The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2015, ••, ••–••. With 3 figures.

The emerging phylogenetic pattern of parthenogenesis in snakes

WARREN BOOTH[1,2,3]* and GORDON W. SCHUETT[2,3,4]

[1]*Department of Biological Sciences, The University of Tulsa, Tulsa, OK, 74104, USA*
[2]*The Copperhead Institute, PO Box 6755, Spartanburg, SC, 29304, USA*
[3]*Chiricahua Desert Museum, PO Box 376, Rodeo, NM, 88056, USA*
[4]*Department of Biology and Neuroscience Institute, Georgia State University, Atlanta, GA, 30303, USA*

Received 11 September 2015; revised 3 November 2015; accepted for publication 3 November 2015

Parthenogenesis occurs across a variety of vertebrate taxa. Within squamate reptiles (lizards and snakes), a group for which the largest number of cases has been documented, both obligate and facultative types of parthenogenesis exists, although the obligate form in snakes appears to be restricted to a single basal species of blind snake, *Indotyphlops braminus*. By contrast, a number of snake species that otherwise reproduce sexually have been found capable of facultative parthenogenesis. Because the original documentation of this phenomenon was restricted to subjects held in captivity and isolated from males, facultative parthenogenesis was attributed as a captive syndrome. However, its recent discovery in nature shifts the paradigm and identifies this form of reproduction as a potentially important feature of vertebrate evolution. In light of the growing number of documented cases of parthenogenesis, it is now possible to review the phylogenetic distribution in snakes and thus identify subtle variations and commonalities that may exist through the characterization of its emerging properties. Based on our findings, we propose partitioning facultative parthenogenesis in snakes into two categories, type A and type B, based on the sex of the progeny produced, their viability, sex chromosome morphology, and ploidy, as well as their phylogenetic position. Furthermore, we introduce a hypothesis (directionality of heterogamety hypothesis) to explain the production of female-only parthenogens in basal alethinophidian snakes and male-only parthenogens in caenophidian (advanced) snakes. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **00**, 000–000.

ADDITIONAL KEYWORDS: automixis – facultative and obligate parthenogenesis – Serpentes – sex chromosomes morphology – sex determination – squamates.

INTRODUCTION

Parthenogenesis, or virgin birth, has been documented across a diverse group of vertebrate taxa, particularly birds, elasmobranchs (sharks and rays), and squamate reptiles (Olsen, 1975; Darevsky, Kupriyanova & Uzzell, 1985; Dubach, Sajewicz & Pawley, 1997; Schuett *et al.*, 1997; Avise, 2008). Two evolutionarily divergent types of parthenogenesis are recognized among these vertebrates: obligate and facultative (Lampert, 2008). Obligate parthenogenesis (OP) occurs primarily in lizards and is characterized by all-female (unisexual) populations; hence, reproduction occurs in the absence of any paternal genetic contribution (Dawley & Bogart, 1989; Kearney, Fujita & Ridenour, 2009; Sinclair *et al.*, 2010; Neaves & Baumann, 2011; Sites, Reeder & Wiens, 2011). These unisexual populations have been the focus of considerable research efforts, particularly with respect to understanding their evolutionary origin and phylogenetic distribution (Dawley & Bogart, 1989; Reeder, Cole & Dessauer, 2002; Fujita & Moritz, 2009; Sinclair *et al.*, 2010; Lutes *et al.*, 2011; Cole *et al.*, 2014; Grismer *et al.*, 2014). Within

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*Corresponding author. E-mail: warren-booth@utulsa.edu snakes, however, OP is documented only in a single typhlopid species, the Brahminy blind snake (*Indotyphlops braminus*), formerly known as *Ramphotyphlops braminus* (Nussbaum, 1980; Wynn, Cole & Gardner, 1987; Ota *et al.*, 1991). Despite its worldwide distribution, attributed to the commercial nursery business (hence, its common moniker, the flower pot snake), surprisingly little is known about the parthenogenetic characteristics of this diminutive species, including the mechanism of reproduction. With a single exception (Sinclair *et al.*, 2010), all cases of OP in squamate reptiles (lizards) appear to be the result of interspecific hybridization (Cole, Dessauer & Barrowclough, 1988; Moritz *et al.*, 1989). Although it is currently unconfirmed, it is assumed that this hybrid mechanism may explain the heteromorphic triploid karyotype of the *I. braminus* (Wynn *et al.*, 1987). Parthenogenesis is also assumed given the failure to find males within any populations sampled to date and a lack of molecular tests, such as DNA-based analyses (McDowell, 1974; Nussbaum, 1980; Wynn *et al.*, 1987; Ota *et al.*, 1991).

Facultative parthenogenesis (FP), asexual reproduction in an otherwise sexually reproducing species, has been documented in a variety of vertebrates (Avise, 2008, 2015; Lampert, 2008) but was initially discovered in certain strains of turkeys and chickens in the 1950s (Olsen, 1975). Approximately 50 years after the discovery of FP in domestic fowl, several species of snakes were found to reproduce via FP (Dubach *et al.*, 1997; Schuett *et al.*, 1997), which was documented using DNA-based methods not available in the early work on birds. Subsequent instances of FP were documented in varanid (Varanidae) lizards (Lenk *et al.*, 2005; Watts *et al.*, 2006; Hennessy, 2010; Wiechmann, 2012; Grabbe & Koch, 2014) and in elasmobranch fishes (Chapman *et al.*, 2007; Chapman, Firchau & Shivji, 2008; Feldheim *et al.*, 2010; Robinson *et al.*, 2011; Fields *et al.*, 2015, Harmon *et al.*, 2015). Nonetheless, the greatest wealth of genetically confirmed and anecdotal cases of FP has been reported in snakes (Dubach *et al.*, 1997; Schuett *et al.*, 1997, 1998; Murphy & Curry, 2000; Groot, Bruins & Breeuwer, 2003; Germano & Smith, 2010; Booth & Schuett, 2011; Booth *et al.*, 2011a, b, 2012, 2014; Kinney *et al.*, 2013; Reynolds *et al.*, 2012; Lara-Resendiz *et al.*, 2013; Vaughan & Steele, 2014; Jordan, Perrine-Ripplinger & Carter, 2015) (Table 1). The number of examples of FP may actually be much greater; when cases previously attributed to long-term sperm storage or other mechanisms (Magnusson, 1979; Scalka & Vozenilek, 1986) are re-evaluated, FP is often a more viable hypothesis (Booth & Schuett, 2011).

When FP is evaluated across vertebrates, we see a set of commonalities. For example, FP appears to be phylogenetically widespread, with instances reported in the Galliformes, Columbiformes, and Passeriformes lineages of birds (Bartelmez & Riddle, 1924; Olsen & Marsden, 1954; Schut, Hemmings & Birkhead, 2008); the Orectolobiformes, Carcharhiniformes, Pristoformes, and Myliobatiformes lineages of elasmobranchs (Chapman *et al.*, 2007, 2008; Feldheim *et al.*, 2010; Robinson *et al.*, 2011; Fields *et al.*, 2015); and Boidae, Pythonidae, Acrochordidae, Natricinae, and Crotalinae lineages of snakes (Dubach *et al.*, 1997; Schuett *et al.*, 1997, 1998; Booth & Schuett, 2011; Booth *et al.*, 2011a, b, 2012, 2014; Reynolds *et al.*, 2012; Vaughan & Steele, 2014). In lizards, however, most cases of FP are limited to varanids (Lampert, 2008).

In snakes and elasmobranch fishes, FP has been documented in both viviparous and oviparous taxa. With two exceptions (Groot *et al.*, 2003; Portnoy *et al.*, 2014; but see also Booth *et al.*, 2014), parthenogenesis appears to result from automixis; specifically, it is mostly attributed to terminal fusion, the process whereby the egg nucleus fuses with a second polar body and diploidy is restored (Lampert, 2008) (Fig. 1). However, we argue that, without confirmation of heterozygosity of progeny suspected to be parthenogens via FP, gamete duplication (a form of automixis resulting from the division of a haploid egg to produce cleaved nuclei which fuse to produce a diploid nucleus; Stenberg & Saura, 2009) (Fig. 1) cannot be rejected as a plausible cellular mechanism. Detecting heterozygosity within progeny produced by FP is difficult using conventional molecular tools [e.g. microsatellites, amplified fragment length polymorphisms (AFLPs)] given that recombination generating heterozygosity may be restricted to the terminal tips of the chromosomes (Lampert, 2008); accordingly, the detection of FP is possible only if the marker employed is situated within a region of recombination. Heterozygosity has been documented in parthenogens of two species (Robinson *et al.*, 2011; Reynolds *et al.*, 2012), supporting terminal fusion automixis. Regardless of the actual mechanism, both forms of automixis result in genome-wide or near genome-wide homozygosity and an individual that is highly inbred (Hedrick, 2007; Pearcy, Hardy & Aron, 2011). Under automixis, the offspring are half-clones of the mother; thus, in sharks, all parthenogens are female given female homogamety (XX) of the sex chromosomes (Chapman *et al.*, 2007), whereas, in birds and varanid lizards, the offspring are male owing to heterogamety (ZW) in the female sex (Olsen, 1975; Watts *et al.*, 2006). In snakes, which are considered to exhibit female heterogamety (ZW), both male and female parthenogens are documented via FP (Booth & Schuett, 2011).

Until recently, FP had been considered as an evolutionary novelty of minor significance largely

Table 1. Genetically confirmed cases (*) and those supported by captive history (†) of facultative parthenogenesis in snakes

Species	Origin	Captive history	Number of eggs/ offspring	Sex of offspring	Viability	Parthenogenetic mode	Reference
Boa constrictor (*Boa constrictor*)	CB	Previous history of sexual reproduction. Housed with 4 males	22	Female	22 live young	Automixis	Booth *et al.* (2011a)*
Colombian rainbow boa (*Epicrates maurus*)	CB	Isolated from males from birth	13+ unreported number of yolked ova	Female	Six born alive of which four later died because of failure to establish feeding	Automixis	Booth *et al.* (2011b)*
Brazilian rainbow boa (*Epicrates cenchria*)	CB	Housed with a male vasectomized 59 months earlier	Four offspring	Female	Three live, one stillborn	Automixis	Kinney *et al.* (2013)*
Green anaconda (*Eunectes murinus*)	CB	Isolated from males from birth	Eight offspring	Female	Three live (one later died),	Untested	M. O'Shea (pers. comm.)†
Burmese python (*Python bivittatus*)	CB	Isolated from males for several years	Multiple clutches of which 25% appear viable	Female	No eggs incubated full term. Seven eggs developed outwardly normal embryos	Apomixis, premeiotic doubling, or central fusion automixis.	Groot *et al.* (2003)*
	CB	Unknown	Unknown	Female	Two live	Automixis	T. V. Groot (pers. comm.)*
Reticulated python (*Malopython reticulatus*)	CB	Isolated from males for more than 2 years	61 eggs	Female	~50% of clutch appeared viable and was incubated. Eventually culled to six eggs. Culled eggs contained a mix of healthy and malformed embryos	Automixis	Booth *et al.* (2014)*

Table 1. *Continued*

Species	Origin	Captive history	Number of eggs/ offspring	Sex of offspring	Viability	Parthenogenetic mode	Reference
Royal python (*Python regius*)	CB	Observed copulating with a male	Three eggs	Female	Three live	Automixis	Booth *et al.* (2014)*
	CB	Observed copulating with a male	Six eggs	Female	Six live	Automixis	Booth *et al.* (2014)*
	CB	Isolated from males for over 20 years	Four eggs (two viable)	Female	Two live	Automixis	Booth *et al.* (2014)*
Blood python (*Python brongersmai*)	CB	Housed with males – observed breeding	Ten eggs	Female	Eight live, two deformed and killed	Untested	D. Barker & T. Barker (pers. comm.)†
Arafuran filesnake (*Acrochordus arafurae*)	CB	Isolated from males since birth	Five offspring	Male	Two live (later died), three stillborn	Automixis	Dubach *et al.* (1997)*
Wandering garter snake (*Thamnophis elegans vagrans*)	WC	Isolated from males for 4 years	Nine offspring, 21 yolked ova – multiple litters	Not recorded	Four live, five dead (two normal in appearance, two partially formed, one developmental abnormalities)	Automixis	Schuett *et al.* (1997)*
Checkered garter snake (*Thamnophis marcianus*)	WC	Isolated from males since capture	Fourteen offspring, 27 yolked ova – multiple litters	Male	Three born alive, 11 stillborn/ deformed	Terminal fusion automixis	Schuett *et al.* (1997) Reynolds *et al.* (2012)*
Plains garter snake (*Thamnophis radix*)	CB	Isolated from males since birth	Six offspring, multiple yolked ova	Unsexed	Four live (one later died), three stillborn	Untested	Murphy & Curry (2000)†
Sierra garter snake (*Thamnophis couchii*)	WC (collected as juvenile)	Isolated from males for 6 years	Three offspring, no further reproductive products noted	Unsexed	One live, two stillborn	Automixis (however, molecular data inconclusive as a result of homozygosity across all samples)	Germano & Smith (2010)*
Northern water snake (*Nerodia sipedon*)	WC (collected as an adult)	Isolated from males for ~2 years	Four offspring, 42 yolked ova	One male, three unknown	One live, three stillborn	Automixis	W. Booth & G. W. Schuett (unpubl. data)*

Table 1. *Continued*

Species	Origin	Captive history	Number of eggs/ offspring	Sex of offspring	Viability	Parthenogenetic mode	Reference
Timber rattlesnake (*Crotalus horridus*	CB	Isolated from males for 14 years	Three offspring, three yolked ova	Male	One live, two stillborn	Automixis	Schuett *et al.* (1997)*
Aruba Island rattlesnake (*Crotalus unicolor*)	CB	Isolated from males since birth	Two offspring (not fully developed), three yolked ova	Unknown	None viable	Untested	Schuett *et al.* (1997)†
Prairie rattlesnake (*Crotalus viridis*)	WC (collected as a baby)	Isolated from males since capture (time unknown)	One offspring, multiple yolked ova	Male	One live (facial deformity and later died)	Automixis	W. Booth (unpubl. data)*
Copperhead (*Agkistrodon contortrix*)	CB	Isolated from males for 5 years	Four offspring, 12 yolked ova	Male	Two alive, two stillborn, one alive	Automixis	Booth & Schuett (2011)*
	Wild	~1 : 1 population sex ratio	One offspring, multiple yolked ova	Male	One alive	Automixis	Booth *et al.* (2012)*
	WC (9 years prior to birth)	Isolated from males since capture	One offspring, four yolked ova	Male	One stillborn	Automixis	Jordan *et al.* (2015)*
Cottonmouth (*Agkistrodon piscivorus*)	WC	Isolated from males since capture	Four offspring, 13 yolked ova	Male	One live, two stillborn, one under-developed	Automixis	Booth *et al.* (2012)*
	Wild	Isolated from males since capture	One offspring, multiple yolked ova	Male	One live, two stillborn, one under-developed	Automixis	Booth *et al.* (2012)*
Terciopelo (*Bothrops asper*)	CB	Isolated from males since birth	One offspring, 11 yolked ova	Male	One stillborn	Untested	Vaughan & Steele (2014)†
	CB	Isolated from males since birth	Four offspring, multiple yolked ova – two litters	Male	Two live, one stillborn, one under-developed	Untested	Z. Marchetti (pers. comm.)†
	WC	Isolated from males for 5 years prior to birth	Three offspring, 12 yolked ova	Male	Three live	Untested	R. Labanowski (pers. comm.)†

WC, wild-collected; CB, captive born.

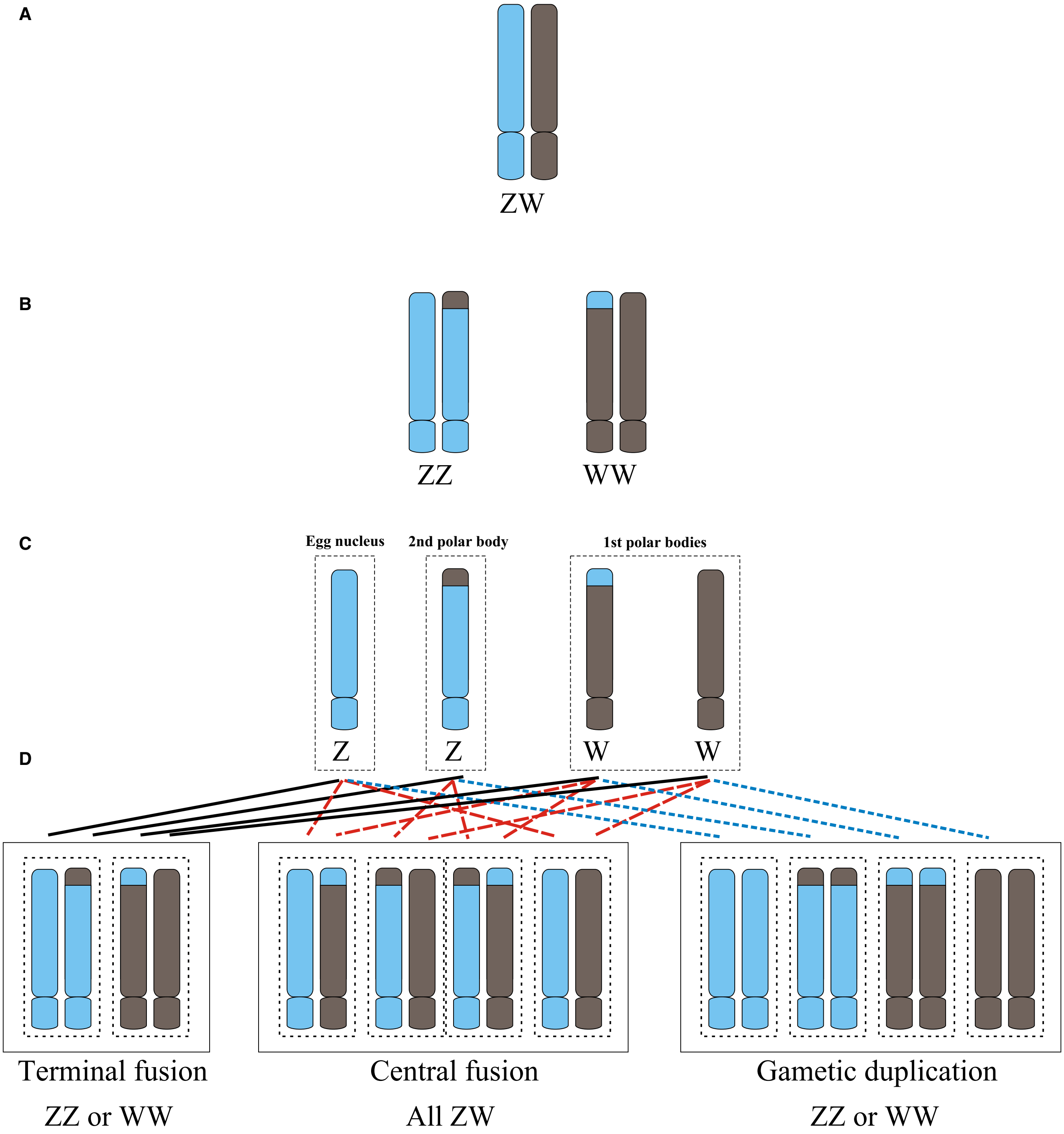

Figure 1. Mechanisms of automixis with ZW sex chromosomes. A, primordial germ cell. B, meiotic products following DNA replication and recombination during the first cell division. C, meiotic products following second cell division. D, potential sex chromosomal arrangements following terminal fusion (black lines, solid), central fusion (red line lines, dashes), and gametic duplication (blue lines, dotted). Each chromosome pair is denoted by a dashed box.

because all records resulted from animals held in captive isolation for prolonged periods. FP was therefore considered to represent a captive syndrome with little relevance to the natural world (Booth *et al.*, 2012). Two studies concerning pitviper snakes (genus *Agkistrodon*) have subsequently been published documenting parthenogenetic reproduction occurring in natural populations (Booth *et al.*, 2012), and a third one has documented the presence of free-living parthenogens within a population of smalltooth sawfish, *Pristis pectinate* (Fields *et al.*, 2015). In these cases, the population sex ratios of adults were found to be at or close to unity (1 : 1), suggesting that a shortage of mates was not a driving force of this alternative reproductive strategy. Consequently, with the discovery of FP in nature, the paradigm is shifting toward understanding the role of FP in vertebrate evolution (Booth *et al.*, 2012; Fields *et al.*, 2015). Although the identification of FP in natural populations was previously considered to comprise a formidable task (Avise, 2008), re-analysis of the available population datasets, which have employed variable nuclear markers (microsatellites, restriction site-associated DNA sequencing or RAD-seq), focusing on estimates of internal relatedness (Fields *et al.*, 2015), may greatly expand the number of cases currently known within natural populations and increase its significance as an alternative reproductive strategy in the evolution of vertebrates.

In light of the growing number of cases of parthenogenesis in snakes, along with the confirmation of its occurrence in natural populations, such a taxonomic spread across the lineages of snakes allows for a review of its emerging properties from a phylogenetic perspective. Specifically, we address five characteristics commonly discussed in reports of parthenogenesis in snakes: (1) parthenogenetic mode (FP or OP); (2) ploidy; (3) sex chromosome morphology; (4) mode of parity; and (5) the sex and viability of the parthenogens produced. These properties are interpreted based on several recent, large-scale phylogenetic analyses of squamate reptiles that were conducted using morphological characters of extant and extinct taxa (Gauthier *et al.*, 2012; Longrich, Bhullar & Gauthier, 2012), or molecular (DNA-based) characters (Wiens *et al.*, 2008, 2012; Pyron, Burbrink & Wiens, 2013). Because the trees recovered in the morphological and molecular analyses were distinctly discordant in topology, which is significant with respect to understanding evolutionary trends (Losos, Hillis & Greene, 2012), we opted to use both interpretations of the historical relationships of snakes and their squamate relatives.

EMERGING PHYLOGENETIC PATTERNS

Parthenogenetic mode

Obligate parthenogenesis is present in early snake history and in only a single lineage (putative autapomorphy) of an extant basal scolecophidian, the Brahminy blindsnake, *I. braminus* (Fig. 2). Facultative parthenogenesis appears early in snake evolution, first in the basal alethinophidian lineages Boidae (*Boa constrictor*, *Epicrates* spp.) and Pythonidae (*Python* spp.) (Groot *et al.*, 2003; Booth *et al.*, 2011a, b, 2014; Kinney *et al.*, 2013). In the morphological phylogenetic tree (Fig. 2A), boids (mostly viviparous) and pythonids (all oviparous) are sisters and thus are derived from a recent most-common ancestor, whereas, in the molecular phylogenetic tree (Fig. 2B), boids and pythonids were recovered as more distantly related. Facultative parthenogenesis next appears in the viviparous basal caenophidian *Acrochordus arafurae* (Dubach *et al.*, 1997), and it is currently documented in two large colubroid lineages, the crotalines (pitvipers) and natricines (garter snakes and water snakes), from North America (Fig. 2 and Table 1) (Schuett *et al.*, 1997; Murphy & Curry, 2000; Germano & Smith, 2010; Booth & Schuett, 2011; Reynolds *et al.*, 2012; Vaughan & Steele, 2014; Jordan *et al.*, 2015). At present, we have no recorded evidence of FP in snakes basal to boids and pythonids (e.g. cylindrophiids, uropeltids). In caenophidians (‘advanced snakes’), we lack evidence for FP in the elapids, colubrids, and other lineages. As such, the conclusions we report here are based on evidence derived from a somewhat limited number of basal alethinophidian taxa (boids, pythonids), *Acrochordus*, and several caenophidian lineages (i.e. Crotalinae and Natricinae), which we mention above. Although re-evaluation of long-term sperm storage suggests that FP may be present in other caenophidian lineages (Booth & Schuett, 2011), genetic confirmation is lacking. Accordingly, we recommend that our results should not be extrapolated widely to additional lineages until multiple lines of evidence, including molecular analysis, become available.

Ploidy

Although information is sparse, the Brahaminy blindsnake (*I. braminus*), which is capable of OP, is highly suspected to be triploid (3*n*) based on the unusual karyotype and electrophoretic allozyme patterns of specimens collected in the USA and the Seychelles (Wynn *et al.*, 1987). Subsequent to karyotyping of specimens collected in Asia, Ota *et al.* (1991) presented comparable results (42 chromosomes arranged as 14 triplets). With respect to ploidy in snakes, all other taxa (> 3000 species) are typically diploid (Oguiura, Ferrarezzi & Batistic, 2009); however, the occurrence of a spontaneous triploid has been reported in the Cottonmouth (*Agkistrodon piscivorus*), a North American pitviper (Tiersch & Figiel, 1991), and a species where FP has been documented in both captive and wild individuals (Booth *et al.*, 2012).

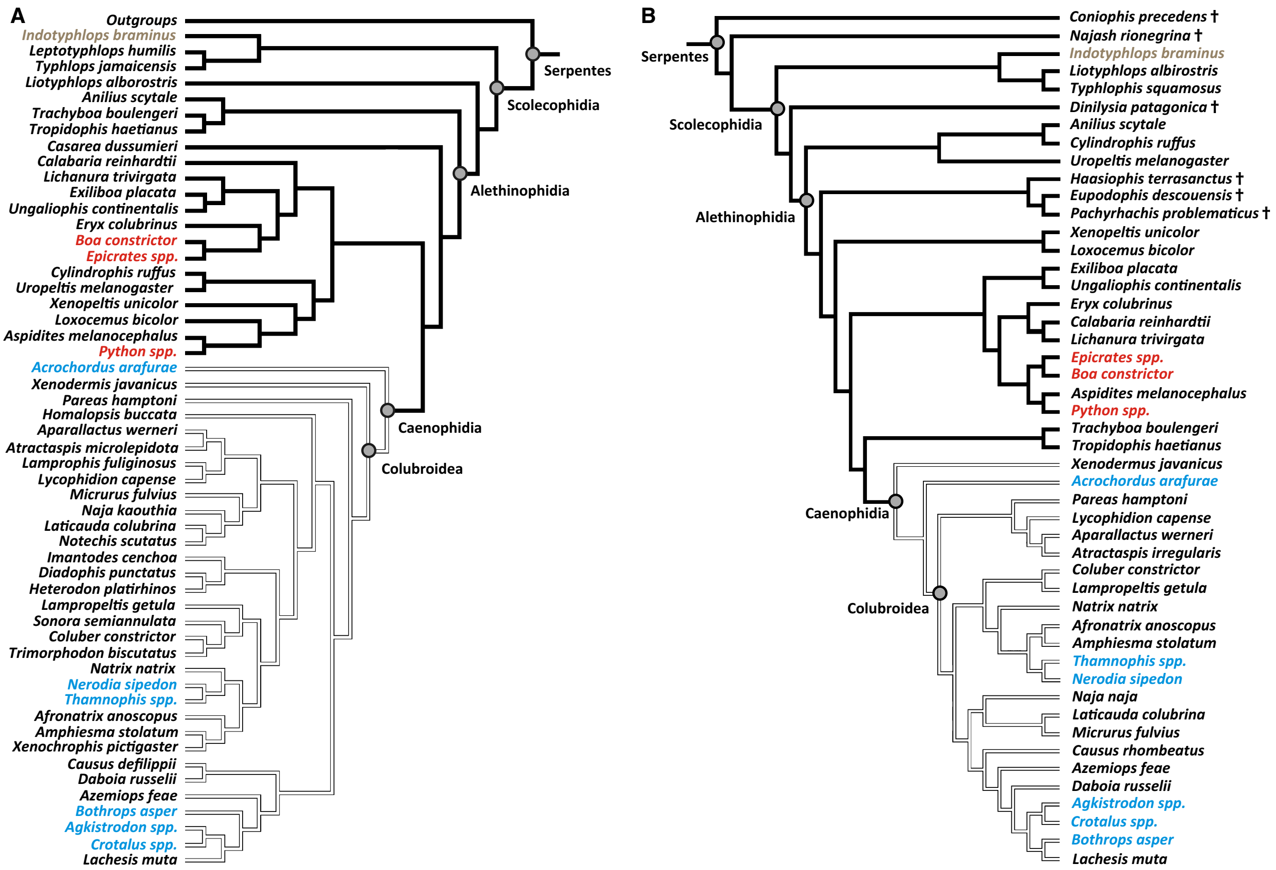

Figure 2. Phylogeny of snakes and pattern of parthenogenesis. A, phylogeny constructed from molecular characters (primarily nuclear DNA). Modified from Wiens *et al.* (2008, 2012) and Pyron *et al.* (2013). B, phylogeny constructed from morphological and fossil (extinct taxa) characters. Modified from Gauthier *et al.* (2012) and Longrich *et al.* (2012). Nodes identify Scolecophidia, Alethinophidia, Caenophidia, and Colubroidea. Where possible, species documented as being parthenogenetic are indicated in brown (obligate), red (facultative, type A), and blue (facultative, type B).

MODE OF PARITY

Across the phylogenetic tree of snakes, the mode of parity varies (oviparous, viviparous) and is the result of multiple independent transitions from oviparity → viviparity and the reverse (Pyron & Burbrink, 2014; but, for concerns related to viviparity → oviparity transitions, see Blackburn, 2015; Griffith *et al.*, 2015). Obligate parthenogenesis in snakes, however, has been found only in a single oviparous species, whereas FP has been reported in both viviparous and oviparous species. Oviparity has been documented in boid lineages; namely, a single species of sand boa (genus *Eryx*) and the African *Calabaria reinhardtii*. Oviparity also occurs in several distant boid relatives, such as *Casarea dussumieri* from Round Island (Lynch & Wagner, 2010; Reynolds, Niemiller & Revell, 2014). However, all boid species for which FP has been observed are viviparous (Booth *et al.*, 2011a, b; Kinney *et al.*, 2013; M. O'Shea. pers. comm.). All pythonids are oviparous and FP has been genetically confirmed in three species (Groot *et al.*, 2003; Booth *et al.*, 2014). FP is suspected in two other species based on captive history (*Python brongersmai*, D. Barker & T. Barker, pers. comm.; *Anteresia childreni*, W. Booth, unpubl. data). In advanced snakes (caenophidians), all instances of FP reported are in species that are viviparous (Dubach *et al.*, 1997; Schuett *et al.*, 1997; Murphy & Curry, 2000; Germano & Smith, 2010; Booth & Schuett, 2011; Booth *et al.*, 2012; Reynolds *et al.*, 2012; Vaughan & Steele, 2014; Jordan *et al.*, 2015).

SEX CHROMOSOME MORPHOLOGY

To date, all snakes appear to show some type of the ZZ/ZW chromosomal system. As such, females are the heterogametic (ZW) sex (Lampert, 2008; Oguiura *et al.*, 2009; Graves, 2013). Early work on the karyotypes of snakes suggests, however, that, across taxa, significant variation exists with regard to W chromosome degeneration (Ohno, 1967; Beçak & Beçak, 1969; Vicoso, Kaiser & Bachtrog, 2013). For example, the sex chromosomes of boids and pythonids appear to be undifferentiated (homomorphic) at the karyotypic level and, in the former, at least for now, also at the genomic level (Vicoso *et al.*, 2013). By contrast, various levels of degeneration have been reported in the 'advanced snakes' or caenophidians (Ohno, 1967; Beçak & Beçak, 1969; Matsubara *et al.*, 2006; Vicoso *et al.*, 2013). As such, the sex chromosomes in caenophidian snakes are termed heteromorphic as a result of the observable size differentiation of Z and W sex chromosomes. It should be noted that the sex chromosome arrangement of WW was considered nonviable until recent molecular evidence provided some support for it in the parthenogens of several basal alethinophidian taxa (Booth *et al.*, 2011a, b, 2014; Kinney *et al.*, 2013). The hypothesis of WW parthenogens in snakes requires further testing.

THE SEX OF PARTHENOGENS AND VIABILITY

Parthenogenetic progeny of boids and pythonids produced in captivity are female, exhibit high viability at birth (few stillborn with morphological defects), and litters or clutches tend to be large (Booth *et al.*, 2011a, b, 2014; Kinney *et al.*, 2013; D. Barker & T. Barker, pers. comm; W. Booth & G. W. Schuett, unpubl. data). A small number of malformed embryos and stillborn young, however, were reported in one species of python, *Malayopython reticulatus* (Booth *et al.*, 2014) and two species of the boid genus *Epicrates* (Booth *et al.*, 2011b; Kinney *et al.*, 2013). Facultative parthenogenesis within caenophidian snakes is characterized by all-male offspring, low viability (single or few offspring), extreme developmental abnormalities (Fig. 3), and high numbers of infertile ova (Schuett *et al.*, 1997; Murphy & Curry, 2000; Germano & Smith, 2010; Booth & Schuett, 2011; Booth *et al.*, 2012; Reynolds *et al.*, 2012; Vaughan & Steele, 2014; Jordan *et al.*, 2015; W. Booth & G. W. Schuett, unpubl. data).

DISCUSSION

PHYLOGENETIC PATTERN OF PARTHENOGENESIS IN SNAKES

Our review and synthesis of obligate and FP and associated traits in snakes reveals several intriguing properties not yet explicitly discussed in our previous studies (Booth & Schuett, 2011; Booth *et al.*, 2011a, b, 2012, 2014; Reynolds *et al.*, 2012), nor in other studies as far as we are aware (Avise, 2015). Here, we show that OP is present only in the basal scolecophidian *I. braminus*, a diminutive unisexual species close to the root of serpent evolution. It is this obligate parthenogenetic mode that likely explains establishment success across its cosmopolitan distribution, which is the most extensive terrestrial distribution of any snake species (Nussbaum, 1980; Greene, 1997). By sharp contrast, FP appears to be widespread across the phylogeny of snakes, occurring in the ancestral boids and pythonids, a basal caenophidian (*Acrochordus*), and two large lineages (natricines, crotalines) of higher-level snakes

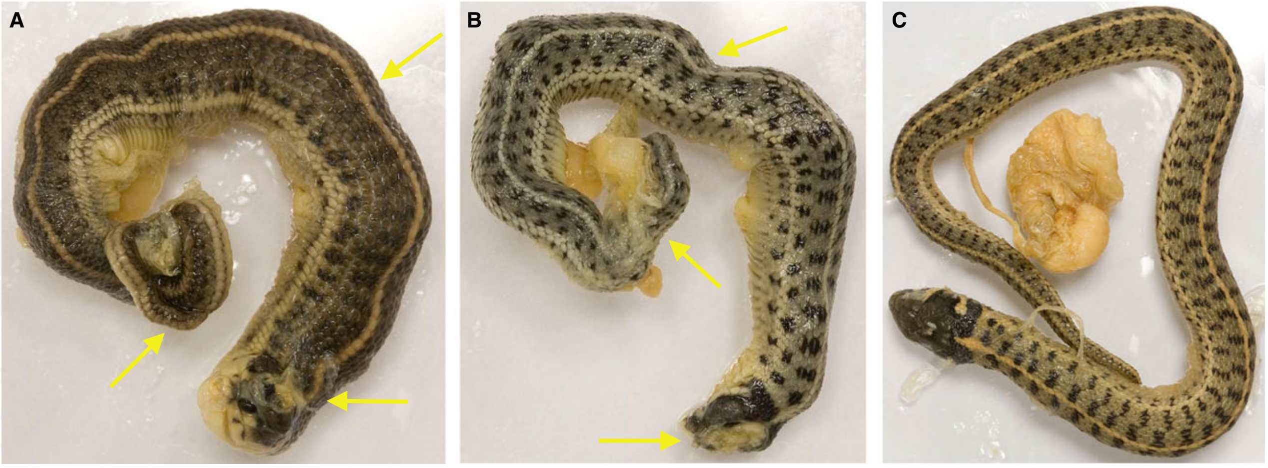

Figure 3. Stillborn parthenogen specimens of the checkered garter snake, *Thamnophis marcianus* (Reynolds *et al.*, 2012). A, B, yellow arrows denote where significant brain case and post-cranial deformities occur. These kinds of developmental deformities are common in cases of facultative parthenogenesis in caenophidian snakes (Schuett *et al.*, 1997; Booth & Schuett, 2011). C, developmentally normal but stillborn offspring.

(Colubroidea). Across this phylogeny, FP has been documented primarily in viviparous taxa.

We consider the most striking and intriguing result of our review is that the major characteristics of FP differ sharply between the lineages of ancient and advanced snakes. Specifically, this shift appears to occur at the node splitting alethinophidians from higher-level snakes, the caenophidians (Fig. 2). Furthermore, these FP characters appear to be conserved within the lineages. In the Boidae and Pythonidae (basal alethinophidians), the available evidence thus far suggests that FP is by automixis (likely terminal fusion) and results only in female progeny with an arrangement of sex chromosomes presumed to be WW (Booth *et al.*, 2011a, b, 2014; Kinney *et al.*, 2013; D. Barker & T. Barker, pers. comm; M. O'Shea, pers. comm; W. Booth & G. W. Schuett, unpubl. data). Based on the abovementioned characteristics, we proposed that FP in boids and pythonids be referred to as a category of FP we term type A.

Other than in a single record (Groot *et al.*, 2003), ZW female parthenogens have yet to be conclusively substantiated in any snake species of (Schuett *et al.*, 1997; Booth *et al.*, 2011a, b, 2014; Kinney *et al.*, 2013). In their unique case, Groot *et al.* (2003) presented molecular evidence supporting apparently clonal offspring produced by a Burmese python, *Python bivittatus*, held in captivity at the Artis Zoo (The Netherlands). Although no offspring were incubated to hatching, and thus viability could not be determined, the sex of the offspring was female and all (mother and seven offspring) exhibited homozygous microsatellite profiles and identical genotypes across AFLP loci. This apparent clonal production, suggestive of ZW females, excludes terminal fusion automixis as a parthenogenetic mechanism. The actual mode of FP could not be determined accurately; however, apomixis, premeiotic doubling of chromosomes, and central fusion automixis were proposed as possible mechanisms (Groot *et al.*, 2003). This apparent clonal reproduction contrasts starkly with cases subsequently described in other basal alethinophidian and caenophidian taxa (Booth *et al.*, 2011a, b, 2014; Kinney *et al.*, 2013) (i.e. FP resulting from automixis).

Recently, Booth *et al.* (2014) reported multiple instances of FP in two species of pythons closely related to *P. bivittatus*: the reticulated python (*M. reticulatus*) and royal python (*Python regius*). Evidence provided for these species, representing three independent cases in *P. regius* and one case in *M. reticulatus*, supports the conclusions presented for other basal alethinophidian snakes (namely *B. constrictor* and *Epicrates* spp.), that FP reproduction was by automixis with viable, female offspring. Booth *et al.* (2014) presented the hypothesis that the case documented by Groot *et al.* (2003) may represent secondary parthenogenesis by a female itself produced by parthenogenesis. Consequently, if the adult female *P. bivitattus* was herself produced by FP, any offspring produced by her under FP would appear as clonal. This hypothesis has yet to be tested. By way of a personal communication with T. V. M. Groot (University of Amsterdam), we know that a second *P. bivittatus* parthenogenetic clutch presented to him was found to feature characteristics comparable to those described in all other basal alethinophidian FP events (as we have described above). The findings of this later case remain unpublished. Given this degree of uncertainty resulting from Groot *et al.* (2003), additional research concerning the mode of FP in this taxon is warranted.

In higher-level snakes (Caenophidia + Colubroidea), a clade that contains the majority of extant species (Greene, 1997; see http://www.reptile-database.org), FP appears to occur by automixis (likely terminal fusion) and results only in male progeny with ZZ sex chromosomes (Dubach *et al.*, 1997; Schuett *et al.*, 1997; Germano & Smith, 2010; Booth & Schuett, 2011; Booth *et al.*, 2012; Reynolds *et al.*, 2012; Vaughan & Steele, 2014; Jordan *et al.*, 2015). To date, female progeny (ZW or WW) are not known to occur by FP in higher-level snakes (Booth & Schuett, 2011). To differentiate these characteristics from those exhibited by basal alethinophidian taxa (type A), we propose that FP in caenophidian snakes be referred to as type B.

A secondary characteristic, which appears conserved within each form of FP, relates to the viability of progeny. Although type A FP often results in large, outwardly viable litters or clutches (Booth *et al.*, 2011a, b, 2014), type B FP produces progeny often with severe deformities (Fig. 3) and litters that exhibit low viability, typically containing many undeveloped ova (Schuett *et al.*, 1997; Germano & Smith, 2010; Booth & Schuett, 2011; Booth *et al.*, 2012; Reynolds *et al.*, 2012; Vaughan & Steele, 2014; Jordan *et al.*, 2015). In the absence of fertilization, it is known that follicles may undergo atresia or become reabsorbed (Ford & Karges, 1987) or, if ovulated, they are evacuated following a gestation period (Gibbons, 1972). Consequently, we speculate that the switch influencing the nucleus of each independent egg to fuse with its second polar body, and thus undergo automixis, may be recognized across many ova in ancestral lineages of snakes (basal alethinophidians), although in only a few ova in advanced lineages (caenophidians). The factors or conditions that would mediate this outcome are entirely unknown at present, and understanding the proximate control of this particular phenomenon is likely a rich area for future research.

In progeny of FP type B, deformities of the genitalia have been reported in some cases (Schuett *et al.*, 1997), possibly resulting from the severe inbreeding depression that terminal fusion automixis essential represents; yet 'normal-appearing spermatozoa' have been reported in others (Reynolds *et al.*, 2012; W. Booth & G. W. Schuett, unpubl. data). Nonetheless, the existence of outwardly healthy parthenogens of boas and pythons (basal alethinophidians) and some caenophidian taxa in captivity substantially increases the likelihood that the question of reproductive competence will be answered in the near future.

With respect to sex chromosome morphology and karyotypic patterns, substantial differences exist among the three major lineages (Scolecophidia, Alethinophidia, and Caenophidia). The sex chromosomes of blind snakes (basal scolecophidians) and most basal alethinophidians, for example, do not vary in morphology (homomorphic), whereas they are variably heteromorphic in most members of the clade Caenophidia + Colubroidea (Ohno, 1967; Gorman & Gress, 1970; Oguiura *et al.*, 2009). Interestingly, the heteromorphic condition is not present in all colubroids, thus indicating some evidence for reversals. The spectacled cobra (*Naja naja*), an elapid from India and Sri Lanka, possesses homomorphic sex chromosomes (Singh, Sharma & Ray-Chaudhuri, 1970; Ray-Chaudhuri, Singh & Sharma, 1971). Whether these are homologous to those found in the basal alethinophidians or result from subsequent degradation of the W chromosome followed by heterochromatization is not known. Additionally, females of the Indian krait (*Bungarus caeruleus*), another elapid, possess variable diploid numbers (43, 44, and 45) and multiple sex chromosomes (e.g. Z_1Z_2W) (Singh *et al.*, 1970). Whether these cytogenetic systems influence FP is not known because we are currently unaware of any published cases of FP in any elapid snake. Genomic comparisons across snake lineages may hold the key to understanding the influence of both female heterogamety and sex chromosome morphological variation on the parthenogenetic mode and sex outcome.

SEX DETERMINATION IN PARTHENOGENESIS

The cytogenic mechanism(s) involved in FP to explain the absence of male parthenogens in basal alethinophidians is not understood. Similarly, the specific genes involved in sex determination and their locations remain a mystery (Vicoso *et al.*, 2013). Despite these uncertainties, however, two characteristics of FP in basal alethinophidians already discussed (i.e. production of female-only parthenogens and homomorphic sex chromosomes) lend insight to an alternative view that we designate as the directionality of heterogamety hypothesis. This perspective invokes that evolutionary transitions occur between XY and ZW sex chromosomes in snake lineages. Within reptiles, a diversity of sex-determining mechanisms have been reported, including those regulated by temperature (commonly observed within some lizards, chelonians, crocodilians, and Tuatara) and those regulated genetically (all snakes, some chelonians and lizards) (Modi & Crews, 2005). Within lizards, the direction of heterogamety determines whether the sex chromosomes are considered ZW or XY. Female heterogamety determines whether the sex chromosome system is ZW (♀) : ZZ (♂), whereas male heterogamety identifies a XY (♂) : XX (♀) system (Bull, 1980). In both chelonians (turtles) and the lizards, multiple independent transitions between both temperature-dependent and genetic-dependent sex determination and between male heterogamety and female heterogamety are documented (Bull, 1980; Ewart & Nelson, 1991; Pokorná & Kratochvíl, 2009; Sarre, Ezaz & Georges, 2011; Holleley *et al.*, 2015).

For example, in Gekkota (gecko lizards), temperature-dependent and genetically-determined mechanisms have been reported. Furthermore, both XY and ZW sex chromosomes have been identified (Pokorná & Kratochvíl, 2009). Similarly, in Agamidae (agamid lizards), both temperature-dependent and genetic mechanisms are documented (Pokorná & Kratochvíl, 2009); transitions between genetic- and temperature-dependent mechanisms have been reported recently in natural populations of the Australian agamid lizard, *Pogona vitticeps* (Holleley *et al.*, 2015). With regard to sex chromosome transitions between female and male heterogamety, Pokorná & Kratochvíl (2009) detected two such transitions that appear to be independent. The first concerned the common ancestor of Scincidae (scincid lizards) and the second concerned the common ancestor of two other lineages of lizards (Gymnophthalmidae, Teiidae). Such transitions may affect the directionality of heterogamety. Where present, however, these transitions appear to be restricted to cases where the sex chromosomes are newly emerged and not fully differentiated, and in which YY and WW individuals remain fertile and viable (Miura, 2008).

In light of recent evidence of transitions among sex-determining mechanisms and the direction of heterogamety in other reptiles, as well as recent evidence of sex chromosome morphology and the production of all-female FP progeny of basal alethinophidians (e.g. boids and pythonids), we propose that the most parsimonious explanation is a transition in the direction of heterogamety existing across the snake phylogeny. Although our data are restricted to boids and pythonids, it suggests that male heterogamety (i.e. XY sex chromosome system) may best explain our earlier findings (Booth *et al.*, 2014). With heteromorphism occurring in caenophidian snakes, combined with the finding of all-male parthenogenetic offspring, we consider that this is best explained by female heterogamety, thus providing support for the accepted ZW sex chromosome system. With the limited karyotypic data available for basal snake lineages, the widespread nature of homomorphism is poorly understood. Nonetheless, with recent advances in genomic procedures, such as RAD-seq, it may now be possible to identify transitions among sex determination systems more readily, and specifically those with cryptic, homomorphic sex chromosomes (Gamble & Zarkower, 2014). Using this approach, Gamble *et al.* (2015) recently investigated the directionality of heterogamety among 12 species of gecko. Their findings revealed multiple transitions among sex-determining systems, outlining the potential application of this approach to other species, including snakes. With such transitions evident across the phylogenies of other reptiles, we question why such transitions may not also be present across the phylogeny of snakes.

TESTING FOR FP IN SNAKES LINEAGES

Facultative parthenogenesis has yet to be documented in any members of the highly speciose lineage Scolecophidia. Furthermore, outside of the clades Boidae and Pythonidae, FP is not documented in any other basal alethinophidian species. We are thus keen to see documentation of FP in members of Scolecophidia, Aniliidae, Cylindrophiidae, Loxocemidae, Tropidophiidae, Uropeltidae, and Xenopeltidae. We consider these taxa especially important to the study of FP because they will strengthen our understanding of phylogenetic distribution and provide information on proximate mechanisms of sex determination. For example, is FP type A, which we describe for boids and pythonids, present in the earliest snakes (basal scolecophidians and basal alethinophidians)? The primary difficulty in studying these somewhat obscure taxa lies in obtaining the animals themselves; unfortunately, they are rarely maintained in zoological collections and most are absent from the pet trade. Wherever these species are maintained in zoos or private collections, we are hopeful that cases of suspected FP will be reported to researchers, which thus far has been a profitable way of studying FP in snakes.

In higher-level taxa (Caenophidia + Colubroidea), FP has been reported in *A. arafurae* (Dubach *et al.*, 1997; FP is highly suspected in *Achrochordus javanicus*, see Booth & Schuett, 2011) and a variety of North American natricines and pitvipers (crotalines), all of which are viviparous (Schuett *et al.*, 1997; Murphy & Curry, 2000; Germano & Smith, 2010; Booth & Schuett, 2011; Booth *et al.*, 2012; Reynolds *et al.*, 2012; Vaughan & Steele, 2014; Jordan *et al.*, 2015; W. Booth & G. W. Schuett, unpubl. data). Future emphasis, therefore, should be placed on documenting FP in other major colubroid lineages, such as Colubridae, Elapidae, and Lamprophiidae, where oviparity is the dominant mode.

CONCLUSIONS

Obligate parthenogenesis is extremely rare in snakes (Darevsky *et al.*, 1985; Dawley & Bogart, 1989; Avise, 2008) and, presently, our understanding is that it is limited to a single taxon of basal scolecophidian. The presence of OP in other species of *Indotyphlops*, other members of blindsnakes, and other taxa remains for future investigations. By sharp contrast, we show that FP appears to be widespread throughout other lineages of snakes and is likely more common than reports thus far indicate (Booth & Schuett, 2011). Because there appear to be no genetic barriers to FP in snakes and other squamates (e.g. genomic imprinting), and because most molecular tools are now within reach of many investigators, including next-generation sequencing methods, we contend that more taxa will be revealed with additional research and progress will be achieved concerning proximate mechanisms.

Finally, we emphasize that our review of the emerging properties of parthenogenesis in snakes is preliminary because our understanding of these reproductive phenomena is in its infancy. Nonetheless, our review provides the necessary first steps for phylogenetic interpretation of the origin and evolution of parthenogenesis in snakes and indicates precisely where further research would be most beneficially applied.

ACKNOWLEDGEMENTS

WB was supported by a Faculty Summer Research Fellowship awarded by The University of Tulsa, Oklahoma, as well as a Genomics Education Matching Funds grant by LiCor BioSciences. GWS was supported by Georgia State University and the Chiricahua Desert Museum. We thank Mark O'Shea and Tom V. M. Groot for discussing their findings with us. We thank the anonymous reviewer for providing valuable comments.

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