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Microscopic studies of *Neoechinorhynchus agilis* (Acanthocephala: Neoechinorhynchidae) infecting the Thinlip mullet *Liza ramada* (Mugilidae)

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ABSTRACT

Acanthocephalan parasite, *Neoechinorhynchus agilis*, is recovered and isolated from the Thinlip mullet *Liza ramada* obtained from the Abu Qir Coasts, Alexandria City, Egypt. Infection with this parasite species has been observed in the intestine for the examined fish. Morphological characterization was performed using light and scanning electron microscopic studies to determine the most characteristic features of the recovered Eoacanthocephalan parasite, such as the presence of globular proboscis with three rows of 6 hooks on each row, single-walled proboscis receptacle inserted at the proboscis base, and long lemnisci. Male worms characterized by testes of tandem position, large cement gland, and saefftigen's pouch that underlying ducts of seminal vesicle and cement gland. While, female worms have a vagina with vaginal sphincter and opening by funnel into the uterus, selector apparatus between the uterus and uterine ball, ovarian mass fills the space of uterine ball, a genital pore is subterminal and provided with caudal papillae. Furthermore, it compared morphometrically with other *Neoechinorhynchus agilis*, which had previously described and showed little difference in measurements for different body parts.
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Introduction

Acanthocephalans is the smallest parasite group of nearly 1100 species (Bush et al., 2001). More than half of these worms are found in wild and captive fish (Nickol, 2006). The life cycle of acanthocephalans is indirect and is based on the food chain, which means using arthropods as an intermediate hosts, vertebrates as final hosts, and sometimes paratenic hosts (Santos et al., 2013; Mikhailova & Kusenko, 2018; Chagas et al., 2019).

The genus *Neoechinorhynchus* Stiles and Hassall, 1905 is one of the most diverse genera within Acanthocephala with approximately 101 species described from mostly fish, some reptiles and amphibians throughout the world (Amin, 1985, 2002; Shih, 2004; García-Varela et al., 2005; Smith et al., 2005; Pinacho-Pinacho et al., 2017; Amin et al., 2018). These species are characterized by a small globular or sub-cylindrical proboscis that armed with hooks arranged in three circles, and cerebral ganglion at the base of the proboscis receptacle with a single-walled. In addition, male worms have two spherical to oblique testes that are equatorial or post-equatorial with a single syncytial cement gland, the eggs being elliptical or elongated with concentric shells or with polar expansion of fertilization membrane, and genital pore terminal in both sexes or sub-terminal in females (Amin & Heckmann, 1992).

Therefore, the objective of the present investigation was to detect morphological and morphometric characterizations of acanthocephalan parasite infecting the Thinlip mullet *Liza ramada*.

Materials and methods

Twenty specimens of the Thinlip mullet *Liza ramada* (Family: Mugilidae) were obtained in 2019 on the coasts of Abu Qir, Alexandria City, Egypt (longitude 29° 47.1′–29° 50.4′ E and latitude 31° 7.5′–31° 09′ N). The fish samples were transferred to the

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Parasitology Research Laboratory at Zoology Department, Faculty of Science, Cairo University, Egypt. Fish were dissected and body cavity was thoroughly examined for parasitic infections. The intestine was placed in petri dishes containing normal saline. The observed acanthocephalan parasites were left in the refrigerator overnight to allow protrusion of proboscis to come out then fixed with hot glycerol-alcohol (5% glycerin in 70% ethyl alcohol), cleared in clove oil, stained with Semichon's acetocarmine, and then

Fig. 1. Photomicrographs of *N. agilis* infecting *L. ramada* showing: A The whole female worm. B–G High magnifications showing: B, C The anterior end. D, E The middle part of male worm. F The posterior end of male. G The posterior end of female. Note: BU, bursa; CA, cap; CG, cement gland; CSD, common sperm duct; F, folds; GP, genital pore; HO, hooks; L1, L2, lemnisci; OM, ovarian mass; PR, proboscis; RP, proboscis receptacle; SP, saefftigen's pouch; SV, seminal vesicle; T, trunk; TE, testes; U, uterus; UB, uterine ball; VA, vagina.

examined for internal details. Photomicrographs were taken using a Leica DM 2500 microscope (NIS ELEMENTS software, ver. 3.8). The isolated parasites were fixed with 4% glutaraldehyde for scanning electron microscopy (SEM), then washed with cacodylate buffer, fixed with aqueous OsO_4 for 4 h, dehydrated with acetone, and dried with liquid CO_2, placed on an aluminum stub, covered with gold palladium, and then examined using Etec Autoscan JEOL scanning electron microscope (JSM-6060LV). The measurements are shown in millimeters as the range followed by means ± SD in parentheses.

Results

Of the twenty specimens of Thinlip mullet *Liza ramada*, eleven (55%) were infected with *Neoechinorhynchus agilis*. The infection with the parasite was reported in the infected fish's intestine. Seasonally, the infection increased to 70% (7 out of 10) in summer and fell to 40% (4 out of 10) in winter.

Microscopic examination

The body of the worm was long-cylindrical with truncated ends. It's covered with thick folds of tegument. The proboscis was small and globular, with 18 hooks arranged in three spiral rows, with six hooks in each row. The first row's hooks were the largest. The proboscis receptacle was thin, single-layer, cylindrical inserted at the base of the proboscis. Proboscis followed by neck region. Lemnisci long, unequal, rod-shaped but narrow anteriorly (Figs. 1 and 2).

The body of a male worm

The length of proboscis was 0.092–0.108 (0.101 ± 0.01) mm by 0.123–0.146 (0.137 ± 0.001) mm wide. Apical proboscis hooks were 0.088–0.098 (0.090 ± 0.001) mm long, middle hooks measured 0.049–0.053 (0.050 ± 0.001) mm long, and basal hooks measured 0.040–0.049 (0.045 ± 0.001) mm long. The trunk length was 5.98–8.12 (6.93 ± 0.1) mm. Testes were elongated oval and tandem. The length of the anterior testis was 0.821–1.11 (0.983 ± 0.1) mm. While, the length of the posterior testis was 0.722–1.01 (0.921 ± 0.1) mm. Cement gland large, rectangular and packed with giant nuclei. The cement gland reservoir was a small rounded sac that overlapped the cement gland. The seminal vesicle elongated and opening separately into genital bursa. Saefftigen's pouch underlying ducts of seminal vesicle and cement gland. Muscular bursa provided with many sensory papillae and 0.138–0.304 (0.222 ± 0.1) mm long. Cirrus sac in bursa. Genital pore was terminal.

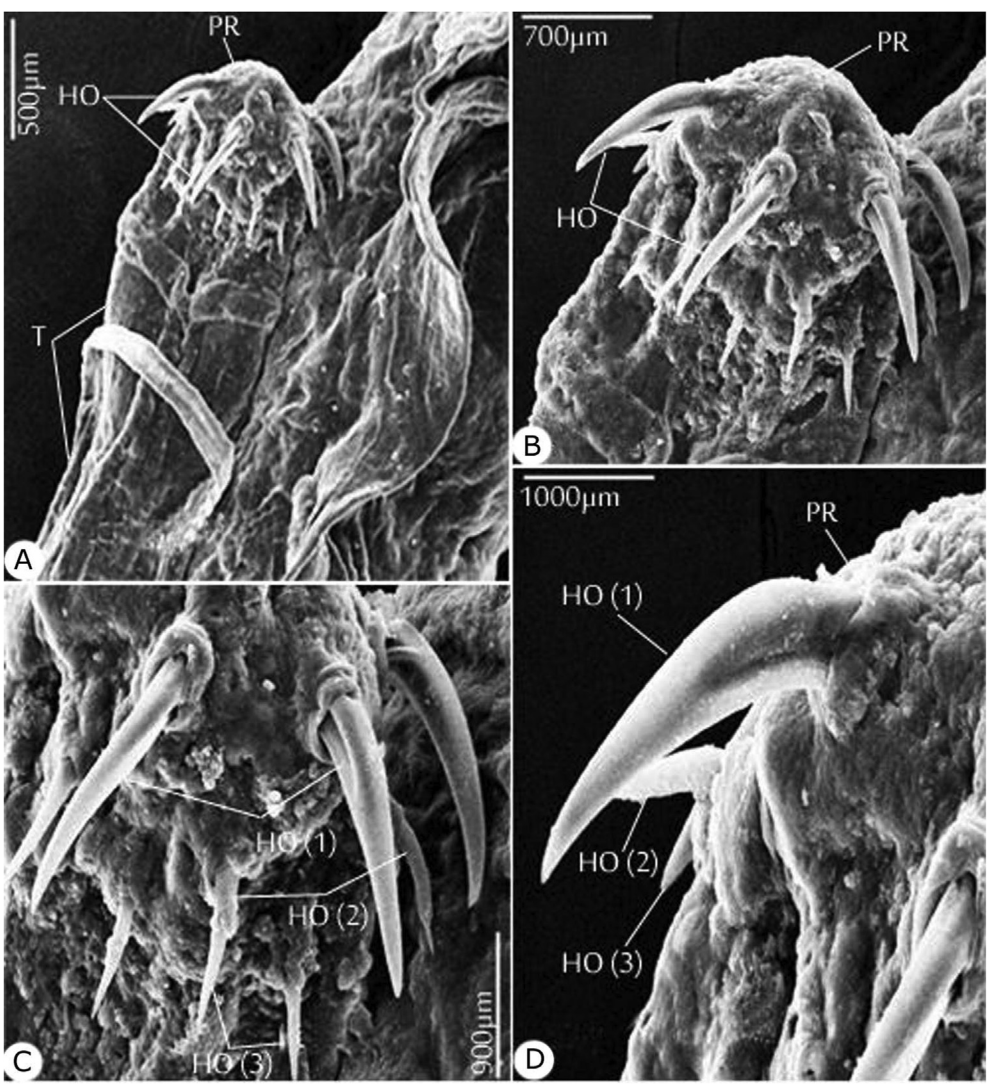

Fig. 2. Scanning electron micrographs of *N. agilis* infecting *L. ramada* showing: A The worm with proboscis (PR), hooks (HO) followed by trunk (T). B–D High magnifications of the proboscis (PR) provided with hooks (HO) arranged in three rows.

The body of a female worm

Proboscis was 0.123–0.223 (0.193 ± 0.01) mm long and 0.081–0.150 (0.198 ± 0.01) mm wide. Apical proboscis hooks were 0.042–0.077 (0.059 ± 0.01) mm long, middle hooks 0.023–0.057 (0.043 ± 0.001) mm long, and basal hooks 0.020–0.034 (0.030 ± 0.001) mm long. The trunk was 4.93–18.24 (8.42 ± 0.1) mm long. Vagina with vaginal sphincter opening by funnel into uterus. There is a selector apparatus between the uterus and the uterine ball. The ovarian mass filled the uterine ball space. Genital pore was subterminal and provided with caudal papillae. Tables 1 and 2 show the maximum and minimum values, as well as the mean values, of the various body parts of this species compared to *Neoechinorhynchus agilis* previously described.

Discussion

Rudolphi's (1819) created a classification of *Neoechinorhynchus agilis* based on nine specimens from the intestine of mullet fish in Spezia, Italy. According to Rudolphi (1819), specimens of *N. agilis* were collected from *Mugil cephalus*, but only three species of mullets were identified from the Mediterranean Sea at that time, namely *M. cephalus*, *Liza aurata* and *L. saliens*, but only *Chelon labrosus* was described by Risso (1827). Therefore, it is not possible to classify the type host for the specimens of Rudolphi's (1819). Amin (2002) categorized the genus *Neoechinorhynchus* into two subgenera based on egg characteristics. Based on the data of Yamaguti (1935), *N. agilis* has been assigned to the *Hebesoma* subgenus. The present study has clearly shown that this species has eggs with concentric shells, but does not have polar prolongations of fertilization membrane. Therefore, it should be transferred to the subgenus *Neoechinorhynchus* and classified as *N. agilis*.

In this study, *N. agilis* has been reported in *L. ramada* with an infection percentage of 55%. These results are higher than the data of Al-Zubaidy and Mhaisen (2012) who reported infection with *S. sagittifer* from 4% to 24% in *Pomadasys argenteus*. The present results have shown that summer is the most prevalent season of infection, as agreed with Ali et al. (2010), who recorded 21–40% of the three species of the genus *Schizothorax* (*S. niger, S. esocinus,* and *S. curvifrons*) infected with *Pomphorhynchus kashmirensis*, and the seasonal cycle with the highest proportion (40%) was observed in summer, and the lowest prevalence in winter (10%). In addition, similar results by Radwan et al. (2012) who reported that *Sphaeriro strispicae* (Centrorhynchidae) was infecting the hooded crow (*Corvus corone cornix*) and reached the highest percentage of infections during the summer season and the lowest levels in the winter season.

The morphology of the present species of *Neoechinorhynchus* has similar generic diagnostic features. It has been found to be closely related to the previously described *N. agilis* due to it has all the characteristics of this parasite species with some differences in the size of different body parts, host species, and geographical location. In addition, the following combination of characters distinguish it from all other species of the genus *Neoechinorhynchus*: i) all proboscis hooks in each row are equal in length and all hooks are rooted; ii) thin neck ring; iii) presence of six dorsal and two hypodermal ventral giant nuclei; iv) lemnisci are clearly unequal and distant from the anterior testis; v) the male reproductive system occupies the posterior half of the body; vi) the posterior end of the female has a moderate caudal papillae. The latter feature has a special value to distinguish *N. agilis*. The revision by

Table 1
Comparative measurements (in millimeters) of the present female *Neoechinorhynchus agilis* with previously described species.

Species	Parameters							
	Host species	* Dimensions of different body parts						Female gonopore
		Proboscis L	Proboscis W	Proboscis hooks			Trunk L	
				I	II	III		
Neoechinorhynchus agilis Tepe and Oğuz (2013)	*Liza aurata*	0.137–0.234 (0.183 ± 0.003)	0.097–0.162 (0.128 ± 0.02)	0.049–0.087 (0.63 ± 0.018)	0.026–0.061 (0.041 ± 0.014)	0.024–0.037 (0.029 ± 0.0006)	4.852–26.12 (12.49 ± 1.0)	Subterminal
Neoechinorhynchus agilis Tkach et al. (2014)	*Chelon labrosus*	0.093–0.125 (0.105 ± 0.01)	0.140–0.168 (0.150 ± 0.008)	0.093–0.110 (0.099 ± 0.005)	0.053–0.063 (0.057 ± 0.003)	0.043–0.050 (0.046 ± 0.003)	5.950–8.949 (7.535 ± 0.9)	Subterminal
Neoechinorhynchus agilis (Present study)	***Liza ramada***	**0.123–0.223 (0.193 ± 0.01)**	**0.081–0.150 (0.198 ± 0.01)**	**0.042–0.077 (0.059 ± 0.01)**	**0.023–0.057 (0.043 ± 0.01)**	**0.020–0.034 (0.030 ± 0.001)**	**4.93–18.24 (8.42 ± 0.1)**	**Subterminal**

*Values represent range followed by means ± SD in parentheses.

Table 2
Comparative measurements (in millimeters) of the present male *Neoechinorhynchus agilis* with previously described species.

Species	Parameters								
	Host species	* Dimensions of different body parts						Testis L	
		Proboscis L	Proboscis W	Proboscis hooks			Trunk L	Anterior	Posterior
				I	II	III			
Neoechinorhynchus agilis Tepe and Oğuz (2013)	*Liza aurata*	0.137–0.194 (0.168 ± 0.002)	0.097–0.170 (0.127 ± 0.002)	0.041–0.087 (0.064 ± 0.02)	0.024–0.061 (0.041 ± 0.02)	0.022–0.041 (0.033 ± 0.010)	4.12–7.51 (5.64 ± 0.11)	0.121–0.670 (0.394 ± 0.2)	0.145–1.07 (0.449 ± 0.340)
Neoechinorhynchus agilis Tkach et al. (2014)	*Chelon labrosus*	0.100–0.112 (0.105 ± 0.006)	0.133–0.150 (0.141 ± 0.007)	0.100	0.050–0.056 (0.054 ± 0.003)	0.045–0.050 (0.047 ± 0.002)	5.85–8.04 (6.69 ± 0.8)	0.420–1.30 (0.838 ± 0.3)	0.400–1.100 (0.684 ± 0.26)
Neoechinorhynchus agilis (Present study)	***Liza ramada***	**0.092–0.108 (0.101 ± 0.01)**	**0.123–0.146 (0.137 ± 0.001)**	**0.088–0.098 (0.090 ± 0.001)**	**0.049–0.053 (0.050 ± 0.001)**	**0.040–0.049 (0.045 ± 0.001)**	**5.98–8.12 (6.93 ± 0.1)**	**0.821–1.11 (0.983 ± 0.1)**	**0.722–1.01 (0.921 ± 0.1)**

*Values represent range followed by means ± SD in parentheses.

Amin (2002) described four species of *Neoechinorhynchus* with caudal papillae: *N. cylindratus* Van Cleave, 1913; *N. stunkardi* Cable & Fisher, 1961; *N. magnapapillosus* Johnson, 1969; and *N. chelonos* Schmidt et al., 1970. The size of the caudal papillae of *N. agilis* is different from that of *N. stunkardi* and *N. chelonos* (larger in *N. stunkardi* and smaller in *N. chelonos*); the same size as of the proboscis hooks in each row (rather than lateral anterior proboscis hooks larger in the same row) by *N. chelonos* and *N. stunkardi*; *N. cylindratus* (instead of unrooted middle and posterior proboscis hooks) due to the presence of roots in each row of hooks; from *N. cylindratus* with an attenuating trunk (instead of cylindrical and elongated, with almost parallel sides in the latter species); and from *N. stunkardi* by the straight trunk on the posterior end of the female (instead of swollen posterior end) (comparable data from Cable & Fisher, 1961; Amin, 2002).

Conclusion

According to the present study, *N. agilis* has been reported from *L. ramada*, which has been proposed herein to be an excellent host for this acanthocephalan species. Further molecular studies have been suggested to elucidate the classical status of this parasite.

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