

Axinoides euzeti n. sp. (Monogenea: Axinidae) from the gills of the needlefish *Tylosurus acus imperialis* (Rafinesque) (Belonidae) off Tunisia, with an updated list of hosts and localities for *Axinoides* spp.

Manel Châari · Hela Derbel · Lassâd Neifar

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Abstract A new axinid monogenean species, Axinoides euzeti n. sp. from the gills of the Mediterranean needlefish Tylosurus acus imperialis (Rafinesque) off the Tunisian coast is described. The new species is most similar in general morphology to A. meservei Price, 1946 from Tylosurus crocodilus fodiator Jordan & Gilbert off the Atlantic Ocean and to A. kola Unnithan, 1957 from Ablennes hians (Valenciennes) off the Indian Ocean. It can be differentiated from A. meservei by the shape of anterior male genital complex armed with several rows of curved spines on the cirrus rather than 3 to 4 rows of slender spines, in having a fewer testes (87-94 vs more than 100 in the drawing of A. meservei) and a body size twice longer than wide. It differs also from A. kola which have a single row of small spines in the cirrus, in having fewer and wider clamps and fewer and smaller testes. A. euzeti represents the first record of a representative of this genus in the Mediterranean Sea. In addition, taxonomic keys and an updated list of hosts and geographic localities for Axinoides species is presented.

Introduction

The taxonomic status of the family Axinidae Unnithan, 1957 has been reviewed several times (Yamaguti, 1963; Mamaev & Lebedev, 1979; Spencer Jones & Gibson, 1990; Zhang et al., 2003) but the system proposed by Price (1962) for these monogeneans has been the most followed (Zhang et al., 2003). Within the Axinidae, the subfamily Axinoidinae Price, 1962 was erected by Price (1962) for species characterised by numerous haptoral clamps in two unequal rows, an unarmed genital atrium and usually an armed cirrus to accommodate the genus Axinoides Yamaguti, 1938. Axinoides was proposed as a subgenus of Axine Abildgaard, 1794 by Yamaguti (1938), elevated to full generic status by Price (1946) and later by Sproston (1946) based on the dorsomedian position of the vaginal aperture. In this genus, eleven species have been reported mainly from belonids off the Atlantic, Indian and the Pacific coasts (Gibson, 2015). As far as we are aware, there are no records of the genus Axinoides in the Mediterranean Sea.

In this paper we describe a new species of *Axinoides* collected during a parasitological survey on the Mediterranean needlefish *Tylosurus acus imperialis* (Rafinesque) off Tunisia. Taxonomic keys are provided herein for the species of *Axinoides* based on morphological characteristics sourced from their original descriptions and previously used to differentiate them. We also provide updated lists of hosts and geographic localities for *Axinoides* spp.

M. Châari (\boxtimes) · H. Derbel · L. Neifar

Laboratoire de Biodiversité et Ecosystèmes Aquatiques, Département des Sciences de la vie, Faculté des Sciences de Sfax, Université de Sfax, BP 1171, 3000 Sfax, Tunisie e-mail: htchaari.m@gmail.com

Materials and methods

A total of 126 specimens of Tylosurus acus imperialis was collected between May and July during 2004–2009. Fish were caught by local fishermen using gillnets from off the eastern Tunisian coast, at the central area [Mahdia (35°30'N, 11°3'E), Sousse (35°50'N, 10°38'E) and Chebba (35°14'N, 11°8'E)] and at the southern area [Sfax (34°47′N, 10°49′E), Kerkennah (34°37'N, 11°6'E), Skhira (34°5'N, 10°1'E) and Zarzis (33°30'N, 11°7'E)]. Specimens were identified using Collette & Parin (1970) and Bauchot (1987). Fish were dissected and examined a few hours after capture. The operculum and gill arches were separated and placed in Petri dishes with filtered seawater using filter paper. Monogeneans were detected using a stereomicroscope, detached from the gills and operculum and transferred to a dish containing filtered seawater. They were studied either live or fixed under a coverslip in 70% alcohol. Some fixed specimens were stained with Semichon's acetic carmine. Other specimens were double-stained with light green and Semichon's acetic carmine to study the morphology of the clamps. After dehydration through a graded ethanol series, specimens were cleared with clove oil and mounted in Canada balsam. Some fixed specimens were mounted in Berlese's fluid in order to study the haptoral sclerites and the genital armature.

Illustrations and measurements of stained specimens were made with the aid of a Leitz microscope equipped with a drawing tube, then scanned and redrawn on a computer with Corel Draw Software. All measurements are given in micrometres unless otherwise stated as the range followed by the mean and the number of measurements (n) in parentheses.

Family Axinidae Unnithan, 1957 Subfamily Axinoidinae Price, 1962 Genus *Axinoides* Yamaguti, 1938

Axinoides euzeti n. sp.

Type-host: Tylosurus acus imperialis (Rafinesque) (Beloniformes: Belonidae).

Type-locality: Off Mahdia (35°30'N, 11°3'E), Tunisia. *Other localities*: Off Chebba (35°14'N, 11°8'E) and Zarzis (33°30'N, 11°7'E), Tunisia.

Type-material: Holotype (MNHN HEL566) deposited at the Muséum National d'Histoire Naturelle, Paris, France; paratype (NHMUK 2016.8.3.1) deposited at the Natural History Museum, London.

Site in host: Gill filaments, between secondary lamellae. *Prevalence and intensity*: In 4 fishes; 1–3 specimens per infected fish.

Etymology: The species is named in memory of late Professor Louis Euzet from the University of Montpellier, France.

Description (Figs. 1, 2)

[Based on six whole-mounted specimens.] Body flattened dorsoventrally; 2.6-3.6 (3.26) mm long, 750-900 (842) wide at level of ovary. Haptor asymmetrical, triangular, 700-1,140 (973) in width, elongated with straight posterior base bordered by single row of 36-60 (47) clamps (Fig. 1). Half of specimens with haptor directed to right, line of clamps located on same side as dextral genito-intestinal canal and remainders with haptor directed to left, line of clamps facing the opposite genito-intestinal canal. Clamps 20-45 (33) long, 50-60 (53) wide; each clamp of "Axine-type" with marginal sclerites of anterior and posterior jaw, in two parts (Fig. 2A). Number of clamps at right side of hamuli and uncinuli 17-26 (22), at left side 19-33 (25). Two pairs of hamuli and one pair of uncinuli situated at 310-450(390)(n = 4) from posterior extremity of haptor. Median hamuli falciform 46–58 (53) (n = 4) long; blade, Y: 8–12 (11) (n = 4) articulated with long straight handle, X: 38-46 (43) (n = 4). Lateral hamuli 27–40 (35) (n = 6) long, with wide guard, bent shaft and short pointed blade, Y: 22–28 (24) (n = 6), handle, X: 12–17 (15) (n = 6). Postero-lateral uncinuli 8-10 (10) (n = 4) long, between median and lateral hamuli (Fig. 2B). Anterior end bi-lobed, buccal suckers 2, muscular, subcircular, aseptate, $36-60(50)(n = 6) \log_{10}(30-45(40))(n = 6)$ wide. Mouth ventro-subterminal; pharynx oval, 20-45 $(35) (n = 3) \log_{10} (15-35) (27) (n = 3) wide (Fig. 2C).$ Oesophagus long, caeca with dendritic diverticles laterally and medially, not extending into haptor.

Testes intercaecal, in several rows in median field, 87–94 (90) (n = 2) in number, 10–12 (11) (n = 19) long, 8–11 (9) (n = 19) wide. Genital atrium unarmed, located slightly anterior to intestinal bifurcation. Cirrus armed with numerous crowns of curved



spines (Fig. 2D). Cirrus pouch with folded muscular wall 195–200 (197) (n = 2) long, 40–65 (50) (n = 3) in maximum width, followed with anterior part of vas deferens with tubular, muscular wall; medio-dorsal, narrowed and sinuous in posterior part. Genital complex surrounded by glandular cells.

Ovary pre-testicular, U-shaped, with distal limbs lobated, intercaecal, almost equatorial, 270-400 (317) (n = 5) long. Oviduct directed anteriorly, emerging from distal extremity of ovary. Dextral genito-intestinal canal branching off near distal end of oviduct. Ovovitelline duct loops posteriorly, ascending limb widens to form oötype. Mehlis' glands not observed. Vitelline follicles lateral, surrounding dendritic intestinal caeca, extending anteriorly slightly posterior to vaginal aperture and posteriorly to level of haptoral region. Transverse vitelloducts near halfway between vagina and ovary, joining at midline to form dorsomedian vitelloduct opening posteriorly at distal end of oviduct. Vagina, 40 (40) (n = 2) in maximum width; dorsal, posterior to genital atrium, opening into the median vitelloduct. Vaginal pore, dorso-median, located at 21-28 (25)% of length from apical margin of body followed with a tiny sclerotised duct. Vaginal chamber funnel-shaped wide in its distal portion, armed with sclerotised lamellae in the inner wall, tapering in the proximal portion joining median vitelloduct. Uterus ventral, in midline, opening at level of genital atrium in common genital pore. Egg fusiform, 70–80 (75) (n = 2) wide with two polar filaments of unequal length and curved at tips. Abopercular filament 170(170)(n = 2) long, opercular filament $290 (290) (n = 2) \log (Fig. 2E).$

Remarks

The diagnostic features of *Axinoides euzeti* n. sp. comprise the plump body shape, the shape of the anterior male genital complex armed with several rows of curved spines, the number of testes of about 90 arranged in 7 or 8 longitudinal rows and an average clamp number of 47.

The new species is morphologically similar to *A. meservei* Price, 1946 in having a plump body but can be easily distinguished from the latter by the





Fig. 2 Axinoides euzeti n. sp. ex Tylosurus acus imperialis (Rafinesque). A, Closed clamp; B, Hamuli and uncinuli; C, Anterior extremity; D, Dorsal view of anterior part of reproductive system with partly evaginated cirrus; E, Egg. *Abbreviations*: C, cirrus; Cp, cirrus pouch; Ga, genital atrium; Lh, lateral hamuli; Mh, median hamuli; M, mouth; Ms, marginal sclerite; Os, oral sucker; Ph, pharynx; Un, postero-lateral uncinuli; Ut, uterus; X, length of handle; Y, length of blade. *Scale-bars*: 50 µm

possession of several rows of curved spines on the cirrus (vs a corona of three or four alternating rows of slender spines in A. meservei), fewer testes (vs numerous testes, more than 100 testes in the drawing of A. meservei), as well as in the body length to width ratio, A. euzeti is approximately four times longer than wide (vs two times in A. meservei) and in the medial position of larval hooks (vs 7th and 8th clamps from the left in A. meservei) (Price, 1962).

The new species mainly differs from *A. aberrans* (Goto, 1864) Price, 1946 in the anterior extent of the vitellarium (to about the level of the vaginal aperture in *A euzeti vs* midway between the vaginal and genital aperture in *A. aberrans*) (Goto, 1894).

Axinoides euzeti n. sp. closely resembles to A. kola Unnithan, 1957 in the body shape and size but differs in the shape of the male genital complex armed with several rows of curved spines of the cirrus rather than a cirrus with a crown of small spines (as described by Yamaguti, 1968) and a single row of 25–40 spines (as described by Gupta & Khanna, 1975). *Axinoides euzeti* n. sp. can be also distinguished from *A. kola* in having fewer and smaller testes and fewer and wider clamps (Table 1).

According to Price (1962), A. kola from Ablennes hians (Valenciennes) from the Indian Ocean resembles A. meservei from Tylosurus crocodilus fodiator Jordan & Gilbert from the Atlantic Ocean in body form and in most other respects. However, A. kola is a much larger species and with cirrus spines arranged in a single row. The occurrence of A. kola on a host belonging to a different genus than that on which A. meservei was found suggests that this species should be regarded as distinct (Price, 1962). Axinoides euzeti n. sp. differs from A. meservei with a body size twice

Species Source Host	A. euzeti n. sp. Present study Tylosurus acus imperialis (Rafinesque)		A. kola Unnithan, 1957		A. meservei Price, 1946	
			Yamaguti (1968) Ablennes hians (Valenciennes)	Gupta & Khanna (1975) Ablennes hians (Valenciennes)	Price (1962) Tylosurus crocodilus fodiator Jordan & Gilbert	
	Body length	2,600-3,600	3,260	3,860–4,350	2,495–3,044	2,000–2,300
Body width	750–900	842	700–900	376–961	1,100	
Haptor width	700-1140	973	-	_	1,200	
Buccal sucker length	36–60	50	40-68	36–52	45	
Buccal sucker width	30–45	40	32–50	32–56	38	
Clamps number	36-60	47	69–75	62–73	41	
Clamps length	20-45	33	-	36–54	-	
Clamps width	50-60	53	-	24–50	40	
Lateral hamuli length	27–40	35	-	-	33	
Median hamuli length	46–58	53	-	-	40	
Testes number	87–94	90	Very numerous	90–120	Numerous	
Testes length	10-12	11	-	16–20	-	
Testes width	8-11	9	-	12–14	-	
Cirrus length	195–200	197	-	-	-	
Cirrus width	40-65	50	-	-	-	
Egg width	70–80	75	-	-	-	

Table 1 Morphometric data for Axinoides euzeti n. sp., Axinoides kola Unnithan, 1957 and Axinoides meservei Price, 1946

longer than wide (Table 1). Comparative metrical and morphological data for the new species, *A. kola* and *A. meservei* reported by Gupta & Khanna (1975) and by Price (1962) are listed in Table 1.

The new species also resembles *A. sebatisci* Yamaguti, 1958 in having a plump body but the body of *A. sebatisci* is broad at the posterior extremity, tapers gradually to the level of anterior part of the vitellarium then abruptly narrows to a slender bluntpointed anterior extremity. *Axinoides euzeti* n. sp. is clearly different in having more testes and clamps (*vs* two fields of 14–30 testes and 38–42 clamps in *A. sebatisci*) and a disc-shaped cirrus provided at its central opening with 2–3 circular rows of spines (Yamaguti, 1958).

Axinoides euzeti n. sp. appears to be similar to A. jimenezi Caballero y & Bravo Hollis, 1969 and to A. raphidoma Hargis, 1956 in the clamp number but is easily distinguished by its plump body shape rather than a slender body with a high ratio between length and width in A. jimenezi and A. raphidoma (almost four times longer than wide in A. euzeti vs seven times longer than wide in A. jimenezi and A. raphidoma). It can be further distinguished by the greater number of testes and their arrangement (90 testes arranged in 7 or 8 longitudinal rows in A. euzeti n. sp. vs 61 testes in the drawing of A. jimenezi and 18 testes in A. raphidoma arranged in two or three rows) (Caballero y & Bravo Hollis, 1969). The new species differs in having a small haptor with respect to the total body (almost 1/3 of body length in A. euzeti vs longer haptor almost 1/2 of body length in A. jimenezi and A. raphidoma). The new species differs in the shape of the anterior male genital complex armed with several rows of curved spines (vs a small ovoid cirrus pouch, containing a small short conical cirrus, armed with slightly sclerotised fine papillae, the seminal vesicle was not observed and numerous fusiform cells form pars prostatica in A. jimenezi); in A. raphidoma the anterior part of the cirrus pouch is occupied by a long and thick copulatory organ provided in the proximal part with numerous long slightly sclerotised papillae extending from the inner wall towards the centre of the genital atrium, the cirrus pouch is wide and conical, the pars prostatica occupies the almost spherical central part of the cirrus pouch which is separated from the anterior seminal vesicle by a constriction and the tusk-shaped seminal vesicle.

We believe that the above mentioned morphological and metrical differences between *A. euzeti* and its closest relatives *A. meservei* and *A. kola* (Table 1) and parasitism in a different host from a distinct geographical area are sufficient to justify the description of *A. euzeti* n. sp.

Discussion

Axinoides now consists of twelve nominal species, including the new species described herein (Gibson, 2015). This genus has never been recorded in the Mediterranean Sea. The present study off the Tunisian coast also represents the first record of this genus in the Mediterranean needlefish *T. a. imperialis*. This is probably due to the lack of investigations on the monogenean parasites of this host in the Mediterranean Sea.

According to Price (1962), species that may be included in the genus Axinoides are: A. abberans (Goto, 1864) Price, 1946, A. kola, A. meservei, A. oceanicum Caballero, Bravo Hollis & Grocott, 1953, A. raphidoma, A. sebatisci, A. strongylurae Price, 1962, A. tylosuri Yamaguti, 1938. Since then, Yamaguti (1968) reported two additional species: A. bulbous Yamaguti, 1968 from Platybelone argalus trachura (Valenciennes) and A. diploporus Yamaguti, 1968 from A. hians in the Pacific Ocean. Caballero y & Bravo Hollis (1969) found A. jimenezi and A. raphidoma in Tylosurus crocodilus crocodilus (Péron & Lsueur) from the Atlantic Ocean. Gupta & Khanna (1975) reported A. kola and A. synorchis Gupta & Khanna, 1975 from the carangid Chorinemus sp. (Oligoplites or Scomberoides, actually valid name) in the Indian Ocean. Recently, Hadi & Bilgees (2014) described A. belangerii Hadi & Bilqees, 2014 from the sciaenid Johnius belangerii (Cuvier) in the northern Indian Ocean off Pakistan.

Yamaguti (1968) proposed a key for species of *Axinoides* from Hawaii based on the position of the genital pore, the number of testes and clamps and the shape of the body. We herein provide and updated key to the species of *Axinoides* based on morphological characteristics used previously by Yamaguti (1968) and sourced from the original descriptions. *Axinoides belangerii* is not included in this key due to the lack of information about the genital pore in the description and the drawings. This species differs in having a slender body and a fewer testes (10–11) arranged in two rows.

Table 2 Fish hosts and geographical localities for species of Axinoides Yamaguti, 1938 (Monogenea: Axinidae)

Species	Host	Locality	Reference
A. aberrans (Goto, 1864) Price, 1946	Tylosurus schismatorhynchus (Bleeker) [as Tylosurus crocodilus (Pèron & Lesueur)]	Pacific Ocean	Goto (1894)
		Atlantic Ocean	Price (1962)
A. belangerii Hadi & Bilqees, 2014	Johnius belangerii (Cuvier)	Arabian Sea	Hadi & Bilqees (2014)
A. bulbous Yamaguti, 1968	Belone platyura Bennett [as Platybelone argalus trachura (Valenciennes)]	Pacific Ocean	Yamaguti (1968)
A. diploporus Yamaguti, 1968	Ablennes hians (Valenciennes)	Pacific Ocean	Yamaguti (1968)
A. euzeti n. sp.	Tylosurus acus imperialis (Rafinesque)	Mediterranean Sea	Present study
A. gracilis (Linton, 1940) [as Nudaciraxine gracilis (Linton, 1940) Price 1962]	Strongylura marina (Walbaum)	Atlantic Ocean	Price (1962)
A. jimenezi Caballero y& Bravo Hollis (1969)	Tylosurus raphidoma (Ranzani) [as Tylosurus crocodilus crocodilus (Péron & Lesueur)]	Gulf of Mexico	Caballero y& Bravo Hollis (1969);
			Kohn et al. (2006)
A. kola Unnithan, 1957	A. hians (Valenciennes)	Pacific Ocean	Yamaguti (1968)
		Indian Ocean	Gupta & Khanna (1975)
		China Sea	Zhang et al. (2003)
A. meservei Price, 1946	Tylosurus fodiator Jordan & Gilbert [as Tylosurus crocodilus fodiator Jordan & Gilbert]	Atlantic Ocean	Price (1962); Kohn & Cohen
A. oceanicum Caballero, Bravo Hollis & Grocott, 1953	<i>T. fodiator</i> Jordan & Gilbert [as <i>T. c. fodiator</i> Jordan & Gilbert]	Panama Canal	(1998) Price (1962); Kohn et al. (2006)
A. raphidoma Hargis, 1956	Tylosurus acus melanotus (Bleeker)	Gulf of Mexico	Caballero y & Bravo Hollis (1969)
	T. raphidoma (Ranzani) [as T. c. crocodilus (Péron & Lesueur)]	Gulf of Mexico	Price (1962)
			Pérez-Ponce de León et al. (1999)
			Kohn et al. (2006)
A. sebatisci Yamaguti, 1958 [as Axine sebatisci Yamaguti, 1958]	Sebastiscus marmoratus (Cuvier)	Inland sea	Yamaguti (1958)
A. strongylurae Price, 1962	S. marina (Walbaum)	Gulf of Mexico	Price (1962)
	Strongylura gigantea (Temminck & Schlegel) [as T. c. crocodilus (Péron & Lesueur, 1821)]	Pacific Ocean	Yamaguti (1968)
A. synorchis Gupta & Khanna, 1975	Chorinemus sp.	Indian Ocean	Gupta & Khanna (1975)
A. tylosuri Yamaguti, 1938	<i>T. schismatorhynchus</i> (Bleeker) [as <i>T. crocodilus</i> (Pèron & Lesueur)]	Pacific Ocean	Yamaguti (1938)

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The majority of *Axinoides* spp. were reported from belonid hosts mainly of the genus *Tylosurus* Cocco (Table 2). Nevertheless, some species such as *A. sebatisci*, *A. synorchis* and *A. belangerii* were reported from the scorpaeniform *Sebatiscus marmoratus* (Cuvier), the carangid *Chorinemus* sp. (i.e. *Oligoplites* or *Scomberoides*) and the sciaenid *J. belangerii*, respectively.

The new monogenean species described here cooccurs on the gill filaments of the Mediterranean needlefish T. a. imperialis with another axinid monogenean, Nudaciraxine imperium Châari, Derbel & Neifar, 2010 (see Châari et al., 2010). Similarly, in fishes of the genus Tylosurus from the Gulf of Mexico, two monogeneans species of the genus Axinoides were described: A. gracilis (Linton, 1940) [as Nudaciraxine gracilis (Linton, 1940) Price 1962] and A. raphidoma. Price (1962) initially established for the former species Nudaciraxine Price, 1962 considering that the genital atrium and cirrus are unarmed and that the positions of the vagina and genital pore are different (Caballero & Bravo Hollis, 1969). Furthermore, the low prevalence of infection with this monogenean species can be explained by co-speciation between the host species and the axinid monogenean parasite. A phylogenetic study is needed to confirm the validity of this hypothesis. Numerous studies have investigated co-speciation between monogenan parasites and their hosts (see Desdevises et al., 2000; Desdevises et al., 2002, Mendlová & Šimková, 2014; Mendlová et al., 2012). The relationship between host range and species diversification should be related to the mechanisms of speciation (Thompson, 1994). According to Sasal et al. (1998), strong arguments support the view that co-speciation occurs more frequently with highly specific parasites.

In spite of our extensive sampling, we did not find *A. euzeti* n. sp. in fishes from the southern areas such as Sfax, Kerkennah and Skhira; this was found in fishes from the centre off Mahdia, Chebba and Sousse and off the southernmost Tunisian coast at Zarzis (distant by at least 100 km). Furthermore, the transmission of monogeneans may be related to differences in the density of the host population from *T. a. imperialis* in different areas.

The absence of this monogenean in the southern coast at Sfax, Kerkennah and Skhira could be explained by local abiotic factors such as salinity, temperature and depth (Williams & Jones, 1994).

Abiotic factors affect the completion of the life-cycle of the parasite. Indeed, several studies have proven that infection parameters of monogenean ectoparasites depend on the water temperature, salinity and depth (Munroe et al., 1981; Blažek et al., 2008; Blahoua et al., 2009; Antonelli & Marchand, 2012). For axinid monogeneans, studies on hatching time of eggs *in vitro* have been conducted by Tubbs et al. (2005) and showed that the eggs hatch after a few days to several weeks depending on the temperature of the water.

Key to the species of Axinoides

1a	Male and female genital pore separate
	A. diploporus
1b	Male and female genital pore common 2
2a	Body margin bulging out symmetrically at level
	of genital pore; body slender; testes 12-17 in
	number, arranged in two longitudinal rows
	A. bulbous
2b	Body margin not bulging out symmetrically at
	level of genital pore
3a	Body slender
3b	Body plump
4a	Testes < 50 in number
4b	Testes > 50 in number
5a	Testes 18; clamps 44 A. raphidoma
5b	Testes 21–35; clamps 21–27
	A. strongylura
6a	$Clamps \le 50 \dots 7$
6b	Clamps > 50
7a	Clamps 36; haptor small in relation to the total
	body length, with typical folded shape
7b	Clamps 40-50; haptor long, 1/2 of total body
	length A. jimenezi
8a	Testes 60–75; clamps 70
8b	Testes > 100; clamps 60–62
	A. synorchis
9a	Testes 14–30, arranged in two longitudinal rows
9b	Testes numerous, arranged in several longitu-
	dinal rows 10
10a	Cirrus with a single row of small spines: clamps
	> 60 A. kola

10b	Clamps < 60 11
11a	Clamps 25; anterior extent of vitellarium
	between vaginal and genital aperture
	A. aberrans
11b	Clamps around 40; anterior extent of vitellar-
	ium at level of vaginal aperture 12
12a	Cirrus with a corona of 3 or 4 alternating rows of

- between length and width c.4 A. euzeti

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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