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Freshwater sponge-dwelling Chironomidae (Insecta, Diptera) in northeastern Brazil

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Resumo

Chironomidae habitantes de esponjas de água doce no nordeste do Brasil. A região Neotropical possui uma rica fauna de esponjas de água doce, porém o conhecimento sobre sua taxonomia, bem como sobre as comunidades dentro delas permanece fragmentado. Larvas de Chironomidae (Insecta, Diptera) associadas a esponjas de água doce têm sido amplamente relatadas na literatura, com alguns gêneros que são reconhecidos como contendo espécies exclusivamente dependentes desses organismos. Neste estudo, analisamos a ocorrência de larvas de Chironomidae associadas a esponjas de água doce (Spongillidae) no nordeste do Brasil. As amostras foram coletadas em dois sistemas aquáticos em Pernambuco, no período de fevereiro de 2011 a outubro de 2012. No Rio Sirinhaém, *Corvoheteromeyenia australis* (Bonetto & Ezcurra de Drago) foi a única esponja coletada, sendo que *Polypedilum* Kieffer, *Goeldichironomus* Fittkau, *Xenochironomus* Kieffer e *Cricotopus* Van der Wulp foram os Chironomidae registrados como representantes dessa fauna associada. Por outro lado, no tanque de piscicultura, *Heteromeyenia cristalina* Batista, Volkmer-Ribeiro & Melão, *Radiospongilla inesi* Nicacio & Pinheiro e *Tubella variabilis* Boneto & Ezcurra de Drago foram as esponjas coletadas, e *Polypedilum*, *Chironomus* Meigen, *Dicrotendipes* Kieffer e *Goeldichironomus* foram as larvas associadas a essas espécies de esponjas. Embora apenas espécies pertencentes ao gênero *Xenochironomus* sejam relatadas em estreita interação com esponjas de água doce, aqui documentamos outros gêneros de Chironomidae também associados a esses organismos. No entanto, é necessária uma investigação mais aprofundada para entender melhor como as larvas de Chironomidae usam esponjas como abrigo e/ou prevenção de predação de forma facultativa. O objetivo do presente estudo foi analisar a ocorrência de habitantes de Chironomidae de esponjas de água doce em sistemas aquáticos no estado de Pernambuco, a fim de incrementar o conhecimento de Chironomidae habitantes de esponjas no nordeste do Brasil.

Palavras-chave: Insetos aquáticos; Interação interespecífica; Porifera; Esponjas; Neotropical

Abstract

The Neotropical region possesses a rich freshwater sponge fauna; however, knowledge about its taxonomy and the community inside the sponges remains fragmentary. Chironomidae (Insecta, Diptera) larvae associated with freshwater sponges have been largely reported in literature and some genera are recognized as containing species exclusively dependent on these organisms. In this study, we analyze the occurrence of chironomid larvae associated with freshwater sponges (Spongillidae) in northeastern Brazil. Samples were collected in two aquatic systems in Pernambuco, Brazil, in February 2011 and October 2012. In the Rio Sirinhaém, *Corvoheteromeyenia australis* (Bonetto & Ezcurra de Drago) was the only sponge collected, and *Polypedilum* Kieffer, *Goeldichironomus* Fittkau, *Xenochironomus* Kieffer and *Cricotopus* Van der Wulp were the chironomids found as its associated fauna. On the other hand, in the fishpond, *Heteromeyenia cristalina* Batista, Volkmer-Ribeiro & Melão, *Radiospongilla inesi* Nicacio & Pinheiro and *Tubella variabilis* Boneto & Ezcurra de Drago were the collected sponges, and *Polypedilum*, *Chironomus* Meigen, *Dicrotendipes* Kieffer and *Goeldichironomus* were the larvae associated with these species. Although only species of *Xenochironomus* are reported to have close interactions with freshwater sponges, herein we document other Chironomidae genera associated with these organisms. Nevertheless, further investigations are needed to better understand how chironomid larvae use sponges as shelter and/or to avoid predation in a facultative way. The objective of the present study was to analyze the occurrence of Chironomidae inhabitants of freshwater sponges in aquatic systems in the state of Pernambuco, in order to contribute to the knowledge of sponge-dwelling chironomids in northeastern Brazil.

Key words: Aquatic insects; Interspecific interaction; Porifera; Sponges; Neotropical

Introduction

The non-biting midges Chironomidae (Insecta: Diptera) are true flies and the most widely distributed free-living holometabolous insects (CRANSTON, 1982; FERRINGTON, 2008). Species of Chironomidae inhabit an enormous variety of aquatic ecosystems, ranging from moist soils to pools in tree holes, and from low-oxygen lake sediments to fast-flowing mountain streams (FERRINGTON et al., 2008). For chironomids, the adult life stage is ephemeral and most of the lifespan is spent as a larva (THIENEMANN, 1954; TOKESHI, 1995). The numerous species and habitat diversity make taxa of this family valuable indicator species for lentic and lotic aquatic ecosystems, and particularly appropriate for phylogenetic and biogeographical research (SILVA; EKREM, 2016). While recent years have seen increased activity concerning the study of Chironomidae fauna in the Neotropical region (e.g., OLIVEIRA et al., 2013; TRIVINHO-STRIXINO et al., 2013; 2015; ANDERSEN; PINHO, 2014; SILVA et al., 2014a; 2014b; ANDERSEN et al., 2015; SILVA; WIEDENBRUG, 2015; SIRI et al., 2015; PARISE; PINHO, 2016; SILVA; OLIVEIRA, 2016; SILVA; FARRELL, 2017; SILVA; FERRINGTON, 2018), what is known about the interactions between some species of Chironomidae and their hosts remains fragmentary (TRIVINHO-STRIXINO et al., 2012).

Interactions between organisms, such as competition and predation, have been reported as factors that regulate communities (RICKLEFS, 1987). However, interactions, such as commensalism, have been briefly described and still require further investigation, as indicated by Holomuzki et al. (2010). Freshwater sponges (Spongillidae, Porifera) comprise a group of utmost significance for the metabolism of ecosystems, acting as important aquatic filter-feeding organisms. The association between chironomids and these organisms has been occasionally mentioned in the literature (STEFFAN, 1967; ROBACK, 1968; MATTESON; JACOBI, 1980; TOKESHI, 1993; 1995; MELÃO; ROCHA, 1996; ROQUE et al., 2004; ROQUE; TRIVINHO-STRIXINO, 2005; FUSARI et al., 2012; 2013; 2014). However, in the Neotropics only three genera are recognized as having this type of interaction: *Ablabesmyia* Johannsen; *Xenochironomus* Kieffer; and *Oukuriella* Epler (FUSARI et al., 2012; 2013). Additionally, questions addressing whether larvae of other species of Chironomidae can inhabit freshwater sponges without necessarily having an obligate association are still unclarified. In this study,

we analyze the occurrence of chironomid inhabitants of freshwater sponges in aquatic systems in the state of Pernambuco, Brazil.

Material and Methods

Study area

The Rio Sirinhaém originates in the municipality of Camocim de São Félix, Pernambuco, Brazil, and extends about 158 km in a northwest-southeast direction. It also crosses the municipalities of Gameleira and Cortes, where part of this study was conducted (08°30'S, 35º31'W). The second sampling site was in a fishpond located on the campus of the Federal Rural University of Pernambuco (8°01'S, 34°56'W). This system presents different types of sediments (sand and clay) at the bottom and is formed by water flowing from Plata Creek, located in Parque Dois Irmãos, in northwestern Recife, which had its course changed to supply the fishpond to farm tilapia for scientific research.

Collection and identification

Collections were made in February 2011 in Rio Sirinhaém and in October 2012 in the fishpond. In each of these environments, we searched for sponges on several potential substrates, such as decaying wood, rocks, and macrophytes. All samples were field-preserved using 70% ethanol. In the laboratory, the sponges were dissected and slide-mounted following the procedures described in Hajdu et al. (2011). Identifications were made by the team of the Laboratory of Porifera, Federal University of Pernambuco, based on Manconi and Pronzato (2002) and Nicacio and Pinheiro (2015), mainly using sponge spicules. Larval chironomids associated with different sponges were sorted using a stereomicroscope and slide-mounted following the procedure outlined by Pinder (1983). The material was morphologically identified to the lowest taxonomic level possible based on the keys in Wiederholm (1983), Epler (2001), Andersen et al. (2013), Trivinho-Strixino (2014) and Silva et al. (2018), and occasionally original descriptions. Gut content examination was made according to Mcshaffrey and Olive (1985), based on previously prepared slides of larvae. No attempt was made during collection to prevent regurgitation of food in the gut; although, all analyzed larvae had some gut content. Guts were left in the bodies and examined microscopically by transparency through the cuticle (for more details see SILVA et al., 2008a; 2008b). Voucher specimens are deposited in the Laboratory of Assessment, Recovery, and Restoration of Aquatic Ecosystems, at the Federal University of Pernambuco.

Results

In total, 266 representatives of subfamilies Chironominae and Orthocladiinae were recorded inhabiting four species of freshwater sponges (Table 1). Six genera were identified. *Polypedilum* Kieffer and *Goeldichironomus* Fittkau were the most abundant genera, predominating in all samples. *Xenochironomus* was only sampled in the Rio Sirinhaém, while *Chironomus* Meigen and *Dicrotendipes* Kieffer occurred entirely in the fishpond. Only one genus was recorded for Orthocladiinae, *Cricotopus* Van der Wulp. In the Rio Sirinhaém the chironomid fauna were exclusively associated with *Corvoheteromeyenia australis* (Bonetto; Ezcurra de Drago). Other species of freshwater sponges were not found in this environment. On the other hand, in the fishpond, Chironomidae larvae were collected in association with *Heteromeyenia cristalina* Batista, Volkmer-Ribeiro & Melão, *Radiospongilla inesi* Nicacio & Pinheiro and *Tubella variabilis* Bonetto & Ezcurra de Drago. This aquatic ecosystem presented not only the highest diversity of freshwater sponges, but also the highest abundance of chironomids (Table 1). In general, the gut content of the analyzed larvae was predominantly diatoms and fine organic matter. Spicules of *H. crystalina* were found in larvae of *Goeldichironomus*, while spicules of *C. australis* were observed in larval *Cricotopus*.

TABLE 1: Absolute (N) and relative (%) abundance of chironomid larvae in freshwater sponges in the Rio Sirinhaém and the fishpond, Pernambuco, Brazil. *Corvoheteromeyenia australis* (A), *Heteromeyenia cristalina* (B), *Radiospongilla inesi* (C) and *Tubella variabilis* (D).

Diptera: Chironomidae		Rio Sirinhaém		Sponge	Fishpond		Sponge
Subfamily	**Taxa**	**N**	**%**		**N**	**%**	
Chironominae	*Polypedilum (Asheum)* sp.	20	30.3	A	25	12.5	A-C
	Chironomus sp.	0	0.0	-	15	7.5	A-D
	Dicrotendipes sp.	0	0.0	-	17	8.5	B-C
	Goeldichironomus neopictus	17	25.8	A	143	71.5	A-C
	Xenochironomus sp.	14	21.2	A	0	0.0	A
Orthocladiinae	*Cricotopus* sp.	15	22.7	A	0	0.0	A
Total		**66**	**100**		**200**	**100**	

Chironomus Meigen, 1800

Chironomus is a cosmopolitan genus with several hundred species. Numerous species of this group are very abundant in heavily polluted standing or running waters, whereas some species usually colonize small water bodies, such as fish breeding tanks, where they complete their life cycle in a few days (CORREIA et al., 2013). Spies and Reiss (1996) catalogued 19 species from the Neotropical region. However, this number clearly represents outdated knowledge and significantly underestimates the real diversity of *Chironomus* in the Neotropics, considering that several new species have been described more recently (CORREIA; TRIVINHO-STRIXINO 2005; 2007; CORREIA et al., 2005; 2006; 2013). In our study, larvae of *Chironomus* (see Figure 1a) were found associated with the following freshwater sponges: *Corvoheteromeyenia australis* (Bonetto; Ezcurra de Drago), *Heteromeyenia cristalina* Batista, Volkmer-Ribeiro & Melão, *Radiospongilla inesi* Nicacio & Pinheiro and *Tubella variabilis* Boneto & Ezcurra de Drago. None of the larvae analyzed in the present study had any spicules in their digestive tracts, which were filled with debris, sand and diatoms.

Cricotopus Van der Wulp, 1874

This genus is one of the largest in subfamily Orthocladiinae, comprising five subgenera, and has a worldwide distribution (CRANSTON et al., 1989; ASHE; O'CONNOR, 2009). Larval *Cricotopus* are known to inhabit a wide range of water bodies, from pristine streams and brooks to eutrophic ponds and brackish estuaries (HIRVENOJA, 1973; BOESEL, 1983). Certain species may become so abundant in eutrophic waters that adult swarms become a nuisance (SPIES, 2000; HIRABAYASHI et al., 2004). The group is often recorded in association with aquatic macrophytes, algae and sometimes cyanobacteria. Some larvae mine in macrophytes, while many graze on the surfaces (CRANSTON et al., 1983), and according to Roback (1968) they are capable of living in and on sponges as well as other habitats. In our study, larvae of *Cricotopus* (Figures 1b; 1c) were associated with *Corvoheteromeyenia australis*. The digestive tract was filled with debris and spicules of the aforementioned freshwater sponge (Figure 1d).

Dicrotendipes Kieffer, 1913

Dicrotendipes is a large group with a worldwide distribution. It is commonly found in littoral sediments of brackish and fresh water, in lotic and lentic conditions, in pristine or degraded habitats (EPLER, 2001), and in many cases it is the most abundant organism. Although larvae occur in sediments, they are most often encountered on vegetation (EPLER, 2001). The group is considered pollution-sensitive to most forms of toxic pollution but thrives in areas containing high levels of nutrients or organic wastes (SIMPSON; BODE, 1980). Its most common associates in such situations are oligochaetes. In our study, larval *Dicrotendipes* were found in association with *Heteromeyenia cristalina* and

Radiospongilla inesi (Figure 1e). None of the larvae analyzed in the present study had any spicules in their digestive tracts. This might be due to the fact that the larvae here examined may not have achieved their full body growth, since it seems that the presence of spicules in the gut of sponge-dwelling chironomid mainly occurs in final instars (ROBACK, 1968).

Goeldichironomus Fittkau, 1965

Goeldichironomus is a Pan-American genus, which commonly occurs in eutrophic standing and slow-moving water, where it is found in or on sediments and aquatic plants (EPLER, 2001). Immatures of this genus favor floating and drifting vegetation of small standing water bodies. Several species are miners that form small tunnels in the petioles of macrophytes and decaying wood (REISS, 1974; TRIVINHO-STRIXINO, 2014). They also live in burrows in plant material made by other organisms (EPLER, 2001). The larvae analyzed in our study were identified as *Goeldichironomus neopictus* Trivinho-Strixino and Strixino (Figure 1f). These were the most common larvae and were encountered in association with *Corvoheteromeyenia australis*, *Heteromeyenia cristalina* and *Radiospongilla inesi*. Larval *Goeldichironomus* were found throughout the body of the sponge. The digestive tract contained many sponge spicules of *H. cristalina* (Figures 1g; 1h), indicating that the larva was probably feeding on this sponge and its presence may not have been merely accidental.

Polypedilum Kieffer, 1912

This genus is one of the largest in the Chironominae and has eight subgenera, a worldwide distribution, and about 440 described species (SÆTHER et al., 2010). Larvae of *Polypedilum* occur in nearly all types of still and flowing waters, ranging from pristine to heavily degraded, except for in the arctic and high mountains. Sediments are the preferred substratum. Some species are also found on hard substrata, as well as mining aquatic plants and in bromeliad tanks (CRANSTON; EPLER, 2013). Larval *Polypedilum* are filter-feeders, and their occurrence seems to be governed more by current speed and the amount of suspended food particles than by water quality (SIMPSON; BODE, 1980). The immatures analyzed in our study were identified as *Polypedilum* (*Asheum*) sp. Sublette (Figures 1i; 1j), which is commonly found in lakes, reservoirs and rivers, mainly in association with aquatic macrophytes (TRIVINHO-STRIXINO, 2014). The taxon was previously recorded for southeastern Brazil by Silva and Farrell (2017). Herein, the species was found interacting with *Corvoheteromeyenia australis*, *Heteromeyenia cristalina* and *Radiospongilla inesi*. None of the larvae examined had any spicules in their digestive tracts, which were basically filled with debris.

Xenochironomus Kieffer, 1912

This is a small genus with species distributed in the Afrotropical, Nearctic and Neotropical regions. Larvae of *Xenochironomus* are obligate miners in freshwater sponges in both standing and flowing waters (CRANSTON; EPLER, 2013). The immatures of *Xenochironomus* can be found in freshwater sponges with the exception of *Xenochironomus canterburyensis* (FREEMAN, 1959), which has been reported in association with Mollusca (FORSYTH; MCCALLUM, 1978). This is the first record of *Xenochironomus* from the Northeast Region of Brazil. The larvae have very large salivary glands and line the tubes they create with silk as they move along (ROBACK, 1968). The immatures analyzed here were identified as *Xenochironomus* (Figures 1k; 1l) and were found associated only with *Corvoheteromeyenia australis*. Some were in gently curved tubes, both ends of which opened on the surface of the sponge, and others were in the internal cavities of the sponge. None of the larvae found in the present study had any spicules in their digestive tracts.

FIGURE 1: Freshwater sponge-dwelling Chironomidae (Insecta, Diptera) in northeastern Brazil.

Legend: **a)** *Chironomus* Meigen. **b-c)** *Cricotopus* Van der Wulp. **d)** sponge spicules of *Corvoheteromeyenia australis* (Bonetto & Ezcurra Drago) in *Cricotopus* Van der Wulp. **e)** *Dicrotendipes* Kieffer. **f)** *Goeldichironomus* Fittkau. **g-h)** sponge spicules of *Heteromeyenia crystalline* Baptist, Melon & Volkmer-Ribeiro in *Goeldichironomus* Fittkau. **i-j)** *Polypedilum* Kieffer. **k-l)** *Xenochironomus* Kieffer.

Discussion

Spongillidae has an interspecific relationship with the family Chironomidae. In our study, we record for the first time the larvae of *Polypedilum*, *Chironomus*, *Cricotopus*, *Dicrotendipes* and *Goeldichironomus* associated with freshwater sponges. To most insects, such as baetid and heptageniid mayfly nymphs, hydropsychid caddisfly larvae, as well as larval chironomids, the sponges seem to be merely another substrate (ROBACK, 1968), since there was no evidence indicating an obligate association of chironomids with the sponges, despite the fact that larvae were found living within the sponges and/or feeding on them. However, the association between chironomid larvae and freshwater sponges might benefit the chironomids by improving feeding opportunity, providing protection from disturbances and decreasing predation risks. This appears to be the scenario in the fishpond, where the association of larval chironomids with sponges may be related to a defensive strategy and/or predation avoidance (mainly from tilapia), since these taxa were not found free-living or on the bottom and walls of this environment. Moreover, the higher diversity of sponges in the fishpond suggests that this environment provides more adequate conditions for the development of these organisms, which may be related to the lentic character of the system compared to the Rio Sirinhaém with lotic characteristics and lower diversity.

Despite the presence of spicules in the gut content of some examined larvae, this association might be considered facultative, since except for *Xenochironomus,* all the other genera are widely recorded in different environments. Furthermore, the amount of spicules observed does not seem to be enough to assure this association as an obligate relationship, since spicules were found associated with other types of food, such as detritus and diatoms, which are commonly recorded in the Chironomidae diet. Roque and Trivinho-Strixino (2005) described *Xenochironomus ceciliae* as a freshwater sponge-dwelling Chironomidae in the upper Paraná River. We recorded larvae of *Xenochironomus* in association with *C. australis*. Moreover, the water quality of the Rio Sirinhaém, which is rich in fine particles of organic matter, seems to be suitable for the development of the sponge and the observed interspecific interaction with chironomids. Larvae of *Xenochironomus* have a brush of numerous long labral setae, which indicates a filter-feeding habit, likely related to the particles originating from the aquifer system of sponges (ROQUE; TRIVINHO-STRIXINO, 2005).

When comparing our results with Roback (1968) and Fusari et al. (2008), who recorded several larvae of Chironomidae associated with different species of freshwater sponges, it is possible that some species of the following genera might still be found interacting with sponges: *Oukuriella* Epler, *Orthocladius* Van der Wulp and *Stenochironomus* Kieffer. Even though our inventory documents 266 representatives of two subfamilies living in four species of freshwater sponges, we consider this a small freshwater sponge-dwelling chironomid community for northeastern Brazil. We believe that collections in different periods and broadening the variety of sampling habitats and geographic area may reveal a greater diversity than currently recorded. Despite the increased activity in studying the Neotropical chironomid fauna in recent years, data on the interaction between chironomids and freshwater sponges remain fragmentary. Thus, additional inventories are needed to improve our understanding of the insects that might be associated and to add to the information about those already known to be associated. Studies on interspecific interactions between chironomids and sponges remain incipient and, due to a lack of information, the relevance of obligatory or optional associations for Spongillidae and Chironomidae larvae is still unclarified. This study reports for the first time the larvae of *Polypedilum*, *Chironomus*, *Dicrotendipes* and *Goeldichironomus* living inside freshwater sponges. Despite that the association between these organisms might benefit the chironomids by improving feeding opportunity and providing protection, no evidence indicates an obligate relationship between these taxa and the different species of freshwater sponges recorded.

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