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Perlesta armitagei n. sp. (Plecoptera: Perlidae): More cryptic diversity in darkly pigmented *Perlesta* from the eastern Nearctic

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Abstract

Perlesta Banks, 1906 (Plecoptera: Perlidae) is a genus of small, summer-emergent stoneflies known primarily from the eastern Nearctic. Thirty-two species are currently recognized, including two from China and nymphs have been reported from Costa Rica. We report here on some cryptic diversity within a small group of *Perlesta* with dark wings and bodies. *Perlesta armitagei* **sp. nov.** is described from the adult male, adult female, and egg. Diagnostic characters are presented with light microscope and scanning electron microscope (SEM) photomicrographs. *Perlesta armitagei* **sp. nov.** most closely resembles *P. browni* Stark, 1989 and *P. cinctipes* (Banks, 1905), two species distributed mainly within the Interior Highland Region. *Perlesta armitagei* **sp. nov.** is known currently within the Ohio River drainage from Indiana eastward to western Pennsylvania and southward into central Kentucky. Comparative light microscope and SEM images are also provided for *P. adena* Stark, 1989, *P. browni*, *P. cinctipes*, and *P. xube* Stark and Rhodes, 1997 in an effort to better define the morphological concepts of these dark colored species.

Key words: Plecoptera, Perlidae, *Perlesta*, new species, Nearctic

Introduction

Stark (1989) used a holomorphological approach to revise the stonefly genus *Perlesta* Banks, 1906 (Plecoptera, Perlidae), defining 12 species using a combination of internal (*e.g.* male aedeagus) and external adult genitalic characteristics (*e.g.* male paraprocts, female subgenital plate) plus structural features of mature eggs. Stark's revision sparked a modest revolution in the description of new species and refinement of morphology-based species concepts (DeWalt *et al.* 1998, Grubbs 2005, 2012, 2018, Grubbs & DeWalt 2011, 2012, Kirchner & Kondratieff 1997, Kondratieff & Baumann 1999, Kondratieff & Kirchner 2002, 2003, Kondratieff *et al.* 2006, 2008, 2011, Poulton & Stewart 1991, Stark 2004, 2007, Stark & Rhodes 1997). Currently, 30 valid species are recognized across the central and eastern Nearctic (DeWalt *et al.* 2018), two species are known from China (Murányi & Li 2016), and nymphs have been reported from Costa Rica (Gutiérrez-Fonseca & Springer 2011).

As new taxa are described, however, the delineation between species using morphological characteristics is becoming increasingly narrower. The main difficulty of *Perlesta* taxonomy lies in that several life stages are typically required, and minimally in near perfect condition, to confidently identify existing species or to describe as new. Two examples make this point clear. First, the paraprocts of *P. cinctipes* (Banks, 1905) are superficially similar to *P. browni* Stark, 1989 but these sympatric species can be readily separated using head and femur pigmentation patterns, egg ultrastructure, subgenital plate differences, and aedeagal characteristics (Stark 2004). Second, *P. decipiens* (Walsh, 1862), *P. ephelida* Grubbs & DeWalt, 2012, and *P. mihucorum* Kondratieff & Myers, 2011 are easily differentiated using aedeagal, paraproct, and subgenital characteristics yet the eggs are inseparable.

Stark (2004) reported *P. cinctipes* from two locations in southern Ohio. This represented a large eastern range extension from a single locality reported from western Illinois (DeWalt *et al.* 2001). Up to that point, *P. cinctipes* was known from Iowa and Kansas south to Arkansas, Missouri, and Oklahoma (Stark 1989, Poulton & Stewart

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1991). This raised the question of the identity of the Ohio *P. cinctipes* specimens. Although *P. cinctipes* has since also been reported from Kentucky (Tarter *et al.* 2006) and West Virginia (Tarter & Nelson 2006), the lack of recent or historical populations from Indiana (Grubbs 2004, DeWalt & Grubbs 2011) and most of Illinois (DeWalt & Grubbs 2011; see one exception above) has further raised doubts about the identity of eastern specimens determined as *P. cinctipes*.

Collecting by the first author at a location reported in Stark (2004; Ohio, Ross Co., Deer Creek) produced material that superficially resembled adult males of *P. cinctipes* yet associated females had mature eggs that were not of this species. The associated females were likewise distinct from *P. cinctipes*. Several other specimens of this apparently cryptic species resulted from examination of Ohio Biological Survey specimens that were included in DeWalt *et al.* (2012, 2016) under the temporary name *Perlesta* I-4, plus additional collected material from Indiana, Kentucky, Ohio, and Pennsylvania. The main intent of this study was to use a holomorphological approach to resolve the identity of these specimens of "*P. cinctipes*" and "*P.* I-4". During the course of this study, however, it became apparent that a broader comparative approach was required to refine the morphological concept of *P. cinctipes* due to superficial resemblances with other species sharing similar characteristics (*e.g. P. browni*).

Material and methods

Specimens examined in this study were obtained from the Monte L. Bean Museum, Brigham Young University, Provo, Utah (BYU), Canadian National Collection of Insects, Ottawa (CNC), C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins (CSUIC), Donald C. Tarter collection, Marshall University, Huntington, West Virginia (DCTC), Illinois Natural History Survey, Champaign (INHS), Ohio Biological Survey Collection, Columbus (OBS—all now housed permanently at INHS), and Western Kentucky University, Bowling Green (WKUC). Other codens used in this paper were Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts (MCZ), United States National Museum, Washington, D.C. (USNM), and West Virginia University Collection, Morgantown (WVUC).

Fresh adult *Perlesta* were collected mainly with a beating sheet to dislodge individuals from living vegetation and light trapping. Location data (in decimal degrees) for each specimen record were recorded directly with either a portable GPS unit or georeferenced from label data using Acme Mapper (http://mapper.acme.com). All specimen data are available as a comma separate values file prepared in Darwin Core Archive file format. Specimens for SEM analyses were serially dehydrated through a series of 75%, 95%, and 100% ethanol for 10 minutes each and placed in hexamethyldisilizane for 30 minutes. Dehydrated specimens were attached to aluminum stubs with double-stick tape and coated with gold-palladium using an Emscope SC500. Coated specimens were examined using a Jeol JSM-6510LV scanning electron microscope and digital images were captured with an IXRF system. Digital images were also obtained using Auto-Montage software with a Leica MZ16 stereomicroscope equipped with a JVC KY-F75U digital camera.

Results and discussion

Perlesta adena Stark, 1989

Adena Stone
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:1240
(Figs. 1 & 11)

Perlesta adena Stark 1989:277. Holotype ♂ (USNM), Stillwater River, Miami Co., Ohio.
Perlesta adena: Stark 2004:90

Distribution. USA: IN, KY, OH, TN (DeWalt *et al.* 2018)

Remarks. Males and females of *P. adena* have a dark head mask (Figs. 1a–1f) that is superficially similar to *P. xube* (Figs. 2a–2e), but easily distinguished from sympatric populations of *P. armitagei* **sp. nov.** from Indiana, Kentucky, and Ohio (Figs. 6a–6f). Published line drawings of the aedeagi of *P. adena* (Stark 1989, his Fig. 76, Stark 2004, his Figs. 273–274) and *P. xube* (Stark and Rhodes 1997, their Fig. 6, Stark 2004, his Figs. 370–371) make it easy to differentiate between males of these two species.

Females of *P. adena* and *P. xube* are difficult to separate due to subtle characteristics of their subgenital plates (Stark 2004). Eggs are likewise superficially too similar in appearance even with SEM to differentiate to species (Stark 2004, his Fig. 7.400 for *P. adena* and Fig. 7.404 for *P. xube*).

Perlesta adena and *P. xube* overlap in distribution mainly in Ohio (Fig. 11a). *Perlesta adena* is distributed in the lower Ohio River, Cumberland River, and Tennessee River basins plus a few Lake Erie drainages in northern Ohio (Fig. 11a).

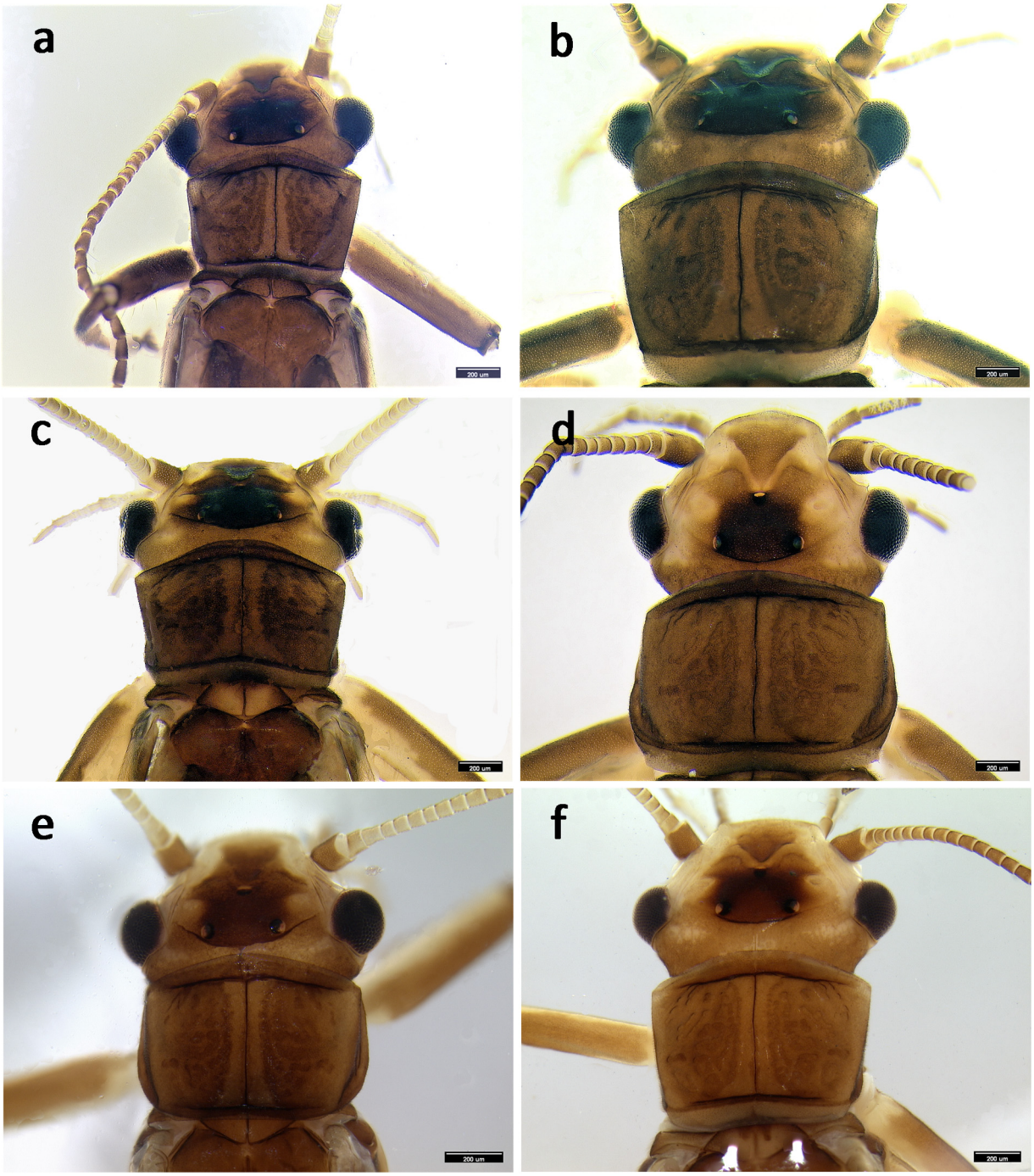

FIGURE 1. *Perlesta adena*, a–b, Indiana, West Fork Tanners Creek; c–d, Kentucky, Little Trammel Creek; e–f, Tennessee, Rocky Creek. a, c, e, male, head + pronotum, dorsal profile; b, d, f, female, head + pronotum, dorsal profile.

***Perlesta xube* Stark & Rhodes 1997**
Pawnee Stone
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:1241
(Figs. 2 & 11)

Perlesta xube Stark & Rhodes 1997:92. Holotype ♂ (USNM), Dry Creek, Merriman, Cherry Co., Nebraska.
Perlesta xube: Stark 2004:95

Distribution. USA: IA, IL, ND, NE, OH (DeWalt *et al.* 2018), IN (new state record)

Remarks. The head masks of males and females of *P. xube* (Figs. 2a–2e) are readily distinguished from *P. armitagei* **sp. nov.** (Figs. 6a–6h) and *P. cinctipes* (Figs. 5a–5d). The specimens (all males) determined as *P. cinctipes* in Stark (2004) were not of *P. armitagei* **sp. nov.** but of *P. xube*. Males of *P. xube* and *P. cinctipes* key to couplet 6 in Stark (2004). This couplet focuses on whether the dorsal patch is "wider at apex than at base" (*P. xube*, Stark 2004, his Fig. 7.370–7.371) or "narrow throughout most of length" (*P. cinctipes*, Stark 2004, his Fig. 7.300). *Perlesta cinctipes* is one of few *Perlesta* species where the fully-extruded male aedeagus has yet to be illustrated with a line drawing or SEM. The length of the sac is not fully understood, whether in relation to the length of the tube or length and shape of the caecum. An additional aedeagal characteristic is useful in differentiating between these two species. The dorsal patch of *P. xube* is expanded at the base and body of the caecum but not apically (Stark & Rhodes 1997, their Fig. 6; Stark 2004, his Figs. 7.370–7.371). The dorsal patch of *P. cinctipes*, however, expands well onto the caecum (Stark 2004, his Fig. 7.299).

Females of *P. xube* can be easily differentiated from *P. cinctipes* using Stark (2004) and from *P. armitagei* **sp. nov.** here with subgenital plate characteristics. Eggs of *P. xube* (Stark & Rhodes 1997, their Fig. 8, Stark 2004, his Fig. 7.404) are also distinct from both *P. cinctipes* (Stark 1989, his Fig. 17, Stark 2004, his Fig. 7.394–7.395) and *P. armitagei* **sp. nov.** (Figs. 10a–10d).

Perlesta xube overlaps in distribution with *P. armitagei* **sp. nov.** from southern Indiana east to Ohio and with *P. cinctipes* to date only in southern Iowa (Figs. 11a–11b).

***Perlesta browni* Stark, 1989**
Toothed Stone
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:1237
(Figs. 3–4 & 11)

Perlesta browni Stark 1989:279. Holotype ♂ (USNM), Rock Creek, Latimer Co., Oklahoma.
Perlesta browni: Poulton & Stewart 1991:39
Perlesta browni: Stark 2004:91
Perlesta browni: Kondratieff *et al.* 2011:300

Distribution. USA: AR, MO, OK, VA (DeWalt *et al.* 2018)

Remarks. Adults of *P. browni* display golden-brown background color with darker pigmentation on the head, thorax (Figs. 3a–3d), and abdomen. Whereas the abdominal terga for females are nearly uniform in color throughout their length, by comparison male terga 6–10 are markedly darker than the anterior five segments. The posterolateral corners of the male 9th sternum are clothed by a dense matting of long hairs, noticeably longer and denser than the anterolateral and medial portions of the segment (Fig. 4d). Males have long, slender, tubular paraprocts with a mesoapical tooth (Figs. 4a–4c) that are similar to sympatric *P. cinctipes* (Figs. 5e–5f) but longer than those of *P. armitagei* **sp. nov.** (Figs. 7a, 7c). In addition, Poulton & Stewart (1991) showed that *P. browni* femora are pigmented only in the distal third, the brown color extending to the articulation with the tibia (their Fig. 344). Conversely, *P. cinctipes* femora are pigmented in approximately the middle 40%, the articulations with the coxae and tibiae are unpigmented (Poulton & Stewart 1991, their Fig. 342).

Females can be separated by characteristics of their subgenital plate lobes and median notch (Stark 2004). The eggs of *P. browni* are easily differentiated from *P. cinctipes*. The egg of *P. browni* has a short, slightly distally-flanged ribbed collar and the chorionic surface is covered by fine punctations except near the eclosion line (Figs. 4e–4f; Stark 1989, his Fig. 15). The egg of *P. cinctipes* has a similar collar but the chorionic surface is coarsely pitted except for the smooth eclosion line region (Stark 1989, his Fig. 17; Stark 2004, his Figs. 7.394–7.396).

Perlesta browni was considered endemic to the Interior Highland region (Fig. 11a; Poulton & Stewart 1991, Stark 2004) until specimens seemingly indistinguishable from this species were reported from a single locality in eastern Virginia (Kondratieff *et al.* 2011). *Perlesta browni* is sympatric with *P. cinctipes* mainly in the Ouachita Mountain region of western Arkansas and eastern Oklahoma (Fig. 11a).

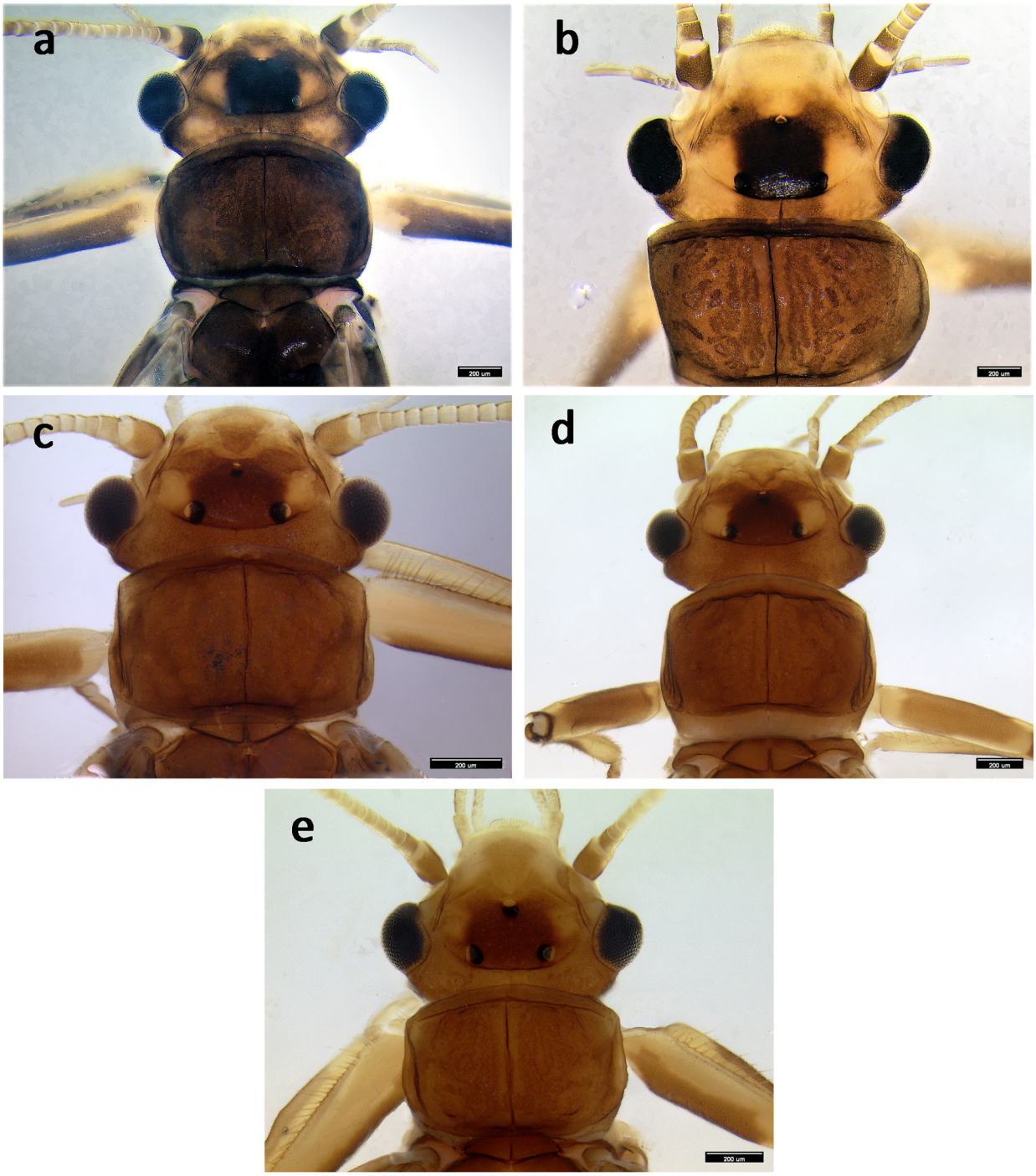

FIGURE 2. *Perlesta xube*, a–b, Illinois, Little Saline River; c–d, Nebraska, Cherry Creek (paratypes); 11, Ohio, Pee Pee Creek. a, c, e, male, head + pronotum, dorsal profile; b, d, female, head + pronotum, dorsal profile.

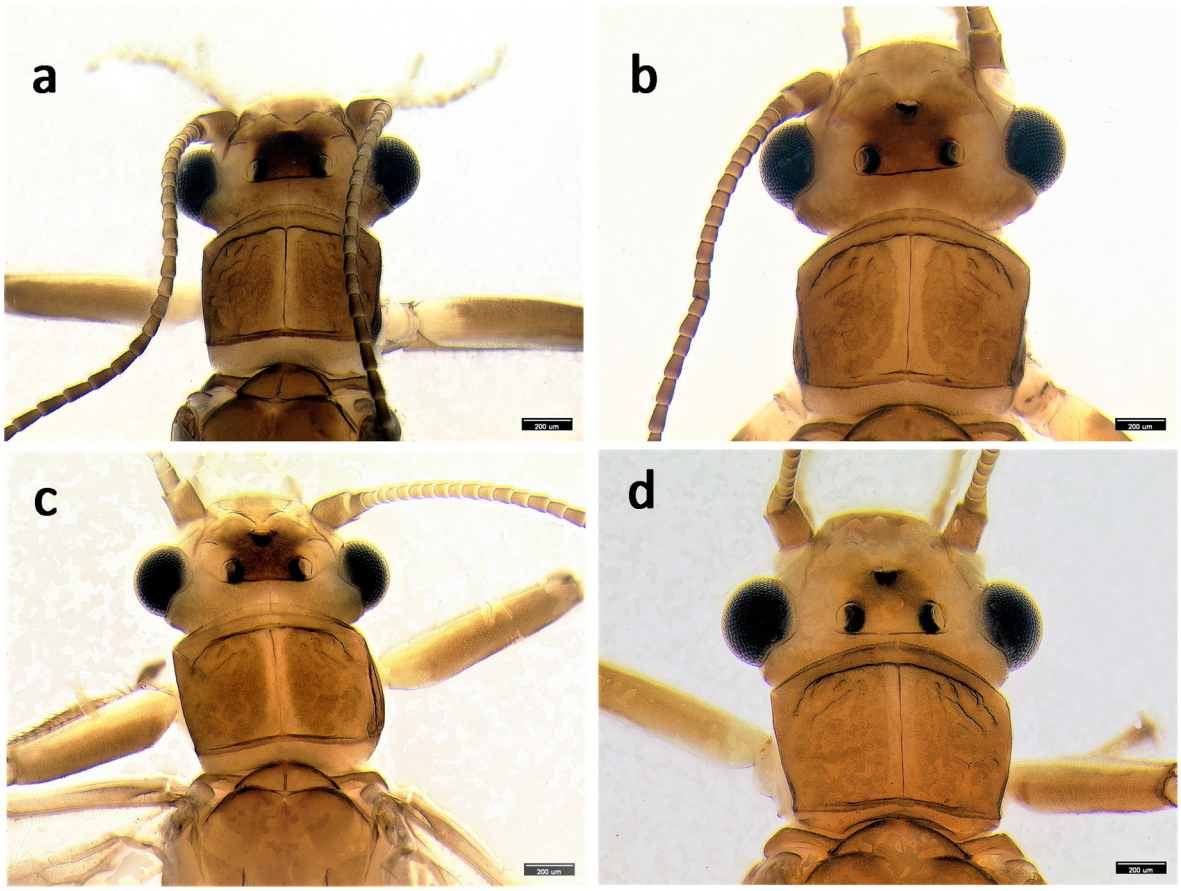

FIGURE 3. *Perlesta browni*. a–b, Arkansas, Fourche La Fave River; c–d, Arkansas, Ouachita River; a, c, male, head + pronotum, dorsal profile; b, d, female, head + pronotum, dorsal profile.

***Perlesta cinctipes* (Banks, 1905)**
Plains Stone
http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:1211
(Figs. 5 & 11)

Perlinella cinctipes Banks 1905:56. Lectotype ♂ (MCZC), Onaga, (Pottawatomie Co.), Kansas.
Perlesta cinctipes: Stark 1989:270
Perlesta cinctipes: Poulton & Stewart 1991:39
Perlesta cinctipes: Stark 2004:91

Distribution. USA: AR, IA, IL, KS, MO, NE, OK (DeWalt *et al.* 2018)

Remarks. *Perlesta cinctipes* is distributed from Nebraska south into the Interior Highlands region of Arkansas, Missouri and Oklahoma and east only to western Illinois (Fig. 11a; DeWalt *et al.* 2018). All previously published records from Indiana (DeWalt & Grubbs 2011), Kentucky (Tarter *et al.* 2006, 2015), Ohio (Stark 2004, Grubbs *et al.* 2013, DeWalt *et al.* 2016), and West Virginia (Tarter & Nelson 2006), either as *P. cinctipes* or *P.* I-4, now refer to *P. armitagei* **sp. nov.** (see below). The record of *P. cinctipes* from Illinois as noted above represents the eastern known extent of this species range (DeWalt *et al.* 2001).

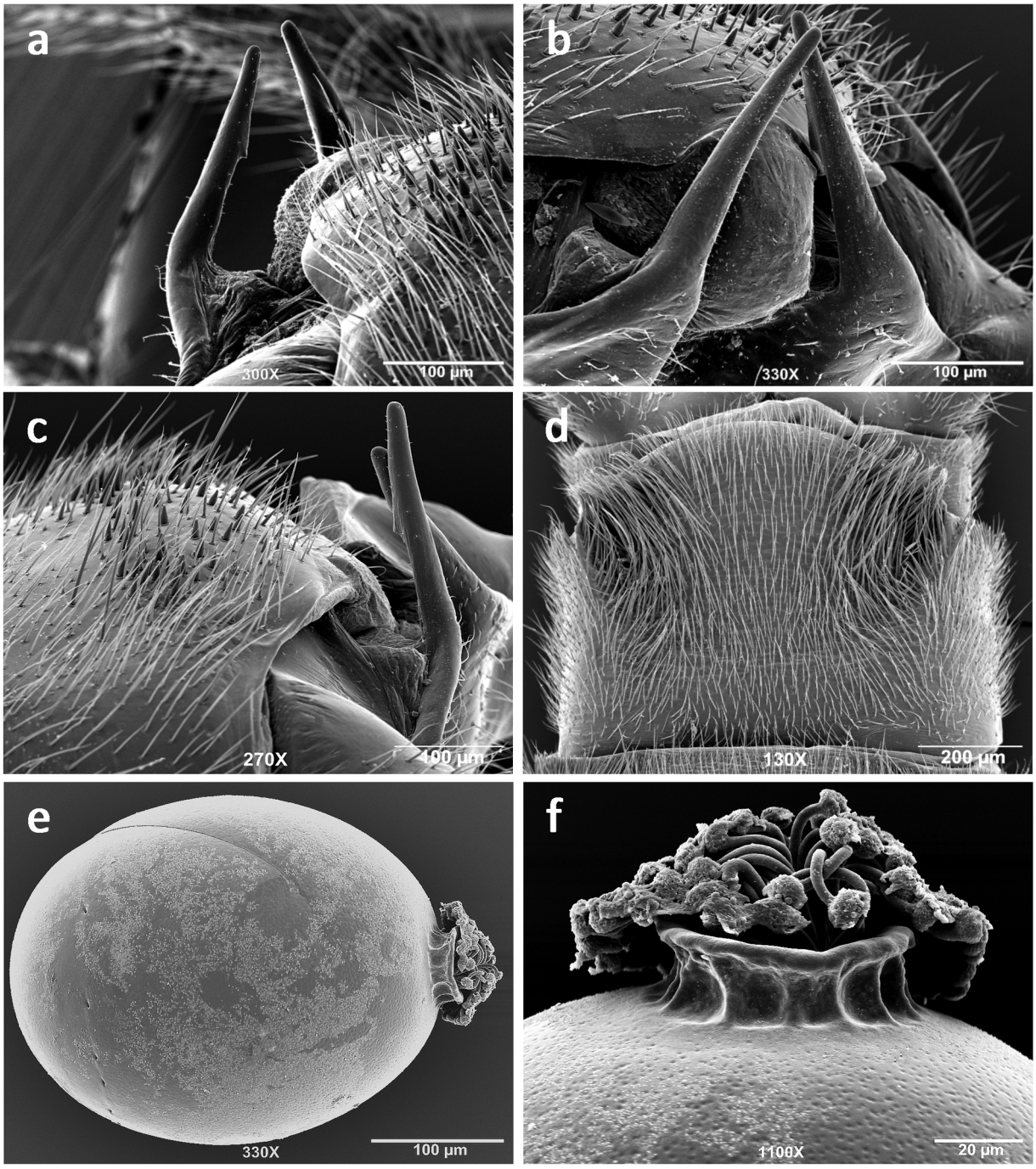

FIGURE 4. *Perlesta browni*, Arkansas, Fourche La Fave River. a, male, paraprocts, lateral view, 300X; b, male, paraprocts, caudal (left) and mostly lateral (right) views, 330X; c, male, right paraproct, lateral view 270X; d, male, 9th sternum, 130X; e, egg, 330X; f, egg collar, anterior pole, 1100X.

***Perlesta armitagei* sp. nov.**

http://lsid.speciesfile.org/urn:lsid:Plecoptera.speciesfile.org:TaxonName:502858

(Figs. 6–11)

Description. Male forewing length 8.0–10.0 mm (n = 29). Head pale yellow with a dark brown subquadrate ocellar patch and a small, light brown triangular patch anterior to the median ocellus, usually separated by a pale M-line;

epicranial suture extends laterally, slightly beyond lateral ocelli (Figs. 6a, 6c, 6e, 6g). Pronotum brown with a faint yellow medial stripe (Figs. 6a, 6c, 6e, 6g). Female forewing length 10.0–11.5 mm (n = 25). Color pattern similar to male but typically lighter and with less ocellar and epicranial suture pigmentation (Figs. 6b, 6d, 6f, 6h). Wing membrane and veins amber except for pale white costal region. Femora brown dorsally extending to articulation with tibiae (Figs. 6a–6h), dorsum of tibiae brown.

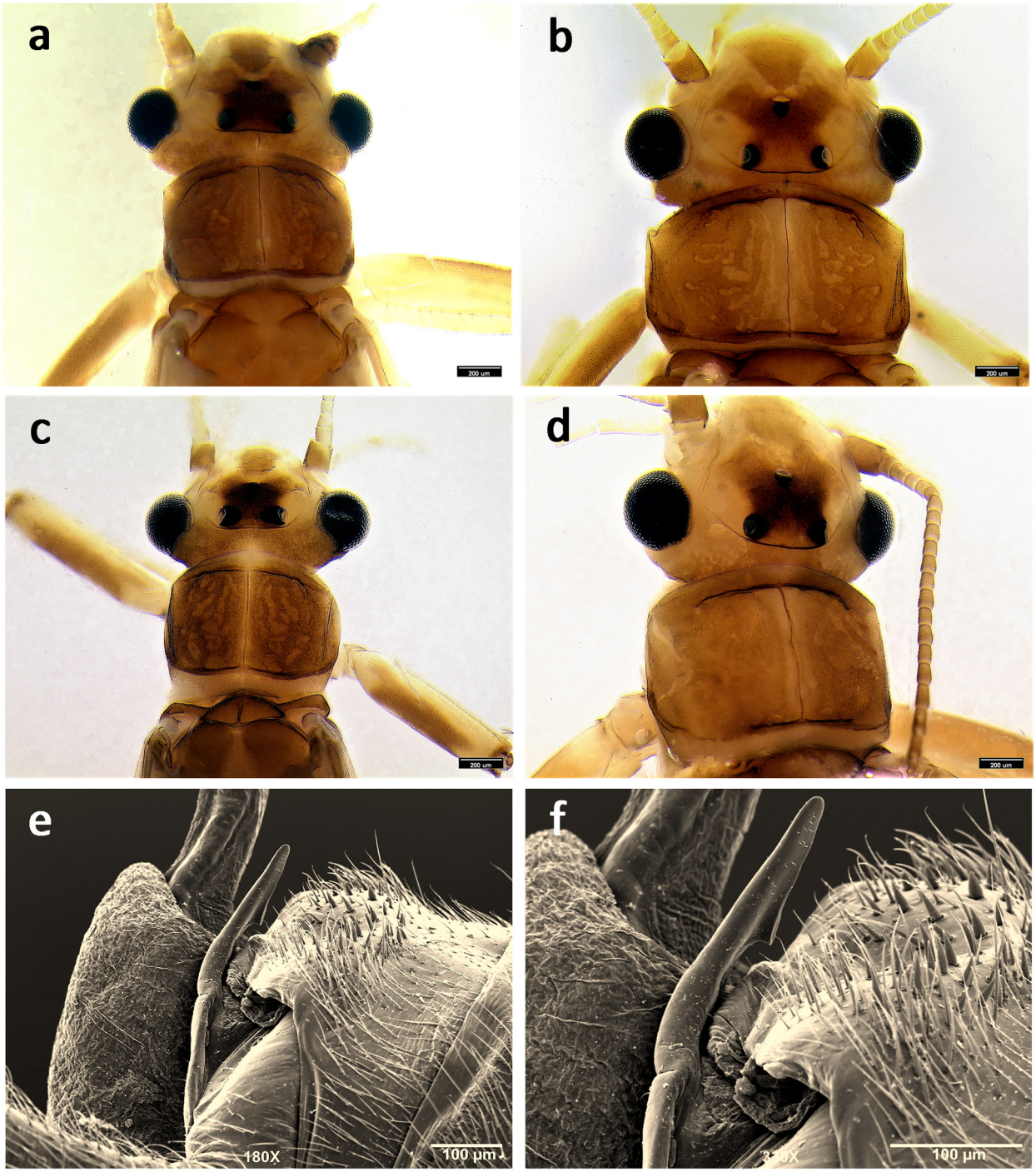

FIGURE 5. *Perlesta cinctipes*. a–b, Arkansas, unknown locality; c–f, Missouri, South Moreau Creek; a, c, male, head + pronotum, dorsal profile; b, d, female, head + pronotum, dorsal profile; e, right paraproct, lateral view, 180X; f, right paraproct, lateral view, 330X.

Male (Figs. 6–8). Sternum 9 ca. evenly and sparsely clothed by long hairs across most of the segment (Fig. 7f). Abdominal terga brown, sterna yellow. Cerci pale yellow proximally, brown distally. Tergum 10 mesal sclerites

brown, sensilla basiconica restricted to posterolateral margins with interspersed long hairs (Figs. 7d–7e). Paraprocts long and slender, bearing a prominent mesoapical tooth in lateral aspect and directed anteriorly, rounded apically (Figs. 7a, 7c–7d), not visible in caudal aspect (Fig. 7b, 7e). Penis tube + sac long, caecum prominent and thumb-like (Figs. 8a–8c), sac nearly as long as tube (Figs. 8a–8b, 8e), lateral sclerite often lightly sclerotized, located in proximal ¼ of tube (Fig. 8a), dorsal patch broad basally, narrowing apically throughout length of sac, completely covering caecum (Figs. 8d–8f).

Female (Figs. 6, 9). Subgenital plate, ca. 1/2 width of 8th abdominal sternum (Fig. 9a), lacking pigmentation; lobes subtruncate and rounded both medially and laterally (Figs. 9b–9d), with some populations with a slight concavity near medial corners (Figs. 9b, 9d); lobes bordered posteriorly by a diffuse row of stiff bristle-like hairs; separated by an oval-shaped notch that is expanded medially (Figs. 9b–9d).

Egg (Fig. 10). Oval. Collar distinctly stalked, wide, ribbed, and flanged at apex (Figs. 10a–10c). Chorion smooth with very fine shallow impressions visible only under high magnification (Figs. 10a, 10d). Micropyles located ca. ¼ from anterior pole (Fig. 10d).

Larva. Unknown.

Type material. Holotype male (INHS): USA, **Ohio**, **Ross Co.**, Deer Creek, 15 km NNE Chillicothe, 39.46120, -83.02120, 28 June 2010, S.A. Grubbs. Paratypes: USA, **Indiana, Bartholomew Co.**, East Fork White River, Azalia Bridge, 1.5 km SW Azalia, 39.0849, -85.8598, 11 June 2000, S.A. Grubbs, 3 males, 3 females (WKUC); **Daviess Co.**, East Fork White River, 14 km S Washington, 38.5447, -87.2138, 7 June 2009, S.A. Grubbs, 8 males, 1 female (WKUC); **Greene Co.**, West Fork White River, Rte. 157, 2 km SE Worthington, 39.1109, -86.9632, 7 June 2009, S.A. Grubbs, 3 males, 16 females (WKUC); **Harrison Co.**, Blue River, 6 km NE Leavenworth, Stagestop Canoe Access Site, Harrison-Crawford State Forest, 38.2153, -86.2718, 18 May 2000, S.A. Grubbs, 15 males, 7 females (WKUC); same but 9 June 2000, S.A. Grubbs, 2 males, 1 female (WKUC); Ohio River, Leavenworth, 38.1966, -86.3504, 28 June 2002, S.A. Grubbs, 3 males (WKUC); **Jackson Co.**, Little Salt River, Houston, 39.0342, -86.1679, 22 June 2008, R.E. DeWalt, 1 male (INHS); **Martin Co.**, East Fork White River, 2 km NNE Shoals, 38.7013, -86.7674, 7 June 2009, S.A. Grubbs, 2 males, 4 females (WKUC); **Washington Co.**, Blue River, Rte. 150, Fredericksburg, 38.4338, -86.1918, 28 June 2010, S.A. Grubbs, 3 males, 3 females (WKUC). **Kentucky**, **Adair Co.**, Russell Creek, nr. Rte. 768, Milltown, 37.1235, -85.4046, 19 June 2008, S.A. Grubbs, 2 males, 5 females (WKUC); **Green Co.**, Green River, at mouth of Big Pitman Creek, 7.5 km SE Summersville, 37.2851, -85.5819, 22 May 2002, S.A. Grubbs, 4 males, 1 female (WKUC); Big Pitman Creek, Narrows of Pitman, 5 km WNW Greensburg, 37.2833, -85.5576, 21 May 2002, S.A. Grubbs, 4 males, 5 females (WKUC). **Ohio**, **Ross Co.**, same as holotype but 1 July 2007, S.A. Grubbs, 1 female (WKUC); same but 28 June 2010, S.A. Grubbs, 7 males, 9 females (WKUC). **Pennsylvania**, **Crawford Co.**, French Creek, Meadville Access Site, 3 mi SE Meadville, 41.5914, -80.1449, 10 June 1998, S.A. Grubbs, 14 males, 9 females (WKUC); same but 1 July 1998, S.A. Grubbs, 1 male (WKUC).

Additional material examined. Indiana, **Harrison Co.**, Blue River, White Cloud, 38.2290, -86.2254, 25 May 1949, W.E. Ricker, 1 male (CNCI). **Kentucky**, **Fayette Co.**, Lexington, 7–9 June 1970, 1 male (DCTC). **Ohio**, **Butler Co.**, Seven Mile Creek, Hwy 127 Collinsville, 39.5050, -84.5946, 28 May 1953, A.R. Gaufin, 1 male, 1 female (BYU); Seven Mile Creek, New Miami, 39.4316, -84.5441, 28 May 1953, A.R. Gaufin, 1 male (BYU); **Coshocton Co.**, Beaver Run, 2 km NW Warsaw at Twp. 348 Bridge, 40.3495¸ -82.0134, 26 June 1999, S.W. Chordas III and J. Thompson, 1 male (INHS); Mohawk Creek, 0.5 km E Mohawk Village at Co. Rd. 82 Bridge, 40.3205, -82.0741, 26 June 1999, S.W. Chordas III and J. Thompson, 2 males (INHS); Killbuck Creek, 2 km NNW Randle at Co. Rd. 28 Bridge, 40.3338, -81.9457, 20 July 1999, S.W. Chordas III and J. Thompson, 1 male (INHS); **Miami Co.**, Stillwater River, Hwy 718, 40.0521, -84.3659, 3 June 1953, A.R. Gaufin, 1 male (BYU); **Warren Co.**, Little Miami River, Morrow, 39.3568, -84.1288, 28 June 1952, A.R. Gaufin, 1 male (BYU). **Pennsylvania**, **Crawford Co.**, French Creek, SR 1002 bridge, Venango, 41.7716, -80.1083, 10 June 1991, S.A. Grubbs, 16 females (WKUC).

Etymology. This species is named in honor of Dr. Brian J. Armitage, former Director of the Ohio Biological Survey and now "retired" in Boquete, Panama. Brian tirelessly performed design, layout, and other production activities for several books through the Ohio Biological Survey and Caddis Press. He improved our understanding of the modern day composition and distribution of aquatic insects within Ohio and secured funding for statewide surveys of aquatic insects that trained several young entomologists. The common name "Brian's Stone" is proposed for this species (Stark et al. 2012).

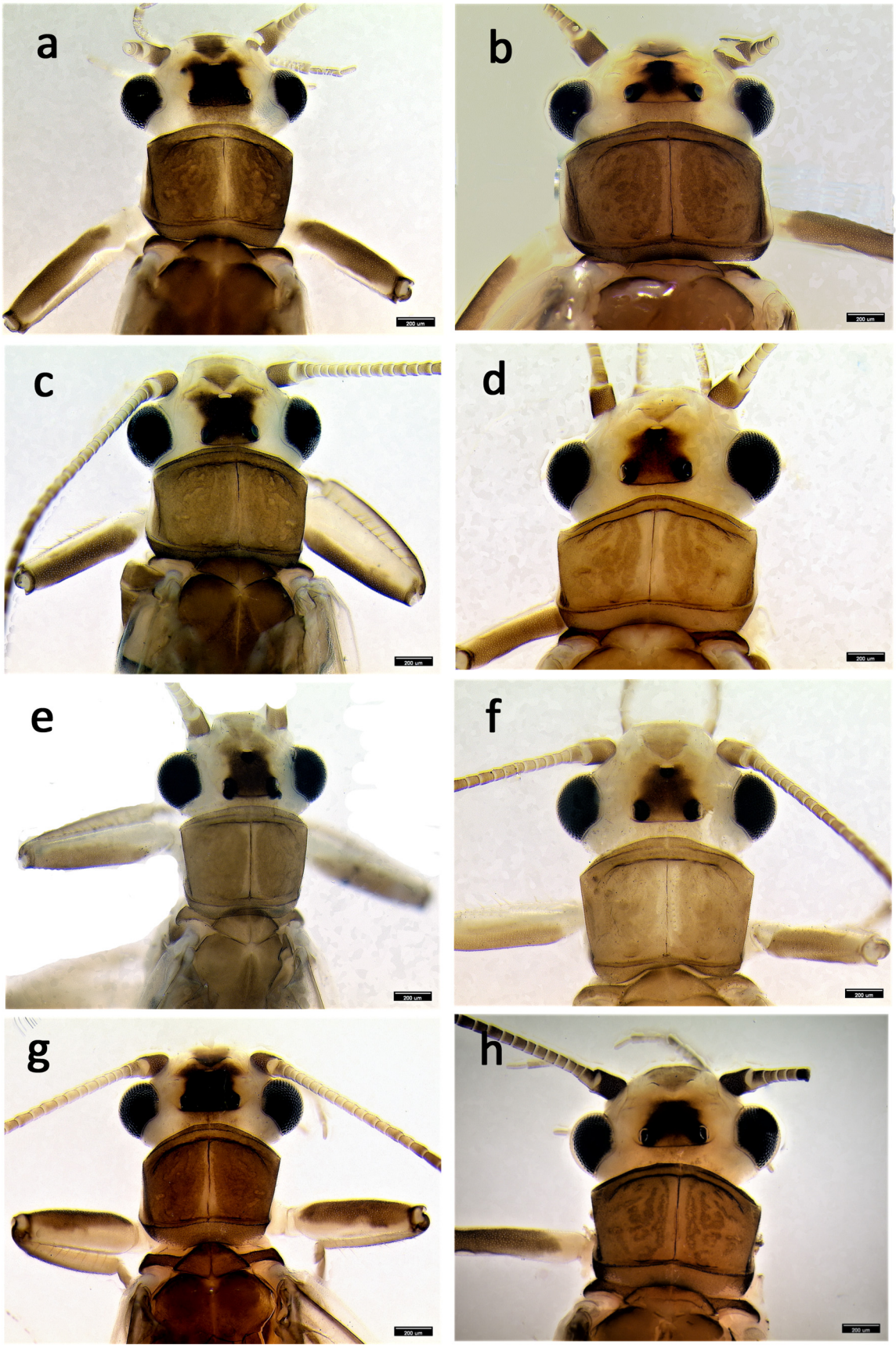

FIGURE 6. *Perlesta armitagei* **sp. nov.** a–b, Ohio, Deer Creek; c–d, Indiana, Blue River; e–f, Kentucky, Russell Creek; g–h, Pennsylvania, French Creek; a, c, e, g, male, head + pronotum, dorsal profile; b, d, f, h. female, head + pronotum, dorsal profile.

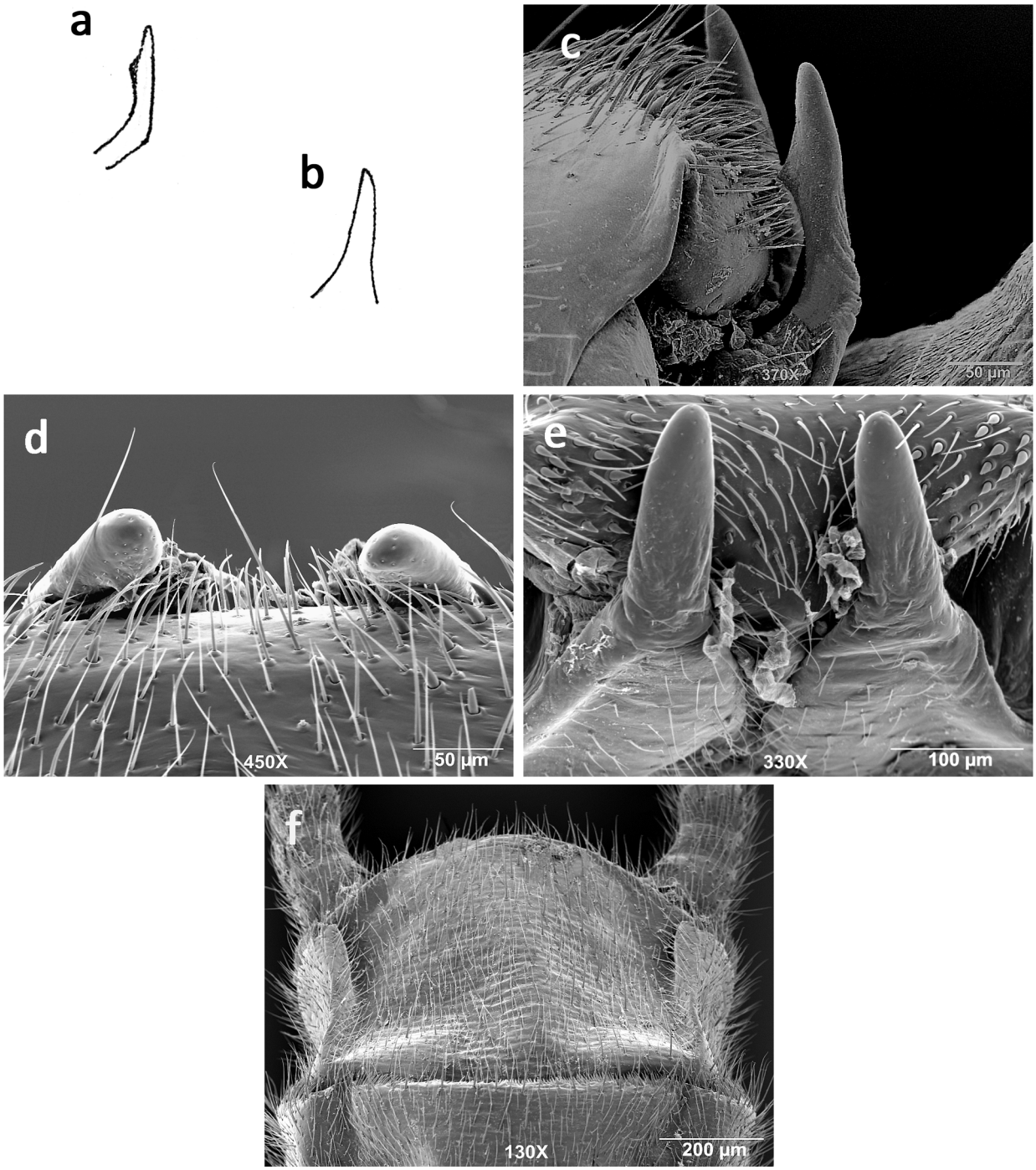

FIGURE 7. *Perlesta armitagei* **sp. nov.**, male.; a, Ohio, Deer Creek, male, 9th sternum, 130X; b, Indiana, Blue River, right paraproct, lateral, 370X; c, Pennsylvania, French Creek, paraprocts, dorsal, 450X; d, Pennsylvania, French Creek, paraprocts, caudal, 330X.

Diagnosis. Males of *P. armitagei* possess a well-developed dorsal caecum (Figs. 8a–8c), which initially takes this species to couplet 2 in Stark (2004, pg. 88). The first choice in couplet 2 reads "epicranial suture arms extend well beyond ocelli as a distinct dark line" (Stark 2004). Couplet 2, however, can be problematic for specimens that are lightly pigmented either due to (a) bleaching following long-term wet storage, or (b) adults preserved while still teneral. Five midwestern and Interior Highland species exhibit this characteristic: *P. adena*, *P. baumanni* Stark, 1989, *P. cinctipes*, *P. fusca* Poulton & Stewart, 1991, and *P. xube*. Material of *P. adena* (Figs. 1a, 1c, 1e), *P.*

cinctipes (Figs. 5a, 5c), and *P. xube* (Figs. 2a, 2c, 2e) examined in this study exhibit this characteristic. *Perlesta armitagei* (Figs. 6a, 6c, 6e, 6g) and *P. browni* (Figs. 3a, 3c), however, generally lack this feature.

Males of *P. armitagei* will key most closely to *P. decipiens* (Walsh, 1862) in Stark (2004, see couplet 12) and Grubbs & DeWalt (2012, a modified couplet 12), with a paraproct spine visible in lateral view and directed forward (Figs. 7a, 7c–7d). Although the dorsal aedeagal patch of *P. armitagei* (Figs. 8d–8e) is similar to *P. decipiens* (Stark 2004, his Fig. 7.306), the mesoapical paraproct tooth of *P. armitagei* (Fig. 7a, 7c) is easily contrasted from the antepical paraproct tooth of *P. decipiens* (Stark 1989, his Figs. 35–36, 38–41).

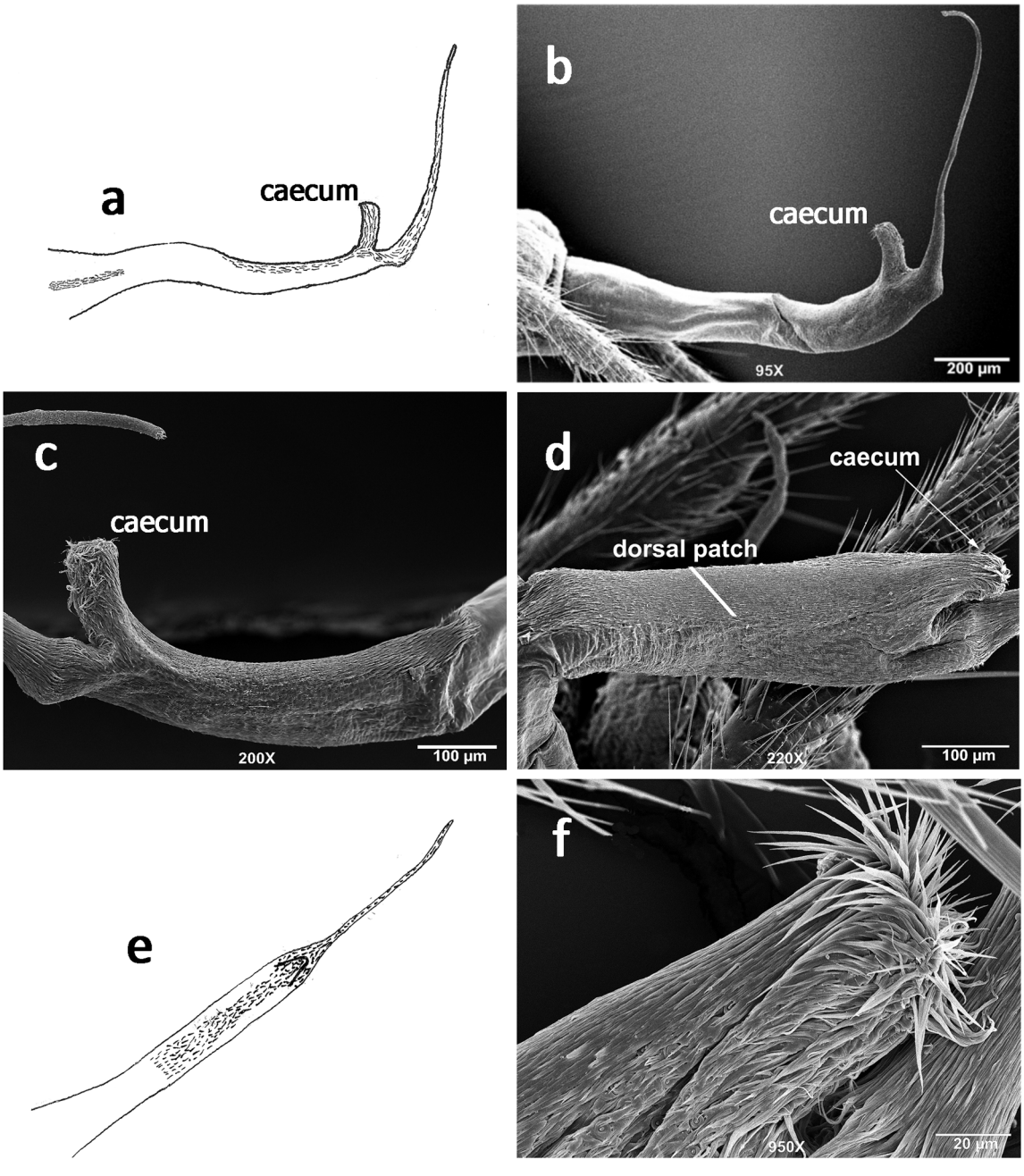

FIGURE 8. *Perlesta armitagei* **sp. nov.**, male, aedeagus. a, Ohio, Deer Creek, lateral; b, Indiana, Blue River, lateral, 95X; c, Ohio, Deer Creek, lateral, 200X; d, Ohio Deer Creek, dorsal, 220X; e, Ohio Deer Creek, dorsal; f, Pennsylvania, French Creek, details, of caecum, 950X.

The paraprocts of *P. armitagei* (Fig. 7c) more closely resemble those of *P. cinctipes* (Figs. 5e–5f) and *P. browni* (Figs. 4a–4c) but are not as tubular or elongate as either species. *Perlesta armitagei* is more readily distinguished from *P. cinctipes* and *P. browni* by aedeagal characteristics. The aedeagi of *P. armitagei* and *P. cinctipes* are similar

in that both species have a long tube + sac and well-developed caecum. The dorsal patch of *P. cinctipes* is very narrow (Stark 2004, his Fig. 300) whereas the dorsal patch of *P. armitagei* is markedly wider (Figs. 8d–8e). Although the epicranial suture arms of *P. browni* extend little laterad of the ocelli (Figs. 3a, 3c), and similar to *P. armitagei* (Figs. 6a, 6c, 6e, 6g), the aedeagi of the two species are distinct. The aedeagal tube + sac of *P. browni* (Stark 1989, his Fig. 80; Stark 2004, his Fig. 7.297) is much shorter than that of *P. armitagei* (Figs. 8a–8b).

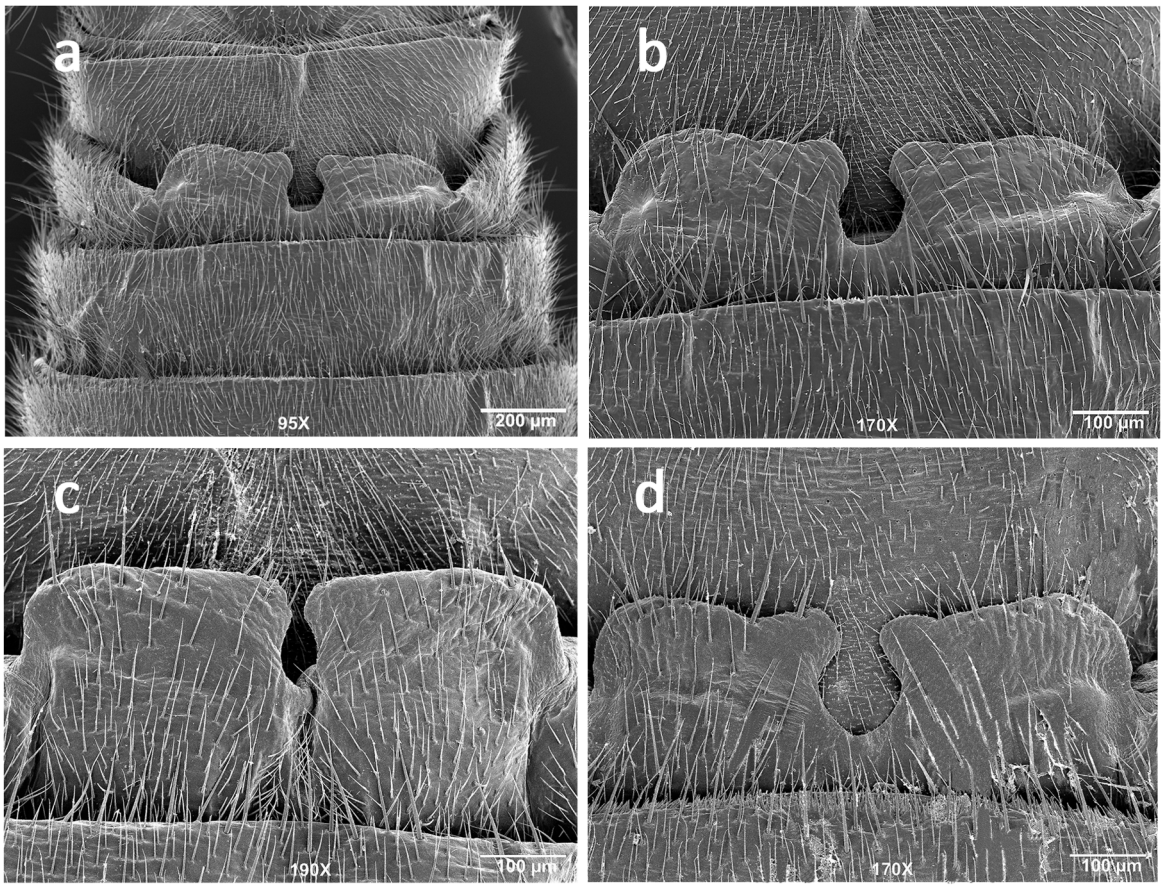

FIGURE 9. *Perlesta armitagei* **sp. nov.**, female, subgenital plate, ventral profile. a, Ohio, Deer Creek, 95X; b, Ohio, Deer Creek, 170X; c, Indiana, Blue River, 190X; d, Kentucky, Russell Creek, 170X.

One additional male characteristic appears worthy of further study and may provide diagnostic information. On *P. browni* the posterolateral corners of the male 9th sternum are clothed by a dense matting of long hairs (Fig. 4d). These are visible under standard light microscopy and appear brush-like and denser than the remaining portions of the segment. In contrast, the male 9th sternum of *P. armitagei* is only sparsely clothed with long hairs (Fig. 7f).

The female of *P. armitagei* can be confidently identified if mature eggs are present and associated with a male with a fully extruded aedeagus. Females will key to couplet 14 in Stark (2004), a dichotomy with *P. shubuta* Stark, 1989 and *P. decipiens*. *Perlesta ephelida* Grubbs & DeWalt, 2012 also keys to couplet 14, but the subgenital plate of these three species are easily differentiated using standard light microscopy. The subgenital plate notch of *P. armitagei* is ovoid and expanded medially (Figs. 9b–9d). In contrast, the subgenital plate notches of *P. ephelida* (Grubbs & DeWalt 2012, their Fig. 7), *P. shubuta* (Stark 1989, his Fig. 91; Stark 2004, his Fig. 7.391), and *P. decipiens* (Stark 1989, his Figs. 44, 46, 48; Stark 2004, his Fig. 7.379) are variable but typically v-shaped.

The egg is only partially diagnostic for *P. armitagei*. The egg of *P. armitagei* possesses a wide, well developed, and distally flanged collar (Figs. 10a–10c), resembling *P. decipiens* (Stark 1989, his Figs. 12–13; Stark 2004, his Figs. 7.397–7.398), *P. ephelida* (Grubbs & DeWalt 2012, their Figs. 14–21), and *P. mihucorum* Kondratieff & Myers, 2011 (their Figs. 9–14), but easily contrasted from the coarsely and near-completely punctate egg chorion (except for the eclosion line) of *P. cinctipes* (Stark 1989, his Fig. 17; Stark 2004, his Figs. 7.394–7.396).

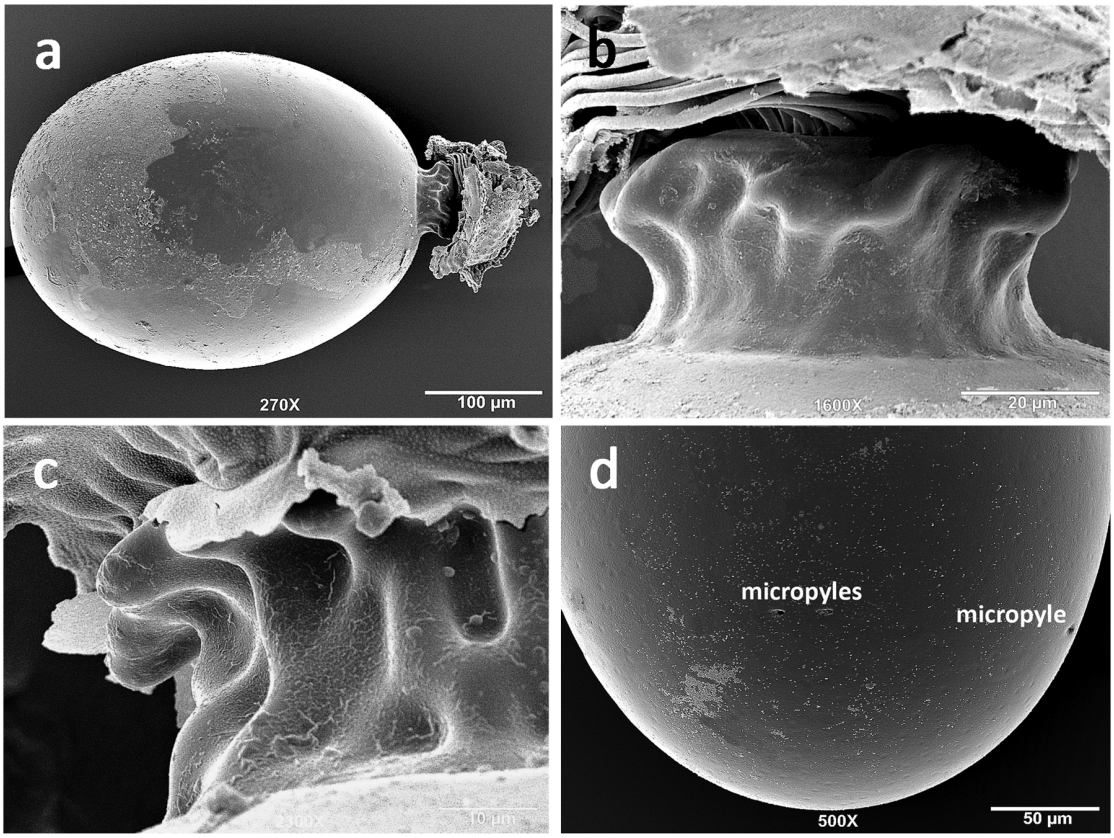

FIGURE 10. *Perlesta armitagei* **sp. nov.**, egg. a, Kentucky, Russell Creek, entire egg, 270X; b, Kentucky, Russell Creek, anterior pole and collar, 1600X; c, Ohio, Deer Creek, details of collar, 2300X; d, Kentucky, Russell Creek, posterior pole and micropyles, 500X.

Remarks. The type locality is in southern Ohio at the southern edge of EPA Level IV Ecoregion 55b (Loamy, High Lime Till Plains). All streams where *P. armitagei* has been collected are mid-order systems with partially- to fully-open canopies. *Allocapnia granulata* (Claassen, 1924), *A. vivipara* (Claassen, 1924), and *Taeniopteryx burksi* Ricker & Ross, 1968 were the only other stonefly species collected at the type locality.

The plotted distribution of *P. armitagei* (Fig. 11b) is conditional since it only includes the specimens examined in this study. This distribution, however, suggests an Ohio River basin species. We have not found this species during our extensive fieldwork and examination of museum material from Illinois (DeWalt & Grubbs 2011), Michigan (Grubbs et al. 2012), and Ontario (unpublished data). The plotted records from Indiana may represent the western edge the range.

The species reported as *Perlesta* sp. I-4 from Ohio in DeWalt *et al.* (2012, 2016) is now regarded as *P. armitagei*. The specimen determined as *P. cinctipes* from Kentucky (Fayette Co., Tarter *et al.* 2006), and subsequently carried forward in the updated state checklist (Tarter *et al.* 2015), was reexamined and determined as *P. armitagei*. The specimens determined as *P. cinctipes* from West Virginia (Monongalia Co., Tarter & Nelson 2006) were not available for study but the location was still plotted (Fig. 11b) and the inclusion in this map should be considered conditional.

Conclusions

Perlesta is rapidly approaching the species richness of eastern *Isoperla* Banks, 1906 as recently revised by Szczytko & Kondratieff (2015). Discussions with our colleagues suggest that many more undescribed species exist in their collections, including several between the authors. Boris C. Kondratieff (Fort Collins, Colorado State

University) likewise has several species awaiting additional material and time for formal descriptions. These additional cryptic species could easily push the total to 40. We surmise that rigorous collecting across the Interior Highlands region of Arkansas, Missouri, and Oklahoma, the Gulf Coastal Plain and Atlantic Coastal Plain states, and the unglaciated sections of the Appalachian Mountains and foothills would result in many more previously unknown species.

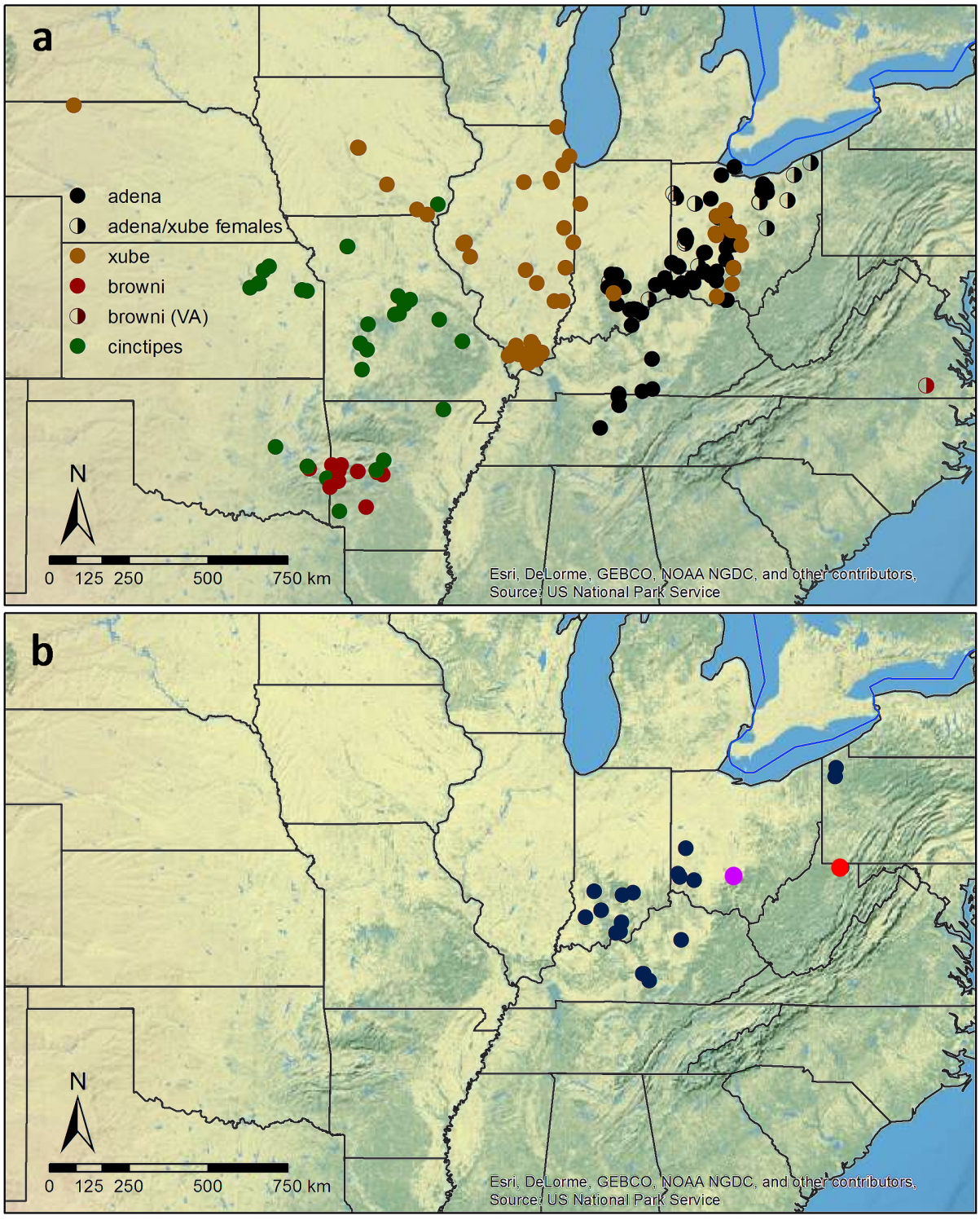

FIGURE 11. Distribution maps of *Perlesta* based on material examined during this study. a, *P. adena*, *P. browni*, *P. cinctipes*, and *P. xube*; b, *P. armitagei* **sp. nov.** The record from northern West Virginia (red circle; Tarter & Nelson 2006) is conditional since that material was not available for study. The type locality is represented by the purple circle.

The time has come for a multi-laboratory approach to revise the genus once again, using both morphological and molecular characters. Included in this study should be the compilation of a matrix of traditional characters and a host of other characters that have not been critically assessed. This work must include collection of new material that will allow for proper preparation of fresh specimens and extraction of DNA. Multiple molecular markers will likely be necessary to produce a robust phylogeny of the genus. To make the project attractive to funding agencies, females and larvae should be associated with males and larval keys constructed that allow positive identification of species found by aquatic biomonitoring agencies.

Reassessment of the environmental sensitivity of *Perlesta* larvae, namely at the species level, is needed. The catch-all species "*Perlesta placida*" has been historically used by biomonitoring agencies and private contractors for decades. Note that taxonomic concept of *P. placida* was revised nearly 30 years ago (Stark 1989). Hilsenhoff (1987) and Lenat (1993) list most *Perlesta* as moderately tolerant to pollution. This is surely an oversimplification given the range of habitats and water quality conditions in which we find *Perlesta*, a fact that argues for species-level identification to improve the precision of biomonitoring efforts.

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Dr. John Andersland (Western Kentucky University) assisted with SEM and Auto-Montage imaging. Dr. Boris Kondratieff (Colorado State University, Fort Collins, Colorado) made material of *Perlesta* available for study. Elise D. Snyder, undergraduate student at the University of Illinois, digitized the Barry C. Poulton Arkansas and Missouri locations that had been listed only as codes with the specimens. With Ms. Snyder's help, RED linked these locations to several specimens used in this study. These specimens were part of the Kenneth W. Stewart Collection that were recently incorporated into the INHS Insect Collection, along with those of the Stanley W. Szczytko collection, and many specimens from the Ohio Biological Survey donated by Brian J. Armitage. Incorporation and digitization of specimens have been partially funded by an NSF Collections in Support Biological Research grant (NSF DBI 14-58285) to RED, and by NSF DEB 09–18805 ARRA, that funded digitization of specimens and preliminary distribution modeling of stoneflies in the Midwest, USA.

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