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The life cycle of *Bucephalus margaritae* Ozaki & Ishibashi, 1934 (Digenea, Bucephalidae) from the coast of Santa Catarina State, Brazil

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ABSTRACT. The orange disease is considered the main parasitosis in *Perna perna* mussel. It is caused by a complex life cycle bucephalid, involving three hosts, among them mollusks and fishes. With the aim of contributing to the knowledge of orange disease in mussel culture, the parasite life cycle was investigated. Experimental studies and monthly samples in the study area allowed the identification and characterization of the *Bucephalus margaritae* life cycle. Larvae and adults of *B. margaritae* were fixed in 5% formaldehyde, stained with Gomori's trichrome, clarified in creosote and mounted in Canada balsam. The cercariae are found in the first intermediate host *P. perna* inside the sporocysts, which have the form of orange and ramified filaments. The metacercariae encysts in the gills and gill cavity of the second intermediate host, the blenniid *Hypleurochilus fissicornis*. The definitive host *Menticirrhus americanus* is infected ingesting blenniids parasitized with metacercariae. The high parasitological indexes of *B. margaritae* suggests that *M. americanus* and *H. fissicornis* act as the main definitive and intermediate hosts, respectively, in the trematode life cycle. The blenniid *H. fissicornis* is a new intermediate host to the species.

Key words: *Bucephalus margaritae*, *Hypleurochilus fissicornis*, life cycle, orange disease, *Perna perna*.

RESUMO. O ciclo de vida de *Bucephalus margaritae* Ozaki e Ishibashi, 1934 (Digenea, Bucephalidae) da costa do Estado de Santa Catarina, Brasil. A bucefalose é considerada a principal parasitose do mexilhão *Perna perna*. É causada por um Bucephalidae, de ciclo de vida complexo, envolvendo três hospedeiros, entre eles, moluscos e peixes. Com o objetivo de se contribuir para o conhecimento da bucefalose em mexilhões de cultivo, foi investigado o ciclo de vida deste parasito. Estudos experimentais e coletas mensais na área de estudo permitiram caracterizar o ciclo de vida de *Bucephalus margaritae*. Larvas e adultos do parasito foram fixados em formol 5%, corados com tricrômico de Gômori, clarificados em creosoto e montados em lâminas permanentes com bálsamo do Canadá. As cercárias ocorrem no primeiro hospedeiro intermediário *P. perna,* no interior dos esporocistos que têm a forma de filamentos ramificados e alaranjados. As metacercárias encistam nas brânquias e cavidade branquial do segundo hospedeiro intermediário, o blenídeo *Hypleurochilus fissicornis*. O hospedeiro definitivo, *Menticirrhus americanus,* é infectado quando ingere blenídeos com metacercárias. Os elevados índices parasitológicos de *B. margaritae* sugerem que *M. americanus e H. fissicornis* sejam os principais hospedeiros definitivo e intermediário, respectivamente, deste tremátodeo. O blenídeo *H. fissicornis* é um novo hospedeiro intermediário para a espécie.

Palavras-chave: *Bucephalus margaritae*, *Hypleurochilus fissicornis*, ciclo de vida, enfermidade laranja, *Perna perna*.

Introduction

Bucephalus sp. larvae have been reported in *Perna perna* (Linnaeus, 1758) mussel from the coast of Santa Catarina and can cause severe impact on its mitiliculture and commercial production due to its elevated degree of pathogenicity (SILVA et al., 2002). Bucephalosis, or orange disease, destroys the reproductive tissues of the host, disables its gametogenesis, leads it to castration (CALVO-UGARTEBURU; McQUAID, 1998; COUSTAU et al., 1990) and, possibly to the bivalve's death (LAUCKNER, 1983; SILVA et al., 2002). Some *Bucephalus* species have had their life cycle studied (MATTHEWS,

1973; TASKINEN et al., 1991; ABDALLAH; MAAMOURI, 2002; 2005). In Brazil, two *Bucephalus* species are reported: *Bucephalus solitarius* Kohn, 1968 in *Caranx crysos* (Mitchill, 1815), and *Bucephalus margaritae* Ozaki et Ishibashi, 1934, registered in ten fish species (KOHN et al., 2007).

With the aim of contributing to bucephalosis knowledge, *B. margaritae* life cycle was established, with recognition of the species utilized as hosts at Sambaqui region, Florianópolis/Santa Catarina State and also its developmental stages described.

Material and methods

Locality. Ponta do Sambaqui is situated at the North Bay of Santa Catarina island, Florianópolis (27°29'S, 48°33'W) and shelters the cultures of *P. perna* from Marine Mollusks Laboratory (LMM) of Federal University of Santa Catarina (UFSC). The culture system is longline.

Observations in naturally infected hosts. Samples of 30 *P. perna* mussels were monthly collected, between november 2006 to november 2007 from the UFSC experimental cultures. The hosts were dissected and examined for bucephalid's larval stages. *Hypleurochilus fissicornis* (Quoy & Gaimard, 1824) (n = 51) were collected along the rope cultured mussels between december 2006 to october 2007 and examined under a stereosmicroscope for the presence of metacercariae. When present, the larvae were desencysted with the assistance of histological needles or by applying a slight pressure on the cyst. Between november 2006 to july 2007, trematode potential definitive hosts were collected for further investigation of its adult form. This was verified for fishes *Trichiurus lepturus* Linnaeus, 1758 (n = 5), *Dicentrarchus labrax* (Linnaeus, 1758) (n = 7), *Sciades herzbergii* (Bloch, 1794) (n = 12), *Archosargus rhomboidalis* (Linnaeus, 1758) (n = 9), *Mycteroperca bonaci* (Poey, 1960) (n = 1), *Spheroides testudineus* (Linnaeus, 1758) (n = 5), *Micropogonias furnieri* (Desmarest, 1823) (n = 11), *Menticirrhus americanus* (Linnaeus, 1758) (n = 32), *Balistes capriscus* (Gmelin, 1788) (n = 8) and *Epinephelus gigas* (Brunnich, 1768) (n = 1). For all parasitized fish species it was calculated the parasitological indexes of prevalence and mean intensity of infection (MII) according to Bush et al. (1997).

Morphology. The morphology of adults and metacercariae were studied in 5% formaldehyde fixed specimens. Fixed parasites were washed in distilled water, stained in Gomori's trichrome, clarified in creosote and mounted in Canada balsam. Cercariae observations were made in live specimens. Sporocysts were disrupted with the assistance of histological needles for larvae release. Morphological helminth characters measured with the aid of a camera lucida are in micrometers. Measurements are presented as follows: mean ± standard deviation and minimum and maximum values in parenthesis, when the case numbers are higher than three.

Observations of cercariae longevity. A hundred cercariae were obtained through ruptured sporocysts from *P. perna* mussels and maintained in Petri dishes at room temperature (20-23°C) and its survival monitored up to 72 hours.

Observations in experimentally infected second intermediate host. Specimens of *H. fissicornis* were separated in two aquaria, one exposed to *B. margaritae* cercariae released from infected mussels (n = 15) and the other acting as a control group (n = 15). Two replicates of seven animals for each aquarium were done. Temperature and salinity were controlled and maintained constant at 20°C and 34‰, respectively. The period of exposition was 24 hours.

Observations in experimentally infected definitive hosts. Ten *Trachinotus goodei* (Jordan & Evermann, 1896) were collected at Barra da Lagoa (27°34'S, 48°33'W), east side of Santa Catarina island. The animals were fed with blenniid´s infected gills and 16 days after, necropsied for the presence of adult bucephalids inside the digestory tract of fish.

Results

Sporocyst (Figure 1A)

From 360 collected mussels 21.6% were parasitized by helminth sporocysts.

The sporocysts presented ramifications with dilatations occuring in the mantle, gonads and digestive gland of *P. perna* mussel. Sporocysts held numerous cercariae in different developmental stages.

Cercariae (Figures 1B and 2A)

After released from the sporocysts, the larvae were observed at the bottom of a Petri dish and realized body contractions, including rotatory moviments around its own axis and the furcae.

Survival time of most cercariae was about 48 hours. In the absence of a second intermediate host, the process of larvae encystment did not occur.

Description based on 20 specimens: Body small and thin 288.3 ± 45.0 (220.0 – 360.0) long and 54.4 ± 19.7 (20.1 – 90.0) wide, dorso-ventrally flattened with thorny surface. The rinchus 74.3 ± 11.5 (50.0 – 100.2) of long and 31.1 ± 7.4 (20.1 – 40.2) wide, has a bulb shape and contains numerous glandular cells. The mouth, shaped as a small gap, is situated next to the larva's body equator and is strongly muscular. The pharynx is short and the intestine has an irregular sac shape 94.8 ± 31.7 (60.6 – 120.0). Primordiums of the reproductive system are not evidenced or visible. The furcae is characterized by two long projections which, when extended, surpass up to 9.5 times larva body, with transversal folds all over its surface and ending into a small sucker that surrounds the excretory pores.
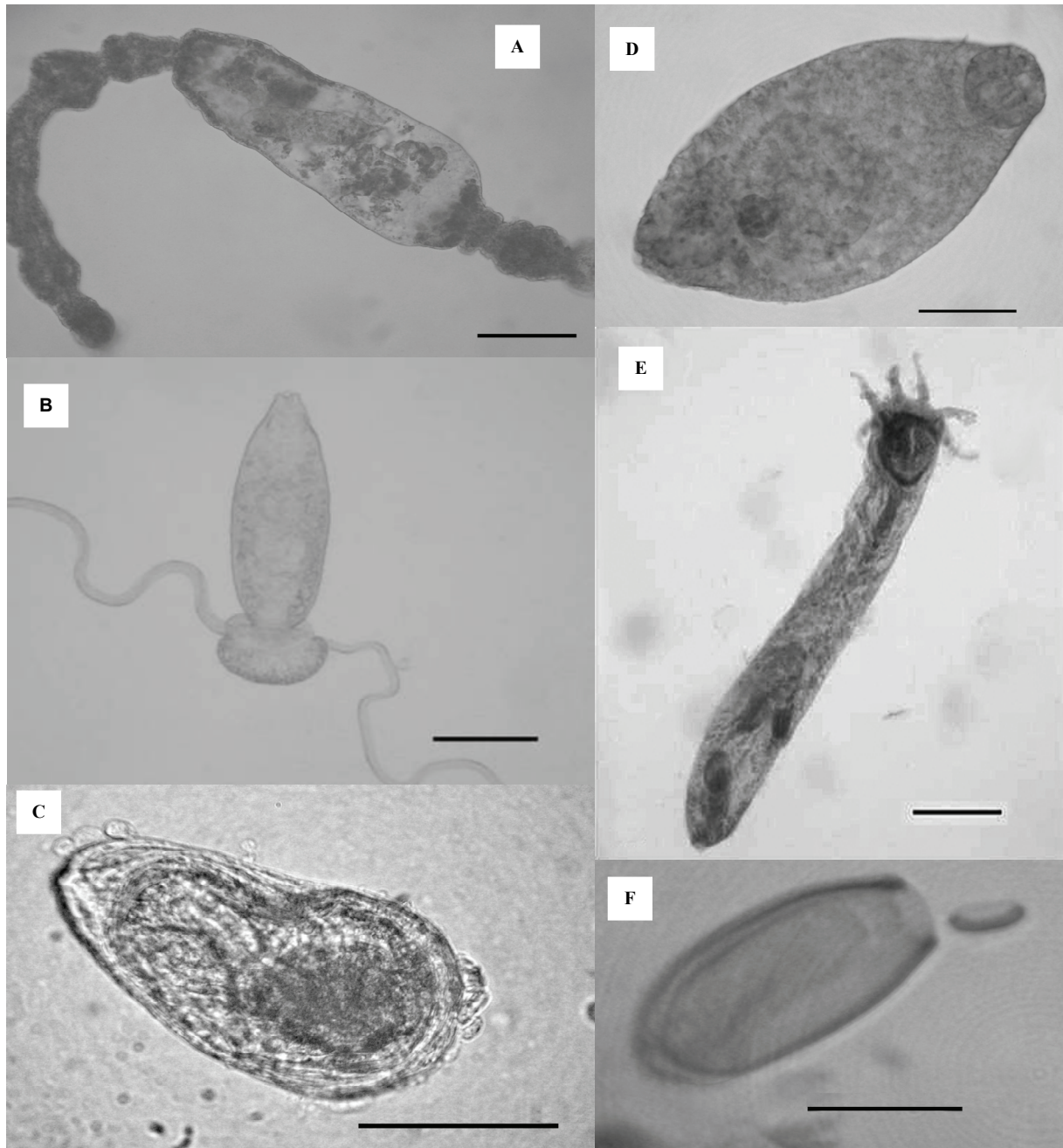

Figure 1. *Bucephalus margaritae* from the coast of Santa Catarina State. **A-** Sporocyst. Bar: 100 μm. **B-** Cercariae. Bar: 100 μm. **C-** Metacercariae cyst. Bar: 20 μm. **D-** Three-year-old metacercariae. Bar: 30 μm. **E-** Adult. Bar: 100 μm. **F-** Egg. Bar: 10 μm.
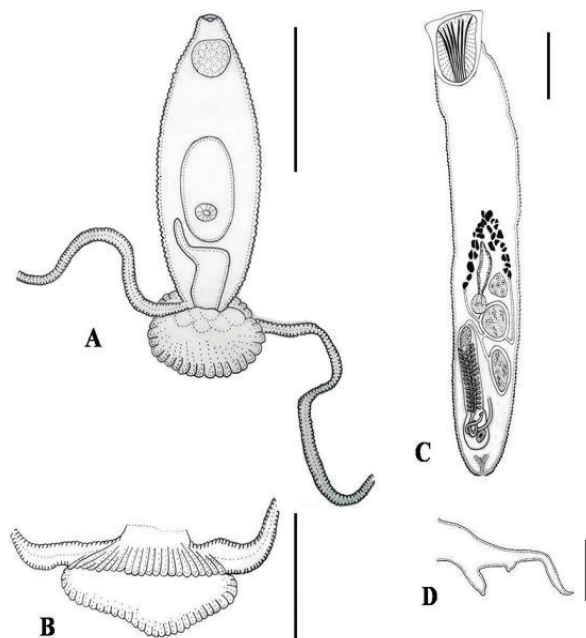
Figure 2. *Bucephalus margaritae* from the coast of Santa Catarina State. **A-** Cercariae. Bar: 100 μm. **B-** Posterior region in detail. Bar: 10 μm. **C-** Adult, ventral side, with the testicles introverted. Bar: 100 μm. **D-** Adult tentacle. Bar: 10 μm.

Metacercariae (Figures 1C and D)

Encysted metacercariae of *B. margaritae* were found in the blenniid´s gill cavity, including the filaments and mainly at the base of gill arch. The prevalence of infected animals by metacercariae was 75% the mean intensity of infection ranged from 2 to 352 larvae per host. The blenniids are easily found among the rope cultured mussels or even inside the shell of dead bivalves commonly used for the fish spawning.

Cysts are ovoid with 43.7 $\pm$ 5.7 (30.0 – 60.2) long and 28.7 $\pm$ 3.3 (22.5 – 40.5) μm wide, composed by two membranes: the inner and thinner one is secreted by the parasite´s cystogenic cells and the other, capsular and thicker, is possibly formed by its host. Metacercariae 181.6 $\pm$ 14.2 (160.8 – 200.4) long and 71.8 $\pm$ 16.9 (45.8 – 90.8) μm wide (n = 15). Body covered by thin spines. Rinchus composed by seven tentacles with two projections each: the bigger is basal, and the smaller is distal (Figure 3A). The mouth is localized a little down from the ventral surface of larva's equator. Pharynx short. The excretory vesicle is situated in the posterior third of the body. Gonads poorly developed.

Adult (Figures 1E and 2C)

Bucephalus margaritae adult forms were found in the intestine and pyloric caecum of *M. americanus* (n = 32). The prevalence of infected animals was 93% and mean intensity of 29.54.
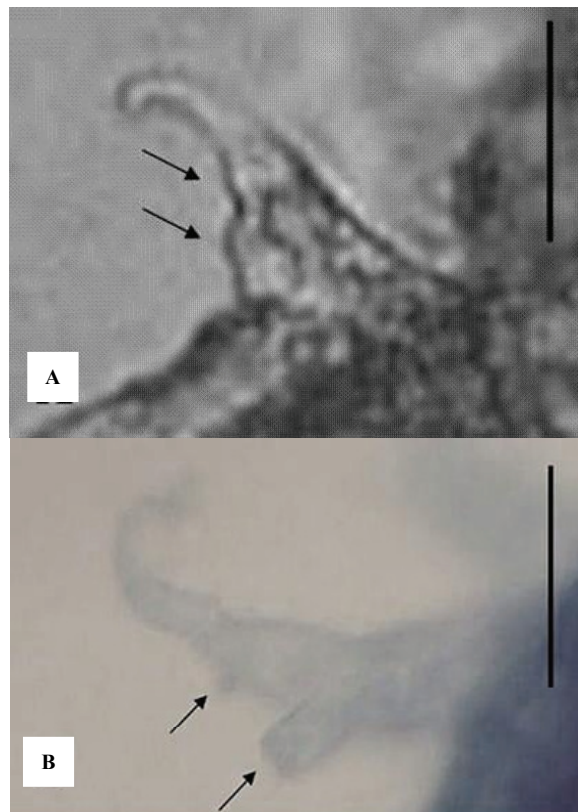

Figure 3. *Bucephalus margaritae*. Projections of the tentacle. **A-** Metacercariae. **B-** Adult. Bar: 10 μm.

Description of the studied specimens: (Based on 10 specimens): Elongated body with 513.7 $\pm$ 131.7 (323.0 – 814.7) long and 81.1 $\pm$ 24.1 (52.2 – 164.1) wide. Spined tegument, with small spines all over the body. Funnel-like apical rinchus with concave bottom and seven marginal tentacles, retractiles, telescopics, with reduced witdh in its extremity and with two projections, one proximal and the other distal (Figures 2D and 3B). The proximal, with 12.5 long, presents an estimated angle of 90º with the tentacle axis, and has obtuse extremity. The distal one, discrete, with acute extremity and 2.5 long, is five times smaller than the proximal. Oral sucker post equatorial, circular, muscular, 339.5 $\pm$ 27.2 (220.3 – 546.3) from the anterior extremity; it includes the mouth as an horizontal gap on its center. Small pharynx, with 10.7 (6.4 – 16.0) width. Numerous rounded vitellaria distributed in the median equatorial body region and laterally in two convergents camps, with 14 to 19 each side. The intestine is simple, sac shaped, anteriorly projected among vitellaria space. The ovary, globular, pre-testicular and pre-pharinx is smaller than the testicles, with 30.3 $\pm$ 11. 3 (22.3 – 44.3) long and 52.9 (30.0 – 114.2) wide. Mehlis gland and Laurer´s canal not evidenced. Uterine loops exceed the

anterior limit of vitellaria and posteriorly reaches the body´s posterior extremity. Numerous eggs, ovoids and operculated with 21.5 ± 4.6 (14.0 – 28.2) long and 9.0 ± 4.3 (4.4 – 19.2) wide (Figure 1F).

Two testicles, posterior to oral sucker, one after the other, envolved by a thick capsule (not included in the measurements), globular with 41.0 ± 9.7 (34.0 – 64.2) long and 26.7 ± 6.6 (20.2 – 44.1) wide (anterior) and 36.4 (22.3 – 82.2) long and 38.5 (28.0 – 76.0) wide (posterior). From each testicle appears a vas efferens which come together anteriorly and forms a single vas deferens that opens into the cirrus sac. Cirrus sac elongated, localized in the posterior third of the body beginning at the level of first testicle with 100.5 ± 17.9 (78.2 – 200.3) long. It shelters the circular seminal vesicle, the pars prostatica with lots of small prostatic cells and the genital atrium,which includes the finger-like and sinuous cirrus and the genital pore, surrounded by a discrete musculature. Uterous final portion is sinuous and partially involves the cirrus inside´s genital atrium. Terminal excretory pore.

Taxonomic summary of Bucephalus margaritae Ozaki et Ishibashi, 1934

Host: *Menticirrhus americanus* (Sciaenidae).

Locality: Ponta do Sambaqui, Florianópolis, Santa catarina State (27°29'S, 48° 33'W).

Sites of infection in the definitive host: intestine and pyloric caecum.

Synonyms: *Prosorhynchus margaritae* (Ozaki et Ishibashi, 1934) Ozaki, 1960; *Bucephalus polymorphus* von Baer, 1827; *Bucephalus varicus* Manter, 1940; *Bucephalus retractilis* Yamaguti, 1952; *Bucephalus pseudovaricus* Velásquez, 1959; *Bucephalus carangoides* Yamaguti, 1970; *Bucephalus ulua* Yamaguti, 1970.

Species distribution: *Bucephalus margaritae* has wide distribution and is found in tropical and subtropical waters being registered in the caribbean sea, pacific coast of Panama, Mexico, Brazil, Venezuela, Guinea-Bissau, China, mediterranean coast of Israel, Kuwait, Red Sea, Arabian Sea, Hawaii, India and Japan (AMATO, 1982; CHINCHILLA et al., 2006; NAHHAS et al., 2006).

Regardings: Reported as *B. varicus* in Kohn (1968), Madhavi (1974), Amato (1982), Takemoto et al. (1995; 1996), Pereira Junior et al. (1996), Chaves and Luque (1998), Luque et al. (2000), Luque et Alves (2001) and Alves et al. (2004); *Bucephalus polymorphus* in Caballero et al. (1953) and Baturo (1977); *Bucephalus pseudovaricus* in Velasquez (1959) and Yamaguti (1971); *Bucephalus retractilis* in Yamaguti (1952; 1958; 1971); *Bucephalus carangoides* in Yamaguti (1971); *Bucephalus ulua* in Yamaguti (1971) and *B. margaritae* in Bray (1984), Yamaguti (1958; 1971), Nahhas et al. (2006), Chinchilla et al. (2006) and Kohn et al. (2007).

Table 1. Known hosts (organized by Families) for *Bucephalus margaritae*.

Hosts	References
Carangidae	
Alectis spp.	Bray (1984)
Atropus atropos (Bloch & Schneider, 1801)	Nahhas et al. (2006)
Caranx crysus (Mitchill, 1815)	Luque et al. (2000), Luque et Alves (2001) and Kohn et al. (2007)
Caranx hippos (L.)	Luque et al. (2000), Luque et Alves (2001) and Kohn et al. (2007)
Caranx latus Agassiz, 1831	Luque et al. (2000), Luque et Alves (2001) and Kohn et al. (2007)
Caranx sem Cuvier, 1833	Bray (1984)
Caranx ignobilis (Forsskal, 1755)	Bray (1984)
Caranx sexfasciatus Quoy & Gaimard, 1825	Parukhin (1970) apud Bray (1984)
Carangoides chrysophrys (Cuvier, 1833)	Madhavi (1974)
Carangoides malabaricus (Bloch & Schneider, 1801)	Madhavi (1974) and Nahhas et al. (2006)
Carangoides hedlandensis (Whitley, 1934)	Bray (1984)
Chloroscombrus crysurus (Linnaeus, 1766)	Amato (1982)
Chorinemus spp.	Bray (1984)
Oligoplistes palometa (Cuvier, 1832)	Takemoto et al. (1995; 1996) and Kohn et al. (2007)
Oligoplistes saurus (Bloch & Schneider, 1801)	Takemoto et al. (1995; 1996) and Kohn et al. (2007)
Oligoplistes saliens (Bloch, 1793)	Takemoto et al. (1995; 1996) and Kohn et al. (2007)
Scomberoides commersonianus Lacépède, 1801	Nahhas et al. (2006)
Trachinotus spp.	Bray (1984)
Zonichthys spp.	Bray (1984)
Gerreidae	
Gerres filamentosus Cuvier, 1829	Nahhas et al. (2006)
Sphyraenidae	
Sphyraena jello Cuvier, 1829	Nahhas et al. (2006)
Sphyraena picudilla Poey, 1860	Chinchilla et al. (2006)
Gadidae	
Urophycis brasiliensis	Pereira Junior et al. (1996), Alves et al. (2004) and Kohn et al. (2007)
Sciaenidae	
Menticirrhus americanus (Linnaeus, 1758)	Kohn (1968), Amato (1982), Chaves and Luque (1998) and Kohn et al. (2007)
Menticirrhus littoralis (Holbrook, 1847)	Amato (1982) and Kohn et al. (2007)
Micropogonias furnieri (Desmarest, 1823)	Pereira Junior et al. (1996) and Kohn et al. (2007).

Observations in experimental infections

Experimentally infected *Hypleurochilus fissicornis* (n = 30) were necropsied for parasite's larval stages. The metacercariae were found encysted in the host's gill cavity. The site with higher cyst concentration was at the base of gill arches and also the filaments. Mean intensity of infection by encysted metacercariae in experimentally infected blenniids was high, with means of 136 cysts per fish ± 117.24 (25 – 332), differently from the control group which was observed means of 8.28 ± 13.43 (1-41).

Experimentally infected *Trachinotus goodei* were necropsied for the parasite´s adult form. It was not registered adult specimens of *B. margaritae* in none of the examined fishes. All hosts were examined in the same day and this way it was not observed if time could influence in those infection indexes.

Discussion

Bucephalosis is considered the main parasitosis in *P. perna* mussel culture (BOWER et al., 1994; COCHÔA; MAGALHÃES, 2008). Many of the damages caused by this parasite are known (SILVA et al., 2002).

The elevated prevalence indexes registered in *H. fissicornis* and *M. americanus* in this study suggests that populations from these species acts as the main definitive and intermediate hosts, respectively, in the life cycle of *B. margaritae* from the coast of Santa Catarina State.

The blenniid *H. fissicornis* utilizes bivalve's valves as a place for spawning besides feeding actively of *P. perna* tissues, as observed in this study. This is an important data for the local mitiliculture since its niche is already integrated to this activity.

In Brazil, *M. americanus* is a common definitive host for adults of *B. margaritae* (KOHN, 1968; AMATO, 1982; CHAVES; LUQUE, 1998; KOHN et al., 2007). In the coast of Santa Catarina State, Amato (1982) registered *B. margaritae* (= *B. varicus*) in *M. americanus* with both measures and description very close from our results. This work register, for the first time, the blenniid *H. fissicornis* hosting cysts of *B. margaritae* metacercariae. It is hence considered a new host record.

According to Nahhas et al. (2006), the diagnostic character of greater relevance for *B. margaritae* is the presence of seven tentacles, each one with two projections: one big and basal and the other small and distal. According to Spakulová et al. (2002), 12 *Bucephalus* species has a rinchus surrounded by seven tentacles with two projections. *Bucephalus priacanthi* Manter, 1940 and *Bucephalus scorpaenae* Manter, 1940 are described from marine fishes of

Florida and *B. varicus* in marine fishes from Brazil. *Bucephalus elegans* Woodhead, 1930 is registered from freshwater fishes of United States; *Bucephalus fragilis* Velásquez, 1959 and *Bucephalus uranoscopi* Yamaguti, 1934 are described from marine fishes of the Philippines and Japan and *Bucephalus anguillae* Spakulová, Macko, Berrili et Dezfuli, 2002 in *Anguilla anguilla* (Linnaeus, 1758) of Adriatic Sea. *Bucephalus minimus* (Stossich, 1887), *Bucephalus blanchardi* (Stossich, 1898) and *Bucephalus labracis* Paggi et Orecchia, 1965 were described from *D. labrax* of Mediterranean Sea. *Bucephalus minimus* was transferred by Yamaguti (1971) to *Bucephalopsis* Yamaguti, 1971. Bartoli et al. (2005) reported *Bucephalus gorgon* (Linton, 1905) from carangids of West mediterranean also containing seven tentacles in its rinchus. However, besides their register of approximately 11 to 14 papillae at the basis of the seven tentacles, this species does not present the small distal projection characteristic of *B. margaritae*. In addition, the only species from the cited above that clearly presents the ovary in a pre-pharynx position is *B. margaritae* (SPAKULOVÁ et al., 2002).

Another diagnostic character that varies in *B. margaritae* is the egg size. Nahhas et al. (2006) registered eggs of this species with 13-20 μm long and 10-18 μm wide. In Brazil, Amato (1982) described eggs of *B. varicus* with 18-20 by 10-12 μm; Kohn (1968), while studying *B. margaritae* collected from the esophagus of *M. americanus,* registered eggs with 21-27 by 11-12 μm. Takemoto et al. (1995), studied the trematode fauna of *Oligoplites palometa*, *O. saurus* and *O. saliens,* and registered eggs of *B. varicus* with 16-20 by 9-11 μm. Nahhas et al. (2006) affirmed that the means of *B. margaritae*´s eggs measured found in the literature were 14-27 by 10-13 μm, values very close from what observed in this study.

An historical revision of *B. margaritae* and its relation with *B. polymorphus* was done by Bray (1984). Despite the morphological resemblance between the two species, leading some authors to indicate them as synonyms (NAHHAS et al., 2006; BRAY, 1984), some differences with respect to larvae ecology are evident. Parasite from freshwater fishes (SCHUSTER et al., 2001; DILER; YILDIRIM, 2003), encysted *B. polymorphus* metacercariae are registered by Baturo (1977) in the musculature and fins of its hosts, differently from the reports of *B. margaritae* metacercariae, so far always sited at the gills of marine hosts. Future studies might show if the species are, in fact, synonyms.

Abdallah and Maamouri (2005) concluded that, in only ten days, *B. labracis* completed its development reaching sexual maturity with consequent eggs formation in its final host. The

experimentally infected definitive hosts in this study were necropsied in the 16º day post-infection. However, as in Taskinen et al. (1991), it was not registered the helminth adult stage in this period. It was not possible to explain this result.

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