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INGRID COSTA MARÇAL

EGLÍDEOS (CRUSTACEA, ANOMURA) DA BACIA DO RIO TIBAGI:

RIQUEZA, DISTRIBUIÇÃO E TAXONOMIA INTEGRATIVA

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Tese apresentada ao Programa de Pós-graduação em Ciências Biológicas da Universidade Estadual de Londrina - UEL, como requisito parcial para a obtenção do título de Doutor.

Orientador: Prof. Dr. Gustavo Monteiro Teixeira
Coorientadora: Dra. Lenice Souza-Shibatta

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RESUMO

Os crustáceos dulcícolas do gênero *Aegla* são endêmicos do sul da América do Sul. A grande maioria das espécies está restrita a cursos d'água de baixa ordem, sendo altamente suscetíveis às alterações ambientais. No sul do Brasil, o estado do Paraná é a região menos estudada, mas demonstra grande potencial para abrigar uma elevada diversidade de *Aegla*. Na bacia do rio Tibagi, por exemplo, quatro espécies foram registradas até agora (*Aegla castro*, *A. schmitti*, *A. lata* e *A. jacutinga*). A necessidade de inventários e estudos taxonômicos sobre este gênero, como uma medida de conservação da biodiversidade, é evidente. No entanto, a morfologia do grupo é conservadora e caracterizações moleculares têm sido utilizadas para a realização de interpretações seguras da diversidade, incluindo a detecção de espécies crípticas. Diante disso, o presente trabalho foi desenvolvido com o objetivo de contribuir com o conhecimento da riqueza, distribuição geográfica e taxonomia do gênero *Aegla*. Os resultados deste trabalho foram organizados em três capítulos. No primeiro discorremos sobre a diversidade críptica de *Aegla* no estado do Paraná, apresentando as descrições de três novas espécies coletadas na bacia do rio Tibagi. De acordo com os critérios da IUCN, uma das novas espécies pode ser classificada como "Em perigo" e as outras duas como "Vulneráveis". O segundo capítulo é dedicado a atualizar a distribuição geográfica dos eglídeos da bacia do rio Tibagi. Foram analisados espécimes depositados na Coleção de Crustáceos do Museu de Zoologia da Universidade Estadual de Londrina. Novos registros de ocorrência foram obtidos para *A. castro* (10), *A. lata* (5) e *A. jacutinga* (10). Análises morfológicas e moleculares permitiram identificar duas novas espécies para a região do Médio Tibagi. As novas espécies têm distribuição limitada e são categorizadas como "Em perigo" de acordo com os critérios da IUCN. Além disso, uma chave de identificação para as espécies de *Aegla* da bacia do rio Tibagi é fornecida. No terceiro capítulo avaliamos a eficácia da região barcode do gene COI na discriminação de espécies de *Aegla*, baseado na diferença dos valores de divergência intra e interespecíficas. Das 74 espécies analisadas, 68 (92%) foram corretamente identificadas por suas sequências, confirmando a eficácia da região barcode do COI na identificação de espécies de *Aegla*. Além disso, a aplicação do limiar de distância genética de 2% revelou possíveis táxons crípticos e linhagens geneticamente divergentes em 22 espécies investigadas. Espera-se que os dados fornecidos por nossa pesquisa contribuam para revelar a real diversidade e padrões de distribuição dos eglídeos. Em suma, nossos resultados enfatizam a importância da bacia do rio Tibagi para a conservação dos eglídeos e reforçam o alto potencial de descoberta de espécies novas no estado do Paraná.

Palavras-chave: aeglidae; COI; conservação; diversidade críptica; identificação de espécies.

MARÇAL, Ingrid Costa. **Aeglids (Crustacea, Anomura) from the Tibagi River basin**: richness, distribution and integrative taxonomy. 2021. 172 p. Thesis (Doctorate degree in Biological Sciences) – Universidade Estadual de Londrina, Londrina, 2021.

ABSTRACT

Freshwater crustaceans of the genus *Aegla* are endemic to southern South America. Most species are restricted to low-order watercourses, being highly susceptible to environmental changes. In south Brazil, the Paraná state is the least studied region but shows great potential for harboring a high diversity of *Aegla*. For example, in the Tibagi River basin, four species have been recorded so far (*Aegla castro*, *A. schmitti*, *A. lata*, and *A. jacutinga*). The need for inventories and taxonomic studies on this genus as a measure of biodiversity conservation is evident. However, the morphology of the group is conservative and molecular characterizations have been used to make safe interpretations of diversity, including the detection of cryptic species. Thus, the present work aimed to contribute to the knowledge of the richness, geographic distribution, and taxonomy of the genus *Aegla*. The results of this work were organized into three chapters. In the first, we discuss the cryptic diversity of *Aegla* in the state of Paraná, presenting the descriptions of three new species collected in the Tibagi River basin. According to IUCN criteria, one of the new species can be classified as "Endangered" and the other two as "Vulnerable". The second chapter is dedicated to updating the geographic distribution of the aeglids from the Tibagi River basin. Specimens deposited at the Crustacean Collection of the Museu de Zoologia of Universidade Estadual de Londrina were analyzed. New records of occurrence were obtained for *A. castro* (10), *A. lata* (5), and *A. jacutinga* (10). Morphological and molecular analyzes allowed to identify two new species for the Middle Tibagi region. New species have limited distribution and are categorized as "Endangered" according to IUCN criteria. In addition, a diagnostic key for *Aegla* species from the Tibagi River basin is provided. In the third chapter, we evaluated the effectiveness of the COI gene barcode in discriminating *Aegla* species based on the difference in intra and interspecific divergence values. Of the 74 species analyzed, 68 (92%) were correctly identified by their sequences, confirming the effectiveness of the COI barcode in identifying *Aegla* species. In addition, the 2% genetic distance threshold application revealed possible cryptic taxa and genetically divergent lineages in 22 investigated species. We hope that the data provided by our research will contribute to the real diversity and distribution patterns of aeglids. In summary, our results emphasize the importance of the Tibagi River basin for the conservation of aeglids and reinforce the high potential of finding new species in the Paraná state.

Key words: aeglidae; COI; conservation; cryptic diversity; species identification.

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LISTA DE ABREVIATURAS E SIGLAS

AAD	Marcação areolar anterior
AH	Altura da aréola
APM	Margem posterior da aréola
bp	Pares de bases
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CL	Comprimento da carapaça incluindo o rostro
CLE	Comprimento da carapaça sem o rostro
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
COI	Citocromo c Oxidase subunidade I
CW	Largura da carapaça
DNA	Deoxyribonucleic acid
EN	Em perigo
GMYC	Generalized Mixed Yule-coalescent
HWT	Metade da largura máxima do telson
IUCN	International Union for Conservation of Nature
K2P	Modelo Kimura-2-Parâmetros
LabIAS	Laboratório de Invertebrados Aquáticos e Simbiontes
LMR	Margem lateral do rostro
LT	Baixo Tibagi
MGSP	Parque Estadual Mata dos Godoy
MHNCI	Museu de História Natural Capão da Imbuia
MN/UFRJ	Museu Nacional da Universidade Federal do Rio de Janeiro
m.s.n.m.	Metros sobre o nível do mar
MT	Médio Tibagi
MZUEL	Museu de Zoologia da Universidade Estadual de Londrina
MZUSP	Museu de Zoologia da Universidade de São Paulo
NJ	Neighbor-joining tree
NMNH	National Museum of Natural History of the Smithsonian Institution
PCR	Polymerase Chain Reaction
PCW	Largura pré-cervical
PMC	Região mesial da margem posterior do cefalotórax
RBW	Largura da base do rostro

TDL	Comprimento da linha dorsal transversal
UFRGS	Universidade Federal do Rio Grande do Sul
UT	Alto Tibagi
VU	Vulneravél
WU	Largura máxima do endopodito

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1. INTRODUÇÃO GERAL

Com base em extensa pesquisa na literatura, De Grave et al. (2009) estimaram que a ordem Decapoda consiste em 233 famílias, 2.725 gêneros e 17.635 espécies, sendo 14.756 espécies existentes e 2.979 espécies fósseis. A infraordem Anomura representa em torno de 15% desta diversidade, tendo 2.470 espécies existentes e 230 espécies fósseis (De Grave et al., 2009). Apesar das relações filogenéticas entre os anomuros serem debatidas há muitos anos, estudos recentes baseados tanto em dados morfológicos como moleculares têm contribuído para decifrar essas relações (De Grave et al., 2009). McLaughlin et al. (2007), por exemplo, revisaram amplamente os caracteres morfológicos externos dos representantes da infraordem Anomura e propuseram que a mesma fosse dividida em sete superfamílias (Paguroidea, Kiwaoidea, Lomisoidea, Galatheoidea, Aegloidea, Lithodoidea e Hippoidea). Desta forma, a família Aeglidae foi elevada à classificação de superfamília (McLaughlin et al., 2007).

Aeglidae é a única família que constitui a superfamília Aegloidea (McLaughlin et al., 2010). Das 92 espécies que compõe a família Aeglidae, 90 são espécies e subespécies do gênero *Aegla* Leach, 1820 (Moraes et al., 2017; Jara et al., 2018; Páez et al., 2018; Trombetta et al., 2019; Marçal et al., 2020, 2021) e duas são fósseis. *Haumuriaegla glaessneri* Feldmann, 1984 foi descoberta em rochas marinhas na Nova Zelândia (Feldmann, 1984) e *Protaegla minuscula* Feldmann, Veja, Applegate & Bishop, 1998 foi encontrada em rochas marinhas no México (Feldmann et al., 1998). Diferentemente destas espécies fósseis, os representantes do gênero *Aegla* são exclusivamente de águas continentais do sul da América do Sul, ocorrendo nas bacias hidrográficas do Brasil, Chile, Argentina, Uruguai, Paraguai e Bolívia (Bond-Buckup & Buckup, 1994; Bond-Buckup & Buckup, 1999; Bond-Buckup, 2003).

Os registros fósseis (Feldmann, 1984; Feldmann et al., 1998) sugerem que a origem de

Aeglidae ocorreu em um ambiente marinho e posteriormente houve uma dispersão em direção ao ambiente dulcícola. Diferentes trabalhos debatem sobre qual teria sido a direção da dispersão desses crustáceos nos habitats de água doce do sul da América do Sul. Alguns pesquisadores postularam que essa dispersão ocorreu a partir do oceano Atlântico (Schmitt, 1942; Ringuelet, 1948; Morrone & Loppretto, 1994). Entretanto, estudos sobre os padrões de distribuição (Ortmann, 1902), evidências fósseis (Feldmann, 1984; Feldmann et al., 1998), biogeografia e sistemática molecular de *Aegla* (Pérez-Losada et al., 2004) dão maior suporte a hipótese de que essa dispersão ocorreu a partir do oceano Pacífico.

Os padrões de distribuição geográfica atuais de *Aegla* têm sido associados à fatores históricos, como transgressões marinhas e elevações tectônicas (Pérez-Losada et al., 2004), glaciação e a formação de refúgios glaciais (Xu et al., 2009; Oyanedel et al., 2011). Trevisan et al. (2009) associaram a distribuição de *Aegla platensis* Schmitt, 1942 e *Aegla singularis* Ringuelet, 1948 ao longo da bacia do rio Jacutinga, no estado do Rio Grande do Sul (Brasil), ao uso e ocupação do solo. Estes autores sugeriram que a predominância de *A. platensis* em nascentes de rios e pastagens e de *A. singularis* em áreas urbanas e agrícolas podem estar relacionadas à cobertura de mata ciliar, grau de erosão das margens, disponibilidade de alimento, diversidade de habitats, competição interespecífica, entre outros. Tumini et al. (2016) atestaram que as distribuições de decápodes de água doce no sul da América do Sul (Argentina) são fortemente influenciadas por variáveis ambientais locais, como altitude, estabilidade do corpo d’água, faixa anual de temperatura, pH e condutividade. Além disso, Gonçalves et al. (2018) usaram a modelagem de distribuição de espécies de *Aegla* para identificar e propor áreas prioritárias para conservação em quatro ecorregiões de água doce na América do Sul, mais especificamente no extremo sul do Brasil e em áreas adjacentes na Argentina e no Uruguai.

De acordo com a literatura, a área de distribuição de *Aegla* é limitada ao norte pelo registro de *Aegla franca* Schmitt, 1942 no município de Claraval, estado de Minas Gerais, Brasil (20°18′47″S; 47°16′37″O) e ao sul pelo relato de *Aegla alacalufi* Jara & López, 1981 na ilha de Duque de York, Chile (50°33′31″S; 75°19′17″O) (Bueno et al., 2007; Oyanedel et al., 2011). Embora algumas espécies tenham registro de ocorrência em mais de uma bacia hidrográfica (Schmitt, 1942; Bond-Buckup & Buckup, 1994; Jara & Palacios, 1999; Oyanedel et al., 2011), a maioria é conhecida por ocorrer em afluentes de um mesmo rio ou somente em sua localidade tipo (Buckup & Rossi, 1977; Jara, 1982; Rocha & Bueno, 2011; Santos et al., 2009, 2010, 2012, 2013, 2014, 2015; Moraes et al., 2016, 2017; Bueno et al., 2017; Jara et al., 2018; Páez et al., 2018; Trombetta et al., 2019; Marçal et al., 2020, 2021).

Esses anomuros ocorrem geralmente em riachos de cabeceira, com águas correntes e bem oxigenadas e são considerados bons bioindicadores da qualidade do habitat (Bond-Buckup & Buckup, 1994; Buckup & Bond-Buckup, 1999; Bortoluzzi et al., 2007; Correa-Araneda et al., 2010; Santos et al., 2017). Algumas espécies, no entanto, são habitantes obrigatórias de cavernas no sudeste do Brasil (Bueno et al., 2017). Em seu habitat natural, os eglídeos[1] podem ser encontrados embaixo de rochas, pedaços de tronco e folhas, ou seja, são animais de hábitos bentônicos (Bond-Buckup, 2003). Esses crustáceos são considerados elos importantes nas cadeias alimentares dos ecossistemas aquáticos, pois servem de alimento para diversos vertebrados (Arenas, 1976; Medina, 1998; Cassini et al., 2009) e, por serem onívoros, generalistas e oportunistas, desempenham um papel importante na ciclagem de nutrientes (Bueno & Bond-Buckup, 2004; Castro-Souza & Bueno, 2004; Santos et al., 2008; Williner, 2010; Cogo et al., 2018; Collins, 2020; Almeida et al., 2021).

Das 90 espécies e subespécies de *Aegla* descritas, 58 ocorrem no Brasil, 23 no Chile, 14 na Argentina, 4 no Uruguai, 1 no Paraguai e 1 na Bolívia (Bueno et al., 2017; Santos et al., 2017; Moraes et al., 2017; Jara et al., 2018; Páez et al., 2018; Trombetta et al., 2019; Marçal

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[1] O termo “eglídeos” será aqui utilizado para se referir exclusivamente às espécies e subespécies do gênero *Aegla*.

et al., 2020, 2021). Apesar da alta diversidade, os eglídeos provavelmente são os crustáceos decápodes de água doce mais ameaçados na América do Sul (Bueno et al., 2016). Entre as 58 espécies que ocorrem no Brasil, 39 são enquadradas em algum grau de ameaça, sendo 13 vulneráveis, 12 em perigo e 14 criticamente em perigo de acordo com critérios da IUCN (Santos et al., 2017). A distribuição restrita de muitas populações, o alto grau de endemismo e o fato de serem suscetíveis a impactos antrópicos são fatores que contribuem para esse quadro (Bond-Buckup & Buckup, 1994; Bueno et al., 2016; Santos et al., 2017). Desta forma, os eglídeos fazem parte de um contexto preocupante, em que a biodiversidade global nos ambientes de água doce tem declinado em taxas mais elevadas que em outros ambientes (Sala et al., 2000; Dudgeon et al., 2006; WWF, 2012).

Este cenário é agravado no frágil mosaico de fragmentos florestais do estado do Paraná. De acordo com um recente mapeamento de uso e cobertura do solo, apenas 29,12% dos 19.987.990,3 hectares deste estado contêm floresta nativa, a maioria cercada por pastagens ou plantações agrícolas (IAT, 2020). Além disso, esta região é a menos investigada e com menos estudos taxonômicos sobre *Aegla* no Brasil. Mesmo assim, há grande potencial para descoberta de espécies novas e ampliação da distribuição de espécies já conhecidas (Santos et al., 2015). Recentemente, *Aegla okora* Páez & Teixeira, 2018 foi descrita para a porção média do rio Iguaçu (Páez et al., 2018) e *Aegla nebeccana* Trombetta, Páez, Santos & Teixeira, 2019 para a bacia do rio Ivaí (Trombetta et al., 2019). Estudos investigando outras populações de *Aegla*, morfologicamente semelhantes, revelaram a existência de três espécies novas para as bacias do rio Tibagi (*Aegla jacutinga* Marçal & Teixeira, 2020, Marçal et al., 2020), rio das Cinzas (*Aegla buenoi* Marçal & Teixeira, 2021, Marçal et al., 2021) e rio Pirapó (Marçal et al., em preparação).

A bacia do rio Tibagi é uma das maiores bacias da drenagem do Alto Paraná no estado do Paraná, cobrindo aproximadamente 25.000 km^2 (SEMA-PR, 2010). O rio Tibagi tem uma extensão de 550 quilômetros com 65 tributários diretos e centenas de subafluentes (Maack, 2002). Os principais afluentes na margem direita são os rios Iapó, São Jerônimo e Congonhas, já na margem esquerda os rios Taquara, ribeirão dos Apertados e ribeirão Três Bocas são os maiores contribuintes (SEMA-PR, 2010). Além de possuir mais de 90 saltos e cachoeiras, o rio Tibagi apresenta características geomorfológicas distintas ao longo do seu curso (Parolin et al., 2010). A altitude, por exemplo, tem uma queda total de 792 metros, desde 1.100 metros sobre o nível do mar (m.s.n.m.) em sua nascente na Serra das Almas (Arroio da Invernada), no município de Ponta Grossa, até 298 m.s.n.m. quando deságua no reservatório da Usina Hidrelétrica de Capivara no rio Paranapanema (Maack, 2002; Parolin et al., 2010). A existência dessa diferença de altitude em seu percurso atribui à bacia um grande potencial hidroenergético (Medri et al., 2002). Na bacia do rio Tibagi existem cinco usinas hidrelétricas, Presidente Vargas e Mauá no rio Tibagi, Apucaraninha no rio Apucaraninha, Pitangui e São Jorge no rio Pitangui (Parolin et al., 2010).

Em função das variações do clima, da altitude e do tipo de solo e vegetação, a bacia do rio Tibagi pode ser dividida em três sub-regiões: alto, médio e baixo Tibagi (Anjos et al., 1997). O alto Tibagi é a região localizada mais ao sul da bacia (> 800 m.s.n.m.), onde há predomínio da vegetação do tipo estepe gramíneo-lenhosa (os Campos Gerais) e a temperatura média anual é mais baixa (Anjos et al., 1997; Torezan, 2002). No médio Tibagi, a região intermediária (800–600 m.s.n.m.), é possível encontrar a floresta ombrófila mista (ou floresta de araucária) (Torezan, 2002). Já o baixo Tibagi, a região mais ao norte (600–300 m.s.n.m.), é caracterizado pela floresta estacional semidecidual com temperatura média anual mais alta comparada à região sul da bacia (Anjos et al., 1997; Torezan, 2002).

Grande parte da bacia é ocupada por áreas de agricultura intensiva, com cultivo de soja, milho, trigo e café (SEMA-PR, 2010). O alto volume de agrotóxicos comercializados, a presença de indústrias com alto potencial contaminante e o déficit na infraestrutura urbana quanto aos sistemas de drenagem e esgoto, representam elevado risco de contaminação aos cursos de água da bacia, afetando diretamente o meio ambiente e as populações humanas (SEMA-PR, 2010). Além disso, com o intenso processo de desmatamento no estado do Paraná, a cobertura florestal nativa da bacia do rio Tibagi apresenta-se bastante fragmentada (Torezan, 2002). Atualmente, restam somente alguns remanescentes de floresta estacional semidecidual (p. ex. Parque Estadual Mata dos Godoy), de floresta ombrófila mista (p. ex. Parque Ecológico da Klabin) e de vegetação rasteira dos Campos Gerais (p. ex. Parque Estadual de Vila Velha) (Parolin et al., 2010).

Para muitas espécies de decápodes de água doce o risco de extinção é alto, devido tanto à carência de planos de conservação direcionados ao grupo, quanto ao estágio avançado de degradação dos ecossistemas límnicos (Cumberlidge et al., 2009). A necessidade de inventários e estudos taxonômicos sobre *Aegla* como uma medida de conservação da biodiversidade é evidente. Até agora, quatro espécies de eglídeos foram registradas na bacia do rio Tibagi, *Aegla castro* Schmitt, 1942 (Schmitt, 1942), *Aegla schmitti* Hobbs, 1979 (Bond-Buckup & Buckup, 1994), *Aegla lata* Bond-Buckup & Buckup, 1994 (Bond-Buckup & Buckup, 1994) e *A. jacutinga* (Marçal et al., 2020). O aumento dos esforços de pesquisa tem elevado potencial para a ampliação do número de espécies conhecidas, bem como das áreas de distribuição das espécies já descritas. Além disso, informações sobre distribuições geográficas são cruciais para avaliar o risco de extinção das espécies (Boos et al., 2012). No entanto, um fato complicador é a morfologia altamente conservadora dentro do gênero (Schmitt, 1942; Ringuelet, 1948; Martin & Abele, 1988).

Vários taxonomistas têm usado técnicas moleculares juntamente com dados morfológicos, em um panorama taxonômico integrativo, para discriminação mais precisa das espécies (Jara et al., 2003; Bond-Buckup et al., 2010; Santos et al., 2009, 2010, 2012, 2013, 2015; Moraes et al., 2016; Jara et al., 2018; Páez et al., 2018; Trombetta et al., 2019; Marçal et al., 2020, 2021). Segundo Hillis (1987), as técnicas moleculares podem revelar níveis de diferenciação genética escondidos em populações morfologicamente semelhantes. *Aegla paulensis* Schmitt, 1942, por exemplo, foi reportada como um complexo com sete espécies crípticas (Santos et al., 2015; Moraes et al., 2016). A população da bacia do Ribeira de Iguape foi descrita como *Aegla lancinhas* Bond-Buckup & Buckup, 2015 em Santos et al., 2015, e quatro populações da bacia do Tietê foram descritas como novas espécies (Moraes et al., 2016).

Existem vários outros exemplos de espécies crípticas dentro dos eglídeos. Moraes et al. (2017) descreveram *Aegla quilombola* Moraes, Tavares & Bueno, 2017 a partir de uma das populações anteriormente conhecida como *Aegla marginata* Bond-Buckup & Buckup, 1994. Crivellaro et al. (2017) analisaram 17 populações atribuídas a *Aegla longirostri* Bond-Buckup & Buckup, 1994, usando dois métodos de delimitação de espécies, e identificaram pelo menos 14 potenciais espécies. Jara et al. (2018) descreveram *Aegla chilota* Jara, Pérez-Losada & Crandall, 2018 a partir de uma população designada preliminarmente como *Aegla araucaniensis* Jara, 1980. Espécies crípticas também foram relatadas entre as populações de *A. platensis* e *A. uruguayana* Schmitt, 1942 (Zimmermann et al., 2018, 2021). Além disso, *A. jacutinga* foi descrita a partir de populações morfologicamente semelhantes a *A. lata* (Marçal et al., 2020).

Uma técnica molecular comumente utilizada na identificação de espécies é o DNA barcode, em que cada táxon seria identificado por ter uma sequência única de DNA (Hebert et al., 2003). A região barcode do gene Citocromo c Oxidase subunidade I (COI) têm sido usada eficazmente na discriminação de lepidópteros (Hebert et al., 2003; Janzen et al., 2005), pássaros (Hebert et al., 2004), aranhas (Barrett & Hebert, 2005), formigas (Smith et al., 2005) al., 2005), peixes (Ward et al., 2005; Pereira et al., 2011, 2013; Souza-Shibatta et al., 2018), crustáceos (Lefébure et al., 2006; Costa et al., 2007; Havermans et al., 2011; Matzen da Silva et al., 2011) e outros.

O método clássico de distância de pares baseado no modelo Kimura-2-Parâmetros (K2P) é a abordagem predominante usada para analisar os padrões de diversidade com a região barcode do COI (Matzen da Silva et al., 2011). Este método pressupõe que a distância genética entre indivíduos da mesma espécie (divergência intraespecífica) é menor do que a variação entre espécies diferentes (divergência interespecífica) (Hebert et al., 2004; Ribolli et al., 2017). Assumindo um limiar intraespecífico de no máximo 2%, Matzen da Silva et al. (2011) tiveram em torno de 98% de sucesso no reconhecimento de decápodes marinhos. Lefébure et al. (2006) analisaram sequências COI de Aeglidae com outras 17 famílias diferentes de Crustacea e propuseram um limiar molecular para ajudar na delimitação de espécies. No entanto, esse limiar foi calculado excluindo as sequências do gênero *Aegla* da análise. Desta forma, nenhum estudo analisou distâncias genéticas e avaliou a eficácia da região barcode do COI para delimitar eglídeos.

Este trabalho de tese foi desenvolvido com o objetivo de contribuir com o conhecimento da riqueza, distribuição geográfica e taxonomia dos crustáceos de água doce do gênero *Aegla*. Dentre as perguntas desta tese estão:

1. As diversas populações morfologicamente semelhantes à *Aegla lata*, coletadas dentro de sua ampla área de distribuição, constituem espécies crípticas? (Capítulo 1)
2. Qual a riqueza de *Aegla* na bacia do rio Tibagi e qual a distribuição e grau de endemismo destas espécies? (Capítulo 2)
3. A análise morfológica permite a distinção segura entre espécies morfologicamente semelhantes? (Capítulo 2)
4. A região barcode do COI permite discriminar com eficácia as espécies de *Aegla*? (Capítulo 3)

5. O limiar de distância genética de 2% pode ser aplicado para a delimitação de espécies de *Aegla*? (Capítulo 3)

Os resultados deste trabalho foram divididos em três capítulos em formato de artigo. O primeiro intitulado "Cryptic diversity among populations of *Aegla* Leach, 1820 (Decapoda: Anomura: Aeglidae) from Tibagi River basin, Paraná state, Brazil, with descriptions of three new species" foi submetido à revista *Journal of Natural History*. O segundo capítulo intitulado "Diversity and geographical distribution of aeglids crabs (Crustacea, Anomura, Aeglidae) from Tibagi River basin, Paraná state, Brazil" encontra-se nas normas da revista *Zoological Journal of the Linnean Society*. O terceiro intitulado "The effectiveness of DNA barcoding as a tool in *Aegla* diversity (Crustacea, Aeglidae)" foi submetido à revista *Zoologica Scripta*. A fim de facilitar a compreensão e permitir uma leitura contínua, dispomos as figuras e tabelas próximas ao local em que foram citadas pela primeira vez no texto, diferindo das normas de submissão.

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2. CAPÍTULO 1

CRYPTIC DIVERSITY AMONG POPULATIONS OF *AEGLA* LEACH, 1820 (DECAPODA: ANOMURA: AEGLIDAE) FROM TIBAGI RIVER BASIN, PARANÁ STATE, BRAZIL, WITH DESCRIPTIONS OF THREE NEW SPECIES

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ABSTRACT

Freshwater crustaceans of the genus *Aegla* are endemic to southern South America. In Brazil, the greatest diversity of species of the aeglid species occurs in river basins in the Rio Grande do Sul state. Nevertheless, recent studies have shown that the potential for discovering new species of *Aegla* in the Paraná state is huge. In recent years, for example, seven new species have been described for the state of Paraná. Furthermore, there is a need to solve problems related to cryptic species and refine taxonomic information about the group. Here we expand our current knowledge of the freshwater biodiversity of *Aegla* in the region by describing three new species. *Aegla* sp. n. 1, *Aegla* sp. n. 2, and *Aegla* sp. n. 3 were discriminated based on morphological and molecular evidence. Particular morphological characters of the carapace, chelipeds, epimeron and uropods distinguished the new taxa. Molecular results also support the separation of the new species from the closely related species *A. jacutinga*, *A. castro* and *A. lata*. The new species have limited distribution and are categorized as endangered (*Aegla* sp. n. 1) and vulnerable (*Aegla* sp. n. 2 and *Aegla* sp. n. 3) according to International Union for Conservation of Nature Red List Categories and Criteria.

Keywords: COI gene; morphology; species delimitation; taxonomy; threatened species

INTRODUCTION

Freshwater crustaceans of the genus *Aegla* Leach, 1820 are endemic to southern South America (Schmitt 1942). These anomuran crabs usually occur in headwater streams with running and well-oxygenated waters, being quite susceptible to environmental disturbances and therefore considered good bioindicators of water quality (Bond-Buckup and Buckup 1994; Bortoluzzi *et al*. 2007; Correa-Araneda *et al*. 2010; Santos *et al*. 2017). Of the 90 described species, approximately 70% are under some level of threat of extinction (Moraes *et al*. 2017; Santos *et al*. 2017; Jara *et al*. 2018). The conservation of this fauna, therefore, must be considered of extremely high priority.

This scenario is potentially aggravated in the fragile mosaic of forest fragments in the Paraná state, southern Brazil. According to a recent mapping of land use and coverage, only 29.12% of the 19,987,990.3 hectares of this state contains native forest, most of which is surrounded by pastures or agricultural plantations (IAT 2020). Moreover, this region is the least explored and has been subject to the fewest taxonomic studies on *Aegla* in Brazil. Even so, recent work suggests that the potential to discover new species of *Aegla* in Paraná state is huge (Santos *et al*. 2015). Notably, seven new species were described in the last 6 years: *Aegla meloi* Bond-Buckup and Santos, 2015 (Santos *et al*. 2015), *Aegla loyolai* Bond-Buckup and Santos, 2015 (Santos *et al*. 2015), *Aegla lancinhas* Bond-Buckup and Buckup, 2015 (Santos *et al*. 2015), *Aegla okora* Páez and Teixeira, 2018 (Páez *et al*. 2018), *Aegla nebeccana* Trombetta, Páez, Santos and Teixeira (Trombetta *et al*. 2019), *Aegla jacutinga* Marçal and Teixeira, 2020 (Marçal *et al*. 2020) and *Aegla buenoi* Marçal and Teixeira, 2021 (Marçal *et al*. 2021) – the penultime species described for the Tibagi River basin.

The Tibagi River basin is one of the largest basins of the Upper Paraná drainage in the Paraná state, covering approximately 25,000 km^2 (SEMA-PR 2010). Its main river starts in the Serra das Almas, Ponta Grossa city, and flows into the Paranapanema River (Maack 2002; Parolin *et al*. 2010). According to Pérez-Losada *et al*. (2009), the rivers located in the Paranapanema River basin, in the Upper Paraná region, support high biodiversity of aeglids compared with other rivers from the same ecoregion. In the Tibagi River basin, for example, four species of aeglids were recorded until now, namely *Aegla castro* Schmitt, 1942 (see Schmitt 1942), *Aegla schmitti* Hobbs, 1979 (see Bond-Buckup and Buckup 1994), *Aegla lata* Bond-Buckup and Buckup, 1994 (see Bond-Buckup and Buckup 1994) and *A. jacutinga* (see Marçal *et al*. 2020).

Since 1991 the Museu de Zoologia of the Universidade Estadual de Londrina (MZUEL) has made a relevant collection of freshwater decapod crabs of Paraná state, primarily Aeglidae crabs. Some of this material has already been studied by Galves *et al*. (2007), Silva *et al*. (2017), Marçal *et al*. (2018), Páez *et al*. (2018), Chaves *et al*. (2019), Trombetta *et al*. (2019), Marçal *et al*. (2020), Almeida *et al*. (2021) and Silva *et al*. (2021). Galves *et al*. (2007) recorded the occurrence of *A. lata* in two streams in the city of Londrina, in the northern region of Paraná state. As a result, the distribution area of the species, which was previously known only for the type locality in the city of Ponta Grossa, was expanded. Within this wide area of distribution, there are diverse populations morphologically similar to *A. lata*. However, some of these specimens probably constitute cryptic species. *Aegla jacutinga*, for example, was described from two populations collected in sub-tributaries that flow into the left bank of the Tibagi River, confirming this assumption (Marçal *et al*. 2020). Surprisingly, *A. lata* is considered 'critically endangered' (Santos *et al*. 2017), and is highly likely to be extinct in its type locality (Pérez-Losada *et al*. 2009). Thus, the discovery of cryptic species in the *A. lata* distribution area, as well as the confident identification of other populations of *A. lata*, plays a critical role in the conservation assignment and maintenance of aeglid diversity. Here, we present the description of three new species of *Aegla* from the Tibagi River basin, Paraná state, Brazil. Both morphological and molecular approaches were used to compare and delimit species. We also evaluate the conservation status of the new species according to the criteria established by the International Union for Conservation of Nature (IUCN 2019).

MATERIAL AND METHODS

Sampling

Specimens of the new species were collected in watercourses of the Tibagi River basin (Figure 1) during 16 sampling campaigns. To obtain fresh material for molecular analysis, additional collections were carried out at the type localities. Submerged pebbles and small rocks were manually removed to dislodge aeglids from their hidings and to subsequently be captured by a hand net (90 cm diameter, 4 mm mesh size) immediately positioned and against the water flow. Ten sampled specimens per locality were placed on crushed ice to be anesthetised. Then, abdominal muscle tissue was removed and fixed in absolute alcohol for DNA extraction. The remaining specimens were preserved in 70% alcohol for morphological analysis. All sampled specimens are deposited in the Crustacean Collection of MZUEL.

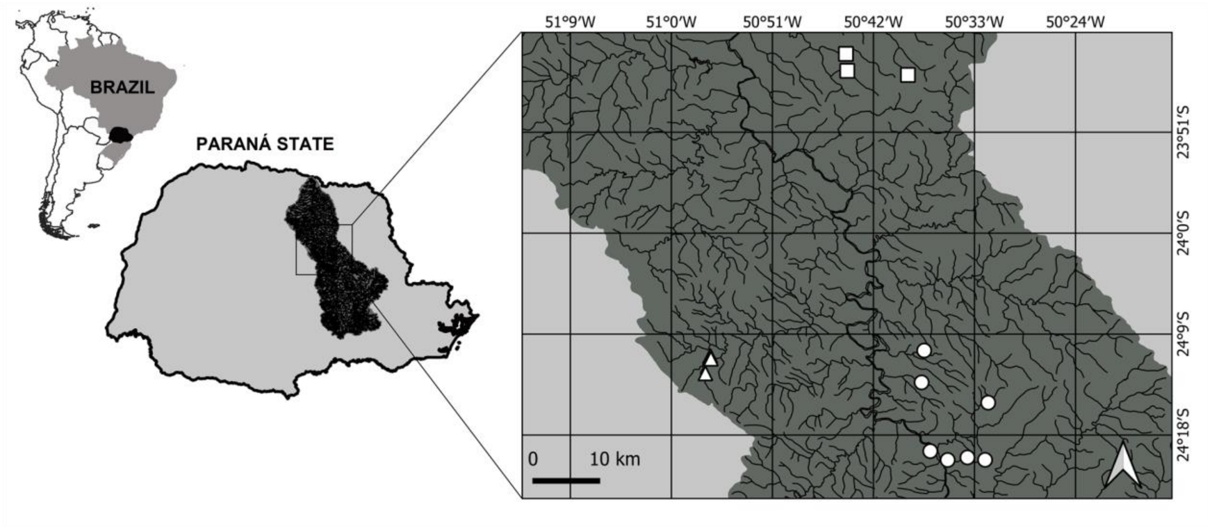

Figure 1. Map showing the distribution of *Aegla* sp. n. 1 (squares), *Aegla* sp. n. 2 (triangles) and *Aegla* sp. n. 3 **(**circles) in the Paraná state, Brazil. The dark gray area indicates the Tibagi River basin.

Morphological Analysis

The descriptions are based on morphological characters of the holotypes. The variations are based on examination of the paratypes. The morphological terms used in the descriptions follow Martin and Abele (1988), Bond-Buckup and Buckup (1994), and Moraes *et al*. (2016). We adopted the methods of Bond-Buckup and Buckup (1994) and Moraes *et al*. (2016) to take the measurements with a calliper (0.01 mm precision). The following abbreviations are employed in the text: AAD, areolar anterior demarcation; AH, areolar height; APM, areolar posterior margin; CL, carapace length including rostrum; CLE, carapace length excluding rostrum; CW, carapace width; HWT, half the maximum width of telson; LMR, lateral margin of rostrum; PCW, pre-cervical width; PMC, mesial region of the posterior margin of the cephalothorax; RBW, rostral base width; TDL, transversal dorsal linea length; WU, width of uropod. The shape of the rostrum, areola, cardiac area, and uropods was determined through morphometric relationships, according to Moraes *et al*. (2016). The eyestalks were positioned in parallel alignment with the rostrum to determine the extent of the apex of the anterolateral spine relative to the basal margin of the cornea (Moraes *et al*. 2016). We used a stereomicroscope equipped with a program for stacking images and a digital camera with a 4 megapixel frame to take photographs.

Molecular Analysis

Genomic DNA was extracted from preserved tissues following the method described by

Shiozawa *et al*. (1992), with some modifications. Approximately 100 mg of tissue was minced with scissors into a micro-centrifuge tube containing 200 µl of TE9 buffer (100 µl Tris [pH 8.0], 71.6 µl H_2O, 8 µl 0.5 M EDTA, 0.4 µl 5 M NaCl, 20 µl SDS). Soon after, 5 µl of proteinase K (Invitrogen) was added to each sample and they were incubated for 4 hours at 63 °C. Following incubation, the DNA was purified by successive washes with equal volumes of phenol, chlorophane (1 phenol: 1 chlorophyll), and chlorophyll (24 chloroform: 1 isoamyl alcohol). The DNA was precipitated with 10% of 3 M NaCl and 2 ½ of the volume of absolute ethanol and stored at -20 °C for 12 hours. After that, the DNA was washed with 70% ethanol and resuspended in 200 µl of Tris-EDTA buffer. The region of the mitochondrial cytochrome c oxidase subunit I (COI, ± 600 bp) gene was amplified using primers COI-f and COI-r (van Syoc 1995), following the PCR conditions proposed by Pérez-Losada *et al*. (2002). PCR products were visualized on a 0.8% agarose gel, stained with SYBR® Safe DNA Gel Stain (Life Technologies), following the manufacturer's protocol, and purified using ExoSAP IT® (Prodimol Biotecnologia). The samples were then sequenced bidirectionally in independent reactions and the product of the sequencing reactions was analyzed in an ABI 3500 XL automatic sequencer (Applied Biosystems Inc., CA, USA) using ABI Big Dye Terminator v 3.1. The quality of the sequences was verified using the Eletropherogram Quality Analysis software (Togawa and Brigido 2003), also used to produce consensus sequences between forward and reverse. The sequences generated in this study were deposited in Bold Systems under access numbers AEGLA003-21, AEGLA004-21, AEGLA005-21, AEGLA006-21, AEGLA009-21 and AEGLA010-21. These sequences were aligned and edited in the MEGA v. 6 software (Tamura *et al*. 2013), together with other sequences of the *Aegla* species that are morphologically similar and that occur in the Tibagi River basin. The sequences included in the analyses were retrieved from GenBank or the Bold database (*Aegla odebrechtii* Müller, 1876: AY595571, AY595572; *A. castro*: AY595561, AY595586; *A. strinatii* Türkay, 1972: AY595560, AY595640; *A. perobae* Hebling and Rodrigues, 1977: AY595587, AY595588; *A. schmitti*: AY595563, AY595583; *A. lata*: AEGPR001-18, AEGPR002-18; *A. jarai* Bond-Buckup and Buckup, 1994: AY595581, AY595595; *A. jacutinga*: AEGPR006-18, AEGPR021-19). Intraspecific and interspecific genetic distances were calculated based on the Kimura-two-parameter model (Kimura 1980). To test the hypothesis of new species, an *a posteriori* species delimitation strategy, based on the topology of phylogenetic trees, was used. The Generalized Mixed Yule Coalescent (GMYC) method was performed in the Splits package (Pons *et al*. 2006; Fujisawa and Barraclough 2013) with the R 3.5.1 program, from an ultrametric tree. Using this method it is possible to calculate the number of clusters, classifying the bifurcation rates of a phylogram as resulting from the processes of interspecific or intraspecific ramifications of the lineages (Pons *et al*. 2006; Fujisawa and Barraclough 2013). The ultrametric tree was obtained via Bayesian inference in the BEAST v. 2.6.2 program (Bouckaert *et al*. 2019) with a strict molecular clock and a birth-death model (Heled and Drummond 2010). The data were analyzed as a single partition and the evolutionary model used was HKY+I+G (proportion of invariant sites = 0.620; gamma shape = 0.629), previously specified by the jModelTest2 (Darriba *et al*. 2012). The analyses were carried out for 10 million generations, sampling a tree every 1000 generations. The TRACER v. 1.5 (BEAST package) was used to verify the subsequent probability of sufficient minimum effective size (ESS> 200) and a significant estimate of the parameters and the average age of the nodes, considering a burn-in of 25 million generations. The trees were summarized and visualized using the programs TREEANNOTATOR 1.5.3 and FIGTREE 1.3.1, respectively (Drummond and Rambaut 2007; Rambaut 2009).

RESULTS

Family Aeglidae Dana, 1852

***Aegla* sp. n. 1** Marçal and Teixeira

(Figures 2–5)

urn:lsid:zoobank.org:act:A3164F39-FA92-479B-8A0D-7D947309D8F2

Holotype

Male [CLE 19.9 mm], Brazil, Paraná, São Jerônimo da Serra, Paranapanema River basin, Tibagi River sub-basin, Tigre River, 23°44′00.50″S, 50°44′26.74″W, altitude 836 m, F.C. Jerep, E. Santana and A. de Souza coll., 9 January 2019 (MZUEL 513).

Paratypes

5 females [CLE 14.9–16.5 mm], same data as holotype (MZUEL 487). 3 males [CLE 14.5–25.6 mm] and 6 females [CLE 14.2–16.8 mm], Brazil, Paraná, São Jerônimo da Serra, Paranapanema River basin, Tibagi River sub-basin, Tigre River, 23°45′53.90″S, 50°38′55.49″W, altitude 1034 m, G.M. Teixeira and I.C. Marçal coll., 17 March 2020 (MZUEL 502).

Additional material examined

2 males [size not recorded] and 7 females [size not recorded], Brazil, Paraná, São Jerônimo da

Serra, Paranapanema River basin, Tibagi River sub-basin, Tigre River, 23°45′53.90″S, 50°38′55.49″W, altitude 1034 m, G.M. Teixeira and I.C. Marçal coll., 17 March 2020 (MZUEL 507, genetic vouchers: Bold Systems access AEGLA009-21, AEGLA010-21). 4 males [CLE 12.8–16.3 mm] and 2 females [CLE 14.1–14.9 mm], Brazil, Paraná, São Jerônimo da Serra, Paranapanema River basin, Tibagi River sub-basin, Pilões stream, 23°45'31.25"S, 50°44'19.70"W, altitude 912 m, F.C. Jerep, E. Santana and A. de Souza coll., 1 August 2018 (MZUEL 465).

Diagnosis

Rostrum triangular, narrow base, extending beyond distal apex of compound eyes, carinate along entire length. Subrostral process well developed, occupying proximal third of subrostral margin, anterior and posterior margins forming obtuse angle. Orbital and extra-orbital sinuses deep. Anterolateral spines not reaching basal margin of cornea. Protogastric lobes pronounced. Cervical groove U-shaped. Areola trapezoidal. Cardiac area trapezoidal. Epibranchial area strongly elongated. Palmar crest disciform, outer surface excavated, margin poorly serrated. Subterminal lobe of carpus well defined, pointed. Carpal ridge high on outer surface of carpus. Ventromesial border of cheliped ischium with 4 or 5 tubercles. Anteromesial region of third thoracic sternite tapered. Rudimentary pleopods in adult male specimens. Anterolateral angle of second abdominal epimeron unarmed. Anterior margin of second abdominal epimeron almost straight. Uropods wide.

Description of holotype

Carapace moderately convex, gastric region convex, dorsal surface scabrous, covered with punctations, anterior and posterior branchial areas expanded laterally (CW/CLE = 1.01) (Figure 2A). Rostrum triangular, narrow base (RBW/LMR = 0.91), extending beyond distal apex of compound eyes, carinate along entire length, small corneous scales on lateral margins and tip; ventral portion of rostrum much higher than dorsal in profile. Rostral carina beginning at level of protogastric lobes, with one row of corneous scales extending next to apex. Subrostral process well developed, occupying proximal third of subrostral margin, tip rounded, anterior and posterior margins forming obtuse angle (131°) (Figure 3A).

Eyestalk and cornea well developed. Orbital and extra-orbital sinuses deep. Orbital sinus U-shaped. Orbital spines well developed, with small terminal corneous scale. Anterolateral spines acuminate apically with small corneous scales terminally, not reaching basal margin of cornea.

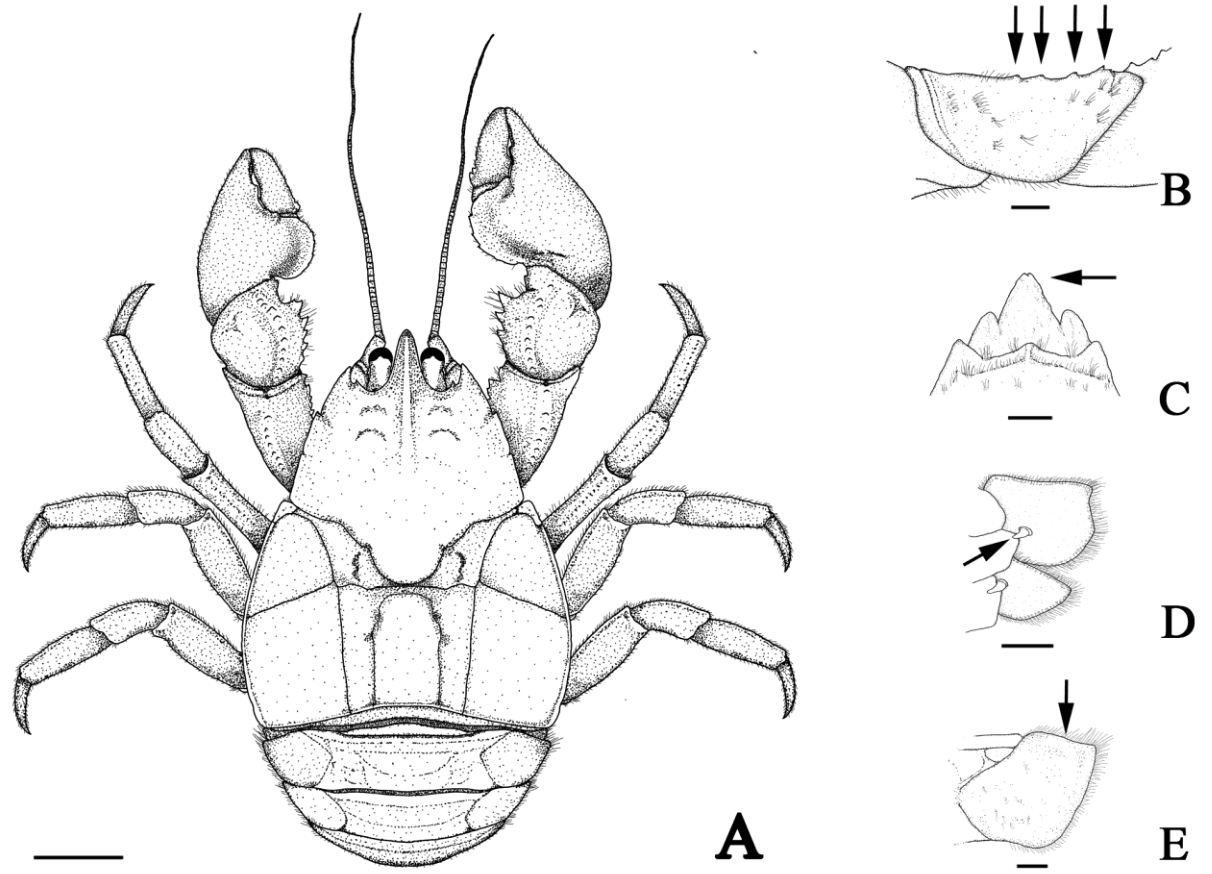

Figure 2. *Aegla* sp. n. 1, male holotype, CLE 19.9 mm, Tigre River, São Jerônimo da Serra, Paraná, Brazil (MZUEL 513). (a) dorsal view of the cephalothorax and anterior portion of the abdomen. (b) ventral view of ischium of minor cheliped (left) showing four tubercles (arrows). (c) third thoracic sternite tapered (arrow). (d) ventral view of pleon showing rudimentary pleopods (arrow). (e) dorsal view of second abdominal epimeron (right side) showing anterior margin almost straight (arrow). Scale bars: a = 5.0 mm; b−e = 1.0 mm.

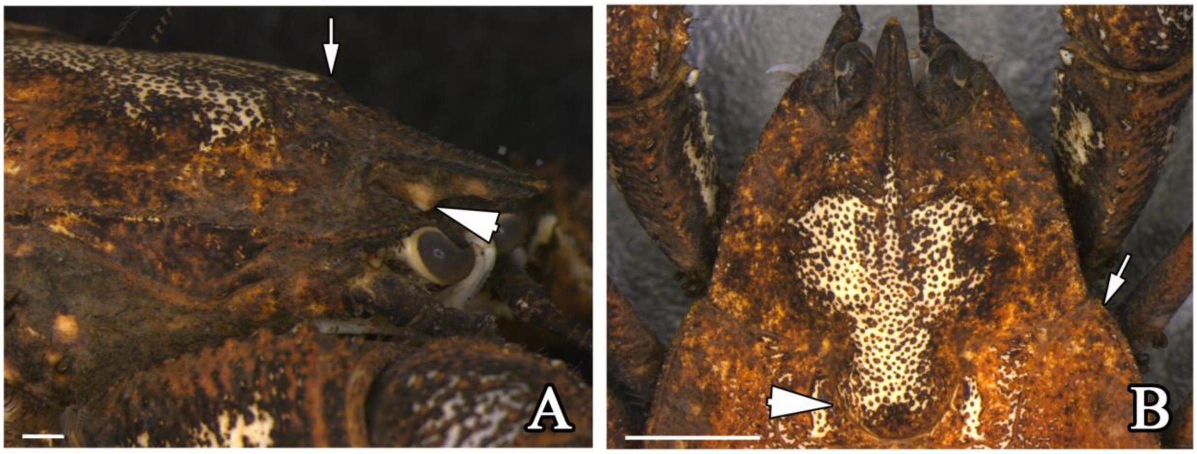

Figure 3. *Aegla* sp. n. 1, male holotype, CLE 19.9 mm (MZUEL 513). (a) external surface view of the anterior portion of the cephalothorax showing pronounced protogastric lobes (thin arrow) and well-developed subrostral process (thick arrow). (b) dorsal view of the anterior region of the cephalothorax showing U-shaped cervical groove (thick arrow) and strongly elongated epibranchial area (thin arrow). Scale bars: a = 1.0 mm; b = 5.0 mm.

Epigastric prominences pronounced, with corneous scales. Protogastric lobes pronounced, with corneous scales (Figure 3A). Gastric area elevated in relation to hepatic lobes and rostrum in lateral view. Demarcation between the first and the second hepatic lobe well defined, between the second and the third hepatic lobe weakly delimited. Lateral margins of hepatic lobes with small corneous scales and small setae.

Cervical groove U-shaped (Figure 3B). Transverse dorsal linea sinuous along its extension. Areola trapezoidal (APM/AAD = 1.95). Cardiac area trapezoidal (TDL/PMC = 1.39). Epibranchial area strongly elongated, anterolateral angle with corneous scale, lateral margin with row of corneous scales and small setae (Figure 3B). Lateral margins of anterior and posterior branchial areas with row of corneous scales and small setae.

Chelipeds unequal. Right cheliped largest (Figures 4A, B). Dactylus: dorsal margin and outer surface with small corneous scales and small setae, inner surface with setal tufts and scales. Proximal dorsal margin with moderate-sized lobe. Cutting margin with well-developed lobular basal tooth, followed by row of corneous scales to distal end. Row of small tufts of long setae next to cutting margin. Pre-dactylar lobe well developed, rounded, without corneous scales. Propodus: outer surface granular, globose aspect. Palmar crest disciform with outer surface excavated, margin poorly serrated, covered by corneous scales. Cutting margin of fixed finger with well-developed lobular basal tooth, followed by row of corneous scales to distal end. Inner and outer surfaces of fixed finger with rows of long setal tufts next to cutting margin. Carpus: dorsal margin with 2 proximal tubercles, median spine, 2 distal spines, each tubercle or spine with small setae and terminal corneous scale, subterminal lobe well defined, pointed, with small corneous scales and setae. Inner surface with 3 tubercles, long setae next to dorsal margin. Outer surface with carpal ridge high, with small corneous scales. Merus: dorsolateral edge with distal tubercle, with corneous scale terminally, followed by row of tubercles decreasing in size proximally. Ventromesial edge with 2 distal spines, with corneous scale terminally, followed by 4 tubercles of similar size, with corneous scale. Ventrolateral border with distal tubercle, with terminal corneous scale, followed by several small tubercles proximally. Ischium: dorsolateral edge with spine, with corneous scale terminally. Ventromesial border with proximal tubercle, 2 small median tubercles and large distal tubercle, each with terminal corneous scale. Ventrolateral border smooth.

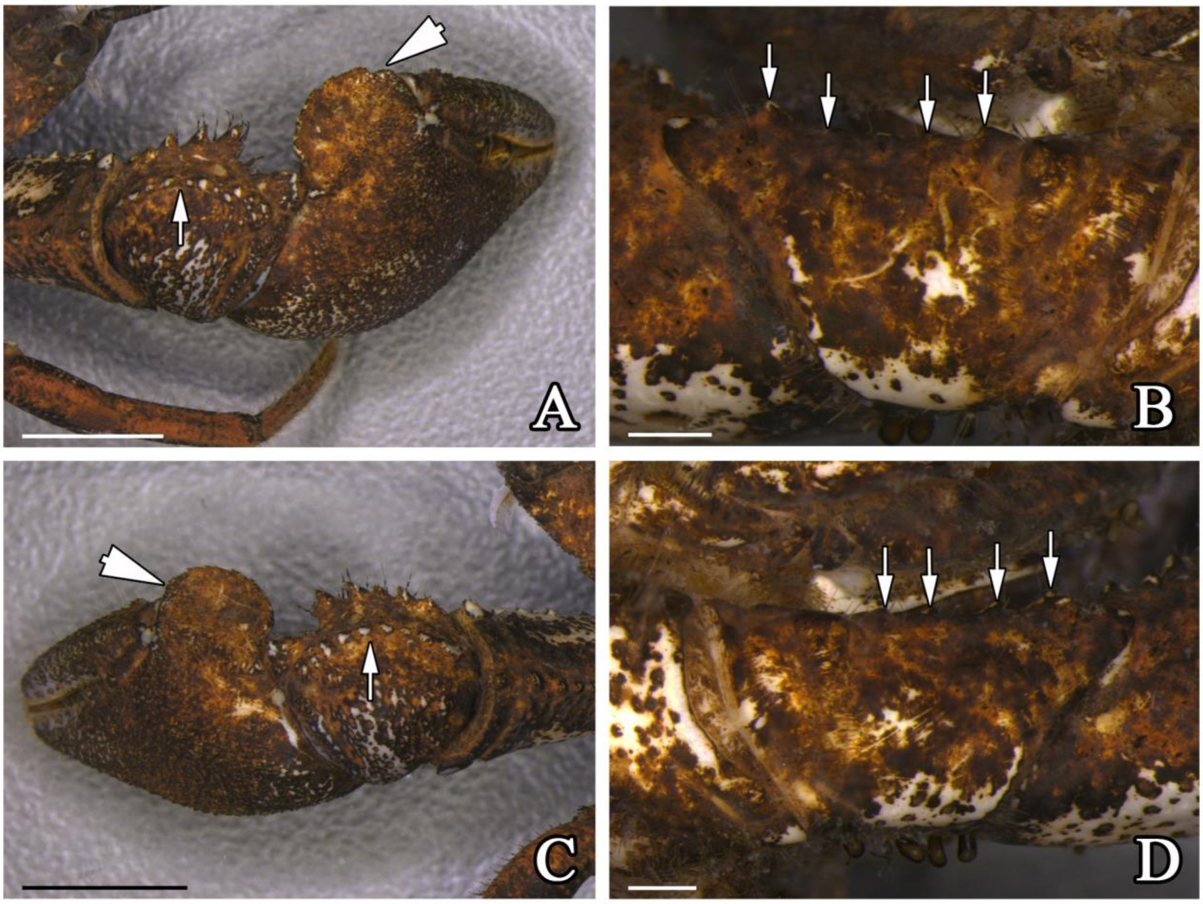

Figure 4. *Aegla* sp. n. 1, male holotype, CLE 19.9 mm (MZUEL 513). (a) dorsal view of the dactylus, propodus, and carpus of major cheliped (right) showing disciform palmar crest (thick arrow) and high carpal ridge (thin arrow). (b) ventral view of ischium of major cheliped (right) with four tubercles (arrows). (c) dorsal view of the dactylus, propodus, and carpus of minor cheliped (left) showing disciform palmar crest (thick arrow) and high carpal ridge (thin

arrow). (d) ventral view of ischium of minor cheliped (left) with four tubercles (arrows). Scale bars: a, c = 5.0 mm; b, d = 1.0 mm.

Minor cheliped (left) similar to major cheliped except as noted hereafter (Figures 4C, D). Dactylus: cutting margin with rudimentary lobular basal tooth. Propodus: cutting margin with rudimentary lobular basal tooth. Carpus: dorsal margin with proximal tubercle, 3 median spines, distal spine, double-tipped, each tubercle or spine with terminal corneous scale. Inner surface with 5 tubercles, corneous scale terminally. Merus: ventromesial edge with distal tubercle, double-tipped, followed by 5 tubercles, each with terminal corneous scale. Ischium (Figure 2B): ventromesial border with 4 tubercles, each with terminal corneous scale, distal tubercle double-tipped.

Second, third and fourth pereiopods similar. Dactyli, propodi, carpi, meri and ischii with several rows of setal tufts and small scales on surface. Carpi and meri with row of small tubercles with terminal corneous scale along dorsal margin. Meri with row of small tubercles with terminal corneous scale along ventral margin. Meri and ischii with long setae concentrated along dorsal margin.

Anteromesial region of third thoracic sternite tapered, projecting between coxae of third maxillipeds, with 2 terminal small corneous scales and scattered setae (Figure 2C). Fourth thoracic sternite with anterolateral angles produced anteriorly, with scattered setae (Figure 5A). Pleopods 2–5 rudimentary (Figure 2D). Anterolateral angle of second abdominal epimeron well defined, unarmed (Figure 5B). Ventral angles of third and fourth abdominal epimeron well defined, fourth abdominal epimeron with corneous scales apically. Anterior margin of second abdominal epimeron almost straight (Figures 2E, 5B). Uropods well developed, wide (WU/HWT = 1.06). Telson divided by longitudinal suture. Anterolateral and posterolateral margins well differentiated.

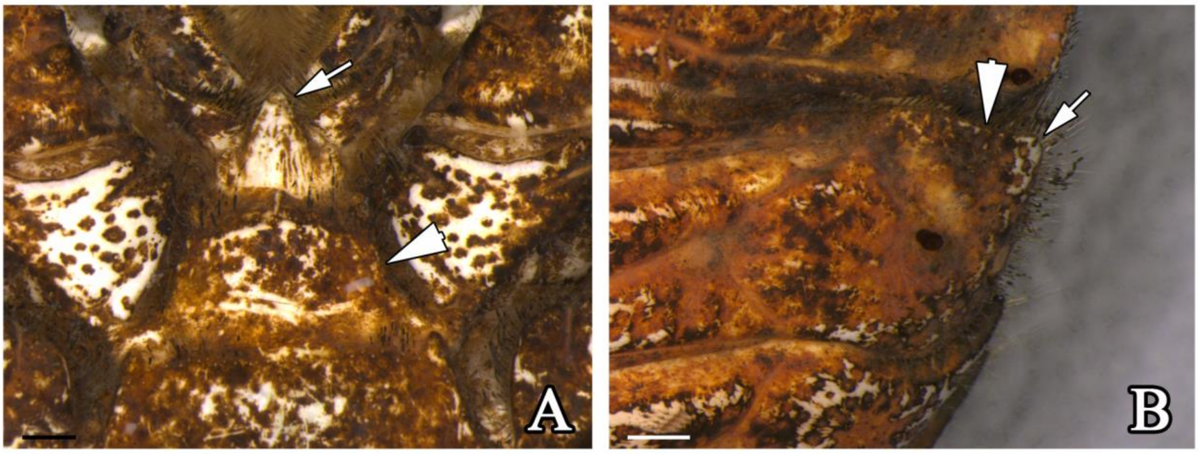

Figure 5. *Aegla* sp. n. 1, male holotype, CLE 19.9 mm (MZUEL 513). (a) third (thin arrow) and fourth (thick arrow) thoracic sternites. (b) dorsal view of second abdominal epimeron (right side) showing almost straight anterior margin (thick arrow) and unarmed anterolateral angle (thin arrow). Scale bars = 1.0 mm.

Variations

The epigastric prominences may be poorly pronounced in some paratypes. The areola can be trapezoidal (n = 9), rectangular (n = 3) or subrectangular (n = 2). The cardiac area varies from trapezoidal (n = 11) to subrectangular (n = 3). The proximal lobe on the dorsal margin of the dactylus of the major and minor chela may be less developed than in the holotype. The palmar crest is rectangular instead of disciform in female paratypes. The ventromesial border of ischium may present 5 tubercles instead of 4 as in the holotype. In some specimens, the ventral angle of the fourth abdominal epimeron is devoid of corneous scales. The uropods are narrow in a few specimens (n = 4). All measurements are summarized in Table 1.

Distribution

The new species has been collected at two watercourses within the Tibagi River sub-basin and the Paranapanema River basin. These watercourses are located in the city of São Jerônimo da Serra, Paraná state, Brazil.

Conservation status

The threatened category ‘Endangered’ (EN) is herein suggested for *Aegla* sp. n. 1 under criteria B1, B2ab(iii) as defined by the IUCN (2019). *Aegla* sp. n. 1 has been recorded from two localities only (B2a), the known extent of occurrence is estimated to be less than 100 km^2 (B1), and these sites of occurrence are in agricultural and urbanized areas (B2b(iii)).

Table 1. Measurements (mm) and morphometric relationships of the type material of the new *Aegla* species. The measurements of the paratypes are shown as mean (standard deviation). AAD, areolar anterior demarcation; AH, areolar height; APM, areolar posterior margin; CL, carapace length including rostrum; CLE, carapace length excluding rostrum; CW, carapace width; HWT, half the maximum width of telson; LMR, lateral margin of rostrum; PCW, pre-cervical width; PMC, mesial region of the posterior margin of the cephalothorax; RBW, rostral base width; TDL, transversal dorsal linea length; WU, width of uropod.

	Aegla sp. n. 1		*Aegla* sp. n. 2		*Aegla* sp. n. 3	
	Holotype	Paratypes (n = 14)	Holotype	Paratypes (n = 13)	Holotype	Paratypes (n = 12)
CL	23.5	19.50(3.29)	19.6	18.25(3.82)	23.1	18.86(2.63)
CLE	19.9	16.50(2.80)	16.2	15.22(3.16)	19.3	15.77(2.12)
CW	20.0	16.97(2.95)	15.7	15.04(3.22)	20.0	16.03(2.18)
PCW	14.1	12.09(1.89)	11.8	11.15(2.13)	14.3	11.54(1.59)
RBW	3.9	3.09(0.43)	3.1	3.10(0.54)	4.1	3.24(0.44)
LMR	4.3	3.37(0.50)	3.9	3.45(0.75)	4.4	3.59(0.57)
APM	3.9	3.11(0.52)	3.0	2.97(0.53)	3.6	3.03(0.37)
AAD	2.0	1.79(0.26)	1.9	1.90(0.41)	1.8	1.56(0.27)
AH	6.9	5.72(1.11)	5.4	5.14(1.13)	7.0	5.48(0.77)
TDL	7.9	6.56(0.97)	6.4	6.15(1.10)	7.3	6.13(0.84)
PMC	5.7	4.74(0.77)	4.6	4.67(0.84)	5.9	4.57(0.56)
WU	3.6	3.05(0.51)	2.8	2.81(0.60)	3.6	2.98(0.35)
HWT	3.4	3.09(0.49)	2.7	2.75(0.52)	3.4	2.84(0.45)
CW/CLE	1.01	1.03(0.02)	0.97	0.99(0.03)	1.04	1.02(0.03)
PCW/CW	0.71	0.71(0.02)	0.75	0.74(0.02)	0.72	0.72(0.02)
RBW/LMR	0.91	0.92(0.05)	0.79	0.91(0.06)	0.93	0.91(0.06)
APM/AAD	1.95	1.75(0.19)	1.58	1.58(0.18)	2.00	1.96(0.20)
AH/[(APM+AAD)/2]	—	2.34(0.14)	2.20	2.12(0.19)	—	2.44(0.30)
TDL/PMC	1.39	1.39(0.07)	1.39	1.32(0.06)	1.24	1.35(0.14)
WU/HWT	1.06	0.99(0.06)	1.04	1.02(0.06)	1.06	1.06(0.10)

Remarks

The disciform palmar crest of *Aegla* sp. n. 1 is shared with *A. odebrechtii*, *A. castro, A. strinatii*, *A. schmitti*, *A. lata*, *A. jarai*, *Aegla jaragua* Moraes, Tavares and Bueno, 2016 and *A. jacutinga*. Notwithstanding, the new species can be differentiated from its congeners in having: subrostral process well developed (undeveloped in *A. strinatii*); orbital and extra-orbital sinuses deep (shallow in *A. odebrechtii*); protogastric lobes pronounced (poorly pronounced in *A. odebrechtii*, *A. lata*, *A. jarai* and *A. jaragua*); subterminal lobe of carpus well defined, pointed (poorly defined in *A. strinatii*); outer surface of carpus with carpal ridge high (low in *A. jacutinga*); dorsal margin of carpus and merus of second pereiopods with small tubercles with terminal corneous scale (with distal spine followed by tubercles decreasing in size proximally in *A. jarai*); ventral margin of merus of second pereiopods with small tubercles with terminal corneous scale (one or two small spines in *A. castro* and *A. schmitti*); uropods wide (narrow in *A. lata* and *A. jaragua*).

Aegla sp. n. 1 can be further differentiated from these species by the: cardiac area trapezoidal (subrectangular in *Aegla* sp. n. 2 and *Aegla* sp. n. 3); epibranchial area strongly elongated (shortened in *Aegla* sp. n. 2, slightly elongated in *A. jaragua* and *Aegla* sp. n. 3); proximal dorsal margin of dactylus with moderate-sized lobe (without lobe in *A. strinatii*, rudimentary in *Aegla* sp. n. 2, rudimentary or absent lobe in *A. jacutinga* and *Aegla* sp. n. 3, pronounced and surmounted by tubercles in *A. castro* and *A. schmitti*); anterolateral angle of second abdominal epimeron unarmed (with spine in *A. castro* and *A. schmitti*); anterior margin of second abdominal epimeron almost straight (concave in *A. castro* and *A. schmitti* and slightly concave in *A. jacutinga*).

Another unusual character of *Aegla* sp. n. 1 is the presence of rudimentary pleopods in adult male specimens. Until now, this morphological trait has been described only for *A. perobae* (see Moraes and Bueno 2015) and partially developed pleopods 2-5 have been observed in adult males of *Aegla charon* Bueno and Moraes, 2017 (Bueno *et al*. 2017). *Aegla* sp. n. 1, however, differs from these species in having: subrostral process occupying proximal third of subrostral margin (on proximal half of subrostral margin in *A. charon*); protogastric lobes pronounced (rudimentary in *A. charon*); areola trapezoidal (rectangular in *A. perobae*); proximal dorsal margin of dactylus with moderate-sized lobe (without lobe in *A. perobae*); palmar crest disciform (rectangular in *A. charon* and subrectangular in *A. perobae*); outer surface of carpus with carpal ridge high (low in *A. perobae*); anteromesial region of third thoracic sternite tapered (abrupt in *A. charon*).

The genetic distance between *Aegla* sp. n. 1 and its congeners ranged from 1.2% to

5.8% (Table 2). *Aegla* sp. n. 2 showed the lowest divergence relative to *Aegla* sp. n. 1. As shown in Figure 6, most of the analyzed sequences grouped in a clade with high nodal support. This clade is composed of two major subclades, both maximally supported. The new species clustered in one of these subclades, along with *A. castro*, *A. lata* and *A. jacutinga*. Furthermore, our Bayesian tree suggests *Aegla* sp. n. 2 is the sister species to *Aegla* sp. n. 1.

Table 2. Interspecific and intraspecific genetic distances based on mitochondrial gene cytochrome c oxidase I (COI). Values are shown as percentages. Intraspecific distances are in bold.

Species	1	2	3	4	5	6	7	8	9	10	11
1. *Aegla odebrechtii*	**0.3**										
2. *Aegla castro*	4.4	**1.9**									
3. *Aegla strinatii*	4.1	5.5	**2.7**								
4. *Aegla perobae*	6.3	6.5	7.3	**0.1**							
5. *Aegla schmitti*	2.9	4.2	3.9	6.4	**2.7**						
6. *Aegla lata*	6.0	3.0	6.9	7.4	5.3	**0.0**					
7. *Aegla jarai*	3.3	4.4	4.5	6.9	3.1	5.3	**3.4**				
8. *Aegla jacutinga*	4.2	1.8	5.5	5.9	3.7	3.2	4.1	**0.1**			
9. *Aegla* sp. n. 1	4.0	2.2	5.2	5.8	4.0	3.4	4.0	1.8	**0.2**		
10. *Aegla* sp. n. 2	4.4	2.2	5.6	6.3	4.3	3.6	4.1	1.7	1.2	**0.2**	
11. *Aegla* sp. n. 3	4.7	2.3	5.7	6.5	4.1	3.5	4.5	2.0	2.6	2.2	**0.0**

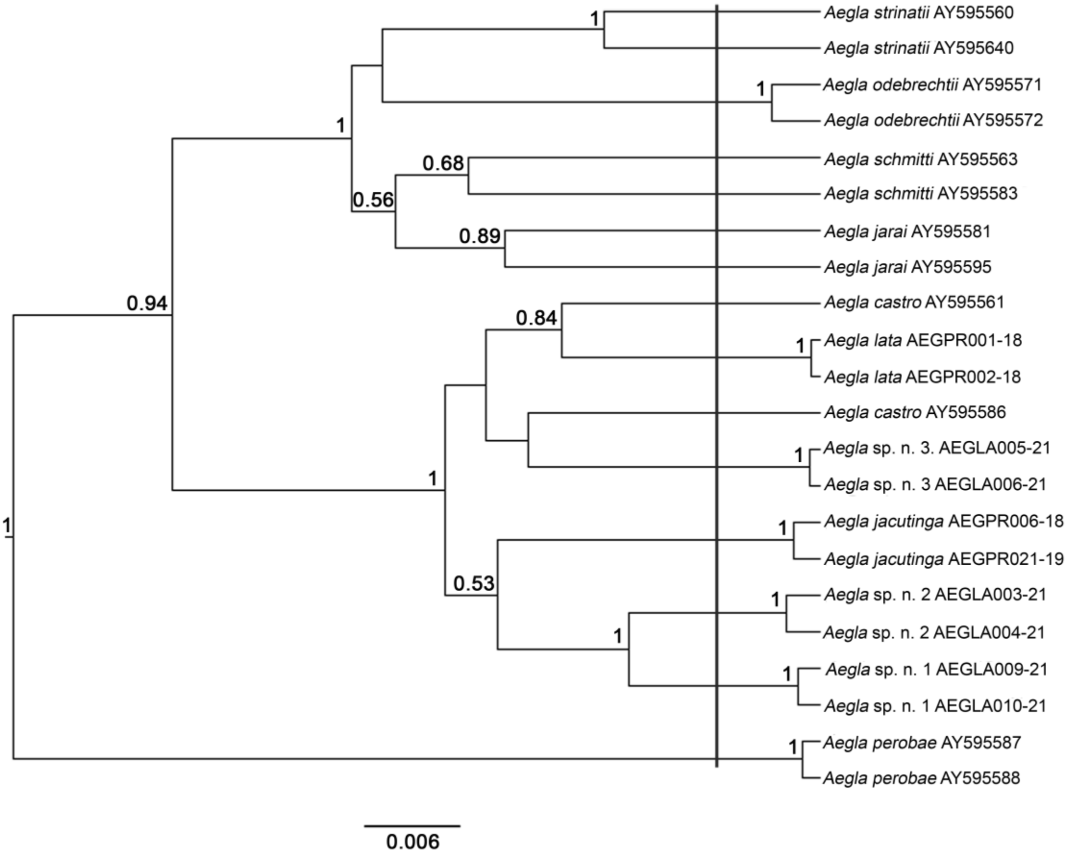

Figure 6. Bayesian tree (HKY+I+G) for *Aegla* species based on partial sequences of the COI gene. The numbers above the branches represent the posterior probability (values < 0.5 are not indicated), and the vertical line indicates the species delimitation assigned in the GMYC analysis.

Aegla sp. n. 2 Marçal and Teixeira

(Figures 7–10)

urn:lsid:zoobank.org:act:8DC448C9-6106-447A-90E0-9B143E120783

Holotype

Male [CLE 16.2 mm], Brazil, Paraná, Ortigueira, Paranapanema River basin, Tibagi River sub-basin, Formiga River, 24°11'11.77"S, 50°56'28.60"W, altitude 725 m, G.M. Teixeira, I.C. Marçal and R.S. Vieira coll., 10 March 2020 (MZUEL 515).

Paratypes

5 males [CLE 12.8–24.4 mm] and 8 females [CLE 12.5–18.2 mm], same data as holotype (MZUEL 499).

Additional material examined

4 males [size not recorded] and 6 females [size not recorded], same data as holotype (MZUEL 504, genetic vouchers: Bold Systems access AEGLA003-21, AEGLA004-21). 1 male [CLE 16.7 mm], Brazil, Paraná, Ortigueira, Paranapanema River basin, Tibagi River sub-basin, tributary of the Formiga River, 24°10'56.50"S, 50°56'31.50"W, altitude 726 m, O.A. Shibatta coll., August 2014 (MZUEL 388). 1 male [CLE 20.1 mm] and 6 females [CLE 13.6–19.4 mm], Brazil, Paraná, Ortigueira, Paranapanema River basin, Tibagi River sub-basin, Piquira River, coordinates and coll. unknown, 02 February 2001 (MZUEL 440). 1 male [CLE 20.1 mm] and 1 female [CLE 19.2 mm], same locality, coordinates and coll. unknown, 25 April 2002 (MZUEL 441).

Diagnosis

Rostrum triangular, narrow base, extending beyond distal apex of compound eyes, carinate along entire length. Subrostral process well developed, occupying proximal half of subrostral margin, anterior and posterior margins forming acute angle. Orbital and extra-orbital sinuses deep. Anterolateral spines reaching basal margin of cornea. Epigastric prominences pronounced. Protogastric lobes pronounced. Cervical groove trapezoidal. Areola subrectangular. Epibranchial area shortened. Lobe on proximal dorsal margin of dactylus rudimentary. Carpal ridge high on outer surface of carpus. Ventromesial border of cheliped ischium with 4 or 5 tubercles. Anteromesial region of third thoracic sternite abrupt. Anterior margin of second abdominal epimeron almost straight. Uropods wide.

Description of holotype

Carapace moderately convex, gastric region convex, dorsal surface scabrous, covered with punctations (Figure 7A). Rostrum triangular, narrow base (RBW/LMR = 0.79), extending beyond distal apex of compound eyes, carinate along entire length, small corneous scales on lateral margins and tip; ventral portion of rostrum much higher than dorsal in profile. Rostral carina beginning at level of protogastric lobes, with 2 rows of corneous scales extending next to apex. Subrostral process well developed, occupying proximal half of subrostral margin, triangular, tip rounded, anterior and posterior margins forming acute angle (88°) (Figure 8A).

Eyestalk and cornea well developed. Orbital and extra-orbital sinuses deep. Orbital sinus U-shaped. Orbital spines well developed, with small terminal corneous scale. Anterolateral spines acuminate apically with small corneous scales terminally, reaching basal margin of cornea.

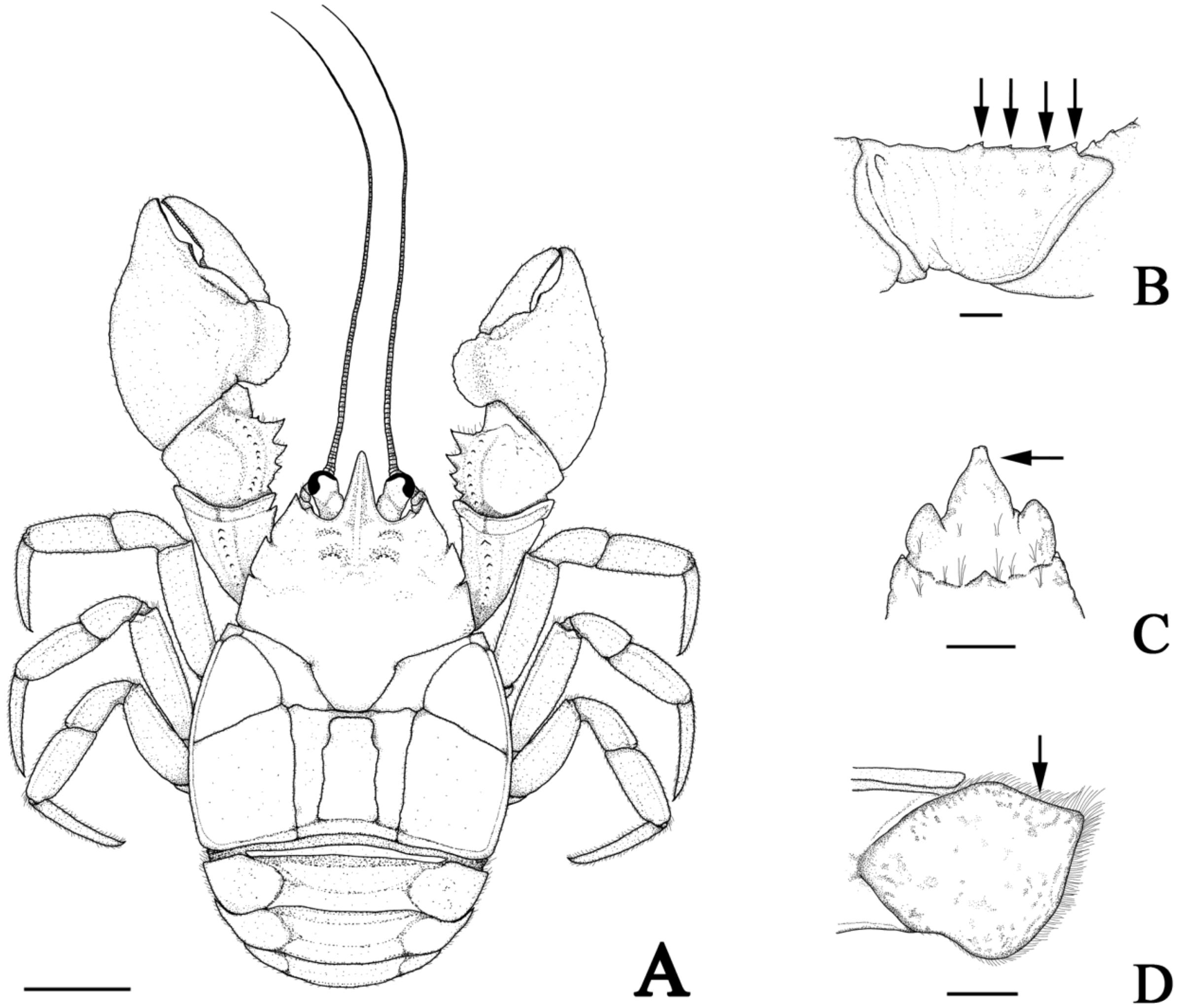

Figure 7. *Aegla* sp. n. 2, male holotype, CLE 16.2 mm, Formiga River, Ortigueira, Paraná, Brazil (MZUEL 515). (a) dorsal view of the cephalothorax and anterior portion of the abdomen. (b) ventral view of ischium of major cheliped (left) showing four tubercles (arrows). (c) third thoracic sternite abrupt (arrow). (d) dorsal view of second abdominal epimeron (right side) showing anterior margin almost straight (arrow). Scale bars: a = 5.0 mm; b−d = 1.0 mm.

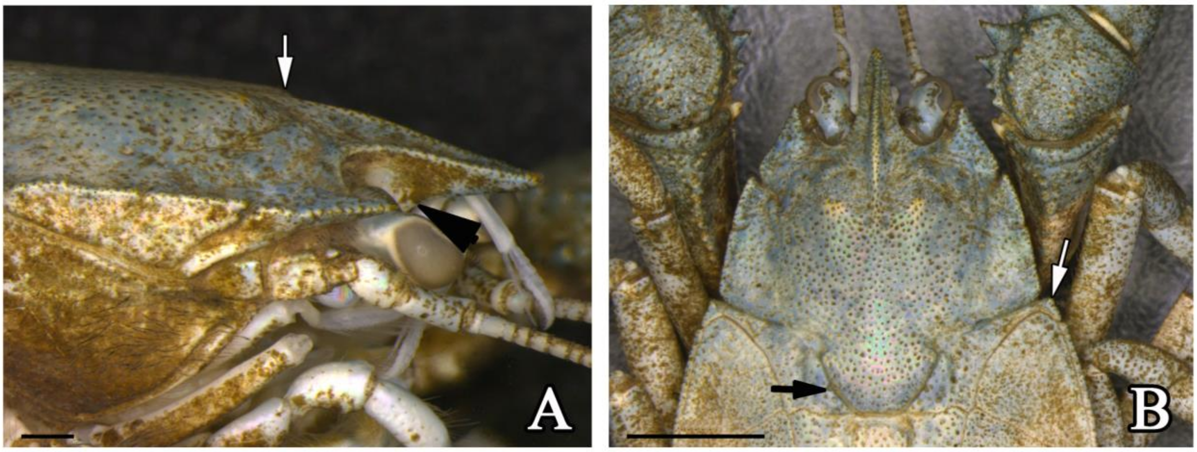

Figure 8. *Aegla* sp. n. 2, male holotype, CLE 16.2 mm (MZUEL 515). (a) external surface view of the anterior portion of the cephalothorax showing pronounced protogastric lobes (white arrow) and well-developed subrostral process (black arrow). (b) dorsal view of the anterior region of the cephalothorax showing trapezoidal cervical groove (black arrow) and shortened epibranchial area (white arrow). Scale bars: a = 1.0 mm; b = 5.0 mm.

Epigastric prominences pronounced, with corneous scales. Protogastric lobes pronounced, with corneous scales (Figure 8A). Gastric area elevated in relation to hepatic lobes and rostrum in lateral view. Demarcation between the first and the second hepatic lobes well defined, between the second and the third hepatic lobes weakly delimited. Lateral margins of hepatic lobes with small corneous scales and small setae.

Cervical groove trapezoidal (Figure 8B). Transverse dorsal linea sinuous along its extension. Areola subrectangular (AH/[(APM+AAD)/2] = 2.20). Cardiac area trapezoidal (TDL/PMC = 1.39). Epibranchial area shortened, triangular, anterolateral angle with corneous scale, lateral margin with row of corneous scales and small setae (Figure 8B). Lateral margins of anterior and posterior branchial areas with row of corneous scales and small setae.

Chelipeds unequal. Left cheliped largest (Figures 9A, B). Dactylus: dorsal margin and outer surface with small corneous scales, inner surface with setal tufts and scales. Proximal dorsal margin with rudimentary lobe. Cutting margin with well-developed lobular basal tooth, followed by row of corneous scales to distal end. Row of small tufts of long setae next to cutting margin. Pre-dactylar lobe well developed, rounded, without corneous scales. Propodus: outer surface granular, globose aspect. Palmar crest subdisciform with outer surface excavated, margin poorly serrated, covered by acuminate corneous scales. Cutting margin of fixed finger with well-developed lobular basal tooth, followed by row of corneous scales to distal end. Inner and outer surfaces of fixed finger with rows of long setal tufts next to cutting margin. Carpus: dorsal margin with 2 proximal tubercles, median spine, distal spine longest, each tubercle or spine with small setae and terminal corneous scale, subterminal lobe well defined, pointed, with small corneous scales and setae. Inner surface with large tubercle, long setae next to dorsal margin. Outer surface with carpal ridge high, with small corneous scales. Merus: dorsolateral edge with distal tubercle, with corneous scale terminally, followed by row of tubercles decreasing in size proximally. Ventromesial edge with distal spine, with corneous scale terminally, followed by 4 tubercles of similar size, with corneous scale. Ventrolateral border with distal tubercle, with terminal corneous scale, followed by row of small tubercles. Ischium (Figure 7B): dorsolateral edge with spine, with corneous scale terminally. Ventromesial border with proximal tubercle, 2 median tubercles and large distal tubercle, each with terminal corneous scale. Ventrolateral border smooth.

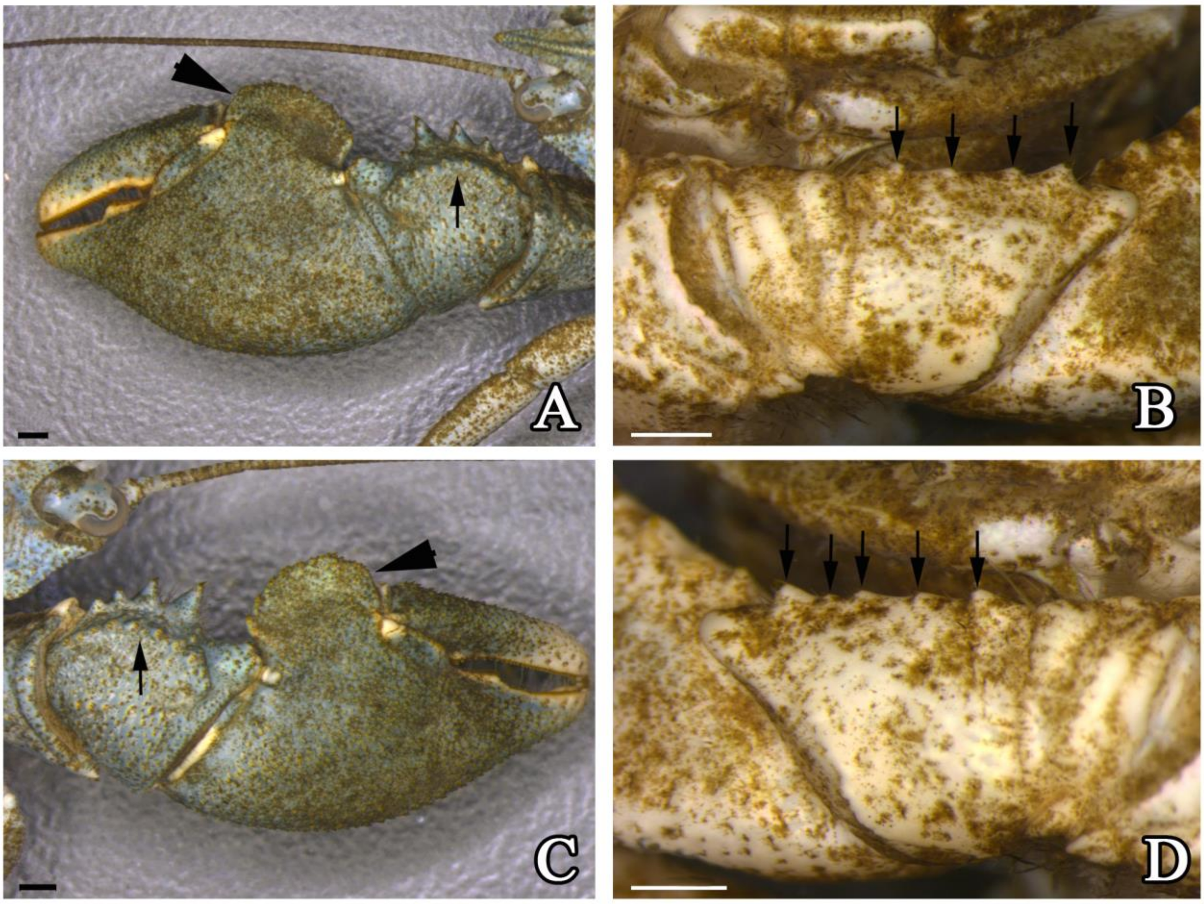

Figure 9. *Aegla* sp. n. 2, male holotype, CLE 16.2 mm (MZUEL 515). (a) dorsal view of the dactylus, propodus, and carpus of major cheliped (left) showing subdisciform palmar crest (thick arrow) and high carpal ridge (thin arrow). (b) ventral view of ischium of major cheliped (left) with four tubercles (arrows). (c) dorsal view of the dactylus, propodus, and carpus of minor cheliped (right) showing subdisciform palmar crest (thick arrow) and high carpal ridge

(thin arrow). (d) ventral view of ischium of minor cheliped (right) with five tubercles (arrows). Scale bars = 1.0 mm.

Minor cheliped (right) similar to major cheliped except as noted hereafter (Figures 9C, D). Dactylus: cutting margin with rudimentary lobular basal tooth. Propodus: cutting margin with rudimentary lobular basal tooth. Ischium: ventromesial border with proximal tubercle, 3 small median tubercles and large distal tubercle, each with terminal corneous scale.

Second, third and fourth pereiopods similar. Dactyli, propodi, carpi, meri and ischii with several rows of setal tufts and small scales on surface. Carpi and meri with row of small tubercles with terminal corneous scale along dorsal margin. Meri with row of small tubercles with terminal corneous scale along ventral margin. Meri and ischii with long setae concentrated along dorsal margin.

Anteromesial region of third thoracic sternite abrupt, projecting between coxae of third maxillipeds, with scattered setae (Figure 7C). Fourth thoracic sternite with anterolateral angles produced anteriorly, with scattered setae (Figure 10A). Pleopods 2–5 absent. Anterolateral angle of second abdominal epimeron well defined, unarmed (Figure 10B). Ventral angle of third abdominal epimeron well defined with small corneous scales apically. Ventral angle of fourth abdominal epimeron well defined, unarmed. Anterior margin of second abdominal epimeron almost straight (Figures 7D, 10B). Uropods well developed, wide (WU/HWT = 1.04). Telson divided by longitudinal suture. Anterolateral and posterolateral margins well differentiated.

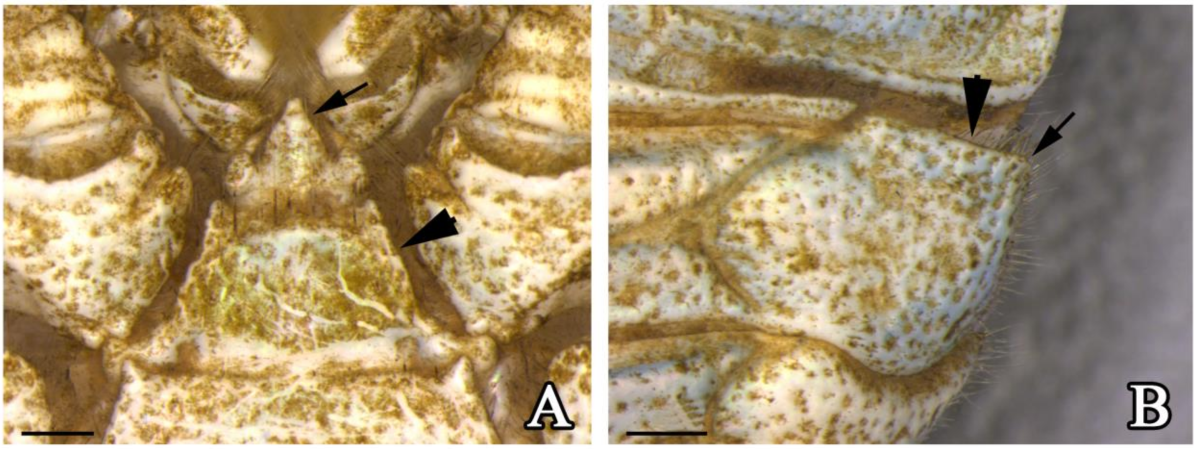

Figure 10. *Aegla* sp. n. 2, male holotype, CLE 16.2 mm (MZUEL 515). (a) third (thin arrow) and fourth (thick arrow) thoracic sternites. (b) dorsal view of second abdominal epimeron (right side) showing almost straight anterior margin (thick arrow) and unarmed anterolateral

angle (thin arrow). Scale bars = 1.0 mm.

Variations

The areola can be subrectangular (n = 10), trapezoidal (n = 2) or rectangular (n = 1). The cardiac area varies from subrectangular (n = 9) to trapezoidal (n = 4). The palmar crest may appear rectangular instead of subdisciform. The anteromesial region of third thoracic sternite can be abrupt (n = 8) or tapered (n = 5). The anterolateral angle of second abdominal epimeron may present apical corneous scales instead of being naked as in the holotype. In most specimens, the ventral angle of the third abdominal epimeron is devoid of corneous scales. The uropods are narrow in a few specimens (n = 2). All measurements are summarized in Table 1.

Distribution

The new species has been collected in three watercourses within the Tibagi River sub-basin, Paranapanema River basin. These watercourses are located in the city of Ortigueira, Paraná state, Brazil.

Conservation status

We suggest that *Aegla* sp. n. 2 be assigned as ‘Vulnerable’ (VU) under criteria B1, B2ab(iii) as defined by the IUCN (2019). This species has been recorded in three localities (B2a) and the known extent of occurrence is estimated to be less than 500 km^2 (B1). The streams where the specimens of *Aegla* sp. n. 2 were found are in agricultural and urbanized areas (B2b(iii)).

Remarks

Aegla sp. n. 2 can be separated from its congeners, mainly by, among other characters, subrostral process well developed (undeveloped in *A. strinatii*), occupying proximal half of subrostral margin (on proximal third of subrostral margin in *A. lata*, *A. jacutinga*, *Aegla* sp. n. 3 and *Aegla* sp. n. 1, and occupying median portion of subrostral margin in *A. jaragua*); anterior and posterior margins of subrostral process forming acute angle (right angle in *A. lata* and *A. jacutinga*, and obtuse angle in *A. jaragua*, *Aegla* sp. n. 3 and *Aegla* sp. n. 1); orbital and extra-orbital sinuses deep (shallow in *A. odebrechtii*); epigastric prominences pronounced (poorly pronounced in *A. lata* and *A. jacutinga*); protogastric lobes pronounced (poorly pronounced in *A. odebrechtii*, *A. lata*, *A. jarai* and *A. jaragua*); cervical groove trapezoidal (U-shaped in *A. lata* and *Aegla* sp. n. 1); subterminal lobe of carpus well defined, pointed (poorly defined in *A. strinatii*); outer surface of carpus with carpal ridge high (low in *A.*

jacutinga); dorsal margin of carpus and merus of second pereiopods with small tubercles with terminal corneous scale (with distal spine followed by tubercles decreasing in size proximally in *A. jarai*); ventral margin of merus of second pereiopods with small tubercles with terminal corneous scale (one or two small spines in *A. castro* and *A. schmitti*); anteromesial region of third thoracic sternite abrupt (tapered or truncate in *A. lata*, truncate in *A. jaragua*, and tapered in *A. jacutinga*, *Aegla* sp. n. 3 and *Aegla* sp. n. 1); anterior margin of second abdominal epimeron almost straight (concave in *A. castro* and *A. schmitti* and slightly concave in *A. jacutinga*); uropods wide (narrow in *A. lata* and *A. jaragua*).

Our Bayesian tree based on the COI mitochondrial gene (Figure 6) recovered *Aegla* sp. n. 2 as the sister species to *Aegla* sp. n. 1, and both clustered in the same subclade as *A. jacutinga*. The interspecific genetic distances (Table 2) reinforce the relationships evidenced in the Bayesian analysis.

***Aegla* sp. n. 3** Marçal and Teixeira

(Figures 11–14)

urn:lsid:zoobank.org:act:5751F246-51C1-4BC3-BBD5-CE21CB09CEC5

Holotype

Male [CLE 19.3 mm], Brazil, Paraná, Telêmaco Borba, Paranapanema River basin, Tibagi River sub-basin, Codorna Branca stream, 24°15'06.70"S, 50°31'46.58"W, altitude 792 m, G.M. Teixeira, I.C. Marçal and P.F. da Silva coll., 11 March 2020 (MZUEL 511).

Paratypes

5 males [CLE 13.4–20.9 mm] and 5 females [CLE 14.1–16.0 mm], same data as holotype (MZUEL 500). 1 male [CLE 17.0 mm] and 1 female [CLE 16.9 mm], same locality, J.F.M. da Silva, E.S. da Silva and S.T. Bennemann coll., 07 August 2014 (MZUEL 470).

Additional material examined

7 males [size not recorded] and 3 females [size not recorded], same data as holotype (MZUEL 505, genetic vouchers: Bold Systems access AEGLA005-21, AEGLA006-21). 1 male [CLE 13.3 mm], Brazil, Paraná, Telêmaco Borba, Paranapanema River basin, Tibagi River sub-basin, Varanal stream, 24°19'59.70"S, 50°33'37.20"W, altitude 761 m, S.T. Bennemann coll., 19 May 2005 (MZUEL 183). 3 males [CLE 12.7–16.8 mm], same locality and coll., March 2004 (MZUEL 349). 1 male [CLE 20.5 mm], same locality and coll., 24°20'12.70"S, 50°32'03.20"W, altitude 802 m, 11 February 2005 (MZUEL 332). 1 male [CLE 16.4 mm],

same locality and coll., 24°20'13.40"S, 50°35'23.10"W, altitude 687 m, 9 November 2005 (MZUEL 344). 1 female [CLE 17.0 mm], Brazil, Paraná, Telêmaco Borba, Paranapanema River basin, Tibagi River sub-basin, Harmonia stream, coordinates and coll. unknown, 20 June 2006 (MZUEL 414). 4 females [CLE 12.5–15.8 mm], same locality, coordinates and coll. unknown, 16 March 2012 (MZUEL 415). 1 male [CLE 15.2 mm] and 1 female [CLE 15.4 mm], Brazil, Paraná, Telêmaco Borba, Paranapanema River basin, Tibagi River sub-basin, Moinho Velho stream, 24°13'18.34"S, 50°37'42.42"W, altitude 739 m, S.T. Bennemann coll., 7 August 2014 (MZUEL 426). 1 male [CLE 25.3 mm], same locality, J.F.M. da Silva coll., 3 November 2014 (MZUEL 427). 2 females [CLE 14.4–15.0 mm], Brazil, Paraná, Telêmaco Borba, Paranapanema River basin, Tibagi River sub-basin, Colônia stream, 24°10'27.79"S, 50°37'28.45"W, altitude 736 m, S.T. Bennemann coll., 7 August 2014 (MZUEL 437).

Diagnosis

Rostrum triangular, narrow base, extending beyond distal apex of compound eyes, carinate along entire length. Subrostral process well developed, occupying proximal third of subrostral margin, anterior and posterior margins forming obtuse angle. Orbital and extra-orbital sinuses deep. Anterolateral spines reaching basal margin of cornea. Epigastric prominences pronounced. Protogastric lobes pronounced. Areola trapezoidal. Cardiac area subrectangular. Epibranchial area slightly elongated. Lobe on proximal dorsal margin of dactylus rudimentary or absent. Carpal ridge high on outer surface of carpus. Ventromesial border of cheliped ischium with 4 or 5 tubercles. Ventral angles of third and fourth abdominal epimeron with corneous scales apically. Uropods wide.

Description of holotype

Carapace moderately convex, gastric region convex, dorsal surface scabrous, covered with punctations (Figure 11A). Rostrum triangular, narrow base (RBW/LMR = 0.93), extending beyond distal apex of compound eyes, carinate along entire length, small corneous scales on lateral margins and tip; ventral portion of rostrum slightly higher than dorsal in profile. Rostral carina beginning at level of protogastric lobes, with 2 rows of corneous scales extending next to apex. Subrostral process well developed, occupying proximal third of subrostral margin, produced ventrally, triangular, tip rounded, anterior and posterior margins forming obtuse angle (95°) (Figure 12A).

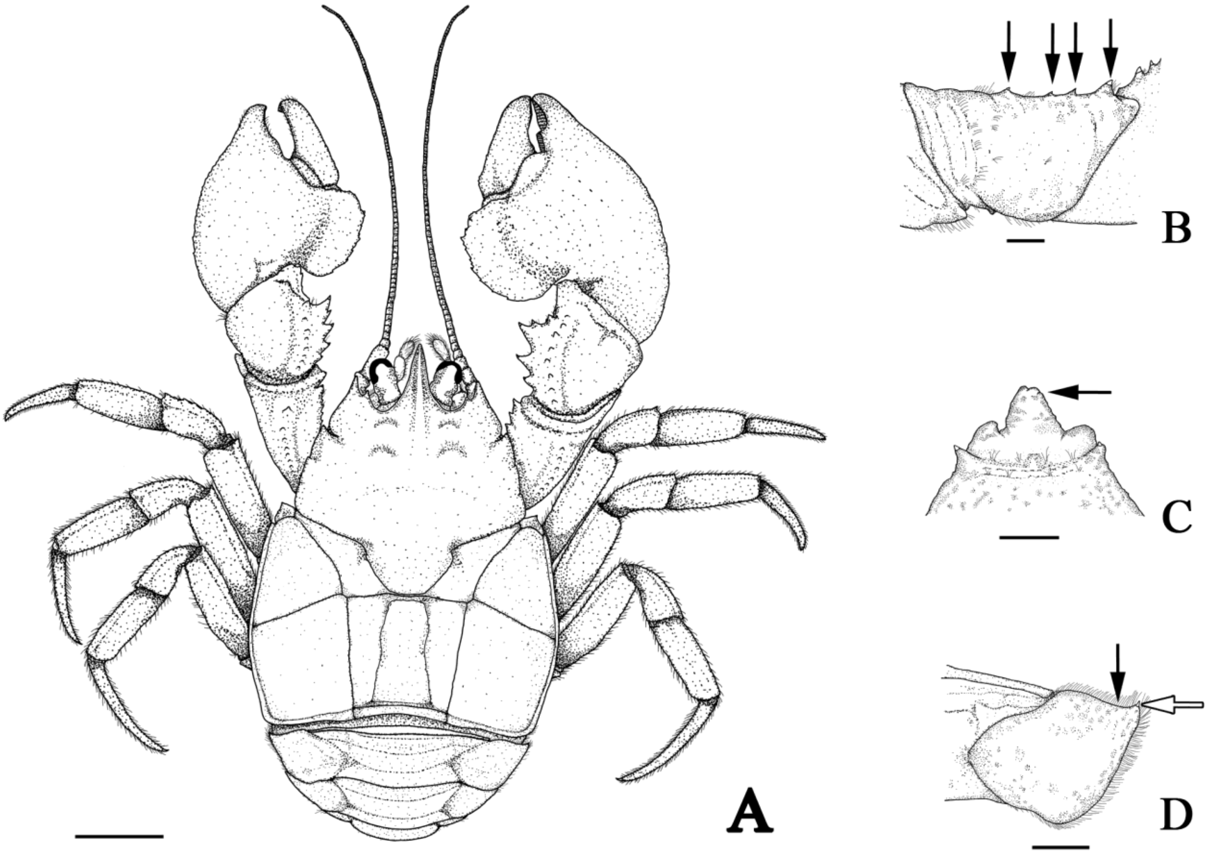

Figure 11. *Aegla* sp. n. 3, male holotype, CLE 19.3 mm, Codorna Branca stream, Telêmaco Borba, Paraná, Brazil (MZUEL 511). (a) dorsal view of the cephalothorax and anterior portion of the abdomen. (b) ventral view of ischium of minor cheliped (left) showing four tubercles (arrow). (c) third thoracic sternite truncate (arrow). (d) dorsal view of second abdominal epimeron (right side) showing anterolateral angle with corneous scale (white arrow) and anterior margin slightly concave (black arrow). Scale bars: a = 5.0 mm. b–d = 1.0 mm.

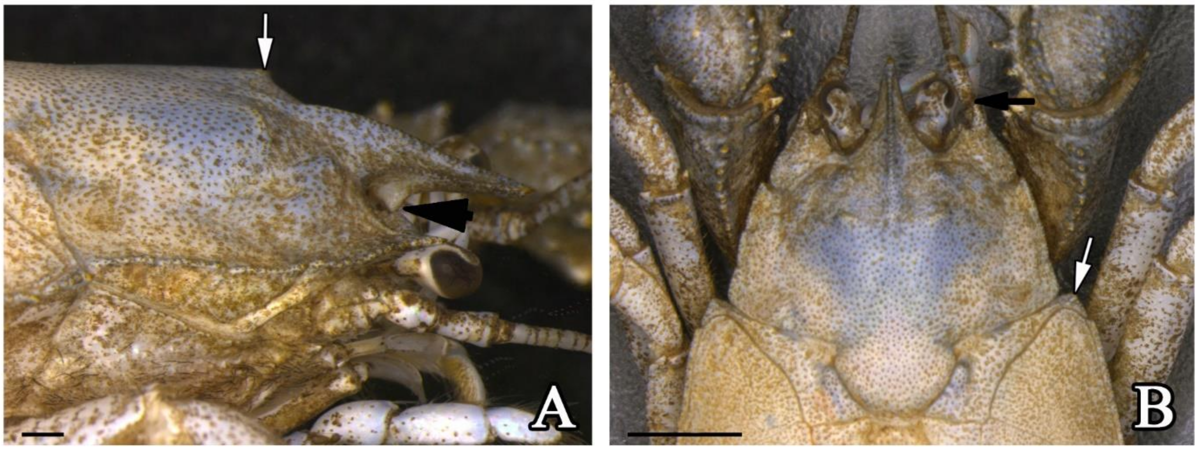

Figure 12. *Aegla* sp. n. 3, male holotype, CLE 19.3 mm (MZUEL 511). (a) external surface

view of the anterior portion of the cephalothorax showing pronounced protogastric lobes (white arrow) and well-developed subrostral process (black arrow). (b) dorsal view of the anterior region of the cephalothorax showing anterolateral spine reaching basal margin of the cornea (black arrow) and slightly elongated epibranchial area (white arrow). Scale bars: a = 1.0 mm; b = 5.0 mm.

Eyestalk and cornea well developed. Orbital and extra-orbital sinuses deep. Orbital sinus U-shaped. Orbital spines well developed, with small terminal corneous scale. Anterolateral spines acuminate apically with small corneous scales terminally, reaching basal margin of cornea (Figure 12B).

Epigastric prominences pronounced, with corneous scales. Protogastric lobes pronounced, with corneous scales (Figure 12A). Gastric area elevated in relation to hepatic lobes and rostrum in lateral view. Demarcation between hepatic lobes well defined. Lateral margins of hepatic lobes with small corneous scales and small setae.

Cervical groove trapezoidal. Transverse dorsal linea sinuous along its extension. Areola trapezoidal (APM/AAD = 2.00). Cardiac area subrectangular (TDL/PMC = 1.24). Epibranchial area slightly elongated, triangular, anterolateral angle with corneous scale, lateral margin with row of corneous scales and small setae (Figure 12B). Lateral margins of anterior and posterior branchial areas with row of corneous scales and small setae.

Chelipeds unequal. Right cheliped largest (Figures 13A, B). Dactylus: dorsal margin and outer surface with small corneous scales, inner surface with setal tufts and scales. Proximal dorsal margin with rudimentary lobe. Cutting margin with well-developed lobular basal tooth, followed by row of corneous scales to distal end. Row of small tufts of long setae next to cutting margin. Pre-dactylar lobe well developed, rounded, without corneous scales. Propodus: outer surface granular, globose aspect. Palmar crest disciform with outer surface excavated, margin poorly serrated, covered by acuminate corneous scales. Cutting margin of fixed finger with well-developed lobular basal tooth, followed by row of corneous scales to distal end. Inner and outer surfaces of fixed finger with rows of long setal tufts next to cutting margin. Carpus: dorsal margin with 2 proximal tubercles, median spine, distal spine longest, double-tipped, each tubercle or spine with terminal corneous scale, subterminal lobe well defined, pointed, with small corneous scales and setae. Inner surface with spine, long setae next to dorsal margin. Outer surface with carpal ridge high, with small corneous scales. Merus: dorsolateral edge with distal tubercle, with corneous scale terminally, followed by row of tubercles decreasing in size proximally. Ventromesial edge with distal spine, with corneous scale terminally, followed by 4 tubercles of similar size, with corneous scale. Ventrolateral border with distal tubercle, with terminal corneous scale, followed by row of tubercles decreasing in size proximally. Ischium: dorsolateral edge with spine, with corneous scale terminally. Ventromesial border with proximal tubercle, 2 small median tubercles and large distal tubercle, each with terminal corneous scale. Ventrolateral border smooth.

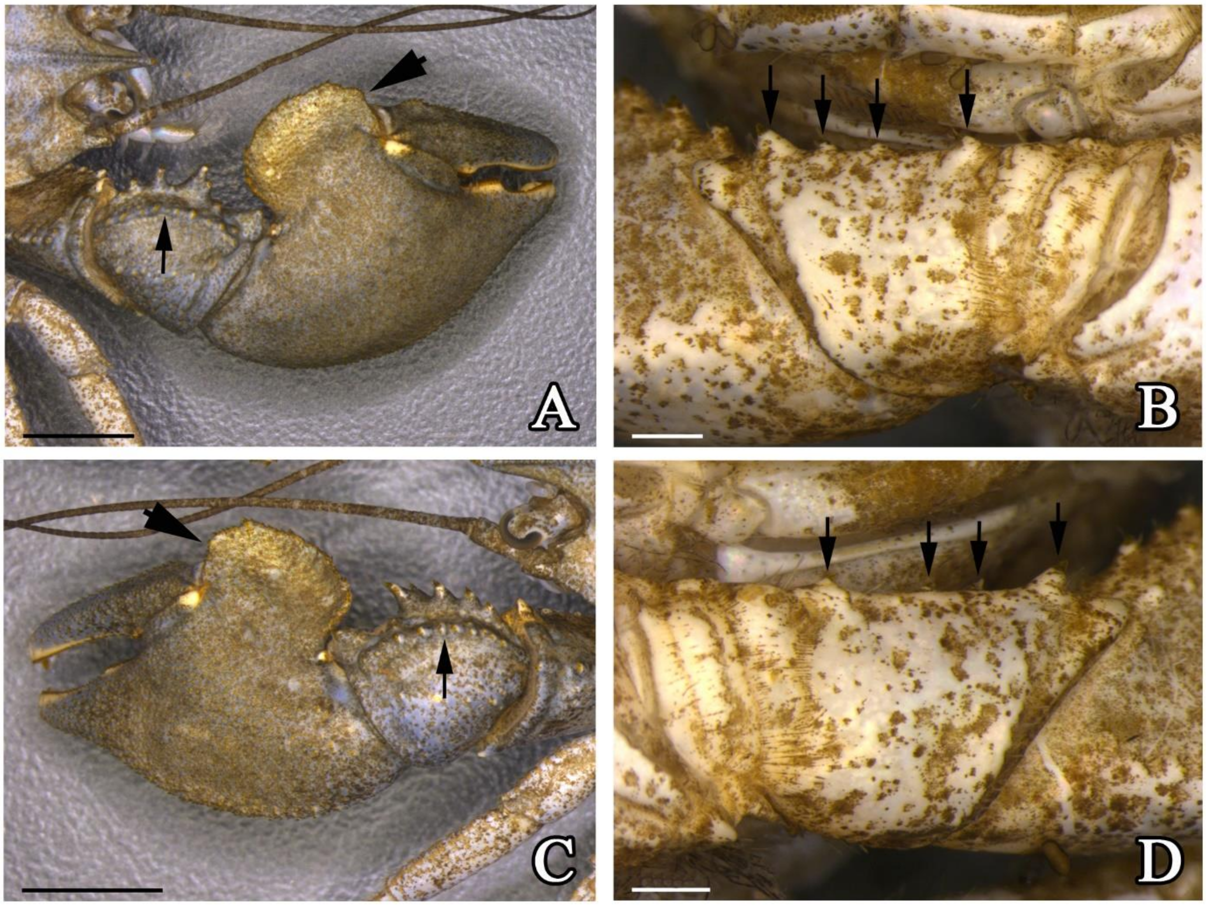

Figure 13. *Aegla* sp. n. 3, male holotype, CLE 19.3 mm (MZUEL 511). (a) dorsal view of the dactylus, propodus, and carpus of major cheliped (right) showing disciform palmar crest (thick arrow) and high carpal ridge (thin arrow). (b) ventral view of ischium of major cheliped (right) with four tubercles (arrows). (c) dorsal view of the dactylus, propodus, and carpus of minor cheliped (left) showing disciform palmar crest (thick arrow) and high carpal ridge (thin arrow). (d) ventral view of ischium of minor cheliped (left) with four tubercles (arrows). Scale bars: a, c = 5.0 mm; b, d = 1.0 mm.

Minor cheliped (left) similar to major cheliped except as noted hereafter (Figures 11B, 13C, D). Dactylus: proximal lobe on dorsal margin absent. Cutting margin with rudimentary lobular basal tooth. Propodus: cutting margin with rudimentary lobular basal tooth. Carpus:

dorsal margin with 2 proximal tubercles, median tubercle, distal spine longest, double-tipped, each tubercle or spine with small setae and terminal corneous scale. Inner surface with 2 tubercles, largest tubercle with double-tipped, corneous scale terminally. Merus: ventromesial edge with 2 distal tubercles, followed by 4 tubercles, each with terminal corneous scale.

Second, third and fourth pereiopods similar. Dactyli, propodi, carpi, meri and ischii with several rows of setal tufts and small scales on surface. Carpi and meri with row of small tubercles with terminal corneous scale along dorsal margin. Meri with row of small corneous scales along ventral margin. Meri and ischii with long setae concentrated along dorsal margin.

Anteromesial region of third thoracic sternite truncate, projecting between coxae of third maxillipeds, with 2 terminal small corneous scales and scattered setae (Figure 11C). Fourth thoracic sternite with anterolateral angles produced anteriorly, with scattered setae (Figure 14A). Pleopods 2–5 absent. Anterolateral angle of second abdominal epimeron and ventral angles of third and fourth abdominal epimeron well defined, with corneous scales apically. Anterior margin of second abdominal epimeron slightly concave (Figures 11D, 14B). Uropods well developed, wide (WU/HWT = 1.06). Telson divided by longitudinal suture. Anterolateral and posterolateral margins well differentiated.

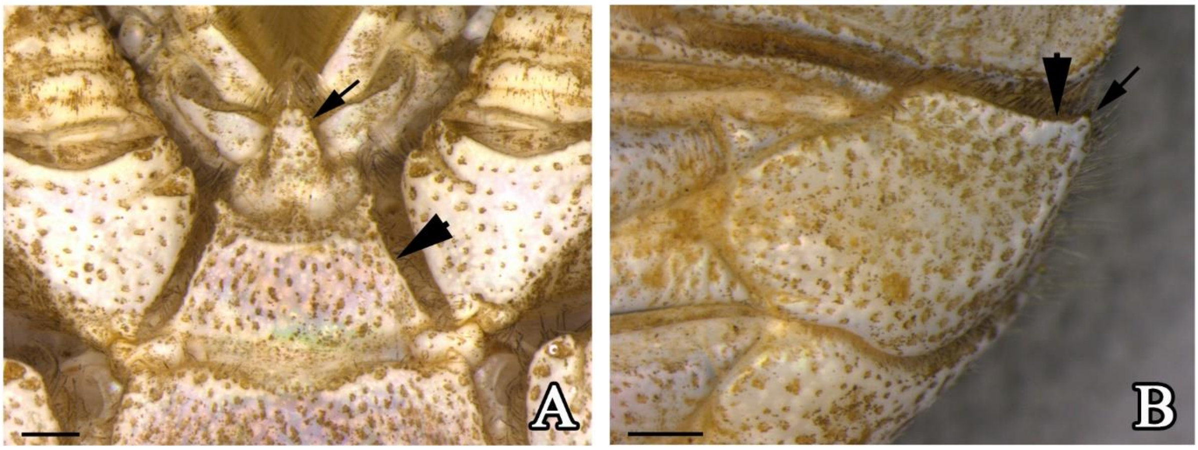

Figure 14. *Aegla* sp. n. 3, male holotype, CLE 19.3 mm (MZUEL 511). (a) third (thin arrow) and fourth (thick arrow) thoracic sternites. (b) dorsal view of second abdominal epimeron (right side) showing slightly concave anterior margin (thick arrow) and anterolateral angle with corneous scales (thin arrow). Scale bars = 1.0 mm.

Variations

The areola can be trapezoidal (n = 10), rectangular (n = 1) or subrectangular (n = 1). The cardiac area varies from subrectangular (n = 7) to trapezoidal (n = 5). The palmar crest may appear rectangular instead of disciform. The ventromesial border of ischium may present 5 tubercles instead of 4 as in the holotype. In all paratypes, the third thoracic sternite is tapered instead of being truncate. The anterolateral angle of second abdominal epimeron is devoid of corneous scales in most specimens. Also, the anterior margin of second abdominal epimeron may be almost straight. The uropods are narrow in a few specimens (n = 3). All measurements are summarized in Table 1.

Geographical distribution

The new species has been collected in five streams within the Tibagi River sub-basin, Paranapanema River basin. These streams are located in the city of Telêmaco Borba, Paraná state, Brazil.

Conservation status

The conservation status of *Aegla* sp. n. 3 may, for the moment, be assessed as ‘Vulnerable’ (VU) under criteria B1, B2ab(iii) as defined by the IUCN (2019). Since the known extent of occurrence is estimated to be less than 500 km^2, the record of occurrence is limited to ≤ 5 locations, and the decline in quality of habitat is observed because of the agricultural matrix.

Remarks

Aegla sp. n. 3 can be distinguished from its congeners because it has: rostrum carinate along entire length (without carina in the distal third in *A. odebrechtii*); subrostral process well developed (undeveloped in *A. strinatii*); orbital and extra-orbital sinuses deep (shallow in *A. odebrechtii*); anterolateral spines reaching basal margin of cornea (not reaching basal margin of cornea in *A. strinatii*, *A. jaragua*, *A. jacutinga* and *Aegla* sp. n. 1, and extending beyond basal margin of cornea in *A. odebrechtii*, *A. castro*, *A. schmitti* and *A. jarai*); epigastric prominences pronounced (poorly pronounced in *A. lata* and *A. jacutinga*); protogastric lobes pronounced (poorly pronounced in *A. odebrechtii*, *A. lata*, *A. jarai* and *A. jaragua*); areola trapezoidal (rectangular in *A. lata*, and subrectangular in *A. jaragua, A. jacutinga* and *Aegla* sp. n. 2); subterminal lobe of carpus well defined, pointed (poorly defined in *A. strinatii*); outer surface of carpus with carpal ridge high (low in *A. jacutinga*); dorsal margin of carpus and merus of second pereiopods with small tubercles with terminal corneous scale (with distal spine followed by tubercles decreasing in size proximally in *A. jarai*); ventral margin of merus of second pereiopods with small tubercles with terminal corneous scale (one or two small spines in *A. castro* and *A. schmitti*); ventral angles of third and fourth abdominal epimeron with corneous scales apically (unarmed in *Aegla* sp. n. 2); uropods wide (narrow in

A. lata and *A. jaragua*).

Based on the Bayesian tree (Figure 6), *Aegla* sp. n. 3 is the sister of *A. castro* and *Aegla* sp. n. 3 + *A. castro* is the sister group of *A. lata* + *A. castro*.

DISCUSSION

In general, the aeglids are very similar to each other (Schmitt 1942). Notwithstanding, the detailed analysis of the morphology allowed us to identify several morphological characters that differentiate the new species from their congeners. Some structures, such as the rostrum or the palmar crest, are more easily recognized and have been used as diagnostic characters for many decades (Schmitt 1942; Jara 1977; Jara 1982; Bond-Buckup and Buckup 1994; Santos *et al*. 2015; Jara *et al*. 2018; Páez *et al*. 2018; Trombetta *et al*. 2019; Marçal *et al*. 2020). Other structures, such as the epibranchial area, are more subtle and have been used to compare species in more recent studies (Moraes *et al*. 2016; Moraes *et al*. 2017).

Considering all the species that occur in the Tibagi River basin, *Aegla* sp. n. 1 is unique in having the following combination of characters: anterolateral spines not reaching basal margin of the cornea, cervical groove U-shaped, cardiac area trapezoidal, epibranchial area strongly elongated and rudimentary pleopods in adult male specimens. By the same token, *Aegla* sp. n. 2 can be distinguished from its congeners by the following combination of characters: subrostral process occupying proximal half of the subrostral margin, anterior and posterior margins of subrostral process forming an acute angle, cervical groove trapezoidal, epibranchial area shortened and anteromesial region of third thoracic sternite abrupt. *Aegla* sp. n. 3 can be separated from its congeners by a unique combination of characters that includes the subrostral process occupying the proximal third of the subrostral margin, the anterolateral spines reaching the basal margin of the cornea, the cardiac area subrectangular, the epibranchial area slightly elongated and the ventral angles of third and fourth abdominal epimeron with corneous scales apically.

The diagnostic morphological characters listed here are clear in indicating that *Aegla* sp. n. 1, *Aegla* sp. n. 2, and *Aegla* sp. n. 3 are new species. In addition, the maximum nucleotide divergence values obtained in intraspecific comparisons do not overlap with the lowest values found in interspecific comparisons (Table 2), clearly indicating an interval between them. According to Meyer and Paulay (2005), if there is an interval between intraspecific and interspecific distance, a cut value for species delimitation can be established in the data. Similar values were observed for other species of *Aegla* (Jara *et al*. 2003; Páez *et* *al*. 2018; Marçal *et al*. 2020). However, *A. castro*, *A. strinatii*, *A. schmitti* and *A. jarai* exhibited intraspecific values that overlap with interspecific values. The GMYC analysis indicated that these species correspond to non-monophyletic groups, as observed by Pérez-Losada *et al*. (2004), Páez *et al*. (2018), Trombetta *et al*. (2019), and Marçal *et al*. (2020). Molecular data also indicate a probable phylogenetic relationship among the species, suggesting the new species belong to the same subclade as *A. castro*, *A. lata* and *A. jacutinga* (see Figure 6). This subclade is strongly supported, and all these species have been recorded in the Tibagi River basin. Furthermore, *A. castro*, *A. lata* and *A. jacutinga* are grouped in clade C of the aeglid phylogeny as proposed by Pérez-Losada *et al*. (2004) (see Marçal *et al*. 2020). Thus, we can assume that the new species also belong to clade C, which includes species from the Paraná River. However, to corroborate the phylogenetic relationships of the new species, it is recommended to include more genes in the analysis, both mitochondrial and nuclear genes.

The three new species of *Aegla* described here, all occurring in watercourses in the Tibagi River basin, Paraná state, southern Brazil, expand the diversity of aeglids in this hydrographic basin from four to seven species. Furthermore, our results raise the number of known *Aegla* species to 93. Nevertheless, the new species have limited distributions and occur in habitats impacted by urbanization or agriculture. The rivers of the Paraná state have been degraded because of the intense deforestation in this region (Santos *et al*. 2015). According to IAT (2020), approximately 60% of the state's area is used as pasture and agricultural plantations. Despite the efforts of various groups of researchers, we believe that our knowledge about the diversity, biology and ecology of *Aegla* species in this region is still scarce. There is a vast field of work to be explored in future studies. Given these points, the current study emphasizes the importance of the Tibagi River basin for the conservation of aeglids and reinforces the high potential for finding new species in the Paraná state.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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3. CAPÍTULO 2

DIVERSITY AND GEOGRAPHICAL DISTRIBUTION OF AEGLIDS CRABS (CRUSTACEA, ANOMURA, AEGLIDAE) FROM TIBAGI RIVER BASIN, PARANÁ STATE, BRAZIL

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ABSTRACT

This study updates the geographical distribution of the *Aegla* species from the Tibagi River basin, Paraná state, Brazil. We analyzed specimens deposited at the Crustacean Collection of the Museu de Zoologia of Universidade Estadual de Londrina (MZUEL). The taxonomic determination was done following the identification keys and descriptions. New records of occurrence were obtained for *Aegla castro* (10), *A. lata* (5), and *A. jacutinga* (10). The collections also allowed us to identify two new species. Here *Aegla* n. sp. 4 and *Aegla* n. sp. 5 are described and differentiated from their congeners based on morphological and molecular evidence (COI mitochondrial DNA). The conservation status of the new species is discussed and a diagnostic key to all currently recognized species of *Aegla* from the Tibagi River basin is provided. Our results reveal that most studied species have a restricted distribution and occur in streams impacted by urbanization, industry, and agriculture. Of the nine species that occur in the Tibagi River basin, seven are endemic to this basin. Given these points, this hydrographic basin has a great diversity of aeglids and, therefore, needs to be protected.

ADDITIONAL KEYWORDS: *Aegla*, description, new records, Paraná River basin, taxonomy

INTRODUCTION

Aeglidae is the only family that constitutes the superfamily Aegloidea (McLaughlin *et al*., 2010). Of the 95 species that compound the Aeglidae family, 93 are species and subspecies of the genus *Aegla* Leach, 1820 (Moraes *et al*., 2017; Jara *et al*., 2018; Páez *et al*., 2018; Trombetta *et al*., 2019; Marçal *et al*., 2020, 2021; Marçal *et al*., in press) and two are fossils. *Haumuriaegla glaessneri* Feldmann, 1984 was discovered in marine rocks in New Zealand (Feldmann, 1984) while *Protaegla minuscula* Feldmann, Veja, Applegate & Bishop, 1998 was found in marine rocks in Mexico (Feldmann *et al*., 1998). On the other hand, the representatives of the genus *Aegla* are exclusively from continental waters of southern South America, occurring in the hydrographic basins of Brazil, Chile, Argentina, Uruguay, Paraguay, and Bolivia (Bond-Buckup & Buckup, 1994; Bond-Buckup & Buckup, 1999; Bond-Buckup, 2003).

The fossil records (Feldmann, 1984; Feldmann *et al*., 1998) suggest that the origin of Aeglidae occurred in a marine environment and, later there was a dispersion towards the freshwater environment. Different works debate about the direction of the dispersion of these crustaceans in the freshwater habitats of southern South America. Some researchers postulated that this dispersion occurred from the Atlantic Ocean (Schmitt, 1942; Ringuelet, 1948; Morrone & Loppretto, 1994). However, studies on distribution patterns (Ortmann, 1902), fossil evidence (Feldmann, 1984; Feldmann *et al*., 1998), biogeography and molecular systematics of *Aegla* (Pérez-Losada *et al*., 2004) give greater support to the hypothesis that this dispersion occurred from the Pacific Ocean.

Phylogenetic analysis of *Aegla* suggests that the colonization of southern South America began at least 60 Mya (Pérez-Losada *et al*., 2004). This analysis clustered *Aegla* species into five large groups informally named clades A, B, C, D, and E. The species that occur in the Paraná River basin (including those from the Tibagi River basin) are encompassed in clade C, whose origin was estimated to be 33.2 ± 1.8 Mya. Pérez-Losada *et al*. (2004) postulated that the primary radiation of the aeglids along this drainage occurred before the formation of the Paranan Sea and the final uplift of the Serra do Mar (~12 Mya). Existing studies recognize that the final uplift of Serra do Mar had dramatic effects on drainage patterns because it altered the course of some tributaries of rivers (Potter, 1997; Pérez-Losada *et al*., 2004). Thus, more recent taxa probably speciated after the regression of the Paranan Sea (Pérez-Losada *et al*., 2004).

According to the literature, the distribution area of *Aegla* is limited to the north by the record of *Aegla franca* Schmitt, 1942 in the city of Claraval, state of Minas Gerais, Brazil (20°18′47″S, 47°16′37″W) and to the south by the report of *Aegla alacalufi* Jara & López, 1981 on the island of Duke of York, Chile (50°33′31″S, 75°19′17″W) (Bueno *et al*., 2007; Oyanedel *et al*., 2011). Although some species have been reported in more than one hydrographic basin (Schmitt, 1942; Bond-Buckup & Buckup, 1994; Jara & Palacios, 1999; Oyanedel *et al*., 2011), most are known to occur in tributaries of the same river or only in its type locality (Buckup & Rossi, 1977; Jara, 1982; Rocha & Bueno, 2011; Santos *et al*., 2009, 2010, 2012, 2013, 2014, 2015; Moraes *et al*., 2016, 2017; Bueno *et al*., 2017; Jara *et al*., 2018; Páez *et al*., 2018; Trombetta *et al*., 2019; Marçal *et al*., 2020, 2021).

Current geographic distribution patterns of *Aegla* have been associated with historical factors, such as marine transgressions, tectonic elevations (Pérez-Losada *et al*., 2004), glaciation, and the formation of glacial refuges (Xu *et al*., 2009; Oyanedel *et al*., 2011). Trevisan *et al*. (2009) associated the distribution of *Aegla platensis* Schmitt, 1942 and *Aegla singularis* Ringuelet, 1948 along the Jacutinga River basin, in the state of Rio Grande do Sul (Brazil), to land use and occupation. These authors suggested that the predominance of *A. platensis* in river and pasture springs and *A. singularis* in urban and agricultural areas may be related to riparian coverage, degree of margin erosion, food availability, habitat diversity, competition interspecific, among others. Tumini *et al*. (2016) attested that the distribution of freshwater decapods in southern South America (Argentina) is strongly influenced by local environmental variables, such as altitude, water body stability, annual temperature range, pH, and conductivity. Furthermore, Gonçalves *et al*. (2018) used *Aegla* species distribution modeling to identify and propose priority areas for conservation within four freshwater ecoregions in South America, specifically in extreme south Brazil and adjacent areas in Argentina and Uruguay.

Of the 61 species of *Aegla* recorded in Brazil, 19 occur in the Paraná River basin, seven of which are reported for the Tibagi River basin (Santos *et al*., 2017; Páez *et al*., 2018; Trombetta *et al*., 2019; Marçal *et al*., 2020, 2021; Marçal *et al*., in press). The Tibagi River basin covers approximately 25,000 km^2 of the state of Paraná (SEMA-PR, 2010). Despite the large area and importance of this basin, the aeglids of this region have only recently begun to be studied more extensively (Galves *et al*. 2007; Silva *et al*., 2017; Marçal *et al*., 2018; Chaves *et al*., 2019; Marçal *et al*., 2020; Almeida *et al*., 2021; Silva *et al*., 2021; Marçal *et al*., in press). Probably aeglids are the most threatened freshwater decapod crabs in South America (Bueno *et al*., 2016), and knowledge about the number of species and their geographic ranges is crucial to assess the risk of extinction of these crab species (Boos *et al*., 2012).

Based on our inventory and literature records of *Aegla* species (Schmitt, 1942; Bond-Buckup & Buckup, 1994; Galves *et al*., 2007; Trevisan & Masunari, 2010; Santos *et al*., 2017; Marçal *et al*., 2018; Chaves *et al*., 2019; Marçal *et al*., 2020; Marçal *et al*., in press), the present work aimed to update the geographical distribution of *Aegla* species from the Tibagi River basin, adding new records and describing two new species. The conservation status of the new species was assessed according to criteria established by the International Union for Conservation of Nature (IUCN, 2019). Finally, a diagnostic key to all currently recognized species of *Aegla* from this hydrographic basin is provided.

MATERIAL AND METHODS

Study Area And Data Collection

Tibagi River is a tributary of Paranapanema river, which in turn, drains into the Paraná River. Its main tributaries on the right bank are the Iapó, São Jerônimo, and Congonhas rivers, while on the left bank are the Taquara, Apertados, and Três Bocas rivers (SEMA-PR, 2010). According to Mendonça & Danni-Oliveira (2002), the Tibagi River integrates the region of the Paraná River basin in an area of transition between subtropical and tropical climates. Surprisingly, the Tibagi River has an altitude drop of about 792 m, from its source in Serra das Almas (1.100 m above sea level) until it flows into the Paranapanema River (298 m above sea level) (Maack, 2002; Parolin *et al*., 2010). Because of variations in altitude, climate, and type of vegetation, the Tibagi River basin has been traditionally divided into Upper, Middle, and Lower Tibagi (Anjos *et al*., 1997; Medri *et al*., 2002). Upper Tibagi is the southernmost region of the basin (> 800 m above sea level), where there is a predominance of grassy-woody steppe vegetation and the average annual temperature is lower (Anjos *et al*., 1997; Torezan, 2002). The intermediate region, Middle Tibagi is constituted of the mixed ombrophilous forest (or Aracauria forest) (Torezan, 2002). Finally, the Lower Tibagi, the northernmost region (600–300 m above sea level), is characterized by the seasonal semidecidous forest and the highest average annual temperature (Anjos *et al*., 1997; Torezan, 2002).

Streams and tributary rivers of the Tibagi River (between 23°13′01.24″S, 51°12′58.19″W and 24°40′09.00″S, 50°53′20.80″W), central-eastern portion of Paraná state, Brazil were sampled (Fig. 1). Aeglids deposited in the Crustacean Collection of the Museu de Zoologia, Universidade Estadual de Londrina (MZUEL) were analyzed and identified. Additional collections were made at the type localities of the new species to obtain fresh material for molecular analysis. Specimens were collected by a hand net (90 cm diameter, 4 mm mesh size) and fixed in 70% or absolute alcohol. Sampled specimens are deposited in the Crustacean Collection of the MZUEL.

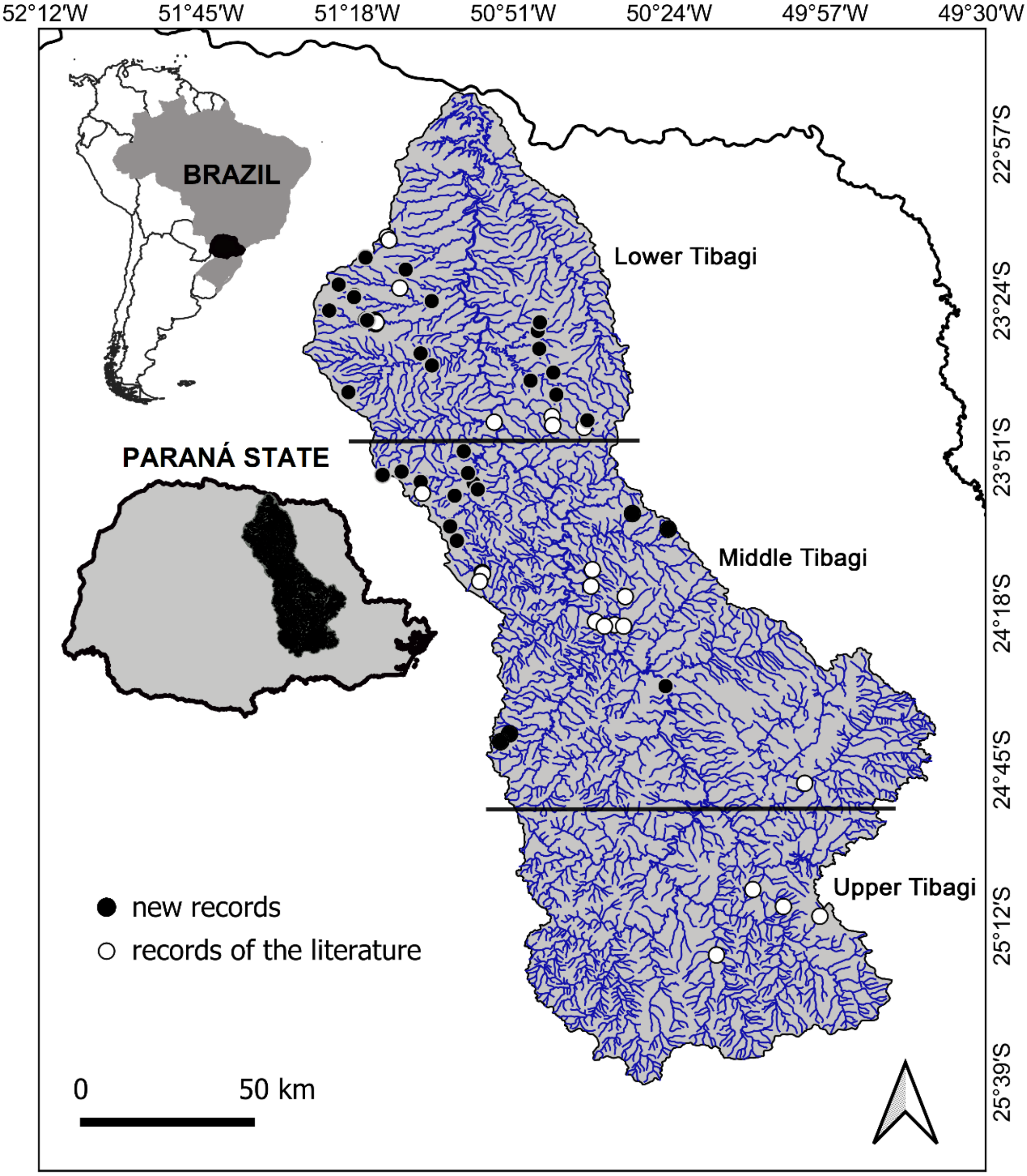

Figure 1. Map of the records of occurrence of *Aegla* in the Tibagi River basin, Paraná state, Brazil. Black circles represent the new records of occurrences, while white correspond to the records reported in the literature.

Morphological Analysis

The taxonomic determination was done following the identification keys (Bond-Buckup & Buckup, 1994) and descriptions (Schmitt, 1942; Santos *et al*., 2015; Moraes *et al*. 2016, 2017; Páez *et al*., 2018; Trombetta *et al*., 2019; Marçal *et al*., 2020, 2021; Marçal *et al*., in press). Sex was recognized by the presence (females) or absence (males) of pleopods and by the position of the gonopore openings (coxa of the third pair of pereiopods in females and of the fifth pair in males) (Martin & Abele, 1988).

Descriptions of the new species use the standard terminology for the carapace and pleon (Martin & Abele, 1988; Bond-Buckup & Buckup, 1994; Moraes *et al*., 2016). Characters of the carapace, chelipeds, thoracic sternite and epimeron were examined in detail, and photographs were taken using a Leica stereomicroscope (model M205, Leica Biosystems, Wetzlar, Germany). The following abbreviations are used: areolar anterior demarcation (AAD), areolar height (AH), areolar posterior margin (APM), carapace length including rostrum (CL), carapace length excluding rostrum (CLE), carapace width (CW), half the maximum width of telson (HWT), lateral margin of rostrum (LMR), pre-cervical width (PCW), mesial region of the posterior margin of the cephalothorax (PMC), rostral base width (RBW), transversal dorsal linea length (TDL), width of uropod (WU). All measurements (in mm) were taken using a 0.01 mm precision caliper. The morphometric ratios RBW/LMR, APM/AAD, AH/[(APM+AAD)/2], TDL/PMC, and WU/HWT were calculated according to Moraes *et al*. (2016).

Molecular Analysis

DNA was extracted from muscle tissues following the protocol described by Marçal *et al*. in press. Amplification of partial sequences of the COI gene was carried out by polymerase chain reaction (PCR) using the primers of van Syoc (1995), following the PCR method proposed by Pérez-Losada *et al*. (2002). Prior to sequencing PCR products were cleaned with ExoSAP IT® (Prodimol Biotecnologia, Belo Horizonte, MG, Brazil). Purified PCR products were sequenced in both directions on an ABI 3500xL automated sequencer using ABI Big Dye Terminator v 3.1. Sequences were assembled into contigs using Electropherogram Quality Analysis software (Togawa & Brigido, 2003). Contigs were aligned using ClustalW implemented in MEGA 6.0 (Tamura *et al*., 2013). We adjusted our dataset with the sequences available in the GenBank or BOLD database for *Aegla* species that are morphologically similar to the new species or occur in the Tibagi River basin. Genetic distances (intra and interspecific) were calculated in MEGA 6.0 based on Kimura-2-parameter model (Kimura, 1980). The HKY+I model (proportion of invariant sites = 0.802) was selected by jModelTest2 (Darriba *et al*., 2012) as the most adequate mutation model for the data. Bayesian analysis was then conducted in BEAST 2.6.2 (Bouckaert *et al*., 2019), sampling one tree every 1,000 generations per 100,000,000 generations. Posterior probabilities were calculated with a burn-in of 25%. Finally, the Generalized Mixed Yule Coalescent (GMYC) model was carried out in Splits package (Fujisawa & Barraclough, 2013; Pons *et al*., 2006) with R 3.5.1. program, using the unique threshold method to delimit the species.

Geographical Distribution

We researched data on the occurrence of aeglids in the Tibagi River basin from four scientific collections of crustaceans (MN/UFRJ, MZUSP, MHNCI, UFRGS) and one invertebrate zoology collection (NMNH). We used data from the collections because they include the records not published and those cited in the literature. These records were added to those obtained in the present work to make maps of species geographic distribution. Localities were georeferenced using Google Earth® software. If the collection site coordinates were not available, we used the city coordinate as the reference point. We obtained the city coordinates using GeoNames (http://www.geonames.org/). The geographic distribution maps of the species were prepared using the free software QGIS v.3.10.7 (https://www.qgis.org/pt_BR/site/).

RESULTS

Surveys resulted in 51 records of Aeglidae crabs in the study area (Table 1), comprising nine species, of which two are morphologically and genetically distinct new species. Considering the Tibagi River basin regions, three species are present in the Upper Tibagi (*Aegla castro* Schmitt, 1942, *Aegla schmitti* Hobbs, 1979, and *Aegla lata* Bond-Buckup & Buckup, 1994), five in the Middle Tibagi (*A. castro*, *Aegla* n. sp. 2 Marçal & Teixeira in press, *Aegla* n. sp. 3 Marçal & Teixeira in press, *Aegla* n. sp. 4, and *Aegla* n. sp. 5) and four species occur in the Lower Tibagi (*A. castro*, *A. lata*, *Aegla jacutinga* Marçal & Teixeira, 2020, and *Aegla* n. sp. 1 Marçal & Teixeira in press). In addition, 25 new records of occurrence of three aeglids species, previously described, were added (Fig. 2). One of them is *A. castro*, species widely distributed throughout the Tibagi River basin, occurring from the Apucararinha River (Londrina city) to the Guaraúna Creek (Ponta Grossa city). However, the ten new records are within the already known geographical distribution (Fig. 2A). It is worth mentioning that Fig. 2A does not represent all the distribution sites of *A. castro*, as this species also occurs on the right bank of the Paranapanema River. *Aegla lata*, in turn, is known from the southeastern and northwestern portions of the Tibagi River basin, occurring in streams in the cities of Ponta Grossa and Londrina. The five new records extend its range towards the city of Califórnia, north of Paraná state (Fig. 2C). Finally, *A. jacutinga* is known from the northwestern portions of the Tibagi River basin. Although this species had already been recorded in streams in the city of Londrina, its distribution was described only for two streams. Herein, we report five new populations found in Londrina and surrounding cities. Besides, five localities in the cities of São Sebastião da Amoreira, Nova Santa Bárbara, and São Jerônimo da Serra are new records and broaden the geographical distribution of *A. jacutinga* towards the northeastern of the Tibagi basin (Fig. 2D). The specimens examined are very similar to those described by Marçal *et al.* (2020), notwithstanding they can present more pronounced epigastric prominences and an almost straight anterior margin of the second abdominal epimeron. We believe, however, that such differences are subtle and can be considered as intraspecific variability.

Table 1. Records of occurrence of *Aegla* species in the Tibagi River basin, Paraná state, Brazil.

Species	Region	City	Locality	Geographical coordinates
Aegla jacutinga Marçal & Teixeira, 2020[7]	LT	Londrina	Jacutinga stream	23°13′31.05″S, 51°12′39.47″W
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	Cambé	Tributary of the Cafezal stream	23°16′33.00″S, 51°16′42.00″W*
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	Londrina	Lindóia stream	23°18′37.00″S, 51°09′46.00″W*
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	Rolândia	Cavalaris stream	23°21′12.90″S, 51°21′22.50″W
Aegla jacutinga Marçal & Teixeira, 2020[7]	LT	Londrina	Tributary of the Cafezal stream	23°21′48.90″S, 51°10′47.10″W
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	Rolândia	Três Bocas Creek	23°22′59.90″S, 51°18′37.60″W
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	Londrina	Tributary of the Três Bocas Creek	23°23′23.00″S, 51°18′41.80″W
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	São Sebastião da Amoreira	Três Barras stream	23°29′12.83″S, 50°46′55.74″W
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	Nova Santa Bárbara	José Maria River	23°32′17.54″S, 50°46′39.76″W
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	Nova Santa Bárbara	Tigre stream	23°36′24.28″S, 50°44′13.82″W
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	São Jerônimo da Serra	Matão stream	23°37′47.93″S, 50°48′9.49″W
Aegla jacutinga Marçal & Teixeira, 2020[9]	LT	São Jerônimo da Serra	São Jerônimo River	23°44′39.16″S, 50°38′19.98″W
Aegla lata Bond-Buckup & Buckup, 1994[9]	LT	Londrina	Tributary of the Apertados Creek	23°25′41.60″S, 51°23′00.20″W
Aegla lata Bond-Buckup & Buckup, 1994[6]	LT	Londrina	Bule stream	23°27′20.86″S, 51°16′32.44″W
Aegla lata Bond-Buckup & Buckup, 1994[9]	LT	Londrina	Coqueiros River	23°27′22.08″S, 51°16′25.07″W
Aegla lata Bond-Buckup & Buckup, 1994[4]	LT	Londrina	Stream 1 of the MGSP	23°27′23.50″S, 51°15′05.50″W
Aegla lata Bond-Buckup & Buckup, 1994[4]	LT	Londrina	Stream 2 of the MGSP	23°27′45.60″S, 51°14′52.30″W
Aegla lata Bond-Buckup & Buckup, 1994[9]	LT	Londrina	Tributary of the Taquara River	23°33′06.10″S, 51°07′10.80″W
Aegla lata Bond-Buckup & Buckup, 1994[9]	LT	Londrina	Marrequinha stream	23°35′11.00″S, 51°05′14.00″W*
Aegla lata Bond-Buckup & Buckup, 1994[9]	LT	Califórnia	Jacucaca stream	23°39′48.00″S, 51°19′41.00″W*
Aegla lata Bond-Buckup & Buckup, 1994[2]	UT	Ponta Grossa	Pinto River	25°5′42.00″S, 50°9′43.00″W*
Aegla n. sp. 1 Marçal & Teixeira in press[8]	LT	São Jerônimo da Serra	Tigre River	23°44′00.50″S, 50°44′26.74″W
Aegla n. sp. 1 Marçal & Teixeira in press[8]	LT	São Jerônimo da Serra	Pilões stream	23°45′31.25″S, 50°44′19.70″W
Aegla castro Schmitt, 1942[2]	LT	Londrina	Apucaraninha River	23°44′58.62″S, 50°54′27.01″W*
Aegla castro Schmitt, 1942[9]	MT	Tamarana	Encontro stream	23°50′01.10″S, 50°59′43.70″W
Aegla castro Schmitt, 1942[9]	MT	Mauá da Serra	Recanto Pinhão stream	23°53′31.19″S, 51°10′29.54″W
Aegla castro Schmitt, 1942[9]	MT	Mauá da Serra	Unnamed stream	23°54′05.00″S, 51°13′46.00″W*
Aegla castro Schmitt, 1942[9]	MT	Tamarana	Água Branca River	23°55′30.91″S, 50°58′01.32″W
Aegla castro Schmitt, 1942[9]	MT	Mauá da Serra	Meio River	23°55′17.10″S, 51°07′09.80″W

Aegla castro Schmitt, 1942[9]	MT	Tamarana	Água do Anta River	23°56′35.97″S, 50°57′17.81″W
Aegla castro Schmitt, 1942[5]	MT	Mauá da Serra	Couro River	23°57′15.60″S, 51°06′53.80″W
Aegla castro Schmitt, 1942[9]	MT	Mauá da Serra	Preto River	23°57′23.10″S, 51°06′50.30″W
Aegla castro Schmitt, 1942[9]	MT	Tamarana	Água Boa stream	23°57′40.43″S, 51°01′17.46″W
Aegla castro Schmitt, 1942[9]	MT	Ortigueira	Apucarana River	24°05′26.82″S, 51°00′54.66″W
Aegla castro Schmitt, 1942[9]	MT	Tibagi	Rosas stream	24°30′34.00″S, 50°24′49.00″W*
Aegla castro Schmitt, 1942[1]	MT	Castro	Iapó River	24°47′21.00″S, 50°0′44.00″W*
Aegla castro Schmitt, 1942[2]	UT	Ponta Grossa	Quebra-Perna River	25°10′18.33″S, 49°58′6.64″W*
Aegla castro Schmitt, 1942[3]	UT	Ponta Grossa	Guaraúna Creek	25°17′00.00″S, 50°16′00.00″W
Aegla n. sp. 4[9]	MT	Curiúva	Unnamed stream	24°00′45.39″S, 50°30′32.44″W
Aegla n. sp. 4[9]	MT	Curiúva	Água das Pombas stream	24°03′27.84″S, 50°24′20.34″W
Aegla n. sp. 2 Marçal & Teixeira in press[8]	MT	Ortigueira	Tributary of the Formiga River	24°10′56.50″S, 50°56′31.50″W
Aegla n. sp. 2 Marçal & Teixeira in press[8]	MT	Ortigueira	Formiga River	24°11′11.77″S, 50°56′28.60″W
Aegla n. sp. 2 Marçal & Teixeira in press[8]	MT	Ortigueira	Piquira River	24°12′30.00″S, 50°56′58.00″W*
Aegla n. sp. 3 Marçal & Teixeira in press[8]	MT	Telêmaco Borba	Colônia stream	24°10′27.79″S, 50°37′28.45″W
Aegla n. sp. 3 Marçal & Teixeira in press[8]	MT	Telêmaco Borba	Moinho velho stream	24°13′18.34″S, 50°37′42.42″W
Aegla n. sp. 3 Marçal & Teixeira in press[8]	MT	Telêmaco Borba	Codorna Branca stream	24°15′06.70″S, 50°31′46.58″W
Aegla n. sp. 3 Marçal & Teixeira in press[8]	MT	Telêmaco Borba	Harmonia stream	24°19′26.00″S, 50°36′56.00″W*
Aegla n. sp. 3 Marçal & Teixeira in press[8]	MT	Telêmaco Borba	Varanal stream	24°20′12.70″S, 50°32′03.20″W
Aegla n. sp. 5[9]	MT	Reserva	Tributary of the Tibagi River	24°38′47.70″S, 50°51′48.10″W
Aegla n. sp. 5[9]	MT	Reserva	Maromba stream	24°40′09.00″S, 50°53′20.80″W
Aegla schmitti Hobbs, 1979[2]	UT	Ponta Grossa	Roça Velha River	25°08′38.00″S, 50°04′25.00″W*

LT, Lower Tibagi; MGSP, Mata dos Godoy State Park; MT, Middle Tibagi; UT, Upper Tibagi. The number above species name indicates the reference: 1 = Schmitt (1942); 2 = Bond-Buckup & Buckup (1994); 3 = Pérez-Losada *et al*. (2004); 4 = Galves *et al*. (2007); 5 = Marçal *et al*. (2018); 6 = Chaves *et al*. (2019); 7 = Marçal *et al*. (2020); 8 = Marçal *et al*. in press; 9 = Present work. Underlined localities are the type localities. The type locality of *Aegla schmitti* is in the Iguaçu River basin. The asterisk represents the use of city coordinate as the reference point.

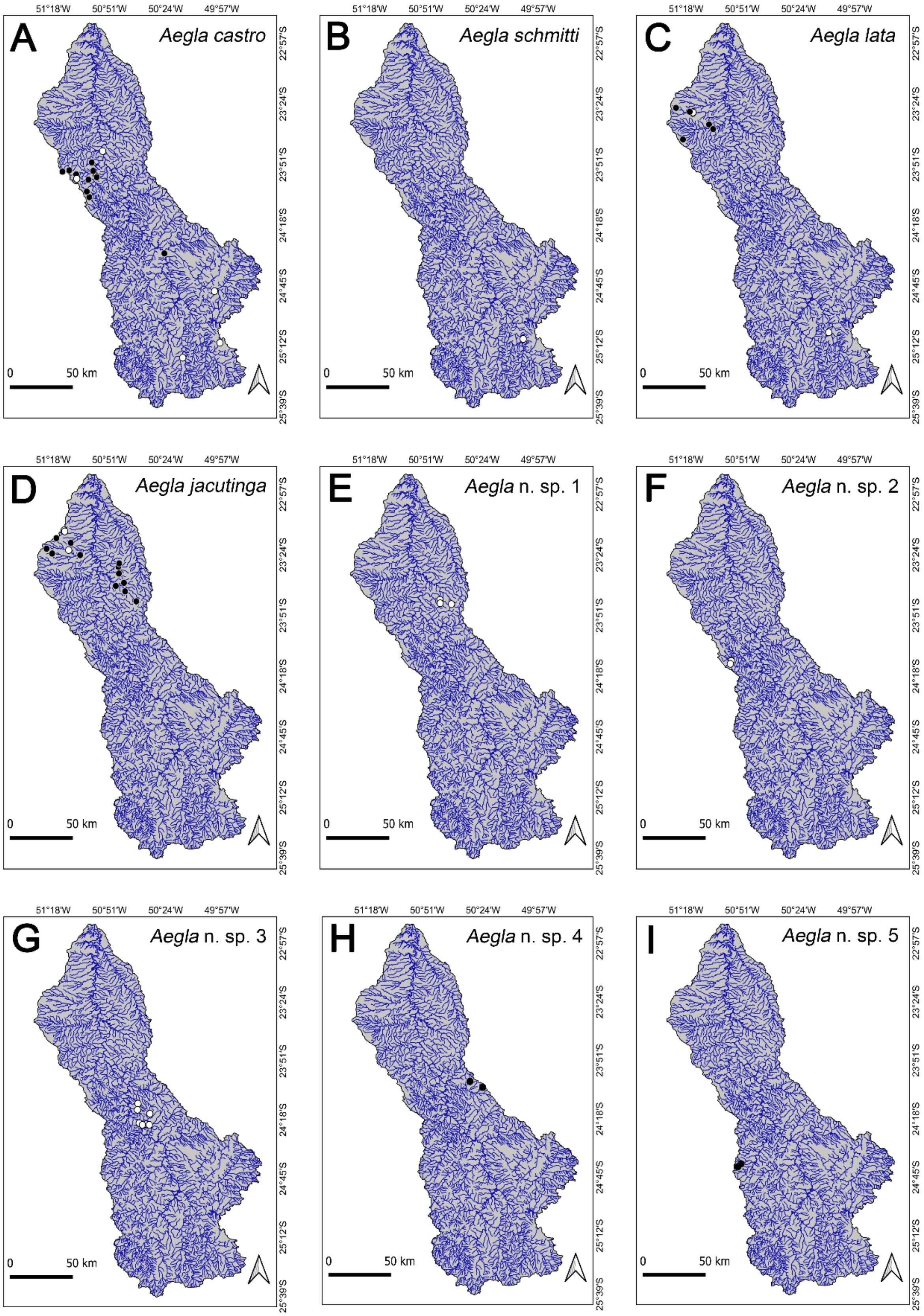

Figure 2. Distribution map of *Aegla* species in the Tibagi River basin. A, Aegla *castro*. B, *Aegla schmitti*. C, *Aegla lata*. D, *Aegla jacutinga*. E, *Aegla* n. sp. 1. F, *Aegla* n. sp. 2. G, *Aegla* n. sp. 3. H, *Aegla* n. sp. 4. I, *Aegla* n. sp. 5. Black circles represent the new records of

occurrences, while white correspond to the records reported in the literature.

TAXONOMY

Family Aeglidae Dana, 1852

Genus *Aegla* Leach, 1820

***Aegla* n. sp. 4**

(FIGS 3–6)

Type material: Holotype. male, Brazil, Paraná, Curiúva, Tibagi River basin, Água das Pombas stream, 24°03′27.84″S, 50°24′20.34″W, 769 m, 26 June 2014, coll. unknown (MZUEL 512). Paratypes. 2 males, same data as the holotype (MZUEL 390); 2 males and 3 females, same locality as holotype, 11 March 2020, G.M. Teixeira, I.C. Marçal & P.F. da Silva coll. (MZUEL 506, genetic vouchers: Bold Systems access AEGLA001-21, AEGLA002-21).

Additional material examined: 1 male and 1 female, Brazil, Paraná, Curiúva, Tibagi River basin, Unnamed stream, 24°00′45.39″S, 50°30′32.44″W, 697 m, 19 August 2014, J.F.M. da Silva, F.C. Jerep & R.H.C. Nascimento coll. (MZUEL 391).

Diagnosis: Rostrum triangular, extending beyond distal apex of compound eyes, carinate along entire length. Subrostral process well developed, occupying proximal half of subrostral margin, anterior and posterior margins forming obtuse angle. Orbital sinus deep, extra-orbital sinus shallow. Epigastric prominences pronounced. Protogastric lobes pronounced. Epibranchial area strongly elongated. Proximal dorsal margin of dactylus with moderate size lobe. Palmar crest rectangular, outer surface slightly excavated, margin serrated. Outer surface of carpus with carpal ridge high. Ventromesial border of cheliped ischium with 3 to 5 tubercles. Anteromesial region of third thoracic sternite tapered. Anterolateral angle of second abdominal epimeron unarmed. Ventral angles of third and fourth abdominal epimeron with corneous scales apically. Anterior margin of second abdominal epimeron almost straight. Uropods wide.

Description of holotype: Carapace moderately convex, gastric region convex, dorsal surface scabrous, covered with punctations (Fig. 3). Rostrum triangular, wide base (RBW/LMR = 1.00), reaching distal apex of compound eyes, carinate along entire length, small corneous scales on lateral margins and tip; ventral portion of rostrum much higher than dorsal in profile. Rostral carina beginning at level of protogastric lobes, with 2 rows of corneous scales extending next to apex. Subrostral process well developed, occupying proximal half of subrostral margin, produced ventrally, triangular shaped, tip rounded, anterior and posterior margins forming obtuse angle (113°) (Fig. 4A).

Eyestalk and cornea well developed. Orbital sinus deep, extra-orbital sinus shallow (Fig. 4B). Orbital sinus U-shaped. Orbital spines well developed, with small terminal corneous scale. Anterolateral spines acuminate apically with small corneous scales terminally, extending beyond basal margin of cornea (Fig. 4B).

Epigastric prominences pronounced, with corneous scales. Protogastric lobes pronounced, with corneous scales (Fig. 4A). Gastric area elevated in relation to hepatic lobes and rostrum in lateral view. Demarcation between the first and the second hepatic lobe well defined, between the second and the third hepatic lobe weakly delimited. Lateral margins of hepatic lobes with small corneous scales and small setae.

Cervical groove trapezoidal. Transverse dorsal linea sinuous along its extension. Areola trapezoidal (APM/AAD = 2.00). Cardiac area subrectangular (TDL/PMC = 1.31). Epibranchial area strongly elongated, anterolateral angle with corneous scale, lateral margin with row of corneous scales and small setae (Fig. 4B). Lateral margins of anterior and posterior branchial areas with row of corneous scales and small setae.

Figure 3. *Aegla* n. sp. 4, male holotype, MZUEL 512. Specimen collected in the Água das Pombas stream, Curiúva, Paraná, Brazil. Dorsal view of the cephalothorax and abdomen. Scale bar = 5.0 mm.

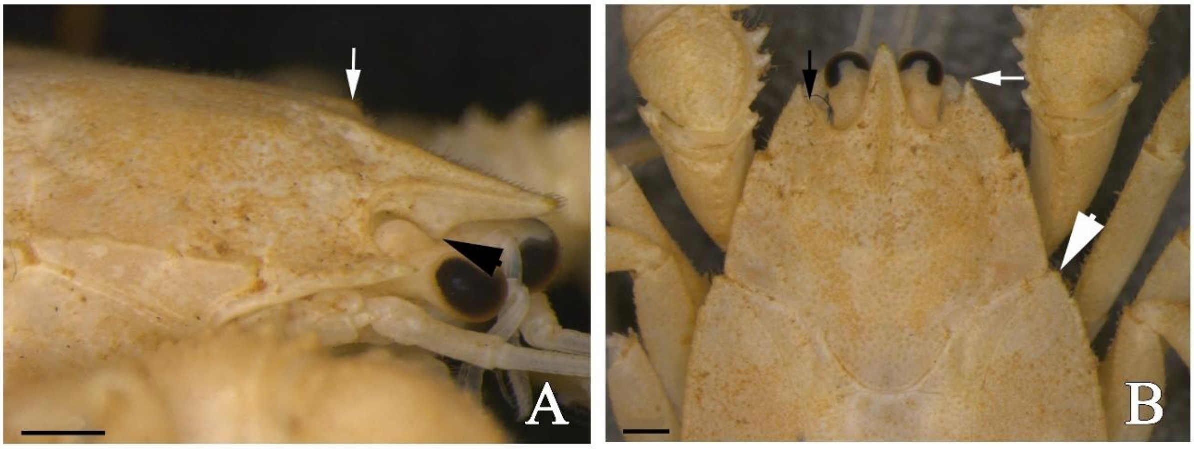

Figure 4. *Aegla* n. sp. 4, male holotype, MZUEL 512. A, external surface view of the anterior portion of the cephalothorax showing pronounced protogastric lobes (white arrow) and well developed subrostral process (black arrow). B, dorsal view of the anterior region of the cephalothorax showing shallow extra-orbital sinus (black arrow), anterolateral spine extending beyond basal margin of cornea (thin white arrow) and strongly elongated epibranchial area (thick white arrow). Scale bars = 1.0 mm.

Chelipeds unequal. Left cheliped largest (Figs 5A, B). Dactylus: dorsal margin and outer surface with small corneous scales, inner surface with setal tufts and scales. Proximal dorsal margin with moderate size lobe. Cutting margin with well-developed lobular basal tooth, followed by row of corneous scales up to distal end. Row of small tufts of long setae next to cutting margin. Pre-dactylar lobe well developed, without corneous scales. Propodus: outer surface granular, globose aspect. Palmar crest rectangular with outer surface slightly excavated, margin serrated, covered by acuminate corneous scales. Cutting margin of fixed finger with well-developed lobular basal tooth, followed by row of corneous scales up to distal end. Inner and outer surfaces of fixed finger with rows of long setae tufts next to cutting margin. Carpus: dorsal margin with proximal tubercle and 3 spines, distal spine longest, each spine with small setae and terminal corneous scale, subterminal lobe well defined, pointed, with small corneous scales and setae. Inner surface with 2 small tubercles, long setae next to dorsal margin. Outer surface with carpal ridge high, with small corneous scales. Merus:

dorsolateral edge with distal tubercle, with corneous scale terminally, followed by row of tubercles decreasing in size proximally. Ventromesial edge with distal spine, with corneous scale terminally, followed by 3 tubercles of similar size, with corneous scale. Ventrolateral border with distal tubercle, with terminal corneous scale, followed by row of tubercles decreasing in size proximally. Ischium: dorsolateral edge with tubercle, with corneous scale terminally. Ventromesial border with proximal tubercle, small median tubercle and large distal tubercle, each with terminal corneous scale. Ventrolateral border smooth.

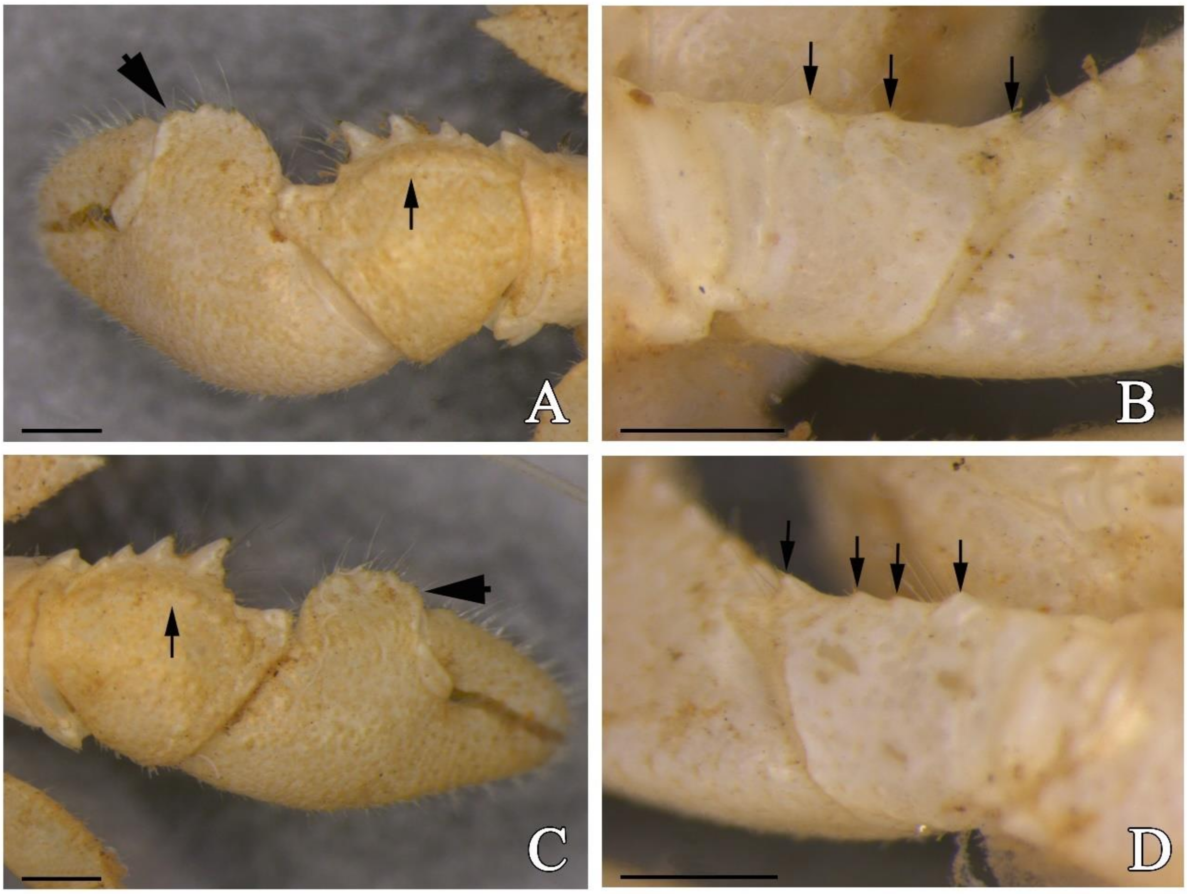

Figure 5. *Aegla* n. sp. 4, male holotype, MZUEL 512. A, dorsal view of the dactylus, propodus, and carpus of major cheliped (left) showing rectangular palmar crest (thick arrow) and high carpal ridge (thin arrow). B, ventral view of ischium of major cheliped (left) with three tubercles (arrows). C, dorsal view of the dactylus, propodus, and carpus of minor cheliped (right) showing rectangular palmar crest (thick arrow) and high carpal ridge (thin arrow). D, ventral view of ischium of minor cheliped (right) with four tubercles (arrows). Scale bars = 1.0 mm.

Minor cheliped (right) similar to major cheliped except as noted hereafter (Figs 5C, D). Dactylus: proximal dorsal margin with rudimentary lobe. Cutting margin with rudimentary lobular basal tooth. Propodus: cutting margin with rudimentary lobular basal tooth. Carpus: dorsal margin with 2 proximal tubercles, median tubercle, distal spine longest, each tubercle or spine with small setae and terminal corneous scale. Merus: ventromesial edge with distal spine, followed by 4 tubercles, each with terminal corneous scale. Ischium: ventromesial border with proximal tubercle, 2 small median tubercles and large distal tubercle, each with terminal corneous scale.

Second, third, and fourth pereiopods morphologically similar. Dactyli, propodi, carpi, meri and ischii with several rows of setal tufts and small scales on surface. Carpi and meri with row of small tubercles with terminal corneous scale along dorsal margin. Meri with row of small tubercles with terminal corneous scale along ventral margin. Meri and ischii with long setae concentrated along dorsal margin.

Anteromesial region of third thoracic sternite tapered, projecting between coxae of third maxillipeds, with scattered setae. Fourth thoracic sternite with anterolateral angles produced anteriorly, with scattered setae (Fig. 6A). Pleopods 2–5 absent. Anterolateral angle of second abdominal epimeron well defined, unarmed (Fig. 6B). Ventral angles of third and fourth abdominal epimeron well defined, with corneous scales apically. Anterior margin of second abdominal epimeron almost straight (Fig. 6B). Uropods well developed, wide (WU/HWT = 1.06). Telson divided by longitudinal suture. Anterolateral and posterolateral margins well differentiated.

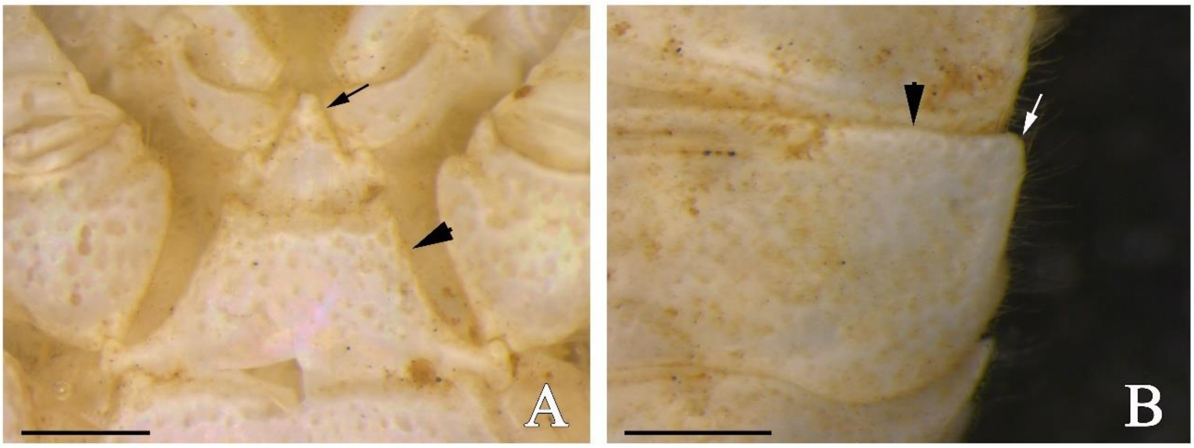

Figure 6. *Aegla* n. sp. 4, male holotype, MZUEL 512. A, third (thin arrow) and fourth (thick arrow) thoracic sternites. B, dorsal view of second abdominal epimeron (right side) showing almost straight anterior margin (black arrow) and unarmed anterolateral angle (white arrow). Scale bars = 1.0 mm.

Variations: The base of the rostrum can be narrow (n = 3) or wide (n = 3). The areola can be trapezoidal (n = 3), subrectangular (n = 3), or rectangular (n = 1). The cardiac area varies from trapezoidal (n = 5) to subrectangular (n = 2). The ventromesial border of ischium may present five tubercles instead of three or four as in the holotype. In most specimens, the anteromesial region of third thoracic sternite is bifurcated, with two corneous scales terminally. Measurements and morphometric relationships of the type series specimens are summarized in Table 2.

Table 2. General measurements and morphometric ratios of the type material of *Aegla* n. sp. 4 and *Aegla* n. sp. 5.

	Aegla n. sp. 4		*Aegla* n. sp. 5	
	Holotype	Paratypes (n = 7)	Holotype	Paratypes (n = 6)
CL	11.6	14.04(2.26)	13.7	17.40(3.94)
CLE	9.6	11.90(2.22)	11.5	14.63(3.17)
CW	9.3	11.90(2.28)	11.2	14.63(3.50)
PCW	7.0	8.93(1.69)	8.2	10.47(2.46)
RBW	2.2	2.50(0.37)	2.5	2.95(0.60)
LMR	2.2	2.50(0.39)	2.7	3.22(0.74)
APM	2.0	2.20(0.56)	2.0	2.82(0.86)
AAD	1.0	1.29(0.29)	1.1	1.43(0.42)
AH	3.2	3.84(0.72)	4.0	5.20(1.11)
TDL	3.8	4.86(0.79)	4.4	5.92(1.33)
PMC	2.9	3.41(0.76)	3.0	4.32(1.30)
WU	1.8	2.30(0.37)	2.2	2.92(0.71)
HWT	1.7	2.17(0.35)	1.9	2.70(0.78)
CW/CLE	0.97	0.99(0.04)	0.97	1.00(0.03)
PCW/CW	0.75	0.75(0.02)	0.73	0.72(0.01)
RBW/LMR	1.00	0.97(0.03)	0.93	0.92(0.04)
APM/AAD	2.00	1.71(0.18)	1.82	1.97(0.18)
AH/[(APM+AAD)/2]	—	2.25(0.27)	—	—
TDL/PMC	1.31	1.44(0.10)	1.47	1.40(0.12)
WU/HWT	1.06	1.06(0.03)	1.16	1.09(0.07)

All measurements in mm. The measures of the paratypes are shown as mean (standard deviation). AAD, areolar anterior demarcation; AH, areolar height; APM, areolar posterior margin; CL, carapace length including rostrum; CLE, carapace length excluding rostrum; CW, carapace width; HWT, half the maximum width of telson; LMR, lateral margin of rostrum; PCW, pre-cervical width; PMC, mesial region of the posterior margin of the cephalothorax; RBW, rostral base width; TDL, transversal dorsal linea length; WU, width of uropod.

Remarks: Aegla n. sp. 4 can be easily differentiated from its congeners from the Tibagi River basin by several morphological characteristics. The new species have a rectangular palmar crest, diverging from the subdisciform to the disciform palmar crest of *A. castro*, *A. schmitti*, *A. lata*, *A. jacutinga*, and *Aegla* n. sp. 1. Moreover, the subrostral process occupies the proximal half of the subrostral margin in *Aegla* n. sp. 4 whereas in *A. lata*, *A. jacutinga*, *Aegla* n. sp. 1, and *Aegla* n. sp. 3 is on the proximal third of subrostral margin. The pronounced protogastric lobes of *Aegla* n. sp. 4 are shared with *A. castro*, *A. schmitti*, *A. jacutinga*, *Aegla*

n. sp. 1, *Aegla* n. sp. 2, and *Aegla* n. sp. 3. However, the new species can be differentiated from *A. castro*, *A. schmitti*, and *A. jacutinga* by present the anterior margin of the second abdominal epimeron almost straight. The epigastric prominences and the carpal ridge of *A. jacutinga* are poorly pronounced and low, distinguishing it from *Aegla* n. sp. 4. Besides, *A. castro* and *A. schmitti* exhibit a spine on the anterolateral angle of the second abdominal epimeron while this structure is unarmed in the new species. An unusual feature that discriminates the new species from *Aegla* n. sp. 1 is the presence of rudimentary pleopods in adult male specimens of the latter species. Morphologic characters that distinguish *Aegla* n. sp. 4 from *Aegla* n. sp. 2 are the shortened epibranchial area, the rudimentary lobe on the proximal dorsal margin of the dactylus, and the anteromesial region of the third thoracic sternite of the abrupt format in *Aegla* n. sp. 2. Similarly, *Aegla* n. sp. 4 can be separated from *Aegla* n. sp. 3 by the slightly elongated epibranchial area and the rudimentary or absent lobe on the proximal dorsal margin of the dactylus in *Aegla* n. sp. 3. *Aegla* n. sp. 4 and *A. lata* share the presence of corneous scales in the ventral angles of the third and fourth abdominal epimeron. Notwithstanding, the new species differs from *A. lata* in having pronounced epigastric prominences, pronounced protogastric lobes, and wide uropods. Another morphological characteristic, the shallow extra-orbital sinus of *Aegla* n. sp. 4, is not reported for other species of the same basin.

Although they occur in different hydrographic basins, *Aegla parva* Bond-Buckup & Buckup, 1994, *Aegla meloi* Bond-Buckup & Santos, 2015, *Aegla okora* Páez & Teixeira, 2018, and *Aegla nebeccana* Trombetta, Páez, Santos & Teixeira, 2019 resemble the new species in the shape of the palmar crest of cheliped. Nevertheless, *Aegla* n. sp. 4 can be distinguished from these species especially by the: 1) epigastric prominences absent in *A. meloi*, poorly pronounced in *A. parva* and inconspicuous in *A. nebeccana*; 2) proximal dorsal margin of dactylus without lobe in *A. parva*, *A. meloi*, *A. okora*, and *A. nebeccana*; 3) ventromesial border of cheliped ischium with a distal tubercle in *A. meloi*; 4) anterolateral angle of second abdominal epimeron with a spine in *A. parva*; 5) anterior margin of second abdominal epimeron slightly concave in *A. parva*, *A. okora*, and *A. nebeccana*; and 6) narrow uropods in *A. nebeccana*.

Geographical distribution: The new species has been collected in two streams within the Tibagi River basin. Both streams are located in the city of Curiúva, Paraná state, Brazil (Fig. 2H).

***Aegla* n. sp. 5**

(FIGS 7–10)

Type material: Holotype. male, Brazil, Paraná, Reserva, Tibagi River basin, tributary of the Tibagi River, 24°38′47.70″S, 50°51′48.10″W, 901 m, 10 March 2020, G.M. Teixeira, I.C. Marçal & R.S. Vieira coll. (MZUEL 514). Paratypes. 2 males and 2 females, same data as for holotype (MZUEL 503, genetic vouchers: Bold Systems access AEGLA007-21, AEGLA008-21); 1 male and 1 female, same locality as holotype, 09 April 2015, A.C. Hoffmann coll. (MZUEL 463).

Additional material examined: 6 males and 4 females, Brazil, Paraná, Reserva, Tibagi River basin, Maromba stream, 24°40′09.00″S, 50°53′20.80″W, 911 m, 09 April 2015, A.C. Hoffmann coll. (MZUEL 462).

Diagnosis: Rostrum triangular, narrow base, extending beyond distal apex of compound eyes, carinate along entire length. Subrostral process well developed, occupying proximal half of subrostral margin, anterior and posterior margins forming obtuse angle. Orbital and extra-orbital sinuses deep. Epigastric prominences pronounced. Protogastric lobes pronounced. Cervical groove U-shaped. Areola trapezoidal. Epibranchial area slightly elongated. Proximal dorsal margin of dactylus with rudimentary lobe. Palmar crest rectangular, outer surface slightly excavated, margin poorly serrated. Outer surface of carpus with carpal ridge high. Ventromesial border of cheliped ischium with 4 tubercles. Anterolateral angle of second abdominal epimeron unarmed. Ventral angles of third and fourth abdominal epimeron with corneous scales apically. Anterior margin of second abdominal epimeron almost straight. Uropods wide.

Description of holotype: Carapace moderately convex, gastric region convex, dorsal surface scabrous, covered with punctations (Fig. 7). Rostrum triangular, narrow base (RBW/LMR = 0.93), extending beyond distal apex of compound eyes, carinate along entire length, small corneous scales on lateral margins and tip; ventral portion of rostrum much higher than dorsal in profile. Rostral carina beginning at level of protogastric lobes, with 2 rows of corneous scales extending next to apex. Subrostral process well developed and covered by small setae, occupying proximal half of subrostral margin, tip rounded, anterior and posterior margins forming obtuse angle (126°) (Fig. 8A).

Eyestalk and cornea well developed. Orbital and extra-orbital sinuses deep. Orbital sinus U-shaped. Orbital spines well developed, with small terminal corneous scale. Anterolateral spines acuminate apically with small corneous scales terminally, reaching basal margin of cornea.

Epigastric prominences pronounced, with corneous scales. Protogastric lobes pronounced, with corneous scales (Fig. 8A). Gastric area elevated in relation to hepatic lobes and rostrum in lateral view. Demarcation between the first and the second hepatic lobe well defined, between the second and the third hepatic lobe weakly delimited. Lateral margins of hepatic lobes with small corneous scales and small setae.

Cervical groove U-shaped (Fig. 8B). Transverse dorsal linea sinuous along its extension. Areola trapezoidal (APM/AAD = 1.82). Cardiac area trapezoidal (TDL/PMC = 1.47). Epibranchial area slightly elongated, triangular shaped, anterolateral angle with corneous scale, lateral margin with row of corneous scales and small setae (Fig. 8B). Lateral margins of anterior and posterior branchial areas with row of corneous scales and small setae.

Figure 7. *Aegla* n. sp. 5, male holotype, MZUEL 514. Specimen collected in the tributary of the Tibagi River, Reserva, Paraná, Brazil. Dorsal view of the cephalothorax and abdomen. Scale bar = 5.0 mm.

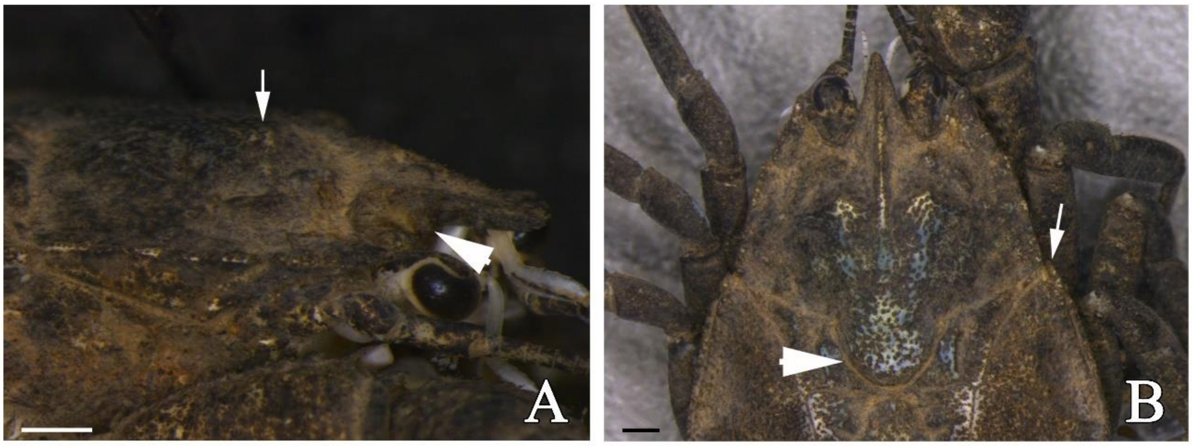

Figure 8. *Aegla* n. sp. 5, male holotype, MZUEL 514. A, external surface view of the anterior portion of the cephalothorax showing pronounced protogastric lobes (thin arrow) and well developed subrostral process (thick arrow). B, dorsal view of the anterior region of the cephalothorax showing U-shaped cervical groove (thick arrow) and slightly elongated epibranchial area (thin arrow). Scale bars = 1.0 mm.

Chelipeds unequal. Left cheliped largest (Figs 9A, B). Dactylus: dorsal margin and outer surface with small corneous scales, inner surface with setal tufts and scales. Proximal dorsal margin with rudimentary lobe. Cutting margin with well-developed lobular basal tooth, followed by row of corneous scales up to distal end. Row of small tufts of long setae next to cutting margin. Pre-dactylar lobe well developed, rounded, without corneous scales. Propodus: outer surface granular, globose aspect. Palmar crest rectangular with outer surface slightly excavated, margin poorly serrated, covered by acuminate corneous scales. Cutting margin of fixed finger with well-developed lobular basal tooth, followed by row of corneous scales up to distal end. Inner and outer surfaces of fixed finger with rows of long setae tufts next to cutting margin. Carpus: dorsal margin with proximal tubercle, 2 median spines, distal spine longest, each tubercle or spine with small setae and terminal corneous scale, subterminal lobe well defined, pointed, with small corneous scales and setae. Inner surface with large tubercle, long setae next to dorsal margin. Outer surface with carpal ridge high, with small corneous scales. Merus: dorsolateral edge with distal tubercle, with corneous scale terminally, followed by row of tubercles decreasing in size proximally. Ventromesial edge with 2 distal spines, with corneous scale terminally, followed by 3 tubercles of similar size, with corneous scale. Ventrolateral border with distal tubercle, with terminal corneous scale, followed by row of tubercles decreasing in size proximally. Ischium: dorsolateral edge with large tubercle, with corneous scale terminally. Ventromesial border with 3 similar size tubercles and large distal tubercle, each with terminal corneous scale. Ventrolateral border smooth.

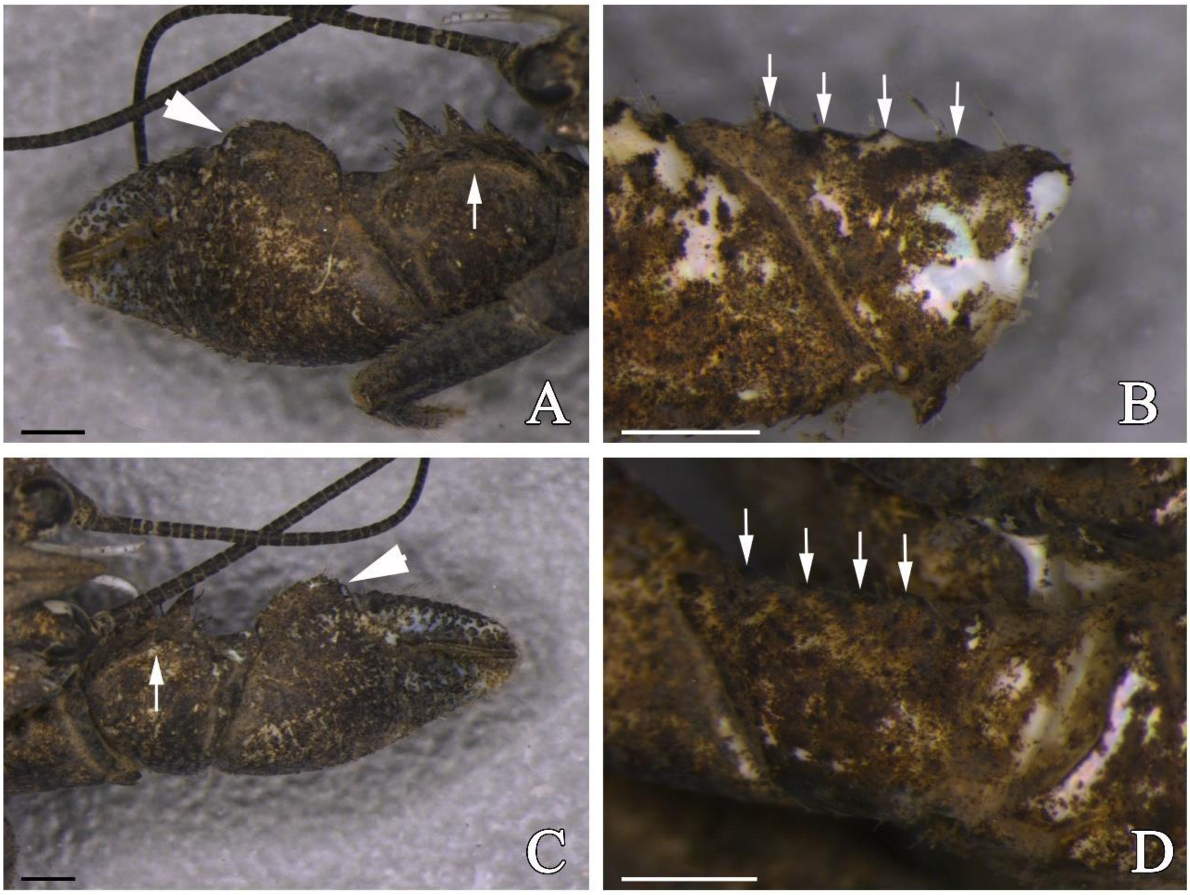

Figure 9. *Aegla* n. sp. 5, male holotype, MZUEL 514. A, dorsal view of the dactylus, propodus, and carpus of major cheliped (left) showing rectangular palmar crest (thick arrow) and high carpal ridge (thin arrow). B, ventral view of ischium of major cheliped (left) with four tubercles (arrows). C, dorsal view of the dactylus, propodus, and carpus of minor cheliped (right) showing rectangular palmar crest (thick arrow) and high carpal ridge (thin arrow). D, ventral view of ischium of minor cheliped (right) with four tubercles (arrows). Scale bars = 1.0 mm.

Minor cheliped (right) similar to major cheliped except as noted hereafter (Figs 9C, D). Dactylus: cutting margin with rudimentary lobular basal tooth. Propodus: cutting margin with rudimentary lobular basal tooth. Merus: ventromesial edge with distal spine, followed by 3 tubercles, each with terminal corneous scale.

Second, third, and fourth pereiopods morphologically similar. Dactyli, propodi, carpi, meri and ischii with several rows of setal tufts and small scales on surface. Carpi and meri with row of small tubercles with terminal corneous scale along dorsal margin. Meri with row of small tubercles with terminal corneous scale along ventral margin. Meri and ischii with long setae concentrated along dorsal margin.

Anteromesial region of third thoracic sternite tapered, projecting between coxae of third maxillipeds, with scattered setae. Fourth thoracic sternite with anterolateral angles produced anteriorly, with scattered setae (Fig. 10A). Pleopods 2–5 absent. Anterolateral angle of second abdominal epimeron well defined, unarmed (Fig. 10B). Ventral angles of third and fourth abdominal epimeron well defined, with corneous scales apically. Anterior margin of second abdominal epimeron almost straight (Fig. 10B). Uropods well developed, wide (WU/HWT = 1.16). Telson divided by longitudinal suture. Anterolateral and posterolateral margins well differentiated.

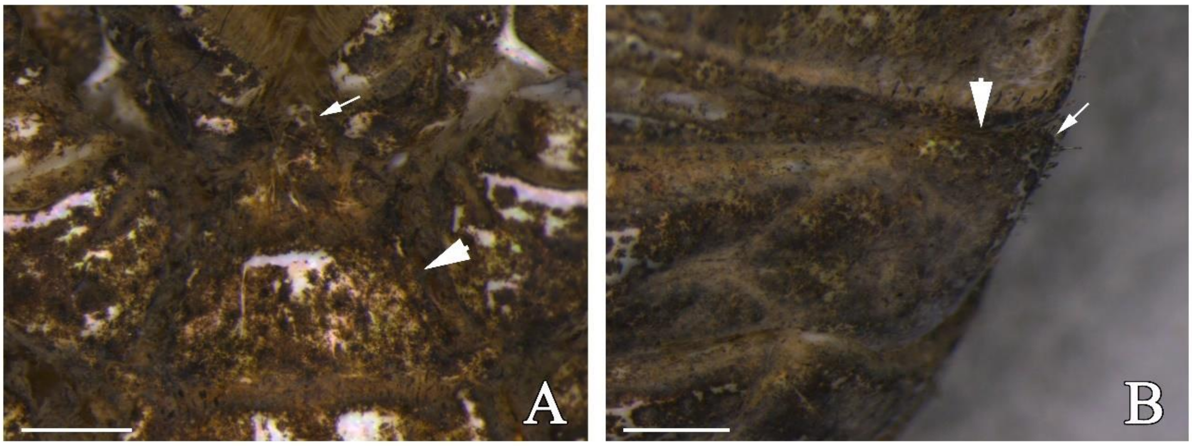

Figure 10. *Aegla* n. sp. 5, male holotype, MZUEL 514. A, third (thin arrow) and fourth (thick arrow) thoracic sternites. B, dorsal view of second abdominal epimeron (right side) showing

almost straight anterior margin (thick arrow) and unarmed anterolateral angle (thin arrow). Scale bars = 1.0 mm.

Variations: In some specimens, the anterolateral spines can extend beyond the basal margin of the cornea. The area cardiac varies from trapezoidal (n = 3) to subrectangular (n = 3). The anteromesial region of third thoracic sternite can be tapered (n = 4) or abrupt (n = 2). Measurements and morphometric relationships of the type series specimens are summarized in Table 2.

Remarks: Several morphological features differentiate *Aegla* n. sp. 5 from the species that occur in the Tibagi River basin. For example, the subrostral process occupies the proximal half of the subrostral margin in *Aegla* n. sp. 5 whereas *A. lata*, *A. jacutinga*, *Aegla* n. sp. 1, and *Aegla* n. sp. 3 is on the proximal third of the subrostral margin. The lobe on the proximal dorsal margin of the dactylus is rudimentary in *Aegla* n. sp. 5 but of moderate size in *Aegla* n. sp. 1 and *Aegla* n. sp. 4, and pronounced and surmounted by tubercles in *A. castro* and *A. schmitti*. *Aegla* n. sp. 5 has a cervical groove U-shaped whereas *A. jacutinga*, *Aegla* n. sp. 2, and *Aegla* n. sp. 3 has a cervical groove trapezoidal-shaped. The anterior margin of the second abdominal epimeron is almost straight in *Aegla* n. sp. 5, slightly concave in *A. jacutinga*, and concave in *A. castro* and *A. schmitti*. The new species can be further separated from *A. jacutinga* by the pronounced epigastric prominences and the high carpal ridge on the outer surface of the carpus. Epibranchial area is slightly elongated in *Aegla* n. sp. 5, shortened in *Aegla* n. sp. 2, and strongly elongated in *Aegla* n. sp. 1 and *Aegla* n. sp. 4. *Aegla* n. sp. 5 has a trapezoidal areola while *A. lata* has a rectangular areola and *Aegla* n. sp. 2 has a subrectangular areola. *Aegla* n. sp. 5 can be also distinguished from *A. lata* in having pronounced epigastric prominences, pronounced protogastric lobes, and wide uropods. The anterolateral angle of the second abdominal epimeron is inermis in *Aegla* n. sp. 5, while in *A.*

castro and *A. schmitti* is projected in a spine. In *Aegla* n. sp. 5, the ventral angles of the third and fourth abdominal epimeron are marked by corneous scales apically, while in *Aegla* n. sp. 2 is unarmed. Moreover, the margin of the palmar crest is poorly serrated in *Aegla* n. sp. 5 but serrated in *Aegla* n. sp. 4.

The rectangular palmar crest of the new species is shared with *A. parva*, *A. meloi*, *A. okora*, and *A. nebeccana*. Notwithstanding, *Aegla* n. sp. 5 differs from these species in having a rudimentary lobe on the proximal dorsal margin of the dactylus. The epigastric prominences are pronounced in *Aegla* n. sp. 5, absent in *A. meloi*, poorly pronounced in *A. parva*, and inconspicuous in *A. nebeccana*. Furthermore, the anterior margin of the second abdominal epimeron is almost straight in *Aegla* n. sp. 5, whereas in *A. parva*, *A. okora*, and *A. nebeccana* is slightly concave. *Aegla* n. sp. 5 has four tubercles on the ventromesial border of cheliped ischium while *A. meloi* has only a distal tubercle. The anterolateral angle of the second abdominal epimeron is inermis in *Aegla* n. sp. 5 but is marked by a spine in *A. parva*. In *Aegla* n. sp. 5, the uropods are wide, whereas in *A. nebeccana* are narrow.

Geographical distribution: The new species has been collected in two streams within the Tibagi River basin. Both streams are located in the city of Reserva, Paraná state, Brazil (Fig. 2I).

MOLECULAR DATA

Altogether we analyzed 1231 base pairs (bp) of the mitochondrial gene COI. The mean base frequencies were: A = 0.2954, C = 0.1403, G = 0.1479, T = 0.4164. The transition/transversion ratio was 3.1669. The genetic distances ranged from 1.4% to 5.9% among species of *Aegla* and 0% to 2.8% within species (Table 3). Concerning the new species, *Aegla* n. sp. 4 showed the smallest genetic distance relative to *A. lata* and the highest with *A. okora*. *Aegla* n. sp. 5 presented the lowest and highest divergence relative to *A.*

jacutinga and *A. okora*, respectively. Our Bayesian inference tree (Fig. 11) shows that *A. lata* is the sister species of *Aegla* n. sp. 4. Furthermore, both are grouped in the same subclade as *A. castro* with high nodal support. The *Aegla* n. sp. 5 specimens formed a clade sister related to *A. jacutinga* (Pp = 0.57). Based on the estimated phylogenetic tree, *Aegla* n. sp. 5 + *A. jacutinga* is the sister group of *Aegla* n. sp. 1 + *Aegla* n. sp. 2. The GMYC analysis indicated the presence of 15 independent strains within the analyzed samples. Moreover, all species were separated from each other by this species delimitation analysis.

Table 3. Genetic distances (%) of the COI partial sequences of species of *Aegla*.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *Aegla castro*	**2.8**												
2. *Aegla schmitti*	4.0	**2.4**											
3. *Aegla lata*	3.9	5.3	**0.0**										
4. *Aegla jacutinga*	2.2	3.5	3.8	**0.2**									
5. *Aegla parva*	3.1	2.8	4.7	2.3	**0.0**								
6. *Aegla meloi*	3.3	3.6	4.8	2.4	2.3	**0.2**							
7. *Aegla okora*	4.2	3.2	5.6	3.4	3.1	4.3	**0.4**						
8. *Aegla nebeccana*	4.7	4.3	5.6	4.1	3.5	5.0	5.9	**0.0**					
9. *Aegla* n. sp. 1	3.2	3.6	4.3	2.2	3.2	3.1	4.6	4.3	**0.2**				
10. *Aegla* n. sp. 2	3.1	4.0	4.6	1.8	3.7	3.2	4.7	4.9	1.4	**0.4**			
11. *Aegla* n. sp. 3	2.9	3.7	4.0	2.1	3.7	3.2	4.5	4.7	3.2	2.4	**0.0**		
12. *Aegla* n. sp. 4	2.3	3.6	1.7	2.1	3.3	3.0	4.5	4.2	2.7	2.8	2.2	**0.0**	
13. *Aegla* n. sp. 5	2.8	3.8	3.3	1.7	3.0	3.1	4.2	3.9	2.8	2.7	2.7	2.3	**0.4**

The intraspecific distances are in bold.

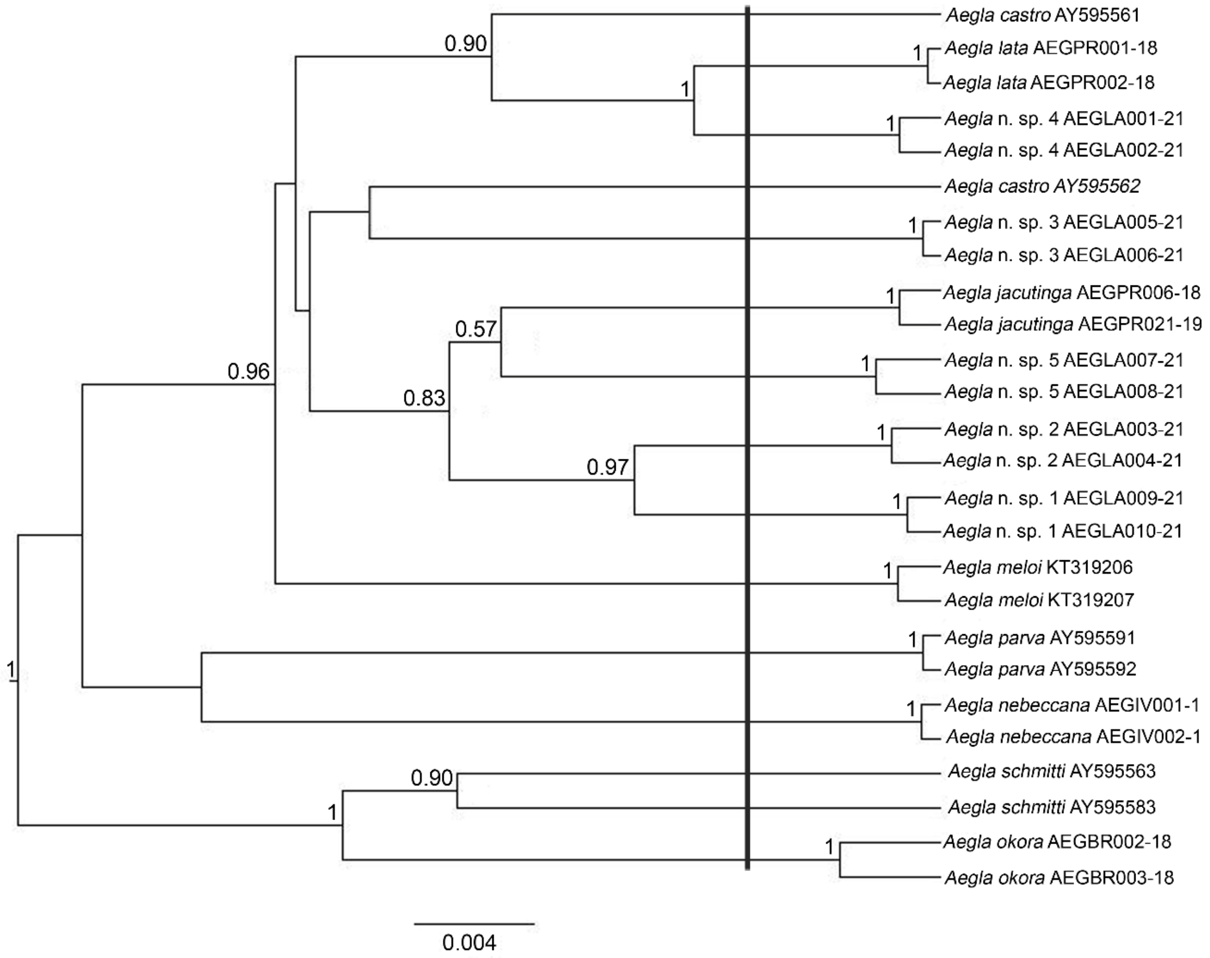

Figure 11. Bayesian inference tree of the *Aegla* spp. analyzed in this study. Numbers at the corresponding nodes represent the posterior probability (values < 0.5 are not indicated). The vertical line indicates the species delimitation assigned by the single-threshold method in GMYC analysis.

DISCUSSION

For over 70 years, researchers have carried out surveys in the Tibagi River basin, generating information on aeglids’ taxonomy and distribution. The first report came in 1942, with the description of *A. castro* from Iapó River, Castro city (Schmitt, 1942). More than 50 years later, Bond-Buckup & Buckup (1994) described *A. lata* from Pinto River, Ponta Grossa city. These authors also reported new records of occurrence for *A. castro* and *A. schmitti* in this hydrographic basin. Several other works recorded the presence of these three species in tributaries of the Tibagi River (Swiech-Ayoub & Masunari, 2001; Galves *et al.*, 2007;

Trevisan & Masurani, 2010; Silva *et al*., 2017; Marçal *et al*., 2018; Chaves *et al*., 2019). Later, Marçal *et al*. (2020) described *A. jacutinga* from specimens collected in two streams in Londrina city. More recently, one new species from the Lower Tibagi region and two from the Middle Tibagi region have been described (Marçal *et al*., in press).

Aegla castro and *A. schmitti* have wide geographical distribution in the sub-basins of the Paraná River, southeastern and southern Brazil. The known localities of *A. castro* are concentrated in tributaries of the Tibagi River basin (Paraná state), including its type locality (Schmitt, 1942; Bond-Buckup & Buckup, 1994; Swiech-Ayoub & Masunari, 2001; Silva *et al*., 2017; Marçal *et al*., 2018; Almeida *et al*., 2021; Silva *et al*., 2021). Besides, there are several records of *A. castro* in the south of São Paulo state, on the right bank of the Paranapanema River (Bond-Buckup & Buckup, 1994; Fransozo *et al*., 2003; Takano *et al*., 2016). Similarly, *A. schmitti* is mainly distributed in the Paraná state (Trevisan & Masunari, 2010; Trevisan *et al*., 2021). However, the northern limit of the geographic distribution of *A. schmitti* is in the Iporanga city, São Paulo state, and the southern limit is in the municipality of Canoinhas, Santa Catarina state (Bond-Buckup & Buckup, 1994; Trevisan & Masunari, 2010). Interestingly, molecular studies indicate that *A. castro* and *A. schmitti* are cryptic species complexes (Páez *et al*., 2018; Trombetta *et al*., 2019; Marçal *et al*., 2020, 2021; Marçal *et al*., in prep). Therefore, its geographic distribution ranges must be interpreted with caution as they may be narrower than currently recognized.

Aegla lata, *A. jacutinga*, *Aegla* n. sp. 1, *Aegla* n. sp. 2, *Aegla* n. sp. 3, *Aegla* n. sp. 4, and *Aegla* n. sp. 5, on the other hand, have a restricted distribution and are probably endemic to the Tibagi River basin. Literature reveals that *A. lata* is distributed in rivers and small streams that drain into the Tibagi River (Bond-Buckup & Buckup, 1994; Galves *et al*., 2007; Chaves *et al*., 2019; Marçal *et al*., 2020), and it is extinct in the type locality (Pérez-Losada *et al*., 2004, Pérez-Losada *et al*., 2009). The new records reported on here place *A. lata* in the tributaries of the Tibagi River that drain waters of the Califórnia city (23°39′S, 51°19′W), in north Paraná. Although this species occurs between Londrina city (23°25′S, 51°23′W) and Ponta Grossa city (25°5′S, 50°9′W), its distribution can be considered as fragmented, since there is no record of occurrence in the Middle Tibagi region (see Fig. 2C). Similarly, *A. jacutinga* has been recorded along the left bank of the Tibagi River basin (Marçal *et al.*, 2020). Our data extend the known distribution of this species to the right bank of the Tibagi River but maintain records only in the Lower Tibagi region (see Fig. 2D).

SYSTEMATICS

The Bayesian inference tree depicts *A. lata* as sister species to *Aegla* n. sp. 4 and places *Aegla* n. sp. 5 in a subclade with *A. jacutinga* (Fig. 11). Both species belong to clade C of the phylogeny presented in Pérez-Losada *et al.* (2004) (see Marçal *et al.*, 2020). Therefore, it is likely that the new species also belong to clade C. Furthermore, molecular evidence clearly shows that *Aegla* n. sp. 4 and *Aegla* n. sp. 5 are distinct phylogenetic lineage, supporting the morphological differentiation observed. In future investigations, analyzes with other mitochondrial and nuclear genes may be carried out to confirm our hypothesis on the phylogenetic position of the new species.

CONSERVATION AND DIVERSITY

Regarding the conservation status of the new species, we suggest that *Aegla* n. sp. 4 and *Aegla* n. sp. 5 be classified as “Endangered” (EN) under criteria B1, B2abiii as defined by the International Union for Conservation of Nature (IUCN, 2019). Both species have been recorded in only two localities (B2a) and the known extent of occurrence is estimated to be less than 100 km2 (B1). The streams where the specimens of *Aegla* n. sp. 5 were found are located in agricultural and urbanized areas (B2biii). Moreover, in a recent sampling at the type-locality of *Aegla* n. sp. 4 we did not find adult specimens and noticed that the stream is heavily impacted, with hogs being raised next to the watercourse (B2biii).

According to our results and extensive literature research, the Tibagi River basin hosts nine species of *Aegla*. This basin, therefore, has a greater number of aeglids species compared to other hydrographic basins in Paraná state, such as Iguaçu (five species), Ivaí (four species), and Cinzas (one species). Raio & Bennemann (2010) revealed that the Middle Tibagi region has the highest fish biodiversity in the state of Paraná and recommend that all tributaries in this region be inventoried, as each one has a different composition. Pérez-Losada *et al*. (2009) assessed the conservation priority of 18 freshwater ecoregions in southern South America based on the diversity and genetic distribution of *Aegla*. This study supports that the Paranapanema River (Upper Paraná ecoregion) contains high biodiversity and identifies it as one of the top priorities for conservation. Considering recent descriptions (Marçal *et al*., 2020, 2021; Marçal *et al*., in press), the number of *Aegla* species for the Paranapanema River basin increased from 4 to 11. In contrast, most streams sampled in the present study have only small remnants as a riparian forest. Santos *et al*. (2015) reported that the rivers in this region are being degraded by intense deforestation of the banks and the dumping of residues from lumber industries. As a consequence, most species that occur in this basin are under some degree of extinction threat. Given the above considerations, it can be said that the Tibagi River basin is essential for the conservation of the neotropical freshwater biota and, therefore, must be protected.

In summary, we have improved the knowledge of the aeglids of the Tibagi River basin. The distributions of *A. castro*, *A. lata*, and *A. jacutinga* were reviewed and extended. Besides, we described two new *Aegla* species from the Middle Tibagi. Of the nine species that occur in the Tibagi River basin, seven are endemic to this basin. Although morphologically conservative, the detailed analysis of the morphology allowed the distinction of the species as well as the construction of a diagnostic key. We emphasize that, for comparison purposes, it is important to have adult male specimens because the full development of chelipeds, spines, and tubercles in these individuals allows a safer interpretation of the diagnostic characters of the species. Despite several watercourses of the Middle and Lower Tibagi River were sampled in this study, many parts of the Upper Tibagi have either only a few or no records of aeglids. We recognize that this inventory represents a small fraction of what remains to be sampled within the Paraná River basin. However, it is hoped that the data provided by our research will contribute to revealing the real diversity and distribution patterns of aeglids from the Paraná River basin.

KEY TO THE SPECIES OF *AEGLA* FROM TIBAGI RIVER BASIN

1\. Palmar crest of the chelipeds rectangular 2

\- Palmar crest of the chelipeds subdisciform to disciform 3

2\. Extra-orbital sinus shallow. Epibranchial area strongly elongated. Proximal dorsal margin of dactylus with moderate size lobe .... *Aegla* n. sp. 4

\- Extra-orbital sinus deep. Epibranchial area slightly elongated. Proximal dorsal margin of dactylus with rudimentary lobe .... *Aegla* n. sp. 5

3\. Protogastric lobes poorly pronounced. Uropods narrow .... *Aegla lata*

\- Protogastric lobes pronounced. Uropods wide 4

4\. Anterolateral spines not reaching basal margin of cornea 5

\- Anterolateral spines reaching or extending beyond basal margin of cornea 6

5\. Cervical groove U-shaped. Areola trapezoidal in shape. Rudimentary pleopods in adult male specimens .... *Aegla* n. sp. 1

\- Cervical groove trapezoidal. Areola subrectangular in shape. Pleopods absent in adult male specimens .... *Aegla jacutinga*

6\. Proximal dorsal margin of dactylus with pronounced lobe 7

\- Proximal dorsal margin of dactylus with rudimentary or absent lobe 8

7. Epigastric prominences poorly pronounced. Chelipeds broadly ovate and inflated. Palmar crest dorsally excavate .... *Aegla schmitti*

\- Epigastric prominences pronounced. Chelipeds moderately inflated, somewhat elongated. Palmar crest shallowly impressed or excavate .... *Aegla castro*

8. Subrostral process occupying proximal half of subrostral margin. Areola subrectangular in shape. Epibranchial area shortened... *Aegla* n. sp. 2

\- Subrostral process occupying proximal third of subrostral margin. Areola trapezoidal in shape. Epibranchial area slightly elongated.. *Aegla* n. sp. 3

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4. CAPÍTULO 3

THE EFFECTIVENESS OF DNA BARCODING AS A TOOL IN *AEGLA* DIVERSITY (CRUSTACEA, AEGLIDAE)

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ABSTRACT

The barcode region of the cytochrome c oxidase I gene (COI) has been widely used to identify animal taxa. Here, we examine the effectiveness of the COI barcode in discriminating *Aegla* species, freshwater crustacean's endemic to southern South American. We calculated the intra- and interspecific genetic distances based on the Kimura 2-parameter (K2P) model. The Generalized Mixed Yule Coalescent (GMYC) model was also employed to delimit the species. K2P genetic distances were on average 0.2% within species and 5.8% between species. Species that exhibited high intraspecific divergence (>2%) were split into at least two clusters by GMYC analysis, suggesting the presence of cryptic genetic diversity. The levels of nucleotide divergence among the species were from 5 to 29 times greater than the intraspecific divergences. Of the 74 species analyzed, 68 (92%) were correctly identified by their barcode sequences. Thus, our results confirm the effectiveness of the COI barcode in identifying *Aegla* species. In addition, possible cryptic taxa and genetically divergent lineages were revealed in 22 investigated species. This study provides the first comprehensive assessment of the genetic distances of aeglids species and therefore lays the groundwork for future research. Given the richness, ecological relevance, high degree of endemism, and threat of the *Aegla* genus, the validation, and standardization of tools such as the DNA barcode are essential for the correct interpretation of diversity and the conservation of these crustaceans.

KEYWORDS: COI, cryptic species, genetic distance, species identification, molecular taxonomy

INTRODUCTION

Aegla Leach, 1820 is currently represented by 90 species and subspecies (Trombetta et al., 2019; Marçal et al., 2020, 2021) exclusively from freshwater of the temperate and subtropical regions of southern South America (Schmitt, 1942a). The first described species was named *Galathea laevis* Latreille, 1818, among marine crustaceans (Schmitt, 1942a). Through a more accurate examination of the specimens deposited at the Muséum National D´Histoire Naturelle in 1820, Leach proposed the genus *Aegla* for the species of Latreille (Bond-Buckup & Buckup, 1994). For many years, several authors argued that the genus *Aegla* would have a single species with wide distribution, occurring in watercourses in southern Brazil, Argentina, and Chile (Bond-Buckup & Buckup, 1994). However, in 1942, Schmitt described 17 new species and validated two other species, in addition to *Aegla laevis* (Latreille, 1818) (see Schmitt 1942a, 1942b), demonstrating that the morphological differences noted in the analyzed specimens could not be attributed to variations in a single species. Since then, several studies have shown subtle differences in the morphological characters of the aeglids (Buckup & Rossi, 1977; Jara, 1977, 1982; Jara & López, 1981; Bond-Buckup & Buckup, 1994; Jara & Palacios, 1999).

For more accurate discrimination of many aeglid species, several taxonomists have used molecular techniques along with morphological data (Jara et al., 2003; Bond-Buckup et al., 2010; Santos et al., 2009, 2010, 2012, 2013, 2015; Moraes et al., 2016; Jara et al., 2018; Páez et al., 2018; Trombetta et al., 2019; Marçal et al., 2020, 2021). According to Hillis (1987), molecular techniques can potentially reveal levels of genetic differentiation hidden in morphologically similar populations. The phylogeny presented by Pérez-Losada et al. (2004), for example, indicates that *Aegla cholchol* Jara & Palacios, 1999, *Aegla jarai* Bond-Buckup & Buckup, 1994, *Aegla parana* Schmitt, 1942, *Aegla marginata* Bond-Buckup & Buckup, 1994, *Aegla platensis* Schmitt, 1942 and *Aegla franciscana* Buckup & Rossi, 1977 form non-monophyletic groups. Pérez-Losada et al. (2002) had already made similar observations for *A. cholchol*. Under Cracraft's (1983) species concepts, populations that do not form monophyletic groups qualify as distinct species. Thus, according to Pérez-Losada et al. (2004), the conflict between alpha taxonomy and the results of gene trees may represent unrecognized species (cryptic species). This assumption was later confirmed with the description of *Aegla quilombola* Moraes, Tavares & Bueno, 2017, from one of the populations formerly designated as *A. marginata* (Moraes et al., 2017).

There are various other examples of cryptic species within aeglids. For example, morphological and molecular analyses showed that *Aegla paulensis* Schmitt, 1942 was a complex with at least seven different species (Santos et al., 2015; Moraes et al., 2016). The Ribeira de Iguape basin population was described as *Aegla lancinhas* Bond-Buckup & Buckup, 2015 in Santos et al., 2015, and four populations of the Tietê basin were described as new species (Moraes et al., 2016). Crivellaro et al. (2017) analyzed 17 populations attributed to *Aegla longirostri* Bond-Buckup & Buckup, 1994, using two species delimitation methods, and identified at least 14 potential species. Jara et al. (2018) described *Aegla chilota* Jara, Pérez-Losada & Crandall, 2018 from a population preliminary designate as *Aegla araucaniensis* Jara, 1980. Overlooked species were also reported between populations of *A. platensis* and *Aegla uruguayana* Schmitt, 1942 (Zimmermann et al., 2018, 2021). Furthermore, *Aegla jacutinga* Marçal & Teixeira, 2020 was described from populations morphologically similar to *Aegla lata* Bond-Bukup & Buckup, 1994 (Marçal et al., 2020).

A molecular technique commonly used in species identification is DNA barcode, in which each taxon would be identified by having a unique DNA sequence (Hebert et al., 2003). Surveys such as that conducted by Hebert et al. (2003) have shown that the barcode region of the cytochrome c oxidase I gene (COI) is a successful taxonomic tool for identifications of known animal species and the recognition of undescribed ones. This technique has been effectively used to discriminated species of lepidopterans (Hebert et al., 2003; Janzen et al., 2005), birds (Hebert et al., 2004), spiders (Barrett & Hebert, 2005), ants (Smith et al., 2005), fishes (Ward et al., 2005; Pereira et al., 2011, 2013; Souza-Shibatta et al., 2018), crustaceans (Lefébure et al., 2006; Costa et al., 2007; Havermans et al., 2011; Matzen da Silva et al., 2011) and others. The predominant approach used to analyze diversity patterns with the COI barcode region is the classic pairwise distance method based on the Kimura 2-parameter (K2P) model (Matzen da Silva et al., 2011). This method premise that genetic distance between individuals of the same species (intraspecific divergence) is smaller than the variation between different species (interspecific divergence) (Hebert et al., 2004; Ribolli et al., 2017). Thus, a standard sequence threshold of ten times the mean intraspecific variation was proposed by Hebert et al. (2004) and has been successfully employed in the delimitation of some groups (Hebert et al., 2004; Witt et al., 2006; Ferri et al., 2009). Matzen da Silva et al. (2011), assuming an intraspecific barcode threshold of a maximum of 2%, had success around 98% in recognizing marine decapods. Lefébure et al. (2006) analyzed Aeglidae COI sequences with another 17 different families of Crustacea and proposed a molecular threshold to help species delimitation. However, this threshold was calculated excluding *Aegla* genus sequences from the analysis. Thus, no study has evaluated genetic distances and proved the success of the COI barcode for delimiting aeglids. Currently, 74 of the 90 morphologically described species of *Aegla* have at least one COI sequence deposited in GenBank or BOLD databases. Therefore, the present study aims to examine the effectiveness of the COI barcode for species recognition in *Aegla*. We tested whether the 2% genetic distance threshold can also be applied for *Aegla* delimitation.

MATERIAL AND METHODS

COI Profile

Aegla species COI sequences were accessed from the GenBank and BOLD databases. At least five sequences per species were determined to maximize the recovery of high intraspecific variation (Meyer & Pauley, 2005). Nevertheless, to avoid an imbalance in the representation of some taxa, the maximum limit of ten sequences per species was established (Lefébure et al., 2006). Sixteen species of *Aegla* (*Aegla charon* Bueno & Moraes, 2017, *A. expansa* Jara, 1992, *A. intermedia* Girard, 1855, *A. japi* Moraes, Tavares & Bueno, 2016, *A. jaragua* Moraes, Tavares & Bueno, 2016, *A. jundiai* Moraes, Tavares & Bueno, 2016, *A. leachi* Bond-Buckup & Buckup, 2012, *A. microphthalma* Bond-Buckup & Buckup, 1994, *A. muelleri* Bond-Buckup & Buckup, 2010, *A. oblata* Bond-Buckup & Santos, 2012, *A. occidentalis* Jara, Pérez-Losada & Crandall, 2003, *A. pomerana* Bond-Buckup & Buckup, 2010, *A. renana* Bond-Buckup & Santos, 2010, *A. rosanae* Campos Jr., 1998, *A. saltensis* Bond-Buckup & Jara, 2010, and *A. vanini* Moraes, Tavares & Bueno, 2016) do not have COI sequences available and were not included in the analyses. Overall, 263 DNA sequences were selected to establish a COI profile referred to here as dataset A.

Data Analysis

DNA sequences were aligned using the ClustalW algorithm in the MEGA v6 software (Tamura et al., 2013). Genetic distances within species (intraspecific) and between species (interspecific) were calculated based on the Kimura-2-parameter model (Kimura, 1980). Histograms of the frequency distribution of intra- and interspecific distances from dataset A were made. A Neighbor-joining (NJ) tree was created to provide a graphic representation of the relationships among specimens.

The second method used to detect the transition point between intra- and interspecific relations was the Generalized Mixed Yule Coalescent (GMYC) approach based on single-locus data, a relatively robust tool for species delimitation (Pons et al., 2006; Fujisawa & Barraclough, 2013). First, an ultrametric tree was generated using Bayesian inference. Then, Bayesian analysis was conducted in BEAST 2.6.2 (Bouckaert et al., 2019) with a strict molecular clock, a Birth-Death model, and a sequence evolution model estimated by jModelTest2 (Darriba et al., 2012). If the best-fit model is not implemented in BEAST, we replaced it with the closest over-parameterized model following Lecocq et al. (2013). A run of 10,000,000 generations, sampling one tree every 1,000 generations, was performed. The subsequent probability of sufficient minimum effective sample size (ESS> 200) was verified in TRACER 1.6. The TREEANNOTATOR 2.6.2 summarized the trees, with a burn-in of 25%. Finally, GMYC was performed from the ultrametric tree in the “Splits” package (Pons et al., 2006; Fujisawa & Barraclough, 2013) with the R 3.5.1 program.

We identified the sequences of possible cryptic species, i.e., those that formed non-monophyletic clades and/or presented intraspecific distances values ≥ of 2%. Then, we calculated the genetic distances considering each cryptic species as an independent genetic unit. This dataset is here called B. In dataset B, the species delimitation performed by GMYC analysis was considered. Therefore, species that were grouped with congeners were treated as being only one.

RESULTS

COI sequences ranged in length from 615 to 1045 base pairs (bp), comprising 1292 bp in alignment. No stop codons, deletions, or insertions were observed. A few species (*Aegla bahamondei* Jara, 1982, *A. carinata* Bond-Buckup & Loureiro, 2014, *A. spectabilis* Jara, 1986, *A. franca* Schmitt, 1942, *A. singularis* Ringuelet, 1948, and *A. inconspicua* Bond-Buckup & Buckup, 1994) had only one sequence available in the databases. However, two to four sequences were obtained for most species (56) and five or more for 12 species.

Analysis Of The Molecular Dataset A

The genetic distance matrix of dataset A (Table 1) showed that the divergences between conspecific individuals were usually small, ranging from 0.0% to 8.9% (mean = 1.1%). The frequency distribution graph of K2P divergence values showed that 74% of the intraspecific comparisons were less than 2% (Figure 1). In contrast, 21 species exhibited intraspecific distance values that exceeded 2%, suggesting the presence of cryptic genetic diversity (Table 1). GMYC analysis confirmed at least two different strains among these species. These cases raise the global mean intraspecific divergence and are responsible for the slight overlap between intra- and interspecific variations (Figure 1). Moreover, of the 17 species that formed non-monophyletic groups in the Neighbor-joining tree (Figure 2), 16 presented high within-species distances (values > 2%). The exception is *Aegla brevipalma* Bond-Buckup & Santos, 2012, with a maximum intraspecific K2P distance of 1.5%.

TABLE 1. Pairwise COI barcode nucleotide divergences for *Aegla* species using K2P distances (%). N represents the number of analyzed sequences.

Species	N	Intraspecific distance			Interspecific distance		
		Min.	Mean	Max.	Min.	Mean	Max.
Aegla abtao	8	0.0	2.2	3.7	1.5	5.7	9.6
Aegla affinis	4	0.0	0.2	0.3	3.3	6.2	9.8
Aegla alacalufi	10	0.0	0.2	0.6	1.5	5.4	8.8
Aegla araucaniensis	2	0.9	0.9	0.9	2.1	4.8	9.5
Aegla bahamondei	1	–	–	–	2.7	5.4	7.9
Aegla brevipalma	2	1.5	1.5	1.5	0.9	4.5	8.5
Aegla buenoi	4	0.0	0.0	0.0	0.9	5.2	9.6
Aegla camargoi	4	0.3	2.7	3.7	2.1	5.6	10.2
Aegla carinata	1	–	–	–	3.0	5.1	7.9
Aegla castro	3	2.1	2.8	3.6	1.2	5.7	10.2
Aegla cavernicola	2	0.0	0.0	0.0	1.5	4.6	8.5
Aegla chilota	10	0.0	0.1	0.6	2.7	6.1	9.3
Aegla chochol	10	0.0	1.5	3.7	2.4	5.9	9.5
Aegla concepcionensis	2	0.3	0.3	0.3	2.7	5.5	8.6
Aegla denticulata denticulata	2	0.0	0.0	0.0	0.0	5.6	8.5
Aegla denticulata lacustris	2	0.0	0.0	0.0	0.0	5.6	8.5

Aegla franca	1	–	–	–	3.4	5.9	9.6
Aegla franciscana	3	0.6	2.0	3.0	0.9	5.0	9.2
Aegla georginae	10	0.0	2.9	4.9	4.3	7.3	11.4
Aegla grisella	4	0.6	3.3	4.6	2.7	6.0	10.6
Aegla hueicollensis	2	0.0	0.0	0.0	2.7	6.5	9.2
Aegla humahuaca	2	0.0	0.0	0.0	2.4	5.5	8.5
Aegla inconspicua	1	–	–	–	3.7	6.6	9.2
Aegla inermis	2	0.3	0.3	0.3	3.0	6.5	10.9
Aegla intercalata	3	3.0	3.1	3.3	2.1	5.3	8.9
Aegla itacolomiensis	3	0.0	0.0	0.0	3.3	5.5	10.6
Aegla jacutinga	4	0.0	0.2	0.3	0.9	5.6	10.2
Aegla jarai	10	0.0	2.4	4.0	0.0	4.8	9.6
Aegla jujuyana	2	0.6	0.6	0.6	2.1	6.7	10.2
Aegla laevis	2	0.0	0.0	0.0	2.4	5.1	8.8
Aegla lancinhas	2	0.9	0.9	0.9	2.1	5.5	9.3
Aegla lata	4	0.0	0.0	0.0	3.3	7.2	11.6
Aegla leptochela	2	0.0	0.0	0.0	0.0	6.2	10.2
Aegla leptodactyla	2	0.9	0.9	0.9	3.0	5.7	9.5
Aegla ligulata	3	0.0	0.6	0.9	0.9	6.0	9.5
Aegla longirostri	10	0.0	4.5	8.9	2.4	6.6	12.0
Aegla loyolai	3	0.0	0.0	0.0	2.4	5.6	8.9
Aegla ludwigi	10	0.0	0.2	0.6	4.0	6.4	10.7
Aegla manni	3	0.0	1.4	2.1	1.8	5.7	9.3
Aegla manuinflata	3	0.0	0.2	0.3	3.0	5.0	7.8
Aegla marginata	2	0.0	0.0	0.0	4.6	6.4	8.9
Aegla meloi	3	0.0	0.2	0.3	2.4	5.1	8.5
Aegla nebeccana	2	0.0	0.0	0.0	3.6	6.7	10.6
Aegla neuquensis	2	0.0	0.0	0.0	2.4	6.5	9.9
Aegla obstipa	2	0.3	0.3	0.3	3.7	6.5	9.9
Aegla odebrechtii	2	0.9	0.9	0.9	1.8	4.6	7.9
Aegla okora	4	0.0	0.0	0.0	3.0	5.8	9.5
Aegla papudo	2	0.0	0.0	0.0	4.7	7.4	11.1
Aegla parana	2	4.6	4.6	4.6	1.2	5.5	10.2
Aegla parva	2	0.0	0.0	0.0	2.1	5.0	8.9
Aegla paulensis	2	1.2	1.2	1.2	2.1	5.0	9.9
Aegla perobae	2	0.3	0.3	0.3	4.0	7.5	11.4
Aegla pewenchae	3	0.0	0.0	0.0	3.7	6.4	10.3
Aegla plana	2	0.0	0.0	0.0	2.7	5.8	9.2
Aegla platensis	10	0.0	3.8	6.8	0.6	7.4	12.0
Aegla prado	2	0.0	0.0	0.0	4.0	6.6	9.6
Aegla quilombola	2	0.3	0.3	0.3	0.0	6.3	10.2
Aegla ringueleti	2	0.3	0.3	0.3	4.6	7.6	10.3
Aegla riolimayana	4	0.0	0.0	0.0	1.5	4.6	7.9
Aegla rossiana	2	0.0	0.0	0.0	2.4	5.6	7.9

Aegla rostrata	10	0.0	0.1	0.3	3.0	6.1	9.2
Aegla sanlorenzo	2	3.0	3.0	3.0	2.1	6.7	10.9
Aegla scamosa	3	0.3	3.3	4.9	3.7	6.3	9.2
Aegla schmitti	2	2.1	2.1	2.1	1.5	4.6	8.5
Aegla septentrionalis	2	0.0	0.0	0.0	3.7	6.4	10.5
Aegla serrana	3	1.2	3.5	4.6	2.7	5.9	9.3
Aegla singularis	1	–	–	–	4.0	8.5	11.1
Aegla spectabilis	1	–	–	–	1.8	4.6	8.2
Aegla spinipalma	4	0.0	0.3	0.6	3.3	6.5	11.6
Aegla spinosa	2	0.3	0.3	0.3	0.0	4.6	8.9
Aegla strinatii	3	0.3	1.8	2.7	1.5	5.5	9.9
Aegla talcahuano	3	1.2	3.5	5.2	2.4	5.7	9.6
Aegla uruguayana	10	0.0	4.2	7.4	0.6	6.5	9.6
Aegla violacea	5	0.0	2.2	3.7	2.4	5.6	9.2

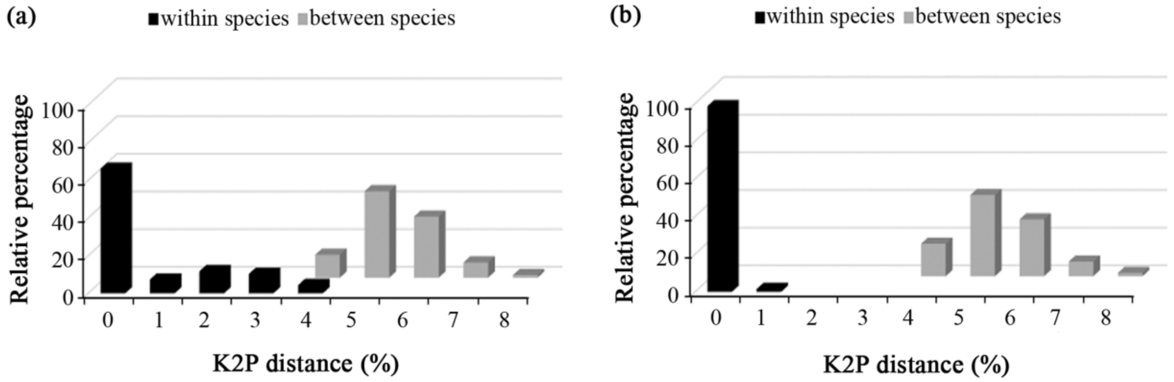

Figure 1. Frequency distribution of mean intraspecific (black bars) and interspecific (gray bars) distances for *Aegla* species: (a) dataset A; (b) dataset B.

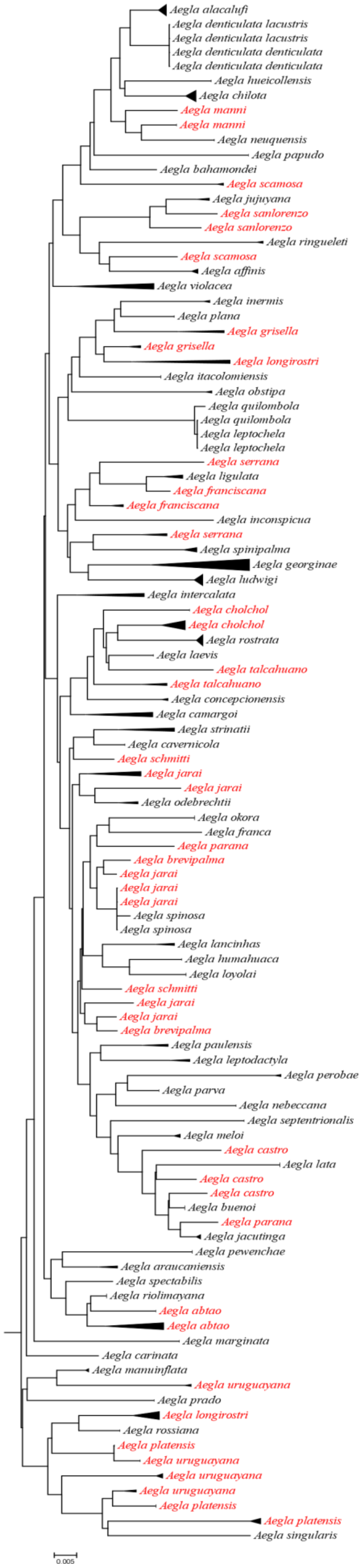

Figure 2. Neighbor-joining tree based on analysis of 263 COI sequences from 74 species of *Aegla*. Clusters with sequences of the same species are collapsed. Non-monophyletic taxa are colored in red. The branch length represents the Kimura 2-Parameter (K2P) distances.

The K2P genetic distance between species ranged from 0.0% to 12.0% (mean = 5.9%), about five times greater than the intraspecific variation. Most species had a minimum interspecific distance above 2%, which allowed correct discrimination of 92% of analyzed species (68 of 74). In three exceptional cases, the interspecific distances were 0.0%. They included the divergence between *Aegla denticulata denticulata* Nicolet, 1849 and *A. denticulata lacustris* Jara, 1986; two sequences of *A. jarai* (AY595594, AY595595) and *A. spinosa* Bond-Buckup & Buckup, 1994; *A. leptochela* Bond-Buckup & Buckup, 1994 and *A. quilombola*. GMYC analysis joined these sequences into three clades as if they represented only three species instead of six. Additionally, two sequences of *A. platensis* (AY595627, AY595628) and *A. uruguayana* (MT489799, MT489800) presented 0.6% of the genetic distance between them and were grouped by the delimitation analysis. Another 16 species showed low divergences (0.9–1.8%) with some congeners. Nevertheless, in all cases, they were delimited from their congeners by the GMYC method.

The model of sequence evolution applied in dataset A was GTR + I + G ($\alpha = 0.6480$, proportion of invariant sites = 0.5570). Mean base frequencies were A: 0.2864, C: 0.1279, G: 0.1455, T: 0.4402. Transition/transversion rates were AC: 2.6831, AG: 19.2160, AT: 1.4111, CG: 1.6919, CT: 16.2188, GT: 1.0000. Concerning species delimitation, the GMYC analysis indicated the presence of 116 independent strains. Twenty-three species were split into two clusters or even more (Figure 3). Surprisingly, the ten *A. jarai* sequences were separated into nine strains. Data analysis in R is available as Supporting information Text S1.

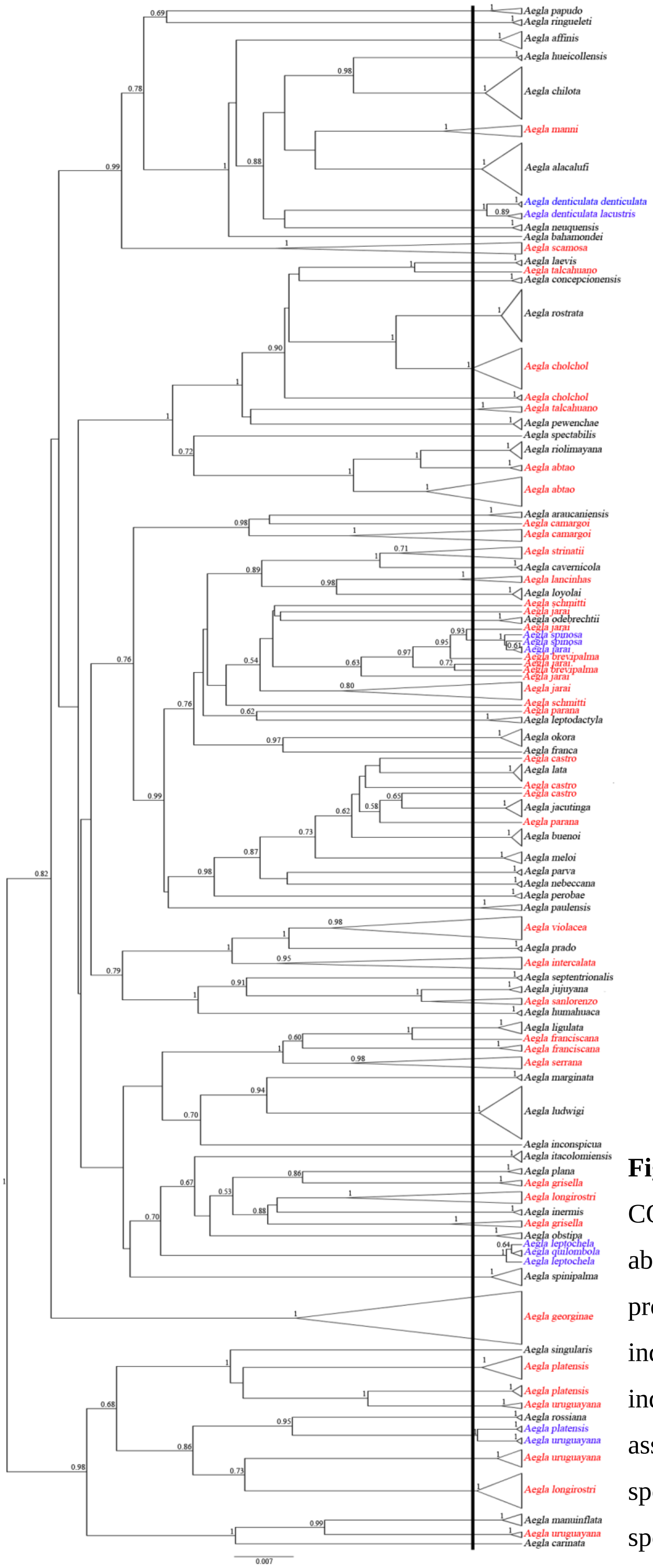

Figure 3. Bayesian tree based on COI sequences of *Aegla*. Numbers above branches are posterior probabilities (values < 0.5 are not indicated). The vertical line indicates species delimitation assigned in GMYC analysis. Split species are colored in red. Grouped species are colored in blue.

Analysis Of The Molecular Dataset B

The average K2P distance within species was only 0.2%, while congeneric divergence averaged 5.8% (Table S1). Considering these values, the barcode gap among congeneric and intraspecific comparisons increases to 29 times. Furthermore, intraspecific divergences were below 1% for 99% of the within-species comparisons in this dataset (Figure 1B). By contrast, more than 82% of interspecific distance values exceeded 5%. As shown in Figure 1, there was no overlap between the intra- and interspecific distance values for dataset B, contrary to dataset A.

DISCUSSION

This study provides the first comprehensive assessment of the genetic distances of aeglids species and includes sequences from 263 individuals in 74 supposed taxa, representing about 82% of the aeglids species recognized to date. Observed divergences were below 1% within species of *Aegla* for 66% of analyzed taxa in dataset A. This finding is consistent with those of Costa et al. (2007) for species of decapods (87%), *Gammarus* (75%), and *Daphnia* (59%). However, with the approach used in dataset B, the intraspecific distance was less than 1% in 99% of cases. The average intraspecific distance calculated for dataset A was 1.1%. In some cases, this divergence between individuals of the same species was greater than 2%. We believe these high values are due to the presence of unrecognized cryptic species. When these cryptic species complexes are considered independent genetic units (dataset B), the mean K2P intraspecific divergence is 0.2%. These results align with data reported for other crustaceans (Costa et al., 2007; Havermans et al., 2011; Matzen da Silva et al., 2011).

Extensive research has shown that several *Aegla* species represent in fact complexes of morphologically similar (cryptic) species with restricted distribution (Pérez-Losada et al., 2002, 2004; Santos et al., 2015; Moraes et al., 2016, 2017; Crivellaro et al., 2017; Jara et al., 2018; Marçal et al., 2020; Zimmermann et al., 2021). Our study supports evidence from previous observations. For example, the deep divergence observed combined with phylogenetic and geographic distribution data (Pérez-Losada et al., 2002) reinforces the possibility of overlooked species within *A. cholchol* and *A. talcahuano* Schmitt, 1942. Based on the high intraspecific distances and the separation into two clusters by GMYC analysis, the *A. franciscana*, *A. parana*, and *A. schmitti* Hobbs, 1979 complexes seem to consist of two different species. These results corroborate the findings of Pérez-Losada et al. (2004), Páez et al. (2018) and Marçal et al. (2020). Under the present results, previous molecular analyzes have demonstrated the non-monophyly of *A. brevipalma*, *A. castro* Schmitt, 1942, *A. grisella* Bond-Buckup & Buckup, 1994, and *A. jarai* (Pérez-Losada et al., 2004; Trombetta et al. 2019). The deep barcode divergences within *A. longirostri*, *A. platensis*, and *A. uruguayana* members suggest the presence of overlooked species, confirming similar observations of Crivellaro et al. (2017), Zimmermann et al. (2018), and Zimmermann et al. (2021). Additionally, possible cryptic taxa and genetically divergent lineages were revealed in *A. abtao* Schmitt, 1942, *A. camargoi* Buckup & Rossi, 1977, *A. georginae* Santos & Jara, 2013, *A. intercalata* Bond-Buckup & Buckup, 1994, *A. manni* Jara, 1980, *A. sanlorenzo* Schmitt, 1942, *A. scamosa* Ringuelet, 1948, *A. serrana* Buckup & Rossi, 1977, *A. strinatii* Türkay, 1972, and *A. violacea* Bond-Buckup & Buckup, 1994.

Our genetic distance matrix also indicated that divergence values between species are ordinarily greater than 2%. When this value was employed as a threshold for species diagnosis, 50 out of the 74 species were recognized. The exceptions comprise six species that showed 0.0% of the minimum interspecific distance, two species that presented 0.6% of the distance between some specimens, and 16 congeneric pairs that were genetically distinct but showed low (0.9–1.8%) divergences. In the first case, the COI barcode grouped the two subspecies *A. denticulata denticulata* and *A. denticulata lacustris*, with 0.0% distance. Such genetic similarity indicates that these subspecies should be maintained as a single species. Together, their similar morphological characteristics and the lack of barcode divergence suggest that *A. denticulata lacustris* is a synonym of *A. denticulata denticulata*. The 0.0% distance was also observed between *A. leptochela* and *A. quilombola*. Morphologically, *A. quilombola* can be distinguished from related species *A. leptochela* by the well-developed cornea, whereas *A. leptochela* possesses reduced cornea, an adaptation to obligatory cave-dwelling (Bond-Buckup & Buckup, 1994; Moraes et al., 2017). However, both species were collected in the same cave, "Caverna dos Paiva", Ribeira do Iguape, Brazil (see Pérez-Losada et al., 2004), and do not show genetic divergence, suggesting that they are a single species. Likewise, we point out that one population of *A. jarai* (AY595594, AY595595) and *A. spinosa* (AY595576, AY595577) are synonymous. Furthermore, there was confirmation of a single species, through GMYC analysis, in both cases. It is noteworthy that the sequences referred to here as *A. quilombola* were deposited in GenBank as *A. marginata* and later described as *A. quilombola* (see Moraes et al., 2017).

Aegla uruguayana and *A. platensis* have a wide distribution, occurring in streams in Argentina, Brazil, and Uruguay (Schmitt, 1942a). Most *A. platensis* populations belong to a single species, but two potentially new species were reported (Zimmermann et al., 2018). On the other hand, *A. uruguayana* was suggested as a complex of at least ten cryptic species (Zimmermann et al., 2021). Thus, in addition to occurring in the same hydrographic basins, both species were suggested as a species complex (Zimmermann et al., 2018, 2021). In our study, only two sequences of each species showed a low genetic distance (0.6%) and were grouped in the GMYC analysis. It may be the case, therefore, that these sequences represent a cryptic species not yet described. However, detailed morphological and molecular analyzes are needed to support this hypothesis.

The low interspecific divergences presented by the 16 congeneric pairs may indicate more recent speciation. According to a phylogenetic study of *Aegla*, the radiation of aeglids towards the freshwater habitats began at least 60 Mya, but some taxa show a more recent age than their sister relatives (Pérez-Losada et al., 2004). Interestingly, these species have low interspecific distances with congeners that belong to the same clade. For example, *A. riolimayana* Schmitt, 1942, and *A. abtao* have 1.5% K2P distance, and both are grouped in clade A of the phylogeny presented in Pérez-Losada et al. (2004). Similar values of low divergence between closely related *Aegla* species were observed by Jara et al. (2003) and Páez et al. (2018).

Several studies have demonstrated that genetic divergence is much lower within species than among species (Hebert et al., 2003; Hebert et al., 2004; Lefébure et al., 2006; Costa et al., 2007; Ferri et al., 2009; Tang et al., 2009; Havermans et al., 2011; Matzen da Silva et al., 2011; Pereira et al., 2011, 2013). In particular, studies with crustaceans have reported nucleotide sequence divergence levels from 6 to 48 times greater among congeneric than in intraspecific variation (Costa et al., 2007; Havermans et al., 2011; Matzen da Silva et al., 2011). Consistent with the literature, the divergence between closely related *Aegla* species was 5 to 29 times higher than the genetic distances within species.

In general, our results based on DNA barcoding show that genetic divergences do not correspond to all currently morphological classifications of the *Aegla* species. Some species formed non-monophyletic groups in the NJ tree and showed unusually high intraspecific distances, comprising a complex of cryptic species. When considering species complexes (dataset A), it is possible to observe an overlap between intra- and inter-specific divergences, but a barcoding gap exists between independent genetic units (dataset B). By the way, the occurrence of cryptic species can change species richness, endemism, and, consequently, the importance of a given region for conservation. Therefore, recognize the cryptic species is crucial to understanding the diversity and distributional patterns of *Aegla*.

In summary, accurate discrimination of 92% of the analyzed species confirms the effectiveness of the COI barcode in identifying *Aegla* species. Furthermore, the application of the 2% threshold revealed many overlooked species. Our analyses suggest that the 74 supposed taxa possibly represent 116 species, pointing to at least 40 candidates for new species. Nevertheless, caution must be taken to establish that value as a threshold to delimit *Aegla* species, as a few species exhibit low interspecific divergences. According to Hebert et al. (2003), an identification system based on COI provides a higher taxonomic resolution than morphological studies achieve. However, this does not mean that DNA barcoding should be considered a substitute for conventional taxonomic approaches (Costa et al., 2007; Havermans et al., 2011). On the contrary, this tool should collaborate with morphological and ecological data in an integrative taxonomic framework to delimit species. Considering the above, we conclude that the barcode region of COI has considerable potential as a species identification system for *Aegla* and recommend a critical examination of the morphology of the specimens belonging to the species complexes recognized here to define new species.

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SUPPORTING INFORMATION

S1 Text. Statistical analyses in R.

```
> library(splits)
Loading required package: ape
Loading required package: MASS
Loading required package: paran
Warning messages:
1: package ‘ape’ was built under R version 3.5.3
2: package ‘MASS’ was built under R version 3.5.3
3: package ‘paran’ was built under R version 3.5.3
> setwd("C:/Users/Ingrid/Desktop/ANALISES/datasetA")
> list.files()
[1] "datasetA.fas"                  "datasetA.fas.jmodeltest.console"
[3] "datasetA.log"                  "datasetA.tre"
[5] "datasetA.trees"                "datasetA.xml"
[7] "datasetA.xml.state"
> tree<-read.nexus("datasetA.tre")
> plot(tree)
> result<-gmyc(tree)
node     T       loglik
2 -0.05602779 2081.693
3 -0.05511339 2082.087
4 -0.05275027 2079.416
5 -0.05243123 2080.026
6 -0.05172594 2083.238
7 -0.05122257 2083.74
8 -0.04819222 2091.224
9 -0.04755885 2092.846
10 -0.04745702 2094.322
11 -0.04734627 2097.092
12 -0.04657236 2096.316
13 -0.04621604 2094.516
```

```
14 -0.04493611 2098.331
15 -0.04293636 2097.82
16 -0.04277671 2097.051
17 -0.04254546 2098.444
18 -0.04222827 2110.291
19 -0.04197059 2109.367
20 -0.04150058 2109.998
21 -0.03909064 2109.126
22 -0.03896916 2112.115
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Wed Jul 21 17:48:33 2021
finish.
> summary(result)
Result of GMYC species delimitation

        method: single
        likelihood of null model:       2079.694
        maximum likelihood of GMYC model:       2168.666
        likelihood ratio:       177.943
        result of LR test:      0***

        number of ML clusters:  68
        confidence interval:    67-72

        number of ML entities:  116
        confidence interval:    109-127

        threshold time: -0.005222857

> plot(result)
Waiting to confirm page change...
Waiting to confirm page change...
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Waiting to confirm page change...
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2          1           AY050078_Aegla_papudo
3          2      AY595656.1_Aegla_ringueleti
4          2      AY595657.1_Aegla_ringueleti
5          3         AY050079.1_Aegla_affinis
6          3         AY050080.1_Aegla_affinis
7          3         AY050081.1_Aegla_affinis
8          3         AY050082.1_Aegla_affinis
9          4  AY050102.1_Aegla_hueicollensis
10         4  AY050103.1_Aegla_hueicollensis
11         5         FJ471847.1_Aegla_chilota
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14         5         FJ471853.1_Aegla_chilota
15         5         FJ471854.1_Aegla_chilota
16         5         FJ471848.1_Aegla_chilota
17         5         FJ471850.1_Aegla_chilota
18         5         FJ471849.1_Aegla_chilota
19         5         FJ471851.1_Aegla_chilota
20         5         FJ471852.1_Aegla_chilota
21         6          AY050116.1_Aegla_manni
22         6          AY050118.1_Aegla_manni
23         7      FJ471822.1_Aegla_alacalufi
24         7      FJ471826.1_Aegla_alacalufi
25         7      FJ471823.1_Aegla_alacalufi
26         7      FJ471824.1_Aegla_alacalufi
27         7      FJ471825.1_Aegla_alacalufi
28         7      FJ471827.1_Aegla_alacalufi
29         7      FJ471830.1_Aegla_alacalufi
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31         7      FJ471828.1_Aegla_alacalufi
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36	8	AY050115.1_Aegla_denticulata_lacustris
37	9	AY595667_Aegla_neuquensis
38	9	AY595668_Aegla_neuquensis
39	10	AY595648.1_Aegla_scamosa
40	10	AY595649.1_Aegla_scamosa
41	11	AY050083.1_Aegla_laevis
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43	12	FJ360704.1_Aegla_concepcionensis
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65	16	AY050101.1_Aegla_pewenchae

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8	73	19	AY050108.1_Aegla_abtao
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11	76	20	AY050111.1_Aegla_abtao
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223	76	AY595561_Aegla_castro
224	77	AY595562_Aegla_castro
225	78	AY595563_Aegla_schmitti
226	79	AY595564_Aegla_parana
227	80	AY595565_Aegla_parana
228	81	AY595568_Aegla_jarai
229	82	AY595569_Aegla_jarai
230	83	AY595570_Aegla_jarai
231	84	AY595573_Aegla_jarai
232	85	AY595578_Aegla_jarai
233	86	AY595579_Aegla_jarai
234	87	AY595580.1_Aegla_camargoi
235	88	AY595581_Aegla_jarai

236	89	AY595582_Aegla_jarai
237	90	AY595583_Aegla_schmitti
238	91	AY595586_Aegla_castro
239	92	AY595593.1_Aegla_serrana
240	93	AY595604.1_Aegla_inconspicua
241	94	AY595605.1_Aegla_franciscana
242	95	AY595607.1_Aegla_serrana
243	96	AY595608.1_Aegla_longirostri
244	97	AY595609.1_Aegla_longirostri
245	98	AY595610.1_Aegla_longirostri
246	99	AY595611.1_Aegla_grisella
247	100	AY595612.1_Aegla_grisella
248	101	AY595614.1_Aegla_singularis
249	102	AY595617.1_Aegla_serrana
250	103	AY595620.1_Aegla_camargoi
251	104	AY595647.1_Aegla_scamosa
252	105	AY595654.1_Aegla_sanlorenzo
253	106	AY595655.1_Aegla_sanlorenzo
254	107	AY595664.1_Aegla_intercalata
255	108	AY595665.1_Aegla_intercalata
256	109	AY595666.1_Aegla_intercalata
257	110	FJ360706.1_Aegla_lancinhas
258	111	FJ360707.1_Aegla_lancinhas
259	112	HQ236289.1_Aegla_cholchol
260	113	MH998634.1_Aegla_brevipalma
261	114	MH998635.1_Aegla_brevipalma
262	115	MH998636.1_Aegla_carinata
263	116	MH998637.1_Aegla_franca

TABLE S1. Average pairwise divergences within and between each cryptic species of *Aegla* identified in dataset B. The asterisk after taxon names indicates the species grouped by the GMYC analysis. N represents the number of analyzed sequences. ** Accession numbers correspond to the BOLD System database. Genetic distances values are shown as percentages.

Species	**N**	**Genbank accession number**	**Intraspecific distance**	**Interspecific distance**
Aegla abtao species complex 1	2	AY050112, AY050113	0.0	5.5
Aegla abtao species complex 2	2	AY050108, AY050109	0.0	5.7
Aegla abtao species complex 3	2	AY050110, AY050111	0.3	5.1
Aegla abtao species complex 4	2	KX910287, KX910288	0.0	6.0
Aegla affinis	4	AY050079-AY050082	0.2	6.3
Aegla alacalufi	10	FJ471822-FJ471831	0.2	5.5
Aegla araucaniensis	2	AY050088, AY050089	0.9	4.7
Aegla bahamondei	1	AY050090	–	5.4
Aegla brevipalma species complex 1	1	MH998634	–	4.6
Aegla brevipalma species complex 2	1	MH998635	–	4.2
Aegla buenoi	4	AEGPR016-18-AEGPR020-18**	0.0	5.1
Aegla camargoi species complex 1	2	AY595589, AY595590	0.3	6.0
Aegla camargoi species complex 2	1	AY595580	–	5.4
Aegla camargoi species complex 3	1	AY595620	–	4.6
Aegla carinata	1	MH998636	–	5.0
Aegla castro species complex 1	1	AY595561	–	5.4
Aegla castro species complex 2	1	AY595562	–	6.0
Aegla castro species complex 3	1	AY595586	–	5.6
Aegla cavernicola	2	AY595635, AY595636	0.0	4.5
Aegla chilota	10	FJ471847-FJ471856	0.1	6.3
Aegla cholchol species complex 1	7	HQ236287, HQ236290-HQ236292, HQ236294-HQ236296	0.2	5.9
Aegla cholchol species complex 2	2	HQ236288, HQ236293	0.0	5.8
Aegla cholchol species complex 3	1	HQ236289	–	5.5

Aegla concepcionensis	2	FJ360704, FJ360705	0.3	5.4
*Aegla denticulata denticulata/lacustris**	4	AY050106, AY050107, AY050114, AY050115	0.0	5.7
Aegla franca	1	MH998637	–	5.9
Aegla franciscana species complex 1	2	AY595603, AY595606	0.6	4.6
Aegla franciscana species complex 2	1	AY595605	–	5.6
Aegla georginae species complex 1	4	MG581889-MG581892	0.0	7.1
Aegla georginae species complex 2	4	MH212137, MH212138, MH998638, MH998639	0.0	7.0
Aegla georginae species complex 3	2	MH212139, MH998640	0.0	7.9
Aegla grisella species complex 1	2	AY595613, AY595616	0.6	5.0
Aegla grisella species complex 2	1	AY595611	–	6.9
Aegla grisella species complex 3	1	AY595612	–	6.7
Aegla hueicollensis	2	AY050102, AY050103	0.0	6.6
Aegla humahuaca	2	AY595660, AY595661	0.0	5.4
Aegla inconspicua	1	AY595604	–	6.6
Aegla inermis	2	AY595621, AY595622	0.3	6.5
Aegla intercalata species complex 1	1	AY595664	–	4.7
Aegla intercalata species complex 2	1	AY595665	–	5.4
Aegla intercalata species complex 3	1	AY595666	–	5.3
Aegla itacolomiensis	3	AY595598-AY595600	0.0	5.5
Aegla jacutinga	4	AEGPR006-18, AEGPR010-18, AEGPR021-19, AEGPR022-19**	0.2	5.5
Aegla jarai species complex 1	1	AY595568	–	4.4
Aegla jarai species complex 2	1	AY595569	–	4.7
Aegla jarai species complex 3	1	AY595570	–	4.8
Aegla jarai species complex 4	1	AY595573	–	5.5
Aegla jarai species complex 5	1	AY595578	–	4.5
Aegla jarai species complex 6	1	AY595579	–	5.0
Aegla jarai species complex 7	1	AY595581	–	5.3

Aegla jarai species complex 8	1	AY595582	–	4.0
Aegla jujuyana	2	AY595652, AY595653	0.6	6.6
Aegla laevis	2	AY050083, AY050084	0.0	5.0
Aegla lancinhas species complex 1	1	FJ360706	–	5.2
Aegla lancinhas species complex 2	1	FJ360707	–	5.5
Aegla lata	4	AEGPR001-18-AEGPR004-18**	0.0	7.2
*Aegla leptochela/quilombola**	4	AY595637, AY595638, AY595641, AY595642	0.1	6.3
Aegla leptodactyla	2	AY595618, AY595619	0.9	5.6
Aegla ligulata	3	AY595555- AY595557	0.6	5.9
Aegla longirostri species complex 1	5	KX910280, KX910281, KX910283, KX910285, KX910286	0.0	6.3
Aegla longirostri species complex 2	2	KX910282, KX910284	0.0	6.4
Aegla longirostri species complex 3	1	AY595608	–	6.9
Aegla longirostri species complex 4	1	AY595609	–	7.0
Aegla longirostri species complex 5	1	AY595610	–	6.9
Aegla loyolai	3	KT319209-KT319211	0.0	5.6
Aegla ludwigi	10	MG581884-MG581887, MH212140-MH212142, MH998641-MH998643	0.2	6.4
Aegla manni species complex 1	2	AY050116, AY050118	0.0	5.8
Aegla manni species complex 2	1	AY050117	–	5.8
Aegla manuinflata	3	MF442423-MF442425	0.2	5.0
Aegla marginata	2	AY595558, AY595559	0.0	6.4
Aegla meloi	3	KT319206-KT319208	0.2	5.1
Aegla nebeccana	2	AEGIV001-19, AEGIV002-19**	0.0	6.6
Aegla neuquensis	2	AY595667, AY595668	0.0	6.6
Aegla obstipa	2	AY595645, AY595646	0.3	6.5
Aegla odebrechtii	2	AY595571, AY595572	0.9	4.5
Aegla okora	4	AEGBR001-18-AEGBR004-18**	0.0	5.7
Aegla papudo	2	AY050077, AY050078	0.0	7.5

Aegla parana species complex 1	1	AY595564	–	5.8
Aegla parana species complex 2	1	AY595565	–	5.1
Aegla parva	2	AY595591, AY595592	0.0	4.9
Aegla paulensis	2	AY595584, AY595585	1.2	5.0
Aegla perobae	2	AY595587, AY595588	0.3	7.5
Aegla pewenchae	3	AY050099, AY050100, AY050101	0.0	6.4
Aegla plana	2	AY595601, AY595602	0.0	5.7
*Aegla platensis/uruguayana**	4	AY595627, AY595628, MT489799, MT489800	0.4	5.8
Aegla platensis species complex 1	5	AY595662, AY595663, MF448838-MF448840	0.4	8.7
Aegla platensis species complex 2	3	MF448831-MF448833	0.0	6.3
Aegla prado	2	AY595633, AY595634	0.0	6.5
Aegla ringueleti	2	AY595656, AY595657	0.3	7.7
Aegla riolimayana	4	AY595548, AY050098, KX910289, KX910290	0.0	4.5
Aegla rossiana	2	AY595623, AY595624	0.0	5.5
Aegla rostrata	10	HQ236248-HQ236257	0.1	6.0
Aegla sanlorenzo species complex 1	1	AY595654	–	6.4
Aegla sanlorenzo species complex 2	1	AY595655	–	6.8
Aegla scamosa species complex 1	2	AY595648, AY595649	0.3	6.7
Aegla scamosa species complex 2	1	AY595647	–	5.8
Aegla schmitti species complex 1	1	AY595583	–	4.4
Aegla schmitti species complex 2	1	AY595563	–	4.5
Aegla septentrionalis	2	AY595650, AY595651	0.0	6.4
Aegla serrana species complex 1	1	AY595593	–	5.6
Aegla serrana species complex 2	1	AY595607	–	6.3
Aegla serrana species complex 3	1	AY595617	–	5.6
Aegla singularis	1	AY595614	–	8.4
Aegla spectabilis	1	AY050097	–	4.6
Aegla spinipalma	4	AY595615, KX910291-KX910293	0.3	6.4

*Aegla spinosa/jarai**	4	AY595576, AY595577, AY595594, AY595595	0.1	4.4
Aegla strinatii species complex 1	2	AY595639, AY595640	0.3	5.1
Aegla strinatii species complex 2	1	AY595560	–	6.2
Aegla talcahuano species complex 1	1	AY050085	–	6.2
Aegla talcahuano species complex 2	1	AY050086	–	4.9
Aegla talcahuano species complex 3	1	AY050087	–	5.9
Aegla uruguayana species complex 1	4	AY595631, AY595632, MT489834, MT489835	0.2	6.5
Aegla uruguayana species complex 2	2	MT489824, MT489825	0.3	7.1
Aegla uruguayana species complex 3	2	MT489826, MT489827	0.6	5.9
Aegla violacea species complex 1	3	AY595552-AY595554	0.0	6.0
Aegla violacea species complex 2	2	AY595625, AY595626	0.0	5.0

5. CONCLUSÃO GERAL

A análise de uma grande quantidade de amostras de diferentes períodos e regiões da bacia do rio Tibagi, tanto de coleção quanto amostras recém coletadas, possibilitou aprimorar o conhecimento da diversidade e padrões de distribuição dos eglídeos desta região. As distribuições de *A. castro*, *A. lata* e *A. jacutinga* foram revisadas e ampliadas. Além disso, descrevemos cinco espécies novas; uma da região do Baixo Tibagi e as outras quatro da região do Médio Tibagi. Com isso, a diversidade de eglídeos nesta bacia hidrográfica passa de 4 para 9 espécies e o número de espécies de *Aegla* conhecidas aumenta para 95. No entanto, as espécies novas têm distribuições limitadas e ocorrem em habitats impactados pela urbanização ou agricultura.

Das nove espécies de *Aegla* que ocorrem na bacia do rio Tibagi, sete são endêmicas dessa bacia. Embora morfologicamente conservativos, a análise minuciosa da morfologia permitiu a distinção das espécies bem como a construção de uma chave de identificação. Ressaltamos que, para efeito de comparação, é importante ter espécimes machos adultos, pois nestes indivíduos o desenvolvimento completo dos quelípodos, espinhos e tubérculos permite uma interpretação mais segura dos caracteres diagnósticos das espécies.

Apesar de vários cursos de água do Médio e Baixo Rio Tibagi terem sido amostrados neste estudo, muitas partes do Alto Tibagi têm apenas alguns ou nenhum registro de eglídeos, indicando uma área prioritária a ser explorada em estudos futuros. Assim, os nossos resultados demonstram a importância da bacia do rio Tibagi para a conservação dos eglídeos e reforçam o alto potencial de descoberta de espécies novas no estado do Paraná.

A discriminação precisa de 92% das espécies analisadas confirma a eficácia da região barcode do COI na identificação de espécies *Aegla*. Nossa matriz de distância genética indicou que os valores de divergência entre as espécies são normalmente maiores que 2%. Poucos pares de espécies (16 pares) asseguradas como válidas, tanto pela análise morfológica quanto pelo GMYC, apresentaram divergências interespecíficas inferiores a 2%, provavelmente em decorrência de especiações recentes. Desta forma, ressaltamos o cuidado ao estabelecer esse valor como um limiar para delimitar as espécies de *Aegla*. Nossas análises sugerem que os 74 supostos táxons possivelmente representam 116 espécies, apontando para pelo menos 40 candidatos para espécies novas. Contudo, recomendamos um exame crítico da morfologia dos espécimes pertencentes a estes complexos para dar suporte à identificação de novas espécies com base no DNA barcode.