PHYLOGENETIC RELATIONSHIPS OF NEW WORLD PITVIPERS AS INFERRED FROM ANATOMICAL EVIDENCE

RONALD L. GUTBERLET, JR.[1,2] AND MICHAEL B. HARVEY[1,3]

ABSTRACT: Phylogenetic studies continue to improve our understanding of New World pitviper biogeography and evolution. A classification consistent with these snakes' phylogeny has nearly been achieved, although polyphyly of the genus *Porthidium* has been problematic throughout the last decade. We conducted phylogenetic analyses of 32 pitviper species representing every New World genus. The analyses were based on 76 characters from crania, vertebrae, hemipenes, scalation, and color pattern. By including every known species of *Porthidium*, we provide the first test of *Porthidium* monophyly. With the removal of *P. hyoprora*, monophyly of *Porthidium* is strongly supported by numerous synapomorphies, including an apical papilla on each lobe of the hemipenis, few gulars between the chin shields and first ventral, an orange middorsal stripe, and an anteriorly positioned choanal process of the palatine bone. The anatomical data strongly suggest that *P. hyoprora* is closely related to two other South American pitvipers, *Bothrops campbelli* and *B. microphthalmus*; these three species are now included in the genus *Bothrocophias* Gutberlet and Campbell (2001), along with the newly described *B. myersi*. Monophyly of each Middle American genus is well-supported at this time, though relationships among these genera remain poorly resolved. A monophyletic North American group (*Agkistrodon*, *Crotalus*, and *Sistrurus*) and a monophyletic Neotropical group are supported in most analyses. Within the Neotropical group, a potentially monophyletic South American group (*Bothriopsis*, *Bothrops*, *Bothrocophias*, and *Lachesis*) deserves further study.

INTRODUCTION

Reconstructing the phylogenetic history of New World pitvipers has been a gradual process, but recent studies show that impressive progress has been achieved. Burger's (1971) dissertation, though never formally published, was ground-breaking in delimiting many hypothetically monophyletic groups and in describing many characters of potential use in pitviper systematics. Burger divided the speciose and morphologically diverse genus *Bothrops* into five genera: *Bothriechis, Bothriopsis, Bothrops, Ophryacus,* and *Porthidium.* These genera continue to be recognized, although subsequent studies have called for some revision of their content as well as the recognition of new genera.

Of particular interest has been Burger's concept of the genus *Porthidium,* which was polyphyletic. Werman (1992) addressed part of the problem by removing three species (*P. nummifer*, *P. olmec*, and *P. picadoi*) to a new genus, *Atropoides.* Campbell and Lamar (1992) later recognized another new genus, *Cerrophidion*, for a clade of three montane species that had been included in *Porthidium.* Gutberlet (1998a) removed *P. melanurum* from *Porthidium*, and placed it with its closest extant relative in the genus *Ophryacus*. Using molecular data, Kraus et al. (1996) and Parkinson (1999) found that *Porthidium hyoprora* was closely related to species of *Bothrops*, and did not form a monophyletic group with other *Porthidium* species. McDiarmid et al. (1999) assigned *P. hyoprora* to the genus *Bothrops*, and Gutberlet and Campbell (2001) subsequently erected a new genus *Bothrocophias* to accommodate *Bothrops campbelli*, *B. hyoprora*, *B. microphthalmus*, and the newly recognized *Bothrocophias myersi*.

Thus *Porthidium* currently contains seven species; eight other species have been allocated to different genera (Werman, 1992; Campbell and Lamar, 1992; Gutberlet, 1998a; Gutberlet and Campbell, 2001) and one new species (*P. volcanicum*) was recently described (Solórzano, 1994). *Porthidium almawebi* Schätti and Kramer (1993) was shown to be a junior synonym of *Bothrocophias campbelli* (Freire-Lascano, 1991; Kuch, 1997; Gutberlet and Harvey, 1998; Wüster, 1998; International Commission on Zoological Nomenclature, 1999). Phylogenetic analyses based on allozymes and morphology (Werman, 1992), mitochondrial DNA (Kraus et al., 1996; Parkinson, 1999; Parkinson et al., this volume), and morphology only (Gutberlet, 1998a) have convincingly demonstrated that *P. nasutum* and *P. ophryomegas* are closely related, but no study has attempted to determine whether all seven species of *Porthidium* form a monophyletic group. One purpose of this study was to critically evaluate *Porthidium* monophyly through phylogenetic analyses of anatomical features of representative New World crotaline snakes. This is the first phylogenetic study to include every species of *Porthidium*, hence it provides a unique opportunity to

[1]Department of Biology, The University of Texas at Arlington, Arlington, Texas 76019, USA

[2]Present Address: Department of Biology, The University of Texas at Tyler, 3900 University Blvd., Tyler, Texas 75799, USA
E-mail: Ronald_Gutberlet@mail.uttyl.edu

[3]Present Address: Department of Biological Sciences, Box 70703, East Tennessee State University, Johnson City, Tennessee 37614-0703, USA

Table 1. Summary of phylogenetic analyses of New World pitvipers.

Analysis number	Analysis description
1	Maximum ordering, all characters included, all taxa included
2	Maximum ordering, meristic characters excluded, all taxa included
3	Maximum ordering, all characters included, taxa with missing osteological data excluded
4	Maximum ordering, meristic characters excluded, taxa with missing osteological data excluded
5	Minimum ordering, all characters included, all taxa included
6	Minimum ordering, meristic characters excluded, all taxa included
7	Minimum ordering, all characters included, taxa with missing osteological data excluded
8	Minimum ordering, meristic characters excluded, taxa with missing osteological data excluded

make a definitive statement about the monophyly of this heretofore problematic genus.

The monophyly of *Atropoides* has recently been questioned. Phylogenetic analyses of nucleotide sequences from the mitochondrial ND4 gene (Kraus et al., 1996) suggested that *Atropoides* may be paraphyletic; however, phylogenetic analyses of sequences from 12S and 16S rRNA genes (Parkinson, 1999) supported the monophyly of *Atropoides*. Another purpose of our study is to revisit the question of *Atropoides* monophyly with an anatomical data set.

Despite recent success in identifying terminal clades (i.e., genera) of New World pitvipers, little resolution of generic relationships has been achieved. We hope that our analyses of new data from pitviper anatomy will contribute to the research cycle that seeks clarification of deeper relationships within the New World pitviper radiation. Such clarification is a prerequisite for a fuller understanding of the historical biogeography and evolution of these fascinating snakes.

MATERIALS AND METHODS

Our phylogenetic analyses included every species of *Porthidium* and representative species from every New World pitviper genus. Kraus et al. (1996), Vidal et al. (1997), Parkinson (1999), and Parkinson et al. (this volume) provided evidence that supports the monophyly of New World pitvipers. Among Old World pitvipers, species of *Gloydius* are closely related to New World pitvipers (Parkinson et al., this volume); thus, we used *Gloydius blomhoffii* as an outgroup.

Fifty-two of the anatomical characters included in this study were used by Gutberlet (1998a) in his study on the pitviper genus *Ophryacus.* An additional 24 anatomical characters described for this study are included. The following references provided important information for many of the anatomical characters: Burger (1971), Campbell (1976), Campbell and Lamar (1989), Campbell and Solórzano (1992), Crother et al. (1992), Dorcas (1992), Gloyd and Conant (1990), Kardong (1990), Klauber (1972), Malnate (1990), and Werman (1992). Characters were scored by examining museum specimens (Appendix I). Terminology for squamation and crania is mostly that of Klauber (1972), for vertebrae that of Hofstetter and Gasc (1969), and for hemipenes that of Dowling and Savage (1960).

Parsimony analyses (Farris, 1983; Farris and Kluge, 1985, 1986; Kluge and Farris, 1969) were implemented with the PAUP 3.1 computer program (Swofford, 1993) to estimate phylogenetic relationships of the ingroup taxa. Character polarities were determined by PAUP through outgroup rooting (Nixon and Carpenter, 1993; Swofford, 1993). The large number of taxa and characters included in this study required use of heuristic tree searches. These searches consisted of 200 replicates, each using TBR (tree-bisection-reconnection) branch-swapping. When more than one equally parsimonious tree resulted from a search, a strict consensus tree (Sokal and Rohlf, 1981) was used to summarize the results, thus depicting only the clades shared among all shortest trees.

Alternative Analyses

In order to minimize methodological concerns and to make the results as transparent as possible, an approach of alternative analysis was used. Whenever a particular aspect of methodology is controversial (e.g., ordered vs unordered treatment of multistate characters; Slowinski, 1993), multiple analyses are used so that results can be interpreted under a variety of assumptions. Congruence among results of alternative analyses circumvents methodological controversy. Incongruence of alternative analyses may require a more careful evaluation of assumptions or require acceptance of less phylogenetic resolution than might have resulted through ignoring alternative assumptions. The various analyses used in this study are summarized in Table 1 and fully described below.

Table 2. Frequency bins used for coding polymorphic characters (after Wiens, 1995).

Character/subcharacter state	Frequency range (%)
a	0–3
b	4–7
c	8–11
d	12–15
e	16–19
f	20–23
g	24–27
h	28–31
i	32–35
j	36–39
k	40–43
l	44–47
m	48–51
n	52–55
o	56–59
p	60–63
q	64–67
r	68–71
s	72–75
t	76–79
u	80–83
v	84–87
w	88–91
x	92–95
y	96–100

Multistate Characters

Thirty-five multistate characters (1–10, 12, 14, 16, 22, 26, 28–31, 36–41, 44–45, 47, 49, 52, 55, 61–62, 72, 74) are included in this study. Fourteen of these (1–10, 28–31, 62) are overlapping meristic characters and are discussed below under the heading Polymorphic Multistate Characters. That section also covers the three conventional multistate characters (16, 38, 52) that exhibit polymorphism in at least one taxon. Non-polymorphic multistates were treated in two ways, using maximum ordering and minimum ordering (Gutberlet, 1998a) in separate analyses (Table 1). Under maximum ordering, morphological intermediacy and adjacency are used to order characters. Under minimum ordering, intermediacy and adjacency are excluded as justification for ordering, but partial ordering is maintained when: (1) two or more states clearly represent modifications of the same trait, and (2) that trait is completely absent in some taxa (Campbell and Frost, 1993). Clades supported under both maximum and minimum ordering are robust to assumptions inherent in the use of each type of ordering.

Polymorphic Characters

The frequency bins approach (Wiens, 1993, 1995) was used to code the 10 binary characters (11, 18-19, 35, 42, 51, 53, 58–60) that exhibited polymorphism. For these characters, each taxon was assigned a letter from a to y, representing the observed frequency of the state presumed to be derived. Each letter represents a frequency range of 4% (except y, which encompasses 5%) from a (= derived state present in 0–3% of specimens examined) to y (= derived state present in 96–100% of specimens examined; see Table 2).

Seventeen polymorphic multistate characters are included in this study. Three of these characters (16, 38, 52) are conventional qualitative characters while 14 (1–10, 28–30, 62) are meristic characters—specifically scale and tooth counts—that exhibit intertaxonomic overlap in variation. In order to extract as much phylogenetic signal from these characters as possible, generalized frequency coding (GFC; Smith and Gutberlet, 2001) was used.

Using GFC, polymorphic multistate characters were divided into subcharacters so that each observable state could be treated separately. Variation within each subcharacter was then coded using frequency bins. Weighting was used so that the contribution of the set of subcharacters for a given character was equal to the contribution of one nonpolymorphic character. The computer program CodeThis! (Gutberlet et al., 2000) was used to transform raw data into GFC codes. (Codes for unordered treatment of characters 16 and 52 available from senior author).

The meristic characters included in the present study appear to be phylogenetically informative (Gutberlet, 1998a; Smith and Gutberlet, 2001). However, criticism of such characters (Crisp and Weston, 1987; Pimentel and Riggins, 1987) warrants caution with their use, so separate analyses were run with and without these characters (Table 1).

Character Weighting

Due to the complex character coding involved in this study (i.e., frequency bins coding and GFC), it was necessary to employ weighting to ensure that each character contributed equally to the analysis. This was accomplished by assigning a base weight of 32,767 (the greatest allowable weight in PAUP 3.1) to every character or subcharacter. Because weights in PAUP 3.1 must be whole numbers, use of the largest

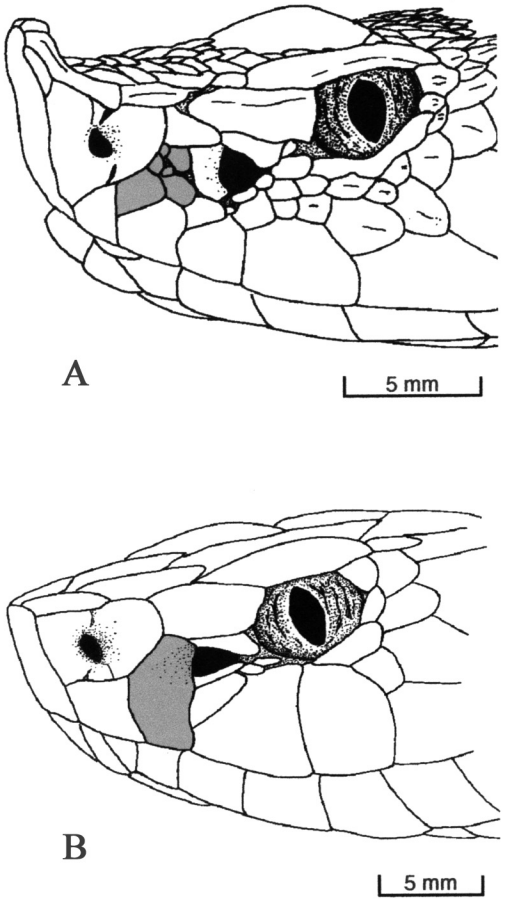

Fig. 1. Differences in number of interoculabials (character 1), number of prefoveals (character 2, shaded in A, *Porthidium nasutum*, UTA R-25372), condition of prelacunal (character 16, shaded in B, *Agkistrodon piscivorus*, UTA R-28771), and forward extension of suboculars (character 57).

allowable base weight results in the most exact relative weighting possible.

Subcharacters and polymorphic binary characters were subsequently down-weighted. Because subcharacters and polymorphic binary characters are divided into many ordered states, they will receive greater weight than conventionally coded characters (Wiens, 1995). To account for this potential bias, the base weight of each subcharacter and polymorphic binary character was divided by the number of steps between the lowest and highest frequency bins included in it (unequal subcharacter weighting; Smith and Gutberlet, 2001). Additionally, the weight of each subcharacter was divided by the total number of informative subcharacters used to represent the single character of which it is a part (Appendix II).

Missing Data

Owing to the rarity of some species included in this study, scoring every character for every species was not possible. Osteological data are missing for *Bothrocophias campbelli, Porthidium hespere, P. lansbergii, P. volcanicum,* and *P. yucatanicum,* and hemipenial data are missing for *B. campbelli*, *B. microphthalmus*, *P. hespere*, and *P. volcanicum*. All

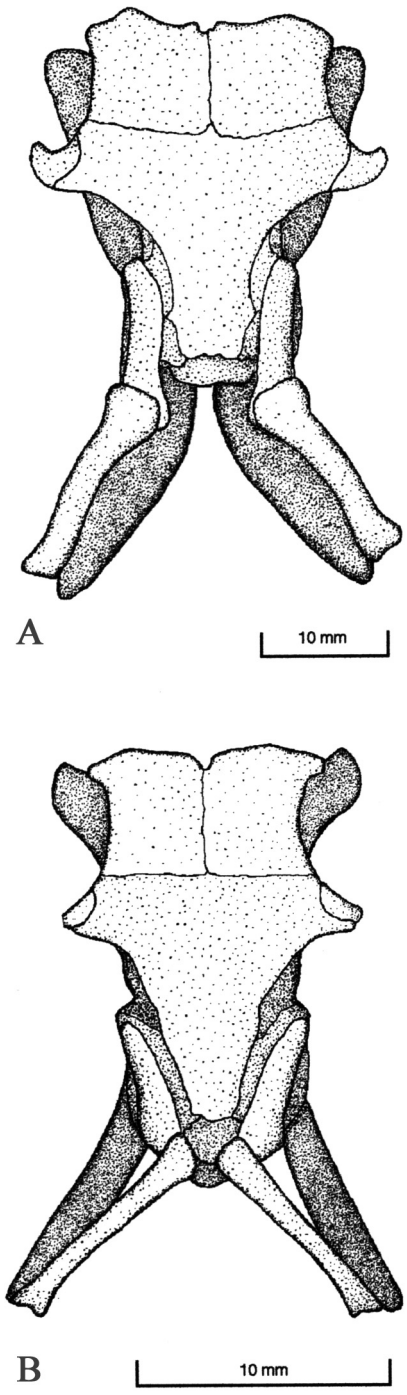

Fig. 2. Dorsal aspect of skulls of (A) *Atropoides picadoi* (UTA R-15617) and (B) *Cerrophidion godmani* (UTA R-38106), illustrating minimum width across both frontal bones (character 45) and shape of the postfrontal bones (character 75). Bones anterior to the frontals are not illustrated.

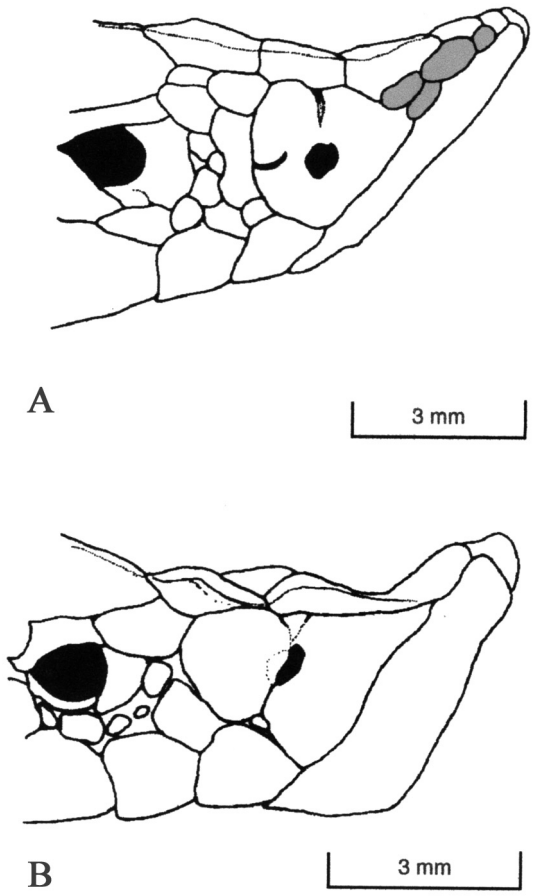

Fig. 3. Snouts of *Bothrocophias hyoprora* (A, FMNH 56171) and *Porthidium nasutum* (B, UTA R-31057), illustrating presence (A, shaded) and absence (B) of canthorostrals (character 53).

other anatomical data are included. To investigate the effect of missing data for these species, alternative analyses were run that excluded them. Wiens and Reeder (1995) supported the inclusion of taxa with incomplete data but noted that inclusion of such taxa may result in decreased phylogenetic accuracy. Wiens and Reeder (1997) suggested that relationships recovered regardless of inclusion or exclusion of incomplete taxa can be interpreted as well supported, whereas relationships that vary with respect to inclusion or exclusion of taxa require further study.

Evaluation of Phylogenetic Results

Nonparametric bootstrapping (Felsenstein, 1985) was used to estimate support for individual branches of the phylogenies recovered in this study. Hillis and Bull (1993) provided evidence that clades with bootstrap proportions of 70% or greater are likely to be accurate. Arguments against bootstrapping phylogenies (Campbell and Frost, 1993:58, and citations therein) should be considered when evaluating bootstrap results.

Bootstrap analyses were based on 100 pseudoreplicates. Within each pseudoreplicate, PAUP sampled characters with equal probability and then applied weights after the data matrix was complete. Uninformative characters were not sampled. Heuristic searches were used within each pseudoreplicate, which consisted of two random taxon addition tree searches with TBR branch swapping. Phylogenies generated by analyses 2 and 6 could not be bootstrapped within a reasonable amount of time—possibly due to many missing cells in the data matrix.

CHARACTER DESCRIPTIONS

The characters used in this study are features of squamation (1–21, 53–62), hemipenes (22–24, 71–73), vertebrae (25–26), crania (27–52, 74–76), color pattern (67–70), and other miscellaneous features of anatomy (63–66). Gutberlet (1998a) provided definitions for the first 52 characters, and additional details about these characters are available in Gutberlet (1998b). Characters 53–76 have been added for this study and are described below. Those characters adapted from Werman (1992) are so indicated and labeled with the number used by Werman. Owing to the greater number of taxa in the present study, it was necessary to add new states for some of the previously described characters. (Character state assignments for all characters available from senior author).

Characters

1. Number of interoculabials (Fig. 1).
2. Number of prefoveals (Werman no. 37, in part; (Fig. 1).
3. Number of suboculars.
4. Number of supralabials (Werman no. 26).
5. Number of canthals (Werman no. 32).
6. Number of intersupraoculars (Werman no. 25).
7. Number of interrictals.
8. Number of gulars between the chin shields and the first ventral (first ventral in the sense of Klauber, 1972).
9. Number of ventrals.
10. Number of middorsal scale rows.
11. Loreal: (a) entire, (y) fragmented vertically (Werman no. 31 in part).
12. Rostral: (0) broader than high, (1) approximatelyas broad as high, (2) higher than broad.
13. Upper preocular: (0) entire, (1) divided.
14. Supraocular horn: (0) absent, (1) present, one per side, (2) present, two per side (Werman no. 33).
15. Canthals: (0) flat, (1) raised into small horns.

16. Prelacunal and second supralabial: (0) fused (Fig.1b), (1) not fused, subfoveals absent, (2) separated by one row of subfoveals (Fig. 1a), (3) separated by two rows of subfoveals (Werman no. 23 and 29).
17. Scales in parietal region: (0) keeled, (1) tuberculate.
18. Middle preocular and supralacunal: (a) fused, (y) not fused (Werman no. 36).
19. Sublacunal: (a) entire, (y) divided, with an anterior and posterior component (Werman no. 35).
20. Canthus rostralis: (0) not elevated, (1) elevated, forms a ridge (Werman no. 38).
21. Loreals: (0) not projecting laterally, (1) projecting laterally (Werman no. 39).
22. Subcaudals: (0) divided, (1) divided and entire, (2) entire (Werman no. 24).
23. Papilla protruding from apex of hemipenis: (0) absent, (1) present.
24. Basal and lateral hemipenial spines: (0) many, densely distributed, (1) few, widely spaced.
25. Calyces on lateral surfaces of hemipenial lobes: (0) restricted to distal portion of lobe, (1) extending proximally to level of crotch.
26. Pleurapophyses of midcaudal vertebrae: (0) long and slender, (1) short and slender, (2) short and wide.
27. Haemapophyses of midcaudal vertebrae: (0) not in contact distally, (1) in contact distally.
28. Number of palatine teeth.
29. Number of pterygoid teeth (Werman no. 51, in part).
30. Number of dentary teeth.
31. Length of maxillary fang: (0) short, maximum length only slightly greater than height of maxilla, (1) moderate, approximately 1.5 times longer than height of maxilla, (2) long, approximately two times longer than height of maxilla. (Werman no. 43).
32. Medial wall of pit cavity in maxilla: (0) weakly developed to almost absent, (1) well-developed (Werman no. 40).
33. Small pit in anterolateral wall of pit cavity in maxilla: (0) absent, (1) present (Werman no. 41).
34. Anterior foramina of prootic: (0) separated by a bony partition, (1) not separated by a bony partition (Werman no. 61).
35. Foramen in ventral surface of lateral process of prootic: (a) absent, (y) present.
36. Lateral portion of head of ectopterygoid in dorsal view: (0) broad, (1) intermediate, (2) narrow (Werman no. 42 and 46).
37. Shaft of ectopterygoid: (0) flat, broad, does not taper posteriorly, (1) flat, gradually tapers posteriorly, (2) narrow, does not taper posteriorly (Werman no. 47).
38. Pits at point of attachment of ectopterygoid retractors on posterior surface of anterior end of ectopterygoid: (0) absent, (1) single, (2) paired (Werman no. 48).
39. Base of ectopterygoid at point of articulation with pterygoid: (0) with a short, well-defined, finger- like projection that articulates with pterygoid, (1) with an elongate, less defined projection that broadly overlaps pterygoid, (2) elongate projection present but not set off from rest of bone, i.e, spatulate (Werman no. 49).
40. Ectopterygoid: (0) shorter than base of pterygoid, (1) approximately equal in length to base of pterygoid, (2) longer than base of pterygoid (Werman no. 50).
41. Choanal process of palatine: (0) positioned anteriorly, (1) positioned medially, (2) positioned posteriorly (Werman no. 52, in part).
42. Ventral process of basioccipital: (a) single, (y) bifurcates distally.
43. Lateral processes of prefrontal: (0) directed laterally, (1) directed ventrally (Werman no. 53 and 54).
44. Medial margin of dorsal portion of prefrontal: (0) strongly concave, (1) moderately concave, (2) weakly concave (Werman no. 55).
45. Minimum width across both frontals: (0) less than (Fig. 2b), (1) equal to, or (2) greater than width of skull at anterior end of supratemporals (Fig. 2a) (Werman no. 56).
46. Dorsal surface of frontals: (0) predominantly flat, (1) with elevated lateral margins (Werman no. 57).
47. Posterolateral edges of dorsal surface of parietal: (0) slope ventrolaterally, (1) intermediate, with a small lateral shelf of bone, (2) flare laterally and slightly dorsad (Werman no. 58, in part).
48. Size of postfrontal: (0) large, contributing as much or more to the dorsal margin of the orbit than the parietal does, (1) small, contributing less to the dorsal margin of the orbit than the parietal does (Werman no. 60).
49. Supratemporal: (0) expanded posteriorly but lacking a distinct projection, (1) with small posterolateral projection, (2) with large, hook-like posterolateral projection (Werman no. 59).

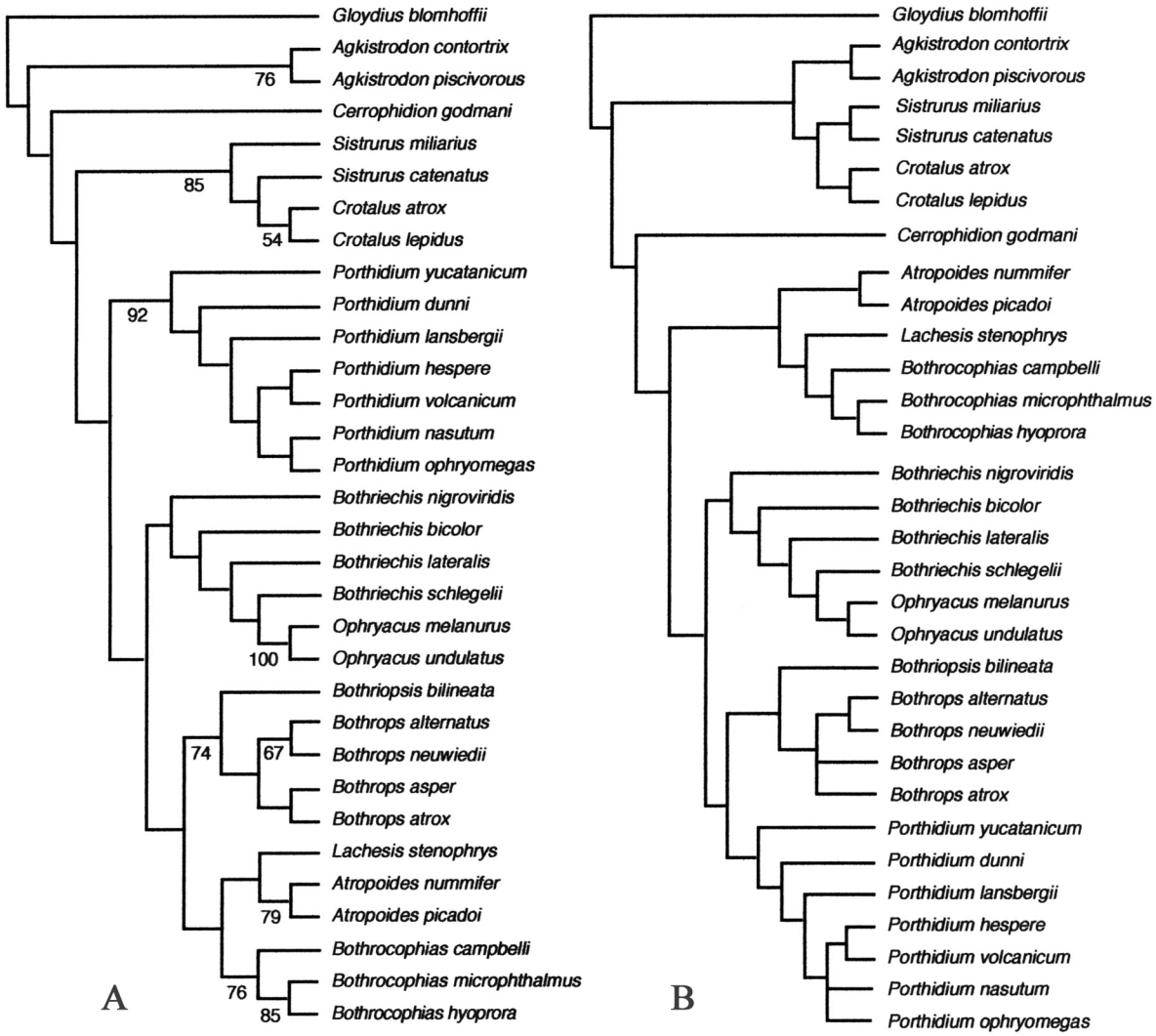

Fig. 4. (A) Single shortest tree (8,653,823 weighted steps, consistency index = 0.364, retention index = 0.653) recovered by Analysis 1 (maximum ordering, all characters included, all taxa included). Numbers below the branches are bootstrap proportions. (B) Strict consensus of 3 shortest trees (6,992,876 weighted steps, consistency index = 0.384, retention index = 0.681) recovered by Analysis 2 (maximum ordering, meristic characters excluded, all taxa included).

50. Supratemporal: (0) thick with a rounded dorsal surface, (1) thin with a flat dorsal surface.
51. Meckellian foramen: (a) completely or partially divided into two foramina, (y) single foramen, not divided.
52. Angular and splenial: (0) separate, (1) partially fused, (2) completely fused (Werman no. 44 and 45).
53. Canthorostrals: (a) absent (Fig. 3b), (y) present (Fig. 3a). Canthorostrals are tiny scales positioned between the rostral and the internasals. Of the taxa studied, only *B. microphthalmus* and *B. hyoprora* exhibit the derived condition. Both of these taxa are polymorphic for this trait.
54. Dorsal head scales: (0) smooth, (1) keeled (Werman no. 28). Gutberlet (1998a) excluded this character, because among the taxa included in that study keeling of dorsal head scales is directly correlated with fragmentation of head shields. Fragmentation of head shields is already coded in character 6 above (number of intersupraoculars). The larger taxonomic sample used in this study includes some taxa with head scales that are both fragmented and smooth; thus, the inclusion of this separate character is both justified and warranted.

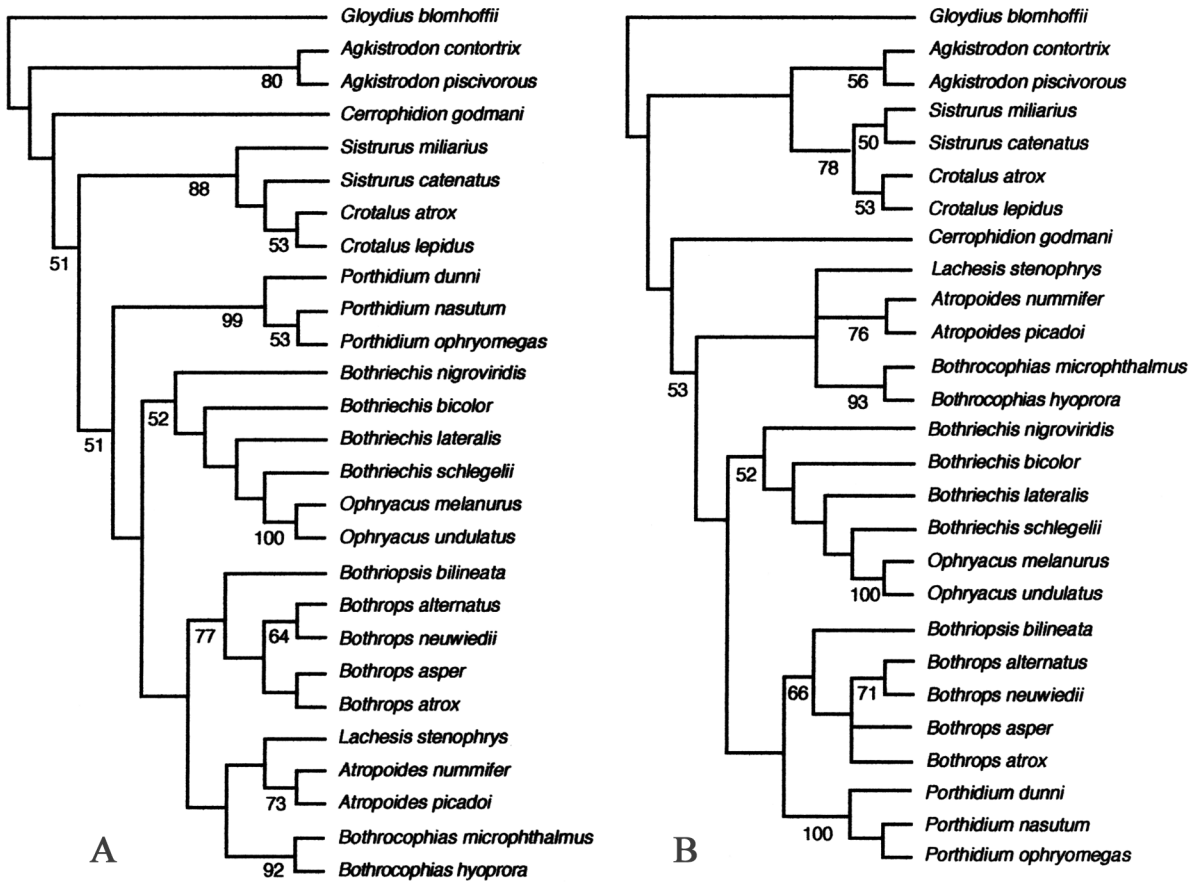

Fig. 5. (A) Single shortest tree (8,479,323 weighted steps, consistency index = 0.371, retention index = 0.637) recovered by Analysis 3 (maximum ordering, all characters included, taxa with missing osteological data excluded). (B) Strict consensus of 6 shortest trees (6,939,641 weighted steps, consistency index = 0.387, retention index = 0.663) recovered by Analysis 4 (maximum ordering, meristic characters excluded, taxa with missing osteological data excluded). Numbers below the branches are bootstrap proportions.

55. Keel on dorsal scales: (0) typical thin ridge, (1) tuberculate on dorsals on caudal part of body, (2) tuberculate on all dorsals. Based on morphological intermediacy, it can be argued that 0→1→2 constitutes an ordered transformation series.
56. Keel on parasubcaudals: (0) present, (1) absent. The parasubcaudals are the scales on the lateral surface of the tail that contact the subcaudals.
57. Suboculars: (0) excluded from anteroventral corner of orbit, (1) extend to anteroventral corner of orbit (Werman no. 34 in part). In *G. blomhoffii* and *Agkistrodon piscivorus,* a supralabial scale contacts the orbit, occupying its anteroventral corner.
58. Sublacunal: (a) entire, (y) divided, with an internal and external component. In several taxa, the portion of the sublacunal inside the loreal pit is separated from the external portion by a complete suture. In other taxa studied, the internal and external portions of this scale are completely fused. Some, but not all, of the taxa exhibiting an internal-external division also exhibit the anterior-posterior division (character 19). Species of *Bothriechis* exhibit the anterior-posterior division but lack the internal-external division.
59. Loreal: (a)entire, (y) divided horizontally (Werman no. 31 in part). Taxa with state y exhibit two loreals on each side of the head, one dorsal to the other. *Crotalus atrox* is polymorphic for this character.
60. Loreal: (a) contacts canthals, (y) does not contact canthals. *Crotalus atrox* is polymorphic for this character.
61. Loreal: (0) longer than high, (1) approximately as long as high, (2) higher than long. Based on morphological intermediacy, it can be argued that 0→1→2 constitutes an ordered transformation series.

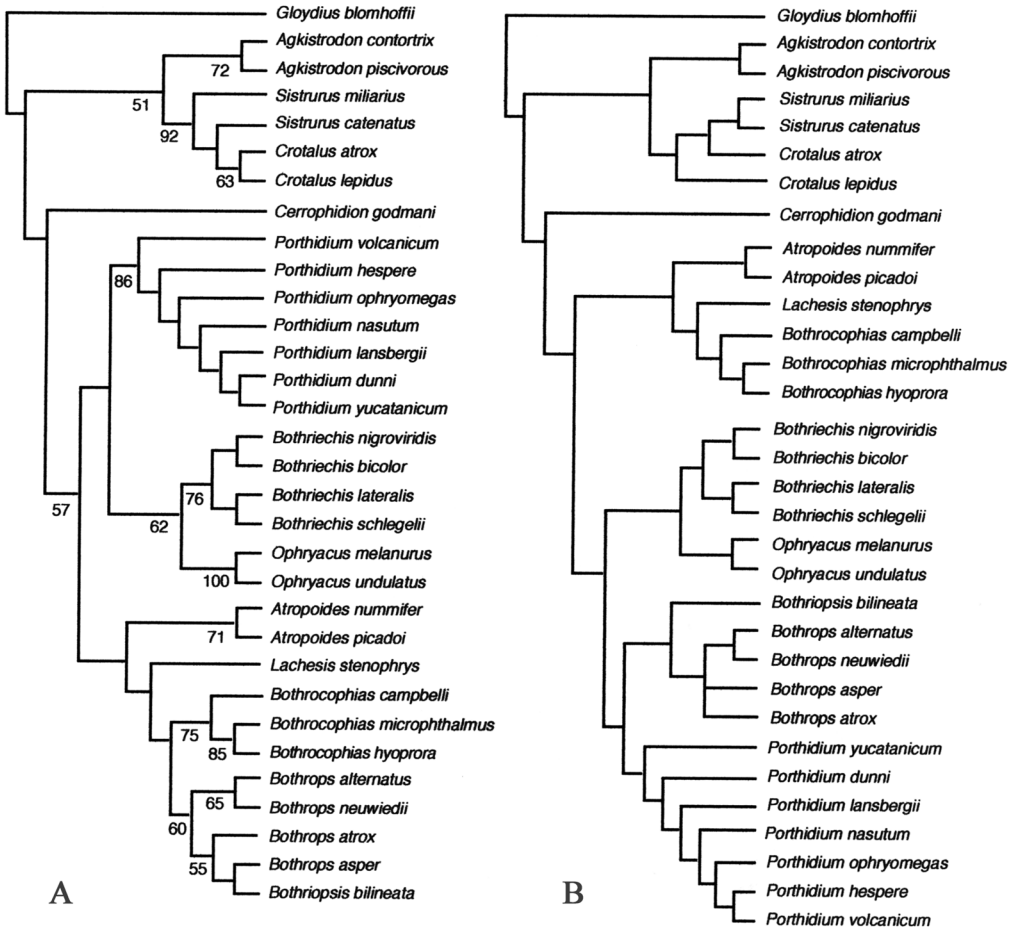

Fig. 6. (A) Single shortest tree (8,152,383 weighted steps, consistency index = 0.382, retention index = 0.647) recovered by Analysis 5 (minimum ordering, all characters included, all taxa included). Numbers below the branches are bootstrap proportions. (B) Strict consensus of 3 shortest trees (6,459,633 weighted steps, consistency index = 0.411, retention index = 0.681) recovered by Analysis 6 (minimum ordering, meristic characters excluded, all taxa included).

62. Number of subcaudals: Subcaudals are the rectangular scales on the ventral surface of the tail.
63. Nasal pore: (0) present, (1) absent. The nasal pore is a tiny opening inside the nostril of most snakes. This pore opens into a duct that leads to the lateral nasal gland (Burger, 1971) and has been described by Kathariner (1900). Maslin (1942) reported that the nasal pore is absent in *Tropidolaemus wagleri* and several species of *Bothrops* (*sensu lato*). Burger (1971) found that in fact the nasal pore is present in most New World pitvipers including species of *Bothrops*. Observations in our study are in agreement with those of Burger: only the four rattlesnake species studied lacked this structure.
64. Loreal pit: (0) crossed by naso-orbital line, (1) ventral to naso-orbital line. The naso-orbital line is an imaginary line that extends between the ventral edge of the eye and the ventral edge of the nostril and was used by Burger (1971) to discuss the position of the loreal pit.
65. Rattle: (0) absent, (1) present.
66. Tail: (0) not prehensile, (1) prehensile.
67. Distinct white spots on posterior infralabials and gulars: (0) absent, (1) present. Taxa assigned state 1 have small white spots with dark borders on the chin scales.
68. Orange middorsal stripe: (0) absent, (1) present. Many populations of *Sistrurus miliarius* and *Crotalus horridus* exhibit some orange coloration dorsally, but this color does not usually form a distinct line: *S. miliarius* is coded with state 0.
69. Tail pattern: (0) not banded, (1) banded.
70. Dorsum with green ground color: (0) absent, (1) present.

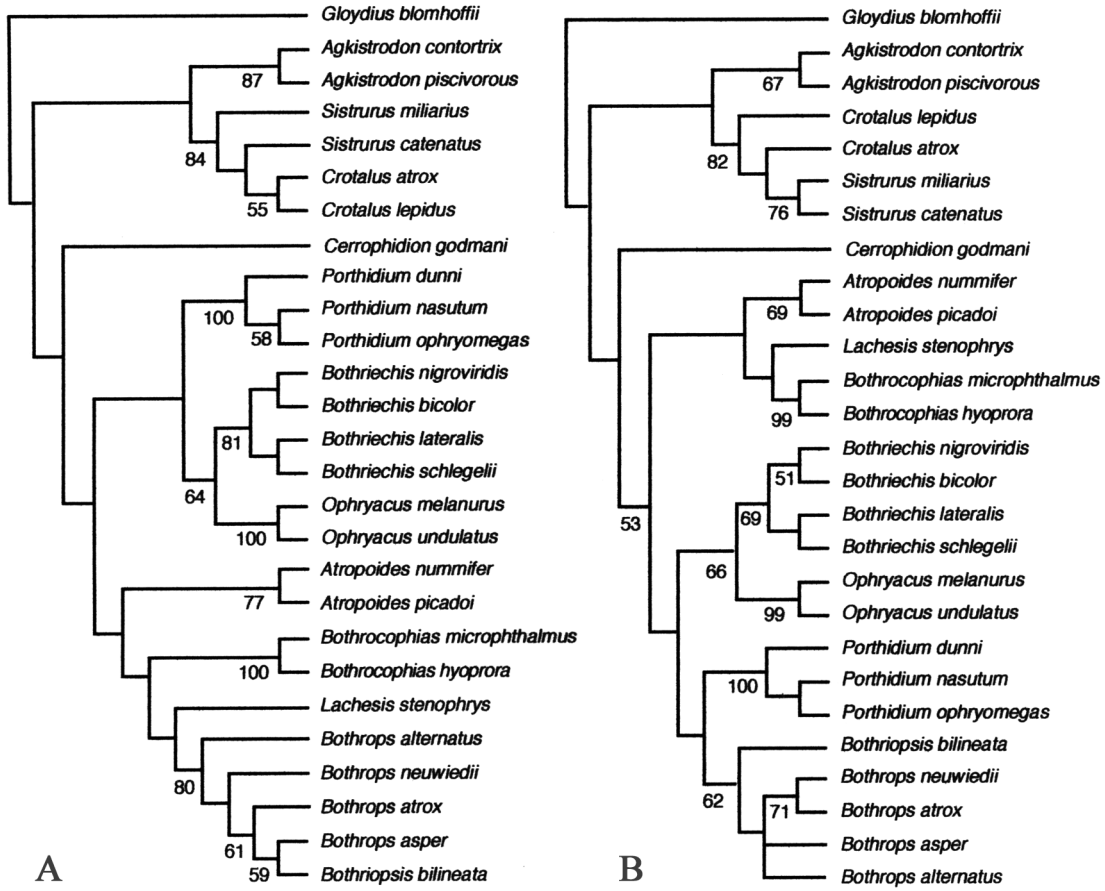

Fig. 7. (A) Single shortest tree (7,958,179 weighted steps, consistency index = 0.391, retention index = 0.631) recovered by Analysis 7 (minimum ordering, all characters included, taxa with missing osteological data excluded). (B) Strict consensus of 3 shortest trees (6,372,616 weighted steps, consistency index = 0.416, retention index = 0.665) recovered by Analysis 8 (minimum ordering, meristic characters excluded, taxa with missing osteological data excluded). Numbers below the branches are bootstrap proportions.

71. Mesial spines on hemipenial lobes: (0) absent, (1) present. Taxa assigned state 0 exhibit only low calyces on the mesial surface of each hemipenial lobe.
72. Hemipenial lobes: (0) deeply divided, greater than two times longer than base, (1) moderately divided, approximately two times longer than base, (2) partially divided, approximately as long as base, (3) weakly divided, shorter than base.
73. Calyces on hemipenial lobes: (0) spinulate, (1) smooth. On the hemipenes of most taxa studied, calyx ridges are adorned with tiny spinules, but in *Bothrops alternatus, B. neuwiedii,* and *Lachesis stenophrys* calyx ridges are smooth.
74. Size of choanal process of palatine: (0) greatly reduced, (1) reduced, (2) moderate, (3) attenuate (Werman 52, in part). The choanal process is rounded and almost indistinguishable from the rest of the bone in taxa with state 0. The choanal process is low and rounded distally in taxa with state 1. The choanal process is triangular in taxa with state 2. The choanal process tapers to a thin point distally in taxa with state 3. Based on morphological intermediacy, it can be argued that 0→1→2→3 constitutes an ordered transformation series.
75. Postfrontal: (0) curves posterolaterally (Fig. 2b), (1) angles anteriorly (Fig. 2a). In the two species of *Atropoides* included in this study, the posterior portion of the postfrontal bone angles anteriorly rather than laterally.
76. Medial process at posterior end of ectopterygoid: (0) weakly developed, (1) large and prominent.

RESULTS

Selected clades recovered by analyses 1–8 are summarized in Table 3, and a more detailed presentation of the results of these analyses will be found in

Table 3. Major clades of New World pitvipers recovered in phylogenetic analyses.

Clade	Number of analyses	Range of bootstrap proportions
Agkistrodon	8/8	56–87
Atropoides	8/8	69–79
Bothriechis	4/8	65–81
Bothrops[a] + *Bothriopsis*	8/8	60–80
Bothrocophias	8/8	75–100
Ophryacus	8/8	99–100
Porthidium (*sensu stricto*)[b]	8/8	86–100
Crotalus + *Sistrurus*	8/8	78–92
Bothriechis + *Ophryacus*	8/8	< 50–66
Bothrocophias + *Bothrops/Bothriopsis*	1/8	< 50
Agkistrodon + *Crotalus/Sistrurus*	6/8	< 50–51

[a]*Bothrops* includes only *B. alternatus*, *B. asper*, *B. atrox*, and *B. neuwiedii*.
[b]*Porthidium* (*sensu stricto*) includes all *Porthidium* species, but not the recently reallocated *Bothrocophias hyoprora*.

Figs. 4–7. Analysis 1 (Table 1) generated a single shortest tree (Fig. 4a), with 8,653,823 weighted steps, a consistency index (CI) of 0.364, and a retention index (RI) of 0.653. Analysis 2 generated three shortest trees (Fig. 4b), with 6,992,876 weighted steps, a CI of 0.384, and a RI of 0.681. Analysis 3 generated a single shortest tree (Fig. 5a), with 8,479,323 weighted steps, a CI of 0.371, and a RI of 0.637. Analysis 4 generated six shortest trees (Fig. 5b), with 6,939,641 weighted steps, a CI of 0.387, and a RI of 0.663. Analysis 5 generated a single shortest tree (Fig. 6a), with 8,152,383 weighted steps, a CI of 0.382, and a RI of 0.647. Analysis 6 generated three shortest trees (Fig. 6b), with 6,459,633 weighted steps, a CI of 0.411, and a RI of 0.681. Analysis 7 generated a single shortest tree (Fig. 7a), with 7,958,179 weighted steps, a CI of 0.391, and a RI of 0.631. Analysis 8 generated three shortest trees (Fig. 7b), with 6,372,616 weighted steps, a CI of 0.416, and a RI of 0.665.

In every analysis, the seven species currently included in *Porthidium* formed a monophyletic group. Unique, derived features shared by species in this genus include few (usually 2) gulars between the chin shields and first ventral (Fig. 1 in Gutberlet, 1998a), an apical papilla on each lobe of the hemipenis (Fig. 2 in Gutberlet, 1998a), an orange middorsal stripe, and an anteriorly positioned choanal process of the palatine bone. Other derived characters, though not unique to the genus, include a single row of subfoveals between the prelacunal and second supralabial (Fig. 1a), 2–8 prefoveals (Fig. 1a), and interior-exterior division of the sublacunal.

Bothrocophias hyoprora always formed a monophyletic group with *B. campbelli* and *B. microphthalmus* in the analyses that included these species. Synapomorphies of *Bothrocophias* include distinctive white spots on gular and infralabial scales and tuberculate keels on the caudal portion of the dorsum. Species of this genus are unusual in that the small intersupraocular scales are not keeled. Many specimens of *B. hyoprora* and *B. microphthalmus* have canthorostral scales (Fig. 3a), but these scales have been observed in neither *B. campbelli* nor *B. myersi* (Gutberlet and Campbell, 2001).

The monophyly of *Atropoides* was supported in every analysis. Derived characters shared by *A. nummifer* and *A. picadoi* include a high number of intersupraocular scales (typically 8–10) and postfrontal bones that angle anteriorly (Fig. 2a).

DISCUSSION

In drawing conclusions about the evolutionary history of New World pitvipers from the eight phylogenetic analyses we conducted herein, we note that a clade's appearance in a single shortest tree may not itself represent strong evidence that the clade is valid. The likelihood of recovering a single shortest tree increases as the number of characters increases relative to the number of taxa. Even large, artificial data sets containing random information can generate a single shortest tree (Hillis and Huelsenbeck, 1992). Furthermore, the ordered treatment of finely divided characters, as occurs with frequency bins coding and GFC, increases the likelihood of recovering a single shortest tree regardless of the quality of the data. Thus, measures must be taken to evaluate accuracy of putative clades.

Two useful criteria for assessing accuracy of recovered clades are (1) the clade's robustness to various analytical assumptions, and (2) the frequency with which the clade is recovered in nonparametric bootstrap analyses. These criteria also provide an indication of the overall strength of phylogenetic signal within the data set. In this study, clades are judged to be well-supported when they were recovered in a majority of the analyses, and when they were supported by bootstrap proportions of ≥ 70% (Table 3).

Minimum Ordering vs Maximum Ordering

A comparison of the shortest trees recovered in analyses 1–4 with those recovered in analyses 5–8 demonstrates a high degree of congruence between maximum and minimum ordering analyses. Congruence is nearly complete when only clades supported by bootstrap proportions of ≥ 70% are considered (Table 3, Figs. 4–7).

One result in all maximum ordering analyses (1–4) that is inconsistent with the results of former studies (Crother et al., 1992; Werman, 1992; Gutberlet, 1998a) is that *Bothriechis* is paraphyletic with respect to *Ophryacus*. This result is unlikely to be accurate because it does not occur in any of the minimum ordering analyses and has no bootstrap support in the maximum ordering analyses (e.g., Fig. 4a). This may indicate that ordering assumptions were flawed for one or more characters. The extensive congruence between maximum and minimum ordering analyses, however, suggests that most characters were ordered correctly or that the phylogenetic signal in the data set is strong enough to overcome any faulty character ordering. Clades that are robust to ordering assumptions include *Agkistrodon*, *Atropoides*, *Crotalus* + *Sistrurus*, *Ophryacus*, *Porthidium*, *Bothrocophias*, and *Bothrops* + *Bothriopsis.*

Overlapping Meristic Characters

The effect of excluding meristic characters can be evaluated by comparing the following pairs of analyses: 1 and 2, 3 and 4, 5 and 6, 7 and 8. In general, most terminal clades (see Table 3 and Figs. 4–7) are robust to inclusion or exclusion of meristic data, however deeper relationships depicted in the shortest trees are markedly different between analyses including and excluding these data. None of these deeper relationships has strong bootstrap support, so the importance of these differences may be minor.

Several factors indicate that meristic data contribute important phylogenetic information to the study. When the meristic data are analyzed alone, they recover several clades that are well-supported by the entire study as well as other studies using molecular data. Additionally, bootstrap support for terminal clades is noticeably greater overall when the meristic data are included.

Complete Data vs Incomplete Data

The effect of including taxa with missing data can be evaluated by comparing the following pairs of analyses: 1 and 3, 2 and 4, 5 and 7, 6 and 8. Results within each pair of analyses are nearly identical; thus, inclusion of taxa with missing osteological data does not appear to have any effect on phylogenetic accuracy in this study. The analyses that include these taxa are preferred, because they allow phylogenetic placement of the incomplete taxa (Wiens and Reeder, 1997). Bootstrap proportions are slightly greater overall in the analyses that exclude taxa with missing data.

Taxonomy

The seven species of *Porthidium* form a well-supported, monophyletic group (Table 3, Figs. 4–7). The anatomical data strongly suggest that *Bothrocophias hyoprora*, despite its convergent snout morphology and its mostly entire subcaudals, is not a member of this clade. This result is consistent with the findings of Kraus et al. (1996) and Parkinson (1999). The phylogenetic affinities of *B. hyoprora* clearly lie with three other South American pitviper species now assigned to the genus *Bothrocophias*. Interestingly, this newly recognized lineage (Gutberlet and Campbell, 2001) may not form a monophyletic group with species of *Bothrops* and *Bothriopsis* (Figs. 4–7, Table 3).

A growing number of studies (Campbell and Lamar, 1992; Kraus et al., 1996; Werman, 1992; Salomão et al., 1997; Vidal et al., 1997; Parkinson, 1999; Parkinson et al., this volume) demonstrate that species of *Bothriopsis* are more closely related to species of *Bothrops* than they are to species of *Bothriechis*. Ours is the first study to include characters obviously related to arboreality (e.g., green dorsal coloration and prehensile tail) in phylogenetic analyses of these snakes. The parsimony analyses provide strong evidence that these features evolved independently in *Bothriechis* and *Bothriopsis;* many other derived features establish them as different lineages (Figs. 4–7).

Based on species included in this study, monophyly of the following genera is well-supported: *Agkistrodon*, *Atropoides*, *Bothriechis*, *Bothrocophias*, *Crotalus*,

Ophryacus, and *Porthidium* (*sensu stricto*). Rattlesnake monophyly is supported. The monophyly of *Sistrurus* was strongly supported in only two analyses (7 and 8), but too few rattlesnake species were included in this study to interpret this result. *Bothrops* is paraphyletic with respect to *Bothriopsis.*

Intrageneric and Deeper Relationships

Relationships within genera and among deeper branches of the phylogeny were poorly resolved in this study. This problem is common to other studies (e.g., Kraus et al., 1996; Wiens and Reeder, 1997; Gutberlet, 1998a) and is often attributed to rapid speciation (Lanyon, 1988; Kraus and Miyamoto, 1991; Donoghue and Sanderson, 1992). Kraus and Miyamoto (1991) suggested that extensive DNA sequence data from relatively slowly evolving genes may be necessary to provide greater resolution in deeper regions of phylogenies.

Despite the overall lack of resolution of intergeneric relationships, some information about deeper relationships was gained. The most parsimonius explanation of the data in six of eight analyses indicates that *Agkistrodon* and the rattlesnakes (*Crotalus* and *Sistrurus*) form a clade. Though this result is not strongly supported by the bootstrap (Table 3), it is consistent with the findings of Kraus et al. (1996), Parkinson (1999), and Parkinson et al. (this volume).

Werman (1999) noted that recent studies have presented conflicting hypotheses about the relationships of *Atropoides*, *Cerrophidion*, *Porthidium*, and the *Bothrops-Bothriopsis* clade. Studies by Kraus et al. (1996), Parkinson (1999), and Parkinson et al. (this volume) show that *Atropoides, Cerrophidion,* and *Porthidium* are closely related, but other studies (Werman, 1992; Gutberlet, 1998a) suggested that *Porthidium* is more closely related to *Bothrops-Bothriopsis*. Although *Porthidium* grouped with *Bothrops-Bothriopsis* in the shortest trees generated by several of our analyses, this relationship was never supported by our bootstrap analyses. We argue that the lack of strong support for a *Bothrops-Bothriopsis-Porthidium* clade lends support to Werman's (1999) hypothesis that similar features of skull anatomy in *Porthidium* and several species of *Bothrops* may be the result of convergent evolution, and not evidence of a close relationship between these genera.

Historical Biogeography

Prior to the discovery that *Bothrocophias hyoprora* should not be included in the genus *Porthidium* (Kraus et al., 1996; Parkinson, 1999; Gutberlet and Campbell, 2001; Parkinson et al., this volume; this study), the historical biogeography of *Porthidium* was difficult to explain. The Amazonian distribution of *hyoprora* was anomalous relative to the predominantly Middle American distribution of other *Porthidium* species. Our finding that *B. hyoprora* is closely related to *B. microphthalmus* is consistent with the geographic distribution of these snakes, since the latter occurs on the eastern slopes of the Andes in Colombia, Ecuador, Peru, and Bolivia. *Porthidium* as now recognized represents a geographically cohesive group occupying much of Middle America and northern South America north and west of the Andes.

Parkinson (1999) identified two monophyletic groups of New World pitvipers: (1) a North American group containing *Agkistrodon*, *Crotalus*, and *Sistrurus*, and (2) a Neotropical group containing the other New World genera. Our study supports Parkinson's hypothesis, which is also appealing on the basis of geography alone. Our study hints that there may be a monophyletic South American group within the Neotropical clade, but this hypothesis is preliminary at best. This study and others (Campbell and Lamar, 1992; Werman, 1992; Kraus et al., 1996; Salomão et al., 1997; Vidal et al., 1997; Parkinson, 1999; Parkinson et al., this volume) strongly support the close relationship of the South American genera *Bothrops* and *Bothriopsis*. Two other clades, *Bothrocophias* and *Lachesis*, may be the closest relatives of *Bothrops-Bothriopsis*. The phylogenetic position of *Lachesis* is often close to *Bothrocophias* in this study, and together these clades often group with *Bothrops-Bothriopsis*. An unexpected result is that *Atropoides* is sometimes included in this clade. Though this putative South American group (*Bothriopsis*, *Bothrocophias*, *Bothrops*, and *Lachesis*) makes sense geographically, it was not supported by bootstrapping. Additional studies are needed to evaluate this tentative hypothesis, especially since the data of Parkinson (1999) and Parkinson et al. (this volume) suggest that *Lachesis* may be more closely related to the Middle American taxa.

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APPENDIX I

Specimens examined.

Specimens examined for this study are housed in the American Museum of Natural History (AMNH), California Academy of Science (CAS), Carnegie Museum of Natural History (CM), Field Museum of Natural History (FMNH), United States National Museum (USNM), University of Kansas Museum of Natural History (KU), and The University of Texas at Arlington Collection of Vertebrates (UTA).

Alcohol-Preserved Specimens.—*Agkistrodon contortrix* (UTA R-426, 14724, 15619, 15621, 15623–24, 15626–27, 16851, 19339, 22438, 24533). *Agkistrodon piscivorus* (UTA R-1449–51, 1456, 2206, 17043, 17057–59). *Atropoides nummifer* (KU 191206, UTA R-12943, 14234, 24842–43, 25864–65). *Atropoides picadoi* (UTA R-16111–12, 18215, 23838, 23848–49, 24834). *Bothriechis bicolor* (UTA R-24758–59, 31977, 34535, 39414, 39419). *Bothriechis lateralis* (KU 180261, UTA R-16069, 16071–74, 16350). *Bothriechis nigroviridis* (UTA R-2801, 2808, 7463, 9637, 10433, 24841). *Bothriechis schlegelii* (KU 112607, 179509, UTA R-12956, 21864–66, 21868–71, 26395, 28553, 28555, 28557). *Bothriopsis bilineata* (KU 112296, UTA R-6310, 15650, 16084, 19490, 31019, 39101). *Bothrocophias campbelli* (AMNH 22094, USNM 165322, 165340). *Bothrocophias hyoprora* (FMNH 27597, 56171, 83079, 165849, 197880, KU 140418, 222208–09, USNM 165297, 165299, 165301–02, 165307, 165309, 165311, 165313, UTA R-3768). *Bothrocophias microphthalmus* (FMNH 5580, 40242, 63740, KU 209540, 211621, UTA R-23530). *Bothrocophias myersi* (AMNH 107919–20, 109812, FMNH 165586–96, USNM 151708, 154051, UTA R-21689). *Bothrops alternatus* (UTA R-2848, 5602, 32427, 37709, 38293–94). *Bothrops asper* (UTA R-8834, 14531, 15651, 17095, 21904–05, 33128). *Bothrops atrox* (UTA R-

3377–78, 3771, 3852, 5848, 5850). *Bothrops neuwiedii* (UTA R-34559–60, 38036–37, 38075, 39112). *Cerrophidion godmani* (KU 187380, UTA R-5917, 5919–22, 6529, 6577, 6583, 6635, 6643, 7693, 7706, 8781). *Crotalus atrox* (UTA R-1330, 2339, 12767, 28793, 30458, 30722, 32432, 32671, 44289). *Crotalus lepidus* (UTA R-7433–34, 8691, 9307, 11001, 28912). *Gloydius blomhoffii* (FMNH 73968–71, UTA R-16873, 18698–99). *Lachesis stenophrys* (USNM 165966, 192284, UTA R-12944, 16086, 16090–92, 16601). *Ophryacus melanurus* (KU 191916, UTA R-5563–66, 5811, 5815, 6118, 6155, 6224, 6644, 6817–18, 7731, 9610–11, 12554–59, 13032, 14496, 16309, 21927, 22257, 22402, 22450–53). *Ophryacus undulatus* (UTA R-2851, 3836, 4108, 4517–18, 4644–51, 4832, 4834–36, 4914, 5538–40, 5632, 5810, 6154, 6651–52, 6688, 6824, 8120, 9023, 9861, 15788, 16094–95, 18026, 18422, 19603, 22370, 24749–51, 25115, 30825, 30868, 32426. *Porthidium dunni* (UTA R-4367, 8354–55, 8816, 9090, 12553). *Porthidium hespere* (UTA R-4443). *Porthidium lansbergii* (UTA R-3676, 3678–80, 4993–95). *Porthidium nasutum* (KU 55705, UTA R-14181, 15292, 16099–100, 19605–06, 19608, 19611, 21928–39, 22229, 22231–32, 22234, 22252, 23066, 24515–16, 26408–10, 30830, 31057). *Porthidium ophryomegas* (UTA R-16102–03, 22228, 28563–65, 29970–71, 39217, 39600, 39755). *Porthidium volcanicum* (UTA R-24828–30). *Porthidium yucatanicum* (UTA R-16960). *Sistrurus catenatus* (UTA R-12681, 12772, 14082–83, 32386, 32435). *Sistrurus miliarius* (UTA R-18364, 30732, 32165, 34172, 40389–90).

Osteological Preparations.—*Agkistrodon contortrix* (UTA R-38098, 38113, 40755–56, 40961–62). *Agkistrodon piscivorus* (UTA R-34944–45, 40717). *Atropoides nummifer* (KU 55707, UTA R-6738, 7430). *Atropoides picadoi* (UTA R-15617, 40481). *Bothriechis bicolor* (UTA R-9353, 18365). *Bothriechis lateralis* (UTA R-2811, 3660, 14537). *Bothriechis nigroviridis* (USNM 76408, UTA R-9635–36). *Bothriechis schlegelii* (USNM 319276, UTA R-5124, 7982, 32143). *Bothriopsis bilineata* (AMNH 53422, 140856, 140859). *Bothrocophias hyoprora* (AMNH 54141). *Bothrocophias microphthalmus* (FMNH 63740). *Bothrops alternatus* (AMNH 31737, 74441, 75479, 76209). *Bothrops asper* (USNM 319235–36, UTA R-16961, 41282). *Bothrops atrox* (AMNH 56195, 62581, CM 112360). *Bothrops neuwiedii* (AMNH 29256). *Cerrophidion godmani* (KU 117478, UTA R-6205, 7772, 7776, 7778, 14534–35, 38106–12). *Crotalus atrox* (UTA R-35363, 40712, 40715). *Crotalus lepidus* (UTA R-8275, 40483–84). *Gloydius blomhoffii* (CAS 14622, 16097, FMNH 73969, 73971, USNM 17847). *Lachesis stenophrys* (UTA R-40468, 40526). *Ophryacus melanurus* (UTA R-34604–06, 40412). *Ophryacus undulatus* (UTA R-4640–01, 4643). *Porthidium dunni* (AMNH 65874, 65877). *Porthidium nasutum* (AMNH 46958, KU 35734, UTA R-31057). *Porthidium ophryomegas* (UTA R-14532). *Sistrurus catenatus* (UTA R-8727, 8730, 16600, 38105, 40471–72, 40522). *Sistrurus miliarius* (UTA R-39909)

APPENDIX II

Character Weights

Character number	Character number in PAUP matrix	Weight
1	1–3	455
2	4–8	137
	9–10	149
	11	193
	12	234
	13	252
3	18–19	341
	20	410
	21	1365
4	22–28	171
	29	205
5	33–36	228
	37	237
	38	420
6	40–52	105

7	62	96
	63–73	76
	74	91
	75–76	114
	77–78	228
	79	455
8	81	585
	82–87	195
9	90	95
	91	54
	92–93	29
	94	24
	95	21
	96–97	18
	98–172	16
	173–174	19
	175	25
10	183–196	98
11	200	1365
12–15	201–204	32767
16	205–207	455
17	208	32767
18	209	1365
19	210	1365
20–26	211–218	32767
28	220–223	273
	224	819
29	225	210
	226–235	105
	236	168
	237	210
30	240–248	124
	249	175
	250	596
31–34	252–255	32767
35	256	1365
36–37	257–258	32767
38	259–260	683
39–41	261–263	32767
42	264	1365
43–50	265–272	32767
51	273	1365
52	274	910
	275	683
53	276	1425
54–57	277–280	32767
58	281	1365
59	282	1365
60	283	1365
61	284	32767
62	288–291	54
	292	40
	293–331	27
	332–333	36
	334–337	40
	338	80
63–76	340–353	32767