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ORIGINAL RESEARCH ARTICLE

Host and geographical distribution of *Pomphorhynchus spindletrancatus* (Acanthocephala: Pomphorhynchidae) in Turkey, with enhanced description from new fish and amphibian hosts using SEM, and histopathological notes

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Abstract. This is the first report of *Pomphorhynchus spindletruncatus* Amin, Abdullah and Mhaisen, 2003 from new fish and amphibian hosts in Turkey since its original description from 2 freshwater fish species, *Aspius vorax* and *Barbus xanthopterus* (Cyprinidae) in the Greater and Lesser Zab rivers of Northern Iraq in 2003. The Turkish fish hosts included 6 species: the Kura bleak *Alburnus filippii*, the Italian barbell *Barbus plebejus*, the Transcaucasian barb *Capoeta capoeta*, the Anatolian khramulya *Capoeta tinca*, the Crusian carp *Carassius* sp., and the European chub *Leuciscus cephalus* collected from 3 locations in the Aras River, the Karasu River, and Tortum Lake. The intestine of 1 amphibian species, the marsh frog *Pelophylax ridibundus* from Işikli Lake was rather heavily infected with only juveniles. Worms from all 6 fish species were immature adults, with a partial exception in *Carassius* sp. from Tortum Lake where 3 gravid females were found. Measurements for worms from all fish and amphibian hosts were compared with those in the original description. Juveniles from frogs were the smallest. Immature specimens from *B. plebejus* were the largest and compared well with mature worms in the original description. Immature worms from other fish species were intermediate in size. Posterior hooks were longest in juveniles from frogs, and a new complete ring of larger hooks at the posterior end of the proboscis is reported for the first time. SEM pictures also revealed new features including sensory pits in the neck, epidermal micro-pores only in the trunk, and sensory papillae in the bursa. The proboscis proved to be longitudinally ribbed and with bald anterior tip.

Keywords: Acanthocephala; *Pomphorhynchus spindletruncatus*; Fish; Amphibians; Turkey; SEM; Pathology.

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Introduction

This is the first report on *Pomphorhynchus spindletruncatus* Amin, Abdullah and Mhaisen, 2003 since its original description from 2 fish species in the Greater and Lesser Zab rivers in Northern Iraq in 2003. Routine parasitological surveys in Turkey revealed the presence of this acanthocephalan in 6 additional species of fish and one new amphibian host from 4 different waterways in Turkey. This report documents the expanded geographical and host distribution of *P. spindletruncatus* in Turkey, well beyond its originally reported presence in 2 connected streams in Northern Iraq.

Materials and methods

Six species of fish and 1 amphibian species were examined for parasites between April 2008 and July 2009 in 4 Turkish waterways (Table 1) at collecting sites in Tortum Lake (40°35'43''N 41°36'59'E'), the Aras River (39°57'40''N 41°51'26''E), the Karasu River (39°55'22''N 40°39'52''E), and Işikli Lake (38°19'19''N 29°51'12''E).

The altitude of Tortum lake is 1010m. It is 11 km long, 700 to 1000m wide, and about 110 m deep (Altuner, 1982). The Aras River rises from Bingöl province at an altitude of 2990m and drains into Caspian Lake in Azerbaijan. It is 1072 km long. The general surface area of the basin is 101900 km^2 (Güney, 2004). The Karasu River is a northern branch of Firat (Euphrates) River. It is 971 km long. It rises from the Kargapazari mountains which is located north east of the Erzurum Province (Güney 2004). Işıklı Lake is a freshwater lake in the Inner West Anatolia Region (the eastern part of Aegean Region) Turkey, extending on the Çivril Plain between the provinces of Denizli and Afyonkarahisar. The altitude of Işıklı lake is 825 m and it covers an area is approximately 73 km^2 (Kir and Ozan, 2005).

Fish were placed in plastic containers containing fresh water and then transferred to the laboratory of the Biology Department of Ataturk University Erzurum. They were kept in tanks until examined within 24 hours of collection. Frogs were caught, brought to, and examined at the Biology Department of Pamukkale University, Denizli.

Methods used for necropsy, and later for the analysis, were adapted from Pritchard and Kruse (1982). Parasites were fixed with AFA then carried through ethanol 50% then 35%, then distilled water, and stained in Mayer's carmine. They were then placed in distilled water, then in increasing concentrations of ethanol till 100%, cleared in xylene, and mounted in Canada balsam.

For histological examination, infected tissues, from both *B. plebejus* and *P. ridibundus*, received in 70% ethyl alcohol were transferred to 10% buffered formalin. The host intestinal tissue was dehydrated and blocked in paraffin using standard methods (Bancroft and Gamble, 2001) and then sectioned at 4-6 micrometers. Sections were placed on glass slides and stained with hemotoxylin and eosin (HE) and Mallory's triple. Slides were viewed with LSM laser (Carl Zeiss, Thornwood, New York) equipped compound light microscope and representative pictures were taken at varying magnifications with a digital camera. For comparison, host tissue without the helminth was also sectioned and viewed. HE is a common stain for tissue and Mallory's richrome stain is used to differentiate granular tissue typical of parasite infections.

Data for the prevalence, intensity and abundance were used as per Bush et al. (1997). The specimens are deposited in the Biology Department, Faculty of Science, Ataturk University.

Results and discussion

Seventeen of 130 examined fish and 25 of 34 examined frogs were found infected with *P. spindletruncatus* with a total of 210 parasites, 48 from fish, 162 from frogs (table 1).

All specimens were found in the intestinal tract of their respective hosts with the proboscis deeply embedded in the intestinal wall which often bulged into the body cavity at the site of infection, especially in frogs (figure 1).

Table 1. Infection parameters of specimens of *P. spindletruncatus* collected from fish and frogs in Turkey

Locality	Host	Hosts examined	Hosts infected	Parasites collected	Prevalence (%)	Mean intensity
Tortum Lake	*Carassius* sp.	1	1	20	100	20.0
Tortum Lake	*Capoeta tinca* (Heckel, 1843)	1	1	4	100	4.0
Aras River	*Capoeta capoeta* (Berg, 1914)	102	6	9	6	1.5
Aras River	*Barbus plebejus* Bonaparte, 1839	13	2	3	15	1.5
Aras River	*Leuciscus cephalus* (Linnaeus, 1758)	5	3	6	60	2.0
Aras River	*Alburnus filippii* Kessler, 1877	2	1	2	50	2.0
Karasu River	*Barbus plebejus*	6	3	4	50	1.3
Işikli Lake	*Pelophylax ridibundus* (Pallas, 1771)	34	25	162	74	6.5

The parasites

Comparative measurements (table 2) and SEM pictures (figures 2-13) of the new material from fish and frogs contributed new information enhancing the original description of *P. spindletruncatus* by Amin et al. (2003). This information included: (1) the ribbed appearance of the proboscis (figure 2) and its bald unarmed anterior tip (figure 3); (2) the presence of a complete ring (corona) of hooks at the posterior-most end of the proboscis at its juncture with the bulb and appearing as though it emerges from it (figure 6); (3) a pair of lateral sensory pits on the neck (figure 8); (4) the presence of micro-pores on the tegument of the trunk that decrease in density from posterior (figure 9) to anterior (figure 10) and their absence from the neck, with a clear cut transitional area (figure 11); (5) the presence of sensory papillae on the inner surface of the bursa (figure 13). Some of these new features are of significant taxonomic importance especially the ring of hooks at the posterior end of the proboscis which further distinguishes *P. spindletruncatus* from all other species of the genus. These hooks are somewhat larger than the next posterior hooks and a distance away from them.

Worms from the reported collections were more elongate than the extremely wider spindle shaped worms reported in the original description. That statement also applies to the only 3 gravid females collected from *Carassius* sp. in Tortum Lake (not included in table 2) (trunk: 13.12-16.75 x 3.6-4.12 mm; proboscis: 520-750 x 275-333 µm; bulb: 1.67-2.32 x 1.60-2.97 mm; neck: 2.55-3.15 x 0.87-1.02 mm; egg: 70-75 x 11-15 µm). These measurements are similar to those of the largest gravid females in the original description, except for their more moderate width. All other worms collected in Turkish localities were either small juveniles with incompletely developed reproductive system from frogs or the relatively larger immature adults from fish hosts (table 2). The largest specimens from fish and the closest to those described from Iraq (Amin et al., 2003) were those collected from *B. plebejus* in the Karasu River (males: 8.04-8.93 X 1.87-2.03 mm, females: 6.66-6.80 X 2.07-2.21 mm). We assume that this difference is more due to locality associations rather than to host species because worms from the same host from the Aras River were smaller. The Karasu River was and remains connected to the Zab River from which the species was originally described in Iraq. The lemnisci were somewhat shorter in the new collections from all hosts compared to those reported earlier by Amin et al. (2003) but the posterior hooks were longest in the smallest worms from frogs. Juvenile worms from frogs had the smallest reproductive system organs.

Some specimens had as many as 10 proboscis hooks per row, especially in frogs. The original range was reported to be 7-9 hooks per row (Amin et al., 2003).

Geographical distribution

All 3 fish collection sites, the Aras and Karasu rivers and Tortum Lake are located in Eastern Anatolia while Işıklı Lake from which frogs were collected is located in Western Anatolia.

Table 2. Morphological data for *P. spindletruncatus* collected in Turkish localities compared to the original description

Parameter	Fish - Amin et al. 2003		Fish - present study		Frog - present study	
	13 ♂♂	**20 ♀♀**	**9 ♂♂**	**12 ♀♀**	**15 ♂♂**	**21 ♀♀**
TL	7.75 (5.70-9.35)	9.30 (6.25-13.50)	6.43±2.37 (2.74-9.66)	5.73±2.21 (1.26-11.98)	4.20±0.74 (2.66-5.39)	4.29±0.85 (2.82-6.21)
TW	2.57 (1.87-3.27)	3.15 (2.20-4.50)	1.72±0.48 (1.04-2.56)	1.66±0.46 (0.85-2.33)	1.22±0.19 (0.91-1.60)	1.21±0.20 (0.92-1.60)
PL	0.53 (0.38-0.62)	0.56 (0.41-0.72)	0.59±0.13 (0.26-0.75)	0.56±0.15 (0.24-0.81)	0.56±0.11 (0.36-0.75)	0.60±0.12 (0.41-0.81)
PW	0.29 (0.26-0.37)	0.31 (0.22-0.36)	0.28±0.08 (0.08-0.39)	0.30±0.07 (0.18-0.43)	0.34±0.07 (0.16-0.41)	0.36±0.09 (0.20-0.487)
PRL	2.64 (1.72-3.37)	2.60 (1.82-3.47)	2.53±0.63 (1.77-3.80)	2.34±0.74 (1.42-4.59)	2.86±0.57 (2.05-3.57)	2.61±0.49 (1.93-3.86)
PRW	0.19 (0.10-0.32)	0.19 (0.12-0.32)	0.21±0.06 (0.14-0.30)	0.15±0.06 (0.06-0.30)	0.20±0.09 (0.08-0.34)	0.18±0.07 (0.08-0.32)
BLBL	1.11 (0.75-1.50)	1.04 (0.75-1.50)	1.01±0.35 (0.53-1.73)	1.00±0.33 (0.53-1.61)	0.98±0.28 (0.49-1.48)	0.87±0.24 (0.39-1.44)
BLBW	1.07 (0.65-1.55)	1.19 (0.85-1.75)	1.07±0.36 (0.57-1.71)	1.15±0.369 (0.61-2.03)	1.11±0.504 (0.49-1.75)	0.96±0.303 (0.59-1.58)
NL	2.76 (2.12-3.30)	2.94 (2.12-3.75)	2.15±0.82 (0.71-3.65)	2.34±1.67 (0.69-7.51)	1.78±0.55 (0.49-2.46)	1.79±0.33 (1.12-2.23)
NW1	0.30 (0.42-0.17)	0.26 (0.17-0.35)	0.34±0.10 (0.20-0.51)	0.41±0.11 (0.24-0.57)	0.38±0.13 (0.18-0.61)	0.39±0.12 (0.16-0.63)
NW2	0.67 (0.55-0.75)	0.75 (0.62-0.90)	0.56±0.23 (0.36-1.20)	0.711±0.39 (0.39-1.52)	0.501±0.10 (0.32-0.65)	0.58±0.12 (0.43-0.79)
LL1	1.66 (1.12-2.5)	1.87 (1.15-2.6)	1.18±0.33 (0.53-1.70)	1.01±0.41 (0.59-2.17)	1.29±0.20 (0.95-1.68)	1.33±0.23 (0.95-1.56)
LW1	0.31 (0.12-0.60)	0.29 (0.20-0.50)	0.25±0.13 (0.08-0.53)	0.30±0.12 (0.14-0.61)	0.28±0.07 (0.14-0.41)	0.29±0.09 (0.14-0.43)
LL2	1.42 (0.99-1.95)	1.67 (0.97-2.62)	1.12±0.46 (0.43-1.66)	0.82±0.41 (0.36-1.97)	1.13±0.25 (0.75-1.66)	1.33±0.21 (0.97-1.70)
LW2	0.31 (0.15-0.67)	0.32 (0.12-0.50)	0.25±0.11 (0.10-0.43)	0.23±0.08 (0.12-0.39)	0.31±0.08 (0.18-0.43)	0.31±0.06 (0.24-0.43)
H1	41 (40-50)	45 (40-50)	44±8 (33-56)	46±14 (28-81)	41±9 (31-57)	40±9 (26-59)
H2	47 (40-50)	53 (40-60)	55±9 (45-71)	55±14 (37-81)	48±13 (28-67)	50±10 (28-71)
H3	42 (30-50)	42 (30-40)	52±11 (39-71)	54±14 (32-81)	49±7 (41-61)	51±10 (35-69)
H4	32 (30-40)	34 (20-40)	48±13 (31-69)	48±12 (28-69)	45±7 (31-55)	48±7 (31-57)
H5	30 (20-30)	30 (20-30)	42±11 (22-59)	41±9 (26-59)	44±8 (31-53)	45±7 (31-53)
H6	30 (20-30)	30 (30-40)	34±8 (24-51)	35±6 (26-47)	40±6 (28-49)	39±6 (28-49)
H7	30 (20-40)	29 (20-30)	32±6 (22-39)	33±6 (22-45)	41±6 (32-51)	41±5 (33-49)
H8	30 (29-37)	30 (25-32)	35±8 (24-51)	35±7 (22-53)	44±5 (32-51)	43±7 (31-55)
H9	30 (30-30)	-	35±8 (20-49)	36±7 (20-51)	49±7 (37-63)	49±8 (35-61)
ATL	1.04 (0.67-1.42)	-	0.84±0.42 (0.36-2.01)	-	0.41±0.07 (0.32-0.53)	-
ATW	0.63 (0.30-1.10)	-	0.55±0.28 (0.16-1.40)	-	0.25±0.05 (0.18-0.36)	-
PTL	(0.62-1.50)	-	0.79±0.38 (0.34-1.64)	-	0.43±0.08 (0.34-0.61)	-
PTW	0.60 (0.35-1.17)	-	0.54±0.32 (0.16-1.46)	-	0.24±0.06 (0.12-0.34)	-
BL	-	-	0.63	-	-	-
BW	-	-	0.87	-	-	-
CgL	0.5768 (0.37-0.87)	-	0.49±0.28 (0.18-1.06)	-	0.18±0.04 (0.12-0.24)	-
CgW	0.35 (0.22-0.77)	-	0.24±0.18 (0.12-0.85)	-	0.09±0.02 (0.06-0.12)	-
SL	1.27 (1.05-1.50)	-	0.70±0.29 (0.22-1.06)	-	0.45±0.14 (0.30-0.71)	-
SW	0.26 (0.17-0.37)	-	0.27±0.08 (0.08-0.36)	-	0.21±0.08 (0.12-0.36)	-
EL	79 (70-90)	-	-	-	-	-
EW	14 (11-17)	-	-	-	-	-

ATL: anterior testis length. ATW: anterior testis width. BL: bursa copulatrix length. BW: bursa copulatrix width. BLBL: bulb length. BLBW: bulb width. CgL: cement gland length. CgW: cement gland width. EL: Egg length. EW: Egg width. H1, H2, H3, H4, H5, H6, H7, H8, H9: hook length from anterior to posterior. LL1: long lemniscus length. LW1: long lemniscus width. LL2: short lemniscus length. LW2: short lemniscus width. NL: neck length. NW1: neck width at middle. NW2: neck width at base. PRL: proboscis receptacle length. PRW: proboscis receptacle width. PL: proboscis length. PW: proboscis width. PTL: posterior testis length. PTW: posterior testis width. SL: Saefftigen pouch length. SW: Saefftigen pouch width. TL: trunk length. TW: trunk width.
* All worms from fish were immature adults except for 3 large gravid females (not included in table) from *Carassius* sp.

During glacial Pleistocene times (0.5-3 million years ago), both regions of Anatolia were connected with a large freshwater lake representing a bridge between European waterways into Western Anatolia and Eastern Anatolia into South Syria and Philistine (including the Zab River system from which *P. spindletruncatus* was originally described). That large lake was apparently the water source for rivers flowing into the Agean Sea, Sea of Marmara, and the Black Sea. During those times, the rivers making the basin of the Euphrates, Karasu and Tigris systems flew into the freshwater lake in Western Anatolia (Demirsoy, 1996). The glacial map of Turkish and Persian Gulf waterways may account for the present day distribution of *P. spindletruncatus*. Of these geological water connections, one remains today. The northern branch of the Euphrates, the Karasu River, from which infected *B. plebejus* were found is connected to the Zab River which originates in southeast Anatolia where it is connected to the Tigris which, in turn, is connected to the Euphrates in Shutt El Arab in Southern Iraq.

It is interesting to note that worms from *B. plebejus* found in the Karasu River were closest to those from *Barbus xanthopterus* (and *Aspius vorax*) from the Zab River which remains connected to the Karasu River to date. Other Turkish waterways reported herein have been isolated from each other and from the Zab River probably since glacial times. It will not be surprising to discover further extensions of the geographical range of *P. spindletruncatus* in fishes west and north of the large glacial freshwater lake in Western Anatolia at least within the range of the associated waterways connecting Europe with Asia Minor.

Many families and genera of fish in the Tigris-Euphrates basin are also found farther north in the Black-Caspian sea and neighboring Iranian basins, e.g., the genera *Alburnoides,* Jeitteles, 1861, *Alburnus*, Rafinesque, 1820, *Aspius,* Agassiz, 1832, *Chondrostoma,* Agassiz, 1832, and *Squalius,* Bonaparte, 1837, suggesting that the Tigris-Euphrates basin fishes were enriched from the north (Coad, 2010).

Fish host associations

Immature adult females of *P. spindletruncatus* with unripe eggs or ovarian balls, as well as males, mostly with sperm, were found in all fish species examined. One specimen of *Carassius* sp. included 3 large gravid females. The parasite appears to develop and cycle in all 6 species of fish that are presently distributed in Eurasian waterways. The transcaucasian barb, *C. capoeta*, is widely distributed from Afghanistan to Aegean coastal in Turkey and reaches 70 cm in length (Elp and Sen, 2009). Of the 11 subspecies known throughout its range, 4: *C. c. capoeta C. c. kosswigi*, *C. c. sieboldi*, and *C. c. umbla* are known in our research locality in the Aras River (Kuru, 1975).

Capoeta capoeta may also serve as a post-cyclic host for *P. spindletruncatus* when fed upon by predatory fishes like the brown trout, *Salmo trutta* Linnaeus, (see Kara and Alp, 2005) and the transcaspian marinka, *Schizothorax pelzami* Kessler (see Aliev et al., 1988), none of which was examined in our present study. Similar patterns have been demonstrated elsewhere, e.g., rainbow smelt, *Osmerus mordax* (Mitchill, 1814) is a post-cyclic host for *Echinorhynchus salmonis* Müller, 1784 when fed upon by large salmonid fishes in Lake Michigan, USA (Amin and Burrows, 1977).

Role reversal may also occur if omnivorous *Capoeta* spp. feed on smaller fish infected with *P. spindletruncatus*. Some fish host categories are known to change with certain fish species assuming one role in one habitat and another role in a different habitat (Amin, 1987).

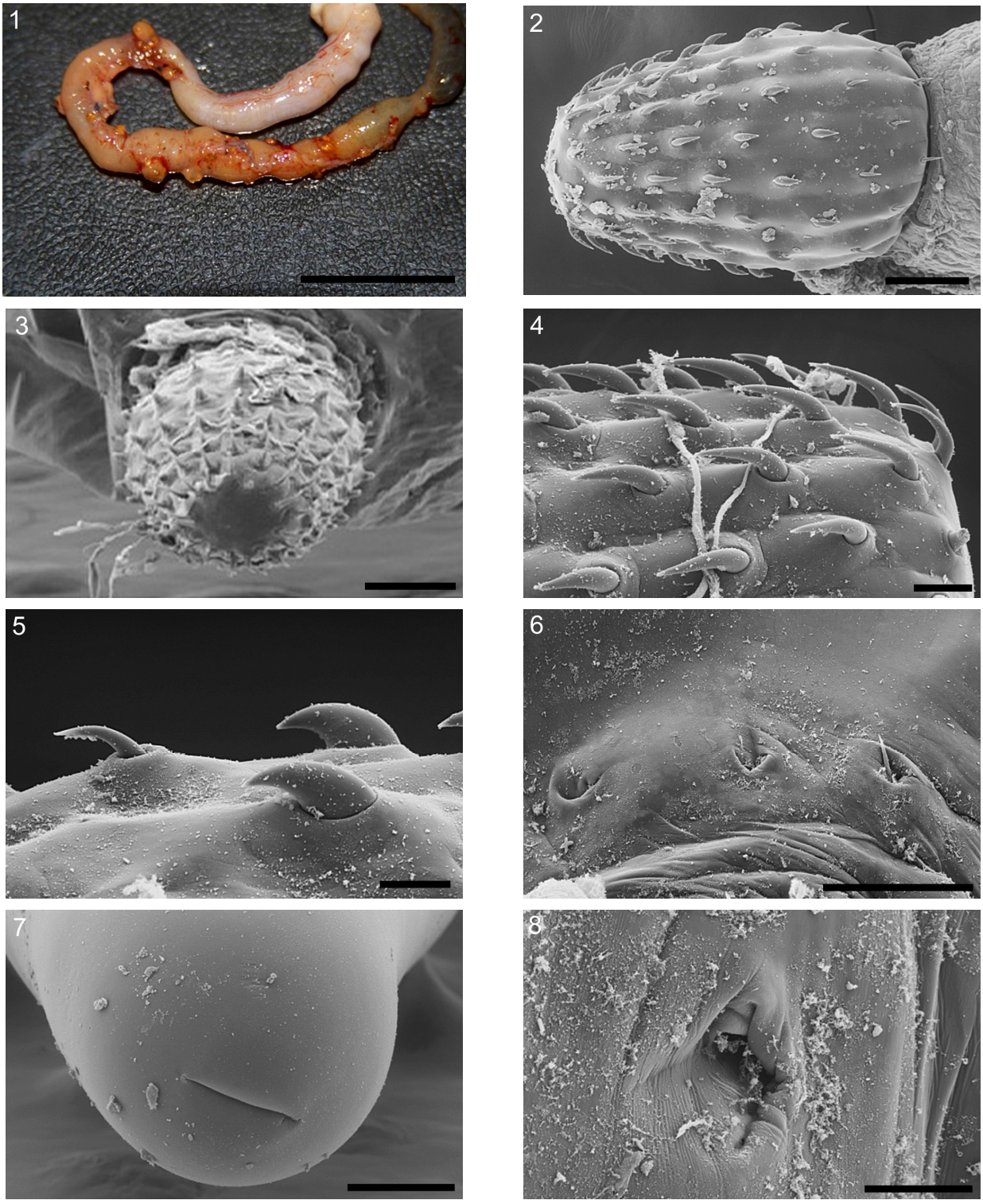
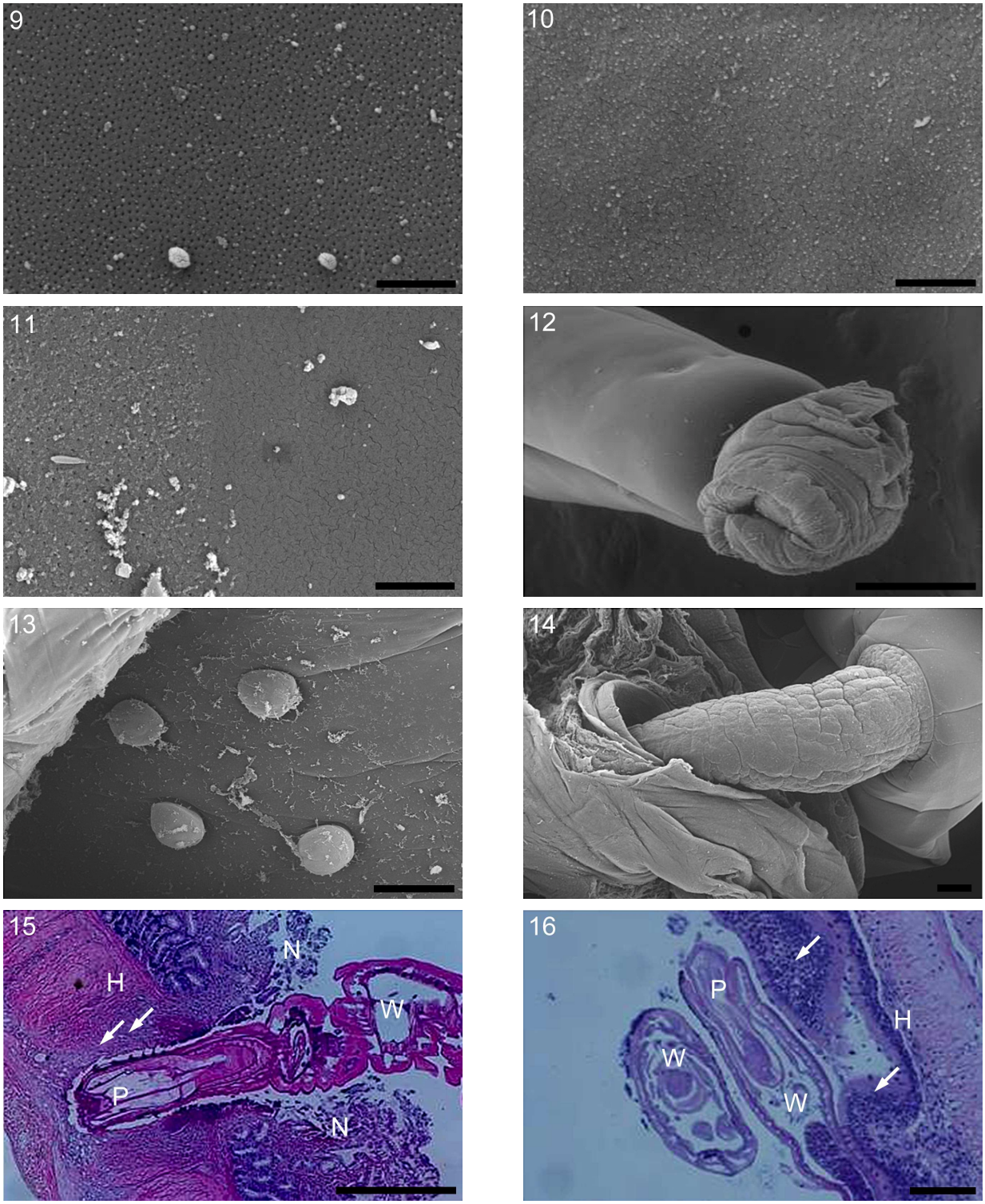


Figure 1. Infected intestine of *P. ridibundus* studded with bulges of worms protruding into the body cavity (bar=1 cm). **Figures 2-8.** SEM of *Pomphorhynchus spindletruncatus* from *P. ridibundus* and *Carassius* sp. in Turkey: 2. A proboscis (slightly constricted posteriorly) of a worm showing armature and ribbed appearance (bar=100 µm); 3. Front view of a proboscis showing the bald flattened anterior tip (bar=150 µm); 4. Part of the anterior end of a proboscis showing the small anterior hooks and the gradual increase in hook size posteriorly (bar=30 µm); 5. A profile about the middle of the proboscis showing the robust hooks characteristic of *P. spindletruncatus* (bar=30 µm); 6. A few hooks in the posteriormost circle of hooks near the interface with the bulb (bar=50 µm); 7. A basal view of the slit-like female gonopore (bar=100 µm); 8. A sensory pit near the base of the neck (bar=40 µm).



Figures 9-16. SEM and histopathological sections of *P. spindletruncatus* from *P. ridibundus* and *Carassius* sp. in Turkey: 9. Epidermis at posterior trunk showing high density of micropores (bar= 1 µm); 10. Epidermis at mid trunk showing lower density of micropores (bar= 1 µm); 11. Transition area between the anterior trunk with least dense micropores (left) and the neck with no micropores (right) (bar= 1 µm); 12. Somewhat contracted bursa (bar= 50 µm); 13. Higher magnification of the sensory papillae inside the bursa (bar= 20 µm); 14. Proboscis inserted into the intestinal wall of a fish (bar= 20 µm); 15. Adult worm (W) from *B. plebejus*; proboscis (P) is inserted into the intestine extending to the muscularis externa but not breaking into the peritoneal cavity; extensive hemorrhage (H) around the proboscis. Proboscis hooks (arrows) are embedding into gut wall; necrotic tissue (N) in the area of attachment (bar= 180 µm); 16. Two immature worms (W), one with visible proboscis (P) within the intestine of *P. ridibundus* host (H); prominent compression on the intestinal wall with extensive hemorrhage (arrows) near the parasite; No reproductive structures are visible within the worms (bar= 180 µm).

The Anatolian khramulya, *C. tinca* is a benthopelagic freshwater fish found only in Turkey, with the exception of an introduced population in Taiwan (Turan et al., 2006). *Capoeta tinca* is primarily a herbivorous fish that adapts easily to changes in aquatic environments and occurs both in lotic and lentic habitats (Ekmekci and Ozeren, 2003).

The Kura bleak, *A. filippii*, is a native widely distributed benthopelagic freshwater fish in the Kura-Aras basin where it can be found from the headwaters to the lower reaches and tributaries. It is also known from lakes Cildir and Chaldyr-gel in Turkey and Arpilitsh in Armenia as well as from Iran and Georgia (Berg, 1964; Bogutskaya, 1997). Iranian specimens contain insect remains, crustaceans, and sand grains. Samples from the Qareh Su north of Ardebil had also been feeding on water beetles (Hydrophilidae), spiders, and scarab beetles (*Euoniticellus* sp.) (Coad, 1995).

The Italian barbell, *B. plebejus*, is a large size barbell (reaching 55cm) that inhabits rivers and freshwater lakes in Turkey, Switzerland, Slovenia, Croatia, Iran, and Italy. It has been classified as a "large-sized, warm water adapted species" of Barbinae fishes in Southern Europe (Bianco, 1998). The crustacean component of its diet prompts its infection with an assortment of helminth parasites (cestodes, nematodes, trematodes), at least in the Turkish Doganci (Bursa) Dam Lake, but no acanthocephalans have been reported there (Aydogdu et al., 2002).

The European chub, *L. cephalus*, is a generalist and opportunistic feeder as observed in Southern Italy (Balestrieri et al., 2006) reaching 40cm in length in the Karasu River, Turkey (Sen and Saygin, 2008). A total of 13 species of helminth parasites were reported from the chub in Italy (Tieri et al., 2006) and Bulgaria (Kirin, 2002a, b) indicating a marked utilization of the rich and diverse invertebrate fauna in the streams surveyed. The reported parasites included 4 species of trematodes (1 from Italy and 3 different ones from Bulgaria), 3 species of cestodes (1 from Italy and the same and 2 others from Bulgaria), 1 species of nematode (the same in both countries), and 5 species of acanthocephalans. The acanthocephalans from Italy were *Pomphorhynchus laevis* (Zoega in O.F. Müller, 1776) Van Cleave, 1924, *Neoechinorhynchus rutili* (O. F. Müller, 1780) Hamann, 1892 in Stiles and Hassal, 1905, and *Acanthocephalus clavula* (Dujardin, 1845) Grabda-Kazubska and Chub, 1968. Those from Bulgaria included 2 different species, *Acanthocephalus anguillae* (O. F. Müller, 1780) Lühe, 1911 and *Acanthocephalus tenuirostris* (Achmerov and Dombrowskaja-Achmerova, 1941) Yamaguti, 1963. The effects of geographic range and feeding habits of hosts accounted for 73% of the variation in total parasite species number per host species (Price and Clancy, 1983). It appears that the European chub is susceptible to a wide assortment of helminth species that will vary depending on the geographical diversity of the invertebrate fauna in surveyed streams. It is probable that *P. spindletruncatus* may either be absent in the reported waters or has been overlooked or misidentified.

The carp, *Carassius* sp., probably the Crucian carp, *Carassius crassius*, is widely distributed in the Eurasian region. Like carps, *Carassius* sp. commonly lives in ponds and shallow waters in vegetated coastal zone. Their main food consists of plants, larvae of mayfly and diptera and small zooplanktons (Geldiay and Balik, 1996).

Amphibian host associations

The presence of an acanthocephalan of fish in amphibian hosts, especially in such large numbers as reported herein for *P. spindletruncatus* in *P. ridibundus* (table 1), is a highly unusual finding. *Pelophylax ridibundus* is the largest and most widely distributed frog in Europe that spreads east into East Asia and south into North Africa with a vertical distribution of up to 2500 m (Basoglu and Ozeti, 1994). In Turkey, this species is known in all suitable habitats except for a portion of the Lakes District (Baran and Atatür, 1998; Budak and Göçmen, 2008). It is a semi-aquatic and highly opportunistic amphibian that lives in mixed deciduous forests, steppe, semi-desert and desert zones. The tadpoles consume detritus, algae, and a wide variety of invertebrates. Adults often eat conspecific and other amphibians, reptiles, and even small birds (Basoglu and Ozeti, 1994; Tarkhnishvili et al.,

1999; Tok et al., 2000). In Turkey, the crustacean component of the invertebrate diet includes isopodes (*Oniscus* sp.: Oniscidae, and *Philoscia* sp.: Philosciidae) and amphipodes (*Elasmopus* sp.: Gammaridae) (Çiçek and Mermer, 2006). The marsh frog appears to acquire infection with larval *P. spindletruncatus* from its intermediate host(s) in the same manner as fish hosts do. The fact that the parasites never reach sexual maturity in frogs classifies the frogs as occasional hosts with no contribution in the biological transmission route to the definitive host system (Amin, 1987) but with a marked role in extending the distribution of the parasite over a large geographical range. That ecological role is suggested by the fact that large fish predator populations are known to feed on the frog (Ayaz et al., 2007) making them post-cyclic hosts. The distribution of *P. spindletruncatus* may be affected by the distribution of the amphibian and crustacean hosts alike as associated with fish predatory activities; the diet of some large fish may not include many crustaceans.

A comparable situation occurs in specimens of *Pallisentis* (*Pallisentis*) *celatus* Van Cleave, 1928 that infect the intestine of 3 species of occasional fish hosts as juveniles/immatures where they never reach sexual maturity. They were not found encysted in the body cavity, as in paratenic hosts and thus did not represent dead-end infections but had a secondary route accessible at least to the definitive hosts (Amin et al., 2004).

Histopathology

Figures 14, 15 and 16 display the invasive action of the organism both in the immature and mature stages.

The SEM micrograph (figure 14) shows the proboscis extended into the host tissue embedded into the outer layers of the intestine and extended to the outer muscle layers (figure 16). There is a prominent compression on the intestinal wall of both the frog and fish hosts with extensive hemorrhaging especially around the area of the proboscis. Hemorrhaging is characterized by the nucleated red blood cells with the expected movement of granulocytes into the infected tissue. Figure 15 represents the immature stages of *P. spindletruncatus* invading the intestine of *P. ridibundus.* The proboscis is visible in both figures 15 and 16. The typical columnar cells for the intestine of the fish (*B. plebejus*) that line the villi are lost in the area of parasite attachment and the proboscis hooks are visible (figure 16). Necrosis of host intestinal cells is visible (figures 15 and 16). Obstruction and blockage of the intestinal tract is also prominent (figures 15 and 16).

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