

Realities of rarity: climatically and ecologically restricted, critically endangered

Kandian 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, following several years of surveying, a few *A. kandianus* were found in 2012 and referred to as "the world's rarest toad." However, tadpoles of *A. kandianus* bearing unique ventral suckers were soon discovered, but the rarity of the adult and the profusion of tadpoles were never explained. Here, using ecological methods, niche modeling and DNA-barcoding, we aim to understand the ecology, natural history and distribution of this rare toad. Following a two-year study of occurrence, habits and habitat associations of adults and larvae, we show this to be a secretive species with a patchy distribution. During non-mating periods female toads ($N = 23$) were found in primary forests habitat up to 650 m away from the breeding streams, and predominantly males in the riparian zone (12 males, 2 females). Following heavy rain they form large ($N = 388$) but patchy mating congregations in torrential streams (six sites; range 0–95 mating pairs; mean = 25, SD = 38.16, CV = 152%). Amplexed pairs swim synchronously, enabling them to traverse fast currents. Egg-laying sites remain unknown, but ability to dive, vocalize underwater, and characteristics of the eggs, suggests that they lay eggs in dark recesses of the stream. Quadrat sampling of tadpoles show microhabitat partitioning (in depth, flow-rate and substrate conditions) within the stream: the greatest diversity of larval developmental stages (25–42) in slow-flowing (depth, 0.75–1.5 m) rocky areas; more robust stages (31–39) bearing sucker discs utilize rocky-rapids (depth, 0.25–0.75 m); metamorphic stages (43–45) use stream margins (depth, <0.25 m); slow flowing silt covered areas of the stream were unoccupied, irrespective of the depth. DNA barcoding of the 16S rRNA gene fragment from the two known localities confirms the identity of the Pedro population also as *A. kandianus*. The uncorrected pairwise genetic distance of 0.1–0.7% suggests historical gene flow between the two populations. Distribution modeling (using MaxEnt), with forest-cover layers added, predicts a very small remaining area of suitable habitats (an area of occupancy of 16 km² and an extent of occurrence of 128 km²) isolated by habitats that are not conducive to these toads. While the healthy population recorded at one site gives hope for the survival of the species, long-term conservation of this climatically and

ecologically restricted species hinges largely on the preservation of cloud and riparian forests and the unpolluted high-flow torrents.

Introduction

The Sri Lankan torrent toads of the genus *Adenomus* are known from two species, *A. kelaartii* and *A. kandianus*. The former is relatively well known from several populations, the latter has had a checkered history, having been known, from the time of its original description in 1872, from only its type specimens. Although the species was finally declared extinct in 2008 (Stuart et al. 2008), it turned out that it had been rediscovered near Adam's Peak by Manamendra-Arachchi and Pethiyagoda (1998) and wrongly described as a new species, *A. dasi*, distinguished from the type specimens of *A. kandianus* by the pattern of dorsal tuberculation and the extent of pedal webbing. This error was finally pointed out by Meegaskumbura et al. (2015) in a study that involved a morphological, morphometric, genetic, bioacoustic, osteological and ecological analyses of both metamorphs and larvae of *A. kelaartii*, *A. kandianus* and '*A. dasi*'.

Meanwhile falling into the same error as Manamendra-Arachchi and Pethiyagoda (1998), Wickramasinghe et al. (2012) had announced the rediscovery of *A. kandianus* from the same locality as '*A. dasi*' and heralded it as "world's rarest toad" on the brink of extinction. Subsequently, Gabadage et al. (2014) reported *A. kandianus* (fully-webbed) and '*A. dasi*' (partially-webbed) from a locality close to Nuwara-Eliya, about 20 km from the Adam's Peak population.

Adenomus kandianus nevertheless remains a rare, critically endangered species, restricted to a handful of montane and submontane forest patches.

Discovered only recently (Meegaskumbura et al. 2015), endowed with a ventral suctorial disc, the tadpoles of *A. kandianus* show distinct adaptations for torrent life; they are highlighted as being gastromyzophorous larvae among the very few currently known. Sucker discs and ventrally-oriented mouths enable them to feed on aufwuchs (surface films constituting of algae and other minute organisms growing on submerged substrates) while being attached to submerged substrates in torrents (Meegaskumbura et al. 2015). Nothing, however, is known about stream-microhabitat partitioning of different life-history stages of these larvae, knowledge that is important for focused conservation efforts.

The abundance of tadpoles and the rarity of adults prompt several questions about both the breeding patterns of the mature toads and the fate of larvae following metamorphosis. The abundance of larvae in streams can be explained either by a few highly fecund mating pairs laying large numbers

of eggs or by the reproducing population being much greater than presently estimated. If metamorphs are abundant, where are they? When, where and how do they breed? Generation of such knowledge would provide insights as to why this remarkable toad remained lost for well over a century. Here we use ecological methods, niche modeling and DNA-barcoding, to better understand the ecology, natural history and the distribution of this rare toad.

We show that *Adenomus kandianus* is not in fact extremely rare as hitherto feared, once its habits and habitat-occupation patterns are understood. These toads occupy undisturbed primary forests and riparian habitats bordering unpolluted streams, but are specialized, secretive and nocturnal (except when forming mating congregations). Following surveys and natural-history observations of both adults and larvae over a period of more than two years, we report synchronous mating congregations, discuss their natural history and resource partitioning by tadpoles of various stages. We point out knowledge gaps to facilitate their long-term conservation.

Material and methods

Fieldwork was carried out under the research permission (permit # WL/3/2/13/13) provided by The Department of Wildlife Conservation of Sri Lanka. This study has been cleared by the Ethical Clearance Committee, Postgraduate Institute of Science, University of Peradeniya at its 18th meeting held on 19th May 2015.

Sampling adult toads

Over a period of about two years, we carried out surveys in the search of *Adenomus kandianus* adults and tadpoles (14–16 May 2012; 28–31 March 2013; 25–27 July 2013; 20–21 September 2013; 29 July–2 August 2014). We studied their patterns of occurrence, habitat associations, resource partitioning, microhabitat utilization, and natural history.

General surveys along a 4.5 km long trail were carried out, across an altitudinal (1300–2000 m) and habitat gradient (streams and riparian habitats, tea plantations, montane and submontane forests, grasslands, bamboo forests, isolated rock-pools and ponds) representative of the major habitat types in the Peak Wilderness Protected Area (Adam's Peak), a part of the UNESCO Central Highlands World

Heritage site (Fig. S1). The five predominantly nocturnal surveys were conducted between 1600–0400 hours; the habitat types, microhabitats and elevations at which toads were found were noted.

Sampling tadpoles

During daytime (1000–1400 hours), six stations along a large mountain stream were sampled for larval stages and mating occurrences (Fig. S1). After the presence of tadpoles was established, 1×1 m quadrats ($N = 36$), representative of six microhabitat types in the stream, based on the flow conditions (surface flow rates above 0.2 m/s were considered rapids, determined using the distance a styrofoam float travelled in 5s), substrate type and/or stream edge associations (slow-rocky, slow-silty, slow-sandy, rapid-rocky, stream-edge, shore-litter, shore-sandy), were sampled for various life-history stages during the 3 sampling periods: 14–16 May 2012, 28–31 March 2013 and 29 July–2 August 2014. Six stations (each of 50 m) along the stream, at least 50 m apart from each other, were scrutinized during each of these visits. Within each 1×1 m quadrat, tadpoles were counted, collected, staged according to Gosner (1960) and were released. A few ($N = 3$) tadpoles escaped in the rapids, where net handling was awkward; we were unable to stage these, as staging requires closer scrutiny. The depth at the middle of the quadrat was approximated using a 2 m long probe, marked every 0.25 m. Deeper areas (1–2 m) were investigated by snorkeling.

Mating observations

When a breeding congregation was unexpectedly observed during our last visit (29 July–2 August 2014), initially at tadpole sampling station II, all six tadpole-sampling stations along the stream were scrutinized for breeding activity. Due to the patchy distribution of the congregations within each station, a non-random 5×5 m plot was demarcated and surveyed, focusing on the numbers of amplexed pairs, free males and females. The characteristics of the mating sites were also noted (water depth, substrate and current speed) and contrasted with surrounding areas to identify the stream refuges these toads use as congregation points.

Nocturnal observations were carried out by two team members (MM and GS) for 4 hours. A search for ovipositing pairs or deposited eggs, which also included snorkeling, began in the morning at 0600 hours. Underwater behavior was recorded using a GoPro camera set in its watertight casing.

Surface videos and photographs were made using a Canon 60D camera and a Canon 100 mm macro-lens.

Water temperature, pH and conductivity at the four mating sites were measured directly using an Extech DO700 meter. Water samples from these sites were measured for total hardness, nitrites, nitrates, ammonia and sulphates using a YSI 9500 direct-reading photometer.

DNA barcoding to identify the new population

To confirm the species identification of the newly-discovered *Adenomus* populations (Gabadage et al. 2014), two tiny toe clips from each morphospecies (mature males) were obtained, preserved in absolute ethanol, and stored at -20°C . DNA was extracted using Qiagen tissue-extraction kits, following manufacture protocols. A fragment of ca 600 bp of 16S rRNA was PCR-amplified (Palumbi 1996). Sequencing was done using an ABI 3730 automated DNA sequencer (Applied Biosystems). Sequences were assembled in 4peaks v.1.7.1. Subsequently, the sequences of the new population were compared against a dataset consisting of *Adenomus kanidanus* and *A. kelaartii* obtained from Meegaskumbura et al. (2015). Sequences were aligned using ClustalW as implemented in MEGA 5.0 (Tamura et al. 2011). Uncorrected pairwise distances were calculated using PAUP* 4.0b10 (Swofford, 2002). Newly generated sequences are deposited in GenBank under accession numbers xxxxxx, xxxxxx <upon acceptance>.

Niche Models

Based on field records from this study and published literature (Gabadage et al. 2014; Karunaratna et al. 2012; Wickramasinghe et al. 2012), seven presence locations from two regions (Table S1) were used to produce the predicted distribution map of *Adenomus kandianus* using MaxEnt version 3.3.3k (Phillips et al. 2004). Bioclimatic layers ($N = 19$) and an altitude layer with a 0.0083° ($\sim 1\text{ km}^2$) resolution downloaded from WorldClim database (<http://www.worldclim.org>) were clipped to our study region bounded by $5^{\circ}908' \text{ N}$ to $9^{\circ}842' \text{ N}$ and $79^{\circ}516' \text{ E}$ to $81^{\circ}891' \text{ E}$ (i.e. Sri Lanka) prior to being used to build the predicted distribution map. Correlation of the climatic variables across the study sites were assessed by computing pairwise squared Spearman's rank correlation coefficient. Variables having $R^2 > 0.75$ were excluded from the analysis and only six variables were selected to establish the

final model. The following settings were used in MaxEnt: automatic mode with jackknife validation, random seed with 25 random test percentage and ten replicates using bootstrap function. The average model was selected and minimum presence threshold (46%) was applied to demarcate presence and absence. As the toad is a forest-associated species requiring canopy cover, the predicted range was further refined using the tree cover layer downloaded from the Google Earth engine database (Hansen et al. 2013). Using a uniform grid with a grid size of 2×2 km, area of occupancy (AOO) and extent of occurrence (EOO), in the context of IUCN Red Listing criteria (IUCN, 2001) were estimated using ArcGIS version 10.2.

Results

Adult toad sampling

Surveys with an effort of 300 person-hours during the non-mating period, only yielded 37 individuals ($N = 25$ females, $N = 12$ males). Non-mating adult toads were found in undisturbed and unpolluted habitats; males were exclusively 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 not observed this 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 not recorded in isolated rock pools or ponds.

The female toads were observed to emerge from under rock crevices ($N = 2$) and spaces under large roots ($N = 2$), between 1900–1930 hours within the primary-forest habitat. On both occasions females were observed, it was drizzling. Males emerged from earth-crevices ($N = 3$) and exposed root formations ($N = 1$) on the steep banks of riparian habitat between 1900–1930 hours. 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 hours), which swelled the water level of the stream by about 0.5 m, the first breeding pair was observed at 2030 hours 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 were observed 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). This preliminary nocturnal data was not used in the assessment of the abundance of mating pairs at the six sampling sites.

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 (31st July, 2013) by the rest of the team (24 person-hours) helped locate many more breeding pairs (Fig. 1; Video S1; Table S3). Associated with the breeding activity were 388 toads (208 males and 180 females, a ratio close to 1:1); 300 mating toads (150 mating pairs) spread across 4 of the six sites that were sampled.

Mating pairs showed a patchy distribution. Between 0600–1000 hours, a total of 95 breeding pairs were counted from sampling station II. Mating congregations were also observed in three other tadpole-sampling stations: station III had a 44-pair congregation; this had a deep pool and a shallow area similar to that of sampling station II. Sampling stations I and IV had fewer pairs (3 and 8, respectively). Two of the tadpole-sampling stations did not contain any mating pairs, but several individuals of both sexes were observed (Table S3).

Eggs were not observed, despite five days of scrutiny. The bottoms of the deeper pools could not be investigated due to the lack of underwater lights and diving gear.

Underwater video (using surface lights) of amplexed pairs clearly showed synchronized diving by the male and the female (Video S1). Swimming strokes by the male caused an inverted, V-shaped scar mark on the backs of many females after a few hours in amplexus.

Initial observations during daytime at station II showed the largest congregation of amplexed pairs, together with many unamplexed males and females. The deepest regions of the stream occur in curved areas of the stream through which the main current flows; the breeding congregation was found in the area opposite this, a region shadowed from the main current. These areas always had a sandy substrate, at a depth of 0.25–1.5 m. There was also a large submerged log, providing further protection from the current (Video S1).

Two female toads were found dead in the evening (31 July 2013) at station II. Dissection showed 180–200 pale grey eggs in each specimen, left and right ovaries were mature in both. Attempts to disturb amplexed pairs resting on the sandy bottom (underwater) by satellite males were restricted to brief pouncing on the pairs (Video S1); the amplexed males always warded off the invading male by frantic kicking ($N = 5$).

Water from the four sites from which mating pairs were observed showed the following water-quality parameters: pH 6.5; conductivity 9.67–10.17 μS , total hardness of 5 mg/L CaCO_3 , extremely

low levels of nitrites (10–17 μL), absence of ammonia, low levels of nitrates (0.348–0.510 mg/L), and absence of sulphates. The temperature of the water at each site was 16.2 °C (at 1100 hours).

Tadpole sampling

A total of 97 tadpoles were observed in the 1×1 m plots sampled from the 7 stream microhabitats from the six tadpole-sampling stations during three visits. Slow-rocky ($N = 40$) and rapid-rocky ($N = 28$) stream habitats harbored the highest number of individuals; slow-silty ($N = 1$), slow-sandy ($N = 3$), stream edge ($N = 7$), stream shore-sandy ($N = 3$) and stream shore-litter ($N = 15$). The distribution of larval stages showed several patterns: initial postembryonic stages (stage 25, 26) were found mostly in slow-rocky habitats, in an area with a water depth of between 0.5–1.5 m; only the larger-bodied, robust swimming morphs were found in the rocky-rapids (0.25–0.75 m); the metamorphic stages (stage 44, 45) were found in calmer and shallower (less than 0.25 m deep) water closer to the stream edge. The greatest diversity of stages was found in slow-rocky areas that were less than 1.5 m in depth (Fig. 2); the slow-silty and slow-sandy (deeper than 0.25 m) areas had the least abundance and diversity of stages. We assume that the three tadpoles that escaped being netted in the rapids are also between stages 31–39; however, given their negligible numbers, they were not included in the assessment.

Observations of many of the small shallow streams, rock pools and puddles across the altitudinal gradient did not show tadpoles of *A. kandianus*.

Other natural history observations

Males call above water and also underwater, a soft call, but very well heard underwater. These calls were mostly heard when snorkeling, closer to the congregations of pairs in the slow sandy area.

Amplexed males disturbed by satellite males always called loudly.

DNA barcoding

Uncorrected pairwise distances between the recently discovered population of *Adenomus* from Pedro was confirmed as *A. kandianus* with pairwise percentage distance range of 0.1–0.7% for the 16S rRNA gene fragment (Table S2).

Niche modeling

The AUC value (ranging between 1 and 0), which is commonly used to determine the predictive accuracy of the model, was 0.996. Altitude and Bio4 (Temperature Seasonality) mostly contributed to the prediction; percentage contribution of each variable used to establish the model is shown in Table S4.

Based on the MaxEnt models, the predicted climatic niche of the species within the island is 670 km², distributed on the upper reaches of the Central Hills (Fig. 3). Since the tree cover percentage in all presence records for the species were above 89%, refined distribution within forest habitats was calculated as 420 km² (i.e. the predicted distribution where the tree cover was above 89%). AOO and EOO were 16 km² and 128 km² respectively.

Discussion

Many larger toads are tolerant of various environmental stressors and have expansive distributions (Van Bocxlaer et al. 2009). *Adenomus kandianus*, however, are habitat specialists, both as adults and as larvae, exclusively occupying primary forests, riparian and suitable lotic habitats. Hence they are not only torrent toads, as their common name implies, but also forest toads that travel far from streams for poorly understood reasons.

The total number of toads (breeding aggregations and non-mating individuals) observed during this study ($N = 425$) from the Peak Wilderness protected area was disproportionately large when compared against the number of individuals ($N < 5$) observed in previous surveys/ studies (Manamendra-Arachchi & Pethiyagoda 1998, Wickremasinghe et al. 2012). However, much of this ($N = 388$) was due to the rare one-time observation of the large breeding congregation.

Adult toads were only observed in riverine habitats and primary tropical montane cloud-forest habitats suggesting they are habitat and climate specialists. They were not observed in grasslands, tea plantations or scrubland situated at a similar elevation, suggesting that these toads require an undisturbed forest canopy that preserves humidity and maintains a lower temperature, factors that reduce desiccation.

Only the larger-bodied female toads seem to wander, as much as 650 m away from the torrential breeding streams, while male toads seem to occupy primarily a riparian habitat. This pattern suggests sex-specific resource partitioning, seen also in many other toad species (Schmidt 2012). Often, females occupy a different habitat from that of males to prevent premature amplexus, to reduce stress and to avoid competition for food. This suggests that, larger-bodied females (Meegaskumbura et al. 2015) probably wander far from the breeding streams, foraging to build up energy reserves to produce their substantial clutches of eggs and to undergo the strenuous torrent diving activity, while the smaller-bodied males live in forested riparian habitats, awaiting a chance to mate.

Since females hide in rock and root crevices in primary forest habitats during daytime, diurnal encounter-surveys may not be successful in uncovering them; however, quadrat sampling and focused-searches on spaces beneath objects such as stones and logs may be useful to locate them. Males also hide among crevices in stream banks during daytime, underlining that these toads are predominantly nocturnal and secretive. This, together with their patchy distributions, may explain why they are observed in such low numbers.

High tadpole abundance almost throughout the year suggests a thriving population of toads that mate profusely. It shows that these toads are breeding, and the conditions necessary for population viability is currently present. On two occasions (sampling periods), tadpoles of two strikingly different developmental stages were observed. This again suggests multiple breeding events during a breeding season.

On one sampling attempt (20–21 September 2013), tadpoles were not observed. This was a rainy period and the entire stream was unusually swollen and torrential, with water levels about 2 m higher than at times when tadpoles were observed. We may not have observed the tadpoles on this occasion, as they may have been swept downstream, since stream characteristics can change dramatically with changes in flow volume (e.g., Ficklin et al. 2013; Yeh et al. 2015). They may also have hidden in calm water refuges or may not have bred at the full progression of the monsoon.

These toads are not explosive breeders: the two naturally-dead females contained only 180–200 eggs each. Therefore, many toads would need to mate to give rise to the large number of tadpoles, as observed in the present study. Hence the large breeding congregations of moderately fecund females would explain the prevalence of tadpoles in streams.

The water quality parameters at the breeding site indicate a dependence on unpolluted conditions for breeding. The low nitrate, nitrite and phosphate concentrations indicate that there is little or no eutrophication or runoff of nutrients from tea fields to impact stream chemistry. Given that the headwaters of the stream originate within the cloud forest and our sampling stations were located at the upper boundaries of the tea fields, this is to be expected.

The sites of egg deposition remain unknown. Nevertheless, based on several observations, we presume that these toads oviposit at the bottom of deep pools (beyond 2 m depth) or the spaces under sunken boulders or rocks. About twenty pairs of toads in amplexus were observed diving into the deeper regions of pools that also contained boulders and a rocky bottom; non-amplexed females that were not egg-heavy were observed swimming from the deep pool towards shallow areas. Next, at two of the sites of the largest congregations, satellite males formed a crescent between the deeper pool and the congregation of amplexed pairs in the shallows, suggesting that they may attempt amplexus by pouncing on already amplexed-males, ready to make the journey into the depths.

The egg type can also help predict the oviposition site. The related common species, *A. kelaartii*, has been bred in captivity outside its natural range (Hass et al. 1997). This toad has pale non-pigmented eggs embedded in gelatinous strings. If character-sharing due to common ancestry is assumed, we could infer that *A. kandianus* also lays pale eggs in strings. Pale eggs are found in species that often lay eggs underground or in dark recesses, as penetrating solar radiation can damage the developing embryos (eggs laid in well-lit areas have darker pigmentation; Liht, 2003; Palen et al. 2005). In fact, dissection of the two dead females showed pale eggs within their ovaries, but these observations do not inform whether eggs are laid in strings or not, as gelatinous layers are made within the oviduct, when eggs are being laid; hence the possibility of eggs of these toads being in strings is present. Strings of pale grey eggs laid in deep, dark water among debris and boulders at the bottoms of pools or under boulders will both be protected from sunlight and from being washed away. We suspect these to be the oviposition sites of *A. kandianus*.

The other option for an amplexed pair is to lay their pale grey eggs under rocks and litter on stream edges, under the protection of shade. This unusual mode of reproduction seen in at least one megophryid frog (*Leptolalax khasiorum*), which also has pale cream-colored eggs (Tron et al. 2015). But for *Adenomus kandianus*, toads that are specialized for swimming and diving, this will necessitate

crawling in very shallow water, risking predation. Heavily-webbed feet, underwater vocalization ability and synchronous diving of the amplexed pairs suggest that they are well adapted to an aquatic life, hence also aquatic egg-laying.

Dark-pigmented, stage 25 and above tadpoles were the least developed stage observed in the stream, suggesting that the hatching stage is around 25, and that dark pigmentation develops long after the (pale) eggs are laid. This again suggests that eggs are laid in the dark recesses of streams or its edges. It is noteworthy that the oviposition site of *A. kelaartii* too, remains unknown in the wild.

Many of the major stream microhabitats are utilized by various life-history stages of these toads. The rocky substrates of the fast-flowing areas are dominated by the larval stages that have a well-developed suckers and a dorsoventrally flattened body that probably enables them to secure themselves onto substrates and also traverse fast currents. Slow-flowing, rocky areas of the stream seem to be important for the early (25, 26) and late developmental stages (stage 44, 45) to feed. Both these stages seem to lack the fast-swimming and clinging abilities needed to exploit rapid-rocky habitats. Slow-sandy areas near the edge are occupied by stages with diminishing larval mouthparts, including the oral sucker, that are close to completing metamorphosis. Tadpoles do not use the deeper sandy areas of the stream, as the sucker would prevent them from burrowing and feeding, as do many other sand-inhabiting tadpoles. Moreover, shifting sand is a poor growth substrate for aufwuchs. However, as noted above, the sandy regions between 0.5–1.5 m are important areas for the breeding congregations of adult toads. These seem to act as staging areas to make egg-laying forays into the deeper and darker waters and submerged boulders/rocks.

The stream-associated microhabitat utilization and resource partitioning among various developmental stages within the stream are different, which suggests that these toads, despite their abundance, have specialized requirements. Tadpole stages having the ventral sucker disc were found predominantly attached to rocky substrates, feeding on aufwuchs; these surface films cannot grow on constantly moving objects (e.g. sand, detritus). This could be the primary reason for these tadpoles to feed on rocky surfaces. Within the two rocky surfaces available, depending on the flow rate, more tadpoles were observed in slow-flowing water than in rapids. Feeding in the rapids is energetically more expensive, but there could also be more food there owing to reduced competition.

Once the ventral sucker disc diminishes with advancement to metamorphosis, the larvae migrate to the shallow areas at the stream's edge, where water flow is slower. Especially in areas that had a layer of detritus, substantial numbers of almost metamorphosed froglets (stage 46) were observed along the stream edge, suggesting that many of the tadpoles survive up to a well-developed metamorph.

Compared to some other toads in which mass mortality is prevalent (Franz et al. 2013; Santos et al. 2007), we witnessed only two females who seem to have died in association with breeding activities; both these bore the swimming scar made by the male during amplexed swimming. However, it is possible that some may have died and been washed away by the current. The scarring of the back may increase the incidence of infection, and females may die several days after mating occurs.

Pollution and the opening up of the canopy above streams may have reduced the area available for the survival of these highly specialized toads (both as tadpoles and adults). However, we do not know if what we are observing at present are the last few populations of the Kandian Torrent Toad. A single thriving population is insufficient to guarantee the survival of this species even through the next few decades; it has been witnessed limited numbers of thriving populations being wiped out within short periods of time (Corn & Fogleman 1984). It remains to be established how the other known populations of these toads are faring, especially the recently discovered population (Gabadage et al. 2014) at Pedro.

Barcoding of the Pedro population confirms the species identity as *A. kandianus*. The genetic distances (0.1–0.7%) for 16S rRNA suggest within-species level divergences (Vences et al. 2005), highlighting historical gene flow between Peak Wilderness and Pedro populations. This would have been facilitated by the habit of at least adult female movement across large distances under primary forest cover. However, fragmentation of habitats will pose a serious impediment to this movement.

Improved niche models that take into consideration all known populations of *A. kandianus* suggest a fairly large area of occupancy (ca. 670 km²) within the Central Hills (Fig. 3). Though this region was historically forested, most of these natural habitats have since been replaced by tea, vegetable farms and home gardens that are not conducive to the persistence of these toads; the specific habitats (primary forest, riparian or forest-shaded torrent habitats) required are not present in much of this area. Only the region of Pedro and Adam's peak harbor substantial primary and riparian forests and the large streams that are needed in combination to help these toads survive. Habitat barriers separate

these two populations from each other. When forest cover is overlaid on the predicted area of the model, only about 420 km² appears suitable for the species. The two distantly-placed populations, Pedro and Adam's Peak, provide an extent of occurrence of 128 km², but a very small area of occupancy of only 16 km². However the predicted distributions suggest a high probability of the existence of another population in the western slopes of the Horton Plains National park (the forested area, having large unpolluted streams, between Pedro and Adam's Peak). Daytime searches for suckered tadpoles in these streams (especially in rocky, slow-flowing areas and rocky rapids) may uncover further populations of this secretive and nocturnal toad.

A single stochastic event could destroy any of these two existing populations. At breeding time, given their propensity to congregate in suitable streams, even a short-term threat can wipe out a significant portion of the population, driving the population into an irreversible extinction vortex. Here we have used, adult and tadpole surveys, a rare mating observation, DNA-barcoding, and presence-based ecological niche modeling to better understand a species that was until recently thought to be extinct. We show that *Adenomus kandianus*, a species that was once thought to be extinct and since considered extremely rare (the "world's rarest toad"), is in fact not so rare when it comes to mating. With the accumulation of survey data and natural history observations of both adults and larvae over a period of more than two years, we report several large synchronous-mating congregations of the species. Metamorphs, however, are nocturnal and secretive during non-breeding periods. We suggest that these toads are climate and habitat specialists both as adults and as larvae, and that they occupy undisturbed primary forests and riparian and rock outlined stream habitats. We worry that the pollution of streams, loss of stream microhabitat diversity due to siltation, and the degradation of riparian and primary forest habitats and their inter connections must be addressed if future declines are to be prevented. Our findings underline that generation of new knowledge is of vital importance for long-term conservation of biodiversity.

Acknowledgements

We thank: XXXXXXXXX for helpful comments to improve the manuscript and support; Department of Wildlife Conservation and Forest Department of Sri Lanka for research permits; RohanPethiyagoda for his support in preparing the manuscript; Kelum Manamendra-Arachchi, Don Church, Robin Moore and James Lewis for their support and discussions regarding amphibian conservation activities; C Bandara, A Gunarathne and B Jayawardena for involvement in some of the fieldwork; T Gunatilleke, W Agalawatta and S Hewapathirana for their assistance with a 4.5 km general collection. We acknowledge the University of Peradeniya Research Grant (RG/2012/45/S) for fieldwork and National Research Council Grant (11-124) of Sri Lanka for graduate student support and lab work.

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Figure 1. 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. 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 a few tadpoles. Earliest stage observed within the stream is stage 25, in slow-rocky habitats.

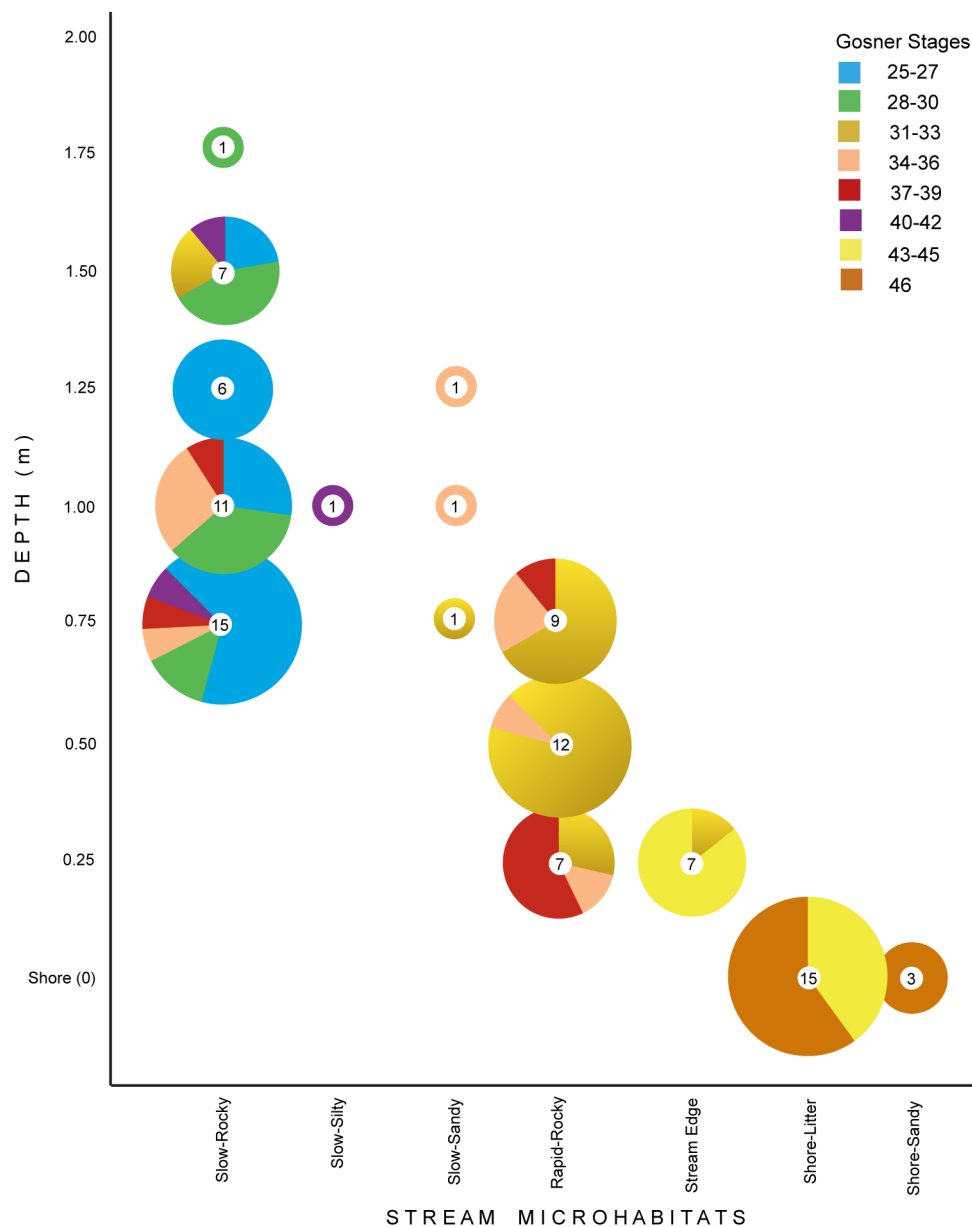


Figure 3. Distribution modeling of *Adenomus kandianus* using MaxEnt.

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.

