Sexual dichromatism in the neotropical genus *Mannophryne* (Anura: Aromobatidae)

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ABSTRACT

Recent reviews on sexual dichromatism in frogs included *Mannophryne trinitatis* as the only example they could find of dynamic dichromatism (males turn black when calling) within the family Aromobatidae and found no example of ontogenetic dichromatism in this group. We demonstrate ontogenetic dichromatism in *M. trinitatis* by rearing post-metamorphic froglets to near maturity: the throats of all individuals started as grey coloured; at around seven weeks, the throat became pale yellow in some, and more strongly yellow as development proceeded; the throats of adults are grey in males and variably bright yellow in females, backed by a dark collar. We demonstrated the degree of throat colour variability by analysing a large sample of females. The red: green (R:G) ratio ranged from ~1.1 to 1.4, reflecting variation from yellow to yellow/orange, and there was also variation in the tone and width of the dark collar, and in the extent to which the yellow colouration occurred posterior to the collar. Female *M. trinitatis* are known to be territorial in behaviour. We show a positive relationship between throat colour (R:G ratio) and escape performance, as a proxy for

quality. Our field observations on Tobago's *M*. *olmonae* showed variability in female throat colour and confirmed that males in this species also turn black when calling. Our literature review of the 20 *Mannophryne* species so far named showed that all females have yellow throats with dark collars, and that male colour change to black when calling has been reported in eight species; in the remaining 12 species, descriptions of males calling are usually lacking so far. We predict that both dynamic and ontogenetic sexual dichromatism are universal in this genus and provide discussion of the ecological role of dichromatism in this genus of predominantly diurnal, non-toxic frogs, with strong paternal care of offspring.

Keywords: Aromobatidae, Anurans, Mannophryne, sexual dichromatism, sexual signalling

Introduction

It is well established that most anuran amphibians are active mainly at night and that intra-specific communication is mediated by auditory signals. However, as more complexity in anuran behaviour is found, a wide diversity of visual signalling (movements, colours, patterns, shapes) both in diurnal and in nocturnal species is becoming established. For example, Rojas (1) reviewed the roles of colours and patterns, Hodl and Amezquita (2) reviewed and classified the variety of visual signals, and Starnberger *et al*. (3) discussed the multimodal roles of the vocal sac in signalling: not only auditory, but also visual and chemical in some cases. One category of visual signals involves sexual dichromatism, reviewed by Bell and Zamudio (4). They distinguished two types. First, dynamic dichromatism, restricted to males, where the male develops a temporary colour signal related to courtship and breeding. The review identified 31 species in nine families where this occurred. Second, ontogenetic dichromatism, where either males or females develop a permanent colour difference as they mature. The review found this reported from 92 species in 18 families. Bell *et al.* (5) extended the dataset for dynamic dichromatism to 178 species in 15 families and subfamilies. Bell and Zamudio (4) found no species reported as having both sexual and ontogenetic dichromatism.

Dynamic sexual dichromatism is likely to be an aspect of sexual selection, where the male's temporary colour in some way attracts females. Although most frogs attract mates through acoustic signals and breed at night, with a colour signal appearing not to be useful, some species are diurnal and there is evidence that colour can be used by females to assess male quality in nocturnal breeding aggregations where discrimination of acoustic signals is difficult (6) .

Bell and Zamudio (4) included two classes of ontogenetic dichromatism. In the first, where males develop more conspicuous colouration than females, sexual selection is likely to be the main driver, with an expectation that the permanent colour should not be at significant cost. An example is the poison frog *Oophaga pumilio* where the male's bright colour attracts females but also acts as an aposematic signal, deterring predators (7). In the second class, females develop brighter colours than the males: this may be explicable through sexual selection, but an alternative may be sexual niche partitioning, where the two sexes occupy different niches, and colour contributes in some way to successful occupation.

In this paper, we report on the occurrence of both dynamic and ontogenetic sexual dichromatism in frogs of the neotropical genus *Mannophryne.* Frost (8) lists 38 species in the subfamily Aromobatinae of which 20 belong to the genus *Mannophryne*. *Mannophryne* are ground-living frogs inhabiting the environs of mountain streams in Venezuela and the Caribbean islands of Trinidad and Tobago (West Indies). They are cryptically coloured, with dorsal sides mostly mottled grey and brown, and they lack the poison gland protection found in dendrobatids, to which they are closely related (9,10). Most accounts find *Mannophryne* to be day active frogs, but there are occasional reports of them remaining active after dusk (11,12, this paper).

We focus on sexual dichromatism in two species, *M. trinitatis* and *M. olmonae*, but also review literature reports from other *Mannophryne* species. Kenny (13) noticed that when male *M. trinitatis* are calling, their colour changes from the normal cryptic mottled brown/grey to jet black. Wells (14) reported that the colour change is very rapid, occurring over 1-10 minutes, both at the start of

74 calling and at the end of an episode of calling when the colour reverts to normal. Bell and Zamudio
75 (4) included this as their only example of dynamic sexual dichromatism in the family Dendrobatidae
76 (we follow Frost (8) in placing *Mannophryne* in the family Aromobatidae). Wells (14) noticed
77 another form of sexual dichromatism in *M. trinitatis*. Females aggressively defend territories and
78 display their pulsating bright yellow throats when they do so. The yellow patch is posteriorly
79 bounded by a narrow dark collar (a pigmented band of variable tone and width, extending across the
80 ventral surface at the level of the forelimbs). Males possess the collar, but the throat is grey and
81 Wells comments that it is not pulsated during aggressive encounters between males. Bright yellows
82 are often based on carotenoid pigments, regarded as costly to synthesise, and in many taxa,
83 including some anurans, they have been associated with signals of fitness (reviewed by Olson &
84 Owens (15); tree frog example: Richardson *et al* (16)).

85 In this paper, we assess the variability of the yellow pigmentation in a large sample of *M. trinitatis*
86 females, and report on an experiment where we used escape responses as a proxy for fitness. We
87 also document the development of the yellow throat colour as metamorphs grow towards maturity.
88 Finally, we provide field observations on the behaviour of both *M. trinitatis* and *M. olmonae,* and
89 review what has been reported on sexual dichromatism in other *Mannophryne* species.

90 ## Materials and Methods

91 ### Colour variability in female *Mannophryne trinitatis*

92 A large sample (n=500) of adult female *M. trinitatis* was collected from different localities (Fig 1, S1
93 Table) in Trinidad's Northern Range during June to August 2015 and 2016. Females, recognised by
94 having yellow throats, were caught either by hand or with the aid of small hand-nets and placed for
95 transport into small polyethylene bags containing a little damp forest leaf litter. They were kept
96 overnight in a holding tank furnished with damp leaf litter, at the University of the West Indies, St.
97 Augustine. The frogs were photographed and measured (snout-vent length [SVL] to 0.1 mm using

98 dial callipers) on the day after collection, and then returned to their collection sites. Despite an
99 earlier report of the presence of *Batrachochytrium dendrobatidis* (*Bd*) in the *M. trinitatis* population,
100 Greener *et al.* (17) found the infection to be absent. Capturing and returning the frogs should
101 therefore not risk spreading infection.

102 **Figure 1. Map of sites used in field studies.** *M. trinitatis* sampling sites, as detailed in S1 Table.
103 Adapted from Greener *et al.* (17).

104 To photograph the chin, each frog was carefully held upside down and the ventral side
105 photographed. All photographs were taken using a Sony ILCE-6000 with an attached Sony E-Mount
106 SEL 55-200mm lens and lens hood (2015) or SEL 18-55mm lens (2016). The camera was set with F8.0
107 aperture, and shutter speed adjusted to give an exposure of 0.0. Light was provided from two small
108 LED lamps. As in Stevens *et al.* (18), RAW format was chosen, as opposed to JPEG – as used by
109 Bergman and Beehner (19) – as this prevents loss of information due to compression, allows for
110 later adjustment, and due to current storage capabilities, file size was not an issue. The photograph
111 from the first frog at each site was used to create a colour profile using the software accompanying
112 an X-Rite Colorchecker passport colour rendition chart (X-Rite Inc., Michigan, USA). This profile was
113 then applied to all photographs in Adobe Photoshop CC 2015, and white balance corrected for each
114 photograph. The area of interest was selected, and average blurred before red (R) and green (G)
115 values were recorded. These values were then used to generate R:G ratios. To allow comparison
116 between sites, we combined the relationship between R:G ratio and the corresponding SVL of the
117 individual (SVL*R:G ratio), creating a throat colour to body length metric. We modelled the
118 relationship between R:G ratio and SVL in all sites individually and combined, using linear regression.
119 For comparison of the relationship between SVL and R:G ratio between sites, we used a non-
120 parametric Kruskal-Wallis test followed by pairwise Mann-Whitney U tests, Benjamini- Hochberg
121 corrected for multiple testing. The relationship between R:G ratio or SVL and presence of colour
122 posterior to the collar was examined by Mann-Whitney U tests. The presence of colour posterior to

123 the collar was then analysed for each site by Mann-Whitney U tests. All analysis was conducted in
124 RStudio 1.1.463 environment R3.5.2 (20).

125 **Colour development in juvenile *Mannophryne trinitatis***

126 A sample of about 60 *M. trinitatis* tadpoles was collected in early July 2016 by hand netting from a
127 pool in a stream beside the Arima-Blanchisseuse Road in Trinidad's Northern Range mountains. The
128 tadpoles were transferred to the University of Glasgow, Scotland in two-litre polyethylene
129 containers with the tadpoles resting on damp cotton cloth. Downie and Smith (21) showed that
130 these tadpoles survive well under such conditions. In Glasgow, the tadpoles were grown at an initial
131 density of 30 individuals per tank in plastic aquaria 32x18x18 cm in dechlorinated tap water at a
132 depth of 10 cm and with a constant air supply delivered through a submerged air-stone at one end
133 of the tank. The room where the aquaria were located had a 12:12h light/dark cycle and an air
134 temperature of 23-24^{0}C. The tadpoles were fed daily with aquarium fish food flakes (New Era brand),
135 and the water was changed weekly to avoid the build-up of waste. Each day, the aquaria were
136 checked for tadpoles showing forelimb emergence, the sign of metamorphosis beginning. The first
137 metamorph was found on 8th July and the last on 16th September. Each metamorph was caught by
138 hand-net and transferred to an individual translucent 22x15x8 cm polyethylene container with an
139 opaque lid. Each container was provided with a 'shelf' of washed gravel at one end, about 18 cm
140 wide and 1-2 cm deep, and dechlorinated tap water to a depth of about 0.5 cm. Downie *et al.* (22)
141 have shown that *M. trinitatis* take 6-7 days to complete metamorphosis and that they hide in gravel
142 during that time.

143 After completion of metamorphosis, each froglet was provided twice a week with live *Drosophila*
144 *melanogaster* as food, supplied in 5 cm long tubes, which the frogs could enter to forage. Froglets
145 were measured (SVL, using callipers accurate to 0.1 mm) and their throat patterns noted and/or
146 photographed, starting about two weeks after forelimb emergence and continuing at approximately
147 four-week intervals for 16 weeks or so. For this purpose, froglets were captured by hand, transferred

to a transparent 9 cm diameter petri dish, and measured from below. Once the 16-week growth period was complete, each frog was transferred to a communal tank for rearing to adulthood.

Escape responses in *Mannophryne trinitatis* in relation to female throat colour

Adult female (n=81) *M. trinitatis* were captured with the aid of small hand-nets from five sites across Trinidad's Northern Range (Fig 1, S1 Table), during mornings over six weeks, June to August 2018. Sites were visited in rotation, with 5-6 frogs captured at each visit. Frogs were transported to our laboratory at the William Beebe Tropical Research Station (Simla) and housed individually in plastic aquaria furnished with a thick layer of damp forest leaf litter. Frogs were measured (SVL to 0.1 mm with dial callipers; weight to 0.01g using an electronic balance) and any with SVL <16mm were excluded from further study since classed as juveniles. The day after collection, frogs were photographed in the morning and repeated at night around 21.00h: three pictures of the throat were taken using a Canon PowerShot s110 digital camera, all under identical lighting conditions; dorsal sides of each frog were also photographed to identify frogs, so as to ensure that no frog was used more than once, following subsequent collections (dorsal patterns are individually variable). Throat colour, as R:G ratio was measured as described earlier.

Escape responses were assessed two mornings after collection (09.00 -12.00h) in a specially constructed outdoor arena set in a shaded area: this had wooden sides about 0.8m high (to prevent frogs escaping) and enclosed an area of short grass 1.5x1.5m. Each frog was put into a 9cm diameter plastic petri dish and placed at the centre of the arena; it was left there for 30 seconds with the lid off to acclimatise. If the frog jumped before 30 seconds were over, it was recaptured and left a further 30 seconds. The frog was then stimulated by a light tap to the rear using a metre stick. Each frog's responses, three times for each frog, were recorded for 20 seconds using a GoPro HERO6 video camera set above the arena. After each response, each frog was given at least 30 minutes to recover before being stimulated to jump again. Air temperature (^{0}C) and relative humidity (%) within

172 the arena were recorded using an ETI pocket thermo-hygrometer at the same times as each set of
173 responses.

174 Image J (v1.52a) was used to measure the distance of each jump. From the recordings, we calculated
175 maximum and minimum distances of each jump made and the total distance travelled in each trial
176 (i.e. different measures of escape performance). Using RStudio 1.1.463 environment R3.5.2 (20),
177 general linear models were used to test relationships between escape performance and size (SVL),
178 site, humidity, temperature and throat colour (as R:G ratio). We expected escape performance (for
179 example total distance jumped, or initial jump length) to be positively related both to size and throat
180 colour, but to be independent of collection site, humidity and temperature.

181 **New field observations on *Mannophryne trinitatis***

182 While assessing the population status of *M. trinitatis* (17), we made occasional observations
183 relevant to colour and behaviour, both during the day and at dusk/night.

184 **Sexual dichromatism in *Mannophryne olmonae***

185 Field observations on *M. olmonae* were made at several small un-named streams in northeast
186 Tobago in June to August 2014 and 2015. In 2015, female *M. olmonae* were captured using hand-
187 nets and transferred in individual containers to accommodation in Charlotteville. Here, they were
188 photographed alongside an X-rite Colour Checker rendition chart, under identical lighting conditions,
189 using a Canon EOS Rebel T3i. Throat colour, as R:G ratio, was measured as described earlier and
190 frogs were returned to their collection sites. As in the case of *M. trinitatis*, Thomson *et al.* (2018)
191 have shown *Bd* to be absent from the *M. olmonae* population.

192 **Comparison of sexual dichromatism across the genus *Mannophryne***

193 We checked the information provided on male and female colours in life in all *Mannophryne* species
194 so far described. The black colour in calling males can only be seen when observing males calling in

195 the field; the female yellow throat colour fades in preservative. In some cases, colours in life are not
196 presented in the original species descriptions, but we were often able to find later accounts of
197 colours in life.

198 **Ehtics Statement**

199 Field research permit was provided by Government's Wildlife Section, Special Export License 001192
200 (29/6/16). No ethical approval was required.

201 ## Results

202 **Throat colour variability in female *Mannophryne trinitatis***

203 We found no significant difference in the relationship between SVL and R:G between years (p >0.05),
204 so years were grouped for all further analysis. Larger females were found to be more likely to have a
205 higher R:G ratio on their throat patch. This was found across all sites individually (p < 0.001 for all
206 sites), and when combined (F=153.5, p <0.001) (Figure 2). We found inter-site variation in the
207 relationship between SVL and R:G ratio (chi squared = 41.531, df = 6, p <0.001). Post hoc testing
208 revealed groups of sites that varied significantly from each other (Figure 3). However, no site was
209 found to be significantly different from all others.

210 **Figure 2. The relationship between throat patch R:G ratio and SVL (mm) in *M. trinitatis* for**
211 **all sites.** Line indicates linear regression of all sites combined. The shaded area indicates the
212 95% confidence interval.

213 **Figure 3. SVL*R:G comparison for all sites.** The boxes indicate 25th and 75th percentiles;
214 the thick central lines indicate the means; the bars indicate the 95th percentiles; and points
215 indicate individual samples. Shared letters indicate non-significant relationships.

216

The colouring of the throat area differed between individuals not only in R:G ratio but also in the relative size of the throat patch, the intensity and width of the dark collar, and the extent to which the yellow colour extended posterior to the collar (Figure 4). Larger females, and/or females with high R:G ratios were found to be more likely to have colour posterior to the collar (SVL, R:G and SVL*R:G all p <0.001). The correlation between SVL and R:G ratio had a different relationship with the presence of colour posterior to the collar in some sites, but not all (Figure 5).

Figure 4. Variation in the throat colouration of female *M. trinitatis*. Variability in throat patch relative size and shape, collar width and intensity, and extension of the colour posterior to the collar. R:G denotes the Red:Green ratio as extracted from photographs.

Figure 5. SVL*R:G comparison of *M. trinitatis* sites with respect to colour posterior to the collar. The boxes indicate 25th and 75th percentiles; the thick central lines indicate the means; the bars indicate the 95th percentiles; and points indicate individual samples. * indicates significant difference between pair.

Colour development in juvenile *Mannophryne trinitatis*

Forty-four of the tadpoles reached metamorphosis. Of these, 25 developed long enough for their sex to be distinguished by throat colour differences, 20 as females and five as males. This sex ratio is significantly biased towards females (chi squared= 15.8; p< 0.001). Table 1 shows size and colour development data for all froglets recorded beyond 90 days post metamorphosis (omitting two females that escaped at around 80 days). Dark collars developed in both males and females. Although these were variable in width and shade, we saw no consistent difference between males and females. Throat colour started and remained grey in five individuals throughout the observation period and these were classed as males (we did not check sex by examining gonads). In individuals developing as females, throat colour started as grey, became pale yellow at around seven weeks post metamorphosis, and either remained pale or became more brightly yellow around nine weeks

(Figure 6). Since we did not assess throat colour weekly (too frequent disturbance could be stressful, and risked escapes), we cannot tell precisely when the yellow colour first became apparent. However, throats were pale grey in all individuals at the first set of observations (2-3 weeks after metamorphosis began) and remained grey at 5-6 weeks in individuals that developed as females. These results indicate that throat colour is ontogenetically sexually dichromatic, developing in the juvenile phase, well before female maturation (mature females are around 20mm SVL; the yellow throat was distinguishable at around 15.5 mm).

Table 1. The appearance of coloured throats in post-metamorphic *M. trinitatis* reared in captivity.

Sex	Days followed post metamorphosis (mean +/- SD	Final SVL (mm:mean +/- SD)	Yellow colour first seen	
			Days	SVL
Females (n= 18)	110.2 +/- 12.0	16.4 +/- 1.0	52.2 +/- 11.1	15.5 +/- 1.0
Males (n= 5)	132.4 +/- 8.2	17.3 +/- 0.9	NA	NA

Fig 6. Throat colour patch development in a selection of post-metamorphic *M. trinitatis* reared in captivity. Early development, left hand column; later stages of the same froglets to the right. A,B: frog 1, 30 and 119 days post metamorphosis, 15.6 and 17.8mm SVL respectively; C,D: frog 1, 20 and 109 days, 14.0 and 18.0mm; E,F: frog 3, 17 and 106 days, 14.1 and 17.0mm; G,H: frog 4, 17 and 106 days, 12.5 and 16.2mm; I,J: frog 5, 40 and 138 days, 14.9 and 16.2mm. Early stages show slight or no yellow pigmentation. Later stages all with yellow throats, female, except frog 5 J, male.

Escape responses in *Mannophryne trinitatis* in relation to female throat colour

Responses to stimulation were quite varied. Some frogs made only a few jumps before stopping on the grass; others jumped to the edge of the arena and climbed some way up the wall (*Mannophryne*

have adhesive toe pads); the direction of jumping was also variable but most tended to maintain more or less the same direction once they set off.

Comparison of morning and night photographs of frog throats indicated that the colour was stable, with no diurnal variation. Table 2 shows female frog sizes, colour variability and escape performance. There were no significant differences between collection sites and escape performance (p>0.05 for all measures). Air temperature during the trials had a range of only 1.8^{0}C (26.9-28.7^{0}C), but humidity varied more widely (53-86%). There were no significant relationships between temperature or humidity and escape performance (all p>0.05). Also, there was no significant relationship between collection site and throat colour (R:G ratio: p>0.05). However, there were strong and significant positive relationships between R:G ratio and frog size (weight: F=18.42, p<0.001; SVL: F=19.07, p< 0.001). Weight and SVL were also strongly correlated with one another (not shown).

Table 2. Mean(+/-SD) values for frog size, R:G ratio and measures of escape response.

Samples	SVL (mm)	Weight (g)	R:G	Total escape distance	Mean distance per jump	Minimum jump	Maximum jump	Initial jump
81	21.2 +/- 2.6	1.2 +/- 0.4	1.2 +/- 0.1	85.6 +/- 40.3	18.4 +/- 6.2	5.1 +/- 5.6	35.8 +/- 10.0	24.2 +/- 8.0

We found two significant relationships between throat colour and escape performance. First, there was a positive relationship between R:G ratio and the maximum distance travelled in a single jump (F= 4.57, p=0.036); second, a positive relationship between R:G ratio and total distance travelled (F=6.98, p<0.001). However, there were no significant relationships between R:G ratio and three other measures of escape performance: minimum distance in a single jump; mean distance per jump; initial jump distance (p>0.05 in all cases).

In addition, we found significant positive relationships between SVL and both maximum distance travelled in a single jump and total distance travelled (F=7.33, p=0.008; F=6.98, p<0.001 respectively). A two-way ANOVA test was carried out to assess whether there was an interaction between size (SVL) and throat colour concerning their relationships to escape responses: this interaction was non-significant. S2 Table summarises the statistical results on escape performance.

New field observations on *Mannophryne trinitatis*

We observed *M. trinitatis* sites at dusk and during the hour after sunset. Some male *Mannophryne* remained active, calling, feeding and transporting tadpoles. Although numbers were fewer and calling less frequent than during the day, frogs were out in the open and not hard to find. During a day-time survey, we noticed a male in normal non-calling colouration calling occasionally i.e. not at the usual high frequency. As we watched, this frog began to change to black and over the same time, its calling frequency increased, the full change taking about 20 minutes.

Sexual dichromatism in *Mannophryne olmonae*

Hardy's (23) original description of *M. olmonae* made limited reference to colours in life and no mention of sexual dichromatism. There was doubt about the distinctness of this species until DNA sequencing evidence (24) showed that *M. olmonae* is a distinct species and that it is more closely related to the mainland species *M. riveroi* than to the neighbouring *M. trinitatis.* Lehtinen *et al.* (25) established that *M. olmonae* is more widely and abundantly distributed in Tobago than previously reported.

Alemu *et al.* (26) found *M. olmonae* along forested streams in northeast Tobago. Individuals were within 10 m of stream edges, except calling males which were sometimes more distant. They noted that adult females had yellow throat and belly colouration, and that adult males had grey ventral colouration, changing to black when calling.

Our field observations confirm that *M. olmonae* males are black when calling. On a late afternoon in June 2014 we first observed a group of three males on a rock by a stream near the Charlotteville-Bloody Bay road (Tobago). All three were calling and all were black. One soon hopped away. The other two called facing one another until one other hopped away, leaving the 'victor' of the encounter (Figure 7 a,b). We made many similar observations over the next four weeks, but did not actually observe the colour transformation from brownish to black, although we did see a calling male that was mainly brown dorsally and possibly at the start or end of the transition (Figure 7 c,d). Of 47 calling males observed, 68% were on rocks, 17% on leaves and only 15% in crevices. In a further visit in 2015, we captured 12 adult females and photographed their throats for colour analysis. All had yellow throat patches with a narrow brownish collar and an R:G ratio ranging from 1.03 to 1.104.

Figure 7. Males of *M. olmonae*. (A) two males, jet black all over, soon after a third male had hopped away. (B) the remaining male after the second one in (A) had hopped away; black colour already diminished. (C,D) males with inflated throats, but not yet black all over.

Comparison of sexual dichromatism across the genus *Mannophryne*

S3 Table shows the results of our literature search for evidence of both dynamic sexual dichromatism in males and sex differences in throat colour, assumed to be ontogenetic. In a few cases, colour change in males has not been observed, but this is generally in species where calling has not been seen. The table includes any information found on the speed of colour change in males, but this has only been reported in a few species so far.

All data used within paper can be found in supporting tables 4-6.

Discussion

In their review of sexual dichromatism in anuran amphibians, Bell and Zamudio (4) found 31 species where males undergo a short-term change related to courtship and breeding; Bell *et al.* (5) extended these cases of dynamic dichromatism to 178 species in 15 families and subfamilies, but noted that their conservative methodology probably meant that other cases would be found. Bell and Zamudio (4) also found 92 species in 18 families showing ontogenetic dichromatism where adult males or females developed a permanent colour difference between the sexes, with one of them essentially retaining juvenile colouration. These reviews did not report any species where both dynamic and ontogenetic dichromatism occur.

In this paper, focussed on the Trinidad stream frog *Mannophryne trinitatis,* with additional observations on the Tobago stream frog *M. olmonae*, and a review of the colour descriptions of the other *Mannophryne* species so far identified, we show that a) dynamic sexual dichromatism is widespread in the genus, and b) that ontogenetic sexual dichromatism, principally involving the development of a bright yellow throat patch occurs throughout the genus in females. We also show that the yellow throat patch is highly variable in *M. trinitatis* and *M. olmonae* and provide a test of the hypothesis that throat colour provides a signal of female quality.

Reviews of the occurrence of conspicuous colouration in frogs (1,2) emphasise two general cases. First, aposematic (warning) signals to other species indicate that these frogs are well protected by toxins. A complication may arise where harmless species evolve to mimic the toxic species, gaining protection without incurring the costs of producing toxins. Second, to protect the frog from drawing the unwelcome attention of predators, the conspicuously coloured element is either temporary or concealed, except from the intended receiver.

Dynamic sexual dichromatism in male *Mannophryne*

Bell *et al*. (5) found a relationship between dynamic sexual dichromatism and explosive breeding aggregations in hylids, bufonids and some other groups, and suggested that colour change may

assist mate recognition in such situations. However, they also noted that dynamic dichromatism occurs in the absence of breeding aggregations in some species, and related it to intraspecific competition, such as territory defence, in these cases. Colour change in frogs is generally found to be slow (hours to days) and mediated by hormones. However, Kindermann *et al.* (27) found that a dorsal change from brown to yellow in amplexing male *Litoria wilcoxii* took around 5 minutes, and that it could be induced in non-amplexing males by epinephrine injection, implying a neuroendocrine mechanism. Wells (14) reported that male *M. trinitatis* begin calling and then change to black within 1-10 minutes; we also noticed that males start to call before changing to black and that the change occurred over minutes. In other *Mannophryne* species, La Marca {LaMarca:1994uk} reported *M. cordilleriana* changing to black a few seconds after starting to call, and Rojas-Runjaic *et al.* (12) made a similar observation on *M. molinai*. In other species where a change has been seen (8 out of 20 species, plus probably *M. riveroi*: S3 Table), the rate of change has not been noted. In a few accounts, the return from black to brown has been reported to be similarly fast. Although no research has been reported on the mechanism of colour change in male *Mannophryne*, the speed implies a neuroendocrine process, and a study to test this is needed.

The function of the male change to black is unclear. *Mannophryne trinitatis* is not an explosive breeder: frogs are distributed along stream sides, with females holding long-term territories. Breeding can occur throughout the long wet season, presumably dependent on females having ripe egg clutches (13,14). *Mannophryne,* although closely related to dendrobatids, lack toxic protection and are generally cryptic in colouration and behaviour. Their usual habitat is the margins of rocky streams in tropical forest, and they are mainly active during the day. Light levels are low, and the habitat provides abundant shaded crevices where frogs with mottled dorsal colouration of browns, greys and blacks are well concealed. Wells (14)found that males with normal brownish colouration never attacked other males, nor were they attacked by calling males. However, aggressive encounters between black calling males were common. Calling males did not appear to be

374 particularly territorial in their behaviour, often changing calling site, usually a conspicuous position
375 such as a rock or log (however, we have often seen males calling from shaded crevices). Since both
376 calling and black colouration make the males conspicuous, it is unclear why both signals are needed,
377 especially when they likely increase the risk of predation. Mimicry is unlikely to be at work here.
378 Although some toxic frogs are conspicuously black (for example, dendrobatids of the genus
379 *Ameerega*), their ranges do not appear to overlap with those of *Mannophryne* (29). The key to the
380 dynamic black signal in males may lie in the unusual territorial behaviour of female *Mannophryne*
381 (see later). In order to attract a female, the males may need to demonstrate their own quality by
382 having visibly successful encounters with other males, or by being conspicuous (colour and sound)
383 for an extended period. Zahavi's (30) handicap principle could be at work here.

384 Bell *et al*. (5) checked 13 species of *Mannophryne* for the occurrence of colour change in the males
385 and found that only *M.trinitatis* fitted their criteria, which required photographic evidence.
386 However, we read original species descriptions and some later reports. Since the occurrence of the
387 black colour is transient and not found in preserved specimens, definitive sightings require field
388 observations of calling males. These are often lacking in reports and colour change is often only
389 briefly referred to, since it cannot be used as a species identification criterion in preserved
390 specimens. Of the 12 species reviewed by Bell *et al.* (5) as lacking colour change, we found colour
391 change descriptions in three cases: *herminae, olmonae* and *venezuelensis*. In addition, the seven
392 species not covered by Bell *et al.* yielded three cases of colour change: *cordilleriana, larandina* and
393 *molinai*.

394 If male colour change occurs in some *Mannophryne* but not others, a possible explanation is
395 phylogeny. Manzanilla *et al.* (31) analysed mitochondrial DNA sequences from 13 of the 15
396 *Mannophryne* then known. They identified three clades of five, one and three species respectively.
397 More recently, Grant *et al.* (10) analysed 14 species and essentially confirmed Manzanilla *et al.*'s
398 clades, though they noted some anomalies in the species so far identified. Male colour change while

399 calling has been reported in species belonging to both of the larger clades, and phylogeny therefore
400 provides no explanation for the distribution of this trait as reported so far. Given that all
401 *Mannophryne* species appear to live in similar habitats with similar behavioural ecology, our
402 hypothesis is that colour change in calling males is likely to occur throughout the genus, and that
403 those species where it has not been reported have not yet been adequately observed in the field.

404 **Ontogenetic sexual dichromatism in *Mannophryne***

405 Amongst the anurans, the group previously shown to commonly undergo ontogenetic sexual
406 dichromatism, with females developing a bright colour while the males remain dull, is the
407 hyperoliids (reed frogs), where it has been found in 35 of 215 species (4,32). As we have shown,
408 ontogenetic dichromatism in female throat colour appears to occur throughout *Mannophryne* (S2
409 Table). Females have yellow throat patches of varying size and shade, backed by a dark collar of
410 variable width and tone; the yellow patches sometimes extend beyond the collar on to the
411 abdomen. Males may have a collar, but their throats are invariably grey to black, never yellow. Some
412 species accounts refer to the colour patterns of juveniles, but our study presents the first account of
413 ontogenetic changes in throat colour from the end of metamorphosis to near maturity, and it is clear
414 that the adult sexual dichromatism is ontogenetic. Wells (14) found that female *M. trinitatis*
415 aggressively defend their territories and that the signal used to denote a territory holder is pulsation
416 of the throat with the head held high so that the yellow throat patch, pulsation and dark collar are
417 clearly visible to any approaching conspecific. Durant and Dole (33) described similar behaviour in
418 female *Colostethus* (now *Mannophryne*) *collaris*. Exposure of a coloured signal that is normally
419 concealed has been infrequently reported in frogs. One other example is the brightly coloured foot
420 webbing in the foot-flagging frog *Staurois parvus*, where males extend and rotate their legs,
421 displaying the colours, during social interactions (34).

422 To our knowledge, no-one has previously suggested that variation in the yellow colour acts as a
423 signal of female quality. However, across the animal kingdom, bright yellow patches are often used

in this way, related to the cost of synthesising the carotenoids on which yellow colours are often based, and to the role of carotenoids in immune system function (15). In anurans, a relevant example is the orange-coloured (carotenoid-based) vocal sac of chorusing tree frogs, where females prefer males with colourful compared to pale vocal sacs (16,35).

Our data show considerable individual differences in female throat colour in both *M. trinitatis* and *M. olmonae*. To test whether throat colour differences act as signals of female quality, it would be best to recover the winners and losers after territorial encounters and measure their throat colours. However, in practice, the combination of the need to observe from a distance (to prevent disturbance) and the nature of the terrain (rocks with abundant deep crevices used by frogs for concealment) made this unfeasible. We used a proxy for quality instead. Royan *et al.* (36) reported experiments where the escape response trajectories of captured *M. trinitatis* were measured using an outdoor arena. They found that angle of escape was variable, indicating a degree of unpredictability, which could help individuals to escape potential predators. We reasoned that differences in escape response might provide as good a measure of quality as the results of territorial encounters. Our results showed a positive relationship between R:G ratio and two measures of escape performance, providing evidence that throat patch colour is indeed a signal of quality. However, the signal emitted by females is more than simply the colour of the throat. Our measurements over a large population of female *M. trinitatis* and a small sample of *M. olmonae* show variation in throat colour, patch size, the width and colour of the collar, and the extent of the yellow patch posterior to the collar so the quality of the signal may include all these components.

Conclusion and a hypothesis

The adaptive significance of female sexual dichromatism in *Mannophryne* seems clear: it is associated with territorial defence. Territorial behaviour in dendrobatids is common, but mainly involves males (1). Long-term defence of a territory by female frogs, as occurs in *Mannophryne*

(extrapolating from the species where it has been demonstrated, such as *M. trinitatis* and *M. collaris*), is very rare. The obvious suggestion is that females are defending resources, most likely food. Frogs captured in late afternoon showed that females had significantly fuller stomachs (small insects, arachnids and occasional snails) than males (37). It is not known whether food resources are patchily distributed or simply related to area: a study on territory size in relation to throat colour would be helpful. If we are correct in concluding that throat colour differences in females provide a measure of quality, then it is likely to be beneficial to the males to choose the best possible quality of mate, demonstrated by their visually striking territorial defence. Females also need to select the best possible mate: males guard the eggs produced throughout incubation and then transport the hatchlings to a suitable body of water. Downie *et al.* (38) found that transporting males may take several days to locate a suitable pool, ideally one lacking predators. This post-hatching transportation phase probably makes it unfeasible for male *Mannophryne* to guard multiple egg clutches, as occurs in some other clutch-guarders, such as glass frogs (39). Males therefore have to be sure that the quality of the eggs is high enough to justify the considerable investment in time involved in incubation and transportation. Males can demonstrate their quality by calling for long periods, and by turning conspicuously black, both hazardous and possibly energetically expensive activities. The mating dances the males perform also contribute (14). Our hypothesis therefore is that the occurrence of sexual dichromatism in both sexes derives from the resource-based territoriality of the females, and strong selection for quality in both sexes. Males avoid the predation costs of conspicuousness by their colour signal being temporary and quickly turned off and on; for females, the colour signal is concealed, except when used against conspecific receivers.

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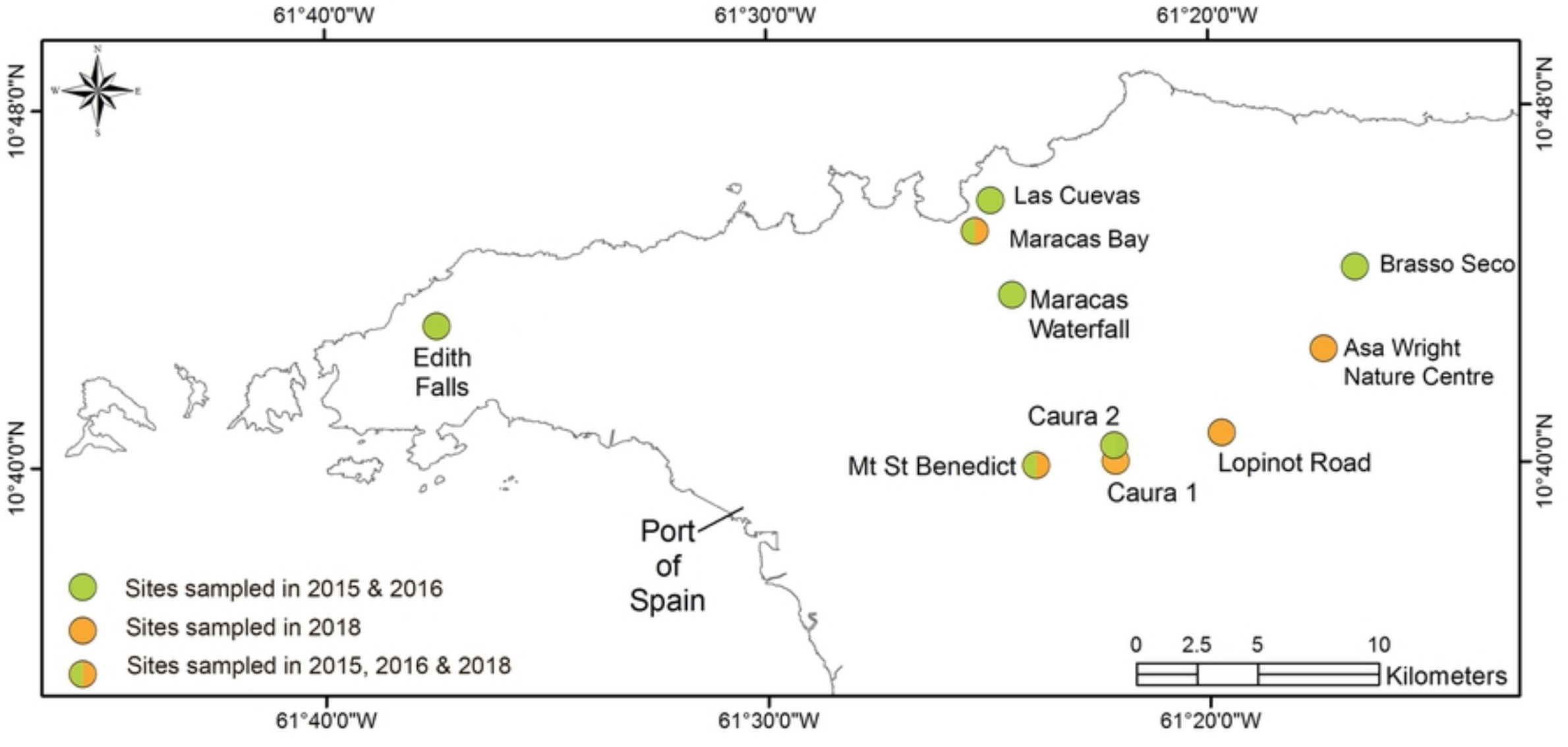
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Edith Falls
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Mt St Benedict
Lopinot Road
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Sites sampled in 2015 & 2016
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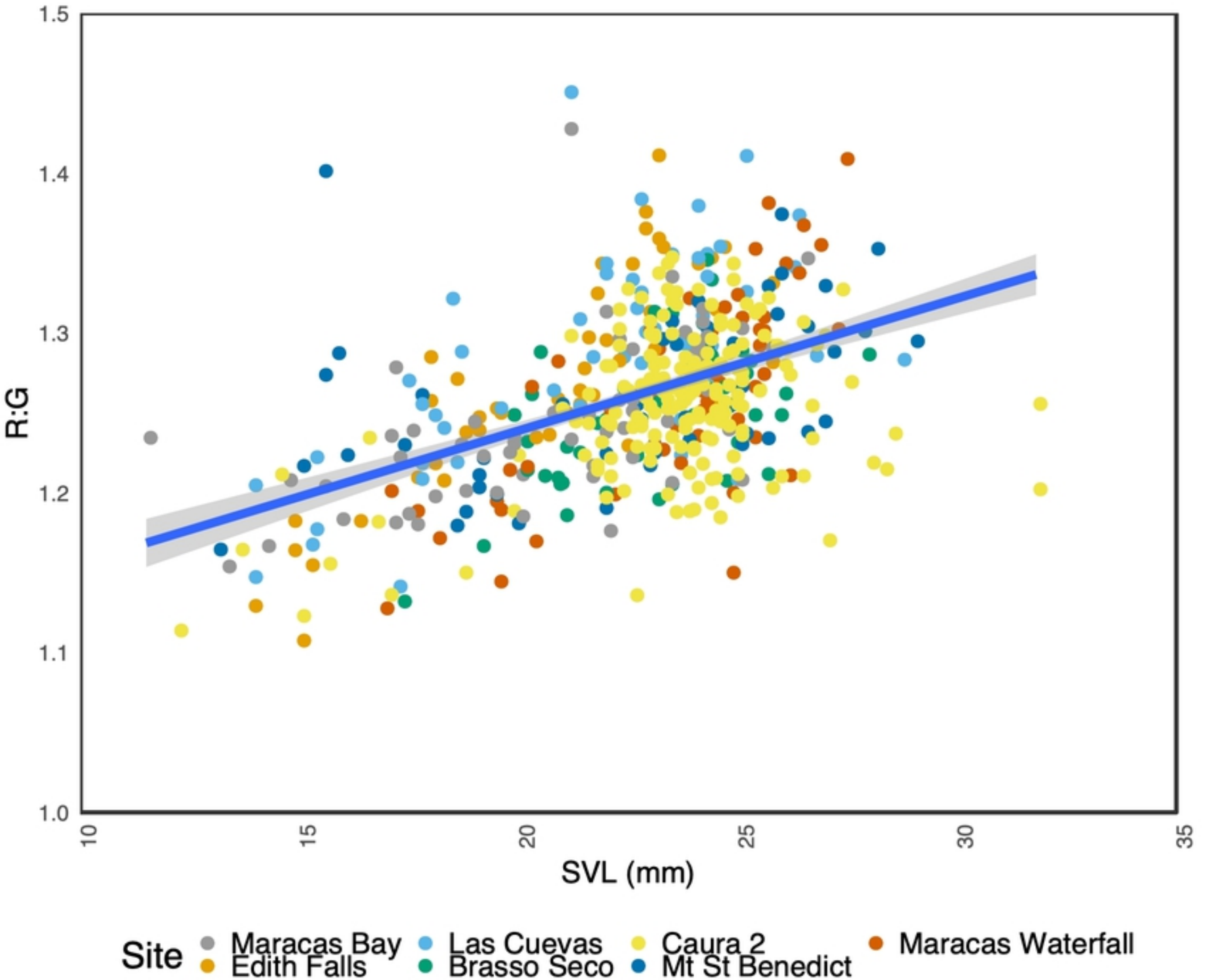
Figure 1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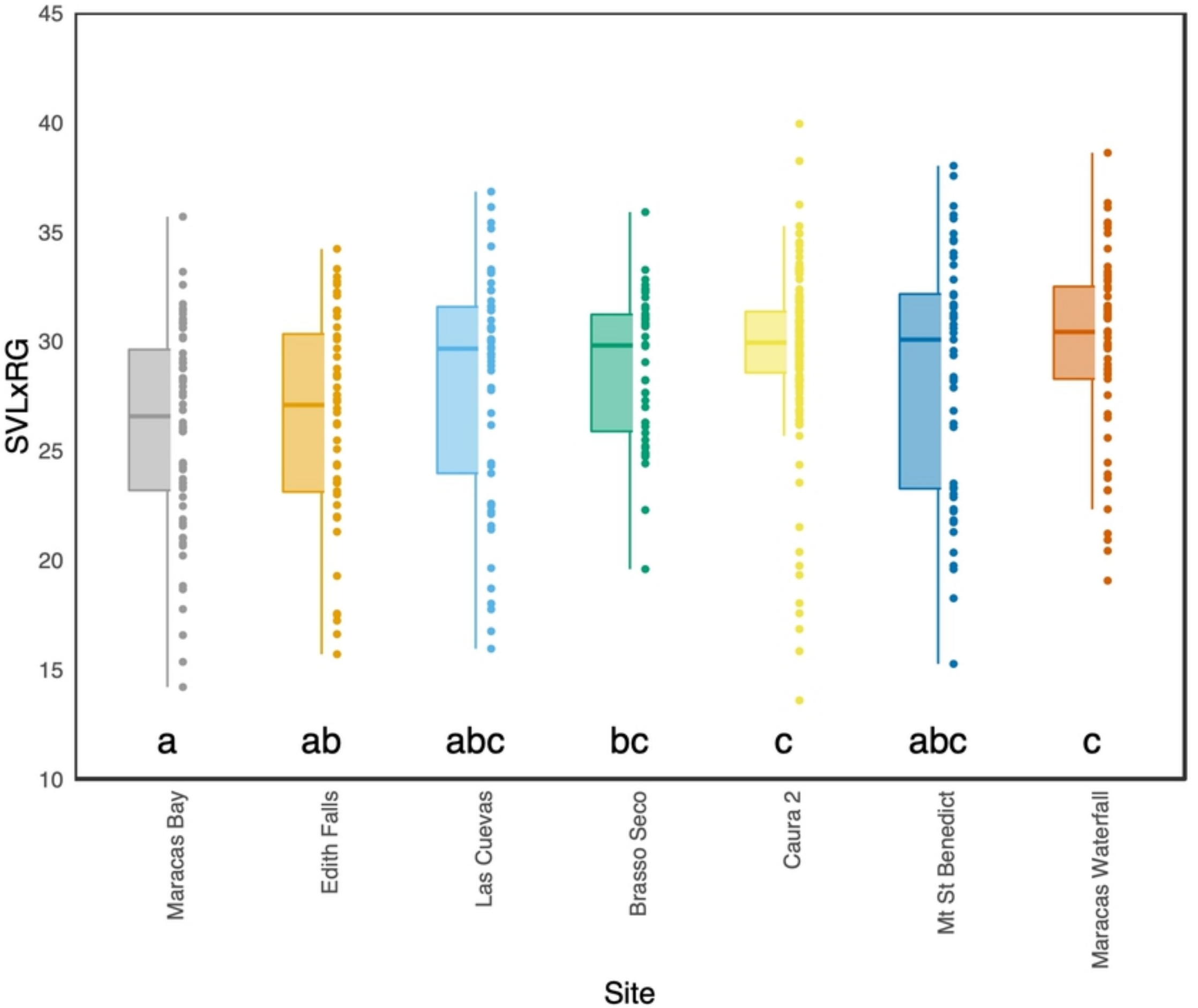
Edith Falls
Brasso Seco
Mt St Benedict

Figure 2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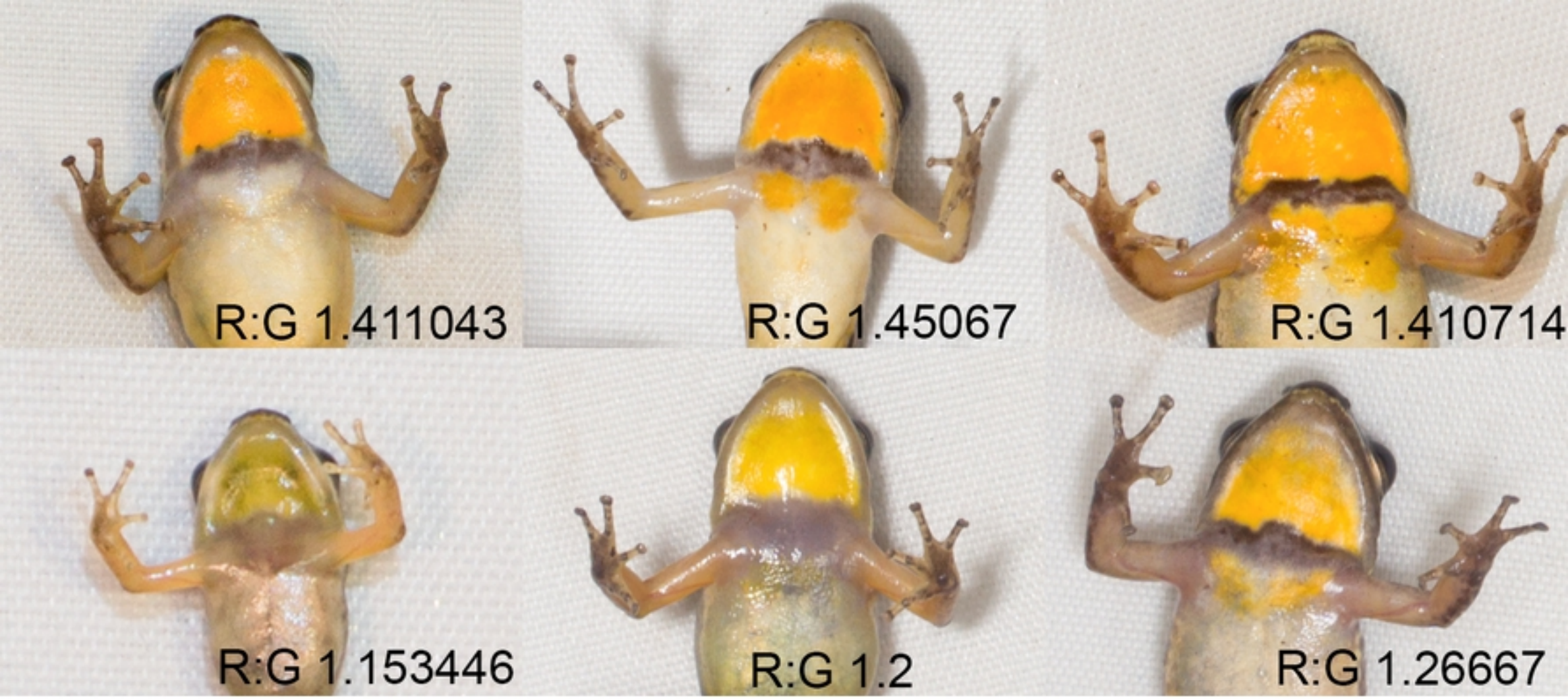
Maracas Waterfall
Site

Figure 3



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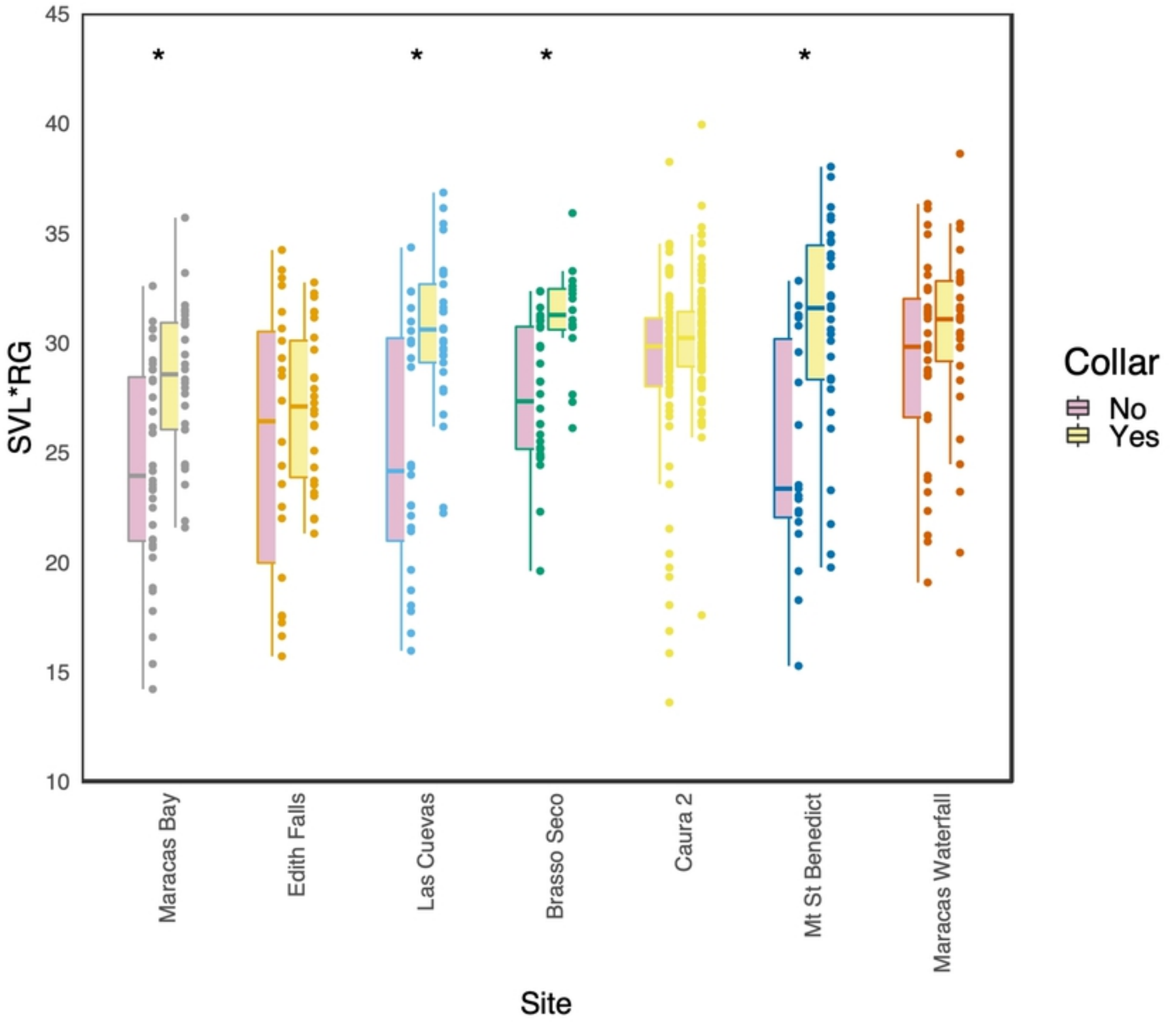

Figure 5

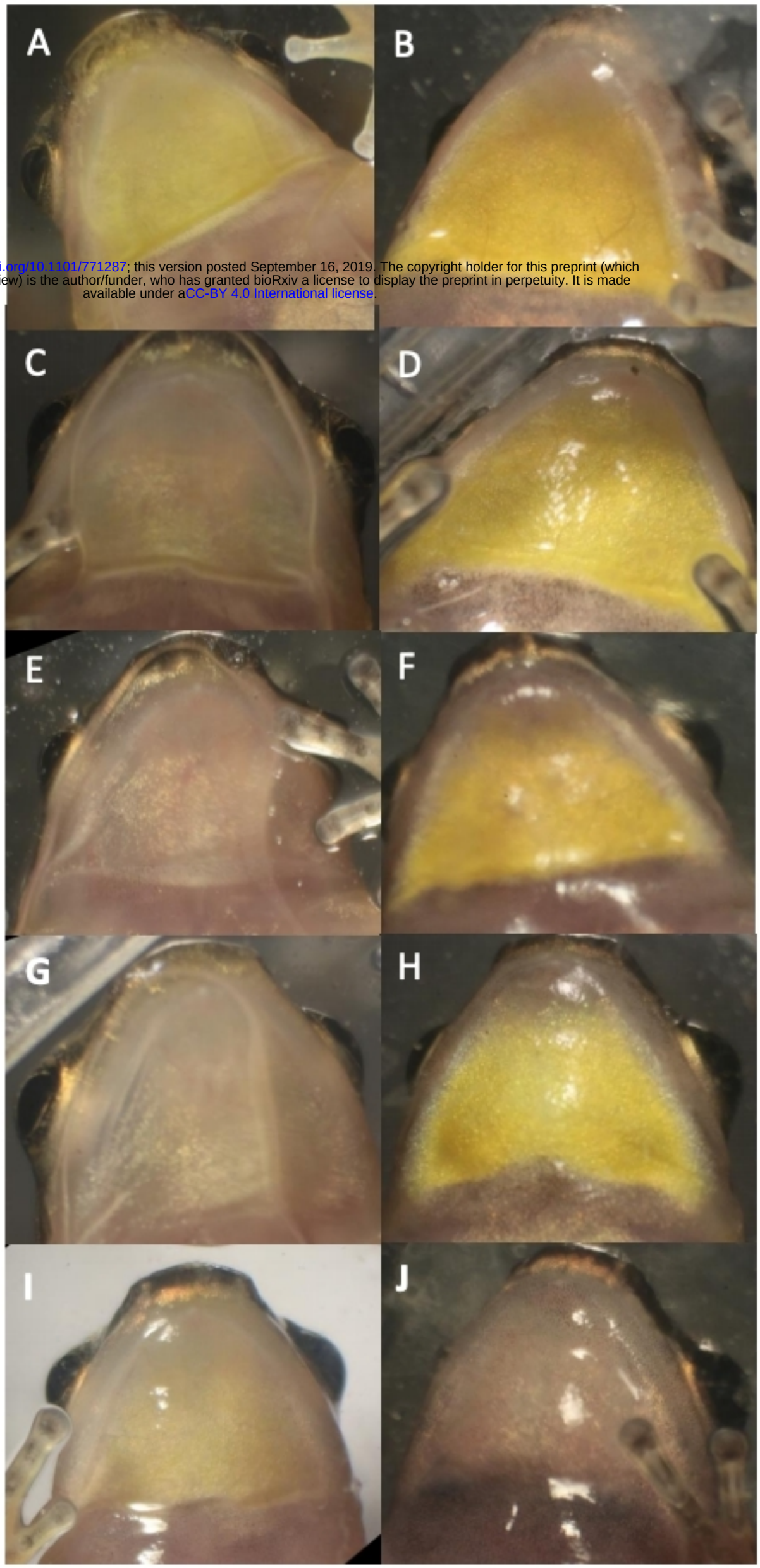

Figure 6

Figure 7