago Pago Harbor	14°06.82′S	170°40.07′W	2001	FJ621421
S. gravis	RMNH Coel. 38736	Palau, Peleliu, Ngermoket	6°58′36.9″N	134°13′20″E	2005	FJ621422
S. halversoni	NTM C13848	Fiji, Vanubalavu Lagoon	17°10.27′S	179°01.30′W	1996	FJ621423
S. halversoni	NTM C14058	Vanuatu, Santo, Malo Seamount	15°38.10′S	167°18.72′E	2000	FJ621424
S. heterospiculata	NTM C13968	Mauritius	20°14.38′S	57°22.80′E	1999	FJ621425
S. heterospiculata	NTM C14003	Vanuatu, Konanda Reef	17°45.17′S	168°17.28′E	2000	FJ621426
S. hirta	RMNH Coel. 38399	Indonesia, Ambon, Manuala Beach	3°35′S	128°05′E	1996	FJ621427

Appendix 1. (cont'd).

Species	Museum Cat. No.	Collection location	Latitude	Longitude	Date	GenBank no.
S. hirta	ZMTAU CO 34100	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621428
S. hirta	ZMTAU CO 34103	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621429
S. hirta	ZMTAU CO 34148	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621430
S. humesi	UF3500	Guam, Piti Bay			1998	FJ621431
S. humilis	RMNH Coel. 38737	Palau, Koror, Wonder Channel	7°10′53.3″N	134°21′38.6″E	2005	FJ621432
S. intacta	NTM C12479	India, Gulf of Mannar	9°16′N	79°12′E	1991	FJ621433
S. lamellata	NTM C13824	Palau, E Babeldaob, Ngchesar, RRII Outlet	07°25.07′N	134°35.98′E	1996	FJ621434
S. lamellata	NTM C13931	Papua New Guinea, Milne Bay, Alotau 4	10°22.63′S	150°23.05′E	1998	FJ621435
S. leptoclados	NTM C5421	Australia, WA, Broome, Roebuck Bay			1987	FJ621436
S. leptoclados	NTM C14492	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621437
S. leptoclados	NTM C14519	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621438
S. leptoclados	ZMTAU CO 34095	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621439
S. linnei	NTM C14480	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621440
S. longula	RMNH Coel. 38439	Indonesia, Ambon, Seri Bay	3°45′S	128°09′E	1996	FJ621441
S. loyai	ZMTAU CO 34154	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621442
S. luxuriosa	RMNH Coel. 38742	Palau, Koror, Toachel Mid	7°20′21.9″N	134°31′03.8″E	2005	FJ621443
S. mammifera	NTM C14198	Vanuatu, Konanda Reef	17°45.17′S	168°17.28′E	2000	FJ621444
S. mauritiana	NTM C13852	Fiji, Vanua Levu, Viani Bay	16°45.50′S	179°55.10′E	1996	FJ621445
S. maxima	NTM C14001	Vanuatu, Konanda Reef	17°45.17′S	168°17.28′E	2000	FJ621446
S. maxima	NTM C14255	India, Gulf of Mannar	19°10′N	09°20′E	2001	FJ621447
S. maxima		Guam, Piti Bay			1998	DQ302813
S. maxima	NTM C14512	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621448
S. molesta	RMNH Coel. 38440	Indonesia, Ambon, E of Cape Nusanive	3°48′S	128°06′E	1996	FJ621449
S. muralis	NTM C13978	Mauritius	19°58.44′S	57°38.89′E	1999	FJ621450
S. nanolobata	RMNH Coel. 38441	Indonesia, Ambon, near Morela	3°33′S	128°12′E	1996	FJ621451

Appendix 1. (cont'd).

Species	Museum Cat. No.	Collection location	Latitude	Longitude	Date	GenBank no.
S. notanda	NTM C14555	Australia, Gulf of Carpentaria, W. Woody I.	12°11.10′S	136°40.29′E	2003	FJ621452
S. numerosa	NTM C13790	Palau, Lighthouse Channel marker 3	7°17.21′N	134°27.77′E	1995	FJ621453
S. ornata	NTM C13971	Mauritius	20°19.23′S	57°22.01′E	1999	FJ621454
S. ornata	NTM C14138	American Samoa, W end Ofu I.	14°00.95′S	169°40.85′W	2001	FJ621455
S. papula	NTM C14527	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621456
S. parulekari	NTM C13989	Mauritius	19°56.75′S	57°37.24′E	1999	FJ621457
S. parulekari	NTM C14249	India, Gulf of Mannar	19°10′N	9°20′E	2001	FJ621458
S. pavida	RMNH Coel. 38744	Palau, Koror, Uchelbeluu Reef	7°16′25.2″N	134°31′27″E	2005	FJ621459
S. peculiaris	NTM C13959	Palau, Angauri 2	6°53.94′N	134°07.35′E	2000	FJ621460
S. peculiaris	NTM C14092	Micronesia, Yap, Ulithi Atoll	9°59.73′N	139°40.05′E	2000	FJ621461
S. polydactyla	NTM C14142	American Samoa, Ofu, Ofu Harbor	14°00.138′S	169°40.86′W	2001	FJ621462
S. polydactyla	NTM C14173	Papua New Guinea, Normanby I.	9°43.93′S	150°44.77′E	2002	FJ621463
S. polydactyla	RMNH Coel. 38442	Indonesia, Ambon, Ambon Bay, E of Erie	3°45′S	128°08′E	1996	FJ621464
S. polydactyla	ZMTAU CO 34106	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621465
S. polydactyla	ZMTAU CO 34138	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621466
S. procera	RMNH Coel. 38386	Indonesia, Ambon, W of Sahuru	3°40′S	128°09′E	1996	FJ621467
S. querciformis	NTM C14019	Vanuatu, Efate, Paul Reef	17°39.87′S	168°10.85′E	2000	FJ621468
S. querciformis	ZMTAU CO 34096	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621469
S. querciformis	ZMTAU CO 34191	Red Sea, Israel, Eilat, Princess Beach	29°29.77′N	34°54.53′E	2007	FJ621470
S. rigida	NTM C13937	Papua New Guinea, E Fields 02	10°00.66′S	145°39.90′E	1998	FJ621471
S. rigida	NTM C14141	Am. Samoa, Olosega I., SE Asaga Strait	14°00.95′S	169°37.67′W	2001	FJ621472
S. robusta	NTM C14518	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621473
S. robusta	NTM C14526	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	FJ621474
S. rotundata	NTM C3755	Australia, Cobourg Peninsula, Orontes Reef	11°04′S	132°09′E	1982	FJ621475
S. scabra	NTM C14043	Vanuatu, Santo, Tutuba	15°33.32′S	167°16.59′E	2000	FJ621476
S. siaesensis	RMNH Coel. 38746	Palau, Koror, Siaes Reef	7°10′56.5″N	134°21′38.7″E	2005	FJ621477

Appendix 1. (cont'd).

Species	Museum Cat. No.	Collection location	Latitude	Longitude	Date	GenBank no.
S. siaesensis	RMNH Coel. 38747	Palau, Koror, Siaes Reef	7°10′56.5″N	134°21′38.7″E	2005	FJ621478
S. sobolifera	RMNH Coel. 38748	Palau, Koror, Wonder Channel	7°10′56.5″N	134°21′38.7″E	2005	FJ621479
S. sublimis	RMNH Coel. 38750	Palau, Koror, Siaes Reef	7°10′56.5″N	134°21′38.7″E	2005	FJ621480
S. terspilli	ZMTAU CO 34156	Red Sea, Israel, Eilat, Nature Reserve	29°30.6′N	34°55.35′E	2007	FJ621481
S. tumulosa	RMNH Coel. 38751	Palau, Peleliu, Ngermoket	6°58′36.9″N	134°13′20″E	2005	FJ621482
S. ultima	RMNH Coel. 38752	Palau, Koror, Uchelbeluu Reef	7°16′25.2″N	134°31′27″E	2005	FJ621483
S. uniformis	RMNH Coel. 38753	Palau, Babeldaob, Toagel Mlungi Channel	7°32′33″N	134°28′06.6″E	2005	FJ621484
S. variabilis	NTM C14134	American Samoa, Ofu, W of Nuutele I.	14°00.46′S	169°41.16′W	2001	FJ621485
S. variabilis	NTM C14164	Papua New Guinea, Woodlark I.	9°12.51′S	151°55.28′E	2002	FJ621486
S. verruca	RMNH Coel. 38754	Palau, Koror, Siaes Reef	7°10′56.5″N	134°21′38.7″E	2005	FJ621487
S. verseveldti	NTM C13520	Malaysia, Sabah, Semporna I.	4°36.40′N	118°46.63′E	1999	FJ621488
S. vrijmoethi	NTM C14095	Micronesia, Yap, Ulithi Atoll	10°00.79′N	139°47.33′E	2000	FJ621489
S. woodyensis	NTM C14557	Australia, Gulf of Carpentaria, W. Woody I.	12°11.10′S	136°40.29′E	2003	FJ621490
D. pocilloporae-formis	NTM C5805	Australia, WA, Rowley Shoals	17°07.70′S	119°20.20′E	1987	DQ280593
Sar. ehrenbergi	NTM C11208	Indonesia, Central Java Sea, Jepara	6°35.00′S	110°39.00′E	1992	DQ280512
Sar. troche-liophorum	NTM C14469	Australia, Gulf of Carpentaria, Bremer I.	12°05.66′S	136°47.75′E	2003	DQ280549
L. compactum	NTM C11566	Australia, GBR, Orpheus I.	18°33′S	146°30′E	1992	DQ280559

Appendix 2.   Key to the clades of *Sinularia*.

Clade 1: Polyps with tentacle scales but without collaret or points; clubs with extreme
   wide heads (see Verseveldt 1980: fig. 2D)
Clade 2: Polyps with collaret, points and tentacle rods; clubs with central wart indistinct
   because the central wart and the three warts below it are close together
Clade 3: Polyps with indistinct points and no collaret; clubs with small central wart giving
   the clubs a triangular form
Clade 4: Polyps without collaret or tentacle sclerites; clubs with central wart
   4A: Polyps without sclerites; clubs with central wart indistinct because of leaf-like
      processes, or clubs absent
   4B: Polyps with point sclerites; clubs with central wart distinct, or clubs absent
   4C, 4D: Polyps without sclerites; clubs with central wart distinct
Clade 5: Polyps with collaret and point sclerites
   5A: Polyps with tentacle scales; clubs with central wart distinct
   5B: Polyps with tentacle rods; clubs with central wart distinct
   5C: Leptoclados-type clubs

1. No collaret or point sclerites	2
Collaret and/or points present	4
2. Scales in tentacles, extremely wide club heads	Clade 1
Rods or no sclerites in tentacles	3
3. Central wart with leaf-like processes, or clubs absent	Clade 4A
Central wart distinct	Clade 4C(4D)
4. Only point sclerites present	5
Collaret and points present	6
5. Points indistinct, point sclerites rod-like, clubs triangular	Clade 3
Points distinct, point sclerites club-like	Clade 4B
6. Central wart indistinct	7
Central wart distinct	8
7. Central wart indistinct, clubs not leptoclados-type	Clade 2
Leptoclados-type clubs	Clade 5C
8. Scales in tentacles	Clade 5A
Rods in tentacles	Clade 5B