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Short Note

Solo Spawning, Egg Guarding by a Female and Remarks on Vocal Signalling and Colour Change for the Freshwater Puffer *Pao suvattii* in Captivity

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Pufferfish (Tetraodontidae) are a diverse family with nearly 200 species in 29 genera showing a circumglobal distribution[1,2]. They do not only inhabit marine and brackish water environments but often live temporarily in estuaries, rivers, or streams during the juvenile phase. Some pufferfish can be found predominantly in freshwater habitats and are therefore particularly of interest for the aquarium trade, especially species of the genera *Tetraodon* from Africa, *Colomesus* from South America as well as *Auriglobus*, *Carinotetraodon*, *Dichotomyctere*, *Leiodon* and *Pao* from Asia[3,4]. Before Kottelat (2013)[5] revised the nomenclature of this group, most of these freshwater pufferfish were all placed within the genus *Tetraodon* that is now restricted to the African representatives only. Among those newly established genera is the South-East Asian group *Pao* that comprises 14 species and can be characterized by the presence of a unique colour pattern and elongated premaxillary pedicels[6]. The species that are most common in the aquarium trade are *Pao baileyi* (Sontirat, 1985), *P. cochinchinensis* (Steindachner, 1866), *P. leiurus* (Bleeker, 1850), *P. palembangensis* (Bleeker, 1851) and *P. suvattii* (Sontirat & Soonthornsatit, 1985).

The latter – being also known as the Pignose Puffer (or Arrowhead puffer) – *P. suvattii* is a particular interesting and fascinating freshwater pufferfish to be kept and studied in captivity. This species inhabits the Mekong River and its tributaries in Laos and Thailand and prefers muddy or rocky substrates[7,8]. *P. suvattii* has a characteristic depressed head terminating in a long snout with thick lips and an upturned mouth. It greatly varies in colouration by showing a greyish to brownish ground colouration with small darkish spots dorsally and darkish ocellae with whitish spots laterally and ventrolaterally in the posterior part of the body. Similarly, to its congeners *P. suvattii* feeds predominantly on smaller fish, although also shrimps and mussel flesh are accepted in captivity. As a sit-and-wait predator they are not very active swimmers, only when prey items are spotted the pufferfish rapidly moves towards them, grabs them with its protruding snout and either swallows them entirely or bites larger pieces out of them. In captivity *P. suvattii* is known to be territorial and might show aggressive display towards conspecifics[3].

Spawning in captivity has already been described for *P. suvattii* and others (*P. cochinchinensis*, *P. palembangensis* and *P. turgidus* (Kottelat, 2000))[3,4]. Erroneously the first record of captive spawning for *P. suvattii* by Recher (1974)[9] was assigned to its African equivalent, *Tetraodon miurus* Boulenger, 1902 from the Congo Basin, since this species shows a considerable convergence regarding its phenotypical appearance, feeding habits and lifestyle. Ebert (2001)[3] reported that *P. suvattii* spawn's large eggs in batches directly on the substrate or hidden in a cavity and the male protecting them until hatching. A more detailed description of the spawing behaviour of this species is given by Schoopp Jensen (2007)[10] (a video of the courtship behaviour and spawning can be accessed on YouTube, see 'Arrowhead Puffer (Tetraodon Suvattii) spawning'. Accessed 02. Nov. 2021. available at https://www.youtube.com/watch?v=9X9l99oHbWY):
After a period of courtship lasting two to four days the eggs are deposited in a spawning pit that is maintained and cleaned. Subsequent to spawning the male shows very aggressive behaviour and protects the eggs – also from the female – until hatching after 9 to 12 days.
In the following, captive spawning is reported for the first time for an uncopulated female *P. suvattii* kept without contact to a mature male individual. Here, this phenomenon is referred to as 'solo spawning'.

A single female Pignose Puffer *P. suvattii* obtained from the pet trade as an adult was kept isolated in a 180 l tank since June 2017. The aquarium was structured with a large wooden root as a hiding place and a close arrangement of underwater plants. Very fine-grained gravel was used as a substrate. The water temperature was maintained at 24-26 °C and the aquarium water was changed partially (15%) once in the month (untreated tap water, 6°dH). The specimen was fed targeted by using a tweezer every two to three days predominantly with defrosted smelt (*Osmerus eperlanus*), rarely with defrosted shrimps, mussel meat or living mealworms.

The described specimen of *P. suvattii* first showed solo spawning in the evening (18.00 h) of the 26 June 2020 about 3 years after being kept isolated. In a corner of the aquarium, hidden underneath the aquarium filter and a wooden root, the female burrowed in the gravel a spawning pit (Fig. 1A) (the exact start of the construction of the spawing site was not observed). Interestingly, the cleaning of the spawning pit was continued to be carried out also after the eggs were laid, causing the partial displacement of the spawn. The female blowed water target-oriented at the spawning pit and removed dropping gravel and plant material. About 60 eggs were recorded in proximity to the spawning site and retained in the tank for four days (Fig. 1B). None of the eggs was fertilized and developed further.

During the time the eggs were left in the spawning pit the female *P. suvattii* strikingly differed in its behaviour. The specimen showed a guarding behaviour by remaining in the spawning pit. When disturbed (e.g., when perceiving movements of people close to the spawning site, even outside of the tank), it responded with aggressive display, starting from spreading its pelvic fins over swimming by threatening and being inflated with water (Fig. 2) to attacking and biting at the aquarium glass. No food was taken up during the entire time of egg guarding (for 8 days, then the eggs were removed). A second solo spawning event occurred in the evening (19.30 h) of the 27 July 2020 with a spawning interval of about a month. Here, the number of eggs laid was lower than in the first solo spawning; only about 30 unfertilized eggs were recorded (Fig. 1C). Considering to the spawning behaviour of *P. suvattii* under normal conditions, viz. the presence of both male and female, in the case described, the single female seemed to take over the

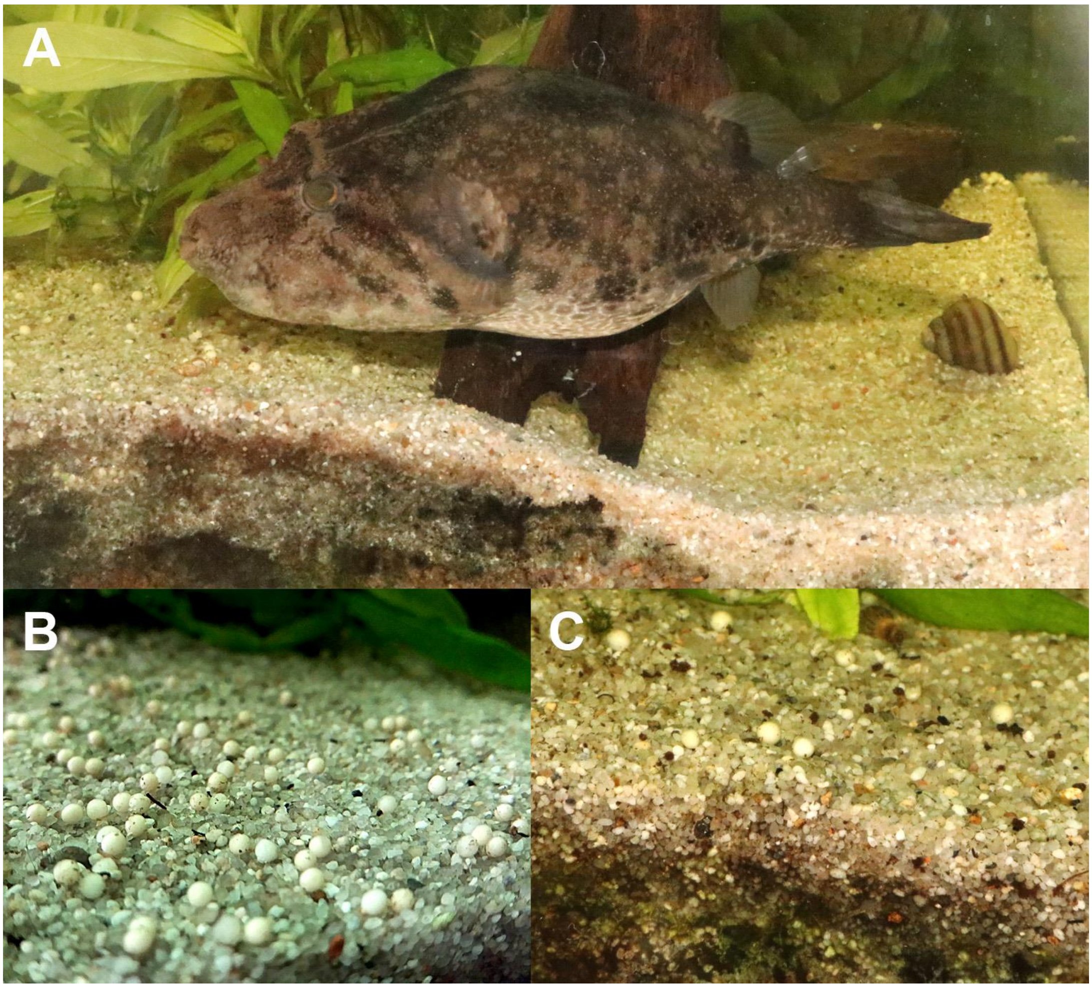

FIGURE 1. Solo spawning of *Pao suvattii;* A. The single female of the Pignose Puffer *Pao suvattii* showed egg guarding behaviour at the spawning pit; B. Eggs deposited on 26. June 2020; C. Eggs deposited on 27. July 2020.

FIGURE 2. Aggressive behaviour: The egg-guarding female Pignose Puffer *Pao suvattii s*wimming along the aquarium glass being inflated with water.

male's role in egg guarding and protection of the spawning pit. However, it is clearly recognisable that the behavioural patterns associated with the spawning event are not as coordinated as they would be under normal circumstances, e.g., shown by the dispersion of the eggs due to a continued and extensive cleaning of the spawning pit.

Descriptions of multiple solo spawning events had only been recorded for uncopulated oval squids *Sepioteuthis lessoniana*[11], but have not been scientifically reported for fish species in captivity in more detail. However, there are at least observations referring to entries of fish keepers in online forums of female captive angelfish *Pterophyllum scalare* depositing infertile eggs and subsequently consuming these in the absence of males[12]. Nevertheless, records for other fish species are rare, and solo spawning is not known for puffer fish yet. Therefore, although observed in captivity, this occurrence of solo spawning in *Pao suvattii* is an interesting new record.

Although for some pufferfish, e.g., *Dichotomyctere nigroviridis*, induced spawning techniques were used for stimulating captive spawning[13], several freshwater puffer fish including *P. suvattii* successfully reproduce in captivity without artificial stimulation[3,4,9,14]. Therefore, it can conclude that the preferences and requirements of inducing spawning in those species can be fulfilled easily also in captivity. In this case of spawning by an uncopulated and isolated puffer fish female it can be only speculated what has caused this behaviour in detail. Perhaps a continuous supply with food in combination with a sudden and short-termed rise of the water temperature from 24-26°C during single hot summer days to 28-30°C were affecting and promoting the egg production. Nevertheless, the absence of further records for other species of ornamental fish reveals that this phenomenon is still rare – even in captivity. The capacity of solo spawning for *P. suvattii* may be an indicative that reproducing this species in captivity may be triggered more easily

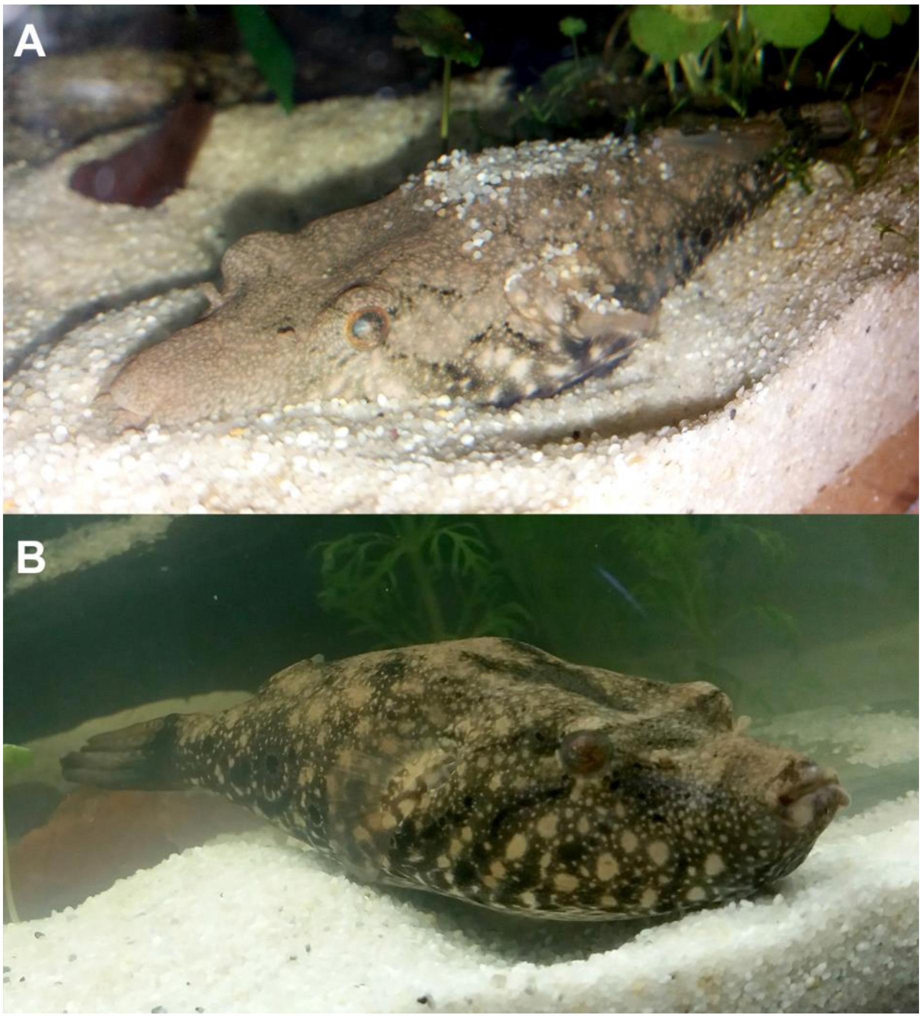

FIGURE 3. Colour change in the Pignose Puffer *Pao suvattii*; A. Pale beige with dissolved dorsal pattern when burrowed in the sand; B. A high contrast darkish brown colouration when resting.

than expected, which could be further explored to produce juvenile fish for aquaculture purposes and should motivate fish keepers to investigate possibilities of keeping and rearing a couple and not only solitary individuals in captivity.

Independently from this solo spawning event, this Pignose puffer was observed to show a change of its colouration and patterns. This colour change was induced when the specimen was stressed, e.g., by showing suddenly a lightened-up colouration and disappearance of prominent dark brownish patterns, or was associated with camouflage, e.g., by changing the colouration similar to the substrate colour when burrowing into it. The ground colouration changed from a pale creamy beige to darkish brown (Fig. 3A-B). When hiding in the substrate, particularly the dorsal patterns appeared more inconspicuous and dissolved (Fig. 3A). With observations based on a single individual only, it remains unclear whether this colour change might be also associated with intraspecific communication and more specifically sexual signalling.[15]

Also, vocal signals could be detected for this specimen of *P. suvattii*. When resting on the substrate, sounds were produced by rapidly moving the jaws against each other and protruding the mouth opening. The resulting vocal signals were clearly perceptible even outside the aquarium and consisted of five to six cracking or cough-like sounds. Since they did not occur only after feeding, but also on days where no prey was available, they were not caused indirectly e.g., by crushing hard-shelled food items.

Sound production and acoustic communication can be found in various fish families[16]. In the context of the diversity of fish species that can emit vocal signals, these sounds are produced in various ways, e.g., by using swim-bladders, pharyngeal jaws or pectoral structures[17]. Records of sound production in puffer fish are not well-known and have been reported only for some marine species, e.g., *Sphoeroides maculatus* or *S. nephelus*[18,19]. Most sounds of tetraodontids were thought to be produced only indirectly during treatment of hard-shelled food objects[20]. The record of vocal signalling in a captive *Pao suvattii* clearly show that suchlike sounds are not only associated with food update. They might also play a substantial role in territorial and aggressive behaviour and intraspecific communication.

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