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DARWIN'S *PYRGOMA* (CIRRIPEDIA) REVISITED: REVISION OF THE *SAVIGNIUM* GROUP, MOLECULAR ANALYSIS AND DESCRIPTION OF NEW SPECIES

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ABSTRACT

Darwin recognized the coral inhabiting barnacles as congeneric belonging to *Pyrgoma*. Ross and Newman (1973) assigned those with fused shell and elongated scuta to the genus *Savignium*. Later those with tooth like articular projection on tergum were assigned to *Trevathana dentata*. We sequenced and analyzed the divergence of three mithochondrial genes (12S rDNA, 16S rDNA, COI) of *Savignium crenatum* from two host coral, of *Neotrevathana elongata* from *Echinopra* and of *T. dentata* from five host corals genera, *Leptastrea*, *Platygyra*, *Favia*, *Favites*, and *Cyphastrea*. Based on the molecular analysis of these genes we show that members of populations of the nominal species *T. dentata* occupying different host corals display clear genetic differences, suggesting that the barnacles from these five different coral genera represent five distinct biological species. These species display host specificity at the generic level. Barnacles colleted from the same host genus but different geographical regions, clustered in the same clades. We described morphological characters of the shell, opercular valves, and limb characteristics, trophi and cirri, of barnacles from these host coral. We concluded that the material extracted from *Leptastrea* conform with Darwin's type and the specific epithet of *Trevathana* from *Leptastrea* should continue to be *dentata*, specimens from the four other host corals are four new species of *Trevathana*; namely *T. mizrachae* n. sp. from *Platygyra*, *T. margaretae* n. sp. from *Favia*, *T. jensi* n. sp. from *Favites*, and *T. sarae* n. sp. from *Cyphastrea*. The morphological data confirm the results of the molecular analysis that species of coral inhabiting barnacles are highly genus-level host-specific.

KEY WORDS: Cirripedia, coral-inhabiting barnacles, Pyrgomatidae, *Savignium*, *Trevathana*

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INTRODUCTION

Symbiotic interactions are critical in coral reef communities; in the marine realm, reef corals support the highest diversity of symbiotic relationships. Host mediated evolution is an important driving force for the diversity of symbiotic animals on the reefs, which may lead to host-symbiont species specificity through reproductive isolation (Knowlton and Jackson, 1994; Duff, 1996). In order to avoid competition, many symbionts exhibit ecological adaptation to a specific host.

In his monograph on the barnacles, Darwin (1854) recognized the coral inhabiting barnacles as congeneric belonging to *Pyrgoma*. Ross and Newman (1973) assigned those species with fused shell and elongated scuta to the genus *Savignium*; this genus was later divided into four genera: *Savignium*, *Trevathana*, *Neotrevathana*, and *Wanella* (Anderson, 1992; Ross and Newman, 1999). In a previous study (Simon-Blecher et al., 2007), *Wanella*, which exhibits these morphological features but inhabits the hydrocoral, *Millepora*, was found to fit into a separate clade from all other pyrgomatids. More than 15 genera of corals have been reported to serve as hosts of these three genera of pyrgomatids (Ogawa and Matsuzaki, 1992; Asami and Yamaguchi, 2001). Examination of coral collections in Naturalis (Leyden, The Netherlands) and in the Museum National Histoire Naturelle (Paris) (Achituv, unpublished) indicated that the number of hosts might be even higher.

One of the most important adaptations of coral-inhabiting barnacles is the recognition of the host coral during settlement. In free-living barnacles, chemical cues induce settlement of cyprids (for review see Hui and Moyse, 1987). In addition, barnacles probably recognize the bacterial flora characteristic of the potentially suitable settlement habitat (Strathmann and Branscomb, 1979). Nauplii of the coral inhabiting barnacle *Trevathana dentata* (Darwin, 1854), from *Cyphastrea*, are able to not only recognize and locate their target host in still water using chemotaxis, but also perform efficient host location in flow using odor-modulated rheotaxis (Pasternak et al., 2004). Once settled, coral barnacles must keep pace with the growth of the host or grow even more rapidly above the coral surface; otherwise they might be entombed within the coral skeleton (Ross and Newman, 1973). This specific adaptation may lead to "allopatric" speciation, where a host taxon with specific chemical properties and growth rate serves as a unique substratum suitable for settlement of an adapted barnacle species. Mokady et al. (1999) already raised the possibility based on differences in 12S rDNA that *T. dentatum* actually consists of several cryptic species with individualized host specificity; each cryptic species is adapted to a particular host coral.

In the present study, we investigated the hypothesis that pyrgomatids of the *Savignium* group encompass several cryptic species that exhibit host specificity. We used the mitochondrial genes 12S rDNA, 16S rDNA and cytochrome oxidase subunit 1 (COI) to infer divergence among these barnacles. These techniques were previously shown to be an effective tool for studying phylogeny and inferring the presence of cryptic barnacle species (Mokady and Brickner, 2001; Wares, 2001; Chan et al., 2007a, b; Tsang et al., 2008). Based on information gained from these analyses, we suggest that the *Savignium* represent a group of cryptic species and requires redescription. For the description of the barnacles from different hosts, we used high resolution techniques and described the morphological characteristics of both hard and soft parts. These techniques were only recently introduced to the description of morphology of coral barnacles (Anderson, 1992; Ross and Newman, 1999, 2000; Achituv and Newman, 2002; Tsang et al., 2009). We confirmed the molecular results, which suggested that barnacles collected from different genera of corals should be assigned to different species. However, both our morphological and molecular analyses are limited, due to the restricted availability of material suitable for molecular study and for describing soft parts of the *Savignium* group.

Materials and Methods

Species Sampling

Most of the barnacles used in this study were collected on the corals adjacent to the Interuniversity Institute (IUI) of Marine Sciences, Eilat, Red Sea. Coral barnacles were also collected from Heron Island, The Great Barrier Reef, and Seychelles. The barnacles were removed from the host coral and preserved in ethanol. The number of barnacles studied was dependent on availability of material.

We examined Darwin's barnacle collection in the Natural History Museum, London (NHM); this contains only dry material. We also extracted barnacles from corals in the coral collection of the Museum National d'Histoire Naturelle Paris and the coral collection of Naturalis, Netherlands National Museum of Natural History, Leiden. This material contains the hard parts of barnacles attached to the coral hosts.

DNA Extraction, Amplification, Sequencing, and Phylogenetic Analysis

For molecular studies, DNA was extracted from the alcohol-preserved specimens using the High Pure PCR template kit (Roche; Germany). DNA (50 ng) was used for amplification by the polymerase-chain-reaction (PCR) (Saiki et al., 1988). For amplification and sequencing of the 12S subunit of mitochondrial rDNA, we used the primer set of Kocher et al. (1989) as modified by Mokady et al. (1999). Primers 16SAR and SBR of Palumbi (1996) were used for 16S rDNA gene fragments. Universal primers HCOI2198 5′- and LCOI1490 (Folmer et al., 1994) were used for PCR and sequencing of the Cytochrome Oxidase subunit I (COI). Amplification was carried out in a personal combi-thermocycler (Biometra, Germany). The 12S rDNA was amplified by performing 40 cycles of 30 sec at 94°C, 20 sec at 48°C and 20 sec at 72°C, followed by a final extension for 7 min at 72°C. The 16S rDNA and COI were amplified by performing 35 cycles of 30 sec at 94°C, 30 sec at 48°C and 20 sec at 72°C, followed by a final extension of 7 min at 72°C. PCR products were purified by centrifugation through a High Pure PCR product purification kit (Roche Diagnostics GmbH, Mannheim, Germany).

PCR products were sequenced on both strands using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) at Macrogene Inc., Seoul (Korea). Sequences were then manually inspected and edited using the BioEdit program. All sequences have been deposited in GenBank; accession numbers are given in the supplementary material (Supplementary material, Appendix 1, access through DOI: 10.1651/09-3152.1.)

Sequences were aligned in Clustal X. The alignments were then manually corrected and sites carrying gaps in more than 25% of the taxa were removed from the analyses (alignment available as Supplementary material Appendix 2 accessed through DOI: 10.1651/09-3152.1). Maximum likelihood searches were performed in RAxML ver7.0.3 under mixmodels and with 100 randomized MP starting trees. The GTR + G + I model was chosen for each data partition. Clade support was assessed using the nonparametric bootstrap procedure with 1,000 bootstrap replicates and one random addition per replicate.

Morphological Analysis

For the morphological study, we used material from the Red Sea, from the same coral genera used for the molecular study. The wall plates and opercular valves were removed from the coral, immersed for about two hours in household bleach, rinsed in tap water followed by distilled water, and then dried on a small hot plate at 80°C. The specimens were examined under the dissecting microscope, and adhering chitin was removed using needles and fine forceps. Dried samples were mounted on brass stubs, coated with gold, and examined with a JEOL scanning electron microscope at 25 kV. Images were stored using Autobeam software. In addition, we examined Darwin's coral barnacle collection at the Natural History Museum, London (NHM).

Soft parts were dissected under the microscope using fine needles. The mouth parts and cirri were mounted in gelatin-glycerin mounting media and were observed and photographed using Zeiss Axiovax Microscope.

Holotypes and paratypes were deposited in the Zoological Museum Tel Aviv University, (TAU), additional paratypes were deposit in the Natural History Museum, London (BMNH).

Results

Animal Collection

Trevathana was found on five genera of corals from the Red Sea, namely *Leptastrea*, *Platygyra*, *Favites*, *Favia*, and *Cyphastrea*. Additional material was obtained from the Great Barrier Reef and from Seychelles. *Neotrevathana* was extracted from *Echinopora gammacea* Lamarck, 1816, and *Savignium crenatum* (Sowerby, 1823) was extracted from *Platygyra* and from *Acantastrea*. Voucher specimens have been deposited at the Zoological Museum Tel Aviv University (TAU). As an out-group, we used sequences of *Cantellius pallidus* (Broch, 1931) generated by us in a previous study (Simon-Blecher et al., 2007) (AM497881; AM497880; FJ481645).

DNA Sequencing and Barnacle Phylogeny

PCR products of the mitochondrial genes 12S rRNA, 16S rRNA and COI were successfully amplified from each specimen as indicated in the supplementary material, Appendix 1. The aligned fragment of the 12S rRNA gene was 327 characters long, the 16S rRNA gene was 507 characters long; the COI fragment was 670 characters in length As might be expected, given their co-linearity, there was no significant incongruence between 12S and 16S and COI genes ($P = 0.595$).

The phylogenetic tree generated by RaxML for the combined is presented in figure 1. The tree was rooted with *Cantellius* as the out-group, according to Simon-Blecher et al. (2007). In the ML tree, the in-groups were divided into two main clades, *Savignium* from two host corals, *Acantastrea* and *Platygyra*, on one clade, and *Trevathana* and *Neotrevathana* on the second clade (Fig. 1). The Bayesian tree supports the same topology. The single specimen of *Neotrevathana elongata* (Hiro, 1935) obtained from *Echinopora gamacea* was placed at the base of the *Neotrevathana-Trevathana* clade. Within the *Trevathana* clade, there are five subclades, the sequences of each

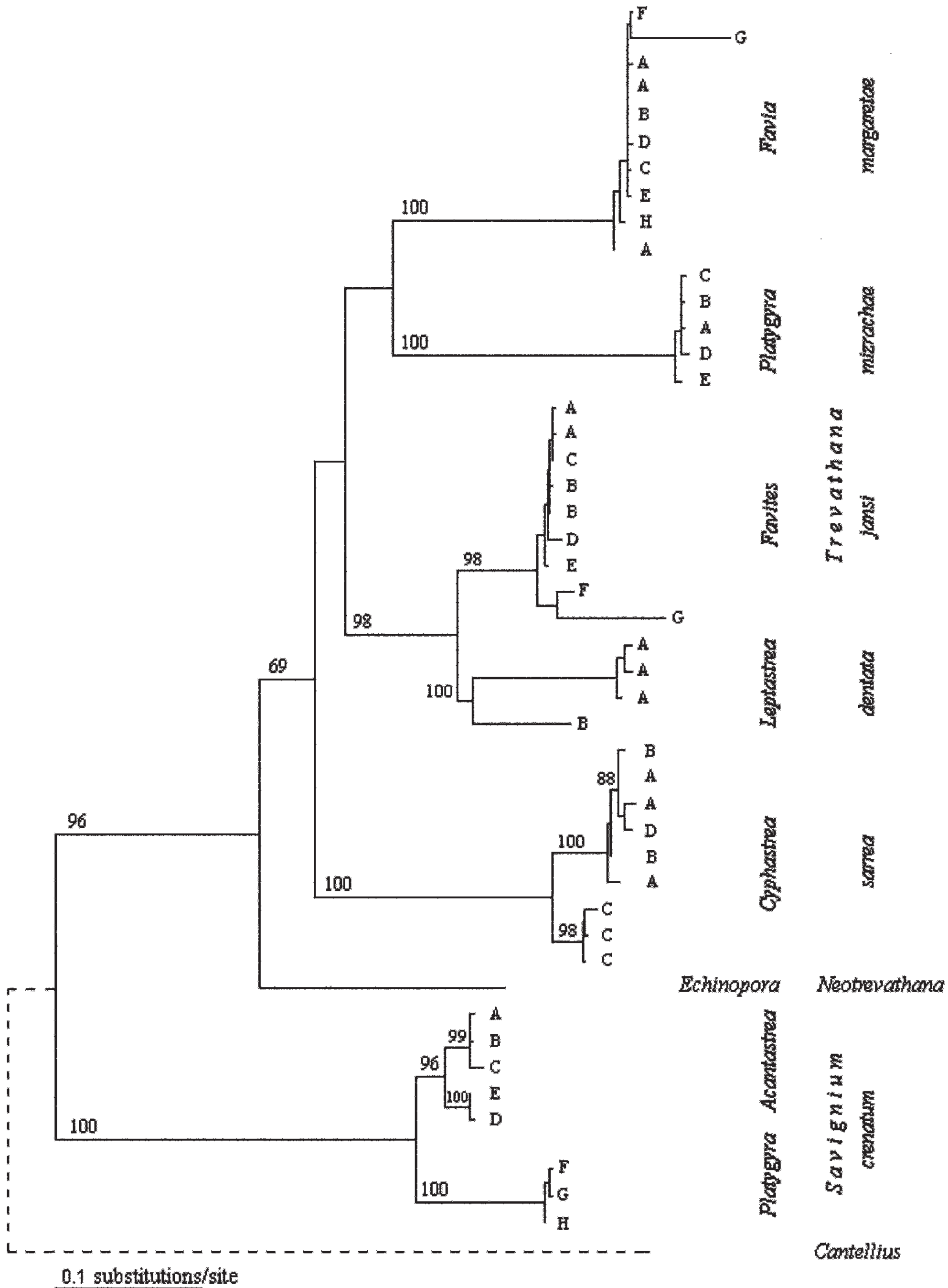

Fig. 1. Maximum likelihood (ML) tree based on concatenated 12S rRNA, 16S rRNA and COI sequences of barnacles of the "*Savignium*" group. For each node, the ML bootstrap percentage higher than 80 are given. Genus of host coral and proposed specific name of the existing and new species of barnacles is indicated. Barnacles extracted from the same coral colony are indicated by the same latter (for details see Appendix 1).

resulted from the same genus of host coral; however, barnacles extracted from *Cyphastrea* were separated from those collected from *Favia*, *Favites*, *Leptastrea* and *Platygyra*.

Savignium barnacles extracted from *Platygyra* and *Acantastrea* are sister groups. This finding may indicate that *Savignium* also exhibits host specificity at the host genus level, but our sample included only two host corals and a limited number of barnacles, which was too small to confirm this suggestion.

Systematics and Description of New Species of *Trevathana*

Our analyses strongly support the concept that *T. dentata* isolated from different host corals represent a series of distinct sister clades. Confirming this hypothesis required morphological study and comparison of material from different host corals to the type specimens of *Pyrgoma dentatum* (= *Trevathana dentata*).

In Darwin's barnacle collection at the Natural History Museum, London, there are three samples labeled *P. dentatum* that might represent the three varieties described

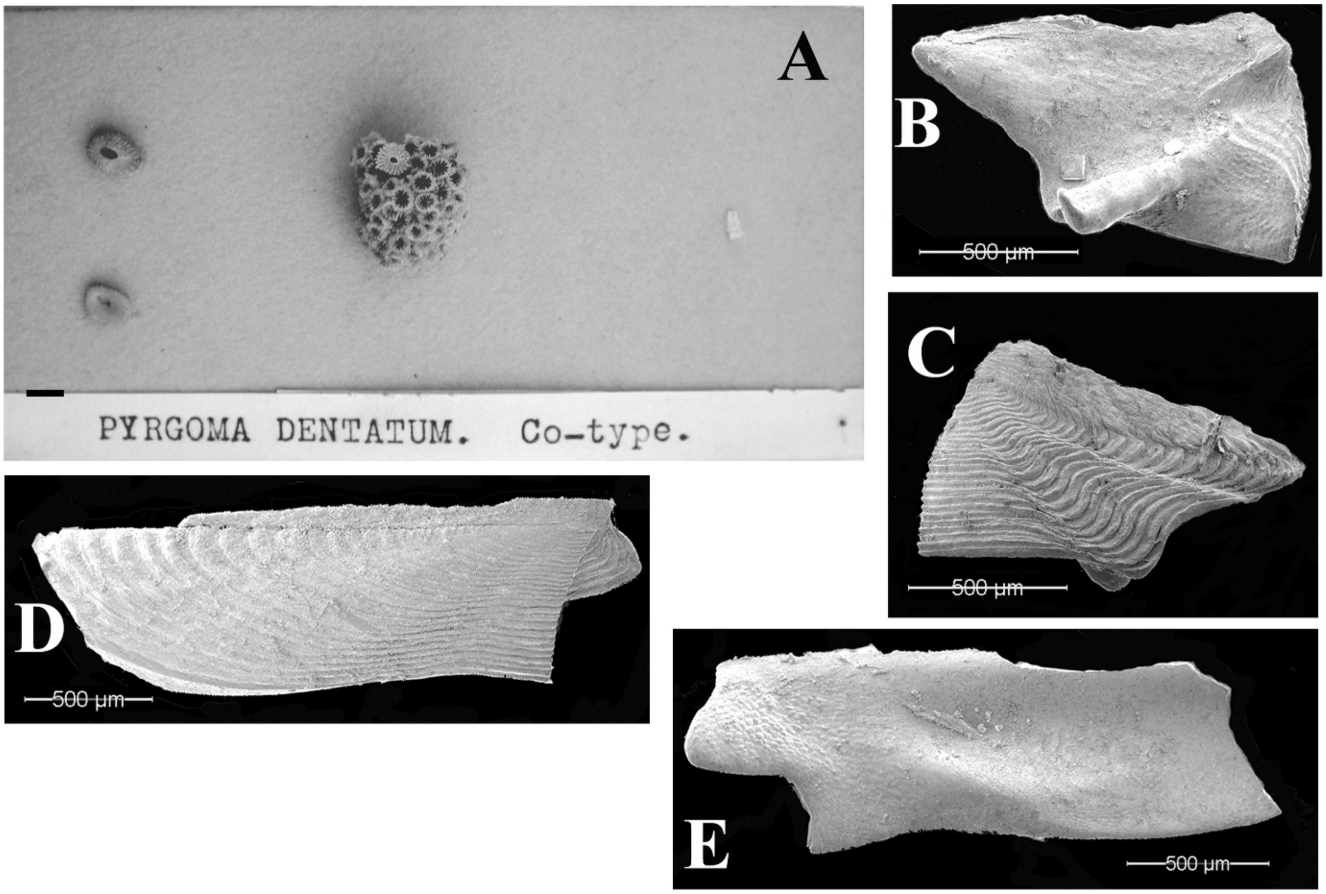

Fig. 2. *Trevethana dentata* from Darwin's collection, Natural History Museum, London. A, Cardboard with a piece of host coral, *Leptastrea inequalis*, with one specimen of barnacle attached to it, two shell plates showing inner and outer view of shell and one broken scutum. B, internal view of tergum released from the attached barnacle. C, external view of B. D, external view of scutum released from the attached barnacle. E, internal view of scutum released from the attached barnacle.

by Darwin (1854). Two are labeled ''*Pyrgoma dentatum* var.'' and one (Fig. 2A) ''*Pyrgoma dentatum* co-type.'' Unfortunately the two samples labeled ''var.'' do not carry any terga, and the scuta, when present, are covered with glue, making it impossible to observe their details. The sample labeled ''co-type'' carries a piece of the host coral, *Leptastrea inequalis* Klunzinger, 1879, to which a single barnacle is still attached, two shells plates and a broken scutum (Fig. 2A). A layer of glue covers the scutum and it was impossible to make out its details. By gentle shaking, it was possible to retrieve from the barnacle that was still attached to the coral, two scuta, one broken, and a single tergum. These opercular valves were used for SEM study and could be compared to opercular valves from other barnacles.

On the surface of shells of Darwin's co-type, there are 32 radiating ribs (Fig. 2A) carrying rows of projections; the primary rows contain 4 projections, while the incomplete secondary rows carry 2-3 projections. Darwin did not describe the shell of each of the three varieties; the separation of the three varieties is based mainly on the characteristics of the terga. The tergum from the co-type, carry a sharp internal tooth (Fig. 2B, C), this agrees with the description of variety I of *Pyrgoma dentatum* (Fig. 3-3g).

A sample of *Leptastrea transversa* Klunzinger 1879 collected at Nabeq in the Gulf of Eilat (Aqaba) on the south of Sinai Peninsula carried barnacles that resemble the Darwin co-type.

We assigned the material from *Leptastrea* from the Red Sea to Darwin's variety I, but there are some differences, between the material from the Red Sea and Darwin's co-type, such as the blunt rostral angle in the co-type rather than a pointed one in the material from the Red Sea. Since there is only one pair of scuta in Darwin's sample (Fig. 2 D, E), and in one of them the rostral angle is broken, and the other one might be worn, it is therefore not possible to see how constant these differences are. The number of radiating ribs of the shell plate of *Trevathana* from *Leptastrea* was 32, closer to that found on Darwin's co-type while the number of ribs on shell from other corals checked by us, never exceeded 22 the number found in *Trevathana* from *Cyphastrea*.

Barnacles that match Darwin's description of *T. dentata* were also found on other host corals. Examination of these barnacles, hard parts and arthropordial parts, trophi and cirri reveal that they differ from the sample from *Leptastrea*. We redescribe *T. dentata* and describe four new species based on soft and hard parts. Comparison of the morphological characters of barnacles from the five host corals are presented in Table 1.

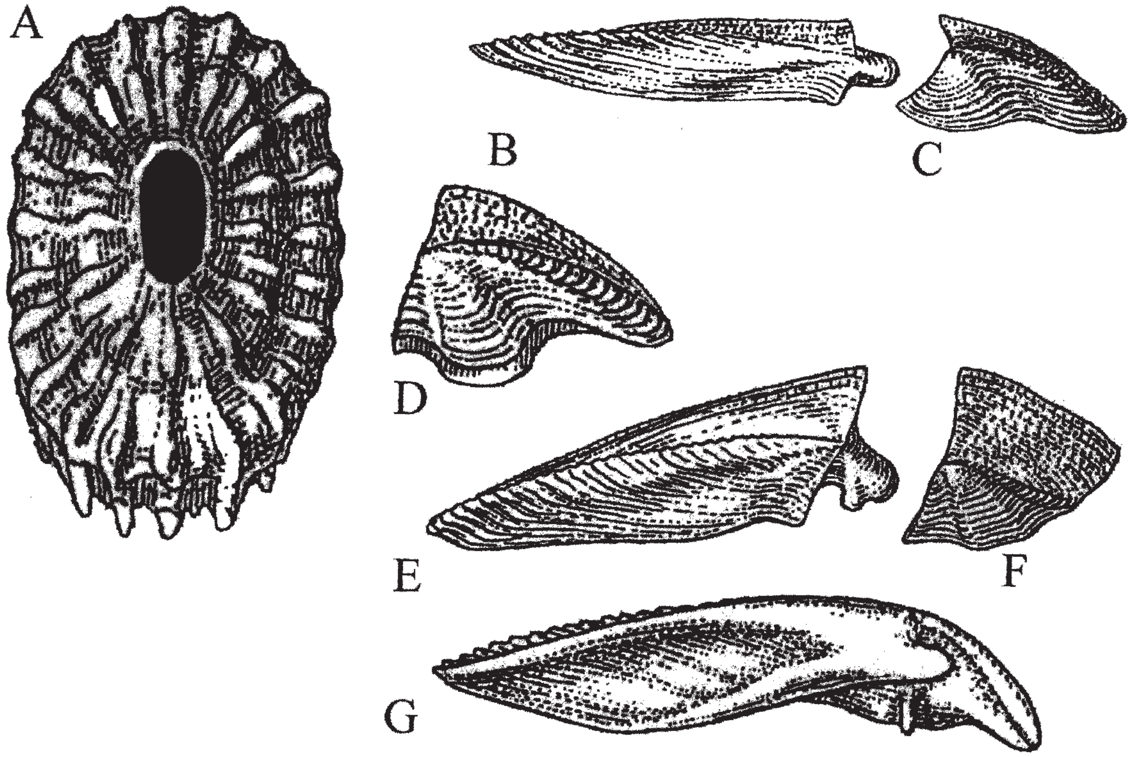

Fig. 3. *Trevethana dentata* (= *Pyrgoma dentatum*), adapted from Darwin's Plate 13; Fig. 3, (Darwin, 1854). A, shell, external view. B, external view of scutum, *var. 1*. C, external view of tergum, *var. 1*. D, external view of tergum. *var. 2*. E, external view of scutum, *var. 3*. 3b, external view of tergum, *var. 3*. F, external view of scutum, *var. 1*. G, internal view of scutum and tergum of *var. 1*, joint together.

Systematics

Balanoidea Leach, 1817
Pyrgomatidae Gay, 1825
Pyrgoma Darwin, 1854
Trevathana dentata (Darwin, 1854)

Pyrgoma dentatum Darwin, 1854: Plate 13 3a-3g.
Pyrgoma dentatum—Hiro, 1935: Fig. 6
Pyrgoma dentatum—Hiro, 1938: Fig. 8C, D, E.
Savignium dentatum—Ross and Newman, 1973.
Trevathana dentatum—Anderson, 1992.
Trevathana dentata—Ross and Newman, 1999.

Diagnosis of genus.—Balanoid with elliptical, concrescent shell, basis calcareous cup shape, separate opercular plates, scutum transversely elongated, tooth like articular projection on tergum, internal inward projecting tooth.

Table 1. Comparison of morphological and morphometric characters of different species of *Trevathana dentata* from different host corals. Abbreviation: bm = basal margins.

Species:	*T. dentata*	*T. mizrachae*	*T. margaretae*	*T. jensi*	*T. sarae*
Host coral:	*Leptastrea*	*Platygyraa*	*Favia*	*Favites*	*Cyphastrea*
Maximum rostro-carinal diameter (mm)	5	7	5	> 7	4
Maximum number of radial ribs	35	21	18	17	22
Tergum					
Position of internal tooth	Parallel to spur axis	Perpendicular to spur axis	Perpendicular to spur axis	Parallel to spur axis	Parallel to spur axis
	Perpendicular to basal margins	Parallel to basal margins	Parallel to basal margins	Perpendicular to basal margins	Perpendicular to basal margins
Trophi					
Number of teeth on labrum	1	2	1-3	8	1
Maxilla I, Cutting edge	No notch	With notch	With notch	With notch	No notch
Cirri					
Pigmentation of cirri	Present	Present	Present	Present	No pigmentation
Exopod of Cirrus I antenniform	No	No	No	No	Yes
Conical spines on endopod II	No	Present	Present	Present	No
Conical spines on endopod III	Present	Present	Present	Present	No
Conical spines on exopod III	No	Present	Present	No	No
Apical setae endopod II	bipectinate	multifurcate	multifurcate	bipectinate	bipectinate

Material Examined.—Five barnacles extracted from *Leptastrea transversa* collected on the reef flat at Nabeq, South of Gulf of Eilat (Aqaba), Red Sea. The host coral carrying a few barnacles, shell plates and operculum valves mounted on SEM stabs and microscopic slides carrying cirri and trophi are deposited at the Zoological Museum Tel Aviv University, Israel, museum number TAU–AR 27812; AR - 28923.

Diagnosis.—*Trevathana* with up to 35 radial ribs with rows of projection, short spur, internal tooth of tergum perpendicular to basal margins.

Redescription (Figs. 4-7).—Shell white, fused, flat, elliptic, maximum rostro-carinal diameter 5 mm. Lateral diameter about 2/3 of rostro-carinal diameter (Fig. 5A). Surface of shell with radiating ribs carrying rows of small projections, covered with thin layer of deposition of host coral skeleton. Up to four projections in row, maximum number of primary and secondary ribs 35. Shell tubiferous, number of radiating tubes equal to number of ribs. Sheat, white, covers most of the inside side of shell concentric growth ridges (Fig. 4B).

Orifice elliptic located close to carinal edge of shell, rostro-carinal diameter of aperture about 1/3 of rostro-carinal diameter of shell (Fig. 4A, B). Length to width ratio 2:1. Basis white, tapering.

Scutum and tergum white, separated. Scutum (Fig. 4D, F) transversally elongated. Outer surface with growth ridges, median closed furrow nearly parallel to occludent margins, occludent margins three times longer than tergal margins. Tergal margins at about 45° to basal margins. Tooth projecting at tergal margin, located closer to occludent margins than to basal margins, width about half of tergal margins length. Adductor and lateral depressor pits shallow, adductor ridge only slightly prominent, slightly projecting beyond basal margins. Tergum (Fig. 4C, E) triangular, on outer surface growth ridges, spur distinct, with shallow furrow. A longitudinal furrow from middle of scutal margins to carino-basal angle, a small notch in middle of tergal margins. Inner side depression accommodates scutal tooth, growth lines inside depression. A thin, pointed, inward projecting tooth, located on spur, perpendicular to basal margins (Fig. 4C, G).

Labrum (Figs. 5B, 6E) bilobed, lobes oval, one tooth on each lobe, a broad V shape notch, about 90°, between lobes, short hairs on inside margins of notch. Palpi elongated oval (Figs. 5B, 6E) on distal margins' pinnate spines.

Mandible (Figs. 5C, 6A, B) five teeth along cutting edge, distance between teeth is not equal. The upper three teeth occupy two third of the cutting edge, the gap between first and second tooth smaller than half of cutting edge. All teeth, except first, bifid. Inferior angle with short spines, surface of mandible bearing rows of short sharp spines.

Maxilla I (Figs. 5D, 6C) with ten sharp teeth of equal size, cutting edge without notch inferior angle two to three spines half size of other teeth. Few simple setae on upper and lower margins. Distal surface of maxilla two rows of short sharp setae.

Maxilla II (Figs. 5A, 6D) bilobed, proximal lob round and bigger than elongated distal one. Both lobes with setae, distal setae simple, pinnate setae on proximal end.

Cirri: range of number of articles is presented in Table 2.

Cirrus I (Fig. 7A) rami of different size, number of articles of exopod twice than that of endopod, ranging from 9-12; 4-5 on endopod, on apex bipectinate setae, on proximal articles small number of pinnate setae (Fig. 7F).

Cirrus II (Fig. 7B) with most setae on anterior of rami pinnate, setae on terminal article bipectinate.

Cirrus III (Fig. 7C) number of articles on two rami equal or slightly greater on exopod, spines pinnate, on terminal articles bipectinate. Conical spines on posterior of articles of endopod, (Fig. 7I) spines on distal articles unidentate (Fig. 7G).

Cirri IV-VI (Fig. 7D) number of articles not more than 17. On distal articles, three pairs of simple setae, on proximal articles four pairs, of which two most proximal are shorter. Long setae are bifurcate, terminal ones simple.

Penis long, annulated, short simple setae scattered along penis, point of basis conical (Fig. 7E, H)

Trevathana mizrachae n. sp.
Figs. 8-10

Host coral.—*Platygyra lamellina* (Ehrenberg, 1834)

Etymology.—Named in the honor of the late Ms Lea Mizrahi in appreciation of her devoted help during many years in the Faculty of Life Sciences, Bar Ilan University.

Material examined.—Barnacle extracted from a colony of *Platygyra lamellina* collected in shallow water in front of the Interuniversity Institute, Eilat, Israel. Holotype: TAU-AR 28615 paratypes: TAU AR-28927; BMNH 2009.20.

Diagnosis.—*Trevathana* with up to 21 low radial ribs with up to three projection on rib, spur short and broad, internal tooth of tergum broad parallel to basal margins.

Description.—Shell white, fused, flat, elliptic, maximum rostro-carinal diameter 7 mm. Lateral diameter about half that of rostro-carinal diameter. Surface of shell with low radiating ribs, maximum number of ribs 21, only part of them reaching the orifice; ribs carry up to three fingerlike projections, covered with thin layer of deposition of host coral skeleton (Fig. 8A). Shell tubiferous, number of longitudinal septa equal to number of ribs. Sheath, pear-like, rostral side width larger than carinal width, white, covers more than half of inside side of shell, concentric growth lines on sheath (Fig. 8B). In newly settled specimens shell is thin with a small round orifice (Fig. 8C).

Orifice elliptic located close to carinal edge of shell, rostro-carinal diameter of aperture about a third of rostro-carinal diameter of shell. Basis tapering, maximal depth 13 mm.

Scutum and tergum white, separated. Scutum (Fig. 8D, F) transversally elongated, outer surface with growth ridges, median closed furrow nearly parallel to occludent margins, occludent margins 4.5 times longer than tergal margins. Tergal margins at about 45° to occludent margins. Tooth projecting at tergal margin, width about half of tergal

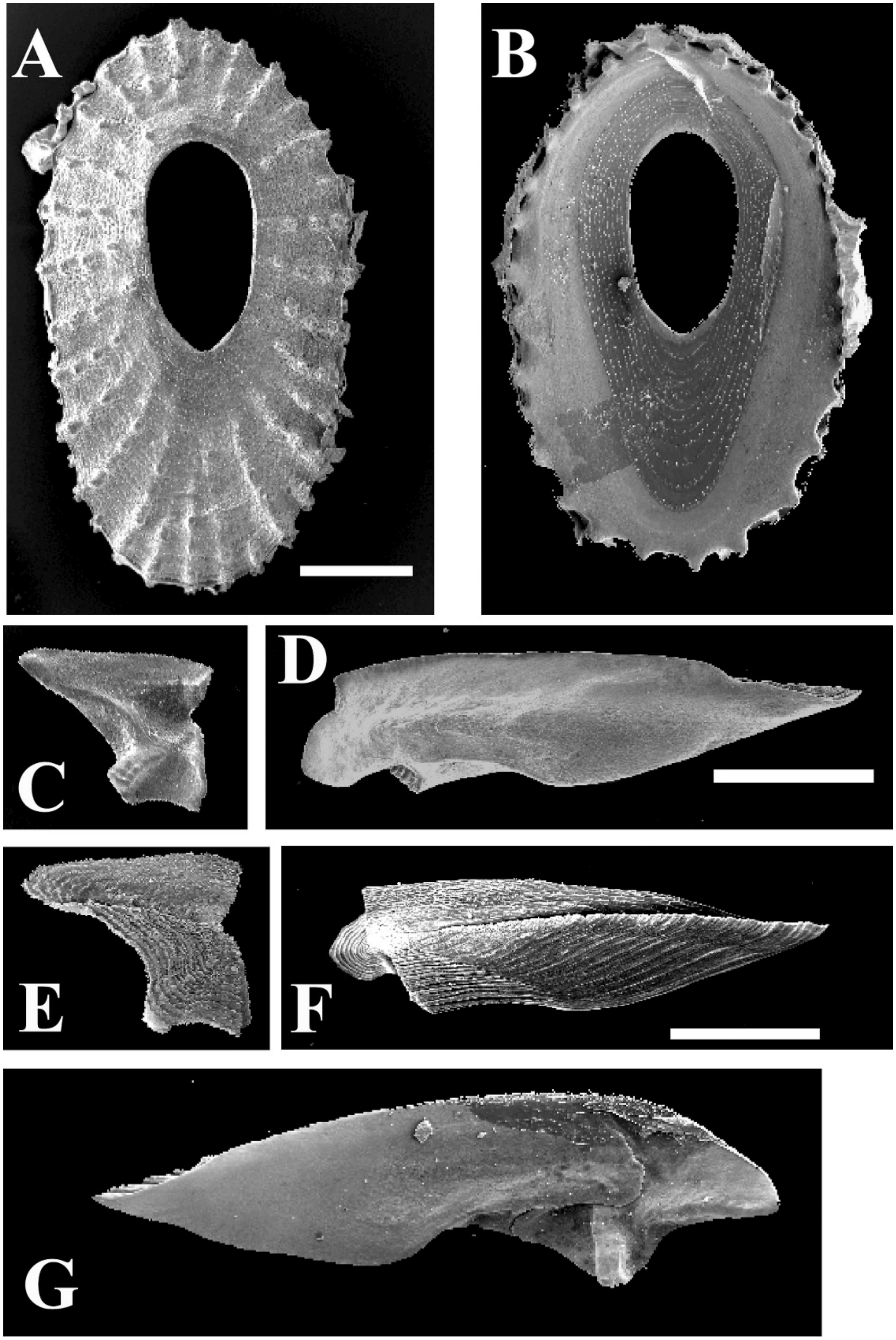

Fig. 4. Hard parts of *Trevethana dentata*, host coral *Leptastrea transversa* from the Red Sea. A, external view of shell surface. B, internal view of shell plate. C, internal view of tergum, D, internal view of scutum. E, external view of tergum. F, external view of scutum. G, internal view of scutum and tergum joint together. Scale bars: A-B = 1 mm; D, F = 0.5 mm, scales of D and F apply also to C, E, G.

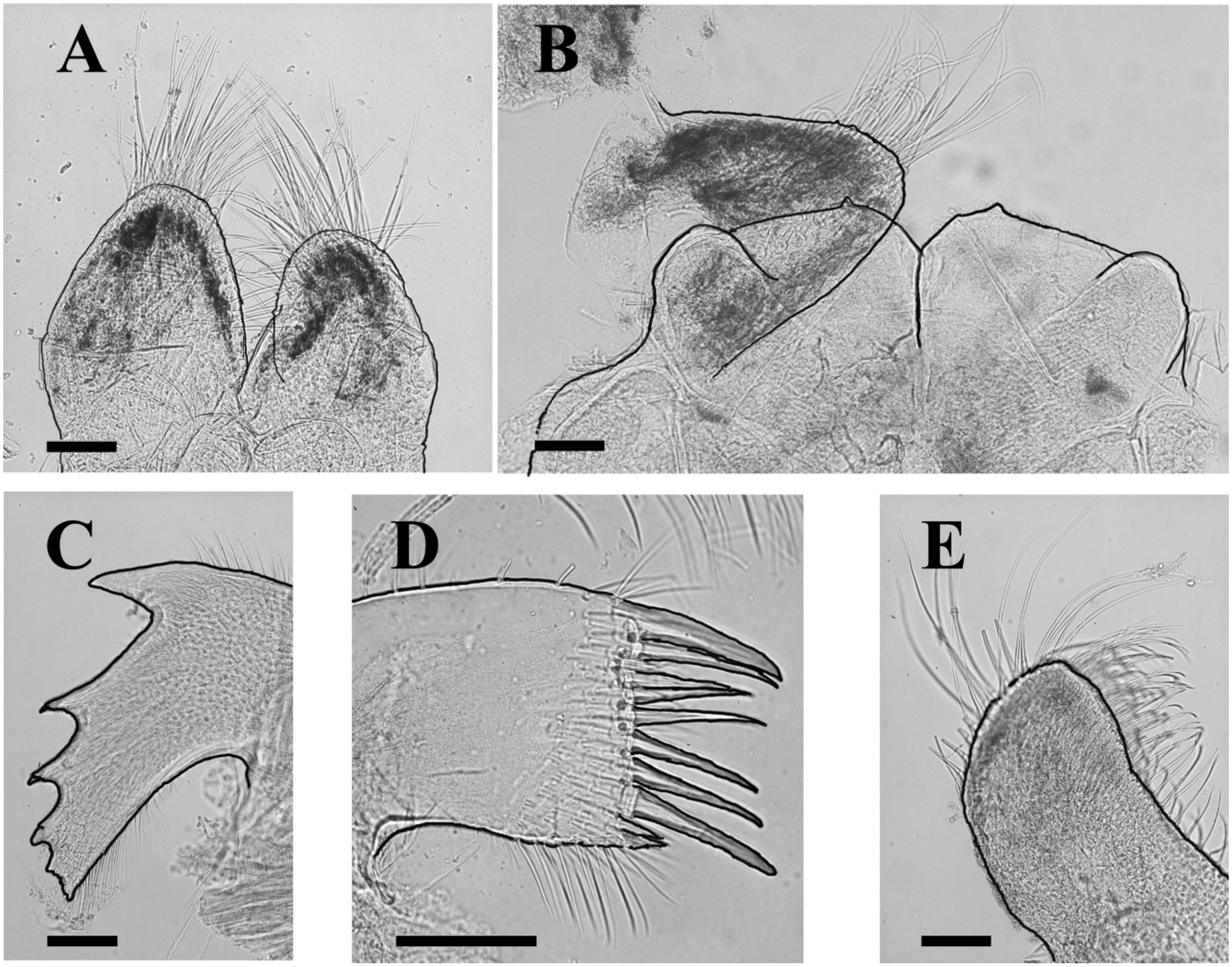

Fig. 5. Micrograph of Trophi of *Trevethana dentata*, host coral *Leptastrea transversa* from Red Sea. A, maxillae I. B, labrum and palp. C, mandibule. D, maxilla II, E, maxilla I. Scale bars = 100 µm.

margins; length, centrally located on tergal margins. Adductor and lateral depressor pits shallow, low adductor ridge parallel to basal margins but not projecting beyond basal margins. Tergum (Fig. 8E, G) triangular, on outer surface growth ridges, spur short and broad, shallow furrow from middle of scutal margins, along spur, to basal margin, scutal margins obtuse angle. Inner side concave, depression accommodates the scutal tooth, growth lines inside depression. A broad inward projecting tooth, located on margins of spur, long axis of tooth parallel to basal margins.

Labrum bilobed (Fig. 9E), lobes oval nearly quadrangular, deep notch between lobes, one tooth on each lobe, short hairs on inside margins of notch. Palpus pigmented, elongated club-like, long spines on distal and proximal margins pinnate.

Mandible (Fig. 9D) five teeth along cutting edge, distance between teeth not equal. Upper three teeth occupy two thirds of the cutting edge, gap between first and second tooth smaller than half of cutting edge. All teeth, except first, bifid. Inferior angle with short spines, surface of mandible bearing combs made of short sharp spines as well as single spines.

Maxilla I (Fig. 9B) bilobed, proximal lob round and bigger than elongated distal one. Distal lobe pigmented, both lobes with pinnate spines.

Maxilla II (Fig. 9C) cutting edge with ten to thirteen sharp teeth of unequal size, upper two teeth bigger than others, a gap between second and third tooth, inferior angle two to three spines slightly shorter than other teeth. Spines close to superior margins simple, some of those close to inferior edge splinted. Marginal surface of maxilla parallel rows of short sharp spines.

Ranges of number of articles of cirri are presented in Table 2.

Cirrus I (Fig. 10A) rami of different size, number of articles of exopod twice than that of endopod, 12-14 in exopod, 6-8 in endopod. Proximal articles of exopod and all article of endopod, apart of distal one, pigmented blots. Protrusions on frontal side articles of endopod. Long pinnate setae with short pinnae on proximal end of each

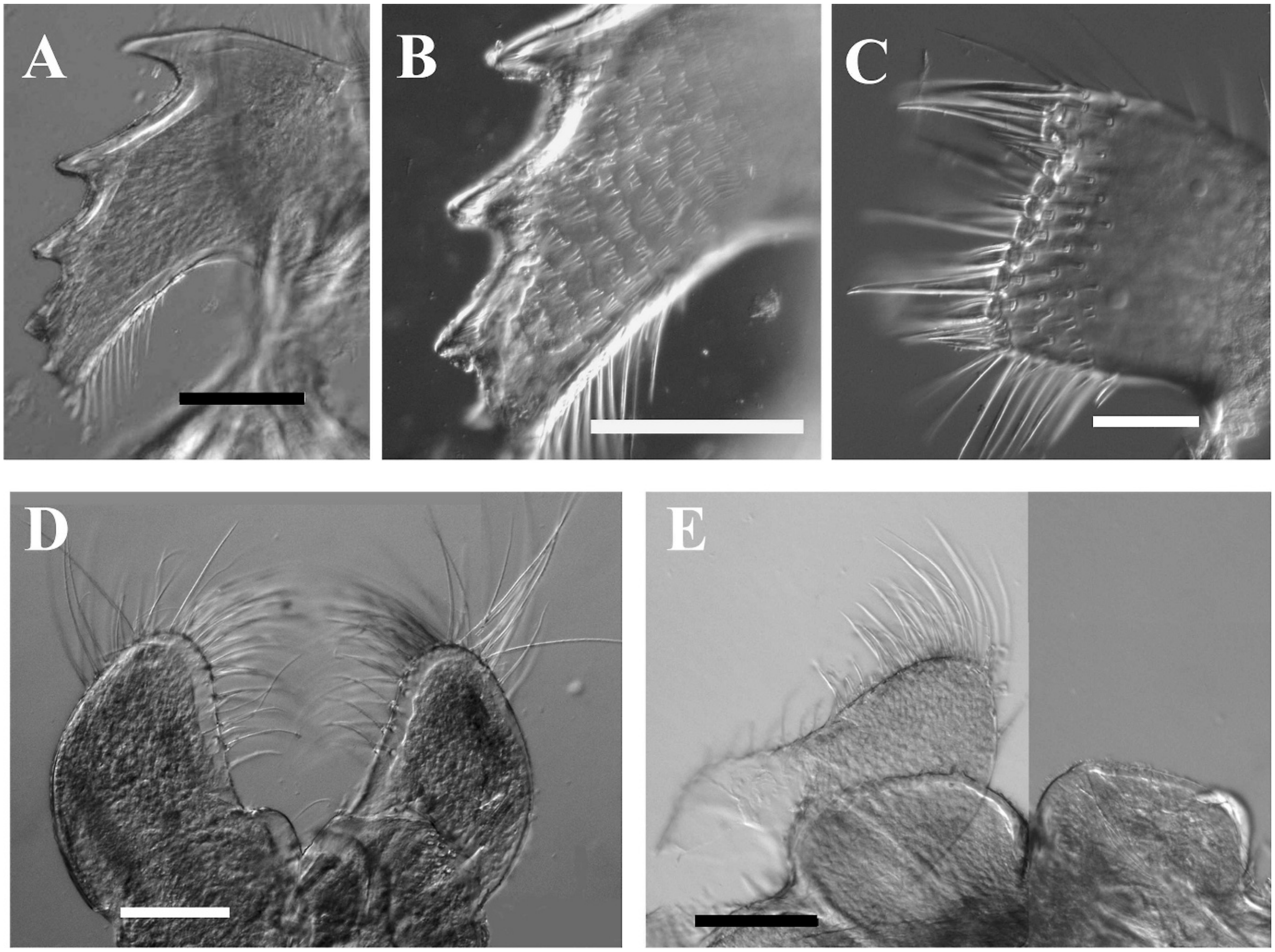

Fig. 6. Differential interference contrast (Nomarski) micrograph of Trophi of *Trevethana dentata*, host coral *Leptastrea transversa* from Red Sea. A and B, mandibule. C, maxilla II. D, maxillae I. E, labrum and palp. Scale bars = 100 μm.

article. Apical setae on distal articles pinnate, with long pinnae, setation of endopod heavier than on exopod; in a few cases, bipectinate setae present.

Cirrus II (Fig. 10B) number of articles in exopod 8-9 endopod 6-7. Anterior margins of articles of both rami protuberant on protrusions pinnate setae. Articles, except distal ones, with pigmentated blots. Row of conical spines on posterior side of two distal articles of endopod (Fig. 10E). Apical setae of two types, most bipectinate and some pinnate.

Cirrus III number of articles on two rami equal or slightly bigger on exopod, 10-12 in exopod, 8-10 in endopod. Protopod and rami with pigmented blots on posterior part, on both rami rows of spines like that described on endopod of cirrus II; these are more developed on distal articles. On apical articles, bipectinate and pinnate setae. Protrusions on frontal side of exopod, protrusions carry pinnate setae. On articles of endopod two types of setae, pinnate and bipectinate.

Cirri IV-VI long, number of articles up to 22. On distal articles, three pairs of setae; on proximal articles four pairs of pinnate setae, two distal of each article are shorter. Long setae are bifurcate, apical setae multifurcate.

Penis (Fig. 10F, G) long, annulated, short setae along penis, basi-dorsal point conical.

Trevathana margaretae n. sp.
Plates 11-14

Host coral.—*Favia favus* (Forskål, 1775)

Etymology.—Named in honor of Dr. Margaret Barnes in recognition and appreciation of long years of contribution to the study of barnacles.

Material Examined.—Barnacle extracted from a colony of *Favia favus* collected in shallow water in front of the Interuniversity Institute, Eilat, Israel. Holotype: TAU-AR 28613, paratypes: TAU- AR-28926; BMNH 2009.21

Diagnosis.—*Trevathana* with thin outer lamina, radial ribs noticeable only as small folds at the perimeter of the outer lamina of shell, spur short and broad, internal tooth of tergum broad parallel to basal margins.

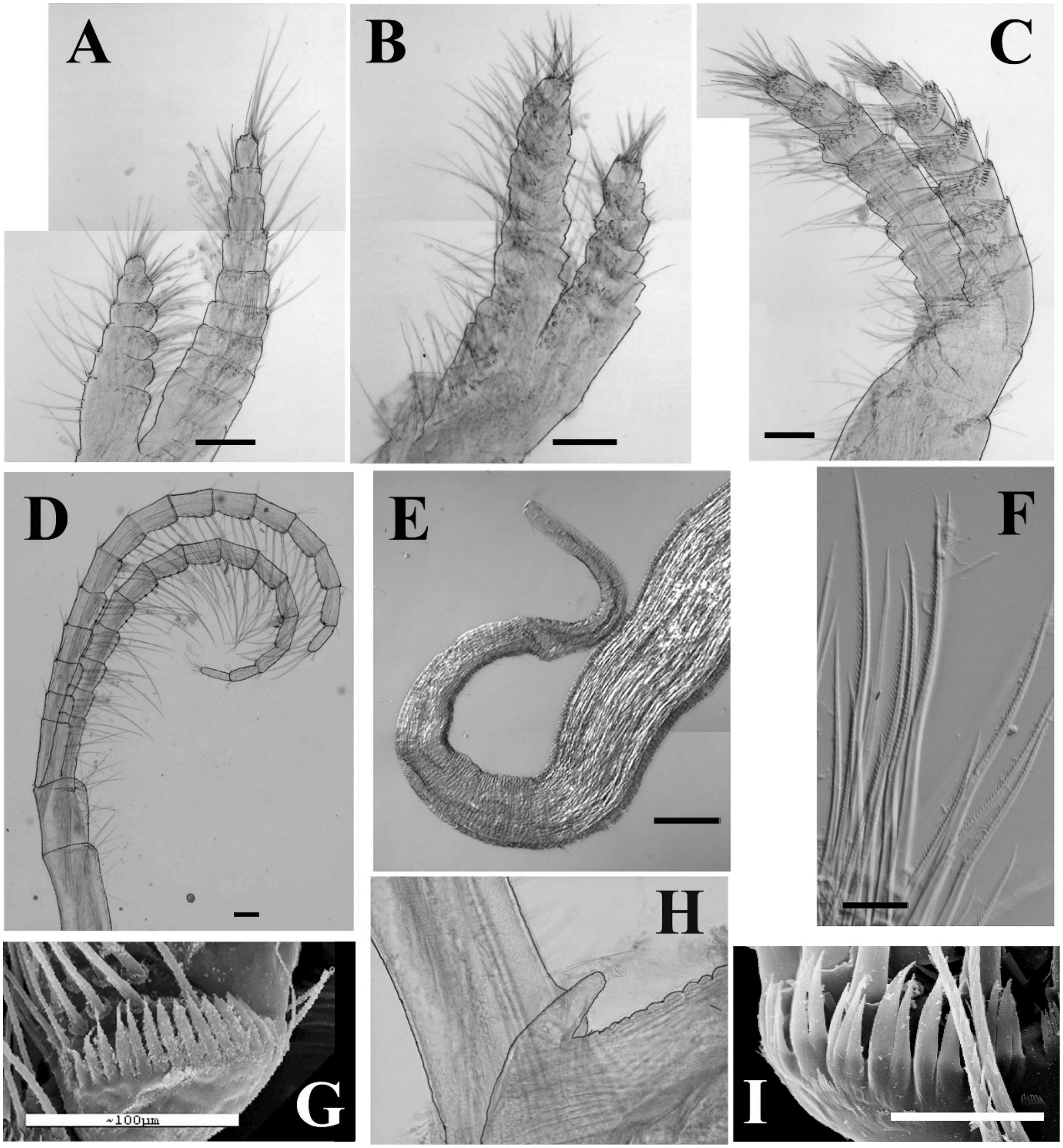

Fig. 7. Micrograph of cirri of *Trevethana dentata*, host coral *Leptastrea transversa* from the Red Sea. A, cirrus I. B, cirrus II. C, cirrus III. D, cirrus IV; E, tip of penis. F, terminal setae of cirrus I. G, conical spines on distal posterior articles of endopod of cirrus III H, basis of penis I. I, trident spines on proximal posterior articles of endopod of cirrus III. Scale bars: A-E, G, I = 100 µm; F = 20 µm (scale bar of F also applies to H).

Description.—Shell white, fused, flat, elliptic, maximum rostro-carinal diameter 5 mm, outer lamina thin, smaller than inner lamina (Fig. 11A). Lateral diameter less than half of rostro-carinal diameter. Radiating ribs distinct only as small folds at the perimeter of outer lamina, do not reach the orifice, maximum number of ribs 18. Shell tubiferous, longitudinal septa and inner lamina project beyond outer lamina (Fig. 13B, H). Sheath, white, covers more than half of the inside side of shell, pear-like, rostral side wider than carinal side, inside concentric growth lines. Orifice elliptic located close to carinal edge of shell, rostro-carinal diameter of aperture about half that of rostro-carinal

Table 2. Range of number of cirral articles of *Trevathana* from different host corals.

Host coral		Cirrus I	Cirrus II	Cirrus III	Cirrus IV	Cirrus V	Cirrus VI
Leptastrea transversa	endopod	6 (n = 2)	5 (n = 2)	7 (n = 2)	16-20 (n = 3)	16-20 (n = 3)	18-21 (n = 3)
	exopod	10 (n = 2)	6 (n = 2)	6 (n = 2)	14-19 (n = 3)	17-20 (n = 3)	17-22 (n = 3)
Favites abdita	endopod	6-7 (n = 7)	6-7 (n = 7)	8-9 (n = 6)	19-23 (n = 6)	21-26 (n = 7)	23-27 (n = 7)
	exopod	11-13 (n = 7)	8 (n = 7)	20-24 (n = 7)	23-25 (n = 7)	23-28 (n = 7)	23-28 (n = 7)
Favia favus	endopod	5-7 (n = 5)	5-7 (n = 5)	6-7 (n = 5)	15-18 (n = 5)	17-21 (n = 5)	19-20 (n = 4)
	exopod	11-14 (n = 5)	7-9 (n = 5)	8-9 (n = 5)	17-20 (n = 5)	18-21 (n = 5)	21-23 (n = 4)
Platygyra lamellina	endopod	6-8 (n = 5)	6-7 (n = 5)	9-10 (n = 5)	14-19 (n = 5)	17-22 (n = 5)	19-23 (n = 5)
	exopod	12-14 (n = 5)	8-9 (n = 5)	11-12 (n = 5)	16-19 (n = 5)	19-24 (n = 5)	20-24 (n = 5)
Cyphastrea chalcidicum	endopod	4-5 (n = 7)	5-6 (n = 7)	6-9 (n = 7)	9-12 (n = 6)	12-15 (n = 6)	15-16 (n = 6)
	exopod	9-12 (n = 7)	6-8 (n = 7)	7-9 (n = 7)	13-14 (n = 6)	14-17 (n = 6)	16-17 (n = 6)

diameter of shell. Length to width ratio 2:1. Basis tapering, with radial ribs, maximal depth 7 mm.

Scutum and tergum white, separated, tightly interlocked. (Fig. 11G). Scutum (Fig. 11E, F) transversally elongated, growth ridges on outer surface, median furrow closed, oblique from middle of occludent margin to middle of tergal margin. Occludent margins five times longer than tergal margins. Tergal margins perpendicular to occludent margins. Blunt tooth, centrally located on tergal margins, width more than half of tergal margins. Adductor and lateral depressor pits shallow, low adductor ridge, not extending beyond basal margins. Tergum triangular, basi-occludent angle acute, on outer surface growth ridges, spur short, close to basal-scutal margins. Broad, hardly distinct furrow from middle of scutal margins to basal margin. Inner side on scutal margins depression that accommodates the tergal tooth, growth lines inside depression. An inward projecting tooth, located on margins of spur, long axis of tooth parallel to basal margins (Fig. 11C, D).

Labrum (Fig. 12A) bilobed, lobes nearly quadrangular, deep V shaped notch between lobes, one to three pointed teeth on each lobe, short setae inside margins of notch. Palpus wide, rectangular pigmented, long pinnate spines on proximal margins, simple setae on distal margins.

Mandible (Fig. 12D) five teeth, unequally distributed along cutting edge. Upper three teeth occupy two thirds of cutting edge, gap between first and second tooth smaller than half of cutting edge. All teeth, except first, bifid. Inferior angle with short teeth, on inferior margins short simple setae. Short sharp spines and combs on surface of mandible.

Maxilla I (Fig. 12C) on cutting edge, nine to ten pointed big teeth of unequal size, upper two teeth bigger than others, a gap between second and third tooth, shorter spines on inferior angle. Simple setae on superior and inferior margins. On surface of maxilla parallel rows of short sharp spines, longest on distal raw.

Maxilla II (Fig. 12B) bilobed, proximal lobe round and bigger than distal one, which is elongated and pigmented, pinnate spines on both lobes.

Cirri long, range of count of articles is presented in Table 2.

Cirrus I (Fig. 13A) rami of different size, number of articles exopod 11-14, endopod 5-7. Proximal articles of exopod and all article of endopod, apart from the distal one pigmented. Setation of endopod heavier than on exopod. Protrusions on frontal side of articles of endopod. Two types of pinnate setae, setae with short pinnae on distal margins of each articles of endopod, and setae with dense long pinnae on protrusions, in several cases, bipectinate setae also present. Apical articles with pinnate and bipectinate setae.

Cirrus II (Fig. 13B) number of articles exopod 7-9, endopod 5-7. Articles of both rami with frontal protrusions, pigmentation on all articles except two distal. On posterior side of two distal article of endopod, row of conical spines, located on distal end of article. Apical setae of both rami of two types, most bipectinate and some pinnate. On protuberating side of exopod, pinnate setae with long pinnae.

Cirrus III (Fig. 13C) articles on exopod 8-9, on endopod 6-7. Posterior side of basis and rami pigmented, row of conical spines on distal articles of both rami, apical setae of endopod bipectinate, those of exopod pinnated and bipectinate. On protrusions of exopod, pinnate setae with short pinnae.

Cirri IV-VI long, number of articles on longest ramus more than 22. On distal articles, three pairs of setae, on proximal articles four pairs, of which the two distal are shorter (Fig. 13D). In cirrus VI, number of pairs of setae five. Long setae pinnate, terminal setae multi-furcate.

Penis (Fig. 13E) annulated short seta scattered along penis, basi-dorsal point non distinct.

Trevathana jensi n. sp.
Plates 14-16

Host coral.—*Favites abdita* (Ellis and Solander, 1786)

Etymology.—Named in honor of Dr. Jens Høeg in recognition of his contribution to the study of Cirripedia.

Material Examined.—Barnacle extracted from a colony of *Favites abdita* collected at 3m depth in front of the Interuniversity Institute, Eilat, Red Sea, Israel. Holotype: TAU-AR 28612, paratypes: TAU- AR - 28924; AR – 28925; BMNH 2009.19.

Diagnosis.—*Trevathana* with up to 17 low radial ribs with tow projection on rib, spur short and broad, internal tooth of tergum short, broad parallel to basal margins. Scutal margins with deep articular furrow.

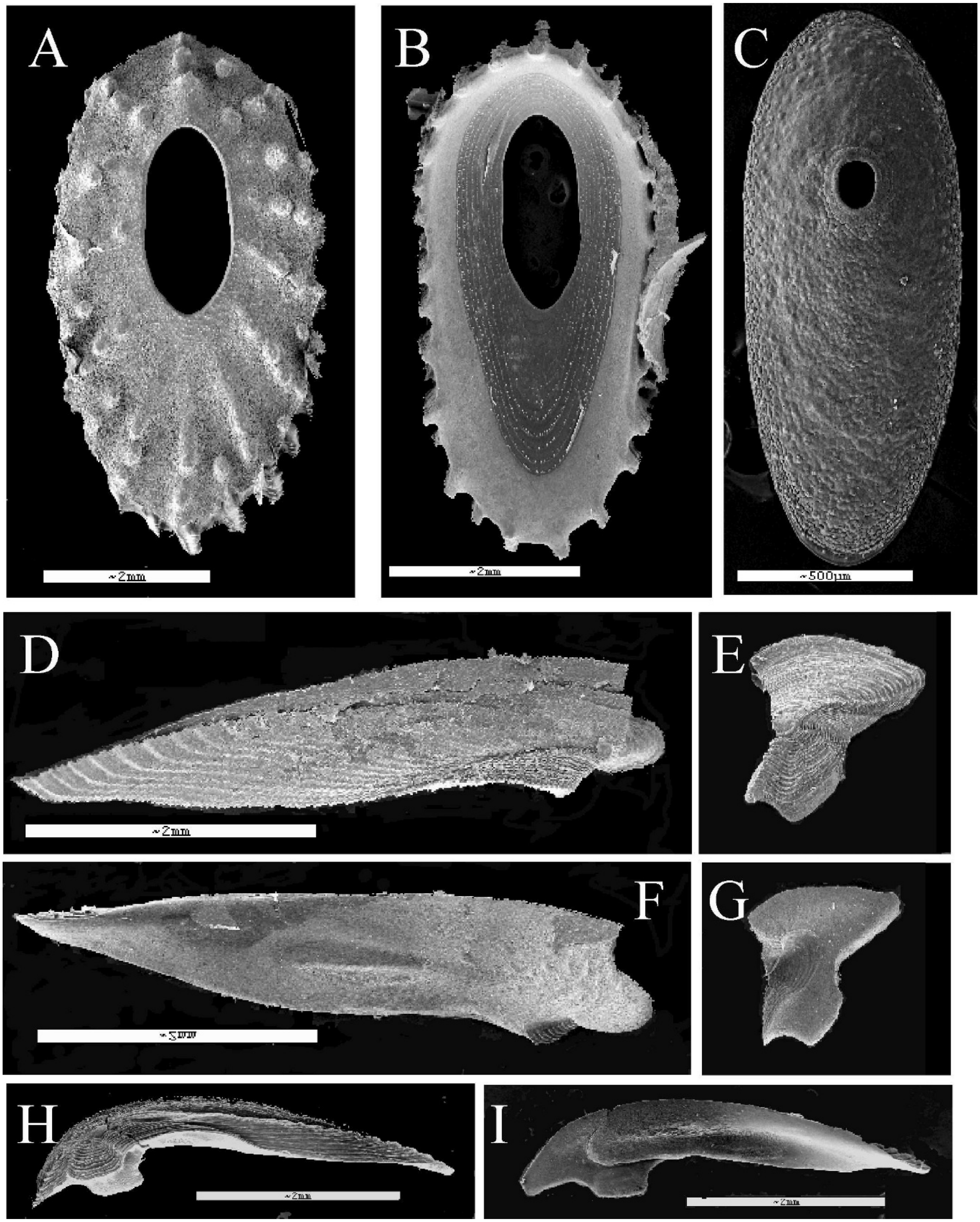

Fig. 8. Hard parts of *Trevethana mizrachae* n. sp., host coral *Platygyra lamellina* from the Red Sea. A, shell plate, outer view. B, shell plate, inner view. C, shell plate of newly settled specimen, outer view. D, scutum, outer view. E, tergum, outer view. F, scutum inner view. G, tergum outer view. H, outer view of scutum and tergum joint together. I, inner view of scutum and tergum joint together. Scale bars: A, B, D, I = 2 mm; C = 0.5 mm. Scales of D and F also apply to E, G.

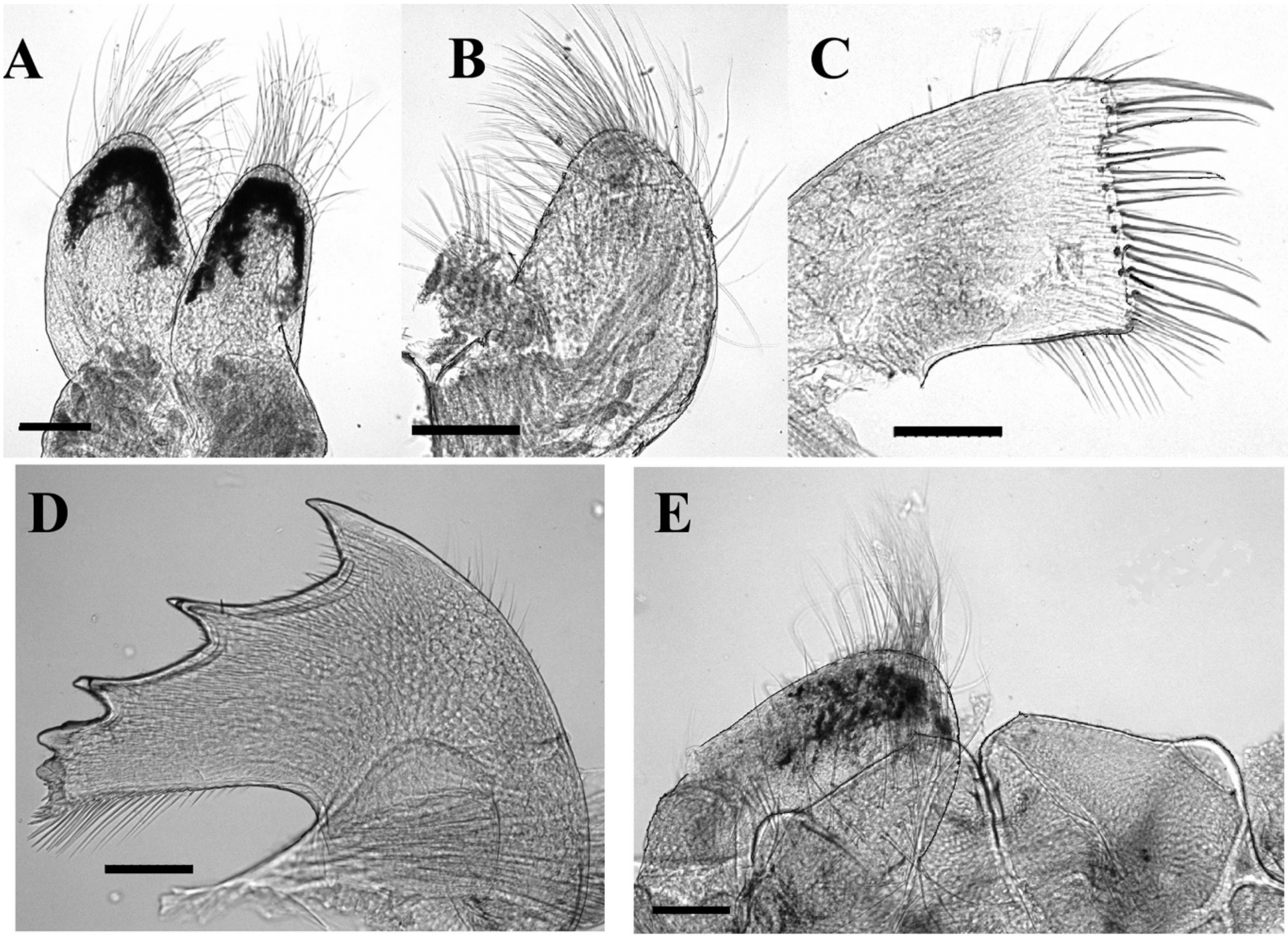

Fig. 9. Micrograph of Trophi of *Trevethana mizrachae* n. sp. host coral *Platygyra lamellina* from the Red Sea. A, B, maxillae II. C, maxilla I. D, mandibule. E, labrum and palp. Scale bars = 100 μm.

Description.—Shell white, fused, flat, elliptic, maximum rostro-carinal diameter more than 7 mm. Outer lateral diameter more than half of rostro-carinal diameter. (Fig. 14A). Up to 17 radiating ribs reaching the orifice, carrying up to two short projections. Shell tubiferous, longitudinal septa and inner lamina project beyond outer lamina. Sheath, white, covers most of the inner side of shell, pear-like, carinal end wider than rostral, inside concentric growth lines (Fig. 14B). Orifice elliptic located close to carinal edge of shell, rostro-carinal diameter of orifice less than half that of rostro-carinal diameter of shell. Shell length to width ratio more than 2:1. Basis tapering, with radial ribs; maximal depth 18 mm.

Scutum and tergum white, separated. Scutum transversally elongated, outer surface with growth ridges, median furrow closed, oblique from near basi-occludent to middle of tergal margin. Occludent margins about three times longer than tergal margins. Tergal margins perpendicular to occludent margins, but curving at its middle, angle between tergal margins and basal margin obtuse. (Fig. 14C, F). Prominent tooth, centrally located on tergal margins, width more than half of tergal margins. Adductor and lateral depressor pits deep, low adductor ridge, extending beyond basal margins. Tergum triangular, inner part concave, basi-occludent angle acute, on outer surface growth ridges, spur short, close to basal-scutal margins. Inner side on scutal margins deep depression that accommodates the tergal tooth, growth lines inside depression. In large, mature animals, middle of depression is eroded (Fig. 14D, G). A short inward projecting tooth, located on margins of spur, long axis of tooth parallel to basal margins (Fig. 14G, H).

Labrum (Fig. 16A) bilobed, lobes nearly quadrangular, deep V shape notch between lobes, blunt tooth on each lobe, number of teeth on lobes variable, maximal number of teeth nine, short setae inside margins of notch. Palpus elongated (Fig. 16B), pigmented, long pinnate spines on distal margins.

Mandible (Fig. 15D) with five teeth, not equally distributed along cutting edge. Upper three teeth occupy 2/5 of the cutting edge, gap between first and second tooth smaller than half of cutting edge. All teeth, except first, bifid. On surface of mandible, short sharp spines arranged as combs, three to four spines in a comb. On inferior margins short simple setae.

Maxilla I (Fig. 15E) cutting edge, eleven pointed teeth of unequal size, upper two and lower two bigger than others, a gap between second and third tooth, few short simple spines on inferior angle. On surface of maxilla parallel rows of short sharp spines, longest on distal row.

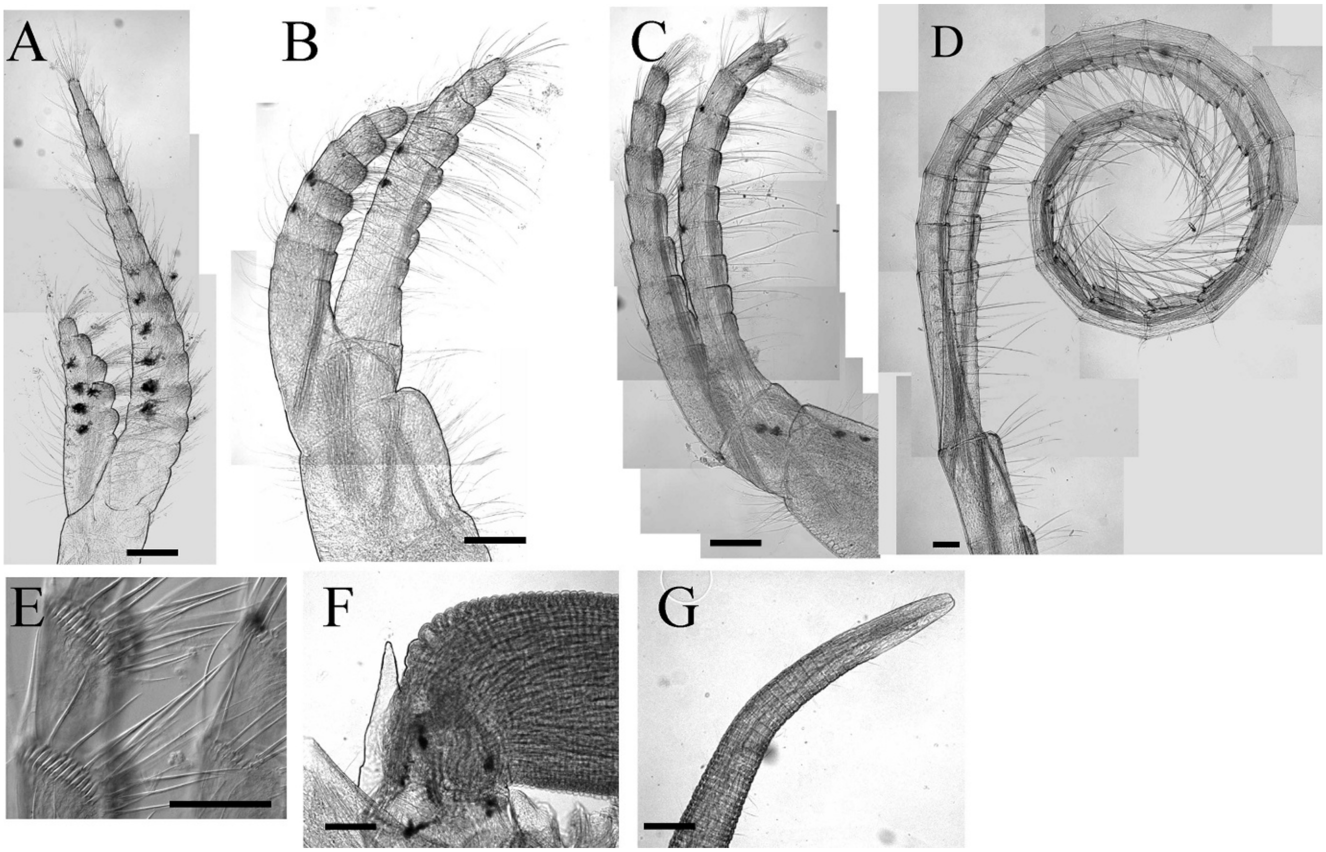

Fig. 10. Micrograph of cirri of *Trevethana mizrachae* n. sp. host coral *Platygyra lamellina* from the Red Sea. A, cirrus I. B, cirrus II. C, cirrus III; D, cirrus IV. E, conical spines on distal posterior articles of endopod of cirrus III. F, basis of penis. G, tip of penis. Scale bars = 100 μm.

Maxilla II bilobed, proximal lobe round and bigger than elongated distal one, pinnate spines on both lobes.

Cirri short, range of count of articles is presented in Table 2.

Cirrus I (Fig. 16A) rami of different size, number of articles exopod 11-13, endopod 6-7. Articles of exopod and all article of endopod (except distal one) with pigmented patches. Frontal side of articles of endopod with protrusions. On distal margins of each article long pinnate setae. Apical setae of both rami bipectinate.

Cirrus II (Fig. 16B) number of articles exopod 8, endopod 6-7. Articles of both rami with frontal protrusions. On rear of two distal articles of endopod row of conical spines, located at distal end of article. Apical setae of endopod bipectinate on other articles, only pinnate seta. Apical setae of expopod and of three distal articles of two types pinnae and bipectinate.

Cirrus III (Fig. 16C) number of articles of exopod 8-9, of endopod 9-11. Frontal side of articles with protrusions. Raw of conical spines on rear of distal articles of endopod (Fig. 16D), apical setae bipectinate, on protrusions two types of setae, pinnae and bipectinate. Apical setae of expopod bipectinate, on protrusions pinnate setae, on basis pinnate setae.

Cirri IV-VI (Fig. 16E) long, number of articles on longest ramus more than 22. On distal articles three pairs of setae, on proximal articles four pairs, of which the two distal are shorter, long setae pinnate. On apical articles two to three bifurcate setae.

Penis (Fig. 16F) annulated, short seta scattered along penis, non-distinct basi-dorsal point.

Trevathana sarae n. sp.
Plates 17-19

Host coral.—*Cyphastrea chalcidicum* (Forskål, 1775)

Material Examined.—Barnacle extracted from a colony of *Cyphastrea chalcidicum* collected in shallow water in front of the Interuniversity Institute, Eilat, Israel. Holotype: TAU- TAU- AR 28614, paratypes: TAU- AR-28922; TAU-AR-28617; TAU-AR-28619; BMNH 2009.18.

Etymology.—Named in honour of Mrs. Sara Brickner in recognition of her help to the present study.

Diagnosis.—*Trevathana* with up to 22 radial ribs with rows of projection, spur hardly distinct, pointed internal tooth of tergum on inner side of spur.

Description.—Shell white, fused, flat, elliptic, (Fig. 17A) maximum rostro-carinal diameter 4 mm. Lateral diameter about 2/3 of rostro-carinal diameter. Surface of shell with radiating ribs carrying rows of finger-like projections, covered with thin layer of deposition of host coral

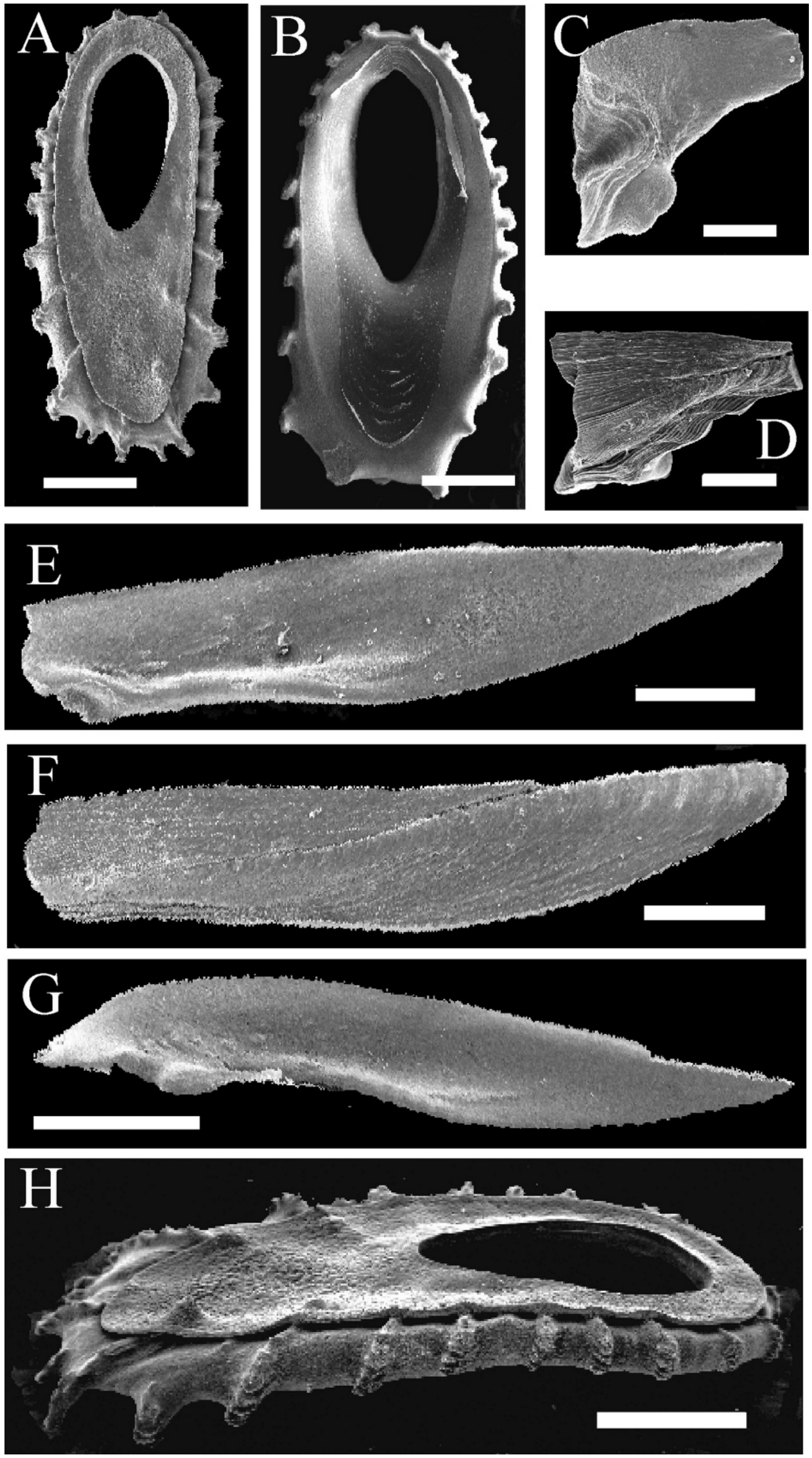

Fig. 11. Hard parts of *Trevethana margaretae* n. sp. host coral *Favia favus* from the Red Sea. A, shell plate, external view. B, shell plate, internal view. C, tergum, external view. D, tergum, inner view. E, scutum, inner view. F, scutum, external view. G, internal view of scutum and tergum joint together. H, side view of shell plate. Scale bars: A, B, G, H = 1 mm. C, D = 0.25 mm. E, F, = 0.5 mm.

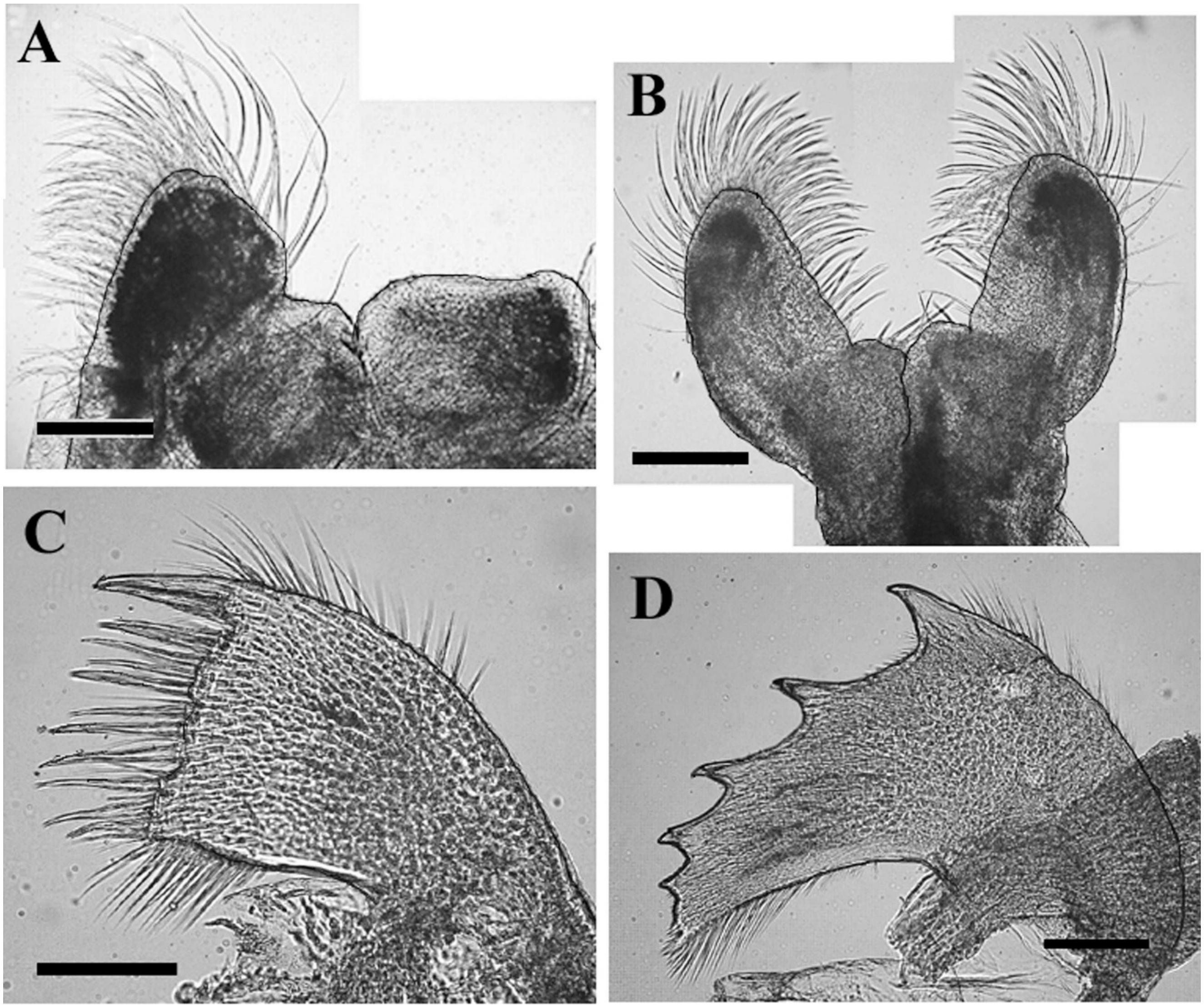

Fig. 12. Micrograph of trophi of *Trevethana margaretae* n. sp. host coral *Favia favus* from the Red Sea. A, labrum and palpi. B, labium showing pair of Maxillae II. C, maxilla I. D, mandible. Scale bars = 100 µm.

skeleton, U shape furrow between ribs. Up to four projections in row, maximum number of ribs 22. Shell tubiferous, number of radiating tubes equal to number of ribs. Sheat, white, covers most of the inside side of shell concentric growth ridges. (Fig. 17B)

Orifice elliptic located close to carinal edge of shell rostro-carinal diameter of aperture about 1/3 of rostro-carinal diameter of shell. Length to width ratio 2:1. Basis tapering, maximal depth 17 mm.

Scutum and tergum white, separated. Scutum transversally elongated (Fig. 17C). Outer surface with growth ridges, median closed furrow nearly parallel to occludent margins, occludent margins three times longer than tergal margins. Angle between tergal margins and occludent margins about 45°. Tooth projecting at the tergal margin, located closer to occludent margins than to basal margins, width about half of tergal margins length. Adductor and lateral depressor pits shallow, adductor ridge only slightly prominent, not projecting beyond basal margins (Fig. 17D). Tergum triangular, on outer surface growth ridges, spur hardly distinct, shallow longitudinal furrow from middle of scutal margins to carino-basal angle (Fig. 17E). Inner side depression accommodates the scutal tooth, growth lines inside depression. A pointed thin inward projecting tooth on inner side of spur, located at middle of basal margins, axis of internal tooth parallel to axis of spur (Fig. 17F).

Labrum bilobed (Fig. 18B), lobes oval, one tooth on each lobe, V shape notch between lobes, short hairs on inside margins of notch. Palpus, elongated, club-like on distal margins pinnate spines.

Mandible (Fig. 18C) five teeth along cutting edge, distance between teeth is not equal. The upper three teeth occupy 2/3 of cutting edge, gap between first and second tooth smaller than half of cutting edge. All teeth, except first, bifid. Inferior angle with short spines, surface of mandible bearing rows of short sharp spines.

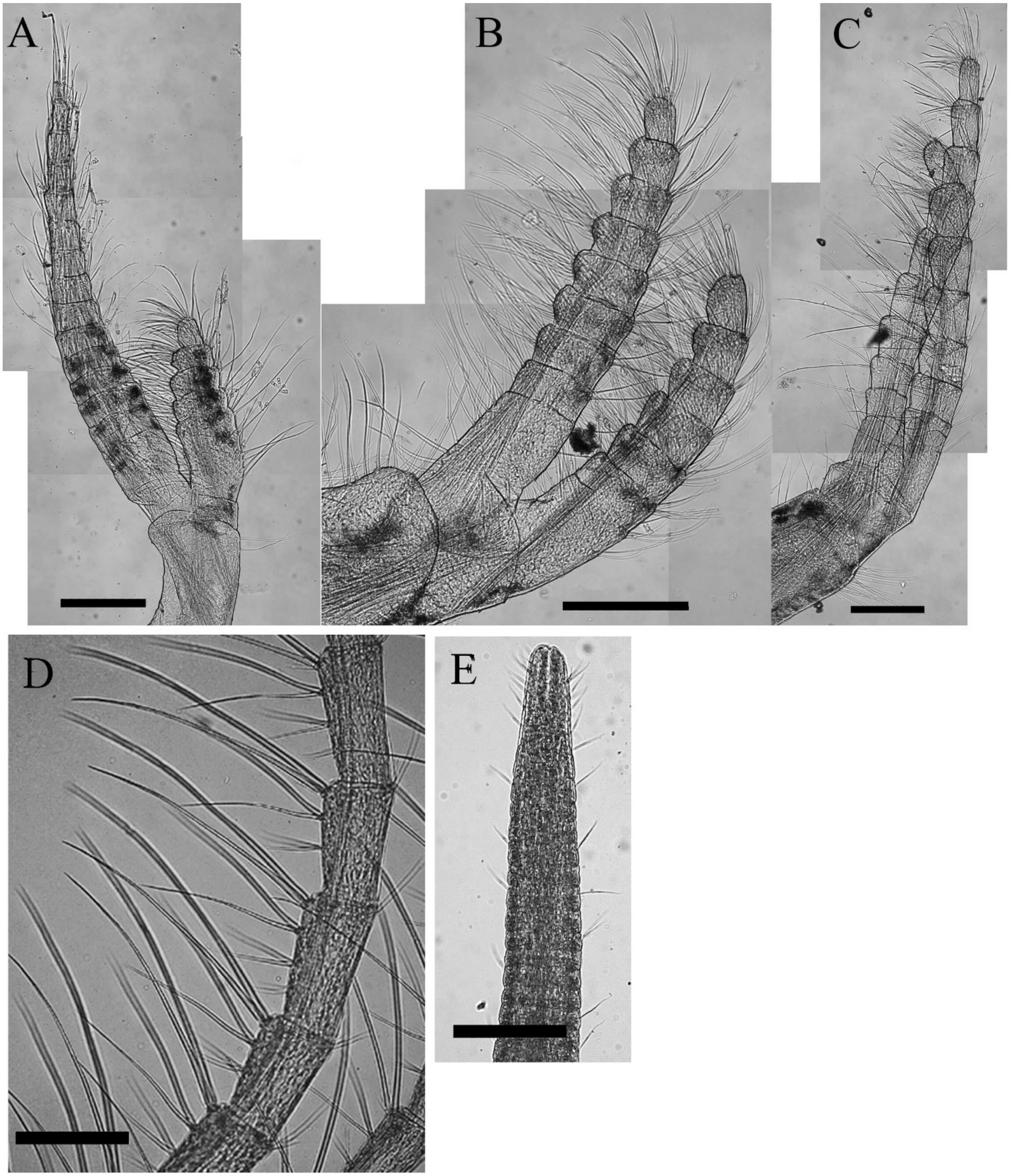

Fig. 13. Micrograph of cirri of *Trevethana margaretae* n. sp., host coral *Favia favus* from the Red Sea. A, cirrus I. B, cirrus II. C, cirrus III. D, section of cirrus IV. E, tip of penis. Scale bars = 100 μm.

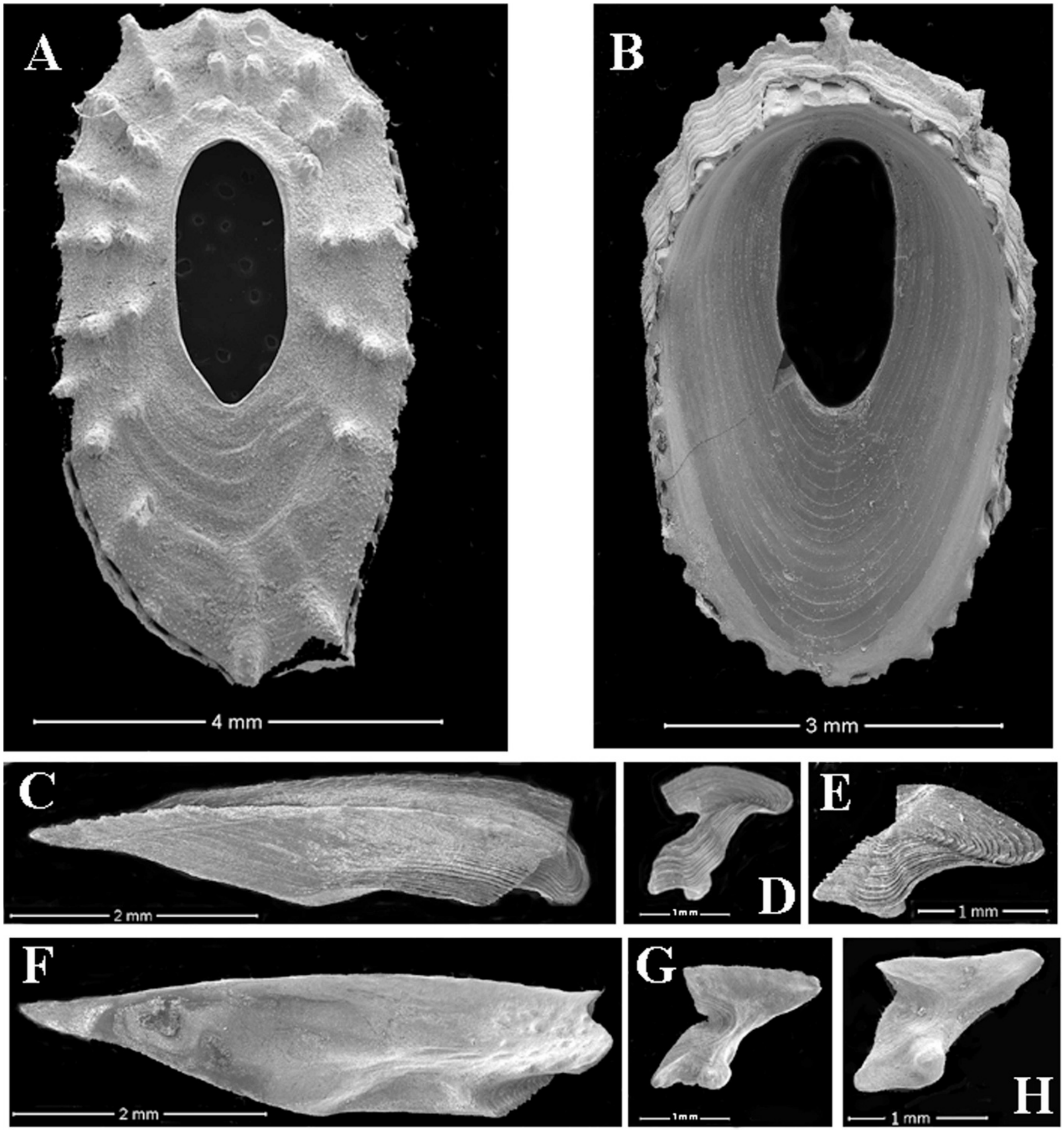

Fig. 14. Hard parts of *Trevethana jensi* n. sp. host coral *Favites abdita* from the Red Sea. A, shell plate, outer view. B, shell plate, internal view. C, scutum external view. D, tergum of old, eroded specimen, outer view. E, tergum of young specimen, external view. F, scutum internal view. G, tergum of eroded specimen, inner view. H, tergum of young specimen, inner view. Scale bars: A = 4 mm, B = 3 mm, C, F = 2 mm. D, E, G, H = 1 mm.

Maxilla I (Fig. 18D) ten sharp teeth of equal size on cutting edge, cutting edge without notch at inferior angle two to three short spines. Few simple setae on upper and lower margins. Surface of maxilla, close to teeth row, two rows of short sharp spines.

Maxilla II (Fig. 18A) bilobed, proximal round and larger than elongated distal one. Both lobes with spines, spines on distal lobe simple, on proximal pinnate.

Cirri short, spines on terminal articles usually bipectinate; range of number of articles is presented in Table 2.

Cirrus I (Fig. 19A) rami of different size, exopod antenniform long and slender, number of articles twice than that of endopod, 9-12 in exopod, 4-5 in endopod. Apical setae on exopod bipectinate, on proximal articles small number of pinnate setae.

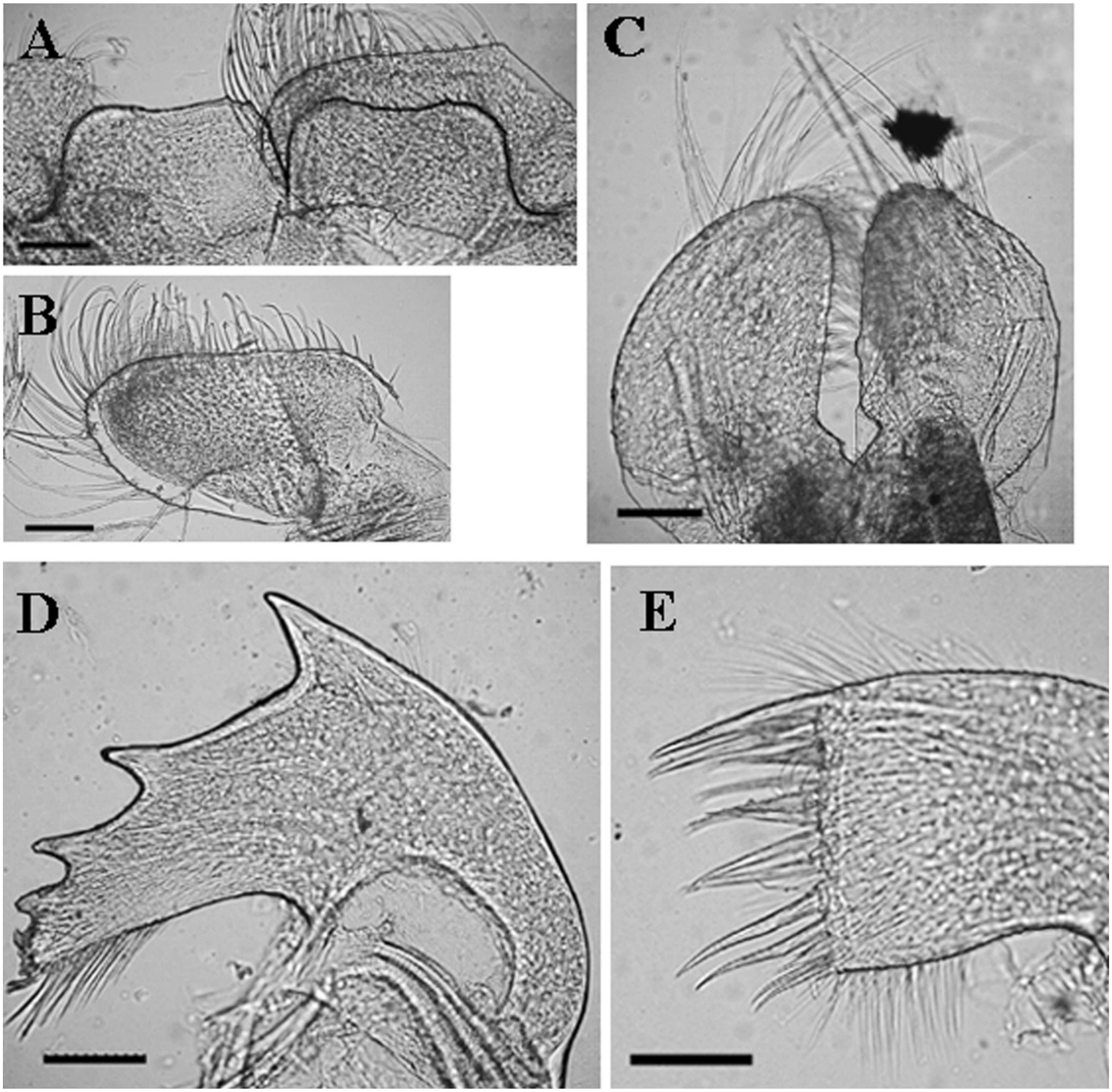

Fig. 15. Micrograph of Trophi of *Trevethana jensi* n. sp. host coral *Favites abdita* from the Red Sea. A, maxillae I. B, labrum and palp. C, mandibule. D, maxilla II. Scale bars = 100 μm.

Cirrus II (Fig. 19B) number of articles in exopod 6-8, 5-6 in endopod. Pinnate spines mainly on anterior face of rami, terminal articles with bipectinate setae.

Cirrus III (Fig. 19C) number of articles on two rami equal, 6-9, or slightly bigger on exopod, setae pinnate, on terminal articles bipectinate setae.

Cirri IV-VI short, number of articles not more than 17. On distal articles three pairs of setae, on proximal articles four pairs, of which the two distal are shorter. Long setae are bifurcate, terminal setae simple.

Penis (Fig. 19D) annulated short seta scattered along penis, short basi-dorsal point.

Discussion

Genetic differences revealed by molecular techniques have proven to be useful in separating closely related species and proposing the presence of cryptic species. Cryptic speciation was found in a wide range of marine invertebrates such as crustaceans, molluscs, annelids, sipunculans, and sponges (Knowlton, 2000). In coral inhabiting barnacles, Mokady et al. (1999) raised the possibility, based on genetic and morphological differences, that *T. dentatum* actually consists of several cryptic species with differing host specificity. In the hydrocoral barnacle, *Wanella milleporae* (Darwin, 1854), Mokady and Brickner (2001)

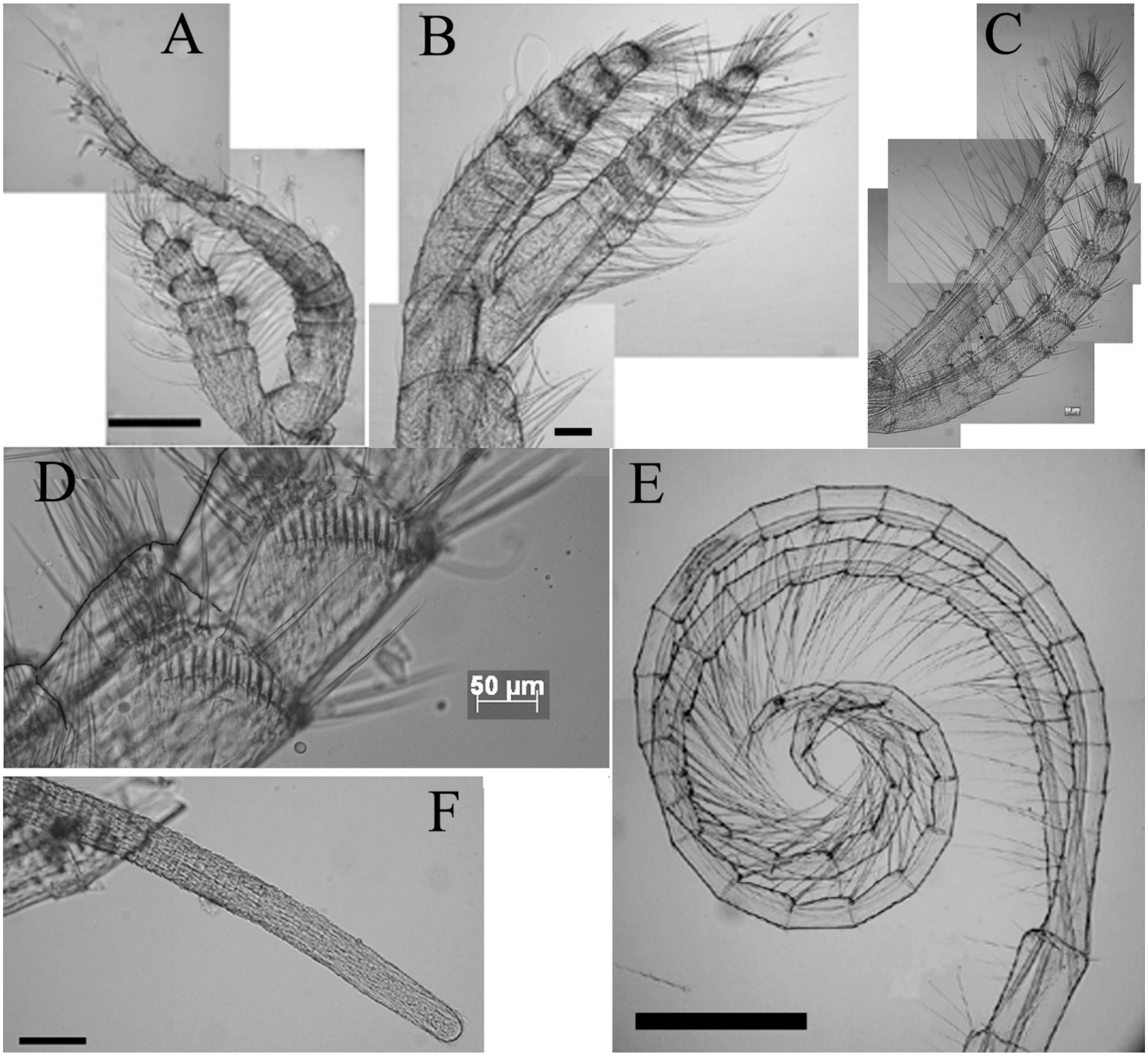

Fig. 16. Micrograph of Cirri of *Trevethana jensi* n. sp. host coral *Favites abdita* from the Red Sea. A, cirrus I. B, cirrus II. C, cirrus III. D, cirrus IV. E, section of posterior ramus of cirrus III showing conical spines. F, tip of penis. Scale bars A, B, F = 100 µm (scale of B also applies to C), D = 50 µm E = 500 µm.

demonstrated that specimens extracted from two ecotypes of *Millepora dichotoma* Forskål, 1775 are sibling species. Based on the ecological and morphological features of the two hydrocoral ecotypes of *M. dichotoma*, Meroz-Fine et al. (2003) concluded that the two ecotypes represent two separate species. The partition of barnacle species into two species parallel to the host hydrocoral partition suggests host specificity. Tsang et al. (2009), on the other hand, working on the genetic and morphological variations of *Wanella* in Taiwan, suggested that the population of *Wanella* from this region probably consists of several cryptic species, which are not species-specific to their hydrocoral host.

Our molecular analyses based on three genes and a larger set of specimens support the partition of barnacles of the *"Savignium"* group into different species. Our results also support paraphyly of *Trevathana*. The clade that includes *Savignium* extracted from *Acantastrea* and *Platygyra*, forms a sister-group to the clade that comprises *Neotrevathana* and *Trevathana*. The clustering of *Neotrevathana* with *Trevathana* in the same clade indicates their close relationship, and these two genera should probably be included in a single genus. Examination of the valves of *Neotrevathana* from *Echinopora* indicated that the fusion of scutum and tergum is noticeable as a fissure between these valves, and it is similar to the tooth that articulates the

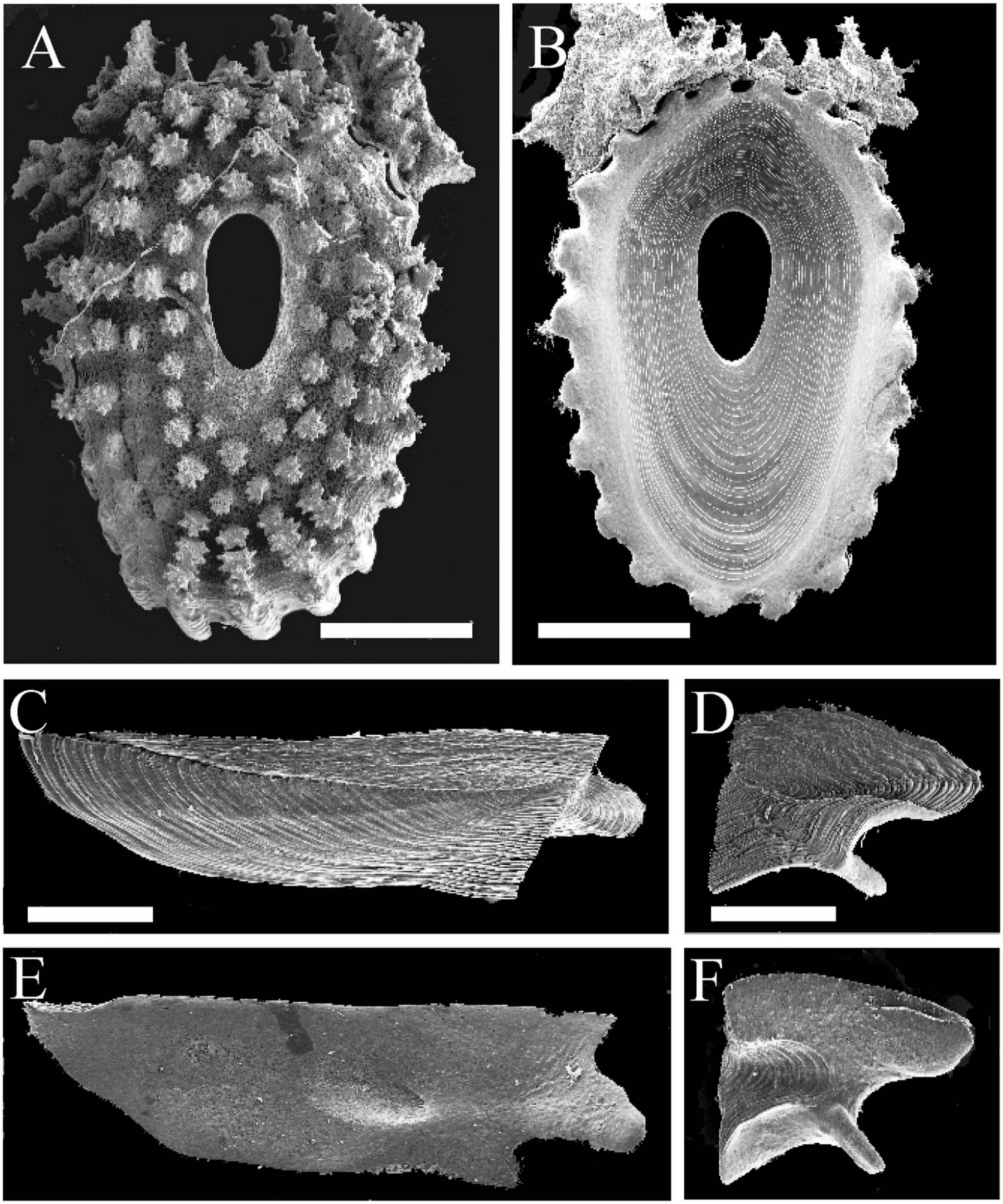

Fig. 17. Hard parts of *Trevethana sarae* n. sp. host coral *Cyphastrea chalcidicum* from the Red Sea. A, shell plate, external view. B, shell plate, inner view. C, scutum, external view. D, tergum, external view. E, scutum, inner view. F, tergum, inner view. Scale bars: = A, B, C, D = 1 mm. Scales of C apply to E and F.

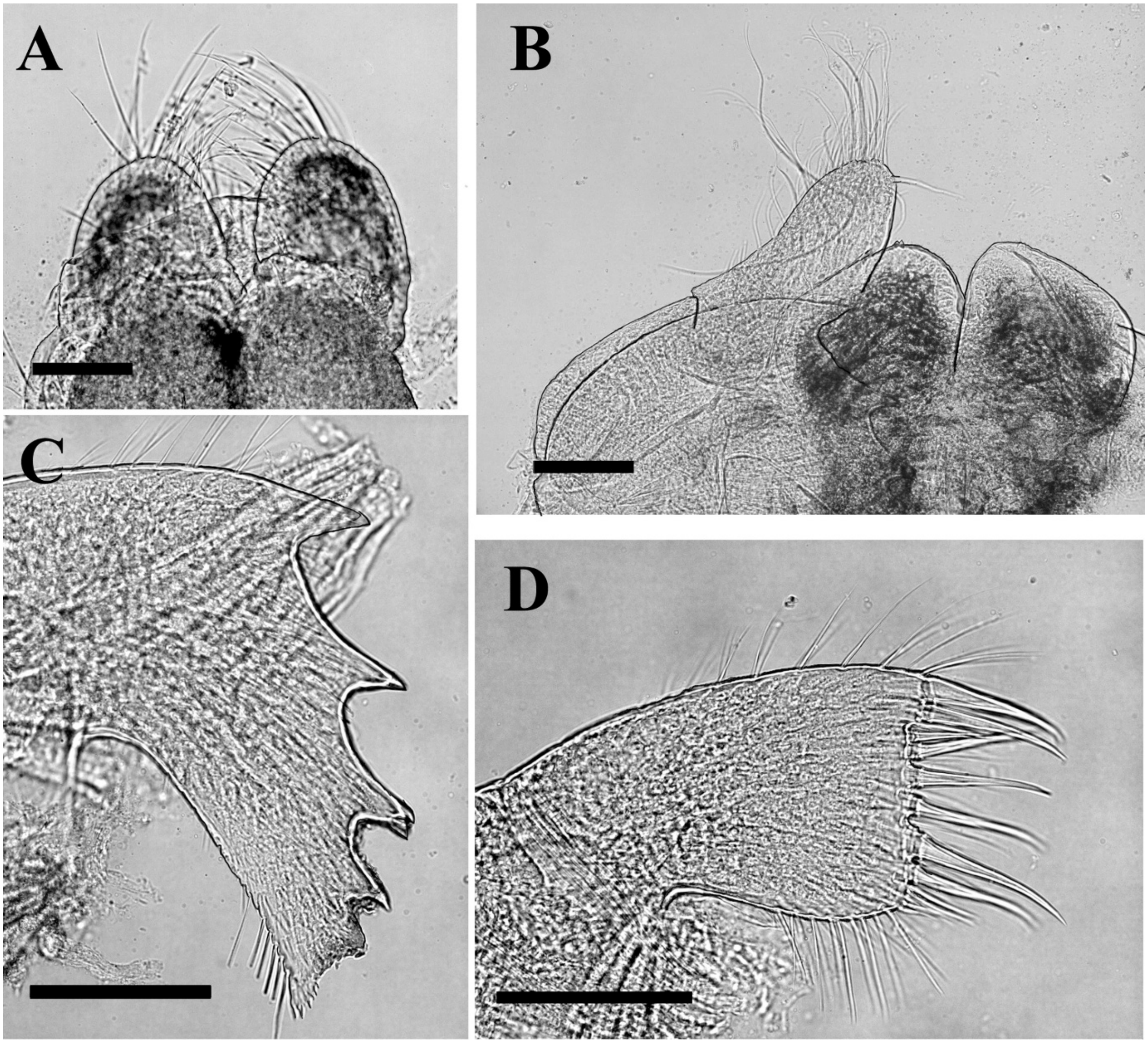

Fig. 18. Micrograph of Trophi of *Trevethana sarae* n. sp. host coral *Cyphastrea chalcidicum* from the Red Sea. A, maxillae II. B, labrum and palp. C, mandibule. D, maxilla I. Scale bars = 100 µm.

scutum and tergum in *Trevathana* (Mokady et al., 1999). Within *Trevathana*, we identified two main subclades; one consists of barnacles extracted from *Cyphastrea* and the other includes barnacles from the other four host corals. These are distributed on subclades according to the genus of the host. The morphological data also support this separation, and the absence of conical spines on cirri II and cirri III is a unique characteristic of *Cyphasrea* barnacles (Table 2).

The origin of the sequences of *Trevathana* from *Favites* is from two congeneric species of host, from two geographical regions, the Red Sea and the Great Barrier Reef (see Appendix 1). In *Cyphastrea* from the Red Sea, we could not differentiate barnacles from two species of corals, *C. chalcidicum* and *C. serialia* (Forskål, 1775). We also extracted three specimens from a colony of unidentified *Cyphastrea* from Seychelles (Fig. 1, marked by C); these form a sub-clade within the *Cyphastrea* clade. It is possible that these barnacles were extracted from a different species of *Cyphastrea*. In *Favites*, we found similar results using *F. abdita* and *F. halicora* (Ehrenberg, 1834), from the Red Sea and the Great Barrier Reef (see Appendix 1). The biogeographic uniformity on one hand and the distinctive specificity to a unique coral genus on the other hand, indicate that divergence and speciation in *Trevathana* is host dependent and not due to geographical separation.

Darwin (1854; p. 369) described three varieties of *P. dentatum*. He noticed that there are variations in the scuta, such as the ratio between length and width, the width of the occludent ledge, the adductor ridge, the scutal tooth, and

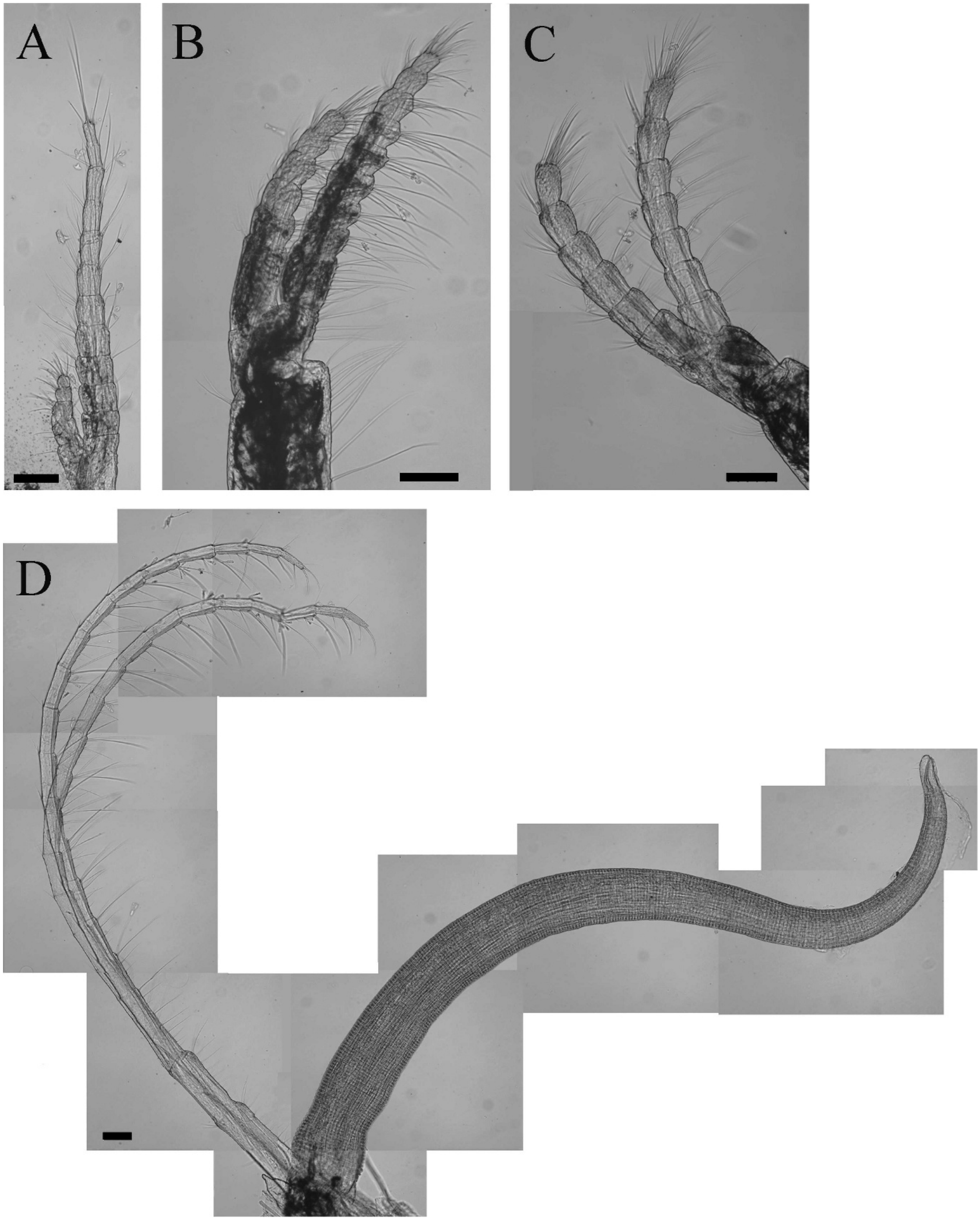

Fig. 19. Micrograph of Cirri of *Trevethana sarae* n. sp. host coral *Cyphastrea chalcidicum* from the Red Sea. A, cirrus I. B, cirrus II. C, cirrus III. D, cirrus VI and penis. Scale bars = 100 μm.

the interlock between the tergum and scutum, but he did not use these parameters as a basis for separation of his varieties. Darwin did not refer to the shape of the shell and to the podial characters, cirri and trophi. His separation of the three varieties is based mainly on the differences in the structure of terga (Fig. 3, depicted after Plate 13; 3a-3g). In the first variety, the spur is hardly developed; the internal tooth is long and sharp. In the second variety there is flexure separating the spur from the basi-carinal angle, the internal tooth is broad and its long axis is parallel to the margins of the spur. In the third variety, there is no sign of a spur and the internal tooth is very small. Referring to the characteristics used by Darwin, differences in the scuta, our material can be assigned to two of his varieties. *Trevathana* from *Leptastrea*, *Cyphastrea* and *Favites* fit into the description of the first variety, while those from *Platygyra* and *Favia* resemble the second variety. This agrees with the classification of Soong and Chang (1983) who noted that in *T. dentata* from *Platygyra* the scutum is more slender than that described by Darwin (1854). Hiro (1935) noted that the tergum of *T. dentate* from *Platygyra* resembles that of Darwin's variety 2. We did not find material that can be assigned to Darwin's third variety. However our findings suggest that in addition to the differences in the form of the scuta and the terga, there are distinct differences in the shape of the shell and the arthropordial characters of *Trevathana* from different genera of host coral. These differences justify the separation of Darwin's original *T. dentata* to several discrete species. We therefore erected four new species that accommodate *Trevathana* from different hosts.

The separation of *S. crenatum* extracted from *Acantastrea* and *Platygyra* into two subclades according to their host coral might indicate that in this species is also a group of cryptic species that are specific to their coral host. However we do not have enough data to support this assumption, and it require material from more host corals.

One of the drawbacks of the study of coral barnacles is the limited availability of suitable material for soft-parts morphological description and for molecular analysis. Most of the information on the taxonomy of Pyrgomatidae was gained from collection from dry corals, which cannot render molecular information or information on the podial characters. *Trevathana* is not limited to the five genera of corals discussed in the present paper. However, the limited range of host corals in which we could examine both the hard parts and the podial characters does not enable us to clarify whether barnacles from other host corals should be assigned to different species of *Trevathana*, or if the same species can be found on a variety of hosts.

A compilation by Ogawa and Matsuzaki (1992) reports additional host genera of *T. dentata*, namely *Goniastrea sp.*, *Madrepora* sp., *Pectinia lactuca* (Pallas, 1766), *Plesiastrea versipora* (Lamarck, 1816) and *Symphyllia* sp. Asami and Yamaguchi (2001) described a new species of *Trevathana*, *T. paulayi* Asami and Yamaguchi, 2001 inhabiting *Acantastrea echinata* (Dana, 1846) from Guam. Paulay and Ross (2003) suggested that *T. paulayi* infests most colonies of *Acanthastrea echinata* on Guam. Ren (1986) described a new species of *Trevathana*, from *Goniastrea sp*., which he named *Savignium orientale* Ren 1986; this species closely resembles *T. dentata*. Achituv and Langsam (2005) described *T. tuariae* Achituv, 2005 from *Goniastrea*, another species of *Trevathana*, *T. niuea* Achituv, 2004 was described from *Goniopora* sp. from Niue Islands (Achituv, 2004). The material used for the description of these new species came from dried corals and the characterization is based only on the calcareous parts of the barnacles, the shell, scuta, and terga, and not on the podial characters. Additional coral genera hosting *T. dentata* were found by us (unpublished) in the coral collections of Naturalis, The Royal Natural History Museum, Leiden, the Netherlands, and in the collection of the Museum National d'Histoire Naturelle, Paris. These genera include *Acantastrea*, *Echinopora*, *Goniastrea*, *Montastrea*, and *Leptoria*. The absence of soft parts of these barnacles and of material suitable for molecular analyses does not allow us to determine whether these barnacles belong to one of the species describe previously or to a new species. In the absence of these components, and until sufficient evidence distinguishing these structures will be obtained, these barnacles will continue to be assigned to one of Darwin's varieties of *T. dentata*.

Unlike many other studies on coral barnacles that are based only on dry material, the present study is based on both soft parts and hard parts; this enables molecular analysis and the examination of podial characters. Our analysis of these characteristics supports the notion that barnacles collected from five host genera show that host specificity in *Trevathana* is determined at the level of genus. Similar material from other host corals is needed to ensure that this specificity is a general trend in *Trevathana*. On the other hand, it should be noted that we cannot reject the option that there may be cases in which different barnacle species occupy the same host genus, or that same barnacle species occupies hosts of two different genera.

Darwin used the barnacles to demonstrate variability within species; the recognition of the presence of varieties within species provided him with means of testing his views on the species question. Many of his varieties were proved later to be valid species (Newman, 1993). In the present paper, we show that there is a solid basis to the idea that Darwin's varieties of coral barnacles and especially those found on different corals are different species, and some might be cryptic species. A consequential result of our study may indicate that the high biodiversity found in reef corals is higher than estimated, and, for the most part, is driven by host-symbiont specificity.

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APPENDIX 1.

GenBank accession numbers of sequences of barnacles from different host corals and localities, and codes for the alignments used for constructing ML presented in Supplementary Material, Appendix 2, DOI: 10.1651/09-3152.1.

APPENDIX 2.

Alignment of DNA sequences used for molecular analysis, DOI: 10.1651/09-3152.1.