

A new species of *Prosopistoma* Latreille, 1833 (Ephemeroptera: Prosopistomatidae) from northwestern Turkey

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The new mayfly species *Prosopistoma orhanelicum* sp. n. (Ephemeroptera: Prosopistomatidae) was collected from Orhaneli stream, northwestern Anatolia, Turkey. The main diagnostic larval characters are described and compared with two species which are found in nearby geographic regions. The larvae of *P. orhanelicum* sp. n. are differentiated by the number of antennal segments, antennal segment 2/3-5 ratio, maxillary palp seg 1/seg 2 ratio, width/length ratio of the head and posterolateral projections on abdominal segments 7-9.

Keywords: Ephemeroptera; Prosopistomatidae; Prosopistoma orhanelicum; new species; Turkey

Introduction

The Prosopistomatidae Lameere, 1917 is a small and little known family of Ephemeroptera. The larvae of the sole genus *Prosopistoma* Latreille, 1833 show atypical characters like a notal shield, resembling aquatic beetles. Because of their unique shape, *Prosopistoma* larvae were first described as a crustaceans (Geoffroy 1762). However, later research has proved that it is a mayfly taxon (Joly 1871; Joly and Joly 1872). To date, 19 *Prosopistoma* species have been described in the larval stage (Barber-James, Gattoliat, Sartori and Hubbard 2008), while only a few *Prosopistoma* adults have been described (Vayssière 1881; Gillies 1954; Campbell and Hubbard 1998). Five of the 19 species are found in the Palaearctic realm (Barber-James et al. 2008), two of them being found in nearby geographic regions of Turkey. *Prosopistoma pennigerum* (Müller, 1785) (syn: *P. foliaceum* (Fourcroy, 1785); see Hubbard 1979) is widespread in Europe (Eaton 1883–1888). *Prosopistoma phoenicum* Alouf, 1977 was first recorded in Lebanon (Alouf 1977) and later also reported from Syria and Israel (Koch 1988). The other three Palaearctic realm species are found in China (Tong and Dudgeon 2000; Zhou and Zheng 2004).

Prosopistoma was first recorded from Turkey by Koch (1985). He collected 15 larvae of *P. pennigerum* from Dicle (Tigris) River in Diyarbakır, southeastern Anatolia. Dalkıran (2006) also collected specimens of Prosopistomatidae larvae in Orhaneli Stream from

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Turkey. The diagnostic characters show that these specimens are new to science. In this paper, a new species of *Prosopistoma* is described from northwestern Anatolia, Turkey.

Methods

The material was collected using the kick-net method suggested by Klemm, Lazorchak and Peck (2000). Collected kick-net samples were fixed in 4% formaldehyde solution in the field. Benthic macroinvertebrate samples were picked by using a Prior stereo microscope and preserved in 70% ethanol. Thirty two specimens were dissected and mounted on permanent slides using Entellan. In three of the 32 specimens, both antennae had six segments, including the holotype. The drawings were made by means of a camera lucida attached to a Zeiss Axioplan research microscope. The measurements were made by using a micrometric ocular in the Prior light microscope with 10×40 magnifications. The main diagnostic characters were compared with notal shield (carapace) length using Pearson correlation analysis.

An air-drying method (Ubero-Pascal, Fortuno and De Los Angeles Puig 2005) was carried out to prepare the notal shield before the scanning electron microscopy (SEM) procedure. In this method, absolute ethanol was used as the intermediate liquid and tetramethylsilane (TMS) as the transition liquid. The notal shield was transferred directly to a double-sided tape-affixed to a stub and coated with gold–palladium in an AL–TEC SCD 005 Sputter Coater. The material was observed by SEM (EVO 40/CARL ZEISS) with working voltages of 20 KV.

Taxonomy

Prosopistoma orhanelicum sp. n. (Figures 1–12)

Material examined. Holotype: mature \bigcirc larva: northwestern Turkey, Bursa province, Orhaneli district, Orhaneli stream, Deliballılar site (39°55′56″ N, 28°58′21″ E), 01.XI.2001. 345 m a.s.l.

Paratypes: Northwestern Turkey, Bursa province, Orhaneli district, Orhaneli stream, Deliballılar site (39°55′56″ N, 28°58′21″ E), altitude 345 m: 1 larva 18.IV.2001; 4 larvae 24.V.2001; 7 larvae 26.VI.2001; 33 larvae 26.VII.2001; 2 larvae 28.VIII.2001; 27 larvae 25.IX.2001; 34 larvae 01.XI.2001; 12 larvae 22.XI.2001; 8 larvae 30.V.2002.

Çınarcık site (40°01'23" N, 28°47'53" E) altitude 220 m: 5 larvae 18.IV.2001; 7 larvae 26. VI.2001; 6 larvae 26.VII.2001; 2 larvae 28.VIII.2001; 14 larvae 25.IX.2001; 19 larvae 01.XI.2001.

Kestelek I site (39°57′28″ N, 28°35′24″ E) altitude 75 m: 1 larva 21.VI.2001; 1 larva 23. VIII.2001; 1 larva 24.IX.2001; 4 larvae 30.X.2001; 1 larva 19.XI.2001.

Kestelek II site (39°56'49" N, 28°32'14" E) altitude 50 m: 1 larva 23. VII.2001; 3 larva
e 24. IX.20011.

All specimens are deposited in Uludağ University, Art and Science Faculty, Biology Department, Hydrobiology Section, Aquatic Insect Collection, Turkey.

Description of the mature larva (holotype)

The notal shield (carapace) length along the median suture is 3.62 mm. The total length of the larva is 5.25 mm, excluding cerci (Figure 1). The width of the notal shield is $1.15 \times$ length. General colour is light brown. Darker brown markings on the notal shield are observed. The width of head is $2.3 \times$ length. The shape of the compound eyes is ovate, lateral ocelli are comma-shaped and median ocellus is triangular in shape



Figure 1. Prosopistoma orhanelicum sp. n., dorsal view of mature larva.

(Figure 2). Two symmetric lines cross the central part of the lateral ocelli (Figure 2). Dorsal face of labrum is densely covered with micropores, anterior edge convex (Figure 2). Circular ornamentations are observed on all body parts except legs (Figures 3 and 12c). Short, slender hairs, arising in pairs, from the same point, finely cover the notal shield and head (Figures 3 and 12e). Rarely, single and short slender hairs are observed on the notal shield (Figure 12f) and head. Both antennae are six-segmented, the second segment being longer than segments 3 and 4 together, while shorter than segments 3 to 5 together (Figure 4d). Antennae do not extend beyond the anterior margin of the head.

Labium is as in Figure 5. Anterior edge of labium is finely covered with simple short hairs. Labial palp is 3 segmented, segment 1 is the longest. Segment 2 is $0.79 \times \text{length of segment 1}$, segment 3 is $0.33 \times \text{segment 1}$.

Outer canine of mandible has three apical teeth, inner tooth slightly longer, inner margin of the outer canine has 6–7 and outer margin has 7–8 subapical small teeth (Figure 6a). Inner canine of mandible is shorter than outer canine, with two apical teeth. Inner margin has three subapical teeth and outer margin has 3–4 subapical teeth. Seven serrated long bristles arise from the base of the inner canine. A single unserrated bristle arises from the middle of the outer margin of the mandible.



Figures 2–5. *Prosopistoma orhanelicum* sp. n. (2) Dorsal view of head (half-grown larva); (3) view of ornamentations observed on all body parts except legs; (4) antennae, notal shield length (a) 1.4 mm, (b) 1.62 mm, (c) 2.63 mm, (d) 3.75 mm; (5) labium, anterior portion dorsal view, posterior portion ventral view. Scale lines 0.1 mm.

Maxilla has four long dentisetae and three long serrated bristles which arise from the base of apical dentiseta on galea-lacinia (Figure 7). A single unserrated short bristle arises below the base of apical bristles near the base of galea-lacinia. The maxillary palp is



Figures 6–9. *Prosopistoma orhanelicum* sp. n. (6) Mandible of (a) mature larva, (b) half-grown larva (antennae four-segmented); (7) maxilla of mature larva; (8) dorsal view of abdominal segments VII-X; (9) general view of egg dissected from mature larva. Scale lines 0.1 mm.

three-segmented, segment 2 is the longest, segment 1 is $0.63 \times \text{length}$ of segment 2, segment 3 is $0.22 \times \text{segment} 2$.

The inner margin of foretibiae has 7–8 spines (Figures 10b and 10d). Five of them are serrated and three of them are not serrated. The one additional apical serrated spine is located near the first spine, side by side. These serrated spines are found on the front half of the foretibiae (Figure 10b). The middle and hind tibiae have two apical serrated spines.

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One of these is located near the first spines, side by side as on the foretibiae. Diagonal ornamentations are observed on the femora (Figure 9c). Short, slender hairs, two of which emerge from the same point, finely cover the femora, tarsi and dorsal face of the tibiae, also seen on the notal shield and head. The ventral faces of the tibiae have 5–6 short, thick hairs reaching in the same direction (Figure 10d). Posterolateral projections on abdominal segments VII and VIII are truncate, on segment IX pointed (Figure 8). Six pairs of abdominal gills; the illustrations of gills 1–5 are given in Figure 11.

Description of paratypes (half-grown larvae and mature larvae)

General characters are same as holotype. Some descriptive characters are given in Table 1. The notal shield length along the median suture is 1.1-3.75 mm. The total length of the



Figure 10. *Prosopistoma orhanelicum* sp. n.; foreleg, (a) ventral view, half-grown larva (antennae four-segmented); (b) dorsal view, mature larva; (c) ornamentations observed on femora; (d) ventral view, mature larva; Scale lines 0.1 mm.

	<i>P. pennigerum</i> (Müller, 1785)	P. phoenicum Alouf, 1977	P. orhanelicum sp. n.
Antennae Antenna	5-segmented extending beyond anterior margin of head	5-segmented (rarely 4) not extending**	4–6 segmented not extending
Length ratio of antenna segment 2 to 3–5	equal	longer than other 4 segments (Alouf 1977)	shorter
Total length or notal shield length along median suture	total length 3–5 mm (Lafon 1952)	notal shield 2.5–3.0 mm (Alouf 1977) notal shield up to 3.3 mm (Thomas et al. 1988)	notal shield up to 3.75 mm total length up to 5.4 mm, excluding cerci
notal shield width/ length	1.2–1.38 (Lieftinck 1932) 1.0–1.3 (Lafon 1952)	1.0-1.1**	1.08–1.36
Head width/length Head with two symmetric lines Number of maxillary bristles	1.6–2.0 (Lafon 1952) crossing under lateral ocelli 2–3 (Eaton 1883–1888) 4 (Kluge 2004)	2.0 (Alouf 1977) not described 3	2.03–2.50 crossing central part of lateral ocelli 3 (rarely 2)
Outer margin of outer canine		6–9 subapical teeth (Alouf 1977) 6–11 subapical teeth (Thomas et al. 1988)	2-8 subapical teeth
Inner margin of outer canine		4–7 (Thomas et al. 1988) 4–9 subapical teeth (Alouf 1977)	2-7 subapical teeth
Outer margin of inner canine		2–3 subapical teeth (Alouf 1977)	1-4 subapical teeth
Inner margin of inner canine		2 subapical teeth (Alouf 1977)	1-3 subapical teeth
Number of mandible bristles	5–6 (Eaton 1883–1888)	5-8 (Thomas et al. 1988)	4-7 (rarely 8)
Maxillary palp seg 1/seg 2	Ratio: 1:1.5 (0.67) (Lieftinck 1932)	0.88–1.0 (Alouf 1977)	0.48-0.73
Labial palp seg 2/seg 1	Subequal (Lieftinck 1932)	0.75–0.77 (Alouf 1977)	0.73–0.92
Number of spines on foretibiae	7–8 (Vayssière 1890*)	3-7 (Thomas et al. 1988)	3-9 (rarely 10)
Ornamentations on femora	not described	not described	diagonal
Additional apical serrated spines on tibiae	described (Eaton 1883–1888)	not described	described
Double short hairs	present (Vayssière 1890*)	present**	present
Posterolateral projections on abdominal segments VII–IX	angulate, apex more pronounced (Lieftinck 1932)	apex pointed**	7 and 8 truncate 9 pointed

Table 1. Some descriptive characters of P. orhanelicum sp. n. compared with two related species.

*Observed from Vayssière's drawings, **observed from Alouf's (1977) drawings.



Figure 11. *Prosopistoma orhanelicum* sp. n.; gills; (a–e) gills I to V of half-grown larva (antennae five-segmented); 11(f, g) gill I and II of mature larva. Scale lines 0.1 mm.

larvae is up to 5.4 mm, excluding cerci. The width of notal shield is 1.08 to $1.36 \times$ length (Figures 1 and 12a). General colour is yellowish brown to light brown. Darker brown markings on the notal shield were observed but these markings varied from specimen to specimen. The width of head is 2.03 to $2.5 \times$ length. Antennae with four, five or six segments (Figure 4). In specimens with four antennal segments, the second segment is longer than segments 3 and 4 together (Figure 4b) and rarely equal length to segments 3 and 4 together (Figure 4a). In specimens with five and six antennal segments, the second segment is longer than segments 3 and 4 together, while shorter than segments 3 to 5 together (Figures 4c and 4d). Sometimes the left and right antennae do not contain the same antennal segment numbers in the same specimen, in combinations of 4 and 5 or 5 and



Figure 12. *Prosopistoma orhanelicum* sp. n. SEMs of notal shield; (a, b) general views; (c, d) two different types of circular ornamentation; (e, f) the hair types covering notal shield.

6. Labial palp three-segmented, segment 1 is the longest. Segment 2 is 0.73 to $0.92 \times \text{length of segment 1}$, segment 3 is 0.28 to 0.48 \times segment 1.

The inner margin of the outer canine has 2–7, the outer margin of the outer canine has 2–8 small subapical teeth. The inner margin of inner canine has 1–3, the outer margin of inner canine has 1–4 subapical small teeth. Four to seven (rarely eight) serrated long bristles arise from the base of the inner canine. The specimens which have four antennal segments mostly have five, sometimes 4–6 serrated long bristles on the mandible. However, the specimens which have five and six antennal segments contain six to seven, rarely eight serrated long bristles (Figures 6a and 6b). Maxillae have four long dentisetae and three (rarely two) long serrated bristles arising from the base of the apical spines.

The inner margin of foretibiae has 3–9, rarely 10 serrated and unserrated spines. The specimens which have four antennal segments contain 3–5 serrated fore-tibial spines (Figure 10a). However, the specimens which have five and six antennal segments contain

4–9, rarely 10 serrated spines. Sometimes the last spines are minute and not serrated. The serrated spines are found on the front $\frac{1}{4}$ to $\frac{1}{2}$ of the foretibiae (Figures 10a and 10b). The additional apical serrated spines are located near the first spine on each pair of tibiae. Sometimes these additional apical spines are not observed, perhaps due to the posture angle on each leg pair.

Paired, short, slender hairs cover the femora, tarsus and dorsal face of tibiae but their numbers increase as the notal shield length increases. The ventral faces of tibiae have 0–6 short, thick hairs reaching in the same direction (Figures 10a and 10d). Their numbers increase as the notal shield length increases. Asymmetries are observed in subapical teeth and serrated bristle numbers on the left and right mandibles, antennal segment lengths, and labial and maxillary palp lengths, and the foretibiae spines in one specimen.

The first gill of mature and half-grown larvae appears similar except for the last two branches at the anterior site (Figures 11a and 11f). The first two extensions are not branched in half-grown larvae (Figure 11a) while dichotomously branched in mature larvae (Figure 11f). This finding indicates that dichotomous branching is formed as notal shield length increases.

Pearson correlation analysis results show that head width and length, antennal segment length, antennal segment number, subapical teeth number of inner and outer canine of mandible, mandibular bristle number, foretibiae seta number, labial and maxillary palp length, labial palp segment 3/segment 1 ratio, maxillary palp segment 3/ segment 2 ratio and the spine number of ventral faces of tibiae all increase in relation to increased notal shield length (p < 0.01). However, the notal shield width/length ratio, head width/length ratio, antennal segment 2/segment 3 + segment 4 ratio, labial palp segment 2/segment 1 ratio and maxillary palp segment 1/segment 2 ratio and maxillary bristle numbers show insignificant correlations compared with notal shield length (p > 0.05). Maxillary palp seg 1/seg 2 ratio (r: 0.070, p: 0.708), antennal seg 2/seg 3 + 4 ratio (r: 0.106, p: 0.565) and the number of maxillary bristles seem to be the most stable diagnostic characters (r: 0.120, p: 0.513).

Eggs

The eggs were dissected from completely mature larva. The shapes of the eggs are oblong to oblong-ovate (Figure 9). The size of the egg is up to 90 \times 45 μ m.

Adult

Unknown.

Etymology

The name orhanelicum is derived from the name of Orhaneli stream and Orhaneli district.

Discussion

Alouf (1977) described two new *Prosopistoma* species, *P. oronti* Alouf and *P. phoenicum* Alouf. However, Thomas, Dia and Moubayed (1988) combined these two *Prosopistoma* species under the name of *P. phoenicum* Alouf. They also found that this combined species showed great diagnostic variation (Thomas et al. 1988). This great variation also appeared in some diagnostic characters of *P. orhanelicum* sp. n.

Gillies (1954) discussed the value of some larval diagnostic structures used as taxonomic characters. Gillies (1954) suggested that the markings of the notal shield were an important distinctive character. Gillies (1954) examined over 50 specimens of P. africanum Gillies and observed that two lateral pale areas were nearly always present. However, Thomas et al. (1988) observed that the notal shield markings varied from specimen to specimen. This variation was also observed in P. orhanelicum sp. n. When examining whole larvae under the stereo microscope, the notal shield darkening was observed. However, in the permanent slide, investigation of the notal shield under the compound light microscope, mostly no pigmentation, or rarely, indistinctive but irregular pigmentation was observed. This darkening may be observed due to shadowing of the muscle and internal organs of P. orhanelicum sp. n. However, SEMs indicated two different ornamentation types on the notal shield. The first type was circular and observed in all body parts except legs (Figures 3, 12b and 12c). The second type was smoother than the first type and never contained slender hairs (Figure 12d). Detailed microscopic research indicated that the second type ornamentation also varied from specimen to specimen. Because of these findings, the notal shield darkening or markings are not used as a distinctive character in *P. orhanelicum* sp. n. and it is also stated that *P. orhanelicum* sp. n. does not contain any distinctive notal shield markings.

Antennal segment number is one of the most distinctive taxonomic characters. Lieftinck's (1932) figure of the antennae of *P. wouterae* Lieftinck indicates a possible small, apical, sixth segment. Peters (1967) observed four-segmented antennae in three oriental species and six-segmented antennae in *P. lieftincki* Peters. According to these findings, Peters (1967) interpreted that the number of antennal segments varies within a species. Tong and Dudgeon (2000) also observed four antennal segments in the mature larva of *P. sinense* Tong & Dudgeon.

Gillies (1954) believed that antennal seg 2/seg 3+4+5 ratio form another easily distinctive character. Statistical analysis showed that antennal seg 2/seg 3+4+5 ratio was the second most important distinctive character. Antennal seg 2/seg 3+4 ratio was a more useful character than seg 2/seg 3+4+5 ratio, because some half-grown larvae contain four antennal segments.

Gillies (1954) believed that the foretibiae spine number, apical teeth number of inner and outer canine of mandible and mandible bristle number were the other important distinctive characters. In my opinion, these characters are distinctive characters but the values observed for each antennal segment number must be used. For example, *P. orhanelicum* sp. n. specimens which have four antennal segments usually have five, sometimes four and six mandible bristles and 3-5 foretibiae spines.

The main diagnostic larval characters are described and compared with two species which are found in nearby geographic regions (Table 1). This comparison shows that *P. orhanelicum* sp. n. is different from two related species by antennal segment number, antennal seg 2/seg 3+4+5 ratio, maxillary palp seg 1/seg 2 ratio, head width/length ratio and posterolateral projections on abdominal segments VII–IX.

P. orhanelicum sp. n. is distinctively different from *P. phoenicum* and *P. pennigerum* by antennal segment numbers. In this study, specimens which have six antennal segments are observed to have a notal shield length which varies between 3 and 3.75 mm. The notal shield lengths of the related species *P. phoenicum* were up to 3.3 mm (Thomas et al. 1988) while no findings were given as to any increase in antennal segment numbers. *P. pennigerum* has five antennal segments (e.g. Eaton 1883–1888; Lafon 1952). However, Katschalova (1965) reported six antennal segments in *P. pennigerum* which was collected in Daugava River in European part of former USSR (Latvia). However, illustrated figures

indicate that these specimens are different from *P. pennigerum* by the presence of additional serrated spines in middle and hind tibiae and two symmetric lines which cross the central part of the lateral ocelli.

The shape of mandible of *P. orhanelicum* sp. n. is also distinctively different from *P. phoenicum*. The joining point of inner and outer canines is at a lower point in *P. orhanelicum* sp. n. The subapical teeth of outer canine are smaller and more slender in *P. orhanelicum* sp. n. compared with *P. phoenicum*.

The one additional apical serrated spine is not reported in *P. phoenicum* (Alouf 1977; Thomas et al. 1988) while it was observed in *P. sinense* (Tong and Dudgeon 2000) and *P. lieftincki* (Peters 1967). However, Eaton (1883–1888) illustrated two spines in a figure of the mid leg in *P. pennigerum*.

In this study, statistical analysis showed that antennal segment number, foretibiae spine number, apical teeth number of inner and outer canine of mandible and mandible bristle number, increases as the notal shield length increases. This finding proved that increasing these characters as the notal shield length increases are related to the stages of maturation of *P. orhanelicum* sp. n.

Biology

This species was collected from Orhaneli stream from April to October 2001 and May 2002 at four sites, at altitudes ranging from 50 m to 354 m. The highest specimen numbers were collected in September 2001 at Delliballılar (35 specimens) and Çınarcık (19 specimens) sites. A few specimens were collected in some months at the last two stations. During the study period, the specimens were continuously observed at Deliballılar site. At this site, the water temperature was measured as $7.7-24.3^{\circ}$ C, the stream flow was $2.9-9.6 \text{ m}^3$ /s and pH was 7.8-8.6 from April to October 2001 (Dalkıran 2006).

Chironomidae head capsules were observed in the intestinal system of two of 32 dissected specimens of *P. orhanelicum* sp. n. This finding shows that *P. orhanelicum* sp. n. prefers carnivorous feeding.

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