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Evidence of phenotypic plasticity of larvae of *Simulium subpallidum* Lutz in different streams from the Brazilian Cerrado

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ABSTRACT

In this paper, the overall morphological differences between populations of *Simulium subpallidum* Lutz, 1909 are studied. Several studies found in the literature point to a relationship between the labral fans and body size and the habitat where blackfly larvae occur. However, other characteristics potentially related to the microhabitat, such as abdominal hook circlet morphology, which is used for larvae to fix themselves in the substratum, and thoracic prolegs morphology, which help larvae move in the substratum, were analyzed in three different populations of *S. subpallidum*, one of which occupied a faster flow. The results suggest phenotypic plasticity in *S. subpallidum* and a tendency toward larger structures in faster flows.

Introduction

Organisms often show environmentally-specific phenotypes selected to variable conditions for success in habitats with high environmental variation (Agrawal, 2001; Piersma and Drent, 2003). Phenotypic plasticity is the ability of organisms to alter their physiology or morphology according to the ambient conditions (Nylin and Gotthard, 1998; West-Eberhard, 1989). Morphological variation of the feeding apparatus reflects the selection pressure with respect to food availability (Schluter, 1996), but other environmental factors can also be responsible for the final outcome. Phenotypic plasticity of the feeding apparatus can affect the foraging function, which in turn influences the organism's growth.

In aquatic invertebrates, the effects of multiple environmental factors on the phenotypic plasticity of the feeding apparatus have not been well studied. Within the biological filtration theory, the current velocity, the food particles and the filter structure determine the feeding mechanisms of suspension feeders (Cheer and Koehl, 1987a, b). Empirical studies have indicated that variations in hydrodynamic conditions are key influences on suspension feeding invertebrates, in terms of their feeding structures and function (Koehl, 1996, 2004), such as blackfly larvae in flowing waters (Zhang and Malmqvist, 1996).

Black fly larvae are often considered classic examples of filter feeding organisms (Burgherr et al., 2001). These organisms employ their cephalic labral fans to capture food in lotic environments, and play the role of engineering species in such ecosystems, once they ingest fine particle organic matter (FPOM) and excrete larger fecal pellets, still useful as resource for other organisms due to their low digestive efficiency (Malmqvist et al., 2001; Wotton et al., 1998; Zhang, 2006).

The characteristics of black fly larvae are often related to larval microhabitat features, such as water flow velocity and food concentration (Craig and Chance, 1982; Currie and Craig, 1987; Malmqvist et al., 1999; Palmer and Craig, 2000; Santos-Jr. et al., 2007; Zhang, 2000; Zhang and Malmqvist, 1996, 1997). Some black fly species may occur only in the riffles of large rivers, while others are restricted to small streams of slower water currents (Bertazo and Figueiró, 2012; Figueiró et al., 2006, 2008, 2014; Hamada et al., 2002; Shelley et al., 2000; Zhang and Malmqvist, 1996). Ecological theory and some empirical studies suggest that the habitat choice may be directly influenced by labral fan morphology (Carlsson, 1962; Grenier, 1949; Kurtak, 1978; Lewis, 1953), with black fly species from faster water velocities tending to have smaller labral fans,

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with stout rays, while species that occur in slower water current velocities tend to have longer labral fans and more delicate rays (Malmqvist et al., 1999; Palmer and Craig, 2000; Zhang and Malmqvist, 1996).

Although there are few studies relating labral fan morphology to body size and habitat type (Zhang and Malmqvist, 1996, 1997), other morphological characteristics potentially related to microhabitat associations are neglected in the literature. Figueiró and Gil-Azevedo (2010) reported the scarcity of studies on microhabitat requirements of Neotropical black flies and the lack of studies relating labral morphology to microhabitat type in the Neotropics.

Figueiró et al. (2012) recently observed that *Simulium subpallidum* Lutz, 1909, in the presence of *Simulium nigrimanum* Macquart, 1838, was restricted to velocities between 0.19 $m.s^{-1}$ and 0.88 $m.s^{-1}$, while the later occupied velocities between 0.99 $m.s^{-1}$ and 1.32 $m.s^{-1}$. However, when *S. nigrimanum* was absent, *S. subpallidum* occupied the same water velocity range that the former species would.

The aim of the present study was to compare the morphology of structures potentially related to the water current velocity ranges occupied by *S. subpallidum*, and so investigate if the distributional patterns observed in Figueiró et al. (2012) may reflect phenotypic plasticity in larvae of *S. subpallidum*. Thus, we tested the hypotheses that anal disk diameters should be larger in larvae that occur in faster flowing sites due to surface area for fixation requirements, as well as proleg diameters and areas should be larger and larvae body sizes should be lengthier, in order to resist water current. Another tested hypothesis was the well-established pattern that larvae from faster current sites should present smaller labral fans. Although this pattern is often observed in Holarctic black flies, it has not been investigated for the Neotropical black fly fauna.

Material and methods

Due to the reduced number of last instar larvae among the sampled material, three groups of 15 last instar larvae of *S. subpallidum* were separated, one from each of the three sampling sites from the state of Tocantins, Brazil: Córrego do Mato (S12°39'33.0" W48°18'27.3"), Piabanha (S12°45'07.8" W48°17'16.6"), and Ribeirão do Lages (S12°35'7.7" W48°2'29.2").

Larvae were sorted in morphotypes, and their final instar specimens were dissected and identified using direct comparison with pupae collected in the sites and with the material deposited at the Laboratório de Simulídeos e Oncocercose/Instituto Oswaldo Cruz (LSO-IOC), and with the aid of taxonomic bibliography (*e.g.* Coscarón and Coscarón-Arias, 2007; Hamada and Adler, 2001). The specimens are currently deposited at LSO-IOC.

Hence, three populations of *S. subpallidum* from the sites Córrego do Mato, Piabanha and Ribeirão do Lages were compared with each other, as their larvae were sorted and photographed in a stereoscopic microscope and later measured with the use of CMEIAS software (Liu et al., 2001) (Fig. 1).

The labral fans were separated and photographed in slides, in order to have them open and facilitate their measuring. Each labral fan had one of their central rays measured, in order to estimate its size. The anal disks had their diameters measured as a form of estimating the surface for their fixation to the substrate, since it should be expected that, in faster currents, larvae would have more surface area for fixation in order to resist the water velocity. The prolegs had their sclerotized basal diameter measured, as a way of estimating their stoutness, since it would be expected that, in fast flowing microhabitats, stronger prolegs should be demanded. Additional measurements of proleg area and labral fan area were also taken (Fig. 1).

The measured groups from the different populations were compared with each other through the Kruskal-Wallis test, and each measured characteristic was tested, in order to verify their correlations with each other, using the Spearman correlation coefficient, since the normality tests indicated that data diverged significantly from a Gaussian distribution.

Results

The larvae of *S. subpallidum* from Ribeirão do Lages had its body size significantly larger than that of the other two populations, which co-occurred with *S. nigrimanum* (Fig. 2A), while the diameter and the area of the anal disk varied among *S. subpallidum* populations, as Ribeirão do Lages' differed significantly from the other populations (Fig. 2B, 3B).

The proleg diameter showed the same pattern of the previously mentioned characteristics, with the *S. subpallidum* population from Ribeirão do Lages being significantly different from the rest of the populations of the same species (Fig. 2C), and the same was true for proleg areas (Fig. 3A), while labral fan size of the *S. subpallidum* population from Ribeirão do Lages was significantly smaller than the others (Fig. 2D, Table 1), as were their labral fan areas (Fig. 3C).

Discussion

The population shown in the study of Figueiró et al. (2012), associated to faster currents, showed larger bodies and anal disk and proleg diameters, corroborating the hypothesis that these structures showed morphological differences among sites.

The *S. subpallidum* population from Ribeirão do Lages, which Figueiró et al. (2012) showed to be associated to the same current

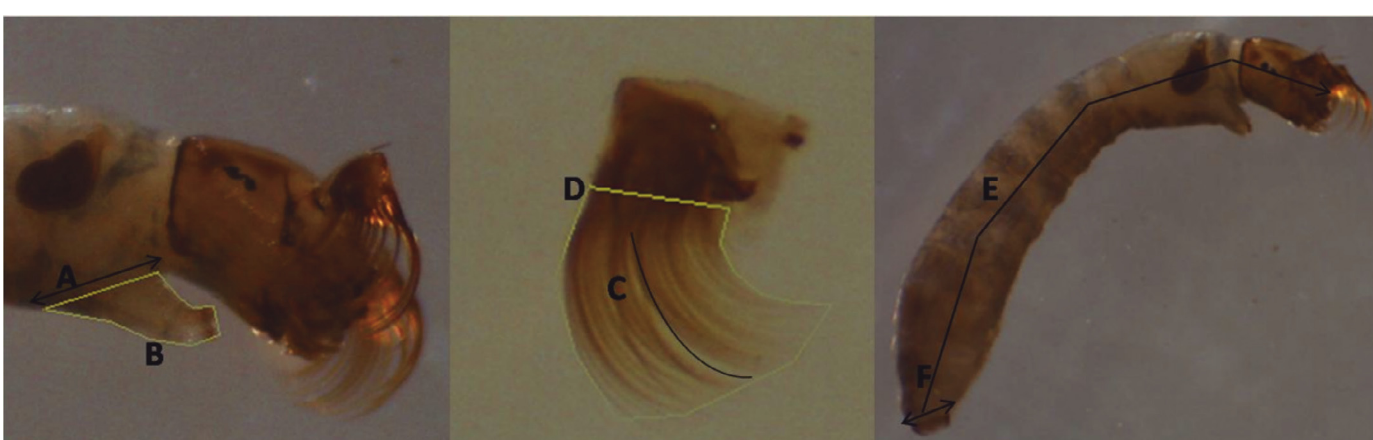

Figure 1. Measurements of black fly larvae morphology taken using CMEIAS: (A) Proleg diameter, (B) Proleg area, (C) Labral fan length, (D) Labral fans area, (E) Body length, (F) Anal disk diameter.

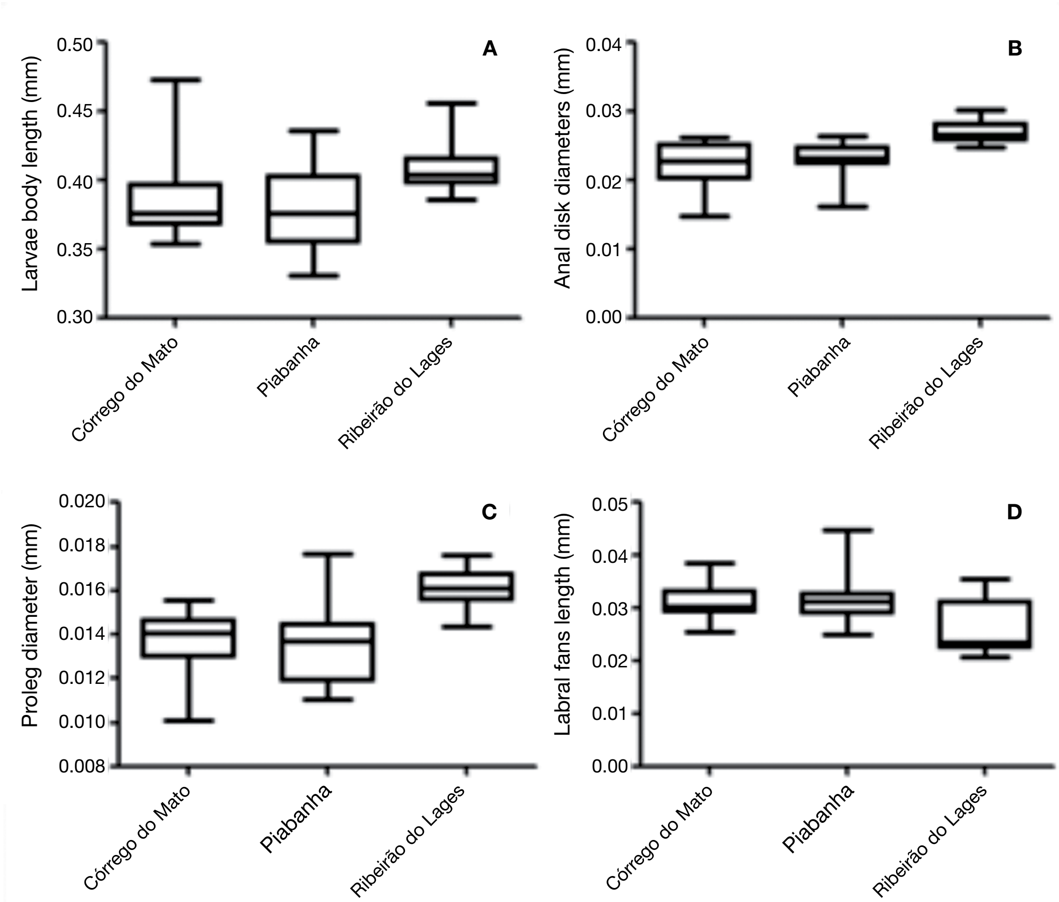

Figure 2. Variation of body length (A), anal disk diameters (B), proleg diameters (C), and labral fans length (D), indicating the significant differences ($p < 0.05$) among the *S. subpallidum* population from Ribeirão do Lages and the other conspecific populations.

Table 1.
Correlations between the morphological characteristics of Simuliidae larvae significant at $p < 0.05$.

	Anal disk	Length	Proleg	Labral fans
Anal disk	-	0.907517	0.745671	0.397057
Length	0.907517	-	0.751661	0.414658
Proleg	0.745671	0.751661	-	0.425113
Labral fans	0.397057	0.414658	0.425113	-

range than *S. nigrimanum* in the absence of the latter, differed significantly from the other populations of the same species, which may suggest that a character displacement process could be occurring in this population of *S. subpallidum*.

The *S. subpallidum* population from Ribeirão do Lages had smaller labral fans than the other populations of the same species, corroborating the pattern established in literature: studies with *Simulium lundstromi* (Enderlein, 1921), for example, showed a phenotypic plasticity of the fan structure with a similar pattern to the one already described for *S. noelleri* Friederichs, 1920 in response to different current velocities (Zhang and Malmqvist, 1997). In this perspective, morphological adaptations enable feeding at different flow regimes by balancing increasing particle capture in slow currents and reducing drag force cost on fans in fast currents (Zhang, 2000).

On the other hand, another study by Lucas and Hunter (1999) demonstrated that the ray number of *S. rostratum* (Lundström, 1911) and *S. decorum* Walker, 1848 decreased with food supply increase in a laboratory experiment, which may suggest that our patterns may have been influenced by food supply as well, although this variable was not measured in our experiment.

The results of the present study suggest that phenotypic plasticity among *S. subpallidum* occurring in different habitats and taxocenoses could represent a character displacement, which would allow the coexistence of species that would normally explore very similar niches, and thus exclude each other by the competitive exclusion principle, while the positive correlation among the measured morphological characteristics points towards an ensemble response of these structures to habitat conditions and/or competition.

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Conflicts of interest

The authors declare no conflicts of interest.

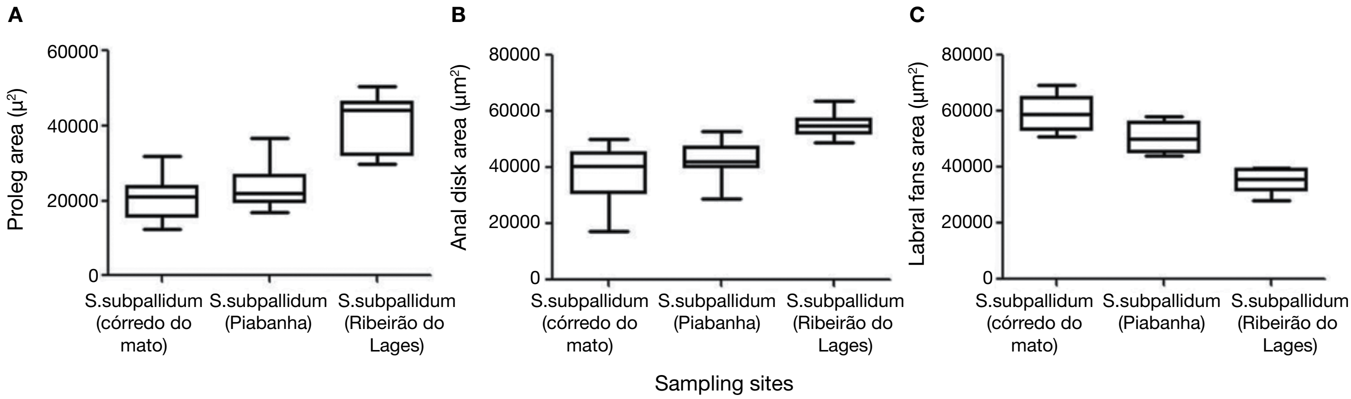

Figure 3. Variations in µm² of proleg area (A), anal disk area (B) and labral fans area (C), indicating the significant ($p < 0.05$) differences among the *S. subpallidum* population from Ribeirão do Lages and the other conspecific populations.

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