Ophiotaenia tessellata sp. n. (Eucestoda: Proteocephalinae) from Natrix tessellata (Laurenti, 1768) (Serpentes: Colubridae) in Egypt

Irene S. Gamil*1 and Dalia Fouad1,2

1Zoology and Entomology Department, Faculty of Science, Helwan University, Helwan, Egypt;
2Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

ABSTRACT

Ophiotaenia tessellata sp. n. (Proteocephalidea: Proteocephalinae) is described from the intestine of the dice water snake Natrix tessellata (Laurenti, 1768) (Serpentes: Colubridae) collected from El Fayoum Governorate, Egypt. Standard methods of collection of the snakes and examination of the cestode tapeworms for taxonomic studies were used. Ophiotaenia tessellata sp. n. was identified and being separable from Ophiotaenia species found in African snakes as well as those from colubrid snakes based on many morphological characteristics. Analysis of a dataset based on 473 bp of its 18S rRNA gene regions was carried out to determine the phylogenetic position of the new species among other proteocephalideans. Ophiotaenia tessellata sp. n. shows a close relationship to O. lapata Rambeloson, Ramavoson and de Chambrier, 2012 parasite of the endemic snake Madagascarophis colubrinus from Madagascar; both infect African colubrid snakes.

INTRODUCTION

Tapeworms of the order (Proteocephalidea Mola (1928) (currently part of the erected order Onchoproteocephalidea Caira, Jensen, Waeschenbach (Caire et al., 2014) based on molecular data only) are frequent and widely distributed parasites of freshwater fishes, amphibians and reptiles (Rego, 1994). To date, there are 14 proteocephalidean genera with their species being recorded from reptiles: Crepidobothrium Monticelli, 1900, Acanthotaenia von Linstow, 1903, Ophiotaenia La Rue, 1911, Deblocktaenia Odening, 1963, Kapsulotaenia Freze, 1963, Rostellotaenia Freze, 1963, Macrobothriotaenia Freze, 1965, Tejidotaenia Freze, 1965, Testudotaenia Freze, 1965, Vaucherella de Chambrier, 1987, Cairaella Coquille and de Chambrier, 2000, Australotaenia de Chambrier and de Chambrier, 2008, Ophiotaenia La Rue, 1911 is the second most speciose genus of proteocephalidean tapeworms, and up to date, more than 60 species of Ophiotaenia have been recorded from reptiles in all zoogeographical regions (see de Chambrier et al., 2010). In Africa, the most recorded proteocephalidean cestodes parasitize catfishes (de Chambrier et al., 2009b, 2011; Scholz et al., 2009), while Deblocktaenia ventosaloculata (Deblock, Rosé and Broussart, 1962) and about 15 species of Ophiotaenia commonly infect snakes (see Rudin, 1917; Deblock et al., 1962; Ammann and de Chambrier, 2008; Coquille and de Chambrier, 2008; de Chambrier et al., 2010; Rambeloson et al., 2012). According to Freze (1965), the genus Ophiotaenia differs from Proteocephalus in possessing a preformed uterus in mature proglottides and parasitism in reptiles, while Brooks (1978) considered Ophiotaenia a junior synonym of Proteocephalus.

Most species of Ophiotaenia are strictly host-specific (oioxenous sensu Euzet and Combes, 1980), infecting only one species of definitive host (see de Chambrier et al., 2006; Ammann and de Chambrier, 2008; Rambeloson et al., 2012; Scholz et al., 2013) and limited in their distribution to individual continents and/or zoogeographical regions (Freze, 1965). Molecular data have revealed this genus...
as polyphyletic and as many as 10 distinct lineages of *Ophiotaenia* were found (see de Chambrier *et al.*, 2015). In the present paper, a new species of *Ophiotaenia* is described from the dice water snake *Natrix tessellata* (Laurenti, 1768) (Serpentes: Colubridae) in Egypt. New morphological data as revealed by SEM and TEM are provided. To determine the position of the present worm within order Proteocephalidea, we aligned its 18S rRNA gene with a representative selection of proteocephalidean tapeworms.

**MATERIALS AND METHODS**

A total of 10 dice water snake *Natrix tessellata* (Laurenti, 1768) were collected from the irrigation canals and the surrounding terrestrial habitat from El Fayoum Governorate, Egypt, (29°19'38.3“ N 30°51'03.4”E) by professional hunters during summer 2015, average temperature 34/21°C (day/night). The snakes were transported alive to the laboratory, where they were euthanized and dissected. Intestinal tapeworms were collected, washed in physiological saline, fixed in 4% hot neutral formalin solution and subsequently stored in 70% ethanol, then stained with hematoxylin and eosin, dehydrated in an ethanol series, cleared with eugenol and mounted in Canada balsam as permanent preparations. Pieces of the strobila were embedded in paraffin wax, longitudinally and transversely sectioned at 12-15μm and stained with hematoxylin and eosin. Eggs were studied in distilled water and illustrated.

In preparation for electron microscopy studies, some tapeworms were transferred to 4% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated through a graded ethanol series; critically point dried. The scolex with the proliferative zone and parts of the strobila were coated with gold and examined using a JEOL scanning electron microscope (JSM–5500LV) at an accelerating voltage of 20kV. Parts of mature tapeworm proglottides were embedded in epoxy resin and a series of ultrathin sections were cut (for further spermatogenesis study) with Leica EM UC6 ultramicrotome, post-stained with uranyl acetate and lead citrate lead citrate. Next, they were examined using a transmission electron microscope (JSM–5500LV) at an accelerating voltage of 80kV. Microthrix terminology follows that described by Chervy (2009).

All measurements are given in micrometres unless otherwise indicated. Abbreviations used in the description are as follows: x, mean; n, number of measurements; CS, relative size of the cirrus sac expressed as percentage of its length to the width of the proglottis; GP, genital pore position expressed as percentage of its position to the proglottis length; OV, percent of ovary width to width of the proglottis; ROV, relative size of the ovary, defined as the proportion of its size to the size of the proglottis (see de Chambrier *et al.*, 2012). Paratype of the whole cestode worm has been deposited in the Institute of Parasitology, České Budějovice, Czech Republic (IPCAS) with a collection number C-825.

**DNA extraction and gene amplification**

The total genomic DNA was extracted from 96% ethanol-preserved cestode worm specimens using the All Prep DNA/RNA Mini kit (Qiagen, Cat#80204, Ambion, Courtabeuf, France) following the manufacturer’s instructions. PCR primers were designed to amplify 455 bp for 18S ribosomal subunits genes. The used forward and reverse primers were 18SF 5‘-CCA GCC GCG GTA ACT CCA-3‘; and 18SR 5‘-CCC CCG CCT GTC TCT TTG GAT-3‘ (IDT, Coralville, Iowa 52241, USA).

Gradient temperature PCR runs were conducted in the range of 50°C to 60°C to determine the optimal annealing temperature in the final volume of 50μl. The reaction mixture contained 25μl of GoTaq® Green Master Mix (Promega, Cat #M712c, Madison, USA), 5 μl of genomic DNA, 3 μl of each forward and reverse primers (30 pmole), and an amount of nuclease free water corresponding to a final volume of 50μl. The cycling conditions were as follows: 1 cycle at 95°C for 1 min followed by 40 cycles at 94°C for 30 sec, 50-65°C for 45 sec, and 68°C for 60 sec. A final extension was carried out at 68°C for 5 min followed by cooling to 4°C. The amplified DNA regions were analysed with electrophoresis in 1.5% agarose gel in TAE buffer. The most effective annealing temperature was found to be 61.5°C for 18S primers. The separated DNA bands of the expected size were cut from the gel and purified using the QiAquick gel extraction kit (Qiagen, Cat # 28706, Ambion, Courtabeuf, France). The fragments were then cloned into the pGEM®-T Easy vector (Promega, Cat # A1360, Madison, USA) following the manufacturer’s instructions. The ligation mixture was used to transform *Escherichia coli* JM 109 competent cells according to Sambrook *et al.* (1989), and the positive clones were screened in selective LB/IPTG/X-gal/Ampicillin/agar plates. The plasmids of the mostly white colonies were extracted using the PureYield™ Plasmid Miniprep system (Promega, Cat # 1222, Madison, USA), and the presence of the correct insert was judged by PCR using T7 and SP6 universal primers at an annealing temperature of 55°C as described previously.

**Sequence alignment and phylogenetic analysis**

Sequencing was performed in both directions using either the T7 or SP6 primers obtained from Macrogen Inc. (http://www.macrogen.com/en/main/index.php).
The sequences were analysed in both directions and assembled using the Seqman PROGRAM (Seqman, version 5.07; DNASTAR, Inc., Madison, WI, USA, 2003). A BLAST search (ver.2.2.30; http://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed to search for similarities between the obtained sequence and previously deposited sequences in the GenBank database. Data derived from the 18S sequence were aligned using CLUSTAL-X multiple sequence alignment according to Thompson et al. (1997) and compared with previously recorded data from GenBank. The alignments were manually corrected using the alignment editor in BioEdit 4.8.9 according to Hall (1999). A phylogenetic tree was constructed using maximum parsimony (neighbour-interchange [CNI] level 3, random addition trees 100). To evaluate the robustness of tree topologies, a bootstrap analysis was performed based on 1000 replicates using MEGA 4.0 according to Tamura et al. (2007).

A total of 473 bp of the 18S rRNA gene regions of the examined tapeworm parasite were deposited in GenBank with accession number KJ917783.1. A list of species with hosts, collection sites, associated GenBank accession numbers and percent identity is provided in Table IV, however, few taxonomical changes have taken place: Ophiotaenia gallardi is now Austalophiotaenia gallardi (Johnston, 1911) n. comb. (see de Chambrier et al., 2018).

**Ophiotaenia tessellata sp. n.**

**Description**

Large-sized cestode worms 230–550 mm long with a maximum width of 1 mm, flattened dorsoventrally. Strobila acraspedote, anapolytic, consisting of 240–580 (x= 400, n= 10) proglottides. Immature proglottides wider than long to longer than wide (length: width ratio 0.12–1.47), mature and gravid proglottides longer than wide (length: width ratio 1.19–2.32 and 1.59–2.78, respectively).

Scolex 280–320 (x= 306, n= 5) long and 300–370 (x= 342, n= 8) wide with 4 unarmed large suckers and tapsers anteriorly. Suckers uniloculate, situated anterolaterally, 100–140 (x= 121, n= 8) in diameter. Scolex lacks an apical organ but provided with a concentration of cells with granular contents situated postero-medially to the suckers, which may represent gland cells (Fig. 1A). Using SEM, wrinkles and folds were observed, which give the scolex a rough appearance; a transverse slit-like structure was also observed in the anterior margin of each sucker (Fig. 3A, B). Proliferative zone 270–310 (x= 300, n= 10) wide with wrinkles and transverse folds, covered with acicular filitriches, and showed scattered pores (seem to be excretory pores) and tumuli ( mound-like structures) of the tegument, such tumuli burst and form large pores (Fig. 3C-E). Acicular filitriches cover immature, mature and gravid proglottides; few gladiate spinitriches, observed by TEM, cover also mature proglottides (Fig. 3F, G, J). Additionally, few tumuli were observed scattered on the whole strobila (Fig. 3I).

Subtegumetal muscles developed with a band of longitudinal muscular fibers surrounding the genital organs and vitelline follicles (Fig. 2A, B). Ventral osmoregulatory canals 20–40 (x= 29, n= 25) much wider than dorsal ones 9–12 (x= 10, n= 25) in diameter, with narrow secondary osmoregulatory canals directed externally (Fig. 1C) and open to the surface, through fine ducts, by conspicuous pores covering the whole worm (Fig. 3H, J).

Testes medullary, in one layer arranged in two lateral fields, not overlapping the cirrus-sac, vagina or vas deferens and occupying 83–92% (x= 88%, n= 10) of the total length

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**Fig. 1. Ophiotaenia tessellata sp. n.** from Natrix tessellata, Egypt. (A) Scolex, dorsoventral view; (B) mature proglottis, dorsal view; (C) gravid proglottis, ventral view; (D) vagina and cirrus sac region, dorsal view. **Abbreviations:** ci, cirrus; cs, cirrus sac, do, dorsal osmoregulatory canal; mg, Mehlis' gland, ov, ovary; te, testes; ud, uterine diverticula; up, uterine pore; ut, uterus; vd, vas deferens; vc, vaginal canal; vf, vitelline follicles; vs, ventral osmoregulatory canal; vs, vaginal sphincter. **Scale-bars:** A= 100 μm; B, C = 500μm; D= 250μm.
of proglottis. Testes spherical to oval (polygonal), with many developmental stages, reaching the vitelline follicles laterally and the anterior margin of the ovary. Testes 65–135 \( (x= 92, n= 16) \) in number with 30–64 \( (x= 45) \) aporal testes, 16–36 \( (x= 25) \) preporal testes and 15–35 \( (x= 20) \) postporal testes. Each testis 30–70 long and 30–60 \( (x= 53 \times 42, n= 40) \) and still present in gravid proglottides (Figs. 1B-D, 2B). Cirrus sac elongate to slightly pyriform, thick-walled 130–200 long and 75–94 \( (x= 163 x 84, n= 10) \) wide; CS 21–33\% \( (x= 27\%, n= 20) \). Cirrus thick-walled strongly muscular enveloped by numerous dark staining cells and occupies more than half of the cirrus sac length (Fig. 1D).

Vas deferens (external sperm duct) wide, strongly coiled, situated between proximal part of cirrus sac and midline of proglottides, and does not normally extend beyond the middle of the proglottis, it occupies up to 19–27\% \( (x= 23\%, n= 16) \) of proglottis width and makes up to 25 coils completely filled with spermatozoa. Internal vas deferens forming few loops (up to 7 coils). Genital atrium shallow containing separate apertures for male and female genital ducts. Genital pores are alternating irregularly; GP 41–51\% \( (x= 46\%, n= 15) \) (Fig. 1B-D).

Ovary medullary, bilobed, follicular with wide lateral wings of irregular shape, 340–500 wide \( (x= 427, n= 13) \) and up to 90 in length; OV 61–76\% \( (x= 68\%, n= 13) \) and ROV 4.1\%. Mehlis’ gland 55–90 \( (x= 72, n= 17) \) in diameter, representing 11–17\% \( (x= 13\%, n= 6) \) of proglottis width (Figs. 1B, C, 2A). Vaginal canal 12–25 \( (x= 17, n= 12) \) wide, anterior or posterior to cirrus sac, with terminal part near genital atrium surrounded by intensely staining cells and a circular vaginal sphincter 39–67 \( (x= 51, n= 9) \) in diameter that exhibits a ratio of 3:1 to the vaginal canal width (Fig. 1D). Vagina anterior (40\%) or posterior (60\%) \( (n= 56) \) to cirrus sac. Vitelline follicles round to oval, medullary occupying 70–88\% \( (x= 80\%, n= 9) \) of the proglottis length, interrupted at level of cirrus-sac, and extending at a distance of 40–85 \( (x= 59, n= 39) \) from the lateral margin of the proglottis and parallel to it. They reach the posterior end of the ovary (Figs. 1B-D, 2A, B).

![Fig. 2. Ophiotaenia tessellata sp. n. from Natrix tessellata, Egypt. (A) Mature proglottis, transverse section at ovarian level; (B) cross-section of gravid proglottis, at level of anterior part; (C) eggs drawn after examination in distilled water. Abbreviations: do, dorsal osmoregulatory canal; dp, digitiform projections; em, embryophore; lm, internal longitudinal musculature; ln, longitudinal lateral nerves; mg, Mehlis’ gland; oe, outer envelope; om, oncospheral membrane; on, oncosphere; ov, ovary; te, testes; ut, uterus; vd, vitelline duct; vf, vitelline follicles; vo, ventral osmoregulatory canal. Scale-bars: A= 500μm; B= 250μm; C= 50μm.](image)

![Fig. 3. (A-F, H-K) Scanning and, (G, L) Transmission electron micrographs of Ophiotaenia tessellata sp. n. from Natrix tessellata, Egypt. (A) Scolex with 4 suckers showing wrinkles and folds; (B) an enlarged sucker showing the transverse slit-like structure (sl); (C) acicular filitriches covering the proliferative zone; (D) a tumulus on the proliferative zone; (E) Burst of the tumulus on the proliferative zone forming large pore. Note also the excretory pore (ex); (F) acicular filitriches covering immature proglottis; (G) acicular filitriches and few gladiate spinriches covering mature proglottis; (H) excretory pores scattered on mature proglottis; (I) a tumulus on mature proglottis; (J) acicular filitriches covering gravid proglottis; (K) one uterine pore; (L) an egg in a stage of development showing the oncosphere (on), oncospheral membrane (om) and bi-layered embryophore (em). Scale-bars: A= 50μm; B, K= 10μm; C, J= 1μm; D-F, L= 2μm; G= 500nm; H= 20μm; I= 5μm.](image)
Ophiotaenia tessellata sp. n. (Eucestoda: Proteocephalinae) from Natrix tessellata

Uterus medullary showing type 1 of development according to de Chambrier et al. (2004b). In immature proglottides, uterus stem is a medial straight longitudinal tube of intensely staining cells. Lumen of uterus appearing in first mature proglottides. Uterine diverticula formed before first eggs appear in uterine stem. In gravid proglottides, uterus occupying up to 47% of proglottis width and reach up to 97% of proglottis length, with 18–30 (x= 23, n= 22) lateral uterine diverticula on each side. Uterus opening ventrally by 4–5 uterine pores in gravid proglottides (Figs. 1B, C, 2B, 3K).

Eggs spherical, with thin hyaline outer envelope 80–160 (x= 128, n= 22) in diameter. Embryophore thick, round to oval, consisting of two layers; outer layer 29–33 (x= 31, n= 15) in diameter, bearing on its external surface layer small digitiform projections, and larger than a nuclei-containing envelope irregular in shape 27–30 (x= 29, n= 8). Oncosphere spherical to oval 12–19 (x= 15, n= 10) in diameter with 6 hooklets 6–8 long, and surrounded by an oncospheral membrane (Figs. 2C, 3L).

Type locality: El Faiyoum Governorate, Egypt.

Prevalence: 8/10 (80%) during summer 2015.

Voucher specimens: Paratype of the whole cestode worm has been deposited in the Institute of Parasitology, České Budějovice, Czech Republic (IPCAS) with a collection number C-825.

Etymology: The specific name is derived from the host specific name.

Differential diagnosis

The new species is placed in the genus Ophiotaenia La Rue, 1911 (Subfamily: Proteocephalinae) on the basis of the medullary position of the reproductive organs and vitellaria, the unarmed scolex with four simple uniloculate suckers of normal type and the distribution of testes in two lateral fields (La Rue, 1911; Freze, 1965; Schmidt, 1986; Rego, 1994).

The new species is separable from Ophiotaenia species found in African snakes in the followings: This species is marked by (1) a transverse slit-like structure in the anterior margin of each sucker which could be recognized...
as the opening of gland cells located posteromedially to the suckers, (2) the tumuli formed by the apocrine-type secretion of gland cells which are abundant in the proliferative zone as also observed in the whole strobila, and (3) the digital projections on the embryophore.

*Ophiotaenia tessellata* sp. n. can be distinguished from *O. europaea* Odening, 1963, both infecting *Natrix tessellata*, in the possession of a vaginal sphencter, smaller body width (1 versus 2.3 mm), fewer testes (65-135 versus 115-344), smaller ratio of ovary width to proglottis width (61-76% versus 86%) and lower relative size of the ovary (4.1% versus 12.7%) (see de Chambrier et al., 2012), in addition to different type of uterus formation (type 1 versus type 2, see de Chambrier et al., 2004b) (Table I).

Although *O. tessellata* sp. n. resembles *O. georgievi* de Chambrier et al., 2010 and *O. lapata* Rambeloson et al., 2012 infecting African colubrid snakes in the number of testes, the relative size of the cirrus sac, percent of ovary width to proglottis width and the presence of vaginal sphencter, it differs from both species in the scolex width and by the number of embryophore layers around the oncosphere, and from *O. georgievi* by the presence of digitiform projections on the external surface of the embryophore and from *O. lapata* by the absence of apical organ and fewer uterine diverticula (18-30 versus 41-68) (Tables I, III). The new species differs from *O. congolensis* Southwell and Lake, 1939 in the higher egg diameter (29-33 versus 15) and from *O. crotaphopeltis* Sandground, 1928, *O. nybelini* Hilmy, 1936 and *sanbernardinensis* Rudin, 1917 by a higher scolex width. It differs also from *O. dubinini* Freze and Scharpilo, 1965 and *O. viperis* (Beddard, 1913) in the position of the genital pore as well as by a lower percent of ovary width to proglottis width. *O. tessellata* sp. n. can be distinguished from *O. faranciae* (MacCallum, 1921), *O. gilberti* Ammann and de Chambrier, 2008 and *O. joanae* (de Chambrier and Paulino, 1997) by the absence of apical organ and by the scolex width. It differs from *O. flavo* Rudin, 1917 and *O. hyalina* Rudin, 1917 by a narrower scolex width and the relative size of the cirrus sac but possesses a greater testes number. *O. tessellata* sp. n. can be differentiated from *O. nankingensis* Hsu, 1935 based on fewer testes (65-135 versus 147-166) and fewer uterine diverticula (18-30 versus 36-40) and from *O. nattereri* (Parona, 1901) and *O. racemosa* (Rudolphi, 1819) by a narrower scolex width. Finally, *O. tessellata* sp. n differs from *O. paraguayensis* Rudin, 1917 by a larger scolex width (300-370 versus 240), higher relative size of the cirrus sac (21-33% versus 12-19%), genital pore position to the proglottis length (41-51% versus 27-39%) and larger egg diameter (29-33 versus 21-24) and by fewer testes (65-135 versus 238-344) (Table I).

**Table II** shows a high resemblance between *O. tessellata* sp. n. and *O. theleri* Rudin, 1917 and *O. zschokkei* Rudin, 1917 infecting the Egyptian cobra *Naja haje* in Africa; *O. tessellata* sp. n. differs from both species by the lower body width, lower number and dimension of testes and fewer uterine diverticula.

**Phylogenetic analysis**

In the present study, the maximum likelihood method was used to construct the phylogenetic tree and representatives of proteocephalidea, tetraphyllidea, phyllobothriidea and cyclophyllidea, with strongly supported independent clades. Our phylogenetic analysis, incorporating new and existing data investigated the placement of the examined proteocephalid species within Proteocephalidea.

Pairwise comparison of the isolated genomic sequence from *Ophiotaenia tessellata* sp. n. with a variety of species and genotypes disclosed unique genetic sequences. Comparison of this novel genetic sequence with others retrieved from GenBank demonstrated a high degree of similarity with range of 91%–99% for 18S rRNA. The results showed that the sequence exhibited the highest percent identity (99%) with *Ophiotaenia lapata* Rambeloson et al., 2012 (Table IV). Molecular phylogenetic analysis based on 18S rRNA gene sequence of *Ophiotaenia tessellata* sp. n. shows that it is closely related to *O. lapata* parasite of the endemic snake *Madagascarophis colubrinus* from Madagascar and grouped with *Australophiotaenia gallardi* (Johnston, 1911) n. comb. and an assembly of four *Ophiotaenia* species infecting snakes (Fig. 4) with a total of three *Ophiotaenia* species infecting colubrid snakes, in addition, the highest BLAST scores with the lowest divergence values were recorded for *O. lapata*.

**DISCUSSION**

*Ophiotaenia tessellata* sp. n. is the first proteocephalid species described infecting *Natrix tessellata* in Egypt. Only *O. nybelini* Hilmy, 1936 has been recorded from the colubrid snake *Coronella coronata* from Liberia and Egypt (see Freze, 1965) while *O. europaea Odening, 1963* is the only *Ophiotaenia* species recorded from *Natrix natrix* and *N. tessellata* in Germany (Odening, 1963), other European countries and Turkey (see Yildirimhan et al., 2007), Georgia and Asian countries (see Halajian et al., 2013; Al-Moussawi, 2014).

In the present study, although, the present worm records a large total body length in comparison with other *Ophiotaenia* species found in colubrid snakes and...
Table I. Comparative measurements of some species of Ophiotaenia in colubrid snakes and *Ophiotaenia tessellata* sp. n.

| Species                  | Locality                      | Body length (mm) | Scolex width | Apical organ | Num-ber of testis | CSGPOV | ROV | Vaginal sphincter | Uterine diverticula/ | Uterine pore | Egg diameter | Vitelline follicles | Reference                  |
|--------------------------|-------------------------------|------------------|--------------|--------------|-------------------|--------|-----|-------------------|----------------------|--------------|---------------|--------------------|--------------------------|
| O. congolensis           | Congo basin                   | Up to 80--65     | < 25%        | 45%**        | 75%**             | (4.3%) |     | -15-20            | -15                   | 87% ap**     | 81% po**      | 87% ap**        | Southwell and Lake (1939) |
| O. crotaphopeltis        | Lake Tanganyika, Kenya        | -160-180         | a94-98       | 16%¤         | 52%¤              | 65%¤   | (3.8%) | -15-18            | 26                   | 92% ap¤      | 86% po¤       | 92% ap¤        | Freze (1965)               |
| O. dubinini             | Russia                        | 202-283          |              |              |                   |        |     |                   |                      |              |               |                   | Freze (1965)               |
| O. faranciae            | North America                 | More than 180    | 500-390      | 29%¤         | 17-25%            | 82%¤   | (2.1%) | a30-50-29-34       | 17-23        | 91% ap¤      | 84% po¤       | Freze (1965)               |
| O. flava                 | Brazil                        | 50-60            | 500-600      | 50%          | 20-40             | 71%    |      |                   |                      | 85% ap¤      | 70% po¤       |                   | Freze (1965)               |
| O. georgievi            | Madagascar                    | Up to 572        | 225-235      | 19-32%       | 44-56%            | 71-76% |      | p23-28-31         | 10-14        | 91-96%       |                  | de Chambrier et al. (2010) |
| O. gilberti             | Paraguay                      | 60-170           | 140-145      | 15-23%       | 42-50%            | 56-69% | (3.7%) | p28-41            | some 12-15    | 90-94% ap    | 85-96% po    | Ammann and de Chambrier (2008) |
| O. hyalina              | Brazil                        | -680-800         | a≈ 50-55     | ≈ 37-55       | 50%               | 33%    |      |                   |                      | 82% ap¤      | 70% po¤       |                   | Freze (1965)               |
| O. joanae               | Brazil                        | 140-250           | 480-790      | 147-210      | 28-56%            | 39-56% | (3.1%) | p26-49            | 26-30        | 79-88% ap    | 75-88% po    | de Chambrier and Paulino (1997) |
| O. lapata               | Madagascar                    | Up to 295        | 190-280      | 19-26%       | 43-53%            | 68-81% | (2.8%) | p41-68            | 41-15        | 90-95%       |                  | Rambeloson et al. (2012)  |
| O. nankingensis         | China, India                  | 105-124           | 320-166      | 147-166      | 44%‡              | 69%‡   |      |                   |                      | 94% ap¤      | 86% po¤       |                   | Freze (1965)               |
| O. nattereri            | Cuba                          | 80-500            | -           | -            | -                 | -      |     |                   |                      | 80-90%       |                  |                   | Freze and Ryšavý (1976)    |
| O. tessellata           | India                         | 105-124           | 320-166      | 147-166      | 44%‡              | 69%‡   |      |                   |                      | 94% ap¤      | 86% po¤       |                   | Freze (1965)               |
| Species                  | Locality            | Length (mm) | Scolex Width | Apical Organ | Num. of Testis | CSGPOV (ROV) | Vaginal Sphincter | Uterine Diverticula// | Uterine Pore | Egg Diameter‡ | Vitelline Follicles † | Reference |
|-------------------------|---------------------|-------------|--------------|--------------|----------------|---------------|-------------------|----------------------|--------------|----------------|-------------------------|-----------|
| O. nybelini (Hilmy, 1936) | Liberia and Egypt | 52105a67-90 | 16-20%       | 46%          | 75% (3.6%)     | 54-70%        | 25-40-25          | 12-19%               | 16%          | 94% ap || 86% po || 54-70% pop | Freze (1965) |
| O. paraguayensis (Rudin, 1917) | Paraguay | 550-600 | 240a238-344 | 12-19%       | 27-39%         | 62-68%       | 54-70%        | 30-100%              | 12-14        | 81% ap || 79% po | Redescription of de Chambrier (1990) |
| O. racemosa (Rudolphi, 1819; La Rue, 1911) | Brazil, Ukraine, Volga Delta | 160-540 | 24-36       | 37-66        | 137             | 25-33%       | 40-50-24           | 70-88%              | 97% ap || 96% po | Freze (1965) |
| O. sanbernardensis (Rudin, 1917) | Paraguay | 100-120 | 228-247 | 70-102 | 50% (5%) | 40% | 27-33 | 21-24 | 81% ap || 79% po | Freze (1965) |
| O. viperis (Beddard, 1913; Rudin, 1917) | Cuba | 24-36 | 37-66 | 137 | 25-33% | 92% | --- | --- | 88% ap || 70% po | Freze and Ryšavý (1976) |
| Ophiotaenia tessellata sp. n. | Egypt | 230-550 | 300-370 | 21-33% | 61-135 | 41-61% | 67-76% | 41-51% | 61-76% | 4.1% | --- | Present study |

Abbreviations: a, absent; gp, gravid proglottis; mp, mature proglottis; on, oncosphere diameter; p, present; CS, relative size of the cirrus-sac expressed as percentage of its length to the width of the proglottis; GP, genital pore position expressed as percentage of its position to the length of the proglottis (see Table II in Ammann and de Chambrier, 2008 and Table I in de Chambrier et al., 2012) written in parentheses; //, number of lateral uterine diverticula on each side; ‡, diameter of the external layer of embryophore; †, ratio of the length of lateral bands of vitelline follicles to proglottis length and clarified as aporal (ap), poral (po), postporal (pop), preporal (pp); **, taken from figures in Southwell and Lake (1939); ¤, taken from figures in Freze (1965); †, after Coquille and de Chambrier (2008); ¶, after Brooks (1978); ‏, after de Chambrier et al. (2015); §, taken from figures in Freze and Ryšavý (1976); /, after La Rue (1914); ●, the uterine aperture is of Crepidobothrium type (see de Chambrier, 1989b).
represents the largest one in all species of *Ophiotaenia* infecting African colubrid snakes (Table I), its width is somewhat small in relation to its length. *O. tessellata* sp. n. lacks an apical organ. Similarly, most species of *Ophiotaenia* infecting colubrid snakes (Table I) as well as those infecting African snakes (see de Chambrier et al., 2010; Rambeloson et al., 2012) lack the apical organ. On the contrary, the apical organ was recorded in proteocephalidean tapeworms infecting amphibians, fishes, lizards and snakes (Table III). Numerous acicular filitriches and few gladiate spinitriches were observed covering *O. tessellata* sp. n. Arredondo et al. (2013) recorded that the gladiate spinitriches is the most frequent microthrix type on the surface of the scolex and strobila in proteocephalidean cestodes and may be either alone or interspersed with filitriches. Filitriches are thought to contribute to the amplification of the absorptive surface of the tegument while the spinitriches in fixation (see Scholz et al., 1999; Žd’árská et al., 2004).

The scolex of *O. tessellata* sp. n. is characterized by the presence of a concentration of cells with granular contents, which may represent gland cells, situated posteromedially to the suckers, with secretions that may participate in the attachment of the scolex between the villi of the host intestine, as previously suggested (see Befus and Freeman, 1973), and recorded (see Gamil, 2012) and explaining the observation of the transverse slit-like structure of the suckers. Cells with granular contents differently located in the scolex and proliferative zone or evidently defined as glands (sometimes as eccrine glands) with ducts transporting their secretory products to the tegument surface have been recorded in the proteocephalideans *Nomimoscolex suspectus*, *Proteocephalus exigus*, *P. macrocephalus*, *P. percae*, *P. soniae*, *P. torulosus*, *Silurotaenia siluri* (de Chambrier and Vaucher, 1994; Scholz et al., 1998, 1999; Žd’árská and Nebesářová, 1999; Zehnder et al., 2000; Žd’árská et al., 2004); all these proteocephalideans are fish parasites, in addition to *Austalophiotaenia mjobergi* (Nybelin, 1917) and *Ophiotaenia azevedoi* (de Chambrier et al., 1992) infecting snakes (de Chambrier et al., 1992, 2018).

Table II. Comparative measurements of *Ophiotaenia* species infecting the Egyptian cobra and *Ophiotaenia tessellata* sp. n.

| Species | Reference | Host | Locality | Body length (mm) | Body width (mm) | Scolex width | Sucker diameter | Apical organ | Excretory pores | Testis number | Testis dimensions | CS | GP | OV | ROV | Vaginal sphincter | Uterine diverticula/ | Uterine pore | Vitelline follicles | Egg outer envelope | No. of embryophore layers | Oncosphere diameter |
|---------|-----------|------|----------|-----------------|-----------------|--------------|----------------|--------------|---------------|--------------|-----------------|-----|-----|-----|-----|-----------------|-------------------|-------------|------------------|------------------|---------------------|------------------|
| *O. theileri* (Rudin, 1917) | Freze (1965) | *Naja haje* | Africa | exceeds 300 | 2.5-4 | 400 | 150 | a | p | 160-310 | 85 in diameter | 20-25% | 40% | 68-75% | 4.5% | p | p | 1 or 15 consecutive apertures | Short slits | 91% ap | 81% po | - | - | 18 |
| *O. zschokkei* (Rudin, 1917) | Freze (1965) | *Naja haje* | South Africa | estimated 550-600 | 2 | 400 | - | a | p | 160-300 | 90 in diameter | 20-25% | 50% | 82% | 6.4% | p | p | - | 80 | 93% ap | 85% po | - | - | 18-30 |
| *Ophiotaenia tessellata* sp. n. | Present study | *Natrix tessellata* | Egypt | 230-550 | 1 | 300-370 | 100-140 | a | p | 230-550 | 2 | 100-140 | 21-33% | 41-51% | 61-76% | 4.1% | p | 80-160 | 2-layered +1 om | 12-19 |

Abbreviations: a, absent; om, oncospheral membrane; p, present; CS, relative size of the cirrus-sac expressed as percentage of its length to the width of the proglottis; GP, genital pore position expressed as percentage of its position to the proglottis length; OV, percent of ovary width to width of the proglottis; ROV, relative size of the ovary defined as the proportion of its size to the size of the proglottis (see Table I in de Chambrier et al., 2012); // number of lateral uterine diverticula on each side; † ratio of the length of lateral bands of vitelline follicles to proglottis length and clarified as aporal (ap) and poral (po); ‡ taken from figures in Freze (1965).
| Species | Host | Type of uterus formation* | NO of uterine diverticula on each side | NO. of uterine pore ** | Egg diameter‡ | NO. of embryophore layers | References |
|---------|------|---------------------------|--------------------------------------|----------------------|---------------|--------------------------|------------|
| Australotaenia hylae | (ao) Amphibians | Litoria aurea | Type 2 | 10-171 | ● | 13-14 (60-75) | 2-layered | ade Chambrier (2004) |
| Nomimoscolex touzeti | (ao) Ceratophrys cornuta | Type 2 | 6-24 (70-120) | 2-layered | 1 | 13-14 (60-75) | 2-layered | Chambrier and Vaucher (1992) |
| Ophiotaenia alessandrae | Hyla boans | Type 1 | 18-25 | several | 2-32 (up to 50) | 2-layered +1 | ade Chambrier and de Pertierra (2012) |
| O. bonneti | Rana vaillanti | Type 2 | 18-32 | 1-25-30 (50-70) | 2-layered | +1 | ade Chambrier et al. (2006) |
| O. oumanskyi | (ao) Lepidobatrachus laevis | Type 1 | 18-25 | several | 23-26 (up to 55) | 2-layered | ade Chambrier and Gil de Pertierra (2012) |
| Testudotaenia testudo | Apalone spinifera | Type 2 | 17-28 | 1-23-25 | ● | 23-25-28 | ade Chambrier et al. (2009a) |
| Barsonella lafoni | (ao) Fishes | Clarias cf. anguillaris | Type 1 | 12-22 | 7-13-24 (40-54) | 2-layered | ade Chambrier et al. (2009b) |
| Cichlidocestus gillesi | (ao) Cichlasoma amazonicum | Type 2 | 16-21 | 4-5-32 (up to 40) | 2-layered | | ade Chambrier et al. (2017) |
| Electrotaenia malopteruri | (ao) Malapterurus electricus | Type 1 | 18-30 | Groove-like pore | 32-36 (80-115) | 2-layered | ade Chambrier et al. (2004a) |
| Gangesia oligonchis | Tachysurus fulvidraco | Type 1 | 18-25 | 27-32 (up to 70) | 2-layered | | Ash et al. (2015) |
| Nomimoscolex suspectus | (ao) Brachyplatystoma filamentosum | Type 2 | 10-18 | 1 or 2 | | 2-layered +1 | Zehnder et al. (2000) |
| Proteocephalus Synodontis | (ao) Synodontis schall | Type 1 | 14-16 | 2-32 | 18-20 | | ade Chambrier et al. (2011) |
| Pseudocrepidobothrium chanaorum | Pseudoplatystoma reticulatum | Type 1 | 10-18 | 11-19 | 15-20 (30-40) | 2-layered | Arredondo et al. (2014) |
| Scholzia emarginata | Phractocephalus hemioliopterus | Type 1 | 8-15 | ● | 18-20 (55) | 2-layered | ade Chambrier et al. (2005) |
| Cairaella henrii | (ao) Lizards | Norops trachyderma | Type 1 | 13-17 | 3-5-37 (70-105) | 2-layered | Coquille and de Chambrier (2008) |
| Kapsulotaenia chisholmiae | (ao) Varanus spenceri | Type 1 | -- | 37-45 (100-125) | 3-layered | | Jones and de Chambrier (2016) |
| K. sandgroundi | Varanus komodoensis | Type 1 | 11-31 | 1-25-30 (90-110) | 3-layered | | ade Chambrier (2006) |
| Tejidotaenia appendiculata | Tupinambis teguixin | Type 1 | 16-20 | several | 30-32 (up to 50) | 2-layered | Rego and de Chambrier (2000) |
| Thaumasioscolex didelphidis | Didelphis marsupialis | Type 1* | 12-22 | 130-33 (160-420) | 2-layered | | Cañeda-Guzmán et al. (2001) |
| Species Host Type | Echinostoma Host Type | NO. uterine diverticula | NO. uterine pore | Egg diameter | NO. embryophore layers | Digiti-form projections |
|------------------|----------------------|------------------------|----------------|-------------|------------------------|------------------------|
| Australophiotaenia galardi | (ao) Snakes | | | | | |
| Australophiotaenia longmani | § | | | | | |
| Australotaenia bunthangi | (ao) | | | | | |
| Crepidothorium gerrardi | (ao) | | | | | |
| Ophiotaenia azevedoi | § (ao) | | | | | |
| Ophiotaenia bungari | (ao) | | | | | |
| Ophiotaenia georgievi | | | | | | |
| Ophiotaenia gilberti | | | | | | |
| Ophiotaenia jarara | (ao) | | | | | |
| Ophiotaenia joanae | § (ao) | | | | | |
| Ophiotaenia lapata | (ao) | | | | | |
| Ophiotaenia paraguayensis | | | | | | |
| Ophiotaenia tessellata | sp. n. | | | | | |
| Vandiermenia beveridgei | (ao) | | | | | |
| Vaucheriella bicheti | (ao) | | | | | |

**Abbreviations:**
- **a:** absent
- **ao:** species with apical organ
- **ap:** aporal
- **Int.:** Intermediate type of uterus formation according to de Chambrier et al. (2017a)
- **M:** mammal
- **om:** oncospheral membrane
- **p:** present
- **po:** poral
- ***,** according to de Chambrier et al. (2004b)
- **,** in pregravid and/or gravid proglottides
- **‡:** diameter of the external layer of embryophore, the diameter of the hyaline outer envelope is written in parentheses
- **^:** on the external surface of the embryophore
- **§:** new combination
- **●:** the uterine aperture is of Crepidothorium type (see de Chambrier, 1989b) defined as a single longitudinal pore occupying the whole length of the gravid proglottis with narrow internal uterine pores
- **†:** illustrated but not described in text
- **African species are in bold.**

The hosts from *Litoria aurea* to *Apalone spinifera* are amphibians, and from *Clarias cf. anguillaris* to *Phractocephalus hemioliopterus* are fishes, and from *Norops trachyderma* to *Tupinambis teguixin* are lizards, and from *Pseudechis porphyriacus* to *Tropidophis cf. taczanowskyi* are snakes.

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**Ophiotaenia tessellata** sp. n. (Eucestoda: Proteocephalinae) from *Natrix tessellata*
Table IV. Cestode species used in the phylogenetic analysis of *Ophiotaenia tessellata* sp. n. using 18S rRNA gene sequence.

| Species                              | Host                | Country     | GenBank accession No. | Percent identity (%) | Reference               |
|--------------------------------------|---------------------|-------------|-----------------------|----------------------|-------------------------|
| Amphoteromorphus piraeeba (P/M)      | *Brachyplatystoma filamentosum* | Brazil      | AY551104.1            | 93%                  | Hypša et al. (2005)     |
| **Barsonella lafoni** (P/P)          | *Clarias gariepinus* | Ethiopia    | KC786005.1            | 91%                  | Scholz et al. (2013)    |
| Choanoscolex absicus (P/M)           | *Pseudoplatystoma coruscans* | Paraguay    | AY551105.1            | 94%                  | Hypša et al. (2005)     |
| Endorchis piraeba (P/M)              | *Brachyplatystoma filamentosum* | Brazil      | AY551107.1            | 93%                  | Hypša et al. (2005)     |
| Gibsoniela meursaulti (P/M)          | *Ageneiosus brevifilis* | Paraguay    | AY551109.1            | 93%                  | Hypša et al. (2005)     |
| Monticellia sp. (P/M)                | *Ophisternon aenigmaticum* | Mexico      | AF267296.1            | 94%                  | Kodedová et al. (2000)  |
| Myzophorus pirarara (P/M)            | *Pterois plumbea*    | Brazil      | AY551112.1            | 92%                  | Hypša et al. (2005)     |
| Nominoscolex lenha (P/M)             | *Sorubimichthys planiceps* | Brazil      | AY551115.1            | 94%                  | Hypša et al. (2005)     |
| Proteocephalus brooksi (P/P)         | *Rhamdia guatemalensis* | Mexico      | AY551124.1            | 94%                  | Hypša et al. (2005)     |
| Rudolfiella lobosa (P/M)             | *Megalonema platanum* | Paraguay    | AY551134.1            | 91%                  | Hypša et al. (2005)     |
| Scholzia emarginata (P/P)            | *Phractocephalus hemioliopetrus* | Brazil     | KC786006.1            | 92%                  | Scholz et al. (2013)    |
| Zygothirum megacephalam (P/M)        | *Phractocephalus hemioliopetrus* | Brazil     | AF286991.1            | 91%                  | Olson et al. (2001)     |
| Australophiotaenia gallardi n. comb. (P/P) | *Pseudechis porphyriacus* | Australia   | KC786014.1            | 98%                  | Scholz et al. (2013)    |
| Ophiotaenia bungari (P/P)            | *Bungarus fasciatus* | Vietnam     | KC786011.1            | 98%                  | Scholz et al. (2013)    |
| O. grandis (P/P)                     | *Agkistrodon piscivorus* (C) | USA        | AY551128.1            | 97%                  | Hypša et al. (2005)     |
| O. lapata (P/P)                      | *Madagascarophis colubrinus* (C) | Madagascar | KC786010.1            | 99%                  | Scholz et al. (2013)    |
| Ophiotaenia sp. (P/P)                | *Antaresia maculosa* | Australia   | KC786013.1            | 98%                  | Scholz et al. (2013)    |
| Ophiotaenia sp. (P/P)                | *Compsisophis* (C)   | Madagascar  | KC786012.1            | 98%                  | Scholz et al. (2013)    |
| Ophiotaenia tessellata sp. n.        | *Natrix tessellata* (C) | Egypt      | KJ917783.1            | 100%                 | Present study           |
| Out Acanthobothrium sp. Group (T/O)  | *Dasyatis longus*    | Mexico      | AF286993.1            | 90%                  | Olson et al. (2001)     |
| Calyptrobothrium sp. (Ph/Ph)         | *Toxopelobus* nobiliana | USA        | KF685848.1            | 89%                  | Caira et al. (2014)     |
| Clistobothrium sp. (Ph/Ph)           | *Arctocephalus pusillus pusillus* | Germany    | KU724058.2            | 88%                  | Klotz et al. (2018)     |
| Pachybothrium hutsoni (T/O)          | *Nebras ferrugineus* | Australia   | EF095246.1            | 90%                  | Waeschenbach et al. (2007) |

**Abbreviations:** P/M, Proteocephalidea/ Monticellidae; P/P, Proteocephalidea/ Proteocephalidae; Ph/Ph, Phyllobothriidea, Phyllobothriidae; T/O, Tetraphyllidea, Onchobothriidae; C, Colubridae; E, Elasmobranch; M, Mammal; //, *Ophiotaenia gallardi* is now Australophiotaenia gallardi (see de Chambrier et al., 2018); African species are in bold. The hosts from *Brachyplatystoma filamentosum* to *Phractocephalus hemioliopetrus* are fishes and from *Pseudechis porphyriacus* to *Natrix tessellata* are snakes.

with the assumed attachment function. Proteolytic, adhesive and protective functions for cestode scolex gland secretions have also been suggested (see McCullough and Fairweather, 1989). Notably, *O. euzeti* (de Chambrier et al., 1992) infecting *Bothrops jararaca* is the only species of *Ophiotaenia* sharing the present worm the posteromedially situation of gland cells to the suckers (although there are no detectable pores to the surface) (de
Chambrier et al., 1992). Such situation and distribution of gland cells was never recorded in any proteocephalidean species infecting colubrid snakes generally and represents one of the three types of gland cells described in proteocephalidean scoleces (see de Chambrier et al., 2017a).

Another character for *O. tessellata* sp. n. is the tumuli (mound-like structures) which are more abundant on the proliferative zone than the whole strobila; tumuli burst and form large pores which seem to be that of gland cells that discharge their secretions by apocrine-like mechanism. Such structures were firstly observed in *O. tessellata* sp. n. and require further studies for their nature and function. A similar structure has been observed earlier by Threadgold (1965) who had proposed that the bulbous evaginations of the proteocephalid *P. pollanicoli* tegument exhibited either excretory or secretory activity; some evaginations swell, burst, and release their contents or are separated as spherical bodies. Tumuli have been also observed on the monticellideans *Spatulifer* cf. *maringaensis* Pavanelli and Rego, 1989 and *Synbranchiella mabelae* Arredondo et al., 2017, and supposed to be formed by the secretory products of unicellular glands (Arredondo and Gil de Pertierra, 2008; Arredondo et al., 2017); Note that all are fish parasites. Žďárská and Nebesářová (1997, 1999) had observed in the apocrine gland type the destruction of secretory projections accompanied with the discharge of the secretion which form a part of the secretory materials. It should also be mentioned that the eccrine-like mechanism of releasing secretory materials is the only type of releasing recorded in proteocephalideans to date (which is elaborated upon earlier in this section).

In Proteocephalidea, secondary canals branch from ventral osmoregulatory canals and extend mainly in proglottides; they are rarely observed in the scolex and proliferative zone parts (žďárská and nebesářová, 2006). In the present study, secondary osmoregulatory canals are observed in the scolex, proliferative zone and the strobila; such canals open outside by fine pores irregularly scattered all over the worm’s body. Interestingly, such excretory pores have been recorded previously in *O. nankinensis* Hsu, 1935 and *O. sanbernardiniensis* Rudin, 1917, both infecting colubrid snakes, as well as *O. adiposaj Rudin, 1917* infecting the African snake *Bitis arietans* (see Freze, 1965) in addition to *Ophiotaina* species infecting the Brazilian snake *Bothrops jararaca* (de Chambrier et al., 1991, 1992).

The relative ovarian size in *Ophiotaina* sp. n. is 4.1%; such percent is used recently as a novel and useful diagnostic character for proteocephalidean tapeworms by Ammann and de Chambrier (2008) and de Chambrier et al. (2012) who found that the relative ovarian size in species of *Ophiotaina* from reptiles from all parts of the world except Europe ranges between 1.5% and 6.7%. The relative ovarian size of *O. tessellata* sp. n. (4.1%) falls into the range reported for *Ophiotaina* spp. It is to be noted that the relative ovarian size in *Ophiotaina* species infecting colubrid snakes from all parts of the world ranges from 2.1% in *O. faranciae* to 9.8% in *O. dubinini* (Table I), and that in *Ophiotaina* infecting African snakes from 2.1% to 6.4% (see de Chambrier et al., 2012).

In *O. tessellata* sp. n., type 1 of uterus development is recorded and has 18-30 uterine diverticula on each side; Table III shows the presence of type 1, type 2 in addition to the intermediate type of uterus formation (recorded by de Chambrier et al., 2017) in some proteocephalidean tapeworms infecting amphibians, fishes and snakes. In fact, there is a divergent uterine diverticula-range observed in *Ophiotaina* species infecting snakes (Tables I, II) and (Table I in Rambeloson et al., 2012). The uterus of *O. tessellata* sp. n. exhibits 4-5 uterine pores in gravid proglottides for the exit of eggs; the uterine pores have been shown in only *O. cotaphophelites*, *O. europaee*, *O. gilberti*, *O. paraguayensis* and *O. sanbernardiniensis* infecting colubrid snakes (Table I) and *O. theileri* and *O. zschokkei* infecting the Egyptian cobra (Table II). It is worthwhile noting that no precise number of uterine pores is observed, and this number ranges from one to more in proteocephalidean tapeworms from different hosts (Table III), in addition to the specified uterine aperture typical of *Crepidobothrium* species (see Beddard, 1913; de Chambrier, 1988, 1989a, b, 1990; de Chambrier et al., 1991).

Although the oncosphere diameter of *O. tessellata* sp. n. is more or less similar in most species of *Ophiotaina* infecting colubrid snakes and the Egyptian cobra (Tables I, II), it is easy to detect that *O. tessellata* sp. n. exhibits a greater range of hyaline outer envelope compared to many proteocephalidean tapeworms infecting snakes (Table III). *O. tessellata* sp. n. is with a 2-layered embryophore sharing *O. conglolensis*, *O. cotaphophelites* and *O. nybelini* (see Southwell and Lake, 1939; Freze, 1965) infecting African colubrid snakes, but it is interesting to highlight that the 3-layered embryophore (provided with an additional thick supplementary layer) has been also recorded in *O. georgievii* and *O. lapata* (de Chambrier et al., 2010, Rambeloson et al., 2012), both are also infecting African colubrid snakes. The variation in the number of embryophore layers is clearly observable in proteocephalidean tapeworms infecting amphibians, lizards and snakes (Table III) and considered by Rambeloson et al. (2012) as a good discriminant character.

*Ophiotaina tessellata* sp. n. shows an evident oncospheral membrane, observed by TEM, surrounding
the oncosphere. Although this membrane is one of the four envelopes that are classically described to surround the proteocephalidean eggs (see de Chambrier, 2006), it is poorly recorded or illustrated (Table III) and defined as a thin supplementary layer between the embryophore and oncosphere of Sandonella sandoni eggs (see de Chambrier et al., 2008) which must be differed from the thick layer forming the 3-layered embryophore. The small digitiform projections (outgrowths) on the external surface of the embryophore in O. tessellata sp. n. have been recorded in only two species of Ophiotaenia infecting colubrid snakes: O. lapata and O. nattereri (as fine hooklets or processes, see La Rue, 1914), as well as in few proteocephalideans infecting amphibians, lizards, snakes and the only species infecting a mammal (Table III). The cluster form of the egg recorded in the majority of Australian proteocephalideans from varanids and snakes (see de Chambrier, 2006; de Chambrier and de Chambrier, 2010; Jones and de Chambrier, 2016; de Chambrier et al., 2018) and the only species infecting mammal (Cañeda-Guzmán et al., 2001) was not observed from any species of Ophiotaenia from colubrid snakes.

Ophiotaenia is a species-rich genus, and in particular, phylogenetic analyses indicate that the genus is polyphyletic and include assemblages of distantly related taxa with similar morphology, apparently as a result of convergent evolution (de Chambrier et al., 2017b). The most comprehensive molecular phylogenetic analysis, based on 28S rDNA revealed the grouping of Ophiotaenia spp. in three main clades by de Chambrier et al. (2015) who added that all these Ophiotaenia species do not differ significantly in their morphology except for that the species of Ophiotaenia of clade K possess type 1 uterus whereas those in the other two clades (N and O) have type 2. A major interest has been in determining that Ophiotaenia tessellata sp. n. seems to belong to the clade K, whereas O. europaea parasitizing Natrix maura in Europe belongs to the clade O (de Chambrier et al., 2015) and previously it did not group with any other species from its genus (Zehnder and Mariaux, 1999). In the present study, a close phylogenetic relationship between O. tessellata sp. n. and O. lapata (both are found in African colubrid snakes) was observed. The 18S rRNA regions yielded congruent results for the taxonomic position of O. tessellata sp. n.; it has a unique genetic sequence embedded in the genus Ophiotaenia and exhibits a close relationship with O. lapata as a putative sister taxon.

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Statement of conflict of interest

The authors have declared no conflict of interests.

Ethical approval

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

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