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Molecular phylogeny and shell microstructure of *Fungiacava eilatensis* Goreau *et al.* 1968, boring into mushroom corals (Scleractinia: Fungiidae), in relation to other mussels (Bivalvia: Mytilidae)

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Abstract

Research on the evolution of the symbiosis between the boring mussel *Fungiacava eilatensis* (Bivalvia: Mytilidae) and its mushroom coral hosts (Scleractinia: Fungiidae), which requires phylogenetic reconstructions of both the Mytilidae and the Fungiidae, contributes to the understanding of the complexity of coral reef ecosystems. Previously, *Fungiacava* was regarded as a genus that had descended from *Leiosolenus* or as belonging to the subfamily Crenellinae, but no phylogenetic support has been obtained for this hypothesis. In the present study, the 18s rRNA sequences of ten mytilid species and the shell microstructures of 12 mytilids were investigated. The phylogenetic position of *F. eilatensis* is discussed in relation to its associations with its host species. The results of the molecular phylogenetic analysis indicate that *F. eilatensis* forms a sister group with *Leiosolenus simplex*, a live-coral-boring species within the *Leiosolenus* clade. Scanning electron microscope observations indicate that the shell of *F. eilatensis* is constructed of 3 layers: the outer shell layer with a homogeneous structure, the middle shell layer as a sheet nacreous structure, and the inner shell layer with an irregular simple prism structure. This shell microstructure of *F. eilatensis* is similar to that of *Leiosolenus malaccanus* and *L. simplex*. These findings show that *F. eilatensis* has descended from a coral-boring *Leiosolenus* species and that it would be adequate for *Fungiacava* to be treated as *Leiosolenus*. However, because of its extraordinary shell shape it has been kept as a separate genus.

Contents

Introduction

Bivalves of the genera *Fungiacava* and *Leiosolenus* are endosymbiotic mytilids that bore into living corals (Goreau *et al*., 1970; Mokady *et al*., 1998). Shells of *Fungiacava* are typically flat and heart-shaped (Hoeksema and Kleemann, 2002), whereas those of *Lithophaga* are more or less cylindrical and torpedo-shaped (Kleemann and Hoeksema, 2002). As endosymbionts they show very little intraspecific variation in shell morphology in comparison to attached species of, for instance, *Mytilus* (Groenenberg *et al*., 2011). *Fungiacava eilatensis* Goreau, Goreau and Neumann, 1968, bores into 14 species of living corals that belonged to four genera and six subgenera of Fungiidae (Hoeksema and Achituv, 1993; Hoeksema and Kleemann, 2002; Hoeksema and Gittenberger, 2008), which presently are considered eight genera together (Gittenberger *et al*., 2011). *Leiosolenus punctatus* (Kleemann & Hoeksema, 2002), exclusively recorded from Fungiidae, and *Leiosolenus laevigatus* (Quoy & Gaimard, 1835), infesting many species of living corals, bore into six species of Fungiidae and nine species of five scleractinian families, respectively (Kleemann, 1980; Kleemann and Hoeksema, 2002). Consequently, among the Mytilidae, *F. eilatensis* has the largest number of confirmed host coral species and the largest number of hosts belonging to a single coral family, which comprises at least 50 species (listed by Gittenberger *et al*., 2011).

Research on the evolution of symbiosis between the monospecific genus *Fungiacava* and the Fungiidae is relevant to the understanding of the complexity of interspecific associations in coral reef ecosystems, for which phylogeny reconstructions of both the Mytilidae and the Fungiidae are necessary. Although phylogeny reconstructions of fungiid corals are available

(Hoeksema, 1989, 1991a, 1993; Gittenberger *et al.*, 2011), the phylogenetic position of *F. eilatensis* is not yet fully investigated. In addition, the classification of *F. eilatensis* is not consistent among researchers. For example, Goreau *et al.* (1969) described *F. eilatensis* as a species of the subfamily Crenellinae based on the shell form, whereas others treated *Fungiacava* as belonging to the subfamily Lithophaginae based on the mode of life, along with other genera of boring mussels (Morton, 1990). Therefore, the phylogenetic position of *F. eilatensis* needs to be analysed on the basis of morphological characters and DNA sequences that strongly reflect the mytilid phylogeny.

It is known that shell microstructure characters are generally consistent within each family. In the Mytilidae, however, the shell microstructure characters are relatively variable (Carter, 1990; Kobayashi, 1998, 2004; Taylor *et al.*, 1969, 1973). This variation may support the classification of *F. eilatensis*. Furthermore, the 18s rRNA sequences of many species were investigated and deposited in the DNA Data Bank of Japan. The accumulated data are useful to reconstruct the phylogenetic position of *F. eilatensis*. In the present study, 18s rRNA sequences and the shell microstructures of ten and 12 mytilid species, respectively, were investigated in order to reconstruct the phylogenetic position of *F. eilatensis* and to classify the genus *Fungiacava* in relation to other mytilids.

Material and methods

Analysis of molecular phylogeny

Specimens of *F. eilatensis* were obtained from two specimens of the mushroom coral *Sandalolitha robusta* (Quelch, 1886) collected at Ternate, northern Molluccas, Indonesia (October 2009). After sampling, the specimens were directly fixed in 95% ethanol. Total DNA was extracted from the fixed soft tissue by using DNeasy Blood & Tissue Kit (QIAGEN). The sequence encoding the 18s rRNA was amplified using Premix Ex Taq and the polymerase chain reaction (PCR) thermal cycler PERSONAL (TaKaRa). The PCR amplification conditions consisted of denaturation (94°C and 30 s), annealing (58°C and 30 s), extension (72°C and 1 min) and 30 cycles. Used primers are given in Table 1. The PCR products were purified using ExoSAP-IT (USB Corporation), and the sequencing reaction was performed using BigDye Terminator v3.1 Cycle Sequencing Kit (ABI PRISM). The determination of DNA sequence was carried out using 3130 Genetic Analyzer (ABI PRISM).

Table 1. Amplification and sequencing primers for 18s rRNA sequence. 22F and 1789R were used to amplify in the PCR.

Primer	5'-3' sequence
22F	WAYCTGGTTGATCCTGCCAGT
393F	AGGGTTYGRYTCCGGAGA
393R	TCTCCGGAATCGAACCCT
762F	AGTGTCCTGGGTGGCCRGAAMGTTTA
762R	TAAACKTTCYGGCCACCCGAGACACT
1000F	CTGACCATAAACGATGCC
1100R	GCTATKGRARYAACKCCGSC
1418F	KTTYAGCCACACGAGATYGAGC
1418R	GCTCRATCTCGTGTGGCTRAAM
1789R	GATCCTTCYGCAGGTTCACCTAC

The sequence data were aligned using ClustalW version 1.8 (Thompson *et al.*, 1997); gaps and sites without confirmed homology were excluded from the analyses. Thirteen species belonging to nine families that are related to Mytilidae were used as outgroups. A molecular phylogenetic tree was constructed using both the maximum likelihood and Bayesian methods, and a strict consensus tree was computed from the two trees. The model for maximum likelihood analysis was determined using Modeltest version 3.7 (Posada and Crandall, 1998), and the tree was constructed using the likelihood ratchet algorithm (Vos, 2003), which was run 2000 times by using PAUP* version 4.0b10 (Swofford, 1997); the bootstrap replication number was 1000 times. The model for Bayesian analysis was determined using Mrmodeltest version 2.3 (Posada and Buckley, 2004), and the tree was constructed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). This program was run for 5,000,000 generations, with sampling every 1000th generation. The 18s rRNA-encoding sequences of 10 species that were determined in this study were registered in the DNA Data Bank of Japan. Species names and accession numbers of the analysed samples are given in Table 2.

Scanning electron microscope observations

After removal of soft tissues, the shells were cleaned and desiccated, and mechanically fragmented with a hammer. The fracture surfaces were coated with platinum by using ion coater JFC-1600 (JEOL) and observed under a scanning electron microscope (SEM) JSM-T20 (JEOL). The shell microstructure was identified on the

Table 2. Species of bivalves used for molecular phylogenetic analysis.

Family	Species	"DDBJ/EMBI/GenBank accession number"	Reference
Mytilidae	*Adipicola arcuatilis* Dell, 1995	AF221644	Distel *et al*. (2000)
	Adula aff. *californiensis* (Philippi, 1847)	AB569155	this study
	Adula falcatoides Habe, 1955	AB569156	this study
	Bathymodiolus childressi Gustafson *et al*., 1998	AF221641	Distel *et al*. (2000)
	Benthomodiolus lignocola Dell, 1987	AF221648	Distel *et al*. (2000)
	Botula fusca (Gmelin, 1791)	AB569157	this study
	Brachidontes exustus (Linnaeus, 1758)	AF229623	Campbell (2000)
	Fungiacava eilatensis Goreau *et al*., 1968	AB569158	this study
	Geukensia demissa (Dillwyn, 1817)	L33450	Kenchington *et al*. (1995)
	Gigantidas gladius Cosel & Marshall, 2003	AY649821	Jones *et al*. (2006)
	Hormomya domingensis (Lamarck, 1819)	AF117736	Distel (2000)
	Hormomya mutabilis (Gould, 1861)	AB201233	Owada (2007)
	Idas arcuatilis (Dell, 1995)	AF221643	Distel *et al*. (2000)
	Idas macdonaldi Gustafson *et al*., 1998	AF221647	Distel *et al*. (2000)
	Idas washingtonia (Bernard, 1978)	AF221645	Distel *et al*. (2000)
	Leiosolenus curtus (Liscke, 1874)	AB201235	Owada (2007)
	Leiosolenus hanleyanus (Reeve, 1857)	AB569159	this study
	Leiosolenus lithura (Pilsbry, 1905)	AB201236	Owada (2007)
	Leiosolenus malaccanus (Reeve, 1857)	AB569160	this study
	Leiosolenus nasutus (Philippi, 1846)	AB569161	this study
	Leiosolenus obesus (Philippi, 1847)	AB201237	Owada (2007)
	Leiosolenus plumula (Hanley, 1843)	AB569162	this study
	Leiosolenus simplex (Iredale, 1939)	AB569163	this study
	Lithophaga antillarum (d'Orbigny, 1853)	AB201234	Owada (2007)
	Lithophaga lithophaga (Linnaeus, 1758)	AF124208	Distel (2000)
	Lithophaga nigra (d'Orbigny, 1853)	AF124209	Distel (2000)
	Modiolus modiolus (Linnaeus, 1758)	AF124210	Distel (2000)
	Modiolus auriculatus (Krauss, 1848)	AF117735	Distel (2000)
	Modiolus philippinarum (Hanley, 1843)	AB201232	Owada (2007)
	Musculus cupreus (Gould, 1861)	AB569164	this study
	Musculus discors (Linnaeus, 1767)	AF124206	Distel (2000)
	Musculista senhousia (Benson, 1842)	AF124207	Distel (2000)
	Myrina pacifica Dall *et al*., 1938	AF221646	Distel *et al*. (2000)
	Mytilus edulis Linnaeus, 1758	L33448	Kenchington *et al*. (1995)
	Mytilus galloprovincialis Lamarck, 1819	L33452	Kenchington *et al*. (1995)
	Mytilus trossulus Gould, 1850	L33453	Kenchington *et al*. (1995)
	Septifer bilocularis (Linnaeus, 1758)	AF229622	Campbell (2000)
	Tamu fisheri Gustafson *et al*., 1998	AF221642	Distel *et al*. (2000)
Arcidae	*Arca noae* Linnaeus, 1758	X90960	unpublidhed data
	Barbatia barbata (Linnaeus, 1758)	AF207646	unpublished data
Noetiidae	*Striarca lactea* (Linnaeus, 1758)	AF120531	Giribet and Wheeler (2002)
Glycymerididae	*Glycymeris insubrica* (Brocchi, 1814)	AF207647	unpublished data
Pteriidae	*Pinctada fucata* (Gould, 1850)	AY028625	unpublished data
	Pteria hirundo (Linnaeus, 1758)	AF120532	Giribet and Wheeler (2002)
Isognomonidae	*Isognomon isognomum* (Linnaeus, 1758)	AF229621	Campbell (2000)
Pinnidae	*Atrina pectinata* (Linnaeus, 1767)	X90961	unpublished data
Limidae	*Lima lima* (Linnaeus, 1758)	AF120533	Giribet and Wheeler (2002)
Ostreidae	*Crassostrea gigas* (Thunberg, 1793)	AB064942	unpublished data
	Ostrea edulis Linnaeus, 1758	L49052	Frischer *et al*. (1998)
Pectinidae	*Chlamys islandica* (Müller, 1776)	L11232	Kenchington *et al*. (1993)
	Pecten maximus (Linnaeus, 1758)	L49053	Frischer *et al*. (1998)

Table 3. Sampling localities and patterns of shell microstructures. The symbol † indicates species described by Owada (2009). FP: fibrous prism structure, Ho: homogeneous structure, ISP: irregular simple prism structure, ISpP: irregular spherulitic prism structure, SN: sheet nacreous structure.

Species	Locality	Outer shell layer	Middle shell layer	Inner shell layer
Adula aff. *californiensis*	Waiheke island, New Zealand	ISP	SN	ISP
Adula falcatoides†	Wakkanai, Hokkaido, Japan	ISP+Ho	SN	ISP
Botula fusca†	Haneji inland sea, Okinawa, Japan	Ho	SN	ISP
Brachidontes setigera	Haneji inland sea, Okinawa, Japan	ISP	SN	ISP
Fungiacava eilatensis	Ternate, northern Moluccas, Indonesia	Ho	SN	ISP
Hormomya mutabilis	Miyako island, Okinawa, Japan	Ho+ISP	SN	ISP+SN
Leiosolenus curtus†	Oiso, Kanagawa, Japan	ISP+Ho	SN	ISP
Leiosolenus hanleyanus†	Malakal islet, Palau islands	Ho	SN	ISP
Leiosolenus lithura†	Kita-Nashiro, Okinawa, Japan	Ho	SN	ISP
Leiosolenus malaccanus†	Kita-Nashiro, Okinawa, Japan	Ho	SN	ISP
Leiosolenus nasutus†	Malakal islet, Palau islands	ISP+Ho	SN	ISP
Leiosolenus obesus†	Kita-Nashiro, Okinawa, Japan	ISP+Ho	SN	ISP
Leiosolenus plumula†	Kita-Nashiro, Okinawa, Japan	Ho	SN	ISP
Leiosolenus simplex	Minamata, Kumamoto, Japan	Ho	SN	ISP
Lithophaga antillarum†	Kyota, Okinawa, Japan	ISP+ISpP	SN	ISP
Lithophaga nigra†	Kita-Nashiro, Okinawa, Japan	ISP+ISpP	SN	ISP
Modiolus auriculatus	Nashiro, Okinawa, Japan	Ho+ISP	SN	ISP
Modiolus flavidus	Haneji inland sea, Okinawa, Japan	Ho	SN	ISP
Modiolus philippinarum	Awase tideland, Okinawa, Japan	Ho+ISP	SN	ISP
Musculus cupreus	Miura, Kanagawa, Japan	Ho	SN	ISP
Musculista senhousia	Kanazawa-Hakkei, Kanagawa, Japan	Ho	SN	ISP
Mytilus galloprovincialis	Kanazawa-Hakkei, Kanagawa, Japan	FP	SN	SN
Septifer bilocularis	Untenbaru, Okinawa, Japan	ISP	SN	ISP+SN

basis of the features described by Taylor *et al.* (1969, 1973) and Carter (1990). Species names and sampling localities of the observed samples are given in Table 3.

Results

Molecular phylogeny

The 18s rRNA-encoding sequences consisted of 1721-1742 bp long. After alignment, the analysed sequences were 1681 bp long, with 361 variable sites. In the maximum likelihood analysis, the TrNef + I + G model was selected by Modeltest, and the likelihood index (-ln *L*) of the constructed tree was 6968.1327. In the Bayesian method, the SYM + I + G model was selected by Mrmodeltest. A strict consensus tree was computed from these 2 trees (Fig. 1).

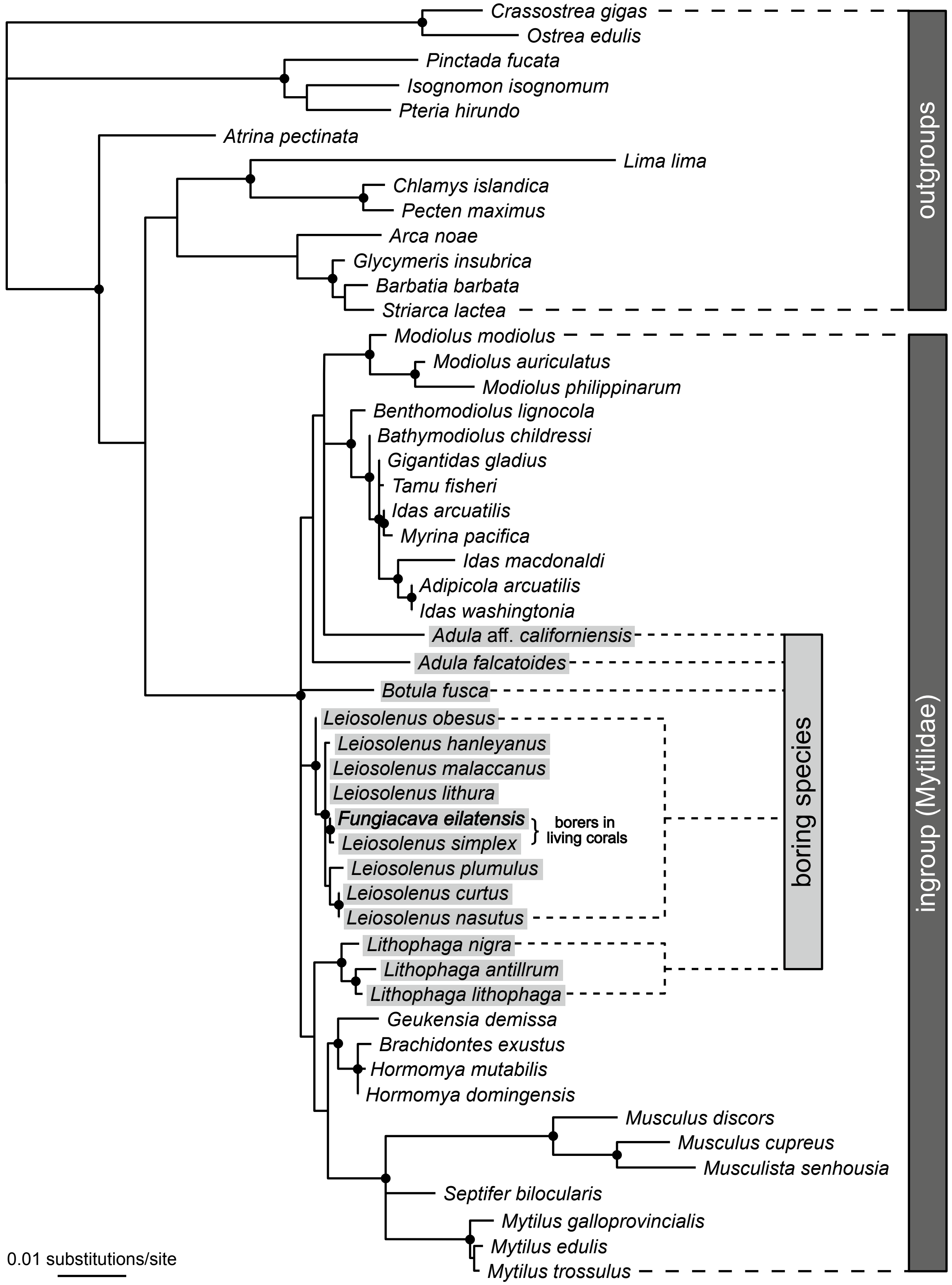
The strict consensus tree topology indicates that the phylogenetic relationships between subfamilies are not clear although the Mytilidae appears to be monophyletic (bootstrap value > 50% and Bayesian posterior probability > 0.90). *Lithophaga*, *Modiolus*, and *Mytilus* are monophyletic, but *Idas*, *Leiosolenus*, and *Musculus* not. Furthermore, the groups [*Adipicola* + *Bathymodiolus* + *Benthomodiolus* + *Gigantidas* + *Idas* + *Myrina* + *Tamu*], [*Brachidontes* + *Geukensia* + *Hormomya*], [*Fungiacava* + *Leiosolenus*], and [*Musculista* + *Musculus* + *Mytilus* + *Septifer*] also exhibit monophyly. In the present tree, *F. eilatensis* is a sister taxon of *Leiosolenus simplex*.

Shell microstructure

Shells of all of the observed species consisted of three layers: outer, middle, and inner shell layers. The outer shell layer was composed of either one or two of the following three shell microstructures: fibrous prism

Fig. 1. The strict consensus tree of the mytilid genera composed from the 2 trees constructed using the maximum likelihood and Bayesian methods. The nodes on which the topologies of the 2 trees were not concordant are indicated as polytomies. The mark ● on a node indicates bootstrap value > 50% and Bayesian posterior probability > 0.90 for the respective monophyletic clade. The branch length was calculated using the maximum likelihood method.

▶


Crassostrea gigas
Ostrea edulis
Pinctada fucata
Isognomon isognomum
Pteria hirundo
Atrina pectinata
Lima lima
Chlamys islandica
Pecten maximus
Arca noae
Glycymeris insubrica
Barbatia barbata
Striarca lactea
outgroups
Modiolus modiolus
Modiolus auriculatus
Modiolus philippinarum
Benthomodiolus lignocola
Bathymodiolus childressi
Gigantidas gladius
Tamu fisheri
Idas arcuatilis
Myrina pacifica
Idas macdonaldi
Adipicola arcuatilis
Idas washingtonia
Adula aff. californiensis
Adula falcatoides
Botula fusca
Leiosolenus obesus
Leiosolenus hanleyanus
Leiosolenus malaccanus
Leiosolenus lithura
Fungiacava eilatensis
Leiosolenus simplex
borers in living corals
Leiosolenus plumulus
Leiosolenus curtus
Leiosolenus nasutus
Lithophaga nigra
Lithophaga antillrum
Lithophaga lithophaga
boring species
Geukensia demissa
Brachidontes exustus
Hormomya mutabilis
Hormomya domingensis
Musculus discors
Musculus cupreus
Musculista senhousia
Septifer bilocularis
Mytilus galloprovincialis
Mytilus edulis
Mytilus trossulus
ingroup (Mytilidae)
0.01 substitutions/site

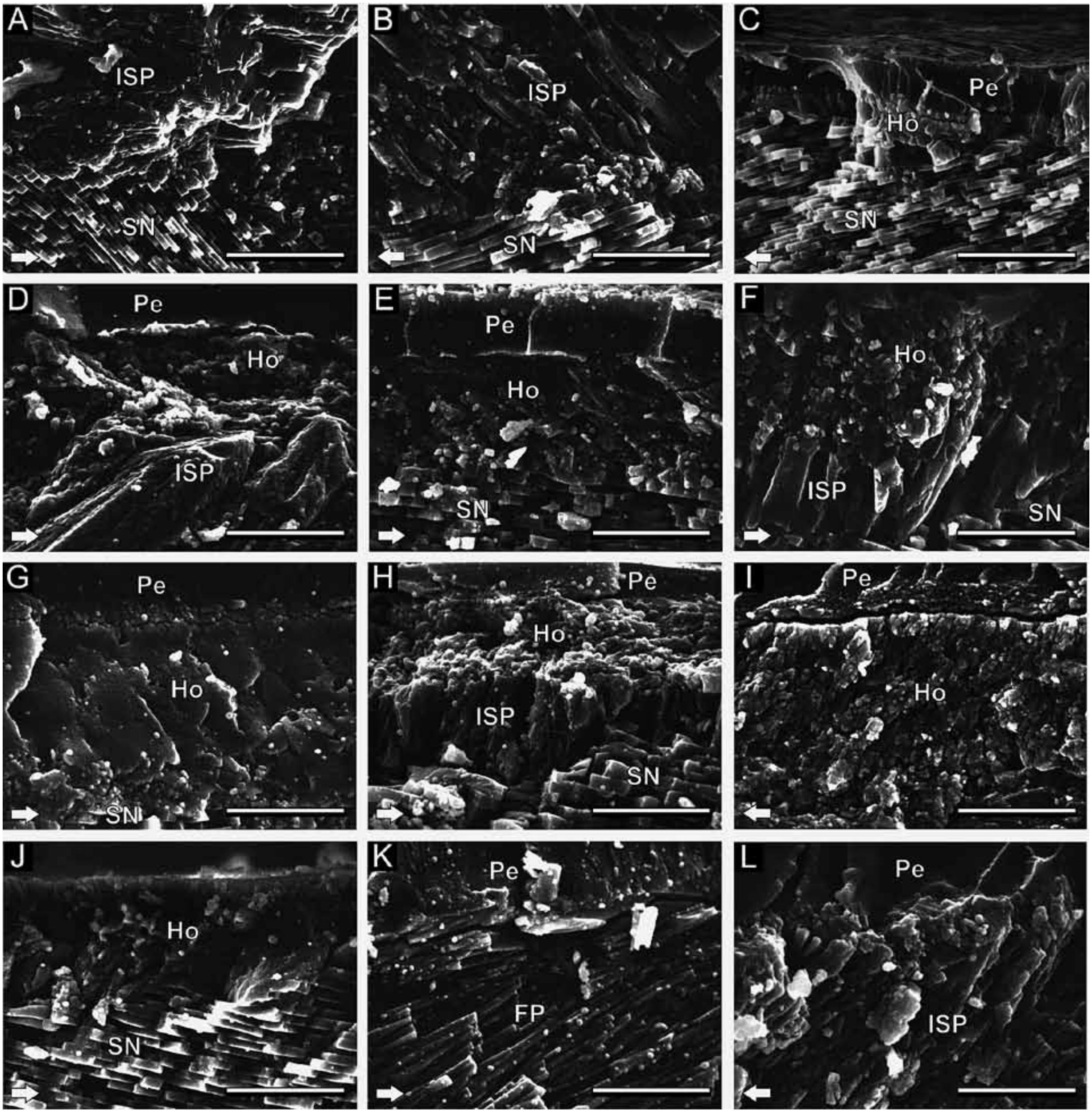


Fig. 2. Shell microstructures observed using SEM. The outer shell layer is partially shown. The arrows indicate the direction of the umbo. The scale bars indicate 10 µm. FP: fibrous prism structure, Ho: homogeneous structure, ISP: irregular simple prism structure, Pe: periostracum, SN: sheet nacreous structure. A: *Adula* aff. *californiensis*, B: *Brachidontes setigera* (Dunker, 1856), C: *Fungiacava eilatensis*, D: *Hormomya mutabilis*, E: *Leiosolenus simplex*, F: *Modiolus auriculatus*, G: *Modiolus flavidus* (Dunker, 1857), H: *Modiolus philippinarum*, I: *Musculus cupreus*, J: *Musculus senhousia*, K: *Mytilus galloprovincialis*, L: *Septifer bilocularis*.

(FP) structure, homogeneous (Ho) structure, and irregular simple prism (ISP) structure (Fig. 2, Table 3). The ISP structure of the outer shell layer was consistently observed in *Adula*, *Leiosolenus*, *Lithophaga*, and *Modiolus*, though it was absent in some species of *Leiosolenus* and *Modiolus*. The middle shell layer was composed of the sheet nacreous (SN) structure in all observed species. The inner shell layer of most species was composed of the ISP structure, except in *Hormomya mutabilis* and *Septifer bilocularis*, in which the inner shell layer was composed of alternating ISP and SN structures. The ISP structure was absent in *Mytilus galloprovincialis*. The outer shell layer of *F. eilatensis* was Ho structure, and the shell microstructure of each layer was similar to that of each layer of *Leiosolenus malaccanus* and *L. simplex* (see Fig. 4-E in Owada, 2009).

Discussion

Phylogeny of Fungiacava *and* Leiosolenus

Fungiacava was included in the *Leiosolenus* clade of mussels that live as endosymbionts in living corals, and forms a sister taxon of *L. simplex*. *Musculus* and *Musculista*, which belong to the Crenellinae, did not form a clade with *Fungiacava*. Furthermore, the shell microstructure of *F. eilatensis* was more similar to that of *L. malaccanus* and *L. simplex* than that of *Musculus cupreus* and *Musculista senhousia*. These observations suggest that *F. eilatensis* has descended from a coral-boring *Leiosolenus* species.

The fossil record suggests that *Leiosolenus* dates back to the Jurassic and that the symbiosis between *Leiosolenus* and the corals was established since the Cretaceous (Kleemann, 1983, 1990, 1994, 1996). Fungiid corals appeared since the Paleocene, and the number of species has increased since the Miocene (Wells, 1966; Hoeksema, 1989). Furthermore, the symbiosis between *Fungiacava* and the fungiid corals was not established before the Pleistocene (Goreau *et al*., 1976). The time period of the establishment of symbiosis between *Leiosolenus* and fungiid corals is uncertain. However, one of the *Leiosolenus* lineages must have diverged into *Fungiacava* between the Paleocene, the period to which the earliest known presence of Fungiidae has been attributed, and the Pleistocene, the period from which the earliest specimens of *Fungiacava* has been recorded. Since *Fungiacava* is found in 14 mushroom coral species (Hoeksema and Kleemann, 2002) distributed over eight genera (Gittenberger *et al.,* 2011), it is likely that the association between *Fungiacava* and the Fungiidae evolved early in this period, unless *Fungiacava* has only recently become a generalist with regard to its host preferences.

Juvenile free-living fungiids are attached to a hard substratum and break loose before they reach their adult stage (Hoeksema, 1989; Yamashiro and Yamazato, 1996; Vizel *et al*., 2009). Their mode of life is quite different from that of the attached fungiid species (see *e.g.* Hoeksema, 2009) since they can move towards different types of substrata, including sand and silt (Hoeksema and Moka, 1989; Hoeksema and Koh, 2009). Of the 14 recorded mushroom coral hosts of *F. eilatensis*, only two species are unable to detach from the substratum. Because these attached species are closely related to free-living host species (Hoeksema, 1989, 1991a; Gittenberger *et al*., 2011), the original appearance of *Fungiacava* in the evolutionary history of the Fungiidae appears to be related to the detached growth form of mushroom corals. Owing to their mobility, mushroom corals may form mixed multi-species assemblages (Hoeksema, 1991b; Hoeksema and Koh, 2009; Hoeksema and Matthews, 2011), and the boring mussels move along with their hosts over the reef bottom. This may facilitate their infestation success, especially if the symbionts are not host-specific. On the other hand, it is remarkable that some mushroom coral species that are known to reach high population densities, such as *Zoopilus echinatus* Dana, 1846, *Halomitra clavator* Hoeksema, 1989, *Heliofungia actiniformis* (Quoy & Gaimard, 1833) and *Heliofungia fralinae* (Nemenzo, 1955) are not infested by *Fungiacava*. *Z. echinatus* and *H. clavator* are thin mushroom corals, which can form large aggregations of loose fragments (Hoeksema and Gittenberger, 2010). They usually do not offer much space to boring organisms and when they break, endosymbionts may become more easily exposed to predators. Furthermore, the mushroom coral species *H. actiniformis* and *H. fralinae* both have fleshy polyps and may also form large aggregations (Hoeksema, 1989). They may reproduce asexually by growing many buds on vacated stalks in *H. actiniformis* (Hoeksema, 1989; Knittweis *et al*., 2009) or on dying parent corals in *H. fralinae* (Hoeksema, 2004). Mushroom corals with fleshy polyps may be difficult to infest by coral-boring mussels, and since live-coral-boring bivalves benefit from a long life span of their host corals for their own survival, corals that die easily are not a suitable habitat for them. Therefore, the success of the host selection for *Fungiacava* may be related to the special niche that they have entered with their free-living hosts.

Taxonomical position of Fungiacava

In the Mytilidae, both *Leiosolenus* and *Lithophaga* are characterized by an elongated cylindrical shell form (Newell, 1969). In addition, *Leiosolenus* is classified by the calcareous incrustations covering the periostracum, while *Lithophaga* lacks such incrustations (Oliver, 1992). The shell microstructure characters of the two genera are quite different (Table 3), and the genetic distance is also large (Owada, 2007, 2009). The shell form of *Fungiacava* looks like that of *Solamen* (Crenellinae) rather than that of the two aforementioned genera, and the calcareous incrustations are not observed on the periostracum (Goreau *et al*., 1969). However, the shell microstructure characters of *Fungiacava* are very similar to those of *Leiosolenus* (Table 3), and *Fungiacava* was included in the *Leiosolenus* clade of the present molecular phylogenetic tree (Fig. 1). Therefore, it would be adequate for *Fungiacava* to be treated as *Leiosolenus*, but its unique heart-shaped shell form is a reason to keep it a separate genus, as originally proposed by its authors (Goreau *et al*., 1968, 1969).

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