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 We use this protocol and it's working

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## Protocol for breeding *Hymenochirus boettgeri* in captivity

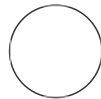
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Chytrid Fungi - Molecular and Genomic Techniques

Symbiosis Model Systems



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### ABSTRACT

*Hymenochirus boettgeri* is a sexually dimorphic species, with females typically exhibiting a larger oval-shaped body (up to 35 mm in snout-vent length (SVL)), while males possess a large obiculated tympanum and a lateral post-axillary subdermal gland that increases in size and vascularity during sexual activity (Figure 1).



Figure 1. *H. boettgeri* female (superior) and male (inferior) adult individuals. Black arrow points to post-axillary subdermal gland and black bar represent 1 cm scale.

GUIDELINES

**Conclusion:**

This protocol outlines the steps for breeding *H. boettgeri* in captivity. Careful selection of breeding pairs, proper feeding, and attentive observation are essential for successful breeding and egg collection. Regular water changes help maintain water quality during the breeding period.

MATERIALS


- Male *H. boettgeri* specimens
- Female *H. boettgeri* specimens
- Penn Plax Deluxe Net Breeder (17.15 cm × 12.07 cm × 13.34 cm; Hauppauge, NY, USA)
- 38 L glass tanks (25 × 30 × 50 cm) with plastic lids containing holes smaller than 1 cm in diameter for air circulation
- Aquarium heater (EHEIM thermocontrol 75 W, Deizisau, Germany)
- Aquarium filter (Tetra, Blacksburg, VA, USA)
- EcoTestr CTS1 meter (Oakton, Vernon Hills, IL, USA)
- Water testing kit (API Liquid Test Kits, API®, Chalfont, PA, USA)
- Gravel substrate (diameter > 1 cm to prevent ingestion by the animals)
- Artificial plants
- PVC pipes
- Reverse osmosis (RO) water
- Synthetic sea salt mixture (Instant Ocean® Sea Salt, Blacksburg, VA, USA)
- Sodium bicarbonate
- Brine shrimp (*Artemia* sp., BIO-MARINE®, Hawthorne, CA, USA)
- Frozen gamma-irradiated bloodworms (*Chironomus* sp., Tropical Marine Center, UK)
- Powdered fry food (Sera® Micron Nature, Heinsberg, Germany)
- Small-granule fish food (BUG BITES™ Tropical Formula, Mansfield, MA, USA)
- Nitrifying bacterial suspension (ProLine® Aquaculture Bacteria Concentrate, Apopka, FL, USA)
- Tricaine methanesulfonate (MS-222; Syndel, Ferndale, WA, USA)
- Virkon™ Aquatic (LANXESS, Pittsburgh, PA, USA)

**Sexual selection**

- 1 Select male *H. boettgeri* specimens with heavily vascularized glands and the largest females for breeding.

**Note**

Sexual maturity will depend on the conditions in which the animals were raised (e.g., space, diet, temperature). Observing the glands in males and an increased body size in females will indicate that they are sexually mature. In our systems, the male glands were first observed at 5 months of age, and females spawned for the first time at a minimum of 6 months of age.

- 2 Keep males and females in separate 38 L glass tanks by sex at  25 °C with conditioned water as described in the “Housing and Care for *Hymenochirus boettgeri*” protocol and 13:11 light-dark cycle.

## Feeding

- 3 Feed the frogs ad libitum twice per day for ten consecutive days, providing newly hatched brine shrimp as the first meal of the day, and offer either frozen gamma-irradiated bloodworms, live blackworms, or small-granule fish food as the second meal of the day, changing the combination daily if possible.

## Pair selection

- 4 After 10 days of enhanced feeding, choose breeding pairs consisting of one male and one female each.

## Mating tanks

- 5 Using an aquarium net, place each breeding pair in separate 8 L plastic tanks at 21 °C, with a minimum water volume of 2 L per individual.

### Note

These tanks should not have any enrichment, filters, or heaters to facilitate egg visualization and collection.

- 6 Allow the breeding pairs to remain together for one to four days, during which mating behavior is expected.
- 7 Observe the frogs closely during this period. If the pair does not reproduce, consider replacing one of them with another animal to create a new pair combination.

## Egg Collection and maintenance

- 8 After mating, collect the eggs as they are laid using a sterile siphon or spoon and transfer them to net breeder tanks as described in “Housing and Care for *Hymenochirus boettgeri*” protocol.

- 9 Check the eggs daily and remove any that contain fungi growing or that haven't hatched after 4 days of being laid.

## Water safety

- 10 During the breeding period, feed the animals and, if necessary, perform a water change as described in the "Housing and Care for *Hymenochirus boettgeri*" protocol.