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Biological Conservation 105 (2002) 345–353

BIOLOGICAL CONSERVATION

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Conservation phylogenetics of Chilean freshwater crabs *Aegla* (Anomura, Aeglidae): assigning priorities for aquatic habitat protection

Marcos Pérez-Losada[a,*], C.G. Jara[b], G. Bond-Buckup[c], K.A. Crandall[a]

[a]*Department of Zoology and Monte L. Bean Museum, 574 Widtsoe Building, Brigham Young University, Provo, UT 84602-5255, USA*
[b]*Instituto de Zoología, Casilla 567, Universidad Austral de Chile, Valdivia, Chile*
[c]*Departamento de Zoologia, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, Brazil*

Received 6 April 2001; received in revised form 27 August 2001; accepted 11 September 2001

Abstract

Recently, a molecular phylogeny based on four mtDNA gene regions has been estimated for 17 species and subspecies of *Aegla* freshwater crabs from Chile. With this phylogenetic hypothesis and information on geographic distribution, environmental conditions, habitat requirements, and population abundance, Chilean aeglids were assessed for conservation status based on the criteria included in the IUCN Red List Categories (2001). *Aegla concepcionensis* and *Aegla expansa* qualify as "Extinct in the Wild" and nine other taxa fall within the threatened category: three as "Critically Endangered" (*A. laevis laevis*, *A. papudo* and *A. spectabilis*) and six as "Vulnerable" (*A. alacalufi*, *A. bahamondei*, *A. cholchol*, *A. hueicollensis*, *A. laevis talcahuano* and *A. manni*). Six hydrographic regions within temperate Chile were ranked for conservation priority using species richness, and phylogenetic and genetic diversity indices. The hydrographic region made up of the Tucapel, Imperial, and Toltén Basins was found to rank highest in terms of conservation priorities. Conservation of this region and the regions ranging between the Aconcagua and Mataquito Basins, and the Valdivia and Maullín Basins, would preserve almost all the *Aegla* diversity present in Chile.

Keywords: Aeglidae; Phylogeny; Endangered species; Chile; Phylogenetic diversity; Conservation

1. Introduction

The Aeglidae Dana, 1852 are freshwater decapod crustaceans endemic to the Neotropical region of South America. They presently belong to a single genus, *Aegla* Leach, consisting of approximately 70 recognized species and subspecies (Bond-Buckup and Buckup, 1994) distributed among Chile, Brazil, Argentina, Uruguay, and Bolivia. Chile supports 19 *Aegla* species and subspecies, including 16 endemic taxa. Over the last 25 years these species have experienced progressive reductions in their populations due to severe deterioration of the Chilean stream environments (Jara, 1996; Bahamonde et al., 1998), leading in two cases (*Aegla concepcionensis* and *Aegla expansa*) to the extinction of the populations in their known extents of occurrence (Jara, personal observation). Therefore, we believe the Chilean aeglids must be prioritized for conservation efforts. Recently, 17 of the 19 Chilean *Aegla* species were assessed for endangered status (Bahamonde et al., 1998). However, the criteria and categories used in this preliminary study were different from those adopted by the IUCN Council in 1994. We think that to create a meaningful list of threatened species, those applying the classification system must have a common understanding of the categories of threat. The 1996 IUCN Red List categories and criteria, and subsequent updated versions, were created under this premise, providing a clear quantitative framework for the categories of threat. Within this framework, the objectivity of the listing process and the consistency in its application can be increased. Therefore, this study assesses the status of the Chilean aeglids based on the new criteria included in the IUCN Red List Categories (2001).

Chilean Aeglidae freshwater crabs range latitudinally from the Choapa River (~31°S) to the Insular Territory

* Corresponding author. Tel.: +1-801-378-9378; fax: +1-801-378-7423.
E-mail address: mp323@email.byu.edu (M. Pérez-Losada).

0006-3207/02/$ - see front matter
PII: S0006-3207(01)00218-X

(~50°S; Fig. 1), covering all of temperate Chile. In this region these crabs are the most broadly distributed macroinvertebrate (Bond-Buckup and Buckup, 1994; Jara, 1996). Thus, we think *Aegla* is well suited for the study of conservation biology of the Chilean temperate freshwater streams. Temperate Chile is of specific concern for conservation because it represents approximately 90% of the freshwater environment in this country. However, as far as we know there are no studies setting objective conservation priorities for Chile on a large scale. No biogeographic regionalization of freshwater ecosystems representing unique habitats has been done. Therefore, for lack of a better framework, we have divided the temperate Chile region into six areas of study using the available data on climate, lithology/geology and river regimens presented by Santis (1975) and the Instituto Hidrográfico de la Armada (1980; Fig. 1, regions A–F). These six areas correspond to well defined hydrographic regions and include all the main basins in temperate Chile. Using *Aegla* systematic relationships from Pérez-Losada et al. (2002) and geographic distributions from Bond-Buckup and Buckup (1994) and Jara (1996), this study assigns conservation priorities to these six hydrographic regions, which may correlate to different habitats and ecosystems.

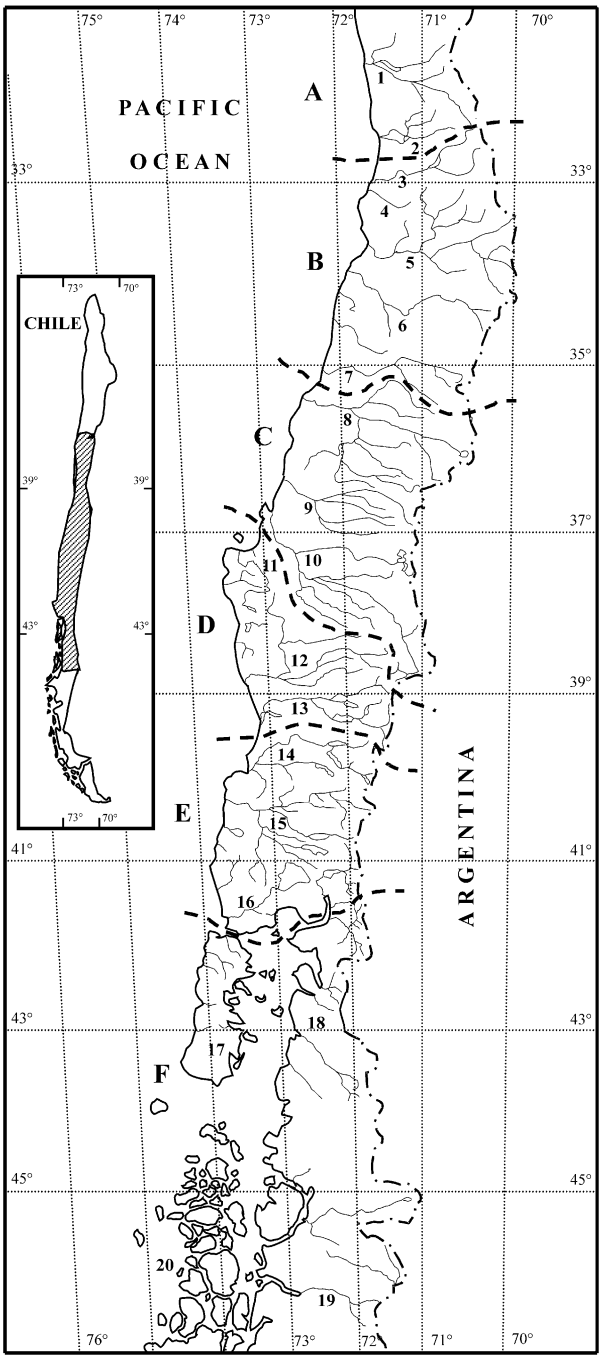

Fig. 1. Map of Chile showing the studied hydrographic regions (A–F) and main basins: 1, Choapa; 2, La Ligua; 3, Aconcagua; 4, Valparaiso; 5, Maipo; 6, Rapel; 7, Mataquito; 8, Maule; 9, Ñuble; 10, Bío Bío; 11, Tucapel; 12, Imperial; 13, Toltén; 14, Valdivia; 15, Bueno; 16, Maullín; 17, Chiloé Island; 18, Chiloé Continental; 19, Aysén Province; 20, Insular Territory (Archipelagos between 44°S and 50°S are only partially shown in the map).

The determination of species boundaries is perhaps the most important area of application of phylogeny to conservation biology. Many species concepts have phylogenetic relatedness as the central focus in the determination of species, e.g. phylogenetic species concept (Cracraft, 1983), cohesion species concept (Templeton, 1989), and evolutionary species concept (Wiley, 1978). Furthermore, phylogeny can be used to assess the degree of genetic isolation between two populations (Slatkin and Maddison, 1989), and partition historical and repeated events shaping population structure (Templeton, 1998). It is of primary importance to establish whether or not distinct populations are distinct species within an hypothesis testing framework (Sites and Crandall, 1997). Furthermore, phylogenetic approaches are used extensively to identify highly differentiated subpopulations of a species in need of conservation action (Crandall et al., 2000). The delineation of species and distinct populations greatly influences conservation policy in terms of introductions and listings as rare and endangered. In this study, we also explore the species status of some *Aegla* populations based on the phylogenetic information available.

2. Materials and methods

2.1. Study area

Temperate Chile encompasses 20 different main basins with the Choapa River (approximately 31°S) the northernmost basin and the Insular Territory (between 44°S and 50°S) the southernmost basins (Fig. 1). Based on the available data on climate, lithology/geology and river regimens, these basins have been divided in six hydrographic regions: region A, rivers of snowy and pluvious regimen; region B, rivers of snowy regimen

with torrential draining; region C, rivers with snowy regimen and fast flood; region D, transition rivers; region E, rivers of constant flow and light slope; region F, Patagonian Rivers (Fig. 1). These six regions will be assessed for conservation priorities based on *Aegla* distributions and systematics.

2.2. Species richness

Until the implementation of phylogenetic methods for measuring biodiversity, conservation priorities were based solely on methods like species richness, all species being considered of equal value. Here, species richness was used in an attempt to compare the standard species counts to the more recent phylogenetic measures. Distributions of every *Aegla* species compiled from the general reviews developed by Bond-Buckup and Buckup (1994) and Jara (1996) were mapped against the 20 basins and recorded as present or absent in each hydrographic region. Tallies were made of total numbers of species present in each region.

2.3. Phylogenetic diversity

Methods for evaluation of biodiversity based on phylogeny have played a large role in recent advances in conservation biology, being particularly useful for a preliminary assessment of conservation priorities and endangered species, and to identify populations or species for which more detailed studies are needed (Templeton, 1991; Eldredge, 1992; Cracraft, 1994; Krajewski, 1994; Greene, 1994; Crandall, 1998; Crandall et al., 2000; Whiting et al., 2000). These phylogenetic methods have typically been separated into two 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 organisms that evolved earliest with the highest priority regardless of divergence between species (Vane-Wright et al., 1991; Nixon and Wheeler, 1992). 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 (Crozier, 1997; Krajewski, 1994; Faith, 1992). It has been generally agreed that it is more appropriate for conservation purposes to use unrooted trees, and that branch length measures more accurately assess genetic diversity (Crozier, 1997). For these reasons, Conserve 3.2.1 (Agapow and Crozier, 1998) was used to compute two different distance measures, the phylogenetic diversity (PD; Faith, 1992), and the genetic diversity (GD; Crozier, 1992). The unrooted maximum likelihood tree based on the ribosomal 12S and 16S, and the cytochrome oxidase I (COI) and II (COII) mitochondrial genes (approximately 2500 bp) collected from a representative sampling of Chilean aeglids estimated by Pérez-Losada et al. (2002) was used to compute both diversity measures. In this study we have collapsed all the monophyletic clades representing samples or specimens from the same species for an easier visualization. To compute PD values we followed the method suggested by Faith (1992, 1994) and used corrected branch lengths based on the model of evolution justified in Pérez-Losada et al. (2002). We used uncorrected proportional distance to compute GD values as suggested by Crozier and Kusmierski (1994).

For those taxa not forming monophyletic clades (i.e. *Aegla laevis talcahuano* and *Aegla cholchol*) only one sample was used to compute PD and GD for each region. PD and GD values were standardized by finding the quotient value that resulted in the smallest PD or GD value being equal to 1.0. PD and GD values were then computed for each region by summing up the PD and GD values for all the species represented in that region.

For those taxa representing non-monophyletic groups, alternative maximum likelihood tree topologies were searched using heuristic searches in PAUP* (Swofford, 2000) but constraining every non-monophyletic group to be monophyletic. Then, for every species, both phylogenetic hypotheses were tested for significant differences using the Shimodaira and Hasegawa (1999) method implemented in the program SHTest v1.0 (http://evolve.zoo.ox.ac.uk/software.html). This test is a more conservative modification of the Kishino and Hasegawa (1989) test. The use of the Kishino and Hasegawa test leads to overconfidence for a wrong tree when many topologies are compared because the sampling error due to the selection of the topology is overlooked (Goldman et al., 2000). This new test estimates the confidence limit of every topology within a likelihood framework by taking into account a multiplicity of testing. There are two kinds of SHTests, the RELL test and the FULL test. The FULL model re-optimizes the branch lengths and other parameters of each tree for each bootstrap replicate. The RELL model simply resamples the partial likelihoods for each site, meaning it is faster than the FULL model but approximate. Because we are testing for every non-monophyletic group a high number of trees which also have a high number of taxa (see later), we used the RELL model to avoid extremely long computational times. The $TVM+\Gamma+I$ model of evolution specified in Pérez-Losada et al. (2002) was used to set up the parameters that the SHTest requires.

3. Results

3.1. Systematic implications

The phylogeny determined by 12S, 16S, COI, and COII mtDNA genes shown in Fig. 2 places *A. cholchol* and *A. laevis* in non-monophyletic groups. To test the

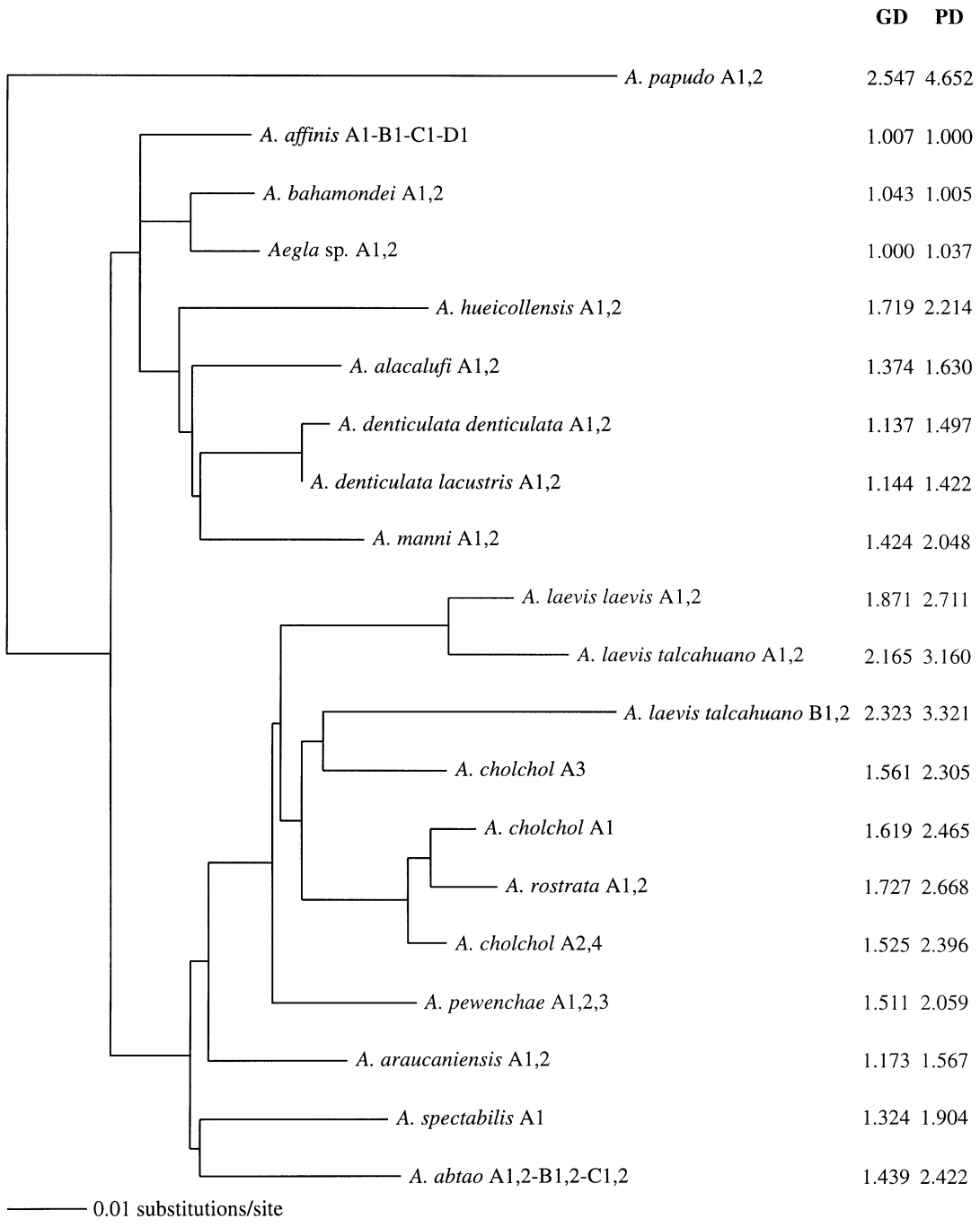

Fig. 2. Maximum likelihood tree based on 12S, 16S, COI, and COII mtDNA genes (drawn from Pérez-Losada et al., 2001). Capital letters (A–D) and numbers (1–4) after the species names represent different samples and specimens, respectively, collected within every species. All the monophyletic clades representing samples or specimens from the same species were collapsed for an easier visualization. Genetic diversity (GD) and phylogenetic diversity (PD) values for each taxon are also indicated.

monophyly of these species, alternative topologies with the species in question as a monophyletic group were compared to the tree in Fig. 2 using the Shimodaira and Hasegawa (1999) test. The results of these comparisons are shown in Table 1. *A. cholchol* forms statistically significant non-monophyletic relationships ($P<0.001$) for the 27 alternative monophyletic hypotheses tested but *A. laevis* does not ($P>0.3$). However, the two samples of *A. laevis talcahuano* from Maule (sample A1,2) and Ñuble (sample B1,2) Basins form a statistically significant non-monophyletic clade ($P<0.001$; Table 1).

3.2. Phylogenetic diversity and species richness

Phylogenetic diversity (PD) was found to be the highest in hydrographic region D (from Tucapel to

Table 1
Testing monophyletic groups—1000 bootstrap replicates were performed under the RELL test (Goldman et al., 2000)

Taxonomic group	*n* trees	ln L	delta	*P* (delta)
A. chochol	27	<-8693	>55	<0.001
A. laevis	27	>-8645	<7.7	>0.30
A. laevis talcahuano	27	<-8729	>91	<0.001

Toltén Basins) with a value of 16.56 (Table 2A), thus it was considered as top conservation priority (rank 1 at Table 2B). Region E (from Valdivia to Maullín Basins) was next in priority with a value of 13.84, thus it was given second priority conservation ranking. Regions D and E had also the first and the second highest genetic diversity (GD) values as well (11.88 and 9.76, respectively; Table 2A). The results from the species richness measure were identical to the results based on PD and GD indices for designating first and second regional priorities. Regions D and E were considered as first and second priority conservation for SR with nine and seven species, respectively (Table 2A). The largest deviation between the species richness and the phylogeny-based methods was region B (from Aconcagua to Mataquito Basins); this region has only four species, but a PD of 12.58 and a GD of 8.09 (Table 2A). This reflects the fact that the species in region B represent more phylogenetically distinct taxa than those found in other regions.

For conservation purposes it is important to identify areas that represent similar species richness thereby eliminating redundancy. We, therefore, performed a complimentarity analysis where the region with the highest rank was given first priority, then all taxa represented in that area were deleted from the analysis, and the rankings were recomputed (Table 2C). For example, region E contains seven species, but in the complimentarity analysis, it only contains three unique species because the other four also occur in the highest priority region D. In this analysis region D (from Tucapel to Toltén Basins) contains the most species, followed by region B (from Aconcagua to Mataquito Basins).

Table 2
Phylogenetic diversity and species richness

	A (1–2)	B (3–7)	C (8–10)	D (11–13)	E (14–16)	F (17–20)
(A) Chilean hydrographic regions						
PD	4.65	12.58	9.28	16.56	13.84	7.12
GD	2.55	8.09	6.99	11.88	9.76	5.12
SR	1	4	6	9	7	4
(B) Chilean hydrographic region ranks						
PD	6	3	4	1	2	5
GD	6	3	4	1	2	5
SR	6	4.5	3	1	2	4.5
(C) Complimentarity analysis						
PD	4.65	10.52	4.16	16.56	5.72	1.63
GD	2.55	6.58	3.17	11.88	4.29	1.37
SR	1	3	2	9	3	1

(A) Values for phylogenetic diversity (PD), genetic diversity (GD) and species richness (SR) associated with the Chilean hydrographic regions (main basins included). (B) Chilean hydrographic region ranks for all indices. (C) Complimentarity analysis. See Fig. 1 for a key to the Chilean regions and basins (in parentheses).

4. Discussion

4.1. Systematic implications

A. cholchol and *A. laevis talcahuano* form significant non-monophyletic groups. Under certain species concepts (e.g. Cracraft, 1983), species must form monophyletic groupings. Other concepts (e.g. Templeton, 1998, 2001) allow for non-monophyletic relationships within a species. One explanation for the non-monophyly of mtDNA haplotypes within a species is the problem of gene trees reflecting species (or population) trees. The results of theoretical population genetic work indicate that for some time after the divergence of two or more populations, there is a high probability that populations may show non-monophyletic relationships for a specific gene; therefore, the gene genealogy may not accurately reflect the population divergence (Neigel and Avise, 1986; Takahata and Slatkin, 1990). Thus, although these results are based on four different mtDNA genes that gave the same non-monophyletic pattern when they were analyzed individually (Pérez-Losada et al., 2002), unlinked nuclear genes should also be included in the analysis. Our current sequencing efforts are focussed on this question.

A. cholchol occurs in two different aquatic environments on the bottom of the Chol-Chol River (Imperial Basin), one is stony and pebbly and the other is sandy (Jara, 1996). Moreover, different morphotypes have been observed for this species, although their assignment to one of these two habitats has not been studied (Jara, personal observation). Given this suggestive ecological and morphological differentiation, and the genetic results presented here, a process of sympatric speciation via habitat partitioning could be postulated for *A. cholchol* in this area.

A. cholchol and its sister taxa *A. rostrata* and *A. laevis talcahuno* have allopatric distributions within temperate Chile (i.e. the Imperial Basin; Toltén and Valdivia Basins; and Rapel, Mataquito and Maule Basins, respectively; Fig. 1). Moreover, *A. cholchol* individuals show extensive genetic differentiation with both sister taxa. *A. cholchol* A1 presents 30 nucleotide differences with *A. rostrata* A1,2, and *A. cholchol* A3 presents 89 nucleotide differences with *A. laevis talcahuno* B1,2 for the mtDNA regions analyzed. Therefore, all these

results suggest that this sample of *A. cholchol* pass species criteria for the phylogenetic and evolutionary concepts of species. The species status via the cohesion concept, recognition concept, and the biological species concept will require further study.

The putative *A. laevis talcahuano* B1,2 sample was collected from outside its recognized area of occurrence (i.e. from the Rapel to the Maule Rivers), and it was classified as similar to *A. laevis talcahuano* because the sample shares several morphological characters with this named species, although it has some autapomorphies that differentiate it from the other Chilean aeglids. The significant placement of the two *A. laevis talcahuano* samples (A1,2 and B1,2) in two distinct areas in the phylogeny suggests the possibility of sufficient divergence to be considered distinct species. Obviously, morphological and ecological studies of these samples, and more population genetic analyses of the *A. laevis* subspecies are warranted to determine species status.

4.2. Species conservation status

The qualification of the aeglids for the criteria included in the IUCN Red List Categories (2001) is a primary necessity for the conservation of these unique freshwater crabs. Conservation status for every *Aegla* taxon present in Chile was assessed using geographic range, habitat descriptions, and population analyses compiled from the literature since 1959 (see Jara, 1996; Bahamonde et al., 1998; Jara and Palacios, 1999) and field observations made by the authors during the last 25 years. Geographic ranges were calculated from species distribution maps provided by Bond-Buckup and Buckup (1994) and Jara (1996). Table 3 shows that two species, *Aegla concepcionensis* and *Aegla expansa*, can be presumed "Extinct in the Wild" (EW) because exhaustive surveys during several years in known and expected habitats throughout their historical ranges have failed to record any individuals. The extent of occurrence of both species has been severely altered by forest exploitation and urbanization (Jara, 1986, 1992, 1996). Another nine taxa fall within the threatened category. *Aegla laevis laevis*, *Aegla papudo* and *Aegla spectabilis* qualified as "Critically Endangered" (CR) for two criteria (i.e. suspected population reduction of at least 80% and extent of occurrence estimated to be less than 100 km^2). *A. laevis laevis* and *A. papudo* populations have experienced significant reductions (direct observation) due to severe water level diminution of the streams in their areas of occupancy caused by irrigation farming. *A. papudo* no longer exists in its type locality and several populations of both species from the Maipo River are extinct due to dumping of toxic waters from Santiago de Chile (López, 1959; Bahamonde and Atria, 1976; Jara et al., 1995; Jara, 1996). Moreover, these two species have high phylogenetic diversity indices (Fig. 2), which highlights their importance for conservation. *A. spectabilis* is known to exist in a single location from the Chol-Chol River, which over the last few years has been polluted by organic detritus from cattle farms and chemical compounds from agricultural exploitation. In the last survey made in this area by the authors (February 2000), only a single mature male was collected and no juveniles. Furthermore, this species has a very restricted habitat specificity and small population sizes (Jara, 1986, 1996). The other six species have been considered "Vulnerable" (VU) for different criteria. *Aegla alacalufi* has been described in four South Chilean basins; however, it qualified as "Vulnerable" because a population reduction of at least 30% has been projected to occur over the next ten years (Jara, 1996). The continental extent of occurrence of this species has been deeply altered due to deforestation and drainage sedimentation produced by the occurrence of large fires in this area during the last decades. This has provoked habitat fragmentation and isolation of populations that have already started to experience size reductions (Jara and Lopez, 1981; Jara, 1996). *Aegla bahamondei* and *Aegla manni* also were considered "Vulnerable" because their fragmented populations are very restricted in their area of occupancy and number of locations (only four locations are recognized for both species; Jara, 1980, 1982, 1996). *A. cholchol* and *A. laevis talcahuano* qualified as "Vulnerable" due to an observed population reduction of at least 30%. In addition, both species have experienced a decline of their areas of occupancy due to

Table 3
Status of the Chilean *Aegla* for the criteria included in the IUCN Red List Categories (2001)

Species	Category[a]	Criteria[b]
A. abtao	LC	
A. affinis	LC	
A. alacalufi	VU	A3c
A. araucaniensis	LC	
A. bahamondei	VU	D2
A. concepcionensis	EW	
A. cholchol	VU	A2ae
A. denticulata denticulata	LC	
A. denticulata lacustris	NT	
A. expansa	EW	
A. hueicollensis	VU	B1ab(iii,iv)
A. laevis laevis	CR	A2ae
A. laevis talcahuano	VU	A2ae
A. manni	VU	D2
A. neuquensis	LC	
A. papudo	CR	A2ae
A. pewenchae	LC	
A. rostrata	LC	
A. spectabilis	CR	A2ae; B1ab(i,iii,v)
Aegla sp.	DD	

[a] CR, Critically Endangered; DD, Data Deficient; EW, Extinct in the Wild; LC, Least Concern; NT, Near Threatened; VU, Vulnerable.
[b] See text for a key to the criteria.

contamination, habitat alteration, and water level diminution provoked by intensive agricultural exploitation, irrigation farming and urbanization (Jara, 1996; Bahamonde et al., 1998; Jara and Palacios, 1999). Moreover, *A. laevis talcahuano* also presents high phylogenetic diversity indices (Fig. 2). *Aegla hueicollensis* occurs mainly in the streams from the West Pelada Cordillera (i.e. extent of occurrence estimated to be less than 20,000 km^2), where it has a severely fragmented distribution (Jara, 1996; Jara and Palacios, 1999). Recently this area of native forest has been exploited by timber farms. Presumably this could alter the freshwater environments and provoke an important reduction in the *A. hueicollensis* populations as has been already observed for other *Aegla* species (e.g. *A. concepcionensis* from Concepción River). Therefore, this species also qualified as "Vulnerable". The other eight recognized species did not satisfy the criteria for any of the threatened categories: seven were considered "Least Concern" (LC) and *Aegla denticulata lacustris* was considered "Near Threatened" (NT) because it was close to qualifying for "Vulnerable" (Jara, 1977, 1980, 1989, 1994, 1996). *Aegla* sp. was recently collected in the Tucapel River and represents a morphologically unrecognized taxon, according to the most recent taxonomic classification developed by Jara (1996). Therefore, for this possible new species there is not enough information to make an assessment of its risk of extinction ("Data Deficient"—DD).

4.3. Regional priorities

When the resulting ranks from the various biodiversity indices are compared, there is little difference between the traditional species counts and the newer phylogenetic diversity measures (Table 2). When they are used for ranking regions for conservation priorities the top ranks are the same. Crandall (1998) and Whiting et al. (2000) found similar results when comparing phylogenetic and traditional methods in the north-eastern USA and Australia, respectively, based on freshwater crayfish. Given unlimited resources, we think the optimal way to estimate conservation rankings is to use phylogenies for computing genetic diversity indices. PD and GD allow for more accurate assessments of the species (e.g. *A. papudo* or *A. laevis*) and ranking of regions for conservation priorities (e.g. region B in Table 2A). However, the differences between the traditional and phylogenetic methods do not appear to be sufficient to warrant the added expense of obtaining sequence data for every taxon. Therefore we suggest that in cases of limited resources a species count be taken first, and then sequence data can be obtained to compute phylogenetic diversity measures.

In the complimentarity analysis (Table 2C), it is interesting to note that region B has the same number of species as region E, however the former has a much higher PD value (10.52) than the latter (5.72). This is due to the presence of the most divergent species (i.e. *A. papudo* and *A. laevis*) in the former region. This result addresses the conceptual differences between traditional species counts and the newer phylogenetic methods for assigning conservation priorities to specific taxa (see also Crandall, 1998; Whiting et al., 2000). Nevertheless, region E still has importance for conservation because it has three endemic species. Within regions D and B most of the *Aegla* species are represented. Only those ranging in the southernmost part of temperate Chile (i.e. *Aegla manni*, *A. hueicollensis*, *A. denticulata lacustris* and *A. alacalufi*) are not included.

4.3.1. Recommendations

When all of the diversity indices are considered in combination with the complimentarity analysis, the data suggest that the middle part of temperate Chile (region D), the smallest in area, represented by the transition rivers Tucapel, Imperial, and Toltén should receive top conservation priority. Regions B and E were found to be second and third priority in conservation, respectively. We hope this assessment of conservation priorities based on *Aegla* phylogeny and species distribution reflects overall species richness in freshwater ecosystems. We look forward to comparative studies of other freshwater stream organisms to confirm or reject this assumption.

1. Region D ranges between the Tucapel and the Toltén Basins and is the first priority in the conservation of temperate Chilean freshwater systems. It ranks first according to species richness (9), phylogenetic diversity (16.56) and genetic diversity (11.88) indices.
2. Region E (from Valdivia to Maullín Basins) and region B (from Aconcagua to Mataquito Basins) have similar phylogenetic indices, but region E has higher SR than region B. However, in the complimentarity analysis region B ranks much higher than region E. This reflects the redundancy of species richness in region E and stresses the importance of conservation for *A. papudo* and *A. laevis* (region B). Therefore, we suggest region B as second priority in conservation. Nevertheless, we still think region E is an important area for conservation because it contains three endemic species.

Conservation of regions D, B and E would preserve almost all the diversity found in the middle, north and south temperate Chile, respectively. Only *Aegla affinis*, which also occurs in Argentina, and *Aegla alacalufi*, which occurs in all basins from region F, are not included.

Acknowledgements

We would like to thank Alejandro Riedemann for assistance in collecting specimens and Megan Porter for her valuable suggestions to improve the manuscript. This study was funded by a grant from the National Science Foundation (NSF 0075600). MP-L was supported by the Fulbright Commission for Cultural, Educational and Scientific Exchange between the United States of America and Spain.

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