

Grillotia erinaceus (van Beneden, 1858) (Cestoda:Trypanorhyncha) from whiting in the Black Sea, with observations on seasonality and host-parasite interrelationship

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Abstract

The genus *Grillotia* Guiart, 1927 is cosmopolitan in its distribution and the type-species, *G. erinaceus* (van Beneden, 1858), has been relatively well studied. However, this study provides infection indices of *Grillotia erinaceus* from southern and northern Black Sea whiting *Merlangius merlangus* for the first time. The specimens of *Grillotia erinaceus* were obtained from subserosa of the anterior oesophagus, stomach, pyloric caeca, liver, ovaries and mesenterium of whiting caught by commercial fishing vessels off Sinop, Turkey and off Balaklava, Ukraine. Fish were examined during the period from May 2011 to April 2012. Prevalence and mean intensity values in 268 fish collected off Sinop in the Black Sea were 18.66% and 1.82 ± 0.16 parasites per infected fish, respectively. In Ukrainian 166 whiting samples collected off Balaklava in the Black Sea, however, *G. erinaceus* plerocercus infection prevalence was 10.24% and mean intensity 1.71 ± 0.75 parasites per infected fish. Infection parameters were also determined at both sampling sites in relation with host length, sex and season.

Keywords

Grillotia erinaceus, whiting, Black Sea, Sinop, Balaklava

Introduction

Trypanorhynch cestodes are amongst the most common metazoan parasites of marine fish (Palm and Caira, 2008). Whilst the adults are typically found in the stomach and intestine of sharks and rays, larval forms infect a wide variety of marine invertebrates and teleosts. Larval worms, especially, have low host specificity (Palm and Caira, 2008) and a wide zoogeographical, or even cosmopolitan, distribution (Palm 2004, Palm *et al.* 2007). Being widely distributed from brackish waters into the deep sea, the highest species diversity of trypanorhynchs can be found in coastal tropical waters of the Indo-Australian region (Palm *et al.* 2009).

Grillotia plerocercus, in the form of white spherical or ovoid cysts approximately 2 mm long, are easily visible and occur attached to the serosal surface or embedded in the wall of the oesophagus, stomach, pyloric caeca or intestine (Lubieniecki 1976). Calanoid copepods are known to be the first intermedi-

ate hosts (Ruszkowski 1934) while the second intermediate hosts are gadoids, heterosomates, scombroids and some other groups of marine fish (Lubieniecki 1976). As shown experimentally by Young (1955), the elasmobranch final host becomes infested by feeding on the teleost intermediate host (Lubieniecki 1976). *Grillotia erinaceus* was described first from species of *Raja* Linnaeus off the coast of Belgium but was subsequently reported from various species of rays on both sides of the north Atlantic (Dollfus 1942 – cited by Beveridge and Campbell (2007). There have been several studies on the presence of different *Grillotia* species in their hosts in relation with some host characteristics such as length and sex (Lubieniecki 1976, Sanmartin *et al.* 2000, Timi *et al.* 2005).

The aim of present study was to provide the first comparative infection parameters of *Grillotia erinaceus* (van Beneden, 1858) plerocercus in whiting, *Merlangius merlangus*, at southern and northern parts of the Black Sea, thus providing the first data on this species in Turkish Black Sea waters.

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Materials and Methods

Specimens of *G. erinaceus* were obtained from the subserosa layer of the anterior oesophagus, stomach, pyloric caeca, liver, ovaries and mesenterium of whiting caught by commercial fishing vessels in the Black Sea off Sinop in Turkey and off Balaklava in Ukraine during the period from May 2011 to April 2012. Total length and sex were recorded for each fish. Fish were then examined for parasites using standard methods. The number of *G. erinaceus* plerocerci was counted and the site of infection recorded. When calculating infection prevalence (%) and mean intensity, we followed the recommendations of Bush *et al.* (1997). Briefly, the prevalence (%) was calculated as the percentage of the total number of fish infected and the mean intensity was calculated as the average number of parasites in the total number of infected fish.

A Kruskal-Wallis test (nonparametric ANOVA) was performed to determine significant differences in the mean intensity values of *G. erinaceus* for length classes of fish and the seasons in which this study was conducted. The difference between parasite loads in male and female fish was tested by the Mann-Whitney U test. All statistical analyses were performed at the significance level of 5% using the statistical program GraphPad InStat 3.00.

Results

Grillotia erinaceus plerocerci were found in the form of white ovoid cysts approximately 6 mm long and were easily visible and occurred attached to the serosal surface or embedded in the wall of the anterior oesophagus, stomach and pyloric caeca, liver, ovaries and mesenterium. Paratypes were deposited at the museum collection of parasites of hydrobionts at the Institute of the Biology of the Southern Seas, Ukraine with the numbers C.901.018.16; C.902.018.17; C.903.018.18.

Overall infection prevalence (%) and mean intensity levels of *G. erinaceus*

Throughout the investigation period, *G. erinaceus* was found to be the only cestode plerocercus infecting *M. merlangus* at both sampling sites. Of the 268 and 166 fish specimens examined in Sinop and Balaklava, 50 and 17 were infected with the prevalences of 18.7% and 10.2%, mean intensity values of 1.82 ± 0.16 and 1.71 ± 0.75 per infected fish, respectively (Table II). The differences between overall mean intensity values determined at both sampling sites were not statistically significant ($P > 0.05$) (Table I).

The distribution of *G. erinaceus* with respect to season

Seasonal prevalence and mean intensities of *G. erinaceus* infecting *M. merlangus* at both sampling sites from Turkish and Ukrainian waters of the Black Sea are presented in Table II. Despite differences recorded between seasons, they were not statistically significant either between seasons at each sampling sites or between sampling sites in Turkey and Ukraine ($P > 0.05$) (Table I).

The distribution of *G. erinaceus* with respect to the length classes of the host fish

Prevalence (%) and mean intensity of infection in different fish length classes from both sampling sites were determined and compared. Within the three different fish length classes studied (Table I), there was a progressive increase in infection prevalence with increasing fish length in Turkish samples; the lowest (15.3%) in the shortest length class (<15 cm) and the highest (31.0%) in the largest length class. However, the maximum mean intensity value (1.88 ± 0.21) was in the middle length class and the minimum (1.67 ± 0.44) in the largest length class (Table I). On the other hand, both infection preva-

Table I. Infection parameters of *Grillotia erinaceus* from whiting, *Merlangius merlangus* from southern and northern parts of the Black Sea

	Turkish Coasts of the Black Sea		Ukrainian Coasts of the Black Sea	
	Prevalence (%)	Mean Intensity $\pm$ S.E.	Prevalence (%)	Mean Intensity $\pm$ S.E.
Seasons				
Summer	7.68 (n:26)	2.00 ± 0.00^a		
Autumn	34.57 (n:81)	2.00 ± 0.23^a		
Winter	18.31 (n:71)	1.54 ± 0.27^a	15.00 (n:40)	1.83 ± 0.47^a
Spring	7.78 (n:90)	1.57 ± 0.57^a	8.73 (n:126)	1.63 ± 0.30^a
Fish Length Classes				
<15 cm	15.25 (n:59)	1.78 ± 0.36^a	13.69 (n:73)	1.70 ± 0.33^a
15–18 cm	17.77 (n:180)	1.88 ± 0.21^a	6.32 (n:79)	1.40 ± 0.24^a
18> cm	31.03 (n:29)	1.67 ± 0.44^a	14.28 (n:14)	$2.50 \pm 1.50^*$
Fish Sex				
Male	23.08 (n:65)	2.53 ± 0.40^a	4.76 (n:21)	$1.00 \pm 0.00^*$
Female	17.24 (n:203)	1.51 ± 0.14^b	11.03 (n:145)	1.75 ± 0.27
Overall	18.66	1.82 ± 0.16	10.24	1.71 ± 0.75

Values followed by the same superscript letter are not significantly different, analysis of variance-protected Tukey – Kramer, $\alpha = 0.05$.

*Too few values for statistical analysis

lence and mean intensities showed a maximum in middle sized fish with the lowest and the largest sized fish with the highest values in Ukrainian samples. Despite the differences in distribution of *G. erinaceus* with respect to length classes of the host fish, they were not statistically significant at both sampling sites ($P > 0.05$).

The distribution of *G. erinaceus* in host fish of different sexes

The prevalence and mean intensity were both higher in males than in females in Turkish samples and the difference between mean intensity values was statistically significant ($P < 0.05$) (Table I). On the other hand, the situation was reversed in Ukrainian samples. As only one male fish was found infected, it was not possible to make a statistical comparison between male and female fish.

Discussion

Merlangius merlangus, commonly known as whiting is widely distributed in the eastern North Atlantic Ocean, the northern Mediterranean, western Baltic and the Black Sea and it is a coldwater fish and prefers temperature 5–15°C and depths up to 200 m (ICES 2006). In the Black Sea, it is distributed everywhere – near north, north-western, western (Romanian and Bulgarian) and eastern (Caucasus) coasts, along all the Anatolian shelf zone (Burdak 1960, Svetnovidov 1964) and is abundant near the Sinop coast (Gönener and Bilgin 2010). Black Sea whiting do not form large stocks; nevertheless, it is an important part of the diet of dolphins and other predatory fishes (especially turbot and rays) (Popovici 1941, Probatov and Uralskaya 1957, Svetnovidov 1964) and is one of the economically important fish species in the Black Sea, especially in Sinop, Turkey and in Balaklava, Ukraine. This fish species was found to be infected with *Grillotia plerocerci* at the same organs those of reported by Lubieniecki (1976) and Brickle *et al.* (2006). Lubieniecki (1976) reported this parasite along the entire length of the gut, in the present study, however, it was located only in some parts of the gut possibly as a result of lower infection parameters. Sometimes, larvae were found on the serosal surface of the liver, ovaries and on mesenteries. *Grillotia erinaceus* is one of the well-known species of the genus and this species was first reported by Korniyushin and Solonchenko (1978) from the Black Sea in the cartilaginous fish *Raja clavata* L., 1758 and whiting collected in the vicinities of Sevastopol and Karadag where this study was conducted. On the other hand, this is the first report of this parasite species in Turkey.

Prevalence, mean intensity and abundance values of several *Grillotia* species in the plerocercus stage in different fish host reported by several authors from different locations are shown in Table II. In general, overall infection prevalence (%), mean intensity and abundance values of *Grillotia* spp. reported

by several authors are higher than those of the present study (Table II). Overall infection prevalence of *G. erinaceus* was at least two-fold higher when compared with our data (Tables I and II). On the other hand, the overall mean intensity value reported by Brickle *et al.* (2006) was similar, though the range was higher than that of this study (Table II). It is obvious from Table II that the members of *Grillotia* may have wide range of infection prevalence from 8.75% to 100% and intensity from 0.10 ± 0.30 to 638 ± 635 . Hence, our findings are somehow lower than those reported in Table II. Some effects of larval cestodes on the host fish include growth retardation and a lower condition factor (Hoffmann *et al.* 1986), tissue disruption, metabolic disturbances (Richards and Arme 1981, Rosen and Dick 1984), reduced swimming speed in infected fish (Sprengel and Luchtenberg, 1991) and even mortality in heavy infections (Adjei *et al.* 1986). However, Lubieniecki (1976) indicated that *G. erinaceus* migrated only a short distance into or through its host's gut wall and became bound in a cyst of parasite and host origin, thus not being able to stimulate a detectable humoral response. So, it seems unlikely that this parasite alone causes any adverse effects on host fish.

Ruszkowski (1934) showed experimentally that four calanoid copepod species (*Acartia longiremis*, *Pseudocalanus eleongatus*, *Paracalanus parvus* and *Temora longicornis*) can act as first intermediate hosts of *G. erinaceus* (? *pseudoerinaceus*). Teleost fish act as second intermediate hosts and many species of rays may be final hosts (Dollfus 1942). Svetnovidov (1964, 1986) and Burdak (1960) reported that young pelagic whiting from the Black Sea also feeds on copepods (*Calanus helgolandicus*, *Temora longicornis*, *Pseudocalanus elongates* and *Acartia* spp.) but adult whiting feed dominantly on fish (it consists about 80% of food), decapods and mysids; food composition depends on the region of the Black Sea and on a season. Pelagic larvae of whiting in North Sea start feeding when they are 2.4 mm long, and their main prey are the nauplii and copepodite stages of copepods (Last 1978). Hamerlynck and Hostens (1993) determined that 0+ age whiting from 50 mm feed exclusively on calanoid copepods then shift to fish, mysids and amphipods and, beyond 100 mm, feed nearly exclusively on shrimp and small fish. Immature whiting (<20 cm) feed on crustaceans such as euphausiids, mysids and crangonid shrimps. Whiting >30 cm feed almost entirely on fish, including a variety of small species such as Norwegian pout, sprat, and sandeel, and the younger age classes of larger species such as herring, cod, and haddock (ICES 2006). Samsun *et al.* (2011) reported that bony fishes constitute a large percentage of the nutrient group, followed by crustaceans, in the stomach of whiting caught off Sinop in the Black Sea, where this study partially was conducted. The percentage distribution of bony fishes were 61.6% for anchovy (*Engraulis encrasicolus* L., 1758), 15.7% for horse mackerel (*Trachurus mediterraneus* Steindachner, 1836), 13.8% for whiting (*M. merlangus*), 6.3%, for goby (*Gobius* spp.), 1.9% for sprat (*Spratus spratus* L., 1758) and 0.75% for pipefish (*Syngnathus acus* L., 1758). Samsun *et al.* (2011) also reported that sprat

Table II. Infection prevalence (%), mean intensity and abundance values of several *Grillotia* species in different host fish reported by several authors from different locations

Parasite species	Host species	Prevalence (%)	Range (Mean Intensity)	Mean Abundance $\pm$ SD	Location	Author(s)
<i>Grillotia</i> sp.	<i>Percophis brasiliensis</i> Quoy and Gaimard, 1894	Zone I – IV 100–100	–	Zone I – IV 142 $\pm$ 191 – 638 $\pm$ 635	South-west Atlantic	Braicovich and Timi (2008)
<i>Grillotia</i> sp1 pterocercus	<i>Merluccius hubbsi</i> Marini, 1933	Zone I – IV 8.75–84.3	–	Zone I – IV 0.5 $\pm$ 2.2 – 14.0 $\pm$ 29.7	South-west Atlantic Ocean	Sardella and Timi (2004)
<i>Grillotia</i> sp2 pterocercus	<i>Merluccius hubbsi</i> Marini, 1933	Zone I – IV 0–12.5	–	Zone I – IV 0–2.2 $\pm$ 0.7		
<i>Grillotia</i> sp	<i>Aphos porosus</i> (Valenciennes, 1837)	1999–2007 4.6–14.3	–	1999–2007 0.05 $\pm$ 0.21 – 0.14 $\pm$ 0.35	El Tabo, Central Chile	Cortes and Munoz (2009)
<i>Grillotia</i> sp.	<i>Micromesistius australis</i> Norman, 1937	9.8	–	0.10 $\pm$ 0.30	South-west Atlantic	Niklitschek <i>et al.</i> (2010)
<i>Grillotia carvajalregorum</i> pterocercus	<i>Nemadactylus bergi</i> (Norman, 1937)	Zone I – V 81.3–100	–	Zone I – V 16.00 $\pm$ 22.1–25.5 $\pm$ 35.3	Mar del Plata, Argentina	Rossin and Timi (2010)
	<i>Melanogrammus</i> <i>aeglefinus</i> L. 1758	45.13	–	1 – 30	Northern North Sea	
<i>Grillotia erinaceus</i>	<i>Gadus morhua</i> L., 1758	46.87	–	–	Aberdeen Bay	Lubieniecki (1976)
	<i>Pollachius virens</i> L., 1758	80.00	–	–	Firth of Clyde	
<i>Grillotia erinaceus</i>	<i>Dissostichus eleginoides</i> Smitt, 1898	38.10	1–23 (3.45)	1.31	Falkland Islands	Brickle <i>et al.</i> (2006)

and pipefish were consumed by small length whiting, anchovy and horse mackerel were consumed by all sizes of whiting, being preferred by the length group of 14–16 cm. On the other hand, whiting and goby were mostly consumed by whiting at the length group of 16–18 cm.

The results of this study show that infection with *Grillotia erinaceus* increases with fish length, so that parasites are still being acquired after the fish have apparently ceased to feed on copepods at both sampling sites in northern and southern Black Sea. *Merlangius merlangus* are unlikely to become infected with *G. erinaceus* by feeding only on copepods and some larger invertebrates might have acted as first intermediate hosts. Moreover, whiting might have consumed the above mentioned fish species as acting second host in the life cycle that had recently acquired parasites but which had not yet developed beyond the plerocercus stage. Rae (1958) reported similar findings in halibut *Hippoglossus hippoglossus* L., 1758 for *G. erinaceus* infections in the flesh of Atlantic halibut caught on certain grounds in the northeast Atlantic. However, it must be noted that, as far as we are aware of, no *G. erinaceus* infections have been reported from anchovy, horse mackerel, goby, sprat and pipe fish so far. On the other hand, small sized whiting were found in the stomach contents of larger whittings as food fish and it is more likely that the whiting studied in the present study might have got *G. erinaceus* infections from small sized whiting that fed on calanoid copepods carrying the first developmental stage. Lubieniecki (1976) showed experimentally that transfer of *G. erinaceus* from haddock to haddock was unsuccessful possibly as a result of fully developed plerocerci but he did not rule of the possibility of, as suggested by Rae (1958), that young plerocerci may be able to transfer from prey to predator fish.

Infection parameters of *G. erinaceus* from whiting, *M. merlangus* from the southern and northern parts of the Black Sea with respect to season showed similar pattern at both sampling sites, however it must be noted that fish were collected only in two seasons in Ukraine samples. Despite a clear picture of a peak in autumn and then a gradual decrease in infection prevalence, mean intensity of infection showed little variation between seasons. The lack of seasonal variations in infection with parasites could be a result of the availability of infected intermediate host in most time of year or to prolonged of plerocercus life span that may be as long of host age, that is probable more than seven years.

The prevalence and mean intensity levels in males and females had different patterns in Turkish and Ukrainian samples, the difference between mean intensity values being statistically significant in favour of male fish in Turkish samples. It is known that female whiting grow to a larger size than males (Probatov and Uralskaya 1957, Burdak 1960, Oven et al. 1995) and this affects the feeding habits of fish. Langton and Bowman (1978) reported that 13 gadoid fishes (e.g. *Gadus morhua* L., 1758, *Pollachius virens* L., 1758, *Merluccius bilinearis* Mitchell, 1814) in northwest Atlantic had dif-

ferences in the composition of diet consumed by male and female fish with a shift from crustaceans being the major prey of the males to fish being the major prey of the females. As growth is faster in females than males, the longer period of feeding of males on crustaceans that act as the first intermediate hosts before shifting to fish as the main prey could influence prevalence. However, in Ukrainian samples, infection prevalence and mean intensity values were reversed with females having higher values than those of males. We can not rule out that this difference at both sampling sites could be a result of an insufficient number of fish sampled to provide a clear picture on the effects of sex on *G. erinaceus* infections. Worth also noting that no significant differences have been reported in a diet composition of males and females of the Black Sea whiting (Probatov and Uralskaya 1957).

Conclusion

Here in the present study, we provide the first comparative infection parameters of *G. erinaceus* plerocerci in whiting, *M. merlangus*, at southern and northern parts of the Black Sea, thus providing the first data on this species in Turkish Black Sea waters.

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