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# New insights on facultative parthenogenesis in pythons

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In vertebrates, facultative parthenogenesis (i.e. asexual reproduction by a sexually reproducing species) has been documented in four diverse taxonomic groups, namely sharks, birds, lizards, and snakes. With a single exception, the mode is terminal fusion automixis, where the second polar body fuses with the nucleus of the gamete, restoring diploidy and triggering cell division. The deviating case involves a report of a captive Burmese python (*Python bivittatus*), a giant Asiatic species common in zoological gardens and the pet trade. Although terminal fusion automixis produces half-clones of the mother, under this unique case in *P. bivittatus*, the foetuses were reported as full clones. This conclusion is an apparent anomaly with respect to the mechanism of facultative parthenogenesis reported in all other snakes. In the present study, using genotyping methods, we analyze facultative parthenogenesis in two other species of pythonids and report results that challenge the abovementioned conclusions regarding clonality. Specifically, we report new findings comparable to those reported in other primitive snakes (namely boids), which support the hypothesis of terminal fusion automixis as the mode of facultative parthenogenesis. Furthermore, in light of our new data, we re-examine the previous report of facultative parthenogenesis in the Burmese python and suggest an intriguing alternative explanation for the earlier findings.  *Biological Journal of the Linnean Society*, 2014, ••, ••–••.

ADDITONAL KEYWORDS: *Malayopython reticulatus* – microsatellite genotyping – *Python bivittattus* – *Python regius* – reptilia – Serpentes – terminal fusion automixis.

## INTRODUCTION

Facultative parthenogenesis (FP) (i.e. asexual reproduction by a sexually reproducing species) has been documented in numerous species of invertebrate (Suomalainen, 1962; Beaton & Hebert, 1988; Matsuura *et al*., 2009) and across four highly diverse vertebrate clades, namely sharks, birds, lizards, and snakes (see Supporting information, Table S1). Within each of these groups, examples of FP have been discovered across multiple lineages. Within birds, the first vertebrate group for which FP was described (Oellacher, 1872), it has been studied extensively in domesticated chickens and turkeys, and also documented in pigeons, Chinese painted quail, and the zebra finch (Bartelmez & Riddle, 1924; Olsen & Marsden, 1954; Sarvella, 1973; Parker & McDaniel, 2010). Interestingly, these represent three divergent lineages, namely the galliforms, columbiforms, and

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passerines. Within sharks, FP has been documented in the orectolobiform (Feldheim *et al*., 2010; Robinson *et al*., 2011 and carcharhiniform (Chapman *et al*., 2007; Chapman, Firchau & Shivji, 2008) lineages. In snakes, although a single basal scolecophidian species is known to reproduce through obligate parthenogenesis, the Brahminy blind snake, *Indotyphlops braminus* (Nussbaum, 1980), FP is phylogenetically widespread, having been detected early in serpent evolution in the ancient boas (Boidae) and pythons (Pythonidae), and also in several lineages of 'advanced' snakes (Booth *et al*., 2012). In all but one instance, the parthenogenetic mode has been attributed to terminal fusion automixis, a mechanism were the second polar body, a meiotic product that typically degenerates, essentially behaves as a spermatozoon to activate and fertilize the ovum and restore diploidy (Lampert, 2008). Under this mode of parthenogenesis, progeny are essentially half clones of the mother because of the resulting genome wide homozygosity (Lampert, 2008; Booth & Schuett, 2011; Booth *et al*., 2011a, b). Consequently, if multiple offspring are produced, they will not be genetically identical to each other, or to their mother (Booth & Schuett, 2011; Booth *et al*., 2011a, b). Given that all snake species have the ZZ/ZW genetic sex determination system with female heterogamy (ZW), offspring resulting from FP will be either male (ZZ) (Booth & Schuett, 2011; Booth *et al*., 2012; Reynolds *et al*., 2012) or female (WW) (Booth *et al*., 2011a, b). In FP, the ZW (female) condition is theoretically possible under modes other than terminal fusion automixis (Lampert, 2008); however, convincing evidence of these individuals has yet to be reported.

Interestingly, the one example that deviates from this pattern of development is that of the Burmese python, *Python bivittatus* (Groot, Bruins & Breeuwer, 2003). In this case, a female *P. bivittatus*, reared in captivity at the Artis Zoo (The Netherlands), in isolation from males, produced annual clutches of eggs from 1997 to 2002, of which 25–30% appeared to contain healthy embryos. Zoo policy prohibited the incubation of these eggs to full term; thus, offspring viability was never determined. Through the use of both microsatellite and amplified fragment length polymorphism (AFLP) screening, genetic testing was performed on this female and seven of her foetuses, 24 days into incubation. Full term requires approximately 55–70 days of incubation at 88–90 °F (31.1–32.2 °C). The results revealed homozygous microsatellite profiles for the mother and all seven foetuses, and identical genotypes across AFLP loci. A half-sibling sister to the mother and an unrelated female revealed variation at the AFLP loci amplified. These embryos were surgically sexed as female, based on the presence of ovaries and the absence of testes. Given the identical maternal-offspring genotypes and the sex of the offspring, Groot *et al*. (2003) concluded that, unlike the first two original reports of FP in snakes (Dubach, Sajewicz & Pawley, 1997; Schuett *et al*., 1997), this female had reproduced clonally, which thus excluded terminal fusion automixis as a viable mechanism for development. Although the parthenogenetic mechanism could not be ascertained definitively, Groot *et al*. (2003) conjectured it to be premeiotic doubling of chromosomes, apomixis or central fusion automixis, all of which would result in offspring sharing maternal diversity and thus be clones of their mother.

With the recent identification of additional species of snakes reproducing through FP, and specifically all through terminal fusion automixis [Booth & Schuett, 2011; Booth *et al*., 2011a, b, 2012; Kinney *et al*., 2012; Reynolds *et al*., 2012), it is apparent that the initial *P. bivittatus* case represents an anomaly. Unlike all other snake species for which FP has been documented, all species of pythons are oviparous; thus, the variation in mode could indeed be real. However, a similar comparison can be found in certain species of orectolobiform (oviviparous) and carcharhiniforms (viviparous) sharks, although both share terminal fusion automixis (Chapman *et al*., 2007, 2008; Feldheim *et al*., 2010; Robinson *et al*., 2011). Given that all other cases of FP in vertebrates appear to follow terminal fusion automixis (Lampert, 2008), the report by Groot *et al*. (2003) poses this hypothesis: do pythons utilize an alternative mode of parthenogenesis to all other vertebrate species for which FP has been reported?

In the present study, and in the absence of cases of FP in *P. bivittatus*, we addressed the above hypothesis using microsatellite DNA genotyping of the clutches of two closely-related python species (Reynolds, Niemiller & Revell, 2014), the reticulated python (*Malayopython reticulatus*) from Asia, the world's longest extant snake, with individuals attaining maximum lengths of 25 ft (7.62 m) or greater (Headland & Greene, 2011), and the royal python (*P. regius*) from Africa, which attains maximum lengths approaching 5 ft (1.52 m) (Barker & Barker, 2006). Similar to *P. bivittatus*, these two species are frequently maintained in zoological gardens but are also kept privately as pets. Furthermore, they exhibit a variety of heritable colour and pattern traits (autosomal and expressed as simple Mendelian recessive or incomplete dominance) that permit easy identification of unusual progeny and reproductive events even to the most novice in genetics (Ihle, Schuett & Hughes, 2000; Barker & Barker, 2006). Similar to other species for which FP has been documented, such as domestic fowl, captive snakes represent an invaluable research tool for investigating FP (Dubach

*et al*., 1997; Schuett *et al*., 1997; Groot *et al*., 2003; Booth & Schuett, 2011; Booth *et al*., 2011a, b; Kinney *et al*., 2012; Reynolds *et al*., 2012). Given that FP has now been discovered in wild individuals of two species of North American pitvipers (Booth *et al*., 2012), cases of FP in zoos and private collections should not be overlooked as representing a captive syndrome but, instead, as additional instances of the diversity and distribution of FP in snakes. Given the phylogenetic diversity over which FP has been documented in snakes, resolution of apparent inconsistencies may remove noise that otherwise would cloud future efforts aimed at understanding this reproductive behaviour in vertebrates.

## MATERIAL AND METHODS

### Sample collection

From four clutches (one *M. reticulatus* and three *P. regius*), 18 offspring, the associated female parent, and the putative male parents were known and available for molecular screening. All offspring were female and represented reproductive anomalies from those expected based on captive history and/or resulting colour pattern phenotypes (for specific details, see below).

### *Malayopython reticulatus*

In the collection of the Louisville Zoological Gardens, a 6-m female 'Tiger' morph *M. reticulatus* (incomplete dominant colour and pattern mutation; Fig. 1A) was maintained in a 36-$m^2$ enclosure with a confirmed female conspecific. On 28 June 2012, this female produced a clutch of 61 eggs despite the absence of a male for more than 2 years. Approximately half of the clutch appeared viable and were artificially incubated. Owing to resource constraints, eggs were culled periodically and embryonic development was monitored. These eggs contained a mix of healthy and malformed embryos. The clutch was further culled to six eggs from which six healthy females hatched on 10 September 2012. Of these, three were wild-type (Fig. 1B) and three were Super-Tiger (homozygous form of the Tiger phenotype; Fig. 1C). Shed skins were collected from the mother and live offspring, and whole embryos were saved from the culling processes for tissue harvesting and visualization of gonads for definitive sex determination.

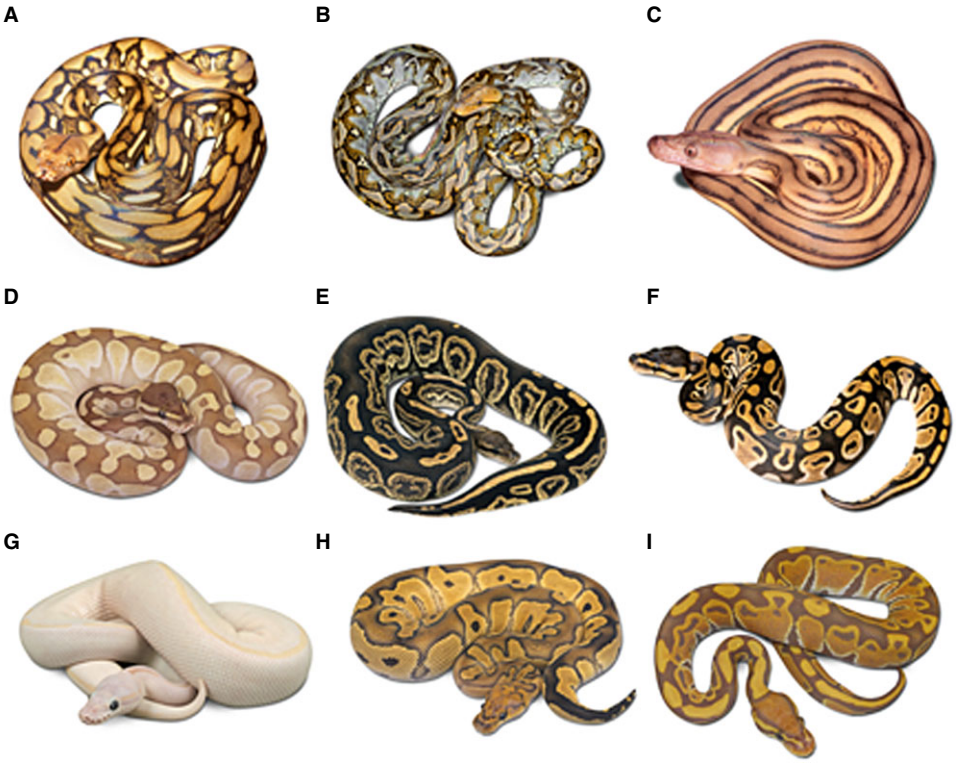


**Figure 1.** Examples of python morphs: *Malayopython reticulatus* – A, tiger (incomplete dominance heterozygous); B, wild-type; C, super-tiger (incomplete dominance homozygous); *Python regius* – D, lesser platinum (incomplete dominance heterozygous); E, black pastel (incomplete dominance heterozygous); F, wild-type; G, leucistic (incomplete dominance homozygous); H, clown (homozygous recessive); I, ultramel albino (homozygous recessive).

### *Python regius*

Samples were collected from three unrelated clutches produced in three private collections. The first clutch consisted of three eggs laid by a female 'Lesser Platinum' (incomplete dominance; Fig. 1D), after she was observed copulating with a male 'Black Pastel' (incomplete dominance; Fig. 1E). From this clutch of three eggs, all hatched females: two wild-type (Fig. 1F) and one leucistic (homozygous form of Lesser Platinum, Fig. 1G). The second clutch consisted of six eggs laid by a female 'Clown' (homozygous recessive; Fig. 1H), after copulation with an 'Ultramel Albino' male (homozygous recessive; Fig. 1I). From this clutch of six eggs, all hatched females of the clown phenotype. The third clutch consisted of four eggs laid by a wild-type female, which was housed in strict isolation from other snakes for more than 20 years. Of these, two eggs appeared viable and were artificially incubated, and two hatched females of wild-type. In clutches one and two, the phenotypes of the offspring did not correspond to the expected phenotypes (based on parental phenotypes) and thus this indicated the potential for genetic anomalies. In clutch three, the prolonged isolation from a male of the same species indicated a reproductive anomaly (e.g. long-term sperm storage or parthenogenesis).

### Microsatellite locus identification and primer design

We used the PALFINDER (Castoe *et al*., 2012; based on PRIMERDESIGNER, Castoe *et al*., 2010) to identify microsatellite-containing reads from previously published data from *P. bivittatus* (Castoe *et al*., 2010, NCBI SRA accession number: SRA029568.1). PALFINDER essentially identifies microsatellite reads present in raw next-generation datasets, and identifies and designs primer sequences flanking these microsatellite repeats, as well as the characteristics of the microsatellite repeat and the flanking primer sequences. These data analyzed for microsatellites represent approximately 60 Mbp of raw reads from a shotgun genomic library sequenced using a Roche 454, with a mean individual length of approximately 260 bp.

Parameter settings for PALFINDER were set to defaults. These choices resulted in the targeting of 3mer and 4mer tandem repeats because they are more accurately scored compared to 2mer repeats, which resulted in the identification of loci with a relatively large (> 6 repeated units) number of repeats because these are more likely to be variable. We further filtered the output of PALFNINDER to include only loci in which both primers per locus were found to occur a single time in the remaining shotgun dataset (and thus are unlikely to fall within repeat elements). This resulted in a total of 147 candidate 3mer and 183 candidate 4mer loci with associated flanking primers. From this set, 20 were chosen for use in screening. Eight were found to yield consistent unambiguous products across both species tested (for primer sequences and expected product size, see Table 1).

### DNA extraction, microsatellite genotyping, and determination of parthenogenesis

Total genomic DNA was extracted from shed skins (living specimens) or muscle tissue (deceased embryos)

**Table 1.** Characteristics of eight microsatellite DNA loci developed for *Python bivittatus*

| Locus | Repeat motif | Sequence | Fragment size (bp) |
|---|---|---|---|
| Pmo-1335 | $ATC^{(15)}$ | F: GATGACCAGCAACAAGGTGG<br>R: CTCTTCTTCCAATGTGGCCC | 178–199 |
| Pmo-2346 | $ATT^{(18)}$ | F: CAGTCCTTCAAACAGTGGGC<br>R: TCGTTGTGGGAAAATAGGAGG | 131–155 |
| Pmo-1654 | $ATT^{(19)}$ | F: TCTTTCAAGGGTGAGGTGTAAGC<br>R: CATTGGGTTACAACATATTCATGC | 281–302 |
| Pmo_0554 | $AATG^{(15)}$ | F: GGGGAGGCCACAAACTAAGG<br>R: TCATCATCACACTGTACCAGATGC | 168–222 |
| Pmo_0426 | $ATCT^{(16)}$ | F: CCAAGTCCCTATTATATTTTATTCCTGC<br>R: GTCCATAGTTCAAGGCCAGG | 170–180 |
| Pmo_0810 | $ATCT^{(16)}$ | F: TGAGGTTCAAAGGATGAAATACACC<br>R: ACAATTCATGGCCTTGGTCC | 152–156 |
| Pmo_0337 | $ATCT^{(17)}$ | F: GAATGACTCTCCCAGAAATCAGC<br>R: GCTTATAGAAGTCAAGGGAAGGG | 110–138 |
| Pmo_1068 | $ATCT^{(19)}$ | F: CAAACTTGGTAAAACACACACGC<br>R: CGCTACCGCTGTCTTCTGG | 145–161 |

using the PUREGENE DNA isolation procedure (Gentra systems Inc.). Samples were screened at eight microsatellite loci described above. Polymerase chain reactions (PCR) were carried out in 12-μL total volumes, each containing 1 × PCR buffer, 1.75 mM $MgCl_2$, 100 mM dNTPs, approximately 20 ng of DNA template, 1 pmol of primer, 0.5 U of Apex *Taq* DNA polymerase (Genesee Scientific), and PCR-grade $H_2O$ to 12 μL. The forward primer of each pair was end-labelled with a M13F-29 IRDye tag (LI-COR Biosciences). PCR cycling conditions comprised an initial denaturation stage of 3 min at 95 °C, followed by 28 cycles consisting of 30 s denaturation at 95 °C, 30 s at an optimal annealing temperature of 59 °C, and 30 s of extension at 72 °C. After PCR, 4 μL of stop solution (95% formamide, 20 mM ethylenediaminetetraacetic acid, bromophenol blue) was added to each 12-μL reaction. Reactions were subsequently denatured at 90 °C for 4 min and approximately 1 μL was loaded onto 25-cm 6% 1 × TBE polyacrylamide gels, mounted on a Li-Cor 4300 automated DNA sequencer (Li-Cor Biosciences). Loci were sized using a 50–350 bp standard (Li-Cor Biosciences). Gels were run at a constant power of 40 W at 50 °C for 2 h. The results were analyzed using SAGA-GT (LiCor Biosciences).

Assuming the alternative explanation (i.e. clutches resulted from the long-term storage of sperm from previous mating events), the probability of each clutch being produced sexually was calculated: the number of maternally homozygous and heterozygous loci were identified and identical paternal genotypes were assumed at each. As such, per offspring, a probability of 0.5 was assumed per locus if maternally homozygous and offspring mirrored the maternal genotype; and a probability of 0.25 per locus, per offspring, was assumed if the offspring was homozygous for one of the heterozygous mother's alleles. The resulting combined probability of obtaining the observed genotype per individual was then used to determine the probability of the entire clutch appearing to have resulted from sexual reproduction [i.e. $(\text{overall } P \text{ per individual})^{\text{number of individuals in the clutch}}$].

## RESULTS AND DISCUSSION

Homozygosity was observed in all offspring of both python species at all loci, including those for which the respective mother was heterozygous (Table 2). The probability of the offspring from each clutch resulting from long-term sperm storage (LTSS), a

**Table 2.** Genotypes of the mother, potential sires, and female offspring of *Malayopython reticulatus* and *Python regius* at eight microsatellite loci

| Species | Individual | Pmo-1335 | Pmo-2346 | Pmo-1654 | Pmo-0554 | Pmo-0426 | Pmo-0810 | Pmo-0337 | Pmo-1068 |
|---|---|---|---|---|---|---|---|---|---|
| *Malayopython reticulatus* | Female | 199/199 | 155/155 | 299/302 | 168/168 | 172/180 | 152/156 | 122/138 | 145/149 |
| | O/S-1 | 199/199 | 155/155 | 299/299 | 168/168 | 180/180 | 152/152 | 122/122 | 149/149 |
| | O/S-2 | 199/199 | 155/155 | 302/302 | 168/168 | 180/180 | 152/152 | 122/122 | 145/145 |
| | O/S-3 | 199/199 | 155/155 | 302/302 | 168/168 | 172/172 | 152/152 | 122/122 | 145/145 |
| | O/S-4 | 199/199 | 155/155 | 302/302 | 168/168 | 172/172 | 156/156 | 138/138 | 145/145 |
| | O/S-5 | 199/199 | 155/155 | 299/299 | 168/168 | 172/172 | 156/156 | 138/138 | 145/145 |
| | O/S-6 | 199/199 | 155/155 | 299/299 | 168/168 | 180/180 | 156/156 | 138/138 | 145/145 |
| | O/S-7 | 199/199 | 155/155 | 299/299 | 168/168 | 172/172 | 152/152 | 122/122 | 145/145 |
| *Python regius* | Female-1 | 178/199 | 131/134 | 281/290 | 214/218 | 174/178 | 152/156 | 110/110 | 157/161 |
| | Male-1 | 178/178 | 131/134 | 281/290 | 202/222 | 170/170 | 152/160 | 110/110 | 153/169 |
| | O/S-1.1 | 199/199 | 134/134 | 281/281 | 214/214 | 174/174 | 156/156 | 110/110 | 157/157 |
| | O/S-1.2 | 199/199 | 134/134 | 290/290 | 214/214 | 178/178 | 156/156 | 110/110 | 161/161 |
| | O/S-1.3 | 178/178 | 134/134 | 281/281 | 218/218 | 174/174 | 152/152 | 110/110 | 161/161 |
| *Python regius* | Female-2 | 178/178 | 131/131 | 287/302 | 218/222 | 170/170 | 152/152 | 110/110 | 153/157 |
| | Male-2 | 178/178 | 131/131 | 290/290 | 206/218 | 174/174 | 156/156 | 110/110 | 153/165 |
| | O/S-2.1 | 178/178 | 131/131 | 302/302 | 218/218 | 170/170 | 152/152 | 110/110 | 153/153 |
| | O/S-2.2 | 178/178 | 131/131 | 302/302 | 222/222 | 170/170 | 152/152 | 110/110 | 153/153 |
| | O/S-2.3 | 178/178 | 131/131 | 302/302 | 222/222 | 170/170 | 152/152 | 110/110 | 153/153 |
| | O/S-2.4 | 178/178 | 131/131 | 302/302 | 222/222 | 170/170 | 152/152 | 110/110 | 157/157 |
| | O/S-2.5 | 178/178 | 131/131 | 302/302 | 222/222 | 170/170 | 152/152 | 110/110 | 157/157 |
| | O/S-2.6 | 178/178 | 131/131 | 302/302 | 218/218 | 170/170 | 152/152 | 110/110 | 157/157 |
| *Python regius* | Female-3 | 178/193 | 131/134 | 284/284 | 198/210 | 170/174 | 152/156 | 110/110 | 153/157 |
| | O/S-3.1 | 178/178 | 131/131 | 284/284 | 210/210 | 174/174 | 156/156 | 110/110 | 153/153 |
| | O/S-3.2 | 193/193 | 134/134 | 284/284 | 210/210 | 170/170 | 152/152 | 110/110 | 157/157 |

**Table 3.** Clutch details for two species of python, *Malayopython reticulatus* and *Python regius*, and the probability of sexual reproduction explaining each individual and clutch combined

| Species | Number of offspring | Number of maternally homozygous loci | Number of maternally heterozygous loci | Probability of long-term sperm storage (per individual) | Probability of long-term sperm storage (per clutch) |
|---|---|---|---|---|---|
| *Malayopython reticulatus* | 7 | 3 | 5 | 0.0001 | $1.00 \times 10^{-28}$ |
| *Python regius* – clutch1 | 3 | 1 | 7 | 0.00003 | $2.7 \times 10^{-14}$ |
| *Python regius* – clutch2 | 6 | 5 | 3 | 0.0005 | $1.56 \times 10^{-20}$ |
| *Python regius* – clutch3 | 2 | 2 | 6 | 0.00006 | $3.6 \times 10^{-9}$ |

reproductive mode recently documented via genotypic analysis in the eastern diamond-backed rattlesnake, *Crotalus adamanteus* (Booth & Schuett, 2011), ranged from $3.6 \times 10^{-9}$ to $1 \times 10^{-28}$ (Table 3). Thus, with such infinitesimally small probabilities of LTSS, in concert with genomic incompatibilities of the respective traits in producing the present offspring and their captive histories, we propose that all offspring in each of the clutches resulted from FP. Given the observed homozygosity, we conclude that, as in other vertebrates for which FP has been reported (see Supporting information, Table S1), and specifically for all other snake species, excluding the previous report in *P. bivittatus* (Groot *et al*., 2003), the mode of FP is terminal fusion automixis.

We contend that the study of Groot *et al*. (2003) deserved specific investigation with respect to interpretation. It was discovered that a second instance of FP in *P. bivittatus* was presented to T. V. M. Groot (University of Amsterdam) in 2003, subsequent to the publication of their initial findings. In this second case, a female *P. bivittatus*, held in isolation in a private collection, deposited 25 eggs of which three offspring (females) successfully hatched. Genetic screening, in accordance with the method of Groot *et al*. (2003), revealed that the offspring appeared to lack paternal DNA but, in contrast to the previous case, the offspring did not exhibit identical genotypes to the mother and, instead, only a subset of the maternal genotype (T. V. M. Groot, University of Amsterdam; W. Spencer, Artis Zoo, Amsterdam. pers. comm.). The possession of only a subset of maternal DNA follows the characteristics expected under terminal fusion automixis (Lampert, 2008), which thus challenges the previously reported findings of Groot *et al*. (2003).

We were informed that the results of this second case of FP in *P. bivittatus* remain unpublished; however, they pose a tantalizing question regarding the apparent anomalies in the initial case. Based on data generated in the present study, and excluding scientific error on the behalf of Groot *et al*. (2003), we propose that the foetuses produced by the female described by Groot *et al*. (2003) resulted from secondary FP (i.e. parthenogenetic reproduction by a parthenogen). If this is indeed the case, this would represent the first documentation of such reproductive competence of FP in vertebrates. Our reasoning for this includes the clonal similarity of all offspring to the mother, a condition that would result after terminal fusion automictic reproduction by an individual with genome wide homozygosity, and their confirmed sex as being female. Additionally, unlike all other instances of FP in snakes, the offspring themselves appear genetically identical to each other. This would be possible through secondary FP given that the mother herself would be homozygous across the majority of her genome; thus, during gametogenesis, regardless of which chromosome is passed to each egg, all progeny would be identical. An alternative, albeit less plausible explanation may be that the female Burmese python is herself highly inbred and reproduced sexually with a highly related individual prior to entry into the zoological collection, and thus we see the combined results of extreme inbreeding and long-term sperm storage. However, given that a half-sibling to the mother revealed genetic diversity, as did an unrelated individual, at both microsatellite and AFLP loci, we consider that the likelihood of genome wide homozygosity of the mother and of a potential father is slim.

Although reproductive competency in reptiles produced by FP has yet to be conclusively confirmed, the appearance of viable spermatozoa has been previously observed in two colubroid (natricine) snakes: *Thamnophis marcianus* (Reynolds *et al*., 2012) and *Nerodia sipedon* (W. Booth, G. W. Schuett, pers. observ.). Interestingly, it has been observed in several colubroid snakes (stillborns) that genital morphology may be deformed (Schuett *et al*., 1997); thus, reproductive competence in colubroid parthenogens remains in question. Survival to adulthood and the onset of sexual activity (i.e. courting) has been observed in an adult female *Boa imperator* that was

produced via facultative parthenogenesis (W. Booth, pers. observ.). The potential for reproductive competency in a parthenogenetically produced *P. bivittatus* therefore represents a further difference in FP between 'primitive' (basal alethinophidian) and 'advanced' (caenophidian) snake lineages.

## Conclusions

In the present study, our results of FP in two species of pythons support the general hypothesis that the mode of FP is the same as documented in other alethinophidian snakes, such as *B. imperator* and *Epicrates* spp. (Booth *et al*., 2011a, b; Kinney *et al*., 2012). Consequently, although we cannot reject outright the possibility that the Burmese python did in fact produce clonal offspring, given the more substantial evidence reported in the present study for two closely-related python species covering a total of four clutches, the additional case described by T. V. M. Groot that remains unpublished, and the alternative explanation offered, we recommend that the case presented by Groot *et al*. (2003) be viewed cautiously when considering parthenogenetic modes in vertebrates and, instead, the results of the present study be considered as an alternative, more plausible viewpoint. Given the apparent widespread occurrence of FP across the phylogeny of snakes (see Supporting information, Table S1), the growing number of cases of FP recently reported along with their associated characteristics permits an initial investigation of the emerging phylogenetic patterns of FP in snakes. With its occurrence documented in wild individuals and the conservation of parthenogenetic mode observed across all snake species demonstrating facultative parthenogenesis, and indeed all vertebrates exhibiting FP, the growing number of reports in snakes warrants the establishment of a focused research programme, specifically investigating the proximate and genetic mechanisms driving the 'sexual–asexual switch'. Undoubtedly, additional cases of FP will be reported in the coming years given the ease of molecular screening for confirmation; thus, concerted efforts should now be focused on a deeper understanding of this alternative reproductive strategy in evolutionary theory.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Currently known cases of facultative parthenogenesis in vertebrates.