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Instituto de Ciências Biológicas

Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre

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AUMENTO DE ASSIMETRIA FLUTUANTE INDUZIDO POR REJEITO DE MINERAÇÃO EM *Smicridea* (*Rhyacophylax*) *coronata* (Trichoptera)

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre da Universidade Federal de Minas Gerais como requisito parcial para obtenção do título de Mestre em Ecologia, Conservação e Manejo da Vida Silvestre.

Orientador: Prof. Dr. Geraldo Wilson Fernandes

Coorientadora: Dra. Isabela Cristina Rocha

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"Aumento de assimetria flutuante induzido por rejeito de mineração em Smicridea (Rhyacophylax) coronata (Trichoptera)"

HELENA MAURA DE ANDRADE SOARES

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"O desenvolvimento de um organismo não é um desdobramento de um programa interno autônomo, mas a consequência de uma interação entre os padrões internos de resposta do organismo e seu ambiente externo."

(Levins & Lewontin, 1997).

RESUMO GERAL

O rompimento da barragem de Fundão, pertencente à Samarco/Vale/BHP ocorreu em Minas Gerais, Brasil, em 2015, depositando altas concentrações de metais e metaloides na bacia hidrográfica do Rio Doce e impactando gravemente os ecossistemas de água doce. Para avaliar a instabilidade do desenvolvimento de Trichoptera em resposta aos impactos ambientais do derramamento de rejeito, avaliamos a assimetria flutuante (AF) na espécie *Smicridea* (*Rhyacophylax*) *coronata* (Trichoptera: Hydropsychidae). A AF foi estimada em escalas individual e populacional usando métodos de morfometria geométrica na cápsula cefálica e mandíbulas das larvas e no par de asas mesotorácicas de adultos, ambos coletados na condição impactada (CI) e condição menos perturbada (CMP). Os níveis de AF aumentaram em resposta a estressores ambientais tanto nas asas mesotorácicas, em escala populacional, quanto nas mandíbulas, a nível individual. A variação morfológica detectada nesses caracteres nos estágios larval e adulto podem levar a efeitos prejudiciais e resultar em altas taxas de mortalidade e uma redução do *fitness* de adultos. As asas mesotorácicas em Trichoptera foram indicadas como caractere adequado para avaliações de AF, tendo potencial para aplicações em programas de biomonitoramento. Nossos resultados validam a relação entre os impactos do rompimento da barragem de Fundão e um aumento na instabilidade do desenvolvimento nessa espécie. Nós levantamos a hipótese de que correlações negativas entre níveis de AF e impactos ambientais podem ser explicadas pelo efeito legado da bacia hidrográfica do Rio Doce.

Palavras-chave: derramamento de rejeito de mineração; rompimento da barragem de Fundão; morfometria geométrica; estresse ambiental; bioindicador; desastre ambiental.

ABSTRACT

The Samarco/Vale/BHP mine tailing dam breach occurred in Minas Gerais, southeastern Brazil, in 2015, depositing high concentrations of metals and metalloids in the Doce river basin and severely impacting freshwater ecosystems. To assess developmental instability of caddisflies in response to the environmental impacts of the dam breach, we investigated fluctuating asymmetry (FA) in the species *Smicridea* (*Rhyacophylax*) *coronata* (Trichoptera: Hydropsychidae). FA was assessed at individual and populational scales using geometric morphometric methods in the cephalic capsule and mandibles of larvae and also on the forewings of adults, both belonging to the impacted condition (IC) and least disturbed condition (LDC). The levels of FA increased in response to stressors on the forewings at a populational scale, and on the mandibles, at individual scale. These morphological variations in the larval and adult stages may lead to detrimental effects and result in high mortality rates as well as lower adult fitness. Trichoptera forewings are revealed as suitable traits for assessing FA, holding potential for applications in biomonitoring programs. Our results validate the relationship between the impacts from the Fundão dam breach and increased developmental instability in this species. We hypothesize that negative correlations between FA levels and environmental impacts can be explained by the legacy effect in the Doce river basin.

Keywords: mine tailing failure; Fundão dam breach; geometric morphometrics; environmental stress; bioindicator; environmental disaster.

SUMÁRIO

INTRODUÇÃO GERAL

A bacia hidrográfica do Rio Doce é uma das bacias mais importantes do Brasil, principalmente para a região sudeste. 86% da sua área de drenagem pertence ao estado de Minas Gerais, onde se originam suas nascentes, e 14% ao estado do Espírito Santo, onde seu curso principal deságua no oceano Atlântico (Coelho, 2009). A bacia tem grande importância socioeconômica mundial por conta da extração em larga escala de minério de ferro no Quadrilátero Ferrífero e exploração de ouro há mais de 200 anos (G. Santos et al., 2023), bem como local, por conta de suas comunidades tradicionais, atividades de pesca, agricultura e provisão de água (Fernandes et al., 2016). Além disso, a bacia se distribui entre os biomas Mata Atlântica (98% de sua área) e Cerrado (2%), de modo que está integralmente inserida nos dois únicos *hotspots* mundiais de conservação do Brasil (Myers et al., 2000), cenário que demanda esforços para conservar sua biodiversidade.

O rompimento da barragem de Fundão, pertencente às empresas Samarco/Vale/BHP no município de Mariana, Minas Gerais, despejou mais de 50 milhões de metros cúbicos de rejeitos de mineração nas águas da bacia hidrográfica do Rio Doce (Fernandes et al., 2016). Os rejeitos, compostos por altos índices de sílica, arsênio e metais como ferro, alumínio e chumbo (Gomes et al., 2017; Queiroz et al., 2018), se somaram às consequências históricas da exploração minerária sobre a bacia, que já apresentava concentrações anômalas de metais previamente ao desastre (A. Costa, 2001; Rhodes et al., 2018). Contaminantes foram amplamente carregados pela extensão da bacia e, ao longo dos anos, continuam sendo ressuspensos no período chuvoso (P. Costa et al., 2022; Felippe & Mendes, 2022; Santana et al., 2021). Consequentemente, metais e metaloides foram detectados em concentrações elevadas em sistemas de água doce e marinhos, impactando diretamente invertebrados aquáticos e peixes (P. Costa et al., 2022; Francini-Filho et al., 2019).

Além de ter provocado a mortalidade instantânea da fauna aquática (Felippe et al., 2016; Fernandes et al., 2016), a desposição de rejeitos representa riscos mais profundos à biota em escala espaço-temporal. A biodiversidade aquática da bacia é ainda pouco documentada e pode sofrer grandes alterações na composição de espécies em decorrência de mudanças biogeoquímicas causadas pelos rejeitos (Gomes et al., 2017). Os metais depositados na bacia são tóxicos para invertebrados bênticos e apresentam altos riscos de bioacumulação através do contato com sedimento ou ingestão de partículas e presas contaminadas (Bernardino et al., 2019; Gabriel et al., 2020). Tal cenário demanda esforços para avaliar impactos da deposição de rejeitos sobre organismos aquáticos, a fim de subsidiar ações de recuperação ambiental (Freitas et al., 2016).

Dentre as ferramentas utilizadas no biomonitoramento, destaca-se a avaliação da assimetria flutuante (AF) como um método rápido e eficiente (Clarke, 1993; Henriques & Cornelissen, 2018; Sanseverino & Nessimian, 2008). A AF consiste em pequenos e aleatórios desvios de simetria entre os lados esquerdo e direito em organismos bilaterais (Klingenberg, 2015; Nunes & Souto, 2021). Essas alterações morfológicas sutis, mas desviantes do fenótipo esperado, são comumente atribuídas à sinergia entre estressores genéticos e ambientais, provocando imprecisões nos processos de desenvolvimento (Benítez et al., 2020; Van Valen, 1962). Altos índices de AF podem impactar negativamente processos relacionados à reprodução e sobrevivência de espécies, de modo que a assimetria é considerada deletéria ao fitness (Nunes & Souto, 2021). Por essa razão, a AF é frequentemente considerada como biomarcador de instabilidades do desenvolvimento induzidas por perturbações ambientais (Beasley et al., 2013; Palmer & Strobeck, 1986).

O estudo da AF é particularmente relevante em contextos de contaminação intensa, sendo capaz de detectar efeitos de poluentes sobre o desenvolvimento dos organismos antes que os contaminantes atinjam níveis suficientemente altos para causar diferenças perceptíveis na riqueza ou abundância de espécies, afetando a sobrevivência das comunidades (Valentine & Soulé, 1973). Frente à necessidade de mensurar a condição ecológica de ecossistemas aquáticos, a assimetria flutuante é frequentemente estudada como indicadora de impactos ambientais em insetos aquáticos, organismos sensíveis a estressores e amplamente utilizados em estudos de biomonitoramento (Sanseverino & Nessimian, 2008). O estudo de AF e deformações em estruturas cefálicas de mosquitos da família Chironomidae são o indicador mais comum de estressores ambientais no meio aquático, como contaminação por metais (Bhattacharyay et al., 2005; Groenendijk et al., 1998; Ilyashuk et al., 2003; Martinez et al., 2004; Montaño-Campaz et al., 2019) e resíduos de fábricas (Clarke, 1993; Servia et al., 2004).

As Ordens Ephemeroptera, Plecoptera e Trichoptera (EPT) se destacam entre os insetos aquáticos por constituírem os grupos mais sensíveis a perturbações ambientais (Barbour et al., 1999). A Ordem Trichoptera Kirby, 1813 é composta por insetos holometábolos com estágios de ovo, larva e pupa aquáticas e fase adulta aérea-terrestre (Paprocki, 2012). Trichoptera é considerada a sétima maior ordem de insetos, com cerca de 16.000 espécies descritas mundialmente (Morse, 2022) e 903 espécies registradas no Brasil (A. Santos et al., 2023). Os tricópteros em estágios imaturos são encontrados principalmente em ambientes de água doce, sendo especialmente abundantes em água corrente. Na fase larval, constroem abrigos fixos ou portáteis feitos de seda, quase sempre incorporando diferentes tipos de materiais encontrados no leito dos rios, como grãos de areia, fragmentos de folha, gravetos, algas, entre outros (Pes et al., 2019; Wiggins, 2004). Através do comportamento de construção de abrigos e de seus hábitos alimentares, larvas de Trichoptera mantêm uma relação íntima com a água, o sedimento e a matéria orgânica dos ecossistemas aquáticos.

O contato com partículas contaminadas pode provocar alterações morfológicas em insetos aquáticos, a exemplo daquelas observadas em Chironomidae em resposta a estressores como contaminação por metais (Groenendijk et al., 1998; Montaño-Campaz, 2019) e a radiação em Chernobyl (D. Williams et al., 2001). Desse modo, larvas de Trichoptera em ambientes impactados também podem estar sujeitas a instabilidades no desenvolvimento. Apesar da alta sensibilidade desses insetos a impactos ambientais (Holzenthal et al., 2015; Pereira et al., 2012; Rosenberg & Resh, 1993; Spies et al., 2006), estudos sobre AF em Trichoptera são raros. A AF foi estudada em resposta a impactos antrópicos apenas em larvas das espécies *Hydropsyche morosa* (Bonada & Williams 2002)*, Hydropsyche exocellata* (Bonada et al., 2005) e *Hydropsyche doctersi* (Hamid et al., 2020), sendo registradas nos três estudos correlações entre aumento de AF nas fases larvais e impactos na qualidade da água.

A fase adulta do desenvolvimento é particularmente negligenciada nos estudos sobre AF em Trichoptera. Essa lacuna reflete a relação pouco consensual entre AF e ontogenia na literatura: por um lado, há evidências de que AF causada por contaminação seria mais dificilmente detectada em adultos, uma vez que esses apresentam taxas metabólicas mais baixas que larvas e, por isso, podem ser menos capazes de refletir rapidamente os efeitos de perturbações ambientais (Dobrin & Corkum, 1999; Parsons, 1992); por outro lado, o estudo conduzido por Arambourou et al. (2012) em larvas de Chironomidae levanta a hipótese de que o estágio larval pode apresentar maior estabilidade do desenvolvimento, de modo que larvas seriam menos sensíveis a impactos ambientais e, consequentemente, adultos seriam mais adequados para avaliar AF. Contudo, esses autores não desenvolveram uma possível explicação para a maior estabilidade do estágio larval. A única análise de AF em Trichoptera adultos (Piscart et al., 2005) avaliou populações naturais e está em conformidade com a primeira hipótese (Dobrin & Corkum, 1999; Parsons, 1992), que propõe que a AF é mais detectável no estágio larval. No entanto, o estudo aponta que o nível de AF não se altera entre o último ínstar larval e a fase adulta (Piscart et al., 2005). Desse modo, considerando que o conhecimento acerca da relação entre AF e a ontogenia continua incipiente para os insetos aquáticos, torna-se necessário que estudos sobre AF nesses grupos incluam dados morfológicos de diferentes estágios do desenvolvimento, possibilitando avaliações sobre a adequabilidade de diferentes caracteres e fases de desenvolvimento para o estudo de AF.

Apesar da adequabilidade do grupo como indicador de instabilidade do desenvolvimento causada por perturbações ambientais, a relação entre AF e Trichoptera permanece pouco explorada. Os estudos existentes (Bonada & Williams, 2002; Bonada et al., 2005; Hamid et al., 2020; Piscart et al., 2005) avaliam AF em resposta a impactos difusos e raramente exploram os efeitos de AF sobre Trichoptera adultos. Considerando essas lacunas de conhecimento e a necessidade ainda premente de se avaliar os impactos da deposição de rejeitos da barragem de Fundão sobre os organismos aquáticos, avaliamos a AF nos estágios larval e adulto do desenvolvimento na espécie *Smicridea* (*Rhyacophylax*) *coronata* na bacia hidrográfica do Rio Doce. Nossa hipótese de trabalho afirma que a perturbação ambiental causada pelo rompimento da barragem de Fundão induz um aumento de AF em Trichoptera, fazendo com que índices de AF sejam maiores em áreas impactadas pelo rejeito. Adicionalmente, objetivamos descrever as mudanças morfológicas relacionadas à assimetria esperada em *S. coronata*, bem como identificar caracteres adequados para a detecção de AF em cada fase do desenvolvimento e avaliar a adequabilidade de AF em Trichoptera como bioindicadora de impacto por metais e metaloides. Em consonância com o estudo de Piscart et al. (2005), esperamos que as larvas apresentassem índices mais altos de AF que adultos. Este é o primeiro estudo de AF em resposta a impactos ambientais em Trichoptera adultos.

CAPÍTULO I

Artigo a ser submetido no periódico Environmental Monitoring and Assessment.

Title: Mining tailings alter insects: revealing fluctuating asymmetry in the caddisfly *Smicridea* (*Rhyacophylax*) *coronata*

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Introduction

Developmental instability is characterized by abrupt deviations from the manner an organism responds to disturbances, and as such has been widely used as an indirect estimator of fitness for several species (Sanseverino & Nessimian, 2008; Van Dongen, 2006). In symmetric organisms, developmental instability can be exhibited through directional asymmetry, antisymmetry or fluctuating asymmetry (FA) (Graham et al., 2010; Nunes & Souto, 2021). While antisymmetry corresponds to a pattern of deviations in symmetry with differing directions of asymmetries, and directional asymmetry is characterized by a trait presenting one consistently asymmetric side, FA consists of small and random deviations from symmetry (Klingenberg, 2015; Palmer & Strobeck, 1986; Van Valen, 1962). FA is often associated with genetic, environmental or combined factors (Klingenberg, 2003; Palmer & Strobeck, 2003) and is strongly correlated with developmental instability through the hypothesis that the exposure to stressors leads to an increase of developmental noise and a reduced capacity of buffering against developmental perturbations (Klingenberg, 2015). The study of FA is therefore broadly applied as a bioindicator of environmental quality, being able to provide early warning signs of environmental stress before more widespread effects inflict upon the populations (Beasley et al., 2013; Benítez et al., 2020; Clarke, 1993). FA analyses have been historically refined through the use of geometric morphometric methods, which have supplied new analytical tools, as well as conceptual additions to this study field (Benítez et al., 2020; Klingenberg, 2015).

In freshwater ecosystems, FA has been mostly evaluated in insects in response to the effects of many stressors, including industrial chemicals (Dobrin & Corkum, 1999; Odume et al., 2016; Servia et al., 2004), factory effluents (Clarke, 1993), temperature increases (Hogg et al., 2001), agricultural activities (Pinto et al., 2012), and metal contamination (Al-Shami et al., 2010; Arambourou et al., 2012, 2014; Bhattacharyay et al., 2005; Callisto et al., 2000; Groenendijk et al., 1998). In spite of being a highly diverse aquatic insect Order with more than 16,000 described species, the caddisflies (Trichoptera) have been the subject of few studies addressing developmental instability. Therefore, this may represent an important gap to be filled as they are central to the functioning of freshwater ecosystems and are in direct contact with the pollutants that enter these systems (Morse et al., 2019). An increase in caddisfly FA was recorded in the legs of *Hydropsyche morosa, H. exocellata, and H. doctersi* in response to various human activities in the Palearctic, Nearctic and Oriental regions (Bonada & Williams, 2002; Bonada et al., 2005; Hamid et al., 2020). On the other hand, fluctuating asymmetry in caddisflies has been identified in natural populations as well, with a decrease in asymmetry along the larval stages. Lower FA levels were detected in adults, possibly due to natural selection of larvae that exhibited extreme levels of FA (Piscart et al., 2005).

Caddisflies are indicated as suitable organisms for assessing FA in disturbed environments (Bonada & Williams, 2002), as they are well-known bioindicators of water quality due to their diversity, abundance in various habitats, and sensitivity to changes in physical and chemical conditions of waters (Holzenthal et al., 2015; Pereira et al., 2012; Rosenberg & Resh, 1993; Spies et al., 2006). In this study, we examined FA in caddisflies in the context of the Samarco/Vale/BHP mine tailing dam failure, the largest socio-environmental disaster in Brazil's history (Escobar, 2015; Fernandes et al., 2016). This tailing dam's rupture took place in the Minas Gerais state, southeastern Brazil, in 2015. Over 50 million cubic meters of mining tailings were deposited into the Doce river basin, a watershed situated in Brazil's only two biodiversity hotspots for conservation (Myers et al., 2000), contaminating its waters with heavy metals and metalloids; thus, drastically impacting the riverine ecosystems (Fernandes et al., 2016; Freitas et al., 2016; Gabriel et al., 2020; Gomes et al., 2017).

Despite having occurred almost nine years ago, the effects of the dam rupture have recent repercussions. Over the years, the tailings were not diluted and dispersed homogeneously along the basin, but accumulated within sediment and continually remobilized because of the natural seasonality of the waters, thus metal concentrations increase after rains (Felippe & Mendes, 2022; Santana et al., 2021). Consequently, effects of the tailing spillage remain latent through the years, with serious implications such as the contamination of groundwater and bioaccumulation by aquatic biota (P. Costa et al., 2022; Felippe & Mendes, 2022). The extensive contamination of this watershed with metals and other pollutants may affect benthic fauna through sediment toxicity, biogeochemical changes and shifts in macrofaunal assemblages (Gomes et al., 2017), with still unknown effects through the several layers of the food web and species interactions.

This paper examines FA as an indicator of environmental stress in larval and adult stages of *Smicridea* (*Rhyacophylax*) *coronata* Flint 1980 (Trichoptera: Hydropsychidae), a species belonging to a remarkably diverse and widespread genus (Vilarino et al., 2019), often studied in the context of bioindication (Biasus et al., 2019). Neotropical *Smicridea* are often considered collector-filterers (Henriques & Nessimian, 2010; Tamaris-Turizo et al., 2020; Tomanova et al., 2006), a functional feeding group that is directly exposed to suspended particulate matter and, for this reason, presents some of the highest levels of mercury accumulation (Marle et al., 2022). *Smicridea coronata* is widely distributed in the Atlantic Forest biome in southeastern Brazil, presenting distribution records to the biomes of Cerrado and Pampas as well. This species is distributed in median and low altitudes, in areas of strong river currents, with rocky or sandy riverbeds (Desidério, 2016). Although caddisflies are a generally sensitive group to environmental disturbances, tolerance levels vary within families and genera (Lenat & Resh, 2001; Pereira et al., 2012) and *S. coronata* is considered a relatively tolerant species to environmental impacts (Desidério, 2016). We aimed to investigate whether the environmental impacts of the Samarco/Vale/BHP dam breach increased FA in *S. coronata*, characterizing levels of FA in larval and adult stages of development. We hypothesized that individuals collected from impacted areas would present higher levels of FA than individuals collected from areas unaffected by the tailings. FA levels in larvae were also expected to be higher than adult FA levels (see Piscart et al., 2005). This is the first study to apply geometric morphometric methods in a FA assessment in caddisflies, and as far as we know, the first to evaluate FA in Neotropical representatives of this order.

Material and methods

Study sites

Caddisflies were sampled in the Doce river basin in two different environmental conditions within a radius of 20 kilometers from location of the tailing dam breach in the municipality of Mariana, Minas Gerais, Brazil (see Fernandes et al., 2016). Study sites were separated by a minimum of 5 kilometers within-river and divided between the impacted condition (IC) affected directly by the tailing deposit and sites in the least disturbed conditions (LDC). The latter are described by Stoddard (2006) as the best available habitat conditions in an area with extensive human disturbance (see also Toma et al., 2023). Although not directly affected by the catastrophic deposit of mine tailings, LDC sites are historically impacted by regular human activities such as agriculture, as well as intense gold and iron mining activities (Rhodes et al., 2018).

Three sampling sites in each condition were selected with a minimum distance of 10 kilometers between each other (Fig. 1). The IC sites were located downstream from the dam failure site, in Gualaxo do Norte river, and the LDC sites were located upstream from the dam failure site, in Gualaxo do Norte river and two tributaries. In each sampling site, a Rapid

Assessment Protocol of habitat diversity (Callisto et al., 2002) was filled out in order to categorize sampling sites within the natural, altered or impacted conditions. Protocol scores ranging from 0 to 40, 41 to 60 and 61 to 100 represented, respectively, impacted, altered, and natural stretches of river.

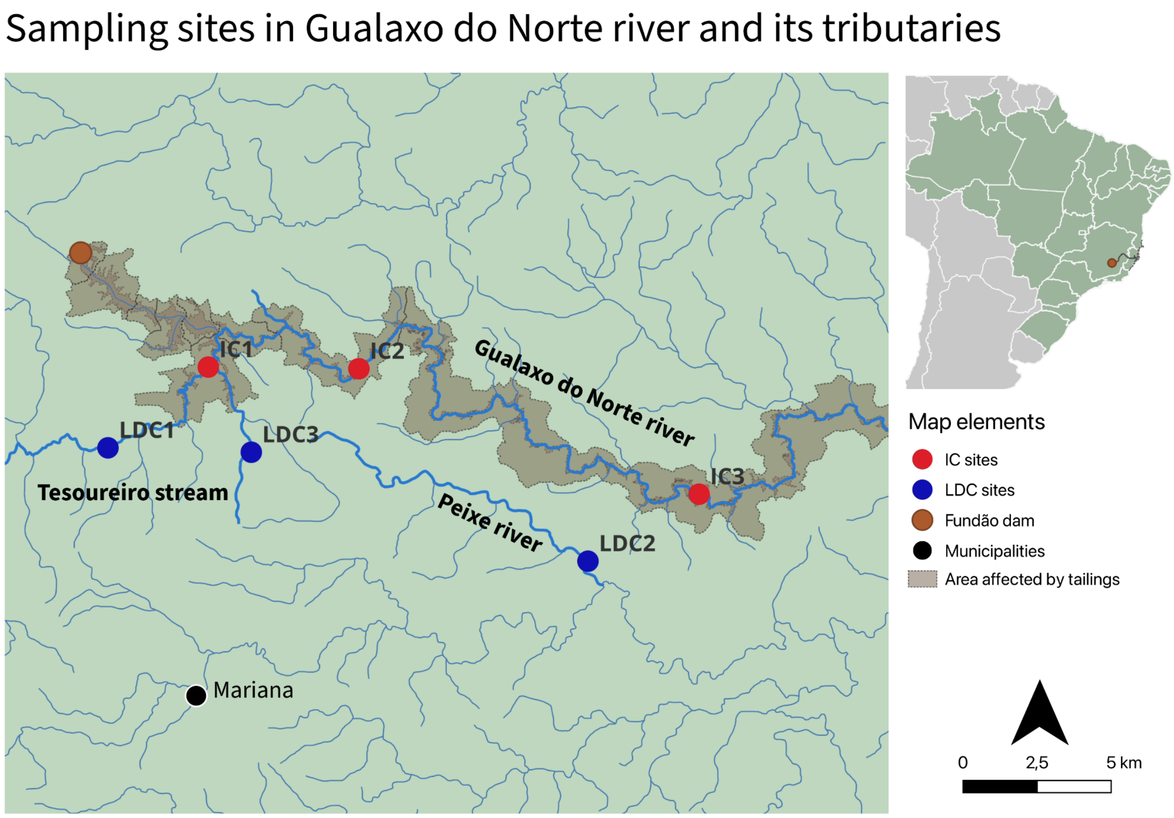

Figure 1. Sampling sites in the Impacted Condition (IC) and Least Disturbed Condition (LDC) distributed along the study area in the Doce river basin (Mariana, Minas Gerais, Brazil).

Smicridea (*Rhyacophylax*) *coronata* sampling and processing

Smicridea coronata sampling was conducted between September and December 2022. Caddisfly larvae were sampled with Surber samplers and manual collections aided by sieves. Adults were collected between 6 and 8 PM with timer-automated pan traps filled with alcohol, coupled with white and UV lights. Adult sampling using white sheets and white and UV lights were used as complementary methods. All collected individuals were preserved in 70% ethanol for sorting and identification. The criteria for selecting the studied species was the abundance of individuals.

Adults of *S. coronata* were identified by comparing descriptions and illustrations provided for male individuals of the species in the genus according to the available bibliography. Larvae of *S. coronata* were not formally associated with the described adult, but we decided to name the immatures studied here as *S. coronata* following the preliminary study provided by Desidério (2016). All specimens were deposited in the entomological collection of the Museu de Ciências Naturais PUC Minas, Minas Gerais state, Brazil.

Individuals were photographed using a Zeiss Stemi 508 stereoscope coupled with an Axiocam 208 camera. All photographs of each morphological trait were taken with standardized scaling and magnification settings. As measurement error is undesirable, yet inevitable in morphometric analyses, replicate digitizing procedures were performed by the same person in order to minimize errors (Arnqvist & Matersson, 1998; Benítez et al., 2020; Mikac et al., 2016). Traits were photographed twice for each individual, then each picture was doubled in order to replicate photograph and landmark digitization, allowing the measurement of each digitization error (Klingenberg, 2015).

Parallel and equidistant lines were inserted on the images of wings and larvae cephalic capsules with the aid of the software MakeFan6, allowing the positioning of homologous landmarks on curved structures. All landmarks were digitized with tpsDig2 and geometric morphometric analyses were conducted with MorphoJ (Klingenberg, 2011).

Analyzed traits

We selected a multiple-trait approach to the assessment of FA with the purpose of improving the estimate of developmental instability (Klingenberg, 2015). Traits of more and less functional importance were selected for landmark placement, as both are considered useful for the study of FA. That occurs because FA in functionally important traits allows a better understanding of the possible effects of asymmetry in a population, yet the selective pressure for symmetry is stronger on more important functional traits, thus these traits may not always exhibit FA (Anciães & Marini, 2000; Balmford et al., 1993; Nunes & Souto, 2021).

The analyzed traits in the larval stage were the cephalic capsule and the mandibles. Uneven sample sizes were analyzed, as caddisfly abundance tended to be higher in the IC in both larvae and adults. Cephalic capsules (N_{IC} = 30, N_{LDC} = 15) were photographed dorsally and eleven landmarks were distributed on each photograph (Fig. 2A, supplementary table S1). The left and right mandibles (N_{IC} = 27, N_{LDC} = 13) were detached from the individual's cephalic capsule, mounted on microscope slides with glycerin and photographed. Nine landmarks were placed on each mandible photograph (Fig. 2B, supplementary table S2). The forewings (N_{IC} = 60, N_{LDC} = 60) were the analyzed traits in adults. Left and right forewings were gently detached from the individual's bodies, mounted on microscope slides with glycerin and photographed. Twenty landmarks were inserted in each photograph (Fig. 2C, supplementary table S3). Individuals that presented damage on the analyzed traits and adult *Smicridea* females were discarded from the analyses.

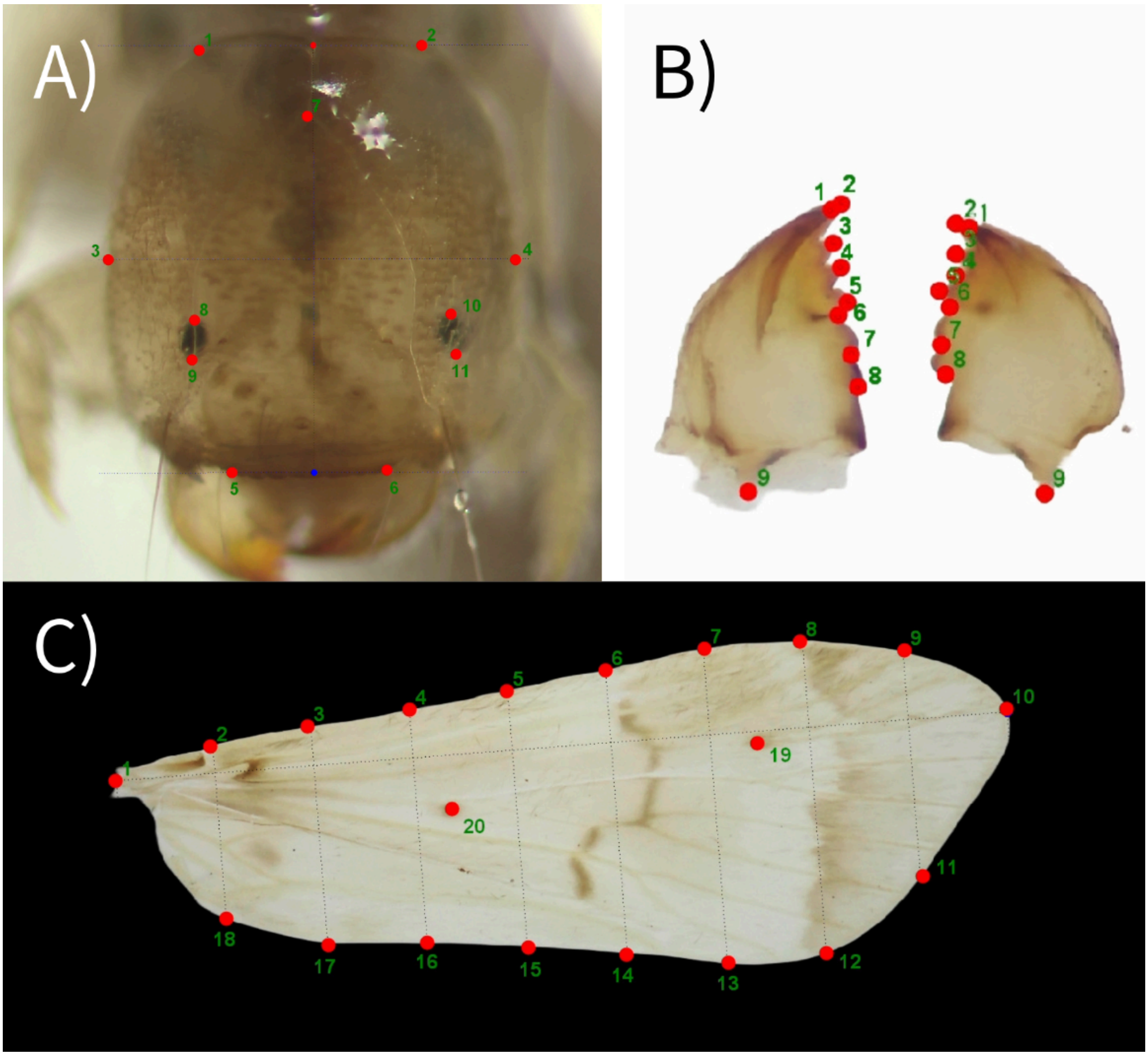

Figure 2. Configuration of landmarks for morphometric analyses of *Smicridea* (*Rhyacophylax*) *coronata*. A) Cephalic capsule; B) Mandibles; C) Left forewing.

Data analyses

Preliminary procedures performed for each trait in MorphoJ included a generalized Procrustes fit to configure the coordinates of each landmark, superimposing configurations of all analyzed individuals as closely as possible to their overall average shape. This process removes the components of variation that are not part of shape from the coordinate data (e.g. variation in scaling, position and rotation) (Klingenberg, 2015). We performed a Procrustes analysis of variation (ANOVA) to measure the errors in photograph and landmark digitization, as well as population estimates of directional and fluctuating asymmetry, for each condition (IC and LDC) in every analyzed trait. For each trait that presented negligible measurement error, IC and LDC datasets were combined in a single dataset for subsequent analyses (Benítez et al., 2020).

Allometry analyses were performed to test the effects of size on shape variation, in order to disregard morphometric changes due to size-dependency. For this purpose, multivariate regression analyses of each trait were carried out using individual values of centroid size as the independent variable and the individual asymmetric component as the dependent variable (Klingenberg & Marugán-Lobón, 2013). Size-dependent data were corrected in the following analyses with the use of regression residuals (Klingenberg, 2016).

Shape asymmetry was evaluated in three ways. First, the Procrustes ANOVAs for each trait in both environmental conditions calculated populational FA as comparable mean square (MS) and significance values of differences among individuals in their left-right asymmetries (individual-by-side interaction) (Benítez et al., 2020). As a second step, we constructed a covariance matrix from the individual asymmetric component to perform a principal component analysis (PCA) for each trait, as an exploratory analysis to describe asymmetric shape variation of individuals from the two different conditions (Montaño-Campaz et al., 2019; Zelditch et al., 2004). Lastly, individual Mahalanobis distances between left and right sides were extracted to evaluate asymmetric variations considering the non-isotropic nature of FA. This metric is suitable for FA analyses as it represents left-right distances in a transformed morphospace, disregarding the directionality of data whilst maintaining magnitude information (Klingenberg & Monteiro, 2005). Mahalanobis distances were therefore used as representatives of the individual distance between groups relative to the within-group variation (Klingenberg, 2015).

To account for variation within environmental conditions and evaluate the effect of both conditions upon individual FA, we then used Mahalanobis distances to build a generalized linear mixed model (GLMM) using the conditions as independent variables, Mahalanobis distances as dependent variables and sampling sites as random effects. As mandible data were concentrated on single IC and LDC sites, a general linear model (GLM) was used to test the effect of environmental conditions on Mahalanobis distances. When individual distributions made it possible, the effect of sampling site variation on Mahalanobis distances within each environmental condition was tested through a GLM. ANOVAs were used to test all models for significance. Linear models were built and tested in R (R Core Team, 2023).

Results

Rapid Assessment Protocol of habitat diversity

Rapid Assessment Protocol of habitat diversity (Callisto et al., 2002) values indicated all Impacted Condition sites as altered (score values: IC_1 = 50, IC_2 = 41, IC_3 = 46) and all sites classified as the Least Disturbed Condition as natural environments (score values: LDC_1 = 71, LDC_2 = 80, LDC_3 = 63), indicating they indeed represent different environmental conditions for ecological comparisons.

Procrustes ANOVA: measurement error and asymmetry values

Measurement error was considered negligible for the mandibles and forewings, as mean squares (MS) values for errors were lower than FA values (see Klingenberg, 2015). Error negligibility was suggested for all traits due to the higher proportion of variation between individuals compared to photograph and digitization variations (for complete results of Procrustes ANOVAS see supplementary tables S4, S5 and S6). However, the cephalic capsule of LDC individuals presented lower FA values than the variation in photograph digitizations and this result remained unchanged after successive digitization attempts. Therefore, measurement error for the cephalic capsule of LDC individuals was not negligible and this trait was not included in subsequent analyses.

Although cephalic capsule data were considered unreliable, this trait exhibited levels of populational FA over 1.5 times greater in the IC (MS_{IC} = 0.00023; Table 1) than in the LDC (MS_{LDC} = 0.00014), as predicted by our hypothesis. The forewings presented the same pattern, with IC levels of FA 1.7 times greater than LDC levels (MS_{IC} = 0.000034, MS_{LDC} = 0.00002). FA values for the mandibles were the highest of all analyzed traits, yet exhibited a decrease in asymmetry in the impacted area, with LDC values exceeding IC values by 50% (MS_{IC} = 0.00091, MS_{LDC} = 0.00145).

Table 1. Mean squares (MS), degrees of freedom (df), F statistics and p-values of estimates of populational FA calculated using the Procrustes ANOVA. Metrics refer to the cephalic capsule, mandibles and forewings of individuals belonging to IC and LDC in Gualaxo do Norte river and its tributaries. Significant p-values are represented as * ($p < 0.0001$) and ** ($p < 0.05$), non-significant p-values are presented as *** ($p > 0.05$)

Trait	Condition	MS	df	F
Cephalic capsule	Impacted	0.000236	270	1.28**
	Least disturbed	0.000149	126	0.8***
Mandibles	Impacted	0.000910	350	11.51*
	Least disturbed	0.00145	168	23.19*
Forewings	Impacted	0.0000340	2088	5.09*
	Least disturbed	0.0000223	2124	3.7*

FA values were significantly lower than directional asymmetry (DA) for all analyzed traits ($p < 0.001$ in all Procrustes ANOVAs). The greatest disparity was found in the Procrustes ANOVAs of the mandibles, as FA values in this trait were exceeded by DA values by 62 (IC) and 21 (LDC) times. DA values were 15 and 10 times greater than FA values in the cephalic capsule from the IC and LDC, respectively. Forewing FA values were 2.9 (IC) and 6.2 (LDC) times lower than DA values. DA values increased in the impacted area in traits from the larval stage, with cephalic capsule (MS_{IC} = 0.003, MS_{LDC} = 0.001) and mandible (MS_{IC} = 0.056, MS_{LDC} = 0.019) DA values roughly three times greater in the IC than in the LDC. Directional asymmetry in forewings was 30% greater in the least disturbed condition (MS_{LDC} = 0.00013) than in the impacted condition (MS_{IC} = 0.00010).

Allometric effect

Regression analyses of individual size on asymmetry revealed no significant allometry for larvae mandibles ($p = 0.63$), indicating no size-dependency of the asymmetric changes. The permutation test associated with the regression indicated that individual size variance influenced the forewing asymmetry ($p = 0.02$), explaining only 2.16% of asymmetric shape changes. We used the residuals of the regression performed for forewings for the subsequent analyses, in order to disregard the allometric effect.

Asymmetry analyses

The first four principal components of the PCA of asymmetric mandible shape variation were responsible for 85.6% of all variation. Though the first two principal components (see scores in the supplementary figure S1) explain a higher percentage of shape variation, patterns were best observed in the first and third principal components. Shape changes are represented through orange lines illustrating the consensus of shape configured from the Procrustes fit, and the green lines represent disparities of shape corresponding to the extreme of each axis. Though individuals from different environmental conditions tended to be mostly overlapped, IC individuals were grouped along the positive extreme of the first principal component (PC1 = 49.5% of shape variation). This extreme exhibited subtle vertical shifts of teeth position, with shifts in the mesal points between teeth and molar lobes, as well as a shortening of the length of the medial process of the posterior margin (Fig. 3).

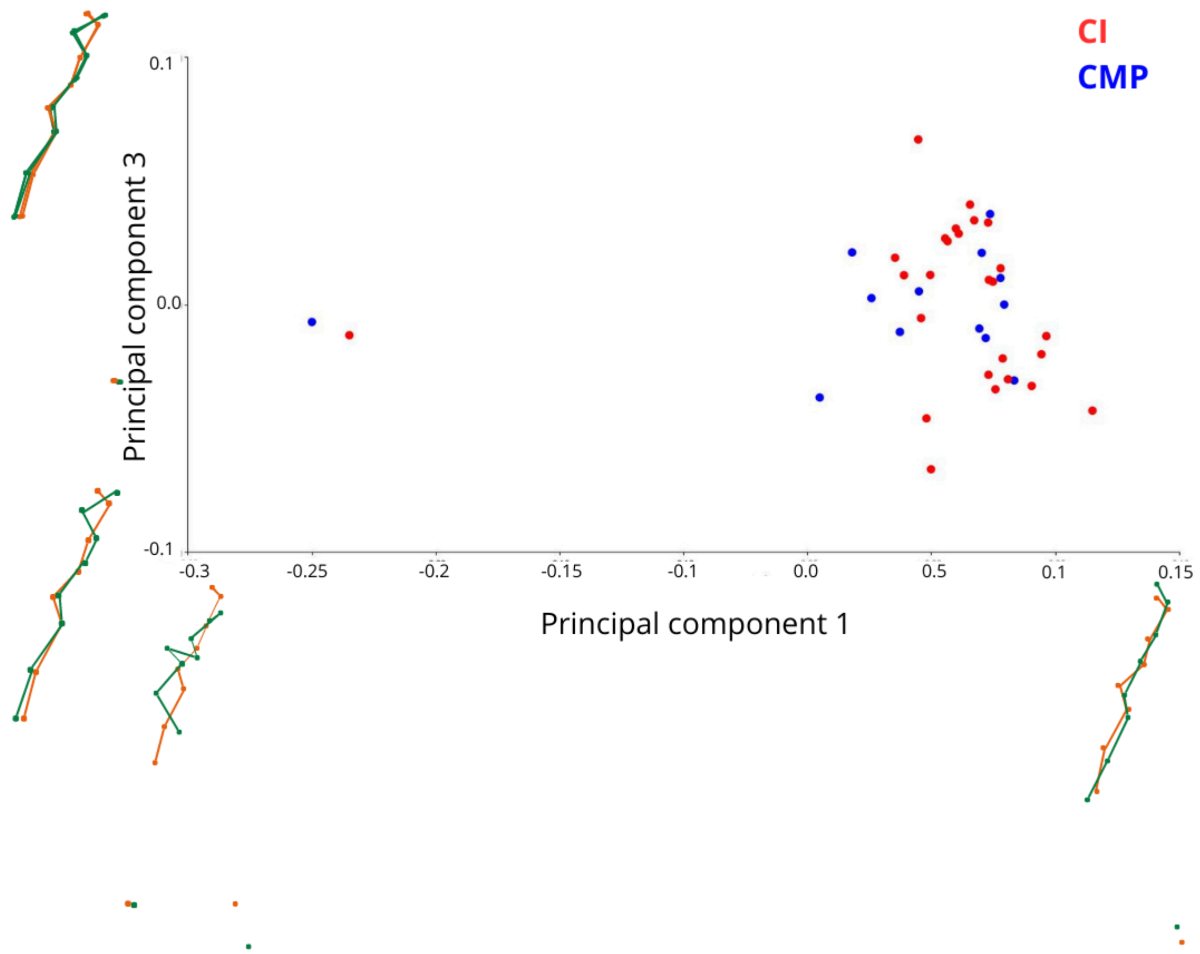

Figure 3. Principal component analysis of the mandibles of *Smicridea coronata* showing the asymmetric shape changes along the distribution of individuals from impacted (IC) and least disturbed conditions (LDC) in Gualaxo do Norte river and its tributaries. The phenotypes corresponding to the extremes of each principal component axis are represented, with the consensus of shape configured from the Procrustes fit illustrated by the orange lines. Green lines represent disparities of shape of individuals in the extremes of each principal component axis.

The first four axes of the principal component analysis of asymmetric shape changes in forewings explained 78% of the total variance. Individuals belonging to the LDC were grouped around the center of both axes and both IC and LDC individuals tended slightly towards the positive end of the PC1 (38% of shape variation), which reflects a decrease in the width of the forewings (Fig. 4). IC individuals were also subtly distributed towards the negative end of the PC2 (22.3% of total shape variation), presenting phenotypes of irregularities in the anterior margin of the forewings, with more curved and decreased forewing widths.

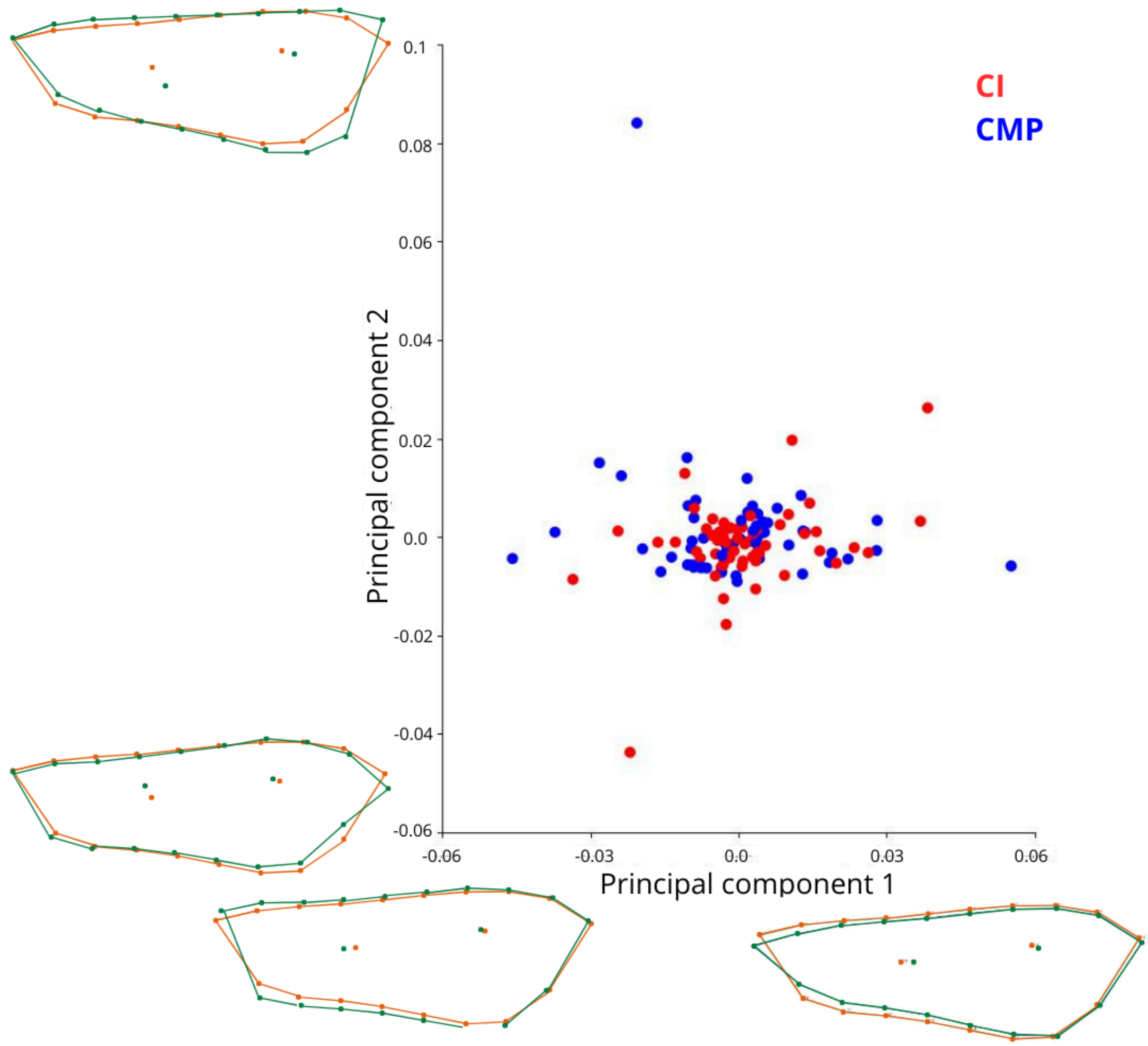

Figure 4. Principal component analysis of the forewings of *Smicridea* (*Rhyacophylax*) *coronata* showing the asymmetric shape changes along the distribution of individuals from impacted (IC) and least disturbed conditions (LDC) in Gualaxo do Norte river and its tributaries. The phenotypes corresponding to the extremes of each principal component axis are represented, with the consensus of shape configured from the Procrustes fit illustrated by the orange lines. Green lines represent disparities of shape of individuals in the extremes of each principal component axis.

Individual FA estimates were significantly influenced by variation in the environmental condition in the mandible data ($p = 2.2 \times 10^{-16}$), but not in the forewings ($p = 0.87$), as indicated by the ANOVAS for the GLM for mandibles and GLMM for forewings (Fig. 5). The GLM for influence of sampling site variation within the IC on Mahalanobis distances in forewings revealed a non-significant influence ($p = 0.22$). The same analysis could not be performed for the forewings from the LDC, as these data were distributed on less than three sampling sites.

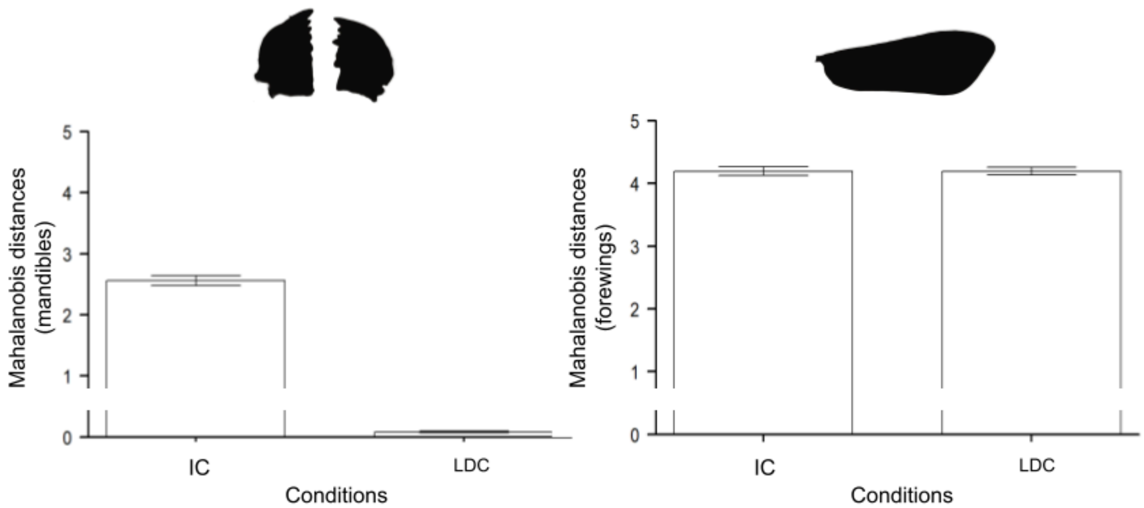

Figure 5. Linear models of the influence of environmental conditions on Mahalanobis distances of *Smicridea coronata* individuals from the IC and LDC in Gualaxo do Norte river and its tributaries. Models represent, respectively, mandibles and forewings.

Discussion

Disturbances can have adverse effects on vital elements during insect development, such as impairing the expansion of wings through the pumping of hemolymph in the transition into adult life (Salcedo et al., 2023). Different studies have demonstrated that insect wing morphology can present asymmetric shape variations in response to deforestation (Pinto et al., 2012), altitudinal variation (Henriques & Cornelissen, 2019; Vaca-Sánchez et al., 2023), forest fragmentation (Pignataro et al., 2023), cattle raising, mining and agricultural activities (Montaño-Campaz et al., 2019). Our study reports an increase of forewing FA in caddisfly populations affected by mining tailings, with a tendency of IC individuals to present more irregular and curved forewing widths. Flight-related traits in Trichoptera, as in most insects, have important roles in dispersal ability, mating, oviposition and predator avoidance (Holzenthal et al., 2015; Müller-Peddinghaus & Hering, 2013; Wiggins, 2004). For this reason, adaptive consequences of asymmetric changes in caddisfly forewings may have strong and similar effects as those reported for other insect taxa, such as reduced mobility, worsened aerodynamics and possibly poor flight performance and lifetime fitness (De Block et al., 2008; Windig et al., 2000).

Forewings were the only analyzed trait in this study to present significant and reliable data that corroborated our hypothesis at the populational level. This outcome is the first to confirm the suitability of caddisfly forewing asymmetry as a measure of contamination-induced developmental instability through geometric morphometric methods. Given that FA in response to disturbances has only been detected in caddisfly larvae (Bonada & Williams, 2002; Bonada et al., 2005; Piscart et al., 2005), this is the first report of adult caddisflies as more appropriate objects for FA studies. This result contradicts the more well-established premise of larvae as a developmental stage that is more capable of reflecting the effects of environmental disturbances due to their high metabolic rate (Dobrin & Corkum, 1999; Parsons, 1992). On the other hand, FA assessments selecting the very initial larval instars to compare larval and adult FA could provide more insight into the relationship between asymmetry and ontogeny in caddisflies, given that FA levels have been reported to remain unchanged from the later larval instars to the adult stage (Piscart et al., 2005).

The suitability of adults for detecting significant increases of FA along an environmental impact gradient may be extremely useful for biomonitoring. As species-level identifications of Trichoptera are highly dependent on the adult morphology, especially the male genitalia, adults are more often precisely identified for biomonitoring studies (Holzenthal et al., 2015). Larvae are often analyzed to a genus level in biomonitoring programs, since interspecific variation in this developmental stage is poorly known, and this nonspecifity could conceal morphological indicators that could be useful in FA studies. Therefore, FA assessments of adults represent an efficient tool for monitoring and providing early warnings for disturbances, which could impact decision-making regarding conservation and restoration of natural ecosystems.

Though morphological variation of the mandibles according to environmental conditions was not extreme, mandibles of individuals from the Impacted Condition tended to exhibit changes in the position of all teeth. Tooth deformities are very commonly reported for Chironomidae through addition, depletion, fusion and fissures of teeth (Arambourou et al., 2012; Montaño-Campaz et al., 2019; Sanseverino & Nessimian, 2008) as a response to various environmental impacts. Similar morphological alterations might occur in caddisflies due to their close relationship with sediment and case-making habits (Wiggins, 2004).

Contrastingly, though the mandibles presented the highest populational FA scores of all traits, lower mandible FA was found in the IC. This result contradicts the reported tendency of freshwater insects in impacted environments to display asymmetries and deformities in the mouthparts (e.g. Callisto et al., 2000; Montaño-Campaz et al, 2019). A possible explanation for this relies on the fact that the literature is heavily based on chironomid larvae (de Bisthoven et al., 2005; Callisto et al., 2000; Hamilton & Saether, 1971; Ilyashuk et al., 2003; Martinez et al., 2004; Montaño-Campaz et al., 2019; Servia et al., 2004; Warwick, 1985; Warwick & Tisdale, 1988), while different taxa could respond in distinct ways. Alternatively, this result could also be an outcome of a stronger selective pressure for symmetry in functionally important structures (Anciães & Marini, 2000; Balmford et al., 1993). As revealed by Piscart et al. (2005), higher FA values can be found in earlier larval instars. Seeing that the mandibles in caddisflies are central to feeding and metamorphosis (Wiggins, 2004), we propose that strong selective pressures for symmetry in this functionally important trait might cause early mortality of extremely asymmetric larvae. Therefore, experimental studies are necessary to better understand this finding.

Directional asymmetry was higher than FA in all traits. Nonetheless, the magnitude of DA levels in relation to FA was considerably higher for the mandibles, with DA levels 62 (IC), and 21 (LDC) times larger than FA values. These results are consonant with the findings of the only other assessment of FA in caddisfly mandibles. The study conducted by Bonada et al. (2005) in a temperate ecoregion detected DA in another pollution-tolerant Hydropsychidae species for all sampled river reaches affected in different degrees by urban and industrial activities in a temperate ecoregion. The high levels of DA and the unexpected decrease in FA presented by the mandibles suggest that this may be a less suitable trait for analyzing FA, possibly due to natural attributes of caddisfly mandibles. Like many caddisfly species, *S. coronata* mandibles are described as asymmetric in the larval stage (Desidério, 2016) and we propose that this asymmetry could be mostly explained by DA, a type of asymmetry not strongly correlated with developmental instability. Different types of asymmetry have been reported to exist in complementary ways and commonly transition among FA, DA or antisymmetry under different stressors (Graham et al., 1998; Van Valen, 1962). Though DA is not perceived as a nuisance in studies of geometric morphometrics (Klingenberg, 2022), it has been hypothesized to be exhibited as an admixture of asymmetries, with FA transitioning to DA in the presence of enhanced disturbance (Lens & Van Dongen, 2000). However, inferences are still anecdotal, seeing that the genetic and developmental basis of DA are yet unclear and in need of further studies (Carter et al., 2009; Klingenberg, 2022).

Measurement error is often a serious concern for the study of FA (Klingenberg, 2015; Palmer & Strobeck, 1986) and we found low contributions of errors for measures of FA variation in the analyzed traits, with the exception of cephalic capsule data. The cephalic capsule is a structure of great functional importance in caddisflies, as the eyes, setae and antennae are related to sensorial abilities that are key for reproduction and foraging (Holzenthal et al., 2015; Wiggins, 2004). Moreover, larval mouthparts located in the cephalic capsule are important to the production of silk, which Hydropsychidae use to spin nets to filter food particles of organic matter (Holzenthal et al., 2015) and build retreats useful for protection from predators (Wiggins, 2004). This trait exhibited a detectable FA increase that could indicate impairments in pivotal mechanisms for the survival of larvae, though data was considered unreliable by the Procrustes ANOVA results. The continuously high measurement error for this trait may be related to the curvature of this structure. Considering that the cephalic capsule was the only analyzed trait that could not be mounted on microscope slides, our results might indicate a need for a highly standardized approach in the image acquisition in order to incisively avoid photographic distortion.

Contrasting results for individual (Mahalanobis distances) and populational (Procrustes ANOVA) estimates of FA were observed, given that the Procrustes ANOVA results showed an increase in FA in the IC for the forewings, yet the linear models revealed that individual Mahalanobis distances for this trait were not significantly influenced by the environmental conditions. This inconsistency may be due to inherent characteristics of each measure of asymmetry. Measures of FA based on Mahalanobis distances are considered as preferred asymmetry measures for the study of FA, especially in observational settings, being able to pick out relatively subtle differences in the amount of asymmetry (Klingenberg, 2015; Klingenberg & McIntyre, 1998; Klingenberg & Monteiro, 2005). However, in order to disregard non-isotropic asymmetries, this approach uses transformed morphospaces to alter data geometry (Klingenberg & Monteiro, 2005) and, for this reason, may not always reflect data realistically.

Contrasting associations of FA and disturbances at the individual and populational scales can perhaps be attributed to the unsuitability of these traits for asymmetry assessments, as demonstrated by Servia et al. (2004) for pecten epipharyngis in chironomids, or in the assessment of FA in mayfly wings and legs by Dobrin & Corkum (1999). We also hypothesize that it is more likely that our results could be associated with the historical human impact of the Doce river basin due to extensive anthropic activities such as mining, silviculture and agriculture (Carvalho et al., 2020; Fernandes et al., 2016).

Characteristic consequences of the legacy effect in rivers include alterations in sedimentation supplies, commonly through the deposit of contaminants from past human activities (Wohl, 2015), and can take part in the structuring of benthic fauna assemblages

(Linares et al., 2023). Therefore, though the Rapid Assessment Protocol of habitat diversity scores clearly separated IC and LDC sites into different groups of environmental impact, pre-existing FA levels or selective pressures from different activities may have taken a role in the obtained results. This does not indicate insignificant effects of mining tailings from the Fundão dam break, since temporal increases in metal and metalloid concentrations are still being recently reported for various aquatic organisms (P. Costa et al., 2022). The selection of the extreme ends of environmental gradients, including sites in the best attainable conditions where the best possible management practices are applied (Stoddard, 2006) might enable a more accurate differentiation between environmental conditions. Alternatively, experimental ecotoxicological approaches that test the effects of specific pollutants under controlled circumstances have been recommended for the study of morphological abnormalities and FA (Beasley et al., 2013; Vuori & Kukkonen, 2002) and could be beneficial in the context of diffuse and synergetic impacts.

Conclusions

Increases of fluctuating asymmetry in the impacted condition were reliably detectable at the individual level in the mandibles of larvae, exhibiting variation in the position of teeth. FA also increased in response to disturbance at the populational level in the forewings of adults of *Smicridea coronata*, showing reduced forewing widths that could be detrimental to fitness in individuals from the IC. The contradicting results for FA in mandibles at individual and populational scales, combined with extremely high directional asymmetry levels for this trait in both conditions, might indicate caddisfly mandibles as less suitable traits for assessing FA. We propose that the predominant type of asymmetry in larvae mandibles is DA and FA in this trait could be channeled as DA in the presence of disturbance. We present unprecedented results about detectability of FA in adult caddisflies, identifying caddisfly forewings as efficient traits to measure FA and revealing the study of morphological variation in adult caddisflies as a valuable tool for biomonitoring. Nevertheless, studies of FA in Trichoptera are lacking and the literature has consistently reported the suitability of larval traits for FA assessments as well. Thus, more morphometric studies of caddisflies in both developmental stages are recommended for a further understanding of the ontogeny of FA and trait suitability for FA evaluation.

We believe that the negative correlations of forewing (individual scale) and mandible (populational scale) FA and environmental impact are likely to be explained by the legacy effect of the Doce river basin, either through pre-existing asymmetry levels or selective pressures in LDC sites. Therefore, a wider environmental gradient, incorporating sites in the best attainable conditions, may be needed for precise assessments of the Fundão dam failure. We also recommend complementary experimental approaches using specific contaminants in controlled concentrations for this purpose.

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Author contribution

Helena Maura de Andrade Soares: design, data acquisition and analysis, interpretation of data, manuscript writing.

Isabela Cristina Rocha: design, taxonomic identification, manuscript reviewing.

Henrique Paprocki: conception, design, manuscript reviewing.

Geraldo Wilson Fernandes: design, funding, manuscript reviewing.

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Data availability

Raw data of this research can be obtained at a reasonable request by correspondence with the first author.

Competing interest

The authors declare no competing interests.

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APÊNDICES

Supplementary information

Supplementary table S1. Description of the landmarks used in the geometric morphometrics analyses of the cephalic capsule of *Smicridea coronata* larvae.

Landmark	Description
1, 2	Posterior-most points on the right and left halves, respectively, of the posterior margin of the cephalic capsule.
3, 4	Lateral-most points of the right and left lateral margins of the cephalic capsule, respectively.
5, 6	Anterior-most points on the right and left halves, respectively, of the anterior margin of the cephalic capsule.
7	Coronal suture bifurcation
8, 10	Posterior-most points of the right and left eyes, respectively.
9, 11	Anterior-most points of the right and left eyes, respectively.

Supplementary table S2. Description of the landmarks used in the geometric morphometrics analyses of the left and right mandibles of *Smicridea* (*Rhyacophylax*) *coronata* larvae.

Landmark	Description
1	Apex of the apicodorsal tooth.
2	Apex of the apical tooth.
3	Apex of the first subapical tooth.
4	Apex of the second subapical tooth.
5	Apex of the third subapical tooth.
6	Mesal point between the third subapical tooth and the molar tooth.
7	Mesal point between the two lobes of the molar tooth.

8	Posterior base of the molar tooth.
9	Apex of the medial process of the posterior margin.

Supplementary table S3. Description of the landmarks used in the geometric morphometrics analyses of the left and right forewings of *Smicridea coronata* adults.

Landmark	Description
1	Basal-most point of the anterior margin of the forewing.
2 - 9	Anterior margin of the forewing.
10	Apex of R5 vein.
11 - 18	Posterior margin of the forewing.
19	Center of the dark spot (nygma) in the base of fork II (R4 + R5 veins).
20	Center of the dark spot (thyridium) in the thyridial cell.

Supplementary table S4. Procrustes ANOVA results for *Smicridea coronata* larval cephalic capsules from the impacted condition (IC) and least disturbed condition (LDC). Sum of squares (SS), mean squares (MS), degree of freedom (DF), F statistics, and P-value calculated by an ANOVA considering the difference of Procrustes coordinates between two photographs of the same individuals and two landmark digitalisations of the same photograph.

Impacted condition					
Effect	**SS**	**MS**	**df**	**F**	**p (param.)**
Individual	0.56	0.00209	270	8.88	< 0.0001
Directional asymmetry	0.033	0.0037	9	15.69	< 0.0001
Fluctuating asymmetry	0.063	0.00023	270	1.28	0.0087
Photograph error	0.103	0.00018	558	8.21	< 0.0001
Landmark error	0.024	0.00002	1098	-	-

Least disturbed condition					
Individual	0.21	0.0017	126	11.57	< 0.0001
Directional asymmetry	0.014	0.0015	9	10.6	< 0.0001
Fluctuating asymmetry	0.018	0.00014	126	0.8	0.9206
Photograph error	0.0503	0.00018	270	2.56	< 0.0001
Landmark error	0.039	0.00007	540	-	-

Supplementary table S5. Procrustes ANOVA results for *Smicridea coronata* larval mandibles from the impacted condition (IC) and least disturbed condition (LDC). Sum of squares (SS), mean squares (MS), degree of freedom (DF), F statistics, and P-value calculated by an ANOVA considering the difference of Procrustes coordinates between two photographs of the same individuals and two landmark digitalisations of the same photograph.

Impacted condition					
Effect	**SS**	**MS**	**df**	**F**	**p (param.)**
Individual	0.33	0.00094	350	1.04	0.359
Directional asymmetry	0.79	0.056	14	62.32	< 0.0001
Fluctuating asymmetry	0.31	0.00091	350	11.51	< 0.0001
Photograph error	0.056	0.000079	714	1.24	0.0003
Landmark error	0.093	0.000063	1470	-	-
Least disturbed condition					
Individual	0.25	0.0209	12	50.83	< 0.0001
Directional asymmetry	0.0088	0.0088	1	21.42	0.0006
Fluctuating	0.0049	0.00041	12	18	< 0.0001

asymmetry					
Photograph error	0.00059	0.000023	26	3.15	0.0002
Landmark error	0.00037	0.000007	52	-	-

Supplementary table S6. Procrustes ANOVA results for *Smicridea coronata* adult forewings from the impacted condition (IC) and least disturbed condition (LDC). Sum of squares (SS), mean squares (MS), degree of freedom (DF), F statistics, and P-value calculated by an ANOVA considering the difference of Procrustes coordinates between two photographs of the same individuals and two landmark digitalisations of the same photograph.

Impacted condition					
Effect	**SS**	**MS**	**df**	**F**	**p (param.)**
Individual	0.14	0.000067	2088	1.99	< 0.0001
Directional asymmetry	0.0036	0.0001	36	2.94	< 0.0001
Fluctuating asymmetry	0.071	0.000034	2088	5.09	< 0.0001
Photograph error	0.028	0.0000066	4212	1.84	< 0.0001
Landmark error	0.0306	0.0000036	8424	-	-
Least disturbed condition					
Individual	0.13	0.000063	2124	2.83	< 0.0001
Directional asymmetry	0.0049	0.00013	36	6.2	< 0.0001
Fluctuating asymmetry	0.047	0.000022	2124	3.7	< 0.0001
Photograph error	0.025	0.00000604	4284	2.68	< 0.0001
Landmark error	0.019	0.0000022	8604	-	-

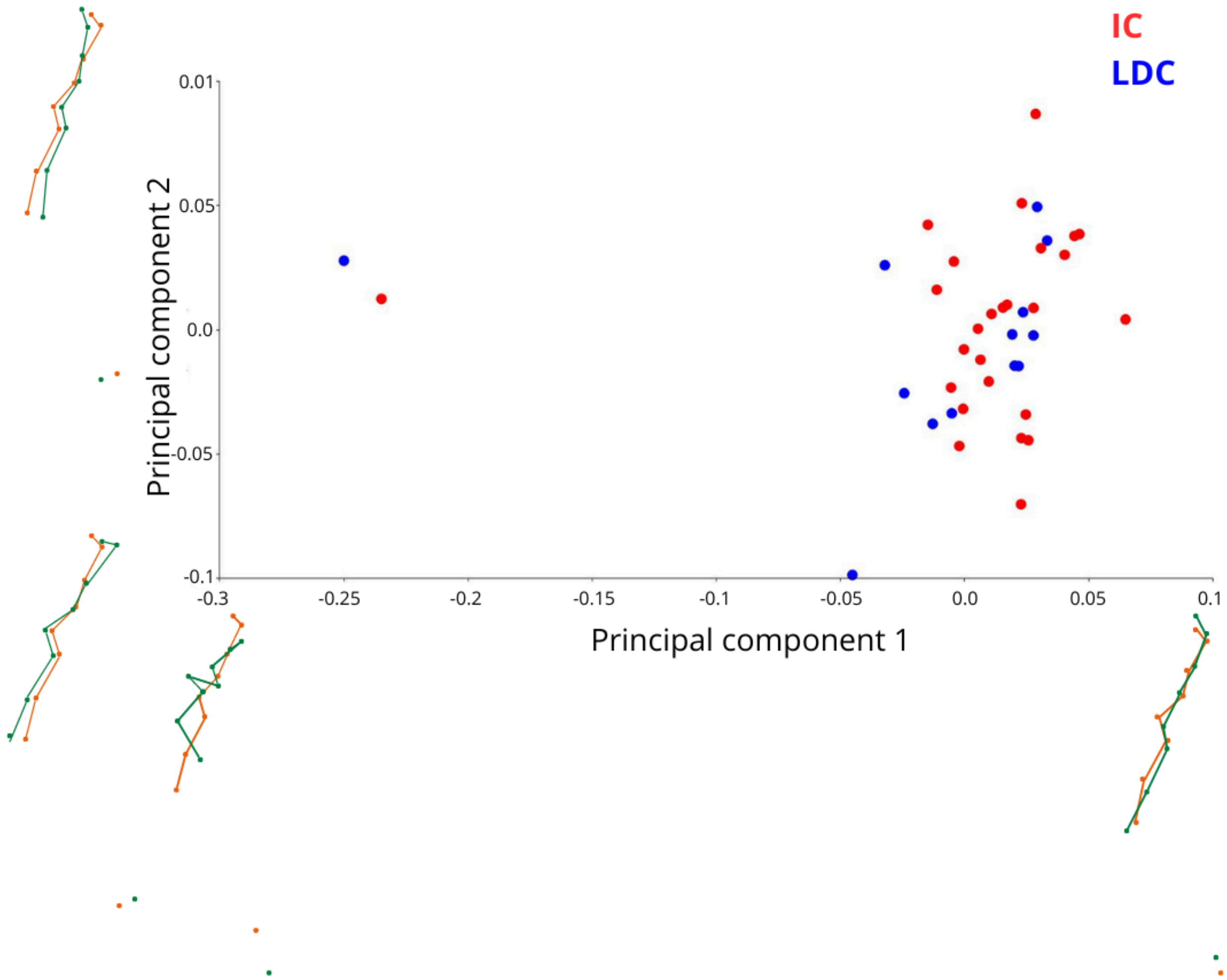

Figure S1. Principal component analysis (PC1 and PC2) of the mandibles of *Smicridea coronata* showing the asymmetric shape changes along the distribution of individuals from Impacted (IC) and Least Disturbed Conditions (LDC) in Gualaxo do Norte river and its tributaries. The phenotypes corresponding to the extremes of each principal component axis are represented, with the consensus of shape configured from the Procrustes fit illustrated by the orange lines. Green lines represent disparities of shape of individuals in the extremes of each principal component axis.