SIERRAPERLA JEWETT, 1954 (PLECOPTERA: PELTOPERLIDAE), DISTRIBUTION, EGG MORPHOLOGY AND DESCRIPTION OF A NEW SPECIES

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ABSTRACT
The genus *Sierraperla* Jewett is reviewed and a new species, *S. tolowa* is recognized. Data supporting recognition of the new species include scanning electron microscopy of egg chorion, adult external terminalia, morphology of internal male genitalia, adult pigment patterns, and DNA barcodes (cytochrome c oxidase I; COI).

Keywords: Plecoptera, Peltoperlidae, *Sierraperla*, egg morphology, scanning electron microscopy, COI gene, cytochrome c oxidase I, DNA barcodes, new species

INTRODUCTION
*Sierraperla* was proposed by Jewett (1954) as a subgenus of *Peltoperla*, primarily on the basis of “…shape and location of the gills…” and “…additional details…” of the larval stage. *Sierraperla* larvae differ in these characters from those of *Yoraperla*, in which the sole species had been placed by Ricker (1952). The subgenus was later given full generic rank by Illies (1966) and has remained monotypic, with *S. cora* (Needham & Smith 1916) the only recognized species. The type locality for this species is given as “Reno, Nevada” by Needham & Smith (1916), but the holotype female was the only specimen known from Nevada until Richard L. Bottorff recently collected larval specimens in Douglas Co. and confirmed larval
records from other sites in Douglas Co., collected by personnel of the Nevada Division of Environmental Protection (Bottorff, personal communication). Needham & Claassen (1925) gave the first California record based on 14 adults collected at Shasta Springs by C.L. Fox and E.P. Van Duzee, and Jewett (1954, 1960) gave records from five California counties (8 total sites) based on 17 specimens. Little additional data have appeared for *Sierraperla*, but Stark & Stewart (1981) examined specimens from Oregon (no specific locality given) and California, in addition to the Nevada holotype, and provided a synopsis of generic characters, and Stewart & Stark (1988, 2002) presented a larval habitus prepared from a specimen collected at Wrangle Camp in Jackson Co., Oregon. Nelson & Stark (1987) reported three additional specimens from California and Stark et al. (2008) reported 22 specimens from six sites in the Warner Mountains of northeastern California. Stark & Stewart (1981) included three scanning electron microscopy images of eggs taken from specimens from the Big Spring, Mt. Shasta City Park, California population. Zeigler & Stewart (1985) provided details of the drumming signals from members of the same population, and Stewart & Stark (1988, 2002) suggested a probable semivoltine cycle with extended emergence for the *Sierraperla* at that locality. Sandberg (2011) revised the *Sierraperla* interval pattern from monophasic to varied beat-interval and detected differences between the signals collected by Zeigler & Stewart (1985) and those collected by Sandberg (2011) from Butte Creek, California.

In this study, we examine egg morphology and male genitalic structures for additional *Sierraperla* populations in order to test the monotypic status for the genus. We also present the first DNA barcodes, and compile published and unpublished records in order to more precisely define the distribution and relative abundance of the genus. Results from these studies suggest the presence of a second species represented by the southern Oregon and northwestern California populations that we describe below.

**MATERIALS AND METHODS**

Much of the material for this study was collected on the following expeditions to California and Oregon.

- **July 1979**: B. Stark, K.W. Stewart
- **May 1982**: Stark, D. Zeigler
- **April 1987**: B. Stark, R.W. Baumann, C.R. Nelson
- **June 1991**: B. Stark, R.W. Baumann, C. Henderson
- **May 1998**: B. Stark, C.R. Nelson, S.W. Szczytko, I. Sivec, R. Bottorff
- **May 2001**: B. Stark, K.W. Stewart, J.B. Sandberg
- **June 2004**: B. Stark, R.W. Baumann
- **May 2007**: B.C. Kondratieff, R.W. Baumann
- **June 2009**: B. Stark, B.C. Kondratieff, J.B. Sandberg, S.W. Szczytko, R.W. Baumann, A.B. Harrison
- **May 2014**: B. Stark, B.C. Kondratieff, J.B. Sandberg, C.J. Verdone, A.B. Harrison

Larval specimens were hand-picked, usually from wood in splash zones and preserved in 95% ethanol or placed in cups of stream water and kept on ice until they could be placed in rearing facilities. Adult specimens were hand-picked from stream-side vegetation, or collected on beating sheets and preserved in 75-80% ethanol.

Tissue samples from 46 specimens of larval *Sierraperla*, one adult male *Soliperla campanula* (Jewett 1954), and one adult female *Yoraperla mariana* (Ricker 1952) were sent to the Canadian Centre for DNA Barcoding (CCDB) for sequencing. Mitochondrial COI sequences (DNA barcodes) were uploaded to the Barcode of Life Database (BOLD; Ratnasingham & Hebert 2007; http://www.boldsystems.org) to the dataset “DS-SIERRA” which has been made publicly available. Additional sequences for *Yoraperla brevis* (Banks) (Process IDs: ABSTO026-09, ABSTO027-09, ABSTO031-09, ABSTO032-09, BB0EPT023-10) were downloaded from BOLD and included in our analysis. All sequences were aligned using MUSCLE (Edgar 2004) in MEGA6 (Tamura et al. 2013). Estimates of genetic distance (divergence) were calculated among specimens using the Kimura-2-parameter model (Kimura 1980) with a pairwise deletion option. Genetic distances at COI among individuals of the same stream insect
species are typically low (usually < 2-3%), whereas those among individuals of different species are much higher (> 2-3% usually ~ 10% for congeneric species; Hebert et al. 2003, Sweeney et al. 2011, Zhou et al. 2009), thus allowing us to delimit putative species by examining levels of divergence at COI among specimens. Mean intraspecific and interspecific genetic distances were estimated for all taxa.

Egg samples were dissected from gravid females, cleaned with fine forceps and dental brushes, placed in an ultrasonic cleaner for 15-30 seconds, removed to acetone and attached to specimen stubs with double stick copper tape. Stubs were sputter coated with gold-palladium and examined with an Amray 1810D scanning electron microscope. Eighteen stubs with 5-12 eggs each were examined from 13 sites and more than 150 individual SEM images were collected for analysis. Egg diameter was estimated based on the average of the length of two diameters taken through the long axis and the short axis of each individual egg. The terminal abdominal segments of male specimens were squeezed under a dissecting microscope and held in hot water briefly to fix the everted aedeagus in a suitable position for study. Terminal abdominal segments were then clipped and placed in cold 10% KOH for one hour, and the internal soft tissues were manually removed using fine tip forceps. Male and female terminalia were examined and drawn using an Olympus SZH10, or a Wild M5A stereomicroscope equipped with drawing tube.

The holotype of *S. tolowa* is deposited in the United States National Museum of Natural History, Washington, D.C. (USNM). Other specimens utilized in this study were obtained from the collections listed below, and are deposited in these collections as indicated in the text. Additional collection sites were provided by Richard L. Bottorff, South Lake Tahoe, CA, Jonathan J. Lee, Eureka, CA, and A. Brady Richards, Chico State University, Chico, CA.

**RESULTS AND DISCUSSION**

*Sierraperla cora* (Needham & Smith)  
(Figs. 1-16, 33)

*Peltoperla cora* Needham & Smith 1916:86. Holotype ♀ (Museum of Comparative Zoology), Reno, [Washoe Co.], Nevada

*Peltoperla cora*: Needham & Claassen, 1925:172, in part  
*Peltoperla* (*Yoraperla*) *cora*: Ricker, 1952:156  

**Published Records:** Adult *Sierraperla* examined in this study indicate *S. cora* is restricted primarily to the Sierra Nevada and Warner Mountains of eastern and northeastern California and potentially western Nevada in the Lake Tahoe area. Other populations in northwestern California and southern Oregon, except one population from Black Butte Spring near Weed, California, are referred to the new species, *S. tolowa*. The following list provides a summary of the previously published sites that we assign, at least tentatively, to *S. cora*. *California:* Jewett (1954) referred to “A nearly mature nymph...” collected by H.P. Chandler in Plumas Co. at Howells [Road?], 29 August 1946. Jewett (1960) gives apparently valid records from Colusa Co., (Paradise Creek), El Dorado Co., (Pyramid Ranger Station), Plumas Co., (2 sites, Howells, and tributaries of Smith Creek near Blairsden), Shasta Co., (2 sites, Hat Creek and Shingletown). Nelson & Stark (1987) list one site from Shasta County, and Stark et al. 2008 report collections from six Modoc Co. sites. *Nevada:* (Needham & Smith 1916; Needham & Claassen 1925) both list the holotype female from Reno, collected in 1878 by Morrison.

Adult habitus. General color yellow-brown patterned with dark brown (Figs. 13, 33). Head mostly yellow brown, usually with an irregular narrow bar extending across median frons forward of ocelli and often with a narrow brown band extending between ocelli; occiput yellow brown, sometimes with obscure dusky pigment; some specimens have more extensive dark pigment forward of ocelli. Pronotum with numerous irregular pale spots, some bearing small dark inclusions, particularly in median half of pronotum. Femora yellow-brown to brown, but sometimes slightly darker along dorsal margin; tibiae usually yellow-brown. Wing membrane brown with darker veins except along C and Sc (Fig. 33).

Male. Forewing length 16-17 mm. Abdominal tergum 10 with extensive pale pigment.

surrounding epiproct sclerite; transverse anterior band brown but slightly paler at midlength (Fig. 14). Epiproct sclerite variable but often with a well-defined triangular notch on anterior margin. Abdominal sternum 9 with a wide oval hammer. Ventral aspect of aedeagus a wide membranous bag without sclerites and bearing sparse thin setal-spines on and adjacent to lateral and mesal lobes; lateral lobes near midlength not bifurcate; smaller mesal lobes triangular in outline and often project toward lateral lobes (Fig. 15); apex bearing two pairs of closely appressed lobes, separated by a shallow notch. **Female.** Forewing length 18-19 mm. Subgenital plate extends over ca. half of sternum 9; lateral margins broadly curved forming a parabolic plate with median apical notch (Fig. 16). **Egg.** Hemispherical in lateral aspect, polar aspects
circular (Fig. 9). Diameter (n = 12) ca. 364-403 μm. Collar a flattened disc covered with shallow pits and reticulations and surrounded by a narrow groove (Fig. 9). Anchor flattened, membranous, ca. 212-235 μm in diameter (n = 5), and bearing clusters of globular bodies around outer margin. Lateral surface of egg covered with pits; micropyles located in an irregular ring nearer collar than anterior pole; orifices smaller in diameter than adjacent pits and surrounded by slightly raised, oval rims (Fig. 10). Anterior surface completely covered with pits, surrounded by raised, follicle cell impression walls; walls smooth and of similar height and thickness on all sides; inner dimensions of pits relatively large giving FCIs an open appearance (Figs. 1-8, 11-12).

**Larva.** Undescribed. The larval descriptions attributed to this species (Stark & Stewart 1981, Stewart & Stark 1988; 2002) are assigned below to *S. tolowa* sp. n. Identification of the larva of *S. cora* is discussed in the diagnosis section of *S. tolowa* sp. n.

**Sierraperla tolowa** Stark & Kondratieff, sp. n. (Figs. 17-32, 34)


**Published Records** (as *S. cora* but presumptively *S. tolowa*): **California**: Needham & Claassen (1925) report 10 males, 4 females from Shasta Springs (Siskiyou Co.), collected 8-29 June [year not given], by C.L. Fox and E.P. Van Duzee. Jewett (1960) includes records from Mt. Shasta and Shasta Springs in Siskiyou Co. Nelson & Stark (1987) include a larval record from Bidden Creek, Hwy 299, W of Cedar Flat in Trinity Co. **Oregon**: Jackson Co., Wrangle Camp, 8 July 1979, B. Stark, K.W. Stewart (collection data listed below larval habitus, Fig. 12.3 in Stewart & Stark 1988, 2002).


**Additional Specimens. California: Del Norte Co.,** Same data as holotype, 4 larvae, 3 in 95% EtOH (BPSC). Small falls above Shelly Creek into Patrick Creek, Patrick Creek Rd, 41° 54.320’N, 123° 51.370’W, 24 May 2014, B. Stark, A.B. Harrison, 6 larvae (95% EtOH) (BPSC). **Humboldt Co.,** Ruby Creek, Hwy 299, 40° 54.470’N, 123° 43.093’W, 25 May 2014, B. Stark, A.B. Harrison, 3 larvae (95% EtOH) (BPSC). Boise Creek, Hwy 299, abv. Boise Creek Campground, 40.94155°N, 123.65765°W, 20 April 2009, J.B. Sandberg, 1 larva (JBS). Small


C. Verdone, 1 larva (95% EtOH) (BPSC). Split Rock Creek, Wagner Gap Rd, 42° 05.676'N, 122° 46.493'W, 22 May 2014, B. Stark, B.C. Kondratieff, J.B. Sandberg, C. Verdone, 5 larvae (95% EtOH), 2 larvae (95% EtOH) (JBSC). Wagner Creek, Wagner Creek Rd crossing, 42° 10.222’N, 122° 47.045’W, 22 May 2014, B. Stark, B.C. Kondratieff, J.B. Sandberg, C. Verdone, 1 larva (95% EtOH) (BPSC), 4 larvae (95% EtOH) (JBSC). Josephine Co., Lake Creek, below Oregon Caves National Monument, 9 July 1979, B. Stark, K.W. Stewart, 4 larvae (BPSC).

**Adult Habitus.** General color black patterned with yellow-brown (Figs. 34). Head mostly yellow-orange but with extensive dark pigment in area surrounding ocelli; interocellar area mostly pale but with a narrow median band separating pale areas around ocelli (Fig. 17); antennal segments 1-7 pale on posterior margin and black on anterior margin, additional segments uniformly black. Pronotum mostly dark except for pale curved band extending from posteromedian line to midlateral margin; an additional pale diamond shaped area located near anteromedian margin, and an intricate pattern of small pale spots and lines occurs adjacent to midline and extends laterally onto discs. Femora brown, darker along dorsum; tibiae dark brown but bearing a narrow pale line on ventral margin. Wing membrane dusky, veins black.

**Male.** Forewing length ca. 15-18 mm. Abdominal tergum 10 yellow over most of surface, but bearing a transverse dark, anterior band which constricts near midlength; epiproct sclerite almost rectangular, but wider near anterior margin and often strongly excavated with a pale V-shaped notch on anteromedian margin (Fig. 18). Abdominal sternum 9 with a wide, oval hammer. Ventral aspect of aedeagus a wide membranous bag without sclerites and with sparse thin setal-spines on lateral lobes; lateral lobes near midlength bifurcate; smaller pair of lobes located between bases of lateral pair; apex broadly and shallowly notched, each lobe consisting of a pair of closely appressed small lobes (Fig. 19).

**Female.** Forewing length ca. 18-21 mm. Subgenital plate extends over ca. half of abdominal sternum 9; lateral margins subparallel for much of plate length; apical margin bearing a slight median notch (Fig. 20).

**Egg.** Hemispherical in lateral aspect, polar aspects circular (Figs. 21, 24, 25). Diameter (n = 16) ca. 376-420 μm. Collar a flattened disc covered with shallow pits and reticulations; collar surrounded by a narrow groove (Fig. 21). Anchor flattened, membranous, and bearing clusters of globular bodies around outer margin; diameter (n = 5) ca. 180-210 μm. Lateral surface of egg covered with pits; micropyles located in an irregular ring nearer collar than anterior pole (Fig. 24); orifices smaller in diameter than adjacent pits and surrounded by slightly raised, oval rims. Anterior surface completely covered with pits, surrounded by raised, follicle cell impression walls; walls thickened, height and thickness irregular; inner dimensions of FCIs relatively small and almost closed in some (Figs. 22-32).

**Etymology.** The species name honors the Native American Tolowa people whose homeland, based in northwestern California and southwestern Oregon, may have encompassed the site in Del Norte County, California where the type specimen was collected.

**Diagnosis.** This species appears distinct by virtue of its dark habitus, the bilobed lateral aedeagal lobes, the chorionic detail of the anterior pole of the egg and the barcode sequence of the COI gene. Females may be separated by comparison of subgenital plate shape and size of the median notch. No morphological characters are known which permit separation of larvae of the two species of *Sierraperla*. Because present data suggests the two species are allopatric we assigned identifications of some larval specimens on this basis when no barcode or adult data were available. *Sierraperla cora* is known from sites in the Sierra Nevada and Warner Mountains of California and Nevada, whereas *S. tolowa* is known from the Coast and Cascade mountains of southern Oregon and northern California. A possible exception to the allopatric distribution occurs in Siskiyou Co., California, where populations of both species were discovered near Mt. Shasta. Adult specimens from...

Big Spring, Mt. Shasta City Park were diagnosed based on the dark habitus, bilobed lateral aedeagal lobes and chorionic detail of the anterior pole (Figs. 24-26), and larval specimens from this site and Black Butte Spring, near Weed were diagnosed by comparing COI sequences. Adults and larvae from the Mt. Shasta City Park site are both identified as *S. tolowa*. Unfortunately, no adult specimens are available from Black Butte Spring but the larval COI sequence from this population is consistent with that of other *S. cora* populations.

**Distribution.** *Sierraperla* is currently known from a region of northern California, southern Oregon and western Nevada. The northernmost known locality is a site in Jackson Co., Oregon at Pearsony Falls on Mill Creek, the southernmost known locality is Bendorf Spring in El Dorado Co., California, the westernmost site is in Elk River Canyon, Curry Co., Oregon, and the easternmost site is in Douglas Co., Nevada (R. Bottorff, personal communication). *Sierraperla* appears to be rare along the western slope of the Coast Range but has been collected from upper Freshwater Creek in Humboldt Co., California by a colleague (J.J. Lee, personal communication).

**DNA barcoding.** Of the 48 specimens submitted, CCDB returned 44 high quality sequences (~92%) meeting the standards for barcode compliance of the Consortium for DNA Barcoding (CBOL) for *Sierraperla cora* (*N* = 19), *S. tolowa* (*N* = 24) and *Soliperla campanula* (*N* = 1). Mean genetic distances among individuals of *S. cora* and *S. tolowa* were 0.3% and 0.8% respectively (very similar). The mean genetic distance among specimens of these two species was 9% (quite divergent). Therefore, low levels of intraspecific and high levels of interspecific divergence of DNA barcode sequences among individuals of these taxa provide further support for the recognition of *S. tolowa* as a species distinct from *S. cora* (Hebert et al. 2003, Sweeney et al. 2011, Zhou et al. 2009). Mean genetic distances among *S. cora*, *S. tolowa* and exemplars of the other western peltoperlid genera (*Soliperla* and *Yoraperla*) calculated here are presented in Table 1 for reference.

**Table 1.** Mean genetic distances (%) based on COI among specimens of *Sierraperla cora*, *Sierraperla tolowa*, *Yoraperla brevis* and *Soliperla campanula*.

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<th><em>Sierraperla tolowa</em></th>
<th><em>Sierraperla cora</em></th>
<th><em>Soliperla campanula</em></th>
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REFERENCES

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