



## EGGS OF WESTERN NEARCTIC ACRONEURIINAE (PLECOPTERA: PERLIDAE)

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### ABSTRACT

Eggs for western Nearctic acroneuriine species of *Calineuria* Ricker, *Doroneuria* Needham & Claassen and *Hesperoperla* Banks are examined and redescribed based on scanning electron microscopy images taken from specimens collected from a substantial portion of each species range. Within genera, species differences in egg morphology are small and not always useful for species recognition, however eggs from one population of *Calineuria* are significantly different from those found in other populations and this population is given informal recognition as a possible new species.

**Keywords:** Plecoptera, *Calineuria*, *Doroneuria*, *Hesperoperla*, Egg morphology, Western Nearctic

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### INTRODUCTION

Scanning electron microscopy (SEM) is often used to elucidate chorionic features for stoneflies (e.g. Baumann 1973; Grubbs 2005; Isobe 1988; Kondratieff 2004; Kondratieff & Kirchner 1996; Nelson 2000; Sivec & Stark 2002; 2008; Stark & Nelson 1994; Stark & Szczytko 1982; 1988; Szczytko & Stewart 1979) and Nearctic Perlidae were among the earliest stoneflies to be studied with this technique (Stark & Gaufin 1976). Unfortunately, the latter study is limited in sample size and in image quality. More recently, eggs of many eastern Nearctic Perlidae have been examined, or re-examined, greatly increasing the number of species studied and improving the image quality for species known from this region (e.g. Kondratieff & Kirchner 1996; Stark 2004; Stark & Lentz 1988), however eggs of the primary western Nearctic Acroneuriinae have not recently been re-examined.

The primary western North American acroneuriine stonefly fauna includes only three genera and five species of Perlidae, although a few representatives of primarily eastern (*Perlesta*, *Acroneuria*) or southern genera (*Anacroneuria*) also

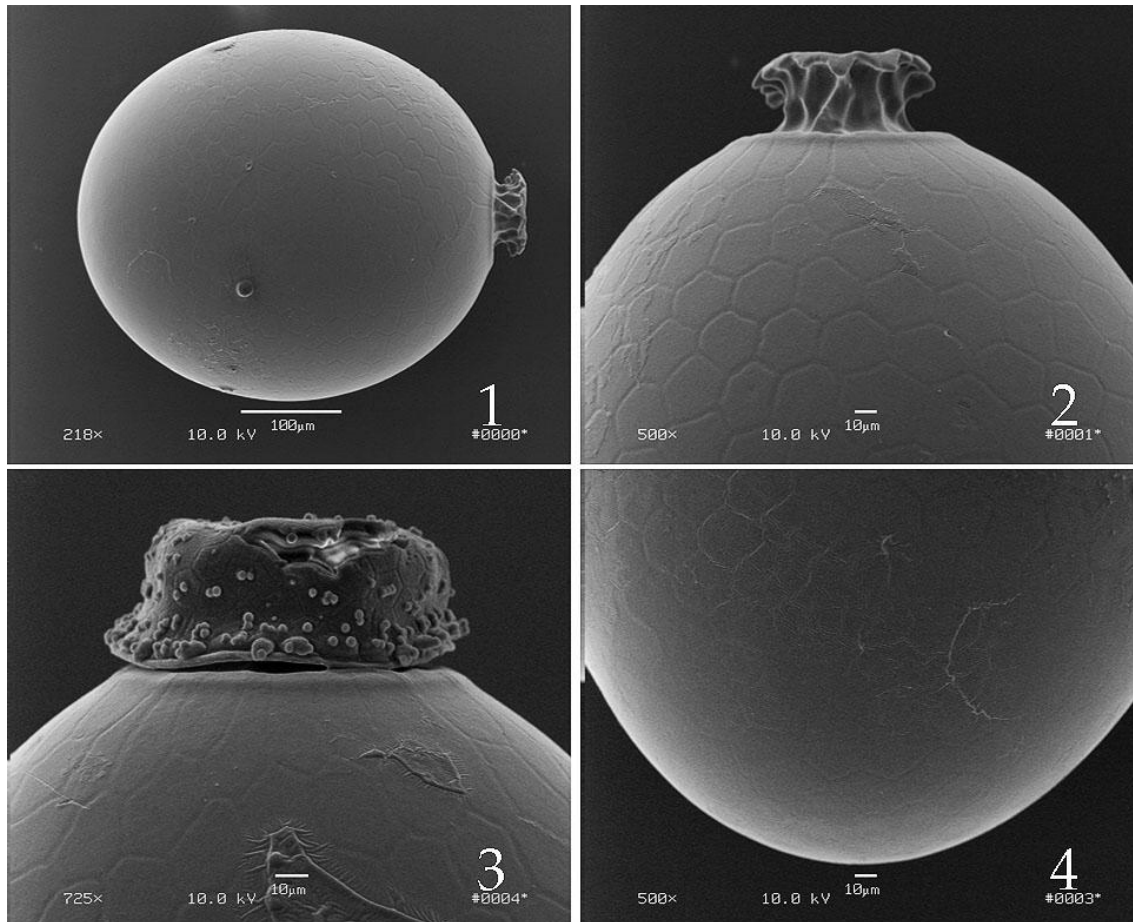
occur in the region (Baumann & Olson 1984; Kondratieff & Baumann 2002; Stark 1989; Stark & Gaufin 1976; Stark & Kondratieff 2004; Zuellig et al. 2006). SEM images for eggs of the primary western acroneuriine genera, *Calineuria* Ricker, *Doroneuria* Needham & Claassen and *Hesperoperla* Banks include single images for each of these genera in Stark & Gaufin (1976), three images of *Hesperoperla hoguei* Baumann & Stark (1980) and three images of *H. pacifica* (Banks) in Isobe (1997). In this study, we examine specimens from populations with available egg samples for each of the five western Nearctic acroneuriine species. Terminology follows Stark & Szczytko (1988) except in the designation of anterior and posterior poles, where Zwick (1982) is followed.

### MATERIALS AND METHODS

Eggs were dissected from female specimens archived in 80% ethanol, cleaned manually with fine tipped forceps, fine dissecting needles and dental brushes and sonicated for 10-20 seconds to remove extra-chorionic membranes. Cleaned eggs were picked up with fine tip forceps, allowed to air dry and placed on specimen stubs covered with double

stick copper tape. Stubs were sputter coated with gold-palladium and examined with an Amray 1810 scanning electron microscope equipped with an Orion digital imaging system. Specimens used in the study are archived in the Stark collection, Mississippi

College, Clinton, Mississippi (BPS), the Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah (BYU), or in the C.P. Gillette Museum, Colorado State University, Fort Collins, Colorado (CSU).



Figs. 1-4. *Calineuria californica* eggs, Montana, Lincoln Co., Yaak River. 1. Egg, lateral aspect. 2. Collar end. 3. Collar end with anchor. 4. Anterior end.

## RESULTS AND DISCUSSION

### *Calineuria californica* (Banks) (Figs. 1-4)

*Perla californica* Banks, 1905:87. Holotype ♀ (Museum of Comparative Zoology), Claremont [Los Angeles Co.], California

**Material examined. California:** Butte Co., Heinz

Creek, Hwy 70, 20 May 1982, B. Stark, D. Ziegler, 1♀ (BPS). Sierra Co., Little Truckee River, Little Truckee Campground, 4 July 1979, B. Stark, K.W. Stewart, 18♂, 3♀ (BPS). **Montana:** Lincoln Co., Yaak River, Yaak River Campground, 19 July 1979, B. Stark, K.W. Stewart, R.W. Baumann, 2♀ (BPS). **Oregon:** Union Co., Grande Ronde River, Hwy 244, 29 May 2000, B. Stark, I. Sivec, M. Zúñiga, 3♂, 4♀ (BPS).

**Egg.** Outline oval with short stalked collar, ends broadly rounded (Fig. 1). Length including collar ca.

430-460  $\mu\text{m}$ , equatorial width ca. 360-390  $\mu\text{m}$ , collar length ca. 35-40  $\mu\text{m}$ , collar width at midlength ca. 60-68  $\mu\text{m}$ . Egg body at base of collar encircled by slightly raised ridge. Sides of collar with irregular, prominent struts (Fig. 2); rim flanged and irregularly incised (Figs. 1-2). Anchor biscuit-shaped, surface with scattered mushroom bodies (Fig. 3). Chorionic surface relatively smooth but with obscure hexagonal follicle cell impressions visible at 500X (Figs. 2, 4). Micropylar row subequatorial.

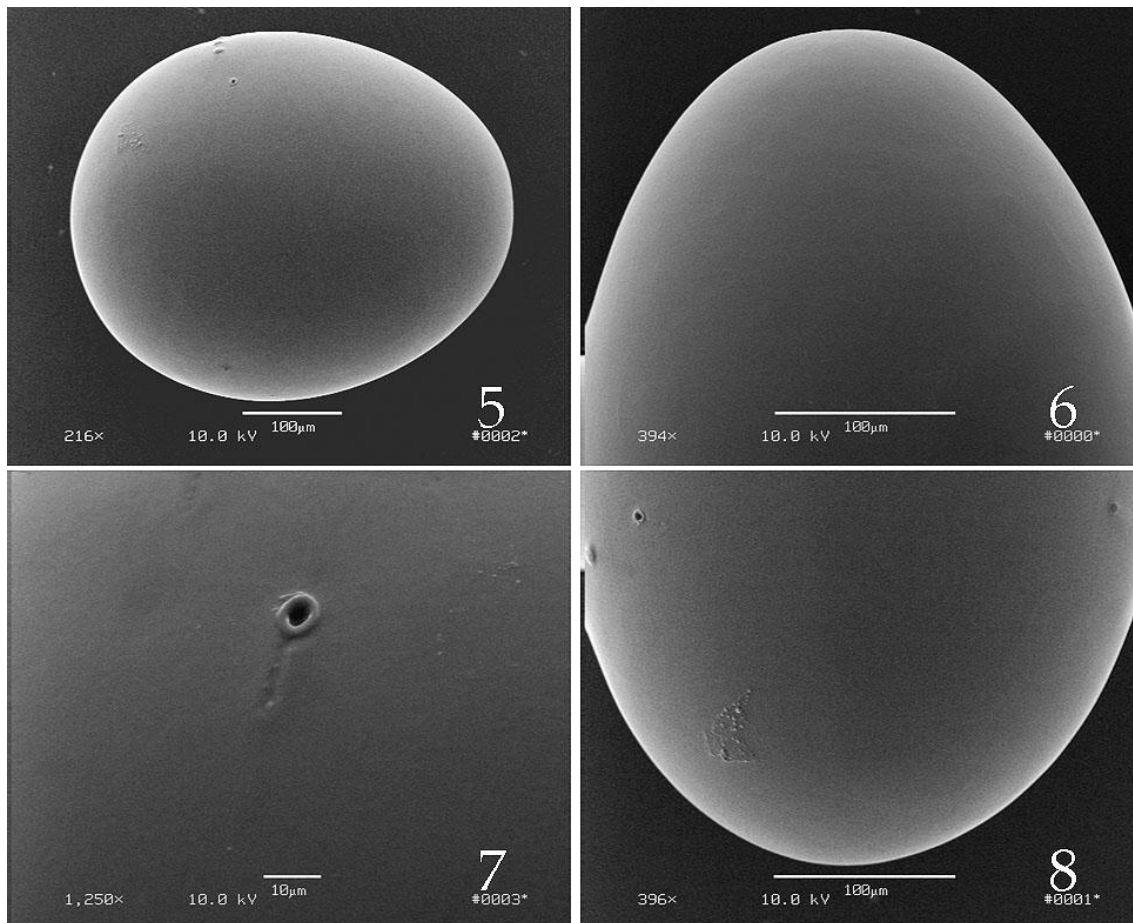
***Calineuria* sp. A**  
(Figs. 5-8)

**Material examined. California:** Marin Co., Muir Woods, Redwood Creek, 21 June 1985, B. Stark, 1♀ (BPS). Same location, 18 June 1966, R.W. Baumann, 1♂, 1♀ (BYU).

**Egg.** Outline oval, collar absent (Fig. 5). Length ca.

440  $\mu\text{m}$ , equatorial width ca. 372  $\mu\text{m}$ . Chorionic surface without punctations or obvious follicle cell impressions (Figs. 6, 8). Micropylar row subequatorial; micropylar orifices with raised rims (Fig. 7).

**Comments.** Eggs of *C. californica* (Figs. 1-4) and the three species of Japanese *Calineuria* (Isobe 1988; 1997) have similar short stalked collars encircled by a low to well developed basal ridge. The chorionic surface is punctate in the Japanese species but smooth or with obscure follicle cell impressions in *C. californica* and *C. sp. A* (Figs. 2, 6). The male and female of *C. sp. A* appear indistinguishable from *C. californica* in color pattern, subgenital plate shape and aedeagal armature, however, the egg has no collar. These specimens may represent an undescribed species, but a larger sample and a comprehensive study of other *Calineuria* specimens is needed in order to evaluate its status. Drumming and molecular data may also be desirable.



Figs. 5-8. *Calineuria* sp. A eggs, California, Marin Co., Redwood Creek, Muir Woods. 5. Egg, lateral aspect. 6. Collar end. 7. Micropyle. 8. Anterior end.

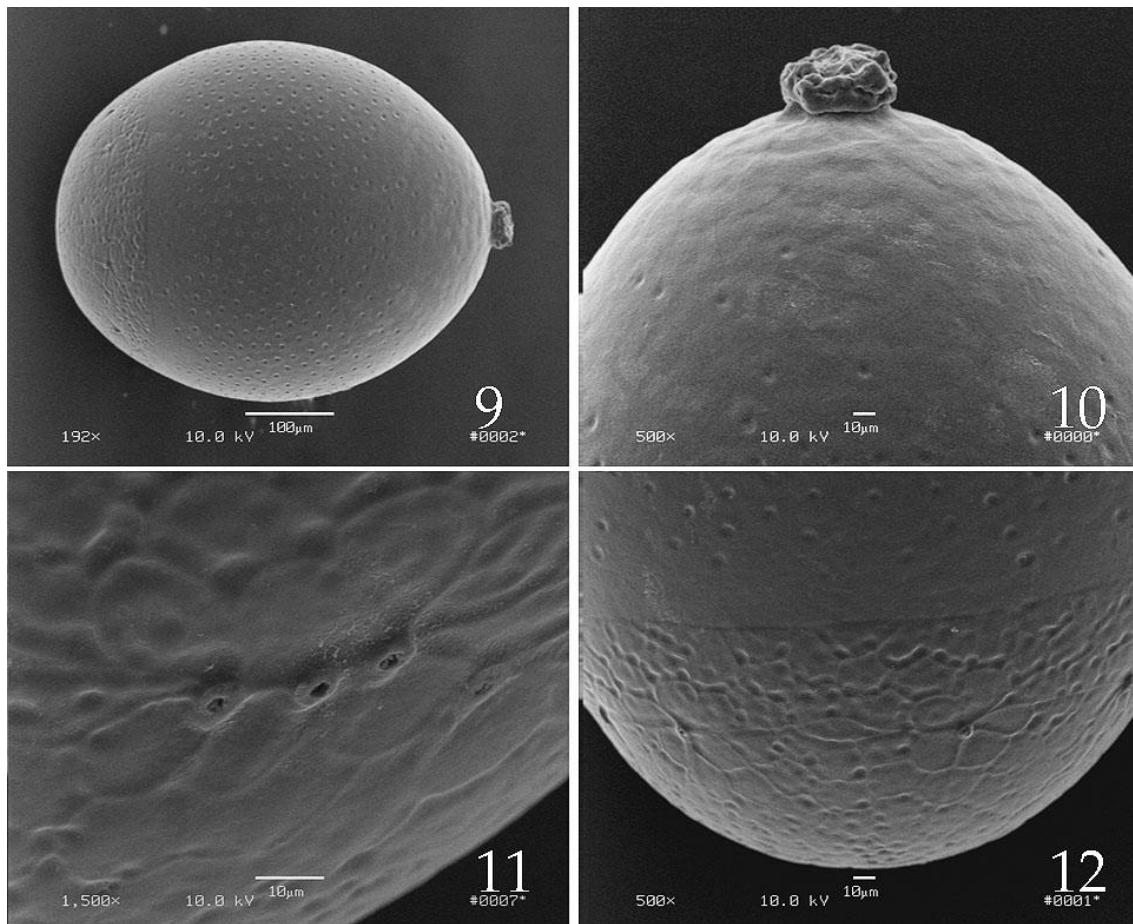
***Doroneuria baumanni* Stark & Gaufin**  
(Figs. 9-12)

*Doroneuria baumanni* Stark & Gaufin, 1974:88. Holotype ♂ (United States National Museum), Burney Falls State Park, Shasta Co., California

**Material examined. California:** Siskiyou Co., Big Springs, Mt. Shasta City Park, 7 July 1979, B. Stark, K.W. Stewart, 1♀ (BPS). **Oregon:** Benton Co., Kiser Creek, Hwy 39, 1 June 2000, B. Stark, I. Sivec, M. Zúñiga, 3♂, 1♀ (BPS). Multnomah Co., Wahkeena Falls, 25 June 1985, B. Stark, 24♂, 4♀ (BPS).

**Washington:** Pierce Co., Carbon River, 16 August 1999, B.C. Kondratieff, 1♂, 3♀ (BPS).

**Egg.** Outline oval with short, narrow collar (Fig. 9). Egg length ca. 500 µm, equatorial width ca. 400 µm, collar length ca. 20 µm, width ca. 50 µm. Chorionic surface coarsely punctate with shallow pits over middle half of egg (Figs 10, 12. ); punctate zone ca. 260 µm wide; area around collar and a narrow opercular ring around lid lack pits; some pits on lid form obscure follicle cell impression walls. Micropylar row located on lid (Fig. 11); orifices raised, canals not clearly visible on surface.



Figs. 9-12. *Doroneuria baumanni* eggs, Oregon, Multnomah Co., Wahkeena Falls. 9. Egg, lateral aspect. 10. Collar end. 11. Micropyles. 12. Anterior end.

***Doroneuria theodora* (Needham & Claassen)**  
(Figs. 13-16)

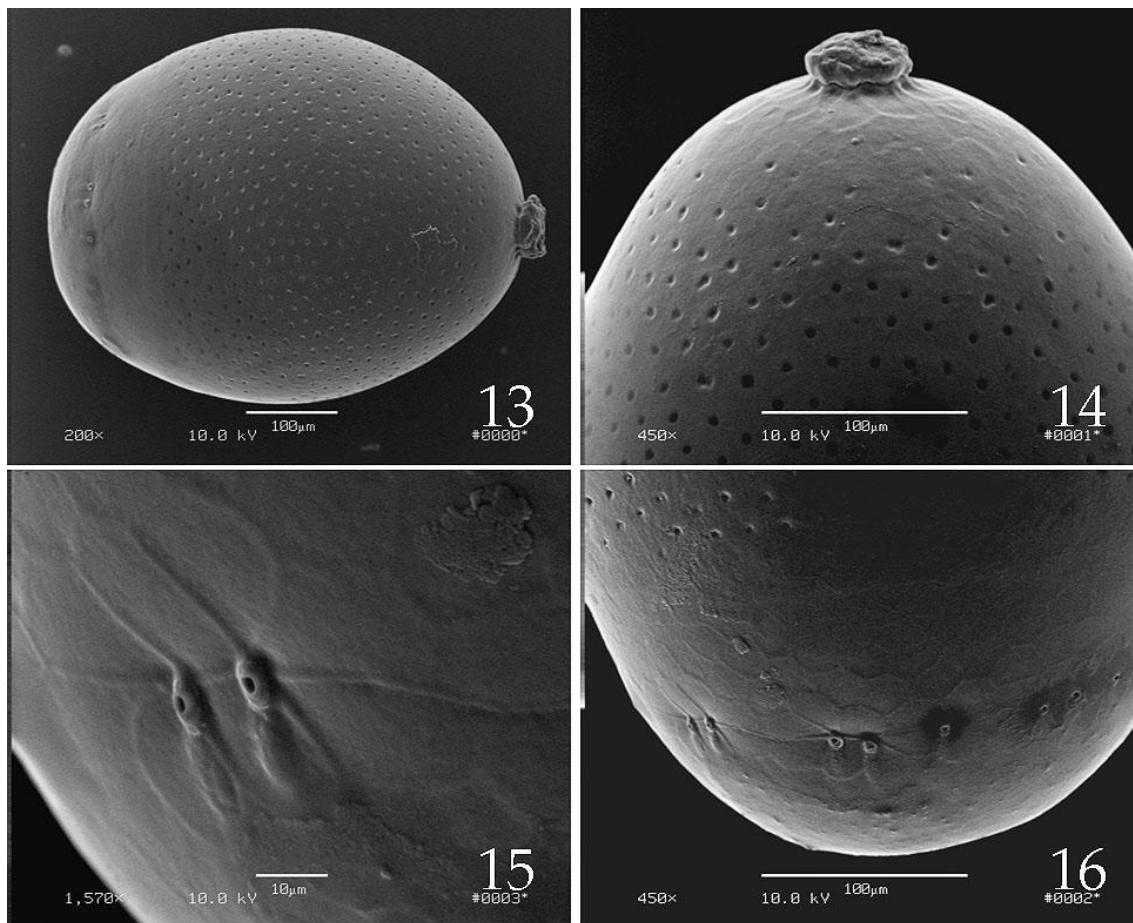
*Acroneuria* (*Doroneuria*) *theodora* Needham & Claassen, 1922:254. Holotype ♀ (Cornell University), Yellowstone National Park, Wyoming

**Material examined. Montana:** Gallatin Co., Hyalite Creek, above Window Rock, 20 August 2004, B. Stark, I. Sivec, 1♀ (BPS). **Wyoming:** Johnson Co., Middle Fork Clear Creek, 17 August 2004, B. Stark, I. Sivec, 2♂, 2♀ (BPS). Park Co., Lake Creek, Hwy 296, 18 August 2004, B. Stark, I. Sivec, 3♂, 3♀ (BPS).

**Egg.** Outline oval with short, narrow collar (Fig. 13). Egg length ca. 530 µm, equatorial width ca. 400 µm, collar length ca. 30 µm, width ca. 60 µm. Chorionic surface coarsely punctate in a band ca. 325 µm wide, extending from near collar (Figs. 14, 16) to smooth

opercular band surrounding lid. Lid with obscure, irregularly shaped follicle cell impressions, but punctations absent (Fig. ). Micropylar row located on lid; orifices raised, canals obscure (Fig. 15).

**Comments.** Eggs of *Doroneuria* are large, oval, and have small collars. The chorionic surface has a broad median zone of shallow pits, a narrow smooth opercular band and micropyles located on the lid near the anterior pole (Figs. 9, 13). The eggs of the two species are quite similar, but differ subtly among specimens examined in two respects. The punctate zone for *D. theodora* eggs extends nearer the collar and is, therefore, considerably broader in that species than in *D. baumanni* (Figs. 10, 14). In addition, the lid of *D. theodora* eggs lacks punctations, whereas in *D. baumanni* this area has obscure punctations which outline the irregular margins of follicle cell impressions (Fig. 12).



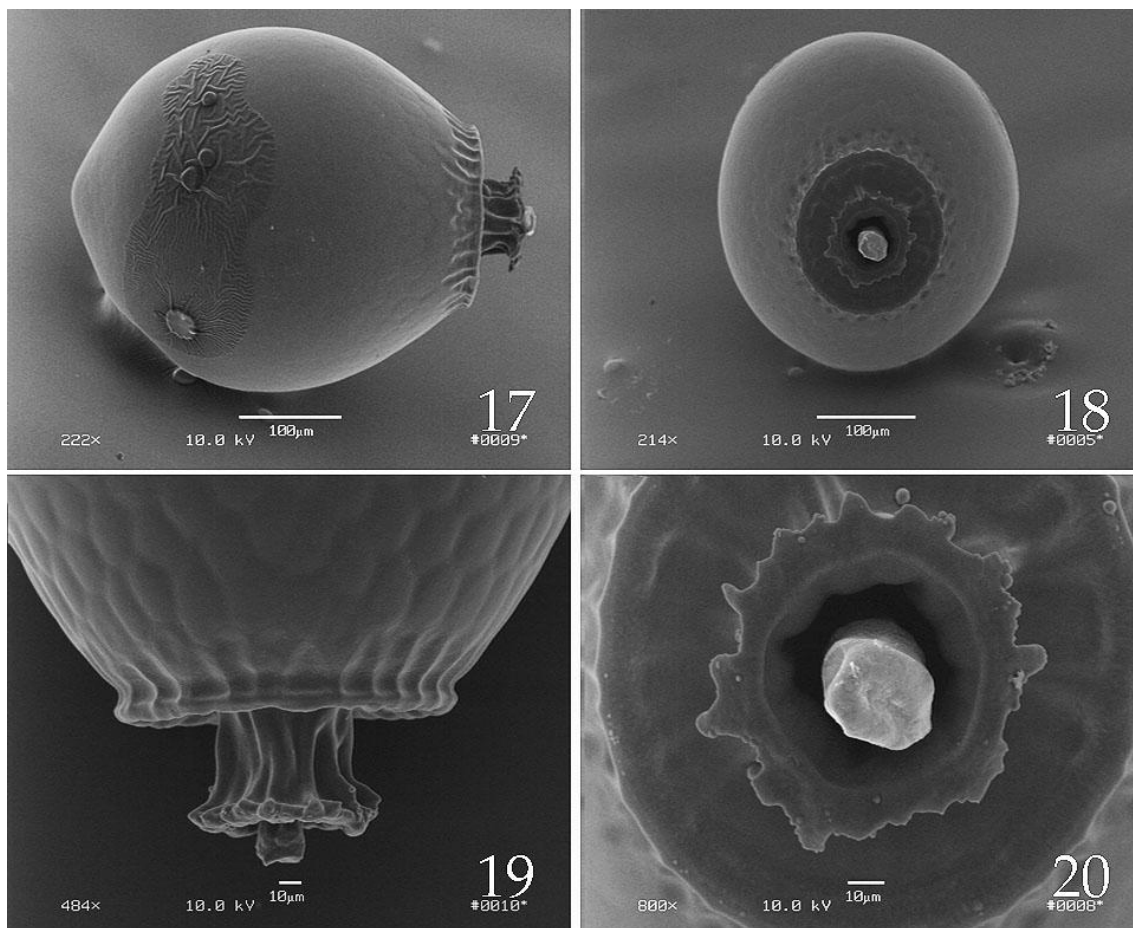
Figs. 13-16. *Doroneuria theodora* eggs, Wyoming, Johnson Co., Middle Fork Clear Creek. 13. Egg, lateral aspect. 14. Collar end. 15. Micropyles. 16. Anterior end.

***Hesperoperla hoguei* Baumann & Stark**  
(Figs. 17-20)

*Hesperoperla hoguei* Baumann & Stark, 1980:63.  
Holotype ♂ (Natural History Museum, Los Angeles County), Gibson Creek, Mendocino Co., California

**Material examined. California:** Plumas Co., Domingo Springs, 8 miles NW Chester, Old Red Bluff Road, 21 September 2007, J. Sandberg, 2♂, 5♀ (BPS). Sierra Co., Big Spring, Hwy 49, 2 miles N Bassetts, 3 July 2010, P.A. Opler, 1♀ (CSU).

**Egg.** Outline oval with short, wide collar (Fig. 17); collar base encircled by prominent raised ridge, sides of egg body adjacent to ridge with a series of vertical struts. Egg length ca. 400 µm, equatorial width ca. 360 µm, collar length ca. 50 µm, median width ca. 75 µm. Sides of collar with several prominent longitudinal ridges (Fig. 19); collar rim flanged and irregularly incised (Figs. 18, 20). Anchor biscuit shaped with moderately long pedicel; surface covered with mushroom bodies. Chorionic surface covered with shallow, hexagonal follicle cell impressions. Micropylar row subequatorial.



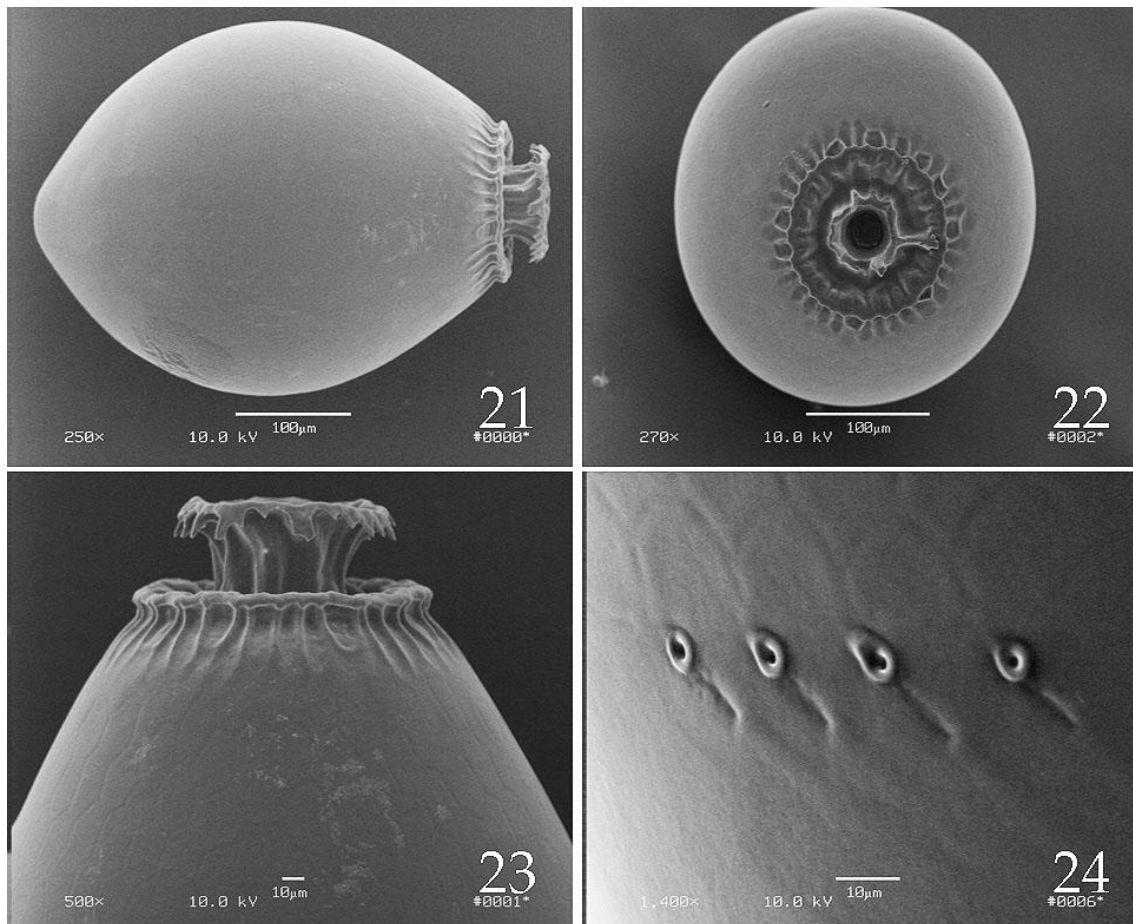
Figs. 17-20. *Hesperoperla hoguei* eggs, California, Sierra Co., Big Spring, Hwy 49, 2 miles north Bassetts. 17. Egg, lateral aspect. 18. Egg, apical aspect. 19. Collar end. 20. Collar detail, apical aspect.

***Hesperoperla pacifica* (Banks)**  
(Figs. 21-30)

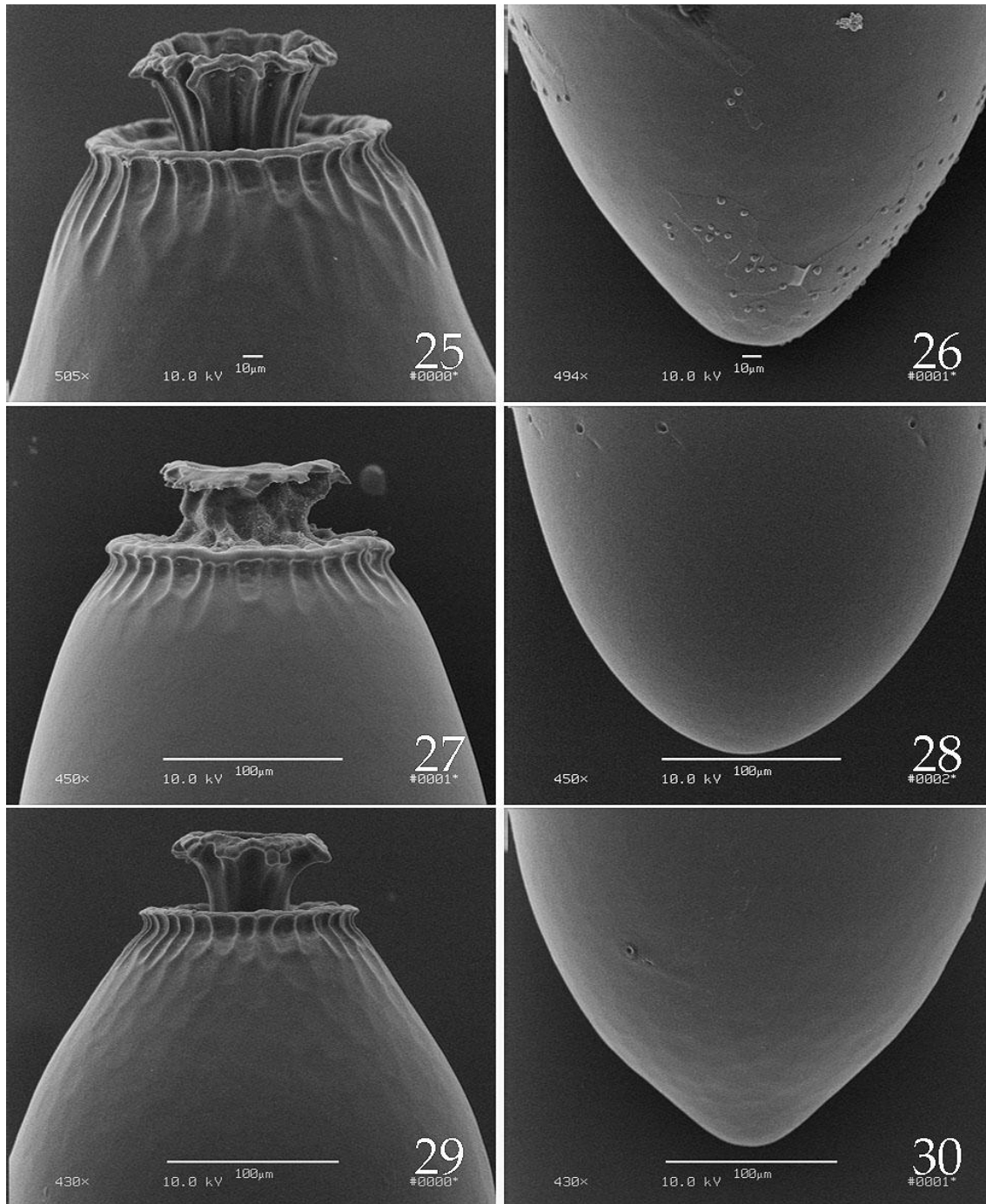
*Acroneuria pacifica* Banks, 1900:242. Holotype ♀ (Museum of Comparative Zoology), Olympia [Thurston Co.], Washington

**Material examined.** **Alberta:** Battle Creek at Reservoir Lake, 23 June 1975, L. Dosdall, 1♀ (BPS). **California:** Humboldt Co., East Fork Willow Creek, Hwy 299, 24 April 1987, B. Stark, R.W. Baumann, C.R. Nelson, 2♀ (BPS). Shasta Co., North Fork Battle Creek, south of Shingletown, 8 June 2004, B. Stark, R.W. Baumann, 1♀ (BPS). Tehama Co., North Fork Battle Creek, Wildcat Road, 26 April 1987, B. Stark, R.W. Baumann, C.R. Nelson, 2♀ (BPS). **Colorado:** Gunnison Co., Gunnison River, Hwy 135, 8 June 2003, B. Stark, 3♂, 2♀ (BPS). **Idaho:** Bonneville Co., Snake River, 4 July 1972, G. Tourtlotte, 1♂, 1♀ (BPS). Fremont Co., Big Springs, 5 miles east Macks Inn, 25 July 1979, B. Stark, K.W. Stewart, R.W. Baumann, 4♀ (BPS). **Montana:** Carbon Co., East Rosebud River, Roscoe, 23 July 1989, B. Stark, 5♂, 3♀ (BPS). Lewis & Clark Co., Trout Creek, Vigilante Campground, 21 August 2004, B. Stark, I. Sivec, 1♂, 1♀ (BPS). **New**

**Mexico:** San Miguel Co., Pecos River, 6 miles north Pecos, 12 July 1973, B. Stark, 2♂, 3♀ (BPS). **Nevada:** Elko Co., Secret Creek, Secret Pass, 15 June 1974, B. Stark, 1♂, 1♀ (BPS). **Oregon:** Jefferson Co., Metolius River at headwater spring source, 11 July 1979, B. Stark, K.W. Stewart, 5♂, 3♀ (BPS). Tillamook Co., Nehalem River at Cronin Creek jct., 4 June 1991, B. Stark, R.W. Baumann, C. Henderson, 1♀ (BPS). **Wyoming:** Park Co., Clarks Fork Yellowstone River, below Cooke City, 20 July 1989, B. Stark, 3♂, 1♀ (BPS). **Egg.** Outline oval with short, wide collar (Fig. 21); collar base encircled by a prominent basal ring, ca. 137-176 µm wide (Figs. 23, 25, 27, 29); sides of egg body along basal ring bearing a series of vertical ridges separated by wide depressions. Egg length ca. 428-484 µm, equatorial width ca. 312-324 µm, collar length ca. 34-55 µm, median collar width ca. 41-58 µm, rim width ca. 89-116 µm. Sides of collar with



Figs. 21-24. *Hesperoperla pacifica* eggs, Idaho, Bonneville Co., Snake River. 21. Egg, lateral aspect. 22. Egg, apical aspect. 23. Collar end. 24. Micropyles.



Figs. 25-30. *Hesperoperla pacifica* eggs. 25-26. Colorado, Gunnison Co., Gunnison River. 25. Collar end. 26. Anterior end. 27-28. Montana, Lewis & Clark Co., Trout Creek. 27. Collar end. 28. Anterior end. 29-30. Oregon, Jefferson Co., headwaters Metolius River. 29. Collar end. 30. Anterior end.

several prominent vertical ridges, rim flanged and irregularly incised. Chorionic surface appearing

smooth (Figs. 21, 26, 28, 30) but with obscure hexagonal follicle cell impressions sometimes

apparent at higher magnifications. Micropyles located near equator; orifices raised slightly (Fig. 24). **Comments.** *Hesperoperla* eggs are generally similar to those of *Calineuria californica* in shape, collar form and in having a basal ridge encircling the collar base (Baumann & Stark 1980; Isobe 1997). The most conspicuous feature distinguishing the eggs of the two genera is the row of vertical struts on the egg body which connect to the basal ridge in *Hesperoperla* (Fig. 25) which are absent in eggs of *Calineuria* (Fig. 2). No consistent morphological differences were found among eggs of different populations of *H. pacifica*, or between eggs of *H. hoguei* and *H. pacifica*. However, variations are evident in the images of *H. pacifica* eggs from different sites (Figs. 21-30), especially in shape of the anterior pole (e.g. Figs. 26, 28), shape of the collar (e.g. Figs. 25, 27) and in length of lines delimiting cells surrounding the basal ridge (= vertical struts). The specimen shown from the Gunnison River, Colorado, for example, has a few long lines of at least 57 µm (Fig. 25), whereas the longest vertical struts on the specimen shown from Trout Creek, Montana (Fig. 27) are only 30 µm.

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