CALCAREA FROM THE GREAT BARRIER REEF. 1: CRYPTIC CALCINEA FROM HERON ISLAND AND WISTARI REEF (CAPRICORN-BUNKER GROUP)

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Fourteen species of calcareous sponges (Porifera: Calcarea) of the subclass Calcinea are described from cryptic and semi-cryptic habitats of Heron Island and Wistari Reef, Capricorn-Bunker Group, southern Great Barrier Reef (GBR). Three are new records for the GBR (*Soleniscus stolonifer* (Dendy, 1891); *Leucaltis clathria* Haeckel, 1872; *Leucetta microraphis* Haeckel, 1872), one a new locality record for Australia (*Levinella prolifera* (Dendy, 1913)), and eight are new to science (*Clathrina heronensis*, *C. wistariensis*, *C. adusta*, *C. parva*, *C. helveola*, *C. luteoculcitella*, *Soleniscus radovani* and *Leucetta villosa* n. spp.). In the taxonomically difficult groups *Clathrina* and *Leucetta* differentiation between sibling species is supported by statistically significant differences in actinal lengths of spicules. Stable isotope analysis of spicules from nine species in both subclasses of Calcarea show a clear distinction between Calcinea (negative $\delta^{13}C$ values) and Calcaronea (positive $\delta^{13}C$ values), indicating that different biocalcification processes might take place in each subclass. ❐ *Porifera, Calcarea, Calcinea, Clathrina, Soleniscus, Levinella, Leucaltis, Leucetta, Pericharax, taxonomy, new species, Great Barrier Reef*.

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Calcarea, or calcareous sponges (Phylum Porifera), have an exclusively calcium carbonate spicule skeleton, clearly differentiating them from the other two poriferan classes Demospongiae and Hexactinellida (with spicules composed of silica). Calcareous sponges have persisted since the early Cambrian, with little apparent morphological divergence, and some (the 'Pharetronids') have contributed significantly to reef-building throughout different periods of the Earth's history (Reitner, 1992). Molecular data show that calcareous sponges might be the link between lower and higher metazoan phyla (Ctenophora/Cnidaria) (Lafay et al., 1992; Cavalier-Smith et al., 1996; van de Peer & de Wachter, 1997) and Calcarea show a unique composition in their skeleton and histological features compared to the other classes: only in Calcarea are all three stages of development of the aquiferous system realised (asconoid-syconoid-leuconoid).

Modern Calcarea are now mostly found hidden in cryptic habitats (e.g. caves, overhangs, coral rubble). This might be explained by early divergence in evolutionary strategies between Demospongiae and Calcarea, expressed by a lower level of chemical activity in calcarean secondary metabolites, as compared to demosponges (van Soest & Braekman, 1999). Cryptic habitats provide a large surface area and different environmental conditions within reef systems, and the highly diverse but so far virtually undescribed sponge fauna (Demospongiae, Calcarea) is one of the main constituents of the sessile macro-benthos within this vast cryptic reef system.

Knowledge of Recent Calcarea worldwide is substantially poorer than for other groups of Porifera, given their relatively poor fossil record, their difficult taxonomy and cryptic life style, and that the state of identification for most of calcarean collections is still relatively rudimentary. In contrast to tropical reefs, Calcarea are relatively well known from temperate Australian waters since the late 1800's (e.g. Carter, 1886; Dendy, 1891, 1892a,b), whereas the tropical fauna has only been investigated relatively haphazardly (e.g. Poléjaeff, 1883; Lendenfeld, 1885a,b; Burton, 1934; Pulitzer-Finali, 1982). It is hypothesised that this latter fauna comprises a vast, relatively unexplored resource. Moreover, existing collections of Calcarea were usually obtained by indirect sampling methods (e.g. dredging), with no data on habitat, ecology or community structure. These existing collections are largely badly preserved, and therefore their descriptions often lack any proper documentation or graphical depiction of cellular and

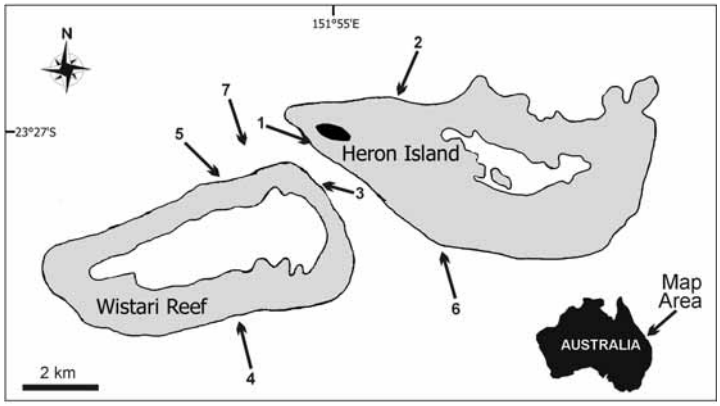

FIG. 1. Sites sampled at Wistari Reef and Heron Island, S GBR. 1, Underside of coral rubble, reef crest of Heron Island at Wistari Channel; 2, Tenements, N side of Heron Island; overhangs under coral bommies; 3, Wistari Reef, E side of Wistari Channel; steep wall with small overhangs; 4, Wistari Reef, S side; small patch reefs with overhangs; 5, Wistari Reef, N side; shallow bommies near reef crest with swim-throughs and overhangs; 6, Heron Island, S side near Wistari Channel; small crevices under bommies; 7, 'The Patch', between Heron Island and Wistari Reef, N of Wistari Channel, overhangs in cemented coral rubble.

skeletal features. These shortcomings are pertinent to our present understanding of calcarean biodiversity throughout Australasia.

Taxonomy of Calcarea is based on a combination of characters obtained from detailed studies of growth form, structure of the aquiferous system, architecture of soft tissue, larval development, cytological characters (e.g. position of the nucleus in choanocytes), morphology and association of calcareous spicules, and their specific arrangement within the soft tissue — features which are difficult or impossible to discern in poorly preserved collections. This makes the examination and documentation of contemporary samples, using a variety of methods, mandatory (e.g. spicule preparations, histological sections, ultra-thin sections for TEM), involving several different procedures for fixation, subsequent preservation, post-fixation, and histological preparation for examination in the laboratory. In this project emphasis was placed on specialised fixation techniques using a variety of methods, to allow subsequent multidisciplinary studies (e.g. light microscopy, SEM, TEM, molecular genetics, biogeochemistry, geochemistry). This present contribution provides a careful and detailed initial taxonomic investigation of GBR Calcarea, as a sound basis for further detailed studies.

This publication is the first comprehensive taxonomic study of Calcarea in Australasia in over 50 years. It documents calcarean biodiversity from cryptic and non-cryptic habitats of the southern Great Barrier Reef. So far only 14 species have been described for the entire GBR (see Hooper & Wiedenmayer, 1994). By comparison, there are now over 1,500 species of Demospongiae collected from the GBR, housed in the collections of the Queensland Museum (Hooper, pers. com.), of which only 428 have been described so far in scientific literature (Hooper & Wiedenmayer, 1994). Present results demonstrate that calcinean Calcarea are highly diverse in cryptic- and semi-cryptic habitats of the S GBR, with 70% of species collected new to science. A second contribution will describe the Calcaronea from this region, with subsequent publications documenting Calcarea from the northern and central sections of the GBR (Wörheide, in prep.).

Since the early works of Haeckel (1872) and Dendy & Row (1913), the supra-specific classification of Calcarea has been a topic of continuous discussion (e.g. Hartman, 1958, 1982; Burton, 1963; Borojévic et al., 1990, in press). The present study follows the supra-specific classification of Borojévic et al. (1990) for Calcinea.

MATERIAL AND METHODS

COLLECTION. Samples of Calcarea were collected from Heron Island and Wistari Reef, S GBR (Fig. 1), from a variety of habitats, using SCUBA, under GBRMPA Permit nos. G98/142 and G98/022.

For each sample collected the depth and habitat were recorded using protocols developed by Wörheide (1998) (Fig. 2). Where possible, underwater photographs of all samples were taken, although this was not always feasible given that most cryptic Calcarea are very small and dwell in small crevices and other cryptic habitats where photography is not possible. Consequently, samples were also photographed

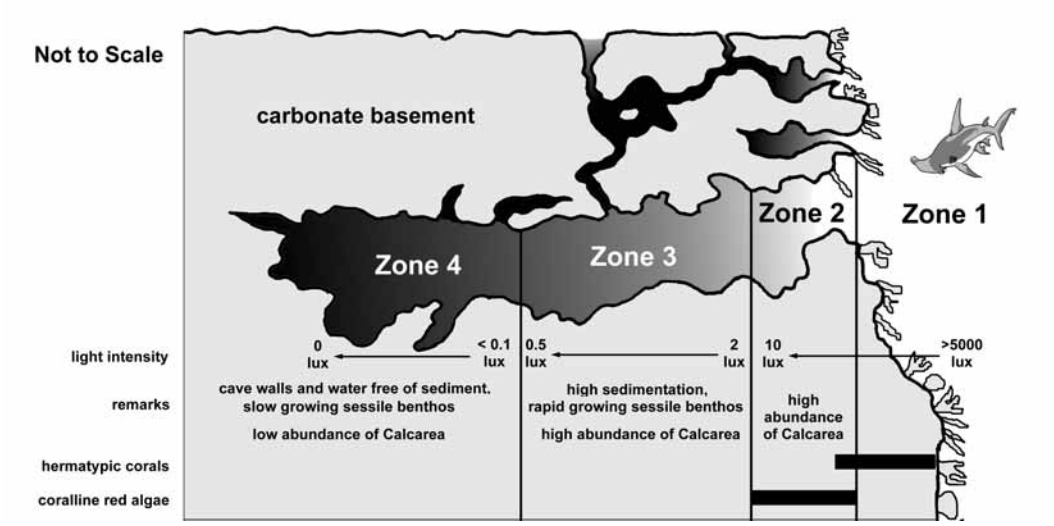

FIG. 2. Schematic model of reef cave zonation, noting the occurrence of Calcarea, habitat characteristics, light regimes, and associated biota (modified from Wörheide, 1998).

post-fixation at the Queensland Museum using a macro-lens camera set-up.

FIXATION OF SPECIMENS. Specimens were fixed shortly after collection, using a variety of methods (depending on purpose of study). In addition to fixation for routine taxonomic identification (see below), subsamples of each specimen were fixed and/or preserved for molecular biology (DNA analysis; Wörheide, 1998) and electron microscopy (TEM/SEM) (Willenz & Hartman, 1989; Wörheide, 1998).

Fixation for histological thick sections and spicule preparations. Samples were fixed for 24hrs in a 4% Glutaraldehyde/sterile filtered seawater solution, buffered with 0.2M sodium cacodylate, supplemented with 0.35M sucrose. After fixation, samples were rinsed three times with unbuffered sterile filtered seawater. Samples were then transferred through a graded EtOH-series and stored in 70% EtOH. At least one large piece of tissue from all specimens collected was treated in this way to ensure proper fixation for subsequent staining, essential to make tissue sections for routine taxonomic identification.

Two standard preparations were applied to each sample for taxonomic identification.

HISTOLOGY. Detailed methods are presented here as these protocols have not been published previously.

Spicule preparations. Descriptions of spicules were made from different regions of the sponge body (e.g. osculum, cortex, atrium), in addition to skeletal architecture, spicule dimensions, and associations between spicules from different regions of the body.

For spicule preparations, small pieces of soft tissue were cut and dissolved in sodium hypochlorite. After complete digestion of the soft tissue the solution was drained and the supernatant washed three times each in distilled water and absolute ethanol. Spicules were then transferred onto microscopic slides and embedded in Canada Balsam. Unused portions of spicule preparations were kept in absolute alcohol for subsequent bio-/geochemical investigations (e.g. stable isotopes, see below).

Sections of sponge tissue. It is mandatory to prepare good anatomical sections of soft tissue and spicules, as Calcarea often show a complex differential distribution of skeletal structures between the cortex, choanosome and/ or central atrial tube. Tissue was stained using acid Fuchsin, and hand sections were made using a scalpel after embedding one or more fragments of sponge in paraffin (after Borojevic, pers. com.). Good sections were obtained mostly in cross-section, allowing examination of skeletal organisation of the cortical, choanosomal and atrial regions. Sections were made at variable thickness depending on the species under examination and the size of their spicules.

Thinner sections are desirable in order to investigate the arrangement of choanocytes within the choanosome, and to recognise details of spicule distributions, whereas thicker sections are essential to reveal relationships among different spicule elements within the sponge wall.

The following protocol was used to prepare sections of the sponge body. Samples were kept in 70% ethanol prior to sectioning. Selected parts of the sponge body containing cortical, choanosomal, and atrial regions (if present), were cut using a scalpel prior to dehydrating. Preparation times given below apply to tissue blocks of 1cm³ maximum volume, whereas most tissue samples were smaller than this.

Dehydrating and staining. Tissue was stained in a saturated solution of acid Fuchsin in 70% EtOH for 60mins; washed three times in 70% EtOH (30mins each); transferred to 90% EtOH (30mins); and two changes of absolute EtOH (30mins each).

Clearing. Tissue was transferred two times into xylene (30mins each).

Infiltration. Tissue was transferred to a mixture of xylene and melted paraffin wax (1:1) in an oven (60°C) for 60mins and two changes of melted paraffin wax in an oven (60°C) (60mins each).

Embedding. Selected moulds were lightly coated with glycerol, enabling solidified paraffin blocks to be easily removed, and moulds filled with molten paraffin wax; tissue placed in wax using warmed forceps and arranged in the correct plane for sectioning (preferably in cross-section allowing cutting of the cortex, choanosome and atrium); when the wax block was partially set (i.e. with a thin scum appearing over the surface of the block), blocks were placed in the refrigerator to ensure rapid cooling and to prevent crystallisation of the wax.

Sectioning. After paraffin had hardened, blocks were released from the mould and trimmed with a scalpel to the desired size and cutting plane; sections of different thickness were cut by hand with a scalpel.

De-paraffinisation. Sections were placed on a microscope slide on a hot plate to allow the paraffin to partially melt again, and thus flattening sections (sections normally curl after sectioning). Excess paraffin was soaked up with paper tissue. Slides were removed from the hot plate to cool and re-harden. Slides were de-paraffinised in two changes of xylene (15mins each).

Mounting. After all paraffin was removed, sections were mounted using Durcupan embedding resin (ACM Fluka) and covered with a cover-glass.

Spicule preparations and tissue sections were examined using an Olympus BH-2 Stereomicroscope equipped with a Panasonic WV-CP410 digital camera and a Snappy video grabber (Play Inc.), providing digital photographs with a resolution of up to 300dpi, a maximum size of 1500 ×1125 pixels, and 24-bit color depth. Measurements were made using a calibrated scale and camera lucida and are presented as range of minimum-(mean)-maximum length × minimum-(mean)-maximum width, in µm (n=30, or as indicated). Statistical analyses were performed using 'Origin 3.0' (MicroCal).

All type material was deposited in the collections of the Queensland Museum, Brisbane. Abbreviations: AM, Australian Museum, Sydney; BMNH, The Natural History Museum, London;GBR, Great Barrier Reef, Qld., Australia; NMV, Museum of Victoria, Melbourne; PMJ, Phyletisches Museum, Jena; Qld., Queensland; QM, Queensland Museum, Brisbane.

STABLE ISOTOPE ANALYSIS OF CALCAREAN SPICULES. Small numbers (100-150µg) of spicules from unused portions of spicule preparations were used as bulk samples for analysis of $\delta^{18}O$ and $\delta^{13}C$ values. Measurements were made on a Finnigan MAT 252 with automated Kiel carbonate device using '100%' phosphoric acid at 75°C. Data were corrected for ^{17}O interference using the method of Santrock et al. (1985) and are reported in permille relative to PDB (Peedee belemnite).

RESULTS

FIELD OBSERVATIONS. Abundance and diversity of Calcarea from cryptic habitats around Heron Island and Wistari Reef was surprisingly high given the few species recorded so far in the literature. Although these reefs contain no known deep caves (i.e. with restricted light regimes, and thus containing a distinct suite of fauna), a large number of specimens were collected from semi-cryptic habitats such as overhangs, small crevices, and swim-throughs (Zones 1-3; Fig. 2). Patchy species distributions were notable, with some species abundant at certain sites but absent elsewhere on the reef. For example, *Soleniscus radovani* sp. nov. was only found at one site on the S side of Wistari Reef,

where it was the most dominant calcareous sponge, but so far not recorded at any of the other sites. Whether these patchy distributions are the result of species adaptations to specialised niche requirements, or a random effect due to haphazard larval settlement etc., cannot be resolved from present data. Generally, however, small and fragile Calcarea appear to be most common in semi-dark/dark and sheltered habitats, whereas more massive species, such as *Pericharax heteroraphis* and *Leucetta chagosensis*, are more abundant in fully illuminated parts of the reef.

Similar patchy species distributions were observed on reefs around Lizard I. and the outer barrier reefs (Ribbon Reef #10, Yonge Reef), with different species dominant at different sites. In this region of the GBR there are several large caves with a distinct zonation of species related to light regimes. Although our data are still preliminary, the calcarean fauna of the N GBR, around Lizard I., differs from the fauna of the S GBR, around Heron I. (Wörheide, unpublished data). This observation supports the findings of Hooper et al. (1999), that the GBR demosponge fauna has distinct latitudinal gradients in species diversity and composition, although more substantial taxonomic studies are required to substantiate this claim for Calcarea.

SYSTEMATICS

Fourteen species of Calcarea (Subclass Calcinea, Order Clathrinida) are described here, from five families and six genera (following the scheme of Borojévic et al., 1990), of which eight are new species, three are new records for the GBR and one new for the Australian fauna. Diagnoses of Calcinea higher taxa follow Borojevic et al. (1990) and Borojevic & Klautau (in press).

Class **Calcarea** Bowerbank, 1864

Exclusively marine Porifera in which the mineral skeleton is composed entirely of calcium carbonate. Spicules are diactines, triactines and tetractines. Calcarea are always viviparous.

Subclass **Calcinea** Bidder, 1898

Calcarea with regular (equiangular and equiactinal), or exceptionally parasagittal or sagittal triactines, and a basal system of tetractines. In terms of ontogeny, triactines are the first spicules to be secreted. Choanocytes are basinucleate with spherical nuclei. The basal body of the flagellum is not adjacent to the nucleus. Calcinea incubate coeloblastula larvae.

Order **Clathrinida** Hartman, 1958

Calcinea with skeleton composed exclusively of free spicules, without hypercalcified non-spicular tracts, calcareous scales, or plates.

Family **Clathrinidae** Minchin, 1900

Clathrinidae with an essentially tubular organisation. A continuous choanoderm lines all the internal cavities. Growth is by longitudinal median divisions and anastomosis of tubes to form large units, called the cormus. There is neither a common cortex nor a well-defined inhalant or exhalant aquiferous system.

Clathrina Gray, 1867

Clathrina Gray, 1867: 557.
Ascetta Haeckel, 1872: 14; Dendy & Row, 1913: 788.
Ascaltis Haeckel, 1872: 51; Dendy & Row, 1913: 787.
Leucopsis Lendenfeld, 1885b: 1089.

Clathrinidae in which the choanoderm is flat, or rarely raised into conuli, by the apical actines of large tetractines, but never forming true folds, at least when the sponge is in the extended state. The cormus is composed of anastomosed tubes. The skeleton contains regular, equiangular and equiradiate triactines and/or tetractines, to which diactines or tripods may be added.

Clathrina heronensis sp. nov.
(Fig. 3A-F, Table 1)

ETYMOLOGY. For the type locality.

MATERIAL. HOLOTYPE: QMG313647, Heron I., at Wistari Channel, GBR, 23°26.9'S, 151°54.6'E, opposite Research Station, 300m S of shipping channel, at reef crest, 21.vi.1998, coll. G. Wörheide (reef-walking).

HABITAT AND DISTRIBUTION. Under rubble on reef crest, intertidal. Heron I., S GBR.

DESCRIPTION. *Growth form*. Mass of loosely anastomosing tubes, approximately 1mm diameter, with fairly large spaces between tubes, cormus 3×2cm, flat.

Colour. White in life, brownish after fixation with Glutaraldehyde in EtOH.

Oscules. Macroscopically not visible; no distinct exhalant system visible.

Texture. Soft, compressible, delicate.

Surface ornamentation. Smooth.

Ectosomal skeleton. No ectosome/cortex

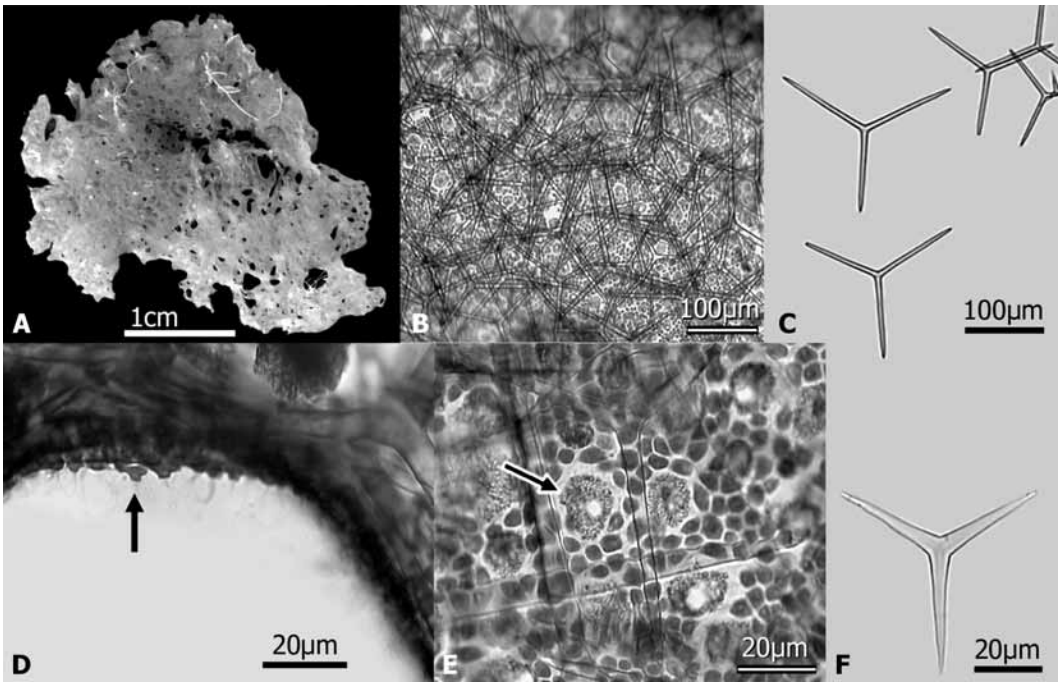

FIG. 3. *Clathrina heronensis* sp. nov. (holotype QMG313647). A, holotype after fixation. B, wall of a tube with irregularly arranged triactines. C, cylindrical triactines. D, cross-section of one tube showing choanocytes (arrow) continuously lining the tube wall. E, magnification of tube wall with ostiae (in porocytes, arrow) and surrounding choanocytes. F, small, actively growing triactine with conical actines.

distinguishable.

Choanosomal skeleton. Irregular layer of triactines, with a mean thickness of 20-30µm. Triactines are tangentially orientated and their actines sometimes overlap, so that a few triactines (mostly not more than three) are stacked above each other. Sometimes the actines of larger triactines are aligned in a parallel pattern (one actine of one triactine is parallel to one actine of a second triactine), but mostly they are arranged irregularly, and are densely packed next to each other. No differentiation or zonation in the skeleton was observed, and the skeleton appears to be uniform throughout the cormus.

Aquiferous system. No defined inhalant or exhalant system is present. The water system has an asconoid grade of construction, with choanocytes continuously lining the internal walls of the tubes.

Soft tissue. The choanoderm has a dense appearance. Choanocytes continuously line the walls of tubes in 'circles' with porocytes in the center. Porocytes contain many granules with a brownish colour (in Fuchsin-stained sections), densely arranged around the small ostiae (1-3µm diameter). Choanocytes have a diameter of 2-3µm and a triangular to hexagonal shape in section parallel to the surface, and 2-5 are located between the porocytes (Fig. 3E). Porocytes have a diameter of 12-20µm in section parallel to the surface.

Spicules. The spicular skeleton consists of only one type of triactines, with no differentiation and/or zonation of spicules in different parts of the skeleton observed. Regular cylindrical triactines possess a more-or-less blunt tip with their actines measuring 84-(107)-126×8-(10)-12µm. A few pointed triactines, with more conical actines (30-70µm long), occur irregularly scattered throughout the skeleton, representing young, growing spicules. See Table 1 for statistics on triactine dimensions.

Clathrina wistariensis sp. nov.
(Fig. 4A-C, Table 1)

ETYMOLOGY. For the type locality.

MATERIAL. HOLOTYPE:. QMG313663, S side of

Wistari Reef, GBR, 23°29.4'S, 151°52.8'E, small reef patches, 18m depth, 07.vii.1998, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. Under overhangs of small coral patches, Zone 2 (Fig. 2), 18m. Wistari Reef, S GBR.

DESCRIPTION. *Growth form*. Dense mass of anastomosing tubes, 1-2mm diameter, no large space between tubes, cormus 2×3cm, flat, attached to a calcareous hydrozoan.

Colour. White in life, brownish after fixation with Glutaraldehyde in EtOH.

Oscules. No oscules visible macroscopically.

Texture. Soft, easily torn.

Surface ornamentation. Smooth.

Ectosomal skeleton. Not distinguishable.

Choanosomal skeleton. Irregular layer of triactines, less than 20µm thick. Triactines are tangentially orientated, but not densely packed, with some space between their actines. Their actines sometimes overlap, so that a few triactines (mostly not more than three) are stacked above each other. Only occasionally are actines of larger triactines aligned in a parallel pattern (one actine of one triactine is parallel to one actine of a second triactine). Mostly they are irregularly arranged. No differentiation or zonation in the skeleton was observed, which appears to be predominantly uniform throughout the cormus.

Aquiferous system. No defined inhalant or exhalant system is present. The water system has an asconoid grade of construction.

Soft tissue. The choanoderm is light in appearance. Choanocytes continuously line the walls of tubes with porocytes in between. Sometimes there is a 'circular' arrangement of choanocytes around the porocytes, but more often choanocytes are clustered together. Porocytes contain few small granules with a brownish colour (in Fuchsin-stained sections), densely arranged around the large ostiae (up to 48µm diameter), in one layer only. Porocytes are up to 50µm diameter (with only one layer of granules), in section parallel to the surface. Granule-containing cells (size of 15-20µm diameter), which are either contracted porocytes or storage cells, are scattered in the choanoderm. Choanocytes have a mean diameter of 2µm and have predominantly a circular shape in section parallel to the surface.

Spicules. The spicular skeleton consists of only one type of triactine, with no differentiation and/or zonation of spicules between different parts of the skeleton. The majority of triactines (actines: 150-(175.16)-230×10-(13.7)-20µm), are regular and possess cylindrical actines with a more-or-less blunt tip; few triactines are slightly parasagittal (angles between actines are equal, but one actine is slightly shorter). Smaller triactines, with more conical actines, are also found, representing young, growing triactines. See Table 1 for statistics on triactine dimensions.

Clathrina adusta sp. nov.
(Fig. 4D-H; Table 1)

ETYMOLOGY. For the post-fixation brown coloration (Latin, *adustus*).

MATERIAL. HOLOTYPE: QMG313665: S side of Wistari Reef, GBR, 23°29.4'S, 151°52.8'E, 18m depth, 07.vii.1998, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. Under overhangs of small coral patches, 18m depth, Zone 2 (Fig. 2). Specimen was overgrowing a calcareous hydrozoan. Wistari Reef, S GBR.

DESCRIPTION. *Growth form*. Dense mass of anastomosing tubes with small tubes (0.5mm diameter). Tubes fused together to form fewer, larger (excurrent) tubes (2-3mm diameter), which end in a terminal osculum. The cormus measures 2×2cm.

Colour. White in life, dark brown after fixation in Glutaraldehyde in EtOH.

Oscules. Few, on top of larger excurrent tubes.

Texture. Relatively firm, harsh.

Surface ornamentation. Smooth.

Ectosomal skeleton. Not differentiated.

Choanosomal skeleton. Sustained by a densely packed irregular layer of tangential triactines and tangentially orientated basal plane of tetractines (same size as triactines), forming the wall of tubes. Apical free actines of the tetractines protrude free into tubes. Actines of both triactines and the basal plane of tetractines are arranged without any order, although sometimes actines are aligned in parallel, but this seems to be random. Actines overlap so that few (not more than five) are stacked above each other. The tube walls have a thickness of up to 40µm. Tetractines are concentrated mostly in the walls of the larger (excurrent) tubes, with fewer present in the walls of smaller tubes.

Aquiferous system. With an asconoid grade of construction, where choanocytes continuously line the internal wall of tubes. Slightly larger excurrent canals do not represent a true atrium,

but are sometimes devoid of choanocytes in their distal parts (showing here only spherical cells with granules).

Soft tissue. The choanoderm appears dense. Choanocytes continuously line the walls of tubes, except in some of the distal parts of the larger excurrent canals. Choanocytes are arranged without any apparent order, sub-spherical and small, with a diameter of about 2µm in section parallel to the surface. Larger, brownish cells with granules are far more obvious, up to 15µm diameter. These cells occur in large numbers in all parts of the soft tissue, and are sometimes, in the distal parts of the larger tubes, the only visible cell type present. Whether these cells are contracted porocytes (no ostiae were visible in sections), a type of storage cell, or an early larval stage, cannot be determined with certainty without higher resolution microscopy.

Spicules. The major part of the skeleton consists of regular triactines with more-or-less cylindrical actines, with a size of 90-(108.13)-142× 12-(14.3)-20µm. The distal part of their actines is sometimes slightly undulated. A few tetractines are present, more abundant in the walls of the larger tubes. These tetractines possess a basal system of the same size as the triactines, with their reduced (thinner and shorter) free apical actine protruding into water canals. Neither triactines nor tetractines are arranged in any apparent order in any part of the skeleton. See Table 1 for statistics on triactine dimensions.

Clathrina parva sp. nov.
(Fig. 4I-K, Table 1)

ETYMOLOGY. For the small size of this species (Latin, *parvus*, little).

MATERIAL. HOLOTYPE: QMG313666, S side of Wistari Reef, GBR, 23°29.4'S, 151°52.8'E, 18m depth, 07.vii.1998, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. Under overhangs of small coral patches, 18m depth, Zone 2 (Fig. 2), growing on a bryozoan. Wistari Reef, S GBR.

DESCRIPTION. *Growth form.* Small anastomosing tubes, with cormus <1×1cm in size.

Colour. White in life and after fixation in Glutaraldehyde in EtOH.

Oscules. A few prominent naked oscules are visible on the top of fused tubes.

Texture. Soft.

Surface ornamentation. Smooth.

Ectosomal skeleton. Not present.

Choanosomal skeleton. Irregular layer of triactines, about 15µm thick. Triactines are orientated tangentially, but not densely packed, with some space between the actines. Actines sometimes overlap, so that a few triactines (mostly not more than three) are stacked above each other. Only occasionally are actines of the larger triactines arranged in a parallel pattern (one actine of one triactine is aligned parallel to one actine of a second triactine), whereas mostly they are irregularly arranged. No differentiation or zonation in the skeleton was observed, which appears to be uniform throughout the cormus.

Aquiferous system. No well-defined inhalant or exhalant system present. The water system has an asconoid grade of construction, with choanocytes continuously lining the internal walls of tubes.

Soft tissue. The choanoderm is light in appearance. Choanocytes continuously line the walls of tubes, with porocytes in between, but are randomly arranged around porocytes, not in circles. Porocytes contain few small granules with a brownish colour (in Fuchsin-stained sections), densely arranged around ostiae (up to 15µm diameter), in only one layer. Porocytes are up to 25µm diameter (with a few layers of granules) in section parallel to the surface. Choanocytes have a mean diameter of 2-3µm and are predominantly circular in shape, parallel to the surface.

Spicules. The spicular skeleton consists of regular cylindrical triactines (actines: 80-(143)-185×10-(13.7)-20µm), with a more-or-less blunt tip, arranged without any apparent order. No zonation and/or differentiation was observed within the cormus. Some triactines are parasagittal (angles between actines equal, but one actine slightly shorter). Many smaller triactines, with more conical actines, were also found, representing young, growing triactines. See Table 1 for statistics on triactine dimensions.

Clathrina helveola sp. nov.
(Fig. 4L-N, Table 1)

ETYMOLOGY. For the pale yellow live coloration (Latin, *helveolus*).

MATERIAL. HOLOTYPE: QMG313680, S side of Heron I., GBR, 23°28.2'S, 151°56.7'E, 17m depth, 08.vii.1998, coll. G. Wörheide (SCUBA). PARATYPE: QMG313805, same locality.

HABITAT AND DISTRIBUTION. Overhangs, under coral bommies, Zone 2 (Fig. 2), 17m depth. Heron I., S GBR.

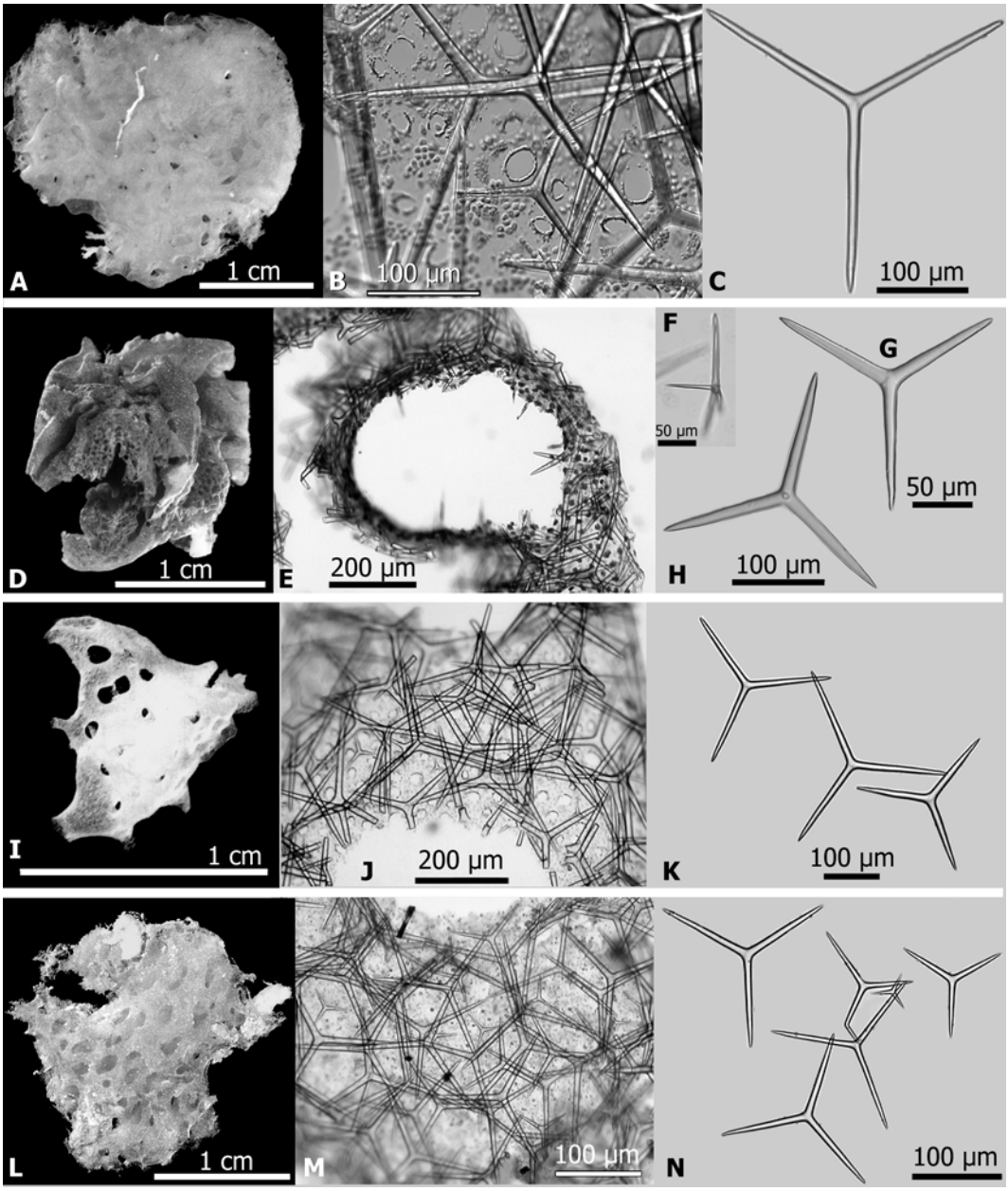

FIG. 4. *Clathrina* spp. A-C, *Clathrina wistariensis* sp. nov. (holotype QMG313663). A, holotype after fixation. B, section of tube wall. C, cylindrical triactine. D-H, *Clathrina adusta* sp. nov. (holotype QMG313665). D, holotype after fixation. E, cross section of tube wall with free actines of tetractines protruding into the tube. F, tetractine showing reduced free actine. G, triactine. H, basal system of tetractine. I-K, *Clathrina parva* sp. nov. (holotype QMG313666). I, holotype after fixation. J, section of tube wall. K, cylindrical triactines. L-N, *Clathrina helveola* sp. nov. (holotype QMG313680). L, holotype after fixation. M, section of tube wall. N, cylindrical triactines.

TABLE 1. Statistical comparisons between common forms of triactines in *Clathrina heronensis*, *C. wistariensis*, *C. adusta*, *C. parva*, *C. helveola* and *C. luteoculcitella* n.spp. Measurements in µm (n=30).

	C. heronensis		*C. wistariensis*		*C. adusta*		*C. parva*		*C. helveola*		*C. luteoculcitella*	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
Mean	107	10	175.16	13.70	108.13	14.13	143	13.7	159.6	16.3	77.7	9.4
St Dev.	12.1	1.39	16.48	2.83	14.26	2.03	21	2.23	20.9	1.8	4.5	1.2
Min.	84	8	150	10.00	90	12	80	10	114	14	68	8
Max.	126	12	230	20.00	142	20	185	20	200	20	84	12

DESCRIPTION. *Growth form*. Mass of anastomosing tubes, each about 1mm diameter; dense cormus with some space between tubes (<2mm), cushion shaped, 2×3cm.

Colour. Yellowish in life, beige after fixation in Glutaraldehyde and in EtOH.

Oscules. None visible macroscopically.

Texture. Soft.

Surface ornamentation. Smooth.

Ectosomal skeleton. Not present.

Choanosomal skeleton. Tangential layer of triactines, irregularly orientated, forming walls of tubes. No differentiation and/or zonation was observed within the skeleton, with the skeleton uniform throughout the cormus. Actines of the tangentially orientated triactines sometimes overlap, so that a few triactines appear to be stacked above each other, but not more than five. Tube walls are 20-40µm thick. Only rarely are the actines of triactines aligned parallel to each other, in which case they form sub-hexagonal to hexagonal patterns. Triactines are not densely arranged in the tube wall.

Aquiferous system. The water system has an asconoid grade of construction, with choanocytes continuously lining internal walls of tubes. No well-defined inhalant or exhalant system present.

Soft tissue. The choanoderm is light in appearance, with cells appearing to be loosely arranged. Small choanocytes (3-4µm diameter), continuously line walls of tubes, without any apparent order, and only a few porocytes are present. Small spherical, sometimes granular, cells or bodies, twice the size of choanocytes, are scattered in large numbers in the soft tissue. Determination of the true nature of these cellular bodies requires TEM examination (in progress).

Spicules. The spicular skeleton consists only of one type of regular cylindrical triactines (actines: 114-(159.6)-200×14-(16.3)-20µm), with no zonation and/or differentiation within the cormus observed. Actines of the triactines are sometimes slightly undulated in their distal part and some possess blunt tips. A few triactines show a bent tip on one of their actines (Fig. 4N). Numerous smaller triactines, with more conical actines and sharp, pointed tips, are irregularly scattered between larger triactines. These small triactines are young, growing spicules.

Clathrina luteoculcitella sp. nov.
(Fig. 5A-E, Table 1)

ETYMOLOGY. For the yellow color and pillow-like shape of the cormus (Latin, *luteus*, yellow; *culcitella*, pillow).

MATERIAL. HOLOTYPE: QMG313684, 'The Patch', at the N end of the channel between Heron I. and Wistari Reef, GBR, 23°26.6'S, 151°53.4'E, 25m depth, 09.vii.1998, coll. G. Wörheide (SCUBA). PARATYPE: QMG313806, same locality.

HABITAT AND DISTRIBUTION. Overhangs in boulders of cemented coral rubble, Zone 3 (Fig. 3), 25m depth. Heron I., S GBR.

DESCRIPTION. *Growth form*. Dense mass of small anastomosing tubes, each with diameter of about 0.5mm, cushion shaped, with only little space between the tubes (<1mm).

Colour. Yellow in life, yellow-beige after fixation in Glutaraldehyde in EtOH.

Oscules. No oscules visible macroscopically.

Texture. Soft.

Surface ornamentation. Smooth.

Ectosomal skeleton. No defined ectosomal skeleton, but diactines are found in some areas perpendicular to, and protruding through, the walls of tubes.

Choanosomal skeleton. Irregular tangential layer of triactines, build the walls of tubes. Tube walls are 20-30µm thick. Triactines are not really densely packed but do overlap with their actines, so that not more than five triactines are partially stacked above each other to form the tube walls. No differentiation and/or zonation of triactines was observed. Diactines are present in some areas of the cormus, perpendicular to the tube

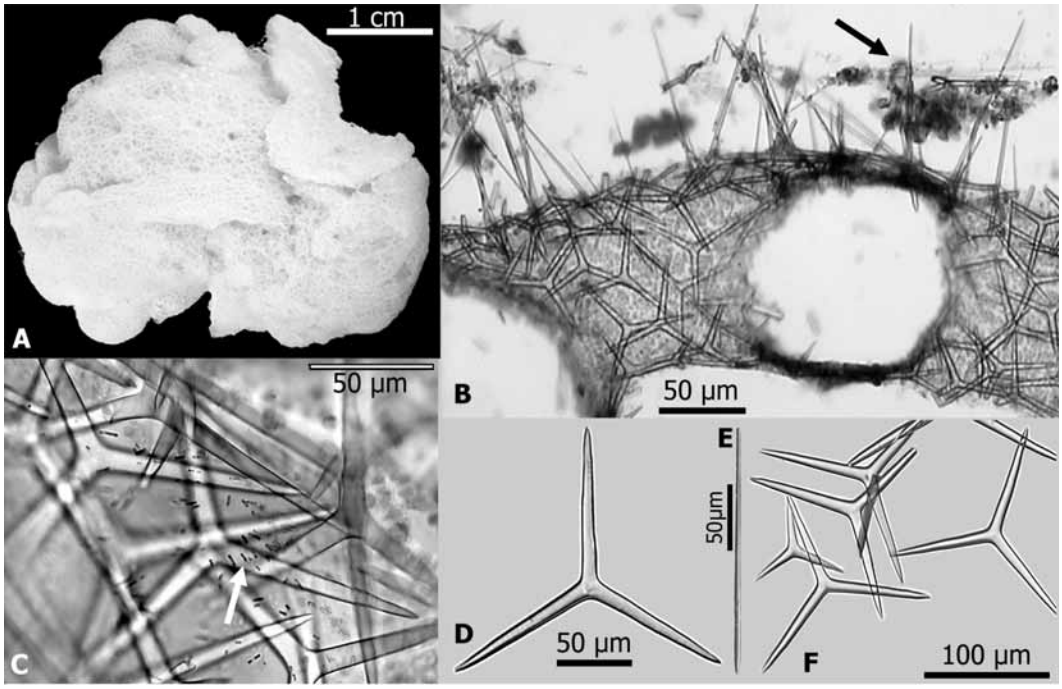

FIG. 5. *Clathrina luteoculcitella* sp. nov. (Holotype QMG313684). A, holotype after fixation. B, cross-section through tube wall with perpendicular diactines (arrow). C, section of tube wall with irregularly arranged triactines and bacteria in the tissue (arrow). D, cylindrical triactine with blunt tips. E, diactine. F, two sizes of triactines.

wall. These diactines protrude through the tube wall with their thicker proximal bases slightly protruding into the tube. Their sharply pointed, thinner distal end sometimes baffles and holds fine grained sediment (Fig. 5B). Diactines are numerous at the external surface of the cormus, but are also found in some areas within the cormus, on the external surfaces of larger tubes, although no distinct pattern of localisation was recognised.

Aquiferous system. With an asconoid grade of construction, with choanocytes continuously lining all internal tube walls. No defined inhalant or exhalant system present.

Soft tissue. The choanoderm has a dense appearance. Subspherical choanocytes, 3-4µm diameter, are arranged without any order or pattern side-by-side, with only few gaps between. Neither porocytes nor granular cells were observed, but rod-like bacteria (possibly cyanobacteria) are rarely present in the soft tissue (Fig. 5C).

Spicules. The spicular skeleton consists of regular, cylindrical triactines of a single size (actines: 68-(77.7)-84×8-(9.4)-12µm), with distal end of actines slightly undulated (Fig. 5D), and diactines (90-(164.4)-220×2-(3.12)-6µm). Diactines have one thin, sharply pointed end (distal) and one thicker, blunt end (proximal). Additionally, a few smaller, conical triactines were found, representing young, growing spicules. No zonation and/or differentiation of triactines was observed. Diactines are concentrated in the external surface but are also found within cavities of the cormus on the external surfaces of tubes.

REMARKS ON *CLATHRINA*

Classification of *Clathrina* is very difficult (Borojevic & Boury-Esnault, 1987), given that most species have only few morphological characters useful for taxonomy (e.g. only a single type of triactine). Studies on Atlantic populations of *Clathrina*, using biochemical and molecular approaches (Solé-Cava et al., 1991; Klautau et al., 1994), showed that very small morphological differences correspond to quite large genetic differences, representing genetically distinct

species. These authors showed that complete genetic separation of sympatric and allopatric populations of *Clathrina* was linked to very small or sometimes undetectable differences in 'classical' taxonomic characters. Taxonomy, based exclusively on these 'classical' morphological characters, appears to be overconservative. Many 'regional species' or populations previously classified as being simple varieties of so-called 'cosmopolitan species', therefore most likely represent distinct taxa. In contrast, Wörheide (1998) demonstrated using molecular approaches (RFLP of rDNA), that geometric differences in spicule morphology of the reef cave-dwelling, ultraconservative, coralline demosponge *Astrosclera willeyana* (with the genus *Astrosclera* present at least since the Triassic; Wörheide, 1998), do not appear to correspond to a genetic separation of allopatric populations across the Indo-Pacific (Red Sea to Fiji). Whether this observation is also valid for other (modern) demosponge or calcarean sponge groups has yet to be tested. Further studies are in progress (see below). For the current classification of *Clathrina* from the GBR, the results of Solé-Cava et al. (1991) and Klautau et al. (1994) are adopted here, with empirical support provided by statistical comparisons of spicule dimensions (Table 1).

Haeckel's (1872) original description of *C. primordialis* is vague concerning the dimensions of spicules (i.e. only one type of triactines, ranging from 100-200µm in length), with nearly the same dimensions recorded for *C. coriacea* (60-120µm, depending on the geographic variety). However, *C. primordialis* is well differentiated from *C. coriacea* by the different shape of actines of the triactines (conical versus cylindrical, respectively; see Haeckel, 1872, Plate 5, Figs 1-2). Haeckel (1872: 22) also noted the high variability in spicule size of *C. primordialis* from different parts of the world, and described several generic and specific varieties based on differences in cormus-shape and spicule size. Haeckel also mentioned an Australian variety with very large spicules (180-200µm length), without naming the variety. Unfortunately he did not describe the type locality for *C. primordialis* or its varieties, nor for *C. coriacea*. After more detailed comparative studies, Borojevic (1971) subsequently designated La Manche (British Channel), as the type locality for *C. coriacea*, and Klautau et al. (1994) nominated Rio de Janeiro (Brazil) as the type locality for *C. primordialis*. Although some species from the GBR show general affinities to both *C. coriacea* and *C. primordialis* (based on the shape of their triactinal actines), these species are described as new taxa, given: 1) the high improbability that *Clathrina* species from the GBR are conspecific with taxa from the North or South Atlantic; and 2) all new species of *Clathrina* described here from the S GBR differ consistently in one or more characters despite their geographic sympatric distribution. Using light microscopy and thick-sectioning (the latter technique essential for determining skeletal structure), it was possible to ascertain that each of these species differed slightly in their cytological features, as judged by the density of choanocytes, patterns of choanocyte arrangement, presence/absence of granular cells, occurrence, size and shape of porocytes. However, more sophisticated, thin- and ultra-sectioning techniques are required to accurately describe and identify the different cell types in each of these species.

Clathrina spp. are also supposedly readily contractile, largely influenced by local environmental conditions (as first observed many years ago by Haeckel, 1872, for *C. clathrus*). Consequently, it is possible that certain cytological features may be altered after fixation (i.e. cell contraction) (Borojevic pers. comm.). It is likely, therefore, that tube wall dimensions may not be an accurate mensural character to differentiate between these alied species, given that this feature is the most likely gross feature to expand and contract in response to local hydrodynamic conditions. More detailed studies on the cell biology and ultrastructure of the soft tissue, using electron microscopy (SEM/TEM), are currently in progress, seeking to answer some of these cytological questions, and to evaluate the use of cytological features as potential taxonomic tools (Wörheide, in prep.).

Statistical analyses of triactine dimensions were compared between species. For species with only one type of spicule (viz. *C. heronensis*, *C. wistariensis*, *C. helveola* and *C. parva*), this is currently the only consistent 'classical' feature to differentiate species. Pairwise-comparisons between spicule actinal-lengths in each species were made using a Student t-test at a confidence level of 99%. This analysis showed consistent and significant differences in spicule lengths between all species ($t=2.663$, $P<0.01$, $n=30$), supporting their recognition as distinct taxa. *Clathrina adusta* and *C. luteoculcitella* also possess a second type of spicule (tetractines and diactines, respectively), making it easy to

differentiate them from the other GBR *Clathrina*. Based on illustrations of Haeckel (1872: Plate 5, Figs 1-2), the shape of actines of triactinal spicules in *C. heronensis*, *C. wistariensis*, *C. parva* and *C. helveola* show superficial similarities to those of *C. coriacea*, having a characteristic cylindrical shape and more-or-less blunt triactine tips. On this basis these species are referred to as members of the 'coriacea-group'.

Yellow *Clathrina* are have been identified as: *C. clathrus*, described by Schmidt (1864) from the Mediterranean (with relatively small triactines; 92×5.5µm; Borojevic & Klautau, in press); *C. aurea*, described by Sole-Cava et al. (1992) from the Atlantic (with even smaller triactines; 72×5.6µm); or more recently as *C. chrysea*, described by Borojevic & Klautau (in press) from New Caledonia (with longer actines; 105×9.8µm). By comparison, *C. helveola* from the GBR has much larger triactines than these three species (with a mean 159.6×16.3µm, n=30), which is distinctive amongst all species. To support the current species classification, additional studies on molecular genetics and systematics of other Indo-Pacific *Clathrina* are in progress, with expectations that results will hopefully clarify questions of species boundaries within this difficult genus, and eventually supporting or refuting the utility of statistical differences in spicule size, as proposed here, to differentiate GBR *Clathrina* spp.

Family **Soleniscidae** Borojevic et al., 1990

Clathrinida with an essentially tubular organisation, growing in the form of an individual olynthus, with several olynthi growing from the basal stolon-like tubes or in the form of distally ramified tubes radially arranged around a central olynthus tube, without any special skeletal differentiation. A continuous choanoderm lines all the internal cavities. Spicules are regular triactines and/or tetractines, to which tripods or diactines may be added.

Soleniscus Borojevic et al., 1990

'*Soleniscus*', 'generic variety' of Haeckel, 1869: 244.
Soleniscus Borojevic et al., 1990:253.

Soleniscidae in the form of an individual olynthus, with several olynthi growing from basal stolon-like tubes, or in the form of distally ramified creeping tubes.

REMARKS. Even though *Soleniscus* has been attributed by authors to Haeckel (1869), Borojevic et al. (1990) correctly note that Haeckel never formally attributed this name at the generic level, but merely mentioned it as a 'generic variety'. Consequently, *Soleniscus* must be attributed to Borojevic et al. (1990).

Soleniscus radovani sp. nov.
(Fig. 6A-H)

ETYMOLOGY. For Radovan Borojevic, in recognition of his substantial and pioneering achievements in calcarean taxonomy.

MATERIAL. HOLOTYPE: QMG313661, S side of Wistari Reef, GBR, 23°29.4'S, 151°52.8'E, 17m depth, 07.vii.1998, coll. G. Wörheide (SCUBA). PARATYPE: QMG313807, same locality.

HABITAT AND DISTRIBUTION. Small patches of coral, under overhangs, Zone 2 (Fig. 2), 17m depth. Wistari Reef, S GBR.

DESCRIPTION. *Growth form*. Aborescent, bushy, with single, delicate tubes branching dichotomously and polychotomously from a few central tubes. The central, proximal tube is larger than distal tubes, and tubes ramify only in the lower part of the sponge 'bush'. Distal parts of the tubes are mostly longer than the ramified parts. Bushy, single tubes are approximately 2mm diameter. Two tubes sometimes fuse together at their distal end, just below the osculum. Size of the sponge 'bush' is <5cm.

Colour. Bright yellow in life, brownish-beige after fixation in Glutaraldehyde in EtOH.

Oscules. One naked osculum occurs on top of each tube, with slightly smaller diameter than the tube itself. When two tubes are fused together, one osculum is located at the distal end of the fused tubes, just above their point of fusion.

Texture. Soft, delicate, easily torn.

Surface ornamentation. Smooth.

Ectosomal skeleton. Not present.

Choanosomal skeleton. The skeleton consists only of sagittal tetractines. Tangentially arranged, parasagittal facial plane of the tetractines forms the wall of tubes, with one (the longer) ray of the basal triradiate system pointing in the direction of the growth axis (i.e. directed towards the central tube). Actines of tetractines overlap, with no more than three stacked above each other to form the tube wall, 15-20µm thick. The curved, free actines of tetractines protrude into the tube, and sometimes a 'tent' like tissue was observed over the tips of the free actines inside the tube (Fig. 6F). Tips of free actines are bent in the

direction of the osculum. No zonation and/or differentiation of tetractines was observed within tubes.

Aquiferous system. Asconoid grade of construction, with choanocytes continuously lining walls of the tubes. The (single) tubes grow in the form of an individual olynthus and several distally ramified olynthi tubes grow radially arranged around a central olynthus tube, with a terminal osculum on top of each tube. No single central atrium is present.

Soft tissue. The choanoderm has a dense appearance. Choanocytes 4-6µm diameter, and partially clustered and partially arranged in circles of 2-3 choanocytes surrounding small ostiae (<2µm diameter), which are not always visible in the center of circles in thick sections. Choanocytes are subspherical to hexagonal in section parallel to the surface, and are basinucleate (with the nucleus at the base of the choanocyte).

Spicules. Only one spicule type is present, consisting of sagittal tetractines with a curved apical actine. No differentiation and/or zonation of spicules was observed within tubes. Basal triradiate system is predominantly parasagittal, with slightly curved paired actines and one longer unpaired basipetal actine. The apical actine is bent at the tip ('whipped'), and is either slightly enlarged or reduced. The non-curved longer unpaired actine of the basal triradiate system measures 120-(152)-190µm×8-(10)-12µm (n=10), and the paired shorter (curved) actines of the pseudosagittal basal plane measure 85-(111)-130×8-(10)-12µm (n=10).

REMARKS. *Soleniscus radovani* is most similar to *S. (Ascilla) japonica* (Haeckel, 1872) from California, although in *S. japonica* all three actines of the basal system are equal. In *S. radovani* the basipetal actine is longer than the other two actines, which is regarded here as a distinctive character to differentiate these species.

Soleniscus stolonifer (Dendy, 1891)
(Fig. 6G-O)

Leucosolenia stolonifer Dendy, 1891: 46, pl. i, fig. 2; pl. vi, figs 1-3; pl. ix, fig. 2; Dendy & Row, 1913: 723; Dendy, 1924: 275; Tanita, 1942: 81; Burton, 1963: 182.
Soleniscus stolonifer; Borojevic et al., 1990: 253.

MATERIAL. LECTOTYPE: BMNH1891.9.19.4 (Dendy collection), near Port Phillip Heads, Victoria, coll. J.B. Wilson. OTHER MATERIAL: QMG313668, N side of Wistari Reef, GBR, 23°27.2'S, 151°53.2'E, 5m depth, 07.vii.1998, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. Shallow swim-throughs between coral bommies, Zone 2 (Fig. 2), 5m depth. Port Phillip Heads (Victoria) and S GBR (Qld); New Zealand.

DESCRIPTION. Growth form. Several olynthus-like tubes grow from basal stolon-like tubes. Distal tubes ramify and the proximal (deeper and older) tubes are larger (0.5cm diameter) than the distal (top) tubes, which are slightly conical (>1mm at the osculum). The size of the specimen is 2×3cm.

Colour. White in life, beige after fixation in Glutaraldehyde in EtOH.

Oscules. One naked osculum is apical at the end of each distal tube.

Texture. Smooth.

Surface ornamentation. No surface ornamentation is visible macroscopically, but fine grained silt and organic material produces a dirty-whitish cover (like dust) on the external surface of tubes.

Ectosomal skeleton. Diactines are arranged perpendicular to the surface of tubes in 'tee-pee'-like bundles, where individual diactines are aligned at an angle of <45° to each other and cross in their proximal lower third. These diactines are covered with a biofilm which contains small sediment particles, diatoms, and foraminiferans (producing the 'dusty' appearance on the external surface). The slightly broader base of diactines sometimes protrudes slightly through the choanosomal skeleton, whereas the proximal part of diactines is mostly anchored between the basal systems of the tetractines, not protruding into the tube.

Choanosomal skeleton. The choanosomal skeleton is sustained by a tangentially arranged basal triradiate system of tetractines of very variable size and form, forming the walls of tubes. Two main types of spicules were distinguished: a larger regular, and a smaller parasagittal-sagittal type of tetractine. Their apical actines, which are sometimes greatly exaggerated in the larger regular type, and reduced in the smaller sagittal type, protrude into the tube. Actines of their basal triradiate systems, which are thicker in the regular type, do overlap, with up to five actines partially stacked above each other, supporting the tube wall. Tube walls have a thickness of about 100µm (excluding the diactines). No differentiation and/or zonation of spicule types was observed.

Aquiferous system. This species has an asconoid grade of construction with choanocytes continuously lining the walls of tubes. Tubes grow in the form of an individual olynthus from

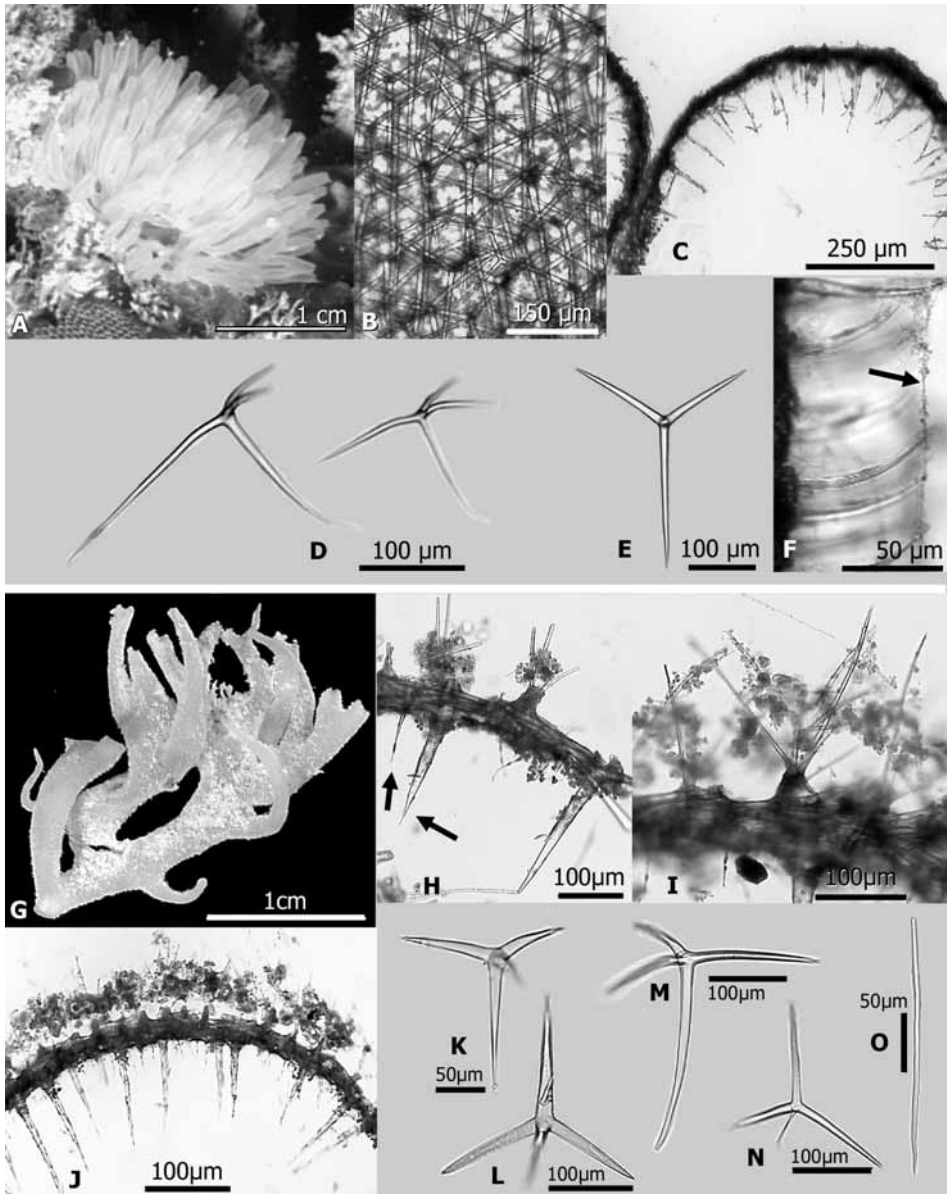

FIG. 6. *Soleniscus* spp. A-H, *Soleniscus radovani* sp. nov. (holotype QMG313661, histological sections and spicules; paratype QMG313807, *in situ* photograph). A, paratype in situ from Wistari Reef. B, section of tube wall with basal system of sagittal tetractines, longer unpaired actines point towards central tube (bottom of image). C, Cross section of one tube with free actines of sagittal tetractines protruding into the tube. D, two sagittal tetractines with curved free actine and longer unpaired actine of basal system. E, basal system of sagittal tetractine with longer unpaired actine. F, cross section of tube with bent free actines and 'tent-like' tissue over their tips. The tips of the free actines are bent in the direction of the osculum (top of image). G-O, *Soleniscus stolonifer* (Dendy, 1891) (specimen QMG313668). G, specimen after fixation. H, cross-section of tube wall showing two sizes of free actines of tetractines building the skeleton of the wall (arrows) (compare with K,N in this figure). I, cross-section of tube wall showing 'tee-pee' like bundled diactines with baffled sediment particles. J, cross-section of tube wall. K, regular tetractine. L, regular tetractine. M, sagittal tetractine with curved free actine. N, tetractine with reduced 'whip-like' apical actine. O, diactine.

the basal stolon-like tube, with a terminal osculum on top of each tube.

Soft tissue. A dense choanoderm covers the internal walls of tubes, which are continuously lined by choanocytes. The choanoderm also covers the lower 10-15% of the proximal part of the free actines of regular larger tetractines, protruding into the tube. Distal parts of these free actines are partly covered with cells, but not with choanocytes, as are the smaller, 'whip-like', free actines of smaller sagittal tetractines. Choanocytes are basinucleate (i.e. with the nucleus at the base of the cell), measuring 4-7µm diameter, and possessing a long flagellum (up to three times the size of the choanocyte). Open spaces within the skeleton, between the choanoderm and external surface (built by the basal triradiate systems of tetractine spicules), is filled with a mesohyle of yet unknown cellular composition. This mesohyl is certainly free of choanocytes and contains some archaeocytes. Ectosomal exopinacoderm covers the basal triradiate systems of choanosomal tetractines on the external surface. The 'tee-pee'-like bundles of ectosomal diactines are also covered in their lower parts, up to their last point of crossing, with exopinacoderm. Ostiae (<10µm diameter) are scattered on the external surface between the bundles of diactines.

Spicules. Regular tetractines with conical actines, sharply pointed, 80-(129.9)-170× 8-(12.2)-21µm, sometimes with an exaggerated apical actine (up to 500µm) (n=25); parasagittal to sagittal tetractines with mostly reduced apical actines, length of shorter paired actines 40-(81.6)-110µm, length of longer unpaired actine 80-(124.4)-160µm, width of actines 8-(9.7)-12µm (n=25); diactines 150-(207.8)-260×4-(5.7)-8µm, sometimes sinused, with one broader part near the proximal end, but both tips sharply pointed, fusiform (n=25).

REMARKS. This is the first record of the species for the GBR. It was initially described by Dendy (1891) as a species of *Leucosolenia* (Calcaronea) from Port Phillip Heads (Victoria), and was later described by Dendy (1924) from New Zealand. However, the species clearly belongs to Calcinea due to its possession of basinucleolate choanocytes (see Dendy, 1891: plate 6, fig. 2), which were also observed in the specimen described here. This species is the types species of *Soleniscus*, as stated by Borojevic et al. (1990), because of its characteristic morphological features (several olynthi arising from basal, stolon-like tubes; its spicule types; asconoid grade of construction; and basinucleolate choanocytes). Although the growth form of the specimen described here (with a wider central tube and conical and thinner distal tubes), is slightly different from that described by Dendy (1891: pl. 1, fig. 2) (with thinner basal tube and wider cylindrical distal tubes), the spicular skeleton is very similar. Dendy (1891: 47) mentioned a large variability in both spicule size and spicule morphology in his specimens, but clearly recognised that the free apical actine of tetractines was sometimes exaggerated, which he regarded as typical and distinctive for this species. This feature was also observed in the specimen studied here. Dendy (1891) also noted, as typical for *S. stolonifer*, the peculiar form of the ectosomal diactines, often being slightly irregularly curved, with one broader proximal end but with both tips sharply pointed. The same form of diactines was observed in the specimen from the GBR, although the diactines observed here are only about half the size of the largest ones described by Dendy (1891) (which were up to 700µm long). Despite differences in spicule sizes (which Dendy suggested may vary greatly in this species), and differences in the growth form, this specimen from the GBR is assigned to *S. stolonifer* identical skeletal structure as described by Dendy (1891) (i.e. only two types of spicules present; some tetractines with exaggerated free apical actines; and peculiarly formed diactines), and the same organisation of the soft tissue (see Dendy, 1891: pl. 6, figs 1,3).

Family **Levinellidae** Borojevic & Boury-Esnault, 1986

Clathrinida with a cormus composed of a central tube which may be ramified, and of diverticuli isolated or grouped in clusters. The skeleton of the central and radial tubes is composed of regular (equiactinal and equiangular), and/or parasagittal spicules. The skeleton of the diverticuli is composed of regular and/or parasagittal spicules, always clearly distinct from spicules which form the skeleton of the central tube. Differences occur in the size of spicules between the diverticuli and those of the central tube, where the latter the spicules are larger. The choanoderm either lines all the central cavity or is restricted to the diverticuli (emend.).

Levinella Borojevic & Boury-Esnault, 1986

Levinella Borojevic & Boury-Esnault, 1986: 444; 1987: 22; Borojevic et al., 1990: 255.

Levinellidae with a cormus divided into a central

tube and external diverticuli. The central tube is not ramified. A choanoderm lines all the internal cavities.

Levinella prolifera (Dendy, 1913)
(Fig. 7A-H)

Dendya prolifera Dendy, 1913: 6, pl. i, figs 3-4; pl. iii, figs 4-5; Dendy & Row, 1913: 728; Burton, 1930: 2, figs 1-2; 1963: 232, text fig. 99.
Levinella prolifera; Borojevic & Boury-Esnault 1986: 447.

MATERIAL. HOLOTYPE: BMNH 1920.12.9.49, 'Sealark' Collection, Amirante Is. (Seychelles), 80m depth. OTHER MATERIAL: QMG313664, S side of Wistari Reef, 23°29.4'S, 151°52.8'E, 18m depth, 07.vii.1998, coll. S.D. Cook & G. Wörheide (SCUBA). Fragment sent to N. Boury-Esnault (Centre d'Océanologique de Marseille, Station marine d'Endoume, Marseille, France).

HABITAT AND DISTRIBUTION. Small patches of coral in 18m depth, under overhangs, Zone 2 (Fig. 2). Indian Ocean (Seychelles); Indonesia; S GBR (Qld, Australia).

DESCRIPTION. *Growth form*. The specimen has a size of about 2.5cm and consists of one central tube (4mm diameter) with clustered diverticuli around the central tube (<2mm size) extending to about 2mm below the osculum. The upper 2mm of the central tube is free of diverticuli.

Colour. Brownish-beige in life and after fixation in Glutaraldehyde in EtOH.

Oscules. One naked osculum at apical end of the central tube.

Texture. Soft.

Surface ornamentation. Clustered diverticuli around the central tube, resembling a 'bunch of grapes'.

Ectosomal skeleton. No distinct ectosomal skeleton.

Choanosomal skeleton. The central tube is not ramified and choanocytes sparsely line the walls of the central tube with distinct ostia between them (up to 40µm diameter). The density of choanocytes in this region is not as high as those in the diverticuli. The wall of the central tube, translucent in the upper part where no diverticuli are found, is sustained by tangential regular and parasagittal to sagittal triactines and the tangentially orientated regular and parasagittal basal system of tetractines, with not more than three overlapping actines forming the thin wall of the tube (thickness 30-50µm). Triactines and tetractines are arranged without any apparent order or direction, scattered irregularly in the wall of the central tube. Some free actines of the tetractines protrude into the tube. Fewer tetractines occur here as compared with those in the proximal parts of the diverticuli. Diverticuli ramify from the central tube into clusters of little tubes. They originate from one cavity/larger tube splitting from the central tube and ramify from this 'proximal diverticuli-tube/cavity'. This latter structure is sometimes developed as a small tube (250µm diameter), branching from the central tube, and sometimes like a bulbous, half spherical, 'chamber-like' extension of the central tube (up to 500µm diameter), from which up to ten other tubes of the diverticuli ramify. The walls of the diverticuli are supported by tangential regular and parasagittal to sagittal triactines and the parasagittal basal systems of tetractines. Two, or less often three overlapping actines form the walls of the diverticuli, 10-20µm thick. Many free actines of tetractines protrude into the 'proximal diverticuli tube', their number decreasing distally, and in the most distal parts of the ramified diverticuli tubes are devoid of free tetractinal actines. Distal parts of diverticuli are constructed by tangential parasagittal and sagittal triactines with their unpaired actines directed towards the distal end of diverticuli, away from the central tube.

Aquiferous system. A choanoderm lines all internal cavities of the sponge (central tube and diverticuli), and although the density of the choanoderm is reduced in the central tube, the central tube is not regarded as a true atrium, because numerous choanocytes are present (whereas the term 'atrium' is restricted to a central exhalant-only cavity, devoid of choanoderm). The aquiferous system has an asconoid grade of construction.

Soft tissue. The soft tissue of the central tube is thin, delicate, slightly translucent and cells are only sparsely present. Choanocytes, 4-6µm diameter, are sometimes clustered together or solitary, but not as densely arranged here as they are in the diverticuli. Their shape is sub-spherical to hexagonal in section parallel to the surface. Amoeboid archaeocytes were observed scattered between choanocytes, slightly smaller than choanocytes. The soft tissue of diverticuli appears to be denser than the soft tissue of the central tube. All walls of diverticuli are lined by choanocytes in rows of 1-3, surrounding the ostiae. Ostiae 10-20µm diameter. Choanocytes have a subspherical, pseudo-hexagonal to hexagonal shape and a size of 4-10µm in section parallel to the

surface. Rod-shaped bacteria (possibly cyanobacteria), 5-8µm long, are sometimes scattered in the soft tissue of diverticuli. Only few amoeboid archaeocytes are present in diverticuli.

Spicules. *Central tube*: regular triactines with cylindrical actines, 70-(110.4)-140×8-(9)-12µm (n=10); parasagittal equiangular triactines with cylindrical actines, longer unpaired actine 120-(229.2)-360×8-(10.64)-15µm, shorter paired actines (curved proximal) 80-(174.8)-290×8-(10.64)-15µm (n=25); parasagittal tetractines with reduced apical actine and cylindrical actines of the basal triradiate system, equiangular basal system similar to the parasagittal triactines, longer unpaired actine 120-(188.4)-290×8-(10.2)-12µm, shorter paired actines (curved proximal) of the basal system 85-(142.2)-190×8-(10.2)-12µm, apical actine reduced; few regular tetractines with cylindrical actines of the triradiate basal system, with curved apical actine, actines of the basal triradiate system 80-(112)-200×8-(9.2)-11µm (n=10). Diverticuli, regular cylindrical triactines 50- (74.2)-100×5-(7.5)-10µm (n=12); parasagittal to sometimes sagittal cylindrical triactines (about 10% are sagittal, e.g. enlarged unpaired angle), longer unpaired actine 70-(116.4)-160×5-(7.64)-10µm, shorter paired actines, mostly proximally slightly curved 60-(79.8)-100×5-(7.64)-10µm (n=25); parasagittal cylindrical tetractines with often reduced apical actine, longer unpaired actine 100-(125.41)-140×7-(8.75)-10µm, shorter paired actines, mostly proximally slightly curved 70-(90)-100×7-(8.75)-10µm (n=12), apical actine 40-(70)-90µm (n=3); rare regular cylindrical tetractines with a curved apical actine 50-(73.3)-100×5-(7.3)-9µm (n=3).

REMARKS. Initially described in *Dendya* (Dendy, 1913), with type locality from the Seychelles, Borojevic & Boury-Esnault (1986) referred it to their new genus *Levinella*, but without comprehensive redescription. *Dendya prolifera* was reported by Burton (1930) from Indonesia, whereas the specimen described here is the first record of this species for Australia. The specimen described by Dendy (1913) had a very characteristic and unique morphology, and the species is readily recognisable in the field, resembling a 'bunch of grapes'. Based on obvious similarities in skeletal features (size and form of spicules, arrangement of diverticuli around the central tube; see Dendy, 1913, pl. 3, fig. 4), the present material from the GBR is clearly conspecific with Dendy's (1913) species, and easily assigned to *Levinella prolifera*. However, in the definition of the genus *Levinella*, Borojevic et al. (1990: 254) described the skeleton of the central and radial tubes as composed of 'regular (equiradiate and equiangular) spicules', whereas this is neither the case in the holotype of *Levinella prolifera* (Dendy, 1913: 8; pl. 3, fig. 5a), nor in the specimen described here. In both specimens the skeleton of the central tube consists partly of parasagittal spicules (such as seen in the diverticuli). Differences in spicules between central tube and diverticuli are mainly in their size (spicules of the central tube are larger), and the definition of the family is emended here accordingly.

Family **Leucaltidae** Dendy & Row, 1913

Clathrinida with tubular, ramified or anastomosed cormus bearing many osculae, or occurring in the form of single tubes each with a single large terminal osculum. The tubes have a large atrium surrounded by a strong wall composed of a distinct cortex and a choanosome. The cortical skeleton is composed of large tangential triactines and/or tetractines. The choanoskeleton may be absent, reduced to apical actines of cortical tetractines, or contains small and irregularly dispersed triactines and tetractines.

Leucaltis Haeckel, 1872

Leucaltis Haeckel, 1872: 142; Dendy & Row, 1913: 737.
Heteropegma Poléjaeff, 1883: 25, 45.

Leucaltidae with a body composed of large, ramified and anastomosed tubes. Each tube has a distinct strong cortex sustained by large triactines and tetractines. The choanosome is organised in elongate, radial chambers, which open to the central atrial cavity. It is supported by apical actines of cortical tetractines and small scattered triactines and tetractines.

Leucaltis clathria Haeckel, 1872
(Fig. 7I-S)

Leucaltis clathria Haeckel, 1872: 159, pl. 26, fig. 3; Dendy & Row, 1913: 738; Dendy, 1913: 16, pl. 2, figs 1-2; Hozawa, 1940: 136, pl. 6, fig. 3; Arndt, 1940: 46; Tanita, 1943: 394, pl. 8, fig. 27; Borojevic & Peixinho, 1976: 1002, fig. 8; Borojevic & Klautau, in press.
Heteropegma nodusgordii Poléjaeff, 1883: 45, pl. 1, fig. 7, pl. 4, fig 1.
Leucaltis bathybia mascarenica Ridley, 1884: 625, Pl. 54a,a'.
Clathrina latitubulata Carter, 1886: 515.
(see Burton, 1963: 549 for detailed synonymy)

MATERIAL. HOLOTYPE: Unknown; BMNH 1956.4.26.42 (slide) fragment of type. OTHER MATERIAL: QMG313676, S side of Heron I., GBR,

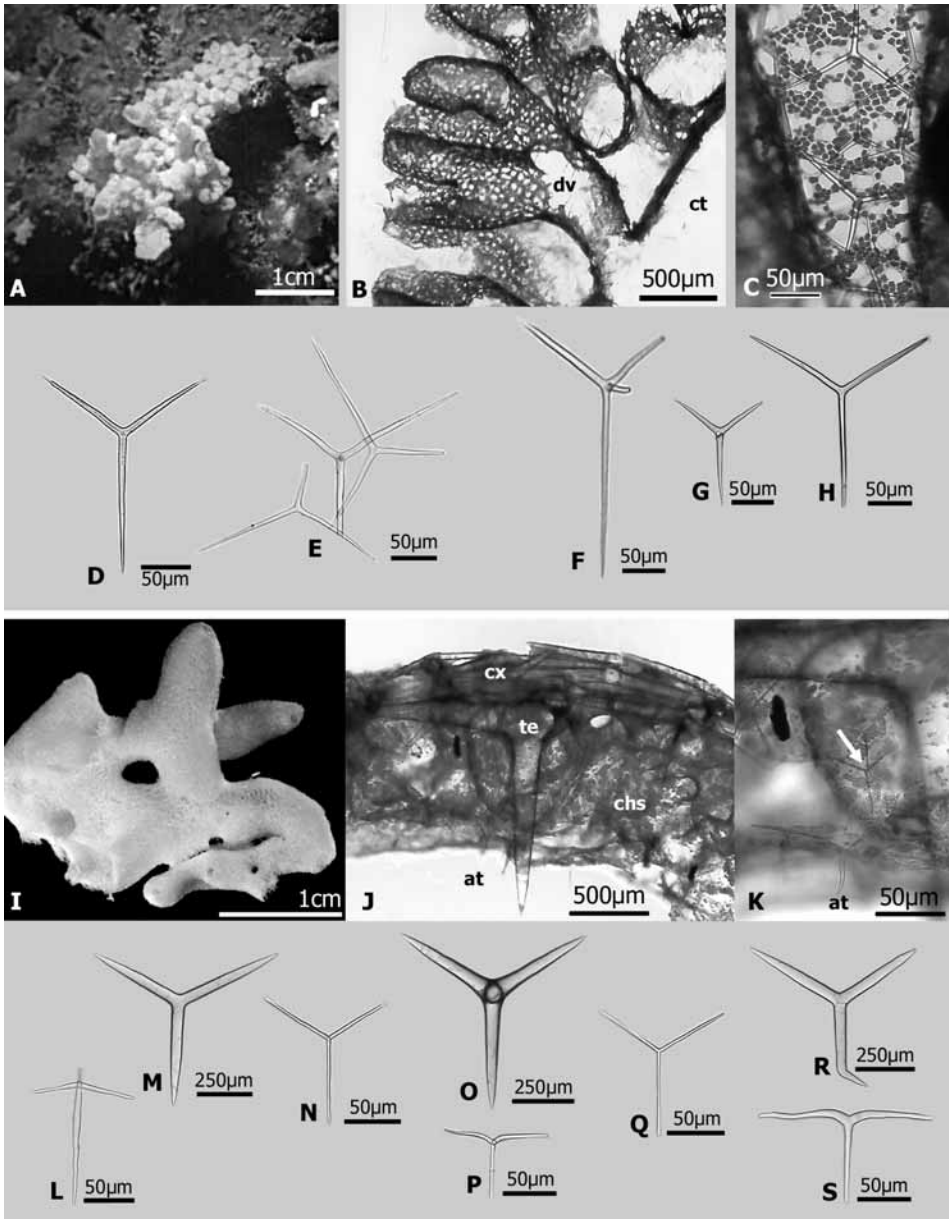

FIG. 7. *Levinella* and *Leucaltis* spp. A-H, *Levinella prolifera* (Dendy, 1913) (specimen QMG313664). A, specimen in situ. B, section through the diverticuli (dv) and part of the central tube (ct). C, section through one diverticuli with ostiae (round holes) and surrounding choanocytes. D, parasagittal triactine of the diverticuli. E, two parasagittal to sagittal triactines and one parasagittal tetractine (center) of the diverticuli. F, parasagittal tetractine of the central tube. G, small parasagittal tetractine of the central tube. H, regular triactine of the central tube. I-S, *Leucaltis clathria* Haeckel, 1872 (specimen QMG313676). I, specimen after fixation. J, cross-section through the tube wall with cortex (cx), choanosome (chs), atrium (at), and a large tetractine (te) which free apical actine protruding through the choanosome into the atrium. K, enlargement of the inner choanosome with triactine (arrow) atrial tetractine (at). L, atrial tetractine. M, cortical triactine., N, choanosomal tetractine. O, large subcortical tetractine. P, atrial tetractine. Q, choanosomal triactine. R, cortical triactine with one bent actine. S, atrial triactine.

23°28.2'S, 151°56.7'E, 08.vii.1998, 17m depth, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. Small crevices under coral bommies in 17m water depth, Zone 2 (Fig. 2). Apparently cosmopolitan. Distribution in Australia: Houtman Abrolhos (Western Australia); Bass Strait (Tasmania); Port Phillip Heads and Westernport Bay (Victoria); Torres Straits, Cape York, and GBR (Queensland); also recorded from the SW Pacific (New Caledonia), NW Pacific (Japan), Indian Ocean (Seychelles, Ceylon (Sri Lanka), and amphi-Atlantic (Florida, Bermudas, Brazil, Portugal).

DESCRIPTION. *Growth form*. The cormus is composed of large anastomosing, round to flattened tubes up to 1cm diameter. The round tubes (<5mm diameter) partly stand vertically about 1cm above the rest of the cormus and possess a terminal naked osculum, about half the size of the tube. The main part of the cormus is flat, with only few naked oscules scattered on ridges of the anastomosing tubes.

Colour. White in life, beige after fixation with Glutaraldehyde in EtOH.

Oscules. A few naked oscules are scattered on the ridges of anastomosing tubes, or are terminal at the top of vertical tubes. The diameter of ridge oscules are smaller (<1.5mm) than terminal oscules (<3mm).

Texture. Harsh, relatively firm, slightly rough.

Surface ornamentation. Surface is unornamented, although it appears coarse due to the presence of large tangential triactines in the cortex.

Ectosomal skeleton. A distinct cortex is developed, 100-170µm thick, consisting of a tangential layer of regular, cylindrical, more-or-less sharply pointed large triactines. The angle between two rays is occasionally greatly enlarged (up to nearly 180°), in which case these triactines become sagittal, or one actine may be sharply bent at its top, or the actines may be slightly undulated. Up to five triactines are densely stacked above each other without any apparent alignment, but in a way that space is maintained the actines for vertical sub-circular inhalant canals. Occasional, large, regular tetractines are regularly dispersed directly beneath the distinct cortex. The basal triradiate systems of these enormous tetractines are tangentially arranged in the subcortical layer, between cortex and choanosome, with their apical actines penetrating the choanosome and protruding through the atrial surface into the atrium.

Choansomal skeleton. The choanosomal skeleton is only supported by small scattered cylindrical regular triactines and tetractines, with a regular basal triradiate system, 300-500µm thick. One actine of the regular triactines often points in the direction of the atrium, but actines are also sometimes irregularly aligned. Apical actines of tetractine spicules often point towards the atrium, but these are also often tangentially aligned around the radially arranged, branching and elongated choanocyte chambers. In this latter case their free apical actines protrude into the choanocyte chambers. Furthermore, a distinct atrial skeleton is supported by small sagittal triactines and tetractines which are embedded in a thin atrial membrane. Their unpaired actines or apical actines, respectively, protrude through the atrial membrane into the atrium. The free apical actine of atrial tetractines is often slightly undulated, and in rare cases it may be slightly enlarged.

Aquiferous system. Numerous inhalant canals, up to 150µm diameter, are located between tangential cortical triactines, suppling choanocyte chambers with water. These inhalant canals are covered by a thin membrane, pierced by several ostiae above each canal. Ostiae have a diameter of 40-60µm. Choanocyte chambers, elongated and branch irregularly, 80-160µm diameter, are radially arranged around the atrium. These open through the atrial membrane into the atrium. The aquiferous system has a syconoid grade of construction, and although choanocyte chambers are irregularly branched, they maintain a general radial organisation.

Soft tissue. Thin ectosomal exopinacoderm covers the tangentially arranged triactines of the cortex and incurrent canals. Above the incurrent canals, the exopinacoderm is pierced by several ostiae (see above for dimensions). Cortical triactines are embedded in a relatively thin mesohyle with only a few visible cells, probably archaeocytes and/or sclerocytes. The choanosome consists of elongated, irregularly branching choanocyte chambers, where choanocytes densely line all walls of chambers. Choanocytes are 4-6µm in diameter, and are sub-spherical, tetragonal to hexagonal in shape in section parallel to the surface. Only few archaeocytes were observed in the choanosome. Choanocyte chambers open via 50-150µm wide openings through the thin atrial membrane (10-15µm thick), into the central atrium. The atrial membrane contains only few cell-types, of which some are amoeboid and may be archaeocytes, and others are more spherical and resemble choanocytes (although no flagella were seen and

it is not possible to determine if they are choanocytes without higher resolution microscopy (in progress).

Spicules. Regular cortical triactines, sometimes sagittal (t-shaped, with unpaired angle up to 180°), 105-(285.2)-470×15-(38.28)-60µm (n=25); Large regular subcortical tetractines, 320-(532.5)-630× 70-(100)-130µm (n=4); Regular choanosomal triactines with cylindrical actines and blunt (rounded) tips, 54-(63.76)-70×2-(2.48)-4µm (n=25); Regular choanosomal tetractines with cylindrical actines, same size and shape of the basal triradiate system as the choanosomal triactines, but with a reduced apical actine; Sagittal atrial triactines with cylindrical actines and blunt (rounded tips), longer paired actines 48-(51.88)-62×2-(4)-7µm (n=25), shorter unpaired actine 32-(40.44)-56 (n=25), the shorter unpaired actine is often slightly thinner than the longer paired actines; Sagittal atrial tetractines, same size and shape of the basal triradiate system as the choanosomal triactines, but with a reduced apical actine.

REMARKS. *Leucaltis clathria* was initially described by Haeckel (1872) from Florida, and subsequently recorded in many parts of the world (see above). It was recorded from the N coast of Qld. (Cape York, Torres Strait) by Poléjaeff (1883) as '*Heteropegma nodus gordii*', which is allegedly synonymous with *L. clathria*. The specimen described here from Heron Island is the first record of this species from the GBR. It is morphologically indistinguishable from *L. clathria*, as described by Haeckel (1872), and *H. nodus gordii*, as described by Poléjaeff (1883), in having very similar skeletal structure, spicule sizes, shapes and varieties, as well as irregular radial choanocyte chambers. *Leucaltis clathria* is allegedly cosmopolitan, with all recorded specimens having these very characteristic and unique morphological and organisational features, despite populations being widely dispersed and discontiguous. Although the taxonomic status of allopatric populations of so-called 'cosmopolitan species' is still not clearly resolved for most sponge groups (e.g. Solé-Cava et al., 1991, Klautau et al., 1994, Wörheide, 1998), we are presently unable to find any morphological characters to differentiate GBR populations of *L. clathria* from other populations in other parts of the world (Haeckel, 1872; Poléjaeff, 1883; Dendy, 1913; Borojevic & Klautau, in press). More sophisticated biological tools (e.g. PCR profiles), are required to resolve this problem, currently beyond the scope of the present project.

Family **Leucettidae** de Laubenfels, 1936

Clathrinida with a solid body. The aquiferous system is always leuconoid. The choanoskeleton is well-developed and in the form of a regular network composed of triactines and/or tetractines. The cortex is thin and composed of spicules similar to those of the choanoskeleton (*sensu* Borojevic, 1968).

Leucetta Haeckel, 1872

Leucetta Haeckel, 1872: 116.
Leucettaga Haeckel, 1872: 117.
Teichonella Carter, 1878: 35.

Leucettidae with a homogeneous organisation of the wall and a typical leuconoid aquiferous system. There is neither a clear distinction between the cortex and the choanoskeleton, nor the presence of a distinct layer of subcortical inhalant cavities. The atrium is frequently reduced to a system of exhalant canals that open directly into the osculum.

Leucetta microraphis Haeckel, 1872
(Fig. 8A-G, Table 2)

Leucetta microraphis Haeckel, 1872: 119, pl. 21, figs 10-17; Dendy & Row, 1913: 734; Dendy & Frederick, 1924: 482; Row & Hozawa, 1931: 746; Tanita, 1942: 111, pl. 6, fig. 4; Borojevic, 1967: 3, fig. 2; Borojevic & Peixinho, 1976: 1003, fig. 9; Pulitzer-Finali, 1982: 48, Fig. 1; Borojevic & Klautau, in press.
Leucetta primigenia microraphis Haeckel, 1872:119, pl. 21, figs 10-17; Ridley, 1884: 482.
Leuconia dura Poléjaeff, 1883: 65, pl. 2, fig.3, pl.7, fig.7.
Pericharax carteri homoraphis Poléjaeff, 1883: 66.
Leucaltis floridana australiensis Carter, 1886: 145.
Leucandra carteri Dendy, 1893: 103.
(see Burton, 1963: 270, and Borojevic, 1967: 3, for detailed synonymy)

MATERIAL. HOLOTYPE: No clear segregation of surviving syntypes into respective type localities. SYNTYPES: PMJ Porif.106, '*Leucetta microraphis* (*L. primigenia* var.)/Mare Rubrum/Frauenfeld' (NMV photos 56/26–29). Type locality: Red Sea, possibly also Gulf of St. Vincent, South Australia. OTHER MATERIAL: QMG313659, Wistari Reef, Wistari Channel, GBR, 23°27.5'S, 151°55'E, 17m depth, 06.vii.1998, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. Steep wall, small crevices under coral, Zone 1 (Fig. 2); also known to live in exposed and semi-shaded areas of coral reefs. Allegedly cosmopolitan. Recorded distribution in Australia: Qld (Torres Strait; Heron I., Wistari Reef, S GBR), SE coast (Bass Strait), New South Wales (Port Jackson to Shark Is), Victoria (Port Phillip Bay), Western Australia (Houtman

TABLE 2. Statistical comparison of the most abundant type of small triactines between *Leucetta microraphis*, *Leucetta chagosensis* and *Leucetta villosa* sp. nov. Measurements in µm (n=30).

Species	*L. microraphis*		*L. chagosensis*						*L. villosa*	
Sample	QMG313659		QMG313654		QMG313658		QMG313667		QMG313662	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
Mean	169.4	18.2	126.4	18.1	134.1	15.4	128	16.4	118.3	16.3
Std Dev.	19.4	2.6	11.9	1.7	11.4	1.7	9.1	1.6	8.3	2.1
Min.	140	14	98	14	118	12	112	14	100	12
Max.	240	28	144	22	158	18	148	20	134	20

Abrolhos, Shark Bay, Geraldton, Bunbury); also reported from the Mediterranean, amphi-Atlantic, S Atlantic, amphi-Pacific, Indian Ocean, S Africa, Red Sea and Antarctica.

DESCRIPTION. *Growth form*. With a massive, convoluted, lamellate growth form, 3×4cm in size, and one 'finger-like' protrusion,1.5cm long, 0.5cm wide.

Colour. Brownish-yellow to greenish-yellow in life, beige after fixation in Glutaraldehyde in EtOH.

Oscules. A few small naked oscules without 'lip' are scattered on ridges of the sponge and terminal on the finger-like protrusion, 1-2mm diameter.

Texture. Firm, harsh.

Surface ornamentation. Smooth.

Ectosomal skeleton. A distinct dense cortex of tangential small regular triactines is developed, with a thickness of 60-100µm. A few tangentially orientated large ('giant') triactines are also present in the cortex. The cortex is followed inwards by a prominent layer of subdermal cavities (40-200µm diameter). This zone of subdermal cavities, up to 250µm thick, is the transition zone between surface and choanoome. The zone of subdermal cavities is composed of irregularly arranged, but mostly perpendicular aligned small triactines, with a few scattered large 'giant' triactines between. A peculiar form of small sagittal triactine and tetractine is only found in the small oscular rim. In this spicule type all three actines have the same total length, and the angles between the actines are equal, but both paired actines of the triactine (or of the basal triradiate system of the tetractine, respectively), are bent from each of their actinal center about 30° towards the unpaired actine (Fig. 8C-F). Sagittal triactines and tetractines have the same dimensions in their triradiate system, and the apical actine of the sagittal tetractine is either straight, or slightly bent at the distal end.

Choanosomal skeleton. Regular triactines of two very different sizes form an irregular meshwork in the choanosome, with a relatively low density, leaving space for the choanocyte chambers. Large 'giant' triactines are present, occasionally scattered throughout the choanosome between the numerous smaller triactines. No pattern of alignment was recognised between both size classes of triactines in the choanosome, although small triactines are found tangentially aligned and densely packed around incurrent canals, where they form the walls of these canals. Similarily, sagittal tetractines are found only around exhalant canals, with their basal triradiate system tangentially arranged, forming the walls. Their free apical actines protrude into the excurrent canal.

Aquiferous system. Water enters the sponge through ostiae (although none were observed in sections studied), via subdermal cavities located below the cortex. These cavities open into larger incurrent canals which lead into the choanosome. The water leaves the choanosome initially through small excurrent canals, which open into larger canals leading to the osculae. These larger canals are often flattened, measuring about 0.5x1mm. Both incurrent and excurrent canals maintain a general radial orientation. The aquiferous system has a leuconoid grade of construction.

Soft tissue. The soft tissue of the cortex has an extremely dense appearance. It is free of choanocytes, but contains numerous amoeboid archaeocytes (up to 14µm diameter). The early stages of spherical oocytes (8-10µm diameter), are far more abundant, mostly opaque, sometimes granular, and rarely translucent. They posses a distinct central area (90% of their diameter), and always have a translucent thin rim (possibly a cell coat). Sometimes two-cell embryos were observed. Oocytes are predominant in, but not restricted to, the cortex, scattered in varing numbers in other parts of the soft tissue. The choanosome is dense, with sub-spherical to

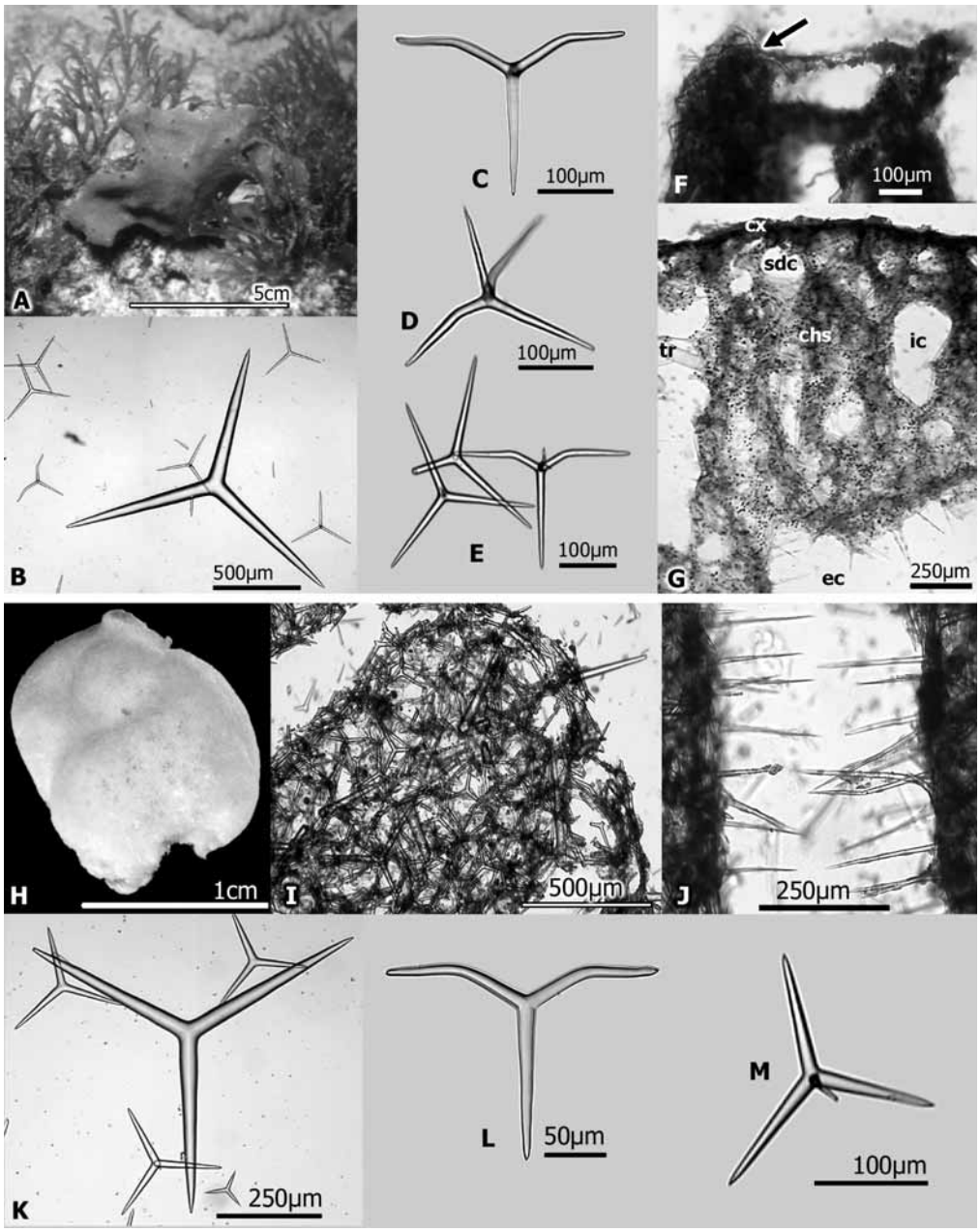

FIG. 8. *Leucetta* spp. A-G, *Leucetta microraphis* Haeckel, 1872 (specimen QMG313659). A, a specimen in situ. B, two sizes of triactines. C, oscular sagittal tetractine. D, oscular sagittal tetractine. E, oscular sagittal tetractine (left) and two regular tetractines. F, cross-section of an osculum with one oscular triactine (arrow). G, cross-section of cortex (cx) and choanosome (chs) with sub-dermal cavities (sdc), a large 'giant' triactine (tr), incurrent (ic) and excurrent (ec) canals. The little black dots are oocytes. H-M, *Leucetta chagosensis* Dendy, 1913 (specimen QMG313654). H, specimen after fixation. I, cross-section of choanosome. J, section of an excurrent canal with free apical actines of tetractines pointing into canal. K, three size classes of triactines (smallest one is young) and one tetractine (bottom center). L, sagittal triactine of the oscular rim ('lip'). M, tetractine of the excurrent canal with reduced apical actine.

spherical choanocyte chambers, 50-60µm diameter. Choanocytes are sub-spherical to spherical, 2-4µm diameter.

Spicules. Large 'giant' regular triactines with conical actines, 460-(705.2)-940×60-(120)-91.2µm (n=25); small triactines with conical actines 140-(169.4)-240×14-(18.2)-28µm (n=25); sagittal tetractines of exhalant canals, with more cylindrical actines, and their free actine curved 110-(143.4)-165×12-(14.4)-20µm (n=25); small sagittal triactines and tetractines of the oscular rim (both the same size) 70-(122.9)-160×10-(12.4)-16µm (n=14). In this latter spicule type all three actines have the same total length, where the unpaired angle is slightly enlarged and both paired actines of the triactine (or of the basal triradiate system of the tetractine, respectively), are bent below their actinal center, about 30° towards the unpaired actine (Fig. 8C-F). Sagittal triactines and tetractines have the same dimensions in their triradiate system, and the apical actine of sagittal tetractines is either straight, or slightly bent at the distal end.

REMARKS. The allegedly cosmopolitan distribution of *L. microraphis* is disputed here, but there is currently no additional empirical support to resolve this problem one way or another, based on 'classical' morphological criteria.

Leucetta chagosensis Dendy, 1913
(Fig. 8H-M, Table 2)

Leucetta chagosensis Dendy, 1913: 10, pl. 1, fig. 6, pl. 4, fig 2; Dendy & Row, 1913: 733; Dendy & Frederick, 1924: 482; Burton, 1963: 241; Borojevic, 1967: 2, fig. 1; Pulitzer-Finali, 1982: 89; Borojevic & Klautau, in press.

Leucetta infrequens Row & Hozawa, 1931: 747, pl. 19, Fig. 4.

Leucetta expansa Row & Hozawa, 1931: 749, pl. 19, fig. 5.

MATERIAL. HOLOTYPE: BMNH1920.12.9.51, 'Sealark Collection' (CXIX 11), Salomon (Chagos Archipelago). OTHER MATERIAL: QMG313654, Tenements, Heron I., GBR, 23°26.05'S 151°57.1'E, 15m depth, 22.vi.1998, coll. G. Wörheide (SCUBA); QMG313658: Wistari Reef, Wistari Channel, GBR, 23°27.5'S, 151°55'E, 17m depth, 06.vii.1998, coll. G. Wörheide (SCUBA); QMG313667, S side of Wistari Reef, GBR, 23°29.4'S, 151°52.8'E, 18m depth, 07.vii.1998, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. In crevices and under overhangs of coral bommies, also abundant in illuminated reef habitats, Zones 1-2 (Fig. 2). Indo-west Pacific: Recorded distribution in Australia: Western Australia (Houtman Abrolhos, Fremantle), Queensland (Heron I., S GBR); also Indian Ocean (Chagos) and W Pacific (New Caledonia).

DESCRIPTION. *Growth form*. Massive, globular, slightly elongated-globular to pyriform, elongate. Specimens range from 1-5cm.

Colour: A distinct bright yellow colour in life, brownish-beige after fixation with Glutaraldehyde in EtOH.

Oscules. Globular to pyriform specimens have one prominent osculum with a naked 'lip' at the apical end of the body. Elongated specimens have a few oscules with a naked 'lip' scattered on the ridge of the sponge body.

Texture. Firm and smooth, not harsh, soft compared to *L. microraphis*, slightly translucent surface.

Surface ornamentation. Surface is unornamented, although sometimes small protuberances are present on elongated specimens.

Ectosomal skeleton. A distinct thin cortex, up to 50µm thick, is supported by tangentially arranged small regular triactines, with some large triactines also scattered tangentially in the cortex. Small sub-dermal cavities (50-150µm diameter) are present, with their walls partially formed by bundled actines of perpendicular triactines of the upper choanosome. The zone of sub-dermal cavities, which is devoid of choanocyte chambers, is up to 250µm thick. Large regular triactines are also found in the zone of sub-dermal cavities, either tangentially aligned, or scattered perpendicular to the surface. A peculiar, special, small sagittal triactine is only found in the oscular rim ('lip'), where both paired actines are bent from each of their actinal center about 30° towards the unpaired actine (Fig. 8L).

Choanosomal skeleton. A dense meshwork of numerous small regular triactines, aligned in such a way that they form an irregular, sometimes sub-hexagonal to hexagonal pattern with their actines. Choanocyte chambers are located within the free space provided within this hexagonal or irregular network. Larger triactines are occasionally scattered and irregularly arranged in the choanosome in variable numbers (usually only a few are present), but never reaching the high numbers of small triactines. Small tetractines are found concentrated in the exhalant canals, with their basal triradiate system tangentially aligned to form the walls of canals, and their mostly straight and elongated free apical actine protrudes into the canal. Tetractines were only very rarely found in the choanosome, and when present, they were in close proximity to a canal. The number of tetractines varies from

specimen to specimen.

Aquiferous system. Water enters the sponge body via ostiae (20-50µm diameter) and incurrent canals, which pierce the cortex in spaces between tangential triactines. These incurrent canals are 50-100µm wide and become wider below the sub-dermal cavities to supply the choanosome with sea water. Walls of the incurrent canals in the choanosome are only rarely formed by tangentially aligned triactines, whereas mostly canals are found between the hexagonal network formed by triactines aligned perpendicular to canals. Walls of exhalant canals, in contrast, are formed by the tangentially aligned basal triradiate system of tetractines. These canals are wider than incurrent canals and open in an irregular pattern into the more-or-less wide atrium, which never reaches the dimensions of those seen in *Pericharax heteroraphis*. The aquiferous system has a leuconoid grade of construction.

Soft tissue. Soft tissue of the cortex is free of choanocytes but contains some scattered amoeboid cells, probably archaeocytes, up to 10-30µm long. The choanosome follows the cortex without a sharp transition. Choanocytes are sub-spherical, 4-8µm diameter in section parallel to the surface, and choanocyte chambers are spherical, 80-100µm diameter. Water canals are lined by the endopinacoderm, which consists of relatively large and stretched pinacocytes (up to 12µm long).

Spicules. Larger regular triactines with conical actines, actines: 250-(361)-500x25-(34.7)-50µm (n=25); smaller regular triactines with conical actines, actines: 98-(129.5)-158x 12-(16.7)-22µm (n=30); regular tetractines of the excurrent canals with cylindrical actines 93-(107.3)-125× 10-(12.8)-18µm (n=25); rare sagittal triactines of oscular rim ('lip') 70-(88.6)-120×9-(11.4)-15µm (n=7). In this latter spicule type all three actines have the same total length, the unpaired angle is slightly enlarged than or equal to the paired angles, and both paired actines of the triactine are each bent from or below their actinal center about 30° towards the unpaired actine (Fig. 8L).

Leucetta villosa sp. nov.
(Fig. 9A-F, Table 2)

ETYMOLOGY. For the hair-like extensions on the sponge surface (Latin, *villosus*).

MATERIAL. HOLOTYPE: QMG313662, S side of Wistari Reef, GBR, 23°29.4'S, 151°52.8'E, 18m depth, 07.vii.1998, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. Coral patches, small crevices and overhangs under coral bommies, Zone 2 (Fig. 2), 18m depth. Wistari Reef, S GBR.

DESCRIPTION. *Growth form*. Massive, convoluted-lobate, 5×5×3cm.

Colour. Yellow-brownish in life, bright beige after fixation in Glutaraldehyde in EtOH.

Oscules. Many oscules, 2-5mm diameter, on ridges of the sponge body. Oscules possess a small naked 'lip'.

Texture. Firm but not harsh, slightly translucent surface.

Surface ornamentation. The surface has characteristic 'hairy' surface extensions, which protrude 1-3mm above the external surface.

Ectosomal skeleton. A distinct, thin cortex, up to 50µm thick, is supported by tangentially arranged small regular triactines, with some large triactines also scattered tangentially in the cortex. Small sub-dermal cavities (50-100µm diameter) are only infrequently developed, and where present, their walls are partially formed by the bundled actines of perpendicular triactines from the upper choanosome. A sub-cortical zone, which is devoid of choanocyte chambers, is present in some parts of the sponge, approximately 100-150µm thick. Where the subcortical zone is absent, the choanosome follows sharply under the cortex. Large regular triactines are also found in places within the sub-dermal zone, and where present, they are either tangentially aligned, or scattered perpendicular to the surface (then protruding into the choanosome). Unlike *L. chagosensis*, there are no peculiar small sagittal triactines in the oscular rim.

Choanosomal skeleton. A dense meshwork of numerous small regular triactines, aligned in such a way that they form an irregular, sometimes sub-hexagonal to hexagonal pattern with their actines. Choanocyte chambers are located in the free space provided within this meshwork, sometimes hexagonal but mostly forming irregular patterns. Larger triactines are scattered and irregularly arranged within the choanosome in moderate numbers, but never reaching the high abundance of small triactines. Small tetractines are not very common and mostly found concentrated in the larger exhalant canals, with their basal triradiate system tangentially aligned to form the walls of canals. Their mostly straight, free apical actine protrudes into the canal. Rare tetractines were also found in the choanosome.

Aquiferous system. Water enters the sponge body via ostiae (although not seen in sections) and

incurrent canals, which pierce the cortex in the space between tangential triactines. These incurrent canals are 50-100µm wide and become wider below the sub-dermal cavities to supply the choanosome with sea water. Walls of the incurrent canals in the choanosome are only rarely formed by tangentially aligned triactines, whereas mostly canals are found between the sub-hexagonal to spherical network produced by triactines aligned perpendicular to the canal. Walls of the exhalant canals, in contrast, are formed by tangential triactines and the tangentially aligned basal triradiate system of tetractines. These canals are wider than incurrent canals and open, in an irregular pattern, into larger excurrent canals (up to 5mm diameter), which may fuse just below the osculum. No single central large atrium is present. The aquiferous system has a leuconoid grade of construction.

Soft tissue. Soft tissue of the cortex is free of choanocytes and only rarely contains amoeboid cells, most likely archaeocytes, up to 6-10µm long. The choanosome follows the cortex without a sharp transition. Choanocytes are sub-spherical, 3-6µm diameter in section parallel to the surface, choanocyte chambers are spherical, 70-100µm diameter.

Spicules. Larger regular triactines with conical actines, sometimes slightly parasagittal (unpaired actine slightly longer): 300-(405.5)-490x30-(37.4)-40µm (n=25); smaller regular triactines with conical actines: 100-(118.3)-134x12-(16.3)-20µm (n=30); few regular conical tetractines with reduced undulated apical actine 95-(105)-120x10-(13.1)-16µm (n=25).

REMARKS. *Leucetta microraphis* and *L. chagosensis* have been previously reported from the GBR (Heron Island and Wistari Reef) by Pulitzer-Finali (1982), and from Torres Strait by Ridley (1884) and Poléjaeff (1883, as *Leuconia dura*), but none of this material was comprehensively or adequately described. Furthermore, it appears that Pulitzer-Finali (1982) misidentified his specimens of '*L. microraphis*'. The specimen depicted in his Figure 1 is more probably *Pericharax heteroraphis*, due to its possession of an irregularly folded external surface and a wide central osculum, both highly characteristic for *P. heteroraphis* (see below). *Leucetta microraphis* is clearly different in having: 1) multiple small oscules (nowhere near the size in the specimen figured by Pulitzer-Finali (1982)); 2) no folded external surface; and 3) no 'ample internal cavity' (Pulitzer-Finali, 1982), but shares the large ('giant') triactines. The peculiar small sagittal triactines and tetractines described here from the oscular rim of the GBR *Leucetta microraphis*, were also described by Borojevic & Peixinho (1976) from their specimen from Brazil, but due to the presence of large 'giant' tetractines in the cortex of the Brazilian specimen, Borojevic & Klautau (in press) suggested it might be more appropriately placed in the Atlantic '*Leucetta floridana*' (Haeckel, 1872) complex (with 'giant' tetractines), which is supposedly a sibling species of *L. microraphis*.

Differentiating between species of *Leucetta* is similar to the problems described above for *Clathrina*, largely due to the possession of only few diagnostically important morphological characters (i.e. two types of triactines). Consequently, statistical analysis of the most frequent category of spicule (small triactines) was undertaken for three specimen *of L. chagosensis*, and one each of *L. microraphis* and *L. villosa* (Table 2). Pairwise comparisons between actinal length of 30 spicules for each specimen, using a Student t-test, showed that the means of G313659 (*L. microraphis*) and G313662 (*L. villosa*) were significantly different at a confidence level of 99% ($t=2.663$, with $P<0.01$) compared to each other, and compared to the means of *L. chagosensis* (G313654, G313658 and G313667). The means of the latter three specimens were not significantly different compared to each other ($t=2.663$, $P>0.01$). These three specimens agree with the description of *L. chagosensis*, based on their morphological features and characteristic bright-yellow color, as described by Dendy (1913) and Borojevic (1967). The peculiar sagittal triactines in the oscular rim of *L. chagosensis*, as described by Borojevic (1967: Fig. 1D) from New Caledonia, were also found in *L. chagosensis* from the GBR. In addition to having significantly different mean dimensions in actinal spicule lengths, *L. microraphis* is clearly characterised by the presence of large ('giant') triactines (not present in other species of *Leucetta*), its distinct growth form, color, texture, and the relatively small size of its choanocyte chambers (50-60µm diameter). The peculiar sagittal triactines and tetractines found around the small oscular rim of *L. microraphis* were also mentioned by Poléjaeff (1983: 65, Pl. 7, Fig 7) in his description of *'Leuconia dura'*, which is a junior synonym of *L. microraphis* (Burton, 1963; Borojevic, 1967). Compared to *L. chagosensis* and *L. microraphis*, *L. villosa* can be

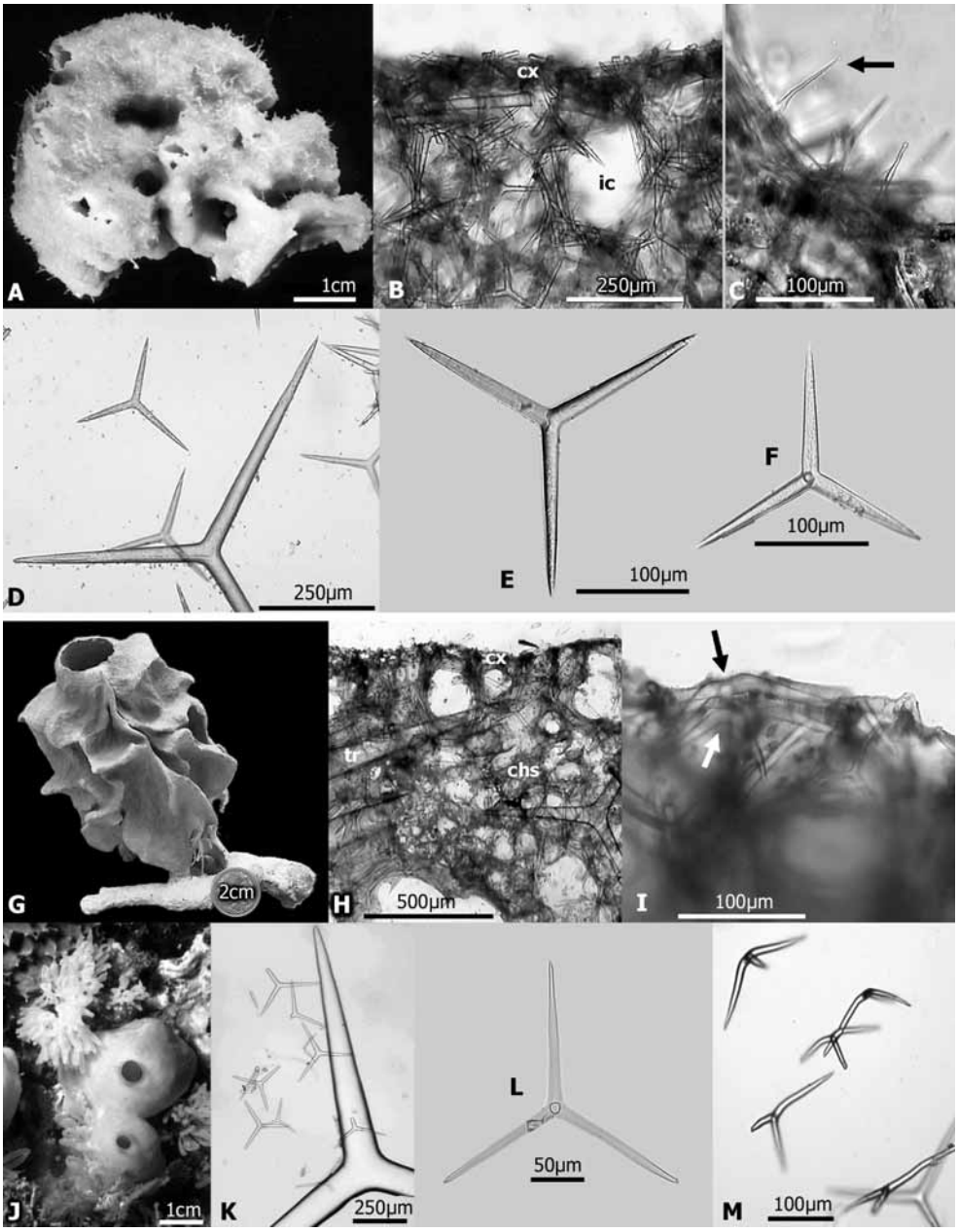

FIG. 9. *Leucetta* and *Pericharax* spp. A-F, *Leucetta villosa* sp. nov. (holotype QMG313662). A, specimen after fixation. B, section through the cortex (cx), choanosome with irregularly arranged triactines and one incurrent canal. C, excurrent canal with free apical actines of tetractines pointing into canal (arrow). D, two size classes of triactines. E, small triactine with conical actines. F, tetractine. G-M, *Pericharax heteroraphis* Poléjaeff, 1883 (specimen QMG313653, in situ photograph, sections, spicules). G, dried larger specimen with irregularly folded external surface and one large central osculum. H, section through cortex (cx) and choanosome (chs) with large triactines (tr). I, enlargement of the cortex with 'tripod-like' triactines (arrows), characteristic for *P. heteroraphis*. J, two small specimen in situ at Wistari Reef (center), together with *Soleniscus radovani* sp. nov. (upper left). K, two size classes of triactines. L, tetractine. M, 'tripod-like' triactines (compare with Fig. 9I).

distinguished by statistically significant differences in the mean actinal lengths of small triactines (at a confidence level of 99%; Students *t*-test, pairwise comparisons, t=2.663, P<0.01), its distinct growth form, color, hirsute surface, the absence of small sagittal triactines in the oscular rim, and the different mean lengths of its large triactines.

Tropical and subtropical species of *Leucetta*, together with *Pericharax heteroraphis*, are the most common and abundant calcareous sponges in the exposed and semi-cryptic habitats of the GBR. Regional populations of these species certainly require a comprehensive morphological and genetic study, and thorough taxonomic revision. These studies, (population genetics, intraspecific morphological variation, molecular and morphological analyses) are currently in progress and will hopefully provide valuable new information on the taxonomy of this important species complex of *Leucetta*, particularly of GBR species.

Pericharax Poléjaeff, 1883

Pericharax Poléjaeff, 1883: 66.

Leucettidae with a large central atrium surrounded by a thick wall. The wall is divided into a choanoderm and a thin subcortical layer of inhalant cavities, supported by a peculiar skeleton partially composed of the centripetal actines of the special cortical triactines.

Pericharax heteroraphis Poléjaeff, 1883 (Fig. 9G-M)

Pericharax carteri heteroraphis Poléjaeff, 1883: 66, pl. 2, fig. 5, pl. 7, fig. 8.
Pericharax heteroraphis; Dendy, 1913: 13; Dendy & Row, 1913: 735; Burton, 1963: 260, text-fig. 126; Wilkinson, 1978a: 162; Wilkinson; 1978b: 172; Wilkinson, 1978c: 178, fig. 1; Wilkinson 1979: 794.
Pericharax peziza Dendy, 1913: 15, pl. 1, fig. 9, pl. 5, figs 3-4; Dendy & Row, 1913: 735; Burton, 1930: 3; Burton, 1934: 518.
Pericharax pyriformis Burton, 1932: 258, pl. 48, figs 1-2.

MATERIAL. HOLOTYPE: BMNH1884.4.22.56-57, Tristan Da Cunha, 'Challenger' Collection. OTHER MATERIAL: QMG313652, QMG313653, Tenements, N side of Heron I., GBR, 23°26.05'S 151°57.1'E, 15m depth, 22.vi.1998, coll. G. Wörheide (SCUBA); QMG313657, Wistari Reef, Wistari Channel, GBR, 23°27.5'S, 151°55'E, 17m depth, 06.vii.1998, coll. G. Wörheide (SCUBA); QMG313660, S side of Wistari Reef, GBR, 23°29.4'S, 151°52.8'E, 18m depth, 07.vii.1998, coll. G. Wörheide (SCUBA).

HABITAT AND DISTRIBUTION. Widely distributed in exposed and semi-shaded habitats of coral reefs, sometimes under overhangs, Zones 1-2 (Fig. 2). Widely distributed, allegedly nearly cosmopolitan. Reported distribution in Australia: GBR (Qld), Pascoe Reef, Papuan Pass; also S Atlantic, Indian Ocean, Indo-Malayan region and Subantarctic.

DESCRIPTION. *Growth form*. Massive, bulbous, rarely clavate. Young (small) specimens pyriform, with external surface not folded; large (older) specimens with characteristic irregularly folded external surface. Maximum size observed for the species was 25cm in height (specimen from Lizard Island, N GBR).

Colour. Yellow-greenish to dark greenish-brown in life and brownish after fixation with Glutaraldehyde in EtOH

Oscules. One large prominent terminal osculum always present, mostly with prominent 'lip'.

Texture. Firm, harsh, surface smooth but brittle due to large triactines protruding through the surface.

Surface ornamentation. Smooth in small specimens, irregularly folded in large specimens.

Ectosomal skeleton. A thin but distinct cortex is present, up to 50µm thick, consisting of tangentially aligned, small, characteristic tripod–like triactines, sometimes sagittal, with curved paired actines. Both small and large triactines are also present, with the former tangential or perpendicular to the surface, and the latter more-or-less tangentially arranged. Subdermal cavities are present in most areas below the cortex (50-200µm diameter), and are supported in their lower regions by perpendicularly 'bundled' actines of small choanosomal triactines, forming a distinct sub-cortical skeleton, devoid of choanocyte chambers. The zone of sub-dermal cavities is up to 400µm thick. Scattered large triactines are also arranged parallel to the surface in the sub-dermal area.

Choanosomal skeleton. A dense meshwork of mainly regular small triactines forms the choanosomal skeleton. These small triactines are aligned in such a way that their actines form an irregular, often sub-hexagonal to hexagonal pattern. choanocyte chambers are located between the sub-hexagonal to hexagonal, or sometimes irregular skeletal meshwork. Some small tetractines are also found scattered throughout the choanosome, without any apparent order or alignment. Many large 'giant' triactines are dispersed throughout the choanosome without any recognisable order. Small tetractines are concentrated at, but not restricted to, the excurrent water canals. Their tangential basal triradiate system

forms the wall of canals, and the shorter and curved free apical actine protrudes into the exhalant water canal and atrium.

Aquiferous system. Water enters the sponge body via ostiae (30-50µm diameter) and incurrent canals, which pierce the cortex in the space between tangential triactines. These incurrent canals, 100-200µm wide, become wider below sub-dermal cavities in order to supply the choanosome with sea water. Walls of the incurrent canals in the choanosome are only rarely formed by tangentially aligned triactines, whereas canals are mostly found between the hexagonal network formed by triactines aligned perpendicular to the canal. Walls of the exhalant canals are more frequently formed by tangentially aligned basal triradiate system of tetractines and by tangential triactines. These excurrent canals are much wider than incurrent canals and open in a regular pattern into the wide central atrium The surface of this central atrium resembles, in larger specimens, a 'sieve'-like surface with regularly sized (0.5mm), and densely arranged openings. The aquiferous system has a leuconoid grade of construction.

Soft tissue. The cortex and the zone of sub-dermal cavities is devoid of choanocyte chambers, but many other cell-types are present. Most of these are spherical or amoeboid, some contain small granules, ranging in size 4-18µm. It is not yet certain but it is likely that the amoeboid cells are archaeocytes, the granular cells are some type of storage cell, and the spherical cells possibly oocytes. Some cells are 'wrapped' and attached to spicules, and are probably sclerocytes. The choanosome follows the cortex with a more-or-less sharp transition and is dense. Spherical choanocyte chambers are 80-120µm diameter, with sub-spherical to polygonal choanocytes (4-6µm diameter in section parallel to the surface). A few apopyles were observed, up to 35µm diameter, lined by two to three elongated apopylar cells.

Spicules. Large regular triactines with conical actines, with actines: 550-(834)-1570×70-(108.6)-180µm (n=25); smaller regular triactines with more-or-less cylindrical actines, with actines: 120-(159.4)-190×15-(18.64)-22µm (n=25); tetractines with a mostly regular basal triradiate system and slightly shorter and curved apical actine, sometimes one or two actines of the basal triradiate system are bent, sometimes the basal triradiate system is slightly parasagittal, with the actines of the regular basal triradiate system: 90-(128.4)-180×10-(12.16)-18µm (n=25); tripod-like cortical sagittal triactines, with curved paired actines: 45-(93.8)-130× 7-(10)-15µm (n=25), with the unpaired actine sometimes slightly either shorter or longer, and the unpaired angle is larger than the paired angles.

REMARKS. *Pericharax heteroraphis* is the most common calcareous sponge in exposed and semi-cryptic habitats throughout the GBR and other tropical seas of the Indo-Pacific. Large specimens of *P. heteroraphis* are easily differentiated macroscopically from the highly abundant *Leucetta* spp. by their distinct greenish-brown color and their irregularly folded external surface. However, small specimens do not show a distinct surface folding and sometimes closely resemble *Leucetta chagosensis* in growth form, although most can be distinguished by their color (*L. chagosensis* is very bright yellow, small *P. heteroraphis* tend to be darker). In most cases, especially for specimens not observed in situ (i.e. dried and preserved specimens), a taxonomic decision is only possible from examination of spicule morphology, where only *P. heteroraphis* possesses the species-characteristic tripod-like small triactines. *Pericharax heteroraphis*, one of the so-called 'circum-Pacific' species, is the current target of studies on population genetics and intra-specific variation in Calcarea throughout the GBR and Coral Sea. This study will investigate whether 'widely distributed' Indo-Pacific species represent genetically distinct, reproductively isolated populations of morphologically closely related taxa ('morphospecies'), or are truly 'widely distributed' with regional populations in genetic contact (Wörheide, in preparation).

RESULTS OF STABLE ISOTOPE ANALYSIS OF CALCAREOUS SPICULES

Bulk samples of calcareous spicules from five species of Calcinea, described in this work, and four species of Calcaronea, to be described in the second part of this series (Wörheide, in preparation), were analysed for their stable isotope values ($\delta^{13}C$, $\delta^{18}O$). These results are presented in Figure 10. Data show that Calcinea have negative $\delta^{13}C$ values and Calcaronea have positive $\delta^{13}C$ values. $\delta^{18}O$ values range from -0.28 to -1.41 in Calcaronea, and -1.39 to -2.39 in Calcinea .

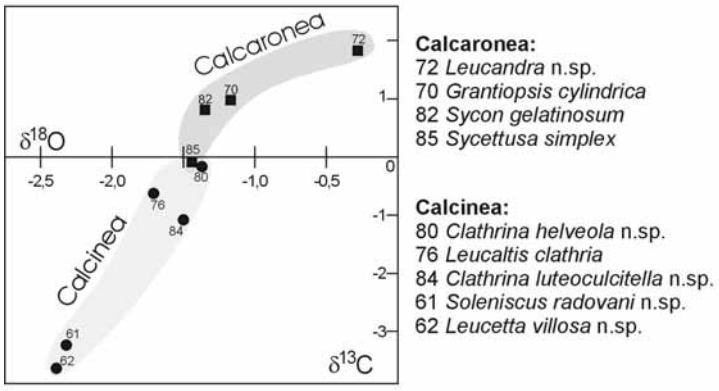

FIG. 10. Stable Isotope values ($\delta^{13}C/\delta^{18}O$) of calcareous spicules of nine Calcarea from Heron Island and Wistari Reef (Capricorn/Bunker Group, GBR).

DISCUSSION

STABLE ISOTOPES. Reitner (1992) first showed that stable isotope values of calcareous spicules ($\delta^{13}C$, $\delta^{18}O$) were useful to differentiate between Calcinea and Calcaronea, which is clearly confirmed by present results. Distinct $\delta^{13}C$ values indicate that different biocalcification processes occur in each subclass, involving different fractionation processes. This is most likely a result of different cellular and/or subcellular mechanisms involved in spicule formation occurring in each subclass, as proposed by Reitner (1992), although no specific mechanisms have yet been considered. Of relevance here is the fact that the most highly developed species (i.e. with most developed canal system), *Leucandra* sp. (no. 72 in Fig. 10) and *Leucetta villosa* (no. 62 in Fig. 10), show the highest and lowest $\delta^{13}C$ values, respectively. Conversely, differences in $\delta^{18}O$ values cannot be explained at the present stage without further investigation. Further stable isotope analyses of calcareous spicules are in progress, investigating the potential value of isotopic data to support differentiation between Calcarea at the subclass, and possibly also lower taxonomic level. Since the work of Ledger (1976) and Ledger & Jones (1977) the biogenesis of calcarean spicule development was thought to be uniform throughout Calcarea, although previous data was only comprehensive for Calcaronea (*Sycon ciliatum*, *Leucosolenia complicata*; see review in Simpson, 1984). Although preliminary, present data strongly indicate the biological basis for differentiating Calcinea and Calcaronea, supporting Borojevic's et al. (1990, in press) proposal based on morphological data. However, our data also emphasise that a comprehensive and detailed re-examination of biocalcification processes is required for Calcarea in general (currently in progress), involving investigations at the cellular (SEM/TEM) and subcellular level (analysis of biogeochemistry: biomarkers, intracrystalline organic matrix; molecular techniques) (Wörheide, in prep.).

TAXONOMY. The present study describes 14 species of calcarean Calcinea,including 8 new species, whereas prior to this work only 4 species had been described for the entire GBR (Hooper & Wiedenmeyer, 1994). We have demonstrated in this preliminary study that the vast world heritage reef system of the GBR is virtually unexplored with regard to calcarean biodiversity. This is perhaps not surprising given that this study is the first comprehensive taxonomic investigation of Calcarea in Australasia in more than 50 years. We have also shown that in two genera, where only few 'classical' morphological characters can be used effectively for species identification, clear differentiation of species is very difficult, reinforcing the problems encountered by previous authors studying Calcarea, and explaining why calcarean systematics is still in flux. Biochemical and molecular studies have shown that very small morphological differences in several species of Calcarea (e.g. Atlantic *Clathrina*) correspond to quite large genetic differences, otherwise undetectable in their 'classical' morphology (Solé-Cava et al., 1991; Klautau et al., 1994). 'Classical' taxonomy thus appears to be in many cases overconservative. Characters used to differentiate species and higher taxa of Calcarea, clearly, require rethinking, making 'integrative taxonomic' approaches essential. 'Integrative taxonomy' combines different multidisciplinary approaches, which in Calcarea would involve examination of soft part ultra-structure, stable isotope and trace element analysis of spicules, molecular techniques, biogeochemical characterisation, and re-evaluation of the importance of certain 'traditional' morphological characters based on the above data.

However, a taxonomy based on, or supported by non-morphological features (e.g. molecular data) should in some way be related back to 'classical' morphology in order to be usable and practical, and this still remains a major challenge for Calcarea.

A major theme during this ongoing project in studying Australasian Calcarea is the application of modern, multidisciplinary, and integrative approaches, searching for valuable new characters in particular. The first results of this approach, presented here (stable isotope values of calcarean spicules), have shown that these new data can contribute significantly to the taxonomy of this important poriferan class, with wider applications also apparent to the biology of Porifera in general.

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