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Marine parasite in a freshwater wetland: new host and geographical records of *Progrillotia dasyatidis* (Cestoda: Trypanorhyncha) from *Gasterosteus aculeatus* (Actinopterygii: Gasterosteidae) in Bulgaria, with comments on its life-cycle

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Research Paper

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Abstract

Plerocerci of the trypanorhynch cestode *Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004 (Progrillotiidae) were isolated from the gallbladder of the three-spined stickleback *Gasterosteus aculeatus* from a small freshwater marsh in the Lake Atanasovsko Wetlands, a coastal area adjacent to the Black Sea coast, Bulgaria. The parasite was recorded in five out of 134 fish individuals studied (prevalence 3.73%, intensity 1–7, mean intensity 2.40 ± 1.17 and mean abundance 0.09 ± 0.06). A description of the plerocerci is presented, expanding data on intraspecific variation. The present report is the first record of *P. dasyatidis* from *G. aculeatus* (new host record) and from Bulgaria (new geographical record). Recording only plerocerci with evaginated scoleces in the sticklebacks is consistent with the hypothesis that teleosts are paratenic hosts and not intermediate hosts of *P. dasyatidis*.

Introduction

The cestodes of the order Trypanorhyncha Diesing, 1863 are cosmopolitan marine fish parasites, unique in having a rhyncheal apparatus (except the genus *Aporhynchus* Nybelin, 1918), comprising four retractable tentacles emerging from four bulbs, armed with hooks arranged in different patterns (Campbell & Beveridge, 1994; Palm, 2004). At present, 345 species are known (World Register of Marine Species, 2021), with complex life-cycles involving a variety of invertebrates and teleost fishes as intermediate hosts and elasmobranchs as definitive hosts (Palm, 2004).

Progrillotia Dollfus, 1946 is the only genus of the family Progrillotiidae Palm, 2004. It consists of three species parasitic in rays as adults: *Progrillotia pastinacae* Dollfus, 1946 (type species); *Progrillotia louiseuzeti* Dollfus, 1969; and *Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004 (Dollfus, 1946, 1969; Beveridge *et al.*, 2004, 2017; Palm, 2004; Marques *et al.*, 2005). Larvae have been recorded in marine demersal and pelagic teleosts from coastal Atlantic waters as well as from the Black Sea, Marmara Sea and the Persian Gulf (Palm, 2004; Marques *et al.*, 2005; Oguz & Bray, 2008; Al-Niaeem *et al.*, 2014; Polyakova *et al.*, 2014, 2017; Özer & Öztürk, 2017; Çelik & Oğuz, 2021). Another species, *Progrillotia dollfusi* Carvajal & Rego, 1983, originally reported as plerocerci from sciaenid fishes from South America (Carvajal & Rego, 1983; Pereira & Boeger, 2005), has been supposed (Beveridge *et al.*, 2004; Palm, 2004) and proved (Menoret & Ivanov, 2009) as a member of the genus *Grillotia* Guiart, 1927 (Lacistorhynchidae), currently known as *Grillotia* (*Christianella*) *carvajalregorum* Menoret & Ivanov, 2009 (see also Beveridge & Campbell, 2010).

The three-spined stickleback *Gasterosteus aculeatus* L. is a small-sized euryhaline fish widespread in temperate and subarctic shallow coastal and inland waters throughout the Holarctic Region (Wootton, 1976, 1984; Mattern, 2007). It has been recorded as an intermediate, paratenic or definitive host of more than 20 cestode species (Wootton, 1976; Ermolenko, 1992; Hoffman, 1999; Palm *et al.*, 1999; Pugachev, 2002; Barber, 2007; Kirjušina & Vismanis, 2007; Poulin *et al.*, 2011). Data on the occurrence of trypanorhynch cestodes from *G. aculeatus* are scarce (Linton, 1924; Palm, 2004; Pospekhov *et al.*, 2010).

In the course of a survey of helminth parasites of fishes from the Lake Atanasovsko Wetlands, Bulgaria (Stoyanov *et al.*, 2015, 2016, 2017a, b, 2018), the trypanorhynch cestode *P. dasyatidis* has been found as a parasite of the three-spined stickleback. In this article, we present the first record of *P. dasyatidis* in *G. aculeatus* and provide data on the morphology of its plerocerci as well as comments on its life-cycle.

Table 1. Metrical data of *Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004 (plerocerci) from different fish species and localities in Europe. For abbreviations, see Materials and Methods section.

Host	Various pleuronectiform and batrachoidiform fishes			*Gasterosteus aculeatus* L.		
Locality	Atlantic Ocean off Portugal			Lake Atanasovsko Wetlands (Bulgaria)		
Source	Marques *et al.* (2005)			Present study		
Characters	Range	Mean ± standard deviation (SD)	*n*	Range	Mean ± SD	*n*
BTL	1462–4699	3047 ± 1026	17	1750–2530	2204	8
SL	504–1378	840 ± 196.0	16	625–915	763	8
PBOL	149–297	213 ± 43.0	18	190–320	230	8
PBOW	86–182	134	18	250–580	348	8
PVL	112–351	165 ± 65.0	12	350–440	371	7
PVW	–	–	–	198–305	233	7
PBL	–	–	–	317–373	343	7
PBW	–	–	–	195–351	253	7
BUL	282–622	392 ± 80.0	20	278–380	332 ± 24.7	30
BUW	43–69	58 ± 6.0	18	45–80	61 ± 8.9	30
PPBL	–	–	–	50–108	78	7
PPBW	–	–	–	175–345	239	7
BLL	–	–	–	1085–1905	1441	8
BLMW	–	–	–	220–520	306	8
HPRL 1(1′)	10–14	12	–	10–16	14	8
HPRB 1(1′)	–	–	–	9–14	12	8
HPRL 2(2′)	10–13	12	–	12–19	15	9
HPRB 2(2′)	–	–	–	5–8	6	9
HPRL 3(3′)	16–18	14	–	11–18	15	12
HPRB 3(3′)	–	–	–	4–6	5	12
HPRL 4(4′)	12–17	14	–	10–17	15	12
HPRB 4(4′)	–	–	–	4–7	5	12
HPRL 5(5′)	2–6	4	–	5–14	12	11
HPRB 5(5′)	–	–	–	4–6	5	11
HPRL 6(6′)	3–5	4	–	6–10	7	9
HPRB 6(6′)	–	–	–	3–6	5	9
HIRL	–	–	–	4–7	5 ± 0.7	39
HIRB	–	–	–	2–4	3 ± 0.4	39
Ratio:						
BBL/BBW	5–9	7 ± 2.0	18	4–7	6 ± 0.6	30

Materials and methods

The present study was carried out in the northern part of the Lake Atanasovsko Wetlands, declared as a Ramsar site (in 1984) and a managed nature reserve (in 1999). These wetlands represent a complex of shallow coastal habitats highly variable in their salinity and hydrological regime, that is, a hyperhaline lagoon partially used in salt production, a system of brackish and freshwater canals and ponds as well as freshwater marshes surrounding the lagoon (Ivanov *et al.*, 1964; Vassilev *et al.*, 2013). A total of 134 individuals of the three-spined stickleback *G. aculeatus* (42 individuals from brackish water and 92 individuals from freshwater) were examined for helminth parasites in May, July and September 2012–2013. These were 33 mature individuals with total length 5.5–8.1 cm (av. ± standard deviation (SD) 6.91 ± 0.67 cm) and 101 immature individuals with total length 1.2–3.6 cm (av. ± SD 2.44 ± 0.59 cm). No fish of this species were caught in September 2012. The fish was caught by seines and traps. The five fish specimens infected with *P. dasyatidis* were sampled on 12–13 May 2013 from a freshwater pond (42°34′45″N, 27°28′29″E) adjacent to the canal connecting the sea and salt ponds.

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Fish specimens were kept alive in containers with aerated water taken from the habitat. In the laboratory, each fish was dissected under a stereomicroscope within the next 24 h. The recovered 12 cestode specimens were fixed in hot saline and transferred to 70% ethanol. Subsequently, six of them were stained in iron acetocarmine (Georgiev *et al.*, 1986), dehydrated in an ascending ethanol series, cleared in dimethyl phthalate and mounted in Canada balsam. In addition, for better visualization of the tentacular armature, four specimens were mounted in Berlese's medium (Swan, 1936). Two specimens were unsuccessfully used for DNA extraction.

The specimens (four slides in Canada balsam and four slides in Berlese's medium) are preserved in the Helminthological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Accession Nos. IBER-BAS C0161.3.1–8. Two slides of specimens mounted in Canada balsam are in the collection of Professor Harry Palm, University of Rostock, Germany.

The measurements taken and the related terminology are as proposed by Palm (2004). The characters measured were: BLL, blastocyst length; BLMW, blastocyst maximum width; BUL, bulb length; BUW, bulb width; BTL, body total length; HIRB, hooks of intercalary rows base length; HIRL, hooks of intercalary rows total length; HPRB, hooks of principal rows base length; HPRL, hooks of principal rows total length; PBOL, pars bothrialis length; PBOW, pars bothrialis width; PPBL, pars postbulbosa length; PPBW, pars postbulbosa width; PVL, pars vaginalis length; PVW, pars vaginalis width; and SL, scolex length. The metrical data are presented as the range, followed by the mean and the number of measurements taken (*n*) (table 1). The standard deviation is given only when $n \geq 30$. All measurements are presented in micrometres.

The infection parameters prevalence (%), intensity (range, mean ± standard error) and abundance (mean ± standard error) follow the definitions by Bush *et al.* (1997).

Results

Progrillotiidae Palm, 2004.

Progrillotia dasyatidis Beveridge, Neifar & Euzet, 2004.

Site of infection: Gallbladder.

Infection characteristics: prevalence 3.73%; intensity 1–7; mean intensity 2.40 ± 1.17; and mean abundance (0.09 ± 0.06).

Description (based on 8 plerocerci with evaginated scolex; figs 1 and 2; for metrical data, see table 1): scolex acraspedote, with maximum width at pars bothrialis, with broadly-rounded anterior margin (fig. 1). Pars bothrialis short, provided with two large, oval bothria, with free non-thickened margins and without posterior notch, opposite one another, one dorsal and one ventral (fig. 1b). Pars vaginalis longer than pars bothrialis (table 1), comprising sinuous tentacular sheaths (fig. 1b). Prebulbar organ present, prominent, at level of tentacle–bulbar junction (fig. 2d). Pars bulbosa containing four bulbs; bulb muscular, thick-walled, sausage-like, comprising retractor muscle originating at basis of bulb, surrounded by aggregations of glandular cells (fig. 2d). Pars postbulbosa very short, lacking in one specimen. Tentacles elongate, protruding beyond anterior margin of scolex (fig. 1); provided with heteroacanthous atypical armature of solid hooks. Metabasal armature consisting of repetitive principal half-rows of six hooks extending from internal to external surface and a single intercalary half-row consisting of tiny hooks, three hooks on antibothridial surface

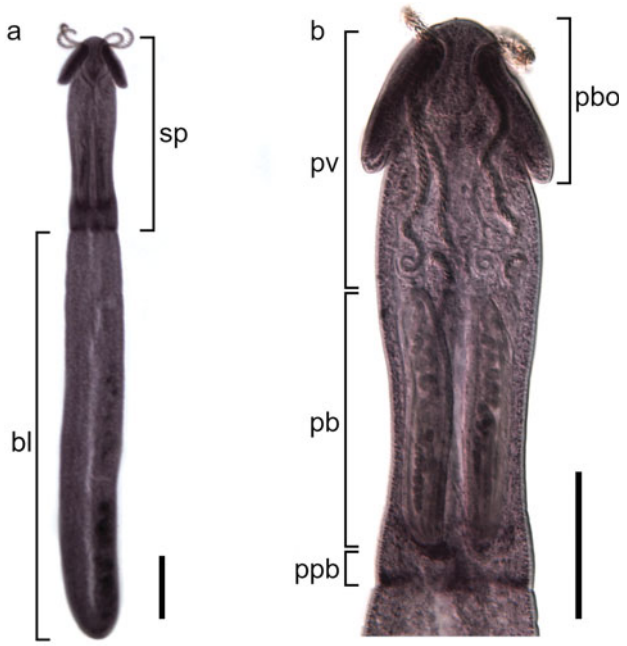

Fig. 1. *Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004, plerocerci from *Gasterosteus aculeatus*, Bulgaria: (a) general view (sp, scolex peduncle; bl, blastocyst); and (b) scolex (pbo, pars bothrialis; pv, pars vaginalis; pb, pars bulbosa; ppb, pars postbulbosa). Scale-bars: (a) 250 μm; (b) 200 μm.

(fig. 2a) and five to seven hooks arranged in arc on external surface of tentacle (fig. 2b). Hooks 1(1′) of principal rows uncinate, with anterior extension of base. Hooks 2(2′) uncinate but with shorter base and more elongate blade than hooks 1(1′). Remaining four hooks falciform (fig. 2c). Hooks of intercalary rows uncinate, with relatively long base; blade fine, slightly curved (fig. 2c). Blastocyst strongly elongated, with maximum width approximately at mid-length; posterior margin broadly-rounded (fig. 1a).

Discussion

The morphology of the present larvae is compatible with the generic characteristics of *Progrillotia* (Campbell & Beveridge, 1994; Beveridge *et al.*, 2004; Palm, 2004). The position of tentacles in the available specimens does not allow observations of the internal surface, thus the characteristic empty field between hooks 1(1′) of the principal rows has not been observed in the present study. The plerocerci from the Lake Atanasovsko Wetlands are identified as *P. dasyatidis* based on the armature comprising a single intercalary half-row of tiny hooks between principal rows. Each half-row consists of three hooks on the antibothridial surface and five to seven hooks on the external surface of the tentacle (fig. 2a, b). The presence of single intercalary rows is the main character distinguishing *P. dasyatidis* from the other two congeners possessing two intercalary rows between principal rows (Beveridge *et al.*, 2004; Palm, 2004; Marques *et al.*, 2005). This species identification is also supported by the presence of overlapping ranges of the main metrical characters with those reported by Marques *et al.* (2005) for plerocerci of *P. dasyatidis* from Atlantic waters (table 1). The only exceptions are the wider pars bothrialis, the longer pars vaginalis and the longer hooks 5(5′) and 6(6′) of the principal rows than those in the

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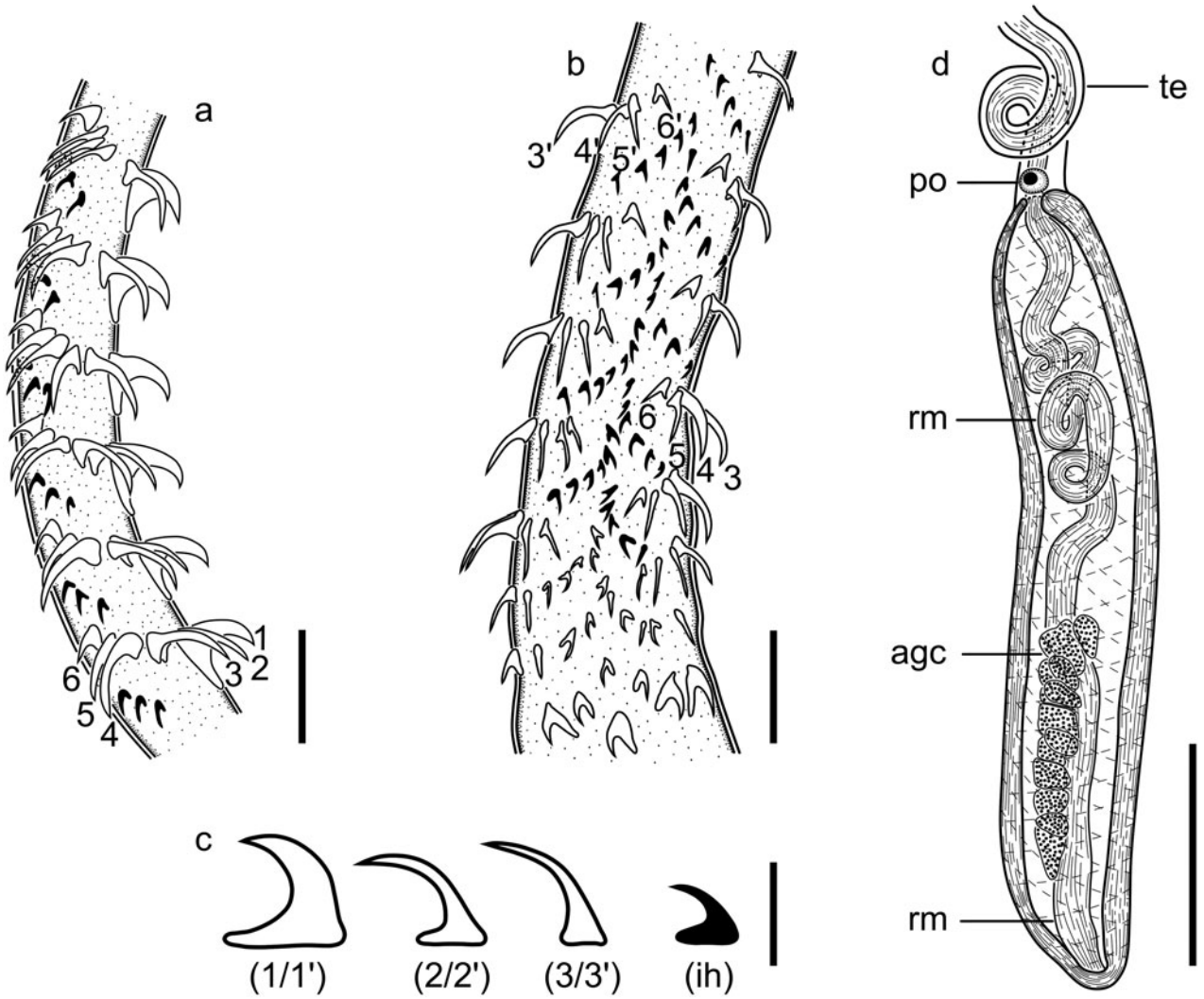

Fig. 2. *Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004, plerocerci from *Gasterosteus aculeatus*, Bulgaria: (a) metabasal armature on antibothridial surface of tentacle; (b) basal and metabasal armature on external surface of tentacle; (c) types of hooks: principal rows (1/1′, uncinate hooks; 2/2′, uncinate but with shorter base hooks; 3/3′ and remaining hooks falciform); intercalary rows (ih, intercalary hook, uncinate); and (d) bulb (agc, aggregation of glandular cells; po, prebulbar organ; rm, retractor muscle; te, tentacle). Scale-bars: (a, b) 20 μm; (c) 10 μm; (d) 100 μm.

previous description of plerocerci (table 1). However, these metrical differences are minor and can be considered as intraspecific variations. In addition, Marques *et al.* (2005) reported maximum width of the scolex at the pars bulbosa vs. maximum width at the pars bothrialis in the present description. It could be attributed to differences in the condition of the specimens studied. The present specimens were recovered from fish hosts kept alive prior to examination and tapeworms were fixed after being relaxed in saline while Marques *et al.* (2005) studied plerocerci sampled from commercially-captured fish.

The plerocerci of *P. dasyatidis* were found in the lumen of the gallbladder of large-sized mature fish individuals from a small freshwater marsh. Most probably, these are anadromous sticklebacks entering coastal wetlands from the nearby sea where definitive hosts (rays) occur. The anadromous mature sticklebacks undertake breeding migrations (March–July) to inland waters (Wootton, 1976, 1984). No cestode infection has been found in any of the small-sized immature fish individuals examined in the same wetlands in the course of the present study.

Progrillotia dasyatidis was originally described from the spiral valve of the Tortonese's stingray *Dasyatis tortonesei* Capapé (Dasyatidae) off the Mediterranean coast of Tunisia (type-host and type-locality) and from *Dasyatis pastinaca* (L.) near Arcachon on the Atlantic coast of France (Beveridge *et al.*, 2004). Marques *et al.* (2005) recorded plerocerci of this species in several pleuronectiform and batrachoidiform hosts off the Atlantic coast of Portugal. As with the vast majority of trypanorhynchs, however, the life-cycles of *Progrillotia* spp. are still insufficiently studied (Palm, 2004; Marques *et al.*, 2005). Due to the close phylogenetic relationships of *Progrillotia* and the genera of the family Eutetrarhynchidae, it is assumed that they have similar three-host life-cycles (Palm, 2004; Marques *et al.*, 2005). Moreover, *Progrillotia* spp. and eutetrarhynchids of the genus *Oncomegas* Dollfus, 1929 develop plerocerci with evaginated scolex in teleost hosts (Palm, 2004; Marques *et al.*, 2005). It is believed that their life-cycle includes copepods as first intermediate hosts, higher crustaceans and other marine invertebrates as second intermediate hosts and elasmobranchs as definitive hosts (Palm, 2004). The teleosts have been suggested to be either

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paratenic hosts or obligatory further intermediate hosts in their life-cycles (Marques *et al.*, 2005). Polyakova *et al.* (2014) considered that the life-cycle of *P. dasyatidis* is successfully completed in higher crustaceans as second intermediate hosts while teleosts are paratenic hosts only. In our study, except plerocerci with evaginated scoleces, no other developmental stages have been observed. This is in agreement with the suggestion that teleosts are paratenic hosts for *P. dasyatidis*, consistent with the developmental model considering teleosts as paratenic hosts (Polyakova *et al.*, 2014). As juveniles, the three-spined sticklebacks feed basically on copepods but, as they grow, the proportion of copepods decreases and the share of higher crustaceans, molluscs and other invertebrates increases in their diet (Wootton, 1976). These invertebrates are believed to be second intermediate hosts of eutetrarhynchids and related groups (Palm, 2004). A similar infection pattern for *P. dasyatidis* has also been observed in the red mullet *Mullus barbatus* L. (Mullidae) off the Anatolian Black Sea coast, which also shift their diet from copepods to bigger invertebrates when growing (Öztürk & Yesil, 2018).

In the Black Sea basin, two congeners, *P. louiseuzeti* and *P. dasyatidis*, have been recorded as adults and plerocerci (Naydenova & Solonchenko, 1989; Polyakova, 2009, 2020; Popjuk, 2009; Gaevskaya, 2012; Polyakova *et al.*, 2014, 2017; Tepe *et al.*, 2014; Kvach & Drobiniak, 2017; Özer & Öztürk, 2017; Çelik & Oğuz, 2021). In addition, Polyakova & Biserova (2016) recorded adults of *Progrillotia* sp. from the thornback ray *Raja clavata* L. (Rajidae) and plerocerci in the gallbladder of the knout goby, *Mesogobius batrachocephalus* (Pallas) (Gobiidae), as a possible fourth representative (not described) of this genus. In the Black Sea basin, *P. dasyatidis* has been recorded from the rays *D. pastinaca* and *R. clavata* (Polyakova *et al.*, 2017) as well as from several demersal and pelagic teleost fishes (Polyakova *et al.*, 2014; Tepe *et al.*, 2014; Kornyychuk *et al.*, 2016a, b, 2022; Öztürk & Yesil, 2018; Polyakova, 2020; Çelik & Oğuz, 2021). Oguz & Bray (2008) recorded *P. dasyatidis* (plerocerci) from the gallbladder of the black goby, *Gobius niger* L. (Gobiidae), from the adjacent Sea of Marmara, Turkey. Until now, no records of *Progrillotia* spp. from Bulgarian waters have been published. In addition, there were no records of this genus from *G. aculeatus*.

Only two trypanorhynch species, that is, *Lacistorhynchus tenuis* (van Beneden, 1858) (syn. *Rhynchobothrium bulbifer* Linton, 1889) (plerocerci) (Lacistorhynchidae) from the North Atlantic Ocean off Woods Hole, Massachusetts (USA) (Linton, 1924; Palm, 2004) and *Nybelinia surmenicola* Okada in Dollfus, 1929 (plerocerci) (Tentaculariidae) from the Gizhiga River, Magadan Oblast, Russia (Pospekhov *et al.*, 2010) have been recorded as parasites of *G. aculeatus*. Therefore, the present study expands the knowledge about the geographical distribution and the host range of the fish parasites of the genus *Progrillotia*.

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Conflicts of interest. None.

Ethical standards. The Scientific Council of the Institute of Biodiversity and Ecosystem Research–Bulgarian Academy of Sciences (IBER-BAS), acting as an ethics board, reviewed and approved the ethical standards applied in this study (Decision 2012/02/14/6).

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