

Phylogeny of Cidaroida (Echinodermata: Echinoidea) based on mitochondrial and nuclear markers

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Abstract We present the first molecular phylogeny of Cidaroida, one of the most problematic groups within the echinoids. Two genes—the nuclear ribosomal gene 28 S rRNA and the mitochondrial protein-encoding gene COI—were obtained from 21 specimens representing 17 genera and 20 species, among which 13 species belong to Cidaroida. Phylogenetic analyses of the combined molecular data using parsimony and maximum likelihood optimality criteria resulted in a well-resolved phylogeny. Our results are broadly compatible (with the notable exception of *Cidaris cidaris*) with previous results obtained from morphological data. We find that Cidaroida represent a monophyletic group sister to the non-cidaroid Echinoidea. The family Cidaridae sensu Mortensen (1928) and Fell (1966) is paraphyletic because of the placement of *Psychocidaris ohshimai* as sister-group to *Histocidaris elegans*. Inside the Stylocidarina, we show that the two Atlantic species *Stylocidaris affinis* and *Stylocidaris lineata* constitute a well-supported clade. However, these two taxa could also represent two morphotypes within a single species showing high morphological variation.

Keywords Cidaroida · Phylogeny · Molecular · 28S rRNA · COI

Introduction

The pencil urchins (Echinoidea, Cidaroida) represent an important group of “regular” echinoids, with 123 extant species in 33 genera (Mortensen 1928; Fell 1966) and an even larger number of fossil representatives. This group occurs throughout all oceans, from shallow waters to great depths. The order Cidaroida has proved to be hard to classify on the basis of morphological characters (Smith and Wright 1989). The most important revision was made by Mortensen (1928). His taxonomy was criticized for being oversplit (e.g., Philip 1964) but formed the basis for the alternative classification proposed by Fell (1966), and is still a reference today. Cidaroida is considered sister-group to the other families of echinoids (Smith et al. 1992; Littlewood and Smith 1995; Smith et al. 2006).

The classification of Cidaroida still relies heavily on the morphological framework established by Mortensen (1928). Mortensen (1903) introduced the structure of pedicellariae as an important set of taxonomic characters used to differentiate between groups at different levels of classification. Following Mortensen, Fell (1966) proposed another classification, splitting Stylocidarina between Cidarina and Rhabdocidarina (Table 1). As a paleontologist, this author questioned the taxonomic value of pedicellariae, which are often missing in fossils, and his classification is based on characters of the plates. Smith and Wright (1989) provided the first comprehensive cladistic analysis of the group. More recently, Kroh and Smith (2010) performed a more thorough analysis that served for the basis of a new classification scheme (Kroh and Mooi 2011).

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Table 1 Classification of Cidaroida according to Mortensen (1928, 1932, 1939, 1951), Fell (1966), and Kroh and Mooi (2011)

Mortensen (1928, 1932, 1939, 1951)	Fell (1966)	Kroh and Mooi (2011)
Family: Cidaridae Gray, 1825		
Sub-family: Stereocidarinae Lambert, 1900		
Sub-tribe: Histocidarina Lambert, 1900	Sub-family: Histocidarinae Lambert, 1900	Family: Histocidaridae Lambert, 1900
<i>Histocidaris</i> Mortensen 1903	<i>Histocidaris</i>	<i>Histocidaris</i>
<i>Poriocidaris</i> Mortensen, 1909	<i>Poriocidaris</i>	
		Family: Cidaridae Gray, 1825
Sub-tribe: Cidarina Gray, 1825	Sub-family: Cidarinae Gray, 1825	Sub-family: Cidarinae Gray, 1825
<i>Cidaris</i> Leske, 1778	<i>Cidaris</i>	<i>Cidaris</i>
<i>Calocidaris</i> Clark 1907	<i>Calocidaris</i>	<i>Calocidaris</i>
<i>Tretocidaris</i> Mortensen, 1903	<i>Tretocidaris</i>	<i>Tretocidaris</i>
<i>Lissocidaris</i> Mortensen, 1939	<i>Lissocidaris</i>	<i>Lissocidaris</i>
Sub-tribe: Stylocidarina Mortensen, 1903		
<i>Centrocidaris</i> Agassiz, 1904	<i>Centrocidaris</i>	<i>Centrocidaris</i>
<i>Eucidaris</i> Pomel, 1883	<i>Eucidaris</i>	<i>Eucidaris</i>
<i>Hesperocidaris</i> Mortensen 1928	<i>Hesperocidaris</i>	<i>Hesperocidaris</i>
<i>Kionocidaris</i> Mortensen, 1932	<i>Kionocidaris</i>	
<i>Stylocidaris</i> Mortensen, 1909	<i>Stylocidaris</i>	<i>Stylocidaris</i>
		<i>Compsocidaris</i>
		<i>Chorocidaris</i>
	Sub-family: Rhabdocidarinae Lambert, 1900	
<i>Acanthocidaris</i> Mortensen, 1903	<i>Acanthocidaris</i>	<i>Acanthocidaris</i>
<i>Actinocidaris</i> Mortensen 1928	<i>Actinocidaris</i>	
<i>Plococidaris</i> Mortensen, 1909	<i>Plococidaris</i>	<i>Plococidaris</i>
<i>Prionocidaris</i> Agassiz, 1863	<i>Prionocidaris</i>	<i>Prionocidaris</i>
Sub-tribe: Rhabdocidarina Lambert, 1900		
<i>Chondrocidaris</i> Agassiz, 1863	<i>Chondrocidaris</i>	<i>Chondrocidaris</i>
<i>Phyllacanthus</i> Brandt, 1835	<i>Phyllacanthus</i>	<i>Phyllacanthus</i>
Sub-tribe: Goniocidarina Mortensen, 1928	Sub-family: Goniocidarinae Mortensen, 1928	Sub-family: Goniocidarinae
<i>Goniocidaris</i> Desor, in Agassiz et Desor, 1846	<i>Goniocidaris</i>	<i>Goniocidaris</i>
<i>Schizocidaris</i> Mortensen 1903	<i>Schizocidaris</i>	<i>Schizocidaris</i>
<i>Rhopalocidaris</i> Mortensen 1927	<i>Rhopalocidaris</i>	<i>Rhopalocidaris</i>
<i>Psilocidaris</i> Mortensen 1927	<i>Psilocidaris</i>	<i>Psilocidaris</i>
		<i>Ogmocidaris</i>
		<i>Austrocidaris</i>
Sub-tribe: Stereocidarina Lambert, 1900	Sub-family: Stereocidarinae Lambert, 1900	Sub-family: Stereocidarinae
<i>Stereocidaris</i> Pomel, 1883	<i>Stereocidaris</i>	<i>Stereocidaris</i>
<i>Compsocidaris</i> Ikeda, 1939	<i>Compsocidaris</i>	
<i>Chorocidaris</i> Ikeda, 1939		
Sub-tribe: Ctenocidarina Mortensen 1928	Sub-family: Ctenocidarinae Mortensen 1928	Family: Ctenocidaridae Mortensen 1928
<i>Ogmocidaris</i> Mortensen, 1921	<i>Ogmocidaris</i>	
<i>Austrocidaris</i> Clark 1907	<i>Austrocidaris</i>	
<i>Rhynchocidaris</i> Mortensen, 1909	<i>Rhynchocidaris</i>	<i>Rhynchocidaris</i>
<i>Ctenocidaris</i> Mortensen, 1910	<i>Ctenocidaris</i>	<i>Ctenocidaris</i>
<i>Notocidaris</i> Mortensen, 1909	<i>Notocidaris</i>	<i>Notocidaris</i>
<i>Aporocidaris</i> Agassiz and Clark, 1907	<i>Aporocidaris</i>	<i>Aporocidaris</i>
<i>Homalocidaris</i> Mortensen 1928	<i>Homalocidaris</i>	<i>Homalocidaris</i>
<i>Eurocidaris</i> Mortensen, 1909	<i>Eurocidaris</i>	
Family: Psychocidaridae Ikeda 1936	Family: Psychocidaridae	Family: Psychocidaridae
<i>Psychocidaris</i> Ikeda 1935	<i>Psychocidaris</i>	<i>Psychocidaris</i>

No phylogeny based on molecular characters has been published to date on the order Cidaroida. However, some authors have demonstrated an interest in molecular data in order to resolve echinoid inter-relationships. Matsuoka (1993) published a review of works on biochemistry and immunology that had been made on several groups of echinoids since the beginning of the 1980s. Other phylogenies were published based on DNA–DNA hybridization (Marshall and Swift 1992) as well as nuclear 28 S and 18 S subunit rRNA genes (hereafter 28 S and 18 S) (Féral and Derelle 1991; Smith et al. 1992, 2006; Féral et al. 1994; Littlewood and Smith 1995; Winchell et al. 2002). Suzuki and Yoshino (1992), Suzuki et al. (1988) and Zigler and Lessios (2003) showed the phylogenetic implications of a DNA-binding protein sequence. Lessios et al. (1999, 2001) made phylogeographic studies on a few echinoid genera based on the mitochondrial cytochrome *c* oxydase subunit I gene (hereafter COI). The first of these studies (Lessios et al. 1999) was made on one genus of Cidaroida (*Eucidaris*). Matsuoka and Inamori (1999) studied phylogenetic relationships among four cidarids based on allozymes. More recently, Lee (2003) used mitochondrial COI, NADH and 12 S genes to study the Strongylocentrotidae family within Echinoidea. Jeffery et al. (2003) analyzed a combined data set including mitochondrial 16 S rRNA [16 S], COI and nuclear 18 S gene sequences, as well as morphological character data to study the evolution of developmental modes in temnopleuroid echinoids. Stockley et al. (2005) constructed a phylogeny of spatangoid sea urchins using data from three genes (16 S, COI and 28 S) and compared their results with morphology-based phylogenies. Hart et al. (2011) used 16 S and COI to study Echinometrid sea urchins. Information on sequences (Janies et al. 2011; Pisani et al. 2012) and on mitochondrial gene order was also used in several studies at the echinoderm level (Cantatore et al. 1989; Smith et al. 1993; De Giorgi et al. 1996; Scouras and Smith 2001).

In the present paper, we used for the first time two genes, one mitochondrial (COI) and one nuclear (28 S), to study the phylogeny of Cidaroida. The goal of this study was twofold: (1) to test the monophyly of Cidaroida, and (2) to determine the relationships within this group. These relationships will be compared to the classifications of Mortensen (1928), Fell (1966) and Kroh and Mooi (2011) (Table 1).

Materials and methods

Taxon sampling

Twenty-one specimens attributed to 20 species in 17 genera were used in this study in order to best reflect the broad

diversity of Mortensen's cidaroid sub-tribes [termed subfamilies in the alternative classification of Fell (1966)]. Thirteen species of Cidaroida representing 9 genera and 2 families form the core of the study (Table 2). To test the monophyly of Cidaroida, five species of non-cidaroid echinoids were included in the analyses: *Heterobrissus niasicus* (Dörderlein 1901), *Maretia* sp., *Brisaster fragilis* (Düben and Koren 1844), *Paracentrotus lividus* (Lamarck 1816) as well as an unidentified juvenile Euechinoidea. Outgroups include one species of Crinoidea, *Florometra serratissima* (Clark 1907), one species of Asteroidea, *Crossaster papposus* (Linnaeus 1767), and one species of Ophiuroidea, *Ophiopholis aculeata* (Linnaeus 1767).

Most of the samples sequenced (11 species out of 16) were taken from specimens collected from 1985 to 2001 and preserved in 70–80 % ethanol in the collection of the Muséum national d'Histoire naturelle (Paris, France). The five remaining samples were taken from fresh material collected during the SALOMON 1 cruise near the Solomon Islands in 2001. Vouchers are deposited at the Muséum national d'Histoire naturelle (Paris, France) under the collection numbers specified in Table 2.

DNA extraction, amplification, and sequencing

DNA extraction was performed with a DNeasy Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Two genes were selected for the analyses: the mitochondrial protein-encoding gene cytochrome *c* oxidase subunit I (COI) and nuclear ribosomal gene 28 S rRNA (28 S). These have been used successfully in earlier studies on echinoids (Ratto and Christen 1990; Smith et al. 1992; Littlewood and Smith 1995; Lessios et al. 1999, 2001). Universal primers (Baroin et al. 1988) were used to amplify a fragment of approximately 350 bp from the 28 S-D1 region. The specific primers COI190L (5'–ATG ATH TTY TTY ATG GTW ATG CC–3') and COI660H (5'–TCT CCT CCT CCT GCW GGG TC–3') (Bonhomme 2002) were used to amplify a fragment of 448 bp from COI, not overlapping with the typical "barcode" region amplified by the Folmer et al. (1994) primers.

Polymerase chain reactions (PCR) included 2 µL template DNA, 1 µL of each 50 µM primer, 2.5 µL DMSO, 5 µL 6.6 mM dinucleotide-triphosphates, 10 µL (0.3 µg/µL) PCR buffer and 1.5 U *Taq* DNA polymerase. PCR reactions were performed in a TRIO-Thermoblock (Biometra, Göttingen, Germany), and involved an initial denaturation step (5 min at 94 °C) followed by 30 cycles including denaturation at 94 °C for 40 s, annealing at 50 °C for 40 s and extension at 72 °C for 40 s, with a final extension step at 72 °C for 7 min.

The double-stranded PCR products were verified by agarose gel electrophoresis (1 % agarose) and purified with the

Table 2 Echinoid species sequenced with distribution and GenBank database accession numbers. Voucher number at the Museum national d'Histoire naturelle (Paris, France). Sequences obtained from GenBank are based on the following studies: Cantatore et al. (1989), Smith et al.

(1992), Smith et al. (1993), Knott and Wray (2000), Scouras and Smith (2001), Winchell et al. (2002), and Stockley et al. (2005). The positions of the sequences used in our study are indicated in *brackets*

Species	Voucher	Location	Cruise	COI accession number	28 S accession number
<i>Florometra serratissima</i>	-	-	-	AF049132-[7445:7891]	AF212168-[479:702]
<i>Crossaster papposus</i>	-	-	-	AF217383-[192:638]	AJ225842-[101:328]
<i>Ophiopholis aculeata</i>	-	-	-	AF314589-[195:641]	AJ225836-[101:324]
<i>Brisaster fragilis</i>	-	-	-	AJ639906-[69:515]	AJ639781-[34:261]
<i>Paracentrotus lividus</i>	-	-	-	J04815-[6029:6475]	AJ225815-[101:324]
<i>Heterobrissus niasicus</i>	-	Indo-west Pacific	Salomon1	JN091889	JN091873
<i>Maretia</i> sp.	-	Indo-west Pacific	Salomon1	JN091890	JN091874
unidentified juvenile Euechinoidea	-	Indo-west Pacific	Salomon1	JN091891	JN091875
<i>Psychocidaris ohshimai</i>	EcEh1272	Indo-west Pacific	Bordau2	JN091892	JN091876
<i>Acanthocidaris curvatispinis</i>	EcEh1271	Indo-west Pacific	Chalcal2	JN091893	JN091877
<i>Cidaris cidaris</i>	EcEh1194	North Atlantic	Seamount2	JN091894	JN091878
<i>Goniocidaris (Discocidaris) peltata</i>	EcEh1207	Indo-west Pacific	Norfolk1	JN091895	JN091879
<i>Goniocidaris (Aspidocidaris) fimbriata</i>	EcEh1267	Indo-west Pacific	Bordau2	JN091896	JN091880
<i>Goniocidaris (Aspidocidaris) sibogae</i>	EcEh1268	Indo-west Pacific	Salomon1	JN091897	JN091881
<i>Goniocidaris (Aspidocidaris) fimbriata</i>	EcEh1275	Indo-west Pacific	Musorstom1	JN091898	JN091882
<i>Histocidaris elegans</i>	EcEh1269	Indo-west Pacific	Musorstom9	JN091899	JN091883
<i>Plococidaris verticillata</i>	EcEh1211	Indo-west Pacific	Richer coll.	JN091900	JN091884
<i>Prionocidaris popeae</i>	EcEh1281	Indo-west Pacific	Musorstom8	JN091901	JN091885
<i>Stereocidaris microtuberculata</i>	EcEh1196	Indo-west Pacific	Salomon1	JN091902	JN091886
<i>Stylocidaris affinis</i>	EcEh1199	North Atlantic	Seamount2	JN091903	JN091887
<i>Stylocidaris lineata</i>	EcEh1198	North Atlantic	Seamount2	JN091904	JN091888

QIAquick PCR purification kit (Qiagen). The purified PCR products were sequenced directly with the same primers used for amplification. All sequencing was performed on a CEQ2000 capillary sequencer (Beckman-Coulter, Fullerton, CA) using CEQ DCTS kits (Beckman-Coulter) with 10 µL reaction volumes (4 µL DCTS master mix, 1 µL primer, 10 µM, 1–5 µL DNA, 0–4 µL water). Sequencing reactions were performed with 30 cycles of denaturation (94 °C, 30 s), annealing (50 °C, 30 s), and elongation (60 °C, 2 min).

Chromatograms were edited with the CEQ software (Beckman-Coulter), and overlapping sequence fragments were assembled with BioEdit 7.0.1 (Hall 1999). BLAST searches (Altschul et al. 1997) were conducted with the Blastn program on the NCBI nucleotide collection (<http://ncbi.nlm.nih.gov/>) to check for putative contamination.

Phylogenetic analyses

All sequences were aligned using MAFFT v6.853b (Katoh et al. 2002; Katoh and Toh 2008) with 1,000 cycles of iterative refinement incorporating local pairwise alignment information with the L-INS-i algorithm (Katoh et al. 2005). A gap opening penalty of 1.53 and offset value (equivalent to a gap extension penalty) of 0.123 were used. No manual

adjustment was performed, and coding sequences were checked for conservation of the reading frame. GBLOCK 0.91b (Castresana 2000) was used to identify regions of potential ambiguous alignment. All the alignments and associated trees can be found online on the TreeBASE database at <http://purl.org/phylo/treebase/phyloids/study/TB2:S11628>.

For the maximum likelihood (ML) analyses, the best-fit model was chosen using the phymtest procedure implemented in the R package APE (Paradis et al. 2004). PHYML 3.0 (Guindon and Gascuel 2003; Guindon et al. 2010) was then used with the appropriate nucleotide model for a more thorough search strategy including ten replicates of randomized starting trees followed by NNI and SPR tree rearrangement. The tree search was followed by 1,000 bootstrap replicates (Felsenstein 1985). All trees are rooted on *Florometra serratissima* and taxonomic names are presented following Mortensen (1928) and Kroh and Mooi (2011). In order to check if our results were subject to a problem of random outgrouping, we performed an additional analysis with only cidaroid species included. For this analysis, trees were rooted on *Histocidaris elegans* as it is placed as the most early branch in previous morphological analyses (Smith and Wright 1989; Kroh and Smith 2010).

In order to evaluate if there is a significant mismatch between our molecular results and previous morphological results (Smith and Wright 1989; Kroh and Smith 2010), tests of alternative tree topologies were performed with the SH-test (Shimodaira and Hasegawa 1999) as implemented in the R package PHANGORN (Schliep 2010) with 10,000 bootstrap replicates. For a given dataset, the SH test uses the difference in log likelihoods of competing topologies as the test statistic, and the null distribution of the test statistic is obtained using nonparametric bootstrapping.

Parsimony analyses were performed using TNT (Goloboff et al. 2008) with gaps treated as missing data to be consistent with ML analyses. A first “classical” search strategy implemented heuristic searches with 1,000 random addition sequence and tree-bisection-reconnection (mult 1000=tbr). Alternatively, we also performed a “new technology” search with an automated procedure of difficulty level 2 (xmult=level 2). The tree search was followed by 1,000 bootstrap replicates.

Results

For the 28 S gene, GBLOCK did not identify any region of ambiguous alignment. Of the 237 positions, there were 113 distinct alignment patterns, of which 72 were parsimony informative. The mean base composition was 16.8 % A, 29.6 %C, 36.7 %G, 16.7 %T, and the best fit model was HKY + Γ . The optimal tree (LogL=−1,200.72275) is depicted in Fig. 1 for the record. For the COI gene, GBLOCK did not identify any region of ambiguous alignment. Of the 447 positions, there were 225 distinct alignment patterns, of which 164 were parsimony informative. The mean base composition was 27.5 % A, 23.8 %C, 17.9 %G, 30.5 %T, and the best-fit model was GTR + I + Γ . The optimal tree (LogL=−3,825.12487) is depicted in Fig. 2 for the record.

For the combined dataset analyzed under a GTR + Γ model, the optimal tree (LogL=−5,265.11487) is depicted in Fig. 3. Cidaroida is monophyletic [62 % bootstrap frequency (BF)] and sister to the non-cidaroid Echinoidea. Cidaroida is divided into two clades. The first clade includes

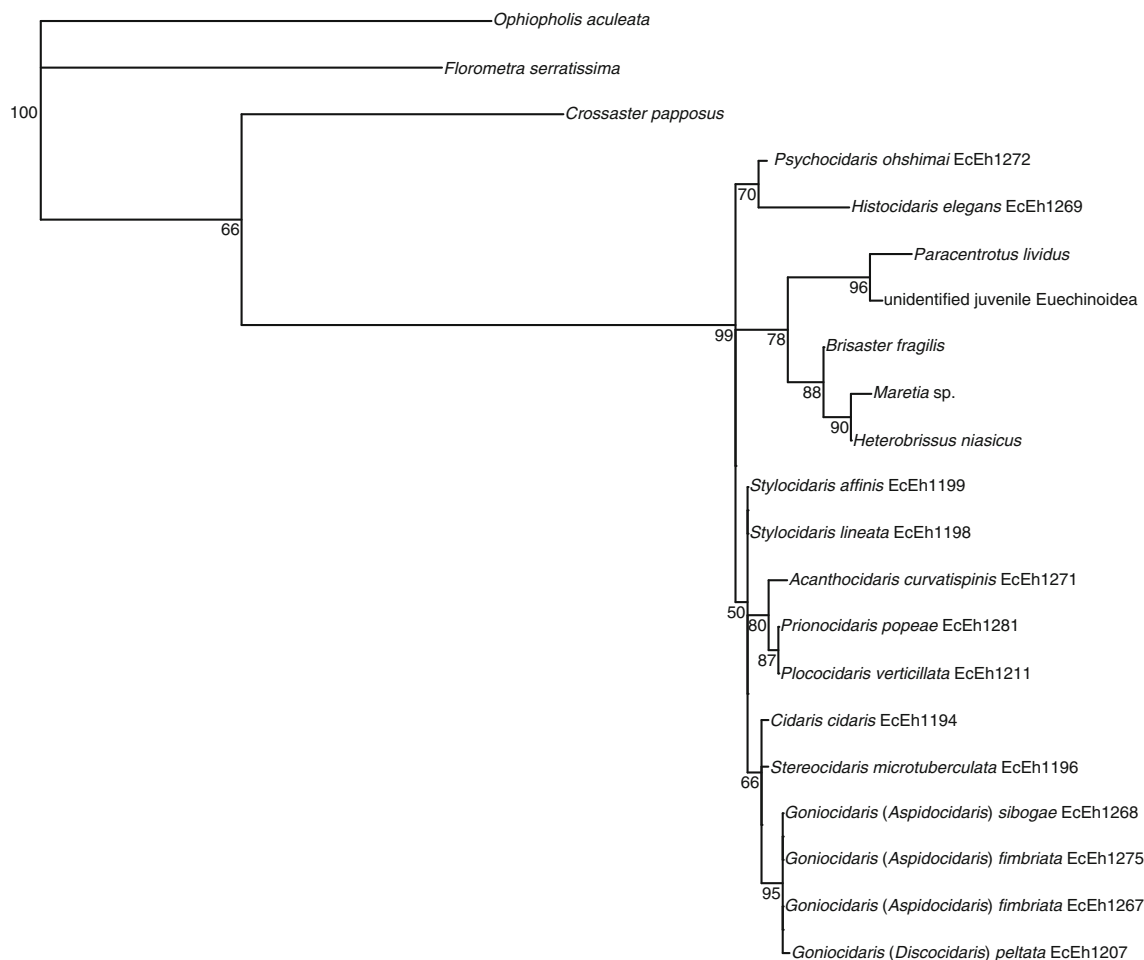


Fig. 1 Optimal tree obtained under maximum likelihood (ML: LogL=−1,200.72275) with PHYML 3.0 for the 28 S gene under a HKY85 + gamma model. Bootstrap frequencies (1,000 replicates) are indicated below nodes

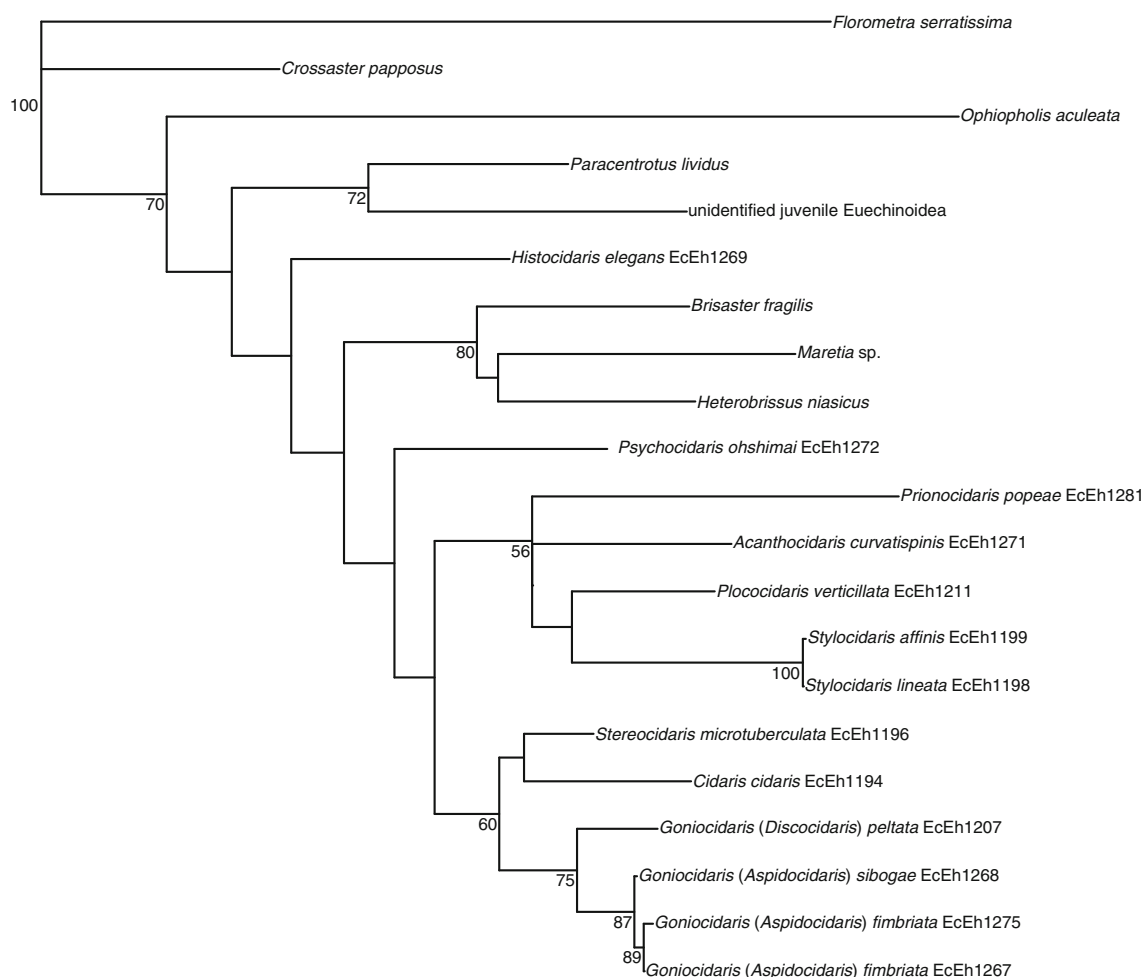


Fig. 2 Optimal tree obtained under ML (LogL=-3,825.12487) with PHYML 3.0 for the mitochondrial cytochrome *c* oxidase subunit I gene (COI) under a GTR + I + gamma model. Bootstrap frequencies (1,000 replicates) are indicated below nodes

Psychocidaris ohshimai (Ikeda 1935), the only extant species of the Psychocidaridae family, as well as *Histocidaris elegans* (Agassiz 1879). The second clade includes all the other taxa and is subdivided into two groups. The first group (76 % BF) includes *Stylocidaris affinis* (Philippi 1845), *Stylocidaris lineata* (Mortensen 1910), *Acanthocidaris curvatispinis* (Bell 1892), *Plococidaris verticillata* (Lamarck 1816) and *Prionocidaris popeae* (Hoggett and Rowe 1986). The second group (85 % BF) comprises *Stereocidaris microtuberculata*, *Cidaris cidaris* (Linnaeus 1758), *Goniocidaris fimbriata* (de Meijere 1904), *Goniocidaris sibogae* (Mortensen 1928) and *Goniocidaris peltata* (Mortensen 1927). The genus *Goniocidaris* is monophyletic with high support value (96 % BF).

We conducted Shimodaira-Hasegawa (SH) tests of three alternative topologies. In the first topology, we constrained *Psychocidaris ohshimai* to be sister to Cidaridae as in Smith and Wright (1989) and Kroh and Smith (2010) rather than sister to *Histocidaris elegans*. We found that this topology was not significantly less likely than the

optimal unconstrained topology (delta=2.82368, $P=0.5476$). In the second topology, we constrained *Cidaris cidaris* to be sister to *Stylocidaris* as in Smith and Wright (1989) rather than sister to *Stereocidaris*. This topology was significantly less likely than the optimal unconstrained topology (delta=24.21709, $P=0.0095$). Finally, in the third topology, both *Psychocidaris ohshimai* and *Cidaris cidaris* were constrained and the resulting topology was significantly less likely than the optimal unconstrained topology (delta=26.98141, $P=0.0038$).

When only cidaroids are included in the analysis and *Histocidaris elegans* is used to root the tree, the optimal tree (LogL=-2,737.32060) shows the same topology (data not shown) with *Psychocidaris ohshimai* sister to the two groups identified previously. However, the bootstrap support values are slightly changed (indicated on the right of the nodes in Fig. 3) with higher values for the deeper nodes. *Cidaris cidaris* is still sister to *Stereocidaris microtuberculata* but with bootstrap values higher than 50 % this time (though still very low). We

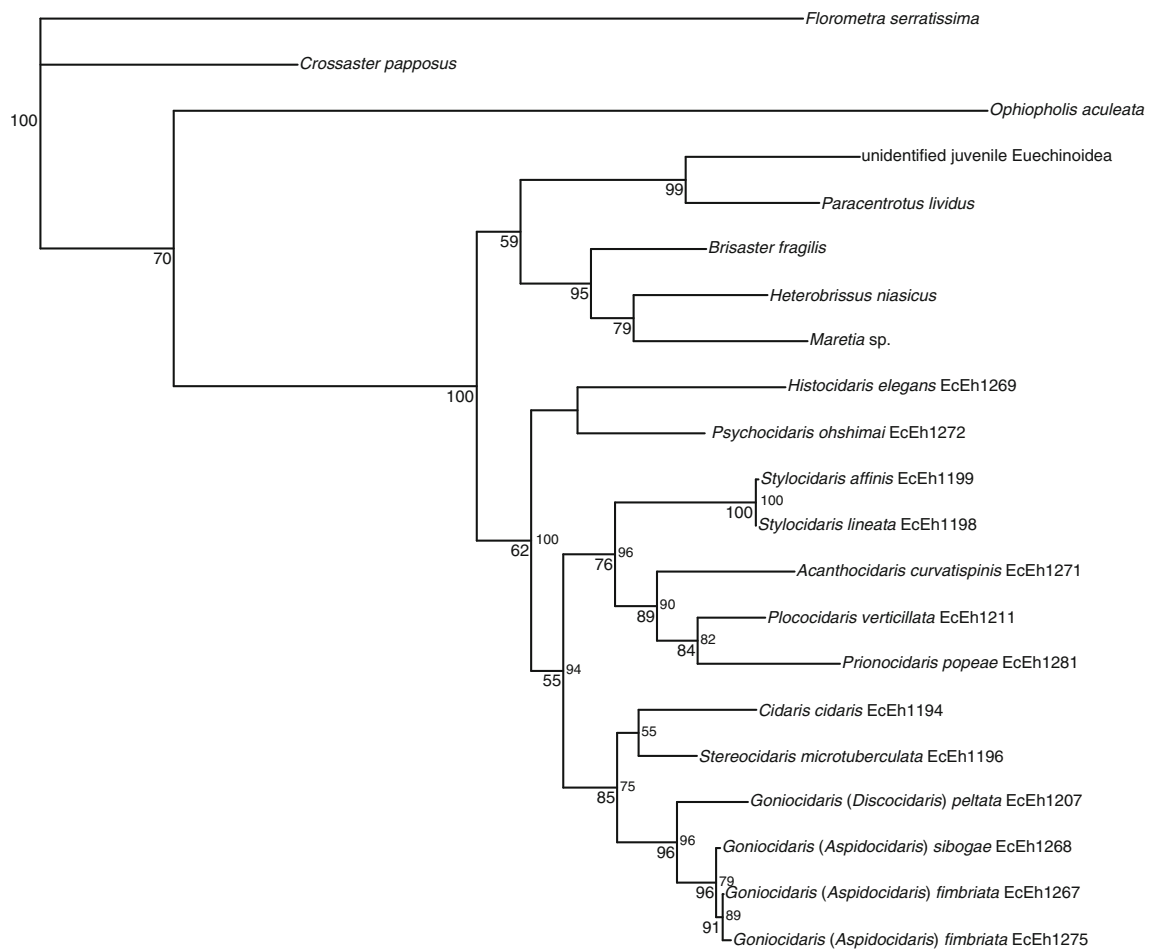


Fig. 3 Optimal tree obtained under ML (LogL=-5,265.11487) with PHYML 3.0 for the 28 S and COI genes concatenated under a GTR + gamma model. Bootstrap frequencies (1,000 replicates) are indicated below nodes. We also report, to the right of the nodes, bootstrap

frequencies (1,000 replicates) obtained when only cidaroid species are included and the tree is rooted on *Histocidaris elegans* (identical topology)

performed the SH-test once again on this reduced dataset by constraining *Cidaris cidaris* to be sister to *Stylocidaris* as in Smith and Wright (1989) rather than sister to *Stereocidaris*. Once again, this topology was significantly less likely than the optimal unconstrained topology ($\Delta=23.51702$, $P=0.0028$).

The parsimony analysis yielded two equally parsimonious trees ($L=1,060$), the strict consensus of which is depicted in Fig. 4. There was no differences in the results obtained with the different search strategies even though the “classical” search performed 4,230,920 rearrangements while the “new technology” search performed 61,138 rearrangements. The parsimony tree differs from the ML tree only in the position of *Cidaris cidaris*. This species is always placed in the same group, but either as sister-group to *Goniocidaris* (BF below 50 %) when using the parsimony criterion (Fig. 4), or as sister-group to *Stereocidaris* (BF below 50 %) in the ML analysis (Fig. 3).

Discussion

Monophyly of Cidaroida

Our molecular-based results clearly support the monophyly of Cidaroida both under maximum likelihood and maximum parsimony optimality criteria. Several morphological characters may constitute autapomorphies of Cidaroida. These include simple ambulacral plating, a perignathic girdle composed of interambulacral apophyses, a peristome margin without buccal notches, a peristome composed of ambulacral and interambulacral plates, and, at the level of Aristotle’s lantern, the summit of the junction of the two hemi-pyramids being higher than the base of the epiphyses (Mortensen 1928; Philip 1964; Fell 1966; Smith and Wright 1989; Kroh and Mooi 2011). This provides additional evidence for retaining Cidaroida and Euechinoidea as subclasses.

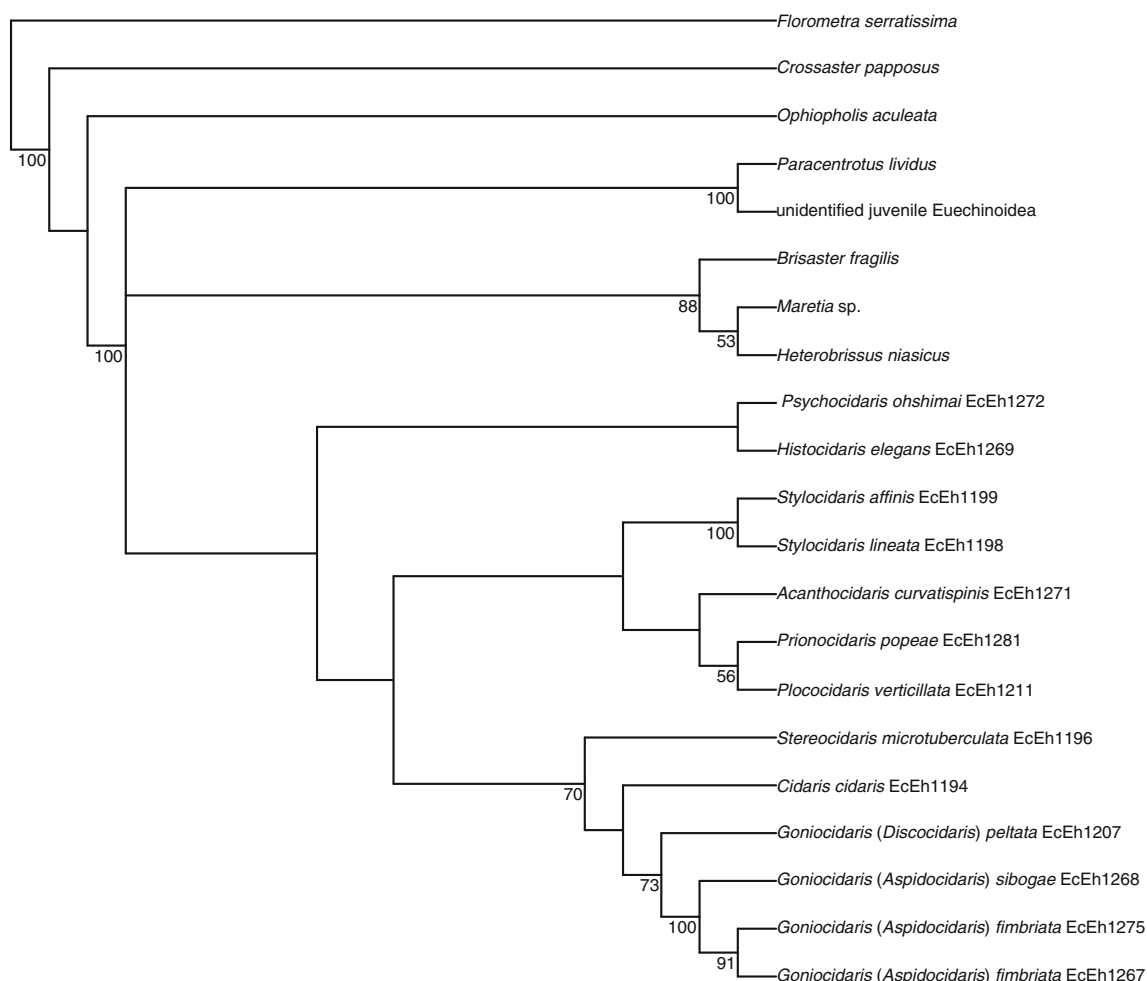


Fig. 4 Strict consensus of the two optimal trees (L=1,060) obtained under maximum parsimony with TNT for the 28 S and COI genes concatenated. Bootstrap frequencies (1,000 replicates) are indicated below nodes

Family level

In our analyses, the family Cidaridae sensu Mortensen (1928) and Fell (1966) appears paraphyletic because of the placement of *Psychocidaris ohshimai* as sister-group to *Histocidaris elegans*. The genus *Psychocidaris* was originally described and placed in the new Psychocidaridae family by Ikeda (1935, 1936). This family was subject to debate. Durham and Melville (1957) proposed to transfer this group to the family Cidaridae, other authors (e.g., Philip 1964; Fell 1966) accepted this monospecific family. Our results suggest that *Psychocidaris ohshimai* could represent a distinct family, even if resampling techniques poorly support its position. In addition, results from the SH test indicate that the position of *Psychocidaris ohshimai* as sister-group to Cidaridae [as found by Smith (2005) and Kroh and Smith (2010)] does not differ significantly from our optimal tree. If the Psychocidaridae is considered to be a valid family, it will be necessary to revise the family rank groups inside Cidaroida, especially

the position of the *Histocidaris* genus. Smith (2005) and Kroh and Smith (2010) consider that the genus *Histocidaris* belongs to the family Histocidaridae and not to Cidaridae. If we accept this classification, then the family Cidaridae is monophyletic, Cidaridae being sister to the clade (Psychocidaridae + Histocidaridae).

Infra-family level

Mortensen (1928), Fell (1966) and Kroh and Mooi (2011) (see Table 1) gave broadly similar classifications of the Cidaroida. One major difference is that Fell (1966) split Mortensen's sub-tribe Stylocidarina into two sub-families and placed the genera *Stylocidaris* and *Cidaris* together. This difference is due to the fact that Mortensen created the Stylocidarina group based on the presence of an end-tooth on the large globiferous pedicellariae, while Fell (1966) emphasized characters of the plates (non-conjugated ambulacral pores). Smith (2005) is in line with Fell (1966), considering the genera *Cidaris* and *Stylocidaris* as belonging

to the Cidarinae sub-family. In addition, following Smith (2005), Kroh and Mooi (2011) groups the sub-families Cidarinae and Rhabdocidarinae of Fell (1966), all the genera considered being placed in the sub-family Cidarinae. This sub-family as considered by Kroh and Mooi (2011) is polyphyletic in our results. The Stylocidarina included in our analyses, *Acanthocidaris*, *Plococidaris*, *Prionocidaris*, and *Stylocidaris* (type genus of the group) are sister-group to a clade including *Stereocidaris*, *Cidaris*, and *Goniocidaris*, these being type genera of the Stereocidarina, Cidarina, and Goniocidarina (sensu Mortensen 1928), respectively. Therefore, our results are in agreement with Mortensen's classification at the infra-family level. This result is further supported by the SH-test, showing that an alternative placement of *Cidaris* as sister to *Stylocidaris* is significantly different.

Species level

Inside the Stylocidarina, the two Atlantic species *Stylocidaris affinis* (from Bermuda, the Caribbean Sea, the Gulf of Mexico, and the East Atlantic, at depths ranging from 30 to 1,000 m) and *Stylocidaris lineata* (from the Caribbean Sea at depths ranging from 100 to 500 m) constitute a well-supported clade. According to Mortensen (1928), *S. lineata* differs from *S. affinis* by its color pattern, a reduced number of tubercles in the interporiferous zones of the ambulacra, and long primary spines with the shaft showing 12 longitudinal serrated ribs in *S. lineata* instead of 18 in *S. affinis*. The neck of the primary spines in *S. lineata* is often smooth and shiny. The tridentate pedicellariae display a broader proximal part of the head and a thinner space between their valves. Some of the discriminating characters used by Mortensen (1928) have been rejected by Phelan (1970): the tuberculation of the interporiferous zones of the ambulacra, the number of longitudinal serrated ribs, the neck of the primary spines, and the space between the valves of the tridentate pedicellariae. Phelan (1970) agrees with Mortensen on the differences in color pattern and the length of the primary spines. He also added the shape of the tubercles of the apical system as a discriminating character, even though he admitted observing a specimen of *S. affinis* with a tuberculation similar to that in *S. lineata*. However, the study of a large number of specimens belonging to each of the morphotypes (Brosseau 2005) showed that the differences put forward by either Mortensen (1928) or Fell (1966) are artificial because numerous individuals display intermediate morphologies. For example, the tuberculation of the apex is highly variable, some specimens showing at the same time round and radially elongated tubercles. The two morphotypes could actually belong to a single species showing high morphological variations as shown in Clark (1918). Since only one difference exists in

the COI gene between the two individuals of *Stylocidaris* sampled for this study (a G at position 324 for EcEh1198 and an A for EcEh1199), it is difficult to favor any of the hypotheses. Future work needs to be done at the population level in order to answer this question.

Likewise, in our results, *Goniocidaris fimbriata* and *Goniocidaris sibogae* group together with very short branch lengths, questioning the existence of separate entities. However, this result is based on fragments of only two genes and in the absence of morphological data. Just as for the species of *Stylocidaris*, further work needs to be done at the species and infra-species level in the genus *Goniocidaris*.

Concluding remarks

Our results based on molecules are broadly compatible (with the notable exception of *Cidaris cidaris*) with previous results obtained from morphological data (Smith and Wright 1989; Kroh and Smith 2010). At the infra-family level, our results are more in agreement with Mortensen's classification. It is now necessary to increase the sampling effort to establish the relationships within this group more precisely. The molecular markers used in this study proved to be valuable when used in combination, even if the sequences were short with relatively few informative sites. However, lengthening of existing markers or designing new ones is required in order to test inter-generic and inter-specific relationships.

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