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A review of *Triplotaenia undosa* Beveridge, 1976 (Cestoda: Anoplocephalidae) from macropodid marsupials, with the erection of *T. macropodis* sp. nov.

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Research Paper

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Abstract

Molecular evidence (28S DNA) has suggested that *Triplotaenia undosa* from macropodid marsupials is a species complex. Additional data (cox 1) presented in this study confirmed the hypothesis and a morphological examination of all available specimens identified a new species, *T. macropodis* sp. nov., in the grey kangaroos *Macropus fuliginosus* and *M. giganteus* as well as the tammar wallaby, *Notamacropus eugenii*, and the red kangaroo, *Osphranter rufus.* The new species differs in the ratio of the number of testes to the number of female genital complexes. Specimens of *T. undosa* from the swamp wallaby, *Wallabia bicolor*, the type host, and the common wallaroo, *Osphranter robustus*, are each genetically distinct, but the fixed material from *O. robustus* is too fragmentary to permit a detailed morphological description. An amended description and new illustrations of *T. undosa* from *W. bicolor* are provided.

Introduction

The anoplocephalid cestode genus *Triplotaenia* Boas, 1902, parasitic in the small intestines of kangaroos and wallabies, is remarkable morphologically in having the strobila divided into two spirally arranged arms and lacking external segmentation (Beveridge 1976). The type species *T. mirabilis* Boas, 1902, was originally described from a rock wallaby, *Petrogale penicillata*, from a zoo in Copenhagen (Boas 1902). The genus was revised by Beveridge (1976) with the recognition of three species: *T. fimbriata* Beveridge, 1976, found primarily in the grey kangaroos (*Macropus fuliginosus* and *M. giganteus*), *T. mirabilis* in rock wallabies (*Petrogale* spp.), and *T. undosa* Beveridge, 1976 in a range of kangaroo and wallaby species. *Triplotaenia fimbriata* is distinguished from its congeners in having the testes internal rather than external to the female genital organs, while *T. mirabilis* and *T. undosa* are distinguishable based on the number of testes per set of female genitalia, with approximately one in *T. mirabilis* and two or more in *T. undosa* (Beveridge 1976).

A molecular study of anoplocephalid cestodes of Australian marsupials, including species of *Triplotaenia*, using the 28S ribosomal gene, provided evidence for the independence of the three described species but also suggested the presence of multiple genotypes within *T. undosa* (Hardman *et al.* 2012). Their study identified one clade (specimens V73, V77, X13 in their Table 1 and Figure 3) in the western grey kangaroo, *Macropus fuliginosus*, and the tammar wallaby, *Notamacropus eugenii*, a second clade in the common wallaroo, *Osphranter robustus* (V52, V92), and a third clade (V54, V86) in the swamp wallaby, *Wallabia bicolor* (V54, V68), although at the time, their sample V54 was incorrectly identified as coming from the eastern grey kangaroo, *Macropus giganteus.* Conspicuously lacking from their analysis was material from *M. giganteus*, a common host of this parasite species (Beveridge 2023).

In the present study, genetic variation within *T. undosa* has been re-assessed using the cox1 gene with the addition of material from *M. giganteus* and additional illustrations are provided for this species. Since their original descriptions or redescription (Beveridge, 1976) a substantial amount of new material of species of *Triplotaenia* has been collected from macropodid hosts around the continent. This material has been incorporated into the current study in a re-assessment of the morphological variation within *T. undosa* from various host species as well as adding the newer host and geographical records.

Materials and methods

Frozen specimens accessed from collections held in the South Australian Museum, Adelaide (SAM) and the School of Veterinary Science at the University of Melbourne were characterised genetically by targeting a region of the mitochondrial cytochrome *c* oxidase I as described

Table 1. Specimens of *Triplotaenia undosa* used for molecular studies with GenBank registration numbers for sequence data and South Australian Museum registration numbers for morphological vouchers

Host species	Locality	GenBank no.	SAM voucher no.
Macropus giganteus	Lara, Victoria	PV247776	34617
	Bacchus Marsh, Victoria	PV247775	34939
	Avalon, Victoria	PV247778	31161
Macropus fuliginosus	Bourke, New South Wales	PV247777	24242
Osphranter robustus	Cloncurry, Queensland	PV247779	34943
Wallabia bicolor	Hoppers Crossing, Victoria	PV247780	34639

previously (Hu *et al.* 2005). Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, USA) following the protocol provided by the manufacturer. PCR was carried out in a 25 µl volume containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl (Promega, USA), 3.5 mM of $MgCl_2$, 200 µM of each deoxynucleotide triphosphate, 50 pmol of each primer JB3 (5'-TTTTTTGGGCATCCTGAGG TTTAT-3'), and JB4.5 (5'–TAAAGAAAGAACATAATGAAAA TG–3') and 1 U of Go*Taq* polymerase (Promega) under the following cycling conditions: 94°C for 5 min (initial denaturation); 35 cycles of 94°C for 30 s (denaturation); 52°C for 30 s (annealing) and 72°C for 30 s (extension); followed by a final extension at 72°C for 5 min. For each PCR, negative (no-DNA) and positive (*Bertiella paraberrata* Beveridge, 1985) controls were included. No amplification was detected in any of the negative control reactions during this study. Amplicons (5 µl) were examined on 1.5% agarose gels stained with Gel Red Nucleic Acid Stain (Biotium, Inc. Hayward, CA, USA). Gels were examined using trans-illumination and were photographed using a GelDoc system (BioRad, Hercules, CA, USA). PCR amplicons were purified using FavorPrep^{TM} GEL/PCR Purification Kit (Favorgen Biotech Corp., Taiwan) prior to automated DNA Sanger sequencing using the primers JB3 and JB4.5 in separate reactions. The quality of each sequence obtained was appraised using the program Geneious Prime 2024.0.7 (Biomatters Ltd., Auckland, New Zealand). The DNA sequences determined herein have been submitted to the GenBank database (see Table 1).

Nucleotide sequences were aligned using the MUSCLE alignment program and adjusted manually by employing the Geneious Prime program. A dataset representing the *cox*1 sequences generated herein was aligned and adjusted manually. *Progamotaenia festiva* (sequence from GenBank AM495468; Beveridge *et al.* 2007) served as the outgroup. Phylogenetic analyses were performed using Bayesian Inference (BI) and Neighbour-Joining (NJ) methods. The BI was conducted using the Monte Carlo Markov Chain (MCMC) analysis in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The likelihood parameters for BI were based on the Akaike Information Criterion (AIC) test in jModeltest v2.1.10 (Darriba *et al.* 2012). AIC revealed the substitution model of evolution with equal sites with gamma distribution (HKY+G) as the 'best' model. Posterior probabilities (pp) were calculated using 2,000,000 generations, employing four simultaneous tree-building chains, with every 100th tree being saved. A consensus tree (50% majority rule) was constructed based upon the remaining trees generated by BI. The NJ analyses were performed using software MEGA11.0.10 (Tamura *et al.* 2021) and the nodes were tested for robustness with 10,000 bootstrap replicates. The phylogenetic trees produced from the BI and NJ analyses were compared for concordance in their topologies.

Specimens of *T. undosa* held in the collections of SAM were re-examined. The specimens within the museum are held in the Australian Helminthological Collection. In some instances, additional wet material (indicated by W) was prepared as slide mounts (indicated by S). Due to the fragmented nature of most specimens and the difficulty of extracting entire specimens, the number of specimens examined at each locality is not provided. Specimens were stained in celestine blue, dehydrated in an ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Drawings were made with a drawing tube attached to an Olympus BH-2 microscope. Measurements of the new species, made with an ocular micrometre, are provided in millimetres as the range followed by the mean and the number of specimens measured (n) in parentheses. Measurements of the type specimens of *T. undosa*, taken from Beveridge (1976), are presented as ranges. Drawings were made with a drawing tube attached to an Olympus BH2 microscope. To determine the ratio of testes to female genital complexes, drawings were made from as long a piece of strobila as possible and the ratio obtained from the drawings. Parts of specimens from *M. giganteus* were embedded in paraffin, and sections, cut at a thickness of 5 µm, were stained with haematoxylin and eosin. Host nomenclature follows Jackson and Groves (2015).

Results

In both the NJ and BI trees of the cox 1 data, the three samples from *M. giganteus* and a fourth from *M. fuliginosus* formed a strongly supported clade, to the exclusion of samples from *O. robustus* and *W. bicolor*, each of which occurred in distinct branches (Figure 1).

The specimens of *T. undosa* from *O. robustus* were too fragmentary and poorly preserved for detailed morphological analysis. Comparisons undertaken between specimens from the type host, *W. bicolor*, and those from *M. giganteus*, *M. fuliginosus, Osphranter rufus*, and *N. eugenii* revealed morphological differences, primarily in the ratio of number of testes to number of female genital complexes. On this basis, combined with the molecular data, the latter are described here as an independent species, *T. macropodis* sp. nov.

Triplotaenia macropodis *sp. nov.*

Triplotaenia undosa Beveridge, 1976 *pro parte*: Beveridge 1976, 73–75, Figures 161–174; Smales and Mawson 1978, 10 (as *Triplotaenia* sp.); Arundel *et al.* 1979, 365; Beveridge and Arundel 1979, 73; Arundel *et al.* 1990, 43; Beveridge *et al.* 1998, 479; Webley *et al.* 2004, 629; Cripps *et al.* 2015, 168; Beveridge 2023, 5.

Type data: holotype SAM 37088; paratype SAM 37089.

Type host: *Macropus giganteus* Shaw (eastern grey kangaroo) (Marsupialia: Macropodidae).

Other hosts: *Macropus fuliginosus* (Desmarest) (western grey kangaroo); *Notamacropus eugenii* (Desmarest) (tammar wallaby); *Osphranter rufus* (Desmarest) (red kangaroo).

Type locality: Picola North, Victoria, Australia (36° S 145° 07'E).

Site in host: small intestine.

DNA sequence data: cox1: PV247775, PV247776, PV247778 (from *M. giganteus*).

Etymology: named after the principal host genus, *Macropus*.

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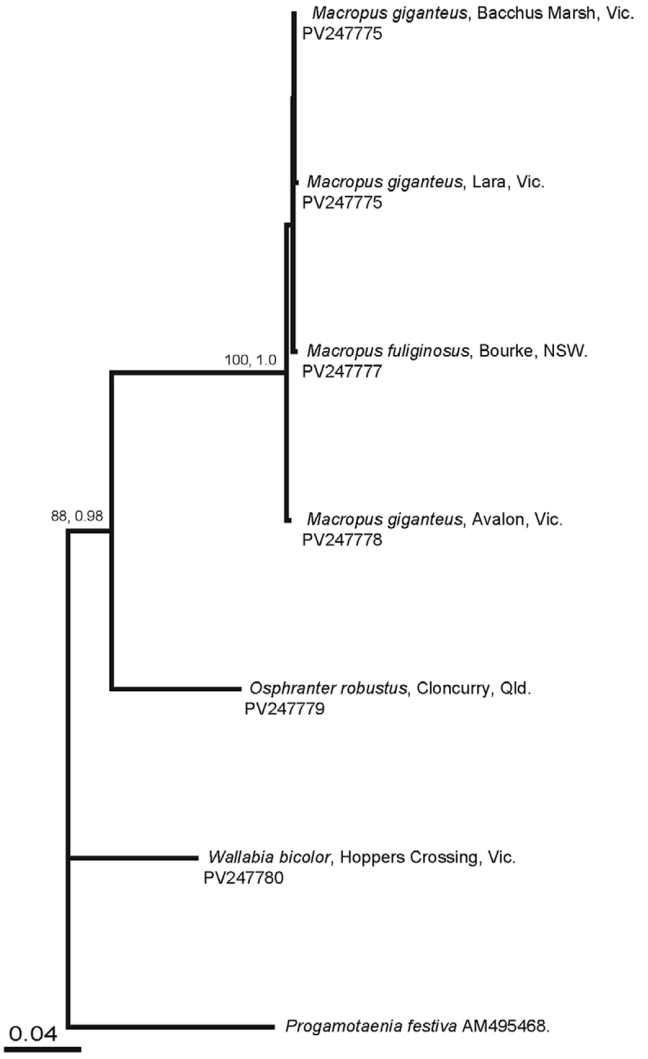

Figure 1. Phylogenetic relationships between specimens identified as *Triplotaenia undosa* from different species of macropodid hosts. Numerals indicate NJ percentages and BI posterior probabilities respectively. State abbreviations and contractions: NSW, New South Wales; Qld, Queensland, Vic, Victoria. *Progamotaenia festiva* was used as the outgroup.

Material examined

From *Macropus giganteus*: New South Wales: Kioloa (SAM 34617 W, 37140 S); Victoria: Picola North (types); Wodonga (SAM 47806 W, 37139 S); Friarstown (SAM 46279 W, 37147 S); Fraser National Park (SAM 37143 S); Yarram Park via Dunkeld (SAM 10887 W, 37142 S); Yan Yean (SAM 8470, 10690, 10175, 10928 W, 21460, 20872 S); Bacchus Marsh (SAM 34939 W, 37146 S); Cardinia (SAM 22939 W, 37145 S); Avalon (SAM 31161 W, 37144 S); Lara (SAM 34617 W, 37125 S); Anglesea (SAM 46003 W, 37132 S); Tasmania: Maria Island (SAM 16549 W);
Zoobank registration: urn:lsid:zoobank.org:pub:917EF94B-A502-4113-8A62-AAF40F111AA1.
Description (Figures 2–3, 5, 6) (based on specimens from *M. giganteus*). Elongate, delicate cestodes often found in convoluted masses (Figure 5); extraction of entire specimens difficult. Strobila divided into two spirally coiled arms (Figure 2A), with genitalia on external surface and with internal surface irregularly fimbriate (Figure 3A); segmentation lacking. Holotype incomplete, entire arm of strobila (terminal region gravid) 123 long. Scolex small, squat, 0.46–0.67 (0.60, n = 7) in diameter; four suckers, oval to circular, 0.20–0.32 (0.25, n = 7) long, 0.20–0.28 (0.23, n = 7) wide. Central strobilar tag highly variable morphologically, entire or lobed (Figures 2B–E). Strobilar arms, narrow at origin from scolex, reaching 0.78–1.20 (0.96, n = 5) wide in gravid regions of strobila; lateral margin of each strobilar arm thickened, folded and coiled irregularly about longitudinal axis (Figure 3A). Genital pores opening on lateral margin; medial margin of strobilar arms relatively straight, diaphanous, with small, irregular fimbriae; genital pores in continuous band, arranged in 4–5 irregular transverse rows (Figures 3C, 6B). Cirrus sacs numerous, 0.065–0.085 (0.073, n = 5) long, 0.015–0.023 (0.018, n = 5) wide (Figure 6A); cirrus unarmed; internal seminal vesicle 0.013–0.023 (0.018, n = 5) in diameter. Testes small, spherical, situated in single row between cirrus sacs and female genitalia (Figure 3B), 0.05–0.07 (0.06, n = 10) in diameter. Female genital organs composed of ovary and adjacent vitellarium in row medial to testes (Figures 3B, 6C). Ovary ovoid, 0.06–0.09 (0.070, n = 10) long, 0.030–0.050 (0.042, n = 10) wide. Vitellarium circular, at anterior margin of ovary, 0.020–0.028 (0.023, n = 5) in diameter; ratio of testes to female genitalia 1.2–2.0 (n = 3). Gravid uteri sacciform, usually narrower distally, arranged at regular intervals along lateral margin of strobila (Figure 3D). Eggs spherical, 0.05–0.07 (0.06, n = 10) in diameter, none with fully formed pyriform apparatus. Longitudinal muscle well developed, in broad bands consisting of large fibres (Figure 6C); transverse musculature poorly developed, consisting of fine, individual fibres; osmoregulatory canals c. 0.01 in diameter.

Other hosts

From *Macropus fuliginosus*: New South Wales: 60 km SE of Bourke (SAM 24242 W, 37122 S); Kinchega National Park (SAM 19231 W, 37118 S); Victoria: Pine Plains Station via Patchewollock (SAM 9109, 10692 W, 20934 S); Hattah National Park (SAM 19928 W, 37121 S); South Australia: Nonning Station via Port Augusta (SAM 7891, 7923 W, 37103, 37104 S); Jabuk (SAM 8815 W, 21390 S); Second Valley (SAM 14906 W, 37110 S); Kangaroo Island (SAM 12540, 31513-5 W, 28560, 29264, 29270 S).
Morphologically similar to material from type host; ratio of no. of testes to female genitalia 1.5.
From *Notamacropus eugenii*: South Australia: Kangaroo Island (SAM 20869, 20871, 21531 S). Morphologically similar to material from type host; ratio of no of testes to female genitalia 1.2.
From *Osphranter rufus*: New South Wales: Menindee (SAM 21512 S). Morphologically similar to material from type host.

Triplotaenia undosa

Triplotaenia undosa: Beveridge 1976, 73–75, Figures 161–174; Schmidt 1986, 416; Smales 1987, 129 (host misidentification); Beveridge 1994, 329, Figures 17.1–17.3; Beveridge 2016, 210.

Material examined

From *Wallabia bicolor*: Victoria: Dartmouth (SAM 9532 W, 20962 S, types); Bonang (SAM 10506 W, 20833 S); Kyneton (SAM 20872 S); Buangor (6692, 33983 W); Beaufort (SAM 44458 W); Healesville (SAM 33994, 45601 W, 29267 S); Hoppers Crossing (SAM 34639 W); Portland (SAM 46178 W).
DNA sequence data: cox1: PV247780.

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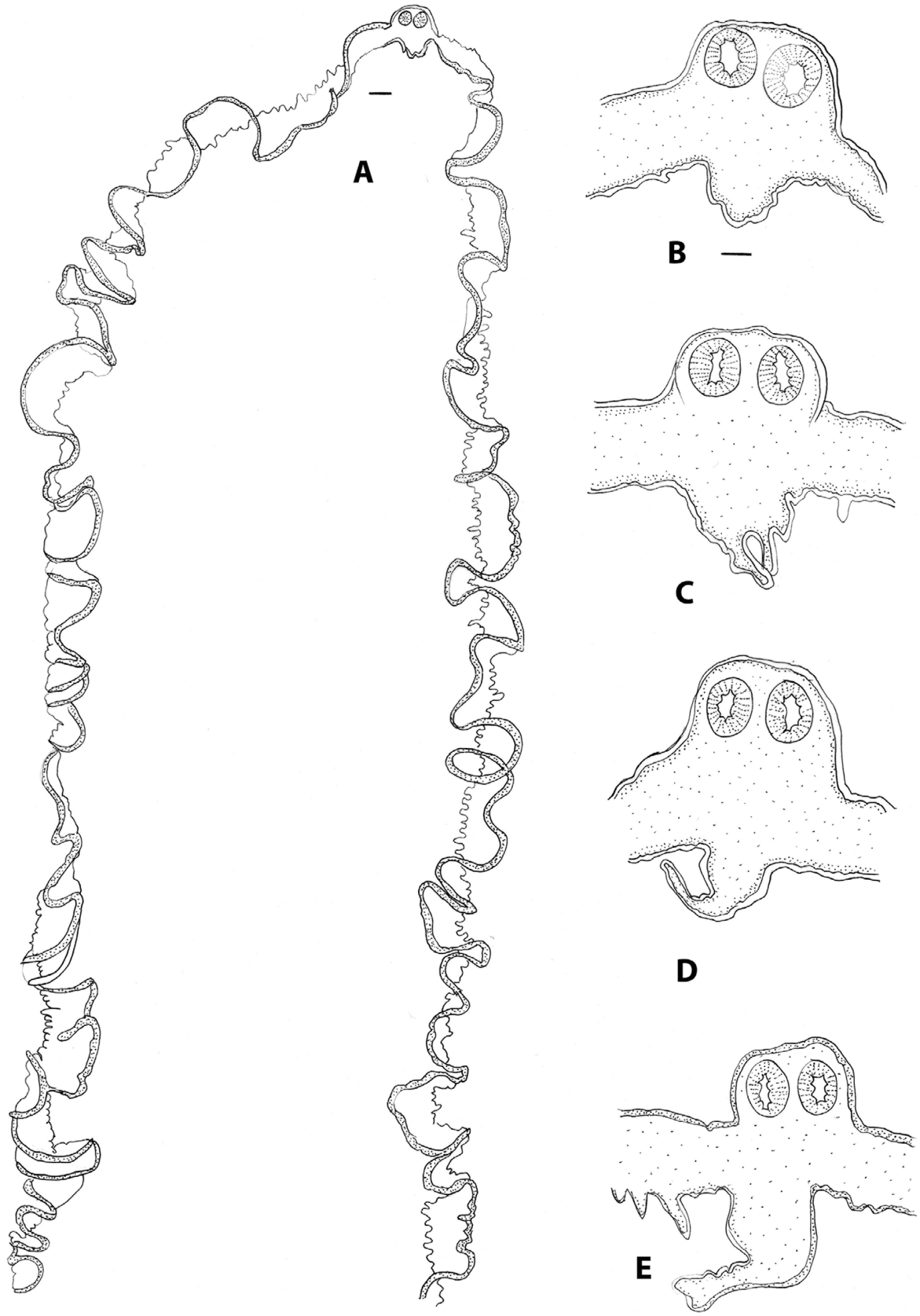

Figure 2. *Triplotaenia macropodis* sp. nov. from *Macropus giganteus.* A, anterior region of strobila of holotype; B–E, scoleces showing variation in morphology of scolex. Scale bars: A, 1.0 mm; B–E, 0.1 mm.

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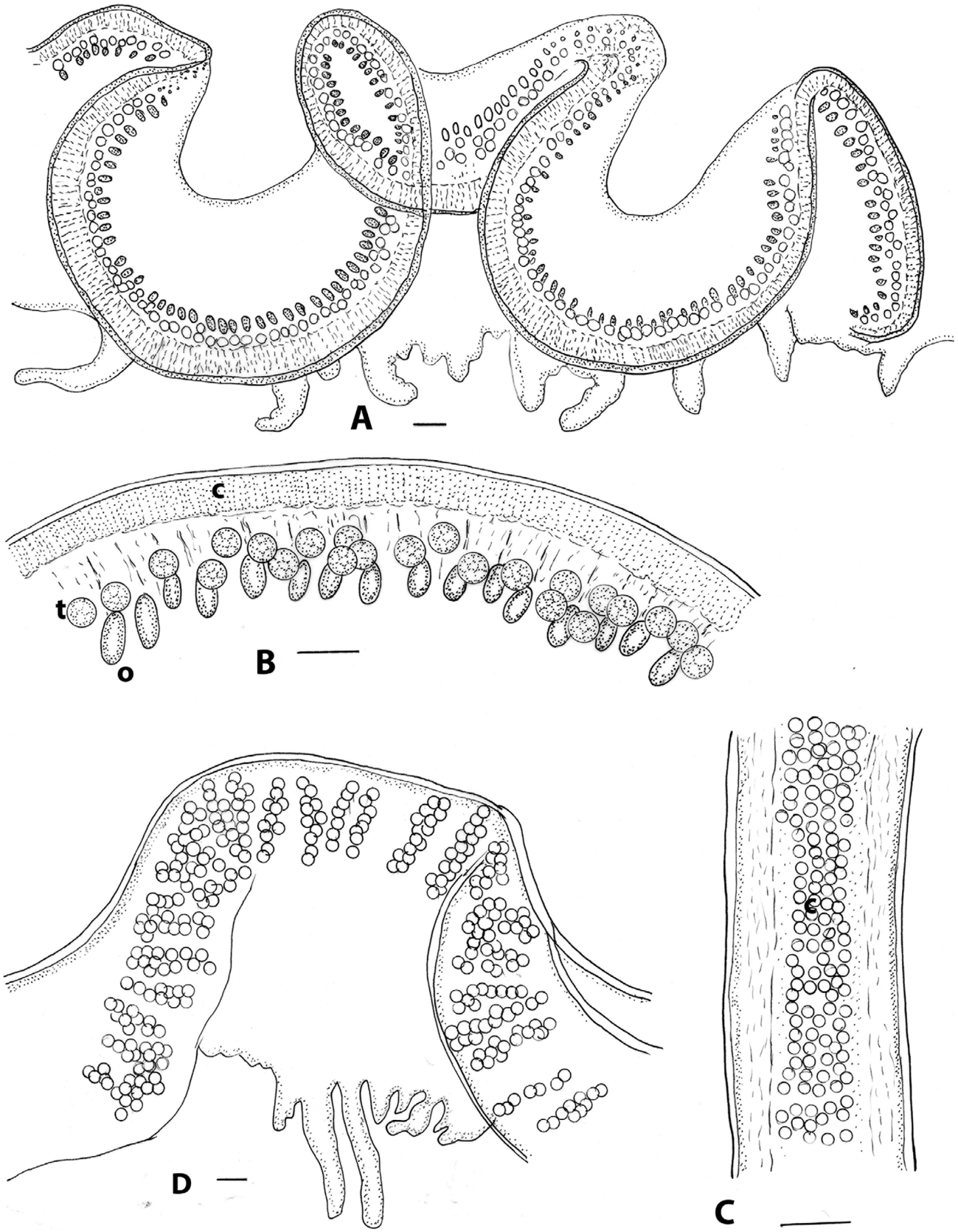

Figure 3. *Triplotaenia macropodis* sp. nov. from *Macropus giganteus.* A, mature region of strobila showing convoluted lateral region with genitalia and fringed medial region; B, lateral region of strobila showing genitalia; C, longitudinal section through lateral region with cross-sections of numerous cirrus sacs. D, gravid region of strobila with uteri containing eggs. Scale bars: 0.1 mm. Abbreviations: c, cirrus sacs; o, ovary; t, testis.

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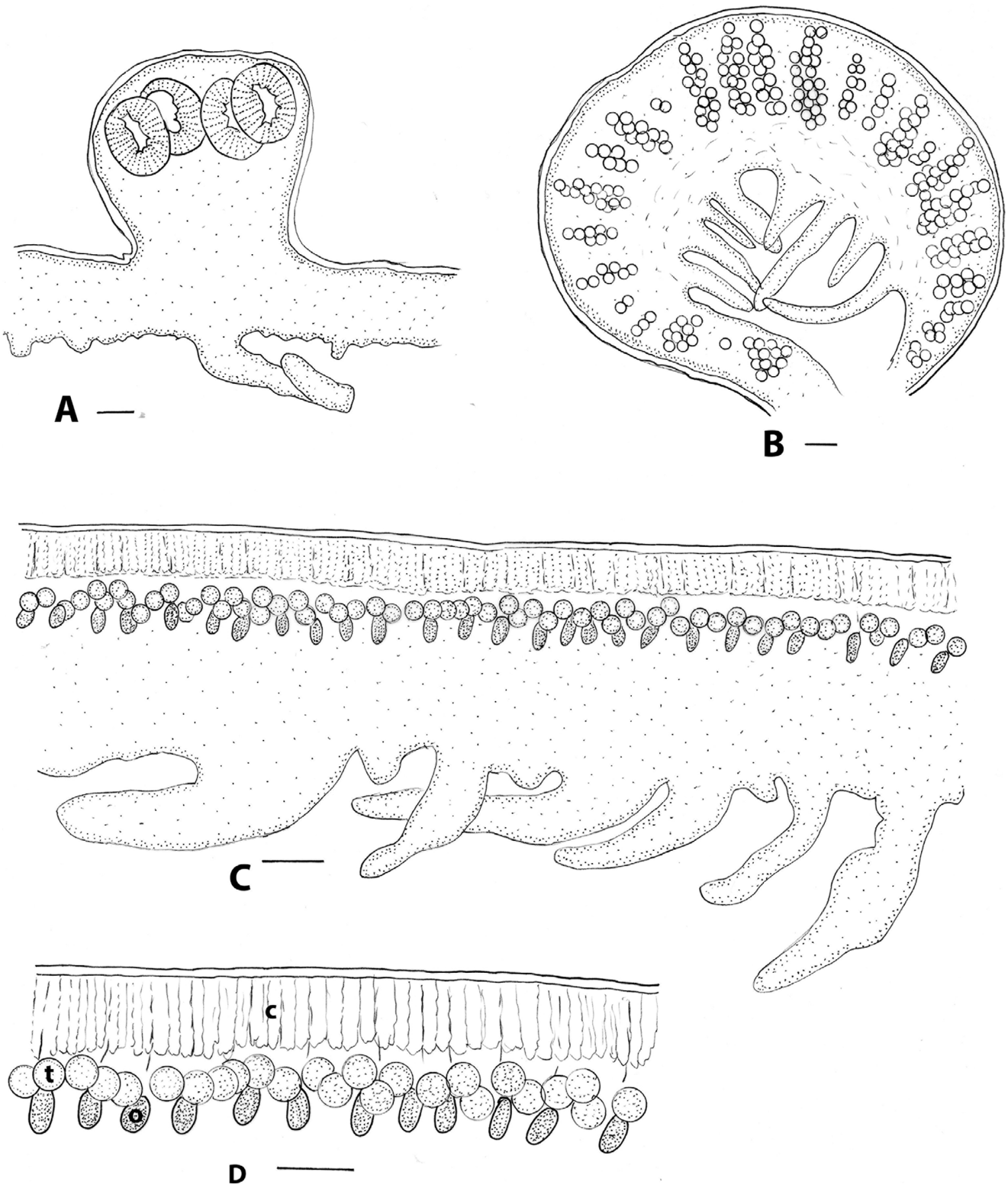

Figure 4. *Triplotaenia undosa* Beveridge, 1976 from *Wallabia bicolor*, illustrations from type specimens. A, scolex; B, gravid region of strobila with uteri containing eggs; C, mature region of strobila showing fimbriated internal region; D, lateral region of strobila showing genitalia. Scale bars: 0.1 mm. Abbreviations: c, cirrus sacs; o, ovary; t, testis.

Amended description (Figure 4)

Elongate, delicate cestodes; strobila divided into two spirally coiled arms, 58–250 long, with genitalia on external surface and with internal surface irregularly fimbriate (Figure 4C). Segmentation lacking. Scolex small, squat, 0.52–0.75 in diameter; four suckers, oval to circular, 0.23–0.32 long, 0.20–0.27 wide; central strobilar tag highly variable morphologically, entire (Figure 4A). Genital pores opening on lateral margin; medial margin of strobilar arms relatively straight, diaphanous, with small, irregular fimbriae; genital

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Figure 5. Mass of intertwined *Triplotaenia macropodis* sp. nov. strobilae from the duodenum of an eastern grey kangaroo, *Macropus giganteus.* Scale bar: 10 mm.

pores in continuous band, arranged in 3–6 irregular transverse rows. Cirrus sacs numerous, 0.07–0.12 long, 0.030–0.035 wide; cirrus unarmed; internal seminal vesicle 0.045–0.060 long, 0.025–0.030 wide. Testes small, spherical, situated in single row between cirrus sacs and female genitalia (Figure 4C), 0.035–0.040 in diameter. Female genital organs composed of ovary and adjacent vitellarium in row medial to testes (Figures 4C, D). Ovary ovoid, 0.040–0.065 long, 0.036–0.060 wide. Vitellarium circular, at anterior margin of ovary, 0.025–0.035 in diameter; ratio of testes to female genitalia 1:2.3–2.4. Gravid uteri sacciform, usually narrower distally, arranged at regular intervals along lateral margin of strobila (Figure 4B). Eggs spherical, 0.04–0.08 in diameter; pyriform apparatus 0.010–0.015 long, terminating in two horns with numerous filaments. Longitudinal muscles well developed, in broad bands consisting of large fibres; transverse musculature poorly developed, consisting of fine, individual fibres. Osmoregulatory canals paired, aporal to genitalia; canals anastomose in scolex.

Remarks: the new material examined complied with the original description and measurements. Additional illustrations of *T. undosa* from the type host are provided (Figure 4). New estimates of the ratio of testes to female genitalia were 1:2.3–2.4. The material examined expands the number of geographical localities at which this species has been found, but it still appears to be restricted to Victoria, with no specimens found in extensive sampling of the host species from New South Wales and Queensland (Beveridge 2016).

Triplotaenia sp.

Triplotaenia undosa Beveridge, 1976: Beveridge 2020b, 4.

Material examined

From *Osphranter robustus*: Queensland: Mingela (SAM 21326 S); Warrawee Station via Charters Towers (SAM 19758 S); Cloncurry (SAM 34943 W, 29263 S).

DNA sequence data: cox1: PV247779.

From *Macropus giganteus*: Queensland: Mingela (SAM 21327 S).

Remarks: the fixed material was too fragmentary and poorly preserved for detailed morphological examination. The taxonomic status of the specimens of *Triplotaenia* occurring in these hosts requires further studies based on newly collected and well-preserved specimens.

Discussion

The phylogenetic study of Australian marsupial cestodes using the 28S ribosomal gene (Hardman *et al.* 2012), which included species of *Triplotaenia*, provided the first indication that *T. undosa* was a species complex. In this report, cox 1 data are added which support the original findings and include specimens from an additional common host species, *M. giganteus*, to the available data. The cox1 phylogenetic tree is similar to the data provided by the 28S tree, but shows, in addition, that specimens from *M. giganteus* are identical to those in *M. fuliginosus*, while those from *Wallabia* and *Osphranter* occur on independent branches. Specimens from *N. eugenii* were not available for the cox1 study but evidence from the 28S tree indicated that they were identical to specimens from *M. fuliginosus.*

Based on the combined evidence from the two molecular studies, the morphology of *T. undosa* was re-examined in its principal host species. Specimens from *Osphranter robustus* were too fragmented and poorly preserved to permit a detailed morphological examination, and while the material from other hosts was better preserved, it was usually fragmented and often stained poorly.

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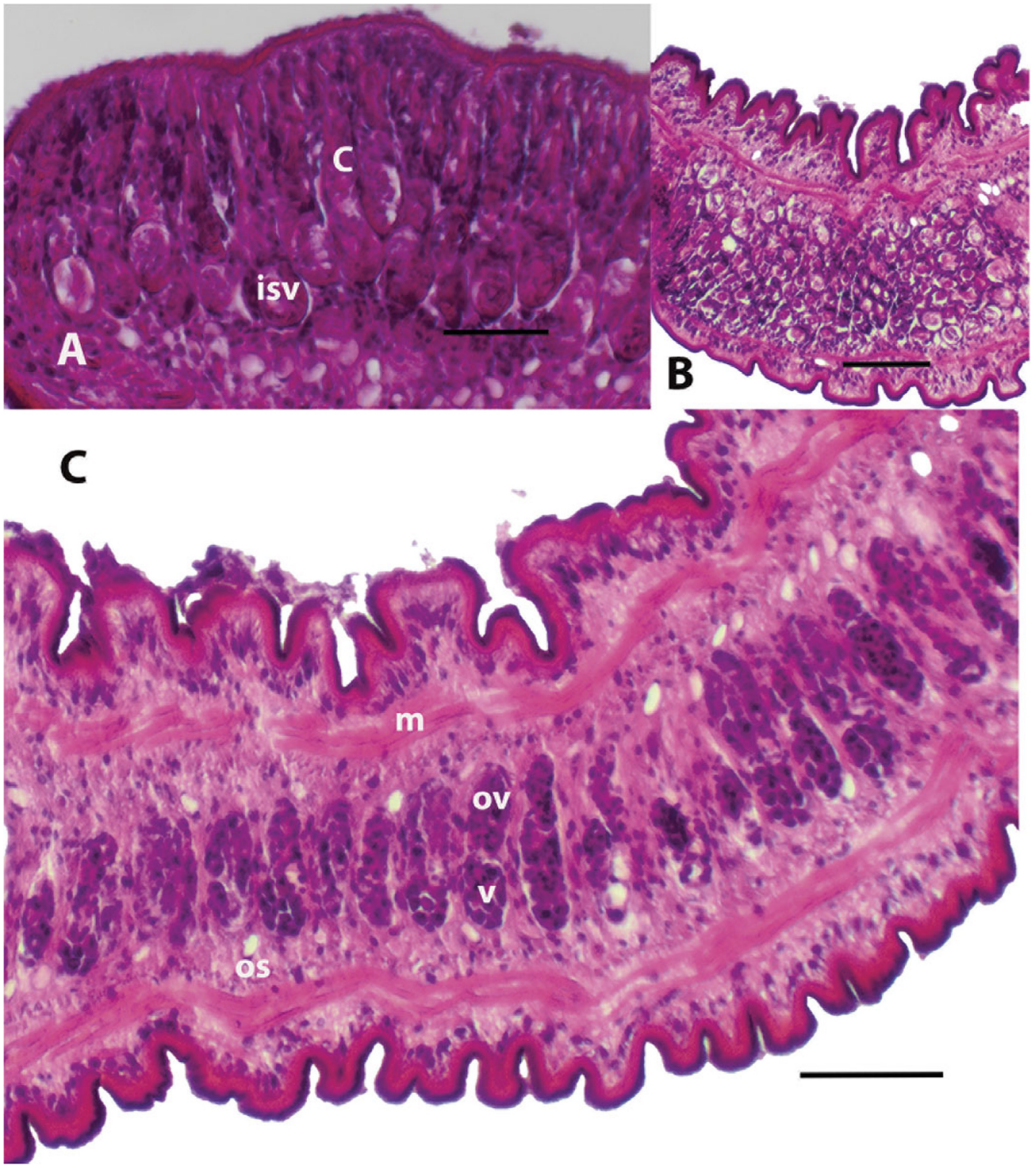

Figure 6. Histological details of *Triplotaenia macropodis* sp. nov. from the duodenum of an eastern grey kangaroo, *Macropus giganteus.* A, Longitudinal section of strobila through cirrus sacs (c) and internal seminal vesicles (isv); B, Transverse section of strobila through cirrus sacs showing arrangement in numerous transverse rows; C, Oblique transverse section of strobila, showing ovaries (ov), vitellaria (v), longitudinal musculature (m) and osmoregulatory canals (os). Scale bars: A, B, 50 μm, C, 10 μm.

In addition, the coiled nature of the strobilar arms and the small size of the genital organs makes these species very difficult to examine other than with serial histological sections. From re-examining the specimens of *T. undosa*, it became evident that while Beveridge (1976) gave a relatively detailed description of the species, it is a composite, based on measurements from cestodes from the type host, *W. bicolor*, but with most of the illustrations based on specimens from *M. giganteus* and a few from *M. fuliginosus* and *N. eugenii.* In the illustrations of this species (Beveridge 1976, p. 109), Figures 161–164 (scoleces), 170 (gravid strobila) and 173 (mature strobila) are from *M. giganteus*, 165 (egg) and 168 (gravid strobila) are from *M. fuliginosus*, and 174 (gross features of strobila) and 166 (scolex osmoregulatory system) are from *N. eugenii.* For the reconstruction of the genital system (Figures 171, 172), the host species was not indicated either in the publication or in unpublished data, and could not be determined with certainty, but neither of the illustrations is likely to be from the type host, *W. bicolor*, based on the provenance of the remaining specimens and are probably also from *M. giganteus.* The original slides of the serial sections could not be located. Some illustrations of the morphological specimens from *W. bicolor* had been made but were not published in the revision of Beveridge (1976); they are included here (Figure 4) together with the amended description.

While the results from the combined 28S and cox1 genetic data clearly distinguish three genotypes, one in *W. bicolor*, a second in *M. fuliginosus*, *M. giganteus* and *N. eugenii*, and a third in *O. robustus*, recognition of these genotypes as independent species is hampered in part by minimal morphological differences and in the case of *O. robustus*, by the lack of adequately preserved material for a detailed morphological examination. Although *O. robustus* occurs across much of inland Australia, *T. undosa* has been found in it only in a restricted area in northern Queensland (Beveridge *et al.* 1998; Beveridge 2020a).

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Comparison of specimens of *T. undosa* from *W. bicolor*, *Macropus* spp. and *N. eugenii* revealed only minor differences, essentially in the average number of testes per set of female genitalia with 1:2.3–2.4 in *W. bicolor* and 1:1.2–2.0 in *M. giganteus*, *M. fuliginosus*, and *N. eugenii*. However, since this ratio was the principal morphological feature differentiating *T. undosa* from the genetically independent species *T. mirabilis*, variation in this character was investigated in greater detail. In Figure 173 of Beveridge (1976), the ratio in a specimen from *M. giganteus* is 2.7 but attempts to confirm this using the type specimens resulted rather in ratios of 1.2 and 1.5 respectively. This difference could be due to the length of strobila examined or the difficulty in identifying poorly staining testes. Two illustrations of the mature region of the strobila of *T. mirabilis* were illustrated by Beveridge (1976), with testis to female genitalia ratios being 1.06 in a region of strobila with about 16 testes (Figure 155) but 1.5 in a shorter region of 12 testes (Figure 156), a figure specifically included to show the extreme range of variation. The text indicates that there was usually one testis per female genital complex, with occasional supernumerary testes. Specimens of *T. mirabilis* from *Petrogale* spp. were re-examined with ratios of 1.0 and 1.2 in *P. lateralis* from two localities, 1.0 in *P. assimilis* and *P. inornata*, and 1.2 in *P. purpureicollis*.

Although a variable character and sometimes not possible to determine in poorly preserved or immature specimens, the difference in the ratio between the number of testes and the number of female genital complexes remains the sole observable character differentiating genotypes. However, together with the genetic data, this appears to warrant the separation of these taxa. It has been expressed here as a ratio between the number of testes compared with the number of female genital complexes over as wide a range of the strobila as possible. On this basis, but together with the genetic data, a description of a new species from *M. giganteus*, *M. fuliginosus*, and *N. eugenii* is described here as *T. macropodis* sp. nov. No material was available for molecular study from the single collection from *O. rufus*. The occurrence of this species in *O. rufus* was at a very low prevalence (0.8% of 115 kangaroos examined) at a single site in New South Wales (Arundel *et al.* 1979) and was not encountered at all in 106 additional kangaroos examined over a wide geographical range by Beveridge (2020b). Its occurrence is probably attributable to a transfer from sympatric *M. fuliginosus*, in which it was also present in considerable numbers at the same locality (Arundel *et al.* 1979). It seems likely that its occurrence in *N. eugenii* is also a case of a host transfer as *N. eugenii* and *M. fuliginosus* occur in close sympatry on Kangaroo Island in South Australia and share a number of parasites (Webley *et al.* 2004).

Triplotaenia macropodis was found commonly in the southern states (Victoria, South Australia, New South Wales), but only a single collection of *Triplotaenia* was made in Queensland at Mingela in *M. giganteus*, from 24 kangaroos examined there (Beveridge *et al.* 1998). This collection was 1,200 km north of the second most northerly collection of *T. macropodis* sp. nov.in *M. fuliginosus* at Bourke in New South Wales. In addition, *Triplotaenia* was also found in *O. robustus* in the same region, also at a low prevalence (7% of 30 *O. robustus*) (Beveridge *et al.* 1998). North Queensland is the only region in Australia in which *T. undosa* occurs in the latter host species (Beveridge 2020a). Given the poor quality of the material available from *M. giganteus* from this region and the inability to provide a reliable description of this species from *O. robustus*, the identification of this specimen is given as *Triplotaenia* sp. Hardman *et al.* (2012) included sequence data of specimens of *T. undosa* from *M. fuliginosus* from Western Australia. However, no voucher specimen was deposited, and without this, Western Australia has been excluded from the distribution of *T. macropodis*, although its occurrence in this region is highly likely. While the description of the new species, *T. macropodis*, is based on minor morphological differences supported by genetic data, the status of specimens from *O. robustus* remains indeterminate and requires additional collections to confirm its identity.

In spite of the abundance of mounted gravid specimens, no pyriform apparatus was detected in the new species. Additional gravid regions of strobilae examined from spirit specimens also failed to provide eggs with a pyriform apparatus although this structure was illustrated by Beveridge (1976) in material from *M. fuliginosus*. No explanation was provided as to how the latter material was obtained, but it may have been from fresh pieces of detached strobilae in the faeces in which additional development of the pyriform apparatus had occurred.

Considerable additional data are needed to resolve the issue of the number of species present in the *T. undosa* species complex, their host distribution, and their morphological differentiation. Molecular data appear to be essential, but in addition, carefully collected and preserved morphological specimens are required to further elucidate the morphological features of the species and to identify additional, reliable morphological characters which align with the molecular data

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Competing interests. The authors declare no conflicts of interest.

Ethical standard. This study was conducted entirely on museum specimens.

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