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#### **ABSTRACT**

Amongst echinoderms, the Holothuroidea represents the class that is the most infested by parasites. Parasites of holothuroids are Bacteria, Protozoa and Metazoa. There are about 150 species of metazoans which parasite holothuroids. Most of them are turbellarians, gastropods, copepods, crabs or fishes. The main body compartments suffering of the infestations are the digestive system and the coelom. The diseases induced by metazoan parasites are mostly structural: they create galls at the surface of the epidermis, pierce the respiratory tree or dig into the body wall down to the coelom. Most metazoans that live in the digestive system do not induce obvious diseases and their relationship with their hosts is probably close to commensalism. Most Protozoa that parasite holothuroids are sporozoans. They occur mainly in the coelom and/or the haemal system, one species having been reported infesting the gonads. Even in heavily infested hosts, the signs of disease induced by sporozoans are low: at most, host haemal lacuna is occluded by trophozoites or cysts are formed into the coelomic epithelium.

The most pathogen agents reported from cultured sea cucumbers are Bacteria. Cultivated holothuroids may suffer from a bacterial disease, called skin ulceration disease, that affects their body wall. In particular, juvenile *Holothuria scabra* reared in the Aqua-Lab hatchery of Toliara, Madagascar, suffered from such a disease that caused death within three days. The first sign of the infection is a white spot that appears on the integument of individuals, close to the cloacal aperture. The spot extends quickly onto the whole integument leading to the death of individuals. The lesions consist in a zone where the epidermis is totally destroyed and where collagen fibres and ossicles are exposed to the external medium. This zone is surrounded by a border line where degrading epidermis is mixed with connective tissue. Lesions include three bacterial morphotypes: rod-shaped bacteria, rough ovoid bacteria, and smooth ovoid bacteria. Three species of bacteria have also been put in evidence in the white spot lesions thanks to biomolecular analyses (DGGE and sequencing): *Vibrio* sp., *Bacteroides* sp., and an  $\alpha$ -Proteobacterium.

Keywords: Parasitoses; Bacteria; Holothuroidea

# INTRODUCTION

Many organisms are reported to infest echinoderms, especially holothuroids. Holothuroid associates are either commensals or parasites. The first are harmless for the hosts: they often live at the surface of holothuroids that act as protective substrates against eventual predators. For the second, holothuroids also represent biotic substrates where they can live but yet serve sometimes as a source of food and/or as a source of developmental stimuli without which they cannot complete their life cycle. Parasites are always harmful for their hosts and they induce diseases that are non lethal most of the time but that

can cause the host's dead in a few cases. According to a review on echinoderm diseases (Jangoux 1990), more than one third of echinoderm parasites live in or on holothuroids. No bacteria had been recorded at that time and only protozoan and ten metazoan taxa of high rank in the Linnean hierarchy were known to infest holothuroids. According to Jangoux (1990), most parasitic animals were Platyhelminthes (39 turbellarian species and 3 flukes), then Mollusca (33 gastropods and 4 bivalves), Arthropoda (one tardigrade, 22 copepods, 8 crabs and 2 pycnogonids), Pisces (9 carapids), Bryozoa (4 species), Annelida (2 polychaetes) and finally Porifera, Cnidaria, Entoprocta (1 species each) and Nematoda (undetermined number of species).

The present report aims at synthesising the main parasites and biotic diseases of sea cucumbers and includes the recent studies on the subject since the work of Jangoux (1990). It particularly emphasises new bacterial parasites and the disease that they induce.

# **BACTERIA**

Almost nothing is known about bacterial parasites and diseases in holothuroids. Morgan (2000) reported that a broodstock of *Holothuria scabra* from Bribie Island (Australia) suffered from a bacterial disease. The infection provoked the loss of epidermal pigmentation associated with the presence of huge amount of viscous mucus. The lesions first arose around the mouth and/or cloacal opening of animals and then spread on both sides of the individuals before possibly encompassing the whole body. If infected animals were not eliminated from the broodstock, up to 95% of the reared individuals died (Morgan, 2000). According to this author, Vibrio harveyi was the predominant bacterium in the lesions of affected holothuroids. Becker (2002) reported on a disease, called the skin ulceration disease, affecting juvenile *H. scabra* reared in the hatchery of Toliara, Madagascar. Microscopic and biomolecular techniques (denaturing gradient gel electrophoresis and sequencing) together with bacterial cultures and infection assays were used to characterise the disease and to investigate the microbial communities of the lesions.

The skin ulceration disease, that affects the body wall of juvenile *Holothuria scabra* at Toliara, appeared for the first time in September 2000 and broke out in February 2001, May 2001, February 2002 and July 2002. The infection extended very quickly in the aquaria, being highly contagious: in February 2001, the skin ulceration disease reached a peak and affected two third of the juveniles two days after its appearance. The disease was also highly virulent causing death three days after detection of the first symptoms.

The first obvious sign of the disease was the appearance of little, white, rounded spots of ca. 1 mm in diameter corresponding to areas where the epidermis was destroyed. The white colour of the lesions is due to the exposure of the connective tissue that follows the destruction of the cuticle, the epidermis and the upper part of the connective tissue. Yet, the mesothelium, the muscles and the internal organs remained unaffected. The first white spots always appeared close to the cloacal opening and this was quickly followed by the outbreak of other spots similar in size, shape and colour in the posterior half of the individuals. The rest of the body remained healthy and the behaviour of diseased and healthy holothuroids was unchanged except that the former did not seem to burrow anymore. Twenty-four hours later, white spots spread and joined together to form a large, posterior lesion which covered about a fifth of the body surface (Fig. 1). Podia inside this lesion were totally destroyed. Other spots then appeared on the anterior part of the body and ossicles became visible externally, some of them separating from the integument. Forty-eight hours later, more than a half of the body surface was affected. Juveniles were much less active and became almost translucent, their internal organs being seen through the body wall. Three days after the beginning of the disease, the whole body surface was affected. Dead individuals were reduced to pellets of tissues heavily infested by microorganisms.

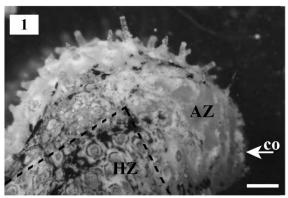


Figure 1. Juvenile of *Holothuria scabra* affected by the skin ulceration disease (O.M.). AZ: affected zone; CO: cloacal orifice; HZ: healthy zone. Scale  $bar = 100 \mu m$ 

With the use of scanning electron mycroscopy, two different zones of the body surface may be observed in the white spot lesions of diseased holothuroids: (1) the affected zone, colonised by microorganisms, where the epidermis and cuticle is totally destroyed and where a disorganised connective tissue is exposed to the medium and (2) a borderline of a few tens of micrometers wide where the surface is colonised by microorganisms and where patches of degrading epidermis are mixed with degrading, exposed connective tissue. In the affected zone, collagen fibres are running in all directions, breaking off from each other and ossicles, some of them being highly degraded, are exposed to the external medium (Fig. 2). Three bacterial morphotypes are observed in these two zones: rod-shaped bacteria, rough ovoid bacteria and smooth ovoid bacteria. The last were observed degrading holothuroid ossicles (Fig. 3).

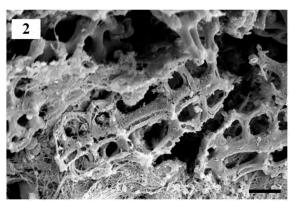


Figure 2. S.E.M. views of a lesion of a Holothuria scabra juvenile affected by the skin ulceration disease showing degraded ossicles in the affected zone and smooth ovoid bacteria on an ossicle, respectively. Scale bars =  $10 \mu m$ .

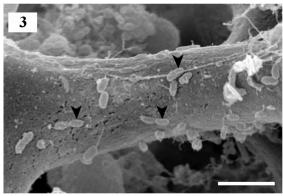


Figure 3. S.E.M. views of a lesion of a Holothuria scabra juvenile affected by the skin ulceration disease showing smooth ovoid bacteria on a degrading ossicle. Scale bars =  $5 \mu m$ .

The biomolecular techniques identified three species of bacteria in the lesions. The first, Vibrio sp., has been isolated in bacterial cultures of sea cucumber lesions and identified by DNA sequencing. The two other bacteria, Bacteroides sp. and an  $\alpha$ -Proteobacterium, have been detected by the denaturing gradient gel electrophoresis method. From the three bacteria, Vibrio sp. is the best candidate as the etiological agent of the skin ulceration disease. It is indeed close to Vibrio harveyi and V. alginolyticus, two well-known pathogenic bacteria. Vibrio harveyi is responsible of numerous infections to both vertebrates and invertebrates.

# **PROTOZOA**

Ciliates living in the digestive tract and in the respiratory trees of holothuroids have been reported by Barel and Kramer (1977). Holothuroids are otherwise mainly infested by members of five gregarine genera (13 described species) - Cystobia, Diplodina, Goniospora, Lithocystis and Urospora - and one species of coccidia (i.e., Ixoreis psychropotae). Deposit-feeding holothuroids are very sensitive to gregarine infestations. Kroll and Jangoux (1989), for example, observed that 90% of the 50 Holothuria tubulosa collected in the bed of Posidonia at Castello (Ischia Island, Italy) were infested by gregarines. The high infestations of holothuroids by gregarines can be explained by the fact that echinoderms may infest themselves simply by swallowing sediment that contains mature sporocysts. The life cycle of gregarines is complex and includes six developmental stages - sporocysts, sporozoites, trophozoites, gamontes, gametocysts, and sporogonia - that are all observed within the holothuroids. Once in the digestive system, sporocysts are broken down and sporozoites are liberated owing to the physicochemical properties of the digestive fluid (Jangoux 1990). Generally, they penetrate cells of the digestive system and migrate into the haemal system or the coelom. A more or less prolonged stay in the host's haemal system is necessary for a few species, probably for most of them, but the described species are often known only from one stage that infested a specific host's body part. Trophozoites and subsequent stages are extracellular and found in the haemal lacuna, attached to the coelomic epithelium or embedded into coelomic brown bodies. The escape of gregarines from the hosts is unknown though it is generally accepted that they leave holothuroids through evisceration or at the host's death. Recently, Doignon et al. (2003) observed that the coelomic content (brown bodies and parasites) of *Holothuria tubulosa* is purged seasonally, a phenomenon that could exist in other species. To explain this coelomic purge, the main hypothesis was that it would be an indirect result of the host's spawning as it occurs at that time: to discharge their gametes, holothuroids raise their body, adhere on the substratum by their posterior end and then contract muscles. This behaviour would cause an overpressure in the peri-cloacal end of the animal and consequently the expulsion of the coelomic fluids through the coelo-cloacal ducts, carrying the coelomic contents, brown bodies and parasites, away to the environment.

Diseases induced by protozoans to holothuroids are reduced to small internal wounds and no lethality due to gregarines has been described in literature. Growth of the Cystobia trophozoites progressively occludes the host's haemal lacuna, and a particular haemal outgrowth is formed like a bell-clapper protruding into the coelomic cavity; the clapper represents the so-called "stalked gregarine" that is formed by an evagination of the underlying haemal lacuna whose distal end encloses an enlarged trophozoite or a cyst (Jangoux 1990). One species, Diplodina gonadipertha, infests the gonads of Cucumaria frondosa and could partially destroy the gonads (Djakonov 1923). The single species of Coccidia, Iroxeis psychropotae, lives in the gut-associated haemal system of deep sea holothuroids (Massin et al., 1978).

#### **METAZOA**

# **Platyhelminthes**

Jangoux (1990) has found 5 Acoela, 34 Umagillidae (Rhabdocoela) and 3 Trematoda infesting holothuroids. Five umagillids have been described since his review (Table 1). The acoels are found mostly in the digestive tract of holothuroids, one species has been observed in the coelom and another one in the respiratory trees. Umagillids infest either the coelom or the digestive tract. Snyder (1980) could determine neither beneficial nor detrimental effects due to the occurrence of gut-associated umagillids. He concluded that these symbionts should be considered simply commensals. In contrast, Shinn (1981) reported that the gut-associated umagillids always compete with their host for nutrients and thus may exert adverse effects. He noted that all the umagillids studied by him ingest intestinal host tissue and he considered that they parasite their hosts to varying degrees. Whether they are living in the gut or in the coelom, they breed in the hosts and release egg capsules. Egg capsules are defecated in gut-associated umagillids or are accumulated in the holothuroid body cavity in coelomassociated umagillids. Coelomic umagillids swim in the host's body cavity and they are thought to ingest host's coelomic fluid together with coelomocytes (Shinn 1983) or to feed on coelom-associated organisms such as ciliates (Jennings 1980).

**Table 1.** New species of Platyhelminthes infesting holothuroids that have been described since Jangoux(1990)'s review.

Turbellaria	Host	Geographical area	Source
Umagillidae			
Anoplodium heronensis	Stichopus sp.	Great Barrer Reef	Canon (1990)
Anoplodium leighi	Stichopus sp.	New Zealand	Canon (1990)
Paranotothrix sp.	various holothuroids	Seychelles	Martens and De Clerck
			(1994)
Anoplodium sp.	Stichopus variegatus	Seychelles	Martens and De Clerck
	Actinopyga miliaris		(1994)
Wahlia westbladi	Stichopus mollis	Australia	Jondelius (1996)

Infestations by umagillids can be very high. The 27 individuals of *Telenota anax*, *Holothuria* fuscogilva, H. coluber and Actinopyga mauritiana collected in Hansa Bay (Papua New Guinea) were all infested by gut-associated umagillids and some of them by more than 200 flatworms (Eeckhaut, pers. obs.). Doignon et al. (2003) studied the infestation of *H. tubulosa* by the coelom-associated umagillid Anoplodium parasita. They observed that 26 holothuroids out of the 202 collected at Banyuls-sur-mer (France) were infested by 1 to 6 adult A. parasita. The infestation of the holothuroids by the turbellarian egg capsules was very important: 128 out of the 202 holothuroids inspected contained at least 6 and up to 10,000 egg capsules (mean of 1,433). Quite uniform throughout the year, the number of egg capsules found in the coelom as well as the number of holothuroids infested by egg capsules fall drastically in July. Interestingly, this period is the one where the gonads of holothuroids

were fully developed. Doignon et al. (2003) suggested that the release of the egg capsules could be made through coelo-rectal ducts and would be an indirect result of the host spawning.

The flukes *Himasthla leptosoma* and *zoogonoides viviparus* infest the body wall, at the base of the buccal tentacles of *Leptosynapta* spp. The first species has also been observed in the brown bodies (Cuénot 1892, 1912; Jangoux 1990). A third species, Tetrarynchus holothuriae would occur in the body wall of Molpadia sp. (Shipley 1903). Holothuroids are probably intermediate hosts for flukes. Sea birds are the definite hosts of *Himasthla leptosoma*.

# Annelida

The most recent review on symbiotic polychaetes is the one of Martin and Britayev (1998). They have found 18 polychaete species commensal of holothuroids and one parasite. The parasite, Ophryotrocha puerilis, occurs in the coelomic cavity of Ocnus planci from Napoli (Monticelli 1892; Barel & Kramer 1977; Jangoux 1990). Ganapati and Rhadakrishna (1962) noted that 50% of the holothuroids Molpadia sp. were infested by the small hesionid Ancistrocyllis sp. either in the digestive tract or respiratory trees. Commensal polychaetes are simply attached by their parapodia to the surface of holothuroids and are considered totally harmless for their hosts. Britayev and Lyskin (2002), however, recently demonstrated that Gastrolepia clavigera, the commonest Indo-Pacific polychaete associated to holothuroids, partially eats the host's integument. Holothuroid spicules represented up to 75% of the polychaete's gut content. These results suggest that symbiotic polychaetes currently considered as commensals could be true parasites.

#### Mollusca

Four Bivalvia and 33 Gastropoda are known to infest holothuroids (Jangoux 1990). Bivalves live in the cloaca or inhabit small pouches dug into the digestive wall of synaptid holothuroids (Jangoux 1990). Gastropods are free on the body surface or induced galls on it; they are also found in the respiratory tree, the digestive tract or the coelom. Ectoparasitic gastropods feed on host's tissues or fluids using their proboscis which penetrates more or less deeply into the holothuroid body wall or crosses it to reach the coelomic cavity or the haemal system (Jangoux 1990). Intradigestive symbionts enter the digestive tract in order to feed by puncturing the digestive wall (Jangoux 1990). A special case of intradigestive symbiosis is the one developed by Megadenus cantharelloides which attaches to the digestive wall of Stichopus chloronotus with its proboscis reaching the host's body wall in order to feed on dermal tissue (Humphreys & Lützen 1972). With the exception of Gasterosiphon deimatis which feed on haemal fluid (Koehler & Vaney 1903), intracoelomic gastropods are believed to derive their energy from the host's coelomic fluid by direct absorption of nutrients through their body wall (Jangoux 1990).

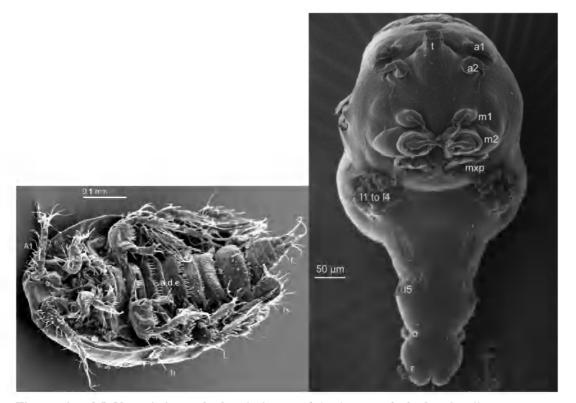
Harmfull effects of parasitic gastropods are not restricted to their feeding activities but they may produce attachment lesions involving host reactions and induction of galls. It is also reported that the intracoelomic Entocolax spp. may castrate their hosts (Heding and Mandahl-Barth 1938). So far, there is no data indicating that parasitic eulimids can seriously affect the holothuroid cycle and the ecological consequences of this parasitism may be quite limited for the holothuroid populations involved (Jangoux 1990).

# Arthropoda

Twelve pinnotherid crabs are associated with holothuroids. Most live in the respiratory trees or the posterior part of the digestive tract. One, *Pinnotheres decanus*, is rarely found in the coelomic cavity. Lissocarcinus orbicularis, the commonest Indo-Pacific crab associated with holothuroids, is often found walking at the host's body surface. Presumably, holothuroid-associated crabs do not feed on host tissues, and most of them do not cause any detrimental effects, except to slightly wound the wall of

respiratory trees or cloaca (Jangoux 1990). A notable exception to this rule is the pea crab *Pinnotheres* halingi which causes a significant atrophy of the right respiratory tree (Hamel et al., 1999).

At least eighty-five cases of association between holothuroids and copepods have been described to date. The symbiotic copepods belong to four classes (Harpacticoida, Cyclopoida, Poecilostomatoida and Siphonostomatoida), and the holothuroid hosts to more than twenty genera. But still, there is little information regarding the relations between hosts and symbionts (see, e.g., Humes 1980). Most associations reported essentially describe the localisation of the symbionts in regards to the host and the presence/absence of conspicuous characteristics that would be induced by the symbiont presence (Jangoux 1990). In the majority of these associations (ca. 60%), copepods are defined as external symbionts, with a rather unclear definition of their relations with the holothuroid host. Since Jangoux (1990) 's review, only the work of Gomes da Silva (2001) focused on the biology of symbiotic copepods, i.e. the poecilostome Synaptiphilus luteus (Fig. 4) and the siphonostome Allantogynus delamarei (Fig. 5). The first has never been reported as free living and shows a wide range of host tolerance (weak specificity). Its hosts are the northern Altlantic synaptid holothuroids *Labidoplax* digitata, Leptosynapta inhaerens, L. gallienei and L. bergensis (Bocquet 1952, Bocquet & Stock 1957, Humes 1980). Gomes da Silva (2001) performed population studies on S. luteus and showed that, if females are encountered throughout the year on their hosts, infestation by males occurs seasonally, the males and females being only present simultaneously on the hosts for a restricted mating period. Gomes da Silva (2001) also studied the coelomocyte reaction induced by Allantogynus delamarei in the body cavity of the Mediterranean aspidochirotes holothuroids H. tubulosa and H. poli. The parasite shows many peculiarities, no male is known, only the females and three larval stages are described (Changeux 1958). The female shows a highly modified morphology, she secretes an incubating envelope in which she continuously secretes ovigerous sacs each containing two embryos. The female and her ovigerous sacs are embedded in this incubating envelope, from which only the buccal tube protrudes to puncture the mesothelium of the host's coelomic cavity.



**Figures 4 and 5.** Ventral views of a female *Synaptiphilus luteus* and of a female *Allantogynus* delamarei, respectively. a1: antennula, a2: antenna, ade: distal endopodite article, m1: maxillula, m2: maxilla, mxp: maxillipede, o: laying orifice, 11 to 14: leg 1 to leg 4, 15: leg 5, r: caudal ramus, t: buccal tube.

# **Pisces**

Although some pearlfish (Carapidae, Ophidiiformes) are free-living, this family includes fishes that are able to live in association with different invertebrates. The origin of "pearlfish" would be the discovery of dead carapid fishes, paralysed and completely covered by nacreous substance in an oyster shell (Ballard 1991). However, the majority of the species belonging to the Carapini tribe (Carapus spp. and Encheliophis spp.) are usually found in the respiratory trees or in the coelomic cavity of holothuroids or in the coelomic cavity of asteroids.

Pearlfishes associated with sea cucumbers are listed in Table 2. Although the fishes are able to enter in different host species, they often show a preference to a specific one (Trott & Trott 1972, Gustato et al. 1979). C. boraborensis, C. homei, C. mourlani and E. gracilis can live in the same host species and are found in the same waters, but they usually do not inhabit the same host individual (Smith 1964, Trott 1981, Shen & Yeh 1987, Vandenspiegel & Jangoux 1989, Markle & Olney 1990). If it exists, a system of sea cucumber recognition is not yet correctly highlighted. These fishes are able to produce sounds (Parmentier et al. 2003). Sounds are not likely to be used by the fish to identify the presence of another fish in the holothuroid before penetration but are produced in the presence of congeneric individuals, inside holothuroids (Parmentier et al. 2003). Pearlfish sound emissions could be a communication system that is used in a place where the visibility is strongly reduced and where chemical communications may be masked by the host.

Table 2. Fishes associated with echinoderms (Parmentier, 2003). (\*) most common host (indicated when known); A: asteroid; B: bivalve; H: holothuroid

Carapidae		Hôte	Auteur
Carapus acus	Н	Stichopus regalis (*)	Arnold (1953, 1957), Gustato et al. (1979), Vitturi &
•			Catalano (1988), Kloss & Pfeiffer (2000)
	Н	Holothuria tubulosa (*)	Arnold (1953, 1956, 1957), Gustato et al. (1979),
			Vitturi & Catalano (1988), Kloss & Pfeiffer (2000)
	Н	Holothuria poli	Arnold (1956, 1957)
	Н	Holothuria helleri	Arnold (1956, 1957)
	Н	Holothuria sanctori	Arnold (1956)
Carapus bermudensis	Н	Actinopyga agassizi (*)	Arnold (1956), Smith & Tyler (1969), Van Meter &
1			Ache (1974), Trott (1970), Smith et al. (1981), Tyler
			et al. (1992)
	Н	Isiostichopus badionatus	Smith and Tyler (1969)
	Н	Astichopus multifudus	Trott (1970)
	Н	Holothuria glaberrima	Trott (1970)
	Н	Holothuria princeps	Dawson (1971)
	Н	Holothuria lentiginosa	Miller & Pawson (1979), Valentine & Goeke (1983),
	Н	Holothuria mexicana	Smith & Tyler (1969), Trott (1970), Tyler et al.
			(1992)
Carapus mourlani	Н	Culcita novaeguineae	Mortensen (1923), Petit (1934), Schultz (1960),
-		1	Smith (1964), Trott (1970), Trott & Trott (1972),
			Meyer-Rochow (1977, 1979), Meyer-Rochow &
			Tiang (1978), Machida (1989)
	Α	Pentaceros hawaiiensis	Markle & Olney (1990)
	Α	Choriaster granulatus	Markle & Olney (1990)
	Α	Acanthaster planci	Cheney (1973), Machida (1989)
	Н	Stichopus variegatus	Markle & Olney (1990)
	Н	Stichopus chloronotus	Markle & Olney (1990)
	Н	Bohadschia argus	Trott (1970), Meyer-Rochow (1977), Machida
		1	(1989), Markle & Olney (1990)
	Н	Actinopyga mauritania	Markle & Olney (1990)
	Н	Holothuria scabra	Markle & Olney (1990)
Carapus homei	Н	Bohadschia argus (*)	Smith (1964), Branch (1969), Trott (1970),
		l ~ ~ ~ ~ ~	VandenSpiegel & Jangoux (1989), Markle & Olney
			(1990)
	Н	Stichopus chloronotus (*)	Smith (1964), Branch (1969), Trott (1970), Trott &
			Garth (1970), Trott & Trott (1972), Seymour &
		1	McCosker (1974), Markle & Olney (1990)

[	Н	Stichopus variegatus	Trott (1970), Meyer-Rochow (1977)
	Н	Thelenota ananas	Markle & Olney (1990)
	Н	Actinopyga echinites	Markle & Olney (1990)
	Η	Holothuria ocellata	VandenSpiegel & Jangoux (1989)
	Α	Culcita schmideliana	Hipeau-Jacquotte (1967), Jangoux (1974)
	Α	Mithrodia fisheri	Jangoux (1974)
	В	Pteria lotorium	Mahadevan (1959)

Carapidae		Hôte	Auteur
Carapus boraborensis	Н	Thelenota ananas	Smith (1964), Trott (1970), Meyer-Rochow (1977), VandenSpiegel & Jangoux (1989),
			Markle & Olney (1990)
	Н	Bohadschia argus	Smith (1964), Markle & Olney (1990),
			VandenSpiegel & Jangoux (1989)
	Н	Stichopus chloronotus	Markle & Olney (1990)
	Н	Pearsonothuria graeffei	VandenSpiegel & Jangoux (1989)
	Н	Thelenota anax	Ballard (1991) – Parmentier et al. (2002)
Encheliophis sagamianus	Н	Holothuria	Tanaka (1908), Arnold (1956)
		mertensiothuria	
	Α	Certonardoa semiregularis	Yosii (1928)
	Α	Nardoa semiregularis	Arnold (1956)
Encheliophis	Н	Holothuria kefersteinii	Trott (1970)
vermicularis	Η	Holothuria leucospilota	Murdy & Cowan (1980), Masuda et al. (1984)
	Н	Holothuria lubrica	Steinbeck & Ricketts (1941)
	Н	Thelenota ananas	Markle & Olney (1990)
	Н	Holothuria atra	Schultz (1960)
	Η	Holothuria scabra	Murdy & Cowan (1980)
Encheliophis gracilis	Н	Holothuria scabra	Arnold (1956)
	Н	Thelenota ananas	Shen & Yeh (1987), Markle & Olney (1990)
	Η	Holothuria atra	Strasburg (1961), Schultz (1960)
	Η	Actynopyga crassa	Markle & Olney (1990)
	Η	Bohadschia vitiensis	Markle & Olney (1990)
	Η	Bohadschia argus	Smith (1964), Trott (1970), Machida (1989),
			Markle & Olney (1990)
	Н	Stichopus chlroronotus	Smith (1964)
	Η	Holothuria chilensis	Trott (1970)
	Η	Stichopus sp.	Markle & Olney (1990)
	Α	Culcita discoidea	Arnold (1956)
	A	Cuclcita novaeguineae)	Branch (1969), Trott (1970)
Encheliophis chardewalli	Н	Actinopyga mauritania	Parmentier (2003)

In most cases, there is only one fish per host. However, specimens of *Encheliophis (E. gracilis, E.* vermicularis and E. sagamianus) were reported to be sexually paired in various holothuroids (Murdy & Cowan 1980, Trott 1981). More than two specimens of C. homei, C. mourlani and C. bermudensis can be observed in a same host (Aronson & Mosher 1951, Meyer-Rochow 1977, Trott & Trott 1972, Smith et al. 1981). Meyer-Rochow (1977) holds the record with fifteen C. mourlani in a specimen of Bohadschia argus. Although no data were reported in the literature, the presence of sexual pairs suggests that sea cucumbers serve also as breeding sites. Sea cucumbers also act as developmental stimulators for some carapid larvae. In Carapus and Encheliophis species, the penetration inside a sea cucumber is followed by heavy transformations during which the fish length is reduced by 60%.

The stomach contents (Trott, 1970; Murdy & Cowan, 1980; VandenSpiegel & Jangoux, 1989) and the musculo-skeletal descriptions of the buccal and pharyngeal jaws (Parmentier et al. 1999, 2000a, 2000b) have suggested different kinds of symbiosis depending on the species. Various authors have suggested that Carapus adults could be parasites (Arnold, 1953; Gustato, 1976; Trott, 1981) while others consider them as commensals, using their hosts as shelters and leaving them to hunt small preys such as annelids, crustaceans (shrimp, decapod, amphipod) and small fishes (Trott, 1970; Meyer-Rochow, 1979; VandenSpiegel & Jangoux, 1989; Parmentier et al., 2000a,b). They can also be cannibals, feeding on other carapids within their host (Parmentier, pers. obs.). On contrast, Encheliophis species are true parasite, feeding on the internal tissues of their host, mainly the gonads (Smith, 1964; Trott, 1970; Trott & Trott, 1972; Murdy & Cowan, 1980; Trott, 1981).

Some individuals can be frequently found in the general cavity of holothuroids, implying a perforation of the respiratory tree or the stomach. This kind of injury, also done when the fish leaves its host, should be negligible as pearlfishes do not always infest the same host. However in a heavily infested population, chances that a given host is infested regularly increase; hence repeated loss of coelomic fluid and successive wound repairs have to be considered (Jangoux, 1990). On the other hand, Cuverian tubules are never exuded and holothuroid eviscerations seem never caused at the time of pearlfish entry.

#### **GENERAL CONSIDERATIONS**

Platyhelminthes, Mollusca and Arthropoda are the taxa that include the highest numbers of holothuroid parasites but low attention has been devoted to holothuroid-associated gregarines and it is very probable that the number of infesting species recorded in the future will be higher than that of metazoan taxa (Fig. 6). The digestive tract (including the cloaca) is the body compartment that is the most infested (Fig. 7) and gut-associated parasites are flatworms, molluscs and crustaceans. Most of them are living free in the digestive tract, gliding on the digestive wall. The symbiotic relations that they develop with the holothuroids are still not well firmly established and their parasitic status relies more on suggestions than on true experiments or valuable observations. The coelom of holothuroids is also well infested and other flatworms, molluscs and crustaceans are living in there but also protozoan gregarines and carapid fishes. Other body compartments - the haemal system, ambulacral system, respiratory trees, gonads and body wall - are less affected by parasites. The body wall, in particular, is mainly affected by gastropods.

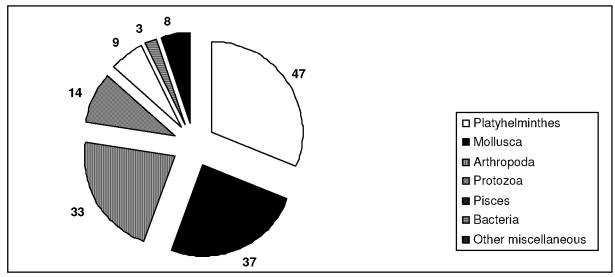
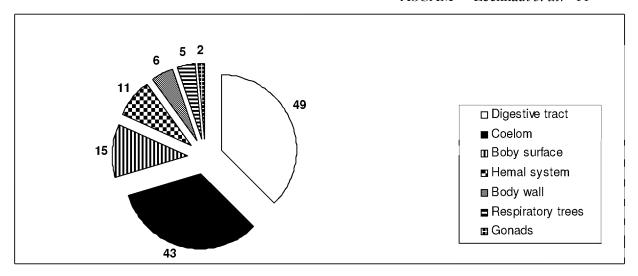


Figure 6. Number of species per taxon infesting holothuroids.



**Figure 7.** Location of the parasites in the holothuroid body. When a parasite infested two body compartments, both have been included in the graph.

Infestation of holothuroids by internal parasites takes place mainly through body openings (mouth and cloacal aperture). Intracoelomic organims reach the coelom in passing through the digestive wall. Intracoelomic copepods enter the coelom by penetrating the anterior part of the gut, larvae of most intracoelomic gastropods enter the coelom through the digestive wall and carapid fishes pierce the cloacal wall (Jangoux 1990). Holothuroid reactions to parasites are of three types: (1) inflammatorylike reactions; (2) connective tissue reactions and (3) coelomocyte reaction (Jangoux 1990). Inflammatory-like reactions consist in the migration of defensive cells (red spherules and coelomocytes) to the site of infection and occur mainly in diseases caused by microorganisms. Connective tissue reactions counteract organisms such as gastropods that tend to stay within the connective tissue layer. Coelomocyte reactions are directed towards intracoelomic parasites and result in the phagocytosis or the walling off of foreign organisms.

All the protozoans and metazoans parasites currently described in scientific literature are not lethal for wild or cultured adult holothuroids. They are thus of no economic importance and their presence in or on adult holothuroids should not alarm the sea cucumber industrial stockbreeders. However, it should be emphasised that the high density of holothuroid populations in cultures could favour overinfestations by parasites and could influence some important parameters in farming such as the growth speed of individuals or the reproductive abilities of breeders. Most alarming are the infections of sea cucumber juveniles by bacteria that can eradicate nearly all individuals of cultured populations in a few days. Parameters that cause the apparition of bacterial diseases are poorly known and we suppose that an increase in temperature and/or a too high density of individuals in aquaria can favour the infection of individuals. The best way to avoid such bacterial disease is to check constantly the health of juveniles and to eliminate sick individuals. Juveniles that are not too infected can recover by treating them with a faint formalin solution in salt water for a few minutes.

#### AKNOWLEDMENTS

Thanks are expressed to Dr. Alessandro Lovatelli for his kind invitation to the ASCAM workshop and to Dr. J.-F. Hamel for having reviewed the manuscript. We are indebted to the Food and Agriculture organization of the United Nations (F.A.O.) for having financed the participation of Dr. I. Eeckhaut to the ASCAM workshop. We also thank the Belgian Coopération Universitaire au développement (C.U.D.) and the Belgian National Fund for Scientific Researches for their financial help during various field trips to Madagascar without which this study would not have been possible. Aknowlegments are finally expressed to Man Wai Rabenevanana and Edouard Mara, Directors of the Institut Halieutique et des Sciences Marines (IH.SM) of Toliara (Madagascar), and to all the people of this institution who participate to this contribution.

#### REFERENCES

Arnold D.C. 1953. Observation on Carapus acus (Brünnich) (Jugulares, Carapidae). Pubblicazioni della Stazione Zoologica di Napoli 24: 152-166.

Arnold D.C. 1956. A systematic revision of the fishes of the teleost family Carapidae (Percomorphi, Blennioidea), with description of two new species. Bulletin of the British Museum (Natural History) 4: 247-307.

Arnold D.C. 1957. Further studies on the behaviour of the fish *Carapus acus* (Brünnich). Pubblicazioni della Stazione Zoologica di Napoli 30: 263-268.

Aronson L.H., and C. Mosher, 1951. Observations on the behaviour and ecology of the West Indian pearlfish. Anatomical Record 111: 489.

Ballard J. 1991. The Pearlfish. African Wild 45(1): 16-19.

Barel C.D., and P.G. Kramer. 1977. A survey of the echinoderm associates of the north-east Atlantic area. Zoologische Verhandelingen, Leiden 156: 1-159.

Becker P. 2002. La maladie de la tache blanche chez l'holothurie comestible commercialisée Holothuria scabra. Université de Mons-Hainaut. Master thesis. 55pp.

Bocquet C. 1952. Copepods semi-parasites et parasites de la région de Roscoff. Description de Lichomolgus asterinae n.sp. Bulletin de la Société Zoologique de France 77: 495-504.

Bocquet C., and J.H. Stock. 1957. Copépodes Parasites d'Invertébrés des Côtes de France, le genre Synaptiphilus Canu et Cuénot. Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen, Amsterdam 60(5): 680-695.

Branch J.B. 1969. Observations on the ecology and behaviour of Guam pearlfishes (Carapidae). Micronesica 24(2): 274.

Britayev T., and S. Lyskin. 2002. Feeding of the symbiotic polychaetes Gastrolepidia clavigera (Polynoidea) and its interactions with its hosts. Doklady Biological Sciences 385: 352-356.

Cannon L.R.G. 1990. Anoplodium (Rhabdocoela, Platyhelminthes) endosymbionts of sea cucumbers from Australia and new Zealand. Zoologica Scripta 19(4): 395-402.

Changeux J.P. 1958. Quelques caractères biologiques d'un copépode parasite d'holothuries: Allantogynus delamarei n.g.n.sp. Comptes Rendus Hebdomadaire des Séances de l'Académie des Sciences, Paris 247: 961-964.

Cheney D.P. 1973. Pearlfish (Carapidae) in Acanthaster planci (L.). Micronesica 9(1): 159.

Cuénot L. 1892. Commensaux et parasites des échinodermes (deuxième note). Revue Biologique du Nord de la France 5: 1-22.

Cuénot L. 1912. Contribution à la faune du Bassin d'Arcachon. V. Echinodermes. Bulletin de la Station biologique d'Arcachon 14: 17-116.

Dawson C.E. 1971. Records on the pearlfish, Carapus bermudensis in the nothern Gulf of Mexico and of a new host species. Copeia 1971: 730-731.

Djakonov M.D. 1923. Diplodina gonadipertha, n. sp. a new neogamus gregarine, parasite of the gonads of Cucumaria frondosa (Gunn.). Russkij Arkhiv Protistologie 2: 127-147.

Doignon G., M. Jangoux, J.P. Féral, and I. Eeckhaut. 2003. Seosonal release of the egg capsules of Anoplodium parasita Shneider, 1858, intracoelomic turbellarian (Platyhelminthes, Rhabdocoel) symbiotic of the sea cucumber *Holothuria tubulosa* Gmelin, 1788 (Echinodermata, Holothuroidea). p.261-264. In: Echinoderm Research 2001. Féral J.P. and B. David (Eds.) Balkema, Rotterdam.

Ganapati P.N., and Y. Radhakrishna. 1962. Inquilinism between a new hesionid polychaete and a holothurian *Molpadia* sp. Current Science 31: 382-383.

Gomes da Silva S. 2001. Particularités de la biologie de deux coépodes symbiotiques d'holothuries : Synaptiphilus luteus Canu et Cuénot, 1892 et Allantogynus delamarei Changeux, 1958. Université Libre de Bruxelles. Master thesis. 56pp.

Gustato G. 1976. Osservazioni sulla biologica e sul comportamento di Carapus acus (Ophioidei, Percomorphi). Bollettino Societa Naturalisti di Napoli 85: 505-535.

Gustato G., A. Villari, and G. Villani. 1979. Ulteriori dati sul comportamento di Carapus acus (Gadiformes, Ophidiodei). Bollettino Societa Naturalisti di Napoli 88: 535-547.

Hamel J.-F., P.K.L. Ng, and A. Mercier. 1999. Life cycle of the pea crab *Pinnotheres halingi*, sp. nov., and obligate symbiont of the sea cucumber *Holothuria scabra* Jaeger. Ophelia 50: 149-175.

Heding S.G., and G. Mandahl-Barth. 1938. Investigations on the anatomy and systematic position of the parasitic snail *Entocolax* Voigtlaender Meddr Groenland 108 (5): 1-40.

Hipeau-Jacquotte R. 1967. Notes de faunistique et biologie marines de Madagascar. IV. Observation sur le comportement du poisson Carapidae : Carapus homei (Richardson, 1844) de Madagascar. Recueils des Travaux de la Station Marine d'Endoume 6: 141-151.

Humes A.G. 1980. A review of the copepods associated with holothurians, including new species from the indo-pacific. Beaufortia 30(4): 31-123.

Humphreys W.F., and J. Lützen. 1972. Studies on parasitic gatsropods from echinoderms. II. On the structure and biology of the parasitic gastropod, Megadenus cantharelloides n. sp. Biologiske Skrifter 19(1): 1-27.

Jangoux M. 1974. Sur l'association entre certaines astéries (Echinodermata) et des poissons Carapidae. Revue Zoologique Afrique 88: 789-796.

Jangoux M. 1990. Diseases of Echinodermata. p.439-567. In: Diseases of Marine Animals. Vol.3. Kinne, O. (Ed.). Biologische Anstalt Helgoland, Hamburg, Germany. 696p.

Jennings J.B. 1980. Nutrition in Symbiotic Turbellaria. p.45-56. In: Nutrition In the Lower Metazoa. Smith D.C. and Y. Tiffon (Eds.). Pergamon Press, Oxford.

Jondelius U. 1996. The echinoderm-inhabiting flatworms (Platyhelminthes, Rhabdocoela) from Western Australia. Belgian Journal of Zoology 126(1): 37-48.

Kloss K., and W. Pfeiffer. 2000. Zur biologie des « eingeweidefisches » C. acus (Brunnich, 1768) (Carapidae, Teleostei), mit hinweisen auf eine nich-parasitische ernähung. Revue Suisse de Zoologie 107(2): 335-349.

Koehler R., and C. Vaney. 1903. Entosiphon deimatis, nouveau mollusque parasite d'une holothurie abyssale. Revue Suisse de Zoologie 11: 23-41.

Kroll A., and M. Jangoux. 1989. Les grégarines (Sporozoea) et les Umagillides (Turbellaria) parasites du coelome et du système hémal de l'holothurie Holothuria tubulosa Gmelin (Echinodermata). Vie marine, Marseille 10: 193-204.

Machida Y. 1989. New distribution of the pearlfish, C. mourlani, with notes on its morphometry. Japanese Journal of Ichthyology 36(3): 363-368.

Mahadevan S.,1959. On the occurence of Fierasfer (Cuvier) homei as a commensal inside the bivalve Pteria lotorium Lamarck. Current Science 3: 129.

Markle D.F., and J.E. Olney. 1990. Systematics of the Pearlfish (Pisces: Carapidae). Bulletin of Marine Science 47(2): 269-410.

Martens E.E., and G.G. De Clerck. 1994. Interstitial and parasitic Platyhelminthes from the coast of the Seychelles. Oceanic reefs of the Seychelles, Netherlands Indian Ocean programme, Cruise reports 2: 97-106.

Martin D., and A. Britayev. 1998. Symbiotic polychaetes: a review of known species. Oceanography and Marine Biology. An Annual Review 36: 217-340.

Massin C., M. Jangoux, and M. Sibuet. 1978. Description d'Ixoreis psychropotae, nov. gen., nov. sp. coccidie parasite du tube digestif de l'holothurie abyssale Psychropotes longicauda Théel Protistologica 14: 253-259.

Meyer-Rochow V.B. 1977. Comparison between 15 Carapus mourlani in a single Holoturian and 19 carapus mourlani from starfish. Copeia 1977(3): 582-585.

Meyer-Rochow V.B. 1979. Stomach and gut content of Carapus mourlani from starfish and a holothurian. Annales Zoologici Fennici 16: 287-289.

Meyer-Rochow V.B., and M.K. Tiang. 1978. Visual behavior, eye and retina of the parasitic fish Carapus mourlani. Biological Bulletin 155: 576-585.

Miller J.E., and D.L. Pawson. 1979. A new subspecies of Holothuria lentiginosa Marenzeller from the western Atlantic Ocean (Echinodermata: Holothuroidea). Proceedings of the Biological Society of Washington 91: 912-922.

Monticelli F.S. 1892. Notizia preliminare intorno ad inquilini degli Holothuroidea del Golfo di Napoli. Monitore zool. ital. 3: 249-256.

Morgan A.D. 2000. Aspects de la gestion des stocks géniteurs d'holothuries de sable (Echinoderme: Holothurides). Secrétariat général de la Communauté du Pacifique: La Bêche-de-mer: Bulletin d'information 13: 2-8.

Mortensen T. 1923. The Danish expedition tp the Kei Islands 1922. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening 76: 55-100.

Murdy E.O., and M.E. Cowan. 1980. Observation on the behaviour and symbiotic relationship of the pearlfish Encheliophis vermicularis (Osteichthys: Carapidae). Kalikasan, Philippine Journal of Biology 9(2-3): 309-312.

Parmentier E. 2003. Contribution à l'étude des relations entre des poissons de la famille des Carapidae et leurs hôtes invertébrés: une approche multidisciplinaire. Université de Liège, PhD thesis. 229 pp.

Parmentier E., M. Chardon, M. Poulicek, J.C. Bussers, and P. Vandewalle. 1999. Morphological particularities of the head in four Carapidae (Ophidiiformes), p.135-146. In: Proc. 5<sup>th</sup> Indo-Pacific Conf., Nouméa. 1997, Séret B and J.Y. Sire (Eds.). Société Française Ichthyologique, Paris. 866p.

Parmentier E., G. Castillo, M. Chardon, and P. Vandewalle. 2000a. Phylogenetic analysis of the pearlfish tribe Carapini (Pisces: Carapidae). Acta Zoologica (Stockholm) 81: 293-306.

Parmentier E., J.L. Castro-Aguirre, and P. Vandewalle. 2000b. Morphological comparison of the buccal apparatus in two bivalve commensal Teleostei: Encheliophis dubius and Onuxodon fowleri (Carapidae, Ophidiiformes). Zoomorphology 2000, 120: 29-37.

Parmentier E., P. Vandewalle, and J.P. Lagardère. 2003. Sound producing mechanisms and recordings in three Carapidae species. Journal of Comparative Physiology 189: 283-292.

Petit M.G. 1934. Un Fierasfer nouveau de Madagascar. Bulletin du Musée d'Histoire Naturelle de Paris 2: 393-397.

Schultz L.P. 1960. Fishes of the Marshall and Marianas islands. Vol. II. Bulletin. United States National Museum 202: 1-418.

Seymour R.S., and J.E. McCosker. 1974. Oxygen consumption of the commensal fish, Carapus homei. Copeia 1974: 971-972.

Shipley A.E. 1903. On the ento-parasites collected by the "Skeat Expedition" to Lower Siam and Malay Peninsula in the years 1899-1900. Proceedings of the Zoological Society of London 2: 145-156.

Shen S.C., and H.S. Yeh. 1987. Study on Pearlfishes (ophidiiformes: Carapidae) of Taïwan. Journal of the Taïwan Museum 40(2): 45-56.

Shinn G.L. 1981. The diet of three species of umagillid neorhabdocoel turbellarians inhabiting the intestine of echinoids. Hydrobiologia 84: 155-162.

Shinn G.L. 1983. Anoplodium hymanae sp. n., an umagillid turbellarian from the coelom of Stichopus californicus, a northeast Pacific holothurian. Canadian Journal of Zoology 61: 750-760.

Smith C.L. 1964. Some Pearlfishes from Guam, with notes on their ecology. Pacific Science 18: 34-

Smith C.L., and J.C. Tyler. 1969. Observations on the commensal relationship of the western Atlantic pearlfish, Carapus bermudensis, and holothurians. Copeia 1969: 206-208.

Smith C.L., J.C. Tyler, and M.N. Feinberg. 1981. Population ecology and biology of the pearlfish (Carapus bermudensis) in the lagoon at Bimini, Bahamas. Bulletin of Marine Science 3: 876-902.

Snyder R.D. 1980. Commensal turbellarians from Bermuda holothurians. Canadian Journal of Zoology 58: 1741-1744.

Strasburg D.W. 1961. Larval carapid fishes from Hawaii, with remarks on the ecology of adults. Copeia 1961: 478-480.

Tanaka S. 1908. Description of eight new species of fishes from Japan. Annotationes Zoologicae Japoneses 7: 27-46.

Trott L.B. 1970. Contribution of the biology of Carapid fishes (Paracanthopterygian: Gadiformes). University California Publications in Zoology 89: 1-41.

Trott L.B. 1981. A general review of the pearlfishes (Pisces, Carapidae). Bulletin of Marine Science 31(3): 623-629.

Trott L.B., and J.S. Garth. 1970. Lissocarcinus orbicularis Dana (Portunidae, Caphyrinidae), commensal with Holothuria argus Jaeger - A new host record; cohabitation with the pearlfish, carapus homei (Richardson). Crustaceana 19: 30-321.

Trott L.B., and Trott E.B. 1972. Pearlfishes (Carapidae: Gadiforme) collected from Puerto Galera, Minobra, Philippines. Copeia 1972: 839-843.

Tyler J.C., C.R. Robins, C.L. Smith, and R.G. Gilmore. 1992. Deepwater populations of the western atlantic pearlfish Carapus bermudensis (Ophidiiformes: Carapidae). Bulletin of Marine Science 51(2): 218-223.

VandenSpiegel D., and M. Jangoux. 1989. La symbiose entre poissons Carapidae et Holothuries autour de l'île de Laing (Mer de Bismarck, Papouasie Nouvelle Guinée). Indo-Malayan Zoology 6: 223-228.

Valentine J.F., and G.D. Goeke. 1983. First record of Holothuria lentiginosa enodis Miller and Pawson (Echinodermata) in the northern Gulf of Mexico. Nirteast Gulf Science 6: 155-156.

Van Meter V.B., and B.W. Ache. 1974. Host location by the pearlfish *Carapus bermudensis*. Marine Biology 26: 379-386.

Vitturi R., and E. Catalanno. 1988. Karyotype of *Carapus acus* (Brünnich, 1768) (Pisces, Carapidae). Caryologia 41: 131-135.

Yosii N. 1928. Note on a *Carapus* in a starfish. Annotationes Zoologicae Japoneses 2: 339-340.