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# MORPHOLOGICAL VARIATION OF *ALLOCREADIUM LOBATUM* (DIGENEA: ALLOCREADIIDAE) IN THE CREEK CHUB, *SEMOTILUS ATROMACULATUS* (OSTEICHTHYES: CYPRINIDAE), IN NEBRASKA, USA

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*ABSTRACT*

*Allocreadium lobatum* Wallin 1909, a parasite of freshwater fish, was originally described as having distinct lobate testes, and subsequent descriptions have left this original description unchanged. The present study quantifies the observation of distinctly non-lobate testes that could be categorized as round or asymmetrical in addition to the previously described lobate testes. Six hundred thirty six *A. lobatum* were collected from 228 *Semotilus atromaculatus* over a 9 month period. Overall, 21.3%, 61.6%, and 16% were found to be round, asymmetrical, and lobate testes respectively ($N$ = 1071). Analysis of testis morphology found the increasing presence of lobate testis as the length of *A. lobatum* increased, being the prominent morph in parasites > 40 mm. Analysis of previous studies in Nebraska and Idaho, USA, confirm the presence of all three testicular categories, with a predominance of lobate testes in 9 of the 12 studies. Complete morphological analyses of *A. lobatum* over this study period are reported and demonstrate greater variation than did previous studies.

† † †

*Allocreadium lobatum* Wallin (Trematoda: Allocreadiidae) was described by Wallin from the host *Semotilus corporalis* (Mitchill), a description that has remained applicable in many freshwater fish species (Camp 1989, DeGuisti 1962, Schell 1985, Willis 2001). In initial studies of *A. lobatum*, we detected distinctly non-lobate testes in both living and fixed specimens. This precipitated categorization of testicular morphology (in addition to the collection of other morphometric data) as this finding was distinctly different from previous descriptions. In fact, the species was originally named because of its lobate testes (Wallin 1909), although this distinguishing feature is not unique in the genus *Allocreadium* in New World fish (Williams and Dyer 1992). To determine if this variability in testicular morphology could be generalized to studies in Nebraska and Idaho by other investigators, specimens from previous studies were re-evaluated, and comparisons to original syntypes and paratypes were made. Lastly, comparisons of morphometric data from this nine-month study to previous descriptions, which included mainly isolated collections, were made.

## MATERIALS AND METHODS

From May to December 1991, 636 *Allocreadium lobatum* were collected from Elk Creek, Lancaster County, as previously described (Willis 2001). Recovered parasites (which were exclusively *A. lobatum*), were counted, fixed in hot AFA (alcohol-formalin-acetic acid, allowed to boil, then cooled for 1–2 minutes at RT), and stored in 70% ethanol. No pressure that may have interfered with morphology was added to specimens. Specimens were hydrated in an alcohol series, stained with Mayer's hematoxylin, dehydrated, cleared in methyl benzoate, and mounted in Canada balsam.

Worm-maturity analysis was performed on 583 specimens, based on criteria established by Bell and Smyth (1958) and later used by Rand and Burt (1985) and Camp (1989): Stage A, immature (vitellaria undeveloped, testes and ovary distinct or indistinct, eggs absent); Stage B, mature (granular vitellaria extending anteriorly at least to the posterior testis, eggs absent); Stage C, gravid (vitellaria thick and follicular, egg(s) present). Testes were classified as round (completely spherical and smooth); asymmetrical (smooth, irregular, often elongate); or lobate (multiple lobes in all dimensions as originally described (Wallin 1909)) or

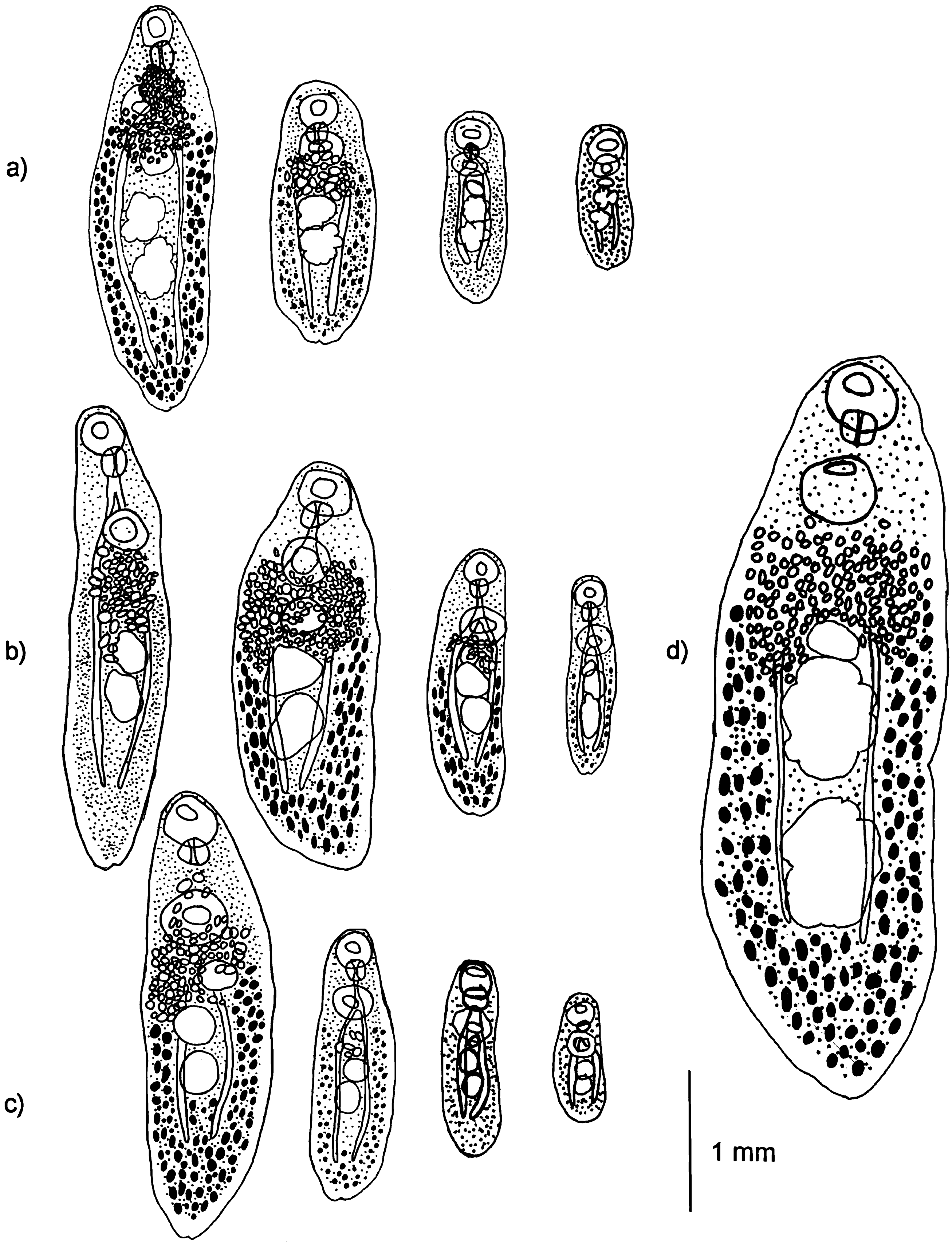


Figure 1. Morphological types in descending size classes of *A. lobatum*: a) lobate (l. to r.) HWML slides 35119 (37-5), 35119 (37-4), 35117 (24-3), 35120 (53-8); b) asymmetrical (l. to r.) slides 35126 (79-2), 35114 (1-10), 35127 (84-2), 35139 (139-3); c) round (l. to r.) slides 35117 (20-1), 35139 (140-1), 35117 (21-13), 35139 (151-7); and d) syntype, slide 35114 (1-5).

with at least one indentation forming a lobe (Fig. 1). These morphological groups were found to be the most descriptive after evaluating all of the specimens. Analyses of other morphometric data (length, width, sucker ratios, etc) used only mature and gravid specimens, to compare properly to previous studies that did the same. Morphometric data were analyzed using Lotus 1-2-3 Version 2 (Lotus Corp., USA) and Quattro Pro 8 (Corel Corporation, Canada).

Syntypes (No. 49986) and paratypes (No. 81415) from Wallin (1909) were mounted and used for comparative purposes and were on loan from the U.S. National Parasite Collection. Voucher specimens from previous studies were from the Harold W. Manter Laboratory (HWML No. (collection date)): 19732 (14 June 1980); 19829 (14 June 1979); 20355-56 (15 May 1974); 20357 (25 May 1974); 20358 (22 May 1974); 20359 (10 May 1974); 20360 (19 April 1974); 20361 (August 1974); 20362 (10 June 1974); 20363 (19 June 1974); 20364 (19 May 1974); 23667 (no collection date available); 35006 (June 1989). Voucher specimens for the present study were deposited at the Harold W. Manter Laboratory (HWML Nos. 35114–35140).

## RESULTS

Initial inquiry into *A. lobatum* testicular morphology (Fig. 1) demonstrated that the most common category was asymmetrical (61.6%; 671 of 1,071 specimens) (Fig. 2). Similarly, when the relationship of maturity (immature and mature/gravid) and testicular morphology were determined, the major testis morphological category was asymmetrical (64.6% (237 of 367 immature specimens, Stage A) and 61.6% (434 of 704 mature/gravid specimens, Stages B and C), respectively) (Fig. 2). The proportion of the round testis phenotype with respect to maturity decreased from 29.7% (109 of 367 immature specimens) to 17% (120 of 704 mature/gravid specimens). Conversely, the proportion of lobed testes increased from 5.7% (21 of 367 immature specimens) to 21.3% (150 of 704 mature/gravid specimens) indicating that as *A. lobatum* matures, the testes become less round and more lobed. Of the 583 specimens used in this analysis, both anterior and posterior testes were of the same phenotype in 505 specimens, and it was found that 11.5% (58/505) had a single lobate testis in addition to one identified as either round or asymmetrical.

We hypothesized that testes morphology would be correlated with maturity (and therefore length) with lobed testes found mostly in mature *A. lobatum*. Therefore, we analyzed testis morphology as a function of length (probably more closely related to age) and found that the smaller *A. lobatum* (0–10 mm) had mainly round and asymmetrical testes (39.8% and 46.9%, respectively, Fig. 3). As the size of the parasite increased, so did the proportion of lobate testes (13.3% in 0–10 mm vs. 100% in 40–50 mm, Fig. 3). The occurrence of round testes conversely decreased from 39.8 % in the 0–10 mm group to 0 % in the 40–50 mm group, although the proportion of parasites with asymmetrical testes was fairly constant in parasites 0–40 mm (43.9–

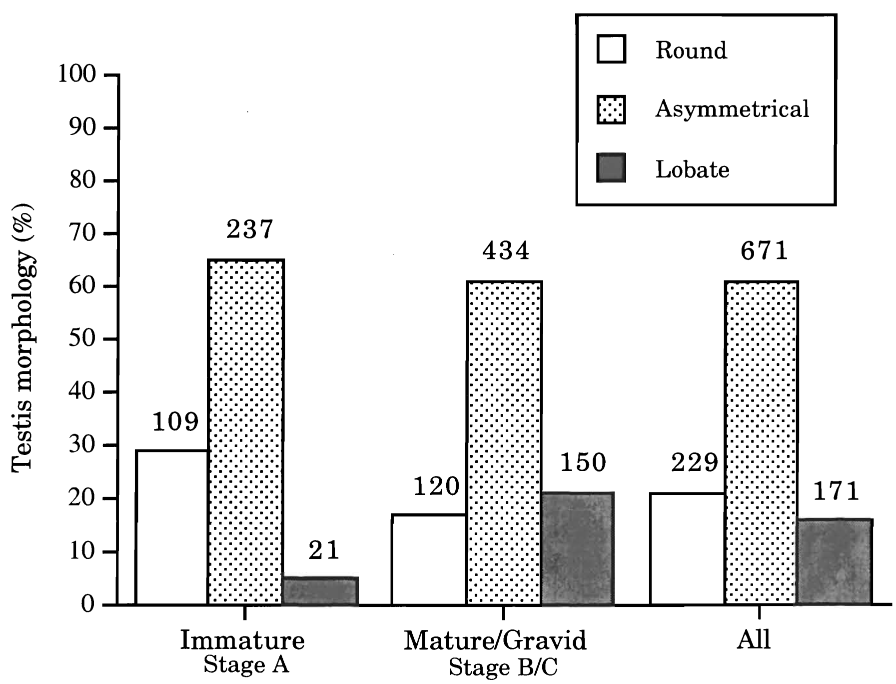


Figure 2. Occurrence of anterior and posterior testis morphological categories in *Allocreadium lobatum* with respect to parasite maturity, Stages A–C. Datum above bar = *N*.

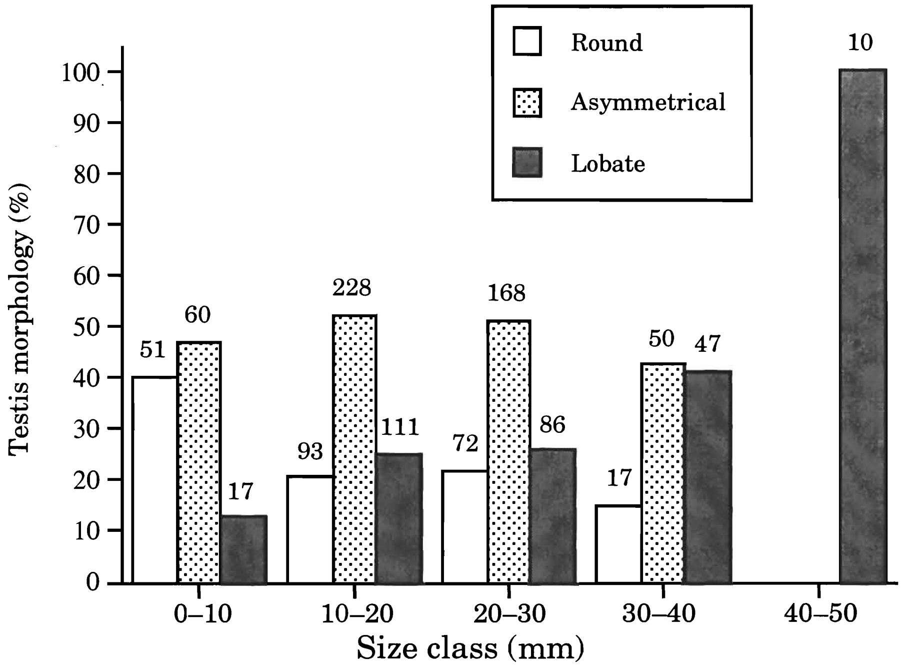


Figure 3. Occurrence of anterior and posterior testis morphological categories in *Allocreadium lobatum* with respect to parasite length. Datum above bar = *N*.

Table 1. Morphologic variation in testes found in mature/gravid (Stages B and C) *Allocreadium lobatum* in *Semotilus atromaculatus* in previous studies in Nebraska.

| **Location in Nebraska** | **Mean length (cm ± SD)** | **Round % (*N*)** | **Asymmetrical % (*N*)** | **Lobate % (*N*)** | **Total *N*** |
|---|---|---|---|---|---|
| Brown County, Willow Creek[1] | 3.6 ± .52 | 8 (2) | 0 (0) | 92 (22) | 24 |
| Brown County, Calamus River[1] | 2.5 ± .36 | 17 (1) | 0 (0) | 83 (5) | 6 |
| Cherry County, Minnechaduza Creek[1] | 2.8 ± .46 | 14 (2) | 14 (2) | 72 (10) | 14 |
| Dawes County, Niobrara River[1] | 3.5 ± 1.0 | 0 (0) | 0 (0) | 100 (14) | 14 |
| Franklin County, Center Creek[1] | 3.5 ± .16 | 25 (1) | 25 (1) | 50 (2) | 4 |
| Franklin County, Little Cottonwood Creek[1] | 3.0 ± .53 | 0 (0) | 14 (2) | 86 (12) | 14 |
| Keya Paha County, Holt Creek[1] | 3.0 ± .70 | 25 (2) | 25 (2) | 50 (4) | 8 |
| Keith County, Lonergan Creek[2] | 4.5 ± .44 | 44 (7) | 31 (5) | 25 (4) | 16 |
| Lancaster County, Elk Creek[3] | 4.5 ± .44 | 64 (9) | 36 (5) | 0 (0) | 14 |
| Richardson County, Easly Creek[1] | 5.6 ± .26 | 0 (0) | 0 (0) | 100 (6) | 6 |
| Richardson County, unnamed creek near Cornhusker Boy Scout Camp[1] | 3.7 ± .15 | 63 (5) | 25 (2) | 12 (1) | 8 |
| Webster County, Republican River[1] | 2.4 ± .52 | 0 (0) | 17 (2) | 83 (10) | 12 |

Mayes, 1976.
[2]UN–L student, 1979.
[3]Parr, 1991.

Table 2. Morphologic variation in testes found in mature/gravid (Stages B and C) *Allocreadium lobatum* in hosts other than *Semotilus atromaculatus* in previous studies.

| **Location** | **Mean length (cm ± SD)** | **Round % (*N*)** | **Asymmetrical % (*N*)** | **Lobate % (*N*)** | ***N*** |
|---|---|---|---|---|---|
| Keith Co., NE[1] | 2.0 ± .22 | 100 (4) | 0 (0) | 0 (0) | 4 |
| Idaho Co., ID[2] | 2.4 ± .37 | 0 (0) | 19 (3) | 81 (13) | 16 |

[1]Host: *Salmo gairdneri* (Richardson), rainbow trout; Keystone Lake (N. Platte River), Keith County, NE (D.A. Peters, 1980)
[2]Host: *Richardsonius balteatus* (Richardson), redside shiner; Selway River, Idaho County, ID (Stewart Schell Collection)

52.8%) and absent in the 40–50 mm group. These data demonstrate that while the asymmetrical testis morphology is the most prevalent in most *A. lobatum* size classes (0–40 mm), there was a trend of decreasing percentage of round morphology as the parasite increased in length, and a conversely increasing percentage of lobate morphology as the parasite increased in length.

In most previous collections (9) of *A. lobatum* in *S. atromaculatus* in Nebraska, the lobate testis morphology was the most common (Table 1), although a few collections (3) did have a predominance of round testes (44–63%; Table 1). When taking parasite length into consideration, we find that most studies were performed on larger *A. lobatum* (average length >2.4 cm), although even in studies with mainly round testis morphology length was >3.7 cm (Table 1). Similarly, morphological variation in testes was not correlated with size in studies of *A. lobatum* in different hosts (Table 2). All of these studies were single collections and included relatively sparse numbers (2–24 specimens) compared to the current study. The most likely reason for this lack of correlation could be sampling error (not enough specimens over a sufficient period of time) or some populations may not demonstrate this variation due to genetic isolation or other factors. Additionally, only mature/gravid specimens were found because at the season when these collections were made (April–June), primarily gravid individuals have been reported in Nebraska (Willis 2001). Seven specimens (of 62) with paired testes demonstrated both single-lobate and either asymmetrical or round morphology in the same specimen.

*Allocreadium lobatum* in hosts other than *S. atromaculatus*, deposited in the Harold W. Manter Laboratory, were analyzed morphologically (Table 2). In *A. lobatum* from a rainbow trout in Keith County, Nebraska, the testes were only round (100%), while testes from *A. lobatum* collected from a redside shiner in Idaho County, Idaho, were primarily lobate (81%) with the others asymmetrical (Table 2). Average parasite length was > 2 cm in both collections. Each of these studies represents a single collection with only one specimen (of 10) demonstrating both a single lobate morphology along with either asymmetrical or round morphology.

Morphometric comparisons of the present and previous studies are reported in Table 3.

## DISCUSSION

Several investigators have reported *A. lobatum* in freshwater fishes in North America and the description has remained fundamentally unchanged (Amin 1982, Mayes 1976, Mueller 1934, Wallin 1909). Distinctly lobate testes were identified in the original description of *A. lobatum* from the fallfish, *Semotilus corporalis* (Mitchill) collected at Sebago Lake, Maine, and were instrumental in the epithet's etymology (Wallin 1909). Variation in *A. lobatum* testes was identified in three distinct categories (round, asymmetrical, lobate), not only in the present study but also in earlier collections.

There are four possible reasons for testis morphological variation identified in this study: 1) differences in mounting technique; 2) species polymorphism; 3) the presence of a species of *Allocreadium* (new or not *A. lobatum*); or 4) testis morphological variation. Differences in mounting technique can likely be ruled out because distinct morphological categories of testis could be identified not only in this study, but in studies performed by other investigators. Additionally, variation in other morphological measurements was not seen in this study, and such variation would probably be present if a technique were to blame.

Species polymorphism has been defined as the discontinuous, discrete differences between individuals of a species (Cabaret and Durette-Desset 1990). The individuals in this study may have discrete categories of testis morphology, but individuals were shown to have multiple morphologies on occasion (i.e. one lobate and one round testis). Multiple morphologies of testes in an individual were similarly found in previous specimens of *A. lobatum*, and thus polymorphism can be ruled out.

Table 3. Morphometric analysis of mature/gravid (Stages B and C) *Allocreadium lobatum* in *Semotilus atromaculatus*.

| Body measurement | | Range (cm) | Average | *N* |
|---|---|---|---|---|
| Length | | 0.7290–5.1876 | 2.36 | 327 |
| Width | | 0.2160–1.6632 | 0.65 | 362 |
| Oral sucker length | | 0.1485–0.6885 | 0.28 | 338 |
| Oral sucker width | | 0.1755–0.5265 | 0.30 | 335 |
| Acetabulum length | | 0.1215–0.5535 | 0.30 | 350 |
| Acetabulum width | | 0.2025–0.5670 | 0.32 | 346 |
| Sucker ratio | | 1:0.68–1:1.79 | 1:1.1 | 333 |
| Pharynx length | | 0.0945–0.2835 | 0.16 | 328 |
| Pharynx width | | 0.0810–0.2700 | 0.15 | 328 |
| Ant. testis | (length) | 0.1080–0.6750 | 0.27 | 348 |
| | (width) | 0.1080–0.8910 | 0.26 | 349 |
| Post. testis | (length) | 0.1215–0.7695 | 0.32 | 350 |
| | (width) | 0.0945–0.6345 | 0.25 | 348 |
| Ovary length | | 0.0540–0.4050 | 0.12 | 352 |
| Ovary width | | 0.0540–0.4725 | 0.10 | 350 |

In New World freshwater fishes, four species of *Allocreadium* are recognized: 1) *A. (Allocreadium) lobatum* Wallin 1909 (USA); 2) *A. (Neoallocreadium) mexicanum* Osorio-Sarabia, Perez-Ponce de Leon, and Salgado-Maldonado 1986 (Michoacan, Mexico), 3) *A. (Neoallocreadium) cetropomi* Fischthal and Nasir 1974 (Venezuela) (Yamaguti 1971) and 4) *A. (Neoallocreadium) lucyae* Williams & Dyer 1992 (Williams and Dyer 1992). Of these four species, *A. lobatum* and *A. (Neoallocreadium) mexicanum* have been reported with lobate testes (Williams and Dyer 1992). *Allocreadium (Neoallocreadium) mexicanum*, however, has been reported with testes in the posterior 1/3 of the body and ceca terminating near the posterior end of the body, whereas *A. lobatum* (and those specimens identified in this study; see Fig. 1) have testes in the middle 1/3 of the body and the ceca terminate at the anterior edge of the posterior testis (Williams and Dyer 1992). Therefore, the specimens in this study do not represent a species other than *A. lobatum*.

In the original description of *A. lobatum*, the testis shape was a distinguishing characteristic in *Allocreadium* in New World freshwater fishes (Wallin 1909). The analysis of syntypes (Fig. 1,d) and paratypes from this original description confirms that indeed only lobate testes were present. Subsequent studies including the present one have demonstrated that the shape of the testes in *A. lobatum* lies in a continuum from round to lobate and represents morphological variation. It is likely that the morphology of the testes of *A. lobatum* is more diverse than previously thought, which may be a result of larger samples collected over longer periods in this study. This finding may help others identify *A. lobatum* in which this morphological variation can be appreciated.

Four studies reported morphometric measurements of *A. lobatum* (Amin 1982, Mayes 1976, Mueller 1934, Wallin 1909). In mature and gravid specimens, the length ranges in this study were 0.73–5.2 cm, which is similar to previously reported ranges: 3–6.7 (Wallin 1909), 2–4 (Mueller 1934), and 2.1–5.4 (Mayes 1976). The average length of *A. lobatum* in this study was 2.4 cm, which compares to previously reported 2.16 cm (Amin 1982). The sizes of *A. lobatum* eggs in the present study were 0.036–0.136 × 0.028–0.140, and the average (0.086 × 0.056 cm) fell within the limits of the combined ranges reported by the above authors (0.048–0.104 × 0.038–0.080 cm). Sucker ratio (anterior:posterior sucker length) in this study averaged 1:1.11, which is similar to that reported by Wallin (1:1.08) and Mayes (1:1.17). The wider ranges of measurements found in the present study (e.g., egg size) as compared to previous studies may be due to: 1) the greater number of mature/gravid specimens (362) that were used (Amin, 2; Mueller, 3; Mayes, 65; Wallin, ?); 2) the time over which specimens were collected (8 months in the present study, compared to single collections) allowing the expression of the full range of developmental sequences; and 3) the objective of the collectors (documentation of the presence of a species vs. in-depth investigation of the expression of species morphology).

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