The taxonomic and phylogenetic status of digeneans from the genus *Timoniella* (Digenea: Cryptogonimidae) in the Black and Baltic seas

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Abstract

Timoniella spp. are cryptogonimid flukes (Digenea: Cryptogonimidae) that parasitize the guts of fish in brackish waters. *Timoniella imbutiforme*, a species from the Mediterranean Sea, is recorded in the Black Sea, while *T. balthica* has been described from the Baltic Sea. In this paper, we clarify the taxonomic status of *Timoniella* populations in the Baltic and Black seas. Adults and metacercariae of *Timoniella* spp. were sampled from localities in the Mediterranean Sea (France), Black Sea (Ukraine) and Baltic Sea (Germany) and subjected to molecular and morphological analysis, including Bayesian phylogenetic reconstruction based on concatenated sequences of ITS1–ITS2–28S. This allowed us to construct a new key to species of the genus *Timoniella*. Our results suggest that *T. balthica* forms part of the Boreal–Atlantic relict fauna of the Black Sea and should now be considered a junior synonym of *T. imbutiforme*.

Introduction

The family Cryptogonimidae Ward, 1917 (Digenea: Trematoda) includes a number of flukes parasitizing the gut and pyloric caeca of poikilothermic vertebrates, including fish, reptiles and amphibians (Miller & Cribb, 2008b). The genus name *Timoniella* was proposed by Rebecq (1960) for *Timoniella atherinae* Rebecq, 1960 based on metacercariae from big-scale sand-smelt (*Atherina boyeri* Risso, 1810) in the Mediterranean Sea. Later, these metacercariae were synonymized with *Timoniella praeterita* (Looss, 1901), which is now the type species for the genus (Maillard, 1974). In all, eight species are now known for the genus, with three being described from European marine/brackish-water fish: *Timoniella balthica* (Reimer *et al.*, 1996), *Timoniella imbutiforme* (Molin, 1859) and *T. praeterita* (Brooks, 1980; Miller & Cribb, 2008b). Adult *T. praeterita* are known only from European seabass (*Dicentrarchus labrax* (L., 1758)) occurring off the Mediterranean coasts of Egypt, France and Italy, the Adriatic Sea, the Norwegian North Sea and the Atlantic coast of Great Britain (Looss, 1901; Nicoll, 1915; Maillard, 1974; Sterud, 2002; Radujković & Šundić, 2014), although *T. praeterita* metacercariae have also been found on the sand-smelt *A. boyeri* and gilthead seabream *Sparus aurata* L., 1758 (Maillard, 1974; Maillard *et al.*, 1980). *Timoniella imbutiforme* is a widely distributed species, known from *D. labrax*, the European flounder (*Platichthyes flesus* L., 1758) and the broad-nosed pipefish (*Syngnathus typhle* L., 1758) in the Mediterranean Sea (Molin, 1859; Looss, 1901; Maillard, 1973; Bartoli & Gibson, 2007; Culurgioni *et al.*, 2014), the Atlantic coast of Europe, the North Sea off Norway and the coast of England (Johnstone, 1906; Nicoll, 1915; McDowall & James, 1988; El-Darsh & Whitfield, 1999; Sterud, 2002). It is also known from the common dentex (*Dentex dentex* L., 1758) in the Adriatic Sea (Marengo *et al.*, 2014; Radujković & Šundić, 2014), while *T. imbutiforme* metacercariae are known from *Pomatoschistus* spp., the black goby (*Gobius niger* L., 1758), *A. boyeri*, the Mediterranean banded killifish (*Aphanius fasciatus* Valenciennes, 1821), *P. flesus*, the common dab (*Limanda limanda* L., 1758) and the common sole (*Solea solea* L., 1758) (Maillard, 1973; Køie, 1983; El-Darsh & Whitfield, 2000; Malek, 2004; Culurgioni *et al.*, 2014). Adults have been registered from *S. typhle* in the Black Sea and *P. flesus* in the Gulf of Odessa and the Sea of Azov (Chernyshenko, 1949; Domnich & Sarabeev, 2000), while metacercariae are often registered on gobiid species from the same localities, and from Sevastopol Bay and lagoons of the north-western Black Sea (Naydenova, 1974; Domnich & Sarabeev, 2000; Kvach, 2005, 2010; Kvach & Oğuz, 2009; Krasnovyd *et al.*, 2012). Finally, adult *T. balthica* have been found on *S. typhle* from the Salzhaff Lagoon in the Baltic Sea (Reimer *et al.*,

1996), while metacercariae have been recorded on many small fishes, including the three-spined stickleback (*Gasterosteus aculeatus* L., 1758), the nine-spined stickleback (*Pungitius pungitius* L., 1758), *S. typhle*, the viviparous eelpout (*Zoarces viviparus* L., 1758), and gobiids and gobionellids (Reimer *et al.*, 1996; Zander *et al.*, 1999, 2002; Zander, 2001, 2003; Zander & Reimer, 2002; Kvach & Winkler, 2011). This relatively newly described species (*T. balthica*) is morphologically very similar to *T. imbutiforme* from the Black Sea, which also uses the same definitive host, i.e. *S. typhle*.

The Black and Baltic seas are both large brackish waterbodies with salinity ranging around 18‰ in the Black Sea and 6–8‰ in the Baltic Sea (Zenkevich, 1963; Dethier, 1992). Forming part of the Mediterranean region, the Black Sea is characterized by the presence of both relict Boreo-Atlantic and Ponto-Caspian faunas, in addition to Mediterranean species (Zaitsev & Mamaev, 1997; Zaitsev, 1998). Cryptogonimids inhabiting the Black Sea are mainly of Mediterranean origin; for example, *Anisocoelium capitellatum* Rudolphi, 1819, *Anisocladium fallax* Rudolphi, 1819 and *A. gracilis* Looss, 1901 are all common parasites of the stargazer (*Uranoscopus scaber* L., 1758) throughout the Mediterranean basin, including the Black Sea (Bartoli & Gibson, 2000). *Metadena pauli* Vlasenko, 1931 was originally described from fish of the Black Sea, although it has since also been recorded in fish species from the eastern and western Mediterranean Sea (Sey, 1970; Naydenova, 1974; Fischthal, 1980; Bartoli & Bray, 1987; Bartoli & Gibson, 1995). On the other hand, *Aphallus tubarium* Rudolphi, 1819, a common species found on many fish in the Mediterranean Sea, has only been recorded in *S. typhle* in the Black Sea (Korniychuk & Gaevskaya, 2004). *Aphalloides coelomicola* Dollfus *et al.*, 1957 is the only Boreal–Atlantic species, its distribution covering both the Mediterranean basin and northern Europe (Kvach *et al.*, 2017).

Due to the overall similarity of the Black and Baltic seas as habitat, and the similarity of representatives of *Timoniella* found within them, the status of these taxa remains unclear. Hence, the aim of this work was to undertake a morphological and genetic comparison of *Timoniella* spp. from different geographical regions (the Baltic, Black and Mediterranean seas) in order to clarify the taxonomic status of the Black Sea and Baltic Sea populations.

Materials and methods

Adults and metacercariae of *Timoniella* spp. were sampled from different localities in the Mediterranean Sea (Saint-Nazaire Lagoon, France), the Black Sea (Budaki Lagoon, Sevastopol Bay and the Gulf of Odessa, Ukraine) and the Baltic Sea (Salzhaff, Germany) (site details are provided in table 1). Examples of each parasite were fixed in pure 96% ethanol for molecular study and hot 4% formaldehyde for morphological study (Cribb & Bray, 2010). Formaldehyde-preserved worms were then stained with iron acetic carmine, dehydrated in ethanol of increasing concentration and mounted in Canada balsam as permanent slides (Georgiev *et al.*, 1986). All metacercariae were identified based on the number of spines in the oral sucker crown (Maillard, 1973; Reimer *et al.*, 1996).

DNA was extracted from individual worms using the JetQuick kit (Genomed, Löhne, Germany), while the KAPA2G Robust HotStart PCR Kit (Kapabiosystems, Wilmington, Massachusetts, USA) was used to amplify the internal transcribed spacer-1–internal transcribed spacer-2–28S rDNA (ITS1–ITS2–28S rDNA) nuclear genomic region (primers and annealing temperatures are detailed in table 2). Sanger sequencing of polymerase chain reaction (PCR) products was performed commercially at GATC Biotech (Konstanz, Germany), with sequences edited and aligned using Geneious 9.0.5 (Kearse *et al.*, 2012). Bayesian phylogenetic reconstruction was based on concatenated ITS1–ITS2–28S sequences of ten newly sequenced *Timoniella* individuals (table 1). This represents the first sequencing of the ITS1–ITS2–28S region in morphologically identified *Timoniella* spp. Samples of five other cryptogonimid species from our own samples (see table 1) were used as an outgroup. Also included in the outgroup were sequences for *Acanthostomum burminis* Bhalerao, 1926 (KC489791; Jayawardena *et al.*, 2013), *Siphoderina jactus* Miller & Cribb, 2008b (EU571263) and *Siphoderina poulini* Miller & Cribb, 2008 (EU571267; Miller & Cribb, 2008a). *Acanthostomum burminis* is presently considered a sister lineage to the remaining cryptogonimids. PartitionFinder 1.0.1 (Lanfear *et al.*, 2012) detected all three regions (ITS1, ITS2, 28S) as forming a single partition, indicating GTR+G as the most suitable substitution model to use. Hence, Bayesian analysis of evolutionary relationships using a single partition with GTR+G model priors was performed in MrBayes 3.2.1 (Ronquist & Huelsenbeck, 2003), as implemented in Geneious 9.0.5.

Descriptions of adult *Timoniella* were used for the morphological study (Chernyshenko, 1949; Maillard, 1973; Reimer *et al.*, 1996). Measurements were made of total body length (TBL), total body width (TBW), forebody length (FBL) and hindbody length (HBL), with all parameters measured in micrometres. TBW was taken as maximum body width for all further calculations. FBL was measured as distance from the anterior extremity to the anterior margin of the ventral sucker, while HBL was measured from the anterior margin of the ventral sucker to the posterior end of the body. FBL was calculated as the percentage of TBL. We also measured the length and width of the oral (OS) and ventral (VS) suckers, the pharynx, the seminal receptacle, the ovary (Ov), the anterior (AT) and posterior (PT) testes, and the egg, along with the length of the prepharynx and oesophagus, the distance from the ovary to the anterior testis (Ov/AT), and distance from the posterior testis to the posterior end of the body (PT/PostB). The number of spines in the oral-sucker spine crown was calculated, as was the length and width of a single spine. Finally, ratios of TBL to TBW (TBL/TBW), OS width to length (OS W/L), VS width to length (VS W/L), and OS to VS length (OS/VS) were calculated as a percentage.

Results

Phylogenetic analysis

Bayesian phylogenetic reconstruction based on the ITS1–ITS2–28S genomic region indicated a highly supported monophyletic group encompassing four cryptogonimid genera in two pairs (posterior probability PP = 1.00); with *Acanthochasmus* as a highly supported sister genus to *Neochasmus* (PP = 1.00), and slightly lower support (PP = 0.79) for a sister relationship between *Timoniella* and *Aphalloides* (fig. 1). The genus *Timoniella* was monophyletic and included all samples from France, Germany and Ukraine (PP = 1.0). *Timoniella* worm sequences from different hosts showed little variability and no clear structure caused by host fish species.

Morphological study

There was no morphological difference between worms sampled from different regions (table 3, fig. 2), although morphometric

Table 1. Samples used for genetic analysis of *Timoniella* spp.

Parasite taxa	Host	Sample date	Locality	Country
Timoniella cf. *imbutiforme* (two samples)	*Pomatoschistus microps*	03.03.2008	Mediterranean Sea, St.-Nazaire Lagoon	France
Timoniella cf. *imbutiforme*	*Atherina boyeri*	05.03.2008	Mediterranean Sea, St.-Nazaire Lagoon	France
Timoniella cf. *imbutiforme*	*Atherina boyeri*	06.03.2008	Mediterranean Sea, St.-Nazaire Lagoon	France
Timoniella cf. *balthica* (two samples)	*Pomatoschistus microps*	10.06.2008	Baltic Sea, Salzhaff	Germany
Timoniella cf. *balthica*	*Syngnathus typhle*	10.06.2008	Baltic Sea, Salzhaff	Germany
Timoniella cf. *imbutiforme*	*Atherina hepsetus*	18.07.2008	Black Sea, Sevastopol Bay	Ukraine
Timoniella cf. *imbutiforme*	*Pomatoschistus marmoratus*	28.07.2008	Black Sea, Budaki Lagoon	Ukraine
Timoniella cf. *imbutiforme*	*Neogobius melanostomus*	03.06.2009	Black Sea, Gulf of Odessa	Ukraine
Allocanthochasmus sp.	*Morone chrysops*	15.11.2006	Lake Erie, Ohio	USA
Neochasmus umbellus	*Morone chrysops*	15.11.2006	Lake Erie, Ohio	USA
Anisocoelium capitellatum	*Uranoscopus scaber*	22.10.2011	Black Sea, Gelendzhik Bay	Russia
Anisocladium fallax	*Uranoscopus scaber*	22.10.2011	Black Sea, Gelendzhik Bay	Russia
Aphalloides coelomicola	*Pomatoschistus microps*	06.03.2008	Mediterranean Sea, St.-Nazaire Lagoon	France

parameters within each region showed high variability. As a result, there is some overlap between regions and with data from previous work, including the original descriptions (see table 3; Chernyshenko, 1949; Maillard, 1973; Reimer *et al.*, 1996). Based on this morphological study, we provide a re-description of *T. imbutiforme* below.

Taxonomic summary

Family Cryptogonimidae Ward, 1917.

Genus *Timoniella* Rebeqc, 1960.

Timoniella imbutiforme (Molin, 1859) Brooks, 1980 (fig. 2).

Synonyms. Distomum imbutiforme Molin, 1859; *Anoikostoma imbutiforme* (Molin, 1859) Stossich, 1899; *Acanthochasmus imbutiformis* (Molin, 1859) Looss, 1901; *Echinostomum imbutiforme* (Molin, 1859) Johnstone, 1906; *Acanthostomum imbutiforme* (Molin, 1859) Gohar, 1934; *Acanthostomum balthicum* Reimer *et al.*, 1996; *Timoniella balthica* (Reimer *et al.*, 1996) Miller & Cribb, 2008.

Type host. Dicentrarchus labrax (L., 1758) (Actinopterygii: Moronidae).

Other hosts. Syngnathus typhle L., 1758, *Syngnathus abaster* Risso, 1826 (Actinopterygii: Syngnathidae), *Dentex dentex* (L., 1758) (Actinopterygii: Sparidae), *Platichthys flesus* L., 1758 (Actinopterygii: Pleuronectidae); metacercariae in *Atherina boyeri*

Risso, 1810 (Actinopterygii: Atherinidae), *Neogobius melanostomus* (Pallas, 1814) (Actinopterygii: Gobiidae), *Pomatoschistus marmoratus* (Risso, 1810), *Pomatoschistus microps* (Krøyer, 1838) (Actinopterygii: Gobionellidae), and many other brackish-water fish species.

Type locality. Mediterranean Sea near Egypt.

Other localities. Gulf of Leon, Nile delta, Adriatic Sea, Black Sea, Sea of Azov, North Sea, Themes delta, Øresund, south-western Baltic Sea.

Site in host. Intestine.

Voucher material. C-616 (Helminthological collection of the Institute of Parasitology of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic).

Representative sequence. MF491832–MF491865 (NCBI GenBank).

Description

Body elongate, 743.5–3944 in length. Forebody usually shorter than hindbody, 22–50% of total body length. Maximum body width in forebody, 113–500, at the level of ventral sucker. Ratio between body length and width 5.3–11.6. Tegument covered with small spines. Two brown eyespots at level of pharynx.

Oral sucker terminal, funnel-shaped, 79–304 × 65–277. Oral sucker with crown of 17–20 spines, 13–78 × 5–10. In specimens fixed in hot formalin, oral sucker partly everted and spines

Table 2. DNA sites and primers used for genetic sequencing of *Timoniella* spp. (T °C = annealing temperature).

Region	Primer	Primer sequence	T °C	Reference
28S	LSU5	TAGGTCGACCCGCTGAAYTTAAGCA	57	Littlewood, 1994
	ECD2	CCTTGGTCCGTGTTTCAAGACGGG		Littlewood *et al.*, 1997
ITS1	BD1	GTCGTAACAAGGTTTCCGTA	57	Luton *et al.*, 1992
	4S	TCTAGATGCGTTCGAARTGTCGATG		Bowles *et al.*, 1993
ITS2	3S	GGTACCGGTGGATCACGTGGCTAGTG	57	Bowles *et al.*, 1993
	ITS2.2	CCTGGTTAGTTTCTTTTCCTCCGC		Cribb *et al.*, 1998

Table 3. Morphological and morphometric parameters used for differentiating adult *Timoniella imbutiforme* sampled from different regions (see 'Materials and methods' for an explanation of the abbreviations used).

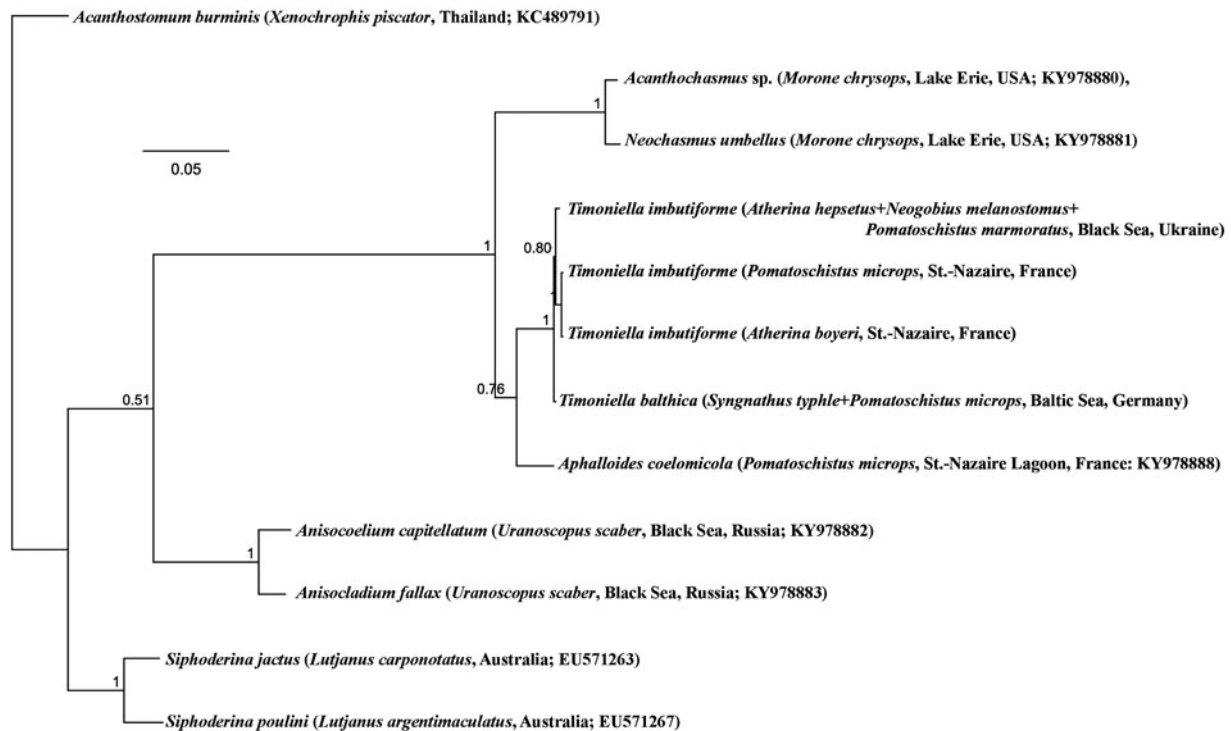|	Baltic Sea		Black Sea		Mediterranean Sea
Host	*Syngnathus typhle*	*Syngnathus typhle*	*Syngnathus typhle*	*Syngnathus typhle*	*Dicentrarchus labrax*
Source	This study	Reimer *et al.*, 1996	This study	Chernyshenko, 1949	Maillard, 1973
TBL	1199 (743.5–1629)	980–1987	1442 (1003–1996)	2200–3000	2978 (2074–3944)
TBW	175 (113–241.5)	154–267	194 (129–290)	360–500	333 (240–442)
FBL	363 (259–432)		428 (180–535)		
HBL	836 (438–1196)		1014 (634–1535)		
TBL/TBW	7.1 (5.3–11.6)	5.0–9.5	7.6 (6.2–9.6)		9.1 (5.8–10.8)
FBL/TBL	31 (26–41)		31 (11–41)		
OS length	101 (79–130)	111–194	132 (101–165)	164–225	255 (200–304)
OS width	93 (64.5–120)	97–199	136 (95.5–170)		229 (166–277)
OS W/L	93.5 (73–113)		102.3 (94.5–111)		
Spine no.	19 (17–20)	17–19	18 (17–20)	18	18 (17–19)
Spine length	24 (20–29)	13–42	41 (26–50)		63 (50–74)
Spine width	7.7 (5–9)		9.5 (7.5–10)		
Prepharynx	129 (58–170)	40–167	192.5 (131–223)		209 (144–255)
Pharynx length	52 (34–64)	70–90	67 (48–79)	123–143	121 (96–151)
Pharynx width	58 (40.5–74)	50–67	74 (55–88)	109–139	
Oesophagus	47 (30–73)	25–50	60 (46–85)		
VS length	96 (69–113)	99–142	128 (89–179)	205–246	263 (216–304)
VS width	100 (73.5–125)	40–142	125 (92–154)		
VS W/L	104 (91–119)		99.5 (86–105)		
OS/VS	105 (88–124)	100	106 (84–116)		80–90
Seminal vesicle		15–19			
Receptaculum semenis length	42 (27.5–70)	52–102	55 (28–83.5)		
Receptaculum semenis width	38 (26–61)	50–72	45 (28–65)		
Receptaculum semenis position	Anterior to ovary; rarely partly overlapped with ovary	Anterior to ovary; partly overlapped with ovary	Anterior to ovary	Anterior to ovary	Anterior to ovary
Ov length	80 (23–129)	82–109	106 (59–136)	150–200	
Ov width	81 (20.5–147)	65–109	64 (19–113)	140–220	
Vitellaria position	From posterior testis to middle of ant. testis/ventral sucker distance	From middle of anterior testis to middle of ant. testis/ventral sucker distance	From posterior testis to middle of ant. testis/ventral sucker distance	From posterior testis to middle of ant. testis/ventral sucker distance	From middle of anterior testis to middle of ant. testis/ventral sucker distance
Ov/AT	6.3 (0–27)		31 (0–137.5)		
AT length	107 (40–159)	137–265	121 (70–200)	300–440	330 (297–411)
AT width	109 (39–172)	72–97	112 (53.5–181)	160–260	193 (158–227)
PT length	127 (47–205)	–	124 (75–217)		363 (297–436)
PT width	110 (40.5–181)	–	111 (58–200)		194 (168–217)
PT/PostB	61 (51–69)		67 (48–94)		
Egg length	25 (20–31)	26–33	25 (23–28)	24	26 (24–27)
Egg width	13 (12–15)	11–15	13 (13–13.5)	12	11 (9–12)

Fig. 1. Cryptogonimid phylogenetic tree based on *Timoniella* sp. ITS–28S rDNA sequences.

curved. Ventral sucker same size as acetabulum, almost round, sometimes prolonged laterally, 69–179 × 40–154 (sometimes up to 205–304). Ratio of oral/ventral sucker length is 80–124%.

Prepharynx 40–255 long, usually winding. Pharynx muscular, elongate–oval, 34–151 × 40–139. Oesophagus commonly shorter than prepharynx, 30–85. Intestinal bifurcation in central forebody, anterior to ventral sucker. Caeca terminate blindly close to posterior extremity. Excretory pore terminal, excretory vesicle Y-shaped.

Genital pore located immediately anterior to ventral sucker. Gonotyle small, simple, located anterior to ventral sucker, associated with ventral sucker. Seminal vesicle tubular, posterior to ventral sucker.

Gonads in posterior hindbody. Testes two, oval, tandem, close to posterior extremity of body. Post-testicular distance about 51–94, measured from last one. Testes of similar size with high variability, 40–436 × 39–227. Ovary entire, globular, 80–200 × 64–220, anterior to testes, contiguous with, or up to about 27–137 from, anterior testes. Seminal receptacle obliquely anterior to ovary, sometimes overlapping, thick-walled, oval to circular, 28–102 × 26–65. Follicular vitellarium in two lateral groups, from posterior testis (rarely from middle of anterior testis) to middle of distance between anterior testis and ventral sucker. Eggs small, elongate–oval, with some variability in size, 20–33 × 9–15, yellowish-brown, not embryonated.

Discussion

Based on the results of this study, we propose *T. balthica* as a junior synonym of *T. imbutiforme*, making this the second Boreal–Atlantic cryptogonimid species registered in the Black Sea fauna. The other species, *A. coelomicola*, was originally described from Mediterranean annual gobies, *Pomatoschistus* spp., and another species, *Aphalloides timmi* Reimer, 1970, from the same host in the Baltic Sea (Dollfus *et al.*, 1957; Reimer, 1970). Kvach *et al.* (2017) later considered these two taxa as the same species, *A. coelomicola*. The sister relationship of *Timoniella* and *Aphalloides* was also confirmed by Kvach *et al.* (2017), suggesting that both species probably form part of the Boreal–Atlantic relict fauna of the Black and Baltic seas, dating from the Upper Miocene.

Unlike *A. coelomicola*, whose life cycle is strongly synchronized with that of *Pomatoschistus* spp. (Pampoulie *et al.*, 2000), the *T. imbutiforme* life cycle includes different definitive and secondary intermediate hosts, with *D. labrax* previously described as the main definitive host and *Pomatoschistus* spp. as the main second intermediate host (Maillard, 1973). In the Black and Baltic seas, however, *S. typhle* is the main definitive host (Chernyshenko, 1949; Reimer *et al.*, 1996), although *Pomatoschistus* spp. remains the main secondary intermediate host (Reimer *et al.*, 1996; Zander *et al.*, 1999, 2002; Zander & Reimer, 2002; Kvach, 2005, 2010; Krasnovyd *et al.*, 2012). Small fish such as *Pomatoschistus* spp. are important dietary items of *S. typhle* (Oliveira *et al.*, 2007). Both main definitive hosts (*D. labrax* and *S. typhle*) are widely distributed along European coasts, stretching from the North Sea off Norway to the Mediterranean and Black seas in the south (note that *D. labrax* is absent from the Baltic Sea) (Dawson, 1986; Smith, 1990). While the main definitive hosts have a Boreal–Atlantic distribution, the first intermediate host of the parasite, mud snails of the *Hydrobia* group (Maillard, 1973; Reimer *et al.*, 1996), are part of a group of European lagoon species distributed throughout the Boreal zone (Barnes, 1989).

Our analysis confirms two European species within the genus *Timoniella*: *T. imbutiforme* and *T. praeterita*. Both representatives appear to be Boreal–Atlantic species, with *T. imbutiforme* having
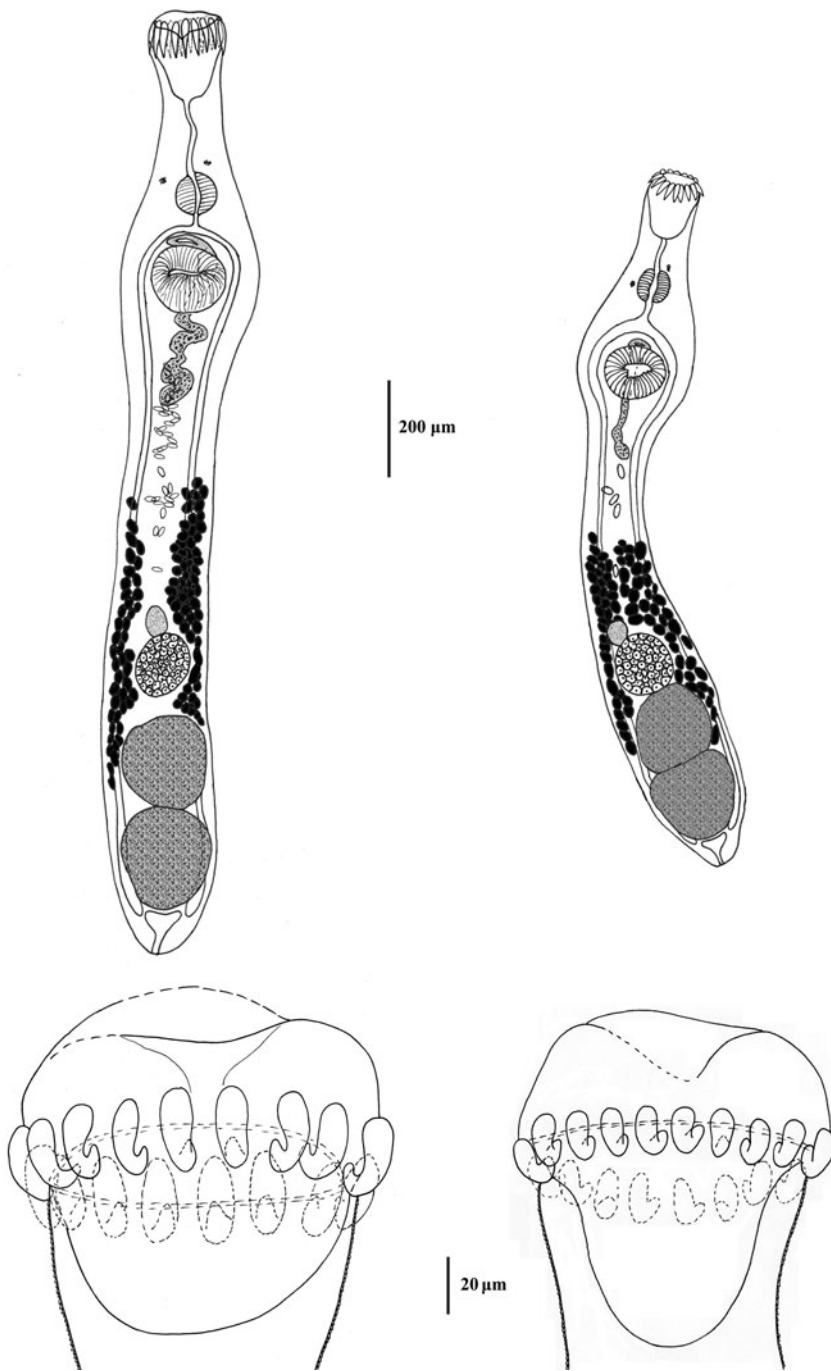
Fig. 2. *Timoniella imbutiforme* ex. *Syngnathus typhle*, overviews (above) and spine crowns (below); (left) from Budaki Lagoon, Ukraine; (right) from Salzhaff.

the wider range. Taking into account the absence of *T. praeterita* in the Black Sea, we suggest that *T. imbutiforme* is of older origin, representing a Boreal relict within the Black Sea fauna. The maritae and metacercariae of these two species can be distinguished by the termination of the intestinal caeca and the oral sucker armature (see Brooks, 1980). We provide (below) a new key for the identification of *Timoniella*.

According to Brooks (1980) the genus was previously considered a representative of the subfamily Acanthostominae. The Acanthostominae, with *Acanthostomum* Looss, 1899 as its type genus, includes both fish and reptile parasites. Based on our own data, *A. burminis*, a parasite of the Asiatic water snake, *Xenochrophis piscator* Schneider, 1799, was found to lie far from the fish parasites (see fig. 1). As such, we consider adult

Key for the identification of *Timoniella* (modified from Brooks, 1980).

1 (2) Intestinal caeca opening into excretory vesicle. Oral sucker armored by 21–23 spines .... *Timoniella praeterita* (Looss, 1901)
2 (1) Intestinal caeca ending blindly near posterior end of body. Oral sucker armored by 17–20 spines .... *Timoniella imbutiforme* (Molin, 1859)

cryptogonimid parasites of fish to constitute a common monophyletic group, while reptile parasites should be considered as a separate group.

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