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Methodological guidelines for minimally invasive tail-clipping: a case study on *Rana huanrenensis* tadpoles

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ABSTRACT

Tail clipping of amphibian tadpoles is one of the minimally invasive, non-lethal procedures, (apart from skin swabbing) used to collect tissues without euthanising the target individual. It is commonly used for species identification, especially when the continuity between the tadpoles and adult stages is not known. However, there is a lack of published standard and safe protocol for tail clipping of anuran tadpoles. To determine the efficiency of the protocol defined herein, we tail clipped 3.0 mm of four *Rana huanrenensis* tadpoles (Fei, Ye & Huang, 1990), two at each of the Gosner stages 34 and 41. We observed the tails resorbing from tail length = 20.625 ± 0.64 mm on day 0 post-clipping to 5.75 ± 3.49 mm on day 6 post-clipping. During this period, metamorphosis progressed for individuals tail-clipped at Gosner stage 34 (total length: 33.75 ± 2.35 mm; day 0 post-clipping) to Gosner stage 43 (total length: 28.5 ± 3.47 mm; day 6 post-clipping); and individuals tail-clipped at Gosner stage 41 (total length: 35.75 ± 0.35 mm; day 0 post-clipping) to Gosner stage 46 (total length: 15.00 ± 0.00 mm; day 6 post-clipping). We did not record any fatality during the experiment. DNA extracted from 3.0 mm of tail tip tissue yielded gDNA concentrations between 10 and 32 ng/µl, a sufficient amount for barcoding and fingerprinting. We conclude that this protocol is adequate for *R. huanrenensis* and Ranidae in general, and it is safe for tadpoles at Gosner stage 34 and above.

Key words: tail clip, Huanren Frog, larvae, DNA collection, minimally invasive