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BRIEF REPORT

Facultative Parthenogenesis in a Zoo-Held Northern Water Snake, *Nerodia sipedon*

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ABSTRACT

Over the past several decades, facultative parthenogenesis (FP)—the ability of a sexually reproducing species to reproduce asexually—in vertebrates has been removed from the realm of obscurity and placed firmly in a position where it warrants focused scientific attention. Likely fueled by increased recognition of the trait, the availability of molecular tools capable of disentangling FP from long-term sperm storage, and the availability of potential cases originating from both zoological and private collections, a wealth of papers has been published revealing the diversity of vertebrate systems in which FP occurs. Specifically, cases have been reported in squamate reptiles (lizards and snakes), crocodiles, birds, and elasmobranch fishes (sharks, rays, and skates). Nonetheless, gaps remain in species documentation, and it is important to analyze and report on new cases. In this paper, we provide a DNA–based analysis confirming FP in a zoo-maintained northern water snake, *Nerodia sipedon*, a viviparous natricine species that is common and widely distributed in North America. Additionally, we provide information on the sexual development and health of the male parthenogen. Encouragingly, zoological institutions, aquaria, university laboratories, and private collections continue to be rich sources for the further study and documentation of FP in vertebrate species, advancing our understanding of this reproductive trait.

1 | Introduction

Facultative parthenogenesis (FP)—asexual reproduction by a sexually reproducing species—was first reported in 1872, with the detection of parthenogenetic development in domestic fowl *Gallus domesticus* (Oellacher 1872). Later, its discovery in domestic pigeons (Bartelmez and Riddle 1924) and subsequently commercial strains of turkey (reviewed in Olsen 1975) led to a decades-long research program on vertebrate FP. This yielded valuable information on the sex of parthenogens, the putative parthenogenetic mechanism, the potential for this unorthodox reproductive mode to be under the control of a single autosomal recessive gene (Olsen, Wilson, and Marks 1968), and the production of viable spermatozoa and thus reproductive competency (Olsen 1975).

Based on individuals maintained in zoos, research institutes, and private collections, the first reports of FP outside of birds were made, with cases described in multiple species of caenophidian snakes (Dubach, Sajewicz, and Pawley 1997; Schuett et al. 1997). These early cases were viewed with skepticism and were instead attributed to female long-term sperm storage (LTSS), a common reproductive strategy in snakes (Schuett 1992; Booth and Schuett 2011). However, as the ease with which FP could be conclusively disentangled from LTSS became possible using molecular markers (Booth and Schuett 2011; Levine, Schuett, and Booth 2021; Levine et al. 2024), it was soon revealed that FP was more common than previously thought (Booth and Schuett 2016).

By 2024, FP had been reported in elasmobranch fishes (Chapman et al. 2007, Chapman, Firchau & Shivji 2008; Feldheim

Research Highlights

- A female northern water snake produced a single male offspring 3 years after capture.
- Genetic analyses confirm facultative parthenogenesis.

et al. 2010; Fields et al. 2015; Harmon et al. 2016; Straube et al. 2016; Dudgeon et al. 2017), birds (Schut, Hemmings, and Birkhead 2008; Ramachandran and McDaniel 2018; Ryder et al. 2021), lizards (Watts et al. 2006, Miller et al. 2019; Kratochvíl et al. 2020), snakes (Groot, Bruins, and Breeuwer 2003; Germano and Smith 2010; Booth, Johnson, et al. 2011; Booth, Million, et al. 2011; Booth et al. 2012, 2014; Booth and Schuett 2011; Kinney et al. 2012; Reynolds et al. 2012; Jordan, Perrine-Ripplinger, and Carter 2015; Shibata et al. 2017; Allen, Sanders, and Thomson 2018; Seixas et al. 2019; Cubides-Cubillos et al. 2020; Card et al. 2021), and crocodilians (Booth et al. 2023). Furthermore, it was discovered in natural populations (Booth et al. 2012; Fields et al. 2015), proving that it is not a captive syndrome. That said, data from captive individuals continue to be important for expanding our understanding of the phylogenetic distribution of FP and the underlying characteristics (Booth and Schuett 2016; Gamble et al. 2017; Calvete et al. 2018). Nonetheless, gaps exist in the phylogeny (Booth and Schuett 2016), and it thus remains important to document new taxa with molecular confirmation. Within zoological collections and for species of conservation concern, where FP and LTSS have potentially far-reaching implications due to their differential impact on reproductive effort and parentage assignment (Levine et al. 2022, 2024), it is important that conclusive determination of reproductive mode is made.

Here, we provide the first DNA–based evidence for FP in a zoo-maintained northern water snake, *Nerodia sipedon*, a viviparous natricine species that is common and widely distributed in North America (Conant and Collins 1998).

2 | Materials

2.1 | Study System

On August 23, 1996, zoo staff of the Milwaukee County Zoological Gardens (MCZG) captured an adult female *N. sipedon* at Vernon Marsh, Waukesha County, Wisconsin. Based on its girth and mass (406 g), the snake was suspected of being gravid. She was brought to the MCZG live collection and processed by the zoo veterinarians (Accession Number H951). On September 7, 1996, 15 days after her capture, H951 produced a litter of 16 healthy offspring and one fully developed but stillborn individual. On May 28, 1997, H951 produced 22 infertile ova and one stillborn individual. Two years later (July 5–14, 1998), female H951 produced 16 infertile ova and three stillborn progeny, and on October 12, 1998, one live, suspected parthenogenetic male (H1098), was born. H1098 exhibited no obvious developmental abnormalities. On May 5, 1999, H951 produced four infertile ova. The reproductive events of female H951 are summarized in Table 1.

TABLE 1 | Reproductive history of the female *Nerodia sipedon* (H951) in this study.

	Progeny		
Births	**Live**	**Stillborn**	**Infertile Ova**
September 7, 1996	16	1	0
May 28, 1997	0	1	22
July 5–14, 1998	0	3	16
October 12, 1998	1 (H1098)	0	0
May 5, 1999	0	0	4

Note: Parenthetical information denotes the Milwaukee County Zoological Gardens (MCZG) accession numbers. Male H1098 is the suspected parthenogen.

2.2 | Molecular Methods and Bioinformatic Analysis

Genomic DNA was extracted from ethanol-preserved whole blood (Qiagen DNEasy blood/tissue kit; Qiagen Inc., Valencia, CA, USA) from the adult female (H951), the suspected male parthenogen (H1098), and one male offspring produced in 1996 (H968). The samples were screened at nine polymorphic microsatellite loci recently developed for the gartersnake *Thamnophis butleri*: *Tbu*-A3, *Tbu*-A62, *Tbu*-A64, *Tbu*-A70, *Tbu*-A74, *Tbu*-A92, *Tbu*-A95, *Tbu*-B12, and *Tbu*-B38 (Sloss et al. 2011), following amplification profiles outlined by the authors. Amplified PCR products were electrophoresed on a Li-Cor 4300 dual-laser DNA sequencer (Li-Cor Inc., Lincoln, NE, USA). Loci were sized using a 50–700 bp standard (Li-Cor). Gels were run at a constant power of 40 W at 50°C for 2 h. Allele sizes were called using the SAGA-GT software (Li-Cor) and confirmed visually for accuracy.

Data were analyzed using *ParthenoGenius* (Levine & Booth in review; https://github.com/brenna-levine/Levine_ParthenoGenius), a software for inferring the presence and mechanism of FP from genetic data sets.

3 | Results

Unambiguous genotypes were generated at all loci, with maternal heterozygosity at seven (Table 2). Unique alleles were detected at six loci in the offspring produced shortly after capture in 1996 (H968) and can be attributed to the non-sampled sire of that litter. Of the remaining three loci, two were monomorphic. In contrast, the suspected parthenogen (H1098) was differentially homozygous at each of the maternally heterozygous loci and identical at the two maternally homozygous loci. Across the nine microsatellite loci, the probability of the suspected parthenogen receiving identical alleles from an unsampled father as those contributed by the mother, and thus the offspring resulting from long-term sperm storage (LTSS) of at least 22 months (assuming an approximate 4-month gestation), is $p = 3.814 \times 10^{-6}$ [i.e., $(0.5)^9$ assuming paternal heterozygosity at all loci]. *ParthenoGenius* confirmed a sexual origin for male H968 and deemed H1098 as a parthenogen produced through

TABLE 2 | Microsatellite genotypes for female northern water snake, *Nerodia sipedon* (H951), a male offspring of H968, and a suspected parthenogenetic male (H1098).

	Tbu-A3	*Tbu*-A62	*Tbu*-A64	*Tbu*-A70	*Tbu*-A74	*Tbu*-A92	*Tbu*-A95	*Tbu*-B12	*Tbu*-B38
Dam (H951)	266/272	327/329	258/266	174/180	310/314	402/402	333/341	373/373	241/253
1996 male offspring (H968)	266/**298**	329/**331**	**254**/258	174/174	310/314	**400**/402	341/341	373/**405**	253/**255**
Parthenogen (H1098)	266/266	329/329	266/266	180/180	310/310	402/402	333/333	373/373	253/253

Note: Genotypes in bold denote paternal alleles, and thus the sexual origin of the offspring.

facultative automictic parthenogenesis. Due to the lack of heterozygosity in the parthenogens microsatellite genotype, the automictic mechanism responsible could not be differentiated between gametic duplication and terminal fusion.

Despite being deemed healthy by zoo veterinarians at nearly 14 months old, on December 11, 1999, the parthenogen began displaying symptoms indicative of ill health (e.g., stiff, wrinkled integument) and was subsequently found dead in its enclosure on January 28, 2000. Its live mass was 52.3 g on January 22, 2000, and 40 g at the time of death. Necropsy reports revealed mostly unremarkable findings of the organ systems with several exceptions. Notably, the intestines showed hypoplasia of the villar architecture along with atrophy of the submucosa. Additionally, the hemipenes showed deformation that was a possible congenital anomaly; however, zoo veterinarians had unsuccessfully attempted to harvest blood from the tail vessels, potentially resulting in damage to the hemipenes. The testes were normal in appearance, though possibly regressed. Histopathology showed that testicular spermatogenesis appeared normal, with progression from spermatogonia to primary and secondary spermatocytes, and then to spermatozoa. Both Sertoli and Leydig cells were present and normal in appearance. The cause of death was declared unknown, but it was possibly related to the intestinal problems already noted and which might have contributed to malabsorption syndrome and secondary wasting, a condition commonly seen in snakes with FP origin (W. Booth, unpublished data).

4 | Discussion

Considering the litter characteristics produced by this female in 1998 (i.e., high number of reproductive failures and a single viable male offspring), the infinitesimally low probability of LTSS, and the genetic data presented, we report here compelling evidence for FP in *N. sipedon*. While the automictic mechanism cannot be conclusively confirmed with our genetic data, recent genomic analyses in snakes (Card et al. 2021) and crocodilians (Booth et al. 2023) suggest that this is likely to be terminal fusion (Lampert 2008).

FP appears to be prevalent within natricine snakes, with multiple reports in the North American garter snakes, *Thamnophis* spp. (Schuett et al. 1997; Murphy and Curry 2000; Germano and Smith 2010; Reynolds et al. 2012), and data supportive of FP in two species of *Nerodia* (Skalka and Voženilek 1986; Gasanov and Katz 2020). Skalka and Voženilek (1986) report a female *N. sipedon* that was purchased as a neonate, reared in isolation, and died accidentally as an adult after 40 months. Dissection revealed six ova and two well-developed fetuses. No molecular analyses were performed. Gasanov and Katz (2020) present polyacrylamide gel electrophoresis data supporting FP in a diamondback water snake, *N. rhombifer*, obtained at 1 month of age, reared in isolation, that later produced four infertile ova, one stillborn male, and one live male, both of which exhibited notably spinal deformities. Booth and Schuett (2011) challenged a range of published records of presumptive LTSS in female snakes. Alternatively, they provided circumstantial evidence for FP, with specific cases including a congener of the present species, *Nerodia erythrogaster* from Mexico (Conant 1965). We highly suspect that additional cases of FP in natricine snakes and other lineages will surface; however, definitive confirmation will require molecular determination (Booth and Schuett 2016).

Encouragingly, zoological facilities, aquaria, university laboratories, and private collections continue to play important roles in the investigation of FP in captive squamates, crocodilians, birds, and fishes, greatly expanding our understanding of the ecological and evolutionary implications of this reproductive trait.

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Ethics Statement

Samples were collected with the approval of Milwaukee County Zoo under Protocol #ISIS-ARKS-3.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw data are available at request from Dr. Warren Booth (warrenbooth@vt.edu). Input and output files for the ParthenoGenius run are available at https://github.com/brenna-levine/LEVINE_ParthenoGenius.

References

Allen, L., K. L. Sanders, and V. A. Thomson. 2018. "Molecular Evidence for the First Records of Facultative Parthenogenesis in Elapid Snakes." *Royal Society Open Science* 5: 171901.

Bartelmez, G. W., and O. Riddle. 1924. "On Parthenogenetic Cleavage and on the Role of Water Absorption by the Ovum in the Formation of

the Subgerminal Cavity in the Pigeon's Egg." *American Journal of Anatomy* 33: 57–66.

Booth, W., D. H. Johnson, S. Moore, C. Schal, and E. L. Vargo. 2011. "Evidence for Viable, Non-Clonal but Fatherless *Boa constrictors*." *Biology Letters* 7: 253–256.

Booth, W., B. A. Levine, J. B. Corush, et al. 2023. "Discovery of Facultative Parthenogenesis in a New World Crocodile." *Biology Letters* 19: 20230129.

Booth, W., L. Million, R. G. Reynolds, et al. 2011. "Consecutive Virgin Births in the New World Boid Snake, the Colombian Rainbow Boa, *Epicrates maurus*." *Journal of Heredity* 102: 759–763.

Booth, W., and G. W. Schuett. 2011. "Molecular Genetic Evidence for Alternative Reproductive Strategies in North American Pitvipers (Serpentes: Viperidae): Long-Term Sperm Storage and Facultative Parthenogenesis." *Biological Journal of the Linnean Society* 104: 934–942.

Booth, W., and G. W. Schuett. 2016. "The Emerging Phylogenetic Pattern of Parthenogenesis in Snakes." *Biological Journal of the Linnean Society* 118: 172–186.

Booth, W., G. W. Schuett, A. Ridgway, et al. 2014. "New Insights on Facultative Parthenogenesis in Pythons." *Biological Journal of the Linnean Society* 112: 461–468.

Booth, W., C. F. Smith, P. H. Eskridge, S. K. Hoss, J. R. Mendelson, and G. W. Schuett. 2012. "Facultative Parthenogenesis Discovered in Wild Vertebrates." *Biology Letters* 8: 983–985.

Calvete, J. J., N. R. Casewell, U. Hernández-Guzmán, et al. 2018. "Venom Complexity in a Pitviper Produced by Facultative Parthenogenesis." *Scientific Reports* 8: 11539.

Card, D. C., F. J. Vonk, S. Smalbrugge, et al. 2021. "Genome-Wide Data Implicate Terminal Fusion Automixis in King Cobra Facultative Parthenogenesis." *Scientific Reports* 11: 7271.

Chapman, D. D., B. Firchau, and M. S. Shivji. 2008. "Parthenogenesis in a Large-Bodied Requiem Shark, the Blacktip *Carcharhinus limbatus*." *Journal of Fish Biology* 73: 1473–1477.

Chapman, D. D., M. S. Shivji, E. Louis, J. Sommer, H. Fletcher, and P. A. Prodöhl. 2007. "Virgin Birth in a Hammerhead Shark." *Biology Letters* 3: 425–427.

Conant, R. 1965. "Notes on Reproduction in Two Natricine Snakes From Mexico." *Copeia* 1965: 140–144.

Conant, R., and J. T. Collins. 1998. *A Field Guide to Reptiles and Amphibians: Eastern and Central North America*. Boston, MA: Houghton Mifflin Harcourt.

Cubides-Cubillos, S. D., J. S. L. Patané, K. M. Pereira da Silva, et al. 2020. "Evidence of Facultative Parthenogenesis in Three Neotropical Pitviper Species of the *Bothrops atrox* Group." *PeerJ* 8: e10097.

Dubach, J., A. Sajewicz, and R. Pawley. 1997. "Parthenogenesis in the Arafuran Filesnake (*Acrochordus arafurae*)." *Herpetological Natural History* 5: 11–18.

Dudgeon, C. L., L. Coulton, R. Bone, J. R. Ovenden, and S. Thomas. 2017. "Switch From Sexual to Parthenogenetic Reproduction in a Zebra Shark." *Scientific Reports* 7: 40537.

Feldheim, K. A., D. D. Chapman, D. Sweet, et al. 2010. "Shark Virgin Birth Produces Multiple Viable Offspring." *Journal of Heredity* 101: 374–377.

Fields, A. T., K. A. Feldheim, G. R. Poulakis, and D. D. Chapman. 2015. "Facultative Parthenogenesis in a Critically Endangered Wild Vertebrate." *Current Biology* 25: R446–R447.

Gamble, T., T. A. Castoe, S. V. Nielsen, et al. 2017. "The Discovery of XY Sex Chromosomes in a *Boa* and *Python*." *Current Biology* 27: 2148–2153.e4.

Gasanov, E. V., and A. V. Katz. 2020. "Facultative Parthenogenesis in a Diamondback Water Snake (*Nerodia rhombifer*)." *Russian Journal of Herpetology* 27: 341–347.

Germano, D. J., and P. T. Smith. 2010. "Molecular Evidence for Parthenogenesis in the Sierra Garter Snake, *Thamnophis couchii* (Colubridae)." *The Southwestern Naturalist* 55: 280–282.

Groot, T. V. M., E. Bruins, and J. A. J. Breeuwer. 2003. "Molecular Genetic Evidence for Parthenogenesis in the Burmese Python, *Python molurus bivittatus*." *Heredity* 90: 130–135.

Harmon, T. S., T. Y. Kamerman, A. L. Corwin, and A. B. Sellas. 2016. "Consecutive Parthenogenetic Births in a Spotted Eagle Ray *Aetobatus narinari*." *Journal of Fish Biology* 88: 741–745.

Jordan, M. A., N. Perrine-Ripplinger, and E. T. Carter. 2015. "An Independent Observation of Facultative Parthenogenesis in the Copperhead (*Agkistrodon contortrix*)." *Journal of Herpetology* 49: 118–121.

Kinney, M. E., R. F. Wack, R. A. Grahn, and L. Lyons. 2012. "Parthenogenesis in a Brazilian Rainbow Boa (*Epicrates cenchria cenchria*)." *Zoo Biology* 32: 172–176.

Kratochvíl, L., J. Vukić, J. Červenka, et al. 2020. "Mixed-Sex Offspring Produced via Cryptic Parthenogenesis in a Lizard." *Molecular Ecology* 29: 4118–4127.

Lampert, K. P. 2008. "Facultative Parthenogenesis in Vertebrates: Reproductive Error or Chance?" *Sexual Development*, 2: 290–301.

Levine, B. A., and W. Booth (in review). "ParthenoGenius: A User-Friendly Heuristic for Inferring Presence and Mechanism of Facultative Parthenogenesis From Genetic and Genomic Data Sets."

Levine, B. A., R. L. Hill, J. R. Mendelson, and W. Booth. 2022. "Parentage Assignment Reveals Multiple Paternity in the Critically Endangered Guatemalan Beaded Lizard (*Heloderma charlesbogerti*)." *Conservation Genetics* 23: 859–863.

Levine, B. A., A. Moresco, T. Trout, G. W. Schuett, and W. Booth. 2024. "Female Long-Term Sperm Storage Results in Viable Offspring in the Himalayan Mountain Pitviper, *Ovophis monticola*." *Zoo Biology* 43: 183–187.

Levine, B. A., G. W. Schuett, and W. Booth. 2021. "Exceptional Long-Term Sperm Storage by a Female Vertebrate." *PLoS One* 16: e0252049.

Miller, K. L., S. Castañeda Rico, C. R. Muletz-Wolz, et al. 2019. "Parthenogenesis in a Captive Asian Water Dragon (*Physignathus cocincinus*) Identified With Novel Microsatellites." *PLoS One* 14, no. 6: e0217489.

Murphy, J. C., and R. M. Curry. 2000. "A Case of Parthenogenesis in the Plains Garter Snake, Thamnophis radix." *Bulletin of the Chicago Herpetological Society* 35: 17–19.

Oellacher, J. 1872. "Die Veränderungen des unbefruchteten Keimes des Hühnereis in Eileiter und bei Bebrütungsversuchen." *Zeitschrift für Wissenschatliche* 22: 181–234.

Olsen, M. W. 1975. *Avian Parthenogenesis*, vol. 65. Beltsville, MD: USDA ARS-NE. 1–82.

Olsen, M. W., S. P. Wilson, and H. L. Marks. 1968. "Genetic Control of Parthenogenesis in Chickens." *Journal of Heredity* 59: 41–42.

Ramachandran, R., and C. D. McDaniel. 2018. "Parthenogenesis in Birds: A Review." *Reproduction* 155: R245–R257.

Reynolds, R. G., W. Booth, G. W. Schuett, B. M. Fitzpatrick, and G. M. Burghardt. 2012. "Successive Virgin Births of Viable Male Progeny in the Checkered Gartersnake, *Thamnophis marcianus*." *Biological Journal of the Linnean Society* 107: 566–572.

Ryder, O. A., S. Thomas, J. M. Judson, et al. 2021. "Facultative Parthenogenesis in California Condors." *Journal of Heredity* 112: 569–574.

Schuett, G. W. 1992. "Is Long-Term Sperm Storage an Important Component of the Reproductive Biology of Temperate Pitvipers?" In *Biology of the Pitvipers*, edited by J. A. Campbell and E. D. Brodie Jr., 169–184. Tyler, TX: Selva.

Schuett, G. W., P. J. Fernandez, W. F. Gergits, et al. 1997. "Production of Offspring in the Absence of Males: Evidence for Facultative Parthenogenesis in Bisexual Snakes." *Herpetological Natural History* 5: 1–10.

Schut, E., N. Hemmings, and T. R. Birkhead. 2008. “Parthenogenesis in a Passerine Bird, the Zebra Finch *Taeniopygia Guttata*.” *Ibis* 150: 197–199.

Seixas, F., F. Morinha, C. Luis, N. Alvura, and M. d. A. Pire. 2019. “DNA–Validated Parthenogenesis: First Case in a Captive Female Cuban Boa (*Chilabothrus angulifer*).” *Salamandra* 56: 83–86.

Shibata, H., S. Sakata, Y. Hirano, E. Nitasaka, and A. Sakabe. 2017. “Facultative Parthenogenesis Validated by DNA Analyses in the Green Anaconda (*Eunectes murinus*).” *PLoS One* 12, no. 12: e0189654.

Skalka, P., and P. Voženilek. 1986. “Případ partenogenese u užovky *Nerodia sipedon*.” *Fauna Bohemiea Septentrionalis* 11: 81–82.

Sloss, B. L., G. W. Schuurman, R. A. Paloski, O. D. Boyle, and J. M. Kapfer. 2011. “Novel Microsatellite Loci for Studies of *Thamnophis* Gartersnake Genetic Identity and Hybridization.” *Conservation Genetics Resources* 4: 383–386.

Straube, N., K. P. Lampert, M. F. Geiger, J. D. Weiß, and J. X. Kirchhauser. 2016. “First Record of Second-Generation Facultative Parthenogenesis in a Vertebrate Species, the Whitespotted Bamblooshark *Chiloscyllium plagiosum*.” *Journal of Fish Biology* 88: 668–675.

Watts, P. C., K. R. Buley, S. Sanderson, W. Boardman, C. Ciofi, and R. Gibson. 2006. “Parthenogenesis in Komodo Dragons.” *Nature* 444: 1021–1022.