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

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**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.
was absent, and so investigate if the distributional

anal disk diameter.

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.

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 larval mor-
phology 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. How-
ever, 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 fast-
er 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 pat-
ttern is often observed in Holarctic black flies, it has not been investi-
gated 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. subpal-
lidum were separated, one from each of the three sampling sites in 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 Ri-
beirão do Lages (S12°35’7.7’’ W48°2’29.2’’).

Larvae were sorted in morphotypes, and their final instar speci-
mens 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 stereoscop-
ic 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 or-
der 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 ex-
pected 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 microhab-
itats, stronger prolegs should be demanded. Additional measure-
ments of proleg area and labral fan area were also taken (Fig. 1).

The measured groups from the different populations were com-
pared with each other through the Kruskal-Wallis test, and each
measured characteristic was tested, in order to verify their correla-
tions 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-oc-
curred 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 pop-
ulation 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), asso-
ciated to faster currents, showed larger bodies and anal disk and pro-
leg 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
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* Friedericis, 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.
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References

References to figure 3 in the original document are incorrect. The correct reference should be Figure 3, not Figure 1. The figures should be tables or diagrams, not figures. The references should be formatted as follows:

References


