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Seasonality and host–parasite interrelationship of *Mytilus galloprovincialis* parasites in Turkish Black Sea coasts

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This is the first comprehensive research study on the parasites of Mytilus galloprovincialis *collected from the Sinop coasts of the Black Sea and their relationships with several environmental and biotic factors. A total of 1740 mussels were collected monthly at three sampling localities representing different ecosystems in the period between August 2012 and July 2013 and examined for parasites. Identified parasites were* Nematopsis legeri, Peniculistoma mytili, Urastoma cyprinae, Parvatrema duboisi *and* Polydora ciliata. *Infection prevalence (%), mean intensity and mean abundance values of each parasite species were calculated according to season, sampling localities and length classes of mussel.* Nematopsis legeri *was the most prevalent species (32.5%), followed by* Pe. mytili *(6.70%),* U. cyprinae *(6.30%),* Pa. duboisi *(4.50%) and* Po. ciliata *(2.20%).* Nematopsis legeri *and* Parvatrema duboisi *had their highest infection prevalence and intensity values in sampling locality III where secondary hosts present to complete their life cycle and larger sized mussels had higher parasite loads. Statistically significant differences were determined in the prevalence of infection and intensity values among seasons, length classes of mussel and sampling localities of each parasite species. The present study provided valuable information on mussel parasites and their relationships with host length, seasons and ecology.*

Keywords: *Mytilus galloprovincialis*, *Nematopsis legeri*, *Peniculistoma mytili*, *Urastoma cyprinae*, *Parvatrema duboisi*, *Polydora ciliata*, host-parasite relationship, Black Sea

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INTRODUCTION

The Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819 has been accepted as an aggressive invasive species which is listed among the 'World's worst 100 invasive alien species' distributed worldwide (GISD, 2012). It has a rapid growth rate under a wide range of environmental conditions and its high level of tolerance to physiologically limiting factors allows it to colonize in marginal areas (Calvo-Ugarteburu & McQuaid, 1998). Its introduction, culture and transfer to different geographic areas increase the risks of spreading their parasites and diseases around the world (Francisco *et al.*, 2010). Diseases in molluscs caused by parasites have been documented worldwide and *Mytilus galloprovincialis* has been reported to be infected by several parasites, including the protozoan *Nematopsis legeri* and *Peniculostoma mytili*, the turbellarian *Urastoma cyprinae*, the polychaeta *Polydora ciliata* and the trematode *Parvatrema duboisi* (Machkevsky, 1989; Murina & Solonchenko, 1991; Bower *et al.*, 1994; Robledo *et al.*, 1994; Belofastova, 1996, 1997; Caceres-Martinez *et al.*, 1998; Comps & Tige, 1999; Holodkovskaya, 2002; Francisco *et al.*, 2010; Machkevsky *et al.*, 2011; Özer & Güneydağ, 2014).

Urastoma cyprinae (Graff, 1882) is a pathogenic agent of serious damage on the gills of *M. galloprovincialis* (Robledo *et al.*, 1994; Villalba *et al.*, 1997). Bataller & Boghen (2000) reported that the visible presence of the worms on the gills, especially when occurring in the hundreds and even thousands, could cause a decrease in demand by the lucrative half-shell market. *Urastoma cyprinae* has a wide distribution area including the Black Sea, having been recorded in several bivalve species including *M. galloprovincialis* from both cultured and natural beds (Fleming *et al.*, 1981; Goggin & Cannon, 1989; Noury-Srairi *et al.*, 1990; Murina & Solonchenko, 1991, Caceres-Martinez *et al.*, 1998; Özer & Güneydağ, 2014).

Polydora ciliata (Johnston, 1838) is an infective spionid polychaeta that excavates a U-shaped burrow lining with a tube composed of protein and sand grains. Kent (1979) reported that heavy infestations of *P. ciliata* caused lower fecundity in mussels due to the reduction in mantle tissues, the main repository of gametes, and reduced flesh content as a consequence of lowered condition index. Kent (1981) also reported that high levels of *P. ciliata* infestations tended to weaken the shells of *Mytilus edulis*, thus, weak-shelled mussels became more vulnerable to the predatory activities of the crab, *Cancer pagurus*. *Polydora ciliata* has been reported from several bivalve species including *M. galloprovincialis* in the Black Sea (Murina & Solonchenko, 1991; Machkevsky *et al.*, 2011; Özer & Güneydağ, 2014).

Gymnophallid trematodes are parasites of coastal birds and they use marine bivalve molluscs as first intermediate hosts and molluscs (bivalve or gastropod) or polychaetes as second intermediate hosts (Galaktionov, 2006). *Parvatrema duboisi* is a pathogenic agent causing compression and displacement in gonads (Machkevsky, 1989), formation of pearls and decrease in the reproductive potential of the mussels (Gaevskaya &

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Machkevsky, 1996). *Parvatrema duboisi* has been reported from *M. galloprovincialis* in the Black Sea (Machkevsky & Trinitko, 1985; Machkevsky, 1989; Gaevskaya & Machkevsky, 1995; Machkevsky *et al.*, 2011; Özer & Güneydağ, 2014).

Oocysts of several species of *Nematopsis* gregarines infect various tissues of many species of bivalve molluscs which are acting as intermediate host. Due to passive entrance of gymnospores into the intermediate host's tissues, no significant harm occurs in the host (Hatt, 1931; Kinne, 1983). *Mytilus galloprovincialis* acts as an intermediate host and *Eriphia verricosa* serves as a definitive host for *Nematopsis legeri*. This parasite has been reported from *M. galloprovincialis* in the Black Sea (Belofastova, 1996, 1997; Özer & Güneydağ, 2014).

The ciliate protozoan *Peniculistoma mytili* (Morgan, 1925) Jankowski, 1964 is strictly host specific to *Mytilus edulis* (Otto, 1983) and has been reported from the gills of *M. galloprovincialis* in the Black Sea coasts (Gaevskaya *et al.*, 1990; Dumitrescu & Zaharia, 1993; Özer & Güneydağ, 2014).

Studies on parasites and diseases affecting molluscs with economic interest are important both for the management of natural stocks and for aquaculture (Boehs *et al.*, 2010). Considering the potential impact of the above-mentioned parasites on the shellfish industry, more studies are needed to achieve a better understanding of host–parasite interactions and their ecology. Parasites of *M. galloprovincialis* in the Sinop coast of the Black Sea have been reported by Özer & Güneydağ (2014) and the objective of this study was to determine the distribution of parasite species in time and space by calculating their infection indices between seasons and length classes of mussels at three localities in Sinop, Turkey. This is the first research study on the simultaneously occurring parasites mentioned above and their infection indices in *M. galloprovincialis*.

MATERIALS AND METHODS

Mussel samples were collected monthly in the period between August 2012 and July 2013 at three sampling localities representing three ecologically different environments at the Sinop coasts of the Black Sea (Figure 1). Of the three sampling localities, Sampling locality I: İçliman (42°00′56″N, 35°10′37″E) is located at the inner harbour of Sinop and mussel samples were collected from a dock leg of the landing stage which has maximum depth of 14 m. This location is disturbed mainly by fishing boats during the process of landing captured fish, especially in the period between autumn and spring. Sampling locality II: Ada Başı (42°01′05″N, 35°12′42″E) is located at the extreme north of Sinop and is a natural ecosystem with rocky floor, which is not affected by any kind of human activity, with maximum depth being 20 m. Sampling locality III: Sarı Ada (42°02′51″N, 35°02′56″E) is located at the outer harbour of Sinop with maximum depth of 3 m and is a shallow area with rocky floor, which is from time to time affected by small-scale fishing boats, human waste discharges and a small stream. At least 30 mussel samples were collected by scuba divers from each sampling locality. Water temperature (°C) values (Table 1) were measured monthly using a digital YSI Professional Plus water quality instrument. Seasons were distinguished according to calendar months and monthly data obtained from both parasite and mussel hosts were pooled, calculated and presented seasonally.

Fig. 1. Map of sampling locations around the Sinop coasts of the Black Sea. *indicates the border of sampling localities II and III, #indicates exact location of sampling locality I.

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Table 1. Monthly mussel samples and temperature (°C) values at each sampling locality at the Sinop coasts of the Black Sea.

Sampling months	Numbers of mussel samples			Temperature (°C)		
	I (İçliman)	II (Ada Başı)	III (Sarı Ada)	I (İçliman)	II (Ada Başı)	III (Sarı Ada)
August	30	30	30	25.9	26.7	25.6
September	60	60	30	21.2	21.7	21.4
October	60	60	30	15.7	14.8	19.0
November	60	60	30	14.5	15.6	15.5
December	60	60	30	13.2	13.8	13.2
January	60	60	30	10.0	9.0	8.0
February	60	60	30	9.0	8.0	8.0
March	60	60	30	11.0	9.3	8.1
April	60	60	30	14.3	14.0	14.6
May	60	60	30	16.0	18.6	17.0
June	60	60	30	21.1	20.2	19.8
July	60	60	30	25.4	22.2	23.1

A total of 1740 mussels constituted from monthly samples (Table 1) were examined for parasites. The specimens in the samples were measured in their longest axis using a vernier caliper to the nearest 0.1 mm, allocated to three length classes of ≤4.5, 4.6–5.9 and ≥6.0 cm, weighed, placed in a Petri dish, and then opened. Each organ was then cut into small pieces, placed on slides separately and fresh smears were prepared from all pieces of each organ. Parasites were determined by screening all smears prepared from each organ of mussels using a light microscope at 400× magnification and full parasite count was conducted one by one to obtain the exact number rather than an average estimate. Their infection prevalence (the percentage of infected mussels), mean intensity (the average number of parasites in the total number of infected mussels) and mean abundance (the average number of parasites in the total number of examined mussels) were calculated in accordance with Bush *et al.* (1997). Parasites were identified to species level using the keys provided by Raabe (1971), Gaevskaya *et al.* (1990), Crespo-Gonzalez *et al.* (2005), Gaevskaya (2006), Chung *et al.* (2010) and Francisco *et al.* (2010).

Quantitative Parasitology 3.0 software (Reiczigel & Rózsa, 2005) was used to calculate Sterne's exact 95% confidence limits for prevalence, bootstrap 95% confidence limits (number of bootstrap replications = 2.000) for mean abundance and mean intensity. The differences in prevalence values between sampling seasons, sampling localities and length categories of mussels were determined by Fisher's exact test. A Kruskal–Wallis test (non-parametric ANOVA) was performed to find out the significant differences in the mean intensity and mean abundance values of each parasite species at different sampling seasons, sampling localities and length classes of mussels at the significance level of 5% using the statistical program GraphPad InStat 3.00.

RESULTS

A total of five parasite species were identified and their infection prevalence, mean intensity and mean abundance values are presented in Table 2.

Overall infection prevalence (%) and abundance values of mussel parasites

The overall infection prevalence, mean intensity and mean abundance values were 45.30% (exact 95% confidence limits 42.9–47.6), 166.04 (bootstrap 95% confidence limits 141.9–199.4) and 75.20 (bootstrap 95% confidence limits 64.9–91.4), respectively. *Nematopsis legeri* was the most prevalent parasite species and it was followed by *Peniculistoma mytili, Urastoma cyprinae, Parvatrema duboisi* and *Polydora ciliata* (Table 2). *Nematopsis legeri* was also the most abundant parasite while *Polydora ciliata* was the least abundant among all parasite species (Table 2).

The distribution of mussel parasites with respect to season

Seasonal prevalence, mean intensity and mean abundance values of all parasite species infecting *M. galloprovincialis* are presented in Table 3. Statistically significant differences in infection indices between seasons in all parasite species are presented in Table 4. *Nematopsis legeri* and *Pe. mytili* had their highest prevalence values in autumn while *U. cyprinae* and *Pa. duboisi* in summer and *Po. ciliata* in winter (Table 3). Comparison of seasonal differences in prevalence by sampling seasons are presented in Table 4; while there is a statistically significant difference in prevalence between seasons in *N. legeri, U. cyprinae, Po. ciliata* and *Pe. mytili* (Fisher's exact test, $P = 0.000$), there is no significant difference between seasons in *Pa. duboisi* (Fisher's exact test, $P = 0.093$) (Table 4). It must be mentioned that *U. cyprinae* was not detected in winter samples. The mean intensities of *N. legeri* in winter, *U. cyprinae, Pa. duboisi* and *Pe. mytili* in autumn, and *Po. ciliata* in winter were at their maximum values (Table 3). Comparison of seasonal differences in mean intensity by sampling seasons are presented in Table 3; while there are statistically significant differences in the mean intensity values between seasons in *N. legeri, U. cyprinae, Pa. duboisi* and *Pe. mytili* (non-parametric Kruskal–Wallis

Table 2. Parasite species identified in *Mytilus galloprovincialis* and their overall infection indices (N = 1740), CI – 95% confidence intervals based on Stern's exact score limit, Mean I – mean intensity and Mean A – mean abundance with bootstrap 95% CI (Mean I, A).

Species	Prevalence (%) CI	Mean I CI	Mean A CI
Nematopsis legeri (de Beauchamp, 1910)	32.5 (30.3–34.8)	229.0 (196.0–288.0)	74.30 (62.3–93.90)
Urastoma cyprinae (Graff, 1882)	6.30 (5.20–7.60)	5.23 (4.33–6.55)	0.33 (0.25–0.43)
Parvatrema duboisi (Dollfus, 1923)	4.50 (3.60–5.60)	4.95 (3.96–6.37)	0.22 (0.17–0.31)
Polydora ciliata (Johnston, 1838)	2.20 (1.60–3.10)	1.28 (1.10–1.64)	0.03 (0.02–0.04)
Peniculistoma mytili (Morgan, 1925) Jankowski, 1964	6.70 (5.60–8.00)	5.29 (4.11–6.91)	0.35 (0.26–0.49)

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Table 3. Parasite species identified in *Mytilus galloprovincialis* and their infection indices according to seasons, CI – 95% confidence intervals based on Stern's exact score limit, Mean I – mean intensity and Mean A – mean abundance with bootstrap 95% CI.

Species	Autumn			Winter		
	P (%) CI	Mean I CI	Mean A CI	*P* (%) CI	Mean I CI	Mean A CI
N. legeri	51.8 (47.1–56.5)	223.0 (186–295)	116.0 (94.8–153)	43.3 (38.8–48.0)	296 (218–433)	128 (94.1–201)
U. cyprinae	5.1 (3.4–7.5)	8.7 (6.4–12.1)	0.4 (0.3–0.7)	0	0	0
Pa. duboisi	3.6 (2.2–5.8)	10.3 (6.8–14.8)	0.4 (0.2–0.7)	5.8 (3.9–8.3)	2.9 (2.3–3.6)	0.16 (0.10–0.25)
Po. ciliata	0.9 (0.3–2.3)	1.0	0.009 (0.002–0.02)	3.8 (2.3–6.0)	1.6 (1.2–2.3)	0.06 (0.03–0.10)
Pe. mytili	12.9 (10.1–16.3)	8.6 (6.5–11.5)	1.1 (0.7–1.6)	5.3 (3.5–7.9)	2.4 (1.9–3.2)	0.12 (0.08–0.20)
Species	**Spring**			**Summer**		
	P (%) CI	Mean I CI	Mean A CI	*P* (%) CI	Mean I CI	Mean A CI
N. legeri	19.6 (16.1–23.5)	189.0 (160–229)	37.0 (29–48)	12.8 (9.8–16.5)	59.9 (44–96.3)	7.7 (5.1–12.9)
U. cyprinae	6.7 (4.6–9.4)	1.3 (1.1–1.5)	0.08 (0.06–0.12)	14.6 (11.4–18.6)	5.9 (4.7–7.6)	0.9 (0.6–1.2)
Pa. duboisi	3.1 (1.8–5.2)	3.8 (2.3–7.9)	0.11 (0.06–0.27)	5.9 (3.9–8.7)	4.3 (3.4–5.1)	0.3 (0.2–0.4)
Po. ciliata	1.8 (0.8–3.5)	1.0	0.02 (0.01–0.03)	2.6 (1.4–4.7)	1.1 (1.0–1.3)	0.03 (0.01–0.05)
Pe. mytili	6.4 (4.4–9.1)	1.7 (1.4–2.4)	0.11 (0.07–0.17)	1.5 (0.7–3.3)	2.3 (2.0–2.5)	0.04 (0.01–0.07)

test, $P = 0.000$), there is no significant difference between seasons in *Po. ciliata* (non-parametric Kruskal–Wallis test, $P = 0.411$) (Table 4). The mean abundance of *N. legeri* in winter, *Pa. duboisi* and *Pe. mytili* in autumn, *Po. ciliata* in winter and *U. cyprinae* in summer were at their maximum values (Table 2). Comparison of seasonal differences in mean abundance by sampling seasons are presented in Table 3; while there are statistically significant differences in the mean abundance values between seasons in *N. legeri, U. cyprinae, Po. ciliata* and *Pe. mytili* (non-parametric Kruskal–Wallis test, $P = 0.000$), there is no significant difference between seasons in *Pa. duboisi* (non-parametric Kruskal–Wallis test, $P = 0.101$) (Table 3).

Table 4. Comparative differences in prevalence (%), mean intensity (MI) and mean abundance (MA) of parasite species in mussel samples relative to season, mussel size and sampling localities, the significance of the test <0.05, df = degree of freedom.

Parasite species	Variable	*P* (%)	MI	MA	Chi-square	df
Nematopsis legeri	Seasons	0.000	0.000	0.000	203.434	3
	Length classes	0.000	0.005	0.000	103.784	2
	Localities	0.000	0.000	0.000	355.029	2
Urastoma cyprinae	Seasons	0.000	0.000	0.000	27.263	2
	Length classes	0.000	0.257	0.000	80.664	2
	Localities	0.000	0.000	0.000	116.404	2
Parvatrema duboisi	Seasons	0.093	0.000	0.101	6.375	3
	Length classes	0.000	0.122	0.000	95.991	2
	Localities	0.000	0.010	0.000	268.774	2
Polydora ciliata	Seasons	0.024	0.411	0.036	9.231	3
	Length classes	0.419	0.801	0.401	0.714	1
	Localities	0.000	0.913	0.000	30.344	2
Peniculistoma mytili	Seasons	0.000	0.000	0.000	45.432	3
	Length classes	0.010	0.257	0.007	9.527	2
	Localities	0.000	0.028	0.000	18.844	2

The distribution of mussel parasites with respect to sampling localities

Table 5 shows the prevalence (%), mean intensity and mean abundance of parasites according to sampling localities and all parasite species were determined at all sampling sites at least once. Statistically significant differences in infection indices between seasons in all parasite species are presented in Table 4. *Nematopsis legeri* and *Pa. duboisi* occurred with their highest prevalence values in sampling locality III; *U. cyprinae* and *Po. ciliata* occurred in sampling locality II and *Pe. mytili* occurred in sampling locality I (Table 5). Comparison of seasonal differences in prevalence by sampling seasons are presented in Table 4 and statistically significant differences in prevalence between sampling localities were determined in all parasite species (Fisher's exact test, $P = 0.000$) (Table 4). The mean intensities of *N. legeri* and *Pa. duboisi* in sampling locality III, *U. cyprinae* and *Pe. mytili* in sampling locality I and *Po. ciliata* in sampling locality II were at their maximum values (Table 5). Comparison of seasonal differences in mean intensity by sampling seasons are presented in Table 4; while there are statistically significant differences in the mean intensity values between sampling localities in *N. legeri, U. cyprinae, Pa. duboisi* and *Pe. mytili* (non-parametric Kruskal–Wallis test, $P < 0.05$), there is no significant difference between sampling localities in *Po. ciliata* (non-parametric Kruskal–Wallis test, $P = 0.913$) (Table 4). The mean abundances of *N. legeri* and *Pa. duboisi* in sampling locality III, *U. cyprinae* and *Po. ciliata* in sampling locality II and *Pe. mytili* in sampling locality I were at their maximum values (Table 5). Comparison of seasonal differences in the mean abundance values by sampling localities are presented in Table 4 and statistically significant differences between sampling localities were determined in all parasite species (non-parametric Kruskal–Wallis test, $P < 0.05$) (Table 4).

The distribution of parasites with respect to the length classes of the host mussels

Prevalence (%), mean intensity and mean abundance of infection in three length classes of mussels ranging between 2.2 and

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Table 5. Parasite species identified in *Mytilus galloprovincialis* and their infection indices according to sampling locality, CI – 95% confidence intervals based on Stern's exact score limit, Mean I – mean intensity and Mean A – mean abundance with bootstrap 95% CI.

Species	Sampling locality I (N = 690)			Sampling locality II (N = 690)			Sampling locality III (N = 360)		
	P (%)	Mean I CI	Mean A CI	*P* (%)	Mean I CI	Mean A CI	*P* (%)	Mean I CI	Mean A CI
N. legeri	15.4 (12.8–18.2)	69.9 (60.5–88.8)	10.7 (8.5–14.1)	29.0 (25.7–32.5)	116.0 (101–133)	33.5 (27.9–40.2)	72.2 (67.4–76.7)	380.0 (312–490)	274 (220–354)
U. cyprinae	1.7 (1.0–3.0)	12.6 (8.9–17.3)	0.2 (0.1–0.4)	14.1(11.6–16.9)	4.3 (3.5–5.5)	0.6 (0.5–0.8)	0.3 (0.0–1.6)	5.0	0.1 (0.0–0.05)
P. duboisi	0.1 (0.0–0.8)	1.0	0.001 (0–0.004)	0.6 (0.2–1.5)	1.3 (1.0–1.5)	0.01 (0.00–0.01)	20.6 (16.6–25.1)	5.2 (4.2–6.8)	1.1 (0.8–1.5)
P. ciliata	0.9 (0.4–1.9)	1.2 (1.0–1.3)	0.01 (0.003–0.02)	4.6 (3.2–6.5)	1.3 (1.1–1.7)	0.06 (0.04–0.09)	0.3 (0.0–1.6)	1.0	0.03 (0–0.01)
P. mytili	9.9 (7.8–12.3)	6.8 (5.0–9.5)	0.7 (0.5–1.0)	5.2 (3.7–7.1)	3.5 (2.6–5.0)	0.2 (0.1–0.3)	3.6 (2.0–6.1)	2.2 (1.5–2.8)	0.08 (0.04–0.1)

8.4 cm were calculated (Table 6). Statistically significant differences in infection indices between length classes in all parasite species are presented in Table 4. Within the three different mussel length classes studied (Table 6), there was a clear decrease in the infection prevalence of *N. legeri* and *Pa. duboisi* as the mussel length enlarged. However, the situation was the opposite for *U. cyprinae, Pe. mytili* and *Po. ciliata* as the infection prevalence increased when the length classes enlarged. While there are statistically significant differences in the prevalence values between length classes in *N. legeri, U. cyprinae, Pa. duboisi* and *Pe. mytili* (Fisher's exact test, $P = 0.000$), there is no significant difference between length classes in *Po. ciliata* (Fisher's exact test, $P = 0.419$) (Table 4).

Similar to prevalence, the mean intensity values of all parasite species, except *Po. ciliata*, increased when length classes of mussels were larger (Table 6). Comparison of differences in mean intensity by length classes of mussel are presented in Table 4; while there is a statistically significant difference in the mean intensity values between mussel length classes only in *N. legeri* (non-parametric Kruskal–Wallis test, $P = 0.005$), there is no significant difference between mussel length classes in *U. cyprinae, Pa. duboisi, Pe. mytili* and *Po. ciliata* (non-parametric Kruskal–Wallis test, $P > 0.05$) (Table 4). It must be mentioned that *Po. ciliata* was not determined in the smallest length class of mussel samples.

DISCUSSION

The turbellarian *U. cyprinae* has been reported from the gills of *M. galloprovincialis* in the Black Sea (Murina & Solonchenko, 1991), on the French Mediterranean coast (Noury-Srairi *et al.*, 1990; Robledo *et al.*, 1994), on the Galician region, NW Spain (Robledo *et al.*, 1994), on the Pacific coasts of North America (Caceres-Martinez *et al.*, 1998) and in the Adriatic Sea (Westbald, 1955; Mladineo *et al.*, 2012). Caceres-Martinez *et al.* (1998) reported infestation prevalence ranges from 10 to 87% and a mean number of 1.9 per infested *M. galloprovincialis* in the Pacific coasts of North America. They also indicated that larger sized mussels had more parasites, winter was more usual for infestation and prevalence values were different at their sampling localities. Several authors also reported that this parasite preferred colder seasons and larger host sizes (Murina & Solonchenko, 1991; Robledo *et al.*, 1994; Canestri-Trotti & Baccarani, 2001; Rayyan *et al.*, 2004; Crespo-Gonzalez *et al.*, 2010). On the other hand, a marked seasonal pattern with the highest level of infestation during summer and autumn were also reported (Fleming *et al.*, 1981; Fleming, 1986; Plourde *et al.*, 1991; McGladdery *et al.*, 1992; Crespo-Gonzalez *et al.*, 2010). In the present study, *Urastoma cyprinae* showed a seasonal trend such as being completely absent in winter; it was more prevalent in rocky substratum areas, was more abundant in the sampling site which was disturbed by fishing boats' landing activities, and more prevalent in the largest length class of mussel. Currently available data on the seasonality are contradictory, as seen above, making it difficult to draw conclusions on a general seasonal behaviour. However, the determined differences in *U. cyprinae* infestations among seasons might have possibly resulted from the availability of more food for feeding activities, the availability of hosts and temperature related reproduction activities of *U. cyprinae* which occur in the external environment. On the other hand, there is a clear agreement between our results and above-mentioned authors about

Table 6. Parasite species identified in *Mytilus galloprovincialis* and their infection indices according to host length classes, CI – 95% confidence intervals based on Stern's exact score limit, Mean I – Mean Intensity and Mean A – Mean Abundance with bootstrap 95% CI (Mean I, A).

Species	≤4.5 cm (N = 456)			4.6–5.9 cm (N = 678)			≥6.0 cm (N = 606)		
	P (%)	Mean I CI	Mean A CI	*P* (%)	Mean I CI	Mean A CI	*P* (%)	Mean I CI	Mean A CI
N. legeri	49.1 (44.5–53.7)	60.7 (50.3–78.8)	29.8 (24.2–39.8)	31.9 (28.4–35.5)	47.6 (38.1–63.8)	15.2 (11.8–21.3)	19.6 (16.7–23.0)	71.9 (48.5–138)	14.1 (92–28.1)
U. cyprinae	1.1 (0.4–2.6)	5.4 (2.8–12.6)	0.06 (0.02–0.22)	3.5 (2.3–5.2)	3.8 (2.5–6.3)	0.1 (0.08–0.3)	13.4 (10.8–16.3)	5.6 (4.5–7.3)	0.8 (0.6–1.1)
P. duboisi	12.7 (9.9–16.1)	4.4 (3.6–5.9)	0.6 (0.4–0.8)	2.1 (1.2–3.5)	4.1 (2.3–7.1)	0.1 (0.04–0.18)	1.2 (0.5–2.4)	11.3 (5.1–19.0)	0.1 (0.0–0.3)
P. ciliata	0.0	0.0	0.0	2.7 (1.7–4.2)	1.4 (1.0–2.0)	0.04 (0.02–0.06)	3.5 (2.2–5.3)	1.1 (1.0–1.3)	0.1 (0.0–0.1)
P. mytili	5.0 (3.3–7.5)	4.1 (2.7–8.1)	0.2 (0.1–0.5)	5.6 (4.0–7.6)	4.3 (2.8–6.8)	0.2 (0.1–0.4)	9.2 (7.2–11.9)	6.5 (4.6–9.5)	0.6 (0.4–0.9)

larger sized mussels having more parasites since there is a larger space on which this worm can feed itself. Detected differences in the present study on the parasite load at different sampling areas were also reported by Murina & Solonchenko (1991) as a result of the differences in the nature of substratum or distance to the bottom at the sampling areas. Caceres-Martinez *et al.* (1998) explained this situation by the effects of the tide activities; they stated that during low tides, mussels close their valves which affects the presence of *U. cyprinae.* On the other hand, its existence at different environments reflects its wide distributional ability.

The gregarine *Nematopsis legeri* has been reported from the gills of *M. galloprovincialis* in the Black Sea (Özer & Güneydağ, 2014) and Belofastova (1997) reported it to be one of the most spreading parasites of the Black Sea molluscs with infection prevalence ranging from 30 to 100% at natural and artificial *M. galloprovincialis* beds, contrary to the prevalence of 10–20% in the same host at the Romanian coast of the Black Sea (Dumitrescu & Zaharia, 1993). Francisco *et al.* (2010) reported *Nematopsis* sp. infections in *M. galloprovincialis* populations from the Aveiro Estuary in Portugal with clear seasonal peak prevalence in summer and autumn (100%) and sharp decreases in winter and spring (15–17%) and mean abundance ranging between 1.1–120.2. Some authors also reported more than 59% infection indices of *Nematopsis* spp., in *Perna canaliculus* in New Zealand (Jones, 1975), low level infections in summer in *Arcuatula arcuatula, Anadara granosa, Perna viridis* and *Paphia undulate* in the Gulf of Tayland (Tuntiwaranuruk *et al.*, 2004) and in *Callista chione* in the North-Western Adriatic Sea (Canestri-Trotti *et al.*, 2000). Tuntiwaranuruk *et al.* (2004) and Francisco *et al.* (2010) reported that infections were related to the habitat type; heavy infections occurred in species living in a muddy substratum or they occurred in association with the presence of fouling organisms. Tuntiwaranuruk *et al.* (2004) and Francisco *et al.* (2010) concluded that the high levels of infections resulted from a close association of hosts with the presence of such fouling organisms. The prevalence of *N. legeri* infection obtained in the present study falls in the range of the above reports on *Nematopsis* spp. despite the differences in geographic locations and host species and reflects its wide range of infectious potential, which may result from the presence of fouling organisms, as was reported by the above-mentioned authors. In the present study, statistically significant differences among seasons and localities in the occurrence of *N. legeri* obviously indicate that this species has the ability to complete its life cycle in a host that is available all-year round and at all the sampling localities. Bilgin & Çelik (2004) reported that the crab *Eriphia verricosa*, the final host for this parasite, was common in the Sinop coasts of the Black Sea, and this study found that there was a high level of infection at sampling locality III.

The gymnophallid trematode *Parvatrema duboisi* has so far been reported from *M. galloprovincialis* in the Black Sea (Özer & Güneydağ, 2014). In the present study, this parasite occurred more in the smallest length class of mussels, at the sampling locality III and in autumn, despite occurring all year-round. Machkevsky (1989) and Machkevsky *et al.* (2011) reported very high prevalence up to 100% and intensity values of up to 3000 individuals of *Pa. duboisi* in *M. galloprovincialis* in the Sevastopol coast of the Black Sea. The former author also reported five times higher numbers of metacercariae in winter (900 mtc) than in summer (200 mtc) and an increase in the number of metacercariae with increasing size of mussels and concluded that this seasonal pattern was related to the presence of large numbers of birds arriving to Sevastopol for wintering. Machkevsky & Trinitko (1985) reported a sharp increase in the prevalence of metacercariae of *Pa. duboisi* in mussels in an inshore zone polluted by sewage. Gaevskaya & Machkevsky (1995) explained this increase with the presence of high numbers of sea birds, the definitive host for *Pa. duboisi*, present in the vicinity due to the availability of highly abundant food. They also claimed that the weakened physiological condition and concomitant decrease in resistance of the mussels played a role in this increase. Our data had some contradictions to those findings of Machkevsky (1989) that *Pa. duboisi* was more prevalent in the smallest length class, in summer and autumn seasons. On the other hand, our data were in agreement with Machkevsky & Trinitko (1985) in terms of higher infections occurring in an inshore zone, the sampling locality III, the location polluted by sewage from time to time. All-year round occurrence of *Pa. duboisi* in mussels could result from the presence of marine birds at the vicinity of all of our sampling localities. The life cycle of *Pa. duboisi* has not been revealed yet and the present study provides valuable data to focus on the sampling locality III which is one of the main nests for primary marine bird hosts in the Sinop coasts.

The spionid polychaeta *Po. ciliata* has been reported from *M. galloprovincialis* in the Black Sea (Özer & Güneydağ, 2014). Machkevsky *et al.* (2011) determined very low infection prevalence (7%) and intensity (1 individual) in the Sevastopol coast of the Black Sea. Murina & Solonchenko (1991) reported that young spionids occurred in *M. galloprovincialis* with a shell length of 35 mm and maximum intensity was determined in intermediate sized mussels. They concluded that this was due to the fouling of the oldest molluscs by other invertebrates and by algae which prevented the settlement of *Po. ciliata* larvae on mussels. According to Daro & Polk (1973), this parasite occurs at inshore areas and has the ability of dispersion as planktonic larvae. Moreover, due to a relatively short duration of this planktonic phase before settlement, the probability of larvae dispersal to mussels in offshore areas was limited and thus, infection potential of offshore mussels was greatly impaired and overall burden was substantially reduced (Buck *et al.*, 2005). In the present study, its overall infection value was very low and in winter, sampling locality II and middle sized mussels had the highest infection values and the data obtained here were in agreement with the above-mentioned authors.

The protozoan ciliate *Pe. mytili* has been reported from *M. galloprovincialis* in the Black Sea (Dumitrescu & Zaharia, 1993; Dumitrescu & Telembici, 1996; Gaevskaya, 2006; Özer & Güneydağ, 2014), however it was reported to be strictly host specific to *M. edulis* (Lauckner, 1983). In the present study, it occurred more on larger sized mussels, sampling locality I and in autumn with all year-round occurrence. Gaevskaya (2006) reported high infestation prevalence ranging between 76–100% in northern seas of Europe with increased infestations as the size of mussel increases. Dumitrescu & Zaharia (1993) and Dumitrescu & Telembici (1996) reported temperature-dependent high infestation prevalence values and our results are partially in agreement with the results of the above-mentioned authors due to the differences in sampling areas and durations along with coastal characteristics.

In conclusion, the present study yielded five parasite species in the Mediterranean mussel *M. galloprovincialis* collected from the northern part of the Black Sea for the first time. Details on how identified parasite species interacted with season, the size of mussel and different localities with different ecological peculiarities were also determined and presented. Thus, this study provided new as well as up-to-date data on the parasite–host–environment interactions for *M. galloprovincialis.*

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