



A revision of *Apteromantis* (Mantodea: Mantidae, Amelinae): A comprehensive approach to manage old taxonomic and conservation problems

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Abstract

The genus *Apteromantis* Werner, 1931 comprises two species of wingless mantids, the Iberian *A. aptera* (Fuente, 1894) and the North African *A. bolivari* (Werner, 1929). Although *A. aptera* and *A. bolivari* have been traditionally considered as separate and valid species, their external appearance is quite similar and no comprehensive taxonomic study has analyzed their morphological and genetic characteristics. This taxonomic uncertainty has important implications for conservation because *A. aptera* is considered an Iberian endemic and the only praying mantis protected by international laws. In this study, we apply a comprehensive approach, including quantitative morphological and molecular analyses, to shed new light on the taxonomic and conservation status of the genus *Apteromantis* and the putative species. We have found that the Iberian and North African specimens analyzed herein significantly differ in female head shape, male genitalia morphology and several other traits related to body size. Molecular data suggest the presence of two main lineages, with sequence divergence rates of approximately 4 %, which are within the range reported for other well defined insect species. Overall, this study supports that *A. aptera* and *A. bolivari* are valid species despite their ecological and morphological similarity and highlights the importance of comprehensive approaches to resolve old taxonomic and conservation problems.

Key words: Mantodea, *Apteromantis*, conservation, evolutionary significant units (ESUs), DNA barcoding, phylogeography

Introduction

The genus *Apteromantis* Werner, 1931 is composed of two species of wingless mantids: *Apteromantis aptera* (Fuente, 1894) distributed in central and south Spain and Portugal, and *Apteromantis bolivari* (Werner, 1929) distributed mostly in the Mediterranean part of Morocco and Algeria. Although the external morphology of these two species is extremely similar (Fig. 1), they have been traditionally considered as separate and valid species (Ehrmann 2002; Battiston *et al.* 2010; Otte *et al.* 2011). The geographical distribution and presumed isolation of these species is the main character used to separate the Iberian *A. aptera* from the North African *A. bolivari*. However, after the original description of these species, no detailed taxonomic study has analyzed the morphological and genetic traits justifying their distinctiveness. This taxonomic uncertainty has important applied implications because *A. aptera* is an Iberian endemic and it is the only mantis protected in Spain (OM. 13682, BOE n. 136, 1988) and by the European community (Annexes II and IV of Habitat Directive 92/43/CE). *Apteromantis aptera* has been also included in the Appendix II of the Bern Convention and in the IUCN red-list of threatened species with the status "Least Concern" (Battiston, in press). The protection status of *A. aptera* is mainly based on its small distribution range and the scarce abundance of its populations (Peinado & Mateos 1998; Pascual 2005; Pascual *et al.* 2008; Pascual 2012). The uncertainty of the status of this may modify the protection and conservation status of *A. aptera*.



FIGURE 1. Left: adult female of *A. bolivari* in Morocco (Fèz); right: adult female of *A. aptera* in Spain (Brunete); Photos by R. Battiston.

While *A. aptera* is quite well represented in museum collections and several new records have been published in recent years (e.g. López-Villalta 2009; Obregón & López 2009; Arizmendi *et al.* 2011; Obregón *et al.* 2013; Marabuto *et al.* 2014), less than 30 specimens of *A. bolivari* (all collected in the first half of the past century) are preserved in natural history museums (R. Battiston, unpublished data). This low number of available specimens dispersed in museums from several countries (Morocco, Spain, Germany, Austria, United Kingdom), together with the lack of fresh tissues required to extract DNA and perform molecular diagnostic analysis, limits the possibility of evaluating the taxonomic status of *A. aptera* and *A. bolivari*. The intricate synonymic history of these two recognized species has also played a role in this taxonomic uncertainty. Bolivar (1898) placed the species *A. aptera* in the genus *Ameles* Burmeister, 1838 and subgenus *Yersinia* Saussure, 1869. Kirby (1904) included all the European species of subgenus *Yersinia* under *Pseudoyersinia* Kirby, 1904. This is likely why Werner (1929) also placed *bolivari* under the genus *Pseudoyersinia*. In 1931, Werner described the genus *Apteromantis* using a specimen from Morocco: *A. bolivari*. However this was a very short note and he gave a more detailed description of the genus and the species *A. bolivari* within his faunistic treatment of Morocco (Werner 1932). We summarize the original descriptions and the synonymic history of both *A. aptera* and *A. bolivari* and the genus *Apteromantis* as follows:

Ameles aptera Fuente, 1884

Pseudoyersinia aptera in Kirby, 1904

Apteromantis aptera in Beier, 1935

Description by Fuente, 1884: “*Major viridis. Latitudo capitis dimidiam longitudinem pronoti haud attingens. Vertex concavus. Oculi conici, valde acuminati. Scutellum frontale supra lateribus rotundatum, medio obtuse angulatum. Pronotum latiusculum, marginibus integerrimis. Elitra nulla, ala nulla. Pedes postici breviter griseo-pilosi. Abdomen ♂ parallelum, ♀ latiusculum, depressiusculum*”.

Pseudoyersinia bolivari Werner, 1929

Apteromantis bolivari in Werner, 1932

Description by Werner, 1929: “♀ Fes, 8. V. Differt a *P. occidentalis* Bol. elytris et alis ♀ nullis, pronoto longiore cercis laminam subgenitalem multo superantibus. An nov gen.? Viridis aut flavescens, unicolor. Vertex distincte excavatus; Oculi mammillati, tuberculo spiniformi terminati. Scutellum frontale pentagonale, cristulis tribus obtusis instructum. Pronotum plusquam duplo longius quam in medio latius marginibus integris, sulco transverso distincte ante medium sito. Abdomen fusiforme. Lamina supraanalis triangularis, medio carinata; lamina subgenitalis elongata, triangularis, profunde incisa; cerci elongati, acuti, laminam subgenitalem multum superantes”.

Genus description:

Apteromantis n. g. für *Pseudoyersinia bolivari* in Werner, 1932

“Elytra et alae in ♂ et ♀ perfecte deficientes; lamina sub-genitalis triangularis, profunde incisa; cerci apicem abdominis multo superantes”.

The original descriptions transcribed above have been traditionally used by the different authors studying those species that conform to the same depiction: greenish colour, conical and acuminate eyes, elongate pronotum, absence of wings in both sexes, frontal shield pentagonal, obtuse and more or less rounded. Besides their distinct distribution, only two characters have been used to discriminate *A. bolivari* from *A. aptera*: 1) overall female size, being *A. bolivari* slightly larger than *A. aptera*; and 2) the shape of the female's vertex of the head, which is concave in *A. aptera* and excavated in *A. bolivari*. Battiston *et al.* (2010) recently proposed a preliminary diagnostic key based on comparative analysis of a number of individuals based on such character differentiation. In addition, the distributions of *A. aptera* and *A. bolivari* have also been used as a character to discriminate between both species, but a specimen with a *bolivari*-like morphology was collected in 1912 in Evora, Portugal (ZMUH). This suggests that the biogeographic character used as a discriminative trait may not be reliable. However, this old record might represent a mislabelled museum specimen as individuals exhibiting such morphology have not been collected again in the Iberian Peninsula, whereas *A. aptera* is frequently reported in Spain (Brenes-Redón 2003; Ruiz-Luque 2004; Cano-Villegas & Zafra de la Haza 2007; Lopez-Villalta 2009; Obregón & López 2009; Arizmendi *et al.* 2011; Obregón *et al.* 2013) and more recently in Portugal (Grosso-Silva & Soares-Vieira 2004; Boeiro *et al.* 2007; Marabuto *et al.* 2014).

The taxonomy and systematics of the subfamily Amelinae remains poorly defined. However, *Apteromantis*, exhibits a straightforward, autapomorphic character: absence of flight organs. Nevertheless, a detailed species-level comparative diagnosis for both *A. aptera* and *A. bolivari* has been lacking. In this paper, we confront the traditional hypothesis of two distinct species (i.e. *A. aptera* and *A. bolivari*) with the assumption that the two are synonyms resulting from poor taxonomic treatments and a historical misinterpretation of morphological characters that may prove to be insufficient for species discrimination. To test the validity of the species we consider a comprehensive approach, including quantitative morphological and molecular analysis, to shed new light on the taxonomic and conservation status of the genus *Apteromantis*.

Materials and Methods

Specimens examined

The material examined belongs to the following collections: British Museum of Natural History, London (BMNH); Museo Nacional de Ciencias Naturales, Madrid (MNCN); Naturhistorischen Museum Wien, Wien (NHMW); Zoologisches Museum Hamburg, Hamburg (ZMUH); and the senior author's collection. The following specimens were examined:

A. aptera: 16♀ 13♂ adults, 1 juvenile, 3 additional specimens of undetermined sex:

SPAIN: Alcalá de Guadaíra, VII 1961, Hopkins (BMNH), 1♀; Alcazar de San Juan, 2007, Cordero, 1juv; Brunete, VI 1936, Morales-Agacino, coll. Bolivar (MNCN), 1♀; Brunete, 19 VI 2011, coll. Battiston, 1♀1♂; Cañada de

Calatrava, 24 VIII 2009, Cordero, 1 ♀ (specimen collected as nymph and reared in captivity until adult phase); Cienvallejos Brunete VI 1927, Morales-Agacino, coll. Bolivar (MNCN), 6 ♂ 9 ♀; Ciudad Real, 13 V 2011, Cordero, 1 ♂; Los Barrios, 12 V 2011, coll. Battiston 2 ♂; Los Barrios, 15 V 2011, 2 ♂; Mora, 02 VI 2008, Cordero, 2 n.d.; Mora, 01 VII 2008, Cordero, 1 n.d.; Pozuelo de Calatrava, Fuente (MNCN), Cotype, 3 ♀, 1 ♂.

A. bolivari: 12 ♀ 6 ♂ adults:

MOROCCO: El Haseb, 22 V 1930, Nadig, NMW, 1 ♀; Fès, 25 V 1930 (BMNH), 1 ♀; Fès, 23 V 1930, coll. Werner (NMW), 1 ♂ 1 ♀; Fès Tag-Hat, 25 V 1930, Ebner, coll. Werner (NMW), 1 ♀ 1 ♂; Fès Zalagh, 23 V 1930, Ebner, coll. Ebner (NMW), 3 ♀; Fès Zalagh, 29-V-2011, Battiston, coll. Battiston, 3 ♂ 2 ♀; Fès Zalagh, 29-V-2011, Battiston, IREC 1 ♀, 2 oothecae; Fès Zalagh, 29-V-2011, Battiston, UM5A 1 ♀; Sidi Allal El Bahraoui, 31-V-2011, Battiston, coll. Battiston, 1 ♀;

Portugal: Evora, 1912 (ZMUH), 1 ♂.

Morphology

We performed Fourier shape analyses for the upper part of the head of females (*A. aptera*: $n = 6$; *A. bolivari*: $n = 9$) and male genitalia (*A. aptera*: $n = 3$; *A. bolivari*: $n = 5$). It should be noted that sample size was limited for these traits because several museum specimens were deformed or broken. We did not consider males for head shape analyses because this character has only been described for females (Battiston *et al.* 2010). Genitalia was extracted from males and prepared as described in Battiston *et al.* (2010). A camera coupled to a binocular stereomicroscope was used to photograph specimens. Each digital image was later computer-processed and converted into uniformly black shapes on white background. For head shape analyses, we focused on the vertex, the area included between the eyes, and the dorsal part of the eyes, all potential diagnostic characters qualitatively described by previous studies (see introduction). For genitalia, we focused on the distal margin of the hypophallus. This is generally a good diagnostic trait in many other mantids, particularly in the Amelinae group (Battiston & Fontana 2005). We performed Fourier analyses using SHAPE software (Iwata & Ukai 2002) to obtain shape related principal components that could discriminate between *A. aptera* and *A. bolivari* (see Battiston & Massa 2008). Finally, we analyzed differences between *A. aptera* and *A. bolivari* in the principal component scores obtained for each trait using one-way ANOVAs in SPSS 19.0.

Using different specimens, we also measured several linear morphometric traits that could be diagnostic, including distance between compound eyes at the apex of the tubercles, maximum length and width of pronotum, length and width of fore femora, length of hind femora and tibiae, and overall body size (measured from the vertex to the distal margin of the supra-anal plate). We took calibrated pictures of all morphometric traits considered for each studied specimen (*A. aptera*: six males and six females; *A. bolivari*: six males and six females) and the images were processed and measured using JMicroVision 1.2.7 (Roudit, 2006). Sample size was slightly lower (*A. aptera*: six males and six females; *A. bolivari*: five males and six females) for eye distance, length and width of fore femur, and hind tibia length because one of the studied museum specimens had broken legs and a deformed head. We analyzed differences in these morphological traits between *A. aptera* and *A. bolivari* performing general linear models (GLMs) that included species, sex and their interaction as fixed factors. GLMs were performed using SPSS 19.0.

Genetic analyses

We sequenced a fragment of the cytochrome oxidase subunit I (COI) gene to study the relationship and estimate the divergence time between *A. aptera* and *A. bolivari*. We used NucleoSpin Tissue (Macherey-Nagel, Düren, Germany) kits to extract and purify genomic DNA from a hind leg of each individual. We used primers LCO1490 (5' -GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5' -TAAACTTCAGGGTGACCAAAAAATCA-3') to amplify a 630 bp fragment of the COI gene (Folmer *et al.* 1994). Approximately 5 ng of template DNA was amplified in 25- μ L reaction volumes containing 1X reaction buffer (67 mM Tris-HCL, pH 8.3, 16 mM (NH₄)₂SO₄, 0.01 % Tween-20, EcoStart Reaction Buffer, Ecogen), 2 mM MgCl₂, 0.2 mM of each dNTP, 0.15 μ M of each primer and 0.1 U of *Taq* DNA EcoStart Polymerase (Ecogen). All reactions were carried out on a Mastercycler EppgradientS (Eppendorf, Hamburg, Germany) thermal cycler. The PCR programme used was 9 min denaturing at

95 °C followed by 40 cycles of 30 s at 94 °C, 45 s at 55 °C and 45 s at 72 °C, ending with a 10 min final elongation stage at 72 °C. PCR products were purified using NucleoSpin Extract II (Macherey-Nagel, Düren, Germany) kits and sequenced on an ABI 310 Genetic Analyser (Applied Biosystems, Foster City, USA). Sequences were edited and aligned using the program BIOEDIT (Hall, 1999). All sequences have been deposited in GenBank (accession numbers, *A. aptera*: JQ041758- JQ041761; *A. bolivari* JQ041757).

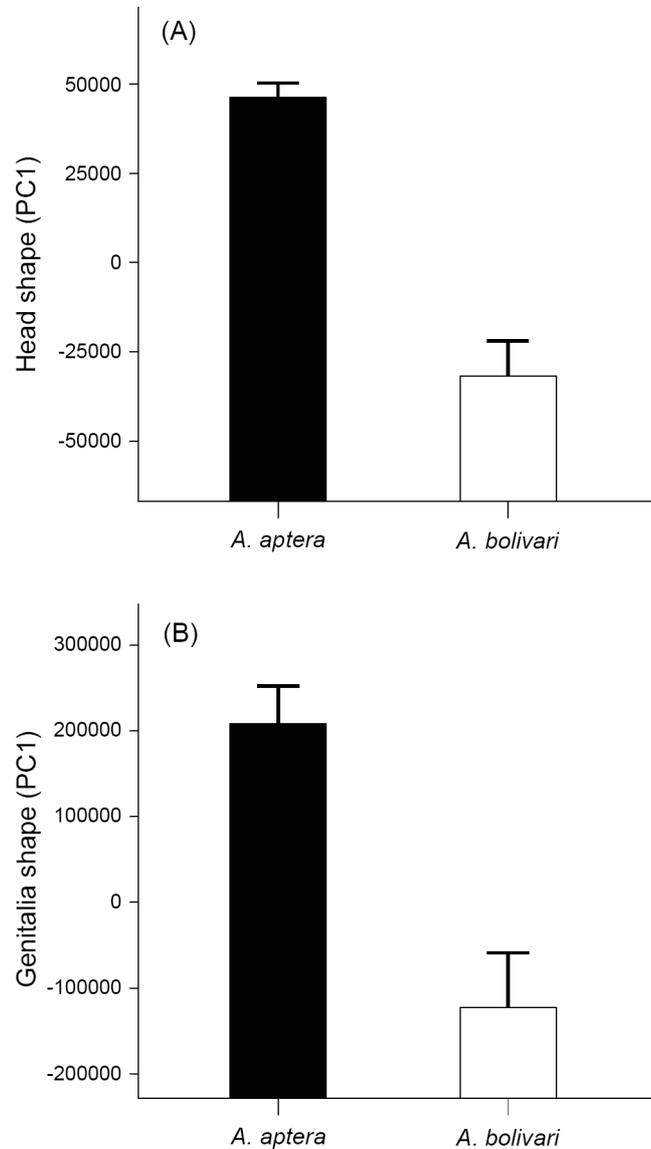


FIGURE 2. Mean (\pm S.E.) of (A) Female head shape and (B) male genitalia shape for *A. aptera* and *A. bolivari* based on the first principal component scores (PC1) extracted from Fourier analyses.

Minimum evolution (ME) and neighbour-joining (NJ) cladograms were constructed using the software MEGA version 3.1 with a Kimura 2-parameter distance matrix (Kumar *et al.* 2008). Node support in both ME and NJ phylogenetic analysis was tested using 1000 bootstrap replicates. Phylogenetic trees were rooted using a sequence of *Ameles* sp. (Mantodea: Amelinae) as outgroup (accession number, JQ041762). We estimated the split time between the main lineages obtained using a sequence divergence rate of ~2% per million years (Myr) for the COI gene as described for other arthropods (Brown *et al.* 1979; Brower 1994; Hewitt 1996; Lunt *et al.* 1998).

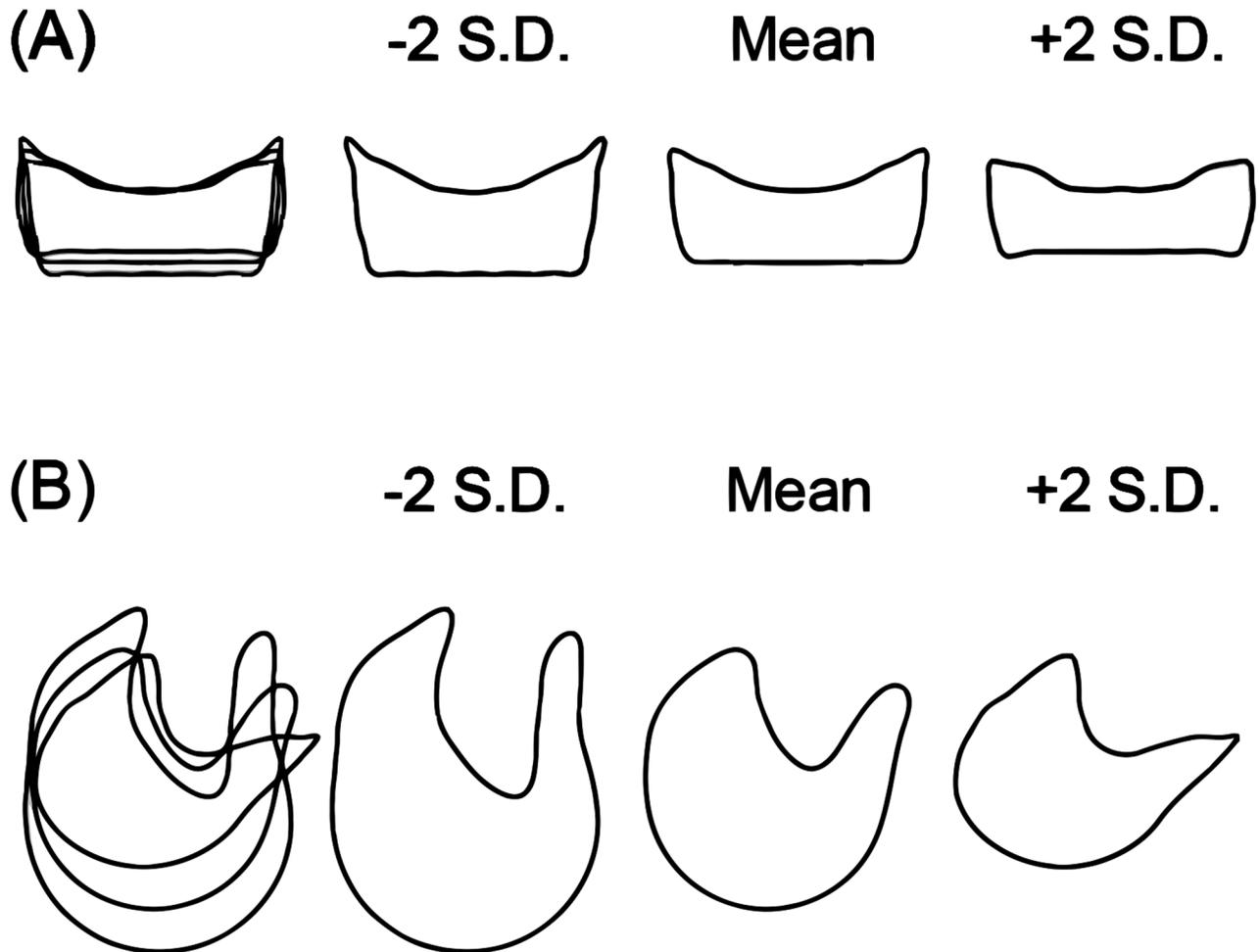


FIGURE 3. Variation in (a) female head and (b) male genitalia shape along the first principal component (PC1) extracted from Fourier analyses. The last three columns show the case where the score takes -2 standard deviations (S.D.), mean, and $+2$ S.D., respectively. The first column shows the overlapped drawing for these three cases.

Ecology

Information on the species ecology (life cycle and habitat use) was obtained from previous published studies (Werner 1932; Peinado & Mateos 1998; Grosso-Silva & Soares-Vieira 2004; Pascual 2005; Arizmendi *et al.* 2011; Pasqual 2012; Marabuto *et al.* 2014) and during field sampling. Since the biology of insects is usually very complex and needs a lot of independent and multidisciplinary studies, we herein focused on descriptive information only. This information is provided in order to contextualize and discuss ecological differentiation as an additional and potentially valid informative character for species delimitation.

Results

Morphology

The first principal component scores (PC1) extracted from Fourier analysis strongly differed between *A. aptera* and *A. bolivari* for the shape of female head ($F_{1,13} = 35.59$, $P < 0.001$; Figs. 2A, 3A) and male genitalia ($F_{1,6} = 12.33$, $P = 0.013$; Figs. 2B, 3B). Other principal components did not significantly differ between both species for any of these two studied character systems (all P s > 0.45). Fourier analyses on the shape of the upper ridge of female head suggest that both species mainly differ on the protrusion of the eyes and apical tubercles described by PC1 (Figs.

2A, 3A). Even with the low available sample sizes, the Fourier analyses on male genitalia also showed a clear separation between the two species (Figs. 2B, 3B). However, these differences are based on a trait (the amplitude and depth of the incision between the two apical spines) that is difficult to detect without direct comparative analyses of specimens representative of both species (Fig. 3B). In such situation, a conspicuous difference can be appreciated at the angle formed at the apex of the lower distal branch of the hypophallus, which tends to be acute in *A. bolivari* and almost right in *A. aptera* (Figs. 4A, 5). It should be noted, that the pseudophallus in this genus is small, thin, lightly sclerotized and very difficult to prepare in old and dry specimens to perform Fourier analyses. However, in *A. bolivari* the pseudophallus tapers and projects into a thinner process, as opposed to what is seen in *A. aptera*, where it is shorter (Fig. 4B).

All the linear morphometric traits studied were significantly larger in *A. bolivari* than in *A. aptera* (all $P_s < 0.003$) (Table 1). Females were also significantly larger than males in both species for all the studied traits (all $P_s < 0.003$) but the interaction between “sex” and “species” was not significant in any analysis (all $P_s > 0.09$) (Table 1). The ranges for some of the studied traits do not overlap in *A. bolivari* and *A. aptera*, indicating that they could be reliable diagnostic characters to distinguish between both species (Table 1). As opposed to what was previously reported by Battiston & Fontana (2005) for other Amelinae, ratios between the studied traits were not capable of separating the species (data not shown).

TABLE 1. Mean \pm S.E. and range for eight linear morphological traits measured for *A. aptera* and *A. bolivari*. Asterisks denote traits that do not overlap between both species.

	<i>A. aptera</i> (males)		<i>A. aptera</i> (females)		<i>A. bolivari</i> (males)		<i>A. bolivari</i> (females)	
	Mean \pm S.E.	Range	Mean \pm S.E.	Range	Mean \pm S.E.	Range	Mean \pm S.E.	Range
Eye distance	3.10 \pm 0.23	2.84-3.47	4.14 \pm 0.21	3.87-4.39	3.41 \pm 0.13	3.20-3.55	4.86 \pm 0.62	4.27-6.02
Pronotum length	5.71 \pm 0.19	5.49-5.95*	7.41 \pm 0.43	6.89-7.86*	7.03 \pm 0.44	6.23-7.56*	9.33 \pm 1.27	7.72-11.26*
Pronotum width	2.03 \pm 0.23	1.71-2.31	3.19 \pm 0.08	3.06-3.30	2.42 \pm 0.16	2.12-2.57	3.35 \pm 0.25	2.90-3.56
Fore femur length	6.00 \pm 0.33	5.70-6.44	7.88 \pm 0.43	7.21-8.32	6.77 \pm 0.31	6.37-7.12	9.65 \pm 1.17	7.91-10.96
Fore femur width	1.07 \pm 0.12	0.92-1.20*	1.58 \pm 0.16	1.35-1.83	1.36 \pm 0.07	1.26-1.45*	1.98 \pm 0.15	1.71-2.14
Hind femur length	8.60 \pm 0.89	7.24-9.52	9.29 \pm 0.19	8.92-9.44*	10.21 \pm 0.92	9.45-11.70	11.95 \pm 1.03	10.25-13.17*
Hind tibia length	9.15 \pm 1.04	7.69-10.33	10.56 \pm 0.21	10.14-10.73*	11.11 \pm 1.28	9.04-12.26	13.83 \pm 1.60	11.58-15.45*
Body length	23.81 \pm 0.76	22.84-24.97*	30.95 \pm 1.02	30.01-32.91*	29.48 \pm 1.27	28.05-31.10*	39.04 \pm 2.93	35.12-43.78*

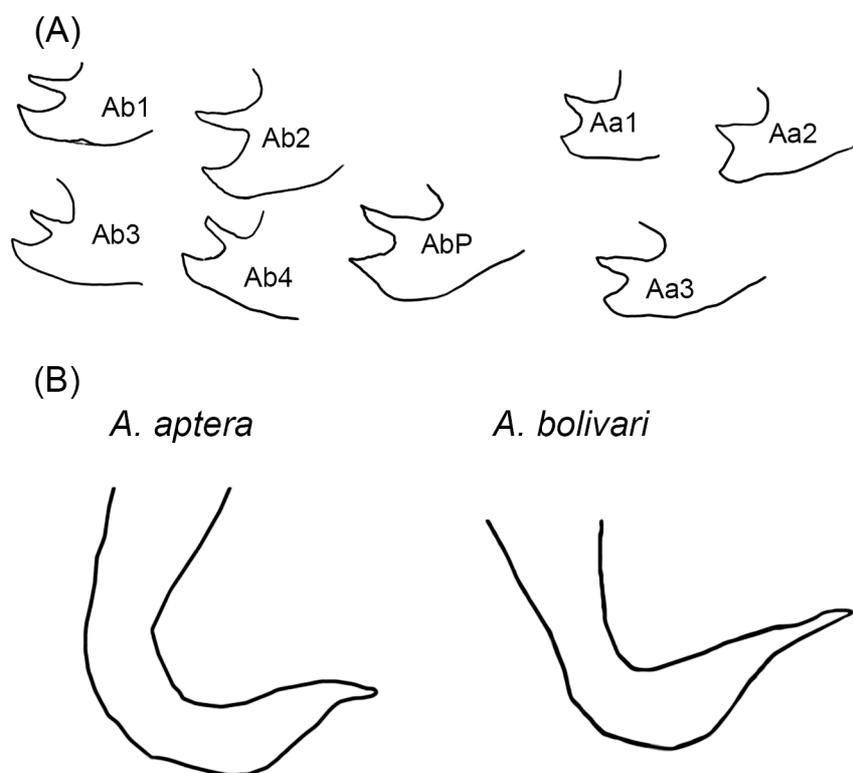


FIGURE 4. (A) Apex of the hypophallus for four specimens of *A. bolivari* from Morocco (Ab1-4), one specimen of *A. bolivari* from Portugal (AbP), and three specimens of *A. aptera* from Spain (Aa1-3). All the drawings have been reduced to the same scale to put in evidence the shape differences. (B) Pseudophallus for *A. bolivari* and *A. aptera*.

We obtained sequences from 7 individuals of *A. aptera* and 2 individuals of *A. bolivari*, recovering 4 and 1 haplotypes, respectively. Both ME and NJ trees recovered very similar tree topologies and suggested the monophyly of *A. aptera* and *A. bolivari* (Fig. 6). Furthermore, phylogenetic analyses also suggested the monophyly of the haplotypes found in the three main geographical areas sampled for *A. aptera* (Fig. 6). Based on sequence divergence rates described for other arthropods (Brown *et al.* 1979; Brower 1994; Hewitt 1996; Lunt *et al.* 1998), we were able to estimate the split time between *A. aptera* and *A. bolivari* around Lower Pleistocene (3.6-4.0 % of sequence divergence; ~1.74-1.98 Myr). We have also found a deep divergence between lineages of *A. aptera* from central (Toledo and Ciudad Real) and south Iberia (Cádiz) that was estimated to have also occurred around Lower Pleistocene (3.0-3.3 % of sequence divergence; ~1.66-1.50 Myr).

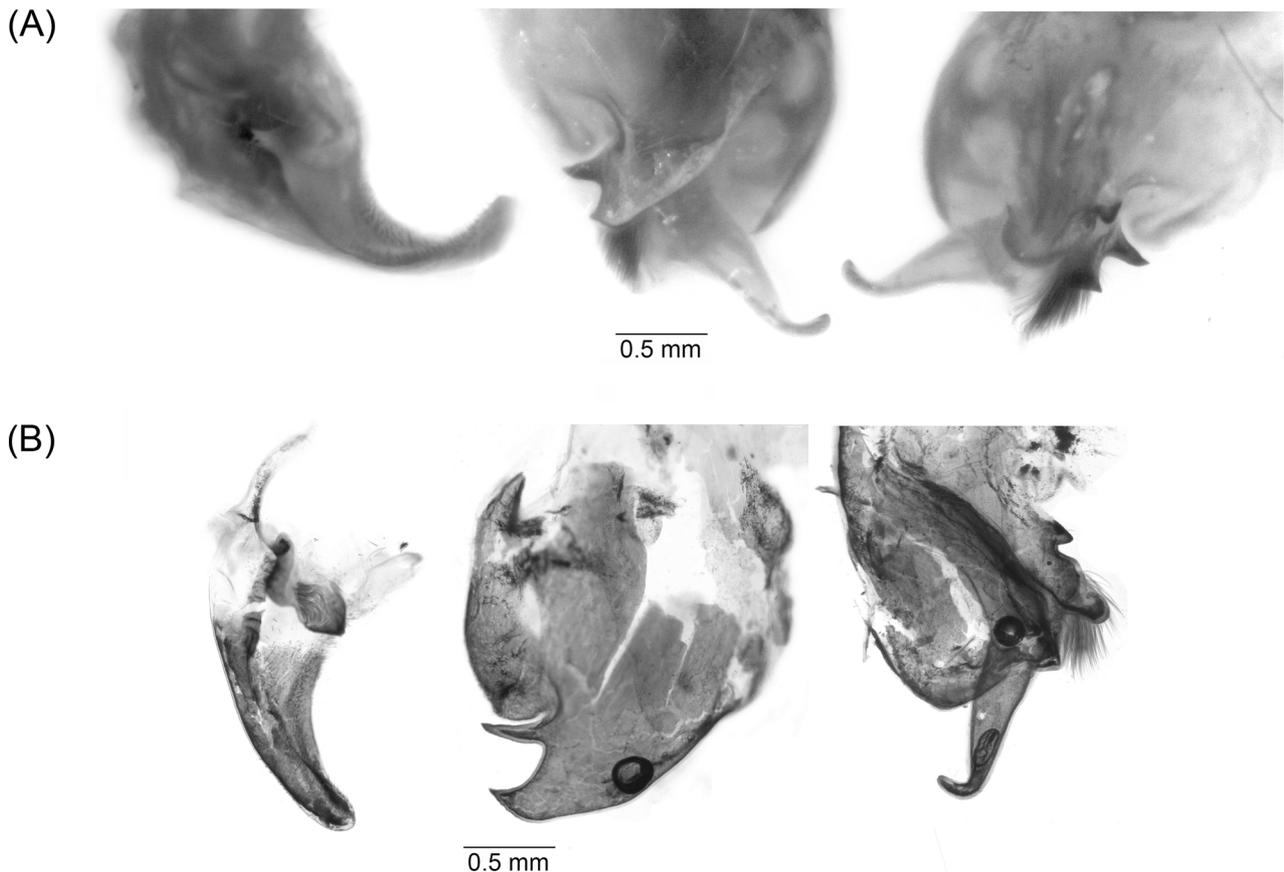


FIGURE 5. Male genitalia of (A) *A. aptera* from Spain (Brunete) and (B) *A. bolivari* from Morocco (Fèz). From left to right: ventral view of right epiphallus, hypophallus and left epiphallus, dorsal view of left epiphallus, and pseudophallus.

Ecology

A. aptera is an entomophagous insect with a univoltine life-cycle. Eggs are laid in a rigid foam ootheca with 30-40 eggs. We have found that nymphs hatch from late June to August. They overwinter as nymphs and become adults in late May or early June of the following year. Some previous studies indicate that adults are present from April to September (Pascual 2005). However, we have only found adult individuals from May to July in Castilla-La Mancha (Central Spain) (P.J. Cordero, unpublished data), a period similarly reported for adult specimens preserved in the entomological collections of the Natural History Museum from Madrid (Peinado & Mateos 1998). Arizmendi *et al.* (2011) reported adult individuals in Toledo (Central Spain) in October, but a more detailed examination of the collected specimens revealed that they were nymphs (I. Arizmendi, pers. com.). Grosso-Silva & Soares-Vieira (2004) reported the presence of adult individuals in early March in southern Portugal. The distribution of *A. aptera* is discontinuous, limited to Central-Southern Spain and Portugal in scrubland, maquis and garrigue-phrygana habitats (Pascual 2005, and 2012; Arizmendi *et al.* 2011; Marabuto *et al.* 2014). *A. aptera*

seems to prefer small plants in dry and sunny hills in a wide altitudinal range, from sea level to 1300 m (Pascual 2012). In Central Spain, we have found that *A. aptera* occurs in thermophile areas, in undisturbed and open patches of *Quercus ilex* “dehesas”, pine forest edges, hedgerows in cereal fields, old vineyards and along roads, and path sides in pseudosteppe agricultural lands. They also occur in patches of old wasteland with mature ruderal vegetation and in treeless areas with herbaceous vegetation around small lagoons and along streams. Microhabitats include tall green and dense grasses, hedgerows and other vegetation patches of old ruderal fields with a wide variety of herbaceous plant species, from communities of *Phoeniculum vulgare*-*Daucus carota* to dry saline steppes of *Suaeda vera*. Most individuals, particularly the females, are generally found on bare ground amid the cover of sparse vegetation (Fig. 1). Paradoxically, the less frequent habitat in which we have found *A. aptera* is the typical garrigue described by Peinado & Mateos (1998). Frequency of encounter is low and *A. aptera* has been usually considered to be locally very rare (Peinado & Mateos 1998; Pascual 2005 and 2012). *Apteromantis aptera* has been found in altitudes ranging between 60 and 1250 m above sea level.

Virtually nothing is known about the biology of *A. bolivari*. Werner (1932) only described its type locality and reported that the species was common in tall grasses along a small river crossing cereal-cultivated fields near Fès (Morocco). According to our own data and studied specimens preserved in museum collections, *A. bolivari* seems to become adult during May. Adult individuals have been reported until July. In 2011, we found *A. bolivari* in small numbers around the same fields of the type locality described by Werner 79 years before. We have confirmed that *A. bolivari* is present in habitats with tall grasses but we have also reported its presence in very hot and dry sandy scrublands, maquis and garrigue-phrygana habitats. *Apteromantis bolivari* has been found in altitudes ranging between 230 and 570 m above sea level.

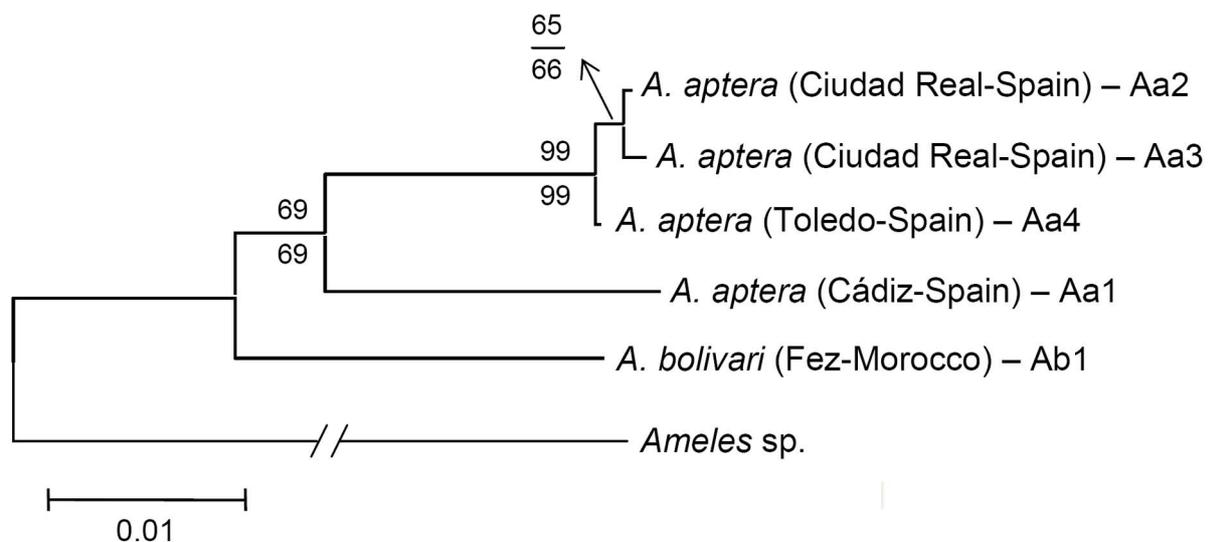


FIGURE 6. Phylograms showing the relationships between *Apteromantis aptera* and *A. bolivari* haplotypes for the COI mitochondrial gene using minimum evolution (ME, above branches) and neighbour-joining (NJ, below branches) methods. Only bootstrap support values over 60% are shown. The tree was rooted with a sequence of *Ameles* sp.

Discussion

Cases of doubtful taxonomy, specimen rarity and difficulties in obtaining large series, lost type specimens or original descriptions based on rather subjective and poorly described characters, are very common in entomology (e.g. Cordero *et al.* 2009). Since protection laws are usually made on the basis of single and well-defined species, the clarification of taxonomic uncertainties may result in influencing existing strategies in conservation biology. The genus *Apteromantis* is a good example of this taxonomic problem. It is comprised of only two, traditionally valid species, one of which is protected by international laws, and thus offers an interesting case-study to apply a

comprehensive approach that can be useful to solve problems regarding the identity of threatened species whose taxonomic status remains doubtful and their conservation.

We have found morphological and genetic evidence indicating that *A. aptera* and *A. bolivari* are distinctive species. Morphological data do support the traditional hypothesis of two distinct species. Elliptical Fourier analyses have also revealed that both species significantly differ in female head shape, particularly in the protrusion of eyes, a character that was previously suggested to be diagnostic (Battiston *et al.* 2010). We have also found significant differences between both species in the shape of male genitalia, a trait associated with reproductive isolation (Jensen *et al.* 2009; Holwell *et al.* 2010) and thus considered highly diagnostic in mantids (e.g. Lombardo 2000; Battiston & Fontana 2005; Jensen *et al.* 2009; Roy & Svenson 2011; Svenson & Roy 2011) and other arthropod groups (e.g. Bond *et al.* 2003; Polihronakis 2009). Finally, morphological divergence in *A. aptera* and *A. bolivari* was also supported by all the studied traits related with body size (Table 1). Even if these traits evolved by environmental selective pressures, differences in body size could also constitute an important reproductive barrier (McKinnon *et al.* 2004; Richmond & Jockusch 2007).

Molecular analyses have shown the presence of three main genetic lineages, two involving the Iberian *A. aptera* and one the North African *A. bolivari* (Fig. 6). Sequence divergence rates between *A. aptera* and *A. bolivari* (~4 %) are within the range reported for the COI mitochondrial gene in other congeneric insect species, supporting the genetic distinctiveness of *A. aptera* and *A. bolivari* (Hebert *et al.* 2003, 2004). Based on sequence divergence rates described for other arthropods, we can estimate the split time between *A. aptera* and *A. bolivari* around Lower Pleistocene. Although our phylogeographical data are very limited, the Pliocene flooding of the Mediterranean basin with the last opening of the Gibraltar strait around 5.33 Ma is expected to have contributed to the fragmentation of a suspected Iberian-North African continuous population and favour a progressive allopatric speciation (Krijgsman *et al.* 1999; Valero-Garcés *et al.* 2000). Molecular data have also revealed strong divergence between *A. aptera* populations from central and south Iberia, indicating the presence of genetically divergent lineages that could be considered evolutionary significant units (ESUs) (Ortego *et al.* 2009, 2010). Pleistocene glaciations are likely to have contributed to the fragmentation of the populations of this thermophilus species in different refugia, which could explain the deep divergence observed between populations of *A. aptera* from central and southern Iberia (Hewitt 1996). Future niche modelling and phylogeographic analyses covering the whole species distribution range would help to determine the historical factors behind this strong divergence and resolve if these divergent lineages are cryptic species that deserve independent conservation strategies (Bickford *et al.* 2007).

Overall, this study highlights the importance of a comprehensive approach to resolve taxonomic and conservation problems. Our data support that *A. aptera* and *A. bolivari* are valid species despite their similar ecology and morphological appearance. Our data also suggest that some morphological traits can retain useful information on genetic divergence and can offer a preliminary/complementary useful criterion for the establishment of management units necessary to guide conservation policies (Ortego *et al.* 2012). Future experiments testing potential interbreeding and offspring viability together with more extensive and detailed genetic studies could help to get a better understanding on the taxonomy and biology of the rare and geographically restricted genus *Apteromantis*.

Key to the species of the genus *Apteromantis* (based on examined specimens):

- 1 Eyes of female moderately conical and projected into a triangular tubercle; area between compound eyes with a flat profile. Angle formed at the apex of the lower distal branch of the hypophallus almost right; tip of pseudophallus almost triangular (specimens from Iberia). *A. aptera*
- Eyes markedly conical and projected into a spiny tubercle; area between compound eyes with a concave profile. Angle formed at the apex of the lower distal branch of the hypophallus acute; tip of pseudophallus very thin, thread-like (specimens from Northwest Africa). *A. bolivari*

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