

DESCRIPTION OF THE PUPA OF “DIAMESINAE GENUS P” DOUGHMAN, 1985 (DIPTERA: CHIRONOMIDAE: DIAMESINAE), WITH COMMENTS ON HABITAT, DISTRIBUTION AND FURTHER CHARACTERIZATION OF THE LARVA

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Abstract

The pupal stage of “Diamesinae Genus P” Doughman, 1985 is described from specimens collected in northwest Georgia, USA. The pupa is recognized as Diamesinae by dorso-central thoracic setation with no prealar setae and lack of hooklets on tergite II. Also, the anal lobe has apical short tubercles and 3 hooked macrosetae with very distinctive apices. However, leg sheath arrangement differs slightly from described Diamesini with fore and mid leg sheaths directed laterally at the wing apex and frontal setae are lacking, but for now this is considered variation within the tribe. The strikingly unusual larva is recognized as Diamesinae by the annulate third antennal segment as well as characteristics of the premento-hypopharyngeal complex. The larva fits within Diamesini in the most recent keys of Holarctic genera. Currently known distribution, habitat and additional larval morphological details are noted.

Introduction

An unusual and rarely collected Diamesinae larva was characterized by Doughman (1985) in a key to Nearctic Diamesini as “Genus P” from “Southeastern USA.” This initial characterization included a single figure of the distinctive mentum, followed by records from sandy bottom Coastal Plain Province streams in Alabama, Georgia, Florida and North Carolina by Hudson et al. (1990) and Caldwell et al. (1997). For Florida and the southeastern USA, Epler (1992, 1995, 2001) and more recently Sæther and Andersen (2013) repeated these distribution records with further larval characterization.

During biomonitoring of a stream mitigation banking project (administered by the U.S. Army Corps of Engineers), larvae were discovered in February 2019 and December 2020 from multi-habitat samples collected in only one remote small, unnamed tributary stream, informally known for the project

as “Main Creek.” The site is a perennial second order stream in the Etowah River Basin, Dawson County, Georgia. Additional drift and aerial net sampling in February and March 2021 yielded pupal exuviae but no larvae, pupae, or adults. The drainage area at the sampling location is 2.85 km², with mean annual precipitation (1971-2000) of 1.76 m and range of 1.20 to 2.07 m. (Gotvald 2017). The stream is located in the Blue Ridge Physiographic Province and U.S. Environmental Protection Agency Blue Ridge Ecoregion, Southern Metasedimentary Mountains (66g), (Griffith et al. 2001b). The positive association of the pupa and larva was later confirmed in a Florida larva with some discernible developing key pharate pupal features.

Methods

Morphological terminology follows Sæther (1980) and Langton (1994a, 1994b, 2011). Counts and measurements were made following Soponis (1977) except left or right sides of pupal exuviae were utilized. Measurements are in units that are stated, and reported as the respective minimum, maximum and mean value when 3 or more specimens were measured. The number of specimens or structures measured or counted is given in parentheses (n). The letter L is sometimes used for larva(e) with Pex used for pupal exuviae (shed skins - all dissected). Project stream sampling followed Georgia Department of Natural Resources, Environmental Protection Division protocols (2007). Specimens were preserved in approximately 80-90% ethanol and directly mounted in CMC-10 or dehydrated in 95% ethanol and mounted in Canada balsam.

Results

“Diamesinae Genus P” Doughman, 1985

Material examined: USA: 1 L, Florida, Escambia County, Canoe Creek, 7-II-1966, leg. W. Beck; 1 L (w/ pupal characters), Perdido River, Barrineau Park Rd., 25-IV-2018, N30.6903°, W87.4404°, Sample #1987815, leg. N. Mulkey; 1 L, Calhoun County, Juniper Creek, Florida [Highway] 20, 4-XII-1969, leg. W. Beck; 1 L, Georgia, Dawson County, 8-II-2019, “Main Creek”, Site 205, leg. Corblu Ecology Group, LLC; 4 L, same data except, 21-XII-2020; 15L, 15 Pex, same data except 17-II-2021, leg. B. A. Caldwell; 1 Pex, same data except 9-III-2021; 1 L, Harris County, [trib.] Mulberry Creek, 11-II-1981, leg. M. W. Heyn; 1 L, same data, except 14-I-1984. Specimens are in collections of the Florida Department of Environmental Protection (FLDEP), M. W. Heyn or the senior author.

Pupa (Pex, n = 6); small, in comparison to larva, generally about 3.5 – 4.8, 4.0 mm total length, light yellowish color, rather stiff and very buoyant.

Cephalothorax. Frontal apotome with two small,

moderately rugose central areas, few facial creases, without frontal warts, cephalic tubercles, frontal setae or setal sockets. Wide area of prefrons covering labial sheaths projecting posteriorly, ending in a simple point. Thorax (Fig. 1a) mostly rugose anteriorly along middorsal suture with median and posterior areas less rugose with development as a sculptured/reticulate or wrinkled pattern. Anteprepronotum with two very thin median anteprepronotal setae; distance apart 36-52, 41 μm . Three thin precorneal setae present, with Pc_1 and Pc_2 being closest together and Pc_2 thickest of the three. Small socket-like structure close to Pc_1 and Pc_2 . Thoracic horn absent. Dorsocentral setae Dc_1 and Dc_2 rather close together with Dc_1 seta generally longest; distant Sa seta usually most easily discernible. No prealar setae. Fore and mid leg sheaths in close contact, strongly curving laterally near apex of wing sheath, barely exceeding lateral margin (Fig. 1b). Apex of hind leg sheath curved medially, overlying tip of mid leg sheath just past lateral margin of wing sheath. Wing sheaths mostly smooth except for small area of fine wrinkles at anterior base joining thorax.



Figure 1. (a) Pupal thorax, right, lateral view.

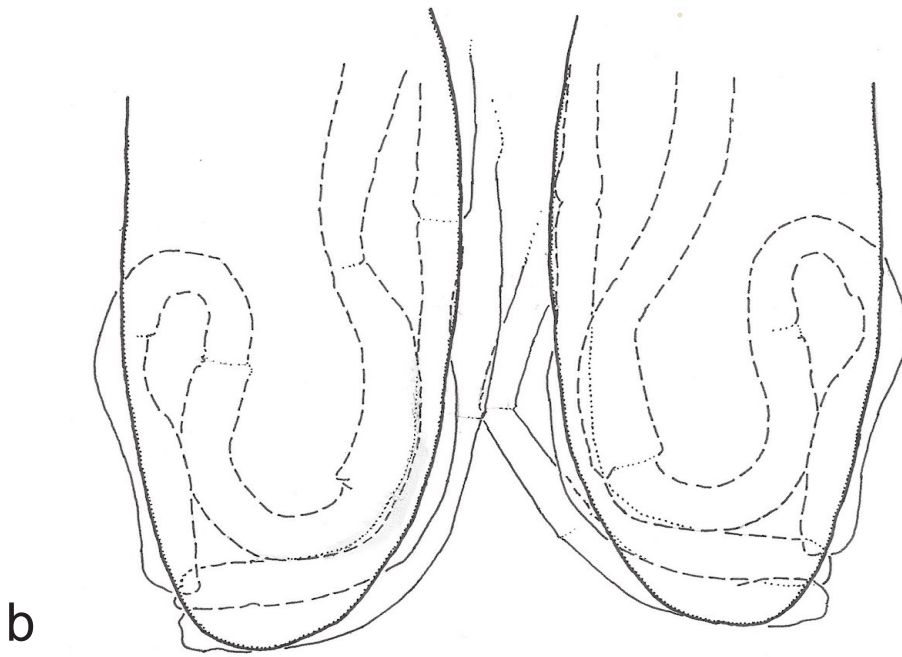


Figure 1 contd. (b) Pupal wing and leg sheaths.

Abdomen. Tergites I–VIII with fine shagreen, most sparse on TI and TVIII. Anterior shagreen points of other tergites merge into curved point rows. TVI and VII with median posterior area rounded and extended, with small, mostly rounded small points on TVI and short, sharp spines on TVII directed posteriorly. Sternite VIII with posterior band of anteriorly directed small spines in the male (Fig. 2a), band divided medially in the female (Fig. 2b). Faint lateral adhesion marks present on all eight abdominal segments. Tergite and sternite setae present, very thin and short but very difficult to discern or accurately count due to orientation or even possible loss. Number of O-setae discernable. All of the lateral setae on segments I–VII very thin, hair-like and short with 2 pair anterior and 2 pair posterior, each pair with one dorsal and one ventral seta. No lateral setae discernible on Segment VIII. Segment VIII with approximate third of posterior outer edge thickened and appearing at the corner as a projecting rough tubercle(s); in all specimens extending posteriorly with a very short knob-like shape. Male anal lobe (Fig. 3a) with posterior tip slightly darkened and extended as a short finger-like projection. Macrosetae (Fig. 3b) strongly hooked, curving medially, apically flared often with very, very slightly concave tips. Male genital sacs relatively straight, exceeding tip of anal lobe spur a short distance.

Larva (n = 10). Medium-sized larva (Fig. 4a), about 6–7, 6.25 mm long (n=4) with general body

coloration slightly yellowish and other features as noted.

Head. Golden-tan with several brownish markings and distinct black occipital margin, often appearing widest in later instars (flattened mounts). Early instar larvae often with at least one additional very small lateral mentum tooth. Mandible in some specimens appearing rounded but slightly angled in apical third. Premandible (Fig. 4b) with large apical tooth, 3 much smaller inner teeth and an elongate, thin, apically pointed lateral spine appearing attached between base and apex. Labroepipharyngeal complex with three “brushes” present but not strongly produced with the two groups of labral lamellae with several spinulae appearing very distinctive (Fig. 4b). Pecten epipharyngis appearing as three elongate pointed scales not readily separable from chaetae laterales (Fig. 4b).

Thorax and abdomen. Body with few scattered, thin, short setae present. Anterior parapods with numerous thin, gently curved claws as well as short strongly hooked, robust claws, some bifid and at least two very large, strongly hooked claws with as many as 3–4 inner teeth. Some very small, slightly curved, mostly simple claws also present basally. Each posterior parapod with a strong sub-basal seta; claws robust, with at least two strongly hooked, others strongly curved. Procercus short, with robust basal/lateral seta and six rather short anal setae; four apically rounded anal tubules, about half as long as posterior proleg.

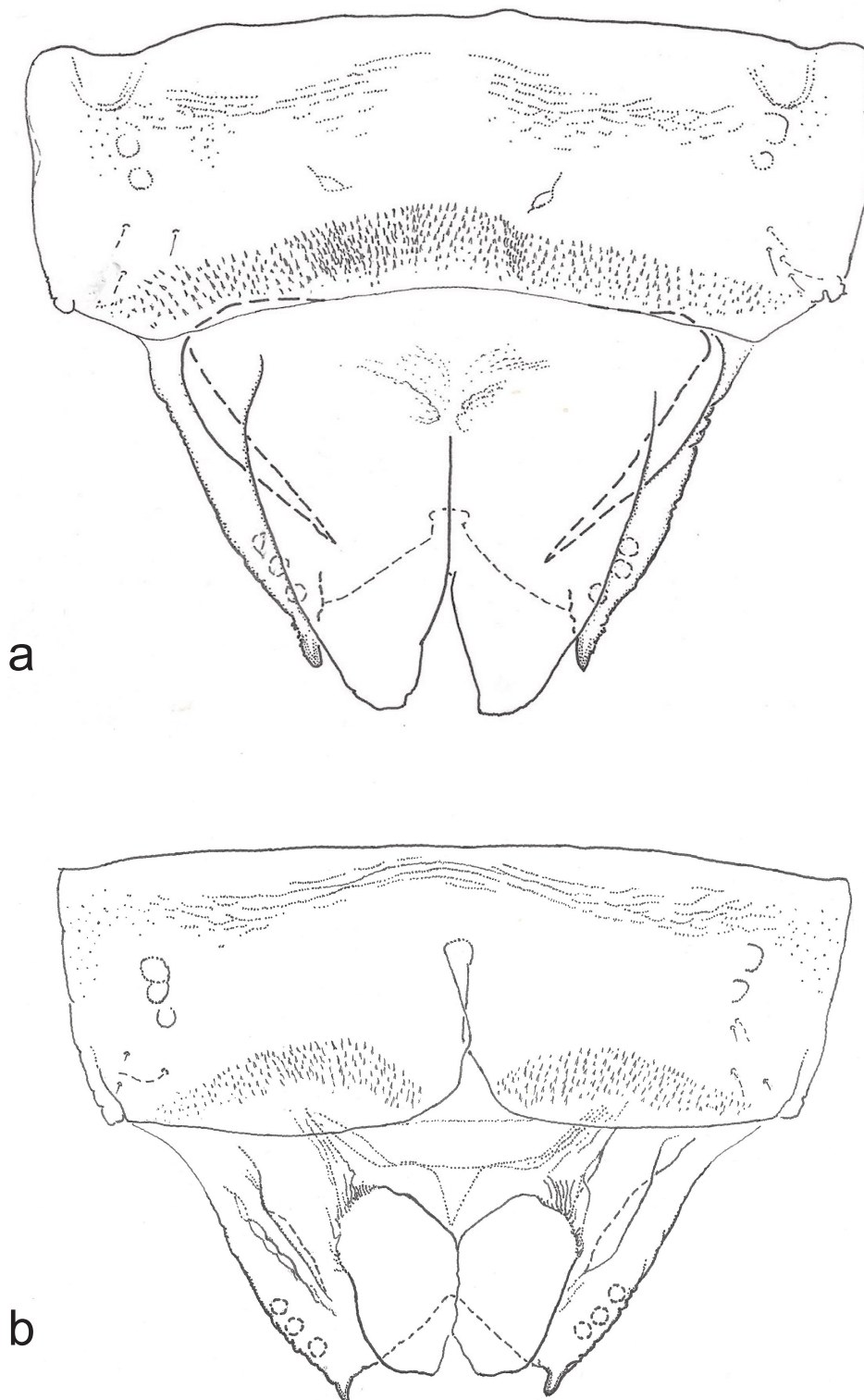


Figure 2. Pupal sternite VIII and anal lobe, macrosetae omitted (a) male; (b) female.

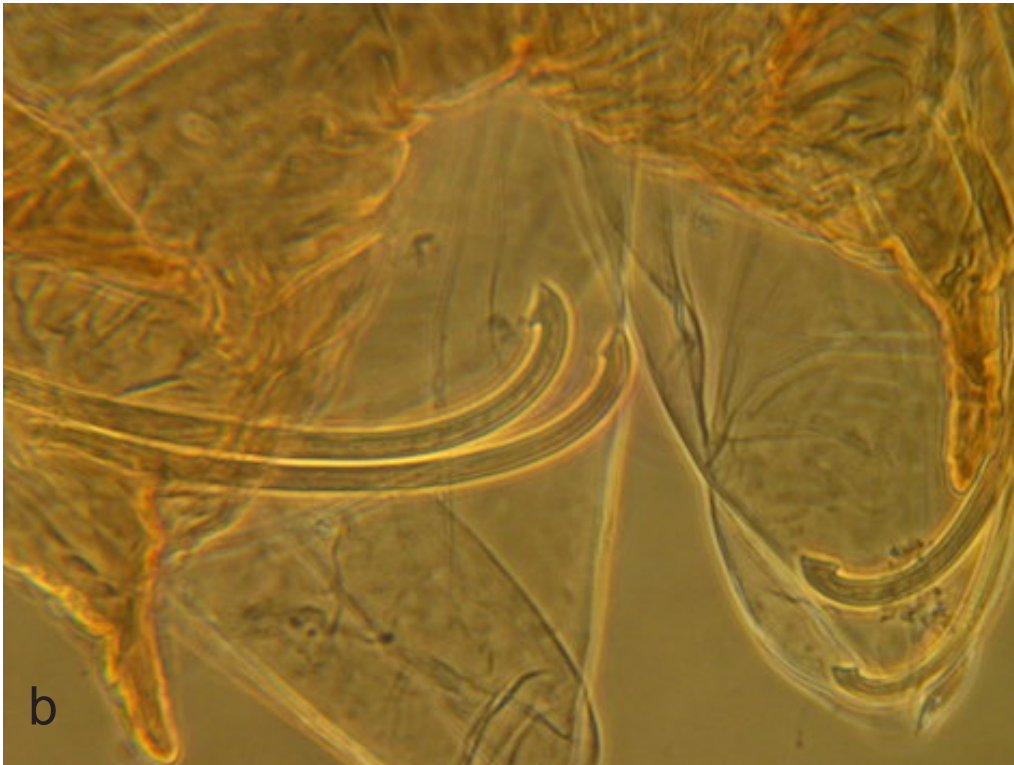
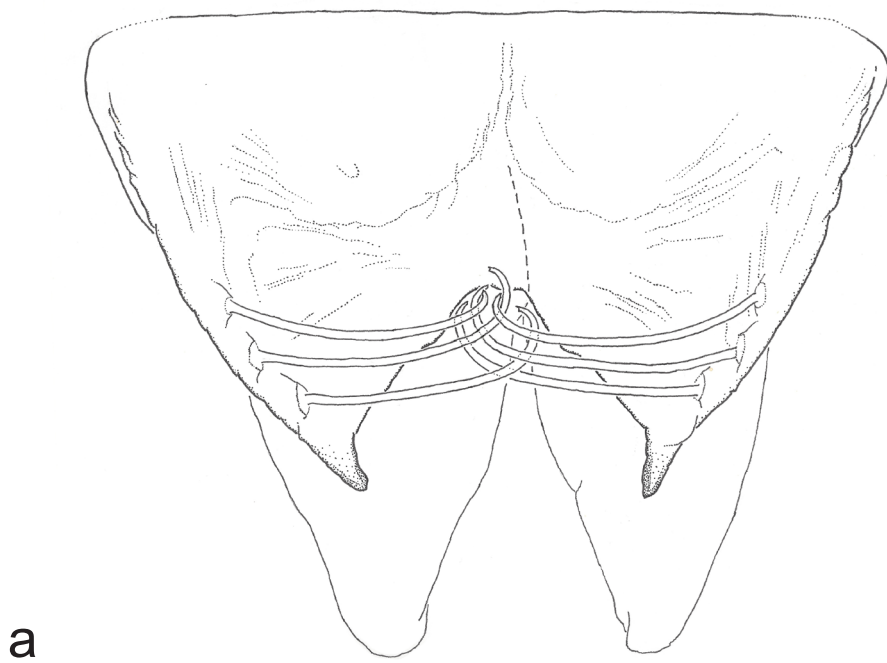


Figure 3. (a) Male pupal anal lobe, dorsal; (b) detail of macrosetae, note apices.

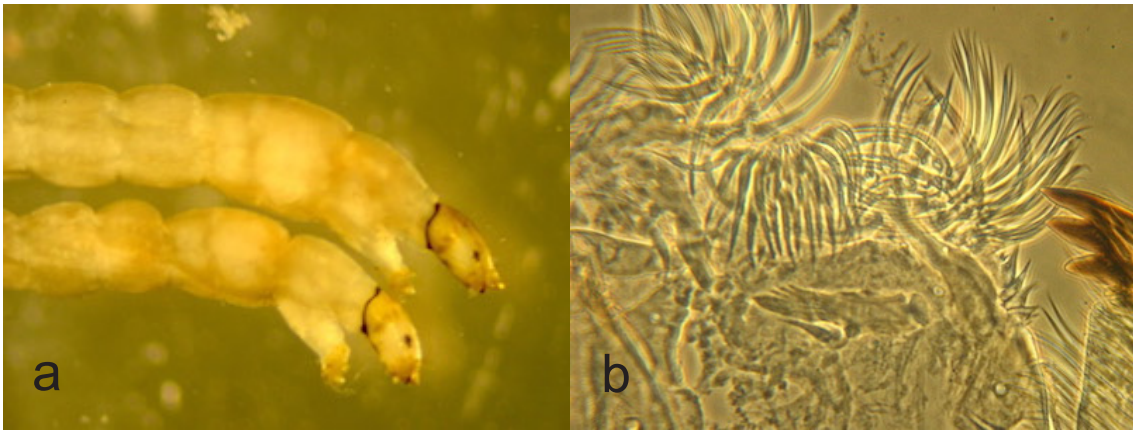


Figure 4. (a) Immature larvae, lateral head and thoracic area; (b) larval labro- epipharyngeal and palatum region, note premandible with sharp lateral spine near lower center.

Discussion

Important morphological features of the “Diamesinae Genus P” pupa include typical thoracic setation without any prealar setae, fine but sparse shagreen on tergites I and VIII, leg sheath arrangement, lack of hook row on tergite II, an anal lobe with macrosetae exhibiting rather uniquely expanded apices, sexual dimorphism in posterior spine row on sternite VIII and lack of thoracic horn. Although the hind leg sheath contacts the mid leg sheath, and fore and mid leg sheaths are oriented laterally at the wing apex, the arrangement is most similar to other Diamesini. However, frontal setae are lacking. Specimens key to couplet 5, Diamesini in Oliver (1986), with Holarctic Boreoheptagyini (*Boreoheptagyia* Brundin) excluded. The larval ventromental plates show similarity to *Pagastia* Oliver and *Potthastia gaedi* group Meigen (Sæther and Andersen 2013) by entirely covering the mentum. The larval labral lamellae are somewhat similar to the long but simple ones of *Xenochironomus* Kieffer but are not on lobes.

“Diamesinae Genus P” larvae and pupae have unique morphological character states (or autapomorphies) within the Diamesini. One pupal character, no frontal setae, is shared with three other Diamesinae tribes - Harrisonini, Lobodiamesini and Heptagyini. Recognition of a possible new genus and species awaits discovery of the adult male/female hopefully with molecular data. See Lin et al. (2022) on use of mitogenomes. Cranston and Krosch (2015) describe a similar situation concerning a formal description of the informal Podonominae taxon, Genus “Chile” Brundin, 1966 as *Podonomopsis* (*Araucanopsis*) *avelasse*, which was inadequately known as a Pex for over half a century.

The “Main Creek” sampling site can be characterized as forested with partly open canopy, including shrubs, grasses and herbaceous plants. Typical sampling reach morphology included about 65% riffles, 30% runs and 5% pools. Bottom substrate was generally a mix of boulders, cobble, gravel and sand, with sand constituting about 15% of the various components. No bedrock, silt or clay was noted. Thus, “Main Creek” is not a totally “sandy bottom” or even sand dominated stream as is found in the southeastern Coastal Plain Province, an area well known for the occurrence of “Diamesinae Genus P”.

The preferred microhabitat of “Diamesinae Genus P” larvae appears to be in smaller streams to moderately wide rivers within relatively stable, sandy substrate. The Pex but no additional L from “Main Creek” were collected by drift net, especially downstream of short, relatively straight sandy stream reaches. Beck and Beck (1974) were unable to rear larvae, even to the initial pupal stage and lack of adults in recent Georgia sampling requires determining adult phenology and terrestrial preferences. Other sand dwelling and sand case making chironomid taxa collected in Main Creek include *Neostempellina reissi* Caldwell, *Stempellinella leptocelloides* (Webb) and *S. boltoni* Ekrem.

Collection data for Florida (M. W. Heyn, personal communication) documents “Diamesinae Genus P” larvae present in all months except June, July and October with one pre-pupal larva collected in late April. Beck and Beck (1974) and Beck (1977) incorrectly regarded Florida larvae as *Sympotthastia* Pagast, and being present only in winter months. Research of records in other southeastern states reveals few larvae mostly in late winter through

early spring, which is typical for other Diamesinae life stages. See Table 1 for historical and updated distribution records. All Florida Department of Environmental Protection (FLDEP) historical and recent records are confined to the western panhandle area of the state (M. W. Heyn, personal communication, including specimens labeled as *Sympotthastia* (sensu Beck and Beck 1974).

Much research of historic and more current distribution records for other southeastern states is presented in Table 1. Information was gathered from the states and included incorporation of United States Environmental Protection Agency (USEPA) Level IV ecoregions and subregions (Griffith et al. 2001a, 2001b, 2002). The USEPA maps are very small scale and in certain instances, especially Georgia, a couple of collection sites are “borderline” and did not allow for more precise placement of these localities for now.

New distribution records include South Carolina presence of “Diamesinae Genus P” formally published for the first time. A new record other than Coastal Plain was found for Alabama, jointly shared with Florida (Perdido River). “Genus P” was found in a taxa list for Mississippi but apparently no specimens were retained (M. Chimahusky and A. Dossett, personal communication). This is the first formally published report of this taxon presence in Mississippi. No records were found for Tennessee although the standard chironomid larval midge key used does not include this taxon (D. Arnwine, personal communication). No other records are known for Tennessee from an additional source (W. Pennington, personal communication). The Kentucky “Master Macroinvertebrate Species List” (2015) does not include this Diamesinae taxon and knowledgeable sources in and outside of the state also knew of no records.

Table 1. Historical and updated distribution records of “Diamesinae Genus P” Doughman, 1985 for eight southeastern states including Physiographic Province and United State Environmental Agency (USEPA) ecoregions and subregions.

State	Physiographic province	USEPA ecoregion	USEPA subregion
Alabama	Coastal Plain	No other data	No other data
	Coastal Plain	Southeastern Plains (65)	Southern Pine Plains and Hills (65f)
Florida	Coastal Plain	Southeastern Plains (65)	Southern Pine Plains and Hills (65f)
		Southern Coastal Plain (75)	Gulf Coast Flatwoods (75a)
Georgia*	Coastal Plain	No other data	No other data
	Piedmont	Piedmont (45)	Pine Mountain Ridges (45h)
	Blue Ridge	Blue Ridge (66)	S Medisedimentary Mountains (66g)
Mississippi	Coastal Plain	In taxa list only	No other data
North Carolina	Coastal Plain	Southeastern Plains (65)	Sand Hills (65c)
	Piedmont	Piedmont (45)	Carolina Slate Belt (45c) N. Inner Piedmont (45e)
	Blue Ridge	Blue Ridge (66)	S Crystalline Ridges and Mountains (66d)
South Carolina	Coastal Plain	Middle Atlantic Coastal Plain (63)	Carolina Flatwoods (63h)
		S. E. Plains (65)	Sand Hills (65c)
	Piedmont	Piedmont (45)	Carolina Slate Belt (45c)
	Blue Ridge	Blue Ridge (66)	S Crystalline Ridges and Mountains (66d)
Kentucky	No records in state database. No other records known.		
Tennessee	No records in state database. “Diamesinae Genus P” not included in currently used taxonomic keys. No other records known.		

*NOTE: The Georgia record for subregion (45h) is very near Southern Outer Piedmont (45b) and a record for subregion (66g) is very near Southern Inner Piedmont (45a). Larger scale Georgia Department of Transportation County maps were used in conjunction with a very small-scale color-coded USEPA ecoregion/subregion map of Georgia to assist in determining subregion placements.

Acknowledgements

The authors especially thank Rick Whiteside and Matt Otto of Corblu Ecology Group, LLC, Woodstock, and Lawrenceville, Georgia respectively, for arranging additional sampling and site data for “Main Creek”. We thank Michael W. Heyn and Todd Risk, Florida Department of Environmental Protection, for providing additional state larval collection data and specimens. We also thank Lisa Huff, Alabama Department of Environmental Management, Steve Beaty, North Carolina Department of Environmental and Natural Resources, Division of Water Quality, David Eargle, South Carolina Department of Environmental Services, Bureau of Water, Debbie Arnwine, Tennessee Department of Environment & Conservation, Division of Water Resources, Melody Chimahusky and Alice Dossett, Mississippi Department of Environmental Quality and Cody Jones, Georgia Department of Natural Resources, Environmental Protection Division for collection records for their respective states. Thanks are also due to Wendell Pennington, Pennington and Associates, for input on other possible Tennessee records. Morris Flexner, USEPA, Athens, GA, Greg Pond, USEPA, Wheeling, WV, Robert Johnson and Logan Phelps, Kentucky Energy and Environmental Cabinet, Division of Water are thanked for their help with possible Kentucky records. Much appreciation is expressed to J. H. Epler, Crawfordville, Florida and Eugenyi A. Makarchenko, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of Russian Academy of Sciences, Vladivostok, Russia as well as two anonymous reviewers for reviews of earlier drafts of the manuscript with very useful comments and suggestions for improvement.

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Article submitted 01. July 2024, accepted by Alyssa M. Anderson 22. November 2024, published 03. December 2024.