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Natural History Notes and New County Records for Ozarkian Millipeds (Arthropoda: Diplododa) from Arkansas, Kansas and Missouri

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Running Title: Natural History Notes on Millipeds

Over the past decade, there have been numerous new geographic records documented for milliped distributions in Arkansas (see McAllister et al. 2013 and refs.) and, to a lesser degree, for Kansas and Missouri (Gunthorp 1913, 1921, Chamberlin 1928, Shelley and Snyder 2012), but little is known about their natural history in these states (Youngsteadt 2008, 2009). Here we summarize observations on several Ozark millipeds within six orders and nine families that the senior author made over the last eight years, and add several new geographic distribution records.

Unless noted, specimens were collected from under logs or rocks in woodland habitat. Specimens were maintained in 11 or 16 cm diameter clear-plastic deli dishes provided with clay, wood, rock, and/or soil as a substrate to approximate the natural microhabitat. Millipeds were kept in a general purpose room that had windows, but was also artificially lighted when too dark for other purposes. The temperature varied with time of day and season from about 13 to 29°C (55 to 85°F). The most common food items provided were baker's yeast and compost in the blackened stage that was derived largely from oak/sweet gum leaves and scrap fruits and vegetables. Tetramin® tropical fish food was sometimes provided and, occasionally, carrot or potato peels, raw ground beef, or freshly killed insects. Photographs/photomicrographs were taken with a Canon Power Shot SX-100, 110 or 160 IS digital camera, either directly or through an ocular of a stereomicroscope. Some were taken through the clear plastic of the deli dish. Each annotated account below begins with the taxon studied along with the longest time one of the individuals lived, the collection sites and dates the millipeds were collected, Voucher specimens (photovouchers) of millipeds representing new county records are on deposit in the Sam Noble Oklahoma Museum of Natural History, Norman, Oklahoma.

Platydesmida: Andrognathidae

Brachycybe lecontii Wood - lived at least 1.3 yrs. Lake Leatherwood Park, Eureka Springs, Carroll County, Arkansas (27 Nov., 29 Dec. 2011); ca. 16 km SSE of Ozark, Christian County, Missouri (22 Apr., 11 May 2013. These pink to red millipeds were about 2 cm long and found in colonies under logs. They apparently feed on the microorganisms that live on the rotting wood. To molt, these millipeds curled up in a protected place and shed their skins after about 10 days. They did not eat their exoskeletons. Mating and egg-laying were not observed, but about 24 eggs appeared that were being tended by an adult that had its anterior half wrapped around them (Fig. 1A). This adult was not sexed, but male brooding of eggs has been documented in several Brachycybe species (Shear 1999, Kudo et al. 2010). The eggs hatched in 21 days, during which time the adult remained in the same place with them. The eggs were 0.6 mm in diameter, but swelled a bit before hatching. The hatchlings young (Figs. 1B-C) had five pairs of legs (Fig. 1C) and were 2.0 to 2.5 mm long; an older hatchling is shown in Fig. 1D. None survived more than several days.



Figure 1. *Brachycybe lecontii*. A. Adult brooding eggs. B. Newlyhatched young. C. Six days old. D. New hatchling.

We also documented a new county record for *B. lecontii* collected on 22 Apr. 2013, ca. 16 km SSE of Ozark, Christian County, Missouri (see Shelley et al. 2005b).

Chordeumatida: Cleidogonidae

Various species. – lived 1-2 mos. Greene and Taney cos., Missouri and Carroll Co., Arkansas (Jan. -Apr., Oct.-Dec., 2007-2013). *Tiganogona* is a common local genus, but some of the specimens may have belonged to other genera (i.e., *Cleidogona*). The longest mating (Fig. 2A) lasted at least 18 hrs during which the female would sometimes walk around with the male twisting his body and using his posterior and middle legs to keep up. Although no eggs were observed, hatchlings (Fig. 2B) that appeared in March were 1.0-1.2 mm long, had five pairs of legs, eye spots, and curved setae. None lived long enough to molt.



Figure 2. A. Cleidogonid mating pair. B. Hatchling; scale bar = 500 $\mu m.$

Trichopetalidae

Trigenotyla parca Causey. – Nov.; lived 5 months. Lake Leatherwood Park, Eureka Springs, Carroll County, Arkansas (27 Nov. 2011). This single male was 0.8 cm long and it had sticky droplets at the bases of its long segmental setae. If the sticky droplets were touched with a needle, they would stretch into strands as the needle was withdrawn as they did in the closely related *Causeyella* (Youngsteadt 2008). Shear (2008) discussed milliped spinnerets and a possible homology between the segmental setae (with their sticky droplets) and the spinnerets.

Callipodida: Abacionidae

Abacion spp. – lived one yr. E of Springfield near Turners, Greene County, Missouri (17, 20 Apr., 12 Jun. 2007); N side of McDaniel Lake, N of Springfield, Greene County, Missouri (15 Apr. 2009); N of Wakefield, Clay County, Kansas (28 May 2011); Lake Leatherwood Park, Eureka Springs, Carroll County, Arkansas (28 Oct. 2011). Two of five *Abacion texense* collected in Kansas produced silken molting cocoons (Fig. 3), but their construction was not observed and both died in the cocoons before molting. Enghoff and Akkari (2011) described a dense callipodidan cocoon in more detail and noted that it was the first such report since 1874. That gap between publications tends to characterize the present state of milliped biology. Enghoff and Akkari had hoped others would look for similar cocoons and add more detail.

The *A. texense* from northcentral Kansas represent a new county record for Clay County (see McAllister and Shelley 2010). They were collected from under rocks in prairie habitat.



Figure 3. Abacion texense showing silky cocoon.

Polydesmida: Xystodesmidae

Apheloria virginiensis reducta Chamberlin. – lived four mos. Ca. 16 km SSE of Ozark Christian County, Missouri (23 May 2007, 18 May 2011); Beachler Ridge, ca. 19 km SSE of Ozark, Christian County, Missouri (2 Apr., 26 Oct. 2010, 3 Mar., 6 May 2011). These four cm long millipeds were black with bright yellow markings. Data suggested a one yr life cycle: adults were collected in the spring, mating occurred in May and June, young appeared in July (and lived three mos), adults died in late spring or summer, and subadults were collected in October.

When males attempted to mate, the females attempted to avoid them. If the male did attain the belly to belly mating position, he curled his head and anterior segments around the front of the female's head and apparently tried to push it back, while the female tried to keep her head tucked. Such female resistance resulted in many unsuccessful mating attempts. In general, matings were relatively short, the longest lasting about 30 min.

Although eggs were never seen, young did appear in a dish that also contained other kinds of millipeds. Since the young did not resemble the hatchlings of the other kinds, it was assumed they were *Apheloria*.

The first instars moved slowly and spent most of their time in the soil. They were white, 1.2-1.5 mm long, had three pairs of legs and seven segments counting the epiproct. They had longer setae than the first instars of the other polydesmidans; the lateral midbody setae were about two-thirds the width of the body. There were three of these setae per side per segment including dorso-laterals, laterals, and ventrolaterals. The laterals stuck straight out with only a slight curve. Second instars were 2.1-2.2 mm long, had 10 segments and six pairs of legs. Third instars were about 3.2 mm long with 11 pairs of legs. A possible fourth instar was 3.6 mm long with mid-body setae about one-fourth the width of the body. Stages progressed as follows: a first instar was molting on 16 Aug.; a second instar was seen on 20 Aug.; a third instar was seen on 14 Sept.; the possible fourth instar was seen on 19 Oct.

All specimens from Christian County, Missouri document a new county record; previous reports from the state include Barry, Cole, Franklin, Howell, Oregon, Pulaski, Shannon St. Louis, Stone and Taney counties (see Shelley and McAllister 2007).

Euryuridae

Auturus evides (Bollman). – lived five mos. Hatchlings (Fig. 4A) appeared in June and lived two yrs. E of Springfield near Turners, Greene County, Missouri (30 Mar., 18 May 2007, sometime before 9 May 2008, inadvertently introduced with wood); N side of Fellows Lake, Greene County, Missouri (1 Jan. 2012). These 3.5 cm long millipeds mated frequently from March to May in a manner typical of polydesmidans: belly to belly with the front of the male curled over the head of the female and his legs firmly enclosing her, particularly toward the front. If the animal was upside down, it was not unusual for the posterior part to be twisted so the legs were on the ground. Mating lasted for over an hour.

Eggs were laid in hollows beneath the soil in clusters of 12 to 30. They were tan and about 0.45 mm in diameter. They swelled somewhat before hatching and became more grayish and translucent. Hatching was not synchronous, but proceeded for two or more weeks from a given clutch. The hatchlings (Fig. 4A) were slow and lethargic compared to those of *Pseudopolydesmus pinetorum* (herein), and did not form a flock. A few survived to adulthood.

Molting took place in an igloo-like chamber constructed of fecal pellets shaped by the everted rectum; the chambers sometimes had a chimney-like structure on the side (Fig. 4B). Chambers varied with the size of the builder, but one with an outside diameter of 14 mm had walls 2 mm thick. Construction took about a day and the milliped spent about 10 days in it before the skin was shed. It might spend another three days in the chamber before exiting, usually without eating the skin.

We document a new county record (Greene County) in Missouri for *A. evides*. Shelley (1982) previously reported this milliped from 25 other counties of the state.



Figure 4. Auturus evides. A. Hatchling; scale bar = 1 mm. B. Molting chamber; scale bar = 5 mm.

Polydesmidae

Scytonotus granulatus (Say). – lived three mos. Young appeared in April and May. E of Springfield near Turners, Greene County, Missouri (30 Mar. 2007, 4 Feb., 13 Nov. 2008); N side of McDaniel Lake, Greene County, Missouri (20 Feb. 2009); Beachler Ridge, ca. 19 km SSE of Ozark, Christian County, Missouri (2 Apr. 2010). These bumpy-backed millipeds were about 1.2 cm long. Mating occurred around spring, and in one observed case the male mounted the female from behind, crawled forward, and then turned belly to belly with his anterior end curled around her head. His legs completely surrounded her, including her legs. The longest mating lasted at least 3.5 hrs.

On 22 Apr., a female had built an open-topped igloo-like egg chamber, apparently of fecal material (construction was not observed) in which she laid 12 eggs. The eggs were white to tan, spherical, and had a diameter of 0.4 mm. By 3 May they were less spherical, enlarged to about 0.5 mm in diameter, grayer and more translucent, and embryonic structure was apparent in some. By 5 May, one egg was somewhat crescent shaped and about 0.8 mm long. They all

hatched later that day (except one that was apparently infertile) and the hatchlings dispersed.

The first instars were white, 1.1 to 1.4 mm long, had seven segments counting the epiproct, and three pairs of legs. Like the adults, they had bumps on their backs but were covered with short, hooked setae. Successive instars were increasingly coordinated, but remained white with hooked setae through the fifth instar, at which the last one died at about three months old.

We document a new county record (Greene County) for *S. granulatus* in Missouri. Shelley et al. (2005a) previously reported this milliped from Boone, Calloway, Chariton, Christian, Cole, Dent and Phelps counties.

Polydesmidae

Pseudopolydesmus pinetorum (Bollman). Springfield, Greene County, Missouri (20 Aug. 2007, 13 Mar. 2011); Beachler Ridge, ca. 19 km SSE of Ozark, Christian County, Missouri (13 Oct. 2008); N of Wakefield, Clay County, Kansas (28 May 2011). This account summarizes some observations made by Youngsteadt (2009) and adds new information. These millipeds were about 2 cm long and had a one yr life cycle. The females built igloo-like egg chambers constructed of fecal material and laid several clutches of eggs in the spring (Figs. 5A and 5B) before they and the males died in the summer. The fecal material used for chamber construction was shaped by the everted rectum and the eggs were laid in the chamber as construction proceeded. The eggs hatched synchronously after about 8 to 18 days, depending on temperature, after which the hatchlings spent another two days in the chamber before one of them made a hole in the chamber wall through which they all departed, one after another (Fig. 5D). These first instars stayed together as a flock (Fig. 5C). Molting took about 10 days and occurred in chambers comparable to egg chambers. Adulthood was reached after seven molts. Some reached this stage and mated in the fall, but others overwintered in their sixth or seventh stadia to become adult and mated in the spring. Regardless, egg laying was centered near springtime. Mating in this species lasted up to two days.

If prodded with a paintbrush bristle, the first instars produced a clear secretion from a pair of stalked lateral pores on the fifth segment; the secretion was assumed to be repellant. More recent testing demonstrated that if an object was inoculated with the secretion and held in front of a first instar, the first instar stopped moving, laid its antennae back, and either retreated or changed direction. Second and third instars and adults also responded, but first and second instars of *A. evides* (herein) did not obviously do so.



Figure 5. *Pseudopolydesmus pinetorum*. A. Female laying eggs (arrow). B. Egg chamber with hatchlings. C. Flock of first instars. D. Hatchlings exiting egg chamber.

With continued prodding, a first instar also produced what looked like a clear droplet of fluid from its rectum, and this was reported as a droplet by Youngsteadt (2009). However, further observation suggested that the "droplet" was more likely an everted rectum. In addition to being everted when individuals were prodded, they were also commonly everted as individuals moved about in a flock; every once in a while an individual would lift its abdomen a bit and rather quickly evert and retract its rectum. Since these millipeds are blind, the idea emerges that the everted rectum might emit a signaling substance that helps organize the flock. However, no individual movement or movement of the flock as a whole seemed to be influenced by the eversions.

As a test for possible cryptic species, males and females from different locations were placed together to see if they would mate; all did. The most distant locations were separated by about 422 km (264 mi), and included a male from Springfield, Missouri (Greene County) and two females from Wakefield, Kansas (Clay County). Offspring were also produced, but since one of the females laid eggs prior to the

Journal of the Arkansas Academy of Science, Vol. 68, 2014 180

mating, neither was likely virgin. Overall, the longestsurviving colony of this species died out in its third generation.

As previous records of *P. pinetorum* reported by Withrow (1988) are from an unpublished dissertation, we take this opportunity to document new county records for *P. pinetorum* from Christian and Greene counties, Missouri, and Clay County, Kansas.

Julida: Parajulidae

Species 1 – lived two months and was released. N side of McDaniel Lake, Greene County, Missouri (20, 25 Feb. 2009). These 4 cm long millipeds (Fig. 6) resembled snakes in the way they twisted and curled, particularly during mating, which lasted just over an hour for the longest record.



Figure 6. Parajulids mating.

Species 2 – lived two mos. Greenway trail by prairie plots ca. 0.8 km S of Pershing School, Springfield, Greene County, Missouri (8, 12 Nov. 2011). These 4.5 cm long millipeds were generally similar to species 1 above. Four matings or attempted matings were observed in November but none lasted more than a few minutes. In one case when a male met a female, they quickly coiled into a mating knot, but then quickly separated. The knotting was almost snap quick when they met. Later, two were observed to touch, but one quickly retreated.

Spirobolida: Spirobolidae

Narceus americanus (Palisot de Beauvois). – the original adults lived six mos, but one juvenile was released when nearing three years old. E of

Springfield near Turners, Greene County, Missouri (17 Apr., 10 Aug. 2007). One young that appeared in Apr. lived about 11 mos; one that appeared in Aug. was released when nearing 3 years old. These 5 or 6 cm long millipeds ate rotting wood and compost and their fecal pellets, the color and texture of wood, suggested that wood was the major component. Juveniles reduced dead oak leaves to veins but left most other kinds uneaten.

Mating was observed in Sept.; the longest lasted at least five hours. Coupling was typical for millipeds with the head and anterior part of the male curved over the head of the female, but as mating progressed, the male moved its anterior part slowly back and forth over the female's head in approximately two-second cycles.

A single egg appeared in May. It was grayishwhite, slightly ovoid, and the long dimension was about 1.5-1.6 mm. It was deposited on the bottom of the dish under a mud capsule. The smallest juveniles observed were 1 to 1.5 cm long.

These millipeds (Fig. 7) coiled in a protected spot to molt, which took about 10 days for juveniles and 20 for adults. In one case in which a 1.5-2 cm long individual was observed, its skin finally split between the head and collum and the milliped crawled out through the slit. About 5 segments were added during the molt: it emerged with 40-42 segments counting the collum and epiproct from an exoskeleton that had 35-36.



Figure 7. Narceus americanus.

Regarding growth, a 1.5 cm long juvenile grew to 3.5 - 4 cm in about 11 months; another that was 1 + cm long grew to about 5.5 cm in almost three years.

We document a new county record for N. *americanus* in Greene County. Missouri. Shelley et al. (2006) previously reported this milliped from 22

Journal of the Arkansas Academy of Science, Vol. 68, 2014 181

Missouri counties.

In summary, we have provided some new natural history information for several millipeds collected from three states that help augment previously published accounts. In addition, we report nine new geographic distribution records for some of these millipeds. We still need more information on milliped ecology and natural history and undoubtedly, with additional study, that should become available to diplopodologists in the near future.

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Journal of the Arkansas Academy of Science, Vol. 68, 2014 182