

## Oviposition sites of the Hoang Lien Horned Frog, *Megophrys hoanglienensis* (Tapley et al., 2018)

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The genus *Megophrys* Kuhl & Van Hasselt, 1822 comprises 111 species in seven subgenera (Mahony et al., 2017; Frost, 2021). Other than advertisement calls and larval descriptions there are very few published observations of breeding behaviour and oviposition sites of *Megophrys*. Male frogs of the subgenus *Brachytarsophrys* are reported to call from within deep crevices between boulders in streams (Smith, 1921; Tapley, 2011) and guard egg clutches that are laid in water (Stuart, 2005). *Megophrys (Brachytarsophrys) platyparietus* is known to oviposit eggs on the underside of boulders (Li et al., 2020). *Megophrys (Xenophrys) cf. major* have been reported to lay clutches with hundreds of white eggs, each with a diameter of approximately 1.9 mm (Vassilieva et al., 2016). *Megophrys (Ophryophryne) synoria* lay clutches of more than 800 white eggs with a diameter of 2.5–2.6 mm that are attached to stones or leaf litter on the bottom of streams (Vassilieva et al., 2016). For *M. (Pelobatrachus) nasuta*, clutches of approximately 500–1800 large white eggs on oviposition sites such as pieces of wood protruding from water have been documented; eggs measure approximately 2 mm in diameter and are covered by a gelatinous layer (Schmidt, 1976; Selveindran, 2014). Lastly, *M. (Panophrys) omeimontis* are reported to lay 282–429 eggs in a clump that is adhered to the bottom of the stream (Fei et al., 2012).

*Megophrys (Panophrys) hoanglienensis* (Fig. 1A) is endemic to the Hoang Lien Range in northwestern

Vietnam and likely qualifies for an assessment as Endangered (Tapley et al., 2018). The species is associated with streams in broadleaf forests at elevations from 1898–2242 m (Tapley et al., 2018). Males are known to call from streamside vegetation and rocks in June, and larvae at stage 26 (Gosner, 1960) have been collected in a 5 m wide stream with rocky substrate in September (Tapley et al., 2020).

On 11 June 2018 we encountered several clusters of eggs on Mount Fansipan, at a site bordering Sa Pa District, Lao Cai Province, and Tam Duong District, Lai Chau Province (22.3422°N, 103.7764°E; elevation 2000 m). One cluster of eggs was adhered to the underside of a muddy bolder overhanging a seepage with a gravel substrate (Fig. 1B). Another cluster of eggs was floating in very shallow water nearby. Several clusters of eggs were found submerged in shallow water in a swampy area beside the same seepage (Fig. 1C). Eggs within each cluster adhered together (Fig. 1D).

To measure the eggs, we used ImageJ 1.49 (Schneider et al., 2012) on photographs of freshly collected eggs that were taken next to a scale. Mean egg diameter was 2.73 mm (2.65–2.82 mm,  $n = 20$ ). Eggs were a uniform cream colour and surrounded by a fluid filled capsule approximately 0.8 mm wide (Fig. 1D). There was no indication that the eggs had started to develop. One of the clusters was collected as a voucher, with several eggs preserved in ethanol; the remaining eggs were preserved in formalin. Vouchers were deposited at the Vietnam National Museum of Nature (VNMN 010911).

Species identity of the eggs was confirmed using molecular analysis of one of the ethanol-preserved egg specimens (following the methodology of Rowley et al., 2017). The new 16S rDNA sequence generated from the specimen was identical to that from the holotype of *M. hoanglienensis* collected 800 m away on Mount Fansipan (GenBank accession number MH514889.1). The new sequence was deposited in GenBank under the accession number MW536998.

This observation confirms that *M. hoanglienensis* breeds in June, coinciding with observations of male *M.*

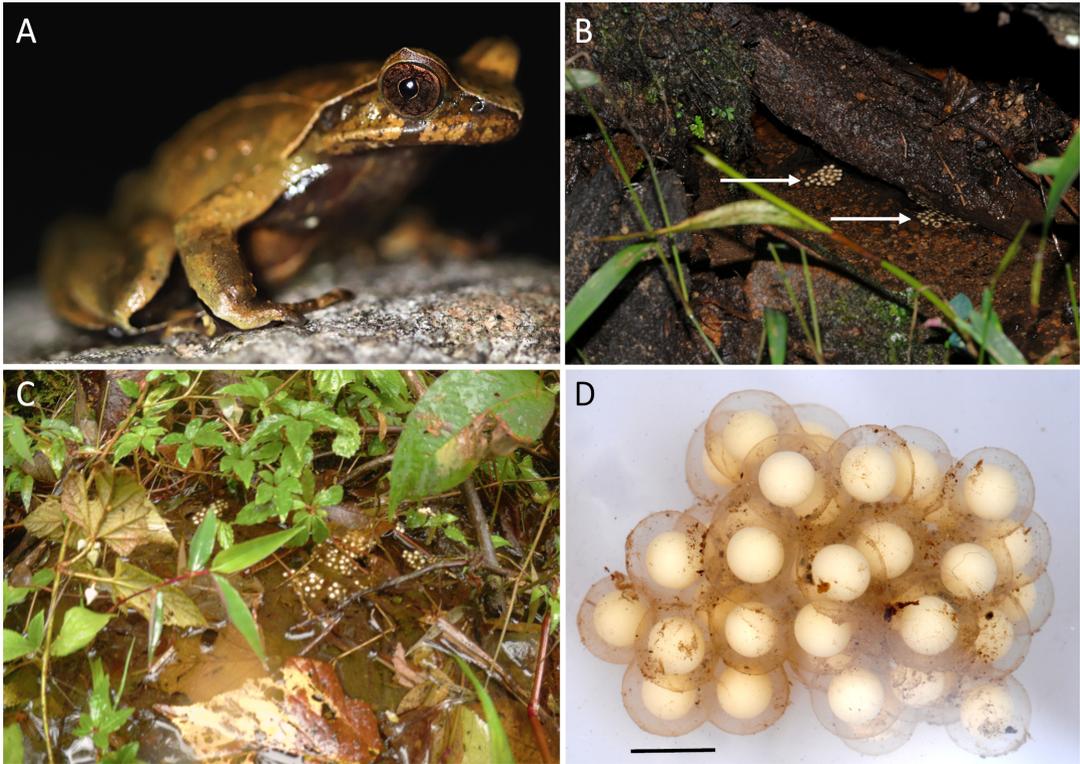
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**Figure 1.** (A) Adult male *Megophrys hoanglienensis* in situ, Mount Fansipan, Lao Cai Province, Vietnam; (B) *Megophrys hoanglienensis* eggs at the presumed oviposition site, Mount Fansipan, Lao Cai Province, Vietnam. White arrows highlight the position of the clutches; (C) *Megophrys hoanglienensis* eggs that may have been washed away from the oviposition site; (D) *Megophrys hoanglienensis* eggs. Scale = 5 mm. Photographs by Benjamin Tapley and Luan Thanh Nguyen.

*hoanglienensis* calling during this month (Tapley et al., 2018). We are unable to comment on clutch size as it was not possible to determine whether clumps of eggs represented single clutches or not. It is possible that the clumps of eggs that were found floating in water had become detached from a substrate to which they had previously been adhered, possibly by heavy rain. Eggs of *M. nasuta* were washed away from oviposition sites in Singapore (Selveindran, 2014). The oviposition site was 3.0 m away from a stream and, given that tadpoles of *M. hoanglienensis* have been found in large streams (Tapley et al., 2020), they may be inadvertently flushed into streams by rainfall. Alternatively, tadpoles may deliberately access streams via the seepages where they complete larval development. It is also possible that *M. hoanglienensis* may lay eggs in large streams or on stream banks. This observation provides important new life history information on this potentially highly threatened amphibian.

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