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Larval redescription of *Prosopistoma pennigerum* (MÜLLER, 1785) from the River Volga near Rzhev, Tver Region, Russia (Insecta: Ephemeroptera)

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Abstract

Prosopistomatidae are a monotypic mayfly family, comprising at present about 25 nominal species, distributed throughout the Palaearctic, Oriental, Australian and Afrotropical Realms, but missing from the Nearctic and Neotropics. Larvae of *Prosopistoma pennigerum* (the species name '*pennigerum*' relating to the feather-like caudal filaments) had been discovered in 1762 by Étienne Louis Geoffroy in the neighbourhood of Paris and initially considered to represent a taxon related to *Triops* SCHRANK (Crustacea: Notostraca). Only in 1871, more than 100 years later, Émile Joly realized that the *'binocle à queue en plumet'* was in fact a mayfly larva. Although recorded from various rivers throughout Europe in the past, nowadays most populations are considered lost or extinct. In 2006 the species was found in the Volga River, which represented the first record from the Russian Federation. On the basis of this material a detailed larval redescription of *P. pennigerum* is provided and discriminating characters and their variation are compared with previous descriptions and illustrations. The morphological description includes micrographs of relevant features, produced with special image processing software. Presumptive larval habitat requirements of *P. pennigerum* as observed at the R. Volga are summarized and discussed in comparison with earlier records.

Key words: *Prosopistoma pennigerum*, larval characters, variation, taxonomy, ecology, Prosopistomatidae, Ephemeroptera

Introduction

A review of the mayfly family Prosopistomatidae, including 20 species, was provided by Schletterer and Füreder (2008). Barber-James *et al.* (2013) listed 22 species. Meanwhile, several new species have been described, i.e. *Prosopistoma amanzamnyama* Barber-James 2010, *P. mccaffertyi* Barber-James 2010, *P. ocellatum* Shi and Tong 2013, *P. alaini* Bojková and Soldán 2015 and *P. helenae* Bojková and Soldán 2015. The presently described number of species has increased from 20 (Schletterer & Füreder 2008) to 25 (including the recently described species as listed above). Prosopistomatidae occur throughout the Palaearctic (8 spp.), Oriental (9 spp.), Australian (2 spp.) and Afrotropical Realms (6 spp.), but they are entirely lacking in the Nearctic and Neotropics. Barber-James and de Moor (subm.) indicate that a total of 39 species are now recognised, though not all yet described, many of these from the Afrotropical realm (Barber-James 2009). An additional species could be expected from the Hindu Kush-Himalayan region (Schletterer & Füreder 2009).

Larvae of *Prosopistoma pennigerum* (the species name '*pennigerum*' relating to the feather-like caudal filaments) had been discovered in 1762 by Étienne Louis Geoffroy (1725–1810) in the neighbourhood of Paris ("environs de Paris") and initially considered to represent a taxon related to *Triops* SCHRANK (Crustacea: Notostraca). Only in 1871, more than 100 years later, Émile Joly realized that the *'binocle à queue en plumet'* was in fact a mayfly larva. Although recorded from various rivers in countries throughout Europe (France, Luxembourg, Netherlands, Germany, Austria, Portugal, Spain, Italy, Macedonia, Greece, Turkey, Algeria, Sweden, Czech Republic, Hungary, Romania, Georgia, Latvia and Russia) in the past, nowadays most populations are considered lost or extinct (Schletterer & Füreder 2009).

Morphological descriptions have been provided (among others) by Joly and Joly (1872), Vayssière (1890, 1925), Trägårdh (1911), Lafon (1952), Landa (1969), and Bojková and Soldán (2015); the latter providing a tabular summary of characters for all West Palaearctic representatives of *Prosopistoma*. The publication of Vayssière (1890) provided a comprehensive description of *P. pennigerum*, however the type material (collected by Geoffroy) is considered lost. A detailed and updated diagnosis of Prosopistomatidae Lameere, 1917 and *Prosopistoma* Latreille, 1833 were established by Barber-James (2010). The state of knowledge about synonyms, distribution and biology of *P. pennigerum* was reviewed and summarized by Bauernfeind and Soldán (2012). Recently, a micro-CT study of the anatomy of the nymph of *Prosopistoma pennigerum* was carried out based on Spanish material (Alba-Tercedor 2012; 2014). Molecular analysis (16S, 18S, Histone3) of specimens from the Volga River (Russia) and the Segura River (Spain) suggests that despite the different habitats the material is conspecific. In the same study, a comparison of different *Prosopistoma* spp. showed that there was a close relationship between *P. pennigerum* and the South African species *P. crassi* Gillies, 1954 (Barber-James *et al*. 2015).

In this paper we provide a redescription of the larva of *Prosopistoma pennigerum* (O.F. Müller, 1785) including a comparison of discriminating characters as figured by Vayssière (1890) with micrographs based on material from the Volga River. Further, we describe the habitat of *Prosopistoma pennigerum* in the Volga River and the associated zoobenthic fauna.

Material and Methods

Prosopistoma pennigerum was found in the headwaters of the Volga River in 2006, which represents the first record of this species from the Russian Federation (Schletterer & Kuzovlev 2007) and subsequent surveys revealed a viable population of this flagship species among freshwater invertebrates.

Fieldwork was carried out within the framework of the REFCOND_VOLGA project, a joint Russian-Austrian monitoring initiative in the headwaters of the Volga River. Herein we present data from annual surveys between 2006 and 2014. Samples were taken with a multi-habitat sampling method (modified after Hering *et al*. 2003). The material was rinsed through a 500 µm net and stored in ethanol (Table 1). In general *Prosopistoma* was sorted immediately after sampling and preserved in 96% ethanol, however sometimes this was not possible (e.g. for juveniles in June 2013). All samples were collected by M. Schletterer.

TABLE 1: Overview on the specimens collected from the Volga River at Rzhev with multi-habitat sampling and the calculated density (ind. m²); all sampling dates are indicated, in spring no larvae were found ("-"); some remarks are provided: "+" indicates that additional larvae are available from a certain date (i.e. additional qualitative sampling)

Date	Number of larvae in quantitative samples	Calculated density [ind. m²]	Remarks
2006-08-15	2	9	
2007-05-20	-	-	
2007-08-19	3	13	
2008-05-09	-	-	
2008-08-17	2	9	
2009-06-10	-	-	
2010-08-26	2	9	+
2011-08-07	5	22	
2012-08-20	3	13	+
2013-06-15	17	84	only juvenile larvae
2014-08-28	7	39	+

Specimens were deposited in the Natural History Museum, Vienna [NMW, partly on slides], the Albany Museum, Grahamstown South Africa [AMGS], the Tyrolean State Museum Ferdinandeum, Innsbruck [TSMF], the Zoological Institute of the Russian Academy of Sciences, St. Petersburg [RAS], as well as at Tver State Technical University [TSTU] and in the first authors private collection [SCHM, partly on slides]. Additionally micrographs of numerous morphological structures are available from the digital reference collection EUTAXA (Bauernfeind & Lechthaler 2014).

For mounting, specimens were dissected with head, body segments, legs and mouthparts removed and transferred into 10 % potassium hydroxide solution (KOH) to macerate muscles and other soft tissues. The remaining cuticular structures were embedded on slides in mounting medium (Euparal, Liquid de Faure), and (after drying) photographed under the microscope.

The images presented herein were created using the program Olympus Analysis Docu 5.1 (Extended Focal Imaging). Specimens were photographed with an Olympus XC30 digital camera, mounted on an Olympus SZX12 Stereo microscope and on a Nikon E600 light microscope.

Locality, associated zoobenthic fauna and ecological considerations

The locality (Plate 1: a, b) in the Volga River (56°15′31.89′′ N, 34°19′13.16′′ E) is situated about 1.0 km upstream of the main bridge of the city Rzhev (Russian, Tver Prov., Rzhev Distr.). The gauging station Rzhev is situated close to the locality, at river km 3274 (i.e. 257 km from the source): the altitude is 154.2 m, the catchment area 12.200 km² and the mean annual discharge amounts 94.24 m³/s (Shiklomanov 1999). The hydrological characteristics are given in Table 2. *Prosopistoma pennigerum* occurs in a fast flowing section (about 0.6 m s^{-1}) in approximately 0.5 m depth, where the substrate is formed by gravel and small stones (littoral).

TABLE 2: Hydrological characteristics (MMQ = mean monthly discharge, NMQ = lowest monthly discharge, HMQ = highest monthly discharge) at Rzhev (1891–1985; data source: Shiklomanov 1999).

	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
MMQ	43.6	37.5	49.1	262.3	165.2	117.7	86.0	58.0	68.2	93.8	88.9	60.3
NMQ	10.4	12.7	16.1	105.0	54.5	41.2	19.4	15.7	19.7	23.1	16.7	13.1
HMQ	109.0	102.0	150.0	547.0	485.0	228.0	185.0	195.0	201.0	219.0	309.0	228.0

The associated macrozoobenthic fauna at the locality Rzhev (Table 3) underlines the high integrity of this river reach and its importance as a European refugium for potamal fauna (Schletterer & Füreder 2009; 2013), as outstanding taxa like the stonefly *Xanthoperla apicalis* and the mayflies *Isonychia ignota*, *Ephoron virgo*, *Potamanthus luteus* as well as *P. pennigerum* occur there. Interestingly it seems that *P. pennigerum* is restricted to a small stretch of the River Volga (a meandering section at Rzhev), whereas it was not found in similar habitats up- or downstream, nor in the main tributaries.

A literature review revealed that most records of *P. pennigerum* were made up to a depth of 1.5 m (Schletterer & Füreder 2009). From River Daugava the species was recorded in the littoral in 0.3 to 1 m depth in the summer period and in 4.4 m depth in November, thus Kačalova (1965) hypothesized that the species is migrating to deeper areas during winter. In November the larvae were 3.5 mm long (without cerci), in May their size ranged from 4–5 mm and in June 5–5.6 mm, these larvae were already ready for emergence (Kačalova 1965). Within the present study extensive sampling activities were carried out in May and August (2006–2014), however *P. pennigerum* was not recorded in spring and only in August it was found with bodysize between 4.3 and 5.2 mm. Thus a migration to deeper zones at least in some live stages could be expected.

From the River Rhone subimagines were collected in June and July (Fontaine 1955), this would be in accordance with the observations of Kačalova (1965) in River Daugava. However the larvae from Volga River likely emerge in late August or September, since large larvae were only found in August. Since no adults were recorded from River Daugava it could be expected that (despite large larvae were present in June) emergence also took place later.

TABLE 3: Associated zoobenthic fauna (EPT taxa) found at the site Rzhev within the sampling campaigns between 2005 and 2014 together with *P. pennigerum.*

Group	Taxa
Ephemeroptera	Baetidae spp., *Baetis muticus* (LINNAEUS, 1758), *B. tracheatus* KEFFERMÜLLER & MACHEL, 1967, *Baetis tricolor* TSHERNOVA, 1928, *Caenis lactea* PICTET, 1843, *Caenis luctuosa* (BURMEISTER, 1839), *Caenis pseudorivulorum* KEFFERMÜLLER, 1960, *Caenis* spp. STEPHENS, 1835, *Centroptilum luteolum* (O.F. MÜLLER, 1776), *Cloeon dipterum* (LINNAEUS, 1761), *Ecdyonurus insignis* (EATON, 1870), *Ephemera lineata* EATON, 1870, *Ephemerella (Serratella) ignita* (PODA, 1761), *Ephoron virgo* (OLIVIER, 1781), *Heptagenia sulphurea* (MÜLLER, 1776), *Isonychia ignota* (WALKER, 1853), *Paraleptophlebia cincta* (RETZIUS, 1783), *P. submarginata* (STEPHENS, 1835), *Potamanthus luteus* (LINNAEUS, 1767), *Procloeon bifidum* (BENGTSSON, 1912), *Siphlonurus aestivalis* (EATON, 1903)
Plecoptera	*Amphinemura cf. sulcicollis* (STEPHENS, 1836), Chloroperlidae spp., *Leuctra fusca* (LINNAEUS, 1758), *Isoperla* BANKS, 1906, *Isoperla obscura* (ZETTERSTEDT, 1840), *Diura bicaudata* (LINNAEUS, 1758), *Xanthoperla apicalis* (NEWMAN, 1836)
Trichoptera	*Brachycentrus subnubilis* CURTIS, 1834, *Ceraclea annulicornis* (STEPHENS, 1836), *Cheumatopsyche lepida* (PICTET, 1834), *Hydropsyche contubernalis* MCLACHLAN, 1865, *Hydropsyche ornatula* MCLACHLAN 1878, Limnephilidae, *Molanna angustata* CURTIS, 1834, *Polycentropus flavomaculatus* (PICTET, 1834), *Psychomyia pusilla* (FABRICIUS, 1781), Goeridae spp.

It is emphasised that *P. pennigerum* is predacious at least in some life stages (Schletterer & Füreder 2009), because although Fontaine (1980) claimed non-predacious feeding habits for *P. pennigerum*, other authors reported that the species is feeding on chironomids and oligochaetes (Trägårdh 1911).

In a review about the ecology of larvae of the family Prosopistomatidae, substratum (lithophilous larvae) and current (rheophilous larvae) were identified as key factors for their distribution (Schletterer & Füreder 2009). At the locality at Rzhev in the Volga River suitable habitats are present, as described above. The organisation of the larval body supports the life in such habitats: between carapax and sternum two strong muscle strings are located, that support the "suction cup effect", i.e. the larvae can cling to the stone. In combination with the stream-lined body this enables *Prosopistoma* to inhabit stony substrate in areas with strong current.

In addition the distribution of *P. pennigerum* is also considered to be linked to basin scale and temperature regime (potamophilous larvae) (Schletterer & Füreder 2009). The factor temperature regime applies for all habitats across Europe, i.e. suitable temperatures can be found in potamalic sections of large rivers, but also in smaller (mediterranean) rivers. As the most frequent records of *P. pennigerum* came from potamalic water bodies in France, Germany and Latvia, basin scale was considered as a trigger factor for their presence. This is not always the case, as the species is also found in smaller rivers. However, it can be assumed that pollution in potamalic water bodies reduced the populations in the large European rivers (Schletterer & Füreder 2009) and some populations survived in refugial habitats, i.e. smaller rivers.

The disappearance throughout Europe is possibly linked with structural characteristics of the species. The gills are located in a gill chamber, which is divided by gill II into an upper and lower chamber: water is sucked up from the dorsal side into the gill chamber and emitted through two orifices on the ventral side. This indicates that the species needs oxygen-rich water, thus we assume that pollution and subsequent oxygen reduction was the critical point for populations in potamalic water bodies across Europe. The controlled emission of water from the gill chamber also enables fast swimming ("kickback") of the specimens in the free-water, which was documented while specimens were observed in an aquarium in the field (right after sampling). This may be a useful feature for switching between micro-habitats or to avoid predators.

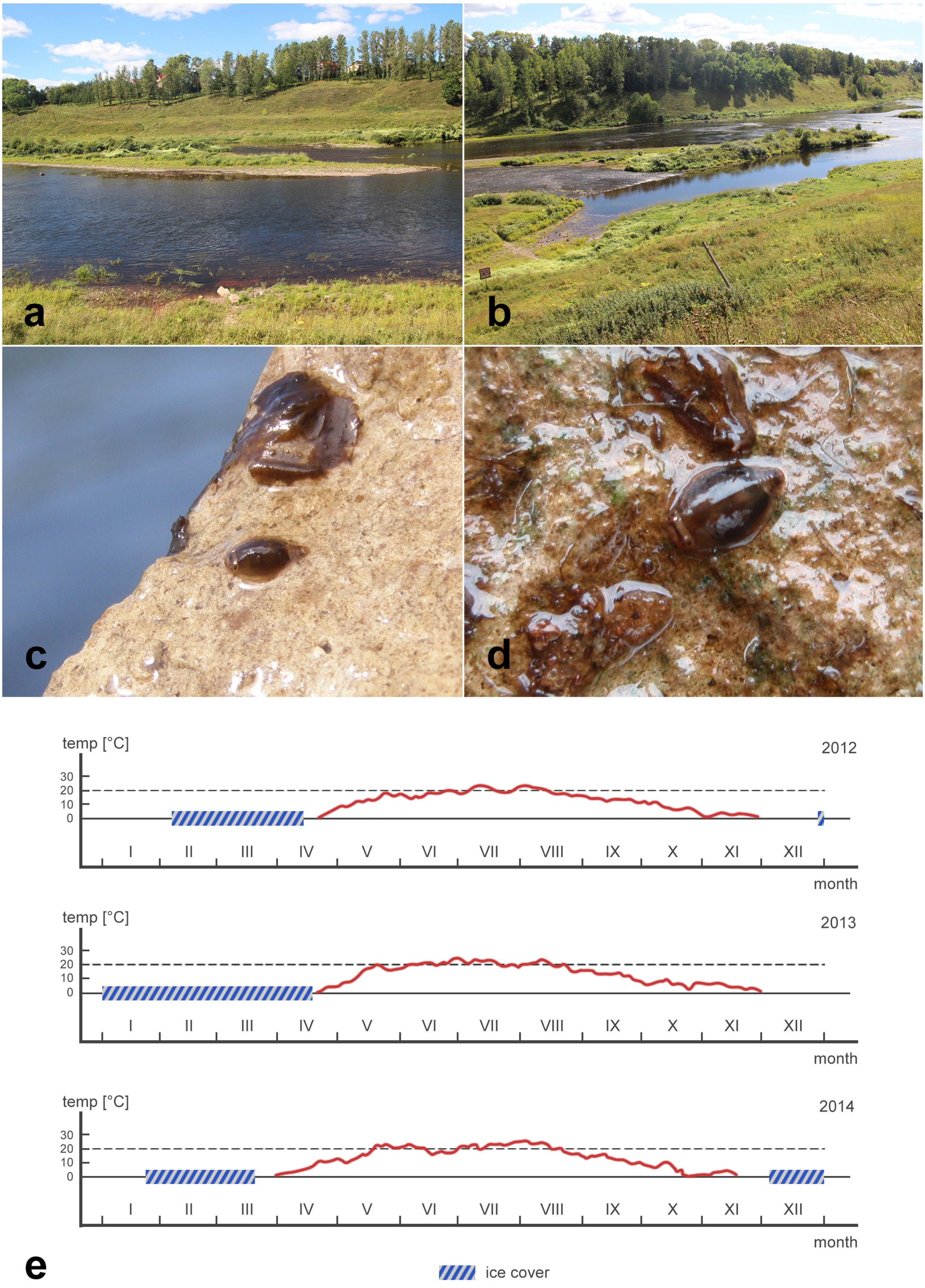

PLATE 1. Volga River at Rzhev (a, b), larva of *P. pennigerum in situ*, clinging to stone surface (c, d), water temperatures (redrawn from Roshydromet data) for the years 2012–2014 (e).

Results

Descriptions

Mature female larva.
Colouration dark olive-brown in life (Plate 1: c, d), brownish to cream-coloured in preserved specimens. Coloration pattern rather variable (Plate 2). Head usually lighter, yellowish, a darker brownish angular transversal band connecting the bases of antennae with the middle ocellus (and sometimes extending towards the posterior margin of head capsule). Carapace frequently with a light marginal area (creating a flange or rim-like impression), more central area variously darker but without distinct pattern.

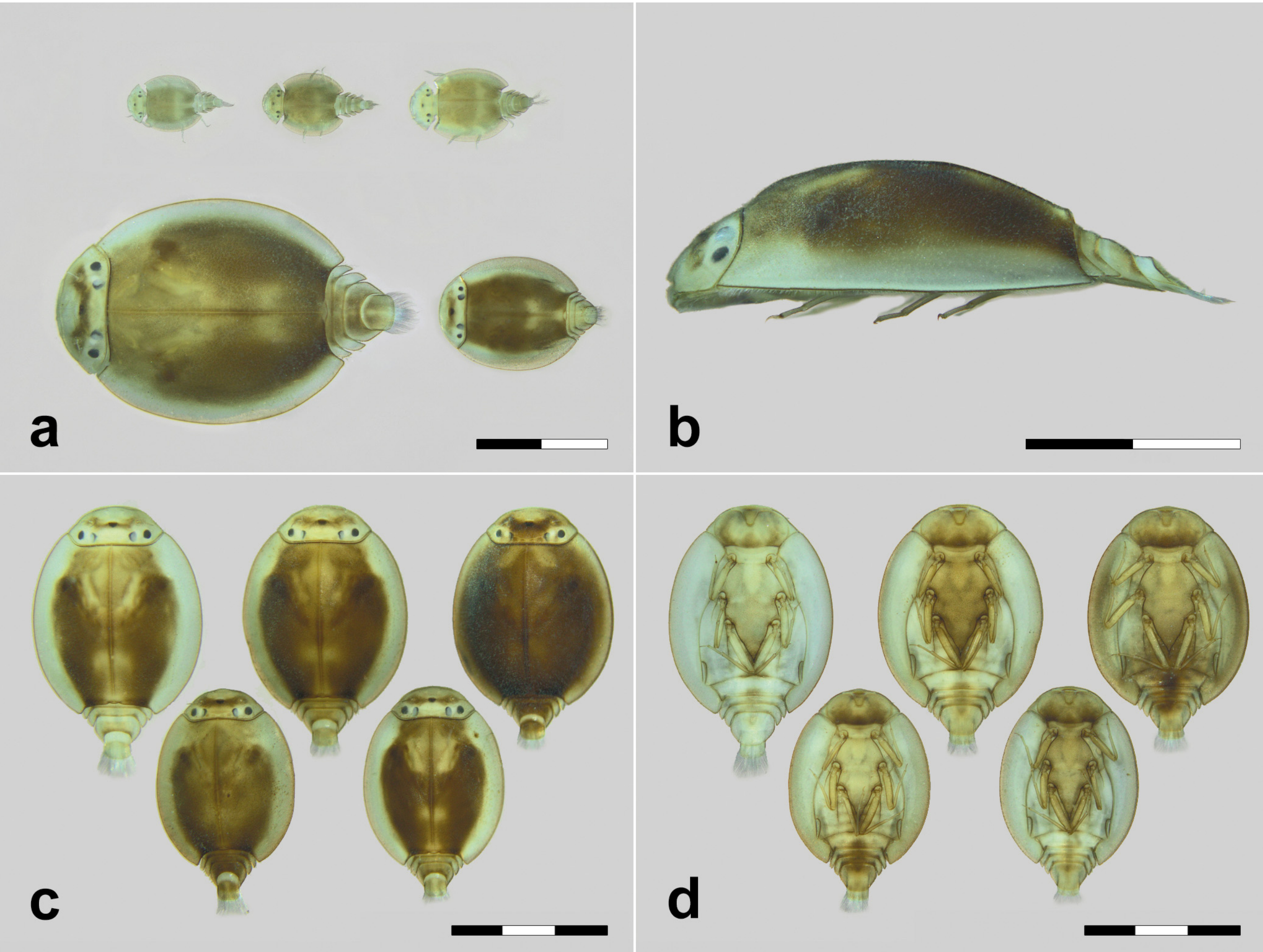

PLATE 2. *Prosopistoma pennigerum*: size range and colouration pattern of material from the Volga River (a). Mature larva, habitus in lateral view (b), full grown larvae in dorsal view (c) and ventral view (d). Scale bar divided into 1 mm sections.

Body length 4.10–5.20 mm (n: 7), without cerci. Head two times wider than long. Compound eyes round, of the same size as ocelli. Antennae relatively short, not reaching margin of head (but may appear to protrude, depending on angle of vision). Antennae 6- or 7-segmented (Plate 3: b1, b2) sometimes differing between left and right antenna, 7th segment very short), scape frequently retracted and not visible from above. Segment 3 approximately as long as segments 4–6 together (Plate 3: b2), ultimate segment with subapical sensory bristle. Epicranial suture closely anterior to lateral ocelli (but may appear to divide ocellus in slide preparations due to dislocated dark pigment). Carapace slightly wider than long, length-to-width ratio 0.92–0.94 (n: 7). Posterior dorsal margin of carapace in midline with a slight, rounded notch (as e.g., in *P. crassi* Gillies, 1954) above exhalent orifice. In lateral view (Plate 2: b), carapace strongly convex, depth of carapace divided by length of carapace is 0.33–0.34 (n: 7). Lateral rim or flange poorly developed (contrary to impression suggested by lighter margin when seen from above). Surface of head, carapace and sternal plate with characteristic scale-like structure (chagrin structure), protuberances either pointed or rounded and accordingly creating a punctured or pitted impression with interspersed simple (especially along margins), bifid (on the surface) or multifid (along margins) setae. Sternal plate elongate triangular, tapering posterior end reaching the distal quarter of hind femora.

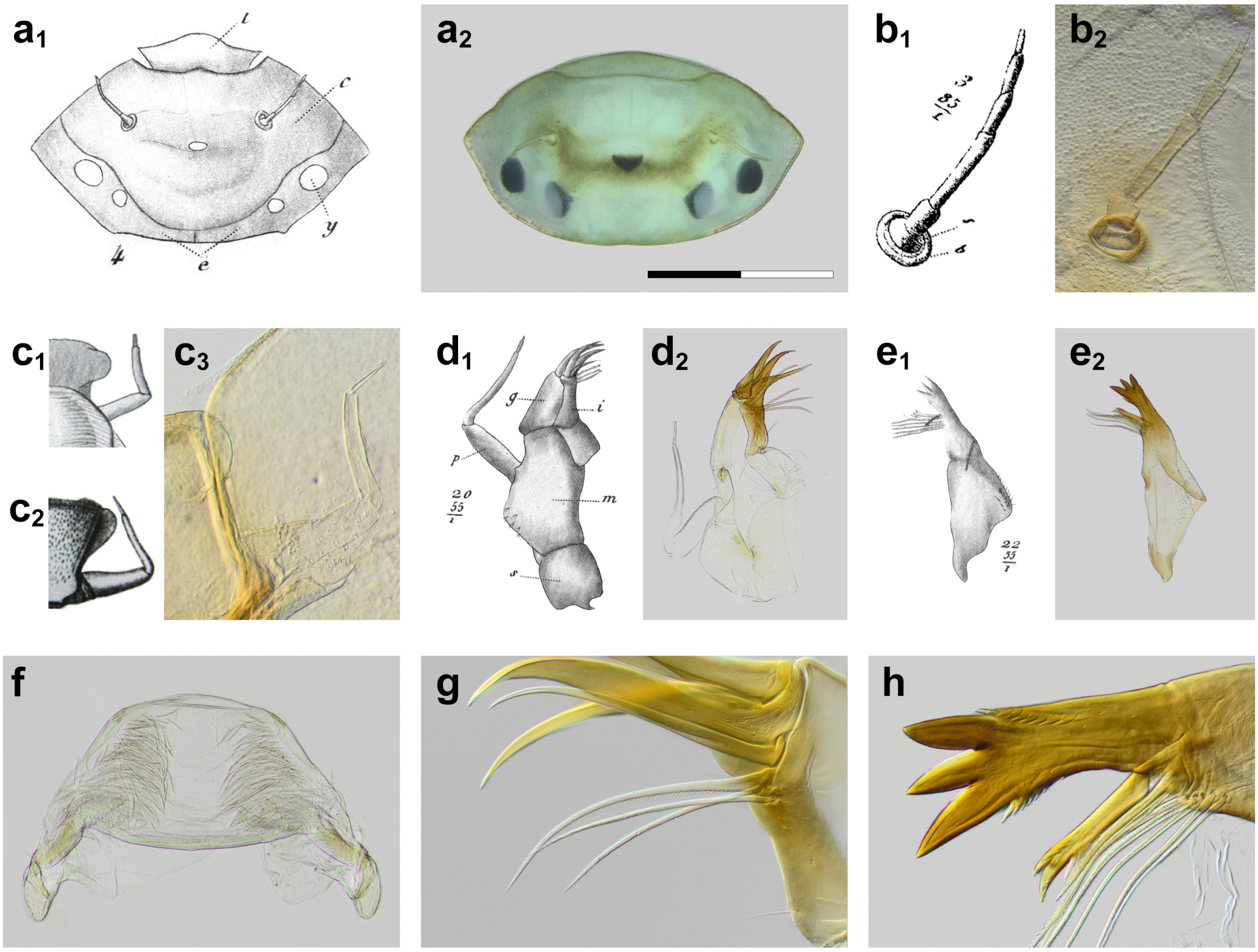

PLATE 3. a1: Head in dorsal view (Vayssière 1890); a2: head in dorsal view (scale bar divided into 500 µm sections); b1: right antenna (Vayssière 1890); b2: right antenna; c1: left labial palp (Vayssière 1890); c2: labial palp (Eaton 1884); c3 labial palp; d1: right maxilla (Vayssière 1890); d2: maxilla; e1: left mandible (Vayssière 1890); e2: mandible; f: hypopharynx; g: subapical bristles on maxilla (detail); h: apical part of mandible (detail); all micrographs from Volga specimens.

Mouth parts. **Labrum** short, almost three times wider than long, with strongly sclerotized margins and pitted surface. Anterolaterally a dense fringe of fine, long and short multifid setae, the anteromedial part slightly convex with only few short multifid marginal setae and numerous peg-shaped sensory structures along the ventral margin. **Mandible** slender, outer canine with three strong teeth of approximately equal length, inner and outer margin with 7–8 small subapical teeth (Plate 3: e2). Inner canine distinctly narrower, about 1/3 of outer canine width, and 1/3 shorter, the tip with two teeth and inner and outer margin equally with 3–4 minute subapical teeth. A row of 7–8 long bristles with serrate margin arises from the base of the inner canine (Plate 3: h). A single strong bristle (with finely feathered margins and bifid tip) is situated lateromedially near a dense field of sensory pits, peg-shaped sensilla and multifid setae. Left and right mandible isomorphic. **Maxilla** apically with canine and three moveable strong dentisetae of subequal length, third dentiseta distinctly narrower (Plate 3: d2). A longitudinal row of three strong bristles (Plate 3: g), the first finely serrated, is situated closely below the dentisetae. A single, much finer, bristle situated on inner margin in proximal part of sclerotised galea. Maxillary palp 3-segmented, terminal segment with apical and subapical sensory bristle. Ratio segments 1 : 2 : 3 = 3 : 4.5 : 1. **Hypopharynx** broad and comparatively short, superlinguae dorsolaterally fused with lingua, inner margins beset with long fine setae (Plate 3: f). **Labium** (Plate 5: c) with mentum, glossae and paraglossae fused and forming an integral distally widened plate (terminology follows Barber-James 2006). Anterior margin of prementum with a few multifid setae near lateral corners, central area of margin without bristles or setae but with an irregular row of peg-shaped sensilla. Dorsal surface with a few scattered peg-shaped sensilla and a prominent stout single multifid bristle near anterior corners, ventral surface with chagrin-structure and scattered fine multifid setae. Anterior margin of postmentum fringed with long fine multifid or multiciliate setae. Labial palps rather delicate (Plate 3: c3), 3-segmented, ratio segments 1 : 2 : 3 = 3 : 2.5 : 1, tip of terminal segment with subapical sensory bristle.

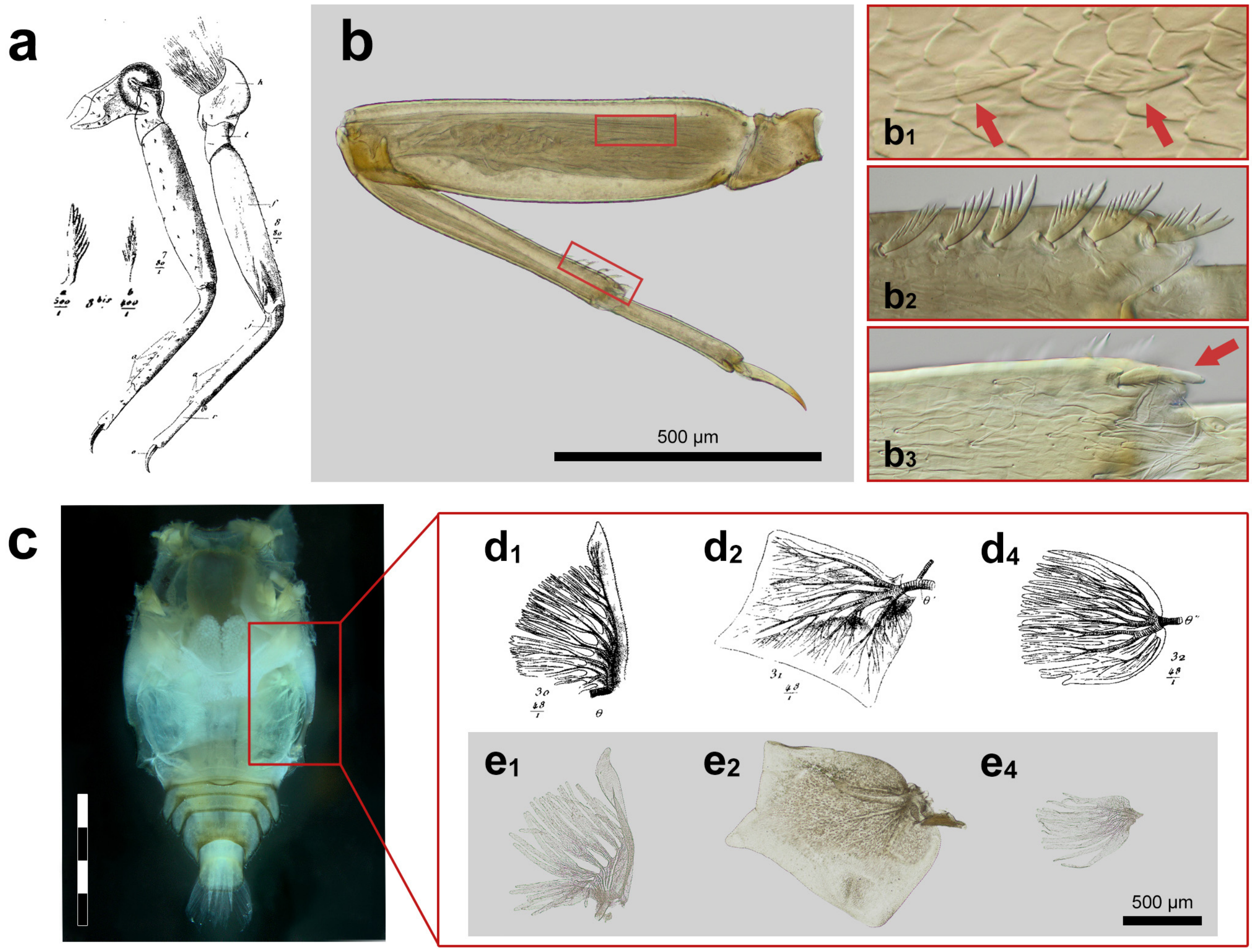

PLATE 4. Fore leg in dorsal and ventral view, drawings from Vayssière (1890: pl. 2) (a). Fore leg (b): chagrin pattern and bristles (arrows) on femur dorsal surface (b1), bristles on the anterior third of ventral margin of fore tibia (b2) and terminal broad and serrated bristle (arrow) on inner side (b3). Gill chamber (carapace removed; scale bar divided into 500 µm sections) in dorsal view (c), gill 1 (d1 / e1), gill 2 (d2 / e2), gill 4 (d4 / e4) (drawings from Vayssière (1890: pl. 5); all micrographs from Volga specimens.

Legs (Plate 4: a, b). Surface of coxae and femora with chagrin-structure. Fore femur basomarginally with 5–7 multifid bristles followed by approximately 10 strong pointed bristles along dorsal margin, submarginally a few stout multifid bristles in proximal third (Plate 4: b1), surface with a few scattered single pointed bristles. Tibia with long bifid bristles on inner side and forming a group of 4–6 near apex, outer side with a few (2–5) single strong bristles forming an indistinct row. Distal third of ventral margin of tibia with a row of conspicuous bristles, consisting of 2–4 simple strong bristles and 5–8 strong unilaterally deeply serrated bristles (Plate 4: b2), terminally an additional broad and serrated bristle on the inner side (Plate 4: b3). Tarsus with bifid bristles, tarsal claw slender and regularly curved. Mid and hind legs with successively decreasing number of marginal bristles on femur, ventral margin of tibia with a terminal pair of serrated bristles (one apically pointed on outer side, the second apically truncate or rounded on inner side of tibia).

Gills (Plate 4: c, d1- d4, e1–e4). Gill 1 with long and slender laminate dorsal part with finely serrated margins and prolonged rounded tip, ventral part filamentous, consisting of approximately 10 main stems further divided into 24–28 filaments. Gill 2 roughly quadrangular or trapezoidal with concave outer margin, forming a broad lamella which covers gills 3–5. Gills 3–5 successively slightly decreasing in size, filamentous, the filaments dichotomous; gill 3 with 6–7 main stems (app. 18 filaments), gill 4 and 5 with 4–5 main stems (app. 14 filaments). Gill 6 very small, inserted nearer the midline, somewhat pediculate with distal part very delicate, irregular quadrangular (app. ¼ the length of gill 5).

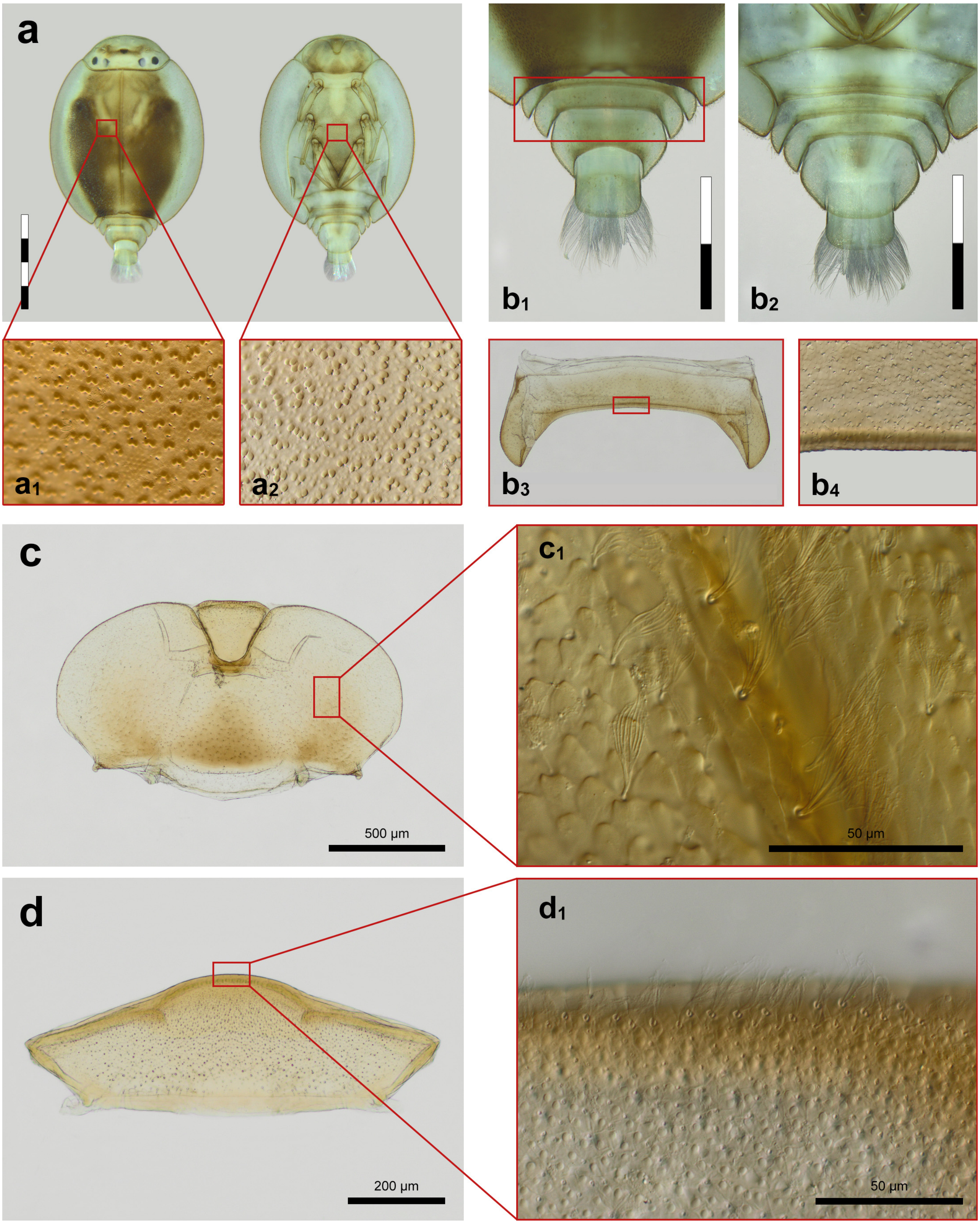

PLATE 5. *P. pennigerum* , habitus in dorsal and ventral view (a), carapace surface (a1) and sternum surface (a2). Abdominal segments 7–10 in dorsal and ventral view (b1, b2), tergum 8 (b3) and hind margin tergum 8 under higher magnification (b4). Labium (c) and ventral surface structure (c1). Labrum (d) and frontal margin with dorsal surface structure (d1); all micrographs from Volga specimens. Scale bar (in a, b1 + b2) divided into 500 µm sections.

Abdomen. Abdominal segments 1–6 fused, terga 7–10 separated and visible from above. Surface with characteristic scale-like structure (chagrin structure), with scattered (?) chloride cells, simple and bifid setae. Lateral processes on segments 7–9 more or less bluntly pointed *in situ* seen from above (Plate 5: b1) tips of segments 7 and 8 appear truncate if flattened in slide preparations (Plate 5: b3). Paraproct plates rectangular, length to width app. 2.2 : 1, margin with strong and pointed bristles. Cerci about twice longer than tergum 10, terminal filament subequal to cerci. A dense row of long pointed setae present along inner and outer margin of cerci and paracercus.

Younger larvae. The number of larval instars is not known for *P. pennigerum*. In the material examined considerable structural differences were observed between larvae with carapace length above (respectively, below) 2.5 mm. In larvae with carapace length between 1.8–2.5 mm (n: 6) the length-to-width ratio of carapace was 0.72–0.85, antennae were 4- or 5-segmented, mandible with a row of 3–6 strong bristles, subapical teeth on margins of outer incisor 3–4, on margins of inner incisor 0–1. Number of strong bristles along margin of fore tibia ranged between 1–4, and bristles were mostly not pectinate but rather club-shaped with margin entire. Length ratio of segments of maxillary palp and labial palp varied considerably depending on size of larva.

Discussion

We described only female larvae, as no mature male larvae were present in our material. This could indicate geographic parthenogenesis in *P. pennigerum*. Previously, Campbell and Hubbard (1998) hypothesised that *P. pearsonorum* could have a parthenogenetic life cycle, as no male imagines or subimagines were ever collected.

Most structural characters from material collected from the Volga River agree in detail with those described and figured by Vayssière (1890). The only discrepancies concern overall body shape (length-to-width ratio of carapace 0.76) and relative length of terminal segment of labial palp (segment 2 : segment 3 = 4 : 1). Most probably, both differences may be attributed to inaccuracies[1] of the engraver: the drawings in Eaton (1884, pl. XLIII), based on material from Vayssière, exhibit a length-to-width ratio of carapace of 0.90 (labial palp segments 2 : 3 = 3 : 1) and also the measurements by Lafon (1952: 433), material originating from the Rhone River near Lyon, agree well with our material.

In their tabular comparison of West Palaearctic representatives of *Prosopistoma* (Bojková & Soldán 2015: 114, Table 1), a total of 19 characters were listed and compared. Again, material from the Volga River agrees with most of the characters given for *P. pennigerum*, whereas differences concern the colour pattern of carapax (Bojková & Soldán 2015: 'distinct') and the relative length of antennae (Bojková & Soldán 2015: 'reaching anterior margin of head'). The number of unilaterally serrated bristles on fore tibia (Bojková & Soldán 2015: 'No. of pectinate spines [sic] on inner [= ventral] margin of fore tibia: 10–11') obviously includes bristles situated along the outer side (9) and apically on inner side (2) of the ventral margin, exactly as already distinguished by Vayssière (1890: 52 and pl. 2, figs 7, 8).

Characters frequently used for identifying species-group taxa (e.g., Peters 1967; Soldán & Braasch 1984; Dalkiran 2009; Barber-James 2010; Shi & Tong 2013; Bojková & Soldán 2015) within *Prosopistoma* include:

1. **Relation length : width of head capsule**
2. **Relation length of outer and inner mandibular canine**
3. **Outer and inner mandibular canine (shape and number of apical teeth)**
4. **Number and structure of long bristles arising from base of apical spines of galea-lacinia**
5. **Relation mesonotum maximal width : length (measured along median suture)**
6. **Posterior dorsal margin of carapace in midline (straight, with concave notch , with convex projection)**
7. **Posterolateral projections on abdominal segments 7–9 (apex rounded, truncate or pointed)**
8. Relative length of antennae (reaching / not reaching anterior margin of head capsule)
9. Number of antennal segments
10. Relation length antennal segment 3 : combined length of remaining antennal segments 4–6 (7)
11. Number and structure of long bristles arising from base of inner mandibular canine

1. Segments may have not been arranged strictly in a plane or the drawings had been based on larvae of different age classes.

12. Outer and inner mandibular canine (relative length and number of inner & outer marginal teeth)
13. Relation length of segments of maxillary palp
14. Relation length of segments of labial palp
15. Number and position of bristles on ventral margin of prothoracic tibia (serrated & simple strong bristles)
16. Colour pattern of carapace
17. Shape of dorsal part of gills 1 and 2

Our analysis confirms that some of the characters (**1–7**) listed above show little or no intraspecific variation or age-related developmental changes in larvae of *P. pennigerum*, whereas characters 8–17 are subject to considerable age-related developmental changes. Nothing is so far known about possible differences between male and female larvae, if indeed male larvae exist.

TABLE 4: Comparison of structural characters for *P. pennigerum* (data from Vayssière 1890 and from the present study)

Structural character	Material / source	
	Vayssière 1890	present study (specimens from R. Volga; n: 13)
carapace length	3.36 mm	0.7–3.5 mm
length-to-width ratio of carapace	0.76	0.90
antenna-segm.	6	4–7
maxilla-bristles	3	3
mandible-bristles	5	3–7
maxillary palp; segm. 3 : segm. 2	1 : 5	1 : 3–5
labial palp; segm. 2 : segm. 3	4 : 1 Lafon* (1952) 2 : 1 * pl. 3, photograph	1.5–2.5 : 1
fore tibia–bristles (ventral, inside)	9	1–9

Conclusions

Variation in structural characters within full grown female larvae was rather insignificant (with possible differences between sexes still unknown). However, considering the notable age-dependent structural changes in larvae (pointed out already by Thomas *et al*. 1988; Dalkiran 2009; Bojková & Soldán 2015), identification of species group-taxa in *Prosopistoma* is only possible if specimens of approximately the same stage of development (age class) are compared. More or less full grown larvae of *P. pennigerum* are characterized by carapace length > 3.0 mm, carapace length-to-width ratio (female larvae) ≥ 0.90 and antennae with ≥ 6 segments. The combination of characters as detailed in the description above, may presumably be considered species-specific for *P. pennigerum*, despite the comparatively small sample.

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