Bol. Mus. Nac. Hist. Nat. Parag. Vol. 14 (1-2), Setiembre 2002, pp. 43 - 48

REARING *CULICOIDES BAMBUSICOLA* (LUTZ, 1913) (DIPTERA, CERATOPOGONIDAE) IN LABORATORY. OBSERVATIONS AND NEW RECORDS

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Abstract.- Culicoides bambusicola Lutz, 1913 biting midges (Diptera, Ceratopogonidae) has immature stages that breed in tacuara cane internodes, and in artificial microholders. They were reared from larvae I to adult in petri dishes on substrate containing water from their natural environment. They exhibit the ultrastructural characters of *Culicoides* larvae that breed in tree holes. In this paper reports on larval growth, feeding behavior, locomotion behavior and the duration of immature stages are provided. The technique has significant advantages for small colony maintenance in the laboratory and it allows permanent observation of developing immatures in experimental studies. Besides, this species is recorded for the first time in the Province of Buenos Aires (Argentina).

Resumen.- Culicoides bambusícola Lutz, 1913 (Diptera, Ceratopogonidae) presenta los estados inmaduros que críán en los internodos de cañas de tacuara y microcontenedores artificales. Fueron criados desde larva I a adultos en pequeñas cápsulas de petri utilizando como sustrato el agua de su ambiente natural. La larva de *Culicoides bambusicola* exibe los caracteres ultraestructurales de las larvas de *Culicoides* que crían en huecos de árboles. En este trabajo se da a conocer el desarrollo larval, comportamiento alimenticio, locomoción y duración de los estados inmaduros. La técnica demuestra ventajas significativas sobre el mantenimiento de colonias pequeñas en laboratorio y permitió la permanente observación del desarrollo de los estados preimaginales en estudios experimentales. Además, se da a conocer por primera vez el registro de esta especie para la Provincia de Buenos Aires (Argentina).

Scientific knowledge concerning adults of biting midges, *Culicoides* Latreille, is advanced in comparison to what is known of immature stages. Despite the interest in the genus, there is comparatively little knowledge about them. In most cases, the larval habitats are largely unknown, and details concerning to larval biology and feeding behavior remained obscure. The accumulation of data and observations during the past years have contributed significantly to the knowledge of the genus. Most information on breeding sites is based on studies on North American species: Thomsen's (1937), Jamback (1965), Battle & Turner (1971, 1972), Jones (1961), Blanton & Wirth (1979), Knausenberger (1986 dissert.), Mullen & Hribar (1988), and more recently, Hribar & Mullen (1991).

The larval biology of Neotropical *Culicoides* biting midges while receiving considerable study, remains for the most part unknown. Wirth & Blanton (1959) described the breeding sites for only 15 out of the 88 reported Panamanian species of *Culicoides*, some of which are biologically closely associated to the local flora, in which the immature stages are found in plants, plant products, or in the remaining water associated with them. Williams (1964) made a significant contribution to the biology of Neotropical *Culicoides*, reporting the larval habitats for 24 species in Trinidad, from which 15 species were found associated with plant materials. Clastrier (1971) reared one species from moss growing on the palm trunk and from moist material accumulated in the axils of palm leaves in French Guinea. Vitale *et al*. (1981) reported results of a study on rearing from arboreal habitats in Panama.

There have been relatively few morphological studies of larval *Culicoides*, especially for the neotropical fauna (being described only the larvae of 12 of the 271 known species, Borkent & Spinelli (2000). The major work is on North American species. It was done by Murphee & Mullen (1991), but little is known about the ultrastructure of the larvae of biting midges, in relation to their biology. Later, Hribar (1993) discussed the relationship between feeding behavior and morphology of mouthparts.

The larva and pupa of *Culicoides bambusicola* have recently been redescribed by Ronderos & Spinelli (2000), from material collected in the area influenced by the Yacyreta dam lake in the Paraná River (Argentina-Paraguay).

The purpose of this paper is to provide data on the biology traits: breeding location, locomotion and feeding behavior of the larvae *Culicoides bambusicola.*, we describe the technique for rearing in the lab, and we also present here the first record of thus species in the Province of Buenos Aires.

MATERIALS AND METHODS

A total (colony) of 108 larvae, and 8 adults of *Culicoides bambusicola* were used in this study. They were collected in sites where sampled, from tacuara internodes (Argentina, Corrientes, Garapé, 17 VI 1999; Corpus, Pto. Maní, V 2000; II 2001), and in artificial microholders (Argentina, Buenos Aires). Standing water and/or moist organic matter were removed from tacuara and were aspirated through pipettes and carried to the laboratory in tubes containing water from their natural environment. In laboratory, they were placed in styrofoam cups and covered with glass lids to prevent adults from escaping. They were observed daily to record different phases of development, and water containing nutrients, Oligochaeta (immature stages of Tubicidae) and detritus were added, and observed daily for pupation. The pupae were extracted from samples by micropipette, and placed individually in small cotton-stoppered test tubes with ca. 5 ml of water. The tubes were checked daily for emergence of adults.

The remaining sample was transferred. Larvae were cleared in a saturated solution of phenol in ethanol. The some ethanol-preserved specimens were cleared in 10 percent K(OH) for 24-48 hours. These larvae were mounted on microscope slides in Canada balsam Wirth & Marston (1968). Larvae and pupae were also examined using scanning electron microscopy (SEM). For that purpose, they were mounted by using the techniques of Ronderos *et al*. (2000).

All emerging arthropods were collected and stored individually in vials of 70% alcohol for later observation, by means of an isolating individual rearing method association of larval skin and pupal case with a specific adult specimen. It also permitted emergence of a large number of adults from the less-disturbed, nutrient-containing original sample.

The material examined was deposited in the collection of the Museo de La Plata, Argentina in 70% alcohol in hemolysis tubes, some others on microscope slides, and on SEM stubs.

The description, illustration and SEM microphotographs, of the larva, pupa and adult of *Culicoides bambusicola* can be seen in Ronderos & Spinelli (2000).

RESULTS

Larval habitats: *Culicoides bambusicola* larvae were collected from holes of tacuara cane *Guada trini* (Ness) Ness et Rupr, at different heights, and in closed and open tacuara, and in very small artificial microholders in tree holes. This is the first species from the *cotocripus* species group found where breeding. Generally *Culicoides* larvae occur in many aquatic and semiaquatic habitats Mullen & Hribar (1988*)*, *Culicoides* genus is also adapted to utilizing smaller ecological habitats. Therefore it is difficult to identify breeding sites, and to collect and associate larvae with adults Hynes (1984). The new record in Gonnet was collected with *Dasyhelea necrophila* Spinelli & Rodriguez, 1999 (Ronderos *et al*. In press).

In some cases, associations are evident between certain taxonomic structures and larval habitats. Ronderos & Spinelli (2000) mention that *Culicoides bambusicola* exhibits: the same stated characters by Murphree & Mullen (1991) for the larvae of tree hole breeding species: diffuse yellowish brown thoracic pigmentation, the extensive epipharingeal lateral curtains, and large setae of the caudal segment. The greatest variation is in OLR (ratio of caudal segment–length to length of setae "o") and average of caudal setae. In particular, the average on caudal setae ö" and "ï" differs considerably from typical average.

Larval food resources: *C. bambusicola* feed from Oligochaeta (immature stages of the Tubicidae family). These larvae have long, narrow heads with the mouthparts directed anteriorly, a sclerotized labium, present a lightly built pharynge and hooked mandible, broad and pointed, it has smooth and finely serrate hypostomata single-toothed mandibles. Significant variation in form and arrangement of the palatal sensilla and appendages exits among *Culicoides bambusicola* in relation to feeding. *C. bambusicola* was fed by a supply, of small aquatic organisms as potential prey. Tissues were removed from the mandibles and taken out from its exoskeleton. Movements of the epipharynx cause the suction inside the cibarium and from the stomodeum by peristalsis.

Larval locomotion: *C. bambusicola* shows a good adaptation for swimming and typical active movement, the young larvae (I-II) moves much more slowly, but the later instar (III-IV) exhibits serpentine locomotion. Larvae are positively phototrofic, their activity notably grows when lay under the ME light and their positive phototropismo come down into term to pupae state (notable difference in relation to larvae *Culicoides insignis* Lutz, 1913) (Ronderos personal observation, 2000).

Developmental times**:** Adult phase is very short (5-10 days) comparing to larval phase lifetime that varies between 30-35 days. The first larval instar is the longest in life cycle of. C. *bambusicola*, Its development requires two or three weeks, but from the fourth larval instar to pupation requires one week. *C. bambusicola* completes its development in as little as six days when placed in cotton-stoppered vials individually, from two to three days when reared at 27 ° C in the same place. Larval cycle lasted 30 days more or less in the lab.

Therefore, larval lifetime represents more than 80% in biotic cycle of this species without egg time.

New Records: *Culicoides bambusicola* (Lutz): Argentina, Buenos Aires, Gonnet, XII-2000, Ronderos-Baran, 1 larva; City Bell, 6-III-2001, 2 larvae, col. Ronderos, reared in laboratory.

DISCUSSION

This work tries to find a solution to the relative difficulty in locating developmental sites or collecting and associating adults. Factors which have contributed to difficult collecting are: small size (3-6 mm), colour (whitish), difficulty in identifying breeding sites and in collecting and associating larvae to adults Hynes (1984).

However, significant progress has been made in recent years in the larval taxonomy and habitat associations. The few *Culicoides* larvae described in detail from tree holes *C. hoffmani* Fox, 1946 , *C. paraensis* (Goeldi),1905 and *C. furens* (Poey, 1851) are unique in possessing four pairs of long perianal bristles Linley & Kettle (1960) and Murphree & Mullen (1991). These are present in many other Ceratopogonid genera but have not been recorded in many species of *Culicoides.* It would be interesting to find out whether other tree-hole breeding species have similar perianal bristles.

From a morphobehavioral perspective this variation is most evident in the mandibles, epipharynx and hypostoma. Larvae that posses a heavily sclerotized epipharynx also posses a heavily sclerotized hypopharynx.

One of the least known aspects of *Culicoides* larvae is the nature of their food and feeding behavior. Direct feeding observations have been made for only a few representative species. The species whose larvae have lightly built pharynges may feed on algae

(*Culicoides* sp.), or on detritus, or conversely they are carnivorous (*C. furens* can be reared on free-living nematodes) (Bildlingmayer, personal observation). Variety in larval feeding shows variety in feeding habitat. It were observed oligochaetes, fungi and non-identificable particles in the alimentary tract with optical microscope oil immersion (100x) with phase contrast. Other authors (Mullens & Velten, 1994; Breidenbaugh & Mullens, 1999) offered several food sources, including a rich nutrient liquid diet consisting of bacteria, algae and added nematodes.

This work indicates that *C. bambusicola* feeds exclusively on microorganisms carried in the standing water and organic matter of their natural environment.

Regarding the locomotion, *Culicoides* larvae are generally considered to be good swimmers, although detailed observations have been made for only a few species. Linley (1986) has demonstrated that swimming ability is highly influenced by the instar and size of larvae, water temperature, and viscosity of the medium. *C .bambusicola* larvae use their specialized posterior setae for their strong activity when swimming; the reason may reside in the cleanliness and tranquility of water in the tree holes.

Development times for *Culicoides* larvae are highly variable depending on the species, temperature, water quality, and availability of qualified food.

This bloodsucking genus *Culicoides* is an important pest for man and domestic animals, but the information on the life cycle and taxonomy of the immature stages of *Culicoides* is so limited that progress on its knowledge is very limited.

Furthermore, it would be important to remark that two light traps set during two years near tacuara canes in the sampling stations, did not collect specimens of *C. bambusicola*, suggesting that the species is poorly attracted by light. The previous valid record for *C. bambusicola* is also based on specimens collected in cane internodes.

This is one of the first studies of the breeding sites of the different species of the genus, and the relationship existing with the taxonomic ultrastructure. Further taxonomic work with the immature stages will, no doubt enhance the prospects for more detailed characterizations of the larval biology and breeding, locomotion and feeding behavior of *Culicoides* species in the future.

This record of *C. bambusicola* in Gonnet was collected with other Ceratopogonidae: *Dasyhelea necrophila* Spinelli & Rodriguez (1999). The larva of this species exhibits the character states typical of "herbivorous" ceratopogonids (Ronderos *et al.*, in press). Precisely specific adaptations to different larval habitats will allow researchers to set limits on the actual distribution of certain species, since immature states can show different morphoecological types.

ACKNOWLEDGMENTS

We gratefully acknowledge the critiques and helpful suggestions by Dra. María Marta Cigliano, Dr. Gustavo Spinelli.

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