

# Development of assisted reproductive technologies for the conservation of *Atelopus* sp. (spumarius complex)

[Renato E Naranjo](#)<sup>1</sup>, [Elena Naydenova](#)<sup>2</sup>, [Carolina Proaño-Bolaños](#)<sup>3</sup>, [Karla Vizúete](#)<sup>4</sup>, [Alexis Debut](#)<sup>4</sup>, [Marbel Torres Arias](#)<sup>5</sup>, [Luis A Coloma](#)<sup>2</sup>

Affiliations collapse

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- <sup>1</sup>Centro Jambatu de Investigación y Conservación de Anfibios, Fundación Jambatu, Giovanni Farina 566 y Baltra, 171102, San Rafael, Quito, Ecuador; Dirección Nacional de Biodiversidad, Ministerio del Ambiente, Agua y Transición Ecológica, Madrid 1159 y Andalucía, 170525, Quito, Ecuador. Electronic address: renato3141@hotmail.com.
- <sup>2</sup>Centro Jambatu de Investigación y Conservación de Anfibios, Fundación Jambatu, Giovanni Farina 566 y Baltra, 171102, San Rafael, Quito, Ecuador.
- <sup>3</sup>Biomolecules Discovery Group, Laboratory of Molecular Biology and Biochemistry, Universidad Regional Amazónica Ikiam, km 7 ½ vía Muyuna, 150150, Tena, Ecuador.
- <sup>4</sup>Centro de Investigación en Nanociencia y Nanotecnología, Universidad de las Fuerzas Armadas ESPE, Sangolquí, 171103, Ecuador.
- <sup>5</sup>Laboratorio de Inmunología y Virología, Departamento de Ciencias de la Vida y Agricultura, Cencinat, Gisah, Universidad de las Fuerzas Armadas ESPE, Sangolquí, Ecuador.
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## Abstract

Amphibians are in peril, given the ongoing sixth mass extinction of wildlife. Thus, Conservation Breeding Programs (CBPs) are attempting to breed some species under laboratory conditions. The incorporation of assisted reproduction technologies (ARTs), such as hormonal stimulation, sperm collection and cryopreservation, and in vitro fertilization is contributing to successful CBPs. The objective of this study was to apply ARTs in sexually mature individuals of an undescribed species of *Atelopus* (spumarius complex) (harlequin frog). Our procedure involves hormonal induction of gametogenesis in this species. We were able to induce gamete release through administration of human chorionic gonadotropin (hCG) in males, and in females this has

been achieved through the sequential administration of hCG (priming doses), and combinations of hCG with gonadotropin releasing hormone analogue, GnRHa (ovulatory dose). We standardized sperm cryopreservation by performing toxicity tests of cryoprotectants, fast/slow freezing and thawing, as well as supplementation of non-penetrating cryoprotectants (sugars). Next, we performed in vitro fertilization, evaluated the fertilization capacity of the cryopreserved sperm, and describe external features of fresh and cryopreserved sperm. We found that 10 IU/g hCG induced the release of the highest sperm concentrations between 3 and 5 h post-injection, while 2.5 IU/g hCG induced the release of eggs in most treated females. Under cryopreservation conditions, the highest recovery of forward progressive motility or FPM was  $26.3 \pm 3.5\%$ , which was obtained in cryosuspensions prepared with the 5% DMF and 2.5% sucrose. Cryopreserved sperm showed narrower mitochondrial vesicles after thawing, while in frozen samples without cryodiluent showed 31% of spermatozoa lost their tails. In most cases, our attempts of in vitro fertilization were successful. However, only ~10% of embryos were viable. Overall, our study demonstrates that the development of ARTs in individuals of *Atelopus* sp. (spumarius complex) bred in laboratory can be successful, which result in viable offspring through in vitro fertilization. Our study provides a baseline for assisted breeding protocols applicable to other harlequin frogs of the genus *Atelopus*.

**Keywords:** *Atelopus*; Cryopreservation; Hormonal induction; In vitro fertilization; Spermic urine.