First recorded birth of suspected non-identical twins in the Cuban boa *Chilabothrus angulifer*

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The occurrence of identical (monozygotic) or non-identical (dizygotic) twins in snakes is a rare phenomenon, reported in less than 1 % of more than 3,970 currently recognised species (Smith, 1999; Wallach, 2018; Uetz et al., 2022). Even rarer are twins in viviparous snakes, being recorded in only seven species belonging to seven families: *Boa constrictor* (Boidae), *Coronella austriaca* (Colubridae), *Diadophis punctatus* (Dipsadidae), *Erpeton tentaculatum* (Homalopsidae), *Pseudechis colletti* (Elapidae), *Thamnophis sirtalis* (Natricidae) and *Vipera berus* (Viperidae) (for reviews see Smith, 1999; Wallach, 2018). Smith (1999) reported the only known twins in the family Boidae (*B. constrictor*), accounting for one case in approximately 600 litters obtained in captivity by that author, evidencing its very low frequency. Herein we report the first case of twins, suspected to be non-identical, in the Cuban boa (*Chilabothrus angulifer*), which represents also the second case of twinning in the family Boidae and the eighth species of viviparous snake in which this phenomenon has been recorded.

On 30 June 2021, we rescued a gravid female *C. angulifer* (1,800 mm SVL, 200 mm tail length) that had been collected by local people about three weeks earlier at Cuatro Caminos town (22° 53'34" W, 82° 22'44" N; 75 m a.s.l.; WGS 84), Bejucal Municipality, Mayabeque Province, Cuba. According to the collectors, the snake was in a tree hole by day when first seen. Its mouth was severely damaged, apparently because of the poor conditions in which it had been kept for three weeks, and also showed clear signs of pregnancy. We kept it to treat the mouth injury with antibiotics and to collect data related to the birth and the neonates. We placed it in a plastic, well-ventilated container (700 x 350 x 350 mm) with newspaper and chopped dry banana leaves as substrate, and offered water ad libitum. Temperature during the time it spent in that container varied from 24.7–35.3 °C. It only accepted food once during the time from its collection to birth: it ate two freshly euthanised adult lab house mice (*Mus musculus*) about one month before it gave birth. On 18 July it shed its skin (53 days before birth).

We took measurements of the neonate snakes to the nearest millimeter from photographs using the application Snake Measurements v1.0 for Android (https://apkpure.com/snake-measurement/com.theultimatelabs.snake), which proved to be highly accurate when compared to the string method (Rivas et al., 2008). Body mass was recorded to the nearest gram with a WeiHeng® digital scale in the case of the mother and the normal siblings, and to the nearest 0.1 g with a TANITA® digital scale in the case of the twins. We recorded the body measurements and masses of the neonates twice: one day after birth (with the yolk inside) and after the neonatal shed. We made all comparisons taking into account the measurements one day after birth, but for masses we waited until after the neonatal shed, in order to avoid any possible bias because of the absorbed yolk. Using metallic probes, we recorded the sex based on the distance of penetration (females: < 6 subcaudals; males: > 7 subcaudals; Frynta et al., 2016).

After the neonatal shed, we placed the baby boas in separate containers. As a first and second meal we offered each several food options at the same time, differing in size, texture and other features to test for food preferences. The prey options were: lab house mice (pinky: <25 mm body length, <3.0 g; hopper: 40–50 mm body length, 7.0–9.0 g; and weaned: 60–80 mm body length, 14.0–15.0 g), fuzzy lab brown rats (*Rattus norvegicus*: 60 mm body length, 9.0 g), adult anoles (male *Anolis porcatus*: 60 mm SVL, 4.0 g; female *A. porcatus* 55 mm SVL, 3.0 g; or male *A. sagrei*: 50 mm SVL, 4.0 g), and Cuban treefrogs (*Osteopilus septentrionalis*: 40 mm SVL, 3.0 g). During the subsequent months we continued offering food to the four siblings on a weekly basis, each time offering mice as the first option and then anoles if the mice were refused.

During the afternoon of 9 September (71 days after rescue) the female gave birth to two normal babies (within the range of sizes reported for neonates of this species, see references below), a pair of twins and five infertile ova (data on neonates in Table 1) (Fig. 1). The twins were encapsulated in the same fetal membranes (Fig. 1) and were connected to a single yolk sac by separate umbilical cords (Fig. 2). We left the four neonates, as well as the infertile ova, with their mother overnight. The next day we moved them to a smaller container with paper towel as substrate and a water bowl.

The twins were the most delayed of the four siblings in both umbilical cord detachment and first shed, and thus in the taking of their first meal. The two larger babies absorbed their yolks completely within the 24 h following birth (Fig. 3), which was not the case with the twins. One twin absorbed a small amount of yolk (as suggested by its slightly swollen abdomen) and the other one showed no visible signs of having absorbed

Figure 1. Litter of *Chilabothrus angulifer* with its mother shortly after birth. Notice the infertile ova and some of the babies still encapsulated in the fetal membranes. The arrow indicates a single fetal membrane encapsulating the twins.

Figure 3. A normal sibling of *Chilabothrus angulifer* one day after birth (No. 2; Table 1). Notice the umbilical cord ending in the empty yolk sac and the swollen abdomen due to the absorbed yolk.

Figure 2. Twins of *Chilabothrus angulifer* one day after birth: left No. 3, right No. 4 (Table 1). Notice the common yolk sac (still not absorbed) and the separate umbilical cords.

yolk; their common yolk sac remained almost as large as the day before (Fig. 2). After that time and in order to avoid any infections, we decided to tie off the twins' umbilical cords close to the yolk sac and cut them off. The umbilical cords dried and detached from the neonates 3–5 days later. During the period prior to the neonatal shed, the four babies did not ingest food, some of them struck at us when disturbed, and some occasionally spent time in the water bowl when their

skins turned dull. The neonatal shed of the two normal siblings occurred between 17–18 days after birth, but that of the twins occurred between 57–58 days after birth (Table 1). Indeed, the two normal siblings shed twice during the period in which the twins shed only once (Table 1). The period between the first and second shed of the twins was also considerably longer (>3 times) than that of the normal siblings (Table 1). Of the two normal siblings, one kept its body mass and the other lost 15 g between birth and the neonatal shed (Table 1). By the time the twins had completed their neonatal shed, they had lost 11 and 14 g respectively when compared to their masses one day after birth. Nonetheless, the twins showed good vitality and behaved similarly to their normal siblings. The normal siblings accepted their first meals between 1–16 days after the neonatal shed, which consisted of hopper and weaned mice and a Cuban treefrog (Table 1). The larger twin accepted its first meal five days after the neonatal shed (63 days after birth), eating a female *A. porcatus* (No. 3, Table 1) and 17 days later it accepted a hopper mouse. The smaller twin accepted its first meal (a frog) 46 days after the neonatal shed and 103 days after birth (No. 4, Table 1) and it took its second meal (a male *A. sagrei*) five days later.

The four babies continued feeding on a weekly basis after

Table 1. Data on the four neonates of *Chilabothrus angulifer*, two of which were twins (Nos. 3 and 4), born in captivity on 9 September 2021. For each variable we present two values: one day after birth/after the neonatal shed. Abbreviations: SVL = snout-vent length, TL = tail length, HL = head length. The number of days since birth (1st shed) or since the previous shed (2nd shed) are in parenthesis. All dates refer to 2021 except the second sheds of the pair of twins that occurred in 2022.

No.	Sex	SVL (mm)	TL (mm)	HL (mm)	Mass (g)	1st shed (days)	2nd shed (days)	1st and 2nd meals (prey type)
1	M	552/580	48/50	27/28	165/150	27 Sept (18)	25 Oct (28)	13 Oct (hopper mouse and frog) 20 Oct (weaned mouse)
2	F	534/561	46/49	27/28	140/140	26 Sept (17)	1 Nov (37)	27 Sept (hopper mouse) 13 Oct (hopper mouse)
3	F	402/457	38/43	25/26	54.8/40.9	6 Nov (58)	27 Feb (113)	11 Nov (female Anolis porcatus) 28 Nov (hopper mouse)
4	F	384/430	36/40	23/24	42.5/31.6	5 Nov (57)	2 Mar (119)	21 Dec (frog) 26 Dec (male Anolis sagrei)

their neonatal shed, until we measured them again on 1 April 2022 (ca. 7 months after birth). During this period the twins always refused mice as their first food option but readily took anoles instead, whereas the normal siblings accepted mice as their first food options most of the time (accepted anoles a couple of times only). The smaller twin increased 16 mm in length and 10.5 g in mass with respect to the previous measurement; the larger twin increased 20 mm and 15.5 g. In contrast, the normal siblings increased from 80–106 mm in length and from 80–90 g in mass with respect to the previous measurement.

The mother did not consume any of the infertile ova during the first night or thereafter. It weighed 3,330 g on 23 August (17 days before birth) and 2,130 g a few hours after birth (the neonates, infertile ova and fetal membranes summed up together ca. 1,200 g). It accepted a young Guinea pig (*Cavia porcellus*; 150 g) as food two days after birth, and four days later it accepted an adult lab brown rat (200 g). After that, it started to show signs of being close to shed and we stopped offering food.

The twins reported here resembled each other closely and were both females, but they showed strong phenotypic discordance in colour pattern, and to a lesser degree in size, suggesting they were dizygotic twins (Figs. 4, 5 & 6). Phenotypic discordance in twin snakes has been largely attributed to cases of non-identical twins. Dizygotic twins are encapsulated in the same fetal membranes in the oviduct and are initially attached to separate yolk sacs with their own umbilical cords, but the yolk masses and even the umbilical cords may eventually fuse together forming a single yolk mass (Marion & Nowak, 1980; Wallach, 2007, 2018). Fusion of non-identical twins is thought to go even further, since conjoined dizygotic twins (even of separate sexes) have been reported in *Crotalus durisus*, *V. berus*, *V. ursinii* and *T. sirtalis* (Dorner, 1873; Vanzolini, 1947; Tóth et al., 2005; Wallach, 2007). The set of twins of *B. constrictor* reported by Smith (1999) were attached to the same yolk sac by separate umbilical cords, similar to the twins of *C. angulifer*, but they showed high pattern concordance, which led Smith to assume that they were monozygotic. Thus, if our and Smith's (1999) hypothesis are correct, the twins of *C. angulifer* reported here would be the first dizygotic twins documented in the family Boidae.

Twin snakes can be up to nearly 50 % shorter and weigh up to nearly 70 % less than their normal siblings (Wallach, 2007), probably because of resource sharing. Smith (1999) commented that the twins of *B. constrictor* apparently had no birth defects other than a much smaller size than their normal siblings, but they were poor feeders and died some months later. The twins of *C. angulifer* were also smaller than their normal siblings between 24–30 % in length and between 71–79 % in mass (Fig. 5, Table 1) and apparently had difficulties in absorbing the yolk during the time span in which their normal siblings did. We suspect that the lack of that first boost provided by the absorbed yolk in the twins of *C. angulifer* apparently affected their further performance and development, hence the delay in the neonatal shed. The longest period before the neonatal shed previously reported for the species was 37 days (20 days less than the minimum time in the twins reported here), in a neonate from an apparently normal litter, where

Figure 4. Detail of the anterior part of the body of the twins of *Chilabothrus angulifer* in dorsolateral view. Notice the similar but not identical spot pattern.

Figure 5. The four neonates of *Chilabothrus angulifer* four days after birth. Notice the considerable difference in size between the twins (centre, right) and the normal siblings (lower left, upper centre).

the total lengths ranged between 600–610 mm (Sheplan & Schwartz, 1974); neonates of *C. angulifer* frequently exceed 600 mm SVL and 146.6 g (see Rodríguez-Cabrera et al., 2015 and references therein). The twins of *C. angulifer* represent the smallest viable individuals of this species ever reported (Fig. 6), being 103 mm (20 %) and 121 mm (24 %) shorter, and weighed 39 g (49 %) and 48 g (60 %) less than the previous minimum size record for the species, respectively (505 mm SVL, 80 g; Rodríguez-Cabrera et al., 2015).

Considering that the twins absorbed a minimal amount of yolk, as evidenced by the slight or no visible swelling of the abdomens and the almost intact yolk mass one day after birth (Fig. 2), it is unsurprising that they lost mass during the unusually prolonged period before their neonatal shed (Fig. 6). During this period one of the normal siblings remained at the same mass while the other lost 15 g only (Table 1),

Figure 6. Twins of *Chilabothrus angulifer* on 7 November 2021 (shortly after the neonatal shed, nearly two months after birth): left No. 3, right No. 4 (Table 1). Notice the skinny appearance, suggestive of having lost mass. They both represent minimum size records for the species with the smaller twin (No. 4) being the smallest individual of *C. angulifer* ever reported.

suggesting that yolk was converted to tissue but also there was some weight loss; the two normal siblings increased 30 mm in total length during this period. The twins increased 50–60 mm in total length from birth to the neonatal shed, but during a much longer period (57–58 days vs. 17–18 days in the normal siblings). Even after several months with regular food intake, the increase in total length and mass of the twins was considerably less than that of the normal siblings (4–6 times in total length and 5–9 times in mass).

The two normal siblings took both endothermic and/or ectothermic prey as their first and second meals (Table 1) and continued to do so in subsequent meals (not recorded here), but they took ectothermic prey only sporadically. This is consistent with Rodríguez-Cabrera et al. (2015, 2020) who presented compelling evidence for neonates of *C. angulifer* being large enough and equipped with specialised structures (heat-sensing labial pits) to consume mammals. Despite their very small size, heat sensing labial pits are present and presumably functional, indeed at least the largest twin accepted both ectothermic and endothermic prey as its first two meals (Table 1). Nevertheless both twins showed strong preferences for ectothermic prey during the subsequent months, a behaviour possibly related to their small size.

Chilobothrus angulifer is a Cuban endemic species widely distributed in the archipelago (Rodríguez et al., 2013). The apparent absence of substantially smaller neonates (potential twins) of *C. angulifer* in the wild suggests that twinning might represent a disadvantage with respect to normal neonates, possibly decreasing their fitness. To the best of our knowledge, no data on longevity have been recorded for twin snakes, but it has been documented that some anomalous snakes (e.g., dicephalous) may live for up to more than 22 years in captivity (Wallach, 2007). Nonetheless, it seems unlikely that the twins reported here would have survived for very long in the wild given their small size. Free-ranging neonates of *C. angulifer* from the same litter tend to stay together during the period between birth and the first shed, sometimes not as well hidden as expected for an individual of this species (T.M. Rodríguez-Cabrera, unpublished data). During this period, they do not eat or drink water, living off their yolk reserves only. They seem highly vulnerable to potential predators during this time and consequently any elongation of this period, as observed in the twins reported here, would likely be detrimental to their survival.

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