

## Phylogenetic relationships within the tropical soft coral genera *Sarcophyton* and *Lobophytum* (Anthozoa, Octocorallia)

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**Abstract.** The alcyonacean soft coral genera *Sarcophyton* and *Lobophytum* are conspicuous, ecologically important members of shallow reef communities throughout the Indo-West Pacific. Study of their ecology is, however, hindered by incomplete knowledge of their taxonomy: most species cannot be identified in the field and the two genera cannot always be distinguished reliably. We used a 735-bp fragment of the octocoral-specific mitochondrial protein-coding gene *msh1* to construct a phylogeny for 92 specimens identified to 19 species of *Lobophytum* and 16 species of *Sarcophyton*. All phylogenetic methods used recovered a tree with three strongly supported clades. One clade included only morphologically typical *Sarcophyton* species with a stalk distinct from the polypary, poorly formed club-shaped sclerites in the colony surface, and large spindles in the interior of the stalk. A second clade included only morphologically typical *Lobophytum* colonies with lobes and ridges on the colony surface, poorly formed clubs in the colony surface, and interior sclerites consisting of oval forms with regular girdles of ornamental warts. The third distinct clade included a mix of *Sarcophyton* and *Lobophytum* nominal species with intermediate morphologies. Most of the species in this mixed clade had a polypary that was not distinct from the stalk, and the sclerites in the colony surface were clubs with well-defined heads. Within the *Sarcophyton* clade, specimens identified as *Sarcophyton glaucum* belonged to six very distinct genetic sub-clades, suggesting that this morphologically heterogeneous species is actually a cryptic species complex. Our results highlight the need for a complete taxonomic revision of these genera, using molecular data to help confirm species boundaries as well as to guide higher taxonomic decisions.

*Additional key words:* Alcyonacea, cryptic species, mitochondrial DNA, *msh1*, sclerite

Alcyonacean soft corals are abundant and ecologically important members of coral reef communities throughout the Indo-West Pacific, often equaling or exceeding scleractinian corals in percent cover of available primary space (Tursch & Tursch 1982; Dinesen 1983; Dai 1988; Riegl et al. 1995; Fabricius 1997). Species belonging to the alcyoniid genera *Sarcophyton* LESSON 1834, *Lobophytum* VON MARENZELLER 1886, and *Simularia* MAY 1898 are particularly common and conspicuous in shallow near-shore reef flat habitats where they often form large monospecific aggregations (Benayahu & Loya 1977; Benayahu 1995; Fabricius 1998; Fabricius & Dom-

misse 2000). Species in these genera are known to be long-lived, competitive dominants that grow slowly, are resistant to predation, and have infrequent larval recruitment (Benayahu & Loya 1986; Fabricius 1995; Bastidas et al. 2004). All three taxa are zooxanthellate, and as a result have been heavily impacted by recent coral bleaching events, with mortality exceeding 90% in some areas of the Indo-West Pacific (Fabricius 1999; Bruno et al. 2001; Loya et al. 2001).

Despite their abundance and importance, these three genera have been the subject of few ecological studies (Fabricius 1995, 1997, 1998; Fabricius & Dommissie 2000; Bastidas et al. 2004), due in part to the difficulties of identifying species in the field. Colony growth morphology can be highly variable (Benayahu 1998; Benayahu et al. 1998), and most species can be distinguished only by microscopic

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examination of the CaCO<sub>3</sub> sclerites found within the coenenchymal tissue. Despite fairly recent taxonomic revisions of all three genera (Verseveldt 1980, 1982, 1983), many species remain undescribed. In this article, we use molecular markers to examine the phylogenetic and taxonomic relationships among species belonging to the genera *Sarcophyton* and *Lobophytum*. Although these two genera are quite easily distinguished from *Simularia* by the presence of siphonozooids, the morphological distinctions between *Sarcophyton* and *Lobophytum* are less obvious, and preliminary molecular data suggest that their relationship may be paraphyletic. Moreover, members of the two genera have been documented to hybridize in aquaria, producing viable offspring (K. Michalek-Wagner, pers. comm.).

Following taxonomic revisions of both genera, Verseveldt (1982, 1983) considered there to be 35 valid species of *Sarcophyton* and 46 valid species of *Lobophytum*; an additional five species of *Sarcophyton* and 12 of *Lobophytum* have been described subsequently (Verseveldt & Benayahu 1983; Li 1984; Alderslade & Shirwaiker 1991; Alderslade 1993; Benayahu 1995; Ofwegen 1999; Benayahu & Perkol-Finkel 2004). In keys to the octocoral family Alcyoniidae, these two genera are distinguished from one another primarily by colony growth morphology (Bayer 1981; Verseveldt 1982). *Sarcophyton* typically forms colonies in which the polyp-bearing region (disc or polypary) is raised on a prominent stalk, often resembling a mushroom (Fig. 1A). Polyps are only found on the upper surface of the disc, which is typically smooth, wider than the stalk, and may have a highly folded margin (Fig. 1B).

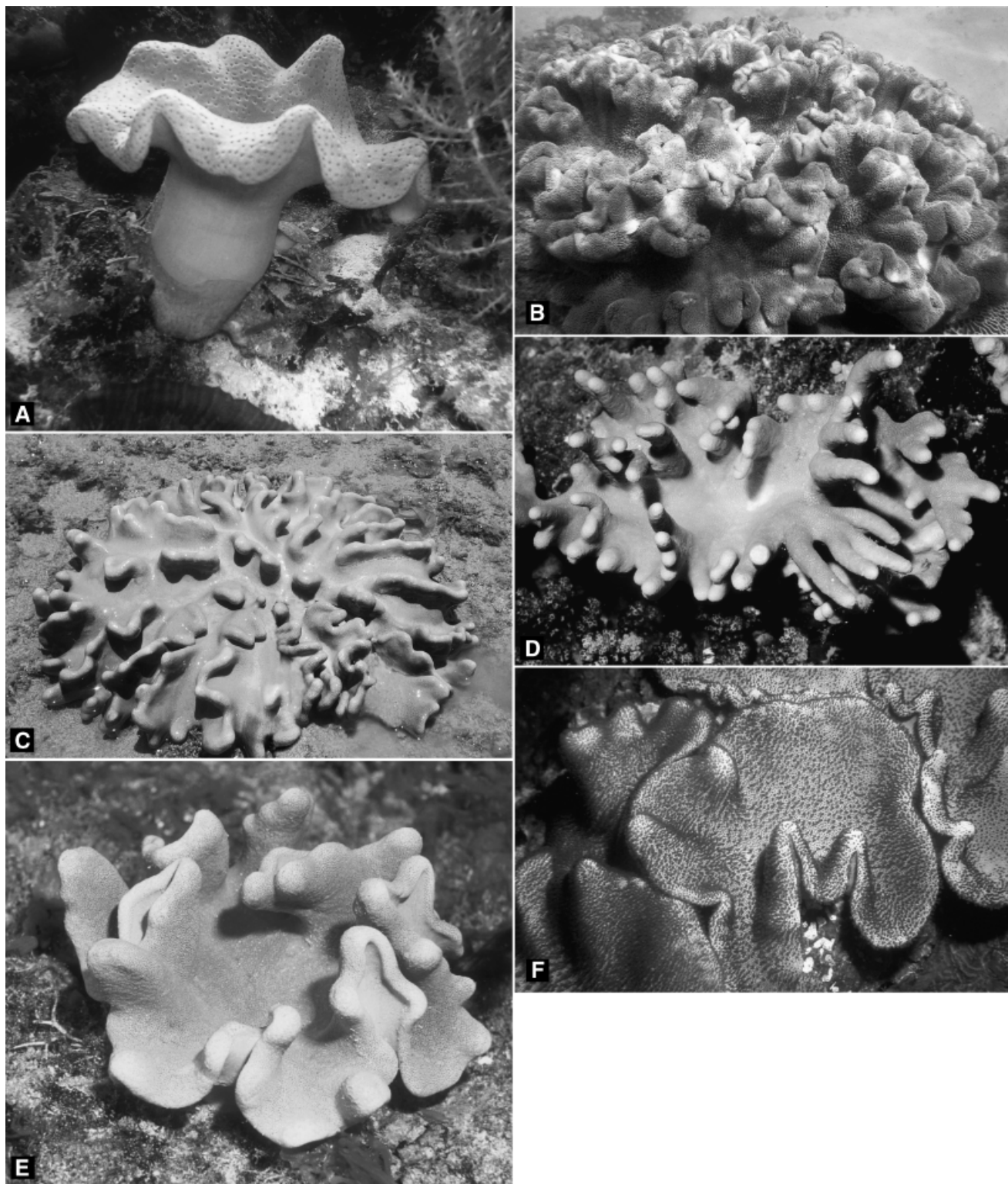
In contrast, in species of *Lobophytum* the polyp-bearing area is commonly not much wider than the stalk and usually has prominent lobes or ridges on its upper surface; colonies are often thick and plate-like (Fig. 1C,D). The two genera can be distinguished further by the form of the sclerites that are found in the interior coenenchymal tissue of the colony stalk or base. In *Sarcophyton*, these sclerites are usually large (to 2.0 mm) and spindle shaped and commonly have irregularly distributed tubercular ornamentation, while the interior sclerites of *Lobophytum* tend to be smaller (<0.5 mm) and oval, with the tubercles arranged in regular, transverse girdles. Both genera have club-shaped sclerites in the colony surface, although in *Lobophytum* the heads of these clubs tend to be poorly defined relative to those of *Sarcophyton* (Verseveldt 1983). These distinctions between the genera blur, however, in cases where colony growth form is intermediate (Fig. 1E,F), or in which species with the growth form typical of one genus have scler-

ites more typical of the other. In the course of his revisions, Verseveldt (1982, 1983) transferred five nominal *Lobophytum* species to *Sarcophyton* (four of them synonymized with *Sarcophyton crassocaule*) and one *Sarcophyton* to *Lobophytum*. However, a number of the species he retained within *Lobophytum* on the basis of their colony growth form have typical *Sarcophyton* sclerites, while those he synonymized with *S. crassocaule* have fairly typical *Lobophytum* sclerites.

In this study, we use DNA sequence data to examine the phylogenetic relationships among species in the two genera, in particular those taxa with intermediate morphologies. Most mitochondrial genes evolve very slowly in octocorals, and therefore lack sufficient variation to use for intrageneric studies (France & Hoover 2001, 2002; McFadden et al. 2004). The protein-coding gene *msh1*, a homolog to the bacterial DNA repair protein MutS that is found uniquely in the octocoral mitochondrial genome (Pont-Kingdon et al. 1995, 1998; France & Hoover 2001), is, however, sufficiently variable to discriminate species in some octocoral genera, including *Sarcophyton* and *Lobophytum*. We have used a 735-bp fragment of this gene to construct a molecular phylogeny for 92 specimens of *Lobophytum* and *Sarcophyton* that had previously been identified to species on the basis of morphology. Subsequently, we have re-examined the morphology of specimens included in the phylogeny in order to identify morphological characters that are congruent with the observed patterns of genetic differentiation within and between the two genera.

## Methods

We obtained tissue samples from specimens of *Lobophytum* and *Sarcophyton* housed at the Museum and Art Gallery of the Northern Territory (Darwin, Australia) that had been collected within the past 20 years and identified to species. A majority of this material was collected throughout the Indo-West Pacific in 1995–2004 by the Coral Reef Research Foundation (Palau). Additional material was collected in 2003–2005 from NE Kalimantan, Indonesia; Gulf of Carpentaria, Australia; Republic of Palau; and Okinawa, Japan. Specimens were collected by hand while snorkeling or using SCUBA, and were preserved and stored in 70–95% EtOH. Vouchers of all new specimens were deposited in the collections of the Museum and Art Gallery of the Northern Territory (NTM), the National Museum of Natural History, formerly Rijksmuseum van Natuurlijke Historie, Leiden (RMNH), and the Florida Museum of Natural History (UF) (Appendix A).



### DNA extraction and sequencing

Before DNA extraction, samples were soaked for 24 h in  $2 \times$  CTAB buffer with several solution changes to remove EtOH from the tissue. DNA was extracted from polypoid tissue using the method of McFadden et al. (2001) with several modifications. For older museum specimens ( $>2$ – $3$  years), incubation with proteinase K was extended to 24 h at  $55^\circ\text{C}$  (e.g., Berntson & France 2001), and for all samples, Nucleon Phytopure<sup>®</sup> (Amersham Biosciences), a resin designed to remove excess polysaccharides from plant tissue extracts, was added to the final chloroform extraction step. Following extraction, DNA was run on a 1% agarose gel to estimate concentration, and was diluted 1:10 or 1:100 before polymerase chain reaction (PCR) amplification. The 5' end of the mitochondrial *msh1* gene was amplified by PCR using the primers ND42599F and Mut2458R (Sánchez et al. 2003) in a reaction with  $10 \times$  PCR buffer ( $100 \text{ mmol L}^{-1}$  Tris-HCl pH 8.3,  $250 \text{ mmol L}^{-1}$  KCl),  $3.5 \text{ mmol L}^{-1}$   $\text{MgCl}_2$ ,  $2 \text{ mmol L}^{-1}$  dNTPs, 20 pmol each primer, 0.5 U *Taq* polymerase, and  $1 \mu\text{L}$  DNA. For specimens that yielded no or only faintly visible PCR products, we ran a second PCR reaction using an internal forward primer (ND42625F: 5'-TACGTGGYACAATTGCTG-3') (Lepard 2003) and  $1 \mu\text{L}$  of the original PCR product as a template (e.g., Berntson & France 2001). To check for sample contamination,  $1 \mu\text{L}$  of the negative (no DNA) control from the first reaction was re-amplified in the second reaction. PCR products were purified by precipitation with 20% (w/v) polyethyleneglycol 8000 in  $2.5 \text{ mol L}^{-1}$  NaCl (Sánchez et al. 2003), cycle sequenced (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit, PE Applied Biosystems, Foster City, CA, USA), and run on an ABI 3100 automated gene analyzer.

### Morphological characters

Morphological character states for each species were obtained either by direct observation or from Verseveldt (1982, 1983). Colony growth morphology

when contracted was scored as disc wider than stalk, disc not wider than stalk, or plate-like (disc not wider than very short stalk or no stalk). The presence of lobes or ridges on the polypary surface was noted, as was the shape (convex or concave) of the contracted disc in stalked forms. Small ( $\sim 1 \text{ mm}^2$ ) tissue samples were removed from the surface and interior of the polypary, and the surface and interior of the stalk (or base) of the colony, and dissolved in 10% sodium hypochlorite (concentrated bleach) to extract sclerites. Several whole polyps were removed from each colony and treated likewise. Sclerites were observed under a compound microscope at  $\times 4$ – $40$  magnification, and a camera lucida was used to draw a sample of representative sclerites from each anatomical region of each specimen. Sclerite size was estimated to the nearest 0.005 mm, and sclerite form was determined using the definitions of Bayer et al. (1983). In addition, several polyps from each colony were soaked in 1% KOH for 2–4 d to clear the tissue, and the arrangement of sclerites in the anthocodia and tentacles was drawn at  $\times 10$  magnification.

### Phylogenetic analysis

Nucleotide sequences were assembled and proof-read using LaserGene software, and aligned using ClustalX v. 1.81 (Thompson et al. 1997). Bayesian phylogenetic analyses were conducted using MrBayes v. 3.04 (Huelsenbeck & Ronquist 2001) with a GTR+I+ $\Gamma$  model run for  $1.5 \times 10^6$  generations (burn-in = 3750 generations). Maximum likelihood and maximum parsimony analyses were run using PAUP\* v. 4.0b10 (Swofford 2002). Modeltest v. 3.06 (Posada & Crandall 1998) was used to select the best model for maximum likelihood (TVM+G). Analyses were run using the heuristic search option with simple sequence addition and TBR branch swapping; 100 bootstrap replicates were run for maximum parsimony, but computational and time limitations prevented bootstrapping of maximum likelihood analyses. Members of the genera *Simularia* and *Dampia*,

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**Fig. 1.** *In situ* photographs of colonies of *Sarcophyton* and *Lobophytum*, illustrating a range of colony growth morphologies. **A.** *Sarcophyton glaucum* (NTM-C014912), a colony with a mushroom growth form typical of the genus *Sarcophyton*. **B.** *S. trocheliophorum* (NTM-C001732), a colony with a highly folded margin and broad, low base, also typical of many species of *Sarcophyton*. **C.** *Lobophytum crassum*, a typical *Lobophytum* colony with ridges on the polypary surface. **D.** *L. hirsutum* (NTM-C014761), a typical *Lobophytum* form with finger-like lobes. **E.** *L. sarcophytoides* (NTM-C014661), a species that forms bowl-shaped colonies with a highly folded edge where some folds have fused to form lobes around the periphery of the polypary. **F.** *L. schoedei* (NTM-C014149), a rather flat colony with a few small lobes and some folding of the polypary margin. Photos: A, C: S. McFadden; B, C: P. Alderslade; D–F: Courtesy of Coral Reef Research Foundation.

shown by prior phylogenetic analysis of the subclass Octocorallia to be the sister clade to *Lobophytum*+*Sarcophyton* (McFadden et al., in press), were included as outgroup taxa to root all trees.

## Results

### Molecular phylogeny

We obtained a 735-bp fragment of the 5' end of *msh1* for all specimens, with the exception of one individual of *S. glaucum* (NTM-C014113) that had a 5-amino-acid (15-bp) deletion and a single specimen of *Lobophytum* (NTM-C014937) with a 1-amino-acid (3-bp) insertion. All sequences could be aligned unambiguously. The final alignment included 738 nucleotide positions, of which 508 were invariant, 230 were variable, and 88 were parsimony informative. Pairwise genetic distances (uncorrected p) among taxa of *Lobophytum* and *Sarcophyton* ranged 0–0.093, excluding *S. glaucum* NTM-C014113, which differed from other taxa by  $\leq 0.158$ .

All three phylogenetic methods yielded trees with virtually identical topology, and most of the nodes that were supported by high ( $> 90\%$ ) posterior probabilities in the Bayesian analysis were also supported by high ( $> 70\%$ ) bootstrap values using maximum parsimony (Fig. 2). A majority of the *Sarcophyton* and *Lobophytum* specimens belonged to three large clades that were well supported by all analyses. Trees differed only in support for several of the sub-clades within the three major clades (specifically, three sub-clades of *Lobophytum* that were supported well by Bayesian but not by maximum parsimony analyses) and in the placement of the very long branch to *S. glaucum* NTM-C014113. Bayesian and maximum likelihood analyses suggested a sister relationship between this specimen and *S. regulare* NTM-C014871 (Fig. 2), while maximum parsimony positioned NTM-C014113 as a sister taxon to the D clade of *S. glaucum*.

Twenty-six specimens identified to 13 species of *Lobophytum* (Table 1) formed one very well-supported clade (Fig. 2). Within this *Lobophytum* clade, there was, however, little internal structure, and many specimens identified to different species had sequences that were identical to one another.

A second well-supported clade included 41 specimens identified to 12 species of *Sarcophyton* (Table 1). In contrast to the *Lobophytum* clade, this clade had a significant internal structure with a number of well-supported, genetically distinct sub-clades (Fig. 2). Six of these sub-clades (A–F), including those separated by the longest branch lengths, included speci-

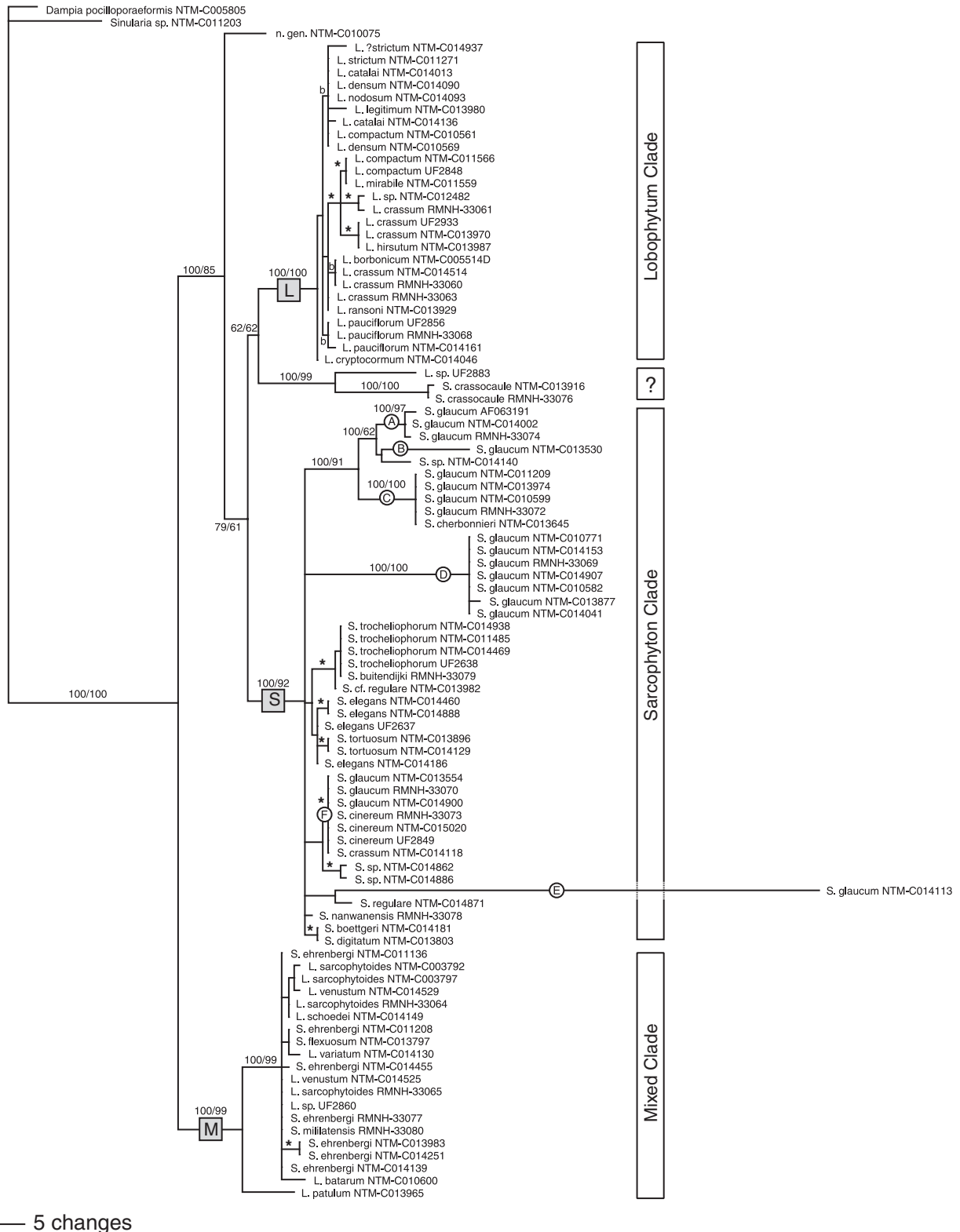
mens identified to a single species; *S. glaucum*. *S. cherbonnieri*, *S. crassum*, and *S. cinereum* fell within two of these distinct *S. glaucum* clades (C and F). Several geographically widespread specimens of *S. trocheliophorum* formed another strongly supported sub-clade, although *S. buitendijki* and a specimen identified as *S. cf. regulare* were also included within this clade.

The third major clade formed a sister group to the *Lobophytum* and *Sarcophyton* clades, and included a mix of nominal species from each of the two genera (Fig. 2). The 21 specimens in this clade represented three *Sarcophyton* and six *Lobophytum* species (Table 1). *Lobophytum patulum* was significantly differentiated from all other species in this mixed clade. However, with the exception of two specimens of *S. ehrenbergi* that formed a well-supported sub-clade, the rest of the specimens were virtually identical to one another genetically and there was no significant phylogenetic structure among them.

The only two species of *Sarcophyton* and *Lobophytum* that did not fall into one of these three major clades were *Sarcophyton crassocaule* and an unidentified *Lobophytum* species from Okinawa (UF2883). These two taxa formed a clade with 100% support, but their position relative to the *Sarcophyton* and *Lobophytum* clades was poorly resolved. Although all three phylogenetic analyses positioned this clade as a sister group to the *Lobophytum* clade, support for such a relationship was only weak (posterior probability = 62%, bootstrap value = 62%).

### Morphology

The three major clades of *Sarcophyton* and *Lobophytum* represented in the molecular phylogeny can also be distinguished from one another morphologically using a combination of colony growth form, polyp, and sclerite characters (Table 2). All of the specimens in the *Lobophytum* clade exhibit the typical *Lobophytum* growth morphology with prominent lobes or ridges on the polypary surface (Fig. 1C,D). Species in this clade also have typical *Lobophytum* sclerites. Sclerites in the surface tissues are fairly small ( $< 0.27$  mm) poorly formed clubs with relatively large tubercular warts often arranged in girdles around the club “handle” (Fig. 3A). Basal interior sclerites are short ( $< 0.5$  mm) oval or cylindrical forms with regular girdles of warts (Fig. 4A). Polyp armature varies from no sclerites in the polyps (e.g., *L. compactum*) to prominent points formed by rods to 0.30 mm length (e.g., *L. crassum*) (Fig. 3D). When present, sclerites in the tentacles are short



**Fig. 2.** Consensus phylogram (50% majority rule) of 12,251 Bayesian likelihood trees (burn-in = 3750). Numbers at nodes indicate Bayesian posterior probabilities, followed by bootstrap percentages from maximum parsimony analysis (100 bootstrap replicates). Where there was insufficient space to include a number, asterisks indicate nodes with posterior probability > 90 and bootstrap percentage > 70. Nodes labeled b were supported by Bayesian posterior probabilities > 90, but had bootstrap values < 70%. Circled letters A–F indicate clades containing specimens identified as *Sarcophyton glaucum*.

**Table 1.** Species of *Lobophytum* and *Sarcophyton* included in each of the three major clades of the *msh1* phylogeny (Fig. 2).

Lobophytum clade	Sarcophyton clade	Mixed clade
<i>Lobophytum borbonicum</i> VON MARENZELLER 1886	<i>Sarcophyton boettgeri</i> SCHENK 1896	<i>L. batarum</i> MOSER 1919
<i>L. catalai</i> TIXIER-DURIVAUT 1957	<i>S. buitendijki</i> VERSEVELDT 1982	<i>L. patulum</i> TIXIER-DURIVAUT 1956
<i>L. compactum</i> TIXIER-DURIVAUT 1956	<i>S. cherbonnieri</i> TIXIER-DURIVAUT 1958	<i>L. sarcophytoides</i> MOSER 1919
<i>L. crassum</i> VON MARENZELLER 1886	<i>S. cinereum</i> TIXIER-DURIVAUT 1946	<i>L. schoedei</i> MOSER 1919
<i>L. cryptocormum</i> VERSEVELDT & TURSCH 1979	<i>S. crassum</i> TIXIER-DURIVAUT 1946	<i>L. variatum</i> TIXIER-DURIVAUT 1957
<i>L. densum</i> TIXIER-DURIVAUT 1970	<i>S. digitatum</i> MOSER 1919	<i>L. venustum</i> TIXIER-DURIVAUT 1957
<i>L. hirsutum</i> TIXIER-DURIVAUT 1956	<i>S. elegans</i> MOSER 1919	<i>S. ehrenbergi</i> VON MARENZELLER 1886
<i>L. legitimum</i> TIXIER-DURIVAUT 1970	<i>S. glaucum</i> (QUOY & GAIMARD 1833)	<i>S. flexuosum</i> TIXIER-DURIVAUT 1966
<i>L. mirabile</i> TIXIER-DURIVAUT 1956	<i>S. nanwanensis</i> BENAYAHU & PERKOL-FINKEL 2004	<i>S. mililatensis</i> VERSEVELDT & TURSCH 1979
<i>L. nodosum</i> TIXIER-DURIVAUT 1969	<i>S. regulare</i> TIXIER-DURIVAUT 1946	
<i>L. pauciflorum</i> (EHRENBERG 1834)	<i>S. tortuosum</i> TIXIER-DURIVAUT 1946	
<i>L. ransonii</i> TIXIER-DURIVAUT 1957	<i>S. trocheliophorum</i> Von Marenzeller 1886	
<i>L. strictum</i> TIXIER-DURIVAUT 1957		

(<0.11 mm) rods with sparse, conical, or blunt projections.

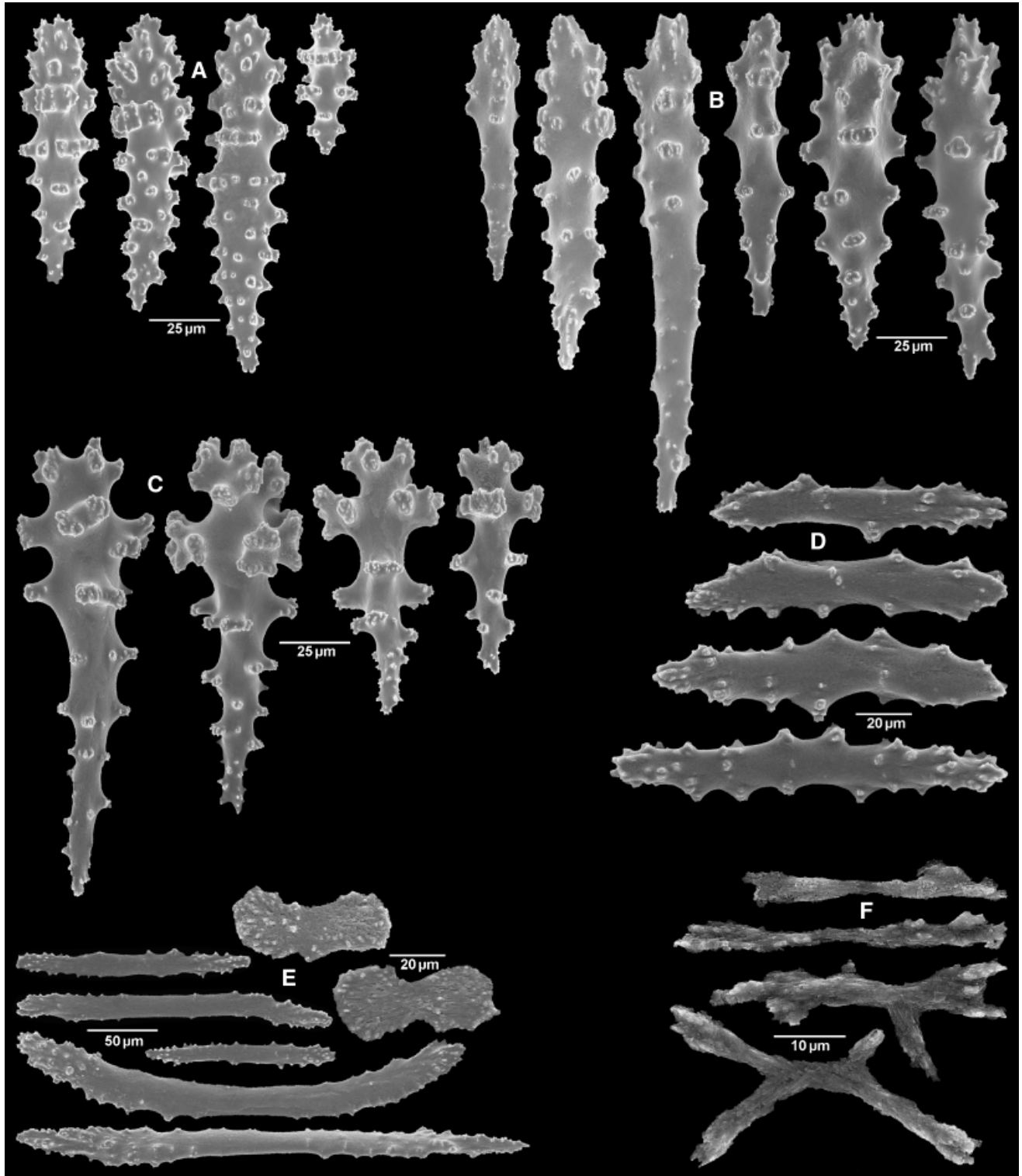
Specimens in the *Sarcophyton* clade have a growth form in which the polypary consists of a smooth, marginally folded disc that projects beyond a clearly differentiated base or stalk (Fig. 1A,B). When fully contracted or preserved, the edges of the disc, which may be highly convoluted, fold down around the top of the stalk. Surface sclerites are usually long-handled clubs (0.25–0.77 mm) with poorly differentiated heads and fairly sparse, simple ornamentation (Fig. 3B). Most of the species in this clade have long (0.5–2.5 mm) spindle-shaped sclerites in the

interior of the stalk, with tubercles distributed randomly rather than in regular girdles (Fig. 4B). With a single known exception, sclerites are always present in the polyps, although armature ranges from sparse points formed by a few small rods to a very heavy collaret and points of large ( $\leq 0.9$  mm) rods, often with transverse rows of rods distributed down the anthocodia. Flat scales (<0.1 mm) are almost always present in the tentacles, usually double-ended forms or “butterflies” (Fig. 3E).

Within the *Sarcophyton* clade, *S. nanwanensis* represents an exception to most of the generalities described above and in Table 2. This species lacks polyp

**Table 2.** Summary table of morphological differences among the three genetically distinct clades of *Sarcophyton* and *Lobophytum* in the *msh1* phylogeny (Fig. 2).

Character	Sarcophyton clade	Lobophytum clade	Mixed clade
Colony growth morphology	Smooth disc distinct from base or stalk; may resemble a mushroom	Disc surface with prominent lobes, ridges, or fingers	Disc not distinct from stalk, may have partial ridges or edges fused into lobes
Polyp armature	Rods forming points	Variable	None
Tentacle sclerites	Flat scales	Small rods, if present	Small rods, if present
Surface clubs	Poorly developed, with simple ornamentation	Poorly developed, with large warts in girdles	Well developed, often with central wart
Size of surface clubs	0.25 to 0.77 mm	<0.27 mm	<0.25 mm
Disc interior	Spindles or rods with short thorns	Fat spindles or ovals with girdles of warts	Spindles, often with antler or rose thorn tubercles
Stalk interior	Large spindles with dense warts	Ovals, cylinders with girdles of warts	Spindles or elongated ovals
Size of interior sclerites	$\gg 0.5$ –2.5 mm	<0.5 mm	<0.5 mm



**Fig. 3.** Typical morphology of sclerites from the colony surface and polyps of *Sarcophyton* and *Lobophytum*. A–C. surface sclerites; **A.** *Lobophytum crassum*, RMNH-Coel. 33061. **B.** *Sarcophyton glaucum*, RMNH-Coel. 33072. **C.** *L. sarcophytoides*, RMNH-Coel. 33064. D–F. polyp sclerites; **D.** *L. crassum*, RMNH-Coel. 33061. **E.** *S. glaucum*, RMNH-Coel. 33072. **F.** *S. ehrenbergi*, RMNH-Coel. 33077. Photos taken using a scanning electron microscope.

sclerites entirely, has short, club-like oval sclerites rather than long-handled clubs in the colony surface, and small (<0.3 mm) cylinders and capstans in the stalk interior (Benayahu & Perkol-Finkel 2004). Other species that also present some exceptions are *S. elegans*, whose surface clubs have a large, rounded head that is clearly differentiated from the handle and is ornamented with dense warts, and *S. troche-liophorum* and *S. buitendijki*, whose characteristic basal interior sclerites are short (0.3–0.55 mm) ovals rather than long spindles (Verseveldt 1982). In contrast to the typical *Lobophytum* sclerites, however, the tubercles on these ovals are not arranged in regular girdles, although they may be distributed on either side of a median constriction.

The mixed clade includes specimens with several different colony growth forms. Species such as *S. ehrenbergi* and *S. mililatisensis* tend to form bowl- or funnel-shaped colonies in which the disc is not clearly differentiated from and barely protrudes beyond the stalk, and the colony surface is concave when contracted. A second growth form within this clade is represented by species such as *L. patulum* and *L. variatum*, which form flat plates with or without a few low lobes or ridges on their surface. In species such as *L. schoedei* and *L. sarcophytoides*, the folded edges of the disc may fuse to form lobes or ridges (Fig. 1E,F). The sclerites in the surface tissue of species in this clade are small (<0.25 mm) well-formed clubs with a head that is distinct from the handle; the head often consists of a prominent central wart surrounded by a girdle of large warts (Fig. 3C). The sclerites of the colony interior are relatively small (<0.5 mm) spindles whose ornamentation often includes antler-like projections, recurved thorns (“rose thorns”), or square-ended processes (Fig. 4C). Some species, however, have cylinders or elongated ovals with tubercles arranged in girdles, similar to those found in the *Lobophytum* clade. Species in the mixed clade can usually be distinguished from those in the *Sarcophyton* clade by the near or complete absence of sclerites in the polyps. Mixed clade species never have points or a collaret, and they lack flat scales in the tentacles. When a few sclerites are present in the tentacles, they are always small (<0.1 mm) rods with sparse prominences that are usually arranged in two sub-terminal girdles (Fig. 3F).

The only two species to fall outside of the three major genetic clades, *S. crassocaula* and *Lobophytum* sp. UF2883, have little in common with one another morphologically. *Sarcophyton crassocaula* is like *Sarcophyton*-clade species in that it forms colonies with a clearly differentiated stalk and smooth-surfaced disc, and the surface sclerites are poorly formed clubs with sparse, simple ornamentation. The sclerites in the

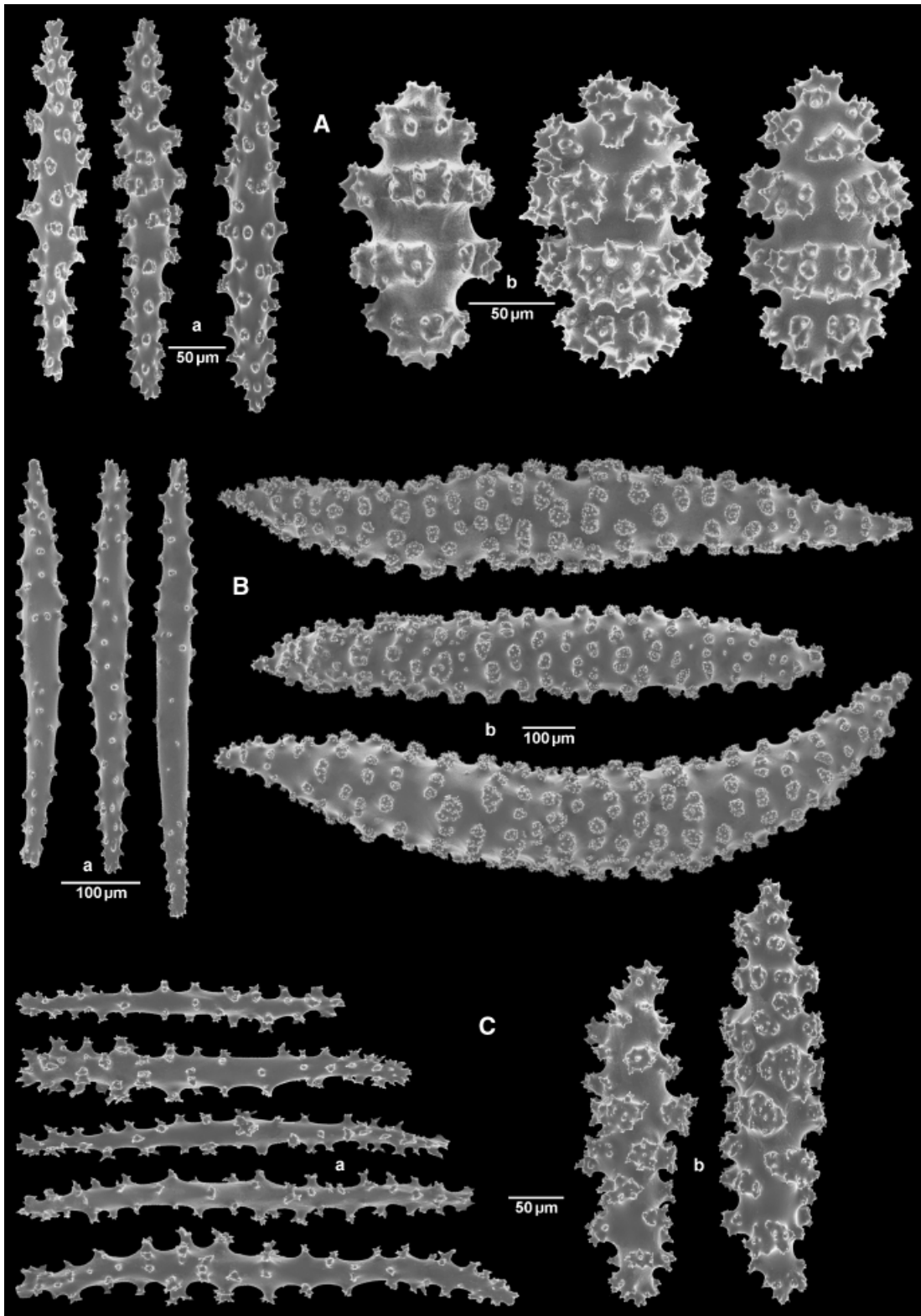
interior of the stalk, however, are not the typical large spindles of the *Sarcophyton* clade, but include both small spindles (<0.32 mm) and ovals (<0.25 mm) with warts arranged in girdles similar to those of the *Lobophytum* clade. The polyps of *S. crassocaula* have large ( $\leq 0.52$  mm), very smooth rods but lack the flat scales in the tentacles that are characteristic of the *Sarcophyton* clade. Morphologically, therefore, *S. crassocaula* appears to share some character states with the *Sarcophyton* clade and some with the *Lobophytum* clade.

*Lobophytum* sp. UF2883, on the other hand, shares most of its morphological character states with the *Lobophytum* clade. The polypary is not differentiated from the stalk, and large, laterally flattened lobes arise from the colony surface. The surface sclerites are poorly formed clubs with regular girdles of tubercles, and the interior sclerites are short ovals, also with tubercles arranged in regular girdles. The polyps contain short rods with numerous conical projections. Based on morphology, therefore, this species would appear to belong to the *Lobophytum* clade rather than to any morphologically intermediate clade.

## Discussion

Our molecular phylogenetic analysis suggests that the conventional taxonomic division between *Lobophytum* and *Sarcophyton* does not reflect the evolutionary relationships among species assigned to these two genera. Three very well-supported clades are evident in our analysis: one encompassing species with typical *Sarcophyton* morphological characters, one comprising morphologically typical *Lobophytum* species, and a third distinct clade that includes a mix of nominal species from each genus. This mixed clade includes a number of taxa that have traditionally been assigned to *Lobophytum* on the basis of colony growth morphology but whose interior sclerites bear closer resemblance to those of *Sarcophyton*. Based on morphology, we predict that an additional three to four species not included in our molecular phylogeny—*Sarcophyton infundibuliforme* TIXIER-DURIVALT 1958, *S. roseum* PRATT 1903, *Lobophytum denticulatum* TIXIER-DURIVALT 1956, and perhaps *L. latilobatum* VERSEVELDT 1971—also belong to the mixed clade. Despite having morphological characters that might be considered intermediate to typical *Sarcophyton* and *Lobophytum*, this mixed clade is not phylogenetically intermediate but is genetically very well separated from and a sister to both of those clades (Fig. 2).

Genetic differentiation at the *msh1* locus is much greater among the three clades of *Sarcophyton* and *Lobophytum* than it is among numerous genera in



**Fig. 4.** Typical morphology of sclerites from the interior tissues of *Sarcophyton* and *Lobophytum*. **A.** *Lobophytum crassum*, RMNH-Coel. 33061. **B.** *Sarcophyton glaucum*, RMNH-Coel. 33072. **C.** *L. sarcophytoides*, RMNH-Coel. 33064. a, interior of polypary; b, interior of colony base. Photos taken using a scanning electron microscope.

other families of soft corals. Pairwise genetic distances (uncorrected *p*) for *msh1* range 0–0.03 among seven genera in family Nephtheidae and 0–0.02 among six genera of Xeniidae (C.S. McFadden, unpubl. data). For comparison, the average pairwise distance among the three *Sarcophyton*–*Lobophytum* clades ranges 0.05–0.07. In combination with morphological characters that are diagnostic for each clade (Table 2), such large genetic differences argue for division of the two genera into three. The mixed clade is particularly distinct genetically, and the relative lack of genetic differentiation among the species within this clade, some currently assigned to *Sarcophyton* and some to *Lobophytum*, certainly argues for taxonomic revision. This has significant implications for the genus *Sarcophyton*, whose type species *S. ehrenbergi* belongs to this mixed clade.

The morphological distinctions between the *Lobophytum* and *Sarcophyton* clades become very clear once the species with intermediate morphologies are recognized as belonging to a separate clade (Table 2). These two clades are less well separated from one another genetically, however, than either is from the mixed clade. Especially troublesome is the small clade formed by *S. crassocaule* and *Lobophytum* sp. UF2883, whose phylogenetic position relative to the *Sarcophyton* and *Lobophytum* clades is poorly resolved. Morphologically, *S. crassocaule* appears to be a fairly typical *Sarcophyton*-clade species, while *L.* sp. UF2883 has typical *Lobophytum*-clade characters. No obvious morphological clues suggest why either of these two species should fall outside of those clades or why they would be united with one another genetically. Additional sequence data will be necessary to determine whether these species might actually belong to the *Lobophytum* clade, as the posterior probabilities and bootstrap values both weakly suggest. Although the interior basal sclerites of *S. crassocaule* bear some resemblance to those typical of *Lobophytum* (Verveveldt 1982), little else about this species suggests that it might be an aberrant member of that clade.

The only other specimen to fall outside of any of the three major clades is NTM-C010075. This undescribed species has a unique, globose colony morphology unlike that of any other species of *Sarcophyton* or *Lobophytum*. Before this study, this species had already been proposed as a new genus (P. Alderslade, unpubl. data), a designation supported by its clear genetic separation from all three *Sarcophyton*–*Lobophytum* clades.

### Morphological variation and species boundaries

Within each of the three well-defined clades in our phylogeny, relatively few species form well-

supported, monophyletic groups. For instance, within each clade are multiple examples of specimens identified to different species that nonetheless share identical *msh1* haplotypes. Conversely, some specimens identified as belonging to the same species fall into different genetic sub-clades. This latter result is especially pronounced for specimens identified as *S. glaucum*, and will be discussed in a separate section below.

The lack of clear genetic distinctions among species in each clade is likely the combined result of two factors, the most obvious of which is that the *msh1* gene simply may not be sufficiently variable to distinguish closely related species within these genera. Within the mixed and *Lobophytum* clades, in particular, there is little genetic differentiation among most of the species belonging to the clade, suggesting that *msh1* is an inappropriate marker for intra-clade phylogenetics. This problem can be remedied by using a more variable molecular marker to examine the phylogenetic relationships of species within each clade. Preliminary data suggest that the rDNA internal transcribed spacers (ITS) may be useful for further discrimination of genetic differences among nominal species of *Sarcophyton* and *Lobophytum* (C.S. McFadden & H. Johnsen, unpubl. data).

The second, more serious factor contributing to the confused relationships among species within clades is our lack of understanding of intraspecific morphological variation and species boundaries in *Sarcophyton* and *Lobophytum*. Verveveldt's (1982, 1983) taxonomic revisions are the sole authoritative sources available for identification of species in these genera. Verveveldt examined and illustrated type material only, however, basing most of his species descriptions on a single specimen; he included multiple specimens only when they represented taxonomically recognized, named variants. The number of morphological characters that he used for taxonomic classification was also small, including only (1) colony growth form based on preserved specimens (no live material was examined), (2) spacing of autozooids and siphonozooids on the colony surface, and (3) the size and shape (judged subjectively) of sclerites from the (a) polypary surface, (b) interior of the polypary, (c) surface of the stalk or base, and (d) interior of the stalk or base. For each species, his description includes only a photograph of the preserved type specimen(s) and hand-drawn illustrations of no more than six to eight sclerites from each anatomical region of the colony. Identifying a colony of *Sarcophyton* or *Lobophytum* to species therefore requires matching sclerite samples to the limited selection illustrated for the type specimen. An exact match is

rarely possible (like snowflakes, no two sclerites are identical), and species identification becomes a subjective process of determining whether or not a specimen resembles the type of one species more closely than another. Most often, positive identification to species is simply not possible using this limited typological approach.

Comparison of the original type material with Verseveldt's (1982, 1983) species descriptions has revealed some additional inadequacies that further impact our ability to identify nominal species of *Sarcophyton* and *Lobophytum* with certainty. Our comparisons of Verseveldt's sclerite slides with slides we have prepared subsequently from the same type material suggest that his methods of slide preparation inadvertently resulted in the loss of many of the smaller sclerite forms. Moreover, comparison of his original slides with the illustrations he made from them indicates that he did not always illustrate the full range of sclerite types found on a slide, and occasionally omitted forms that could be species diagnostic. The omission of small sclerites and other possibly unique sclerite forms from his revisions compounds the difficulty of typologically matching specimens to his descriptions.

To date, only one study has examined the range of intraspecific variation in both sclerite morphology and colony growth form for any alcyoniid soft coral (*Simularia brassica*; Benayahu et al. 1998). Our lack of understanding of intraspecific variation makes it impossible to determine whether cases in which specimens identified to different species nonetheless share identical genotypes are due to (1) insufficient resolution of the genetic markers to distinguish closely related species, (2) incorrect species identifications, or (3) morphological variants of the same species having been classified as distinct species. Preliminary comparisons among individuals of *Sarcophyton* with similar colony growth forms and identical *msh1* haplotypes collected from the same reef suggest that differences in sclerite morphology among (presumably) conspecific individuals may be greater than the differences among type specimens attributed to different species (C.S. McFadden, unpubl. data).

### ***Sarcophyton glaucum*: a cryptic species complex?**

In contrast to the *Lobophytum* and mixed clades within which there is little phylogenetic structure, the *Sarcophyton* clade includes a number of sub-clades that are very well differentiated from one another genetically. Six of these sub-clades include specimens identified as *S. glaucum*, and the genetic distances separating them are greater than those among any of

the other nominal species in our tree. NTM-C014113, a specimen of *S. glaucum* from American Samoa, is particularly noteworthy for the extreme degree of genetic differentiation separating it from other clades (Fig. 2).

Verseveldt (1982) and other taxonomists who preceded him recognized *S. glaucum* as a problematic taxon: "Many investigators already pointed out the great variability found in this species, especially with respect to shape and size of the colony, and shape and dimensions of the coenenchymal sclerites in the stalk. This variability explains why so many authors established new species and varieties, which after all must be referred to a single species: *S. glaucum*" (pp. 53–54). Verseveldt synonymized eight nominal species and three recognized variants with *S. glaucum*, but in his revision included illustrations of sclerites from only two specimens. Moreover, he did not explain how or why he chose these two particular specimens to represent the species: the type specimen of *S. glaucum* has been lost, and presumably only previous descriptions of the species, none based on type material, would have been available to him. We included in our study numerous specimens that we had assigned to *S. glaucum sensu* Verseveldt (1982) on the basis of their general similarity to the material he illustrated, but among which we nonetheless recognized considerable variation in sclerite complement. Our genetic data suggest that this morphologically variable taxon actually represents a complex of at least six genetically distinct species.

As a result of the confused state of the species-level taxonomy in this genus, we cannot yet say whether the phylogenetically distinct clades that comprise *Sarcophyton glaucum* in our tree represent cryptic species that have not been recognized previously, or whether they represent other nominal species with which *S. glaucum* may be confused or has previously been synonymized. Specimens identified as *S. cinereum* and *S. crassum* both fall into clade F, raising the possibility that the specimens in that clade identified as *S. glaucum* might actually belong to one of these other morphologically similar species. Likewise, the *S. glaucum* specimens in clade C are genetically identical to a specimen identified as *S. cherbonnieri*. Other species that are morphologically similar to *S. glaucum* and difficult to distinguish from it include *S. solidum* TIXIER-DURIVault 1958 and *S. subviride* TIXIER-DURIVault 1958. Examination of type material for all of these similar forms as well as the species synonymized with *S. glaucum* by Verseveldt (1982) will be required in order to determine whether the clades we have distinguished genetically represent new or previously described morphospecies.

In many geographical areas, *S. glaucum sensu lato* is one of the most common and conspicuous alcyoniid species on shallow reefs (Benayahu & Loya 1977, 1986; Schleyer et al. 2004). It is of some importance, therefore, to understand the ecological and geographical distributions of the genetically distinct clades comprising this species complex. Preliminary data on the geographic ranges of the six distinct *S. glaucum* clades suggest that they are broadly sympatric over large areas of the Indo-West Pacific and that multiple clades co-occur on the same reefs (C.S. McFadden, unpubl. data). Co-occurrence of multiple morphologically cryptic forms at a single location may help to explain the high diversity of chemical compounds (Tanaka et al. 2005) and variation in reproductive biology (Schleyer et al. 2004) that have been observed in some populations of *S. glaucum*.

### Conclusions

The confused state of taxonomic relationships within and between the genera *Sarcophyton* and *Lobophytum* is a combined product of (1) the relatively few morphological characters available for study in these organisms, (2) our present lack of understanding of intraspecific variation in those morphological characters, and (3) a historical lack of taxonomic and ecological work on alcyonacean octocorals in general. The paucity of ecological work on these common and conspicuous genera stems in part from an inability to distinguish species reliably, but has also contributed to lack of progress in that area. Ecological, reproductive, and behavioral differences can provide important taxonomic characters that may help to distinguish morphologically cryptic yet genetically distinct forms (McFadden 1999), but until more ecological and reproductive work is conducted—in conjunction with the study of morphological and genetic variation—such characters will remain unavailable for taxonomy. Much progress is being made in our understanding of species boundaries in scleractinian corals through the combined study of genetics, reproduction, ecology, and morphology (Stobart 2000; Carlon & Budd 2002; Wolstenholme et al. 2003; Fukami et al. 2004). Octocoral taxonomy will certainly benefit from a similarly holistic approach.

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## Appendix A

**Table A1.** Collection information for specimens included in molecular phylogeny. NTM = Museum and Art Gallery of the Northern Territory, Darwin; RMNH = National Museum of Natural History (NNM), formerly Rijksmuseum van Natuurlijke Historie, Leiden, The Netherlands; UF = Florida Museum of Natural History, Gainesville.

Species	Accession no.	Site	Latitude	Longitude	Date	GenBank #
<i>Sarcophyton boettgeri</i>	NTM-C014181	Normany I., Papua New Guinea	10°00.96'S	150°57.49'E	2002	DQ280501
<i>S. buitendijki</i>	RMNH Coel33079	Berau I., NE Kalimantan, Indonesia	02°14.22'N	118°05.36'E	2003	DQ280502
<i>S. cherbonnieri</i>	NTM-C013645	Stanley Reef, GBR, Australia	19°17'S	148°08'E	2002	DQ280503
<i>S. cinereum</i>	NTM-C015020	Darwin, NT, Australia	12°18.88'S	130°54.26'E	2002	DQ280504
<i>S. cinereum</i>	RMNH Coel33073	Berau I., NE Kalimantan, Indonesia	02°19.15'N	118°06.32'E	2003	DQ280505
<i>S. cinereum</i>	UF2849	Ie I., Okinawa, Japan	26°44.35'N	127°48.61'E	2004	DQ280506
<i>S. crassocaula</i>	NTM-C013916	Milne Bay, Papua New Guinea	10°36.72'S	152°32.29'E	1998	DQ280507
<i>S. crassocaula</i>	RMNH Coel33076	Berau I., NE Kalimantan, Indonesia	02°17.41'N	118°13.52'E	2003	DQ280508
<i>S. crassum</i>	NTM-C014118	Pago Pago Harbor, American Samoa	14°07.22'S	170°14.70'W	2001	DQ280509
<i>S. digitatum</i>	NTM-C013803	West Babeldaob, Palau	07°29.80'N	134°25.92'E	1995	DQ280510
<i>S. ehrenbergi</i>	NTM-C011136	Magnetic I., GBR, Australia	19°09'S	146°52'E	1989	DQ280511
<i>S. ehrenbergi</i>	NTM-C011208	Jepara, Central Java Sea	06°35.00'S	110°39.00'E	1992	DQ280512
<i>S. ehrenbergi</i>	NTM-C013983	Mauritius 16, Mauritius	19°56.61'S	57°36.96'E	1999	DQ280513
<i>S. ehrenbergi</i>	NTM-C014139	Ofu I., American Samoa	14°00.95'S	169°40.85'W	2001	DQ280514
<i>S. ehrenbergi</i>	NTM-C014251	Gulf of Mannar, India	19°10'N	9°20'E	2001	DQ280515
<i>S. ehrenbergi</i>	NTM-C014455	Gulf of Carpentaria, Australia	12°05.66'S	136°47.75'E	2003	DQ280516
<i>S. ehrenbergi</i>	RMNH Coel33077	Berau I., NE Kalimantan, Indonesia	02°25.09'N	118°07.22'E	2003	DQ280517
<i>S. elegans</i>	NTM-C014186	Cape Nelson, Papua New Guinea	09°07.94'S	149°29.32'E	2002	DQ280518
<i>S. elegans</i>	NTM-C014460	Gulf of Carpentaria, NT	12°05.66'S	136°47.75'E	2003	DQ280519
<i>S. elegans</i>	UF2637	Sesoko I., Okinawa, Japan	26°38'N	127°52'E	2004	DQ280520
<i>S. elegans</i>	NTM-C014888	Wonder Channel, Palau	7°10.83'N	134°21.67'E	2005	DQ280521
<i>S. elegans</i>	NTM-C013797	Ngel Channel, Palau	07°18.69'N	134°28.24'E	1995	DQ280522
<i>S. flexuosum</i>		Townsville, Australia				AF063191
<i>S. glaucum</i>	NTM-C010599	Yanutha I., Fiji	18°20'S	178°E	1986	DQ280523
<i>S. glaucum</i>	NTM-C010582	Yanuca I., Fiji	18°20'S	178°E	1987	DQ280524
<i>S. glaucum</i>	NTM-C010771	Rowley Shoals, WA, Australia	17°07.07'S	119°20.02'E	1987	DQ280525
<i>S. glaucum</i>	NTM-C011209	Karimunjawa I., Central Java Sea			1991	DQ280526
<i>S. glaucum</i>	NTM-C013877	N. Male Atoll, Maldives	04°18.36'N	73°30.52'E	1997	DQ280527
<i>S. glaucum</i>	NTM-C013530	Semporna I., Sabah, Malaysia	04°38.06'N	118°42.58'E	1999	DQ280528
<i>S. glaucum</i>	NTM-C013554	Semporna I., Sabah, Malaysia	04°34.12'N	118°44.29'E	1999	DQ280529
<i>S. glaucum</i>	NTM-C013974	Mauritius 11, Mauritius	19°56.75'S	57°37.24'E	1999	DQ280530
<i>S. glaucum</i>	NTM-C014002	Konanda Reef, Vanuatu	17°45.17'S	168°17.28'E	2000	DQ280531

Table A1. Continued

Species	Accession no.	Site	Latitude	Longitude	Date	GenBank #
<i>S. glaucum</i>	NTM-C014041	Tutuba, Santo, Vanuatu	15°33.32'S	167°16.59'E	2000	DQ280532
<i>S. glaucum</i>	NTM-C014113	Pago Pago Harbour, American Samoa	14°06.82'S	170°40.07'W	2001	DQ280533
<i>S. glaucum</i>	NTM-C014153	Woodlark I., Papua New Guinea	09°13.89'S	152°25.56'E	2002	DQ280534
<i>S. glaucum</i>	RMNH Coel33069	Berau I., NE Kalimantan, Indonesia	02°09.47'N	118°32.03'E	2003	DQ280535
<i>S. glaucum</i>	RMNH Coel33070	Berau I., NE Kalimantan, Indonesia	02°12.08'N	118°11.34'E	2003	DQ280536
<i>S. glaucum</i>	RMNH Coel33072	Berau I., NE Kalimantan, Indonesia	02°17.40'N	118°13.52'E	2003	DQ280537
<i>S. glaucum</i>	RMNH Coel33074	Berau I., NE Kalimantan, Indonesia	02°04.45'N	118°24.11'E	2003	DQ280538
<i>S. glaucum</i>	NTM-C014900	West Channel, Palau	07°32.50'N	134°28.35'E	2005	DQ280539
<i>S. glaucum</i>	NTM-C014907	West Channel, Palau	07°32.50'N	134°28.35'E	2005	DQ280540
<i>S. militatensis</i>	RMNH Coel33080	Berau I., NE Kalimantan, Indonesia	02°20.16'N	118°08.12'E	2003	DQ280541
<i>S. nanwanensis</i>	RMNH Coel33078	Berau I., NE Kalimantan, Indonesia	02°12.08'N	118°11.34'E	2003	DQ280542
<i>S. cf. regulare</i>	NTM-C013982	Mauritius 13, Mauritius	19°58.04'S	57°38.01'E	1999	DQ280543
<i>S. regulare</i>	NTM-C014871	Mutremdiu, Palau	07°16.41'N	134°31.43'E	2005	DQ280544
<i>S. tortuosum</i>	NTM-C013896	Vavau, Tonga	18°40.28'S	174°03.37'W	1997	DQ280545
<i>S. tortuosum</i>	NTM-C014129	Tutuila, American Samoa	14°07.19'S	170°43.97'W	2001	DQ280546
<i>S. trocheliophorum</i>	NTM-C011485	NW Eil Malk, Palau	07°11'N	134°22'E	1990	DQ280547
<i>S. trocheliophorum</i>	NTM-C014938	Piti Bay, Guam			1998	DQ280548
<i>S. trocheliophorum</i>	NTM-C014469	Gulf of Carpentaria, Australia	12°05.66'S	136°47.75'E	2003	DQ280549
<i>S. trocheliophorum</i>	UF2638	Ginowan City, Okinawa, Japan	26°17'N	127°43'E	2004	DQ280550
<i>Sarcophyton</i> sp.	NTM-C014140	Ofu I., American Samoa	14°00.95'S	169°40.85'W	2001	DQ280551
<i>Sarcophyton</i> sp.	NTM-C014862	Mutremdiu, Palau	07°16.41'N	134°31.43'E	2005	DQ280552
<i>Sarcophyton</i> sp.	NTM-C014886	Wonder Channel, Palau	07°10.83'N	134°21.67'E	2005	DQ280553
<i>Lobophytum batarum</i>	NTM-C010600	Viti Levu, Fiji	18°20'S	178°E	1986	DQ280554
<i>L. borbonicum</i>	NTM-C005514-D	Darwin, NT, Australia	12°29.50'S	130°53.50'E	1988	DQ280555
<i>L. catalai</i>	NTM-C014013	Tukituki, Vanuatu	17°42.49'S	168°09.35'E	2000	DQ280556
<i>L. catalai</i>	NTM-C014136	Ofu I., American Samoa	14°00.46'S	169°41.16'W	2001	DQ280557
<i>L. compactum</i>	NTM-C010561	Motupore I., Papua New Guinea	09°60'S	147°20'E	1988	DQ280558
<i>L. compactum</i>	NTM-C011566	Orpheus I., GBR, Australia	18°33'S	146°30'E	1992	DQ280559
<i>L. compactum</i>	UF2848	Ginowan City, Okinawa, Japan	26°17'N	127°43'E	2004	DQ280560
<i>L. crassum</i>	NTM-C013970	Mauritius 7, Mauritius	20°19.23'S	57°22.01'E	1999	DQ280561
<i>L. crassum</i>	NTM-C014514	Gulf of Carpentaria, Australia	12°05.66'S	136°47.75'E	2003	DQ280562
<i>L. crassum</i>	RMNH Coel33060	Berau I., NE Kalimantan, Indonesia	02°17.03'N	118°14.48'E	2003	DQ280563
<i>L. crassum</i>	RMNH Coel33061	Berau I., NE Kalimantan, Indonesia	02°04.53'N	118°24.29'E	2003	DQ280564
<i>L. crassum</i>	RMNH Coel33063	Berau I., NE Kalimantan, Indonesia	02°18.34'N	118°15.16'E	2003	DQ280565
<i>L. crassum</i>	UF2933	Ginowan City, Okinawa, Japan	26°17'N	127°43'E	2004	DQ280566
<i>L. cryptocormum</i>	NTM-C014046	Tutuba, Santo, Vanuatu	15°33.32'S	167°16.59'E	2000	DQ280567
<i>L. densum</i>	NTM-C010569	South Male Atoll, Maldives	04°07'N	73°28'E	1988	DQ280568
<i>L. densum</i>	NTM-C014090	Ulithi Atoll, Yap, Micronesia	10°01.08'N	139°47.18'E	2000	DQ280569
<i>L. hirsutum</i>	NTM-C013987	Mauritius 11, Mauritius	19°56.75'S	57°37.24'E	1999	DQ280570
<i>L. legitimum</i>	NTM-C013980	Mauritius 12, Mauritius	19°58.44'S	57°38.89'E	1999	DQ280571

Table A1. Continued

Species	Accession no.	Site	Latitude	Longitude	Date	GenBank #
<i>L. mirabile</i>	NTM-C011559	Orpheus I., GBR, Australia	18°33'S	146°30'E	1992	DQ280572
<i>L. nodosum</i>	NTM-C014093	Ulithi Atoll, Yap, Micronesia	09°59.73'N	139°40.05'E	2000	DQ280573
<i>L. patulum</i>	NTM-C013965	Mauritius 04, Mauritius	20°14.38'S	57°22.80'E	1999	DQ280574
<i>L. pauciflorum</i>	NTM-C014161	Milne Bay, Papua New Guinea	10°36.72'S	152°32.39'E	2002	DQ280575
<i>L. pauciflorum</i>	RMNH Coel33068	Berau I., NE Kalimantan, Indonesia	02°04.53'N	118°24.29'E	2003	DQ280576
<i>L. pauciflorum</i>	UF2856	Ginowan City, Okinawa, Japan	26°17'N	127°43'E	2004	DQ280577
<i>L. ransonii</i>	NTM-C013929	Milne Bay, Papua New Guinea	10°18.03'S	151°03.94'E	1998	DQ280578
<i>L. sarcophytoides</i>	NTM-C003792	Darwin, NT, Australia	12°33.10'S	130°52.30'E	1982	DQ280579
<i>L. sarcophytoides</i>	NTM-C003797	Darwin, NT, Australia	12°33.10'S	130°52.30'E	1982	DQ280580
<i>L. sarcophytoides</i>	RMNH Coel33064	Berau I., NE Kalimantan, Indonesia	02°14.22'N	118°05.36'E	2003	DQ280581
<i>L. sarcophytoides</i>	RMNH Coel33065	Berau I., NE Kalimantan, Indonesia	02°07.07'N	118°20.31'E	2003	DQ280582
<i>L. schoedei</i>	NTM-C014149	Tutuila I, American Samoa	14°11.94'S	170°44.97'W	2001	DQ280583
<i>L. strictum</i>	NTM-C011271	Andaman and Nicobar Islands			1991	DQ280584
<i>L. strictum</i>	NTM-C014937	Agat Bay, Guam	13°40'N	144°65'E	1998	DQ280585
<i>L. variatum</i>	NTM-C014130	Tutuila, American Samoa	14°07.98'S	170°45.15'W	2001	DQ280586
<i>L. venustum</i>	NTM-C014525	Gulf of Carpentaria, Australia	12°05.66'S	136°47.75'E	2003	DQ280587
<i>L. venustum</i>	NTM-C014529	Gulf of Carpentaria, Australia	12°05.66'S	136°47.75'E	2003	DQ280588
<i>Lobophytum</i> sp.	NTM-C012482	Andaman and Nicobar Islands			?	DQ280589
<i>Lobophytum</i> sp.	UF2860	Ginowan City, Okinawa, Japan	26°17'N	127°43'E	2004	DQ280590
<i>Lobophytum</i> sp.	UF2883	Ginowan City, Okinawa, Japan	26°17'N	127°43'E	2004	DQ280591
n. gen.	NTM-C010075	Darwin, NT, Australia	12°29.80'S	130°53.50'E	1990	DQ280592
<i>Dampiera pocilloporaeformis</i>	NTM-C005805	Rowley Shoals, WA, Australia	17°07.70'S	119°20.20'E	1987	DQ280593
<i>Simularia</i> sp.	NTM-C011203	Jepara, Central Java Sea	06°35.00'S	110°39.00'E	1992	DQ280594