Ophiotaenia echidis n. sp. (Cestoda: Proteocephalidae) from the saw-scaled viper, *Echis carinatus sochureki* Stemmler (Ophidia: Viperidae), one of the world's deadliest snakes, from the United Arab Emirates

Alain de Chambrier [a], Philippe V. Alves [b], Rolf K. Schuster [c], Tomáš Scholz [d,*]

[a] *Natural History Museum, PO Box 6434, CH-1211, Geneva 6, Switzerland*
[b] *Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[c] *Central Veterinary Research Laboratory, Dubai, PO Box 597, United Arab Emirates*
[d] *Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, 370 05, České Budějovice, Czech Republic*

ARTICLE INFO

ABSTRACT

Ophiotaenia echidis n. sp. (Cestoda: Proteocephalidae) is described from the intestine of one of the world's deadliest snakes, the saw-scaled viper *Echis carinatus sochureki* Stemmler (Ophidia: Viperidae) in the United Arab Emirates. The new species differs from other species of the non-monophyletic *Ophiotaenia* by the position of testes in two longitudinal lines on both sides of the uterus, and by the large size of an embryophore (diameter of 44–55 μm *versus* less than 40 μm in other species). Phylogenetic reconstructions based on *lsr*DNA and concatenated *lsr*DNA + COI datasets place the new species among proteocephalids from unrelated zoogeographical realms but mostly infecting venomous snakes. In all analyses, *O. echidis* n. sp. exhibited a strongly supported sister relationship with *O. lapata* Rambeloson, Ranaivoson et de Chambrier, 2012, a parasite of a pseudoxyrhophiid snake endemic to Madagascar. Despite a shared close evolutionary history between these taxa, morphological synapomorphies remain unclear, which impedes the erection of a new genus to accommodate them. A list of the 71 tapeworms of the former, non-monophyletic subfamily Proteocephalinae, parasitising snakes and lizards, including *species inquirendae*, and the phylogenetically closely related *Thaumasioscolex didelphidis* from opossum, with selected characteristics, is also provided, together with a checklist of helminth parasites reported from *E. carinatus*.

1. Introduction

Ophiotaenia La Rue, 1911 is the most species-rich genus of proteocephalid cestodes (Onchoproteocephalidea), with a hundred species (88 according to de Chambrier et al., 2017 plus 12 taxa considered *species inquirendae*) parasitising reptiles and amphibians throughout the world (Rego, 1994; de Chambrier et al., 2017). Despite the overall morphological uniformity of most species of *Ophiotaenia*, the most comprehensive phylogenetic analysis (*lsr*DNA-based) for proteocephalids assessed by de Chambrier et al. (2015), revealed multiple colonisation events in snakes worldwide. These authors recognised three main clades containing species of *Ophiotaenia*. The generic name should be restricted to species of Clade O of de Chambrier et al. (2015), which includes the type species *Ophiotaenia perspicua* La Rue (1911) from North American colubrid snakes (La Rue, 1911, 1914), and also *Ophiotaenia europaea*

Odening, 1963 from Palaearctic colubrids (de Chambrier et al., 2015).

Viperid snakes of the genus *Echis* Merrem, also known as saw-scaled vipers, are small to medium-sized animals widely distributed across xeric areas from Africa (countries north to the Equator) to India and Sri Lanka, including most regions of the Middle East (Pook et al., 2009); they exhibit a diversified diet usually corresponding to a particular species of the genus, e.g., vipers of the *E. carinatus* (Schneider) and *E. pyramidum* (Geoffroy Saint-Hilaire) groups prey mostly upon arthropods, whereas species of the *E. coloratus* Günther group feed almost exclusively on vertebrates (Barlow et al., 2009).

The World Health Organization (2020) classified most species of *Echis* within Category 1 of risk, i.e., of the highest medical importance, which is reasonable since these vipers are presumably responsible for the greatest number of snakebite deaths per year in Africa and also being important causes of mortality and morbidity in India (see Pook et al.,

2009 and references therein). Despite the relevance of *Echis* spp. to human health, their cestode fauna is poorly known, with just a few reports in faunistic surveys (see Sharpilo, 1976; Gibson et al., 2005). Data on helminth parasites of the saw-scaled viper are also scarce and most of them were published by Soviet authors in the 1950's and 1960's (see Sharpilo, 1976). Since most papers were written in Russian and are not easily accessible, a checklist of helminths parasitising *Echis carinatus* is provided to facilitate availability of information largely published in local journals and proceedings ('sbornik').

In the present paper, a new species of *Ophiotaenia* from the saw-scaled viper, *Echis carinatus sochureki* Stemmler, in the United Arab Emirates is described and its phylogenetic position is discussed.

2. Materials and methods

2.1. Morphological data

Tapeworms were found in the small intestine of six saw-scaled vipers, *Echis carinatus sochureki* Kemmler found in different localities of the United Arab Emirates and examined from 2003 to 2020. This subspecies occurs in northern India, Bangladesh, southern Afghanistan, Pakistan, central Iran, southern Iraq and the United Arab Emirates, with an isolated population in southeastern Arabian Peninsula (Uetz et al., 2020). The nominotypical subspecies *E. carinatus carinatus* (Schneider) is limited in its distribution to peninsular India (Uetz et al., 2020). None of 33 other snakes examined was infected, namely one *Echis omanensis* Babocsay, five *Cerastes gasperettii* Leviton et Anderson (both Viperidae), ten *Eryx jayakari* Boulenger (Boidae), one *Malpolon moilensis* (Reuss), 13 *Psammophis schokari* (Forskål) (both Psammophiidae), and three *Platyceps rhodorachis* (Jan) (Colubriidae). A total of 58 tapeworms in different states of maturity were found, but some specimens were kept in tap water until their strobilae were totally relaxed. Therefore, only a few specimens collected in a fresh snake (host code No. UAE 4) fixed in 4% hot formalin were suitable for morphological evaluation (see below).

Tapeworms were stained with Mayer's carmine, dehydrated in an ethanol series, clarified by eugenol (clove oil) and mounted in Canada balsam as permanent preparations. For histology, pieces of strobila were embedded in paraffin, transversely sectioned at 12–15 μm intervals, stained with Weigert's hematoxylin, and counterstained with 1% eosin B (acidified with five drops of pure acetic acid for 100 ml solution) (see de Chambrier, 2001). Eggs were studied in distilled water. For scanning electron microscopy (SEM) observations, one scolex was dehydrated through a graded ethanol series, dried in hexamethyldisilazane, coated with gold (thickness of 10–20 nm) and examined in a JEOL JSM-740 1F scanning electron microscope at the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences. All measurements in morphological description are given in micrometres unless otherwise indicated. Abbreviations used in description (usually if the number of measurements was >5) are: x = mean; n = number of measurements. Host and zoogeographical realm classifications follow Uetz et al. (2020) and Holt et al. (2013), respectively. Material studied is deposited in the Natural History Museum, Geneva, Switzerland (acronym MHNG-PLAT), and in the Helminthological Collection of the Institute of Parasitology, České Budějovice, Czech Republic (IPCAS).

A piece of another specimen (paragenophore from host UAE 03; MHNG-PLAT-120508) was used for DNA sequencing (courtesy of J. Brabec) of the large subunit nuclear ribosomal RNA (*lsr*DNA; D1–D3 domains) and the partial mitochondrial cytochrome *c* oxidase subunit I (COI) following the methodology outlined by de Chambrier et al. (2019). The sequences were assembled and inspected for errors using Geneious version R11 (Kearse et al., 2012), and submitted to GenBank (MW703700 – *lsr*DNA; MW703548 – COI); COI gene assembly was trimmed to the protein-coding region using the echinoderm translational code. Two alignments were created using the newly obtained sequences and selected members of the Proteocephalidae mostly corresponding to Clade K of de Chambrier et al. (2015) (these taxa were

informed by a more comprehensive analysis of currently unpublished data) using default parameters of MAFFT (Katoh and Standley 2013) implemented in the Guidance2 web server (http://guidance.tau.ac.il/; Sela et al. 2015): (i) alignment including only the *lsr*DNA sequence data, and (ii) a concatenated alignment (*lsr*DNA + COI) including only those representatives with available sequences for both markers (see Table 1). Unreliable positions in the alignments were identified and removed using the Gblock web server (https://ngphylogeny.fr/; Dereeper et al., 2008) with less stringent settings.

Phylogenetic reconstructions were performed with the maximum likelihood (ML) criterion using the evolutionary models implemented in ModelFinder (Kalyaanamoorthy et al., 2017) within IQ-TREE (Trifino-poulos et al., 2016), based on the small sample size corrected Akaike Information Criterion (AICc). The models chosen were as follows: TIM3 + F + R2 for the *lsr*DNA dataset alone; K3P + I for 1st codon position and GTR + F + G4 for *lsr*DNA, 2nd and 3rd codon positions using the concatenated dataset. The ML trees were generated via IQ-TREE and clade supports were estimated with 5000 replicates of the ultrafast bootstrap (UFBoot – Minh et al., 2013) and an SH-aLRT test with 5000 replicates (Guindon et al., 2010). To avoid overestimation of UFBoot, we used a hill-climbing nearest neighbour interchange (NNI), as recently recommended by Hoang et al. (2018). Clades with support values of both UFBoot $\geq$ 95 and SH-aLRT $\geq$ 80 were considered strongly supported, while clades with only one of UFBoot $\geq$95 or SH-aLRT $\geq$ 80 were weakly supported; nodes with both UFBoot <95 or SH-aLRT < 80 were unsupported. All the above-mentioned analyses were run on the computational resource CIPRES (Miller et al., 2010).

3. Results

3.1. Ophiotaenia echidis n. sp. Figs. 1–3

Type- and only host: *Echis carinatus sochureki* Stemmler, 1969 (Serpentes: Viperidae).

Type-locality: Houbara Breeding Centre (25.10418; 55.11283), Dubai, United Arab Emirates.

Other localities: Camel Reproduction Centre, Dubai (25.08752; 55.38819); Dubai Safari (25.17971; 55.45056); Emirates Industry for Camel Milk Products, Dubai (25.02290; 55.41492), all in the United Arab Emirates.

Prevalence: Six snakes of 20 examined (i.e., 30%) in March 2007, July 2014, July and December 2017, and June 2020 were positive.

Intensity of infection: A total of 58 tapeworms were found in six positive hosts, i.e., mean intensity of infection was 10 tapeworms/infected host; minimum four tapeworms in June 2020, maximum 21 specimens in December 2017.

Site of infection: Small intestine.

Distribution: United Arab Emirates.

Type-material: Holotype MHNG-PLAT-0137383, 18 slides (complete whole-mounted specimen on three slides and 15 slides with serial sections) and two slides (with serial sections) IPCAS C-876/1 from host field number UAE 04, Houbara Breeding Centre, collected in July 2017; one paratype MHNG-PLAT-0137384, 21 slides (one complete whole-mounted specimens on three slides and 18 slides with serial sections), and two slides (with serial sections) IPCAS C-876/1 field number UAE 04, Houbara Breeding Centre (July 2017); two paratypes, IPCAS C-876/1 (two complete whole-mounted specimens on three and two slides, field number UAE 04-T2a-3a), Houbara Breeding Centre (July 2017), all Emirates Industry for Camel Milk Products in Dubai, examined by R. Schuster in June 2020.

Other material: Vouchers MHNG-PLAT-120508 (paragenophores), 14 slides (seven slides with whole-mounted specimen and seven slides serial sections from host field number UAE 03, Dubai Safari; IPCAS C-876/1 (one whole-mounted specimen); MHNG-PLAT-88912, Houbara Breeding Centre (March 2007), one specimen without scolex, 17 slides (three whole-mount and 14 serial sections), field number UAE 01;

Table 1

Summary data for the isolates used in the molecular analyses sorted by zoogeographical realms. GenBank numbers in bold indicate sequences obtained as part of this study.

Region/Taxon	Host species (family) [group]	Country (isolate)	Accession number	
			*lsr*DNA	COI
Afrotropical				
Ophiotaenia ophiodex	*Causus maculatus* (Viperidae) [R]	Ivory Coast (MHNG-PLAT-25962)	AJ388620	N/A
Australian				
Australophiotaenia gallardi	*Pseudechis porphyriacus* (Elapidae) [R]	Australia (NHMUK: PBI-518)	KC786025	KC785990
Australophiotaenia sp. 1	*Antaresia maculosa* (Pythonidae) [R]	Australia (NHMUK: PBI-513)	KC786024	KC785988
Madagascan				
Ophiotaenia lapata	*Madagascarophis colubrinus* (Pseudoxyrhophiidae) [R]	Madagascar (NHMUK: PBI-677)	KC786021	KC785985
Ophiotaenia sp.	*Compsophis* sp. (Pseudoxyrhophiidae) [R]	Madagascar (NHMUK: PBI-678)	KC786023	KC785986
Nearctic				
Megathylacoides giganteum[a]	*Ictalurus punctatus* (Ictaluridae) [T]	USA (US 685a1)	MT193860	MT193845
Megathylacoides lamothei[a]	*Ictalurus furcatus* (Ictaluridae) [T]	USA (US 688-1)	MT193867	MT193852
Ophitaenia grandis	*Agkistrodon piscivorus* (Viperidae) [R]	USA (N/A)	AJ388632	N/A
Neotropical				
Crepidobothrium gerrardii	*Boa constrictor* (Boidae) [R]	Peru (NHMUK: PBI-516)	KC786018	N/A
Ophiotaenia jararaca	*Bothrops jararaca* (Viperidae) [R]	Brazil (MHNG-PLAT-12393)	AJ388607	N/A
Panamanian				
Thaumasioscolex didelphidis	*Didelphis marsupialis* (Didelphidae) [M]	Mexico (NHMUK: PBI-646)	KC786019	KC785983
Oriental				
Macrobothriotaenia ficta	*Xenopeltis unicolor* (Xenopeltidae) [R]	Vietnam (NHMUK: PBI-491)	KC786020	KC785984
Ophiotaenia bungari	*Bungarus fasciatus* (Elapidae) [R]	Vietnam (NHMUK: PBI-500)	KC786022	KC785987
Saharo-Arabian				
Ophiotaenia echidis n. sp.	*Echis carinatus sochureki* (Viperidae) [R]	United Arab Emirates (MHNG-PLAT-120508)	**MW703700**	**MW703548**

[a] Outgroups. Abbreviations: N/A – not available; MHNG-PLAT – Natural History Museum, Geneva, Switzerland; NHMUK – Natural History Museum, London, UK; PBI-No. – unique specimen ID, see http://www.tapewormdb.uconn.edu/index.php/parasites/molecular_search/; M – mammals; R – reptiles; T – teleosts.

MHNG-PLAT-120507, one specimen without scolex, 35 slides (four whole-mounts and 31 with serial sections) Houbara Breeding Centre, field number UAE 02; MHNG-PLAT-0137385, Houbara Breeding Centre (July 2017) (one whole-mounted specimens without scolex, on two slides and 23 slides serial sections) and MHNG-PLAT-0137386, Houbara Breeding Centre (July 2017) (one whole-mounted specimens without scolex, on three slides), field number UAE 04, all from *E. carinatus*.

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

Representative DNA sequences and phylogenetic relationships: one isolate (MHNG-PLAT-120508) yielded partial *lsr*DNA (MW703700; 1489 bp long) and COI (MW703548; 921 bp long) sequences. The final alignment of only *lsr*DNA dataset comprised 1012 positions including 124 parsimony informative sites, whereas the concatenated alignment comprised 1615 positions (1012 of *lsr*DNA and 603 of COI) including 250 parsimony-informative sites. The trees resulting from the ML analyses of both single and concatenated datasets showed a strongly supported sister relationship between *O. echidis* n. sp. and *Ophiotaenia lapata* Rambeloson, Ranaivoson et de Chambrier, 2012, from *Madagascarophis colubrinus* Schlegel (Pseudoxyrhophiidae) endemic to Madagascar (Fig. 4) (Rambeloson et al., 2012). The analysis of *lsr*DNA alone further shows these species within a polytomy (Clade Q) together with other proteocephalids from distantly related groups of snakes (families Elapidae, Pseudoxyrhophiidae, Pythonidae and Xenopeltidae) from unrelated zoogeographical realms (Afrotropical, Australian, Madagascan and Oriental). While clade K of de Chambrier et al. (2015) is well supported (posterior probability = 1) in their analysis, our results show only weak support for this group (Fig. 4) that encompasses, besides snake cestodes, the only proteocephalid from a mammal, *Thaumasioscolex didelphidis* Cañeda-Guzmán, de Chambrier et Scholz, 2001, from the common opossum (*Didelphis marsupialis* Linnaeus) in Neotropical Mexico (Veracruz).

3.2. Description (Figs. 1–3)

(Based on four entire specimens and part of another two worms)

Proteocephalidae. Large worms, 230–275 mm long, up to 1.2 mm wide, flattened dorsoventrally, with proglottids greatly elongated, up to 6.6 mm long. Strobila acraspedote, anapolytic, with 96–105 immature proglottids (up to appearance of spermatozoa in vas deferens), 5–6 mature proglottids (up to appearance of eggs in uterus), 3–5 pregravid proglottids (up to appearance of hooks in oncospheres), 105–118 proglottids in total. Immature proglottids wider than long to longer than wide (length: width ratio 1: 0.5–6.0); mature, pregravid and gravid proglottids much longer than wide. Terminal proglottids much longer than wide (Fig. 3), length: width ratio up to 1: 6.4. Tegument 7.5–10 thick.

Scolex spherical, aspinose (Fig. 1A–C, 2A), 190–450 long (measured from anterior extremity to posterior margin of suckers; x = 235, n = 3), 400–725 (x = 475, n = 3) wide, wider than neck, with four uniloculate, spherical suckers (Fig. 1A–C, 2A), 165–210 in diameter, representing 30–45% of scolex width; apical sucker or organ absent (Fig. 2A). Neck, i. e., unsegmented zone posterior to scolex to first recognisable proglottids elongate (up to 3.3 mm), up to 540 wide. Apex of scolex and luminal surface of suckers covered with capilliform filitriches (Fig. 1D, G); external (non-adherent) surface of suckers and between suckers covered with gladiate spinitriches (Fig. 1E, I); upper and lower rims of suckers and neck covered with gladiate spinitriches, interspersed with few capilliform filitriches (Fig. 1F, H, J).

Internal longitudinal musculature weakly developed, consisting of few narrow muscle fibres, more numerous along lateral margins of proglottid (Fig. 2D). Osmoregulatory canals run along vitelline follicles, sometimes overlapping them (Fig. 2B). Ventral canals, 10–20 in diameter, with numerous secondary canals directed externally. Dorsal canals thick-walled, 2.5–4 in diameter, situated alongside and median to vitelline follicles (Fig. 2C, F, G). Genital ducts run between osmoregulatory canals (Fig. 2F and G).

Testes spherical, 30–50 in diameter, 118–205 in number (x = 162, n = 20), in single layer and in two longitudinal columns on both sides of uterine stem, composed of single line of testes, situated at about 30% from lateral side of proglottids (Figs. 2B and 3). Anteriorly, testes do not reach anterior margin of proglottids, starting at distance of 2–5% of proglottid length, i.e., slightly posterior to anterior-most vitelline follicles (Fig. 3). Posteriorly, testes never reach ovary, with terminal testes at distance of 9–13% of proglottid length (Figs. 2B and 3).

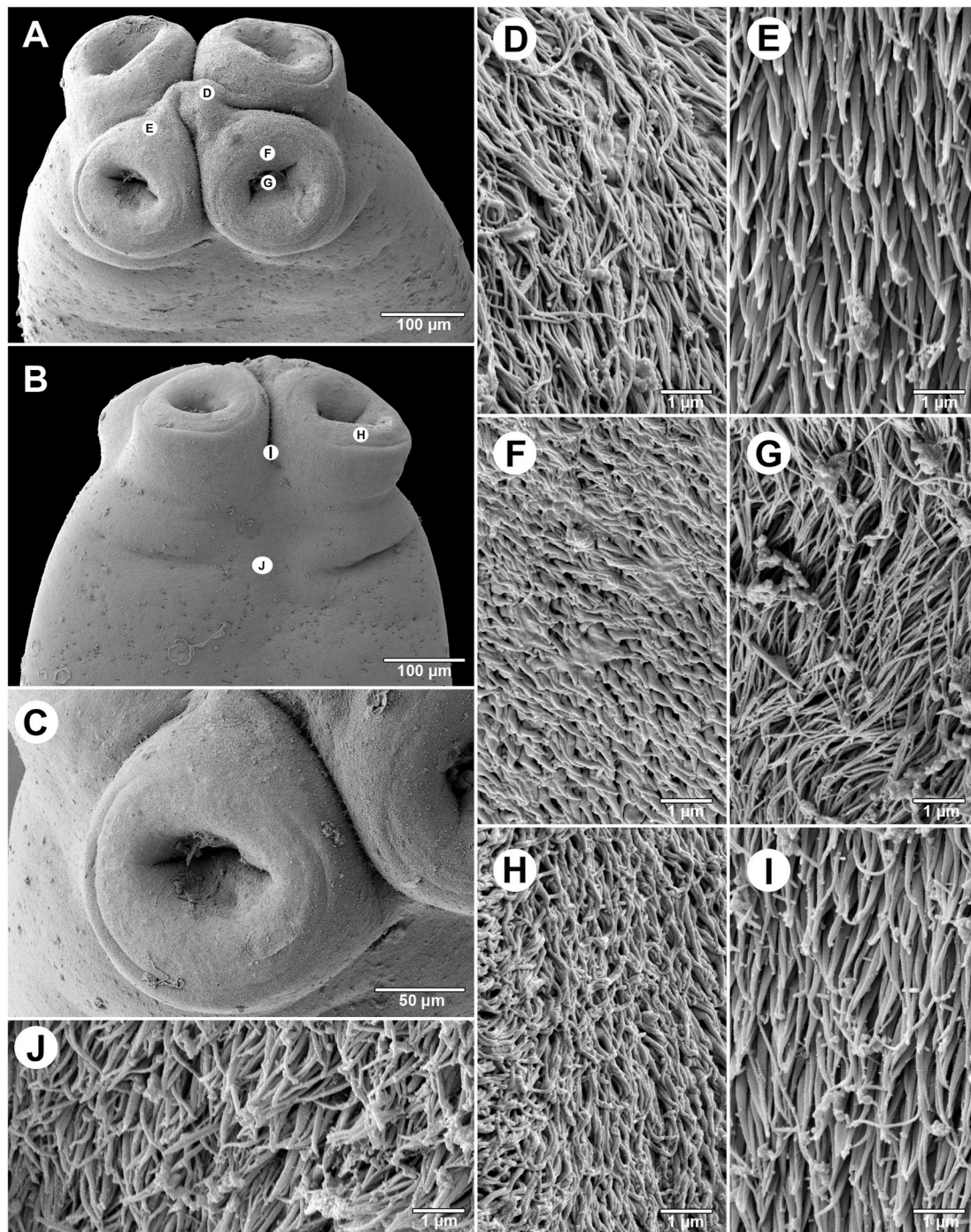Vas deferens strongly coiled, reaching almost to mid-line of

Fig. 1. Scanning electron micrographs of *Ophiotaenia echidis* n. sp. from *Echis carinatus sochureki,* United Arab Emirates. A – subapical view of the scolex. B – frontal view of the scolex. C – detail of suckers. D–J – microtriches on the apex of the scolex, external (non-adherent) surface of suckers, upper rim, luminal surface and lower rim of suckers, between suckers and neck, respectively. Note: small black letters in A and B correspond to the figures showing higher magnification images of these surfaces.

proglottid, occupying very small area (Fig. 2C, G, 3). Cirrus-sac ovoid, thick-walled, 220–290 long, i.e. 20–34% (x = 24%, n = 22) of proglottid width, 100–140 wide (Fig. 2G). Cirrus occupies 73–80% of length of cirrus-sac (Fig. 2C). Genital atrium narrow; genital pores irregularly alternating, slightly postequatorial to equatorial, situated at 49–61% (x = 56%, n = 21) of proglottid length (Fig. 3).

Ovary small, bilobed, 370–540 wide, occupying 52–60% (x = 57%, n = 20) of proglottid width (Figs. 2B and 3); relative size of ovary, i.e., ratio of surface of ovary to surface of proglottid (see de Chambrier et al., 2012), 1.1–1.6% of proglottid size. Ovary length represents 2.5–4.2% of

proglottid length. Vagina predominantly anterior (79%), rarely posterior (21%, n = 24) to cirrus-sac, lined with intensely stained cells in its terminal (distal) part and surrounded by small circular sphincter (diameter of 70–80) near genital atrium (Fig. 2C, F). Mehlis' gland 70–100 in diameter, representing 8–13% of proglottid width.

Vitelline follicles oval, very small (30–60 long, 30–60 wide), arranged in two lateral, longitudinal columns on dorsal side of proglottid (Fig. 3), not interrupted on poral side dorsally at level of terminal genitalia (cirrus-sac and vagina – Fig. 2G). Follicles not reaching anterior margin of proglottids (Fig. 3); posteriorly, follicles may reach ovary,
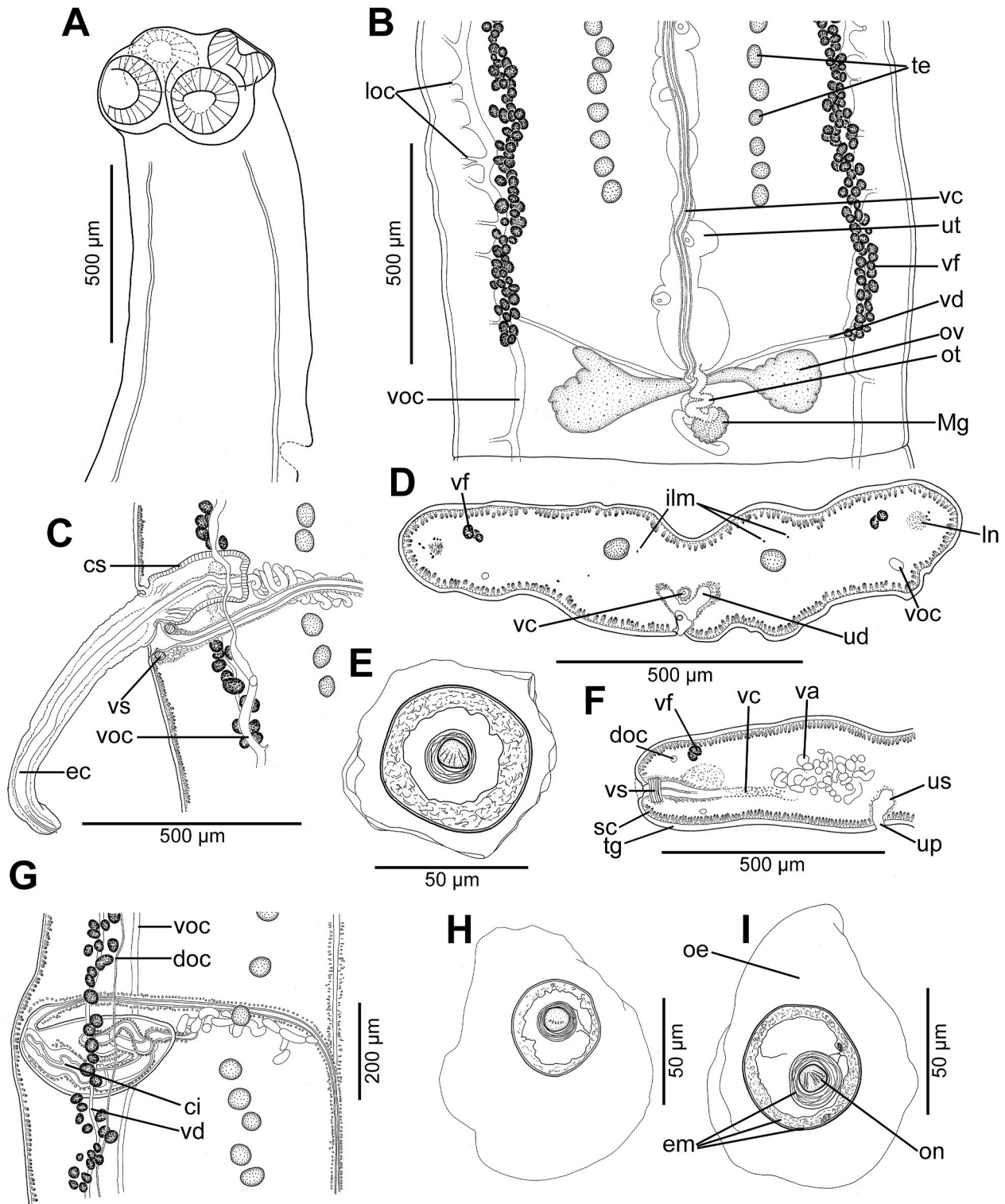
Fig. 2. *Ophiotaenia echidis* n. sp. from *Echis carinatus sochureki*, United Arab Emirates. A – scolex, IPCAS C-876/1 (UAE 04). B – posterior part of proglottid, holotype, MHNG-PLAT-0137383, dorsal view. C – terminal genitalia with evaginated cirrus and vaginal sphincter, MHNG-PLAT-0137385, ventral view. D – cross section at level of anterior part of pregravid proglottid, MHNG-PLAT-0120507. E – egg with a trilayered embryophore, drawn in distilled water, MHNG-PLAT-0120507. F – cross section at level of the vagina, showing the vaginal sphincter, mature proglottid, MHNG-PLAT-0120507. G – terminal genitalia, C-876/1 (UAE 04 TS), dorsal view.

H, I – eggs with a trilayered embryophore, drawn in distilled water, MHNG-PLAT-0120507. Abbrevations: ci: cirrus; cs: cirrus-sac; doc: dorsal osmoregulatory canal; ec: ejaculatory duct; em: trilayered embryophore; ilm: internal longitudinal musculature; ln: longitudinal nerve cord; loc: lateral osmoregulatory canal; Mg: Mehlis' glands; oc: secondary osmoregulatory canals; oe: outer envelope; on: oncosphere; ot: ootype; ov: ovary; sc: subtegumental cells; te: testes; tg: tegument; ud: uterine diverticula; up: uterine pore; us: uterine stem; ut: uterus; va: vas deferens; vc: vaginal canal; vd: vitelline duct; vf: vitelline follicles; voc: ventral osmoregulatory canal; vs: vaginal sphincter.
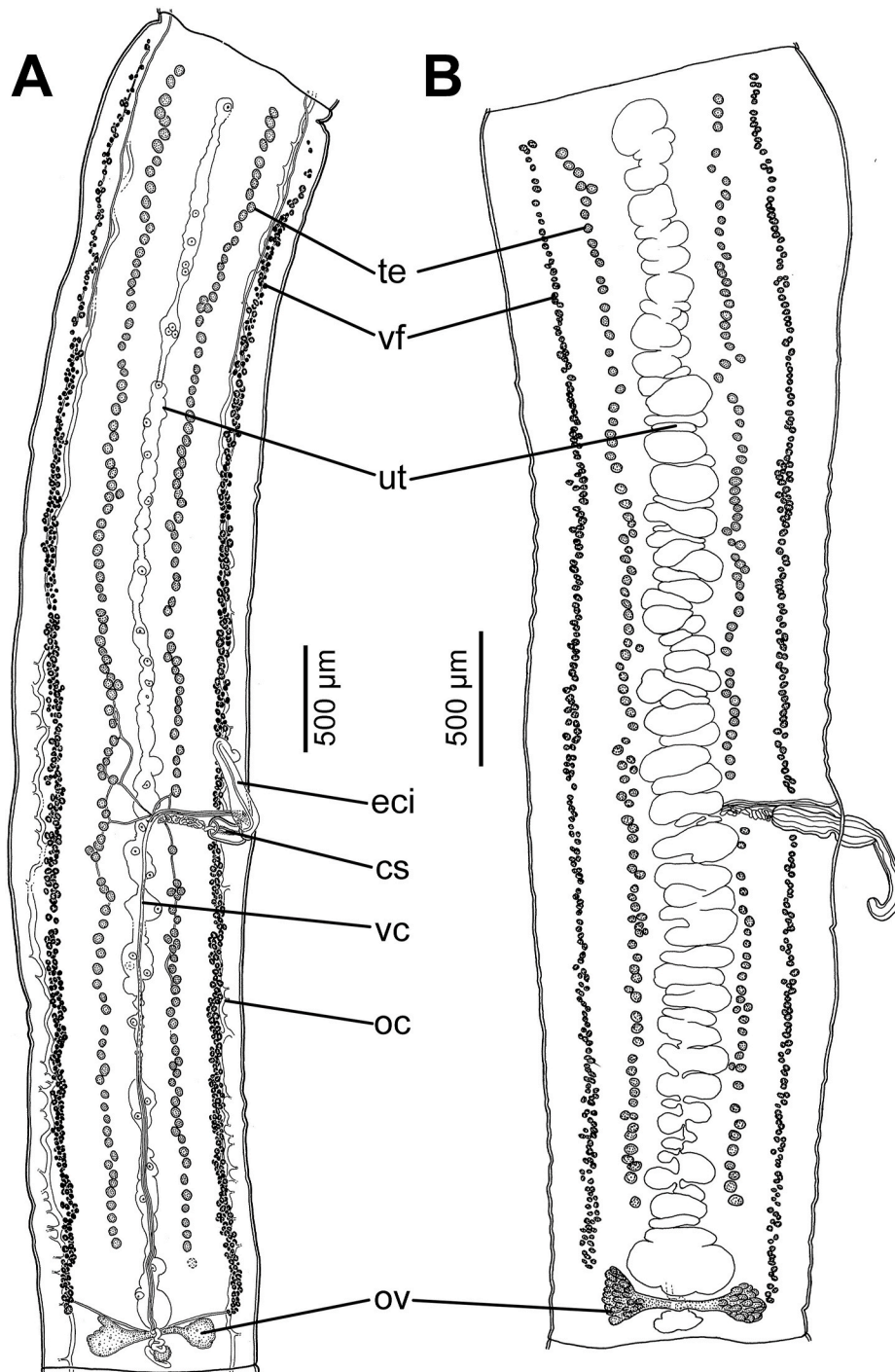
Fig. 3. *Ophiotaenia echidis* n. sp. from *Echis carinatus sochureki,* United Arab Emirates. A - pregravid proglottid, holotype, MHNG-PLAT-0137383, dorsal view. B – gravid proglottid, paratype, IPCAS C-876/1, ventral view. Abbrevations: cs: cirrus-sac; eci: everted cirrus; oc: osmoregulatory canal; ov: ovary; te: testes; ut: uterus; vc: vaginal canal; vf: vitelline follicles.

occupying porally 89–94% and aporally 88–92% of proglottid length, respectively.

Primordium of uterine stem ventral, with 47–55 (x = 50, n = 8) lateral branches (diverticula) on each side (Fig. 3B), never reaching posteriorly beyond ovarian isthmus (Fig. 2B). Formation of uterus of type 1 of de Chambrier et al. (2004) as follows: Uterine stem present as undifferentiated longitudinal median concentration of chromophilic cells in immature proglottids. In mature proglottids, uterine stem straight, occupying almost entire length of proglottids. Lumen appears in first mature proglottids. Lateral branches formed when eggs appear in

uterus. Thin-walled lateral branches grow in pregravid and gravid proglottids (Fig. 3B), occupying up to 72% of proglottid width, and opening by elongate, slit-like uterine pore.

Eggs large, oval to spherical, with thin, hyaline outer envelope, up to 120 (n = 12) in diameter, somewhat collapsed in distilled water (Fig. 2E, H, I). Inner envelope consists of trilayered embryophore, with thick external layer, 44–53 in diameter, and nucleated envelope of irregular shape, 40–49 in diameter. Oncosphere surrounded by additional thick layer, 20–23 in diameter; oncospheres spherical, 11–12 in diameter, with 3 pairs of embryonic hooks 5–6 long (Fig. 2E, H, I).
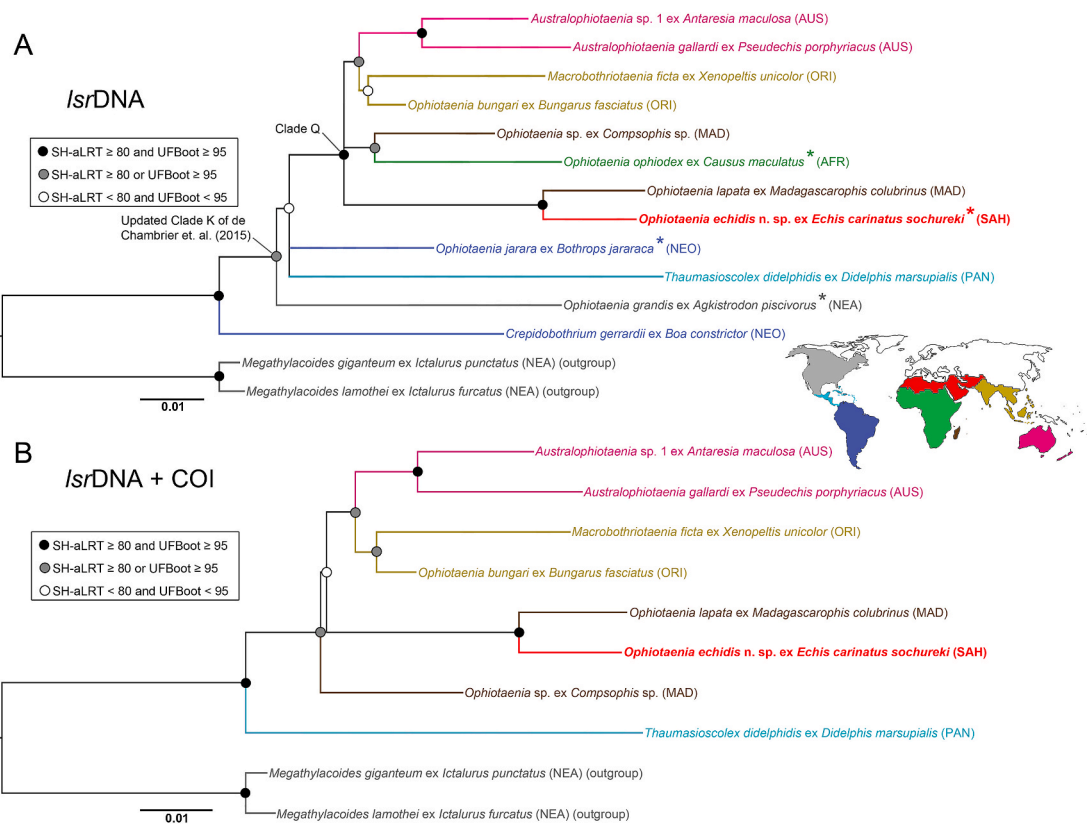
Fig. 4. Maximum likelihood phylograms of the relationships of *Ophiotaenia echidis* n. sp. among selected Proteocephalidae inferred from partial *lsr*DNA (A), and concatenated *lsr*DNA + COI datasets (B). Colours of branch and species names correspond to the zoogeographical realms of the isolates as proposed by Holt et al. (2013) (see inset). Branch length scale bar indicates number of substitutions per site. Abbreviations: AUS, Australian; AFR, Afrotropical; MAD, Madagascan; NEA, Nearctic; NEO, Neotropical; PAN, Panamanian; ORI, Oriental; SAH, Saharo-Arabian; * indicates tapeworms from viperid snakes (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Differential diagnosis

The new species is placed in *Ophiotaenia* (the former subfamily Proteocephalinae) because of the medullary position of the vitelline follicles, the unarmed scolex with uniloculate suckers and testes forming two separate fields (Rego, 1994). Based on molecular data, the new species belongs to Clade K of de Chambrier et al. (2015), which currently includes species of (non-monophyletic) *Ophiotaenia* (= *Ophiotaenia* sensu lato); *Australophiotaenia* de Chambrier, Beveridge and Scholz (2018); *Macrobothriotaenia* Freze, 1965; and *Thaumasioscolex* Cañeda-Guzmán, de Chambrier et Scholz, 2001 (de Chambrier et al., 2015).

The new species differs from all species of *Ophiotaenia* (sensu lato) by the position of testes in single lines alongside the uterus, and by a large embryophore (44–53 μm *versus* less than 40 μm) (see Table 2).

In addition, *O. echidis* n. sp. possesses a trilayered embryophore, which is present only in a few species of *Ophiotaenia* from snakes, such as *Ophiotaenia lapata* Rambeloson, Ranaivoson et de Chambrier, 2012 from *Madagascarophis colubrinus* (Schlegel), *Ophiotaenia georgievi* de Chambrier, Ammann et Scholz, 2010 from *Leioheterodon geayi* (Mocquard) (both host (Pseudoxyrphophiidae) and *O. bungari* de Chambrier, Binh et Scholz, 2012 from *Bungarus fasciatus* (Schneider) (Elapidae) (see de Chambrier et al., 2010; 2012; Rambeloson et al., 2012).

Ophiotaenia echidis differs from the closely related *O. lapata* (see Fig. 4) by the absence of an apical organ and by the larger size of the scolex (400–725 μm *versus* 190–280 μm). The new species can be differentiated from *O. bungari* by the position of the genital pore (at 49–61% of the proglottid length *versus* 29–48%) and by the smaller relative size of the ovary (1.1–1.6% *versus* 3.3%). *Ophiotaenia echidis* differs from *O. georgievi* by the number of uterine diverticula (47–55 *versus* 23–28) and smaller relative size of the ovary (1.1–1.6% *versus*

2.5–4.3%).

Eight species that belong to Clade Q in Fig. 4 (subclade of Clade K of de Chambrier et al., 2015) share the presence of a third embryophoric layer, in contrast to the three remaining species in the Clade K (Fig. 4), namely *O. jarara* (Fuhrmann, 1927), *O. grandis* La Rue, 1911, and *T. didelphis*, which possess only a bilayered embryophore (Table 2). Interestingly, all but one species of Clade K listed above have an embryophore of a similar size (diameter always less than 34 μm), the only exception being the new species, *O. echidis*, with extraordinarily large embryophore (diameter 44–53 μm). The presence of a third layer in the embryophore is not common in the Proteocephalidae and has been observed only in some proteocephalids parasitising reptiles from different genera, such as *Australophiotaenia*, *Cairaella* Coquille et de Chambrier, 2008, *Kapsulotaenia* Freze, 1963, *Macrobothriotaenia* and *Ophiotaenia* from the Old World (Asia, Australia and Madagascar), and in *Cairaella henrii* Coquille et de Chambrier, 2008, a parasite of the poly-chrotid lizard *Norops trachyderma* (= *Anolis trachyderma* Cope) in Ecuador (Coquille and de Chambrier, 2008; Scholz et al., 2013; de Chambrier et al., 2018, 2020). The only proteocephalid parasitising amphibians that possesses a third embryophore layer is *Ophiotaenia alessandrae* Marsella et de Chambrier, 2008 from *Hyla boans* (= *Boana boans* (Linnaeus)) in Ecuador (Marsella and de Chambrier, 2008). This indicates that the presence of a trilayered embryophore is a homoplastic character that has evolved in several groups of proteocephalids parasitising tetrapods independently.

3.4. Checklist of helminth parasites of the saw-scaled viper, Echis carinatus

The present checklist is primarily based on data compiled by

Table 2

List of tapeworms of the former, non-monophyletic subfamily Proteocephalinae, parasitising snakes and lizards, including *species inquirendae*, and phylogenetically closely related *Thaumasioscolex didelphidis* from opossum with selected characteristics (see de Chambrier et al., 2017).

Species (clade/subclade)[a]	Type host	Country	Total length (mm)	Width of scolex (μm)	Testis number	Relative cirrus-sac length[b]	Genital pore position[c]	Position of vagina	Vaginal sphincter	No. uterine diverticula	Apical organ	Ovary surface[d]	Diameter of embryophore	Embryophore (no. layers)
Australophiotaenia amphiboluri (Nybelin, 1917)	*Pogona barbata*	Australia	?	absent	127–152	10–14%	62–73%	ant–post	present	29–32	?	6.7%	30–35	bilayered
A. gallardi (Johnston, 1911) (clade K & subclade Q)	*Pseudechis porphyriacus*	Australia	400	960	72–80	25%	50%	anterior	absent	numerous	present	3.2%	?	trilayered
A. longmani (Johnston, 1916) (clade K & subclade Q; not included in our analyses – no *lsr*DNA data)	*Aspidites ramsayi*	Australia	94	800	?	25%	50%	anterior	present	25–35	absent	2.3%	32–36	trilayered
A. mjobergi (Nybelin, 1917)	*Demansia psammophis*	Australia	150	700–850	200–300	?35%	50%	ant–post	present	30–40	absent	4.7%	30–34	trilayered
A. striata (Johnston, 1914)	*Lialis burtonis*	Australia	33	330–465	64–83	13–17%	48–51%	ant–post	absent	18–27	absent	1.5%	27–30	?
Crepidobothrium gerrardi (clade B)	*Boa constrictor*	Brazil	560	1820–2120	237–370	23–33%	35–53%	ant–post	present	18–35	present	?	17–23	?
Macrobothriotaenia ficta (Meggitt, 1931) (clade K & subclade Q)	*Xenopeltis unicolor*	Burma	51–62	525–1290	43–63	34–48%	44–62%	ant–post	present	26–37	absent	4.1%	33–39	trilayered
Ophiotaenia adiposa Rudin, 1917[f]	*Bitis arietans*	Africa	300–400	55–600	170–220	20–25%	50%	ant–post	present	40–50	present	2.1%	?	?
O. agkistrodontis (Harwood, 1933)[f]	*Agkistrodon piscivorus*	USA	small	850–950	90–110	20–25%	20–25%	ant–post	?	25–30	absent	5.1%	?	?
O. andersoni Jensen, Schmidt et Kuntz, 1983[f]	*Trimeresurus stejnegeri*	Taiwan	130	220–450	42–116	40–45%	42–43%	?	present	about 50	present	2.1%	23–31	?
O. arandasi Santos et Rolas, 1973 *species inquirenda*	*Erythrolamprus miliaris*	Brazil	80	390	70	41–42%	35–45%	ant–post	?	about 60	absent	5.1%	?	?
O. atretiumi (Devi, 1973)	*Atretium schistosum*	India	160–190	210–280	70–95	17–20%	45–59%	ant–post	absent	10–15	present	6.5–8.5%	15	?
O. azevedoi de Chambrier et Vaucher, 1992[f]	*Bothrops jararaca*	Brazil	415	630–735	88–212	17–26%	40–55%	ant–post	present	45–61	present	1.9%	27–31	bilayered
O. barbouri Vigueras, 1934	*Tretanorhinus variabilis*	Cuba	16–18	730	46–58	?	33%	posterior	?	18–22	absent	2.9%	26–28	?
O. bungari de Chambrier, Binh et Scholz, 2012 (clade K & subclade Q)	*Bungarus fasciatus*	Vietnam	240	360–420	100–150	29–30%	29–48%	ant–post	present	50–65	present	3–3.3%	28–32	trilayered
O. calmettei (Barrois, 1898)[f]	*Bothrops lanceolatus*	Martinique	270–800	1000–1300	130–160	17–25%	50%	posterior	present	24–35	absent	2.2%	?	?
O. catzeflisi de Chambrier et Vaucher, 1992[f]	*Bothrops jararaca*	Brazil	124–240	990–1220	107–158	14–22%	40–54%	ant–post	present	numerous	present	2.1%	15–23	bilayered
O. chattoraji Srivastava, 1980	*Naja naja*	India	24–26	300–360	140–160	18%	45–47%	?	?	10–26	present	2.9%	27–30	?
O. congolensis (Southwell et Lake, 1939)	*Boaedon olivaceus*	Zaire	80	?	65	25%	50%	ant–post	?	15–20	?	4.3%	15	?
O. crotali Lopez-Neyra et Diaz–Ungria, 1958[f]	*Crotalus durissus*	Venezuela	520	absent	308–412	7–12%	41–43%	?	present	.8–16	?	2.8%	27–30	?
			?	210–260	94–98	19%	50%	posterior	?	15–18	absent	3.8%	26	?

(continued on next page)

Table 2 (*continued*)

Species (clade/subclade)[a]	Type host	Country	Total length (mm)	Width of scolex (µm)	Testis number	Relative cirrus-sac length[b]	Genital pore position[c]	Position of vagina	Vaginal sphincter	No. uterine diverticula	Apical organ	Ovary surface[d]	Diameter of embryophore	Embryophore (no. layers)
O. crotaphopeltis Sandground, 1928	*Crotaphopeltis tornieri*	Lake Tanganyika												
O. dubinini Freze et Sharpilo, 1967	*Coronella austriaca*	Ukraine	130	202–283	87–166	25%	33–(? 66%)	ant-post	absent	20–26	absent	9.8%	?	?
Ophiotaenia echidis n. sp.[f] (clade K & subclade Q)	***Echis carinatus sochureki***	**UAE**	**230–275**	**400–725**	**118–205**	**20–34%**	**49–61%**	**ant–post**	**present**	**47–55**	**absent**	**1.1–1.6%**	**44–55**	**trilayered**
O. elapsoidaea Sandground, 1928	*Elapsoidea guentherii*	Lake Tanganyika	150	1000–1100	100–125	30%	50%	posterior	? present	48–55	absent	3.4%	?	?
O. elongata Fuhrmann, 1927	'a small snake'	Brazil	30–40	?	26–44	?	?50%	?	?	13–16	?	2.5%	19	?
O. europaea Odening, 1963 (clade O)	*Natrix natrix*	Europe	280–540	222–313	189–344	25–43%	33–66%	posterior	absent	28–73	absent	12.7%	18–22	?
O. euzeti de Chambrier et Vaucher, 1992[f]	*Bothrops jararaca*	Brazil	45	300–310	116–141	25–34	24–42%	ant–post	present	?	absent	2.2%	?	?
O. faranciae (MacCallum, 1921)	*Farancia abacura*	USA	>180	500	390–420	?25%	16–25%	posterior	?	30–50	present	2.1%	?	?
O. fima (Meggitt, 1927)	*Amphiesma stolatum*	India	?	200	68–89	25–33%	?35%	posterior	?	27–33	absent	3.4%	?	?
O. fixa (Meggitt, 1927) *species inquirenda*	*Amphiesma stolatum*	India	50	absent	71–94	20%	33–40%	ant–post	?	20–24	?	3.8%	?	?
O. flava Rudin, 1917	*Coluber* sp.	Brazil	50–60	500–600	45–60	50%	20–40%	ant–post	absent	?	absent	3.6%	28–30	?
O. gabonica (Beddard, 1913)[f]	*Bitis gabonica*	Africa	380	300	130–170	20%	?43%	posterior	present	38–46	absent	2.8%	39	?
O. georgievi de Chambrier, Ammann et Scholz, 2010	*Leioheterodon geayi*	Madagascar	57	225–235	92–140	19–32%	44–56%	ant–post	present	23–28	absent	2.5–4.3%	31–35	trilayered
O. gilberti Ammann et de Chambrier, 2008	*Thamnodynastes pallidus*	Paraguay	60–120	140–145	57–91	15–23%	42–50%	ant–post	present	28–41	present	3.7%	27–28	bilayered
O. grandis La Rue, 1911 [f] (clade K)	*Agkistrodon piscivorus*	USA	300–400	1000–1200	200–250	20–33%	?50%	ant–post	present	40–60	absent	2.1%	21–23	bilayered
O. greeri (Bursey, Goldberg et Kraus, 2006)	*Sphenomorphus aignanus*	Papua New Guinea	7–15	205–410	29–51	15–17%	38–47%	anterior	present	14–24	present	6.8%	15–18	?
O. habanensis Freze et Rysavy, 1976	*Tropidophis pardalis*	Cuba	57–67	360	31–51	>50%	env.60%	?	present	26–32	absent	2.7%	22–28	?
O. hyalina Rudin, 1917	*Coluber* sp. (*Liophis* ?)	Brazil	120	680–800	50–55	50%	?33%	ant–post	present	?	absent	5.5%	?	?
O. indica Johri, 1955	*Naja naja*	India	110–180	200–370	31–32	33%	50%	?anterior	?	15–17	absent	5.4%	24–30	?
O. japonensis Yamaguti, 1935	*Rhabdophis tigrinus*	Japan	400	330–500	90–130	?25%	50%	ant–post	present	20–30	absent	1.9%	39	?
O. jarara Fuhrmann, 1927[f] (clade K)	*Bothrops jarara*	Brazil	140	1100–1200	150	27–34%	50%	anterior	absent	27–32	? absent	2.4%	23	bilayered
O. joanae de Chambrier et Paulino, 1997	*Xenodon neuwiedii*	Brazil	140–250	480–790	147–210	14–25%	28–56%	ant–post	present	26–49	present	3.1%	26–30	bilayered
O. kuantanensis Yeh, 1956	*Ophiophagus hannah*	Malaysia	100	2400	250–350	20%	<50%	ant–post	?	35–40	absent	5.1%	33	?
O. lapata Rambeloson, Ranaivoson et de Chambrier, 2012 (clade K & subclade Q)	*Madagascarophis colubrinus*	Madagascar	295	190–280	89–170	19–25%	43–53	ant–post	present	41–68	present	2.1–2.9%	34–39	trilayered
O. lactea (Leidy, 1855) *species inquirenda*	*Nerodia sipedon*	USA	?	?	?	?	50%	?	?	?	absent	?	?	?

Table 2 (*continued*)

Species (clade/subclade)[a]	Type host	Country	Total length (mm)	Width of scolex (μm)	Testis number	Relative cirrus-sac length[b]	Genital pore position[c]	Position of vagina	Vaginal sphincter	No. uterine diverticula	Apical organ	Ovary surface[d]	Diameter of embryophore	Embryophore (no. layers)
O. macrobothria Rudin, 1917	*Micrurus corallinus*	Brazil	50	400–500	50–60	>50%	20–33%	ant–post	absent	?	absent	4.4%	?	?
O. marenzelleri (Barrois, 1898)[f]	*Agkistrodon piscivorus*	USA	400	1200–2000	150–240	25–33%	50%	anterior	?	20–30	absent	2.6%	20	?
O. meggitti Hilmy, 1936[f] *species inquirenda*	*Atheris chlorechis*	Liberia	68	absent	86–104	33%	>50%	ant–post	?	35.00	?	2.3%	?	?
O. micruricola (Shoop et Corkum, 1982)	*Micrurus diastema*	Mexico	259–290	720–760	121–169	14–20%	48–56%	posterior	absent	35–53	absent	3.2%	20–23	?
O. monnigi Fuhrmann, 1924	*Crotaphopeltis hotamboeia*	Africa	50	?	80	10%	50%	anterior	present	50–57	?	3.1%	25	bilayered
O. najae (Beddard, 1913)	*Naja tripudians*[e]	India	180	248–303	120–(165)	25–33%	?50%	anterior	absent	14–25	absent	3.5%	25	?
O. nankingensis (Hsü, 1935)	*Ptyas dhumnades*	China	105–124	320	147–166	?17%	<50%	ant–post	present	36–40	absent	3.0%	?	?
O. nattereri (Parona, 1901)	*Coluber* sp.	Brazil	75–250	250	80–100	28–33%	<50%	ant–post	present	15–30	absent	?	22–26	?
O. nicolae Coquille et de Chambrier, 2008	*Thecadactylus rapicauda*	Ecuador	230–515	325–340	142–204	21–33%	34–53%	ant–post	present	13–27	present	4.9%	32–36	bilayered
O. nigricollis Mettrick, 1963	*Naja nigricollis*	Zimbabwe	170	300–310	176–210	~25%	~35%	ant–post	?	16–20	?	6.3%	26–33	?
O. nybelini Hilmy, 1936	*Meizodon coronatus*	Africa	52	105	67–90	16–20%	?50%	ant–post	?	25–40	absent	3.6%	25	?
O. ophiodex Mettrick, 1960[f] (clade K & subclade Q)	*Causus rhombeatus*	Zimbabwe	210–270	790–1140	110–120	25%	45–54%	ant-post	present	30–42	absent	?	27–36	?
O. paraguayensis Rudin, 1917 (clade N)	*Hydrodynastes gigas*	Paraguay	550–600	240	238–344	12–19%	27–39%	ant–post	present	20–36	absent	3.3%	21–24	?
O. perspicua La Rue, 1911 (clade O; type species)	*Nerodia rhombifer*	USA	360	255–410	150–215	25–33%	33–50%	anterior	present	20–30	present	2.3%	?	?
O. phillipsi (Burt, 1937)[f]	*Trimeresurus trigonocephalus*	Sri Lanka	400–920	670–1400	170–230	~ 50%	~50%	posterior	present	60–80	absent	2.1%	24–30	?
O. racemosa (Rudolphi, 1819)	*Coluber* sp.	Brazil	160	540–650	80–120	~33%	~33%	ant–post	present	40–50	?	4.3%	24	?
O. rhabdophidis (Burt, 1937)	*Amphiesma stolatum*	Sri Lanka	250	130–187	110–140	20–25%	33–50%	ant–post	?	30–45	present	3.1%	22–27	?
O. sanbernardinensis Rudin, 1917 (clade N)	*Helicops leopardinus*	Paraguay	100–120	230–250	70–102	?	20–40%	ant–post	present	27–33	absent	5.0%	22–23	?
O. sinensis Cheng et Lin, 2002	*Rhabdophis tigrinus*	China	460–694	247–325	256–320	~25%	~45%	?	?	?	?	2.5%	?	?
O. southwelli Freze, 1965 [f]	*Causus rhombeatus*	Africa	90	1500	170–230	?	~50%	ant–post	?	8–12	?	3.4%	30	bilayered
O. spasskyi Freze and Sharpilo, 1967[f]	*Vipera berus*	Russia	70–90	295–355	179–271	20–25%	~30%	ant–post	present	19–31	?	9.1%	?	?
O. theileri Rudin, 1917	*Naja haje*	Africa	300	400	160–310	20–25%	~50%	ant–post	absent	35–40	absent	4.5%	?	?
O. trimeresuri (Parona, 1898)[f]	*Trimeresurus formosus* (= *T. sumatranus*)	Mentawai Islands	105	750	100–108	20–30%	~50%	ant–post	present	20–30	absent	3.6%	?	?
O. variabilis Brooks, 1978	*Nerodia cyclopion*	North America	300	170–200	77–253	22–33%	15–30%	ant–post	absent	25–45	absent	2.8%	?	?
O. wuyiensis Cheng, Yuguang et Zao He, 2007[f]	*Trimeresurus gramineus*	China	460–700	245–325	256–320	23%	~45%	?	?	?	?	2.8%	?	?
O. zschokkei Rudin, 1917	*Naja haje*	South Africa	~550–600	400	160–200	20–25%	~50%	ant–post	?	?	absent	6.4%	?	?

Table 2 (*continued*)

Species (clade/subclade)[a]	Type host	Country	Total length (mm)	Width of scolex (µm)	Testis number	Relative cirrus-sac length[b]	Genital pore position[c]	Position of vagina	Vaginal sphincter	No. uterine diverticula	Apical organ	Ovary surface[d]	Diameter of embryophore	Embryophore (no. layers)
Thaumasioscolex didelphidis Cañeda-Guzmán, de Chambrier et Scholz, 2001 (clade K)	*Didelphis marsupialis*	Mexico	640–1045	655–1040	432–548	11–19%	31–44%	ant–post	present	12–22	absent	8.5%	30–33	bilayered

[a] Clades – see de Chambrier et al. (2015); subclade Q – see the text.
[b] Ratio of the cirrus-sac length to the proglottid width (in %).
[c] Ratio of the genital pore position to the proglottid length (in %).
[d] Ratio of the ovary surface to the proglottid surface (in %; see de Chambrier et al., 2012).
[e] *Naja tripudians* is currently not recognised and it may be *Naja kaouthia* Lesson or *Naja naja* (Linnaeus) (Uetz et al., 2020).
[f] Indicates species of *Ophiotaenia* from viperid snakes.

Sharpilo (1976) and Gibson et al. (2005), with a subsequent search of primary, especially literature written in Russian.

Cestoda – tapeworms

Cyclophyllidea: Anoplocephalidae Cholodkovsky, 1902

1. *Oochoristica fedtschenkoi* Bogdanov et Markov, 1955
   This species was described from several species of viperid snakes, including *E. carinatus*, from Turkmenistan and Uzbekistan. The highest infection rate was reported for the saw-scaled viper (prevalence of 4.2%, mean intensity of infection 26, range 1–50 worms) (Bogdanov and Markov, 1955; Markov et al., 1970; Sharpilo, 1976).

2. *Oochoristica sindensis* Farooq, Khanum et Ansar, 1983
   This cestode was described from the saw-scaled viper in Pakistan by Farooq et al. (1983).

Cyclophyllidea: Dipylidiidae Stiles, 1896

3. *Diplopylidium acanthotetra* (Parona, 1898) – larvae
   Metacestodes of this tapeworm were found in the body cavity of *E. carinatus* in Turkmenistan (Markov et al., 1970). The most common intermediate host of *D. acanthoptera* is the Caspian bent-toed gecko, *Tenuidactylus caspius* (Eichmann), and its metacestodes were found in Algeria, Egypt, Israel, Iraq and Italy (Sharpilo, 1976).

4. *Diplopylidium noelleri* (Skrjabin, 1924) – larvae
   Larvae of this cestode occur in the liver, intestinal wall and body cavity of reptiles of distantly related groups, such as geckoes, lacertids, agamas and colubrid snakes (Sharpilo, 1976). They were also found in *E. carinatus* from Turkmenistan (Markov et al., 1970).

5. *Joyeuxiella echinorhynchoides* (Sonsino, 1889) – larvae
   Metacestodes are frequently found in the liver, intestinal wall and body cavity of a wide spectrum of reptiles, including viperid snakes (Sharpilo, 1976). Markov et al. (1970) found *J. echinorhynchoides* in *E. carinatus* from Turkmenistan.

Onchoproteocephalidea: Proteocephalidae La Rue, 1911

6. *Ophiotaenia* sp.
   Markov et al. (1970) reported unidentified species of *Ophiotaenia* from *E. carinatus* in Turkmenistan. However, the authors did not provide morphological characterisation and illustrations, and no voucher specimens have been deposited. Therefore, it is impossible to compare tapeworms found in Middle Asia with those found in the United Arab Emirates by the present authors.

Acanthocephala – spiny-headed worms

Archiacanthocephala: Giganthorhynchidae Hamann, 1892

7. *Centrorhynchus aluconis* (Müller, 1780) – larvae
   Cystacanths of this very common parasite of owls have also been found in *E. carinatus* from Turkmenistan (Markov et al., 1970).

Archiacanthocephala: Oligacanthorhynchidae Southwell et Macfie, 1924

8. *Centrorhynchus globocaudatus* (Zeder, 1800) – larvae
   Markov et al. (1970) reported cystacanths of this parasite of owl and raptors from *E. carinatus* in Turkmenistan, but Sharpilo (1976) did not list this species in his survey of helminth parasites of reptiles in the former USSR.

9. *Centrorhynchus* sp. – larvae

Unidentified larvae (cystacanths) of another species of *Centrorhynchus* Lühe, 1911 were found in *E. carinatus* from Turkmenistan by Markov et al. (1970). Their identification is not possible because the authors did not provide any morphological data and illustrations.

10. *Oligacanthorhynchus* sp. – larvae

Cystacanths of unidentified species of *Oligacanthorhynchus* Travassos, 1915 were found in the body cavity and liver of various reptiles, including *E. carinatus* from Turkmenistan (Markov et al., 1970).

Nematoda – nematodes (roundworms)

Rhabditida: Ascarididae Blanchard, 1849

11. *Hexametra skrjabini* Markov et Bogdanov, 1960

This nematode was described from the small intestine and stomach of the spotted desert racer, *Coluber karelini* Brandt (= *Platyceps karelini*) (Colubridae), dwarf sand boa, *Eryx miliaris* (Pallas) (Boidae), and *E. carinatus* from Dagestan (Russia) and Turkmenistan (Markov and Bogdanov, 1960; Markov et al., 1970).

Rhabditida: Habronematidae Chitwood et Wehr, 1932

12. *Agamospirura* sp. – larvae

Markov et al. (1970) reported larvae of unidentified species of *Agamospirura* Henry et Sisoff, 1913 from *E. carinatus* in Turkmenistan.

Rhabditida: Pharyngodonidae Travassos, 1919

13. *Spaulingodon auziensis* (Seurat, 1917)

Only immature worms were found in *E. carinatus* from Turkmenistan by Bogdanov and Markov (1955, 1960), and Markov and Bogdanov (1956). This record of the nematode that occurs in Algeria, Egypt and France needs verification (Sharpilo, 1976).

Rhabditida: Physalopteridae Railliet, 1893

14. *Abbreviata uzbekistana* Bogdanov and Markov (1955)

This parasite of the stomach, rarely of the oesophagus and intestine, was described by Bogdanov and Markov (1955). It has been found in several distantly related reptiles, such as agamas, geckoes and the saw-scaled viper from Dagestan (Russia), Turkmenistan and Uzbekistan (Sharpilo, 1976). *Echis carinatus* is probably postcyclic, not definitive host of this spirurine nematode.

In addition, larvae of the following nematodes unidentified to the genus or even family level were reported from *E. carinatus*: Acuaroidea gen. sp. in Turkmenistan (Velikanov, 1984); Omeiinae gen. sp. (Quimperiidae) in Turkmenistan (Markov et al., 1970). One of the present authors (R.K.S.) found the following helminths in the saw-scaled viper from the United Arab Emirates, but these records were presented only at an international conference (Schuster and Sivakumar, 2013): cestodes *Joyeuxiella* sp., most probably *J. pasqualei* (Diamare, 1893), and *Diplopylidium noelleri* (Cestoda); acanthocephalan larvae *Centrorhynchus aluconis* and *Macracanthorhynchus catulinus* Kostylew, 1927; and larvae of spirurine nematodes, most probably *Physocephalus dromedarii* Muschkambarova, 1967.

4. Discussion

Ophiotaenia is a composite genus pending taxonomic revision. Validity of its species should be re-assessed and poorly characterised taxa redescribed. However, this task is difficult because of the large number of species (88 according to de Chambrier et al., 2017 plus 12 *species inquirendae*) and difficulties in obtaining new, properly fixed material suitable for molecular studies. The generic name *Ophiotaenia* should be retained only for taxa of Clade O of de Chambrier et al. (2015), because it includes the type species *O. perspicua* from colubrid snakes in North America. Interestingly, species of this clade have proportionally larger ovaries than those in the other species of *Ophiotaenia* (Clades K and N of de Chambrier et al., 2015). Species in individual clades also differ in uterine development: Type 1 according to de Chambrier et al. (2004) is present in species of Clade K, whereas a Type 2 uterus occurs in species of Clades N and O (de Chambrier et al., 2015). It is clear that species of Clades K and N need to be allocated to other (new) genera, which also affects the new species described in the present paper.

However, there are two principal obstacles in erecting a new genus for the new species and the closely related *O. lapata*: (i) molecular data are available for a limited number of species of *Ophiotaenia* only; and (ii) the absence of obvious morphological synapomorphies of the new species and *O. lapata*, which would unequivocally differentiate them from the other taxa of Clade K. For example, the scoleces of most species of all but two genera are rather uniform; only species of monotypic *Macrobothriotaenia* and *Thaumasioscolex* have distinct scoleces (Cañeda-Guzmán et al., 2001; Scholz et al., 2013). In addition, some species of *Australophiotaenia* and *M. ficta* (Meggitt, 1931) have a trilayered embryophore, similar to *O. echidis* n. sp. (see de Chambrier et al., 2018). Therefore, the new species is provisionally placed in *Ophiotaenia*, even though this genus is not monophyletic (de Chambrier et al., 2015).

Clade K (updated in this study in Fig. 4) is composed almost exclusively of parasites of snakes, namely Boidae, Elapidae, Pseudoxyrhophiidae, Pythonidae, Viperidae (three of the 18 species of *Ophiotaenia* from viperids sequenced), and Xenopeltidae, throughout the world and the only proteocephalid parasitising a mammal, the common opossum (*D. marsupialis*), in Neotropical Mexico in the case of *T. didelphidis*. According to de Chambrier et al. (2015), species of Clade K may represent a relatively recent colonisation of unrelated groups of snakes in most continents or a trace of a very ancient colonisation of these snake hosts. Based on the most informative tree presented here (using only the *lsr*DNA dataset), the early colonisation of Clade K representatives in vertebrates (snakes and the common opossum) currently distributed across the Americas is hypothesised, followed by colonisation of snakes in the Afrotropical, Australian, Madagascan, Oriental, and Saharo-Arabian zoogeographical realms (see Fig. 4). Nevertheless, for proposing a more precise scenario of the evolution of this clade of *Ophiotaenia*, a broader spectrum of related taxa should be analysed, including diverse proteocephalids in Australian reptiles (de Chambrier et al., 2018), which might provide stronger statistical support for internal nodes.

Recently, de Chambrier et al. (2018) erected a new genus, *Australophiotaenia*, to accommodate species of *Ophiotaenia* from snakes in the Australian region, including *A. gallardi* (Johnston, 1911) from Clade K of de Chambrier et al. (2015). In the present analyses, two species of this genus, *A. gallardi* and *Australophiotaenia* sp. 1, grouped together (Fig. 4). *Australophiotaenia* is characterised by the following characteristics: a trilayered, thick-walled embryophore, a scolex with large, anteriorly directed suckers, an exclusively dorsal and paramuscular position of vitelline follicles (i.e., follicles may penetrate into the medulla between fibres of the inner longitudinal musculature), and a postequatorial to equatorial genital pore in most species (de Chambrier et al., 2018).

The present study supports the assumption about strict (oioxenous) host specificity of species of *Ophiotaenia* from reptilian hosts (see de Chambrier et al., 2010, 2017, 2020) because the new species was found only in *E. carinatus sochukeri*, but not in any of 33 other snakes of six

species, including the congeneric Oman saw-scaled viper, *Echis omanensis*. It is worth noting that the latter species belongs to the *E. coloratus* group *sensu* Pook et al. (2009), species of which feed almost exclusively on vertebrates. In contrast, *E. carinatus*, which belongs to the *E. carinatus* group, chiefly feeds on scorpions and other arthropods (Barlow et al., 2009).

Information on proteocephalid life-cycles is scant (Scholz and de Chambrier 2003), but it is postulated that species of *Ophiotaenia* exhibit two types of life-cycle strategies, i.e., a three-host cycle including planktonic copepods (first intermediate host), fish or amphibian tadpoles (second intermediate host) and reptiles (definitive host), and a two-host cycle with a tissue phase of development in the final host and posterior migration to the intestine, with no involvement of intermediate or paratenic vertebrate host (Biserkov and Kostadinova, 1997). It remains unclear how *O. echidis* n. sp. is transmitted to its definitive host, but considering the similar diet of vipers of the *E. carinatus* and *E. pyramidum* groups (Barlow et al., 2009), new studies should focus on these potential hosts.

The taxonomic history of *Echis* has been convoluted, which has direct implications for human health, given that venom composition is highly variable among species resulting in several cases of a lack of antivenom cross-neutralisation (Pook et al. 2009). That having been said and considering the assumption of oioxenous host specificity, the fauna of proteocephalids of these venomous snakes may help to inform us about distinct evolutionary lineages of their hosts, which ultimately may be used to guide searches for specific antivenoms. In the most comprehensive phylogeny of viperid snakes by Alencar et al. (2016), *Echis* appeared as sister to *Cerastes* Laurenti, but species of the latter genus genus have never been found infected with tapeworms of *Ophiotaenia* (in the present study, we examined five specimens of *Cerastes gasperettii* but none was infected). The common European adder, *Vipera berus* (Linnaeus), which is the definitive host of *Ophiotaenia spasskyi* Freze et Sharpilo, 1967 in Europe, clustered with species of *Echis* in a weakly supported clade (Alencar et al., 2016). Therefore such comparison is not informative enough.

Literary search for data on the helminth parasites of the saw-scaled viper has revealed that most information was accumulated by Soviet researchers, especially G.S. Markov and O.P. Bogdanov, from Turkmenistan in the 1950's and 1960's. More recent data are scarce and include just a few reports (Farooq et al., 1983; Nama, 1984; Velikanov, 1984). The helminth fauna of *E. carinatus* includes 14 species and another two larvae of nematodes unidentified to the genus level. This fauna is composed of adult cestodes (3 spp.), their larvae (3 spp.), larval acanthocephalans (4 spp.), adult nematodes (3 spp.) and their larvae (3 spp.). Noteworthy is the complete absence of any trematode (Digenea) (Sharpilo, 1976; Gibson et al., 2005).

Declaration of competing interest

There is no conflict of interest.

Acknowledgements

References

Alencar, L.R.V., Quental, T.B., Grazziotin, F.G., Alfaro, M.L., Martins, M., Venzon, M., Zaherb, H., 2016. Diversification in vipers: phylogenetic relationships, time of divergence and shifts in speciation rates. Mol. Phylogenet. Evol. 105, 50–62.

Barlow, A., Pook, C.E., Harrison, R.A., Wüster, W., 2009. Coevolution of diet and prey specific venom activity supports the role of selection in snake venom evolution. Proc. Roy. Soc. B 276, 2443–2449.

Biserkov, V., Kostadinova, A., 1997. Development of the plerocercoid I of *Ophiotaenia europaea* in reptiles. Int. J. Parasitol. 27, 1513–1516.

Bogdanov, O.P., Markov, G.S., 1955. Parasitic worms of some reptiles of Middle Asia. Izv. Akad. Nauk Uzb. SSR 8, 59–68 (In Russian).

Bogdanov, O.P., Markov, G.S., 1960. Parasite fauna of venomous snakes of Middle Asia and Kazakhstan. In: Venomous Animals of Middle Asia and their Venoms. Tashkent, pp. 38–45 (In Russian).

Cañeda-Guzmán, I.C., de Chambrier, A., Scholz, T., 2001. *Thaumasioscolex didelphidis* n. gen. and n. sp. (Cestoda: Proteocephalidae) from the black-eared opossum *Didelphis marsupialis* from Mexico, the first proteocephalidean tapeworm from a mammal. J. Parasitol. 87, 639–647.

de Chambrier, A., 2001. A new tapeworm from the Amazon, *Amazotaenia yvettae* n. gen., n. sp. (Eucestoda: Proteocephalidea) from the siluriform fishes *Brachyplatystoma filamentosum* and *B. vaillanti* (Pimelodidae). Rev. Suisse Zool. 108, 303–316.

de Chambrier, A., Zehnder, M.P., Vaucher, C., Mariaux, J., 2004. The evolution of the Proteocephalidea (Platyhelminthes, Eucestoda) based on an enlarged molecular phylogeny, with comments on their uterine development. Syst. Parasitol. 57, 159–171.

de Chambrier, A., Ammann, M., Scholz, T., 2010. First species of *Ophiotaenia* (Cestoda: Proteocephalidea) from Madagascar: *O. georgievi* n. sp., a parasite of the endemic snake *Leioheterodon geayi* (Colubridae). Folia Parasitol. 57, 197–205.

de Chambrier, A., Binh, T.T., Scholz, T., 2012. *Ophiotaenia bungari* n. sp. (Cestoda), a parasite of *Bungarus fasciatus* (Schneider) (Ophidia: Elapidae) from Vietnam, with comments on relative ovarian size as a new and potentially useful diagnostic character for proteocephalidean tapeworms. Syst. Parasitol. 81, 39–50.

de Chambrier, A., Waeschenbach, A., Fisseha, M., Scholz, T., Mariaux, J., 2015. A large 28S rDNA-based phylogeny confirms the limitations of established morphological characters for classification of proteocephalidean tapeworms (Platyhelminthes, Cestoda). ZooKeys 500, 25–59.

de Chambrier, A., Scholz, T., Mariaux, J., Kuchta, R., 2017. Onchoproteocephalidea I Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014. In: Caira, J.N., Jensen, K. (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, pp. 251–277.

de Chambrier, A., Beveridge, I., Scholz, T., 2018. Tapeworms (Cestoda: Proteocephalidae) of Australian reptiles: hidden diversity of strictly host-specific parasites. Zootaxa 4461, 477–498.

de Chambrier, A., Brabec, J., Tran, B.T., Scholz, T., 2019. Revision of *Acanthotaenia* von Linstow, 1903 (Cestoda: Proteocephalidae), parasites of monitors (*Varanus* spp.), based on morphological and molecular data. Parasitol. Res. 118, 1761–1783.

de Chambrier, A., Brabec, J., Scholz, T., 2020. Molecular data reveal unexpected species diversity of tapeworms of Australasian reptiles: revision of *Kapsulotaenia* (Cestoda: Proteocephalidae). Zootaxa 4869, 529–561.

Coquille, S.C., de Chambrier, A., 2008. *Cairaella henrii* gen. n., sp. n., a parasite of *Norops trachyderma* (Polychrotidae), and *Ophiotaenia nicoleae* sp. n. (Eucestoda: Proteocephalidea), a parasite of *Thecadactylus rapicauda* (Gekkonidae), in Ecuador. Folia Parasitol. 55, 197–206.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.M., Gascuel, O., 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36, W465–W469.

Farooq, M., Khanum, Z., Ansar, R., 1983. Helminth parasites of fresh water tortoise and poisonous snake from Sind, Pakistan. Pakistan J. Zool. 15, 31–37.

Freze, V.I., 1965. Essentials of Cestodology, vol. V [Proteocephalata in fish, amphibians and reptiles.] Nauka, 538 pp. (In Russian: English translation: Israel Program of Scientific Translation, Jerusalem, 1969, Cat. No. 1853, v + 597pp.).

Gibson, D.I., Bray, R.A., Harris, E.A. (Compilers), 2005. Host-Parasite Database of the Natural History Museum. London. http://www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/. accessed on December 2020.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.-S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol. Biol. Evol. 35, 518–522.

Holt, B.G., Lessard, J.-P., Borregaard, M.K., Fritz, S.A., Araújo, M.B., Dimitrov, D., Fabre, P.-H., Graham, C.-H., Graves, G.R., Jønsson, K.A., Nogués-Bravo, D., Wang, Z., Whittaker, R.J., Fjeldså, J., Rahbek, C., 2013. An update of Wallace's zoogeographic regions of the world. Science 339, 74–78.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Markov, G.S., Bogdanov, O.P., 1956. Parasite fauna of reptiles of Turkmenistan. Tr. Inst. Biol. Akad. Nauk Turkm. SSR 4, 260–277 (In Russian).

Markov, G.S., Bogdanov, O.P., 1960. Helminths and ticks – parasites of snakes of Middle Asia. Uzb. Biol. Zhurnal 2, 35–41 (In Russian).

Markov, G.S., Bogdanov, O.P., Persianova, L.A., 1970. Helminth fauna of saw-scaled viper. In: Venomous Animals of Middle Asia and their Venoms. Tashkent, pp. 46–60 (In Russian).

Marsella, C.M., de Chambrier, A., 2008. *Ophiotaenia alessandrae* sp. n. (Eucestoda: Proteocephalidea), a parasite of *Hyla boans* (Anura: Hylidae) from Amazonia in Ecuador. Rev. Suisse Zool. 115, 553–563.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop. GCE, pp. 1–8, 14 Nov. 2010.

Minh, B.Q., Nguyen, M.A.T., von Haeseler, A., 2013. Ultrafast approximation for phylogenetic bootstrap. Mol. Biol. Evol. 30, 1188–1195.

Nama, H.S., 1984. Faunistic survey of helminth parasites of vertebrates of Rajasthan with special reference to arid zone. Sci. Rev. Arid Zone Res. 2, 187–225.

Pook, C.E., Joger, U., Stümpel, N., Wüster, W., 2009. When continents collide: phylogeny, historical biogeography and systematics of the medically important viper genus *Echis* (Squamata: Serpentes: Viperidae). Mol. Phylogenet. Evol. 53, 792–807.

Rambeloson, V.R., Ranaivoson, H.C., de Chambrier, A., 2012. *Ophiotaenia lapata* sp. n. (Eucestoda: Proteocephalidea) from Madagascar: a parasite of the endemic snake *Madagascarophis colubrinus* (Colubridae). Rev. Suisse Zool. 119, 547–559.

Rego, A.A., 1994. Order Proteocephalidea Mola, 1928. In: Khalil, L.F., Jones, A., Bray, R. A. (Eds.), Keys to the Cestode Parasites of Vertebrates. CAB International, Wallingford, pp. 257–293.

La Rue, G.R., 1911. A revision of the cestode family Proteocephalidae. Zool. Anz. 38, 473–482.

La Rue, G.R., 1914. A revision of the cestode family Proteocephalidae. Ill Biol. Monogr. 1 (1–2), 3–351.

Scholz, T., de Chambrier, A., 2003. Taxonomy and biology of proteocephalidean cestodes: current state and perspectives. Helminthologia 40, 65–77.

Scholz, T., de Chambrier, A., Kuchta, R., Littlewood, D.T.J., Waeschenbach, A., 2013. *Macrobothriotaenia ficta* (Cestoda: Proteocephalidea), a parasite of sunbeam snake (*Xenopeltis unicolor*): example of convergent evolution. Zootaxa 3640, 485–499.

Schuster, R.K., Sivakumar, S., 2013. Reptiles as paratenic hosts of feline parasites. In: 24th Conference of the World Association for the Advancement of Veterinary Parasitology, 25–29 August 2013, Perth, Australia.

Sela, I., Ashkenazy, H., Katoh, K., Pupko, T., 2015. GUIDANCE2: accurate detection of unreliable alignment regions accounting for the uncertainty of multiple parameters. Nucleic Acids Res. 43, W7–W14.

Sharpilo, V.P., 1976. Parasitic Worms of Reptiles of the Fauna of the USSR. Systematics, Chorology, Biology. Naukova Dumka, Kiev, p. 288 (In Russian).

Trifinopoulos, J., Nguyen, L.T., von Haeseler, A., Minh, B.Q., 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44, W232–W235.

Uetz, P., Freed, P., Hošek, J. (Eds.), 2020. The Reptile Database. Available. http://www.reptile-database.org. (Accessed 2 December 2020).

Velikanov, V.P., 1984. Larval forms of the Acuarioidea superfamily (Nematoda, Spirurata). Parasites of reptiles in Turkmenistan. Vestnik Zool 1984 (1), 3–8 (In Russian).

World Health Organization, 2020. Venomous snakes distribution and species risk categories. Available. https://apps.who.int/bloodproducts/snakeantivenoms/database/default.htm.