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# On the Morphology and Taxonomy of *Griphobilharzia amoena* Platt and Blair, 1991 (Schistosomatoidea), a Dioecious Digenetic Trematode Parasite of the Freshwater Crocodile, *Crocodylus johnstoni*, in Australia [Critical Comment]

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**CRITICAL COMMENT...**

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## On the Morphology and Taxonomy of *Griphobilharzia amoena* Platt and Blair, 1991 (Schistosomatoidea), a Dioecious Digenetic Trematode Parasite of the Freshwater Crocodile, *Crocodylus johnstoni*, in Australia

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**ABSTRACT:** *Griphobilharzia amoena* Platt and Blair, 1991 was originally described as a dioecious trematode, parasitic in the circulatory system of the Australian freshwater crocodile, *Crocodylus johnstoni*, with the female completely enclosed in a gynecophoric chamber of the male and the 2 worms oriented anti-parallel to each other. A recent publication questions the original description, arguing that *G. amoena* is monoecious and, as a consequence, the species was transferred to *Vasotrema* Stunkard, 1928 (Spirorchidae) as *Vasotrema amoena* n. comb. We provide photomicrographic evidence that the original description of *G. amoena* is correct and that *Griphobilharzia* Platt and Blair, 1991, is a valid monotypic genus containing *G. amoena*. An accurate understanding of the anatomy of *G. amoena* is not trivial and has implications for revealing the complex origins and evolution of the dioecious condition within the Schistosomatoidea.

*Griphobilharzia amoena* Platt and Blair, 1991, was described as a new genus and species (Platt et al., 1991) in the Schistosomatidae Poche, 1926, from the circulatory system of the freshwater crocodile, *Crocodylus johnstoni* Krefft, 1873. The species was defined as dioecious, with the female oriented anti-parallel to the male and completely enclosed in a gynecophoric chamber within the body of the male. Due to its dioecious condition and unique morphology, Platt et al. (1991) erected *Griphobilharzia* Platt and Blair, 1991 and Griphobilharziinae Platt and Blair, 1991 to accommodate the new species. This general concept for *Griphobilharzia* and the placement of Griphobilharziinae based on a suite of unique morphological attributes among the schistosomatoids has gained recognition and acceptance since the original proposal in 1991 (e.g., Khalil, 2002; Smith, 2002).

In contrast to the original proposal and current concepts for *Griphobilharzia*, Azimov et al. (2011) characterized *G. amoena* as monoecious. Further, they outlined numerous purported errors in the original description and recommended that the species be transferred to *Vasotrema* Stunkard, 1926 [sic], as *Vasotrema amoena* (Platt, Blair, Purdie et Melville, 1991 [sic]) n. comb. The proposed taxonomic revision followed the examination of type specimens by Azimov and colleagues during a visit to the U.S. National Parasite Collection (USNPC), Beltsville, Maryland and access to a large series of photomicrographs of type material taken and provided by EPH and LAC.

In the present study we re-examined the holotype, allotype, and paratypes of *G. amoena*. We refute the interpretations of Azimov et al. (2011) and present evidence to demonstrate that the original morphological description by Platt et al. (1991) was correct in its entirety, reconfirming that *Griphobilharzia*, which contains the single species *G. amoena*, is valid.

### MATERIALS AND METHODS

The holotype, allotype, and 2 paratypes of *G. amoena* deposited at SAMA (Nos. AHC 44145 and AHC 18841, respectively), and 4 paratypes

deposited in the USNPC (No. 81290), were examined and photographed by LAC and EPH, respectively. The photomicrographs described in the current study and others archived in the USNPC were made available to Prof. Azimov and his colleagues for their analysis with the exception of Figure 5, which was taken by TRP at an earlier date, and Figure 6 which is new.

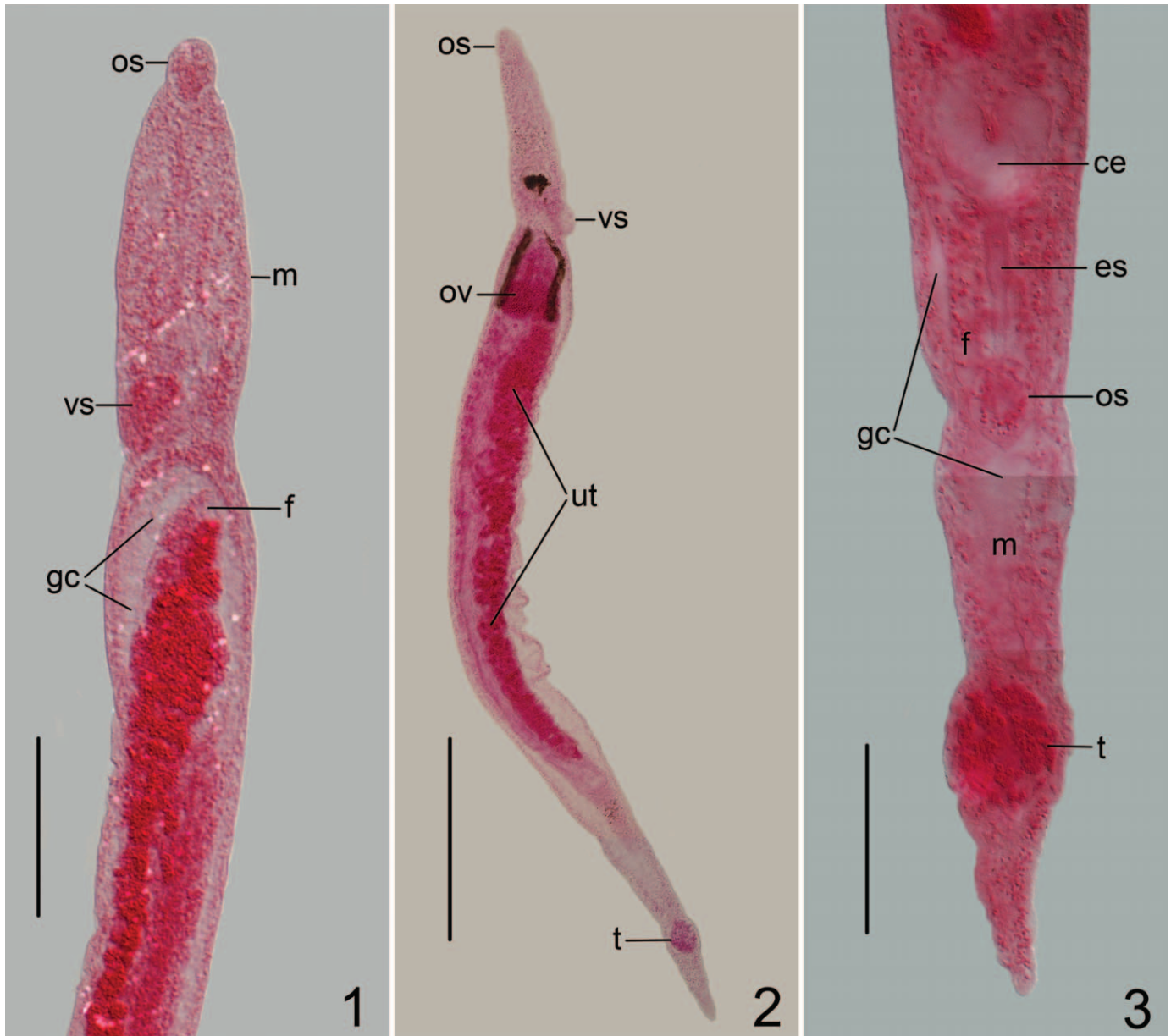
Paratypes examined by EPH were evaluated under differential interference optics across a range of magnifications ( $\times 100$ – $\times 400$ ) with a Zeiss Axiophot microscope (Carl Zeiss, Jena, Germany), and images were acquired with a Nikon DXM 1200F digital system (Nikon Corporation, Tokyo, Japan). SAMA material was examined using a Nikon Eclipse 80i compound microscope equipped with differential interference optics and photographed using a Nikon Coolpix 4500 digital camera (Nikon Corporation). A large series of photomicrographs, including those used in the current study, are archived in the USNPC.

### RESULTS AND DISCUSSION

Azimov et al. (2011) listed several points of error in the original description of *G. amoena* in their Results and Discussion. To support their conclusions regarding the morphology of *G. amoena*, Azimov et al. (2011) only presented line drawings which appear to have been redrawn and relabeled from the original description of Platt et al. (1991); new comparative morphological data were not presented. They made no reference to the photomicrographs provided by the curators of the USNPC or SAMA (also used here; Figs. 1–4). Figure 2 is identical to the image sent to Azimov and co-workers except it was retaken by LAC at a higher resolution for publication purposes.

The photomicrographs of the type specimens of *G. amoena* clearly show the dioecious nature of these worms, with the female oriented anti-parallel to the male and enclosed within a gynecophoric chamber (Figs. 1–3). The male gynecophoric chamber and the anterior end of the enclosed female are also distinctly visible in the paratypes (Figs. 3, 4) as well as the cecae of the female terminating at the level of the ovary (Fig. 5). These images and the original description unequivocally refute the claim by Azimov et al. (2011) that *G. amoena* is a monoecious species.

Azimov et al. (2011) stated that the structure identified as the “testis” in the original description of *G. amoena* is actually a lymphatic vessel, although they provided no evidence to support their interpretation. Stunkard (1923), in his assessment of *Spirorchis* MacCallum, 1918, indicated “at the posterior end of the body... there is a large, coiled vesicle... [t]he contents are fluid, and stain like a secretion” and “may prove to be a lymphatic vesicle.” While the precise nature of the structure has not been identified among the spirorchids, it may represent a component of the excretory system. This structure has been reported in many species of spirorchids and has been described as elongate and either straight or coiled. In contrast, the organ in question in *G. amoena* (Figs. 2, 3) is ovoid, cellular in nature, near terminal in position, has a single duct running anteriorly, and could only be a testis; it is not consistent with a coiled lymphatic vesicle as described by Stunkard (1923) or as proposed by Azimov et al. (2011).



FIGURES 1–3. Photomicrographs of *Griphobilharzia amoena*; (1) Composite image of whole mount of paratype (USNPC 81920) showing anterior of male fluke and posterior of female in gynecophoric chamber. Bar = 200  $\mu$ m. (2) Composite image of whole mount of holotype male and allotype female (SAMA AHC 44145) showing relative positions and distribution of organs. Bar = 500  $\mu$ m. (3) Composite image of whole mount of paratype (USNPC 81920) showing posterior of male fluke, and relative position of anterior of female in gynecophoric chamber. Note terminal testis in male and prominent oral sucker, esophagus, and cecal system of female. Bar = 100  $\mu$ m. Abbreviations: ce – intestinal caeca; es – esophagus; f – female; gc – gynecophoric chamber; m – male; os – oral sucker; ov – ovary; t – testis; ut – uterus; vs – ventral sucker (male).

Azimov et al. (2011) interpreted the uterus as the testis and stated that no eggs were present; however, again no evidence was provided to support this claim. EPH re-examined the paratypes at the USNPC and determined that this structure is a voluminous uterus (Fig. 2) which contains numerous collapsed eggs (Fig. 6).

Azimov et al. (2011) were unable to locate the oral sucker of the female worm, but it is distinct in the type material (Figs. 3, 4). Figure 3 is a ventral view of the anterior end of the female in which the oral sucker, esophagus, and cecal bifurcation are clearly visible. This is near the posterior end of the male, just anterior to the constriction of the male tail that occurs anterior to the testis. The oral sucker of the female is also obvious in

lateral perspective (Fig. 4), which further demonstrates the presence of the gynecophoric chamber of the male (also see Figs. 1, 3).

Azimov et al. (2011) stated, “The ovary conventionally depicted with large cells as is typical in the presentation of this organ in trematodes... is situated in the anterior end of the body, while in the description it is in the posterior end of the body.” We re-confirm that the ovary is near the posterior end of the female (Figs. 2, 5); however, as the male and female are oriented anti-parallel, the ovary is located closer to the anterior end of the male.

Finally, Azimov et al. (2011) stated “Intestinal branches are blind near the posterior part of the body...,” which is incorrect. We observed that the intestinal caeca of the male are short, terminating at the extreme anterior

end of the gynecophoric chamber (Fig. 2), while the longer cecae of the female (Fig. 5) terminate just anterior to the ovary at the posterior end of the female but near the ventral sucker of the male.

Our observations, based on re-examination of the type series, conclusively corroborate the original description in its entirety and support recognition of *G. amoena* as a dioecious schistosomatoid digenean. We confirm a large male contains a smaller female within a gynecophoric chamber as originally described, and this constitutes a unique body plan for dioecious flukes among the schistosomatoids. The emended taxonomy is as follows:

***Griphobilharzia* Platt and Blair, 1991**

*Syn. Vasotrema* sensu Azimov, Filimonova, Shakarboev, and Akramova, 2011; nec Stunkard, 1928.

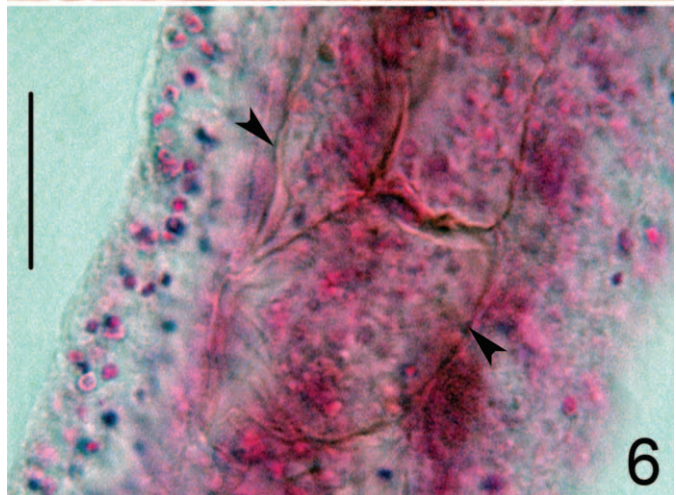
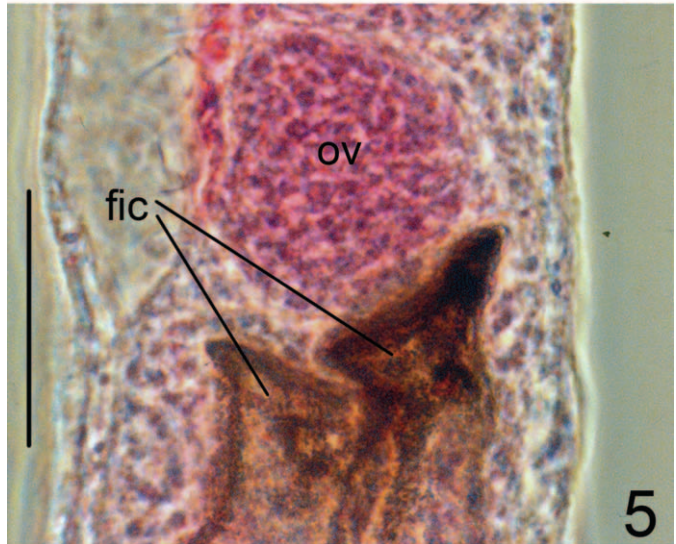
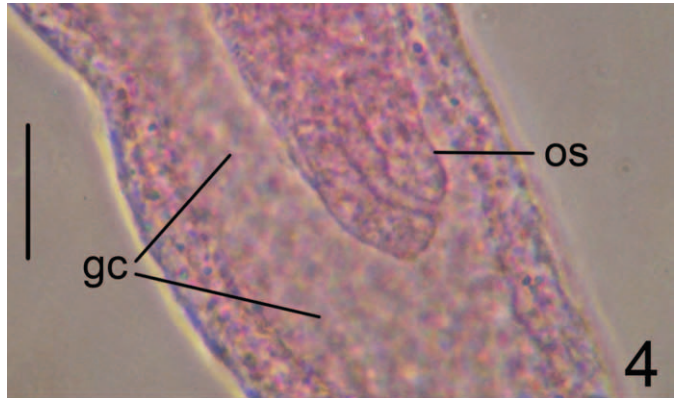
***Griphobilharzia amoena* Platt and Blair, 1991**

*Syn. Vasotrema amoena* Azimov, Filimonova, Shakarboev, and Akramova, 2011.

**The implications of incorrect interpretations**

The conclusion of Azimov et al. (2011) that *Griphobilharzia* is monoecious has implications for hypotheses on the evolution of the dioecious form and patterns of life history among the Schistosomatoidea (e.g., Platt and Brooks, 1997). Monophyly for the blood flukes has been firmly established and the monoecious “spirorchiids,” now recognized as paraphyletic, are considered to represent the basal lineages within the Schistosomatoidea (Platt and Brooks, 1997; Smith, 2002; Snyder, 2004). In the most-recent molecular-based phylogenies, *Griphobilharzia* is aligned within this basal assemblage of spirorchiids (Brant and Loker, 2005). As a consequence, 2 distinct and phylogenetically disparate lineages, including the crown and monophyletic Schistosomatidae and *Griphobilharzia* as the putative sister of *Hapalorhynchus*, are characterized by dioecious body plans which are morphologically divergent. This observation is consistent with a complex history involving varying levels of host colonization in diversification (e.g., Hoberg and Brooks, 2008) and independent origins of the dioecious condition among the schistosomatoids, which are distributed among avian and mammalian hosts (Schistosomatidae) and non-avian archosaurs (*Griphobilharzia*) (Brant and Loker, 2005).

Generally these parasite groups are archaic, and *Griphobilharzia* may be relictual in extant crocodylians depending on the timing of colonization of early non-avian archosaurs which have temporal durations over about 250 MY extending into the early Triassic (Nesbitt, 2011). Thus, *Griphobilharzia* may be representative of a once-broader assemblage where diversity has been secondarily reduced through extinction events in the late Cretaceous and early Tertiary for its primary host group. The contemporary host taxon and living crocodylians are among the surviving members of the once highly diverse Pseudosuchia and Suchia (crocodile-line archosaurs) that radiated and attained dominance in the late Triassic and Jurassic and were subsequently influenced by episodes of extinction through the later Mesozoic (e.g., Nesbitt, 2011). Episodic processes of extinction (and ecological perturbation), colonization, and radiation have been explored as primary determinants of diversity among complex host-parasite assemblages (e.g., Hoberg and Brooks, 2008, 2010) and appear to have influenced contemporary diversity among



FIGURES 4–6. Photomicrographs of *Griphobilharzia amoena*. (4) Whole mount of holotype and allotype (SAMA AHC 44145) showing anterior end of female, oral sucker (os), and gynecophoric chamber (gc). Bar = 30  $\mu$ m. (5) Posterior end of female (USNPC 106896) showing termination of intestinal ceca (fic) at level of ovary (ov). Bar = 100  $\mu$ m. (6) Uterus of female of paratype (SAMA 18841) showing thin egg shells (arrowheads). Bar = 50  $\mu$ m.

schistosomatoids in vertebrate hosts including fishes, crocodylians, birds, and mammals (Snyder, 2004; Brant and Loker, 2005). Further survey of crocodylians and other tetrapods remains necessary to explore the limits of contemporary host distribution for this assemblage. The confusion over the morphology of *Griphobilharzia* introduced by Azimov et al. (2011) and an erroneous depiction of these digeneans as monoecious, however, would substantially impede further evaluation of hypotheses for both diversification and the evolution of life history of the Schistosomatoidea with tetrapod hosts.

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