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Aspects of the reproductive biology of *Chitra vandijki* with a description of neonates

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The Burmese Narrow-headed Softshell Turtle (*Chitra vandijki* McCord & Pritchard, 2003) is a large (straight-line carapace length [CL] to ca. 1000 mm) softshell turtle endemic to Myanmar where confirmed records are available from the Ayeyarwady-Chindwin River system, and lower Sittang and Thanlwin rivers (Kuchling et al. 2004, Platt et al. 2005, 2014, 2018). *Chitra vandijki* is subject to intense subsistence exploitation by rural villagers, large numbers are taken for illegal export to wildlife markets in southern China, eggs are widely collected for domestic consumption, and habitat degradation due to gold-mining and conversion of sandbanks to seasonal agricultural fields is occurring on many rivers (Kuchling et al., 2004, Platt et al. 2014, 2017b). Consequently, populations of *C. vandijki* throughout Myanmar have declined precipitously (Platt et al. 2014), even within some protected areas (Platt et al. 2017b). As a result, *C. vandijki* is now in the process of being classified as Critically Endangered on the IUCN Redlist and ranked among the 25 most endangered chelonians in the world (Stanford et al. 2018).

Despite this parlous conservation situation, virtually nothing is known concerning the ecology of *C. vandijki* (Platt et al. 2014). This is certainly the case with regards to reproduction (Platt et al. 2014); with the exception of a single clutch of eggs obtained from a villager and described by Platt et al. (2018), information on the reproductive ecology of *C. vandijki* is non-existent. Furthermore, descriptions and photographs of neonate C. *vandijki* are unavailable in the literature (Platt et al. 2014). This dearth of information is lamentable because such basic natural history data are a necessary prerequisite for designing and implementing effective conservation strategies for endangered species (Dayton 2003). We here report on aspects of the reproductive ecology (phenology, nesting habitat, clutch and egg attributes, and incubation period) of *C. vandijki* in the upper Chindwin River of Myanmar, and provide the first published description of neonates with accompanying photographs.

The Chindwin River arises in the Hukaung Valley of northern Myanmar and flows southward for about 1200 km before debouching into the Ayeyarwady River near Pakokku (Gresswell & Huxley 1965). Much of the upper (upstream from Homalin) Chindwin River is characterized by wide meanders with extensive sandbanks and point bars, although some stretches are confined to a deeply incised channel with small sandbanks nestled in pockets eroded from the bedrock (Platt et al. 2013, 2017a). The regional climate is tropical monsoonal with an annual wet season extending from late May–early June through late September, followed by a dry season from mid-October to May (Terra 1944, Platt et al. 2013). River levels reflect the monsoonal cycle with peak water levels occurring during the mid- to late wet season (Platt et al. 2013, 2017a).

Our observations of *C. vandijki* reproduction were made in conjunction with a conservation project focused on the critically endangered Burmese Roofed Turtle (*Batagur trivittata* [Duméril & Bibron, 1835]) in the Chindwin River (reviewed by Platt & Platt 2018). We obtained clutches of *C. vandijki* eggs 3–24 hours after being notified by Community Conservation Cadres (CCC) employed to monitor sandbanks along the river for signs (e.g., trackways) of turtle nesting activity. At each nest, we first measured the distance from the approximate center of the nest to the river. We then began excavating the nest until reach-

Table 1. Laying and hatching dates, distance to water, and clutch attributes of three *Chitra vandijki* nests found on sandbanks along the upper Chindwin River, Sagaing Region, Myanmar in 2018.

Nest	Laying date	Hatching date	To river (m)	Clutch size	Egg diameter (mm) Mean ± 1SD	Range
1	21 July 2018	30 September–1 October 2018	21	76	28.3±0.4	27.1–29.6
2	15 August 2018	29 October–5 November 2018	31	102	29.5±1.0	27–36.6
3	7 September 2018	Undetermined (see text)	20	89	26.0±3.2	20.1–30.5

ing the uppermost eggs in the clutch and measured the vertical distance from the sand surface to the eggs. We removed the clutch (taking care to maintain the upright orientation of each egg), counted the eggs, and measured the vertical distance from the surface to the floor of the nest cavity. We measured the diameter of each egg using dial calipers (± 0.1 mm), packed the eggs in a sand-filled plastic box and transported them by boat to a secure husbandry facility at Limpha Village (25°48'19" N, 95°31'44" E, 132 m above sea level). Clutches were incubated under ambient conditions in either a sand-filled Styrofoam Box (1 clutch) or artificial sand bed surrounded by a tightly woven bamboo fence lined with plastic sheeting to prevent the escape of hatchlings (two clutches).

We measured neonates within 24 hours of emerging from the nest and if the laying date was known, calculated the incubation period. Using dial calipers, we measured (to the nearest 0.1 mm) CL, maximum carapace width (CW), mid-line plastron length (PL), and shell depth (SD; from plastron to highest point of carapace) of each neonate. Our measurements include the pliable margins of the bony carapace (Leathery Carapace Length of Pritchard 2001) and plastron. Neonates were maintained in captivity for about six months (head-started) and then released into the wild. We also searched for turtle eggs during infrequent visits to an urban market in Khamti (25°59'48" N, 95°42'06" E, 144 m above sea level). Eggs purchased in the market were returned to Limpha, measured, and incubated as described above. River level and air temperature data were obtained from the Department of Meteorology and Hydrology, Ministry of Transport and Communication in Khamati and Homalin, respectively (air temperature is not recorded at Khamti station). Mean values are presented as ± 1SD.

We found three *C. vandijki* nests on two sandbanks along the western shore of the Chindwin River near Sein Naing Village (25°15'39" N, 95°10'12" E; 140 m above sea level) during 2018 (Table 1). Conspicuous trackways left by nesting female turtles led from the river to each nest. The sandbanks used for nesting by female *C. vandijki* are adjacent to deep holes in the riverbed, and composed largely of medium to fine-grained metamorphic sands, a well-drained, porous substrate that permits gaseous diffusion and heats rapidly, thereby enhancing embryo growth (Platt et al. 2017a). Vegetative cover was absent at the nest sites, which were exposed to direct sunlight for most of the day. Nests were deep holes of approximately uniform depth (ca. 300 mm to eggs and 450 mm to bottom of hole) excavated 20–31 m from the edge of the river. Although positioned on level (slope < 5°) microsites near the highest part of the sandbank, a slight rise in river levels (ca. 30–45 cm) would probably have inundated all three nests. Female *B. trivittata* have used the same sandbanks for nesting during February and March (peak months of dry season) in previous years (Platt et al. 2017a).

The three clutches of *C. vandijki* eggs that we found were deposited from 21 July to 7 September 2018 (Table 1). We also purchased 15 eggs in the Khamti Market on 16 August 2018. Because turtle eggs are unlikely to remain unsold for more than a few days, we assume this clutch was deposited around 14–15 August 2018. Previously, we obtained a clutch of 56 *C. vandijki* eggs from a villager in Padumone (26°00'55" N, 95°52'09" E, 183 m above sea level) on 25 August 2016 that was harvested 1–2 days earlier (Platt et al. 2018). Collectively, these data indicate that egg-laying by *C. vandijki* occurs during a two-month period from mid-July through early September, a time that coincides with near-maximal river levels of the late wet season (Fig. 1). Given this phenology, *C. vandijki* nests are probably at risk of being lost to flooding during most years.

We calculated mean clutch size for *C. vandijki* based on the three nests found along the Chindwin River in 2018 (Table 1) and another clutch of 56 eggs from Padumone (Platt et al. 2018). Eggs purchased in the Khamti Market were not included in our analysis because turtle eggs are sold individually and those we obtained were almost certainly part of a larger clutch from which eggs had already been removed. The mean size of the four complete clutches in our sample was 80.7 ± 19.6 eggs (range = 56–102 eggs). *Chitra vandijki* eggs are spherical in shape with a leathery shell that becomes increasingly hardened as eggs dry during incubation. The mean egg diameter for our complete sample (including eggs from Khamti Market) was 28.2 ± 2.3 mm (n = 338; range = 20.1–36.6 mm).

We incubated 282 *C. vandijki* eggs (267 collected from the wild and 15 purchased in market) of which 150 (53.1%) successfully hatched. None of the eggs we obtained in the Khamti Market hatched, most likely as the result of mishandling prior to our purchase. Confusion as to when hatchlings first emerged from eggs collected on 7 September 2018 precluded an accurate determination of the incubation period for that clutch. Otherwise, eggs deposited in July and August hatched in late September, and late October–early November after incubation periods ranging from 71–72 and 75–81 days, respectively (Table 1). Similarly, Platt et al. (2018) reported an incubation period of 81 days for a clutch of *C. vandijki* eggs collected in August 2016. Air temperatures were relatively stable during the incubation period (21 July through 5 November 2018); minimum and maximum air temperatures averaged 23.9 ± 2.0°C and 31.6 ± 2.7 °C, respectively. For the most part, river levels gradually declined during the incubation period and were approaching the seasonal minima when hatchlings emerged from the nests (Fig. 1).

Morphometric data were obtained from 150 neonate *C. vandijki* that emerged from eggs we collected along the Chindwin River (Table 2). Assuming an upper asymptotic CL of 1000 mm for adult *C. vandijki* (Pritchard 2001, Platt et al. 2014), the mean CL of neonates is 3.5% of the maximum adult body size. Neonate *C. vandijki* are characterized by a brown-green carapace covered with lighter (green-olive), irregular reticulations (Fig. 2a). The asymmetrical dark vertical streaks over the costal bones on the carapace of large juveniles and adults are absent in neonates. As in the adults, distinct longitudinal stripes with a V-shaped mark are obvious on the head and neck of neonates and dark splotches surround the eyes and proboscis (Fig. 2b). The legs and feet are green-brown with lighter reticulations consistent with similar markings on the carapace. The plastron of neonates is uniform whitish-grey and the underside of the carapace margin is greyish. Our observations in captivity suggest neonate *C. vandijki* are ambush predators, which inhabit shallow water, remain buried or partially buried just beneath the substrate, and wait for prey (e.g., small fish). As such, the cryptic body coloration of neonates provides concealment from prey and also lessens the chances of detection (especially when neonates are partially buried in the substrate or swimming) by predators that forage in shallow water (e.g., wading birds). Similar to other hatchling trionychids (Muller 1921, Webb 1962; Greenbaum & Carr 2002), a caruncle (egg-tooth) is present on the maxilla immediately ventral to the proboscis of neonate *C. vandijki* (Fig. 2b). The caruncle was still present on some neonates as late as mid-December 2018, which is somewhat surprising given that others report relatively brief retention periods of ≤ 7 days (Muller 1921, Lovich et al. 2014). We speculate the lengthy retention of the caruncle is an artefact of captivity, perhaps influenced by the nutritional status of the neonates.

Table 2. Morphometric measurements of 150 neonate Burmese Narrow-headed Softshell Turtles (*Chitra vandijki*) hatched from eggs collected along the upper Chindwin River, Sagaing Region, Myanmar.

Attribute	Mean ± 1SD (mm)	Range (mm)
Carapace length	35.2 ± 3.5	27.3–41.0
Carapace width	33.9 ± 2.6	27.5–38.6
Plastron length	30.3 ± 1.6	24.9–34.6
Shell depth	9.4 ± 1.0	7.6–13.2

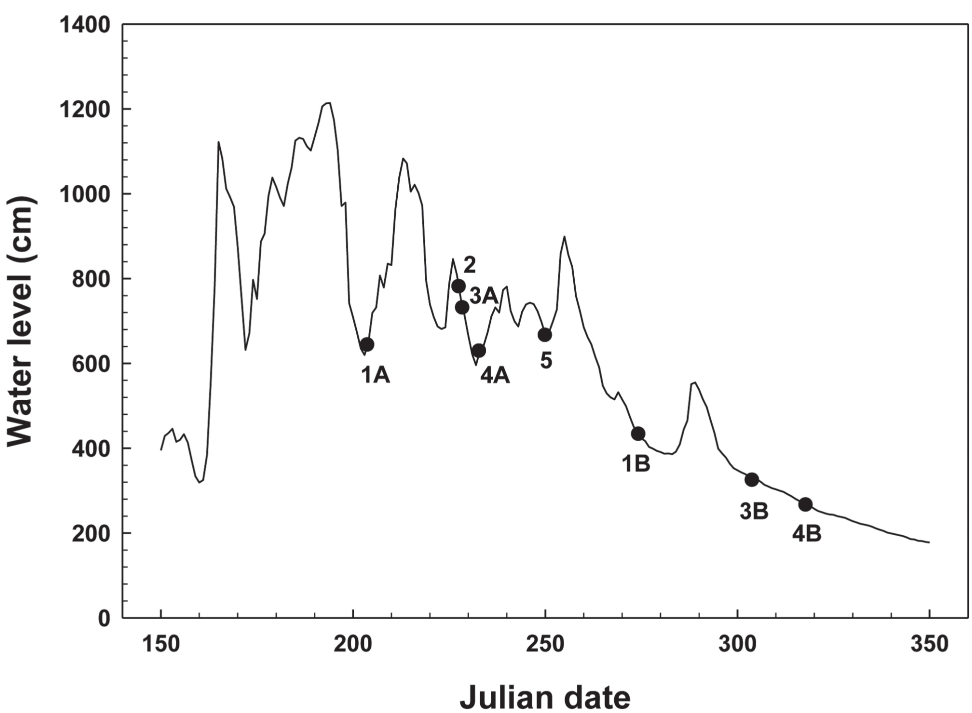

Figure 1. Laying and hatching dates for *Chitra vandijki* eggs in relation to water levels in the Chindwin River, Myanmar (2018). Nest number corresponds to Table 1. 1 = Laying date (A) and hatching date (B) for eggs in Nest 1. 2 = Eggs obtained in Khamti Market. 3 = Laying date (A) and hatching date (B) for eggs in Nest 2. 4 = Probable laying date (A) and hatching date (B) for clutch reported by Platt et al. (2018). 5 = Clutch in Nest 3 deposited (hatching date uncertain; see text). River level data courtesy Department of Meteorology and Hydrology, Ministry of Transport and Communication, Government of Myanmar.

In summary, our observations along the Chindwin River indicate that *C. vandijki* deposits eggs late in the wet season when river levels are near the seasonal maximum and hatching occurs in the late-wet and early-dry seasons as river levels are falling. This phenological pattern differs from that of other sympatric river turtles (e.g., *Batagur trivittata* and *Nilssonia formosa* [Gray, 1869]) in central and northern Myanmar, which deposit eggs during the mid-dry season with hatching timed to coincide with rising river levels at the onset of the wet season (Platt et al. 2016, 2017a, 2018). Our earlier speculation that *C. vandijki* followed this pattern (Platt et al., 2014) was obviously in err, and here stands corrected. Our observations also confirm that like other congeners, *C. vandijki* produces large clutches of relatively small eggs (Nutaphand 1979, Pritchard 2001, Kitimasak et al. 2005). Finally, our observations (Platt et al. 2017a; this study) and those of Kuchling et al. (2004, 2006), further high-light the importance of undisturbed sandbanks as critical nesting habitat for endangered chelonians (e.g., *C. vandijki*, *N. formosa*, and *B. trivittata*) in the upper Chindwin and other rivers in Myanmar where these species occur. Successful conservation therefore depends in part on effectively protecting these habitats from conversion to other uses, such as seasonal agricultural fields and sand-mining.

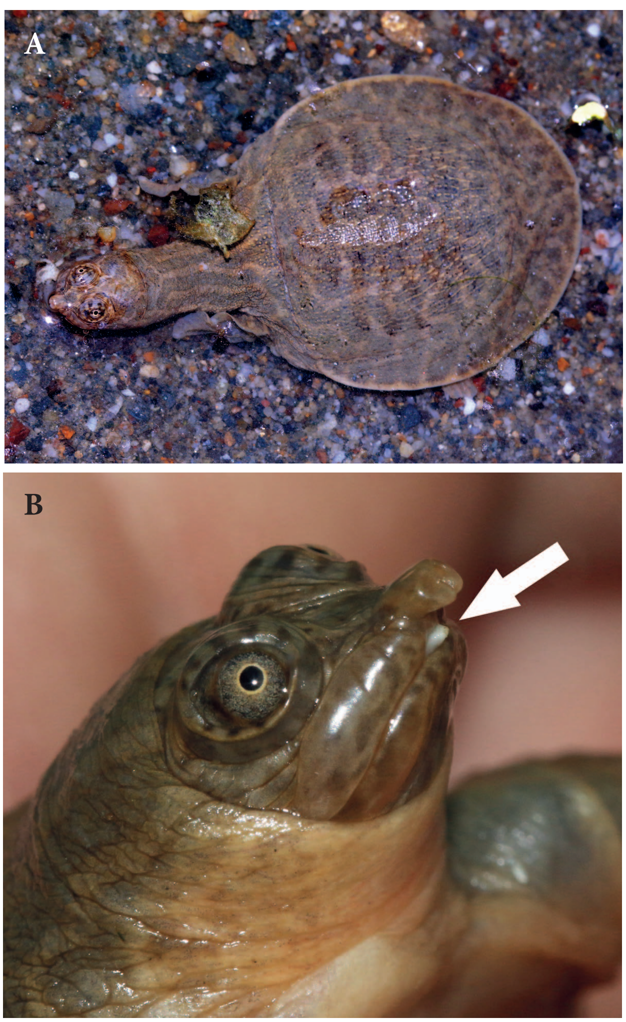

Figure 2. Neonate *Chitra vandijki* hatched from eggs collected along the upper Chindwin River in Myanmar. (A) View of carapace and head. (B) Caruncle below proboscis (white arrow). Photographs by N. A. Haislip.

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