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Phylogeny, evolutionary morphology, and hemipenis descriptions of the Middle American jumping pitvipers (Serpentes: Crotalinae: *Atropoides*)

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Abstract

The jumping pitvipers, genus *Atropoides*, occur at low to middle elevations throughout Middle America. Recent molecular phylogenetic analyses have included all six species of *Atropoides*, but only two studies have found *Atropoides* to be monophyletic and questions persist about relationships within the *A. nummifer* complex. In this study, our phylogenetic analyses of morphological data provide strong support for the monophyly of *Atropoides* and recover relationships within the genus that are mostly congruent with those of recent molecular studies, further supporting the evolutionary and biogeographic hypotheses proposed in those studies. Our analyses find support for a sister relationship between *A. picadoi* and the other *Atropoides* species and an *A. occiduus–A. indomitus* clade sister to an *A. nummifer–A. mexicanus–A. olmec* clade. Within the *A. nummifer* complex, we find *A. mexicanus* and *A. olmec* to be sister species to the exclusion of *A. nummifer*. We include morphological synapomorphies to support each clade within *Atropoides* and describe and illustrate the hemipenes of each species. In addition, we discuss the importance of morphological phylogenetics and the functionality and limitations of hemipenial data in systematics.

Key words: *Cerrophidion* – coding – frequency coding – hemipenes – morphological phylogenetics – *Porthidium* – snake – Viperidae

Introduction

The jumping pitvipers of the genus *Atropoides* are endemic to Middle America, inhabiting low to middle elevation habitats from northeastern Mexico to central Panama (distribution maps are found in Castoe et al. 2003; Campbell and Lamar 2004; Smith and Ferrari-Castro 2008). Along with the genera *Cerrophidion* and *Porthidium* – the montane and hognose pitvipers, respectively – *Atropoides* is part of the pitviper clade known as the *Porthidium* group (Castoe et al. 2005) and has received much attention in both morphological (Campbell and Lamar 1992; Werman 1992; Gutberlet 1998; Gutberlet and Harvey 2002) and molecular phylogenetic studies (Kraus et al. 1996; Parkinson et al. 2002; Castoe et al. 2003, 2005, 2009). Werman (1992) erected the genus *Atropoides* to encompass three species of pitvipers (*A. olmec*, *A. picadoi*, *A. nummifer*) previously assigned to the genus *Porthidium* (Campbell and Lamar 1989). Castoe et al. (2003) found that the wide-ranging *A. nummifer* actually contained several species-level lineages (formerly subspecies, *A. n. mexicanus*, *A. n. nummifer*, and *A. n. occiduus*). In accord with these findings, *A. mexicanus* and *A. occiduus* were recognized by Campbell and Lamar (2004), and most recently a population of *Atropoides* discovered in Honduras was described as a distinct species – *A. indomitus* (Smith and Ferrari-Castro 2008).

Only with low support, using a Bayesian criterion and a 'mixed models' approach for the different genes, has *A. picadoi* been shown to form part of a monophyletic genus *Atropoides* (Castoe et al. 2005, 2009). Even recently, under both parsimony and Bayesian criteria, *Atropoides* monophyly was not supported using molecular data, owing to the placement of *A. picadoi* as sister to a *Cerrophidion–Porthidium* clade (Castoe and Parkinson 2006). In addition, alternative hypotheses within the *A. nummifer* complex have proposed a sister relationship between *A. mexicanus* and *A. nummifer* to the exclusion of *A. olmec* (Castoe et al. 2003, 2005; Castoe and Parkinson 2006) or a sister relationship between *A. mexicanus* and *A. olmec* to the exclusion of *A. nummifer* (Castoe et al. 2005, 2009). These few inconsistencies demonstrate a need for further investigation of these relationships with additional data.

In this study, we used morphological data to conduct two phylogenetic analyses to examine relationships within *Atropoides* and to further evaluate the question of *Atropoides* monophyly. We compare our tree topologies to previously published molecular trees (see references above) and describe morphological synapomorphies for all clades found in our analyses. The everted hemipenes of all species of *Atropoides* are described and illustrated, with *in situ* descriptions for the hemipenes of three species. Finally, we discuss the historical biogeography of the genus, the importance of morphology in phylogenetics, and the utility of hemipenial character data in systematics.

Materials and methods

Phylogenetic analyses

Morphological characters were scored from 159 alcohol-preserved specimens (Appendices S1 and S2). In each of two analyses, all six species of *Atropoides* were included and *Agkistrodon contortrix* was used as an outgroup to root the tree. Multiple specimens per species were used to evaluate morphological polymorphism. Former studies of DNA sequence data have revealed little genetic variation within species of *Atropoides* (Castoe et al. 2003, 2005); therefore multiple operational taxonomic units (OTUs) were not used for any particular species.

The dataset for phylogenetic analysis comprised thirty-five characters of scalation and hemipenial morphology (Appendix S3). Descriptions for characters are derived from Werman (1992), Wüster et al. (1996), Klauber (1997), Gutberlet (1998), Gutberlet and Harvey (2002), Jadin (2007), and Fenwick et al. (2009). The numbering of characters 1–10 and 12 is congruent with Gutberlet (1998) and Gutberlet and Harvey (2002). All characters from Gutberlet (1998) and Gutberlet and Harvey

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(2002) were examined in this study, however within the *Porthidium* group most of these characters were found to be uninformative (e.g. presence or absence of a rattle). Characters 14–16, 18–22, 26–29, and 31–35 herein are modified from studies above or are original to this study; characters 1–11, 14–21, 28, 29, 33, and 34 are meristic; characters 12, 22–27, 30–32, and 35 are qualitative and multistate.

Multistate characters were treated as ordered, and polymorphic characters were coded using generalized frequency coding (Smith and Gutberlet 2001) with unequal subcharacter weighting. Morphological data were entered into the software program *FastMorphologyGFC* (Chang and Smith 2003) to convert raw data into a nexus file that could be used in PAUP*. To determine whether the morphological data contained phylogenetic signal, we calculated a g1 value for the three data matrices based on 1 000 000 randomly generated trees (Hillis and Huelsenbeck 1992). For each analysis, we used PAUP*v4.0b10 (Swofford 2002) to conduct heuristic searches under a parsimony criterion with 10 000 random-taxon-addition sequences and tree bisection reconnection (TBR) branch-swapping. To assess confidence in the relationships depicted by the shortest tree, nonparametric bootstrapping (Felsenstein 1985) was applied using 2000 full heuristic pseudoreplicates and two random-taxon-addition sequence replicates per pseudoreplicate.

To investigate intergeneric relationships within the *Porthidium* group, monophyly of *Atropoides*, and relationships within *Atropoides*, we conducted two analyses which included all six species of *Atropoides*, two species of *Cerrophidion* (*godmani* and *tzotzilorum*), and eight species of *Porthidium* (*dunni*, *hespere*, *lansbergii*, *nasutum*, *ophryomegas*, *porrasi*, *volcanicum*, and *yucatanicum*). To assess the impact and utility of hemipenial character data, we analysed external morphology only (characters 1–31) in analysis 1 and external morphology plus four hemipenial characters (32–35) in analysis 2.

Hemipenes

Hemipenial morphology was examined in 16 alcohol-preserved specimens of *Atropoides* from the Amphibian and Reptile Diversity Research Center (UTA) (Appendix S2). Using the methods of Myers and Cadle (2003) and Zaher and Prudente (2003), the hemipenes of *A. indomitus*, *A. mexicanus*, *A. nummifer*, *A. occiduus*, *A. olmec*, and *A. picadoi* were fully everted and illustrated. Additionally, the tails of specimens (three species) with uneverted hemipenes were dissected to examine *in situ* characteristics. Only a single male specimen is available for the newly described *A. indomitus*; this specimen had both hemipenes everted at the time of fixation (Smith and Ferrari-Castro 2008), and no specimens of *A. picadoi* or *A. nummifer* at UTA had uneverted hemipenes. Therefore, *in situ* examinations of hemipenes were not conducted for these three species. Specimen measurements of snout-vent length (SVL) and tail length (TL) as well as counts of subcaudals (SC) and the side (left or right) of hemipenis extraction (SHE) were taken. Terminology follows that of Dowling and Savage (1960).

Hemipenial character definitions and abbreviations for everted hemipenes are as follows: hemipenis length (HL), distance from the cloaca to tip of the lobes while fully everted; hemipenis width (HW), maximum distance across hemipenis when fully everted; position of the largest hook on the hemipenis (PLH), see character 32 (Appendix S3); number of spine and hook rows on hemipenis (NSHR), see character 33 (Appendix S3); number of spines on each lobe (NSL); number of hooks on each lobe (NHL), hooks defined as any spine reaching a length at least two-thirds the length of the third parasubcaudal from the vent; length of longest hook (LLH), maximum distance of the longest hook on the hemipenis from base to tip; number of spines around the base of each lobe (NSBL), counted at the area of bifurcation in a circle around each lobe; number of spines and hooks around the base of the calyces (NSBC), number of spines and hooks around the lobe counted directly below the calyces; measurement of the spinous region (MSR), measurement in millimetres on the sulcate side, from the first to the last row of spines on the lobes; number of rows of calyces (NRC), see character 34 (Appendix S3); measurement of the calyx region (MCR), measurement in millimeters on the asulcate side, from the first row of calyces to the tip of the lobes; shape of the ridges of calyces (SCR), which can be papillate, scalloped, smooth, or spinulate (Dowling and Savage 1960); distance between *sulcus spermaticus* bifurcation and bilobation (DSBB), measurement from the point of *sulcus* bifurcation to the crotch, which is the area of hemipenis bilobation; spines on the border of the *sulcus spermaticus* (SBSS), see character 35 (Appendix S3).

Abbreviations and definitions for *in situ* hemipenis descriptions are as follows: cloacal scent gland subcaudal extent (CSGE), as the number of subcaudals encompassed by the cloacal scent gland; hemipenis bifurcation level (HBL), measured as the number of subcaudal from vent at which hemipenis bifurcates; proximal level of spines (PLS), measured as number of subcaudals from vent at which first spine is encountered; proximal level of calyces (PLC), measured as subcaudal number at which first calyx is encountered, proximally; hemipenial extent (HE), measured as number of subcaudals from vent to end of hemipenis; level of fusion of *musculus retractor penis magnus* (FRPM), measured as subcaudal number at which hemipenial lobe-branches of the *musculus retractor penis magnus* fuse before vertebral insertion; insertion of *musculus retractor penis magnus* (IRPM), measured as number of subcaudals from vent at the point of vertebral insertion.

Results

Phylogenetic relationships

Our phylogenetic analyses of the *Porthidium* group strongly support the monophyly of *Atropoides* (Figs 1 and 2). An *Atropoides–Porthidium* clade, to the exclusion of *Cerrophidion*, was strongly supported in our analyses (in contradiction to the findings of Castoe et al. 2005, 2009 and Castoe and Parkinson 2006). Within the jumping pitvipers, both of our analyses strongly support a basal split between *A. picadoi* and the remaining *Atropoides* species; an *A. nummifer–A. olmec–A. mexicanus* clade with *A. nummifer* sister to *A. olmec* and *A. mexicanus* was also recovered (Figs 1 and 2). The only discrepancy between the two phylogenetic hypotheses is that analysis 1 shows subtending relationships within *Atropoides* inferring that *A. indomitus* is sister to the *A. nummifer–A. olmec–A. mexicanus* clade, with *A. occiduus* sister to these four species (Fig. 1). Analysis 2 shows a sister relationship between *A. occiduus* and *A. indomitus*, which together are sister to the *A. nummifer–A. olmec–A. mexicanus* clade (Fig. 2). Analysis 2 included a greater number of informative characters

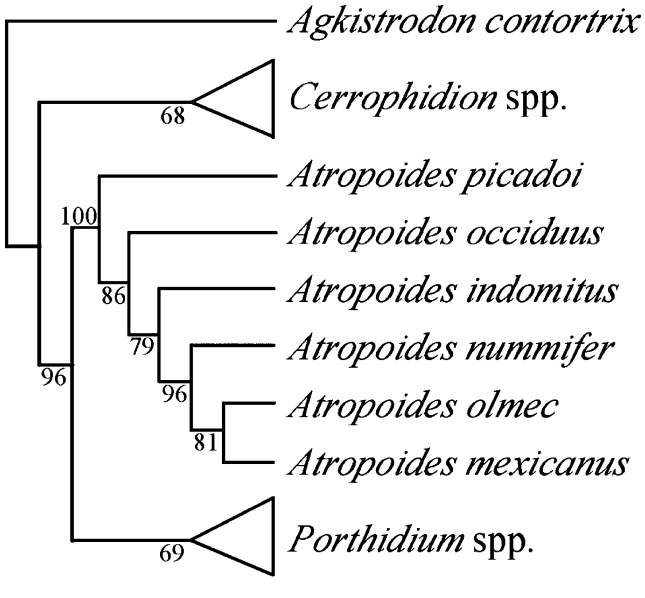

Fig. 1. Single shortest tree (2 006 808 weighted steps, CI = 0.5062, RI = 0.6391, g1 = −0.580609) of the *Porthidium* group recovered from analysis 1 (all taxa, characters 1–31 included)

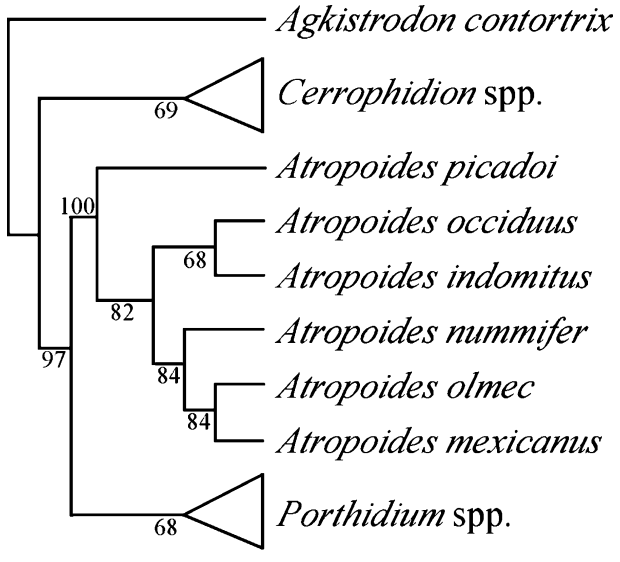

Fig. 2. Single shortest tree (2 227 734 weighted steps, CI = 0.5148, RI = 0.6245, g1 = −0.567221) of the *Porthidium* group recovered from analysis 2 (all taxa, characters 1–35 included)

and produced results that are identical to those recovered by the Bayesian analyses of Castoe et al. (2005, 2009); see discussion.

External synapomorphies

Although molecular data have had difficulty resolving the monophyly of *Atropoides* (e.g. Kraus et al. 1996; Parkinson et al. 2002; Castoe et al. 2003, 2005; Castoe and Parkinson 2006) due to the problematic placement of *A. picadoi*, morphological synapomorphies supporting this clade are distinctive. Species of *Atropoides* have tuberculate scales, narrow supraoculars, and most have nasorostrals – which can be used for species identification (Campbell and Lamar 2004). Additionally, species of *Atropoides* also have greater numbers of interoculabials, intersupraoculars (probably influenced in part by the narrower supraoculars), prefoveals, subfoveal rows, and posterior intercanthals compared to other species within the *Porthidium* group. Species of *Atropoides* also tend to be stouter than most other pitvipers and have a very characteristic long, narrow, and dark postocular stripe.

Atropoides picadoi is found to be sister to all other species of *Atropoides* and differs greatly from its congeners in averaging fewer prefoveals, supralabials, gulars, subfoveal rows, interrictals, and middorsal scale rows, and higher numbers of ventrals, subcaudals, and posterior intercanthals (Table 1). In addition, *A. picadoi* attains a large size, commonly reaching lengths of 75–95 cm, whereas the other species tend to be smaller (adults average 35–70 cm depending on the species; Campbell and Lamar 2004). Although these body lengths are considered fairly short for most viperids, species of *Atropoides* are thick-bodied and attain a rather large body mass. The *A. nummifer–A. olmec–A. mexicanus* clade is supported by a greater average number of interoculabials and fewer scales contacting the supraocular; however, the most obvious synapomorphy of this clade is the high number of nasorostrals (averaging 4.7–6.5). Nasorostrals are likely a derived feature as these scales are not found in the outgroup *Agkistrodon contortrix*, nor are they found in species of *Porthidium* or *Cerrophidion*; in fact, nasorostrals are absent from most pitviper species (Werman 1992). *Atropoides picadoi* has no nasorostrals, *A. occiduus* normally has no nasorostrals but can have two, and of the two specimens of *A. indomitus*, one has no nasorostrals and the other has two. The *A. occiduus–A. indomitus* clade is supported by a greater average number of suboculars and interrictals. The *A. mexicanus–A. olmec* clade is supported by averaging fewer ventrals and body blotches and more subfoveal rows than all other *Atropoides* species. In addition, specimens of *A. mexicanus* and *A. olmec* occasionally have divided supraoculars, a feature that appears to be unique to these species. See Table 1 for a summary of the averages of many of the meristic characters used in this study.

Hemipenial descriptions and synapomorphies

Like most, if not all, pitvipers, *Atropoides* has deeply forked hemipenes which lack pedicels. Each hemipenis includes a short naked base, followed briefly by minute papillae or spines, and long lobes that extend further than twice the length of the base. Hemipenes of *Atropoides* bear calyces distally and lack apical papillae, which are present in species of *Porthidium* (Campbell and Lamar 1989, 2004; Gutberlet 1998: Fig. 2). The more basal calyces are arranged in rows, but calyces form crisscrossing patterns distally near and on the apex.

Hemipenes show little intraspecific variation or side bias between left and right hemipenes, however much variation occurs among species (Fig. 3a–f; Tables S1 and S2). Each clade within *Atropoides* is supported by several distinct features or hemipenial synapomorphies. The most pronounced is *A. picadoi* which has a short and stocky hemipenis and large wide spines (Fig. 3a), with its largest hook at the base of the hemipenial lobe, differing from the other species which possess much narrower hemipenial lobes and spines. Members of the

Table 1. Mean values for most overlapping meristic characters evaluated in this study. These characters represent counts of (1) interoculabials, (2) prefoveals, (3) suboculars, (4) supralabials, (6) intersupraoculars, (7) interrictals, (8) gulars, (9) ventrals, (10) middorsal scale rows, (11) subcaudals, (13) scales contacting supraocular, (16) postoculars, (17) subfoveal rows, (18) posterior intercanthals, (19) body blotches, and (28) nasorostrals

	Characters															
Taxa	1	2	3	4	6	7	8	9	10	11	13	16	17	18	19	28
Agk. contortrix	2	0	2.1	7.7	1	26.3	3.4	147.4	23	43.1	6.4	2	0	2.4	13.3	0
A. indomitus	4.5	7	3.5	10.5	10.5	27	4	141	24	33	8.5	3	2	10.5	27.5	1
A. mexicanus	4.8	7.2	2.1	10	8	24.1	3.8	127	25.1	33.5	6.5	3.3	2.4	9.1	19	6.5
A. nummifer	4.6	7.2	2.4	10	9.2	25.2	4.2	132.3	24.3	31.8	7.5	2.8	2	9.5	23	4.7
A. occiduus	4.2	4.4	3.6	9.6	9.4	28	4.8	133.8	25	26.2	8.4	3.4	1.8	9.2	23	0.4
A. olmec	5.5	5.8	3	10.3	9.5	25.5	4.8	117.8	24	31.8	6.7	3	2.8	10	22	6.3
A. picadoi	4.3	3.8	2.3	9.3	9.3	25.3	3	146.5	24.5	38.5	8.5	3	1.3	11.3	27.3	0

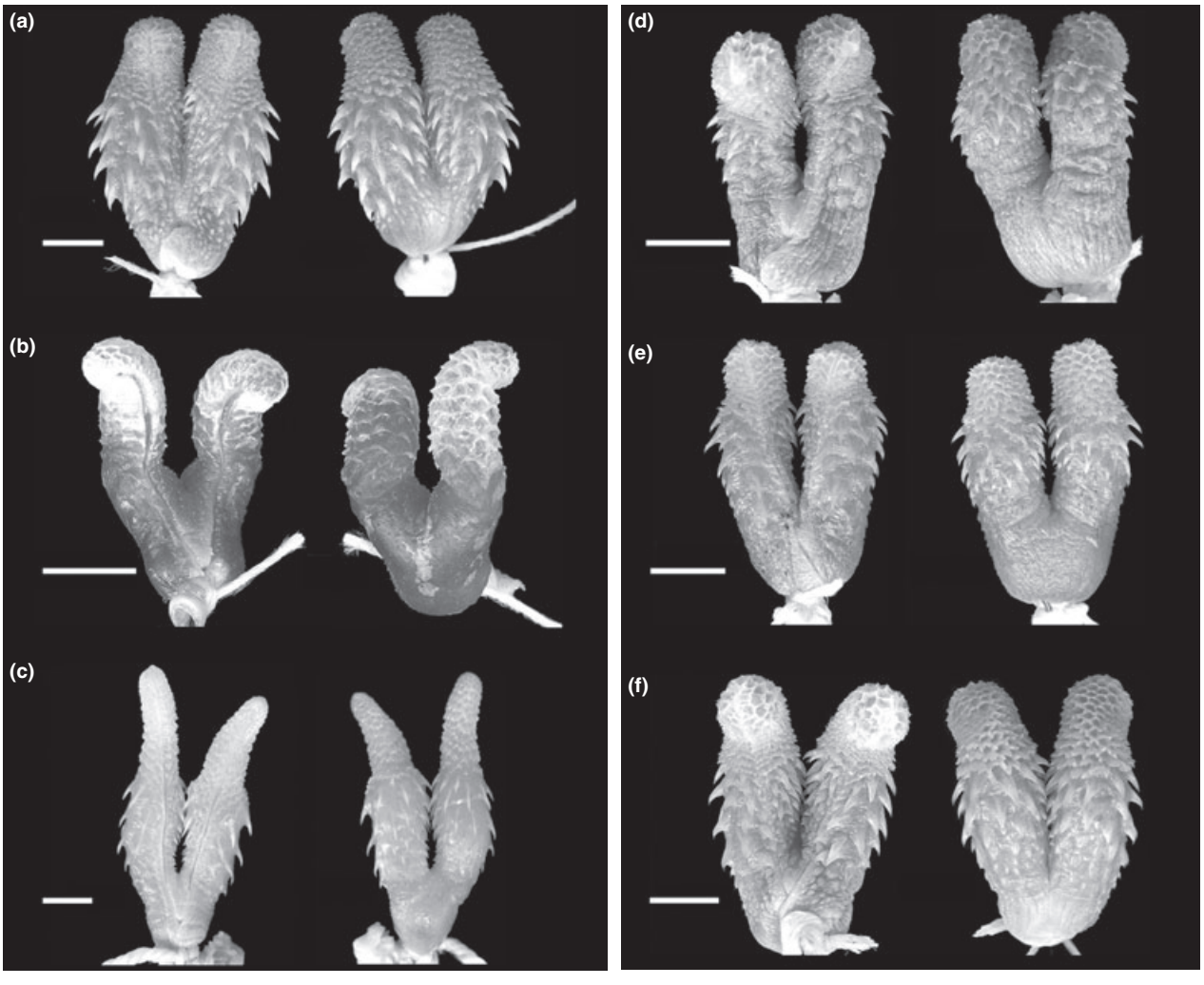

Fig. 3. Sulcate (left) and asulcate (right) view of hemipenes. (a) *Atropoides picadoi* (UTA R-18215). (b) *A. occiduus* (UTA R-9089). (c) holotype of *A. indomitus* (UTA R-52952). (d) *A. nummifer* (UTA R-24842). (e) *A. olmec* (UTA R-25113). (f) *A. mexicanus* (UTA R-45500). See Table S1 for descriptions of each hemipenis. Scale bar = 5 mm

A. occiduus–A. indomitus clade exhibit proportionally longer and thinner hemipenial lobes, much longer calyculate areas that are slightly scalloped to smooth, and – distinctively – a lack of spines on the edge of the *sulcus spermaticus* and on basal calyculate rows (Fig. 3b,c). *Atropoides occiduus*, at least the specimens we examined, lacks spines on its hemipenes (Fig. 3b), an unusual condition among pitvipers. The everted hemipenes of several other specimens of *A. occiduus* were examined (UTA R-29680, 26415, 6227, 16107) for detection of spines. These additional specimens showed little variation in relation to the specimen described in Table S1 (UTA R-9089) and none contained spines on the hemipenes. The *A. nummifer–A. olmec–A. mexicanus* clade is distinct in having similar numbers of spines at the base of lobes (3–5) and a short distance from bifurcation of the *sulcus spermaticus* to the point of bilobation (Fig. 3d–f).

The three species examined *in situ* all share an elongated cloacal scent gland that is pointed caudally. For the right hemipenes of *A. mexicanus* and *A. olmec* the *sulcus spermaticus* originates ventrally and curves ventro-diestrally from the organ's base to the level of the end of subcaudal 3 and middle of subcaudal 4, respectively. This differs from *A. occiduus* whose right hemipenis exhibits a *sulcus spermaticus* that curves ventro-sinistrally from the organ's base to the end of subcaudal 4, after which each *sulcus spermaticus* divides dorsally and ventrally, and proceeds toward the tip of each lobe. The calyces for all three species start at similar positions in the tail; however, the hemipenis of *A. occiduus* extends much further showing the greater proportional length of the calyces and hemipenis.

Discussion

Evolutionary relationships and biogeographic hypotheses

The phylogenetic consensus of both morphological (this study) and molecular data (Castoe et al. 2005, 2009) supports some of the proposed biogeographic hypotheses given (i.e. Castoe et al. 2003, 2005, 2009; Werman 2005). These hypotheses state that the basal split within *Atropoides* occurred near the Nicaraguan Depression between the common ancestor of *A. picadoi* and the common ancestor of the remaining species (Castoe et al. 2003, 2009; Werman 2005). The problematic placement of *A. picadoi* (see Introduction) and unique aspects of its morphology (see Results) suggest that there is a more interesting evolutionary history for this species than is currently known.

Werman (2005: 328) states that 'it is unclear if *A. n. nummifer* and *A. n. occiduus* were separated as a unit or were separated

independently from an *A. n. mexicanus–A. olmec* clade'. Both molecules (Castoe et al. 2003, 2005, 2009) and morphology (this study) strongly support an *A. nummifer–A. mexicanus–A. olmec* clade to the exclusion of *A. occiduus* and do not support the hypothesis that *A. nummifer* and *A. occiduus* form a clade and therefore separated independently. In addition, both types of data (Castoe et al. 2005, 2009; this study) show support for an *A. occiduus–A. indomitus* clade that diverged sister to an *A. nummifer–A. mexicanus–A. olmec* clade. This may have been caused by the uplift of nuclear Central American highlands (Savage 1966); however, corridors may have led to a dispersal event of *A. indomitus* into Honduras, leading to speciation. Although most molecular analyses show weak support for a sister relationship between *A. nummifer* and *A. mexicanus* to the exclusion of *A. olmec* within the '*nummifer* complex' (Castoe et al. 2003, 2005; Castoe and Parkinson 2006); our findings, as well as the analysis using the more complex models of molecular evolution from Castoe et al. (2005, 2009), support a sister relationship between *A. mexicanus* and *A. olmec* to the exclusion of *A. nummifer*. It is likely that the most accurate phylogeny is derived from analyses which utilized the most complex model of molecular evolution (T.A. Castoe, pers. comm.) and there are several morphological synapomorphies supporting this relationship (see Results). However, the slight incongruence found in the molecular analyses and the low support found from the morphology aids the hypothesis proposed by Werman (2005) that *A. mexicanus* and *A. olmec* are the most recent phylogenetic split within *Atropoides* possibly caused by the 'uplift of the Sierra de Los Tuxtlas in Veracruz and habitat changes associated with Neogene glacial episodes' (Werman 2005: 329).

Although it has a wide range throughout Middle America, *A. mexicanus* has shown little molecular variation among the populations throughout its range (Castoe et al. 2005). The apparent disjunct distribution of *A. mexicanus* between northern and southern Central America is likely a factor of incomplete sampling (J.A. Campbell, pers. comm.). This study shows extensive variation among the hemipenes of *Atropoides* species (Fig. 3), and the apparent lack of significant difference between the hemipenes of *A. mexicanus* from Costa Rica and Guatemala (Table S1) provides additional support for the hypothesis that there has been recent gene flow between the populations.

Morphological phylogenetics

Although recent studies have criticized the use of morphology in computer-based phylogenetic analyses (e.g. Scotland et al. 2003; Wortley and Scotland 2006) many systematists defend its use, even in the current age of genomics (see Jenner 2004; Wiens 2004, 2008). The number of possible rooted trees for six OTUs is 945 ($(2n - 3!)/[2^{n-2}(n - 2)!]$; Cavalli-Sforza and Edwards 1967); thus if our morphological data lack phylogenetic information, our results represent a coincidence with a probability of 1 in 945. We believe that this type of phylogenetic congruence, even at the intrageneric level, is evidence that morphological phylogenetics should be considered useful for investigating evolutionary relationships and emphasize that most phylogenetic studies, including this one, hardly investigate all possible morphological characters of an organism. More importantly, the great morphological and genetic diversity of life renders meaningless any general appraisal of the phylogenetic utility of morphological and molecular data. No category of data is useful for every phylogenetic study, and anecdotes about the failure of one 'class' of data hardly disqualify that class from future studies with different organisms and different characters. Rather, extent of intra- and interspecific variation in potential characters (molecular or morphological) should be considered in study design.

Hemipenial characters

Without hemipenial characters, the phylogenetic hypothesis in Fig. 2 would not have been generated. Hemipenial characters can be phylogenetically informative and even unique to particular clades (e.g. presence of apical papillae in species of *Porthidium*). However, other hemipenial characters may be highly variable and thus may provide limited phylogenetic information. This variation highlights the need to examine multiple individuals per OTU and to assess the utility of phylogenetic characters on a case by case basis.

Another challenge of using hemipenial characters in phylogenetic analyses is finding characters that are independent of each other. An interesting character might be a measure of how much space the calyculate or spinous areas occupy on the hemipenis but these characters are directly correlated with numbers of hooks, spines, and calyculate rows. In any case, hemipenial characters should be analysed after extraction and eversion, as well as *in situ* (Dowling and Savage 1960). In addition, hemipenes should be compared to closely related species to better understand common ancestry and patterns of evolution. Schargel et al. (2005) suggested that caution be implemented when describing new genera based in part on hemipenial morphology, and this study provides additional evidence revealing extensive hemipenial variation among species of the same genus.

Acknowledgments

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Resumen

Filogenia, morfología evolutiva, y descripción de hemipenes de las serpientes Mano de Piedra de América Central (Serpentes: Crotalinae: Atropoides*)*

Las víboras mano de piedra, género *Atropoides*, habitan elevaciones bajas y medias en Centro América y México. Análisis filogenéticos moleculares recientes han incluido a las seis especies de *Atropoides*, de ellos solamente dos han soportado la monofilia del género y siguen habiendo relaciones poco respaldadas dentro del complejo de *A. nummifer*. En este estudio nuestro análisis filogenético basado en morfología provee un alto soporte a la monofilia de *Atropoides* y muestra relaciones intragenéricas que son mayormente congruentes con aquellas de estudios moleculares recientes. De esta manera se respaldan las últimas hipótesis biogeográficas propuestas para el género. Nuestros análisis encuentran que *A. picadoi* es un taxón

hermano a las otras especies del genero y que el clado *A. occidus–A. indomitus* es hermano del clado *A. nummifer–A. mexicanus–A. olmec*. Dentro del complejo de *A. nummifer* encontramos que *A. mexicanus* y *A. olmec* son especies hermanas, excluyendo a *A. nummifer*. Incluimos sinapomorfías morfológicas que soportan a cada clado de *Atropoides* y describimos e ilustramos los hemipénes de cada especie. Además, discutimos la importancia de la filogenética morfológica y el uso y limitaciones de datos hemipeniales en sistemática.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Descriptions of everted hemipenes of the genus *Atropoides*. Measurements in mm.

Table S2. Descriptions of *in situ* hemipenes of three species within the genus *Atropoides*. Abbreviations at the front of subcaudal count are: the beginning of (b), the middle of (m), and the end of (e).

Appendix S1. Specimens examined for comparative external morphology and phylogenetic characters. Institutional abbreviations, except UTT (University of Texas at Tyler), follow Leviton et al. (1985).

Appendix S2. Specimens examined for hemipenial characters and morphology.

Appendix S3. Characters used in the phylogenetic analyses.

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