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Nematode and Acanthocephalan Parasites of Confiscated Sunda pangolins, *Manis javanica* Desmarest, 1822 (Mammalia: Pholidota: Manidae), with an Updated List of the Parasites of Pangolins

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Abstract: Background: The Sunda pangolin, *Manis javanica* Desmarest, 1822, is a critically endangered species of pangolin that occurs from Indonesia to southern China. Knowledge of the biology and ecology of *M. javanica* is limited, however there have been previous reports of parasites, including nematodes, protozoans, ticks, and a cestode. Methods: An illegal shipment of 88 *M. javanica* carcasses, originally collected from wild populations throughout southeast Asia, were intercepted by Hong Kong border authorities (AFCD) and confiscated in 2018. Results: During necropsy, two different types of parasites were collected from four infected pangolins. The parasites were identified as the nematode *Gendrespirura* cf. *zschokkei* (Meyer, 1896) Chabaud 1958, which were embedded in the stomach wall, and the acanthocephalan, Oligacanthorhynchidae sp., collected from the intestine. Morphological descriptions and molecular characterization for each parasite type is provided. Conclusions: In addition, an updated list of parasites from pangolins, incorporating current taxonomic identifications and publications is presented.

Keywords: pangolin; parasites; checklist

check for updates

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1. Introduction

The Sunda pangolin, *Manis javanica* Desmarest, 1822, is one of four species of pangolin that occur in the Asian area, with a distribution from Indonesia to southern China and is listed as critically endangered due, primarily, to hunting for meat and traditional medicines [1,2]. *Manis javanica* is an arboreal, solitary nocturnal animal with a diet dominated by ants and termites [1]. Even with the unfortunate legacy of being the most trafficked animal in the world [1], little information is known about the biology and ecology of most species of pangolin in the wild [2,3].

Although a range of parasites and bacteria have been reported from pangolins [3,4], only five species of nematodes were reported from *M. javanica* by Mohapatra et al. [3] and Wicker et al. [4]: the blood-borne filarial *Brugia malayi* S.L. Brug, 1927 and *Brugia pahangi* (Buckley and Edeson, 1956), the intestinal *Necator americanus* Stiles, 1902, a strongyle-type nematode and a larval *Gendrespirura* sp. However, *Gendrespirura* (originally reported as *Habronema*) *hamospiculata* (Neveu-Lemaire, 1927) had also been reported from *M. javanica* in Vietnam [5] and Borneo [6]. In addition to the nematodes, Mohapatra et al. [3] and Wicker et al. [4] also recorded protozoans (*Babesia* spp. and *Eimeria* spp., including

E. tenggilingi Else and Colley, 1976), bacteria (*Anaplasma pangolinii* Koh, Koh, Panchadcharam, Sitam and Tay, 2016, *Mycoplasma* sp.) and a number of ticks (*Amblyomma cordiferum* Neumann, 1899, *Amblyomma javanense* Supino, 1897, *Amblyomma* (was *Aponomma) variensis* (Supino, 1897)) from *M. javanica*. Since these checklists, a further three reports of parasites and bacteria from *M. javanica* have been published: the tick *A. javanense* transmitting the bacterium *Ehrlichia* spp. [7], the blood-borne protozoan *Babesia* spp. [8], and the intestinal nematode, *Ancylostoma* sp. [9]. Of the intestinal nematodes, *N. americanus* and *Ancylostoma* sp. are common parasites of various mammals, including humans [9,10]. The larval stage of *Gendrespirura* sp. caused polypoid gastritis in a captive *M. javanica* [3,11], and due to being a larval stage, was not able to be identified any further. The adult *G. hamospiculata* described by Hsü [5] were collected from a single *M. javanica* with no record of any associated pathology. The report by Myers and Kuntz [6] contained no information with regard to infection level or effect on the host. Rescued *M. javanica* in southern China have been reported to be infected with ectoparasites and some had evidence of infection with internal parasites, although no identification of the parasites had been attempted [2].

No species of acanthocephalan were listed by Mohapatra et al. [3] and Wicker et al. [4] only included three reports, although there were a number of records of acanthocephalans infecting pangolins [12–14]. *Nephridiacanthus* (originally reported as *Nephridiorhynchus*) *palawanesis* (Tubangui and Masiluñgan, 1938) was originally reported from *M. javanica* in the Philippines [12], however it is now recognised that *M. javanica* does not occur in the Philippines [1], so this record needs to be assigned to the Philippine pangolin, *Manis culionensis* (de Elera, 1915). Two other acanthocephalans, *Nephridiacanthus* (originally reported as *Oligacanthorhynchus*) *gerberi* (Baer, 1959) and *Nephridiacanthus* (originally reported as *Oligacanthorhynchus*) *manisensis* Meyer, 1931, collected from *Smutsia gigantea* (Illiger, 1815) and *Phataginus tricuspis* Rafinesque, 1821, respectively, in Africa were also reported [14]. *Paraprosthenorchis ornatus* Amin, Van Ha and Heckmann, 2008 was described from *Manis pentadactyla* Linnaeus, 1758 collected in northern Vietnam [13]. Subsequent to the checklists there have been additional records of an acanthocephalan (*Intraproboscis sanghae* Amin, Heckmann, Sist and Basso, 2021) from African pangolins [15,16]. However, at this point, there are no records of acanthocephalans infecting *M. javanica*.

An illegal shipment of *M. javanica* carcasses, with origins determined to be from wild populations throughout southeast Asia (based on results provided in Worthington et al. (In Review)), were intercepted by Hong Kong border authorities and the Agriculture, Fisheries and Conservation Department (AFCD) of the Government of the Hong Kong SAR and confiscated in 2018. During necropsy, parasites were found in the intestinal system and are presented here.

2. Materials and Methods

A total of 88 carcasses were intercepted and were frozen until necropsied. At the time of necropsy, it was noted that each body had been descaled and cleaned, with the internal organs removed, bagged and placed inside the abdominal cavity (Figure 1). As the carcasses had been frozen, this prevented histological examination of organs and collection of blood samples; internal organs and the intestinal system were examined grossly. The overall body condition of the pangolins was noted as healthy, with body condition scores of 4/5.

Figure 1. Carcass of pangolin, *Manis javanica*, at time of necropsy; note internal organs in bag in body cavity.

Parasites were collected and preserved in 80% ethanol and sent to the Shamsi Parasitology Laboratory at Charles Sturt University, Australia, for identification.

Parasites were examined under a dissector microscope and identified to type prior to excision of a small piece of tissue for molecular characterisation. The remaining sections of parasites were mounted in lactophenol/glycerol for morphological characterisation.

Measurements were made using a micrometer eyepiece. Photographs were taken using a 9-MP microscope digital camera (AmScope Model MU900, USA). Parasite specimens have been deposited in the Australian Helminthological Collection at the South Australian Museum.

Molecular Analysis

Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Three gene regions were attempted for both species, including small subunit ribosomal RNA (18S rRNA), Internal Transcribed Spacer (ITS) and Cytochrome c oxidase I (*CoxI*). PCR amplification was only successful for the 18S region for Oligacanthorhynchidae sp. and the *CoxI* gene for *Gendrespirura* cf. *zschokkei* using the primer pairs (SSU_F04 GCTTGTCTCAAAGATTAAGCC [17] and 1270R CCGTCAATTCCTTTAAGTTT [18]), and (NTF TGATTGGTGGTTTTGGTAA and NTR ATAAGTACGAGTATCAATATC [19]), respectively. PCR were conducted in a 25 µL system containing 1× buffer, 2.5 mM MgCl, 0.4 µM of each dNTP, 500 nM of each primer and 0.25 µL of GoTaq polymerase (Promega, Madison, WI, USA). PCR cycles were initiated with a 95 °C denaturing for 2 min, followed by 40 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min. The cycle is concluded with a final extension at 72 °C for 10 min. The PCR products were checked by electrophoresis and single band products were sequenced with the same primer at Australian Genome Research Facility (Brisbane). All our sequences were quality checked in the original chromatogram using SeqMan Pro ver. 8.1.0(3) (DNASTAR, Inc.). Primers were removed from the sequences and all mutations were double-checked. Sequences of closely related species were obtained from GenBank and aligned using MAFFT [20]. HKY + G and GTR + G were chosen as the best fit evolutionary model for Oligacanthorhynchidae sp. and *G.* cf. *zschokkei* using Jmodeltest 2 [21]. Phylogenetic trees were inferred using MrBayes [22] with 2,000,000 generations and visualized by Figtree V 1.4.3 [23]. Sequences generated in this study were deposited in GenBank with accession numbers OP605522 (Oligacanthorhynchidae gen. sp.) and OP618835-OP618841 (*G.* cf. *zschokkei*).

3. Results

Two different types of parasites were collected from the intestinal systems of 4 of the 88 pangolin carcasses (Table 1). Two pangolins were infected with nematodes, identified as *Gendrespirura* cf. *zschokkei*, which were collected from the stomach, and two were infected with acanthocephalans, identified as Oligacanthorhynchidae sp., collected from the intestine.

Table 1. Host information. AFCD case reference number for all pangolins: CPM/4/55/18. HKU, Hong Kong University; SPL, Shamsi Parasitology Laboratory.

HKU Number	Geographical Origin [a]	SPL Number	Total Length	Weight	Sex	Parasites (Number Collected)	Location in Host
HKU41-2018	Borneo	1292	100 cm	3.2 kg	Female	Nematodes (4)	Embedded in stomach wall
HKU46-2018	Borneo	1295	134.5 cm	7.6 kg	Male	Acanthocephala (3)	Faecal extrusion
HKU51-2018	Borneo	1293	113.5 cm	4.16 kg	Female	Nematodes (5)	Stomach
HKU74-2018	Java, Indonesia	1294	95.5 cm	3.1 kg	Female	Acanthocephala (3)	Small intestine

[a] Origin determined via population genomics study presented in Worthington et al. (In Review).

Phylum Nematoda
Family Habronematidae Ivaschkin, 1961
Genus *Gendrespirura* Chabaud, 1958
Gendrespirura cf. *zschokkei* (Meyer, 1896) Chabaud 1958
Host: *Manis javanica* Desmarest, 1822
Locality: Southeast Asia: Borneo
Site of infection: Stomach
Museum specimens: Voucher specimens: AHC 49285-49286
Description (Figures 2 and 3; Table 2)

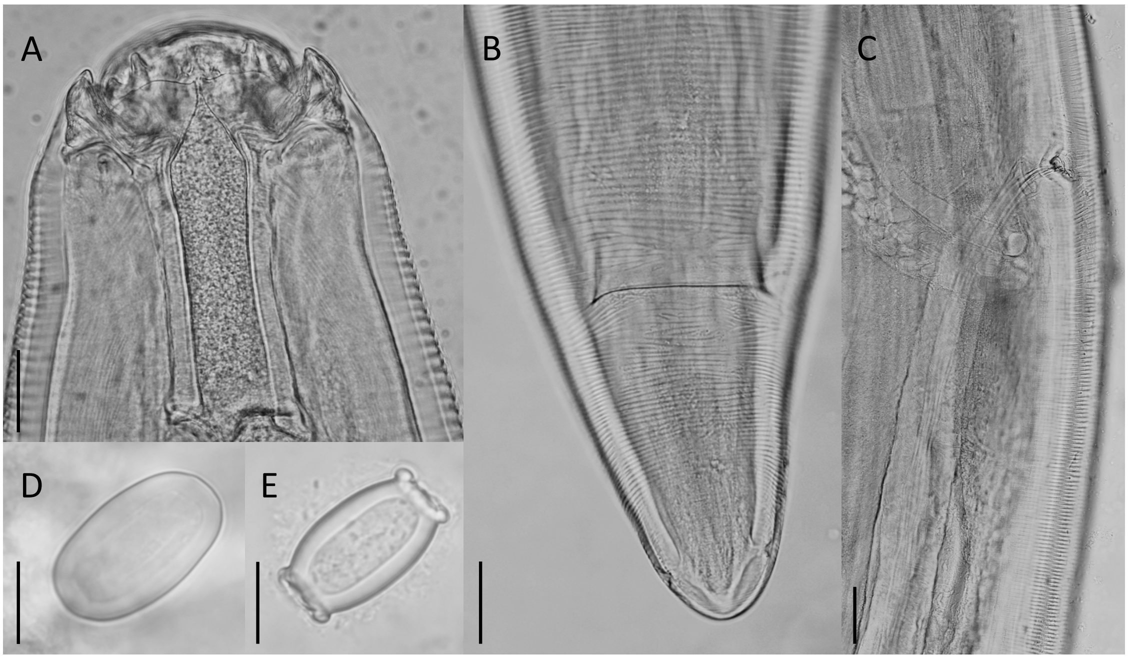

Figure 2. *Gendrespirura* cf. *zschokkei* collected from *Manis javanica*. Female specimens. (**A**) Anterior end, showing teeth on lateral lips and chitinised vestibule, median view. (**B**) Posterior end, showing anus and caudal papillae, ventral view; note striated cuticle. (**C**) Vulva opening with vagina running posteriorly, lateral view. (**D**) Immature egg, dissected from uterus. (**E**) Mature egg, showing characteristic barrel-shape, dissected from uterus. Scale bars: (**A**) 50 µm. (**B**) 50 µm. (**C**) 100 µm. (**D**) 20 µm. (**E**) 20 µm.

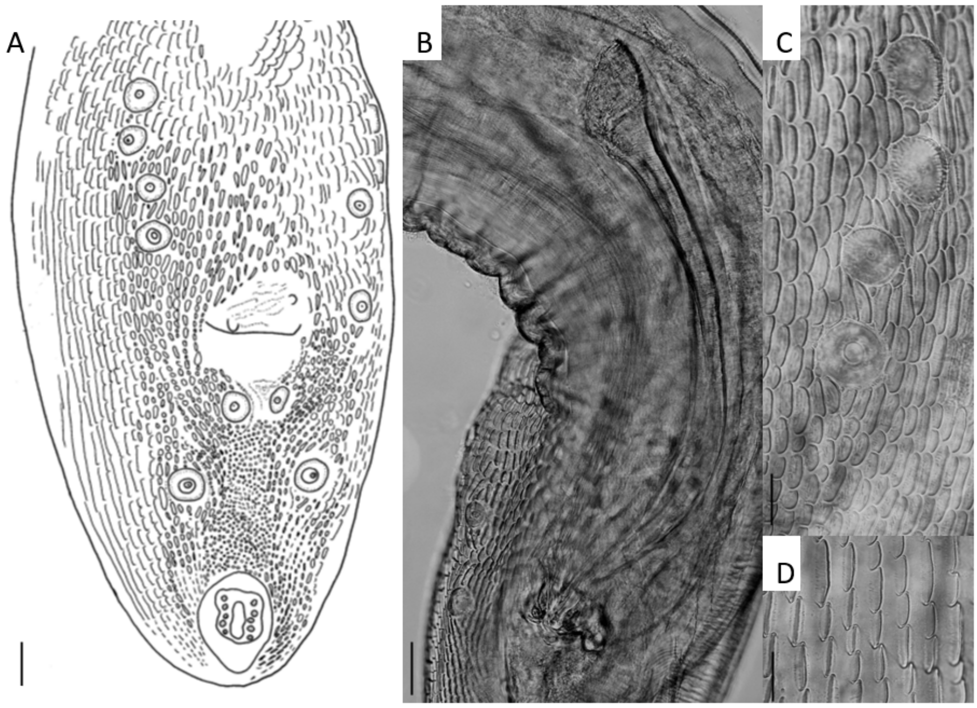

Figure 3. *Gendrespirura* cf. *zschokkei* collected from *Manis javanica*. Male specimen. (**A**) Line drawing of posterior end, showing caudal alae, pre- and post-cloacal papillae, pattern of ventral ridges and cloaca, ventral view. (**B**) Photomicrograph of right spicule in situ, ventral view. (**C**) Photomicrograph of pre-cloacal papillae. (**D**) Photomicrograph of ridges on ventral surface of nematode, anterior to cloacal region. Scale bars: (**A**) 50 µm. (**B**) 50 µm. (**C**) 25 µm. (**D**) 20 µm.

Table 2. Measurements of female *Gendrespirura* cf. *zschokkei* collected from *Manis javanica* in this study compared to previous reports of specimens collected from various pangolins. Species names listed by their original described name; currently all species are considered within *Gendrespirura*. Measurements are presented in micrometers.

Original Identification	*Gendrespirura* cf. *zschokkei*	*Habronema hamospiculatum*	*Protospirura hamospiculata*	*Filariazschokkei*	*Chenospirura kwangtungensis*	*Gendrespirura* sp.
Host	*Manis javanica*	*Manis javanica*	*Smutsia temminckii*	*Manis crassicaudata*	*Manis pentadactyla*	*Manis crassicaudata*
Location	Malaysia	Vietnam	Central African Republic	Sri Lanka	China	Sri Lanka
Reference	This study	Hsü [5]	Neveu-Lemaire [24]	Meyer [25]	Kou [26]	von Linstow [27]
Body length	17,685 (7525–25,375) Mature only: 23,662.5 (21,950–25,375)	15,700–23,800	18,000–23,000	32,000 (25,000–35,000)	25,810–29,510	32,000
Body width	453.1 (337.5–550)	500–800	590–680	1300	770–885	950
No. pairs of teeth on pseudolabia	3	3	7 [a]	-	7	Not stated
Nerve ring to anterior end	400 (350–450)	260–310	350–400		330–370	
Cervical papillae to anterior end	292.5 (230–390)				265–306	
Excretory pore to anterior end	540 (250–900)		402			
Vestibule length	120 (105–135)	150–180	130–150	114		200
Oesophagus length	3940 (2430–5100)	3800–3920	3800–4300	~6400 (5000–7000)	4500–5370	9143 [b]
Vulva to anterior end	6243.3 (5180–7850)	5380 (in 22,000 specimen)	9000	8000 (1/4 body length)	7350–7750	Behind mid-body (18,667 [b])
Egg length × width	Mature: 46.7 (45–47.5) × 25.4 (25–26.25) Immature: 35.6 (32.5–37.5) × 22.8 (21.25 × 25)	42–43 × 24–26	44–46 × 23–24	43 × 23–34	46–47 × 26–27	47 × 29
Tail length	233.8 (155–305)	280–440	250–300	330–380		485 [b]

[a] Specimens re-examined by Baylis [28]. [b] Figures calculated from data presented in [27].

General. Body stout, medium-sized. Cuticle faintly transversely striated along entire body length (Figure 2B). Lateral alae absent. Cephalic region (Figure 2A) with two large lateral pseudolabia, each with three teeth on inner surface; central tooth more prominent than lateral teeth; two small lips; single pair subventral amphids. Buccal capsule cylindrical, well-developed, strongly chitinised. Oesophagus long, with shorter narrow muscular portion and longer wider glandular portion. Cervical papillae anterior to nerve ring; excretory pore posterior to nerve ring.

Female (based on 2 mature specimens and 4 immature specimens; Figure 2): Vulva in anterior part of body; small circular opening (Figure 2C). Vagina backwards from vulva with single uterus in most of body space. Mature eggs small, with thick wall, polar ends with thickened cuticle forming raised hoops, giving a barrel-like appearance (Figure 2E). Immature eggs without thickened hoops (Figure 2D). Tail curved, with blunt rounded end; phasmids near tip (Figure 2B); ratio of tail length to total length 0.016 (0.013–0.021)

Male (based on a single specimen; Figure 3): Posterior extremity spirally coiled, caudal alae well developed, symmetrical. Four pairs of preanal pedunculate papillae (Figure 3C), 1 pair of preanal sessile papilla, and 2 pairs post-anal papillae (Figure 3A). Pre-anal and post-anal pedunculate papillae approximately equal in size, 32 (28.25–35) µm long by 31 (27.5–37.5) µm wide. Spicules uneven (Figure 3B); left spicule very long and slender with terminal hook (ratio of length of spicule to total length 0.254); right spicule short and stout (ratio of length of spicule to total length 0.054); ratio of length of left spicule to right spicule 4.69. Gubernaculum present. Cluster of 5 pairs of small papillae present at posterior extremity (Figure 3A). Posterior papillae 7.5 µm long. Cloacal region, and ventral surface of body immediately anterior, covered with small cuticular elongate ridges or bosses (Figure 3D); post-cloacal median region covered with small cuticular rounded bosses.

Molecular characterization: Only Cox1 sequences were successfully obtained from seven specimens of *G.* cf. *zschokkei*. The length of the Cox1 sequences is 649 bp. The intraspecific variation ranged from 0–0.22% among the sequences. There are no other sequences for species of *Gendrespirura* available in GenBank. *Gendrespirura* cf. *zschokkei* sequences formed a highly supported and distinct group in the phylogenetic tree (Figure 4A) and were closely related to *Tetrameres grusi* (Shumakovich, 1946), collected from red-necked cranes in China [29] within a clade of species of *Habronema* Diesing, 1861.

Table 3. Measurements of male *Gendrespirura* cf. *zschokkei* collected from *Manis javanica* in this study compared to previous reports of specimens collected from various pangolins. Species names listed by their original described name; currently all species are considered within *Gendrespirura*. Measurements are presented in micrometers.

Original Identification	***Gendrespirura* cf. *zschokkei***	***Habronema hamospiculatum***	***Protospirura hamospiculata***	***Filaria zschokkei***	***Chenospirura kwangtungensis***	***Gendrespirura* sp.**
Host	*Manis javanica*	*Manis javanica*	*Smutsia temminckii*	*Manis crassicaudata*	*Manis pentadactyla*	*Manis crassicaudata*
Location	Malaysia	Vietnam	Central African Republic	Sri Lanka	China	Sri Lanka
Reference	This study	Hsü [5]	Neveu-Lemaire [24]	Meyer [25]	Kou [26]	von Linstow [27]
Body length	13,375	11,400–16,100	15,000–17,000	21,000 (19,000–24,000)	18,250–22,380	25,000
Body width	500	380–590	525–570	1100	664–758	710
No. pairs of teeth on pseudolabia	3	3	7 [a]	-	7	Not stated
Nerve ring to anterior end		230–250	325		324–367	
Cervical papillae to anterior end	230				265–278	
Excretory pore to anterior end			230			
Buccal cavity length	130	132–145	130	114		200
Oesophagus length	3800	3240–3900	3300–3700	~4200 (3800–4800)	3337–4651	8333 [b]
Left spicule length	3400	3170–3450	2300	4700–5300	3010–3230	3740
Right spicule length	725	550–620	600	1800–2000	650–710	570
Gubernaculum length	110	70				
Tail length	370	360–450	370	490		481

[a] Specimens re-examined by Baylis [28]. [b] Figures calculated from data presented in [27].

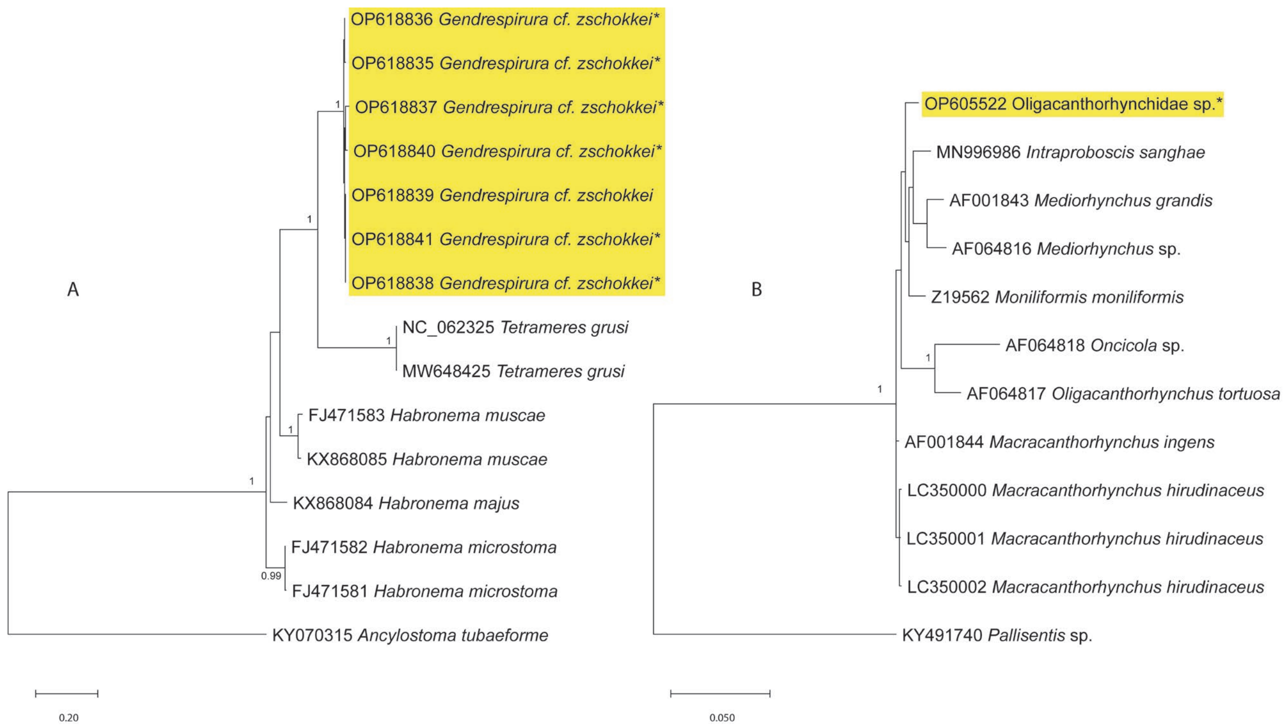

Figure 4. Phylogenetic placement of *Gendrespirura* cf. *zschokkei* (**A**) and Oligacanthorhynchidae sp. (**B**) based on *Cox1* and 18S rRNA sequences, respectively. Branch supports (shown as posterior probabilities > 0.9) are indicated on the branches. Sequences generated in this study are highlighted and indicated with an *.

Phylum Acanthocephala
Family Oligacanthorhynchidae Southwell and Macfie, 1925
Oligacanthorhynchidae sp.
Host: *Manis javanica* Desmarest, 1822
Locality: Southeast Asia: Borneo and Java
Site of infection: Intestine
Museum specimens: Voucher specimens: AHC 49287-49288
Description (Figure 5)

General. Trunk long, slender, anterior end narrower than posterior end, usually fixed with numerous transverse wrinkles. Proboscis subspherical (Figure 5A), 300 long, 340 wide. Proboscis hooks arranged in 5 longitudinal rows of 6 hooks each, giving about 30 hooks in total. Hooks gradually decreasing in size from anterior to posterior with anterior hook 60 long, posterior hook 40 long. Hooks, not barbed, with flange giving hook overall globular appearance, in papillae in proboscis (Figure 5B).

Male specimens not collected.

Female (based on 2 mature specimens and 2 immature specimens): Trunk > 20 cm long, 3–5 mm wide. Uterine bell present. Mature eggs dissected from body oval, 72.5 (65–80) µm long by 45 (50–50) µm wide (Figure 5D).

Molecular characterization: Only 18S sequence was obtained from a single specimen of Oligacanthorhynchidae sp. The length of the 18S sequences was 1022 bp. The phylogenetic tree placed the sequence obtained in this study as basal to a group containing a number of Archiacanthocepahalans, including *I. sanghae*, collected from the African pangolin, *Phataginus tetradactyla* (Linnaeus, 1766) [16] (Figure 4B). However, the support for the groupings was not high, and the phylogenetic relationship among the species within the family are not resolved.

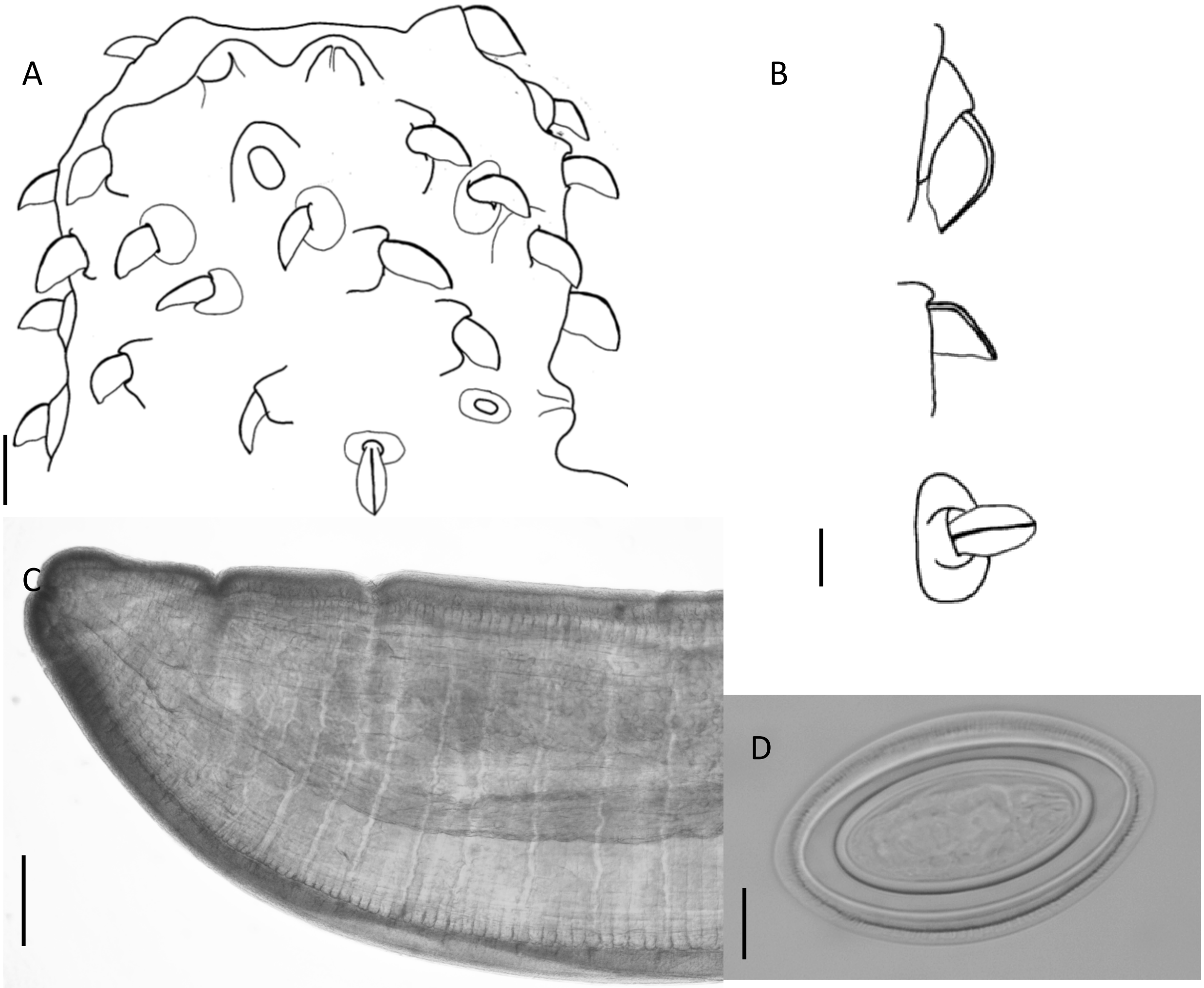

Figure 5. Oligachanthorhynchidae sp. collected from *Manis javanica*. Female specimens. (**A**) Line drawing of roboscis showing pattern of hooks. (**B**) Line drawing of hooks on proboscis; bottom hook is flattened against the proboscis. (**C**) Photomicrograph of posterior end, showing uterine bell. (**D**) Photomicrograph of egg dissected from body. Scale bars: (**A**). 40 µm. (**B**). 40 µm. (**C**). 500 µm. (**D**). 20 µm.

4. Discussion

This study presents two species of intestinal helminths from the critically endangered *M. javanica* from Southeast Asia which updates the number of intestinal helminths reported from this host to nine [3,5,6,9,30].

Habronematoid nematodes have been collected from various species of pangolin under a variety of generic names. The nematode *Filaria zschokkei* Meyer, 1896 was collected from the intestinal system of *M. pentadactyla* collected in Sri Lanka [25] with notes of an earlier finding of a "vast number of the *Ascaris* genera" present within a stomach cyst (by Jansson, 1830 in [25]). This record was subsequently referred to as *Ascaris manidis* Diesing, 1851, but this was refuted as, without a formal description, whether the nematodes were ascarids or habronematids remained unknown [28,31]. *Spiroptera orca* von Linstow, 1906 was subsequently reported from the stomach of *M. pentadactyla*, also collected in Sri Lanka [27], with an immature *F. zschokkei* from the peritoneum of *M. pentadactyla* also listed (Esslinger [32], however, stated that von Linstow [27] examined specimens that had been collected by Meyer [25], so this was not a new record). Esslinger [32] stated that the specimens of *F. zschokkei* collected by Meyer [25] were more likely a spiruroid, not a filaroid, based on the description, but did not officially transfer the species. Nematodes collected from Temminck's pangolin, *Smutsia temminckii* (Smuts, 1832), in Africa, were originally referred to *S. orca* (Monnig (1924) in [28]). Subsequently, however, with fresh collections, nematodes collected from *S. temminckii* were determined to be a different species, *Protospirura hamospiculata* Neveu-Lemaire, 1927 [24]. All these nematodes listed above were then referred to the genus *Habronema* [28]. The African specimens were considered to be the single species, *Habronema hamospiculatum* (Neveu–Lemaire, 1927), while the specimens reported by von Linstow [27] (Baylis [28] did not refer to the specimens from Meyer [25]) were also considered to belong to the same genus, despite a very different described egg morphology. The eggs for *H. hamospiculatum* were slightly asymmetrical and flattened at the poles [28], whereas the eggs for the von Linstow [27] specimens had thickened ridges at the polar ends of the eggs, forming raised hoops and giving a "barrel-like" appearance. Specimens of *H. hamospiculatum* from *M. javanica* were collected from the area of Ho Chi Minh City, Vietnam [5], with differences from the description provided for the African species [28] noted with regard to the teeth morphology and location of vulva opening. *Habronema hamospiculatum* was also reported from *M. javanica* collected in Borneo with no description provided [6]. Nematodes collected from *P. tricuspis* in Africa were named *Habronema congolese* Vuylsteke, 1936 but were found to be identical to the specimens collected by Neveu-Lemaire [31]. Baylis [33], once aware of the specimens from Meyer [25], subsequently referred all the Asian specimens to *Habronema zschokkei* (Meyer, 1896). Similar barrel-shaped eggs had been reported [25] and were also reported for nematodes collected from *M. pentadactyla* in southern China, with the description of *Chenospirura kwangtungensis* Kou, 1958 [26]. The genus *Gendrespirura* Chabaud, 1958 was erected for species with an elongated buccal capsule and two strong median teeth that parasitised toothless mammals (pangolins and aardvarks) [27]. *Gendrespirura hamospiculata* (Neveu-Lemaire, 1927) was designated as the type species, although the validity of the species was questioned due to inconsistencies in the description of the tip of the left copulatory spicule and the observation of barrel-like eggs in nematodes collected from an African pangolin [31]. As the description of the eggs was the character differentiating the African and Asian species [28], this was considered no longer valid as a differentiating characteristic, but the species were not formally synonymized [31]. *Chenospirura kwangtungensis* was not included in the initial generic description [31], but was later included within the synonymies of *Gendrespirura* [34]. Only one species within the genus *Gendrespirura* is currently listed [35], but without details or explanation. However, if the species are to be synonymized, *G. zschokkei* (Meyer, 1896) should be the designated species due to the International Code of Zoological Nomenclature Principle of Priority. Consequently, the specimens collected in this study are referred to *G.* cf. *zschokkei*.

We concur that *Gendrespirura* is a valid genus, however further research is required to re-examine the various "species" of nematodes described from the different host species in their geographical locations. For example, many of the Asian specimens were described from *M. pentadactyla* in Sri Lanka [25,27]. This species of pangolin is no longer recognised in Sri Lanka [1] and these records must now be referred to the Indian pangolin, *Manis crassicaudata* E. Geoffroy, 1803. Similarly, although the overall body measurements of the specimens collected in this study matched those for the *H. hamospiculatum* described by Hsü [5], the measurements for the left spicule were much larger than for the description of *P. hamospiculata* [24] (Table 3). Although this is not enough to justify the erection of a new species, the potential for different species of nematodes to be infecting the different species of pangolins is a possibility (see Tables 2 and 3 for comparison of measurements across the different hosts), and future research may determine the nematodes in *M. javanica* to be a new species. Future research should attempt to obtain molecular sequences for specimens from all pangolin hosts across a broad geographic distribution to determine if there is only the one species infecting all. At the present time the only molecular sequences for a species of *Gendrespirura* are those obtained in this study. Given the possible pathological implications of infection with these nematodes [11], a better understanding of the actual species infecting the hosts is required.

The molecular results obtained placed the specimens sequenced in this study as sister to species of *Tetrameres* Creplin, 1846 within a clade of species of *Habronema*. However, without sequences from other habronematid nematodes collected from pangolins a more detailed phylogenetic analysis is not possible. The life cycle of *Gendrespirura* has not yet been documented [36]. However, the life cycles of species of *Habronema* involve transmission by muscid flies, with infective larvae being released onto the host's face (e.g., near the mouth or nose) as the fly feeds [36]. From there, the larvae move into the mouth, are swallowed and develop within the stomach of the host, where females occur in small tumours in the stomach wall [36], similar to previous reports [11] and what was found in this study. The life cycle of terrestrial species of *Tetrameres*, the genus which grouped with *G.* cf. *zschokkei* in the phylogenetic tree, utilises insects as intermediate hosts, which are ingested by the definitive hosts [36]. Similar to the other genera, species of *Tetrameres* embed in gastric glands within the proventriculus of their bird hosts [36]. Given the predominant ant and termite diet of pangolins [1], it is possible that these are the intermediate hosts; however, given the low occurrence of these parasites (for example, only 2 of 88 *M. javanica* were infected), the intermediate hosts may be other insects which are incidentally ingested [37] or are muscid flies as for *Habronema*.

Acanthocephalans have been infrequently reported from pangolins, with all but one species belonging to the family Oligacanthorhynchidae [13,15], but none were listed in the checklist of Mohapatra et al. [3]. Within Asia, the two reported acanthocephalan parasites, *N. palawanensis* from *M. culionensis* [12] and *P. ornatus* from *M. pentadactyla* in Vietnam [13], are both within the family Oligacanthorhynchidae, as are the specimens collected in this study. However, due to the condition of the specimens collected (faecal extrusion and pulled from the small intestine) and the lack of male specimens, combined with the unique structure of the proboscis hooks, they cannot be identified to any genus so far described within the Oligacanthorhynchidae [38]. Acanthocephalans within this group are renowned for penetration of the intestinal wall, causing secondary septic peritonitis [16]. Given the diet of pangolins, it is not surprising that they are infected with acanthocephalans which are known to utilise insects, such as ants and termites, as intermediate hosts [13].

The molecular results found in this study suggested support for previous phylogenetic analyses of acanthocephalans [39,40], finding the representatives of the Archiacanthocephala to be monophyletic. However, the values of support for the various branches were low. The results of our analyses, however, did show that the Oligacanthorhynchidae sp. collected from *M. javanica* was separate to all other available sequences. The only other acanthocephalan from a pangolin that has been sequenced, *I. sanghae* [16] to be closest to *Moniliformis moniliformis* (Bremser, 1811), collected from a laboratory rat [41], which was supported in the phylogenetic analyses in this study. As for the nematodes, future research should concentrate on the collection of molecular sequences across a range of genes to determine the range of acanthocephalan species across the various host species.

Wicker et al. [4] found that the prevalence of parasites in recently capture pangolins is extremely high, causing fatalities in debilitated animals. The low level of infection with parasites found in this study should be treated with caution as the history of the animals prior to examination is not known. It is possible that pangolins are kept in captivity prior to their illegal trade, either as live or dead animals [1], thus it is possible that, through a combination of time in captivity, change in diet and/or other physiological factors (e.g., stress) that the parasite levels found are not reflective of wild populations. Additionally, due to the treatment of the carcasses prior to necropsy, a full necropsy (including histological examination and blood samples) could not be performed. Thus, it is very likely that a number of parasites may have been overlooked during the examination.

Helminth Parasites of Pangolins: An Updated List of Species

The taxonomic history of most parasites reported from pangolins is complicated, especially with changes in host taxonomy. As such, some of the parasite taxonomy presented in Mohapatra et al. [3] and Wicker et al. [4] is incorrect. Although not directly related to this study, we have included them here to ensure that knowledge regarding the parasites of pangolins is as up to date as possible (Table 4; Supplementary Table S1) and to attempt to unravel some of the complex, and complicated, taxonomy that researchers may come across.

Table 4. An updated checklist of helminths and pentastomes of pangolins. Note that the protozoans, ectoparasites and bacteria listed in Mohapatra et al. [3] and Wicker et al. [4] have not been included in this update. If there are no changes from the list as presented in Mohapatra et al. [3] and/or Wicker et al. [4], that is the reference listed; see Mohapatra et al. [3] and/or Wicker et al. [4] for the specific references to those parasites.

Pangolin Host (Scientific Name)	Parasite Type	Parasite Species	References
Asian pangolins			
Chinese pangolin (*Manis pentadactyla*)	Acanthocephala	*Paraprosthenorchis ornatus*	Amin et al. [13]
	Cestoda	*Raillietina* sp.	Tuli et al. [42]
	Nematoda	*Acanthocheilonema (Chenofilaria) fausti* (Esslinger, 1966)	Chabaud and Bain [43]
		Ancylostoma sp.	Wicker et al. [4]
		Capillaria spp.	Wicker et al. [4]
		Cylicospirura sp.	Mohapatra et al. [3], Wicker et al. [4]
		Gendrespirura zschokkei	Chabaud [31]
		Necator americanus	Mohapatra et al. [3], Wicker et al. [4]
		Strongyle type	Wicker et al. [4]
		Strongyloides spp.	Wicker et al. [4]
		Trichochenia cantonensis	Chabaud et al. [44]
		Trichochenia manisa	Chabaud et al. [44]
		Trichochaenia meyeri	Chabaud et al. [44]
		Trichochenia papillosa	Chabaud et al. [44]
Indian pangolin (*Manis crassicaudata*)	Cestoda	*Diorchiraillietina contorta*	Baer and Fain [45], Yamaguti [46]
		Echinococcus sp.	Mohapatra et al. [3], Wicker et al. [4]
	Nematoda	*Gendrespirura zschokkei*	Chabaud [31]
		Leipernema leiperi	Singh [47]
		Necator americanus	Mohapatra et al. [3]
		Trichochenia meyeri	Mohapatra et al. [3], Wicker et al. [4]
		Trichochenia mucronata	Singh [48], Chabaud et al. [44]
Philippine pangolin (*Manis culionensis*)	Acanthocephala	*Nephridiacanthus palawanesis*	Tubangui and Masiluñgan [12]
Malayan/Sunda pangolin (*Manis javanica*)	Acanthocephala	*Oligacanthorhynchidae* gen. sp.	This study
	Cestoda	Cestode sp.	Zhang et al. [49]
		Diorchiraillietina contorta	Baer and Fain [45], Yamaguti [46]
		Raillietina (Paroniella) bovieni	Baer & Fain [45]

Table 4. *Cont.*

Pangolin Host (Scientific Name)	Parasite Type	Parasite Species	References
	Nematoda	*Ancylostoma* sp.	Tuli et al. [9]
		Brugia malayi	Mohapatra et al. [3], Wicker et al. [4]
		Brugia pahangi	Mohapatra et al. [3], Wicker et al. [4]
		Gendrespirura zschokkei	Chabaud [31], This study
		Gendrespirura sp.	Mohapatra et al. [3]
		Necator americanus	Mohapatra et al. [3], Wicker et al. [4]
		Strongyle type	Wicker et al. [4]
	Pentastomida	Pentastomida sp.	Zhang et al. [49]
African pangolins			
Giant ground pangolin (*Smutsia gigantea*)	Acanthocephala	*Nephridiacanthus gerberi*	Baer [50]
	Cestode	*Baerfinia anoplocephaloides* (Baer and Fain, 1955)	Baer and Fain [45], Yamaguti [46]
		Hymenolepis manidis	Baer and Fain [45]
		Metadavainea aelleni	Baer and Fain [45]
	Nematoda	*Ancylostoma sp.*	Mohapatra et al. [3], Wicker et al. [4]
Temminck's ground pangolin (*Smutsia temminckii*)	Cestoda	*Inermicapsifer rhodiensis*	Mettrick [51]
		Metadavainea sp.	Okonkwo and Okaka [52]
		Oochoristica sp.	Ugiagbe and Awharitome [53]
	Nematoda	*Gendrespirura hamospiculata*	Chabaud [31]
		Parastrongyloides sp.	Wicker et al. [4]
		Strongylidae sp.	Ugiagbe and Awharitome [53]
		Strongyle type	Wicker et al. [4]
	Pentastomida	*Armillifer* sp.	Ugiagbe and Awharitome [53]
Tree/White-bellied pangolin (*Phataginus tricuspis*)	Acanthocephala	*Macracanthorhynchus* sp.	Amin et al. [15]
		Nephridiacanthus manisensis	Amin et al. [15]
		Oncicola sp.	Amin et al. [15]
	Cestoda	*Baerfinia anoplocephaloides*	Baer and Fain [45], Yamaguti [46]
		Cestodes (unidentified)	Chabaud et al. [44]
		Metadavainea aelleni	Baer and Fain [45]
		Metadavainea sp.	Wicker et al. [4]
		Raillietina (Raillietina) rahmi	Baer and Fain [45]
	Nematoda	*Ancylostoma* sp.	Wicker et al. [4]
		Gendrespirura hamospiculata	Chabaud [31]
		Microfilaria lukakae Pais Caeiro, 1959	Wicker et al. [4]
		Microfilaria lundae Pais Caeiro, 1959	Wicker et al. [4]
		Microfilaria nobrei Pais Caeiro, 1959	Wicker et al. [4]
		Microfilaria vilhenae Pais Caeiro, 1959	Wicker et al. [4]
		Parastrongyloides sp.	Wicker et al. [4]

Table 4. *Cont.*

Pangolin Host (Scientific Name)	Parasite Type	Parasite Species	References
		Trichochenia armata	Chabaud et al. [44]
		Trichochenia conincki	Chabaud et al. [44]
		Trichochenia manidis	Chabaud et al. [44]
		Trichochenia rousseloti	Chabaud et al. [44]
	Pentastomida	Unidentified pentastome	Wicker et al. [4]
Black-bellied pangolin (*Phataginus tetradactyla)*	Acanthocephala	*Intraproboscis sanghae*	Amin et al. [15]

Nematodes of the superfamily Trichostrongyloidea have been reported from a number of pangolins under a variety of generic names. *Strongylus costatus* Meyer, 1896, was originally reported from *M. pentadactyla* in Sri Lanka [25]. Following collection of specimens in China, this was subsequently referred to *Trichoskrjabinia costata* (Meyer, 1896) [54]. However, as the genus *Trichoskrjabinia* Travassos, 1937 was a reptile-specific nematode, this was later referred to *Manistrongylus meyeri* [55]. Prior to that, however, *Trichochenia mucronata* Singh, 1958, was described from a pangolin in India [48]. At the same time, reference was made to three species collected from *M. pentadactyla* in China: *T. cantonensis* Kou, 1958, *T. papillosa* Kou, 1958 and *T. manisa* Kou, 1958, although the third species was considered a synonym of *T. cantonensis* [48]. It was also suggested that *S. costatus* was most likely a species of *Trichochenia* Kou, 1958, but further work was required to confirm this [48]. Two trichostrongyles, *Manistrongylus manidis* Baer, 1959 and *Pholidostrongylus armatus* Baer, 1959, were subsequently described from *P. tricuspis* in the Democratic Republic of Congo [50]. *Trichochenia conincki* Chabaud, Bain and Puylaert, 1967 was then described based on a single male specimen collected from a *P. tricuspis* in Gabon in a co-infection with *T. rousseloti* Biocca, 1959, both of which were found in faecal material attached to the external surface of cestodes [44,56]. Additional specimens collected in Sri Lanka (Naidu and Naidu, 1981 in [4]) were referred to *Trichochenia meyeri* (Travassos, 1937). Durette-Desset [57] listed the genera *Manistrongylus* (there were two separate descriptions for this genus by Baer, 1959 [50] and Cameron and Meyers, 1960 [55]; both were included within the synonymy) and *Pholidostrongylus* Baer, 1959 as synonyms of *Trichochenia*. Subsequently, 5 species of *Trichochenia* were listed from Asian pangolins (*T. meyeri*, *T. mucronata*, *T. cantonensis*, *T. manisa* and *T. papillosa*) and 4 species from African pangolins (*T. manidis* (Baer, 1959), *T. armata* (Baer, 1959), *T. rousseloti* and *T. conincki*) [44]. *Mainspinostrongylus* Kalyanker and Palladwar, 1989 was erected for nematodes collected from "*M. crassifialia*" in India [58]; this is a misspelling of the host species and should be corrected to *M. crassicaudata*. *Mainspinostrongylus* was moved, due to its similarity with *Trichochenia*, into the family Molineidae within the Trichostrongyloidea [58]. *Mainspinostrongylus* is currently listed with 1 valid species, *M. crassifialia*, see [59] (within the subfamily Trichostrongylinae) and *Trichochenia* is currently listed with only 3 valid species (*T. costata*, *T. meyeri*, and *T. rousseloti* (see [60]) (within the subfamily Molineinae) within the family Trichostrongylidae [35]. No information on the fates of the other species could be found. Thus, with regard to the records presented by Mohapatra et al. [3], Mohapatra et al. [61] and Wicker et al. [4], the records of *Manistrongylus meyeri* from *M. pentadactyla* and *T. meyeri* from *M. crassicaudata*, should both be referred to as *T. meyeri*, following the current taxonomic knowledge. The other species of *Trichochenia* have been listed in Table 4 until knowledge of their true taxonomic status is found. It is apparent, however, that the trichostrongyloid fauna of pangolins needs urgent re-examination, with new specimens collected for molecular characterisation in combination with morphological measurements to ensure an accurate understanding of the number and distribution of species.

Chenofilaria filaria Kou, 1958 was described from *M. pentadactyla* in China [53,61]. *Dipatelonema fausti* Esslinger, 1966 was subsequently reported from the same host species, also in China (as listed in [3]), but there was no mention of the earlier description. Both species were subsequently synonymised and placed within *Acanthocheilonema* (*Chenofilaria*) Cobbold, 1870, which comprised of species described from pangolins and Australian marsupials [43].

Leipernema leiperi Singh, 1976 was originally described from a *M. pentadactyla* that had died in captivity in India [47]. As for *T. mucronata* [48], the origin of the pangolin was not stated but the collection locality was given as Hyderabad and this record has been referred to *M. crassicaudata*. The report of *Strongyloides* sp. attributed to Singh [47] by Mohapatra et al. [3] is actually a misrepresentation of the information provided where the specimens were referred to as resembling *Strongyloides* sp. [47]. Wicker et al. [4] did not refer to the record of *Strongyloides* sp. (and mis-referenced the *L. leiperi* record as Singh (2009)) but did refer to a report as "*Strongyle* type" [62] which is how it is referred to in Table 4.

The only cestode listed by Mohapatra et al. [3] was the larval stage of *Echinoccocus* sp. infecting *M. crassicaudata*. Wicker et al. [4] listed a number of cestodes infecting pangolins, although with some incorrect taxonomy and host–parasite records as described below.

The taeniid cestode, *Davainea contorta* Zschokke, 1895, was reported as a parasite of *M. crassicaudata* (as *M. pentadactyla*) in Sri Lanka [25]. This species was subsequently transferred to the genus *Raillietina* Fuhrmann, 1920 and reported from *M. javanica* [45]. The genus *Diorchiraillietina* Yamaguti, 1959 was erected with a single species, *D. contorta* (Zschokke, 1895), within the Family Davaineidae [63], for these records. *Diorchiraillietina contorta* was also reported in *M. javanica* collected from Malaysia [30].

Five species of cestodes collected from African pangolins were described: *Raillietina bovieni* Baer and Fain, 1956 from *M. javanica* from Indonesia; *Raillietina anoplocephaloides* Baer and Fain, 1956 from *P. tricuspis* and *S. gigantea* from Ivory Coast, Rwanda and Burundi; *R. rahmi* Baer and Fain, 1956 from *P. tricuspis* from Ivory Coast; *Metadavainea aelleni* Baer and Fain, 1956 from *P. tricuspis* and *S. gigantea* from Ivory Coast, Rwanda and Burundi; and *Hymenolepis manidis* Baer and Fain, 1956 from *S. gigantea* from Rwanda and Burundi [45]. The Ivory Coast specimens were provided by Rahm [64] to Baer and Fain [45] for description and have mistakenly been attributed to infecting *P. tetradactyla* by Wicker et al. [4]. The genus *Baerfainia* Yamaguti, 1959 was erected with the single species *B. anoplocephaloides* to replace *R. anoplocephaloides* [46]; the possibility that this species belonged to a different genus had been flagged [45], due to differences in the morphology of the scolex, but the differences were not considered sufficient at the time. *Baerfainia* has been transferred to different families based on its unusual reproductive structures [65] but has been returned to the family Davaineidae due to its close resemblance to the other cestodes described from pangolins [63,65]. The genus *Manitaurus* Spasskaya and Spasski, 1971 was erected for *R. rahmi* [65]. Jones and Bray [65] refuted a synonymy of this genus with *Diorchiraillietina* (by Schmidt in 1986) due to differences in the number of eggs per segment and confirmed *Manitaurus* as a valid genus (see [63]).

Specimens of a *Metadavainea* sp. were collected from *S. teminckii* in Nigeria with a 38% infection across six pangolins, and a mean intensity of 22 cestodes per infected host animal [52]. A novel species of *Raillietina* was also reported [42] from *M. pentadactyla* in China; no morphological description was provided. This was the first molecular sequence provided for a cestode collected from a pangolin, which confirmed its placement within the family Davaineidae.

Inermicapsifer rhodiensis Mettrick, 1959 (family Anoplocephalidae) was described from *P. temminckii* in Zimbabwe [51] but this report has not beein included in either of the previous checklists. Chabaud et al. [44] also reported cestodes in *P. tricuspis* in Gabon, Africa (the nematodes that were described were originally found in faecal matter attached to the cestodes); however, no name or description of the cestodes was provided.

Although reporting parasites from *M. javanica*, Zhang et al. [49] did not provide identifications. They did, however, provide photographs which could be identified to parasite type: the intestinal parasite (Figure 5A in 2) was a cestode (identifiable by the body segmentation) and the mesenteric parasite (Figure 5B in 2) appeared to be a larval pentastomid (identifiable by shape and body segmentation). Additional larval pentastomids have also been reported from *S. temminckii* [64] and *P. tricuspis* [4].

Interestingly, many of the cestode genera listed above have representatives that are zoonotic [66]. Additionally, the pentastomid genus *Armillifer* Sambon, 1922 (as found in *S. temminckii*), is also zoonotic [67]. As described herein, the taxonomic history of many of these species is complicated and combined with a lack of molecular data to help differentiate species [63], their true zoonotic potential may not be realised [66]. As human–wildlife interactions increase, the possibility of zoonotic infections with these parasites, especially due to the amount of traffic of pangolin meat, will also increase [66,68,69].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14121039/s1, Table S1: List of synonyms of parasite names mentioned in the text. Original parasite names are listed alphabetically.

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