Realities of rarity: climatically and ecologically restricted, critically endangered Kandian Torrent Toads (*Adenomus kandianus*) breed *en masse*

Madhava Meegaskumbura, Nayana Wijayathilaka, Nirodha Abayalath, Gayani Senevirathne

Endemic to Sri Lanka, genus *Adenomus* contains two torrent-associated toad species whose ecology and natural history in the wild is virtually unknown. *Adenomus kelaartii* is relatively common, with a wide geographic distribution. Its sister species, *A. kandianus*, however, is restricted to two isolated populations in fast-disappearing montane and sub-montane forests. Formally declared extinct after not being recorded for over a century, a few *A. kandianus* were rediscovered in 2012 and redescribed as "the world's rarest toad". Here we report the results of a two-year study of the occurrence, habits and habitat associations of adult and larval *A. kandianus* using both general surveys and quadrat sampling. We show this to be a secretive species with a patchy distribution. Non-breeding female toads dwell in primary-forest habitats, but after heavy and sudden downpours they form large mating congregations in large streams. Amplexed pairs swim synchronously, enabling them to traverse fast currents. Egg-laying sites remain unknown, but the ability to dive and vocalize underwater, and characteristics of the eggs, suggest that they lay eggs in dark recesses of the stream. Tadpoles show microhabitat partitioning within the stream, with the greatest diversity of stages in slow-flowing rocky areas. The more robust stages possessing sucker discs exploit rocky-rapids, while metamorphic stages inhabit stream margins. We use DNA-barcoding to show the existence of two disparate toad populations. Distribution modeling with forest-cover layers added, predict a very small remaining area of suitable habitats. Conservation of this climatically and ecologically restricted species hinge largely on the preservation of high-elevation primary and riparian forests and unpolluted torrents.

1   **Realities of rarity: climatically and ecologically restricted, critically endangered Kandian**

2   **Torrent Toads (*Adenomus kandianus*) breed *en masse***

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Abstract

Endemic to Sri Lanka, genus *Adenomus* contains two torrent-associated toad species whose ecology and natural history in the wild is virtually unknown. *Adenomus kelaartii* is relatively common, with a wide geographic distribution. Its sister species, *A. kandianus*, however, is restricted to two isolated populations in fast-disappearing montane and sub-montane forests. Formally declared extinct after not being recorded for over a century, a few *A. kandianus* were rediscovered in 2012 and redescribed as "the world's rarest toad". Here we report the results of a two-year study of the occurrence, habits and habitat associations of adult and larval *A. kandianus* using both general surveys and quadrat sampling. We show this to be a secretive species with a patchy distribution. Non-breeding female toads dwell in primary-forest habitats, but after heavy and sudden downpours they form large mating congregations in large streams. Amplexed pairs swim synchronously, enabling them to traverse fast currents. Egg-laying sites remain unknown, but the ability to dive and vocalize underwater, and characteristics of the eggs, suggest that they lay eggs in dark recesses of the stream. Tadpoles show microhabitat partitioning within the stream, with the greatest diversity of stages in slow-flowing rocky areas. The more robust stages possessing sucker discs exploit rocky-rapids, while metamorphic stages inhabit stream margins. We use DNA-barcoding to show the existence of two disparate toad populations. Distribution modeling with forest-cover layers added, predict a very small remaining area of suitable habitats. Conservation of this climatically and ecologically restricted species hinge largely on the preservation of high-elevation primary and riparian forests and unpolluted torrents.

Introduction

30

31 The Sri Lankan torrent toads of the genus *Adenomus* are known from two species, *A. kelaartii* and *A. kandianus*. The

32 former is relatively well known from several populations, the latter has had a checkered history, having been known,

33 from the time of its original description in 1872, from only its type specimens. Although the species was finally

34 declared extinct in 2008 (Stuart et al. 2008), it turned out that it had been rediscovered near Adam's Peak by

35 Manamendra-Arachchi and Pethiyagoda (1998) and wrongly described as a new species, *A. dasi*, distinguished from

36 the type specimens of *A. kandianus* by the pattern of dorsal tuberculation and the extent of pedal webbing. This error

37 was finally pointed out by Meegaskumbura et al. (2015) in a study that involved a morphological, morphometric,

38 genetic, bioacoustic, osteological and ecological analyses of both metamorphs and larvae of *A. kelaartii*, *A.*

39 *kandianus* and '*A. dasi*'.

40   Meanwhile falling into the same error as Manamendra-Arachchi and Pethiyagoda (1998), Wickramasinghe et

41 al. (2012) had announced the rediscovery of *A. kandianus* from the same locality as '*A. dasi*' and heralded it as

42 "world's rarest toad" on the brink of extinction. Subsequently, Gabadage et al. (2014) reported *A. kandianus* (fully-

43 webbed) and '*A. dasi*' (partially webbed) from a locality close to Nuwara-Eliya, about 20 km from the Adam's Peak

44 population.

45   *Adenomus kandianus* nevertheless remains a rare, critically endangered species, restricted to a handful of

46 montane and submontane forest patches.

47   Discovered only recently (Meegaskumbura et al. 2015), the tadpoles of *A. kandianus* show distinct

48 adaptations for torrent life, most prominently a ventral suctorial disc, highlighting them as among the very few

49 gastromyzophorous tadpoles currently known. Sucker discs and ventrally-oriented mouths enable them to feed on

50 aufwuchs (surface films constituting of algae and other minute organisms growing on submerged substrates) while

51 being attached to submerged substrates in torrents (Meegaskumbura et al. 2015). Nothing, however, is known about

52 stream-microhabitat partitioning of different life-history stages of these larvae, knowledge that is important for

53 focused conservation efforts.

54   The abundance of tadpoles and the rarity of adults prompts several questions about both the breeding patterns

55 of the mature toads and the fate of larvae following metamorphosis. The abundance of larvae in streams can be

56 explained either by a few highly fecund mating pairs laying large numbers of eggs or by the reproducing population

57 being much greater than presently estimated. If metamorphs are abundant, where are they? When, where and how do

58   they breed? Generation of knowledge such knowledge would provide insights as to why this remarkable toad

59   remained lost for well over a century.

60   Here we show that *Adenomus kandianus* is not in fact as rare as hitherto feared, once its habitats and habitat-

61   occupation patterns are understood. These toads occupy undisturbed primary forests and riparian habitats bordering

62   unpolluted streams, but are specialized, secretive and nocturnal. Following surveys and natural-history observations

63   of both adults and larvae over a period of more than two years we report synchronous mating congregations, discuss

64   their natural history, and point out knowledge gaps.

65

Material and methods

67   Fieldwork was carried out under the research permission (permit # WL/3/2/13/13) provided by The Department of

68   Wildlife Conservation of Sri Lanka. This study has been cleared by the Ethical Clearance Committee, Postgraduate

69   Institute of Science, University of Peradeniya at its 18th meeting held on 19th May 2015.

70

Sampling adult toads

72   Over a period of about two years, we carried out a survey of *Adenomus kandianus* adults and tadpoles (14–16

73   May 2012; 28–31 March 2013; 25–27 July 2013; 20–21 September 2013; 29 July–2 August 2014). We studied their

74   patterns of occurrence, habitat associations, resource partitioning, microhabitat utilization, and natural history.

75   General surveys along a 4.5 km long trail, across an altitudinal (1300–2000 m) and habitat gradient (streams

76   and riparian habitats, tea plantations, montane and submontane forests, grasslands, bamboo forests, isolated rock-

77   pools and ponds) representative of the major habitat types in the Peak Wilderness (Adam's Peak) Strict Nature

78   Reserve, were carried out (Fig. S1). The five predominantly nocturnal surveys were conducted between 1600–0400

79   h; the habitat types, microhabitats and elevations at which toads were found were noted.

80

Sampling tadpoles

82   During daytime (1000–1400 h), six stations along a large mountain stream were also sampled for larval stages and

83   mating occurrences (Fig. S1). After the presence of tadpoles was established, 1×1 m quadrats ($N = 36$),

84   representative of six microhabitat types in the stream, based on flow conditions (surface flow rates above 0.2 m/s

85   were considered rapids, determined using the distance a styrofoam float travelled in 5s), substrate type and/or stream

86   edge associations (slow-rocky, slow-silty, slow-sandy, rapid-rocky, stream-edge, shore-litter, shore-sandy), were

87   sampled for various life-history stages in the 3 periods 14–16 May 2012, 28–31 March 2013 and 29 July–2 August

88   2014. Six stations (each of 50 m) along the stream, at least 50 m apart from each other, were scrutinized during each

89   of these visit. Within each 1×1 m quadrat tadpoles were counted, collected (a few tadpoles escaped, especially in the

90   rapids, where net-handling was awkward), staged according to Gosner (1960) and released. The depth at the middle

91   of the quadrat was approximated using a 2 m long probe marked every 0.25 m. Deeper areas (1–2 m) were

92   investigated by snorkeling.

93

94   **Mating observations**

95   When a breeding congregation was unexpectedly observed during the last visit (29 July–2 August 2014), initially at

96   tadpole sampling station II, all six tadpole-sampling stations along the stream were scrutinized for breeding activity.

97   Due to the patchy distribution of the congregations within each station, a non-random 5×5 m plot was demarcated

98   and surveyed, focusing on the numbers of amplexed pairs and free males and females. The characteristics of the

99   mating sites were noted (water depth, substrate and current speed) and contrasted with surrounding areas to identify

100   the stream refuges these toads use as congregation points.

101   Initial nocturnal observations were carried out by two team members (MM and GS) for 4 hours. A search for

102   ovipositing pairs or deposited eggs, which also included snorkeling, began in the morning at 0600 h. Underwater

103   behavior was recorded using a GoPro camera set in its watertight casing. Surface videos and photographs were made

104   using a Canon 60D camera and a Canon 100 mm macro lens.

105   Water temperature, pH and conductivity at the four mating sites were measured directly using an Extech

106   DO700 meter. Water samples from these sites were measured for total hardness, nitrites, nitrates, ammonia and

107   sulphates using a YSI 9500 direct-reading photometer.

108

109   **DNA barcoding to identify the new population**

110   To confirm the species identification of the newly discovered *Adenomus* populations (Gabadage et al. 2014), two

111   tiny toe clips from each morphospecies (mature males) were obtained, preserved in absolute ethanol, and stored at –

112   20° C. DNA was extracted using Qiagen tissue-extraction kits, following manufacture protocols. A fragment of ca

113   600 bp of 16S rRNA was PCR-amplified (Palumbi 1996). Sequencing was done using an ABI 3730 automated DNA

114   sequencer (Applied Biosystems). Sequences were assembled in 4peaks v.1.7.1. Subsequently, the sequences of the

115   new population were compared against a dataset consisting of *Adenomus kanidanus* and *A. kelaartii* obtained from

116   Meegaskumbura et al. (2015). Sequences were aligned using ClustalW as implemented in MEGA 5.0 (Tamura et al.

117   2011). Uncorrected pairwise distances were calculated using PAUP* 4.0b10 (Swofford, 2002). Newly generated

118   sequences are deposited in GenBank under accession numbers xxxxxx, xxxxxx <upon acceptance>.

119

120   **Niche Models**

121   Based on field records from this study and published literature (Gabadage et al. 2014; Karunarathna et al. 2012;

122   Wickramasinghe et al. 2012), seven presence locations from two regions (Table S1) were used to produce the

123   predicted distribution map of *A. kandianus* using MaxEnt version 3.3.3k (Phillips et al. 2004). Bioclimatic layers (N

124   = 19) and an altitude layer with a 0.0083° (~1 km^2) resolution downloaded from WorldClim database

125   (http://www.worldclim.org) were clipped to our study region bounded by 5°908′ N to 9°842′ N and 79°516′ E to

126   81°891′ E (i.e. Sri Lanka) prior to being used to build the predicted distribution map. The following settings were

127   used in MaxEnt: automatic mode with jackknife validation, random seed with 25 random test percentage and ten

128   replicates using bootstrap function. The average model was selected and minimum presence threshold (46%) was

129   applied to demarcate presence and absence. As the toad is a forest-associated species, requiring canopy cover, the

130   predicted range was further refined using the tree cover layer downloaded from the Google Earth engine database

131   (Hansen et al. 2013). Using a uniform grid with a grid size of 2×2 km, area of occupancy (AOO) and extent of

132   occurrence (EOO), in the context of IUCN Red Listing criteria (IUCN, 2001) were estimated using ArcGIS version

133   10.2.

134

Results

Adult toad sampling

Non-mating adult toads were found in undisturbed and unpolluted habitats; males were found in riparian areas bordering large streams and females mainly in primary-forest habitats during non-mating periods. In primary forest habitats, females were found in two large but scattered groups numbering 8 and 15 individuals (within an area of 50–100 m), 400 and 650 m away from the nearest stream. Male toads were never observed so deep within the primary forest. In riparian habitats, males predominated females (12 – males; 2 – females). Neither males nor females were observed in tea plantations, grasslands, or bamboo forest habitats; they were also never recorded in isolated rock pools or ponds.

Female toads were observed to emerge from under rock crevices ($N = 2$) and spaces under large roots ($N = 2$), between 1900–1930 h within the primary-forest habitat. On both occasions females were observed, it was drizzling. Males emerged from earth-crevices ($N = 3$) and root formations ($N = 1$) on the steep banks of riparian habitat between 1900–1930 h. Neither females nor males were ever observed on vegetation.

Mating toads

On 30 July 2013, after a burst of heavy rain lasting about four hours (1330–1730 h), which swelled the water level of the stream by about 0.5 m, the first breeding pair was observed at 2030 h near tadpole-sampling station II. They were diving just below the surface in a slow-flowing area of the stream. At this instance, all efforts were directed at documenting (photographing and video recording) this rare occurrence. Soon, two more amplexed pairs were observed, following which a further 12 within an hour of the first observation. There were 6 non-amplexed males and 3 non-amplexed females, also in the stream (8 person-hours).

Many of these amplexed pairs were swimming either to or from a large (15×10 m) 4–5 m deep pool in the stream. Amplexed-pairs diving towards the deeper regions of the large pool were clearly observed. The pairs at the shallow end (0.25–1 m) were resting on the sandy bottom.

The search in the morning by the rest of the team (24 person-hours) helped locate many more breeding pairs (Fig. 1; Video S1). Between 0600–1000 h, a total of 95 breeding pairs were counted from sampling station II; some were returning from the deeper pool while others were diving towards it. Females ($N = 17$) without males, but

163   showing amplexus-scars, were observed coming towards the shore from the deeper (12 m) region. Pairs in amplexus

164   (N = 22) were also observed, departing to the deeper waters.

165   Eggs were never observed in the stream, despite five days of scrutiny. The bottoms of deeper pools could not

166   be investigated due to the lack of underwater lights and diving gear.

167   Underwater video (using surface lights) of amplexed pairs clearly showed synchronized diving by the male

168   and the female (Video S1). Swimming strokes by the male caused an inverted V-shaped scar mark on the backs of

169   many females after a few hours in amplexus.

170   Initial observations during daytime at tadpole station II showed the largest congregation of amplexed pairs,

171   together with many unamplexed males and females. The deepest regions of the stream occur in curved areas of the

172   stream through which the main current flows; the breeding congregation was found in the area opposite this, a

173   region shadowed from the main current. These areas always had a sandy substrate, at a depth of 0.25–1.5 m. There

174   was also a large submerged log, providing further protection from the current (Video S1).

175   Mating congregations were also observed in three other tadpole-sampling stations: station III had a 44-pair

176   congregation; this had a deep pool and a shallow area similar to that of sampling station II. Sampling stations I and

177   IV had fewer pairs (3 and 8, respectively), both had the deeper area towards the middle of the stream and were not

178   placed in bends in the stream. Two of the tadpole-sampling stations did not contain any mating pairs, but several

179   individuals of both sexes were observed.

180   Two female toads were found dead in the evening. Dissection showed 180 and 200 pale grey eggs in each

181   specimen, both ovaries were mature in both. Attempts to disturb amplexed pairs resting on the sandy bottom

182   (underwater) by satellite males were restricted to brief pouncing on the pairs; the amplexed males always warded off

183   the invading male by frantic kicking (N = 5).

184   Water from the four sites from which mating pairs were observed showed the following water-quality

185   parameters: pH 6.5; conductivity 9.67–10.17 μS; total hardness of 5 mg/L $CaCO_3$; and extremely low levels of

186   nitrites (10–17 μ/L; absence of ammonia; low levels of nitrates (0.348–0.510 mg/L); and absence of sulphates. The

187   temperature of the water at each site was 16.2 °C (at 1100 h).

188

189   **Tadpole sampling**

190   A total of 97 tadpoles were observed in the 1×1 m plots sampled from the 7 stream microhabitats from the six

191   tadpole-sampling stations during three visits. Slow-rocky ($N = 40$) and rapid-rocky ($N = 28$) stream habitats

192   harbored the highest number of individuals; slow-silty ($N = 1$), slow-sandy ($N = 3$), stream edge ($N = 7$), stream

193   shore-sandy ($N = 3$) and stream shore-litter ($N = 15$). The distribution of larval stages showed several patterns: initial

194   postembryonic stages (stage 25,26) were found mostly in slow-rocky habitats, in an area with a water depth of

195   between 0.5–1.5 m; only the larger-bodied, robust swimming morphs were found in the rocky-rapids (0.25–0.75 m);

196   the metamorphic stages (stage 44, 45) were found in calmer and shallower (less than 0.25 m deep) water closer to

197   the stream edge. The greatest diversity of stages was found in slow-rocky areas that were less than 1.5 m in depth

198   (Fig. 2); the slow-silty and slow-sandy (deeper than 0.25 m) areas had the least abundance and diversity of stages.

199   Cursory observations of many of the small shallow streams, rock pools and puddles in this area did not show

200   any tadpoles of these toads.

201

202   **Other natural history observations**

203   Males call above water and also underwater, a soft call, but very well heard underwater. These calls were mostly

204   heard when snorkeling, closer to the congregations of pairs in the slow sandy area. Amplexed males disturbed by

205   satellite males always called loudly.

206

207   **DNA barcoding**

208   Uncorrected pairwise distances between the recently discovered population of *Adenomus* from Pedro was confirmed

209   as *A. kandianus* with pairwise percentage distance range of 0.1−0.7% for the 16S rRNA gene fragment (Table S2).

210

211   **Niche modeling**

212   The AUC value (ranging between 1 and 0), which is commonly used to determine the predictive accuracy of the

213   model was 0.997. Altitude, precipitation in coldest quarter and mean temperature of driest quarter were the three

214   variables that contributed mostly to the prediction.

215   Based on the MaxEnt models, the predicted climatic niche of the species within the island is 652 km^2

216   distributed on the upper reaches of the Central Hills (Fig. 3). Since the tree cover percentage in all presence records

217 for the species were above 89%, refined distribution within forest habitats was calculated as 380 km^2 (i.e. the

218 predicted distribution where the tree cover was above 89%). AOO and EOO were 20 km^2 and 128 km^2 respectively.

219

Discussion

221 Many larger toads are tolerant of various environmental stressors and have expansive distributions (Van Bocxlaer et

222 al. 2009). *Adenomus kandianus*, however, are habitat specialists, both as adults and as larvae, exclusively occupying

223 primary forests, riparian and suitable lotic habitats. Hence they are not only torrent toads, as their common name

224 implies, but also forest toads that travel far from streams, for poorly understood reasons.

225   Adult toads were only observed in riverine habitats and primary tropical montane cloud-forest habitats, which

226 suggests they are habitat and climate specialists. They were not observed in grasslands, tea plantations or scrubland

227 situated at a similar elevation, suggesting that these toads require an undisturbed forest canopy that preserves

228 humidity and maintains a lower temperature, factors that reduce desiccation.

229   Only the larger-bodied female toads, however, show this primary-forest affinity, as much as 650 m away

230 from the breeding streams, while male toads occupy primarily a riparian habitat within dense forest cover. This

231 pattern suggests sex-specific resource allocation, seen also in many other toad species (Schmidt 2012). Often,

232 females occupy a different habitat from that of males to prevent premature amplexus, to reduce stress and to avoid

233 competition for food. This suggests that, larger-bodied females (Meegaskumbura et al. 2015) probably wander far

234 from the breeding streams foraging to build up energy reserves to produce their substantial clutches of eggs and to

235 undergo the strenuous torrent diving activity, while the smaller-bodied males live in forested riparian habitats,

236 awaiting a chance to mate.

237   Since females hide in rock and root crevices in primary forest habitats during daytime, diurnal encounter-

238 surveys may not be successful in observing them; however, quadrat sampling and focusing on spaces beneath

239 objects such as stones and logs may be useful to locate them. Males also hide among crevices in stream banks during

240 daytime, underlining that these toads are predominantly nocturnal and secretive. This, together with their patchy

241 distributions, may explain why they are observed in such low numbers.

242   High tadpole abundance almost throughout the year suggests a thriving population of toads that mate

243 profusely. It shows that these toads are breeding, and the conditions necessary for population viability is currently

244 present. On two occasions (sampling periods), tadpoles of two strikingly different developmental stages were

245 observed. This again suggests multiple breeding events during a breeding season.

246   On one occasion (20–21 September 2013), tadpoles were not observed. This was a rainy period and the entire

247 stream was unusually swollen and torrential, with water levels about 2 m higher than at times tadpoles were

248 observed. We may not have observed the tadpoles on this occasion as they may have been swept downstream, since

249 stream characteristics can change dramatically with changes in flow volume (e.g. Ficklin et al. 2013; Yeh et al.

250 2015). They may also have hidden in calm water refuges or may not have bred at the full progression of the

251 monsoon.

252   These toads are not explosive breeders: the two naturally-dead females contained only 180−200 eggs each.

253 Therefore, many toads would need to mate to give rise to the large number of tadpoles, as was observed in the

254 present study. Hence the large breeding congregations of moderately fecund females would explain the prevalence

255 of tadpoles in streams.

256   The water quality parameters at the breeding site indicate a dependence on unpolluted conditions for

257 breeding. The low nitrate, nitrite and phosphate concentrations indicate that there is little or no eutrophication or

258 runoff of nutrients from tea fields to impact stream chemistry. Given that the headwaters of the stream originate

259 within the cloud forest and our sampling stations were located at the upper boundaries of the tea fields, this is to be

260 expected.

261   The sites of egg deposition remain unknown. Nevertheless, based on several observations, we presume that

262 these toads oviposit at the bottom of deep pools (beyond 2 m depth) or the spaces under sunken boulders or rocks.

263 About twenty pairs of toads in amplexus were observed diving into the deeper regions of pools that also contained

264 boulders and a rocky bottom; non-amplexed females that were not egg-heavy were observed swimming from the

265 deep pool towards shallow areas. Next, at two of the sites of the largest congregations, satellite males formed a

266 crescent between the deeper pool and the congregation of amplexed pairs in the shallows, suggesting that they may

267 attempt amplexus by pouncing on already amplexed-males, ready to make the journey into the depths.

268   The egg type can also help predict the oviposition site. The related common species, *A. kelaartii,* has been

269 bred in captivity outside its natural range (Hass et al. 1997). This toad has pale non-pigmented eggs embedded in

270 gelatinous strings. If character-sharing due to common ancestry is assumed, we could infer that *A. kandianus* also

271 lays pale eggs in strings. Pale eggs are found in species that often lay eggs underground or in dark recesses, as

272   penetrating solar radiation can damage the developing embryos (eggs laid in well-lit areas have darker pigmentation;

273   Liht, 2003; Palen et al. 2005). In fact, dissection of the two dead females showed pale eggs within their ovaries, but

274   these observations do not inform whether eggs are laid in strings or not, as gelatinous layers are made within the

275   oviduct, when eggs are being laid; hence the possibility of eggs of these frogs being in strings is present. Strings of

276   pale grey eggs laid in deep, dark water among debris and boulders at the bottoms of pools or under boulders will

277   both be protected from sunlight and from being washed away. We suspect these to be the oviposition sites of *A.*

278   *kandianus*.

279   The other option for an amplexed pair is to lay their pale grey eggs under rocks and litter on stream edges,

280   under the protection of shade. This unusual mode of reproduction seen in at least one megophryid frog (*Leptolalax*

281   *khasiorum*), which also has pale cream-colored eggs (Tron et al. 2015). But this will necessitate these toads, which

282   are specialized for swimming and diving, having to crawl on the edges of the main stream, risking predation.

283   Heavily webbed feet, underwater vocalization ability and synchronous diving of the amplexed pairs suggests that

284   they are well adapted to an aquatic life, hence also aquatic egg laying.

285   Dark-pigmented, stage 25 and above tadpoles were the least developed stage observed in the stream,

286   suggesting that the hatching stage is around 25, and that dark pigmentation develops long after the (pale) eggs are

287   laid. This again suggests that eggs are laid in the dark recesses of streams or its edges. It is noteworthy too, that the

288   oviposition site of *A. kelaartii* too, remains unknown in the wild.

289   Many of the major stream microhabitats are utilized by various life-history stages of these toads. The rocky

290   substrates of the fastest flowing areas are dominated by the larval stages that have a well-developed sucker and a

291   dorsoventrally flattened body that probably enables them to secure themselves onto substrates and also traverse fast

292   currents. Slow-flowing, rocky areas of the stream seem to be important for the early (25, 26) and late developmental

293   stages (stage 42, 43) to feed. Both these stages seem to lack the fast-swimming and clinging abilities needed to

294   exploit rapid-rocky habitats. Slow-sandy areas near the edge are occupied by stages with diminishing larval

295   mouthparts, including the oral sucker, that are close to completing metamorphosis. Tadpoles do not use the deeper

296   sandy areas of the stream, as the sucker would prevent them from burrowing and feeding as do many other sand-

297   inhabiting tadpoles. Moreover, shifting sand is a poor growth substrate for aufwuchs. However, as noted above, the

298   sandy regions between 0.5−1.5 m are important areas for the breeding congregations of adult toads. These seem to

299   act as staging areas to make egg-laying forays into the deeper and darker waters and submerged boulders/rocks.

300   The stream-associated microhabitat utilization and resource partitioning among various developmental stages

301   within the stream are different, which suggests that these toads, despite their abundance, have specialized

302   requirements. Tadpole stages having the ventral sucker disc were found predominantly attached to rocky substrates,

303   feeding on aufwuchs; these surface films cannot grow on constantly moving objects (e.g. sand, detritus). This could

304   be the primary reason for these tadpoles to feed on rocky surfaces. Within the two rocky surfaces available,

305   depending on the flow rate, more tadpoles were observed in slow-flowing water than in rapids. Feeding in the rapids

306   is energetically more expensive, but there could also be more food there owing to reduced competition.

307   Once the ventral sucker disc diminishes with advancement to metamorphosis, the larvae migrate to the

308   shallow areas at the stream's edge, where water flow is slower. Especially in areas that had a layer of detritus,

309   substantial numbers of almost metamorphosed toadlets (stage 46) were observed along the stream edge. This

310   suggests that many of the tadpoles survive up to toadlet stage.

311   Compared to some other toads in which mass mortality is prevalent (Franz et al. 2013; Santos et al. 2007), we

312   witnessed only two females who seem to have died in association with breeding activities; both these bore the

313   swimming scar made by the male during amplexed swimming. However, it is possible that some may have died and

314   been washed away by the current. The scarring of the back may increase the incidence of infection, and females may

315   die several days after mating occurs.

316   Pollution and the opening up of the canopy above streams may have reduced the area available for the

317   survival of these highly specialized (both as tadpoles and as adults) toads. However, we do not know if what we are

318   observing at present are the last few populations of the Kandian Torrent Toad. A single thriving population is

319   insufficient to guarantee the survival of this species even through the next few decades; it has been witnessed limited

320   numbers of thriving populations being wiped out within short periods of time (Corn and Fogleman, 1984). It remains

321   to be established how the other known populations of these toads are faring, especially the recently discovered

322   (Gabadage et al. 2014) population at Pedro.

323   Barcoding of the Pedro population confirms the species identity as *A. kandianus*. The genetic distances

324   (0.1–0.7%) for 16S rRNA suggest within-species level divergences (Vences et al. 2005), highlighting historical gene

325   flow between Peak Wilderness and Pedro populations. This would have been facilitated by the habit of at least adult

326   female movement across large distances under primary forest cover. However, fragmentation of habitats will pose a

327   serious impediment to this movement.

328   Improved niche models that take into consideration all known populations of *A. kandianus* suggest a fairly

329   large area of occupancy (ca. 650 km^2) within the Central Hills (Fig. 3). Though this region was historically forested,

330   most of these natural habitats have since been replaced by tea, vegetable farms and home gardens that are not

331   conducive to the persistence of these toads; the specific habitats (primary forest, riparian or forest-shaded torrent

332   habitats) required are not present in much of this area. Only the region of Pedro and Adam's peak harbor substantial

333   primary and riparian forests and the large streams that are needed in combination to help these toads survive. Habitat

334   barriers separate these two populations from each other. When forest cover is overlaid on the predicted area of the

335   model, only about 380 km^2 appears suitable for the species. The two distantly-placed populations, Pedro and

336   Adam's Peak, provide an extent of occurrence of 128 km^2, but a very small area of occupancy of only 20 km^2.

337   However the predicted distributions suggest a high probability of the existence of another population in the western

338   slopes of the Horton Plains National park (the forested area, having large unpolluted streams, between Pedro and

339   Adam's Peak). Daytime searches for suckered tadpoles in these streams (especially in rocky, slow-flowing areas and

340   rocky rapids) may uncover further populations of this secretive and nocturnal toad.

341   A single stochastic event could destroy any of these two existing populations. At breeding time, given their

342   propensity to congregate in suitable streams, even a short-term threat can wipe out a significant portion of the

343   population, driving the population into an irreversible extinction vortex.

344   We show that *Adenomas kandianus*, a species that was once thought to be extinct and since considered

345   extremely rare (the "world's rarest toad"), is in fact not so rare when it comes to mating. With the accumulation of

346   survey data and natural history observations of both adults and larvae over a period of more than two years, we

347   report several large synchronous-mating congregations of the species. Metamorphs, however, are nocturnal and

348   secretive during non-breeding periods. We suggest that these toads are climate and habitat specialists both as adults

349   and as larvae, and that they occupy undisturbed primary forests and riparian and rock outlined stream habitats. We

350   worry that the pollution of streams, loss of of stream microhabitat diversity due to siltation, and the degradation of

351   riparian and primary forest habitats and their inter connections must be addressed if future declines are to be

352   prevented. Our findings underline that generation of new knowledge is of vital importance for long-term

353   conservation of biodiversity.

354

355   **Acknowledgements**

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Figure 1(on next page)

Amplexus and mating aggregations in *Adenomus kandianus*.

(a) An amplexed pair out of water, showing axillary amplexus. (b–d) A large mating aggregation in slow water, sandy-bottomed refuge in a stream bordering the Peak Wilderness sanctuary (6°7946′ N; 80°5232′ E).

Figure 2(on next page)

Distribution of tadpole stages at various depths across the stream microhabitats.

Size of pie charts depicts the total number of tadpoles, developmental stages are indicated in different colors. The greatest diversity of stages occupy slow-rocky habitats between 0.75–1.75 m; most robust swimming morphs with well developed ventral sucker occupy rapid-rocky areas between 0.25–0.75 m; late developmental stages occupy the stream edges; shore litter harbour more froglets than shore-sandy areas; slow-silty and slow-sandy areas have only only a few tadpoles. Earliest stage observed within the stream is stage 25, in slow-rocky habitats.
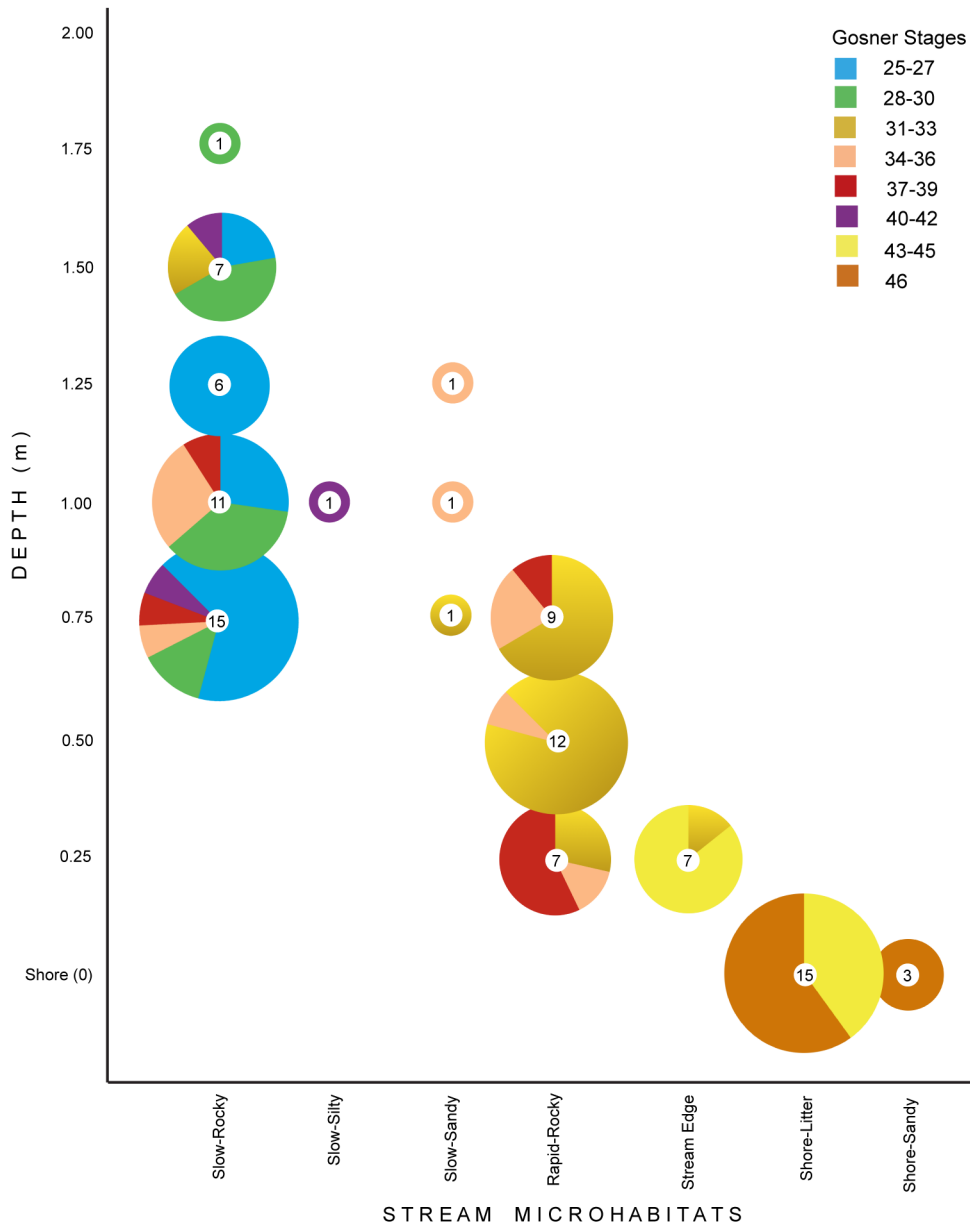
Figure 3(on next page)

Distribution modeling of *Adenomus kandianus* using MaxEnt.

Predicted distribution of *Adenomus kandianus* based on maximum entropy (MaxEnt) models constructed using Lowest Presence Threshold criteria (a, b). (c) Predicted distribution where the tree cover above 89%. Red dots indicate the presence locations used for model the construction.