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Description of immature stages of *Ectemnostega* (*Ectemnostega*) *quadrata* (Signoret, 1885) (Heteroptera: Corixidae), with notes on ecological requirements of the species

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The family Corixidae comprises a small group of true bugs that live in aquatic environments with abundant macrophytes. The genus *Ectemnostega* Enderlein includes two subgenera: *Ectemnostega* and *Ectemnostegella*, both known from South America, ranging from Ecuador to Tierra del Fuego. *Ectemnostega (Ectemnostega) quadrata* (Signoret, 1885) is distinguished by the widened posterior half of hemelytra, and the protibia of males prolonged over the base of pala. This species is distributed in Argentina and Chile along the Andes mountain range, from high altitudes in San Juan to sea level in Cabo Hornos, Tierra del Fuego. Adults and larvae were collected in association with *Cladophora* sp. and *Chara* sp. in northwestern Mendoza, from Los Horcones Lagoon (2891 m a.s.l.) and from ponds on southwestern slopes of Mendoza (Las Loicas locality, 1772 m a.s.l.). Larval stadia of *Ectemnostega* are described for the first time. Distributional and life habitats data of this species are provided.

Keywords: Andean region; aquatic insects; Argentina; larval stadia; habitats; morphology

Introduction

The family Corixidae comprises a group of small bugs, exclusively aquatic throughout their life cycle, inhabiting shallow areas of lotic waters and temporarily stable lentic environments with abundant submerged aquatic macrophytes.

Corixid communities are regulated by ecological variables of the lentic environment, as substrate type, percent vegetation cover and size/stability of the systems (Tully, McCarthy and O'Donnell 1991). Chemical characteristics, such as salinity, hardness and oxygenation also decide on Corixidae distribution. Some Corixidae species are present in almost unoxygenated waters, or with extreme salinity, however, high water temperature makes them migrate to more favourable environments (Bachmann 1981). Water pH plays an important role because living

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in acid waters is almost impossible for unspecialised corixids for these kind of environments (Wollmann 2000). This family generally inhabits waters with neutral to alkaline pH.

Adults and larvae of Corixidae cohabit. According to Bachmann (1981) immatures develop their wing pads at the third instar. Several studies were carried out on the larval morphology, usually of a single species (Hagemann 1910; Abbott 1912; Hungerford 1917, 1919, 1948; Peneau 1921; Butler 1923; Caroli 1925; Lindberg 1935; Griffith 1945; Sutton 1947; Bobb 1953; Poisson 1924; Wroblewski 1958; Bachmann 1981); however, none of them listed characters that allow species identification (Cobben and Moller-Pillot 1960).

The genus *Ectemnostega* was created by Enderlein in 1912 to include *Corixa quadrata* Signoret, 1885. Several authors redescribed and added new characters to distinguish the species (Breddin 1897; Jaczewski 1927). *Ectemnostega* comprises two subgenenera: *Ectemnostega* including only the type species *E.* (*E.*) *quadrata* Signoret 1885 and *Ectemnostegella* including nine species. *Ectemnostega* (*Ectemnostega*) can be easily distinguished by the shape of the frons, convex on females and slightly concave in males; the slightly concave posterior border of the sternite VII of females; male protibiae with a carina covering the basal region of tarsus; and the anterior region of profemur without spines. This subgenus is distributed in Argentina and Chile, from San Juan (30° S) to Cabo de Hornos (56° S). *Ectemnostega (E.) quadrata* shows hemelytral polymorphism and, according to Bachmann (1960), the most common form is the brachypterous one.

Because *Ectemnostega* species live in habitats where water temperature is low, the taxon is considered to be cold stenothermal and restricted to the Andean region and to the South American Transition zone at high altitudes (Morrone 2006). *Ectemnostega (E.) quadrata* has also been found on borders of mountain streams. The aim of this study is to provide a morphological description of larval stadia as well the physico-chemical characteristics of the preferred habitats of this species.

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Materials and methods

Study areas

The province of Mendoza (Argentina) stretches from the base of the Andes to the western flatland of Argentina between 32°–37° 35′ S and 66° 30′–70° 35′ W, over an area of 150,839 km^2. Mendoza presents an arid to semiarid climate, with an annual rainfall average of 250 mm. Snow is the predominant precipitation on the Andes mountain range, with an annual rainfall of 300–1000 mm (Departamento General de Irrigación 1997, 1999).

The specimens were collected in two sampling stations in Mendoza, Argentina: Horcones Lagoon and Loicas locality (Figure 1).

Horcones Lagoon

The Horcones Lagoon (HORC; 32° 48′ 21.1″ S–69° 56′ 35.2″ W) is located in the Aconcagua Provincial Park at 2891 m a.s.l. The Aconcagua Provincial Park is part of the protected areas programme of Mendoza province and includes Mount Aconcagua (6962 m a.s.l.); it is of interest to mountaineers, tourists and scientists (Corte and Espizua 1981; Milana 2004; Méndez, Martínez Carretero and Peralta

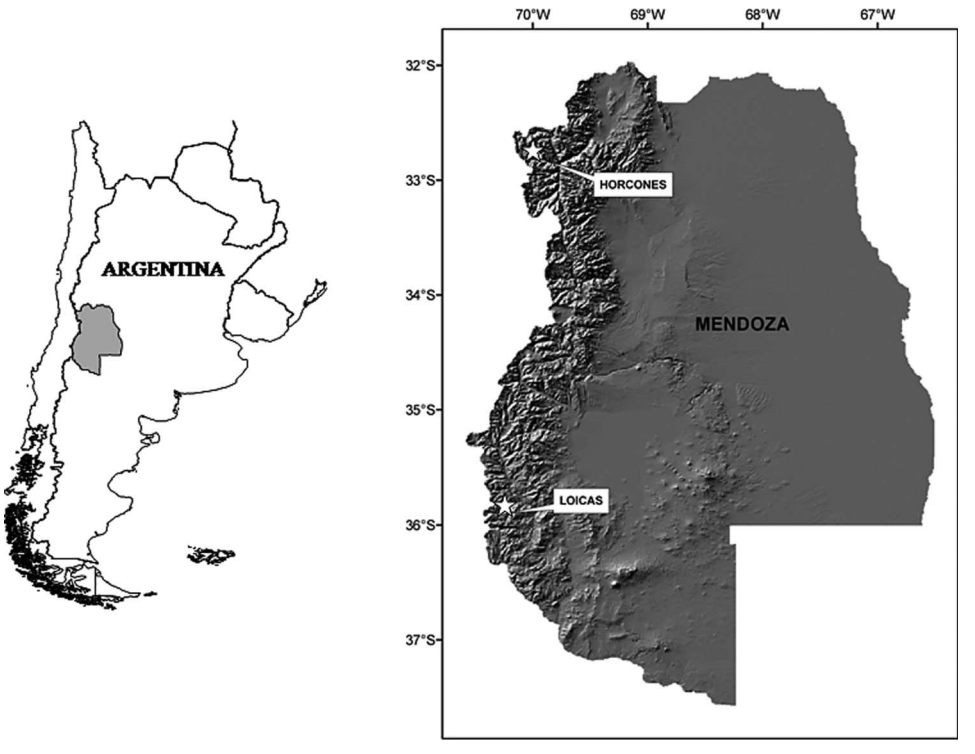

Figure 1. Location of the study sites where *Ectemnostega (Ectemnostega) quadrata* (Signoret, 1885) was found.

2006). The wetland studied is located in the High Andean zone, in the Cordillera Principal in the north west of Mendoza province, Las Heras Department (32° 48′ 21.1″ S–69° 56′ 35.2″ W). The vegetation is mainly composed of *Adesmia aegiceras* (Fabaceae) (Méndez et al. 2006).

Las Loicas

This locality is in southwestern Mendoza, department of Malargüe (35° 49′ 1.52″ S–70° 5′ 52.53″ W), 40 km from the Pehuenche International Crossing to Chile. Las Loicas (LOICAS) is located in the high Andes (35° 52′ 43.1″ S–70° 11′ 40.8″ W) at 1772 m a.s.l, and belongs to the High Andean steppe ecoregion. It is covered by permanent snow and the climate is cold with wide daily temperature amplitude. Annual precipitation averages 100–200 mm, mainly in the form of snow (Subsecretaría de Recursos Hídricos 2002, 2004). Temporary ponds were situated upstream between Las Loicas and the Pehuenche International Crossing, national route 145, parallel to Chico River (12.81 m^3 s^{-1} annual average river discharge).

Sampling

Samples taken from HORC are part of a monthly sampling study conducted over a full year (from October 2007 through September 2008). During the winter

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months (from June to September 2008), the HORC site could not be sampled because the lagoon surface was frozen. The LOICAS site was sampled only once (16 December 2007).

On each sampled site, the following environmental parameters were measured: pH (Hanna pH meter, HI 9025), conductivity (μS cm^{-1}; Hanna conductivity meter HI 9033), water and air temperature (°C; mercury thermometer), environmental humidity (%), transparency (m; Secchi disk), average depth (m; calibrated stick), and presence of aquatic macrophytes; however, percent O_2 saturation (% sat.O_2) was only measured at HORC.

Corixids were caught with a hand-held net (900 μm mesh size), from within the vegetation and through the bottom of the lagoon/pond at 0.4 m of maximum depth. All material collected was fixed in situ with 90% alcohol.

Specimens were measured with a Zenith MBS-10 binocular microscope, and photographs were taken with a JVC KY-F75U camera. An air-dried fifth instar was mounted on a metallic holder, sputter-coated with Au-Pd alloy, and examined using a Jeol 6361 LV scanning electron microscope. Measurements are given in millimetres. Specimens were deposited in the Entomological Collection of La Plata Museum (Argentina), and in the Entomological Laboratory of CCT Mendoza CONICET (Argentina).

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Results

Ectemnostega (Ectemnostega) quadrata (Signoret, 1885)

1885 *Corixa quadrata* Signoret, ser. 6, 5: 68–69; Breddin 1897, 13- 14; Reed 1901, 5(5-6): 110.
1895 *Corisa quadrata*: Berg, 4: 196; Berg 1896, 5: 137.
1912 *Ectemnostega quadrata*: Enderlein, 48(3): 115–116; Jaczewski 1927, 48(3): 146; Jaczewski 1928, 25: 214; Jacsewski 1931, 44: 145; Hungerford 1948, 32: 200; Bachmann 1960, 22(1–4): 34; Bachmann 1961, 3: 13–14; Bachmann 1962, 18: 144; Bachmann 1962, 8(25): 18; Bachmann 1962, 23(64): 23; Bachmann 1962, 23(64): 103; Bachmann 1963, 24(67): 35.
1948 *Ectemnostega darwini* Hungerford, 32: 203.
1979 *Ectemnostega (Ectemnostega) quadrata*: Bachmann, 35: 321; Bachmann 1982, 41(1–4): 198; Contartese & Bachmann 1986, 43(105): 90; Muzón et al. 2005, 64(3–4): 55.

Description of immature stages

Measurements of all instars are shown in Table 1.

First instar (n = 10) (Figure 2a)

Colour generally pale brown, posterior margin of abdominal tergites IV and V with a heart-shaped patch (Figure 2a), just before scent gland openings. Eyes reddish brown.

Head. Widely fused to thorax; vertex obtusely rounded, posterior margin concave; frons convex with long setae. *Rostrum* short with only two transverse bars, long setae present on lateral margins. *Antenna* bisegmented. Scapus shorter than pedicellus, posterior border of pedicellus straight with long setae, anterior border slightly convex.

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Table 1. Measurements of immature stages of *Ectemnostega (Ectemnostega) quadrata* (Signoret, 1885).

	First instar			Second instar			Third instar			Fourth instar			Fifth instar		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Total length	1.60	1.75	1.64	2.13	2.38	2.25	2.75	3.03	2.87	3.50	4.20	3.84	4.85	7.50	5.82
Max. width	0.90	1.03	0.98	1.25	1.38	1.33	1.53	1.75	1.65	1.90	2.18	2.07	2.35	2.55	2.50
Head width	0.65	0.78	0.73	0.98	1.05	1.02	1.25	1.40	1.33	1.63	1.85	1.73	1.95	2.15	2.03
Syntlipsis	0.33	0.48	0.40	0.40	0.60	0.50	0.60	0.73	0.64	0.75	0.98	0.84	0.95	1.10	1.03
Posterior border of eye width	0.13	0.23	0.18	0.23	0.35	0.29	0.38	0.48	0.42	0.43	0.58	0.50	0.60	0.75	0.67
Profemur length	0.23	0.28	0.26	0.30	0.40	0.36	0.45	0.53	0.50	0.63	0.75	0.68	0.75	0.90	0.82
Tibiatarsus length	0.35	0.40	0.37	0.43	0.55	0.50	0.58	0.78	0.70	0.83	0.95	0.91	1.00	1.15	1.08
Mesofemur length	0.48	0.58	0.53	0.80	0.90	0.85	1.20	1.38	1.27	1.45	1.85	1.62	2.15	2.25	2.22
Mesotibia length	0.25	0.30	0.27	0.40	0.48	0.45	0.55	0.68	0.62	0.80	1.00	0.89	1.00	1.20	1.12
Mesotarsus length	0.25	0.33	0.29	0.40	0.48	0.43	0.55	0.65	0.61	0.70	0.88	0.80	0.90	1.05	1.00
Median claw length	0.30	0.38	0.36	0.38	0.50	0.45	0.50	0.63	0.59	0.70	0.80	0.75	0.75	1.00	0.89
Metafemur length	0.48	0.50	0.49	0.53	0.75	0.63	0.75	0.88	0.82	0.95	1.18	1.06	1.00	1.30	1.24
Metatibia length	0.38	0.43	0.40	0.53	0.60	0.56	0.73	0.80	0.77	0.93	1.08	0.98	1.15	1.30	1.25
Metatarsus length	0.60	0.70	0.66	0.83	1.00	0.92	1.15	1.35	1.27	1.58	1.80	1.68	2.00	2.15	2.08
Posterior claw length	0.13	0.20	0.16	0.13	0.18	0.15	0.10	0.18	0.14	0.13	0.18	0.14	0.15	0.25	0.20
Anterior wing pad length	–	–	–	0.05	0.13	0.08	0.30	0.55	0.39	0.58	0.88	0.75	1.10	1.50	1.40
Posterior wing pad length	–	–	–	–	–	–	0.10	0.18	0.13	0.13	0.40	0.26	0.60	0.85	0.63

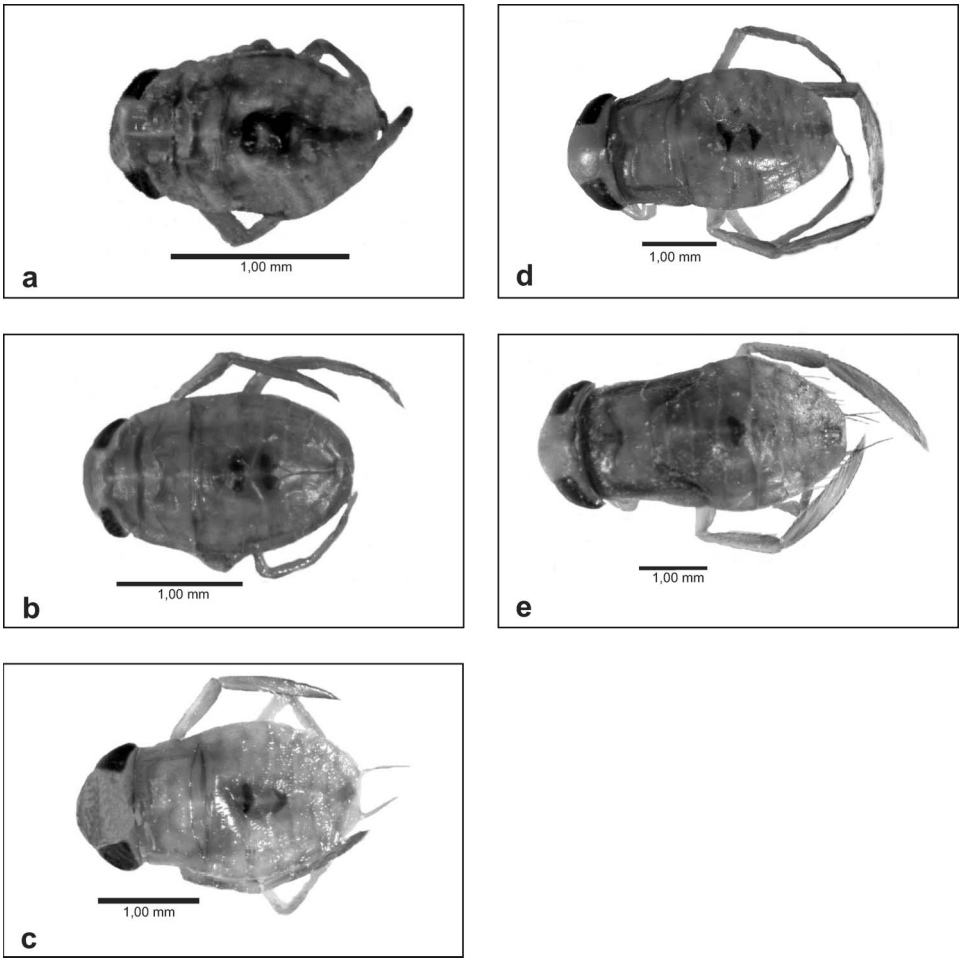

Figure 2. *Ectemnostega (E.) quadrata* (Signoret, 1885), (a) first instar, (b) second instar, (c) third instar, (d) fourth instar, (e) fifth instar.

Thorax. Pronotum conspicuous, as long as metanotum, posterior margin straight. Mesonotum short, posterior margin undulate. Metanotum posterior border straight. Wing pads undeveloped, and hydrophobic setae absent. Metasternum, triangular longer than wide, apex rounded.

Legs. Forelegs short and robust with sparse erect setae; tibia and tarsus fused to tibiotarsus, distal region spoon-shaped with long erect setae on the margins; middle and hind legs with sparse long erect setae; metatarsus not paddle-shaped as in last instars.

Abdomen. Rounded, posterior margin straight. Paired scent gland openings on posterior margin of tergites III, IV and V, anterior openings more contiguous with each other than middle and posterior ones. Lateral margins with long setae, one larger setae on posterior lateral margin of each segment. Posterior margin of abdomen with two tufts of long, thick setae.

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Second instar (n = 10) (Figure 2b)

Larger and more pigmented, but with a similar shape to the first instar larva. Rostrum with three transverse bars. Pronotum shorter, mesonotal posterior margin more undulated than previous instar. Metanotum more elongated. Legs similar to previous instar but more setose. Scent glands as first instar.

Third instar (n = 10) (Figure 2c)

Frons slightly concave with long setae. Rostrum with five transverse bars. Interocular region without a U-shaped row of punctures. Occipital region more developed, posterior margin sinuate. Pronotum covered by head. Mesothoracic wing pads well developed, reaching posterior margin of metanotum; metathoracic wing pads slightly insinuated. Wing pads with abundant setae, longer on inner margins. Lanceolate setae present on inner margins of anterior wing pad, and on middle region of mesonotum. Legs: metafemur with hydrophobic setae on proximal region, metatarsus more flattened and setose than previous instar. Chaetotaxy of legs similar to previous instars in all aspects, but more abundant. Scent glands as second instar.

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Fourth instar (n = 10) (Figure 2d)

Very similar in shape to previous instar, but the abdomen more elongated. Mesothoracic wing pads shorter than metathoracic ones, reaching middle of abdominal tergite I. Metathoracic wing pad surpassing the posterior margin of tergite I. Setae on wing pads more abundant, with same pattern as third instar.

Hydrophobic setae present on coxa, basal region of femur I, III, and on abdominal sternites. Fore and hind legs with similar pattern of setae as third instar; middle legs with similar pattern but setae shorter and more abundant. Metacoxae with a tuft of minute spines on lateral surface. Scent glands as in third instar.

Fifth instar (n = 10) (Figures 2e, 3a–f)

Occipital margin of head sinuate, slightly invaginated at middle, with long setae on the sides of the invagination. Mesonotum expanded medially with abundant short decumbent setae (Figure 3a); wing pads with more abundant setae than previous instar, longer setae on the inner margins. Lanceolate setae as in Figures 3a, b. Metanotum with sparse short decumbent setae, longer near anterior wing pads. Wing pads of the same length, reaching the middle of the abdominal tergite II. Metasternum as Figure 3c. Legs: similar in shape and chaetotaxy to the adult's legs. Profemora with a few stiff setae on ventral distal half and some minute spines scattered among them; hydrophobic setae covering the surface on the proximal third (Figure 3d). In anterior legs tibiotarsus present, elongate; distal region with two outer rows of long setae and an inner row of short setae (Figure 3e); one terminal claw. Mesofemora with longitudinal rows of short spiniform setae, mesotibiae with rows of longer setae, one-segmented tarsi, two terminal claws. Posterior legs compressed; metafemora with hydrophobic setae reaching proximal half of ventral surface (Figure 3f), and minute spines distally,

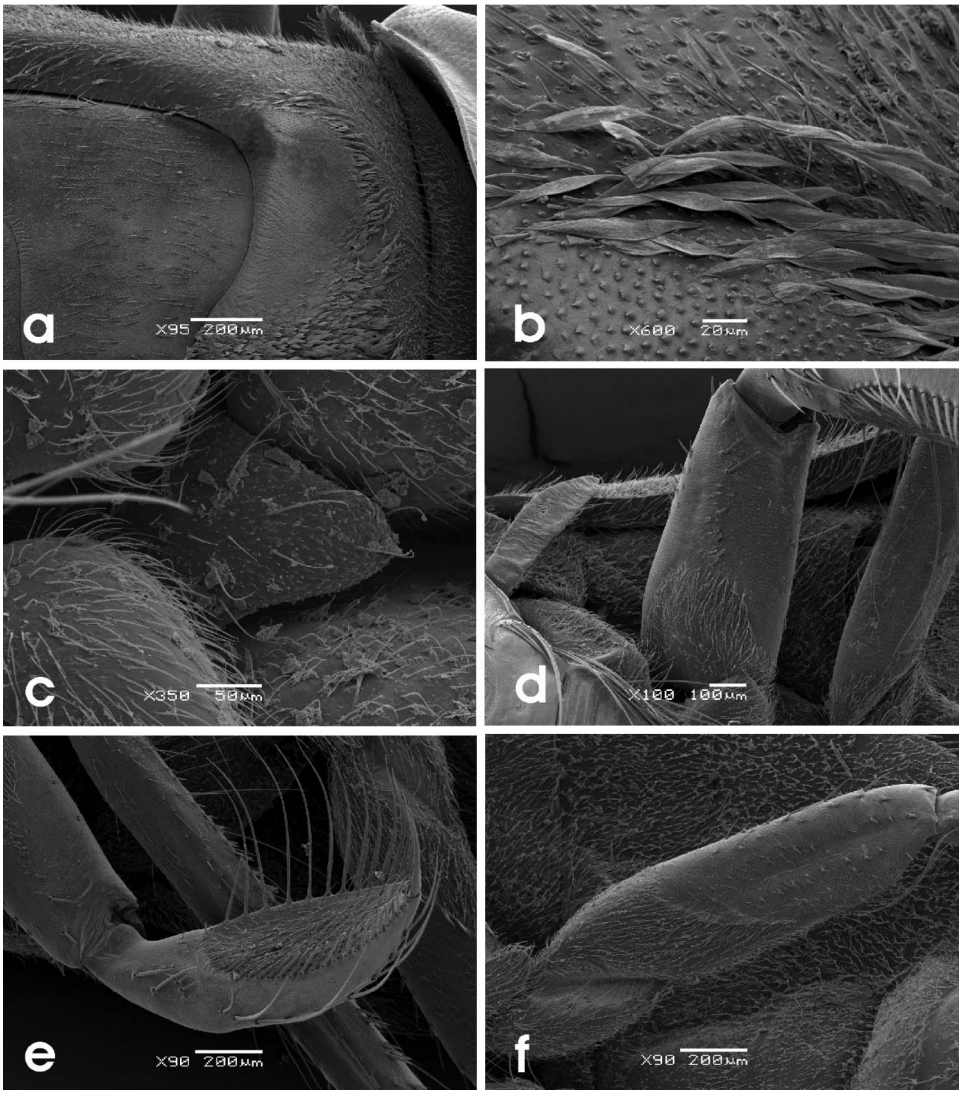

Figure 3. *Ectemnostega (E.) quadrata* (Signoret, 1885), fifth instar, (a) mesonotum; (b) detail of lanceolate setae on mesonotum; (c) metasternum; (d) profemur, inner view; (e) tibiotarsus of foreleg, ventral view; (f) metafemur, inner view.

with a tuft of long erect setae apically. Metatibiae with long slightly decumbent spiniform setae on dorsal surface, glabrous laterally, and with long erect spiniform setae ventrally, with a tuft of longer setae basally. Metatarsi paddle-shape with a row of spiniform setae on ventral and dorsal surface, ventral region with additional conspicuously long setae, and a row of long setae close to the ventral row of spiniferous tubercles. One terminal claw. Tibial grooming comb present on meso- and metatibiae.

Scent gland openings on tergite III covered by an evident expansion on tergites IV and V. Tergites with short decumbent setae.

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Remarks

Throughout the larval development we observed a remarkable change from second to third instar. As stated by Popham (1960), at this point the larva begins to breathe through the tracheal system (evidenced by the opening of the spiracles and the appearance of the hydrophobic setae on the abdominal tergites and basal region of the legs); the development of wing pads; and the pronounced modification of the metalegs for swimming (evidenced by the paddle-shaped tarsus and the more abundant setae on the metaleg).

Examination of the larvae revealed the absence of some characters present in adults, especially those related to sexual dimorphism, such as the dorsal crest on the protibia of males (protibia fused to tarsus in larvae), and the small invagination on the sternite VII of females. Nevertheless, there are some characters present both in adults and larvae: reduced pilose area of profemora with only a few stiff setae, metasternum longer than wide, and hydrophobic setae on metafemur reduced (Figure 3f).

The diagnostic characters for the *Ectemnostega* genus are exclusively found in the adults (male genitalia asymmetry, structure of male protibia and protarsus, female shape of genital segments). The only feature we could observe in larvae is the shortly extended hydrophobic setae at the base of the metafemur. As there is no other description of the larvae of this genus, we were not able to assure that any of the characters studied has a specific value.

Bachmann (1981) stated that the first pair of scent gland openings in the Heterocorixinae and Corixinae are reduced to a short slit; the study of the larvae with the scanning electron microscope showed that the openings on tergite III are almost covered by a small projection of posterior margin of this tergite.

The analysis of the measurements revealed that there is no allometric growth in the measured structures, but we could observe a more pronounced increase of the total length between instars 4 to 5.

Environmental features

Both larvae and adults of *E. (E.) quadrata* were found in the pleuston of HORC and LOICAS, associated with the presence of mats of aquatic macrophytes such as *Chara* sp (Characeae).

The Horcones Lagoon is characterised by having alkaline waters, moderately mineralised, transparent, oxygenated, with a substrate composed of clay and silt. Among other macrophytes associated with charophytes, we can mention Cyperaceae species, such as *Carex aff. gayana* E. Desv. and *Schoennoplectus pungens* (Vahl) Palla, and some Poaceae species, either submerged or as part of the surrounding vegetation. Chlorophytic algae (*Cladophora* sp.) were also found, but to a lesser extent. Water pH increased gradually from neutral in spring (average 7.5) to alkaline in summer and autumn (average 8.9). Environmental temperature was on average 18.5°C (range 8.4–28.7°C) and water temperature was 14.8°C (4.7–21°C). Conductivity showed a range between 730 and 574 μS cm^{-1}, but without a clear pattern. The percentage of oxygen saturation fluctuated from 32 to 45% in early spring, which is the time when the lagoon ice melts and macrophytes are mostly rotten, to nearly 85% in late spring, summer and autumn. Water transparency and depth averaged 0.22 m.

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Temporary ponds of the LOICAS site were sampled in late spring; they covered an area of 30 m^2, environmental temperature was 35.9°C and water temperature 16.3°C. Water pH was neutral (7.45), ponds presented higher salinity (1600 $\mu S\ cm^{-1}$), less transparency (0.10 m water transparency; 0.20 depth) than HORC, and a sand and clay substrate. The surrounding vegetation was composed of *Cortadeira* sp. Aquatic macrophytes included Charophyceae and Cyperaceae intertwined with abundant algae (*Cladophora* sp.).

Association with macroinvertebrates

Among macroinvertebrates associated with *E. (E.) quadrata* at the HORC site, we can mention *Hyalella* sp. (Amphipoda), which was found at high densities, *Heleobia* sp. (Mollusca, Cochliopidae), and representatives of the family Physidae (Mollusca), both at very low densities. Also other insect orders were found in association with *E. (E.) quadrata*, such as larvae and adults of Coleoptera (*Liodessus* sp. and *Lancetes* sp., Dysticidae) and larvae of Odonata, Aeshnidae (*Rhionaeschna variegata*). Densities of both larvae and adults of *E. (E.) quadrata* increased during summer, with the concomitant increase of water temperature. In Horcones Lagoon, adults were recorded during the whole sampling period (from October to May); instars 1–3 in December, all five larval stages during January and February, instars 3 and 4 in March, during April only one specimen of instar 3, and in May only one specimen of instar 4. From June to September the lagoon remained frozen.

The following species were found at LOICAS site: *Hyalella* sp. (Amphipoda: Hyalellidae), *Cyanallagma interruptum*, *Andinagrion peterseni* (Odonata: Coenagrionidae), *Rhionaeschna variegata* (Odonata: Aeshnidae), larvae and adults of *Tropisternus* sp. (Coleoptera: Hydrophilidae), *Lancetes varius* (Fabricius) (Coleoptera: Dysticidae), *Liodessus flavofasciatus* (Steinheil) (Coleoptera: Dysticidae), and larvae of Chironomidae (Diptera).

Distributional patterns

In northern Mendoza *E. (E.) quadrata* was found in Horcones Lagoon at 2891 m a.s.l., below this altitude it is replaced by *Sigara (Tropocorixa) jensenhaarupi* Jaczewski; from Dique Tambillos (2458 m a.s.l.) to lower altitudes near Uspallata stream (1890 m a.s.l.), Potrerillos (1407 m a.s.l.) and El Viborón Lagoon (630 m a.s.l.). In southern Mendoza province, at Loicas site, two samplings were perfomed: upstream (1772 m a.s.l.) and downstream (1661 m a.s.l.); with the same results as in the north of this province. Thus, we can state that in Mendoza province (Argentina) the species shows two altitudinal ranges: one in the northern region of the province over 2800 m a.s.l., and the other in the southern region over 1700 m a.s.l.; below these altitudes it is replaced by *S. (T.) jensenhaarupi* Jaczewski.

Discussion

Ectemnostega (E.) quadrata was found in clear waters with a broad range of salinity and a neutral to alkaline pH, in both permanent and temporary lentic environments of Mendoza province (Andean region) associated with high densities of *Chara* sp. (Characeae). It was also found in the same province in lotic environments, alongside High Andean streams at high altitudes (Bachmann 1981). This species was found

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also at lower altitudes in Patagonia, in the Somuncura Plateau located between 40° 20′–41° 30′ S and 65° 55′–70° 10′ W, in the transition area between Patagonia and Monte regions, in an altitudinal range of 150–1060 m a.s.l., demonstrating that the altitude is not limiting for the distribution of this species, and that it can live in different mountain habitats (Muzón et al. 2005).

According to Breddin (1897), in Ushuaia (Tierra del Fuego) there is only one generation per year; Bachmann (1981) found two generations per year and no larvae in winter in Neuquén, Río Negro and Chubut (Argentina). From the results obtained from an annual sample, it can be concluded that there is only one generation per year and probably the adults overwinter under the frozen surface in Horcones Lagoon. This was also observed in *Hesperocorixa interrupta* (Say) by Bobb (1953) in Virginia (USA).

Bachmann (1981) found macropterous and brachypterous forms cohabiting, with the brachypterous form being the most abundant; in the samples obtained in Mendoza we found the same situation.

The scarce information available on the immature stages of the Corixidae makes it difficult to state that certain characters are species diagnostic. We expect that future studies on this topic will permit us to make more a comprehensive comparison of the larvae.

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