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DESCRIÇÃO DE TRÊS NOVAS ESPÉCIES DE *HYALELLA* (CRUSTACEA, AMPHIPODA, HYALELLIDAE) DA REGIÃO NOROESTE DO ESTADO DO RIO GRANDE DO SUL, BRASIL

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Biodiversidade Animal, da Universidade Federal de Santa Maria, como requisito parcial para obtenção do título de **Mestre em Ciências Biológicas – Área Biodiversidade Animal.**

Orientadora: Prof. Dra. Daniela da Silva Castiglioni

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Aprovada em 12 de agosto de 2016:

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Santa Maria,RS, Brasil
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Aos meus pais Aurélio e

Giovane e minha irmã Mônica.

E ao meu noivo Fábio.

Amo vocês!

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“O que vale na vida não é o ponto de partida, e sim a caminhada. Caminhando e semeando, no fim terás o que colher.”

(Cora Carolina)

RESUMO

DESCRIÇÃO DE TRÊS NOVAS ESPÉCIES DE *Hyalella* (CRUSTACEA, AMPHIPODA, HYALELLIDAE) DA REGIÃO NOROESTE DO ESTADO DO RIO GRANDE DO SUL, BRASIL

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Atualmente existem 68 espécies descritas do gênero *Hyalella,* sendo que na América do Sul, tem-se a maior diversidade, com registro de 52 espécies. No Estado do Rio Grande do Sul, nove espécies do gênero foram registradas. Com base em amostragens de coletas durante um longo período, o objetivo do presente estudo foi descrever três novas espécies de *Hyalella* com ocorrência na Região Noroeste do Estado do Rio Grande do Sul, sul do Brasil. Os exemplares foram amostrados em uma nascente, um açude artifical e um açude natural, no municipio de Palmeira das Missões. As três espécies possuem uma seta curva no ramo interno do urópodo 1 com uma seta acessória nos machos. *Hyalella* sp. nov. 1 possui o dáctilo do gnatópodo 2 mais curto que o própodo, apresentando setas apicais simples no ramo externo do urópodo 3, além de possuir 9 setas serradas na face interna do própodo do gnatópodo 1 e um padrão peculiar de cerdas no pedúnculo do ramo do urópodo 3. Este espécie difere de *H. montenegrinae* e *H. carstica* apresentando brânquias no esterno nos segmentos de 2 a 7 e mais de duas setas apicais no télson. Já *Hyalella* sp. nov. 2 assemelha-se as espécies *H. bonariensis*, *H. castroi*, *H. curvispina*, *H. kaigang*, *H. pampeana* e *H. pleoacuta* por possuir brânquias no esterno nos segmentos de 2 a 7. No entanto, *Hyalella* sp. nov. 2 difere da *H. pleoacuta, H. kaingang* e *H. pseudoazteca* por ausência de flanges dorsal sobre péreon e pleonitos. Por fim, a *Hyalella* sp. nov. 3 difere de outras espécies encontradas no Estado do Rio Grande do Sul (*H. bonariensis, H. castroi, H. kaigang, H. imbya, H. montenegrinae* e *H. pampeana*) no tipo e número de setas nos urópodos, télson e face interna do gnatópodo 1. Apesar da semelhança com *H. curvispina, Hyalella* sp. nov. 3 possui apenas duas cerdas simples no télson, face interna do gnatópodo 1 com cinco setas serradas e dentículos em "comb scales" apenas na margem disto-posterior e, especialmente, por possui brânquias esternais nos segmentos 3 a 7. A partir deste trabalho, o número de espécies de *Hyalella* encontradas no Brasil aumenta para 26 e para 71 espécies para o gênero.

Palavras-chaves: Biodiversidade. Anfípodos de água doce. Taxonomia. Diversidade.

ABSTRACT

DESCRIPTION OF THREE NEW SPECIES OF HYALELLA (CRUSTACEA, AMPHIPODA, HYALELLIDAE) FROM NORWEST REGION OF RIO GRANDE DO SUL STATE, SOUTHERN BRAZIL

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Currently there are 68 species of *Hyalella* gender described, whereas in South America, the largest diversity of species is observed, 52 record species. The State of Rio Grande do Sul has nine species of this gender recorded. Through an extensive sampling period, the current study objectives to described three new species of *Hyalella* observed at the Northwest Region of Rio Grande do Sul State, South region of Brazil. The specimens were sampled at a small spring, an artificial pond and a natural pond, in the municipality of Palmeira das Missões. The three species have a curved seta on the inner ramus of uropod 1 and an accessory seta for the males. *Hyalella* sp. nov. 1 has dactylus of gnathopod 2 shorter than the propodus palm, showing simple apical setae on outer ramus of uropod 3, in addition of having 9 serrate setae inner face of propodus of gnathopod 1 and a peculiar pattern of setae on peduncle of uropod 3 ramus. This species differs from *H. montenegrinae* and *H. carstica* by showing sternal gills on segments 2 to 7 and more than two apical setae on telson. The *Hyalella* sp. nov. 2 resembles the species *H. bonariensis*, *H. castroi*, *H. curvispina*, *H. kaigang*, *H. pampeana* e *H. pleoacuta* by having gills on sternum of segments 2 to 7. This specie differs from *H. pleoacuta, H. kaingang* and *H. pseudoazteca* by lacking dorsal flanges on pereions and pleonites. Finally, *Hyalella* sp. nov. 3 differs from others species found in the State of Rio Grande do Sul (*H. bonariensis, H. castroi, H. kaigang, H. imbya, H. montenegrinae and H. pampeana*) in type and number of setae on uropods, telson and inner face of gnathopod 1. Despite the resemblance with *H. curvispina, Hyalella* sp. nov. 3 has only two simple setae on telson, inner face of gnathopod 1 with five serrate setae and denticles in comb-scales only at the posterior distal margin, and also has sternal gills on segments 3 to 7. After this study, the number of *Hyalella* species found in Brazil raises to 26 and to 71 worldwide for the gender.

Key words: Biodiversity. Freshwater amphipods. Taxonomy. Diversity.

SUMÁRIO

1. INTRODUÇÃO

Aspectos Gerais e Taxonomia

Os crustáceos são animais que formam um grupo de muito sucesso devido principalmente à diversificação morfológica, permitindo sua ocupação em diversos ecossistemas no planeta (DUFFY; THIEL, 2007; MARTIN; DAVIS, 2001). Os crustáceos compartilham muitas características com os demais representantes do Filo Arthropoda como exoesqueleto quitinoso e apêndices articulados, entretanto, possuem características exclusivas, como dois pares de antenas e apêndices birremes (MARTIN; DAVIS, 2001). Na classificação de Martin & Davis (2001) o Subfilo Crustacea possui seis classes: Remipedia, Cephalocarida, Branchiopoda, Ostracoda, Maxillopoda e Malacostraca. Dentre estas, a Classe Malacostraca é a mais representativa, possuindo uma grande diversidade taxonômica e ecológica (HAYWARD et al., 1995). Dentro dos Malacostraca, encontra-se a Superordem Peracarida, que juntamente com a Superordem Eucarida, representam a maioria dos Malacostraca e de fato a maioria dos crustáceos conhecidos (MARTIN; DAVIS, 2001).

Os crustáceos da Superordem Peracarida possuem como característica diagnóstica, a presença de um marsúpio nas fêmeas (MORRIT; SPICER, 1996; POORE, 2005), local onde ocorre a fertilização e incubação dos ovos (BOROWSKY, 1991), também chamado de pericárdio (do latim "*peracadium*"), que dá o nome a superordem (BOROWSKY, 1991; CHAPMAN, 2007; DUFFY; THIEL, 2007). O marsúpio é formado pela superfície ventral dos segmentos torácicos e pelos oostegitos, entre o segundo e quinto segmento, na base dos pereiópodos, no qual o conjunto dessas expansões forma uma bolsa incubadora onde os ovos são fecundados e armazenados até a eclosão dos embriões (GARCIA- SCHROEDER; ARAUJO, 2009; MORRIT; SPICER, 1996; POORE, 2005). A Superordem Peracarida está representada por nove ordens: Spelaeogriphacea, Thermosbaenacea, Lophogastrida, Mysida, Mictacea, Amphipoda, Isopoda, Tanaidacea e Cumacea. Das ordens de peracáridos, a maior riqueza é observada em Amphipoda e Isopoda (HINOJOSA; THIEL, 2009; MARTIN; DAVIS; 2001).

A Ordem Amphipoda é considerada a segunda maior Ordem da Superordem Peracarida (BELLAN-SANTINI, 1999), sendo uma das ordens de crustáceos mais bem-sucedida, podendo ser encontrados em todo o planeta e sua diversidade é majoritariamente

marinha (RODRIGUES, 2016). No entanto, muitas espécies ocorrem em diferentes habitats dulcícolas e são abundantes em águas correntes frias e subterrâneas de regiões temperadas. Das 9.100 espécies da ordem, 20% são encontradas em ambientes de água doce e ainda possuem representantes terrestres, a maioria alocada na família Talitridae Rafinesque, 1815 (VAINOLA et al., 2008).

Devido à ausência de um estágio larval nos crustáceos da Ordem Amphipoda, a capacidade de dispersão por longas distâncias torna-se limitada, ocasionando o isolamento geográfico de muitas populações. Sendo assim, as espécies de água doce, muitas vezes são endêmicas ou possuem distribuição geográfica restrita (BARNARD, 1982; RODRIGUES, 2016). A diversidade de anfípodos de água doce na América do Sul é reduzida, quando comparada com outras regiões, ocorrendo apenas 10 famílias, 22 gêneros e 74 espécies (FISER et al., 2013). Entretanto, no Lago Titicaca localizado entre o Peru e a Bolívia, concentra o maior número de espécies de *Hyalella* vivendo de forma simpátrica, 14 no total (GONZÁLES; WATLING, 2003; RODRIGUES, 2016;). Além disso, este lago é considerado um centro de radiação com existência de aproximadamente 100 espécies ainda não descritas, todas depositadas em Museus (GONZÁLES; WATLING, 2003).

Os crustáceos peracáridos da Ordem Amphipoda eram distribuídos anteriormente em quatro subordens, Gammaridea, Caprellidea, Hyperiidea e Ingolfiellidea (MARTIN; DAVIS, 2001). Numa recente revisão, Lowry & Myers (2013) sugeriram a criação de uma nova subordem nomeada Senticaudata caracterizada pela presença de fortes cerdas apicais no 1º e 2º urópodos, que compreende todas as espécies de anfípodos de água doce e algumas marinhas, que pertenciam à antiga Subordem Gammaridea. Estes autores afirmam que, a Subordem Senticaudata divide-se em seis Infraordens: Carangoliopsida (duas famílias), Talitrida (15 famílias), Corophiida (21 famílias), Hadziida (12 famílias), Bogidieliida (três famílias) e Gammarida (40 famílias).

Na Infraordem Talitrida, recentemente foi criada a Parvordem Talitridira, na qual a Superfamília Talitroidea insere-se e consequentemente a Família Hyalellidae e então o gênero *Hyalella*. Cabe ressaltar que numa classificação mais antiga da Ordem Amphipoda, Serejo (2004) determinou o gênero *Hyalella* Smith, 1874 na Superfamília Talitroidea, na Família Dogielinotidae, e fez uma divisão em três subfamílias, entre elas Hyalellidae. Entretanto, recentemente Hyalellidae foi novamente elevada ao nível de família (LOWRY; MYERS, 2013). Portanto, atualmente o gênero *Hyalella* está inserido no Reino Animalia, Filo

Arthopoda, Subfilo Crustacea, Classe Malacostraca, Superordem Peracarida, Ordem Amphipoda, Subordem Senticaudata, Infraordem Talitrida e Família Hyalellidae (LOWRY; MYERS, 2013).

Na América do Sul, a Ordem Amphipoda está representada por espécies que habitam águas superficiais e subterrâneas (VÄINÖLA et al., 2008) e a baixa representatividade nos trópicos pode ser resultado de um menor número de estudos nessa região e não necessariamente a ausência dos organismos (TORRES, 2012). Os anfípodos são encontrados geralmente em ecossistemas dulcícolas e frequentemente dominam a comunidade bentônica de lagos, e devido a seus hábitos primariamente herbívoros e detritívoros possuem grande relevância ecológica (COTHRAN, 2007; ISHIKAWA; URABE, 2002). Além disso, possuem um papel essencial nas cadeias tróficas, proporcionando a transferência de energia entre os níveis tróficos, atuam na conversão de detritos em matéria orgânica, servem de alimento para aves aquáticas e peixes, e muitas vezes tornam-se hospedeiros intermediários de parasitas de vertebrados. (BUENO et al., 2014; CASTIGLIONI, 2007; COOPER, 1965; DUDGEON, 2006; MUSKÓ, 1993, 1990; PILGRIM; BURT, 1993).

A Família Hyalellidae está representada apenas pelo gênero *Hyalella*, sendo endêmico das Américas e ocorrendo nas regiões Neártica e Neotropical, desde o sul da Patagônia até a região central do Canadá (BUENO et al., 2014; GONZÁLEZ; WATLING, 2003). Atualmente existem 68 espécies de *Hyalella* descritas, sendo que a maior diversidade de espécies encontra-se na América do Sul, com registro de 52 espécies (CARDOSO et al., 2014; COLLA; CÉSAR, 2015; RODRIGUES et al., 2014; SOUCKER et al., 2015). No Brasil são conhecidas 23 espécies de *Hyalella* com registro somente para regiões sudeste e sul. Na região sudeste tem-se o registro de 12 espécies, sendo que dessas, seis ocorrem no Estado de Minas Gerais (*H. warmingi*, *H. gracilicornis*, *H. longistila*, *H. carstica*, *H. minensis*, *H. xakriaba*), seis no Estado de São Paulo (*H. meinerti*, *H. warmingi*, *H. dielaii*, *H. caeca*, *H. spelaea*, *H. epikarstica*), sendo estas três últimas cavernícolas, e duas no Estado do Rio de Janeiro (*H. longistila*, *H. gracilicornis*) (BUENO et al., 2014; CARDOSO et., 2014; RODRIGUES et al., 2014). No entanto, a região sul possui a maior diversidade com registro de 11 espécies, duas no Estado Paraná (*H. brasiliensis* e *H. formosa*) e nove no Estado Rio Grande do Sul (BASTOS-PEREIRA; BUENO, 2013; BUENO et al., 2013; BUENO et al., 2014; CARDOSO et al., 2014; MARRÓN-BECERRA et al., 2014; RODRIGUES et al., 2014). As espécies registradas para o Estado do Rio Grande do Sul são: *H. bonariensis* Bond-Buckup, Araujo & Santos, 2008; *H. castroi* González, Bond-Buckup & Araujo, 2006; *H.*

curvispina Shomaker, 1942; *H. imbya* Rodrigues & Bueno, 2012; *H. kaingang* Bueno & Araujo, 2013, *H. montenegrinae* Bond-Buckup & Araujo, 1998; *H. pampeana* Cavalieri, 1968; *H. pleoacuta* González, Bond-Buckup & Araujo, 2006; *H. pseudoazteca* González & Watling, 2003 (BASTOS-PEREIRA; BUENO, 2013; BUENO et al., 2013; BUENO et al., 2014; CARDOSO et al., 2014; MARRÓN-BECERRA et al., 2014; RODRIGUES et al., 2014), e podem ocorrer em quase todos os tipos de habitats: rios, riachos, lagos e áreas úmidas (BUENO et al, 2013; GROSSO; PERALTA, 1999).

Muitos morfotipos de *Hyalella* que ocorrem no Brasil e ainda não foram descritos são morfologicamente muito semelhantes (BUENO; RODRIGUES; ARAUJO, 2014), tornando difícil separá-los usando apenas sua taxonomia, por isso, para evitar erros de identificação e futuras descrições de espécies, é necessário à realização da filogenia molecular, pois assim não existirão dúvidas sobre a validade desses táxons (RODRIGUES, 2016). Cabe salientar ainda que muitas descrições de espécies do gênero *Hyalella* são muito sucintas, com ilustrações incompletas e muitas destas são antigas (BUENO; RODRIGUES; ARAUJO, 2014). Witt & Hebert (2000) descobriram através de dados moleculares que populações de *Hyalella* consideradas morfologicamente como sendo *H. azteca*, eram pelo menos 10 novas espécies, todas escondidas sob a forma de espécies crípticas.

Os complexos de espécies de *Hyalella* são grupos constituídos por espécies morfologicamente semelhantes, sendo impossível a diferenciação utilizando a taxonomia, entretanto, são geneticamente distintas (STOK; PLATVOET, 1991). No caso do gênero *Hyalella*, as espécies que compõem um complexo, ocupam uma mesma região geográfica ou bacia hidrográfica (RODRIGUES, 2016). Muitas vezes, espécimes são identificados de forma errônea devido a essa semelhança morfológica e posteriormente são consideradas como sinônimos juniores (GONZÁLEZ, 2001; WITT; THRELOFF; HEBERT, 2006). Baseando-se unicamente na morfologia, González (2001), sugere a existência do complexo "*H. curvispina"* e "*H. patagonica*", entretanto, essa hipótese não foi confirmada, pois nunca foram realizadas análises moleculares (RODRIGUES, 2006). O complexo "*H. curvispina"* incluiria espécies com distribuição no Uruguai, Argentina e Sul do Brasil, entretanto, espécies descritas recentemente com características morfológicas e ocorrência nessas regiões se encaixariam no complexo, no entanto, ainda precisa ser revisto (RODRIGUES, 2016). Por isso, a melhor maneira para solucionar os complexos tem sido a utilização de análises moleculares, para diferenciar geneticamente as espécies que são semelhantes morfologicamente (RODRIGUES, 2016).

Biologia e Ecologia do gênero *Hyalella*

Devido as grandes reservas de água doce da América e à diversidade de *Hyalella* ocorrerem no Brasil, o país possui um potencial para a realização de estudos ecológicos com anfípodos dulcícolas (CARDOSO, 2013; RODRIGUES, 2016). No entanto poucos trabalhos foram conduzidos sobre esta temática. Os trabalhos de cunho ecológico existentes no país restringem-se a poucos estudos sobre biologia populacional e reprodutiva com as espécies *H. pleoacuta e H. castroi* no Estado do Rio Grande do Sul (CASTIGLIONI; BOND-BOCKUP, 2009; CASTIGLIONI; BOND-BOCKUP, 2008a; CASTIGLIONI; BOND-BOCKUP, 2008b; CASTIGLIONI, 2007; CASTIGLIONI; BOND-BOCKUP, 2007; CASTIGLIONI et al., 2007). No entanto, este cenário vem mudando com a realização de mais trabalhos acadêmicos sobre ecologia de algumas populações de *Hyalella* realizados nos Estados de Minas Gerais e Rio Grande do Sul. Rodrigues (2011) realizou estudo com espécies de *Hyalella* que ocorrem nas áreas úmidas do Estado do Rio Grande do Sul, no qual foram analisados aspectos populacionais, distribuição geográfica e possíveis fatores que influenciavam a riqueza de espécies, abundância e padrões de distribuição. Neste estudo, foi possível concluir que houve uma maior ocorrência das espécies em áreas permanentes com vegetação emergente ou herbácea, além disso, houve diferença significativa entre os tamanhos corporais das espécies, a relação sexual favoreceu os machos, a estrutura das populações foi bimodal e, as maiores riquezas de espécies foram encontradas na região hidrográfica do Uruguai e na província geomorfológica do Planalto (RODRIGUES, 2011).

Numa dissertação realizada por Ozga (2014) foi caracterizada a estrutura populacional e a biologia reprodutiva de duas espécies do gênero *Hyalella* (desconhecidas da ciência), na região noroeste do Rio Grande do Sul, onde foi possível observar que as espécies apresentavam uma dinâmica populacional e reprodutiva diferentes, o que pode ser decorrência das estratégias de vida adotadas e das adaptações ao tipo de ambiente em que vivem.

Antunes (2015) analisou a estruturação de comunidades de macroinvertabrados de água doce, com ênfase no gênero *Hyalella* e identificou a influência de variáveis ambientais sobre as comunidades, entre ambientes lóticos e lênticos na região central do Rio Grande do Sul, onde constatou que as variações dentro do mesmo ambiente não é o que, de fato, interfere na presença e na abundância de *Hyalella*, e sim, a variação entre os ambientes (lênticos naturais e artificiais e lóticos). Além disso, observou uma forte influência do ambiente na ocorrência e distribuição das espécies de *Hyalella* identificadas para a região amostrada.

Torres et al. (2015) avaliou a fecundidade, o padrão de pareamento e o período de reprodução de *Hyalella carstica* Bastos-Pereira e Bueno, 2012, no oeste do Estado de Minas Gerais. Além disto, este autores analisaram a capacidade da população em se manter ao longo do tempo em um determinado ambiente, e assim contribuir para discussões de políticas conservacionistas). Recentemente, Rodrigues (2016) realizou estudos sobre aspectos populacionais de quatro espécies de *Hyalella* no Brasil, oriundas de diferentes latitudes e biomas, onde concluiu que a reprodução de espécies próximas aos trópicos era continua ao longo do ano e das espécies de altas latitudes a reprodução foi sazonal. Além disso, o tamanho corporal de indivíduos de latitudes altas era maior do que dos indivíduos das espécies de latitudes mais baixas. A fecundidade das espécies parecia estar mais relacionada ao tamanho corporal da fêmea do que com o clima e latitude (RODRIGUES, 2016).

Os anfípodos estão sendo utilizados em testes ecotoxicológicos devido as suas sensibilidades a distúrbios ambientais, como poluição e contaminantes agrícolas, sendo considerados bioindicadores de boa qualidade de água (DING et al., 2011; NEUPARTH et al., 2002; WILCOXEN et al., 2003). Os anfípodos de água doce possuem características distintas e essenciais para estudos como bioindicadores da qualidade de água, como fácil manuseio e coleta, sensibilidade a poluentes e metais pesados, ciclo de vida curto, com desenvolvimento direto, sendo facilmente cultivados em laboratório (DUAN et al., 1997; KRUSCHWITZ, 1978). Entretanto, poucas espécies são padronizadas paras esses testes, na maioria das vezes apenas as espécies *Hyalella azteca* (Saussure, 1858) e *Gammarus lacustres* Sars, 1863, espécies que ocorrem somente no Hemisfério Norte (BUYLE, 1989; DING et al., 2011; DUAN et al., 1997; DUTRA et al., 2009, 2008; GUST, 2006; MORRIS et al., 2002; NELSON; BRUNSON, 1995; NEUPARTH et al., 2002; RINDERHAGEN et al., 2000; WILCOXEN et al., 2003).

No Brasil, nos últimos anos foram realizados alguns ensaios ecotoxicológicos com espécies nativas, como *H. pleoacuta* González, Bond- Buckup & Araujo, 2006, *H. curvispina* Shoemaker, 1942 (DUTRA et al., 2008) e *H. castroi* Gonzáles, Bond-Buckup & Araujo, 2006 (DUTRA et al., 2009). Provavelmente o número de espécies padronizadas para testes de toxicidades permanece limitado devido ao fato de que a maioria dos testes implica em cultivos contínuos de organismos-teste em estado saudável e em número suficiente, o que restringe a seleção das espécies utilizadas (BRENDONCK; PERSOONE, 1993). Por isso, fazem-se necessários mais estudos sobre a dinâmica populacional das espécies nativas de anfípodos dulcícolas do Brasil, para que programas de conservação dos ecossistemas

aquáticos no país possam ser implantados, além de protocolos de avaliação da qualidade ambiental (RODRIGUES, 2016).

As espécies brasileiras do gênero *Hyalella* ocorrem em quase todos os biomas do país, como a Caatinga, Mata Atlântica, Cerrado e Pampa. Suas populações podem ser encontradas tanto em ecossistemas dulcícolas preservados quanto em regiões antropizadas, o que infelizmente é a realidade da maioria (BUENO; RODRIGUES; ARAUJO, 2014; RODRIGUES, 2016). Geralmente as espécies de *Hyalella* são encontradas associadas à macrófitas ou junto ao sedimento em riachos, lagos, áreas úmidas ou ambientes subterrâneos (GROSSO; PERALTA, 1999; BUENO; RODRIGUES; ARAUJO, 2014).

Devido à ação antrópica desenvolvida acentuadamente nos últimos anos, diversos ambientes dulcícolas estão sendo poluídos, e provavelmente a fauna e entre ela, as espécies de *Hyalella*, podem estar sendo eliminadas dos ambientes, mesmo antes de serem conhecidas pela ciência, pois a perda de biodiversidade na água doce é muito superior a dos ambientes marinhos e terrestres (RODRIGUES, 2016). Os ecossistemas dulcícolas são considerados os ambientes que mais sofrem ameaças mundialmente, devido principalmente à atividade humana e dentre as principais ameaças destacam-se a alteração de habitat e degradação, geralmente causados por atividades agrícolas (eutrofização, acidificação, sedimentação, aumento da turbidez, canalização e remoção da vegetação ripária), contaminação por substâncias tóxicas, introdução de espécies exóticas, uso na irrigação e remoção de águas subterrâneas, sobre-exploração de espécies com importância econômica e pressões globais (CARDOSO et al., 2014; DUDGEON et al., 2006; SUSKI; COOKE, 2006; RODRIGUES, 2016). Por isso, o reconhecimento das novas espécies, permitirá uma contribuição importante para a biodiversidade e conservação destes ambientes dulcícolas, especialmente no Estado do Rio Grande do Sul, no qual as atividades agrícolas são a principal fonte de renda da população.

Além disso, devido à alta endemicidade, as espécies de *Hyalella* podem ajudar a criar programas de conservação de ambientes aquáticos dulcícolas ameaçados (RODRIGUES, 2016). Witt, Threloff & Herbet (2006) demostraram esse potencial para conservação ao verificar a existência de 35 novas espécies de *Hyalella* em áreas pequenas dos desertos dos estados da Califórnia e Nevada, nos Estado Unidos. Estima-se que existam pelo menos 500 espécies novas de *Hyalella* a serem descritas somente na América do Norte, o que tornaria o gênero o mais diverso entre os anfípodos de água doce na região (RODRIGUES, 2016;

VÄINÖLÄ et al., 2008).

Sendo assim, devido à importância do gênero *Hyalella* para os ecossistemas aquáticos dulcícolas, o objetivo deste trabalho foi estudar a diversidade do gênero Hyal*ella* no município de Palmeira das Missões, região noroeste do Estado do Rio Grande do Sul, sul do Brasil. O reconhecimento das espécies representará uma contribuição importante para a biodiversidade e conservação dos ambientes aquáticos límnicos do Brasil, especialmente no Estado do Rio Grande do Sul.

1.1 OBJETIVOS

1.1.1 OBJETIVO GERAL

Avaliar a diversidade de crustáceos anfípodos do gênero *Hyalella* encontradas no município de Palmeira das Missões, região Noroeste do Estado do Rio Grande do Sul, Brasil.

1.1.2 OBJETIVOS ESPECÍFICOS

1. Descrever espécies desconhecidas da ciência pertencentes ao gênero *Hyalella* provenientes do município de Palmeira das Missões, Região Noroeste do Estado do Rio Grande do Sul, sul do Brasil;
2. Contribuir para o aumento do conhecimento sobre taxonomia do gênero *Hyalella* no Brasil, especialmente no Estado do Rio Grande do Sul.

1.2 REFERÊNCIAS

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2. ARTIGO 1 Submetido para Zootaxa em 18/12/2015

Two new species of *Hyalella* (Crustacea, Amphipoda, Hyalellidae) from state of Rio Grande do Sul, Southern Brazil

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Abstract

There are 68 known species of *Hyalella* worldwide, with 23 occurring in Brazil. The state of Rio Grande do Sul, Southern Brazil, has the largest diversity of the genus in the country, with nine species recorded. The current study aimed to describe two new species of *Hyalella* from state of Rio Grande do Sul, both of them in the Northwest region of the state, one found in a small spring and another in an artificial pond. *Hyalella* sp. nov. 1 presents several clusters of simple setae on antenna 2, maxilliped very slender, gnathopod 2 dactylus not reaching the lobe of propodus, pleopods rami with short plumose setae and a peculiar pattern of setae on uropods and telson. *Hyalella* sp. nov. 2 presents antenna 2 with few setae, maxilliped very slender, gnathopod 2 dactylus reaching the lobe of propodus and pleopods rami with long plumose setae. From this work, the number of *Hyalella* species found in Brazil increases to 25 and 70 for the genus.

Key words: freshwater crustaceans, diversity, Peracarida, South America, taxonomy

Introduction

The freshwater crustaceans of the Hyalellidae family are represented only by the genus *Hyalella* Smith, 1874. This genus is endemic to the American continent, with 68 species registered and the highest diversity occurs in South America, with 52 species described (Rodrigues *et al.* 2014). Currently, Brazil is the country with the greatest number of species in the world, 23 in total (Bastos-Pereira & Bueno, 2013; Bueno *et al.* 2013, 2014; Cardoso *et al.* 2014; Rodrigues *et al*. 2014).

In Brazil *Hyalella* is known only for the South and Southeast regions, being the last one the most diverse (Bueno *et al.* 2014). However, state of Rio Grande do Sul (Southern Brazil) has highest diversity in the country, with nine species described according to Bueno *et al*. (2014): *H. bonariensis* Bond-Buckup, Araujo & Santos, 2008; *H. castroi* González, Bond-Buckup & Araujo, 2006; *H. curvispina* Shoemaker, 1942; *H. imbya* Rodrigues & Bueno, 2012; *H. kaingang* Araujo & Cardoso, 2013; *H. montenegrinae* Bond-Buckup & Araujo, 1998; *H. pampeana* Cavalieri, 1968; *H. pleoacuta* González, Bond-Buckup & Araujo, 2006 and *H. pseudoazteca* González & Watling, 2003.

The recent researches about the description of new species of *Hyalella* indicate that the diversity of the genus and its distribution is probably underestimated in Brazil (Cardoso *et al*. 2011; Bastos-Pereira & Bueno, 2012; Rodrigues *et al*. 2012; Bueno *et al.* 2013; Cardoso *et al*. 2014; Rodrigues *et al*. 2014). Through an extensive sampling period, the current study objectives to described two new species of *Hyalella* from state of Rio Grande do Sul, Southern Brazil.

Material and methods

The specimens of the two new species were sampled in August 2012, using a hand net, with authorizations of the Instituto Chico Mendes de Conservação da Biodiversidade (MMA; ICM-Bio; SISBIO n° 32726-1). Both species were found in a rural property called Sítio Taqui (for details of each species, see "Habitat"), located in Palmeira das Missões municipality, Northwest region of state of Rio Grande do Sul, Southern Brazil.

The head length and total body size of all specimens were measured using a stereoscopic microscope with a milimetric scale. Adult males and females were preserved in

ethanol 70%, colored with Rose Bengal and dissected. The appendages were mounted on permanent slides and used for the confection of the drawings and for the description of the new species, which were based on the slides of the male paratype and the female allotype.
The descriptions were based on the main morphological characteristics of the genus, such as the size, shape and arrangement of the setae of the appendages, as gnathopods, uropods and telson, based on previous descriptions of *Hyalella* species (González *et al.* 2006; Santos *et al.*, 2008; Rodrigues *et al.* 2012; Bueno *et al*. 2013; Rodrigues *et al.* 2014). The terminology for setae follows Zimmer *et al.* (2009). The type material is deposited in the Museu Nacional do Rio de Janeiro (MNRJ) and the paratypes are in the Coleção de Crustáceos da Universidade Federal de Lavras (CCUFLA).

Taxonomy

Order Amphipoda Latreille, 1816

Suborder Senticaudata Lowry & Myers, 2013

Family Hyalellidae Bulycheva, 1957

Genus *Hyalella* S. I. Smith, 1874

***Hyalella* sp. nov. 1 Streck & Castiglioni**

Material examined: Holotype male, body length = 11.28 mm, head length = 0.90 mm (MNRJ 25898); Allotype female (MNRJ 25899). Brazil, state of Rio Grande do Sul, Palmeira das Missões municipality, Sítio Taqui (27°57'52.79"S-53°14'02.59"W), August, 10, 2012, Castiglioni, D.S. coll.

Paratypes: MNRJ 25900 (20 males and 20 females), CCUFLA 0391. All specimens with the same data as the holotype, August, 10, 2012, Castiglioni, D.S. coll. Mean body length of male paratypes: 11.82 ± 2.88 mm and female paratypes: 7.84 ± 1.31 mm.

Type-locality. Brazil, state of Rio Grande do Sul, Sítio Taqui (27°57'52.79"S-53°14'02.59"W), state of Rio Grande do Sul, Palmeira das Missões municipality, small spring (Várzea river basin), 540 meters of altitude, August, 10, 2012.

Diagnosis. Body surface smooth. Eyes round, pigmented. Antenna 1 smaller than antenna 2, flagellum with 15 articles, article 2 with plumose setae. Antenna 2 peduncle with several clusters of simple setae, flagellum with 16 articles. Maxilla 2 inner plate with 9-10 serrulate setae and two robust papposerrate apical setae. Maxilliped very slender, with very few setae. Gnathopod 1 posterior lobe of carpus with one row of serrate setae and without

comb-scales, propodus inner face with nine serrate setae, hammer-shaped, posterior and anterior distal margins with comb-scales. Gnathopod 2 carpus wider than long, posterior lobe elongated, with pectinate border and one row of serrate setae, without polygonal pattern or comb-scales; propodus longer than wide and with posterior margin of lobe covered by denticles in comb-scales; palm smooth and sub-equal to posterior margin of propodus, slope oblique, palm margin convex; dactylus claw-like, short, not reaching the lobe of propodus. Pleopods rami with short plumose setae. Uropod 1 inner ramus of male with curved setae followed by a row of four small cuspidate setae with an accessory seta. Uropod 2 inner ramus apex with seven cuspidate setae. Uropod 3 peduncle with nine cuspidate setae with accessory setae apically, small cuspidate setae and simple setae distributed along the peduncle and ramus. Telson as long as wide, with seven apical cuspidate setae with an accessory seta distributed in two clusters (two setae and other side five four setae) and four plumose setae laterally. Coxal gills sac-like present on pereionites 2 to 6 and sternal gills tubular on pereionites 2 to 7.

Description of male. (Fig. 1) Mean body length: 11.82 ± 2.88 mm, mean head length: 0.93 ± 0.24 mm (n=20). Body surface smooth; epimeral plates not acuminate.

Head longer than the first pereon segment, rostrum absent. Eyes round, pigmented (Fig. 1).

Antenna 1 (Fig. 2) shorter than antenna 2, less than half the body length; peduncle not surpassing head length, article 2 with two distal plumose setae; flagellum with 15 articles, longer than peduncle; two aesthetascs per article occurring distally on flagellum after article 2.

Antenna 2 (Fig. 3) peduncle not surpassing the first pereionite, less than half body length, article 1 and 2 with a cluster of simple setae occurring distally, article 2 with one plumose seta on the median margin, article 3 with a cluster of simple setae occurring distally and several simple setae distributed along the article; flagellum with 16 articles, longer than peduncle, basal article elongated.

Upper lip (Fig. 4) margin rounded, distal border covered by several setules on dorsal and ventral faces.

Basic amphipodan mandible (Fig. 5) (in sensu of Watling 1993), without palp; incisor toothed; left *lacinia mobilis* with five teeth and setae row on left mandible with three pappose setae; molar process large, cylindrical and with large accessory seta.

Lower lip (Fig. 6) lobes rounded, with several setules on dorsal and ventral faces.

Maxilla 1 (Fig. 7) palp uniarticulate, short, longer than wide, reaching less than half length the distance between the base of the palp and tip of setae on outer plate; inner plate slender, shorter than outer plate, with two papposerrate apical setae, and setules on the inner margin; outer plate with nine long serrate setae.

Maxilla 2 (Fig. 8) inner and outer plates of similar sizes; inner plate with ten serrulate and several simple apical setae and two robust papposerrate apical setae; outer plate with several apical simple setae; outer and inner plates with several setules.

Maxilliped (Fig. 9) slender, with few setae; inner plate apically rounded, longer than wide, with three cuspidate setae apically, with few pappose and simple setae on apical and inner margins; outer plate with simple setae apically; palp with four articles, with simple setae and few pappose setae; dactylus unguiform, shorter than propodus, with simple setae.

Gnathopod 1 (Fig. 10) subchelate; coxal plate wider than long; basis, ischium and merus with simple setae dorsally, with comb-scales posterodistally; carpus longer than wide, slightly longer than propodus, with strong lateral distal lobe produced and forming a scoop-like structure, posterior lobe of carpus with one row of serrate setae and without comb-scales; propodus longer than wide, propodus width about 3⁄4 of maximum length (rectangular), hammer-shaped, inner face with nine serrate setae and several simple triangular setae, posterior and anterior distal margins with denticles in comb-scales and simple setae, disto-anterior border with a cluster of simple setae; palm slope oblique with a cluster of simple setae on the posterior distal corner; dactylus claw-like, with denticles in comb-scales and one plumose seta dorsally.

Gnathopod 2 (Fig. 11) subchelate; coxal plate wider than long; ischium and merus with few simple setae on disto-posterior margin; basis, ischium and merus with one row of denticles in comb-scales on posterior margin; carpus wider than long, posterior lobe elongated, with pectinate border and one row of serrate setae, without polygonal pattern or comb-scales; propodus longer than wide, propodus length 1.5 times the maximum width, posterior margin of lobe covered by denticles in comb-scales; palm smooth and sub-equal to posterior margin of propodus, slope oblique, palm margin convex, with several cuspidate setae and simple setae, posterior distal corner with few cuspidate setae and simple setae; dactylus claw-like, short, not reaching the lobe of propodus, with one plumose seta dorsally, without comb-scales.

Peraeopods 3 to 7 (Figs. 12 - 16) simple, with several simple setae on border; ischium, merus, carpus and propodus posterior margin with cluster of simple and cuspidate setae; dactylus half-length of propodus, unguiform; peraeopod 3 and 4 with similar sizes; peraeopod

5 smaller than others; peraeopod 6 smaller than peraeopod 7.

Pleopods (Fig. 17) peduncle shorter than rami, with two coupling spines; both rami with several short plumose setae.

Uropod 1 (Fig. 18) peduncle slightly (1.2 times) longer than rami, with six cuspidate setae with an accessory seta dorsally; inner ramus longer than outer ramus; inner ramus with four dorsal cuspidate setae with an accessory seta, male with a curved seta followed by a row of four small cuspidate setae with an accessory seta, four cuspidate setae apically (one of them with an accessory seta); outer ramus with five dorsal cuspidate setae with an accessory seta and four cuspidate setae apically (two of them with an accessory seta).

Uropod 2 (Fig. 19) shorter than uropod 1; peduncle rectangular with similar size as rami, with six cuspidate setae dorsally; inner ramus slightly longer than outer ramus, with five dorsal cuspidate setae, apex with seven cuspidate setae; outer ramus with six cuspidate setae dorsally and apex with four cuspidate setae.

Uropod 3 (Fig. 20) shorter than other uropods; peduncle slightly longer than wide, wider than ramus, with nine cuspidate setae with an accessory setae and few small cuspidate setae apically, few small cuspidate setae distributed along the peduncle; inner ramus absent; outer ramus uniarticulate, sub-equal to peduncle, with eight simple setae and one short cuspidate seta apically, two cuspidate setae with an accessory seta and five simple setae distributed along the ramus.

Telson (Fig. 21) entire, apically rounded, as wide as long, with seven apical cuspidate setae with an accessory seta distributed in two clusters (two setae and other side five four setae) and four plumose setae laterally.

Coxal gills sac-like present on pereionites 2 to 6 and sternal gills tubular on pereionites 2 to 7.

Female. Mean body length: 7.84 ± 1.32 mm, mean head length: 0.84 ± 0.14 mm (n=20). Antenna 1 similar shape to male but with 12 articles. Antenna 2 similar shape to male but with 13 articles. Gnathopod 1 (Fig. 22) similar to male gnathopod 1, shorter than gnathopod 2, but more wider and carpus longer than wide, with lateral distal lobe produced and forming a scoope-like strucutre, posterior lobe with polygonal pattern and one row of serrate setae and with denticles in comb-scales on the margin; propodus longer than wide, hammer-shaped, inner face with ten serrate setae and some simple setae, anterior and posterior distal margins with comb-scales, one strong cuspidate setae and some simple setae on the posterior distal corner; dactylus with comb-scales and one plumose seta. Gnathopod 2 (Fig. 23) similar in size and shape to gnathopod 1; different to male gnathopod 2 in shape and smaller; carpus longer

than wide, posterior lobe with polygonal pattern and one row of serrate setae but without denticles in comb-scales; propodus rectangular, longer than wide, inner face with seven serrate setae and several simple setae, palm transverse, posterior distal margin with comb-scales, one short cuspidate setae and some simple setae on the posterior distal corner, anterior distal margin without comb-scales; dactylus with comb-scales and one plumose seta. Telson similar in shape to male, but with distinct pattern of distribution of the cuspidate setae with an accessory seta (two clusters with three setae each, and the other side with four setae) and four plumose setae laterally.

Habitat. Freshwater, epigean. The specimens of *H.* sp. nov. 1 were found in a spring shaded by small trees and surrounded by grasses and pteridophytes (Fig. 47). Despite macrophytes of the genus *Lemna* sp. occur in the spring, the specimens of the new species were found associated to the sediment.

Conservation. The spring where *H.* sp. nov. 1 was found is well preserved and it is located in a rural property, which there is no cultivation of soybeans, wheat or barley (the most commons crops in the region). Furthermore, the water from the spring is collected to be used for human consumption, so is beneficial for the residents of the property keep this environment protected.

Remarks. We noted that *Hyalella* sp. nov. 1 differs from all Brazilian species of *Hyalella* using the taxonomic key of Rodrigues *et al.* (2014). The new species resembles to *H. montenegrinae*, *H. curvispina*, *H. castroi*, *H. pseudoazteca*, *H. kaingang*, *H. pleoacuta*, *H. carstica* Bastos-Pereira & Bueno, 2012 and *H. xakriaba* Bueno & Araujo, 2013 by present a curved seta on the inner ramus of uropod 1 of the males. However, *Hyalella* sp. nov. 1 differs from *H. montenegrinae* and *H. carstica* by presenting sternal gills on segments 2-7 and more than two apical setae on telson, respectively. The new species differs from *H. curvispina*, *H. castroi*, *H. pseudoazteca*, *H. kaingang* and *H. pleoacuta* by the fact that the dactylus of gnathopod 2 is shorter than propodus palm. Despite *Hyalella* sp. nov. 1 resembles in the length of the dactylus of gnathopod 2 to *H. xakriaba* and *H. brasiliensis*, the new species differs from *H. xakriaba* by presenting simple apical setae on outer ramus of uropod 3 and from *H. brasiliensis* by showing nine serrate setae on inner face of propodus of gnathopod 1. Besides, *Hyalella* sp. nov. 1 has a peculiar pattern of setae on peduncle and ramus of uropod 3.

***Hyalella* sp. nov. 2 Streck & Castiglioni**

Material examined: Holotype male, body length = 6.00 mm, head length = 0.64 mm (MNRJ 25901); Allotype female (MNRJ 25902). Brazil, state of Rio Grande do Sul, Palmeira das Missões municipality, Sítio Taqui (27°57'54.30"S 53°14'03.09"W), August, 10, 2012, Castiglioni, D.S. coll.

Paratypes: MNRJ 25903 (20 males and 20 females), CCUFLA 0390. All specimens with the same data as the holotype, August, 10, 2012, Castiglioni, D.S. coll. Mean body length of male paratypes: 10.78 ± 1.88 mm and female paratypes: 5.52 ± 1.40 mm.

Type-locality. Brazil, state of Rio Grande do Sul: Sítio Taqui (27°57'54.30"S 53°14'03.09"W, state of Rio Grande do Sul, Palmeira das Missões municipality, artificial pond (Várzea river basin), 539 meters of altitude, August, 10, 2012.

Diagnosis. Body surface smooth. Eyes round, pigmented. Antenna 1 smaller than antenna 2, flagellum with 11 articles. Antenna 2 peduncle slender, with very few setae, flagellum with 15 articles. Maxilla 2 inner plate with 10 serrulate setae and two robust papposerrate apical setae. Maxilliped very slender, with very few setae. Gnathopod 1 posterior lobe of carpus with polygonal pattern with one row of serrate setae and one row of denticles in comb-scales, propodus inner face with five long serrate setae and 9-10 short serrate setae, hammer-shaped, posterior and anterior distal margins with comb-scales. Gnathopod 2 carpus wider than long, posterior lobe with polygonal pattern and one row of comb-scales and serrate setae and one row of denticles in comb-scales, propodus ovate, longer than wide, palm longer than posterior margin of propodus, slope oblique, palm margin convex, posterior distal corner with a cup for dactylus, dactylus reaching the lobe of propodus. Pleopods rami with long plumose setae. Uropod 1 inner ramus of male with a long curved setae followed by a row of three small cuspidate setae. Uropod 2 inner ramus apex with 11 cuspidate setae. Uropod 3 peduncle with six cuspidate setae apically. Telson (male) as long as wide, with six apical cuspidate setae distributed in three clusters of two setae each, six plumose setae laterally. Coxal gills sac-like present on pereionites 2 to 6 and sternal gills tubular on pereionites 2 to 7.

Description of male. (Fig. 24) Mean body length: 10.78 ± 1.88 mm, mean head length: 0.70 ± 0.18 mm (n=20). Body surface smooth; epimeral plates not acuminate.

Head longer than the first thoracic segments, rostrum absent. Eyes round, pigmented

(Fig. 24).

Antenna 1 (Fig. 25) shorter than antenna 2, less than half body length; peduncle not surpassing head length, flagellum with 11 articles, longer than peduncle; aesthetascs occurring distally on flagellum after article 3.

Antenna 2 (Fig. 26) peduncle surpassing the second pereionite, less than half body length, peduncle slender, with very few setae; flagellum with 15 articles and longer than peduncle.

Upper lip (Fig. 27) margin rounded, distal border covered by several short setules on dorsal and ventral faces.

Basic amphipodan mandible (Fig. 28) (in sensu of Watling 1993), without palp; incisor toothed; left *lacinia mobilis* with five teeth and setae row of left mandible with three long and three short pappose setae; molar process large, cylindrical and with one long accessory seta.

Lower lip (Fig. 29) lobes rounded, with several setules on dorsal and ventral faces.

Maxilla 1 (Fig. 30) palp uniarticulate, short, longer than wide, reaching less than half length the distance between the base of the palp and tip of setae on outer plate; inner plate shorter and slender than outer plate, with two long apical papposerrate setae, and some setules on the inner margin; outer plate with seven serrate setae.

Maxilla 2 (Fig. 31) inner and outer plates of similar sizes; inner plate with several simple setae, ten serrulate setae and two papposerrate apical setae; outer plate with several simple long setae apically; outer and inner plates with several setules.

Maxilliped (Fig. 32) very slender, with very few setae; inner plate apically rounded, longer than wide, with three apical cuspidate setae, few simple setae apically and several pappose setae on inner margin; outer plate with simple apical setae; palp with four articles, with simple setae and few pappose setae; dactylus unguiform, shorter than propodus, with simple and pappose setae and comb-scales.

Gnathopod 1 (Fig. 33) subchelate; coxal plate wider than long; basis and ischium with few simple setae dorsally; merus without setae; basis, ischium and merus with comb-scales posterodistally; carpus longer than wide, longer than propodus, with strong lateral distal lobe produced and forming a scoop-like structure with polygonal pattern, with pectinate border, one row of serrate setae and one row of denticles in comb-scales; propodus longer than wide, propodus length 1.5 times the maximum width (rectangular), hammer-shaped, inner face with five long serrate setae and 9-10 short serrate setae, posterior and anterior distal margins with denticles in comb-scales, disto-anterior border with a cluster of simple setae, palm slope

oblique with few simple setae on the posterior distal corner; dactylus claw-like, with denticles in comb-scales and one plumose seta dorsally.

Gnathopod 2 (Fig. 34) subchelate; coxal plate wider than long; basis, ischium and merus with simple setae and comb-scales on the disto-posterior margin; carpus wider than long, posterior lobe elongated with polygonal pattern, with pectinate border, one row of serrate setae and one row of denticles in comb-scales, disto-anterior margin with serrate setae; propodus ovate, longer than wide, propodus length 1.4 times the maximum width, posterior margin of lobe covered by denticles in comb-scales and with one strong cuspidate setae; palm smooth and longer than posterior margin of propodus, slope oblique, palm margin convex with several simple setae, posterior distal corner with a cup for dactylus; dactylus claw-like, reaching the lobe of propodus, with one plumose seta dorsally, without comb-scales.

Peraeopods 3 to 7 (Figs. 35 - 39) simple, with several simple setae on border; ischium, merus, carpus and propodus posterior margin with cluster of simple and cuspidate setae; dactylus less than half length of propodus, unguiform; peraeopod 3 and 4 with similar sizes; peraeopod 5 smaller than others; peraeopod 6 smaller than peraeopod 7.

Pleopods (Fig. 40) peduncle shorter than rami, with two coupling spines; both rami with long plumose setae.

Uropod 1 (Fig. 41) peduncle 1.3 times longer than rami, with four cuspidate setae dorsally; inner ramus longer than outer ramus; inner ramus with three dorsal cuspidate setae, male with a long curved seta followed by a row of three small cuspidate setae, four cuspidate setae apically (two of them longer than others); outer ramus with four dorsal cuspidate setae and three cuspidate setae apically.

Uropod 2 (Fig. 42) shorter than uropod 1; peduncle rectangular with similar size as rami, with two cuspidate setae dorsally; inner ramus slightly longer than outer ramus, with three dorsal cuspidate setae and apex with seven cuspidate setae and one row of four short cuspidate setae; outer ramus with three cuspidate seta dorsally and apex with four cuspidate setae.

Uropod 3 (Fig. 43) shorter than other uropods; peduncle longer than wide, wider than ramus, with six cuspidate setae apically; inner ramus absent; outer ramus uniarticulate, sub-equal to peduncle, with eight simple setae apically (two of them shorter than others).

Telson (Fig. 44) entire, apically rounded, as wide as long, with six cuspidate setae apically distributed in three clusters of two setae each, six plumose setae laterally.

Coxal gills sac-like present on pereionites 2 to 6 and sternal gills tubular on pereionites 2 to 7.

Female. Mean body length: 5.52 ± 1.40 mm, mean head length: 0.59 ± 0.15 mm (n=20). Antenna 1 similar shape to male but with 10 articles. Antenna 2 similar shape to male but with 13 articles. Gnathopod 1 (Fig. 45) similar to male gnathopod 1; similar size to gnathopod 2; carpus longer than wide, with lateral distal lobe produced and forming a scoope-like strucutre, posterior lobe margin with polygonal pattern and with serrate setae and denticles in comb-scales on the margin; propodus longer than wide, hammer-shaped, inner face with six serrate setae and some simple setae, anterior and posterior distal margins with comb-scales; dactylus with comb-scales and one plumose seta. Gnathopod 2 (Fig. 46) similar in size to gnathopod 1, but the propodus of gnathopod 2 is longer than gnathopod 1; different to male gnathopod 2 in shape and smaller; carpus longer than wide, posterior lobe with polygonal pattern and denticles in comb-scales, with one row of serrate setae on the margin, anterior distal margin with few simple setae; propodus rectangular, longer than wide, inner face with four serrate setae and few simple setae, palm transverse, posterior distal corner with one strong cuspidate seta, posterior and anterior distal margins with comb-scales; dactylus with comb-scales and one plumose seta. Telson similar in shape to male, but with four cuspidate setae and six plumose setae laterally.

Habitat. Freshwater, epigean. Hyalella sp. nov. 2 was found in an artificial pond of shallow depth (around 30 cm) (Fig. 48). The pond presented a large amount of macrophytes of the genus *Salvinia*, which were used as shelter for the specimens of *H.* sp. nov. 2.

Conservation. *Hyalella* sp. nov. 2. occurs in an artificial pond that was built by the residents of the property for fish farming. To build the pond, the residents use the water from the spring where *Hyalella* sp. nov. 2 inhabits. In the surroundings of the pond, there is no cultivation of any type of crops, which could be considered a threat to *Hyalella* sp. nov. 2, as the pesticides used in these cultures can be harmful to the amphipods. However, as this is an artificial pond, it is under the risk of drying out, in the case of the residents interrupt the water flow coming from the spring, which may cause the local extinction of the population of *Hyalella* sp. nov. 2.

Remarks. Using the taxonomic key of Rodrigues *et al.* (2014), we observed that *Hyallla* sp. nov. 2 differ from the other species of the genus in Brazil. As *H.* sp. nov. 1, *Hyalella* sp. nov. 2 resembles to *H. montenegrinae*, *H. curvispina*, *H. castroi*, *H. pseudoazteca, H. kaingang, H. pleoacuta*, *H. carstica* and *H. xakriaba* by present a curved seta on the inner ramus of uropod 1 of the males. The species *Hyalella* sp. nov. 2 is different from *H. pleoacuta, H. kaingang* and *H. pseudoazteca* by lacking dorsal flanges on pereions and pleonites. The new species differs from *H. montenegrinae, H. curvispina, H. castroi*, *H.*

carstica and *H. xakriaba* in the type and number of setae on uropods, telson and inner face of gnathopod 1, as well as the shape and size of ganthopod 2 propodus. *Hyalella* sp. nov. 1 and *Hyalella* sp. nov. 2 differ from each other in the number of serrate setae on the inner face of gnathopod 1, in the shape of gnathopod 2 propodus, in the number of setae on antenna 2, in the size of the plumose setae on pleopods and in the type and number of setae on uropods and telson.

Discussion

Hyalella sp. nov. 1 and *Hyalella* sp. nov. 2 are the first species of the genus described for the Várzea River basin, located in the Northwestern region of the state of Rio Grande do Sul. These two species are the second and third described for this region of the state, followed by *H. imbya* (Rodrigues *et al.* 2012). After the descriptions of this research, the Southern region of Brazil becomes the most diverse of *Hyalella* species in the country, with 13 species described, 11 of them for the state of Rio Grande do Sul, followed by the Southeastern, with 12 species recorded (Bueno *et al.* 2014; Cardoso *et al.* 2014; Rodrigues *et al.* 2014).

The description of *Hyalella* sp. nov. 1 and *Hyalella* sp. nov. 2 increases to 70 the number of known species for the genus and to 25 the species that occur in Brazil. In addition, we have performed new collections in the Northwest and central regions of Rio Grande do Sul and apparently the diversity of *Hyalella* is much higher than reported so far.

It is possible that the morphology of the two new species is related to the habitat in which one inhabits. *Hyalella* sp. nov. 1 may have developed features that allow its association to the substrate in a lotic environment (spring), such as the uncommon high number of setae on uropods and telson. Moreover, the plumose setae of the pleopods are extremely reduced, suggesting that the species should not present a great natatory capacity, and is actually adapted to inhabit the sediment. In contrast, *Hyalella* sp. nov. 2 lives associated to macrophytes in an artificial pond, and presents uropods with fewer number of setae and pleopods with long plumose setae, which certainly aid in swimming and exploring the lentic habitat.

Despite being distant only about (Fig. 49) 15 meters apart, *Hyalella* sp. nov. 1 and *Hyalella* sp. nov. 2 are certainly distinct species, due to the vast morphological differences from each other and to the distinct habit they occupy.

Our PCR trial on the morphotypes from this study showed all COI amplicons presented a single band of 960 bp, as expected. Analysis by sequencing confirmed the identity

of the gene and showed that our samples do not match perfectly with previous characterized haplotypes in described species. Additionally, each morphotype in our sample harbor diverse nucleotide substitutions, suggesting they are different taxon. This data could suggest that the insular characteristic of the habitat can provide geographic isolation and lead to rapid genetic diversification.

New collections should be performed across the state of Rio Grande do Sul, as well as in other regions in Brazil, in order to better understand the biodiversity of *Hyalella* in the country, which is certainly underestimated.

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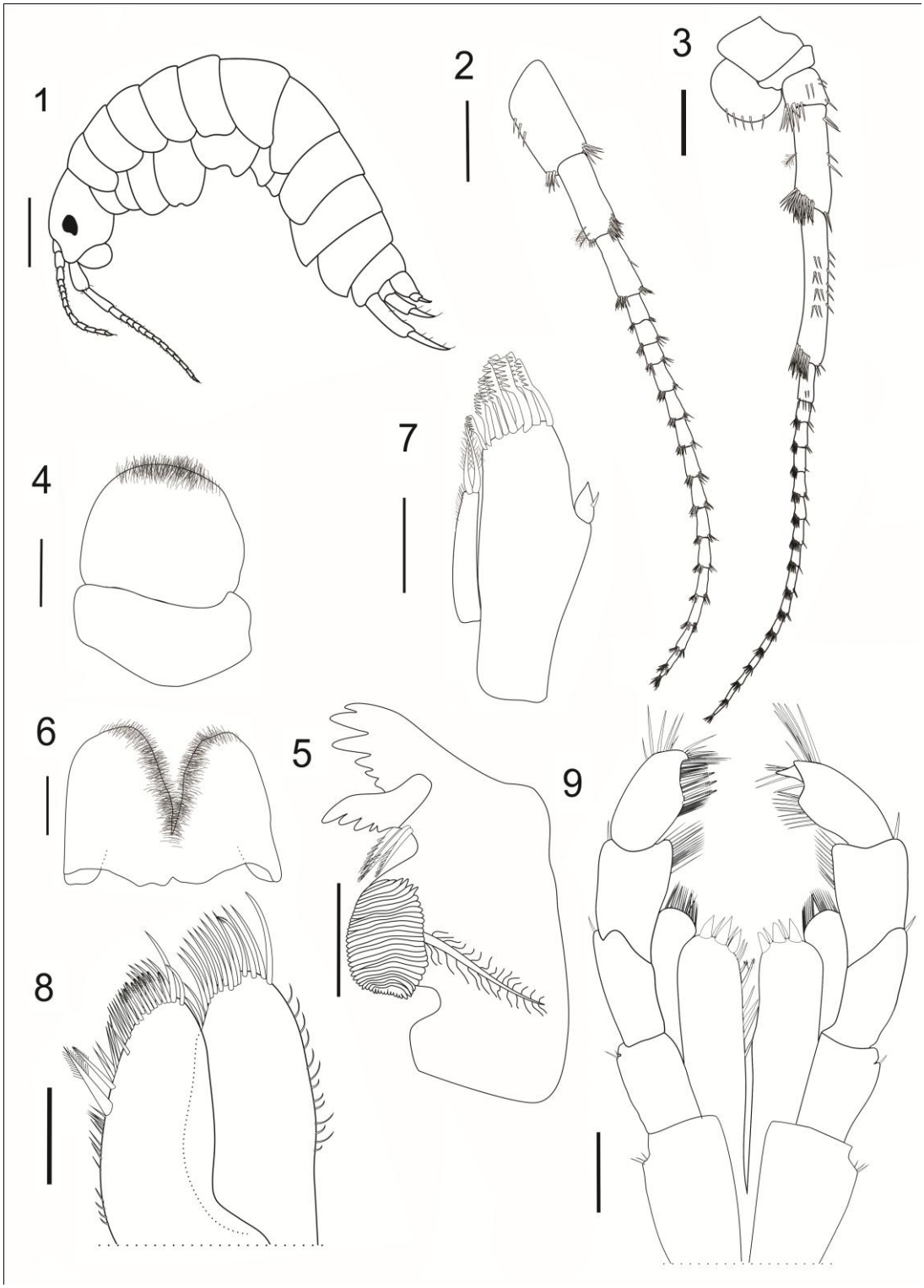
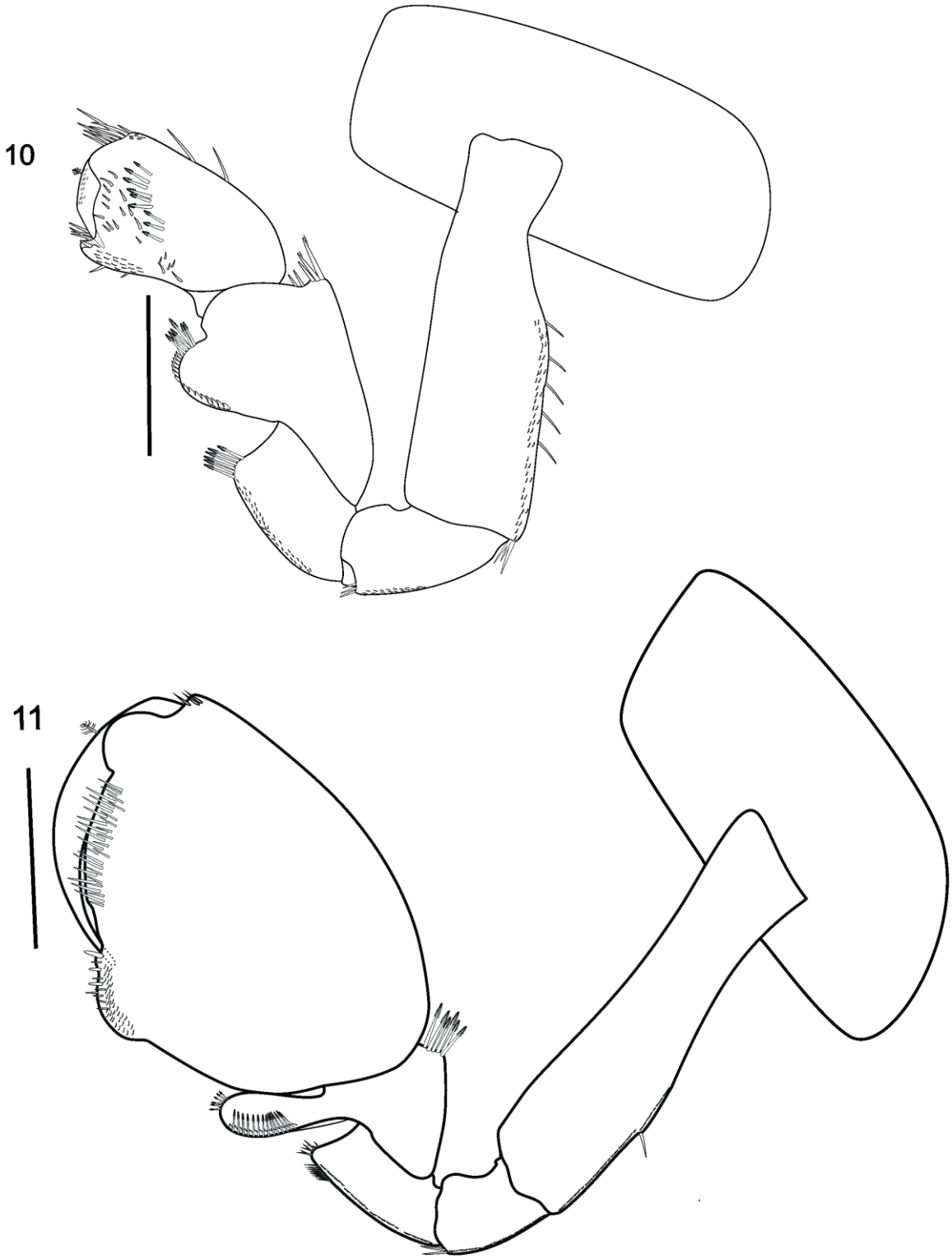
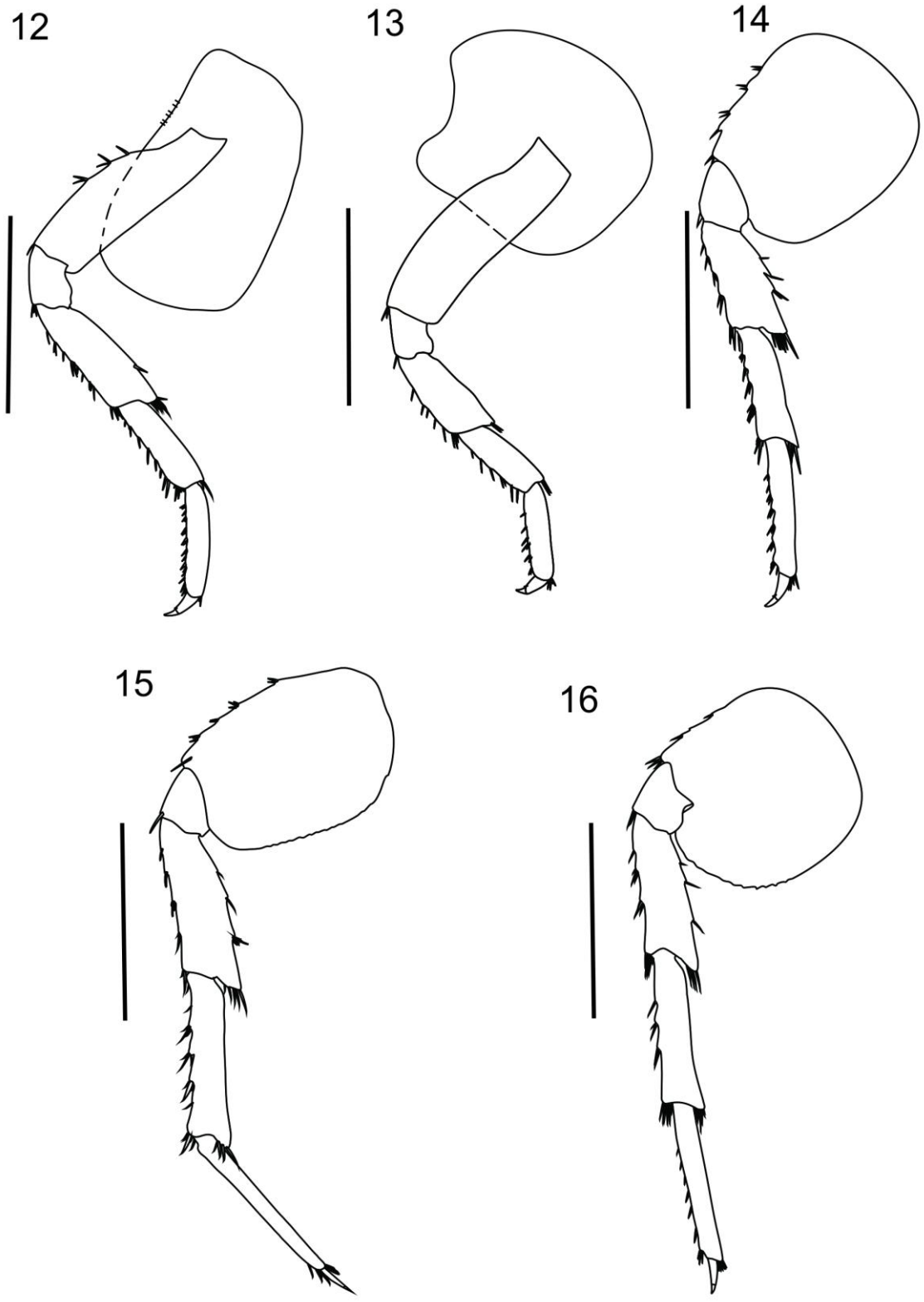


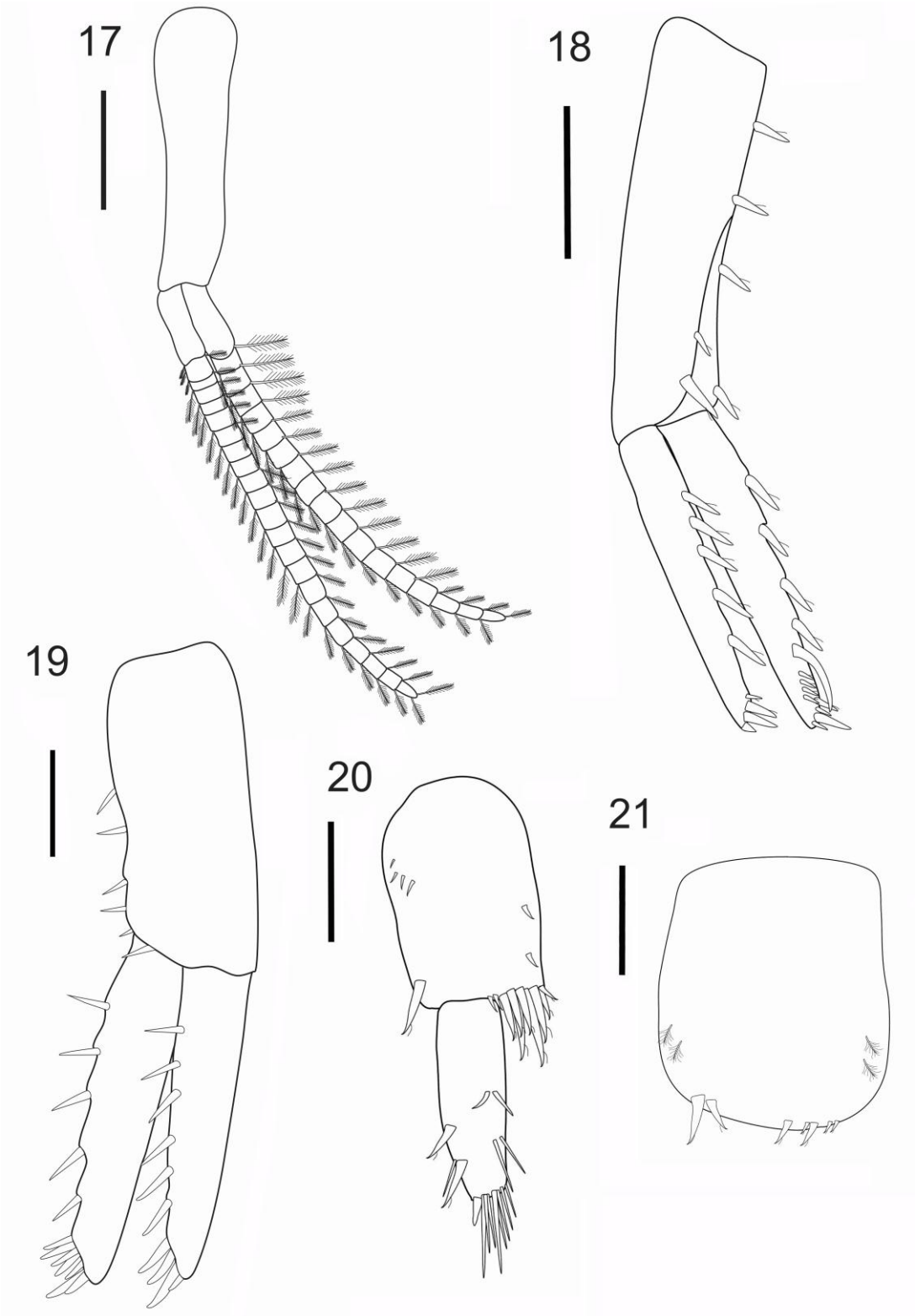
Figures 1-9. *Hyalella* sp. nov. 1. Paratype male (MNRJ 25900): (1) Habitus from holotype (MNRJ 25989), body length: 11.28 mm; (2) Antenna 1; (3) Antenna 2; (4) Upper lip; (5) Mandible; (6) Lower lip; (7) Maxilla 1; (8) Maxilla 2; (9) Maxilliped; Scales: (1-3) represents 0.5 mm; (9) 0.5 mm; (4-8) 0.2 mm.



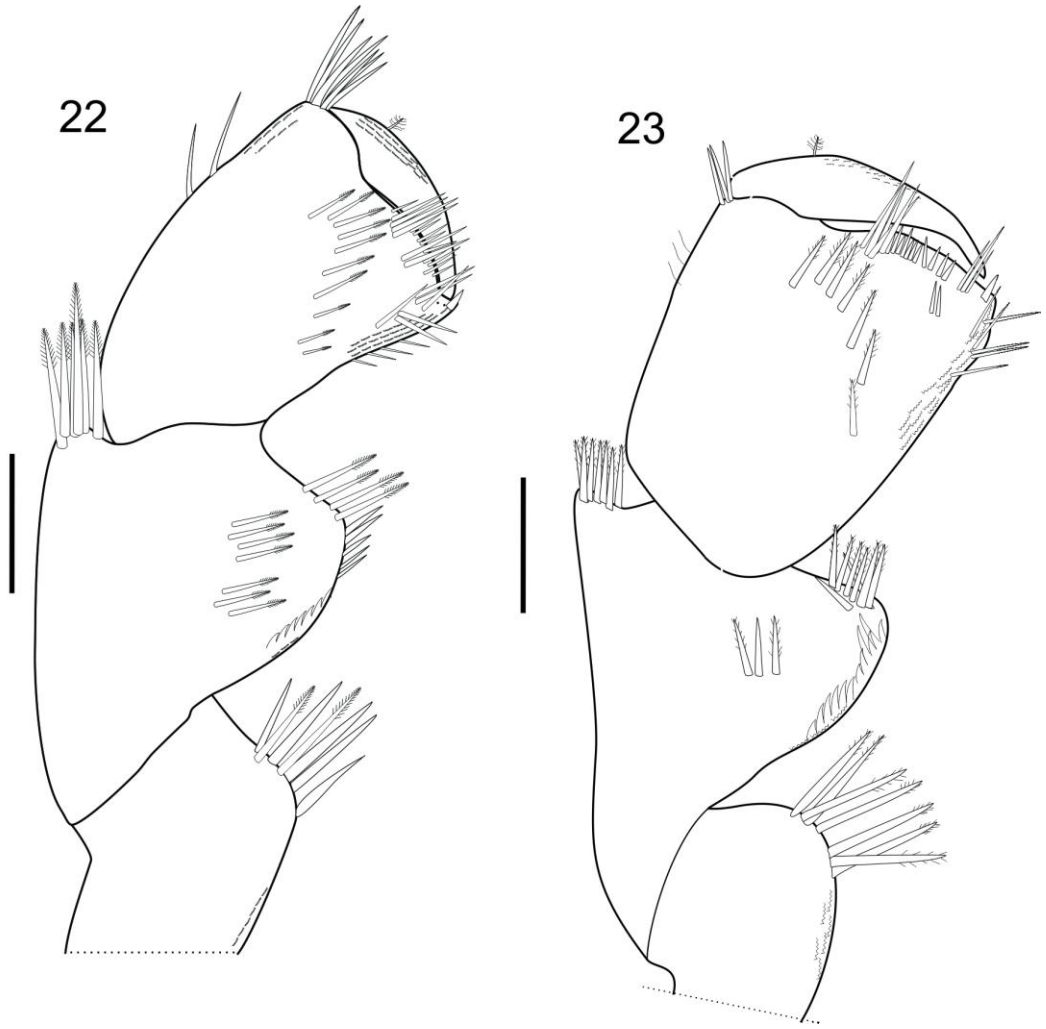
Figures 10-11. *Hyalella* sp. nov. 1. Paratype male (MNRJ 25900); (10) Gnathopod 1; (11) Gnathopod 2; Scales: (10 -11) represents 0.5 mm.



Figures 12-16. *Hyalella* sp. nov. 1. Paratype male (MNRJ 25900); (12) Peraeopod 3; (13) Peraeopod 4; (14) Peraeopod 5; (15) Peraeopod 6; (16) Peraeopod 7; Scales: (12 16) represents 0.5 mm.



Figures 17-21. *Hyalella* sp. nov. 1. Paratype male (MNRJ 25900); (17) Pleopod; (18) Uropod 1; (19) Uropod 2; (20) Uropod 3; (21) Telson; Scales: (17) represents 0.5 mm; (18-21) 0.2 mm.



Figures 22-23. *Hyalella* sp. nov. 1. Allotype female (MNRJ 25899); (22) Gnathopod 1; (23) Gnathopod 2; Scales: (22–23) represents 0.5 mm.

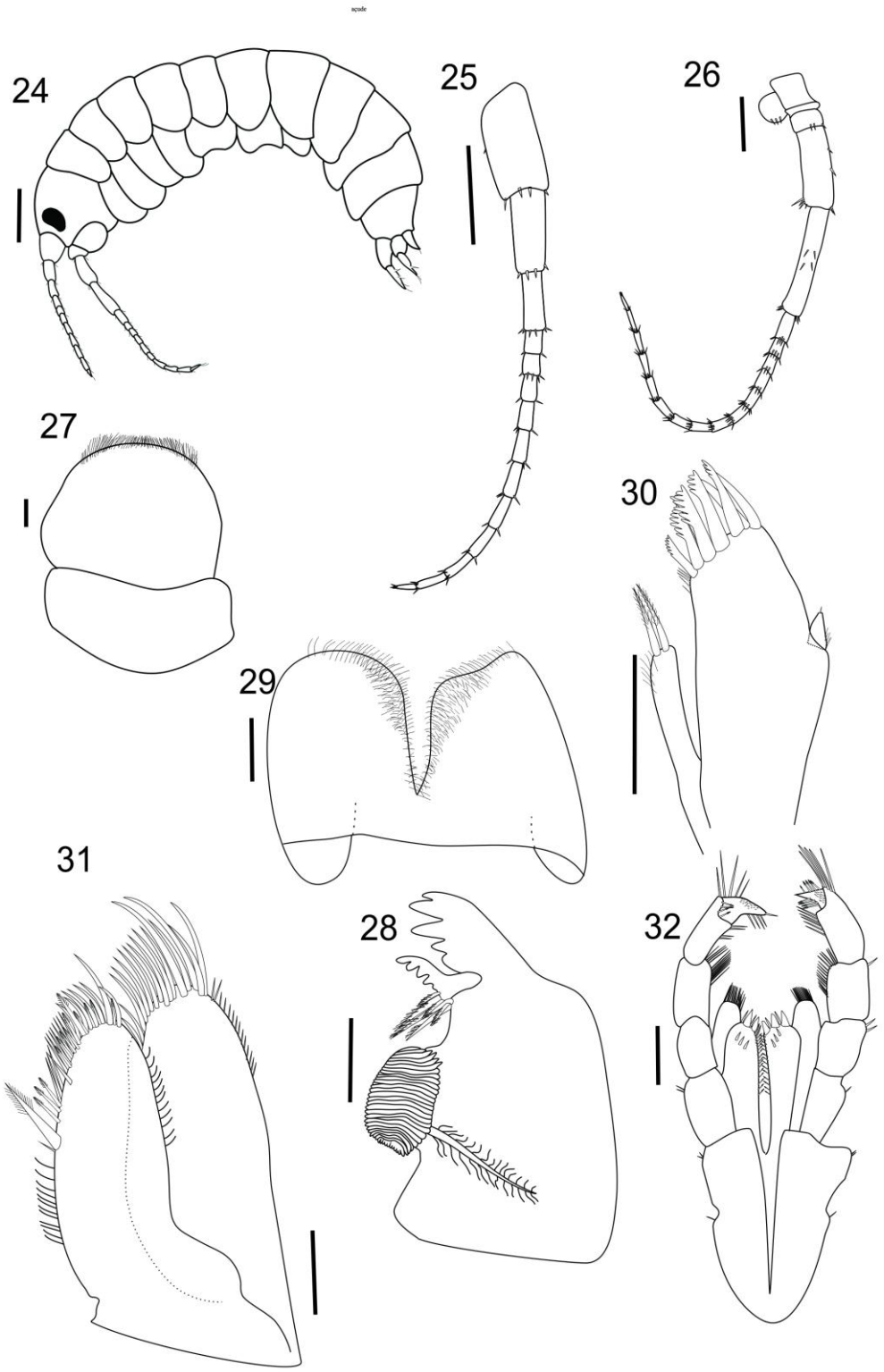
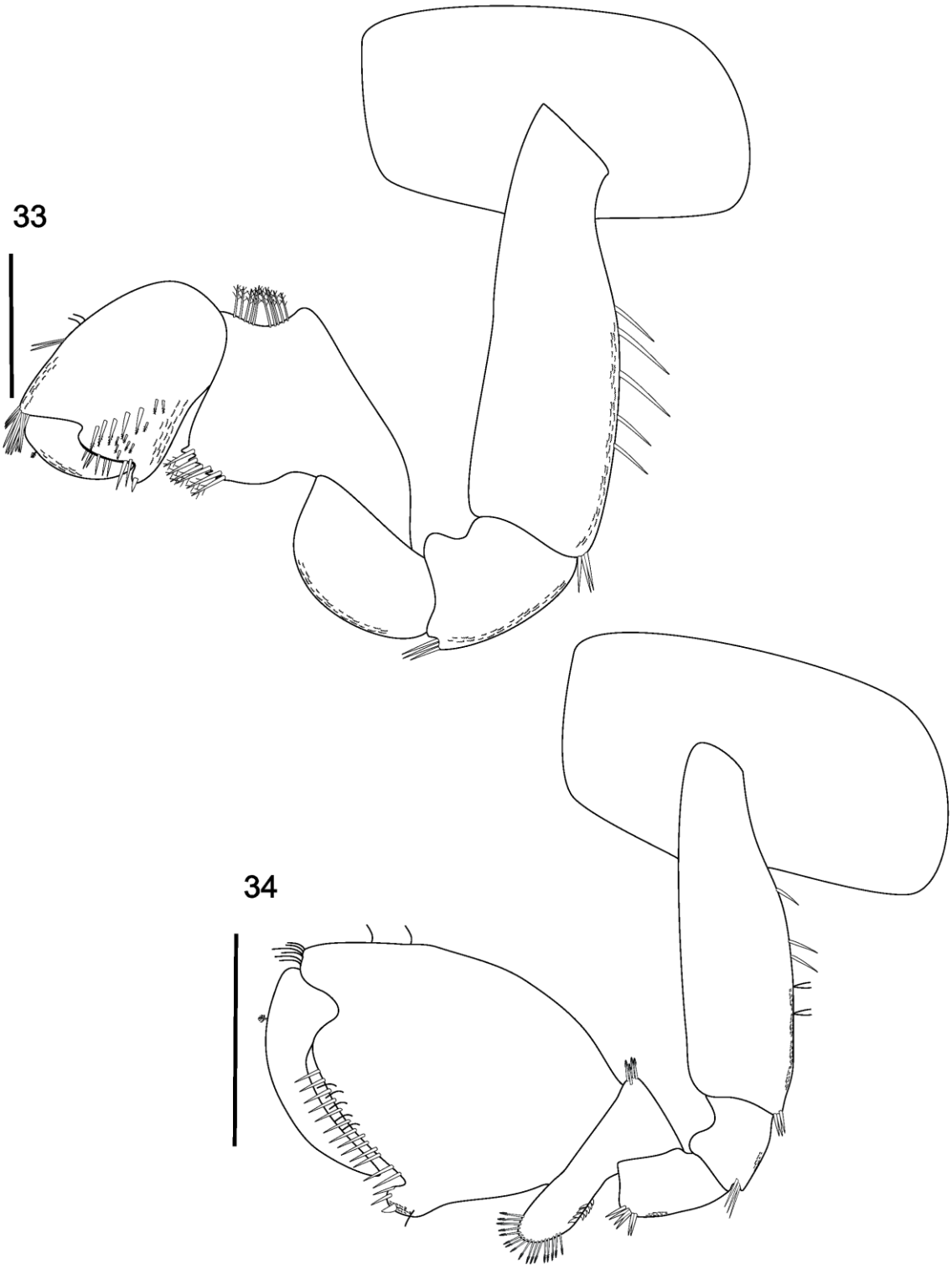
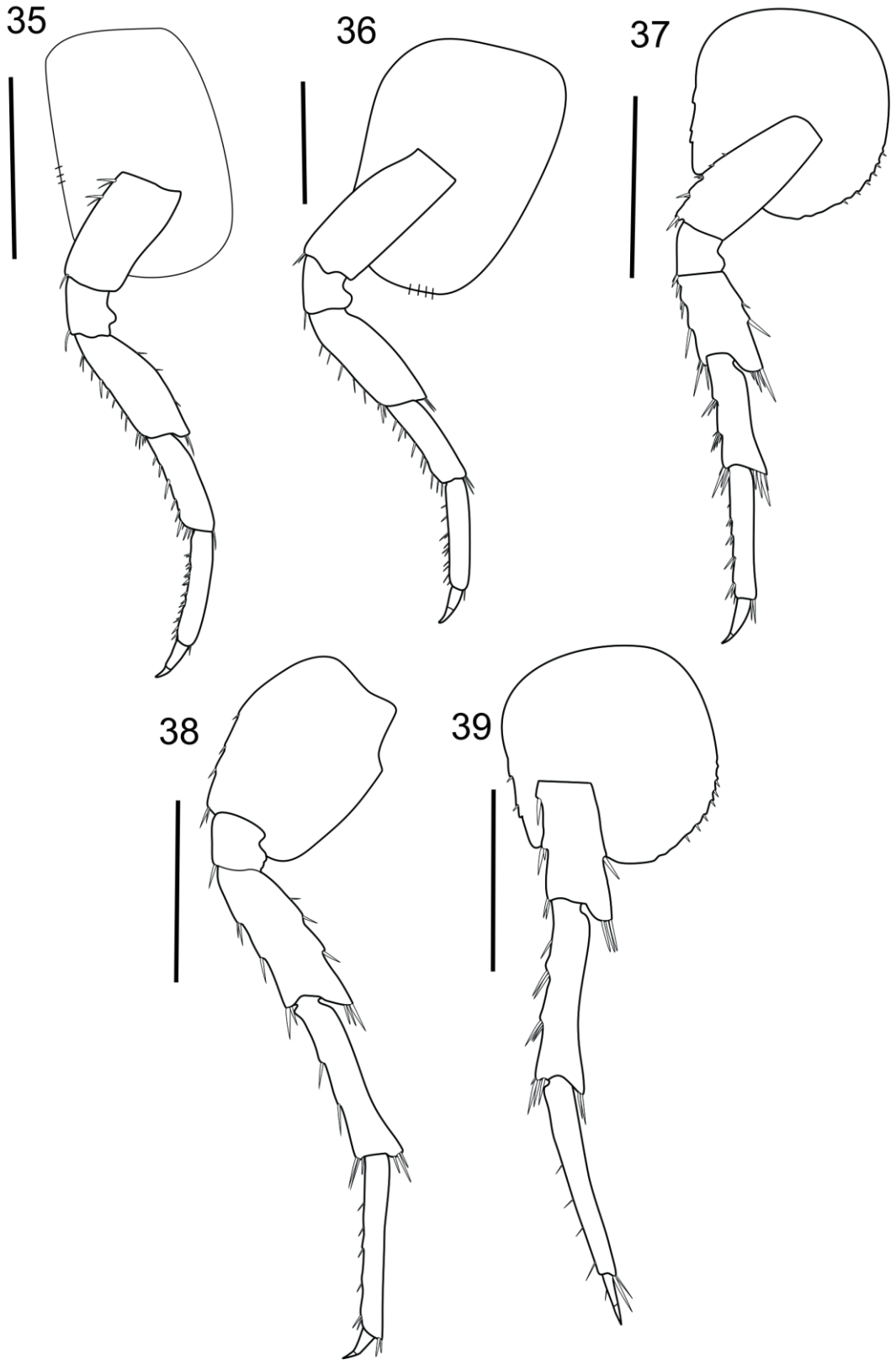


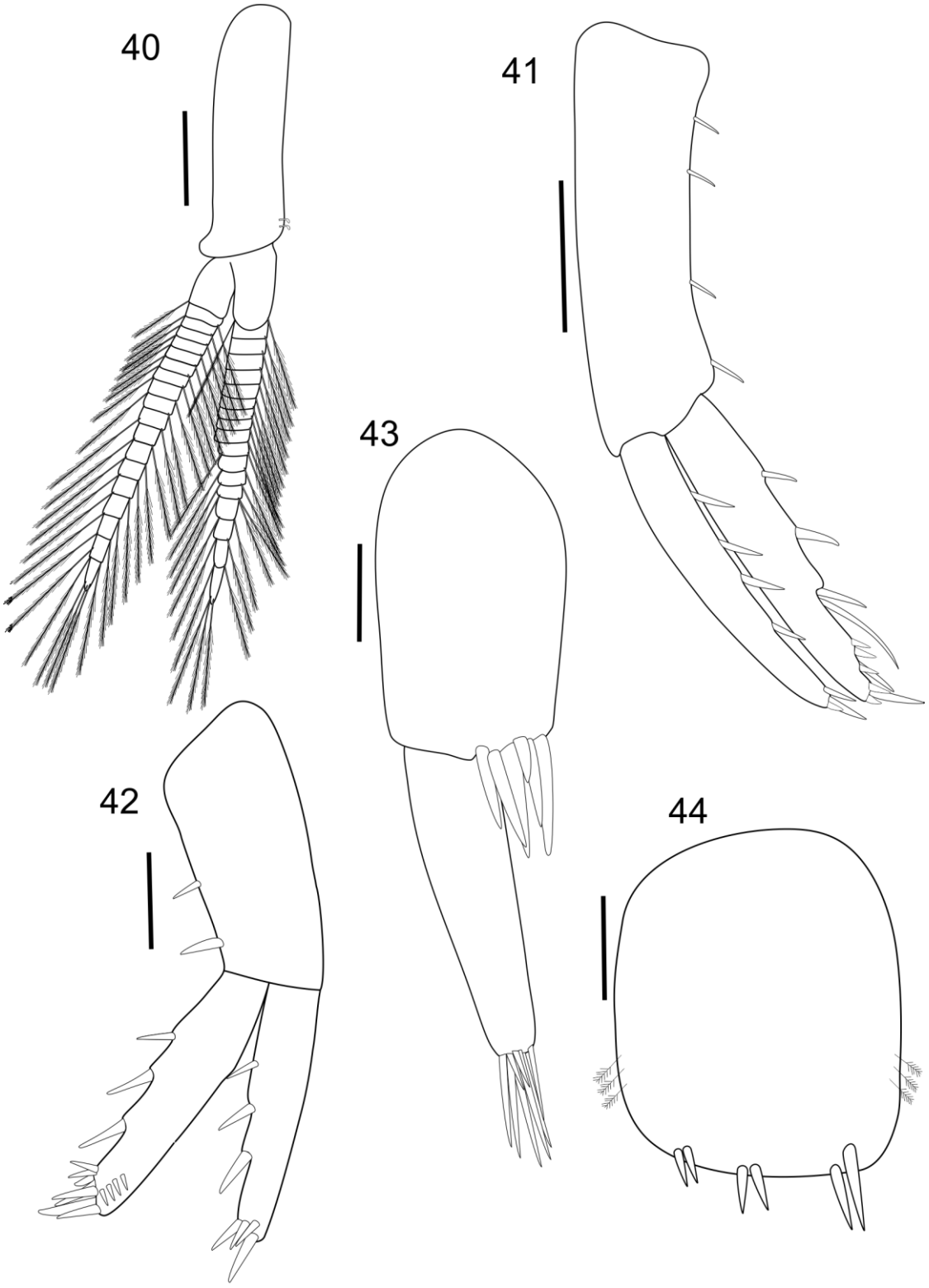
Figure 24-32. *Hyalella* sp. nov. 2. Paratype male (MNRJ 25903); (24) Habitus from holotype (MNRJ 25901), body length: 6.00 mm; (25) Antenna 1; (26) Antenna 2; (27) Upper lip; (28) Mandible; (29) Lower lip; (30) Maxilla 1; (31) Maxilla 2; (32) Maxilliped; Scales: (24, 25, 26, 32) represents 0.5mm; (27, 28, 29, 30, 31) represents 0.2 mm.



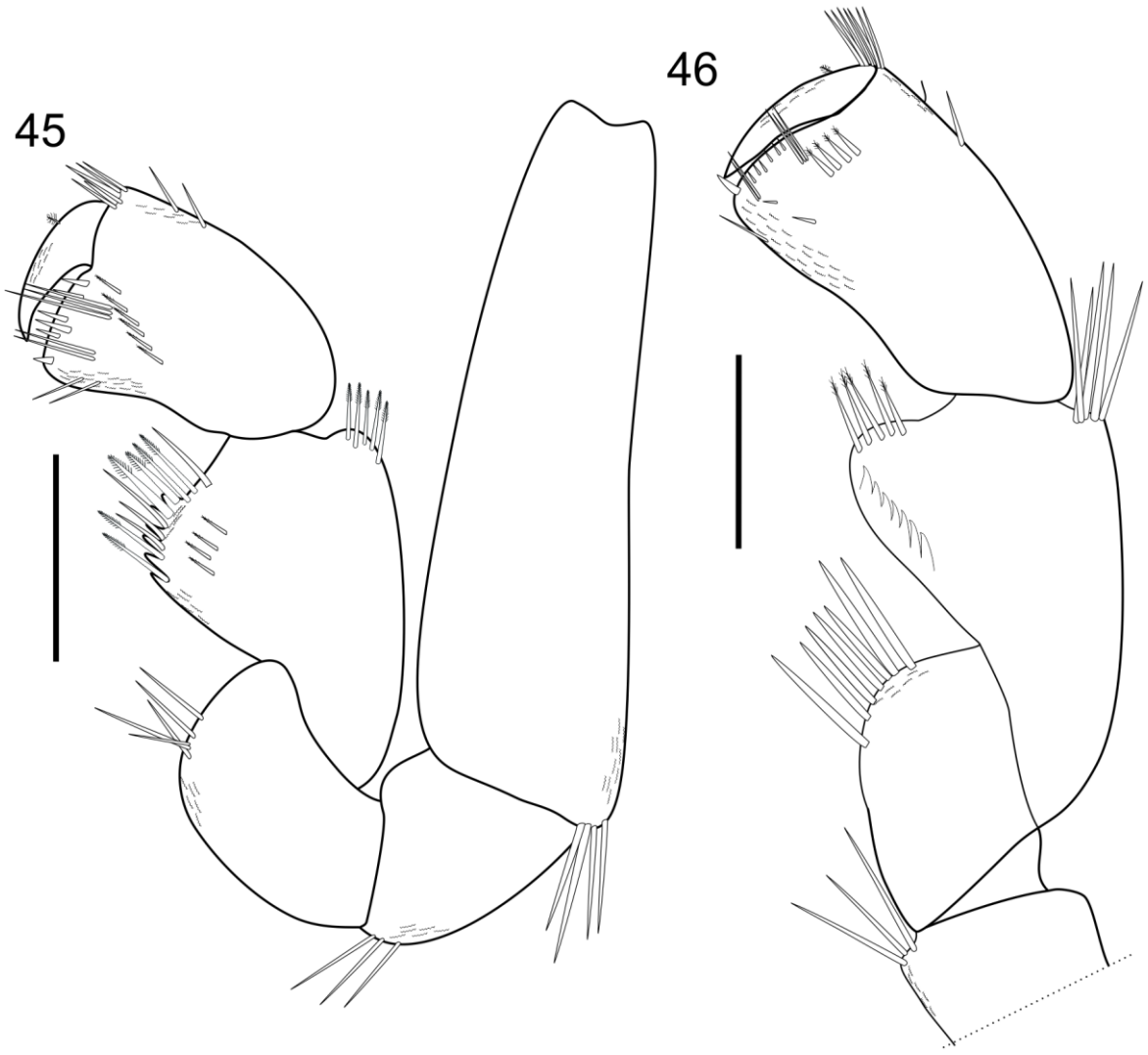
Figures 33-34. *Hyalella* sp. nov. 2. Paratype male (MNRJ25903); (33) Gnathopod 1; (34) Gnathopod 2; Scales: (33- 34) represents 0.5 mm.



Figures 35-39. *Hyalella* sp. nov. 2. Paratype male (MNRJ 25903); (35) Peraeopods (36) Peraeopod 4; (37) Peraeopod 5; (38) Peraeopod 6; (39) Peraeopod 7. Scales: (35-39) represents 0.5 mm.



Figures 40-44. *Hyalella* sp. nov. 2. Paratype male (MNRJ 25903); (40) Pleopod; (41) Uropod 1; (42) Uropod 2; (43) Uropod 3; (44) Telson; Scales: (40) represents 0.5 mm; (41-44) represents 0.2 mm.



Figures 45-46. *Hyalella* sp. nov. 2. Allotype female (MNRJ 25902); (45) Gnathopod 1; (46) Gnathopod 2; Scales: (45-46) represents 0.5 mm.

Figure 47- 48. (47) Type-locality of *Hyalella* sp. nov.1 Streck & Castiglioni, showing the spring shaded by trees and surrounded by grasses and pteridophytes. Note that water of the spring is collected through a pipe by local residents for human consumption. (48)Type-locality of *Hyalella* sp. nov. 2. Streck & Castiglioni, showing the artificial pond of shallow depth Note the large amount of macrophytes of the genus *Salvinia*, which are used as shelter for the specimens of the new species.

Figure 49. Type-locality of *Hyalella* sp. nov.1 Streck & Castiglioni (spring) and *Hyalella* sp. nov. 2 Streck & Castiglioni (artificial pond) in Palmeira das Missões municipality, state of Rio Grande do Sul, Southern Brazil. Note that the distance between the locations is only about 15 meters.

3. ARTIGO 2 Submetido para Zootaxa em 01/07/2016

A new species of freshwater amphipod (Crustacea, Hyalellidae) from state of Rio Grande do Sul, Southern Brazil

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Abstract

The current study aimed to describe a new species of *Hyalella* from state of Rio Grande do Sul, in the Northwest region of the state, found in an natural pond at the municipality of Palmeira das Missões, located at the Northwest region of state of Rio Grande do Sul, Southern Brazil. The new species differs from others species found in state of Rio Grande do Sul (*H. bonariensis*, *H.* castroi, *H. kaigang*, *H. imbya*, *H.* sp. nov. 1, *H.* sp. nov. 2, *H. montenegrinae* and *H. pampeana*) in the type and number of setae on uropods, telson and inner face of gnathopod 1. Besides, *Hyalella* sp. nov. 3 is morphologically very similar to *H. curvispina*, especially in the shape and number of setae of uropods. However, *Hyalella* sp. nov. 3 differs of *H. curvispina* by having only two simple setae on telson, inner face of gnathopod 1 with five serrate setae and only the posterior distal margin with denticles in comb-scales, and especially by sternal gills tubular, present on segments 3 to 7. These new records increase from 11 to 12 the number of species knows from state of Rio Grande do Sul, southern Brazil.

Key words: freshwater crustaceans, *Hyalella*, Peracarida, taxonomy.

Introduction

The Order Amphipoda is considered a successful group of peracarid crustaceans known in several aquatic environments, exerting dominance of the benthic community of lakes (Ishikawa & Urabe, 2002; Rodrigues, 2016). The latest classification of the Order Amphipoda is shown by Lowry & Myers (2013), and these authors presented a new Suborder called Senticaudata, comprising almost all freshwater Amphipoda species and some marine species that previously belong to the ancient Suborder Gammaridea.

In South America, the Order Amphipoda is represented by species inhabiting groundwaters and surface waters (Väinöla et al., 2008). In the state of Rio Grande do Sul, Brazil, crustaceans belonging to seven different Families of the Suborder Senticaudata are observed: Corophiidae, Stenothoidae, Hyalidae, Ischyriceridae, Gammaridae, Talitridae e Hyalellidae (Lowry & Myers, 2013).

The Family Hyalellidae is represented only by the gender *Hyalella* Smith, 1874, which occurs throughout the Neoartic and Neotropical biogeographic regions, and it is endemic of the Americas (Baldinger, 2004). There are 68 limnic crustacean species of the gender *Hyalella*, however the highest diversity of species occurs in South America, where 52 species are currently described (Rodrigues et al., 2014).

Brazil has the greatest number of *Hyalella* species described in the world, 23 in total (Bastos-Pereira & Bueno, 2013; Bueno et al., 2013; Bastos-Pereira 2014; Bueno et al., 2014; Cardoso et al., 2014; Rodrigues et al., 2014). According to Bueno et al. (2014) and Streck et al. (in press) the state of Rio Grande do Sul (Southern Brazil) is the region with the highest species diversity in the country, counting 11 described species. With the finding of the specie *Hyalella* sp. nov. 3 the state of Rio Grande do Sul account now with 12 Hyalella species, the same number of species observed in all Southeast region of Brazil.

A new species of the genus was found in the municipality of Palmeira das Missões, Northwest region of state of Rio Grande do Sul, southern Brazil. Thus, the aim of this work was to describe a new species of freshwater amphipod of the genus *Hyalella*.

Material and Methods

The municipality of Palmeira das Missões is located at the Northwest region of state of Rio Grande do Sul, Southern Brazil. The specimens were collected in August/2012 and the sampling was made with the aid of a hand net, with authorizations of the Instituto Chico

Mendes de Conservação da Biodiversidade (MMA; ICMBio; SISBIO n° 32726-1).

Head length of the animals was measured through an optic microscope with a milimetric scale. Adult males and females were preserved in ethanol 70%, colored with Rose Bengal and dissected. A total of 15 individuals (10 males and 05 females) were dissected and the appendices were mounted on permanent slides and used for the confection of the drawings and for the description of the new species, which were based on the slides of the male paratype and the female allotype.

The description was made based on main morphological characteristics such as the gnathopods, uropods and telson, according to González et al. (2003a, 2003b), González et al. (2006), Cardoso et al. (2011), and Bastos-Pereira & Bueno (2012).The terminology used for the setae of the appendices follows Zimmer et al. (2009).

The type material and the paratypes are deposited in the Museu Nacional do Rio de Janeiro (MNRJ).

Results

Taxonomy

Order Amphipoda Latreille, 1816

Suborder Senticaudata Lowry & Myers, 2013

Family Hyalellidae Bulycheva, 1957

Genus *Hyalella* S. I. Smith, 1874

***Hyalella* sp. nov. 3 Streck & Castiglioni**

Type material: Holotype male, total body length = 5.32 mm, head length = 0.50 mm (MNRJ 26584); Allotype female (MNRJ 26585). Brazil, state of Rio Grande do Sul, Palmeira das Missões municipality (27º 56.949’ S – 53º 19.613’ W), August, 24, 2012, Castiglioni, D.S. coll.

Paratypes: Mean total body length of male paratypes: 5.50 ± 0.78 mm and female paratypes: 5.00 ± 0.50 (20 males and 20 females) (MNRJ 26586), same date as holotype, Castiglioni, D.S. coll.

Type-locality. Brazil, state of Rio Grande do Sul, (27º 56.949’ S – 53º 19.613’ W), state of Rio Grande do Sul, Palmeira das Missões municipality, natural pond, 560 m, August, 24, 2012, Castiglioni, D.S. coll.

Diagnosis. Body surface smooth. Eyes round, pigmented. Antenna 1 longer than

antenna 2, flagellum with 11/12 articles and longer than peduncle, two aesthetascs occurring distally on flagellum after article 5. Antenna 2 flagellum with 10/11 articles and longer than peduncle. Maxilla 1 palp uniarticulate, short, longer than wide, inner plate shorter and slender than outer plate, with two long apical papposerrate setae, and some setules on the inner margin; outer plate with nine serrate setae. Maxilla 2 inner plate with only one long and strong papposerrate seta and several simple and serrate setae. Gnathopod 1 with inner face with five serrate setae, posterior distal margin with denticles in comb-scales, one cuspidate setae and some simple setae. Gnathopod 2 propodus longer than wide and with posterior margin of small lobe covered by denticles in comb-scales; palm smooth and sub-equal to posterior margin of propodus, slope oblique, palm margin convex; dactylus claw-like, short, not reaching the lobe of propodus; carpus wider than long, posterior lobe elongated, with pectinate border and one row of serrate setae, without polygonal pattern or comb-scales. Pleopods rami with long plumose setae. Uropod 1 inner ramus with a long curved setae followed by a row of five small cuspidate setae with an accessory seta. Uropod 2 inner ramus apex with five cuspidate setae with an accessory seta and two cuspidate simple setae. Uropod 3 peduncle with three cuspidate setae with accessory setae apically, small cuspidate setae and simple setae distributed along the peduncle and ramus. Telson (male) as long as wide, with two apical simple setae, six plumose setae laterally. Coxal gills sac-like present on segments 2 to 6 and sternal gills tubular, presents on segments 3 to 7.

Description of male. (Fig.1) Mean body length: 5.50 ± 0.78**,** mean head length: 0.52 ± 0.07 mm (n=20). Head longer than the first thoracic segments, rostrum absent. Eyes round, pigmented.

Antenna 1 (Fig. 2) longer than antenna 2, peduncle not surpassing head length, flagellum with 11/12 articles, longer than peduncle; aesthetascs occurring distally on flagellum after article 5.

Antenna 2 (Fig 3) peduncle less than half body length, with very few setae; flagellum with 10/12 articles and longer than peduncle.

Upper lip (Fig. 4) margin rounded, distal border covered by several setules on dorsal and ventral faces.

Basic amphipodan mandible (Fig. 5) (in sensu of Watling 1993), without palp; incisor toothed; *lacinia mobilis* with five teeth on mandible and with three pappose setae; molar process large, cylindrical and with large accessory seta.

Lower lip (Fig. 6) outer lobes rounded without notches or excavation, with several setules on dorsal and ventral faces.

Maxilla1 (Fig. 7) inner plate uniarticulate, shorter than outer plate, with long and stout pappose setae. Outer plate uniarticulate, with 9 serrate setae.

Maxilla 2 (Fig. 8) inner plate with one long and strong papposerrate seta, several simple and serrate setae; outer plate with one row of simple setae.

Maxilliped (Fig. 9) inner plates apically truncated, with three connate setae and pappose and simple setae apically and medially; outer plates larger than inner plates, apically trun- cated, apical, medial, and facial setae simple. Palp of four articles with simple setae and few pappose setae; dactylus unguiform, shorter than propodus, with simple setae and comb-scales.

Gnathopod 1 (Fig. 10) subchelate; dactylus claw-like, with denticles in comb-scales and one plumose seta dorsally; propodus longer than wide, propodus width about 3⁄4 of maximum length (rectangular), hammer-shaped, palm slope slightly oblique and margin sinuose with several simple setae, posterior distal margins with comb scales, one cuspidate setae and some simple setae, disto- anterior margin without comb scales, but with six or seven simple setae, palm inner face (ventral) with five serrate setae; carpus longer than wide, slightly longer than propodus, with strong lateral distal lobe produced and forming a scoop-like structure, posterior lobe of carpus with one row of serrate setae and without comb-scales some specimens have comb-scales; basis, ischium and merus with simple setae dorsally, without comb-scales posterodistally some specimens have comb-scales ; coxal plates longer than wider wide.

Gnathopod 2 (Fig. 11) subchelate; dactylus claw-like, short, not reaching the lobe of propodus, with one plumose seta dorsally, without comb-scales; propodus longer than wide (ovate), propodus length 1.5 times the maximum width, posterior margin of lobe almost straight and covered with denticles in comb-scales; palm margin convex, with several cuspidate setae with accessory setae and simple setae, posterior distal corner with few simple setae; carpus wider than long, posterior lobe elongated, with pectinate border with one row of serrate setae and without polygonal pattern or comb-scales some specimens have comb-scales; ischium and merus with few simple setae on disto-posterior margin; basis, ischium and merus without comb-scales on posterior margin some specimens have comb-scales; coxal plate wider than long.

Peraeopods 3 to 7 (Fig. 12- 16) dactylus, propodus, carpus, merus, ischium and basis with simple and cuspidate setae with accessory seta. All coxal plates with simple setae on the border.

Pleopods (Fig 17) not modified, peduncle slender smaller than flagellum rami, and two

coupling spines distally; flagellum with long plumose setae.

Uropod 1 (Fig. 18) peduncle slightly (1.2 times) longer than rami, with four cuspidate setae with an accessory seta dorsally; inner ramus longer than outer ramus; inner ramus with two dorsal cuspidate setae with an accessory seta, male with a curved seta followed by a row of five small cuspidate setae with an accessory seta, two cuspidate setae apically; outer ramus with three dorsal cuspidate setae with an accessory seta and four cuspidate setae apically (two of them with an accessory seta).

Uropod 2 (Fig. 19) shorter than uropod 1; peduncle rectangular with similar size as rami, with four cuspidate setae with an accessory seta dorsally; inner ramus slightly longer than outer ramus, with two dorsal cuspidate setae with an accessory seta and seven cuspidate setae apically (five of them with an accessory seta); outer ramus with two dorsal cuspidate setae with an accessory seta and apex with four cuspidate setae (two of them with an accessory seta).

Uropod 3 (Fig. 20) peduncle slightly longer than wide, wider than ramus, with three cuspidate setae with an accessory setae and one simple setae apically, two simples setae distributed along the peduncle; ramus with five simple setae and an short and strong cuspidate setae.

Telson (Fig. 21) as long as wide, with two long simple setae and six plumose setae laterally.

Coxal gills sac-like present on segments 2 to 6. Sternal gills tubular, present on segments 3 to 7.

Female. Mean total body length: 5.00 ± 0.50, mean head length: 0.47 ± 0.05 (n= 20). Antenna 1 similar in shape to male but with flagellum of 7-8 articles. Antenna 2 similar in shape to male, flagellum of 8-9 articles. Gnathopod 1 (Fig. 22) similar to male gnathopod 1, similar size to gnathopod 2; dactylus with comb-scales and one plumose seta; propodus longer than wide, hammer-shaped, inner face with five serrate setae and two long simple setae and several short simple setae , anterior distal margin without comb-scales but two simple setae and distally with six or seven short simple setae, posterior distal margin with comb-scales and two simple setae and posterior distal corner with one strong cuspidate seta; carpus longer than wide, with lateral distal lobe produced and forming a scoope-like strucutre, posterior lobe margin with polygonal pattern and with serrate setae and without comb-scales some specimens have comb-scales; basis, merus and ischium without comb-scales on posterior margin some specimens have comb-scales. Gnathopod 2 (Fig. 23) similar in size to gnathopod 1, but the propodus of gnathopod 2 is slightly longer than gnathopod 1; different to

male gnathopod 2 in shape and smaller; dactylus with comb-scales and one plumose seta; propodus rectangular, longer than wide, inner face with three serrate setae and two long simple setae and several short simple setae, palm transverse, posterior distal corner with one strong cuspidate seta, posterior distal margins with comb-scales and two simple setae and anterior margin without comb-scales; basis, merus and ischum without comb-scales on posterior margin some specimens have comb-scales. Telson similar in shape to male, with two long simple setae and six plumose setae laterally.

Habitat. Freshwater, epigean. *Hyalella* sp. nov. 3 was found in a natural pond of shallow depth (around 40 cm) (Fig. 24). The pond presented a large amount of macrophytes of the genus *Egeria* which were used as shelter for the specimens of *Hyalella*sp. nov. 3.

Conservation: *Hyalella* sp. nov. 3 occurs in a natural pond surrounding cultivation of any type of crops, which could be considered a threat to this species as the pesticides used in these cultures can be harmful to the amphipods (Fig. 25).

Remark: We noted using the taxonomic key of Rodrigues *et al.* (2014) that *Hyalella* sp. nov. 3 differ from the other species of the genus in Brazil. *Hyalella* sp. nov. 3 resemble to *H. montenegrinae*, *H. curvispina*, *H. castroi*, *H. pseudoazteca*, *H. kaingang*, *H. pleoacuta*, *H. carstica and H. xakriaba*, by have a curved seta on the inner ramus of uropod 1 of the males. Besides, *Hyalella* sp. nov. 3 resembles to *H. montenegrinae* and *H. carstica* by presenting sternal gills on segments 3-7. The new species resembles to *H. curvispina*, *H. castroi*, *H. pseudoazteca*, *H. kaingang* and *H. pleoacuta* by the fact that the dactylus of gnathopod 2 is shorter than propodus palm. The new species differs from *H. bonariensis*, *H. castroi*, *H. kaigang H. imbya*, *H. montenegrinae* and *H. pampeana* in the type and number of setae on uropods, telson and inner face of gnathopod 1. *Hyalella* sp. nov. 3 is very morphological similar to *H. curvispina*, especially in the shape and number of setae of uropods. However, *Hyalella* sp. nov. 3 differs of *H. curvispina* by showing only two simple setae on telson, inner face of gnathopod 1 with five serrate setae and only the posterior distal margin with denticles in comb-scales, and especially by sternal gills tubular, present on segments 3 to 7. Comparisons between morphological characters of *Hyalella* species of Rio Grande do Sul are presented in Table 1.

Discussion

The new specie described in this research, will improve the diversity of *Hyalella* in the Southern region of Brazil, increasing the number of species found in the region, with 14

species described, whereas 12 of them are observed in the state of Rio Grande do Sul, the same number of all Southeastern region of Brazil (Bueno et al. 2014; Cardoso et al. 2014; Rodrigues et al. 2014; Streck et al. in press).

Hyalella sp. nov. 3 lives associated to macrophytes in a natural pond of shallow depth. The pond presented a large amount of macrophytes of the genus *Egeria,* which were used as shelter for the specimens. However, *Hyalella* sp. nov. 3 occurs in a natural pond surrounding cultivation of any type of crops, which could be considered a threat to this species as the pesticides used in these cultures can be harmful to the amphipods. The agricultural expansion is one of the main factors that affect and hinders the conservation (Rodrigues et al. 2012).

It is estimated that 90% of areas in state of Rio Grande do Sul (Southern Brazil) have already disappeared due to urban development, construction of dams and reservoirs, primarily with the expansion of areas of agriculture, especially rice and soybean, causing fragmentation and deterioration of these ecosystems (Maltchik and Rolon 2010; Rodrigues, 2012).

Studies have shown that less than 0.1% of the applied pesticides on crops reach their target organisms, while the remaining 99.9% has potential to move to another environmental spheres, such as surface waters and groundwaters (Sabik et al. 2000). Invertebrates can accumulate pesticides levels in their organisms above the ones present in the water, once these compounds are attached to particulate materials ingested (Nimmo et al. 1985). Therefore, the severe impacts caused by the use of pesticides may have altered the habitat of *Hyalella* sp. nov. 3, in such level that extinction cannot be disregarded, once these animals are very sensitive to environmental changes.

The species *Hyalella* sp. nov. 3 resembles to *H. misionensis* Colla & César, 2015, from the Province of Misiones in Argentina, telson (male) with two long apical simple setae. However, differs of curved seta on the inner ramus of uropod 1, inner face of propodus of gnathopod 1 and sternal gills. The study area where *H. misionensis* was found is geographically close to Brazil, where species of the genus have been reported (Bueno et al. 2013).

Table 1. Main morphological differences between *Hyalella* sp. nov. 3 and eleven *Hyalella* species from nearby areas in state of Rio Grande do Sul, Southern, Brazil.

Species	***Body surface***	***Inner margin of maxila 2***	***Articles of flagellum of antenna 1***	***Articles of flagellum of antenna 2***	***Inner face of propodus of gnathopod 1***	***Curved setae in inner ramus of uropod 1***	***Setae on peduncle of uropod 3***	***Telson***	***Sternal gills***	***Tipe locality***
H. bonariensis	Smooth	Two pappose setae	9–12	12–15	5 setae	Present	6 setae	Quadrangular, with 2–3 cuspidate setae apically	2–7	Province of Buenos Aires, Salto, Argentina
H. castroi	Smooth	One pappose setae	10-17	14-18	More than 10 serrate setae	Present	7 setae	Wider than long, with 8 setae	2-7	Vale of trout, Rio Grande do Sul, Brazil
H. curvispina	Smooth	Two plumose setae	11	13	5–7 setae	Present	3 setae	Wider than long, with 3 simple spines	2-7	Montevidéu, Uruguay
H. sp. nov 1	Smooth	Two robust papposerrate apical setae	15	16	9 serrate setae	Present	9 setae	Wider than long, with 7 apical cuspidate setae with an accessory seta, and 4 plumose setae laterally	2-7	Palmeira das Missões, Rio Grande do Sul, Brazil
H. sp. nov. 2	Smooth	Two papposerrate apical setae	11	15	9-20 serrate setae	Present	6 setae	Wider than long, with 6 apical cuspidate setae, and 6 plumose setae laterally	2-7	Palmeira das Missões, Rio Grande do Sul, Brazil

H. imbya	Smooth	Two papposerrate setae	18-23	14-16	7 serrate setae	Present	1 seta	Two long simple apical setae	3-7	Roque Gonzales, Rio Grande do Sul, Brazil
H. kaigang	With dorsal flanges on pleonites 1-2	Two papposerrate setae	17-18	18-24	2 rows of serrate setae	Present	6 setae	Wider than long, with 6-7 cuspidate seta with acessory setae	2-7	Garapiá stream, municipality of São Franciso de Paula, Rio Grande do Sul, Brazil
H. montenegrinae	Smooth	Two plumose setae	14-16	14-19	9-10 plumose setae	Present	4 or 5 setae	Wider than long, 7 to 9 setae	3-7	São José dos Ausentes, Rio Grande do Sul, Brazil
H. pampeana	Smooth	Two bipectinada setae	11–12	Up to 18	5–6 setae	Present	5 to 7 setae	As wide as long, apically rounded, with 2–5 spines	2–7	Province of Buenos Aires, Argentina
H. pleoacuta	With dorsal flanges on some segments	Two pappose setae	14	15	9 serrate setae	Present	4 setae	As long as wide, with 2 simple setae	2-7	Vale of trout, Rio Grande do Sul, Brazil
H. pseoudoazteca	With dorsal flanges on some segments	One pappose seta	10	8-9	1-3 pappose setae	Present	1 seta	Wide as long, 2 closely spaced, long simple setae	3-7	Taim Ecological Reserve , Rio Grande do Sul, Brazil
H. sp. nov. 3	Smooth	One pappose setae	11-12	10-11	5 serrate setae	Present	3 setae	As long as wide, with 2 long simple setae and 6 plumose setae laterally	3-7	Palmeira das Missões, Rio Grande do Sul, Brazil

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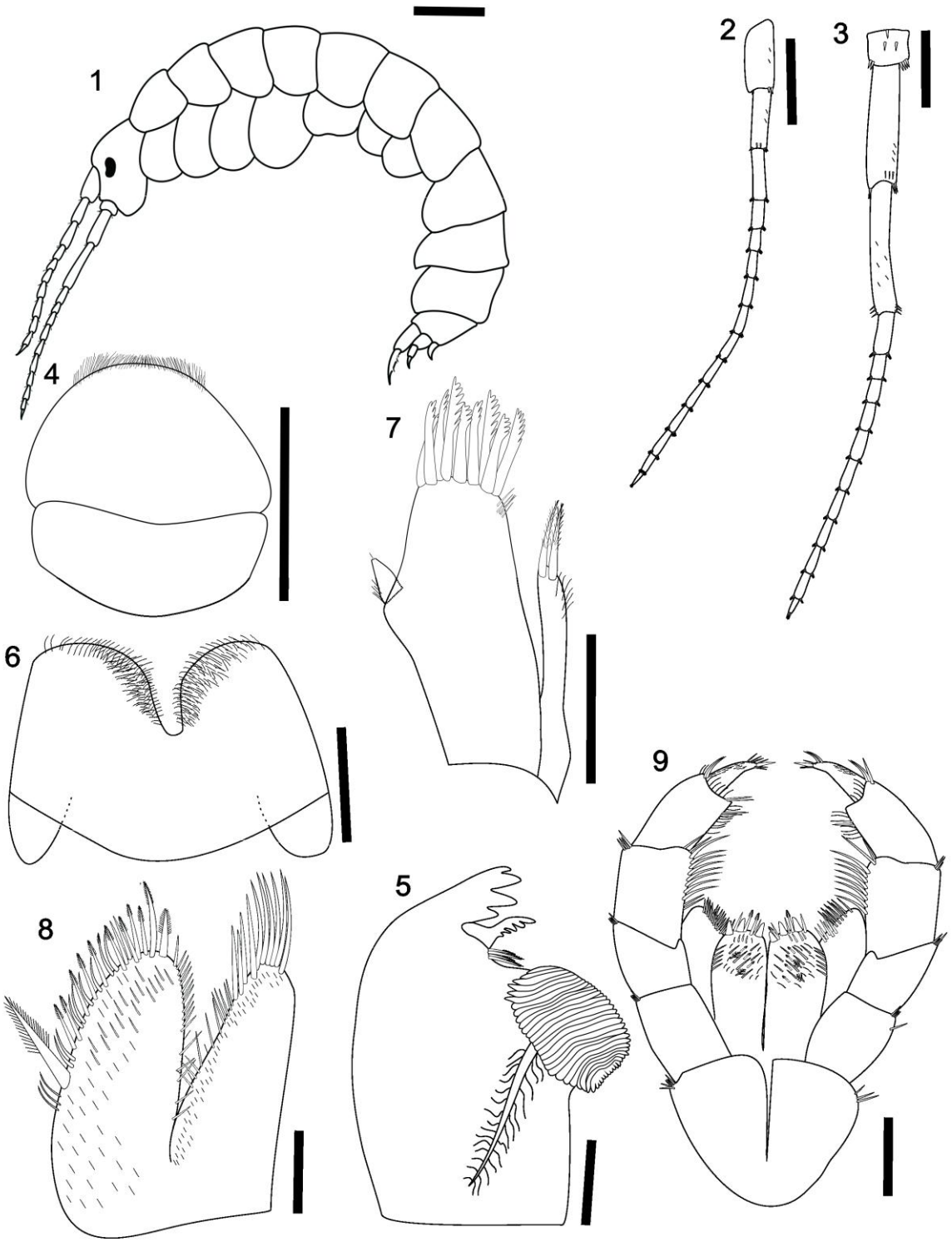
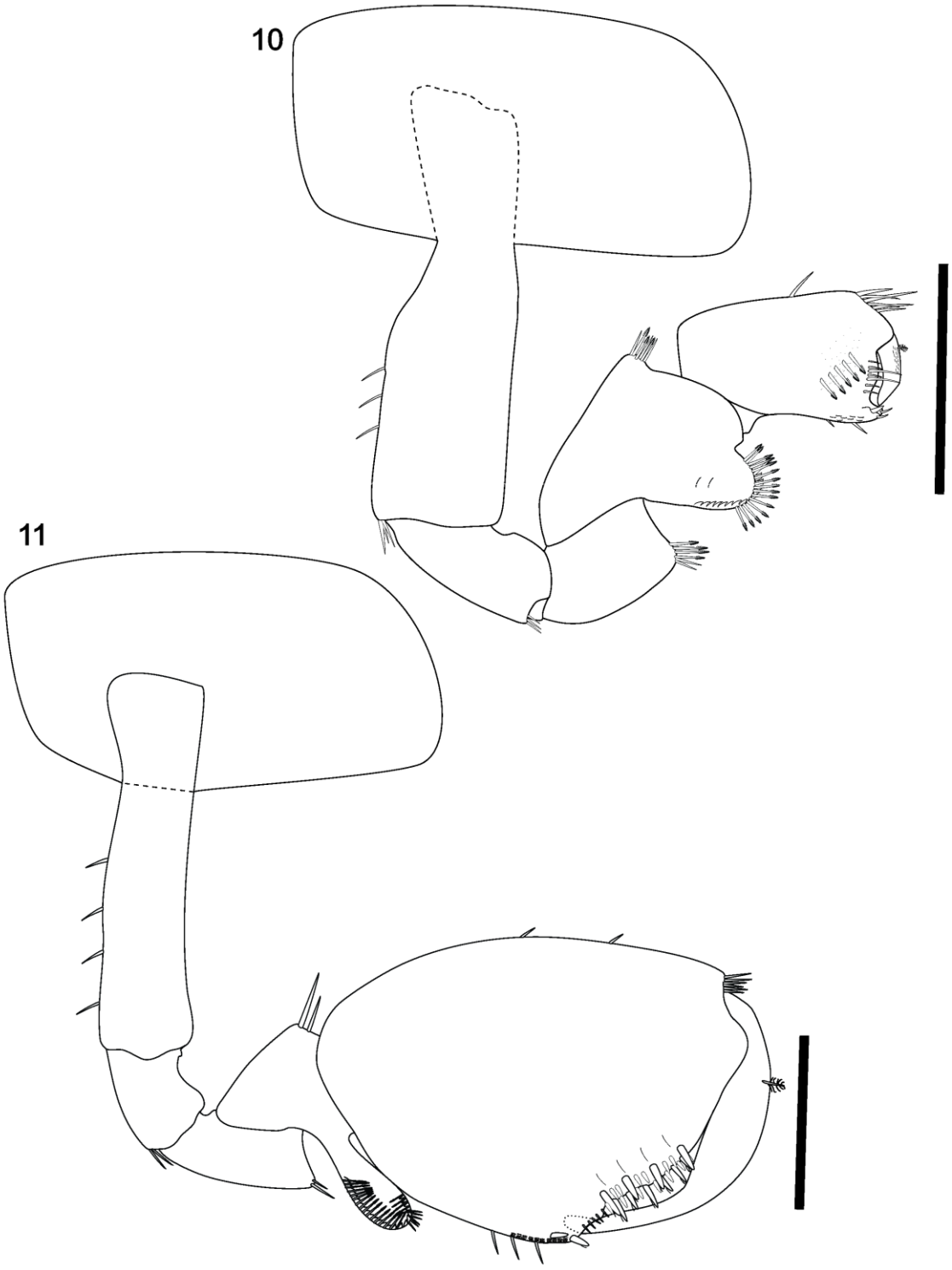
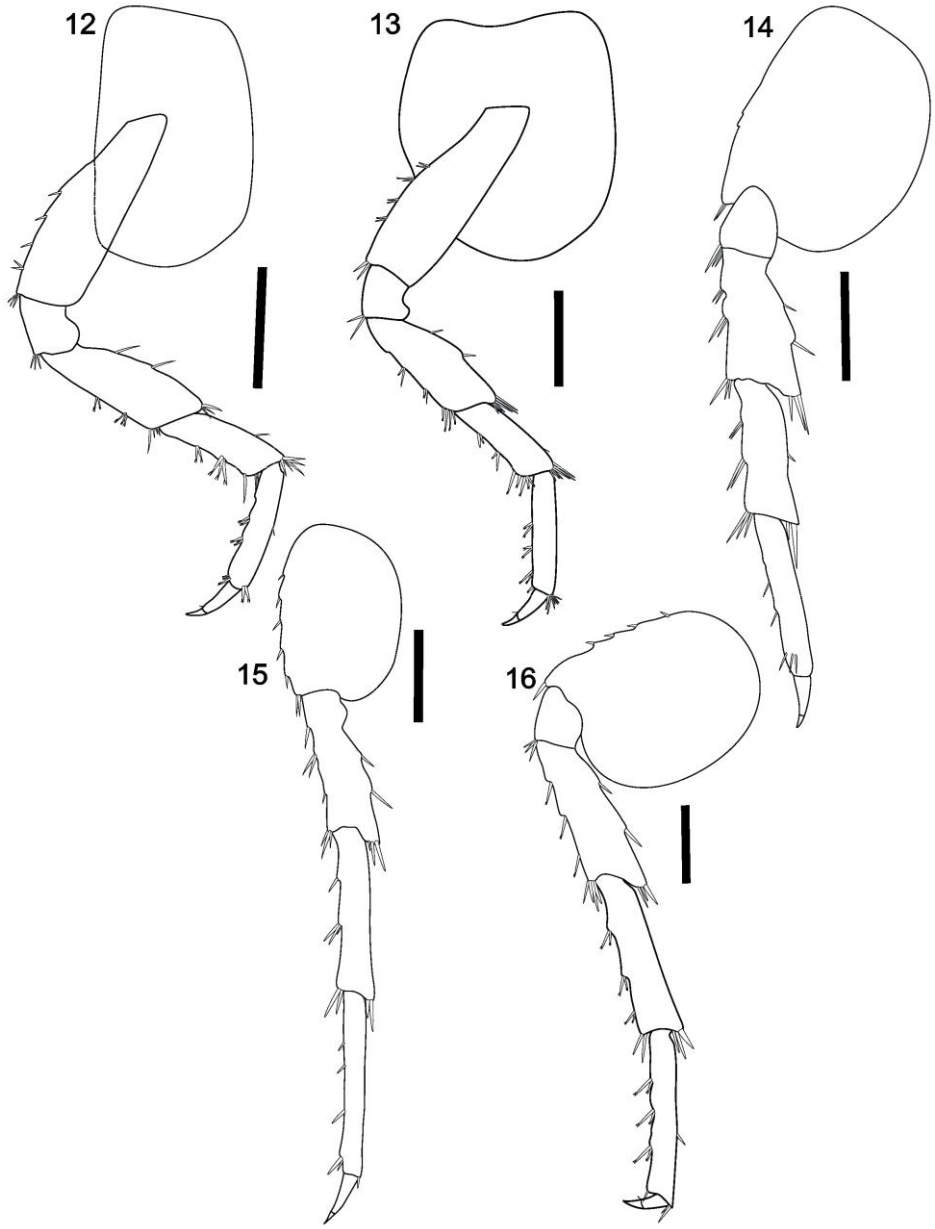


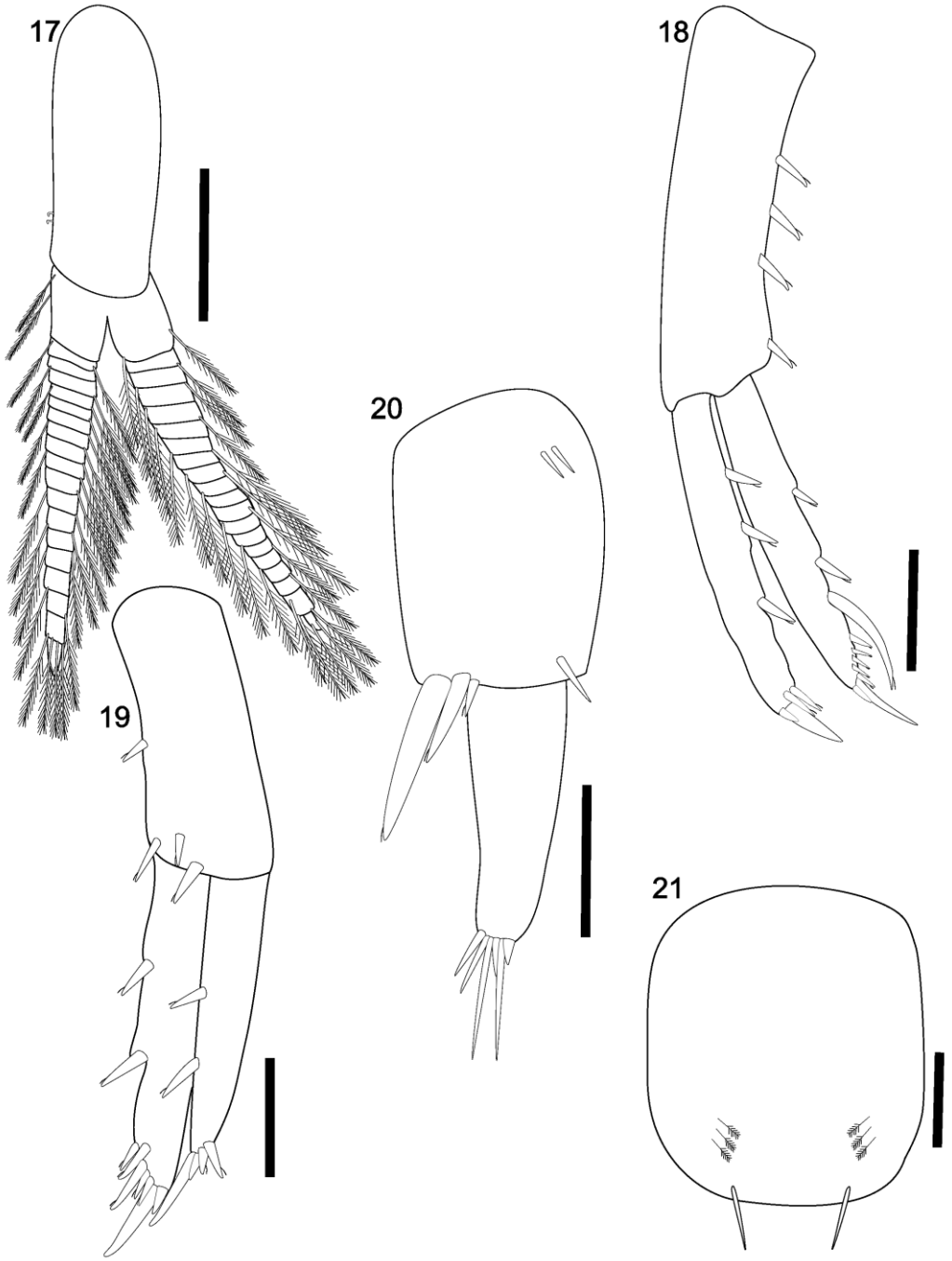
Figures 1-9. ***Hyalella*** **sp. nov. 3.** Paratype male (MNRJ 26586): (1) Habitus from holotype (MNRJ 26584), body length: 5.32mm; (2) Antenna 1; (3) Antenna 2; (4) Upper lip; (5) Mandible; (6) Lower lip; (7) Maxilla 1; (8) Maxilla 2; (9) Maxilliped; Scales: (1-3) represents 0.5 mm; (4-7) 0.2 mm; (8) 0.05 mm; (9) 0.2 mm.



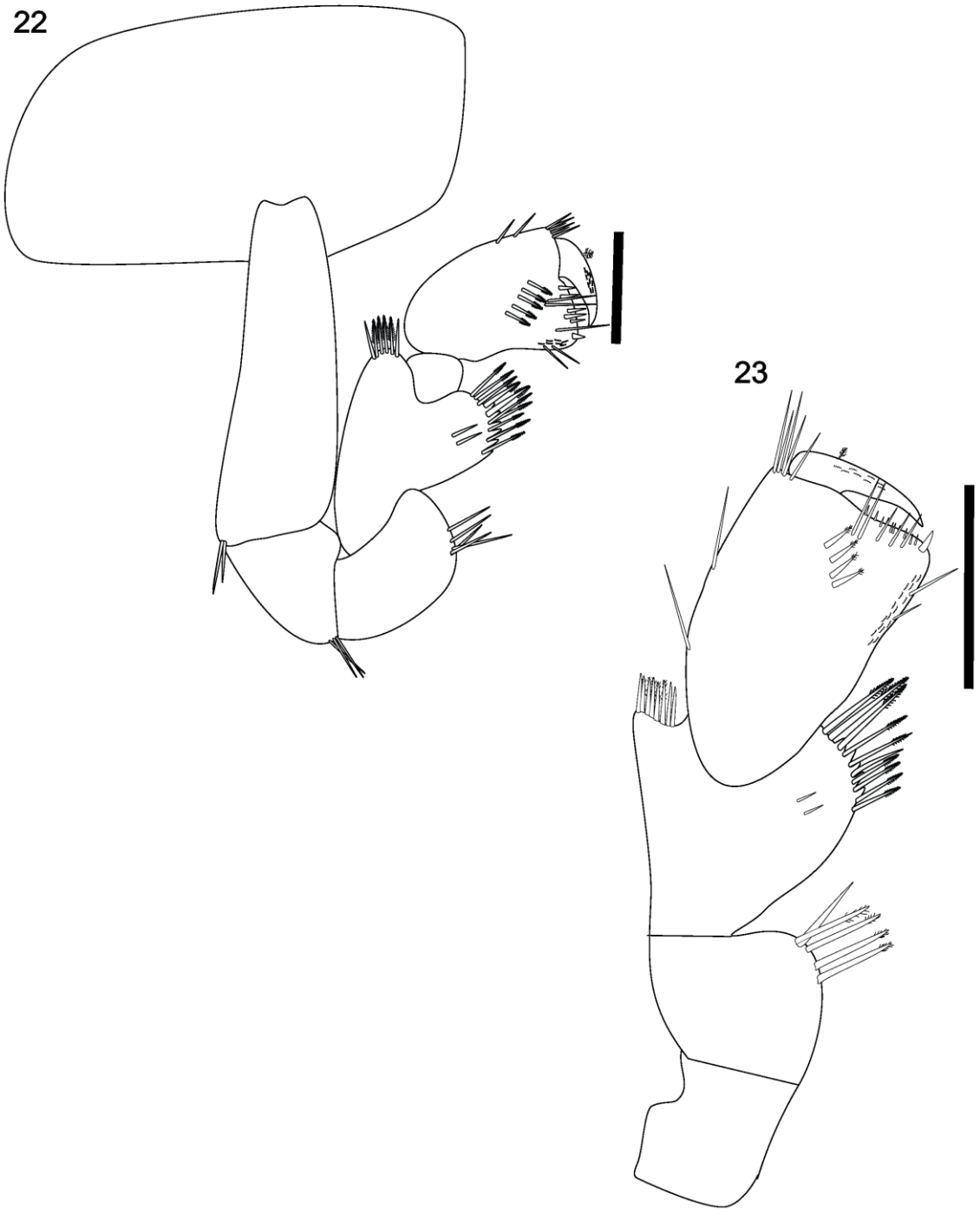
Figures 10-11. *Hyalella* sp. nov. 3. Paratype male (MNRJ 26586); (10) Gnathopod 1; (11) Gnathopod 2; Scales: (10-11) represents 0.5 mm.



Figures 12- 16. *Hyalella* sp. nov. 3. Paratype male (MNRJ 26586); (12) Peraeopod 3; (13) Peraeopod 4; (14) Peraeopod 5; (15) Peraeopod 6; (16) Peraeopod 7; Scales: (12-16) represents 0.5 mm.



Figures 17-21. *Hyalella* sp. nov. 3. Paratype male (MNRJ 26586); (17) Pleopod; (18) Uropod 1; (19) Uropod 2; (20) Uropod 3; (21) Telson; Scales: (17) represents 0.5 mm; (18-20) 0.2 mm; (21) 0.05 mm.



Figures 22-23. *Hyalella* sp. nov. 3. Allotype female (MNRJ 26585); (22) Gnathopod 1; (23) Gnathopod (2) Scales: (22–23) represents 0.2 mm.

Figures 24-25. Type-locality of. *Hyalella* sp. nov. 3 Streck & Castiglioni; (24) Showing the natural pond of shallow depth (around 40 cm). Note the large amount of macrophytes of the genus *Egeria*, which are used as shelter for the specimens of the new species; (25) Showing the natural pond of shallow and surrounding cultivation of any type of crops, which could be considered a threat to this species as the pesticides used in these cultures can be harmful to the amphipods.

4. CONSIDERAÇÕES FINAIS

Os resultados dessa pesquisa ampliaram o conhecimento da diversidade de espécies de *Hyalell*a para o território brasileiro, mais especificamente para a Região Sul, elevando para 26 espécies no país e 12 no Estado do Rio Grande do Sul.

As três espécies descritas no presente trabalho possuem como característica comum uma seta curva no ramo interno do urópodo 1 com uma seta acessória nos machos. A espécie *Hyalella* sp. nov. 1 difere de *H. montenegrinae*, *H. pseoudoazteca* e *H. imbya* apresentando brânquias no esterno nos segmentos 2 a 7 e por possuir duas cerdas apicais no télson. Além disso, possui um padrão peculiar de cerdas no pedúnculo e ramo do urópodo 3. Já *Hyalella* sp. nov. 2 difere de *H. pleoacuta, H. kaingang* e *H. pseudoazteca* por ausência de flanges dorsal sobre péreon e pleonitos. Também difere da *H. montenegrina*e, *H. curvispina* e *H. castroi* em relação ao tipo e número de cerdas nos urópodos, télson e face interna da gnatópodo 1, bem como na forma e tamanho do própodo do gnatópodo 2. Além disso, *Hyalella* sp. nov. 2 possui poucas cerdas na antena 2, maxilípodo muito fino e pleópodo com cerdas longas e plumosas.

A espécie *Hyalella* sp. nov. 3 é morfologicamente muito similar à *H. curvispina* principalmente devido ao número de setas nos urópodos. Entretanto, difere de *H. curvispina* especialmente por possui brânquias esternais nos segmentos 3 a 7. Além disto, *Hyalella* sp. nov. 3 difere de *H. bonariensis*, *H. castroi*, *H. kaigang*, *H. imbya*, *H. pampeana*, *H. montenegrinae* principalmente em relação ao número de setas no télson e na face interna do gnatópodo 1.

Cabe ressaltar que *Hyalella* sp. nov. 1 e *Hyalella* sp. nov. 2 assemelham-se a *H. misionensis* encontrada na Argentina, apresentando brânquias esternais nos segmentos de 2 a 7, e por possuir duas setas apicais paposerradas na margem interna da maxila 2, diferente da *Hyalella* sp. nov. 3 que possuis brânquias nos segmentos de 3 a 7 e apenas uma seta paposa na margem interna da maxila 2. No entanto, *H. misionensis* possui o télson muito similar ao da *H.* sp. nov. 3, com duas setas apicais e seis plumosas laterais. A área de estudo onde *H. misionensis* foi encontrada é geograficamente próximo ao Brasil, local onde quatorze espécies do gênero já foi descritas (Bueno et al, 2013; Colla et al, 2015).

As três espécies descritas no presente estudo, *Hyalella* sp. nov. 1, *Hyalella* sp. nov. 2 e *Hyalella* sp. nov. 3 encontram-se inseridas em propriedades privadas, sendo assim recomendado o desenvolvimento de estratégias junto aos moradores relacionadas à educação para conservação do ambiente e seu entorno. Sendo assim, recomenda-se a ampliação de pesquisas e estudos biológicos e ecológicos das espécies para aplicação de manejo e

conservação adequada. Além disso, deve-se ressaltar junto à comunidade rural a necessidade do desenvolvimento de ações políticas e públicas relacionadas à conservação dos ecossistemas dulcícolas e destas espécies de anfípodos, uma vez que qualquer alteração no meio pode ser refletida diretamente em seu ambiente, sendo sujeitos a diversos tipos de ameaças, como o desmatamento e poluição, mas principalmente a influência de pesticidas utilizados na agricultura.

5. ANEXO A- Normas para publicação na Revista Zootaxa

Information for authors

Information for authors

- **Aim and scope**
    - Research article
    - Correspondence
    - Special issues with collected papers (e.g. Festschrift)
- **Preparation of manuscripts**
- **Submission of manuscripts**
- **Review process**
- **Publication**
    - Page charge and colour plates
    - Open access
    - Reprints

Aim and scope

Zootaxa is a peer-reviewed international journal for rapid publication of high quality papers on any aspect of systematic zoology, with a preference for large taxonomic works such as monographs and revisions. *Zootaxa* considers papers on all animal taxa, both living and fossil, and especially encourages descriptions of new taxa. All types of taxonomic papers are considered, including theories and methods of systematics and phylogeny, taxonomic monographs, revisions and reviews, catalogues/checklists, biographies and bibliographies, identification guides, analysis of characters, phylogenetic relationships and zoogeographical patterns of distribution, descriptions of taxa, and nomenclature. Open access publishing option is strongly encouraged for authors with research grants and other funds. For those without grants/funds, all accepted manuscripts will be published but access is secured for subscribers only. All manuscripts will be subjected to peer review before acceptance. *Zootaxa* aims to publish each paper within one month after the acceptance by editors.

Based on length, two categories of papers are considered.

1) **Research article**

Research articles are significant papers of four or more printed pages reporting original research. Papers between 4 and 59 printed pages are published in multi-paper issues of 60, 64 or 68 pages. Monographs (60 or more pages) are individually issued and bound, with ISBNs.

Zootaxa encourages large comprehensive taxonomic works. There is no upper limit on the length of manuscripts, although authors are advised to break monographs of over 1000 pages into a multi-volume contribution simply because books over 1000 pages are difficult to bind and too heavy to hold.

Very short manuscripts with isolated descriptions of a single species are generally discouraged, especially for taxa with large number of undescribed species. These short manuscripts may be returned to authors without consideration. Short papers on species of economic, environmental or phylogenetic importance may be accepted at the discretion of editors, who will generally encourage and advise authors to add value to the paper by providing more information (e.g. checklist of or key to species of the genus, biological information.....). Short papers of 4 or 5 pages accepted for publication may be shortened for publication in the Correspondence section.

2) **Correspondence**

High quality and important short manuscripts of normally 1 to 4 pages are considered to fill blank pages in multi-paper issues. Zootaxa publishes the following six types of correspondence:

- opinions and views on current issues of interests to systematic zoologists (e.g.Zootaxa 1577: 1-2)
- commentary on or additions/corrections to papers previously published in *Zootaxa*(e.g. Zootaxa 1494: 67-68)
- obituary in memory of deceased systematic zoologists (e.g. Zootaxa 545: 67-68)
- taxonomic/nomenclatural notes of importance
- book reviews meant to introduce readers to new or rare taxonomic monographs (interested authors/publishers must write to subject editors before submitting books for review; editors then prepare the book review or invite colleagues to prepare the review; unsolicited reviews are not published)
- and short papers converted from manuscripts submitted as research articles but are too short to qualify as formal research articles.

These short contributions should have no more than **20 references** and its **total length should not exceed four printed pages (except editorials).** Neither an abstract nor a list of key words is needed; major headings (Introduction, Material and methods...) should NOT be used, except for new taxon heading and references. A typical correspondence should consist of (1) a short and concise title, (2) author name and address (email address), (3) a series of paragraphs of the main text,and (4) a list of references if any. For correspondence of 3 or 4 pages, the first or last paragraph may be a summary.

Commentaries on published papers are intended for scholarly exchange of different views or interpretations of published data and should not contain personal attack; authors of concerned papers may be invited to reply to comments on their papers.

Special issues

Special issues with collected papers such as a Festschrift (see Zootaxa 1325 and Zootaxa 1599) within the scope of the journal are occasionally published. Guest editors should send the proposal to the chief editor for approval and instructions. Although guest editors for special issues are responsible for organising the peer review of papers collected within these issues, they must follow Zootaxa's style, stardard and peer review procedures. If any papers by the guest editors are to be included in the special issue, then these papers must be handled by editors/colleagues other than the editor(s) involved. Special issues must be 60 or more pages. Normally funding is required to offset part of the production cost. Author payment for open access is strongly encouraged. Reprints can be ordered for the entire issue or for individual papers.

Preparation of manuscripts

1) *General.* All papers must be in English. Authors whose native language is not English are encouraged to have their manuscripts read by a native English-speaking colleague before submission. Nomenclature must be in agreement with the *International Code of Zoological Nomenclature* (4th edition 1999), which came into force on 1 January 2000. Author(s) of species name must be provided when the scientific name of any animal species is first mentioned (the year of publication needs not be given; if you give it, then provide a full reference of this in the reference list). Authors of plant species names need not be given. Metric systems should be used. If possible, use the common font New Times Roman and use as little formatting as possible (use only **bold** and *italics* where necessary and indentions of paragraphs except the first). Special symbols (e.g. male or female sign) should be avoided because they are likely to be altered when files are read on different machines (Mac versus PC with different language systems). You can code them as m# and f#, which can be replaced during page setting. The style of each author is generally respected but they must follow the following general guidelines.

2) The **title** should be concise and informative. The higher taxa containing the taxa dealt with in the paper should be indicated in parentheses: e.g. A taxonomic revision of the genus *Aus* (Order: family).

3) The **name(s) of all authors** of the paper must be given and should be typed in the upper case (e.g. ADAM SMITH, BRIAN SMITH & CAROL SMITH). The address of each author should be given in *italics* each starting a separate line. E-mail address(es) should be provided if available.

4) The **abstract** should be concise and informative. Any new names or new combinations proposed in the paper should be mentioned. Abstracts in other languages may also be included in addition to English abstract. The abstract should be followed by a list of **key words** that are not present in the title. Abstract and key words are not needed in short correspondence.

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Author (2002) Title of website, database or other resources, Publisher name and location (if indicated), number of pages (if known). Available from: http://xxx.xxx.xxx/ (Date of access).

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