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**Ostracoda (Crustacea) biodiversity: a taxonomic and functional approach**

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2023

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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para a obtenção do título de Doutor em Ecologia e Limnologia.  
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# **Biodiversidade de Ostracoda (Crustacea): uma abordagem taxonômica e funcional**

## **RESUMO**

O impacto de ações humanas, tais como desflorestamento, poluição e introdução de espécies vêm causando efeitos negativos no meio ambiente. A extinção de espécies e mudanças nas áreas de distribuição das espécies, são alguns desses problemas, principalmente no meio aquático onde é observada uma elevada riqueza de espécies comparada a sua extensão. Portanto, o conhecimento acerca da biodiversidade é de extrema importância para a conservação dos ecossistemas. Os ostracodes são um exemplo de grupo que está sendo afetado por mudanças climáticas e, no entanto, são pouco estudados. Realizou-se uma revisão de Cyprettinae s.l. de regiões circumtropicais, usando a taxonomia morfológica. A primeira etapa na revisão de Cyprettinae s.l. foi redescrever a espécie tipo de *Cypretta* Vávra, 1985 a partir do material tipo original, e discutir a morfologia e diagnóstico do gênero. Após, descreveu-se duas novas espécies de *Pseudocypretta* Klie, 1932 da África do Sul e do Brasil; e um novo gênero e quatro novas espécies do Brasil. Com a redescritção da espécie tipo de *Cypretta* nós determinamos os caracteres diagnósticos da espécie e do gênero. Analisando as características das novas espécies de *Pseudocypretta* moveu-se esse gênero da subfamília Cyprettinae para a subfamília Cypridopsinae, e expandiu-se a distribuição desse gênero para as regiões Afrotropical e Neotropical. O novo gênero e novas espécies foram registradas nas quatro principais planícies de inundação brasileiras, e foram classificados na subfamília Cyprettinae. Ambos *Cypretta* e *Pseudocypretta* e novo gênero são caracterizados pela presença de septas em suas valvas, no entanto variam em nível de desenvolvimento (completas ou incompletas) e disposição ao longo das margens das valvas. Usando um banco de dados de ostracoda e modelos de nicho ecológico, avaliaram-se os efeitos das mudanças climáticas na diversidade funcional de ostracoda no Cone Sul da América do Sul, em cenários moderados-otimista e pessimista dos anos de 2050 e 2080. É projetado que a diversidade funcional de ostracoda sofrerá uma gradual redução em 2050 e 2080 em ambos os cenários de emissão de carbono, mas principalmente no cenário pessimista de 2080. Os resultados destacam a importância de avaliar a diversidade funcional para a conservação de ostracodes, desde que algumas áreas reportadas e possuem baixa riqueza taxonômica suportam intermediários a elevados valores de diversidade funcional.

**Palavras-chave:** Morfologia comparativa. Não-marinho. Cyprettinae. Diversidade funcional. Mudanças climáticas.

## Ostracoda (Crustacea) biodiversity: a taxonomic and functional approach

### ABSTRACT

The impacts of human actions, as deforestation, pollution and introduction of species have been causing negative effects on the environment. The extinction of species, and change of distribution range, are some of them, especially in the aquatic environment where it is observed a high richness compared to its extension. Thus, the knowledge about biodiversity is of extreme importance for conservation of ecosystems. Ostracods are one example of a group constantly affected but ill studied. It was performed a revision of Cypridinae s.l. from circumtropical regions, using morphological taxonomy. The first step in the revision of Cypridinae s.l. was to redescribe the type species of *Cypridina* Vávra, 1895 from the original type material, discuss the morphology and diagnosis of the genus. After that it was described two new species from *Pseudocypridina* Klie, 1932 genus from South Africa and Brazil; and described a new genus and four new species from Brazil. With the redescription of the type species of the *Cypridina* the diagnosis characters for the species and genus were delimited. Analyzing the features of the new species of *Pseudocypridina* this genus was moved from the Cypridinae subfamily to the Cypridopsinae subfamily, and expanded the distribution of this genus to the Afrotropical and Neotropical regions. The new genus and new species were recorded from the four main floodplains of Brazil and were designated to the Cypridinae subfamily. Both *Cypridina*, *Pseudocypridina* and new genus are characterized by presence of septas in its valves, however they vary in level of development (complete or incomplete) and disposition along the margins of the valves. Using a database of ostracod distribution and ecological niche modelling (ENMs) the effects of climate change were evaluated to the functional diversity of ostracods in the South Cone of South America, in the moderate-optimistic and pessimistic scenarios of 2050 and 2080. It is projected that the ostracod functional diversity will suffer a gradual reduction in 2050 and 2080 on both scenarios of carbon emission, but especially in the pessimistic scenario of 2080. The results highlight the importance of evaluating the functional diversity for ostracod conservation, since several areas marked to have low taxonomic richness supported intermediate and high levels of functional diversity.

**Keywords:** Comparative morphology. Non-marine. Cypridinae. Functional diversity. Climate change.

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## 1 GENERAL INTRODUCTION

Freshwater environments sustain a rich biodiversity of species, and due its dendritic nature they hold a diversity of habitats (COLLEN et al. 2014). These environments cover ca. 2% of the Earth surface, however they concentrate ca. 12% of all species known so far (BALIAN et al., 2008; DUDGEON 2019; ALBERT et al. 2020). The impacts of human activities such as deforestation, pollution, and introduction of species on ecosystems (and communities) have intensified and few policy actions have been taken in order to protect them (ALBERT et al. 2020; SUNDAR et al. 2020). Analysis of global trends shows that freshwater ecosystems are being depleted even faster than terrestrial ecosystems, and consequently leading a greater biodiversity loss (ALBERT et al. 2020).

In freshwater ecosystems the invertebrates play important ecosystems services (DUDGEON et al. 2006; SCHMERA et al. 2017). The invertebrates feed on algae, and organic matter, and also provide food for higher trophic levels (i.e. contributing to nutrient cycle) (SCHMERA et al. 2017). Thus, the changes in diversity and distribution could trigger negative impacts on freshwater ecosystems. Amongst the invertebrates ostracod (bivalved crustaceans), is a group with high diversity (MEISCH et al. 2019), but generally are overlooked in ecological assessments, and recent study showed that these organisms are subject to have its suitable areas reduced in face of climate change (CONCEIÇÃO et al. 2023).

Ostracods are small crustaceans, and its body size range between 0.3 and 5mm (HIGUTI & MARTENS 2020). The carapace of ostracods covers the entire body. These organisms inhabit marine and non-marine aquatic environments, and semi (terrestrial) environments (HIGUTI & MARTENS 2020). In freshwater aquatic environments they can be found in the pleuston of aquatic plants, and on the sediment (HIGUTI et al. 2009). Ostracods have different types of reproduction, for example, some species are fully sexual, other fully asexual and other species have mixed reproduction (SCHMIT et al. 2013). They feed on algae and debris and are usually predated by other invertebrates and fishes (Smith et al. 2015). Studies have been reported the ostracods as the main food item, especially for

fishes, including species with socio-economic importance (BATISTA & PETRERE JR. 2003; HANH 2004; PEREIRA et al. 2011).

The last global assessment reported more than 2300 non-marine ostracod species (MEISCH et al. 2019). However, the south hemisphere is ill known about its biodiversity, when compared to the north hemisphere. In groups where the fauna is well known globally, it is observed that in the south could hold even a higher diversity (BALIAN et al. 2008). In fact, in the South America, several works have described new species of ostracod in the last decades (see PINTO et al. 2005; DIAZ & LOPRETTO 2011; HIGUTI & MARTENS 2014; FERREIRA et al. 2020).

Here we used the ostracods to exemplify the little knowledge about biodiversity. One example is the Cyprittinae subfamily that recently was reported new species in different zoogeographical regions. This subfamily is composed by two genera, *Cypritta* (Vávra 1895) and *Pseudocypritta* Klie 1932, which their type species were described in a superficial way. *Cypritta tenuicauda* (Vávra 1895), the type species of *Cypritta*, has few morphological characters in the original description (overall drawings of the carapace and caudal ramus). In *Pseudocypritta maculata* Klie 1932, the type species of *Pseudocypritta*, the description provide only outlines of carapace and valves, and few appendages. These incomplete descriptions can cause misinterpretations when assigning new species to the genus. Thus, we revisit the subfamily Cyprittinae s.l, (re)-describing morphologically new species and a new genus of ostracod.

The morphology of ostracods can provide important characters for taxonomical and ecological purposes since species response to the environment depends on the combination of several traits. For example, body size (related to energy requirements, see MERCKX et al. 2018); presence of natatory setae in the antenna (related to the local dispersal of individuals, CAMPOS et al. 2018); carapace shape (related to the habitat use, MARMONIER et al. 1994). The functional diversity approach considers the role of species in the environment (MCGILL et al. 2006) and has become a routine for some groups (plants and insects), however, for ostracods this approach is still scarce. To fill this gap we evaluate the functional diversity of ostracods under climate change scenarios considering

how human development may evolve and affect the emission of greenhouse gases and air pollutants (van Vuuren et al. 2011).

Thus, this thesis comprises five manuscripts. In the first manuscript we redescribe the type species of the *Cypretta* genus, from the type material. In addition, we describe two new species from *Pseudocypretta* genus from South Africa (second manuscript) and Brazil (third manuscript), discussing the morphology of *Pseudocypretta*, and expanding the distribution range of this genus to the Neotropical and Afrotropical regions. In the fourth manuscript we describe a new genus and four new species of Cyprētinae subfamily from Brazil. Finally, in the fifth manuscript, using a database of ostracods distribution from South America and trait categories, we modelled the effects of climate change on the ostracod functional diversity in the South Cone of South America, considering moderate-optimistic and pessimistic scenarios of carbon emissions, in 2050 and 2080.

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## **2 REDESCRIPTION OF THE TYPE SPECIES OF THE GENUS *CYPRETTA* (OSTRACODA, CRUSTACEA), WITH NOTES ON THE TAXONOMY OF THE GENUS**

### **ABSTRACT**

With 53 formally described species, the genus *Cypretta* is one of the most common freshwater ostracod genera in the world. It has a mainly circumtropical distribution. The type species, *Cypretta tenuicauda* (Vávra, 1895), was described from Zanzibar (Africa) in a superficial way. Therefore, the morphology and identity of this species and of the genus remained problematic until today.

Here, we redescribe *Cypretta tenuicauda* from the original type material and discuss the morphology of the species and the diagnosis of the genus. The species is characterized by the presence of anterior marginal septa in both valves, the sub-triangular carapace shape in lateral view, the right valve overlapping the left valve, the generally wide carapace and the presence of a serrated posteroventral inner list in the right valve. In addition, both  $\alpha$  and  $\beta$  setae on the mandibular palp are long and thin, claws  $G_a$  and  $G_p$  on the caudal ramus are elongated and seta-like, while the caudal ramus itself is equally slender. The caudal ramus attachment is reduced to a simple branch.

The present redescription of the type species will assist in creating order in what is now a taxonomically confused genus.

**Keywords:** circumtropical, comparative morphology, Cyprettinae, non-marine.

## 2.1 Introduction

After transferring the genus *Pseudocypretta* Klie, 1932 from the subfamily Cyprettinae to Cypridopsinae (Savatenalinton *et al.* 2022), the genus *Cypretta* Vávra, 1895 is thus far the only genus of the subfamily Cyprettinae (Meisch *et al.* 2019; Savatenalinton *et al.* 2022). The type species of this genus, *Cypretta tenuicauda* (Vávra, 1895) G.W. Müller, 1898, was described by Vávra (1895) in the genus *Cypridopsis* and the subgenus *Cypretta*. Later, *Cypretta* was elevated to the rank of genus by G.W. Müller (1898). The original description is quite rudimentary, as is usual for many descriptions of that age. The only illustrations consist of (very small) drawings of the carapace in dorsal and lateral views (in which the characteristic marginal septa of the valves are not visible), and of one of the caudal rami.

The genus *Cypretta* presently holds 53 species worldwide and is considered a mainly circumtropical genus, owing to the high diversity of species in the tropical and subtropical regions (Meisch *et al.* 2019). However, as the type species remains ill-known, there is no detailed information on the characteristics of this genus, making it difficult to morphologically delimit the species in this genus.

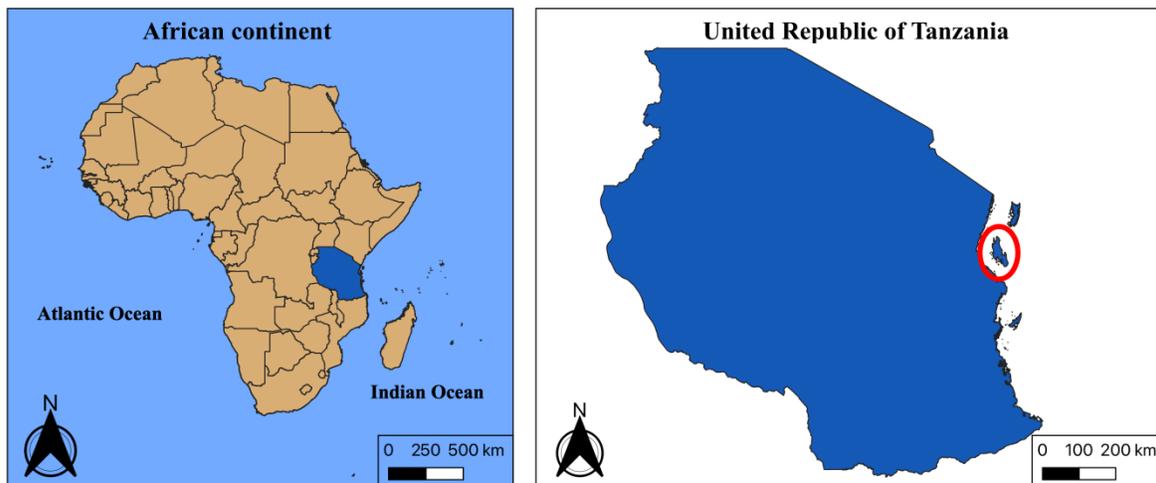
Here, we redescribe the type species of *Cypretta*, *C. tenuicauda*, on the original type material of Vávra (1895) and discuss the morphology of the species and of the genus. The present redescription of the typespecies will contribute to a better understanding of the taxonomic position of the species presently allocated to this genus.

## 2.2 Material and Methods

### 2.2.1 Material

We used the type material of *Cypretta tenuicauda*. The sampling was performed by Dr. Stuhlmann in Zanzibar in 1888 (Fig. 1). The material was sent to the National History Museum of Hamburg (Germany), whose director at that time, Prof. Dr. Kraepelin, delegated Dr Vávra, then affiliated to the Zoological Museum in Prague, to

describe the ostracod species. The material of *C. tenuicauda*, provided to us by the National History Museum of Hamburg, was conserved in alcohol, with 22 female adults and six juveniles in good condition (Vávra 1895).



**FIGURE 1.** Locality where *Cypretta tenuicauda* (Vávra, 1895) was first recorded. Red circle indicates the island of Zanzibar, in the United Republic of Tanzania.

### 2.2.2 Preparation and illustration of soft parts and valves

The specimens were dissected using dissection needles under a binocular stereomicroscope. The valves were removed from the rest of the body by opening the carapace with the dissection needles. Soft parts were dissected in a drop of glycerine on a glass slide. The dissected appendages were covered with a coverslip and sealed with several layers of transparent nail polish. Valves were stored dry in a micropaleontological slide. Drawings of the appendages were made using a camera lucida attached to an optical (transmission) microscope (Leica DM2500 M). Carapaces and valves were illustrated and measured using Scanning Electron Microscopy (SEM; Fei Qanta 200 ESEM) in different views (internal, lateral, dorsal, ventral and details). The preparation and illustration of soft parts and valves was performed in the Royal Belgian Institute of Natural Sciences, Brussels, Belgium (RBINS).

The lectotype and paralectotypes remain stored in the collections of the Zoological Museum of the University of Hamburg, Germany.

### 2.2.3 Abbreviations used in text and figures

A1, antennula; A2, antenna; Cms, central muscle scars; Cp, carapace; CpD, carapace dorsal view; CpLL, carapace left lateral view; CpRL, carapace right lateral view; CpV, carapace ventral view; CR, caudal ramus; H, height; il, inner list; L, length; LV, left valve; LVi, left valve inner view; MdCox, mandibular coxa; MdPalp, mandibular palp; Mx1, maxillula; RO, Rome organ; RV, right valve; RVi, right valve inner view; sl, selvage; T1, first thoracopod; T2, second thoracopod; T3, third thoracopod; W, width; WO, Wouters organ.

The description of the length of the setae (changed to short, medium length and long), as well as the terminology of the thoracopods follows Broodbakker & Danielopol (1982), that of the second antenna follows the revised model proposed by Martens (1987), and of the second and third thoracopods the nomenclature of Meisch (2000). Higher taxonomy of the Ostracoda follows Horne *et al.* (2002) and Meisch *et al.* (2019), with the exception of the authorship of the Cyprettinae (see below).

## 2.3 Results

Class	Ostracoda Latreille, 1802
Subclass	Podocopa Sars, 1866
Order	Podocopida Sars, 1866
Suborder	Cypridocopina Baird, 1845
Superfamily	Cypridoidea Baird, 1845
Family	Cyprididae Baird, 1845
Subfamily	Cyprettinae Hartmann, 1963
Genus	<i>Cypretta</i> Vávra, 1895

**Type species:** *Cypretta tenuicauda* (Vávra, 1895) G.W. Müller 1898

**Other species:** see Meisch *et al.* (2019) and Savatnalinton (2018).

**Diagnosis** (based on the type species)

CpL1 triangular, with rounded dorsal margin in lateral view, Cp wide in dorsal and ventral views, RV anteriorly, ventrally and posteriorly overlapping the LV, most pronounced at the anterior side; both valves with a large outer list. RV with large il, creating a *Strandesia*-like groove along anterior, ventral and posterior margins. This il crenulated in postero-ventral corner. LV with a weak anterior il and a strongly inwardly displaced sl along the entire posterior margin. Both valves with strong anterior marginal septa; posterior septa and lunules possible. Calcified inner lamella in both valves wide anteriorly and narrow posteriorly. Cms consisting of five small, semi-circular scars, two anteriorly, three posteriorly and two anteroventral mandibular scars.

A1 with five distal-most segments relatively short. A2 with large and narrow claws and natatory setae long, reaching tips of end claws. MdCox relatively slender, MdPalp with  $\alpha$  seta of medium length and narrow,  $\beta$  seta of medium length, stout, and hirsute,  $\gamma$  seta short and slender, also hirsute. Mx1 with second palp segment ca. twice as long as basal width; third endite with two *Zahnborsten* distally set with few spines; two side-ways directed bristles of medium length and hirsute. T1 with two short a setae and one short d seta. T2 with setae  $d_1$  and  $d_2$  short; f seta long. T3 a cleaning leg with pincer-shaped distal part; seta  $d_p$  longer than  $d_1$  and  $d_2$ , setae e, f and  $h_1$ -3 short. CR with slender and short ramus, claws  $G_a$  (longest) and  $G_p$  seta-like. CR attachment a single rod.

**Remarks:**

Several species presently allocated to the genus *Cypretta* do not fully fit this diagnosis. Some characters will be discussed below, but a fuller appraisal of the taxonomy of the genus will be presented elsewhere.

### 2.3.1 *Cypretta tenuicauda* (Vávra, 1895)

(Figs 2–6)

- 1895 *Cypridopsis* (*Cypretta*) *tenuicauda* Vávra: p. 7, figs 2 (1–3) (Zanzibar).  
 1936 *Cypretta tenuicauda* Vávra, in : Lowndes, p. 28 (Lake George, Uganda).  
 1937 non *Cypretta tenuicauda* Vávra, fide : Tressler, p. 203 (Bali, Indonesia)

#### 2.3.1.1 *Type locality*

Pool near a leak in a water supply pipeline of the city of Zanzibar, United Republic of Tanzania (Fig. 1). Collection made on 16 June 1888 by Dr Stuhlmann.

Approximate coordinates: -6.16394S, 39.19793E. (from <https://www.geodatos.net/en/coordinates/tanzania/zanzibar->)

#### 2.3.1.2 *Type Material*

*Lectotype* (here designated):

1 ♀, with soft parts dissected in glycerine in a sealed slide and with valves stored dry in a micropaleontological slide (ZMH K-62047).

*Paralectotypes*:

2 ♀ with soft parts dissected as the lectotype (ZMH K-62049 and ZMH K-62048), 3 ♀ carapaces stored dry in micropaleontological slides after use for SEM illustrations (ZMH K-62050), 17 female adults and six juveniles in the original tube in EtOH (ZMH K-19017).

#### 2.3.1.3 *Diagnosis*

CpL1 triangular, L/H ratio ca. 1.4, with rounded dorsal margin and greatest height in the middle, Cp wide in dorsal and ventral views, greatest width situated slightly behind the middle; RV anteriorly, ventrally and posteriorly overlapping the LV, most pronounced at the anterior side. RV with large il crenulated in the postero-ventral corner. LV with a weak anterior il and a strongly inwardly displaced sl along the entire posterior margin, this sl

smooth, not crenulated. Both valves with strong anterior marginal septa and ca. 15 lunules (chambers); LV also with weakly developed

posterior septa and lunules. Calcified inner lamella in both valves wide anteriorly and narrow posteriorly. External valve surface weakly pitted.

A1 with a short RO. A2 with a small g seta, reaching halfway the accompanying seta of aesthetasc y3. MdPalp with a medium  $\alpha$  seta on the first segment and a hirsute  $\beta$  seta of medium length on the second segment. Mx1 with two distally serrated *Zahnborsten* on the third endite and two sideways directed bristles of medium length, second palp segment *ca* twice as long as basal width. T1 with setae b and c absent. T2 with seta d<sub>1</sub> and d<sub>2</sub> both short; penultimate segment divided. T3 with a short medial f seta. CR with claws G<sub>a</sub> and G<sub>p</sub> seta-like; seta S<sub>p</sub> short; seta S<sub>a</sub> absent. CR attachment a single rod.

#### 2.3.1.4 Measurements

See Table 1

**TABLE 1.** Measurements (in  $\mu\text{m}$ ) of specimens of *Cypretta tenuicauda*.

<b>View</b>	<b>Slide #</b>	<b>L</b>	<b>H</b>	<b>W</b>
<b>LVi</b>	ZMH K-62049	612.5	414.2	
<b>RVi</b>	ZMH K-62049	629.2	425.0	
<b>LVi</b>	ZMH K-62047	612.5	400.0	
<b>RVi</b>	ZMH K-62047	623.3	430.8	
<b>CpLi</b>	ZMH K-62050	623.4	430.0	
<b>CpV</b>	ZMH K-62050	621.7		475.0
<b>CpD</b>	ZMH K-62050	630.0		479.2

#### 2.3.1.5 Description of female

LVi (Figs 2A, C) with calcified inner lamella wide along anterior margin, and narrow along ventral, and posterior margins; weak il running along full anterior and half of

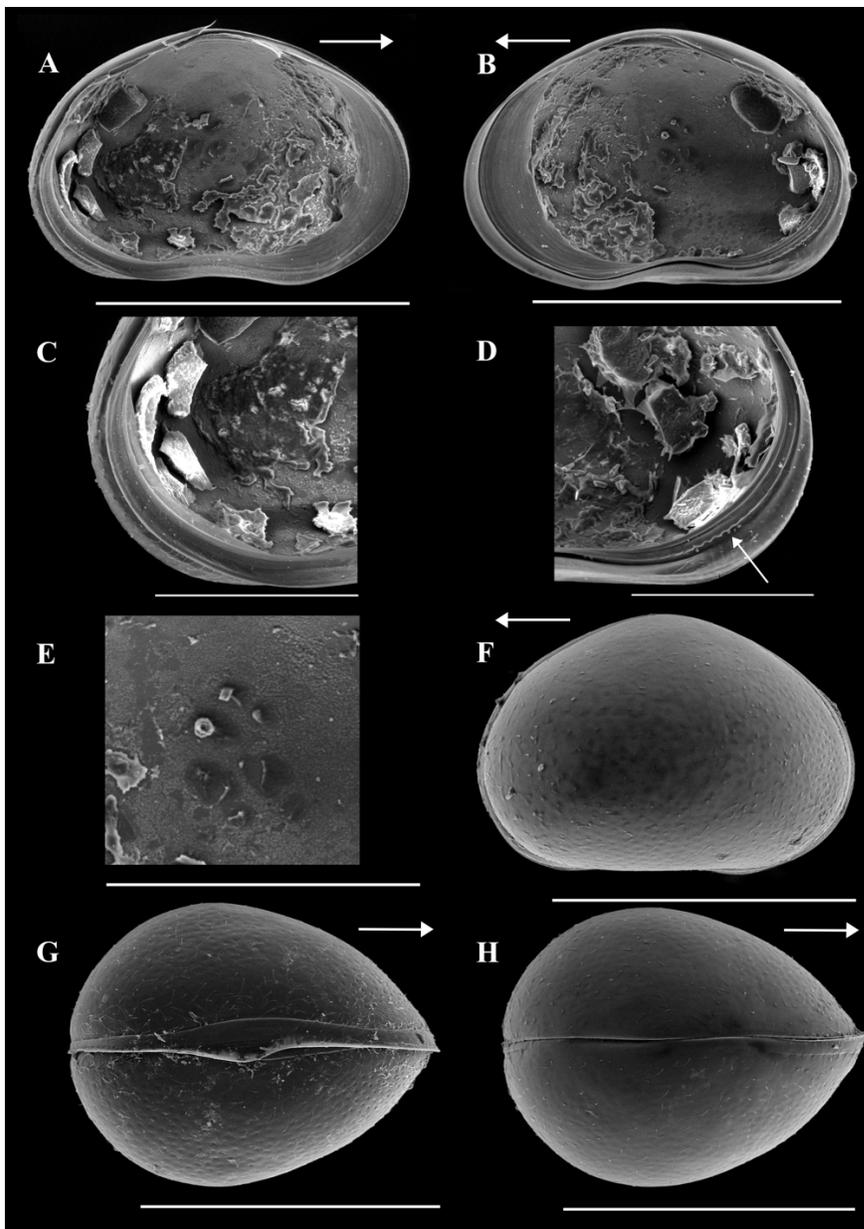
ventral margins; posteriorly with a strongly inwardly displaced sl, the latter smooth (Fig. 2C); with ca. 15 septa along anterior margin and weakly developed ones along postero-ventral margin (Fig. 3A).

RVi (Figs 2B, D) with calcified inner lamella wide along anterior margin and narrow along ventral and posterior margins; strong il forming a *Strandesia*-like groove along anterior, ventral, and posterior margins, this il crenulated in posteroventral part; with ca. 15 septa along the anterior margin (Fig. 3B).

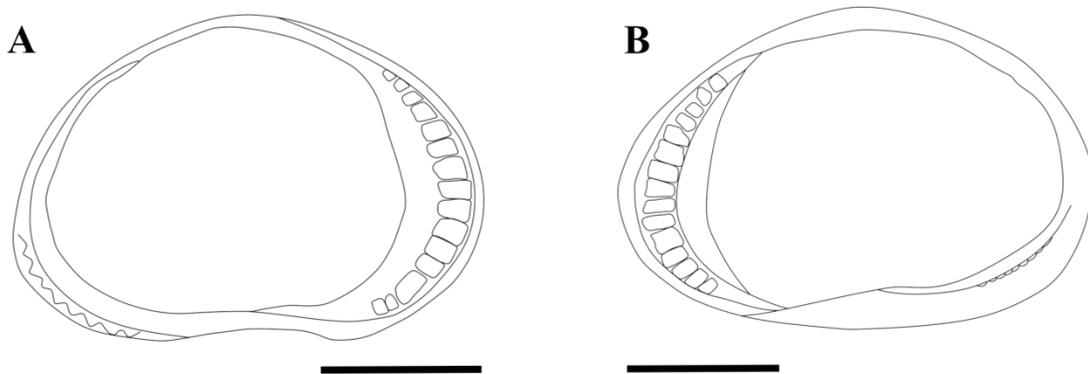
Cms (Fig. 2E) forming a simplified paw-print pattern, consisting of an anterior pair of small scars, posteriorly with a row of three small scars, below with two relatively small mandibular scars.

CpL1 (Fig. 2F) with a subtriangular shape, L/H ratio ca. 1.4, with rounded dorsal margin, greatest height situated in the middle; RV overlapping LV along antero-dorsal, anterior, postero ventral and posterior margins.

CpV and CpD (Figs 2G, H) with oval shape, posterior margin broadly rounded, anterior margin pointed; RV overlapping LV mostly anteriorly, but also ventrally (with large expansion in the central part) and posteriorly; greatest width situated slightly behind the middle; large outer lists on both valves.



**FIGURE 2.** Carapace and valves of *Cypretta tenuicauda* (Vávra, 1895) from Zanzibar. **A.** LVi (ZMH K-62049). **B.** RVi (ZMH K-62049). **C.** LVi, detail of posteroventral margin, showing the sockets of denticules (ZMH K-62049). **D.** RVi, detail of posteroventral margin, showing denticulated inner list (marked by white arrow) (ZMH K-62047). **E.** detail showing the adductor muscles scars (ZMH K-62049). **F.** CpLl (ZMH K-62050). **G.** CpV (ZMH K-62050). **H.** CpD (ZMH K-62050). Scale bars: A–B; F–H = 500  $\mu\text{m}$ ; C–E = 200  $\mu\text{m}$ . A, B, F–H, arrows indicate anterior direction.



**FIGURE 3.** Valves, inner views of *Cypretta tenuicauda* (Vávra, 1895) from Zanzibar, showing the septa. **A.** LVi (ZMH K-62048). **B.** RVi (ZMH K-62048). Scale bars: 200  $\mu\text{m}$ .

A1 (Fig. 4A) composed of seven segments. First segment with two large ventral and one dorsal seta of medium length, the latter ca. 1/3 the length of ventral setae; WO not seen. Second segment with one short dorsal seta (ca. 3/4 the length of the third segment) and a small lateral RO. Third segment with one short ventro-apical seta and one short dorsal seta, almost reaching end of fifth segment. Fourth segment with one short ventral seta (almost reaching end of the sixth segment) and two long dorsal setae. Fifth segment with one short ventral seta and two long dorsal setae. Sixth segment with four long apical setae. Terminal segment with long aesthetasc  $y_a$ , one short seta, half-length of  $y_a$ , two long seta (one slightly longer than  $y_a$ ).

A2 (Figs. 4B, C) composed of six segments: two-segmented protopodite, one-segmented exopodite and three endopodal segments. Protopodite carrying three short and one medium-sized ventral setae, the latter ca. 3 times the length of the short ones. Exopodite consisting of a small plate with two unequally short setae and one seta of medium length. First endopodal segment with ventral aesthetasc Y, ca. half the length of the segment, five long hirsute natatory setae reaching the tips of end claws and one accompanying seta of medium length almost reaching the tip of second endopodal segment. Second endopodal segment with a group of four ventral t setae ( $t_1$ - $t_4$ ) of unequal length (one short, three of medium length), two medio-dorsal setae of unequal, medium

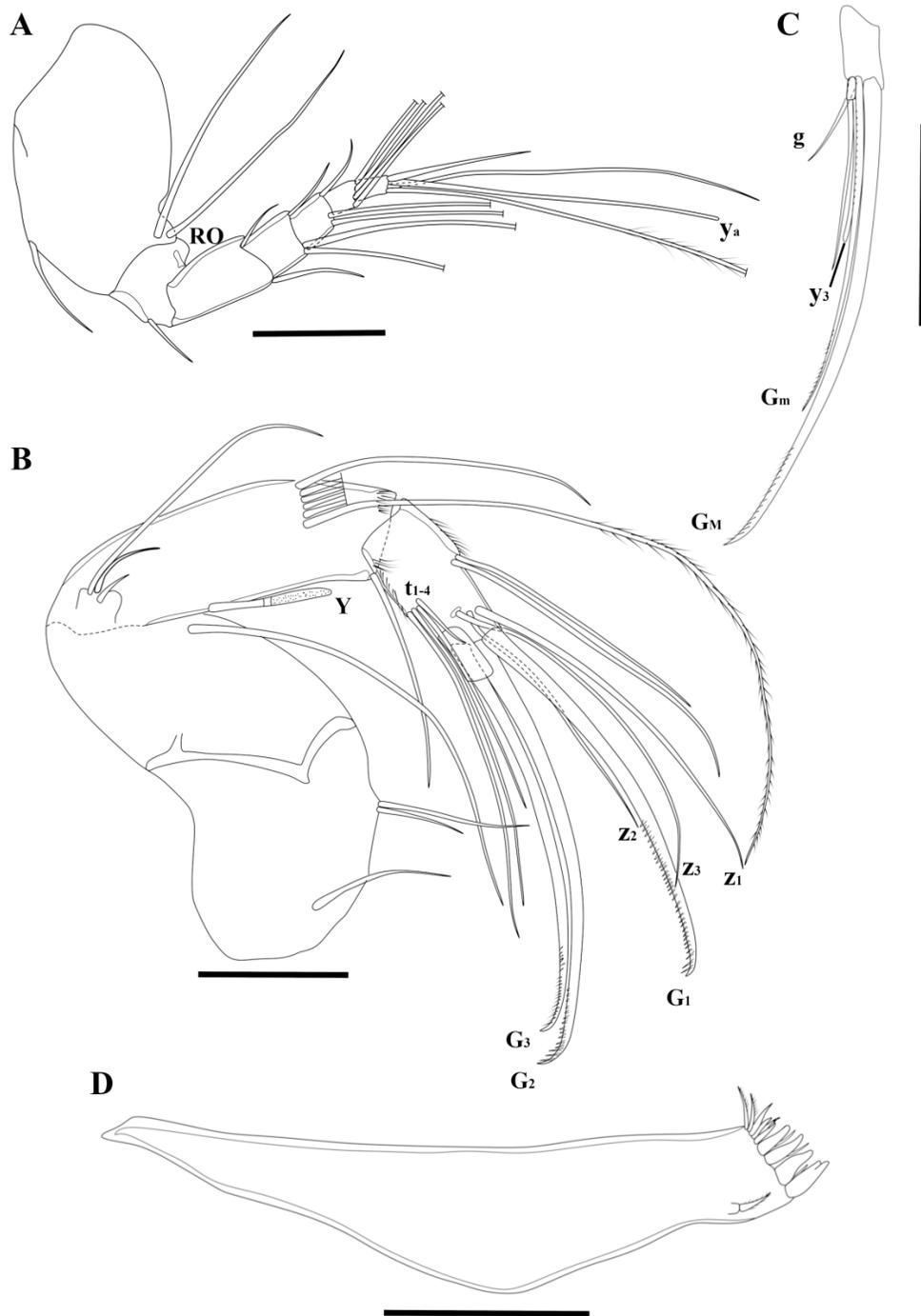
length, three medium-sized z setae ( $z_1$ -  $z_3$ ); and three claws ( $G_1$ -  $G_3$ ,  $G_2$  the longest,  $G_1$  the shortest). Terminal segment (Fig. 4C) with claw  $G_M$  of medium length and claw  $G_m$  short, both weakly serrated; accompanying seta of aesthetasc  $y_3$  slightly longer than aesthetasc, and g seta reaching halfway of  $y_3$  accompanying seta.

MdCox (Fig. 4D) rather slender, with ca. seven apical teeth intercalated with setae; one short sub-apical hirsute seta on the dorsal margin, not reaching the base of the teeth.

MdPalp (Figs. 5A, B) four segmented. First segment ventrally with plumose setae  $S_1$  and  $S_2$ , one long smooth seta and  $\alpha$  seta of medium length (ca. half the length of the long smooth seta). Second segment ventrally with stout and hirsute  $\beta$  seta of medium length and three apical smooth setae of medium length; dorsally with a group of three subequal setae (one long, one of medium length and one short and hirsute). Third segment dorsally with a group of four setae of medium length; apically with three setae of medium length, two short setae and short hirsute seta  $\gamma$ . Last segment (Fig. 5B) with three claws and three setae.

Mx1 (Fig. 5C) composed of two-segmented palp, three endites and large respiratory plate (the latter not illustrated). Basal segment of palp with a group of four short subapical setae and one (sub-) apical seta of medium length, one lateral seta of medium length and one isolated subapical seta. Terminal segment elongated (L ca. twice W), apically with three claws and two setae. Third endite with two large bristles strongly serrated; and one medio-lateral seta (ca. slightly shorter than the endite). First endite with two distally hirsute sideways directed bristles of medium length.

T1 (Fig. 5D) composed of an exopodite (not illustrated), endopodite and protopodite. Endopodite (Fig. 5E) a palp with a conical shape, apically with three unequally long hirsute setae. Protopodite with two equally short a setae and 10 apical and three subapical, unequally short, setae; short seta d present; seta b missing.



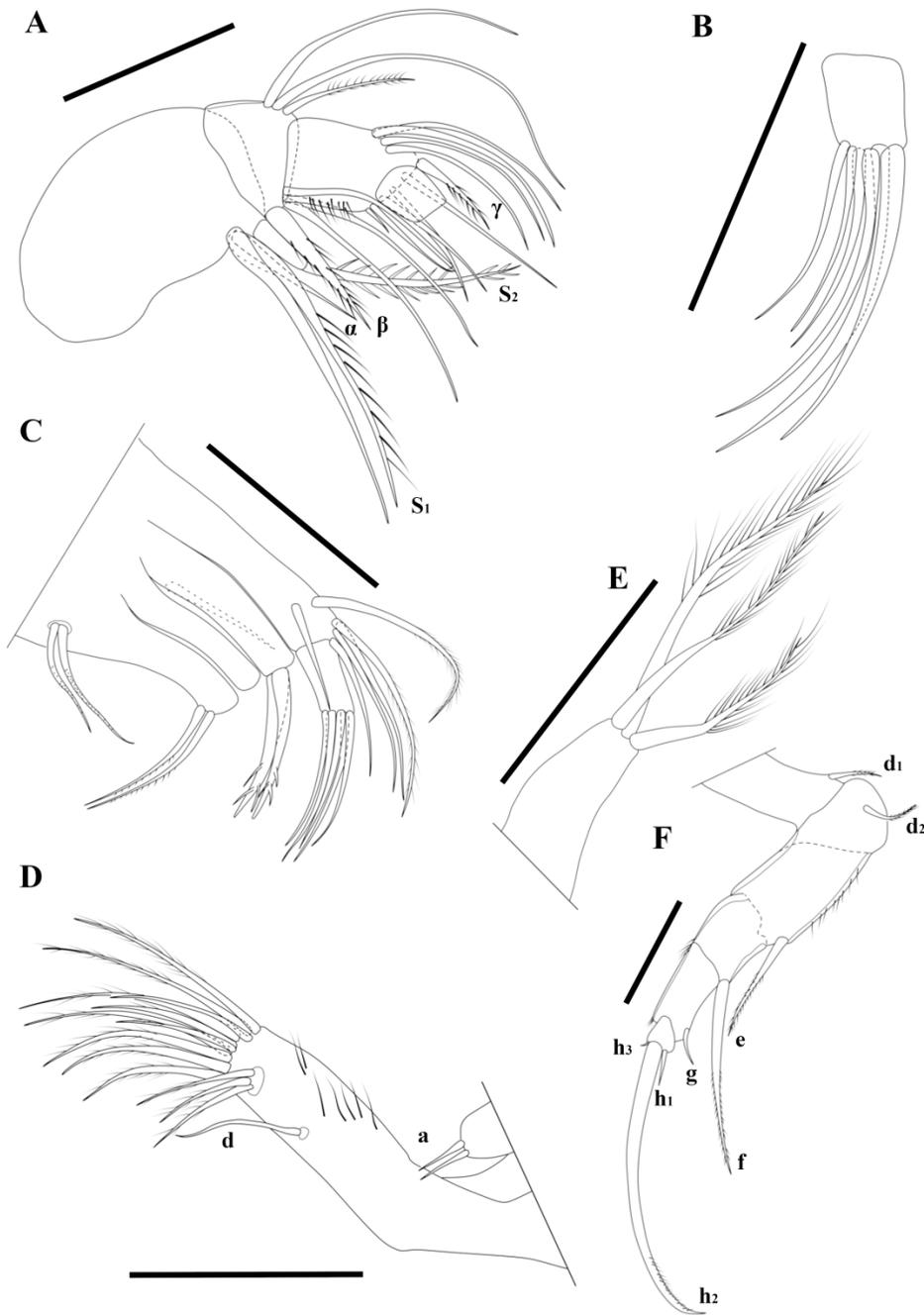
**FIGURE 4.** Append ages of *Cypretta tenuicauda* (Vávra, 1895) from Zanzibar. **A.** A1 (ZMH K-62047). **B.** A2 (ZMH K-62047). **C.** A2 terminal segment (ZMH K-62047). **D.** MdCox (ZMH K-62047). Scale bars: A–D = 50  $\mu$ m.

T2 (Fig. 5F) composed of five segments. First segment with short and hirsute seta  $d_1$ . Second segment with equally short hirsute seta  $d_2$ . Third segment with apical; hirsute and seta  $e$  of medium length, reaching beyond the middle of fourth segment. Fourth segment medially with hirsute and unusually long seta  $f$ , reaching halfway end claw  $h_2$ , apically with small  $g$  seta, reaching the end of terminal segment. Terminal segment apically with short ventral seta  $h_1$ , long but slender and strongly curved claw  $h_2$ , and short dorsal seta  $h_3$ , ca.  $\frac{1}{4}$  the length of seta  $h_1$ .

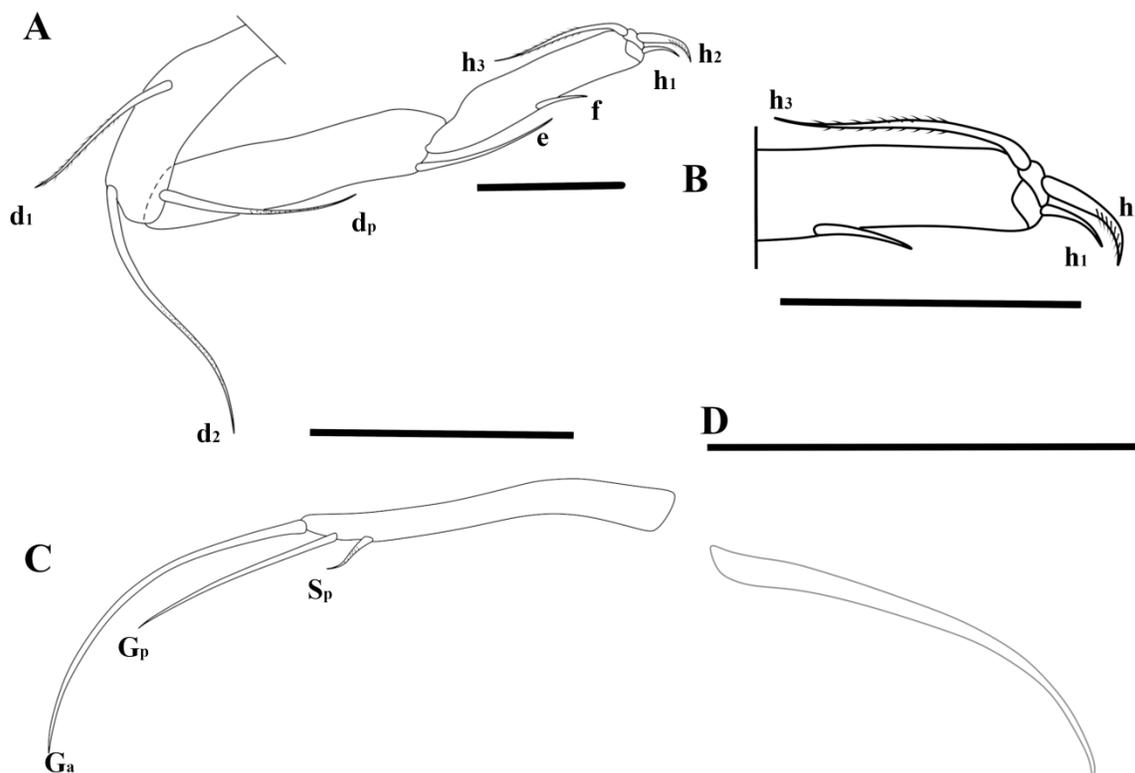
T3 (Figs. 6A, B) composed of four segments. First segment elongated, with hirsute seta  $d_1$  of medium length and two slightly longer and hirsute setae  $d_2$  and  $d_p$ , the latter the longest. Second segment elongated, with short hirsute, apical  $e$  seta reaching halfway third segment. Third segment with short  $f$  seta, ca.  $\frac{1}{4}$  the length of the segment. Terminal segment (Fig. 6B) with pincer-like shape, with short seta  $h_1$ , slightly longer seta  $h_2$ , and short and hirsute reflexed seta  $h_3$ .

CR (Fig. 6C) composed of short and weakly sclerified ramus, long apical claw  $G_a$ , shorter subapical claw  $G_p$ , both slender and seta-like, and short subapical hirsute seta  $S_p$ . Seta  $S_a$  fully absent. CR-attachment (Fig. 6D) reduced to a short and curved rod.

Male unknown.



**FIGURE 5.** Appendages of *Cypretta tenuicauda* (Vávra, 1895) from Zanzibar. **A.** MdPalp (ZMH K-62049). **B.** MdPalp last segment (ZMH K-62049). **C.** Mx1 (ZMH K-62048). **D.** T1 (ZMH K-62048). **E.** T1 endopodite (ZMH K-62048). **F.** T2 (ZMH K-62049). Scale bars: 50  $\mu\text{m}$ .



**FIGURE 6.** Appendages of *Cypretta tenuicauda* (Vávra, 1895) from Zanzibar. **A.** T3 (ZMH K-62047). **B.** T3 pincer (ZMH K-62047). **C.** CR (ZMH K-62049). **D.** CR attachment (ZMH K-62047). Scale bars: 50  $\mu$ m.

## 2.4 Discussion

### 2.4.1 The identity of *Cypretta tenuicauda*

*Cypretta tenuicauda* was described by Vávra (1895) from a pool formed by a leak in the water supply of Zanzibar city. As was pointed out above, the original description was restricted to the point that no trustworthy identification could be made based upon it. This is why the record of this species by Tressler (1937) from Indonesia, is highly unlikely. As Tressler (*loc.cit.*) also provided no illustrations or description of his specimens (“A few females were collected in Akemhoela Lake, Groot Sanghir in June 1932”), this record must be rejected. Some material from Singapore identified as *C. tenuicauda* from the collections

of the Natural History Museum (London, UK) was screened by us and most likely also belongs to a different species.

Lowndes (1936) reported the species from Lake George in Uganda, which is zoogeographically much closer to Zanzibar. We have been able to investigate some of his specimens stored in the collections of the Natural History Museum (London, UK) and can confirm that, most likely, this record is correct.

*Cypretta tenuicauda* is therefore seemingly restricted in its distribution to East Africa.

#### 2.4.2 *Cypretta tenuicauda* as the type species of the genus *Cypretta*

Vávra (1895) described *Cypretta*, albeit as a subgenus of *Cypridopsis*, based on the species *Cypridopsis (Cypretta) tenuicauda* from Zanzibar (Africa). G.W. Müller (1898) raised *Cypretta* to the rank of genus and described his species *Cypretta costata* G.W. Müller, 1898 from Madagascar (Africa). Vávra (1895) had described the marginal septa in *C. tenuicauda* as “grobe, weit voneinander stehende Porenkanäle” (large, widely spaced pore canals), while Sars (1889) described them as “distant transverse grooves” in his species *Cypridopsis globulus* Sars, 1889 from Australia. G.W. Müller (*loc.cit.*) was the first to recognize these structures as transversal septa between the outer lamella and the inner calcified lamella of the valves (see also Sohn & Kornicker 1973), which he observed and described from his species *C. costata*. He then postulated that both his species *C. costata* and *C. globulus* belonged in the same genus as *C. tenuicauda*, namely *Cypretta*. In a footnote, he also referred to the illustrations of *Cypridopsis minna* King, 1855 by Sars (1894) from New Zealand. Most likely independently from G.W. Müller (1898), Daday (1900) in his monograph on freshwater ostracods from Hungary, referred *Cypris viridis* Thomson, 1879 from Australia, *Cypridopsis minna* King, 1855 from New Zealand, *Cypridopsis turgida* Sars, 1896 from Australia and *Pionocypris assimilis* Sars, 1895 from South Africa to the same genus as Vávra’s *C. tenuicauda*, namely *Cypretta*. Whereas G.W. Müller (1898) was correct that *C. costata* and *C. globulus* both belong to *Cypretta s.l.*, Daday (1900) misinterpreted at least the position of *P. assimilis*, which is now considered

to belong to the genus *Cypridopsis* Brady, 1867 and is seen as a synonym of *Cypridopsis vidua* (O.F. Müller, 1776) (see Meisch *et al.* 2019).

Thanks to the interpretation (and illustration) of the characteristic marginal septa in both valves by G.W. Müller, and immediately afterwards the recognition of this character by Daday (1900), the genus *Cypretta* became recognizable, even though *C. tenuicauda* as a species remained unidentifiable.

#### 2.4.3 The morphology of *Cypretta* species

The present paper redescribes the type material of *Cypretta tenuicauda* and provides a new diagnosis for the genus based on the newly designated lectotype. This diagnosis is rather exclusive and not all species presently assigned to *Cypretta* comply with it. Further contributions to a taxonomic revision of the genus *Cypretta* will be presented elsewhere (Ferreira *et al.* in prep.), but some remarks on deviating morphologies are already discussed here. Several of these have already been foreshadowed by Savatnalinton (2018). The characters that seem universally accepted as typical of *Cypretta* are the globular shape of the carapace in dorsal/ ventral views, the mainly triangular shape in lateral view, the presence of anterior marginal septa in both valves and the slender CR and CR attachment.

##### *Marginal septa in both valves*

The presence of septa along the anterior margins of both valves is a characteristic of *Cyprettas.s.* However, such marginal septa also occur in other genera, namely *Pseudocypretta* Klie, 1932, *Zonocypretta* De Deckker, 1981, *Paracypretta* Sars, 1924, *Bradycypris* Sars, 1924, *Batucypretta* Victor & Fernando, 1981a, and were recently also reported from the cypridopsine genus *Cyprettadopsis* Savatnalinton, 2020 (Savatnalinton 2020). They also occur in the completely unrelated genus *Stenocypris* Sars, 1889. However, in *Cypretta* these septa are largely restricted to the anterior margins where they are fully developed, while in other genera they can also occur along the posterior margins (e.g. *Cyprettadopsis*) or can be incomplete in the LV (e.g. *Pseudocypretta*) (Ferreira *et al.* 2022) or occur in one valve only (e.g. *Bradycypris* Sars, 1924).

### *Valve overlap*

In the majority of the species presently allocated to *Cypretta*, the RV overlaps the LV, at least anteriorly. Three species described by Furtos (1934, 1936) from North America have a reversed valve overlap: *Cypretta brevisaepta* Furtos, 1934 (including the subspecies *Cypretta brevisaepta sarta* Furtos, 1936), *Cypretta bilicis* Furtos, 1936 and *Cypretta nigra* Furtos, 1936, all from Florida. Most likely, all (or most) of these nominal species are actually the same species. *Cypretta campechensis* Cohuo-Durán *et al.*, 2013 from Yucatán (Mexico) almost certainly also belongs to this species group, judging from the valve overlap, the shape of the valves and especially of the hemipenis, which is structurally very different from the one described by Farkas (1959) for *Cypretta schubarti* Farkas, 1959 from Brazil. These taxa with the LV>RV overlap (here, we assume that the LV>RV is also ventral based on the anterior overlap of the original descriptions) belong to a different genus, possibly *Neocypridella* Hartmann & Puri, 1974 or a related genus (Savatenalinton *et al.* 2022).

A reversed valve overlap is often considered a good reason to divide species over different genera, for example *Cyprinotus* Brady, 1886 and *Heterocypris* Claus, 1892 versus *Hemicypris* Sars, 1903 in the Cyprinotinae and the two types in *Cyclocypris*, where Krstić (1995) created the subgenus *Laevicypris* for the species *Cyclocypris laevis* (O.F. Müller, 1776) which has a LV > RV overlap which is reversed from other species in this genus (but see Matzke-Karasz *et al.* 2004). Also in this case, we propose that only species with the RV overlapping the LV anteriorly and ventrally can be lodged in the genus *Cypretta*.

### *Valve shape and ornamentation*

Whereas most species presently assigned to *Cypretta* are (sub-) triangular, others have different valve shapes in lateral and dorsal views. For example, *Cypretta triangulata* Savatenalinton, 2018 has valves which are quite elongated in lateral view, whereas in dorsal view, the greatest width of the Cp in this species is situated strongly posteriorly from the middle. Other, very highly arched species also exist, such as *Cypretta minna* (King,

1855) from New Zealand, presumably also found and redescribed by Sars (1924) from South Africa.

Most species of *Cypretta* have moderate valve ornamentation (pitted, reticulated, sparsely set with setae), but some have more pronounced ornamentation, with spine-like bristles, highly dense setae, shallow or deep pits, tubercles and sockets such as *Cypretta vivacis* Würdig & Pinto, 1993 from Brazil, *Cypretta spinosa* Cohuo-Durán *et al.*, 2013 from Yucatán and *Cypretta aculeata* Savatnalinton, 2018 from Thailand and others (Furtos 1936; Victor & Fernando 1981b; Macário-Gonzalez *et al.* 2018 and others).

In general, such special shapes and ornamentation are accepted as intra-generic variability, see for example the sometimes large and stout spines occurring on *Sarscypriopsis aculeata* (Costa, 1847) where as most other species in this genus have smooth valve surfaces. However, if such aberrant morphologies are accompanied by other deviating characters, the taxonomic position still has to be reconsidered (see below).

#### *Internal valve anatomy*

Most *Cypretta* species conform to the body plan in which the RV has a conspicuous circum-marginal il which is crenulated in the postero-ventral part, and where the LV has a widely inwardly displaced posterior selvage. In some species, the crenulation on the postero-ventral part of the il in the RV appears to be missing, but in some cases it might simply have been missed in the illustrations. In other cases, it might be indicative of generic misplacement, such as in *C. campechensis* where the valve reversal also caused the LV to have the circum-marginal il with the postero-ventral crenulation.

#### *Chaetotaxy*

De Deckker (1979) listed a number of soft part features that appear to be consistent in higher level taxonomy. Some of these have more recently been confirmed, for example the importance of the Triebel's loop in the CR attachment for the Cypricercinae (Savatnalinton & Martens 2009, 2010). Other recently established relevant characters are, for example, the presence of the c-seta in the Eucypridinae (Martens 1989), the relative

lengths and presence/ absence of the setae  $d_1$  and  $d_2$  on the T2 of the Cypridinae (Martens 1990, 1992) and others. Here, we assess a number of characters that might be of relevance to establish the taxonomy of *Cypretta* s.s. based on the morphology in *C. tenuicauda*.

MdPalp:  $\alpha$ ,  $\beta$  and  $\gamma$  setae are long and relatively slender, the latter two are also hirsute while the  $\alpha$  seta is smooth. However, some species have a short  $\alpha$  and long  $\beta$  seta, as in *C. triangulata* and *C. spinosa*, while others have both short  $\alpha$  and  $\beta$  setae, such as in *Cypretta maya* Cohuo-Durán *et al.*, 2013: (see Cohuo-Dúran *et al.* 2013; Savatentalinton 2018; Macário-Gonzalez *et al.* 2018). As the shape and structure of these setae are generally considered to be constant within a genus, the position of these species must be revised.

T1: apart from the 10 apical and three subapical setae, also the d seta and a pair of a setae are present. However, in *C. campechensis*, the b seta is reported, whereas the d seta is assumed missing, while in *C. maya*, an additional c seta next to the d seta is illustrated (Cohuo-Dúran *et al.* 2013). This, together with the aberrant  $\alpha$  and  $\beta$  setae on the MdPalp, the unusually long setae  $d_1$  and  $d_2$  on T2 and the reversed valve overlap cause serious doubts on the allocation of at least *C. campechensis* to *Cypretta*.

T2: setae  $d_1$  and  $d_2$  are equally short, however the lengths can differ in some other species. For example, in *C. aculeata* and *Cypretta elongata* Macario-González *et al.*, 2018 the setae are longer, and both are equal in length, while in *C. triangulata*, seta  $d_1$  is half the length of seta  $d_2$  (Savatentalinton 2018). The long seta f on the fourth segment in *C. tenuicauda* (reaching halfway claw  $h_2$  on the fifth segment) is unusual, and occurs also in several other species, e.g. *C. aculeata* and *C. triangulata* and even in *C. campechensis*, *C. spinosa* and *C. maya* (Cohuo-Dúran *et al.* 2013). This should be considered a generic character, as pointed out by Savatentalinton (2018), maybe even a supra-generic one.

T3: the pattern of setae  $d_1$ ,  $d_2$  and  $d_p$  appears to be quite stable, with seta  $d_p$  being the longest. However, here the seta f can have variable length amongst species.

CR: according to Sohn & Kornicker (1973) the CR of *Cypretta* consists of two thin terminal claws and two thinner setae. *Cypretta tenuicauda* has a long claw  $G_a$ , a shorter subapical claw  $G_p$ , and a very short sub-apical seta  $S_p$  with seta  $S_a$  being fully absent. As this is a fragile structure, CR setae and claws can be broken during the dissections. However, seta  $S_a$  was not observed in all dissections we performed. Vávra (1895) also did not illustrate seta  $S_a$ . In other species presently allocated to the genus, different lengths of claws and setae have been reported, and in some of them, e.g., *Cypretta aculeata* Savatentalinton, 2018 and *Cypretta elongata* Macario-González *et al.*, 2018, a short seta  $S_a$  is reported. We refer to Savatentalinton (2018) for a more complete discussion on the variability of the chaetotaxy of the CR in *Cypretta* species.

Generally, ostracod soft part morphology amongst species of the same genus is rather conservative, while the carapace and valves show more plasticity and as such are more useful to delimit species (e.g. *Strandesia*) (see Ferreira *et al.* 2020). However, in *Cypretta* both carapace / valve and soft part morphology are highly variable between species. This can mean that the species are in deed simply more variable in this genus than in many others, but it can also be indicative of the fact that the genus is not monophyletic and that several species are allocated to it which do not belong to the same phylogenetic clade.

## 2.5 Conclusion

The present redescription of *Cypretta tenuicauda* highlights some of the main features characterizing the genus *Cypretta*s.s., but also allows to appraise if all 53 species presently assigned to it actually do belong there. This is the first contribution towards a full revision of the genus *Cypretta*, which is a step-wise process and for which other contributions are forthcoming (Ferreira *et al.* in prep.). A useful addition to this process will be a molecular approach, for which Savatentalinton *et al.* (2022) have made a first step.

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### 3 ON A NEW SPECIES OF *PSEUDOCYPRETTA* KLIE, 1932 (CRUSTACEA, OSTRACODA) FROM THE NEOTROPICAL REGION, WITH A DISCUSSION ON THE POSITION OF THE GENUS

#### ABSTRACT

*Pseudocyprretta amor* sp. nov. (named after the carapace spots resembling the word “Love”) is here described from all-female populations from the four major floodplains in Brazil. The new species is compared to the other two known species in the genus, *P. maculata* Klie (1932), the type species, and *P. lineata* Ma and Yu (2020). As the latter two species are thus far found exclusively in South East Asia and China, respectively, the present extension of the area of the genus to South America is considerable. Several morphological characters in this genus and species are discussed, especially the presence of marginal septa in the valves, the candonid type T3 with 3rd and 4th segment separated (candonid type) and the caudal ramus which is reduced to a flagellum (cypridopsine type) or is fully absent. Based on the combination of these and other characters, the genus *Pseudocyprretta* is here transferred from the Cyprettinae to the tribe Cyprettadopsini in the Cypridopsinae, as it is closely related to the genus *Cyprettadopsis* Savatnalinton, 2020. The presence of the candonid type T3 in Cyprididae and Notodromadidae, where the T3 generally has a pincer-shaped tip by the fusion of the 3rd and the 4th segment, is further discussed.

**Keywords:** Areal extension, Neotropics, Comparative morphology, Taxonomy, Circumtropical.

### 3.1 Background

The subfamily Cyprerinae Hartmann, 1963 comprises two genera: *Cyprer* Vávra, 1895 and *Pseudocyprer* Klie (1932) (Meisch et al. 2019). *Cyprer* presently holds 52 species worldwide and is considered a circumtropical genus, owing to the high diversity of species in the tropical and subtropical regions (Cohuo-Durán et al. 2013; Meisch et al. 2019). *Pseudocyprer*, on the other hand, comprises only two species: *Pseudocyprer maculata* Klie (1932) and *Pseudocyprer lineata* Ma and Yu (2020), both from the Oriental region, inhabiting rice fields, pools, lakes and streams (Klie 1932; Savatnalinton 2018; Ma and Yu 2020). The Neotropical region has a high aquatic biodiversity (Balian et al. 2008). However, the ostracod fauna is still largely overlooked in most biological surveys. Recent sampling efforts discovered several new taxa from various environments, such as semi-terrestrial and river-floodplain systems (e.g., Pinto et al. 2003 2005; Higuti et al. 2013; Ferreira et al. 2019 2020; Almeida et al. 2021). While sampling in the four major Brazilian floodplains during the SISBIOTA project (2010–2014), several species of the genus *Cyprer* were collected (Pereira et al. 2017 and unpublished data), and one was referred to as *Cyprer* sp.3 in Higuti et al. (2010) because of its overall morphological similarity to other *Cyprer* species, namely the general globular shape of the carapace and the presence of marginal septa along the (anterior) valve margins of both valves. However, after more detailed morphological analyses of the valves and appendages, the species was identified as belonging to the genus *Pseudocyprer*. *Pseudocyprer* species also have a globular carapace with a length between 0.4 and 0.5 mm, with marginal septa along anterior and posterior margins in both valves and thus, with these characters, resemble species of the

genus *Cypretta*. But in the two species of *Pseudocypretta*, the left valve overlaps the right valve anteriorly (inverse in *Cypretta*), while they also have a third thoracopod marked by a separate fourth segment (fused into a pincer-shaped organ in *Cypretta*, and in most subfamilies in the Cyprididae) and a caudal ramus which is reduced to a flagellum-like structure, the so called cypridopsine type (the CR is more developed with ramus, claws and setae in *Cypretta*). Here, we describe a new species of *Pseudocypretta*, and discuss the morphology of the species in this genus. We also discuss the distribution of this species and genus respectively across Neotropical Brazilian floodplains and globally.

## 3.2 Material and Methods

### 3.2.1 Study area

The study was conducted in the main four Brazilian floodplains: Amazon (3°02'–3°34'S, 60°50'–60°10'W), Araguaia (12°50'–13°20'S, 50°40'–50°30'W), Pantanal (18°50'–19°30'S, 57°40'–57°00'W), and Upper Paraná (22°40'–24°00'S, 54°20'–53°00'W) (Fig. 1). Together, these floodplains comprise a large area of Brazil and hold different types of habitats such as rivers, channels, backwaters, and open and closed lakes (Agostinho et al. 2004; Barros et al. 2004; Harris et al. 2005; Latrubesse et al. 2009). The Amazon is located in the circumtropical belt of evergreen tropical rainforest at the equator region. It is distributed along several countries in South America, such as Brazil, Bolivia, Colombia, Ecuador, French Guiana, Peru, Suriname and Guyana (Junk et al. 2011). The Amazon River floodplain is in the north region of Brazil, and is composed by a mosaic of extensive rivers, such as the Negro River and Solimões River, as well as the Amazon River itself and

lakes. Over the last several decades, a great area of tropical forest has been destroyed by logging and replaced by herbaceous vegetation (Junk et al. 2011). All samples were taken from sites near Manaus city. The Araguaia River floodplain is situated in the central region of Brazil, in the transition zone between the Amazon Forest and the tropical savanna forest. Its surroundings have experienced extensive landscape changes owing to expanding farming and logging activities, making it a priority area for conservation (Latrubesse et al. 2009). Samples were collected in the central stretch of the Araguaia River. The Pantanal, which contains the Paraguay River, is part of one of the largest wetlands in the world, distributed across the Brazilian states of Mato Grosso and Mato Grosso do Sul, as well as parts of Bolivia and Paraguay. This region holds high biodiversity and is equally a priority in conservation strategies (Barros et al. 2004). Samples were collected in the Pantanal of the Mato Grosso do Sul State. Finally, the Upper Paraná River floodplain is located in a region with one of the highest population densities of Brazil, thus suffering from human impacts such as tourism, overfishing, pollution and extensive flow regulation by damming (Agostinho et al. 2004). It comprises three conservation units: “Área de Proteção Ambiental das Ilhas e Várzeas do Rio Paraná”, the “Parque Nacional de Ilha Grande”, and the “Parque Estadual do Ivinheima”, which were created to preserve the high biodiversity found in this region (Agostinho et al. 2004). Samples were collected in the stretch between Porto Primavera and Itaipu dams.

### 3.2.2 Sampling

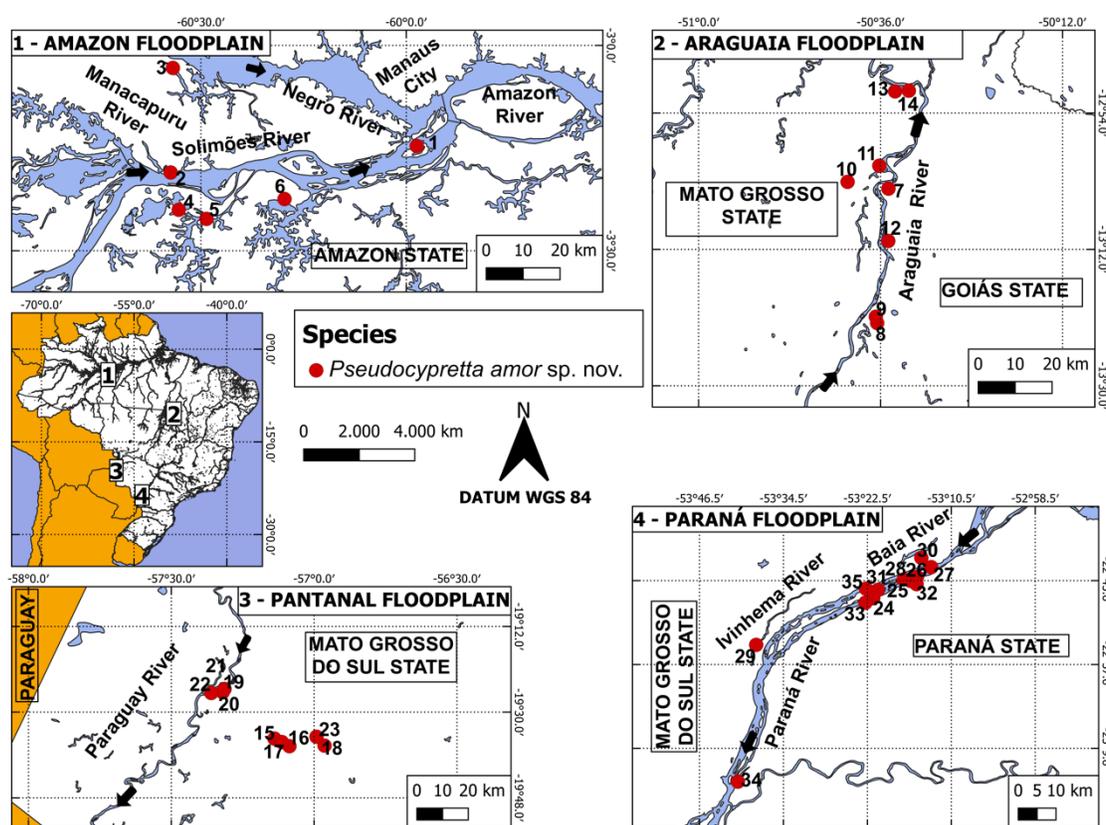
Sampling was performed between 2004 and 2020 in the Upper Paraná River floodplain, and between 2011 and 2012 in the Amazon, Araguaia and Pantanal floodplains.

In the Pantanal floodplain, sampling was also done in 2003. Samples were taken amongst aquatic plants as well as sediment in the littoral region. The macrophytes were hand-collected, and whole plants or roots were washed in a bucket to remove the ostracods (see Campos et al. 2017). This material was then filtered in a net of 160  $\mu\text{m}$  mesh size and preserved in 70% ethanol buffered with sodium tetraborate. Samples from the littoral region were performed *in situ* with a rectangular hand net (28 cm  $\times$  14 cm, mesh size approximately 160  $\mu\text{m}$ ). Environmental variables (water temperature (WT) and dissolved oxygen (DO) concentration (YSI 550A oximeter), pH (pHmeter Digimed) and electrical conductivity (EC) (conductivimeter-Digimed), were measured *in situ*, close to the macrophytes and/or sediment.

### 3.2.3 Preparation and illustration of soft parts and valves

Specimens were dissected with hand-held small needles under a binocular microscope. The ostracod carapace was first opened, and the valves were separated from the soft parts. Soft parts were then dissected in a drop of glycerine on a glass slide. The dissected appendages were covered with cover-slip and sealed with transparent nail polish. Valves were stored dry in a micropaleontological slide. Drawings of the appendages were made using a camera lucida (Olympus U-DA) attached to an optical microscope (Olympus CX-41). Carapaces and valves were illustrated and measured using Scanning Electron Microscopy (SEM; Fei Qanta 200 ESEM, - Royal Belgian Institute of Natural Sciences, Brussels, Belgium) in different views (internal, lateral, dorsal, ventral and frontal). To illustrate the septa on the margin of the valves, both valves were placed on a concave microscope slide in glycerine sealed with cover slip and drawn using a camera lucida

attached to an optical microscope (see above). The type material and illustrated specimens are stored in the collection of the Museum of Zoology of the University of São Paulo (São Paulo, Brazil – MZUSP). Thoracopod terminology follows Broodbakker and Danielopol (1982), second antenna terminology follows the revised model proposed by Martens (1987), and of the second and third thoracopods terminology follows Meisch's nomenclature (2000). Higher taxonomy of the Ostracoda follows Horne et al. (2002) and Meisch et al. (2019).



**Fig. 1.** Map of the study area of the Brazilian floodplains and the occurrences of *Pseudocyprretta amor* sp. nov.

### 3.3 Results

**Class Ostracoda Latreille, 1802**  
**Subclass Podocopa G.O. Sars, 1866**

**Order Podocopida G.O. Sars, 1866**  
**Suborder Cypridocopina G.O. Sars, 1866**  
**Superfamily Cypridoidea Baird, 1845**  
**Family Cyprididae Baird, 1845**  
**Subfamily Cypridopsinae Kaufmann, 1900**  
**Tribe Cyprettadopsini Savatentalinton, 2020**  
**Genus *Pseudocypretta* Klie, 1932**

*Diagnosis:* Cp highly arched, sub-triangular in lateral view, rounded in dorsal and ventral views; LV overlapping RV at least anteriorly and ventrally. Coloration patchy and variable. RV with fully developed marginal septa along anterior and posterior margin; LV with incomplete marginal septa. LV with a large inner list, running parallel to (part of) the anterior margin, straight and obliquely away from the postero ventral margin. RV with selvage weakly inwardly displaced along the postero-ventral margin. Both valves with external lists, most developed on LV; these lists not perforated by pores. A1 with relatively short segments. A2 with natatory setae extending beyond tips of distal claws, claw G2 more strongly developed and serrated than the other claws. Md-palps with alpha seta short, slender and smooth; beta seta short, stout and hirsute; gamma seta long, slender and hirsute. Mx1 with second palp segment rectangular, tooth bristles on third endite smooth. T2 with penultimate segment divided; seta d2 well-developed; seta d1 absent; claw h2 unusually strongly curved. T3 with 4th segment separate from 3rd, distally with seta h2 relatively short. CR minute, with base and distal seta fused, or fully absent. Male unknown. *Type species:* *Pseudocypretta maculata* Klie (1932). *Other species:* *P. lineata* Ma and Yu (2020), *P. amor* sp. nov. *Differential diagnosis:* *Pseudocypretta* is closely related to *Cyprettadopsis* and shares many characters and character states, but the two genera also have some important morphological differences. *Cyprettadopsis* is much more elongated than the three

species of *Pseudocyprretta*, it has a different arrangement of the marginal septa (anteriorly incompletely developed and posteriorly fully developed in both valves in *Cyprrettadopsis*, fully developed in the RV and incompletely developed in the LV in *Pseudocyprretta*), the external lists in the two genera are differently developed, with *Cyprrettadopsis* having additional pores there, and the inner list in the LV has a posteroventral deviation from the valve margin which is significantly larger in *Pseudocyprretta* than in *Cyprrettadopsis* (a longer part is straight and not following the curve of the valve margin).

### 3.3.1 *Pseudocyprretta amorsp.* nov.

(Figs. 2-9)

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“*Cyprretta*” sp.3 – Higuti et al. 2009: 664, Table 1; 2010: 267, Table 2.

“*Cyprretta*” n. sp. – Matsuda et al. 2015: 326, Table 1; Higuti et al. 2017a: 5, Apêndice 1.

“*Cyprretta*” sp.3 n. sp. – Higuti et al. 2017b: e120, Table 2.

“*Cyprretta*” n.sp. 3 – Pereira et al. 2017: 327, Table 2; Campos et al. 2021: 27, Table 1.

“*Cyprretta*” sp. 2 n. sp. – Campos et al. 2018: 6, Table 2.

#### 3.3.1.1 *Type locality*

Garças Lake (PAR 982) in the Upper Paraná River floodplain. Coordinates: 22°43'31.1"S, 53°13'08.4"W.

### 3.3.1.2 *Material examined*

Holotype: 1 ♀, with soft parts dissected in glycerine in a sealed slide and with valves stored dry in a micropaleontological slide (MZUSP 43014), collected in February 2014, in Garças Lake (PAR 982) by Janet Higuti and Eliezer de Oliveira da Conceição. Paratypes: 3 ♀ with soft parts dissected as the holotype (MZUSP 43008, MZUSP 43015 and MZUSP 43016), 6 ♀ carapaces stored dry in micropaleontological slides after use for SEM illustrations (MZUSP 43009, MZUSP 43010, MZUSP 43011, MZUSP 43012, MZUSP 43013 and MZUSP 43017), collected in February 2014 in Garças Lake (PAR 982) by Janet Higuti and Eliezer de Oliveira da Conceição.

### 3.3.1.3 *Other material illustrated*

Amazon River floodplain: 1 ♀ with soft parts dissected as the holotype (MZUSP 43018); 3 ♀ carapaces stored dry in micropaleontological slides after use for SEM illustrations (MZUSP 43019, MZUSP 43020 and MZUSP 43021), collected in May 2012 in Poço Curuça Lake (AMA AMA59, 60) by Janet Higuti. Araguaia River floodplain: 1 ♀ with soft parts dissected as the holotype (MZUSP 43022); 5 ♀ carapaces stored dry in micropaleontological slides after use for SEM illustrations (MZUSP 43023, MZUSP 43024, MZUSP 43025, MZUSP 43026 and MZUSP 43027), collected in March 2012 in Varal Lake (ARA80) by Janet Higuti and Koen Martens. Pantanal: 2 ♀ with soft parts dissected as the holotype (MZUSP 43032 and MZUSP 43028); 3 ♀ carapaces stored dry in micropaleontological slides after use for SEM illustrations (MZUSP 43029, MZUSP 43030

and MZUSP 43031), collected in June 2003 in Corumbá Road II temporary pool (PAN 15) by Janet Higuti, Koen Martens and Kennedy Francis Roche.

#### 3.3.1.4 *Etymology*

The species is named after its colour pattern on the carapace in dorsal view, which looks like the word “love” (*amor* is “love” in Latin) (Fig.2).

#### 3.3.1.5 *Diagnosis*

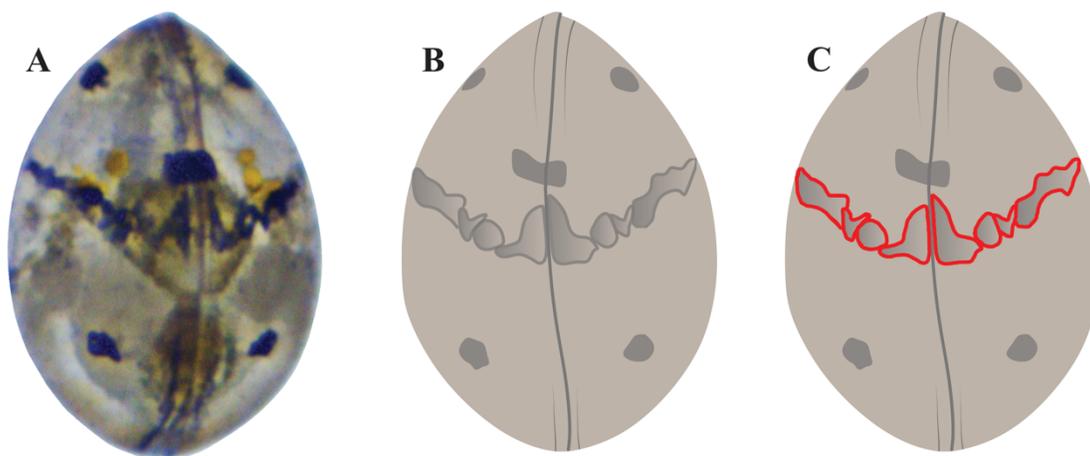
Carapace rounded in dorsal and ventral views and with sub-triangular shape in lateral view, carapace surface set with a few shallow pits; LV overlapping RV along anterior and ventral margins; LV with large outer list along anterior, ventral and posterior margins; RV with ca. 11 fully developed marginal septa; LV with septa incompletely developed. A2 with claw G2 stronger developed and serrated than other claws. Mx1 with sideways directed bristles absent; first segment of Mx1-palp with sub-apical seta present; second segment elongated, L c. twice W; first endite with three apical claws; third endite with two smooth bristles. Md-palp third segment with three dorsal setae. T1 with setae b and d absent. T2 with seta d<sub>1</sub> absent and penultimate segment undivided, claw h<sub>2</sub> unusually strongly curved. T3 with fourth segment not fused with third segment and carrying three apical setae. CR fully absent. Male unknown.

### 3.3.1.6 *Description of female*

LVi (Fig. 3A, 4A) with inner lamella wide along anterior margin, absent along ventral, and narrow along posterior margins; inner groove running parallel to the ventral margin; large outer list running to halfway the anterior margin, almost parallel along the ventral margin and forming an inner groove there, and straight, not parallel to the ventro-posterior margin, showing an outward doubling on this straight part; ca. 12 septa weakly developed along the anterior margin and ca. 5 septa weakly developed along the posterior margin (Fig. 3G, H – indicated by white arrows - 4A). RVi (Fig. 3B, 4B) with inner lamella wide along anterior margin, absent along ventral and narrow along posterior margins; with a weak inner list along anterior-ventral margin, both inner list and inner groove along ventral margin absent; posterior margin with an inwardly displaced selvage; ca. 11 septa along the anterior margin and ca. five septa along the posterior margin (Fig. 4B). Central muscle scars (Figs. 3A, B) forming a simplified paw-print pattern, consisting of an anterior (oblique) row of 3 rounded scars of intermediate size, one large rounded scar posterior of this row and two small rounded scars below these four larger scars. CpRl (Fig. 3C) with a rounded, subtriangular shape; greatest height situated in the middle; LV overlapping RV at the anterior, dorsal and ventral margins. CpD and CpV (Fig. 3D-E) with oval shape, posteriorly slightly broader than anteriorly, the latter bluntly pointed; greatest width situated slightly posteriorly to the middle. CpV with LV overlapping RV, in the middle region with a rounded expansion; both valves with clear external list, the one on the LV valve being the largest and running along anterior, posterior and ventral valve margins. CpFr (Fig. 3F), with LV overlapping

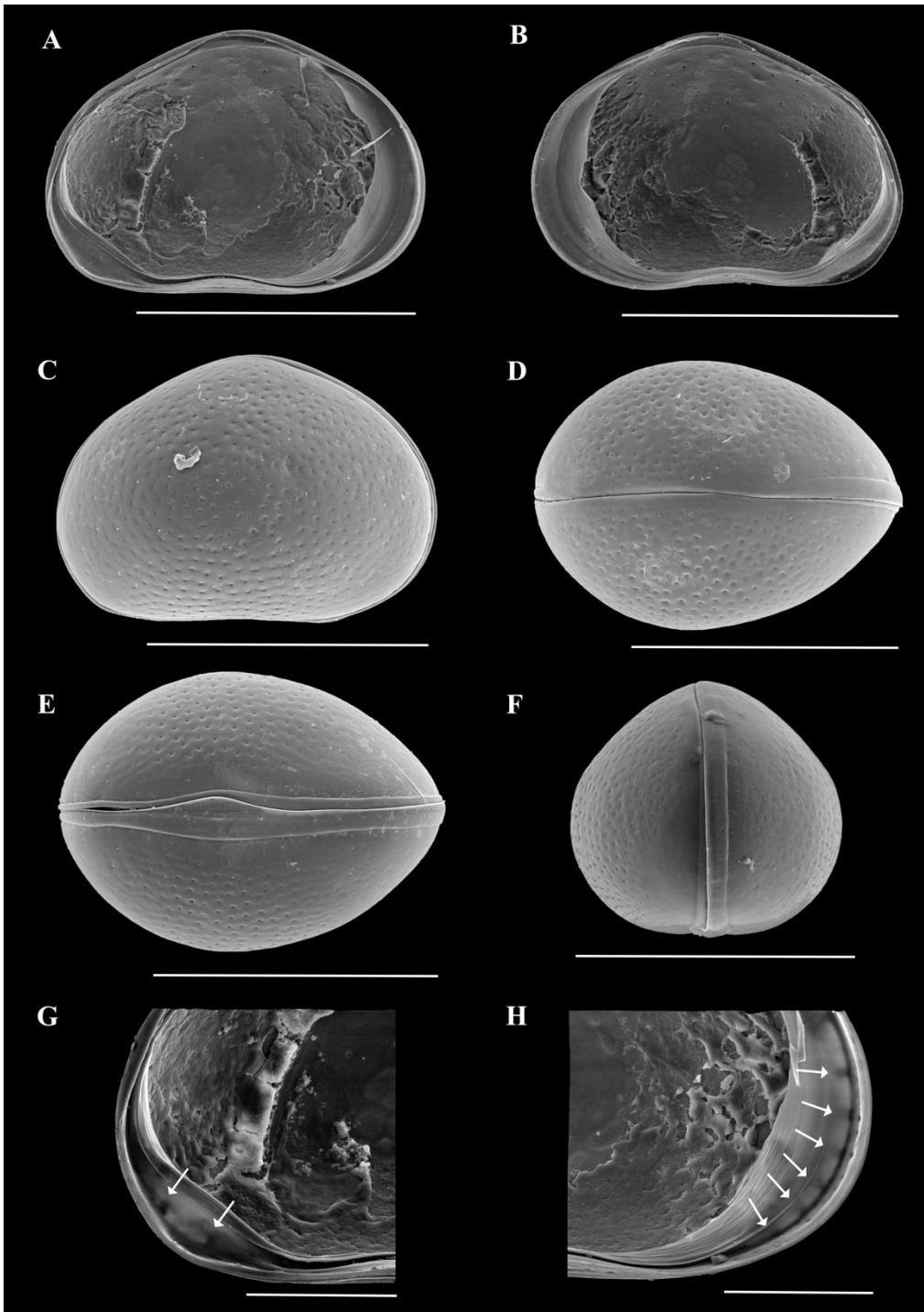
RV, showing the robust external list on the LV. Cp surface (Fig. 3C-F) with a few shallow pits and no clear setae.

A1 (Fig. 5A) composed of seven segments. First segment with two long ventral and one short dorsal setae (ca. 1/3 the length of ventral setae). Second segment with one dorsal seta (ca. 3/4 the length of the third segment) and an elongated lateral R, only slightly shorter than the segment. Third segment with one short ventro-apical seta and one long dorsal seta (almost reaching end of terminal segment). Fourth segment with two short ventral setae (the shortest ca. 3/4 of length of the longest) and two long dorsal setae. Fifth segment with two long dorsal setae. Sixth segment with four long apical setae. Terminal segment with one long aesthetasc (Ya), one short seta (with the same length of Ya) and two long setae. WO not seen.



**Fig. 2.** A, coloured photo of *Pseudocypretta amor* sp. nov.; B, illustration showing the word “love” on the carapace surface; C, illustration highlighting the letters of the word “love”.

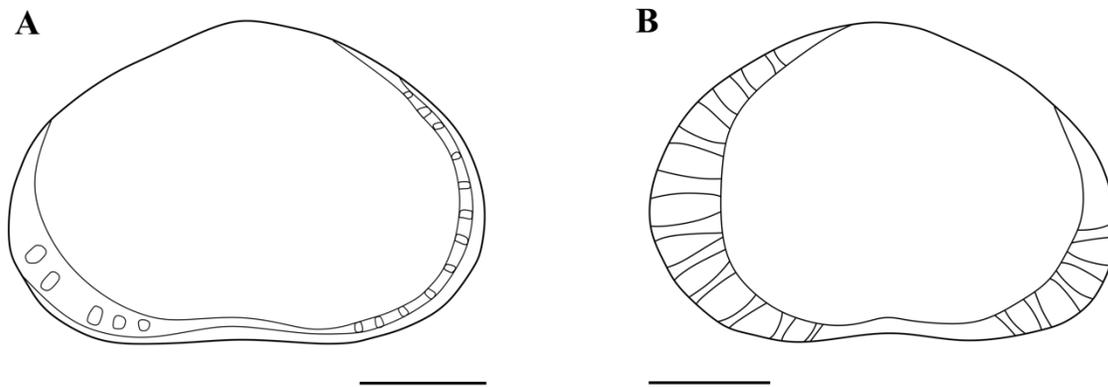
A2 (Fig. 5B, C) composed of five segments (one protopodite, one reduced exopodite and three endopodite segment). Protopodite carrying two short and one long ventral seta (ca. twice the length of the short ones). Exopodite consisting of a small plate with three setae, two short and one long (reaching beyond the tip of the second endopodite). First endopodal



**Fig. 3.** Carapace and valves of *Pseudocypretta amor* sp. nov. from Upper Paraná River floodplain, Garças Lake (PAR 982). A, LVi (MZUSP 43014); B, RVi (MZUSP 43014); C, CpRl (MZUSP 43009); D, CpD (MZUSP 43010); E, CpV (MZUSP 43011); F, CpFr

(MZUSP 43012); G, LVi, detail of septae on posterior margin (MZUSP 43014); H, LVi, detail of septae on anterior margin (MZUSP 43014). Scale bars: A–F = 300  $\mu\text{m}$ ; G–H = 100  $\mu\text{m}$ .

segment with one ventral aesthetasc Y, ca. half the length of the segment; one long ventro-apical seta ca. the length of the segment and five long natatory setae (reaching beyond the tips of the G claws) and one short seta (almost reaching the tip of the second endopodal segment). Second endopodal segment with a group of four medio-ventral t setae of unequal length (two reaching halfway z2, one slightly shorter and one short), and a group of two unequally short medio-dorsal setae; three long z setae (z1, z2 and z3); and three claws (G1, G2 and G3); claw G2 stronger developed and serrated than the other two claws. Terminal segment (Fig. 5C) with one long claw GM, and one short Gm; one aesthetasc y3 and its accompanying seta (slightly longer than y3); seta g absent.



**Fig. 4.** Valves inner view of *Pseudocypretta amor* sp. nov. from Upper Paraná River floodplain, Garças Lake (PAR 982), indicating the septae. A, LVi (MZUSP 43017); B, RVi (MZUSP 43017). Scale bars: A–B = 100  $\mu\text{m}$ .

MdCoxa (Fig. 5D) a plate with ca. six apical teeth intercalated with setae; one hirsute sub-apical seta on the dorsal margin.

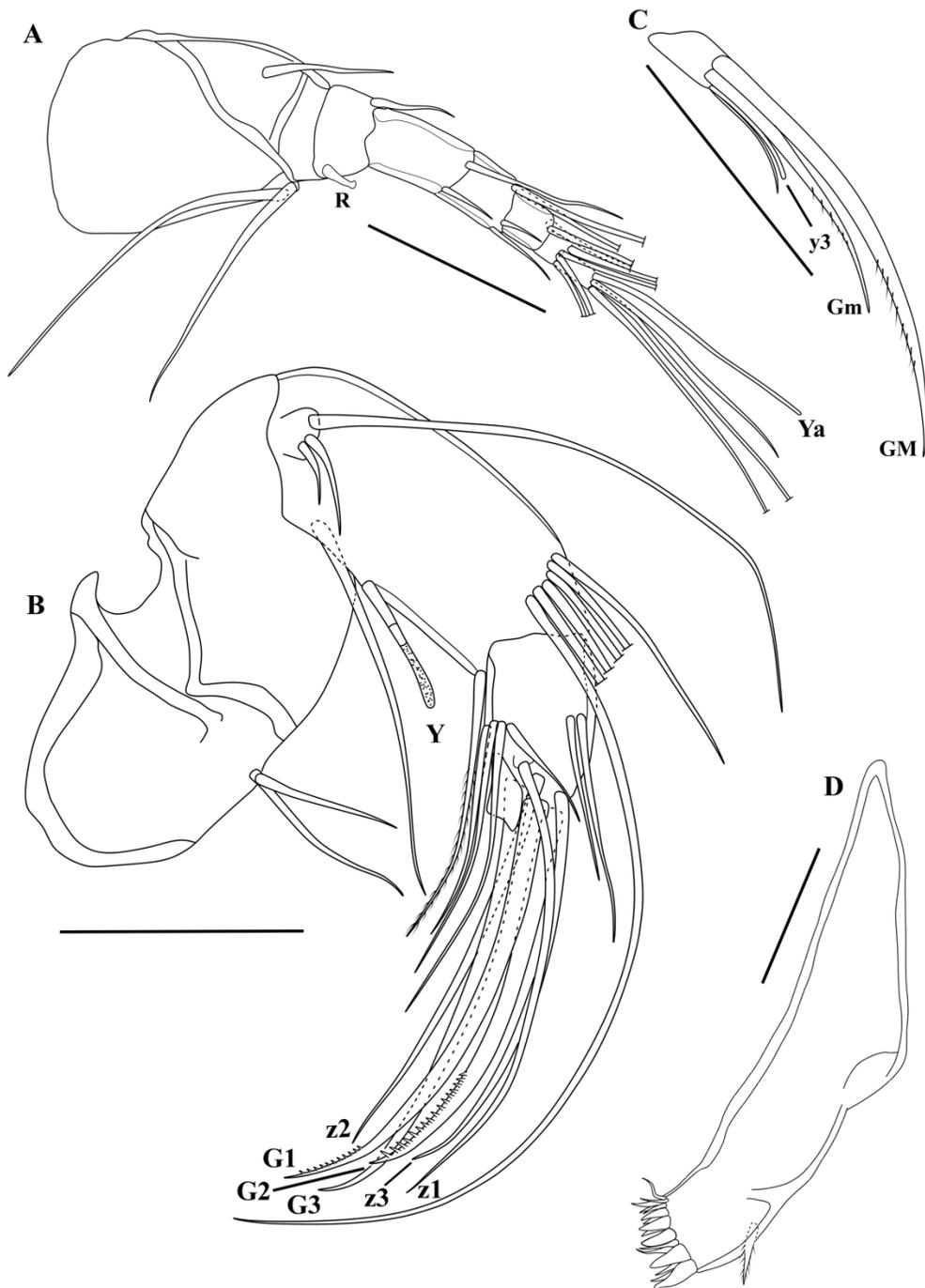
MdPalp (Fig. 6B, C) four segmented. First segment ventrally with plumose setae  $S_1$  and  $S_2$ ; one long smooth seta and one short  $\alpha$  seta (ca.  $1/6$  the length of the smooth seta). Second segment ventrally with a short cone-shaped and hirsute  $\beta$  seta and three long smooth setae; dorsally with a group of three subequal but long setae. Third segment dorsally with a group of four subequal setae; apically with three long setae and one long hirsute seta  $\gamma$ . Last segment (Fig. 6C) with three claws and one seta.

Mx1 (Fig. 6A) composed of a two-segmented palp, three endites and a large respiratory plate (the latter not illustrated). Basal segment of the palp with a group of five unequally long apical setae and one lateral seta. Terminal segment elongated (L ca. twice W), apically with three claws. First endite with two large bristles; and one medio-lateral seta (ca. slightly longer than the endite). Third endite with two basal setae. (Remark: chaetotaxy of endites incomplete, also in Fig. 6A, only major features described).

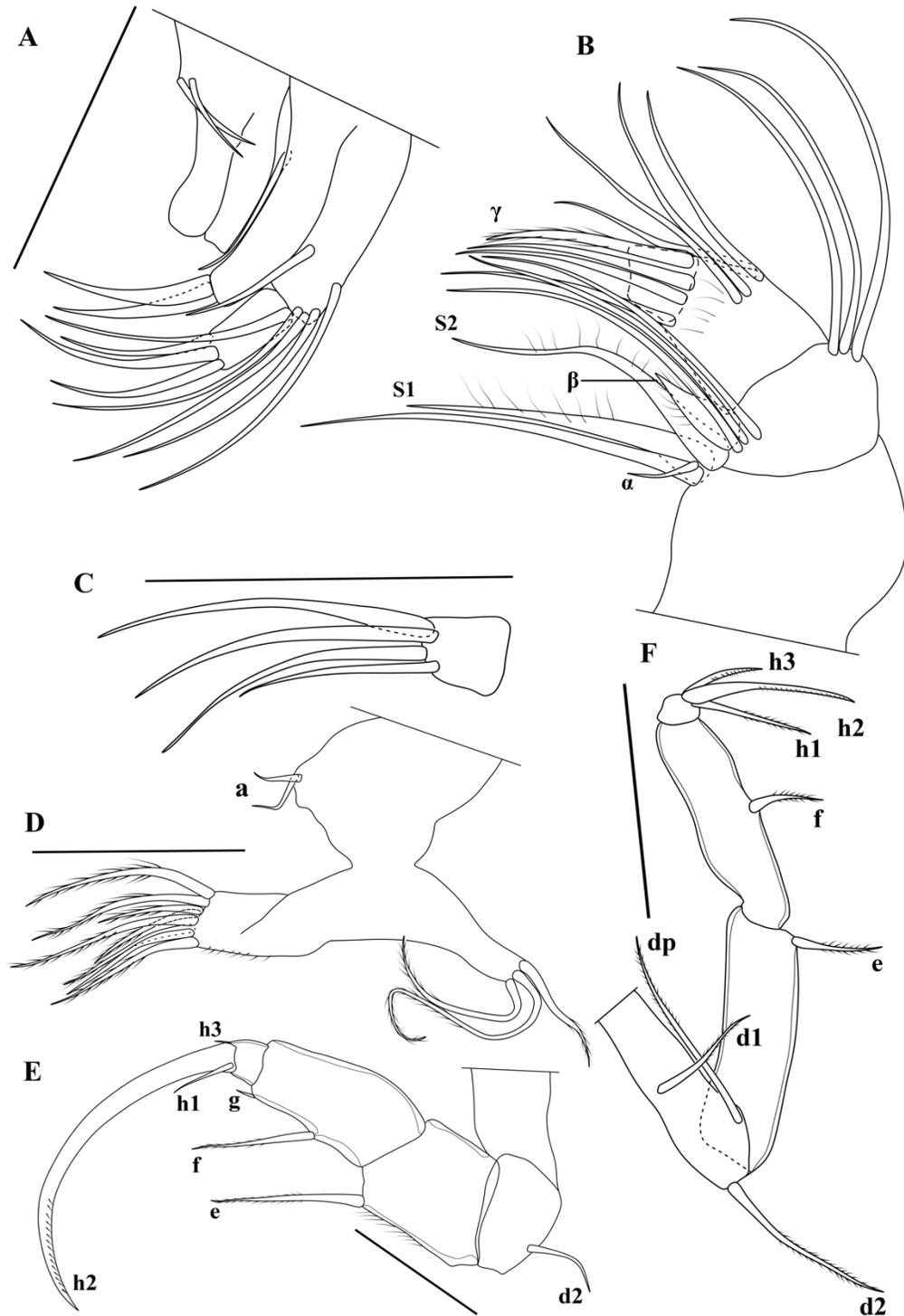
T1 (Fig. 6D) composed of an endopodite and a protopodite. Endopodite a conical palp, apically with three hirsute setae, two equally long and one short (ca.  $2/3$  the length of the longer ones). Protopodite with two equally short a-setae, and eight apical hirsute and unequally long setae; setae b and d missing.

T2 (Fig. 6E) composed of a five segmented walking leg. First segment with seta  $d_1$  absent. Second segment with a smooth seta  $d_2$ . Third segment with apical seta e hirsute and long

(reaching beyond the middle of the fourth segment). Fourth segment medially with seta f  
hirsute and long; and apically with a small seta g (reaching half the length of terminal



**Fig. 5.** Appendages of *Pseudocypretta amor* sp. nov. from the Upper Paraná River floodplain, Garças Lake (PAR 982). A, A1 (MZUSP 43015); B, A2 (MZUSP 43015); C, A2 terminal segment (MZUSP 43015); D, MdCoxa (MZUSP 43015). Scale bars = 50  $\mu$ m.



**Fig. 6.** Appendages of *Pseudocypretta amor* sp. nov. from the Upper Paraná River floodplain, Garças Lake (PAR982). A, Mx1 (MZUSP 43015); B, MdPalp (MZUSP 43008); C, MdPalp terminal segment (MZUSP 43008); D, T1 (MZUSP 43016); E, T2 (MZUSP 43008); F, T3 (MZUSP 43014). Scale bars = 50  $\mu$ m.

segment). Terminal segment rectangular, apically with one short ventral setae  $h_1$ , a long and strongly curved claw  $h_2$ , and a short dorsal seta  $h_3$ .

T3 (Fig. 6F) composed of four segments. First segment elongated, with one short hirsute seta  $d_1$  and two slightly longer and hirsute setae  $dp$  and  $d_2$ . Second segment elongated, with a short hirsute apical seta  $e$ . Third segment with small hirsute sub-apical seta  $f$ , (1/4 the length of the segment). Terminal (4<sup>th</sup>) segment separated from penultimate (3<sup>rd</sup>) segment (candonid type), with one long, thin and hirsute seta  $h_1$ , one claw-like seta  $h_2$  and one short hirsute seta  $h_3$ , the latter not reflexed.

CR fully absent.

Male unknown.

#### 3.3.1.7 *Measurements of illustrated specimens*

See Table 1

#### 3.3.1.8 *Remarks*

For comparative purposes, additional illustrations of valves and carapaces are given for populations from the Amazon River floodplain (Fig. 7), the Araguaia River floodplain (Fig. 8) and the Pantanal (Fig. 9). The individuals of these Brazilian floodplains share the same characteristics in the carapace, valves and appendages, despite some small size differences observed (see Table 1). The LVi of *Pseudocypretta amor* sp. nov. from the Pantanal shows

some damage in the dorsal position, resulted from the dissection. Several trials were made with other individuals to obtain an undamaged LVi but were unsuccessful owing to the fact that these valves were slightly decalcified and the soft parts were stuck by the adductor muscles to this valve. The decalcification of these valves, however, allows to see the marginal septa in the LV (Figs. 9A, H), even with non-transparent microscopy.

**Table 1.** Measurements (in  $\mu\text{m}$ ) of carapaces and valves of specimens of *Pseudocypretta amor* sp. nov. in different views.

Locality name	Sample number	Code	Valve/Cp	Length	Height	Width
Garças	PAR 982	MZUSP 43014	RVi	411	289	
Garças	PAR 982	MZUSP 43014	LVi			
Garças	PAR 982	MZUSP 43009	CpRl	406	286	
Garças	PAR 982	MZUSP 43010	CpD	415		304
Garças	PAR 982	MZUSP 43011	CpV	408		296
Garças	PAR 982	MZUSP 43012	CpFr		284	293
Poço Curuça	AMA 59	MZUSP 43018	RVi	406	284	
Poço Curuça	AMA 59	MZUSP 43018	LVi	410	286	
Poço Curuça	AMA 60	MZUSP 43019	CpRl	410	285	
Poço Curuça	AMA 60	MZUSP 43020	CpD	409		310
Poço Curuça	AMA 60	MZUSP 43021	CpV	406		304
Poço Curuça	AMA 60	MZUSP 43019	CpFr		303	311
Varal	ARA 80	MZUSP 43022	RVi	404	267	
Varal	ARA 80	MZUSP 43022	LVi	411	290	
Varal	ARA 80	MZUSP 43027	CpRl	409	286	
Varal	ARA 80	MZUSP 43027	CpD	416		310
Varal	ARA 80	MZUSP 43027	CpV	398		295
Varal	ARA 80	MZUSP 43026	CpFr		291	294
Corumba Road II	PAN 15	MZUSP 43028	RVi	407	282	
Corumba Road II	PAN 15	MZUSP 43028	LVi	409	292	

Corumba Road II	PAN 15	MZUSP 43029	CpRl	417	297	
Corumba Road II	PAN 15	MZUSP 43030	CpD	413		308
Corumba Road II	PAN 15	MZUSP 43031	CpV	402		304
Corumba Road II	PAN 15	MZUSP 43030	CpFr		304	307

### 3.3.1.9 *Differential diagnosis*

The new species can be distinguished from *Pseudocypretta lineata* by the presence of linear ridges on the carapace surface in *P. lineata* (valve are smooth in *P. amor* sp. nov.); the anterior part of the inner list in the LV runs much higher in *P. lineata*; the Cp is wider in *P. lineata* and the greatest width is situated behind the middle, while also the posterior part is somewhat invaginated; the setae on the last segment of T3 are shorter and the CR is completely absent in *P. amor* sp. nov.

The new species is more similar to *P. maculata*, but differs from it by the fact that in *P. maculata* the RV clearly overlaps the LV at the posterior region, while in *Pseudocypretta amor* sp. nov. both valves extend equally there; the Cp is more highly arched in *P. maculate* and the colouration is reduced to isolated spots; the A2 on *P. maculata* has a short terminal segment (L less than 1.5x W), while in *P. amor* sp. nov. the L is ca. 1.5x W; the last segment of the T3 has a long-reflexed seta h<sub>3</sub> in *P. maculata*, while in *Pseudocypretta amor* sp. nov., this seta h<sub>3</sub> is short and is not reflexed; *P. maculata* has a CR as a reduced flagellum, while the CR is fully absent in *P. amor* sp. nov.

These differences are summarized in Table 2.

**Table 2.** Comparative morphology of the three known species of *Pseudocypretta*, based on the original descriptions.

Species	Cp measurement s (in $\mu\text{m}$ )	A1	A2	Mx1	T2	T3	CR
<i>Pseudocypretta maculata</i> Klie (1932) redescription by Savatnalinton <i>et al.</i> (2022)	L: 0.490 H: 0.350 W: 0.320	Wouter organ present; Rome organ present; length of the short seta ca 2/5 of length of Ya.	natatory setae exceeding the terminal claws; penultimate segment shortened.	Mx2- bristles with teeth.	d1 seta absent; d2 seta present; third segment undivided; long h2 claw on last segment strongly curved.	h1 short; h2 long; h3 long.	Reduced CR
<i>Pseudocypretta lineata</i> Ma and Yu (2020)	L: 0.428-0.437 H: 0.295-0.389 W: 0.372-0.382	Wouter organ present; Rome organ present; Ya 2x the length of the short seta.	natatory setae exceeding the terminal claws; penultimate segment not shortened.	Mx2- bristles smooth.	d1 seta present; d2 seta absent; third segment undivided; long h2 claw on last segment strongly curved.	h1 long; h2 short; h3 long.	Reduced CR
<i>Pseudocypretta amor</i> sp. nov.	L: 0.398-0.415 H: 0.267-0.303 W: 0.293-0.311	Wouter organ absent; Rome organ present; Ya same length of the short seta.	natatory setae exceeding the terminal claws; penultimate segment not shortened.	Mx2- bristles smooth.	d1 seta absent; d2 seta present; third segment undivided; long h2 claw on last segment	h1 short; h2 long; h3 short.	CR absent

strongly  
curved.

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#### 3.3.1.10 *Ecology and distribution*

*Pseudocypretta amor* sp. nov. was recorded in the four main Brazilian floodplains, Amazon, Araguaia, Pantanal, and Paraná, with the major distance between two of these floodplains of 2.300km (Fig. 1). The species was recorded in a wide range of environmental variables, such as 17.1-35°C for WT, 5.71-8.23 for pH, 8.1-222.5  $\mu\text{S}\cdot\text{cm}^{-1}$  for EC and 1-7.28  $\text{mg}\cdot\text{L}^{-1}$  for DO. The species was associated with the root systems of aquatic macrophytes and was also collected from sediment. Detailed information about environmental data is listed in Table 3.

**Table 3.** Environment type, substrate and abiotic variables of localities from where *Pseudocypretta amor* sp. nov. was recorded in the river-floodplain system of Amazon, Araguaia, Pantanal, and Paraná. Type locality in bold. WT, water temperature; EC, electrical conductivity; DO, dissolved oxygen; Az, *Azolla* sp.; Cf, *Cabomba furcata* Schult. and Schult; Ea, *Eichornia azurea* (Sw.) Kunth; Ec, *Eichornia crassipes* (Mart.) Solms; Es, *Eichornia* sp.; Lw, *Ludwigia* sp.; Oc, *Oxycaryum cubense* (Poepp. and Kunth) Palla; Pan, *Paspalum notatum* Flugge.; Pasp, *Paspalum* sp.; Ps, *Pistia stratiotes* L.; Sa, *Salvinia auriculata* Aubl.; Sm, *Salvinia minima* Baker; Sp, *Salvinia* sp.; Uf, *Utricularia foliosa* L.; Se, sediment; NA, information not available.

Locality name	Sample	Date	Environment	Substrate	WT (°C)	pH	EC ( $\mu\text{s.cm}^{-1}$ )	OD ( $\text{mg.L}^{-1}$ )
1. Comprido	AMA 03	10.2011	open lake	Pan, Sm, Ps	35.0	8.2	222.5	9.9
1. Comprido	AMA 82, 84	05.2012	open lake	Ec	31.5	6.7	65.1	1.8
2. Grande	AMA 52, 54, 55	05.2012	open lake	Ec, Ps, As	31.5	6.6	54.1	1.2
3. Poço Curuça	AMA 59, 60	05.2012	open lake	Ec, Sa, Lw, Az, Pan, Uf	31.5	6.6	51.8	1.4
4. Cadete	AMA 62	05.2012	open lake	Pan	32.0	6.7	48.9	1.7
5. Jacaqui	AMA 67	05.2012	open lake	Ec	32.9	6.5	41.5	41.5
6. Grande II	AMA 71	05.2012	open lake	Ec	32.3	6.5	42.5	3.0
7. Piratinga	ARA 24, 25, 26	04.2011	open lake	Pan, Ec	29.5	7.0	26.5	6.9
7. Piratinga	ARA 83, 84	03.2012	open lake	Pan	29.5	7.0	26.5	6.9
8. Crixas II	ARA 46	03.2012	open lake	Uf	29.4	6.9	46.6	6.3
9. Crixas IV	ARA 54, 55, 56	03.2012	open lake	Pan, Ps, Uf	30.0	6.8	54.4	6.7
9. Crixas IV	ARA 63, 64	03.2012	open lake	Ec	29.8	7.0	50.2	5.8
10. Piranha	ARA 75, 76	03.2012	open lake	Pan	29.3	6.8	46.5	3.9
11. Varal	ARA 79, 80, 81	03.2012	open lake	Pan	28.5	6.6	39.1	3.9
12. Brito	ARA 89, 90	03.2012	open lake	Pan	30.9	7.0	31.1	7.4
13. Comprido II	ARA 99	03.2012	open lake	Pan	29.5	6.6	27.6	6.2
14. Goiaba	101, 102, 103	03.2012	open lake	Ec	30.5	6.6	39.0	7.2

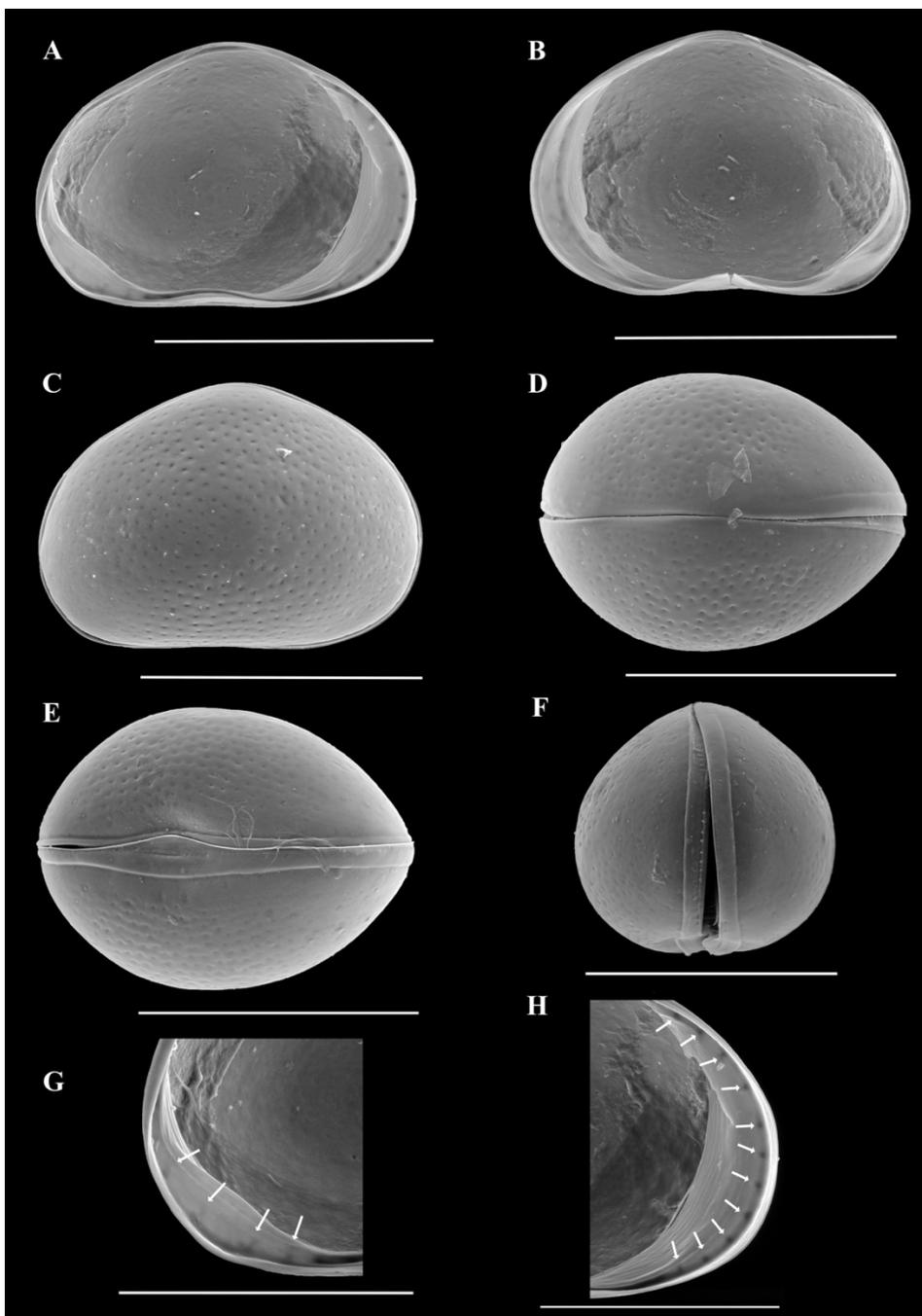
15. Corumba Road I	PAN 12	06.2003	temporary pool	Se	20.8	7.0	NA	NA
16. Corumba Road II	PAN 15	06.2003	temporary pool	Se	18.5	7.0	NA	NA
17. Corumba Road III	PAN 16	06.2003	temporary pool	Se	21.1	7.0	NA	NA
18. Vermelho	PAN 23, 24	06.2003	river	Se	20.0	6.0	NA	NA
19. Miranda II	PAN 66	08.2011	lake	Ea, Pasp, Sp, Lw	19.7	8.1	140.0	4.5
19. Miranda II	PAN 115	03.2012	lake	Ec	29.7	7.4	165.0	6.2
20. Miranda IV	PAN 73	08.2011	lake	Sp, Ec	21.0	7.8	2.4	3.6
21. Miranda I	PAN 117	03.2012	lake	Pasp, Es	29.5	7.0	166.0	2.8
22. Ilha Grande	PAN 142	03.2012	lake	Ec, Pasp, Lw	29.5	6.4	48.8	4.5
23. Rebojão	PAN 154	03.2012	lake	Lw, Pasp	24.7	6.5	118.5	1.6
24. Pombas	PAR 30	03.2004	open lake	Ec	26.6	6.5	96.6	4.4
24. Pombas	PAR 970	02.2014	open lake	Ea	29.6	8.1	67.3	7.8
24. Pombas	PAR 971	02.2014	open lake	Ea	29.9	7.9	68.2	6.9
24. Pombas	PAR 1297	02.2015	open lake	Ea	28.8	7.6	63.5	2.7
24. Pombas	PAR 1395	02.2015	open lake	Ea	19.6	7.1	55.7	4.1
24. Pombas	PAR 1396	02.2015	open lake	Ea	23.5	7.9	59.5	6.5
25. Leopoldo	PAR 36, 37	03.2004	open lake	Oc, Ea	29.3	6.2	80.4	2.5
26. Pau Véio	PAR 43	03.2004	open lake	Se	29.8	6.3	68.2	4.3
26. Pau Véio	PAR 172	11.2004	open lake	Ea	26.7	5.8	67.5	2.3
26. Pau Véio	PAR 1433	03.2017	backwater	Ea	28.9	6.8	71.5	3.7
26. Pau Véio	PAR 1541	03.2018	backwater	Ea	28.8	6.1	63.0	1.2
26. Pau Véio	PAR	03.2020	backwater	Ea	27.4	6.6	8.1	4.4

	1651							
27. Garças	PAR 45	03.2004	open lake	floating	32.1	6.5	68.2	4.7
27. Garças	PAR 176	11.2004	open lake	Sp	27.3	6.3	64.1	3.9
27. Garças	PAR 177	11.2004	open lake	Ea	27.3	6.3	64.1	1.0
27. Garças	PAR 617	07.2012	open lake	Sa	20.6	6.2	49.8	6.9
<b>27. Garças</b>	<b>PAR 982</b>	<b>02.2014</b>	<b>open lake</b>	<b>Ea</b>	<b>30.4</b>	<b>7.7</b>	<b>60.6</b>	<b>5.5</b>
27. Garças	PAR 983	02.2014	open lake	Ea	30.4	7.5	60.8	5.5
27. Garças	PAR 984	02.2014	open lake	Ea	30.3	7.2	59.7	4.0
27. Garças	PAR 1083	05.2014	open lake	Ea	25.4	7.3	53.7	6.7
27. Garças	PAR 1403	02.2015	open lake	Ea	23.5	6.7	44.9	7.9
27. Garças	PAR 1404	02.2015	open lake	Ea	22.4	7.3	53.6	7.5
27. Garças	PAR 1535	03.2018	open lake	Ea	31.3	6.5	57.0	4.8
27. Garças	PAR 1536	03.2018	open lake	Sa	31.3	6.5	57.0	3.6
27. Garças	PAR 1647	03.2020	open lake	Ea	27.5	5.7	8.1	3.0
28. Bilé	PAR 49	03.2004	open lake	Se	32.8	7.2	70.7	2.6
28. Bilé	PAR 611	07.2012	open lake	Ec	21.3	6.3	52.1	6.0
29. Ivinhema	PAR 56	03.2004	river	Sp	30.5	7.0	46.6	3.2
29. Ivinhema	PAR 58	03.2004	river	Ec	30.5	7.0	46.6	7.9
30. Porcos	PAR 90	03.2004	open lake	Ec	29.6	6.1	41.3	5.8
31. Manezinho	PAR 99	03.2004	open lake	Ec	31.6	7.5	65.2	3.9
31. Manezinho	PAR 211	11.2004	open lake	Ec	26.6	6.0	58.8	1.8
31. Manezinho	PAR 610	07.2012	open lake	Cf	21.1	6.1	51.1	6.9
32. Caracu	PAR 100	03.2004	stream	Se	27.2	6.9	54.3	2.1
33. Cortado	PAR 233, 235	11.2004	channel	Sp, Ec	25.1	6.3	61.5	3.1
34. Paraná	PAR 1281	02.2015	river	Ea, Sa	29.5	7.2	68.8	6.3
35. Xirica	PAR 1390	02.2015	open lake	Ea	17.1	7.9	45.3	4.8
35. Xirica	PAR 1391	02.2015	open lake	Ea	19.4	7.2	42.6	5.8

### 3.4 Discussion

#### 3.4.1 Occurrence of the genus *Pseudocyprretta*

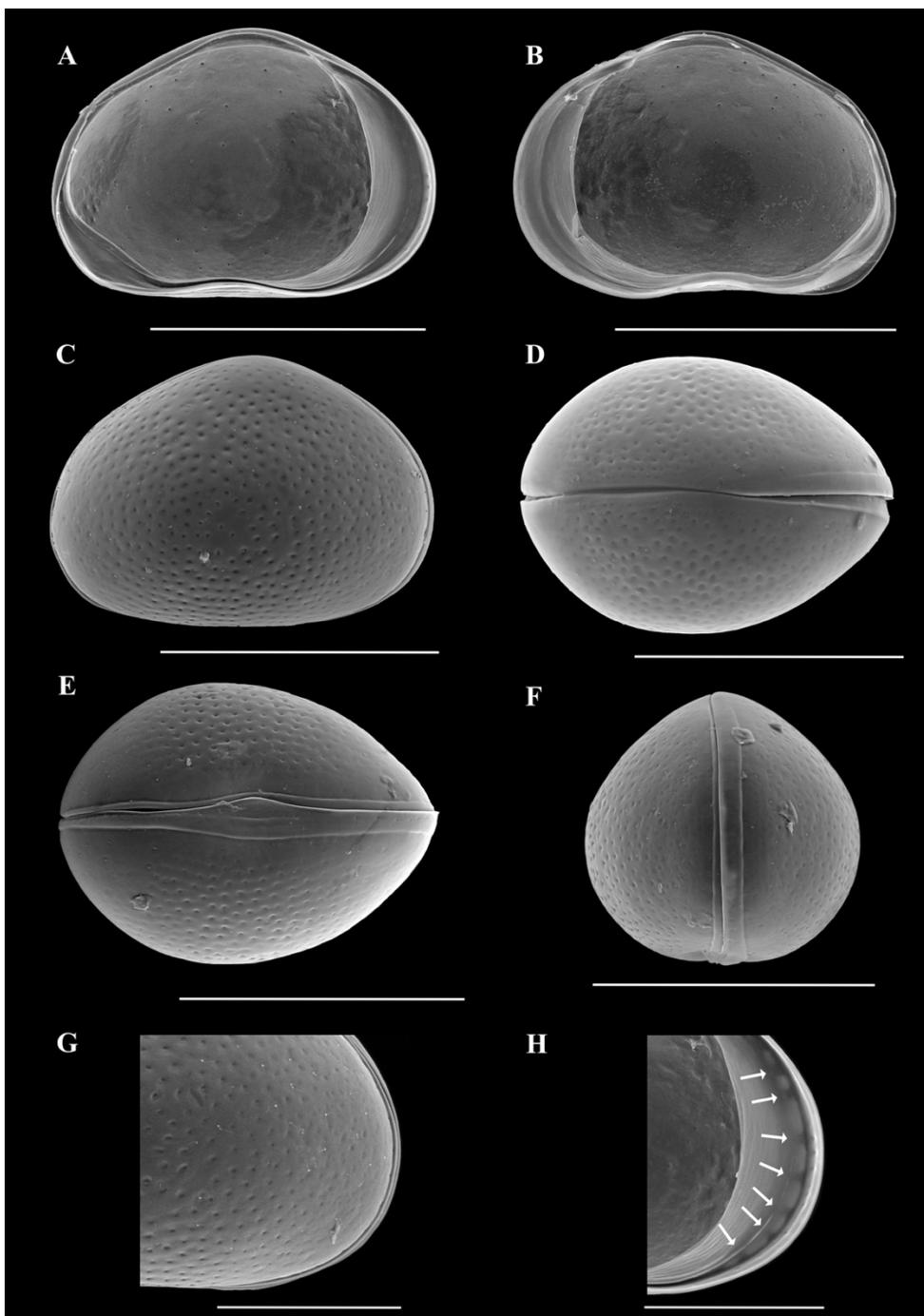
*Pseudocyprretta maculata* was described by Klie (1932) from lakes and rice fields of Sumatra and Java. The original description lacks details about the species' morphology, with illustrations of only carapace, antenna, second and third thoracopod and caudal ramus. Later, Battish (1978, 1982) and Karuthapandi et al. (2014) reported this species from India; Victor and Fernando (1981) from Malaysia and Neale (1984) from Sri Lanka, while Savatnalinton and Martens (2009, 2010), Savatnalinton (2014, 2017) and Savatnalinton and Suttajit (2016) reported *P. maculata* from various habitats in Thailand, indicating that this species is quite common in South East Asia (Savatnalinton 2017, Smith et al. 2018). Ma and Yu (2020) described the second species of *Pseudocyprretta*, *P. lineata*, from Hanan Island, southern China. Both *Pseudocyprretta* species were reported from similar habitats such as wetlands, ponds, steams, rice fields, and this generally in association with aquatic macrophytes. The same is observed for *Pseudocyprretta amor* sp. nov., which is commonly associated with aquatic macrophytes but also occurs on sediment in rivers, open lakes, and



**Figure 7.** Carapace and valves of *Pseudocypretta amor* sp. nov. from Amazon River floodplain, Poço Curuça Lake (AMA59, 60). A, LVi (MZUSP 43018); B, RVi (MZUSP 43018); C, CpRl (MZUSP 43019); D, CpD (MZUSP 43020); E, CpV (MZUSP 43021); F, CpFr (MZUSP 43019); G, Cp, LVi, detail of septae on posterior margin (MZUSP 43018); H, LVi, detail of septae on anterior margin (MZUSP 43018). Scale bars: A-F, 300  $\mu$ m; G-H, 200  $\mu$ m.

backwaters in the four main Brazilian floodplains. The importance of aquatic macrophytes for (macro) invertebrates is well established, as these plants provide resources such as food, shelter and substrate for reproduction for these organisms (Thomaz and Cunha 2010; Matsuda et al. 2015; Campos et al. 2017).

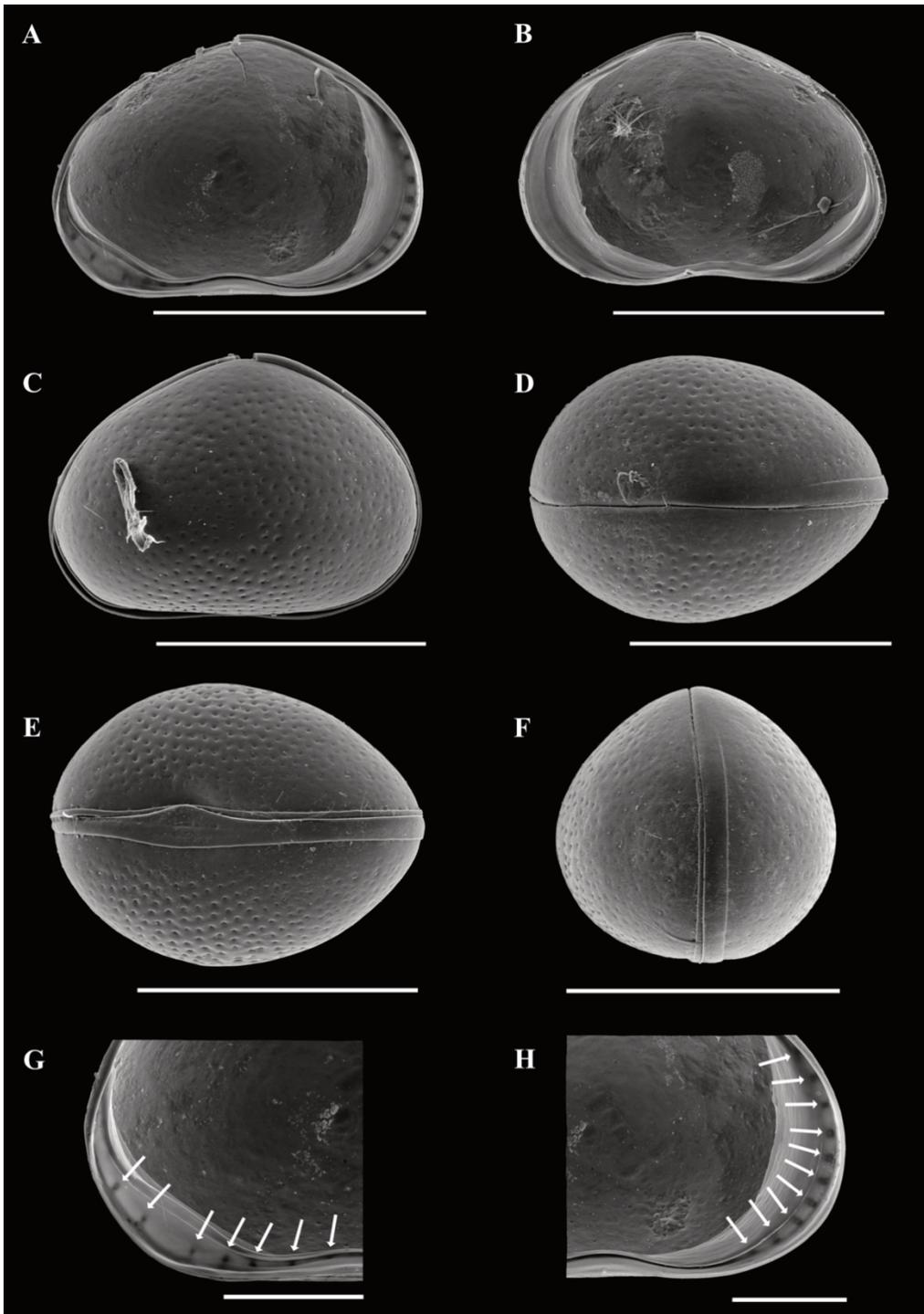
Janz (1997) reported on a fossil species, *Pseudocyprretta* sp., from the oldest sediments of a Miocene (Tertiary) crater lake near Steinheim am Albuch (Germany). The accompanying ostracod fauna (*Strandesia*, *Cyprinotus*, ...) indeed indicate a warmer, maybe subtropical, climate in which *Pseudocyprretta* species could have occurred. However, the single SEM figure of a lateral view of a carapace of this species (Janz 1997, plate 12, fig. 10) clearly indicates that this specimen belongs to a different genus. The specimen does show a triangular, highly arched shape, but the anterior overlap of the RV by the LV is much larger than in any of the recent species. Janz (*loc. cit.*) also mentioned that radial septa are visible through the closed valves, but clearly states that these are visible along the ventral margin, which is not the case in *Pseudocyprretta*.



**Figure 8.** Carapace and valves of *Pseudocypretta amor* sp. nov. from Araguaia River floodplain, Varal Lake (ARA80). A, LVi (MZUSP 43022); B, RVi (MZUSP 43022); C, CpRl (MZUSP 43027); D, CpD (MZUSP 43027); E, CpV (MZUSP 43026); F, CpFr (MZUSP 43026); G, CpRl, detail of anterior margin (MZUSP 43027); H, LVi, detail of septae on anterior margin (MZUSP 43027). Scale bars: A-H, 300  $\mu$ m; G-H, 150  $\mu$ m.

### 3.4.2 Expansion of the distribution area

There are at present 2330 subjective species of living non-marine ostracods, of which almost 90% are known from one zoogeographical region; only six species are presently known from at least six zoogeographical regions and can be considered truly cosmopolitan (Meisch et al. 2019). This in spite of the fact that most species of at least the Cypridoidea (ca. 75% of all known species) have a combination of characters that would allow long-distance dispersion (LDD), namely the potential to reproduce asexually, the production of drought-resistant eggs and (mostly) the potential of free-swimming (McKenzie 1971; Horne and Martens 1998; Schön et al. 2018). Other, non-cypridoid, species add brooding to the list of biological specialisations that would facilitate establishing populations after an LDD event (e.g., Darwinulidae, Timiriaseviinae). In spite of these apparent advantages, inter-continental LDD appears to be rare, although such events within zoogeographical regions might be more common, as was shown for a species complex in the genus *Strandesia* (Schön et al. 2018). The discovery of a new species of the genus *Pseudocyprretta*, thus far thought to be typical of South East Asia and China, in the Neotropical region therefore constitutes a significant range expansion of the genus. However, it could be that *Pseudocyprretta* is in fact a circumtropical genus, such as *Stenocypris* Sars, 1889, *Cyprretta* Vavra, 1895 and others



**Figure 9.** Carapace and valves of *Pseudocypretta amorsp. nov.* from Pantanal, Corumba Road II (PAN15). A, LVi (MZUSP 43028); B, RVi (MZUSP 43028); C, CpRl (MZUSP 43029); D, CpD (MZUSP 43030); E, CpV (MZUSP 43031); F, CpFr (MZUSP 43030); G, LVi, detail of septae on posterior margin (MZUSP 43028); H, LVi, detail of septae on anterior margin (MZUSP 43028). Scale bars: A-F, 300 μm; G-H, 100 μm.

(Meisch *et al.* 2019). In that case, it would be expected that species of this genus could also be found in tropical Africa and Australia.

### 3.4.3 Comparative morphology of *Pseudocyprretta* species

Of the three species presently assigned to *Pseudocyprretta*, only *P. lineata* and *P. amor* sp. nov. have received an extensive description, illustrating most of the morphological characters presently deemed relevant in freshwater ostracod taxonomy. Although rather well-described and illustrated for that time, the description of *P. maculata* by Klie (1932) lacks several characters that would be needed to make a full comparative analysis of the three species. A redescription of the type species of the genus is therefore needed. Here, we will discuss several morphological characters relevant to the identity of the genus and the three congeneric species. A summary is given in Table 2.

In all three species of *Pseudocyprretta*, the LV overlaps the RV at least anteriorly and ventrally, whereas the valve overlap in *Cyprretta* is inverse. Victor and Fernando (1979), Battish (1978, 1982) and Neale (1984) have all identified specimens from South East Asia as *P. maculata*, but in their specimens, the RV is reported to overlap the LV frontally. These identifications are therefore doubtful and might concern species of *Cyprretta*.

Marginal septa are calcified walls between the external lamella and the calcified internal lamellae of the valves, running through the vestibulum and thus strengthening the margins of the valves. They are not uncommon in several (related and unrelated) ostracod lineages, such as the genera *Oncocypris* G.W. Müller (1898) (Oncocypridinae De Deckker (1979) in

Notodromadidae), *Stenocypris* Sars (1889) (Herpetocypridinae Kaufmann (1900) in Cyprididae) and closer to the present genus also *Cypretta* Vavra (1895) (Cyprettinae Hartmann (1963) in Cyprididae), *Batucypretta* Victor and Fernando (1981) (Batucyprettinae Victor and Fernando (1981) in Cyprididae), *Bradycypris* Sars (1925), *Paracyprretta* Sars (1924) and *Zonocyprretta* De Deckker (1981) (Bradycypridinae Hartmann and Puri (1974) in Cyprididae) and *Cyprettadopsis* Savatentalinton (2020) (Cypridopsinae Kaufmann (1900) in Cyprididae).

For *P. maculata*, Klie (1932) illustrated (fig. 67) septa in the RV, but not in the LV (fig. 66), and also only described them for the RV (“... die von Scheidewänden durchsetzt wird, sie lassen den Schalenrand radiär gestreift erscheinen.” – p. 485). Ma and Yu (2020) illustrated well-developed septa for the RV (fig. 6F), and incompletely developed ones for the LV (fig. 6G). The same pattern is true in *P. amor* sp. nov.: well developed septa in the RV, incompletely developed ones in the LV. It would thus appear that Klie (1932) missed the incompletely developed septa in the LV and that all three species could share this character.

The LV has an inner list which postero-ventrally runs straight and not parallel to the valve margin in all three species of *Pseudocypretta* (Klie 1932, fig. 66; Ma and Yu 2020, fig. 6c, present paper, Fig. 3A). This character is also apparent in some species of *Cypridopsis* Brady, 1867, most notably in *Cypridopsis vidua* (O.F. Müller, 1776).

Ma and Yu (2020, fig. 7B) illustrated claw G2 on the A2 of *P. lineata* as being much stronger serrated than the other claws and Klie (1932, fig. 69) showed the same feature for *P. maculata*. This character is generally typical for the taxa in the tribe Zonocypridini

Higuti and Martens, 2012, namely for the species in the genera *Zonocypris* G.W. Müller, 1898 and *Cabelodopsis* Higuti and Martens, 2012. However, Savatanalinton (2020) also illustrated such a strongly serrated claw G2 in the genus *Cyprettadopsis* Savatanalinton, 2020, albeit with a different appearance (see below).

In all three species, the second palp segment of the Mx1 is cylinder-shaped (rectangular in the drawings) and about twice as long as the basal width. Both *P. lineata* and *P. amor* sp. nov. have smooth tooth-bristles on the third endite. However, whereas Ma and Yu (2020, p. 216) indicate that *P. maculata* has serrated tooth-bristles, Klie (1932, p. 486) clearly states “...die beiden zahnartige verstärkten Borsten ungefedert.” (both tooth-like enforced setae not serrated: ungefedert = unfeathered). Therefore, all three species appear to have smooth tooth-bristles.

In all three species of *Pseudocypretta*, the penultimate segment of the T2 is undivided, while this segment is clearly divided in species of *Cypretta*. The presence or absence of setae  $d_1$  and  $d_2$  on T2 in *P. maculata* are unknown, but Ma and Yu (2020, fig. 8A) call the single seta on one of the basal segments of T2, seta  $d_1$ . However, our interpretation is that this seta is clearly inserted on the ‘knee’ segment of this limb, in which case it is seta  $d_2$ , just as in *P. amor* sp. nov. (Fig. 6E)

The third thoracopod (T3) is a walking leg in the Cytheroidea and the Darwinuloidea. Within the Cypridoidea, this leg is a cleaning leg with the tip of the third segment and the fourth segment fused into a pincer in the Cyprididae, whereas the third and the fourth segments are clearly separated in Candonidae (hence the name candonid type), Ilyocyprididae and Notodromadidae. However, there are clear exceptions in several

lineages of Cypridoidea which have the candonid type T3. These are: *Oncocypris* G.W. Müller, 1898 and *Neozonocypris* Klie (1944) in the subfamily Oncocypridinae in the Notodromadidae Kaufmann (1900); *Callistocypris* Shornikov, 1980 in the Callistocypridinae Shornikov, 1980 (see also Pinto et al. 2005; Savatnalinton and Martens, 2013); *Cyprettadopsis* and *Neocypridopsis* Klie (1940) in the Cypridopsinae Kaufmann (1900); *Batucyprretta* in the Batucyprrettinae and *Pseudocyprretta* in the Cyprettinae, all in the Cyprididae. The wider taxonomic significance of this phenomenon will be discussed elsewhere, but the significance for the genus *Pseudocyprretta* is further explained below. The enigmatic *Batucyprretta* needs urgent redescription. Ma and Yu (2020) briefly described what they call a different species from *Batucyprretta* from Hanan Island (southern China), but from their illustrations it is clear that these are specimens of *Cyprettadopsis sutura* Savatnalinton (2020). Ma and Yu (2020) could not have been aware of this species as their paper was published on April 24<sup>th</sup>, 2020, while the paper Savatnalinton (2020) was published only 3 days earlier, on April 21<sup>st</sup>, 2020! This was the first time that *C. sutura* Savatnalinton (2020) was reported outside of Thailand.

A flagellar caudal ramus has been reported for *P. maculata* and *P. lineata*. In both cases, the CR is small, has a short lateral seta, while the ramus is distally fused with the terminal claw. In *Pseudocyprretta amor* sp. nov. it was not possible to clearly observe a structure that resembles the CR, this despite a long series of dissections. This leads us to decide that this structure is either fully absent in *Pseudocyprretta amor* sp. nov., or so small that it is undetectable. Klie (1932) described the CR on *P. maculata* as small and flagellum-like and difficult to observe. In *P. lineata*, the CR has the same morphology as in *P. maculata* but is

not so small. Based on the illustrations by Ma and Yu (2020, fig. 8B) it has almost the same length as the T2 (fig. 8A). This could be an error of scale, but it should be re-checked as it is unlikely.

#### 3.4.4 Taxonomic position of *Pseudocyprretta*

Klie (1932) compared his new genus to genera from Cypridopsinae, because of the reduced CR (including at that stage also *Oncocypris*, this genus is now in the Notodromadidae, but see below), as well as to genera with marginal septae such as *Cypretta*, *Paracyprretta* and *Bradycypris*, but offered no clear opinion as to where to lodge his new genus in a taxonomic classification. Hartmann and Puri (1974) listed *Pseudocyprretta* in the Cypridopsinae, without further discussion. McKenzie (1982, footnote pp 768-769) wrote:

“*Pseudocyprretta* has a smooth shell, the inner lamella bears radial septa (in the right valve only), the maxillule 3rd lobe has 2 Zahnborsten, the furca is reduced as in other cypridopsids. Because the shell bears radial septa, this genus is considered to be transitional to the family Cyprettidae, in which all known genera have such septa. On these grounds, *Pseudocyprretta* likewise merits its own subfamily, herein named Pseudocyprettinae, new subfamily.”

Meanwhile, the family Cyprettidae cited by McKenzie (*loc. cit.*) is lowered to the rank of subfamily within the family Cyprididae, in which *Pseudocyprretta* is included (Martens and

Savatenalinton 2011; Meisch et al.2019) and the subfamily Pseudocypridinae has not been recognized in any global taxonomic overview of freshwater ostracods.

Karanovic (2012) mentioned the genus *Pseudocypridina* in passing in a brief remark, while discussing the subfamily Oncocypridinae (in Notodromadidae), and by making a brief comparison to *Neozonocypris*). She concluded that the position of *Pseudocypridina* is doubtful and that it needs further clarification.

Savatenalinton (2020), while describing the new genus *Cypridopsis*, extensively discussed the characters mentioned above (valve overlap, T3, CR, ..), but mostly in comparison with what she called “the *Cypridina*-group” (*Cypridina*, *Batocypridina*, ...), with several genera of Cypridopsinae and with briefly also with the two genera in the Oncocypridinae, but only mentioned *Pseudocypridina* in passing, this in spite of the fact that both taxa are quite similar (see differential diagnosis above and Table 4).

*Pseudocypridina* differs from *Cypridina* in a number of important characters, such as the anterior valve overlap, the fact that the LV has only incompletely developed marginal septa, the shape of the posteroventral inner list in the LV, in the absence of seta d on T1, of seta d<sub>1</sub> on T2, the separate segment 4 on the T3 and the highly reduce CR. On the other hand, in almost all of these characters, *Pseudocypridina* agrees with the morphology of *Cypridopsis*. Differences between the two genera are listed above in the differential diagnosis but are far less important than the differences between both of these genera and *Cypridina*.

By previously lodging *Pseudocypridina* in the Cypridinae together with *Cypridina*, priority was clearly given to the stability of the presence of marginal septa over the fact that the CR

in the two genera is so very different. Admittedly, several species of *Cypretta* also have a CR which is already partly reduced from the basic cypridid pattern with setae or claws reduced or missing. In Candonidae, several closely related lineages show progressive reduction of the CR (Karanovic, 2007), although this is mostly linked to subterranean or even interstitial life, which causes simplification of limb chaetotaxy (see Danielopol 1978 and a partial revision in Martens, 1992).

However, there are indications that in the present case, maybe the reduced CR is more stable and a more reliable character on which to base classifications. The following arguments should be considered.

(1) Marginal septa occur in various ostracod lineages, some closely, some only distantly related. They are certainly subject to parallel evolution within the Ostracoda (see above).

(2) *Cyprettadopsis*, a genus quite similar to *Pseudocypretta* with both marginal septa and a flagellar CR, was lodged in a separate tribe in the Cypridopsinae, thus giving priority to the flagellar CR over the presence of marginal septa.

(3) The genus *Neocypridopsis* Klie (1940) also has separate fourth segment in the T3 and a flagellar CR and is lodged in the Cypridopsini of the Cypridopsinae. Karanovic and Datry (2009) extensively redescribed *Neocypridopsis albida* (Sars, 1901), and, based on that detailed morphology, maintained the genus in the Cypridopsinae. Karanovic (2012) transferred the genus *Neocypridopsis* to the Oncocypridinae in the Notodromadidae, based on the whip-like CR and the separated fourth segment on the T3, but without demonstrating that it possesses the most important features of the family

and subfamily, namely the separated, not fused, eyes with separate eye tubercles and the presence of four (not two) serrated teeth-bristles on the third endite of the Mx1 (De Deckker 1979). We here maintain *Neocypridopsis* in the Cypridopsinae, albeit maybe not with all species presently allocated to it and not necessarily within the tribe Cypridopsini.

Based on the above arguments, we here assign *Pseudocyprretta* to the tribe Cyprettadopsini. Savatnalinton (2020) extensively discussed the taxonomic consequences of lodging *Cyprettadopsis*, with both complete and incompletely developed marginal septa, in the Cypridopsinae, and all the arguments (pro and contra) she discussed are also valid for *Pseudocyprretta*, so we will not repeat them here, just add one additional remark.

Savatnalinton (2020) is correct in making a distinction between the much larger and more heavily serrated claw G2 on A2 which is typical of the Zonocypridini (*Cabelodopsis* and *Zonocypris s.s.*) and the moderately enlarged claw G2 with a different type of serration as in *Cyprettadopsis* and *Pseudocyprretta*, but also in some (mostly unornamented) species of *Zonocypris l.* The fact that the genus *Zonocypris* is not a monophyletic genus was already foreshadowed by Higuti and Martens (2012). Diaz and Martens (2018) also questioned the position of the Oncocypridinae in the Notodromadidae, as did Karanovic (2012 – see above) and Savatnalinton (2020), but it remains unclear if moving this taxon as a tribe in the Cypridopsinae is really the solution. Maybe acknowledging that *Oncocypris* G.W. Müller, 1898 (with 4-5 teeth bristles on the third endite of the Mx1, a 5-segmented A2 and eye tubercles on the carapace) and *Neozonocypris* Klie (1944) (with 2 teeth bristles, a 4-

segmented A2 and no eye tubercles) (Savatenalinton 2015) do not belong in the same subfamily would be the beginning of a solution.

This seemingly small taxonomic change (moving one genus from one subfamily to another within the same family) might, however, have a nomenclatorial consequence. If *Pseudocypretta* is lodged into the Cypretadopsini, then *Pseudocyprettinae* McKenzie (1982) would, lowered to the rank of tribe to fit the present taxonomy of Meisch et al.(2019), become a senior synonym of *Cyprettadopsini*. However, since the International Code of Zoological Nomenclature (ICZN) does not offer rulings for taxa above the genus-level (only recommendations), we here propose to consider the ill-described and never used subfamily *Pseudocyprettinae* as an unused senior synonym of *Cyprettadopsini* Savatenalinton (2011). To list the extensively described *Cyprettadopsini* as a synonym of *Pseudocyprettinae* would not be in the interest of taxonomic stability.

There are now five lineages with the candonid type T3 in the Cypridoidea, namely one lineage in the Notodromadidae (two genera in the Oncocypridinae, but see above) and four lineages in the Cyprididae (three genera in two tribes in the Cypridopsinae, one genus in the Callistocypridinae and one in the Batucyprettinae).

**Table 4.** Comparative morphology of *Pseudocypretta*, *Cyprettadopsis* and *Cypretta* (mainly based on Klie (1932); Ma and Yu (2020); Savatnalinton (2018), (2020); present paper and various other descriptions of *Cypretta* species).

<b>Character</b>	<b><i>Pseudocypretta</i></b>	<b><i>Cyprettadopsis</i></b>	<b><i>Cypretta</i></b>
Cp anterior overlap	LV>RV	LV>RV	RV>LV
Cp lateral shape	triangular	elongated	variable
RV marginal septa	fully developed	fully developed posteriorly	fully developed
LV marginal septa	incomplete	fully developed posteriorly	fully developed
RV posterior selvage	present	present	absent (variable?)
LV external list	complete, no pores	complete, with pores	incomplete/pores variable?
LV inner list post-vent part	largely straight	partly straight	not straight (variable?)
A2 claw G2	strongly serrate	strongly serrate	normal
T1 seta d	absent	absent	present
T1 seta a	subequal	unequal	subequal
T2 seta d1	absent	absent	present
T2 penultimate segment	undivided	undivided	divided
T3 segment 4	separate	separate	fused with segment 3
CR	strongly reduced/absent	strongly reduced	somewhat reduced (seta or claw missing)

### 3.5 Conclusions

McKenzie (1982) already described several examples in ostracod taxonomy where “homeomorphy is a persistent joker in the taxonomic pack”. Parallel and convergent evolution is known from many animal groups, and ostracod are no exception. It would appear from the above discussion, that for some of the often-used characters in ostracod taxonomy (valve overlap, marginal septa in valves, terminal segment on T3 of the candonid type or with a pincer, CR of the cypridopsine type, reduced to flagellum or not, ...) there is clear evidence of parallel evolution. This leads to a mosaic of characters and character states, and it is the task of the taxonomist to bring order into this chaos, preferably by translating phylogenetic evolution into taxonomic classifications.

The present description of a new species of the previously South East Asian genus *Pseudocyprretta* has allowed us to discuss the relevance of several morphological characters in the taxonomy of cypridoid taxa with a candonid type T3. Given the mosaic development of characters and character states in the Cypridoidea, resulting from homeomorphy and parallel morphological evolution in different lineages, an integrated taxonomic approach, combining morphological and molecular approaches, is the way forward. It is hoped that the advancements in genomic techniques will overcome the difficulties thus far encountered with single genetic marker approaches in the Cyprididae, which thus far never provided a good resolution of the various genera and subfamilies in this family.

**NOTE IN PRESS**

During the review process of the present manuscript, the paper by Savatnalinton *et al.* (2022) was published. This paper redescribed the type species of the genus *Pseudocypretta* and reached very similar conclusions regarding the taxonomic position of this genus to those expressed in the present paper. We have adapted our Table 2 with information from this paper.

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#### **4 ON *PSEUDOCYPRETTA* N. SP. (OSTRACODA, CYPRIDIDAE) FROM LAKE SIBAYA, SOUTH AFRICA**

##### **ABSTRACT**

*Pseudocypretta* n.sp is described from Lake Sibaya in South Africa. This is the first African species of the genus, which has two further Asian and one South American species. Clearly, the genus has a much wider distribution than originally thought and it is postulated that further species could be found in zoogeographical regions from where it is presently unknown. *Pseudocypretta* n.sp. differs from its congeners in the much larger overlap of the right valve by the left valve along anterior, ventral and posterior sides, and by the shape of the valves both in dorsal and in lateral views. The new species completely lacks the caudal rami, just as in the South American *P. amor*, whereas both Asian species do have cypridopsine-type reduced caudal rami.

Key words. — areal extension, Afrotropics, comparative morphology, taxonomy, circumtropical

## 4.1 Introduction

The genus *Pseudocypretta* Klie, 1932 thus far comprises three species: the type species *Pseudocypretta maculata* Klie (1932) from Indonesia, India and Thailand, *P. lineata* Ma & Yu (2020) from Hainan Island, southern China, and *P. amor* Ferreira *et al.*, 2022 from several localities in the four major floodplains of Brazil, South America. All three species have been found inhabiting rice fields, pools, lakes and streams (Klie 1932; Victor & Fernando, 1979; Savatnalinton, 2014, 2015, 2017, 2018, 2021; Savatnalinton *et al.*, 2022; Ma & Yu 2020, Ferreira *et al.*, 2022). Because the first two species were originally exclusively found in South-East Asia, it was assumed that the genus was confined to this region. The discovery of *P. amor* from South America therefore came as a surprise, as it consisted a large range expansion. Here, we describe a fourth species in the genus, *Pseudocypretta*.sp, from Lake Sibaya in South Africa. It appears that the genus *Pseudocypretta* has a much wider distribution in the southern hemisphere than was originally thought.

## 4.2 Material and Methods

### 4.2.1 Study area

The material used in the present study was collected from Lake Sibaya, Maputaland (or Umhlabuyalingana) in Zululand, a permanent freshwater lake situated on the east coast of South Africa. It is part of the Greater St. Lucia Wetland Park, which in December 1999, was declared a UNESCO World Heritage Site. In 2009, it was renamed as the iSimangaliso

Wetland Park. Lake Sibaya is situated on the seaward margin of the Zululand Coastal Plain and approaches to within 1 km of the sea, from which it is cut off by a series of high forested sand dunes. The approximate location is between 27° 15' S – 27° 25' S and 32° 32' E – 32° 44' E. The surface of the lake is about 20 m above sea level, but the bottom extends in places to 20 m below sea level, making its maximum depth c 40 m. Depending on lake level fluctuations, the lake's surface is between 60 and 77 km<sup>2</sup> (Hill, 1979). The lake is situated on Recent and Tertiary sands which are porous, and groundwater can be expected to affect the water budget. Groundwater levels are correlated with lake levels and the lake can be regarded as an exposed portion of the water table (Hill, 1979). The occurrence of well-developed sub-marine canyons is strongly suggestive of a former extension of the Pongolo River to an estuary at Sibaya in Pleistocene time, the existing lake thus presenting the former lagoon of the Pongolo River prior to the development of the river's present course northwards along the eastern foot of the Lebombo Range to join the Ingwavuma and Usutu rivers and to form the Maputo River (South African Wetland Conservation Programme, 1995).

Interpretation of sediment cores reveals that a proto-Lake Sibaya existed on drowned dune topography, during the period ± 43,500 BP to ± 25,500 BP prior to the Last Glacial Maximum. During the early to mid-Holocene the Lake Sibaya site was occupied by a saline lagoon which underwent isolation from the sea ± 5030 BP. Since the mid-Holocene, the lake has evolved to totally freshwater conditions and has undergone little sedimentation (Miller 1998: ii).

#### 4.2.2 Sampling

The present material was collected during a sampling campaign in Kwazulu-Natal in October 1994 (Martens *et al.*, 1998). This campaign was part of a multi-annual survey of the non-marine ostracods of southern Africa. Samples were taken with a rectangular hand net (28cm x 14cm, mesh size approximately 160  $\mu\text{m}$ ), preserved in the field in 4% formalin and later in the lab sorted and transferred to 70% ethanol buffered with sodium tetraborate. Samples were taken amongst macrophytes or by whirling up sediment. Environmental variables: water temperature (WT), pH, and electrical conductivity (EC) were measured *in situ*, close to the macrophytes and/or sediment.

#### 4.2.3 Preparation and illustration of soft parts and valves

Specimens were dissected with hand-held small needles under binocular microscope. The ostracod carapace was first opened, and the valves were separated from the soft parts. Soft parts were then dissected in a drop of glycerine on a glass slide. The dissected appendages were covered with cover-slip and sealed with transparent nail polish. Valves were stored dry in a micropaleontological slide. Drawings of the appendages were made using a camera lucida (Olympus U-DA) attached to an optical microscope (Olympus CX-41). Carapaces and valves were illustrated and measured using Scanning Electron Microscopy (SEM; Fei Qanta 200 ESEM, - Royal Belgian Institute of Natural Sciences, Brussels, Belgium) in different views (internal, lateral, dorsal and ventral). To ascertain the absence of the caudal ramus, critical point dried specimens were also investigated with SEM.

The type material and illustrated specimens are stored in the collection of the Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

#### 4.2.4 Abbreviations used in text and figures

RV, right valve; LV, left valve; LVi, left valve inner view; RVi, right valve inner view; Cp, carapace; CpRl, carapace right lateral view; CpD, carapace dorsal view; CpV, carapace ventral view; L, length; H, height; W, width; A1, antennula; R, Rome organ; WO, Wouters organ; A2, antenna; CR, caudal ramus; Md, mandible; MdPalp, mandibular palp Mx1, maxillula; T1, first thoracopod; T2, second thoracopod; T3, third thoracopod.

Thoracopod terminology follows Broodbakker & Danielopol (1982), of the second antenna the revised model proposed by Martens (1987), and of the second and third thoracopods Meisch's nomenclature (2000). Higher taxonomy of the Ostracoda follows Horne *et al.*, (2002) and Meisch *et al.*(2019).

### 4.3 Results

Class OSTRACODA Latreille, 1802

Subclass PODOCOPA G.O. Sars, 1866

Order PODOCOPIDA G.O. Sars, 1866

Suborder CYPRIDOCOPINA G.O. Sars, 1866

Superfamily CYPRIDOIDEA Baird, 1845

Family CYPRIDIDAE Baird, 1845

Subfamily CYPRIDOPSINAE Kaufmann, 1900

Tribe CYPRETTADOPSINI Savatentalinton, 2020

Genus *PSEUDOCYPRETTA* Klie, 1932

Diagnosis: see Ferreira *et al.* (2022)

Type species: *Pseudocypretta maculata* Klie (1932)

Other species: *P. lineata* Ma & Yu, 2020; *P. amor* Ferreira, Almeida, Higuti & Martens, 2022; *P. n.sp.*

#### 4.3.1 *Pseudocypretta* n.sp.

(figs. 1–2)

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##### 4.3.1.1 *Type locality.*

— RSA/94/071- Lake Sibaya at jetty, Zululand. Approximate coordinates: 27°25'13" S, 32°41'53" E. Collected by Koen Martens, Michelle Hamer & Mike Coke. At the time of collecting, EC = 645  $\mu$ S/cm, WT = 22 °C and pH = 8.6. Accompanying ostracod fauna: *Candonopsis* sp.; *Physocypria* sp.; *Darwinula stevensoni* (Brady & Robertson, 1870); *Perissocytheridea* sp.; *Zonocypris costata* (Vavra, 1897).

#### 4.3.1.2 *Material examined.*

— Holotype: 1 ♀, with soft parts dissected in glycerine in a sealed slide and with valves stored dry in a micropaleontological slide (VF279).

Paratypes: 2 ♀ with soft parts dissected as the holotype (VF272 and VF276), 3 ♀ carapaces stored dry in micropaleontological slides after use for SEM illustrations (VF273, VF274, VF275).

#### 4.3.1.3 *Diagnosis.*

— Cp rounded in dorsal and ventral views and with sub-triangular shape in lateral view, carapace surface set with a many shallow pits; LV largely overlapping RV along anterior, ventral and posterior margins; Both LV and RV with large outer lists along anterior, ventral and posterior margins; RV with ca. 11 fully developed marginal septa; LV with septa incompletely developed. A2 with claw G2 slightly stronger developed and set with stronger teeth than other claws. Md-palp third segment with four dorsal setae. Mx1 with sideways directed bristles absent; first endite with three apical claws; third endite with two smooth bristles. first segment of Mx1-palp with sub-apical seta present; second segment elongated, L c. twice W; T1 with setae b and d absent. T2 with seta d<sub>1</sub> absent and penultimate segment undivided, claw h<sub>2</sub> unusually strongly curved. T3 with fourth segment not fused with third segment and carrying three apical setae. CR fully absent. Male unknown.

#### 4.3.1.4 *Differential diagnosis.*

— *Pseudocypretta* n.sp. can be distinguished from its three congeners most easily by the unusually large overlap of the RV by the LV on all sides, except the dorsal side, resulting in

a large frontal overlap in dorsal and ventral view, by the fact that the greatest width in dorsal and ventral views is situated well beyond the middle and that in the same views the anterior margin is rather pointed, compared to the broadly rounded posterior margin. From *P. maculata*, the new species can also be distinguished by the fact that the highest point in the type species is situated in front of the middle, while in *P. n.sp.* the greatest height is situated almost in the middle. Together with *P. amor*, the new species is also different from the two Asian taxa by the complete absence of the CR.

#### 4.3.1.5 *Description of female.*

— LVi (fig. 1A) with inner lamella wide along anterior margin, narrow along ventral and posterior margins; inner groove running parallel to the ventral margin; large outer list running along valve margin; large inner list running beyond halfway the anterior margin, almost parallel along the ventral margin and forming an inner groove there, and straight, not parallel to the ventro-posterior margin, showing an outward doubling on this straight part, then running dorsally parallel to the valve margin. C. 11 septa along the anterior margin and ca. three septa along posterior margin.

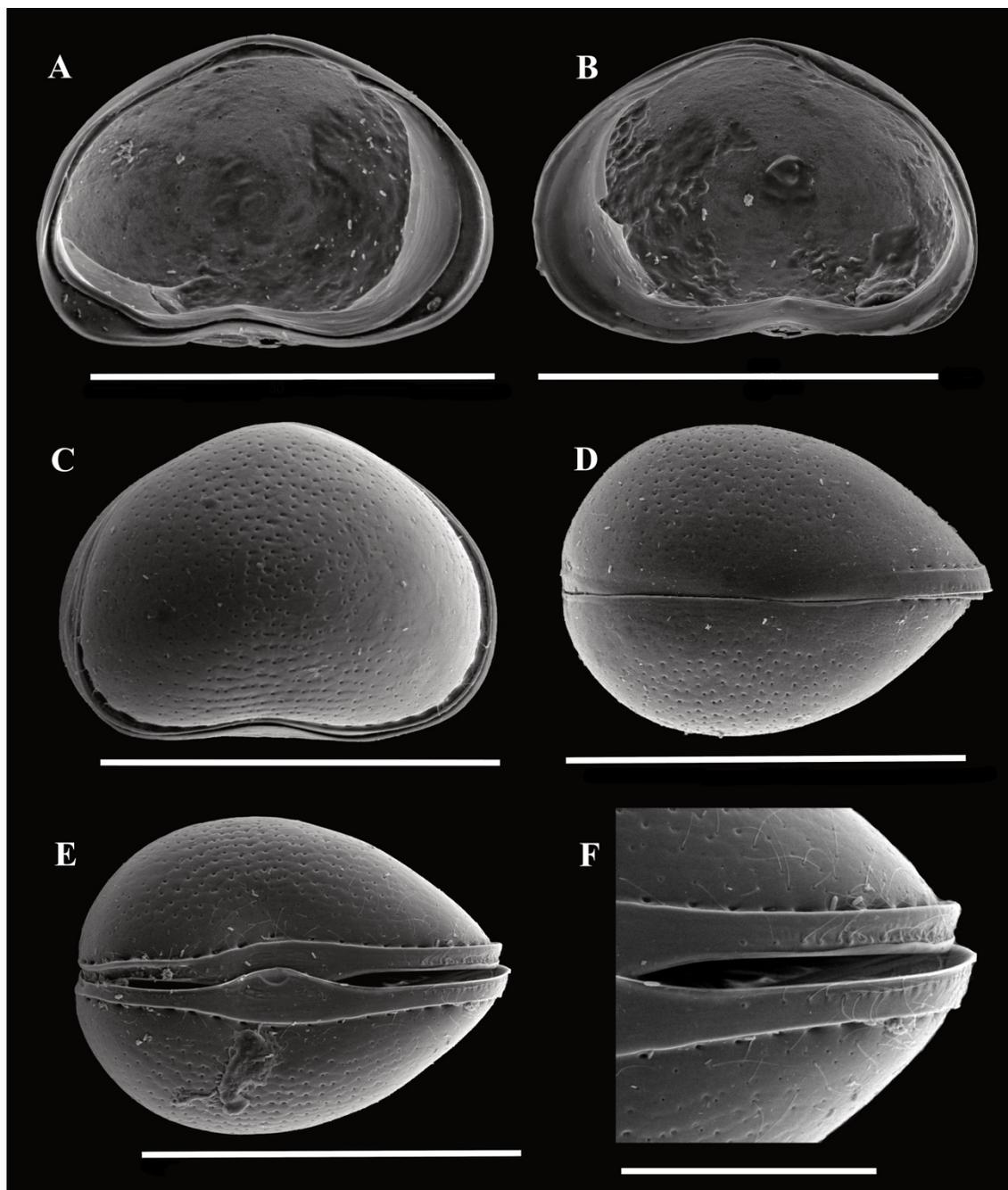
RVi (fig. 1B) with inner lamella wide along anterior margin, and narrow along ventral and posterior margins; with a weak inner list along anterior-ventral margin, both inner list and inner groove along ventral margin absent; weakly inwardly displaced posteroventral selvage. Ca. 10 anterior septa along the anterior margin and ca. three septa along posterior margin. CpRl (fig. 1C) with a rounded, subtriangular shape; greatest height situated almost in the middle; LV strongly overlapping RV at the anterior, posterior and ventral margins and slightly along the dorsal margin. CpD and CpV (fig. 1D–F) with oval shape, posteriorly

more broader rounded than the bluntly pointed anterior side; greatest width situated strongly posteriorly to the middle; CpV with a series of pores (linked to the septa) in both valves along ventral margin extending to the anterior margin of Cp; in the middle region with a rounded expansion in both valves; both valves also with clear external lists.

Table 1. Measurements (in  $\mu\text{m}$ ) of *Pseudocypretta* n.sp.

Cp/Valve view	Specimen #	L	H	W
<b>LVi</b>	VF276b	344.2	263.3	
<b>RVi</b>	VF276b	340.4	233.3	
<b>CpRl</b>	VF274	334.6	245.0	
<b>CpD</b>	VF273	353.3		255.0
<b>CpV</b>	VF275	353.3		257.1

A1 (fig. 2A – chaetotaxy not illustrated): composed of seven segments. First segment with short dorso-subapical (reaching tip of next segment) and two long ventro-apical setae, Wouters organ present. Second segment c twice wider than long, with short dorso-apical seta (reaching 1/4 of next segment) and small Rome organ. Third segment bearing two setae: one long dorso-apical (reaching beyond tip of penultimate segment) and one very short ventro-apical setae (reaching half of next segment). Fourth segment with two long dorsal setae and two short ventral setae (longer seta reaching beyond tip of next segment, length of shortest seta about half of longer one). Fifth segment dorsally with two long setae, ventrally with two (one long, one short) setae, short one reaching half of terminal segment. Penultimate segment with four long apical setae. Terminal segment with three (two long, one short) apical setae and markedly long aesthetasc ya, its length about length of last five segments, length of short seta ca. 2/5 of that of aesthetasc ya.



**Figure 2.** Carapace and valves of *Pseudocypretta* sp1. nov. from South Africa. A, LVi (VF276); B, RVi (VF276); C, CpRl (VF274); D, CpD (VF273); E, CpV (VF275); F, CpV, detail of anterior margin (VF275). Scale bars: A-E, 300  $\mu$ m; F, 100  $\mu$ m.

A2 (fig. 2B-C): composed of five segments (one protopodite, one reduced exopodite and three endopodite segment). Protopodite carrying two short and one long ventral seta (ca. 3x the length of the short one). Exopodite consisting of a small plate with three setae, two short and one long, the latter reaching beyond the tip of the second endopodite. First endopodal segment with a ventral aesthetasc Y, ca. half the length of the segment; one long ventral, sub-apical seta (slightly shorter than the segment), five long hirsute natatory setae (reaching beyond the tips of the z setae) and one short accompanying seta (reaching halfway the second endopodal segment). Second endopodal segment with a group of four medio-ventral t setae of unequal length (two long reaching the tips of G claws one with 1/3 the length of the long ones, and one shorter reaching the tip of the second endopodal segment), and a group of two unequally short medio-dorsal setae; three long z setae (z1, z2 and z3); and three claws (G1, G2 and G3); claw G2 somewhat stronger developed and serrated than the other two claws. Terminal segment (fig. 2C) with one long claw GM, and one slightly shorter and more slender claw Gm; one aesthetasc y3 and its accompanying seta (slightly longer than y3); seta g absent.

MdPalp (not illustrated): first segment with two setae (S1, S2), one long and slender seta and a thin, smooth  $\alpha$  seta. Second segment dorsally with three unequal long apical setae, shortest almost reaching tip of next segment; ventrally with group of 3 three long hirsute setae, one short hirsute seta and small, plumose, cone-shaped  $\beta$  seta with pointed tip. Penultimate segment bearing 3 groups of setae: dorsally with group of four unequal, long, subapical setae; laterally with apical  $\gamma$  seta and three further apical setae, the former slightly plumose (length  $\sim 3.2\times$  terminal segment); ventrally with two subapical setae, one

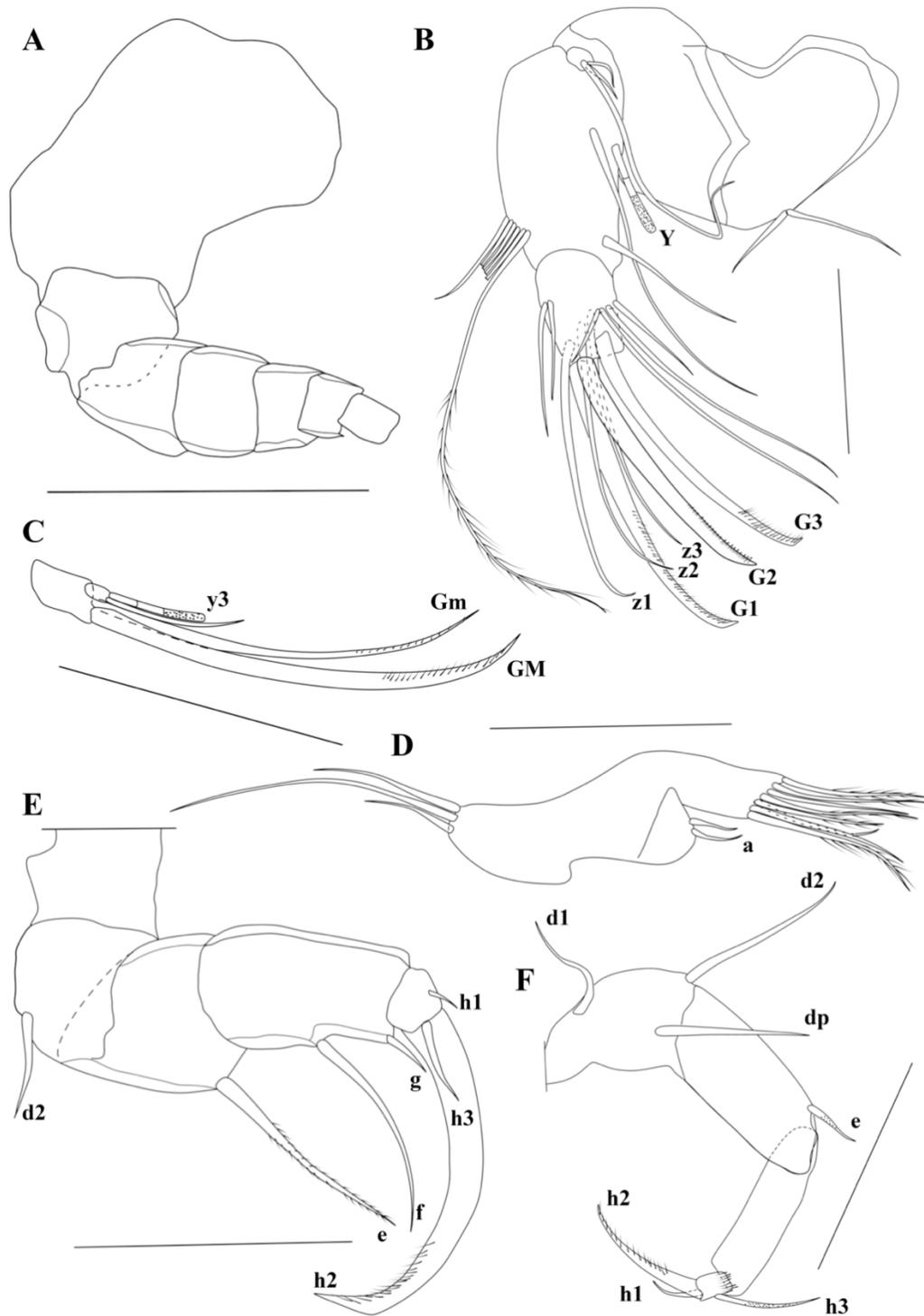
long (reaching tip of terminal claws), one short (almost reaching tip of terminal segment). Terminal segment bearing three large claws and three shorter setae, length of large claws  $\sim 4.2\times$  that of terminal segment.

Mx1 (not illustrated): with two-segmented palp, basal segment of palp dorsally with group of five long, unequal apical setae and long subapical seta; laterally with short subapical seta (reaching  $\sim 1/4$  length of terminal segment), terminal segment elongated (length  $\sim 2\times$  width), apically with three claws and two setae. Two large bristles on third endite smooth, with pointed-tip (without spatula-shaped apex).

T1 (fig. 2D) composed of an endopodite and a protopodite. Endopodite a conical palp, apically with three hirsute setae, two unequally short and one long (more than twice the length of the short one). Protopodite with two equally short a-setae, and eight apical hirsute and unequally long setae; setae b and d missing.

T2 (fig. 2E) composed of a five segmented walking leg. First segment with seta  $d_1$  absent. Second segment with a smooth and stout seta  $d_2$ . Third segment with apical seta e hirsute and long (reaching beyond the middle of the terminal segment). Fourth segment medially with seta f smooth and long; and apically with a short seta g (reaching ca. twice the length of terminal segment). Terminal segment, apically with one short ventral setae  $h_1$  ( $1/3$  the length of  $h_2$ ), a long and strongly curved claw  $h_2$ , and a short dorsal seta  $h_3$ .

T3 (fig. 2F) composed of four segments. First segment elongated, with one short seta  $d_1$  and two slightly longer setae  $d_p$  and  $d_2$ . Second segment elongated, with a short hirsute apical seta e. Third segment with sub-apical seta f absent. Terminal (4<sup>th</sup>) segment separated



**Figure 3.** Appendages of *Pseudocypretta* sp1. nov. from South Africa. A, A1 (VF279); B, A2 (VF279); C, A2 last segment (VF279); D, T1 (VF281); E, T2 (VF279); D, T3 (VF279).

from penultimate (3<sup>rd</sup>) segment (candonid type), with one short and thin seta  $h_1$ , one claw-like seta  $h_2$  and one hirsute seta  $h_3$  (slightly longer than  $h_1$ ) the latter not reflexed.

CR fully absent.

Male unknown.

#### 4.3.1.6 *Remark.*

— In order to confirm the absence of the CR in the new species, we made dissections of several females and investigated complete and critical point dried soft parts with SEM. In none of these specimens the CR could be observed.

## 4.4 Discussion

### Morphology

*Pseudocyprretta* n.sp. fits the diagnosis of the genus in nearly all of its characters and is here assigned to this genus. However, the presence/ absence of the CR is not congruent between the four species. It was found in the females of both *Pseudocyprretta maculata* and *P. lineata*, in spite of the fact that the CR is indeed reduced to a small, cypridopsine-like flagellum in these species (see discussion in Ferreira *et al.*, 2022 about the erroneous scale used for the CR in the illustrations of *P. lineata*). But in spite of extensive efforts, in neither the South American *P. amor*, nor in the South African *P. n.sp.* was the CR found. Normally, the presence or absence of the CR can be considered a character to distinguish taxa at the generic level, as was done when Karanovic (1999) described *Pseudocypridopsis* for two species without CR in both males and females (CR is always missing in male cypridopsines). However, in cypridopsines where the CR is already largely reduced, the

complete disappearance of these structures seems only a small step further. Moreover, with such difficulties to establish presence or absence, the distinction between the two genera would be cumbersome. We thus keep the four species at this stage united in the genus *Pseudocypretta*.

A remarkable structure in all four species is the large and pronounced posterior inner list in the LV, which is running straight in the postero-ventral corner, not parallel to the valve margin. So far, such a structure was thought to be typical of *Cypridopsis vidua* (O.F. Müller, 1776).

#### 4.4.1 Lake Sibaya

Lake Sibaya is the largest natural freshwater lake in southern Africa. In spite of its relatively young age, a variety of endangered or endemic species of reptiles, fish (at least one endemic gobi species was described), birds, mammals and plants occur in Lake Sibaya (Ramsar, 1991). As for microcrustaceans, so far only the copepod *Tropocyclops brevis* Dussart, 1972 was considered to be endemic to the lake (Dussart, 1972). *Pseudocypretta* n. sp. is thus far also only known from this lake but given the fact that at least *P. maculata* and *P. amor* seem to have a wide intra-continental distribution, it seems unlikely that *P. n.* sp. would turn out to be endemic to Lake Sibaya.

At this moment, eleven ostracod species have been reported from Lake Sibaya (Hart 1979, present paper – see Table 2). At least *Cyprideis* sp., *Loxoconcha* sp. and *Perissocytherideacf. estuaria* are remnants of the saline origin of the lake, the other eight species are freshwater taxa.

Table 2. Ostracod species known from Lake Sibaya

Genus	species	present paper	Hart 1979
<i>Candonopsis</i>	sp.	X	
<i>Cyprideis</i>	sp.	X	
<i>Cypridopsis s.l.</i>	sp.	X	
<i>Oncocypris</i>	sp.	X	
<i>Darwinula</i>	<i>stevensoni</i> (Brady & Robertson, 1870)	X	X
<i>Heterocypris</i>	sp.		X
<i>Loxoconcha</i>	sp.		X
<i>Perissocytheridea</i>	<i>cf. estuaria</i> Benson & Maddocks, 1964	X	X
<i>Physocypria</i>	sp.	X	(as <i>Cypria</i> sp. ?)
<i>Pseudocyprretta</i>	n. sp.	X	
<i>Zonocypris</i>	<i>costata</i> (Vavra, 1897)	X	

Verloren Vlei (which translated into English as the “lost lake”) in the Western Cape (South Africa) is a somewhat comparable lagoon, but it is much smaller and still partly connected to the sea. The Verloren Vlei thus has a salinity gradient, which moreover shows significant seasonal fluctuations (Martens, *et al.* 1996). Fifteen species of extant ostracod species were found in this lake, all but one (*Sarscypridopsis aculeata* (Costa, 1847)) being freshwater species (Martens, *et al.* 1996). The survey of the Verloren Vlei was more extensive (16 samples divided over two seasons) than that of Lake Sibaya (four samples on the same day). No species were shared by both lakes with certainty, and it is remarkable that *Cyprideis*, *Loxoconcha* and *Perissocytheridea* are fully absent from the Verloren Vlei ostracod community, in spite of the fact that there is still a salinity gradient in this lake.

#### 4.4.2 Distribution of *Pseudocyprretta*

The global distribution of the four species in the genus *Pseudocypretta* is shown in Fig 3. It is quite clear that the distribution of this genus is much wider than originally assumed. *Pseudocypretta maculata* occurs in South East Asia and India, while *P. lineata* was described from Hainan Island, southern China. Meanwhile, *P. amor* was found to be quite abundant in the four floodplains of Brazil and *P. n. sp.* is here reported from the eastern part of South Africa in Lake Sibaya. Maybe the species of this genus have often been mistaken for juvenile *Cyprretta* as they are generally much smaller than adult *Cyprretta* species, but marginal septa are still visible. The reversed anterior valve overlap (LV>RV in *Pseudocypretta*; RV>LV in *Cyprretta*) might appear to be an easy way to separate the two genera, where it not that several large species with RV>LV overlap have also been assigned to *Cyprretta s.s.* (see discussion in Ferreira *et al.*, 2022).

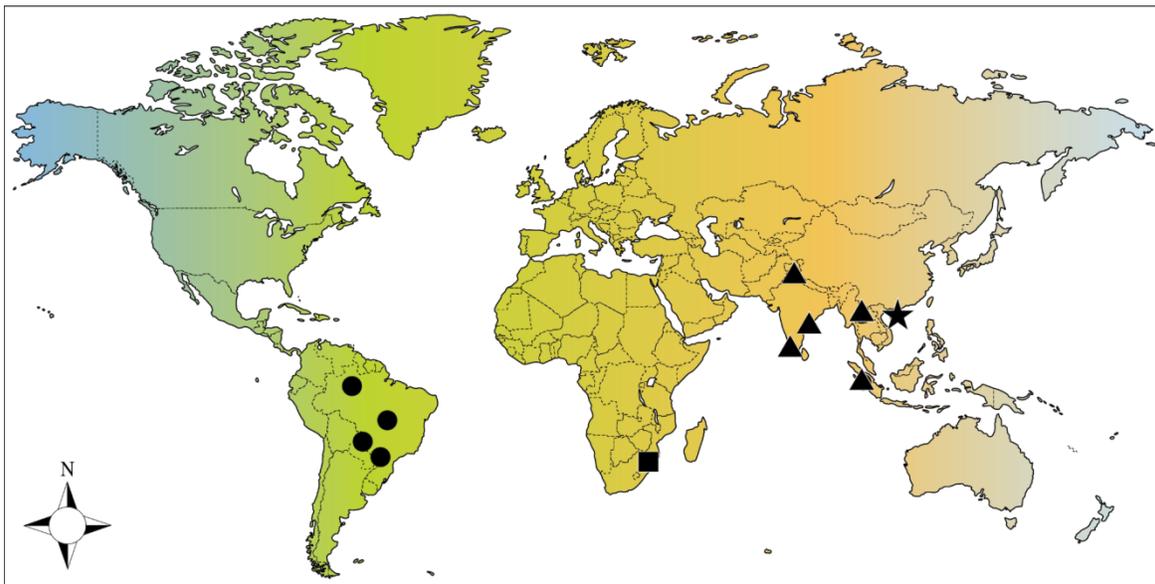


Fig. 3. Global distribution of *Pseudocypretta* species. Dot= *P. amor*; square =*P. franki* n. sp.; triangle = *P. maculata*; star = *P. lineata*.

Based on the recent interest in the genus (Ma & Yu 2020; Savatentalinton *et al.*, 2022; Ferreira *et al.*, 2022; present paper) it will be entirely possible that species of this genus will now also be recognised from other zoogeographical regions from where it is now still missing, i.e., Australasia, Nearctic and Palaearctic.

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## **5 ON A NEW GENUS AND FOUR NEW SPECIES OF THE SUBFAMILY CYPRETTINAE(CRUSTACEA, OSTRACODA) FROM BRAZILLIAN FLOODPLAINS**

### **ABSTRACT**

We describe the new genus Gen. 1 n. gen. and four new species from Brazilian floodplains. Gen. 1 n. gen. sp. 3 n. sp. and Gen. 1 n. gen. sp. 2 n. sp. were described from the Amazon floodplain only, while Gen. 1 n. gen. sp. 4 n. sp. was described from Amazon, Araguaia, and Paraná River floodplains. Gen. 1 n. gen. sp. 1 n. sp. was recorded from all four floodplains: Amazon, Araguaia, Pantanal and Paraná. The new genus is characterized by the triangular shape of the carapace in lateral view, the absence of teeth on the posteroventral inner list in the right valve and the presence of anterior marginal septa in both valves, as well as by the relatively short and thin  $\alpha$  and  $\beta$ -setae on the mandibular palp. All populations found were asexual. Owing to the clear differences in valve anatomy and limb chaetotaxy as compared to species of *Cypretta s.s.*, the four species were allocated to a new genus in the subfamily Cyprettinae.

**Keywords:** Neotropical, comparative morphology, non-marine ostracods, new taxa.

## 5.1 Introduction

With more than 2300 nominal species recorded so far, ostracods have a relatively high species richness in non-marine aquatic environments (Meisch *et al.* 2019). However, in some parts of the world, e.g. in South America, this group is often overlooked, which has led to an underestimation of its diversity (Martens & Behen 1994; Martens *et al.* 1998; Higuti & Martens 2020). In Brazil, five families have been recorded, Candonidae Kaufmann, 1900; Cyprididae Baird, 1845; Cytheridae Baird, 1850; Darwinulidae Brady & Robertson, 1885 and Limnocytheridae Sars, 1925. The Cyprididae is the most species rich with around 50% of all species of the World (Meisch *et al.* 2019). In the Cyprididae, the subfamily Cypridinae Hartmann, 1963 presently consist of one only genus, *Cypridina* Vávra, 1985 after the genus *Pseudocypridina* Klie, 1932 was transferred to the Cypridopsinae Kaufmann, 1900 (Savatentalinton 2022; Ferreira *et al.* 2022). The genus *Cypridina* presently holds 53 species worldwide and it is considered a circumtropical genus, owing to the high diversity of species in the tropical and subtropical regions (Cohuo-Duran *et al.* 2013; Meisch *et al.* 2019; Ferreira *et al.* 2023). In the Neotropical region ca. 16 species are known, of which 12 are endemic to this region (Meisch *et al.* 2019).

The subfamily Cypridinae is characterized by the presence of series of radial septa completely developed along the anterior margin of the valves, and the presence of incomplete septa or their complete absence along the posteroventral margin (Savatentalinton 2022); equally serrated G claws on the antenna (Ferreira *et al.* 2023), and a caudal ramus which is weakly developed, but mostly with full chaetotaxy of two claws and two setae (Cohuo-Duran *et al.* 2013).

Recent collections in Brazilian floodplains yielded four species resembling *Cypridina*. However, detailed observations of valves and appendages show consistent differences with the type species of this genus, *Cypridina tenuicauda* (Vávra, 1895), redescribed by Ferreira *et al.* (2023). Here, we introduce a new genus and four new species from the four main Brazilian floodplains, Amazon, Araguaia, Pantanal, and Paraná; and we describe and discuss the morphology of the new genus and species.

## 5.2 Material and Methods

### 5.2.1 Study area

The study was conducted in the floodplains of the Amazon ( $3^{\circ}02' - 3^{\circ}34' \text{ S}$ ,  $60^{\circ}50' - 60^{\circ}10' \text{ W}$ ), Araguaia ( $12^{\circ}50' - 13^{\circ}20' \text{ S}$ ,  $50^{\circ}40' - 50^{\circ}30' \text{ W}$ ), Upper Paraná Rivers ( $22^{\circ}40' - 24^{\circ}00' \text{ S}$ ,  $54^{\circ}20' - 53^{\circ}00' \text{ W}$ ) and of the South Matogrossense Pantanal ( $18^{\circ}50' - 19^{\circ}30' \text{ S}$ ,  $57^{\circ}40' - 57^{\circ}00' \text{ W}$ ) (Fig. 1). These floodplains comprise a set of different types of habitats, such as lotic: main channel of the rivers and tributaries; and lentic: temporary pools, closed and open lakes etc. (Latrubesse *et al.* 2000; Agostinho *et al.* 2004; Barros *et al.* 2004; Thomaz *et al.* 2007). For more extensive descriptions of these areas, see Ferreira *et al.* (2020).

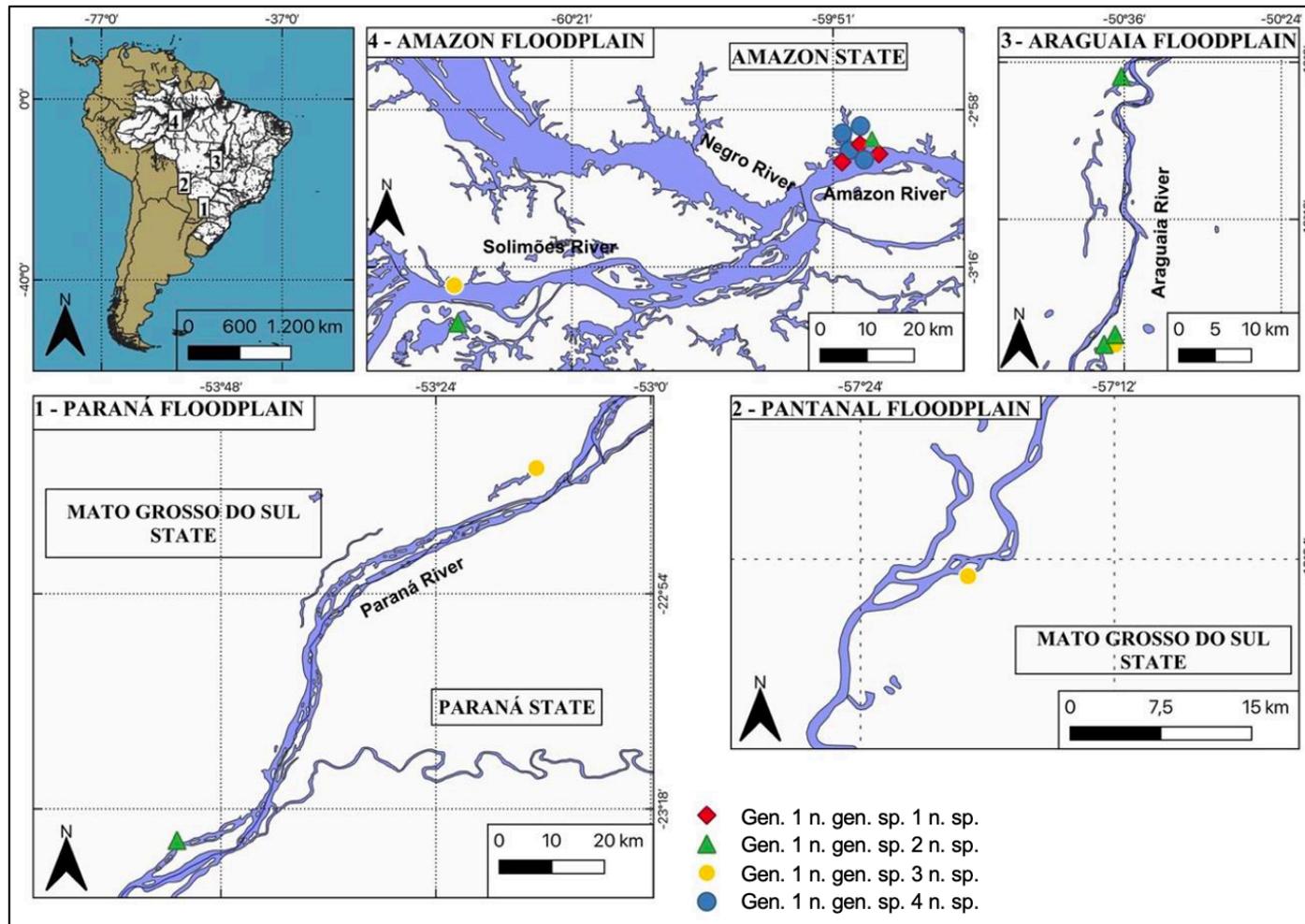


FIGURE 1. Map of Brazil indicating localities where the new species of Gen. 1 n. gen. were recorded.

### 5.2.2 Sampling

Sampling was performed in 2013 and 2018 the Upper Paraná River floodplain; in 2011 and 2012 in the Araguaia River floodplain; and in 2012 in the Amazon and Pantanal. Ostracods were sampled in aquatic vegetation and in the littoral region. The vegetation was hand-collected, and the whole plants or roots were washed in a bucket to remove the ostracods (Campos *et al.* 2017). This material was filtered in a net of 160  $\mu\text{m}$  mesh size, and then preserved in 70% ethanol buffered with sodium tetraborate. For the sediment samples, we used a rectangular hand net (28cm x 14cm, mesh size  $\sim$ 160  $\mu\text{m}$ ).

Environmental variables (Water temperature (WT) and dissolved oxygen (DO) concentration (YSI 550A oxymeter), pH (pHmeter Digimed) and electrical conductivity (EC) (conductivimeter-Digimed), were measured *in situ* close to the macrophytes or sediment.

### 5.2.3 Preparation and illustration of soft parts and valves

The carapace was opened, and the valves separated from the soft parts, using dissection needles. The soft body was dissected in a slide with a drop of glycerine. The dissection was covered with cover-slip and sealed with transparent nail polish. Valves were stored dry in micropaleontological slides. Drawings of soft parts were made using a camera lucida (Olympus U-DA) attached to a microscope (Olympus CX-41). Carapace and valves were illustrated and measured using Scanning Electron Microscopy (SEM; Fei Qanta 200 ESEM, - Royal Belgian Institute of Natural Sciences, Brussels, Belgium) in different views (valves: internal view; carapaces: lateral, dorsal, and ventral views and various details thereof).

The type material and illustrated specimens are stored in the Museum of Zoology of the University of São Paulo (MZUSPxxx).

#### 5.2.4 Abbreviations used in text and figures

A1, antennula; A2, antenna; Cp, carapace; CpD, carapace dorsal view; CpLl, carapace left lateral view; CR, caudal ramus; CpRl, carapace right lateral view; CpV, carapace ventral view; EC, Eliezer de Oliveira da Conceição; H, height; JH, Janet Higuti; JR, Jonathan da Rosa; KM, Koen Martens; L, length; LV, left valve; LVi, left valve inner view; MdCoxa, mandibular coxal plate; MdPalp, mandibular palp; Mx1, maxillula; RC, Ramiro de Campo; RV, right valve; RVi, right valve inner view; T1, first thoracopod; T2, second thoracopod; T3, third thoracopod; VF, Vitor Góis Ferreira; W, width.

The evaluation of the length of the setae (changed to short, medium length and long) as well as the terminology of the thoracopods follows Broodbakker & Danielopol (1982), revised for the A2 by Martens (1987) and T3 by Meisch (2000). Higher taxonomy of the Ostracoda follow the synopsis by Horne *et al.* (2002) and Meisch *et al.* (2019).

### 5.3 Results

Class Ostracoda Latreille, 1802  
 Subclass Podocopa G.O. Sars, 1866  
 Order Podocopida G.O. Sars, 1866  
 Suborder Cypridocopina G.O. Sars, 1866  
 Superfamily Cypridoidea Baird, 1845  
 Family Cyprididae Baird, 1845  
 Subfamily Cyprettinae Hartmann, 1963  
 Genus **Gen. 1 n. gen.**

**Type species** Gen. 1 n. gen. sp. 1 n. sp.

**Other species:** Gen. 1 n. gen. sp. 2 n. sp., Gen. 1 n. gen. sp. 3 n. sp., Gen. 1 n. gen. sp. 4 n. sp.

**Diagnosis:** Cp in lateral view with dorsal margin highly arched; inner list smooth, without the presence of inner tubercules at the posteroventral corner of the RV; A2 with G claws

equally serrated; MdPalp with  $\alpha$  and  $\beta$ -seta short and thin; CR slender and with  $S_a$  seta absent; CR attachment composed by a single branch.

### Differential Diagnosis

The new genus differs from the genus *Cypretta* (the only other genus in the subfamily) by the highly triangular shape of the carapace in lateral view, the absence of teeth on the posteroventral inner list in the RV, as well as by the equally serrated G-claws in the A2 and the relatively shorter and thinner  $\alpha$  and  $\beta$ -setae on the MdPalp.

5.3.1 Gen. 1 n. gen. sp. 1 n. sp.

(Figs. 2–5, 18)

2007 ? *Cypretta* sp.1 Higuti *et al.*: p. 1934, Table 2.

2009 ? *Cypretta* sp.1 Higuti *et al.*: p. 664, Table 1.

2010 ? *Cypretta* sp.1 Higuti *et al.*: p. 644, Table 2.

2010 ? *Cypretta* sp.1 Mormul *et al.*: p. 189.

2015a ? *Cypretta costata* Matusuda *et al.*: p. 119, Table 1.

2015b ? *Cypretta costata* Matsuda *et al.*: p. 325, Table 1.

2016 ? *Cypretta* sp.1 Higuti & Martens: Appendix I.

2017a ? *Cypretta* sp.1 Higuti *et al.*: p. 5, Apêndice I.

2017b ? *Cypretta costata* Higuti *et al.*: p. e120, Table 2.

2017 ? *Cypretta costata* Campos *et al.*: p. 38, Table 2.

2017 ? *Cypretta costata* Conceição *et al.*: p. 329, Table 2.

2017 ? *Cypretta costata* Pereira *et al.*: p. 327, Table 2.

2018 ? *Cypretta costata* Campos *et al.*: p. 6, Table 2.

2018 ? *Cypretta costata* Conceição et al.: Table S1.

2019 ? *Cypretta costata* Campos et al.: p. 375, Table 1.

2020 ? *Cypretta costata* Conceição et al. 2020: p. 1677, Table 2.

2020 ? *Cypretta costata* Higuti et al.: Table S1.

2021 *Cypretta costata* Campos et al.: p. 27, Table 1.

#### 5.3.1.1 *Diagnosis*

Cp with highly arched dorsal margin in lateral view, oval shaped in dorsal view, and anterior margin blunt, not pointed; LV with a protrusion on the postero-ventral margin; RV slightly overlapping LV; Cp surface with setae and shallow pits; T2: d<sub>1</sub> and d<sub>2</sub> with similar length.

#### 5.3.1.2 *Type locality*

Brazil – Paraná River floodplain. Suja Lake (PAR1511), in the roots of *Eichhornia crassipes* Collected on 21.03.2018 by VF, JH, KM, RC, EC and JR. Coordinates: 22°40'03.9"S, 53°12'45.7"W.

#### 5.3.1.3 *Type material*

Holotype: A female, with soft parts dissected in glycerine in a sealed slide and valves stored dry in a micropaleontological slide (VF271).

Paratypes: Two female specimens with soft parts dissected as the holotype (VF116, VF270). Four female carapaces stored dry in micropaleontological slides (VF117, VF118, VF119, VF120).

#### 5.3.1.4 *Other material examined*

Brazil – Amazon River floodplain, Calado Lake (AMA50) Collected by JH & KM on 13.10.2011 in the following plants: *Salvinia auriculata* Aubl.; *Pistia* sp.; *Ludwigia* sp.;

*Azolla* sp. Coordinates: 03°18'30.7"S, 60°34'29.0"W. One female specimen with soft parts dissected as the holotype (VF109) and four female carapaces stored dry in micropaleontological slides (VF110, VF111, VF112, VF113) – Brazil – Araguaia River floodplain, – Japonês Lake I (ARA11) Collected by JH & KM on 11.03.2011 in the roots of *Eichhornia crassipes* (Mart.) Solms. Coordinates: 13°21'33.1"S, 50°36'42.9"W. Two female carapaces stored dry in micropaleontological slides (VF114, VF115). – Brazil – Pantanal, branch of Paraguay River (PAN130) by JH and KM on 27.03.2012 in the roots of *Eichhornia crassipes*. Coordinates: 19°24'48.9"S, 57°18'51.3"W. One female carapace stored dry in micropaleontological slides (VF121).

#### 5.3.1.5 *Other localities*

See Table 2, not all localities included.

#### 5.3.1.6 *Measurements*

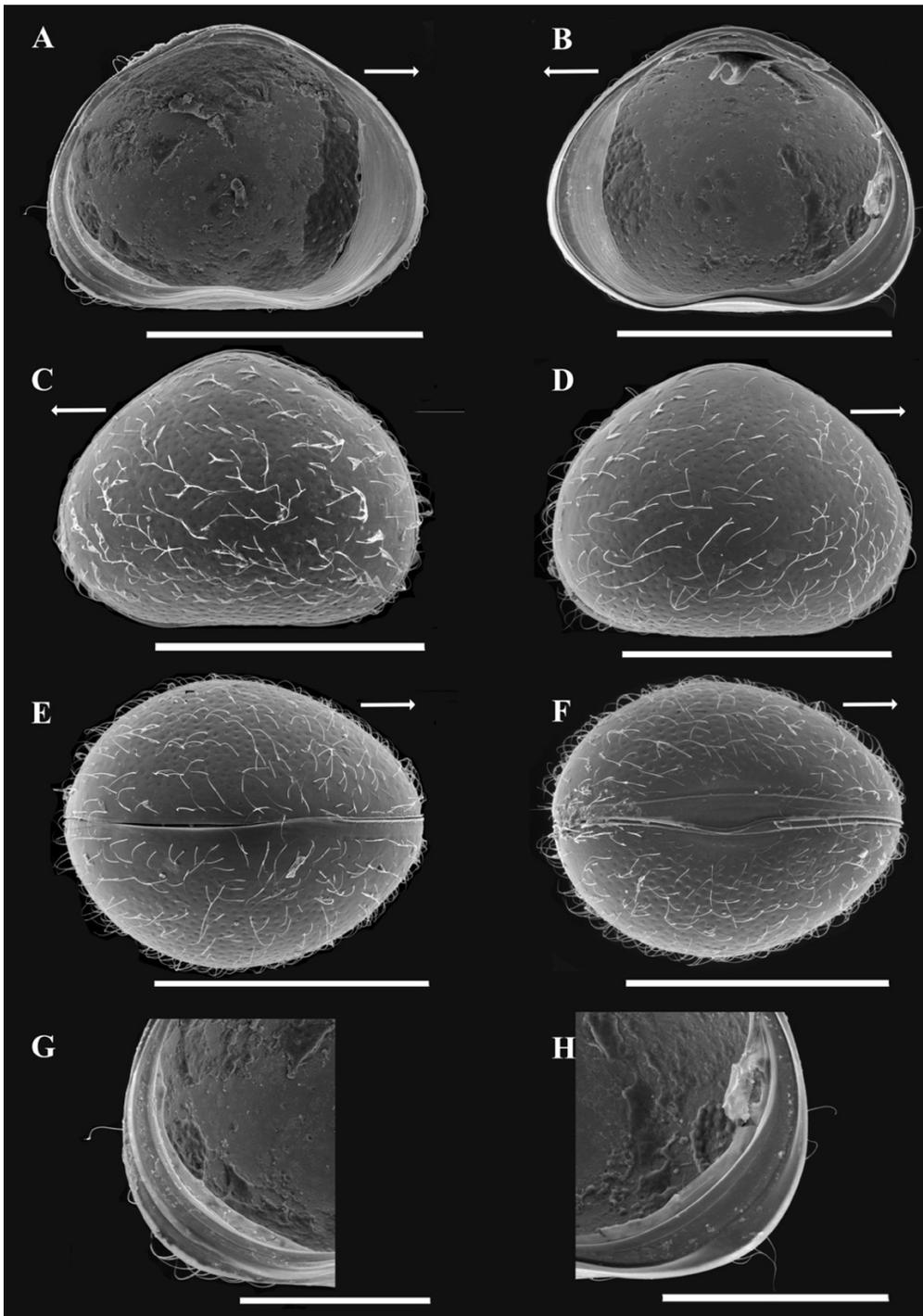
See Table 1

**Table 1.** Measurements of *Gen. 1 n. gen.* species from Brazil. SEM, Scanning Electron Microscopy.

Species	Locality	Slide number	Valve/Cp	L (µm)	H (µm)	W (µm)
Gen. 1 n. gen. sp. 1 n. sp.	PAR1511	VF116	LV	0.670	0.520	
	PAR1511	VF116	RV	0.670	0.520	
	PAR1511	VF118	CpL1	0.690	0.530	
	PAR1511	VF119	CpD	0.690		0.560
	PAR1511	VF120	CpV	0.670		0.530
Gen. 1 n. gen. sp. 2 n. sp.	AMA87	JH848	LV	0.579	0.431	
	AMA87	JH848	RV	0.592	0.452	
	AMA87	JH849	CpL1	0.603	0.462	
	AMA87	JH850	CpD	0.594		0.444
	AMA87	JH851	CpV	0.613		0.459
Gen. 1 n. gen. sp. 3 n. sp.	AMA87	JH852	LV	0.505	0.348	
	AMA87	JH852	RV	0.510	0.364	
	AMA87	JH853	CpL1	0.518	0.373	
	AMA87	JH854	CpD	0.515		0.368
	AMA87	JH855	CpV	0.507		0.367
Gen. 1 n. gen. sp. 4 n. sp.	AMA87	JH856	LV	0.533	0.383	
	AMA87	JH856	RV	0.537	0.385	
	AMA87	JH857	CpL1	0.555	0.409	
	AMA87	JH858	CpD	0.554		0.467
	AMA87	JH859	CpV	0.532		0.416
	ARA52	VF093	LV	0.553	0.382	
	ARA52	VF093	RV	0.556	0.389	
	ARA52	VF094	CpL1	0.552	0.400	
	ARA52	VF095	CpD	0.542		0.433
	ARA52	VF096	CpV	0.550		0.430

### 5.3.1.7 Description

**LVi** (Fig. 2A, G, 18A) very highly arched ( $H \approx 3/4$  of  $L$ ), of triangular shape with greatest height situated in the middle; calcified inner lamella wide along anterior margin, absent along ventral margin and narrow along posterior margin; anteriorly with incomplete inner list (running halfway up the anterior margin); postero-ventrally with a strongly inwardly displaced selvage, not running parallel to valve margin, and with inner margin of the calcified inner lamella fortified by an inner list; in between also with a weakly calcified inner list (Fig. 2G). **RVi** (Fig. 2B, H, 18B) with similar shape as **LVi**, but with posterior calcified inner lamella somewhat wider; with submarginal groove along anterior and ventral margins; posteroventral inner list without denticules. **CpLi** and **CpRl** (Fig. 2C, D) also with a triangular shape, with strongly arched dorsal margin and with greatest height also situated in the middle region; external surface densely set with long setae and shallow pits; RV overlapping LV at the dorsal, ventral, and posterior margins. **CpD** and **CpV** (Fig. 2E, F) with oval shape,  $W \approx 4/5$  of  $L$ , situated in the middle; posterior region more broadly rounded than anterior one; anterior margin with a blunt end, not pointed; **CpV** with RV overlapping LV on all sides, especially in the middle region with a flap.



**FIGURE 2.** Cps and valves of Gen. 1 n. gen. sp. 1 n. sp. **A.** LVi (VF116). **B.** RVi (VF116); **C.** CpLl (VF118). **D.** CpRl (VF117). **E.** CpD (VF119). **F.** CpV (VF120). **G.** LVi, detail of posterior margin (VF116). **H.** RVi, detail of posterior margin. Scale bars: A-F, 500 $\mu$ m; G, H, 250 $\mu$ m.

**A1** (Fig. 3A) consisting of seven segments. The first segment with three setae (two long ventro-apical and one shorter mid-dorsal); Wouters organ not seen. Second segment with one dorsal seta, reaching beyond the middle of the third segment; Rome organ not seen. Third segment with one long (dorsal) and one short (ca. 3/4 of the long one) (ventral) apical setae (both reaching beyond tip of fourth segment). Fourth segment with two unequal short ventro-apical setae and two long dorso-apical setae. Fifth segment with two unequals but short (ventral - the longer one almost reaching the middle of the terminal segment, and the other slightly shorter) and two long (dorsal) apical setae. Sixth segment with four long apical setae. Terminal segment apically carrying one long aesthetasc  $Y_a$ , one short seta (with half the length of  $Y_a$ ) and two long setae.

**A2** (Fig. 3B, C) composed of six segments (one two-segmented protopodite, one exopodite and three endopodites). Protopodite carrying three ventral setae, two mid-ventral (one of medium length, one short) and one long and hirsute ventro-apical seta. Exopodite consisting of a short plate with three setae, two unequal but both short, and one long (reaching the base of the last endopodite). First endopodal segment with one mid-ventral aesthetasc  $Y$  (ca. half the length of the segment); one long and hirsute ventro-apical seta (slightly longer than the segment), five long hirsute natatory setae (reaching the tips of the  $z$  setae) and one medium-long seta accompanying the natatory setae (reaching the tip of the terminal segment). Second endopodal segment with one medium and one long mid-dorsal setae (reaching halfway the  $G$  claws), and a group of four mid-ventral setae  $t$ -setae (two equally long, one medium, and one short); three equally long  $z$ -setae ( $z_1$ ,  $z_2$  and  $z_3$ ); and three claws ( $G_1$  the shorter one;  $G_2$  and  $G_3$ , equally long); aesthetasc  $y_2$  not observed. Terminal segment (Fig. 3 C) with one long claw  $G_M$ , and one medium claw  $G_m$ ; one aesthetasc  $y_3$  and accompanying seta (slightly longer than  $y_3$ ); seta  $g$  not observed.

**MdCoxa** (Fig. 3D) consisting of an elongated plate with ca. seven apical teeth intercalated with short setae; one hirsute sub-apical seta on the dorsal margin. **Mx1** (Fig. 4A) composed of a two segmented palp and three endites. Basal segment of the palp with a group of six setae: three long, three of medium length and one short sub-apical seta. Terminal segment elongated ( $L$  ca.  $1.5 \times W$ ), apically with three claws and three setae. Third endite with two

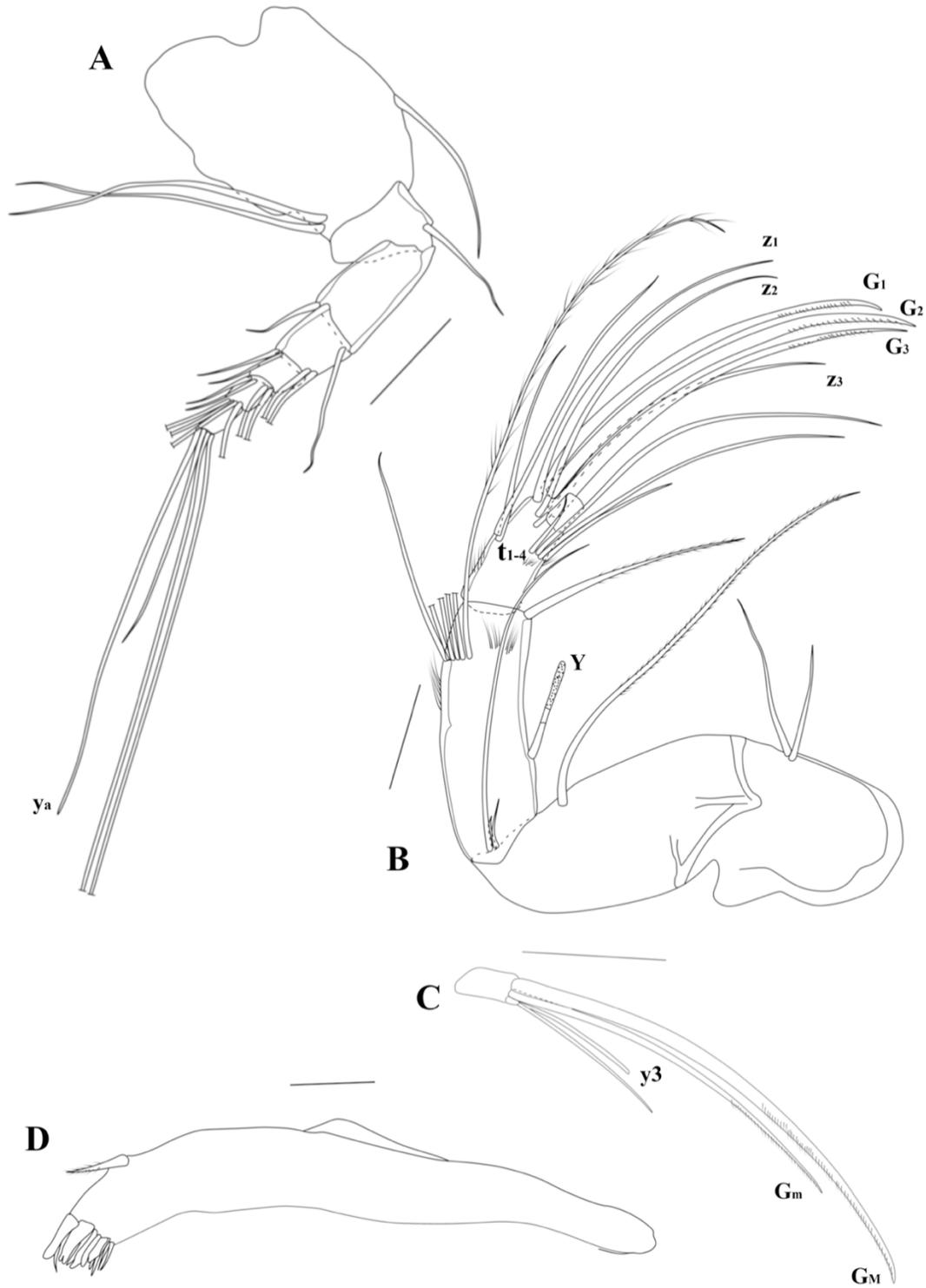
medium length and smooth bristles and one sub-apical seta (reaching beyond the tip of the endite). First endite with two sideways-directed bristles; and two medio-lateral setae of medium length. (Chaetotaxy of endites incompletely described and illustrated).

**MdPalp** (Fig. 4B, C) consisting of four segments. First segment with long setae  $S_1$  and  $S_2$ ; one long smooth seta and a short and narrow smooth  $\alpha$ -seta. Second segment ventrally with a cone-shaped and hirsute  $\beta$ -seta and two long and one medium length setae; dorsally with a group of two unequal but long setae and one short seta (ca. half the length of the long ones). Third segment dorsally with a group of four long setae; apically with four long setae and a cone-shaped, hirsute  $\gamma$ -seta. Last segment (Fig. 4C) with three claws and two setae.

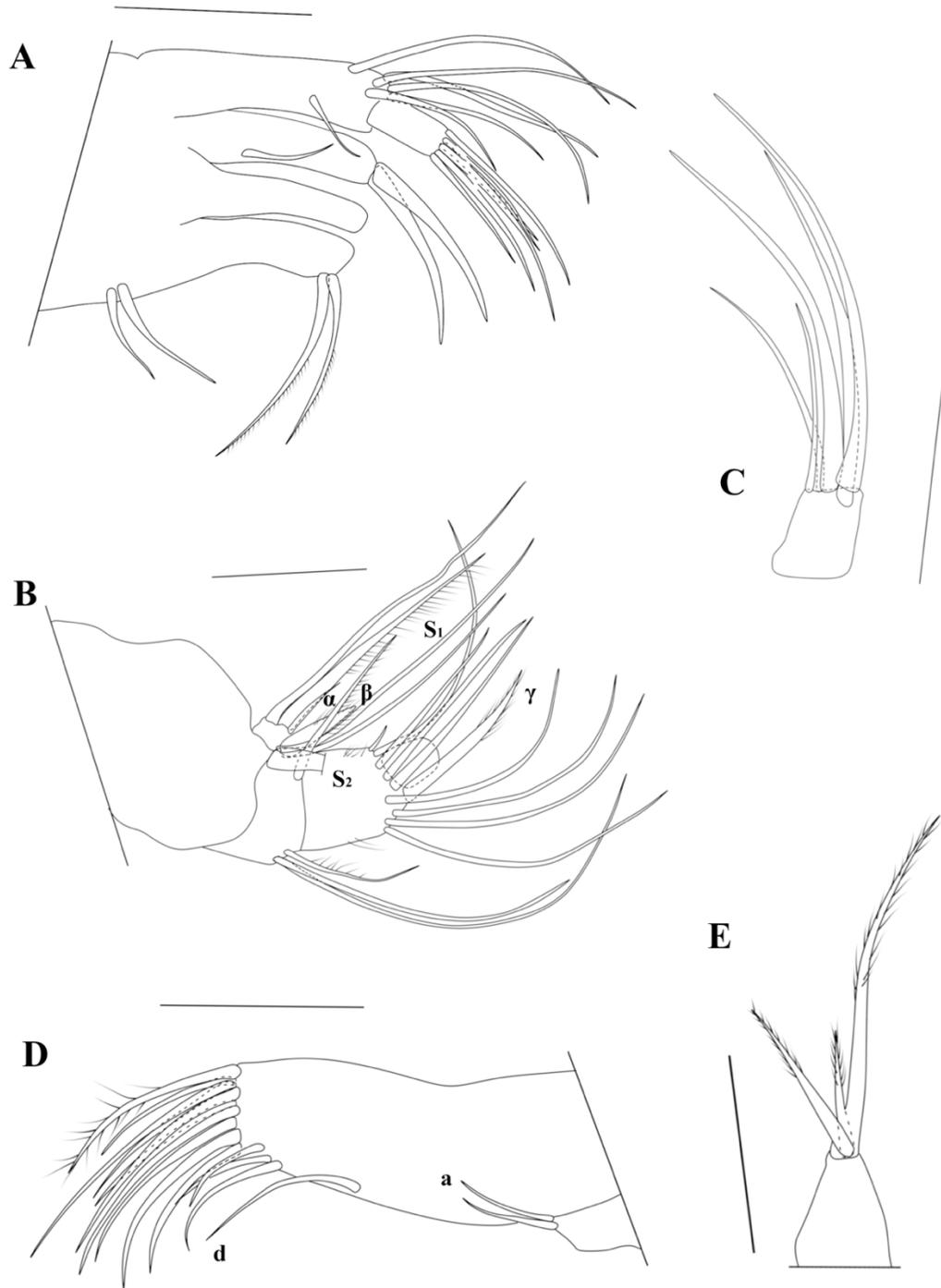
**T1** (Fig. 4D) composed of an endopodite and a protopodite. Endopodite (Fig. 4E) with three apical, hirsute setae, one short, one of medium length and one long. Protopodite with two equally short a-setae and one short d seta; 10 apical hirsute and unequally short setae and three equally short sub-apical setae.

**T2** (Fig. 5A) composed of five segments. First segment with seta  $d_1$  short. Second segment with  $d_2$  slightly shorter than  $d_1$ . Third segment with seta e of medium length (reaching beyond middle of fourth segment). Fourth segment divided in a and b segments; a segment with long apical f seta ventrally serrated; b segment apically with a short g seta (reaching tip of terminal segment). Terminal segment apically with two short setae  $h_1$  and  $h_3$ ; and a longer claw  $h_2$ , the latter weakly serrated in its distal part.

**T3** (Fig. 5B, C) composed of four segments. First segment with three setae of medium length, slightly unequal ( $d_1$ ,  $d_2$  and  $d_p$ ; almost as long as second segment). Second segment with a short apical e seta (reaching halfway the third segment). Third segment with short sub-apical f seta. Third segment (Fig. 5C) with a pincer-like structure, with a short seta  $h_1$ ; longer  $h_2$  claw; and hirsute  $h_3$  seta of medium length.



**FIGURE 3.** Appendages of Gen. 1 n. gen. sp. 1 n. sp. **A.** A1 (VF271). **B.** A2 (VF271). **C.** A2, terminal segment (VF271). **D.** MdCoxa (VF271). Scale bars: 50 $\mu$ m.



**FIGURE 4.** Appendages of Gen. 1 n. gen. sp. 1 n. sp. **A.** Mx1 (VF271). **B.** MdPalp (VF271). **C.** MdPalp last segment (VF271). **D.** T1 protopodite (VF270). **E.** T1 endopodite (VF271). Scale bars: 50 $\mu$ m.

**CR** (Fig. 5D) with long but narrow and weak ramus, two long claws (one apical  $G_a$ , almost as long as ramus, and one shorter  $G_p$  sub-apical); one short seta  $S_p$  on ventral margin, apical seta  $S_a$  absent.

**CR attachment** (Fig. 5E) a single short and narrow rod, without bifurcation.

#### 5.3.1.8 *Differential diagnosis*

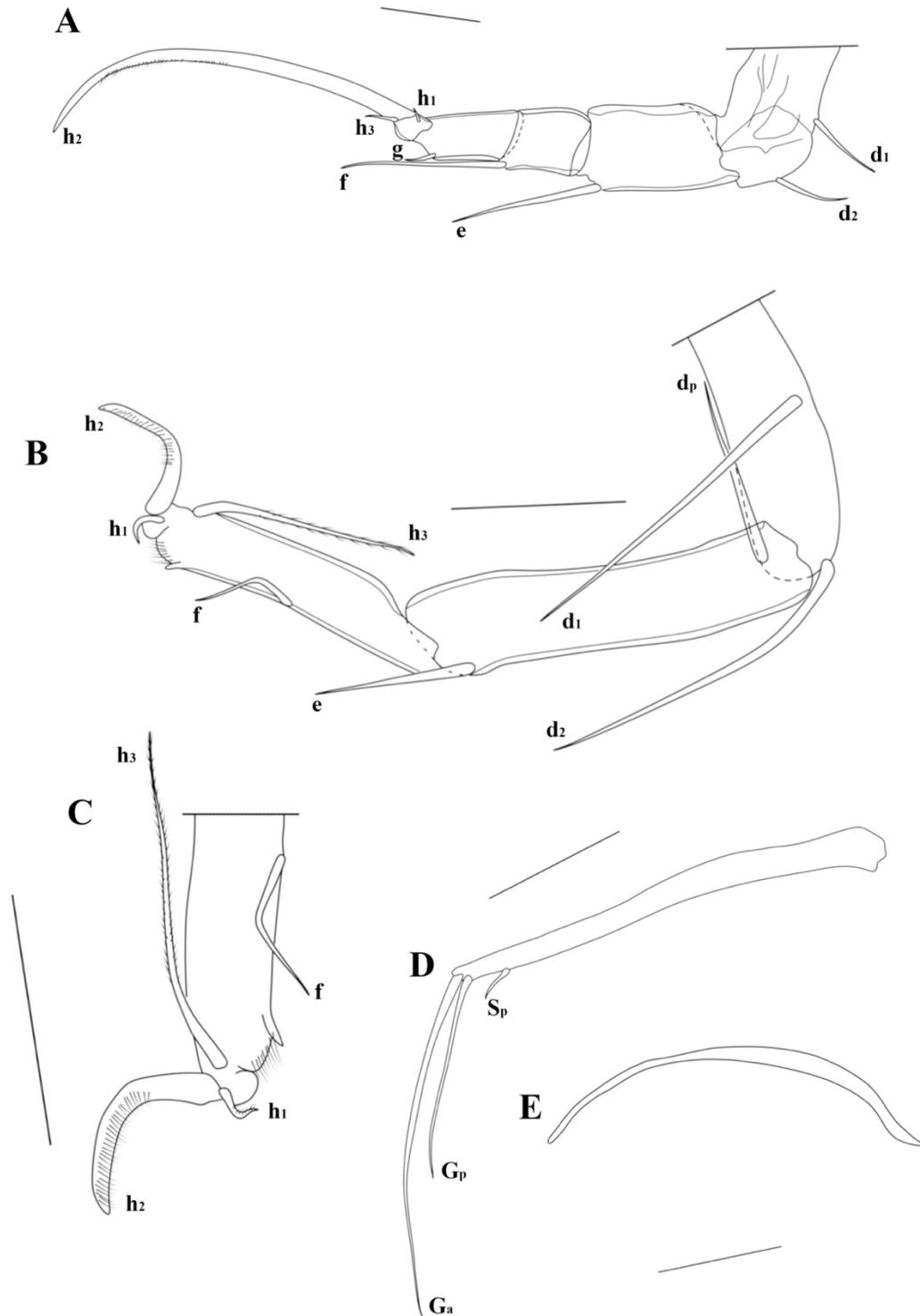
Gen. 1 n. gen. sp. 1 n. sp. is the largest of the four species described here (see Table 1). A clear difference with other species is seen on the CpL1: in the present species as the anterior margins are less pointed. Indeed, Gen. 1 n. gen. sp. 1 n. sp. has an anterior margin which is somehow “straight”, especially in dorsal and ventral views. Compared to Gen. 1 n. gen. sp. 3 n. sp., Gen. 1 n. gen. sp. 1 n. sp. has an oval shape in CpD and CpV, and its more triangular in CpL1 than Gen. 1 n. gen. sp. 4 n. sp. On the T2, the  $d_1$  seta have similar length with  $d_2$ , whereas in the other three species of the new genus the  $d_1$  is shorter than  $d_2$  (especially in Gen. 1 n. gen. sp. 3 n. sp. and Gen. 1 n. gen. sp. 4 n. sp.).

#### 5.3.1.9 *Ecology*

The species was recorded in a wide range of environmental variables: water temperature: 29.5–34.3°C; pH range: 3.1–6.8; electrical conductivity: 17–48.5  $\mu\text{S}\cdot\text{cm}^{-1}$ ; dissolved oxygen: 0.8–6.8  $\text{mg}\cdot\text{L}^{-1}$  (See Table 2, not all localities included).

**Table 2.** Localities and environmental characteristics from where the new species were recorded. Rows in bold means the type locality of the species. AMA, Amazon floodplain; ARA, Araguaia floodplain; PAN, South Matogrossense Pantanal; PAR, Paraná floodplain; WT, water temperature; EC, electrical conductivity; DO, dissolved oxygen; Azo, *Azolla* sp.; Ecr, *Eichhornia crassipes* (Mart.) Solms; Lim, *Limnobium* sp.; Sau, *Salvinia auriculata* Aubl.; She, *Salvinia herzogii* de la Sota; Smi, *Salvinia minima* Baker; Sal, *Salvinia* spp; Pis, *Pistia* sp.; Lud, *Ludwigia* sp.; Pno, *Paspalum notatum* Flugge.; Pas, *Paspalum* sp.; Ocu, *Oxycaryum cubense* (Poepp. & Kunth) Palla.; Nam, *Nymphaea amazonum* Mart. & Zucc.; Nym, *Nymphaea* sp.; Ufo, *Utricularia foliosa* L.; Utr, *Utricularia* sp.; Eaz, *Eichhornia azurea* (Sw.) Kunth; Hve, *Hydrilla verticillata*; Hra, *Hydrocotyle ranunculoides*; Pre, *Paspalum repens* P.J. Bergius; Org, organic matter; Gra, grass; Sed, sediment; Sp.1, Gen. 1 n. gen. sp. 1 n. sp.; Sp.2, Gen. 1 n. gen. sp. 2 n. sp.; Sp.3, Gen. 1 n. gen. sp. 3 n. sp.; Sp.4, Gen. 1 n. gen. sp. 4 n. sp.

Locality name	Sampling year	Locality code	Coordinates	Floodplain	Substrate	WT (° C)	pH	EC (µS.cm <sup>-1</sup> )	DO (mg.L <sup>-1</sup> )	Sp.1	Sp.2	Sp.3	Sp.4
Calado Lake	2012	AMA 50	03°18'30.7"S, 60°34'29.0"W	Amazon	Sau, Pis, Lud, Azo	34.3	3.1	44.5	6.8	X			
<b>Crixas Lake III</b>	<b>2012</b>	<b>ARA 52</b>	<b>13°21'52.6"S, 50°37'20.9"W</b>	<b>Araguaia</b>	<b>Ecr</b>	<b>30.0</b>	<b>7.0</b>	<b>45.7</b>	<b>3.2</b>				<b>X</b>
Crixas Lake IV	2012	ARA 56	13°20'47.5"S, 50°36'42.3"W	Araguaia	Ufo	30.0	6.8	54.4	6.7				X
Varal Lake	2012	ARA 81	13°01'05.2"S, 50°36'13.8"W	Araguaia	Pre	28.5	6.6	39.1	3.9				X
Japones I Lake	2011	ARA 11	13°21'33.1"S, 50°36'42.9"W	Araguaia	Ecr	29.8	6.8	38.8	5.3	X			
Paraguay River	2012	PAN 130	19°24'48.9"S, 57°18'51.3"W	Pantanal	Ecr	29.5	6.1	48.5	0.8	X			
Poço Curuça Lake	2012	AMA 60	03°22'48.9"S, 60°34'03.8"W	Amazonas	Pno, Ufo	31.5	6.6	51.8	1.4				X
Poraquequara Lake IV	2012	AMA 86	03°02'22.7"S, 59°48'03.3"W	Amazonas	Ecr	31.5	9.7	51.5	2.1		X	X	
<b>Poraquequara Lake IV</b>	<b>2012</b>	<b>AMA 87</b>	<b>03°02'20.7"S, 59°47'54.0"W</b>	<b>Amazonas</b>	<b>Ecr, Pis, Sal</b>	<b>31.5</b>	<b>9.7</b>	<b>51.5</b>	<b>2.1</b>		<b>X</b>	<b>X</b>	<b>X</b>
Poraquequara Lake IV	2012	AMA 88	03°02'29.1"S, 59°47'52.2"W	Amazonas	Pno	31.5	9.7	51.5	2.1		X		
Poraquequara Lake III	2012	AMA 90	03°03'03.3"S, 59°49'09.2"W	Amazonas	Ecr	31.5	9.5	51.3	2.9		X	X	
Amambaí River	2013	PAR 791	23°21'29.5"S, 53°52'53.8"W	Paraná	She, Eaz	20.6	7.75	29.8	8.0				X
<b>Suja Lake</b>	<b>2018</b>	<b>PAR1511</b>	<b>22°40'3.9"S, 53°12'45.7"W</b>	<b>Paraná</b>	<b>Ecr</b>	<b>32.8</b>	<b>5.7</b>	<b>17.0</b>	<b>1.8</b>	<b>X</b>			



**FIGURE 5.** Appendages of Gen. 1 n. gen. sp. 1 n. sp. **A.** T2 (VF271). **B.** T3 (VF271). **C.** T3 last segment (VF271). **D.** CR (VF271). **E.** Att (VF270). Scale bars: 50µm.

## 5.3.2 Gen. 1 n. gen. sp. 2 n. sp.

(Figs. 6-9, 18)

5.3.2.1 *Diagnosis*

Cp surface with several long setae and pits; RVi with anterior margin medially produced; RV widely overlapping LV anteriorly, dorsally, and posteriorly; in CpD and CpV views, posterior margin more broadly rounded than anterior one; first endite of Mx1 with two sideways directed bristles; T2 with  $d_1$  and  $d_2$  of similar length.

5.3.2.2 *Type locality*

Brazil – Amazon River floodplain. Poraquequara Lake IV (AMA87), in the roots of *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia* spp. Collected on 18.05.2012 by JH. Coordinates: 03°02'20.7"S, 59°47'54.0"W.

5.3.2.3 *Type material*

Holotype: A female, with soft parts dissected in glycerine in a sealed slide and valves stored dry in a micropaleontological slide (VF090).

Paratypes: Two female specimens with soft parts dissected as the holotype (VF127, VF161). Six female carapaces stored dry in micropaleontological slides (VF147, VF148, JH848, JH849, JH850 and JH851).

5.3.2.4 *Other localities*

Gen. 1 n. gen. sp. 2 n. sp. is restrict to the Amazon River floodplain, occurring there only in lentic environments (See Table 2, not all localities included).

5.3.2.5 *Measurements*

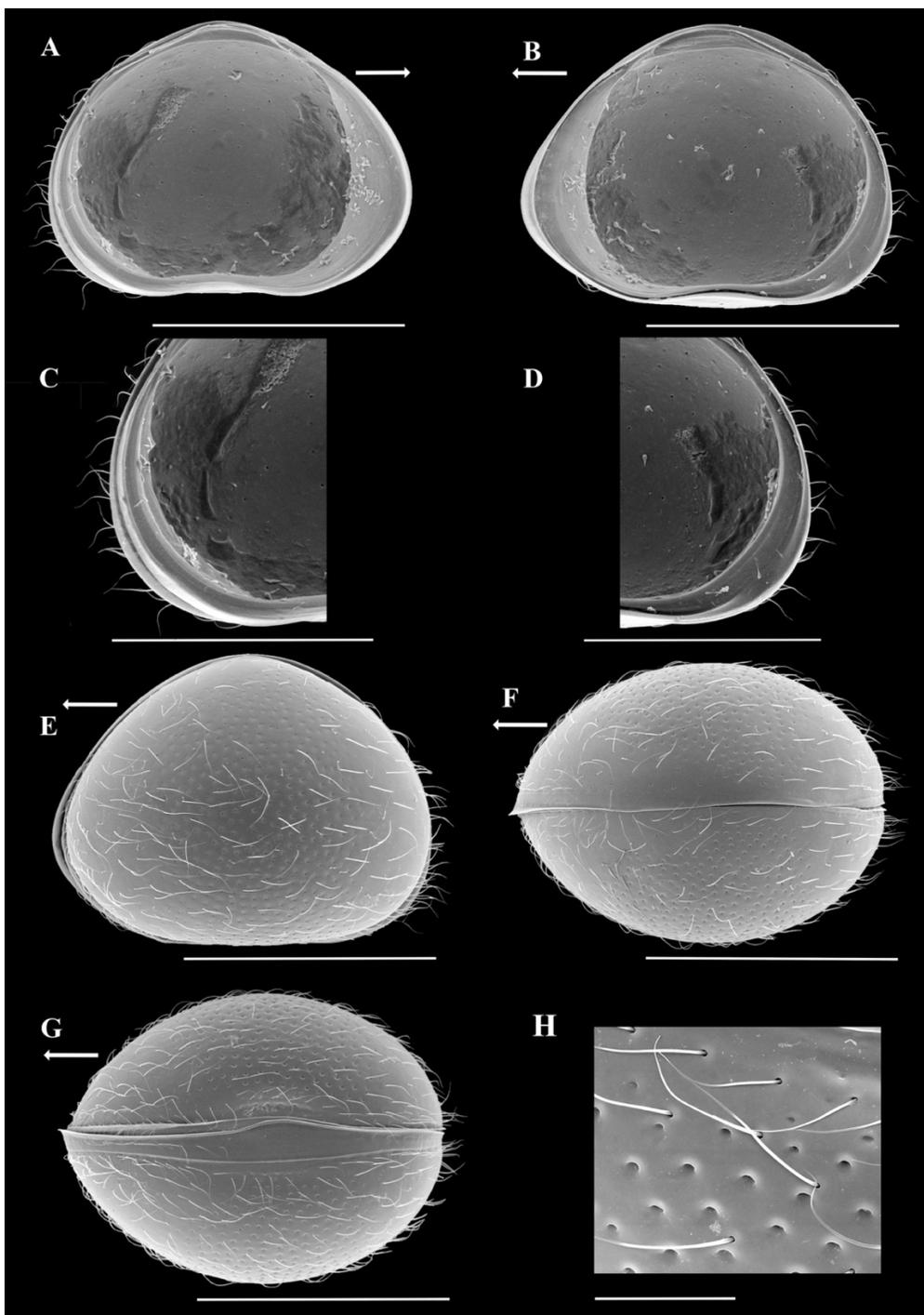
See Table 1

### 5.3.2.6 Description

**LVi** (Fig. 6A, C, 18C) highly arched (H ca.  $3/4$  of L), of triangular shape with greatest height situated in the middle; calcified inner lamella wide along anterior margin and narrow along ventral and posterior margins; anteriorly with incomplete inner list (running halfway up the anterior margin); postero-ventrally with a strongly inwardly displaced selvage, not running parallel to valve margin, and with inner margin of the calcified inner lamella fortified by an inner list; few setae present along posterior margin; ca. 13 septa along the anterior margin. **RVi** (Fig. 6B, D, 18D) with similar shape as LVi, but with a posterior calcified inner lamella somewhat wider; with a submarginal groove along ventral margin; with a more pointed anterior margin; c 13 well-developed septa along anterior margin. **CpLI** (Fig. 6E) also with a triangular shape; with strongly arched dorsal margin and with greatest height also situated in the middle region; external surface densely set with long setae and pits (Fig. 6H); RV overlapping LV at the anterior, dorsal, and posterior margins. **CpD** and **CpV** (Fig. 6F, G) with oval shape, W ca.  $3/4$  of L, situated in the middle; posterior region more broadly rounded than anterior one; anterior margin bluntly pointed; **CpV** with RV overlapping LV on all sides, especially in the middle region with a flap.

**A1** (Fig. 7A) consisting of seven segments. First segment with three setae (two long ventro-apical and one medium dorsal); Wouters organ not seen. Second segment with one dorsal seta (reaching middle of third segment); Rome organ not seen. Third segment with two short apical setae (both reaching beyond tip of fourth segment). Fourth segment with two unequal, short ventro-apical setae (shorter one ca.  $3/4$  length of longer one) and two long dorso-apical setae. Fifth segment with two unequal but short (longer one reaching the middle of terminal segment) and two long (dorsal) apical setae. Sixth segment with four long apical setae, and a short  $\alpha$ -seta reaching tip of terminal segment. Terminal segment apically carrying one long aesthetasc  $Y_a$ , one medium length seta (with half length of  $y_a$ ) and two long setae.

**A2** (Fig. 7B-D) composed of six segments (one two-segmented protopodite, one exopodite and three endopodites). Protopodite carrying three ventral setae, two mid-ventral (unequally

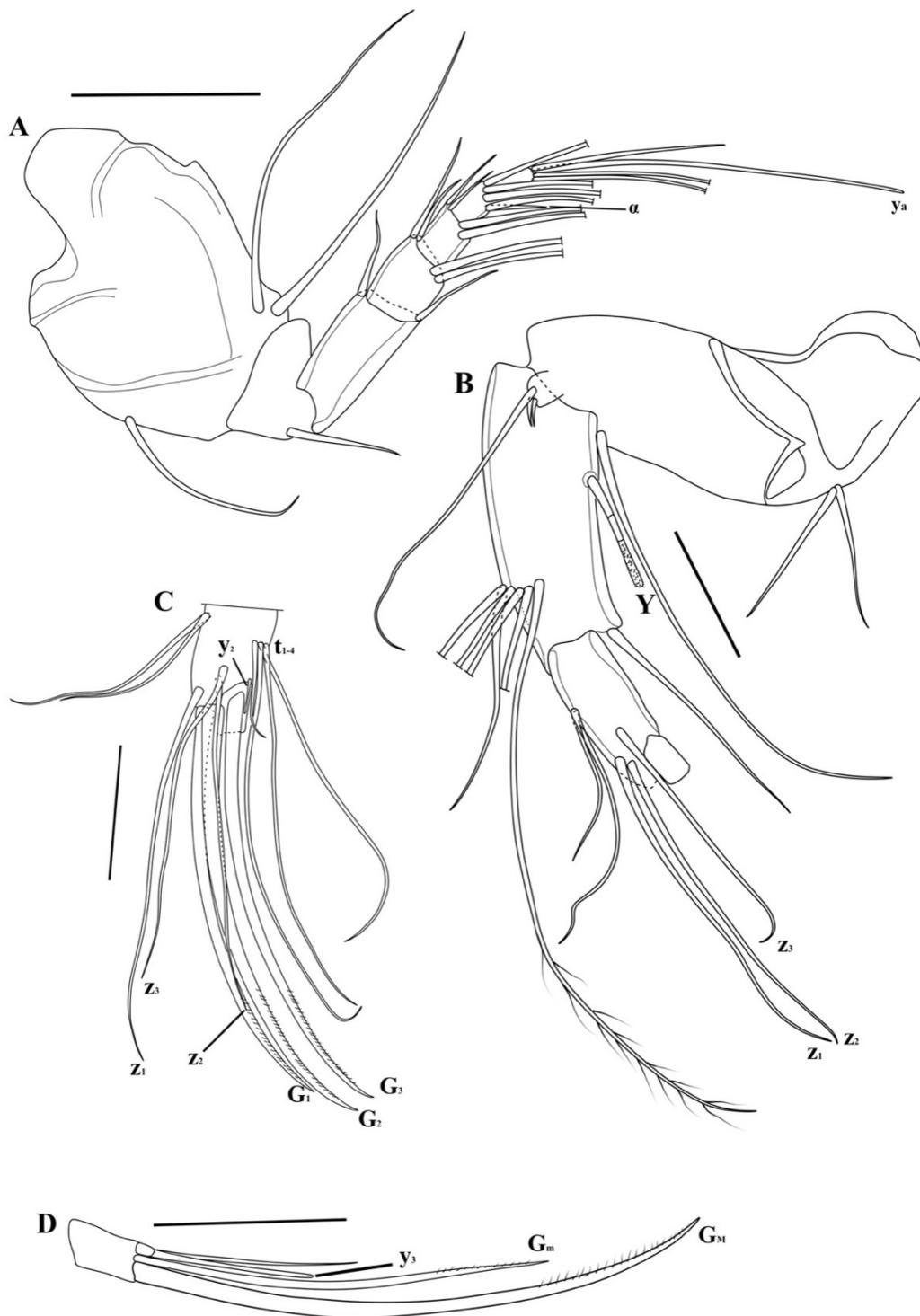


**FIGURE 6.** Cps and valves of Gen. 1 n. gen. sp. 2 n. sp. **A.** LVi (JH848). **B.** RVi (JH848). **C.** LVi, detail of posterior margin (JH848). **D.** RVi, detail of posterior margin (JH848). **E.** CpL1 (JH849). **F.** CpD (JH850). **G.** CpV (JH851). **H.** Cp, detail of surface (JH851). Scale bars: A-B, E-G, 400 $\mu$ m; C-D, 300 $\mu$ m; H, 50 $\mu$ m.

with medium length) and one long apical seta (ca. three times length of short ones). Exopodite consisting of a short plate with three setae, two equally short and one long (reaching beyond tip of first endopodite). First endopodal segment with one mid-ventral aesthetasc Y (ca. half-length of segment); one long ventro-apical seta (about as long as segment), and five long hirsute setae (reaching tips of z setae) and one medium length seta accompanying the natatory setae (almost reaching tip of second endopodal segment). Second endopodal segment (Fig. 7B, C) with one short and one long-mid dorsal setae, and a group of four mid-ventral t-setae (three equally long and one short reaching beyond last segment); three equally long z setae ( $z_1$ ,  $z_2$  and  $z_3$ ); one short aesthetasc  $y_2$  (reaching middle of terminal segment); and three claws ( $G_1$  shorter one;  $G_2$  and  $G_3$ , equally long). Terminal segment (Fig. 7D) with one long claw  $G_M$ , and one medium length claw  $G_m$ ; one aesthetasc  $y_3$  and accompanying seta (slightly longer than  $y_3$ ); seta g not observed.

**MdCoxa** (Fig. 7E) consisting of an elongated plate with ca. seven apical teeth intercalated with short setae; one subapical, dorsal, hirsute seta. **Mx1** (Fig. 8A) composed of a two segmented palp and three endites. Basal segment of palp with a group of six setae: three long, two medium length and one short sub-apical setae. Terminal segment elongated, (L ca. 1.5x W), apically with three claws and three setae. Third endite with two medium length bristles and one medium length sub-apical seta (reaching beyond tip of endite). First endite with two sideways-directed bristles; and two medio-lateral setae of medium length. (Chaetotaxy of endites incompletely described and illustrated).

**MdPalp** (Fig. 8B, C) consisting of four segments. First segment with long setae  $S_1$  and  $S_2$ ; one long smooth seta and a short and narrow smooth  $\alpha$  seta. Second segment ventrally with a cone-shaped and hirsute  $\beta$  seta and three long smooth setae; dorsally with a group of two unequal but long setae and one short seta (ca. half length of long ones). Third segment dorsally with a group of one long and two medium length setae; apically with four medium length setae and a cone-shaped, hirsute  $\gamma$  seta. Last segment (Fig. 8C) with three claws and three setae.



**FIGURE 7.** Appendages of Gen. 1 n. gen. sp. 2 n. sp. **A.** A1 (VF090). **B.** A2 (VF090). **C.** A2 detail of second endopodal segment (VF090). **D.** A2. terminal segment (VF090). Scale bars: 50 $\mu$ m.

**T1** (Fig. 8D, E) composed of an endopodite and a protopodite. Endopodite (Fig. 8E) with two sub-equally medium length hirsute setae and one short hirsute seta. Protopodite with two equally short a-setae; one short d seta; 10 apical hirsute and unequally short setae and three equally short sub-apical setae.

**T2** (Fig. 8F) composed of five segments. First segment with seta  $d_1$  of medium length. Second segment with  $d_2$  of medium length. Third segment with seta e ventrally serrated and with medium length (reaching beyond middle of fourth segment). Fourth segment divided in “a” and “b”-segments; “a” segment with long apical f seta ventrally serrated; “b” segment apically with a short g seta (reaching tip of terminal segment). Terminal segment apically with two short setae  $h_1$  and  $h_3$ ; and a longer claw  $h_2$  weakly serrated in its distal part.

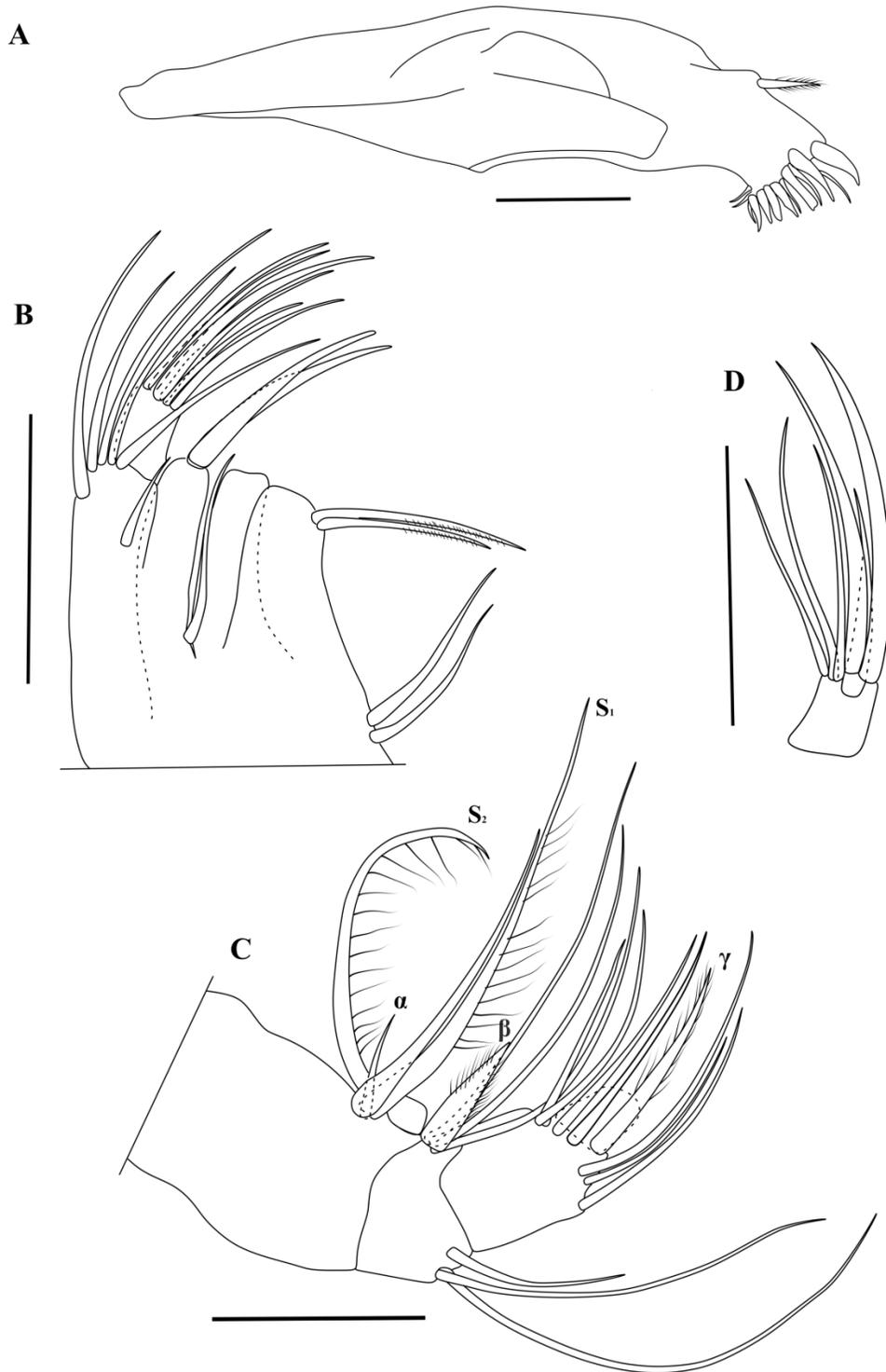
**T3** (Fig. 8G) composed of four segments. First segment with three setae of medium length but slightly unequal ( $d_1$ ,  $d_2$  and  $d_p$ ; all about length of second segment). Second segment with short apical e seta (reaching halfway the third segment). Third segment with short sub-apical f seta, (reaching end of segment). Third segment with a short seta  $h_1$ ; longer  $h_2$  claw; and hirsute  $h_3$  seta of medium length.

**CR** (Fig. 9A) with long but narrow and weak ramus, two long claws (one apical  $G_a$ , almost as long as ramus and one slightly shorter sub-apical  $G_p$ ); one short seta  $S_p$  on the ventral margin, apical seta  $S_a$  absent.

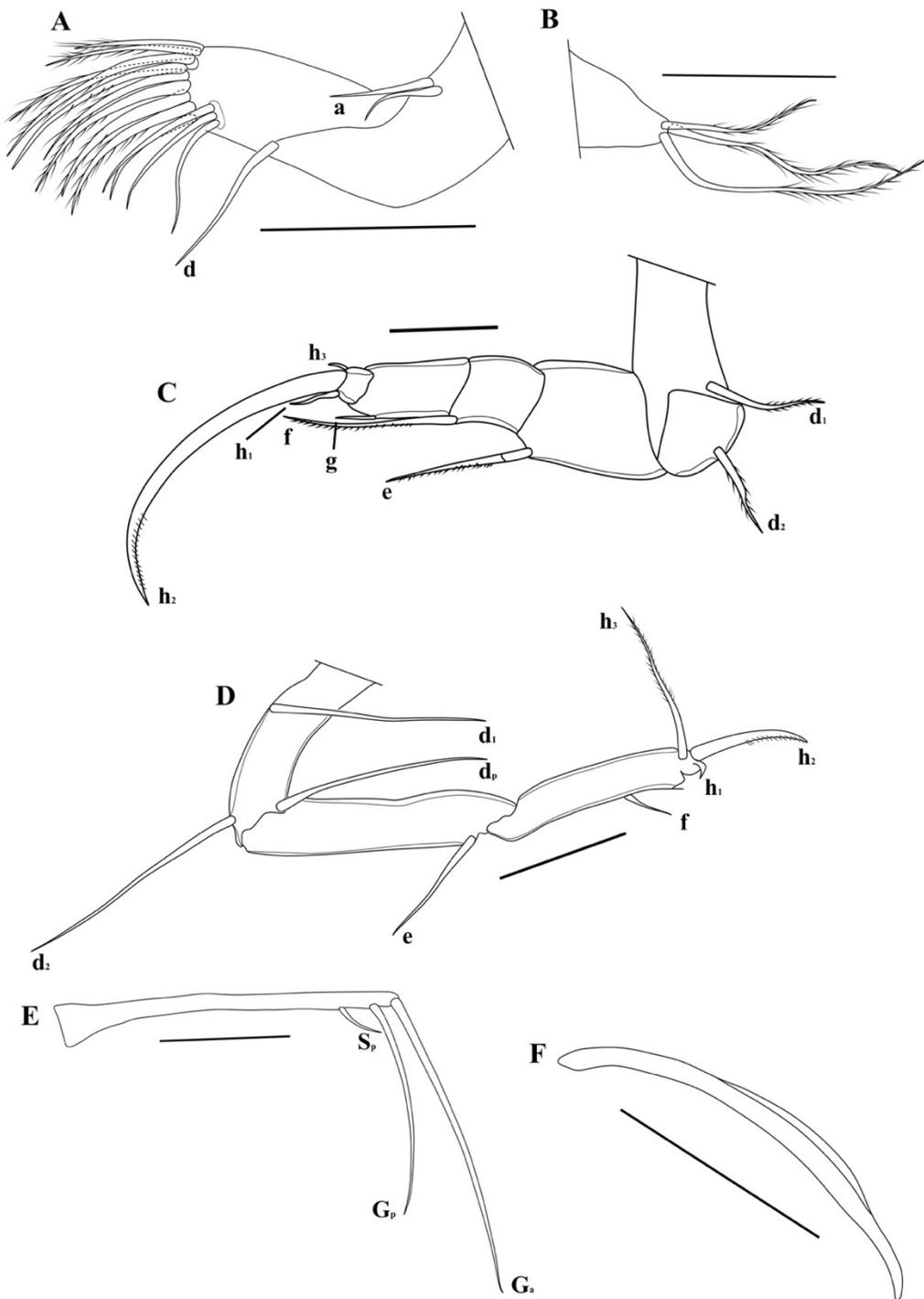
**CR attachment** (Fig. 9B) a single short and narrow rod, without bifurcation.

#### 5.3.2.7 *Differential diagnosis*

Gen. 1 n. gen. sp. 2 n. sp. is the second largest of the species described here (see Table 1). It is slightly larger than Gen. 1 n. gen. sp. 4 n. sp., however there are differences in the surface ornamentation of the Cp (see description of Gen. 1 n. gen. sp. 4 n. sp. below), as it is more hirsute and set with more pits. Another difference, not only with Gen. 1 n. gen. sp. 4 n. sp. but also with Gen. 1 n. gen. sp. 3 n. sp. is the relative length of the  $d_1$  seta on T2. This seta



**FIGURE 8.** Appendages of Gen. 1 n. gen. sp. 2 n. sp. **A.** MdCoxa (VF090). **B.** Mx1 (VF127). **C.** MdPalp (VF127). **D.** MdPalp terminal segment (VF127). Scale bars: 50 $\mu$ m.



**FIGURE 9.** Appendages of Gen. 1 n. gen. sp. 2 n. sp. **A.** T1 protopodite (VF127). **B.** T1 endopodite (VF090). **C.** T2 (VF090). **D.** T3 (VF127). **E.** CR (VF127). **F.** CR attachment (VF090). Scale bars: 50 $\mu$ m.

has almost the same length as the  $d_2$  seta on the T2 of Gen. 1 n. gen. sp. 2 n. sp., while in the other two new species  $d_1$  has half the length of  $d_2$ .

#### 5.3.2.8 Ecology

This species was recorded in a narrow range of environmental variables: water temperature of 31.5 °C; pH range was 9.5–9.7; electrical conductivity range was 51.3–51.5 $\mu$ S.cm<sup>-1</sup>; dissolved oxygen range was 2.1–2.9mg. L<sup>-1</sup>(See Table 2).

#### 5.3.3 Gen. 1 n. gen. sp. 3 n. sp.

**(Figs. 10-13, 18)**

##### 5.3.3.1 Diagnosis

CpL1 with a sub-triangular shape and with ventral margin slightly concave; CpD relatively narrow, Cp surface with several long setae and deep pits; RV clearly overlapping LV along anterior, dorsal, and posterior margins; T2 with  $d_1$  half-length of  $d_2$ .

##### 5.3.3.2 Type locality

Brazil – Amazon River floodplain. Poraquequara Lake IV (AMA87), in the roots of *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia* spp. in the Amazon River floodplain. Collected on 18.05.2012 by JH. Coordinates: 03°02'20.7"S, 59°47'54.0"W.

##### 5.3.3.3 Type material

Holotype: A female with soft parts dissected in glycerine in a sealed slide and valves stored dry in a micropaleontological slide (VF092).

Paratypes: Three female specimens with soft parts dissected as the holotype (VF128, VF129 and VF160). Five specimens with valves stored dry in micropaleontological slide (VF146, JH852, JH853, JH854 and JH855).

#### 5.3.3.4 *Other localities*

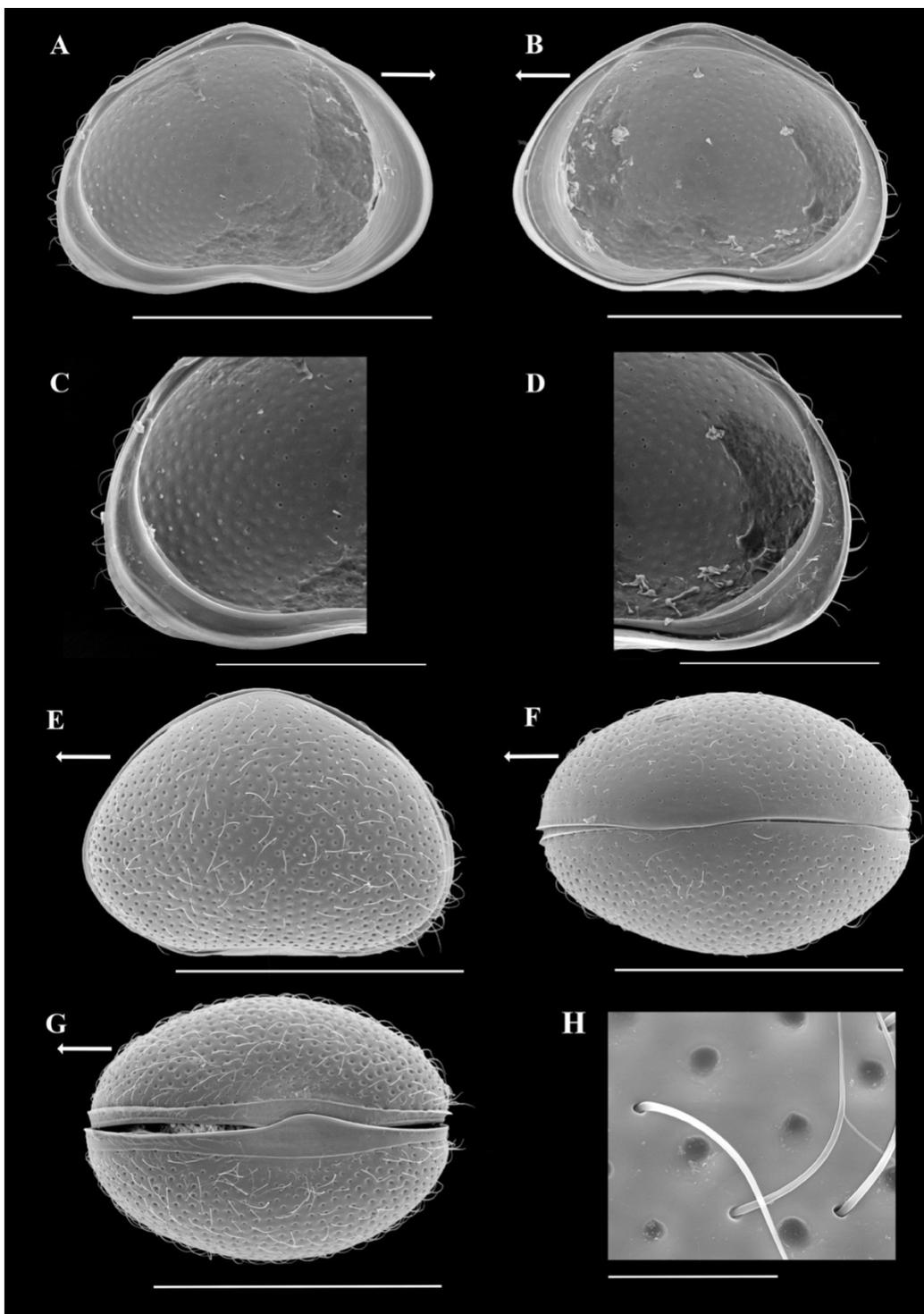
Gen. 1 n. gen. sp. 3 n. sp. is restrict to the Amazon River floodplain, occurring only in lentic environments (See Table 2).

#### 5.3.3.5 *Measurements*

See Table 1

#### 5.3.3.6 *Description*

**LVi** (Fig. 10A, C, 18E) highly arched (H ca.  $2/3$  of L), of a more sub-triangular shape, with greatest height situated in the middle; calcified inner lamella wide along anterior margin and narrow along ventral and posterior margins; anteriorly with incomplete inner list (running halfway up the anterior margin); postero-ventrally with a reduced inwardly displaced selvage, not running parallel to valve margin, and with inner margin of the calcified inner lamella fortified by an inner list; few setae present along posterior margin; ventral margin slightly concave; ca. 15 well-developed setae along anterior margins. **RVi** (Fig. 10B, D, H, 18F) with similar shape as LVi; with a submarginal groove along ventral margin; ca. 14 septa along anterior margins. **CpLI** (Fig. 10E) with a sub-triangular shape; strongly arched dorsally, with greatest height also situated in the middle region; external surface (Fig. 10H) set with long setae and deep pits; RV overlapping LV at the dorsal; posterior and ventral margins. **CpD** and **CpV** (Fig. 10F, G) with relatively narrow, W ca. less than  $2/3$  of L, anterior and posterior margins with blunt ends. **CpV** with RV overlapping LV on all sides, especially in the middle region with a flap.



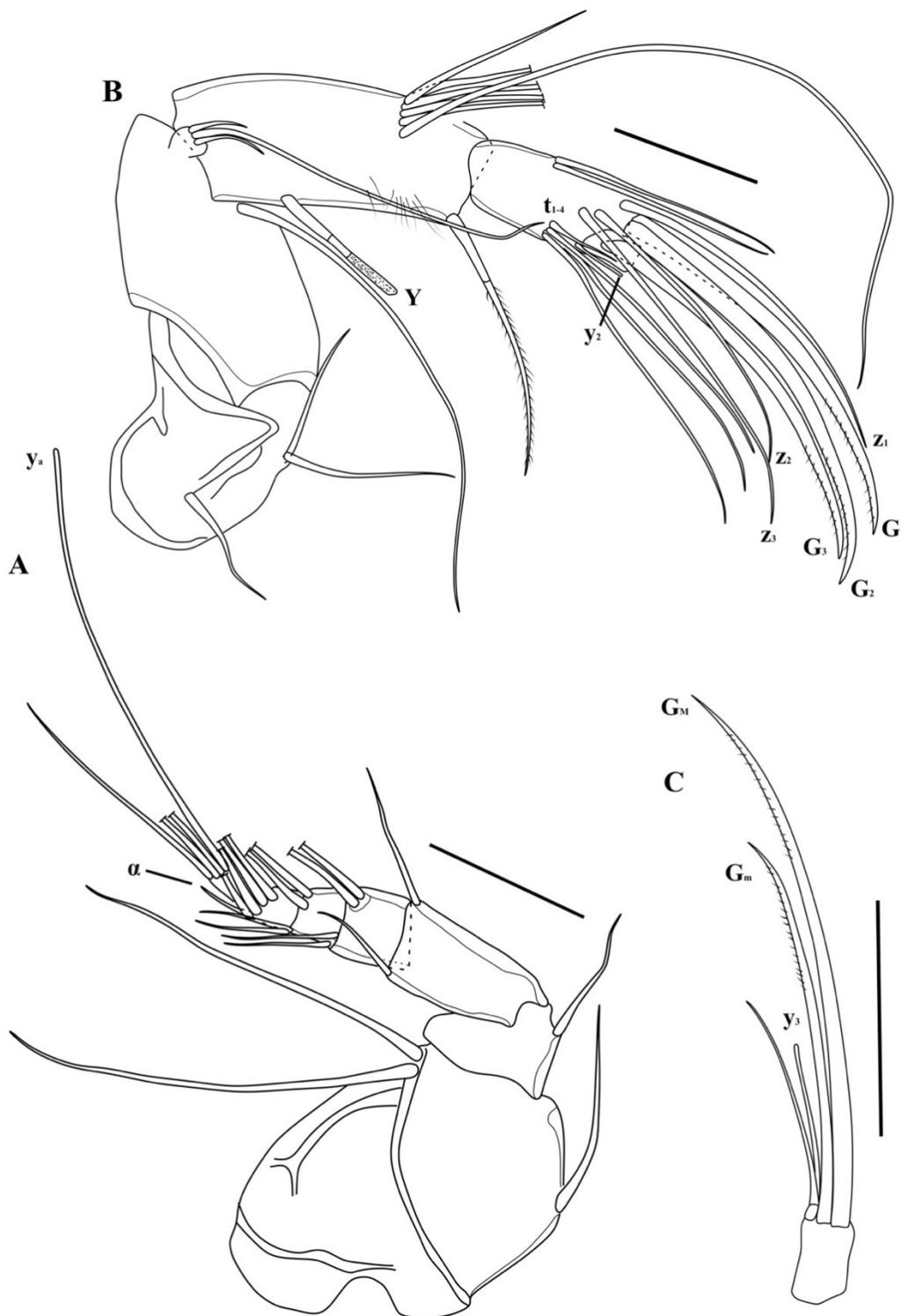
**FIGURE 10.** Cps and valves of *Gen. 1 n. gen. sp. 3 n. sp.* **A.** LVi (JH852). **B.** RVi (JH852). **C.** LVi, detail of posterior margin (JH852). **D.** RVi, detail of posterior margin (JH852). **E.** CpL1 (JH853). **F.** CpD (JH854). **G.** CpV (JH855). **H.** Cp, detail of surface (JH853). Scale bars: A-B, E-G, 400 $\mu$ m; C-D, 200 $\mu$ m; H, 30 $\mu$ m.

**A1** (Fig. 11A) consisting of seven segments. The first segment with three setae (two long ventro-apical and one shorter midorsal); Wouters organ not seen. Second segment with one dorsal seta, reaching middle of third segment; Rome organ not seen. Third segment with one short dorso-apical setae and one short ventro-apical (both reaching beyond tip of fourth segment). Fourth segment with two unequal short ventro-apical short setae and two long dorso-apical setae. Fifth segment with two short (almost reaching tip of the terminal segment) and two long dorso-apical setae. Sixth segment with four long apical setae, and a short  $\alpha$ -seta reaching tip of the terminal segment. Terminal segment apically carrying one long aesthetasc  $Y_a$ , one medium length seta (with half length of  $y_a$ ) and two long setae.

**A2** (Fig. 11B, C) composed of six segments (one two-segmented protopodite, one exopodite and three endopodites). Protopodite carrying three ventral setae, two mid-ventral (unequally with medium length) and one long apical setae (ca. three times length of the short ones). Exopodite consisting of a short plate with three setae, two unequal but short and one long (reaching middle of second endopodite). First endopodal segment with one mid-ventral aesthetasc  $Y$  (ca. half length of segment); one medium length and hirsute ventro-apical seta (ca. length of segment), five long hirsute natatory setae (reaching beyond tips of G claws) and one short seta accompanying the natatory setae (almost reaching tips of second endopodal segment). Second endopodal segment with two equal medium dorsal setae, and a group of four mid-ventral t-setae (three equally medium length and one short); three unequally long z setae ( $z_1$ ,  $z_2$  and  $z_3$ ); and three claws ( $G_1$  and  $G_3$  equally long;  $G_2$  slightly longer); one short aesthetasc  $y_2$ . Terminal segment (Fig. 11C) with one long claw  $G_M$ , and one medium length claw  $G_m$ ; one aesthetasc  $y_3$  and accompanying seta (slightly longer than  $y_3$ ); seta g not observed.

**MdCoxa** (Fig. 12A) consisting of an elongated plate with ca. six apical teeth intercalated with short setae; one hirsute sub-apical seta on dorsal margin. **Mx1** (Fig. 12B) composed of a two segmented palp and three endites. Basal segment of the palp with a group of five unequally long setae and one short sub-apical seta. Terminal segment elongated (L. less than 1.5x W), apically with three claws and three setae. Third endite with two medium

length and smooth bristles and one long sub-apical seta (reaching well beyond tip of the endite). First



**FIGURE 11.** Appendages of *Gen. 1 n. gen. sp. 3 n. sp.* **A.** A1 (VF129). **B.** A2 (VF092). **C.** A2 terminal segment (VF129). Scale bars: 50 $\mu$ m.

endite with two sideways-directed bristles; and two medio-lateral setae of medium length. (Chaetotaxy of endites incompletely described and illustrated).

**MdPalp** (Fig. 12C, D) consisting of four segments. First segment with long setae  $S_1$  and  $S_2$ ; one long smooth seta and short and narrow smooth  $\alpha$ -seta. Second segment ventrally with a cone-shaped and hirsute  $\beta$  seta and three long and one short hirsute setae; dorsally with a group of two unequal but long setae and one short seta (ca. half length of long ones). Third segment dorsally with a group of four long setae; apically with two long and one medium length setae and a cone-shaped, hirsute  $\gamma$  seta. Last segment (Fig. 12D) with three claws and three setae.

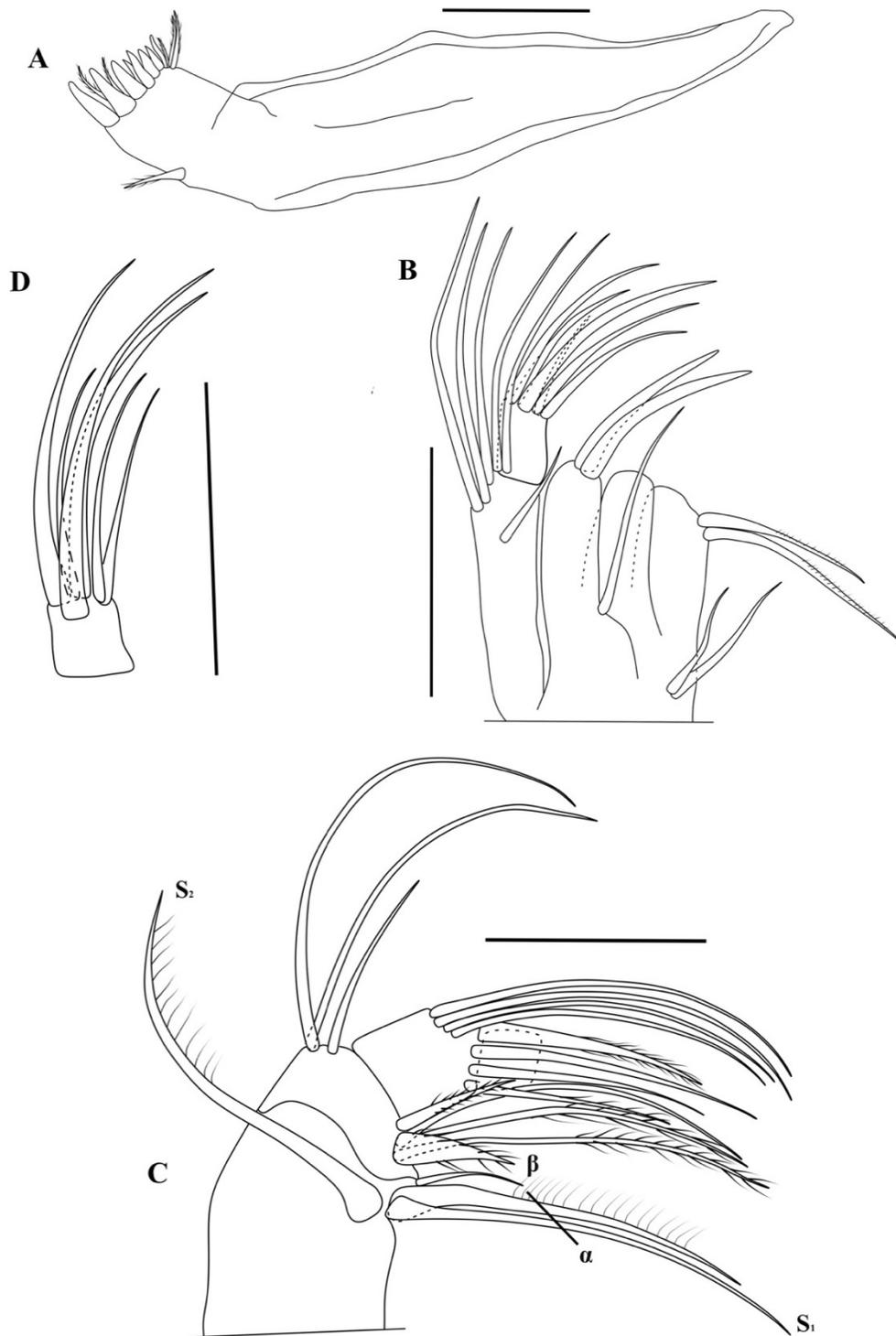
**T1** (Fig. 13A, B) composed of an endopodite and a protopodite. Endopodite (Fig. 13B) with three unequally short apical hirsute setae. Protopodite with two equally short a-setae and one short d seta; 10 apical hirsute and unequally short setae and three equally short sub-apical setae.

**T2** (Fig. 13C) composed of five segments. First segment with seta  $d_1$  short. Second segment with seta  $d_2$  medium length. Third segment with seta  $e$  long. Fourth segment medially divided in a and b segments; a segment with long apical  $f$  seta; b segment apically with a short  $g$  seta. Terminal segment apically with two short setae  $h_1$  and  $h_3$  (ca. half length of  $h_1$ ); and a long claw  $h_2$  weakly serrated in its distal part.

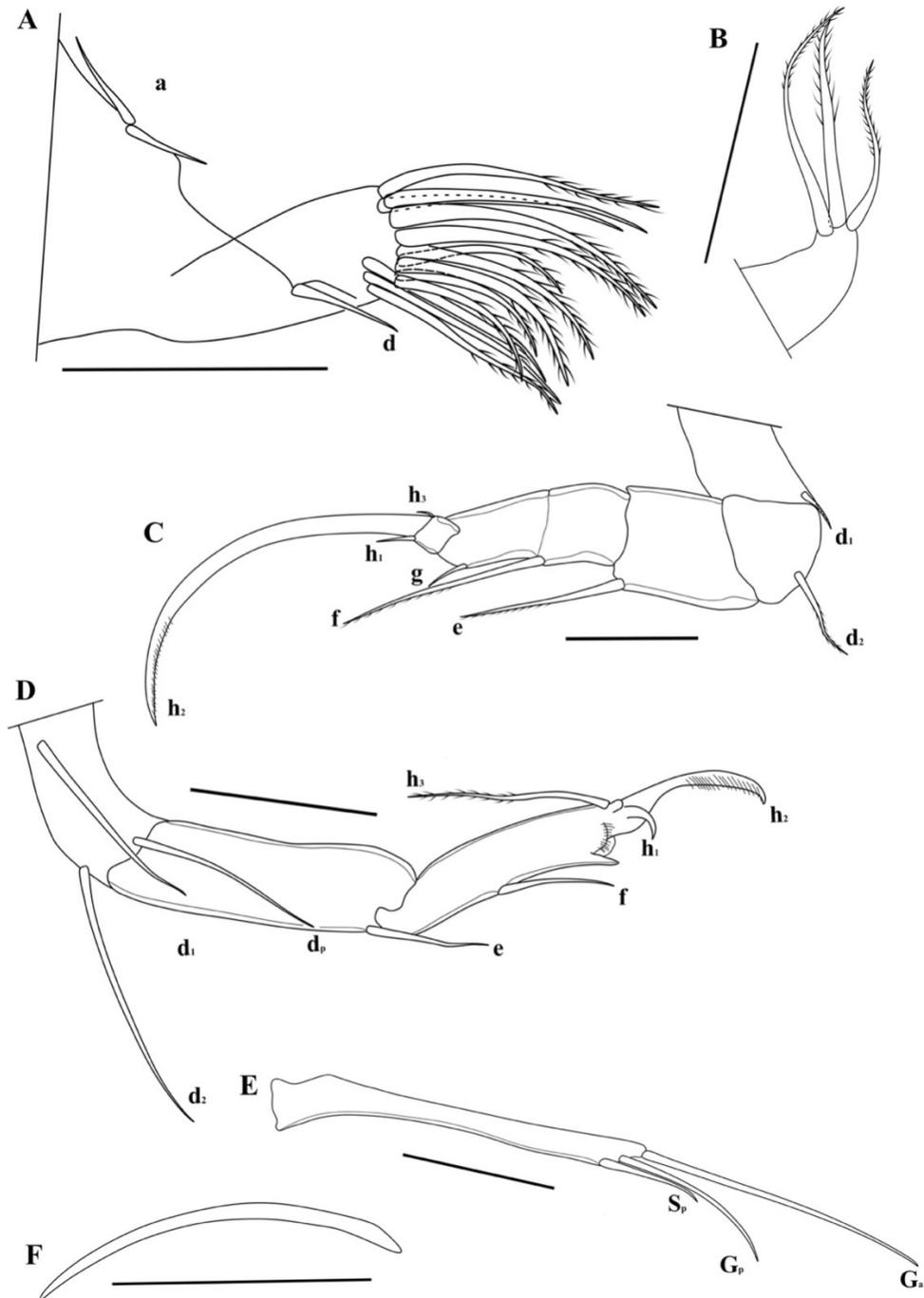
**T3** (Fig. 13D) composed of four segments. First segment with three setae of medium length, slightly unequal ( $d_1$ ,  $d_2$  and  $d_p$ ; almost as long as second segment). Second segment with a short apical  $e$  seta (reaching halfway third segment). Third segment with short sub-apical  $f$  seta. Third segment with a pincer-like structure; with a short seta  $h_1$ ; longer  $h_2$  claw; medium length and hirsute  $h_3$  seta.

**CR** (Fig. 13E) with long and stout ramus, two long claws (one apical  $G_a$  shorter than ramus; and one shorter sub-apical  $G_p$ , slightly longer than half  $G_a$ ); one short and sub-apical seta  $S_p$  (half length of  $G_p$ ), apical seta  $S_a$  absent.

**CR attachment** (Fig. 13F) a single short and narrow rod, without bifurcation.



**FIGURE 12.** Appendages of *Gen. 1 n. gen. sp. 3 n. sp.* **A.** MdCoxa (VF092). **B.** Mx1 (VF128). **C.** MdPalp (VF128). **D.** MdPalp terminal segment (VF128). Scale bars: 50 $\mu$ m.



**FIGURE 13.** Appendages of Gen. 1 n. gen. sp. 3 n. sp. **A.** T1 protopodite (VF092). **B.** T1 endopodite (VF128). **C.** T2 (VF092). **D.** T3 (VF092). **E.** CR (VF128). **F.** CR attachment (VF129). Scale bars: 50 $\mu$ m.

#### 5.3.3.7 *Differential diagnosis:*

Of the four new species described here, Gen. 1 n. gen. sp. 3 n. sp. is the smallest one (see Table 1), and with the most distinguishable carapace shape. It also has the RV overlapping LV, but without a pointed anterior margin of RV, as observed in Gen. 1 n. gen. sp. 2 n. sp. The carapace surface is covered with deep pits and a few setae. In dorsal view, Gen. 1 n. gen. sp. 3 n. sp. is quite distinguishable: whereas the other three species described here share a rounded and broad dorsal shape, Gen. 1 n. gen. sp. 2 n. sp. viewed much narrower, while anterior and posterior ends are bluntly pointed.

The soft part morphology is quite similar to the three other new species described here, the only differences being noticed are in the MdPalp, T2 and CR. The MdPalp is composed by four dorsal setae on the third segment (as in Gen. 1 n. gen. sp. 4 n. sp. see below), which is different from Gen. 1 n. gen. sp. 2 n. sp., which has three dorsal setae. On T2, the  $d_1$  seta is only half the length of  $d_2$  seta in Gen. 1 n. gen. sp. 2 n. sp. (subequal in the other three species).

#### 5.3.3.8 *Ecology*

This species was recorded in a narrow range of environmental variables: water temperature of 31.5°C; pH range was 9.5–9.7; electrical conductivity range was 51.3–51.5  $\mu\text{S}\cdot\text{cm}^{-1}$ ; dissolved oxygen range was 2.1–2.9  $\text{mg}\cdot\text{L}^{-1}$  (See Table 2, not all localities included).

#### 5.3.4 Gen. 1 n. gen. sp. 4 n. sp.

**(Figs. 14-18)**

2017 *Cyprettasp.* 4 n. sp. Higuti et al. : p. e120, Table 2.

2017 *Cypretta n.sp.* 2 Pereira et al. : p. 327, Table 2.

#### 5.3.4.1 *Diagnosis*

RVi anteriorly with ca. 10 septa. CpD ith rounded and broad; Cp surface with few setae and shallow pits; with posterior margin more broadly rounded than anterior margin; RV overlapping LV along the anterior, dorsal, and ventral margins; at the posterior margin, both LV and RV valve inwardly curved; T2 with  $d_1$  half the length of  $d_2$ .

#### 5.3.4.2 *Type locality*

Brazil – Araguaia River floodplain. Crixas Lake III (ARA52), in the roots of *Eichhornia crassipes*, in the Araguaia River floodplain. Collected on 11.02.2012 by JH. Coordinates: 13°21'52.6"S, 50°37'20.9"W.

#### 5.3.4.3 *Type material*

Holotype: A female with soft parts dissected in glycerine in a sealed slide and valves stored dry in a micropaleontological slide (VF093).

Paratypes: A female with soft parts dissected as the holotype (VF130). Five female specimens with valves stored dry in micropaleontological slide (VF094, VF095, VF096, VF142 and VF143).

#### 5.3.4.4 *Other material examined*

Brazil - Amazon River floodplain – Poraquequara IV Lake (AMA87) collected by JH on 18.05.2012 in the following plants: *Eichhornia crassipes*, *Salvinia auriculata*, *Pistia* sp. Coordinates: 03°02'20.7"S, 59°47'54.0"W. One female with soft parts dissected as the holotype (VF159), and a female with valves stored dry in a micropaleontological slide (JH856), five female specimens with carapace stored dry in micropaleontological slide (JH857, JH858, JH859, VF144, VF145).

#### 5.3.4.5 *Other localities*

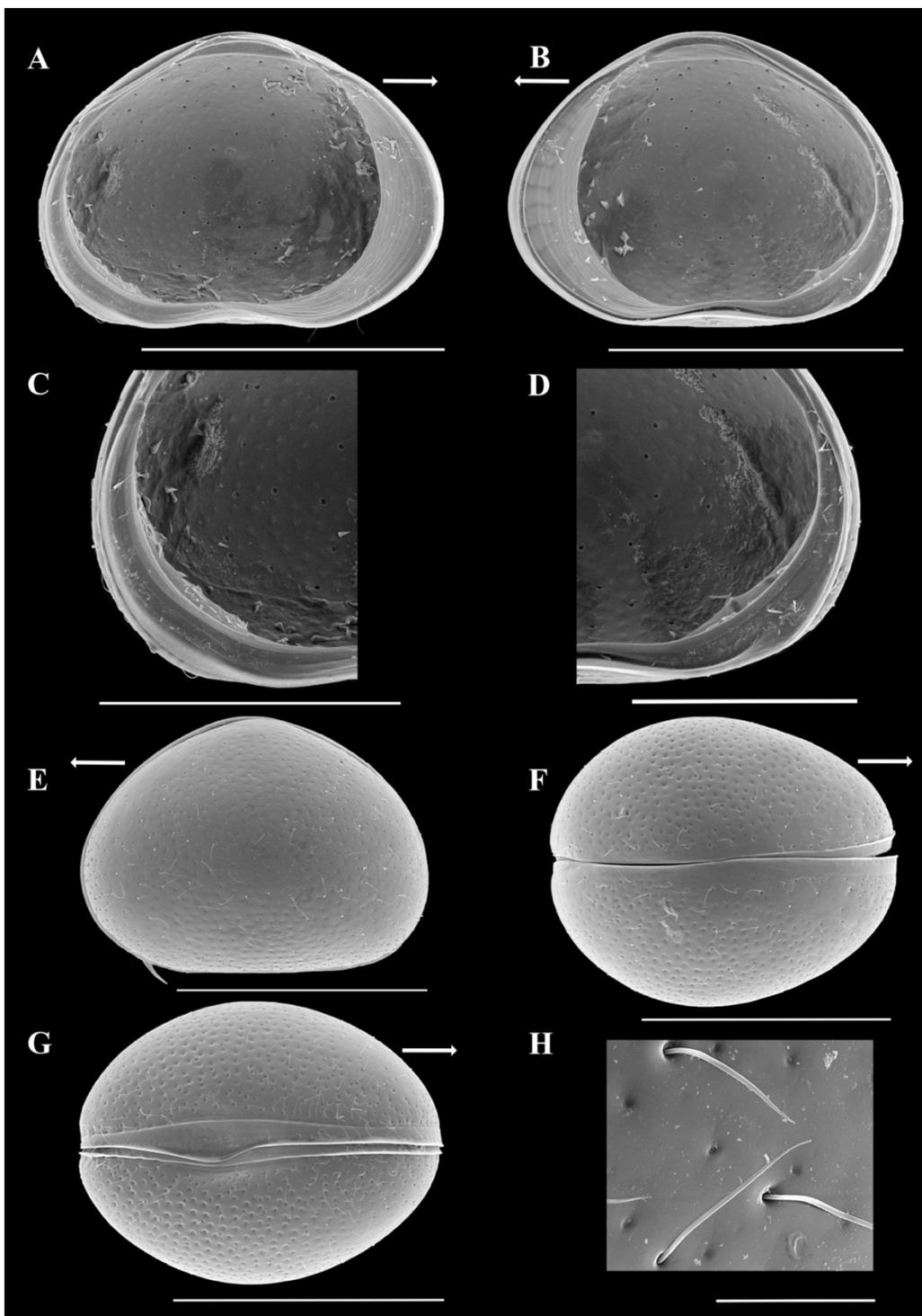
Gen. 1 n. gen. sp. 4 n. sp. occurred in the Amazon and Paraná River floodplains, frequently occurring in lentic, but also in one lotic environment (See Table 2).

#### 5.3.4.6 *Measurements*

See Table 1

#### 5.3.4.7 *Description*

**LVi** (Fig. 14A, C, 18G) with dorsal margin rounded and less arched, of sub-triangular shape only, greatest height situated in the middle; calcified inner lamella wide along anterior margin and narrow along ventral and posterior margins; anteriorly with incomplete inner list (running halfway up the anterior margin); and with inner margin of calcified inner lamella fortified by an inner list; ca. 12 well-developed septa along anterior margin. **RVi** (Fig. 14B, D, 18H) with similar shape as LVi, but with posterior calcified inner lamella somewhat wider; with a submarginal groove along ventral margin; and with ca. 13 anterior septa. **CpLI** (14E) with a sub-triangular shape; dorsally rounded, with greatest height in the middle region; external surface (Fig. 14H) set with a few setae and very shallow pits; RV overlapping LV along anterior and dorsal margins. **CpD** and **CpV** (Fig. 14F, G) broad, with oval shape, W ca. 3/4 of L, posterior margins with LV and RV bending inwardly. **CpV** with RV overlapping LV on all sides, especially in the middle region with a flap.

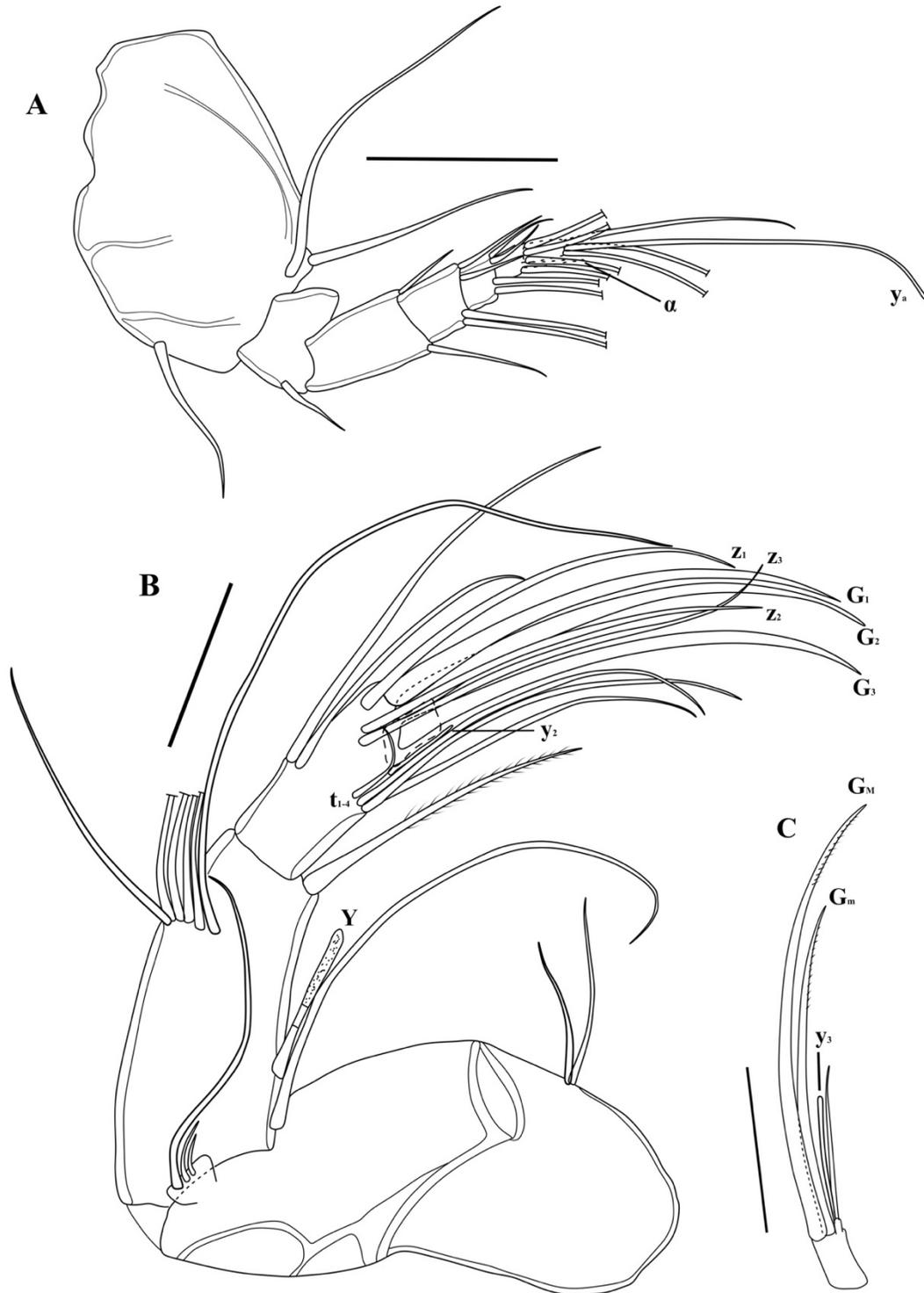


**FIGURE 14.** Cps and valves of Gen. 1 n. gen. sp. 4 n. sp. **A.** LVi (VF093). **B.** RVi (VF093). **C.** LVi, detail of posterior margin (VF093). **D.** RVi, detail of posterior margin (VF093). **E.** CpL1 (VF094). **F.** CpD (VF095). **G.** CpV (VF096). **H.** Cp, detail of surface (JH857). Scale bars: A-B, E-G, 400 $\mu$ m; C-D, 200 $\mu$ m; H, 30 $\mu$ m.

**A1** (Fig. 15A), consisting of seven segments. First segment with three setae (two unequal long ventral setae and a shorter mid-dorsal); Wouters organ not seen. Second segment with one dorsal seta (not reaching middle of third segment); Rome organ not seen. Third segment with one short (dorsal) seta and one short (ventral) (ca. 2/3 length of dorsal one). Fourth segment with two unequal but short ventro-apical setae and two long dorso-apical setae. Fifth segment with two unequal but short ventro-apical setae (longer one reaching beyond middle of terminal segment, and other slightly shorter) and two long dorso-apical setae. Sixth segment with four long apical setae, and a short  $\alpha$  seta reaching beyond the tip of terminal segment. Terminal segment apically carrying one long aesthetasc  $Y_a$ , one medium length seta (with more than half length of  $Y_a$ ) and two long setae.

**A2** (Fig. 15B, C) composed of six segments (one two segmented protopodite, one exopodite and three endopodites). Protopodite carrying three ventral setae, two mid-ventral (unequally with medium length) and one long and hirsute apical setae. Exopodite consisting of a short plate with three setae, two unequal but short, and one medium length (with length of first endopodite). First endopodal segment with one mid-ventral aesthetasc  $Y$  (ca. half length of segment); one medium length and hirsute apical seta with length of segment; and five long hirsute natatory setae (reaching tips of  $z$  setae) and one medium-long seta accompanying natatory setae (almost reaching tip of second endopodal segment). Second endopodal segment with one long and one medium length mid-dorsal setae (medium length one with 2/3 of long one); and a group of four mid-ventral  $t$ -setae (three equally medium length and one short setae); three equally long  $z$  setae ( $z_1$ ,  $z_2$  and  $z_3$ ); and three long claws ( $G_1$  slightly shorter one;  $G_2$  and  $G_3$  equally long); and one short aesthetasc  $y_2$ . Terminal segment (Fig. 15C) with one long claw  $G_M$ , and one medium length claw  $G_m$ ; one aesthetasc  $y_3$  and accompanying seta (slightly longer than  $y_3$ ); seta  $g$  not observed.

**MdCoxa** (Fig. 16A) consisting of an elongated plate with ca. seven apical teeth intercalated with short setae; one hirsute sub-apical seta on dorsal margin. **Mx1** (Fig. 16B) composed of a two segmented palp and three endites. Basal segment of the palp with a group of six setae: three long, two short and one short sub-apical seta. Terminal segment elongated (L ca. 1.5x



**FIGURE 15.** Appendages of Gen. 1 n. gen. sp. 4 n. sp. **A.** A1 (VF130). **B.** A2 (VF130). **C.** A2 terminal segment (VF130). Scale bars: 50 $\mu$ m.

W), apically with three claws and three setae. Third endite with two medium length smooth bristles; and one long sub-apical seta (reaching beyond endite). First endite with twosideways directed bristles; and two medio-lateral setae of medium length. (Chaetotaxy of endites incompletely described and illustrated).

**MdPalp** (Fig. 16C, D) consisting of four segments. First segment with long setae  $S_1$  and  $S_2$ ; one long smooth seta and a short and narrow smooth  $\alpha$ -seta. Second segment ventrally with a cone-shaped and hirsute  $\beta$  seta, two long and one medium length smooth setae, and one medium length and hirsute seta; dorsally with a group of two unequal but long setae and one medium length (ca. half length of long ones). Third segment dorsally with a group of four equally long setae; ventrally with one medium length seta and one short (ca. 1/5 length of medium length one); apically with one long setae, two medium length and one medium length cone-shaped, smooth  $\gamma$ -seta. Last segment (Fig. 16D) with three claws and three setae.

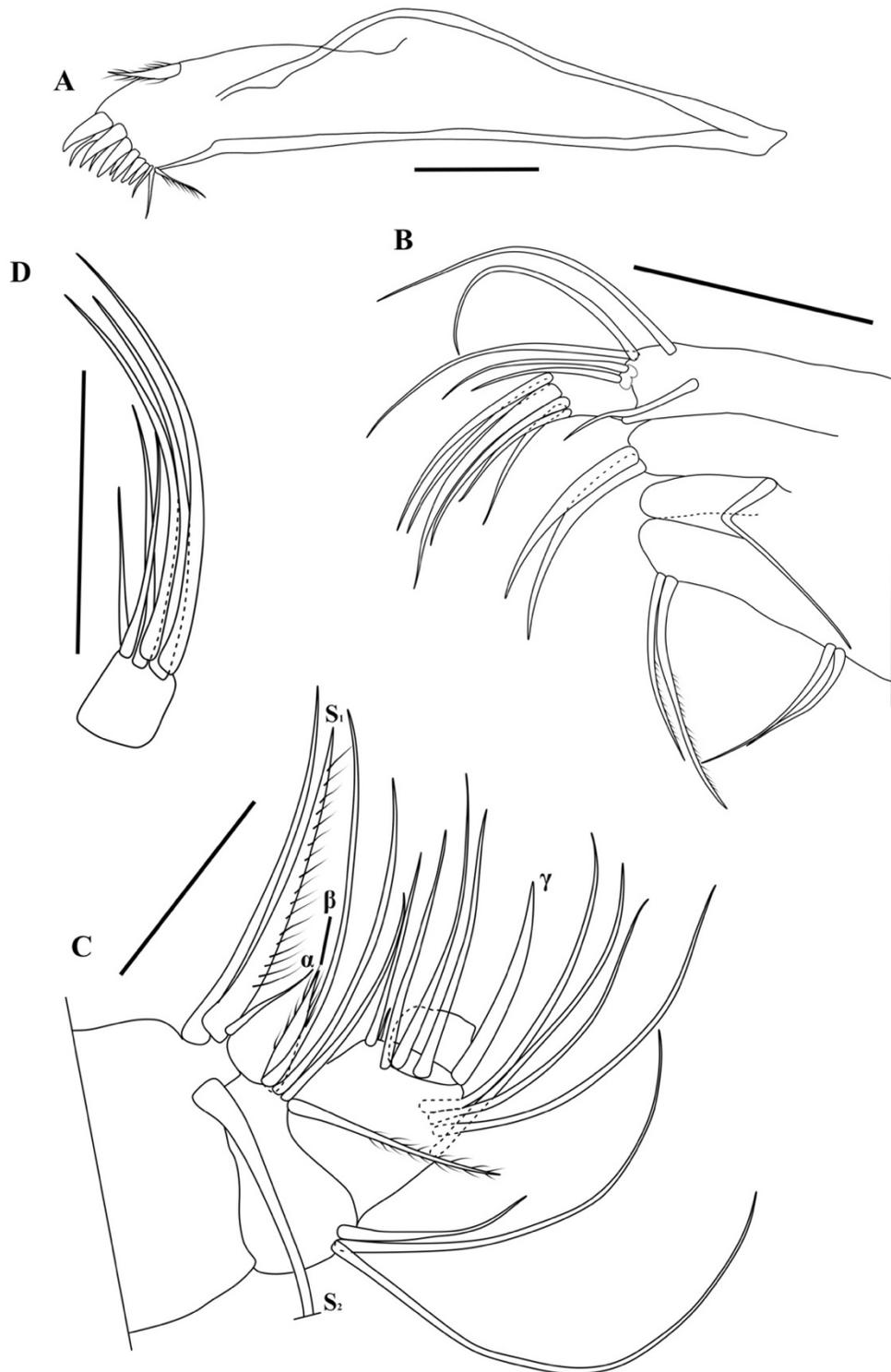
**T1** (Fig. 17A, B) composed of an endopodite and a protopodite. Endopodite with three apical hirsute setae, on short, one of medium length and one long. Protopodite with two equally short a-setae; one short d seta; 10 apical hirsute and unequally short setae and three equally short sub-apical setae.

**T2** (Fig. 17C) composed of five segments. First segment with short seta  $d_1$ . Second segment with seta  $d_2$  twice the length of  $d_1$ . Third segment with seta e of medium length ventrally serrated. Fourth segment divided in a and b segment; a segment with long apical f seta ventrally serrated; b segment apically with a short g seta (almost reaching tip of terminal segment). Terminal segment apically with two short setae  $h_1$  and  $h_3$ ; and a long claw  $h_2$ , weakly serrated in its distal part.

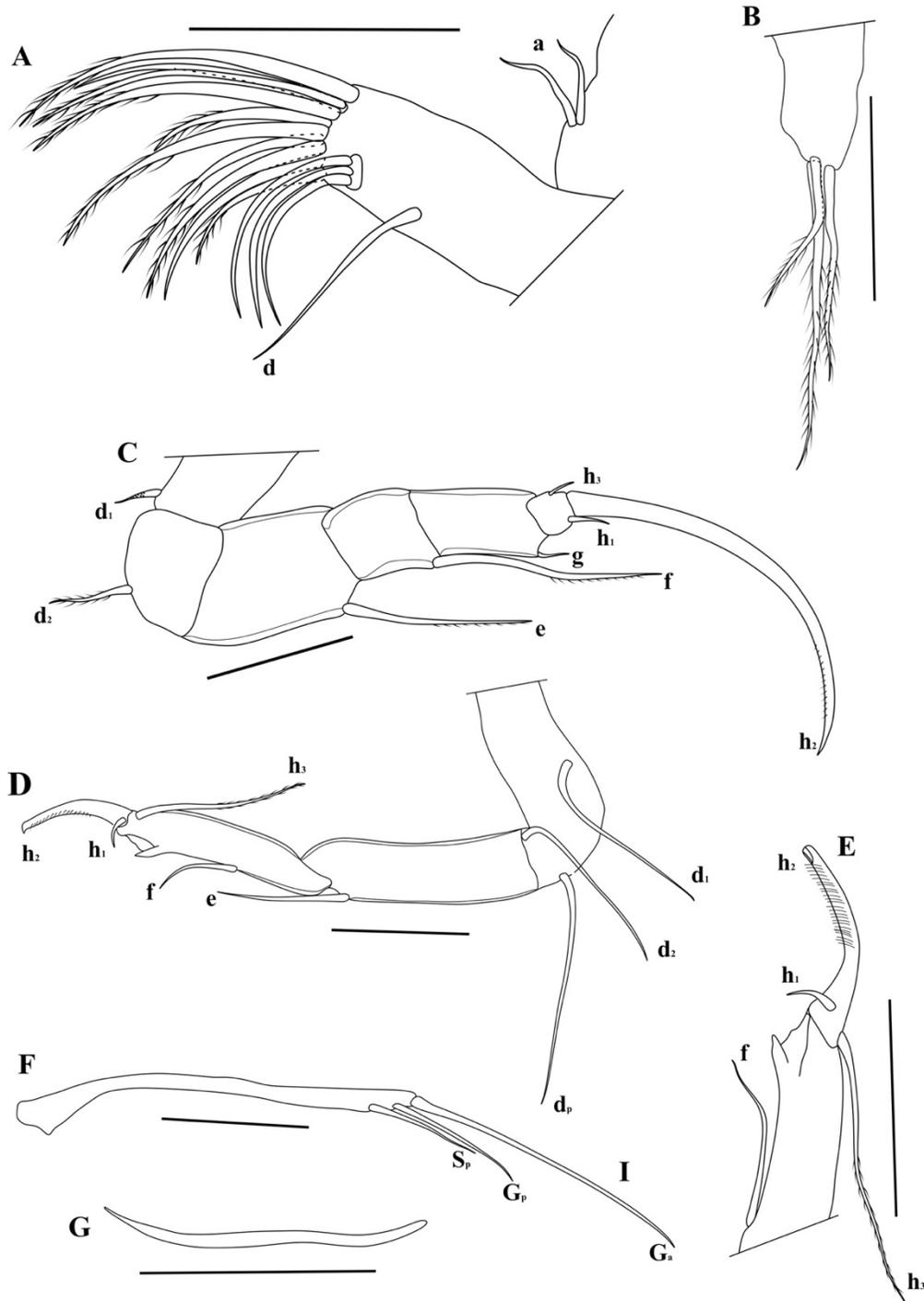
**T3** (Fig. 17D, E) composed of four segments. First segment with three setae of medium length, slightly unequal ( $d_1$ ,  $d_2$  and  $d_p$ ; almost as long as second segment). Second segment with a medium length apical e seta (reaching halfway third segment). Third segment with short sub-apical f seta. Third segment (Fig. 17E) with a pincer-like structure; with a short seta  $h_1$ , longer  $h_2$  claw; and a medium length and hirsute  $h_3$  seta.

**CR** (Fig. 17F) with long but narrow and weak ramus, two long claws (one apical  $G_a$ , shorter than ramus and one sub-apical  $G_p$  with less than half length of  $G_a$ ); one long seta  $S_p$  on ventral margin, apical seta  $S_a$  absent.

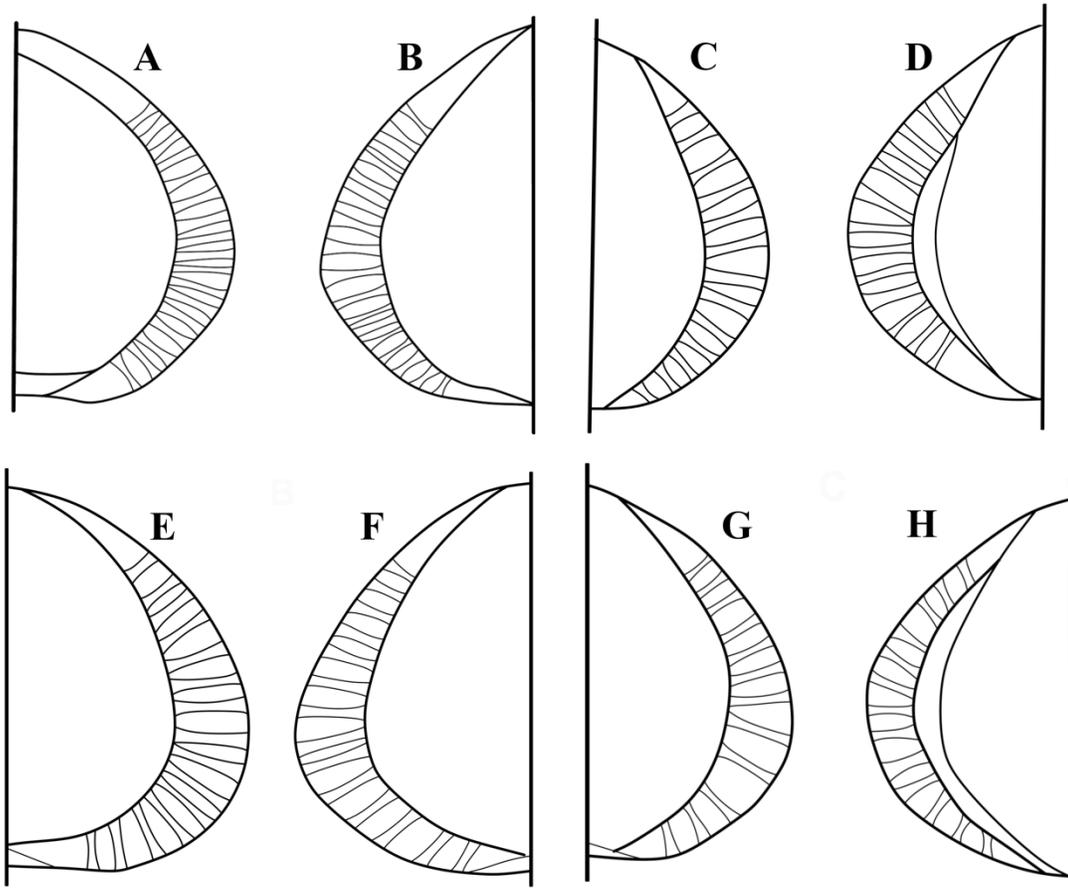
**CR attachment** (Fig. 17G) a single short and narrow rod, without bifurcation.



**FIGURE 16.** Appendages of Gen. 1 n. gen. sp. 4 n. sp. **A.** MdCoxa (VF093). **B.** Mx1 (VF093). **C.** MdPalp (VF093). **D.** MdPalp terminal segment (VF093). Scale bars: 50 $\mu$ m.



**FIGURE 17.** Appendages of Gen. 1 n. gen. sp. 4 n. sp. **A.** T1 protopodite (VF093). **B.** T1 endopodite (VF093). **C.** T2 (VF093). **D.** T3 (VF093). **E.** T3 pincer (VF093). **F.** CR (VF093). **G.** CR attachment (VF093). Scale bars: 50µm.



**FIGURE 18.** Anterior margins of LV(A, C, E, G) and RV (B, D, F, H) in inner views showing the marginal septa. **A-B.**Gen. 1 n. gen. sp. 1 n. sp. **C-D.**Gen. 1 n. gen. sp. 2 n. sp.**E-F.**Gen. 1 n. gen. sp. 3 n. sp.**G-H.**Gen. 1 n. gen. sp. 4 n. sp.

#### 5.3.4.8 Remarks

We dissected individuals from Amazon and Araguaia floodplains, and both valves and soft parts morphology were similar. A few individuals were identified (through stereomicroscope) from Amambáí River, a tributary of Paraná River, however its valves were decalcified and managing them to perform the SEM was not possible.

#### 5.3.4.9 Differential diagnosis

Gen. 1 n. gen. sp. 4 n. sp. has far less valve ornamentation and has much smoother valves than in the other three species. In comparison with the other species described here, the

anterior marginal septa are most visible in the SEM images. It can be distinguished from the other species by the posterior margins of the two valves bending inwardly in dorsal view, giving an image of “buttacks”. Compared with Gen. 1 n. gen. sp. 2 n. sp., Gen. 1 n. gen. sp. 4 n. sp. has a less pointed anterior margin, with the anterior margin more broadly rounded. The soft part morphology is quite similar with the other two new species described here, the only differences are noticed in the MdPalp, T2 and CR (see differential diagnosis above).

The  $d_1$  seta on the T2 has about half the length of seta  $d_2$ , as in Gen. 1 n. gen. sp. 3 n. sp. In Gen. 1 n. gen. sp. 1 n. sp., setae  $d_1$  and  $d_2$  are of subequal length while in Gen. 1 n. gen. sp. 2 n. sp. seta  $d_1$  is slightly shorter than  $d_2$ .

The  $G_a$  claw on the CR has almost the same length of the main branch, similar with what is observed in the other three species. However, the  $G_p$  is half the length of  $G_a$ , while in Gen. 1 n. gen. sp. 1 n. sp., Gen. 1 n. gen. sp. 2 n. sp. and Gen. 1 n. gen. sp. 4 n. sp. it varies around  $3/4$  the length of  $G_a$ .

#### 5.3.4.10 *Ecology*

This species was recorded in a narrow range of environmental variables: temperature range was 20.6–31.5°C; pH range was from 6.6–9.7; electrical conductivity was 29.8–54.4  $\mu\text{S}\cdot\text{cm}^{-1}$ ; dissolved oxygen range was 1.4–8.0 mg.  $\text{L}^{-1}$ . (See Table 2, not all localities included).

## 5.4 Discussion

### 5.4.1 The occurrence of the subfamily Cyprididae in Brazil

With the present description of a new genus and four new species of Cyprididae, this subfamily increased to nine species in Brazil. So far, a few Cyprididae species, from the genus *Cyprid*, had been recorded in Brazil. *Cyprid brevispina* Farkas 1959 with no information about the location; *C. costata* G.W. Müller 1898, recorded by Tressler (1950) in Santarém (Pará State) and Porto Alegre (Rio Grande do Sul State); *C. kawatai* Sohn & Kornicker 1972, described in the United States, however, on specimens derived from sediments collected from Brazil, more specifically from the Minas Gerais State; *C. schubarti* Farkas 1959, described from the Pernambuco State (Farkas, 1959), and *C. vivacis* Würdig & Pinto 1993, described from the Rio Grande do Sul State, south region of Brazil (Würdig & Pinto 1993).

From several of our samples, we previously identified a species as *Cyprid costata*. After the present morphological analysis, it became clear that it does not belong to the genus *Cyprid s.s.*, and it is here described as a new species in a new genus, Gen. 1 n. gen. sp. 1 n. sp. However, we did analyse the type material of *C. costata*, obtained from the Zoological Museum of the University of Greifswald - Germany and it will be redescribed elsewhere (Ferreira *et al.* in prep.).

### 5.4.2 The taxonomic position of Gen. 1 n. gen. in the Cyprididae

The subfamily Cyprididae previously comprised two genera, *Cyprid* and *Pseudocyprid*. However, *Pseudocyprid* was moved to the subfamily Cypridopsinae because of some important differences in soft part morphology. For example, *Pseudocyprid* has some characters as the strong G<sub>2</sub> claw on A<sub>2</sub>; the elongated terminal segment of Mx<sub>1</sub>; the undivided penultimate segment of T<sub>2</sub> and, most importantly, the CR which is reduced to a small flagellum, or even completely absent, which showed its relationships with other genera in the Cypridopsinae (Savatenalinton *et al.* 2022; Ferreira *et al.* 2022). Another particular feature, the separated terminal segment of T<sub>3</sub>, strongly resembling this limb in the Candonidae, remains enigmatic.

However, based on the carapace morphology, the presence of septa along the anterior margins of LV and RV, and the appendage morphology, the four new species and the new genus described share many characteristics with species of the genus *Cypretta*. Valves of the new genus are triangular in lateral view, with greatest height in the middle and have well-developed anterior marginal septae on both valves. All four species have external valve ornamentation consisting of shallow to deep pits, and varying degrees and sizes of setules. However, The RV lack the series of tubercles on the posteroventral part of the inner list which are consistently present in species of *Cypretta s.s.* (Savatenalinton 2018) and also in the type species of the genus, *C. tenuicauda* (see redescription in Ferreira *et al.* 2023). The presence of septa along the anterior margins of the valves has now been observed in nine genera in the Cyprididae Baird, 1845, namely *Cypretta* Vávra, 1895 and Gen. 1 n. gen. nov. (in Cyprettinae Hartmann, 1963), *Stenocypris* Sars, 1889 (in Herpetocypridinae Kaufmann, 1900); *Pseudocypretta* Klie, 1932 and *Cyprettadopsis* Savatenalinton, 2020 (in Cypridopsinae Kaufmann, 1900), *Zonocypretta* De Deckker, 1981, *Paracypretta* Sars, 1924, *Bradycypris* Sars, 1925 (in Bradycypridinae Hartmann & Puri, 1974) and *Batucypris* Victor & Fernando, 1981 (in Batucypridinae Victor & Fernando, 1981) These nine genera are distributed over five subfamilies, and is therefore an excellent example of parallel evolution in one ostracod family. Moreover, such septa also occur outside of the Cyprididae, for example in the Oncocypridinae De Deckker, 1979.

#### 5.4.3 Comparative morphology

The presence / absence, or the length ratios of setae  $d_1$  and  $d_2$  on the second thoracopod T2 have for a long time been considered as informative at the supra-specific level, mostly at the generic level. Examples are the genera in the Cypridini (in Martens 1990, 1992), the Herpetocypridinae (in Martens 2001), the Eucypridinae (Martens *et al.* 2002) and others. In the species of *Cypretta s.s.*, these setae are subequal and short (Ferreira *et al.* 2023). In Gen. 1 n. gen. sp. 1 n. sp. **and in** Gen. 1 n. gen. sp. 2 n. sp., these setae are also short and subequal. But in Gen. 1 n. gen. sp. 3 n. sp. and in Gen. 1 n. gen. sp. 4 n. sp. seta  $d_1$  is much shorter than seta  $d_2$ , about half the length.

The usefulness of the  $\alpha$ ,  $\beta$  and gamma setae on MdPalp for the higher taxonomy of the Cypridoidea was already demonstrated by De Deckker (1979). Gen. 1 n. gen. is characterized by thinner and shorter  $\alpha$  and  $\beta$  setae as compared to those in *Cypretta tenuicauda*, the type species of the genus, where the  $\alpha$  seta is long and narrow while  $\beta$  seta is long and stout (Ferreira *et al.* 2023).

The CR is a relatively fragile structure in the subfamily Cyprettinae, with slender ramus and claws ( $G_a$  and  $G_p$ ) and short and narrow setae  $S_a$  and  $S_p$ . The new species of Gen. 1 n. gen. are characterized by a CR which is even more slender than in the species of *Cypretta* species. Furthermore, seta  $S_a$  is present in some *Cypretta* species (e.g., *C. tenuicauda* in Ferreira *et al.* 2023; *C. aculeata* Savatena linton, 2018) and absent in others (Savatena linton *et al.* 2018). This seta is absent in all four species of Gen. 1 n. gen.

#### 5.4.4 Asexual and sexual reproduction

In the subfamily Cyprettinae, sexual populations have thus far been reported only in very few species of the genus *Cypretta* s.s.. We found no males in any of the populations of the four new species described here. Therefore, the morphological characteristics related to male sexual appendages, which are generally very useful for species identification, could not be used here. However, the four new species described here show a sufficient diversification in morphology and the absence of male appendages does not restrict the identification of these species.

#### 5.4.5 Distribution

The new genus and species are endemic to the Neotropical region. Two of the species described here, Gen. 1 n. gen. sp. 2 n. sp. and Gen. 1 n. gen. sp. 3 n. sp., are restricted to the Amazon River floodplain, while Gen. 1 n. gen. sp. 4 n. sp. was found in the Amazon, Araguaia, and Paraná River floodplain (floodplains which are more than 2000 km apart), and Gen. 1 n. gen. sp. 1 n. sp. is found across the four main Brazilian floodplains. Non-marine ostracod species are generally restricted to one zoogeographical region and very few species can be found on several continents (Martens *et al.* 2008; Meisch *et al.* 2019).

Conceição *et al.* (2019), using Species Distribution Modelling, nevertheless showed that the family Cyprididae, to which Gen. 1 n. gen. belongs, has a great potential for dispersal. The presence of resistant forms such as drought resistant eggs and torpid stages, together with parthenogenetic species and populations, where a single individual can establish a new population, could explain the potential for wide distributions of species in this group (Horne & Martens 1998; Brochet *et al.* 2010). Some notable exceptions indeed have intercontinental distributions, some of which are circumtropical. For example, some species of *Cypretta* have been reported from rice fields and other temporary habitats on different continents (Smith *et al.* 2018; Vendramin *et al.* 2020). It is possible that such a strategy also exists in some of the species of Gen. 1 n. gen.

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## **6 THE EFFECTS OF CLIMATE CHANGE IN THE FUNCTIONAL DIVERSITY OF OSTRACODA (CRUSTACEA) IN THE SOUTH CONE OF SOUTH AMERICA**

### **ABSTRACT**

Climate change is predicted to affect both terrestrial and aquatic environments. In aquatic habitats, extreme events will severely affect the biological communities leading species to extinction. Using species distribution models on two scenarios of carbon emissions, moderate-optimistic (RCP 4.5) and pessimist (RCP 8.5), in 2050 and 2080, we model the taxonomic and functional richness, dispersion, and uniqueness of the ostracod community in the South Cone of South America. Future projections indicate the reduction of taxonomic richness and functional diversity along the basins studied even in the moderate scenario. Areas with higher values of functional and taxonomic richness in the present, such as La Plata basin, will severely lose functional diversity in both future scenarios. Areas with intermediate to low values of taxonomic richness (e.g. Patagonia) will hold intermediate to high levels of functional uniqueness in the future, highlighting the importance of conserving such areas.

**Keywords:** species distribution model, microcrustacean, traits, conservation

## 6.1 Introduction

Human induced climate change is one of the main factors affecting natural ecosystems. The increased levels of greenhouse gases in the atmosphere lead to a rise in global mean temperature and changes in precipitation patterns. As a result, it triggers extreme changes in aquatic environments, both as floods and droughts. These changes affect the composition of biological communities and their distribution ranges (Heino et al., 2009; Sundar et al., 2020). It is predicted that climate change will have more severe impacts on freshwater than on terrestrial or marine ecosystems, owing to the higher connectivity and the responses of aquatic organisms, for example, the changed characteristics of river discharge (Revenga et al., 2005). Furthermore, freshwater environments support a disproportionate biodiversity, especially of invertebrates, considering its reduced spatial coverage compared to the terrestrial environment (Dudgeon et al., 2006; Heino et al., 2009). Freshwater comprises only 0.01% of all unfrozen water on the planet and occupies only 0.8% of the earth's surface, yet it holds about 12% of all known animal species. This incongruence between diversity and occupied surface was labelled by Lévêque et al. (2005) as 'the paradox of freshwater'.

Aquatic invertebrates provide a variety of ecosystem services. They occupy multiple trophic levels, contribute to the carbon and nitrogen cycle (feeding on algae, detritus, and other organic material) and provide food for higher trophic levels (Covich et al., 1999; Prather et al., 2013; Schmera et al., 2017). In general, freshwater invertebrates can be more susceptible to abrupt changes in global climate, owing to their generally limited potential of dispersion (because of their common dependence on active vectors) and their sedentary life cycle, both of which delay the potential response of these organisms to climate change (Fenoglio et al., 2010; Stoks et al., 2014). As a consequence, it is expected that their distribution ranges will decrease because of climate change, thus negatively affecting their contribution to ecosystem services (Covich et al., 1999).

The Neotropical zoogeographical area comprises rich terrestrial and aquatic biodiversity, especially for invertebrates (Barlow et al., 2018). Despite showing higher

diversity, tropical regions do not necessarily ensure higher functional diversity (Mouillot et al., 2014). Functional diversity is a diversity measure that takes into account the role of species in the environment. The functional aspects include morphological, physiological, and behavioural traits, which somehow affect the fitness and survival of species in the environment, as well the ecosystem functioning (McGill et al., 2006; Pacifici et al., 2015; Petchey & Gaston, 2006; Schmera et al., 2017). In the last decades, the use of functional diversity in ecosystem and community research has become a routine. However, such studies are somewhat biased to especially involve certain continents and organisms (i.e. Europe; plants and insects) (Bello et al., 2010; Luiza-Andrade et al., 2017; Schmera et al., 2017). For some groups of aquatic invertebrates and geographical regions, the functional information is scarce (Luiza-Andrade et al., 2017; Pacifici et al., 2015; Schmera et al., 2017; Piano et al., 2020; Mammola et al., 2021). Using only taxonomic richness in biodiversity studies can lead researchers to overlook areas with low (species) richness. However, with functional diversity metrics it is possible to identify areas with low richness which nevertheless support high functional diversity, a pattern which has been called the “conservation paradox” (Braghin et al., 2018).

Some studies indicate that functional redundancy is higher in the tropics, and that places with less taxonomic richness can hold the same amount of functional space than places poorer in taxonomic richness (Mouillot et al., 2014). Furthermore, different traits that are parts of functional diversity are not necessarily equally affected by climate change, with some combinations being more vulnerable than others. This could lead to functional homogenization (i.e., the occurrence of species with higher functional similarity) of communities (Domisch et al., 2013; Mouillot et al., 2014; Sundar et al., 2020). Previous research has identified that loss of taxonomic richness does not necessarily imply changes of functional diversity. For example, in plants a higher value of functional redundancy buffered the impacts of climate change on functional diversity (Villéger et al., 2010; Gallagher et al., 2013).

Considering that climate change affects freshwater ecosystems, and that the forecast for future scenarios is important for conservation measures, the present study has as

objective to assess the impact of climate change on the ostracod (small, bivalved crustaceans) functional diversity in the Southern Cone of South America. Ostracods inhabit aquatic environments, including in the roots of aquatic macrophytes and on sediments (Higuti & Martens, 2020). Although ostracods are generally quite common in aquatic environments, their functional diversity is generally overlooked, as most ecological studies in this have focussed on taxonomical diversity (Meisch et al., 2019; Conceição et al., 2020).

To achieve the above objective, we used ecological niche modelling (ENMs). The ENMs uses environmental data of observed distribution of species in order to predict suitable areas species and communities, allowing to predict future climatic suitable areas, from local to global scales (Pacifici et al., 2015; Guisan et al., 2017; Conceição et al., 2023). As proxies for the functional diversity of non-marine ostracods, we used three indices: functional richness (FRic), functional dispersion (FDis), and functional uniqueness (FUni) (Laliberte & Legendre, 2010; Ricotta et al., 2016). These indices deal with different aspects of functional diversity.

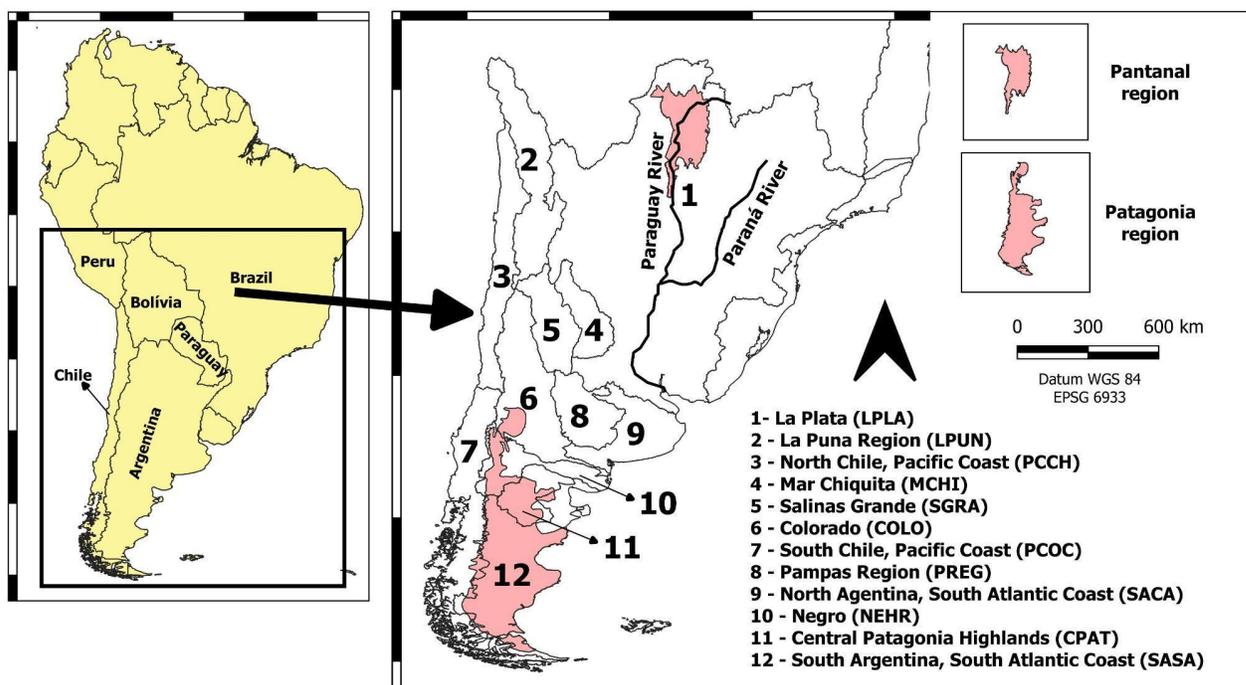
To predict the effects of climate change on the functional diversity of ostracods, we used two scenarios of carbon emissions: (1) moderate-optimistic (Representative Concentration Pathways, RCP 4.5) and (2) pessimistic (RCP 8.5) scenarios for two different years, 2050 and 2080.

## **6.2 Material and Methods**

### **6.2.1 Study Area**

We modelled the distribution of 57 ostracod species in 12 basins of the Southern Cone of South America (Fig 1). Amongst these basins, the larger drainage area is the La Plata basin (LPLA), which drains regions of Argentina, Bolivia, Brazil, Paraguay and Uruguay, maintaining a high human population density (Agostinho et al., 2004; Reis et al., 2016). Furthermore, the Southern Cone of South America comprises different climatic

areas, such as the sub-tropical LPLAbasin, and the temperate climate in the southernmost basins such as those of South Argentina, South Atlantic Coast (SASA), Central Patagonia Highlands (CPAT) and South Chile Pacific Coast (PCOC) (Kitzberger, 2012). The spatial and climatic heterogeneity is important for biodiversity, with some areas holding a larger number of species than others (Lara et al., 2005; Kitzberger 2012; Cusminsky et al., 2020).



**Fig. 1** Map showing the study area and river basins (derived from HydroSHEDS database) from the Southern Cone of South America.

### 6.2.2 Species occurrence data

We compiled a database with 9,772 georeferenced ostracod species occurrences throughout entire South America, except for adjacent islands and archipelagos. The occurrence data were obtained from the Global Biodiversity Information Facility (GBIF), a literature review and from the unpublished databases of the Macroinvertebrate Ecology Laboratory of the Centre of Research in Limnology, Ichthyology and Aquaculture

(Nupélia) of the State University of Maringá (UEM), Brazil. The literature review was performed until April 2021, using Google Scholar, by applying the names of species (and their synonyms), following the checklist of Meisch et al. (2019). The ostracod data of the Macroinvertebrate Ecology laboratory were obtained in the period between 2001 and 2020, from sampling campaigns carried out in the four major Brazilian floodplains (Amazon, Araguaia, Pantanal, and Paraná), and streams and reservoirs of the Paraná State.

### 6.2.3 Ecological niche modelling on taxonomic (species) richness

A grid with spatial resolution of 18 km<sup>2</sup> (24,820 cells for the entire continent) was constructed following the fluvial network of South America (Hydro1K data - <https://lta.cr.usgs.gov/HYDRO1K>, WGS84: EPSG 6933). The species occurrences were used to create a matrix of presence and pseudoabsence (the latter referring to the cells where the species was not recorded but might still occur). This matrix considered only species with occurrences in at least five cells, in order to avoid model bias (Pearson et al., 2006).

We used four Atmospheric-Ocean General Circulation Models (AOGCMs): Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO), Model for Interdisciplinary Research on Climate (MIROC), Meteorological Research Institute (MRI) and National Centre for Atmospheric Research (NCAR). For each AOGCMs we used two Representative Concentration Pathways (RCP), a moderate-optimistic (RCP 4.5) and a pessimist (RCP 8.5) one. The moderate-optimistic RCP means a moderate scenario of carbon accumulation, while the pessimist RCP means a scenario without efforts to contain emissions and consequently higher carbon accumulation (van Vuuren et al., 2011). We used both project scenarios for 2050 and 2080 to predict changes on distribution of both ostracod taxonomic and functional diversity.

For each future target year and for each RCP, we pre-selected the main variables related with freshwater organisms, such as maximum air temperature of the warmest month

(°C; TMAX), minimum air temperature of the coldest month (°C; TMIN), precipitation of the wettest month (mm; PMAX), and the precipitation of the driest month (mm; PMIN). Temperature and precipitation drive wet/drought cycles and affect life cycles of organisms (Ficke et al. 2007; Thomaz et al. 2007). To eliminate redundant variables, we performed a variance inflation factor test (VIF) which indicates the degree to which the standard errors are inflated owing to the levels of multicollinearity, indicating potential problems of collinearity (or redundancy). Creating models with redundant variables can lead to wrong predictions in ENMs (Guisan et al., 2017; Sillero & Barbosa, 2021). We excluded variables with VIF values above 3.1, and in this way TMIN was excluded from our study.

We used two hydrological variables. Firstly, the mean elevation value of the stream segments from node (m; FRMDN), which represent the altitude, and which restricts the dispersion of species. Secondly, the median of Strahler stream order of the segment (STRORD), obtained from the HYDRO1K database. The selected variables were included in our grid to obtain the climatic and environmental layer.

We performed the ENM on the platform BioEnsembles (Diniz-Filho et al., 2009). Six statistically different ENMs were used: Bioclim (BIOC, Busby, 1991, based on bioclimatic envelope logic); Euclidean Distance (EUCDIST—Carpenter et al., 1993); Gower Distance (GOWD, Gower, 1971, based on the environmental distance approach); Ecological Niche Factor Analysis (ENFA, Hirzel et al., 2002); MAXENT (Maximum Entropy, Phillips et al., 2006) and Genetic Algorithm for Rule-set Production (GARP—Stockwell, 1999, based on a machine learning technique). Each ENM results in different predictions of distribution areas. Thus, we employed the ensemble forecasting approach, providing a consensus of multiple models. We only interpreted the majority consensus models (see below), since this model reduces the uncertainty and error associated with different models (Araujo & New, 2007; Diniz-Filho et al. 2010).

In each ENM, species occurrence data were randomly divided in 75% for calibration (training data) and 25% for evaluation (test data). This was repeated 100 times for each model and for each scenario (present, RCP 4.5 and RCP 8.5) (six models x 100

repetitions x four AOGCM). These replications allowed us to generate a frequency of projection, which was then weighted by True Skill Statistics (TSS) (Allouche et al., 2006). The TSS is the sensitivity plus specificity – 1. The values range from -1 (worse than random prediction) to + 1 (ideal prediction). Majority consensus rule was used to build the consensus model, considering species as present only in the cells where 50% of the ENM models predicted the species presence (Diniz-Filho et al., 2009).

#### 6.2.4 Functional trait data analysis

The functional trait information was gathered through an extensive literature review of the 57 modelled species. We used six morphological, behavioural and reproductive traits to characterize the functional ecology of the non-marine ostracod species (APPENDIX A). The traits used were body length, body shape, locomotion mode, reproductive mode, production of resting eggs and presence of an brood pouch, leading to sexual dimorphic carapaces (Table 1). The species traits were classified based on original species descriptions, redescriptions, literature about the biological and morphological aspects (e.g. Martens 1998; Higuti & Martens, 2020). When the trait was not found for a certain species, it was based on the general character state of that trait in the genus or the family. We used in our analysis the so-called *response traits*. These are traits related to the abilities of species to colonize and/or resist environmental changes (Díaz et al., 2013).

For the functional data analyses, we used a species trait matrix (species in the rows x trait in the columns) and a community matrix with the consensus model (each cell of the grid in a row x species in the columns). We transformed the species x trait matrix on a Gower distance matrix, because this distance measure can handle different types of variables (i.e. categorical, quantitative) (Laliberte & Legendre, 2010). From this matrix, we calculated de FRic, FDis, FUni and CWM (community weighted mean, for exploratory purposes) for each cell. The FRic represents the multidimensional volume occupied by the community. While FDis represents the mean distance of species to the centroid of the

community (Laliberte & Legendre, 2010). FUni represents the singularity of each species in the community (Ricotta et al., 2016). As we are dealing with presence-absence data, the CWM shows the most common trait for each cell. The FRic, FDis and CWM indices were calculated with the FD package (Laliberte & Legendre, 2010). The FUni was calculated following the function available in Ricotta et al. (2016). To observe the relationship between taxonomic richness and functional richness we performed a Spearman correlation. To define the functional groups of ostracod we performed the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) using a Gower's distance matrix. All the analysis were performed with the software R (R Studio Team, 2021).

**Table 1.** Functional traits of ostracod species, including trait categories and explanation, used to calculate the functional indices.

<b>Traits</b>	<b>Categories</b>	<b>Functional explanation</b>
Body length	Continuous, in $\mu\text{m}$	Related to the energetic requirements of organisms (Merckx et al., 2018). It may also affect the dispersal of ostracods, owing to the higher potential of larger species to actively disperse (Campos et al., 2018); small-sized species have higher association with more complex habitats (Matsuda et al., 2015).
Body shape	Rounded: largest width >50% of the length  Cylindrical: larger width in posterior region, at least one extremity truncated	Related to habitat use. Ostracods with different shapes occupy different habitats (Marmonier et al., 1994). The body shape also affects predation on ostracods (Hayashi & Ohba, 2018).

	Flattened: largest width in <50% of the length, no extremity truncated	
Locomotion mode	Swimmer: long natatory setae on Antennae.  Non-swimmer: natatory seta absent	Important to the dispersion at smaller spatial scales, in habitat usage and for access to new resources (Petkovski et al., 1993; Campos et al., 2018; Céréghino et al., 2018).
Reproductive mode	Sexual: only sexual populations recorded  Asexual: only asexual populations recorded  Mixed: sexual and asexual populations recorded, or populations with males sexual females and asexual females	The reproductive mode can influence the survival, dispersion, and colonization of species. Sexual species hold a higher adaptation potential to new or changing environments, owing to higher genetic plasticity (Martens, 1998). Asexual individuals are better colonizers than the sexual ones, owing to the potential of one individual to colonize an entirely novel environment (Martens, 1998).
Brooding	Brooding  Non-brooding	The presence of brood chambers in ostracods gives the species the advantage of actively removing defective embryos and reducing the accumulation of deleterious mutations in the offspring (Pinto et al., 2007). Brooding species have less, but better protected, offspring.
Resting eggs	Present	The presence of drought resistant eggs is related to the potential of species to

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Absent

survive in temporary habitat with dry periods, and therefore affects the colonization of the species and environmental resilience (Rosa et al., 2020, 2022).

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## 6.3 Results

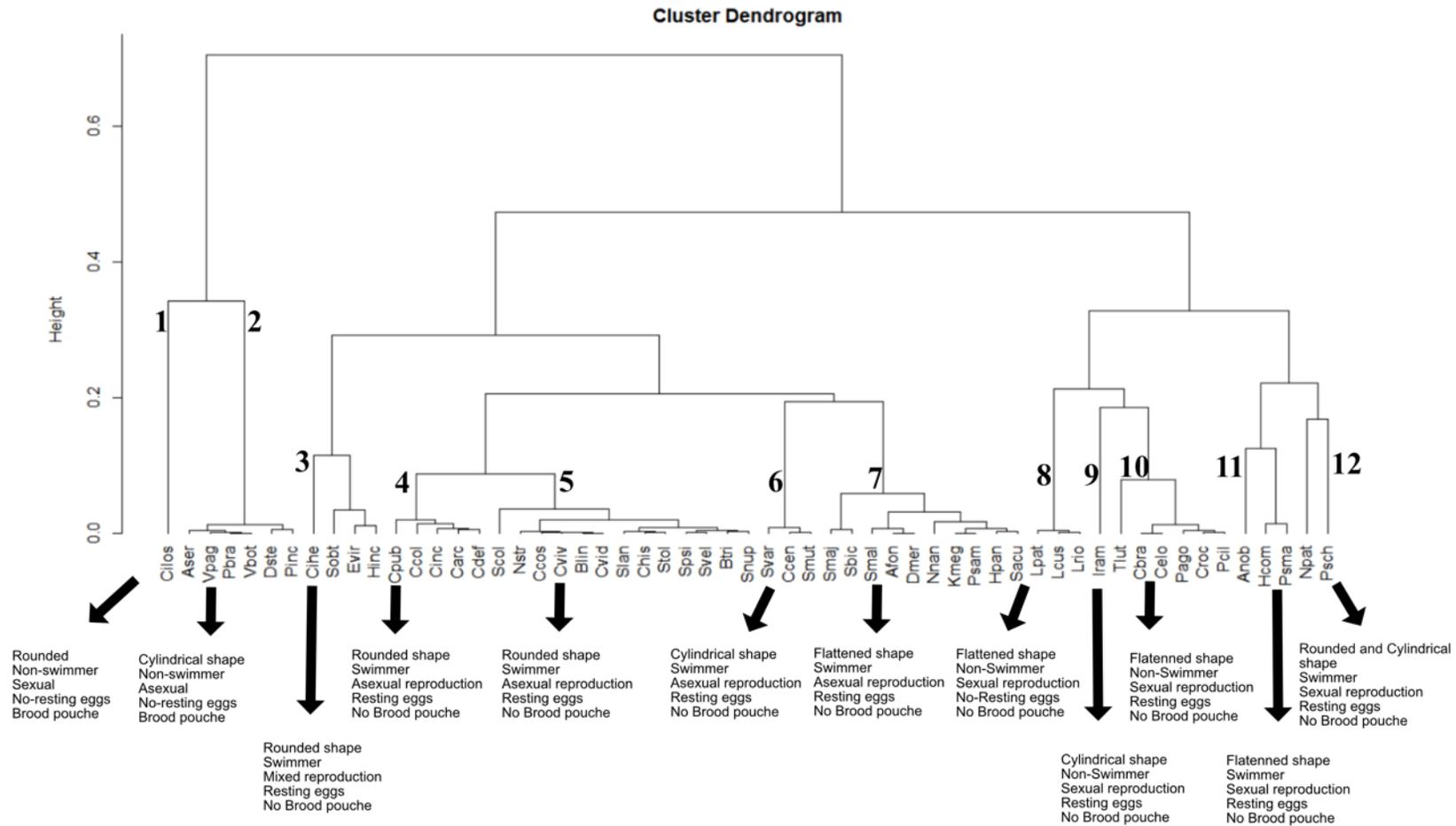
### 6.3.1 Functional groups

A total of 222 species were recorded in our survey, from which 57 species achieved the minimum number of occurrences (at least 5 cells) to perform the ENM. Species from six families were recorded Cyprididae (39), Candonidae (8), Darwinulidae (4), Limnocytheridae (4), Ilyocyprididae (1), Notodromadidae (1) (APPENDIX A). Within these 57 species we identified 12 functional groups with the cluster analysis (Fig. 2). Group five was most species rich (13 species), followed by group seven (10 species). Group 12 has only two species.

### 6.3.2 Taxonomic richness

Based on the consensus model and the overlap of species distribution models in the present, the highest taxonomic richness is found in the LPLA basin, especially in the Paraná River, and the Pantanal region (Table 2). Other areas along the LPLA basin have lower values of species richness, up to four species per cell. The southernmost part of Chile, Argentina, and Patagonia region (PCOC, CPAT, SASA, COLO, NEGR, PREG basins) have low to intermediate values of species richness per cell, up to 12 species (Fig. 3, Table 2).

For all the moderate-optimistic and pessimistic scenarios of 2050 and 2080, a reduction of the taxonomic richness in all studied basins is predicted, but in different intensities (Fig. 3, Table 2). In the moderate-optimistic scenario (RCP 4.5), for both years 2050 and 2080, a intermediate richness reduction is predicted, especially in cells with fewer species (up to four species) in the LPLA basin, and in the richest areas of the Pantanal. In pessimistic scenario (RCP 8.5), the reduction of richness projected is higher than in the moderate-optimistic scenario and occurs mainly in the LPLA basin. The Argentina region (SASA basin) showed a lower reduction of species richness, with areas of intermediate richness (up to twelve species), in both projected years and in both scenarios.



**Fig. 2** Cluster dendrogram of Ostracod functional groups. Species abbreviation: *Alicenula serricaudata* (Aser); *Amphicypris nobilis* (Anob); *Argentocypris fontana* (Afon); *Bradleytriebella lineata* (Blin); *Bradleytriebella trispinosa* (Btri); *Cabelodopsis hispida* (Chis); *Candobrasilopsis brasiliensis* (Cbra); *Candobrasilopsis elongata* (Celo); *Candobrasilopsis rochai* (Croc); *Chlamydotheca arcuata* (Carc); *Chlamydotheca colombiensis* (Ccol); *Chlamydotheca deformis* (Cdef); *Chlamydotheca*

*iheringi* (Cihe); *Chlamydotheca incisa* (Cinc); *Cypretta costata* (Ccos); *Cypretta vivacis* (Cviv); *Cypricercus alfredo* (Ccen); *Cypridopsis vidua* (Cvid); *Cypris pubera* (Cpub); *Cytheridella ilosvayi* (Cilos); *Darwinula stevensoni* (Dste); *Diaphanocypris meridana* (Dmer); *Eucypris virens* (Evir); *Hemicypris communis* (Hcom); *Heterocypris incongruens* (Hinc); *Heterocypris panningi* (Hpan); *Ilyocypris ramirezi* (Iram); *Kapcypridopsis megapodus* (Kmeg); *Limnocythere cusminskyae* (Lcus); *Limnocythere patagonica* (Lpat); *Limnocythere rionegroensis* (Lrio); *Neocypridopsis nana* (Nnan); *Neostrandesia striata* (Nstr); *Newnhamia patagonica* (Npat); *Paranacypris samambaiensis* (Psam); *Penthesilenula brasiliensis* (Pbra); *Penthesilenula incae* (Pinc); *Physocypris schubarti* (Psch); *Potamocypris smaragdina* (Psma); *Pseudocandona agostinhoi* (Pago); *Pseudocandona cillisi* (Pcil); *Sarscypridopsis aculeata* (Sacu); *Stenocypris major* (Smaj); *Stenocypris malayica* (Smal); *Strandesia bicuspis* (Sbic); *Strandesia colombiensis* (Scol); *Strandesia lansactohai* (Slan); *Strandesia mutica* (Smut); *Strandesia nupelia* (Snup); *Strandesia obtusata* (Sobt); *Strandesia psittacea* (Spsi); *Strandesia tolimensis* (Stol); *Strandesia variegata* (Svar); *Strandesia velhoi* (Svel); *Tonnacypris lutaria* (Tlut); *Vestalenula botocuda* (Vbot); *Vestalenula pagliolii* (Vpag).

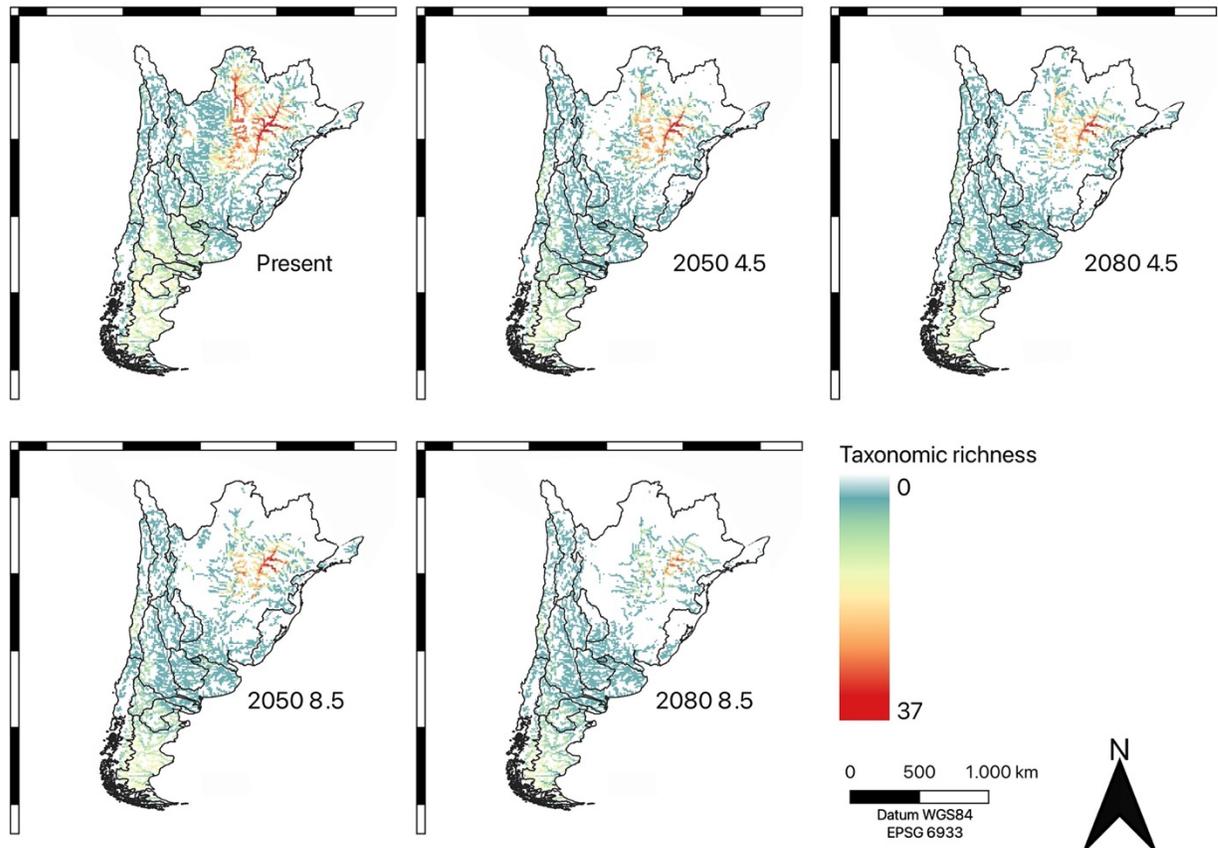
**Table 2.** Mean of taxonomic richness (S) and functional indices per basins, in the present and future times (2050 and 2080) for two scenarios of carbon emissions, moderate-optimistic (RCP 4.5) and pessimistic (RCP 8.5).

	<b>Present</b>				<b>RCP 4.5</b>								<b>RCP 8.5</b>							
					<b>2050</b>				<b>2080</b>				<b>2050</b>				<b>2080</b>			
	<b>S</b>	<b>Fric</b>	<b>Fdis</b>	<b>Funi</b>	<b>S</b>	<b>Fric</b>	<b>Fdis</b>	<b>Funi</b>	<b>S</b>	<b>Fric</b>	<b>Fdis</b>	<b>Funi</b>	<b>S</b>	<b>Fric</b>	<b>Fdis</b>	<b>Funi</b>	<b>S</b>	<b>Fric</b>	<b>Fdis</b>	<b>Funi</b>
<b>LPLA</b>	5.608	0.254	0.141	0.211	3.210	0.138	0.075	0.115	2.577	0.107	0.062	0.096	2.378	0.102	0.060	0.091	1.177	0.036	0.028	0.045
<b>LPUN</b>	0.842	0.002	0.033	0.071	0.957	0.009	0.035	0.064	0.875	0.008	0.028	0.051	0.935	0.010	0.035	0.065	0.917	0.007	0.040	0.073
<b>PCCH</b>	2.951	0.110	0.166	2.046	3.037	0.106	0.182	0.311	2.558	0.084	0.137	0.235	2.381	0.075	0.121	0.200	1.873	0.060	0.101	0.171
<b>MCHI</b>	2.810	0.105	0.136	0.224	1.325	0.011	0.032	0.060	1.010	0.002	0.029	0.057	0.950	0.003	0.025	0.048	0.618	0.000	0.013	0.027
<b>SGRA</b>	2.160	0.235	0.196	0.269	1.290	0.226	0.167	0.065	1.330	0.199	0.174	0.070	1.221	0.164	0.181	0.048	1.043	0.015	0.160	0.023
<b>COLO</b>	4.417	0.435	0.264	0.413	2.441	0.131	0.152	0.254	2.476	0.111	0.173	0.293	1.992	0.099	0.108	0.180	1.676	0.057	0.092	0.157
<b>PCOC</b>	1.692	0.156	0.105	0.165	2.057	0.189	0.097	0.158	2.115	0.167	0.093	0.151	2.409	0.205	0.102	0.164	2.438	0.207	0.106	0.173
<b>PREG</b>	5.404	0.341	0.271	0.408	2.734	0.083	0.158	0.276	1.833	0.018	0.084	0.151	1.843	0.020	0.098	0.172	1.191	0.002	0.029	0.057
<b>SACA</b>	3.484	0.080	0.206	0.329	2.201	0.060	0.103	0.174	2.070	0.031	0.097	0.172	1.917	0.049	0.059	0.106	1.447	0.009	0.045	0.083
<b>NEHR</b>	6.291	0.535	0.304	0.455	3.598	0.192	0.243	0.388	3.492	0.166	0.242	0.386	3.098	0.134	0.227	0.377	2.057	0.092	0.169	0.285
<b>CPAT</b>	7.644	0.616	0.288	0.433	4.573	0.307	0.263	0.413	4.153	0.233	0.262	0.416	4.159	0.216	0.255	0.414	3.408	0.164	0.239	0.399
<b>SASA</b>	7.099	0.608	0.277	0.434	6.034	0.465	0.279	0.425	5.905	0.413	0.280	0.428	6.492	0.429	0.278	0.420	5.288	0.358	0.266	0.412

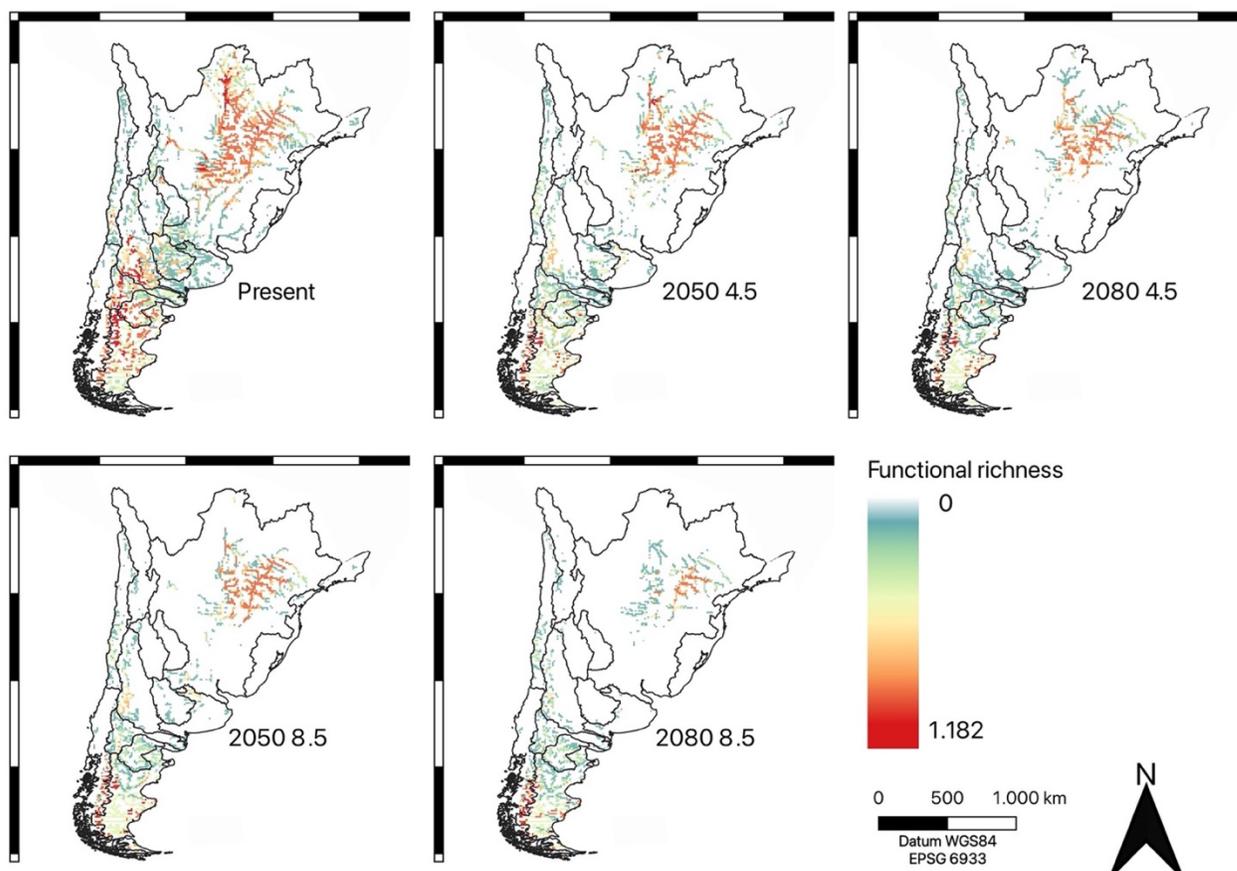
### 6.3.3 Functional richness - FRic

The present situation shows a higher FRic in the LPLA basin (especially in the Pantanal, Paraná River, and its tributaries). Higher values of FRic are also presently found in the Argentina territory (SASA, CPAT, NEHR, and COLO basins) (Fig. 4, Table 2). A gradual reduction of FRic in LPLA basin is predicted for the target year 2050 in both the moderate-optimistic and the pessimistic scenarios as compared to the present situation. Higher values are concentrated in Paraná River and tributaries in general (LPLA basin), but reductions are found in the southernmost part of the river. For 2080, the models also predicted a reduction of FRic in the LPLA basin in both the moderate-optimistic and pessimistic scenarios. In the pessimistic scenario of 2080, the functional richness is reduced to almost zero in the Pantanal region (LPLA basin). Despite the loss of taxonomical richness, the Argentinean and Chilean regions (SASA, PCOC, CPAT, NEHR basins) will sustain low to intermediate values of functional richness, and a few sparse cells with high values in the most pessimist scenario (Fig. 4, Table 2).

The Spearman correlation showed a strong positive correlation between taxonomic richness and FRic in all time scenarios ( $\rho > 0.75$ ,  $p < 0.05$ ).



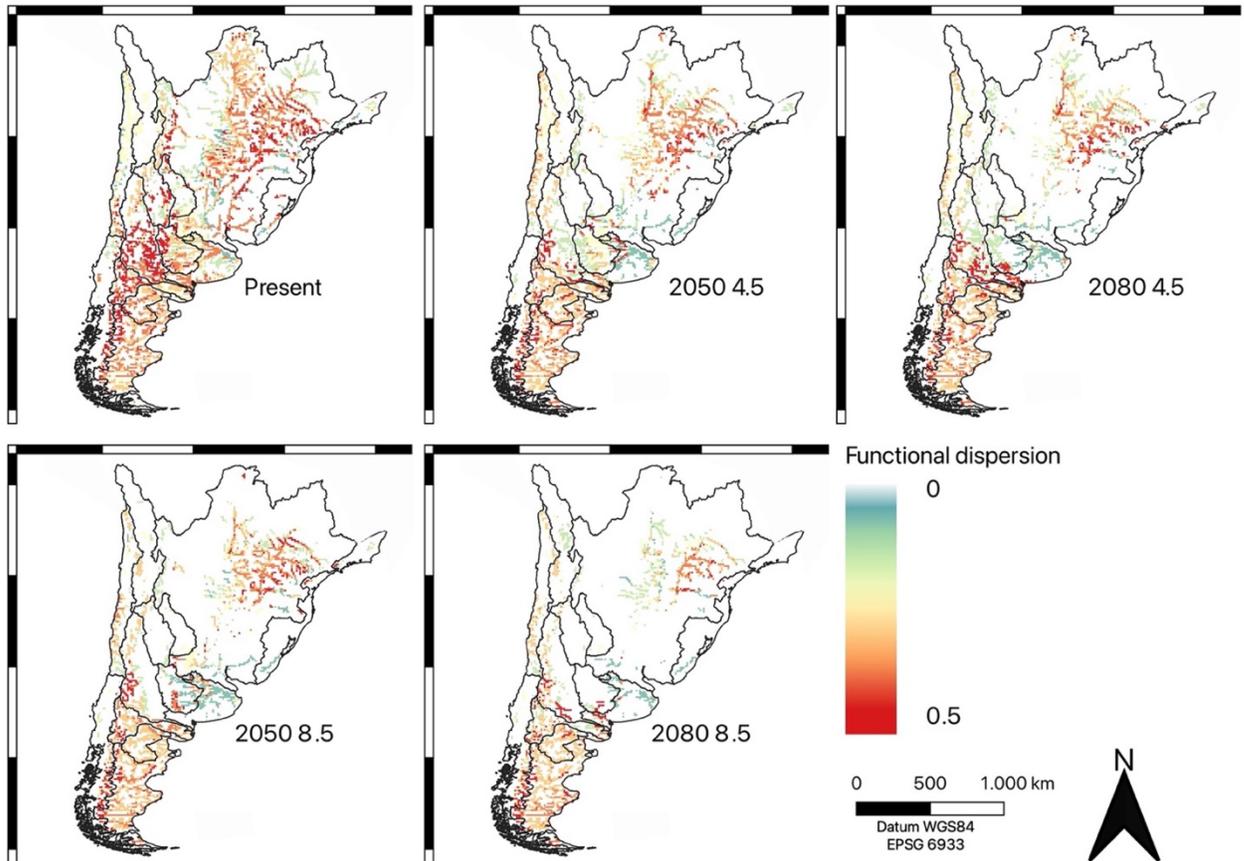
**Figure 3.** Ostracod taxonomic richness in the present and future times (2050 and 2080) for two scenarios of carbon emissions, moderate (RCP 4.5) and pessimist (RCP 8.5).



**Fig. 4** Ostracod FRic in the present and future times (2050 and 2080) for two scenarios of carbon emissions, moderate-optimistic (RCP 4.5) and pessimist (RCP 8.5).

#### 6.3.4 Functional dispersion - FDis

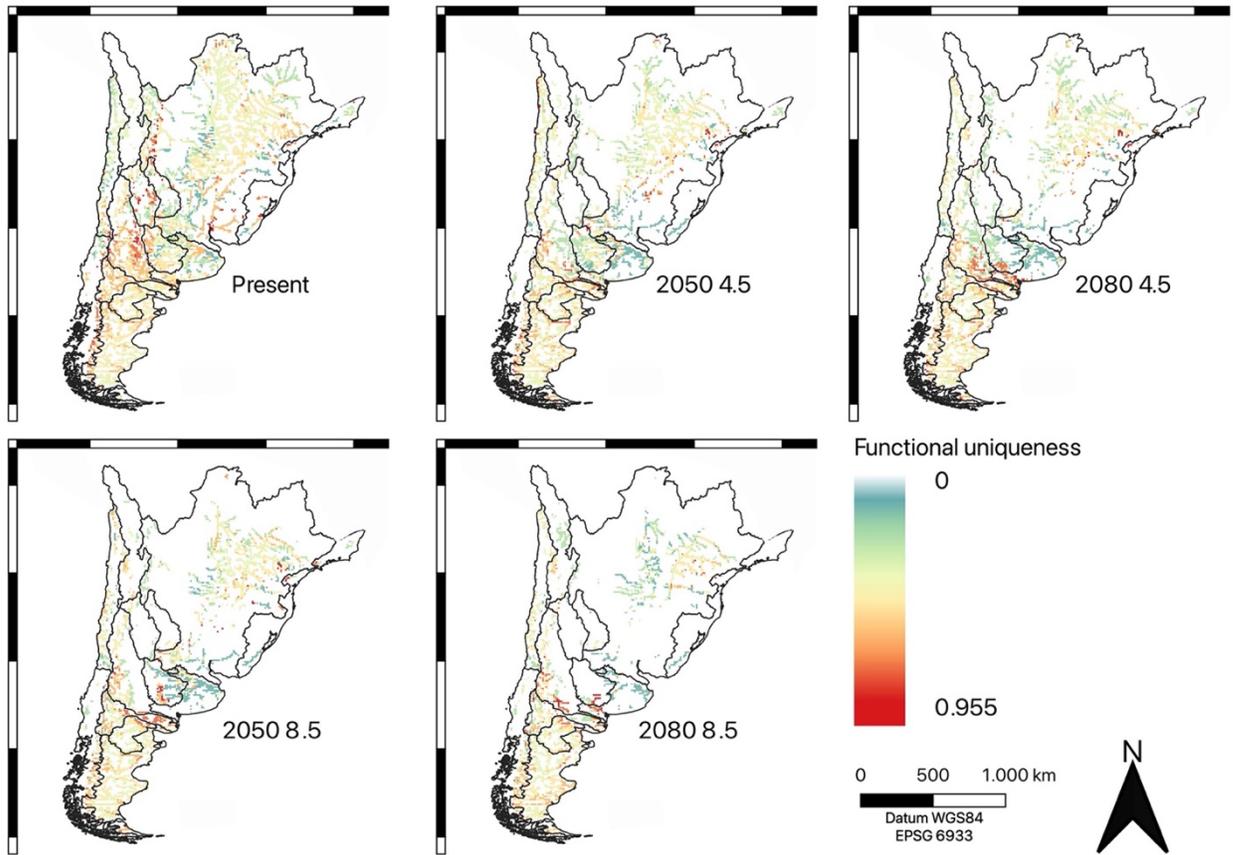
Higher values of FDis are recorded in the Argentinean territory in the present situation (SASA, CPAT, COLO, PREG, LPLA and SGRA basins). In the LPLA basin higher values are recorded in the Paraná River tributaries (Fig. 5, Table 2). For 2050, the models predicted a reduction of FDis in the LPLA, COLO, SGRA and the PREG basins in both the moderate-optimistic and pessimistic scenarios as compared to the present. This reduction is also projected for 2080, especially in the LPLA basin, while in the Argentinean and Chilean territory (SASA, CPAT, PCOC, and COLO basins) intermediate to higher values are still being projected. Intermediate and higher dispersion values are also predicted in the Paraná River and tributaries (LPLA), even in the most pessimist scenario of 2080. A reduction in the Pantanal functional dispersion, with low values being sustained, is also predicted.



**Fig. 5** Ostracod FDis in the present and future times (2050 and 2080) for two scenarios of carbon emissions, moderate-optimistic (RCP 4.5) and pessimist (RCP 8.5).

### 6.3.5 Functional uniqueness - FUni

In the present, for most of the area studied values of FUni are low, while scattered higher values are projected in Argentina (COLO, SGRA, PREG, NEHR basins, and on west of LPLA basin)(Fig. 6, Table 2). For 2050, a reduction of FUni in all basins, but especially in LPLA and COLO, is predicted for both moderate-optimistic and pessimistic scenarios as compared to the present situation. An accentuated reduction of FUni in most cells is projected for the LPLA, and a decline in regions such as the Paraná River and the Pantanal. A reduction is also predicted for 2080, especially with the low and intermediate values concentrated in the southern regions of Argentina and Chile (PCOC, NEHR, CPAT and SASA basins).



**Fig. 6** Ostracod FU in the present and future times (2050 and 2080) for two scenarios of carbon emissions, moderate-optimistic (RCP 4.5) and pessimist (RCP 8.5).

## 6.4 Discussion

Our results show a decrease in suitable areas for ostracod species owing to climate change. For the two future years and in both scenarios of carbon emissions, a gradual reduction of the ostracod functional diversity in the entire South Cone of South America basins is predicted, albeit with different intensities, and with the greatest reduction concentrated in the pessimistic scenario of 2080. These results indicate that both regions with low and high richness of species will have their suitable areas reduced in future scenarios. This could be owing to the substitution of functionally similar species (Mouillot et al., 2014; Ricotta et al., 2016).

The LPLA basin shelters higher ostracod taxonomic richness, although it does not hold higher functional richness. Functional richness depends on mechanisms such as environmental filters, which lead to a limited range of functional characteristics owing to the selection for more functionally similar species (Clavero & Brotons, 2010; Hooper et al., 2005). Although most studies report on correlations between functional and taxonomic richness, the former reaches a maximum faster than taxonomic richness, owing to species functional redundancy (Gerisch et al., 2012; Schmera et al., 2017). When comparing the taxonomical richness and FRic, it was observed a high correlation, which shows that higher values of taxonomic richness also led to high FRic. A similar pattern was also predicted for birds (Bihn et al., 2010) and ants (Petchey et al., 2007).

A mismatch between taxonomic richness and functional diversity was observed mainly in the LPLA basin, more specifically in the Paraná River and Pantanal region. These areas hold high taxonomic richness, and intermediate to higher levels of FRic and FDis. However, they show low to intermediate levels of FUni. This mismatch in combination with the low values of FUni is a sign that most species are performing similar functions, i.e. they hold a similar set of character states for the traits. From 57 modelled species in the present study, 39 belong to Cyprididae (Meisch et al., 2019). Thus, it is expected that phylogenetically closely related species will share similar traits (Campos et al., 2021). Indeed, studies show that most ostracod species recorded in the Pantanal and Paraná River regions belong to the family Cyprididae (Higuti et al., 2017a; Higuti et al., 2017b), and this could explain the intermediate values of FDis and low values for FUni projected to areas as the Pantanal and Paraná River.

Despite the low and intermediate values of taxonomical richness, the projected intermediate and higher values of FDis and FUni in the Patagonian region (CPAT, NEGR and

PREG basins), indicate that these areas are composed of species displaying different character states for the traits, which ensure more ecological functions (e.g. nutrient flux) to the ecosystem functioning (Hopper et al., 2005; Ricotta et al., 2016; Oliveira et al., 2019). In fact, a few endemic species were recorded from the Patagonia region (Ramos et al., 2022), amongst them species with rarer character states for the traits, such as cylindrical body shape, sexual reproduction, and absence of resting eggs. The presence of such character states of the traits makes species less efficient dispersers. For example, the success of sexual reproduction depends on the fact that two individuals of the opposite gender need to find each other in a spatially and temporally diluted environment. In addition, the absence of resting eggs reduces the chances to survive desiccation and reduces the dispersal potential (through dried plants, for example, see Rosa et al., 2022). In addition, owing to the colder climates, many ostracod species of these basins are adapted to lower temperatures (Paruelo et al., 1998; Cusminsky et al., 2020) and with climate change such species will need to migrate towards warmer areas (Conceição et al., 2023), a movement that could be limited by their traits.

The reduction in the FUni in future scenarios, especially in the most pessimistic one for 2080, accompanied by a reduction in taxonomical richness indicate that more unique character states of traits are being lost, leading to an increase in functional redundancy. This is projected for LPLA (especially in the Pantanal, and tributaries of Paraná River), COLO, SGRA basins. Some regions maintained the values for FUni throughout the climate change scenarios, such as the basins in southernmost part of Argentina and Chile (SASA, CPAT, NEHR, PCOC). Evidence from other organisms, such as fish, indicates that human impacts will cause an increase in the similarity in assemblages, by the extinction of species with unique traits (Brandl et al., 2016; Mouillot et al., 2014). On the other hand, the functional redundancy is important to ensure ecosystems functions in the face of species decline (Mouillot et al., 2014).

Although most ostracod species are good regional dispersers through active swimming, they are also dispersed passively (by resting eggs) over longer distances by animals and plants (Sabagh & Rocha, 2014; Higuti & Martens, 2016; Schön et al., 2018, Morais Junior et al., 2019; Rosa et al., 2022). Therefore, the maintenance of places with higher functional diversity is important, as these regions can become a source for these organisms to disperse to other basins. Furthermore, such areas should also be prioritized in conservation efforts, because supporting these unique species and communities could ensure different responses to

disturbances. This could help to stabilize the ecosystems processes and services, owing the species complementarity (Hooper et al., 2005; Buisson et al., 2013; Bruno et al., 2019).

The Pantanal and Paraná River are amongst the largest floodplains in Brazil and are widely known for their high biodiversity and strict conservation measures (Barros et al., 2004; Lozano & Malo, 2013). Because they are located in an area of high human population density, these regions are also affected by factors beyond the climatic change projected here, such as river flow changes owing hydroelectric dam construction, pollution, irrigation systems, and others (Agostinho et al., 2004; Lozano & Malo, 2013). Thus, with the taxonomic richness and functional diversity reduction projected here, these areas are vulnerable to a wide array of human impacts. Furthermore, the Paraguay River is the main tributary of the Paraná River, so the reduction of functional diversity in this river basin will also have consequences for the Paraná River (and tributaries) and all the wetlands associated with them.

## 6.5 Conclusions

The present study is the first approach accessing the impact of climate change on ostracod functional diversity at large geographical scales. As in studies using other organisms (Gallagher et al., 2013; Braghin et al., 2018; Oliveira et al., 2019), we also predict that climate change will seriously impact ostracod functional diversity under both moderate-optimistic and pessimistic scenarios for both years 2050 and 2080. The occurrence of a high functional redundancy in species rich areas can be explained by habitat filtering selecting similar species, the occurrence of phylogenetically closely related species (from the same family, Cyprididae), but also from stochastic and immigration effects, which not analysed here. The use of functional diversity indices is important when analysing ostracod diversity, because of the complementary of these indices to taxonomical measures. Using only the taxonomical information, we would choose to protect only places with higher present diversity (e.g. LPLA basin). However, in doing so we would overlook areas with important functional diversity and redundancy (e.g. NEHR, CPAT and SASA). Thus, the use of different diversity measures is important when considering priority areas for conservation.

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**APPENDIX A-** Functional traits of the 57 modelled ostracod species from South America.

<b>Species</b>	<b>Code</b>	<b>Body length (µm)</b>	<b>Body shape</b>	<b>Locomotion mode</b>	<b>Reproduction mode</b>	<b>Brooding</b>	<b>Resting eggs</b>
<i>Alicenula serricaudata</i> (Klie, 1935) Rossetti & Martens, 1998	Aser	0.62	cylindrical	non-swimmer	asexual	brooding	absent
<i>Amphicypris nobilis</i> Sars, 1901	Anob	3.20	flattened	swimmer	sexual	non-brooding	present
<i>Argentocypris fontana</i> (Graf, 1931) Díaz & Martens, 2014	Afon	1.25	flattened	swimmer	asexual	non-brooding	present
<i>Bradleytriebella lineata</i> (Victor & Fernando, 1981) Savatnalinton & Martens, 2010	Blin	0.64	rounded	swimmer	asexual	non-brooding	present
<i>Bradleytriebella trispinosa</i> (Pinto & Purper, 1965)	Btri	1.09	rounded	swimmer	asexual	non-brooding	present
<i>Cabelodopsis hispida</i> (Sars, 1901) Higuti & Martens, 2012	Chis	0.92	rounded	swimmer	asexual	non-brooding	present
<i>Candobrasilopsis brasiliensis</i> (Sars, 1901) Higuti & Martens, 2012	Cbra	0.97	flattened	non-swimmer	sexual	non-brooding	present
<i>Candobrasilopsis elongata</i> Higuti & Martens, 2014	Celo	0.97	flattened	non-swimmer	sexual	non-brooding	present
<i>Candobrasilopsis rochai</i> Higuti & Martens, 2012	Croc	0.78	flattened	non-swimmer	sexual	non-brooding	present
<i>Chlamydotheca arcuata</i> (Sars, 1901) G.W. Müller, 1912	Carc	2.60	rounded	swimmer	asexual	non-brooding	present
<i>Chlamydotheca colombiensis</i> Roessler, 1985	Ccol	2.88	rounded	swimmer	asexual	non-brooding	present

<i>Chlamydotheca deformis</i> (Farkas, 1958) Kotzian, 1974	Cdef	2.70	rounded	swimmer	asexual	non- brooding	present
<i>Chlamydotheca iheringi</i> (Sars, 1901) Klie, 1930	Cihe	3.62	rounded	swimmer	mixed	non- brooding	present
<i>Chlamydotheca incisa</i> (Claus, 1892) Sharpe, 1910	Cinc	2.50	rounded	swimmer	asexual	non- brooding	present
<i>Cypretta costata</i> G.W. Müller, 1898	Ccos	0.66	rounded	swimmer	asexual	non- brooding	present
<i>Cypretta vivacis</i> Würdig & Pinto, 1993	Cviv	0.62	rounded	swimmer	asexual	non- brooding	present
<i>Cypricercus alfredo</i> Almeida et al. 2021	Ccen	1.50	cylindrical	swimmer	asexual	non- brooding	present
<i>Cypridopsis vidua</i> (O. F. Müller, 1776) Brady, 1867	Cvid	0.64	rounded	swimmer	asexual	non- brooding	present
<i>Cypris pubera</i> O.F. Müller, 1776	Cpub	2.28	rounded	swimmer	asexual	non- brooding	present
<i>Cytheridella ilosvayi</i> Daday, 1905	Cilos	0.78	rounded	non-swimmer	sexual	brooding	absent
<i>Darwinula stevensoni</i> (Brady & Robertson, 1870) Brady & Robertson, 1885	Dste	0.74	cylindrical	non-swimmer	asexual	brooding	absent
<i>Diaphanocypris meridana</i> (Furtos, 1936) Würdig & Pinto, 1990	Dmer	1.24	flattened	swimmer	asexual	non- brooding	present
<i>Eucypris virens</i> (Jurine, 1820) Daday, 1900	Evir	1.55	rounded	swimmer	mixed	non- brooding	present
<i>Hemicypris communis</i> (Klie, 1940) Purper & Würdig-Maciel, 1974	Hcom	0.96	flattened	swimmer	sexual	non- brooding	present
<i>Heterocypris incongruens</i> (Ramdohr, 1808) Claus, 1892	Hinc	1.77	rounded	swimmer	mixed	non- brooding	present
<i>Heterocypris panningi</i> Brehm, 1934	Hpan	0.67	flattened	swimmer	asexual	non- brooding	present

<i>Ilyocypris ramirezi</i> Cusminsky & Whatley, 1996	Iram	0.85	cylindrical	non-swimmer	sexual	non-brooding	present
<i>Kapcypridopsis megapodus</i> Cuminsky et al., 2005	Kmeg	0.82	flattened	swimmer	asexual	non-brooding	present
<i>Limnocythere cusminskyae</i> Ramón Mercau et al. 2014	Lcus	0.62	flattened	non-swimmer	sexual	non-brooding	absent
<i>Limnocythere patagonica</i> Cusminsky & Whatley, 1996	Lpat	0.70	flattened	non-swimmer	sexual	non-brooding	absent
<i>Limnocythere rionegroensis</i> Cusminsky & Whatley, 1996	Lrio	0.60	flattened	non-swimmer	sexual	non-brooding	absent
<i>Neocypridopsis nana</i> (Sars, 1901) Klie, 1940	Nnan	0.44	flattened	swimmer	asexual	non-brooding	present
<i>Neostrandesia striata</i> Ferreira et al. 2019	Nstr	0.69	rounded	swimmer	asexual	non-brooding	present
<i>Newnhamia patagonica</i> (Vávra, 1898) Vávra, 1901	Npat	0.60	cylindrical	swimmer	sexual	non-brooding	present
<i>Paranacypris samambaiensis</i> Higuti et al., 2009	Psam	0.82	flattened	swimmer	asexual	non-brooding	present
<i>Penthesilenula brasiliensis</i> (Pinto & Kotzian, 1961) Rossetti & Martens, 1998	Pbra	0.55	cylindrical	non-swimmer	asexual	brooding	absent
<i>Penthesilenula incae</i> (Delachaux, 1928) Rossetti & Martens, 1998	Pinc	0.87	cylindrical	non-swimmer	asexual	brooding	absent
<i>Physocypris schubarti</i> Farkas, 1958	Psch	0.56	rounded	swimmer	sexual	non-brooding	present
<i>Potamocypris smaragdina</i> (Vávra, 1891) Daday, 1900	Psma	0.68	flattened	swimmer	sexual	non-brooding	present
<i>Pseudocandona agostinhoi</i> Higuti & Martens, 2014	Pago	0.69	flattened	non-swimmer	sexual	non-brooding	present
<i>Pseudocandona cillisi</i> Higuti & Martens, 2014	Pcil	0.75	flattened	non-swimmer	sexual	non-brooding	present

<i>Sarscypridopsis aculeata</i> (Costa, 1847) McKenzie, 1977	Sacu	0.72	flattened	swimmer	asexual	non-brooding	present
<i>Stenocypris major</i> (Baird, 1859) Daday, 1898	Smaj	2.00	flattened	swimmer	asexual	non-brooding	present
<i>Stenocypris malayica</i> Victor & Fernando, 1981	Smal	1.40	flattened	swimmer	asexual	non-brooding	present
<i>Strandesia bicuspis</i> (Claus, 1892) G.W. Müller, 1912	Sbic	2.10	flattened	swimmer	asexual	non-brooding	present
<i>Strandesia colombiensis</i> Roessler 1990 (Ferreira et al. 2020)	Scol	1.56	rounded	swimmer	asexual	non-brooding	present
<i>Strandesia lansactohai</i> Higuti & Martens, 2013	Slan	0.97	rounded	swimmer	asexual	non-brooding	present
<i>Strandesia mutica</i> (Sars, 1901) G.W. Müller, 1912	Smut	1.46	cylindrical	swimmer	asexual	non-brooding	present
<i>Strandesia nupelia</i> Higuti & Martens, 2013	Snup	1.04	rounded	swimmer	asexual	non-brooding	present
<i>Strandesia obtusata</i> (Sars, 1901) G.W. Müller, 1912	Sobt	0.99	rounded	swimmer	mixed	non-brooding	present
<i>Strandesia psittacea</i> (Sars, 1901) Roessler, 1990	Spsi	1.16	rounded	swimmer	asexual	non-brooding	present
<i>Strandesia tolimensis</i> Roessler, 1990	Stol	0.91	rounded	swimmer	asexual	non-brooding	present
<i>Strandesia variegata</i> (Sars, 1901) G.W. Müller, 1912	Svar	1.32	cylindrical	swimmer	asexual	non-brooding	present
<i>Strandesia velhoi</i> Higuti & Martens, 2013	Svel	1.14	rounded	swimmer	asexual	non-brooding	present
<i>Tonnacypris lutaria</i> (Koch, 1838) Diebel & Pietrzeniuk, 1975	Tlut	2.35	flattened	non-swimmer	sexual	non-brooding	present
<i>Vestalenula botocuda</i> Pinto et al., 2003	Vbot	0.56	cylindrical	non-swimmer	asexual	brooding	absent
<i>Vestalenula pagliolii</i> (Pinto & Kotzian, 1961) Rossetti & Martens, 1998	Vpag	0.52	cylindrical	non-swimmer	asexual	brooding	absent

## 7 CONCLUDING REMARKS

In the present thesis we contribute with the increase of the knowledge about the biodiversity of ostracods, and the reduction of the Linnean and Wallacean shortfalls (see HORTAL et al. 2015) describing a new genus and new species from Cyprinae s.l. Despite the effort to know the biodiversity of ostracods, taxonomic attention is still needed owing to the occurrence of new records and the discovery of several new species and new genera. This is especially true for area such as Brazil, with several species being discovered in the last years.

In both moderate-optimistic and pessimistic scenarios of climate change are projected to reduce the ostracod functional diversity along basins of the South Cone of the South America (e.g. La Plata basin), especially in the pessimistic scenario of 2080. Using functional diversity indices showed be important, because with the use of only taxonomical measure we would be overlooking several areas that are not taxonomically rich but sustain high functional diversity.

South America and Africa are regions generally overlooked and possess extensive areas that still do not studied, and probably hold a high biodiversity. Thus, research must be encouraged in these areas, and should also focus on not-so-studied groups, as ostracods. These findings may provide subsidies for managers to make decisions to the conservation of aquatic environments, with the management of preserved areas or with the creating of new ones, since, for instance, species reported here are endemic to unique basin.

**COMPLEMENTARY INFORMATION NOTE–List of papers published or in press, and presented at events during the academic formation.**

**Papers accepted or published**

ALMEIDA, N. M.; FERREIRA, V. G.; HIGUTI, J.; MARTENS, K. Seven new species and two new genera of *Physocypria* sensu lato (Crustacea, Ostracoda) from Brazilian floodplains. *Zootaxa* (Online), 2023. *In press*

FERREIRA, V. G.; ROSA, J.; ALMEIDA, N. M.; PEREIRA, J. S.; SABATER, L. M.; VENDRAMIN, D.; ZHU, H.; MARTENS, K.; HIGUTI, J. A comparison of three main scientific literature databases using a search in aquatic ecology. *Hydrobiologia*, v. 1, p. 1, 2022.

ALMEIDA, N. M.; FERREIRA, V. G.; HIGUTI, J.; MARTENS, K. On two new species of *Cypricercus* Sars, 1895 (Crustacea, Ostracoda) from Brazil with a discussion on the taxonomy of the genus. *Zootaxa* (Online), v. 4938, p. 501-536, 2021.

CAMPOS, R.; ROSA, J.; FERREIRA, V. G.; CONCEIÇÃO, E. O.; MARTENS, K.; HIGUTI, J. Macrophyte life forms influence the effects of environmental and spatial factors on the beta-diversity of associated ostracod communities (Crustacea). *Aquatic Sciences*, v. 83, p. 1-15, 2021.

ALMEIDA, N. M.; HIGUTI, J.; FERREIRA, V. G.; MARTENS, K. A new tribe, two new genera and three new species of Cypridopsinae (Crustacea, Ostracoda, Cyprididae) from Brazil. *European Journal of Taxonomy*, v. 762, p. 1-48, 2021.

HIGUTI, J.; ROSA, J.; FERREIRA, V. G.; CAMPOS, R.; CONCEIÇÃO, E. O.; MARTENS, K. Inter-annual variation of ostracod (Crustacea) communities in the Upper Paraná River Floodplain, Brazil. *Oecologia Australis*, v. 24, p. 474-488, 2020.

**Scientific and Environmental education**

Projeto de extensão “Se todos camPEArem, a conservação acontece” – SOS Riachos de Maringá, PR – 2019.

Mostra Científica promovida pelo PELD CNPq – UEM – Nupélia na Base Avançada do Nupélia, Porto Rico, PR – 2019.

## **Talks**

Palestrante, com a palestra intitulada “Experiências no Doutorado no exterior – Bélgica” – organizada pelo Programa de Pós-graduação em Ecologia de Ambientes Aquáticos Continentais, Maringá, PR – 2022.

Palestrante, com a palestra intitulada “Aspectos gerais e taxonomia morfológica de ostracodes de água doce” – organizado pelo Grupo de Estudos de Ecologia Aquática – 2021.

## **Consultant and Advisor**

Coorientador da aluna de Iniciação Científica Mariana Alice dos Reis Lucio. “Dieta de ostrácodes (Crustacea, Ostracoda) associados a *Eichhornia crassipes* (Mart.) Solms”, Universidade Estadual de Maringá – 2022-2023.

Revisor do periódico *Zootaxa* – 2022.

Consultor Ad Hoc de resumos expandidos dos participantes do 30º Encontro Anual de Iniciação Científica – EAIC e do 10º EAIC Júnior – 2021.

Membro da comissão julgadora de Monografia da aluna Nadiyah Martins de Almeida. “Descrição de duas novas espécies de *Cypricercus* Sars 1895 (Crustacea, Ostracoda) do Brasil” Universidade Estadual de Maringá, Departamento de Biologia – 2020.

Representante discente do Doutorado junto ao Conselho do Programa de Pós-graduação em Ecologia de Ambientes Aquáticos Continentais – 2019-2020.