A REEVALUATION OF THE TAXONOMY OF THE *MESOCOELIUM MONAS* COMPLEX (PLATYHELMINTHES: DIGENEA: MESOCOELIIDAE)

A Thesis

by

DANA MARIE CALHOUN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2011

Major Subject: Wildlife and Fisheries Sciences

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(Platyhelminthes: Digenea: Mesocoeliidae)

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Approved by:

Chair of Committee,	Norman Dronen
Committee Members,	Thomas Craig
	William Neill
Head of Department,	Thomas Lacher

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ABSTRACT

A Reevaluation of the Taxonomy of the *Mesocoelium monas* Complex

(Platyhelminthes: Digenea: Mesoceliidae). (May 2011)

Dana Marie Calhoun, B.S., University of Wisconsin-Stevens Point

Chair of Advisory Committee: Dr. Norman Dronen

Specimens from the National History Museum, London, and from the United States National Parasite Collection, Beltsville, United States of America. , which had all previously been identified as *Mescoelium monas* from a variety of definitive hosts (amphibians, reptiles and/or fish), were evaluated using all available literature and the body-type keys developed by Norman Dronen. None of these 85 putative *M. monas* specimens appeared to fit the original description of *M. monas*. There are likely multiple species of *Mescoelium* worldwide, and the *M. monas* complex, as currently defined, is not a unified monospecific grouping. The present study leads to the conclusion that there can be no fewer than 9 species of *Mescoelium* (6 body types, 3 of which can be divided into 2 separate groups based on the median or submedian placement of the genital pore). The specimens of *M. monas* I examined represented 3 of the 6 body types identified by Norman Dronen: the *M. monas* body type, the *M. lanceatum* body type, and the *M. pesteri* body type. Because *Mesocoelium* is a difficult group within which to distinguish species, it may be expedient in endoparasitic surveys of amphibians, fish and reptiles to consider any specimen of *Mesocoelium* found to be *M. monas*. Because *Mesocoelium* is a difficult group within which to distinguish species, it may be expedient in

endoparasitic surveys of amphibians, fish and reptiles to consider any specimen of *Mesocoelium* found to be *M. monas*. Subsequent reevalations must consider these key characteristics: genital pore placement, cecal length, vitelline follicle distribution, along with other supporting characteristics.

DEDICATION

To the late and great Fudd Calhoun

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I thank my committee chair, Dr. Dronen (without him I would not have produced this thesis), and my committee members, Dr. Craig and Dr. Neill, for their guidance, support and patience throughout the course of this research.

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Lastly, to my eighth-grade, earth-science teacher Mrs. Delo, who first showed me a soil pit that changed my life forever: I can't thank you enough.

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1. INTRODUCTION

Lühe (1901) described *Distomum sociale* Lühe, 1901 from specimens collected in Burma from the Asian black spined or Southeast Asian toad, *Bufo melanostictus* Schneider (Bufonidae). Braun (1901) considered this species to be *Dicrocoelium sociale* (Lühe, 1901) (Dicrocoeliidae Looss, 1899); however, Odhner (1910) established *Mesocoelium* Odhner, 1910 in Dicrocoelidae (Dicrocoelinae Looss, 1899) to accommodate *Mesocoelium sociale* (Lühe, 1901) Odhner, 1910 as the type species for the genus. Johnston (1912) transferred *Mesocoelium* to Branchcoeliinae Looss, 1899 (Dicrocoeliidae). Cort (1919) transferred Branchcoeliinae to Lepodermatidae Odhner, 1910. Based on the posttesticular placement of the ovary in *Mesocoelium*, Travassos (1919) transferred the genus back to Dicrocoeliidae. Dollfus (1929) erected Mesocoeliinae Dollfus, 1929 (Dicrocoeliidae) largely because species of *Mesocoelium* were generally intestinal parasites of amphibians and reptiles rather than being primarily gall bladder parasites of birds and mammals. Dollfus (1933) elevated Mesocoeliinae to Mesocoeliidae Dollfus, 1929 to accommodate species of *Mesocoelium*. Although some authors (e.g. Skrjabin & Morozov 1955; Odening1971) have accepted the assignment of *Mesocoelium* to Mesocoeliidae by Dollfus (1933), Yamaguti (1958; 1971) assigned this genus to Mesocoeliinae within Branchycoeliidae Looss, 1899. Pojmańska (2008) followed Dollfus (1933) by recognizing Mesocoeliidae and assigning *Mesocoelium*, where the 2 testes are adjacent and *Pintneria* Poche, 1907, where the testes are tandem

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This thesis follows the style of *Systematic Parasitology*.

as the only 2 genera in the family. There have been some 41 species described in *Mesocoelium* from the intestines of a variety of reptiles, amphibians and a few limited fish species worldwide; however, members of this genus tend to have extremely similar morphologies, which has made difficult the resolution of species using the key characteristic (e.g. egg size, ratio of suckers) generally used to distinguish species in most fluke genera (e.g. Frietas 1963, Thomas 1965, Mettrick & Dunkley 1968, Nasir and Dìaz 1971, and Goldberg *et al*. 2005). Although a number of synonymies has been suggested (*Mesocoelium meggitti* syn *Mesocoelium sociale*- Chatterji 1940, *Mesocoelium breviacaecum* syn *Mesocoelium lanceatum*- Chen 1960, *Mesocoelium japonicum* syn *Mesocoelium pearsei*- Goto et Ozaki 1930, *Mesocoelium micron* syn *Mesocoleium mesembrinum*- Pereira and Cuocolo 1940, and *Mesocoelium ovatum* syn *Mesocoelim brevicaecum*- Pereia and Cuocolo1940 [exclusive of Frietas 1963]) (Yamaguti, 1971), there remains a lack of consensus among researchers as to the number of valid species in the genus. Freitas (1963) studied the museum specimens available worldwide and based on the presence or absence of tegumental spines, the ratio of the width of the ventral sucker to the width of the oral sucker, egg length and width, and the posterior extent of the uterus recognized only 7 species (*Mesocoelium brevicaecum* Ochi, 1930, *Mesocoelium crossophorum* Pérez Veigueras, 1942, *Mesocoelium danforthi* Hoffman, 1935, *Mesocoelium geomydae* Ozaki, 1936, *Mesocoelium megaloon* Johnston, 1912, *Mesocoelium monas* Rudophi, 1819 and *Mesocoelium sibynomorphi* Ruiz & Leão, 1943 with *M. monas* replacing *M. sociale* as the type species for the genus). Using sucker ratios and egg sizes, Nasir & Dìaz (1971) further reduced the number of species

in the genus to 4 (*M. brevicaecum*; *M. geomydae*; *M. megaloon* and *M. monas*). Goldberg *et al*. (2005) took the reduction of the number of species 1 step further by stating that they suspected that "*Mesocoelium* is represented by a single species, *M. monas*", but provided no explanation for this proposal. It is interesting to note that later Goldberg *et al*. (2009) considered *Mesocoelium malayanum* to be a synonym of *M. monas*, but recognized *M. brevicaecum* as a valid species synonymizing *M. magniovum* and *Mesocoelium tritoni* Matskási, 1990 with it. A comprehensive listing of reports of specimens identified as *M. monas* from fish, amphibians and reptiles has been published by Bursey *et al*. (2007), with updates provided by Goldberg & Bursey (2008) and Goldberg *et al*. (2009). In a recent revision of Mesocoeliidae, Pojmańska (2008) recognized *M. sociale* as a valid species, reestablishing it as the type species in the genus. Although this author did not address the question of the number of valid species in *Mesocoelium*, he did point out that there were at least 2 major body types present in the genus: those with long ceca (represented by *M. sociale*) and those with short ceca (represented by *Mesocoelium elongatum* Goto & Ozaki, 1930).

Most recent authors have accepted the reductions of species in the genus proposed by Freitis (1963) but in fact, there has been a trend in recent surveys to follow the suggestion of Goldberg *et al*. (2005) and identify any specimens of *Mesocoelium* found in amphibians, fishes or reptiles as *M. monas* worldwide (e. g. Ubelaker 1966-Sumatra; Maeder *et al*. 1970-Gabon, Africa; Nasir & Dìaz 1971-Venezuela; Linzey *et al*.1998-Burmuda; Guillen-Hernández *et al.* 2000; Bursey *et* al. 2001-Peru, 2005-Brazil; Golberg *et al*. 1997-Lesser Antilles, 1998-Hispaniola, 2005-Phillippines; Espinoza-

Jiménez *et al*. 2007-Mexico; Espínola-Novelo *et al*. 2008-Mexico; Goldberg & Bursey 1992-U.S.A., 1995-Bermuda, 1996- Lesser Antilles, 2000-Hawaii, 2003-Costa Rica, 2008-Costa Rica).

The specific identity of *M. monas* and the inclusion of all 41 previously described species in the *M. monas* complex is controversial, especially given the unsettled taxonomic history of the genus. I believed that the characteristics that are traditionally used to separate species in *Mesocoeolium* should be reevaluated through a comprehensive literature review of the original species descriptions and a reevaluation of available museum specimens identified as *M. monas*.

Dronen *et al*. (unpublished material, 2011) has identified 6 body types in Mesocoelidae: the *Mesocoelim pesteri* Saoud, 1964 body type (where the ceca do not surpass the ovary posteriorly and the genital pore is prebifurcal); the *Mesocoelium zhejiangensis* body type (where the ceca do not surpass the ovary posteriorly and the genital pore is bifurcal); the *Mesocoelium lanceatum* body type (where the ceca do not surpass the ovary and the genital pore is postbifurcal); the *Mesocoelim monas* body type (where the ceca surpass the ovary posteriorly, extending some distance into postovarian space and the genital pore is prebifurcal); the *Mesocoelium sociale* body type (where the ceca surpass the ovary posteriorly, extending some distance into postovarian space and the genital pore is bifurcal); and the *Mesocoelium carli* André, 1915 body type (where the ceca surpass the ovary posteriorly, extending some distance into postovarian space and the genital pore is postbifurcal). Based on these body types, Dronen *et al*.

(Unpublished material, 2011) have developed a set of comprehensive keys to the species of *Mesocoelium* that considers the 41 species.

The purpose of this study is to reevaluate species that have been previously identified as *M. monas* using the keys developed by Dronen *et* al. (unpublished material, 2011), the available literature and specimens available from the United States National Parasite Collection, Beltsville, U.S.A. (USNPC) and the Natural History Museum, NHM (NHM). The specific objectives are to: 1. evaluate potential specific key charactertics and determine if they are consistent enough to be used to separate *M. monas* from other potential species in the genus; 2. determine if the *M. monas* complex is a monospecific group as currently suggested or a superficial complex composed of more than 1 species; and 3. identify any additional morphological, ecological or biogeographical features that can be added to the existing information to improve the understanding of species within *Mesocoelium*.

2. METHODS AND MATERIALS

The following specimens, which were previously identified as *M. monas*, were examined from USNPC and from NHM: *M. monas* USNPC 072881.00,079344.00, 081918.00, 084288.00, 085478.00, 087204.00, 087355.00, 090332.00, 090333.00, 090334.00, 090335.00, 092185.00, 092774.00 092800.00, 094097.00, 095017.00, 097835.00, 097844.00, 098269.00, 098733.00, 098763.00, 098814.00, 099607.00, NHM 1977.9.28–6, 1978.8.31 48–67, 1979.9.12 17–19, 1980.7.14 50–54, 1980.11.12 1–3, 1980.11.12–4, 1994.6.21–3.These specimens were evaluated using all available literature, and the body-type keys to the species of *Mesocoelium* developed by Dronen *et al.* (Unpublished material, 2011). Measurements are in micrometers (um) and are given as an average, followed by the range of values, in parentheses, if more than 1 specimen was measured. All comparisons made to *M. monas* were taken from original description, redescription and from available type or holotype material. *Mesocoelium monas* measurements were taken from the Frietas (1958) redescription, because the Rudolphi (1819) original description lacked most measurements. Illustrations were done with the aid of a digital camera, drawing tube and Adobe Photoshop 7. All illustrations are presented as composite drawings where some aspects of nonessential features (e.g. the uterine folds) may have been omitted to more clearly show important taxonomic characteristics.

3. RESULTS

Eighty-five specimens (38 slides) were examined. Of these, 6 represented possibly undescribed species (USNPC: 085478.00, 092185.00, 09332.00, 098269.00; NHM: 1980.7.14 50–54, 1980.11.12 1–3); 3 were *M. americanum* (USNPC: 079344.00: NHM 1979.9.12 17–19, 1994.6.21 3); 3 were *M. meggitti* (USNPC: 092800.00, 09335.00; NHM 1977.9.28 6); 1 was *M. burti* (NHM 1980.11.12 4); 1 was *M. crossophorum* (USNPC 072881.00); 1 was *M. lanceatum* (USNPC 084288.00); and, 12 were of poor quality and could not be identified to species (USNPC: 081918.00, 084288.00, 087204.00, 087355.00, 092774.00, 094097.00, 095017.00, 097835.00, 097844.00, 098814.00, 099607.00; NHM 1978. 8.31 48–67). No specimens examined in this study appeared to fit the original description of *M. monas*, as given by Rudolphi (1819) or the redescription by Freitas (1958). Specimens examined represented 3 of the 6 body types defined by Dronen e*t a*l. (Unpublished material, 2011): the *M. monas* body type, the *M. lanceatum* body type, and the *M. pesteri* body type.

It was my experience that the presence or absence of body spines was not a reliable characteristic. In addition, the poor quality of many specimens precluded examination of the details of genitalia and therefore these structures could not be included in the following descriptions. Comparative measurement and morphometric ratios for specimens examined from USNPC and NHM are presented in Appendix B3-B6.

Mesocoeliidea Dollfus, 1933

Mesocoelium *Dollfus*, 1933

Specimens previously identified as *Mescoelium monas* (Rudolphi, 1819) Freitas, 1958

Specimens assigned to the *M. monas* body type

USNPC 098269.00

(Appendix A1)

Host: Cherrie's litter skink, brown forest skink, *Sphenomorphus cherrieri* (Cope) (Squamata: Scincidae)

Locality: Costa Rica

Description: Based on 10 specimens: Body 1,365 (1,196–1,534) long by 743(560–860) wide; forebody 335 (240–420) long, representing about 26% of body length. Mouth subterminal; oral sucker wider than long, 256 (221–290) long by 269 (213–325) wide; prepharynx absent; pharynx wider than long, 90 (88–91) long by 103 (91–107) wide, ratio of width of pharynx to width of oral sucker 1:3.1 (1:3.0–1:3.2); esophagus not visible; ceca surpass ovary posteriorly, cecal ends not visible. Ventral sucker located about one third the distance down the body, wider than long, 135 (109–160) long by 166 (99–232) wide, ratio of ventral sucker width to oral sucker width, 1:2.7. Testes side by side, average left testis, 178 (101–255) long by 158 (117–198) wide and average right testis, 153 (100–226) long by 188 (135–250) wide. Genital pore prebifurcal, submedian

at level of posterior one third of pharynx. Ovary oval to round, wider than long, 155 (140–170) long by 165 (140–190) wide. Postovarian space 780 long, representing about 57% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of anterior margin of oral sucker posteriorly to well past ovary. Eggs 36 (30–42) long by 19 (17–21) wide. Excretory system not visible.

Remarks: Although the specimens described above under USNPC 098269.00 have the ceca surpassing the ovary posteriorly and a prebifurcal genital pore, and thus are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958), because *M. monas* has a: longer body (1,835 [1,260–2,410] compared to 1,365 [1,196–1,534]); longer forebody (970 [about 53% of body length] compared to 335 [240–420] (about 25% of body length); wider pharynx (133 [100–166] compared to 103 [91–107]); smaller ratio of the pharynx width to the oral sucker width, (1:2.2 [1:2.1–1:2.3] compared to 1:3.1 [1:3.0–3.2]); wider ventral sucker (300 compared to 166 [99–232]); smaller ratio of the ventral sucker width to the oral sucker width (1:1.3 compared to 1:2.7); vitelline fields that either terminate near to, or surpass the cecal ends posteriorly (exact location of cecal ends not visible) rather than terminating well anterior to cecal ends; wider ovary (267 compared to 165 [140–190]); wider eggs (24 [22–26] compared to 19 [17–21]); and the genital pore is located at mid-level of the esophagus compared to mid-level of pharynx.

These specimens are similar to *M. monas* by having a: submedian genital pore similar oral sucker width (295 [230–360] compared to 269 [213–325]); moreover, the

eggs of the specimens in USNPC 098269.00 overlap in length with those reported for *M. monas* (39 [34–44] compared to 36 [30–42]).

These specimens appear to be the same species as represented by USNPC 098733.00 and 098763.00 (described separately later) and likely represent a new species of *Mesocoelium*.

USPNC 085478.00

(Appendix A2)

Host: Anguilla bank anole, anguilla anole**,** *Anolis gingivinus* (Cope) (Squamata: Iguanidae)

Locality: Caribbean

Description: Based on 1 specimen: Body 1,738 long by 600 wide; forebody 550 long, representing about 32% of body length. Mouth subterminal; oral sucker circular, 250 long by 250 wide; prepharynx absent; pharynx wider than long, 52 long by 62 wide, ratio of pharynx width to oral sucker width 1:4.0; esophagus not visible; ceca terminating past posterior margin of ovary, occupying about 22% of postovarian space. Ventral sucker one third distance down body from anterior end, wider than long, 200 long by 227 wide, ratio of ventral sucker width to oral sucker width, 1:1.1. Testes nearly side by side, right testis 88 (89–87) by 95 (100–90) and left testis 85 (75–95) long by 92 (90–94) wide. Genital pore prebifurcal, submedian at mid-level of esophagus. Cirrus sac club-shaped 125 long, representing about 7% of body length. Ovary oval to round, longer than wide, 115 long by 93 wide, postovarian space 1,000 long, representing about

58% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of lower margin of oral sucker to reaching near to level of cecal ends. Eggs 46 (43–48) long by 29 (28–29) wide. Excretory system not visible.

Remarks: Although this specimen described above under USNPC 085478.00 has the ceca surpassing the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958)**,**because *M. monas* has a longer forebody (970 [about 53% of body length] compared to 550 [about 32% of body length]); wider pharynx (133 [100–166] compared to 62), smaller ratio of the pharynx width to the oral sucker width (1:2.2 [1:2.1–1:2.3] compared to 1:4.0); wider ventral sucker (300 compared to 227); vitelline fields that terminate well short of cecal end compared to vitelline fields that surpass the cecal ends posteriorly; wider ovary (267 compared to 93); and shorter and thinner eggs (39 [34–44] long by23 [21–25] wide compared to 46 [43–48] by 29 [28–30]).

This specimen is similar to *M. monas* in having a: submedian genital pore, near the mid-level of the esophagus; similar ratio of the ventral sucker width to the oral sucker width (1:1.3 compared to 1:1.1); similar body length (1,835 [1,260–2,410] compared to 1,738); and similar oral sucker width (295 [230–360] compared to 250); however, these characteristics are also shared with *M. americanum* (genital pore submedian and oral sucker width 284 [208–360] compared to 250) as redescribed by Dronen, *et al*. (Unpublished material, 2011)

Mesocoelium americanum also is similar to this specimen in having a: similar host locality (Texas compared to the Caribbean); similar width of the ventral sucker (182 [125–238] compared to 200); similar ratio of the ventral sucker width to the oral sucker width (1:1.6 [1.5–1.7] compared to 1:1.1); similar length of the postovarian space (1,638 [740–2,626] compared to 1,000); similar length of the cirrus sac (176 [about 7% of body length] compared to 125 [about 7% of the body length]); and similar egg length (42 [39–45] compared to 46 [43–48].

The differences between *M. americanum* and USNPC 085478.00 are that *M. americanum* has a longer body (2,682 [1,325–4,038] compared to 1,738); wider pharynx (120 [75–165] compared to 62); smaller ratio of the pharynx width to the oral sucker width (1:2.5 [1:2.2–1:2.7] compared to 1:4.0); smaller egg width (25 [23–26] compared to 29 [28–29]). Based on these differences, this specimen may represent a new species.

USNPC 092800.00

Host: Lion anole, *Anolis lionotus* (Cope) (Squamata: Polychrotidae)

(Appendix A3)

Locality: Panama

Description: Based on 2 specimens: Body 1,846 long by 520 wide; forebody 340 long, representing about 18% of body length. Mouth subterminal; oral sucker circular, 160 long by 160 wide; prepharynx absent; pharynx longer than wide, 52 long by 62 wide, ratio of width of pharynx to width of oral sucker 1:2.4; esophagus 21 long; ceca terminating past posterior margin of ovary occupying at least 20% of postovarian space

(exact cecal ends not visible). Ventral sucker located about one forth the distance down the body, longer than wide, 135 long by 133 wide, ratio of ventral sucker width to oral sucker width 1:1.2. Testes nearly adjacent, left testis, 159 long by 135 wide and right testis, 143 long by 122 wide. Genital pore prebifurcal, median, and postpharyngeal, located near to posterior margin of pharynx. Cirrus sac club shaped, 143 long, representing about 8% of body length. Ovary oval to round, wider than long, 117 long by 143 wide, postovarian space 1,170 long, representing about 63% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of lower margin of oral sucker to level of posterior margin of cecal ends. Eggs 31 (27–35) long by 22 (21–23) wide. Excretory system not visible.

Remarks: The poor quality of these specimens precluded taking measurement of the structures on 1 of these specimens. Although these specimens described above under USNPC 092800.00 have ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958), because *M. monas* has a: longer forebody (970 [about 53% of body length] compared to 340 [about 18% of body length); wider oral sucker (295 [230–360] compared to 160); wider pharynx (133 [100–166] compared to 68); wider ventral sucker (300 compared to 133); vitelline fields that terminate well short of cecal ends compared to vitelline fields that surpass the cecal ends; wider ovary (267 compared to 143); and longer eggs (39 [34–44] compared to 31 [27–35].

These specimens are similar to *M. monas* by having a: genital pore that opens between the level of the upper one third and the lower half of the pharynx; similar body length (1,835 [1,260–2,410] compared to 1,846); similar ratio of the ventral sucker width to the oral sucker width (1:1.3 compared to 1:1.1); similar ratio of the pharynx width to the oral sucker width (1:2.2 [1:2.1–1:2.3]compared to 1:2.4) and similar egg width (23 [21–25] compared to 22 [21–23]); however, these characteristics are also shared with *M. meggetti* (ratio of the ventral sucker width to the oral sucker width 1:1.5, ratio of the pharynx width to the oral sucker width 1:2.3 [1:2.0–1:2.5] and egg width 25 [23–26]) as redescribed by Dronen e*t al*.(Unpublished material, 2011).

Mesocoelium meggitti also is similar to this specimen by having a: similar ventral sucker width (185 [130–240] compared to 133); similar length of the postovarian space (1,340 [880–1,800] long ([about 64% of body length] compared to 1,170 [about 63% of body length]); similar percentage of ceca that surpass the ovary into the postovarian space (30% [28%–31%] compared to at least 20%); similar cirrus sac length (200 long [about 10% of body length] compared to 143 [about 8% of body length]); similar egg length (35 [33-37] compared to 31 [27-35]; and the genital pore is median.

The differences in egg widths seen between the specimens from USNPC 092800.00 and *M. meggetti* are probably due to the unusually large ranges reported by Bhaleraro (1927) in his original species description. Also, one of the specimens from USNPC 092800.00 appear to have the genital pore located slightly submedian rather than median (as seen in *M. meggetti*), but the forebody of this specimen was twisted

(rolled) to one side, which appears to have moved the esophagus and pharynx to one side causing the submedian appearance of the genital pore.

USPNPC 092185.00

Host: Green tree anole, Neotropical green anole, *Anolis biporcatus* (Wiegmann) (Squamata: Polychrotidae**)**

(Appendix A4)

Locality: Panama

Description: Based on 5 specimens: Body 1,807 (1,716–1,898) long by 485 (468–494) wide; forebody 470 long, representing about 26% of body length. Mouth subterminal; oral sucker wider than long, 175 (167–182) long by 184 (172–195) wide; prepharynx absent; pharynx wider than long, 67 (52–81) long by 79 (75–83) wide, ratio of width of pharynx to width of oral sucker 1:2.2 (1:2.0–1:2.3); esophagus not visible; ceca terminating past ovary posteriorly, occupying 20% (17%–23%) of postovarian space. Ventral sucker located in upper third of the body, 133 (123–143) long by 131 (122–140) wide, ratio of ventral sucker width to oral sucker width, 1:1.3 (1:1.2–1:1.4). Testes diagonal, right testis, 176 long by 156 wide and left testis, 176 long by 155 wide. Genital pore prebifurcal, submedian, and postpharyngeal, located near lower margin of pharynx. Cirrus sac club shaped, 149 (141–156) long, representing about 8% of body length. Ovary oval, longer than wide, 158 (146–169) long by 127 (107–146) wide, postovarian space 1,150 (1,020–1,280) long. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of upper margin of pharynx to reaching near to

level of cecal ends. Eggs 33 (29–36) long by 21 (18–23) wide. Excretory system Y-shaped, pore not visible.

Remarks: These specimens were in poor condition with the forebody contracted and the shape of the hindbody distorted suggesting that they may have been fixed without cover slip pressure. Although these specimens described above under USNPC 092185.00 have ceca surpassing the ovary posteriorly and a prebifurcal genital pore, and are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: longer forebody (970 compared to 470); wider oral sucker (295 [230–360] compared to 175 [167–182]); wider pharynx (133 [100–166] compared to 79 [75–83]); wider ventral sucker (300 compared to 131 [122–140]); vitelline fields that terminate well short of ceca ends compared to vitelline fields that terminate nearly surpassing cecal ends; wider ovary (267 compared to 127 [107–146]); and larger eggs (39 [34–44] long by 23 [21–25] wide compared to 33 [29–36] by 21 [18–23]).

These specimens are similar to *M.* monas by having a: submedian genital pore; similar body lengths (1,835 [1,260–2,410] compared to 1,807 [1,716–1,898]); similar ratio of the ventral sucker width to oral sucker width (1:1.3 compared to 1:1.3 [1:1.2–1:1.4]); and a similar ratio of pharynx width compared to width of oral sucker (1:2.2 [1:2.1–1:2.3] compared to 1:2.1 [1:2.0–1:2.3]); however, these characteristics are also shared with *M. monodi* (genital pore submedian, ratio of ventral sucker width to oral sucker width 1:2.7, ratio of pharynx width to oral sucker width 1:1.7) as originally described by Dollfus, 1929.

Mesocoelium monodi is similar to these specimens by having a: similar body length (2,155 [1,310–3,000] compared to 1,807 [1,716–1,898]); similar length of the postovarian space (1,240 [800–1,680] compared to 1,150 [1,020–1,280]); and a similar cirrus sac length (180 [115-244] (about 8% of body length) compared to 149 [141–156] (about 8% of body length).

Mesocoleium monodi differ from these specimens by having a: larger ratio of ventral sucker width to oral sucker width (1:1.7 [1:1.3–1:2.0] compared to 1:1.3 [1:1.2–1:1.4]); larger percentage of ceca in postovarian space (32% [30%–33%] compared to 20% [17%–23%]) and larger eggs (37 [32–41] long by 33 [29–36] wide compared to 25 [20–30] by 21[18–23]).

These specimens likely represent an undescribed species that is closely related to *M. monodi*. Without additional museum-quality specimens this possible new species cannot be adequately documented and described.

USNPC 079344.00

Host: Brown anole**,** *Anolis sagrei* (Dumeril & Bibron) (Squamata: Polychrotidae) (Appendix A5)

Locality: Florida

Description: Based on 1 specimen: Body 2,050 long by 640 wide; forebody 676 long, representing about 33% of body length. Mouth subterminal; oral sucker wider than long, 203 long by 213 wide; prepharynx absent; pharynx wider than long, 85 long by 108 wide, ratio of width of pharynx to width of oral sucker 1:2.1; esophagus 260 long; ceca

terminating past posterior margin of ovary occupying 37% of postovarian space. Ventral sucker located about one fourth distance down the body, wider than long, 163 long by 185 wide, ratio of ventral sucker width to oral sucker width, 1:1.2. Testes adjacent to each other, right testis 138 (136–140) long by 132 (129–135) wide and left testis 135 (130–140) long by 125 (120–130) wide. Genital pore prebifurcal, submedian, and postpharyngial, located upper half of esophagus near posterior margin of pharynx. Cirrus sac club shaped, 200 long, representing about 10% of body length. Ovary circular, 138 long by 138 wide, postovarian space 1,120 long, representing about 55% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of upper margin of pharynx to reaching near to level of cecal ends posteriorly. Eggs 43 [40–45] long by 25 [24–26] wide. Excretory system Y-shaped.

Remarks: Specimen in fair condition, forebody subtlety contracted artificially pulling gonads to the right side of body. Although this specimen described above under USNPC 079344.00 has ceca surpassing the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: longer forebody (970 [about 53% of body length] compared to 676 [about 33% of body length); wider oral sucker (295 [230–360] compared to 213); wider ventral sucker (300 compared to 185); vitelline fields that terminate well short of anterior margins of the ovary compared to vitelline fields that surpass the cecal ends; wider ovary (267 compared to 138); and shorter eggs (39 [34–44] compared to 43 [40–45]).

This specimen is similar to *M. monas* by having a: submedian genital pore; similar body length (1,835 [1,260–2,410] compared to 2,050); similar pharynx width (133 [100–166] compared to 108); similar ratio of pharynx width to oral sucker width (1:2.2 [1:2.1–1:2.3]compared to 1:2.1); similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.2) and similar egg width 23 [21–25] compared to 25 [24–26]); however, these characteristics are also shared with *M. americanum* (genital pore submedian and postpharyngeal, body length 2,682 [1,325–4,038], ratio of ventral sucker width to oral sucker width 1:1.6 [1:1.5–1:1.7], and ratio of pharynx width to oral sucker width 1:2.5 [1:2.2–1:2.7]), as re-described by Dronen *et al*. (Unpublished material, 2011).

Mesocoelium americanum is similar to this specimen by having: similar host localities (Texas compared to Florida); similar width of oral sucker (284 [208–360] compared to 213); similar width of pharynx (120 [75–165] compared to 108); similar ventral sucker width (182 [125–238] compared to 185); similar length of postovarian space (1,638 [740–2,626] [about 61% of body length] compared to 1,120 [about 55% of body length); similar length of cirrus sac (176 [about 7% of body length] compared to 200 [about 8% of body length]); and similar egg sizes (42 [39–45] long by 25 [23–26] wide compared to 43 [40–45] by (25 [24–25]. This specimen 079344.00 compares to *M. americanum* on all key characteristics therefore this specimen is most likely *M. americanum*.

USNPC 072881.00

Host: Cubera snapper, canteen snapper, cuban snapper, gray snapper, *Cutjanus cyanopterus* (Cuvier) (Perciformes: Lutjanidea)

(Appendix A6)

Locality: Venezuela

Description: Based on 1 specimen: Body 1,175 long by 480 wide; forebody 520 long, representing about 42% of body length. Mouth subterminal; oral sucker longer than wide, 175 long by 160 wide; prepharynx absent; pharynx circular, 78 long by 78 wide, ratio of width of pharynx to oral sucker width, 1:2.2; esophagus 39 long; ceca terminating past posterior margin of ovary occupying 20% in postovarian space. Ventral sucker located in posterior margin of upper third of the body, longer than wide, 149 long by 140 wide, ratio of ventral sucker width to oral sucker width, 1:1.3. Testes adjacent, right testis, 80 (75–85) long by 78 (76–80) wide and left testis 76 (74–78) long by 65 (60–70) wide. Genital pore prebifurcal, submedian, and postpharyngial, located near upper margin of cecal bifurcation. Cirrus sac club shaped, 108 long, representing about 9% of body length. Ovary round, 100 long by 96 wide, postovarian space 605 long, representing about 51% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of lower margin of oral sucker to level of cecal ends posteriorly. Eggs 36 [33–38] long by 22 [20–24] wide. Excretory system Y-shaped.

Remarks: Specimen in fair condition, forebody and pharynx contracted artificially pulling gonads to the right side of body. Although this specimen described above under

USNPC 072881.00 has ceca surpassing the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: longer body (1,835 [1,260–2,410] compared to 1,175); longer forebody (970 [about 52 % of body] compared to 520 [about 44% of body]); wider oral sucker (295 [230–360] compared to 175); wider pharynx (133 [100–166] compared to 78); wider ventral sucker (300 compared to 140); vitelline fields that terminate well sort of cecal ends compared to vitelline fields that nearly surpass the cecal ends; wider ovary (267 compared to 96); and longer eggs (39 [34–44] compared to 35 [33–38].

This specimen is similar to *M. monas* by having a: submedian genital pore; similar ratio of pharynx width to oral sucker width (1:2.2 [1:2.1–1:2.3]compared to 1:2.2); similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.3) and similar egg widths 23 [21–25] compared to 22 [20–24]); however, these characteristics are also shared with *M. crossophorum* (genital pore near submedian of esophagus, ratio of ventral sucker width to oral sucker width 1:1.3, ratio of pharynx width to oral sucker width 1:2.6) as originally described by Pérez Vigueras, 1942.

Mesocoelium crossophorum is similar to this specimen by having: similar host localites (Cuba compared to Venezuela); similar body size length (1,050 [1,000–1,100] compared to 1,175); similar width of oral sucker (168 [160–175] compared to 175); similar pharynx width (64 compared to 78); similar ventral sucker width (130 compared to 140); similar length of postovarian space (468 [about 45% of body length] compared to 605 [about 51% of body length); and similar cirrus sac length (114 [about 11% of

body length] compared to 108 [about 9% of body length]). This specimen compares to *M. crossophorum* on all key characteristics except a subtle difference in egg sizes (41 [36–45] long by 27 [24–30] wide compared to 36 [33–38] by 22 [20–24]), therefore 072881.00 is most likely *M. crossophorum*.

USNPC 090335.00

Host: Mediterranean house gecko, turkish gecko, *Hemidactylus turcicus* (Linnaeus) (Squamata: Gekkonidae)

(Appendix A7)

Locality: Louisiana, USA

Description: Based on 1 specimen: Body 2,808 long by 572 wide; forebody 700 long, representing about 25% of body length. Mouth subterminal; oral sucker longer than wide, 290 long by 260 wide; prepharynx absent; pharynx wider than long, 122 long by 143 wide, ratio of width of pharynx to width of oral sucker 1:1.8; esophagus not visible; ceca terminating past posterior margin of ovary occupying at least 26% of postovarian space (exact cecal ends not visible). Ventral sucker located near posterior margin of upper forth of the body, longer than wide, 198 long by 192 wide, ratio of ventral sucker width to oral sucker width, 1:1.4. Testes nearly diagonal, right testis 190 long by 200 wide and left testis 190 long by 150 wide. Genital pore prebifurcal, median and postpharyngial, located near middle of esophagus. Cirrus sac 203 long, representing about 7% of body length. Ovary oval to round, longer than wide, 170 long by 160 wide, postovarian space 1,742 long, representing about 62% of body. Uterus extensive, filling

hindbody. Vitelline follicles distributed in lateral fields from level of lower margin of pharynx to level of ceca posterioly. Eggs 37 (31–42) long by 21 (18–23) wide.

Remark: Specimen appears to be young because of lack of eggs within uterus. Although this specimen described above under USNPC 090335.00 has ceca surpassing the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas has a:* smaller body length (1,835 [1,260–2,410] compared to 2,808); larger forebody (970 [about 52% of body length] compared to 700 [about 25% of body length]); wider ventral sucker (300 compared to 192); vitelline fields that terminate well short of anterior margins of the ovary compared to vitelline fields that surpass the cecal ends; wider ovary (267 compared to 160); and genital pore submedian compared to median.

This specimen is similar to *M. monas* by having a: postpharyngeal genital pore; similar oral sucker width (295 [230–360] compared to 260); similar pharynx width (133 [100–166] compared to 143); similar ratio of pharynx width to width of oral sucker (1:2.5 compared to 1:2.2); similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.3); and similar eggs (39 [34–44] long by 23 [21–25] compared to 35 [33–38] by 21 [18–23]); however these characteristics are also shared with *M. meggitti* (genital pore near mid-level of esophagus, ratio of ventral sucker width to oral sucker width 1:1.5, ratio of pharynx width to oral sucker width 1:2.0) as redescribed by Dronen *et al*. (Unpublished material, 2011)

Mesocoelium meggitti is similar to this specimen by having a: similar body length (2,090 [1,070–3,110] compared to 2,808); similar oral sucker width (260 [200–320] compared to 260); similar pharynx width (120 [100–140] compared to 143); similar ventral sucker width (185 [130–240 compared to 192); similar length of postovarian space (1,340 [880–1,800] [about 64% of body length] compared to 1,742 [about 62% of body length]); similar percentage of ceca that surpass the ovary occupying (30% [28%–31%] postovarian space compared to at least 26% [exact cecal ends not visible]); similar length of cirrus sac (200 [about 10% of body] compared to 203 [about 7% of body]); and similar egg (35 [33–37] long by 25 [23–26] wide compared to 37 [31–42] by 21 [18–23]).This specimen USNPC 090335.00 appears to be most likely *M. meggitti* based on the above comparisons.

USNPC 090332.00

Host: Gulf coast toad, *Bufo valliceps* (Wiegmann) (Anura: Bufonidae)

(Appendix A8)

Locality: Louisiana

Description: Based on 1 specimen: Body 1,456 long by 494 wide; forebody 460 long, representing about 32% of the body length. Mouth subterminal; oral sucker wider than long, 172 long by 169 wide; prepharynx absent; pharynx longer than wide, 75 long by 78 wide, ratio of width of pharynx to width of oral sucker 1:2.2; esophagus not visible; ceca terminating past posterior margin of ovary, occupying 49% of postovarian space. Ventral sucker located anterior margin of upper third of body, wider than long, 140 long by 143

wide, ratio of ventral sucker width to oral sucker width, 1:1.8. Testes adjacent, right testis, 96 long by 120 wide and left testis, 104 long by 101wide. Genital pore prebifurcal, submedian, and located to left of posterior margin of pharynx. Cirrus sac 99 long, representing about 7% of body length. Ovary longer than wide, oval to round, located at posterior margin of Ventral sucker 100 long by 85 wide, postovarian space 790 long, representing about 54% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of lower margin of oral sucker to level of cecal ends posteriorly. Eggs 30 long by 21 wide. Excretory system and pore not visible.

Remarks: Although this specimen described above under USNPC 090332.00 has ceca surpassing the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: larger forebody (970 [about 53% of body] compared to 700 [about 48% of body]); wider oral sucker (295 [230–360] compared to 169); wider pharynx (133 [100–166] compared to 78); wider ventral sucker (300 compared to 143); vitelline follicles terminating well short of cecal ends compared to vitelline follicles terminating near to surpassing cecal ends; smaller percentage of ceca surpass ovary into postovarian space (31% compared to 49%); wider ovary (267 wide compared to 85); wider average testes (170 compared to 111); and larger eggs (39 [34–44] long by 23 [21–25] wide compared to [30 by 21]).

This specimen is similar to *M. monas* by having a: submedian genital pore; similar body length (1,835 [1,260–2,410] compared to 1,456); similar ratio of pharynx

width compared to width of oral sucker (1:2.5 compared to 1:2.2); and similar ratio of ventral sucker width to width of oral sucker (1:1.3 compared to 1:1.8).

This specimen appears to be the same species as represented by USNPC 090333.00 and 090334.00 (described separately later) and likely represent a new species of *Mesocoelium*.

USNPC 090333.00

Host: Green house frog, *Eleutherodacytles planirostris* (Cope) (Anura: Leptodactylidae)

Locality: Lousiana

Description: Based on 1 specimen: Body 1,950 long by 676 wide; forebody 500, representing about 26% of body length. Mouth subterminal; oral sucker wider than long, 263 long by 270 wide; prepharynx absent; pharynx wider than long, 101 long by 109 wide, ratio of width of pharynx to width of oral sucker 1:2.5; esophagus not visible; ceca terminating past posterior margin of ovary occupying 47 % of postovarian space. Ventral sucker located posterior margin of upper third of body length, longer than wide, 179 long by 161 wide, ratio of ventral sucker width to oral sucker width 1:1.7. Testes adjacent, left testis, 130 long by 150 wide and right testis, 140 long by 120 wide. Genital pore prebifurcal, submedian and located on lower third of pharynx. Cirrus sac club shaped, 164 long, representing about 8% of body length. Ovary longer than wide, oval to round, 160 long by 150 wide, postovarian space 1,196 long, representing about 61% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral

fields from level lower margin of oral sucker to level of cecal ends posteriorly. Egg unable to measure. Excretory system not visible.

Remarks: Although this specimen described above under USNPC 090333.00 has ceca surpassing the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: longer forebody (970 [about 53% of body] compared to 500 [about 26% of body]); wider ventral sucker (300 compared to 179); vitelline follicles terminating well short of cecal ends compared to vitelline follicles terminating near to surpassing cecal ends; smaller percentage of ceca surpass ovary into postovarian space (31% compared to 47%); and wider ovary (267 compared to 150).

This specimen is similar to *M. monas* by having a: submedian genital pore; similar body length (1,835 [1,260–2,410] compared to 1,950); similar width of oral sucker (295 [230–360] compared to 270); similar width of pharynx (133 [100–166] compared to 109); similar ratio of pharynx width compared to width of oral sucker (1:2.5 compared to 1:2.5); and similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.7).

This specimen appears to be the same species as represented by USNPC 090332.00 and 090334.00 (described separately) and likely represent a new species of *Mesocoelium*.

USNPC 090334.00

Host: Green anole, American anole, red-throated anole, Carolina anole, *Anolis caronlinensis* (Voigt) (Squamata: Polychrotidae)

Locality: Louisiana

Description: Based on 1 specimen: Body 1,950 long by 646 wide; forebody 550 long, representing about 28% of body length. Mouth subterminal; oral sucker longer than wide, 260 long by 250 wide; prepharynx absent; pharynx wider than long, 99 long by 125 wide, ratio of width of pharynx to width of oral sucker 1:2.0; esophagus not visible; ceca terminating past posterior margin of ovary occupying at least 35% (exact ends of ceca not visible) of postovarian space. Ventral sucker located posterior margin of upper third of body, round, 208 long by 208 wide, ratio of ventral sucker width to oral sucker width 1:1.2. Testes adjacent, right testis, 150 long by 160 wide and left testis, 160 long by 150 wide. Genital pore prebifurcal, submedian and located at level of lower third of pharynx. Cirrus sac not visible. Ovary wider than long, oval to round, 130 long by 150 wide, postovarian space 1,092 long, representing about 56% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from lower margin of oral sucker to level of cecal ends posteriorly. Eggs 38 (36–39) long by 22 (21–23) wide. Excretory system not visible.

Remarks: Although this specimen described above under USNPC 090334.00 has ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: longer forebody (970 [about

53% of body] compared to 550 [about 28% of body]); wider ventral sucker (300 compared to 179); vitelline follicles that terminate well short of cecal ends compared to vitelline that terminate near to surpassing cecal ends; smaller percentage of ceca surpass ovary into postovarian space (31% compared to at least 35% [exact cecal ends not visible]); and wider ovary (267 compared to 150).

This specimen is similar to *M. monas* by having a: submedian genital pore; similar body length (1,835 [1,260–2,410] compared to 1,950); similar oral sucker width (295 [230–360] compared to 270); similar width pharynx (133 [100–166] compared to 109); similar ratio of pharynx width compared to width of oral sucker (1:2.5 compared to 1:2.5); similar ratio ventral sucker width to oral sucker width (1:1.3 compared to 1:1.7); and nearly similar eggs (39 [34–44] long by 23 [21–25] wide compared to 38 [36–39] by 22 [21–23]).

This specimen appears to be the same species as represented by USNPC 090332.00 and 090333.00 (described separately) and likely represent a new species of *Mesocoelium*.

USNPC 097330.00

Host: Golfito robber frog, *Eleutherodacytles tauraus* (Taylor) (Anura: Eleutherodactylidae)

(Appendix A9)

Locality: Costa Rica

Description: Based on 6 specimens: Body 1,131 (962–1,300) long by 801 (754–884) wide; forebody, 364 (312–416) long, representing 32% of body length. Mouth subterminal; oral sucker wider than long, 224 (195–252) long by 241 (208–273) wide; prepharynx absent; pharynx wider than long, 88 (80-96) long by 83 (57–109) wide, ratio of width of pharynx to width of oral sucker 1:3.1 (1:2.0–1:4.1); esophagus not visible; ceca terminating past posterior margin of ovary occupying 13% of postovarian space. Ventral sucker located posterior in upper third of body, wider than long, 183 (157–208) long by 201 (175–226) wide, ratio of ventral sucker width to oral sucker width 1:1.4 (1:1.2–1:1.6). Testes side by side, right testis, 153 (109–205) long by 163 (150–182) wide and left testis, 130 (80–169) long by 183 (150–234). Genital pore prebifurcal, submedian located on upper half to posterior third of pharynx. Ovary oval to round, wider than long, 125 (109–140) long by 165 (140–290) wide, postovarian space 660 long, representing about 58% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of upper margin of oral sucker to posterior level of cecal ends. Eggs 35 (31–39) long by 21 (18–23) wide. Excretory system visible.

Remarks: Although these specimens described above under USNPC 098733.00 have ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a*:* shorter body (1,835 [1,260–2,410] compared to 1,131 [962–1,300]); longer forebody (970 [about 53% of body length] compared to 364 [312–416] [about 32% of body length); smaller ratio of

pharynx width to width of oral sucker (1:2.5 compared to 1:3.1 [1:2.0–1:4.1]); wider ventral sucker (300 compared to 201 [175–226]); vitelline follicles that terminate well short of cecal ends compared to vitelline follicles that near to surpass cecal ends; larger percentage of ceca that surpass ovary into postovarian space (31% compared to 13%); and wider ovary (267 compared to 165 [140–190]).

These specimens are similar to *M. monas* by having a: submedian genital pore; similar oral sucker width (295 [230–360] compared to 241[208–273]); similar width pharynx (133 [100–166] compared to 83 [57–109]); similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.4 (1.2–1.6); similar eggs (39 [34–44] long by 23 [21–25] wide compared to 36 [30–42)] by 21 [18–23]).

These specimens appear to be the same species as represented by USNPC 098269.00 and 098763.00 (described separately) and likely represent a new species of *Mesocoelium*.

USNPC 098763.00

Host: Masked tree frog, Central American smilisca, tarraco treefrog, *Smillisca phaeota (*Cope) (Anura: Hylidae)

Locality: Costa Rica

Description: Based on 1 specimen: Body 1,144 long by 598 wide; forebody 450 long, representing about 39% of body length. Mouth subterminal; oral sucker, longer than wide, 218 long by 208 wide; prepharynx absent; pharynx longer than wide, 78 long by 60 wide, ratio of width of pharynx to width of oral sucker 1:3.4; esophagus not visible;

cecal ends not visible. Ventral sucker located in posterior of upper third of body, longer than wide, 161 long by 148 wide, ratio of ventral sucker width to oral sucker width 1:1.4. Testes not visible. Genital pore prebifurcal, submedian, and located on upper half to posterior third of pharynx. Ovary oval to round, wider than long, 101 long by 125 wide. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of upper margin of oral sucker to near to surpassing cecal ends. Eggs 29 [21–36] long by 21 [20–21] wide. Excretory system not visible.

Remarks: Specimen in poor condition, twisted at pharynx artificially pulling gonads to right. Although this specimen described above under USNPC 098763.00 has ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: longer body (1,835 [1,260–2,410] compared to 1,144); longer forebody (970 [about 53% of body length] compared to 450 [about 25% of body length); wider oral sucker (295 [230–360] compared to 208); wider pharynx (133 [100–166] compared to 60), smaller ratio of pharynx width to width of oral sucker (1:2.5 compared to 1:3.4); wider ventral sucker (300 compared to 148); vitelline follicles that terminate well short of cecal ends to vitelline follicles that terminate near to surpassing the cecal ends; wider ovary (267 compared to 125); and longer egg (39 [34–44] compared to 29 [21–36]).

These specimens are similar to *M. monas* by having a: submedian genital pore; similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.4); and eggs widths (23 [21–25] compared to 21 [20–21]).

These specimens appear to be the same species as represented by USNPC 098269.00 and 098733.00 (described separately) and likely represent a new species of *Mesocoelium*.

NHM 1979.9.12 17–19

Host: Common Indian toad**,** common Asian toad, black-spined toad, *Bufo melanostictus* (Schneider) (Anura: Bufonidae)

(Appendix A10)

Locality: Malaysia, Penang

Description: Based on 1 specimen: Body 2,236 long by 646 wide; forebody, 630 long, representing 28% of body length. Mouth subterminal to terminal; oral sucker, longer than wide, 250 long by 240 wide; prepharynx absent; pharynx wider than long, 105 long by 130 wide, ratio of width of pharynx to width of oral sucker 1:1.8; esophagus not visible; ceca terminating well past posterior margin of ovary occupying 46% of postovarian space. Ventral sucker located in posterior section of upper third of body, longer than wide, 200 long by 190 wide, ratio of ventral sucker width to oral sucker width 1:1.3. Testes side by side, right testis, 117 long by 104 wide, left testis not visible. Genital pore prebifurcal, submedian located lower third of esophagus. Ovary oval to round, wider than long, 151 long by 187 wide, postovarian space 1,300 long, representing about 58% of body length. Uterus extensive, filling hindbody.Vitelline follicles distributed in lateral fields from level of upper margin of oral sucker to level of

cecal ends posteriorly. Eggs 43 (39–47) long by 21 (18–23) wide. Excretory system not visible.

Remarks: Specimen is twisted to right at pharynx and oral sucker. Although this specimen described above under NHM 1979.9.12 17–19 ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: longer forebody (970 compared to 630); larger ratio of pharynx width to width of oral sucker (1:2.2 [1:2.1–1:2.3]compared to 1:1.8); wider ventral sucker (300 wide compared to 200); vitelline follicles that terminate well short of cecal ends compared to vitelline follicles that near to surpassing cecal ends; smaller percentage of ceca that surpass ovary into postovarian space (31% compared to 46%); and wider ovary (267 compared to 187).

This specimens is similar to *M. monas* by having a: submedian genital pore; similar body length (1,835 [1,260–2,410] compared to 2,236); similar wider oral sucker (295 [230–360] compared to 240); wider pharynx (133 [100–166] compared to 130); similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.3); similar eggs (39 [34–44] long by 23 [21–25] wide compared to 43 [39–47] by 21 [18–23]); however these characteristics are shared with *M. americanum* (submedian genital pore, ratio of ventral sucker width to oral sucker width (1:1.6 [1:1.5–1:1.7]) as redescribed by Dronen *et al*. (Unpublished material, 2011).

Mesocoelim americanum is similar to this specimen by having a: similar body length (2,682 [1,325–4,038] long compared to 2,236); similar oral sucker width (284

[208–360] compared to 240); similar pharynx width (120 [75–165] compared to 130); similar ventral sucker width (182 [125–238] compared to 190); similar length of postovarian space (1,638 [740–2,626] compared to 1,300); and similar egg length (42 [39–45] compared to 43 [39–47]).

The differences between *M. americanum* and NHM 1979.9.12 17–19 are, *M. americanum* has a: larger ratio of width of pharynx to oral sucker width (1:2.5 [1:2.2–1:2.7] compared to 1:1.8); wider eggs (25 [23–26] compared to 21[18–23]); and smaller percentage of ceca that surpass the ovary into postovarian space (26% [25%–26%] compared to 46%).Without more quality museum specimen, it remains unclear if this specimen is an undescribed species of *Mesocoelium* or *M. americanum*.

NHM 1978.8.31 48-67

Host: Common Indian toad**,** common Asian toad, black-spined toad, *Bufo melanostictus* (Schneider) (Anura: Bufonidae)

Locality: Malaysia

Description: Based on 4 specimens: Body 1,352 (1,248–1,456) long by 371 (338–416) wide; forebody 385 (320–450) long, representing 28% of body length. Mouth subterminal to terminal; oral sucker round, 200 (190–221) long by 201 (192–210) wide; prepharynx absent; pharynx longer than wide, 90 (88–91) long by 86 (81–86) wide, ratio of width of pharynx to width of oral sucker 1:2.4 (2.3–2.4); esophagus 33 (0–65); ceca terminating well past posterior margin of ovary occupying 58% (53%–63%) of postovarian space. Ventral sucker located in posterior of upper third of body, longer than

wide, 148 (140–156) long by 133(120–146) wide, ratio of ventral sucker width to oral sucker width 1:1.5 (1:1.4–:1.6).Testes side by side, right testis, 108 (91–130) long by 96 (73–104) wide and left testis, 78 long by 117 wide. Genital pore prebifurcal and postphyngeal. Ovary oval to round, longer than wide, 135 (114–156) long by 111 (104–140) wide, postovarian space 390 (60-720) long, representing about 29% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of upper margin of oral sucker to level of cecal ends posteriorly. Eggs 35 (31–39) long by 21 (18–23) wide. Excretory system not visible.

Remarks: Specimens appear to have been fixed without coverslip pressure, altering the body shape, contracting the forebody and twisting of pharynx. No species could be determined, however these specimens described above under NHM 1978.8.31 48–67 have ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: longer body length (1,835 [1,260–2,410] compared to 1,352 [1,248–1,456]); larger forebody (970 compared to 385 [320–450]); wider oral sucker (295 [230–360] compared to 200 [190–221]); wider pharynx (133 [100–166] compared to 86 [81–91]); wider ventral sucker (300 compared to 133 [120–146]); vitelline follicles that terminate well short of cecal ends compared to vitelline follicles that terminate near to surpassing the cecal ends; shorter percentage of ceca that surpass ovary (31% compared to 58% (53%–63%) into the postovarian space); and wider ovary (267 compared to 111 [104–117]).

These specimens are similar to *M. monas* by having a: similar ratio of pharynx width to width of oral sucker (1:2.5 compared to 1:2.4 [1:2.3–1:2.4]); similar ratio of the ventral sucker width to oral sucker width (1:1.3 compared to 1:1.5 [1:1.4–1:1.6]); and similar eggs (39 [34–44] long by 23 [21–25] wide compared to 35 [31–39] by 21 [18–23]).

NHM 1980.7.14 50-54

(Figures 10A and 10B)

Host: Cane Toad, giant neotropical toad, marine toad **,** *Bufo marinus,* (Linnaeus) (Anura; Bufonidae)

Locality: Papua New Guinea

Description: Based on 2 specimens. Body elliptical, tapered posteriorly, 1,131 (1,404–2,784) long by 511 (62–962) wide; forebody 832 long, representing about 74% of body length. Mouth nearly terminal; oral sucker longer than wide, 223 (190–255) long by 22 (21–231) wide; prepharynx absent; pharynx wider than long, 66 (50–81) long by 91 (78–104) wide, ratio of width of pharynx to width of oral sucker 1:2.5 (1:2.2–1:2.7); esophagus not visible; ceca surpassing ovary posteriorly, occupying 42% (37%–47%) of postovarian space. Ventral sucker located third distance down body, wider than long, 146 (110–182) long by 154 (125–182) wide, ratio of width of ventral sucker to width of oral sucker 1:1.5 (1:1.3–1:1.7). Genital pore prebifurcal, submedian near level of posterior margin of pharynx. Ovary oval to round, longer than wide, 148 (114–182) long by 143 (91–195) wide; postovarian space 1,004 (161–1,846) long, representing about

89% or body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of anterior margin of oral sucker to level of cecal ends posteriorly. Eggs 33 (29–36) long by 21 (18–23) wide. Excretory system not visible.

Remarks: Although these specimens described above under NHM 1980.7.14 50–54 have ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: wider oral sucker (295 [230–360] compared to 221 [210–231]); wider pharynx (133 [100–166] compared to 91); wider ventral sucker (300 compared to 154 [125–182]); vitelline follicles that terminate well short of cecal ends compared to vitelline follicles that terminate near to surpassing the cecal ends; smaller percentage of ceca that surpass ovary (31% compared to 42% [37%–47%] into postovarian space); and wider ovary (267 compared to 143[91–195]).

These specimens are similar to *M. monas* by having a: submedian genital pore; similar body length (1,835 [1,260–2,410] compared to 1,144 [1,404–2,784]); similar forebody length (970 [about 53% of body length] compared to 832 [about 72% of body length); similar ratio of pharynx width to width of oral sucker (1:2.5 compared to 1:2.5 [1:2.2–1:2.7]); similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.5 [1:1.3–1:1.7]); and similar eggs (39 [34–44] long by 23 [21–25] wide compared to 33 [29–36] by 21 [18–23]); however, these characteristic are also shared with *M. monodi* (submedian genital pore, ratio of ventral sucker width to oral sucker width 1:1.6 [1:1.3–1:2.0] , and ratio of oral sucker to width of pharynx width 1:2.7 [1:2.7–1:2.8]).

Mesocoelium monodi is similar to these specimens by having a: similar body length (2,155 [1,310–3,000] compared to 2,094 [1,404–2,784]); and similar length of postovarian space (1,240 [800–1,680] compared to 1,004 [161–1,846]).

Given the high degree of similarity, these specimens may represent only one undescribed species; however, the variation in egg size and poor condition of the USNPC specimens make for considerable uncertainty.

Specimen 097835.00 from USNPC compared to these specimen (Appendix B2) both from Papa New Guinea have few different characteristics and many similar characteristics. Specimen 097835.00 is in poor condition which may have resulted in unreliable measurements. These specimens differ; shorter forebody (350 compared 832); ventral sucker width (109 compared to 154 [125–182]), larger ratio of ventral sucker width to oral sucker width (1:2.0 compared to 1:1.5 (1:1.3–1:1.7); and shorter postovarian space (650 compared to 1,004 [161–1,846]). These specimens are similar: similar body length (1,144 compared to [2,094 (1,404–2,784]); similar oral sucker width (218 compared to 221[210–231]); pharynx width (81compared to 91), ratio of width of pharynx to width of oral sucker (1:2.7 compared to 1:2.5 [1.2–1:2.7]); similar ovary width (148 compared to 143 [91–195]); vitelline follicles distributed in lateral fields from level of posterior margin of oral sucker to level past ovary posteriorly; and similar eggs size (36 [29–42] long by 20 [18–21] wide compared to 33[29–36] by 21 [18-23]). The amount of similar characteristics in theses specimens suggest they are the same undescribed species but the condition of the USNPC specimens make that difficult to confirm.

These specimens are most likely an undescribed species that is closely related to *M. monodi* as described by Dollfus (1929) that has been introduced to Papua New Guinea, possibly through the movement of its host, *Bufo marinus.*

With further comparisons of quality specimens a new species could be described but with the quality and number of these specimens, no species can be determined.

NHM 1977.9.28 6

Host: *Mabuya guinguataeniata*

(Appendix A11)

Locality: Rhodesia

Description: Based on 3 specimens: Body 1,924 (1,353– 2,496) long by 537 (416–702) wide; forebody 645 (560–730) long, representing about 34% of body length. Mouth near to terminal; oral sucker oval, 139 (47–230) long by 220 (210–230) wide; prepharynx absent; pharynx wider than long, 77 (73–80) long by 148 (86–210) wide, ratio of width of pharynx to width of oral sucker 1:2.3 (1:2.1–1:2.4); esophagus 107 (0–213); ceca terminating well past posterior margin of ovary occupying 38% in postovarian space. Ventral sucker located anterior portion of middle third of body, longer than wide, 179 (130–227) long by 155 (133–177) wide; ratio of ventral sucker width to oral sucker width 1:1.5 (1.3–1.6). Testes side by side, right testis, 138 (107–169) long by 132 (125–139) wide and left testis, 136 (107–164) long by 165 (156–174) wide. Genital pore prebifurcal, median, and located on mid-level of esophagus. Ovary oval to round, longer than wide, 124 (104–143) long by 122 (104–140) wide, postovarian space 1,031 (580–

1,482) long, representing about 54% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from mid-level of oral sucker to level of cecal ends posteriorly. Eggs 34 (29–39) long by 21(16–26) wide. Excretory system not visible.

Remarks: One specimen is clearly an older animal (far left on slide) than the others, as indicated by larger body size, developed eggs and amount of eggs in uterus compared to two other specimens. (Specimen 2 was unfit to be measured; ovary was outside body cavity due to extreme breakage of body wall on right side). Although these specimens described above under NHM 19877.9.28 6 have ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: submedian gential pore; larger forebody (970 [about 53% of body length] compared to 645 [560–730] [about 34% of body length); wider oral sucker (295 [230–360] compared to 220 [210–230]); wider ventral sucker (300 compared to 179 [130–227]); vitelline follicles that terminate well short of cecal ends compared to vitelline follicles that termiate nearly surpassing cecal ends; smaller percentage of ceca that surpass the ovary occupying (31% compared to 38%) in postovarian space); and wider ovary (267 compared to 122 [104–140]).

These specimens are similar to *M. monas* by having a: similar body length (1,835 [1,260–2,410] compared to 1,924 [1,352– 2,496]); similar pharynx width (133 [100–166] compared to 148 [86–210]); similar ratio of pharynx width to width of oral sucker (1:2.5 compared to 1:2.4 [2.3–2.]); similar ratio of ventral sucker width to oral sucker

width (1:1.3 compared to 1:1.5 (1.3-1.6); and similar eggs (39 [34–44] long by 23 [21–25] wide compared to 34 [29–39] by 21[16–26]); however, these characteristics are shared with *M. meggitti* (genital pore near mid-level of esophagus, ratio of ventral sucker width to oral sucker width 1:1.5, ratio of pharynx width to oral sucker width 1:2.0) as redescribed by Dronen *et al*. (Unpublished material, 2011).

Mesocoelium meggitti is similar to these specimens by having a: similar body size (2,090 [1,070–3,110] compared to 1,924 [1,352–2,496]); similar oral sucker width (260 [200–320] compared to 220 [210–230]; similar pharynx width (120 compared to 148 [86–210]); similar ventral sucker width (185 [130–240] compared to 155 [133–177]); similar length of postovarian space (1,340 [880–1,800] [about 64% of body length] compared to 1,031 [580–1,482][about 54% of body length]); and similar eggs (35[33–37] long by 25 [23–26] wide compared to 33 [29–36] by 21[16–26]). Slight variation in percentage ceca that surpass ovary into postovarian space (30% [28%–31%] compared to greater than 38%), separates these specimens from the redescription by Dronen *et al*. (Unpublished material, 2011).

NHM 1980.11.12 1–3

Host: Cane toad, giant neotropical toad, marine toad **,** *Bufo marinus,* (Linnaeus) (Anura; Bufonidae)

(Appendix A12)

Locality: Jamaica

Description: Based on 1 specimen: Body 1,768 long by 806 wide; forebody 520 long, representing about 29% of body length. Mouth subterminal; oral sucker, oval, 220 long by 221 wide; prepharynx absent; pharynx wider than long, 83 long by 99 wide, ratio of width of pharynx to width of oral sucker 1:2.2; esophagus not visible; ceca terminating well past posterior margin of ovary occupying 28 % in postovarian space. Ventral sucker located in posterior portion of upper third of body, wider than long, 160 long by 170 wide, ratio of ventral sucker width to oral sucker width 1:1.3. Testes diagonal, right testis, 120 long by 143 wide and left testis, 130 long by 120 wide. Genital pore prebifurcal, median located near to posterior margin of pharynx. Ovary oval to round, longer than wide,140 long by 130 wide, postovarian space, 990 long, representing about 56% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from posterior margin of oral sucker to level of ceca ends posteriorly. Eggs 35 (34–39) long by 20 (18–22) wide. Excretory system not visible.

Remarks: Although these specimens described above under NHM 1980.11.12 1–3 have ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: larger forebody (970 compared to 520); wider oral sucker (295 [230–360] compared to 221); wider pharynx (133 [100–166] compared to 83); wider ventral sucker (300 compared to 160); vitelline follicles that terminate well short of cecal ends compared to vitelline follicles that terminate near to surpassing cecal ends; wider ovary (267 compared to 130); submedian genital pore compared to median; and wider eggs (23 [21–25] compared to 18).

These specimens are similar to *M. monas* by having a: similar body length (1,835 [1,260–2,410] compared to 1,768); similar percentage of ceca that surpass ovary occupying (31% compared to 28%) in the postovarian space; similar ratio of pharynx width to width of oral sucker (1:2.5 compared to 1:2.2); similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.3); and similar eggs lengths (39 [34–44] compared to 33 (29–36); however these characteristics are also shared with *M. meggitti* (genital pore near mid-level of esophagus, ratio of ventral sucker width to oral sucker width 1:1.4 (1.3–1.5), ratio of pharynx width to oral sucker width 1:2.0) as redescribed by Dronen *et al*. (Unpublished material, 2011).

Mesocoelium meggitti is similar to these specimens by having a: similar body length (2,090 [1,070– 3,110] compared to 1,768); similar oral sucker width (260 [200–320] compared to 220); pharynx width (120 compared to 99); similar ventral sucker width (185 [130–240] compared to 170); and similar egg length (35 [33–37] compared to 33 [29–36]).

Slight differences in the egg measurement and shorter cecal lengths separate these specimens from the redescription suggesting this is an undescribed species; however, without more quality museum specimen that cannot be determined.

NHM 1994.6.21 3

Host: Mexican tree frog, Baudin's tree frog and Van Vliet’s frog, *Smilisca baudinii* (Duméril & Bibron)

(Appendix A13)

Locality: Mexico

Description: Based on 1 specimen: Body 2,262 long by 936 wide; forebody 780 long, representing about 34% of body. Mouth subterminal; oral sucker round, 270 long by 270 wide; prepharynx absent; pharynx wider than long, 94 long by 104 wide, ratio of width of pharynx to width of oral sucker 1:2.6; esophagus 81 long; ceca terminating well past posterior margin of ovary occupying 37 % in postovarian space. Ventral sucker located in anterior portion of two thirds of body, wider than long, 172 long by 187 wide, ratio of ventral sucker width to oral sucker width 1:1.4. Testes diagonal, right testis, 130 long by 143 wide and left testis, 117 long by 122 wide. Genital pore prebifurcal, submedian, and located near mid-level of esophagus. Ovary oval to round, longer than wide, 220 long by 200 wide, postovarian space long, 1,274 long, representing about 56% of body length. Uterus filling hindbody.Vitelline follicles distributed in lateral fields from anterior margin of oral sucker to nearly surpassing cecal ends; eggs 35(34–39) long by 20 (18–22) wide. Excretory system not visible.

Remarks: Specimen's pharynx and oral sucker are twisted possibly artificially pulling bifurcation to right, along with genital pore. Although this specimen described above under NHM 1994.6.21 3 has ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and is assigned to the *M. monas* body type, it cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: larger forebody (970 compared to 780); wider pharynx (133 [100–166] compared to 104); wider ventral sucker (300 compared to 172); and vitelline follicles

that terminate well short of cecal ends compared to vitelline follicles that terminate near to surpassing the cecal ends.

These specimens are similar to *M. monas* by having a: submedian genital pore; a similar body length (1,835 [1,260–2,410] long compared to 2,262); similar ratio of pharynx width compared to width of oral sucker (1:2.5 compared to 1:2.6); similar percentage of ceca that surpass ovary occupying (31% compared to 37%) in the postovarian space); similar wider oral sucker (295 [230–360] compared to 270); similar ratio of the ventral sucker width to oral sucker width (1:1.3 compared to 1:1.4); similar wider ovary (267 compared to 200); eggs (39 [34–44] long by (23 [21–25] wide compared to 35 [34–39] by 20 [18–22]); however these characteristics are shared with *M. americanum* (genital pore submedian, ratio of pharynx width to oral sucker width 1:2.5 [1:2.2–1:2.7], ratio of ventral sucker width to oral sucker width 1:1.6 [1:1.5–1:1.7]) as redescribed by Dronen *et al*. (Unpublished material, 2011).

Mesocoelium americanum is similar to this specimen by having a: similar body length (2,682 [1,325–4,038] compared to 2,262); similar oral sucker width (284 [208–360] compared to 270); similar pharynx width (120 [75–165] compared to 104); similar ventral sucker width (182 [125–238] compared to 187); and similar length of postovarian body space (1,638 [740–2,626] [about 61% of body] compared to 1,274 [about 55% of body]).

This specimen differs from *M. americanum* only in egg sizes. Egg dimensions are a key characteristic; therefore, this variation may demonstrate that this specimen is

an undescribed species rather than *M. americanum*. Without further specimens this remains problematic.

NHM 1980.11.12 4

Host: Cane toad, giant neotropical toad, marine toad **,** *Bufo marinus,* (Linnaeus) (Anura; Bufonidae)

(Appendix A14)

Locality: Jamaica, Kingston

Description: Based on 5 specimens; only 3 specimen measured: Body 1,378 (1,274–1,482) long by 615 (572–650) wide; forebody 430 (410-450) long, representing about 31% of body length. Mouth subterminal; oral sucker wider than long, 216 (200 –231) long by 228 (216–240) wide; prepharynx absent; pharynx longer than wide, 98 (91–104) long by 81 (78–83) wide, ratio of pharynx width to oral sucker width 1:2.8 (2.6–3.0); esophagus not visible; ceca surpassing ovary posteriorly, occupying about 37 % of postovarian space. Ventral sucker located in posterior section of upper third of body, longer than wide, 172 (166–177) long by 167 (156–177) wide, ratio of ventral sucker width to oral sucker width 1:1.3 (1:1.2–1:1.4). Testes adjacent, right testis, 96 long by 114 wide and left testis, 91 long by 117 wide. Genital pore prebifurcal, median located near level of posterior margin of pharynx. Ovary oval to round, wider than long, 100 (96–104) long by 112 (104–120) wide, postovarian space 94 (91–96) long, representing about 7% of body length. Uterus extensive, filling hindbody. Vitelline follicles

distributed in lateral fields from posterior margin of oral sucker to posterior margin of cecal ends. Eggs 34 (31–36) long by 21(18–24) wide. Excretory system not visible.

Remarks: All specimens forebody was contracted; only 3 specimens could be measured. Although these specimens described above under NHM 1980.11.12 4 have ceca that surpass the ovary posteriorly and a prebifurcal genital pore, and are assigned to the *M. monas* body type, they cannot be assigned to *M. monas*, as described by Rudolphi (1819) and redescribed by Freita (1958) because *M. monas* has a: submedian gential pore compared to a median; longer body (1,835 [1,260–2,410] long compared to 1,378 [1,274–1,482]); longer forebody (970 [about 53% of body length] compared to 430 [410–450] [about 31% of body length]); wider pharynx (133 [100–166] compared to 81 [78–83]); wider ventral sucker (300 compared to 167 [156–177]); vitelline fields terminating well short of cecal ends compared to vitelline fields that surpass the cecal ends; and wider ovary (267 compared to 112 [104–120]).

These specimens are similar to *M. monas* by having a similar ratio of the pharynx width to oral sucker width (1:2.5 compared to 1:2.8 [2.6–3.0]); a similar percentage of ceca that surpass the ovary occupying (31% compared to 37%) in the postovarian space; a similar oral sucker width (295 [230–360] compared to 228 [216–240]); a similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.3 [1:1.2–1:1.4]; and similar egg sizes? (39 [34–44] long by (23 [21–25] wide compared to 34 [31–36] by 21 [18–24]); however, these characteristics are shared with *M. burti* (genital pore prebifurcal, ratio of pharynx width to oral sucker width 1:2.0 and ratio of ventral sucker width to oral sucker width 1:1.5), originally described by Fernando, 1933.

Mesocoelium burti is similar to these specimens by having a similar body length (1,020 [980–1,060] compared to 1,378 [1,274–1,482]); a similar pharynx width (60 compared to 81 [78–83]); and a similar egg size (36 [35–36] long by 21[18–24] wide compared to 34 [31–36] by 21 [19–22]).

The characteristics that differ from *M.burti* and these specimens are not key characteristics and the localities of hosts (Sir Lanka and Jamaica) are similar; most likely theses specimens represent *M. burti*.

USNPC 095017.00

Host: Brown tree climber, diving lizard**,** *Uranoscodon superciliosus* (Linnaeus) (Squamata: Tropiduridae)

Locality: Brazil

Description: Based on 5 specimens: Body elongate, 1,612 long by 650 wide. Mouth subterminal; oral sucker, wider than long, 230 long by 320 wide; pharynx longer than wide, 119 long by 107 wide; ceca surpassing ovary posteriorly a short distance (exact ceca ends not visible). Gential pore prebifurcal, submedian and apparently at level of posterior one forth of oral sucker. Eggs 28–34 long by 21–23 wide.

Remarks: All these specimens were of poor quality, generally contracted and 1 was broken. The far left specimen on the slide was in the best condition, allowing for a few measurements and observations to be taken; however, the forebody of this specimen was contracted, which collapsed the esophagus displacing the cecal bifurcation anteriorly so that it nearly covered the pharynx, pulling the pharynx anterior end inside the oral

sucker. Also, the forebody of this specimen was twisted, resulting in the vitelline fields appearing to be continuously distributed across the forebody at the level of the esophagus.

Although I could not determine the effect of the poor condition of this specimen on my measurements, cannot be assigned to *M. monas* because the vitelline fields surpass both the ovary and the cecal that is visible, whereas in *M. monas* the vitelline fields do not surpass the ovary posteriorly and do not approach the cecal ends posteriorly. If the observation that the vitelline fields are continuous across the forebody, this would also be unlike in *M. monas* where the vitelline fields are completely separated from each other.

Like *M. monas* this specimen is from South America and the genital pore placement is submedian and postphyrangeal, placing it in the *M. monas* body type; however cannot be assigned to *M. monas*.

USPNC 081918.00

Host: Cane toad, giant neotropical toad, marine toad **,** *Bufo marinus,* (Linnaeus) (Anura; Bufonidae)

Locality: Tutulia Island, American Samoa

Description: Based on 13 specimens; measurements from 6 specimens: Body 949 (754-1,144) long; forebody 353 (330-375) long, representing about 37% of body length. Mouth subterminal; oral sucker longer than wide, 242 (234–250) long by 202 (161–242) wide; prepharynx absent; pharynx longer than wide, 106 (104–107) long by 91 (65–117)

wide, ratio of pharynx width to oral sucker width 1:2.3 (1:2.1–1:2.4); esophagus not visible; ceca surpass level of posterior margin of ovary, occupying approximately 15%-16% of the postovarian space (exact cecal ends not visible). Ventral sucker located in upper third of body, longer than wide, 195 (182–208) long by 182 (125–239) wide, ratio of ventral sucker width to oral sucker width 1:1.5 (1:1.0–1:1.2). Ventral sucker average testes 80 long by 130 wide. Genital pore prebifurcal, possibly submedian, located near to posterior margin of pharynx. Ovary oval to round, wider than long, 78 long by 115 (104–125) wide, postovarian space, 750 long, representing about 80% of body length. Uterus filling hindbody.Vitelline follicles distributed in lateral fields from level of posterior margin of oral sucker to level of cecal ends posteriorly. Eggs 34 (21–36) long by 20 (18–21) wide. Excretory system not visible.

Remarks: Specimens were in poor condition with the forebody severely contracted that it is possible the genital pore placement is altered, moving it from a typical location on or near esophagus, to in the oral sucker. Some specimens were also rolled, twisted, broken and poorly stained. The condition of these specimens may have altered measurements making comparisons difficult; however, they can be placed in the *M. monas* body type based on the ceca surpassing the ovary posteriorly and prebifurcal genital pore.

Based on the above description these specimens are most likely not *M. monas* because specimens of *M. monas* have a: longer body (1,835 [1,260–2,410] compared to 949 long); longer forebody (970 compared to 353); wider oral sucker (295 [230–360] compared to 202); wider pharynx (133 [100–166] compared to 91); wider ventral sucker

(300 compared to 182);.longer and wider eggs (39 [34–44] long by 23 [21–25] wide compoared to 34 [21–36] by 20 [18–20]); longer postovarian space (1,070 [about 58% of body length] compared to 750 [about 80% of body length); and wider ovary and testes (ovary 267 compared to 115; average testes 170 compared to 130).

These specimens are similar to *M. monas* by having a: submedian genital pore; similar ratio of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.5); and ratio of pharynx width to oral sucker width (1:2.5 compared to 1:2.3).

Specimen assigned to *M. pesteri* body type

USNPC 089067.00

Host: Brown anole**,** *Anolis sagrei* (Dumeril & Bibron) syn. *Norops sagrei* (Dumeril & Bibron) (Squamata: Polychrotidae)

Locality: Panama

Description: Based on 2 specimens: Body 923 (806–1,040) long by 585 (572–598); forebody 245 long, representing about 27% of body length. Mouth subterminal; oral sucker, wider than long, 216 (208–224) long by 246 (216–276) wide; prepharynx not visible; pharynx not visible; esophagus not visible; ceca terminating near anterior margin of ovary (exact cecal ends not visible, but do not pass ovary).Ventral sucker located in upper fourth of body, wider than long, 148 (135–161) long by 153 (120–182) wide, ratio of ventral sucker width to oral sucker width 1:1.7 (1:1.5–1:1.8). Testes not visible. Genital pore not visible. Cirrus sac not visible. Ovary not visible. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of upper

margins of oral sucker to posterior margin of ovary. Eggs 34 long by 23 wide. Excretory system not visible.

Remarks: Both specimens are in poor condition; forebody extremely contraction pulling oral sucker to within in micrometers of ventral sucker. Although these specimens described above under USNPC 089067.00 have ceca that do not surpass the ovary posteriorly and are assigned to the *M. pesteri* body type. The condition of these specimens precluded identification at the species level; however, egg size and general body morphology suggest that these specimens represent *M. pesteri*. It should be noted that the holotype of *M. pesteri* was in similar poor condition and extremely contracted. Similar to these specimens, the holotype measurements may have been altered because of the body condition, therefore an incorrect identification of a species may have been made.

These specimen are unlike *M. monas* and cannot be assigned to *M. monas* because *M. monas* has a: longer body (1,835 [1,260–2,410] compared to 923 [806–1,040]); larger forebody (970 compared to 245); wider oral sucker (295 [230–360] compared to 246); wider ventral sucker (300 compared to 153 [120–182]); ceca that terminate past posterior margin of ovary compared to cecal that terminate at anterior margin of ovary; and vitelline follicles that terminate well short of cecal ends compared to vitelline follicles that terminate past posterior margin of ovary.

These specimens are similar to *M. monas* by having a: similar ratios of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.7 [1:1.5–1:1.8]); and similar eggs (39 [34–44] long by (23 [21–25] wide compared to 34 by 23).

Specimen assigned to *M. lanceatum* body type

USNPC 084288.00

Host: American toad**,** *Anaxyrus americanus* (Frost *et al*.) syn *Bufo americanus* (Holbrook) (Anura: Bufonidae)

(Appendix A15)

Locality: Arkansas, USA

Description: Based on 1 specimen: Body 1,250 long by 470 wide; forebody 373 long, representing about 30% of body length. Mouth subterminal; oral sucker wider than long, 153 long by 161 wide; prepharynx absent; pharynx longer than wide, 50 long by 48 wide, ratio of pharynx width to oral sucker width 1:3.4; esophagus not visible; ceca terminating anterior to anterior margin of ovary. Ventral sucker located in upper third of body, longer than wide, 123 long by 115 wide, ratio of ventral sucker width to oral sucker width 1:1.4. Testes nearly tandem. Genital pore postbifurcal, submedian, and postpharyngeal. Cirrus sac, 150 long, representing about 12% of body length. Ovary oval to round, wider than long, 105 long by 110 wide, postovarian space, 620 long, representing about 50% of body length. Uterus extensive, filling hindbody. Vitelline follicles distributed in lateral fields from level of lower margin of pharynx to anterior margin not surpassing of ovary. Eggs 45 (44–45) long by 30 (29–30) wide. Excretory system not visible.

Remarks: The specimen was in poor condition; the edges are not flat and are severely folded on the right side, suggesting it may have been fixed without cover slip pressure. Although I could not determine the effect of the poor condition of this specimen on my

measurements, it cannot be assigned to *M. monas* because the vitelline fields surpass the ovary, whereas in *M. monas* the vitelline fields do not surpass the ovary posteriorly and do not approach the cecal ends posteriorly and the ceca do not surpass the ovary, whereas in *M. monas* the ceca surpass the ovary occupying 31% of postovarian space. This specimen, however can be assigned to the *M. lanceatum* body type because it has short ceca that do not surpass ovary and genital pore postbifurcal.

This specimen is unlike *M. monas* because *M. monas* has a prebifurcal genital pore compared to being postbifurcal; longer body (1,835 [1,260–2,410] compared to 1,250); longer forebody (970 [about 53% of body length] compared to 373 [about 30% of body length); wider oral sucker (295 [230–360] compared to 161); wider pharynx (133 [100–166] compared to 48); wider ventral sucker (300 compared to 115); vitelline follicles that terminate well short of anterior margins of the ovary compared to vitelline follicles that terminate past posterior margin of ovary; wider ovary (267 compared to 110); longer and wider eggs (39 [34–44] long by 23 [21–25] wide compared to 45 [44–45] by 30 [29–30]); ceca terminate posterior to ovary compared to ceca that terminate before posterior margin of ovary; and smaller ratio of pharynx width compared to width of oral sucker 1:2.5 compared to 1:3.4).

These specimens are similar to *M. monas* by having a submedian genital pore and similar ratios of ventral sucker width to oral sucker width (1:1.3 compared to 1:1.4); however, these characteristics are shared with *M. lanceatum* (ratio of ventral sucker width to oral sucker width 1:1.7 [1:1.6–1:1.7] and submedian genital pore).

Mesocoleium lanceatum is similar to this specimen by having a: similar ratio of oral sucker width to pharynx width (1:3.2 [1:3.0–1:3.4] compared to 1:3.4); and similar eggs (42[40-44] long by 25[23–27] wide compared to 45 [44–45] by 30 [29–30]).

Mesocoelium lanceatum unlike this specimen has a: longer body length (2,700 [2,100–3,300] compared to 1,250); wider oral sucker, (255 [240–270] compared to 161); wider pharynx (80 [70–90] compared to 48); wider ventral sucker 155 [160–150] compared to 123); and longer postovarian space (1,728 compared to 620).

To date, there has been no *Mesocoelium spp*. found in the Americas that have had short ceca. This specimen is the first report of a species where the ceca do not surpass the ovary posteriorly and it is likely that this specimen is either a new species of *Mesocoelium* or has been introduced into *Anaxyrus (Bufo) americanus* in Arkansas from the Old World. It appears that it may be a new species, but without additional museum quality specimens a new species should not be described.

Specimens of insufficient quality for identification to body type

The poor condition of the specimen listed below made it impossible to identify them to body type, and therefore, I could not compare them *M. monas*.

USNPC 092774.00

Host: Eigenmann's priondactylus, *Cercosaura eigenmanni* (Griffin) (Squamata: Gymnophthalmidae)

Locality: Brazil

USNPC 098814.00

Host: Large-scaled shade lizard, *Alopoglossus angulatus* (Linnaeus) (Squamata: Gymnophthalmidae)

Locality: Ecuador

USNPC 097844.00

Host: Thomson's toothless frog**,** *Genyophryne thomsoni* (Boulenger) (Anura: Microhylidae)

Locality: Papua New Guinea

USNPC 097835.00

Host: Wokan cannibal frog**,** *Lechriodus melanopyga (*Doria) (Anura: Limnodynastidae)

Locality: Papua New Guinea

USNPC 099607.00

Host: Papuascincus stanleyanus (Boulenger)

(Squamata: Scincidae)

Locality: Papua New Guinea

USNPC 087204.00

Host: Johnstone's whistling frog, *Electherodactylus johnstonei* (Barbour) (Anura: Eleutherodacylidea)

Locality: Burmuda

UNSPC 087355.00

Host: *Anolis hendersoni* (Cochran) (Squamata: Polychrotidae)

Locality: Hati

UNSPC 094097.00

Host: Golden skink, many-lined sun skink, many-striped skink, common sun skink, *Mabuya multifasciata* (Kuhl) (Squamata: Scincidae)

Locality: Philippines

4. SUMMARY AND CONCLUSIONS

Consideration of the sweeping synonomies of species of *Mesocoelium* proposed by Frietas (1963) (only 7 valid species) and Nasir & Diaz (1971) (only 4 valid species), and the suggestion of Goldberg *et al*. (2005), that "*Mesocoelium* is represented by a single species worldwide, *M*. *monas*", to me raised a fundamental question: Is it likely that a single species of fluke, such as *M*. *monas*, could have evolved to be the same species worldwide, or otherwise become cosmopolitan in its distribution, given its complex life cycle involving multiple susceptible host species (mollusk, as first and second intermediate hosts, then amphibian, fish or reptile definitive host?)

Pojmańka (2008) pointed out that there are at least 2 basic body morphologies in the genus, those where the ceca are short (not surpassing the ovary posteriorly) and those where the ceca are long (surpassing the ovary posteriorly). Cecal length, especially where differences are of the magnitude pointed out by Pojmańka (2008), has been considered a strong characteristic in nearly all fluke genera and is routinely used in their taxonomic classification. Dronen *et al*. (Unpublished material, 2011) refined the observations of Pojmańka (2008) by identifying 3 body patterns or types in those species where the ceca do not surpass the ovary posteriorly: the *M*. *pesteri* type (where the genital pore is prebifurcal), the *M*. *zhejiangensis* type where the genital pore is bifurcal) and the *M*. *lanceatum* type where the genital pore is postbifurcal), and 3 body patterns or types in those species where the ceca surpass the ovary posteriorly: the *M*. *monas* type (where the genital pore is prebifurcal), the *M*. *sociale* type (where the genital pore is bifurcal) and the *M*. *carli* type (where the genital pore is postbifurcal). Genital pore

placement in relation to the cecal bifurcation in terms of being above the cecal bifurcation, at the level of the cecal bifurcation, or below the cecal bifurcation is a universally used characteristic in flukes in general.

The specimens of *M. monas* I examined represented only 3 of the 6 body types identified by Dronen *et al*. (Unpublished material, 2011): the *M. monas* body type, the *M. lanceatum* body type, and the *M. pesteri* body type. The *M. lanceatum* body type was represented by only 1 specimen (USNPC 084288.00), which was collected from an American toad from Arkansas, U.S.A. This represents an unusual finding, as species of *Mesocoelium* in which the ceca do not surpass the ovary posteriorly have been previously reported only from only the Old World, specifically eastern Asia, eastern India, Indonesia and Africa. This specimen appears either to be young (having few eggs present in the uterus, abnormally developed internal organs and a small body size) or its growth may have been stunted by being in an unsuitable host. However, it fits the description of *M. lanceatum*, suggesting that it likely was introduced into North America from Southern Japan.

The *M. pesteri* body type was also represented by only 2 specimens (USNPC 089067.00), both collected from a brown anole from Panama. Here again, species of *Mesocoelium* in which the ceca do not surpass the ovary posteriorly have been reported previously from only the Old World, and for this body type this has been the only description of *M. pesteri* from central Africa. Although these specimens were of poor quality and a specific identification was not possible, they generally fit the description of

M. pesteri, including egg size, suggesting that this is also a case of importation from the Old World.

Although the remaining 85 (exclusive of the 77 where the quality of the specimens precluded identification of the body type) specimens that I examined, which were previously identified as *M. monas*, were assigned to the *M. monas* body type, none of these specimens were *M. monas*.

Another characteristic commonly used to distinguish species of flukes is the location of the genital pore in reference to the midline of the body, that is, either on the midline (median) or off the midline (submedian). Members of the *M. monas* body type can readily be divided into 2 groups, those where the genital pore is median (*Mesocoelium mesembrinum* Johnston, 1912; *Mesocoelium dubium* Yuen, 1965; *Mesocoelium oligoon* Johnston, 1912, *Mesocoelium cameroonensis* Saoud, 1964; *Mesocoelium scatophagi* Fischthal & Kuntz 1965; *Mesocoelium micron* Nicoll, 1914; *Mesocoelium symbimorphi* Ruiz & Leão, 1943; *M. burti*; *Mesocoelium marsi* Fernando, 1933; *M. meggetti*; and *M. travassosi* Pereira & Cuocola, 1940) and those where the genital pore is submedian (*Mesocoelium gabonensis* Maeder, Combes & Knoepffler, 1969; *Mesocoelium megaloon* Johnston, 1912; *Mesocoleium schwetzi* Dollfus. 1950; *M. monas*; *Mesocoelium melanosticti* Rao, 1989; *Mesocoelium assymmetrovitellarius* Kumari & Verma, 1992; *M. malayanum*; *Mesocoelium georgesblanci* Dollfus, 1954; *M. crossophorum*; *Mesocoelium monodi*; *M. danforthi*; *Mesocoelium brachyenteron* Dollfus, 1954; and *Mesocoelium americanum* Harwood, 1932).

In addition, both the *M. sociale* and *M. carli* body types can also be divided into 2 basic groups, those species with a median genital pore and those with a submedian genital pore. Based only on the proven characteristics described above (length of ceca, position of the genital pore), it is my opinion that there can be no less than 9 species (*M. lanceatum*, *M. zhejiangensis*, *M. pesteri*, *M. mesembrinum*, *M. monas*, *M. brieni*, *M. sociale*, *M. leiperi*, and *M. carli*) of *Mesocoelium* (6 body types, 3 of which can be divided into 2 basic groups based on the median or submedian placement of the genital pore).

My comparative studies of the available literature, including original descriptions and redescriptions of previously recognized species of *Mesocoelium*, and examination of specimens of *Mesocoelium*, including those previously identified as *M. monas* from the holdings of the UNSPC and the NHM, support the proposal that there are likely more than 9 species of *Mesocoelium* worldwide and that the *M. monas* complex, as currently defined, is not a unified monospecific grouping.

Species of *Mesocoelium* tend to have similar morphologies, and therefore, there is a limited number of characteristics that can be used reliably to separate species in the genus. While this is a difficult group in which to distinguish species, and while it may be only a matter of convenience in surveys of amphibians, fish and reptiles for endohelminths to consider any specimen found of *Mesocoelium* to be *M. monas*, I have not encountered the large ranges of variability in the characteristics typically used in most other fluke genera to separate species that have previously been used to support

large scale synonymies of species in *Mesocoelium* proposed by some researchers (e.g. Frietas 1963; Nasir & Diaz 1971).

In addition to the characteristics discussed above, there are a number of additional characteristics that are routinely applied to flukes at the species level. The size of eggs is universally used as a species characteristic in nearly every genus of digenetic trematode, and it has been commonly used in *Mesocoelium*. There are some drawbacks to using egg size in *Mesocoelium*: 1. It appears that freshly produced eggs in the proximal end of the uterus grow as they move towards the distal end of the uterus and the outer protein shell solidifies. 2. I have also noticed that there are nearly always deformed, atypical eggs present in the uterus of species of *Mesocoelium*. 3. Eggs in the uterus are often not in a flat plane, causing them to appear abnormally short, which potentially could provide inaccurate measurements. Any of these drawbacks singularly, or in concert, can provide false measurements of eggs and may be a source of the unusually large ranges for length and width of eggs reported for many species in the genus. Such large ranges reduce the effectiveness of this characteristic in distinguishing species, and I recommend that only fully mature, undamaged, normal-appearing eggs that are in a flat profile be chosen for measurement when species are described or redescribed.

Ratios of sucker width are commonly used to separate species of Digeneans, because these ratios tend to be consistent within a species. I found that although the ratio of the ventral sucker width to the oral sucker width was consistent within species, it was often too similar to those of other species to be used effectively as a distinguishing

characteristic by itself. For example, comparing slide USNPC 090335.00 (ratio of 1:1.4), USNPC 092800.00 (ratio of 1:1.2) and NHM 1980.11.12 1–3 (ratio of 1:1.5 [1:1.3–1:1.6]), all of which are *M. meggitti*, even though USNPC 092800.00 is not a perfect match. More importantly, all species identified within this research have similar sucker width ratios: *M. americanum* (1:1.6 [1:1.5–1:1.7], *M.crossophorum* (1:1.3), *M.burti* (1:1.3), *M. lanceatum* (1:1.7 [1:1.6–1:1.7] and *M. meggetti* (1:1.5). I found it useful to use the ratio of the pharynx width to the oral sucker width in concert with the sucker ratio because it appeared also to be consistent and in many case was a good distinguishing characteristic for species. This characteristic has been previously used effectively in other groups of digeneans, but had apparently not been applied to *Mesocoelium*.

The distribution of the vitelline fields is a characteristic that although effectively used in other groups of Digenea, has been considered to be too variable within species to be used in *Mesocoelium* (e.g. Frietas 1963; Nasir & Diaz 1971). When comparing groups of specimens from the same host where I was confident that only one species was represented, I found that the posterior extent of the vitelline fields was a consistent and effective characteristic to separate species. It appears to me that some confusion in the literature concerning the variability of this characteristic may be due to species being described using specimens representing more than 1 species (e. g. Dollfus 1950 – *M. schwetzi*); redescriptions being based on specimens that did not represent the original species (e.g. Pereira & Cuocolo 1940 – *M. incognitum*; Yuen 1965 – *M. sociale* and *M. incognitum*; Maeder *et* al. 1970 and Nasir & Diaz 1971 – *M. monas*); revisions of the

genus being carried out using specimens from more than 1 species to define a species (Frietas 1963 – revision of Mesocoeliidae); species being described using young, postmetacercarial, but mature, specimens to describe a species (Fernando 1933 – *M. marrsi*); and, occasionally specimens of a given species may have an atypical condition where the vitelline fields may be less extensive on side, or even have 1 of the lateral fields missing (Kumari & Verma, 1992 - *M. assymmetrovitellarius*). I generally expected to see some variability in characteristics like the extent of the 2 vitelline fields, but found that using the longer extent of the vitelline fields expressed in 1 of these atypical specimens usually allowed an effective use of this characteristic.

Although not used much in *Mesocoelium*, I found that the relative lengths of the cirrus sac, forebody and the postovarian space, expressed as a percentage of the body length to accommodate differential growth, showed little variability and provided an effective characteristic in separating species in the genus. I used body size (length) only where specimens were obviously fully mature and where there were large differences in lengths. In my observations, I noted enough inconsistency in specimens of the same species being spinose or aspinose that I did not use this characteristic. Some specimens had the body spines in the mounting medium around the specimen, but none were attached to the tegument. The use of the ratio of the male and female gonads was also not used. Of all the features I examined, the size of the 2 testes was the most variable, and therefore the ratio of the ovary width to the mean width of the testes was not used. I did note that there was the atypical tendency in some species to have an ovary that was larger than the testes, but this was inconsistent, and in my view not reliable. Generally, I

found it best not to rely on any one characteristic, but rather tried to use combinations of characteristics to distinguish species where ever possible.

The biggest problem I encountered, as is obvious from my results section above, was the quality of the specimens available. My experience with the illustrations and, descriptions from the existing literature and specimens from museums leaves no doubt in my mind that this has been probably the most important factor in the misinterpretation of specimens and their misidentification. Beyond damaged and broken specimens, many of the specimens from museums appeared to be poorly fixed without relaxation and/or the application of a minimal amount of coverslip pressure, resulting in contraction of specimens. Some specimens were contracted to such an extent that they were pyriform in shape rather than having the elliptical or elongate shape that is more typical of these ventro-dorsally flattened organisms, suggesting that they were likely removed from a host that previously had been fixed and not removed live from a freshly-dispatched host. Contraction of the body of a specimen pulls the uterus forward, which often covers the gonads, female reproductive system, cecal ends, and the excretory system, making examination and measurement of these structures difficult at best.

This is especially critical in determining the location of the genital pore. If for example, the forebody of a specimen is contracted, the esophagus is generally collapsed, pulling the cecal bifurcation and the ventral sucker anteriorly towards the oral sucker and causing the pharynx to become tilted or even pulled up into the oral sucker. Such conditions mask the true location of the genital pore anteriorly or posteriorly in reference to the cecal bifurcation, and in many cases make it impossible to see the genital pore at

all. Inappropriately fixed specimens also often roll right or left, moving structures like the genital pore that are associated with the ventral surface 1 direction while shifting the structures that are deeper and nearer the dorsal surface of the body the opposite direction. This makes it difficult to determine if the genital pore is median or submedian. Some specimens were apparently over flattened (nearly squashed) during fixation, which spread out all the internal organs and distorted the eggs.

Nearly all potential characteristics will likely appear abnormal when specimens are not collected alive, and correctly relaxed and heat-fixed using a proven technique. Since the fixation techniques are critical to provide specimens that are suitable to analyses of characteristics and to distinguish species in *Mesocoelium*, I have provided an acceptable and application-validated method in Appendix C.

The body-type keys developed by Dronen *et al*. (Unpublished material, 2011) used cecal length (either surpassing the ovary or not surpassing the ovary) and location of the genital pore (either prebifurcal, bifurcal or postbifurcal) as the key characteristics to separate species into the 6 body types. Each body type key then utilized the genital pore placement dextral or sinistral to the body midline as the major characteristic to establish 2 subgroups in each body type. The subgroups were then further broken down using a combination of the key characteristics, as discussed above to distinguish species. These keys proved to be effective in distinguishing species, even when specimens were in poor condition.

I suggest further research needs to be done by reviewing all *Mesocoelium* spp currently in collection and making adjustments to each of these principles. I believe that

this study would show *M. monas* to be found in only a few hosts, and to have a much narrower distribution worldwide than is currently suggested. I believe that *M. monas* is not as common as previously thought, since none of the 86 specimens I examined was *M. monas.*

This study was a morphological evaluation of specimens and in my opinion could be strengthened by additional molecular studies. I feel it would be most beneficial to carry out a comparative study using both molecular and morphological techniques to confirm morphological species identifications and clarify the existing confusion in this genus.

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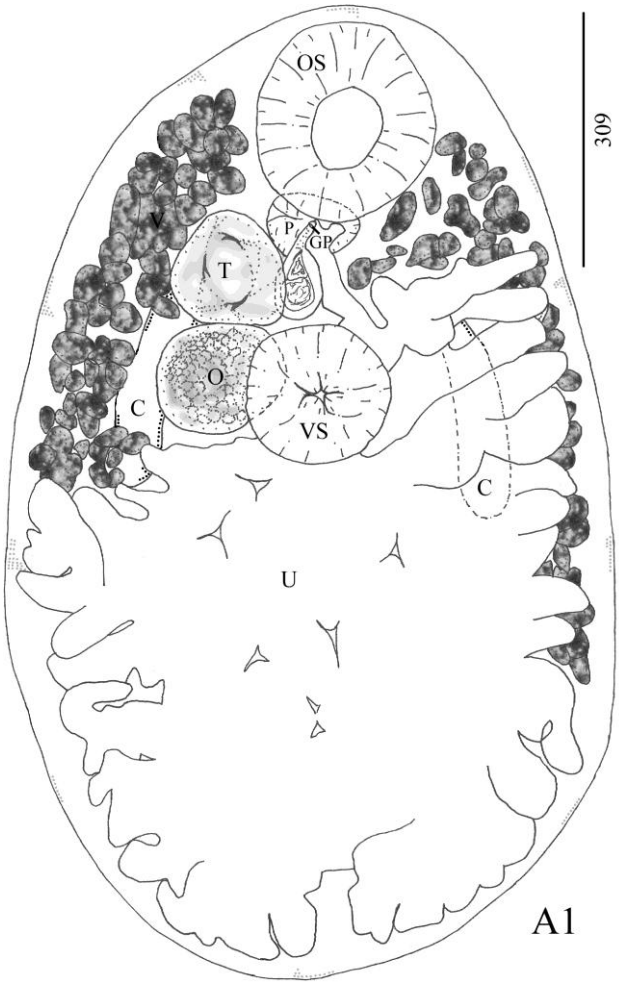
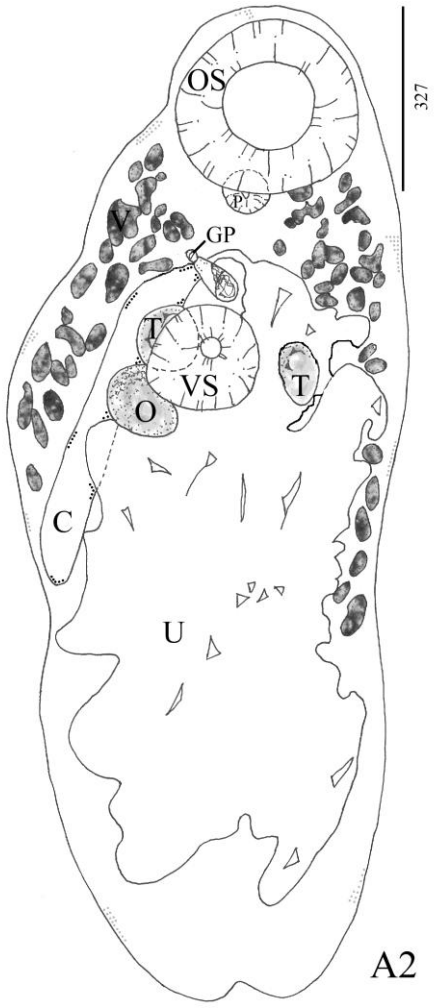
APPENDIX A

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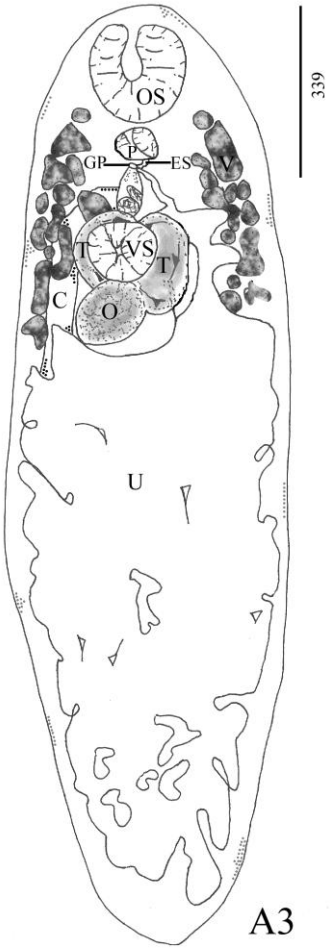
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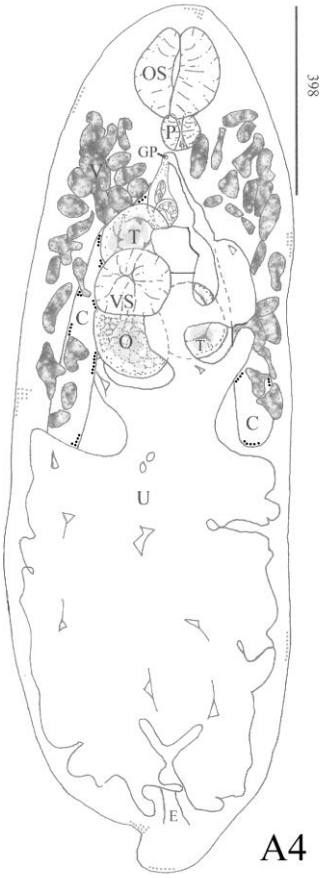
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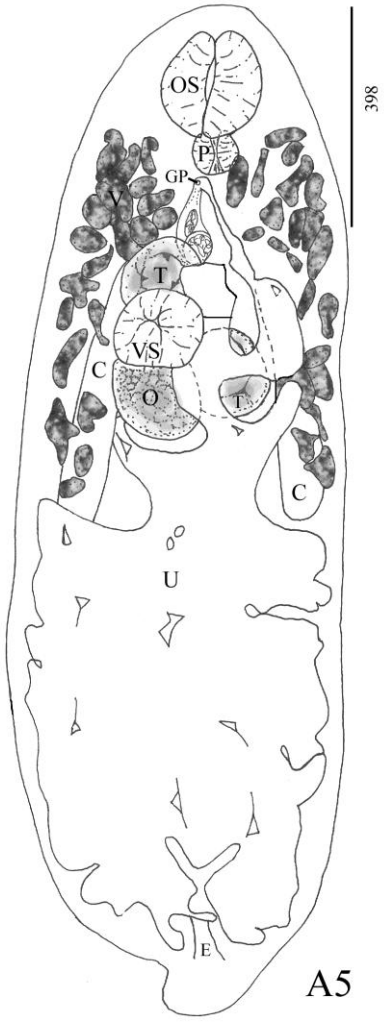

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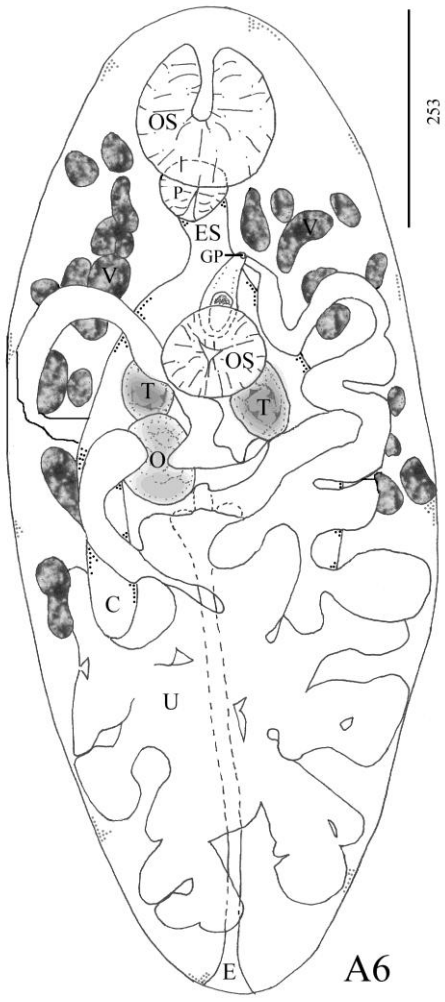
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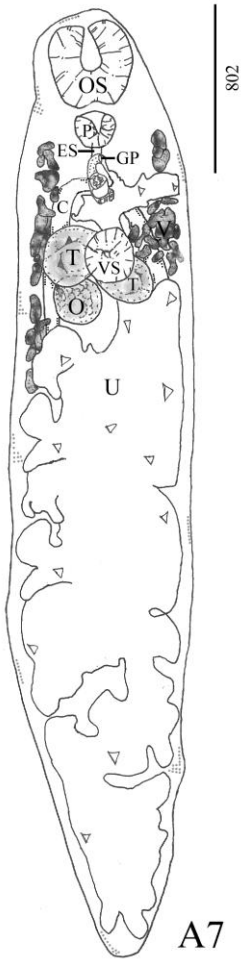
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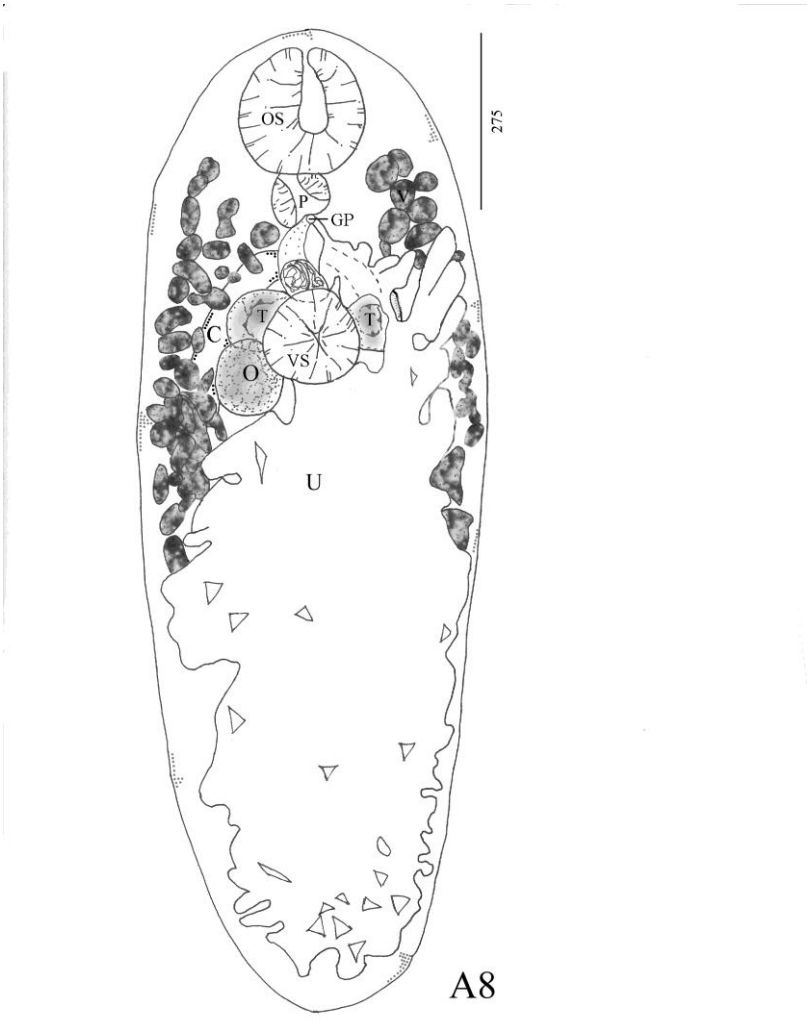
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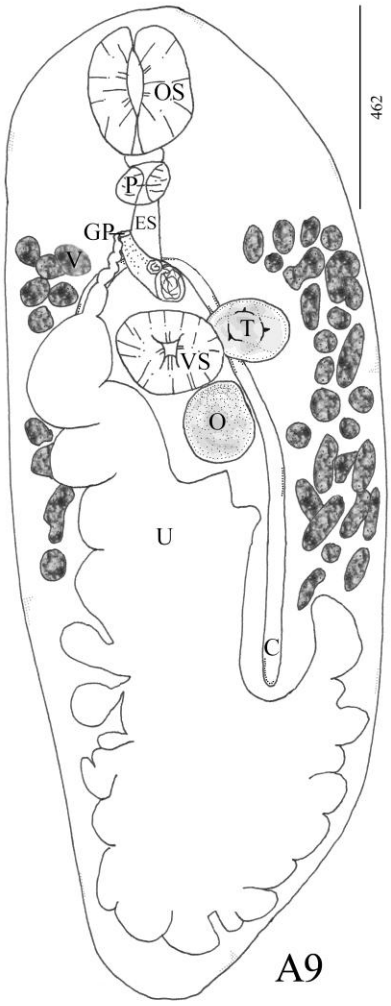
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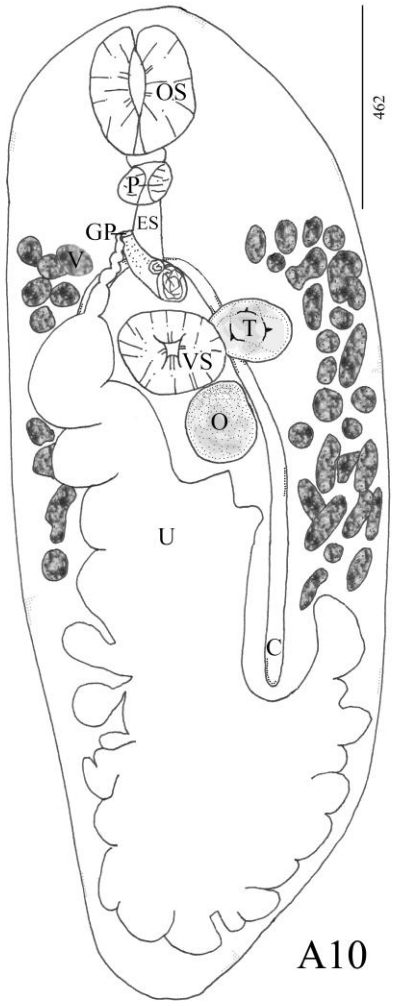
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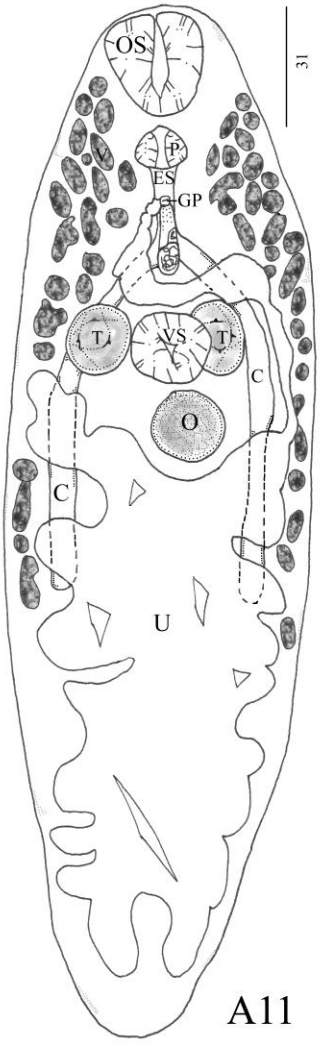

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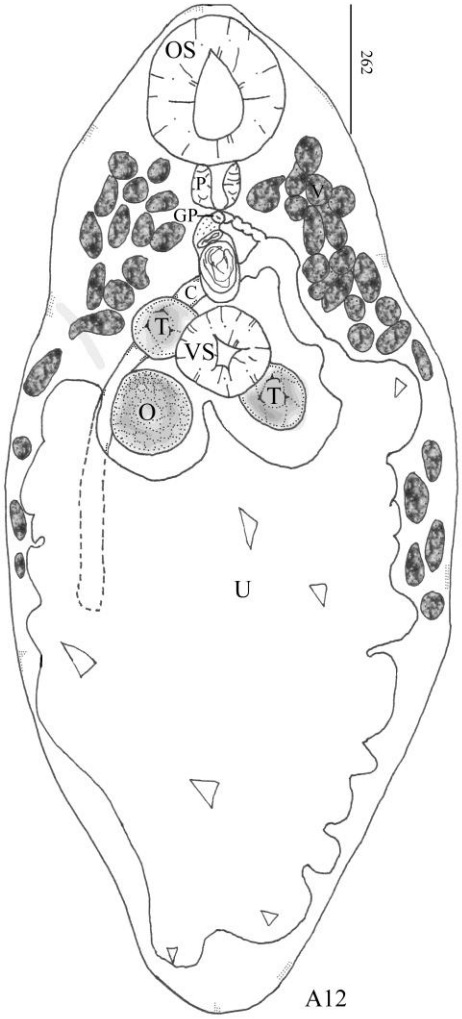
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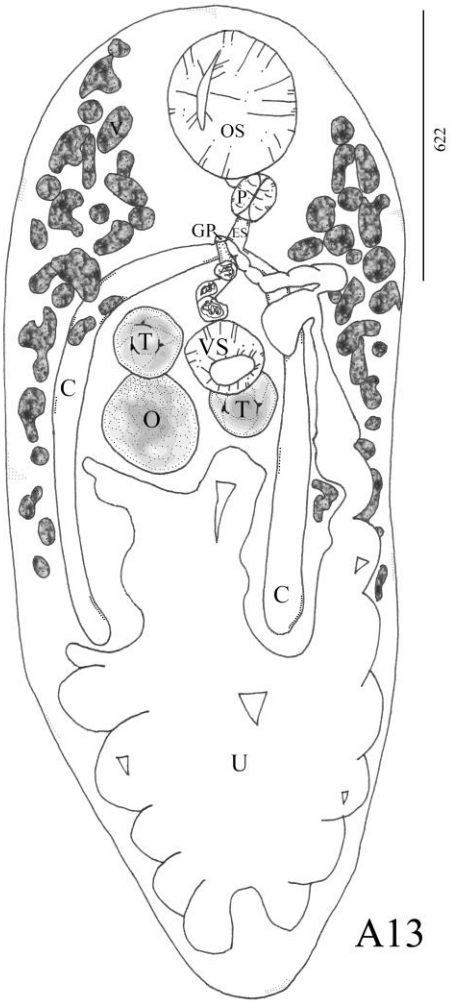
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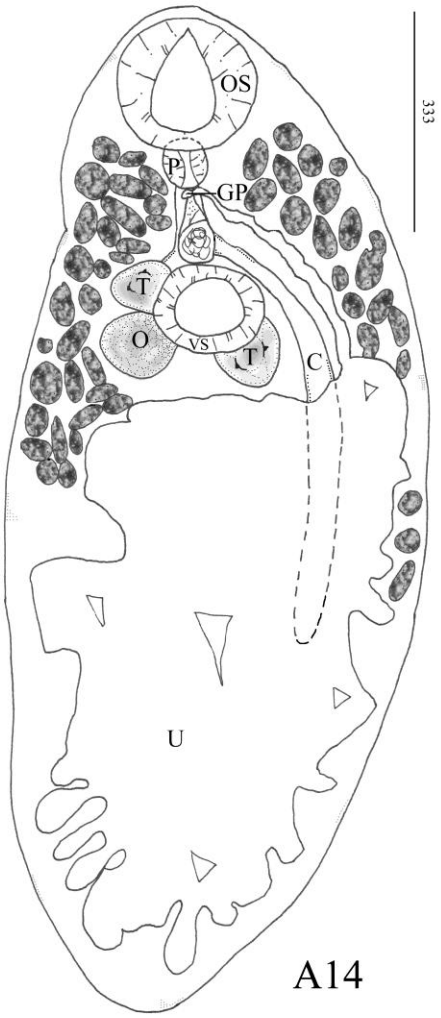
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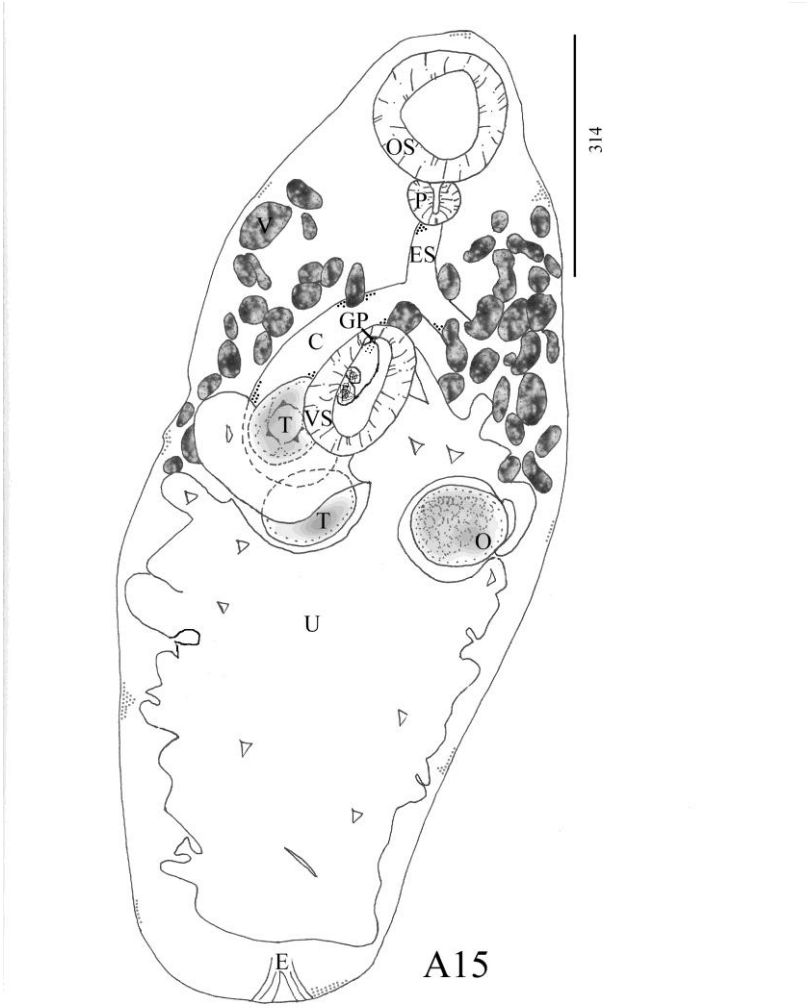
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APPENDIX B

Table B1: Identified Species of *Mesocoelium* Including Original Host(s), Locality and Reference.

Species	Host	Locality	Reference
M. americanum	*Storeria dekayi*, *Leiolopisma laterale*, *Eumeces fasiatus*	South-central U.S.A	Hardwood 1932
M. meggitti	*Mabuia dissimilis*	Union of Myanmar	Bhalerao 1927
M. lanceatum	*Tylototrition andersoni*	Southern Japan	Goto & Ozaki 1930
M. crossophorum	*Bufo peltacephalus*	Cuba	Perez Veigueras 1942
M. burti	*Polypedates maculates*	Sri Lanka	Fernando 1933

Table B2: Measurements and morphometic ratios for species of *Mesocoelium* collected in Papua New Guinea from National History Museum (NHM) and United States Parasitology Collection (USNPC).

	USNPC 097835.00 n=1	USNPC 099607.00 n=3	USNPC 097844.00 n=3	NHM 1980.7.14 50–54 n=2
Body Length (um)	1,144	728 (494–962)	1,235 (1,170–1,300)	2,094 (1,404–2,784)
Forebody Length	350	240	450	832
Oral Sucker Width	218	211 (190–231)	222 (221–223)	221 (210–231)
Oral Sucker Length	182	180 (169–190)	232 (192–273)	223 (190–255)
Pharynx Width	81	82 (81–83)	91	91 (78–104)
Pharynx Length	42	75 (68–81)	73	66 (50–81)
Ventral Sucker Width	109	153	183 (157–208)	154 (125–182)
Ventral Sucker Length	112	164	172 (138–205)	146 (110–182)
Esophagus Length	NV	NV	NV	111 (104–117)
Ovary Width	148	130	94	143 (91–195)
Ovary Length	138	117	88	148 (114–182)
Average Testes Width	NV	140	NV	130 (91–169)
Average Testes Length	NV	100	NV	155 (104–205)
Cirrus Sac Length	122	NV	NV	NV
Postovarian Space	650	500	NV	1,004 (161–1,846)
Cecal Percentage	NV	NV	NV	42% (37%–47%)
Average Egg Width	20 (18–21)	26 (23–29)	25 (21–29)	21 (18–23)
Average Egg Length	36 (29–42)	39 (34–44)	39 (34–44)	33 (29–36)
Oral Sucker Width/ Ventral Sucker Width	1:2.0	1:1.5	1:2.4	1:1.5 (1.3–1.7)
Oral Sucker Width/ Pharynx Width	1:2.7	1:2.5 (2.3–2.7)	1:1.2 (1.1–1.3)	1:2.5 (2.2–2.7)
Host Species	*Lechriddus melanopyga*	*Papuascinus stanleyanus*	*Genyophryne thomsoni*	*Bufo marinus*
	Papua New Guinea	Papua New Guinea	Papua New Guinea	Papua New Guinea

Table B3: Measurements and morphometic ratios for species of *Mesocoelium* from United States Parasitology Collection (USNPC).

	USNPC 085478.00 n=1	USNPC 092800.00 n=2	USNPC 089067.00 n=2	USNPC 084288.00 n=1	USNPC 087204.00 n=1	USNPC 087355.00 n=1	USNPC 094097.00 n=2	USNPC 097600.00 n=1
Body Length (um)	1,738	1,846	923 (806–1,040)	1,250	2,678	1,092	1,378 (1,300–1,456)	560
Forebody Length	550	340	245	373	NV	300	360	NV
Oral Sucker Width	250	160	246 (216–276)	161	NV	255	238 (229–247)	130
Oral Sucker Length	250	160	216 (208–224)	153	NV	NV	275 (268–281	138
Pharynx Width	62	68	NV	48	NV	NV	110 (107–112)	39
Pharynx Length	52	52	NV	50	NV	NV	98 (96–99)	31
Ventral Sucker Width	227	133	153 (120–182)	115	NV	NV	181 (179–182)	81
Ventral Sucker Length	200	135	148 (135–161)	123	NV	NV	177 (169–185)	61
Esophagus Length	NV	21	NV	NV	NV	NV	NV	NV
Ovary Width	93	143	NV	110	NV	187	NV	39
Ovary Length	115	117	NV	105	NV	NV	NV	68
Average Testes Width	95	129 (122–135)	NV	110	NV	167 (146–187)	NV	42 (34–49)
Average Testes Length	88	151 (143–159)	NV	75	NV	NV	NV	75 (68–81)
Cirrus Sac Length	125	143	NV	150	NV	NV	143	NV
Postovarian Space	1,000	1,170	NV	620	NV	620	NV	273
Cecal Percentage	22%	~20% at least	NV	50%	NV	1%	NV	32%
Average Egg Width	29 (28–29)	22 (21–23)	23	30 (29–30)	22 (21–23)	23 (21–25)	22 (21–23)	22 (20–23)
Average Egg Length	46 (43–48)	31 (27–35)	34	45 (44–45)	33 (31–35)	29 (24–34)	33 (31–34)	36 (35–36)
Oral Sucker Width/Ventral Sucker Width	1:1.1	1:1.2	1:1.7 (1.5–1.8)	1:1.4	NV	NV	1:3.5 (1.3–1.4)	1:1.6
Oral Sucker Width/Pharynx Width	1:4.0	1:2.4	NV	1:3.4	NV	NV	1:2.1 (2.0–2.1)	1:3.3
Host Species	*Anolis gingivinus*	*Anolis lionotus*	*Anolis sagrei*	*Anazyrus americanus*	*Eleutherodactylus johnstonei*	*Anolis hendersoni*	*Mabuya multifasciata*	*Eleutherodactylus coqui*
Locality	Caribbean USA	Panama	Hawaii	AR	Bermuda	Haiti	Philippines	Hawaii

Table B4: Measurements and morphometic ratios for species of *Mesocoelium* from United States Parasitology Collection (USNPC).

	USNPC 092774.00 n=1	USNPC 095017.00 n=5	USNPC 098814.00 n=1	USNPC 081918.00 n=13	USNPC 097844.00 n=3	USNPC 097835.00 n=1	USNPC 099607.00 n=3	USNPC 098269.00 n=10
Body Length (um)	754	1,612	910	949 (754–1,144)	1,235 (1,170–1,300)	1,144	728 (494–962)	1,365 (1,196–1,534)
Forebody Length	NV	400	260	353 (330–375)	450	350	240	335 (240–420)
Oral Sucker Width	NV	320	208	202 (161–242)	222 (221–223)	218	211 (190–231)	269 (213–325)
Oral Sucker Length	NV	230	190	242 (234–250)	232 (192–273)	182	180 (169–190)	256 (221–290)
Pharynx Width	NV	107	62	91 (65–117)	91	81	82 (81–83)	103 (99–107)
Pharynx Length	NV	119	65	106 (104–107)	73	42	75 (68–81)	90 (88–91)
Ventral Sucker Width	NV	NV	177	182 (125–239)	183 (157–208)	109	153	166 (99–232)
Ventral Sucker Length	NV	239	169	195 (182–208)	172 (138–205)	112	164	135 (109–160)
Esophagus Length	NV	205	104	NV	NV	NV	NV	NV
Ovary Width	NV	NV	NV	115 (104–125)	94	148	130	165 (140–190)
Ovary Length	NV	NV	NV	78	88	138	117	155 (140–170)
Average Testes Width	NV	NV	NV	130	NV	NV	140	184 (117–250)
Average Testes Length	NV	NV	NV	89 (73–104)	NV	NV	100	178 (100–255)
Cirrus Sac Length	NV	NV	78	NV	NV	122	NV	139 (138–139)
Postovarian Space	NV	NV	NV	750	NV	650	500	78
Cecal Percentage	NV	NV	NV	NV	NV	NV	NV	NV
Average Egg Width	NV	22 (21–23)	22 (21–23)	20 (18–21)	25 (21–29)	20 (18–21)	26 (23–29)	19 (17–21)
Average Egg Length	NV	31 (28–34)	35 (34–36)	34 (31–36)	39 (34–44)	36 (29–42)	39 (34–44)	36 (30–42)
Oral Sucker Width/ Ventral Sucker Width	NV	NV	1:1.2	1:1.1 (1.0–1.2)	1:2.4	1:2.0	1:1.5	1:2.7
Oral Sucker Width/ Pharynx Width	NV	NV	1:3.4	1:2.4 (2.1–2.4)	1:1.2 (1.1–1.3)	1:2.7	1:2.5 (2.3–2.7)	1:3.1 (3.0–3.2)
Host Species	*Prionadactylus eigenmanni*	*Uranoscodon superoiliosus*	*Alopoglossus angulatus*	*Bufo marnius*	*Genyophryne thomsoni*	*Lechriddus melanopyga*	*Papuascinus stanleyanus*	*Sphenomorphus cherrieri*
Locality	Brazil	Brazil	Ecuador	Tutulia Island	Papua New Guinea	Papua New Guinea	Papua New Guinea	Costa Rica

Table B5: Measurements and morphometic ratios for species of *Mesocoelium* from United States Parasitology Collection (USNPC).

	USNPC 092185.00 n= 5	USNPC 079344.00 n=1	USNPC 072881.00 n=1	USNPC 090335.00 n=1	USNPC 090332 n=1	USNPC 090333.00 n=1	USNPC 090334.00 n=1	USNPC 098733.00 n=6	USNPC 098763.00 n=1
Body Length (um)	1,807 (1,716–1,898)	2,050	1,175	2,808	1,456	1,950	1.950	1,131 (962–1,300)	1,144
Forebody Length	470	676	520	700	460	500	550	364 (312–416)	450
Oral Sucker Width	175 (167–182)	213	175	260	169	270	250	241 (208–273)	208
Oral Sucker Length	185 (195–172)	203	160	290	172	263	260	224 (195–252)	218
Pharynx Width	79 (75–83)	108	78	143	78	109	125	83 (57–109)	60
Pharynx Length	67 (52–81)	85	78	122	75	101	99	88 (80–96)	78
Ventral Sucker Width	131 (122–140)	185	140	192	143	161	208	201 (175–226)	148
Ventral Sucker Length	133 (123–143)	163	149	198	140	179	208	183 (157–208)	161
Esophagus Length	NV	260	39	NV	NV	NV	NV	NV	NV
Ovary Width	127 (107–146)	138	96	160	85	150	150	165 (140–190)	125
Ovary Length	158 (146–169)	138	100	170	100	160	130	125 (109–140)	101
Average Testes Width	165 (141–189)	132	77	175	101 (101–120)	135 (120–150)	150–160	170 (106–234)	NV
Average Testes Length	184 (173–195)	138	80	190	99 (96–104)	135 (130–140)	155 (150–160)	143 (80–205)	NV
Cirrus Sac Length	149 (141–156)	200	108	203	99	164	NV	87 (70–104)	NV
Postovarian Space	1,150 (1,020–1,280)	1,120	605	1,742	790	1,196	1,092	660	NV
Cecal Percentage	20% (17%–23%)	37%	20%	~26% or more	49%	47%	~35% or more	13%	NV
Average Egg Width	21 (18–23)	25 (24–26)	22 (20–24)	21 (18–23)	21	NV	22 (21–23)	21 (18–23)	21 (20–21)
Average Egg Length	33 (29–36)	43 (40–45)	36 (33–38)	37 (31–42)	30	NV	38 (36–39)	35 (31–39)	29 (21–36)
Oral Sucker Width/Ventral Sucker Width	1:1.3 (1.2–1.4)	1:1.2	1:1.3	1:1.4	1:1.8	1:1.7	1:1.2	1:1.4 (1.2–1.6)	1:1.4
Oral Sucker Width/Pharynx Width	2.1 (2.0–2.3)	1:2.1	1:2.2	1:1.8	1:2.2	1:2.5	1:2.0	1:3.1 (2.0–4.1)	1:3.4
Host Species	*Anolis biporcatus*	*Anolis Sagrei sagrei*	*Cutjanus cyanopterus*	*Hemidactylus turcicus*	*Bufo valliceps*	*Eleutherodacytles planirostris*	*Anolis carolinensis*	*Eleutherodactyles tauraus*	*Smilisca phaeota*
Locality	Panama	Florida	Venezuela	LA	LA	LA	LA	Costa Rica	Costa Rica

Table B6: Measurements and morphometic ratios for species of *Mesocoelium* from National History Museum (NHM).

	NHM 1980.7.14 50–54 n=2	NHM 1979.9.12 17–19 n=1	NHM 1978.8.31 48–67 n=4	NHM 1977.9.28 6 n=3	NHM 1980.11.12 1–3 n=1	NHM 1994.6.21 3 n=1	NHM 1980.11.12 4 n=5
Body Length (um)	2,094 (1,404–2,784)	2,236	1,352 (1,248–1,456)	1,924 (1,352–2,496)	1,768	2,262	1,378 (1.274–1,482)
Forebody Length	832	630	385 (320–450)	645 (560–730)	520	780	430 (410–450)
Oral Sucker Width	221 (210–231)	240	200 (190–221)	220 (210–230)	221	270	228 (216–240)
Oral Sucker Length	223 (190–255)	250	201 (192–210)	139 (47– 230)	220	270	216 (200–231)
Pharynx Width	91 (78–104)	130	86 (81–91)	148 (86–210)	99	104	81 (78–83)
Pharynx Length	66 (50–81)	105	90 (88–91)	77 (73–80)	83	94	98 (91–104)
Ventral Sucker Width	154 (125–182)	190	133 (120–146)	155 (133–177)	170	187	167 (156–177)
Ventral Sucker Length	146 (110–182)	200	148 (140–156)	179 (130–227)	160	172	172 (166–177)
Esophagus Length	111 (104–117)	NV	33 (0–65)	107 (0–213)	NV	81	NV
Ovary Width	143 (91–195)	187	111 (104–117)	122 (104–140)	130	200	112 (104–120)
Ovary Length	148 (114–182)	151	135 (114–156)	124 (104–143)	140	220	100 (96–104)
Average Testes Width	130 (91–169)	104	111 (104–117)	150 (125–174)	130 (120–140)	133 (122–143)	116 (114–117)
Average Testes Length	155 (104–205)	117	104 (78–130)	138 (107–169)	125 (120–130)	124 (117–130)	94 (91–96)
Cirrus Sac Length	NV	NV	NV	NV	NV	190	NV
Postovarian Space	1,004 (161–1,846)	1,300	390 (60–720)	1,31 (580–1,482)	990	1,274	710 (590–830)
Cecal Percentage	42% (37%–47%)	46%	58% (53%–63%)	38%	28%	37%	37%
Average Egg Width	21 (18–23)	21 (18–23)	21 (18–23)	21 (16–26)	18	20 (18–22)	21 (18–24)
Average Egg Length	33 (29–36)	43 (39–47)	34 (31–39)	34 (29–39)	33 (29–36)	35 (34–39)	34 (31–36)
Oral Sucker Width/ Ventral Sucker Width	1:1.5 (1.3–1.7)	1:1.3	1:1.5 (1.4–1.6)	1:1.5 (1.3–1.6)	1:1.3	1:1.4	1:1.3 (1.2–1.4)
Oral Sucker Width/ Pharynx Width	1:2.5 (2.2–2.7)	1:1.8	1:2.4 (2.3–2.4)	1:2.3 (2.1–2.4)	1:2.2	1:2.6	1:2.8 (2.6–3.0)
Host Species	*Bufo marinus*	*Bufo melanostictus*	*Bufo melanostictus*	*Mabuya guinguataeniata*	*Bufo marinus*	*Smilisca baudinii*	*Bufo marinus*
Locality	Papua New Guinea	Malaysia Penang	Malaysia	Rhodesia	Jamaica	Mexico	Jamaica, Kingston

APPENDIX C

Fixation

1. Live specimens should be relaxed in a wet mount of 0.85% saline and heat killed. If specimens have been already fixed, they should be placed in 70% Ethanol in a stentor dish.

2. Specimens should be left on the slide with coverslip in place and placed in a petri dish in AFA (acid, formaline and alcohol) for 24 hours.

3. After 24 hours the specimens should be removed from the slides and placed in 70% Ethanol in preparation for staining.

Staining

1. Specimens should be placed in a stentor dish in a moderate amount of 70% Ethanol and Semichon's carmine added to fill the container. Specimens should remain in the stain for at least 24 hours so they are overstained. The amount of time specimens should be kept in the stain may depend on the size of the specimens, that is smaller specimens will generally take less time than larger ones.

Destaining

1. Because specimens are overstained they will need to be destrained in acid alcohol as follows:

a. Remove as much of the stain as possible with a pipet and dispose of it into a waste container. Add a generous amount of acid alcohol (70% Ethanol with a small amount of HCL) and watch the destaining process under a dissecting scope. It will

generally be necessary to replace the acid alcohol a number of times to see specimens clearly making a determination as to when to stop the destaining process.

b. When the other tegument has cleared and the specimens have reached a light pink color, remove the acid alcohol and replace it with 70% Ethanol. Then add 5 or 6 drops of sodium bicarbonate to the container and allow about 5 to 10 minutes for it to neutralize the acid in the specimens. Again, the time specimens are left in the sodium bicarbonate may depend on the size of the specimens, but do not leave specimens too long as they can become burned by the sodium bicarbonate.

c. Next, remove all the Ethanol with sodium bicarbonate and add 70% Ethanol.

Dehydration

1. It is necessary to remove all the water for the specimens as follows:

a. Remove all the 70% Ethanol and replace with 80% Ethanol.

b. After about 15 minutes remove the 80% Ethanol and replace with 95% Ethanol.

c. After about 15 minutes remove all the 95% Ethanol and replace with 100% Ethanol.

d. Repeat step c before adding the clearing agent.

Clearing

1. To clear specimens, remove as much of the 100% Ethanol as possible and replace it with Xylene. Specimens may float and will need to be sunk with a wooden applicator stick. This step should be carried out under a hood or in a well ventilated location.

Mounting

1. Specimens should be mounted on a slide directly from the Xylene using either Kleermount or Canada balsam. Slides will need to dry for several days in a flat position so that the specimens do not drift. Canada balsam is preferred if specimens are to be sent to a museum as type material.

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