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NOTE

Monitoring of infections by Protozoa of the genera *Nematopsis*, *Perkinsus* and *Porospora* in the smooth venus clam *Callista chione* from the North-Western Adriatic Sea (Italy)

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ABSTRACT: Marketable smooth venus clams *Callista chione* from natural banks of Chioggia (Venice) and Goro (Ferrara), North-Western Adriatic Sea (Italy), were examined for protozoan parasites from November 1996 to November 1998. Out of the 375 bivalves examined, 149 (39.7%) were infected by *Nematopsis* sp. and 325 (86.7%) by *Porospora* sp. Oocysts of *Nematopsis* sp. were present with a prevalence that varied from 100% in November 1996 to 5% in June 1998; cystic and naked sporozoites of *Porospora* sp. were very common, with a prevalence of 100%. Out of the 229 bivalves examined between January and November 1998, 63 (27.5%) were also infected by *Perkinsus* sp.; the prevalence of *Perkinsus* sp. varied from 9.1% in January to 50% in February. To our knowledge this is the first report of *Porospora* sp. and *Perkinsus* sp. in *C. chione*.

KEY WORDS: *Callista chione* · *Nematopsis* sp. · *Porospora* sp. · *Perkinsus* sp. · Parasitology · Epidemiology

The smooth venus clam *Callista chione* (Bivalvia: Veneridae) (syn.: *Pitaria chione*, *Pitar chione*, *Meretrix chione*, *Cyterea chione*, *Macrocallista chione*; Fasolaro [Italian], Verni [French], braune Venus Muschel [German], Almejon brillante [Spanish]) lives on sandy or muddy bottoms of the Mediterranean Sea. It is actively fished along the Emilia-Romagna, Venetian and Friuli coasts of the Northern Adriatic Sea (Italy). Despite its economic importance, there is a deep lack of information about its health status. To provide a baseline health status of the marketable *C. chione* from the Northern Adriatic Sea, we performed a survey of its protozoan parasites.

Materials and methods. From November 1996 to November 1998, 17 *Callista chione* samples of marketable size (50 to 65 mm) were collected for a total of 375 specimens (aged 4 to 6 yr after Marano et al. 1998): 357 specimens from Chioggia (Venice) and 18 specimens from Goro (Ferrara) (Table 1). Sections (1 cm^2) of gill, mantle and foot tissues were squashed between glass slides and examined for the presence of *Nematopsis* (Apicomplexa: Porosporidae) oocysts and *Porospora* (Apicomplexa: Porosporidae) sporozoites (Bower et al. 1994).

On the grounds that *Perkinsus* (Apicomplexa: Perkinsidae) infections were suspected, between January and November 1998 gill fragments of 229 bivalves were also placed in fluid thioglycollate medium (FTM) and examined microscopically after 10 d for the presence of hypnospores (Ray 1952).

The number of parasites was evaluated per microscope field at 400×.

Results. In the gills and mantle, *Nematopsis* sp.-infected bivalves were identified by the presence of oval oocysts (Fig. 1) measuring 8.1–16.6 × 5.3–11.1 µm and having a thick transparent wall, about 0.5 µm in width. One of the 2 poles of the oocyst appeared slightly pointed because of the presence of an operculum covering the micropyle. A single short and stocky vermiform sporozoite was often easily distinguishable inside the oocyst. The mean prevalence of *Nematopsis* sp. infection was 39.7%, ranging from 100% observed in November 1996 to 5% in June 1998 (Table 1). In infected specimens, the oocysts (n = 1 to 31 per microscope field) appeared irregularly distributed in gill and mantle connective tissue; in the same subjects we observed isolated oocysts or clusters of 10 to 25 parasites.

In gill, mantle and foot connective tissues, *Porospora* sp.-infected bivalves were identified by the presence of either sporozoites enclosed within a thin ellipsoidal cystic membrane, measuring 40.6–131.5 × 14.3–88.6 µm

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Table 1. *Callista chione.* Month, year, locality and number of molluscs examined per sample; prevalence of *Nematopsis* sp., *Porospora* sp. and *Perkinsus* sp.

Month	Year	Locality	No. of molluscs examined	*Nematopsis* sp. infected		*Porospora* sp. infected		*Perkinsus* sp. infected	
				No.	%	No.	%	No.	%
Nov	1996	Chioggia	16	16	100.0	16	100.0	–	–
Dec	1996	Chioggia	15	13	86.7	14	93.3	–	–
Feb	1997	Chioggia	20	4	20.0	19	95.0	–	–
Apr	1997	Chioggia	15	6	40.0	9	60.0	–	–
May	1997	Chioggia	36	14	38.9	36	100.0	–	–
Oct	1997	Chioggia	19	18	94.7	19	100.0	–	–
Nov	1997	Chioggia	25	19	76.0	25	100.0	–	–
Jan	1998	Chioggia	22	8	36.4	22	100.0	2	9.1
Feb	1998	Goro	18	4	22.2	18	100.0	9	50.0
Mar	1998	Chioggia	15	9	60.0	2	13.3	3	20.0
Apr	1998	Chioggia	22	5	22.7	16	72.7	6	27.3
May	1998	Chioggia	40	7	17.5	40	100.0	11	27.5
Jun	1998	Chioggia	20	1	5.0	20	100.0	9	45.0
Jul	1998	Chioggia	18	5	27.8	18	100.0	3	16.7
Aug	1998	Chioggia	30	4	13.3	30	100.0	5	16.7
Oct	1998	Chioggia	22	12	54.5	15	68.2	9	41.0
Nov	1998	Chioggia	22	4	19.2	6	27.3	6	27.3
Total			375	149	39.7	325	86.7	63	27.5

(Fig. 2), or naked vermiform sporozoites, with an evident dome-shaped end, which moved in the host tissue by means of successive contractions and measured about 31 × 5.6 µm (Fig. 3). The mean prevalence of *Porospora* sp. infection was 86.7%, ranging from 100% observed in November 1996, May, October and November 1997, and January, February, May, June, July and August 1998 to 13.3% in March 1998 (Table 1). In

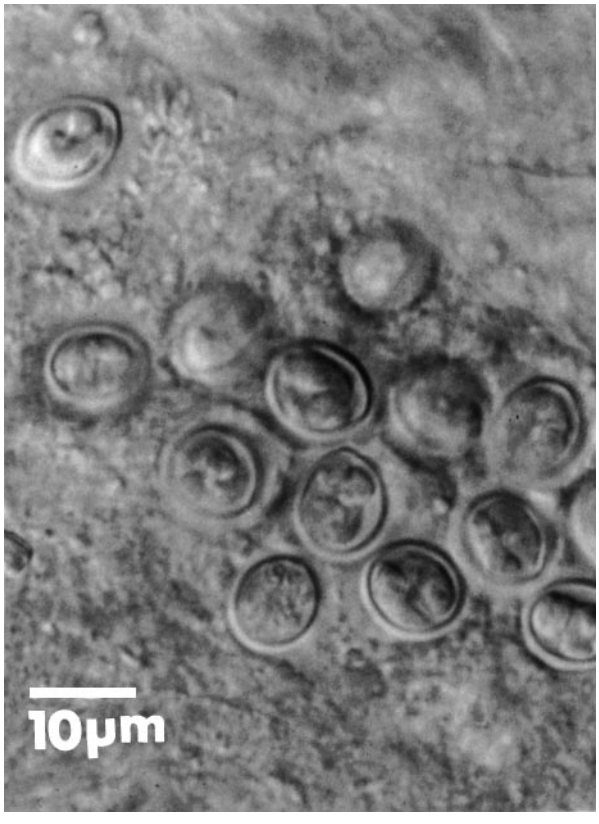

Fig. 1. *Nematopsis* sp. oocysts in *Callista chione*

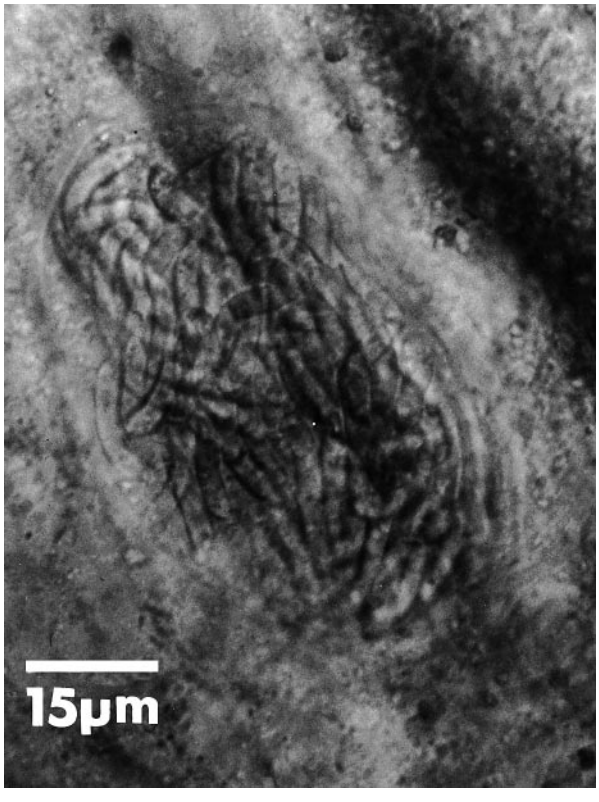

Fig. 2. *Porospora* sp. sporozoites enclosed within a cystic membrane in *Callista chione*

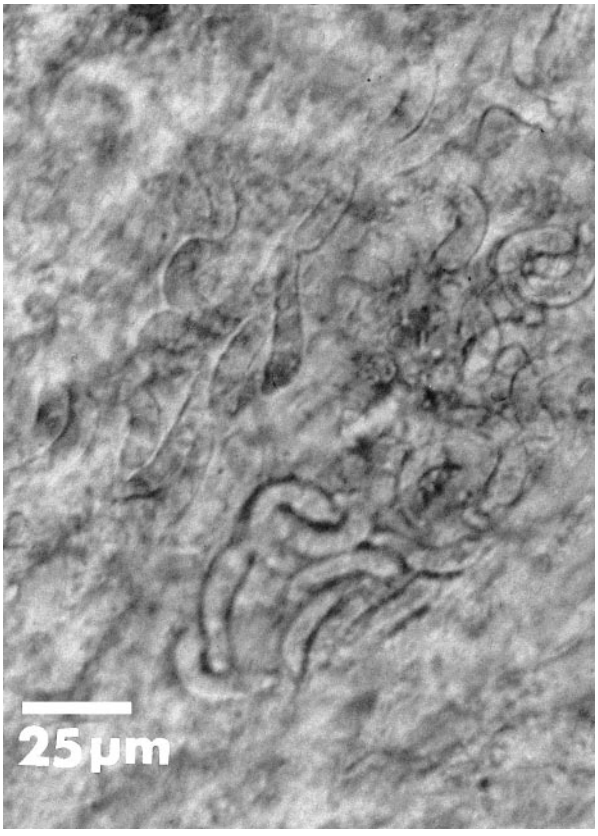

Fig. 3. *Porospora* sp. naked sporozoites in *Callista chione*

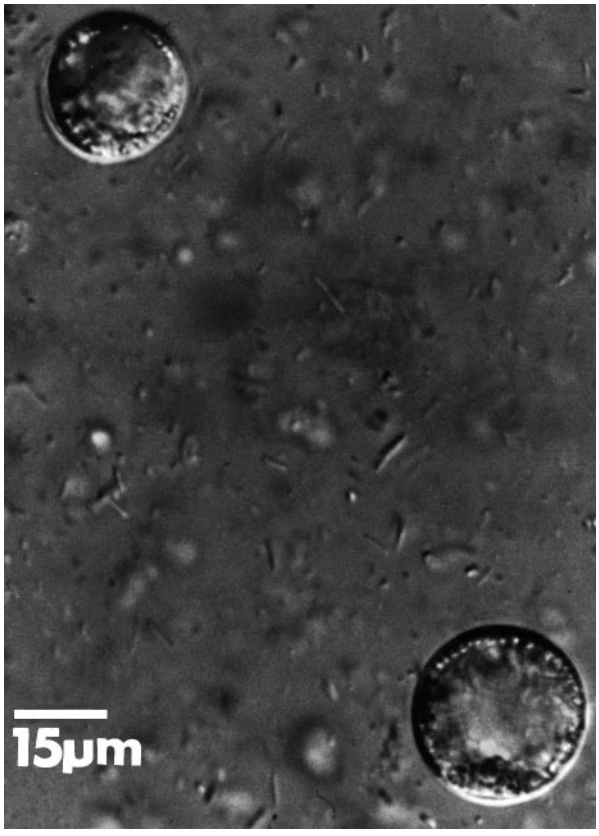

Fig. 4. *Perkinsus* sp. hypnospores in *Callista chione*

infected specimens, *Porospora* cystic sporozoites were frequently seen, while naked vermiform sporozoites (n = 1 to 50 per microscope field) were irregularly distributed in the tissues and rarely observed.

In FTM *Perkinsus* sp.-infected bivalves were identified in the gills by the presence of round bodies (Fig. 4), corresponding to the hypnospore stage (Perkins 1996), measuring about 14.9 to 39.6 µm. The mean prevalence of *Perkinsus* sp. infection was 27.5%, ranging from 9.1% observed in January to 50% in February 1998 (Table 1). The hypnospores (n = 1 to 2 per microscope field) appeared irregularly distributed in the infected specimen tissues.

Out of 375 examined specimens, 128 (34.1%) were simultaneously parasitized by *Nematopsis* sp. and *Porospora* sp. Out of the 229 specimens examined between January and November 1998, 17 (7.4%) were parasitized by both *Nematopsis* sp. and *Perkinsus* sp., 52 (22.7%) by both *Porospora* sp. and *Perkinsus* sp., and 12 (5.2%) by *Nematopsis* sp., *Porospora* sp. and *Perkinsus* sp.

Discussion. *Porospora* sp. was the most common protozoan parasite in *Callista chione* (86.7%), followed by *Nematopsis* sp. (39.7%) and *Perkinsus* sp. (27.5%). The simultaneous presence of 2 or 3 protozoan parasites in the same specimen was also frequent. To our knowledge this is the first report of *Porospora* sp. and *Perkinsus* sp. in *C. chione*. We have not found reports about the presence of *Porospora* sp. infections in edible bivalves from Italian waters.

The genus *Porospora* includes 2 species (Levine 1988): *P. gigantea* and *P. nephropis*. They possess a heteroxenous life cycle that alternates between a decapod crustacean, in which gymnospores develop, and a marine mollusc, the intermediate host. In the latter, a fusiform bundle of sporozoites develops, which is held together by a fragile membrane (Sprague 1970). There are few reports on the pathogenicity of *Porospora* spp. in bivalves. Hatt (1931) reported that the gymnospore causes lesions, disorganization, atrophy and destruction of the epithelial tissue by penetrating the mollusc gill surface.

We observed, very often, a large number of *Porospora* sp. cystic sporozoites in infected specimens and, less frequently, naked vermiform sporozoites. Prevalences were almost always very high (100% or close to 100%) during the years of study (low prevalences were observed only in March and November 1998).

Nematopsis sp. infections were observed for the first time in *Callista chione* from Chioggia (Venice) by

Canestri-Trotti et al. (1998a), where the prevalence was 60.2%. They were also reported in other edible bivalves of Italian waters: *Chamelea gallina*, *Donax semistriatus*, *D. trunculus*, *Ensis siliqua minor*, *Ensis* sp., *Mytilus galloprovincialis*, *Ruditapes philippinarum*, and *Solen marginatus* (Da Ros & Massignan 1985, Costa et al. 1985-1986, Di Cave et al. 1987, Ceccarelli et al. 1988, Bilei et al. 1997, Berilli et al. 1998, Canestri-Trotti et al. 1998a,b, 1999a,b).

The genus *Nematopsis* includes more than 30 species (Levine 1988, Belofastova 1996) that have a heteroxenous life cycle shared between a marine mollusc intermediate host, in the connective tissues of which sporogony takes place, and a decapod crustacean primary host, in the gut of which the sexual and asexual multiplication occurs. There are some discrepancies about the pathological significance of *Nematopsis* infections in bivalves. Recently, Azevedo & Cachola (1992) showed that the infection in *Cerastoderma edule* and *Ruditapes decussatus* causes lysis of the nearby gill cells (suggesting that the parasite does have pathological consequences).

We observed a large number of oocysts in some infected *Callista chione* specimens; however, the branchial tissues of *C. chione* were never as densely occupied by broad oocyst clusters as we frequently saw in *Chamelea gallina* (Canestri-Trotti et al. 1998b). The mean prevalence (39.7%) was lower than that we have reported in *C. gallina* (96.5%); conversely, it was remarkably higher than that reported in *Mytilus galloprovincialis* (7%) (Canestri-Trotti et al. 1999a) and in *Ruditapes philippinarum* (0.6–0.7%) (Canestri-Trotti et al. 1999b). The monthly prevalences were inconstant during the years of study; however, the highest prevalences were reported mainly in autumn to early winter (November and December 1996, October and November 1997 and October 1998) except once in March 1998.

Perkinsus sp. infections have been observed in Italian waters in *Ruditapes decussatus*, *Ostrea edulis*, *Crassostrea gigas*, *Venerupis aurea*, *Venus verrucosa*, *Cerastoderma edule*, *Chamelea gallina* and recently in *Mytilus galloprovincialis* (Breber 1985, Da Ros & Canzonier 1985, Ceschia et al. 1991, Berilli et al. 1998, Canestri-Trotti et al. 1999a). Ceschia et al. (1991) also examined 100 specimens of *Callista chione* sampled from the Northern Adriatic Sea, but none of these proved positive for *Perkinsus* sp.

The genus *Perkinsus* includes *P. marinus*, *P. atlanticus* and *P. olseni* reported in 67 species of molluscs (Perkins 1993), frequently associated with high mortality in bivalves (Lauckner 1983, Bower et al. 1994, Perkins 1996). Transmission of infection is direct from mollusc to mollusc. The parasite apparently penetrates the epithelium of the gill or gut or is carried through it by haemocytes. Multiplication occurs mainly in the connective tissue or between the epithelial cells; a large numbers of parasitic cells may develop and cause extensive tissue damage before death of the mollusc.

While the parasite growth, proliferation and consequent mortality have been correlated with increases in water temperature and salinity (Perkins 1993), our monthly findings were inconstant during the 1998 (highest prevalences in February, June and October). Compared to earlier studies carried out on *Perkinsus* sp. infections in other bivalves living in the Adriatic Sea, the *Perkinsus* sp. prevalence and intensity that we observed in *Callista chione*, in the sampled zones, was relatively low. Ceschia et al. (1991), Ceschia (1995), Orel et al. (1998), Restani et al. (1998) and Canestri-Trotti et al. (1999b) found prevalences of 40–90, 68.2, 80.8, 65–100 and 30.3–50.3% respectively in *Ruditapes philippinarum*. Ceschia & Giorgetti (1998) and Berilli et al. (1998) found prevalences of 92.3 and 30–60–100% respectively in *Chamelea gallina*. Canestri-Trotti et al. (1999a) found a prevalence of 20.5% in *Mytilus galloprovincialis*. There are no data about mass mortality of *C. chione* from the Northern Adriatic Sea; nevertheless, it is known that *Perkinsus*-like organisms are currently the most worrisome of the parasites we observed.

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