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The discovery of *Neoechinorhynchus zabensis* (Acanthocephala: Neoechinorhynchidae) from cyprinid fishes in Turkey and Iran, with special reference to new morphological features revealed by scanning electron microscopy

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Abstract: *Neoechinorhynchus zabensis* Amin, Abdullah, and Mhaisen, 2003 was described from *Capoeta damascina* (Valenciennes, 1842) and *Capoeta trutta* (Heckel, 1843) in the Greater and Lesser Zab rivers of northern Iraq. It is reported here from *C. trutta* in the Dez River, Iran, and from *Capoeta barroisi* Lortet, 1894 (new host record) in the Murat River, Turkey (both are new locality records). *Neoechinorhynchus zabensis* appears to be restricted to the Tigris–Euphrates basin in Iraq, Turkey, and Iran, where it infects native fishes of the genus *Capoeta* Valenciennes in Cuvier and Valenciennes, 1842 (Cyprinidae). Other fishes of the same genus from nearby river systems and fishes of other genera from the Tigris–Euphrates basin were negative for *N. zabensis* infections. The specimens from Iran and Turkey were similar to those described in Iraq; those from *C. barroisi* in the Murat River, Turkey, were somewhat larger. Scanning electron microscopy examination revealed a slightly different size of anterior hook at the anterior and posterior levels, sensory pits at the base of the proboscis, epidermal pores at the surface of the integument that become larger in the middle of trunk, and the oblong shape of the filamented sperms.

Key words: *Neoechinorhynchus zabensis*, Acanthocephala, Iran, Turkey, *Capoeta* spp. new features, scanning electron microscopy

Introduction

Neoechinorhynchus zabensis Amin, Abdullah, and Mhaisen, 2003 was described from *Capoeta damascina* (Valenciennes, 1842) and *Capoeta trutta* (Heckel, 1843) in the Greater and Lesser Zab rivers of northern Iraq. It was reported only once since then, from the same 2 host species, in the Greater Zab River and Dokan Lake on the Lesser Zab River (Abdullah, 2009). That report dealt with host–parasite and seasonal relationships. We have collected *N. zabensis*

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from the same species and from additional species of hosts of the same genus, *Capoeta* Valenciennes in Cuvier and Valenciennes, 1842, in new localities in Iran and Turkey. This report demonstrates that the geographical and host distribution of this acanthocephalan extends well beyond previously known records in Iraq, but only within the basins of the Tigris and Euphrates rivers. In addition, this report provides morphometric observations of the Turkish and Iranian material and gives additional insight into its morphology using scanning electron microscopy (SEM).

Materials and methods

The study was conducted between April of 2008 and July of 2009 in tributaries of the Tigris–Euphrates basin. Fish collected included 15 *Capoeta barroisi* Lortet, 1894 from Turkey in the Murat River (39°29′N, 42°40′E) and 79 *C. trutta* from Iran in the Dez River near the city of Dezful (32°22′42″N, 48°22′93″E) (Table 1). Fish collected also included 111 specimens of *Cyprinion macrostomum* Heckel, 1843 from the Dez River in Iran that were not infected with acanthocephalans and 94 *Capoeta capoeta* (Berg, 1914) from Turkey in the Aras River (39°57′N, 41°51′E) (not a tributary of the Tigris–Euphrates basin) that were infected with another unidentified species of *Neoechinorhynchus* Stiles and Hassall, 1905. Collections of *C. capoeta* from the nearby Aras River and *C. macrostomum* from the Dez River were taken for comparative purposes to see if another species of *Capoeta* from outside the Tigris–Euphrates basin, or fishes of another genus within the Tigris–Euphrates system, were infected with *N. zabensis*. The above collection sites, with the exception of those in the Aras River, Turkey, and those from which *N. zabensis* was originally described in *C. damascina* and *C. trutta*, are all located in tributaries of the Tigris and Euphrates rivers in Iraq, Turkey, and Iran (Figure 1).

Fish hosts were dissected, and acanthocephalans were placed in saline solution and cooled until proboscides everted. They were then fixed in 70% ethanol, 5% formalin, or alcohol–formalin–acetic acid; stained in Mayer's carmine or azocarmine; dehydrated in ethanol series; cleared in xylene; and whole-mounted in Canada balsam.

For SEM, a few male and female specimens of *N. zabensis* previously fixed in 70% ethanol were placed in critical point dryer baskets and dehydrated using the ETOH series of 95% and 3 N 100% for at least 10 min per soak followed by critical point drying (Lee, 1992). Samples were then mounted on SEM sample mounts, gold coated, and observed with a scanning electron microscope (FEI X L30 ESEM-FEG). Digital images of the structures were obtained using computer-based digital imaging software.

Measurements are given in millimeters unless otherwise stated. Range values are followed by the mean in parentheses. Length measurements are given before width; the latter refers to maximum width. Trunk length does not include the neck, proboscis, or bursa. Eggs refer only to fully developed eggs removed from the pseudocoel. Specimens from Turkey were deposited in the Zoology Museum of Ege University, İzmir, Turkey, under ZDEU HEL-15/2008. Specimens from Iran (No. ZUTC Acant.1005) were deposited in the Zoological Museum of the University of Tehran, Iran.

Table 1. Infection of *Capoeta* spp. with *Neoechinorhynchus zabensis* from the Murat River in Turkey and the Dez River in Iran compared to infection in *Capoeta* spp. from the Zab rivers in Iraq.

Collection sites	**Murat River (present study)**	**Dez River (present study)**	**Greater Zab River (Amin et al., 2003)**	**Lesser Zab River (Amin et al., 2003)**
Fish species	*Capoeta barroisi*	*Capoeta trutta*	*Capoeta damascina*	*Capoeta trutta*
Fish length (cm)	16–26 (21)	11–32 (21)	7–42 (28)	6–33 (25)
No. of fish examined	15	79	300	192
No. of fish infected	15	51	280	176
Prevalence (%)	100	64.6	93.3	93.2
Parasites per fish	2–61 (21)	1–90 (14)	0–42 (20)	0–28 (16)

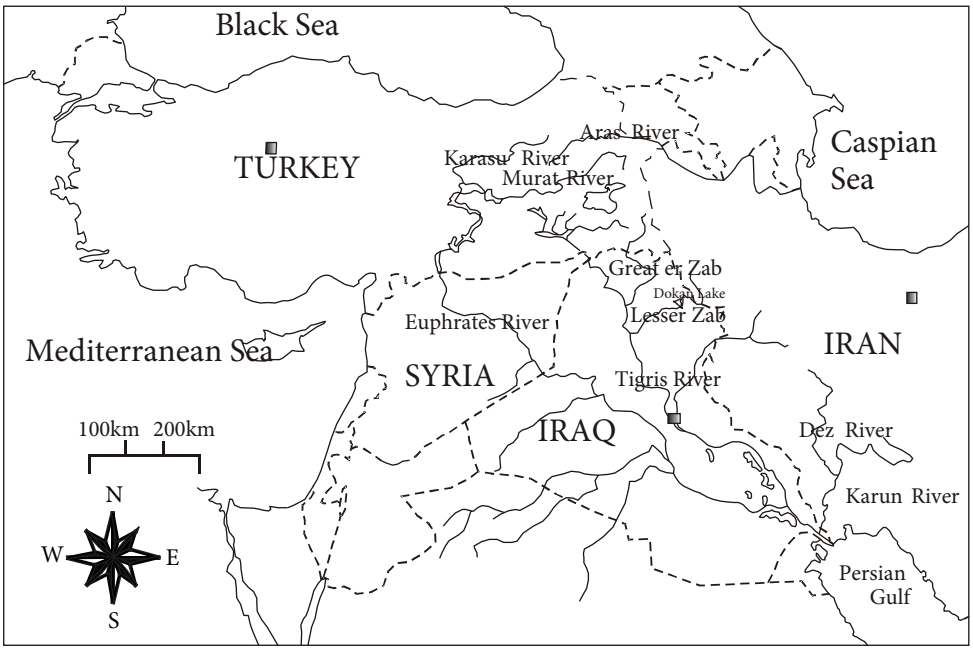

Figure 1. Map of the Tigris–Euphrates streams from which *Neoechinorhynchus zabensis* was collected in Iran, Iraq, and Turkey.

Results and discussion

Description of Iranian and Turkish specimens of *N. zabensis*

In the description of *N. zabensis*, Amin et al. (2003) noted features characteristic of the species that distinguished it from other species of *Neoechinorhynchus* Hamann, 1892 in Stiles and Hassall, 1905. A combination of these features are shared by the new specimens from Iran and Turkey reported herein and include: 1) proboscis about as long as wide, 2) anterior hooks at 2 levels, 3) middle and posterior hooks of equal length (Figure 2), 4) 2 oblong structures located in receptacle wall, 5) receptacle considerably longer than proboscis and with a large oval cephalic ganglion at its base, 6) subequal lemnisci distant from anterior testis and with nuclear fragments in their posterior half, 7) female reproductive system with subterminal gonopore and paired muscular paravaginal appendages, and 8) sinuate uterine wall. In addition, the shape of the trunk and the reproductive structures and eggs were similar, and worms infected only host species of the genus *Capoeta*.

New features observed included: 1) the relatively smaller size of anterior hooks at the second level compared to those at the anterior level (Figure 2), 2) the sensory pits at the base of the proboscis (Figure 3), 3) the epidermal micropores that varied in size and distribution at various trunk locations (Figures 4–6), 4) the rounded posterior trunk in females and the elevated and rounded female gonopore (Figure 7), 5) the shape of the eggs (Figure 8), 6) the presence of many small papillae on the internal rim of the bursa (Figures 9 and 10), and 7) the presence and shape of sperms deep in the bursa (Figure 11). The varied micropore size and distribution suggests differential rates of absorption in different trunk regions (see Amin et al., 2009 for a discussion of porous teguments in the Acanthocephala); the shape of the sperms is reported here for the first time.

Morphometric characteristics of the specimens of *N. zabensis* described in this paper are given in Table 2. Measurements of the Iranian and Turkish specimens were similar to those reported in the original description by Amin et al. (2003). The following variations were, however, noted. Worms from *C. barroisi* in the Murat River, Turkey, were largest and had relatively larger proboscises, testes, and other male reproductive structures (e.g., cement reservoir and Saefftigen's pouch). Eggs were also slightly larger

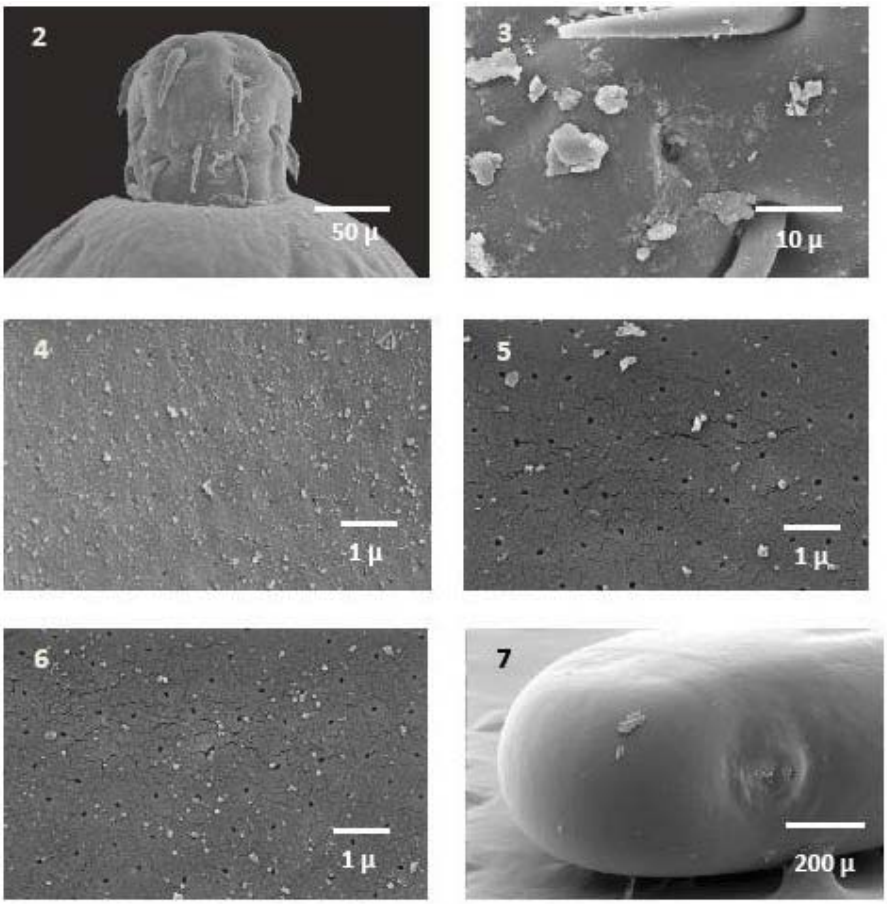

Figure 2. SEM of *Neoechinorhynchus zabensis* specimen from *Capoeta barroisi*: proboscis of a female specimen. Note the different length of anterior hooks at the first and second levels and the close position of the middle and posterior hooks of similar length.

Figure 3. SEM of *Neoechinorhynchus zabensis* specimen from *Capoeta barroisi*: sensory pore near the posterior hooks.

Figure 4. SEM of *Neoechinorhynchus zabensis* specimen from *Capoeta barroisi*: integument and small micropores at the anterior trunk.

Figure 5. SEM of *Neoechinorhynchus zabensis* specimen from *Capoeta barroisi*: integument and larger micropores at middle of trunk.

Figure 6. SEM of *Neoechinorhynchus zabensis* specimen from *Capoeta barroisi*: integument with smaller micropores at the posterior trunk.

Figure 7. SEM of *Neoechinorhynchus zabensis* specimen from *Capoeta barroisi*: rounded posterior end of a female specimen showing the position of the gonopore.

in specimens from *C. barroisi*. Otherwise, specimens from all host species in all localities were similar. It is not known whether size variation in the above-noted structures in the Turkish specimens are related to host species or to geography. Both of these variables have been related to acanthocephalan size; see, for example, Amin and Redlin (1980) and Amin and Dailey (1998).

Geography

Only species of *Capoeta* were found infected with *N. zabensis* and only in tributaries of the Tigris and Euphrates rivers in Iraq, Turkey, and Iran (Table 1 and Figure 1). Tributaries of the Tigris and Euphrates in Syria were not surveyed. The Tigris–Euphrates basin lies primarily in 4 countries: Turkey, Syria, Iraq, and Iran. Both rivers originate in the mountains of

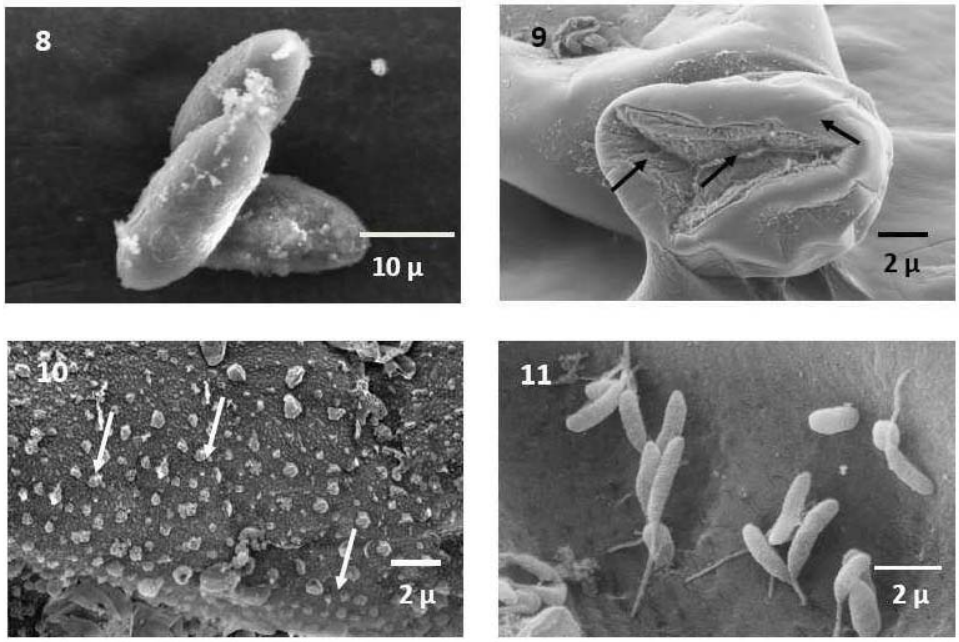

Figure 8. SEM of specimen of *Neoechinorhynchus zabensis* from *Capoeta barroisi*: 3 eggs showing their characteristic shape.

Figure 9. SEM of specimen of *Neoechinorhynchus zabensis* from *Capoeta barroisi*: bursa opening distally along vertical access of worm.

Figure 10. SEM of specimen of *Neoechinorhynchus zabensis* from *Capoeta barroisi*: surface view of a portion of the bursa folds showing many small papillae.

Figure 11. SEM of specimen of *Neoechinorhynchus zabensis* from *Capoeta barroisi*: sperms deep inside a bursa.

Table 2. Comparison of morphometric characteristics of *Neoechinorhynchus zabensis* specimens from hosts in Turkey and Iran and morphometric characteristics described by Amin et al. (2003) in specimens from the Lesser and Greater Zab rivers in Iraq.

	Capoeta barroisi	*Capoeta trutta*	*Capoeta damascina* *Capoeta trutta*		*Capoeta barroisi*	*Capoeta trutta*	*Capoeta damascina* *Capoeta trutta*
	Murat River (Turkey)	Dez River (Iran)	Lesser–Greater Zab rivers (Iraq)		Murat River (Turkey)	Dez River (Iran)	Lesser–Greater Zab rivers (Iraq)
	Present study (n = 20) ♂♂	Present study (n = 10) ♂♂	Amin et al., 2003 (n = 18) ♂♂		Present study (n = 20) ♀♀	Present study (n = 5) ♀♀	Amin et al., 2003 (n = 18) ♀♀
TL*	10.105 ± 2.164 (5.931–13.303)†	7.969 ± 0.419 (6.256–10.205)	7.42 (5.07–10.12)	TL	11.885 ± 3.035 (7.129–18.584)	11.201 ± 0.953 (9.948–13.692)	10.87 (8.82–14.87)
TW	1.391 ± 0.270 (0.914–1.828)	1.124 ± 0.86 (0.86–1.3)	1.12 (0.85–1.50)	TW	1.548 ± 0.388 (1.036–2.701)	1.283 ± 0.105 (0.950–1.6)	1.46 (0.97–2.12)
PL	0.127 ± 0.017 (0.113–0.194)	0.113 ± 0.005 (0.070–0.130)	0.107 (0.088–0.125)	PL	0.124 ± 0.012 (0.105–0.145)	0.124 ± 0.008 (0.101–0.140)	0.112 (0.100–0.125)
PW	0.106 ± 0.008 (0.089–0.121)	0.109 ± 0.003 (0.098–0.128)	0.106 (0.100–0.112)	PW	0.113 ± 0.015 (0.081–0.154)	0.121 ± 0.003 (0.117–0.130)	0.112 (0.100–0.122)
PRL	0.566 ± 0.068 (0.436–0.695)	0.489 ± 0.025 (0.371–0.669)	0.566 (0.426–0.728)	PRL	0.577 ± 0.066 (0.444–0.695)	0.533 ± 0.074 (0.426–0.824)	0.593 (0.510–0.728)
PRW	0.157 ± 0.028 (0.113–0.202)	0.158 ± 0.008 (0.128–0.224)	0.150 (0.125–0.187)	PRW	0.161 ± 0.029 (0.105–0.210)	0.178 ± 0.009 (0.151–0.202)	0.170 (0.135–0.198)

Table 2. Continued.

LL1	3.504 ± 0.754 (2.315–5.219)	3.038 ± 0.124 (2.538–3.897)	2.97 (2.00–3.75)	LL1	3.906 ± 0.682 (2.498–5.179)	3.759 ± 0.254 (3.026–4.410)	3.67 (3.10–4.35)
LW1	0.228 ± 0.053 (0.162–0.323)	0.208 ± 0.014 (0.154–0.282)	0.20 (0.12–0.27)	LW1	0.246 ± 0.044 (0.162–0.323)	0.267 ± 0.036 (0.179–0.385)	0.25 (0.10–0.31)
LL2	3.242 ± 0.666 (2.437–4.874)	2.709 ± 0.188 (1.744–3.513)	2.63 (1.55–3.30)	LL2	3.441 ± 0.765 (2.295–4.935)	3.354 ± 0.361 (2.282–4.154)	3.37 (2.90–4.12)
LW2	0.220 ± 0.050 (0.162–0.307)	0.197 ± 0.017 (0.128–0.282)	0.18 (0.15–0.27)	LW2	0.226 ± 0.063 (0.097–0.315)	0.287 ± 0.045 (0.154–0.385)	0.23 (0.10–0.31)
AHL1	43 ± 3 (39–51)	38.888 ± 1.438 (33.670–49.210)	42 (37–45)	AHL1	45 ± 3 (41–49)	40.792 ± 1.630 (36.260–44.030)	42 (37–46)
AHL2	039 ± 3 (35–45)	33.304 ± 1.208 (30.02–36.26)		AHL2	39 ± 2 (37–42)	35.434 ±1.408 (31.13–39.88)	
MHL	39 ± 2 (34–45)	34.454 ± 0.743 (32.03–36.63)	33 (30–35)	MHL	37 ± 2 (33–43)	36.450 ± 0.533 (35.25–38.12)	35 (30–40)
BHL	32 ± 3 (24–37)	32.732 ± 1.340 (28.490–41.440)	33 (30–35)	BHL	32 ± 2 (26–37)	34.533 ± 2.284 (31.080–38.850)	35 (30–40)
ATL	0.983 ± 0.329 (0.528–1.625)	0.924 ± 0.102 (0.417–1.585)	0.88 (0.50–1.32)	EL	0.029 ± 0.001 (0.026–0.031)	0.025 ± 0.001 (0.020–0.029)	0.030 (0.028–0.035)
ATW	0.751 ± 0.189 (0.427–1.016)	0.622 ± 0.029 (0.54–0.7)	0.54 (0.40–0.75)	EW	0.014 ± 0.004 (0.008– 0.022)	0.011 ± 0.002 (0.005–0.019)	0.014 (0.010–0.017)
PTL	1.081 ± 0.289 (0.649–1.523)	0.739 ± 0.078 (0.365–1.085)	0.88 (0.50–1.30)				
PTW	0.691 ± 0.151 (0.366–0.934)	0.453 ± 0.036 (0.188–0.647)	0.49 (0.35–0.75)				
BL	0.785 ± 0.287 (0.284–1.219)	0.601 ± 0.017 (0.55–0.6)	0.648 (375–925)				
BW	0.703 ± 0.242 (0.325–1.097)	0.656 ± 0.021 (0.59–0.7)	0.570 (350–825)				
CgL	0.882 ± 0.281 (0.447–1.279)	0.526 ± 0.077 (0.250–0.907)	0.73 (0.35–1.20)				
CgW	0.569 ± 0.176 (0.305–0.772)	0.555 ± 0.075 (0.29–0.7)	0.40 (0.27–0.65)				
CrL	0.347 ± 0.107 (0.183–0.589)	0.325 ± 0.058 (0.151–0.483)	0.278 (0.175–0.400)				
CrW	0.268 ± 0.063 (0.162–0.427)	0.299 ± 0.031 (0.146–0.448)	0.179 (0.125–0.250)				
SL	1.056 ± 0.192 (0.751–1.34)	0.762 ± 0.082 (0.541–1.031)	0.73 (0.52–1.02)				
SW	0.298 ± 0.068 (0.162–0.406)	0.273 ± 0.039 (0.094–0.542)	0.15 (0.10–0.21)				
CsdL	0.897 ± 0.299 (0.609–1.217)	0.844 ± 0.079 (0.639–1.101)	0.90 (0.60–1.25)				
CsdW	0.237 ± 0.109 (0.142–0.427)	0.229 ± 0.050 (0.099–0.386)	0.25 (0.10–0.35)				

*AHL1: long apical hook length, AHL2: short apical hook length, ATL: anterior testis length, ATW: anterior testis width, BHL: basal hook length, BL: bursa length, BW: bursa width, CgL: cement gland length, CgW: cement gland width, CrL: cement reservoir length, CrW: cement reservoir width, CsdL: common sperm duct length, CsdW: common sperm duct width, EL: egg length, EW: egg width, LL1: long lemniscus length, LL2: short lemniscus length, LW1: long lemniscus width, LW2: short lemniscus width, MHL: median hook length, PTL: posterior testis length, PTW: posterior testis width, PL: proboscis length, PRL: proboscis receptacle length, PRW: proboscis receptacle width, PW: proboscis width, SL: Saefftigen's pouch length, SW: Saefftigen's pouch width, TL: trunk length, TW: trunk width.

†All measurements are in millimeters except proboscis hook length (μm). Mean ± standard error (range).

southern Turkey and flow south–southeastwards; the Euphrates crosses Syria into Iraq and the Tigris flows directly into Iraq from Turkey. The main stream of the Euphrates in Turkey, the Fırat, has 4 major tributaries: the Karasu, the Murat, the Munzur, and the Peril. However, it has only one large tributary, the Khabur, in Syria. In contrast, the Tigris has 4 main tributaries, all of which unite with the main stream in Iraq. The largest is the Greater Zab River, which originates in Turkey. The Lesser Zab River and the Diyala originate in Iran. Dokan Lake was created by damming the Lesser Zab River. The Dez River rises in the Zagros Range and joins the Karun River, a tributary of the Tigris–Euphrates, at Bamdej. The Karun–Dez basin is the largest river basin in Iran. In Southern Iraq, the Tigris and Euphrates unite to form Shatt al-Arab, which flows into the Persian Gulf. The Aras River, which is not connected to the Tigris–Euphrates basin, rises near Erzurum, Turkey, and flows into Armenia, Iran, and Azerbaijan, where it meets the Kura River before flowing into the Caspian Sea (Kolars and Mitchell, 1991; Mountjoy, 2005; Isaev and Mikhalova, 2009).

Hosts

Only species of *Capoeta* are known to serve as definitive hosts for *N. zabensis* throughout the hosts' native range in the Tigris–Euphrates basin. All fish species infected with *N. zabensis* are benthopelagic freshwater fishes native to this basin. *Capoeta barroisi* and *C. trutta* appear to be confined to the Tigris–Euphrates system (Coad, 1996). The range of *C. damascina*, however, extends into the Jordan River drainage system and the entire Levant (Krupp and Schneider, 1989); the range of *C. capoeta* extends from the Aras River in Turkey east to the Caspian rivers in Iran, Baluchistan, Pakistan, Azerbaijan, Uzbekistan, and the former USSR (Talwar and Jhingran, 1991). The habitats, stream conditions, and diet requirements of these fishes vary considerably. For example, *C. damascina* occurs in lakes, streams with fast- and slow-moving water, and clear muddy water. It is a bottom feeder that scrapes food from the substrate and feeds on algae, aquatic invertebrates, and detritus (Krupp and Schneider, 1989). In contrast, *C. capoeta* inhabits backwaters and channels with weak currents and silt beds, as well as reservoirs (Amanov, 1970), and feeds on detritus, higher plants, blue-green algae, phytoplankton, and small benthic invertebrates including chironomids, Ephemeroptera, and mollusks (Amanov, 1970; Valipour, 2004).

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