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Cladistic, biogeographic and environmental niche analysis of the species of *Agathemera* Stål (Phasmatida, Agathemeridae)

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Abstract

The endemic southern South American genus *Agathemera* Stål, which contains eight species, is analyzed in a cladistic context in order to establish a hypothesis regarding the phylogenetic relationships among its species. The cladistic analysis is based on adult and immature morphological characters belonging to both sexes. A biogeographical analysis is also performed to reconstruct the biogeographic history of the genus, and an environmental niche analysis to determine the potential distribution of the species, estimate niche overlap among species, and to find the most important variables that explain its present distribution. One tree of 51 steps was obtained that supports the monophyly of the genus. The species *A. elegans* and *A. mesoauriculae* distributed in southern Chile are situated at the base of the cladogram and they are the sister group to both the Argentinian (*A. claraziana, A. luteola, A. maculafulgens* and *A. millepunctata*) and the Chilean species (*A. grylloidea* and *A. crassa*). The Biogeographic analysis using DIVA 1.1 found 1 optimal reconstruction that involves a vicariant event at each node. The vicariant event of the most apical node of the tree can be correlated to the uplifting of the Andes. The basal species are distributed in the southern regions of Chile and in the Patagonian Steppe, while the remaining species are distributed in northern highlands. Environmental Niche Models showed that the soil variable was important for all eight species. According to the models, *A. claraziana* and *A. millepunctata* have large potential geographic distribution covering almost all the Patagonian area, and have similar niche requirements, while the six remaining species showed a more restricted geographic distribution.

Key words: Dispersion-vicariance analysis, environmental niche modelling

Introduction

Agathemera Stål, 1875 is a genus of the family Agathemeridae (suborder Agathemerodea) that contains eight endemic species from Southern South America. The monophyly of the genus has clearly been established (Camousseight 1995, 2005); however the phylogenetic relationship among its species has never been treated in a cladistic analysis. Similarly, there are no previous hypotheses regarding the biogeographical history of the species of the genus.

The *Agathemera* species are distributed at both sides of the Andes Mountain range, approximately between parallels 23º and 50º S (Fig. 1), and they are found inhabiting areas at very different altitudes, from near to the sea level to the altitudinal limit of vegetation (4,000 m.a.s.l.). This amplitude in habitats and environmental conditions does not mean that different species can be found intermingled in some areas. There are species geographically closer that must show some environmental separation, making the environmental niche modelling and niche overlap an interesting task.

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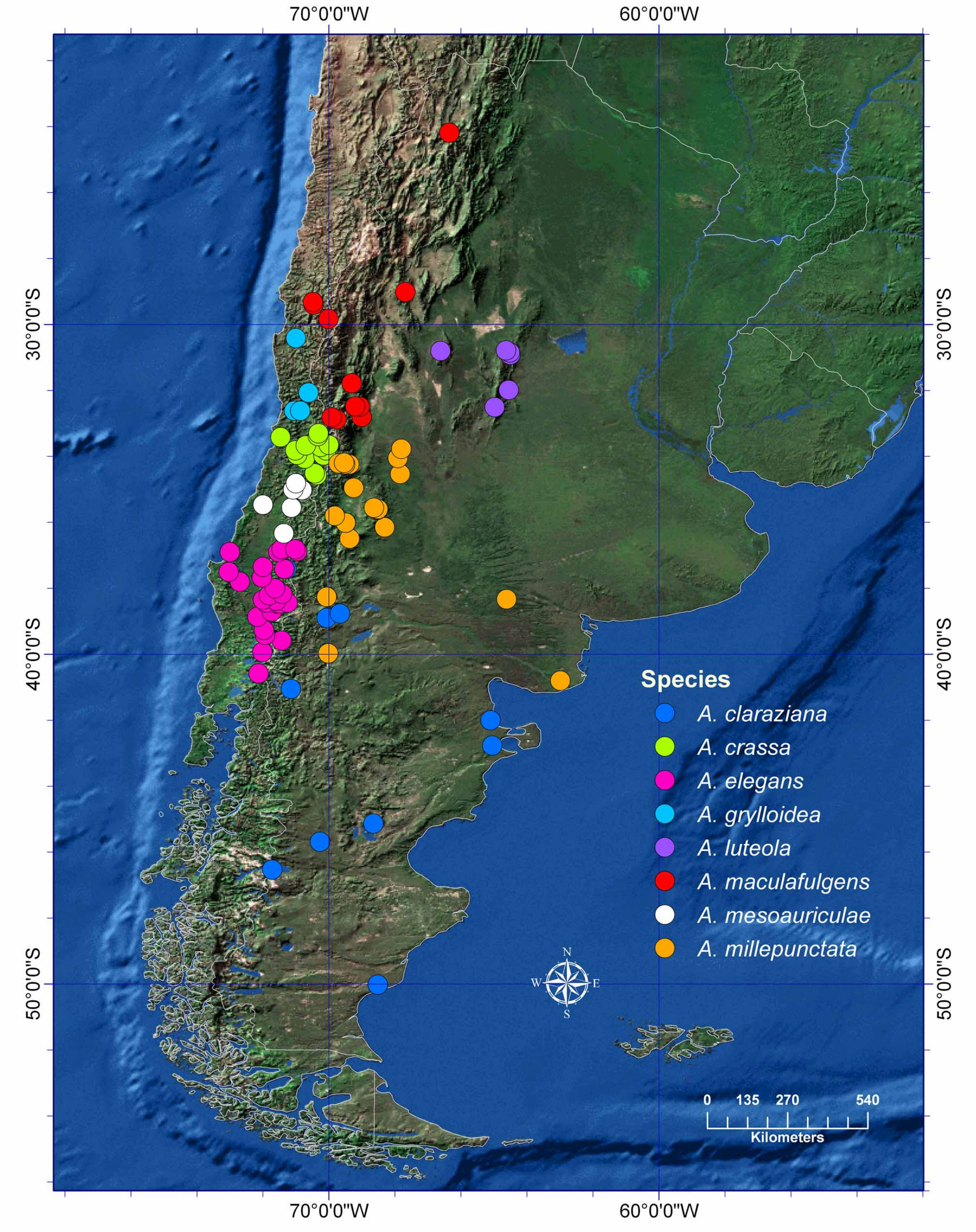


FIGURE 1. Distribution map of the species of the genus.

Phasmatida consists of two extant monophyletic groups: the genus *Timema* Scudder and the remaining species of Phasmida termed the Euphasmida. These two clades diverged at least 50 million years ago (Tilgner 2002) and are phenotypically distinct (Tilgner *et al.* 1999). Bradler (2000) and Bradler *et al.* (2003) hypothesized that the endemic South American genus *Agathemera* is the sister group to the remainder of

Euphasmida. This hypothesis was falsified by Tilgner (2002) who proposed that the subfamily Aschiphasmatinae represents a monophyletic sister group to the remainder of the order. Tilgner (2002) considers *Agathemera* as the most basal clade in the crown group of Euphasmida. Tilgner (2002) was not able to resolve the relationships of the crown group with much certainty. Whiting *et al*. (2003) presented a phylogeny of Euphasmida as part of their hypothesis about wing evolution, but Aschiphasmatinae were not included. Nevertheless, Zompro (2004) subdivided the Phasmatodea into the suborders Agathemerodea with the single genus *Agathemera* Stal, 1875 and the Verophasmatodea, which contain all other recent phasmids and the extinct Archipseudophasmatidae. In summary, according to Bradler *et al.* (2003), Zompro (2004, 2005) and Klug and Bradler (2006), the controversial assumptions on basal splitting events in the Euphasmatodea and the position of *Agathemera* highlight the need for future investigations of phasmid phylogeny.

The genus *Agathemera* presents some of the defensive mechanisms that are displayed by the remaining Phasmida. The species of *Agathemera* are cryptic but a few are aposematically coloured. One aspect of defence that has been studied is a tear gas-like defence spray produced and discharged by a pair of prothoracic exocrine glands (Camousseight 1995). These sprays are effective at deterring both vertebrate and invertebrate predators (Eisner *et al.* 1997). Other defence employed by Phasmida and that also appears in *Agathemera* is purposeful limb loss (i.e. autotomy) (Tilgner 2002).

Males and females are always dimorphic for size and may look dissimilar. Also, size and coloration varies dramatically between individuals of a species, but the details of body morphology, e.g., mouthparts, thoracic structure, male or female genitalia are usually homogenous (Camousseight per. com.). All species of *Agathemera* have nocturnal habits and hide under stones or leaf litter during the day (Camousseight 1995).

Copulation in *Agathemera* involves the transfer of a spermatophore into the genital tract of the female by eversion of balloon-like male copulatory organ (Tilgner 2002). Coupling before and after mating is usually brief, but in few species the male rides on the back of the female and remains coupled by attaching a sclerotized hook-like structure, termed the vomer, into a pregenital pouch located on the female abdomen (Tilgner 2002).

The main objective of this study was to carry out a cladistic analysis based on adult and immature morphological characters belonging to both sexes, a biogeographical analysis to reconstruct the biogeographic history of the genus, and an environmental niche analysis to determine the potential distribution of the species and to find the most important variables that explain its environmental plasticity.

Material and methods

Taxa

The ingroup terminal taxa are the eight known species of *Agathemera*: *A. crassa* (Blanchard), *A. maculafulgens* Camousseight, *A. grylloidea* (Westwood), *A. millepunctata* Redtenbacher, *A. elegans* (Philippi), *A. luteola* Camousseight, *A. claraziana* (Saussure), and *A. mesoauriculae* Camousseight. *Xeropsis crassicornis* (Philippi) and *Spinonemia chilensis* (Westwood) (Phasmatodea: Pseudophasmatidae) were used as outgroups. All included species and their geographical distributions are listed in Appendix 1.

The material used in this study is deposited in the following museums:

IADIZA	Colección Entomológica, Instituto Argentino de Investigaciones de las Zonas Áridas, Mendoza, Argentina.
MNNC	Museo Nacional de Historia Natural, Santiago, Chile.
UCCC	Museo de Zoología de la Universidad de Concepción, Concepción, Chile.
IMLA	Fundación e Instituto Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina.
MACN	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina.

Specimen preparation and study

All characters were studied in at least five adults per species (typically, up to 10–20 individuals per species with an equal proportion of sexes analyzed for external characters). In the case of variable species, different phenotypes were also examined. The material studied was dried, pinned specimens. For genitalic examination, the abdomen was removed from a dry specimen and heated in 10% (OH) K for 10 to 15 minutes, neutralized with acidulated water, and then transferred to glycerine. The post abdominal structures were separated from the rest of the abdomen. Examination of genitalic structures was done using a compound microscope. After examination, the terminalia and the rest of the abdomen were placed in glycerine in a plastic microvial and pinned directly under the specimen.

Characters and character states in the phylogenetic analysis

We coded a total of 25 characters that belong to the male and female adult stage and to the egg structure: from the adult head and its appendages (4), thorax (9), legs (1), abdominal sclerites (1), female terminalia (ovipositor) (3), male terminalia (4) and egg (3). See data matrix (Appendix 2).

Morphological terminology follows Camousseight (1995, 2005), where male and female genitalia are thoroughly explained and illustrated. The distribution of states in the terminal taxa is indicated in the data matrix in Appendix 2.

Phylogenetic analysis

Characters were treated as unordered and inapplicable characters were coded as “–“, whereas unknown character states were coded as “?” (Strong and Lipscomb 1999). We used the program TNT 1.1 (Goloboff *et al.* 2003) to search for optimal trees using the implicit enumeration option. To estimate group support we used Jacknife and Bootstrap, using 500 replicates (p = 0.33). Characters in the text are referred to as numbers with states as superscript (i.e. 25^6)

Characters

Adults:

Head

0. *Head in dorsal area*: (0) concave; (1) slightly depressed; (2) strongly depressed forming a pentagon.
1. *Colour pattern of dorsal area of the head*: (0) absent; (1) present.
2. *Head puncturation*: (0) absent; (1) strongly puncturated; (2) weakly puncturated.
3. *Dorsal conical tubercles in medial area of the head*: (0) present; (1) absent.

Thorax

4. *Shape of prothorax*: (0) 2x as long as broad; (1) sub quadrate; (2) pyramidal; (3) transversally rectangular.
5. *Thorax puncturation*: (0) absent; (1) weakly puncturated; (2) strongly puncturated.
6. *Shape of mesothorax*: (0) more than 4x as long as broad; (1) sub quadrate; (2) transversally rectangular.
7. *Mesonotal expansions*: (0) absent; (1) present.
8. *Internal margin of mesonotal expansions*: (0) separated; (1) joint.
9. *Length of mesonotal expansions with respect to length of metathorax*: (0) entirely covering metathorax ; (1) covering 1/3 of metatorax; (2) covering less than 1/3.
10. *Internal margin of mesonotal expansions*: (1) swollen, lip-shaped; (2) not swollen.
11. *Colour of metathorax*: (0) as remaining; (1) red or yellow.
12. *Inter-segmental colour*: (0) absent; (1) present.

Legs

13. *Leg shape*: (0) leaf shaped; (1) flattened; (2) cylindrical.

Abdomen

14. *Colour patterns*: (0) absent; (1) with darker longitudinal vitta; (2) coloured at posterior apex of each tergite; (3) with small circular markings.

Ovipositor

15. *Gonapofisis (ovipositor valves)*: (0) present; (1) absent.
16. *Spermathecal duct*: (0) short; (1) long.
17. *Shape of spermathecal capsule*: (0) spherical; (1) submoniliform.

Male genitalia

18. *Shape of vomer*: (0) trident; (1) spatulate bearing small teeth on apical margin; (2) semicircular with a central projection.
19. Shape of *central fixed sclerite*: (0) broad, concave, and strongly sclerotized; (1) broad, convex, and strongly sclerotized; (2) thin, weakly sclerotized.
20. *Length of mobile sclerite*: (0) as long as *fixed sclerite*; (1) longer than *fixed sclerite*.
21. *Shape of structure "x"*: (0) posterior arms longer than anterior; (1) anterior and posterior arms of equal length.

Eggs:

22. *Surface of operculum*: (0) smooth; (1) sculptured; (2) puncturated .
23. *Surface of chorion*: (0) smooth; (1) sculptured; (2) smooth except for puncturations on margin of micropylar lamina.
24. *Micropylar lamina*: (0) broad; (1) thin.

Biogeographical analysis

For the biogeographic analysis we used the program DIVA 1.1 (Ronquist 1996, 1997). We replaced the name of each taxon by the biogeographic area that it occupies (Appendix 1). The distribution of each species was based on Camousseight (1995, 2005) and on distributional data of specimens collected at the IADIZA. The areas of endemism to which each species corresponds was estimated from Cabrera and Willink (1980), Roig-Juñent *et al*. (2001), Roig-Juñent and Flores (2001) and Morrone (2001). A total of two hundred and fifty six specimens were considered.

Environmental niche models (ENM) and GIS data layers.

The Environmental Niche Models (ENM) were constructed using Maxent 3.2 software (Phillips & Dudik, 2008). Maxent is a maximum-likelihood method that generates the probability distribution over the pixels in a grid of the modelling area. This software was used because it has demonstrated to perform particularly well in a recent comparison of many ENM construction methods (Elith *et al*. 2006; Wisz *et al*. 2008). This method produces continuously varying, nonnegative suitability scores for each cell in a specified geographic region. We run 50 models using the settings as default, leaving 30% of the records to test the model, and selecting a random seed to change the initial conditions at each run. The resulting suitability models were averaged and only one model is presented here as a consensus. In most cases, species had a lower number of unique records, such as *A. grylloidea* (n=4), *A. luteola* (n=6), *A. mesoauriculae* (n=6), *A. claraziana* (n=10), and *A.maculafulgens* (n=11). For these species in which 70% of the records left for the training models are less than 10 data points, we used the methodology described in Pearson *et al*. (2007), in which each model is built using n-1 records, leaving-one-out at each run. Models were built using 37 environmental variables. From these, 19 were bioclimatic variables, and four were the annual mean values of precipitation, minimum temperature, maximum temperature, and mean temperature (Hijmans *et al*. 2005). Other variables used were: Topography, Frost days, Sunny days, NDVI (Normalized Vegetation Index for the 2007 year), CTI (Compound Topographic Index), Slope, and Aspect (Hydro1k data, http://www.usgs.gov), Altitude (GLOBE project, NOAA, http://www.ngdc.noaa.gov/mgg/topo/globe.html), categories of Land Cover, percentage of bare soil, herbs, and trees cover (Global Land Cover Facility, http://www.landcover.org), and FAO Categories of soils (Global Ecosystems Database Project).

Niche overlap and Identity test

In order to estimate the actual environmental niche overlap among *Agathemera* species, we conducted two analysis using ENM Tools (http://enmtools.com/). The first analysis allows us to measure similarity between predictions of habitat suitability between pair of species. The program calculates a new metric of niche overlap, the statistic *I*, proposed by Warren *et al*. (2008). The second analysis, named Identity test by Warren *et al*. (2008), allows us to test whether two species have statistically significant ecological differences in the ENM that they produce. The program randomizes the identity of occurrence points, while keeping sample sizes constant. We run 100 times this test for each pair of species, obtaining a distribution of niche overlap values that can be used to compare the measured overlap from the first analysis (See Warren *et al*. 2008 for details).

In addition, as another measure of overlapping among geographic distribution of *Agathemera* species, we calculated the distance in kilometres between any pair of data points. To this, we used the Geographic Distance Matrix Generator (http://biodiversityinformatics.amnh.org/open_source/gdmg/index.php) and found the minimum distance among data points for each species pair.

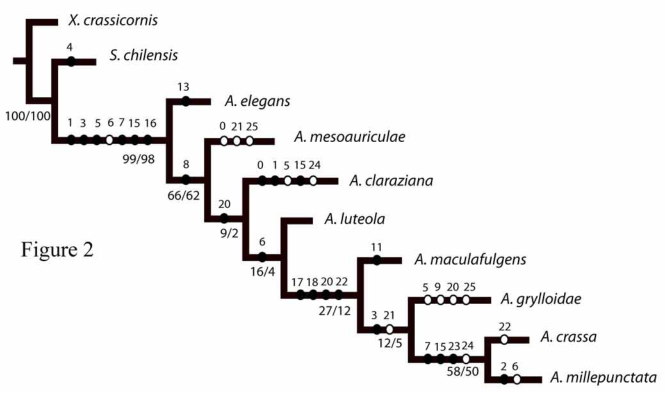


Figure 2

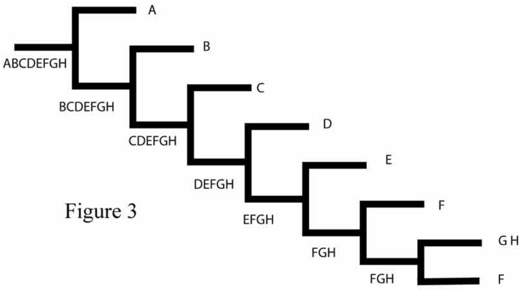


Figure 3

FIGURE 2. Tree obtained using implicit enumeration. Jacknife and, Bootstrap values are shown for each node. Apormophies (exclusive in black and non exclusive in white) are also indicated at each and corresponding character state.

FIGURE 3. Tree with the optimal reconstruction obtained with the DIVA analysis.

Results

One tree of 51 steps was obtained (Fig. 2). This tree supports the monophyly of the genus. Seven characters were found to be synapomorphies of the genus (Fig. 2). The species *A. elegans* and *A. mesoauriculae* distributed in southern Chile are in the base of the cladogram and they are the sister group to both the Argentinian (*A. claraziana, A. luteola*, *A. maculafulgens* and *A. millepunctata*) and the Chilean species (*A. grylloidea* and *A. crassa*).

The Biogeographic analysis using DIVA 1.1 found 1 optimal reconstruction (Fig. 3). The optimal reconstructions involves the occurrence of a vicariant event at each node, each with a frequency = 1, except for the separation of F (Mountainous region of the Central Valley of Chile) from G (Monte) and H (Payunia) that occurred with a frequency = 2, only one dispersion was recorded in this reconstruction.

ENM's showed that the soil variable was important for all eight species, varying their contribution to model building from 13% in *A. mesoauriculae* to 50% in *A. elegans* (Fig. 4). There were differences in the soil categories selected in building the species' ENM's. Species models responded to two to five soil categories, being *A. millepunctata* the species in which its ENM selected more soil categories (Table 1). Similarly, a same category of soil was important to ENM's for one to five species, being Lithosols the most common soil type selected by the models of five species (Table 1). Bioclim 18 (Precipitation of Warmest Quarter) and Bioclim 19 (Precipitation of Coldest Quarter) were also important in predicting distribution of some species (Fig. 4). Bioclim 18 were important for *A. grylloidea* (29.1%), *A. mesoauriculae/* (23.7%) and *A. crassa* (21.3%), while Bioclim 19 were an important variable for *A. elegans* (31.3%), *A. mesoauriculae* (23.7%), *A. crassa* (12.4%), and *A. luteola* (11.9%) (Fig. 4). According to the models, two species have large geographic distribution covering almost all the Patagonian area. These species are *A. claraziana* restricted to the southern part of Patagonia (Fig. 5), and *A. millepunctata* in the northern part of Patagonia, with the highest probability in the Payunia area (Fig 6). The six remaining species showed a more restricted geographic distribution (Figs. 7–12), although the lower number of unique records can affect the performance of models. The most distinctive species in geographic distribution is *A. luteola* (Fig. 7), restricted to the central mountainous area of Argentina, isolated from Andean mountains by large arid lowlands. The ENM for this species (*A. luteola*) predict its presence at the easternmost altitudes of Argentina, from Córdoba province (34° S) to Jujuy province and Bolivia (23° S) (Fig. 7).

Distance among geographic data points measured as the minimum distance between any pair of localities' species, ranges between less than 6 km in the *A. elegans*-*A. claraziana* comparison, to almost 800 km in the *A. claraziana*-*A. luteola* comparison. Results from environmental niche overlap analysis showed that they were lower than expected by chance in all comparisons between pairs of species (Table 2). The highest value of overlap was recorded between *A. crassa* and *A. grylloidea* (I = 0.64) and this value was statistically not significant than expected (P = 0.37) (Table 2). From 28 comparisons, only five showed no statistical difference with the expected estimates of overlapping (Table 2).

TABLE 1. Main FAO soil categories (taken from Global Ecosystems Database Project) selected for each species of *Agathemera* in the ENM analysis.

	Dystric Cambisols	Eutric Cambisols	Haplic Kastanozems	Luvic Kastanozems	Chromic Luvisols	Humic Andosols	Haplic Xerosols	Haplic Yermosols	Eutric Regosols	Eutric Fluvisols	Lithosols	Species frequency
A. elegans	X					X						2
A. mesoauriculae										X	X	2
A. claraziana		X		X		X		X	X			5
A. luteola			X				X			X		3
A. maculafulgens									X		X	2
A. grylloidea					X						X	2
A. crassa					X					X	X	3
A. millepunctata							X	X	X	X	X	5
Soil type frequency	1	1	1	1	2	2	2	2	3	4	5	

TABLE 2. Niche overlap among *Agathemera* species, using the *I* metric from Warren et al. (2008). Figures are: observed *I*/expected *I*. Expected value were calculated from 100 randomized runs using ENMTools (*sensu* Warren et al. 2008). In parentheses is the probability of similitude in niche equivalency between pair of species.

Species pairs	*A. elegans*	*A. mesoauriculae*	*A. claraziana*	*A. luteola*	*A. maculafulgens*	*A. grylloidea*	*A. millepunctata*
A. mesoauriculae	0.49/0.73 (> 0.01)						
A. claraziana	0.46/0.72 (> 0.01)	0.38/0.73 (> 0.01)					
A. luteola	0.32/0.64 (> 0.01)	0.30/0.73 (> 0.01)	0.37/0.67 (> 0.01)				
A. maculafulgens	0.37/0.77 (> 0.01)	0.29/0.69 (> 0.01)	0.44/0.69 (> 0.01)	0.35/0.65 (> 0.01)			
A. grylloidea	0.35/0.68 (> 0.01)	0.34/0.75 (> 0.01)	0.46/0.66 (> 0.01)	0.33/0.73 (> 0.01)	0.44/0.66 (0.09)		
A. crassa	0.39/0.81 (> 0.01)	0.43/0.71 (> 0.01)	0.52/0.73 (> 0.01)	0.31/0.65 (> 0.01)	0.43/0.76 (> 0.01)	0.64/0.71 (0.37)	
A. millepunctata	0.38/0.72 (> 0.01)	0.31/0.55 (> 0.01)	0.55/0.58 (0.17)	0.42/0.55 (0.03)	0.49/0.63 (0.47)	0.39/0.52 (0.17)	0.44/0.66 (> 0.01)

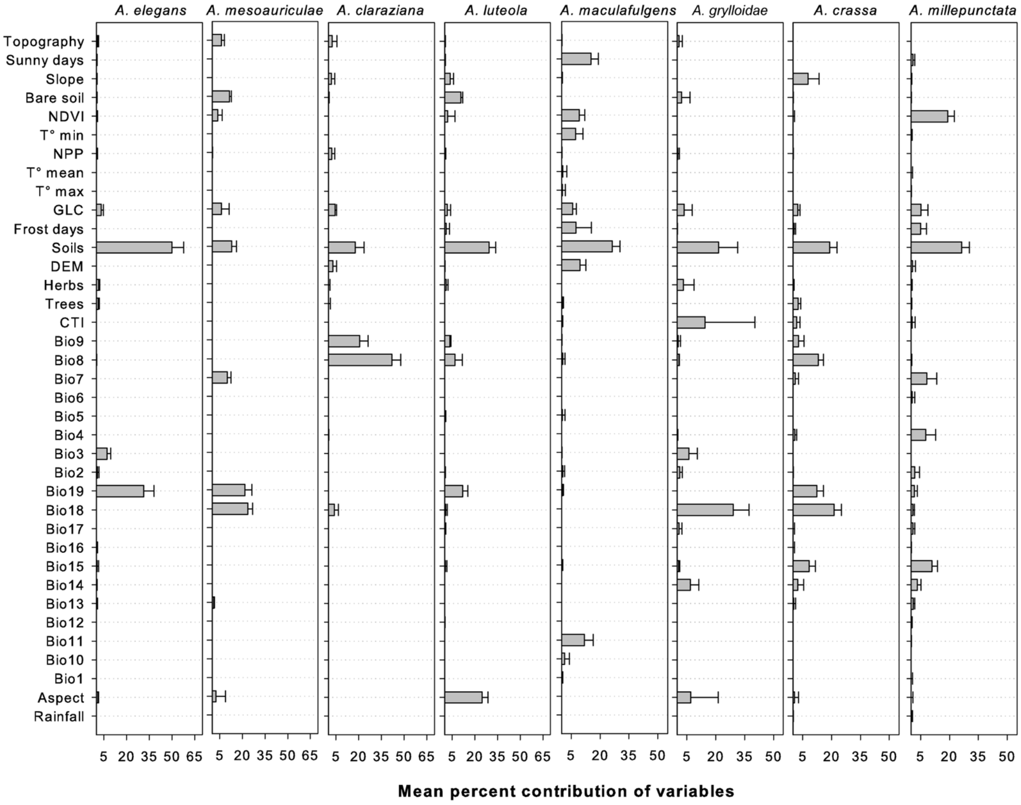


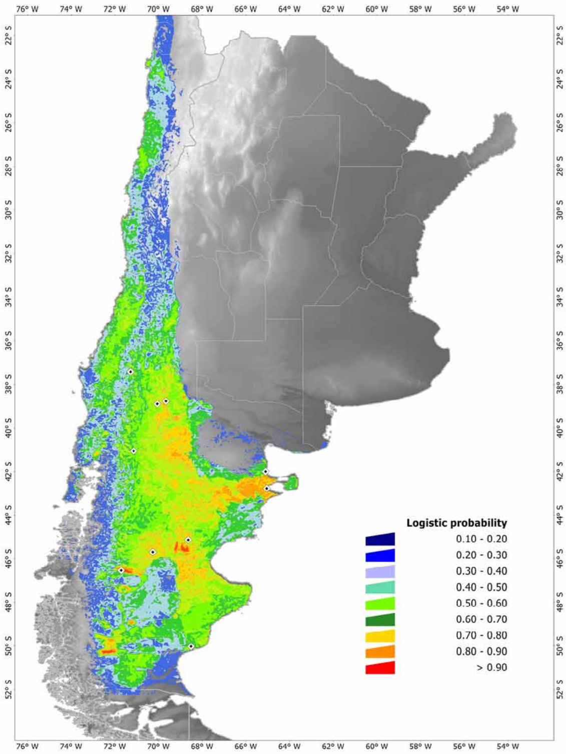
FIGURE 4. Mean percent contribution of variables for each species in the ENM analysis.

Discussion

The tree obtained in the cladistic analysis was fully resolved and the genus *Agathemera* was recovered as a monophyletic unit. The species of *Agathemera* formed a single comb (Fig. 2). Seven synapomorphies support *Agathemera*: 0^1 head slightly depressed in dorsal area; 2^2 head weakly punctuated; 4^1 prothorax sub quadrate; 5^2 thorax strongly punctuated; 6^1 mesothorax subquadrate; 14^2 abdomen coloured at posterior apex of each tergite; 15^1 Gonapofisis (ovipositor valves) absent.

The absence of mesonotal expansions (7^0) separates *A. elegans* from all the remaining species of *Agathemera*, which bears these expansions. It could be interpreted that the acquisition of these structures are a synapomorphy of all the species of *Agathemera* that reverted in *A. elegans.* The following node separates *A. mesoauriculae* from the remaining species of the genus, on the basis of the shape of the fixed sclerite of the male genitalia that is broad, convex and strongly sclerotized (19^0) in *A. elegans* and *A. mesoauriculae*, but in the remaining species it is thin a weakly sclerotized (19^2). This state of the fixed sclerite of the male genitalia varies in the apical species of *Agathemera* (*A. maculafugens*, *A. crassa* and *A. millepuntata*), in which it is broad, concave and strongly sclerotized, and in *A grylloidea* it reverts to broad, convex and strongly sclerotized.

Characters of the female spermathecae provided strong phylogenetical signal in the apical area of the cladogram: the short spermathecal duct 16^0, and the spherical spermathecal capsule 17^0 constitute apomorphies of *A. grylloidea*, *A. crassa* and *A. millepuntata* (Fig. 2). Furthermore, these three species are separated by characters referred to the sculpturing of the egg surface. The surface of the eggs chorion is sculptured 23^1 in all species of *Agathemera*, except for the apical species *A. crassa*, *A. claraziana* and *millepuntata* in which it is smooth.



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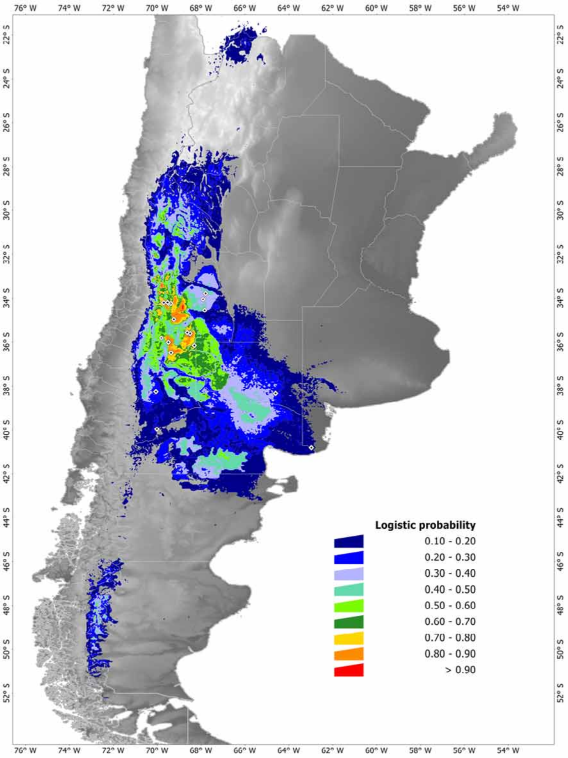

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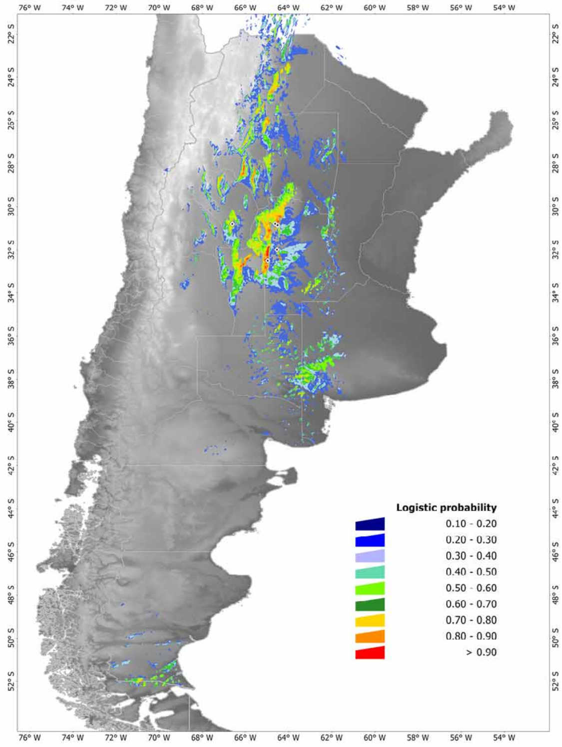

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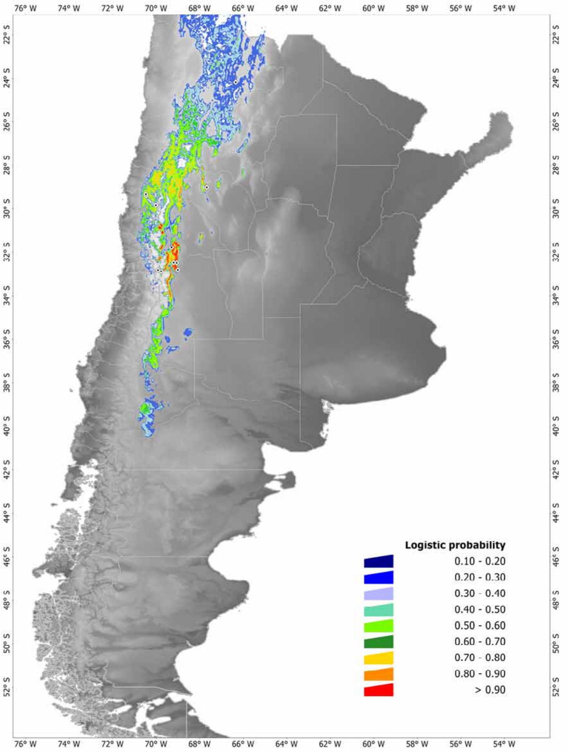
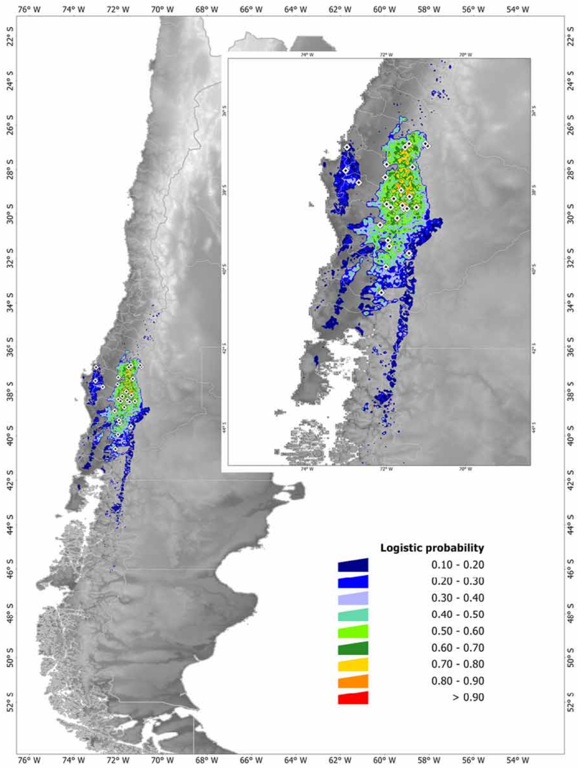


Figure 8

FIGURE 5. Potential geographic distribution estimated for *A. claraziana*.
FIGURE 6. Potential geographic distribution estimated for *A. millepunctata*.
FIGURE 7. Potential geographic distribution estimated for *A. luteola*.
FIGURE 8. Potential geographic distribution estimated for *A. maculafulgens*.



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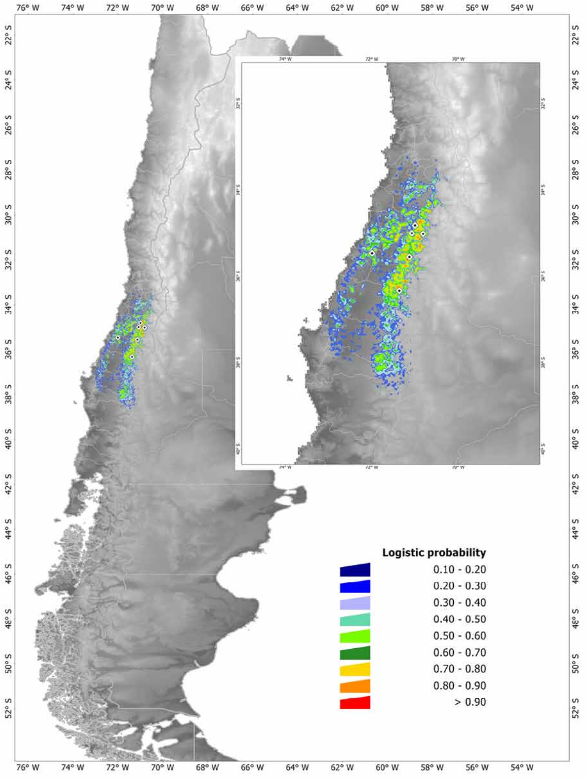

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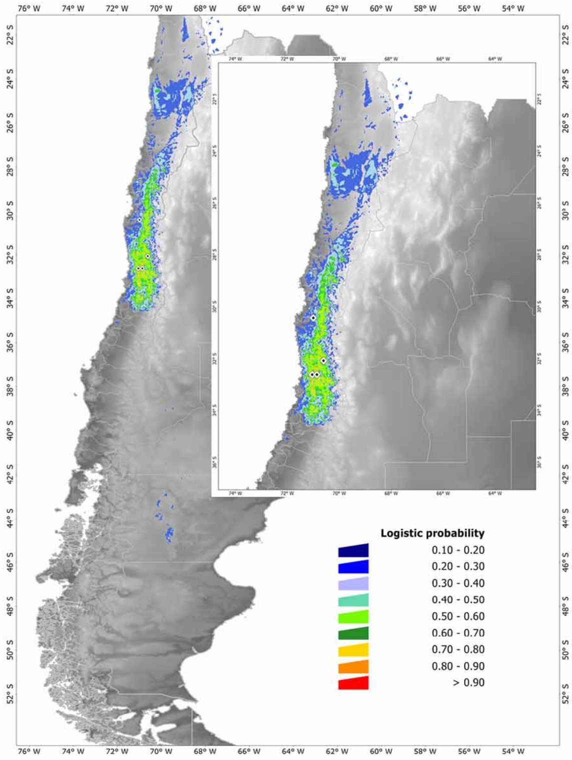

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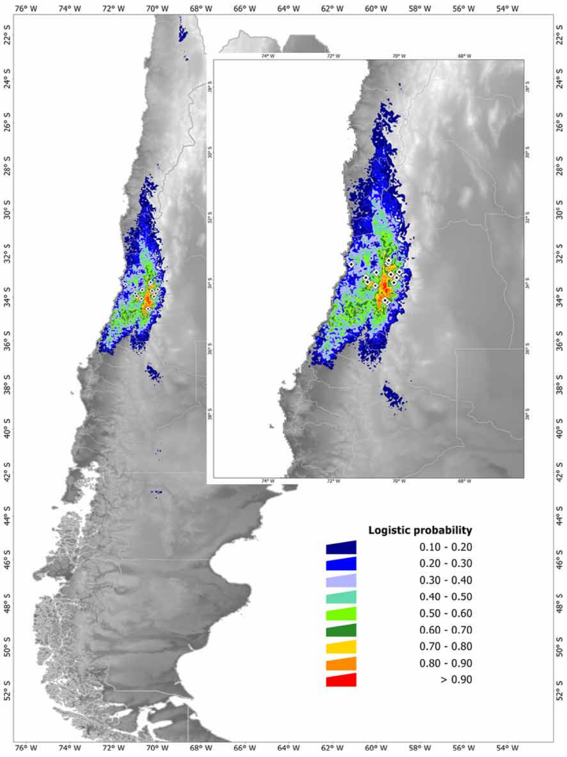

Figure 12

FIGURE 9. Potential geographic distribution estimated for *A. elegans*.

FIGURE 10. Potential geographic distribution estimated for *A. mesoauriculae*.

FIGURE 11. Potential geographic distribution estimated for *A. grylloidea*.

FIGURE 12. Potential geographic distribution estimated for *A. crassa*.

Although the monophyly of the genus *Agathemera* was recovered in this analysis, in order to further test the monophyly of *Agathemera* and to obtain a larger list of synapomorphies, a larger outgroup taxon sampling will be needed in future studies.

According to the results obtained in the biogeographical analysis performed with the program DIVA 1.1, the ancestral area of the genus *Agathemera* is a continuous area that contains all the areas included in the analysis (Fig. 3). At each node an event of vicariance with a frequency = 1 occurs, except for the separation of F (Mountainous region of the Central Valley of Chile) from G (Monte) and H (Payunia) that occurred with a frequency = 2. The area cladogram shows a clear separation of the southern species (*A. elegans*, *A. mesoauriculae* and *A. claraziana*) from the northern species *A. luteola*, *A. maculafulgens*, *A. grylloidea*, *A. crassa* and *A. millepunctata*) (Fig. 3).

The only vicariance that can be related to an historical event is that of the last node: the separation of the species belonging to the central region of Chile from those from Argentina may be due to the uplifting of the Andes, which has had a large impact in the biogeographical history of numerous arthropod groups of South America (Roig-Juñent *et al*. 2006). Nevertheless the uplifting of the Andes can not be recognized as a single event, but as a series of events where different areas related to the uplifting of the mountains have become separate as the mountains became higher.

Therefore the two most apical nodes of the *Agathemera* tree could have been affected by the last period of the uplifting of the Andes. We consider that the Andes mountain ridge became a barrier for this genus when its altitude surpassed 4000 m.a.s.l, (*A. millepunctata* can be found at this altitude). If the apical nodes of the *Agathemera* tree are situated in the final period of the uplifting of the Andes Mountain ridge, this would indicate a very old age for the genus (Fig. 3). Prior to the uplifting of the Andes during the end of the Cretaceous and beginning of the Cenozoic, the only vicariance events that could have affected of South America were marine ingressions and climatic changes that resulted in large latitudinal movements of the biota (Ortiz-Jaureguizar & Cladera 2006). Nevertheless, this event can not be recognized in the *Agathemera* tree because it affected the Caatinga region, in which the genus is absent.

The species *A. elegans*, *A. mesoauriculae* and *A. claraziana* that appear at the base of the tree are distributed in the southern regions of Chile (Valdivian Forest and Maule Region) and in the Patagonian Steppe, while the remaining species of the clade have a northern distribution (Fig. 1). The northern species are distributed in highlands such as the mountain ridges of Pampa de Achala in the province of Cordoba (Argentina), Puna and high altitude grasslands of Argentina (up to the province of Jujuy) or the Mountainous region of the central Valley of Chile.

The pattern of distribution displayed by the species of the genus *Agathemera* is common for many groups of insects of Patagonian origin (Roig-Juñent *et al*. 2008). *Agathemera* is found in biogeographical provinces that belong to the Andino domain (Morrone 2001), or in biogeographical provinces considered as the South American transition zone (Morrone 2004) such as Monte and Puna, and only one species can be found beyond these provinces: *A. luteola* that occupies mountainous environments where numerous organisms with Patagonian origin can be found.

It is common for species of Patagonian origin that are distributed in septentrional areas to occupy mountainous environments, such as the species of *Agathemera* that are found north of parallel 32º S: *A. maculafulgens*, *A. grylloidea* and *A. luteola* (Fig. 1).

Camousseight (1995) refers to this pattern in his description of the distribution of the Chilean species of *Agathemera*. In Chile the species of *Agathemera* are located in mountainous areas that belong to the Andes and Coastal Mountain ridges in the north, and that the southern species are located in lower regions (which also coincides with the lower altitudes of the mountain ridges towards the south). Camousseight (1995) does not refer to this altitudinal pattern in the description of the Argentinean species; nevertheless the distributional pattern of the species of *Agathemera* (1995) resembles that proposed by Noonan (1990, 1992) for the mountain carabid beetles of North America. This author proposed a pattern called "*northern lowland plus southern mountains*" that involves species whose distribution is in lowlands in the north and extends southward along mountains into regions with warmer or more xeric conditions. For the southern hemisphere, this pattern can be called "*southern lowland plus northern mountains*" (SLNM) (Roig-Juñent *et al*. 2008).

If this pattern is considered to explain the distribution of the extant species of *Agathemera*, a negative correlation between altitude and latitude should be expected. But this correlation was not found in this study because all the species of *Agathemera* are distributed in a wide range of altitudes (ranging from 0 to 2000) at same latitudes, especially south of parallel 32ºS. However, the potential suitable area of distribution of northern species likes *A. luteola* (Fig. 7), *A. maculafulgens* (Fig. 8), and *A. millepunctata* (Fig. 6), goes north following the central Argentinean Mountains (mountains that do not belong to the Andean ridge) and the Andes mountains up to 20° S.

Noonan (1992) described isolation as one of the main factors that promote the evolution of new taxa. He explained that mesic refuges in the mountains during periods of drought may have been important centers for diversification of species. He proposed a model in which the new species of carabids evolved from isolated populations. These populations were previously part of an ancestral species that was widely distributed in the lowlands before the montane-vicariance cycle began. For the beetle subgenus *Baripus (Cardiophthalmus)* Roig-Juñent *et al*. (2008) hypothesized that the mountains may have acted as refuge and, later, as centres from which species evolved. Nevertheless, for *Agathemera*, we should expect the southern species to be distributed and restricted to lowlands and the northern species to be distributed in Northern highlands, but as mentioned before this relationship between altitude and latitude was not recovered in our analyses. It could be assumed that the mountain ridges acted as centres of diversification for the species of the genus *Agathemera*, as may have occurred with carabid beetles. In this group of insects (carabid beetles) different species are restricted to high altitudes of different mountain ridges and form distinct populations from other carabid beetles situated at lower altitudes, but this does not occur with the species of *Agathemera* that generally occupy a relatively broad distributional range of a given region. This can be visualized in the potential geographic distribution, since the ENM's of most species occupies large continuous areas that embrace high mountains with very different environmental conditions.

In spite of this relatively broad distribution, the geographic ranges of the species of *Agathemera* have almost no overlap. The highest overlap in the ENM's was observed between *A. millepunctata* (Fig. 6) and *A. claraziana* (Fig. 5), the species with the broadest geographic distribution. However, the core areas (e.g., those areas with highest probability presence) show very low overlap, a pattern also observed in the remaining species. Although some species have been found as close as less than 6km of distance, the coexistence of two or more species has never been registered. This is reinforced by the results obtained using niche identity analysis. All pair of species had lower niche similarity than expected by chance. From all 28 possible combinations of pair of species, only five pairs showed no difference in niche similarity, indicating that each species had different niche requirements. The variable that most contribute to ENM's was soil type, being with land cover the categorical variables that were selected by all eight species ENM's. These two variables could be indicating that the *Agathemera* species have some fidelity to discrete environments (*e.g.* forests, steppes, grasslands) and, if two species respond to different categories of these variables that can be contiguous, to explain why we can not find specimens from different species in a same locality. As an example, *A. claraziana* (Fig. 5) and *A. elegans* (Fig. 9) showed one of the highest expected niche overlap (0.72), the closest minimum distance between localities of different species (5.9 km), and spite of this they had a low niche overlap using 37 environmental variables (0.46).

The fact that this genus is a very ancient one is in concert with the environmental niche differentiation of their species, which can be viewed as a result of an ancient evolution. There is no evidence of a relationship between niche overlap and species position in the phylogenetic tree, as can be expected when species have diverged recently. However, in the present study we found a clear morphological and environmental species separation. More data will be needed to understand the history of this genus; especially molecular data could be useful to explain time divergence among species. Also, new geographic records will be of great utility to better understand the relationships of species with their environment, and to improve the delimitation of their geographic distributions.

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APPENDIX 1. Species of *Agathemera* included in the cladistic analysis and their main geographical distribution according to Camousseight (1995; 2005); Cabrera and Willink (1980); Roig-Juñent *et al*. (2001); Roig-Juñent and Flores (2001) and Morrone (2001) .

Species	Geographical distribution	Reference
A. elegans (Philippi, 1863)	Valvian Forest (Chile)	A
A. mesoauriculae Camousseight, 1995	Maule Region (Chile)	B
A. claraziana (Saussure, 1868)	Patagonian Steppe (Argentina)	C
A. luteola Camousseight, 2005	Pampa de Achala, Sierras of Buenos Aires (Argentina)	D
A. maculafulgens Camousseight, 1995	Puna y and high altitude grasslands (Argentina)	E
A. crassa (Blanchard, 1851)	Mountainous region of the central region (Chile)	F
A. grylloidea (Westwood, 1859)	Mountainous region of the central region (Chile)	F
A. millepunctata Redtenbacher, 1906	Monte Austral, Payunia and high altitude grasslands of the province of Mendoza (Argentina)	G H

APPENDIX 2. Data matrix used in the phylogentic analysis. *Note*: polymorphic entries: a = (13), b = (12), c = (01), d = (23). Inaplicable data = "-" and missing data = "?".

Taxa	Characters		
		1	2
	123456789	0123456789	0123456
X.crassicornis	000010000	- - -00000??	0- - - - - -
S.chilensis	100000000	- - -00100??	1- - - - - -
A.crassa	110111121	1010021100	2000001
A.grilloidea	110112111	01 -0022100	2101110
A.mesoauriculae	01021a2b1	02 -0c22111	2100110
A.elegans	1102112b0	- - -012d111	2110221
A.claraziana	2202122b1	1010023111	2210101
A.maculafulgens	110211111	1121022100	2011???
A.luteola	110211111	1011022111	2210111
A.millepunctata	111111221	1010021100	2001001