

Phylogeny of *Labidodemas* and the Holothuriidae (Holothuroidea: Aspidochirotida) as inferred from morphology

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The Holothuriidae is one of the three established families within the large holothuroid order Aspidochirotida. The approximately 185 recognized species of this family are commonly classified in five nominal genera: *Actinopyga*, *Bohadschia*, *Holothuria*, *Pearsonothuria* and *Labidodemas*. Maximum parsimony analyses on morphological characters, as inferred from type and nontype material of the five genera, revealed that *Labidodemas* comprises highly derived species that arose from within the genus *Holothuria*. The paraphyletic status of the latter, large (148 assumed valid species) and morphologically diverse genus has recently been recognized and is here confirmed and discussed. Nevertheless, we adopt a Darwinian or eclectic classification for *Labidodemas*, which we retain at generic level within the Holothuriidae. We compare our phylogeny of the Holothuriidae with previous classifications of its genera and subgenera, and make suggestions concerning possible systematic changes. © 2005 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2005, 144, 103–120.

ADDITIONAL KEYWORDS: *Actinopyga* – *Bohadschia* – classification – *Holothuria* – *Pearsonothuria* – systematics.

INTRODUCTION

Recently, Massin, Samyn & Thandar (2004) reviewed the holothuroid genus *Labidodemas* Selenka, 1867. They described three new species and transferred two species from *Holothuria* Linnaeus, 1767, the first a synonym of the type species and the second a valid species. They were also the first to discover that one species in *Labidodemas* (*L. americanum* Deichmann, 1938) possesses Cuvierian tubules, a finding which allowed them to question the family rank that James (1981; see also Rowe, 1969 for a more balanced view of ranking) had attributed to the group. As the other four

genera in the Holothuriidae – *Actinopyga* Bronn, 1860, *Bohadschia* Jaeger, 1833, *Pearsonothuria* Levin, 1984 and *Holothuria* – also have representatives that possess this organ, Massin *et al.* (2004) argued that *Labidodemas* is best kept within the Holothuriidae. They regarded the presence of the tubules as a synapomorphic character of the Holothuriidae and their absence as being due to secondary loss. With regard to taxonomic rank, they gave high weight to the ribbon-like form of the calcareous ring and proposed retaining *Labidodemas* at the generic level. However, they urged that a phylogenetic analysis must be carried out to determine the exact systematic position and taxonomic rank of *Labidodemas*. Such studies have been conducted by two independent teams. Kerr *et al.* (2005), on the basis of a 16S mtDNA sequence, and Appeltans (2002), on the basis of morphological characters, who both found that *Labidodemas* indeed falls within the Holothuriidae, more specifically within *Holothuria*. However, as neither Appeltans nor Kerr

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et al. were in a position to include all the currently existing species within *Labidodemas*, they could not accurately test its monophyly or present a phylogeny.

For the present paper, we performed a cladistic analysis on 132 morphological characters drawn from type and nontype species of the five currently recognized holothuriid genera. This analysis allowed objective selection between two recent scenarios that attempted to explain the direction of evolution in *Holothuria*. The first was formulated by Deichmann (1958: 276), who considered *Labidodemas* to be a sister genus of *Holothuria s.l.*, arguing that within *Holothuria* 'most primitive are undoubtedly those with numerous regular tables and regular smooth buttons, somewhat reminiscent of certain synallactid-like members of the Stichopodidae', whilst 'a more advanced stage is indicated by the presence of irregular buttons, or the development of rosettes, or the reduction of the inner layer of spicules, while the tables have become variously modified'. The second is attributed to Rowe (1969), who also regarded *Labidodemas* as a sister clade to the other holothuriid genera (but see also James, 1981; Massin *et al.*, 2004), but contrary to Deichmann (1958), argued that within *Holothuria*, species with plates and without tables or buttons represent the more primitive condition, and those with regular tables and buttons the more advanced forms. Massin, Mercier & Hamel (2000), in their detailed study of the ontogeny of ossicles in *Holothuria (Metriatyla) scabra* Jaeger, 1833, came to the conclusion that the absence of buttons and presence of tables with tall spires are plesiomorph characters in the evolution of the Holothuriidae. From an ecological point of view this implies that, according to Deichmann (1958), holothurian surf-zone species (inhabiting exposed places such as rock-crevices) and rock-clinging species are more advanced, whilst according to Rowe (1969; pers. comm.) the latter forms are considered primitive. Whereas Deichmann (1958) did not provide a satisfying explanation for her line of reasoning, Rowe emphasized that his views are based on the conclusions of Pawson & Fell (1965), who argued that dendrochirots (with dendritic tentacles) are more primitive than aspidochirots (predominantly peltate tentacles). Thus, to Rowe (1969; pers. comm.), holothurians with more dendritiform tentacles (as found in the subgenera *Selenkothuria* Deichmann, 1958 and *Semperothuria* Deichmann, 1958) are to be considered more primitive. With regard to the evolution of the genera, neither author took a position, although Rowe (pers. comm.) nowadays advocates that *Actinopyga* and *Bohadschia* are derived possibly through the *Pearsonothuria* form which is (i) more *Holothuria*-like in body form and (ii) appears to have highly modified tables (raquets) and very complex rosettes.

SYSTEMATICS OF THE HOLOTHURIIDAE THROUGH TIME

Ever since its description, the alpha- and beta-taxonomy of *Labidodemas* Selenka, 1867 has been the subject of considerable and often conflicting debate (see Massin *et al.*, 2004 and references therein). This is hardly surprising, as the taxonomic history of *Holothuria* and the Holothuriidae has itself been the subject of much, at times chaotic, debate which is briefly reviewed here.

At the beginning of the twentieth century, the Holothuriidae were commonly divided on the basis of presence and/or absence of anal teeth and the position of tube feet. Two genera, *Mülleria* Jaeger and *Holothuria* L., were recognized. Pearson (1914), after examination of 'a large number of species', proposed a re-classification, arguing that the number and arrangement of tentacles, Polian vesicles, stone canals and Cuvierian tubules must be disregarded for classification purposes. Instead, he utilized the structure of the calcareous ring, the ossicle assemblage and the arrangement of tube feet and papillae to arrive at a classification that is 'in accordance with relationship [sic]'. Pearson (1914) grouped *Mülleria* and *Holothuria (sensu stricto)* within the single genus *Holothuria (sensu extenso)* wherein he discerned five subgenera: *Actinopyga*, *Argiodia* Pearson, 1914, *Bohadschia*, *Halodeima* Pearson, 1914 and *Thymiosycia* Pearson, 1914. The first two contained species formerly classified under *Mülleria* while the remainder held species formerly classified under *Holothuria s.s.* (Fig. 1).

Pearson (1914) further believed that *Actinopyga* and *Bohadschia* (with the ambulacral appendages more or less arranged in rows, the ossicles in the form of rosettes and rods, the calcareous ring without anterior and posterior projections, but with deep ampullary notches and the interradial pieces almost as high as the radial pieces) represent the primitive condition whereas *Argiodia*, *Halodeima* and *Thymiosycia* (with scattered ambulacral appendages, table and button ossicles and a calcareous ring with pronounced anterior and posterior projections and a deep indentation between the radial and interradial pieces) are the more advanced forms.

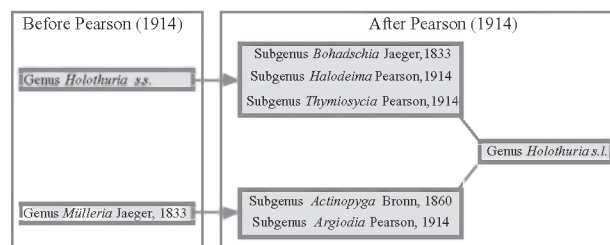


Figure 1. Classification of the genus *Holothuria* before and after Pearson's (1914) revision.

H. L. Clark (1921) completely ignored Pearson's work and opted to follow Fisher's (1907) classification, albeit with some modifications vis-à-vis the rank of the Holothuriidae, which he divided into five genera: *Actinopyga*, *Labidodemas*, *Holothuria*, *Stichopus* Brandt, 1835 and *Thelenota* Brandt, 1835. This classification was largely followed by subsequent authors (Deichmann, 1926; Domantay, 1933), although it is unclear why this generation of taxonomists systematically ignored Haeckel's (1896; see also Östergren, 1907) important revision separating the Stichopodidae from the Holothuriidae.

Panning's (1931b, 1935a, b, c, d) revision of *Holothuria* stands as a series of highly significant works in the classification of *Holothuria*. Not only did he examine practically all the available literature, he also critically analysed most of it and compiled virtually complete synonym lists. Panning's *magnum opus* was perhaps a bit too descriptive and conservative, as noted by Deichmann (1958; see also Rowe, 1969: 121). He tried to follow Pearson's (1914) classification but rapidly (and understandably) came into conflict with the latter's observations.

In his first paper Panning (1931b) listed three pertinent reasons why Pearson's system needed rethinking. First, he argued that Pearson's new taxa were too vaguely described and that it was not clearly stated which species they contained. Second, he noticed that Pearson separated *Thymiosycia* from *Halodeima* on only a single character (i.e. *Halodeima* with tube feet and papillae, *Thymiosycia* with papillae only), which to Panning was insufficient justification (he chose to make *Thymiosycia* a synonym of *Halodeima*). Third, he opposed Pearson's arbitrariness in not considering the geographical distribution (Atlantic vs. Pacific) of the different species, and argued that by doing so Pearson had necessarily overlooked a large number of synonyms.

Panning (1931b) recognized *Actinopyga*, *Bohadschia*, *Halodeima* and *Microthele* Brandt as subgenera in *Holothuria* (*sensu* Pearson). He correctly recognized that *Microthele* has priority over Pearson's (1914) *Argiodia*, although he did not acknowledge it in the original (Brandt, 1835) meaning. By 1935 (a, b, c, d) Panning had altered and refined his classification. On Fisher's advice (see Panning, 1935a: 24) he grouped Pearson's (1914) subgenera *Halodeima* and *Thymiosycia* in the subgenus *Holothuria s.s.* Thus, Panning (1935a, b, c, d) now recognized four subgenera in the genus *Holothuria s.l.*: *Actinopyga*, *Bohadschia*, *Microthele* and *Holothuria s.s.* He believed (Panning, 1935a: 25) that *Actinopyga* was most closely related to *Microthele* and *Bohadschia* to *Holothuria s.s.*

Based upon his studies of the optical properties of ossicles, Panning (1928, 1931a, c, 1933, partially drawing on Hérouard, 1889, 1925 and Perrier, 1902,

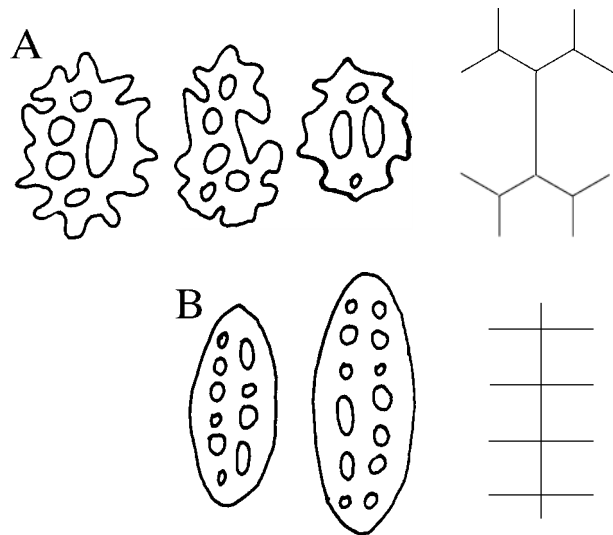


Figure 2. Rosettes vs. buttons according to Panning (1951: 78). A, rosettes of *Holothuria* (*Halodeima*) *grisea* Selenka, 1867, with a schematic representation of the bifurcate rod. B, true buttons of *H. (Platyperona) sanctori* Delle Chiaje, 1823, with a schematic representation of the branching of the primary rod.

but see also Schmidt, 1925, 1932) concluded that *Holothuria* was best split into two groups, those with rosettes and those with true buttons. He defined rosettes as small, thin plates that develop from a rod which bifurcates at each end (*Gabelstab*), the terminal branches growing at an angle of 120° from the rod and eventually anastomosing, thus forming large, lateral perforations, with a pair of terminal holes always present. The central perforations are often rather large and not round, while the branches are generally thin and the overall shape of the rosette is irregular. True buttons on the other hand, even if they are *an sich* also thin plates, arise from a nonterminally branching primary rod (*ungegabelten Primärstab*) that develops lateral projections perpendicular to the primary rod. As such, when these projections bifurcate at their ends and eventually anastomose, pairs of opposite perforations, one on either side of the median rod, are formed; terminal holes are absent. Moreover, in true buttons, the holes are generally smaller and more roundish; their rims (when fully formed) are rather smooth, giving the impression of a 'finished ossicle'. Figure 2 reproduces Panning's (1951) drawings on the position of the optical axes as present in rosette-like buttons and in true buttons as well as scaled drawings of the two ossicle types.

Panning, even though he continually defended his line of reasoning, unfortunately failed to develop his observations into an unambiguous classification of

Holothuria and was drawn to the conclusion that ‘in theory, these are the forms of both ossicle forms, but deviations and intermediates may also be the rule [Our liberal translation from Panning’s (1935a: 25) German]’. Perhaps this is the reason why he stated that he did not want to burden the systematics of the subgenus with yet further names and why he instead created two large groups.

His *Abteilung A* grouped those species with rosettes and rosette-like buttons, while his *Abteilung B* grouped those species that possess true buttons. To the latter he gave the name *Sporadipus*, a designation originally given by Brandt (1835). In these two subdivisions he (Panning, 1935a, b, c, d) discerned several smaller groups, which he based almost exclusively on ossicle assemblages. As such, he created five divisions (*Reiche*) in *Abteilung A* and eight divisions in *Abteilung B* (*Sporadipus*).

By 1940, (Panning, 1940: 523) he realized that: ‘the fact that both subgenera (*Actinopyga* and *Microthele*) possess anal teeth is only a convergence in which we nevertheless cannot go into detail as we have no idea of the function of these structures’ [our liberal translation from Panning’s German, with our brackets] and that thus *Actinopyga* and *Microthele* can no longer be considered closely related taxa. Moreover, as he thought that *Actinopyga* and *Bohadschia* differed from one another only by the presence of anal teeth in *Actinopyga*, he made the former a subgenus of the latter.

In addition, he raised *Abteilung A* to genus level as *Halodeima*, while *Abteilung B* was split into *Microthele* and *Holothuria*. Confusingly, he noted that under certain circumstances *Microthele* has to be seen as ‘only a subgenus of *Holothuria*’ (Panning, 1940: 524, our translation). By 1944, Panning reached his final classification of *Holothuria*, now recognizing *Actinopyga*, *Bohadschia*, *Halodeima*, *Holothuria* and *Microthele*. This classification was accepted and used by subsequent taxonomists, including Tortonese and Cherbonnier. However, H.L. Clark (1946) noted that (our square brackets):

‘the natural classification of this family [Holothuriidae, *our interpolation*] has yet to be discovered. It is a large group with scores of species, but the attempts to break it up into genera have as yet proved unsatisfactory. The genus *Actinopyga* is apparently a natural group and its species are easily recognized. *Labidodemas* is much less satisfactory, and the number of component species is doubtful (monotypic). The rest of the family are best retained in the old genus *Holothuria*. Pearson (1913–14) started out on the task of breaking up the genus, but he made little progress and his work has never been continued. Panning (1931b–1935) attacked the problem *de novo* and gave promise of reaching some helpful conclusions, but he was diverted into a different line of work and his results were incomplete. Neither Pearson nor Panning had access to suffi-

cient material to enable him fully to meet the problems, and it seems best to continue using the name *Holothuria* in the old sense until someone with access to at least half the named species can concentrate on the problem’.

H. L. Clark, who by that time must have examined a huge number of specimens, was thus clearly urging that there be a new start.

Deichmann, who had access to the important collections brought home by the Velero III and IV, took up the challenge and, in 1958, presented a new classification. She rejected the classifications of Pearson, Panning and Clark to a large extent. At the generic level, she accepted Panning’s (1940, 1944) view of *Actinopyga*, *Bohadschia* and *Microthele* (even though she, as Panning before her, unfortunately failed to recognize *Microthele* in Brandt’s (1835) original sense). In addition, she argued that the small genus *Labidodemas* (in which she, twenty years earlier, had described the species *L. americanum*; Deichmann, 1938), did not belong to *Holothuria s.l.* (Panning, 1935c), but stands on its own. Enigmatically, she (Deichmann 1958: 311) changed *Halodeima*, which she ‘accepted with minor changes’ to *Ludwigothuria* Deichmann, 1958. More radical was her decision to split the genus *Holothuria* into several new genera and to abandon the old name *Holothuria* completely. Clark & Rowe (1967) and Rowe (1969; see also Gill, 1907a, b) disagreed with this latter decision: ‘In 1924 (Opinion 80) the generic name *Holothuria* Linnaeus 1767, as restricted by Bruguière 1791, with type-species *H. tremula* Linnaeus 1767 (non Gunnerus 1767) = *H. tubulosa* Gmelin 1790, was placed on the Official List of Generic Names in Zoology’ and ‘this action therefore firmly established the generic name *Holothuria* in the present sense rather than the original one of Linnaeus, 1758’ (Rowe, 1969: 9). Irrespective of the last nomenclatural flaw, it is clear that Deichmann’s (1958) division of *Holothuria* was, as she said herself, ‘foreshadowed in the key which W.K. Fisher made for the Hawaiian holothurians in 1907’. This decision, as noted by Rowe (1969: 122), relied quite heavily on the ecological position of the different taxa she studied. Be that as it may, Deichmann’s (1958) classification was interesting for its novelty and is definitely worthy of critical study.

Rowe (1969) took up the latter challenge when he revised the complete Holothuriidae. Rowe (1969: 119) correctly pointed out that by creating 11 new generic names ‘Deichmann has disregarded a number of appropriate prior genus-group names of Brandt (1835), Jaeger (1833), Haacke (1880) and Pearson (1914) on the grounds of poor definition; most of these names are available under the Rules, being associated with recognized species, those of Jaeger and Brandt needing only designations of type-species in order to qualify for recognition under the Rules’. In the end,

Rowe came to the conclusion that Deichmann's taxonomic groups are best regarded as subgenera of *Holothuria*, which he considered a monophyletic group. He thus largely agreed with Panning's (1940, 1944) generic classification (although he placed *Microthele* and *Halodeima* at the subgeneric level), and with Deichmann (1958) with regard to the position of *Labidodemas*, albeit his phrase 'in truth I think *Labidodemas* may even prove to warrant separation at family level' has caused some commotion during the last two decades (James, 1981; Massin *et al.*, 2004).

Rowe (1969: 122–123) summarized the supraspecific taxa when he presented [our brackets] 'a table of the supraspecific taxa with their type-species represented in her (Deichmann's 1958) paper together with their present disposition'. In addition, he described five new subgenera to accommodate the species that could not be fitted in Deichmann's (1958) revised supraspecific taxa: *Acanthotrapeza* (with type-species *Holothuria pyxis* Selenka, 1867), *Metriatyla* (with type-species *H. scabra* Jaeger, 1833); *Panningothuria* (with type-species *H. forskali* Delle Chiaje, 1823), *Platyperona* (with type-species *H. difficilis* Semper, 1868) and *Stauropora* (with type-species *H. discrepans* Semper, 1868). Based on ossicle complexity, Rowe (1969: 125, text-fig. 1, 165, table 1) constructed a hypothetical evolutionary tree for the genus *Holothuria*. This tree, as he says himself (Rowe, 1969: 124), involves 'speculations that should at least form the basis for future argument'. These speculations are not minor and in fact involve one of the most intricate problems in evolutionary thought, namely the direction of evolution. Synoptically, according to Pearson (1914; partim), Rowe (1969) and later Thandar (1988, 1994), the surf-zone and rock-clinging species (generally characterized by the absence of well developed tables and true buttons) represent the more primitive condition from which evolved the fugitive and fossorial species (generally characterized by well-developed tables and true buttons), whereas according to Deichmann (1958) and later Levin (1999) the reverse scenario is more likely.

MATERIAL AND METHODS

ROOTING

Recent morphological (Kerr & Kim, 2001; Appeltans, 2002) and molecular (Kerr *et al.*, 2005) evidence suggests that the closest extant holothuroid group to the Holothuriidae is the Stichopodidae. Therefore, the type species of two stichopodid genera, *Stichopus chloronotus* Brandt, 1835 and *Thelenota ananas* (Jaeger, 1833), were used to polarize the characters of the ingroup.

TAXON SELECTION

According to recent opinion (Smiley & Pawson, 1990 (unpublished manuscript); Samyn, 2003; Massin *et al.*, 2004) the family Holothuriidae comprises 184 species which are commonly classified into five genera: *Actinopyga* (with 16 species), *Bohadschia* (11), *Holothuria* (148), *Labidodemas* (8) and *Pearsonothuria* (1). It must be noted that these species counts remain tentative; quite a few of them will prove to be synonyms or cryptic species, while new species continue to be described. Characters from three out of five of these genera are taken from their type species. As we lacked specimens of the type of *Bohadschia* (*B. marmorata* Jaeger, 1833, the taxonomy of which is currently under debate), we used the recently described *Bohadschia atra* Massin *et al.*, 1999 as proxy. For *Holothuria* we examined seven of its subgenera (out of the 18 currently recognized; see also Rowe, 1969; Samyn, 2003; Samyn & Massin, 2003). These subgenera were chosen in such a fashion that they reflect the large morphological variation within the large genus *Holothuria* as it is currently perceived. For *Labidodemas* all eight currently known species were included. The selected taxa are listed in Table 1.

CHARACTER SELECTION

Samyn & Massin (2003) recently used the presence of ossicles in the longitudinal muscles to amend the diagnosis of the holothurian subgenus *Mertensiothuria* Deichmann, 1958. To assess the validity of their claim, these authors simultaneously investigated the ossicle content of the longitudinal muscles of *Pearsonothuria graeffei*, three *Actinopyga* species, two *Bohadschia* species, two *Labidodemas* species and one to six species belonging to 18 of the *Holothuria* subgenera. They found that *Actinopyga*, *Pearsonothuria* and four subgenera of *Holothuria* possess ossicles in the longitudinal muscle. Massin *et al.* (2004), in their revision of *Labidodemas*, extended this survey to include the transversal (or circular) and cloacal-retractor muscles and concluded that ossicles are always absent in the musculature of *Labidodemas* spp. The present study includes data of not only the ossicle content of the musculature, but also the presence or absence of ossicles in the gonad and cloaca. As the latter tissues have only sporadically been investigated (Liao, 1980; Cherbonnier & Féral, 1984; Samyn & Massin, 2003; Massin *et al.*, 2004; Rowe, pers. comm.) in terms of ossicle content, we have illustrated some of the ossicles recovered from the cloaca (Fig. 3).

Overall, 132 discrete characters dealing with the gross external and internal morphology (13 characters each), ecology (three characters), ossicle assemblage of the different body parts and organs (102 characters)

Table 1. Taxa used in this study. Only *Bohadschia atra* is not the type species of the genus. For *Holothuria* we utilized the type species of seven representative subgenera

Family Holothuriidae Ludwig, 1874
Genus <i>Actinopyga</i> Bronn, 1860
<i>Actinopyga echinites</i> (Jaeger, 1833)
Genus <i>Bohadschia</i> Jaeger, 1833
<i>Bohadschia atra</i> Massin, Rasolofonirina, Conand & Samyn, 1999
Genus <i>Holothuria</i> Linnaeus, 1767
<i>Holothuria (Cystipus) rigida</i> (Selenka, 1867)
<i>Holothuria (Halodeima) atra</i> Jaeger, 1833
<i>Holothuria (Lessonothuria) pardalis</i> Selenka, 1867
<i>Holothuria (Mertensiothuria) leucospilota</i> (Brandt, 1835)
<i>Holothuria (Metriatyla) scabra</i> Jaeger, 1833
<i>Holothuria (Microthele) nobilis</i> (Selenka, 1867)
<i>Holothuria (Semperothuria) cinerascens</i> Brandt, 1835
Genus <i>Labidodemas</i> Selenka, 1867
<i>Labidodemas americanum</i> Deichmann, 1938
<i>Labidodemas maccullochi</i> (Deichmann, 1958)
<i>Labidodemas pertinax</i> (Ludwig, 1875)
<i>Labidodemas pseudosemperianum</i> Massin, Samyn & Thandar, 2004
<i>Labidodemas quadripartitum</i> Massin, Samyn & Thandar, 2004
<i>Labidodemas rugosum</i> (Ludwig, 1875)
<i>Labidodemas semperianum</i> Selenka, 1867
<i>Labidodemas spineum</i> Massin, Samyn & Thandar, 2004
Genus <i>Pearsonothuria</i> Levin, Kalin & Stonink, 1984
<i>Pearsonothuria graeffei</i> (Semper, 1868)
Family Stichopodidae Haeckel, 1896
Genus <i>Stichopus</i> Brandt, 1835
<i>Stichopus chloronotus</i> Brandt, 1835
Genus <i>Thelenota</i> H.L. Clark, 1921
<i>Thelenota ananas</i> (Jaeger, 1833)

were selected. In addition, one character dealt with the known broad distribution of the investigated taxa (Massin, 1999; Massin *et al.*, 1999, 2004; Samyn, 2003). These characters and their respective states are listed in Table 2. Description of the employed characters and their respective states can be found in Rowe (1969; see also Clark & Rowe, 1971) or in recent monographs on Madagascar (Cherbonnier, 1988), Indonesian (Massin, 1999) or East African (Samyn, 2003) shallow-water holothuroids.

As the recognition of species within holothuriid genera relies heavily on variation in shape of the ossicles, we were obliged to create several characters that refer to the same ossicle type. Such characters (e.g. 30 & 32–44 for the table ossicles of the body wall) were scored with hierarchically related character states, even though this led to character inapplicability in quite a

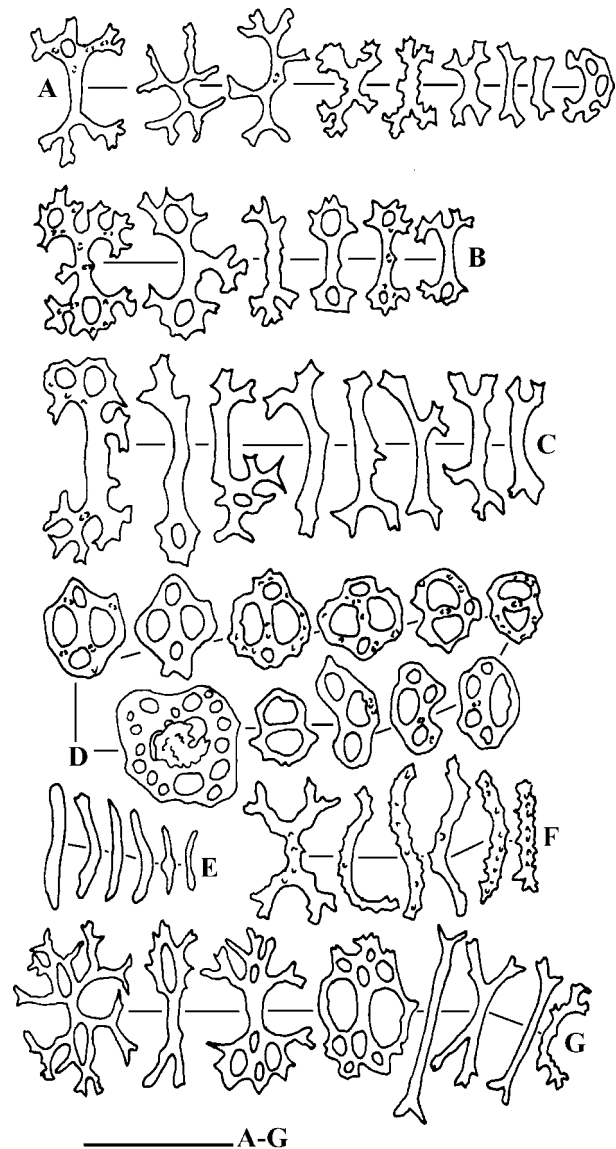


Figure 3. Ossicles of the cloaca. A, *Actionpyga echinites* (Jaeger, 1833). B, *Pearsonothuria graeffei* (Semper, 1868). C, *Bohadschia atra* Massin, Rasolofonirina, Conand & Samyn, 1999. D, *Holothuria (Metriatyla) scabra* Jaeger, 1833. E, *Labidodemas pertinax* (Ludwig, 1875). F, *Stichopus chloronotus* Brandt, 1875. G, *Thelenota ananas* (Jaeger, 1833). Scale bars: A–D, F, G = 100 µm; E = 50 µm.

number of cases. The dataset employed here includes 77 binary and 55 multistate characters (Table 3).

PHYLOGENETIC ANALYSES

Cladistic analyses were performed using PAUP*4.0b10 (Swofford, 2002) for MacIntosh. Due to the high number of taxa and characters, we preferred the heuristic search algorithm to the branch-and-

Table 2. Investigated characters with their respective character states

EXTERNAL MORPHOLOGY

- 1 *Overall shape*: (0) cylindrical; (1) convex; (2) quadrangular.
- 2 *Tentacle size*: (0) small; (1) large.
- 3 *Tentacle shape*: (0) peltate, indentions shallow; (1) peltate, indentions deep; (2) peltate, indentions very deep.
- 4 *Thickness of BW (of live specimens)*: (0) <1 mm; (1) 1–4 mm; (2) +4 mm.
- 5 *Position of mouth*: (0) terminal; (1) ventral.
- 6 *Position of anus*: (0) terminal; (1) superterminal to dorsal.
- 7 *Arrangement of ventral tube feet*: (0) in radial areas only; (1) some also spread in interradial areas; (2) overall spreading.
- 8 *Arrangement of dorsal tube feet and/or papillae*: (0) present, in radial areas only; (1) present, overall spreading.
- 9 *Enlarged dorsal and/or lateral papillae*: (0) absent; (1) present.
- 10 *Anal appendages*: (0) absent; (1) present, anal papillae; (2) present, anal teeth.
- 11 *Collar of papillae around mouth*: (0) absent; (1) present, not fused; (2) present, fused at base.
- 12 *Rugosity of body wall*: (0) smooth; (1) rough to the touch.
- 13 *Firmness of body wall*: (0) firm; (1) very soft (collapsible if animal disturbed).

INTERNAL MORPHOLOGY

- 14 *Proportion of radial/interradial plates (extent to which former is longer than latter)*: (0) up to 1.5×; (1) 1.5–2×; (2) 2–3×; (3) more than 3×.
- 15 *Morphology of radial plates*: (0) posterior side straight or slightly indented or convex; (1) posterior side largely indented, ribbon-like; (2) with posterior medial prolongations.
- 16 *Cuvierian tubules*: (0) absent; (1) present.
- 17 *Expellability of Cuvierian tubules*: (0) nonexpellable; (1) expellable.
- 18 *Adhesivity of Cuvierian tubules*: (0) nonadhesive; (1) adhesive.
- 19 *Appearance of Cuvierian tubules*: (0) long, thin; (1) thick, globulous; (2) short, thin.
- 20 *Structure of Cuvierian tubules*: (0) proximal half of trunk similar in structure to distal half; (1) proximal half of trunk dissimilar in structure to distal half.
- 21 *Number of attachment sites of Cuvierian tubules*: (0) less than 10; (1) more than 10.
- 22 *Number of Polian vesicles*: (0) one; (1) two or more.
- 23 *Length of Polian vesicles*: (0) short (less than 1/12 body length); (1) long (more than 1/12 body length).
- 24 *Number of stone canals*: (0) one; (1) two or more.
- 25 *Length of stone canals*: (0) short (less than 1/12 body length); (1) long (more than 1/12 body length).
- 26 *Gonad tuft, number*: (0) one; (1) two.

ECOLOGY

- 27 *Nature of substratum*: (0) sand and/or rubble; (1) turf algae; (2) living corals and sponges.
- 28 *Hiding behaviour*: (0) body never concealed; (1) body partially concealed; (2) body completely concealed.
- 29 *Host to carapids*: (0) never reported as host to pearlfish; (1) reported as host to pearlfish.

OSSICLE ASSEMBLAGE OF THE BODY WALL

- 30 *Regular tables*: (0) absent; (1) present.
- 31 *Pseudo-tables*: (0) absent; (1) present.
- 32 *Hollow fenestrated spheres*: (0) absent; (1) present.
- 33 *Rim of disc of regular table*: (0) smooth; (1) moderately spiny; (2) spiny.
- 34 *Rugosity of disc of regular table*: (0) smooth; (1) knobbed.
- 35 *Structure of disc of regular table*: (0) flat; (1) raised at rim.
- 36 *Size of table disc of regular table*: (0) reduced; (1) not reduced.
- 37 *Number of central holes in table disc of regular table*: (0) four; (1) variable (never solely four).
- 38 *Number of peripheral holes in table disc of regular table*: (0) absent; (1) present, in one ring; (2) present, in more than one ring.
- 39 *Height of spire of regular table*: (0) low (height less than disc diameter); (1) moderate (up to 1.5× disc diameter); (2) high (more than 2× disc diameter).
- 40 *Number of pillars of regular table*: (0) four; (1) variable (never solely four).
- 41 *Crown of spire of regular table*: (0) absent; (1) present.
- 42 *Structure of crown of spire of regular table*: (0) cluster of spines; (1) regular ring with central opening; (2) irregular ring with one or more central opening(s); (3) Maltese cross-shaped.
- 43 *Length of spines on crown of regular table*: (0) short; (1) medium; (2) long.
- 44 *Bifurcation of spines on crown of regular table*: (0) absent; (1) present.

Table 2. *Continued*

- 45 *Buttons*: (0) absent; (1) present.
- 46 *Ellipsoidal buttons*: (0) absent; (1) present.
- 47 *Rim of buttons*: (0) smooth, not spinose; (1) spinose; (2) undulating.
- 48 *Symmetry of buttons*: (0) regular; (1) irregular.
- 49 *Rugosity of buttons*: (0) smooth; (1) with small knobs; (2) with large knobs.
- 50 *Number of holes in buttons*: (0) generally three pairs of holes; (1) often more than three pairs of holes.
- 51 *Opening of holes in buttons*: (0) open; (1) almost closed.
- 52 *Presence of rods*: (0) absent; (1) present.
- 53 *Rugosity of rods*: (0) smooth; (1) slightly rugose; (2) very rugose.
- 54 *Perforation of rods*: (0) not perforated; (1) perforated distally.
- 55 *Complexity of rods*: (0) nonbranching; (1) branching.
- 56 *C-, S-, X-shaped rods*: (0) absent; (1) present.
- 57 *Rosettes*: (0) absent; (1) button-like; (2) rod-like.
- OSSICLE ASSEMBLAGE TENTACLES
- 58 *Ossicles*: (0) absent; (1) present.
- 59 *Rugosity of tentacle-rods*: (0) smooth; (1) moderately spiny; (2) spiny.
- 60 *Rosette-like branched rods*: (0) absent; (1) present.
- 61 *Other ossicles*: (0) absent; (1) present, irregular plate-like branched rods; (2) present, reduced tables.
- OSSICLE ASSEMBLAGE LONGITUDINAL MUSCLES
- 62 *Ossicles*: (0) absent; (1) present.
- 63 *Structure of ossicles*: (0) nonspinose rods to oblong rings to button-like ossicles (pseudobuttons); (1) ossicles present as spiny rods of various shape; (2) ossicles present as C- to S-shaped rods or derivatives.
- OSSICLE ASSEMBLAGE CLOACAL RETRACTOR MUSCLES
- 64 *Ossicles*: (0) absent; (1) present.
- 65 *Structure of ossicles*: (0) nonspinose rods to oblong rings to button like ossicles (pseudobuttons); (1) reduced tables; (2) spiny rods of various shape.
- OSSICLE ASSEMBLAGE TRANSVERSAL (CIRCULAR) MUSCLES
- 66 *Ossicles*: (0) absent; (1) present.
- 67 *Structure of ossicles*: (0) nonspinose rods to oblong rings to button-like ossicles (pseudobuttons); (1) spiny rods of various shape; (2) C- to S-shaped rods.
- OSSICLE ASSEMBLAGE CLOACA
- 68 *Ossicles*: (0) absent; (1) present.
- 69 *Structure of ossicles*: (0) spiny rods of various shape; (1) C- to S-shaped rods; (2) reduced tables.
- OSSICLE ASSEMBLAGE VENTRAL PEDICELS
- 70 *Regular tables*: (0) absent; (1) present; (2) rare.
- 71 *Pseudo-tables*: (0) absent; (1) present.
- 72 *Rim of disc of regular table*: (0) smooth; (1) moderately spiny; (2) spiny.
- 73 *Rugosity of disc of regular table*: (0) smooth; (1) knobbed.
- 74 *Structure of disc of regular table*: (0) flat; (1) raised at rim.
- 75 *Size of table disc of regular table*: (0) reduced; (1) not reduced.
- 76 *Number of central holes in table disc of regular table*: (0) four; (1) variable (never solely four).
- 77 *Number of peripheral holes in table disc of regular table*: (0) absent; (1) present, in one ring; (2) present, in more than one ring.
- 78 *Height of spire of regular table*: (0) low (height less than disc diameter); (1) moderate (up to 1.5× disc diameter); (2) high (more than 2× disc diameter).
- 79 *Number of pillars of regular table*: (0) four; (1) variable (never solely four).
- 80 *Crown of spire of regular table*: (0) absent; (1) present.
- 81 *Structure of crown of spire of regular table*: (0) cluster of spines; (1) regular ring with central opening; (2) irregular ring with one or more central opening(s); (3) Maltese cross-shaped.
- 82 *Length of spines on crown of regular table*: (0) short; (1) medium; (2) long.
- 83 *Bifurcation of spines on crown of regular table*: (0) absent; (1) present.
- 84 *Regular buttons*: (0) absent; (1) present.
- 85 *Ellipsoidal buttons*: (0) absent; (1) present.
- 86 *Rim of buttons*: (0) smooth, not spinose; (1) spinose; (2) undulating; (3) knobbed.

Table 2. Continued

-
- 87 *Symmetry of buttons*: (0) regular; (1) irregular.
 88 *Rugosity of buttons*: (0) smooth; (1) with small knobs; (2) with large knobs.
 89 *Number of holes in buttons*: (0) generally three pairs of holes; (1) often more than three pairs of holes.
 90 *Opening of holes in buttons*: (0) open; (1) almost closed.
 91 *Plates*: (0) absent; (1) present.
 92 *Structure of plates*: (0) wide, regular; (1) elongate, regular; (2) irregular; (3) pseudoplates.
 93 *Rods*: (0) absent; (1) present.
 94 *Rugosity of rods*: (0) smooth; (1) slightly rugose; (2) very rugose.
 95 *Perforation of rods*: (0) not perforated; (1) perforated distally; (2) perforated overall.
 96 *Complexity of rods*: (0) nonbranching; (1) branching; (2) central and distal widening; (3) central widening only.
 97 *Rosettes*: (0) absent; (1) button-like; (2) rod-like.
 98 *Grains*: (0) absent; (1) present.
- OSSICLE ASSEMBLAGE DORSAL PEDICELS
- 99 *Regular tables*: (0) absent; (1) present.
 100 *Pseudo-tables*: (0) absent; (1) present, with disc; (2) present, without disc.
 101 *Rim of disc of regular table*: (0) smooth; (1) moderately spiny; (2) spiny.
 102 *Rugosity of disc of regular table*: (0) smooth; (1) knobbed.
 103 *Structure of disc of regular table*: (0) flat; (1) raised at rim.
 104 *Size of table disc of regular table*: (0) reduced; (1) not reduced.
 105 *Number of central holes in table disc of regular table*: (0) four; (1) variable (never solely four).
 106 *Number of peripheral holes in table disc of regular table*: (0) absent; (1) present, in one ring; (2) present, in more than one ring.
 107 *Height of spire of regular table*: (0) low (height less than table diameter); (1) moderate (up to 1.5× disc diameter); (2) high (more than 2× disc diameter).
 108 *Number of pillars of regular table*: (0) four; (1) variable (never solely four).
 109 *Crown of spire of regular table*: (0) absent; (1) present.
 110 *Structure of crown of spire of regular table*: (0) cluster of spines; (1) regular ring with central opening; (2) irregular ring with one or more central opening(s); (3) Maltese cross-shaped.
 111 *Length of spines on crown of regular table*: (0) short; (1) medium; (2) long.
 112 *Bifurcation of spines on crown of regular table*: (0) absent; (1) present.
 113 *Buttons*: (0) absent; (1) present.
 114 *Ellipsoidal buttons*: (0) absent; (1) present.
 115 *Rim of buttons*: (0) smooth, not spinose; (1) spinose; (2) undulating.
 116 *Symmetry of buttons*: (0) regular; (1) irregular.
 117 *Rugosity of buttons*: (0) smooth; (1) with small knobs; (2) with large knobs.
 118 *Number of holes in buttons*: (0) generally three pairs of holes; (1) often more than three pairs of holes.
 119 *Opening of holes in buttons*: (0) open; (1) almost closed.
 120 *Plates*: (0) absent; (1) present.
 121 *Structure of plates*: (0) wide regular; (1) elongate regular; (2) irregular.
 122 *Rods*: (0) absent; (1) present, not curved (2) present, curved.
 123 *Rugosity of rods*: (0) smooth; (1) slightly rugose; (2) very rugose.
 124 *Perforation of rods*: (0) not perforated; (1) perforated distally; (2) perforated overall.
 125 *Complexity of rods*: (0) nonbranching; (1) branching; (2) central and distal widening.
 126 *Rosettes*: (0) absent; (1) present, button-like; (2) present, rod-like.
 127 *C-, S-, X-shaped rods*: (0) absent; (1) present.
- OSSICLE ASSEMBLAGE ANAL PAPIILLAE
- 128 *Tables*: (0) absent; (1) present, regular; (2) present, tack-like.
 129 *Buttons*: (0) absent; (1) present.
 130 *Rods*: (0) absent; (1) present.
- OSSICLE ASSEMBLAGE GONAD
- 131 *Gonad*: (0) devoid of ossicles; (1) with ossicles.
- DISTRIBUTION
- 132 *Ocean*: (0) western Indian; (1) Indo-West Pacific; (2) East Pacific.
-

Table 3. Character matrix. '?' code missing; polymorphic states indicated as follows: a = 01, b = 02, c = 012, d = 12, e = 13. Non-applicable characters are indicated by '-'

	1-10	11-20	21-30	31-40	41-50	51-60	61-70
<i>Actinopyga echinites</i>	1102101112	0000010021	0a1a001000	00-----	-----00----	-1a0a02110	0111210100
<i>Bohadrschia atra</i>	110d112100	1000011100	1010000010	00-----	-----00----	-0---02120	00-0-0-100
<i>Holothuria (Cystipus) rigida</i>	1011102101	010200----	-101000201	0101010100	1100112021	00---00100	00-0-0-0-1
<i>Holothuria (Halodeima) atra</i>	1111102100	000200----	-101000001	00100a0110	131100----	-0--01110	00-0-0-0-1
<i>Holothuria (Lessonothuria) pardalis</i>	1011101100	010200----	-110000201	00a01101c0	1100102a00	00---00110	00-0-0-0-1
<i>Holothuria (Mertensiothuria) leucospilota</i>	1111102100	0002011100	1010000101	0010010100	1100100a00	00---000--	-1010100-1
<i>Holothuria (Metriatyla) scabra</i>	110d102100	110200----	-110100001	0000010d10	1100102020	0110100120	00-110-121
<i>Holothuria (Microthele) nobilis</i>	1102112112	0101010110	1aa0000011	0000010110	1a0011b121	00---00120	20-0-0-0-1
<i>Holothuria (Semperothuria) cinerascens</i>	1121001101	100200----	-11aa00101	00100a0a10	131100----	-1200001c1	00-0-0-0-1
<i>Labidodemas americanum</i>	0011000100	0103110?10	1010000201	001a010200	a10000----	-0--00100	00-0-0-0-1
<i>Labidodemas pseudosemperianum</i>	0010000000	010310----	-000000201	0000011101	1221100101	0100000100	00-0-0-0-1
<i>Labidodemas maccullochi</i>	0011000100	010310----	-110000201	0010010220	a12000----	-0---00110	00-0-0-0-1
<i>Labidodemas quadripartitum</i>	1011000000	000310----	-0100?0201	0020000a00	112a00----	-1000000--	-0-0-0-0-1
<i>Labidodemas rugosum</i>	0011001100	010310----	-010000201	0020a10110	aa1010b10a	00---00100	00-0-0-0-1
<i>Labidodemas pertinax</i>	0011000100	010310----	-100000201	0020000010	110000----	-1aaa001a0	00-0-0-101
<i>Labidodemas semperianum</i>	0010000001	010310----	-010100201	00d0011101	12211001a0	0100000100	00-0-0-0-1
<i>Labidodemas spineum</i>	001a000001	010310----	-110000201	00daa11101	1dda101a10	0110a00100	00-0-0-0-1
<i>Pearsonothuria graeffei</i>	1101110100	1000010100	1011002010	10-----	-----00----	-0---021-1	011120-100
<i>Stichopus chloronotus</i>	2101101010	201120----	-a1a010011	0010010110	131a00----	-0---10110	d1d0-121a1
<i>Thelenotia ananas</i>	2102102110	201120----	-101010010	00-----	-----00----	-110100121	1111211100

Table 3. Continued

	71-80	81-90	91-100	101-110	111-120	121-132
<i>Actinopyga echinites</i>	0-----	-----00	0-10012100	-----	--00-----	-100110---11
<i>Bohadschia atra</i>	0-----	-----00	0-10012100	-----	--00-----	-10a110---01
<i>Holothuria (Cystipus) rigida</i>	0010101001	1001020210	0-10220010	0a01010011	001020d100	-20220010101
<i>Holothuria (Halodeima) atra</i>	0a0000aa01	31100-----	130---1010	0000001013	0100-----	-100000---01
<i>Holothuria (Lessonothuria) pardalis</i>	0a0a101a01	10010b1000	1a0---0010	a01a011011	00102a0a00	-201000---01
<i>Holothuria (Mertensiothuria) leucospilota</i>	0100101001	1001000000	1a0---0010	a001010011	0010ba0a00	-10b000---11
<i>Holothuria (Metriatyla) scabra</i>	000010d101	10010b0100	1110220010	00010d1011	0a10201a00	-102200---01
<i>Holothuria (Microthele) nobilis</i>	0000101101	d001100210	1a10b30010	000101101d	0001-----	110b100---01
<i>Holothuria (Semperothuria) cinerascens</i>	000000a101	31100-----	0-1daa00a0	0000001013	1100-----	-12a00000101
<i>Labidodemas americanum</i>	010010c00a	a0000-----	0-0---0010	10000a0a0-	--00-----	-0---0000002
<i>Labidodemas pseudosemperianum</i>	0b0011a01a	d211001000	0-10a00010	b0011a01ad	da00-----	-0---00---01
<i>Labidodemas maccullochi</i>	020010ca0a	a1000-----	0-0---0010	20000a000-	--10ba0a01	1110100---02
<i>Labidodemas quadrupartitum</i>	0200000000	----000----	0-10110010	200000000-	--00-----	-110100---?0
<i>Labidodemas rugosum</i>	020a10100a	1a01021010	110---0010	201a01a011	10102a0100	-102000---01
<i>Labidodemas pertinax</i>	020000000a	10000-----	0-1a010010	d00000000-	--00-----	-110000---01
<i>Labidodemas semperianum</i>	02001100aa	dd100-----	011aa00110	10011a01ad	1a00-----	-10a00000111
<i>Labidodemas spinum</i>	020a11a0aa	d21101ad00	0-11a00010	20aa1a0aad	all01ad000	-11a00010101
<i>Pearsonothuria graeffei</i>	1-----	-----00	120---2001	-----	--00-----	-100d10---11
<i>Stichopus chloronotus</i>	020010100a	10000-----	1210be0010	100001001e	0a00-----	-10001100011
<i>Thelenota ananas</i>	0-----	-----00	1211010002	-----	--00-----	-1d0100---11

bound one. Heuristic searches were carried out with the following options: keep best trees only; starting tree(s) for branch-swapping obtained via stepwise addition, and when multiple starting trees exist, swapping allowed only on the best tree; stepwise addition in a random sequence with 10^5 replicates initiated from a random tree whereby a single tree was held at each step; branch-swapping algorithm set to tree bisection-recognition (TBR) whereby multiple trees were saved (steepest descent not in effect) but swapping allowed only on the best tree. Heuristic searches were run under the maximum parsimony (MP) optimality criterion with the following options: branches collapsed when maximum length is zero. All characters were run unordered, whereby state optimization was achieved through accelerated transformation (ACCTRAN); internal nodes were allowed states that are not observed in terminal taxa; multiple states were treated as polymorphisms; gaps as missing data. Consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated with the minimum possible single-character lengths.

Because we consider equal weighting of characters to be an improbable and unnatural situation (not all characters bear the same information content and/or predictive value), we reweighted on the RC, an action that gives higher relative weight to those characters that are more consistent with initial heuristic cladograms and, as such, a greater weight to parsimony informative characters (Bosselaers & Jocqué, 2000).

Data quality of the best trees was assessed by bootstrapping using 500 randomly obtained replicates under the heuristic search, by examination of the skewness of 10^6 randomly generated trees from the dataset, as well as by the Bremer or decay index (number of evolutionary steps required to break down a clade). To test whether the most parsimonious cladogram(s) as obtained here are statistically superior to the cladograms obtained through classical β -taxonomy (e.g. Rowe, 1969), we analysed them with a normal approximation of a Wilcoxon signed-rank test, as well as with a binomial sign test of winning sites as implemented in PAUP. In all analyses, the outgroup (Stichopodidae) was set as a monophyletic sister group to the ingroup.

RESULTS

PHYLOGENETIC POSITION OF *LABIDODEMAS* AND OTHER HOLOTHURIID GENERA

Of the 132 characters, five proved constant and 28 were parsimony uninformative, leaving 99 informative sites (figures within parentheses show results when uninformative characters are excluded). The equally weighted MP analysis returned four shortest

trees of length 464 (402), a strict consensus of which is presented in Figure 4. This tree has a CI of 0.70 (0.65), a RI of 0.60 and a RC of 0.42 (0.39). The highly left skewed ($g_{(1)} -0.47$; $P < 0.01$) frequency distribution of the tree lengths suggests that our dataset contains considerable hierarchical signal (Hillis & Huelsenbeck, 1992). Unfortunately, however, the bootstrap percentages for many internal nodes were moderate to small (i.e. below 70%). Reweighting on the basis of the RC resulted in a single, fully resolved tree (Fig. 5), which has a length of 93.64 steps (68.64), CI of 0.81 (0.74), RI of 0.82, and RC of 0.65 (0.59).

As can be seen from Figures 4 and 5, each weighting scheme returned *Labidodemas* as a monophyletic clade. The calculated bootstrap and Bremer support proved to be quite high for the *Labidodemas* branch (96% bootstrap support and 4/3.2 decay index for equal and successive weighting, respectively) (Fig. 5). Unfortunately, the relationships between the different *Labidodemas* spp. in some instances received only moderate support. However, a clear pattern is visible: *L. rugosum* occupies the basal position to the clades (*L. pseudosemperianum* (*L. spineum*, *L. semperianum*)) and ((*L. americanum*, *L. maccullochi*), (*L. pertinax*, *L. quadripartitum*)), with the latter two clades sister to each other. However, *Labidodemas* proved to be well nested within the genus *Holothuria* which, if we retain *Labidodemas* as a valid genus, acquires paraphyletic status. Within *Holothuria*, two major, well-supported clades are discernible. The basal one is formed by the subgenera *Halodeima* and *Semperothuria*, while the other one includes *Labidodemas* together with the other examined *Holothuria* subgenera. This again confirms the paraphyletic status of *Holothuria*. The other holothuriid genera, *Actinopyga*, *Bohadschia* and *Pearsonothuria* (the latter two as sister genera, although with low node support) are positioned at the base of the Holothuriidae.

To further test whether *Holothuria* is indeed paraphyletic, we compared the single most parsimonious tree obtained after successive weightings to the shortest tree where *Holothuria* is restrained as a monophyletic group (cf. Rowe, 1969). This tree proved to be significantly longer (reweighted tree length = 71.53; $N = 21$, $z = -2.29$, $P = 0.027$; winning sites = 16, $P = 0.027$). On the other hand, the most parsimonious tree always proved to be identical in length to the tree where subgenera *Halodeima* and *Semperothuria* are set as one clade. As Kerr *et al.* (2005) placed *Bohadschia* and *Pearsonothuria* within *Holothuria*, we tested the length of such a tree. First, we analysed the tree where *Bohadschia*, *Pearsonothuria*, *Labidodemas* and *Holothuria* (without its subgenera *Halodeima* and *Semperothuria*) form one clade. This tree proved to be significantly longer (reweighted tree length = 73.23; $N = 13$, $z = -2.70$, $P = 0.0070$; 11

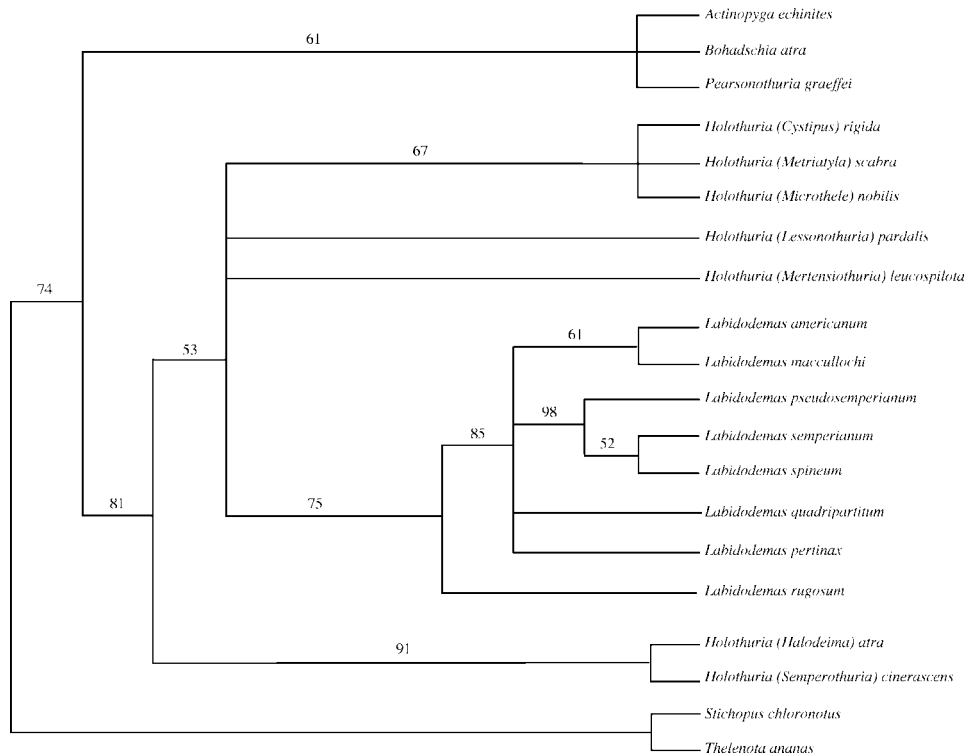


Figure 4. Bootstrap 50% majority rule consensus tree of four trees as recovered under the equal weighting scheme. Values above branches represent bootstrap percentages (500 replicates).

winning sites, $P = 0.025$) than the most parsimonious one. Moreover, the tree where the holothurian subgenera *Halodeima* and *Semperothuria* are included in the *Holothuria* + *Labidodemas* + *Bohadschia* + *Pearsonothuria* clade was not significantly longer (tree length = 70.51; $N = 5$, $z = -0.68$, $P = 0.50$; three winning sites, $P = 1.0$) than the most parsimonious one. On the other hand, the tree where *Halodeima*, *Semperothuria*, *Bohadschia* and *Pearsonothuria* are constrained to form a single clade was, again, significantly longer ($N = 14$, $z = -2.42$, $P = 0.0155$; 11 winning sites, $P = 0.057$).

DISCUSSION

PHYLOGENY

Labidodemas as recently revised by Massin *et al.* (2004) is here confirmed to be a monophyletic lineage. Moreover, as already indicated by Appeltans (2002) and Kerr *et al.* (2005), *Labidodemas* occupies a derived position within *Holothuria*. As we have examined only seven out of the 18 currently assumed valid *Holothuria* subgenera (excluding the nominal subgenus), it is difficult to discuss the phylogeny of the latter genus in all the detail it deserves. Nevertheless, after analysing several constrained topologies, some patterns are readily visible.

First of all, if we accept *Labidodemas* at the generic level, *Holothuria* is clearly paraphyletic. Second, the subgenera *Halodeima* and *Semperothuria* form a distinct clade which is positioned at the base of the [*Holothuria* (partim), *Labidodemas*] clade. This [*Halodeima*, *Semperothuria*] clade is characterized by species which have reduced table ossicles (Fig. 6A, B), button-like rosettes (Fig. 2A), no true buttons (cf. Fig. 2B), rugose rods and irregular plates (Fig. 6C, D). The more derived [*Holothuria* (partim), *Labidodemas*] clade includes those species which generally have well developed and often intricate tables (Fig. 6E, F), true buttons (Fig. 2B), no button-like rosettes (cf. Fig. 2A), less rugose rods and more regular plates (Fig. 6G, H); it is here represented by the other holothurian subgenera as well as by all the *Labidodemas* spp. Thus, these results support the scenario of Rowe (1969) as discussed in the Introduction.

Contrary to Kerr *et al.* (2005), we found no direct evidence that *Bohadschia* and *Pearsonothuria* are derived from within *Holothuria*. Instead, these genera clustered together with *Actinopyga* at the base of the Holothuriidae. While our finding is substantiated by moderate to high bootstrap support (61% in the unweighted and 89% in the weighted analysis), the Bremer support unfortunately proved rather low (1 and 1.9 for equal and successive weighting). Neverthe-

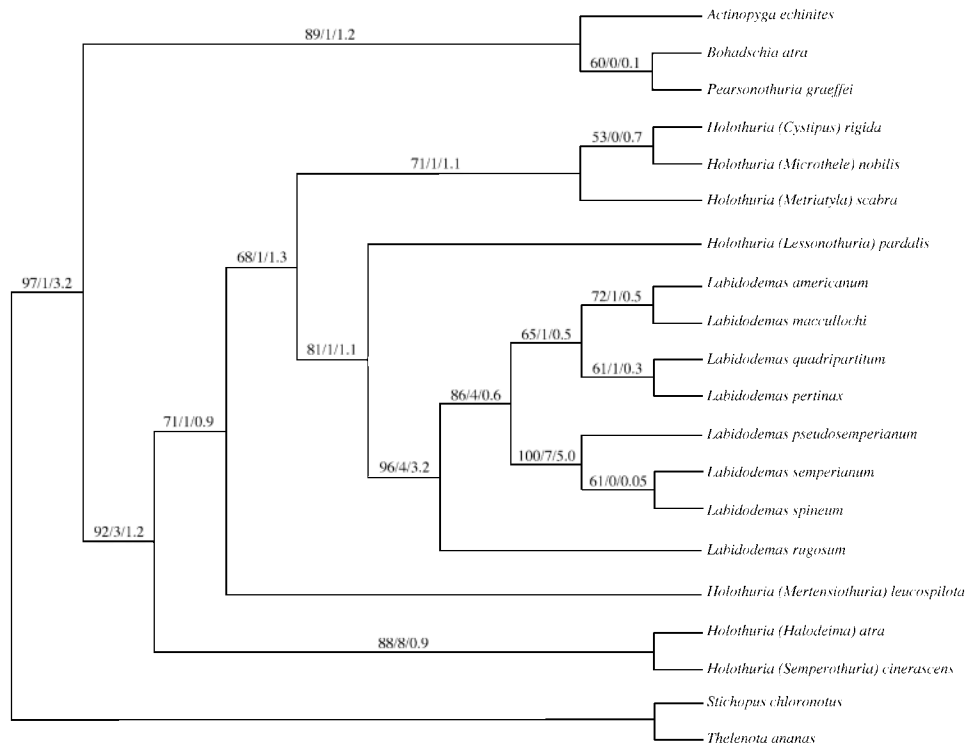


Figure 5. Single most parsimonious tree obtained under successive weighting (on the RC) scheme. Values above the branches indicate per cent bootstrap support (500 replicates)/Bremer support values as obtained under equal/successive weighting.

less, as the tree where all the investigated *Holothuria* subgenera (*Labidodemas*, *Bohadschia* and *Pearsonothuria*) are constrained within one clade is not significantly longer than the most parsimonious one, we cannot deny that *Bohadschia* and *Pearsonothuria* could be closer to certain *Holothuria* subgenera than to *Actinopyga*, as suggested by Kerr *et al.* However, such clustering would imply that: (1) species with regular tables and true buttons have given rise either to species with complex rosettes and racquet-like pseudotables (*Pearsonothuria*) or to species with simple, unbranched solid grains to various dichotomously branched rosettes (*Bohadschia*); (2) the rosettes of *Actinopyga* are analogous to those of *Bohadschia* and *Pearsonothuria*. However, because *Actinopyga*, *Bohadschia* and *Pearsonothuria* share such features as a robust calcareous ring, rod-like rosettes, and absence of regular tables and buttons (cf. Pearson, 1914), we prefer to retain our topology. Further evidence for this comes from the observation that *Actinopyga* and *Pearsonothuria*, just like the outgroup, share the presence of the same type of rods in the musculature, the gonad and the cloaca (*Bohadschia* however, lacks these in the gonad). *Bohadschia* and *Pearsonothuria* are further linked to each other by presenting the same type of

Cuvierian tubules (Vanden Spiegel, 1993, pers. comm.) and in having a superterminal to dorsal anus.

TOWARD A NEW CLASSIFICATION OF THE HOLOTHURIIDAE?

The present phylogeny and the one obtained from molecular data (Kerr *et al.*, in press) agree on two crucial points: (1) a significantly longer tree is required to make *Holothuria* (as defined by Rowe, 1969) monophyletic; (2) *Labidodemas* is firmly nested within *Holothuria*. If we accept our phylogeny and we downgrade *Labidodemas* to the subgeneric level (as a subgenus of *Holothuria*), amendment of the current classification is reduced to nomenclatural changes. However, given the size of *Holothuria* (18 subgenera, comprising some 150 species) and the possibility of deeper paraphyly, we prefer to adopt an eclectic or Darwinian classification (a classification that is based on the criteria of similarity and common descent; see also Mayr, 1982; Mayr & Bock, 2002) and, as such, retain the generic rank of the easily recognized *Labidodemas* (for its synapomorphies, see Massin *et al.*, 2004). This however, necessitates altering the classification of *Holothuria* to retain monophyletic genera.

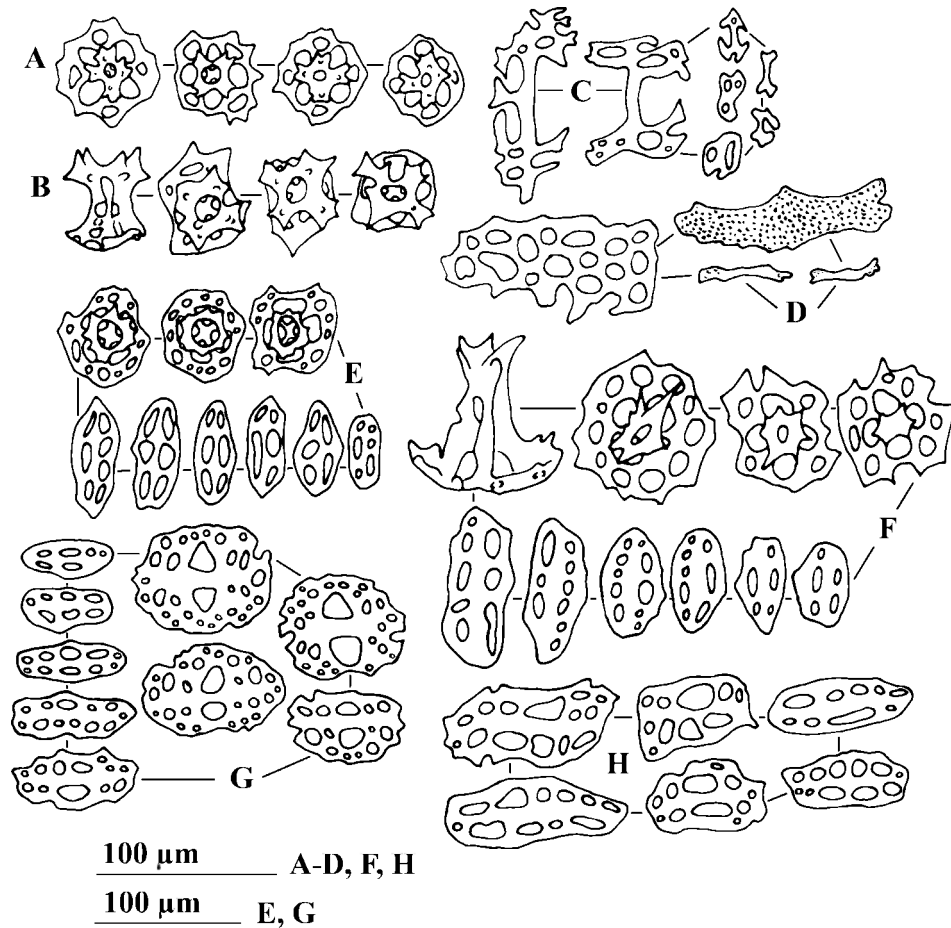


Figure 6. Some representative ossicles as found in the *Holothuria* + *Labidodemas* clade. A, tables of the body wall of *H. (Halodeima) atra* Jaeger, 1833. B, tables of the body wall of *H. (Semperothuria) cinerascans* (Brandt, 1835). C, rosettes and (pseudo)plates of the tube feet of *H. (H.) atra*. D, rods and plates of the tube feet of *H. (S.) cinerascans*. E, tables and buttons of the body wall of *H. (M.) leucospilota* Brandt, 1835. F, tables and buttons of the body wall of *L. rugosum* (Ludwig, 1875). G, buttons to plates of the tube feet of *H. (M.) leucospilota*. H, buttons to plates of the tube feet of *L. rugosum*.

Unfortunately, as we are currently largely in the dark with regards to the phylogenetic position of the type species of *Holothuria* [*Holothuria (Holothuria) tubulosa* Gmelin, 1790], we cannot unambiguously decide to which clade the name *Holothuria* should apply. On the other hand, if the basal position of the clade comprising *Actinopyga*, *Bohadschia* and *Pearsonothuria* can be substantiated with further phylogenetic evidence, there is no reason to deny that clade separate taxonomic rank (family?). It is interesting to note that Panning (1940) appears to have come to the same conclusion when he proposed treating *Actinopyga* as a subgenus of *Bohadschia*. As *Bohadschia* is the earliest generic name, the name Bohadschiidae seems most suitable. In fact, such emendation mirrors Gill's (1907a) suggestion of replacing the name Holothuriidae with Bohadschiidae. As Gill's (1907a)

reasons were nomenclatural (he attacked the validity of the name *Holothuria* and its derivatives) rather than taxonomic or systematic (he did not alter the diagnosis or classification in any way), the name Bohadschiidae remains available. The name Holothuriidae would then apply only to the clade wherein falls the type species, *H. tubulosa*. As such, it would be restricted to the current generic names *Holothuria s.l.* and *Labidodemas*.

Within *Holothuria s.l.* at least two clades are discernible. The first of these comprises the clade (*Halodeima*, *Semperothuria*) to which *Selenkothuria* Deichmann, 1958 and *Acanthotrapeza* Rowe, 1969 most probably also belong (both subgenera hold species with similar button-like rosettes and/or rods, never true buttons); while the second entails the remainder of the *Holothuria* subgenera and *Labidode-*

mas. Such a scenario is reminiscent of Panning's (1935a) splitting of the subgenus *Holothuria* s.s., whereby he termed the group with rosettes *Abteilung A* [to which he later (Panning, 1940) attributed the generic rank *Halodeima*] and the group with buttons *Abteilung B* or *Sporadipus* Brandt, 1835 (a name that has been conclusively rejected by Clark & Rowe, 1967). Thus, a further modification to the current classification possibly entails a re-appraisal of *Halodeima* at the generic rather than subgeneric level as proposed by Rowe (1969).

Panning's (1940) split of *Holothuria* s.s. into the genera *Microthele* and *Holothuria* is, in the light of the present evidence, more problematic. Rowe (1969: 145) was correct to note that neither Panning (1931b, 1940, 1944) nor Deichmann (1958) 'recognized Brandt's (1835) original concept of *H. (Microthele)*' and that as such, this name cannot be attributed to the species for which Panning used this name. Rowe (1969) solved this problem by (1) recognizing the original sense of *Microthele*, and (2) describing a new subgenus (*Platyperona*) for the relevant species. However, given the incompleteness of the present phylogeny (not all current *Holothuria* subgenera and species have been investigated), we cannot fully discuss the intersubgeneric taxonomy of *Holothuria*. For now, it suffices to note that there seems to be a clade (*Microthele* + *Metriatyla* + *Cystipus*) that is characterized by more rugose button- and table-ossicles.

In conclusion, a nomenclatural revision of Holothuriidae will depend on further comparative taxonomic studies as well as on more detailed phylogenetic analysis before any of the changes proposed above can be solidified into a new classification. It is, however, already evident that the latter will by necessity need to incorporate many of the insights expressed by Pearson, Panning, Deichmann and Rowe, as well as novel insights from morphological (Appeltans, 2002; present study) and molecular systematics (Kerr *et al.*, 2005).

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