A lungless frog discovered on Borneo

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A lungless frog discovered on Borneo

David Bickford[1,*], Djoko Iskandar[2], and Anggraini Barlian[2]

The evolution of lunglessness in tetrapods (amphibians, reptiles, birds, and mammals) is an exceedingly rare event. So far lunglessness is known to occur only in amphibians, in particular two families of salamanders [1,2] and a single species of caecilian [3]. Here, we report the first case of complete lunglessness in a frog, *Barbourula kalimantanensis*, from the Indonesian portion of Borneo (Figure 1A). Previously only known from two specimens [4,5], a recent expedition to central Kalimantan on Borneo rediscovered two new populations of this enigmatic aquatic frog (Figure 1B,C). This allowed for a more comprehensive assessment of the species' ecology and anatomy that led to the discovery of its lack of lungs. Loss of lungs in Amphibia is most likely due to their evolutionary history at the interface between aquatic and terrestrial habitats and their ancient ability to respire through the skin [5].

Despite multiple attempts to locate more individuals of *B. kalimantanensis*, prior to 2007, only two specimens of this frog species were known to science [4,5]. In August 2007, we visited the type locality near Nanga Pinoh, Western Kalimantan (0° 44' S; 111° 40' E) but found that illegal gold mining had destroyed all suitable habitats in the vicinity. The originally cool, clear, fast-flowing rivers are now warm and turbid. Water quality around the type locality is no longer suitable for the species, but we were able to discover two new populations of *B. kalimantanensis* upstream of the type locality.

We established the lunglessness of *B. kalimantanensis* specimens through dissections and histological sections of the anterior portion of the coelom (around the heart) that revealed a membrane lining the thoracic cavity, but no evidence of lungs. In all other frogs, there is a protected opening to the airway (the glottis) as the oral cavity narrows to form the esophagus. We found no such opening during dissections of eight specimens of *B. kalimantanesis* (ranging in snout-vent length from 26.9 to 50.5 mm, $\bar{x}$ = 38.3 mm and in mass from 2.2 to 13.5 g, $\bar{x}$ = 6.5 g). However, we did locate a glottis and lungs in a specimen of the only other species in the genus, *Barbourula busuangensis*, and another frog species, *Rana catesbeiana* (Figure 2). With no evidence of any lung tissue and no glottis, *B. kalimantanensis* is thus the first species of frog reported to be lungless.

Among tetrapod vertebrates, lunglessness has only evolved in the amphibians: many salamander species (two species in the family Hynobiidae, genus *Onychodactylus* [2], and more than 350 species in the family Plethodontidae [1]) as well as a single species of caecilian (the other order of amphibians) [3] are lungless. Thus, the complete loss of lungs in tetrapods is a particularly rare evolutionary event. The loss of lungs is a reversal of one of the most important physiological adaptations for terrestrial life and has probably only evolved independently three times. The discovery of lunglessness in a secretive Bornean frog species, supports the idea that lungs are a malleable trait in the Amphibia, the sister group to the rest of the living tetrapods. Amphibians may be more prone to lunglessness since they are known to be able to readily utilize other methods for gas exchange, namely cutaneous, gills, buccopharyngeal and perhaps cloacal (all thin-membrane) gas exchange outside of the lungs [6,7].

Respiration determines much of an organism's inherent biological limits and life history. Hence, the evolution and ecology of lunglessness is a complex physiological development entailing many different mechanisms, possible explanations, and evolutionary and developmental pathways. Trade-offs among kinematic and muscular performance, buoyancy, and metabolic rate somehow reach an evolutionary and ecological balance. In *B. kalimantanensis*, this balance leads to loss of lungs as the main respiratory surface for gas exchange. *B. kalimantanensis* is presumably an ectotherm and lives in cold (14–17°C) fast-flowing (2–5 m/s) water, so loss of lungs may be an adaptation to the combination of higher oxygen content in fast-flowing cold water, the species' presumed low metabolic rate, severe flattening to increase the surface area of the skin (Figure 1B,C), and selection for negative buoyancy.

B. kalimantanensis, the only lungless tetrapod in Southeast Asia, is currently

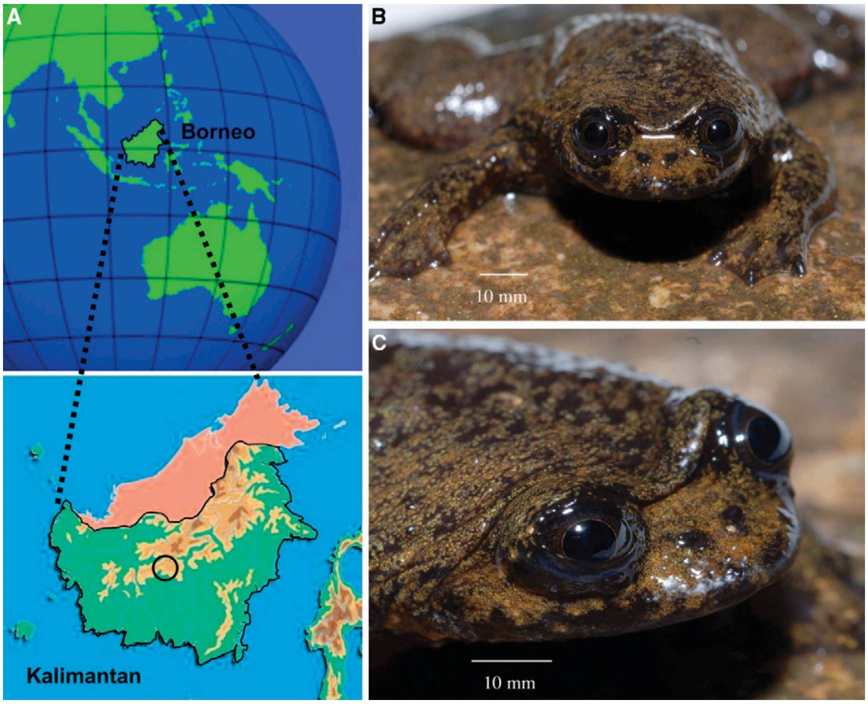

Figure 1. Habitat and appearance of the lungless frog *Barbourula kalimantanensis*.

(A) Map of Borneo, showing the Indonesian portion, Kalimantan, in the South-Central part of the island, and (B) *B. kalimantanensis* in anterior view, and (C) lateral view showing extreme flattening of the body.

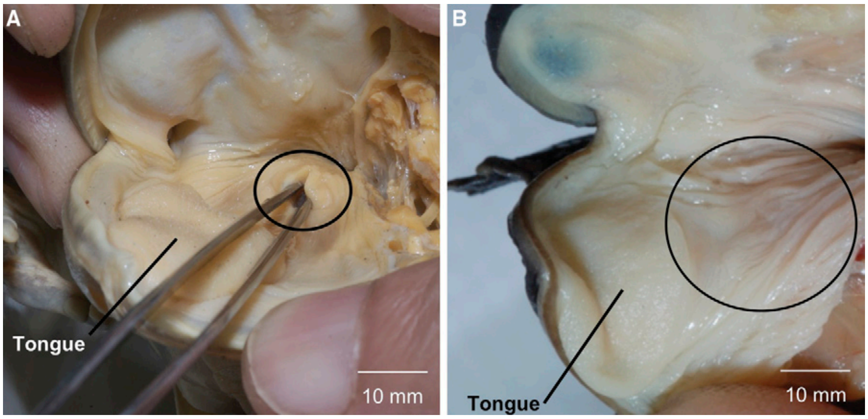

Figure 2. Anatomy of lunglessness.

Comparison of (A) typical frog mouth and pharynx (*Rana catesbeiana*), showing glottis (circled), tongue, and esophageal opening, and (B) *B. kalimantanensis* showing tongue, no glottis (circled), and an enlarged esophageal opening leading directly to the stomach.

listed as endangered [8] and illegal gold mining resulting in increased turbidity and mercury contamination has severely degraded the type locality and much of its presumed former range. Compounding the problem, much of the surrounding terrestrial habitat is also under increasing threat from both legal and illegal logging. Conservation of this evolutionary enigma needs to be prioritized and the remaining habitat in which it can survive needs to be urgently protected. The evolution, development, and maintenance of lunglessness in this frog will become important research foci. How complete loss of lungs evolves and under what kind of selective pressures and genetic mechanisms has been well debated in salamanders [9,10]. However, these are still open and more manageable questions for an aquatic primitive frog. To better understand the extinction risk and endangered status of this species, a much more complete assessment of potential habitats needs to be surveyed and the exact geographic range for the species should be mapped. In addition, virtually nothing is known about how these frogs reproduce, eat and escape predation. Further studies, however, may be hampered by the species' rarity and endangerment. We strongly encourage conservation of the remaining habitats of this species.

Supplemental data

Supplemental data including experimental procedures are available at http://www.current-biology.com/cgi/content/full/18/9/R374/DC1

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Role of fungi in the biogeochemical fate of depleted uranium

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The testing of depleted uranium (DU; a 97.25% U:0.75% Ti alloy) ammunition and its use in recent war campaigns in Iraq (1991 and 2003) and the Balkans (1995 and 1999) has led to dispersion of thermodynamically unstable DU metal into the environment [1–3]. Although less radioactive, DU has the same chemotoxicity as natural uranium and poses a threat to human populations [1]. Uranium tends to form stable aqueous complexes and precipitates with organic ligands [4], suggesting that living organisms could play an important role in geochemical transformations and cycling. Fungi are one of the most biogeochemically active components of the soil microbiota [5], particularly in the aerobic plant-root zone. Although the mutualistic symbiotic associations (mycorrhizas) of fungi with plants are particularly important in mineral transformations [5], fungal effects on metallic DU have not been studied. Here, we report that free-living and plant symbiotic (mycorrhizal) fungi can colonize DU surfaces and transform metallic DU into uranyl phosphate minerals.

Fungal interactions with DU were studied in microcosms simulating a heterogeneous environment (Figure S1A in Supplemental Data, published with this article online). All tested fungi exhibited high DU tolerance and were able to colonize DU surfaces, forming moisture-retaining mycelial biofilms (Figure S1A–D). The fungi also often formed cord-like mycelial structures through aggregation of longitudinally aligned hyphae (Figures 1A,B and S1F,G), commonly interpreted as a survival response to metal stress [6].

DU coupons (triangular sectors of DU alloy of approximate dimensions 15 mm x 15 mm x 11 mm, and 5 mm height, and approximately 6.5–8.5 g in weight) in the microcosms underwent aerobic corrosion forming black and yellow decomposition products