Acta Limnologica Brasiliensia

Publication of the Brazilian Association of Limnology

Original Article
Acta Limnologica Brasiliensia, 2024, vol. 36, e43
https://doi.org/10.1590/S2179-975X4924
ISSN 2179-975X on-line version

Substrate influence on Perlidae (Plecoptera) nymph morphometrics in Parque Nacional da Serra dos Órgãos, Teresópolis, Rio de Janeiro, Brazil

Influência do substrato na forma de ninfas de Perlidae (Plecoptera) no Parque Nacional da Serra dos Órgãos, Teresópolis, Rio de Janeiro, Brasil

Rodrigo Braga Gastaldo[1]*, Willian Fernandes de Azevedo Cunha de Moura[2], Maria Inês da Silva dos Passos[3], Fernanda Avelino-Capistrano[4] and Richieri Antônio Sartori[5]

[1]Programa de Pós-graduação em Entomologia, Universidade Federal de Viçosa – UFV, Vila Matoso, 152-256, Santo Antônio, CEP 36570-000, Viçosa, MG, Brasil

[2]Programa de Pós-graduação em Zoologia – PPGZOO, Museu Nacional – MN, Universidade Federal do Rio de Janeiro – UFRJ, Quinta da Boa Vista, CEP 20940-040, Rio de Janeiro, RJ, Brasil

[3]Departamento de Zoologia, Universidade Federal do Estado do Rio de Janeiro – UNIRIO, Av. Pasteur 458, Botafogo, CEP 22290-255, Rio de Janeiro, RJ, Brasil

[4]Centro Universitário São José, Av. de Santa Cruz, 580, Realengo, CEP 21710-232, Rio de Janeiro, RJ, Brasil

[5]Departamento de Ciências Biológicas, Pontifícia Universidade Católica do Rio de Janeiro, R. Marquês de São Vicente 255, Gávea, CEP 22451-900, Rio de Janeiro, RJ, Brasil

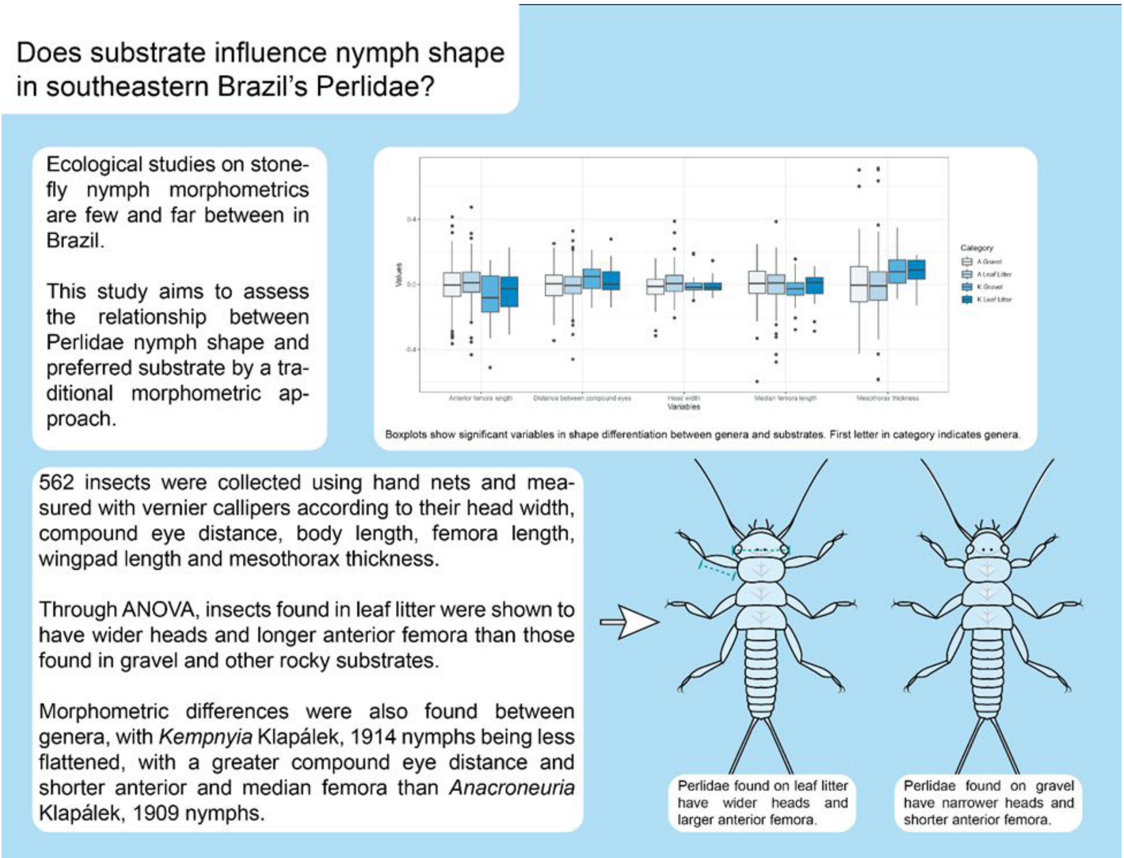
*e-mail: rodrigo.gastaldo@ufv.br

Cite as: Gastaldo, R.B. et al. Substrate influence on Perlidae (Plecoptera) nymph morphometrics in Parque Nacional da Serra dos Órgãos, Teresópolis, Rio de Janeiro, Brazil. *Acta Limnologica Brasiliensia*, 2024, vol. 36, e43. https://doi.org/10.1590/S2179-975X4924

Abstract: Aim: In this study, we determined whether Perlidae nymphs found in leaf litter were morphometrically distinct from those found in stony substrates. **Methods:** Specimens were collected in Parque Nacional da Serra dos Órgãos, Teresópolis, Rio de Janeiro, Brazil. Each insect was measured for femora length, body length, wingpad length, head width, compound eye distance and mesothorax thickness. Data was logarithmically transformed to avoid effects of allometric growth and subjected to a size-free discriminant analysis. Analysis of variance was made to assess the relationship between shape, genus and substrate choice. **Results:** We collected 562 insects belonging to two genera of Perlidae, *Kempnyia* Klapálek, 1914 and *Anacroneuria* Klapálek, 1909. Most occurred primarily in leaf litter (71.9%). We found significant morphometric differences among genera and substrates. Insects occurring in leaf litter were shown to have wider heads and longer anterior femora than those found in stones, suggesting that these characteristics would be important for anchoring to the substrate and resisting stronger water currents. **Conclusions:** This study shows the complex relationship between body shape and substrate choice in neotropical Perlidae. Considering that different substrate bear varying ecological pressures on aquatic insects, morphometrics is a capable tool for assessing ecological relationships between these insects and their environment.

Keywords: traditional morphometrics; aquatic insects; ecology; Atlantic rainforest.

Graphical Abstract



Resumo: Objetivo: Nesse estudo, determinamos se ninfas de Perlidae encontradas em folhiço retido em correnteza são morfometricamente distintas de ninfas encontradas em substratos pedregosos. **Métodos:** Os insetos foram coletados no Parque Nacional da Serra dos Órgãos, Teresópolis, Rio de Janeiro, Brasil. Cada inseto foi medido de acordo com o comprimento dos fêmures, comprimento corporal, tamanho das tecas alares, largura da cabeça, distância entre olhos compostos e espessura do mesotórax. Os dados foram logaritmizados para minimização de efeitos de alometria do crescimento e submetidos a uma análise discriminante livre de tamanho. Por fim, realizamos análises de variância para avaliar a relação entre forma, gênero e opção de substrato das ninfas. **Resultados:** Coletamos ao todo 562 insetos pertencentes a dois gêneros de Perlidae, *Kempnyia* Klapálek, 1914 e *Anacroneuria* Klapálek, 1909. As ninfas ocorreram majoritariamente em folhiço retido em correnteza (71.9%). Encontramos diferenças morfométricas entre gêneros e substratos. Os insetos encontrados em folhiço retido em correnteza apresentaram cabeças mais largas e fêmures anteriores mais longos do que os encontrados em pedras roladas, sugerindo maior necessidade de ancoragem em correntezas mais fortes quando no folhiço. **Conclusões:** Este estudo mostra a relação complexa entre forma e substrato em Perlidae neotropicais. Considerando as pressões ecológicas variáveis exercidas nos insetos aquáticos por diferentes substratos, as técnicas morfométricas se mostram capazes de avaliar as relações ecológicas entre esses insetos e seu ambiente.

Palavras-chave: morfometria tradicional; insetos aquáticos; ecologia; Mata Atlântica.

1. Introduction

Plecoptera is a relatively small order of aquatic insect with approximately 3700 extant species globally (DeWalt & Ower, 2019; DeWalt et al., 2024). Including a cosmopolitan distribution and many endemic taxa, the order is an important component of benthos macrofauna in low order streams (DeWalt et al., 2024). Nymphs are often sensitive to environmental disturbances and anthropogenic effects, and typically inhabit cold and well-oxygenated water (Bispo & Oliveira, 2007; Fochetti & Tierno De Figueroa, 2008).

Stoneflies play varying roles in stream ecology along their development. Nymphs may show detritivory, herbivory or predatory habits, and frequently serve as food for other animals present in streams and rivers (Hynes, 1976). They often choose less exposed environments such as on the underside of rocks, in between decomposing leaf litter or in moss, which facilitates their anchoring to the substrate as well as giving them protection and food (Hynes, 1976).

Most species descriptions are based on male genitalia, which are considered to provide the best characters for discrimination and identification. Since association of nymphs with adults through rearing is difficult given their specific environmental demands of oxygen and temperature, taxonomic knowledge on adults is greater than that of the immature stages. As such, very few species have all of their life stages described (Avelino-Capistrano et al., 2018).

Many Brazilian studies on stoneflies focus on species description and association of nymphs and adults (Almeida & Bispo, 2020). In this way, many research topics are still relatively unexplored. The morphological study of Brazilian stoneflies is often limited to male genitalia description as a means of species identification (Avelino-Capistrano et al., 2014; Avelino-Capistrano et al., 2017). Few studies opt for biometric analysis of different body criteria in association with taxonomy, life stage identification or ecological correlation (De Figueroa & López-Rodríguez, 2005, p. 200; Froehlich, 1999; Zwick, 1991).

Allometric growth – the change of morphological shape accompanying body size growth – is known in nymph development (Fenoglio et al., 2007). It is also known that nymphs may change their preferential substrate according to instar, with older nymphs opting for less stable substrates (Avelino-Capistrano & Barbosa, 2010). Lastly, Perlidae nymphs often vary dietary habits with their development, with younger nymphs being mostly detritivores and older ones being predominantly predatory (DeWalt et al., 2015, p. 201; Miyasaka & Genkai-Kato, 2009).

The many habitats that different substrates offer in a stream put varying ecological pressures on aquatic insects considering the resource availability, hydrodynamics, and refuge from predators, which then affect insect communities in relation to the necessary adaptations for survival in each of these habitats (Fuller & Rand, 1990; Starr et al., 2014). Considering the allometry and change in substrate preference of stoneflies during their growth, we can suggest that insects inhabiting different substrates would show different body shapes, given the different ecological pressures.

Morphometrics is widely used given its quantitative morphological description potential in insects through wing shape and venation pattern (Nguyen et al., 2022). These techniques, however, are rarely used in stonefly ecology, maybe due to most ecological Plecoptera studies focusing on their nymphs, which lack wings. Therefore, the simplest and most widely used morphometric technique in insects wouldn't be available to stonefly nymphs.

Considering the great morphological variation in aquatic insect nymphs according to different ecological stimuli, morphometrics may still prove a capable tool when applied to this life stage (Orlofske & Baird, 2014). Therefore, given the wide scope of possibilities of morphometrics applied to stonefly nymphs and their substrate choice, this study seeks to identify the relationship between Perlidae nymph shape and the substrate they inhabit.

2. Material and Methods

2.1. Study area

The study was conducted at Parque Nacional da Serra dos Órgãos (PARNASO) (Figure 1), Rio de Janeiro, Teresópolis, located at 22º53'S 42º09'W. The park includes over 10 thousand hectares of preserved Atlantic Forest and includes a large net of rivers, streams and rapids, being a place where research on aquatic insects is frequently done (Docile, 2014). The altitude of the park varies between 970m and 2275m, and its placement on a south-facing slope of the Serra dos Órgãos mountain range produces characteristic humid and cold winters. What follows is continuous rain throughout the year, causing a considerable volume of cold water to flow through the rivers and streams in the area. The large declivity also creates large portions of cascading rapids, creating a very suitable environment for stonefly occurrence. The stable, humid weather and the long conservation history of the park ensures the presence of well-developed riparian vegetation, which deposits large amounts of leaf litter in its streams. This way, the streams in PARNASO are rich both in organic and inorganic substrates, and large slates, boulders, sand banks and gravel can be found alongside moss and leaf litter, creating varied shelter for the aquatic insect community (Suguio, 1980).

2.2. Sampling

The material was collected during two expeditions to PARNASO in August and October

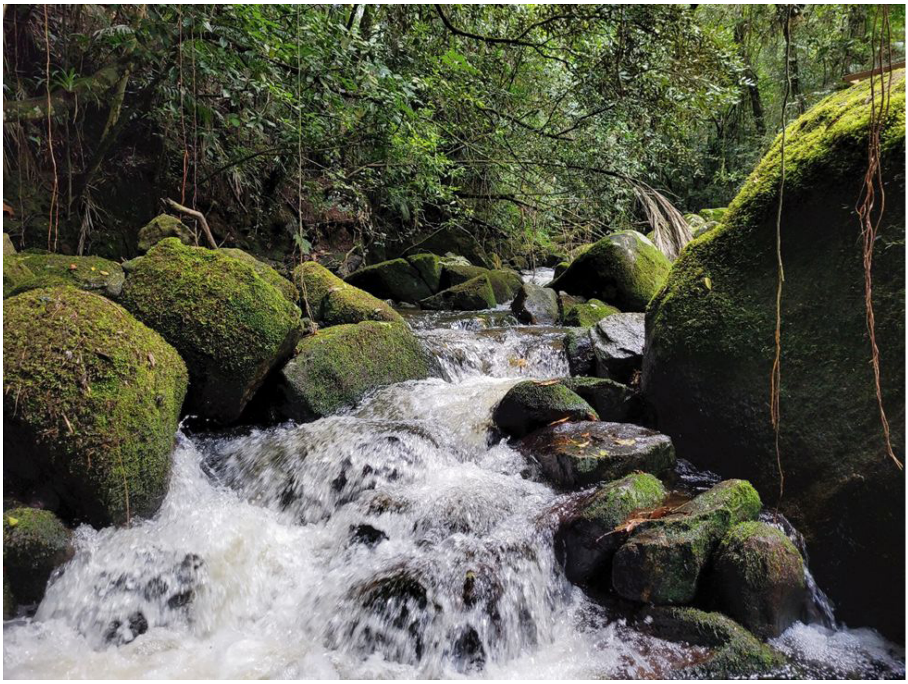


Figure 1. Paquequer river stretch inside Parque Nacional da Serra dos Órgãos.

2022. We didn't sample material during the summer due to the large amounts of rain in the region, which destabilize the aquatic insect community and deeply affect their distribution (Bispo et al., 2001).

In each expedition, we collected 40 samples consisting of aquatic insects present in a 900cm^2 area. The 40 samples were taken from two substrates that offer distinct habitats for stonefly nymphs – 20 samples of retained leaf litter and 20 samples of large gravel – along the riverbed of the Paquequer river. Thus, we amassed 80 samples consisting of a total of 7.2m^2 of sampled area. We chose the Paquequer river due to its conservation, quality of sampling and ease of access. All of the sampling was done on stretches of second and third order inside PARNASO (Figure 2) (Strahler, 1957). The samples were collected with stream nets with a thread of 250 µm primarily upstream and were screened in the field. All aquatic insects found were collected and preserved in 93.7° ethanol. The study material consisted of all the Perlidae nymphs found in both expeditions. All the insects will be deposited in the collection Professor José Alfredo Pinheiro Dutra (DZRJ, UFRJ) and Museu Nacional (MN/UFRJ).

2.3. Data organizing

The samples were identified to genus level following the identification key of Lecci & Froehlich (2007) using a Nikon SMZ745 stereomicroscope. All specimens were measured with a vernier caliper. The following measurements were taken with a precision of 0.05mm – head width, distance between compound eyes, body length, mesothorax wing pad length, length of anterior, median and posterior femora, and mesothorax thickness, following the methodology proposed by Avelino-Capistrano (2014, p. 26) (Figure 3). Images were made using a Leica M205A stereomicroscope with a digital camera with auto montage image software (Leica MC170 HD), treated in Adobe Photoshop 2024 (Adobe Inc., 2019b) and organized into plates in Adobe Illustrator 2022 (Adobe Inc., 2019a). Maps were produced using QGIS 3.28.3 (QGIS.org, 2024).

2.4. Data analysis

We plotted Perlidae sample abundance boxplots by substrate. We tested the distribution of the insects according to the substrate by using Mann-Whitney tests. The morphometric analysis was done by means of traditional morphometrics (Claude, 2008; James Rohlf & Marcus, 1993).

In order to remove the influence of body size from further morphometric analysis, we transformed the data through a Size Free Discriminant Analysis (Reis et al., 1990). To do that, data was logarithmically transformed in order to minimize the effects of allometric growth of the insects (Sokal & Rohlf, 1995). We then executed a Principal Component Analysis on the data, regressing each variable over the first Principal

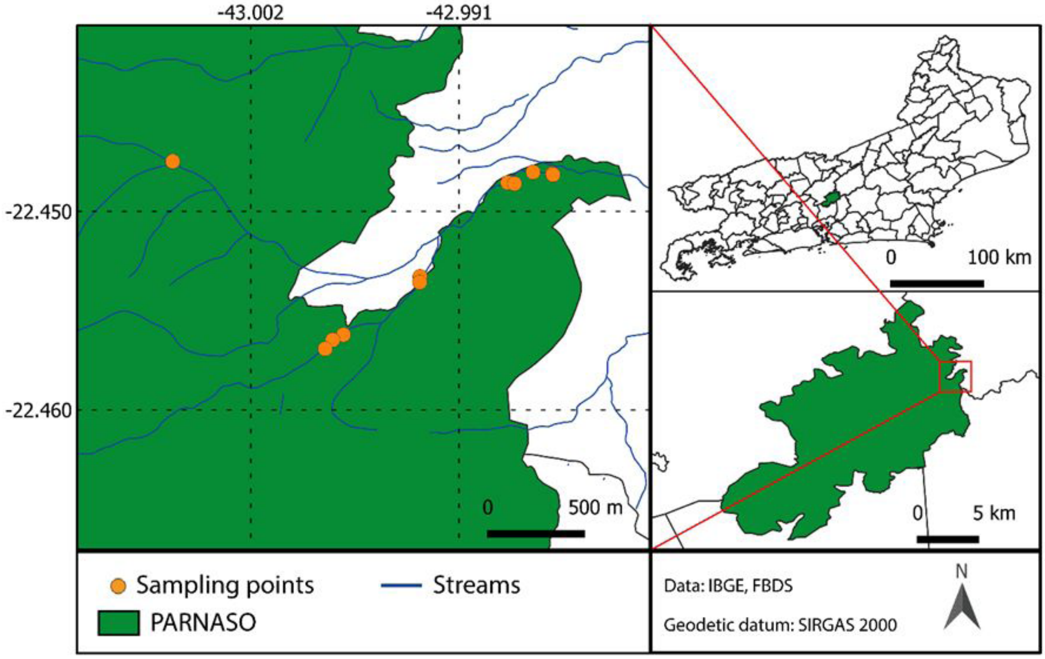


Figure 2. Map disclosing sampling locations; map on top right shows Rio de Janeiro state, while map on bottom right shows Parque Nacional da Serra dos Órgãos (PARNASO). Colored region shows the area occupied by PARNASO, while streams are represented by running lines. Dots show the location where samples were taken from.

Component, since, in this case, the first Principal Component represents mostly the size variation of the insects (Marcus, 1990). This way, the residuals of these regressions represent, for each of the variables, the morphometric variation independent of the size of the insect (Humphries et al., 1981; Marcus, 1990). These residuals were then utilized in morphometric analysis, constituting the morphometric data.

Morphometric data was then subjected through descriptive and analytic statistical tests such as boxplots and two-way Analysis of Variance (ANOVA DBC) to detail and explore the distribution of morphometric data according to each variable for each genus and substrate. The substrates were considered different treatments, and genera as different blocks. The tests compared specimens of each substrate and genus among themselves, relating morphometric data to the environment of specimens. In order to measure differences between the shapes of each insect, we conducted a Linear Discriminant Analysis from the morphometric data of each specimen. Lastly, the results of the analysis were compared with literature on the ecology of Perlidae. All analyses were performed utilizing software RStudio under version 4.2.2 (Posit Team, 2024). The following packages were used for the analysis and plotting: ggplot2 (Wickham, 2016),

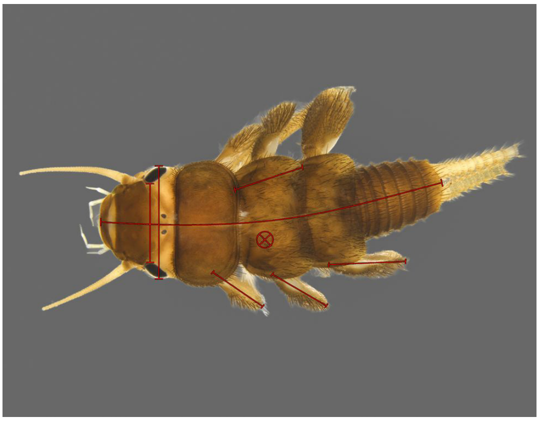


Figure 3. Measurements taken on each Perlidae nymph found; lines show measurements following the plane of the picture, while crossed circle shows the measurement of mesothorax thickness in a dorsoventral direction. From left to right, measurements are: body length, from the anteriormost point of the clypeus to the posteriormost point of the abdominal tergum X; distance between compound eyes, the smallest measurement between the compound eye inner margins; head width, the largest measurement between the compound eye outer margins; anterior, median and posterior femora length, from the dorsal articulation between femur and trochanter to the dorsal articulation between femur and tibia; wingpad length, from the anterior mesonotal suture to the apex of the wingpad; mesothorax thickness, from the median point of the mesonotum to the median point of the mesosternum.

RColorBrewer (Neuwirth, 2022), MASS (Venables & Ripley, 2002), vegan (Oksanen et al., 2022).

3. Results

3.1. Data distribution

Our sampling efforts amassed 562 Perlidae nymphs, 520 of which were measured. The insects that we were not able to measure either lost most of their legs or showed bisected abdomens, making full measurements impossible. Thus, these specimens were removed from the analysis to maintain the number of observations in each variable consistent. 44 specimens (7.8%) belong to *Kempnyia* Klapálek, 1914, while 518 (92.2%) belong to *Anacroneuria*.

Perlidae abundance varied between substrates and genera. 158 specimens were found in gravel (28.1%) while 404 were found in retained leaf litter (71.9%). In gravel, 17.1% of insects belonged to *Kempnyia*, while 82.9% belonged to *Anacroneuria*. In leaf litter, 4.2% belonged to *Kempnyia* and 95.8% to *Anacroneuria*. We tested the disparity of abundance of each genus among samples according to substrate using a Mann-Whitney test (Figure 4), which pointed to a significantly greater abundance of *Anacroneuria* in retained leaf litter ($p < 0.05$). No significant difference was found for *Kempnyia*.

3.2. Morphometric analysis

The morphometric data showed a couple of significant relationships and differences. The LDA, when grouped by genera (Figure 5) shows a large distinction between *Anacroneuria* and *Kempnyia* with reasonable intersection, both due to the large amplitude of shape demonstrated by *Anacroneuria*. We can therefore infer that *Kempnyia* shows limited morphometric variety when compared to *Anacroneuria*, which seems to be a more morphologically plastic genus.

The distribution of morphometric data according to genus and substrate was different for each measured variable (Figure 6). Data on the boxplot values are present at the end of the paper. With the ANOVA, a couple of significant differences between genera and substrates were shown. Distance between compound eyes, anterior and median femora length, as well as mesothorax thickness varied between genera ($p < 0.05$), while head width and anterior femora length varied between substrates ($p < 0.05$). Thus, we found *Kempnyia* to be less flattened, with a greater distance between its compound eyes and shorter anterior and median femora than *Anacroneuria* (Figure 6). As for substrate, Perlidae found in retained leaf litter displayed wider heads and longer anterior femora than those found in gravel (Figure 6).

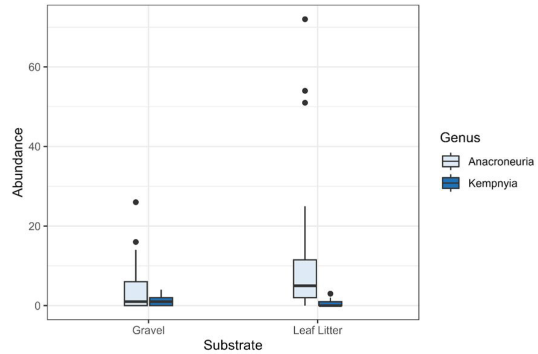


Figure 4. Nymph abundance boxplot of samples in each substrate for each genus. Light plots show abundance of *Anacroneuria* while dark plots show abundance of *Kempnyia*.

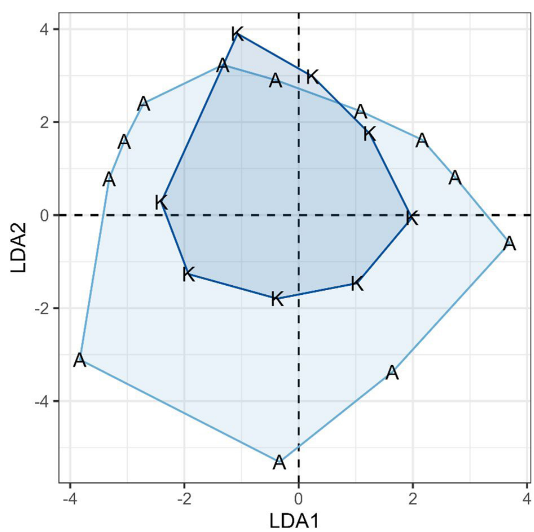


Figure 5. LDA plot from morphometric data. The polygon whose vertices are marked by the letter "K" shows the *Kempnyia* shape diversity, while the polygon with vertices marked by the letter "A" shows the *Anacroneuria* shape diversity.

4. Discussion

The preferred substrate of stoneflies is not homogenous across the world. According to the general ecology of the order, based mainly on Nearctic and Palearctic studies, these insects opt for rocky substrates of variable granulometry (DeWalt et al., 2015). In the Neotropics, however – and particularly in Brazil –, stoneflies seem to prefer leafy and mossy environments, often

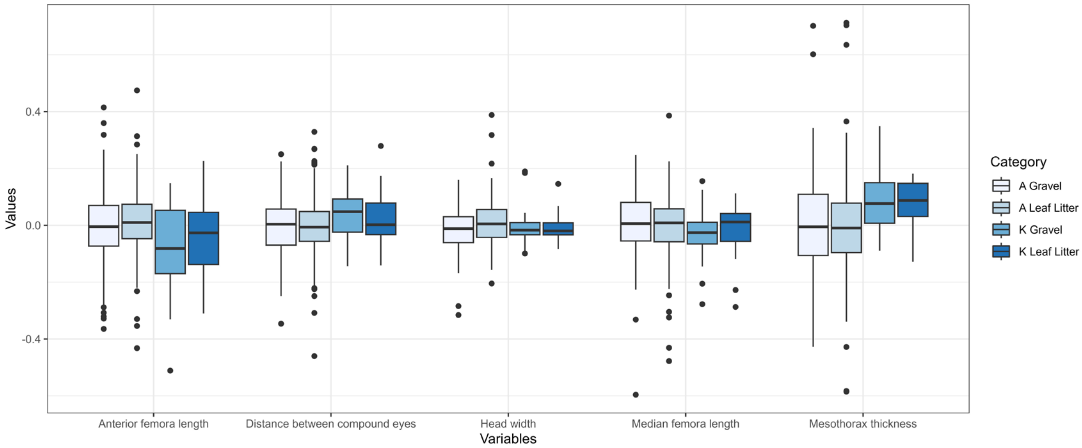


Figure 6. Statistically significant variables boxplot. Each of the boxplot groups show a variable in which either substrate or genus showed significant differences. Each boxplot represents one of the genera in one of the substrates for each variable. Mesothorax thickness, head width, anterior and median femora length were significant in separating genera, while head width and anterior femora length were significant in differentiating substrates ($p < 0.05$). A Gravel: *Anacroneuria* found in gravel substrates; A Leaf Litter: *Anacroneuria* found in riffle retained leaf litter; K Gravel: *Kempnyia* found in gravel substrates; K Leaf Litter: *Kempnyia* found in riffle retained leaf litter.

gathering in these spots (Avelino-Capistrano et al. 2017; Buss et al. 2004). Stonefly congregations in retained leaf litter in riffles are known from different regions in the world (Hynes, 1976). Nonetheless, these congregations seem to be much more frequent in the Neotropics, resulting in a greater abundance of these insects in leaf litter. The greater frequency of congregations may be related to the differences between Neotropical and Nearctic aquatic insect communities and specific ecological interactions with Plecoptera. However, a detailed description and comparison of these effects have not yet been made. Even still, this study, as well as South American tendencies, points to a greater abundance of Perlidae in PARNASO in retained leaf litter than in gravel. We must point out that many other substrates capable of sheltering stonefly nymphs were not sampled in our expeditions, such as moss, streambed leaf litter, or boulders. Still, the pattern we found of greater abundance in organic substrates agrees with what has been found in the region (Brasil et al., 2017; Buss et al., 2004).

Kempnyia showed a less flattened mesothorax, greater distance between the compound eyes, and shorter anterior and median femora than those of *Anacroneuria*. The distance between the compound eyes has been correlated with the dietary habits of Perlidae nymphs (Sheldon, 1969). These results, however, deal with the distance between the compound eyes as an absolute value as a proxy for insect size. Thus, differently-sized Perlidae nymphs would display different dietary habits. In our study, we found variation in the relative distance between compound eyes, independently from insect body size. That means that both younger and older *Kempnyia* nymphs display compound eyes farther apart than those of *Anacroneuria* nymphs, proportionally to their size. While there is a tangential relationship between these results and those of the literature, the inclusion of body size in Sheldon's work (Sheldon, 1969) and the exclusion of it in this study makes direct comparison difficult.

Despite the differences found between genera, we must consider the low specimen count of *Kempnyia* when compared to that of *Anacroneuria*. In this way, while the results show morphometric divergence between genera, maybe these differences do not accurately show the shapes of both genera. Thus, more sampling is necessary for the analysis to be confirmed or refuted according to *Kempnyia* shape in PARNASO.

The morphometric variation according to substrate could be interpreted according to allometric growth. We should consider the South American Perlidae habit of preferential substrate change during development, in which younger nymphs opt for less exposed environments, such as on the underside of rocks, while older nymphs would prefer less stable environments, such as retained leaf litter in riffles (Avelino-Capistrano & Barbosa, 2010). The change of environment may occur according to the different necessities of insects along their development, such as the change in diet and other habits, which would imply the change of shape due to allometric growth (DeWalt et al., 2015; Miyasaka & Genkai-Kato, 2009). However, these effects were minimized during the Size-Free Discriminant Analysis.

Still, other possible differences between Perlidae instars could be identified through the wing pads. As nymphs molt, their wing pads gradually become more developed, eventually forming the wings (DeWalt et al., 2015; Dorvillé & Froehlich, 2001). Considering this, higher instar nymphs should show significant differences in the wing pad variable when compared to lower instars. Such was not the case in our analysis in relation to substrate. Therefore, while the change of habitat may happen following growth, we could not detect that process in our analysis. The only significant differences between insects from different substrates was in head width and anterior femora length.

During our expeditions, we observed how the different sampled stretches of the Paquequer river are heterogeneous. Substrates rarely stretch for over a couple of meters, and sandy, rocky and leafy substrates frequently alternate as in a mosaic (Figure 7). Considering the swimming and crawling displayed by stonefly nymphs (DeWalt et al., 2015), perhaps the frequent substrate mixing in Paquequer river could create an environment in which stonefly nymphs could be able to change substrates with relative ease, which could in turn make substrate choice reversible and temporary. Should we consider this, the effects of development stage in substrate choice would be minimized. Even still, different environments challenge and pressure insects in varying ways, which could still favor certain morphometric tendencies.

The variables in which we found statistically significant difference – head width and anterior femora length – have been associated with hydrodynamics and anchoring capacity in aquatic insects. Wider heads in certain Ephemeroptera genera grant them less resistance in strong currents, while for other insects in this order, shorter legs lead to swimming habits while longer legs lead to crawling and anchoring habits (Dodds & Hisaw, 1924). Considering the different stability, exposure and hydrodynamics of riffle retained leaf litter and

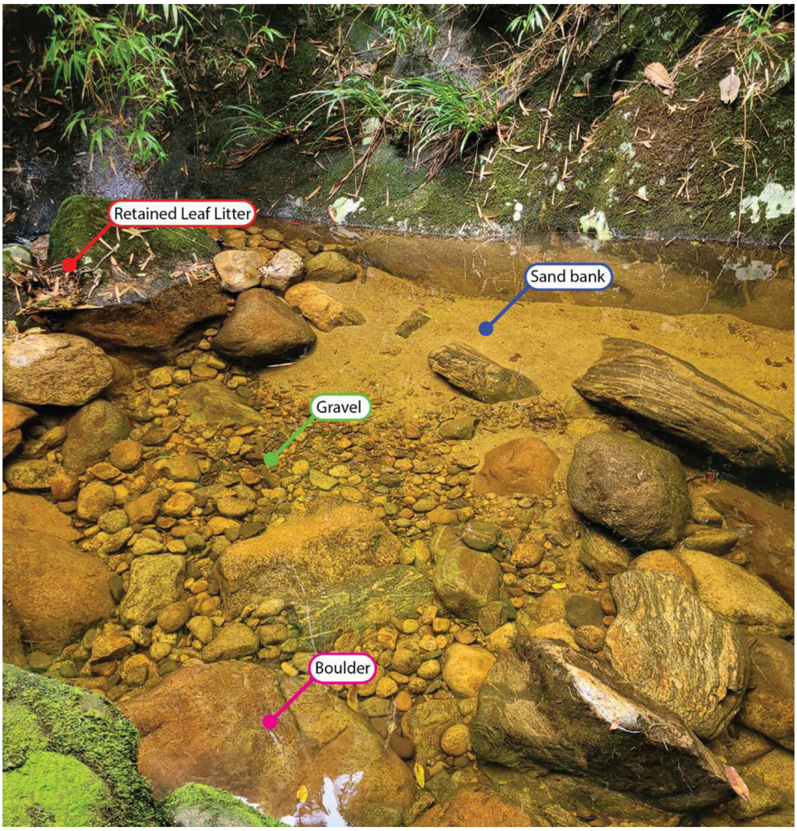


Figure 7. Quick substitution of substrates in a stretch of Paquequer River. Over the few meters that the image spans, three different types of inorganic substrates – sand banks, gravel and boulders – and one type of organic substrate – retained leaf litter – can be seen, showing the rapid succession of substrates in this environment.

gravel, it's possible that these capabilities are tested in different ways in each substrate. Therefore, the ecological pressures generated by each environment would make certain body shapes preferable, possibly selecting specimens.

This study identified novel interactions between substrate and shape of Perlidae nymphs in Brazil. The precise effects that cause these interactions and differences, however, are still in need of further research. Thus, we press for more ecological assessments of nymph shape in Plecoptera to be made, for these relationships to be better understood.

Data Availability Statement

All research data analyzed in the research is available in Google Drive. Access is free for visualization. It can be accessed in: https://drive.google.com/drive/folders/1K1VSz798jTD-1KH_YlxF2o8zDtLwFDm2?usp=sharing

References

Adobe Inc., 2019a. Adobe Illustrator [online]. Retrieved in 2024, August 8, from https://adobe.com/products/illustrator

Adobe Inc., 2019b. Adobe Photoshop [online]. Retrieved in 2024, August 8, from https://www.adobe.com/products/photoshop.html

Almeida, L.H.D., & Bispo, P.C., 2020. Perlidae (Plecoptera) from the Paranapiacaba Mountains, Atlantic Forest, Brazil: diversity and implications of the integrative approach and teneral specimens on taxonomy. PLoS One 15(12), e0243393. PMid:33301489. http://doi.org/10.1371/journal.pone.0243393.

Avelino-Capistrano, F., & Barbosa, L.S., 2010. A idade da ninfa pode estar relacionada com a escolha do habitat? Relação entre o tamanho das ninfas de *Anacroneuria debilis* (Pictet, 1841) (Insecta, Plecoptera, Perlidae) e a escolha de habitat. In: Anais do XXIII Congresso Brasileiro de Entomologia. Natal, Rio Grande do Norte. Viçosa: Sociedade Entomológica Brasileira, pp. 930.

Avelino-Capistrano, F., 2014. Taxonomia integrativa de Plecoptera (Insecta) na Bacia do Rio Macaé, Rio de Janeiro, Brasil. [Tese de Doutorado em Biologia Animal]. Seropédica: Universidade Federal Rural do Rio de Janeiro.

Avelino-Capistrano, F., Almeida, G.L.D., & Barbosa, L.S., 2017. Diversidade e distribuição de ninfas de Plecoptera (Insecta) por substratos em rios do Parque Estadual da Pedra Branca, Rio de Janeiro, Brasil. EntomoBrasilis 10(2), 76-81. http://doi.org/10.12741/ebrasilis.v10i2.680.

Avelino-Capistrano, F., Nessimian, J.L., Santos-Mallet, J.R., & Maeda Takiya, D., 2014. DNA-based identification and descriptions of immatures of *Kempnyia* Klapálek (Insecta:Plecoptera) from Macaé River Basin, Rio de Janeiro State, Brazil. Freshw. Sci. 33(1), 325-337. http://doi.org/10.1086/675226.

Avelino-Capistrano, F., Pessacq, P., & Barbosa, L.S., 2018. Order Plecoptera. In: Hamada, N., Thorp, J.H., & Rogers, D.C., eds. Keys to Neotropical Hexapoda. Thorp and Covich's Freshwater Invertebrates. Cambridge: Academic Press, 119-141, 4. ed., vol. 3. http://doi.org/10.1016/B978-0-12-804223-6.00004-4.

Bispo, P.C., & Oliveira, L.G., 2007. Diversity and structure of Ephemeroptera, Plecoptera and Trichoptera (Insecta) assemblages from riffles in mountain streams of Central Brazil. Rev. Bras. Zool. 24(2), 283-293. http://doi.org/10.1590/S0101-81752007000200004.

Bispo, P.C., Oliveira, L.G., Cresci, V.L., & Silva, M.M., 2001. A pluviosidade como fator de alteração da entomofauna bentônica (Ephemeroptera, Plecoptera e Trichopetera) em córregos do Planalto Central do Brasil. Acta Limnol. Bras. 13(2), 1-9.

Brasil, L.S., Da Silva Giehl, N.F., Darc Batista, J., Oliveira de Resende, B., & Ramos Cabette, H.S., 2017. Aquatic insects in organic and inorganic habitats in the streams on the Central Brazilian savanna. Rev. Colomb. Entomol. 43(2), 286-291. http://doi.org/10.25100/socolen.v43i2.5961.

Buss, D.F., Baptista, D.F., Nessimian, J.L., & Egler, M., 2004. Substrate specificity, environmental degradation and disturbance structuring macroinvertebrate assemblages in neotropical streams. Hydrobiologia 518(1-3), 179-188. http://doi.org/10.1023/B:HYDR.0000025067.66126.1c.

Claude, J., 2008. Morphometrics with R. New York: Springer.

De Figueroa, J.M.T. & López-Rodríguez, M.J., 2005. Biometric relationships among female size, fecundity, and flight period in *Isoperla nevada* Aubert 1952 (Plecoptera: Perlodidae). Ann. Soc. entomol. Fr. (N.S.), 41(1), 3-6. https://doi.org/10.1080/00379271.2005.10697437.

DeWalt, R.E., & Ower, G.D., 2019. Ecosystem services, global diversity, and rate of stonefly species descriptions (Insecta: Plecoptera). Insects 10(4), 99. PMid:30959938. http://doi.org/10.3390/insects10040099.

DeWalt, R.E., Hopkins, H., Neu-Becker, U., & Stueber, G., 2024. Plecoptera Species File [online]. Retrieved in 2024, August 8, from https://plecoptera.speciesfile.org/

DeWalt, R.E., Kondratieff, B.C., & Sandberg, J.B., 2015. Order Plecoptera. In: Thorp, J.H., Rogers, D.C., eds. Thorp and Covich's Freshwater Invertebrates:

ecology and general biology. Cambridge: Academic Press, 933-949, 4. ed., vol. 1. http://doi.org/10.1016/B978-0-12-385026-3.00036-X.

Docile, T.N., 2014. Descrição e comparação das redes tróficas de macroinvertebrados aquáticos em riachos da Bacia do Rio Paquequer, dentro e fora do perímetro urbano da cidade de Teresópolis, RJ [Dissertação de Mestrado em Ciências Biológicas (Ecologia)]. Rio de Janeiro: Universidade Federal do Rio de Janeiro.

Dodds, G.S., & Hisaw, F.L., 1924. Ecological studies of aquatic insects: adaptations of mayfly nymphs to swift streams. Ecology 5(2), 137-148. http://doi.org/10.2307/1929011.

Dorvillé, L.F.M., & Froehlich, C.G., 2001. Description of the Nymph of *Kempnyia tijucana* Dorvillé and Froehlich (Plecoptera, Perlidae), with Notes on Its Development and Biology. In: Domínguez, E., ed. Trends in research in Ephemeroptera and Plecoptera. Boston: Springer, 385-392. http://doi.org/10.1007/978-1-4615-1257-8_45.

Fenoglio, S., Bo, T., & Malacarne, G., 2007. Allometric growth in *Anacroneuria* Klapálek 1909 nymphs (Plecoptera Perlidae). Trop. Zool. 20(1), 109-114.

Fochetti, R., & Tierno De Figueroa, J.M., 2008. Global diversity of stoneflies (Plecoptera; Insecta) in freshwater. Hydrobiologia 595(1), 365-377. http://doi.org/10.1007/s10750-007-9031-3.

Froehlich, C.G., 1999. Biometric notes on *Tupiperla* Froehlich (Plecoptera, Gripopterygidae). Rev. Bras. Zool. 16(Suppl. 2), 181-183. http://doi.org/10.1590/S0101-81751999000600018.

Fuller, R.L., & Rand, P.S., 1990. Influence of Substrate Type on Vulnerability of Prey to Predacious Aquatic Insects. J. N. Am. Benthol. Soc. 9(1), 1-8. http://doi.org/10.2307/1467928.

Humphries, J.M., Bookstein, F.L., Chernoff, B., Smith, G.R., Elder, R.L., & Poss, S.G., 1981. Multivariate Discrimination by Shape in Relation to Size. Syst. Biol. 30(3), 291-308. http://doi.org/10.1093/sysbio/30.3.291.

Hynes, H.B.N., 1976. Biology of Plecoptera. Annu. Rev. Entomol. 21(1), 135-153. http://doi.org/10.1146/annurev.en.21.010176.001031.

James Rohlf, F., & Marcus, L.F., 1993. A revolution morphometrics. Trends Ecol. Evol. 8(4), 129-132. PMid:21236128. http://doi.org/10.1016/0169-5347(93)90024-J.

Lecci, L.S., & Froehlich, C.G., 2007. Plecoptera. In: Froehlich, C.G., org. Guia on-line: identificação de larvas de Insetos Aquáticos do Estado de São Paulo [online]. Ribeirão Preto: USP, pp. 1-10. Retrieved in 2024, August 8, from https://sites.ffclrp.usp.br/aguadoce/guia_online/Guia_identifica%C3%A7%C3%A3o_larvas_Plecoptera.pdf

Marcus, L.F., 1990. Traditional morphometrics. In: Rohlf, F.J., Bookstein, F.L., eds. Proceedings of the Michigan Morphometrics Workshop. Ann Arbor: University of Michigan Museum of Zoology, 77-122.

Miyasaka, H., & Genkai-Kato, M., 2009. Shift between carnivory and omnivory in stream stonefly predators. Ecol. Res. 4(1), 11-19. http://doi.org/10.1007/s11284-008-0475-3.

Neuwirth, E., 2022. RColorBrewer: ColorBrewer Palettes. R package version 1.1-3 [online]. Retrieved in 2024, August 8, from https://CRAN.R-project.org/package=RColorBrewer.

Nguyen, H.H., Ho, B.H., Lai, H.P., Tran, H.T., Bañuls, A.L., Prudhomme, J., & Le, H.T., 2022. A lightweight keypoint matching framework for insect wing morphometric landmark detection. Ecol. Inform. 70, 101694. http://doi.org/10.1016/j.ecoinf.2022.101694.

Oksanen, J., Gavin, L., Simpson, F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.H., Cunha, E.R., Smith, T., Stier, A., Ter Braak, C.J.F., & Weedon, J., 2022. vegan: Community Ecology Package. R package version 2.6-4 [online]. Retrieved in 2024, August 8, from https://CRAN.R-project.org/package=vegan.

Orlofske, J.M., & Baird, D.J., 2014. A geometric morphometric approach to establish body-shape trait criteria for aquatic insects. Freshw. Sci. 33(3), 978-994. http://doi.org/10.1086/676912.

Posit Team, 2024. RStudio: Integrated Development Environment for R [online]. Boston: Posit Software, PBC. Retrieved in 2024, August 8, from http://www.posit.co/.

QGIS.org, 2024. QGIS Geographic Information System [online]. QGIS Association. Retrieved in 2024, August 8, from http://www.qgis.org

Reis, S.F., Pessôa, L.M., & Strauss, R.E., 1990. Application of size-free canonical discriminant analysis to studies of geographic differentiation. Rev. Bras. Genét. 13(3), 509-520.

Sheldon, A.L., 1969. Size relationships of *Acroneuria californica* (Perlidae, Plecoptera) and its prey. Hydrobiologia 34(1), 85-94. http://doi.org/10.1007/BF00040325.

Sokal, R.R., & Rohlf, F.J., 1995. Biometry: the principles and practices of statistics in biological research (3rd ed.). USA: W. H. Freeman.

Starr, S.M., Benstead, J.P., & Sponseller, R.A., 2014. Spatial and temporal organization of macroinvertebrate assemblages in a lowland floodplain ecosystem. Landsc. Ecol. 29(6), 1017-1031. http://doi.org/10.1007/s10980-014-0037-8.

Strahler, A.N., 1957. Quantitative analysis of watershed geomorphology. Eos (Wash. D.C.) 38(6), 913-920. https://doi.org/10.1029/TR038i006p00913.

Suguio, K., 1980. As propriedades dos sedimentos. In: Suguio, K. Geologia sedimentar. São Paulo: Edgard Blucher, 57-83.

Venables, W.N., & Ripley, B.D., 2002. Modern Applied Statistics with S (4th ed.). New York: Springer. http://doi.org/10.1007/978-0-387-21706-2.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag.

Zwick, P., 1991. Biometric studies of the growth and development of two species of *Leuctra* and of *Nemurella pictetii* (Plecoptera: Leuctridae and Nemouridae). In: Alba-Tercedor, J., & Sanches-Ortega, A., eds. Overview and strategies of Ephemeroptera and Plecoptera. England: Sandhill Crane Press, Inc., 515-526.

Received: 20 May 2024
Accepted: 03 October 2024

Associate Editor: Victor Satoru Saito.