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NOTES ON THE GENUS *PHANOPERLA* BANKS FROM SRI LANKA AND INDIA (PLECOPTERA: PERLIDAE)

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ABSTRACT

Eggs of *Phanoperla nana* Zwick 1982a, *P. wedda* Zwick 1982a, and an unassociated female from Sri Lanka are described based on scanning electron micrographs. The putative female and egg are described for *P. peniculus* Kawai 1975, and a revised key is provided for Indian and Sri Lankan *Phanoperla* males.

Keywords: Phanoperla, Plecoptera, Sri Lanka, India, egg morphology, new records

INTRODUCTION

Genus *Phanoperla* Banks 1938, currently includes 49 species (DeWalt et al. 2013) widely distributed over mainland southeast Asia, the Indian Subcontinent and several Asian islands including Borneo, the Philippines and Sri Lanka. The most recent major systematic treatments are those of Zwick (1982a, 1982b) in which 34 species were formally recognized, including a minimum of seven endemic Sri Lankan and eight additional species from India (Table 1). Although recent work on the group has continued in other areas (e.g. Cao & Bae 2009; Sivec & Stark 2010b; 2011; Stark & Sheldon 2009; Stark & Sivec 2007), no additions have been made to the *Phanoperla* species list of India or Sri Lanka.

The work of Zwick (1982a) and others have

amply demonstrated the value of egg morphology in the systematic study of *Phanoperla*. We, therefore, have undertaken a study of eggs available from a series of previously unstudied *Phanoperla* specimens in the United States National Museum and from the Monte L. Bean Life Science Museum, Brigham Young University. Unfortunately, this material did not include numerous female specimens with eggs and we were able to examine egg specimens representing only three Sri Lankan and one Indian species with scanning electron microscopy.

MATERIALS AND METHODS

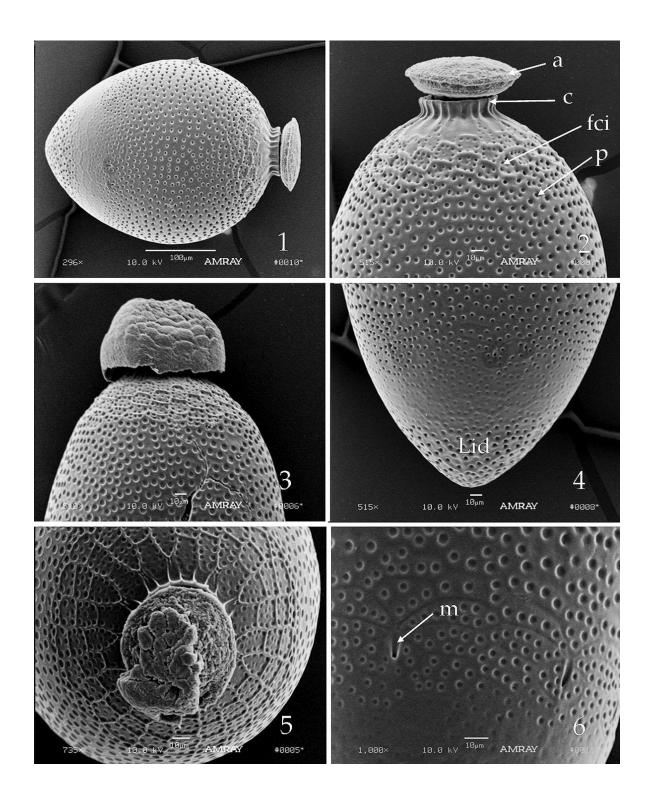
Sri Lankan and Indian *Phanoperla* specimens were obtained on loan from the United States National Museum of Natural History, Washington,

Table 1. Systematic list of *Phanoperla* species known from India and Sri Lanka, including those with informal designations [Note: "species C" was used by Zwick (1982a) to identify female specimens thought to be *P. limosa*]. Distributions are given by country, and by respective state or district for India and Sri Lanka. Species groups are those proposed by Zwick (1982a).

Species	Species Group	Distribution
sertispina Jewett 1975	anomala	INDIA: Tamil Nadu
peniculus Kawai 1968	flaveola	INDIA: Karnataka, Maharashtra, Tamil Nadu
cornuta Zwick 1982a	maindroni	INDIA: Tamil Nadu
himalayana Zwick 1977	maindroni	BHUTAN; INDIA: Assam, West Bengal; NEPAL
maindroni (Navás 1926)	maindroni	INDIA: Tamil Nadu
nana Zwick 1982a	nana	SRI LANKA: Colombo, Kandy, Matale, Ratnapura
parva Zwick 1982a	nana	INDIA: Tamil Nadu
ceylonica Kawai 1975	testacea	SRI LANKA: Kandy
limosa (Hagen 1858)	testacea	SRI LANKA: Galle, Kandy, Nuwara Eliya, Ratnapura
"species C" Zwick 1982a		
nuwara Kawai 1975	testacea	SRI LANKA: Nuwara Eliya
srilanka Zwick 1982a	testacea	SRI LANKA: Badulla
testacea (Hagen 1858)	testacea	SRI LANKA: Badulla, Kandy, Nuwara Eliya
wedda Zwick 1982a	testacea	SRI LANKA: Badulla, Kandy, Kanneliya, Kegalle,
		Matale, Moneragala, Ratnapura
Species A Zwick 1982a	testacea	SRI LANKA: Kandy
Species B Zwick 1982a	testacea	SRI LANKA: Ratnapura
amorpha Zwick 1982a	Incertae sedis	INDIA: Assam
schmidi Zwick 1982a	Incertae sedis	INDIA: Assam
Species D Zwick 1982a	Incertae sedis	INDIA: Assam
Species F Zwick 1982a	Incertae sedis	INDIA: Assam
Species SI-D new designation	Incertae sedis	SRI LANKA: Kandy

D.C. (USNM), and from the Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah (BYUC). The latter collection included specimens originally collected in 95% ethanol and archived under cryogenic conditions. These were sorted and examined in 95% ethanol and placed again in 95% ethanol after study. Male terminalia were examined and drawn using an Olympus SZH10, or a Wild M5A stereo microscope after they were prepared for study using the cold maceration technique of Zwick (1983). Eggs were dissected from female specimens and cleaned with an ultrasonic cleaner. This process sometimes removed the fine marginal ("finger- or tentacle-shaped") structures of Zwick (1982a) from the

anchor. Eggs were placed in acetone, and then transferred to specimen stubs covered with double stick copper tape and coated with gold-palladium using a Hummer sputter coater. Coated specimens were examined using an Amray 1810D scanning electron microscope. Eggs of four species of *Phanoperla* were included in the material examined and these are described, or redescribed below. An informal designation of *Phanoperla* SI-D is used for one species that apparently was not included in the material examined by Zwick (1982a). This designation continues the practice of Zwick (1986) and Sivec & Stark (2010a) in using a two letter code (SI) to represent the country (Sri Lanka) and a single letter to represent the respective species.



Figs. 1-6. *Phanoperla* sp. Sl-D egg. 1. Lateral aspect. 2. Collar end (a = anchor; c = collar; fci = follicle cell impression; p = punctation). 3. Collar end with most of anchor intact. 4. Lid and micropylar zone. 5. Collar and damaged anchor, apical aspect. 6. Micropyles (m = micropyle).

RESULTS AND DISCUSSION

Phanoperla sp. Sl-D (Figs. 1-6)

Material examined. SRI LANKA: Kandy District, Kabargala, Nillomalai, 22-23 March 1975, S. Korunaratne, P.B. Korunaratne, 1♀ (USNM).

Egg. Spindle shaped (Fig. 1). Length ca. 300-330 μm, equatorial width ca. 248-267 μm. Collar ca. 12-15 μm long and ca. 60-73 μm wide, margin relatively smooth, not flanged, sides with short, smooth struts (Fig. 2). Chorion ornate over almost entire surface (Fig. 1); ca. first 40-50 µm adjacent to collar covered with follicle cell impressions (FCIs), most with punctate floors; apical row of FCIs much longer than 2nd and 3rd rows (Figs. 2-3). Beyond the FCI zone, the chorion becomes rather uniformly, coarsely punctate almost to the subequatorial micropylar zone (Figs. 1, 4), however the pores become smaller for a short distance and then disappear for a section in which the chorion is relatively smooth. On the lid, obscure FCIs reappear with coarsely punctate floors extending over the anterior pole (Fig. 4). Micropyles with short sperm guide canals occur in a subequatorial row (Figs. 1, 4, 6).

Comments. In addition to the seven Phanoperla species formally recognized by Zwick (1982a) from Sri Lanka, there are also descriptions for females and eggs of two Sri Lankan species under informal designation (Table 1). The current specimen may represent an unassociated female of a species distinct from any of those considered by Zwick (1982a), or it may be the female of one of the two named Sri Lankan species (P. ceylonica Kawai 1975; P. srilanka Zwick 1982a) for which the female and egg are unknown. The chorionic detail of the current species is similar to that of *P.* sp. A (Zwick 1982a) in having distinct follicle cell impressions limited to the collar area, and in having punctations over much of the remaining chorion. However, as Zwick (1982a) noted in regard to the egg of P. sp. A, the "...upper half of egg unsculptured." The "upper half" refers to the end of the egg opposite the collar, which in the current specimen has conspicuous sculpturing on the lid (Figs. 1, 3). The egg of the current specimen is also similar to that of P. nuwara Kawai 1975 and P. testacea (Hagen 1858) in zonation of chorionic detail, however the image for *P. nuwara* [(Fig. 24e) in Zwick (1982a)], shows an egg with apparent, less prominent follicle cell impressions on both ends, but particularly so near the collar, and the image for P. testacea [(Fig. 24d) in Zwick (1982a)], shows an egg with a linear arrangement of punctations over much of the chorion. In addition, the eggs from our specimen appear to have more densely packed punctations in the equatorial zone. Unfortunately, we returned this single female specimen to the USNM before the subgenital plate was drawn and the specimen may need to be examined later.

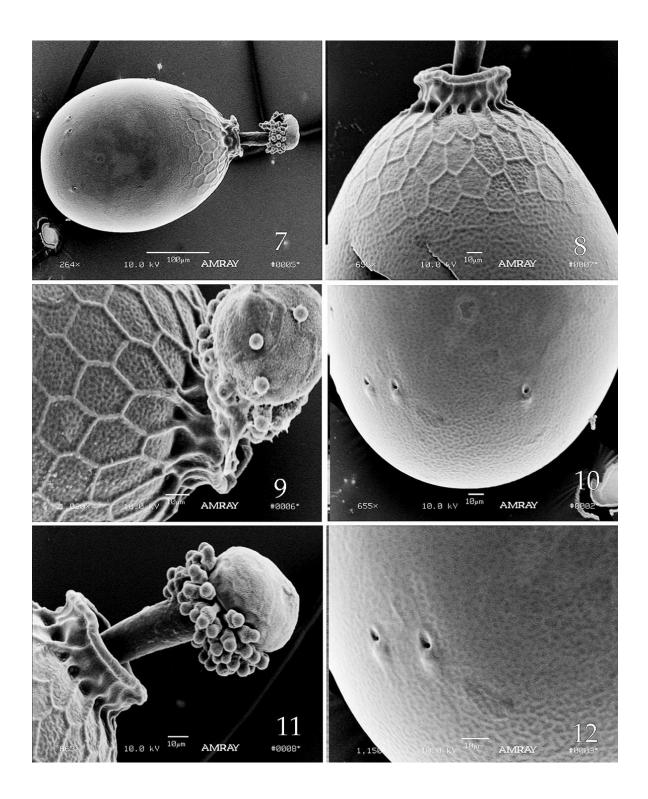
Phanoperla nana Zwick (Figs. 7-12)

Phanoperla nana Zwick 1982a:100. Holotype ♂ (Naturhistorisches Museum, Wien), Peradeniya, [Central Province, Kandy District], Ceylon [Sri Lanka]

Material examined. SRI LANKA: Matale District, Kibissa, 0.5 miles W Sigiriya Jungle, 28 June-4 July 1978, K.V. Krombein, P.B. Karunaratne, T. Wijesinhe, V. Kulasekare, 1♀ (USNM). Ratnapura District, Uggalkaltota, 23-26 June 1978, K.V. Krombein, P.B. Karunaratne, T. Wijesinhe, L. Jayawickrema, N. Karanaratne, 3♂, 8♀ (USNM).

Egg. Outline oval with broadly rounded anterior pole (Fig. 7). Length ca. 318-330 μm, equatorial width ca. 230-240 μm. Collar ca. 18-22 μm long and ca. 55-63 μm wide, margin slightly flanged and irregularly incised, sides covered with two rows of irregular meshes (Figs. 9, 11). Globular anchor subtended by a ring of grape-like clusters of small globular bodies and supported on a long, slender pedicel (Fig. 11). Chorion with shallow, obscure pits over entire surface (Figs. 9-10); follicle cell impressions enclose pits in the chorionic zone adjacent to the collar (Fig. 8), but are not evident over most of surface. Micropyles with slanted, almost circular orifices, without elongate sperm guides (Fig. 12); micropylar row subequatorial.

Comments. The anchor on the eggs of these



Figs. 7-12. Putative *Phanoperla nana* egg. 7. Lateral aspect. 8. Collar end. 9. Chorionic detail surrounding collar. 10. Lid and micropylar zone. 11. Collar and anchor. 12. Micropyles.

specimens is consistent with that of P. nana (compare Fig. 11 with Fig. 8e in Zwick 1982a) and the female is small, has widely separated ocelli, and the subgenital plate is undeveloped. Zwick's (1982a) comment on the P. nana egg chorion indicates the surface is "...very finely and densely punctate..." and in the egg image presented (Fig. 8e) there are no follicle cell impressions shown or mentioned in the description. Our figures show a small zone of follicle cell impressions surrounding the collar (Figs. 8-9), and a surface that is covered throughout with obscure pits. Consequently, these specimens might represent an undescribed sibling species in the P. nana-group, but because the aedeagus of males associated with the females at the Uggalkaltota site are consistent with the descriptions of P. nana provided by Zwick (1982a), we accept these females as the probable females of P. nana.

Phanoperla peniculus Kawai (Figs. 13-20)

Phanoperla peniculus Kawai 1968:115. Holotype ♂ (Limnologische Flussstation, Max-Planck-Instituts für Limnologie), Hogenakal Fall, Cauvery River, Madras, India

Phanoperla peniculus: Zwick, 1982a:106. Redescription

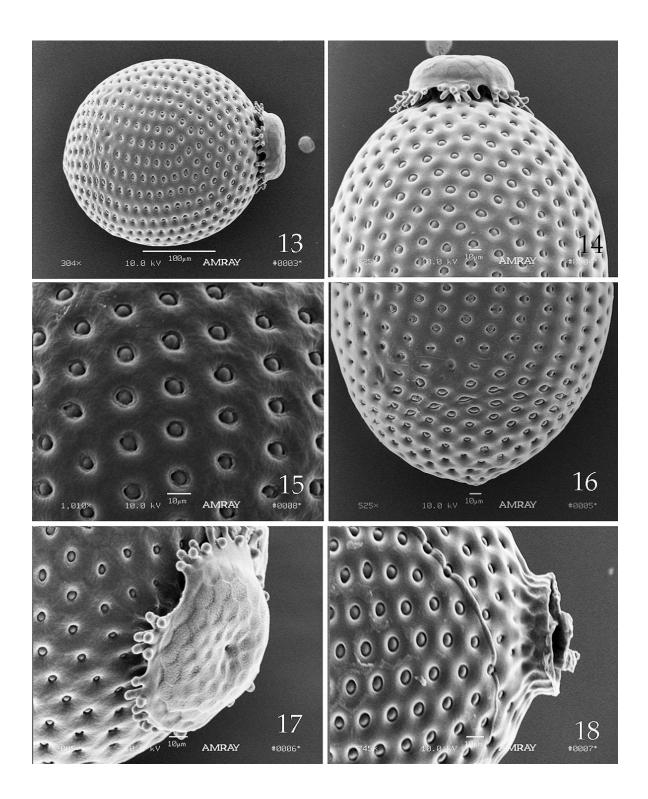
Material examined. INDIA: Karnataka, Agumbe Ghats, 13° 29.386′N, 75° 04.537′E, 9 October 2004, light trap, G. Svenson, 33♂, 24♀ (BYUC). Same site, 11 October 2004, canopy light trap, G. Svenson, 24♂, 11♀ (BYUC). Same site, 10-11 May 2004, canopy light trap, G. Svenson, 1♂ (BYUC).

Adult habitus. General color pale with pale brown markings; color white in alcohol. Head with a small brown area between ocelli, a small, median, curved, brown marking is present on the anterior frons and lappets and antennae are pale brown (Fig. 19). Pronotum with anterior, posterior and median sutures dark brown; disc with a narrow brown band along median suture and additional irregular brown markings on most of disc. Femora pale yellow-brown; tibiae pale brown. Wing membrane transparent; veins brown except C and Sc pale.

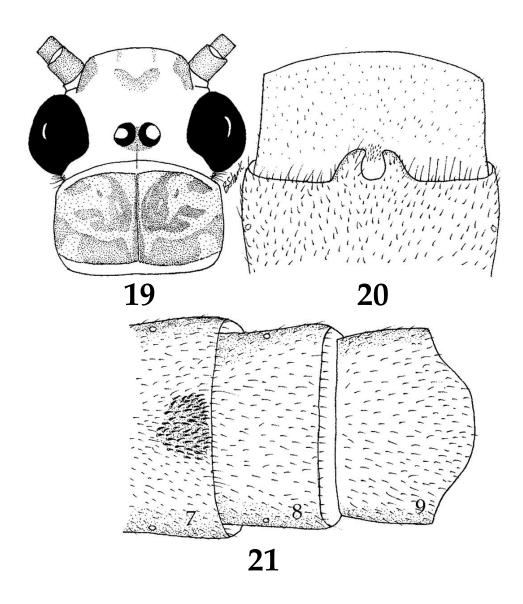
Putative Female. Forewing length 8-9 mm. Subgenital plate bilobed with U-shaped notch between lateral lobes (Fig. 20). Sternum 8 relatively hairy; posterior margin and subgenital plate lobes with long setae; area anterior to subgenital plate bearing a thick patch of shorter setae. Sternum 9 with sparse setation; minute hairs present in a dense patch between projecting subgenital plate lobes.

Egg. Outline oval. Length ca. 300-310 μm, equatorial width ca. 250-265 μm (Fig. 13). Collar short, ca. 12 μ long and ca. 72 μm wide (Fig. 18). Anchor medusa-like with short tentacle-like processes in clusters around the margins (Figs. 14, 17); hexagonal imprints visible in membrane of anchor Fig. 17), but globular bodies absent (perhaps removed during ultrasonic cleaning). Chorion covered throughout with large, ca. 8-9 μm diameter pores (Figs. 13, 15), arranged in curved rows of ca. 15-16 pores between collar and base of lid; pores slightly smaller near collar and on lid. Micropyles not observed.

Comments. In addition to the holotype, Kawai's (1968) type series of this species includes a male and female paratype from the type locality and a paratype male from Chiengmai Province, Thailand. Zwick (1982a) discovered the aedeagus was missing for the paratype from India, and that the Thai specimen represents an entirely different species, P. simplex Zwick 1982a. Zwick (1982a) also reported another male specimen from Bhadravati, Mysore, India, and stated Jewett's (1975) records of this species from Assam, Thailand and Malaysia were in error. In the light trap material provided by the Monte L. Bean Life Science Museum, Brigham Young University, this is the only Phanoperla species represented among more than 50 male specimens collected in the Agumbe Ghats of southern India. A single species is also represented among the female specimens in these samples and we consider it the probable true female for this species. Adult males and females correspond in the obscure color pattern available, in size, and the large sample size comprised of only one type of male and one type of female. Although these data are inconclusive they do support the association.



Figs. 13-18. Putative *Phanoperla peniculus* egg. 13. Lateral aspect. 14. Collar end. 15. Equatorial chorionic detail. 16. Lid. 17. Anchor, oblique apical aspect. 18. Collar, lateral aspect.



Figs. 19-21. *Phanoperla* adult structures. 19. *P. peniculus* head and pronotum. 20. Putative *P. peniculus* female abdominal sterna 8-9. 21. *P. testacea* male abdominal sterna 7-9 showing ventral brush on sternum 7.

Phanoperla testacea (Hagen) (Fig. 21)

Perla testacea Hagen 1858:475. Lectotype ♂, designation Zwick, 1982a, (Museum of Comparative Zoology), Ceylon [Sri Lanka]
Phanoperla testacea: Zwick, 1982a:115

Material examined. SRI LANKA: Kandy District,

Kabaragala, Millomalai, 22-23 March 1975, S. Karunaratne, P.B. Karunaratne, 1♂ (USNM). Nuwara Eliya District, Kande-ela, 14 March 1973, Baumann, Cross, 1♂ (USNM). Nuwara Eliya District, stream below Lover's Leap, 2 April 1979, M.D. Hubbard, T. Wigesiuhe, 2♂ (USNM). Nuwara Eliya District, Horton Plains, 5 April 1978, M.D. Hubbard, T. Wigesiuhe, 1♂ (USNM).

Comments. The *P. testacea*-group proposed by Zwick (1982a) includes six formally recognized species and two recognized by informal designation (Table 1). Zwick (1982a) noted the male "...Ceylonese members of this group, the testacea-group sensu stricto, are the most difficult species in the genus..." to distinguish. He further indicated that specimens of females with eggs are essential for species recognition in the group. All specimens of P. testacea and P. wedda (see below) in this sample were consistent in lacking a sternal brush on segment 8, and in having one on segment 7 (Fig. 21), but the number of Rs veins, the details of the median patch of sensilla basiconica on tergum 9 and the aedeagal features varied, at least subtly, as detailed by Zwick (1982a). It seemed probable, given the number of male specimens we examined from sites where female specimens of *P*. wedda dominate, that Zwick's (1982a) indication, regarding the presence of sternal brushes on male abdominal segment 8 for Sri Lankan members of the testacea-group, might be in error. This has been confirmed now for small samples of P. limosa, P. srilanka, P. testacea and P. wedda (P. Zwick, pers. com., and our observations). Unfortunately, we have not had an opportunity to check this character for specimens of *P. ceylonica*, consequently we have not included this species in our revised key (below) to Sri Lankan and Indian Phanoperla males.

Phanoperla wedda Zwick (Figs. 22-27)

Phanoperla wedda Zwick 1982a:118. Holotype & (United States National Museum), Hasalaka Oya, Kandy District, Ceylon [Sri Lanka]

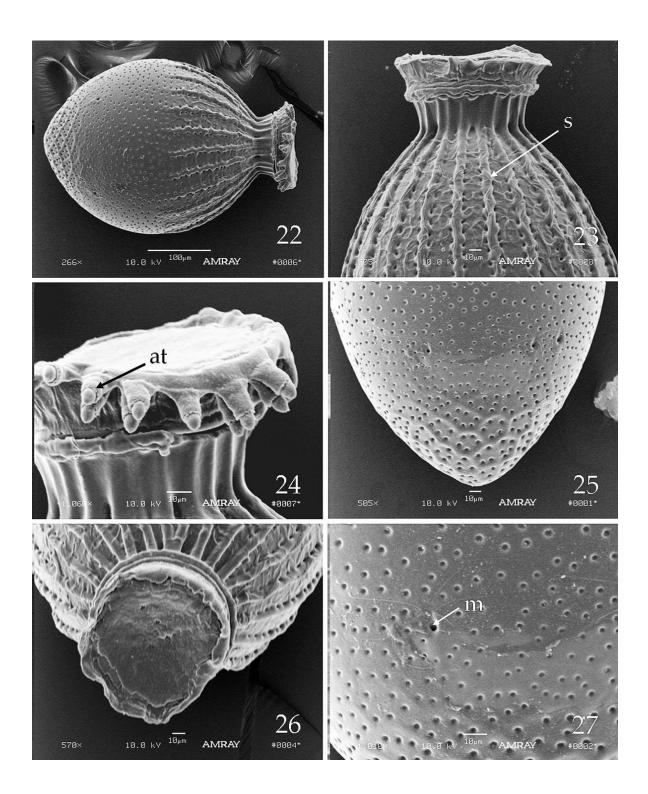
Material examined. SRI LANKA: Matale District, Kibissa, 0.5 miles W of Sigiriya Jungle, 28 June-4 July 1978, K.V. Krombein, 7♂, 7♀ (USNM). Ratnapura District, Balihyloya, 9-10 April 1978, M.D. Hubbard, T. Wijesinhe, 1♀ (USNM). Uggalkaltota, 23-26 June 1978, K.V. Krombein, P.B. Karunaratne, T. Wijesinhe, L. Jayawickrema, N. Karanaratne, 29♂, 15♀ (USNM).

Egg. Spindle shaped. Length ca. 364-381 μ m, equatorial width ca. 270-280 μ m (Fig. 22). Collar ca. 25-29 μ m long and ca. 102-114 μ m wide, margin

slightly flanged and irregularly incised (Figs. 23-24 µm), sides bearing a series of thick vertical ridges (ca. 10 in lateral aspect); ridges continue onto body of egg for ca. 142 µm, forming a series of narrow striae, subtended on each side by a row of punctations; broad sulci between striae without punctations (Figs. 22-23, 26). Mesal chorionic zone (ca. 178 µm wide) consists of rather widely spaced coarse pits which extend over micropylar row and become somewhat finer beyond micropyles (Fig. 25). Lid covered with follicle cell impressions, most containing five pits. Micropylar orifices circular, without prominent sperm guides (Fig. 27).

Comments. The scanning electron micrographs provide a few subtle chorionic details not observed in Zwick's (1982a) description. These include, 1) the extension of chorionic striae (or "ribs") onto the collar (Fig. 8), 2) the presence of FCIs on the lid, and 3) continuation of the fine punctations to the base of lid (not shown in Zwick's Fig. 24c, but noted in his description as "...punctures, very fine along otherwise invisible suture of lid;..."). Although the egg appears distinctive among the Sri Lankan species, several species (e.g. P. huang Sivec & Stark 2010b; P. magnaspina Sivec & Stark 2011; P. uchida Sivec & Stark 2010b) from other areas have eggs with a mixture of similar chorionic striae and punctations (Sivec & Stark 2010b; 2011). The common occurrence of this egg pattern in other species groups raises the question of whether the two named species in the testacea-group with unknown females (P. ceylonica, P. srilanka) might have eggs similar enough to those of P. wedda (also included in this group) to go undetected. We have examined at least one egg from every female listed above with light microscopy and have examined several eggs (ca. 10) from each of 10 females with SEM. No significant variation of the chorionic pattern has been detected but additional samples are needed from more localities to test this hypothesis.

The following key is modified from that of Zwick (1982a) but includes only males of species known from India and Sri Lanka. The Sri Lankan Plecopteran fauna is completely endemic, consequently the initial couplet is based on geographic separation.



Figs. 22-27. *Phanoperla wedda* egg. 22. Lateral aspect. 23. Collar end (s = stria). 24. Anchor and collar (at = anchor tentacle). 25. Lid and micropylar zone. 26. Anchor and collar, apical aspect. 27. Micropyles (m = micropyle).

Key to Males of <i>Phanoperla</i> Species Known from India and Sri Lanka (<i>P. ceylonica</i> not included)	7' Basolateral armature on everted aedeagal sac forms an obscure, small patch of minute spines Zwick 1982a, Fig. 22b); dorsum of everted sac bears a small membranous lobe near distal end
1 Known from Sri Lanka	of basolateral spine patch testacea 8 Hair brushes on abdominal sterna 4-5 well developed, but absent on sternum 7 sertispina
2' Hair brush present on at least abdominal sternum 7 (Fig. 21)	8' Hair brushes absent from abdominal sterna 4-5, but usually well developed on sternum 7 (Fig.
3 Rs vein with 2 branches (Zwick 1982a, Fig. 1e)	21)
3' Rs vein with 3-4 branches	9' Rs vein with 2 branches (Zwick 1982a, Fig. 1e)
subapical grouping of large black spines (Zwick 1982a, Fig. 8d); median sensilla basiconica on tergum 9 united into a single patch (Zwick	10 Aedeagal tube bearing a prominent, sclerotized pair of ventroapical horns (Zwick 1982a, Fig. 7a) cornuta
1982a, Fig. 8a)	10' Aedeagal tube without sclerotized ventroapical horns
of large black spines; median sensilla basiconica on tergum 9 form a pair of narrow elongate	11 Subapical area of everted aedeagal sac armed, in part, with one or more discrete linear or
patches (Zwick 1982a, Fig. 21b)	circumlinear patches of very large black spines (Zwick 1982a, Fig. 9a)
bag-like lobe	form a complete, irregularly double, subapical ring (Zwick 1982a, Fig. 9a)
like lobe	(Zwick 1982a, 6d); aedeagal sac without a membranous dorsal finger-like lobehimalayana
Fig. 21b)	13' Largest black spines on everted aedeagal sac form three widely separated subapical, linear groupings (Zwick 1982a, Fig. 13c); aedeagal sac bearing a membranous, dorsal finger-like lobe
Basolateral armature on everted aedeagal sac forms a long, slender, conspicuously rectangular patch (Zwick 1982a, Fig. 22d); dorsum of everted sac lacks a small membranous lobe located near distal end of basolateral spine patch	peniculus 14 Apical region of everted aedeagal sac bulb- shaped and densely armed with multiple rows of spines which decrease regularly in size approaching apex (Zwick 1982a, Fig. 25d); tube surrounding basal part of sac bears four lobes

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