

## REDESCRIPTION OF THE FROG BLADDER FLUKE *GORGODERINA ATTENUATA* FROM THE NORTHERN LEOPARD FROG, *RANA PIPIENS*

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**ABSTRACT:** Morphological characters used to differentiate North American bladder flukes, *Gorgoderina* spp., are problematic and different authors use different morphological characteristics for distinguishing species. More importantly, no type specimens exist for 4 of the 12 North American species infecting anuran and caudatan hosts. A redescription of *Gorgoderina attenuata* (Stafford, 1902) Stafford, 1905 is based on new collections from 6 species of anurans from Arkansas, Nebraska, New York, and Wisconsin. Morphological comparisons between gravid *G. attenuata* recovered from bullfrogs and northern leopard frogs indicated statistically significant differences in 11 of 28 morphological characters examined. However, there was overlap among all of these characters, and it is unclear whether these morphological types represent host-induced morphological changes in worm morphology or cryptic species. Based on our findings, we suggest that morphological and molecular data from *G. attenuata*-like worms recovered from the other 23 definitive hosts reported for *G. attenuata* need to be collected to resolve this issue.

There are more than 50 species of *Gorgoderina* Looss, 1902 amphibian bladder flukes found worldwide. Twelve species have been described from anuran and caudatan hosts from Canada and the United States (see Mata-López et al., 2005; Mata-López 2006). Stafford (1902) described 3 species of *Gorgoderina*: *Gorgoderina attenuata* (Stafford, 1902) Stafford, 1905, *G. opaca*, (Stafford, 1902) Stafford, 1905, and *G. translucida* (Stafford, 1902) Stafford, 1905; Stafford (1902) also reported on *G. simplex* (Looss, 1899) Looss, 1902 and *Gorgoderina amplivava* Looss 1899 from anurans assumed to be collected in Toronto, Canada. None of these specimens was deposited in any museum. To our knowledge, no type specimens exist for *G. attenuata* or the other *Gorgoderina* spp. described or reported on by Stafford (1902) in the Harold W. Manter Laboratory (HWML) of Parasitology or the U.S. National Parasite Collection, and it is assumed that the types for these 4 North American species of *Gorgoderina*, including *G. attenuata*, have been lost or destroyed. Of the 4 species of *Gorgoderina* reported by Stafford (1902), *G. attenuata* is the most commonly reported bladder fluke in North and Central American anurans and caudatans and it has been reported from 24 amphibian and reptilian hosts (see Brooks, 1976; Mata-López et al., 2002; Bolek and Coggins, 2003; Mata-López and León-Règagnon, 2006; Bolek et al., 2009).

Stafford (1902) described *G. attenuata* from the green frog *Rana virescens*, which is currently a junior synonym of the southern leopard frog *Rana sphenoccephala* Cope 1886. The range of the southern leopard frog does not extend into Canada and it is not clear what species of frog Stafford (1902) was dealing with (Lannoo, 2005). However, *R. virescens* has also been used as a synonym for the northern leopard frog *Rana pipiens* Schreber, 1782 (see Frost et al., 2006) in the older literature, and it is assumed that Stafford's *G. attenuata* was described from the northern leopard frog that occurs in Canada

(see Brooks, 1976). From Stafford's description, the type of locality for *G. attenuata* is also unclear. Stafford (1902) indicated that he collected *G. translucida* from *Bufo lentiginosus* (a junior synonym for *Bufo americanus* LeConte in Cuvier, 1831) and *R. virescens*, and gives Ashbridges Bay, Toronto, as the location for his collection of *B. lentiginosus*, but he provides no other localities for any of the other anurans that he examined. More problematic is the fact that Stafford's description of *G. attenuata* and the other 3 species of *Gorgoderina* are very poor and most morphological characters are omitted.

Due to Stafford's (1902) poor descriptions, morphological characters used to identify *G. attenuata* from the other 11 North American *Gorgoderina* spp. are problematic because different authors use different morphological characteristics in distinguishing among species (see Stafford, 1902; Cort, 1912; Olsen, 1937; Goodchild, 1950; Brooks, 1976; Mata-López et al., 2005). The defining characters in distinguishing *G. attenuata* from most other North American *Gorgoderina* spp. are the attenuated body and the large acetabulum diameter reported as the oral sucker to acetabulum ratio (OS). However, depending on the author, reports of this ratio differ from 1:2.1 to as large as 1:2.85–3.0 (Stafford, 1902; Cort, 1912; Olsen, 1937; Goodchild, 1950; Brooks, 1976; Mata-López et al., 2005). Finally, recent studies on the life cycle of *G. attenuata* by Bolek et al. (2009) indicate that the life cycle of *G. attenuata* from Nebraska differs among northern leopard frogs and bullfrogs *Rana catesbeiana* Shaw 1802 and from the original life cycle description of this species in green frogs *Rana clamitans* Latreille in Sonnini de Manoncourt and Latreille, 1801 and eastern newts *Notophthalmus viridescens* (Rafinesque, 1820) from Massachusetts (Rankin, 1939). These data suggest that *G. attenuata* from different hosts may represent distinct populations, or cryptic species. Taken together, Stafford's (1902) poor description, the questionable identity of the type host, and the lack of a type locality for *G. attenuata*, along with the discovery of life cycle variation by Bolek et al. (2009) warrant the redescription of *G. attenuata*. In this paper, we address these issues by (1) redescrining *G. attenuata* from 6 species of anurans from specimens collected from a variety of locations in North America and designating a neotype specimen of this species; and (2) comparing the morphology of *G. attenuata*-like worms that vary in their recruitment in northern leopard frogs and bullfrogs in Nebraska (Bolek et al., 2009).

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## MATERIALS AND METHODS

Worms used for morphological analysis were collected from a number of frog and toad species and locations during 1996–2007 in Arkansas, Nebraska, New York, and Wisconsin. (See Bolek et al., 2009, for data on population structure among different anuran species.) These included 2 worms recovered from a southern leopard frog (*Rana sphenoccephalus*) collected from a pond in Washington County, Arkansas (35°46.979', -94°14.687'); 8 worms from northern leopard frogs (*Rana pipiens*), 1 worm from bullfrogs (*R. catesbeiana*), and 1 worm from Woodhouse's toads (*Bufo woodhousii*, Girard 1854), all from Cedar Creek, Keith County, Nebraska (41°11.194', -101°21.820'); 9 worms from bullfrogs from Breen's Flyway, Keith County, Nebraska (41°10.914', -101°21.654'); 8 worms from bullfrogs from Elk Creek, Lancaster County, Nebraska (40°53.145', -96°50.048'); 1 worm from a plains leopard frog (*Rana blairi*, Meham, Littlejohn, Oldham, Brown, and Brown, 1973) from Pawnee Lake, Lancaster County, Nebraska (40°51.589', -96°53.468'); 1 worm from a green frog (*Rana clamitans*) from Queechy Lake, Columbia County, New York (42°24.269', -73°25.627'); and 2 worms from northern leopard frogs from Brookfield, Waukesha County, Wisconsin (43°04.082', -88°04.973'). All worms that were used for the redescription were removed from the urinary bladders of anurans, relaxed in tap water, and fixed in alcohol-formalin-acetic acid. Worms were stained with acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Additionally, to obtain a better geographical representation of *G. attenuata*, voucher specimens were borrowed from the HWML, University of Nebraska State Museum. Because not all worms borrowed from the HWML could be identified as *G. attenuata*, we only included 10 worms from a northern leopard frog from Nebraska (HWML 20123), 2 worms from a bullfrog from Nebraska (HWML 20121), and 1 worm from a green frog from Wisconsin (HWML 21344). Based on recent and past *Gorgoderina* spp. descriptions and redescrptions by Brooks (1976) and Mata-López et al. (2005), the following characters were in the descriptions: (1) body shape, length, and width; (2) forebody length and width at cecal bifurcation; (3) hindbody length and width at anterior testis; (4) oral sucker location, length, and width; (5) esophagus length and width; (6) acetabulum length and width; (7) oral sucker/acetabulum length and width ratio (OS/AC); (8) body width to acetabulum width ratio; (9) cecal length from posterior extremity; (10) testes morphology, location, and length and width; (11) seminal vesicle shape, position, and location; (12) genital pore location; (13) ovary morphology and location; (14) vitellaria number and location; (15) uterine loop distribution; and (16) egg length and width.

Student's 2-tailed *t*-test was used to compare morphological characteristics between *G. attenuata* collected from bullfrogs and northern leopard frogs. An approximate *t'*-test was calculated when variances were heteroscedastic (Sokal and Rohlf, 1981). A drawing of a gravid worm from the bladder of a northern leopard frog was done with the aid of a camera lucida. In the redescription, ranges are presented, followed by means in parentheses.

## REDESCRIPTION

### *Gorgoderina attenuata* (Stafford, 1902) Stafford, 1905

(Fig. 1)

**Diagnosis** (based on 46 gravid specimens, from the urinary bladder of 6 species of anurans): Body spindle shaped, blunt anterior end, attenuated end; body length 1.28–5.55 mm (3.23). Forebody 0.20–1.00 mm (0.53) long and 0.15–0.52 mm (0.26) wide at level of cecal bifurcation. Hindbody 0.19–4.03 mm (2.25) long and 0.25–0.61 mm (0.40) wide at widest part of hindbody. Oral sucker subterminal, spherical, 120–520 μm (273) long and 110–460 μm (248) wide. Pharynx absent. Esophagus straight or curved, thick walled, 80–180 μm (120) long, and 30–60 μm (41) wide at widest point. Ceca simple, initial portion wider and sometimes lobed; ceca ending at 5.6–12.0 (7.5% of body length) (n = 20) from posterior extremity. Acetabulum in anterior third of body; 390–940 μm (592) long by 350–900 μm (597) wide. Oral sucker/acetabulum length ratio 0.28–0.76 (0.47); OS/AC width ratio 0.28–0.69 (0.42). Ratio of body width to acetabulum width 0.41–0.96 (0.68). Two testes, oval, sometimes irregular in shape in large individuals, positioned in tandem in midhindbody, anterior testis 40–600 μm (373) long by 100–380 μm (232) wide, and posterior testis 90–980 μm (520) long (n

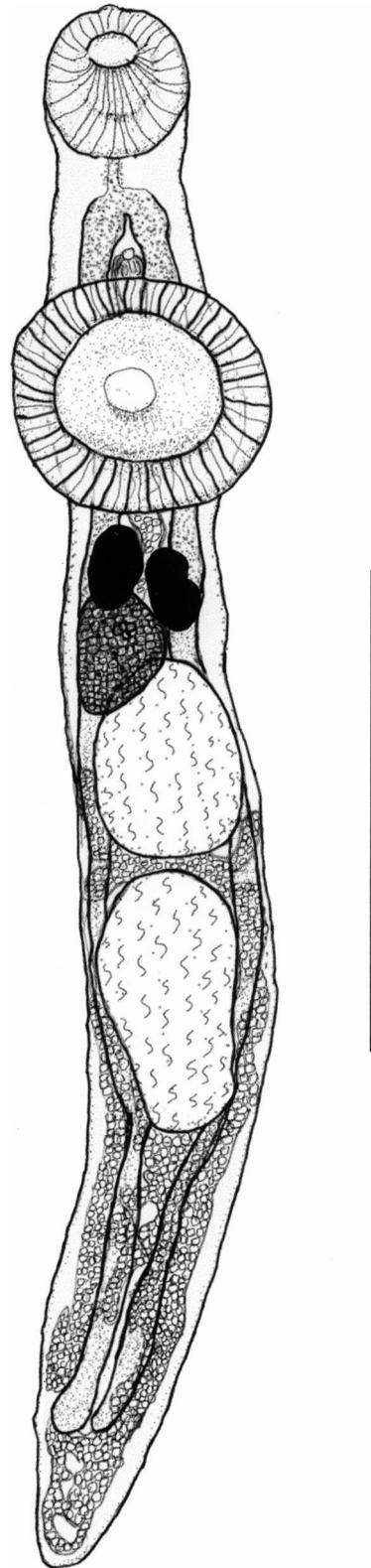


FIGURE 1. Line drawing of the neotype of *G. attenuata* (HWML 49008) from a northern leopard frog (*R. pipiens*) collected from Cedar Creek, Keith County, Nebraska. Scale bar = 1 mm.

TABLE I. Morphological characteristics of adult *G. attenuata* from bullfrogs and northern leopard frogs. Range and means (in parentheses) are given in  $\mu\text{m}$  unless otherwise noted.

	<i>G. attenuata</i> from bullfrogs	<i>G. attenuata</i> from northern leopard frogs	<i>t</i> -Test	<i>P</i> /=/ <i>t</i>
	n = 20 Range (mean)	n = 20 Range (mean)		
Shape	Elongate	Elongate	—	—
Body length	1.3–4.5 mm (2.9)	2.2–5.0 mm (3.3)	–1.5	0.14
Body width	0.25–0.56 mm (0.35)	0.35–0.55 mm (0.44)	–4.18*	0.0002
Forebody length	0.20–0.61 mm (0.45)	0.45–1.0 mm (0.60)	–3.50	0.001
Forebody width at cecal bifurcation	150–400 (220)	190–400 (283)	–3.42	0.001
Hind body length	0.19–3.19 mm (2.04)	1.33–3.37 mm (2.32)	–1.37	0.18
Hind body width	0.25–0.56 mm (0.35)	0.35–0.55 mm (0.44)	–4.18*	0.0002
Oral sucker length	120–300 (230)	200–450 (293)	–3.63	0.0008
Oral sucker width	110–270 (209)	200–370 (262)	–3.93	0.0003
Esophagus length	80–150 (113)	80–180 (124)	–1.17	0.24
Esophagus width	40–60 (43)	30–50 (39)	1.04	0.3
Acetabulum length	320–880 (595)	400–940 (593)	0.03	0.97
Acetabulum width	350–800 (592)	450–900 (604)	–0.30	0.75
OS/AC width ratio	0.3–0.48 (0.36)	0.28–0.56 (0.44)	–3.8	0.0004
Body width/AC width ratio	0.41–0.90 (0.60)	0.53–0.96 (0.74)	–3.69	0.0007
Cecal length from posterior extremity (%)	6.3–7.9 (7.2)	5.6–9.9 (7.4)	–0.36	0.72
Anterior testis length	40–600 (368)	100–520 (366)	0.04	0.96
Anterior testis width	150–300 (214)	100–300 (242)	–1.69	0.09
Posterior testis length	250–980 (553)	90–800 (496)	1.10	0.28
Posterior testis width	150–300 (201)	100–390 (243)	–2.18	0.04
Seminal vesicle length	50–240 (119)	80–230 (152)	–2.74	0.009
Seminal vesicle width	50–190 (93)	50–190 (111)	–1.61	0.11
Ovary length	120–330 (277)	200–400 (284)	–0.40	0.69
Ovary width	100–230 (165)	130–260 (193)	–2.61	0.01
Vitellaria, number and shape	2 kidney-shape lobed	2 kidney-shape lobed	—	—
Uterus	Sinuuous; filling available space posterior to ovary	Sinuuous; filling available space posterior to ovary	—	—
Egg length	25–33 (28)	25–35 (29)	–1.15	0.26
Egg width	17.5–23 (18)	15–23 (17.8)	0.93	0.36

\* Approximate *t*'s, –test.

= 44) by 100–390  $\mu\text{m}$  (223) wide (n = 42). Seminal vesicle oval to round, dorsal to the anterior edge of acetabulum; 50–250  $\mu\text{m}$  (139) long and 50–190  $\mu\text{m}$  (104) wide. Genital pore anterior to ventral sucker, located medially in forebody. Ovary anterior to anterior testis and posterior to vitellaria; sometimes overlaps anterior testis, oval to irregular in shape located on the right or left side of body, 120–430  $\mu\text{m}$  (287) long and 100–320  $\mu\text{m}$  (187) wide (n = 45). Vitellaria 2, compact kidney to oval shape lobed masses; occasionally not compact in some individuals recovered from bullfrogs, immediately posterior to acetabulum on middle line of body. Uterine loops filling postacetabular region and overlapping dorsally, ventrally, and laterally with testes, usually overlap entire posterior testis and partially overlap ovary and vitelline glands in large individuals. Eggs thin shelled and nonoperculated, embryonated, 25–35  $\mu\text{m}$  (29) long, and 15–23  $\mu\text{m}$  (18) wide (N = 45).

#### Taxonomic summary

*Type host*: *Rana pipiens* Schreber 1782, (Anura: Ranidae).

*Other hosts*: *Rana blairi* Mecham, Littlejohn, Oldham, Brown, and Brown, 1973; *R. catesbeiana* Shaw, 1802; *R. chiricahuensis* Platz and Mecham, 1979; *R. clamitans* Latreille in Sonnini de Manoncourt and Latreille, 1801; *R. dunni* Zweifel, 1957; *R. gryllo* Stejneger, 1901; *R. heckscheri* Wright, 1924; *R. megapoda* Taylor, 1942; *R. montezumae* Baird, 1854; *R. neovolcanica* Hillis and Frost, 1985; *R. palustris* LeConte, 1825; *R. septentrionalis* Baird, 1854; *R. sphenoccephala* Cope, 1886; *R. vaillanti* Brocchi, 1877 (Anura: Ranidae); *Bufo americanus* Holbrook, 1836; *B. fowleri* Hinckley, 1882; *B. woodhousii* Girard, 1854 (Anura: Bufonidae); *Leptodactylus melanonotus* (Hallowell, 1861) (Anura: Leptodactylidae); *Notophthalmus viridescens* (Rafinesque, 1820) (Caudata: Salamandridae); *Ambystoma andersoni* Krebs and Brandon,

1984; *A. tigrinum* (Green, 1825); *A. dumerilii* (Dugès, 1870); *A. lermaense* (Taylor, 1940) (Caudata: Ambystomatidae); and *Thamnophis sirtalis* (Linnaeus, 1758) (Squamata: Serpentes).

*Site of infection*: Urinary bladder.

*Type locality*: Cedar Creek, Keith County, Nebraska (*R. pipiens*; *R. catesbeiana*; *B. woodhousii*).

*Other localities*: New Brunswick, Ontario, and Quebec (all in Canada); Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Maine, Massachusetts, Minnesota, Michigan, Nebraska, New York, North Carolina, Ohio, Texas, and Wisconsin (all in the United States); Coahuila, Michoacán; Laguna Escondida, Veracruz; Lerma, Mexico State; Mexico City; Sonora; and Zacapú, Michoacán (all in Mexico); Guatemala (see Brooks, 1976; Bolek and Coggins, 2003; Paredes-Calderón et al., 2004; King et al., 2008; Bolek et al., 2009).

*Neotype*: Deposited at the HWML collection (HWML 49008), University of Nebraska State Museum, Lincoln, Nebraska; vouchers of *G. attenuata* from other anurans were deposited in the H.W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska (accession numbers HWML 48984–48998) by Bolek et al. (2009).

#### Remarks

The few measurements presented by Stafford (1902) fall within the ranges presented in the redescription; all qualitative features (shape, position of the vitellaria, testes, and ovary) observed in the specimens used in this study were consistent with those articulated by Stafford (1902). Of the 12 North American amphibian bladder flukes in the genus *Gorgoderina*, *G. attenuata*, along with *G. tanneri* Olsen, 1937, and *G. aurora* Ingles, 1936, form a group of North American *Gorgoderina* spp. distinguished from the other members of the genus in that

the acetabulum is approximately twice the length and width of the oral sucker. However, *G. tanneri* can be distinguished from *G. attenuata* by its regularly lobed vitellaria, with 3–6 lobes with a slender stem versus compact kidney-shaped lobed masses in *G. attenuata*. *Gorgoderina aurora* differs from *G. attenuata* by being longer (5.0–8.0 mm vs. 1.3–5.0 mm) and wider (0.65 mm vs. 0.40 mm) and having a wider acetabulum (0.65 mm vs. 0.60 mm).

Morphological comparisons between gravid *G. attenuata* recovered from bullfrogs and northern leopard frogs are presented in Table I. Statistically significant differences between worms from bullfrogs and northern leopard frogs existed in body width, forebody length and width, hindbody width, oral sucker length and width, OS/AC width ratio, body width to acetabulum ratio, ovary width, posterior testis width, and seminal vesicle length. Although the differences were significant, there was overlap in the size range of all of these characters.

## DISCUSSION

Stafford (1902) based his original description of *G. attenuata* on worms recovered from *R. virescens* (= *R. pipiens*?) and indicated (but did not provide any measurements) that worms recovered from *R. catesbeiana* were similar to, but morphologically different from, worms recovered from *R. virescens*. Stafford (1902), Cort (1912), and Goodchild (1950) all indicated that *G. attenuata* recovered from bullfrogs were smaller and differed in the morphology of their testes when compared with worms from northern leopard frogs. Additionally, Goodchild (1950) and Bolek et al. (2009) indicated that gravid *G. attenuata* recovered from bullfrogs and northern leopard frogs differ in their location in the host (urinary bladder in northern leopard frogs and urinary bladder and kidneys in bullfrogs). Currently, it is unclear whether *G. attenuata*-like worms recovered from bullfrogs and the 22 other hosts reported for this species are morphologically different from worms recovered from northern leopard frogs due to host-induced morphological variability, represent distinct populations, or are cryptic species. Recent life cycle studies on *G. attenuata* in northern leopard frogs, Woodhouse's toads, and bullfrogs in Nebraska by Bolek et al. (2009) indicate that the life cycle strategies of *G. attenuata* in bullfrogs differ compared with the strategies of *G. attenuata* worms in northern leopard frogs and Woodhouse's toads, as well as from the original life cycle description of this species in green frogs and eastern newts by Rankin (1939). Based on our morphological study and life cycle studies by Bolek et al. (2009), we suggest that morphological and molecular data from multiple genes from *G. attenuata*-like worms collected from bullfrogs and different populations of the 22 other species of definitive hosts will be necessary to resolve this issue.

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