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

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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 Faiyoum 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, Ranaivoson and de Chambrier, 2012 parasite of the endemic snake *Madagascarophis colubrinus* from Madagascar; both infect African colubrid snakes.

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Authors' Contribution
IG designed the experiment, used the standard methods of examination, drew the figures and prepared the plate. DF conducted the experiments, sequencing, bioinformatics studies. Both authors wrote the manuscript.

Key words
Proteocephalidea, Taxonomy, *Ophiotaenia* sp., Snakes, Natrix tessellata

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, *Vaucheriella* de Chambrier, 1987, *Cairaella* Coquille and de Chambrier, 2008, *Australotaenia* de Chambrier and de Chambrier, 2010, *Vandiermenia* de Chambrier and de Chambrier, 2010, and *Australophiotaenia* de Chambrier *et al*., 2018 (Freze, 1965; Schmidt, 1986; de Chambrier, 1987, 1989a, b, 2006; Rego and de Chambrier, 2000; Coquille and de Chambrier, 2008; de Chambrier and de Chambrier, 2010; de Chambrier *et al*., 2010, 2012, 2018, Scholz *et al*., 2013; Jones and de Chambrier, 2016). *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

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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 Faiyoum 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 at 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 GCA GCC GCG GTA ACT CCA-3′; and 18SR 5′-CCC CCG CCT GTC TCT TTT 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 Miniprepsystem (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 tapers 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 posteromedially 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).

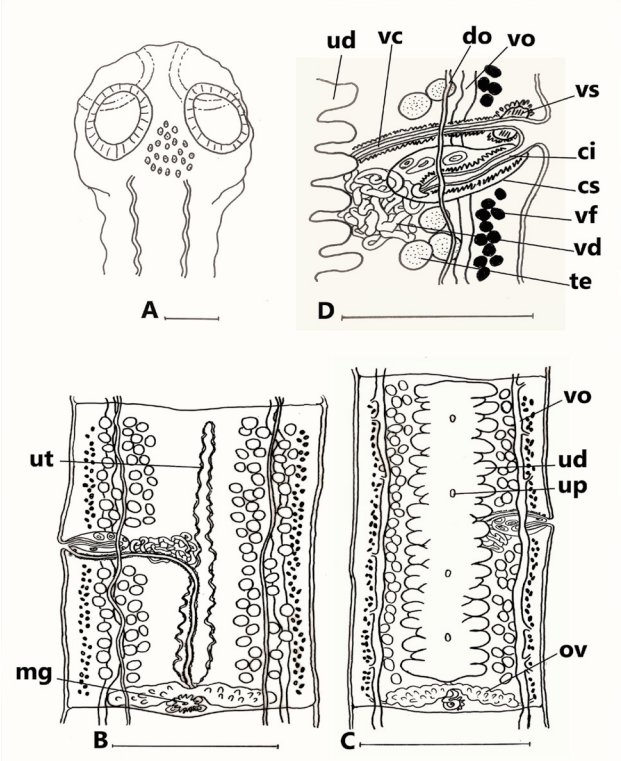

Fig. 1. *Ophiotaenia tessellata* sp. n. from *Natrix tessellata*, Egypt. (A) Scolex, dorsoventral view; (B) mature proglottis, dorsal view; (C) gravid proglottis, ventral view; (D) vagina and cirrus sac region, dorsal view.
Abbreviations: *ci*, cirrus; *cs*, cirrus sac, *do*, dorsal osmoregulatory canal; *mg*, Mehlis' gland, *ov*, ovary; *te*, testes; *ud*, uterine diverticula; *up*, uterine pore; *ut*, uterus; *vd*, vas deferens; *vc*, vaginal canal; *vf*, vitelline follicles; *vo*, ventral osmoregulatory canal; *vs*, vaginal sphincter.
Scale-bars: A= 100 µm; B, C = 500µm; D= 250µm.

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

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 wide (x= 53 x 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).

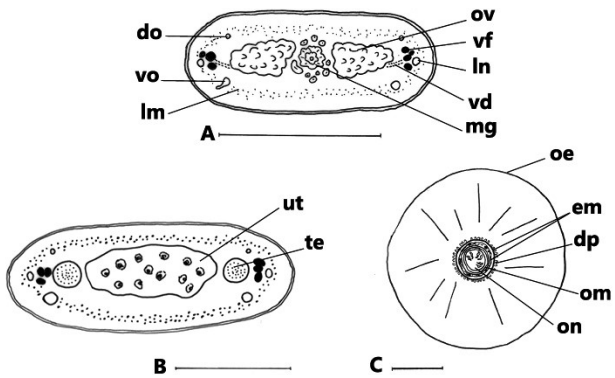

Fig. 2. *Ophiotaenia tessellata* sp. n. from *Natrix tessellata*, Egypt. (A) Mature proglottis, transverse section at ovarian level; (B) cross-section of gravid proglottis, at level of anterior part; (C) eggs drawn after examination in distilled water. **Abbreviations:** *do*, dorsal osmoregulatory canal; *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.

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).

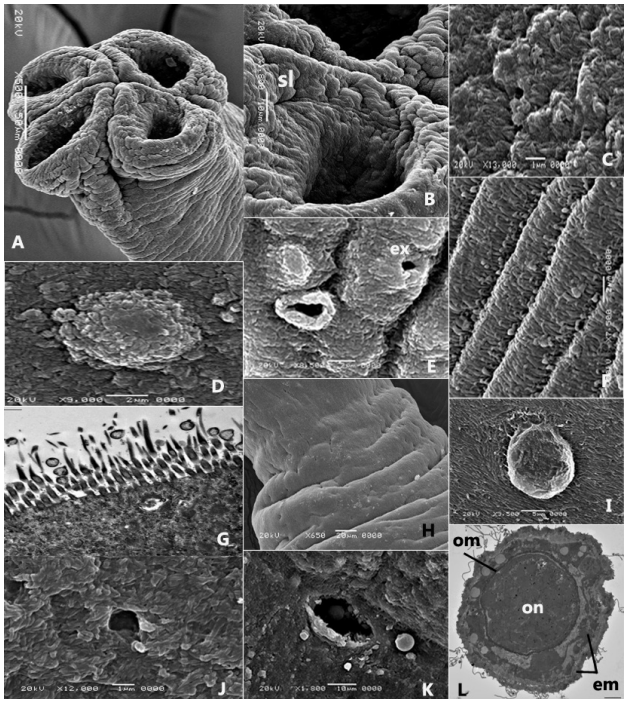

Fig. 3. (A-F, H-K) Scanning and, (G, L) Transmission electron micrographs of *Ophiotaenia tessellata* sp. n. from *Natrix tessellata*, Egypt. (A) Scolex with 4 suckers showing wrinkles and folds; (B) an enlarged sucker showing the transverse slit-like structure (*sl*); (C) acicular filitriches covering 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 spinitriches 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.

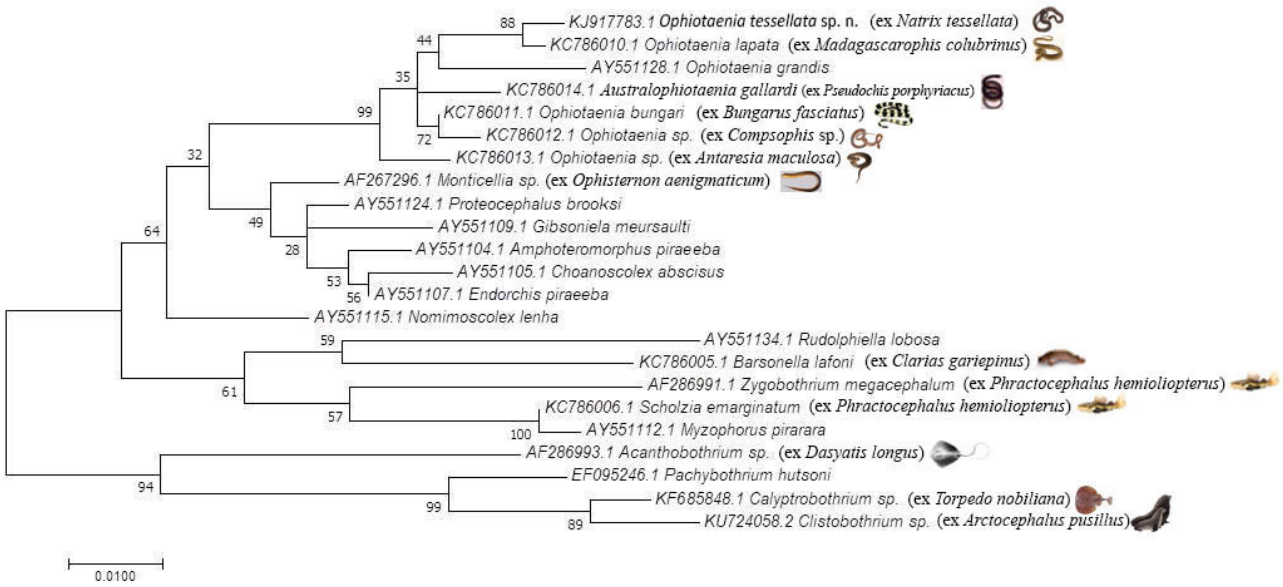

Fig. 4. Molecular Phylogenetic analysis by Maximum Likelihood method based on 18S rRNA gene sequence of *Ophiotaenia tessellata* sp. n. Molecular Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-1795.64) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 453 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Uterus medullary showing type 1 of development according to de Chambrier *et al*. (2004b). In immature proglottides, uterine 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 host: *Natrix tessellata* (Laurenti, 1768) (Serpentes: Colubridae).

Type locality: El Faiyoum Governorate, Egypt.

Site of infection: Intestine.

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 sphincter, 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 sphincter, 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. flava* 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* Hsü, 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. theileri* 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 protocephalidea, 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 proteocephalidean 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	Number of testis	CS	GP	OV (ROV)	Vaginal sphincter	Uterine diverticula//	Uterine pore	Egg diameter‡	Vitelline follicles †	Reference
O. congolensis (Southwell and Lake, 1939)	Congo basin	Up to 80	-	-	65	< 25%	45%**	75%** (4.3%)	-	15-20	-	15	87% ap** 81% po**	Southwell and Lake (1939)
O. crotaphopeltis (Sandground, 1928)	Lake Tanganyika Kenya	-	160-180	a	94-98	16%¤	52%¤	65%¤ (3.8%)	-	15-18	p	26	92% ap¤ 86% po¤	Freze (1965)
O. dubinini (Freze and Scharpilo, 1965)	Russia	-	202-283	-	87-166	25%	23-29%¤	92-95%¤ (9.8%)	-	≈ 22	-	35-57 on:18	85% ap¤ 74-80% po¤	Freze (1965)
O. faranciae (MacCallum, 1921; Hilmy, 1936)	North America	More than 180	500	p	390-420	29%¤	17-25%	82%¤ (2.1%)	a "	30-50	-	29-34¶ on: 17-23¶	91% ap¤ 84% po¤	Freze (1965)
O. flava Rudin, 1917	Brazil	50-60	500-600	-	45-60	50%	20-40 %	71% ¤ (3.6%)	-	-	-	28-30 on:18	85% ap¤ 70% po¤	Freze (1965)
O. georgievi de Chambrier *et al.*, 2010	Madagascar	Up to 57	225-235	a	92-140	19-32%	44-56%	71-76%	p	23-28	-	31-35 on: 10-14	91-96%	de Chambrier *et al.* (2010)
O. gilberti Ammann and de Chambrier, 2008	Paraguay	60-170	140-145	p	57-91	15-23%	42-50%	56-69% (3.7%)	p	28-41	some	27-28 on: 12-15	90-94%ap 85-96%po	Ammann and de Chambrier (2008)
O. hyalina (Rudin, 1917)	Brazil	-	680-800	a	≈ 50-55	50%	33%¤	71%¤ (5.5%)	weak	-	-	-	82% ap¤ 70% po¤	Freze (1965)
O. joanae (de Chambrier and Paulino, 1997) new comb.	Brazil	140-250	480-790	p	147-210	14-25%	28-56%	39-56% (3.1%)	p	26-49	a	26-30 on: 10-12	79-88%ap 75-88%po	de Chambrier and Paulino (1997)
O. lapata Rambeloson *et al.*, 2012	Madagascar	Up to 295	190-280	p	89-170	19-26%	43-53%	68-81% (2.8%)	p	41-68	-	34-39 on: 14-15	90-95%	Rambeloson *et al.* (2012)
O. nankingensis Hsü, 1935	China, India	105-124	320	-	147-166	24%¤	44%¤	69%¤(2.6%)	p	36-40	-	on: 16	94% ap¤ 86% po¤	Freze (1965)
O. nattereri (Parona, 1901) La Rue, 1911	Cuba	80	500	-	92-131	17%§	45%§	63%§	p/	24-35	-	24/	88% ap§ 80% po§	Freze and Ryšavý (1976)

Table continue on next page .....

Species	Locality	Body length (mm)	Scolex width	Apical organ	Number of testis	CS	GP	OV (ROV)	Vaginal sphincter	Uterine diverticula//	Uterine pore	Egg diameter‡	Vitelline follicles †	Reference
O. nybelini (Hilmy, 1936)	Liberia and Egypt	52	105	a	67-90	16-20%	46%¤	75%¤(3.6%)	weak	25-40	-	25 on: 12	94% ap¤ 86% po¤	Freze (1965)
O. paraguayensis (Rudin, 1917)	Paraguay	550-600	240	a	238-344	12-19%	27-39%	62-68% in mp 54-70% in gp (3.3%)	p	20-36	1●	21-24 on: 12-14	89-97%ap 26-43%pp 54-70%pop	Redescription of de Chambrier (1990)
O. racemosa (Rudolphi, 1819; La Rue, 1911)	Brazil Ukraine Volga Delta	160	540-650	-	80-120	33%	33%	76%¤ (4.3%)	weak	40-50	-	24 on: 10	97% ap¤ 96% po¤	Freze (1965)
O. sanbernardinensis (Rudin, 1917)	Paraguay	100-120	228-247	a	70-102	50%	40%¤	70%¤ (5%)	weak	27-33	1	22-23 on: 14-15	81% ap¤ 79% po¤	Freze (1965)
O. viperis (Beddard, 1913; Rudin, 1917)	Cuba	24-36	373	-	66-137	25-33%	33%	92%§	-	-	-	-	88% ap§ 70% po§	Freze and Ryšavý (1976)
Ophiotaenia tessellata sp. n.	Egypt	230-550	300-370	a	65-135	21-33%	41-51%	61-76% (4.1%)	p	18-30	4-5	29-33 on: 12-19	70-88%	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 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 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) defined as a single longitudinal pore occupying the whole length of the gravid proglottis with narrow internal uterine pores. African species are in bold.

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; Žďárská *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 exiguus*, *P. macrocephalus*, *P. percae*, *P. soniae*, *P. torulosus, Silurotaenia siluri* (de Chambrier and Vaucher, 1994; Scholz *et al*., 1998, 1999; Žďárská and Nebesářová, 1999; Zehnder *et al*., 2000; Žďárská *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	***O. theileri* (Rudin, 1917)**	***O. zschokkei (*Rudin, 1917)**	***Ophiotaenia tessellata* sp. n.**
Reference	Freze (1965)	Freze (1965)	Present study
Host	*Naja haje*	*Naja haje*	*Natrix tessellata*
Locality	Africa	South Africa	Egypt
Body length (mm)	exceeds 300	estimated 550-600	230-550
Body width (mm)	3.5-4	2	1
Scolex width	400	400	300-370
Sucker diameter	150	-	100-140
Apical organ	a	a	a
Excretory pores	p	p	p
Testis number	160-310	160-200	65-135
Testis dimensions	85 in diameter	90 in diameter	30-70x30-60
CS	20-25%	20-25%	21-33%
GP	40%¤	50%	41-51%
OV	68-75%¤	82%¤	61-76%
ROV	4.5%	6.4%	4.1%
Vaginal sphincter	p	p	p
Uterine diverticula//	35-40	80	18-30
Uterine pore	1 or 15 consecutive apertures	Short slits	4-5
Vitelline follicles †	91% ap¤ 81% po¤	93% ap¤ 85% po¤	70-88%
Egg outer envelope	-	-	80-160
No. of embryophore layers	-	-	2-layered +1 om
Oncosphere diameter	18	18	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).

Table III. Type of uterus formation*, number of uterine diverticula and uterine pore, and morphological features of the egg in some proteocephalidean species.

Species	Host		Type of uterus formation*	NO of uterine diverticula on each side	NO. of uterine pore **	Egg diameter‡	NO. of embryophore layers	Digitiform projections ^	References
Australotaenia hylae § (ao)	Amphibians	*Litoria aurea*	Type 2	10-17	1●	13-14 (60-75)	2-layered	a	de Chambrier (2004)
Nomimoscolex touzeti (ao)		*Ceratophrys cornuta*	-	24-35	Up to 3	26-29 (70-120)	2-layered	p	de Chambrier and Vaucher (1992)
Ophiotaenia alessandrae		*Hyla boans*	Type 1	18-25	several	22-24 (up to 50)	3-layered	a	Marsella and de Chambrier (2008)
O. bonneti		*Rana vaillanti*	Type 2	18-32	1	25-30 (50-70)	2-layered +1om †	a	de Chambrier *et al*. (2006)
O. oumanskyi (ao)		*Lepidobatrachus laevis*	Type 1	18-25	several	23-26 (up to 55)	2-layered	a	de Chambrier and Gil de Pertierra (2012)
Testudotaenia testudo		*Apalone spinifera*	Type 2	17-28	1●	23-25	-	a	de Chambrier *et al*. (2009a)
Barsonella lafoni (ao)		*Clarias cf. anguillaris*	Type 1	12-22	7-13	21-24 (40-54)	2-layered	a	de Chambrier *et al*. (2009b)
Cichlidocestus gillesi (ao)		*Cichlasoma amazonarum*	Type 2	16-21	4-5	30-33	2-layered	a	de Chambrier *et al*. (2017)
Electrotaenia malopteruri (ao)		*Malapterurus electricus*	Type 1	18-30	Groove-like pore	32-36 (80-115)	2-layered	a	de Chambrier *et al*. (2004a)
Gangesia oligonchis	Fishes	*Tachysurus fulvidraco*	Type 1	18-25	p	27-32 (up to 70)	2-layered	a	Ash *et al*. (2015)
Nomimoscolex suspectus (ao)		*Brachyplatystoma filamentosum*	-	10-18	1 or 2	31-35x 28-30 (> 90)	2-layered +1 om	a	Zehnder *et al*. (2000)
Proteocephalus Synodontis (ao)		*Synodontis schall*	Type 1	4-16	2-3	20-21x 18-20	-	a	de Chambrier *et al*. (2011)
Pseudocrepidobothrium chanaorum		*Pseudoplatystoma reticulatum*	Type 1	10-18 ap 11-19 po	p	15-20 (30-40)	2-layered	a	Arredondo *et al*. (2014)
Scholzia emarginata		*Phractocephalus hemioliopterus*	Type 1	8-15	1●	18-20 (55)	2-layered	a	de Chambrier *et al*. (2005)
Cairaella henrii (ao)		*Norops trachyderma*	Type 1	13-17	p	35-37 (70-105)	3-layered	p	Coquille and de Chambrier (2008)
Kapsulotaenia chisholmae (ao)		*Varanus spenceri*	Type 1	-	-	37-45 (100-125x95-110)	3-layered	a	Jones and de Chambrier (2016)
K. sandgroundi (ao)	Lizards	*Varanus komodoensis*	Type 1	21-31	1●	25-30 (90-110)	3-layered	a	de Chambrier (2006)
Tejidotaenia appendiculata		*Tupinambis teguixin*	-	16-20	several pore-like structures	30-32 (up to 50)	2-layered	a	Rego and de Chambrier (2000)
Thaumasioscolex didelphidis	M	*Didelphis marsupialis*	Type 1*	12-22	1	30-33 (160-420)	2-layered	p	Cañeda-Guzmán *et al*. (2001)

Table continue on next page .....

Species	Host		Type of uterus formation*	NO, of uterine diverticula on each side	NO. of uterine pore **	Egg diameter‡	NO. of embryophore layers	Digitiform projections ^	References
Australophiotaenia gallardi §(ao)	Snakes	*Pseudechis porphyriacus*	Type 1	23-30	pore-like structures	37-40 (110-120)	3-layered	p	de Chambrier *et al*. (2018)
Australophiotaenia longmani §		*Aspidites ramsayi*	Type 1	32-43	-	32-36 (up to 270)	3-layered	-	de Chambrier *et al*. (2018)
Australotaenia bunthangi (ao)		*Enhydris enhydris*	Type Int.	14-24	1	25-27 (60-70)	2-layered	a	de Chambrier and Scholz (2012)
Crepidobothrium gerrardii (ao)		*Boa constrictor L.*	-	18-25	1●	16.5-20.5	-	-	de Chambrier (1989a)
C. lachesidis (ao)		*Eunectes murinus*	-	21-32	1●	24-26 (50-60)	2-layered	a	de Chambrier (1989b)
Macrobothriotaenia ficta		*Xenopeltis unicolor*	Type 1	26-37	-	32-39 (80-120)	3-layered	p	Scholz *et al*. (2013)
O. azevedoi § (ao)		*Bothrops jararaca*	-	45-61	p	32-36 (150)	2-layered +1om †	a	de Chambrier *et al*. (1992)
O. bungari (ao)		*Bungarus fasciatus*	Type 1	50-65	1	28-32 (65-95)	3-layered	a	de Chambrier *et al*. (2012)
O. georgievi		*Leioheterodon geayi*	Type 1	23-28	-	31-35	3-layered	a	de Chambrier *et al*. (2010)
O. gilberti (ao)		*Thamnodynastes pallidus*	Type 1	28-41	some	27-28	2-layered +1om †	a	Ammann and de Chambrier (2008)
O. jarara (ao)		*Bothrops jararaca*	Type 1*	26-29	1●	11-12	2-layered	a	Redescription of de Chambrier *et al*. (1991)
O. joanae § (ao)		*Xenodon neuwiedi*	-	26-49	a	26-30 (up to 90)	2-layered	a	de Chambrier and Paulino (1997)
O. lapata (ao)		*Madagascarophis colubrinus*	Type 1	41-68	-	34-39 (140-165)	3-layered	p	Rambeloson *et al*. (2012)
O. paraguayensis		*Hydrodynastes gigas*	Type 2*	20-36	1●	21-24 (36-40)	-	a	Redescription of de Chambrier (1990)
Ophiotaenia tessellata sp. n.		*Natrix tessellata*	Type 1	18-30	4-5	29-33 (80-160)	2-layered +1 om	p	Present study
Vandiermenia beveridgei (ao)		*Pseudechis porphyriacus*	Type 1	22-26	a	62-70 (85-95)	3-layered	p	de Chambrier and de Chambrier (2010)
Vaucheriella bicheti (ao)		*Tropidophis cf. taczanowskyi*	-	19-34	a	24-26 (51-81x48-57)	2-layered	p †	de Chambrier (1987)

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 *Crepidobothrium* 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.

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 abscisus (P/M)		*Pseudoplatystoma coruscans*	Paraguay	AY551105.1	94%	Hypša *et al*. (2005)
Endorchis piraeeba (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)	Fishes	*Ophisternon aenigmaticum*	Mexico	AF267296.1	94%	Kodedová *et al*. (2000)
Myzophorus pirarara (P/M)		*Phractocephalus hemioliopterus*	-	AY551112.1	92%	Hypša *et al*. (2005)
Nomimoscolex 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)
Rudolphiella lobosa (P/M)		*Megalonema platanum*	Paraguay	AY551134.1	91%	Hypša *et al*. (2005)
Scholzia emarginata (P/P)		*Phractocephalus hemioliopterus*	Brazil	KC786006.1	92%	Scholz *et al*. (2013)
Zygobothrium megacephalum (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)	Snakes	*Madagascarophis colubrinus* (C)	Madagas-car	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)		*Compsophis sp.* (C)	Madagas-car	KC786012.1	98%	Scholz *et al*. (2013)
Ophiotaenia tessellata sp. n.		*Natrix tessellata* (C)	Egypt	KJ917783.1	100%	Present study
Out Group	*Acanthobothrium* sp. (T/O)	E *Dasyatis longus*	Mexico	AF286993.1	90%	Olson *et al*. (2001)
Calyptrobothrium sp. (Ph/Ph)		E *Torpedo nobiliana*	USA	KF685848.1	89%	Caira *et al*. (2014)
Clistobothrium sp. (Ph/Ph)		M *Arctocephalus pusillus pusillus*	Germany	KU724058.2	88%	Klotz *et al*. (2018)
Pachybothrium hutsoni (T/O)		E *Nebrius 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 the snake *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 proteocephalidean *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. nankingensis* Hsü, 1935 and *O. sanbernardinensis* Rudin, 1917, both infecting colubrid snakes, as well as *O. adiposa* Rudin, 1917 infecting the African snake *Bitis arietans* (see Freze, 1965) in addition to *Ophiotaenia* species infecting the Brazilian snake *Bothrops jararaca* (de Chambrier *et al*., 1991, 1992).

The relative ovarian size in *Ophiotaenia* 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 *Ophiotaenia* from reptiles from all parts of the world except Europe ranges between 1.5% and 6.7%. The relative ovarian size of *O. tessellata* spp. n. (4.1%) falls into the range reported for *Ophiotaenia* spp. It is to be noted that the relative ovarian size in *Ophiotaenia* 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 *Ophiotaenia* 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 *Ophiotaenia* 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. crotaphopeltis*, *O. europaea*, *O. gilberti*, *O. paraguayensis* and *O. sanbernardinensis* 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 *Ophiotaenia* 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. congolensis*, *O. crotaphopeltis* 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. georgievi* 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.

Ophiotaenia 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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