Supplemental Information: Imminent extinction in the wild of the world's largest amphibian

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SUPPLEMENTAL FIELDWORK

Site selection. Survey sites within each of the 97 surveyed counties were selected through consultation with local fisheries bureaus, who identified areas of suitable CGS habitat. As any surviving CGS populations are clearly at high risk of continued exploitation, we do not report precise survey localities [S1, S2].

Ethics and permissions. All field research complied with protocols approved by the relevant fisheries and forestry bureaus of each county and province where fieldwork was conducted, and adhered to the legal requirements of the People's Republic of China. The Zoological Society of London's Ethics Committee approved project design (WLE569).

Evidence of poaching. Known methods for harvesting CGS were detected at survey sites in the following counties: **(i) Illegal traps** in Qimen, Xiuning and Yixian (Anhui), Chengkou (Chongqing), Pingnan (Fujian), Badong (Hubei), Jingan (Jiangxi), Mabian and Xingwen (Sichuan), and Yiliang (Yunnan). **(ii) Bow hooks** in Jingde (Anhui), Pengshui (Chongqing), Kaili and Songtao (Guizhou), Badong (Hubei), and Xingwen (Sichuan). **(iii) Evidence of electro-fishing** in Jingde

(Anhui), Jinxiu (Guangxi), Danzhai and Kaili (Guizhou), Xingwen (Sichuan), and Pan'an (Zhejiang). **(iv) Evidence of poison** in Guiping, Xilin and Ziyuan (Guangxi), Huangping (Guizhou), Baokang, Danjiangkou and Macheng (Hubei), Hengshan (Hunan), and Xingwen (Sichuan).

SUPPLEMENTAL INTERVIEW DATA ANALYSIS

Data treatment. Respondents reported sighting records using a variety of different methods for describing the timing of past events, and we converted alternative formats to direct calendar years for analysis using the approach described by ref. S3. As interviews were conducted across multiple years due to the logistical demands of fieldwork, we then converted sighting records to number of years before the date on which interviews were conducted, to allow comparison between sites.

Statistical methods. We used generalised linear models to determine whether there were differences between counties in: **(i)** the number of respondents who reported having seen a wild CGS (using binomial error structure); **(ii)** the time since respondents last saw a wild CGS (using poisson error structure); and **(iii)** the number of respondents who reported that CGS had experienced a local decline (using binomial error structure). In all models, we included respondent age, sex, and whether they were fishers as known covariates that could affect the probability that they had awareness or experience of local CGS populations.

Results. (i) CGS sightings: We found significant differences between counties (X^2=1576.3, p<0.001), and overall the model explained 46.7% of the variation in responses (df=2771, McFadden R^2=0.467), whereas a model without county explained just 7.0% of variation (df=2865, McFadden R^2=0.070). **(ii) CGS**

sighting dates: We found significant differences between counties (X^2=340.5, p<0.001), and overall the model explained 27.8% of the variation in responses (df=1101, McFadden R^2=0.278), whereas a model without county explained just 10.9% of variation (df=1183, McFadden R^2=0.109). **(iii) CGS declines:** We found significant differences between counties (X^2=1594.8, p<0.001), and overall the model explained 43.8% of the variation in responses (df=2771, McFadden R^2=0.438), whereas a model without county explained just 3.0% of variation (df=2865, McFadden R^2=0.030).

The estimates produced by the models were odds ratios, where 1 denotes equal odds. For each county, the estimated difference in CGS sightings and perceptions of decline was calculated in comparison to the estimate for the pooled counties that did have CGS detections. If the 95% confidence interval of this difference did not include 1, we considered that county to be significantly different from counties with CGS detections.

SUPPLEMENTAL GENETIC ANALYSIS

Methods. Non-invasive buccal swab samples were collected from 12 of the wild-caught CGS encountered in our field survey (Liannan, 10; Jiangkou, 1; Zhouzhi, 1). Total genomic DNA was isolated using a TIANamp Swab DNA Kit (Tiangen, Beijing, China) and frozen at -20°C. The mitochondrial cytochrome oxidase I (*COI*) gene was amplified using specific primers (CGSCOIF/CGSCOIFR) designed for CGS [S4]. A polymerase chain reaction (PCR) was carried out in a 25μL reaction including 2.5μL of 10X buffer (with 2mM MgCl2), 1μL of dNTP (0.125mM), 1μL of each primer (3μM), 1UrTaq DNA Polymerase (TaKaRa), 2μL of total DNA (20ng/μL), and sterile water to complete the final volume. The

reactions were performed using the following procedure: initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. The PCR products were purified with a modification of the Exo-SAP method [S5]. The purified products were sequenced in both directions with a BigDye Terminator Cycle Sequencing Kit and an ABI PRISM 3730 (Applied Biosystems) automatic DNA sequencer. Each sequence was proofread and assembled with DNAStar 5.0. The nucleotide sequence dataset was aligned, edited, and trimmed using MEGA 6 [S7]. Haplotype sequences were generated using DNA Sequences Polymorphism (DnaSP) program version 5.10.01 [S7].

Results. Approximately 432 base pairs (bp) of *COI* from the 12 wild-caught CGS individuals were sequenced (GenBank accession numbers XXXX – *to be added upon acceptance of the paper*). Wild CGS populations are known to exhibit geographic structuring associated with different river drainages [5, S4]. However, although the wild-caught CGS individuals sequenced in this study are from three different river drainages (Zhouzhi is in the Yellow River drainage, Jiangkou is in the Yangtze River drainage, and Liannan is in the Pearl River drainage), all of these individuals share the same haplotype, which is native to individuals from the Yellow River (haplotype 2, lineage B in ref. S4).

S1. Meijaard, E., and Nijman, V. (2014). Secrecy considerations for conserving Lazarus species. Biol. Cons. *175*, 21–24.

S2. Yang, J.H. & Chan, B.P. (2016). Two new species of the genus *Goniurosaurus* (Squamata: Sauria: Eublepharidae) from southern China. Zootaxa *3980*, 67–80.

S3. Turvey, S.T., Bryant, J.V., Duncan, C., Wong, M.H.G., Guan, Z., Fei, H., Ma, C., Hong, X., Nash, H.C., Chan, B.P.L., Yang, X., and Fan, P. (2017). How many remnant gibbon populations are left on Hainan? Testing the use of local ecological knowledge to detect cryptic threatened primates. Am. J. Primatol. *79*, e22593.

S4. Yan, F., Lü, J., Zhang, B., Yuan, Z., Zhao, H., Huang, S., Wei, G., Mi, X., Zou, D., Xu, W., Chen, S., Wu, M., Xiao, H., Liang, Z., Tapley, B., Turvey, S.T., Papenfuss, T.J., Cunningham, A.A., Murphy, R.W., Zhang, Y., and Che, J. The Chinese giant salamander exemplifies the hidden extinction of cryptic species. Curr. Biol., submitted.

S5. Werle, E., Schneider, C., Renner, M., Völker, M., and Fiehn, W. (1994). Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acids Res. *22*, 4354–4355.

S6. Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. *30*, 2725–2729.

S7. Librado, P., and Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics *25*, 1451–1452.