Culicidae and Ceratopogonidae (Diptera: Nematocera) inhabiting phytotelmata in Iguazú National Park, Misiones Province, subtropical Argentina

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Culicidae y Ceratopogonidae (Diptera: Nematocera) que habitan en fitotelmata en el Parque Nacional Iguazú, provincia de Misiones, Argentina subtropical

■ **RESUMEN**. Se presenta una lista de las fitotelmata más comunes y los Culicidae y Ceratopogonidae que las habitan, del Parque Nacional Iguazú, Misiones, Argentina; además de observaciones biológicas y de comportamiento. Especies de Poaceae, Bromeliaceae, Apiaceae, Araceae, Urticaceae, Marantaceae, y Arecaceae, fueron identificadas como fitotelmata. Fueron reconocidas 26 especies de culícidos y nueve de Ceratopogonidae. La mayor riqueza de especies de culícidos y ceratopogónidos se registraron en el bambú *Guadua chacoensis* (Poaceae) y en los huecos de los árboles respectivamente. Catorce especies de culícidos y tres de ceratopogónidos habitan bambúes, dos y cinco en los huecos de los árboles, siete y tres en bromelias y nueve y una en otras fitotelmata, respectivamente.

PALABRAS CLAVE. Biodiversidad. Insectos acuáticos. Mosquitos. Jejenes. Larvas de Diptera.

■ **ABSTRACT.** A list of the most common phytotelmata and their Culicidae and Ceratopogonidae inhabitants from Iguazú National Park, Misiones Province, Argentina, is presented, and biological and behavioral observations are also included. Species of Poaceae, Bromeliaceae, Apiaceae, Araceae, Urticaceae, Marantaceae, and Arecaceae, were identified as phytotelmata. Twenty six species of Culicidae and nine of Ceratopogonidae were identified. The highest species richness of culicids and ceratopogonids was recorded from the bamboo *Guadua chacoensis* (Poaceae) and treeholes, respectively. Fourteen species of Culicidae and three of Ceratopogonidae inhabit bamboo, two and five treeholes, seven and three bromeliads, and nine and one, other less known phytotelmata, respectively.

KEY WORDS. Biodiversity. Aquatic insects. Mosquitoes. Gnats. Larvae of Diptera.

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INTRODUCTION

Phytotelmata are aquatic micro-environments formed by accumulation of water in any part of the body of plants such as leaves, flowers, stems or trunks (Frank & Lounibos, 1983). The communities of organisms that live in them are composed mainly of insects, immature stages of Diptera being dominant (Fish, 1983). The fact that phytotelmata are easy to handle small pools of water which allow to design experiments in natural conditions could account for the various studies carried out on them. Furthermore, some phytotelmata are important habitats that produce mosquito vectors of human diseases (Frank *et al.*, 1988; O'Meara *et al.,* 1995). The phytotelmata of Argentina have not been explored in depth, and only a few studies related to the invertebrate community living therein have been published. The first one was conducted in the subtropical region of Corrientes province, regarding the Bromeliaceae *Aechmea distichantha* Lemaire (Torales *et al.*, 1972); it described the physical and chemical characteristics of water, provided an inventory of invertebrates, and highlighted the presence of immature stages of four species of mosquitoes, three of *Culex* L. and one of *Phoniomyia* Theobald (nowadays a subgenus of *Wyeomyia* Theobald). The remaining studies were conducted in temperate areas of Buenos Aires Province: Vucetich & Rossi (1980) described the chemical characteristics of water in the axils of *Eryngium pandanifolium* Cham & Schltdl. (Apiaceae) and presented a list of the micro-organisms collected. Balseiro (1983) made observations on the selectivity of plants as oviposition sites of *Culex castroi* Casal & García. Marti *et al.* (2007) studied the seasonality of the immature stages of the culicid *Isostomyia paranensis* Brèthes living in the axils of the Cyperaceae *Scyrpus giganteus* Kunth. Campos & Lounibos (1999) studied the host-plant specificity, seasonality and population dynamics of *Culex castroi*, *Culex hepperi* Casal & García, and *Culex renatoi* Lane & Ramalho. In a recent study, Campos (2010) analyzed the macro-invertebrate community and food chains of four species of *Eryngium* L. phytotelmata. Finally, Montero *et al.* (2010) studied whether there are differences in species richness, species composition and feeding guild structure between environments (sun and shade) and micro habitats (among or on foliage) of bromeliads. Moreover, they studied whether these differences are similar between seasons.

The knowledge of Ceratopogonidae breeding in phytotelmata in Argentina is scarce, and is mostly limited to isolated citations or descriptions of preimaginal stages that were found in these habitats. Within the haematophagous genus *Culicoides* Latreille, Ronderos & Spinelli (1990) recorded adults of *C. nigrigenus* Wirth & Blanton collected in *A. distichantha* in Salta province, and Ronderos & Spinelli (2000) described the immatures of *C. bambusicola* Lutz from internodes of the bamboo *Guadua trinii* (Nees) Ness ex Ruprecht in northern Corrientes and Misiones provinces, Ronderos *et al.* (2008) of *C. charruus* Spinelli & Martínez from *Eryngium pandanifolium* in Buenos Aires province, and Ronderos *et al.* (2010) of *C. debilipalpis* Lutz from treeholes in Entre Ríos province. Spinelli & Rodríguez (1999) described *Dasyhelea necrophila* on the basis of material collected from flower vases plant-holding containers in La Plata cemetery; the species was found several times in other artificial containers, and likely breeds in phytotelmata in nature. Nothing is known about phytotelmatic ceratopogonids in Iguazú National Park.

Studies in Argentina are quite limited with regard to regions and types of phytotelmata. In this study, we present a list of phytotelmata of Iguazú National Park, located in the subtropical area of Argentina, and its culicid and ceratopogonid inhabitants.

MATERIAL AND METHODS

A field study was conducted from 2005 through 2007 in Iguazú National Park (25° 39′ S, 54° 18′ W), a subtropical area of Argentina. The park, in the Paranense forest

ecoregion (Dinerstein *et al.*, 1995), is located in the northern extreme of Misiones Province, separated from Brazil by the Iguazú River. The topography and drainage pattern of this ecoregion are the typical ones of a basaltic plateau, reaching altitudes of 700m. The annual rainfall varies between 1,500 and 2,000 mm, the dry season takes place in winter and the rainy season in summer. The mean temperature varies between 16ºC in winter and 22ºC in summer, and the dominant vegetation is the typical subtropical forest (APN, 2009).

All phytotelmata except bamboos were sampled by extracting the fluid contents with a pipette attached to a lift pump. After the first extraction, plants were flushed twice with clean tap water, and the aquatic contents extracted with a pipette after each wash. Samples from bamboo were taken by making a hole with a drill in the middle of the internodes, and extracting the content with a tube connected to a lift pump. After the extraction of the sample, the internodes were washed twice with clean water. Stumps (internodes opened after the falling of a bamboo) and broken bamboos were sampled with the same tube and lift pump used for internodes. All insects were killed in the field with 70% commercial ethyl alcohol, and preserved in 80% ethyl alcohol, except some immature dipterans which were carried alive to the laboratory to be reared individually and identified. Culicidae were classified and identified following Lane (1953 a, b), Darsie (1985), and Harbach (2007), and Ceratopogonidae following several publications containing keys and original descriptions of immatures which may be found in Borkent & Spinelli (2007).

RESULTS

Phytotelmata. Fourteen types of phytotelmata were identified in the park. Twelve are native and two introduced, as follows: bamboo *Guadua trinii*; *Guadua chacoensis* (Rojas) Londoño & Peterson; *Merostachys clausseni* Munro (Poaceae); bromeliads *Aechmea distichantha* Lemaire; *Aechmea recurvata* (Klotzsch), L. B. Sm.; *Billbergia nutans* Wendl. ex Regel; *Pseudananas sagenarius* (Arruda) Camargo; *Vriesea friburgensis* Mez. (Bromeliaceae), False Caraguatá *Eryngium* sp. (Apiaceae), elephant ear *Alocasia odora* (Lindl.) Hoch; *Philodendron bipinnatifidum* Schott ex Endl. (Araceae); brave nettles *Urera baccifera* (L.) Gaudich. Ex Wedd. (Urticaceae); *Maranta* sp. (Marantaceae), and the palmettos *Euterpe edulis* Mart. (Arecaceae) of which *P. sagenarius* and *A. odora* are introduced.

Only nine classes of phytotelmata host immatures of Culicidae and Ceratopogonidae (Tables I and II). Bamboos, treeholes and floral bracts of *Euterpe edulis* are woody phytotelmata. *E. edulis* produces phytotelmata when the bracts fall to the ground. The remaining phytotelmata are herbaceous and belong to Apiaceae, Araceae and Bromeliaceae.

No culicids or ceratopogonids were collected from the introduced plants (*P. sagenarius* and *A. odora*), or from *A. recurvata*, *Maranta* sp., and *U. baccifera*. Of these plants, four other than *U. baccifera* hold water in their leaf axils. *Urera baccifera* is the least known as phytotelmata. It is a shrub with spiny and hairy stems usually 1 to 2 m high (maximum 5m), and a cavity capable of containing water is formed in the stems when they break. The hole is small (about 1-2cm in diameter) but because the stems are hollow, the depth can be equal to the length of the branch.

Culicidae (Table I). Immatures were collected from 9 native phytotelmata. Twenty six species grouped into 10 genera were collected. Five species belong to Toxorhynchitinae, 21 to Culicinae, and no species of Anophelinae were recorded. Culicinae was represented by 14 species of Sabethini, five of Culicini and two of Aedini, all of them native except *Aedes aegypti* L.

The highest species richness (n = 10) was recorded from *G. chacoensis*, followed by *G. trinii* and *A. distichantha* (n = 6); *M. clausseni* and *E. edulis* (n = 4); and *P. bipinnatifidum*, *V. friburguensis*, *Eryngium* sp. and treeholes (n = 2).

Table I. Culicidae and host phytotelmata at Iguazú National Park, Misiones Province, Argentina. a: leaf axil, b: fallen floral bract, i : internode, s: stump.

Subfamily - Tribe	Species	Phytotelmata
Toxorhynchitinae		
	Toxorhynchites (Lynchiella) bambusicolus (Lutz & Neiva)	*A. distichantha* (a) *G. chacoensis* (i)
	Toxorhynchites (Lynchiella) guadalupensis (Dyar & Knab)	*G. chacoensis* (i)
	Toxorhynchites (Lynchiella) solstitialis (Lutz)	*A. distichantha* (a) *V. friburgensis* (a)
	Toxorhynchites (Lynchiella) theobaldi (Dyar & Knab)	*M. clausseni* (i)
	Toxorhynchites sp.	*A. distichantha* (a)
Culicinae		
Sabethini	*Limatus durhamii* Theobald	*E. edulis* (b)
	Runchomyia reversa (Lane & Cerqueira)	*G. chacoensis* (i) *G. trinii* (s)
	Sabethes (Sabethes) albiprivus Theobald	Treehole
	Sabethes (Sabethinus) aurescens (Lutz)	*M. clausseni* (i) *G. chacoensis* (i)
	Sabethes (Sabethinus) identicus Dyar & Knab	*G. chacoensis* (i)
	Trichoprosopon(Trichoprosopon) compressum Lutz	*E. edulis* (b)
	Trichoprosopon (Trichoprosopon) pallidiventer (Lutz)	*G. trinii* (s) *M. clausseni* (s) *A. distichantha* (a)
	Wyeomyia (Dendromyia) belkini Casal & García	*P. bipinnatifidum* (a)
	Wyeomyia (Phoniomyia) muhlensis (Petrocchi)	*E. edulis* (b)
	Wyeomyia (Phoniomyia) quasilongirostri Theobald.	*A. distichantha* (a)
	Wyeomyia (Wyeomyia) codiocampa Dyar & Knab	*G. chacoensis* (i) *G. trinii* (i)
	Wyeomyia (Wyeomyia) limai Lane & Cerqueira	*A. distichantha* (a) *M. clausseni* (i)
	Wyeomyia (Wyeomyia) sabethea Lane & Cerqueira	*G. chacoensis* (i) *G. trinii* (i) *M. clausseni* (i)
	Wyeomyia (Wyeomyia) serrata Lutz	*G. chacoensis* (i)
	Wyeomyia sp.1	*G. chacoensis* (i) *G. trinii* (i)
	Wyeomyia sp. 2	*G. chacoensis* (i) *G. trinii* (i)
	Wyeomyia sp.3	*P. bipinnatifidum* (a)
	Wyeomyia sp. 4	*M. clausseni* (i)
Culicini	*Culex (Phytotelmatomyia) castroi* Casal & García	*Eryngium* sp.
	Culex (Phytotelmatomyia) sp.	*Eryngium* sp.
	Culex (Anoedioporpa) chaguanco Casal, García & Fernández	*E. edulis* (b).
	Microculex imitator Theobald	*A. distichantha* (a) *V. friburgensis* (a)
Aedini	*Aedes (Stegomyia) aegypti* L.	*P. bipinnatifidum* (a)
	Haemagogus (Conopostgeus) leococelaenus (Dyar & Shannon)	Treeholes

Table II. Ceratopogonidae and host phytotelmata at Iguazú National Park, Misiones Province, Argentina. a: leaf axil, i : internode; s: stump.

Subfamily - Tribe	Species	Phytotelmata
Forcipomyiinae	*Atrichopogon* sp.	*A. distichantha* (a)
	Forcipomyia (Euprojoannsia) sp.	Treehole
	Forcipomyia (Warmkea) sp.	*E. pandanifolium* (a)
Dasyheleinae	*Dasyhelea* sp.	*A. distichantha* (a)
Ceratopogoninae		
Culicoidini	*Culicoides bambusicola* Lutz	*G. chacoensis* (i) *G. trinii* (i) *M. clausseni* (i,s) *V. friburgensis* (a) Treehole
	Culicoides paraensis (Goeldi)	Treehole
Ceratopogonini	*Stilobezzia* n. sp.	*G. chacoensis* (i,s)
Palpomyiini	*Bezzia snowi* Lane	Treehole
	Palpomyia guarani Lane	*G. chacoensis* (i) Treehole

All species living in the fallen bracts of *E. edulis*, belonging to *Limatus* Theobald, *Trichoprosopon* Theobald, *Wyeomyia*, and *Culex* were found exclusively in that phytotelm. This makes *E. edulis* the phytotelm with the highest number of exclusive species. On the other hand, all species that inhabit *Eryngium* spp. belong to a single subgenus, *Culex (Phytotelmatomyia)* Rossi & Harbach.

The low number of species found in treeholes (n = 1) could be attributed to the fact that this habitat was scarcely explored in the present study (n = 1), in comparison to the other phytotelmata.

Wyeomyia was the best represented genus in the phytotelmata of Iguazú. Of the 11 collected species of this genus (Table I), four were not identified. Two of them were collected in abundance (n > 60) from the internodes of both *Guadua* bamboo, but adults were not obtained from the reared immatures. Although the other two species, collected from the axils of *P. bipinnatifidum* and internodes of *M. clausseni*, were reared and adults were obtained, they could not be identified as any known species from the region.

Ceratopogonidae (Table II). Immatures of Ceratopogonidae were collected from seven native phytotelmata, represented by nine species grouped in six genera. Three species belong to Forcipomyiinae, five to Ceratopogoninae and one to Dasyheleinae.

The highest species richness (n = 5) was recorded from treeholes, following by *G. chacoensis*, (n = 3); *A. distichantha* (n = 2) and *E. pandanifolium*, *V. friburgensis*, *G. trinii* and *M. clausseni* (n = 1). The community of ceratopogonids that live in treeholes exhibits not only the greatest richness in species, but also in genera (n = 4).

Culicoides bambusicola Lutz was the most ubiquitous species, being present in treeholes, bamboo, and bromeliads, contrasting with its congener *C. paranensis* (Goeldi), collected only from treeholes.

DISCUSSION

Culicidae. The community of mosquitoes that inhabits phytotelmata of the subtropical region of Argentina is mainly dominated by species of Sabethini, contrasting with the temperate region where Culicini species are predominant (Campos & Lounibos, 1999; Campos, 2010). This difference could be attributed to the high diversity of phytotelmata of the subtropics in contrast to the temperate region.

It is worth pointing out the close association between species of the subgenus *Culex (Phytotelmatomyia)* with *Eryngium* species, and the fact that they have not been found inhabiting other phytotelmata. On the other hand, it is also remarkable that *Eryngium* is not attractive for species of other genera of mosquitoes. The strong association between mosquitoes of the subgenus *C.* (*Phytotelmatomyia*) and *Eryngium* plants is reaffirmed by our observations in Iguazú Park, where *Eryngium* spp. co-inhabit with bromeliads, and *E. edulis* houses species of *Culex* that belong to other subgenera.

On the basis of the finding of immatures of *Toxorhynchites guadalupensis* (Dyar & Knab) in internodes of *Guadua* bamboo during this study, the geographical distribution and host plants of this species is extended. It was previously reported from the northwestern Provinces of Catamarca, Jujuy, Salta and Tucumán (Mitchell & Darsie, 1985) from axils of *A. distichantha* (Augier *et al.*, 2003). It is also herein extended the range of host plants of *Wyeomyia muhlensis* (Petrocchi) and *Microculex imitator* Theobald, which had been cited previously only from *A. distichantha* (Torales *et al.*, 1972), and *Culex chaguanco* Casal, García & Fernandez, that were collected previously from treeholes (Casal *et al.*, 1968). In this study, we collected immature stages of *W. muhlensis* and *C. chaguanco* from bracts of *E. edulis*, and *M. imitator* from axils of *V. friburguensis*.

Only two invasive species of Aedini are known in Argentina, both residents of phytotelmata, *A. aegypti* and *Aedes albopictus* (Skuse). *Aedes aegypti* was found breeding in axils of *P. bipinnatifidum* as well as in artificial containers in Iguazú National Park. *Aedes albopictus* was not encountered during this study, although this species has been found in the nearby city of Iguazú, breeding in axils of Bromeliaceae (Rossi *et al.*, 1999). *Aedes albopictus* inhabits bromeliads, as well as treeholes in Florida, USA (Hawley, 1988; O'Meara *et al.*, 1995), and Brazil (Cunha *et al.*, 2002), and its invasion in Argentina forests needs extreme caution and further monitoring.

Ceratopogonidae. Several species of ceratopogonids of *Culicoides*, *Dasyhelea* Kieffer, *Forcipomyia* Meigen, and other genera are known to develop in phytotelmata in the Neotropical region (e.g.: Roger, 1964; Yanoviak, 1999; Spinelli *et al.*, 2007), but only *Culicoides* spp. and *Dasyhelea* spp. were reported from such habitats in Argentina (e.g.: Ronderos & Spinelli, 1990; Spinelli & Rodríguez, 1999). Most of the ceratopogonid larvae from the Neotropical region were collected from bromeliads and treeholes (e.g.: Yanoviak, 1999, Ospina-Bautista *et al.*, 2004), and a few from other less known phytotelmata (e.g.: Fish & Soria, 1978; Vitale, 1977; Campos, 2010).

A study carried out in Panama (Vitale, 1977) showed that bromeliads, treeholes and water-containing bamboo internodes are the phytotelmata where the species of *Culicoides* occur more frequently other than in *Heliconia* L. (Musaceae) *Xanthosoma* Schott (Araceae) leaf axils, and *Costus* L. (Costaceae) inflorescences as well as in a water holding palm frond. In accordance with Vitale (1977), during this study the species of *Culicoides* were mainly collected from treeholes and bamboo internodes, and to a lesser extent from other phytotelmata. Vitale (1977) also collected larvae of the predaceous midges genera *Bezzia* Kieffer and *Palpomyia* Meigen from bromeliads, whereas during this study species of both genera were collected from treeholes. Moreover, *Palpomyia guarani* Lane was collected from bamboo internodes.

Observations made by other authors (Vitale, 1977; Fish & Soria, 1978; Ronderos & Spinelli, 2000; Ronderos *et al.*, 2010), as well as during this study, show that the species of *Stilobezzia* whose larvae inhabit phytotelmata have a tendency to select woody plants (dendrotelmata) as their breeding site. Species of *Dasyhelea* and *Atrichopogon* Kieffer, meanwhile, prefer herbaceous plants, while species of *Bezzia*, *Culiciodes*, *Forcipomyia* and *Palpomyia* live in woody and herbaceous plants. More intensive research is needed in order to confirm specificity between species of midges and their host plants.

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