

Conservation Assessment of Southern South American Freshwater Ecoregions on the Basis of the Distribution and Genetic Diversity of Crabs from the Genus *Aegla*

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Abstract: We assessed the conservation priority of 18 freshwater ecoregions in southern South America on the basis of *Aegla* (genus of freshwater crabs) genetic diversity and distribution. Geographical distributions for 66 *Aegla* species were taken from the literature and plotted against ecoregions and main river basins of southern South America. Species richness and number of threatened and endemic species were calculated for each area. To assess taxonomic and phylogenetic diversity, we generated a molecular phylogeny based on DNA sequences for one nuclear (28S) and 4 mitochondrial (12S, 16S, COI, and COII) genes. All species richness and phylogenetic methods agreed, to a large extent, in their rankings of the importance of conservation areas, as indicated by the Spearman's rank correlation coefficient ($p < 0.01$); nonetheless, some of the lowest correlations were observed between taxonomic and phylogenetic diversity indices. The 5 ecoregions of the Laguna dos Patos Basin (Eastern Brazil), Central Chile, South Brazilian Coast, Chilean Lakes, and Subtropical Potamic Axis (northern Argentina and southern Uruguay and Paraguay) had the highest biodiversity scores. Conservation of these regions will preserve the largest number of species and the greatest amount of genetic diversity within the South American freshwater *Aegla* fauna. Biodiversity across rivers and within areas was heterogeneously distributed in the ecoregions of Upper Paraná, Ribeira do Iguape, Upper Uruguay, and South Brazilian Coast (i.e., one river showed significantly more biodiversity than any other river from the same ecoregion), but homogeneously distributed in the other ecoregions. Hence, conservation plans in the former regions will potentially require less effort than plans in the latter regions.

Keywords: *Aegla* conservation, biodiversity, conservation priority setting, freshwater crab, phylogenetic diversity, South America

Evaluación de la Conservación de Ecoregiones Dulceacuícolas del Sur de Sudamérica con Base en la Distribución y Diversidad Genética de Cangrejos del Género *Aegla*

Resumen: Evaluamos la prioridad de conservación de 18 ecoregiones dulceacuícolas en el sur de Sudamérica con base en la diversidad genética y distribución de *Aegla* (género de cangrejos dulceacuícolas). Las distribuciones geográficas de 66 especies de *Aegla* fueron tomadas de la literatura y contrastadas con las ecoregiones y las principales cuencas fluviales del sur de Sudamérica. Se calculó la riqueza de especies y el número de especies amenazadas y endémicas en cada área. Para evaluar la diversidad taxonómica y filogenética, generamos una filogenia molecular basada en secuencias de ADN para un gene nuclear (28S) y

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cuatro mitocondriales (12S, 16S, COI y COII). Todas las riquezas de especies y métodos filogenéticos concordaron, en gran medida, con la clasificación de la importancia de áreas de conservación, como lo indica el coeficiente de correlación de Spearman ($p < 0.01$); sin embargo, algunas de las correlaciones más bajas se observaron entre índices de diversidad taxonómicos y filogenéticos. Las cinco ecoregiones Cuenca Laguna dos Patos (este de Brasil), Centro de Chile, Costa Sur de Brasil, Lagos Chilenos y Cuenca del Paraná (Subtropical Potamic Axis, norte de Argentina y sur de Uruguay y Paraguay) tuvieron los mayores valores de biodiversidad. La conservación de estas regiones preservará el mayor número de especies y la mayor cantidad de diversidad genética de la fauna dulceacuícola Sudamericana de Aegla. La biodiversidad entre ríos y dentro de las áreas se distribuyó heterogéneamente en las ecoregiones Alto Paraná, Ribeira de Iguape, Alto Uruguay y Costa Sur de Brasil (i.e., un río mostró significativamente mayor biodiversidad que cualquier otro río de la misma ecoregión), pero homogéneamente en las otras ecoregiones. Por lo tanto, los planes de conservación en las primeras regiones potencialmente requerirán de menor esfuerzo que los planes en las segundas.

Palabras Clave: biodiversidad, cangrejo de agua dulce, conservación de *Aegla*, definición de prioridades de conservación, diversidad filogenética, Sudamérica

Introduction

The world's freshwater biodiversity is among the most threatened and in the most urgent need of conservation attention (Allan & Flecker 1993; Master et al. 1998; Abell 2002). Within the Neotropics, southern South America outside of the Paraná-Paraguay system has received less attention in global conservation priority-setting exercises than the highly diverse Amazon (Olson et al. 1998), yet at a continental scale the broader southern region stands out for its phylogenetically distinct species and for its high degree of threat (Morrone et al. 1996). Major threats to the southern region's freshwater species and habitats include deforestation, urbanization, farming, overgrazing, and exotic species (Pérez-Losada et al. 2002a). Additionally, southern river systems are on average more fragmented by dams than those in other South American regions (Nilsson et al. 2005).

A first step toward prioritizing conservation investment for southern South America's freshwaters is a realm-specific assessment, a key component of which is the synthesis of the best available species-level distribution data. The World Wildlife Fund's (WWF) new map of South America's freshwater ecoregions provides a coarse framework for such an assessment (Petry et al. 2006; Abell et al. 2008). These freshwater ecoregions, or aquatic zoogeographic units, are vast areas encompassing one or more freshwater systems with a distinct assemblage of natural freshwater communities and species (Abell et al. 2000). The freshwater species, dynamics, and environmental conditions within a given ecoregion are more similar to each other than to those of surrounding ecoregions and together form a conservation unit. Moreover, ecoregion boundaries are not necessarily determined by the turnover of species ranges (McDonald et al. 2005), but are intended to describe broad patterns of species composition and associated ecological and evolutionary processes. Nevertheless, conservation interventions are usually planned for more manageable, smaller units, and

detailed conservation plans are developed on the basis of finer-scale data, for example, from a river basin within an ecoregion (Pérez-Losada et al. 2002a). Examination of these smaller units helps improve knowledge of the distribution of freshwater biodiversity and provides a baseline for conservation planning for freshwater flora and fauna. We used both coarse- and fine-scale data to assess the biological importance of the southern South American freshwater ecoregions as defined by the WWF.

Assessments of areas for determining conservation priorities should be based on taxa that are characteristic of the region and well surveyed. Patterns of species richness, endemism, or vulnerability in these taxa are assumed to be indicative of similar patterns in unsurveyed taxa from the same region (Pearson & Cassola 1992; Reyers & Jaarsveld 2000), but this assumption may be problematic because indicator taxa are sometimes of limited value in biodiversity inventories (e.g., Heino et al. 2005). Conservation assessments of freshwater ecosystems usually rely on fish fauna (Petry et al. 2006), although biogeographic patterns of other freshwater taxa may differ from those of fish in some places. Moreover, because fish tend to have larger distributions than those of other freshwater species, particularly obligate aquatic invertebrate taxa such as *Aegla*, the latter are expected to demonstrate finer-scale distribution patterns within ecoregions.

The freshwater crabs of the genus *Aegla* (Decapoda: Anomura: Aeglididae) are a well-suited invertebrate group for use in a regional assessment of the value of southern South America freshwater ecoregions for the conservation of freshwater crab biodiversity (Bond-Buckup & Buckup 1994; Pérez-Losada et al. 2002a; Bond-Buckup et al. 2007) because they meet most of the criteria for an indicator taxon (Noos 1990; Pearson 1994). Sixty-nine species of *Aegla* are endemic to the Neotropical region of South America, where they represent the most broadly distributed macroinvertebrate group (Bond-Buckup & Buckup 1994; Jara 1996). They are found in all the freshwater ecoregions delimited on the WWF map (except

for the small Fluminense, for which collection data are absent) and all the main river basins of southern South America. Aeglids occupy virtually all kinds of freshwater natural habitats and show individual species adaptation within their habitats. Aeglids are also good indicators because they are readily observed in the field all year, usually in large numbers; they are easy to find and collect (Bond-Buckup & Buckup 1994); they often act as indicator species for stream habitat quality (Bond-Buckup & Buckup 1994); their biology and life history are well understood; and their alpha taxonomy and phylogenetic relationships are well known and stable (Pérez-Losada et al. 2002c; Pérez-Losada et al. 2004). Moreover, conservation of aeglids is also a concern. Several Chilean species are threatened, and some were considered extinct (Pérez-Losada et al. 2002a). If we want to protect them from extinction, their assessment relative to the criteria established by the Species Survival Commission (see IUCN 2007) is a primary necessity.

Within the context of conservation assessment, evaluations of the biological value of a region often incorporate traditional measures of biodiversity such as species richness and endemism and the occurrence of evolutionary phenomena such as species flocking (Abell et al. 2000; Thieme et al. 2005). New genetic analyses permit enhanced evaluations of biodiversity, the results of which can be compared with and potentially used to complement more traditional methods. Key to the evaluation of regions for conservation with phylogenetic methods is a robust phylogeny (rooted if taxonomic diversity methods are used) based on extensive sampling of species and populations (May 1990). In addition, if genetic distance-based methods are applied, the use of multiple gene regions (ideally including mitochondrial and nuclear loci) is recommended for accurately estimating lengths of tree branches (Crozier 1997). We have investigated *Aegla* evolutionary relationships for 10 years with DNA-sequence data (Pérez-Losada et al. 2002a, 2002b, 2002c, 2004). Results of our previous studies and of new phylogenetic analyses presented here are based on multiple nuclear and mitochondrial genes, several outgroup species, and almost all the known aeglids for which several populations and individuals have been collected. These results provide a robust phylogenetic framework for assessing the biological value of the southern South American freshwater ecoregions and defining conservation priorities within the group (Vane-Wright et al. 1991; Crozier 1992).

Methods

South American Freshwater Ecoregions

Ecoregions are widely used planning units for biodiversity conservation and water resource management (Groves 2003; Gallant et al. 2004; Omernik 2004). Ecoregions

have typically been defined on the basis of terrestrial landscapes that represent terrestrial vegetation and biodiversity features. Freshwater ecoregions offer an alternative framework for conservation planning for freshwater biodiversity (Abell 2002), and a global map of freshwater ecoregions has recently been completed by WWF (Petty et al. 2006; Abell et al. 2008). These ecoregions were defined on the basis of freshwater fish biogeography and broad-scale ecological processes and have played an important role in setting global conservation priorities at a broad scale. Ecoregions allow planners to select large areas for protection that maximize conservation effectiveness (i.e., coarse-scale biodiversity analysis). After identification of large areas, fine-scale priority areas within the ecoregions can be delineated (i.e., fine-scale biodiversity analysis). We performed coarse-scale analyses in the South American ecoregions south of the Amazon River (Fig. 1; Petty et al. 2006; Abell et al. 2008) and fine-scale analyses in the main river basins within each of the ecoregions.

Phylogenetic Inference

We updated the extensive phylogenetic analysis of *Aegla* evolutionary relationships (Pérez-Losada et al. 2004) by examining 2 new taxa, *A. conceptionensis*, which was found in a new location outside its known area of distribution, and a new species of *Aegla* from Brazil (*Aegla* n. sp. 7). This represents nearly all the described species and subspecies (59 out of 63) within the genus and 6 new undescribed species. Species not included were *A. expansa* from Chile, formerly considered extinct in the wild (Pérez-Losada et al. 2002a), and *A. franca*, *A. lata*, and *A. microphthalma* from Brazil. *A. franca* was not found at the time of this study, but it has been assessed recently as vulnerable (Bueno et al. 2007). *A. lata* no longer occurs within its native distribution and *A. microphthalma* is a stygobiotic species that only occurs in a single cave that is very difficult to access (G.B.B., personal observation). As in previous studies (Pérez-Losada et al. 2002b, 2004), we collected sequence data for complete nuclear 28S ribosomal and partial mitochondrial 12S, 16S, COI, and COII genes, which yielded a concatenated data set of 5601 nucleotides. This data set was analyzed with model selection, maximum likelihood, and Bayesian phylogenetic methods as described in Pérez-Losada et al. (2004).

Conservation Status, Endemism, and Species Richness

We assessed the conservation status of all known *Aegla* species and subspecies on the basis of categories and criteria of the International Union for Conservation of Nature (see IUCN 2007) and through the use of published habitat descriptions, population analyses, and geographic ranges (Bond-Buckup & Buckup 1994; Jara 1996;

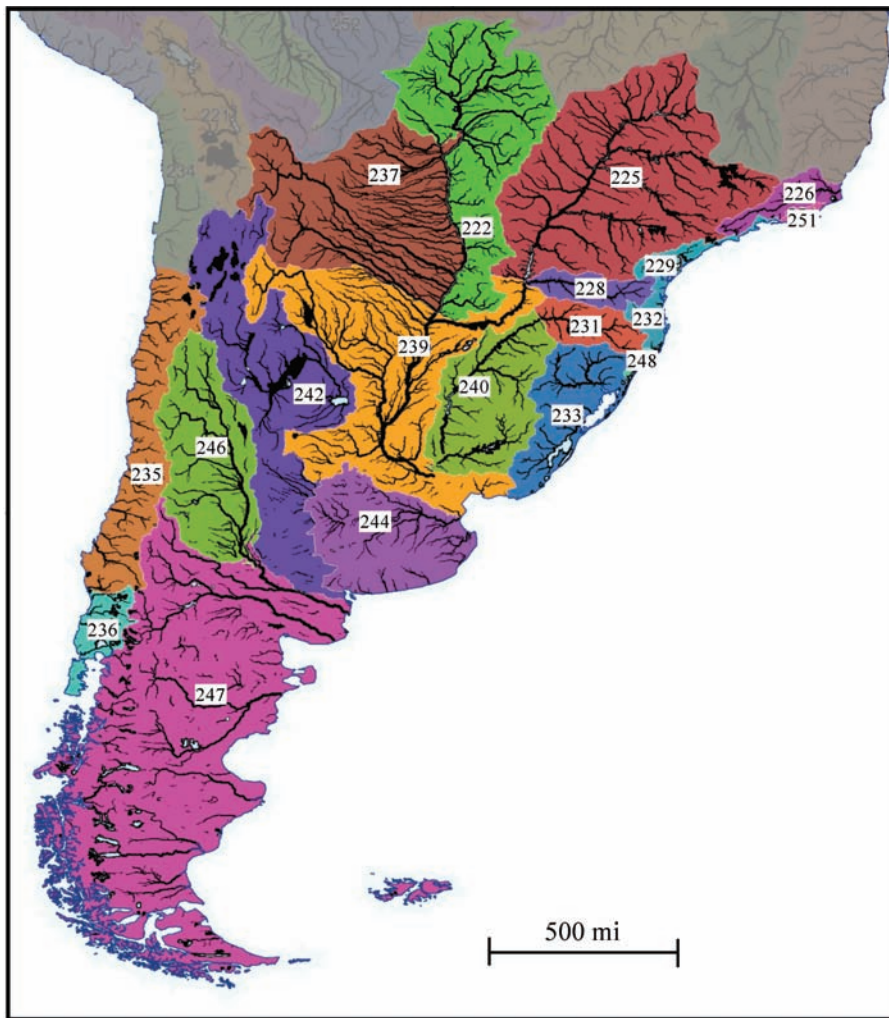


Figure 1. Map of freshwater ecoregions from southern South America (222, Paraguay; 225, Upper Paraná; 226, Paraíba do Sul; 228, Iguaçu; 229, Ribeira do Iguape; 231, Upper Uruguay; 232, South Brazilian Coast; 233, Laguna dos Patos Basin; 235, Central Chile; 236, Chilean Lakes; 237, Cbaco; 239, Subtropical Potamic Axis; 240, Lower Uruguay; 242, Central Endorrheic; 244, Bonaerensean Atlantic Drainage; 246, Subandean-Cuyan; 247, Patagonia; 248, Tramandat-Mampituba; 251, Fluminense). Modified from Petry et al. (2006).

Pérez-Losada et al. 2002a; Jara et al. 2003). Distributions of all *Aegla* species were compiled from the reviews by Bond-Buckup and Buckup (1994) and Jara (1996), delineated on the maps of Petry et al. (2006) and Abell et al. (2008) of freshwater ecoregions (Fig. 1), and recorded as present or absent in each river basin and ecoregion. Tallies were made of total numbers of species and subspecies present in each ecoregion and river drainage. In addition, we counted the number of endemic and threatened taxa per ecoregion. We considered a taxon endemic to an ecoregion if it was present only in that unit. A taxon was considered threatened if it fell within any of the following IUCN Red List categories: critically endangered (CR), endangered (EN), and vulnerable (VU).

Phylogenetic Diversity

Methods for evaluating biodiversity based on phylogenies can be separated into 2 categories: topology dependent and distance dependent (Krajewski 1994). Topology-dependent methods rely on a rooted phylogeny and reflect the branching order and therefore rank those or-

ganisms that evolved earliest with the highest priority regardless of divergence between species (Vane-Wright et al. 1991; Nixon & Wheeler 1992; Posadas et al. 2001). Distance or branch-length-dependent methods sum the branch lengths to derive a phylogenetic diversity for an organism and strive to represent the genetic diversity or divergence between each organism (Faith 1992; Krajewski 1994; Crozier 1997). To estimate phylogenetic diversity per ecoregion we used 4 indices: taxonomic diversity (Vane-Wright et al. 1991); taxonomic endemicity standardized weight (hereafter taxonomic endemicity) by Posadas et al. (2001), which is a modified version of the taxonomic diversity index that accounts for the degree of endemicity of each taxon; phylogenetic diversity (Faith 1992); and genetic diversity (Crozier 1992). The topology-dependent taxonomic diversity (taxonomic distinctness between taxa) and degree of endemicity indices were calculated manually (Posadas et al. 2001) and the branch-length-dependent phylogenetic diversity (length of evolutionary history) and genetic diversity (probability of more than one allele) indices were computed in Conserve 3.2.1 (Agapow & Crozier 1998). We used our

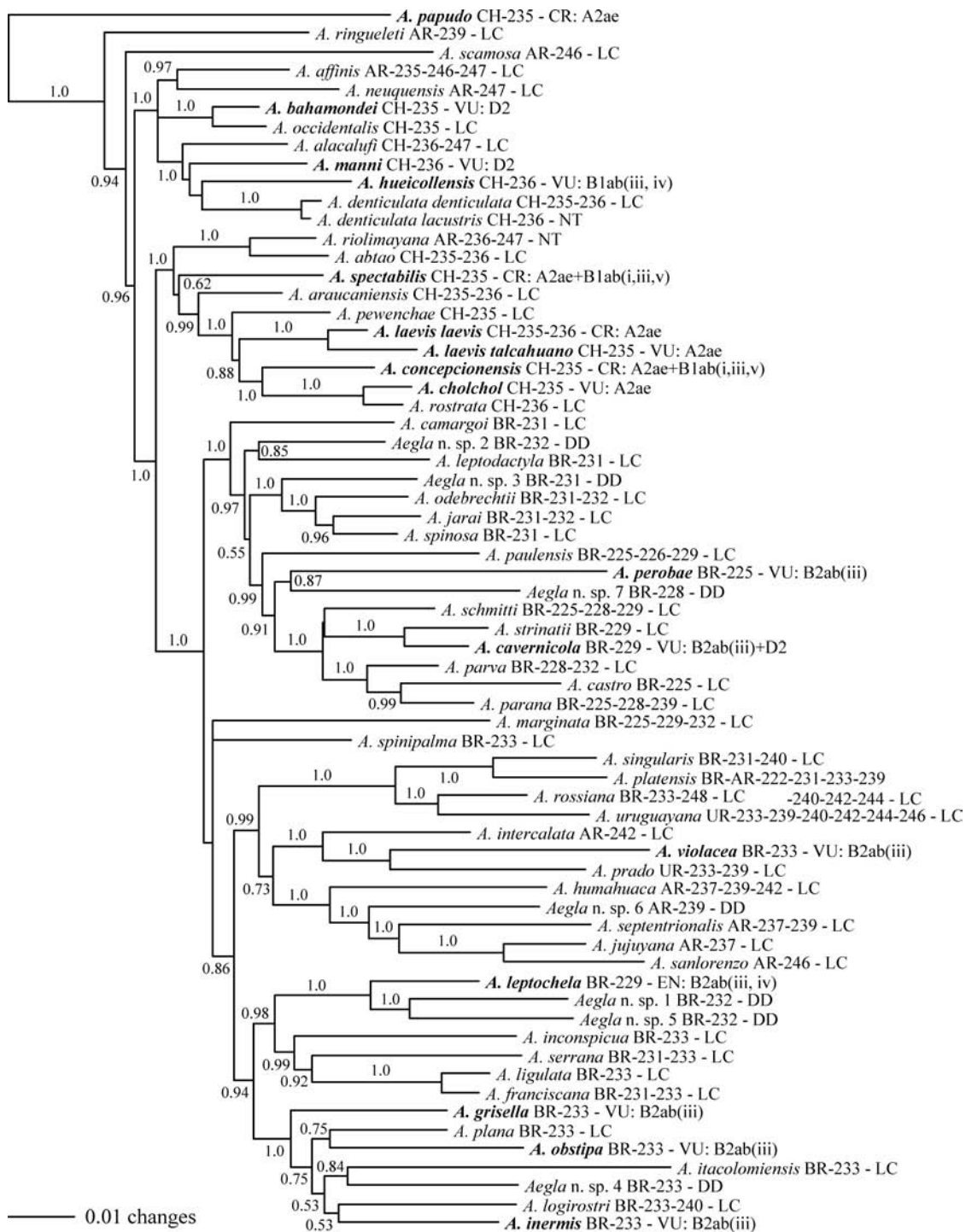


Figure 2. Maximum likelihood and Bayesian trees of phylogenetic relationships among *Aegla* freshwater crabs in southern South America. Posterior probability (if >0.5) for each clade, distribution of species per ecoregion, International Union for Conservation of Nature (IUCN) Red List category (CR, critically endangered; DD, data deficient; EN, endangered; LC, least concern; NT, near threatened; VU, vulnerable), and IUCN criteria (after colon) are indicated. Threatened species (CR, EN, and VU) are indicated in boldface.

best hypothesis of phylogenetic relationships (Fig. 2), including corrected branch lengths under the best-fit model of nucleotide substitution, to calculate all of the above biodiversity measures. For simplicity only one in-

dividual per species was considered when multiple individuals formed a monophyletic species clade. In addition, we estimated phylogenetic diversity and genetic diversity across river basins within ecoregions. We assessed only

ecoregions that included 2 or more sampled rivers and at least one crab species per river.

Complementarity Analyses

It is important to identify areas that complement one another in terms of species composition so as to eliminate redundancy, the so-called complementarity principle (Vane-Wright et al. 1991). Moreover, ranking areas according to their faunistic complementarity may alter the initial ordination based on noncomplementarity information. Area-selection methods based on the complementarity principle rely on criteria such as richness and rarity. Comparative analyses of complementarity approaches based on richness and rarity applied to freshwater ecosystems from the Mediterranean basin show that the richness-based complementarity algorithm is more efficient than the rarity-based algorithm (Abellán et al. 2005). We therefore performed a complementarity analysis of ecoregions for 6 of the 7 species and phylogenetic biodiversity indices applied here; number of endemic species was not included because this index counts unique species in each ecoregion (i.e., there are no endemic species after one round of complementarity analysis). We then deleted all taxa represented in that area and the rankings were recomputed. Because of the small distribution of most *Aegla* species, ecoregions tended to include many unique and few shared species. Consequently, after a first round of complementarity analyses, biodiversity (as indicated by the indices applied here) was still broadly distributed across areas. To correct this, we performed 2 more successive rounds of complementarity analyses so that areas that represent similar biodiversity could be identified and redundancy reduced.

Results

Both maximum likelihood and Bayesian methods generated topologies similar to each other and to earlier estimates of phylogenetic relationships among species of *Aegla* (Fig. 2). *A. conceptionensis* formed a sister clade to *A. cholchol* and *A. rostrata*, and *Aegla* n. sp. 7 was sister to *A. perobae*. All new DNA sequences were deposited in GenBank under the accession numbers FJ360696-FJ360715.

Species Conservation Status

Thirty-two percent of the aeglid species were threatened (CR, EN, or VU), and 7 species were data deficient (Fig. 2). Previous conservation assessments by Pérez-Losada et al. (2002a) assessed *A. conceptionensis* and *A. alacalufi* as extinct in the wild (EW) and VU, respectively. *A. conceptionensis* was recently found in a new location outside of its known area of distribution and was reassessed here as CR. *A. alacalufi* was also found in several new loca-

tions south of its known extent of occurrence and was reevaluated as least concern (LC). *A. franca*, *A. lata*, and *A. microphthalma* from Brazil were not included in our phylogenetic analysis. *A. franca* has been assessed as VU on the basis of criterion B2ab(iii)+D2 (Bueno et al. 2007). *A. lata* was not found despite thorough searching and was assessed as EW, and *A. microphthalma* only occurred in a single cave and its conservation status was assessed as CR on the basis of criterion B2ab(iii) (see IUCN 2007 for details). Out of 70 taxa evaluated 2 were considered extinct in the wild (EW), 18 threatened (CR, VU or EN), 43 unthreatened (LC and near threatened), and 7 could not be assessed (data deficient).

Species Tallies

Ecoregions 233, 235, 231, and 236 had the highest species richness (17, 13, 10, and 10 species, respectively). The same regions had the highest number of endemic species: 233, 10 species; 235, 7; 231, 4; and 236, 4. The highest numbers of threatened species (categories CR, EN, and VU), however, were in regions 235, 233, 236, and 229 (6, 4, 3, and 2 species, respectively). Laguna dos Patos Basin in Brazil (ecoregion 233) and Central Chile (235) had the highest species richness and numbers of endemic and threatened species and thus the highest biological value for aeglid biodiversity. Upper Uruguay (ecoregion 231) and Chilean Lakes (ecoregion 236) were ranked second highest with these 3 indices.

In the major river basins, ecoregions 225, 229, 231, and 232 (Upper Paraná, Ribeira do Iguape, Upper Uruguay, and South Brazilian Coast, respectively) had only one river per region (Parapanema, Iguape, Pelotas, and Itajai-Açu, respectively), contained at least 2 times more species than rivers in the other ecoregions, but in the other ecoregions *Aegla* species were more homogeneously distributed across rivers; hence, no single river accounted for a great deal of species diversity (Table 2).

Phylogenetic Diversity

Taxonomic diversity (TD) and taxonomic endemism (TE) were the highest in Central Chile (ecoregion 235) (35.1 and 31.1, respectively), whereas genetic diversity (GD) and phylogenetic diversity (PD) had the highest scores for the Laguna dos Patos Basin (ecoregion 233) (GD = 0.428 and PD = 0.522) (Table 1). These ecoregions were thus considered to have the highest aeglid genetic diversity values. Ecoregions 233, 236 (Chilean Lakes), and 239 (Subtropical Potamic Axis; northern Argentina and southern Uruguay and Paraguay) had TD-TE values of 20.8–16.1, 17.4–11.9, and 15.4–10.8, respectively, and were ranked second through fourth, respectively (Table 1). As for the phylogenetic indices, ecoregions 239, 235, and 231 (Upper Uruguay) were ranked second through fourth, with GD-PD values of 0.285–0.332, 0.271–0.311, and 0.253–0.289, respectively (Table 1).

Table 1. Estimates of species richness (SR, total number of species), number of endemic species (ES), number of threatened species, taxonomic diversity (TD), taxonomic endemism (TE), genetic diversity (GD), and phylogenetic diversity (PD) for 18 freshwater ecoregions in southern South America.*

Ecoregion	SR	SRr	ES	ESr	TS	TSr	TD	TDr	TE	TEr	GD	GDr	PD	PDr
222	1	16-18	0	14-18	0	6-18	1.2	17-18	0.2	18	0.074	17	0.076	17
225	6	7-8	2	7-9	1	5	7.9	10	4.2	10	0.202	6	0.223	6
226	1	16-18	0	14-18	0	6-18	1.5	16	0.5	16	0.056	18	0.057	18
228	4	9-13	1	10-13	0	6-18	4.5	13	2.6	12	0.114	14	0.12	14
229	6	7-8	3	5-6	2	4	8.1	9	5.1	9	0.183	7	0.2	7
231	10	3-4	4	3-4	0	6-18	13.8	5	9.8	5	0.253	4	0.289	4
232	7	6	3	5-6	0	6-18	9.7	6	6.6	6	0.209	5	0.232	5
233	17	1	10	1	4	2	20.8	2	16.1	2	0.428	1	0.552	1
235	13	2	8	2	6	1	35.1	1	31.1	1	0.271	3	0.311	3
236	10	3-4	4	3-4	3	3	17.4	3	11.9	3	0.178	8	0.195	8
237	4	9-13	2	7-9	0	6-18	4.3	14	2.9	11	0.145	11	0.155	11
239	8	5	2	7-9	0	6-18	15.4	4	10.8	4	0.285	2	0.332	2
240	4	9-13	0	14-18	0	6-18	4.7	12	1.6	14	0.152	10	0.163	10
242	4	9-13	1	10-13	0	6-18	4.8	11	2	13	0.165	9	0.178	9
244	2	15	0	14-18	0	6-18	2.4	15	0.4	17	0.101	15	0.105	15
246	3	14	1	10-13	0	6-18	8.7	8	6	7	0.134	12	0.143	12
247	4	9-13	1	10-13	0	6-18	9.2	7	5.5	8	0.095	16	0.099	16
248	1	16-18	0	14-18	0	6-18	1.2	17-18	0.6	15	0.115	13	0.121	13

*Ranks (τ) are indicated for all indices.

For the major river basins within each ecoregion for which data were available, the distribution of genetic diversity across rivers was heterogeneous for ecoregions 229, 231, and 232 (Ribeira do Iguape, Upper Uruguay, and South Brazilian Coast, respectively), where one river per region (Iguape, Pelotas, and Itajai-Açu, respectively) had at least 3 times more genetic diversity than any other river from the same ecoregion (Table 2). The other ecoregions had a more homogeneous distribution of genetic diversity because no single river had GD or PD estimates 40% higher than any other river in the same ecoregion.

Complementarity Analyses

We performed 3 successive rounds of complementarity analysis for 6 of the biodiversity indices (Table 3). Ecoregions ranked as second in value in the initial analyses showed the same ranking in the complementarity analyses for the species richness, endemic species, threatened species, TD, and TE indices, but not for the GD and PD indices. In the initial analysis, ecoregion 239 was ranked second in GD and PD, but in the complementarity analyses this ecoregion was fourth for the same 2 indices. This is because ecoregion 239 contained 8 species in the initial analysis but only 5 in the complementarity analysis. Ecoregions ranked as third and fourth in value varied between initial and complementarity analyses for all the indices and between indices in the complementarity analyses. Summarizing across all methods, the following ecoregions were ranked the highest in the complementarity analyses for at least 2 of the indices: Laguna dos Patos Basin (233; first and second priority), Central Chile (235; first and second priority), South Brazilian Coast

(232; third and fourth priority), Chilean Lakes (236; third and fourth priority), and Subtropical Potamic Axis (239; third and fourth priority).

Discussion

Method Comparison

There was a strong correlation among rankings of all indices ($r = 0.606-0.997$; $p < 0.01$). When comparing species count-based and phylogeny-based approaches, species richness and endemic species showed greater correlation with GD and PD than with TD and TE (Table 4). Interestingly, the topologically dependent (TD and TE) and distance-dependent (GD and PD) methods showed the lowest correlation (excluding threatened species). Threatened species showed greater correlation with endemic species than species richness. This makes sense because endemic species only includes taxa with narrow distributions that are potentially more susceptible to environmental degradation (i.e., higher risk of extinction) than taxa with wider distributions, whereas species richness counts all taxa. Threatened species showed greater correlation with topologically dependent methods than distance-dependent methods. This is because the most basal taxa in our trees (Chilean aeglids) are also the most threatened. Congruence between species richness and phylogenetic diversity indices has been reported (Whiting et al. 2000; Pérez-Losada et al. 2002a; Pérez-Losada & Crandall 2003). These studies show that although correlation between index estimates is not perfect, when they are used for ranking areas for priority

Table 2. Estimates of species richness (SR, number of species), genetic diversity (GD), and phylogenetic diversity (PD) across rivers and within freshwater ecoregions in southern South America.

Region no.*	River	SR	GD	PD	Region no.	River	SR	GD	PD
225	Paranapanema	4	0.132	0.14	235	La Ligua	1	0.072	0.072
225	Tietê	2	0.105	0.109	235	Lanlahue	1	0.024	0.024
					235	Lleu Lleu	1	0.024	0.024
229	Cubatão	1	0.056	0.057	235	Maipo	1	0.039	0.04
229	Iguape	6	0.183	0.2	235	Malleco	2	0.06	0.062
					235	Maule	3	0.082	0.085
231	Canoas	2	0.056	0.057	235	Aconcagua	1	0.072	0.072
231	Chapecó	1	0.074	0.076	235	Petrohue	1	0.033	0.034
231	Irani	1	0.045	0.046	235	Pocuno	1	0.024	0.024
231	Peixe	1	0.045	0.046	235	Tinguiririca	2	0.06	0.062
231	Pelotas	10	0.253	0.289	235	Tucapel	2	0.031	0.031
232	Itajai-Açu	6	0.174	0.19	236	Bueno	2	0.059	0.06
232	Itapocu	1	0.057	0.058	236	Chiloe Island	3	0.081	0.084
232	Paranaguá	1	0.057	0.058	236	Hueicolla Area	1	0.037	0.037
					236	Mauillin	5	0.086	0.089
233	Antas	5	0.173	0.187	236	Ralun	1	0.027	0.028
233	Cai	6	0.19	0.208	236	Tolten	2	0.066	0.068
233	Camaquã	1	0.071	0.073	236	Valdivia	3	0.09	0.094
233	Dos Sinos	4	0.143	0.153					
233	Gravatai	2	0.134	0.142	237	Bermejo	3	0.135	0.143
233	Guaíba	5	0.221	0.247	237	Grande	2	0.101	0.105
233	Jacui	4	0.152	0.163					
233	Jaguarão	1	0.071	0.073	239	Paraguay	3	0.149	0.155
233	Lagoa Mirim	1	0.07	0.073	239	Parana	3	0.136	0.146
233	Mampituba	1	0.062	0.064	239	Salado	2	0.1	0.104
233	Taquari-Tainhas	8	0.228	0.256	239	Uruguay	2	0.116	0.122
233	Tramandai	1	0.062	0.064					
					240	Ibicui	2	0.113	0.119
235	Aconcagua	1	0.072	0.072	240	Ijui	2	0.088	0.092
235	Cachapoal	2	0.051	0.052	240	Uruguay	1	0.071	0.073
235	Caramavida	2	0.031	0.031					
235	Choapa	1	0.072	0.072	247	Chico-Chubut	1	0.035	0.035
235	Cholchol	1	0.045	0.046	247	Colorado	1	0.028	0.029
235	Concepción Area	1	0.04	0.041	247	Negro	2	0.061	0.062
235	Imperial	2	0.055	0.056	247	Chilean Patagonia	2	0.054	0.055
235	Itata	2	0.06	0.062					

*Only those ecoregions with 2 or more sampled rivers and at least 1 *Aegla* species per river were included in this analysis.

setting the general ranks tend to be the same. Therefore, our results also support the general assertion that taxon richness is a good surrogate for phylogenetic diversity (Polasky et al. 2001; Rodrigues & Gaston 2002; Pérez-Losada & Crandall 2003).

Previous studies also show that phylogenetic diversity can produce quite different results relative to taxonomic diversity (Crozier 1992; Faith 1992, 1994; Krajewski 1994; Pérez-Losada & Crandall 2003). Because the cladistic approach does not take the distinctiveness of particular species (i.e., genetic distances) into account, closely related species can be given greater weight than species whose genetic distance from all others is greater. Moreover, because taxonomic diversity indices only reflect the branching order of evolution (i.e., phylogenetic relatedness), ecoregions supporting basal species will be prone to have higher value ranks than nonbasal species, even if the latter have achieved larger genetic and species differentiation. In our tree of phylogenetic relationships

(Fig. 2), the internal branches connecting the main clades are relatively short compared with the branches leading to the terminal taxa, so the main differences occur at the tips. Moreover, the terminal branches leading to central and southeastern South American clades (*A. camargoi* to *A. inermis*) have on average longer and more geographically dispersed lineages than those leading to southwestern South American clades (*A. papudo* to *A. rostrata*). The genetic distance approaches are sensitive to these features, so the 2 ecoregions with the highest scores are from the central and southeastern South America. On the other hand, the cladistic approach gives the highest rank to ecoregion 235 (Central Chile), which contains deeply rooted and closely related species. We believe that conservation planning should consider not only species relatedness but also the distinctiveness of species, so rankings based on genetic diversity and phylogenetic diversity are preferable relative to those based on TD and TE.

Table 3. Results of complementarity analyses of species richness (SR, number of species), number of threatened species (TS), taxonomic diversity (TD), taxonomic endemism (TE), genetic diversity (GD), and phylogenetic diversity (PD) for 18 freshwater ecoregions from southern South America.*

Ecoregion	SR	SR _r	TS	TS _r	TD	TD _r	TE	TE _r	GD	GD _r	PD	PD _r
222	0	16-18	0	6-18	1.2	14-16	0	17-18	0	16-18	0	16-18
225	6	5-7	1	5	6.8	8	3.8	10	0.166	5	0.180	5
226	1	13-15	0	6-18	1.5	13	0.5	17	0.056	14	0.057	14
228	4	9-10	0	6-18	3.5	11	2.2	11	0.105	10	0.110	10
229	6	5-7	2	3-4	8.1	7	5.1	7	0.147	7	0.158	7
231	7	3-4	0	6-18	10.3	5	8.4	5	0.163	6	0.176	6
232	7	3-4	0	6-18	9.7	6	6.6	6	0.209	3	0.232	3
233	17	1	4	2	20.8	2	16.1	2	0.428	1	0.552	1
235	13	2	6	1	35.1	1	31.1	1	0.271	2	0.311	2
236	6	5-7	2	3-4	10.5	4	8.5	4	0.139	9	0.149	9
237	4	9-10	0	6-18	2	12	2	12	0.145	8	0.155	8
239	5	8	0	6-18	11.9	3	9.8	3	0.179	4	0.195	4
240	1	13-15	0	6-18	1.2	14-16	0.6	14-15	0.072	13	0.074	13
242	2	12	0	6-18	1.2	14-16	1.2	13	0.093	11	0.096	11
244	0	16-18	0	6-18	0	17-18	0	17-18	0	16-18	0	16-18
246	1	13-15	0	6-18	5	10	5	8	0.049	15	0.049	15
247	3	11	0	6-18	6.7	9	4.7	9	0.079	12	0.082	12
248	0	16-18	0	6-18	0	17-18	0.6	14-15	0	16-18	0	16-18

*Ranks (r) are indicated for all indices.

Conservation of Freshwater Ecosystems in Southern South America

Setting broad-scale conservation priorities is a notoriously subjective affair, with no single algorithm or set of criteria receiving universal consensus (Redford et al. 2003). Even putting aside the question of how status and threat should be integrated into priority setting, there are multiple approaches for assessing biological value. Our approach, following Posadas et al. (2001), was to use measures of both species and genetic diversity to yield a more comprehensive result than if either approach were used alone. We focused our analyses only on aeglid crabs, but we think the approach has merit for other groups and potentially for multiple-taxa assessments. Nevertheless, for a broad-scale assessment of freshwater ecosystem priorities, we recommend similar studies be carried out on a variety of freshwater organisms for comparison.

Table 4. Spearman's rank correlation coefficients among estimates of species richness (SR), endemic species (ES), threatened species (TS), taxonomic diversity (TD), taxonomic endemism (TE), genetic diversity (GD), and phylogenetic diversity (PD).*

	SR	ES	TS	TD	TE	GD
ES	0.959***					
TS	0.774***	0.855***				
TD	0.872***	0.863***	0.857***			
TE	0.836***	0.856***	0.869***	0.992***		
GD	0.926***	0.871***	0.606**	0.737***	0.700**	
PD	0.922***	0.877***	0.606**	0.718**	0.684**	0.997***

*The significance of $r = 0$ (** $p < 0.01$; *** $p < 0.001$) after Bonferroni correction is also shown for each pairwise comparison.

When all the biodiversity indices are considered in combination with the complementarity analysis, the data indicated that Laguna dos Patos Basin in Brazil and Central Chile have the highest biological value for aeglid crabs and so should perhaps be a priority for conservation investment. South Brazilian Coast, Chilean Lakes, and Subtropical Potamic Axis were also identified as priorities by 3 of 4 different indices; thus, these 3 ecoregions have considerable value as well. Conservation of these 5 regions will preserve the highest number of species and the greatest amount of genetic diversity within the South American freshwater *Aegla* fauna. When resources are available, we believe the use of phylogenetic-based methods that consider the evolutionary component of biodiversity (i.e., assuring preservation of genetically different and unique species) is crucial. Indeed, because preserving genetic diversity is often a goal in conservation biology (Templeton 1991; Crozier 1992), both relatedness and distinctiveness should be included in the weighting scheme for priority setting. When the results of the genetic-dependent indices, genetic diversity and phylogenetic diversity, were considered alone, only 4 of the 5 areas highlighted via the richness-based complementarity analysis were selected: ecoregion 233, which had the highest rank, and ecoregions 235, 232, and 239, which had the second-, third-, and fourth-highest rankings.

But how well represented is southern South American aeglid biodiversity if only the 5 top ecoregions were protected? These ecoregions represent 48 out of 66 *Aegla* species (73% of the species diversity); hence, a large portion of the *Aegla* biodiversity would be preserved by protecting these ecoregions. Nevertheless, after 3 rounds of complementarity analysis several ecoregions with high

genetic biodiversity (e.g., ecoregions 225 and 229) were not included. This is mainly a consequence of the small distributional ranges of many of the *Aegla* species (several are limited to a single river basin or even a creek), geological features such as the Andean cordillera, the Cordillera de la Costa in Chile, and the Serra do Mar in Brazil, which act as barriers to *Aegla* dispersion, and the large geographical extent of the area (Jara 2005). A more effective assessment of *Aegla* conservation and management would require the split of southern South America into smaller conservation units.

Once global conservation priorities have been established at a broad scale, the next step is to identify fine-scale priority areas within the selected ecoregions. Our analyses of southern South American main river basins with species tallies and phylogenetic indices (Table 2) identified the following Brazilian rivers as the top priority for conservation because of their high biodiversity (as indicated by those indices) compared with other rivers from the same ecoregion: Paranapanema (ecoregion 225), Iguape (ecoregion 229), Pelotas (ecoregion 231), and Itajai-Açu (ecoregion 232). These are relatively small rivers; hence, their conservation seems manageable. *Aegla* biodiversity in Argentinean and Chilean ecoregions was more homogeneously distributed; no single river accounted for a great deal of biodiversity. Conservation plans in these other ecoregions should involve several river basins and will potentially require a greater effort.

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