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THE LARVA AND EGG OF *ALLOPERLA PROGNOIDES* (PLECOPTERA: CHLOROPERLIDAE), WITH ECOLOGICAL NOTES AND NEW STATE RECORDS FROM FLORIDA, U.S.A.

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ABSTRACT

Alloperla prognoides Surdick & Stark, previously reported from Coastal Plain Alabama, is recorded for the first time from Florida (Escambia County). Ecological notes for Florida populations are given. Larvae were collected in 4th to 5th order streams from fast flowing riffles within gravel bottom hyporheic habitats as much as 0.5 m below the stream bed. The egg and larva are described for the first time. The larva of *A. prognoides* has 10-12 cercal segments, whereas its sister species *A. natchez* Surdick & Stark and the larvae of other known species all have at least 13 cercal segments. Chaetotaxic characters were also shown to have diagnostic value.

Keywords: *Alloperla*. hyporheic, Sallfly, stonefly, Southeastern Coastal Plain, streams

INTRODUCTION

The genus *Alloperla* is Nearctic and eastern Palearctic in distribution and contains 35 North American species (Stark et al. 2009), 26 of which occur in eastern North America (Surdick 2004; Kondratieff & Kirchner 2004). Four species are presently known from the Southeastern Coastal Plain: *A. natchez* Surdick & Stark from Mississippi, *A. furcula* Surdick from South Carolina, *A. lenati* Kondratieff & Kirchner from North Carolina, and *A. prognoides* Surdick & Stark from Alabama and Florida. These four closely related species and two others form the *Alloperla leonarda* Group of Willett & Stark (2009) who presented the following sister relationships based on characteristics of the male epiproct: ((*A. leonarda* Ricker/*A. ouachita* Stark &

Stewart)(*A. furcula*/*A. lenati*)(*A. natchez*/*A. prognoides*)).

Within *Alloperla*, larvae of only 8 North American species have been described or partially illustrated (Stewart & Stark 2002); sufficiently detailed larval descriptions exist for only a few species. Deficiencies in current larval taxonomy are due in large part to the fact that larvae of *Alloperla* (and other chloroperlid genera) apparently spend much of their larval life in the hyporheic zone and are not commonly collected from benthic substrates, the target of most macroinvertebrate sampling protocols. In general, hyporheal stoneflies are poorly documented in the scientific literature. One notable exception is the paper by Stanford & Gaufin (1974) documenting the hyporheic community from the Flathead and Tobacco rivers in Montana in which the chloroperlid *Paraperla frontalis* (Banks) was found to be the most abundant stonefly. This species, and other members of the hyporheic community, were found to live as larvae deep within gravel under the stream bed and adjacent floodplain, leaving the hyporheic zone for surface sediments only when preparing to emerge.

The current study was initiated after *Alloperla* females were collected while light trapping along creeks in Escambia County, the westernmost county in the Florida Panhandle, as part of an ongoing survey of the caddisfly fauna of Florida. Only females were collected so we could not make a definitive species determination; however, we suspected they were *A. prognoides*, which was described by Surdick & Stark in Surdick (2004) from specimens collected nearby in southern Alabama. The following objectives were established for this project: i) find and document the larval habitat of *Alloperla* by intensive sampling of hyporheic habitats in areas where adults had been collected and at other stream sites likely to support additional populations; ii) collect, rear and associate the larval and adult stages; and iii) provide detailed descriptions of the egg and larval stages.

MATERIALS AND METHODS

Alloperla larvae were collected at riffle sites using a shovel to dig down into the stream bottom and placing the shoveled gravel/sand material in a 600 µm mesh drift net under water to minimize escape of benthic macroinvertebrates. Each successive shoveling was in the same hole to a depth of approximately 0.5 m. Each sample was washed through 12.5mm, 6.3 mm inch and 600 µm standard testing sieves and the sieved substrate was transferred to white trays for sorting.

Field collected larvae were either kept alive for rearing or preserved in 80% ethanol for morphological study. Larvae with dark wingpads were reared in the laboratory in Styrofoam cups containing stream water and Nitex mesh strips to facilitate larval emergence to the adult. The cups were covered with netting to prevent escape and were kept at 10-20°C in an ice chest. All material was preserved in alcohol for study.

Larvae were examined using a stereomicroscope (Olympus SZX16) and photographed using a Spot Insight 4 digital camera mounted to the microscope. Line drawings were based on photographs with details supplemented under the microscope.

Larval mouthparts (mandible and lacinia), cerci, egg, and male genitalia were examined using Scanning Electron Microscopy. Specimens selected for SEM study were sonicated in 80% ethanol then dehydrated through a series of 90%, 95% and 100% ethanol solutions for 10 minutes each. Dehydrated specimens were placed in Hexamethyldisilizane for 30 minutes, removed to fresh Hexamethyldisilizane for 30 minutes, then blotted dry and mounted on aluminum stubs with double stick copper tape. Mounted specimens were sputter coated with gold-palladium and examined with an Amray 1810 scanning electron microscope. The species identity was confirmed as *Alloperla prognoides* by comparing SEM images of the male epiproct complex with that of SEM images of a paratype specimen of *A. prognoides* figured in Willet and Stark (2009).

Voucher specimens are deposited in the aquatic insect collection at Florida A&M University and the personal collection of Bill P. Stark.

RESULTS AND DISCUSSION

Alloperla prognoides Surdick & Stark
(Figs. 1-21)

Alloperla furcula: *sensu* Stark & Harris, 1986:177, Not

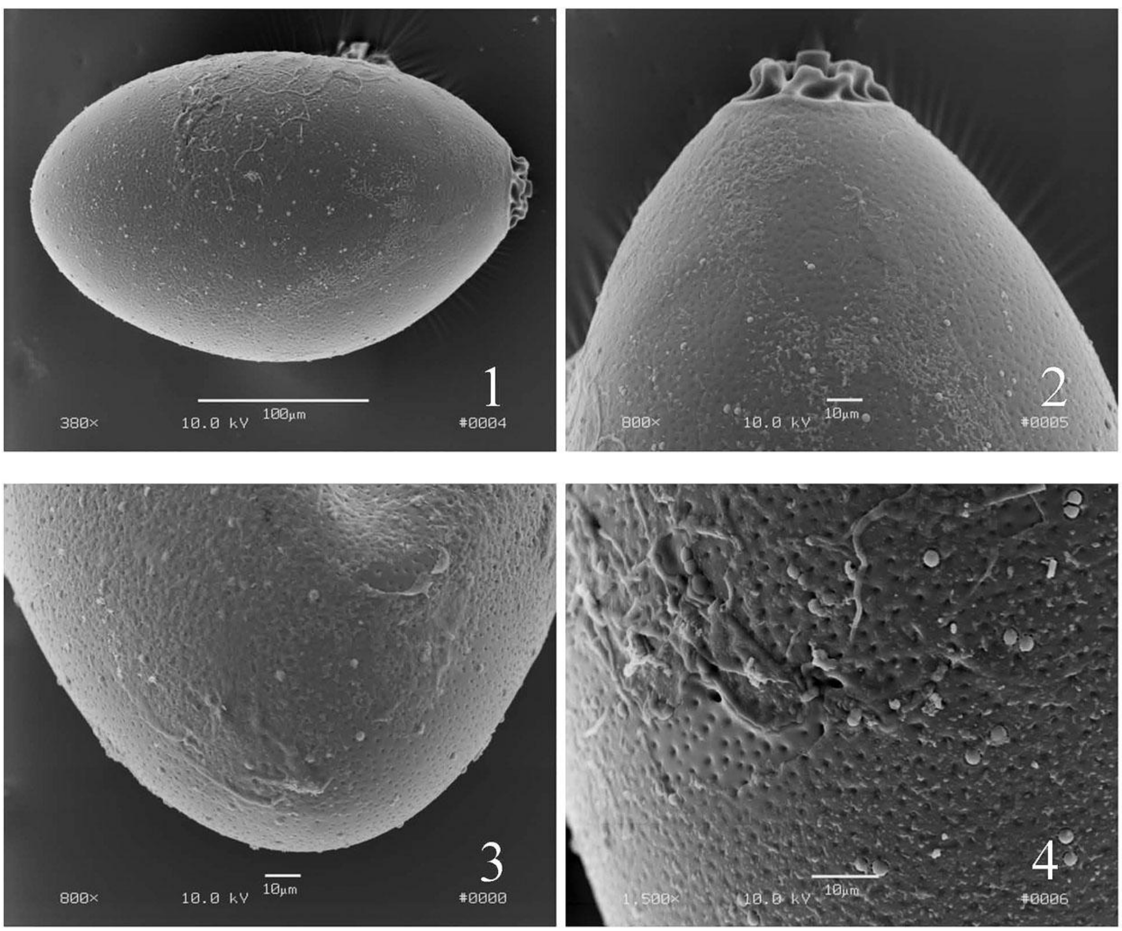
Surdick, 1981.

Alloperla prognoides Surdick & Stark in Surdick, 2004:20. Holotype ♂ (United States National Museum), Little River, Hwy 59, Baldwin Co., Alabama.

Alloperla prognoides: Willet & Stark, 2009:117. Redescription of male epiproct.

Material examined. New State Record. FLORIDA: Escambia Co, Pine Barren Creek, below US Hwy 29, N 30°46′30.04″, W 87°20′15.64″, 30 April 2008, D. Ray, G. Chiello, 3 adult ♂ (reared), 5 larvae. Same, except 22 April 2008 with F. Butera, 5 larvae. Big Escambia Creek, below Fannie Rd, N 30°58′50.4″, W 87°13′43.9″, 25 March 2010, 5 larvae, D. Ray, R. Abad. Same, except 28 April 2010, 1 adult ♂, 1 larva. Canoe Creek, above Bratt Rd, N 30°57′22″, W 87°20′49″ 24, April 2009, D. Ray, R. Abad, 1♂, 3 larvae. Perdido River, 250 m above Brushy Creek confluence, N 30°47′37″, W 87°33′42″, 22 April 2009, D. Ray, R. Abad, 2♂ (reared), 36♀ (2 reared), 4 larvae. Pine Barren Creek, below US Hwy 29, N 30°46′30.04″, W 87°20′15.64″, 28 April 2009, D. Ray, R. Abad, 1♂ (reared), 3♀ (reared). Brushy Creek, above Pineville Road, N 30°47′47.6″, W 87°33′24.4″, 25 February 2008, D. Ray, L. Donelan, G. Chiello, 3 larvae.

Egg. Outline elongate oval. Length ca. 290 µm, width ca. 174 µm. Collar short, ca. 14 µm long and ca. 33 µm wide, and consisting of a single irregular row of large, irregularly shaped meshes (Figs. 1-2). Chorionic surface covered throughout with fine micropunctures (Figs. 2-3). Micropylar orifices somewhat tear drop shaped and at least twice as large as micropunctures (Fig. 4); micropylar canals slanted.



Figs. 1-4. *Alloperla prognoides* egg. 1. Entire egg. 2. Collar end. 3. Micropylar end. 4. Chorionic detail with micropyles.

Larva. Body length (not including cerci) 5.2-7.2 mm (n = 18); body slender, dorsoventrally flattened; general color pale brown, faint brown markings on head, thorax, and abdomen; legs pale; abdominal tergites with brown speckling anteriorly.

Head. Mandible with ca. 5 teeth, apical tooth scoop-shaped; dorsal surface bearing a submarginal setal row more or less parallel to marginal setal row which terminates near base of tooth 4 (Fig. 5); ventral surface with patch of acanthae near base of tooth 5; marginal setal row consisting of ca. 12 major setae. Maxillary laciniae with single apical tooth and prominent row of ca. 12 long setae (Figs. 6-7). Postocular area with 2 long setae (Fig. 11).

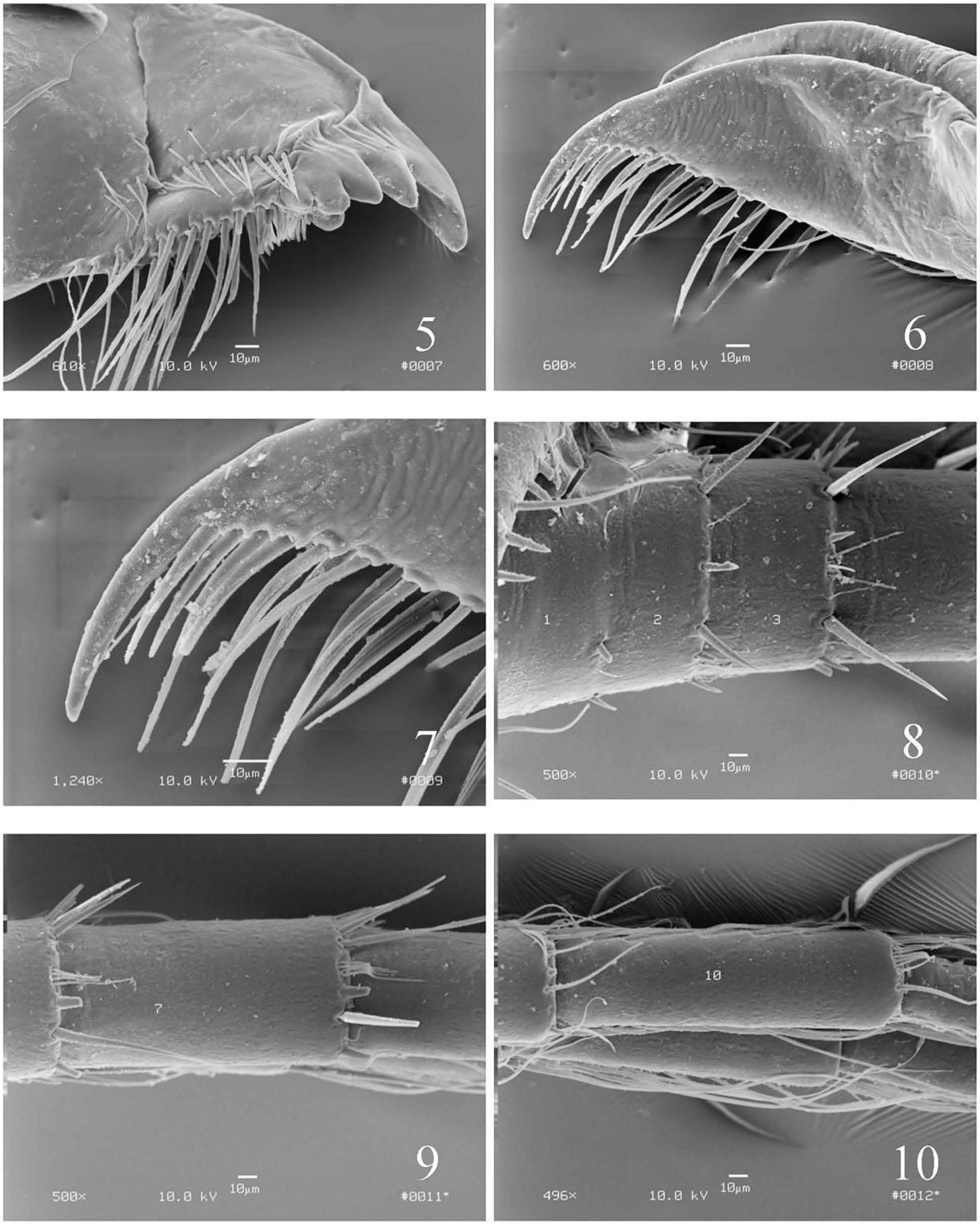
Thorax. Pronotal disk darkened marginally, body of sclerite uniformly pale brown. Pronotal setae arising antero- and posterolaterally; anterolateral setae set in cluster of 1 long seta and 5-7 shorter setae; posterolateral area with 2 widely spaced setae (Fig. 11). Wingpad outer margins broadly rounded, inner margins slightly divergent; short setae scattered along lateral margins (Fig. 12); distinct medial notch apparent during pre-emergent stage. Mesonotal setae of various lengths anterolaterally; group of short setae extending mesally near anterior margin. Foretibia with dorsal fringe of dense, very long hairs; sparse ventral fringe of long hairs, mixed with stout setae (Fig. 13); apex set with several stout spine-like setae ventrally. Forefemora dorsally covered with many spine-like appressed setae; dorsal fringe of long hairs, denser distally; mix of long and short setae ventrally.

Abdomen. Sparse covering of fine, pale clothing hairs; posterior abdominal tergal margins fringed with short setae, incomplete mesally, pair of longer submesal setae, additional longer setae laterally (Fig. 14). Cerci 10-12 segmented; plumate vertical fringe on distal half; dorsal fringe of intercalary hairs on posterior 4-5 segments (Fig. 15); ventral fringe of intercalary hairs on posterior 5-6 segments; fringe somewhat more strongly developed along ventral margin than along dorsal margin; segmental cercal whorls consist of mixed setal types including groups of fine setae and larger, spine-like setae (Figs. 8-10).

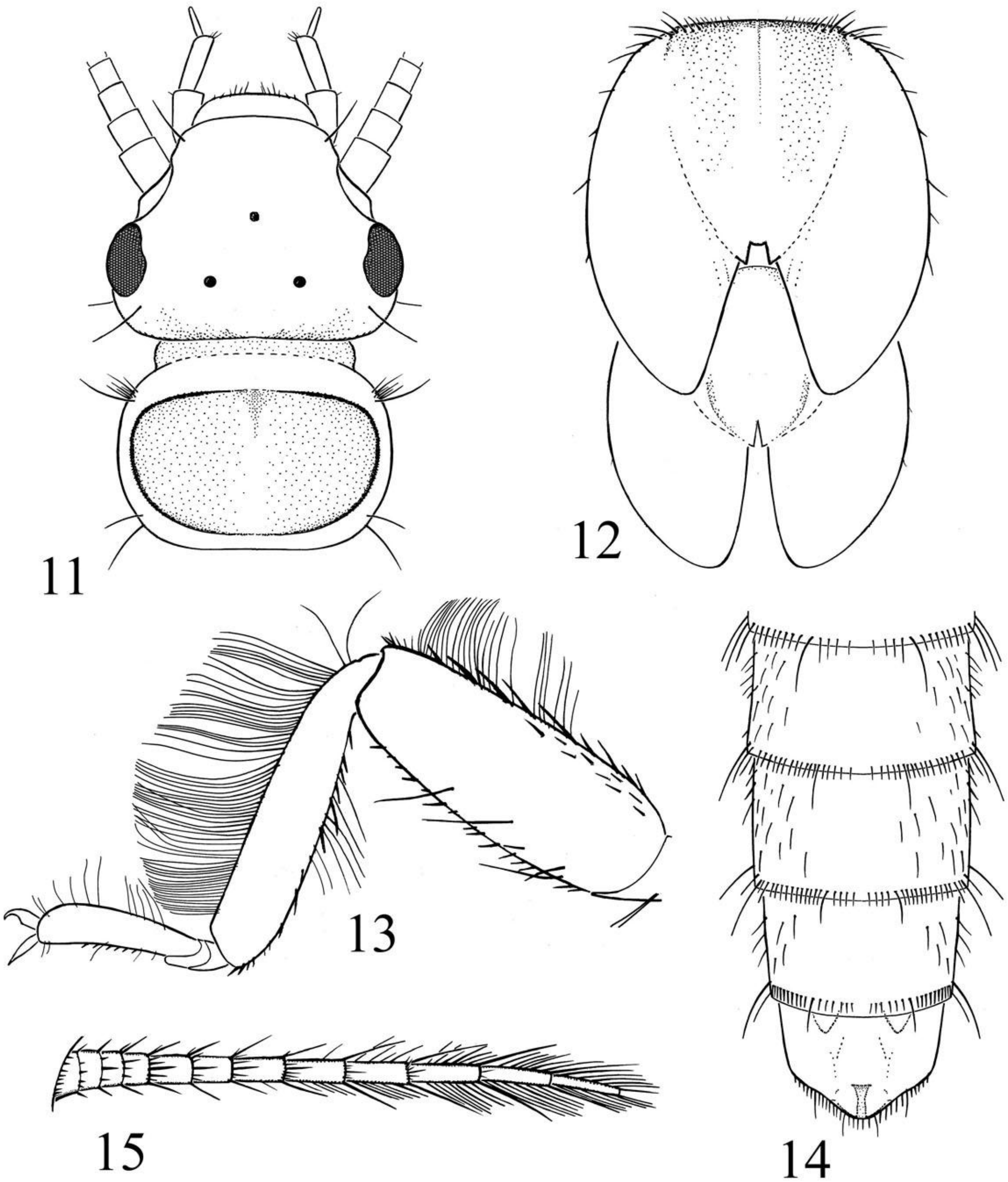
Remarks. As mentioned, larvae of most species of *Alloperla* remain undescribed; therefore, it is presently not possible to provide a definitive diagnosis. However, for the eastern Nearctic species for which detailed illustrations are available, *A. concolor* Ricker by Fiance (1977), *A. natchez* by Brown & Stark (1995), and *A. imbecilla* (Say) by Stewart & Stark (2002), a comparison of their illustrations reveals a number of potentially promising characters for distinguishing larvae of *Alloperla* species (Brown & Stark 1995). Further comparison of the illustrations of the cerci from the publications listed above with Fig. 15 of this study shows that the larvae can be separated based on the number of cercal segments. The cercus comprises 10-12 segments in penultimate and last instar *A. prognoides*, whereas its sister species, *A. natchez* has 13 segments, *A. imbecilla* has 15-16 segments, and *A. concolor* has 18 segments. Additionally, *A. natchez* and *A. prognoides* have a cercal fringe of intercalary hairs which is more strongly developed dorsally than ventrally, whereas the dorsal and ventral fringes appear equally developed for *A. imbecilla* and *A. concolor*. Another character with diagnostic value is the setation of the posterolateral area of the pronotum, which in *A. imbecilla* has 4 posterolateral setae, compared with 2 posterolateral setae as seen in *A. concolor*, *A. natchez*, and *A. prognoides*.

The egg is very similar to that of *A. natchez* (Brown & Stark 1995) in shape, collar form and chorionic detail, although the surface micropores of that species appear finer and more defined than in *A. prognoides*. Images of the male epiproct (Figs. 16-21) are presented for comparison with those in Willett & Stark (2009) made from Alabama specimens.

Distribution. *Alloperla prognoides* is currently known from only Coastal Plain Alabama and the far western Florida panhandle (Escambia County). The species was first reported from Alabama (Baldwin, Escambia, Monroe Cos.) in Stark & Harris (1986) as *A. furcula* based on one male and 13 females collected between 11 May and 24 June. The authors noted that "the epiproct of the single male specimen is shorter and wider than in South Carolina specimens," which suggested the specimen represented an undescribed species. Subsequently, *A. prognoides* was described by Surdick and Stark (2004) based on specimens collected in 1985 (late April-May) by Steve Harris from the Little River (Alabama: Baldwin, Monroe County) and Autauga Creek (Alabama: Autauga

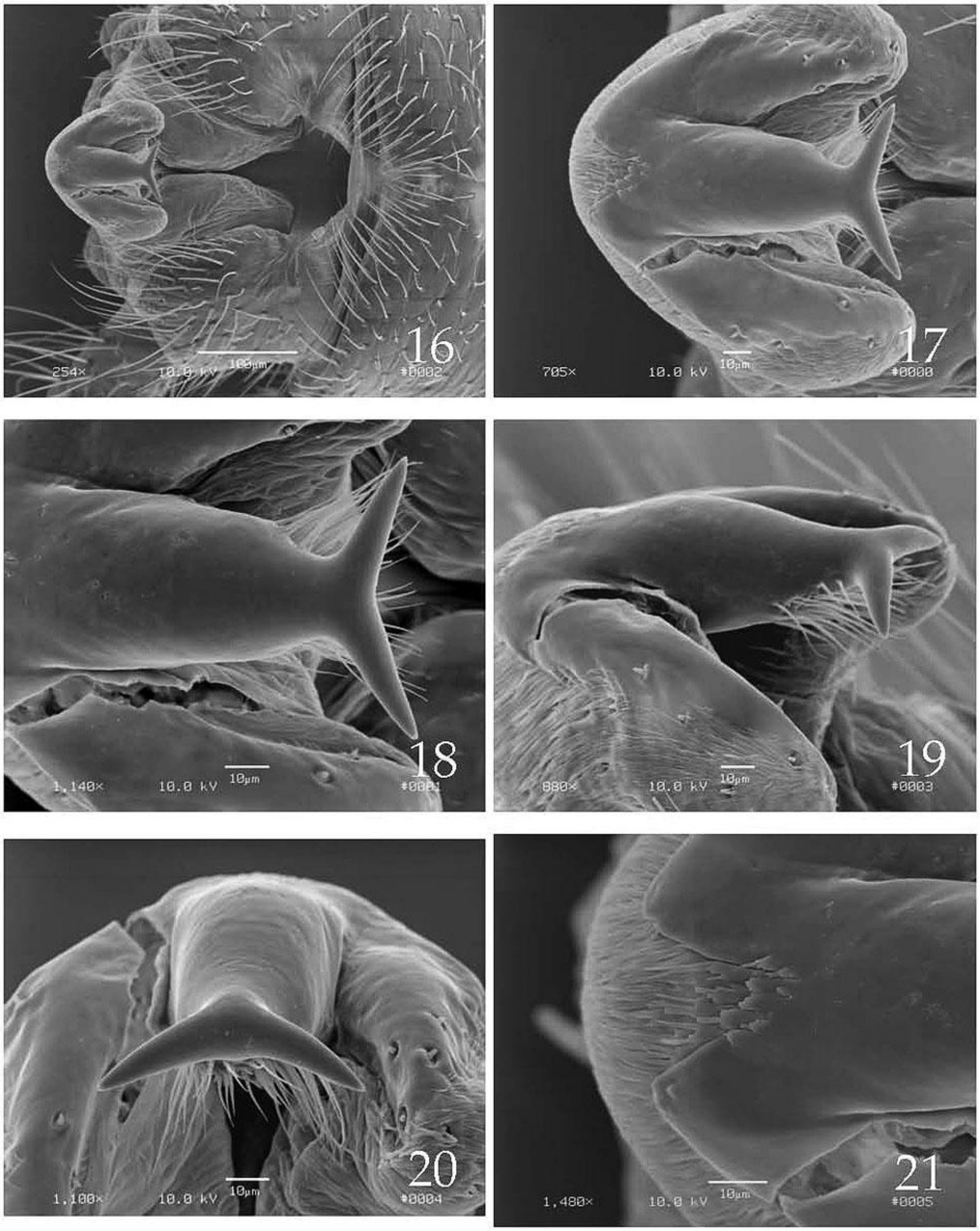


Figs. 5-10. *Alloperla prognoides* nymphal structures. 5. Dorsal aspect of right mandible. 6. Ventral aspect of right lacinia. 7. Detail of lacinial apex. 8. Basal cercal segments, lateral aspect. 9. Cercal segment 7, lateral aspect. 10. Cercal segment 10, lateral aspect.



Figs. 11-15. *Alloperla prognoides* nymphal structures. 11. Head and pronotum. 12. Mesonotum and metanotum. 13. Foreleg. 14. Abdominal segments 7-10, dorsal aspect of male. 15. Cercus, lateral aspect.

County). No other *Alloperla* species have been recorded from Coastal Plain Alabama or Florida. Other *Alloperla* species do occur in Alabama above the Fall Line: *A. atlantica* Baumann, *A. caudata* Frison, *A. hamata* Surdick, *A. idei* (Ricker), and *A. usa* Ricker (Stark & Harris 1986; Surdick 2004). There is always the possibility that one of these species, or another Coastal Plain endemic *of Alloperla*, overlaps in range with *A. prognoides*.



Figs. 16-21. *Alloperla prognoides* epiproct structures, Pine Barren Creek, Escambia Co., FL. 16. Male terminalia, dorsal aspect. 17. Epiproct and cowl, dorsal aspect. 18. Apical half of epiproct. 19. Epiproct and cowl, lateral aspect. 20. Epiproct apex, frontal aspect. 21. Detail of basal epiproct surface.

Ecological Notes on Florida Populations.

To date, *A. prognoides* nymphs have been found within Florida in five 4th to 5th order streams. They are located in Southeastern Plains Ecoregion (65) Southern Pine Plains and Hills (65f) subecoregion (Omernik 1987). These streams all drain from the same general area in the gravel soils north of Atmore, Alabama. The Perdido River and Brushy Creek drain south as part of the Perdido River Basin. Pine Barren Creek, Canoe Creek, and Big Escambia Creek all flow southeast as part of the Escambia River Basin. Land cover for all 5 watersheds is characterized as primarily pine silviculture and agriculture. Forests are located mainly along riparian corridors with gravel mining common in Big Escambia Creek and upper Perdido River basins. The average flow for Perdido River downstream at the Barrineau Park gaging station is 21.4 cms while Boggy Creek was estimated at 4.0 cms at the Walnut Hill gaging site (Musgrove et al. 1965). The average flow for Pine Barren Creek at the Highway 29 gaging station was estimated at 5.9 cms while Canoe Creek basin was estimated at 2.2 cms (Musgrove et al. 1965). Big Escambia Creek's flow averaged 8.1 cms during 2001-2009 upstream at Sardine Road in Alabama (USGS 2010) and was estimated 21 cms below Fanning Road during collections.

All the stream sample reaches containing *A. prognoides* had significant coverage of gravel bed habitat. The streambeds consist of quartz sand and gravel with some sandstone (sand cemented by iron oxides). These streams have a high rate of base flow that comes as seepage from the ground (Musgrove et al.1965). The sand and gravel aquifer that feeds these streams has exceptionally soft and unmineralized water (Musgrove et al. 1965). An exception is the northeast drainage of Big Escambia Creek, which flows over limestone of the harder, more mineralized Floridian aquifer that underlies the sand and gravel aquifer (Musgrove et al. 1965). The pH of the water generally ranges from 5 to 6 in the sand and gravel streams to 6 to 7 in the limestone influenced Big Escambia Creek tributaries to the northeast in Alabama (Kaufman 1975). The limestone provides the circumneutral pH and calcium carbonates required for mussel shell construction. Two native mussels, *Elliptio pullata* (I. Lea) and *Lampsilis straminea* (Conrad) collected on a gravel bar on 28 April 2010 were new records for Big Escambia Creek (Williams 2010). Annual surface water temperatures ranged from 7°C to 25°C in the 5 streams, and ground water temperatures in the sand gravel aquifer (depth of 6-76 m) from 19°C to 23°C (Musgrove et al. 1965). We have no data for the 0.5 m depth, but expect temperatures to be more modulated than surface temperatures.

The *A. prognoides* larvae were collected in fast riffles (0.5 m/sec), mainly created by large, old growth conifer logs. The gravel beds with *A. prognoides* had little sand and no silt filling the interstitial spaces. Fine sand and silt accumulation in the gravel will eliminate interstitial habitat and organic silt will consume dissolved oxygen (Bjornn & Reiser 1991, Chutter 1969, Coble 1961, Cordone & Kelley 1960, Hynes 1970, Scrivener & Brownlee 1981, 1989). The dissolved oxygen concentration was usually near 100% saturation at the gravel riffle sites. Most of the *A. prognoides* were found at the deepest depth achieved with the shovel (0.5 m). This may be dependent on life stage and/or water and habitat quality. While light trapping Big Escambia Creek on 28 April 2010 an adult male *A. prognoides* was caught in flight just before dusk on a gravel bar 2 m from the water. A search of the waterline found a late instar *A. prognoides* larva on woody debris. Other invertebrates found in the hyporheic gravel samples with *A. prognoides* were larval *Haploperla brevis* (Banks), a new Escambia County record, *Perlinella, Stenelmis, Hexatoma,* Chironomidae, and Oligochaeta-Lumbriculidae. Aquatic insects found on the surface gravel were, *Neoperla clymene* (Newman), *N. carlsoni* Stark & Baumann, *Perlesta, Pseudocloeon*, *Boyeria*, *Ophiogomphus australis* Carle, another new Escambia County record and *Dineutus*.

Early instar *Perlinella* were present in run-reach hyporheal samples that contained no *A. prognoides*. The run sites had slower velocities (0.3 m/sec) with a greater proportion of sand to gravel in addition to presence of silt. The dissolved oxygen was usually lower in the gravel run sites with sand and silt accumulation. Two Santa Rosa County gravel bed streams were sampled for *A. prognoides* without success. McCostill Mill Creek was light trapped for adults and gravel beds sampled. This 3rd order stream drains agricultural lands southwest of Jay and flows into the Escambia River. Big Juniper Creek, a 5th order stream above and below Red Rock Road in the Blackwater River State Forest northeast of Whiting Field Naval Air Station was light trapped and gravel beds sampled on 3 different dates. The Big Juniper Creek gravel beds had a high proportion of sand and clay silt. Water quality conditions on both these streams were similar to the Escambia County streams with *A. prognoides.*

Florida *A. prognoides* adults have been found from late April to mid June. Larvae have only been collected from late February to late April. Young larvae (3 mm) without developing wing pads were collected on 25 February. Late instar larvae with darkened wingpads were collected 22 April and emerged to adults the next day. The spring emergence suggests a univoltine life-cycle.

Environmental threats to *A. prognoides* populations include any anthropogenic input that changes the nature of the hyporheic zone. Agriculture, silviculture, mining, roads, and land development have caused sediment problems from storm water runoff and riparian zone deforestation. Chemical threats beside pesticides include fertilizers from municipal waste, row cropping, and livestock production. A nuclear power plant is proposed to be built in north Escambia County during 2012 in the vicinity of Bratt, near the center of *A. prognoides* Florida's range. North Escambia County has many species listed as rare and threatened for Florida including fish, reptiles/amphibians, and a mammal (Gilbert 1992; Moler 1992; Humphrey 1992). The rare seal salamander, *Desmognathus monticola* Dunn, a component of a disjunct fauna common to the Appalachians is only known from the Canoe Creek watershed (Moler 1992). The stonefly *Tallaperla cornelia* (Needham & Smith) discovered in Florida during 1979 after rearing larvae from Pine Barren Creek's Beech-Magnolia forest-covered 1st order tributaries were last collected at those sites in 1982 (Pescador et al. 2000). These tributaries were clear-cut and ditched for pine silviculture and row crops during the 1980's.

Previous monitoring of Brushy Creek below Atmore, Alabama, found water quality problems and silt deposits smothering the gravel streambed (Olsen et al. 1980; Payne 1999; Ray 2005). *Alloperla prognoides* were not found during benthic sampling and light trapping on 20 April 2009 after collecting larvae in 2008 and adults in 2007 at this site. New fine particulate organic material and sand sediment deposits were observed during the 2009 sampling with pools along the bank having a wastewater odor. The adjacent Perdido River had good water quality, but had experienced significant bank erosion from extensive land clearing in the basin.

Escambia County dirt road paving projects have helped reduce sediment runoff to Canoe Creek (Ray 2002a; Ray 2002b) and Pine Barren Creek (Ray 2002c). *A. prognoides* along with the dragonfly *Ophiogomphus australis* Carle, a new Escambia County record, were found at Canoe Creek Bratt Road on 24 April 2009 after road paving reduced the sand and silt runoff smothering the gravel bed at Bratt Road. Habitat smothering from anthropogenic storm water runoff sources is a problem in all the *A. prognoides* watersheds (Ray 1997; Ray 1998a; Ray 1999a; Ray 1999b; Ray 1999c; Ray 1999d; 1999e; Ray 1999f; Ray 2002d). Gravel mining in the Big Escambia Creek watershed has been serious source of sediment pollution (Ray 1998b).

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Ray, D.H. 1999b. Breastworks Branch above Breastworks Road Ecosummary. http://tlhdwf2.dep.state.fl.us/eswizard/esdata/pdfs/267.pdf. FDEP Tallahassee, Florida.

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