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Intraspecific variation in adult *Uvitellina iraquensis* Dronen, Ali & Al-Amura, 2013 (Cyclocoelidae: Haematotrephinae) from two collection sites of white-tailed lapwing, *Vanellus leucurus* (Lichtenstein) (Charadriiformes: Charadriidae), in Iraq

NORMAN O. DRONEN[1], NOTHIALA R. AL-KASSAR[2], ATHEER H. ALI[3], MOHANAD F. ABDULHAMEED[4], BASIM H. ABDULLAH[5] & SABEEH H. AL-MAYAH[5]

[1]*Laboratory of Parasitology, Department of Wildlife and Fisheries Sciences, Texas A&M University, 2258 TAMU, College Station, Texas 77843-2258; USA. E-mail: n-dronen@tamu.edu*

[2]*Department of Anatomy and Histology, Veterinary Medicine College, Thiqar University,Thiqar, Iraq. E-mail: nothialarasheed@yahoo.com*

[3]*Department of Fisheries and Marine resources, College of Agriculture, Basrah University, Basrah, Iraq. E-mail: atheeralibu@gmail.com*

[4]*Department of Microbiology, College of Veterinary Medicine, Basrah University, Basrah, Iraq. E-mail: mohand_amura@yahoo.com*

[5]*Biology Department, Education College for Pure Science, Basrah University, Basrah, Iraq. E-mails: basim_abdulah@yahoo.com; sabeeh.jaid@uobasrah.edu.iq*

Abstract

A total of 19 white-tailed lapwing, *Vanellus leucurus*, were collected from Huwazah Marsh, north-eastern Basrah Province, Iraq from February to March and in October, 2011 (collection site #1) and 60 *V. leucurus* were collected from Al-Hammar Marshes, Thi-Qar Province, southern Iraq from July to November, 2012 (collection site #2), and examined for cyclocoelids. Nineteen *Uvitellina iraquensis* Dronen, Ali & Al-Amura, 2013 from site #1 and 17 specimens from site #2 were fixed with minimal compression for comparisons of morphological characteristics, measurements, morphometric percentages and morphometric ratios commonly used to distinguish species of cyclocoelids. An additional five adult specimens from site #1 were fixed without compression for comparisons. Specimens from site # 1 (n=24) represented only fully-developed, non-senescing adults, while those from site #2 (n=17) could be divided into fully-developed (non-senescing) adults (n=8); younger (smaller, less developed) adults (n=5) and senescing adults (n=4). The following characteristics were relatively consistent, and appeared to be valuable in identifying groups of similar species and distinguishing species in *Uvitellina*: the presence or absence of the oral sucker; the oral sucker/pharynx width ratio; the posterior extent of the cirrus sac relative to the intestinal bifurcation; the position of the genital pore relative to the pharynx; the position of the testes in the body; the length of the intertesticular space; the length of the posttesticular space; the lateral disposition of the uterine loops; the presence of a posteriorly-directed, tail-like extension off the posterior confluence of the vitelline fields; the posterior extent of the uterine loops relative to the gonads; and the size of fully-developed eggs. It may be beneficial to calculate the percentage that measurements represent relative to the body length to provide insight into the relationship of the size of a structure to increased size of the specimens (growth). Specimens fixed without compression appeared to be less uniform, less symmetrical, shorter, more distorted and the internal details were more difficult to see.

Key words: Al-Hammar Marshes, Basrah Province, Charadriidae, Charadriiformes, Cyclocoelidae, egg development and hatching, fluke developmental categories, Haematotrephinae, Huwazah Marsh, intraspecific variability, Iraq, Thi-Qar Province, *Uvitellina iraquensis*, *Vanellus leucurus*, white-tailed lapwing, white-tailed plover

Introduction

The wetlands of Southern Iraq consist of different types of marshes which differ substantially in the number of species and densities of populations they support (Maltby 1994; Partow 2000; Evans 2002 and Alwash and Alwash 2004). The prevailing environment of these marshes differs according to the controlling limiting factors like water

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temperature, salinity, hydro period, availability of nutrients and the level of the functions of these marshes (e.g. primary production, decomposition and major cycles of elements) (Richardson *et al*. 2005; Richardson and Hussain 2006). The southern marshes can be categorized into freshwater, non-tidal marshes like Huwazah; Oligosaline non-tidal marshes like West Hammar and mesosaline, tidal marshes like East Hammar (UNEP, 2004; Hussain *et al*., 2010). Huwazah marsh gets its water from the Tigris River, characterized by low salinity and high nutrient loads. It is considered to be the largest marsh in southern Iraq, being located between 31° and 32° latitudes. The largest part of this marsh is located to the east of the Tigris River in Messan and Basrah, but it extends across the Iraqi-Iranian border. Hammar Marshes are composed of a series of wetlands stretching from between Nasiriyah and Basrah, south of the Euphrates River. West Hammar gets its water from the Euphrates River and is known by its moderate salinity and moderate nutrient loads. East Hammar gets its water mostly from Shatt Al-Arab River known by its moderate salinity and high concentrations of nutrients, and it is affected by semidiurnal tides.

The white-tailed lapwing or plover, *Vanellus leucurus* (Lichtenstein) (syns. *Charadrius leucurus* Lichtenstein; *Chettusia lecura* [Lichtenstein]) (Chardriiformes: Charadriidae), is a relatively common, medium-sized wading bird in middle and southern Iraq, where it is known to breed (Salim *et al*. 2006; Abed 2007). It has a very large range (Red List of least concern) but is predominantly found in shallow water along lakes, river valleys, swamps, marshy meadows and in salt-shrub terrain from the Middle East through North-east Africa into the northern Indian subcontinent (Allouse 1961; BirdLife International, 2016).

This study was carried out to provide a better understanding of the usefulness of the characteristics available for distinguishing *Uvitellina iraquensis* Dronen, Ali & Al-Amura, 2013 (Cyclocoelidae: Haematotrephinae) from other species in *Uvitellina* Witenberg, 1923 and potentially in distinguishing species in other genera of cyclocoelids, and in doing so, add to our understanding of the ranges of variability of these distinguishing characteristics.

Materials and methods

Nineteen white-tailed lapwing, *V. leucurus*, were collected from Huwazah Marsh, north-eastern Basrah Province near Majnoon, southern Iraq (31° 10′ N 47° 39′ E) from February – March and in October, 2011 by A. H. A. and M.F.A (collection site #1) and 60 were collected from Al-Hammar Marshes, Thi-Qar Province, southern Iraq (30° 48′ N 47° 1′ E) from July–November, 2012 by N. R. A. (collection site #2), and examined for endohelminthic parasites. Nineteen cyclocoelids from site #1 were studied alive, relaxed and washed in tap water, heat-fixed in 4% formalin between two glass microscope slides under minimal pressure (referred herein as compression), while 17 specimens from site #2 were fixed with minimal coverslip pressure (compression) in AFA. An additional five adult specimens from site #1 were fixed without compression for comparisons of morphological characteristics, measurements, morphometric percentages and morphometric ratios. All specimens were stained in Semichon's carmine, and mounted in Canada balsam. Specimens from these two collections represented three basic developmental categories as follows: specimens from site #1 were represented only by fully-developed, non-senescing adults (n=24) while specimens from site #2 were subdivided into fully-developed, non-senescing adults (n=8); young adults (n=5) and senescing adults (n=4). Eggs were measured separately from the proximal and distal aspects of the uterus relative to the ovary and only eggs that were in a flat profile were measured for use in comparisons. Photographs were preferentially used in figures; illustrations were used only where the necessary features were not in the same focal plane. Photographs and illustrations were made with the aid of a Zeiss compound microscope using a Pixera Pro 150ES imaging system and a drawing tube. Measurements are in micrometers (μm) unless otherwise stated with the means followed by the ranges in parentheses. Two-dimensional measurements are given with the length before the width. Bird species designations and common names are based on Salim *et al*. (2006) and BirdLife International (2016). Specimens were deposited in the Natural History Museum, London, UK (NHMUK).

Results

Subfamily Haematotrephinae Dollfus, 1948

Genus *Uvitellina* Witenberg, 1923

Uvitellina iraquensis Dronen, Ali & Al-Amura, 2013

Host: white-tailed lapwing or plover, *Vanellus leucurus* (Lichtenstein) (Charadriidae).

Site of infection in hosts: air sacs.

Locality for site #1: Huwazah Marsh, north-eastern Basrah Province near the Majnoon area, southern Iraq (31° 10′ N 47° 39′ E).

Dates of collections at site #1: February–March, 2011 and October, 2011.

Prevalence for site #1: 42% (5 of 12) and 43% (3 of 7), respectively.

Mean intensity for site #1: 7.8 and 10.3, respectively.

Deposited specimens from site #1: NHMUK 2012. 7.
13.1; 2012.7.13.2-3; 2012.7.13.4-6; 2016.11.25.1-8.

Locality for site #2: Al-Hammar Marshes, Thi-Qar Province, southern, Iraq (30°48′ N 47°1′E).

Date of collections at site #2: July–November, 2012.

Prevalence for site #2: 18% (11 of 60).

Mean intensity for site #2: 2.9.

Deposited specimens from site #2: NHMUK 2016.11.25. 9-23.

Three developmental/age categories of *Uvitellina iraquensis*

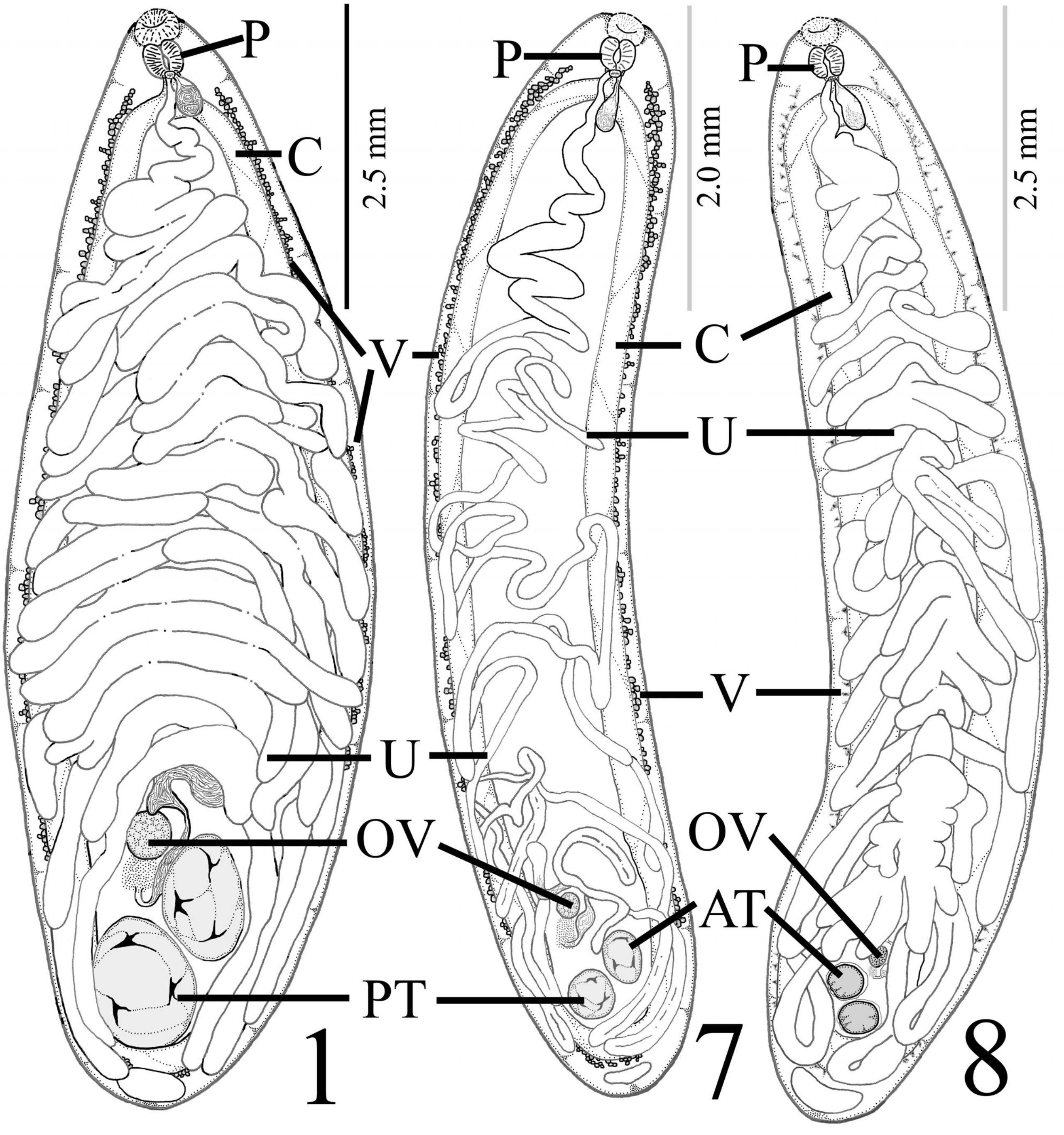
Fully-developed, non-senescing adults

(Figs. 1–6)

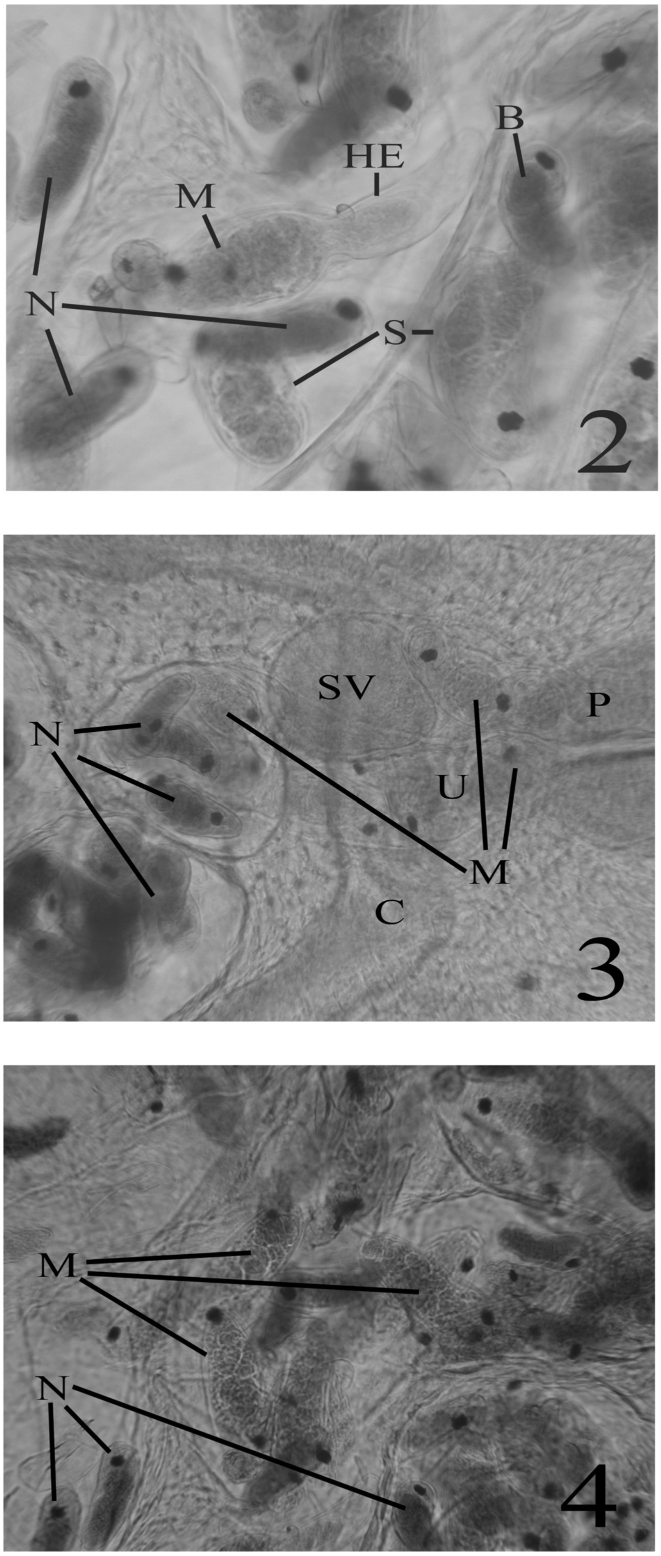
All 24 specimens collected from white-tailed lapwing from site #1 (body size 7,269–10,789 x 1,288–2,525; including both specimens fixed with and without compression) and eight of the 17 specimens from site #2 (7,338–10,802 x 1,749–2.795) represented fully-developed, non-senescing adults (Fig. 1). Characteristically these adults were relatively large (> 7,000 long). There were some swollen eggs (189–225 x 75–95 and 185–215 x 77–94, respectively) that were in the process of hatching (Fig. 2) mixed with the typical fully-developed, non-swollen eggs prior to becoming swollen and hatching (165–217 x 70–84 and 144–197 x 68–87) in the distal half of the uterus, and this developmental category had miracidia (190–225 x 60–84 and 163–224 x 60–99) mainly present in the distal third of the uterus. In these, most eggs had hatched within the distal uterus and miracidia often were present in the area of the metraterm, and occasionally within the genital atrium and anterior aspect of the cirrus sac (Fig. 3). Unhatched eggs typically were not found anterior of the cecal bifurcation. In the largest adults, about 40–50% of eggs had hatched above the level of the development of the most anterior extracecal uterine loops (about a third of the distance posterior to the anterior extremity of the body) (Fig. 4), while only about 10–20% of eggs present immediately posterior to this level had hatched (Fig. 5). Eggs in the proximal aspect of the uterus of fully-developed, non-senescing adults from both collections generally were smaller than those in the distal uterus (107–155 x 44–61 compared with 144–217 x 67–87) and eggs in the proximal aspect of the uterus tended to be collapsed (Fig. 6).

Remarks. In our opinion, these swollen eggs should be considered as a separate category from the smaller, non-hatching eggs in the distal uterus so that a better comparison can be made among species, and that the egg measurements from the proximal and distal uterus also should be considered separately to accommodate intrauterine growth and development. Newly-formed eggs apparently are more delicate and fragile than those in the distal uterus, and therefore tend to collapse during fixation.

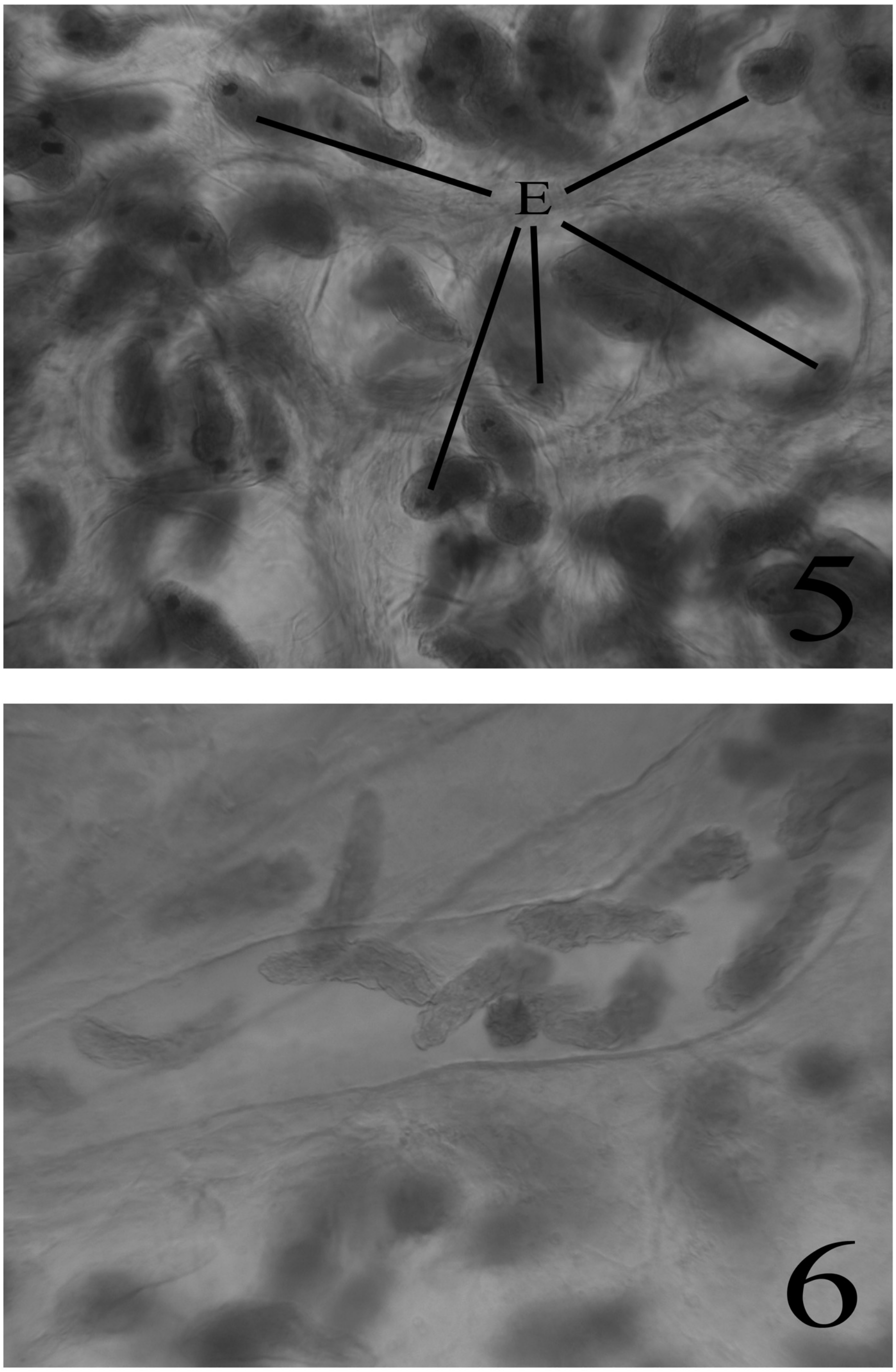
Young adults (Fig. 7). Five of the 17 specimens from site #2 were considered to be young adults because they were generally smaller than fully-developed, non-senescing adults (6,402–6,865 long compared with 7,557–10,789 from site #1 and 7,338–10,802 from site #2), were less developed and young adults on average had fewer, somewhat smaller eggs (131 compared with 189 and 185, respectively) present in the distal uterus (Fig. 7). Most structures were smaller in these young adults than in the fully-developed, non-senescing adults (e.g. prepharynx length 3 compared with 8 and 6; cirrus sac length 342 compared with 396 and 391; intertesticular space length 18 compared with 45 and 31; posttesticular space length 417 compared with 560 and 474); however, the percentages of these measurements relative to body length were relatively similar (Table 1). Although young adults had a limited number of swollen, pre-hatching eggs similar to those described above for the fully-developed, non-senescing specimens mainly present in the distal aspect of the uterus, these eggs tended to be shorter (168–177 compared with 185–225). Also, *in utero* miracidia tended to be uncommon and when present they were shorter than those observed in the fully-developed, non-senescing adults (132–148 compared with 163–225).



FIGURES 1, 7 & 8. 1. Fully-developed, non-senescing adult fixed with minimal compression, ventral view. **7.** Young adult fixed with minimal compression, ventral view. Note that in figures 1 & 7 that the placement of the testes are diagonal with the anterior testis on the right while the posterior testis and ovary are on the left side. In some specimens in this species the position of the gonads may be the opposite of this in a ventral view (mirror image, see fig. 1 of the original description by Dronen *et al*. 2013). **8.** Senescing adult fixed with minimal compression, ventral view. Note the space developing around the testes and the reduced amount of sperm present in the seminal vesicle and receptacle. Abbreviations: AT, anterior testis; C, cecum; OV, ovary; PT, posterior testis; U, uterus; V, vitelline fields.

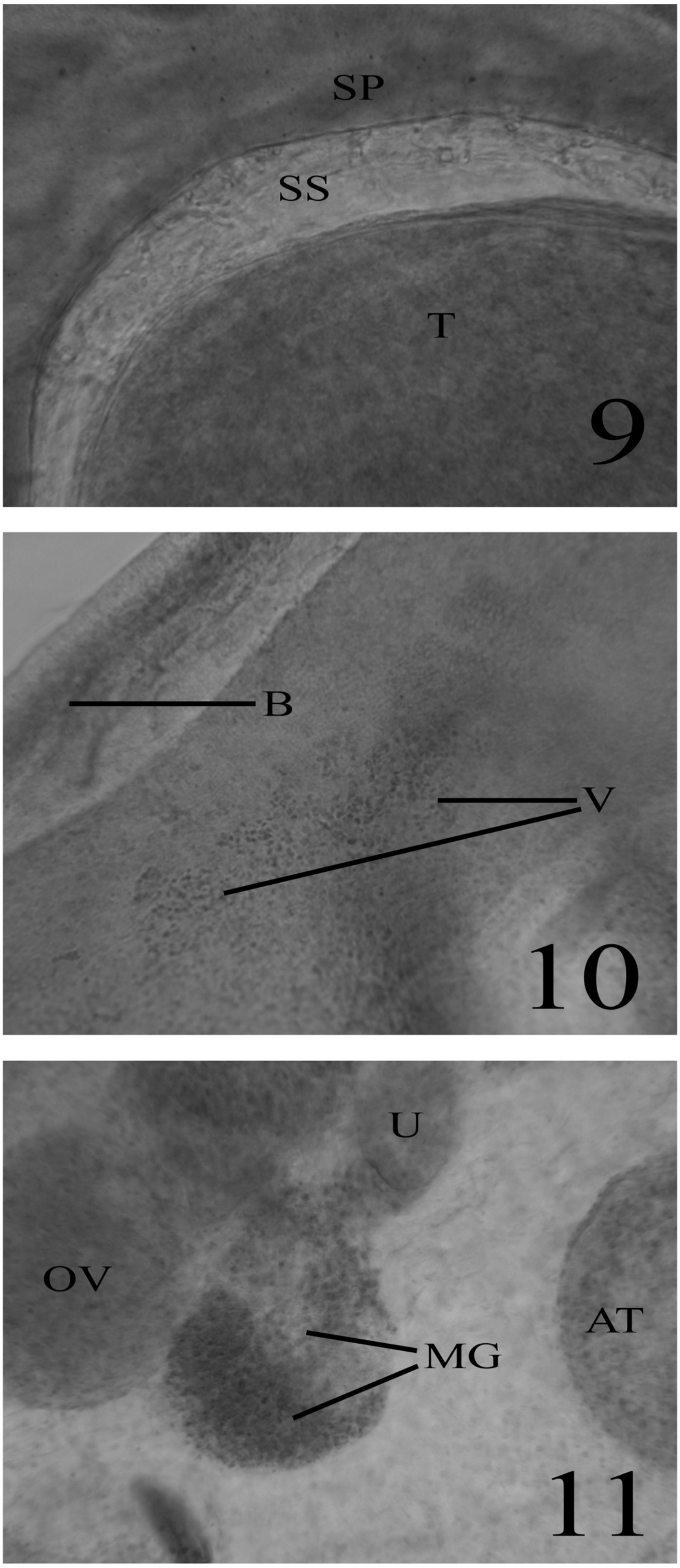


FIGURES 2–4. 2. Eggs and miracidia in distal uterus of a fully-developed, non-senescing adult fixed with minimal compression showing an egg hatching with miracidium being released from the egg shell; examples of fully-developed eggs prior to swelling and hatching and examples of eggs in the process of swelling just prior to hatching. **3.** Area of intestinal bifurcation of a fully-developed, non-senescing adult fixed with minimal compression. **4.** Uterus near anterior third of body of a fully-developed, non-senescing adult fixed with minimal compression. Abbreviations: B, miracidium within egg exerting pressure on shell forming a curved (banana) shape; C, cecum; HE, hatching egg; M, miracidia; N, examples of fully-developed eggs prior to swelling and hatching; P, pharynx; S, eggs in the process of swelling just prior to hatching; SV, seminal vesicle filled with sperm; U, uterus (metraterm).

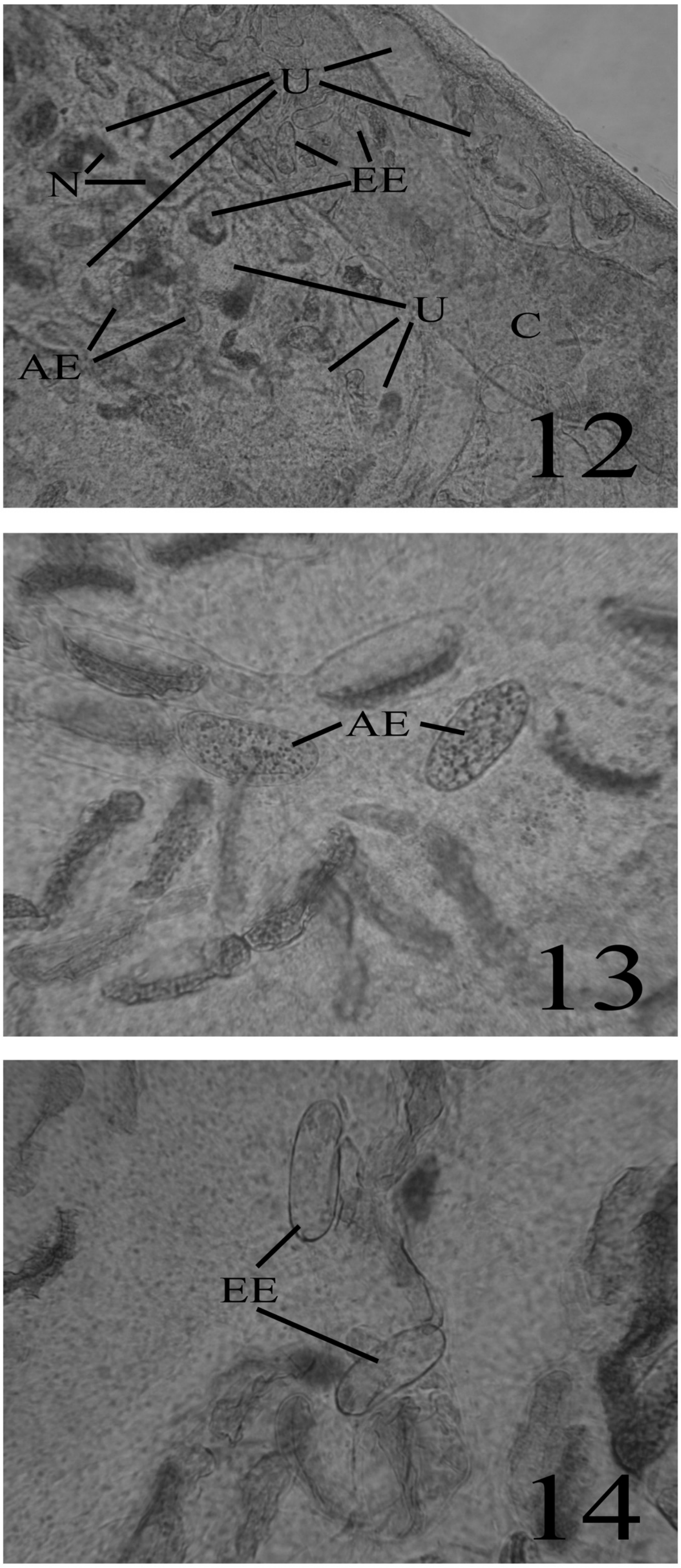


FIGURES 5–6. 5. Uterus immediately posterior to mid-level of body of a fully-developed, non-senescing adult fixed with minimal compression showing first development of miracidial eyespots in eggs and eggs that were not in a flat plane. **6.** Newly-formed eggs of a fully-developed, non-senescing adult fixed with minimal compression from the most proximal aspect of the uterus. Note the tendency for eggs to become collapsed. Abbreviation: E, eggs.

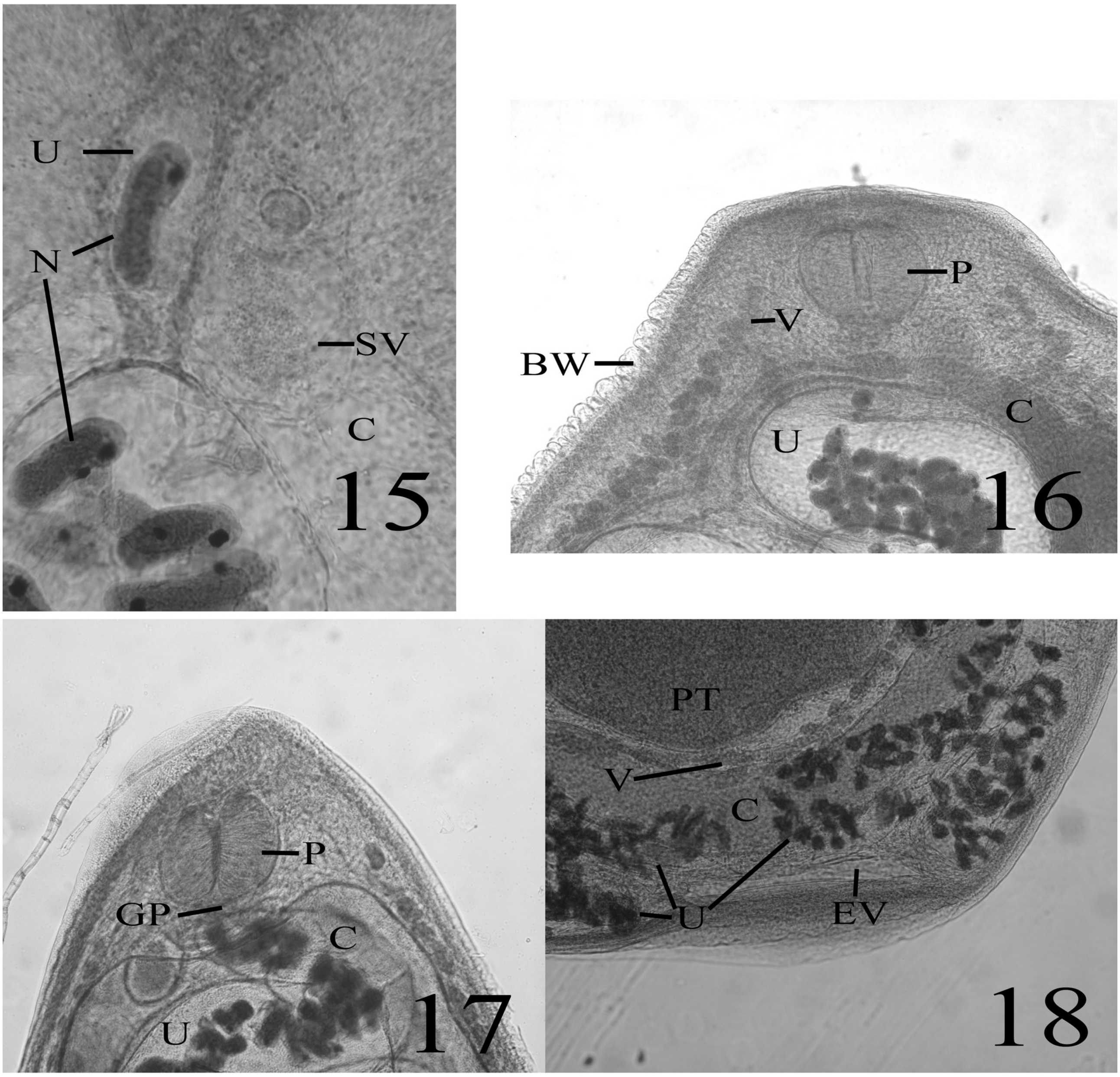
Remarks. Despite the differences observed between young adults from site #2 and fully-developed, non-senescing adults, these smaller (presumably younger) specimens were considered to be representative of the species; however, we encourage future researchers to consider young adults separately from the fully-developed, non-senescing adults in their evaluation of cyclocoelids when this developmental category is present. The similarities in the percentages of structure measurements relative to body length appear to indicate that the apparent differences in sizes likely were related to the size (growth) of the specimens, and we therefore support the use of this percentage when evaluating size ranges of at least some of the structures in cyclocoelids as a rough estimation of size-related differences.



FIGURES 9–11. 9. Posterior region of a senescing adult fixed with minimal compression. **10.** Vitelline fields of a senescing adult fixed with minimal compression showing indistinct follicles in the vitelline fields. **11.** Area of female genital complex from a senescing adult fixed with minimal compression. Note the Mehlis' glands are reduced in number and no longer entirely surrounded the oötype chamber. Abbreviations: AT, anterior testis; B, adjacent to the body wall; MG, Mehlis' glands; OV, ovary; SP, surrounding parenchyma; SS, space of separation of testis from parenchyma; T, testis pulling away from the surrounding parenchyma; U, uterus; V, vitelline fields.



FIGURES 12–14. 14. Middle third of body of senescing adult fixed with minimal compression; photograph taking slightly out of focus to demonstrate the relative position of eggs to the uterine wall. **13.** Abnormally developing eggs in uterus of a senescing adult specimen fixed with minimal compression. **14.** Unhatched eggs in middle third of the uterus of a senescing adult fixed with minimal compression where shells were intact, but there was no contents present within the shell. Abbreviations: AE, abnormally developing eggs in uterus in which there was what appeared to be primordial material and/or yolk material present, but with no miracidial development evident; C, cecum; EE, empty eggs; N, examples of fully-developed eggs prior to swelling and hatching; U, uterus.



FIGURES 15–18. 15. Area of seminal vesicle of a senescing adult fixed with minimal compression. **16.** Contracted anterior end of a fully-developed, non-senescing adult fixed without compression. **17.** Distorted anterior end of a fully-developed, non-senescing adult fixed without compression. Note the inflated uterus, submedian placement of the genital pore, the anterior extent of the vitelline fields are pulled anteriorly to level of pharynx, and the pharynx is rotated off the midline of the body. **18.** Distorted posterior end of a fully-developed, non-senescing adult fixed without compression. Abbreviations: BW, body wall showing the effects of contraction (scalloped to crenated); C, cecum; EV, collapsed excretory vesicle; GP, genital pore; N, examples of fully-developed eggs prior to swelling and hatching; P, pharynx; PT, posterior testis; SV, seminal vesicle with comparatively little sperm present; U, uterus; V, vitelline fields.

Senescing adult (Figs. 8–15). Four of the 17 specimens from site #2 were in the process of senescing (Fig. 8) and provided an opportunity to compare non-senescing to senescing adults. Although the body length of senescing specimens was similar to that of fully-developed, non-senescing specimens (8,554 compared with 8,916 and 8,724, respectively), senescing specimens were narrower (1,547 compared with 2,135 and 2,255), which represented a smaller percentage of body length (17–19% compared with 23–25% and 23–26%). In senescing specimens the prepharynx was absent (too short to measure); the ovary width (214, 2–3% of body length compared with 307, 3–4% and 328, 3–5%) and testes width (anterior testis 303, 3–4% compared with 505, 4–7% and 577, 3–9%; posterior testis width 336, 3–4% compared with 430, 3–7% and 629, 3–9%) were on average smaller and well separated from the testes (219, 2–4% compared with 92, 0–5% and 166, 0.1–4%); the testes appeared to be pulling away from the surrounding parenchyma (Fig. 9); the vitelline fields were indistinct and the individual follicular masses were diffuse so that the individual follicles could no longer be distinguished (Fig. 10); the vitelline reservoir was more diffuse and the contents was atypical in appearance compared with normal vitelline materials; the Mehlis' glands were less dense and no longer entirely surrounded the oötype chamber (Fig. 11); the eggs in the distal uterus were smaller (112–144 long compared with 165–217 and 144–197); and the numbers of eggs in the uterus were noticeably fewer, even though the entire gravid uterus appeared to still be present as in non-senescing adults (Fig. 12). Also, there were eggs present in much of the proximal uterus in which there was what appeared to be primordial material and/or yolk material present, but there was no miracidial development evident in these eggs (98–122 x 48–65) (Fig. 13); there were a number of empty, unhatched eggs observed primarily in the distal and middle thirds of the uterus where the shells were intact, but there was no contents present within the shell (91–148) (Fig. 14); the seminal vesicle had little or no sperm present (Fig. 15); there were no miracidia present in the uterus; and there was little or no sperm visible in the proximal uterus (uterine seminal receptacle). In addition, there was an abundance of empty, collapsed eggs present in the posterior half of the uterus, which may have been the remains of the empty eggs described above, or possibly the shells of eggs left in the uterus after miracidial emergence. Although there were a number of structures that were smaller in senescing specimens when compared with those of fully-developed, non-senescing adults (e.g. pharynx width 267 compared with 324 and 299; cirrus sac length 377 compared with 396 and 391) the percentages of the body length were relatively similar (Table 1). Also, the intertesticular space was 62 long compared with 45 and 31, which represented about the same percentage of body length in all three developmental categories.

Remarks. The appearance of the gonads, the presence of unhatched, empty eggs and unhatched abnormally-developed eggs; the obvious decline in the numbers of eggs present in the uterus; the lack of sperm in the seminal receptacle and vesicle; the absence of miracidia in the uterus; and the other differences described above suggest that these specimens likely were in a state of reproductive decline. We suggest that these senescing specimens be treated separately for comparative purposes concerning this species and likely in other cyclocoelid species.

Specimens fixed without compression
(Fig. 16–19)

The five fully-developed, non-senescing adults from site #1that were fixed without compression were somewhat smaller on average than fully-developed, non-senescing adult specimens that were fixed with compression (7,955 x 1,494 compared with 8,916 x 2,135 from site #1 and 8,724 x 2,255 from site #2), narrower (1,288–1,847, 17–22% of body length compared with 1,800–2,525 wide, 23–25% and 1,749–2,795, 23–26%, respectively), and as a result, although distance from the ventral to dorsal could not be measured, specimens fixed without compression were noticeably thicker. Adults fixed without compression were 961 (306–1,755) or about 11% (4–17%) shorter than those fixed with compression from site #1 and 898 (69–2,107) or 10% (9–20%) from site #2, and were most similar in width to senescing specimens (1,494 compared with 1,547). There were a number of what appeared to be fixation-related differences noted between fully-developed, non-senescing adults fixed without compression when compared with those fixed with compression: specimens fixed without compression were more contracted overall, which appears to have been responsible for the reduced intertesticular space (testes nearly contiguous to overlapping) and the shorter distance from the ovary to the anterior testis (11, 0–0.3% of body length compared to 92, 0–55 and 166, 0.1–4%, respectively). In the specimens fixed without compression egg size was smaller in the distal uterus (134 long compared with 189 and 185) and in the proximal uterus (106 compared with 131 and 127); the internal details of structures were more difficult to see; the boundary of the oral sucker was more indistinct; the body profile tended to be more asymmetrical (less uniform to irregular); the uterus appeared to have become inflated (Fig. 16); the anterior end tended to be noticeably contracted and distorted (Figs. 16; 17); the ceca appeared to have become swollen similarly to what was apparent in senescing adults; the pharynx along with the genital pore appeared to have become rotated and displaced off the midline of the body (Fig. 17); and the posterior end was commonly distorted (Figs. 18; 19). There also were some characteristics that appeared to be less affected by the method of fixation in this species where the ranges of their percentages relative to the body length for specimens fixed without compression remained similar to those fixed with compression: the oral sucker width; the prepharynx length; the pharynx width; the oral sucker/pharynx width ratio; the distance from the pharynx to the intestinal bifurcation; the length of cirrus sac; the width of the ovary; the width of testes; and the size of the vitelline follicles (Table 1).

Remarks. Although a specific body size range was not used in the selection of the five specimens where compression was not employed during fixation, their smaller length suggests that they possibly were among the smaller specimens of the fully-developed, non-senescing adults. While the percentages of body length represented by the body width were consistently similar when comparing fully-developed, non-senescing adults from site #1 (23–25%) and site #2 (23–26%), and the young adults (22–28%), where compression was used in fixation, the range was smaller (17–22%) for those specimens fixed without compression, suggesting that not all of the differences in size seen could be attributed to the non-compressed specimens on average being among the smaller specimens in the fully-developed, non-senescing adult category. In our opinion, the characteristics in the first list above appeared to be most affected by the method of fixation (compression *vs* no compression) and therefore these possible fixation-induced differences should be considered when comparing cyclocoelid specimens fixed in these two different ways. The second list of characteristic above appeared to not be as affected by the method of fixation, and therefore potentially could more easily be used directly to distinguish cyclocoelid species fixed in these two different ways, provided the differences seen are distinctive and their ranges do not overlap extensively.

Characteristics commonly used to distinguish species of cyclocoelids

(Primarily based on the three basic developmental/age categories of specimens in Table 1 where compression was applied during fixation; n=36 with comments on specimens fixed without compression for some characteristics as indicated.)

Body size. As previously mentioned, specimens from site # 1 (n=19) represented only fully-developed, non-senescing adults, while those from site #2 (n=17) were divided into fully-developed (non-senescing) adults (n=8); younger (smaller, less developed) adults (n=5) and senescing adults (n=4). The fully-developed, non-senescing adult specimens from site #1 (8,916 [7,557– 10,789] x 2,135 [1,800–2,525]) were generally similar in size to those from site #2 (8,724 [7,338–10,802] x 2,255 [1,749–2,795]), although the senescing adults were about the same length (8,554 [7,460–9,526]) as full-develop, non-senescing adults, they tended to be narrower (1,547 [1,360–1,713). While young adults (6,748 [6,402–6,865]long) also were similar in width to senescing adults (1,372–1,893), it is interesting to note that the percentage of the width relative to body length was larger than in senescing adults (22–28% compared with 17–19%), but similar to that of fully-developed, non-senescing adults (23–25% and 23–26%).

Remarks. It is our opinion that body size should only be used as a distinguishing characteristic at the species level in cyclocoelids when there are notable and consistent differences demonstrated between the species being compared, and where smaller (likely younger) specimens within a species are recognized and the possibility of there being considerable differences found for morphological features, morphometric ratios and measurements between developmental categories represented in specimens of the same species are considered. In many cases, calculating the measurement of a structure (e.g. the cirrus sac length, sucker width, pharynx width) or distances between structures or parts of the body (e.g. the posttesticular space length, intertesticular space length, distance from ovary to a testis) as a percentage of the body length can provide a rough indication as to whether or not the size of a structure or a particular length measurement may be related to growth (size) of the specimen.

Presence or absence, and size of suckers. *Uvitellina iraquensis* has a rudimentary oral sucker, but it lacks a ventral sucker. In this species the size of the oral sucker appears to be related to the size (age or developmental status) of individual flukes in that size of suckers appeared to increase in our specimens with increased worm size (growth). There was considerable overlap in the width of the oral sucker when comparing fully-developed, non-senescing adults from site #1 (255–420; 3–4% of body length); fully-developed, non-senescing adults (216–357; 3–4%) from site #2; fully-developed; young adults (196–284; 3–5%) and senescing adults (254–304; 3–4%). The size of the oral sucker, as represented by width, appeared to be somewhat larger in the fully-developed adults from site # 1 than in the other categories; however, the percentage of width of the oral sucker relative to body length was about the same in all categories (Table 1). The width of the oral sucker in our specimens was typically more distinct than its length.

Remarks. In our opinion the presence or absence of suckers represent a relatively good distinguishing characteristic. Dronen & Blend (2015) noted that in general the presence of the oral sucker is more easily determined than that of the ventral sucker in cyclocoelids, and that histological sectioning often may be required to detect the presence of the ventral sucker. These authors also pointed out that when a rudimentary oral sucker is present in cyclocoelids, its outer boundary may be vague and difficult to see, and some specimens also may require histological sectioning to access the width and length accurately, often precluding the use of the oral sucker/pharynx width ratio. Although the presence of the oral sucker and or the ventral sucker may be useful in delimiting species and groups of species, we generally would suggest caution in using length and/or width measurements of suckers as a distinguishing characteristic at the species level unless the difference is consistently notable between the species being compared.

Prepharynx length. Generally the length of the prepharynx was similar in fully-developed, non-senescing adults from both site #1 (8 [0–30]; about 0.3% of body length) and site #2 (6 [0–29]; about 0.3%). In young adults where the body was shorter than those of fully-developed, non-senescing adults, the prepharynx length was somewhat smaller (3 [0–8]; 0.1%), while in senescing specimens the length of the prepharynx was undetectable. The prepharynx tended to be short in our specimens and its overall length appeared to be influenced by even minor contraction of the anterior ends of our specimens.

Remarks. Dronen & Blend (2015) indicated that the anterior end of species of cyclocolids have a tendency to contract even when specimens are heat-fixed using an accepted method. The length of the prepharynx was quite variable in our specimens; however, generally the length of the prepharynx beyond being presence or absent, or longer or shorter that the esophagus, is known to be quite variable in many groups of flukes and investigators should be cautious of using measurements of the prepharynx length in separating species and/or genera in the Cyclocoelidae Stossich, 1902.

Pharynx size. The size of the pharynx was generally more distinct than that of oral sucker in our specimens and could be measured more easily. The width of the pharynx, like the width of the oral sucker, was somewhat larger in the specimens from site #1 and the width overlapped between the three developmental categories of specimens fixed with compression; however, the percentage of the width expressed as a percentage of the body length was similar for all three basic categories (fully-developed, non-senescing, young and senescing adults) (Table 1). The relative constancy of this measurement when calculated as a percentage relative to body length among these three categories suggests that this characteristic also may be related to body size (growth) to at least some extent.

Remarks. The size of the pharynx (usually width) is commonly used by researchers in many groups of flukes as a distinguishing characteristic when there are substantial differences between species. In our opinion, the pharynx width by itself is a difficult characteristic to use in separating species of cyclocoelids because the ranges of measurements of it commonly overlap between species, as is the case with *U. iraquensis* when compared with other species in the genus, and in many cases this feature may not be viable as a distinguishing characteristic by itself.

Oral sucker/pharynx width ratio. The oral sucker/pharynx width ratios were somewhat similar in all three categories studies ranging from 1:0.7– 1:1.4. In most cases the pharynx was larger than the oral sucker, but in a few specimens the oral sucker was slightly larger (Table 1). The relative similarities in this ratio in the three categories suggests that this characteristic, as is the case in other digenean groups, has the potential to be a reliable distinguishing feature at the species level in cyclocoelids if the margins of the rudimentary oral sucker are distinct and the width of the oral sucker can be accurately determined.

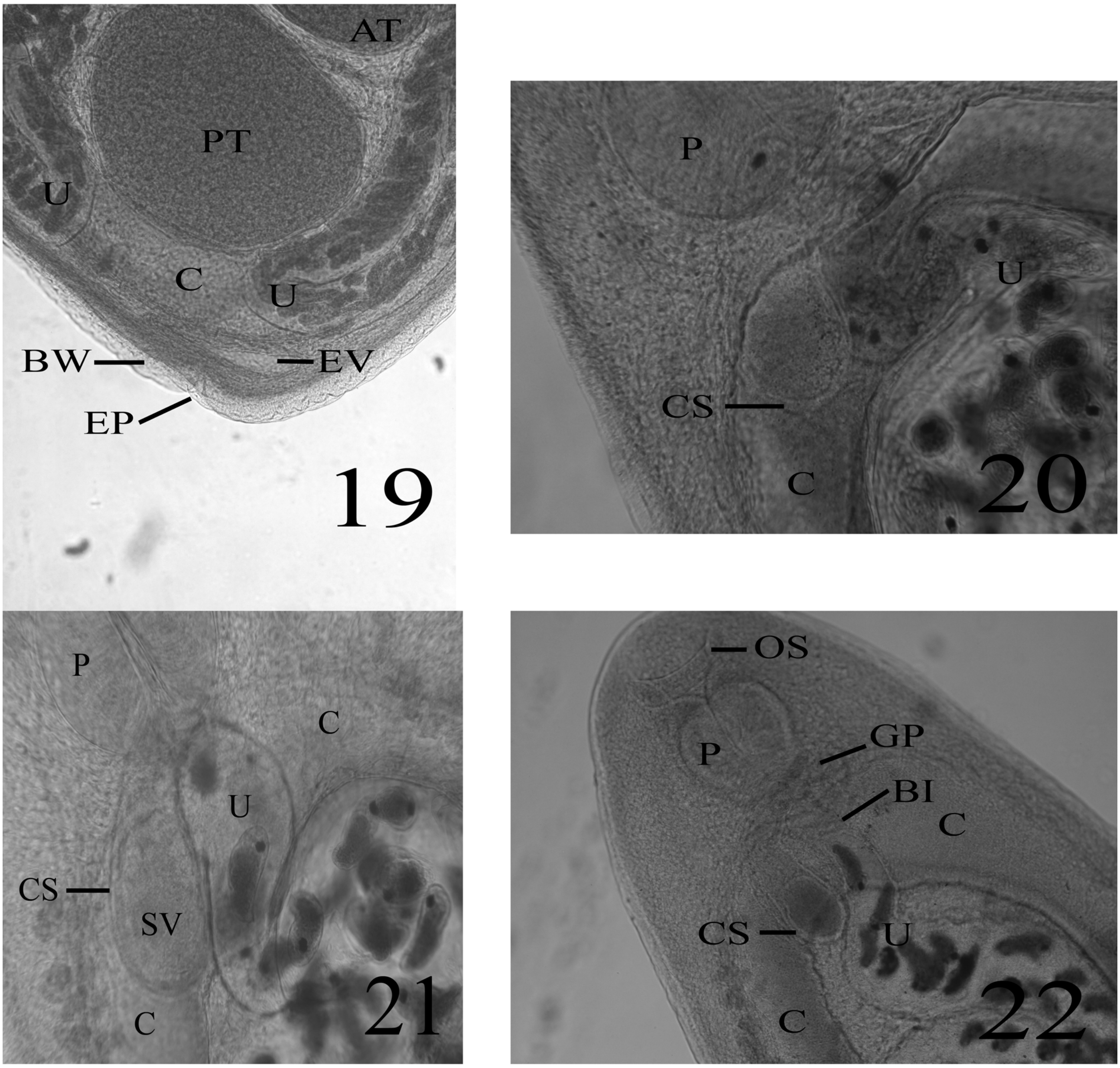
Remarks. Although some cyclocoelids are known to have oral and/or ventral suckers, we have a meager understanding of the normal ranges of variability of morphometric ratios (widths of suckers; the pharynx and oral sucker widths) in species. Although this ratio has the potential to be a distinguishing characteristic at the species level and to some extent genera in the Cyclocoelidae, it has been our experience that this ratio often overlaps among species in the genus, which limits its usefulness. The obvious drawback is our ability to insure accurate measurements of the suckers in species of cyclocoelids; however, the presence or absence of the rudimentary oral sucker appears to us to be of value in distinguishing similar groups in the cyclocoelids, which also can be useful in developing keys in the group.

Distance from pharynx to intestinal bifurcation. We elected to use the distance from the posterior margin of the pharynx to the intestinal bifurcation rather than attempting to measure the actual length of the esophagus because we found that even in heat-fixed specimens where a proven methodology was used that there was usually at least some contraction of the anterior end and that even these slight contractions caused at least some perceived decrease in esophagus length, and sometimes folding and collapse of the esophagus was apparent. Although the distance from the pharynx to the intestinal bifurcation in our specimens was variable and relatively large overall, this distance was shorter on average in specimens from site #1(73) and in all categories in site # 2 (96, 91 and 88) than in specimens fixed without compression (126); however, the percentage of the body length for these specimens was similar in all categories (without compression, 0.5–3% *vs* with compression, 0.4–2%; 0.2–2%, 1–2%, 0.8–2%). Also, the upper end of the ranges of the distance from the pharynx to the intestinal bifurcation for fully-developed, non-senescing adults from sites #1 and #2 (137 and 196) were somewhat larger than those of young adults (127) and senescing adults (118); however, generally the ranges also overlapped and the percentage of the body length were comparable (Table 1). Although our specimens did not show obvious signs of extensive contraction (e.g. tegument irregular [wavy to crenate], pharynx visibly pulled anteriorly into oral sucker to some extent, esophagus completely collapsed, cirrus sac pulled anterior to where the posterior margin of it terminated above mid-level of intestinal bifurcation), it appeared that even minor contraction of the anterior end of specimens often reduced the region from the pharynx to the intestinal bifurcation to some extent in some specimens. In some of our specimens the entire length of the esophagus was difficult to observe because it was masked by the eggs in the distal aspect of the uterus (n=6); however, the distance from the pharynx to the intestinal bifurcation was generally visible in specimens.

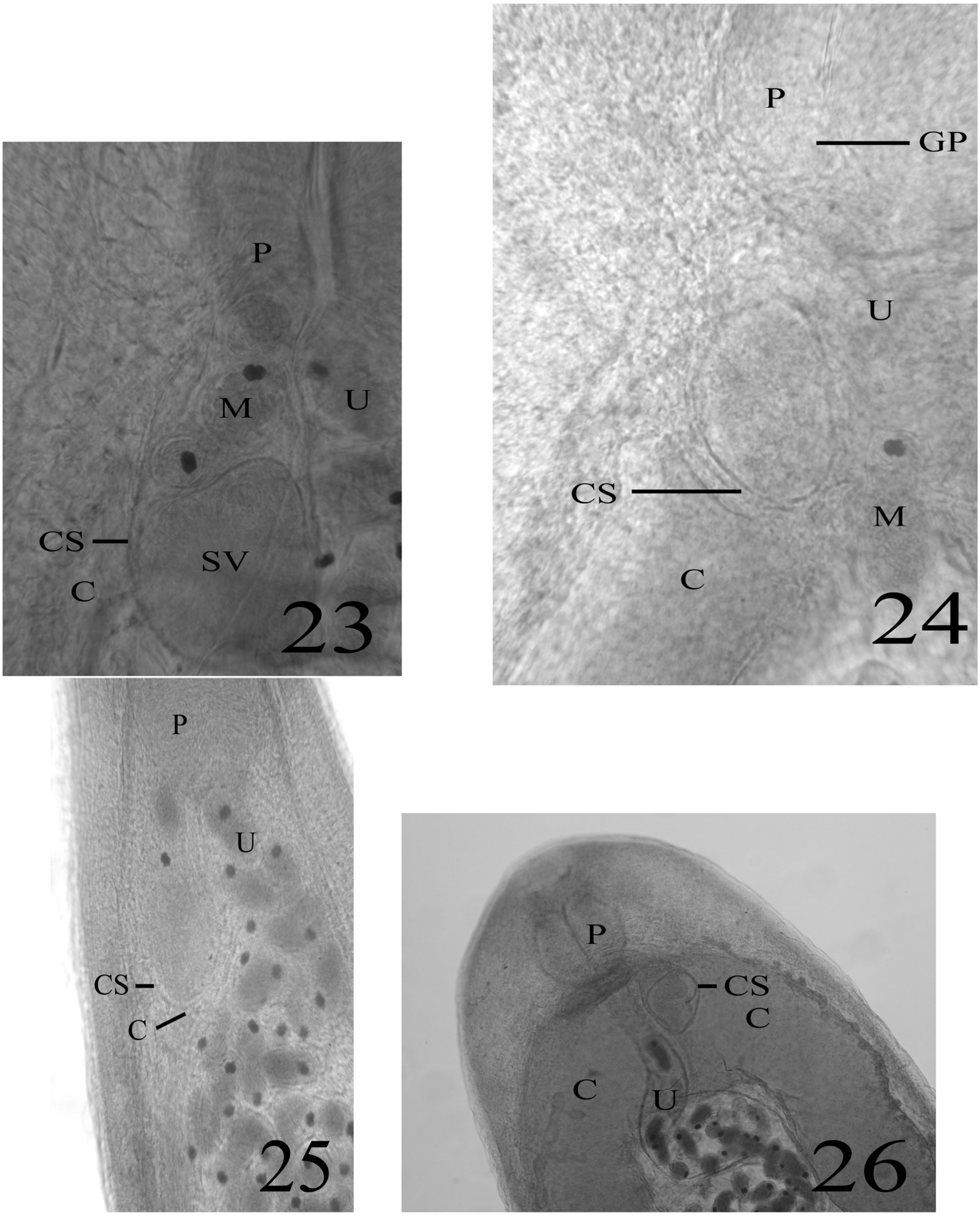
We divided the specimens representing the three developmental categories in Table 1 into three groups based on our perception of the amount of contraction of the anterior end of our specimens: those where the contraction was not detectable (n=13); those where the contraction was minimal (n= 15) and those where the contraction was not dramatic, but was apparent (n=5). In those in the first group the space between the pharynx and the intestinal bifurcation appeared to be little reduced and the esophagus was relatively unaffected (reasonably straight). In the second group the distance between the pharynx and the intestinal bifurcation was reduced so that the esophagus was slightly bent near its mid-level becoming curved. In the third category the distance from the pharynx to the intestinal bifurcation was noticeably reduced to where the esophagus was either bent near its mid-level and folded so that the anterior half of the esophagus was nearly overlaying the posterior half, or where the esophagus became winded forming a partially collapsed spiral. Senescing specimens were not included in this breakdown. Unfortunately, there also were some specimens that did not appear to be at all contracted where the distance from the pharynx to the intestinal bifurcation was reduced (n=4). In these, the intestinal ceca were extremely swollen at the level of the bifurcation or just posterior to it, and surpassed the intestinal bifurcation anteriorly so that the esophagus was overlapped by as much as half its length by the enlarged ceca.

Remarks. Generally we would not encourage the use of measurements of the esophagus length as a distinguishing characteristic in cyclocoelids because esophagus length tends to be quite variable, very susceptible to the influence of body contraction during fixation and sometimes at least some of the esophagus may be masked when the ceca become greatly enlarged (swollen). We found that the esophagus can be relatively short (collapsed) or long (longer than the prepharynx), and in our opinion the length of the esophagus has limited usefulness in comparisons among species. The distance from the pharynx to the intestinal bifurcation in specimens fixed with compression represent a relatively large range in each category; however, the percentage of these measurements relative to body length was relatively similar in all three developmental categories (Table1), suggesting this feature could potentially be a reliable distinguishing characteristic, if the potential effects of contraction are recognized and considered.

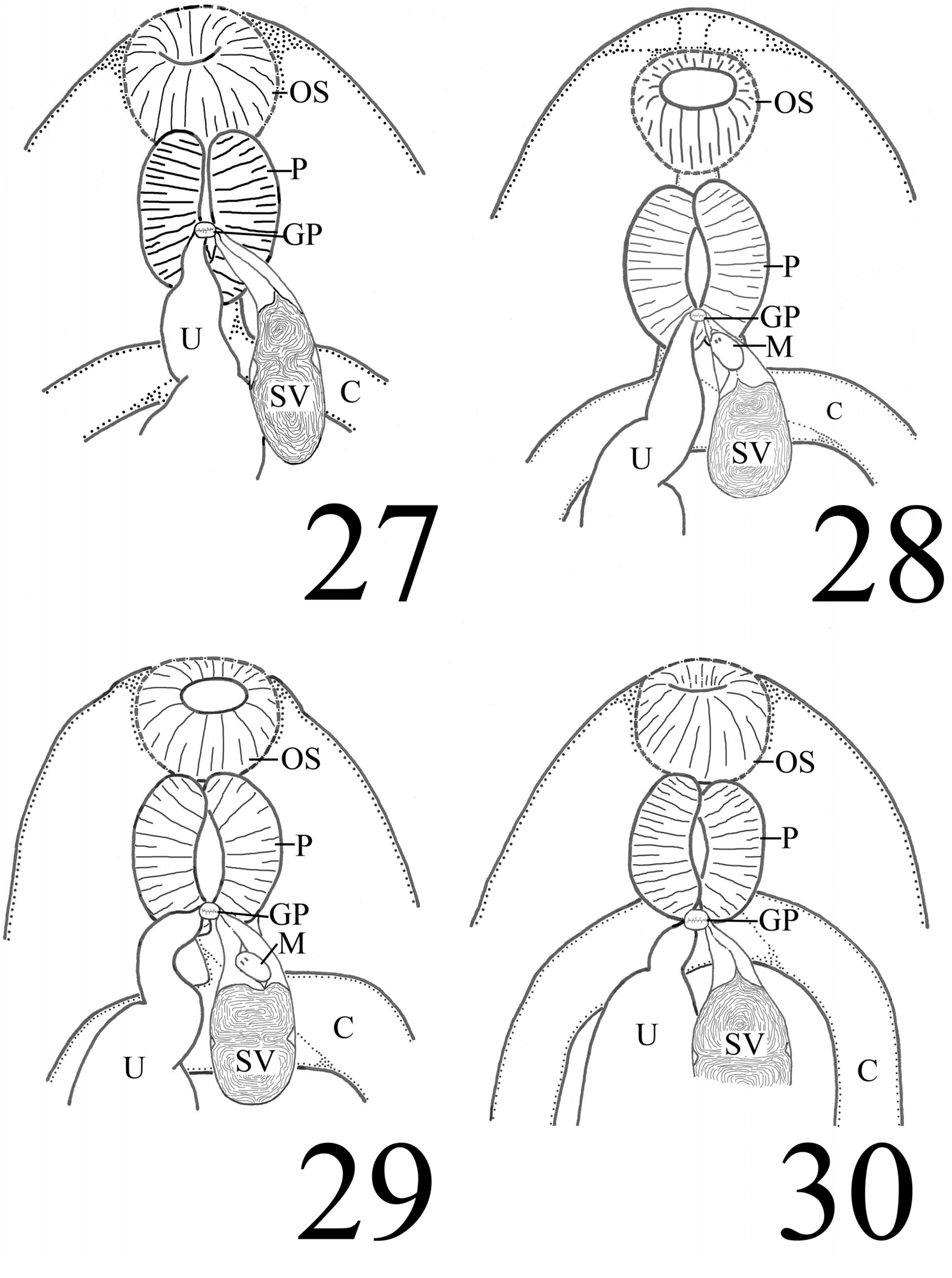
Size and extent of the cirrus sac (Figs. 20–26). In our specimens the posterior extent of the cirrus sac relative to the intestinal bifurcation varied from overreaching about two thirds (Fig. 20) to nearly the entire width of the ceca (Fig. 21) adjacent to the level of the intestinal bifurcation to surpassing the level of the intestinal bifurcation posteriorly by about half of the length of the seminal vesicle (Figs. 22, 23). Larger specimens tended to have longer cirrus sacs than smaller specimens and on average the length of the cirrus sac in young (342 [284–420]) and senescing adults (377 [333–461]) was smaller than in fully-developed, non-senescing adults (396 [300–500] and 391[304–470]). The ranges of these measurements extensively overlapped among the three categories (Table 1), while the percentages of the cirrus sac length relative to the body length (3–7%) were reasonably similar, suggesting that growth and development of the cirrus apparatus may be at least somewhat related to body growth (age). In a few of the specimens (n=2) where the cirrus sac was relatively long, it did not surpass the intestinal bifurcation posteriorly (Fig. 24). While these specimens showed some signs of contraction of the anterior portion of the body, which may have influenced cirrus sac length to some extent, the ceca had become swollen so that the cirrus sac appeared less extensive relative to the width of the ceca at the level of the intestinal bifurcation. One specimen that was fixed with compression appeared to have become elongated, pulling the ceca posteriorly so that the ceca approach the intestinal bifurcation at an unusually steep angle partially overlapping the posterior end of the cirrus sac (Fig. 25). Swollen and irregular-shaped ceca were most common and generally more pronounced in those specimens fixed without compression (Figs. 17, 26).



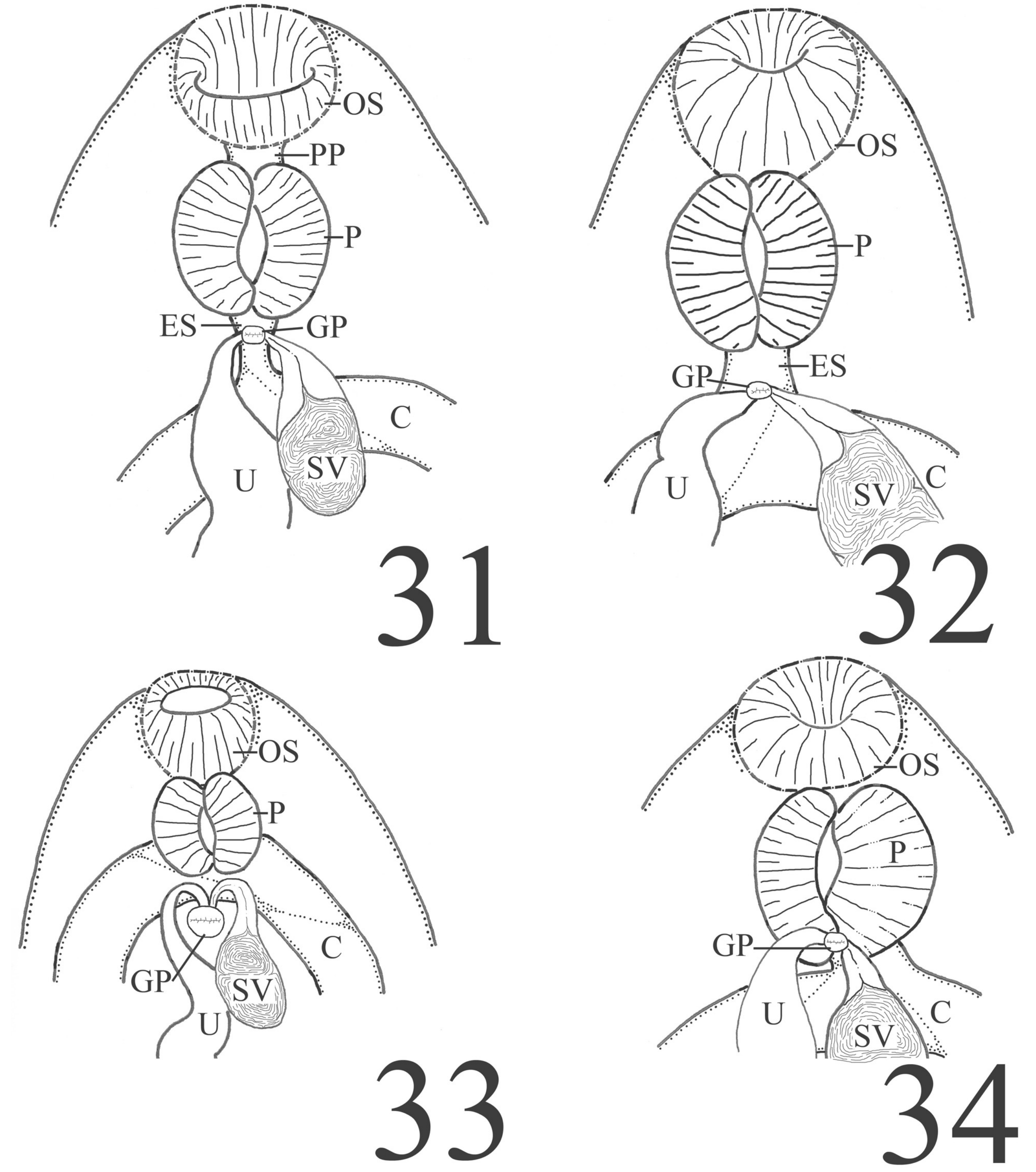
FIGURES 19–22. 19. Distorted posterior end of a fully-developed, non-senescing adult fixed without compression. **20.** Area of intestinal bifurcation of a fully-developed, non-senescing adult fixed with minimal compression. **21.** Area of intestinal bifurcation of a fully-developed, non-senescing adult fixed with minimal compression. Note the posterior extent of cirrus sac overreaches about two thirds of width of cecum. **22.** Ventral view of anterior end of fully-developed, non-senescing adult fixed with minimal compression, dorsal view. Note internally the pharynx has been rolled slightly to the right and shifted slightly posteriorly, while externally the genital pore has been shifted to the right and the cecum on the right is somewhat swollen, and the cirrus sac surpasses the level of the intestinal bifurcation posteriorly. Abbreviations: AT, anterior testis; BI, intestinal bifurcation; BW, body wall; C, cecum; CS, posterior extent of cirrus sac; EP, excretory pore; EV, excretory vesicle; GP, genital pore; OS, oral sucker; P, pharynx; PT, posterior testis; SV, seminal vesicle; U, uterus.



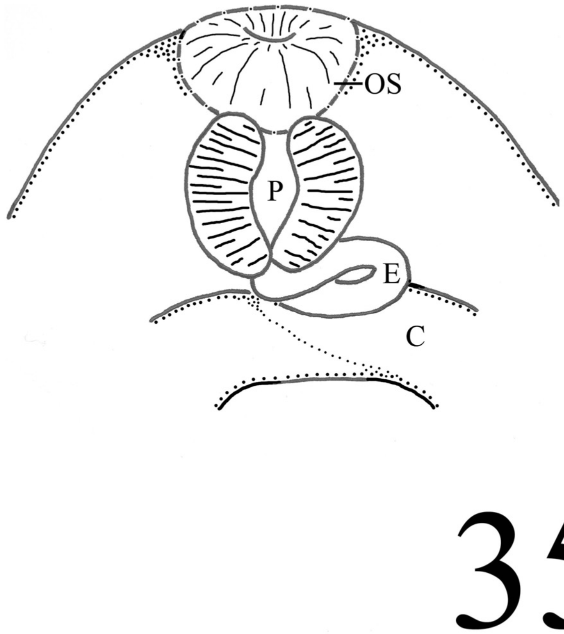
FIGURES 23–26. 23. Region of intestinal bifurcation of fully-developed, non-senescing adult fixed with minimal compression. Note the cirrus sac surpasses the relatively thin cecum. **24.** Region of intestinal bifurcation of a fully-developed, non-senescing adult fixed with minimal compression. Note the extent of cirrus sac relative to width of cecum at level of intestinal bifurcation. **25.** Region of intestinal bifurcation of an unusually elongated, fully-developed, non-senescing adult fixed with minimal compression. Note the extent of cirrus sac relative to width of cecum and the slender ceca. **26.** Region of intestinal bifurcation of a fully-developed, non-senescing adult fixed without compression. Note the extensive contraction of the specimen, the dramatically swollen ceca, and the posterior extent of the cirrus sac relative to the cecum. Abbreviations: C, cecum; CS, cirrus sac; GP, area of genital pore; M, miracidium; P, pharynx; SV, seminal vesicle; U, uterus.

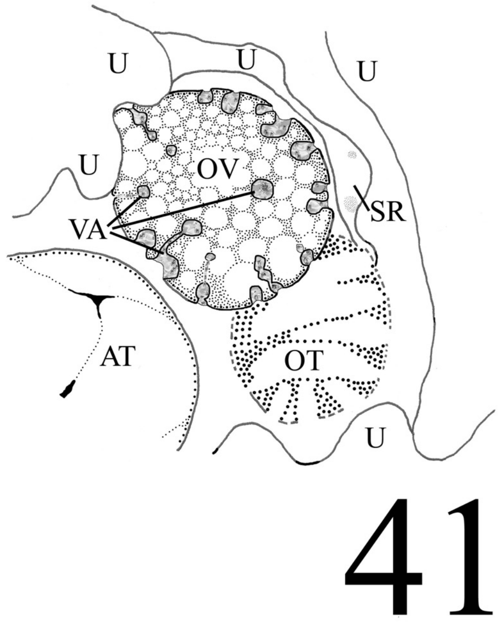


FIGURES 27–30. Diagrammatic representation showing the variability in the location of the genital pore. **27.** Located about a third of distance up pharynx from the esophagus; **28.** Located at about the posterior fourth of pharynx. **29.** Located just anterior to posterior margin of pharynx. **30.** Located at posterior margin of pharynx. Abbreviations: C, cecum; GP, genital pore; M, miracidium; P, pharynx; OS, oral sucker; SV, seminal vesicle; U, uterus.



FIGURES 31–34. Diagrammatic representations showing the variability in the location of the genital pore (cont.). **31.** Located immediately posterior to posterior margin of pharynx or a short distance posterior to it. **32.** Located some distance posterior to the posterior margin of the pharynx near anterior end of esophagus. **33.** Located a short distance posterior to the intestinal bifurcation. Note that the area of terminal genital canals shows the canals extending anteriorly some distance then reflexing posteriorly to join in the genital atrium at, or just posterior to the pharynx giving the impression that the genital pore opening was located more anterior in the posterior aspect of the pharynx. **34.** Located a short distance anterior to the posterior margin of the pharynx and submedian. Note that the area between pharynx and intestinal bifurcation appears to exhibit contraction-induced shortening of the distance from the pharynx to the intestinal bifurcation, and a dextral rotation and displacement of the pharynx posteriorly. Abbreviations: C, cecum; ES, esophagus; GP, genital pore; OS, oral sucker; P, pharynx; PP, prepharynx; SV, seminal vesicle; U, uterus.





FIGURES 35 and 41. 35. Area between pharynx and intestinal bifurcation showing collapsed esophagus. Abbreviations: C, swollen ceca; ES, anterior half of esophagus folded over the posterior half forming an S-shaped configuration; P, pharynx; OS, oral sucker. **41.** Illustration of atrophying ovary from a senescing adult. Abbreviations: AT, anterior testis; OT, oötype; OV, Ovary; SR, seminal receptacle; U, uterus; VA, vacuolization.

Remarks. The length of the cirrus sac and its posterior extent relative to the intestinal bifurcation (or ventral sucker if present) are commonly used as distinguishing characteristics for many species and sometimes genera of digeneans. Based on this study we feel the length of the cirrus sac can be a valuable characteristic for separating species and groups of species in cyclocoelids, especially when the percentage of its length relative to the length of the body also is considered. Generally, we agree with Dronen & Blend (2015) that where consistent differences are apparent in the posterior extent of the cirrus sac relative to the intestinal bifurcation and/or the length of the cirrus sac relative to body length that these characteristics can be valuable tools in distinguishing species of cyclocoelids. It should be kept in mind when comparing specimens where the anterior aspect of the ceca has become swollen, that the increased width of the ceca may alter the perception of the extent that the cirrus sac overlaps the ceca at the level of the intestinal bifurcation.

Position of the genital pore (Figs. 27–35). The genital pore in our specimens was most generally located from just anterior to the posterior margin of the pharynx to a short distance below the pharynx near the midline of the body (94% of our specimens); however, we did encounter some variability form this more typical range. Overall in young and fully-developed, non-senescing specimens fixed with slight compression, the position of the genital pore varied from being located a third of the distance anterior to the posterior margin of the pharynx to near the mid-level of the space from the pharynx to the intestinal bifurcation (genital pore located a third of the distance up the pharynx from the esophagus, n = 1 [Fig. 27]; near the posterior fourth of the pharynx, n = 6 [Fig. 28]; immediately above the posterior margin of the pharynx, n = 12 [Fig. 29]; at the posterior margin of the pharynx, n= 5 [Fig. 30]; immediately posterior to the posterior margin of the pharynx or a short distance posterior to it, n =7 [Fig. 31]; and well below the pharynx near the posterior end of the esophagus (immediately posterior to the level of anterior end of the intestinal bifurcation), n = 1 [Fig. 32]).

Despite carefully relaxation and heat fixation, in five of the specimens where the genital pore was actually located at the posterior margin of the pharynx or just posterior to it, contraction of the anterior end apparently had caused the distal portion of the uterus (region of the metraterm) and the anterior end of the male terminal genitalia to be pulled anteriorly to where it overlapped the posterior end of the pharynx, but the terminal genital canals then reflexed posteriorly joining in the genital atrium at, or just posterior to the pharynx and this configuration gave the impression that the genital pore opening was located more anterior in the posterior aspect of the pharynx (Fig. 33). In one of these specimens this contraction-induced shortening of the distance from the pharynx to the intestinal bifurcation collapsed the esophagus in to a spiral shape and resulted in a dextral rotation and displacement of the pharynx posteriorly (Fig. 34); however, the pharynx remained near the midline of the body. In three of these specimens the position of the genital pore on the ventral surface of these specimens appeared to have been less influenced by the contraction of the anterior end than the internal structures (i.e. pharynx, esophagus, ceca), resulting in the genital pore appearing more anterior than usual relative to the pharynx. Although the genital pore in these three specimens may have become moved anteriorly to some extent due to contraction of the anterior end, the posterior displacement of the pharynx appeared to be the major reason for the unusual anterior placement of the genital pore in these specimens. In these specimens the ceca were markedly swollen near the level of the intestinal bifurcation and pulled forward towards the pharynx to where the intestinal bifurcation overreached the posterior end of the esophagus effectively reducing the area between the pharynx and the intestinal bifurcation and causing the mid-level of the esophagus to bend so that the anterior half folded over the posterior half forming an S-shaped configuration (Fig. 35). In two other fully-developed, non-senescing adult specimens the genital pore appeared to be more than a fourth of the distance up the pharynx from the posterior margin of the pharynx and displaced sinisterly off the midline of the pharynx. In these cases the pharynx had been moved posteriorly with rotation right off the midline of the upper body so that it was no longer appeared centered in the body giving the inaccurate impression that the genital pore was submedian and more anterior in its placement.

In senescing specimens (n = 4) the genital pore was located immediately above the posterior margin of the pharynx (n = 1) at the posterior margin of the pharynx (n =1) immediately posterior to the posterior margin of the pharynx (n = 1) or well below the pharynx near the mid-level of the esophagus (n = 1), while in fully-developed, non-senescing adult specimens fixed without compression (n = 5) the genital pore was located immediately above the posterior margin of the pharynx (n = 1); at the posterior margin of the pharynx (n =1); or immediately posterior to the posterior margin of the pharynx (n = 3).

Specimens fixed without compression appeared to have contracted more than those where compression was used, and in the specimen where the genital pore appeared to be located well below the pharynx, the ventral surface of the body appeared to have contracted more than the dorsal surface moving the genital pore posteriorly and bending the specimens ventrally.

Remarks. The placement of the genital pore has long been an accepted distinguishing characteristic for genera in many groups of digeneans, sometimes being used at the species and subfamily levels for some digenean groups and commonly used to distinguish genera in the Cyclocoelidae. For example, *Uvitellina teesae* Dharejo, Bilqees & Khan, 2007 and *Haematotrephus jaensch* Johnston & Simpson, 1940 in the Haematotrephinae Dollfus, 1948, and *Allopyge undulates* Canavan, 1934 and *Allopyge antigones* Johnston, 1913 in the Hyptiasminae Dollfus, 1948 have an unusually posterior placement of the genital pore where in *U. teesae* and *H. jaensch* it is located at the level of the posterior end of the esophagus and in *A. undulates* and *A. antigones* it is positioned immediately below the intestinal bifurcation.

Digenean practitioners generally are well aware that in their groups of study characteristics such as the position of the genital pore may be somewhat variable either naturally or artificially (removed from live hosts *vs* being removed from previously fixed or frozen hosts) or in the fixation of the specimens (heat fixation of live, relaxed specimens *vs* cold fixation by dropping specimens into a fixative; use of slight coverslip pressure in fixation *vs* fixation of specimens without coverslip pressure) in a collection of the specimens. As with any sample of members of any species, cyclocoelids can exhibit natural variability in the size and/or location of most characteristics (e. g. genital pore, pharynx, esophagus, intestinal bifurcation, ventral sucker, ovary, testes, cirrus sac); however, these differences are not necessarily an indication that such aberrant specimens represent a separate species or a member of another genus. In our opinion, the position of the genital pore is a reliable characteristic when comparing properly-fixed specimens from reasonably large sample sizes, especially at genus and species levels. Although most of our specimens showed relatively few signs of body contraction, there were some instances where there appeared to be subtle contraction-based effects on the perception of the exact location of the genital pore causing the genital pore in these specimens to appear to be outside the most typical range of the placement of the genital pore. Such induced anomalies, and to a lesser extent normal variations, may cause an incorrect interpretation of the location of critical structures in a specimen (i.e. genital pore) that in turn may cause an incorrect identification of a species and/or its placement in the correct genus. Such misinterpretations in conjunction with molecular studies could easily cause taxonomic revisions in which the morphology does not agree with the molecular analyses (different body forms may be placed inadvertently in the same genus and as a consequence be assigned to the wrong subfamily).

Size and shape of ovary and its placement relative to the testes (Figs. 36–41). The ovary in our specimens fixed with compression were relatively small in contrast to the testes (240–480 wide compared to a mean width of 260–922), nearly circular (n=16) to somewhat oval (n=11). The size of the ovary appears to be somewhat variable in all three developmental categories (Table 1); however, the width of the ovary represented as the percentage of the body length generally was similar in the fully-developed, non-senescing adults, young adults and the adults fixed without compression (3–5%; 3–5%; 3–4%, respectively), while slightly smaller in the senescing adults (2–3%).

The position of the ovary relative to the testes in these adults varied from being distinctly pretesticular (n=18) (Figs. 36, 37) to being next to the anterior testes (n=9) (usually across from anterior half of anterior testis, Fig. 38; rarely further posterior, Fig. 39), and the ovary formed a triangle with the testes near the posterior end of the body being most often located a short distance from the anterior testis (n=20), but occasionally contiguous or nearly so with the anterior testis (n=7) (Fig. 40). In two senescing specimens the ovary was vacuolated and appeared to be atrophying (Fig. 41).

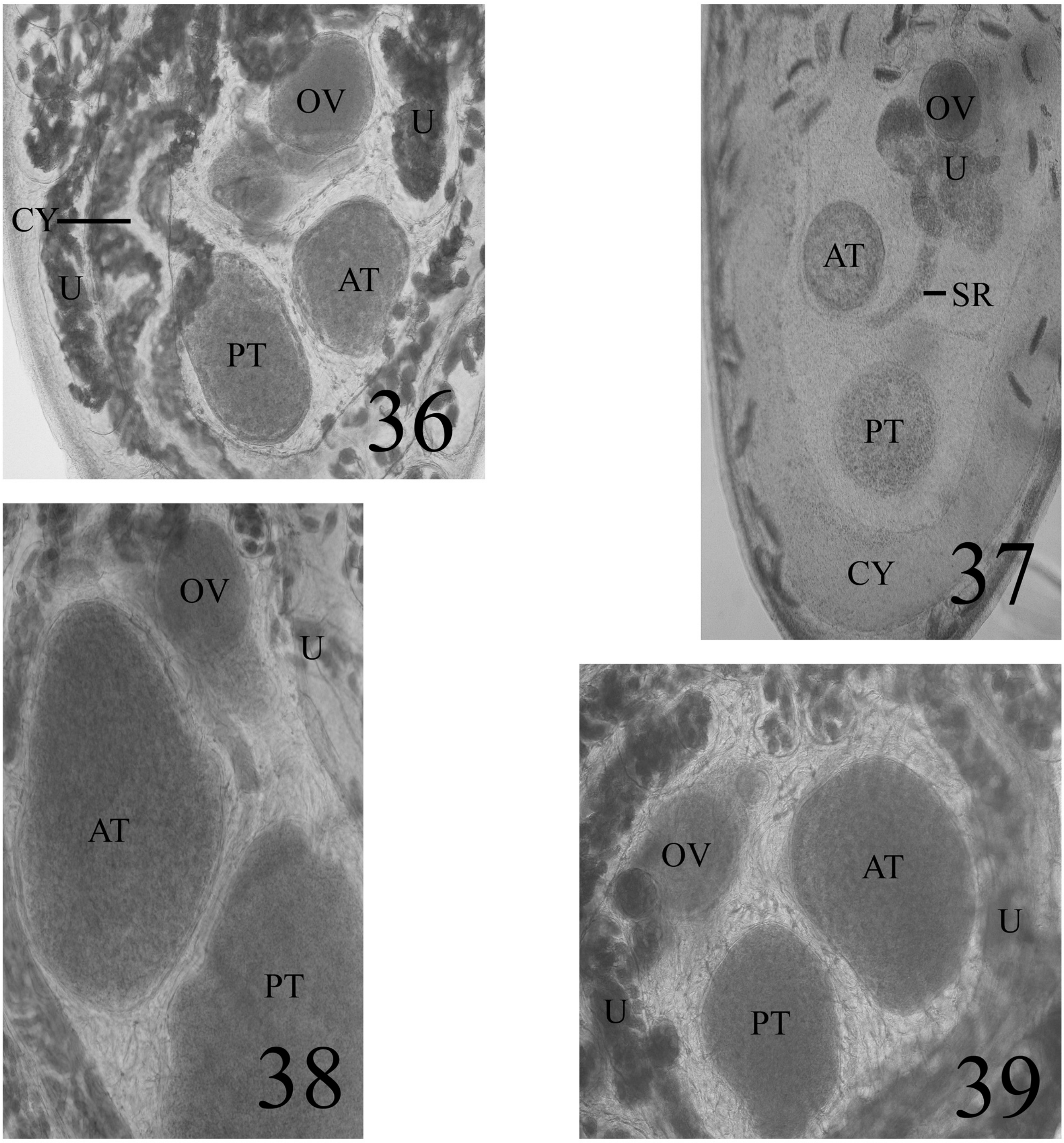
Remarks. Previous authors (e.g. Szidat 1932; Stunkard 1966; Macko *et al*. 2011; Dronen 2002; Dronen & Blend 2015) have noted variability in position and size of the gonads in cyclocoelids. Whereas we consider the placement of the ovary relative to the position of the testes and the location of the gonads in the body relatively strong characteristics to diagnose subfamilies and sometimes genera in this family (e.g. in species of both *Bothriogaster* Dollfus, 1948 and *Spaniometra* Kossack, 1911, the ovary is located near the posterior end of the body, while the testes are located near the mid-body; in *S. mertensisi* Gupta, 1970 the anterior testis is far removed from the posteriorly-placed ovary near the mid-body), it is our opinion that width (size) of the ovary is not overly valuable as a distinguishing characteristic at the species level unless it is dramatically and consistently different. It appears that ovary size (usually width) also may be related to the size (growth) of specimen, although senescing adults had a slightly smaller percentage of the ovary width relative to body length (2–3%).

In specimens where the gonads were crowded together against the cyclocoel arch and were nearly contiguous or overlapped, or where the ovary was located posterior to the anterior margin to the anterior testis there were signs of contraction of the specimens. Here again, it is important that normal and induced variability of characteristics like the position of the ovary relative to the testes be considered when identifying species and determining genus and subfamily placement, especially in studies where molecular analyses are employed, as incorrect interpretation of the morphology could lead to taxonomic revisions and erection of new taxa wherein the morphology does not support the molecular analyses.

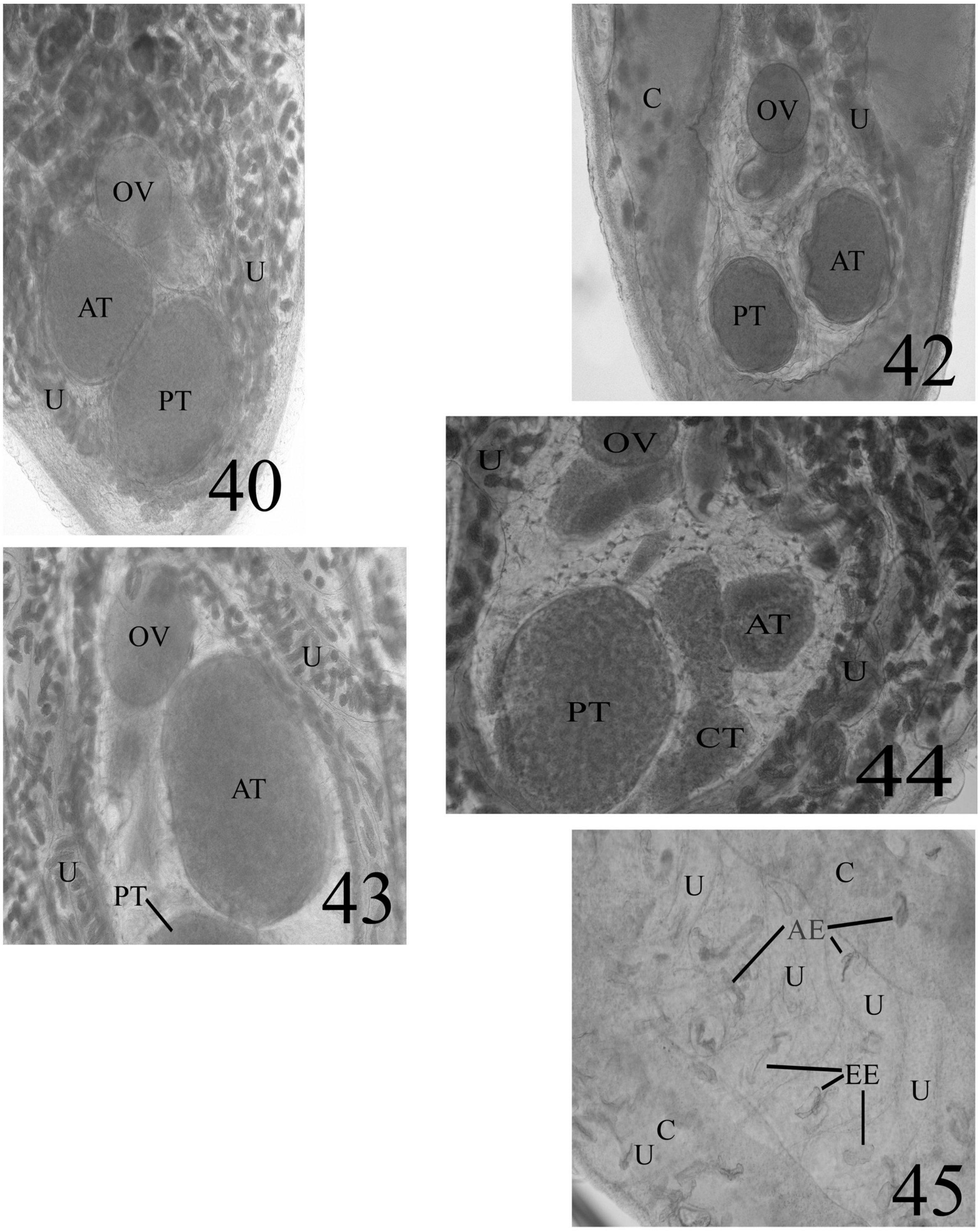
Size, appearance/shape and location of testes (Fig. 43). Measurement of testes were exceptionally variable (mean width 260–922). The width of the testes ranged from 300–690 (3–7% of body length) in fully-developed, non-senescing adults from site #1 and 254–941 (3–9%) from site #2. In the collection from site #2 the width ranged from 245–637 (3–10%) in young adults and 294–363 (3–4%) in senescing adults.

Overall the testes were typically larger than the ovary (testes/ovary width ratios 1:1.1–1:2.9), smooth, spherical to subspherical, oblique, non-contiguous, usually located a short distance anterior to the posterior arch of the cyclocoel in our specimens; however, we observed some variation in the general appearance (shape) and relative placement of testes (e.g. Figs. 37–45). In two senescing specimen the testes obviously were separating from the surrounding parenchyma and both testes were irregular (Fig. 9).

Remarks. Many previous authors have considered the testes size to be one of the most variable characteristics in flukes (e.g. Szidat 1932; Stunkard 1966; Macko *et al*. 2011; Dronen 2002; Dronen & Blend 2015). Dronen (2007) and Dronen & Blend (2015) considered the size, shape and appearance of the testes to be too variable in most cyclocoelids to be effective as a distinguishing characteristic at the species or genus levels. As appears to be the case in most species in the family, investigators can expect to find the size of testes to generally have very large ranges in a comparison between specimens in a reasonable sample size (10 or more) of a species, and should be cautious in the use of testicular measurements to distinguish species. Similarly, as is evidenced above, one can expect to find at least subtle irregularities in the appearance (e.g. indented in part, pyriform, heart-shaped) of testes, especially if specimens are in a state of senescence. In broader terms the general location of testes (e.g. location in the body relative to the posterior arch of the cyclocoel or an unusual placement in the body, obvious differences in the length of the intertesticular space or the length of the posttesticular space) can be of value when comparing genera or in comparing some species. The position of testes relative to the ovary has commonly been used to define subfamilies within the Cyclocoelidae, but most of these characteristic have a more limited value in genera like *Uvitellina* when comparing species.



FIGURES 36–39. 36. Area of testes and female genital complex from a fully-developed, non-senescing adults fixed with minimal compression. Note the ovary is clearly pretesticular and the testes are diagonal and close together. **37.** Area of testes and female genital complex from a young adult. Note the ovary is clearly pretesticular and the testes are nearly tandem and separated by a wide intertesticular space. **38.** Area of testes and female genital complex from a fully-developed, non-senescing adult fixed with minimal compression. Note the prestesticular ovary is located across from anterior margin of anterior testis and the testes are large and contiguous. **39.** Area of testes and female genital complex from a fully-developed, non-senescing adults fixed with minimal compression. Note the ovary is across from the anterior testis. Abbreviations: AT, anterior testis; CY, cyclocoel arch; OV, ovary; PT, posterior testis; SR, seminal receptacle; U, uterus.



FIGURES 40, 42–45. 40. Area of testes and female genital complex from a contracted, fully-developed, non-senescing adults fixed with no compression. Note the close proximity of the ovary to the anterior testis and the overlap of the two testes. **42.** Area of testes and female genital complex of a senescing adult. Note the reduced number of eggs present, the irregular shape of the anterior testis and the separation of the anterior testis from the surrounding parenchyma. **43.** Area of testes and female genital complex from a fully-developed, non-senescing adults fixed with compression. Note the anterior testis is contiguous with both the ovary and the posterior testis. **44.** Area of testes and female genital complex of a fully-developed, non-senescing adult. Note the three different sized and dissimilar testes. **45.** Midlevel region of body of a senescing adult. Note the lack of normally developing eggs and miracidia in the uterus. Abbreviations: AE, abnormally developing eggs in uterus in which there was what appeared to be primordial material and/or yolk material present, but with no miracidial development evident; AT, anterior testis; C, cecum; CT, centrally-located testis-like structure; EE, empty eggs; OV, ovary; PT, posterior testis; U, uterus.

Mean testes/ovary width ratio. Overall the percentages of body length are relatively consistent for the width of the ovary (2–5%), while the percentages for the testes have a much broader range (3–10%). This is reflected in the wide range of the width ratios of the ovary to the testes seen in our specimens (1:1.1–2.9). On average this width ratio is most similar in the young adult and senescing adult specimens (1:1.5) and it is somewhat smaller than those of the fully-developed, non-senescing adults (1:1.8 and 1:2.0, respectively).

Remarks. In our opinion, whereas the width ratio of the oral sucker to the pharynx appears to hold some promise as a distinguishing feature in this species, the large, overlapping ranges of the testes/ovary width ratio suggests it would of little value as a distinguishing tool for species in the cyclocoelids.

Intertesticular space length (Figs. 43–45). Among our 36 specimens fixed with compression 22 specimens had an intertesticular space present (fully-developed, non-senescing adults 16; young adult 3; senescing 3); however, in 10 specimens the testes were contiguous or nearly so (Figs. 38, 43) and in two specimens fixed without compression the testes overlapped and the posterior testes overlapped the cyclocoel arch posteriorly (Fig. 40). There was one very unusual adult that was fixed with compression that appeared to have three different sized and dissimilar testes (Fig. 44). In specimens where the testes were contiguous, there appeared to be slight contraction of the body where the tegument was slightly crenulated, ceca were somewhat irregular (margins wavy and not uniform in width throughout length of body, most were swollen), the gonads often were closely packed together, and the uterus was inflated so that there was noticeable space between the eggs and the uterine wall) (Fig. 45). In one adult from site #1 and one young adult from site #2 the testes overlapped (by 74 and 68, respectively); however, these two specimens showed signs of a moderate amount of contraction (tegument somewhat crenulated, ceca irregular [margins wavy and not uniform in width throughout length of body], gonads tightly grouped together, and swollen uterus). There was one adult and one senescing specimen from site #2 where there was an unusually large intertesticular space (162 long, 2%; 140, 2% of body length, respectively). In these two specimens there were no signs of contraction of the body.

Remarks. A few authors (e.g. Timon-David 1950) have considered the length of the intertesticular space to be too variable to be effective in distinguishing species. Although the intertesticular space showed a large range in length overall (0–240), the percentage of the body length relative to body length was about 0–2% in the three developmental categories. The length of the intertesticular space is best employed where there are large differences apparent between species and where its relationship to body length is considered. For example, *Hyptiasmus magniproles* Witenberg, 1928 has a very extensive intertesticular space that is about 40% of body length. In our specimens, the length of the intertesticular space appeared to be reduced in contracted specimens. Although we feel that this characteristic can be a relatively useful feature in distinguishing species in some genera, we consider it to be most effective as a distinguishing species where the distance between testes is substantial, there are corroborating characteristics available and/or where contraction of the body is minimal.

Posttesticular space length. The posttesticular space in our specimens was quite variable, ranging from 270–1,020 overall in fully-developed, non-senescing adults which represented 3–10% of body length. The fully-developed adults from site # 1 had the largest range for this measurement, but the percentage of the body was about the same as that of the other three groups. For example, the largest specimen (body 10,789) in the collection was in the fully-developed adult category from site #1. This specimen had what appeared to be an unusually long posttesticular space (1,020; 10%); however, the largest specimens in the young adult category (6,865 long) had a posttesticular space that was 628 long, which also represented 10% of body length.

Remarks. Although the ranges of the length of the posttesticular space were quite variable in our specimens, this measurement relative to the body length was reasonably consistent between the categories suggesting that the range of the length of this area expressed as a percentage of the body length may have some value as a distinguishing characteristic at the species level. Although the range of the percentages body length for this measurement were quite consistent in comparing the three developmental categories of *U. iraquensis*, because of this large range of the percentage of body length represented by this characteristic (3–10%), we feel that it would be prudent to have corroborating characteristics if this characteristic is used.

Distance from ovary to anterior testis. Although the range of measurements for the distance from the ovary to the anterior testis appeared to be quite variable and to represent large ranges (0–490 long overall), this measurement represented a relatively small range of the percentage of the body length (0–5%). Young adults (0–108) had the smallest measurements among the three developmental categories and a somewhat smaller percentage relative to body length (0–2%), but overall these percentages were relatively similar in all developmental categories (fully-developed, non-senescing adults from site #1[Huwazah] 0–5%; fully-developed, non-senescing adults from site #2 [East Hammar] 0.1–4%; senescing adults 2–4%).

Remarks. The similarities and small ranges of the percentage of the body length represented by the distance from the ovary to the anterior testis suggest that this feature could be useful in separating species. However, Dronen & Blend (2015) did not use this characteristic in their key to *Uvitellina*. As often is a problem in genera in the Cyclocoelidae, this characteristic overlaps or is similar enough to those of other species that it often provides minimal usefulness in separating species in this genus.

Size of vitelline follicles/distribution of the vitelline fields. The ranges of lengths and widths of follicles overlapped extensively in all three developmental categories. The size of vitelline follicles in the fully-developed, non-senescing (16–99 wide) and young adults (22–72) was variable, and follicles were not consistent in shape (longer or shorter than width). Vitelline follicles were not measured in the senescing adults because the vitelline fields were indistinct and individual follicular masses were diffuse, precluding accurate measurements (Fig.10). Vitelline fields were generally confluent in our specimens. In three senescing specimens the vitelline fields were diffuse and indistinct, giving the impression that they were not confluent posteriorly and in six fully-developed adult specimens the confluence was difficult to see because of the egg-laden uterus and without careful examination from both the ventral and dorsal perspective, these specimens could have easily been misinterpreted as having non-confluent vitelline fields posteriorly. The anterior extent of the vitelline fields in specimens where they both reached anteriorly to about the same level varied from having both fields terminating at the level of the intestinal bifurcation or a little more anterior (n=14) to terminating immediately below the intestinal bifurcation (n=5). There were some specimens where one side of the vitelline fields extended near to the level of the intestinal bifurcation, while in the others they terminated some distance posterior to the intestinal bifurcation (n=9). In one specimen the right vitelline field terminated anteriorly near the mid-level of the esophagus.

Remarks. Although the extent of the vitelline fields (posteriorly and/or anteriorly) has commonly been used to distinguish species in many fluke families, there are a number of studies where doubt has been cast on the usefulness of confluence of vitelline fields anteriorly and/or posteriorly as a distinguishing feature for species and genera in cyclocoelids (e.g. Stunkard 1929; Johnston & Simpson 1940; Dubois 1959, 1965; Macko & Feige 1960; Macko 1964, 1969; Macko *et al*. 2011). Obviously, there is some variability in the anterior extent of the vitelline fields in *U. iraquensis*; however, nearly all of our specimens had one of the vitelline fields terminating near the level of the intestinal bifurcation and in our opinion this characteristic can be used to distinguish species if the anterior extent of the longest vitelline field is considered to be representative of the anterior extent of the vitellarium and researchers anticipate encountering some variability such as seen in the specimens where the anterior extent of one vitelline field was near the mid-level of the esophagus. This characteristic is similar among species of *Uvitellina* and does not provide for a clear separation in the case of *U. iraquensis*. It has been our experience that characteristics such as the posterior extent/distribution of the vitelline fields (confluent or not) can be somewhat variable in cyclocoelids and it is not unexpected to find abnormal expressions of this characteristic in populations. Certainly, we may see some specimens of a species of cyclocoelids where the vitelline fields are expected to be confluent anteriorly and/or posteriorly that may appear to display non-confluence; however, the vast majority of our specimens of *U. iraquensis* have posteriorly confluent vitelline fields. Variations in the appearance of this characteristic could be due to natural variability, improper fixation, the particular developmental state of a specimen or only our inability to accurately assess a masked characteristic. Rarely, we may find some specimens of species that would be expected to lack anteriorly and/or posteriorly confluent vitelline fields, but instead they appear to possess vitelline fields that are either touching or slightly overlapping. While the size and shape of the individual follicles are too variable in cyclocoelids to be an effective tool in separating species, the anterior and posterior extent (confluent or not) of the vitelline fields appears to be a generally consistent characteristic in cyclocoelids, and in our opinion in some cases they can provide a reliable characteristic for the separation of genera within subfamilies.

Disposition of lateral uterine loops. In *U. iraquensis* the uterine loops in egg-bearing specimens consistently overreached the ceca laterally in at least the posterior half to two thirds of the body, frequently reaching to the body wall (Figs. 1, 7). In addition, the most posterior uterine loops generally surrounded the gonads, extending posteriorly into the posttesticular space on both sides (Figs. 18, 19).

Remarks. There are three possible conditions of the posterior and/or lateral extent of the uterine loops in *U.*

iraquensis that we feel are reasonably consistent and are valuable in distinguishing this species and potentially species in other cyclocoelid genera: the lateral extent of the loops relative to the ceca (extracecal or intracecal), the proximity of the uterine loops to the body wall (approaching or not) and the posterior extent of the uterine loops relative to the testis and/or ovary (extending into the posttesticular or postovarian space or not).

Egg size. Generally, the newly-formed eggs of this species (107–155 x 33–61) were found in the proximal region of the uterus near the level of the ovary in fully-developed, non-senescing adults. These young eggs are produced in large numbers, lack eyespots and the primordia of the germinal masses of the future asexual generations in the first intermediate snail host is not yet defined. As these newly-formed eggs move more anteriorly into the middle third of the uterus they continue to grow and develop, and typically produce the miracidial stage where eyespots and the primordia of future germinal masses become more apparent (Fig. 5). As these developing eggs move more anteriorly through the middle third of the uterus into the more distal third of the uterus they become fully-developed reaching their largest size (144–217 x 67–87). In the upper third of the uterus these larger eggs appear to round up and swell (185–225 x 75–95) and eventually the miracidium appears to dramatically elongate within the egg producing pressure on the poles of the egg as part of the hatching process. Fully-developed miracidia are generally most apparent in the distal portion of the uterus. It should be pointed out that some fully-developed eggs, and more rarely miracidia, may be found more posteriorly in the uterus mixed with the smaller, less developed eggs in the middle third of the uterus. Occasionally some smaller, less developed eggs may be found in the anterior third of the uterus. It may be that eggs are sometimes redistributed in the uterus; however, it may also be that a limited number of eggs develop more quickly than others and release miracidia more posteriorly in the uterus. The large number of eggs produced by fully-developed, non-senescing, adults results in the uterus becoming distended and packed with eggs and/or miracidia from immediately posterior to the intestinal bifurcation (Figs. 4, 26) to the posterior end (Figs. 18, 19) with the uterine loops extending laterally approaching the body wall on both sides. Because the lateral uterine loops also extend posteriorly large distances along the body wall older, more developed eggs may be observed in these lateral uterine loops at the same level in the body as smaller, less developed eggs nearer the midline of the body. In addition the uterus winds dorsally and ventrally so that eggs observed may be on a slant or on end, giving the impression that they are shorter. There also may be abnormal eggs present in the uterus. Care should be taken to measure only fully-developed eggs that appear to be normal and that are in a flat profile for comparative purposes. Eggs of this species have a thin, delicate shell, which appears to allow a developing miracidium to redistribute its mass and in doing so, change the shape of the egg, often bending the egg into a banana-like shape (Figs. 2, 3, 5, 21) or causing the anterior end of the eggs to become enlarged (Fig. 21). The smaller, less developed eggs in the proximal aspect of the uterus often appeared to be somewhat collapsed around the developing miracidial mass resulting in a short empty portion of the shell extending ahead of the miracidium and a longer empty portion extending off the posterior end of the miracidium (Fig. 6). These collapsed extensions are often difficult to see and care should be taken to include these extended portions of the eggs shell in length measurements and not to measure just the developing miracidium inside the egg. There were noticeably more hatched miracidia in the distal uterus of the specimens from site #1 as compared with those from site #2. Additionally, senescing adults have an abundance of eggs that are apparently unhatched but empty (Fig.14) and/or contain what appears to be either vitelline materials or undeveloped germinal material (undeveloped miracidia) (Fig. 13) that are not commonly present in any of the other three developmental categories of this species.

Remarks. Generally egg size is considered to be a relatively consistent and reliable distinguishing characteristic in digeneans; however, egg size presents an unusual situation in cyclocoelids like *U. iraquensis*, which appears to utilize a terrestrial-based life cycle (uses a terrestrial snail) because eggs grow and develop in the uterus, often leading to large unusable ranges of measurements (e.g. *Uvitellina teesae* Dharejo, Bilqees & Khan, 2007; 133–216 long), which often overlap between species. Calhoun & Dronen (2012) suggested that when measuring eggs in groups like the Mesocoelidae Dollfus, 1929 where growth and development *in utero* has been documented, that only eggs that are fully-developed (usually in the distal end of the uterus) that are normal in appearance, and that are in a flat profile should be measured for use in species comparisons. We support this approach in the case of the cyclocoelids. Because of the large ranges of egg sizes reported in the literature for species of cyclocoelids, it is often useful to compare the maximum length and/or width of eggs when attempting to distinguish species. The presence of unhatched empty eggs and lack of normally developing miracidia in some eggs in senescing specimens is likely a product of the atrophy of the reproductive systems.

Miracidium size. Generally, eggs typically hatch in the distal uterus of *U. iraquensis* releasing miracidia (Fig.

2). Miracidia are somewhat larger in fully-developed, non-senescing adults (163–225 x 60–99) than in the young adults (132–148 x 53–83), and this stage is not typically found in senescing adults.

Remarks. Miracidia tend to be quite active so that during fixation they may be preserved at varying lengths between being contracted and fully elongated, making only relative estimates of length and width possible. The ranges of lengths and widths of miracidia are large, tend to overlap among the different developmental categories and appear to be shorter in younger specimens when compared with the fully-developed, non-senescing adults. It is our opinion that unless non-overlapping ranges are obvious when comparing species of cyclocoelids, miracidial length and width are of very limited use as a distinguishing characteristic.

Discussion

The most limiting factor in the taxonomy and systematics of cyclocoelids stems from incompletely described species based on a very limited sample size (commonly one specimen). Therefore we have a meager understanding of the variability of the characteristics used to distinguish most species, and to separate genera within subfamilies (Dronen 2007, Dronen & Blend 2015). Our lack of a reasonable understanding of intraspecific variability makes identification of species and their placement in genera and subfamilies more difficult than in many other digenean groups.

The placement of the ovary relative to the testes (i.e. pretesticular, intertesticular or posttesticular; forming a triangle with the testes or in a straight line with the testes) and the position of the testes relative to each other (i.e side by side, tandem or diagonal) historically have been considered to be important considerations in the definition of the subfamilies within the Cyclocoelidae (e.g. Kanev *et al*. 2002; Dronen 2007; Dronen & Blend 2015). Dronen (2007) proposed six subfamilies in the Cyclocoelidae based largely on the position of the ovary relative to the testes: Cyclocoelinae Stossich, 1902 in which the ovary is intertesticular forming a triangle with the testes; Haematotrephinae Dollfus, 1948 in which the position of the ovary ranges from being pretesticular to opposite to the anterior testis forming a triangle with the testes; Szidatitreminae Dronen, 2007 in which the position of the ovary ranges from being posttesticular to opposite to the posterior testis forming a triangle with the testes; Ophthalmophaginae Harrah, 1922 in which the ovary is posttesticular forming a straight, or nearly straight line with the tandem testes; Hyptiasminae Dollfus, 1948 in which the ovary is intertesticular and the testes are tandem to nearly tandem; and Skrjabinocoelinae Dronen, 2007 in which the ovary is intertesticular and nearly in a straight line with the side by side testes (Dronen 2007). In specimens of *Uvitellina* where the ovary is distinctly pretesticular forming a triangle with the testes (Figs. 36, 37, 42), subfamily placement is reasonably unambiguous (Heamatotrephinae); however, one can expect to encounter some variability (natural, but primarily in improperly-fixed specimens) in the placement of the ovary relative to the anterior testis. These anomalies appear to be more pronounced in contracted specimens (usually in those fixed without minimal coverslip pressure, retrieved from fixed host specimens, removed from frozen hosts or in specimens that were cold fixed) where the ovary may be placed across from the anterior testis (Figs. 38, 43) and more rarely somewhat more posteriorly (Fig. 39). In these unusual placements of the ovary, it is often difficult to determine if the ovary should be considered to be pretesticular or intertesticular (Cyclocoelinae). Defining a pretesticular ovary as one that is clearly pretesticular to opposite to the anterior testis can assist those who are not commonly familiar with cyclocoelid morphology and the problems that may be encountered in making a reasonable decision concerning this pattern of placement of the ovary, but if there is doubt about the placement of the ovary, it may be best to consider the specimen as being atypical and not rely heavily on it in a comparative morphological study. The same general problem can occur with specimens that have a posttesticular ovary which forms a triangle with the testes (Szidatitreminae). Here again, variability in the placement of the ovary can be misconstrued as being intertesticular and cause the improper placement of specimens in the appropriate genus and/or subfamily ultimately leading to the misidentification of a species. Definition of the ovary as being distinctly posttesticular to opposite to the posterior testes often assists in the determination of the subfamily placement. Appropriate consideration of the developmental categories one might encounter and the possible influence of contraction of specimens are important in determining the placement of the ovary as well as other structures in the Cyclocoelidae.

Within subfamilies, the genera have been distinguished largely based on the position of the genital pore relative to the pharynx (prepharyngeal [defined as being anterior to the mid-level of the pharynx] or postpharyngeal [being posterior to the mid-level of the pharynx]) and the disposition of the vitelline fields (not confluent at either end [posterior or anterior] of the body; confluent both posteriorly at the posterior arch of the cyclocoel and anteriorly near the intestinal bifurcation; or confluent near the posterior end but not confluent anteriorly in the area of the intestinal bifurcation), and to a lesser extent whether the testes were side by side or diagonal to tandem, or smooth or lobed (Kanev *et al* 2002; Dronen 2007). As previously noted, we encountered some variability (natural and induced during fixation) in these characteristics; however, despite these generally minor differences among the three apparent developmental classes studied herein (fully-developed, non-senescing; young and senescing adults), all of our specimens undoubtedly represented *Uvitellina iraquensis* (pretesticular ovary forming a triangle with testes, Haematotrephinae; postpharyngeal genital pore; vitelline fields confluent posteriorly, *Uvitellina*; rudimentary oral sucker present; vitelline fields confluent posterior but not forming an additional posterior tail-like extension; eggs in distal uterus relatively large, 165–217 µm x 63–88 µm [Dronen & Blend 2015 and the current study]).

There is probably no other family of flukes that could benefit more from molecular studies than the Cyclocoelidae, and there is little doubt that such studies will result in meaningful revisions of subfamilies and genera. But as more molecular studies target cyclocoelids, we cannot emphasize enough that the obvious natural and fixation-induced variations commonly seen in cyclocoelids require caution in evaluating the placement of the testes relative to the ovary when attempting to identify species and assign genera to a subfamily. When conclusions based on DNA sequence data does not support conventional morphological observations, it may be that either pertinent morphological characteristics have been misconstrued in identification of specimens or that researchers may not be looking at the "right genes" to answer a question on the "right level" of taxonomy. In our opinion, all researchers should be keenly aware of the possible influence of contractions of specimens and any possible natural variability in the placement of the gonads in the cyclocoelids, and reluctantly use small sample sizes to make broad generalizations concerning the taxonomy based on molecular data in this morphologically difficult group until there is a better understanding of which genes are most useful in determining phylogenic relationships in the various digenean taxa, especially those like the cyclocoelids where morphology can be difficult and sometimes misleading.

When evaluating possible distinguishing characteristics, a researcher should take in to account how specimens were fixed. Some authors consider heat fixation to promote reduction (shrinkage) in size of the body and in some internal and external structures of flukes. It has also been suggested that the use of compression during fixation alters the size and shape of specimens. Historically, many if not most helminthologists have considered heat fixation to be the most acceptable method for use in flukes (or soft-bodies helminths in general) and minimal coverslip pressure commonly was used by researchers in the preparation of specimens for deposition in collections worldwide. Recently some researchers have preferred to prepare specimens of flukes without compression and/or with cold fixation of specimens as a time-saving procedure (e.g., Crib & Bray 2010). Our concern is that specimens prepared using different methods of fixation could lead to the description of new taxa (i.e. species) where the differences used to distinguish them may be a product of the type of fixation being employed. Admittedly, collection and preparation of specimens in the field is often difficult at best and in many cases heat fixation and/or compression may not always be practical, especially when rare material is encountered, but it is our opinion that researchers need a better understanding of the ramifications of using different methods of fixation in order to facilitate their comparisons of specimens where more than one fixation process is involved. We hope the information herein will be of use to cyclocoelid researchers who may need to compare specimens fixed with compression to those that have been fixed without compression.

It also is reasonable to determine the developmental state (categories) of the individual specimens being examined (fully-developed, young adults, senescing adults), and to consider the potential implications of the developmental state of specimens in regards to distinguishing characteristics. For example, the eggs of this species (and other species of cyclocoelids) grow and develop as they progress from the oötype (proximal end) where they are created to the distal aspect of the uterus, and we therefore recommend that the developmental state of eggs be considered when comparing species. The best comparison would likely be achieved if measurements of eggs that are fully-developed (usually in the distal end of the uterus), normal in appearance, and in a flat profile were compared between species. It should be kept in mind that eggs appear to become more flattened and therefore longer when compression is applied during fixation as compared with those in specimens fixed without compression. Where large ranges in size of eggs are encountered in existing species description, it may be useful to compare maximum egg length and width. Table 1 shows the variations apparent in the three developmental categories observed when comparing the other distinguishing characteristics common used in cyclocoelids.

TABLE 1. Comparative measurements, morphometric percentages, morphometric ratios of adults of *Uvitellina iraquensis* Dronen, Ali & Al Amura, 2013 (Haematotrephinae Dollfus, 1948) from two separate collections of white-tailed lapwings, *Vanellus leucurus* (Lichtenstein) in Iraq. Only mature, normally developed, non-senescing, adults were present in collection #1, while in addition to mature, normally developed, non-senescing, adults (n=8), collection # 2 contained young adult (n=5) and senescing representatives (n=4). Five mature, normally developed, non-senescing, adult specimens from collection # 1 were fixed without cover glass compression for comparison to the 27 specimens from collections #1 (n=19) and #2 (n=8) that were fixed with slight compression. Ranges are shown in parentheses and percentages of body length are shown in brackets.

Locality	Huwazah Marsh, north-eastern Basrah Province near Majnoon, southern Iraq (31° 10' N 47° 39' E)	Huwazah Marsh, north-eastern Basrah Province near Majnoon, southern Iraq (31° 10' N 47° 39' E	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30°48′22″N 47°1′E)	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30°48′22″N 47°1′E)	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30°48′22″N 47°1′E)
Date collected	February–March., 2011	February–March., 2011	July–November, 2012	July–November, 2012	July–November, 2012
	Fully-developed adults from host collection #1 (fixed with slight compression) n=19	Fully-developed adults from host collection #1 (fixed without slight compression) n=5	Fully-developed adults from host collection #2 (fixed with slight coverslip pressure) n=8	Young adults from collection #2 (fixed with slight coverslip pressure) n=5	Senescing adults from host collection #2 (fixed with slight coverslip pressure) n=4
Body length	8,916 (7,557–10,789)	7,955 (7,269–8,695)	8,724 (7,338–10,802)	6,748 (6,402–6,865)	8,554 (7,460–9,526)
Body width	2,135 (1,800–2,525) [23–25%]	1,494 (1,288–1,847) [17–22%]	2,255 (1,749–2,795) [23–26%]	1,744 (1,372–1,893) [22–28%]	1,547 (1,360–1,713) [17–19%]
Oral sucker width	342 (255–420) [3–4%]	249 (211–289) [3–4%]	269 (216–357) [3–4%]	243 (196–284) [3–5%]	285 (254–304) [3–4%]
Prepharynx length	8 (0–30) [0–0.3%]	14 (0–34) [0–0.4%]	6 (0–29) [0–0.3%]	3 (0–8) [0–0.1%]	0
Pharynx width	324 (295–353) [3–4%]	269 (240–309) [3–4%]	299 (255–323) [3–4%]	251 (200–285) [3–5%]	267 (245–284) [2–4%]
Oral sucker/pharynx width ratio	1:1.0 (1:0.7–1:1.4)	1:0.9 (1:0.8–1:1.1)	1:1.0 (1:0.7–1:1.4)	1:0.9 (1:0.4–1:1.1)	1:1.1(1:1.0–1:1.3)
Distance from pharynx to intestinal bifurcation	65 (30–138) [0.4–2%]	126 (34–186) [0.5–3%]	90(19–196) [0.2–2%]	91 (69–127) [1–2%]	88 (67–118) [0.8–2%]
Cirrus sac length	396 (300–500) [3–6%]	411 (353–441) [4–6%]	391 (304–470) [4–5%]	342 (284–420) [4–7%]	377 (333–461) [4–5%]
Ovary width	307 (240–340) [3–4%]	299 (275–333) [3–4%]	328 (244–480) [3–5%]	287 (206–333) [3–5%]	214 (186–250) [2–3%]
Anterior testis width	505 (340–690) [4–7%]	444 (382-519) [5–6%]	577 (254–902) [3–9%]	407 (245–534) [3–8%]	303 (294–353) [3–4%]

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TABLE 1. (Continued)

	Huwazah Marsh, north-eastern Basrah Province near Majnoon, southern Iraq (31° 10’ N 47° 39’ E)	Huwazah Marsh, north-eastern Basrah Province near Majnoon, southern Iraq (31° 10’ N 47° 39’ E	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30º48′22″N 47º1′E)	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30º48′22″N 47º1′E)	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30º48′22″N 47º1′E)
Posterior testis width	430 (300–680) [3–7%]	517 (431–592) [5–7%]	629 (279–941) [3–9%]	484 (269–637) [4–10%]	336 (304–363) [3–4%]
Mean testes width	467 (260–676)	481 (407–525)	610 (276–922)	445 (275–586)	320 (304–353)
Testes width/ovary width	1:1.8 (1:1.3–1:2.1)	1:1.6 (1:1.4–1:1.8)	1:2.0 (1:1.1–1:2.9)	1:1.5 (1:1.2–1:1.8)	1:1.5 (1:1.3–1:1.7)
Intertesticular space length	45 (0–162) [0–2%]	0	31 (0–59) [0–1%]	18 (0–45) [0–1%]	62 (20–140) [0.2–2%]
Posttesticular space length	560 (270–1,020) [3–10%]	580 (559–613) [6–9%]	474 (294–735) [4–7%]	417 (294–628) [4–10%]	572 (480–755) [6–8%]
Distance from ovary to anterior testis	92 (0–490) [0–5%]	11 (0–29) [0–0.3%]	166 (15–343) [0.1–4%]	59 (0–108) [0–2%]	219 (156–294) [2–4%]
Vitelline follicle width	56 (16–99)	46 (29–69)	44 (31–68)	45 (22–72)	NM
Egg length in proximal uterus	131 (107–155)	106 (94–120)	127 (108–132)	113 (101–128)	116 (105–135)
Egg width in proximal uterus	51 (44–60)	38 (29–49)	48 (33–61)	43 (31–51)	47 (40–80)
Egg length in distal uterus	189 (165–217)	134 (122–146)	185 (144–197)	131 (98–156)	130 (112–144)
Egg width in distal uterus	75 (70–84)	69 (66–78)	69 (67–87)	51 (42–58)	51 (48–62)
Swollen, pre-hatched egg length	207 (189–225)	200 (180–220)	199 (185–215)	173 (168–177)	RP

… …*continued on the next page*

TABLE 1. (Continued)

	Huwazah Marsh, north-eastern Basrah Province near Majnoon, southern Iraq (31° 10' N 47° 39' E)	Huwazah Marsh, north-eastern Basrah Province near Majnoon, southern Iraq (31° 10' N 47° 39' E	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30º48′22″N 47º1′E)	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30º48′22″N 47º1′E)	Al-Hammar Marsh, Thi-Qar Province, southern, Iraq (30º48′22″N 47º1′E)
Swollen pre-hatched egg idth	83 (75–95)	85 (74–91)	82 (77–94)	91 (90–92)	RP
Miracidia length	208 (190–225)	184 (170–204)	196 (163–224)	140 (132–148)	RP
Miracidia width	71 (60–84)	83 (77–93)	78 (60–99)	71 (53–83)	RP
Unhatched empty egg length	RP	RP	RP	RP	114 (91–148)
Unhatched empty egg width	RP	RP	RP	RP	54 (38–74)
Unhatched abnormally-developed egg length	RP	RP	RP	RP	109 (98–122)
Unhatched abnormally-developed egg width	RP	RP	RP	RP	51 (48–65)

n=Sample size.
NM=Indistinct; not measurable.
RP = Rarely present.

It also is prudent to recognize likely cases of abnormalities (normal or induced during fixation) of comparative characteristics if encountered. Senescing specimens represent an interesting situation. Although these specimens were somewhat smaller than fully-developed, non-senescing specimens and generally had smaller internal and external structures, many of these measurements represent about the same percentage of body length (e.g. oral sucker width, pharynx width, cirrus sac length), other features were absent (e.g. prepharynx; swollen, pre-hatched eggs; miracidia; unhatched empty eggs; unhatched abnormally-developed eggs) or reduced (i.e. amount of sperm in the vesicles); and the vitelline follicles were indistinct and individual follicular masses were diffuse and could not be measured. The absence or atrophy of these features is likely related to the decline in reproductive capabilities in senescing specimens, but one should expect to encounter some abnormal expressions of otherwise normal appearances of characteristics in some specimens (e.g. confluence of vitelline fields, location of the genital pore, posterior extent of cirrus sac).

In specimens fixed without compression we found that the body was less uniform and symmetrical, shorter, narrower and thicker and showed more signs of distortion and contraction (generally more asymmetrical, often constricted near the mid-body and contracted posteriorly near the level of testes, and the tegument was generally more crenulated); the prepharynx tended to be longer (14 compared with 8 and 6); the pharynx on average was smaller (268 wide compared with 324 and 299); the distance from the pharynx to the intestinal bifurcation was longer (126 compared with 73 and 96, respectively); the testes tended to be contiguous, sometimes overlapping; the ovary was closer to the anterior testis (11 compared with 92 and 166); the eggs were generally shorter in the distal (134 compared with 189 and 185, respectively) and proximal uterus (106 compared with 131 and 127); and internal details were more difficult to see. We found that even though compression during heat fixation may have caused some shrinkage, this shrinkage was much less than that caused by the contractions when specimens were fixed without compression. An advantage to using minimal compression in the fixation of flukes is that these flatter specimens allow observers to more effectively examine internal morphology (e.g. female reproductive system). A disadvantage may be that eggs tend to be larger and more spread out when compression is employed, as appeared to be the case in our specimens of *U. iraquensis*. In addition, the pharynx was often rotated or displaced off the midline of the body causing the genital pore to appear to be somewhat submedian and parts of the body tended to be distorted, especially at the anterior and posterior ends in specimens fixed without compression.

We feel that our comparisons of the specimens from the two collections (Huwazah and East Hammar, respectively) demonstrate that the following characteristics are relatively consistent and potentially valuable in identifying groups of similar species and distinguishing species in *Uvitellina*, and probably in other cyclocoelids: the presence or absence of the oral sucker; the oral sucker/pharynx width ratio; the posterior extent of the cirrus sac relative to the intestinal bifurcation; the position of the genital pore relative to the pharynx; the position of the testes in the body; the length of the intertesticular space (if extensively different); the length of the posttesticular space; the lateral disposition of the uterine loops; the presence of a posteriorly-directed, tail-like extension off the posterior confluence of the vitelline fields where present; the posterior extent of the uterine loops relative to the gonads; and the size of fully-developed eggs. In comparing measurements from a series of adult specimens, it may be beneficial to calculate the percentage that the measurement represents relative to the body length. In many cases, this percentage may provide insight into the relationship of the size of the structure to increased size of the specimens (growth) and establish better grounds for species comparisons. There were a number of characteristics we felt were too variable and inconsistent to be reliable in most comparisons of species in *Uvitellina*: general body size and shape; sucker size; length of the prepharynx; pharynx size; esophagus length; size, appearance and shape of the testes; ovary size and shape; and the anterior extent of the vitelline fields.

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