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Phylogeography reveals unexpectedly low genetic diversity in a widely distributed species: the case of the freshwater crab *Aegla platensis* (Decapoda: Anomura)

BIANCA L. ZIMMERMANN[1*], MARCELO S. CRIVELLARO[1], CAROLINE B. HAUSCHILD[1], MARLISE L. BARTHOLOMEI-SANTOS[1], KEITH A. CRANDALL[2,3], MARCOS PÉREZ-LOSADA[2,3,4], FEDERICO GIRI[5], PABLO COLLINS[5] and SANDRO SANTOS[1]

[1]*Programa de Pós-graduação em Biodiversidade Animal, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil*
[2]*Computational Biology Institute, George Washington University, Ashburn, VA 20147, USA*
[3]*Department of Invertebrate Zoology, US National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA*
[4]*CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, 4485-661 Vairão, Portugal*
[5]*Laboratorio de Macrocrustáceos, Instituto Nacional de Limnología, Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional del Litoral, Santa Fe, CP3000, Argentina*

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Habitat and taxon-specific properties could affect the propensity for cryptic species to be formed. For example, anomurans of the genus *Aegla* possess characteristics that suggest the existence of cryptic diversity. The widely distributed species *Aegla platensis*, besides having been considered paraphyletic, shows a considerable amount of morphological variation in the carapace shape among populations. Thus, the aim of this study was to test the hypothesis that *A. platensis* encompasses a large complex of cryptic species. Seventeen populations of *A. platensis* from Argentina and Brazil were analysed using three molecular markers. Contrary to our expectations, 16 populations seem to belong to a single species. Only one population of *A. platensis* might represent an unrecognized new species. These results are intriguing because they do not fit the phylogeographical pattern seen in other aeglids, which usually have narrow distributions. Although intrinsic characteristics and/or historical biogeographical events could be related to these findings, the factors driving the broad distribution of *A. platensis* still need to be clarified. Finally, we highlight the fact that taxonomic issues in aeglids are far from being fully understood, and the use of a broad population-based sampling can be useful to improve our understanding of the group's systematics and evolution.

ADDITIONAL KEYWORDS: aeglids – cryptic diversity – endangered crustaceans – molecular systematics – South America.

INTRODUCTION

Recent efforts have been devoted to the study, discovery and description of cryptic species (Moraes *et al.*, 2016; Rocha *et al.*, 2016; Domingos *et al.*, 2017). Despite the progress achieved, it is still unclear whether this phenomenon represents the limits of the discriminatory power of traditional taxonomic approaches or a genuine part of biodiversity resulting from recent speciation events (de León & Poulin, 2016). Cryptic species may also have a deep divergence (Bond *et al.*, 2001; Elmer, Dávila & Lougheed, 2007) or may have arisen through evolutionary convergence (Goodman *et al.*, 2009). Mounting evidence indicates the presence of cryptic species in a variety of taxa (Bickford *et al.*, 2007), drawing attention to the fact that this phenomenon is much more common and widespread than previously thought. However, cryptic diversity is not homogeneously distributed among

*Corresponding author. E-mail: bia.lais@gmail.com

metazoans, being particularly common in groups such as amphibians, reptiles and crustaceans (de León & Poulin, 2016). Ecological differences between habitats and intrinsic properties of organisms could affect diversification rates. The discontinuity of freshwater habitats, for example, might explain the disproportionately higher diversification of freshwater organisms (Grosberg, Vermeij & Wainwright, 2012; Poulin & de León, 2017).

One crustacean group that can potentially harbour a large hidden diversity is the freshwater anomurans of the genus *Aegla* Leach, 1820. The extant aeglids are endemic to the temperate and subtropical regions of continental South America (Bond-Buckup *et al.*, 2008), whose origin was estimated at ~60 Mya (Pérez-Losada *et al.*, 2004). The radiation and speciation of aeglids throughout the Cenozoic Era up to the present time corroborate the successful adaptation and colonization of freshwater habitats (Bueno *et al.*, 2016a), culminating in a diversity of 85 currently described species (Moraes, Tavares & Bueno, 2017). However, as the diagnostic characters of these animals are very limited in number and exhibit low variation in terms of character state (Bond-Buckup & Buckup, 1994), the real diversity of *Aegla* might be underestimated. Indeed, several cryptic species were recently found in populations of *Aegla paulensis* Schmitt, 1942 (Moraes *et al.*, 2016) and *Aegla longirostri* Bond-Buckup & Buckup, 1994 (Crivellaro *et al.*, 2017). Researchers also highlight the potential for more cryptic species to be found within this genus (Bueno, Shimizu & Moraes, 2016b; Moraes *et al.*, 2016; Crivellaro *et al.*, 2017).

Aegla platensis Schmitt, 1942 features the widest geographical distribution of all *Aegla* species, occurring in Brazil, Argentina, Uruguay and Paraguay (Santos *et al.*, 2017). Candidates for cryptic species complexes are often concealed within broadly distributed species (Angulo & Icochea, 2010; Florio *et al.*, 2012). In many instances, these species considered to have a wide area of occurrence are in fact a complex of cryptic species (e.g., Lefébure *et al.*, 2007; Manthey, Klicka & Spellman, 2011; Warner, Oppen & Willis, 2015; Dénes *et al.*, 2016). Additionally, specimens of *A. platensis* from Argentina and Brazil were considered paraphyletic in a study performed by Pérez-Losada *et al.* (2004), suggesting the presence of a possible species complex. Lastly, the carapace shape between Argentinean and Brazilian populations of *A. platensis* separated these crabs in two distinct groups, and even within these groups, shape variation was considerable (Marchiori, Fornel & Santos, 2015). Geometric morphometrics also revealed intraspecific variation in the carapace shape of *A. longirostri* (Marchiori, Bartholomei-Santos & Santos, 2014), a species for which a large cryptic diversity was subsequently found (Crivellaro *et al.*, 2017).

The aforementioned evidence indicates that *A. platensis* might exhibit a hidden diversity; nonetheless, no extensive molecular analysis at the population level has been performed for this species. Phylogeographical analyses that cover geographically dense and large sample sizes, alongside molecular genetics-based phylogenies, have made a significant contribution to the understanding of species history and speciation (Hickerson *et al.*, 2010; Tougard *et al.*, 2013; Mráz & Ronikier, 2016). Indeed, phylogeographical studies have revealed cryptic species diversity in a variety of groups (e.g. Rocha, Harris & Posada, 2011; Dincă *et al.*, 2013; Lu, Bi & Fu, 2014; Hassanin *et al.*, 2015; Viñas *et al.*, 2015; DiBattista *et al.*, 2017), including aeglids (Crivellaro *et al.*, 2017). Thus, our objective was to use phylogeographical methods and the focal species *A. platensis* to test the hypothesis that taxa with wide distribution, morphological variation among populations, and propensity to have a hidden diversity could encompass a complex of several cryptic species.

MATERIAL AND METHODS

Sample collection

A total of 179 individuals were sampled, 49 from nine Argentinean locations and 130 from nine Brazilian locations (Table 1); these populations are representative of the main hydrographic basins where the species has been previously described (Santos *et al.*, 2017), the Uruguay River basin (La Plata system) and the endorheic basins of Mar Chiquita and Serrano River (Fig. 1). Specimens were preserved in ethanol and identified on the basis of their external morphology (Bond-Buckup & Buckup, 1994). Tissue (gill or muscle) samples were used for molecular procedures. Genetic vouchers, from which tissue samples were obtained, were deposited at the Crustacean Collection of the Department of Ecology and Evolution, UFSM, Brazil and at the Decapod Collection of the Macrocrustacean Laboratory, Instituto Nacional de Limnología (INALI, CONICET-UNL), Argentina. For outgroup rooting, we included the following representatives of the genus *Aegla* according to the clades identified by Pérez-Losada *et al.* (2004): *Aegla abtao* Schmitt, 1942 and *Aegla riolimayana* Schmitt, 1942 (clade B); *A. longirostri* and *Aegla spinipalma* Bond-Buckup & Buckup, 1994 (clade E); and *A. longirostri sensu lato*, sampled from São João do Polêsine (Crivellaro *et al.*, 2017), which clustered with species from clade D. We also used *A. platensis* sequences from samples from Argentina and Brazil previously analysed by

Table 1. Number of samples, number of sequences, number of haplotypes, and results of analysis of molecular variance and genetic differentiation analyses for the populations examined in this study

Locality	Code	Voucher	*N*	Latitude (S)	Longitude (W)	Elevation (m)	Number of sequences (number of haplotypes)		
							COI	16S	*ANT*
Potiribu River	POTIRIBU BR	307	15	28° 33′ 29″	53° 36′ 36″	407	11 (2)	11 (1)	12 (1)
Tributary of Curuçu River	CURUÇU BR	330	15	29° 13′ 20″	54° 51′ 55″	362	15 (2)	13 (1)	15 (1)
Tributary of Toropí River	TOROPI BR	336	10	29° 26′ 55″	54° 07′ 38″	341	10 (1)	10 (1)	10 (1)
Icamaquazinho River	ICAMAQUAZINHO BR	340	15	28° 46′ 35″	54° 55′ 47″	173	14 (1)	14 (1)	14 (1)
Alegre River	ALEGRE BR	346	15	28° 11′ 16″	53° 25′ 54″	445	12 (1)	11 (2)	15 (1)
Albino Stream	ALBINO BR	353	15	28° 08′ 12″	54° 55′ 31″	167	14 (2)	09 (1)	10 (1)
Tributary of Ijuí River	IJUÍ BR	361	15	28° 10′ 58″	54° 31′ 33″	296	13 (3)	12 (1)	13 (3)
Várzea River	VARZEA BR	380	15	27° 25′ 28″	53° 24′ 41″	486	05 (2)	06 (1)	10 (1)
Sarandi Stream	SARANDI BR	385	15	30° 39′ 53″	55° 52′ 25″	237	03 (1)	11 (2)	15 (1)
Singuil River	SINGUIL AR	AAP1	10	27° 51′ 00″	65° 50′ 03″	1187	—	10 (1)	10 (1)
Los Nogales Stream	LOSNOGALES AR	AAP2	6	28° 13′ 02″	65° 52′ 34″	1323	—	06 (1)	06 (1)
El Tala Stream	ELTALA AR	AAP3	3	28° 27′ 56″	65° 49′ 55″	744	—	03 (1)	03 (1)
Nogales Stream	NOGALES AR	AAP4	3	28° 12′ 58″	65° 52′ 55″	1302	—	03 (1)	03 (1)
Pirquitas Dam	PIRQUITAS DAM AR	AAP5	3	28° 16′ 08″	65° 44′ 22″	716	—	03 (1)	03 (1)
Pirquitas River	PIRQUITAS RIV AR	AAP6	4	28° 17′ 13″	65° 43′ 46″	671	—	03 (1)	04 (1)
La Angostura Dam	LAANGUSTURA AR	AAP10	10	26° 55′ 23″	65° 40′ 55″	1900	08 (2)	10 (1)	10 (1)
Itacuarare River	ITACURARE AR	AAP58	10	27° 52′ 15″	55° 16′ 39″	113	09 (4)	09 (2)	10 (1)
					Haplotype diversity (Hd)		0.932	0.822	0.440
					Nucleotide diversity (π)		0.033	0.007	0.005
					Genetic differentiation (F_{ST})		0.975	0.957	0.885
					Variation among populations		97.54%	95.76%	88.58%
					Variation within populations		2.46%	4.24%	11.42%

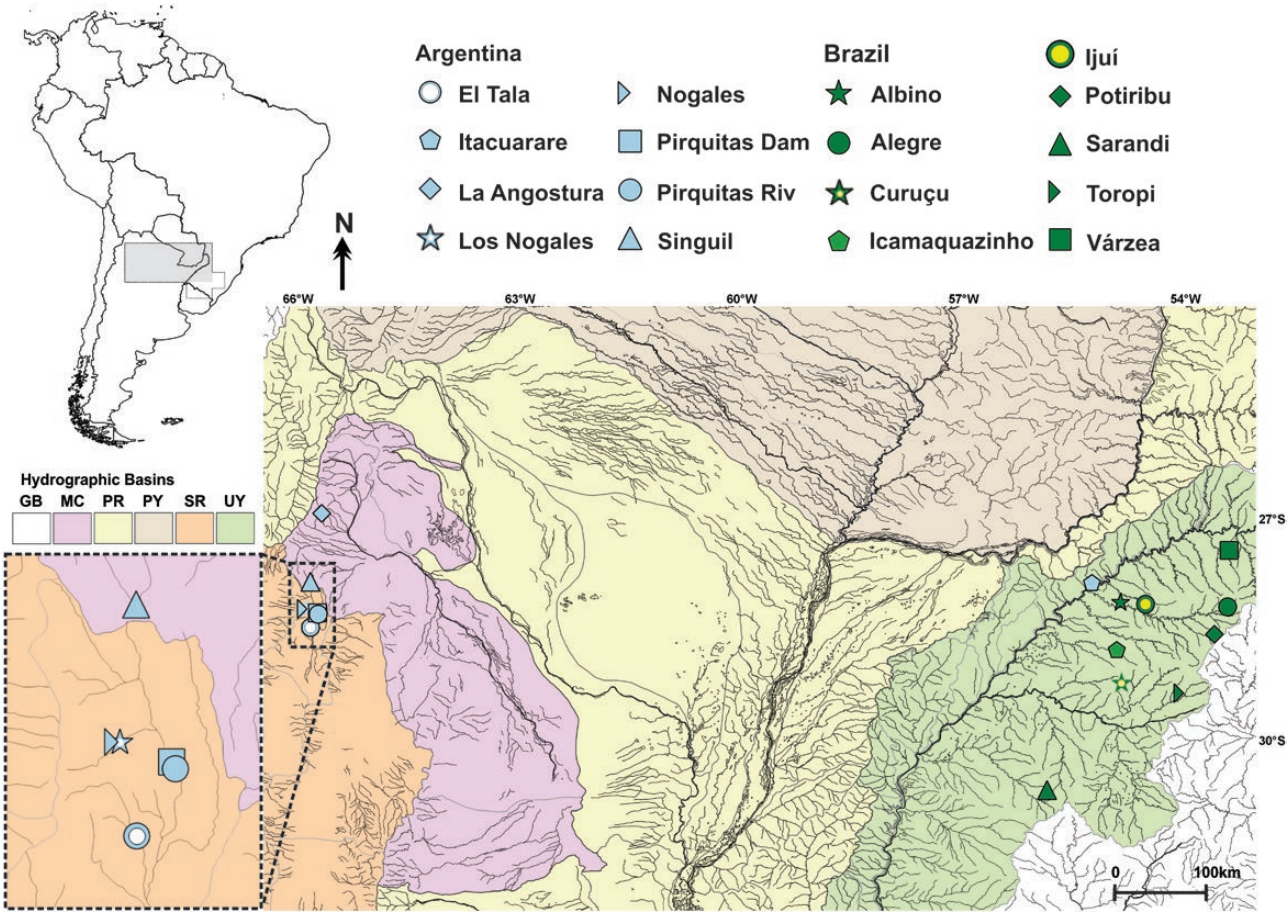


Figure 1. Map showing the sampling locations for *Aegla platensis* in Brazil (green symbols) and Argentina (blue symbols). Hydrographic basins are represented by colours: white for the Guaíba River Basin (GB), yellow for the Paraná River Basin (PR), pink for the Mar Chiquita Basin (MC), beige for the Paraguay River Basin (PY), orange for the Serrano River Basin (SR), and green for the Uruguay River Basin (UY). The dotted area in the map of South America represents the total occurrence range of *A. platensis sensu lato*, and the highlighted area represents the portion of the occurrence range that was sampled.

Pérez-Losada *et al.* (2004), belonging to clade D and considered as non-monophyletic.

DNA EXTRACTION, POLYMERASE CHAIN REACTION AMPLIFICATION AND SEQUENCING

Total DNA was extracted using a QIAamp DNA Micro Kit (Qiagen), following the manufacturer's instructions. Partial fragments of two mitochondrial genes, 16S ribosomal RNA (16S) and cytochrome *c* oxidase subunit I (*COI*), were amplified with primers 16Saeglid-f/16Saeglid-r (Pérez-Losada *et al.*, 2002) and LCOI-f/COIA2-r (Xu *et al.*, 2009). We also amplified a nuclear intron fragment of the adenine nucleotide transporter (*ANT*) gene using the primer pair DecapANTF/ANTir1 (Teske & Beheregaray, 2009; Barber *et al.*, 2012). All the reactions were carried out in volumes of 25 µL, containing 50 ng of DNA, 1.7 U of Taq DNA polymerase, 1× Taq buffer, 4 mM $MgCl_2$, 20 pmol of each primer, 205 µM of each dNTP and 1% DMSO. For the 16S amplifications, the following settings were used: 35 cycles (30 s at 94 °C, 45 s at 54 °C and 1 min at 72 °C), with initial denaturation at 94 °C for 5 min and final extension at 72 °C for 5 min. For the *ANT* gene, the settings were as follows: 35 cycles (30 s at 94 °C, 45 s at 53 °C and 45 s at 72 °C), with initial denaturation at 94 °C for 3 min and final extension at 72 °C for 7 min. For the *COI* amplifications, the settings used were as follows: 35 cycles (30 s at 94 °C, 45 s at 47 °C and 1 min at 72 °C), with initial denaturation at 94 °C for 5 min and final extension at 72 °C for 5 min. The standard polymerase chain reaction (PCR) was run and PCR products were checked by agarose gel electrophoresis, purified and then sequenced. Consensus sequences for both strands were aligned with Muscle (Edgar, 2004). All contiguous

insertion/deletion events (indels) were treated as one mutational step (Simmons, Ochoterena & Carr, 2001), and hypervariable sites were weighted as zero to prevent the inclusion of homoplastic characters.

Phylogenetic analyses

Phylogenetic analyses were conducted for single genes and concatenated data sets using Bayesian (BI) and maximum likelihood (ML) methods. The best-fit models of nucleotide substitution for each gene were selected with JModeltest 2.1.10 (Darriba *et al.*, 2012). Bayesian analyses were performed using the Monte Carlo Markov chain (MCMC) method as implemented in BEAST version 1.8.0 (Drummond *et al.*, 2012). The Bayesian analysis was run for 50 million chains and sampled every 1000 generations. Posterior probabilities were calculated with a burn-in of 5 million states and checked for convergence using Tracer version 1.6 (Rambaut *et al.*, 2014). The ML analyses were performed with raxmlGUI 1.5 (Silvestro & Michalak, 2012), using a GTR + CAT model, with nodal support estimated by 1000 bootstrap replicates. To test the placement of *A. platensis* according to the clades defined by Pérez-Losada *et al.* (2004), we first estimated an ML phylogeny in the raxmlGUI program using mitochondrial sequences (*COI* and 16S) of individuals from this study and from other species of *Aegla* deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/), including representatives from all the clades described. Once the placement of *A. platensis* in clade D was confirmed, we re-estimated a new tree using only the species included in this clade. Phylogenetic analyses were conducted as described previously. All results were visualized and checked with FigTree 1.4.2 (Rambaut, 2014).

Species delimitation

For species delimitation analyses, we used the general mixed Yule coalescent (GMYC) method of Pons *et al.* (2006) and the automatic barcode gap discovery (ABGD) method of Puillandre *et al.* (2012). The GMYC model was conducted using the standard parameters and a single threshold. These analyses were conducted with the package 'splits' (species limits by threshold statistics; http://r-forge.r-project.org/projects/splits) using R v.3.3 (R Development Core Team, 2011). The ABGD method was implemented using the online version of the program (http://wwwabi.snv.jussieu.fr/public/abgd/), with default parameters. Given that it was not possible to obtain *COI* sequences for all tested populations (see below in the Results section), we used a concatenated data set (16S and *ANT* sequences) to test species boundaries. Only the ingroup was considered.

We also used a Bayes factor (BF) approach to test the monophyly of *A. platensis* by comparing species model hypotheses. The tested models were an unconstrained tree *versus* a constrained tree considering *A. platensis* as monophyletic. The BF calculates the ratio of the marginal likelihood of two models, which has the advantage of taking into account priors used in the Bayesian analysis (Xie *et al.*, 2011). The marginal likelihood values of these competing models were estimated using stepping-stone sampling (SS; Xie *et al.*, 2011) in the BEAST package and run for 10 million generations of 30 path-steps. The better model was chosen when twice the natural logarithm of the BF testing statistic ($2lnBf$) was greater than two (Kass & Raftery, 1995). A value greater than ten was assumed to indicate decisive support for distinguishing between competing species-delimitation hypotheses (Grummer, Bryson & Reeder, 2014). All parameters were set up as described in the previous section.

Population genetic, phylogeographical and demographic analysis

The levels of haplotype diversity (Hd) and nucleotide diversity (π) were estimated for each gene sequence using DnaSP v. 4.10.3 (Rozas *et al.*, 2003). To examine the population structure, an analysis of molecular variance (AMOVA) was performed for mitochondrial (16S and *COI*) and nuclear (*ANT*) genes in ARLEQUIN v. 3.1 (Excoffier, Laval & Schneider, 2005). The AMOVA was run with 10 000 permutations and no hierarchical structure (all populations in a single group). Genetic differentiation (F_{ST}) was computed using the same program. Additionally, genetic divergences within and among populations were obtained using the p-distance model with 1000 bootstrap replicates in MEGA 6.0 (Tamura *et al.*, 2013).

Tajima's *D* (Tajima, 1989) and Fu's *Fs* (Fu, 1997) were applied to test the selective neutrality of genetic markers. These estimators are also sensitive to demographic processes, such as recent population expansion or bottlenecks, which we test for below. For each population, Tajima's *D* and Fu's *Fs* were estimated in ARLEQUIN with 10 000 simulations. The genealogical relationships among 16S, *ANT* and *COI* sequences were determined by a haplotype network generated with the median-joining method (Bandelt, Forster & Röhl, 1999) in NETWORK v. 4.6 (http://www.fluxus-engineering.com).

Divergence times [i.e. time to the most recent common ancestor (TMRCA)] among mitochondrial haplotypes were estimated using a Bayesian approach with BEAST 1.8.0 (Drummond *et al.*, 2012). A run of 50 million chains was performed and sampled every 1000 generations. The settings used were the Yule tree prior, the Hasegawa-Kishino-Yano plus invariable

sites model (HKY + I) substitution model (defined by JModeltest model selection) with four gamma categories, and the strict clock model (although the relaxed clock model was slightly better based on Bayes factors, the convergence values were much better for the strict clock model, and both approaches gave similar topologies and divergence times; we therefore chose to use the strict clock; Brown & Yang, 2011). The mitochondrial DNA (mtDNA) substitution rate used was 0.118 substitutions/site/Myr (Xu *et al.*, 2009; Barber *et al.*, 2012), with a standard deviation of 5%. The temporal trends in effective population size were reconstructed with Bayesian Skyride Plot (Minin, Bloomquist & Suchard, 2008) implemented in BEAST using the estimated clock rate and with MCMC simulations and tree sampling as described before.

RESULTS

Phylogenetic analyses

From the 179 individuals sampled, we obtained 16S sequences for 144 specimens (393 bp aligned, including gaps), nuclear *ANT* sequences for 163 specimens (275 bp aligned, including gaps), and *COI* sequences for 114 specimens (723 bp unambiguous alignment). Despite all efforts, we obtained appropriate *COI* sequences for only two populations from Argentina (Table 1). Sequences were deposited in GenBank under the accession numbers MF442420–MF442425 and MF448727–MF449128.

Tree topologies were congruent between maximum likelihood and Bayesian analyses. Their main difference was in clade support, which was generally higher in the BI analysis. Phylogenetic trees generated with single and concatenated (16S + *ANT*) gene sequences showed similar topologies. With the exception of the Potiribu population, all other populations form a well-supported monophyletic clade (Fig. 2). Although incomplete (i.e. missing some populations from Argentina), the tree obtained with the *COI* gene sequences corroborates these results (Supporting information, Fig. S1). Despite the large geographical distance between populations of Argentina and Brazil (~750 km in a straight line, and 1970 km when directly following the stream course), there was no genetic structure according to the geographical origin of the populations.

The phylogenies generated with most of the described *Aegla* species confirmed that all tested populations of *A. platensis* grouped to species belonging to clade D. In addition, these analyses also suggest that the majority of populations (except Potiribu) belong to a monophyletic group (Fig. 3). However, in this same phylogeny, the representative of *Aegla singularis* was positioned among populations of *A. platensis*. Sequences of the Potiribu population, in contrast, were related to some new *Aegla* species (Fig. 3), and it is possible that they represent an unrecognized species of *Aegla*. Regarding the sequences of *A. platensis* previously analysed from Argentina (GenBank vouchers KACa0494 and KACa0495) and Brazil (GenBank vouchers KACa0383, KACa0384, KACa0420, KACa0421, KACapl01, KACapl02 and KACapl03), we observed that the former ones grouped with those of the present study. The latter ones, in turn, were related to the *A. longirostri* species complex from Eldorado do Sul (ES), and possibly represent a new species (Fig. 3).

Species delimitation

The likelihood of the null model in the GMYC analysis (i.e. that all sequences belong to a single species) was not significantly different from the maximum likelihood of species delimitation (1594.19 *versus* 1597.11, ratio = 5.84, $P = 0.0538$). The GMYC analyses indicated the presence of three ML entities [(1) Potiribu; (2) Curuçu and Icamaquazinho; and (3) all remaining populations]. Meanwhile, ABGD analysis with JC69 and K2P (the only models available in this software) produced three recursive partitions ($P = 0.017$, $P = 0.0028$ and $P = 0.0046$) with operational taxonomic units (OTUs) counts of two [(1) Potiribu; and (2) all remaining populations]. Both ABGD and GMYC methods indicated that individuals from the Potiribu population are from a different and new species. The Bayes factor of 131.44 in favour of H1 decisively supports the monophyly of *A. platensis*.

Population genetic, phylogeographical and demographic analysis

The genetic diversity estimates for the populations are summarized in Table 1. We found 21 haplotypes for *COI* sequences. Some populations exhibited more than one haplotype, but no haplotype was shared among populations. For 16S sequences, we found ten haplotypes, with three being shared among populations, and the remaining seven being population exclusive (Fig. 4). For *ANT* sequences, we found five haplotypes, with three being population exclusive (two from Ijuí and one for Potiribu). For all genes tested, most of the genetic diversity was the result of variability among rather than within populations, as reflected in the *F*-statistics (Table 1). Considering *COI* sequences, the mean genetic divergence among populations was 3.3% (3% without the Potiribu population). For 16S sequences, this value was 0.6% (0.4% without the Potiribu population), and for *ANT* sequences, 0.5% (0.2% without the Potiribu population; Supporting information, Table S1).

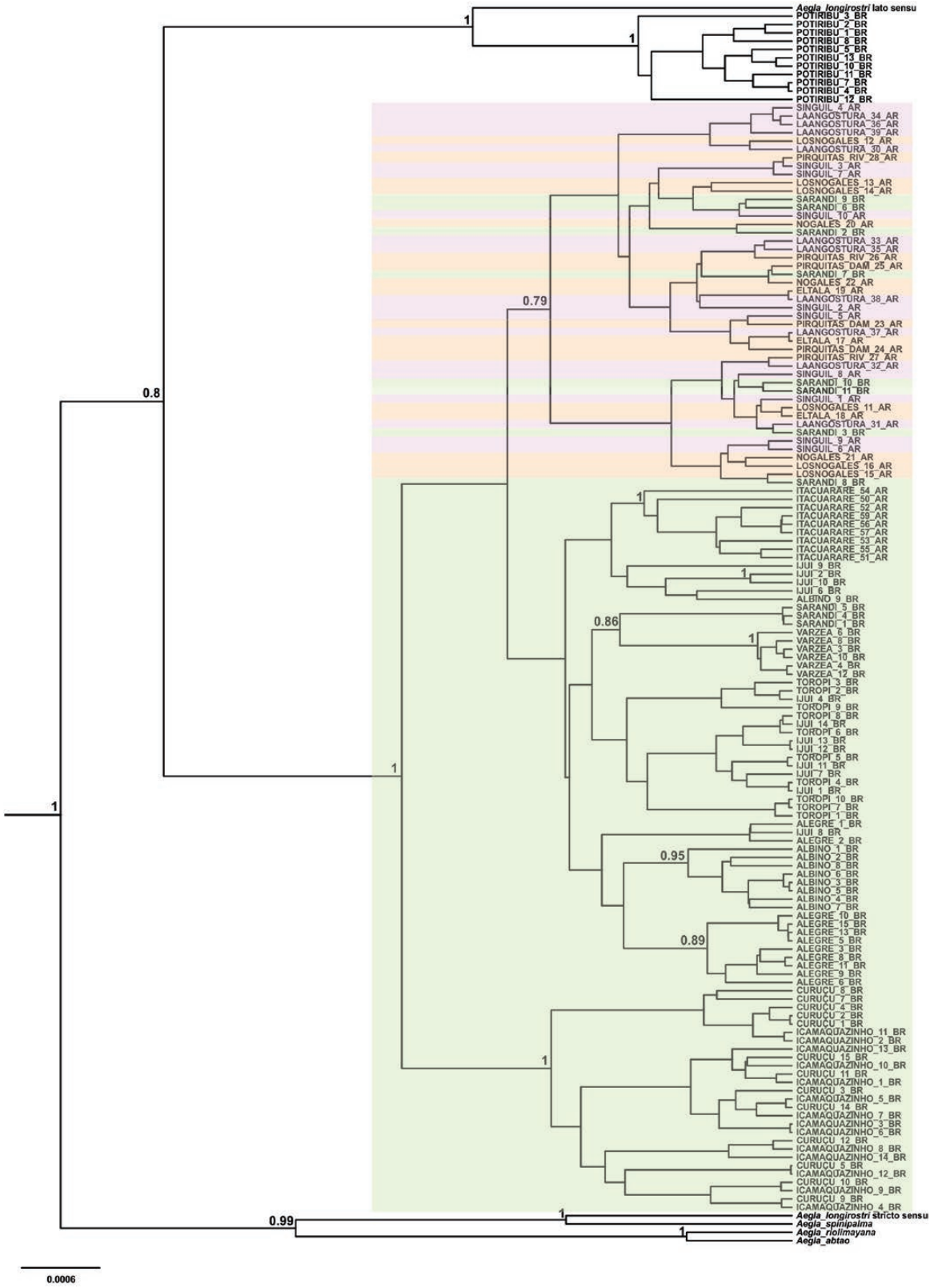


Figure 2. Bayesian tree based on concatenated 16S and *ANT* (mitochondrial DNA + nuclear DNA) sequences of *Aegla platensis*. The Hasegawa-Kishino-Yano (HKY) substitution model was used for the nuclear data and the Hasegawa-Kishino-Yano

For most populations, neutrality tests showed no significant departure from zero ($P > 0.05$), as expected under neutral sequence evolution. The only exceptions were the Argentinean populations of La Angostura and Itacurare, which showed negative values for Tajima's *D* test when considering *COI* sequences (−1.595, $P = 0.03$ and −1.677, $P = 0.026$, respectively). The Bayesian Skyride Plot for the analysed populations (with the exception of Potiribu) indicated that *A. platensis* experienced a long period of demographic stability, followed by a recent reduction, which started ~15 000 years ago (Fig. 5). The haplotype network from 16S sequences shows relationships that are consistent with those recovered in the phylogenetic analyses (Fig. 4); most haplotypes of *A. platensis* from Brazil and Argentina are related to each other, except for the Potiribu population. Although incomplete, the haplotype network from *COI* sequences recovered the same pattern (Supporting information, Fig. S2). The *ANT* gene haplotype network presented similar but much less informative results (data not shown). The TMRCA for the tested populations was estimated at 257 000 years ago. In contrast, the TMRCA for the monophyletic clade (excluding the Potiribu population) was estimated at 208 000 years ago (Supporting information, Fig. S3).

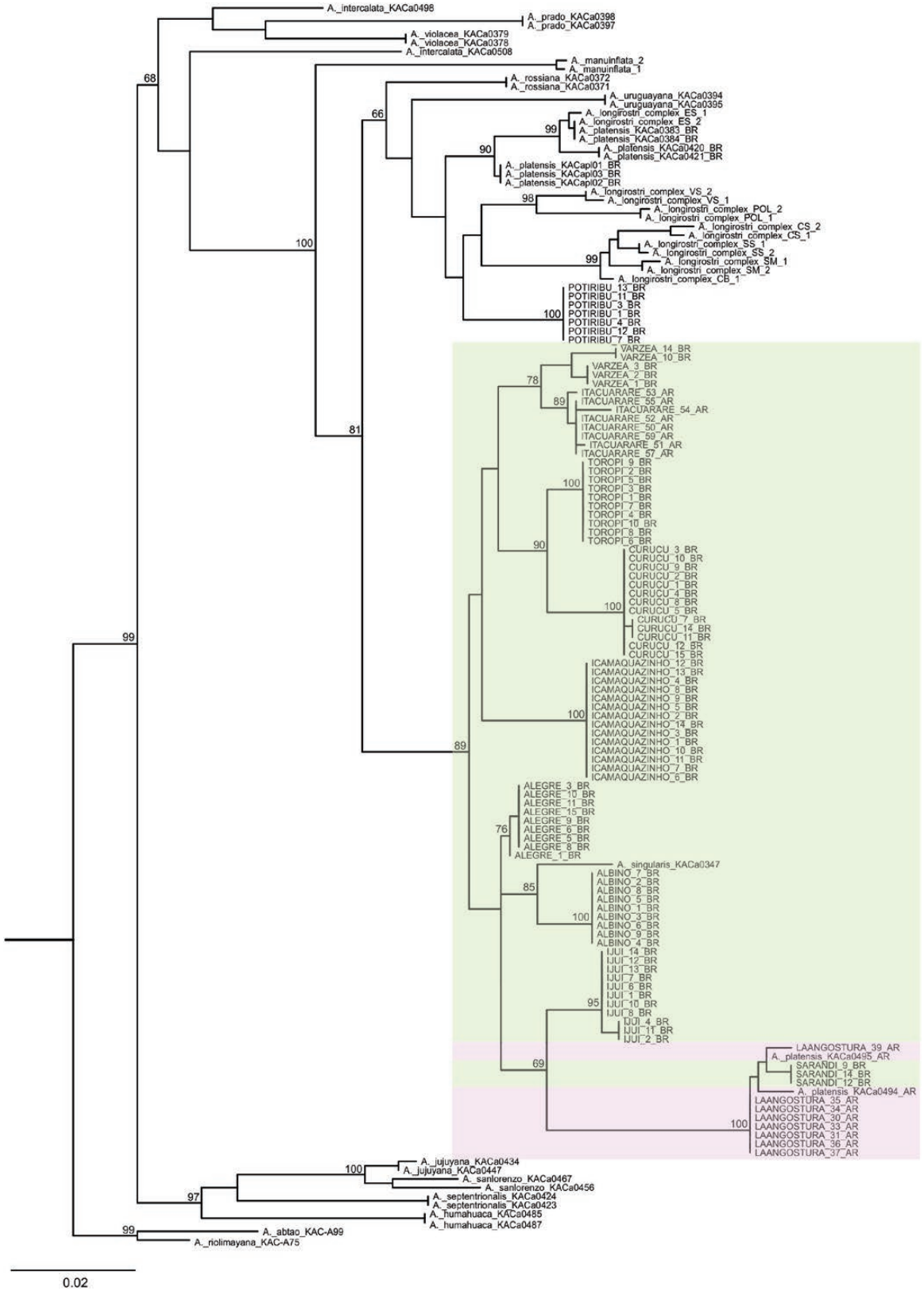
DISCUSSION

Contrary to our expectations, the vast majority of the analysed populations of *A. platensis* seem to belong to a single and well-supported monophyletic group. In general, populations from Argentina and Brazil were genetically related, despite the relatively large geographical distance between them (~1970 km when directly following the stream course). Phylogenies and species delimitation analyses (which used both mitochondrial and nuclear markers) corroborate these findings. In other words, although *A. platensis* presumably composes a species complex, we found evidence for only two potentially unrecognized new species [from Guaíba River Basin (sequences KACa0383, KACa0384, KACa0420, KACa0421, KACapl01, KACapl02 and KACapl03 from Pérez-Losada *et al.*, 2004) and Potiribu River]. These results highlight, as mentioned by Ritchie, Lo & Ho (2016), the importance of considering multiple markers, loci and lines of evidence when performing molecular species delimitation.

Regarding the previously mentioned phylogenetic and morphometric evidence for the presence of cryptic species in *A. platensis*, we propose alternative explanations. First, *A. platensis* was considered paraphyletic by Pérez-Losada *et al.* (2004). Interestingly, the Argentinean specimens used in that study formed a cluster with the sequences of our study; hence, they probably represent *A. platensis sensu stricto*. The type-locality of *A. platensis* ('Isla Flores', Tigre, province of Buenos Aires) is situated in the La Plata River basin, which is geographically close (i.e. neighbouring water systems) to the populations composing the monophyletic group found in the present study (Schmitt, 1942; Ringuelet, 1949; Bond-Buckup & Buckup, 1994). In contrast, Brazilian sequences used by Pérez-Losada *et al.* (2004) from Guaíba basin, as in the case of the Potiribu river population (related to the *A. longirostri* species complex; Crivellaro *et al.*, 2017), are a putative new species. Additional evidence for the last hypothesis comes from the two studies of population biology performed for *A. platensis*. One of them was carried out in the Uruguay River basin (Dalosto *et al.*, 2014; all Brazilian populations analysed in the present study were sampled in this same river basin) and another in the Guaíba River basin (Bueno & Bond-Buckup, 2000), in the same way that the Brazilian specimens analysed by Pérez-Losada *et al.* (2004). *Aegla platensis* presented marked differences between both studies (e.g. sex ratio, sexual dimorphism and size of the largest male and female). Dalosto *et al.* (2014) even suggested that molecular studies were needed to elucidate the taxonomic status of the populations of this species.

Second, considering the differences in carapace shape found by Marchiori *et al.* (2015), the explanation might lie in the phenotypic plasticity of this structure. Metri, Oliveira & Baptista-Metri (2016) used geometric morphometric techniques to understand inter- and intraspecific morphological variability of six species of aeglid crabs. Distinct carapace shapes were found not only between species but also among different populations of the same species. In some cases, phylogenetically unrelated species were more similar in carapace morphology than closely related species, meaning that local adaptation might account for a large amount of the morphological variation found in aeglid populations. Indeed, it seems that population distribution (in different basins and sub-basins) has a significant effect on the variation in carapace shape of *Aegla uruguayana* Schmitt, 1942 (Giri & Collins, 2014).

plus invariable sites model (HKY + I) substitution model for the mitochondrial data (considering gaps and missing data). Numbers above branches represent Bayesian posterior probabilities. Samples from Brazil are followed by 'BR' and those from Argentina by 'AR'. The clade highlighted in the tree corresponds to the monophyletic group of *A. platensis*, and the colours represent the hydrographic basins in which the individuals were collected: pink for the Mar Chiquita Basin (MC), orange for the Serrano River Basin (SR), and green for the Uruguay River Basin (UY).



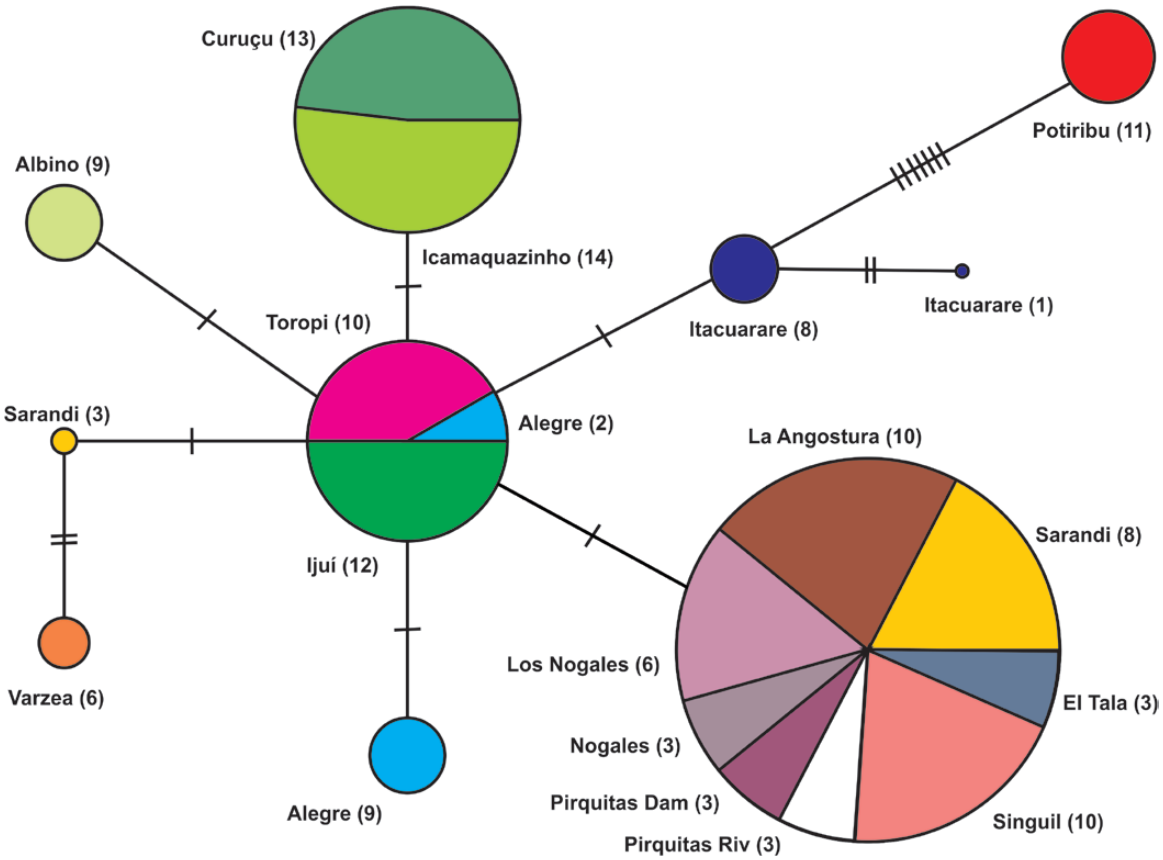


Figure 4. Median-joining haplotype network for 16S sequences from *Aegla platensis*. The area of the circles is proportional to the number of individuals of each haplotype found. Lines between circles represent the number of mutational steps.

An interesting aspect of the present study is the contrasting results compared with those obtained for *A. longirostri*, another species with a relatively wide distribution. In that species, a large hidden diversity was found (Crivellaro *et al.*, 2017), with the presence of at least 14 cryptic species distributed across a geographical area less than half the size of that of *A. platensis*. As stated before, species with wide geographical distributions are generally expected to represent a complex of cryptic species, a pattern already observed for other Brazilian decapods (Carvalho, Pileggi & Mantelatto, 2013; Souza-Carvalho, Magalhães & Mantelatto, 2017). We should mention that aeglids, as well as other groups of freshwater crustaceans, could be particularly prone to the emergence of cryptic species (Poulin & de León, 2017). Indeed, this was already reported for amphipods (Delić *et al.*, 2017), branchiopods (Schwentner *et al.*, 2013), crabs (Phiri & Daniels, 2016), cladocerans (Bekker *et al.*, 2016) and crayfish (Dawkins *et al.*, 2017), among others. Owing to the fragmented nature of freshwater habitats, diversification rates might increase, resulting in a greater frequency of recently diverged species with low morphological differentiation.

In this context, the case of *A. platensis* diversification is very intriguing, because it contrasts with the typical pattern of aeglids. The majority of the > 80 known *Aegla* species have narrow distributions (Santos *et al.*, 2017), and those previously considered as widespread (e.g. *A. longirostri* and *A. paulensis*) are, in

Figure 3. Maximum likelihood tree based on mitochondrial DNA sequences (*COI* + 16S) of *Aegla* from this study and from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) using the GTR + CAT substitution model (considering gaps and missing data). Numbers above branches represent bootstrap support. Only *Aegla* species of clade D were included in the ingroup (according to the clades defined by Pérez-Losada *et al.*, 2004). The clade highlighted in the tree corresponds to the monophyletic group of *Aegla platensis*, and the colours represent the hydrographic basins in which individuals were collected: pink for the Mar Chiquita Basin (MC), orange for the Serrano River Basin (SR), and green for the Uruguay River Basin (UY).

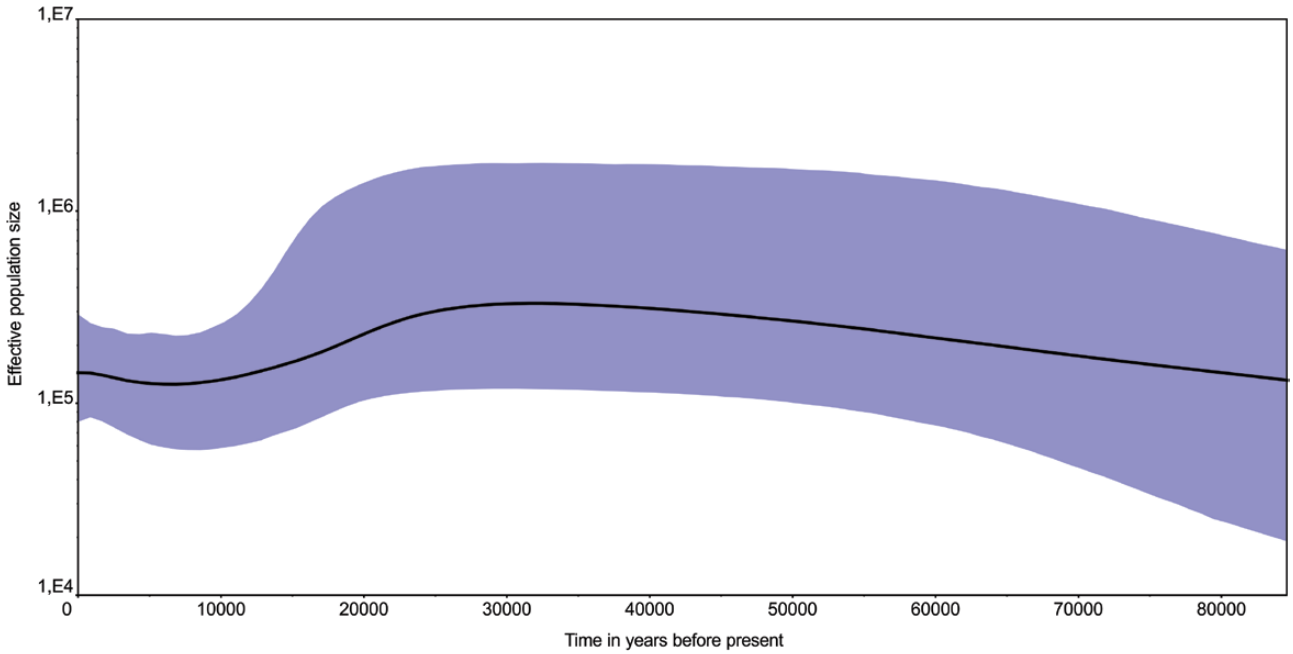


Figure 5. Bayesian Skyride Plot of effective population size through time for *Aegla platensis*, based on mitochondrial DNA sequences (*COI* + 16S). Centre line is the median estimation; upper and lower lines represent limits of the 95% confidence interval.

fact, a complex of cryptic species (Moraes *et al.*, 2016; Crivellaro *et al.*, 2017). In contrasts, *A. platensis* seems to remain, with a few exceptions, a cohesive entity over a wide geographical area. Owing to the lack of more detailed studies on the biology of this species, we can only speculate about the factors behind the wide distribution of *A. platensis*. They could be related, for example, to intrinsic characteristics (higher physiological resilience, greater dispersion capacity, more generalist habits, competitive advantages over other *Aegla* species, etc.) and/or to historical biogeographical events.

In fact, there is some evidence to suggest that differences in the ecological plasticity of the species are related to their geographical range extent (Gaston & Spicer, 2001; Gaston, 2009). For example, when comparing the phylogeography of two freshwater prawn species, the widespread *Macrobrachium australiense* Holthuis, 1950 and the narrow-range endemic *M. koombooloomba* Short, 2004, Bernays *et al.* (2015) observed that *M. australiense* is a better disperser, at least in lowland settings. Upland populations, in contrast, have restricted dispersal as a result of geographical barriers. Regarding historical events, both older and more recent historical processes, including fragmentation on a larger geographical scale and more recent range expansion on a local scale, appear to be responsible for the observed pattern of distribution and genetic variation in *Cherax destructor* Clark, 1936, the most widespread species of freshwater crayfish of Australia (Nguyen *et al.*, 2004). Another hypothesis comes from a study with diving beetles of the *Deronectes* Sharp, 1880 genus (García-Vázquez & Ribera, 2016). Most of the ~60 described species have narrow ranges in the Mediterranean area, with only four species having widespread European distributions. According to the authors, some populations took advantage of a privileged geographical position; that is, those that happened to be at the edge of the newly deglaciated areas during Pleistocene glacial cycles used the optimal ecological conditions to expand their ranges. Although we do not know whether a similar mechanism could have operated in aeglids, it is well established that Pleistocene climatic oscillations contributed to shaping the current diversity and distribution of modern lineages of South America (Collins, Giri & Williner, 2011; Turchetto-Zolet *et al.*, 2013; Cabanne *et al.*, 2016).

Another taxonomic issue that deserves further investigation is the position of *A. singularis*, whose representative fell in the midst of the *A. platensis* populations (Fig. 3). These two species are both morphologically (they share diagnostic characters; see Bond-Buckup & Buckup, 1994) and genetically similar (Pérez-Losada *et al.*, 2004). Besides that, the distributional area of *A. singularis*, although smaller, overlaps with the distributional area of *A. platensis* (Santos *et al.*, 2017). Further analyses using a

higher number of *A. singularis* populations would be necessary to clarify this issue and determine whether *A. singularis* is, in fact, a synonymy of *A. platensis*.

Lastly, although *A. platensis* has a broad distribution, the Bayesian Skyride Plot (Fig. 5) and the low genetic diversity across this broad range suggest that the species has suffered a recent decline (initiated ~15 000 years ago) in both population size and genetic diversity after having experienced a long period of stability. We should highlight that the de la Plata River suffered a post-Last Glacial Maximum marine transgression that began ~18 000 years ago and extended until the lowest sections of the Paraná and Uruguay rivers. Sea level reached its highest position at 6000 years before present, and the subsequent sea-level retreat occurred from 6000 years before present to the present days (Violante & Parker, 2004). It is possible that this event affected the populations of *A. platensis* located in that area. The worrying aspect of these findings is that the demographic reduction could be intensified by the numerous anthropogenic stressors experienced by freshwater fauna in recent years, especially if we consider that *Aegla* is probably the most severely threatened group of all freshwater decapods of South America (Santos *et al.*, 2017). *Aegla platensis* has been recently categorized as having deficient data for conservation status assessment owing to the suspicion that it might be composed of a group of cryptic species (Santos *et al.*, 2017). Our results allow for a new and more accurate assessment of the conservation status of *A. platensis sensu stricto*, which can now be categorized as 'least concern' (LC) based on International Union for Conservation of Nature (IUCN) (2012) criteria, considering that this is a monophyletic group (taxa) with broad distribution and, in general, with abundant populations. Despite this, these crabs will still require attention, especially because our study will lead to the description of two new *Aegla* species (from the Guaíba basin and the Potirubu river), whose conservation status remains to be assessed.

CONCLUSIONS

In summary, our results indicate that *A. platensis* shows low genetic diversity over a broad geographical area. Contrary to most extant aeglids, *A. platensis* seems to be a widely distributed species with low genetic diversity. However, the mechanisms driving its speciation and spread still need to be clarified. In fact, in several lineages, most species have restricted geographical ranges, with only a few reaching widespread distributions; but how these widespread species reached their current ranges is an intriguing biogeographical and evolutionary question (García-Vázquez & Ribera, 2016). We also highlight the fact that *Aegla* taxonomy is far from being fully understood, justifying the need for more studies using phylogenetic approaches. We effectively show that broad population-based sampling can be useful to improve our understanding of the group's systematics and evolution and could, ultimately, facilitate the application of efficacious conservation measures.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Average pairwise differences among and within populations based on 16S, *COI* and *ANT* sequences, respectively. Below diagonal: average number of pairwise differences between populations. Diagonal elements (bold): pairwise differences within populations, when present.

Figure S1. Bayesian tree based on *COI* sequences of *Aegla platensis* using the Hasegawa-Kishino-Yano plus invariable sites model (HKY + I) substitution model (considering gaps and missing data). Numbers above branches represent Bayesian posterior probabilities.

Figure S2. Median-joining haplotype network for *COI* sequences from *Aegla platensis*. Area of the circles is proportional to the number of individuals of each haplotype found. Lines between circles represent the number of mutational steps.

Figure S3. Bayesian tree based on mitochondrial DNA haplotypes (*COI* + 16S) of *Aegla platensis* using the Hasegawa-Kishino-Yano plus invariable sites model (HKY + I) substitution model (considering gaps and missing data). Numbers above branches are the estimates of mean divergence time [thousand years ago (95% highest posterior density intervals)].