



**TÁSSIA RAYANE FERREIRA CHAGAS**

**MACROPHYTES AS DRIVERS OF PLEUSTONIC  
OSTRACODA (CRUSTACEA) IN TROPICAL FLOODPLAIN  
LAKES**

**LAVRAS – MG  
2018**

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Conservação de Recursos Naturais em Ecossistemas Fragmentados e Ecossistemas, para a obtenção do título de Mestra.

Dra. Alessandra Angélica de Pádua Bueno  
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Dra. Janet Higuti  
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## RESUMO

Comunidades de plantas aquáticas, conhecidas como macrófitas, são diversas em planícies de inundação e desempenham papel de agentes estruturadores em tais habitats, proporcionando recursos alimentares, abrigo e habitat para forrageamento para vários organismos. Muitos estudos pelo mundo abordaram macrófitas e seus efeitos de complexidade sobre a biodiversidade, entretando, a maioria deles não considera as diferenças intrínsecas das distintas formas de crescimento das plantas aquáticas. Nós usamos Modelos Lineares Generalizados Mistos, para focar em macrófitas livres flutuantes e seus efeitos nas assembleias dos ostrácodes pleustônicos. Nós testamos duas variáveis preditoras da riqueza e densidade de ostrácodes. A primeira é a espécie da macrófita livre flutuante, representando a relação interespecífica, e a segunda é o volume do sistema radicular, como uma medida da estrutura de habitat. Este estudo foi conduzido em duas lagoas da planície de inundação do Rio Pandeiros, um tributário do Rio São Francisco. A diversidade total encontrada é consideravelmente alta comparada a planícies tropicais (31 espécies – das quais pelo menos quatro espécies, e também dois gêneros, são possíveis novos registros para a ciência). Maiores valores de volume de raiz abrigaram maior riqueza, entretanto, mesmo entre as macrófitas livres flutuantes existem diferenças além da estrutura de habitat proporcionada. Para a densidade de organismos, a espécie da macrófita teve um efeito independente da estrutura de habitat. *P. stratiotes* e *Salvinia* spp. abrigaram maiores densidades de organismos que *E. crassipes*, mesmo não havendo diferença significativa entre os valores de volume das raízes entre as plantas. A baixa densidade de *E. crassipes* encontrada e o efeito negativo desta planta na densidade de ostrácodes é surpreendente. Nossos resultados revelam a existência de links tróficos em um ambiente ameaçado e situado numa ecorregião de alta prioridade de conservação. Nós chamamos atenção para biodiversidade desconhecida que habita as águas do bioma Cerrado e sugerimos futuros estudos na planície do Rio Pandeiros abordando ostrácodes e seus requerimentos específicos dos recursos e condições proporcionados pelas macrófitas.

**Palavras-chave:** Biodiversidade do Cerrado. Macrófitas aquáticas livres flutuantes. Estrutura de habitat. Lagoas conectadas.

## ABSTRACT

Aquatic plants communities, known as macrophytes, are diverse in floodplains and play the role of structuring agents in such habitats, providing availability of food resources, shelter and foraging habitat to several communities. Worldwide, several studies have approached macrophytes and its structure effects on biodiversity, although, most of them do not take in account macrophytes distinct ecologic groups. We used Generalized Linear Mixed Models, to focus on free-floating macrophytes and its effects on pleustonic Ostracoda assemblages. We test two variables as predictors of Ostracoda richness and density. The first is free-floating macrophytes species, representing interspecific relationship, and root system volume, as a habitat structure measure. This study was carried in two lakes in Pandeiros floodplain, a São Francisco River tributaire. Total diversity registered is considerably high compared to tropical floodplains (31 species - which at least four species and also two genera are possibly new to science). Higher roots volume values supported higher richness, however, even amongst free-floating macrophytes there are differences beyond habitat structure provided. For density, macrophytes species effect was independent of habitat structure measure. *P. stratiotes* and *Salvinia* spp. were able to host high organism densities than *E. crassipes*, even though root volumes did not differ amongst them. Low *E. crassipes* density was found and a negative effect of this macrophyte on Ostracoda density is outstanding. Our results reveal the existence of important trophic links in an endangered and high priority ecoregion. We call attention to the undiscovered biodiversity inhabiting wetlands in Cerrado biome and suggest further studies at Pandeiros floodplain targeting ostracods and their specific requirements of resources and conditions provided by macrophytes.

**Keywords:** Cerrado biodiversity. Free-floating aquatic macrophytes. Habitat structure. Connected lakes.



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**PRIMEIRA PARTE**

## 1 INTRODUÇÃO

Macrófitas aquáticas são vegetais que durante o processo evolutivo desenvolveram adaptações que os permitiram voltar ao ambiente aquático. Possuem uma grande heterogeneidade taxonômica e filogenética, incluindo desde macroalgas até angiospermas (Esteves, 1998). São cosmopolitas e colonizam ambientes diversos como fontes termais, lagoas, lagos, estuários e recifes de corais (Esteves, 1998), além de serem muito abundantes em planícies de inundação neotropicais (Pereto et al., 2016).

Plantas aquáticas são classificadas em grupos ecológicos, de acordo com seu biótopo. Elas apresentam um gradiente de adaptações ao ambiente aquático, onde as plantas submersas enraizadas e livres apresentam adaptações mais intensas, como cloroplastos na epiderme superior, e são consideradas totalmente aquáticas. As enraizadas emersas e enraizadas com folhas flutuantes estão num nível intermediário, seguidas pelas flutuantes livres. As principais adaptações desses vegetais são a redução do grau de lignificação e do xilema. Possuem plasticidade tão intensa, que algumas espécies são capazes de se transformar em formas terrestres em períodos de seca, apresentando acentuadas modificações anatômicas, fisiológicas e fenotípicas (Esteves, 1998).

Comunidades de macrófitas habitam a região litorânea de lagos e lagoas rasas, são a principal comunidade produtora de biomassa em tais ambientes e através da produtividade primária tornam este o compartimento mais produtivo do ecossistema (Esteves, 1998). São agentes estruturadores, pois contribuem para o aumento da complexidade do habitat através de diferentes arquiteturas e complexidade estrutural de suas raízes (Bell et al., 1991), atuam no ecossistema provendo uma série de funções ambientais como maior disponibilidade de alimento, abrigo, local de forrageio e estabelecimento de espécies (Matsuda et al., 2015a). Macrófitas têm sido estudadas como elemento estruturador para peixes (Dias et al., 2017),

macroinvertebrados (Ferreiro et al., 2014) e especialmente para organismos fitófilos (Liberto et al., 2012), que vivem associados a plantas aquáticas.

A fauna associada às raízes das plantas livres flutuantes é conhecida como pleuston (Esteves, 1998). Entre os invertebrados pleustônicos destacam-se os ostrácodes que são pequenos crustáceos caracterizados pela presença de carapaça bivalve envolvendo todo o corpo e apêndices (Horne et al., 2002) com comprimento variando entre 0,3 a 5 mm (Meisch, 2000). Alguns estudos têm demonstrado que comunidades de invertebrados associadas a macrófitas complexas apresentam comumente elevada abundância e riqueza (Higuti et al., 2007, 2010)

Ostracoda, uma das classes mais diversa entre os crustáceos, colonizam diferentes habitats como o ambiente terrestre, marinho e também de água doce (Horne et al., 2002). Na região Neotropical, ostrácodes compreendem 13.75% da diversidade global de ostrácodes viventes não marinhos, conhecida atualmente (Martens et al., 2008) e aproximadamente 260 espécies estão descritas na América do Sul (Martens, 1994).

Ostrácodes viventes de água doce podem ser bioindicadores de parâmetros físico-químicos (Ruiz et al., 2013), mas também apresentam certa tolerância a variações de destes e a parâmetros ecológicos, como presença de cobertura vegetal e predação, com diferentes respostas para espécies diferentes (Kiss, 2007). Fatores como tipo de substrato e nicho ecológico da espécie, influenciam mais a abundância e distribuição de ostrácodes do que características espaciais como variações no tipo de ambiente (Matsuda et al., 2015b).

Estes microcrustáceos apresentam uma grande variedade de interações tróficas, podem ser carnívoros ou decompositores, mas também podem ser parasitas, hospedeiros e presas. Devido a grande diversidade de hábitos e formas de interação, ostrácodes são considerados links tróficos entre bactérias, algas, macrófitas, invertebrados e vertebrados (Rodríguez-Peréz and Baltanás, 2008). Além da importância na cadeia trófica e ampla distribuição geográfica, a

sua relação com a estrutura de habitat disponibilizado pelas plantas aquáticas tem sido estudado recentemente (Higuti et al., 2010; Matsuda et al., 2015a), entretanto, não há clareza sobre o quanto dessa influência é devida a fatores bióticos como relações interespecíficas ou a estrutura de habitat que a planta proporciona (Matsuda et al., 2015a).

Os estudos com enfoque na taxonomia e ecologia sobre ostrácodes ainda são escassos no Brasil e estão concentrados na região sul do país. Na região sudeste, apenas um trabalho foi realizado nas lagoas do médio Rio Doce/MG (Miranda, 2008). Este trabalho faz parte do projeto FAPEMIG 03593/12, intitulado “Desenvolvimento de ferramenta para a priorização de descomissionamento de pequenas centrais hidrelétricas (PCH) no estado de Minas Gerais e estudo de caso para a PCH Pandeiros” e foi desenvolvido na planície de inundação do Rio Pandeiros, um tributário do Rio São Francisco, que contribui para a manutenção da flora e fauna de organismos residentes ou migratórios (Nunes et al., 2009). Vários habitats e duas unidades de conservação existem ao longo de sua planície de inundação: a Área de Preservação Ambiental do Rio Pandeiros (APA) e o Refúgio da Vida Silvestre (REVS), com uso sustentável e proteção integral, respectivamente (Pivari et al., 2013).

A biodiversidade da região é tão alta que somente no REVS existem 101 espécies de macrófitas registradas (Pivari et al., 2013). Por conta do alto endemismo de espécies, e por ser um remanescente lótico para peixes, essa região é considerada área prioritária para conservação da biodiversidade da savanna Brasileira (sensu, “bioma cerrado”) (Drummond et al. 2005). Portanto, entender a interação entre ostrácodes e macrófitas no REVS do Rio Pandeiros torna-se relevante para a conservação da biodiversidade aquática do local.

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## **Macrophytes as drivers of pleustonic Ostracoda (Crustacea) in tropical floodplain lakes**

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### **Abstract:**

*Aquatic macrophytes are structuring agents that provide availability of food resources, shelter and foraging habitat to several communities in aquatic habitats. Although several studies have approached aquatic macrophytes and its effects on biodiversity, most of them indistinctly target different ecologic groups. Thus, using the Generalized Linear Mixed Models, we focus on free-floating macrophytes and use pleustonic Ostracoda as model organism. We test two variables as predictors of Ostracoda richness and density: free-floating macrophytes species as an interspecific relationship, and root system volume as a habitat structure measure, in two lakes of a priority area for biodiversity conservation in Cerrado domain. Total diversity is considerably high compared to tropical floodplains (31 species - which at least four are new species and also two genera are new to science). Increasing roots volume provided higher richness, although, even amongst free-floating macrophytes there are differences beyond habitat structure provided. For density, macrophytes species effect was independent of habitat structure measure. *P. stratiotes* and *Salvinia* spp. were able to host higher organism densities than *E. crassipes*, even though root volumes did not differ amongst them. Low occurrence of *E. crassipes* was observed and a negative effect of this macrophyte on ostracods density is surprisingly. Our results reveal the existence of important trophic links in an endangered and high priority ecoregion. We call attention to the undiscovered biodiversity inhabiting wetlands in Cerrado biome and suggest further studies at Pandeiros floodplain targeting ostracods and their specific requirements of resources and conditions provided by macrophytes.*

**Keywords:** Cerrado biodiversity – free-floating aquatic macrophytes – habitat structure – connected lakes

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## **Introduction**

In neotropical floodplains there are high diversity of ecosystems (Osion et al., 1998) and diversity of habitat is one of the factors that maintain and enhance biodiversity (Junk, 2000). Despite conservation efforts, these environments are placed amongst the most threatened ecosystems around world (Millennium Ecosystem Assessment, 2005) by construction and functioning of dams, alteration of floodpulses, degradation of hydromorphic soil areas (known as veredas), withdrawal of riparian vegetation and deforestation (Osion et al., 1998; Nunes et al., 2009. Junk et al., 2014). These impacts, direct or indirectly, promote habitat loss to freshwater organisms.

In the last 15 years, several studies have been conducted in freshwater environments considering influence of habitat structure, including habitat complexity and structural heterogeneity, on assemblages diversity (Taniguchi and Tokeshi, 2004; Pelicice et al., 2008; Choi et al., 2016). The ‘habitat heterogeneity hypothesis’ (see MacArthur and Wilson, 1967) assumes that structural complexity provides more niches and exploiting resources habitats, and therefore, more diverse communities. At local scale, refuge (Mbahinzireki et al., 1991; Warfe and Barmuta, 2004), niche overlap reduction (Vejrikova et al., 2017), availability of small spaces (Tokeshi and Arakake, 2012), particulate organic matter (Stewart, 2003) and biofilm (Balcombe and Closs, 2016) are conditions and resources that maintain biodiversity.

According Tews et al. (2004) “keystone structure” is a distinct spatial structure affording resources, shelter or ecosystem goods and functions crucial for other species. On tropical floodplains, aquatic macrophytes are very abundant (Pereto et al., 2016), they are taxonomic and filogenetic diverse plants which during evolution process returned from terrestrial to aquatic (Esteves, 1998). Thus, they are classified in ecologic groups based on their biotope and on adaptative characteristics to aquatic environment. Submerged plants have more anatomic and fisiologic adaptations compared to free-floating ones and interact distinctly with habitat, the first provide habitat to aquatic organisms with roots, stems and leaves, while the second is not attached to sediment, follow water level and host communities of organisms associated to its roots, which live in the interface water atmosphere, refereed as pleuston (see Esteves, 1998).

Macrophytes are structuring agents that provide availability of food resources, shelter and foraging habitat to several communities, including macroinvertebrates and microinvertebrates (Bell et al., 1991; Kouamé et al., 2011; Lucena-Moya and Duggan, 2011). They act as keystone structure to fishes (Padiál et al., 2009; Dias et al., 2017), macroinvertebrates (Silva and Henry, 2013; Ferreira et al., 2014), periphyton (Hinojosa-Garro, 2010; Hao et al., 2017) and especially to organisms which live associated to aquatic plants roots (Rocha and Por, 1998; Higuti et al., 2007; Liberto et al., 2012).

The habitat structure that macrophytes provide can be measured through several characteristics, as discrete variables, e.g. number of habitat types, or continuous variables, eg. vegetation coverage (Tews et al., 2004) and it has been assessed by both approaches, by considering root cavities (Hull, 1997; Taniguchi and Tokeshi, 2004; Lucena-Moya and Duggan, 2011) or stem per square meter (Gotceitas and Colgan, 1989), and through plant complexity index of natural macrophytes (Dibble et al., 2006) or artificial ones (Warfe and Barmuta, 2006; Padiál et al., 2009), fractal method (Thomaz et al., 2008; Hao et al., 2017) and entire plant dry weight (Dias et al., 2017).

In face of so many approaches to evaluate macrophyte habitat structure and its effects on biodiversity, we recognize macrophytes as keystone structure in aquatic environments, although it is important to consider that the ecological groups, or life forms, have influence in framing associated communities structure (Walker et al., 2013). For example, submerged plants have more anatomic and physiological adaptations to aquatic habitats compared to free-floating ones and interact distinctly with habitat, the first provide habitat to aquatic organisms with roots, stems and leaves, while the second is not attached to sediment and follow water level.

Here we focus on free-floating aquatic macrophytes, they host communities of organisms associated to its roots, which live in the interface water atmosphere, referred as pleuston (see Esteves, 1998). Several taxa comprise pleustonic communities, as Insecta, Mollusca, Oligochaeta and Crustacea (Walker et al., 2013). Among crustaceans, cladocerans, copepods and mainly ostracods are present (Liberto et al., 2012; Mazzini et al., 2014; Higuti et al., 2016; Conceição et al., 2017).

Ostracods, known as “seed shrimp” or “mussel shrimp”, are ubiquitous microcrustaceans protected by bivalve-like valve or “shell” comprising soft body and appendices (Meisch, 2000; Horne et al., 2002). They have a wide variety of trophic interactions as recyclers (Vannier et al., 1998), carnivorous (Otonelo and Romano, 2010; Prokopenko et al., 2017) and parasites (Bennett et al., 1997), but also can be hosts (Abe and Horiuchi, 2000; Gustafson et al., 2016) and most commonly preys (Harding, 1962; Lancaster and Robertson, 1995; Gutierrez et al.,

2016). This diversity place them as trophic links amongst bacteria, algae, aquatic plant, invertebrates and vertebrates (Rodríguez-Peréz and Baltanás, 2008).

Besides trophic importance and ubiquitous distribution, their relationship with aquatic plants complexity has being studied only recently (Higuti et al., 2010; Matsuda et al., 2015a). Biotic factors, as macrophytes specific characteristics, and structural features are amongst variables driving variation on ostracods diversity, but is still not clear how much of this variation is accounted for each variable (Matsuda et al., 2015a). We select ostracods as model organisms due their sensibility to structural habitat changes, since the structural variable depend on model organisms used (Tews et al., 2004).

In this study, we consider that aquatic environments are three-dimensional spaces where organisms interact and thereby they require measuring methods able to represent water-substrate-organisms interaction. Therefore, we test root volume of free-floating macrophytes as a measure of habitat structure because their root system volume, specifically, represents simultaneously an index of area and a depth gradient of three-dimensional space provided as shelter for pleustonic ostracods. This selection also allow us to untangle our results from the influence of different ecological groups on biodiversity.

We aim to assess the effects of macrophytes species characteristics and habitat structure on richness and density of ostracods assemblage in lakes of a tropical floodplain, excluding sazonal effects. In order to do so, we ask two questions: (I) Does root volume affect positively richness and density of ostracods? (II) Is there an effect of macrophytes species on ostracod richness and density?

## **Material and Methods**

### **Study area**

Cerrado area is a global biodiversity hotspot (Strassburg et al., 2017). The second largest Brazilian biome has covered originally approximately 2 millions km<sup>2</sup>, nowadays as little as 20% remains undisturbed and only 6.2% of its area is protected (Myers et al., 2000). In the last 30 years, land use intensification has been altered soil and water parameters (Hunke et al., 2015), and between 2002 and 2011 deforestation rates were 2.5 times higher than in the Amazon (1% per year) (Strassburg et al., 2017). Pandeiros River is a tributary situated on the left margin of the São Francisco and maintains flora and fauna of resident or migratory organisms (Nunes et al., 2009). Because of high endemism of species, especially birds, and for being a lotic remnant for endangered fishes, it is considered a

priority area for biodiversity conservation of the Brazilian Neotropical savanna (sensu, “cerrado biome”), in Minas Gerais state (Drummond et al. 2005). Pandeiros River has two conservation units: the Environmental Protection Area of the Pandeiros River (APA) with sustainable use and the full protection Pandeiros River Wildlife Sanctuary (REVS), which is located at Pandeiros village, Januária/Minas Gerais state, Brazil, occupying a total area of 6,102.75 ha (Pivari et al., 2013).

The floodplain (15° 40' 06'' S and 44° 38' 03'' W) is permanently inundated on wet season (Rodrigues et al., 2009), and its flooding area ranges from approximately 3,000 to 5,000 ha (Nunes et al., 2009). The average flow rate varies between 8 m<sup>3</sup>/s to 24 m<sup>3</sup>/s, during dry and wet seasons, respectively (Fonseca et al., 2008). We selected two permanently connected lakes in the Pandeiros River floodplain apart 4.57 km one from each other, Véio Juca and Torre lakes (Figure 1).

### Sampling procedures

In the entire REVS there are 101 macrophytes species (Pivari et al., 2013), amongst them we selected species of the free-floating ecological group present at these lakes: the angiosperms *Eichhornia crassipes* (Mart.) Solms and *Pistia stratiotes* (L.), and the pteridophyte *Salvinia* spp. To collect pleustonic ostracods, we sampled free-floating macrophytes *E. crassipes*, *P. stratiotes* and *Salvinia* spp. in both margins of Véio Juca and Torre lakes, in March 2016 (wet season) and July 2016 (dry season) in order to represent assemblage variation in both hydrological periods. *E. crassipes* was only found in Torre lake.

We established a total of 16 sites, 8 sites in each lake. They were distributed 100 meters distance apart from each other, 4 sampling sites along each margin (Table 1, Figure 1). Key limnological factors such as pH, dissolved oxygen concentration and water temperature were measured *in situ* at each site. Macrophytes sampling units were collected according to their occurrence over the 16 sites in the lakes. Because macrophytes species were not present at all sites 11 samples of *E. crassipes*, 9 of *P. stratiotes* and 29 samples of *Salvinia* spp., 49 samples in total (Tabela 1) were collected. Macrophytes were sampled manually and placed into plastic bucket (see Higuti et al., 2007; Campos et al., 2017).

Ostracods assemblages

Macrophytes roots were separated from the leaves, washed in the bucket to remove ostracods associated with and the residuals were filtered through a zooplankton net (68  $\mu\text{m}$  mesh size). We preserved the samples in 70% ethanol. Ostracods were sorted under a stereomicroscope and were identified to the higher taxonomic level possible using the specialized literature (Martens & Behen, 1994; Rossetti & Martens, 1998; Higuti & Martens, 2012a, b, 2014; Higuti et al., 2013).

#### Macrophytes root volume

We measured macrophytes root volume (ml) as the displacement of water observed when each root sample was submerged in a 500ml graduated beaker. Then the roots were oven-dried at 60°C for 72h and subsequently weighted to calculate density of organisms as individuals per gram of plant dry mass.

#### Data Analysis

Distinct estimators were used to express values of species richness, in order to evaluate whether observed species richness recorded in this study is representative of the total ostracod diversity for Pandeiros River floodplain. These estimators were Chao 1, Chao 2, first and second orders Jackknife, Bootstrap, ACE and ICE. Chao 1 and ACE estimators are based on species abundance and the other species richness estimators are based on species incidence. Due to the differences in abundance amongst the samples, rarefaction curves were used to compare species richness amongst macrophytes species.

A Principal Component Analysis (PCA) was performed to order sampling sites based on abiotic limnological variables (pH, water temperature and dissolved oxygen). The abiotic matrix was log transformed, except for pH, to minimize the effect of skewed values. Based on PCA results we treated data to following analysis. A nonparametric Kruskal-Wallis variance analysis was used to assess differences of macrophytes root volume amongst macrophytes species/seasons.

We used Generalized Linear Mixed Models (GLMM's), considering season as a random effect - which means do not take in account season effects to evaluate the effects of habitat structure measures (volume) and macrophytes species on the assemblage taxonomic richness and density of ostracods. We constructed one model considering richness as response (count data) variable and another to density (continuous data). Both models

included volume (count data) and macrophytes species (categorical data) as explanatory variables. We used negative binomial error distributions for richness analyses, since our count data showed significant overdispersion, and a Gaussian error distribution for density data as it fitted normality assumptions. Shapiro-Wilk analysis was applied to test normality of models residuals.

To test every possible combinations of the variables included in the global model we used the dredge function from the MUMIN package. Based on the Akaike Information Criterion of Second Order (AICc) we determined which of these explanatory variables have the biggest effect in potential in the assemblage species richness and density, where the lower AICc values (below 2.0) were assumed to indicate the best models (Burnham, Anderson & Huyvaert 2011).

Estimators and rarefaction curves were carried out using the EstimateS statistical software. Graphics were computed using the Systat Software, Inc. SigmaPlot 10.0. Paleontological Statistics Software (PAST) was used to run PCA. GLMM, Kruskal-Wallis and Shapiro-Wilk analysis were performed on R, version 3.2.5 (R Development Core Team. 2009-2017).

## Results

In the three free-floating macrophytes species collected, were recorded 5,972 ostracods individuals distributed within 31 species (Table 2), which at least four species and also two genera are possible new to science. The most abundant species were *Cytheridella ilosvayi* Daday, 1905, *Diaphanocypris meridana* (Furtos, 1936), and *Strandesia lansactohai* Higuti & Martens, 2013.

Based on average richness estimations, our sampling effort has reached 89.32% of total estimated richness. Richness estimators showed values that are similar to observed richness, the estimator which best approached observed species richness were the abundance based ACE and Chao. Hence, total numbers of expected and observed species are closely convergent (Figure 2). *Salvinia* spp. presented 27 ostracods species followed by *E. crassipes* (26) and *P. stratiotes* (25). The rarefaction curves showed that there is tendency to increase richness in all macrophytes according to increase the number of individuals (Figure 3).

For the abiotic data (Table 3) the PCA first two axis explained 97.7% of observed variance (Figure 4). Axes 1 and 2 showed no clustering of seasons or lakes. Kruskal-Wallis could not detect significant differences between both lakes abiotic data ( $p_{(pH)}=0.09$ ;  $p_{(T)}=0.91$ ;  $p_{(O_2)}=0.7$ ;  $p_{(cond)}=0.54$ ). Kruskal-Wallis test showed no

significant difference amongst the volumes of the three macrophytes species ( $p = 0.5633$ )(Figure 5), nevertheless, it changes significantly between seasons ( $p_{(P. stratiotes)} = 0.1761$ ;  $p_{(E. crassipes)} = 0.1839$ ;  $p_{(Salvinia spp.)} = 0.05395$ ).

From the global model constructed to test complexity effects on richness, the best two ones were selected on the AICc bases. The first model has explained 67.7% of the richness variation and included only root volume, it means that root volume affected richness positively, independent of macrophytes species. The second best model explained 25.8% of variation and included root volume and macrophytes. Macrophytes effects were diverse, *Salvinia* spp. showed a negative effect when compared to *P. stratiotes* and *E. crassipes* (Table 5), demonstrating that despite *Salvinia* spp presented the higher total richness of ostracods, it presented lower richness throughout samples (Figure 6).

Taking in account only the best model selected on the AICc bases for density, only macrophytes species was included and explained 74% of the variation on ostracod assemblage. *E. crassipes* surprisingly exhibited a negative influence on density in relation to *Salvinia* spp. and *P. stratiotes* (Table 6). Root volume does not affect ostracod density. Residuals Based on Shapiro-Wilk analysis both models show normality of the residuals ( $p_{\text{resid.rich}} = 0.3841$  and  $p_{\text{resid.dens}} = 0.1604$ ).

## Discussion

Human pressures have been causing biodiversity decline in freshwater ecosystems worldwide (Dudgeon et al., 2006) and our results reveal the existence of important trophic links in an endangered and high priority ecoregion. Wild life sanctuary of Pandeiros River under all threats that are inherent to Cerrado biome, as deforestation, fire, crop production and cattle grazing threats, which cause siltation and water body reduction (Nunes et al., 2009). Besides all these threats, this Conservation Unit shelter high ostracods richness, being one of the best area to biodiversity conservation in Minas Gerais state. We even found possible new species to science, all of them belong to Cyprididae, the most specious ostracod family (1,000 spp) (Martens et al., 2008). Neotropical region account for 13.75% of the known global diversity of living non-marine ostracods (Martens et al., 2008) and these data contribute to highlight the relevance of Conservation Units in Cerrado biome as effective areas to biodiversity conservation. This is the first record of pleustonic ostracods in São Francisco River basin, whose wetlands are considered endangered, regionally outstanding and with high priority for conservation at regional scale (Osion et al., 1998).



High diversity of phytophilous ostracods (associated with aquatic macrophytes) has been recorded in tropical floodplains. For example, in the floodplain of the upper Paraná River and adjacent areas, about 48 species of ostracods were recorded in several environments (over 50), including rivers, channels, isolated and connected lakes (see Higuti et al., 2010, 2013, 2017; Matsuda et al., 2015; Campos et al., 2017), 29 species were recorded in 17 lakes of Araguaia River floodplain (Pereira et al., 2017), and 25 ostracod species were recorded in 13 lakes of Amazon River floodplain (Higuti & Martens, 2016). Thus, when we compare our findings with tropical floodplains, total richness is considerably high (31 species). There are two other studies with pleustonic ostracods around the world, which recorded 20 species from one Hungarian lake (Kiss, 2007) and 11 ostracod species from 13 sampling sites at Caffarella Valley area in Rome (Mazzini et al., 2014). Several theories have been proposed to explain high species richness latitudinal gradient, they are based on climate, area, evolution, random factors or organisms metabolic rates (Wright, 1983; Rohde, 1992; Colwell and Lees, 2000; Wiens and Donoghue, 2004; Whittaker, 2006; Hawkins et al., 2007), however they treat of terrestrial patterns, it seems to be true to aquatic environments, either.

Previous studies have been showed that architectural complexity provided by macrophytes is a driver for aquatic invertebrate richness (Thomaz et al., 2008; Lucena-Moya and Duggan, 2012; St. Pierre and Kovalenko, 2014). In aquatic environments, substrates provide three-dimensional space wherein organisms interact and are essential to enhance diversity (Tokeshi, 1999), as macrophyte stands in freshwaters or sea grasses and macroalgae in shallow coastal waters (Tokeshi and Arakaki, 2012), therefore root volume might represent a proxy of this space for free-floating macrophytes and its associated pleuston.

Similar ostracods richness values registered on each macrophytes species (*Salvinia* spp., 27; *E. crassipes*, 26 and *P. stratiotes*, 25) clearly shows that species richness was not dependent of the number of individuals sampled, since we found significant effects of macrophytes species on organisms density. Similar composition was also observed, indicating no ostracods selectivity related to free-floating macrophytes species (Table 2).

Greater root volumes provided higher richness, and this relationship is macrophytes species-independent. Species richness tends to increase with increasing area in terrestrial habitats, following species area relationship (see Preston, 1960; MacArthur and Wilson, 1967; Fattorini et al., 2017), in aquatic habitats, volume may have an equivalent effect. However, fractal method applied to measure habitat structural complexity of free-floating and submerged macrophytes, a component of habitat structure, showed that it affects both invertebrate's richness and

density (Thomaz et al., 2008), although, only ostracods richness showed positive relationship with root volume. The difference between both results might be due limitations of the complexity measure tested or due intrinsic differences of growth form, at the extent that submerged macrophytes has positive influence on organisms abundance (Walker et al., 2013).

Not only root volume but also macrophytes species was included on the second best model for richness, indicating that even amongst free-floating macrophytes habitat provided there are differences. *E. crassipes* and *P. stratiotes* showed positive effect on ostracod richness as demonstrated by Higuti et al. (2010), their results showed both macrophytes with well developed root system and indicate that size of root systems can affect ostracod assemblages on pleuston. Complexity might be species dependent (Dibble et al., 2006), in spite of that, root volume measured did not show significant differences amongst macrophytes.

For density, macrophytes species was the only variable selected from our global model by the dredge function of MuMin R package, its effect is independent of structural complexity. Although, Ferreiro et al. (2014) showed that macroinvertebrates density might be explained, in part, by a positive effect of complexity on periphyton biomass. In our study, other aspects of specificity of substrate could be determinant to ostracod densities in a greater extent than habitat structure. *P. stratiotes* and *Salvinia* spp. were able to host high organism densities than *E. crassipes*, even though root volumes did not differ amongst them, thus other predictive variables should be addressed in studies to come, as features of productivity (e.g. availability of N) or biotic interactions (e.g. fish and macroinvertebrate predation). Low *E. crassipes* biomass was found in the lakes of Pandeiros floodplain, and a negative effect of this macrophyte on density is surprisingly because finding greater organisms densities associated to *E. crassipes* roots is more common (Kouame et al., 2011) than we recorded in this study. In neotropical environments *E. crassipes* is responsible to host rich pleuston communities and to be an important habitat structuring (Higuti et al., 2007), this aquatic plant usually forms dense large root mats (Mitchell, 1985) that contain muddy sediment and large amounts of autochthonous and allochthonous organic matter (Higuti et al., 2010) and this high complexity condition is known to favor high diversity of invertebrates communities (Warfe et al., 2008).

The high explanation of macrophyte on ostracod density suggests that interaction macrophyte x ostracod is a considerable driver for assemblage structuring in this habitat. Ostracods present habitat preferences shaped by food nutritional value (e.g. periphyton stucked to roots) and food supply (Mbahinzireki et al., 1991, Roca et al., 1993; Balcombe and Closs, 2016).

In this research, we have shown that habitat structure disponibility and inter specific interactions might be predictors to understand pleustonic assemblage structure. Our results indicate root volume as a practical measure of habitat structure amongst free-floating macrophytes, but it is still needed to investigate the inter specific effect of free-floating macrophytes on pleuston densities and carry out studies focusing on the other ecologic groups, because they might lead to different results, since they habit different biotopes. We call attention to the undiscovered biodiversity inhabiting Cerrado wetlands and suggest further studies at Pandeiros floodplain targeting ostracods and their specific requirements of resources and conditions provided by aquatic plants of all ecologic groups. We also reinforce the relevant role that Conservation Units play in maintaining aquatic biodiversity, especially on endangered biomes, because they are able to shelter high diversity of species.

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## Tables

**Table 1. Experimental design in the Pandeiros River floodplain.**

<b>Lake</b>	<b>Season</b>	<b>Macrophyte</b>	<b>Sample unit</b>
Véio Juca	Wet	<i>P. stratiotes</i>	3
		<i>Salvinia</i> spp.	8
	Dry	<i>P. stratiotes</i>	2
		<i>Salvinia</i> spp.	8
Torre	Wet	<i>E. crassipes</i>	7
		<i>P. stratiotes</i>	1
		<i>Salvinia</i> spp.	7
	Dry	<i>E. crassipes</i>	4
		<i>P. stratiotes</i>	3
		<i>Salvinia</i> spp.	6

**Table 2. Faunal inventory of Ostracoda associated with aquatic macrophytes in lakes of Pandeiros****River floodplain.**

	<i>E. crassipes</i>	<i>P. stratiotes</i>	<i>Salvinia</i> spp.
Superfamily Cypridoidea Baird, 1845	X	X	X
Family Candonidae Kaufmann, 1900	X	X	X
<i>Candobrasilopsis brasiliensis</i> Sars, 1901	X	X	X
<i>Candobrasilopsis elongata</i> Higuti & Martens, 2014	X	X	X
<i>Physocypria</i> spp	X		X
Family Cyprididae Baird, 1845	X	X	X
<i>Cabelodopsis hispida</i> Sars, 1901	X	X	X
<i>Chlamydotheca colombiensis</i> Roessler, 1985	X		X
<i>Chlamydotheca deformis</i> Farkas, 1958	X	X	X
<i>Chlamydotheca iheringi</i> (Sars, 1901)	X	X	X
<i>Cypretta costata</i> G.W.Müller, 1898	X	X	X
<i>Cypricercus centrura</i> (Klie, 1940)	X	X	X
" <i>Cypridopsis</i> " n. gen. n. sp.			X
<i>Diaphanocypris meridana</i> (Furtos, 1936)	X	X	X
<i>Hemicypris</i> sp. 1	X	X	
" <i>Ilyodromus</i> " n. gen. n. sp.	X	X	X
<i>Neocypridospis nana</i> (Sars, 1901)	X	X	X
<i>Potamocypris</i> sp. 1	X	X	X
<i>Stenocypris major</i> (Braid, 1859)	X	X	X
<i>Stenocypris malayica</i> Victor & Fernando, 1981	X		X
<i>Stenocypris</i> sp. 1	X	X	X
<i>Strandesia lansactohai</i> Higuti & Martens, 2013 in Higuti et al., 2013	X	X	X
<i>Strandesia mutica</i> (Sars, 1901)		X	X
<i>Strandesia tolimensis</i> Roessler, 1990			X
<i>Strandesia trispinosa</i> (Pinto & Purper, 1965)	X	X	X
<i>Strandesia</i> cf. <i>trispinosa</i>	X	X	X
<i>Strandesia</i> cf. <i>minuta</i>	X	X	
<i>Strandesia</i> n. sp. 1	X		X
<i>Strandesia</i> n. sp. 2	X	X	X
Superfamily Darwinuloidea Brady & Norman, 1889	X	X	X
Family Darwinulidae Brady & Robertson, 1885	X	X	X
<i>Alicenula serricaudata</i> (Klie, 1935)	X	X	X
<i>Darwinula stevensoni</i> (Brady and Robertson, 1870)	X	X	
<i>Penthesilenula brasiliensis</i> (Pinto and Kotzian, 1961)		X	X
<i>Vestalenula pagliolii</i> (Pinto & Kotzian, 1961)	X	X	X
Superfamily Cytheroidea Baird, 1850	X	X	X
Family Limnocytheridae Klie, 1938	X	X	X
<i>Cytheridella ilosvayi</i> Daday, 1905	X	X	X

**Table 3. Environmental characteristics in the lakes of the Pandeiros River floodplain. Water temperature (WT), hydrogen potential (pH) and dissolved oxygen (DO).**

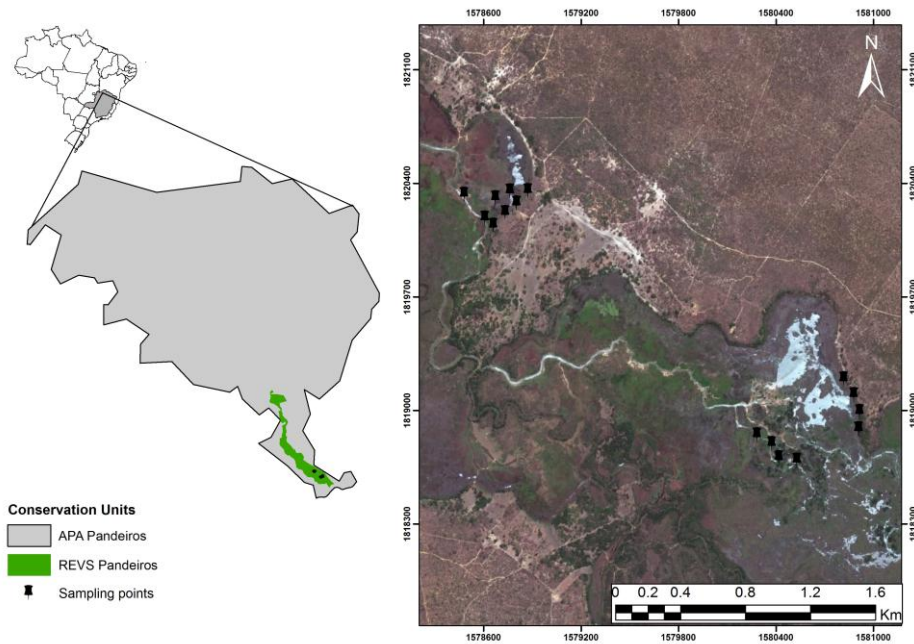
Lake	Season	Sampling sites	pH	WT (°C)	DO (mg.L <sup>-1</sup> )
Véio Juca	Wet	P1	6.31	26.2	7.99
		P2	6.19	27.3	4.94
		P3	6.28	28	3.57
		P4	6.46	26.4	2.31
		P5	6.83	30.5	9.63
		P6	6.59	27.5	6.78
		P7	6.59	29.3	4.25
		P8	6.13	27.9	4.79
	Torre	P9	6.34	28.1	1.36
		P10	6.32	28.2	6.16
		P11	6.55	28.8	6.7
		P12	6.66	28.8	5.3
		P13	6.82	30.9	3.35
		P14	6.98	32.7	7.24
		P15	6.75	30.2	5.02
		P16	6.36	30.2	2.98
Véio Juca	Dry	P1	6.7	28.8	4.16
		P2	6.23	27.4	4.24
		P3	6.6	25.2	3.2
		P4	6.7	26.4	3.37
		P5	6.57	27	5.64
		P6	6.2	24.2	3.33
		P7	6.14	23.9	5.63
		P8	5.92	24.9	3.03
	Torre	P9	6.63	21.3	5.98
		P10	6.43	22.9	6.05
		P11	6.76	21.8	5.84
		P12	6.92	22.2	4.92
		P13	6.19	23.6	4.87
		P14	6.23	23.6	5.43
		P15	6.44	26.3	1.75
		P16	6.45	22.2	4.91

**Table 4. Best Mixed Models selected on AICc bases for ostracods richness, where mcrph: macrophyte; vol: root volume.**

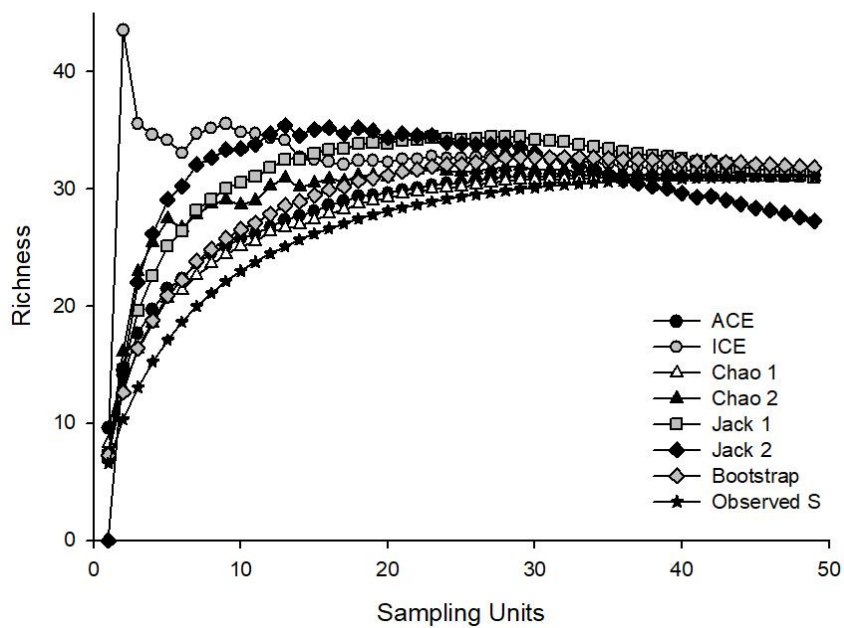
<b>Best Generalized Linear Mixed Models for ostracods richness</b>			
Model	AICc	$\Delta$ AICc	Weight
mcrph + vol	249.5	0	0.677
+			
mcrph + vol	251.4	1.96	0.258
+ +			

**Table 5. Best Mixed Models selected on AICc bases for ostracods density, where mcrph: macrophyte; vol: root volume.**

<b>Best Generalized Linear Mixed Models for ostracods density</b>			
Model	AICc	$\Delta$ AICc	Weight
mcrph + vol	153.2	0	0.740
+			

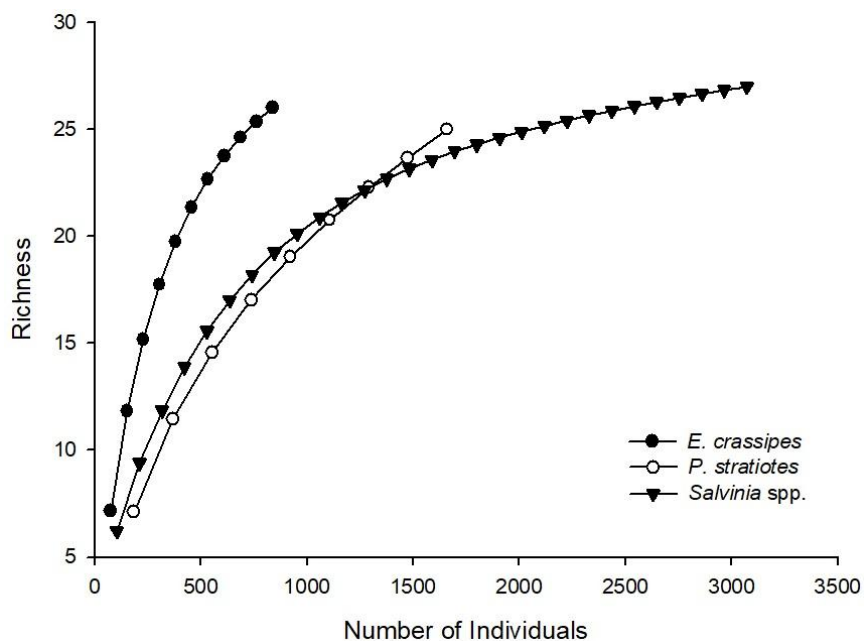


**Figure 1. Location of the lakes in the Pandeiros River floodplain.**

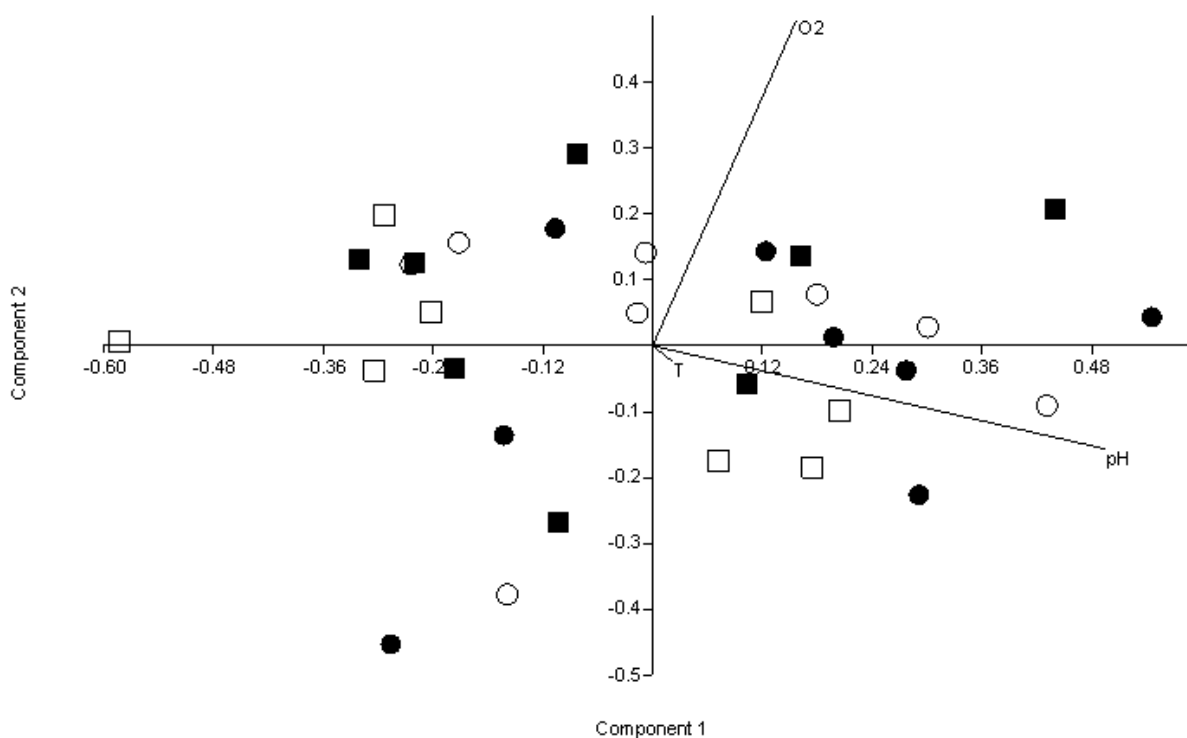


**Figure 2. Species accumulation curve of ostracods resulting from different species richness estimators of the Pandeiros River floodplain.**

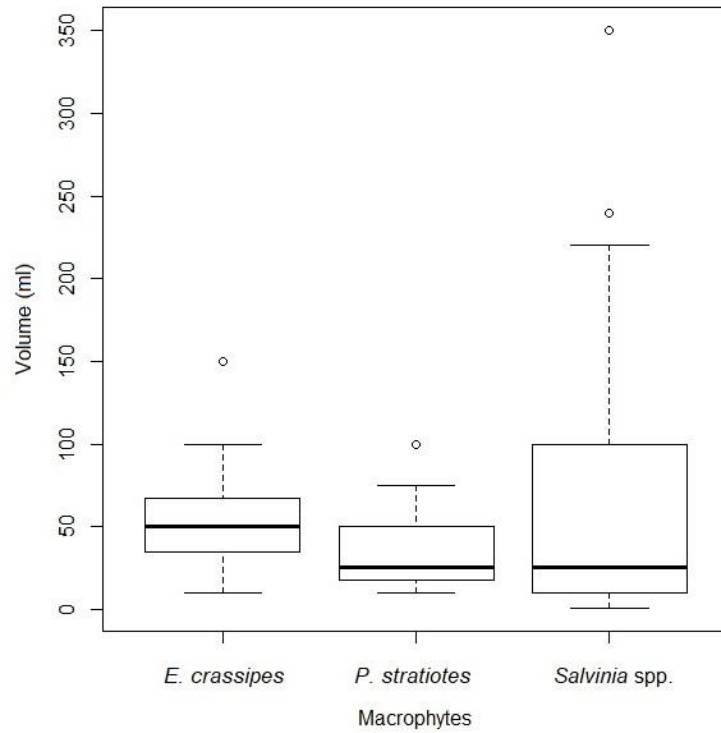




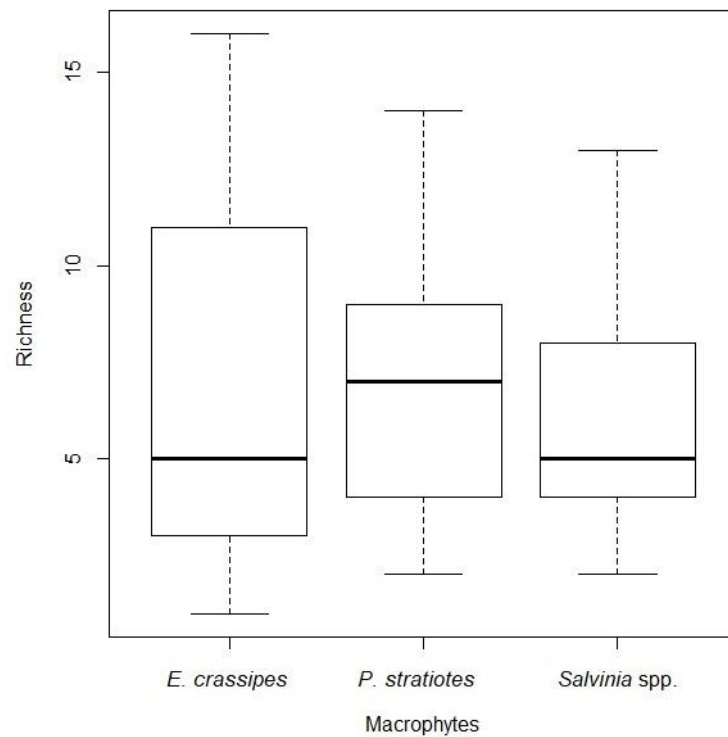
**Figure 3. Rarefaction curves of ostracod assemblage associated with macrophytes in lakes of Pandeiros River floodplain.**



**Figure 4. Results of Principal Components Analysis (PCA) of abiotic data. Filled symbol: wet season, unfilled symbol: dry season, squares: Veio Juca lake, circles: Torre lake.**



**Figure 5. Boxplot of macrophytes root volume data.**

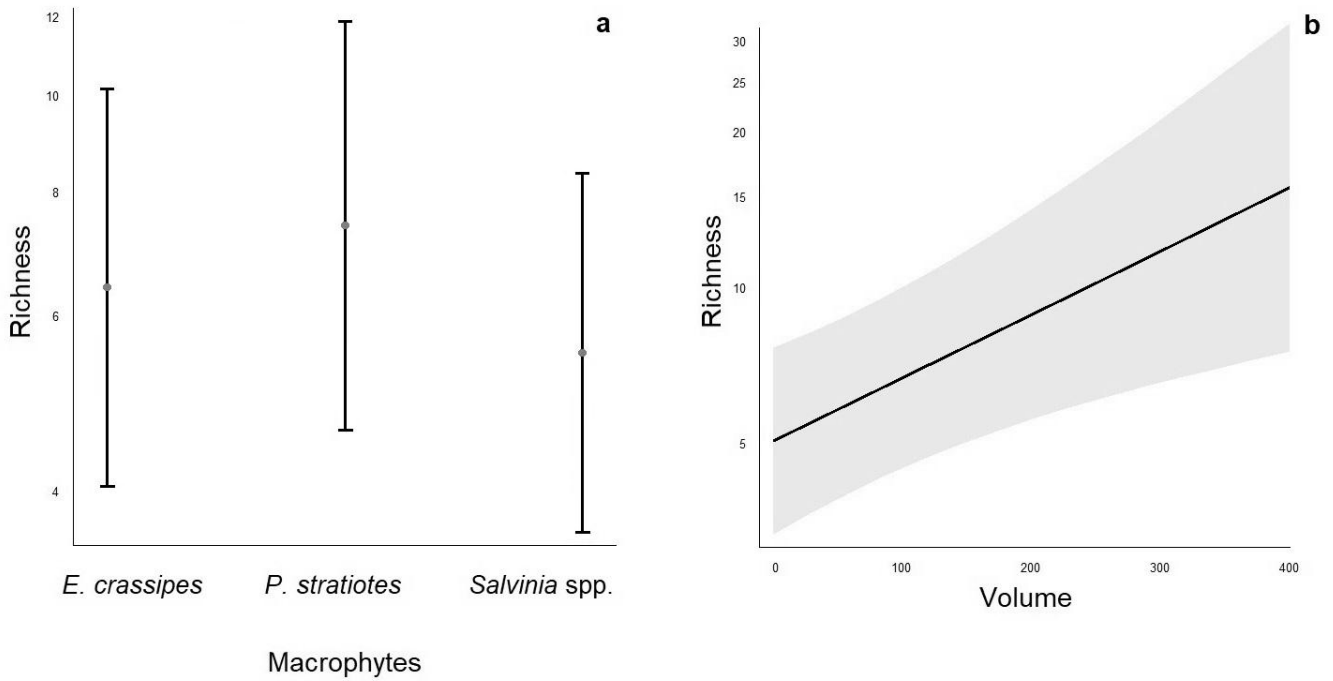


**Figure 6. Boxplot of pleustonic ostracod richness along macrophytes species samples.**

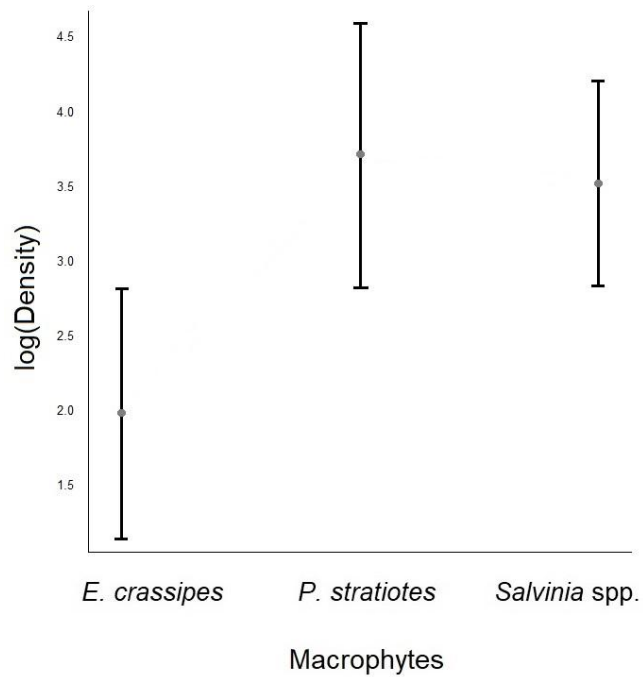
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## Supplementary material



**Figure S1. Effects of (A) macrophyte identity and (B) root volume on ostracods richness.**



**Figure S2. Effects of macrophyte identity on ostracods density.**