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# Husbandry and first record of captive breeding of the Asian giant river toad *Phrynoidis asper*

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**ABSTRACT** – The Asian giant river toad *Phrynoidis asper* is a large species of bufonid from south-east Asia that is apparently obtained by the international exotic-pet trade from wild populations. Captive breeding of this species seems not to have been documented. The donation to Chester Zoo in October 2021 of an adult group of five males and one female of this toad species provided an opportunity to study captive breeding. The specimens were maintained separated by sex until signs of reproductive condition were apparent and then they were placed together in a large breeding enclosure. This had three distinct environmental zones simulating a riverbank. Six days after mixing the sexes a large spawn mass was laid. On hatching, the tadpoles were transferred to a rearing aquarium and then, after reaching Gosner stage 42, the developing toads were transferred to terrestrial vivaria to complete development. Captive breeding of this species could replace collection from the wild.

## INTRODUCTION

The genus *Phrynoidis* (Bufonidae) currently comprises two described species, *Phrynoidis asper* and *Phrynoidis juxtasper* (Frost, 2023) that are widely distributed in south-east Asia (Frost, 2023). *Phrynoidis asper* (Gravenhorst, 1829), commonly known as the spiny river toad or Asian giant river toad, is known to occur in Vietnam, southern Myanmar, western and peninsular Thailand, Malaya, Sumatra, Borneo, and Java (Frost, 2023), where it may be found from sea level up to 1500 m (Inger, 1966).

*Phrynoidis asper* is a large species, with females attaining snout vent lengths (SVL) of 95–215 mm and males 70–100 mm (Inger & Stuebing, 2005) although despite the species' large size in the wild it shows a diet preference for ants and termites (Hui et al., 2014). Throughout its range it is closely associated with rivers and streams and reproduction is believed to be non-seasonal. Males can be observed calling from water bodies year round (Inger & Bacon, 1968), amplexus is axillary, and a single female may deposit up to 12,000 eggs in a single spawning (Inger & Bacon, 1968). Dorsally the colouration is generally brown with green or red flecks, ventrally the colouration is yellowish cream. There are large parotoid glands the size of which varies depending on geographic location (Inger, 1966) and the skin is covered in conical tubercules usually tipped with melanin (Inger, 1966). Skin glands release toxins that predominantly contain bufotelin and have had significant effect on mice in laboratory tests (Daly et al., 2004).

Although populations of *P. asper* have been noted to be declining, the species is assessed as of Least Concern by the IUCN (Chanson et al., 2021). The species is also increasingly present as wild collected individuals within the international exotic pet trade with no evidence of captive breeding (Choquette et al., 2020). This report details captive breeding of *P. asper* at Chester Zoo (Great Britain) during 2022 and may represent the first successful breeding of this species in captivity.

## MATERIALS & METHODS

An adult group of one female and five male *P. asper* were donated to Chester Zoo's Herpetological collection by a private keeper in October 2021. This group is believed to have been collected from the wild for the pet trade at an unknown locality and had been in captivity for two to three years previously. The sexes were initially maintained separately under dry conditions to reduce activity during acclimation, and to begin reproductive cycling, before transfer to a large breeding enclosure as detailed below.

The equipment used to measure the environmental parameters described herein were as follows; Surface temperatures - a Mini RayTemp infrared thermometer; Air temperatures and humidity - a temperature and hygrometer digital probe (Electronic Temperature Instruments Ltd); UVB zones - a Model 6.5R Reptile UV Index Meter (Solar Meter®); and water chemistry parameters - an API Freshwater test kit and colour chart (Aquarium Pharmaceuticals Inc.).

### Dry Period Enclosure

From October 2021 until March 2022 two identical enclosures were used to maintain sexes separately. These consisted of large preformed open top containers measuring (2.4 m long x 1.4 m wide x 1.4 m high) with a substrate of loose soil and gravel, leaf litter, live plants *Dieffenbachia seguine* and *Spathiphylum* sp. for low cover, large cork bark pieces

and a preformed plastic hide box to provide an additional refuge. A large shallow water bowl was always present and was refreshed daily. The specimens were provided with heating and lighting from a 24 w Light wave canopy (Growth Technology Ltd) containing two 6 % T5 Arcadia (Monkfield Nutrition Ltd) UVB emitting lamps combined with a UVB emitting basking spot of a 100w Arcadia D3 basking lamp. This created a localised area of heat and light with a UVB gradient of 0–3.5 and temperature of 28–30 °C across an area of approximately 60 $cm^2$, the photoperiod was 12:12. The ambient temperature was between 23–28 °C during daytime and 18–20 °C at night. The enclosure was lightly misted with water once each evening, the open top enclosure allowed the environment to become dry after a short period following spraying. Food was offered three times per week and consisted of adult black field crickets *Teleogryllus commodus*, adult brown cricket *Acheta domesticus*, locusts *Schistocerca gregaria* and dubia cockroaches *Blaptica dubia*. All food items were gut loaded with Repashy Superload (Repashy Specialty Pet Products ©) 24 hours prior to feeding and dusted with Arcadia Earth Pro A vitamin and mineral supplement before being offered to the toads.

**Breeding Enclosure**

This enclosure simulated a stream and large open water area to induce mating behaviour and oviposition (Fig. 1). It was constructed from a large commercially available paddling pool (4 m long x 2 m wide x 1 m high) and divided into three distinct environmental zones; a land area (1 m x 2 m), an intermediate shallow water area (1 m x 2 m) and a fully aquatic area (2 m x 2 m).

The land area of the enclosure was constructed using plastic pallets to create a raised false flooring. A lower level was added using the same false floor method to create an intermediate shallow water area in a 'step' effect before the drop into the pool. A tarp was used to create a barrier between the false flooring and the substrate, which was a mixture of soil, gravel and leaf litter on the land area, and solely gravel on the intermediate area. These areas were furnished with logs ranging in length from 1–2 m, creating a landscaped environment. A basking zone area of increased heat, light and UVB exposure measuring 45 x 80 cm was provided by an Arcadia Thermal Zoo Pro unit which was fitted with a 50 w Arcadia deep heat projector, 100 w Arcadia incandescent spot lamp, 24 w Arcadia 6 % UVB T5 and a 24w Arcadia Jungle dawn LED. The temperature of the basking zone was 28–32 °C and the UVI range was 1.0–1.8. The ambient daytime temperature range was 23–27 °C and the nighttime temperature was 20–22 °C.

Within the open pool the water depth was approximately 30 cm, emergent large boulders were placed to provide areas above the water line from which males could call. The aquatic plant *Elodea densa* was provided for cover, and as potential egg deposition sites. The water temperature was 18–20 °C. Two circulation pumps were used, the first circulated water from the pool to within a curved, hollow log on the land area which channelled the water back down into the pool, simulating a stream (Syncra Silent 4.0, Sicce S.r.l). The second (Green Line Pond Pump 8000, Velda B. V.) was linked to an

**Figure 1**. The captive breeding enclosure for *Phrynoidis asper*

overhead rain system constructed from plastic pipework with irrigation sprayer nozzles (LBS Worldwide Ltd) placed throughout, which simulated rainfall when the pump was in use. The rainfall covered the entirety of the aquatic section and approximately two thirds of the land area ensuring that specimens had continuous access to some drier areas. The water from simulated rainfall was circulated from the main pool within the enclosure through the rain system, with water being topped up in the pool when required. Netting was used above the enclosure to prevent escape whilst maintaining high air flow.

Five male specimens were introduced to the breeding enclosure on 21 March 2022. The stream remained flowing permanently and the rain system was not used during this time, the land area was sprayed up to twice daily with a pump action spray gun to provide humidity. From 16 April the rain system was turned on 15:00–16:30 h to initiate an environmental change. The female was introduced on 18 April at which point the rain system was turned on for 24 hours. At the time the sexes were together a CamPark T150 trap camera (CamPark Electronics ©) was used to record behaviour and activity of the group both night and day.

**Care of eggs, larvae and metamorphosed toads**

Following egg deposition, the spawn remained in-situ within the breeding pool for the duration of development and hatching, an aquarium air stone was placed by the egg mass to provide additional oxygenation. Upon hatching, tadpoles were transferred into a rearing aquarium (90 cm long x 30 cm wide x 30 cm high) filled to 60 L at a stocking

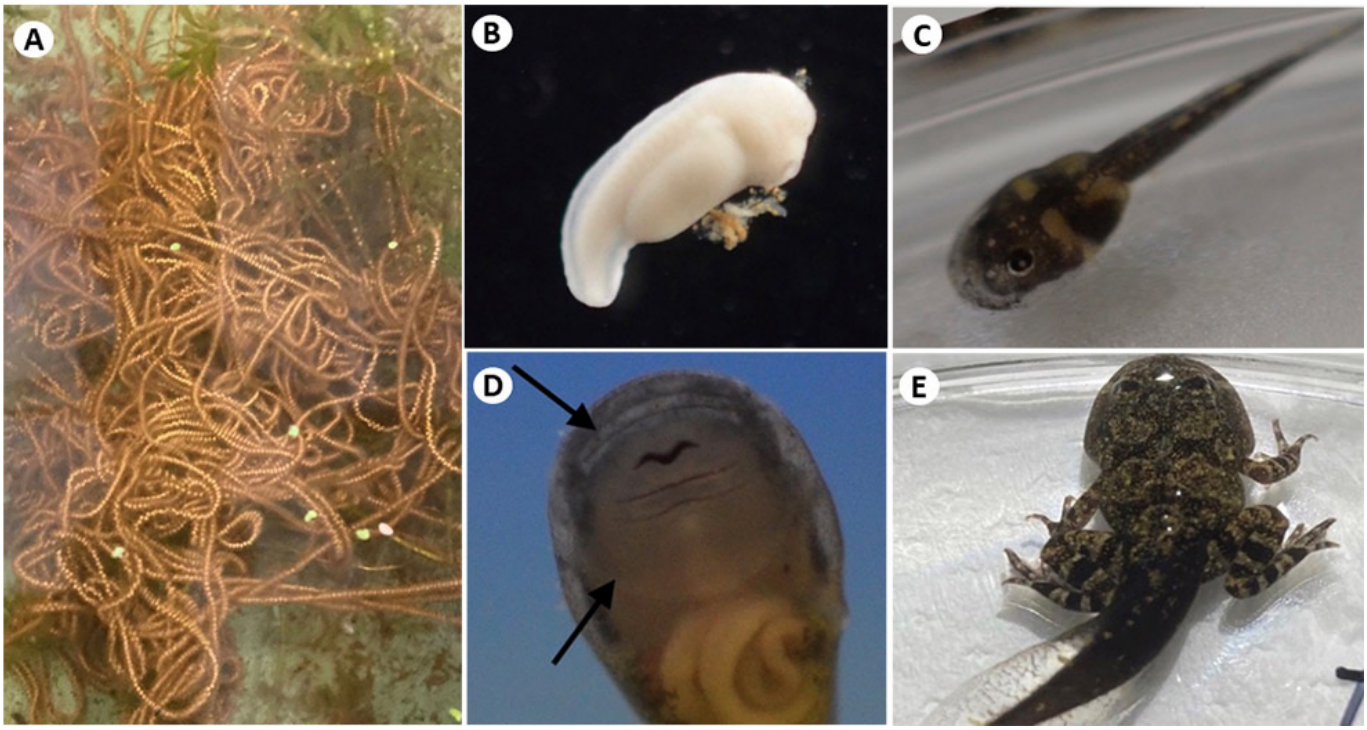


**Figure 2**. Some developmental stages of the captive bred *Phrynoidis asper* - **A.** Spawn mass, **B.** Prematurely hatched tadpole at Gosner stage 19, **C.** Tadpole in dorsal view showing pattern and pigmentation, **D.** Ventral view of tadpole showing the specialised oral disc (arrows), **E.** Developing toad at Gosner stage 42

density of one tadpole per litre. Water was maintained at a temperature of 18.5–21.5 °C, a pH of 7.6–7.8 and general hardness was 143.2 ppm. All water used for aquarium water changes and maintenance was Heavy Metal Axe (HMA) filtered tap water. Filtration was provided by two airline aquarium sponge filters and one Fluval mechanical filter (Rolf C. Hagen Inc.), providing oxygenation and directional water movement across the aquarium, simulating a high flow-rate stream. Lighting was provided by a 15 w 10,000 k Daylight Photon Energy LED (Wave Point® Technology) on a 12:12 photoperiod. Aquarium furnishings consisted of large smooth stones, angled slates providing shelter and a surface for the tadpoles to feed upon and cling to, and cuttings of plastic pipe. *Elodea densa* was provided for cover and the surface of the water was approximately 25 % covered by duckweed (*Lemnoideae* sp.) providing a photo gradient. No substrate was used. Waste was spot cleaned daily, and 25 % water changes took place every 3–5 days.

Food was provided daily and consisted of an Arcadia Amphibi gold pellet which was crumbled into the aquarium and sunk to the floor, generic tropical fish flake, and spirulina powder mixed with water to make a paste which was then dried onto the slates provided as above; Repashy Soilent Green, which was also prepared using the same method as the spirulina slates, and a mixture of Repashy Soilent Green, Repashy Red Rum and Arcadia Amphibi gold pellet ground into a powder with pestle and mortar at a 2:1:1 ratio and then also dried onto slates. All food slates were replaced every 24–48 hours.

At Gosner stage 42 (Gosner, 1960) (Fig. 2E) the developing toadlets were removed from the tadpole rearing aquarium and housed at a density of 20 individuals in an Exo Terra glass vivarium (Rolf C. Hagen Inc.) (45 cm long x 30 cm wide x 30 cm high) with a shallow water body at the front aerated using an aquarium pump and air stone. The land area was provided using an EpiWeb (Dusk Tropic ©) sheet covered with a thin layer of tropical soil seeded with springtails *Siera* sp. for food, and covered with dried leaf litter. Stacks of cork bark were used to provide a humidity gradient and as refuges. Lighting was provided by an Arcadia ProT5 6 % UVB and an Arcadia Jungle Dawn LED. Enclosures were heated using room air conditioning that gave an air temperature gradient of 20–28 °C and a UVB gradient of 0.0–2.0. Specimens were fed daily with a mixture of *Drosophila melanogaster*, *Drosophila hydei*, and 1st instar *A. domesticus* dusted with Repashy Calcium Plus. Approximately two months after metamorphosis the substrate was covered with rehydrated sheet moss and additional cork bark refuges were installed.

After four months specimens were transferred to a larger Exo Terra glass vivarium (60 cm long x 45 cm wide x 30 cm high) with the aerated water bodies replaced by large shallow water bowls. Enclosure furnishings were the same as above although specimens were provided with an Arcadia 35 w halogen basking spot, producing a localised surface temperature of 30 °C. Specimens were observed in groups of up to eight individuals under pieces of cork bark beneath and around the basking spot. The same food was provided as above although cricket instar size offered were larger, between 1st (5 mm) and 3rd (8–10 mm) instar.

## RESULTS

During the initial dry period, weight and visual body scoring was used to determine health status; all the specimens

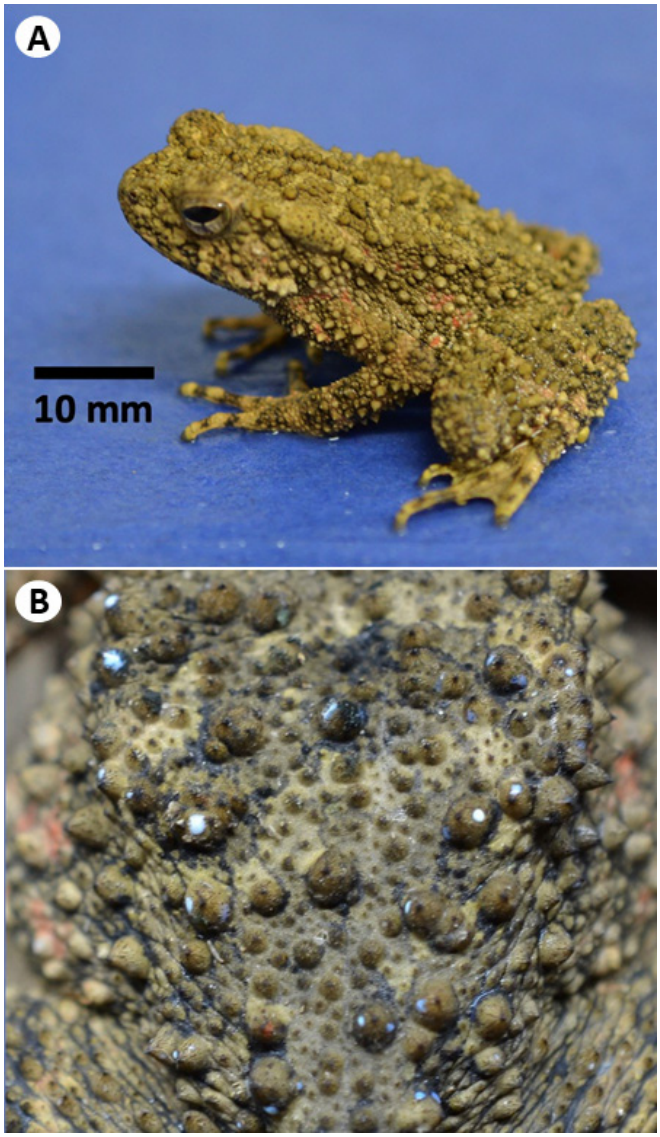


**Figure 3**. **A.** Six-month-old captive bred *Phrynoidis asper*, **B.** Toxic white secretions exuding from tubercules on the skin of a captive bred individual

appeared healthy. The mean ± sd weight of five males was 234.8 g ± 66 g. The female began this period with a weight of 608 g and following the dry period regime weighed 600 g. The consistent weight of the female together with the development of nuptial thumb pads in males was used as an indicator of readiness for a breeding attempt. Upon the initial introduction of the male specimens to the breeding enclosure they appeared to be very active, with specimens noted by keepers to be in different positions of the enclosure each day. Following the introduction of the female to this enclosure on 18 April, coinciding with initiation of rainfall, a high level of nocturnal activity was observed, with males vocalising predominantly from the water's edge but also from emergent rocks within the pool area. This behaviour persisted for an additional five nights.

On the morning of 21 April males were recorded to be very active from 01:00 h, amplexus with the female was initiated early morning at 04:45 h, when the female paired with a male was actively moving around the pool. Subsequently, conspecific males attempted to displace the successful male and over time up to three males were observed in amplexus with the female. From 09:00 h, two males were in amplexus with the female and at approximately 11:00 h spawn was observed in the pool (Fig. 2A); the spawn appeared to have been fertilised by these two males. Following oviposition the two males separated from the female and she was returned to the dry enclosure. At the time of removal from the breeding enclosure the female was weighed and was observed to have lost 56 g as a result of oviposition.

The spawn mass consisted of one continuous string of several thousand individual cream to white eggs measuring approximately 2 mm in diameter. At a water temperature of 18–20 °C the first stages of development were observed within 72 hours. Five days following oviposition at Gosner stage 19 (Gosner, 1960) when the egg mass was disturbed during monitoring, developing larvae were observed to hatch prematurely (Fig. 2B), falling from the egg mass to the floor of the pool, where normal development continued. Eight days following oviposition, those larvae that remained within the egg mass had completed normal stages of development and begun hatching naturally; prematurely hatched larvae had reached the equivalent stage. Hatched larvae used short bursts of movement to disperse from the spawning site but they were then observed remaining motionless attached to the walls and objects within the enclosure such as plants, pipes and rocks. The tadpoles, which still had a pale pigmentation, appeared not to be feeding at this time and were probably relying on the large yolk reserves that could be seen through the ventral body wall. The hatched egg mass was then removed to prevent polluting the water body.

After a further 48 hours the tadpoles had developed dark pigmentation (Fig. 2C) and begun to feed. Their total length was about 5 mm and they possessed a specialised large oral disc (Fig. 2D) that was used to cling to surfaces constantly. Free swimming behaviour was not observed, instead movement was made by short bursts along surfaces whilst clinging by the mouthparts. Individuals fed readily from the food slates provided and upon algae forming on the glass of the aquarium. During the later stages of larval development tadpoles showed a preference for higher protein content foods such as Repashy Soilent Green and slates containing Arcadia Amphibi gold pellets, food slates containing algae alone were visited less frequently at stages close to metamorphosis. The first individual reached Gosner stage 42 (Gosner, 1960) and metamorphosis following 120 days aquatic development with a total length about 20 mm. Upon being moved to the aquatic area of the rearing enclosure, metamorphosing toads were observed on land within 24 hours, completed metamorphosis and feeding in an additional 48 hours, and following absorption of the tail, their total length was about 10 mm. Under the described conditions, at eight months following metamorphosis 38 captive born *P. asper* had a mean ± sd body weight of 12.63 g ± 3.51 and SVL of 46.31 mm ± 3.76 (Fig. 3A).

## DISCUSSION

Despite being a non-seasonal breeder in the wild (Inger & Bacon, 1968), keeping *P. asper* initially in drier conditions

appeared to aid in gaining and maintaining the body condition needed for reproduction. Prior to our study, there were no descriptions of captive breeding for *P. asper*, consequently we devised a breeding enclosure with three zones to simulate as far as possible the toad's ecological niche. Once the toads were introduced into this breeding enclosure this led swiftly to reproduction. However, it should be noted that the introduction of the female to the breeding enclosure coincided with a natural low-pressure weather system. This could have helped induce reproductive behaviour in the males as it is known that captive amphibians may use external changes in barometric pressure as a reproductive cue (Poole & Grow, 2012). Further research at times with low barometric pressure should help clarify this issue.

To improve the success of future captive breeding, we recommend further investigation into the best method of maintaining the spawn, in particular improvements that would avoid premature hatching of the larvae. Premature hatching in amphibians is known to be a defence mechanism (Warkentin, 2011) and although in our study the prematurely hatched larvae continued development they eventually suffered a higher mortality rate than those that hatched at the normal time. A further recommendation is to use matured water bodies with established algae growth upon walls and furnishings. This would allow newly hatched tadpoles to begin feeding when ready and reduce the need to manually rehouse tadpoles during this delicate stage, which risks causing damage to them. It is worth noting that we found that a temporary fault with the aquarium air stone led to some tadpole deaths. This may indicate a particular need for water oxygenation, which would be expected in a species that breeds in streams and rivers.

In the experience of the authors, *P. asper* adapt well to captivity provided that their space requirements are met. Their large size means that they are an engaging species for display in zoological collections, although keepers must exercise caution when handling specimens due to the toxic secretions from the skin (Daly et al., 2004), which are even released by the captive born juveniles, when stressed during handling (Fig. 3B).

The international trade of wild collected amphibian species has been shown to be linked to the spread of pathogens such as the amphibian chytrid fungus *Batrachochytrium dendrobatidis* and ranavirus (Fisher et al., 2007; Picco & Collins, 2008), which presents a risk to zoological collections as well as to wild amphibian populations worldwide (Greenberg & Palen, 2019). Wild collected amphibians may also have high parasite burdens and fail to acclimate to captivity without appropriate specialist care (Write & Whitaker, 2001). A responsibility of modern zoological collections is to establish repeatable methods of captive breeding to reduce these risks by providing sustainable populations without the need for wild collection. Knowledge gained and shared from captive breeding may also, in the future, prove beneficial for the ex-situ conservation of endangered species with similar requirements. Due to the large number of eggs produced by *P. asper*, it would require only relatively few but regular breeding episodes to replace the collection of this species from the wild.

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## REFERENCES

Chanson, J.S., Stuart, B.L., Iskandar, D., Wogan, G., Chan, K.O., Kusrini, M.D., van Dijk, P.P., Inger, R.F., Neang, T., Nguyen, T.Q., Rowley, J.L., Hobin, L. & Cutajar, T. SSC Amphibian Specialist Group. (2021). *Phrynoidis asper*. The IUCN Red List of Threatened Species 2021: e.T54579A87864714.

Choquette, R.E., Angulo, A., Bishop, P.J., Phan, C.T.B. & Rowley, J.J.L. (2020). The internet-based southeast Asia amphibian pet trade. *Traffic Bulletin* 32(2): 69–76.

Daly, J.W., Noimai, N., Kongkathip, B., Kongkathip, N., Wilham, J.M., Garraffo, H.M., Kaneko, T., Spande, T.F., Ninit, Y., Nabhitabhata, J. & Chan-Ard, T. (2004). Biologically active substances from amphibians: preliminary studies on anurans from twenty-one genera of Thailand. *Toxicon* 44: 805–815.

Fisher, M.C. & Garner, T.W.J. (2007). The relationship between the emergence of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduced amphibian species. *Fungal Biology Reviews* 21(1): 2–9.

Frost, D.R. (2023). Amphibian Species of the World: an Online Reference. Version 6.1 (23 April 2023). American Museum of Natural History, New York, USA. Electronic Database.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16(3): 18–190.

Greenberg, D.A. & Palen, P.J. (2019) A deadly amphibian disease goes global. *Science* 363(6434): 1386–1388.

Hui, Y.C., Rahim, N.D.A., Shahrudin, S. & Jaafar, I. (2014) Feeding habits of river toad *Phrynoidis aspera* (Anura: Bufonidae) from lowland dipterocarp forest in Kedah, Malaysia. *Pensee Journal* 76(5): 182–188.

Inger, R.F. (1966) The systematics and zoogeography of the amphibia of Borneo. *Fieldiana: Zoology*. 402 pp.

Inger, R.F. & Bacon, J.P. (1968). Annual reproduction and clutch size in rain forest frogs from Sarawak. *Copeia* 1968: 602–606.

Inger, R.F. & Stuebing, R.B. (2005). *A Field Guide to the Frogs of Borneo, 2nd edition*. Natural History Publications (Borneo), Kota Kinabalu. 209 pp.

Poole, V.A. & Grow, S. (2012). *Amphibian Husbandry Resource Guide, Edition 2.0*. Association of Zoos and Aquariums, Silver Spring, MD. 238 pp.

Warkentin, K.M. (2011). Plasticity of hatching in amphibians: Evolution, trade-offs, cues and mechanisms. *Integrative and Comparative Biology* 51(1): 111–127.

Write, K.M. & Whitaker, B.R. (2001). *Amphibian Medicine and Captive Husbandry*. Krieger publishing company. Philadelphia Zoological Garden, Philadelphia, Pennsylvania, USA. 570 pp.

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