Journal of the Marine Biological Association of the United Kingdom, 2011, 91(2), 329–338. © Marine Biological Association of the United Kingdom, 2010
doi:10.1017/S0025315410001530

A new species of *Triptolemma* (Porifera: Pachastrellidae) from the Pacific Ocean with a revision of the genus

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A new species, T. strongylata *sp. nov. is described on the basis of material collected from the Pacific Ocean. The new species is characterized by the presence of sinuous strongyles. Moreover the incomplete description of* T. simplex *(Sarà, 1959) is implemented on the basis of new abundant material allowing, for the first time, the complete description of the skeleton of a* Triptolemma *species and the detecting of the presence of monaxonic spicules in the spicular complement of the genus. The skeleton is composed of a thick crust of disorderly arranged mesotriaenes and scattered microscleres, supported by diverging spicule tracts formed by oxeas towards the surface. Both species were recorded associated to boring sponges (*Spiroxya *and* Cliona*) in excavations of the calcareous scleraxis of precious corals or in organogenic concretions. In the cavities where the tissue of* Triptolemma *was recorded the wall of the excavation partially lost its typical pattern characterized by ovoid scars and became irregularly eroded. Our hypothesis is that* Triptolemma *insinuates inside the erosions produced by other sponges and it is able to enlarge them by an etching mechanism based on chemical dissolution only.*

Keywords: Demospongiae, *Triptolemma*, boring sponges, new species, precious coral, coralligenous

Submitted 23 March 2010; accepted 30 July 2010; first published online 11 November 2010

INTRODUCTION

Boring aptitude is spread through sponge families. The greatest number of boring species is known in the order Hadromerida where two families, Clionaidae and Alectonidae, are exclusively composed of boring species. Among the non-hadromerid boring sponges, the best known species are those belonging to the phloeodictyid genus *Aka* entirely composed of excavating species (e.g. Schönberg & Tapanila, 2006; Calcinai *et al.*, 2007). In the family Acarnidae the genus *Zyzzya* includes highly destructive species (Calcinai *et al.*, 2000) while *Acarnus nicoleae* van Soest, Hooper & Hiemstra, 1991, is suspected to be boring. Moreover the enigmatic monotypical family Samidae includes the genus *Samus* making tiny holes and corridors in calcareous substrata (Van Soest & Hooper, 2002). Although several astrophorid genera like *Stoeba* and *Jaspis* were suspected to include excavating species (Carter, 1880; Thomas, 1973), the only genus in this order with a recognized boring ability is *Holoxea* with the species *H. excavans* (Calcinai *et al.*, 2001, 2003).

Sponges etch limestone by a two-step (chemical and mechanical) mechanism, based on the activity of peculiar etching cells (Pomponi, 1977), that produce the detachment of chips of carbonates resulting in a typical pitting pattern on the wall of the excavations (Rützler & Rieger, 1973). The uniformity of the morphological pattern observed in many species

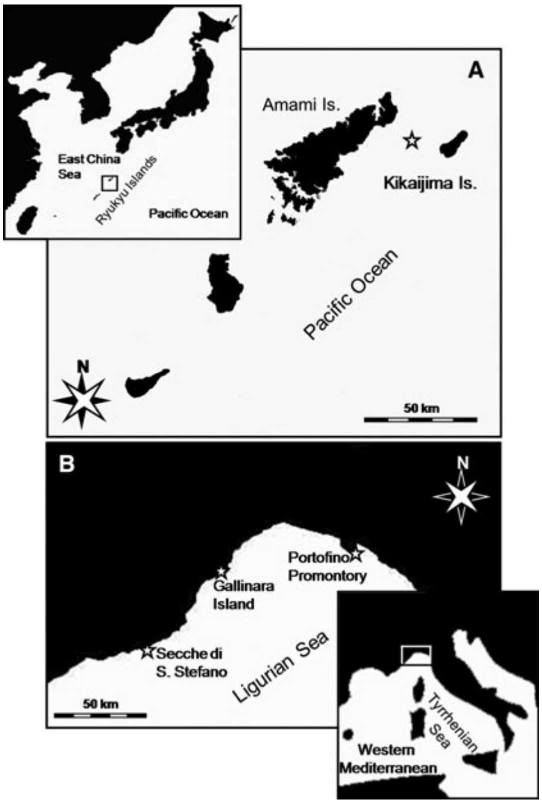

Fig. 1. Sampling areas. (A) Amami Island (Pacific Ocean); (B) Ligurian Sea. The stars indicate the sampling site.

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strongly suggests a homogeneity of the boring process in several, non-taxonomically related sponges. Differences recorded in the micro-morphology of pits that may show a smooth surface or the presence of concentric or sometimes radial scars (Borchiellini *et al*., 2003; Calcinai *et al*., 2003, 2004b), probably reflect differences in etching cell morphology and/or physiology but not in the general excavating mechanism. Recently the relationships between chemical and mechanical action were studied, suggesting that the first step is not negligible and sometimes is more important than the second (Zundelevich *et al*., 2007; Nava & Carballo, 2008).

In the crevices of carbonate substrata a diversified group of insinuating sponges co-exists together with recognized boring species. For example, in the Mediterranean coralligenous conglomerate 26 insinuating species were recorded (Bertolino, unpublished data). These species are known only from a taxonomic point of view while no information is available about their ecology and behaviour. For several of these species the boring aptitude was just hypothesized but never confirmed.

In this paper we describe the new species *Triptolemma strongylata* and implement the incomplete description of *T. simplex* (Sarà, 1959). The new species was recorded in the scleraxis of precious corals from the Pacific Ocean and *T. simplex* in colonies of *Corallium rubrum* (Linnaeus, 1758) and in coralligenous concretions both from the Mediterranean Sea. The SEM analysis of the cavities occupied by both species suggests that they are able to dissolve, probably only by a chemical action, the substratum.

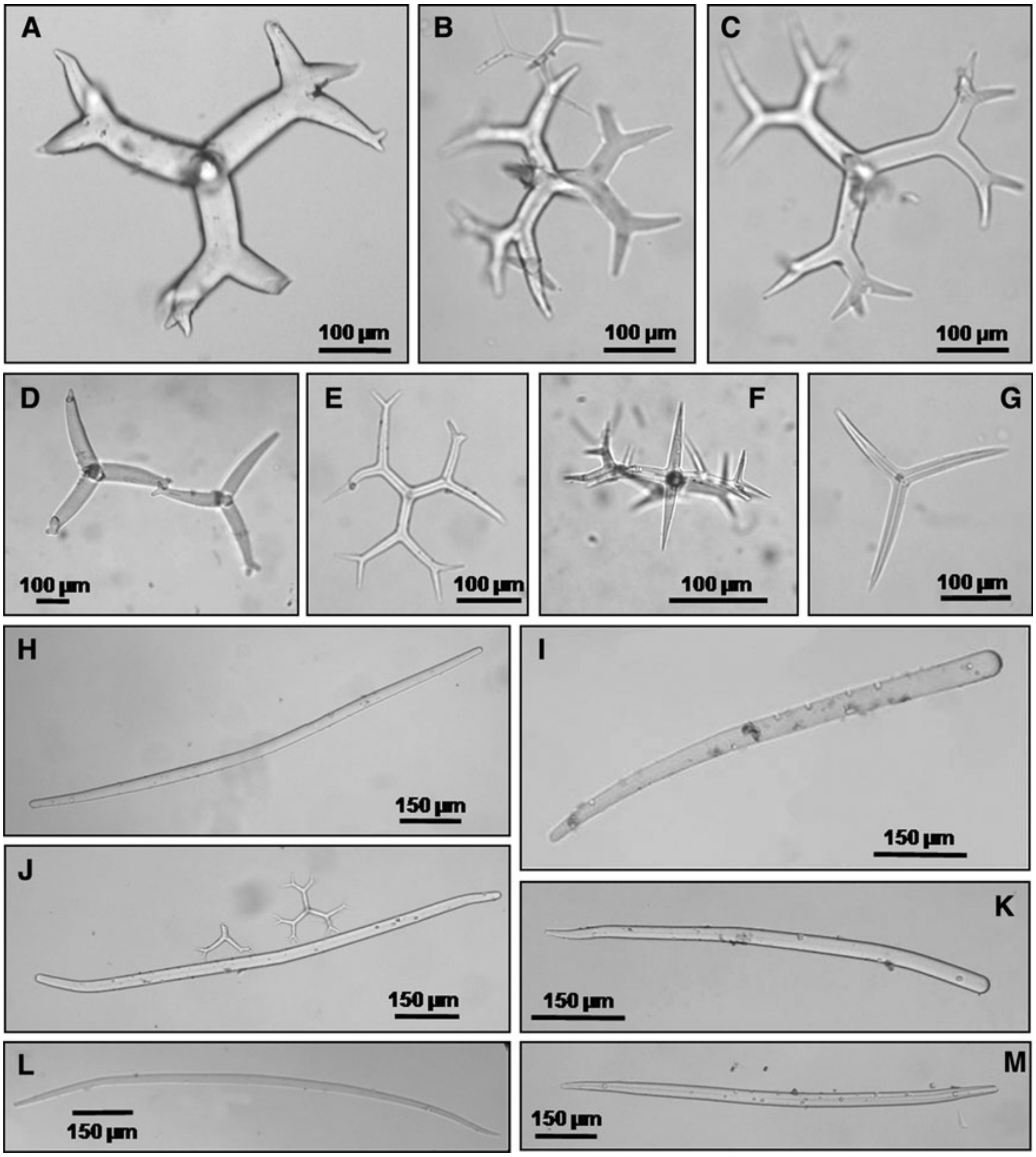

Fig. 2. *Triptolemma strongylata* sp. nov. (A–F) Dichomesotriaenes; (G) mesocalthrop with curved clads; (H–M) sinuous strongyles.

MATERIALS AND METHODS

Specimens were collected from the Pacific Ocean and the Mediterranean Sea (Figure 1A, B). The Pacific Ocean material consisted of colonies of *Paracorallium japonicum* (Kishinouyi, 1903) collected by the ROV 'Hakuyo 2000' during a cruise conducted by the RV 'Shinsei Maru' in March 2009 off Amami Island (Northern Ryukyu Islands, Japan). In this area the flat bottom at 200 m depth is covered by calcareous concretions of coralline algae. The macro-benthic community is composed of a rich population of *P. japonicum* exploited by professional fishermen, with whip black corals of the genus *Stichopathes* sometimes showing high densities, pennatulaceans, gorgonians and stylasterid hydrozoans.

The Mediterranean material was recorded during a study of the coralligenous conglomerates in different localities of the Ligurian Sea (Portofino, Santo Stefano al Mare and Gallinara Island). Blocks of this conglomerate were collected by SCUBA divers at depths between 30 and 40 m. Many organisms with a carbonate skeleton, mainly colonies of the precious coral *Corallium rubrum* (Linnaeus, 1758), were settled on these blocks. Specimens of *Triptolemma* were observed both inside the scleraxis of the red coral and into the calcareous substratum.

Sponge specimens were alcohol (70%) preserved or dried. Skeletal architecture, when possible, was examined by light microscope. Hand-cut sections of the ectosome and choanosome were made following Hooper (2000). Spicule complement was analysed according to Rützler (1978). From 30 measurements for each spicule type, the size-range and mean were obtained. For SEM analyses dissociated spicules were transferred onto stubs and sputtered with gold. These preparations were observed by a Philips XL 20 scanning electron microscope.

The holotypes of *Triptolemma simplex* (Sarà, 1959) (as *Triptolemus simplex*: GG 418), *Triptolemma intexum* (Carter, 1876) (as *Pachastrella intexa*: BMNH

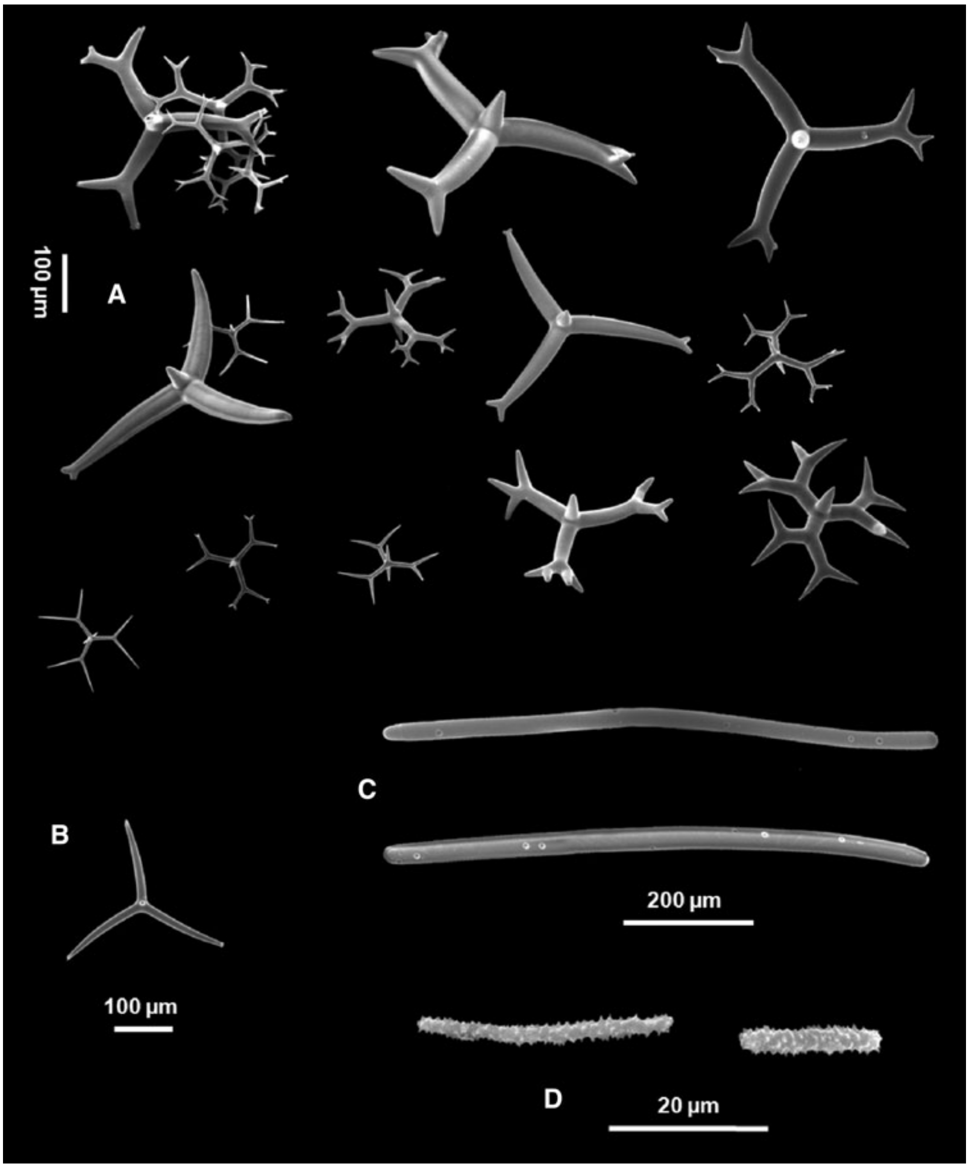

Fig. 3. *Triptolemma strongylata* sp. nov. (A) Dichomesotriaenes, with short, sharp and symmetrical rhabdomes and straight clads in a single plane; (B) mesocalthrop with curved clads; (C) sinuous strongyles; (D) thin, slightly spined microrhabds.

1910.1.1.1693–1697) and *Triptolemma incertum* (Kirkpatrick, 1903) (as *Triptolemus incertus*: BMNH 1902.5.26.5–6) were examined. Moreover, another specimen (GG 1718) labelled by Sarà *Triptolemus simplex*, coming from the type locality of the species, was studied.

RESULTS

SYSTEMATICS
Order ASTROPHORIDA Sollas, 1888
Family PACHASTRELLIDAE Carter, 1875
Genus *Triptolemma* de Laubenfels, 1955

DIAGNOSIS

Cryptic Pachastrellidae with skeleton formed by short-shafted meso(dicho)triaenes with diversely branched clads and frequently by monoaxonic megasclere (oxeas or strongyles) that support the skeleton. Microscleres include amphiasters, metasters transitional to spirasters and spiny microrhabds in one or two categories, which may be partially absent (modified from Maldonado, 2002).

Triptolemma strongylata sp. nov.
(Figures 2–3; Table 1)

TYPE MATERIAL

Holotype: (Museo Civico di Storia Naturale 'G. Doria' of Genoa (MSNG) 55503) AMAMI 15A; a dry specimen and one slide, Amami Island, Japan, Pacific Ocean, depth 200 m, collected 3 March 2009.

DESCRIPTION

Sponge insinuating and burrowing into a fragment of *Paracorallium japonicum* excavated by *Spiroxya* sp. The fragment of coral is completely pervaded by canals and holes. *Triptolemma strongylata* is located in the inner part of the specimen while *Spiroxya* is near the coral surface. The dry preservation of the sample did not allow the detection of sponge consistency and colour.

SKELETON

Not observed.

SPICULES

Megascleres: (i) dichomesotriaenes with a large variability in size and shape (Figures 2A–F & 3A). All the forms show short, sharp and symmetrical rhabdomes. The most frequent form is represented by dichomesotriaenes with cladome in a single plane. Clads are bi- tri- and/or, more rarely, tetrafurcated. Dimensions: cladome 110 (260.5) 450 µm; protoclads 35 (89) 250 µm, deuteroclads 20 (32.6) 50 µm, tritoclads 10 (16.6) 25 µm, tetraclads about 15 µm; rhabdomes 75 (118.3) 145 µm; (ii) mesocalthrops 110 (202) 250 µm, mainly with curved clads (Figures 2G & 3B); (iii) strongyles sinuous, rarely modified into styles, 700 (1018.4) 1337.5 × 15 (29.4) 35 µm (Figures 2H–M & 3C).

Microscleres: microrhabds thin, slightly spined, 17.5 (30) 50 µm (Figure 3D).

ETYMOLOGY

The specific name refers to the presence of sinuous strongyles.

Table 1. Spicular complement of the valid species of the genus *Triptolemma* (measures in µm).

	Monoaxonic megascleres	**Dichomesotriaenes**						**Mesocalthrops**	**Microscleres**
		Cladome	**Clads**				**Rhabdome**		
			Proto-	**Deutero-**	**Trito-**	**Tetra-**			
T. cladosum (Sollas, 1888)	Small oxeas up to 118 × 4	Up to 142	Up to 52 × 21	Up to 55	Up to 27.6	Up to 27.6	Up to 60 × 21	/	Sanidasters up to 27.6	Spirasters up to 11.8
T. endolithicum van Soest, 2009	Oxeas 186 (267) 372 × 10 (17) 28	Up to 400	48 (148.4) 303 × 12 (27.6) 48	12 (33.2) 72 × 6 (10.5) 20	6 (23.5) 60 × 5–7	/	24 (45.9) 62	15-62 × 10 up to 108	Microrhabds 14 (15.8) 18 × 2 (2.9) 4 and 32 (33.7) 37 × 0.5	Amphiasters 9 (11.4) 13
T. incertum (Kirkpatrick, 1903)	Small oxeas 100 × 3 to 180 × 4	/	180	90	/	/	310	/	/	Metasters 10
T. parasiticum (Carter, 1876)	Oxeas 380 × 16	/	60	60	20	/	~120	/	Microrhabds 24	Amphiasters 12
T. simplex (Sarà, 1959).	Large oxeas 240 (295) 350 × 5 (5.8) 7.5 small oxeas 70 (98) 180 × 2.5 (3.7) 5	75 (216) 375	15 (50) 150	10 (34) 75	10 (24) 50	5–10	30–(83)–135	55 (85) 100	Microrhabds 10.5 (13) 15.5 × 2.5 (3.5) 5 and 23.4 (30) 40 × >2	Amphiasters 7.5 (10.5) 15
T. strongylata sp. nov.	Strongyles 700 (1018.4) 1337.5 × 15 (29.4) 35	110 (260.5) 450	35 (89) 250	20 (32.6) 50	10 (16.6) 25	15	75 (118.3) 145	110 (202) 250	Microrhabds 17.5 (30) 50	/

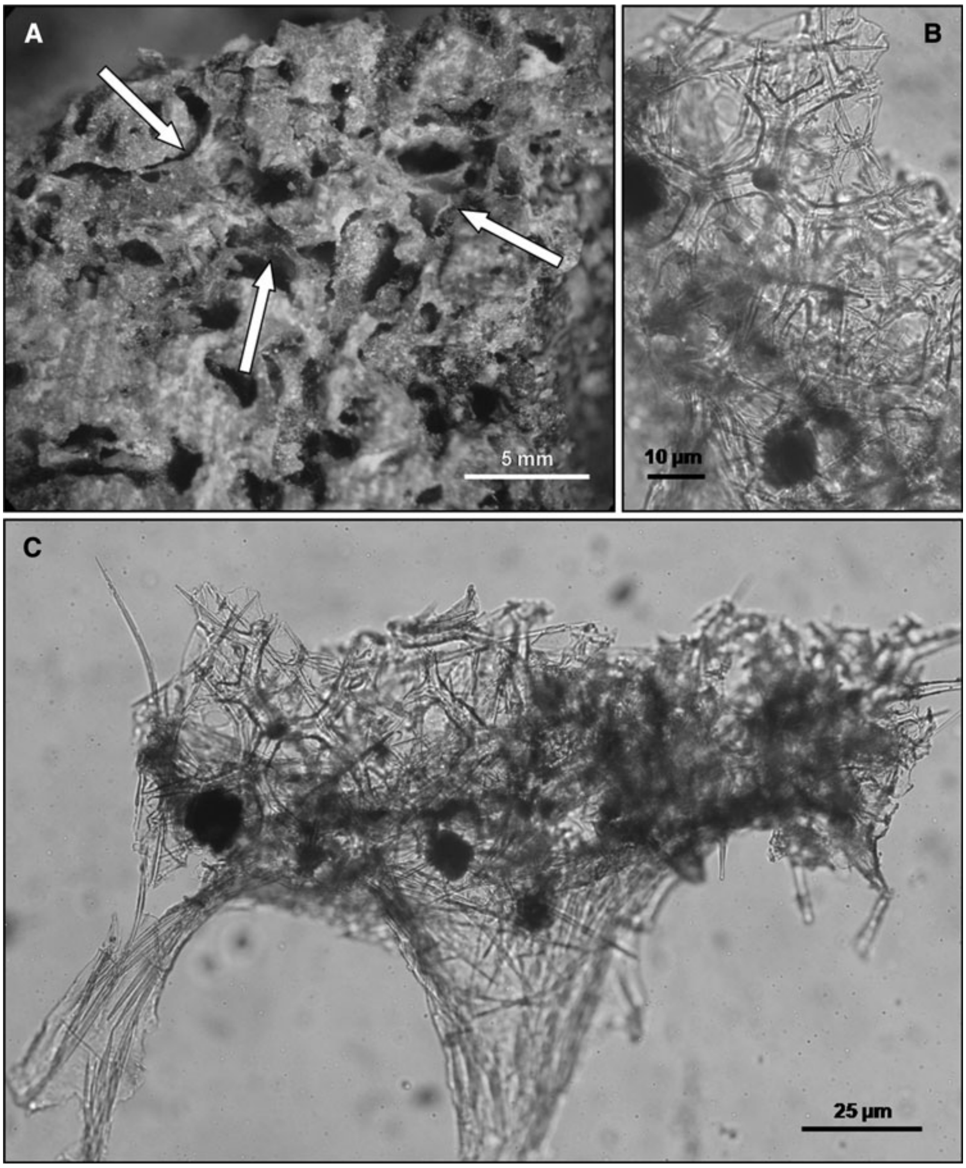

Fig. 4. *Triptolemma simplex* (Sarà, 1959). (A) Sample in the coralligenous accretions (arrows); (B) detail of the sponge ectosome with mesotriaenes layer; (C) choanosome: oxea tracts diverging in bouquets that support the crust of mesotriaenes.

REMARKS

The new species lacks amphiasters. This species, like *T. simplex* and *T. incertum*, has monaxonic megascleres (strongyles) confirming the presence of these elements in the spicular complement of the genus.

This is the first record of the genus *Triptolemma* from Japanese waters.

Triptolemma simplex (Sarà, 1959)
(Figures 4–6; Table 1)

STUDIED MATERIAL

(MSNG 55502), Specimen S.S.S.–Caryon–BL.1–F12A–SPB, dry state, Santo Stefano al Mare, 43°49′N 7°54E, depth 35 m, collected 14 February 2008; Specimen Gall.–Falc.–BL.2–Sciaf.–SP11, spicule slide, Gallinara Island (Falconara) 44°01′22″N 8°13′34″E, depth 35 m, collected 17 June 2009; Specimen PdF–BL1–Sciaf. Inf.-SP7, spicule slide, Portofino (Punta del Faro), 44°18′N 9°B′E, depth 40 m, collected 2 August 2007; (MSNG 55856) GG 1718, spicules slide, Grotta della Gaiola, Gulf of Naples, depth 0–1 m.

Holotype: (MSNG 55843), GG 418, spicules slide of the sponge with *Aaptos aaptos* (Schmidt, 1864), Grotta della Gaiola, Gulf of Naples, depth 0–1 m, collected 22 November 1959.

DESCRIPTION

Sponge is encrusting and penetrating in the basal part of living or dead colonies of *Corallium rubrum*, and in the coralligenous concretions. The colour is beige in alcohol and grey in dry state. Consistency is soft. In *Corallium* colonies the new species was recorded in the cavities produced by *Spiroxya corallophila* (Calcinai, Cerrano & Bavestrello, 2002) while in the conglomerate it was observed in the cavities produced by

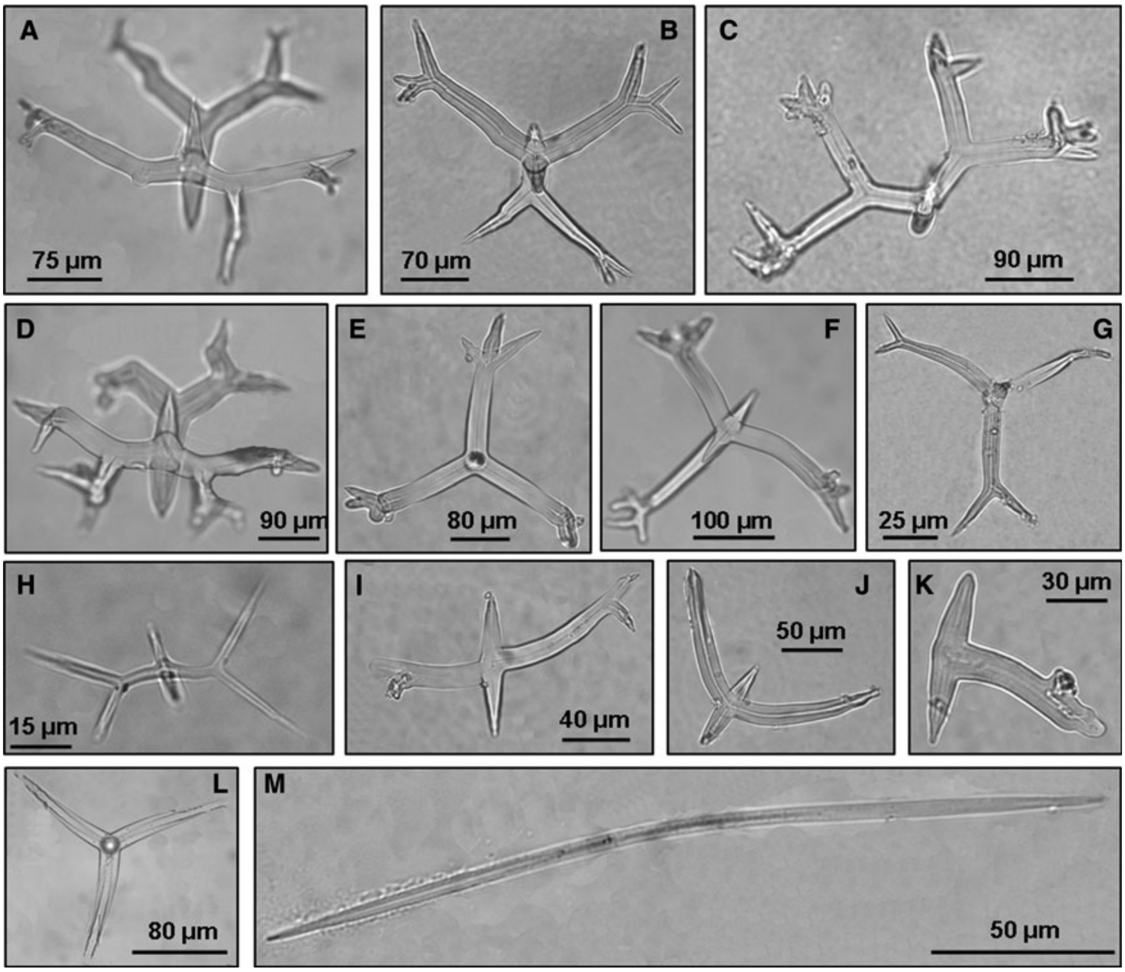

Fig. 5. *Triptolemma simplex* (Sarà, 1959). (A–K) Deformed dichomesotriaenes; (L) mesocalthrop; (M) large oxea.

Cliona sp. (Figure 4A). The ectosome forms a crust on the substratum surface while the choanosome fills holes and galleries, 1–2 mm in diameter, penetrating into the calcareous substrata to a maximum depth of 0.5–1 cm. The excavations occupied by *Triptolemma simplex*, were probably originally produced by the associated sponges.

SKELETON

Ectosome 50–60 µm thick, composed of a layer of disorderly arranged mesotriaenes, forming a superficial crust supported by the choanosomal skeleton (Figure 4B, C).

Choanosome made up by oxea bundles filling the galleries. Toward the surface diverging tracts of oxeas support the crust of mesotriaenes (Figure 4C). Large amount of spiny microrhabds are observed inside the excavations.

SPICULES

Megascleres: (i) large, thick and deformed dichomesotriaenes (Figures 5A–K & 6A) with clads irregularly bi- tri- and/or tetrafurcated at different distances from the centrum of the spicule. Tips may be rounded, acerate, asymmetrical, mucronate or deformed (Figures 5A–J & 6B). Dimension: cladome (of symmetrical spicules) 75 (216) 375; protoclads 15 (50) 150 µm, deuteroclads 10 (34) 75 µm, tritoclads 10 (24) 50 µm, tetraclads rare about 5–10 µm; rhabdome short 30 (83) 135 µm, generally symmetrical; (ii) mesocalthrops (Figures 5L & 6E), rare 55 (85) 100 µm; (iii) oxeas smooth and slightly curved, with hastate tips (Figures 5M & 6E). They may be divided in two size-categories: 240 (295) 350 × 5 (5.8) 7.5 µm; 70 (98) 180 × 2.5 (3.7) 5 µm.

Microscleres: spiny microrhabds in two size-categories (Figure 6E): 10.5 (13) 15.5 × 2.5 (3.5) 5 µm; 23.4 (30) 40 × >2 µm. Amphiasters (Figure 6F) with thin axis 7.5 (10.5) 12.5 µm and two verticils of 6 long, slightly spined rays.

REMARKS

Triptolemma simplex (Sarà, 1959) was described on the base of a slide labelled GG 418 including few spicules of the new species associated with spicules of *Aaptos aaptos*. We have found, in Sarà's collection, another slide (GG 1718) labelled *T. simplex*, deriving from a specimen coming from the same site of the holotype (Grotta della Gaiola, Gulf of Naples) with numerous spicules including deformed dichomesotriaenes and smooth oxeas perfectly fitting with our material. The description of Sarà must be therefore considered incomplete and is integrated in this paper.

Here we describe for the first time the complete skeleton of the genus *Triptolemma*. So far the presence of the monaxonic megascleres has been debatable because it is difficult avoiding contamination with spicules of neighbouring sponges (van

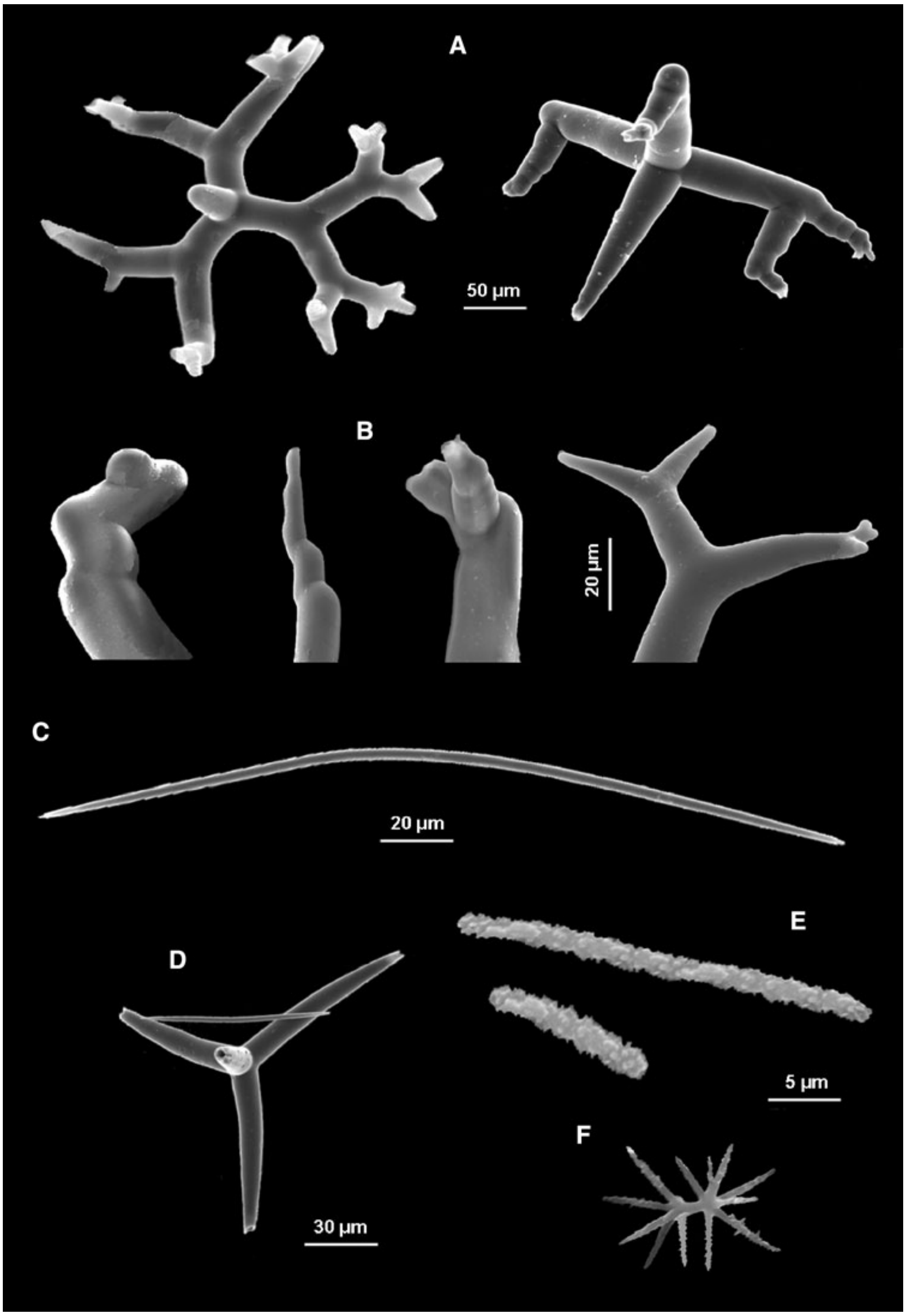

Fig. 6. *Triptolemma simplex* (Sarà, 1959). (A) Deformed dichomesotriaenes; (B) details of the dichomesotriaene tips; (C) large oxea; (D) small oxea and mesocalthrop; (E) spiny microrabd in two size-categories; (F) amphiaster with spiny rays.

Soest, 2009). Maldonado (2002), in fact, considered the oxeas in *T. cladosum* (Sollas, 1888) as foreign and did not include monoaxonic megascleres in the genus definition. Van Soest (2009) recorded oxeas in *T. endolithicum* that, due to their shape and size, were interpreted as reduced triaenes. The observation of oxeas in the skeleton of *Triptolemma simplex* definitively establishes that this genus has monaxonic spicules.

The analysis of the samples of *Triptolemus* sp. (=*Triptolemma*) found in the coralligenous community of Bogliasco (Pansini & Pronzato, 1973), Portofino (Sarà *et al.*, 1978) and Mitigliano cave (Napoli) (Pansini & Pronzato, 1982) indicates that they belong to *T. simplex*.

Triptolemus simplex was collected up to 40 m depth. *Triptolemma endolithicum* van Soest, 2009 was collected in relatively shallow waters, 25 m depth. While other known species were found in deeper waters: *T. cladosum* (Sollas, 1888), 250 m depth, off the Kai Islands, Indonesia; *T. incertum* (Kirkpatrick, 1903), 150–180 m depth, off the east coast of South Africa; *T. strongylata* sp. nov., 200 m depth, Amami Island, Japan.

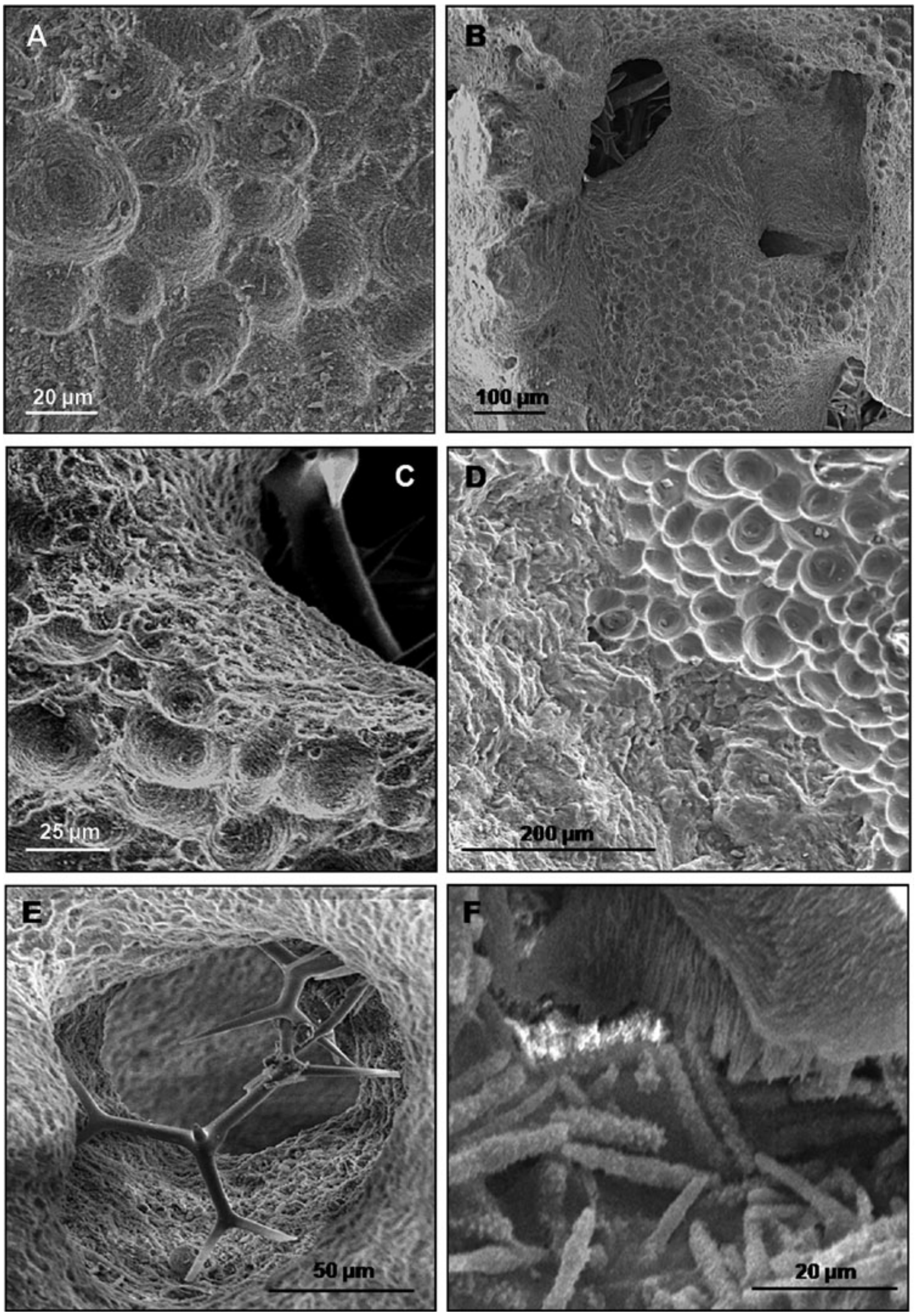

Fig. 7. *Triptolemma* erosion. (A) Pits of *Spiroxya* sp. in the scleraxis of *Paracorallium japonicum*, showing the characteristic pattern of ornamentation; (B) in the cavities colonized by *T. strongylata* (see the spicules in the hole) the erosion pattern of *Spiroxya* is progressively modified giving rise to an irregular etching of the substratum; (C) enlargement of the previous; (D) a similar process is shown by *T. simplex* in *Corallium rubrum*; (E) foramen connected boring chambers of *Spiroxya* irregularly widened and etched by *T. strongylata* (see the dichomesotrianes); (F) detail of coralligenous accretion eroded by *T. simplex* (see the spiny microrhabds). The local carbonate re-crystallization suggests a strong and quick dissolution of the original calcareous matrix.

CONCLUDING REMARKS

The genus *Triptolemus* (=*Triptolemma*) was established by Sollas (1888) for *T. cladosum*. This species was re-described in detail by Maldonado (2002). The other species generally attributed to *Triptolemma* are: *T. intextum* (Carter, 1876 as *Pachastrella intexta*); *T. parasiticum* (Carter, 1876 as *Pachastrella parasitica*); *T. incertum* (Kirkpatrick, 1903 as *Triptolemus incertus*); *T. simplex* (Sarà, 1959 as *Triptolemus simplex*); *T. endolithicum* van Soest, 2009; and *T. strongylata* sp. nov. The differences between the different valid species of the genus are summarized in Table 1.

Triptolemma endolithicum was recently described by van Soest (2009).

Carter (1876) described *P. intexta* associated to a specimen of the lithistid *Corallistes bowerbanki* (Johnson, 1863). The examination of the original slides of the holotype (British Museum, Natural History (BMNH) 1910.1.1.1693–1697) showed the presence of calthrops in large size, almost trifurcated mesotriaenes, two categories of oxeas, oval slightly centrotylote microstrongyles. These spicules indicate that this species must be considered as belonging to the genus *Pachastrella* according to Carter's original description.

Triptolemus parasiticum (Carter, 1876) is poorly described and the loss of the type material does not allow any sure comparison with other co-generic species. The synonymization of this species with *Pachastrella intexta* (Lévi & Lévi, 1983) is highly suspected.

In the BMNH collection, Kirkpatrick deposited three slides (BMNH 1902.5.26.5–6) of *T. incertum*. Two slides show spicules of the new species associated to spicules of *Discodermia natalensis* Kirkpatrick, 1903 as stated by the same author (1903) in the original description. A third slide contains the spicules of *P. intexta* and this fact is confirmed by the contemporaneous presence of spicules of *Corallistes bowerbanki* as in Carter's original description. Probably Kirkpatrick, when describing his species, prepared for comparison a slide from the Carter's material erroneously labelling it as *T. intextum*.

All the species of the genus *Triptolemma* live in association with other sponges and Maldonado (2002) suggested they are able to penetrate the tissues of other species by acting as parasites. Van Soest (2009) described *T. endolithicum* as insinuating inside corridors and holes, approximately 1 mm in diameter and 5–10 mm long, present in a piece of coral covered by *Diplastrella megastellata* Hechtel, 1965.

Triptolemma strongylata sp. nov. and *T. simplex* live inside holes and galleries produced by boring sponges, *Spiroxya* sp. and *S. corallophila* in the precious coral and *Cliona* sp. in the coralligenous concretions.

Spiroxya spp. were identified for the presence of spirasters and for the micro-pattern of the excavation walls characterized by fingerprint like ornamentations (Figure 7A). Nevertheless, in the portion of excavations, where the tissue of *Triptolemma* was recorded, the erosion pattern of *Spiroxya* appears progressively modified (Figure 7B). This corrosion evolved to a complete abrasion of the wall of the cavities that was characterized, at the end of the process, by an irregular corrugation of the substratum (Figure 7 C, D). The foramina connecting the boring chambers of *Spiroxya* are also irregularly widened (Figure 7D). Moreover, in several places, numerous recently formed calcium carbonate crystals probably indicate a local re-crystallization followed by a strong and quick dissolution of the original calcareous matrix (Figure 7E, F).

On the basis of this evidence we hypothesize that *Triptolemma* insinuates into erosions produced by other sponges and it could be able to enlarge them by an etching mechanism based only on chemical dissolution.

Recently all the boring species recorded in the precious coral both in the Mediterranean Sea and the Pacific Ocean were summarized by Calcinai *et al.* (2008). It is interesting that the number of species recorded in *Corallium rubrum* from the Mediterranean Sea (15) is similar to that reported for the Pacific precious coral species (13). All the involved genera (*Holoxea, Alectona, Spiroxya, Cliona, Thoosa, Dotona* and *Aka*) with only the exception of *Delectona* have species associated both to Mediterranean and Pacific precious coral. This fact supports the idea that the sponge species associated to precious corals reflect the same Tethyan relict distribution of the genus *Corallium* (Calcinai *et al.*, 2004a). It is interesting that also the two described species of *Triptolemma* confirm this biogeographical pattern.

ACKNOWLEDGEMENTS

We wish to thank the captain and crew members of RV 'Shinsei Maru' and the operations team of ROV 'Hakuyo 2000' (SNK Ocean Co. Ltd.) for sampling, and Professor Maurizio Pansini (Genova) and the anonymous referees for the helpful suggestions that contributed to improve our manuscript.

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