# *The description and histopathology of* Leptorhynchoides polycristatus *n. sp. (Acanthocephala: Rhadinorhynchidae) from sturgeons,* Acipenser *spp. (Actinopterygii: Acipenseridae) in the Caspian Sea, Iran, with emendation of the generic diagnosis*

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**Parasitology Research**
Founded as Zeitschrift für Parasitenkunde

ISSN 0932-0113
Volume 112
Number 11

Parasitol Res (2013) 112:3873-3882
DOI 10.1007/s00436-013-3576-z


Springer

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ORIGINAL PAPER

# The description and histopathology of *Leptorhynchoides polycristatus* n. sp. (Acanthocephala: Rhadinorhynchidae) from sturgeons, *Acipenser* spp. (Actinopterygii: Acipenseridae) in the Caspian Sea, Iran, with emendation of the generic diagnosis

**Omar M. Amin · Richard A. Heckmann · Ali Halajian · Atif M. El-Naggar · Sareh Tavakol**

Received: 12 July 2013 / Accepted: 6 August 2013 / Published online: 24 August 2013


**Abstract** Of the three known species of *Leptorhynchoides* Kostylew 1924, two are reported from North American fishes: *Leptorhynchoides aphredoderi* Buckner and Buckner 1976 and *Leptorhynchoides thecatus* (Linton 1891) Kostylew 1924. The third species, *Leptorhynchoides plagicephalus* (Westrumb 1821) Kostylew 1924, is commonly found in the Caspian and Black Sea from at least four species of sturgeons including *Acipenser stellatus* Pallas 1771 and the *Acipenser nudiventris* Lovetzsky 1828 from which *Leptorhynchoides polycristatus* n. sp. was found. No taxonomic work has been reported for *L. plagicephalus* for the last 90 years. *L. polycristatus* n. sp. can be readily confused with *L. plagicephalus* because of many superficial similarities. Such similarities include the general shape of the trunk, proboscis, and organ systems. However, *L. polycristatus* is clearly distinguished from the other three species primarily by having (1) 19–20 proboscis hooks per row; (2) the shortest hooks are anterior and the longest at the middle; the opposite is true in *L. plagicephalus*; (3) with a cuticular collar enveloping the base of the proboscis hooks; (4) the surface of its proboscis hooks is ribbed; (5) with a broad collar of multiple rectangular cuticular crests encircling the anterior end of the trunk; this is the only member of *Leptorhynchoides* with such a structure; (6) with many large ovoid uninucleated cells in the subcuticular layer of the trunk; (7) with paired glandular clusters near the male reproductive opening and of suction cup-like sensory structures on the bursa; (8) with dorsoventral ligament across the vagina; (9) cement glands are in a cluster of eight arranged in two horizontal tiers of four glands each; (10) with female gonopore near terminal; (11) with structures interpreted as possible microtriches on the surface of the trunk; (12) and with thinner eggs. *L. polycristatus* caused extensive histopathological damage to host intestinal layers. The armed proboscis invades and attaches to the host mucosa causing villi compression and necrosis of the epithelial lining with subsequent hemorrhaging and granulocyte migration. No encapsulation of the acanthocephalan is visible, and the worm can migrate deep into the smooth muscle layers of the muscularis extrema. The presence of *L. polycristatus* in the lumen of the host intestine obstructs and damages the absorbing surface of the host affecting the nutritional potential. Dead, necrotic host epithelial tissue and remnants of villi and crypts are visible.

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## Introduction

Specimens of a new species of *Leptorhynchoides* Kostylew 1924, *Leptorhynchoides polycristatus* n. sp., were found in the starry sturgeon *Acipenser stellatus* Pallas 1771 and the fringebarbel sturgeon *Acipenser nudiventris* Lovetzsky 1828 from the Caspian Sea, Iran. Of the three other species of *Leptorhynchoides*, one other species, *Leptorhynchoides plagicephalus*, also infects at least four species of sturgeons, including *A. stellatus* and *A. nudiventris* in the Caspian as well as in the Black Sea (Pazooki and Masoumian 2012). Both acanthocephalan species are readily confused because of superficial similarities, and many records of *L. plagicephalus* may actually include specimens of *L. polycristatus*. No new taxonomic accounts of *L. plagicephalus* have been reported since Kostylew's (1924) description, and all subsequent texts reproduced his illustrations and quoted his description, e.g., Petrochenko (1956), Yamaguti (1963), and Golvan (1969). All reports of *L. plagicephalus* during the last 90 years were primarily surveys of ecological nature that lacked any reference to morphological features, except for two studies of its chromosome complement and its spermiogenesis by Fontana et al. (1993) and Foata et al. (2004), respectively. On one occasion, *L. plagicephalus* was reported from barbell, *Barbus barbus* Cuvier and Cloquet 1816, in the Danube River in Bulgaria (Nachev 2010). The crustacean intermediate host was reported to be *Gammarus pulex* Linnaeus 1758 (Gammaridae) by Rasin (1949). *G. pulex* may not constitute a significant portion of the diet of immature sturgeons since juvenile *Acipenser guldenstadtii* Brandt and Ratzeburg 1833 do not normally show infection with *L. plagicephalus* (see Hajimorad Loo and Ghorbani Nasrabadi 2003; Ali Halajian, personal communication).

In the absence of critical taxonomic observations of *Leptorhynchoides* from sturgeons in the Caspian or the Black Sea, it appears that reports simply interpreted all specimens of *Leptorhynchoides* as *L. plagicephalus*. It is not known whether these reported infections included *L. polycristatus*. Reports of *L. plagicephalus* from *A. stellatus* included those of Sattari et al. (2001), Sattari and Mokhayer (2005), Ebrahimi and Malek (2007), Rajabpour et al. (2008), Khara et al. (2011), and Noei (2011). Reports of *L. plagicephalus* from *Acipenser persicus* Borodin 1897 and/or other species of sturgeons included those of Sattari (2005), Masoumzadeh et al. (2007), Bazari Moghaddam et al. (2012), Haghparast et al. (2007), and Pazooki and Masoumian (2012). The present treatment is intended to describe the new species and, in part, to facilitate distinguishing it from *L. plagicephalus* in past and future surveys. Earlier collections of *L. plagicephalus* may need to be re-examined for identity verification. The present collection was made available to the first author for routine diagnostic purposes, and specimens were instantly recognized as representing a new species especially after viewing the SEM images.

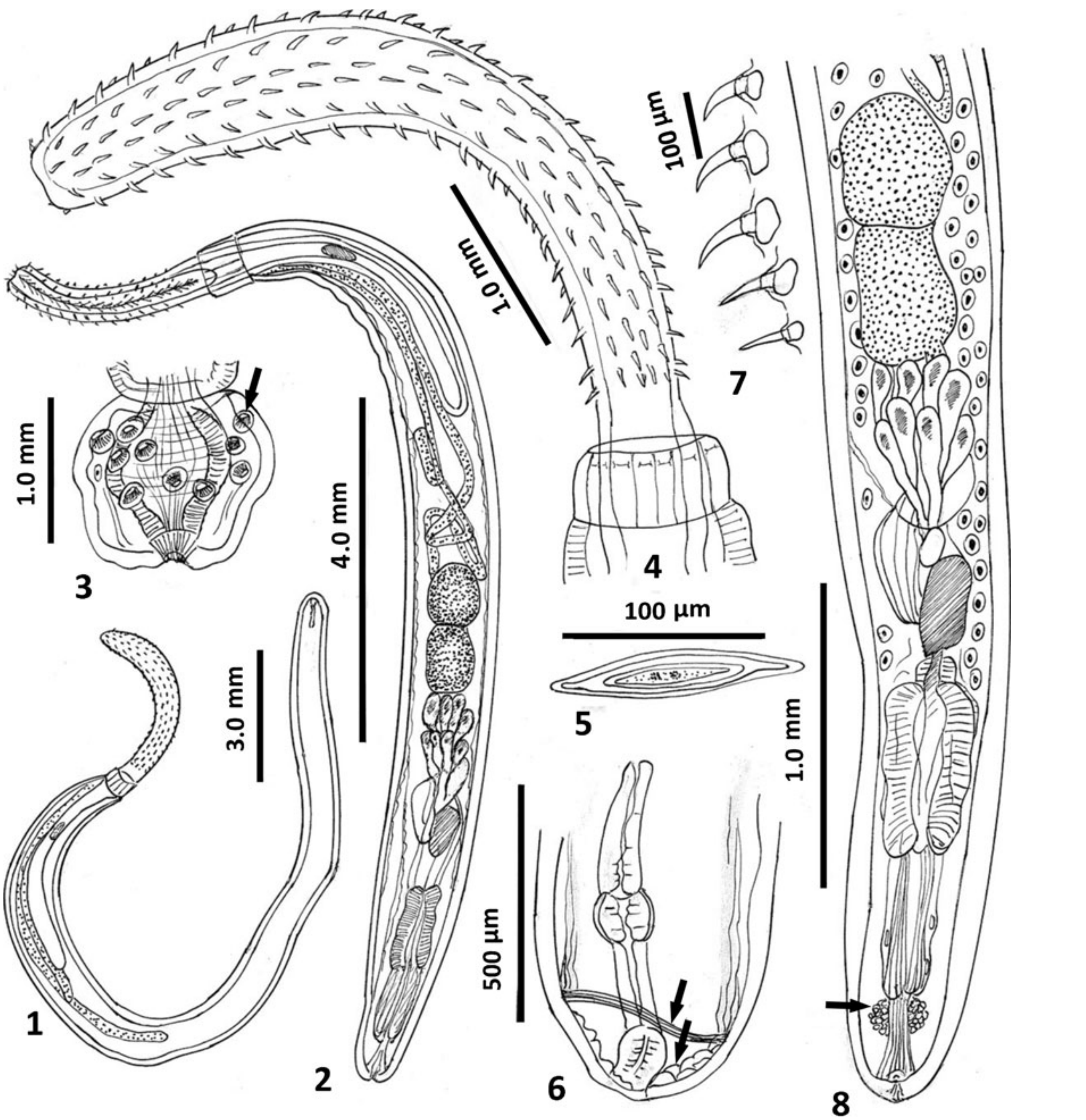
## Materials and methods

Sixteen specimens of acanthocephalans were collected from the intestines of two adult male *A. stellatus* in June 2008 off the southern coast of the Caspian Sea near Ramsar City and ten more specimens from one adult male *A. nudiventris*, collected on 15 October 2009, from Tonekabon City, the Chaparsar area (36°56′56.87″N, 50°38′03.65″E). The GI tracts of the freshly dead fish were obtained from fisheries stations of the Iranian Fisheries Organization (IFO) that were originally caught by certified governmental centers. The IFO program was to catch breeder sturgeons for artificial breeding and release of fingerlings to improve the sturgeon population in the Caspian Sea. Acanthocephalans were fixed and shipped in 70 % ethanol to the Institute of Parasitic Diseases (PCI), Scottsdale, Arizona for optical microscopy for descriptive purposes and to Brigham Young University, Provo, Utah for SEM and histopathological studies.

Upon collection, specimens were placed in refrigerated water for 1–2 days to extend the proboscis then transferred to equally cold 70 % ethanol for fixation. For optical microscopy, 16 worms were punctured with a fine needle and subsequently stained in Mayer's acid carmine, destained in 4 % hydrochloric acid in 70 % ethanol, dehydrated in ascending concentrations of ethanol (24 h each), and cleared in graduated concentrations of terpineol in 100 % ethanol to 100 % terpineol, then 50 % terpineol in 50 % Canada balsam (24 h each). Whole worms were then mounted in Canada balsam. Measurements are in micrometers, unless otherwise noted; the range is followed by the mean values in parentheses. Width measurements represent maximum width. Trunk length does not include proboscis, neck, or bursa. Type specimens were deposited in the University of Nebraska's State Museum's Harold W. Manter Laboratory (HWML) in Lincoln, Nebraska.

For histopathological sections, infected host tissues were fixed in 10 % buffered formalin, and after dehydration and embedding, the specimens were processed using standard methods (Bancroft and Gamble 2001; Kienan 2002). The paraffin-blocked tissue was sectioned at 4–6 μm, placed on glass slides, and stained with hematoxylin and eosin (HE). Additional sections were stained with Mallory's trichrome to emphasize pathological responses to the parasite (Galigher and Kozloff 1971). The prepared glass slides were viewed with an LSM laser (Carl Zeiss, Thornwood, NY, USA) equipped with compound light microscope with representative pictures taken at varying magnifications with a digital camera.

For SEM studies, specimens previously fixed in 70 % ethanol were placed in critical point drying baskets and dehydrated using ethanol series of 95 % and 3 N 100 % for at least 10 min per soak followed by critical point drying (Lee 1992). Samples were mounted on SEM sample mounts, gold coated at 15 nm, and observed with a scanning electron microscope (XL30 ESEMFEG; FEI, Hillsboro, OR, USA). Digital images of the structures were obtained using digital imaging software attached to a computer.




**Figs. 1–8** *Leptorhynchoides polycristatus* from *Acipenser stellatus* from the Caspian Sea. **1** Allotype female; many ovarian balls and hypodermal cells are not shown. **2** Holotype male; hypodermal cells are not shown; bursa is withdrawn. **3** Everted male muscular bursa with many suction cup-like sensory structures (*arrow*). **4** Proboscis of allotype female. Note anterior trunk girdle with longitudinal plates (also in Figs. 1 and 2). **5** Ripe egg. **6** Simple reproductive system of a paratype female. Note the absence of nucleated uterine bell pouches, the near terminal position of the gonopore, the presence of dorsoventral ligament (*upper arrow*), and the posterior cluster of paravaginal cells (*lower arrow*). **7** Ventral proboscis hooks nos. 1 (apical), 2 (sub-apical), 10 (mid), 15 (post), and 19/20 (basal) in a male specimen. Note the short collar of cuticular membrane encircling the base of hooks, the loss of curvature of posterior spine-like hooks, and the shape of the short rounded roots and manubria in anterior and middle and their virtual absence in the basal hooks. **8** Reproductive system of the holotype male in Fig. 2. Note the many hypodermal nucleated cells, the clustering of the cement glands in two tiers of four glands each, and the paired glandular clusters near the reproductive opening (*arrow*)

## Results and discussion

Of the 26 specimens collected, 16 worms (eight males and eight females) collected from two specimens of *A. stellatus* and one specimen of *A. nudiventris* in the southern Caspian Sea were processed and studied microscopically. The specimens proved to represent a new species of *Leptorhynchoides* Kostylew 1924, which may have been previously confused with *L. plagicephalus* because of superficial similarities. Both species are found in Sturgeons in the Caspian Sea. No taxonomic treatment of *L. plagicephalus* has been reported since Koslylew's (1924) description, and all descriptive accounts of this species, e.g., Petrochenko (1956), Yamaguti (1963), and Golvan (1969), quoted Kostylew's description and used his figures. In this treatment of selected specimens from *A. stellatus* and *A. nudiventris* collected in the Caspian Sea in June 2008 and October 2009, respectively, we are describing the new species below. We suspect that careful future taxonomic studies or studies of old collections from sturgeons in the Caspian or the Black Sea will reveal the presence of this new species of *Leptorhynchoides* among specimens previously identified as *L. plagicephalus*.

## Description Leptorhynchoides polycristatus n. sp.

*General* Characteristics of *Leptorhynchoides* (Rhadinorhynchidae) are as emended below. Trunk is cylindrical, slender, tapering towards both ends, rounder posteriorly, and usually with anterior collar, with unique set of multiple rectangular cuticular crests (Figs. 1, 2, 4, 9, 10, 13). Females are about twice as long as males with prominent sexual dimorphism in shared structures. Body wall has many large hypodermal uninucleated ovoid cells (Fig. 8). Tegument has structures interpreted as possible microtriches at anterior trunk (Fig. 14) and micropores in anterior, middle, and posterior trunk regions (Fig. 15–17). The proboscis is long and slightly swollen anteriorly (Figs. 1, 4), with 12 to 15 (usually 12–13) longitudinal rows of 19–20 hooks each. Dorsal proboscis hooks are slightly shorter and less robust than ventral hooks (Fig. 4). All hooks are with longitudinal grooves on ribbed surface (Fig. 12) and short but definite cuticular collar around base (Figs. 7, 11). Apical and subapical hooks are smallest, with longest in the middle; they slightly decrease in length posteriorly. Anterior and middle hook blades are sharply curved posteriorly; posterior hooks are increasingly less

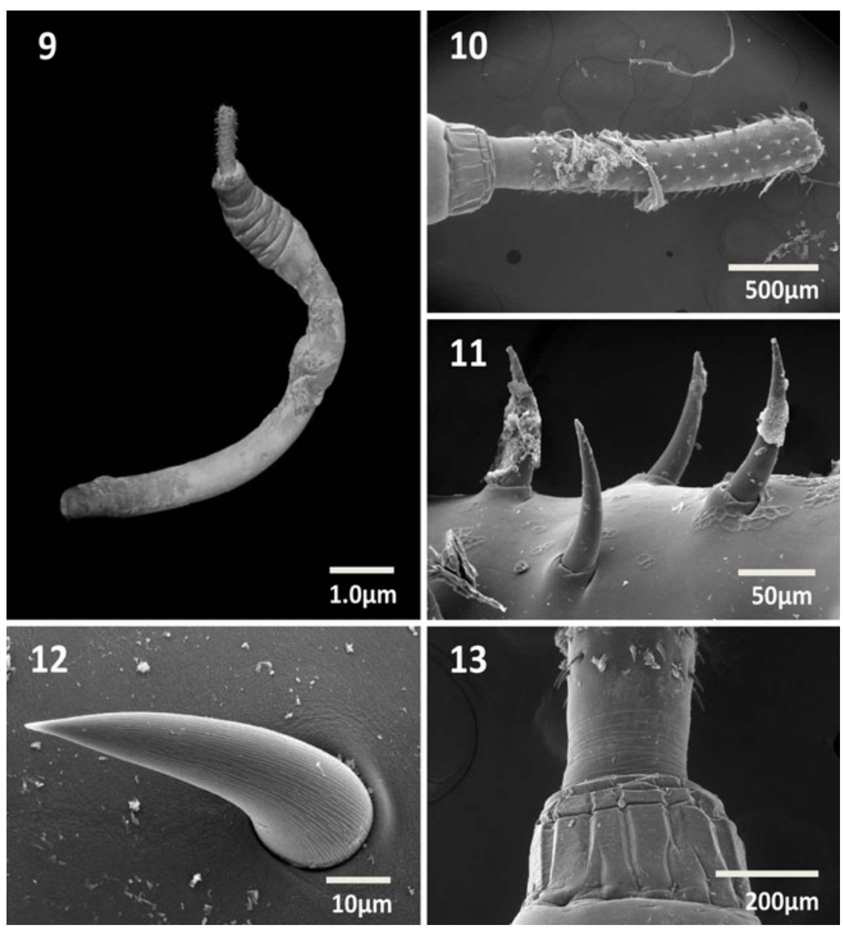



**Fig. 9–13** SEM of *Leptorhynchoides polycristatus* from *Acipenser stellatus* from the Caspian Sea. **9** A composite image of a female showing the proportion of proboscis length to trunk length and the slight anterior enlargement of the trunk. **10** The partially retracted cylindrical proboscis of a paratype male showing the long neck and anterior trunk with cuticular grid and longitudinal plates. **11** A few proboscis hooks at the middle of the proboscis; note their curvature and the short cuticular collar at their base. **12** A high magnification of a middle proboscis hook showing the characteristic serrations on its surface. All proboscis hooks are serrated. **13** An enlargement of the posterior proboscis–anterior trunk region of specimen in Fig. 10 showing the long neck and the anterior cuticular trunk grid with plates

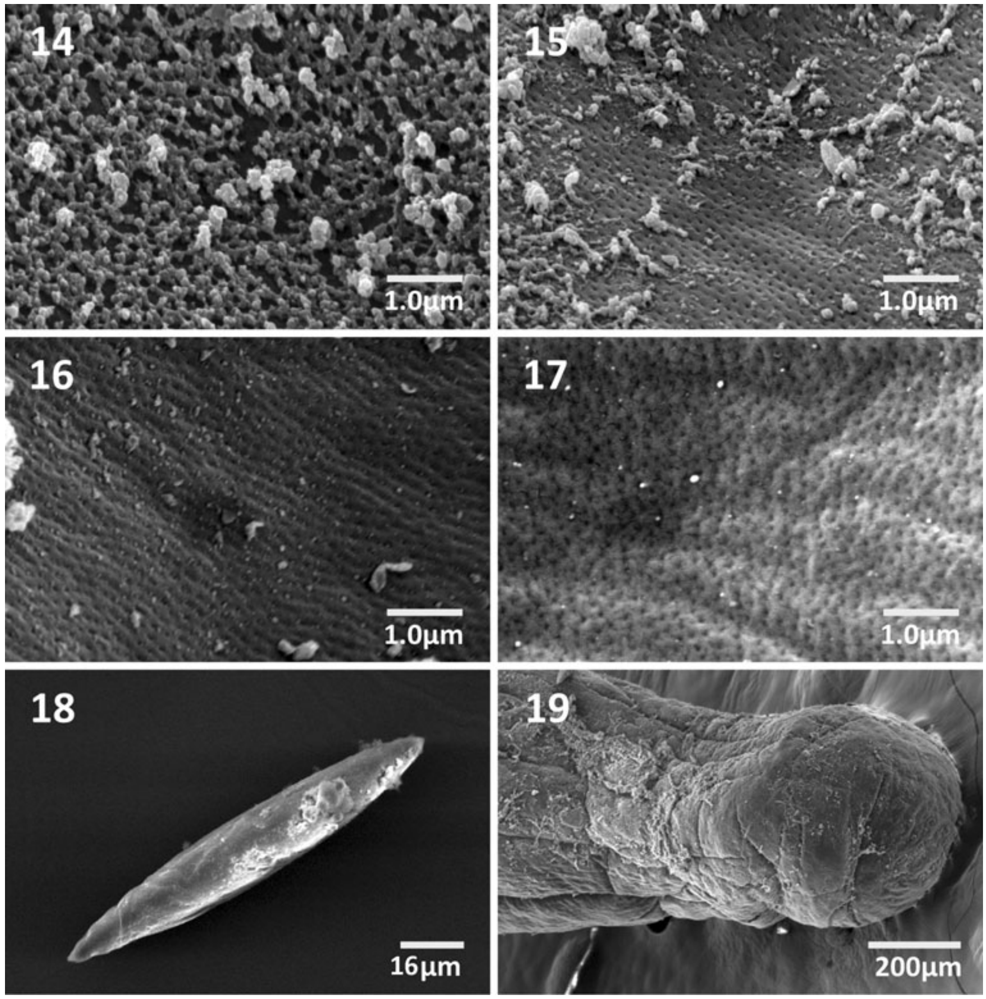



**Fig. 14–19** SEM of *Leptorhynchoides polycristatus* from *Acipenser stellatus* from the Caspian Sea. **14** Microtrich-like structures on the epidermis of the anterior trunk region. **15**–**17** Epidermal micropores at anterior, middle, and posterior trunk, respectively. Note differences in pore diameter and distribution. **18** Egg. **19** The posterior end of a female showing the near terminal slit-shaped gonopore

curved and more straight and spine-like posteriorly and basally. Hook roots are thick and short with manubria; basal hooks are rootless (Fig. 7). Neck is prominent (Figs. 4, 10). Proboscis receptacle is slightly longer than proboscis, with cephalic ganglion in anterior half. Lemnisci are long, slender, and unequal (Figs. 1, 2).

*Male (based on eight mature adults)* Trunk is 5.00–13.00 (9.37)mm long by 0.47–1.00 (0.81)mm wide anteriorly. Proboscis is 2.20–2.90 (2.47)mm long by 0.22–0.35 (0.30)mm wide anteriorly. Apical proboscis hooks are 68–93 (80) long dorsally and 70–100 (83) long ventrally, middle hooks are 92–105 (98) long dorsally and 92–110 (101) long ventrally, and basal hooks are 77–95 (84) long dorsally and 72–100 (85) long ventrally. Neck is 281–325 (308) long by 300–374 (341) wide. Proboscis receptacle is longer than proboscis: 2.50–4.30 (3.70)mm long by 0.20–0.35 (0.28)mm wide anteriorly. Short lemniscus is 2.10–4.50 (2.90)mm long by 0.14–0.17 (0.15) mm wide; long lemniscus is 4.62–6.00 (5.15)mm long by 0.16–0.23 (0.19)mm wide. Reproductive system in posterior half of trunk includes ovoid testes and eight cement glands clustered in two horizontal tiers with four glands in each (Figs. 2, 8). Anterior testis is 312–925 (645) long by 275–525 (426) wide; posterior testis is 240–875 (520) long by 350–675 (474) wide. Posterior end of reproductive track includes two clusters of glandular cells near terminal gonopore (Fig. 8). Bursa has large suction cup-like sensory structures (Fig. 3).

*Female (based on eight adult specimens with eggs and/or ovarian balls)* Trunk is 13.25–22.50 (17.53)mm long by 0.95–1.25 (1.07)mm wide anteriorly. Proboscis is 2.50–3.62 (3.11)mm long by 0.34–0.48 (0.38)mm wide anteriorly. Apical proboscis hooks are 75–114 (96) long dorsally and 82–124 (103) long ventrally, middle hooks are 100–125 (118) long dorsally and 110–146 (125) long ventrally, and basal hooks are 82–114 (97) long dorsally and 95–114 (104) long ventrally. Neck is 312–520 (387) long by 280–406 (359) wide. Anterior trunk collar is 312–354 (326) long by 437–624 (544) wide. Proboscis receptacle is longer than proboscis: 3.50–4.15 (3.82) long by 0.22–0.47 (0.34) wide anteriorly. Short lemniscus is 4.50–6.00 (5.22)mm long by 0.12–0.21 (0.15)mm wide; long lemniscus is 6.00–7.07 (6.61)mm long by 0.16–0.22 (0.18)mm wide. Reproductive system is simple with no prominent uterine bell cells noted (Fig. 6) and is 650 long. Gonopore is near terminal (Figs. 6, 19). Dorsoventral ligament across vagina–uterus junction has less prominent dorsal and ventral branches directed anteriorly (Fig. 6). Fully developed eggs are elongated, smooth, spindle-shaped with polar prolongation of fertilization of fertilization membrane (Figs. 5, 18), and 125–146 (135) long by 21–26 (22) wide.

## Taxonomic summary

*Type host*: Starry sturgeon *A. stellatus* Pallas 1771
*Other host*: Fringebarbel sturgeon *A. nudiventris* Lovetzsky 1828
*Site of infection*: Intestine
*Type locality*: The Caspian Sea near Ramsar City and near Tonekabon City, the Chaparsar area (36°56′56.87″ N, 50°38′03.65″E)
*Type specimens*: HWML coll. no. 49765 (holotype male), 49766 (allotype female), and 49767 (paratypes)
*Etymology*: The new species is named for the multiple cuticular plates of the anterior trunk collar

## Remarks

Of the three known species of *Leptorhynchoides* Kostylew 1924, two are reported from North American fishes: *Leptorhynchoides thecatus* (Linton 1891) Kostylew 1924 is commonly found in at least six families of fishes, especially Centrarchidae in the USA and Canada, and *Leptorhynchoides aphredoderi* Buckner and Buckner 1976 with a limited distribution from the pirate perch, *Aphredoderus sayanus* (Gilliams) in Louisiana. The third species, *L. plagicephalus* (Westrumb 1821) Kostylew 1924, is commonly found in at least four species of sturgeons including *A. stellatus* and *A. nudiventris* from the Caspian and Black seas and associated waters. No taxonomic work has been reported for *L. plagicephalus* for the last 90 years. *L. polycristatus* n. sp. overlaps in host distribution with *L. plagicephalus* because it is also found in the stellate and bastard sturgeons from the Caspian Sea and can be readily be confused with *L. plagicephalus* because of many superficial similarities. Such similarities include the general shape of the trunk, proboscis, and organ systems. An outline of the major characteristics distinguishing the new species from the three other species of *Leptorhynchoides* is presented in Table 1.

*L. polycristatus* n. sp. is easily separated from the two freshwater North American species by having large ovoid uninucleated hypodermal cells instead of branched subcuticular nuclei and considerably more proboscis hooks per row, among other features summarized in Table 1. It is quite important to distinguish the new species from *L. plagicephalus* with which it may have been confused in much of the reported literature. Morphometrically, *L. polycristatus* has fewer proboscis hooks per row and thinner eggs than *L. plagicephalus*. In *L. polycristatus*, proboscis hooks are smallest anteriorly and increase in size to reach 146 in females in mid-proboscis then gradually decrease in size posteriorly. In *L. plagicephalus*, the largest hooks are anterior reaching only 115 in length, and they gradually decrease in size posteriorly (Table 1). Qualitatively, *L. polycristatus* is distinguished by having anatomical structure that are absent in *L. plagicephalus*. Such structures include unique cuticular collar around the base of hooks, anterior trunk collar, hypodermal uninucleated cells, the arrangement of the cement glands in two tiers of four glands each, two glandular clusters near male gonopore, large suction cup-like sensory structures on the bursa, dorsoventral ligament crossing over the vagina–uterus junction with anterior branches, near terminal but not terminal female gonopore, and most importantly, cuticular structures interpreted as possible microtriches. *L. polycristatus* is the only species of the genus that has an anterior cuticular trunk collar.

The surface striations noted on the proboscis hooks have been previously reported in two other species of acanthocephalans: *Dentitruncus truttae* Sinzar 1955 (Palaeacanthocephala) by Dezfuli et al. (2008) and *Rhadinorhynchus ornatus* Van Cleave 1918 (Rhadinorhynchidae) by Amin et al. (2009). Trunk spines in both *D. truttae* and *R. ornatus* had unstriated smooth surfaces. Dezfuli et al. (2008) speculated that hook striations may provide more effective attachment to the host's intestine. It should be noted, however, that most species of acanthocephalans have not been critically examined with SEM and that hook surface striations may be more common than reported.

The anterior trunk collar is a broad collar with a unique set of multiple rectangular cuticular crests encircling the anterior end of the trunk. These crests are more readily seen in SEM images and may not be readily distinguishable or consistently observable by optical microscopy. In the latter cases, specimens of *L. polycristatus* may be readily misidentified as *L. plagicephalus* by the casual observer because of the superficial similarities in the shape of the proboscis, proboscis hooks, eggs, trunk, and organ systems.

Structures interpreted as microtriches have been reported only once before in *R. ornatus* Van Cleave 1918, another rhadinorhynchid acanthocephalan collected from marine fish in the Pacific Ocean (Amin et al. 2009). The entities depicted in Fig. 14 suggest the possibility of microtriches. The verification of these structures, previously only reported in cestodes (Chervy 2009), is a possibility that should be investigated with additional studies using properly fixed material that can provide more details with

**Table 1** Characteristics distinguishing *Leptorhynchoides polycristatus* from the three other species of *Leptorhynchoides*

| Character | *L. thecatus* | *L. aphredoderi* | *L. plagicephalus* | *L. polycristatus* |
|---|---|---|---|---|
| Distribution | North America | Louisiana, USA | Caspian and Black Sea | Caspian and Black Sea |
| Hosts | At least 6 families of fish especially centrarchids | *Aphredoderus sayanus*, localized | Sturgeons, common *Acipenser nudiventris*, *A. stellatus*, *A. persicus*, *A. guldenstadtii* | Sturgeons, common *Acipenser nudiventris*, *A. stellatus* |
| Environment | Freshwater | Freshwater | Marine | Marine |
| Trunk length (mm) | 3.0–13.0 (M), 6.0–26.0 (F) | 3.2–4.4 (M), 2.6–6.4 (F) | 8.0–13.0 (M), 20.0–25.0 (F) | 5.0–13.0 (M), 13.2–22.5 (F) |
| Microtrich-like structures | Absent | Absent | Absent | Prominent on trunk |
| Proboscis hook rows | 12 | 15–18 (usually 17 or 18) | 14 | 12–15 (usually 12–14) |
| No. hooks per row | 11–16 (usually 12–13) | 11–16 (usually 12–14) | 22–24 | 19–20 |
| Collar at hook base | Well-developed | Absent | Absent | Short |
| Surface of hooks | Smooth | Smooth | Smooth | Ribbed |
| Ant., mid., post. hook length | 56–90, 63–97, 34–78 | 40–48, 40–50, 33–45 | 109–115, 66–99, 82 | 68–124, 92–146, 72–114 |
| Lemnisci | Long | Short, equal | Long | Long, unequal |
| Anterior trunk collar | Absent | Absent | Absent | Prominent |
| Subcuticular nuclei | Branched, reticular | Branched, reticular | Branched, reticular | Large uninucleated ovoid cells |
| Cement glands | 8, 2–3 glands at 3–4 levels | 8, clustered | 8, in 4 tandem pairs | 8, in 2 tiers of 4 glands each |
| Glands near male gonopore | None reported | None reported | None reported | 2 clusters |
| Ligament by vagina–uterus across vagina | None reported | None reported | None reported | Present with ant. branches |
| Female gonopore | Terminal | Terminal | Terminal | Near terminal |
| Eggs | 65–110×24–30 | 145–150×20–25 | 136–150×35–39 | 125–146×21–26 |
| Sensory structures on bursa | Absent | Absent | Absent | Prominent |

transmission electron microscopy. Structures interpreted as microtriches in these two species of rhadinorhynchid acanthocephalans do not appear to be homologous with microtriches of cestodes as described by Chervy (2009). See Amin (2013) for a thorough discussion of microtriches and the phylogenetic relationships between Acanthocephalans and cestodes.

A few other acanthocephalans species were observed to have a porous trunk tegumental surface similar to that observed in *L. polycristatus* (Fig. 15–17). A list of these species is included in Amin et al. (2009). Wright and Lumsden (1970) and Byram and Fisher (1973) further reported that these peripheral canals are continuous, with canalicular crypts. These crypts appear to "constitute a huge increase in external surface area…implicated in nutrient uptake." Whitfield (1979) estimated a 44–fold increase at a surface density of 15 invaginations per 1 $\mu m^2$ of the tegumental surface of *Moniliformis moniliformis* (Byram and Fisher 1973). Surface crypts may be involved in pinocytosis and lysosomal activity (Miller and Dunagan 1976).

Generic diagnosis of *Leptorhynchoides*

Using the new information provided by this study, the diagnosis of *Leptorhynchoides* as reported by Petrochenko (1956), Yamaguti (1963), and Golvan (1969) is emended as follows. New diagnostic features are bolded:

Palaeacanthocephala, Rhadinorhynchidae: Trunk cylindrical, aspinose, **occasionally with broad anterior collar having longitudinal plates**. Hypodermis with dendritic nuclei and **occasionally large uni-nucleated ovoid cells**. Longitudinal parietal muscluture in 4 bands. Proboscis subcylindrical, with **12-18 rows** of 11-24 hooks each. **Proboscis hooks smooth or striated; with or without cuticular collar enveloping base**. Proboscis receptacle cylindrical, double-walled; cephalic ganglion in anterior half. Lemnisci tubular to filiform, **equal or unequal**, **very long or short**. Testes contiguous, equatorial or postequatorial. Cement glands 8 in number directly behind testes, **clustered**, **in 4 tandem**

**pairs**, **or in 2 tiers of 4 glands each**. Eggs elongate fusiform with polar prolongation of fertilization membrane. Parasites of freshwater fishes **in North America** and marine fishes **in the Caspian and Black Sea**.

## Histopathology

The results of the histopathology study are presented in Figs. 20–33. Normal intestinal tissue of the host (Fig. 20) characterized by intact villi and deep crypts of the mucosa which are lined with columnar epithelial tissue is invaded by the armed proboscis of *L. polycristatus* (Fig. 21). Figures 21 and 23 shows the hooks of the acanthocephalan attached to the mucosal lining of the intestine causing hemorrhaging, migration of granulocytes, and compaction of the villi. Necrotic epithelial tissue from the mucosal layer of the intestine is evident (Fig. 23). Numerous nucleated red blood cells and granulocytes are visible near the invading proboscis. The worm extends into the crypt (deep mucosa) area (Fig. 22) towards the submucosa and muscularis externa. The presence of *L. polycristatus* in

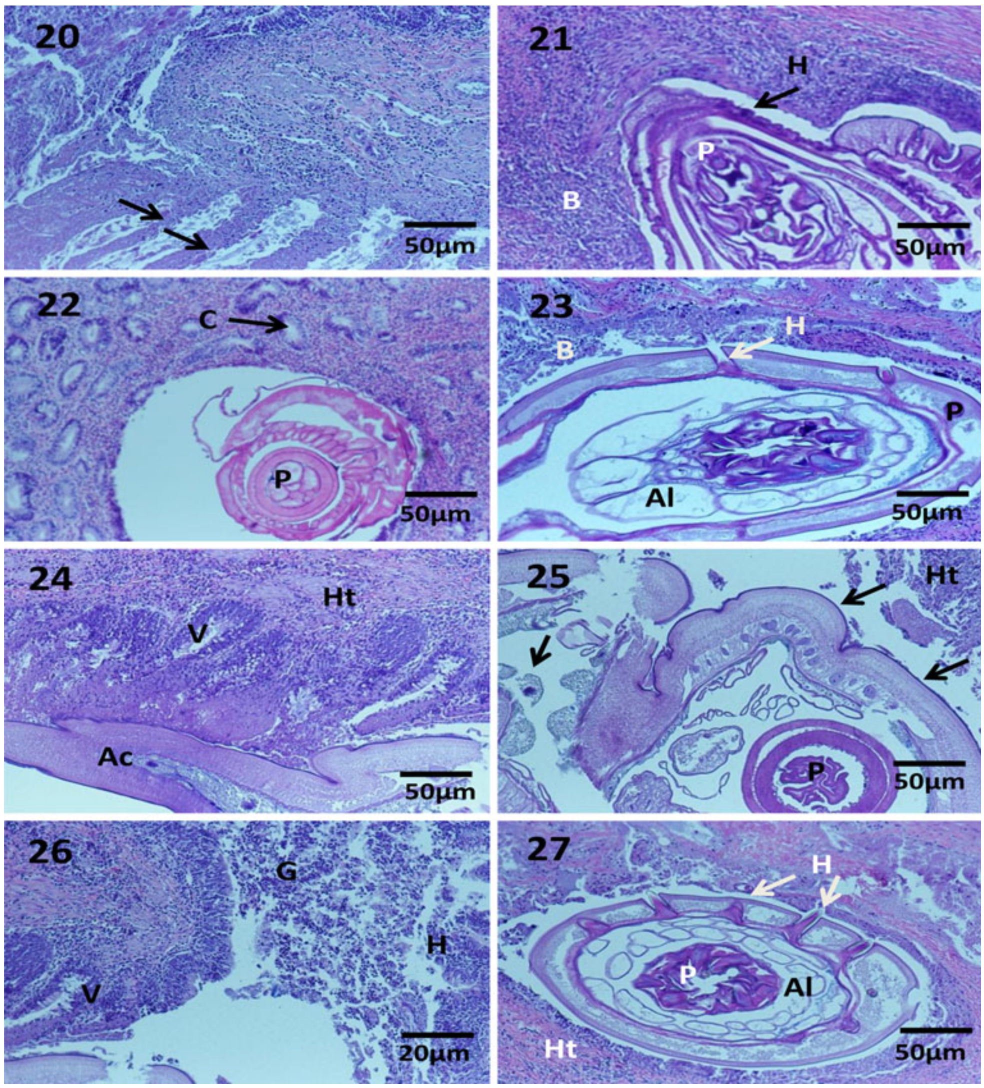



**Fig. 20–27** Histopathological sections of *Leptorhynchoides polycristatus* from *Acipenser stellatus* from the Caspian Sea. **20** Normal host intestinal tissue showing the mucosa and prominent villi (*arrows*) lined with epithelial cells and inner crypts. **21** The proboscis (*P*) of *L. polycristatus* invading host mucosa. Hooks (*H*) are visible with extensive hemorrhaging surrounding the proboscis (*B*) and migrating granulocytes. **22** The proboscis (*P*) has extended into the crypt region (*C*) of the host mucosa. **23** The proboscis (*P*) of a worm invading host mucosa. Hooks (*H*) are visible with subsequent host hemorrhaging (*B*). Note alveolar lobes (*Al*) within the proboscis and the proboscis sheath. **24** A view of parasite compression (*Ac*) of the host tissue (*Ht*) damaging the villi (*V*) of the intestinal mucosa. **25** A cross section of the parasite proboscis (*P*) and parasite tissue (tegument); *arrows to the right* that has invaded the host tissue (*Ht*). Note the necrotic villi in the *upper left-hand corner* and *left side* (*arrow*). **26** Host tissue that has been invaded by *L. polycristatus*. Note necrotic villi (*V*) with extensive hemorrhaging (*H*) and migrating granulocytes (*G*). **27** A cross section of the proboscis (*P*) of *L. polycristatus* inverted into the host mucosal layer (*Ht*). Note damaged and necrotic tissue at *upper right-hand corner*. Proboscis hooks (*H*) and alveolar lobes (*Al*) and the hooks of the tip of the proboscis are visible

the host intestine obstructs and damages the absorbing surface of the host probably affecting the nutritional potential of the mucosal lining (Figs. 24 and 25). The obstruction and congestion of the lumen of the host have been reported by other observers for acanthocephalan infections including those of *Leptorhyn-choides* sp. (Venard and Warfel 1953; Bullock 1963; Amin et al 2012). The alveolar lobes (Al) and proboscis receptacle are visible in these sections (Figs. 23 and 27). Necrotic tissue and hemorrhaging are common near the invading proboscis with villi compression (Figs. 26–28). Nucleated red blood cells and granulocytes are visible near the invading worm (Fig. 28). The outer tegument and cross section of the worm are prominent with subsequent layers of the tegument being visible. Hooks can be seen attaching to the host tissue causing extensive damage (Figs. 28 and 29). Remnants of necrotic villi and connective tissue are common near the armed proboscis. The acanthocephalan migrates through the outer mucosa and submucosal layers into the outer muscular layers (muscularis externa; Fig. 30). Figures 31 and 32 shows the proboscis of *L. polycristatus* deep into the smooth muscle layers. The hooks are visible attached to the smooth muscle fibers with subsequent damage to the capillary network causing hemorrhaging and granulocyte migration. The hooks of the proboscis are occasionally not fully extended (Figs. 30–32), which are also visible on the SEM micrographs (Fig. 10). No encapsulation with collagenous connective tissue is visible; thus, the invading worm continues to migrate into the intestinal host layers (Figs. 31 and 32). Smooth muscle fibers are visible surrounding the proboscis with numerous acanthocephalan hooks disorganizing the host muscle layers (Fig. 33). The general host response to the initial entry of this worm is manifested as hemorrhaging, tissue necrosis, and occlusion of the absorptive surfaces. Common to all sections for the acanthocephalan invasion is extensive hemorrhaging and subsequent necrotic tissue.

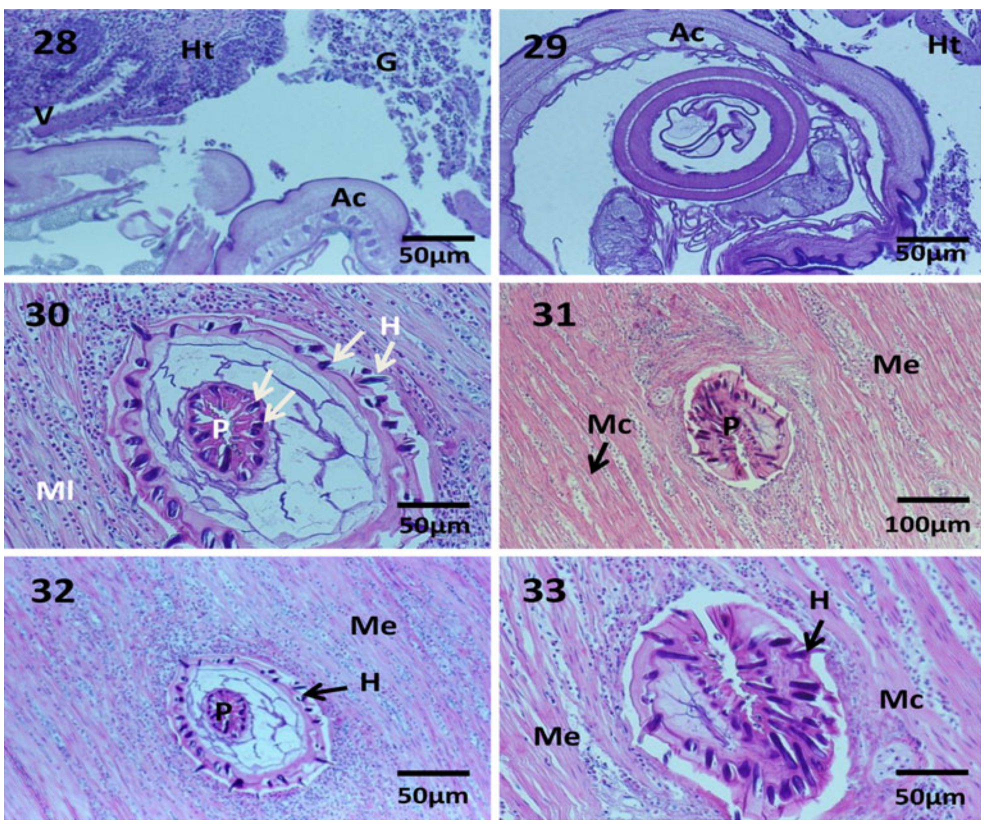



**Fig. 28–33** Histopathological sections of *Leptorhynchoides polycristatus* from *Acipenser stellatus* from the Caspian Sea. **28** Host tissue (*Ht*)–parasite (*Ac*) interface showing necrotic villi (*V*) and granulocytes (*G*). **29** The invading acanthocephalan (*Ac*) has extended further into the lower parts of the mucosa. The hooked proboscis and proboscis receptacle invading the host tissue (*Ht*). Note compressed crypts. **30** Lateral view of an inverted proboscis (*P*) with prominent hooks (*H*) invading the muscularis externa of the host in the process of entering the muscular layers (*Ml*) of the host intestine. Note the terminal hooks (*inner arrows*) are retracted into the proboscis. Hemorrhaging visible immediately around the proboscis. **31** Proboscis (*P*) with prominent hooks that penetrated into the muscle layer of the host intestine. Smooth muscle cells (*Mc*) of the host intestine are visible in the muscularis externa (*Me*). **32** The proboscis (*P*) has invaded the outer muscle layers of the muscularis externa (*Me*). Note the tip of the proboscis is still inverted. Hooks (*H*) are prominent. **33** Higher magnification of Fig. 32 showing the hooks (*H*) of the well-armed proboscis attached to the muscle cells (*Mc*) of the outer muscularis externa (*Me*) of the host intestine. Hemorrhaging is seen surrounding the invasion site. No encapsulation with connective tissue was noted

There is no evidence of encapsulation of the parasite by the host. The invading properties of *L. polycristatus* are well depicted by this study of the infected host intestine representing classic tissue pathology (Bullock 1963).

**Acknowledgments** The authors wish to thank the Histology Laboratory (Mike Downey, Director) at the Provo Utah Valley Regional Medical Center for histopathology slide preparation and Michael Standing, BYU Electron Optics laboratory, for his professional help for the electron optics (SEM) part of the study. Thanks are also extended to the fishing stations in Northern Iran (Mazandaran and Guilan provinces) for providing the opportunity of collecting the gastrointestinal tracts of sturgeons for AH and ST.

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