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Natural history and breeding biology of the poorly known, endangered and endemic, Nicobarese tree frog *Polypedates insularis* Das, 1995

(Lissamphibia, Anura, Rhacophoridae)

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Observations on the natural history of the poorly known, insular rhacophorid species, *Polypedates insularis* Das, 1995 are presented herein for the first time. Courtship, breeding behaviour and calls of this species are described. The morphology of the larvae of *P. insularis* is described in detail across different developmental stages and the structure of their oral apparatus is illustrated. *P. insularis* has a labial tooth row formula 1/4+4//3. Ontogenetic development of the larvae is described. Potential threats to this endangered species are highlighted.

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Introduction

The anuran fauna of the Andaman and Nicobar Islands are one of the least studied among those of South-East Asian regions and our knowledge on biology and natural history of several species still remains scarce. The frog fauna of the Nicobar archipelago has been inventoried in the recent past and has led to the discovery of two new species (Das 1995, 1996a) and a new record (1996b). Although new discoveries have been made, information on the biology and natural history of many of these species are still lacking. Surprisingly, among these, *Polypedates insularis* Das, 1995 happens to be the only representative of the family Rhacophoridae Hoffman, 1932 in the Andaman and Nicobar archipelago, which is normally highly species rich in other regions of insular South East Asia (Borneo, Sumatra, Java, Philippines etc.) (Inger 1999). Das (1995) described *P. insularis* based on a series of eleven specimens collected from Great Nicobar Island. Till date no additional data exists on this species apart from

those presented by Das (1995) upon its description. Current status assessments consider this species to be endangered (Das et al. 2004). Herein, data on adult and larval morphology, natural history and breeding biology of this poorly known species are presented for the first time along with new distribution records.

Materials and methods

Systematic surveys for anurans are being conducted in the Nicobar archipelago since March 2016. The distribution of the target species, *P. insularis*, was mapped based on the geo-coordinates of the regions where the species was detected during the study. Calls of *P. insularis* were recorded using a digital audio recorder and analysed with the software packages Adobe Soundbooth and Audition. Breeding and courtship activities of the target species were observed opportunistically. Nests encountered during the surveys were monitored till the larvae emerged and started metamorphosis. Larvae of early stages observed in water tanks were monitored for observing growth pattern and development. They were

observed to feed on the organic debris at the bottom of the tanks. Single larvae of a specific stage were scooped out into transparent containers and photographed from various angles with a graduated scale placed nearby. After photography, the tadpole was released back into the same source. Measurements of the live tadpoles were made from these photographs using Adobe Photoshop CS3. Three adults and six dead tadpoles of *P. insularis*, belonging to stages between 25 and 43 were collected from dried up water tanks and preserved in ethanol, which are deposited in the collections of the Department of Ocean Studies and Marine Biology, Pondicherry University, Port Blair, under the voucher numbers DOSMB 05008, DOSMB 05009, DOSMB 05021 and DOSMB 05053. They were measured with digital calipers and were examined under a stereo microscope for examination of the oral apparatus.

Abbreviations used for the measurements are: HBL, head-body length; HBW, head-body width; HBD, head-body depth; TAL, tail length; ED, eye diameter; EN, eye-nostril distance; ES, distance from eye to snout tip; IO, inter orbital distance; IN, inter narial distance; ODW, oral disc width; FEL, femur length; TBL, tibia length and FOL, foot length.

Tadpoles were staged according to Gosner's (1960) scheme. Representation of the labial tooth row formula follows Rödel (2000).

Results

During the present study, *Polypedates insularis* was found to be fairly common in all of the study sites (see below) in evergreen forests, plantations and among vegetation near human habitation. Breeding and courtship activities observed in *P. insularis* were found to coincide with both monsoons (late May to September) as well as sporadic, aseasonal rainfall between the months of January and March. Several males (about 3 to 11) of this species were observed to assemble near sources of water such as stagnant rain-water in abandoned barrels (18 occasions), cemented water tanks constructed near seasonal streams inside forests (3 occasions) as well as plastic water tanks in human habitation kept for storage (6 occasions) and to call for attracting females. Perching height of these frogs ranged from 0 (ground level) to about 2.5 m above the ground.

The call described here was recorded in Dec. 2016 at night time (ca. 20:30 h) from near Govind Nagar area at an ambient temperature of 27° C and humidity of 93 %. Calls of this species are composed of a series of interrupted syllables of *trk...trk...trk...* followed by a series of chuckles. The call series recorded and described here lasted for a duration of 49 s. Overall, the calls are of a very low pitch. Dominant frequency of the call was at 2 kHz. The sections of

the call corresponding to grunts (Fig. 1A–B) last for a mean duration of 0.28 ± 0.09 s (range: 0.22–0.45 s; $n=5$) with a mean interval between two consecutive syllables of 4.83 ± 1.89 s (range: 3.5–7 s; $n=3$) and range in amplitude between –25 to –27 dB; while those sections corresponding to chuckles (Fig. 1C–D) range in amplitude between –18 to –24 dB and last for a mean duration of 0.1 ± 0.02 s (range: 0.09–0.13; $n=6$) with a mean interval of 2.05 s (range: 0.22–3.8; $n=6$) between two consecutive syllables. Potential nocturnal ophidian predators of *P. insularis* such as *Boiga wallachi* Das, 1998 ($n=6$) and *Xenochrophis trianguligerus* (Boie, 1827) ($n=2$) were observed on several occasions close to its breeding grounds.

Morphologically, males (mean snout-vent length: 41.95 mm \pm 2.26; range: 39.62–43.28; $n=11$) are normally smaller than females (mean snout-vent length: 70.26 mm \pm 2.77; range: 67.43–73.88; $n=7$). Both males and females sometime lack the characteristic hourglass marking on the back. Males lack an evident external vocal sac and have a thick, white coloured fleshy nuptial pad on the dorsal surface of the first finger which aids in amplexus (Fig. 2). Amplexus in this species is axillary and happens at night time wherein the male mounts on a female usually near a lentic source of water (Fig. 3A). It may last for about an hour by the end of which a creamy white foam nest is made by the female, inside which the eggs are laid. The foam nest made by the female is normally a hemispherical structure measuring about 5–6 cm in diameter. Eggs are yolky and unpigmented, plain white in colour (Fig. 3B). The development of tadpoles may last for a few weeks on average to about a month.

Description of larval stages

Stage 25. Early stage larvae measure about 18–20 mm in total length; tail longer than head-body (mean HBL:TAL=0.69); head-body ovoid; relatively narrow and longer than broad (HBL:HBW=1.83). Head-body flattened; broader than high (HBW:HBD=1.24). Oral disc situated antero-ventrally, 1.2–1.6 mm wide; mouthparts typical of the ‘clasp-ing type’ illustrated by Altig & Johnston (1989). Eyes small (mean ED=0.7); situated laterally; nostrils situated mid-way between eyes and snout tip (EN:ES=0.47); inter-orbital space wider than inter-narial space (IO:IN=1.36). Labial tooth row formula 1/4+4//3. Spiracle sinistral; vent tube dextral in orientation. Overall colouration light brown dorsally and ventrally; caudal musculature poorly developed. Larvae of *P. insularis* belong to the feeding type 1 (bottom suspension feeders) of Inger's (1986) classification scheme.

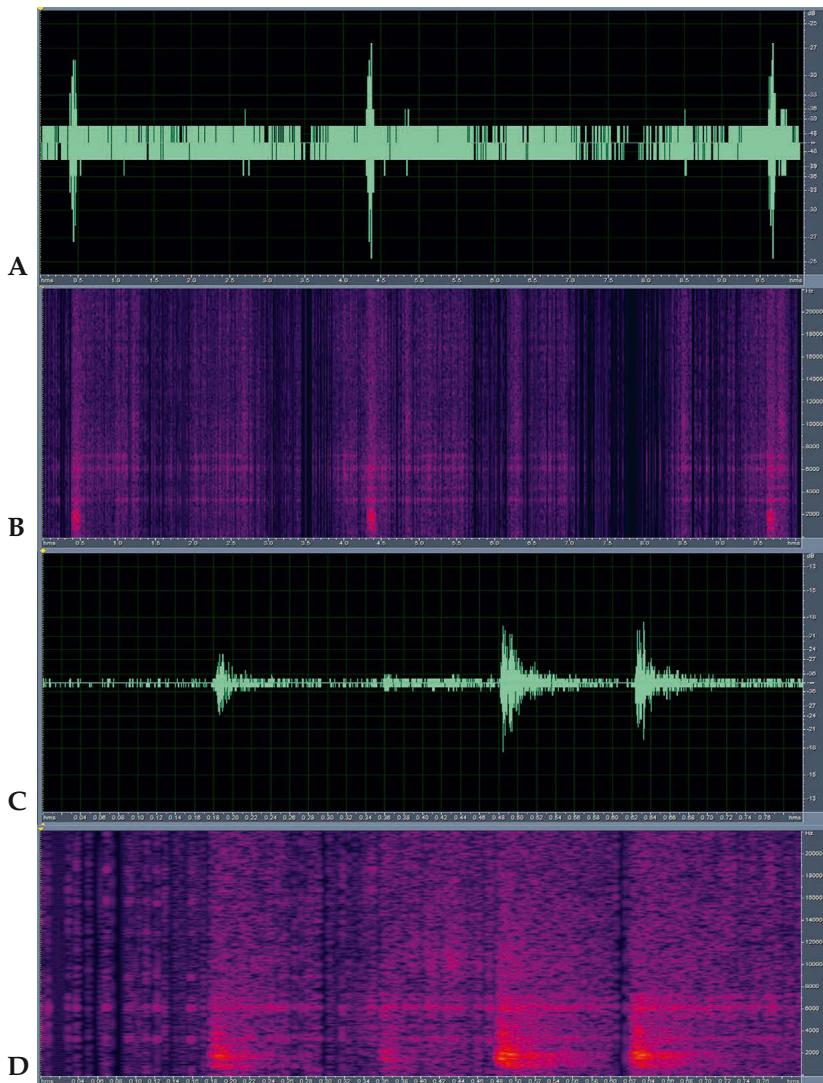


Fig. 1. Spectrogram (A, C) and oscillogram (B, D) of the call of *Polypedates insularis*.

Stage 26 (Fig. 3C). Tadpoles of this stage are a little longer than those of the earlier stage and measure about 24–28 mm in total length; tail length a little reduced (HBL:TAL=0.61); head-body longer than broad (HBL:HBW=1.82); depressed; broader than high (HBW:HBD=1.57). Oral disc a little wider than in the earlier stage, about 1.97 mm. Labial tooth row formula $1/4+4//3$. Eyes lateral in position; a little larger than in the earlier stage (mean ED=0.98); nostrils located midway between snout tip and eyes (EN:ES=0.48); inter orbital space wider than inter-narial space (IO:IN=1.23). Head-body golden brown in colour; tail transparent.

Stage 30 (Fig. 4A–D). At this stage, the tadpoles attain a still greater length, reaching about 30–34 mm; tail reduced in length further (HBL:TAL=0.48); head-body a little more broader than in the previous stage but still longer than broad (HBL:HBW=1.63); a little more broader than high than in the previous stage (HBW:HBD=1.74). Oral disc reaches its maximum width (mean ODW=3.21 mm) and is emarginated; marginal papillae located between anterior and posterior labia; keratodont more developed at this stage than in the earlier stages; labial tooth row formula $1/4+4//3$. Upper and lower jaw sheaths well developed. Eyes larger (mean diameter



Fig. 2. Photographs of *P. insularis*: **A.** an adult male; **B,E.** an adult female and male with the hourglass marking on the back; **C.** nuptial pad on dorsal surface of first finger; **D,F.** an adult male and female without the hourglass marking.



Fig. 3. A. A pair of *P. insularis* in amplexus; B. foam nest of *P. insularis*; C. stage 26 larva; D. stage 30 larva; E. stage 37 larva; F. stage 40 larva and G. stage 43 larva of *P. insularis*.

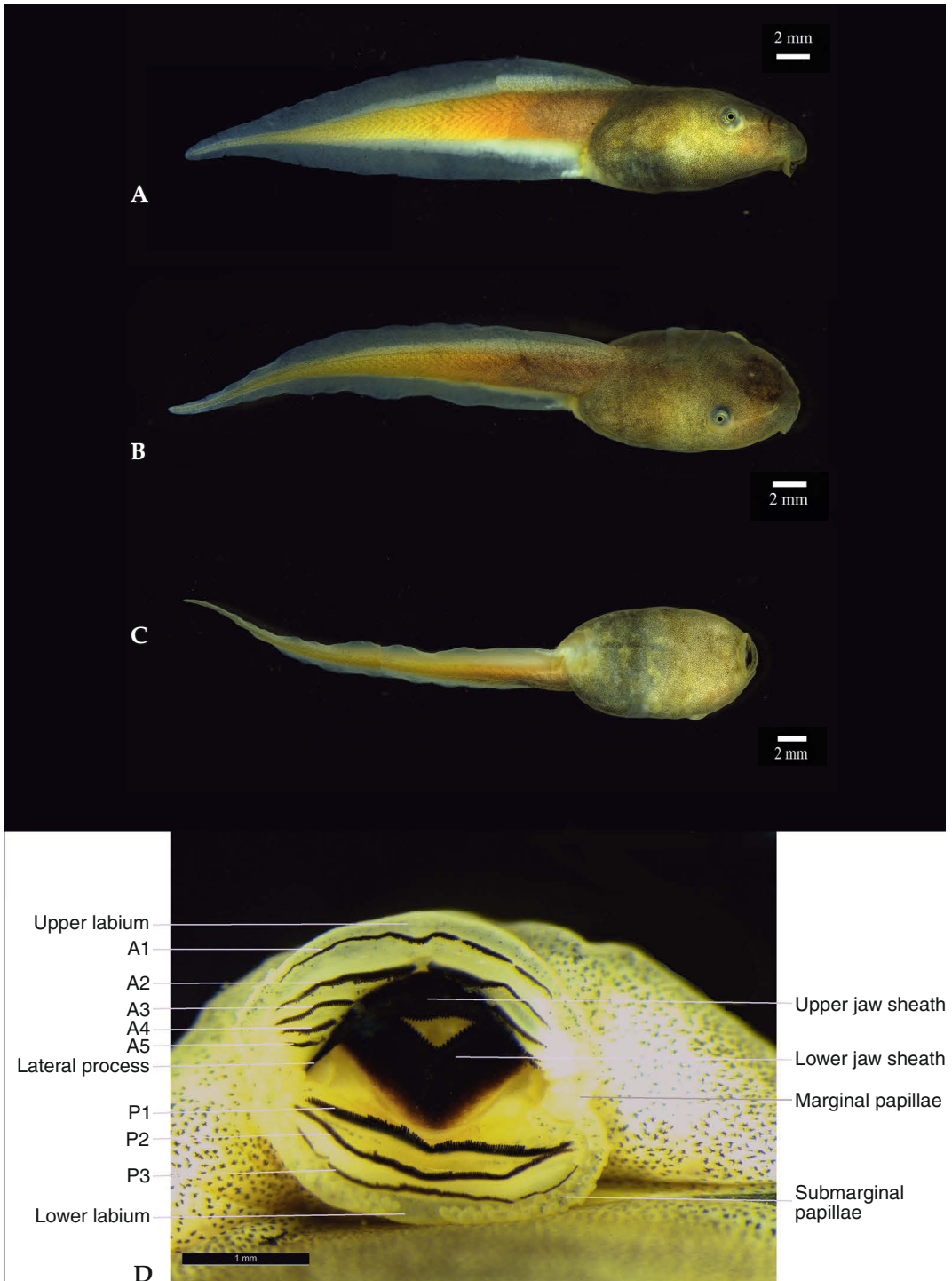


Fig. 4. A. Lateral, B. dorsal and C. ventral views of a stage 30 larva; D. oral disc of a stage 30 larva of *P. insularis*.

=1.49 mm); nostrils a little closer to snout tip than to eyes (EN:ES=0.62); inter orbital space about one and a half times the inter-narial space (IO:IN=1.5). Pigmentation less intense on the tail; body with numerous, minute melanopores.

Stage 37 (Fig. 3E). Upon progressing with metamorphosis, the larvae attaining stage 37 develop minute hind-limbs buds. At this stage the tadpole is about 36 mm long with shorter tail (HBL:TAL=0.6). Head-body attains a greater width (HBL:HBW=2.16) and depth (HBW:HBD=1.52) than in the previous stage; oral disc relatively narrower than in the earlier stage (ODW=2.7 mm). Labial tooth row formula $1/4+4//3$. Nostrils located nearly midway between eyes and snout tip (EN:ES=0.52). Inter orbital space a little less than twice the inter-narial distance (IO:IN=1.87). Thigh longer than tibia (FEL:TBL=1.12) and feet (FOL:FEL=0.63). Dorsal colouration creamy yellow with numerous minute dark spots on the dorsum; abdominal region greyish and posterior part of the tail transparent.

Stage 40 (Fig. 3F). At stage 40, the tadpole develops robust hind-limbs with well differentiated digits but still lack even rudiments of forelimbs. Head-body ovoid (HBL:HBW=2.4) with a pointed posterior end; measuring about 30 mm in length; broader than deep (HBW:HBD=1.53); tail longer than head-body (HBL:TAL=0.69). Nostrils closer to snout tip than to eyes (EN:ES=0.67); inter-orbital space wider than inter-narial space (IO:IN=1.67). Keratodont disappears at this stage and the development of mouth gets initiated. Thigh as long as tibia (FEL:TBL=1.02) and longer than feet (FOL:FEL=0.64). Dorsal pigmentation more intense than in the previous stage; overall colouration dark brown dorsally; hindlimbs creamy white in colour with dark brown transverse bars. Proximal end of the tail with dark brown pigmentation; translucent distally.

Stage 43 (Fig. 3G). At this stage, the tadpole starts decreasing in length; with the tail becoming shorter than in the previous stage (HBL:TAL=1.34). Anterior end of head-body more pointed than in the earlier stage; nostrils located towards the snout tip; inter-orbital space much wider than the distance between nares (IO:IN=2.99). Posterior part of the body streamlined. Forelimbs well developed with well differentiated digits, bearing discs at the terminus. Caudal fin length and height more reduced than in the previous stage. Thigh shorter than tibia (FEL:TBL=0.83) and longer than the feet (FOL:FEL=0.72). Pigmentation more intense on the body; fore-body light brown with darker irregular pattern; forearms and hind-limbs with thin, brown barred pattern. Posterior part of the tail translucent.

Distribution

Polypedates insularis was documented during ongoing surveys in the Nicobar archipelago from the following sites: Campbell Bay (7.018°N, 93.9233°E, 12 m a.s.l.), Govind Nagar (7.0022°N, 93.881°E, 83 m a.s.l.), Gandhi Nagar (6.8273°N, 93.8923°E, 9 m a.s.l.), Shastri Nagar (6.8081°N, 93.8850°E, 0 m a.s.l.), Galathea (6.8205°N, 93.8643°E, 0 m a.s.l.), Navy Dera (7.1353°N, 93.8838°E, 0 m a.s.l.) and Afra Bay (7.1839°N, 93.7369°E, 24 m a.s.l.) in Great Nicobar Island and Makachua (7.4035°N, 93.7133°E, 37 m a.s.l.) and Pulo Ulon (7.2914°N, 93.6857°E, 19 m a.s.l.) in Little Nicobar Island.

Discussion

The larvae of *P. insularis*, the only rhacophorid of the Nicobar Islands, can easily be identified in the field and can be differentiated from those of other sympatric frogs as follows; dorsal body sparsely pigmented (vs. densely pigmented in *Duttaphrynus* and ranids); oral disc positioned antero-ventrally (vs. upturned in *Microhyla* cf. *heymonsi* Vogt, 1911, lacking keratinized mouthparts); eyes positioned laterally (vs. dorsally in *Limnonectes* spp.); labial tooth row formula of $1/4+4//3$ (vs. $1/3+3//1+1/2$ in *Chalcorana* cf. *chalconota* (Schlegel, 1837); $1//1+1/1$ in *Hylarana erythraea* (Schlegel, 1837) and $1//1+1/2$ in *Annirana nicobariensis* (Stoliczka, 1870)) (Malkmus et al. 2002, Bourtamuli et al. 2010). Data on natural history and distribution of the endemic rhacophorid *P. insularis* were limited only to the brief notes provided by Das (1995) in its original description till now. The novel data on its breeding biology, larval development and geographic distribution fills in a vast gap in our knowledge about *P. insularis*. Its occurrence in two different sites within the island of Little Nicobar is noteworthy. However, surveys in the central and northern group of the Nicobar Islands did not record this species, indicating its absence in those regions. Although locally abundant, this species has a very narrow distribution range. A major threat to this species is the lack of prolonged availability of sufficient fresh water sources, which leads to desiccation of nesting sites and consequently, mortality of tadpoles before maturity and recruitment into the breeding population.

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