

# The Chinese giant salamander exemplifies the hidden extinction of cryptic species

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Overexploitation, habitat destruction, human-driven climate change and disease spread are resulting in the extinction of innumerable species, with amphibians being hit harder than most other groups [1]. Few species of amphibians are widespread, and those that apparently are often represent complexes of multiple cryptic species. This is especially true for salamanders, which typically show highly restricted distribution [2]. Here, we test in the widespread and critically endangered Chinese giant salamander (*Andrias davidianus*) how genetically uninformed management efforts can negatively affect species conservation. We show that the Chinese giant salamander appears to consist of at least five species-level lineages. However, the extensive recent exchange of individuals between farms, where the vast majority of extant Chinese giant salamander now live, has resulted in demonstrable genetic homogenization. Mitochondrial DNA (mtDNA) haplotypes of Chinese giant salamander from northern China now predominate in farms across the country. Unfortunately, these hybrids are being released back into the wild under well-intentioned, but misguided, conservation management. Our findings emphasize the necessity of genetic assessments for seemingly well-known, widespread species when proposing and pursuing conservation initiatives. Species serve as the primary unit for protection and management in conservation actions [3], so determining the taxonomic status of threatened species is a major concern, especially for amphibians. The level of threat to amphibians, may be underestimated, and existing conservation strategies may be inadvertently harmful if conducted in the absence of genetic data.

At a length of two meters, the Chinese giant salamander is the largest recognized extant species of amphibian. It is endemic to China and belongs to Cryptobranchidae, which diverged from other amphibians during the Mid-Jurassic Period; there are only two other living species of cryptobranchid. Once common and widespread in China, nowadays it is rare in the wild due to habitat destruction and overexploitation for food [4]. The IUCN lists the Chinese giant salamander as Critically Endangered. It is listed in Appendix I of CITES and in China it is given 'Class II' protection, and artificial breeding has been encouraged as a possible conservation measure. Captive breeding of this species, however, currently takes place almost exclusively in

commercial farms. Second-generation offspring can be traded legally and individuals weighing two kilograms have been sold previously for more than RMB 10,000 (US \$1500). Today, millions of Chinese giant salamanders live in farms and their progeny have been released into local rivers as part of government-promoted conservation action, but without pre-release assessments such as genetic testing or screening for disease [4].

Almost 20 years ago, preliminary molecular analysis revealed that the Chinese giant salamander population in Huangshan, Anhui Province, had divergent mtDNA and apparently fixed allozyme differences compared to samples from elsewhere [5]. Considering its limited ability to disperse, broad distribution and long evolutionary history, it is possible that the Chinese giant salamander is a composite of more than one cryptic species. In such cases, “bad taxonomy can kill” [6], i.e. some cryptic species may go extinct due to a lack of awareness of their existence and conservation requirements.

Over the past 10 years, we acquired tissue samples from 70 wild-caught and 1034 farm-bred individuals to investigate taxonomy of the Chinese giant salamander. Genetic analyses of the wild-caught individuals based on 23,159 SNPs (single nucleotide polymorphism) and mtDNA show that the Chinese giant salamander once consisted of at least five distinctive clusters (Figure 1; Supplemental information), and possibly more unrecognized species (farm-bred individuals grouped to mtDNA haplotype clades U1, U2 in Figure 1A and Tibetan Plateau population; Supplemental information). Species lineages A–E associate with separate river drainages, and which diverged 4.71 to 10.25 million years ago (Mya) (Supplemental information). Distinct species require distinct conservation actions. However, current decision making regarding Chinese giant salamander conservation treats all populations as a single panmictic species. Of greatest concern, Chinese giant salamander farms are the source of animals for reintroduction programmes, and this commercial activity has led to extensive trade and movement of animals between farms across the range of the Chinese giant salamander in China [4]. Genetic analyses based on mtDNA and microsatellite data for farmbred individuals reveal broad genetic mixing (Figure 1C; Supplemental information). In particular, introgression mostly involves species B from the Yellow River in Shaanxi, the site of the earliest and largest commercial breeding center. The mixing of species through farming has, therefore, led to the hybridization of Chinese giant salamander species that diverged over four million years ago. This is not surprising, because hybridization of introduced Chinese giant salamander with Japanese giant salamanders occurs in Japan [7].

Bad taxonomy can drive extinction, and one way in which this can happen is by releasing hybrid and non-native individuals into the wild [6]. Since 2008, at least 72,000 Chinese giant salamanders have been released from farms. To what effect? Individuals recently caught from tributaries of the Pearl and Yangtze rivers were found to possess mitochondrial haplotypes of species B from the Yellow River, but no indigenous haplotypes [8]. By releasing huge numbers of farmed Chinese giant salamander, this genetically uninformed strategy may eradicate the evolutionary uniqueness of native allopatric populations and drive extinction by genetic homogenization [9].

Molecular analyses have revealed that species diversity is underestimated, especially in amphibians [10]. This indicates that many species remain unidentified because of a lack of morphological differentiation, even those as large and seemingly familiar as the Chinese giant salamander. Taxonomic uncertainty may preclude effective conservation, and such questions require answering before investing huge sums of money and effort. Our results indicate that the existing conservation strategy for Chinese giant salamanders, and other highly threatened species, requires urgent updating. We recommend that population genetics be performed for all

threatened taxa, in particular those in current or future conservation breeding programmes.

### Supplemental Information

Supplemental Information including experimental procedures, one figure and one table can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2018.04.004>.

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**Figure 1. Distributions of mtDNA haplotype clades and genetic structure of wild-caught and farm-bred Chinese giant salamanders.** (A) A simplified Bayesian inference tree based on concatenated mtDNA haplotypes. Numbers near branches are posterior probabilities (BPP $\geq$ 0.90). A–E are wild-caught populations and U1 and U2 are known from farms only. The complete tree is given in Figure S1A. (B) Sampling sites for wild-caught individuals. Lower-right insert shows the best genetic clustering (K=5) based on genomic SNPs from localities circled in dash line. (C) Sampling sites for farm-bred individuals where pie-charts show the proportions of mitochondrial haplotypes as grouped in part A. Lower right insert shows the second-best cluster (K=3; optimal K=1, supplemental information) based on microsatellite data for farm-bred individuals from Guizhou (farms circled in dashed line). Colours denote cryptic species lineages.

Figure 1.

