

A description of the larva and pupa of *Culicoides charruus* Spinelli & Martínez (Diptera: Ceratopogonidae) from leaf axils of *Eryngium pandanifolium* (Apiaceae) in Argentina

Описание личинки и куколки *Culicoides charruus* Spinelli & Martínez (Diptera: Ceratopogonidae) из листовых влагалищ *Eryngium pandanifolium* (Apiaceae) в Аргентине

María M. Ronderos¹, Gustavo R. Spinelli¹ & Art Borkent²
Мария М. Рондерос, Густаво Р. Спинелли, Арт Боркент

¹ División Entomología Museo de La Plata, Paseo del Bosque s/n, 1900, La Plata, Argentina. E-mails: ronderos@fcnym.unlp.edu.ar; spinelli@fcnym.unlp.edu.ar

² 691 – 8th Ave. SE, Salmon Arm, British Columbia, V1E 2C2, Canada. E-mail: aborkent@sulnlife.ca

KEY WORDS: *Culicoides*, biting midges, phytotelmata, larval habitats, morphology of larva and pupa.

КЛЮЧЕВЫЕ СЛОВА: *Culicoides*, мокрецы, микроводоёмы на растениях, биотопы личинок, морфология личинки и куколки.

ABSTRACT. The larva of *Culicoides charruus* Spinelli & Martínez is described, the pupa is redescribed, and notes on the bionomics of the immatures are included. The larvae collected in water accumulated in the leaf axils of *Eryngium pandanifolium*, were reared to adults in the laboratory. The morphology of the larva and pupa was studied and illustrated using a scanning electron microscope, a phase-contrast microscope and a binocular microscope. Larvae lack the epipharyngeal lateral curtains; this feature is associated with their habitat (phytotelmata). *C. charruus* is recorded for the first time from the Province of Buenos Aires, Argentina.

РЕЗЮМЕ. Впервые описана личинка и переописана куколка мокреца *Culicoides charruus* Spinelli & Martínez. Приведены заметки по биологии преимагинальных стадий этого вида. Имаго были выведены в лаборатории из личинок, собранных из микроводоёмов во влагалищах листьев синеголовника *Eryngium pandanifolium*. Морфология личинки и куколки *C. charruus* была изучена и проиллюстрирована с использованием сканирующего электронного микроскопа, фазово-контрастного микроскопа и бинокуляра. У личинки отсутствуют латеральные гребни эпифаринкса; эта особенность строения связана с обитанием в микроводоёмах на растениях. *C. charruus* впервые отмечен из провинции Буэнос Айрес (Аргентина).

RESUMEN: Se describe la larva y se redescribe la pupa de *Culicoides charruus* Spinelli & Martínez, y se incluyen comentarios referidos a la biología de los estados inmaduros. Las larvas, recolectadas en agua acumulada en las axilas de las hojas de *Eryngium pandanifolium*, fueron criadas en el laboratorio hasta

adulto. Ambos estados fueron fotografiados mediante Microscopio Electrónico de Barrido, con microscopio de contraste de fase, y con microscopio binocular. Las larvas carecen de cortinas laterales en la epifaringe, condición que está relacionada con su presencia en fitotelmata. Se registra por primera vez a esta especie en la provincia de Buenos Aires, Argentina.

Introduction

Species in the genus *Culicoides* Latreille, 1809 are by far the most notorious members of the 109 currently recognized extant genera of Ceratopogonidae. In spite of their economic, medical and veterinary importance, their preimaginal stages, which breed in aquatic or semiaquatic environments, are relatively poorly known. Of the 267 species recorded from the Neotropical Region, the larvae of only 22 species and the pupae of 34 species are known [Borkent & Spinelli, 2007; Spinelli et al., 2007]. Our knowledge of the habitats of Neotropical species of *Culicoides* is therefore quite limited.

Culicoides charruus Spinelli & Martínez, 1992 is known from Paraguay, northeastern Argentina and Uruguay. The pupa of this species was partially described by Spinelli & Martínez [1992] and Spinelli et al. [1993].

The purpose of this paper is to describe the larva of *C. charruus*, to redescribe the pupa more fully, and to provide data on the life cycle and locomotion of the larva, based on immatures collected in Mar de Ajo, Province of Buenos Aires, Argentina. The larvae were collected from water accumulated in the leaf axils of *Eryngium pandanifolium* Cham & Schlecht, one of 25 species of Apiaceae in Argentina [Cabrera & Zardini, 1978].

Material and methods

Second to fourth instar larvae were collected and placed individually in Petri dishes with water. This water included immatures of Tubificidae (Oligochaeta), upon which the *Culicoides* larvae likely fed. Dishes were checked daily in order to check development and to separate the different larval instars and pupae until the adults emerged.

Larval instars II–IV and pupae were examined and photomicrographed using a JOEL 2000 scanning electron microscope (SEM), using the techniques described by Ronderos et al. [2000]. For observation with a phase-contrast microscope with oil immersion and a binocular microscope, specimens were slide-mounted in Canada Balsam following the technique described by Borkent & Spinelli [2007]. Mounted larval exuviae were placed with their ventral sides upward to facilitate examination of the epipharyngeal combs within the head capsule. Pupal exuviae were mounted dorsoventrally to examine cuticular processes of the cephalothorax, respiratory horns, and abdominal segments. Photomicrographs were taken with a Pentax Optico Power Shot S60 digital camera through a Leitz SM-Lux (10 \times or 40 \times), and the images were assembled in Photoshop 7.0. Measurements are given as mean values generally followed by ranges (in parentheses).

For terminology we followed Díaz et al. [2005], that of the larva was based on Murphree & Mullen [1991], that of the pupa, on Nevill & Dyce [1994]. The material is deposited in the collection of the Museo de La Plata, Argentina (MLPA).

ABBREVIATIONS: ad — anterodorsal tubercle; am — anteromarginal tubercle; AN — antenna; bs — basal sensillum; CO — collar; CS — caudal segment; D — dorsal side of segment; DC — dorsal comb; dl — dorsolateral tubercle; EP — epipharynx; GL — galeolacinia; HP — hypopharynx; HY — hypostoma; LB — labrum; LC1 and LC2 — lacinial sclerites 1 and 2; MD — mandible; MP — palpus; MS — messors; MX — maxilla; os — oval structure; P — pedicel; PL — palatum; PP — posterolateral processes; PRH — respiratory horn; SC — scopa; ss — sensilla styloconica; st — sensilla trichoidea; SCA — sensilla campaniformia; tb — transverse band; V — ventral side of segment; vl — ventrolateral setae; vm — ventromedian setae.

Tubercles of fourth abdominal segment: dasm — dorsal anterosubmarginal tubercles; dpm — dorsal posteromarginal tubercles; lasm — lateral anterosubmarginal tubercle; lpm — lateral posteromarginal tubercles; sp — spot; vpm — ventral posteromarginal tubercles.

Culicoides charruus Spinelli & Martínez Figs 1–25

Culicoides charruus Spinelli & Martínez, 1992: 176 (male, female, pupa; Uruguay); Spinelli et al., 1993: 26 (redescription; in key); Ronderos & Spinelli, 1998: 79, 81 (in key; Argentina record); Spinelli, 1998: 325 (in list; Argentina).

Culicoides charruus: Borkent & Wirth, 1997: 64 (in World catalog); Borkent & Spinelli, 2000: 33 (in catalog south of USA); Spinelli et al., 2005: 139, 142 (in key; Argentina; wing photo); Borkent & Spinelli, 2007: 68 (in Neotropical catalog).

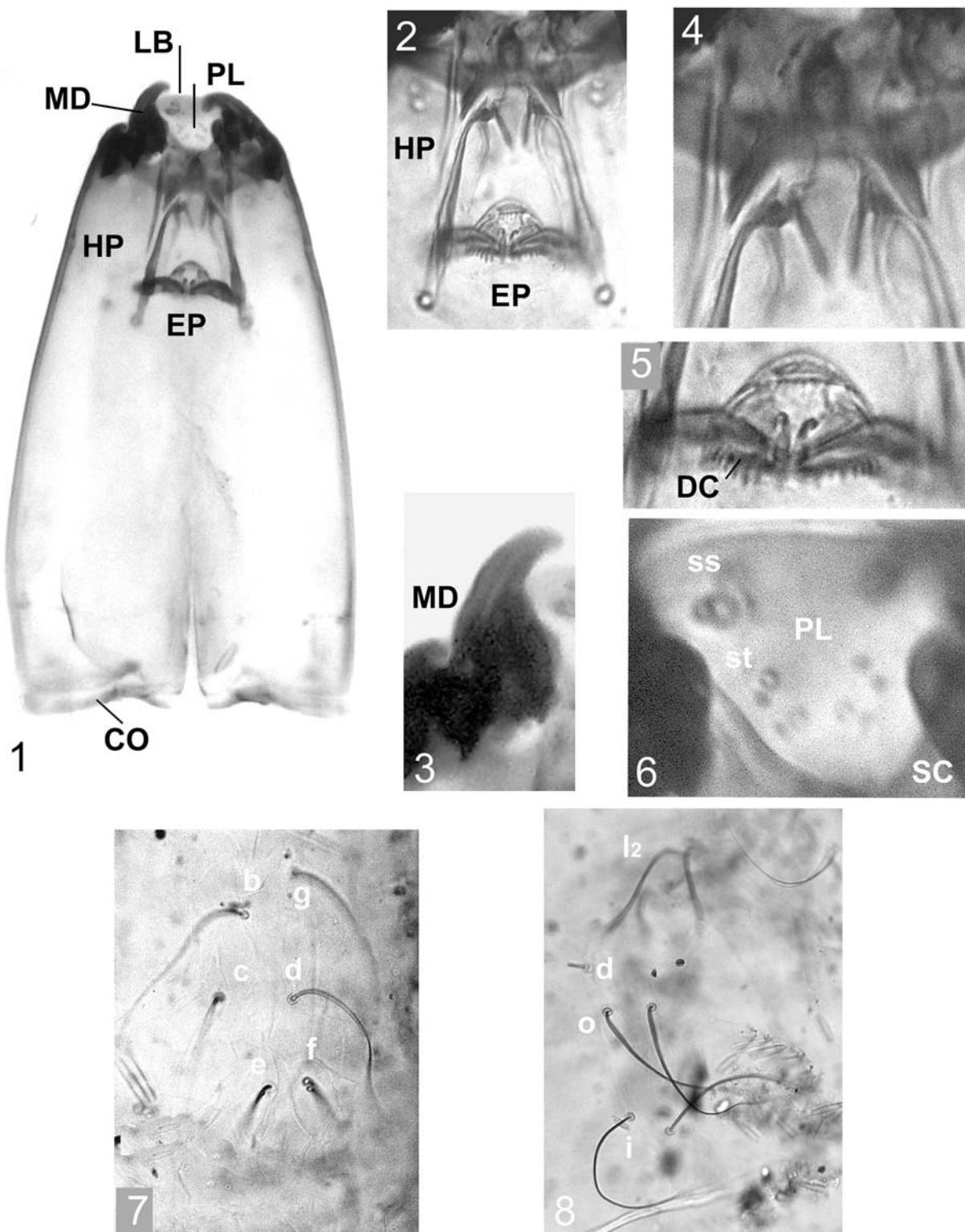
MATERIAL EXAMINED. Argentina, Buenos Aires Province, Mar de Ajo: 1 ♀ (with instar IV larval exuvium and pupal

exuvium), reared in laboratory from instar II larva collected from leaf axil of *Eryngium pandanifolium*, 27.I.2006, M. Donato. Uruguay, Departamento San José, Playa Pascual: 1 ♀ with pupal exuvium (holotype), 1 ♂ with pupal exuvium (paratype), 6.X.1987, M. Martínez. SPECIMENS EXAMINED WITH SEM. Argentina, Buenos Aires Province, Mar de Ajo: 4 larvae (1 instar II, 1 instar III and 2 instar IV larvae), 1 pupa (damaged), 27.I.2006, M. Donato.

DESCRIPTION. Fourth instar larva (Figs 1–16). For measurements and ratios of head capsule and caudal segment, see Table 1. Head capsule (Figs 1, 9, 11) yellowish brown, elongate, apex slightly bent ventrally; setae thin, medium-sized, chaetotaxy as in Figs 9–14. Labrum (Figs 1, 10, 12, 14) as long as its greatest basal width, with a pair of anterolateral sensilla styloconica (Fig. 12); palatum (Figs 1, 6, 12) with 3 pairs of closely-spaced sensilla trichoidea (Figs 6, 12, 14); messors (Fig. 14) small, thin, gently sclerotized, curved; five well-developed scopae (Figs 6, 14). Maxilla (Figs 12–13) with short papillae; maxillary palpus short, cylindrical, with four subapical papillae (Fig. 13); galeolacinia with stout blunt seta (Fig. 13). Mandible (Figs 1, 3, 12) hooked, curved, with broad base and long tooth (Fig. 3); ML 0.06 mm, MW 0.03 mm. Hypostoma (Figs 12, 14) quadrangular, with 25–30 small rounded teeth. Labium small, triangular. Epipharynx (Figs 1–2, 5) moderately developed, with two ventral combs, each with 4–6 stout short angular teeth; dorsal comb sclerites short, broad, with lanceolate long teeth on posterior edge, without auxiliary sclerites near lateral arms, without lateral curtains; LAW 0.06 mm, DCW 0.04 mm. Hypopharynx (Figs 1–2, 4) long, thin, moderately sclerotized; arms slender. Thoracic pigmentation uniformly pale yellowish. Abdominal segments whitish, with dark long thin setae (Fig. 7). Caudal segment (Figs 8, 15–16) with 6 pairs of setae; four of these long, stout, pale brown, other two thin, short, brown.

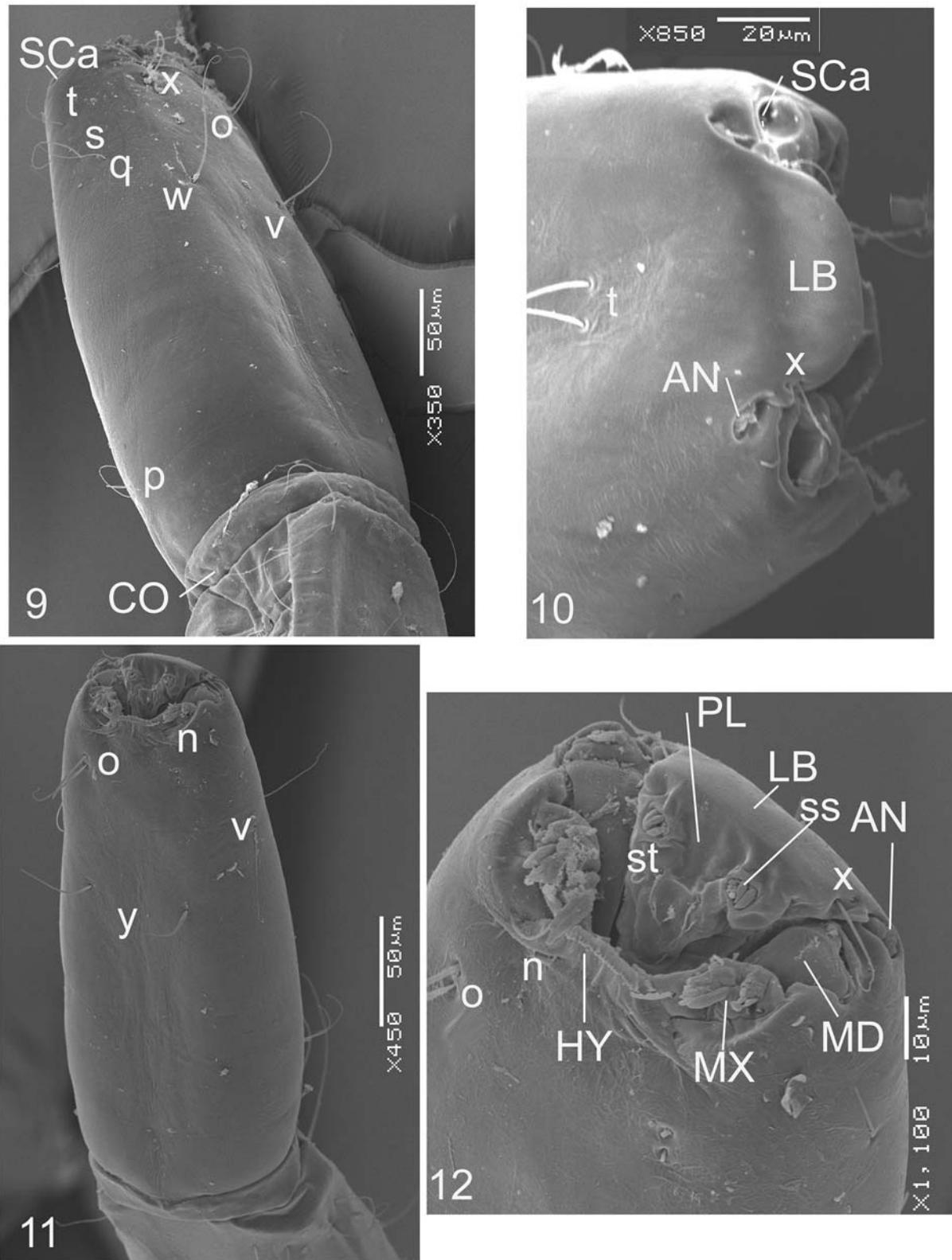
Second and third instar larva. Similar to fourth instar larva but with differences in size and some proportions (Table 1).

Pupa (Figs 17–25). Exuvium pale yellowish brown. Length of cephalothorax 1.27 (1.08–1.47) mm (n=2), its width 0.60 mm. Operculum (Fig. 19) 1.2 times as long as anterior width, blunt distally; surface covered by stout pointed spinules, except anteriorly on disc; anteromarginal tubercle (am) well-developed (Fig. 20), with one stout pointed seta; basal sensillum present; posterior margin slightly convex, smooth except for few rounded pointed spinules posterolateral to am; OL 0.10 mm (n=2); OW 0.18 mm (n=2); OW/OL 1.80 (n=2). Thoracic sensilla as follows: anterodorsal tubercle (ad) prominent, rounded, with two stout, large, subequal, pointed setae (Fig. 22); dorsolateral tubercle (dl) (Fig. 22) strong, conical, with blunt apex and two subbasal, medium-sized thin setae; dorsal tubercles (d) (Fig. 23): i, with short thin seta; ii–iii, with minute seta; iv, with long stout seta; v, as a pore. Respiratory horn (Figs 18–22) slender, yellowish, with distal one-third brown and apex slightly broader, truncate; 12–16 apical spiracles, without lateral spiracles; pedicel short (Figs 19–20, 25); P 0.043 (0.03–0.05) mm (n=3), RH 0.23 (0.19–0.25) mm (n=3), P/H 0.11 (0.15–0.20; n=3). Two ventromedial sensillae (vm), one as a minute seta, other one as a pore (Fig. 24); two ventrolateral setae (vl), anterior one medium-sized, posterior one 3 times as long as anterior one; base of tubercles quadrangular (Fig. 24). Abdominal segments with smooth integument, with only sparse anterior, posteriorly directed spicules; each segment with two pairs of small pigmented anterior spots (sp). Fourth segment with tubercles poorly developed, as follows (Fig. 25): 2 dorsal anterosubmarginal tubercles (dasm), i with medium-sized thin seta, ii with short seta; 5 dorsal posteromarginal tubercles (dpm), i with long thin seta on small rounded base, ii with short seta on small triangular base, iii with



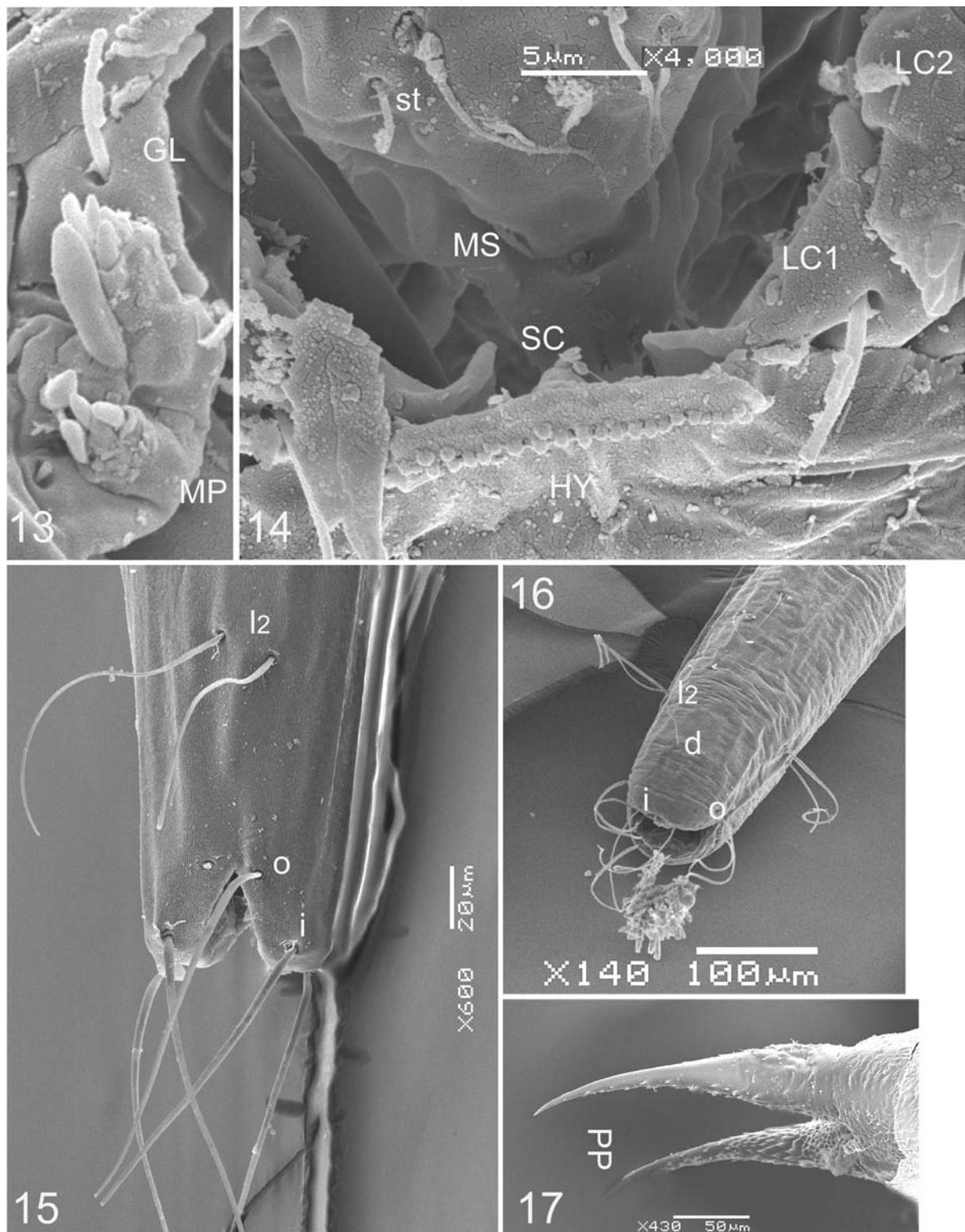
Figs 1–8. *Culicoides charruus* (larval exuvium, instar IV): 1 — head capsule; 2 — pharyngeal apparatus; 3 — mandible; 4 — hypopharynx; 5 — epipharynx; 6 — palatum; 7 — setae on ventral side of caudal abdominal segment; 8 — setae on dorsal side of caudal abdominal segment; 1–7 — ventral view; 8 — dorsal view. Abbreviations see in text; Figs 7–8 also include designations of setae.

Рис. 1–8. *Culicoides charruus* (экзувий личинки 4-го возраста): 1 — головная капсула; 2 — фарингеальный аппарат; 3 — мандибула; 4 — гипофаринкс; 5 — эпифаринкс; 6 — палатум (=вентральная поверхность верхней губы); 7 — щетинки на вентральной стороне анального сегмента; 8 — щетинки на дорсальной стороне анального сегмента; 1–7 — снизу; 8 — сверху. Сокращения см. в конце текста. На рис. 7–8 также приведены обозначения щетинок.



Figs 9–12. *Culicoides charrius* (larval head capsule, instar IV): 9 — dorsolateral view; 10 — dorsofrontal view; 11 — ventral view; 12 — ventrofrontal view. Abbreviations see in text.

Рис. 9–12. *Culicoides charrius* (головная капсула личинки 4-го возраста): 9 — сверху и сбоку; 10 — сверху и спереди; 11 — снизу; 12 — снизу и спереди. Сокращения см. в конце текста.

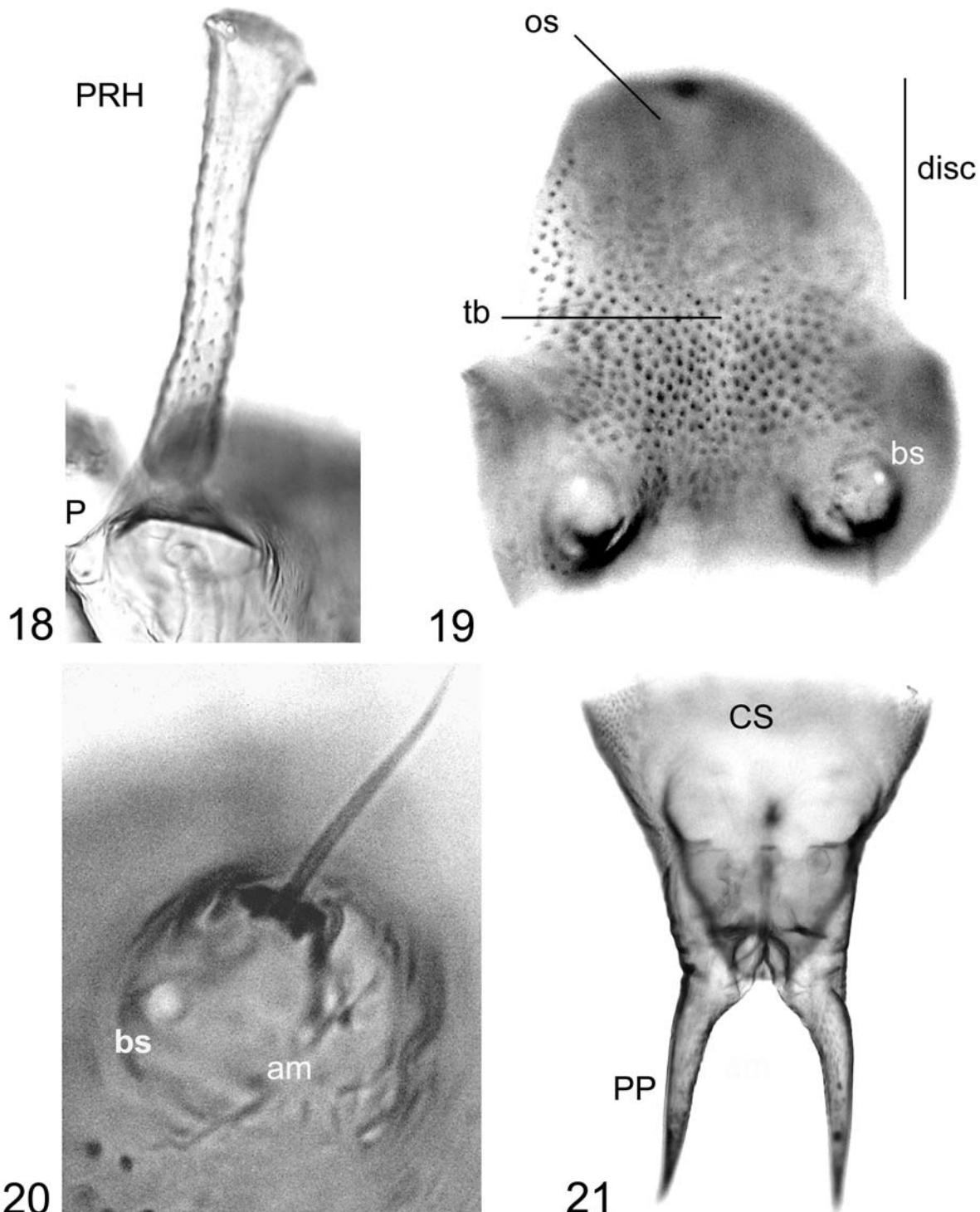


Figs 13–17. *Culicoides charruus*: 13–15 — fourth instar larva; 16 — third instar larva; 17 — pupa; 13 — maxilla; 14 — head capsule (detail); 15 — caudal segment; 16 — caudal segment; 17 — male caudal segment; 13–14 — ventrofrontal view; 15 — dorsal view; 16 — posterodorsal view; 17 — ventrolateral view. Abbreviations see in text; Figs 15–16 also include designations of setae.

Рис. 13–17. *Culicoides charruus*: 13–15 — личинка 4-го возраста; 16 — личинка 3-го возраста; 17 — куколка; 13 — максилла; 14 — детали строения головной капсулы; 15 — анальный (=каудальный) сегмент; 16 — анальный сегмент; 17 — анальный сегмент самца; 13–14 — снизу и спереди; 15 — сверху; 16 — сзади и сверху; 17 — снизу и сбоку. Сокращения см. в конце текста. На рис. 15–16 также приведены обозначения щетинок.

minute seta on small rounded base, iv–v as pores; 3 lateral posteromarginal tubercles (lpm), i and iii with short seta on raised base with bifid pointed tip, ii with very long thin seta on quadrangular base; 1 lateral anterosubmarginal tubercle (lasm) with minute seta on flattened small base; 3 ventral posteromar-

ginal tubercles (vpm) with rounded small bases, i with minute seta, ii with medium-sized seta, iii with short seta. Female caudal segment approximately 1.5 times as long as greatest width; its length 0.37 (0.33–0.41) mm (n=2), width 0.23 (0.21–0.25) mm (n=3); posteriorly directed spicules restricted to narrow anterior



Figs 18–21. *Culicoides charruus* (male pupa): 18 — respiratory horn; 19 — operculum; 20 — anteromarginal tubercle; 21 — caudal segment. 18, 21 — ventral view. Abbreviations see in text.

Рис. 18–21. *Culicoides charruus* (куколка самца): 18 — дыхательная трубка; 19 — лобный щиток; 20 — бугорок АМ; 21 — последний сегмент брюшка; 18, 21 — снизу. Сокращения см. в конце текста.

Table 1. *Culicoides charruus*, larva. Measurements (in mm) and ratios of head capsule and caudal segment.

Таблица 1. *Culicoides charruus*, личинка. Измерения (в мм) и индексы головной капсулы и анального (=каудального) сегмента.

		Larval instars		
		II	III	IV
Head capsule	HL	0.217	0.328	0.428
	HW	0.107	0.169	0.254
	HR	2.02	1.94	1.69
	SGW	0.084	0.126	0.144
Caudal segment	CSL	0.536	0.703	0.803
	CSW	0.093	0.155	0.168
	CSR	5.75	4.53	4.77
	OL	0.216	0.245	0.263
	OD	0.335	0.512	0.535

HL — head length; HW — head width; SGW — subgenal width; HR — head ratio = HL/HW; CSL — caudal segment length; CSW — caudal segment width; CSR — caudal segment ratio = CSL/CSW; OL — length of caudal setae "o"; OD — distance between bases of caudal setae "o".

HL — длина головы; HW — ширина головы; SGW — ширина в области субгенального кольца; HR — индекс длины головы = HL/HW; CSL — длина анального сегмента; CSW — ширина анального сегмента; CSR — индекс анального сегмента = CSL/CSW; OL — длина каудальных щетинок "о"; OD — расстояние между основаниями каудальных щетинок "о".

band and posterolateral processes; ventral surface with spicules present on each side of midline; posterolateral processes of moderate length, subparallel, with pointed tips. Male caudal segment (Figs 17, 21) similar to that of female except for ventral sheath.

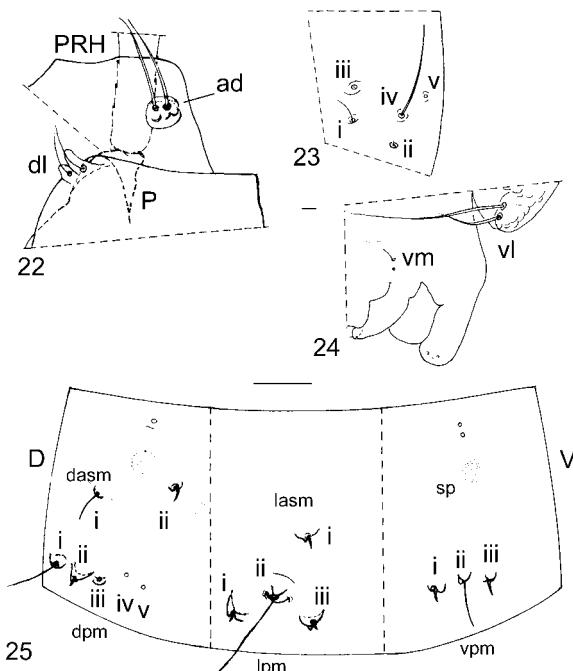
COMPARISON. The pupa of *C. charruus* is very similar to that of *C. albomaculus* Root & Hoffman, 1937, a species that breeds in *Agave* sp. in Mexico, particularly in the shape and spiculate vestiture of the caudal segment. However, the respiratory horn of *C. albomaculus* is more slender, bears a row of 10–12 apical spiracles, and the tubercles of the fourth abdominal segment are stronger. Since the larvae of Neotropical *Culicoides* are poorly known, we cannot provide a comparison of the larva.

DISTRIBUTION. Argentina, Uruguay. The species is recorded for the first time from the Province of Buenos Aires, Argentina.

NOTES ON BIONOMICS. The portion of the life cycle of *Culicoides charruus* from instar II larva to adult lasted approximately 26 days when reared at 27°C in the laboratory, with instar II lasting 9 days, instar III, 2 days, instar IV, 3 days, and the pupal stage, 12 days. The larva exhibited a typical serpentine movement when it moved through substrate, with the younger larvae much more active and rapid than instar IV.

Discussion

The morphological features of the larvae of some species of *Culicoides* are related to their habitat [Kettle & Elson, 1976; Murphree & Mullen, 1991; Díaz et al., 2005]. Murphree & Mullen [1991] reported that diffuse yellowish brown thoracic pigmentation, large setae on the caudal segment, and extensive epipharyngeal lateral curtains are characteristic of tree hole breeding spe-



Figs 22–25. *Culicoides charruus* (пупка): 22 — правый антеродорсальный бугорок и бугорок по боку от дыхательной трубки и её базальная часть (правая сторона тела); 23 — бугорки D (правая сторона тела); 24 — левая вентролатеральная щетинка VL и VM (левая сторона тела); 25 — четвертый сегмент брюшка; 22–23 — сверху; 25 — сбоку. Сокращения см. в конце текста. На рис. 23 и 25 бугорки обозначены римскими цифрами.

Рис. 22–25. *Culicoides charruus* (куколка): 22 — бугорки AD и DL, основание дыхательной трубки и её базальная часть (правая сторона тела); 23 — бугорки D (правая сторона тела); 24 — щетинки VL и VM (левая сторона тела); 25 — 4-й сегмент брюшка; 22–23 — сверху; 25 — сбоку. Сокращения см. в конце текста. На рис. 23 и 25 бугорки обозначены римскими цифрами.

cies of *Culicoides*. Ronderos & Spinelli [2000] and Ronderos & Díaz [2002] corroborate this idea in the case of *C. bambusicola* Lutz, 1913, a South American species that breeds in the internodes of the "tacuara" cane, *Guadua trinii* (Ness) Ness ex Rupr. Huerta et al. [2001] noted the same for the first two characters of *C. albomaculus*, pointing out that the epipharyngeal lateral curtains are reduced. Spinelli et al. [2007] noted the same for *C. felippебauerae* Spinelli, 2007, a species which breeds in the axils of the rhizomatous herb *Phenakospermum guyannense* Endl. in Amazonia, with the epipharynx devoid of curtains. The lack of epipharyngeal lateral curtains in *C. charruus* and *C. felippебauerae*, as well as the reduction of these structures in *C. albomaculus* suggest that the large lateral curtains are characteristic of tree-hole species but not of species whose larvae develop in leaf axils.

Donato & Paggi [2008] described the chironomid *Polypedilum parthenogeneticum* from the material collected in *Eryngium pandanifolium* together with the specimens herein described. In this paper, based on the data published by Vucetich & Rossi [1980] they pointed out that the water impounded in *E. pandanifolium* in Buenos Aires Province had pH 5.6–6.5, temperature 9–12°C in

winter, 21–23°C in spring, and a mean of 26°C in summer, relatively low concentrations of O₂ and high concentrations of dissolved CO₂. Other phytotelmata dipterans collected by Donato & Paggi [2008] from *Eryngium* included the culicids *Culex renatoi* Lane & Ramalho, 1960 and *Culex castroi* Casal & Garcia, 1967, the chironomid *Metricnemus eryngiotelmatus* Donato & Paggi, 2008, and an undetermined larva of Psychodidae.

Of the 35 species of Neotropical *Culicoides* for which at least one immature is recorded, five have been reared from the leaf axils of various plants. Although the present classification of the species of *Culicoides* has not been interpreted cladistically, it is interesting to note that the five species are placed in four different subgenera, as follows: *Anilomyia* Vargas, 1960 (*C. chaverrii* Spinelli & Borkent, 2004), *Haematomyidium* Goeldi, 1905 (*C. paraensis* (Goeldi, 1905) and *C. bayano* Wirth, 1981), *Hoffmania* Fox, 1948 (*C. charruus*), and *Mataemyia* Vargas, 1960 (*C. felippebauerae*). Of the remaining 30 Neotropical *Culicoides* species known as larvae or pupae, immatures develop either in other phytotelmata or in the ground layer habitats [for references, see Borkent & Spinelli, 2007]. This suggests that the leaf axil species in the Neotropical Region have invaded this habitat at least four times. Similar patterns were observed in other regions of the World [Borkent, unpublished data]. Future cladistic analysis and additional identification of larval development sites will establish a more confident interpretation relating to the poignant adaptive radiation seen with the genus *Culicoides*.

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СОКРАЩЕНИЯ: ad — бугорок AD; am — бугорок AM; AN — антenna; bs — базальная сенсилла; CO — воротничок; CS — последний сегмент брюшка; D — дорзальная сторона сегмента; DC — дорзальный гребень эпифаринкса; dl — бугорок DL; EP — эпифаринкс; GL — галеолациния; HP — гипофаринкс; HY — гипостом; LB — верхняя губа; LC1 и LC2 — склериты лацинии 1 и 2; MD — мандибула; MP — щупик; MS — мессор; MX — максилла; os — овальная структура; P — основание дыхательной трубы; PL — палатум; PP — каудальные выросты; PRH — дыхательная трубка; SC — сенсиллы-щеточки; ss — стилоконические сенсиллы; st — триходные сенсиллы; SCa — колоколовидные сенсиллы; tb — попечечная полоса; V — вентральная сторона сегмента; vl — щетинки VL; vm — щетинки VM.

Бугорки 4-го сегмента брюшка: dasm — бугорки DASM, dpm — бугорки DPM, lasm — бугорок LASM, lpm — бугорки LPM, sp — пигментное пятно, vpm — бугорки V.