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## The phylogenetic distribution of the ampulla ureter and ampulla urogenital/uriniferous papilla in the Serpentes

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### Abstract

The ampulla ureter and ampulla urogenital/uriniferous papilla represent differing morphologies of the caudal urogenital ducts in snakes. The ampulla ureter is an enlarged portion of the caudal extremity of the ureter that communicates the cranial regions of the ureter and the ductus deferens/Wolfian duct to the urodaeum. The ampulla urogenital/uriniferous papilla is an enlarged pouch, distinct from the ureter, which communicates the ureter and ductus deferens/Wolfian duct to the urodaeum. Although functional differences of these two structures are unknown, the ampulla urogenital/uriniferous papilla may have evolved for urine storage in males and females, and secondarily evolved a reproductive function in males. The most parsimonious optimization of the ampulla ureter and ampulla urogenital/uriniferous papilla indicates that the ampulla ureter is the ancestral state in snakes. Examining the presence or absence of the ampulla ureter and ampulla urogenital/uriniferous papilla in snakes on conflicting caenophidian phylogenies results in two hypotheses for the evolution of these variant morphologies: (1) The ampulla urogenital/uriniferous papilla evolved from the ampulla ureter independently in the Colubroidea and Elapoidea with subsequent losses of the ampulla urogenital/uriniferous papilla in the Elapoidea and (2) a single transition from the ampulla ureter to the ampulla urogenital/uriniferous papilla on the branch leading to the Colubroidea + Elapoidea with subsequent losses of the ampulla urogenital/uriniferous papilla in the Elapoidea and Colubroidea. The presence of the ampullae urogenital/uriniferous papilla in only the Colubroidea and Elapoidea highlights the affinity of these two taxonomic groups, a relationship that is strongly supported in published cladograms produced with molecular datasets.

**Key words:** Ductus deferens – Wolfian duct – ureter – histology – anatomy

### Introduction

Little data exist on the caudal urogenital ducts in snakes. Recent studies include the description of the ampulla ductus deferens in the Black Swamp Snake (*Seminatrix pygaea*; Sever 2004) and Cottonmouth (*Agkistrodon piscivorus*; Siegel et al. 2009; Trauth and Sever in press), and a review of all the historical literature on the urogenital ducts of snakes (Trauth and Sever in press). In their review, Trauth and Sever (in press) provided novel descriptions of the caudal urogenital ducts in multiple colubroid and viperid taxa and provided an ultrastructural description from a representative species (*Pantherophis obsoletus*). Yet investigation into urogenital duct morphology of snakes began with the work by Martin Saint Ange (1854) and was supplemented greatly by the work of Volsøe (1944).

Martin Saint Ange (1854) provided the first detailed account of the caudal urogenital ducts in snakes. He illustrated the ureters and ductus deferentia communicating with the urodaeum of the cloaca via common pouches in the Grass Snake (*Natrix natrix*; see Fig. 2, plate X in Martin Saint Ange 1854). These pouches were subsequently termed the ampullae urogenital papillae (Aup) in male snakes (Trauth and Sever in press). Equivalent structures have also been described in female Yellowbelly Snakes, *Coniophanes fissidens*, and were termed the ampullae uriniferous papillae (Aunp). This terminology was adopted because in females the Wolfian ducts (vestigial ductus deferentia) and ureters empty into the papillae and, thus, no genital material is passed through these organs (Siegel et al. in press).

In his seminal work on the reproductive system of the male European Viper (*Vipera berus*), Volsøe (1944) described a somewhat different orientation of the caudal urogenital ducts. In *V. berus* he illustrated the ductus deferentia communicating with an enlarged portion of the ureters cranial to communication with the urodaeum of the cloaca (see Fig. 11 in Volsøe 1944). The enlarged portion of the ureters was subsequently termed the ampulla ureter (Trauth and Sever in press). This condition of the caudal urogenital ducts was also described for the Scolecophidia (Fox and Dessauer 1962; Fox 1965).

At first glance, it may appear that the descriptions by Martin Saint Ange (1854) and Volsøe (1944) are in conflict; however, Trauth and Sever (in press) found examples of the differing morphologies of the caudal urogenital ducts in multiple snake taxa. Their results from a sampling of North American colubrids, dipsadids, natricids, and viperids led to the hypothesis that the Aur is a synapomorphy for the Viperidae, whereas the Aup/Aunp is a synapomorphy for the Colubroidea (sensu Zaher et al. 2009). The following investigation was undertaken to describe the phylogenetic distribution of the above structures in the Serpentes in an effort to test the above hypothesis and to stimulate interest in the caudal urogenital/uriniferous ducts of snakes, of which little functional, morphological, and evolutionary details are known.

### Materials and Methods

#### Specimens

Specimens were obtained from the private collections of RD Aldridge, DS Siegel, and SE Trauth (Arkansas State Museum of Zoology; ASUMZ), the Field Museum of Natural History (FMNH), the Sam Noble Oklahoma Museum of Natural History (SNOMNH), and the California Academy of Sciences (CAS). Historical data were also utilized from Fox and Dessauer (1962) and Fox (1965) for the

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Scolecophidia and Gabe and Saint-Girons (1965) for Pythonidae. Representatives of all families were sampled except for the Anomohiliidae, Anomalepididae, and Bolyriidae (see Appendix S1). Agamid and helodermatid specimens were also utilized for outgroup comparison, as these taxa are found in groups that have been implicated to be closely related to snakes (Vidal and Hedges 2004; Eckstut et al. 2009). It is important to note that Gabe and Saint-Girons (1965) investigated the cloacal anatomy of 14 lizard families and that their previous descriptions mirror the morphology of the agamid and helodermatid specimens examined here.

### Microscopy

All specimens were fixed after initial collection and stored in 70% ethanol. Tissues from the ASUMZ and the personal collection of RD Aldridge and DS Siegel were fixed in 10% neutral-buffered formalin for at least 24 h, whereas the fixation method of materials from the FMNH, SNOMNH, and CAS were unknown. Cloacae were removed, dehydrated with a graded series of ethanol (80%, 95%, two changes of 100%), and cleared with two changes of toluene (or xylene). Tissues were subsequently embedded in paraplast, and at least one cloaca from every species was oriented for transverse sectioning. Serial sections 10 µm in thickness were affixed to albumenized slides and stained with hematoxylin and eosin for general histological analysis.

### Character optimization

To examine the presence of variant urogenital duct morphologies in an evolutionary context, character states of the caudal urogenital tract were coded (presence of Aur, 0; presence of Aup/Aunp, 1) and then optimized onto recently published phylogenies to access character state transitions and the ancestral state for different taxonomic groups. The Serpentes branch of the molecular phylogeny of Eckstut et al. (2009) was utilized to analyse the evolution of the caudal urogenital ducts across all snakes, while the molecular phylogenies by Vidal et al. (2008) and Zaher et al. (2009) were used to focus attention on the Caenophidia, for reasons that will become obvious in the subsequent sections. Character optimization analyses were conducted in MacClade 4.06 (Maddison and Maddison 2003). All family level taxonomic groups represented as terminal taxa on the previously published phylogeny by Eckstut et al. (2009) were examined here except for the Anomalepididae and Bolyriidae. These families were left off of the phylogeny produced in MacClade 4.06 for character mapping. All major terminal taxon groups on the caenophidian phylogeny of Vidal et al. (2008) were examined except three: (1) *Prosymna* (Elapoidea insertae sedis; sensu Zaher et al. 2009), of which the state of the urogenital duct morphology of this genus was coded as unknown (?), (2) *Micrelaps* (Atractaspididae), and (3) *Oxyrhabdium* (Elapoidea insertae sedis; sensu Zaher et al. 2009). Vidal et al. (2008; for review see Zaher et al. 2009) considered all three of these taxa as having unknown affinities. Due to the latter two taxa being surrounded by taxa with identical morphologies (e.g., Homalopsidae and Elapidae; see Results), coding the states of *Micrelaps* and *Oxyrhabdium* as '?' on the Vidal et al. (2008) topology would add little to the analysis, as MacClade 4.06 would hypothesize that these taxa have the same caudal urogenital duct morphology as Elapidae and Pseudoxenodontidae. Thus, *Micrelaps* and *Oxyrhabdium* were omitted from the analysis. All of the families distinguished on the phylogeny by Zaher et al. (2009) were represented in the specimen collection.

## Results

### Ampulla ureter

In male and female taxa that possess Aur, the ureters can be seen travelling dorsal to the oviducts (only in females) and urodaeum (Figs 1a–e and 2a–d). The ductus deferentia in males and Wolffian ducts in females are also observed travelling dorsal to the urodaeum in close association with the ureters, but they are encompassed in their own muscularis and pleuroperitoneum (Figs 1a–e and 2a–d). Toward the

caudal extremity of the urodaeum, the ducts become encompassed within the muscularis and visceral pleuroperitoneum of the cloaca (Figs 1d,e and 2c,d). As they approach the cranial extremity of the ampullary papilla(ae) in a dorsal position, the ductus deferentia in males and Wolffian ducts in females merge with the ureters (Figs 1d,e and 2d,e). Immediately caudal to the point of merger, the ureters enlarge and communicate with the urodaeum through the ampullary papilla(ae; Figs 1f and 2e,f). This enlarged area of the ureters is known as the ampulla ureter. The Aur is highly furrowed, possesses a simple to bi-stratified non-ciliated epithelium, and is filled with sperm in males. In females, the duct is empty; however, the Wolffian duct is filled with an eosinophilic secretory material. This condition was found in the lizards examined (*Agama* and *Heloderma*), *Achalinus*, *Acrochordus*, *Anilius*, *Aparallactus*, *Aplopeltura*, boids (*Candoia* and *Eryx*), *Cylindrophis*, elapids (*Laticauda* and *Micrurus*), homalopsids (*Bitia* and *Enhydryis*), *Loxocemus*, *Madagascarophis*, *Morelia* (Gabe and Saint-Girons 1965), *Pseudoxenodon*, *Rhinophis*, scolecophidians (*Leptotyphlops*, *Ramphotyphlops*, and *Typhlops* [Fox and Dessauer 1962]), *Trachyboa*, viperids (*Agkistrodon* and *Azemisops*), and *Xenopeltis*.

### Ampulla urogenital/uriniferous papilla

In male and female taxa that possess Aup or Aunp, respectively, the ureters are observed travelling dorsal to the oviducts (only in females) and urodaeum (Figs 3a–d and 4a–c). As in the case with the Aur condition, the ductus deferentia in males and Wolffian ducts in females are found in close proximity to the ureters but encompassed in their own muscularis and pleuroperitoneum (Figs 3a–d and 4a–c). Yet unlike in the Aur condition, cranial to the vertical plane of the ampullary papilla(ae) the ureters and ductus deferentia, or Wolffian ducts, can be seen travelling dorsal to a third duct that originates within the muscularis and pleuroperitoneum of the ureters (Figs 3b–d and 4b,c). This third duct is blind cranially and is the duct known as the ampulla urogenital/uriniferous papilla. Immediately cranial to the ampullary papilla(ae) in a dorsal position, the ureters communicate with the Aup/Aunp. Towards the caudal extremity of the urodaeum, the Aup/Aunp and ductus deferentia/Wolffian ducts become encompassed in the muscularis and visceral pleuroperitoneum of the cloaca (Figs 3e and 4d,e). The ductus deferentia/Wolffian ducts then communicate with the Aup/Aunp from a ventral position (Figs 3e,f and 4e,f). The Aup is highly furrowed in males, exhibits a non-ciliated simple to bi-stratified epithelium, and is packed with sperm. In females, the lumen of these ducts is empty; however, the Wolffian ducts are filled with an eosinophilic secretory material. This condition was found in *Atractaspis*, calamariids (*Calamaria* and *Pseudorabdion*), colubrids (*Ahaetulla*, *Cemophora*, *Coluber*, *Elaphe*, *Grayia*, *Lampropeltis*, *Masticophis*, *Ophedryx*, *Pantherophis*, *Sonora*, and *Tantilla*), dipsadids (*Carphophis*, *Diadophis*, *Farancia*, *Helicops*, *Heterodon*, *Leptodeira*, *Oxyrhopus*, and *Thamnodynastes*), *Lamprophis*, *Liopholidophis*, natricids (*Natrix*, *Regina*, *Storeria*, and *Thamnophis*), psammophiids (*Malpolon* and *Psammophylax*), and *Pseudaspis*.

### Character optimization

Parsimony optimization of the character states onto the Serpentes phylogeny of Eckstut et al. (2009) resulted in the

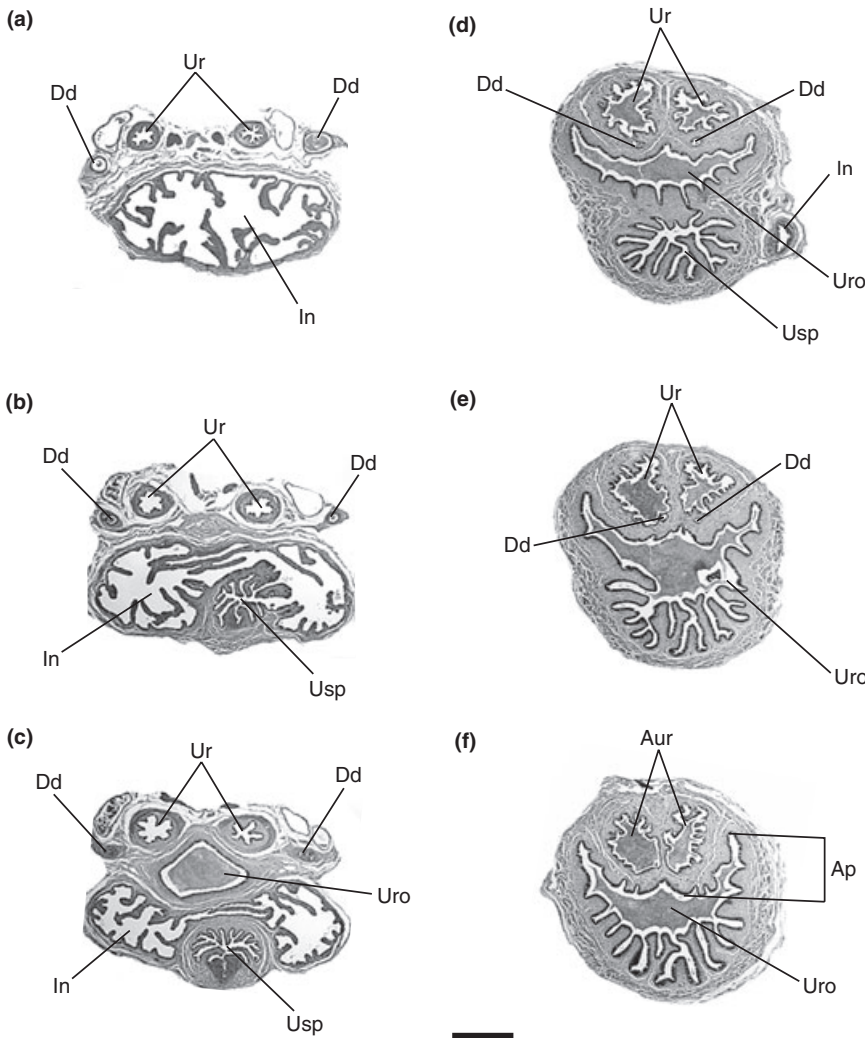


Fig. 1. Transversal sections (cranial to caudal) through the cloaca of male *Sistrurus miliaris* (scale bar = 1000  $\mu\text{m}$ ). (a) Section cranial to the cloaca through the ductus deferentia (Dd), intestine (In), and ureters (Ur). (b) Section through where the intestine communicates with the urodaeal sphincter (Usp). (c) Section through the cranial extremity of the urodaeum (Uro). (d) Section through the urodaeum where the ductus deferentia and ureters are now encompassed in the muscularis and pleuroperitoneum of the cloaca. (e) Section through the urodaeum where the urodaeal sphincter communicates with the urodaeum. (f) Section through the urodaeum immediately caudal to where the ductus deferentia have communicated with the ureters, forming the ampullae ureters (Aur) within the ampullary papillae (Ap)

Aur as being the ancestral condition found in snakes (Fig. 5), as this is the condition found in the lizard outgroups (*Agama* and *Heloderma*), and all of the more basal snake lineages (Acrochordidae, Aniliidae, Boidae, Cyllindrophiiidae, Homalopsidae, Loxocemidae, Pythonidae, Scolecophidia, Tropidopneustidae, Uropeltidae, Viperidae, Xenodermatidae, and Xenopeltidae). Due to the poor resolution of critical nodes in the Eckstut et al. (2009) phylogeny (mainly the intra-relationships of the Elapoidea and Colubroidea at the family level), it appears that the Aup/Aunp urogenital duct system evolved multiple times within the Colubroidea and the Elapoidea (Fig. 5). Yet optimization of the character states onto the fully resolved caenophidian phylogenies of Vidal et al. (2008; Fig. 6a) and Zaher et al. (2009; Fig. 6b) resulted in two opposing hypotheses on the evolution of the caudal urogenital ducts. On the Vidal et al. (2008) phylogeny the Aur transitions to the Aup/Aunp independently in the Colubroidea and the Elapoidea, with subsequent reversals to the Aur condition in the Elapoidea (*Aparallactus capensis* and *Madagascarophis colubrinus*; Fig. 6a). This optimization results in a total number of four evolutionary steps. On the Zaher et al. (2009) phylogeny, the Aur transitions to the Aup/Aunp once on the branch leading to the Colubroidea + Elapoidea, with subsequent reversals in the Colubroidea (*Pseudoxenodon macrops*) and Elapoidea (*Aparallactus capensis*, Elapidae, and

*Madagascarophis colubrinus*; Fig. 6b). This optimization results in a total number of five evolutionary steps.

## Discussion

### Ampulla ureter versus ampulla urogenital/uriniferous papilla

The Aur represents the plesiomorphic condition of the caudal urogenital ducts in snakes, while the Aup/Aunp represents the derived condition. Whereas the Aur is formed from a simple joining of the ductus deferentia or Wolffian ducts with the ureters, the Aup/Aunp is a more complex morphology with all ducts emptying into blind pouches (Aup in males or Aunp in females). Although the functional significance of these two morphological arrangements is unknown, some hypotheses can be made from the inclusion of data from previous work on the reproductive anatomy of snakes.

Considering that both male and female snakes possess an ampulla papilla (Aup/Aunp), it is feasible to believe that this structure evolved for urine storage, or some other function associated with the excretion of nitrogenous waste and resorption of water. From histological study, Trauth and Sever (in press) note the presence of festooned fluctuating hillocks of the apical epithelium that may be indicative of absorption. They also state that uric acid is common in the



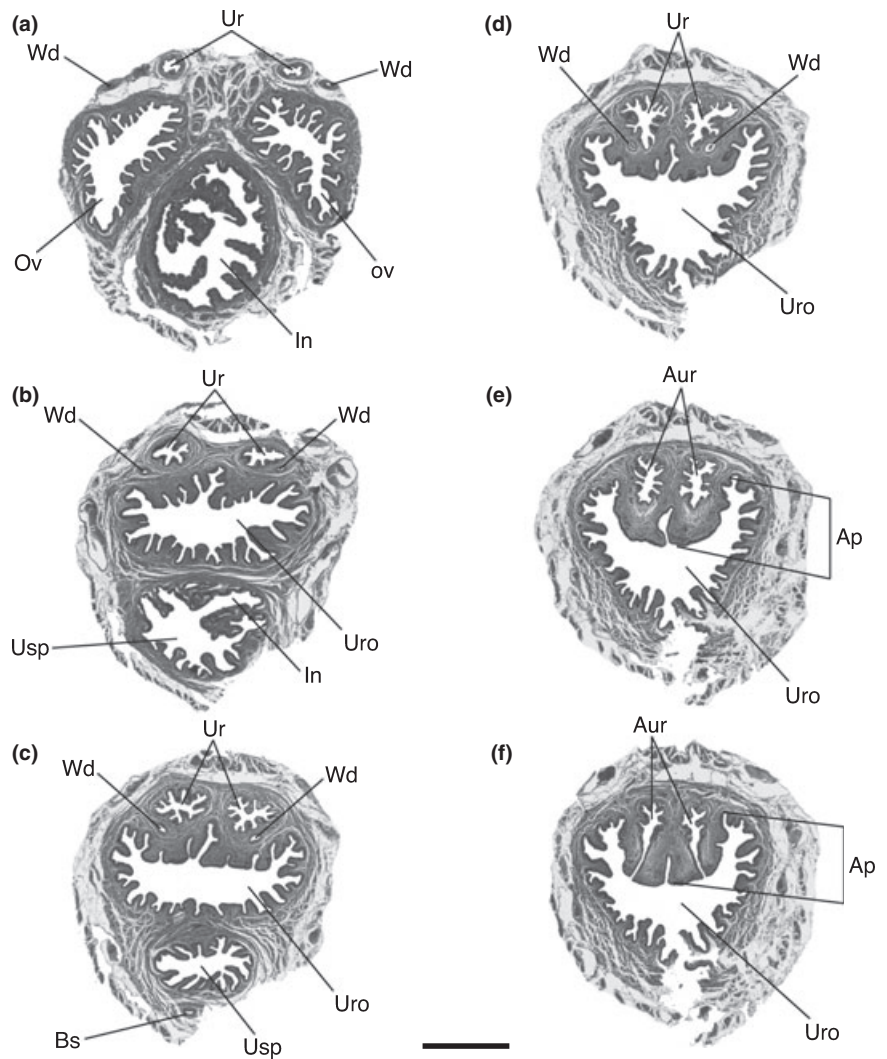


Fig. 2. Transversal sections (cranial to caudal) through the cloaca of female *Achalinus weneri* (scale bar = 700  $\mu$ m). (a) Section cranial to the cloaca through the intestine (In), oviducts (Ov), ureters (Ur), and Wolffian ducts (Wd). (b) Section through the urodaeum where the urodaeal sphincter (Usp) communicates with the urodaeum (Uro). (c) Section through the urodaeum where the ureters and Wolffian ducts are now encompassed in the muscularis and pleuroperitoneum of the cloaca. The bladder stalk (Bs) is observed ventral to the urodaeal sphincter. (d) Section through the urodaeum where the urodaeal sphincter communicates with the urodaeum. (e) Section through the urodaeum immediately caudal to where the Wolffian ducts communicated with the ureters, forming the ampullae ureters (Aur) within the ampullary papillae (Ap). (f) Section through the urodaeum where the ampullae ureters communicate with the urodaeum.

Aup of their small colubroid sample. The urinary bladder was lost early in the snake lineage, as no snake possesses a functional urinary bladder (Gabe and Saint-Girons 1965; Fox 1977). Yet bladder stalks (sensu Mulaik 1946) were described by Gabe and Saint-Girons (1965) in some snakes and can be seen branching from the urodaeal sphincter (Siegel et al. in press) of *Achalinus weneri* in Fig. 2 of this manuscript. Bladder stalks are thought to represent a vestigial remnant of the urinary bladder (Mulaik 1946; Beuchat 1986). Thus, the Aup or Aunp may act as functional urinary bladders in those members of the Colubroidea and Elapoidea that possess these structures. This is an interesting idea as urinary bladders in this position (at the caudal end of the ureters) represent morphology more similar to mammals than to other squamates. The urinary bladders of all other squamates branch from the urodaeal sphincter (Gabe and Saint-Girons 1965) that opens into the intestine more cranially; thus, this type of urinary bladder is not directly associated with the ureteric ducts. Yet Trauth and Sever (in press) state that the size of the two complementary Aup/Aunp pouches is not great enough to consider them important structures in uric acid storage.

The Aup may also represent specializations for sperm maturation, sperm phagocytosis, and sperm storage capability in colubroid and elapoid males and, thus, may have second-

arily evolved reproductive functions. Histochemical and cytological evaluation of the Aup epithelium in *Pantherophis obsoletus* indicated the production and release of neutral carbohydrates and glycosaminoglycans (Trauth and Sever in press). The presence of secretory vacuoles of varying phases of electron densities released into the Aup lumina by exocytosis confirmed this finding. While glycosaminoglycans have not been found previously in sperm storage structures in male or female snakes, neutral carbohydrate secretions have been reported (for review see Siegel et al. 2009). Sperm in the excurrent ducts of males are always associated with copious masses of neutral carbohydrate secretion (for review see Siegel et al. 2009). Whereas secretory activity has been identified in the ampulla ductus deferentis in male viperids (Siegel et al. 2009; Trauth and Sever in press), the ampulla ductus deferens of colubroid snakes has been reported as having little secretory activity (Sever 2004; Trauth and Sever in press). Thus, the Aup may possess a similar function to the ampulla ductus deferentis in non-colubroid/elapoid snakes.

Trauth and Sever (in press) refer to the Aur and Aup as functional equivalents, although the cytology of the Aur in comparison to the Aup is quite different. In the Aur, secretory vesicles are aggregated in the apical cytoplasm and are released into the lumina via large cytoplasmic blebs.

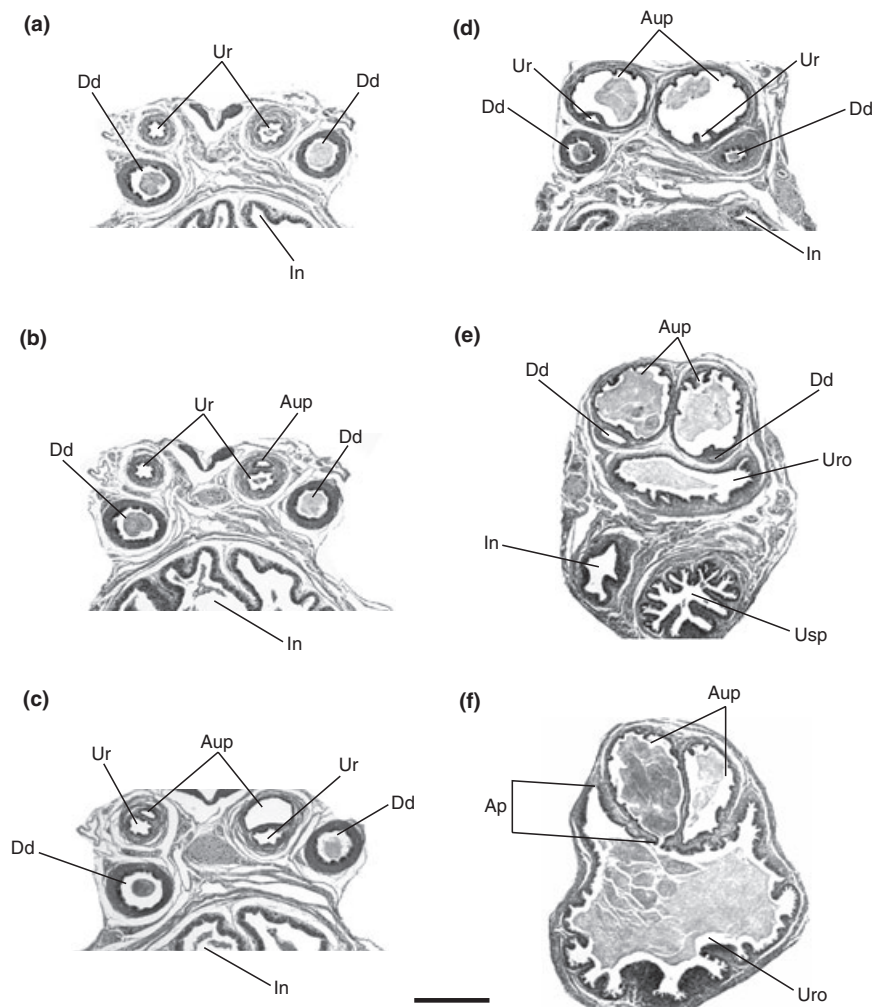


Fig. 3. Transversal sections (cranial to caudal) through the cloaca of male *Pantherophis obsoletus* (scale bar = 500  $\mu$ m). (a) Section cranial to the cloaca through the ductus deferentia (Dd), intestine (In), and ureters (Ur). (b) Section cranial to the cloaca where the right ampulla urogenital papilla (Aup) can first be observed. (c) Section cranial to the cloaca where both ampullae urogenital papillae can be observed. (d) Section cranial to the cloaca where the ureters empty into the ampullae urogenital papillae. (e) Section through the urodaeum where the ductus deferentia and ureters are now encompassed in the muscularis and pleuroperitoneum of the cloaca. The urodaeal sphincter (Usp) is observed in a ventral position to the urodaeum. (f) Section through the urodaeum immediately caudal to where the ductus deferentia communicated with the ampullae urogenital papillae within the ampullary papilla (Ap)

This eocrine process is similar to that observed in the ductus epididymis and ductus deferens (Sever 2010; Trauth and Sever in press) of snakes. Thus, although both structures are secretory, the Aur may have a function more similar to that of the more cranial regions of the genital ducts, whereas the Aup has uniquely derived function. Trauth and Sever (in press) also note the phagocytosis of sperm in the Aup of *P. obsoletus*, a process not identified in the Aur, providing more support for functional differences between the two morphologies and possible reproductive function of the Aup. More ultrastructural studies are needed on a diverse sampling of taxa to uncover the functional differences and similarities between the Aur and Aup.

#### The evolution of the caudal urogenital ducts

Trauth and Sever (in press) described the Aur in viperids and the Aup/Aunp caudal urogenital duct condition in only a limited sample of North American colubroids. This study was undertaken to investigate the possible synapomorphy of these differing urogenital duct morphologies in these two groups. Data presented here reject the previously presented hypothesis that the Aur is a synapomorphy for the viperids, as this condition is found in all basal snake taxa. Data also reject that the Aup/Aunp is a synapomorphy for the Colubroidea, as this condition is also found in some members of the Elapoidea. It

appears that the Aup/Aunp condition is only found within the Colubroidea and Elapoidea, further supporting the monophyly of these two clades (Zaher 1994; Heise et al. 1995; Slowinski and Lawson 2002; Lawson et al. 2004; Vidal et al. 2007; Yan et al. 2008; Zaher et al. 2009).

As mentioned earlier, it is clear that the Aur is the ancestral morphology of the caudal urogenital ducts in snakes, as this structure is found in lizards and all the basal snake lineages. Data presented here support two new hypotheses on the transition of the Aur to the Aup/Aunp condition: (1) two independent transitions to the Aup/Aunp condition and (2) one transition to the Aup/Aunp condition. Based on the phylogeny by Vidal et al. (2008), the most parsimonious evolutionary history of the caudal urogenital duct trajectory is two independent transitions from the Aur to the Aup/Aunp. One transition occurred on the branch leading to the Elapoidea and one on the branch leading to the Colubroidea sans Pseudoxenodontidae. Subsequent losses of the Aup/Aunp occurred multiple times within the Elapoidea. In the Vidal et al. (2008), topology Elapidae is sister to all other Elapoidea members (sans *Micrelaps* and *Oxyrhabdium*) and Pseudoxenodontidae is sister to all other Colubroidea members. Lawson et al. (2005) and Vidal et al. (2007) recovered similar relationships for the Elapoidea.

The utilization of the phylogeny by Zaher et al. (2009) results in the Aup/Aunp caudal urogenital duct system as

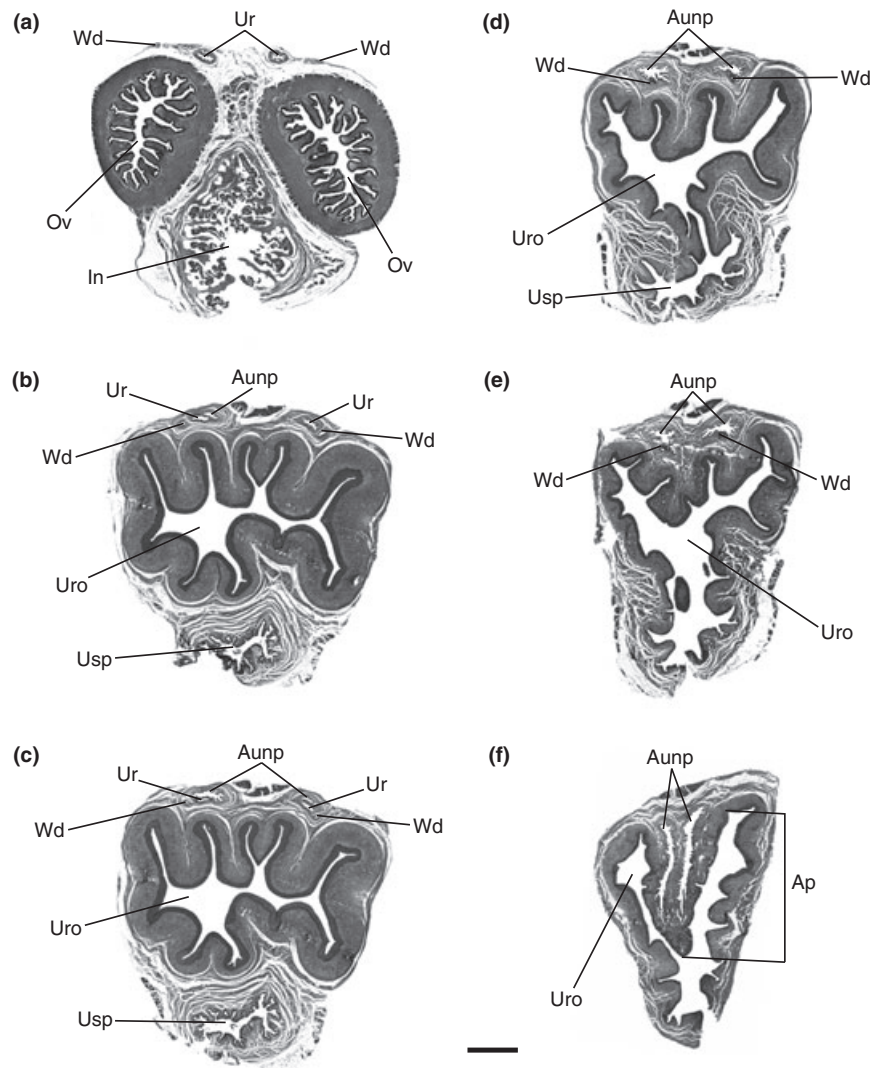


Fig. 4. Transversal sections (cranial to caudal) through the cloaca of female *Liopholidophis lateralis* (scale bar = 800  $\mu$ m). (a) Section cranial to the cloaca through the intestine (In), oviducts (Ov), ureters (Ur), and Wolffian ducts (Wd). (b) Section through the urodaeum where the left ampulla uriniferous papilla (Aunp) can first be observed. The urodaeal sphincter (Usp) is observed in a ventral position to the urodaeum. (c) Section through the urodaeum where both ampullae uriniferous papillae can be observed. (d) Section through the urodaeum where the ampullae uriniferous papillae and Wolffian ducts are now encompassed in the muscularis and pleuroperitoneum of the cloaca. (e) Section through the urodaeum caudal to where the urodaeal sphincter communicated with the urodaeum. (f) Section through the urodaeum caudal to where the ampullae uriniferous papillae communicated with the Wolffian ducts within the ampullary papilla (Ap)

being the ancestral state for the Colubroidea + Elapoidea clade. Thus, this result is the most parsimonious solution (five steps) when Elapidae and Pseudoxenodontidae are embedded deeper in their respective clades, as in the topologies also recently recovered by Kelly et al. (2003, 2009) for the Elapoidea, and Lawson et al. (2004), Vidal et al. (2007), and Kelly et al. (2009) for the Colubroidea.

It is important to note that many of the family level relationships within the Elapoidea were accompanied by poor support in the studies discussed earlier (Kelly et al. 2003, 2009; Lawson et al. 2004; Vidal et al. 2007; Zaher et al. 2009). Only the Vidal et al. (2008) topology provided recent high support for the placement of Pseudoxenodontidae, and in their analysis Pseudoxenodontidae was reconstructed as sister to the remaining Colubroidea. Thus, it is reasonable to believe that with the addition of more taxa and reconstruction of more robust phylogenies, our knowledge of the evolution of the urogenital system in snakes will change considerably. Yet regardless of the current topology utilized, within the Colubroidea and Elapoidea subsequent transitions back to the Aur condition appear to have occurred as some well-supported groups possess taxa with varying urogenital conditions within the Elapoidea; for instance, *Aparallactus* and *Atractaspis* of the Atractaspididae (Lawson et al. 2004;

Vidal et al. 2008; Eckstut et al. 2009; Kelly et al. 2009; Zaher et al. 2009) and *Madagascarophis* and *Liopholidophis* of the Pseudoxyrhopiinae (Vidal et al. 2008; Kelly et al. 2009; Zaher et al. 2009).

The absence of the Aup/Aunp in members of the Elapoidea and Pseudoxenodontidae provides little support for the placement of these taxa in their respective clades with the current sampling of specimens and our current knowledge of snake systematics. It is of interest to note that the Vidal et al. (2008) topology resulted in less evolutionary steps in comparison to the Zaher et al. (2009) topology (four compared to five); however, the finding of the Aup/Aunp condition in *Micrelaps* and *Oxyrhabdium* (the poorly supported basal lineage of Elapoidea in Vidal et al. 2008) would result in five evolutionary steps on the Vidal et al. (2008) topology with the ancestral condition for the Colubroidea + Elapoidea and Colubroidea reconstructed as equivocal. Furthermore, this result would present itself on any reconstruction where the basal lineages of the Elapoidea and Colubroidea possessed different caudal urogenital duct morphologies (given that the Elapoidea and Colubroidea were reconstructed as sister taxa; e.g., Vidal et al. 2007).

Histological and cytological variation was previously found within the Aup/Aunp in a small sample of colubroid taxa



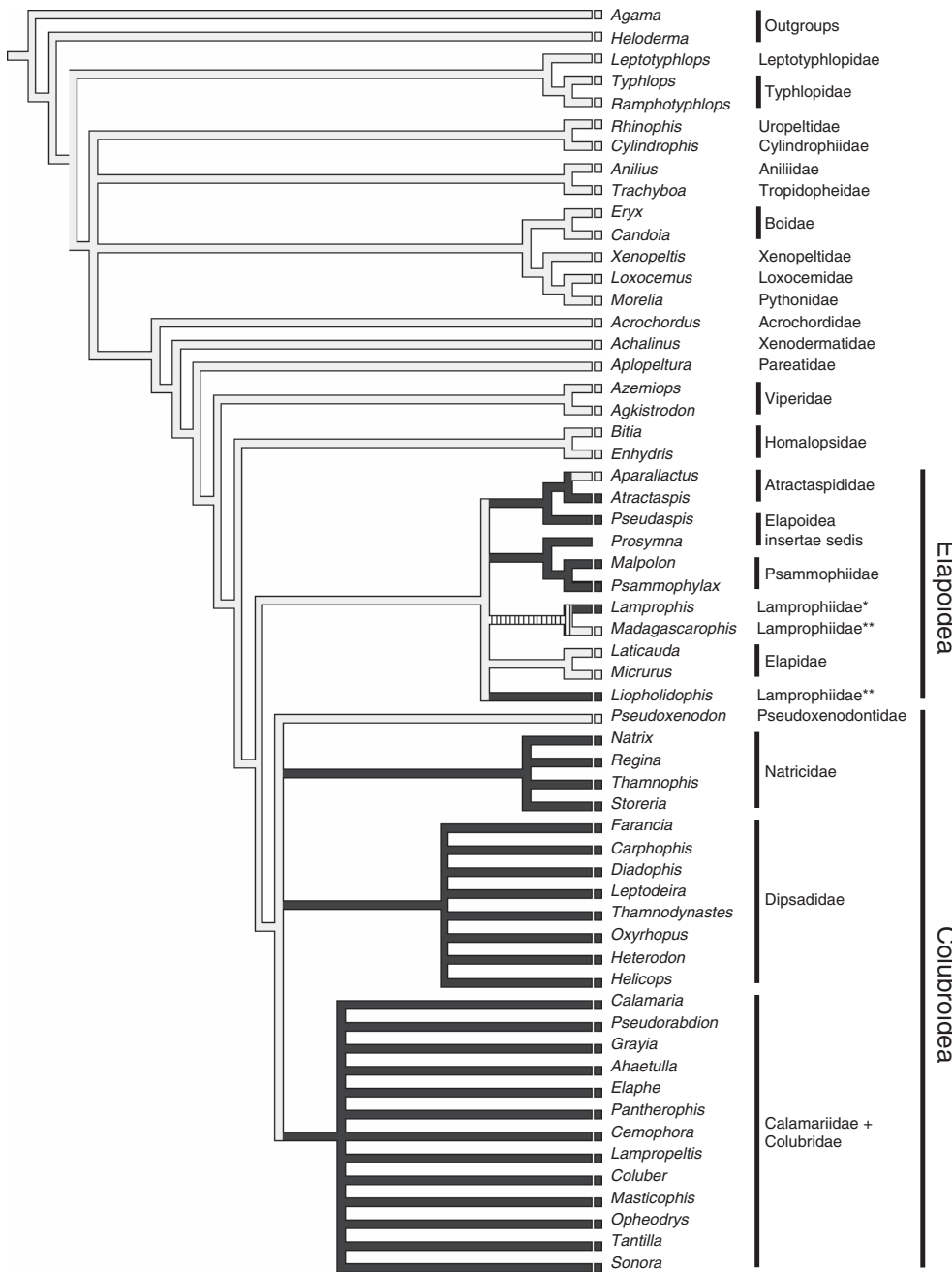


Fig. 5. Serpentes branch of the phylogeny produced by Eckstut et al. (2009) from the nuclear encoding C-mos gene, sans Anomalopidae and Bolyeriidae, with genera examined here inserted as terminal taxa. Grey branches and boxes represent the ampulla ureter urogenital duct morphology, while black branches and boxes represent the ampulla urogenital/uriniferous papilla urogenital duct morphology. The ampullae ureter is the ancestral condition of the caudal urogenital ducts in snakes, while transitions to the ampulla urogenital/uriniferous papilla occurred in the Colubroidea (C) and Elapoidea (E) based on parsimony optimization of character states. Lamprophiinae of the Lamprophiidae is indicated by one asterisk, while two asterisks indicate Pseudoxyrhophiinae of the Lamprophiidae. Family level taxonomy for the Caenophidia follows Zaher et al. (2009)

(Trauth and Sever in press), and further investigation into this organ may result in data more applicable to systematics. Also because of the enigmatic relationship of taxa within the Elapoidea (see Kelly et al. 2003, 2009; Lawson et al. 2004; Vidal et al. 2007, 2008; Eckstut et al. 2009; Zaher et al. 2009), the presence or absence of a transition to the Aup/Aunp morphology may aid in uncovering the evolutionary relationships of the taxa within this clade. Based on these findings, we encourage future investigations aimed at uncovering the evolution of the urogenital system and its possible correlation with the evolutionary history of snakes.

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**Résumé**

*La distribution phylogénique de l'ampoule urétére et de l'ampoule uro/papille urinifères dans les Serpentes*

L'urétére ampoule et ampoule uro/papille urinifères représentent morphologies différentes des voies urogénitales caudale des serpents. L'urétére ampoule est une portion agrandie de l'extrémité caudale de l'urétére qui communique les régions crânienne de l'urétére et le canal déférent/canal de Wolff à l'urodaeuum. L'ampoule uro/papille urinifères

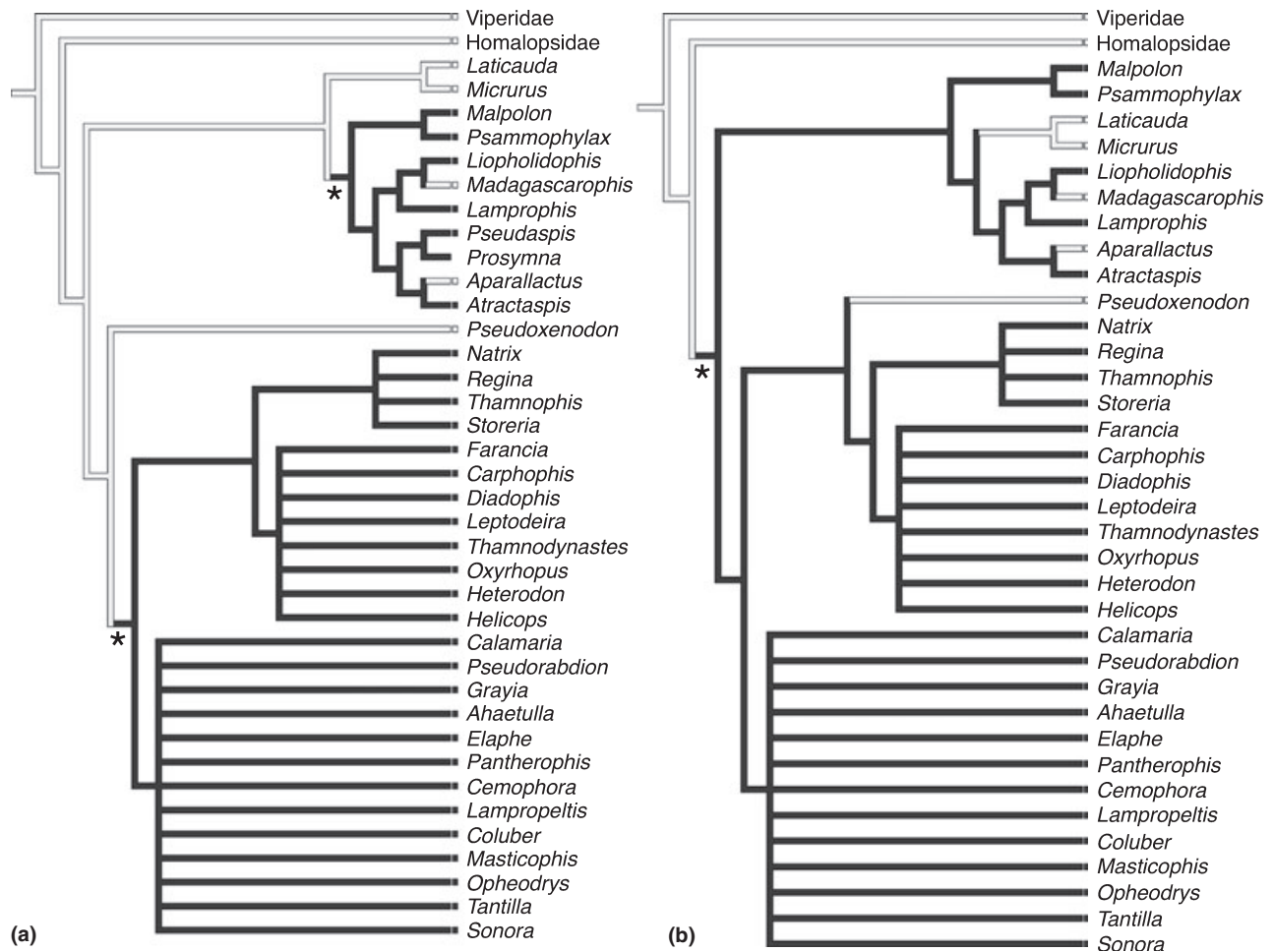


Fig. 6. Comparison of two possible hypotheses for the evolution of the ampulla urogenital/uriniferous papilla in snakes. (a) The Colubroidea + Elapoidea branch of the Vidal et al. (2008) phylogeny reconstructed from the nuclear encoding C-mos and RAG2 genes with genera examined here inserted as terminal taxa. The Elapoidea branch of the phylogeny was oriented to match the topology that Vidal et al. (2008) reconstructed from the combined data set of C-mos, RAG2, 12S & 16S RNA, cytochrome B, and ND4 genes with genera examined here inserted as terminal taxa. The most parsimonious optimization of the caudal urogenital duct character states resulted in two transitions (\*) from the ampulla ureter (grey branches) to the ampulla urogenital/uriniferous papilla (black branches) with subsequent reversals within the Elapoidea. (b) The Colubroidea + Elapoidea branch of the phylogeny produced by Zaher et al. (2009) from C-mos and 12S & 16S RNA with genera examined here inserted as terminal taxa. The most parsimonious optimization of the caudal urogenital duct character states resulted in a single transition (\*) from the ampulla ureter (grey branches) to the ampulla urogenital/uriniferous papilla (black branches), with subsequent reversals within the Elapoidea and Pseudoxenodontidae

est une pochette élargie, distincte de l'uretère, qui communique l'uretère et canal déférent/canal de Wolff à l'urodaeum. Bien que les différences fonctionnelles de ces deux structures ne sont pas connus, l'ampoule uro/papille urinifères peut avoir évolué pour le stockage de l'urine des mâles des femelles, et secondairement avoir évolué en fonction de reproduction chez les mâles. L'optimisation la plus parcimonieuse de l'uretère et de l'ampoule ampoule uro/papille urinifères indique que l'uretère ampoule est l'état ancestral des serpents. L'examen de la présence ou l'absence de l'uretère ampoules et ampoules urogénital/papille urinifères chez les serpents sur contradictoires phylogénies caenophidian résultats dans deux hypothèses pour l'évolution de ces morphologies variante: 1) l'ampoule urogénital/papille urinifères a évolué à partir de l'uretère ampoule de façon autonome dans la Colubroidea et avec des pertes ultérieures Elapoidea de la papille urogénitale/papille urinifères dans le Elapoidea, ou 2) une seule transition de l'uretère ampoule de l'ampoule uro/papille urinifères sur la branche menant à la Elapoidea Colubroidea avec des pertes subséquentes de la papille urogénitale/urinifères papille dans le Elapoidea et Colubroidea. La présence des ampoules uro/papille urinifères que dans la Colubroidea et Elapoidea souligne l'affinité de ces deux groupes taxonomiques, une relation qui est fortement appuyé dans cladogrammes publiée des jeux de données moléculaires.

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## Supporting Information

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

### Appendix S1. Specimens examined.

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