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**Where do we go from here?** Muscle provides an exciting opportunity to explore the molecular mechanisms underlying cell fate and polarity. Specifically, emerging questions involve exploring signalling involved in establishment of spindle orientation, as this is the causal driver of asymmetric division following polarity establishment; defining the hierarchy of fate determinants sequestered in a polarized satellite cell and their function post-mitosis to identify drug-able targets involved in satellite cell commitment; and exploring multifaceted approaches promoting stem cell self-renewal as well as commitment to augment long-term muscle regeneration with age and disease.

#### Where can I find out more?

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# 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 are often represent complexes of multiple cryptic species. This is especially true for range-restricted salamanders [2]. Here, we used the widespread and critically endangered Chinese giant salamander (*Andrias davidianus*) to show how genetically uninformed management efforts can negatively affect species conservation. We find that this salamander consists of at least five species-level lineages. However, the extensive recent translocation of individuals between farms, where the vast majority of extant salamanders now live, has resulted in genetic homogenization. Mitochondrial DNA (mtDNA) haplotypes from northern China now predominate in farms. Unfortunately, hybrid offspring 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 in 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 without genetic assessment.

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 \$1,500). 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.

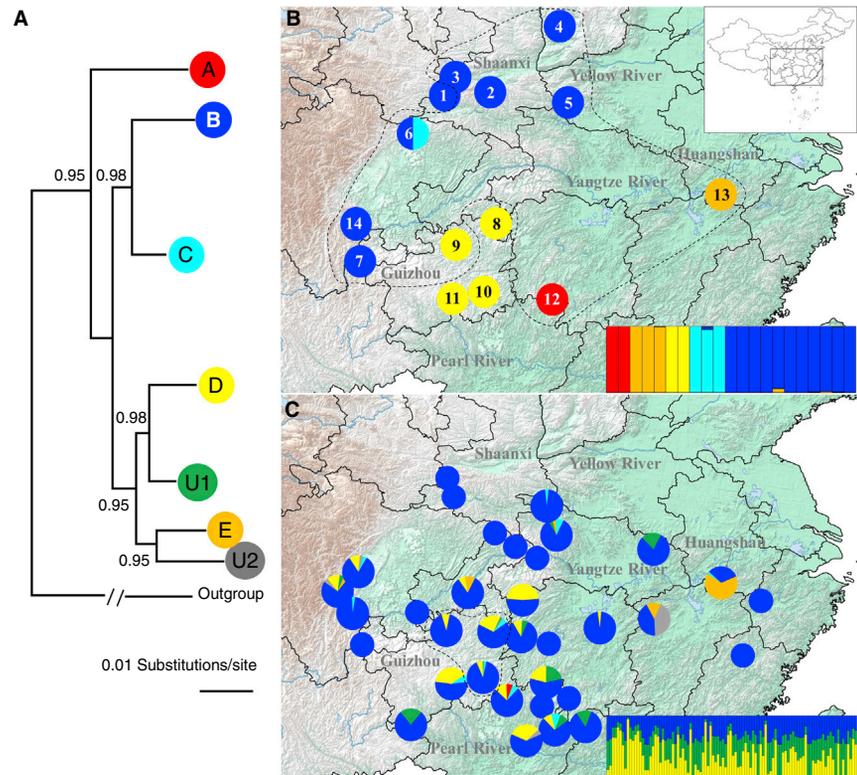
Over the past 10 years, we acquired tissue samples from 70 wild-caught and 1034 farm-bred individuals to investigate taxonomy of the Chinese giant salamander. Genetic analyses



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 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 farm-bred 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



**Figure 1. 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 dashed line. (C) Sampling sites for farm-bred individuals where pie charts show the proportions of mitochondrial haplotypes as grouped in (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.

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, acknowledgments, 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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# Imminent extinction in the wild of the world's largest amphibian

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Species with large geographic ranges are considered resilient to global decline [1]. However, human pressures on biodiversity affect increasingly large areas, in particular across Asia, where market forces drive overexploitation of species [2]. Range-wide threat assessments are often costly and thus extrapolated from non-representative local studies [3]. The Chinese giant salamander (*Andrias davidianus*), the world's largest amphibian, is thought to occur across much of China, but populations are harvested for farming as luxury food [4]. Between 2013 and 2016, we conducted field surveys and 2,872 interviews in possibly the largest wildlife survey conducted in China. This extensive effort revealed that populations of this once-widespread species are now critically depleted or extirpated across all surveyed areas of their range, and illegal poaching is widespread.

Chinese legislation prohibits harvesting wild Chinese giant salamanders, but China's Ministry of Agriculture supports releases of farmed animals, a strategy that fails to meet IUCN reintroduction guidelines and may be harmful to wild populations (e.g., mixing genetic lineages; spreading pathogens) [4]. Chinese giant salamanders now detected in the wild might therefore represent releases or escapes. We conducted a four-year survey to investigate the status of giant salamanders across China at sites in 97 counties selected using historical records or habitat-suitability modelling [3]. All sites contained intact habitat and diverse amphibian faunas. Fieldwork was conducted in

May–October (peak-activity period of the closely-related Japanese giant salamander *A. japonicus* [5]). Surveys covered a cumulative 1 km transect of suitable river habitat at each site and used active (searching) and passive (trapping) techniques [6]. Environmental DNA detection was precluded due to likelihood of false positives from farm discharge. Fieldwork represented 7.20 cumulative person-weeks of active searching and 7.33 person-years of passive searching, and detected 24 Chinese giant salamanders at four sites: Liannan (Guangdong), 17 (active search = 11, passive search = 6); Jiangkou (Guizhou), 1 (passive search); Lüeyang (Shaanxi), 5 (active search = 1, passive search = 4); Zhouzhi (Shaanxi), 1 (passive search). This represents a catch-per-unit-effort (CPU) of 16.23 weeks/Chinese giant salamander (active search CPU = 4.20 days, passive search CPU = 222.97 days) (Figure 1A,B). This effort is substantially greater than for other cryptobranchids (*A. japonicus*, 1.2 hours active searching; hellbender *Cryptobranchus alleganiensis*, 2.2 hours active searching) [5,7]. Illegal traps, bow hooks, and/or evidence of electro-fishing or poison (known methods for harvesting Chinese giant salamanders) were detected at 24 sites, including within protected areas (Supplemental Information).

We verified our findings using local ecological knowledge. Chinese giant salamanders are economically significant and easily identifiable, making them suitable targets for surveys [8]. We conducted interviews within 1 km of surveyed rivers, using a standard questionnaire [6]. Of our respondents, 85.5% recognised Chinese giant salamanders and 46.9% reported sightings, but mean last-sighting date was 18.96 years earlier (range = 0–71 years; SD = 14.7; Supplemental Information). Although 9.6% of reports dated from within 5 years, the temporal distribution of sightings is not unimodal (Hartigan's dip test,  $D = 0.046$ ,  $p < 0.001$ ), a pattern not seen for other declining species [8]; this may represent an older peak of wild sightings and a younger peak of sightings of releases/escapes (Figure 1C). We pooled local ecological knowledge data for the four counties in which Chinese giant salamanders were detected, and compared these pooled data against each of the other

