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Primer

Amphibians

David B. Wake* and Michelle S. Koo

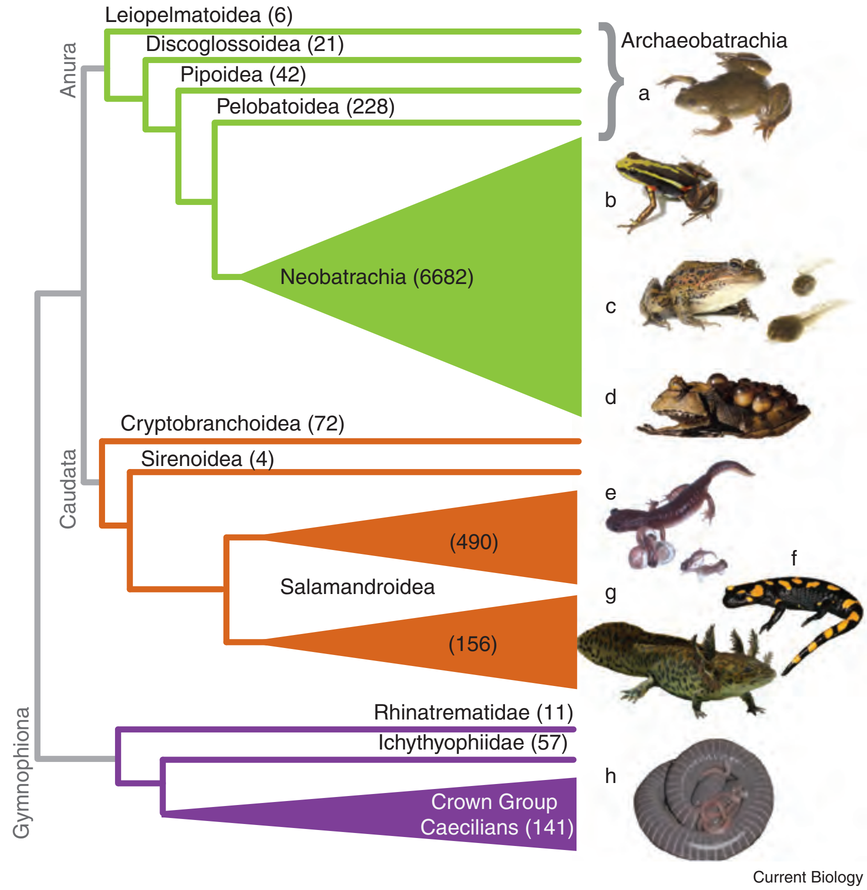

Figure 1. The amphibian tree of life.
The three orders of amphibians differ greatly in numbers, morphology, and reproductive modes. Generalized phylogeny is shown with the number of species for major clades in parentheses. Amphibians shown include: (a) *Xenopus leavis* (Pipidae), a fully aquatic species model organism (photo: B. Gratwicke); (b) *Epipedobates tricolor* (Dendrobatidae), a toxic species that sequesters toxins from its diet (photo: D. Cannatella); (c) *Rana aurora* (Ranidae) has a 'typical' frog life history with eggs laid in a pond where they hatch and grow as tadpoles until metamorphosis into an adult form (photo: B. Freiermuth); (d) females of the frog *Hemiphractus johnsoni* (Hemiphractidae) carry developing eggs on their backs, where they hatch into fully formed miniatures of adults (photo: D. Wake); (e) *Ensatina eschscholtzii* (Plethodontidae) females guard their eggs, which hatch into fully formed miniatures of adults (photo: H. Greene); (f) *Salamandra salamandra* (Salamandridae), the Fire Salamander of European folklore (photo: A. Noellert); (g) *Ambystoma andersonii* (Ambystomidae), a critically endangered permanently larval form closely related to the model organism *A. mexicanum*, the Axolotl (photo: L. Fehlandt); (h) *Siphonops annulatus* (Siphonopidae), a caecilian whose females stay with their eggs until they hatch into miniatures of adults and feed on a lipid secretion produced by the skin (photo: C. Jared).

Since the dawn of history, amphibians have been a part of human culture. Western Europeans built fires for cooking and warmth, adding large logs as needed. What occasionally emerged was astounding: large black animals (which had found shelter in the logs) with four legs and a tail, jet black with striking bright yellow spots. These fire salamanders were variously thought to be the product of the fire itself, or, as Aristotle reported, capable of extinguishing fire. Pliny the Elder is said to have tested this idea by throwing a salamander into flames — the salamander died! — nevertheless the association with fire persisted. Pliny perpetuated other fantastical claims, which spread; even Leonardo da Vinci contributed to the legend, and myths from different regions merged — at one point, asbestos was claimed to be salamander wool. Salamanders were attributed great powers; a single salamander upstream was thought to be sufficient to kill an army. King Francis I. of France chose a salamander as his emblem — a powerful symbol, born of fire, filled with poison, immune from burning, and even able to douse flames. Before the emergence of great cities and conurbations, people grew up surrounded by nature. Salamanders and newts, toads and frogs were all part of normal human experience. Myths such as those surrounding the fire salamanders were commonplace. Shakespeare's witches brewed with an eye of newt and tail of frog. As a child, we raised tadpoles and were taught to shudder at the appearance of a tiger salamander in a root cellar. In general, amphibians are seen as benign and harmless, even helpful as creatures that devour harmful insects and serve as an alternative food source. Thus, it came as a shock to most biologists and to the public at large in the 1980s that amphibians around the world were in decline and that they were at greater risk of extinction as a taxon than any other vertebrate group. A study of every amphibian species known in 2004 showed that on the order of 40% were at high risk of extinction, and by 2008, the decline of amphibians was seen as evidence of an impending sixth mass extinction.

Amphibian taxonomy and anatomy

Amphibians constitute three major taxa: anurans, the frogs and toads; caudates or urodeles, the salamanders and newts; and gymnophiones, the limbless caecilians. Amphibians are an ancient group, representing remnants of the first land vertebrates. Modern taxa are early Mesozoic in origin. The earliest known frog (*Triadobatrachus*) lived about 250 million years ago (early Triassic). They are survivors, with several modern families dating to mid-Cretaceous (110–120 million year ago); amphibians appear to have passed through the mass extinction at the end of the Cretaceous relatively unscathed. Salamanders and frogs are well-known and broadly distributed, but caecilians remain poorly known even to professionals. Caecilians are mainly

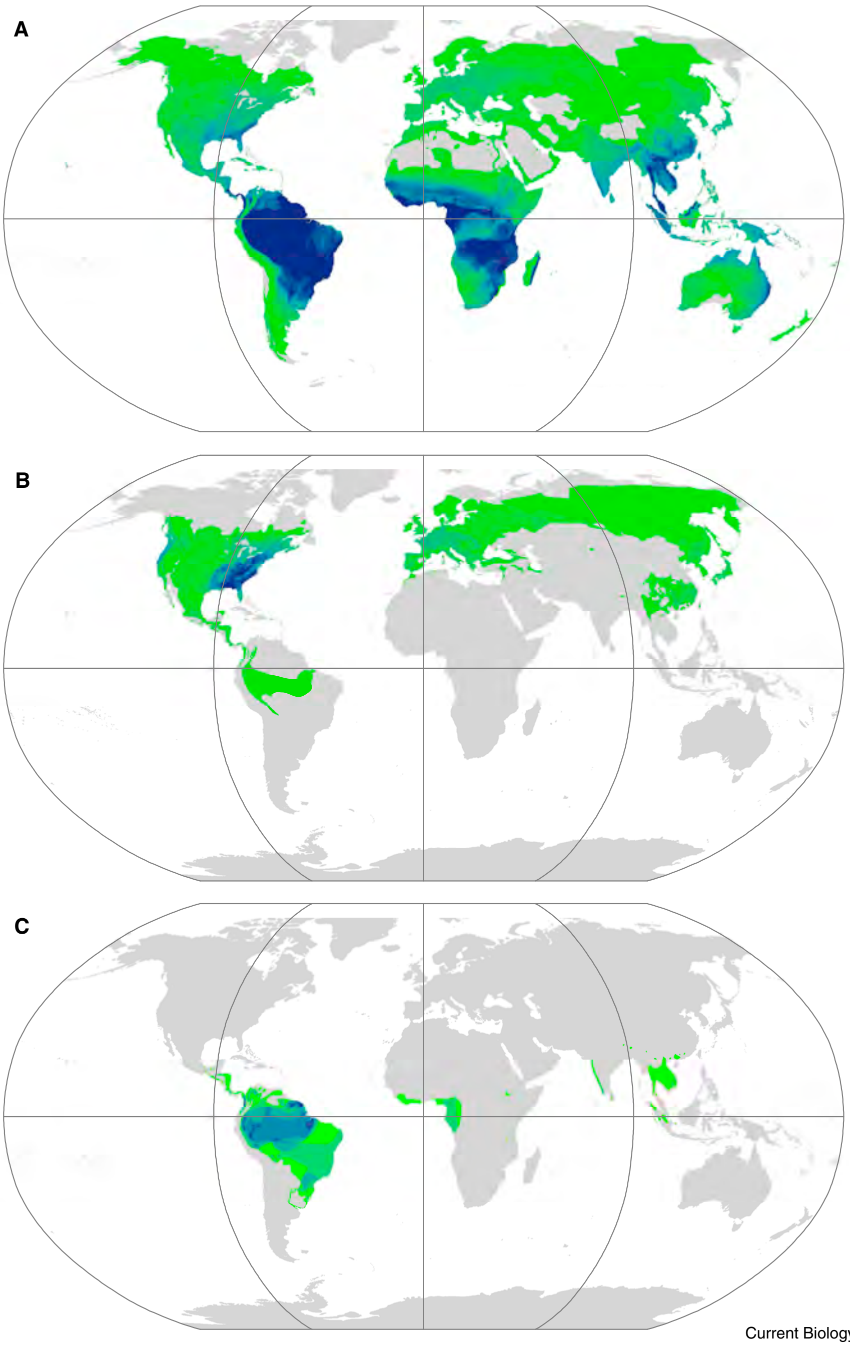

Figure 2. Global distribution of the three orders of amphibians.
The three orders of amphibians differ in distribution with frogs (A) commoner to the south, salamanders (B) to the north, and caecilians (C) restricted to the tropics. Darker colors toward blue indicate higher species richness. Based on data for 7,011 species from AmphibiaWeb and IUCN 2018.

burrowers (fossorial) that rarely are seen on the surface; little is known of their life history. Today nearly 8,000 living amphibian species are known, of which 88% (~7000) are frogs (anurans), about 9% (~700) are salamanders, and about 3% (>200) are caecilians. Frogs and salamanders are more closely related to each other than either is to caecilians (Figure 1). These three groups are distributed very differently around the world (Figure 2). Frogs are found on all continents except Antarctica and on most large and small continental islands in the temperate and tropical regions (for example, they are abundant on Madagascar, the Philippines, Borneo and New Guinea), but absent from all but a very few oceanic islands. They are especially abundant in the tropics. In contrast, salamanders are distributed mainly in northern regions (although they reach below the equator in South America). Salamanders are absent even from most large continental islands (all those mentioned above) and all oceanic islands. Most restricted of all, caecilians are limited to the tropics.

The three groups of amphibians are so distinct in appearance that they never are mistaken for one another. What they most notably share is a moist skin, which serves as the main respiratory organ and produces diverse secretions, many of them poisonous to other organisms. The frog body plan is highly conserved and they are unmistakable for any other vertebrate. Frogs have very short trunks, with nine or fewer vertebrae in front of the sacrum. Their bodies are made larger by the elongated pelvic girdle. All adult frogs lack tails. Forelimbs are relatively short, but hind limbs are long. Frogs have four digits on the forelimb and five on the hind limb, but sometimes one or two are very small. The hind limbs contain four well-defined segments and can be very long, enabling some species to make prodigious leaps. Leaps exceeding two meters have been recorded. However, many species that live mainly underground have short legs and walk or hop, or burrow, rather than leap. Heads of frogs are large relative to body size.

Salamanders typically have the body type of a prototypical, generalized land vertebrate, with well-proportioned heads, limbs of roughly similar length, and a tail about as long as the head plus body. Trunks are of moderate length with between 12 and 22 presacral vertebrae and the tail is sometimes much longer then the body. Limbs are of moderate size and usually overlap when pressed against the trunk; the limbs have three major segments. Salamanders are like frogs in having no more than four digits on the forelimb, while five typically are found on the hind limb. Because of their appearance, people may mistake salamanders for lizards and vice versa. A few exceptions include aquatic amphiumas and sirens, salamanders that have become elongate and eel-like with reduced or absent limbs. Aptly called giant salamanders of China and Japan can exceed 1.5 meters in length. Other salamanders have retained aquatic larval morphology, usually with feathery external gills and keeled tails, throughout life.

Caecilians differ sharply from other amphibians. They are superficially worm-like, with bodies marked by rings. Most are terrestrial burrowers, but a few are aquatic. The head is small and much the same width as the anterior trunk. The very elongated trunk often contains well over 100 vertebrae. The tail is typically absent, but some species have a very short tail. The cloacal opening is usually at the very end of the animal. Eyes are reduced to nearly but never completely absent. The mouth is large with many teeth. The head has a unique chemosensory organ, the tentacle, which lies between the external nostril and the mouth and flicks in and out like a snake's tongue. Limbs or limb rudiments are completely absent, even during development.

Amphibian biology

Water governs the lives of all amphibians, although a few frogs and salamanders have gone to extremes of specialization to live in places where water is scarce. Many frogs and especially the tropical salamanders live on trees.

What is often presented as a typical amphibian life history is strongly biased by the fact that early biologists who first described amphibians resided in temperate zones, where the 'typical' amphibian life history dominates: land-dwelling adults gather at ponds or streams in spring to meet, mate and reproduce. Females lay eggs in water, which are then fertilized by males. Tadpoles (in the case of frogs) or larvae (salamanders) are produced that grow and metamorphose into tiny juveniles, which return to land. Europeans knew about some alternative life histories; the midwife toads (*Alytes*) lay a few large eggs in water, which are collected by males and draped around their hind limbs until they are ready to hatch, when they were returned to ponds.

Figure 3. Amphibian warning colors.
Aposematic (warning) coloration has evolved many times in amphibians, and mimicry is widespread. Top: these poison dart frogs live together in Ecuadorian and northern Peruvian rainforests: *Ameerega bilinguis* (left) is toxic and distasteful to predators, which the harmless Batesian mimic *Allobates zaparo* (right) uses to its advantage. Bottom: the polymorphic salamander *Ensatina eschscholtzii* (right; Plethodontidae) mimics the highly toxic Rough-skinned newt *Taricha granulosa* (left; Salamandridae) in parts of its range where the two species overlap, and only here does it closely resemble the newt. Pictured specimens are displaying their bright eye and ventral coloration (photos: D. Cannatella (top); A. Schusteff (bottom left), H. Rockney (bottom right)).

Thousands of species of frogs, hundreds of salamanders, and nearly all caecilians diverge from the typical life history. The most common difference is loss of the larval stage; eggs that develop directly into miniatures of the adults are laid on land. A stark contrast is found in some salamanders that remain in a larval state (usually with gills and fins) throughout life, a condition often termed paedomorphosis or neoteny. In many caecilians, eggs are retained in modified oviducts and nourished before — sometimes very large — miniatures of adults emerge; this is true viviparity. Both frogs and salamanders have a few viviparous species that give birth to metamorphosed young that have been nourished, but they are uncommon. Instead, especially in frogs, almost endless variations are found on the theme: stay out of water, where danger lurks. Eggs are laid in bromeliads on tree branches, placed in vocal sacs of males, in compartments on the backs of females, even in the stomach! Larvae in bromeliads are fed nutritive eggs, or they have many specializations for living in unusual places. Reproductive diversity is one of the hallmarks of amphibians.

Their skin distinguishes amphibians from other vertebrates. It is well vascularized and serves as the main respiratory organ. It is richly supplied with diverse glands that produce an amazing array of secretions, some of which help moisten the skin while others produce a broad array of poisons and toxins. Chromatophores in the skin produce the often dramatic colors that characterize many species (Figure 3).

Several amphibians qualify as 'model organisms', which are used in experimental biology. The most famous are the Axolotl (*Ambystoma mexicanum*; especially important in studies of limb and tail regeneration) and the tetraploid African clawed frog (*Xenopus laevis*) and its diploid relative *X. tropicalis*, both widely used in studies of developmental and cell biology. A few additional frog and salamander species are widely used, including the Iberian ribbed newt (*Pleurodeles waltl*). There are two major impediments with amphibians as model organisms: husbandry (which explains why there is no caecilian model organism as there is no captive breeding protocol) and lack of complete sequenced genomes. Genomes of the two *Xenopus* have been available for a few years, but two

Figure 4. Amphibian threats.
Amphibians are being hit hard by pathogenic fungi. Top: the blue-tailed firebellied newt (left; *Cynops cyanurus*) and oriental firebellied toad (right; *Bombina orientalis*) are Asian species suspected to be agents in the spread of chytrid fungus worldwide, in part due to their popularity in the pet trade (photos: T. Pierson, T. Leenders). Bottom: the harlequin frog (left; *Atelopus varius*) from Central America through Colombia, and salamandra chica (right; *Bolitoglossa chica*) from northwestern Ecuador have experienced precipitous declines attributed mainly to chytridiomycosis outbreaks (photos: B. Gratwicke, S. Ron).

salamander genomes have become available only now. Few other complete genomes have been obtained because the very large genomes of many frogs and all salamanders are a major impediment. Genome sizes range as high as nearly 15 Gb for frogs and well over 100 Gb for salamanders. Species with very large genomes experience greatly reduced cell cycle times, slow development and long generation times, as well as very low basal metabolic rates. These factors would seem to be highly detrimental and one would expect natural selection to have weeded out the extraordinarily abundant and highly repetitive DNA were it not for some compensating factor. The nature of that compensation, however, remains unknown and constitutes a major research challenge because for the species with the largest genomes the costs are great (e.g., increased developmental time, extended age at first reproduction).

Threats to amphibians

The first worldwide assessment of the status of amphibians by the International Union for Conservation of Nature (IUCN) published in 2004 found an astounding 43% of species to be experiencing population decreases; 7.4% were in the highest category of risk. This is the highest level of endangerment of any major vertebrate taxon and is especially worrisome because 22.5% of the then-known species (5743) were so poorly known that they could not be assessed. These species are also likely to be in high-risk categories. In the intervening years, the situation has only worsened and the proportion of insufficiently known species has increased.

While amphibians might appear to be vulnerable to environmental challenges because of their moist skins and life history traits, they are, in fact, examples of resilience, having survived previous mass extinction events and being successful in diverse habitats from lowland rainforests to deserts and extreme high elevations. Their resilience makes it all the more challenging to explain some of the current declines.

The known causes for amphibian declines are many and complex and include such widely understood factors as habitat destruction, the clearing of forests for timber and replacement with agricultural land, expansion of urbanization and drainage of wetlands. Other factors include the widespread use of pesticides and fertilizers and the influence of increasing environmental temperatures and droughts associated with climate change. What has attracted the greatest attention, however, is a pathogenic chytrid fungus of very wide distribution that has had a demonstrated devastating impact on amphibians worldwide (Figure 4). The pathogen, *Batrachochytrium dendrobatidis* (or Bd), was discovered only at the end of the last century and since then has become a major focus of investigation. The microscopic organism invades the permeable skin and disrupts the osmotic balance of the infected individual, ultimately killing it by inducing heart failure. Bd was first detected in outbreaks of extreme mortality in amphibian-rich environments of the Australian wet tropics and upland cloud forests in Costa Rica and Panama. Massive mortality also occurred in formerly abundant mountain yellow-legged frogs in the high Sierra Nevada in the iconic national parks (Yosemite, Sequoia Kings Canyon) of California. Subsequently, outbreaks have been traced to the early 1970s in Mexico, and there is circumstantial evidence of even earlier events elsewhere. The fungus is likely to have arisen in eastern Asia, where it is endemic and seems to cause little harm, and to have spread out, possibly from Korea, near the middle of the 20th century. Mitigation of the effects of the chytrid has included promising studies of the skin microbial community to enhance the frog's own immune system. In some areas, Australia for example, there has been recovery from the initial outbreaks. At present, however, Bd constitutes the major threat to large numbers of frog species and communities.

Returning to the Fire Salamander of European legend, it is deeply ironic that being the epitome of a survivor, even of fire, they are suffering a steep decline in one of their last redoubts in The Netherlands, a small forested area just north of Maastricht. In this area, researchers recently discovered another new, highly virulent, single-celled chytrid fungus, *Batrachochytrium salamandrivorans* (Bsal). In addition to the chaos caused by Bd, an even more deadly pathogen is now wreaking havoc. Fortunately, the new chytrid fungus is largely restricted to the immediate site of discovery, but in the time since discovery, it has been found in nearby parts of Belgium and Germany and the threat of spread to other species and areas is high. It seems to be endemic and enzootic in species of the Family Salamandridae in eastern Asia and it is likely to have been introduced to Europe through the pet trade. In both Europe and North America, new restrictions have brought importation nearly to a halt. Whereas Bd can kill individual amphibians, some recover, and some immunity has been detected, so far as is known, outside its native range, Bsal is lethal. Other infectious diseases also affect amphibians. While most are relatively poorly known and not associated with any mass mortality events, ranaviruses have been known for decades and they can be a significant factor in local populations.

Amphibian diversity and distribution

Paradoxically, in this era of great concern for amphibian survival, more and more new species are being discovered. Some of these are not new discoveries but rather subdivision of long-known species detected through the use of molecular markers. The majority are new discoveries. The numbers are impressive. As of August 2018, 7900 species of amphibians are known, about twice the species known in 1985. Roughly three new species are being described per week! Many of the new species reflect the greatly increased focus on the study of amphibians and the numbers of scientists being recruited. Field biologists are probing ever deeper, into the last unexplored places on this planet. Areas that are particularly rich in new species include Madagascar, Papua New Guinea and several South American countries. It is not just unexplored areas that are producing new species; in 2017 and 2018, new species were recorded from the United States and many new species were found in urban areas of India and China.

South America is the richest area for frogs, with Brazil having over 1000; Ecuador has 586 frogs, which contrasts with 28 for Spain, a country nearly twice as large. Salamanders are more northern in distribution, with United States having almost 200 species. While salamanders in the Old World barely enter the tropics, the lungless salamanders, Plethodontidae, have undergone an adaptive radiation in the New World tropics (over 40% of all salamanders), where they extend south of the 20th parallel in Bolivia. Caecilians occur only in tropical countries. Perhaps the most surprising center of caecilian diversity is the Seychelles islands (with eight species) in the Indian Ocean; India has the most caecilians (nearly 40 species).

Amphibians are key components of ecosystems throughout the world, and they may be the most abundant vertebrate in many local communities despite escaping notice by most people. They are often the top predator of invertebrates in ecosystems, and the most abundant vertebrates by biomass. Frogs and salamanders are a critical food source for other vertebrates. They are richly represented in tropical regions where they have radiated especially along mountain slopes. Frogs and salamanders are familiar animals, enjoyed by the public, studied by many scientists, and the focus of biomedical and environmental research. Today amphibians are in crisis, often victims of anthropogenic factors and novel pathogens, but they are long-term survivors and many species continue to thrive. Frogs, salamanders and caecilians promise to be with us for the long term.

FURTHER READING

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Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA 94720-3160, USA.
*E-mail: wakelab@berkeley.edu

Correspondence

Orangutan populations are certainly not increasing in the wild

Erik Meijaard[1,2,9,*], Julie Sherman[3], Marc Ancrenaz[1,4], Serge A. Wich[5,6], Truly Santika[2], and Maria Voigt[7,8]

A recent report, published by the Government of Indonesia with support from the Food and Agricultural Organization and Norway's International Climate and Forest Initiative, states that orangutan populations (*Pongo* spp.) have increased by more than 10% in Indonesia from 2015 to 2017, exceeding the government target of an annual 2% population increase [1]. This assessment is in strong contrast with recent publications that showed that the Bornean orangutan (*P. pygmaeus*) lost more than 100,000 individuals in the past 16 years [2] and declined by at least 25% over the past 10 years [3]. Furthermore, recent work has also demonstrated that both Sumatran orangutans (*P. abelii*) and the recently described Tapanuli orangutan (*P. tapanuliensis*) lost more than 60% of their key habitats between 1985 and 2007, and ongoing land use changes are expected to result in an 11–27% decline in their populations by 2020 [4,5]. Most scientific data indicate that the survival of these species continues to be seriously threatened by deforestation and killing [4,6,7] and thus all three are Critically Endangered under the International Union for Conservation of Nature's Red List.

We applaud the Indonesian conservation authorities for providing publicly available documentation on forest management impacts, and for their use of quantitative measures of wildlife conservation progress [1]. Based on the above-mentioned discrepancy, however, we question whether appropriate methods and efforts were employed to assess management impacts on wildlife trends. For orangutan impact monitoring, the Indonesian government reported on nine monitoring sites, including national