SECTION 1. BIOLOGY AND FIELD DATA

BIOLGY

1.1 Taxonomy

Order Anura
Family Hemiphractidae
Subfamily Hemiphractinae
Genus Gastrotheca
Species Gastrotheca gracilis Laurent, 1969
Common name(s) La Banderita Marsupial Frog

1.2 Morphology

Length
Mean snout–vent length of 41.6 mm in males, and 44.9 mm in females (Duellman, 2015).

Colouration
The dorsum is dull grayish brown, with green markings. The postorbital stripe is green; the flanks are cream, with dark brown spots. The posterior surfaces of the thighs are pale brown, with cream flecks; the throat and belly are creamy white, with gray flecks; the ventral surfaces of the limbs are pinkish gray. The labial stripe is cream; the tympanum is bronze. The iris is dull bronze, with black flecks (Duellman, 2015). Dorsal coloration pattern in this species is extremely variable, including individuals with the dorsum completely homogeneous with flashy green coloration (Akmentins et al., 2014).
Description

The body is moderately robust. The head is wider than long, and as wide as the body. The snout is rounded in dorsal view, and is bluntly rounded in profile. The canthus rostralis is nearly straight, and is rounded in cross-section. The loreal region is concave; the lips are thin and rounded. The top of the head is flat. The interorbital distance is slightly greater than the width of the upper eyelid. The internarial area is flat. The nostrils are barely protuberant, and are directed dorsolaterally below the anterior terminus of the canthus rostralis. The diameter of the eye is about equal to its distance from the nostril, and is slightly more than twice its distance from the margin of the jaw. The tympanum is nearly round, and has a distinct smooth annulus; it is separated from the eye by a distance slightly greater than the length of the tympanum, the upper edge of which is obscured by a moderately heavy tubercular supratympanic fold extending from the posterior corner of the orbit to a point posteroverentral to the tympanum. The arm is moderately slender, and lacks distinct ulnar tubercles. The hand is small. The fingers are long, slender, and unwebbed. The discs are moderately small and ovoid; the width of the disc on the third finger is about equal to the length of the tympanum. The relative lengths of fingers are I < II < IV < III. The subarticular tubercles are moderately large and subconical; none are bifid. The supernumerary tubercles are large and subconical, and are numerous on the proximal segments of the digits. The palmar tubercle is elevated and trifid; the prepollical tubercle is elliptical; the thumb bears brown nuptial excrescences in males. The hind limb is moderately slender. The tibia length is about 50% of the snout–vent length; the foot length is about 47% of the snout–vent length. Calcars and tarsal tubercles are absent; an inner tarsal fold is distinct on the distal one-fourth of the tarsus. The outer metatarsal tubercle is small and rounded; the inner metatarsal tubercle is broadly ovoid, and is visible from above. The toes are moderately long. The relative lengths of the toes are I < II < III < V < IV. The outer toes are webbed basally. The subarticular tubercles are moderately large and subconical; the supernumerary tubercles are large, subconical, and present only on the proximal segments of the toes. The skin on the dorsal surfaces of the body and flanks bears low round tubercles; the skin on the throat, chest, belly, and ventral surfaces of the upper arms and thighs is strongly granular; the other surfaces are smooth. A pair of moderately large tubercles is present below the cloacal opening. The opening of the brood pouch is narrowly U-shaped, with the anterior end rounded at the level of the posterior edge of the sacrum (Duellman, 2015).

1.3 Longevity

Unknown in the wild, maximum 6 years in captivity (Akmentins, pers. obs.).

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1.4 Zoogeography/Ecology

Distribution

This species is endemic to Yungas Andean forests of north-western Argentina and is known from several localities in Catamarca and Tucumán Provinces in Argentina. Its elevation range is from 1,200–2,800 m of altitude. Its estimated extent of occurrence (EOO) is 1,407 km2 and all individuals occur in five or fewer threat-defined locations (IUCN, 2020).

Habitat

Adult specimens occur arboreally in tree crowns, tree holes and rock crevices in Yungas Andean forests and high altitude grasslands. This species persists in places with moderate habitat disturbance, such as road edges and secondary Yungas Andean forests (IUCN, 2020).
Population
The tree rediscovered populations seem stables in the last five years, but declining in the entire range (IUCN, 2020).

Conservation status
Endangered (IUCN, 2020); Endangered in the Argentinean red list (Vaira et al., 2012).

Threats in the wild
The major threat is habitat loss caused by human activities as wildfires, deforestation, and road maintenance. Domestic animals can cause mass mortality of tadpoles and reproductive habitat alteration (IUCN, 2020).

1.5 Diets and Feeding Behaviour

Food preference in nature
Unknown

Feeding
Unknown

1.6 Reproduction

Developmental stages to sexual maturity
Tadpoles, metamorphs, juveniles, adults

Age of sexual maturity/first breeding
Males start calling before the year of age (Akmentins, pers. obs.). Unknown for females.

Seasonality of cycling
Gastrotheca gracilis had a winter-spring reproduction (Laurent et al., 1986).

Gestation period/incubation
Females retain the fertilized eggs in the dorsal pouch and the larval development continues inside the pouch until Gosner’s stage 26 (Laurent, 1976). Dorsal pouch gestation last approximately two months (Laurent et al., 1986).

Clutch size
Up to 73 eggs (Laurent et al., 1986)

Birth/hatching details and seasons
Tadpoles are found in the field form November to March, late spring and summer (Akmentins, pers. obs.).
1.7  **Behaviour**  
*Activity*  
Crepuscular and nocturnal. Year-round activity (Laurent et al., 1986).

**Locomotion**  
Jumping and climbing

**Predation**  
Unknown

**Social behaviour**  
Unknown

**Sexual behaviour**  
Calling season of males of *Gastrotheca gracilis* is from May to November (Laurent et al., 1986). This species has a vocal repertoire that includes advertisement call, territorial calls and female reciprocal call (Laurent et al., 1976; Akmentins et al., 2014).

SECTION 2. MANAGEMENT IN CAPTIVITY

2.1  **Enclosure**  
Section 2.1 provides a general guide as to what has been used and found to be appropriate for a particular species without suggesting that these are the only suitable options.

2.1.1  **Dimensions**  
Tanks for tadpoles:  
Tank dimensions: 100 x 50 x 40 cm. The tadpole density was calculated as one tadpole per two litres of water.
Tanks for tadpoles of *Gastrotheca gracilis* in the *ex situ* facilities in Reserva experimental Horco Molle (Universidad Nacional de Tucumán), Tucumán province, Argentina.

Post-metamorphic plastic containers:
Plastic box dimensions were: 52 x 37 x 31 cm (42 lts). Density was up to six individuals per box.

Plastic containers for post-metamorphic individuals of *Gastrotheca gracilis* in the *ex situ* facilities in Reserva experimental Horco Molle (Universidad Nacional de Tucumán), Tucumán province, Argentina.
2.1.2 **Substrate**
The substrate used of the tanks for tadpoles was bare glass.
The substrate used for the post-metamorphic plastic containers was moist paper towels.

2.1.3 **Furnishings and Maintenance**

Tadpole tanks:
Bottom and laterals (up to water level) of the tanks were covered with black plastic tape to avoid reflection to reduce the tadpoles’ stress.
In each thank were placed two clay roof tiles for shelter, one tile was in angle for provide an emergent surface for metamorphic individuals.
Also, in each thank was placed clay tray filled with sterilized river gravel for the biological filtration.

Detail of the furnishings in the tadpole thanks of Gastrotheca gracilis in the ex situ facilities in Reserva experimental Horco Molle (Universidad Nacional de Tucumán), Tucumán province, Argentina.

Post-metamorphic plastic containers:
The best management of post-metamorphic individuals was reached with moist paper towels as substrate. Shelter for frogs was provided with pieces of clay roof tiles. Water in plastic containers. Food was provided in plastic petri dishes.

Detail of the furnishings in the plastic containers for post-metamorphic individuals of Gastrotheca gracilis in the ex situ facilities in Reserva experimental Horco Molle (Universidad Nacional de Tucumán), Tucumán province, Argentina.
2.1.4 Sharing Enclosure with Other Species
If appropriate for the role in captivity, species with which the enclosure might be shared are suggested, and any associated advantages or disadvantages outlined.
No data.

2.2 Temperature, Light and Humidity Requirements (Environment)
Environmental temperature regulated through air conditioner at 24°C. Light with the natural daily cycle, with a combination of natural light (glass widows) and led white lights of 80 W. Environmental relative air humidity. Post-metamorphic individuals were moistened by spray once a day during the morning.

2.3 Quarantine and Biosecurity
All elements for handling and housing the marsupial frogs (hand nets, tadpole tanks, post-metamorphic plastic containers, furnishings) were cleaned with bleach solution.
Post-metamorphic individuals were treated for *Batrachochytrium dendrobatidis* with the standard Itraconazole protocol (0.01% solution for 10 min daily for 7 days). This species tolerated this treatment, without sings of Itraconazole toxicity (Correa, pers. obs.).

2.4 Feeding
Tadpoles were fed with a combination of dry food of flake food for cold water fishes, and fresh food like small cubes of pumpkin. Food was provided every other day and alternating between dry and fresh food. Quantities were approximately 0.05 gr of dry food and 1 gr of fresh food per individual.

The post-metamorphic individuals were fed *ad libitum* every other day with collembolans and drosophila flies. An option is to provide *ad libitum* foraging over compost micro-fauna.

Juveniles and adults were fed *ad libitum* every other day with a combination of drosophila flies, isopods (woodlice) and small crickets (3 o 5 mm).
2.4.1 **Basic Diet**
Specimens maintained in captivity for six years where supplemented with powdered crickets with Reptocal™ once per month and it these not developed metabolic bone diseases or other pathology related with calcium deficiency or excess. This dietary supplementation was complemented with the possibility of natural basking behaviour of the marsupial frogs with direct sun light.

2.4.2 **Special Dietary Requirements**
For young, breeding animals, convalescent animals and due to seasonal variations or physiological intolerance.
No data.

2.4.3 **Method of Feeding**
How often and where (indoors, outdoors, in bowls) feeding occurs. Also how and when food is presented (day/night/etc.).
Tadpoles were feed in the morning.
Post-metamorphic individuals were feed in the morning. Food was presented in Petri dishes.

2.5 **Breeding**
Outlines appropriate breeding techniques such as the use of rain tanks etc. Where appropriate, artificial breeding techniques are described.

2.5.1 **Social Structure**
Details the appropriate male:female ratios.
Unknown.

2.5.2 **Conditioning, Courtship and Spawning**
Details the introduction and segregation of breeding animals and any conditioning related to climate or diet. Also include any special enclosure modifications. Courtship and copulation are described and any changes in behaviour associated with the period of sexual activity (e.g.: increased aggression). Any evidence of seasonality is assessed.
Courtship and copulation are Unknown.
Females of *Gastrotheca gracilis* "give birth" tadpoles in Gosner’s development stage 26 (Laurent, 1976). The expulsion of the tadpoles from the dorsal pouch of the female occurs in the water recipient inside the terrarium and the birth process is helped by the female with its hindlimbs (Laurent et al., 1986).
The complete sequence of the tadpole expulsion by a female of *Gastrotheca gracilis* (Laurent et al., 1986).

### 2.5.3 Egg Laying

When to expect egg deposition or birth. Any special husbandry considerations during incubation or hatching, and details of artificial incubation procedures are outlined.

Eggs in the dorsal pouch of the females.

### 2.5.4 Development and Care of Eggs and Larvae

Conditions for incubation of eggs and time required for hatching is detailed here along with care of early stage larvae or young. Also includes details of neonatal mortality.

For the maintenance of the tadpoles, good results of water quality (turbidity) were obtained with oxygenation with air pump or weekly changing of the 50% of water volume. The employment of water filter is recommended, but not needed.

The mortality rate of the tadpoles is low, with near 90% of survivorship to the metamorphic stage.

### 2.5.5 Rearing of metamorphs and juveniles

Any special considerations to why and when young should be removed, with details of initial care and subsequent rearing.

The metamorphic individuals could get drowned without an emergent surface from water in the tank.
2.6 Handling and Transport

The tadpoles obtained from the field were captured by hand net and transported in large Ziploc bags filled with water of the reproductive site, in a density of 10 tadpoles per bag.

The post-metamorphic individual released into the wild were transported in plastic containers (dimensions were: 52 x 37 x 31 cm; 42 lts). Plastic containers were conditioned with moist paper towels as substrate. With a density of 20 individuals per container.

2.7 Population Management

2.7.1 Individual Identification and Sexing

Accurate sexing and individual identification are particularly important in breeding populations and for some species can be a difficult procedure. Appropriate techniques are described and assessed. The location and type of marker should be standardised per taxon.

Sexing is easy when individuals reach sexual maturity because females develop an open dorsal pouch. The opening of the brood pouch is narrowly U-shaped, with the anterior end rounded at the level of the posterior edge of the sacrum (Duellman, 2015).

Individual identification could be made by photographic registries and identification of the dorsal colouration patterns (Akmentins, pers. obs.).

2.7.2 Managing in Groups or as Individuals

Detail any specific recommendations on maintaining the species in groups or individually and how this relates to managing population genetics and identification.

Unknown.

2.8 Specific Problems: Considerations for Health and Welfare

This section briefly outlines any physical conditions or complaints commonly associated with the species. Requirements for behavioural as well as physical well being are considered.

Symptoms, treatment and prevention of common diseases/conditions are outlined. Common parasites, screening and treatments are outlined (again detailed information on medical procedures not included). Information on causes of adult mortality is also included.

No data.

2.9 Recommended Research

The aim of collating information into the Guidelines format is as much to highlight what information is not available as to present that which is. Additional information is required in a number of areas to fill in obvious gaps or validate existing data, particularly where there are contradictory viewpoints. Section 2.9 highlights this, indicating appropriate areas for further research. Some of the questions raised may be addressed through the use of husbandry questionnaires, with a more in depth assessment of specific aspects carried out through research programmes.

Future research in ex situ breeding of G. gracilis should be concentrated to reach the complete reproductive cycle of this species in captivity. There is a lack of knowledge of the procedures to maintain viable ex situ survival assurance colony and to how to successfully breed this species.

SECTION 3. REFERENCES


