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ORIGINAL RESEARCH

Body size, not habitat or sex, best explains the extent of ultraviolet fluorescence in African dwarf chameleons (*Bradypodion*)

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biofluorescence; chameleons; natural selection; sexual selection; signaling; body size; *Bradypodion*.

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Abstract

It has been hypothesized that biofluorescence is a trait linked to intraspecific signaling in many taxa, especially those with enhanced modes of conspecific signaling in complex habitats. Chameleons possess bone-based fluorescent tubercles (FTs) on their head ornaments that purportedly facilitate intraspecific signaling. We investigated the hypothesis that dwarf chameleons (*Bradypodion*) use biofluorescence for signaling by testing if the number of FTs associated with their ornaments can be explained by sexual dimorphism or ecological variation in five species from various habitats (i.e. fynbos, Afrotemperate forest, and shrublands). If the trait is used for signaling, we would expect males to have more FTs than females due to sexual selection, and/or forest species/populations to have more FTs than open-habitat species/populations via natural selection because forests are expected to be the most conducive terrestrial environment for fluorescent signals. Our results revealed that the number of FTs was greater for the larger sex (regardless of the direction of size dimorphism) but was not significantly different between sexes when adjusted for body size or head area. Forest species had more FTs than smaller-bodied fynbos species but fewer than the large-bodied shrublands species in absolute number, but there were no differences in FTs across species from different habitats when corrected for size and phylogeny. Moreover, there were no differences in FTs between natural and urban populations when correcting for body or head size. These findings suggest that larger-bodied species have more FTs than smaller-bodied species regardless of the conduciveness of their habitats toward facilitating biofluorescence. Therefore, FT trait magnitude is likely explained best by chameleon size rather than natural or sexual selection for increased signaling capability between sexes, species, or populations. We interpret these findings to suggest that it is unlikely that *Bradypodion* use biofluorescence as a signaling mechanism.

Introduction

Animals have evolved a diverse array of visual signals that facilitate important communicative functions such as species recognition, mate attraction, warning conspecifics of danger and intimidating competitors or predators (Endler, 1992, 1993a; Osorio & Vorobyev, 2008; Stuart-Fox et al., 2007). For visual signals to be effective, they must be easily detected by their intended receivers against the backdrop of environmental noise factors that can influence signal perceptibility (e.g. ambient light or sounds, background colors; Cummings & Endler, 2018; Stuart-Fox et al., 2007) within their given habitats (Endler et al., 2005). The sensory drive hypothesis proposes that shifts in environmental conditions within or between habitats should result in selection of the most detectable signals between conspecifics, thus promoting phenotypic divergence at the population level, and ultimately the species level (Boughman, 2002; Endler et al., 2005). Accordingly, closely related species, or populations within species occupying different habitats, are likely to vary in their signaling traits.

Chameleons are well known for their various signaling traits. From their ability to rapidly change body color in response to different ecological or social contexts (Dollion et al., 2022; Stuart-Fox & Moussalli, 2008) to their array of ornate

appendages such as cephalic horns, casques, lobes, and gular scales (Cuadrado, 2000; Ligon & McGraw, 2013), they possess several avenues for intraspecific communication. The bony tubercles on the heads of many chameleon species fluoresce when illuminated with ultraviolet (UV) light in the 320–370 nm range, re-emitting a bright blue color at a longer wavelength (~430 nm) (Prötzel et al., 2018). These fluorescent tubercles (FTs) are covered by a transparent layer of skin lacking chromatophores and melanophores, allowing light to pass through and produce externally visible fluorescence (Prötzel et al., 2018).

Because bone tissue is known to fluoresce under UV light due to the properties of collagen (Bachman & Ellis, 1965), it is unsurprising that bone visible under the skin surface of chameleons near their ornaments would re-emit UV light in the visible spectrum. However, given the tendency for chameleons to use ornamental traits for signaling, it has been proposed that FTs are another trait for signaling, potentially as a secondary system that allows communication between conspecifics while remaining camouflaged to predators (Prötzel et al., 2018). However, the main predators of chameleons include snakes and birds of prey that are able to perceive color within the 400–430 nm range (Crowell et al., 2024; Simões et al., 2016), making it unclear how effective an anti-predatory strategy this would be. In addition, signaling in chameleons is carried out by males and females, and signaling traits relating to reproductive fitness are present in both sexes (Tolley & Burger, 2007; Tolley & Herrel, 2013). Thus, if fluorescence is used for signaling, sexually dimorphic fluorescent morphology may provide evidence of sexual selection. Furthermore, Prötzel et al. (2018) argued that fluorescence is more prevalent in forest chameleon species than those from habitats that lack a canopy, presumably because closed-canopy forests are suitable terrestrial environments for producing natural fluorescence (Lythgoe, 1979; Nicolaï et al., 2024; Prötzel et al., 2018). This supports the notion that there could be differences among species stemming from natural selection via sensory drive.

African dwarf chameleons (*Bradypodion*) provide an opportunity to investigate whether fluorescence is used for signaling by chameleons. Tubercle fluorescence has been documented in all *Bradypodion* species examined to date – *B. pumilum* (Barends & Bester, 2023); *B. damaranum*, *B. occidentale, B. taeniabronchum, B. ventrale* (pers. obs.); *B. thamnobates* (Prötzel et al., 2018 supplementary material) and *B. transvaalense* (Prötzel et al., 2018) – and is likely widespread across the genus as the trait is presumed plesiomorphic to Chamaeleonidae (Prötzel et al., 2018). *Bradypodion* species occur in a variety of habitats throughout temperate and sub-tropical South Africa (Tolley et al., 2023; Tolley & Burger, 2007), ranging from open environments with high incoming light levels, such as fynbos (heathland) or karroid shrublands, to closed canopy Afrotemperate forests that receive diminishing levels of light lower in the canopy (see Fleishman et al., 1993). Forest and shrubland species are generally larger and more ornate compared to those occupying fynbos habitats (Hopkins & Tolley, 2011; Tolley et al., 2022). Males of some *Bradypodion* species have more pronounced ornamentation than females (da Silva & Tolley, 2013; Hopkins & Tolley, 2011), and ornament size relates to sexual selection (Stuart-Fox et al., 2006, 2007). Furthermore, *Bradypodion* species tend to be sexually size dimorphic, but the direction of dimorphism depends on the species (Barends & Tolley, 2024; Stuart-Fox, 2009). In addition, populations of some species also occur in urban greenbelts, municipal parks, gardens and along road verges in towns, with differences in body and head size present in these populations (Barends & Tolley, 2024; Petford et al., 2024).

The prevalence of both sexual dimorphism and ecological (habitat) diversity within *Bradypodion* provides an opportunity to investigate explicit hypotheses regarding UV fluorescence of bony tubercles as a signaling trait, as has been hypothesized (Prötzel et al., 2018). If FTs are used for signaling related to sexual selection, then we would predict that males should have more FTs than females when corrected for body and/or head size. Moreover, if related to sensory drive (Endler, 1992), then forest species should have more FTs than open-habitat species when corrected for body and/or head size. Similarly, we expected that populations in natural environments should have more FTs than those in urban areas as a result of phenotypic plasticity in response to diminished tree cover and concomitant higher levels of light penetrating the vegetation (Petford et al., 2024). To test these hypotheses, we quantified the number of FTs of five species of *Bradypodion* that occur in habitats with differing incoming light levels (closed-canopy and open-canopy) by photographing the head ornamentation while exposed to light from the UV fluorescent spectrum.

Materials and methods

Chameleon sampling

We captured and photographed 289 adult (i.e. SVL > 40 mm) chameleons from five *Bradypodion* species (*B. barbatulum*, *B. damaranum*, *B. pumilum*, *B. taeniabronchum* and *B. ventrale*) from October 2023 through to February 2025 in and around the towns of Betty's Bay, George, Joubertina, Jeffreys Bay, Gqeberha and Stellenbosch in South Africa (Table 1). *Bradypodion barbatulum* and *B. taeniabronchum* occur only in indigenous fynbos vegetation along the Cape Fold Mountains (Tolley et al., 2023), while *B. damaranum* and *B. ventrale* occupy Afrotemperate forests and semi-arid shrublands, respectively, but occur also in urban areas with anthropogenically modified or exotic vegetation (Tolley et al., 2023). For the latter two species, we sampled individuals from both natural and urban populations. *Bradypodion pumilum* occurs as two distinct ecomorphs: a fynbos ecomorph and a forest ecomorph occupying riparian forests, woodlands, wetlands and urban greenbelts (M. A. Petford, A. Herrel, K. A. Tolley, unpublished data; Tolley et al., 2023). While genetically similar, these two ecomorphs are morphologically distinct, with the fynbos (or LBC, i.e., 'little brown chameleon') ecomorph being smaller in size, duller in color and less ornate than the forest ecomorph (M. A. Petford, A. Herrel, K. A. Tolley, unpublished data; Tolley et al., 2023). We sampled *B. pumilum* from fynbos and riparian forest habitats.

Table 1 Sample sizes and localities of populations of five *Bradypodion* species sampled within the Eastern Cape (EC) and Western Cape (WC) provinces of South Africa

Species	Habitat	Locality	Latitude	Longitude	*N*	
					Females	Males
B. barbatulum	Fynbos	Joubertina, Tsitsikamma Mountains, EC	33°50′20.8″ S	23°46′10.2″ E	14	18
B. damaranum	Forest	Groeneweide Forest, George, WC	33°57′24.1″ S	22°32′53.5″ E	14	21
B. damaranum	Urban	Camphersdrift, George, WC	33°57′19.4″ S	22°26′38.4″ E	20	31
B. pumilum	Fynbos	Betty's Bay, Kleinmond, WC	34°19′30.2″ S	18°57′41.8″ E	17	11
B. pumilum	Forest	Brummer Park, Stellenbosch, WC	33°56′22.0″ S	18°53′19.0″ E	22	21
B. taeniabronchum	Fynbos	Lady's Slipper, Port Elizabeth, EC	33°53′09.6″ S	25°15′26.3″ E	5	13
B. ventrale	Shrublands	Papiesfontein, Jeffreys Bay, WC	33°57′56.5″ S	24°58′00.1″ E	30	19
B. ventrale	Urban	Jeffreys Bay Golf Course, Jeffreys Bay, WC	34°02′12.8″ S	24°54′51.5″ E	15	18

All individuals were captured by hand at night when chameleons are easier to locate using torchlight. Capture sites were recorded with a GPS and chameleon perch locations were tagged with chevron flagging tape. Captured chameleons were placed in individually numbered cloth bags with vegetation and brought back to the field station for measurements and UV photography (see below). Chameleons were sexed by visually examining for the presence of hemipenes and measured for snout-vent length (SVL) using digital callipers to the nearest 0.01 mm. After being measured and photographed, each individual was returned to its exact capture site.

UV photography

Chameleons were kept overnight and photographed the following day in a dark room where ambient light was dim. Chameleons were gently restrained by hand and illuminated by a 365 nm UV light emitted from a 36-watt UV torch (model: Alonefire H42, Shenzhen Shiwang Technology Ltd, Shenzhen, China) from a ~30 cm distance. Each individual was photographed from three angles: once laterally on each side of the head and once dorsally (Fig. 1). All photographs were taken with a Canon 70D DSLR camera with a 60 mm f/2.8 macro lens in RAW format. Standardized settings of f/11, ISO 1600, 1/8 s shutter speed were used for each photo. Photographs were processed in Adobe Lightroom software version 12.4 (Adobe Inc., 2023) where we made slight adjustments to the contrast and brightness of each image to improve its clarity before converting it to JPEG format. Exported JPEG photographs were then imported into ImageJ software version 1.42 (Schneider et al., 2012) where we used the multi-point tool to count the numbers of lateral and dorsal FTs of each individual. FTs on the left and right side of the head were averaged to produce a single measure for lateral FTs per individual. FT number was chosen as the focal measure for analysis following Prötzel et al. (2018) who used the same measure to investigate trends in fluorescence across several species of *Calumma*. By using the same measure, our findings can thus be directly compared to those of Prötzel et al. (2018) and provide additional evidence to support or deny the assertion that fluorescence in chameleons is associated with sex or habitat.

Sex-based differences in FTs

All analyses were conducted in R software version 4.4 (R Core Team, 2024). All data were log10-transformed prior to analyses to meet assumptions of normality and equality of variances. To assess the relationships between body and head size with FT number, ordinary least squares linear regressions were run with SVL and head size as independent variables and the numbers of lateral and dorsal FTs as dependent variables. Head sizes were calculated as the total areas of the lateral and dorsal aspects of the head (Fig. 1) taken from the UV photographs using ImageJ following the methods of Measey et al. (2009). Head areas were then linearized via square-root transformation before being log10-transformed. These analyses were conducted separately per sex for each species and each population within species where applicable.

We compared the numbers of FTs between sexes for each species and population within species using separate analyses of variance (ANOVA) tests with sex as the fixed factor, and dorsal and lateral FTs each as dependent variables. However, *Bradypodion* species are sexually dimorphic, with females being larger than males in most species (Stuart-Fox, 2009) but having comparatively smaller heads when accounting for body size (da Silva & Tolley, 2013). Thus, we also corrected for body and head size variation when comparing the numbers of FTs between groups. We used analyses of covariance (ANCOVAs) with sex as the fixed factor and SVL and head size as covariate factors to compare lateral and dorsal FTs across males and females of each species, and populations within species where applicable.

Habitat related differences in FTs

Habitat specific differences in absolute numbers of FTs were compared for each sex separately across our dataset using ANOVAs with habitat type (i.e. forests, fynbos and shrublands) as the fixed factor. Size-adjusted phylogenetic ANOVAs were then used to correct for body and head size variation while accounting for species relatedness for those same comparisons. For these analyses, we first used phylogenetic regressions to produce size-adjusted residuals of FTs against SVL and head

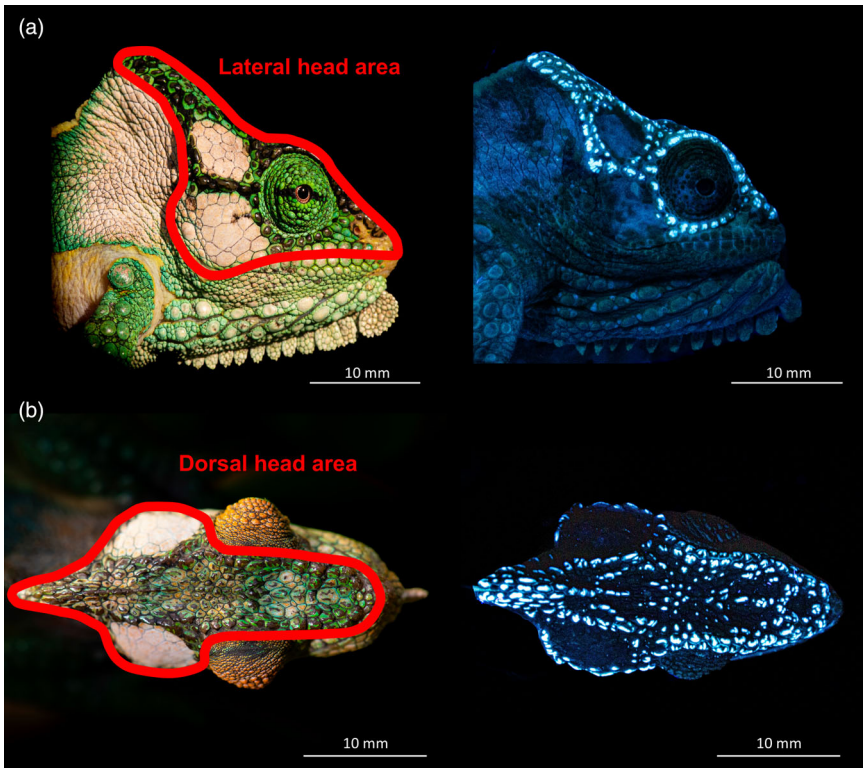

Figure 1 Photographs of lateral (a) and dorsal (b) views of the head of an adult *Bradypodion damaranum* illuminated by visible light (left images) and exposed to 365 nm ultraviolet light (right images). Regions demarcated in red for both (a) and (b) indicated the area of the head that was used as a covariate in statistical analyses. Images on the right show the 'electric blue' coloration of the externally visible fluorescent tubercles. Photographs by JM Barends and WK Stanton-Jones.

area separately via the 'phyl.resid' function in phytools (Revell, 2012). These residuals were then input as the dependent variable in the phylogenetic anova run using the phylANOVA function, over 1000 simulations. A pruned version of the phylogeny of *Bradypodion* from Barends et al. (2024) was used as the input tree for these analyses. Lastly, we used ANOVAs and ANCOVAs to compare intraspecific differences in FTs between natural and urban populations of both *B. damaranum* and *B. ventrale*.

Results

Fluorescence in *Bradypodion*

All photographed chameleons displayed similar patterns of externally visible, bone-based cranial fluorescence upon exposure to 365 nm ultraviolet light as typical of chameleons (Barends & Bester, 2023; Prötzel et al., 2018; Van Wyk & Russell, 2024). Fluorescent tubercles were distributed along the lateral, orbital, parietal, rostral and temporal crests and fluoresced a bright electric blue color (Fig. 1a). Dorsal FTs on the top of the head were ubiquitous across the entirety of the casque, distributed from the snout to the casque peak (Fig. 1b).

Linear regressions showed a strong positive relationship between FT number (laterally and dorsally) and both SVL and head area in most populations of *Bradypodion* in our sample (Figs S1–S4; Tables 2 and 3). In particular, the relationship was strongest in natural and urban populations of *B. damaranum, B. ventrale* and forest *B. pumilum*, and weaker in the fynbos dwelling *B. barbatulum* and *B. taeniabronchum* (Figs S1–S4; Tables 2 and 3).

Sex-based differences in FTs

Females were significantly larger or similar in body size on average than males for each species (ANOVAs of log10-SVL by sex: *B. barbatulum* – $F_{1,30} = 74.468$, $P \leq 0.001$; forest *B. pumilum* – $F_{1,41} = 0.040$, $P = 0.842$; fynbos *B. pumilum* – $F_{1,26} = 0.030$, $P = 0.864$; *B. taeniabronchum* – $F_{1,16} = 15.650$, $P \leq 0.001$; *B. ventrale* – $F_{1,80} = 14.911$, $P \leq 0.05$) except *B. damaranum* ($F_{1,84} = 6.625$, $P \leq 0.05$) which showed the opposite pattern of sexual size dimorphism in body size (Fig. 2). Accordingly, females generally had significantly greater numbers of lateral and dorsal FTs than males on average when comparing absolute measures (Fig. 3; Table 4). However, the mean numbers of lateral and dorsal FTs did not significantly differ between sexes for any of the species or populations

Table 2 Linear relationships between log-transformed numbers of fluorescent tubercles (FTs) and snout-vent lengths of five *Bradypodion* species

			Lateral FTs			Dorsal FTs		
Species	Habitat	Sex	*F*	*P*	R^2	*F*	*P*	R^2
B. barbatulum	Fynbos	F	0.910	0.359	0.071	1.156	0.314	0.126
B. barbatulum	Fynbos	M	**5.095**	**≤0.05**	**0.242**	0.475	0.511	0.056
B. damaranum	Forest	F	**6.401**	**≤0.05**	**0.348**	1.459	0.250	0.108
B. damaranum	Forest	M	**21.783**	**≤0.001**	**0.534**	**5.201**	**≤0.05**	**0.215**
B. damaranum	Urban	F	**16.374**	**≤0.001**	**0.476**	**11.858**	**≤0.05**	**0.397**
B. damaranum	Urban	M	**57.192**	**≤0.001**	**0.664**	**15.283**	**≤0.001**	**0.345**
B. pumilum	Forest	F	**107.488**	**≤0.001**	**0.843**	**133.414**	**≤0.001**	**0.870**
B. pumilum	Forest	M	**47.320**	**≤0.001**	**0.714**	**56.436**	**≤0.001**	**0.748**
B. pumilum	Fynbos	F	**60.454**	**≤0.001**	**0.801**	**70.789**	**≤0.001**	**0.825**
B. pumilum	Fynbos	M	**36.667**	**≤0.001**	**0.803**	**12.874**	**≤0.05**	**0.589**
B. taeniabronchum	Fynbos	F	0.166	0.711	0.052	0.057	0.826	0.019
B. taeniabronchum	Fynbos	M	**5.190**	**≤0.05**	**0.322**	0.443	0.520	0.039
B. ventrale	Shrublands	F	**13.986**	**≤0.001**	**0.333**	**8.838**	**≤0.05**	**0.240**
B. ventrale	Shrublands	M	**12.231**	**≤0.05**	**0.418**	**13.184**	**≤0.05**	**0.437**
B. ventrale	Urban	F	**14.959**	**≤0.05**	**0.535**	**8.207**	**≤0.05**	**0.387**
B. ventrale	Urban	M	**12.856**	**≤0.05**	**0.446**	**14.93**	**≤0.001**	**0.483**

Significant associations are in boldface.

Table 3 Linear relationships between log-transformed numbers of fluorescent tubercles (FTs) and head areas of five *Bradypodion* species

			Lateral FTs			Dorsal FTs		
Species	Habitat	Sex	*F*	*P*	R^2	*F*	*P*	R^2
B. barbatulum	Fynbos	F	0.015	0.905	0.001	2.556	0.149	0.242
B. barbatulum	Fynbos	M	1.703	0.210	0.096	1.409	0.269	0.150
B. damaranum	Forest	F	**8.245**	**≤0.05**	**0.407**	2.765	0.122	0.187
B. damaranum	Forest	M	**25.137**	**≤0.001**	**0.570**	**5.404**	**≤0.05**	**0.221**
B. damaranum	Urban	F	**12.409**	**≤0.05**	**0.408**	4.152	0.057	0.187
B. damaranum	Urban	M	**70.139**	**≤0.001**	**0.707**	**13.381**	**≤0.001**	**0.316**
B. pumilum	Forest	F	**84.005**	**≤0.001**	**0.808**	**85.892**	**≤0.001**	**0.811**
B. pumilum	Forest	M	**65.419**	**≤0.001**	**0.775**	**51.420**	**≤0.001**	**0.730**
B. pumilum	Fynbos	F	**5.601**	**≤0.05**	**0.272**	**9.100**	**≤0.05**	**0.378**
B. pumilum	Fynbos	M	**10.805**	**≤0.05**	**0.546**	**5.770**	**≤0.05**	**0.391**
B. taeniabronchum	Fynbos	F	3.952	0.141	0.568	0.014	0.913	0.005
B. taeniabronchum	Fynbos	M	2.581	0.136	0.190	0.456	0.514	0.040
B. ventrale	Shrublands	F	1.634	0.212	0.055	**4.564**	**≤0.05**	**0.140**
B. ventrale	Shrublands	M	**8.598**	**≤0.05**	**0.336**	3.070	0.098	0.153
B. ventrale	Urban	F	**5.811**	**≤0.05**	**0.309**	4.669	0.078	0.220
B. ventrale	Urban	M	**13.088**	**≤0.05**	**0.451**	3.302	0.088	0.171

Significant results in boldface.

within species when correcting for body size or head size (Table 4).

Habitat related differences in FTs

The results of ANOVA tests with habitat type as a fixed factor showed that the mean numbers of FTs (in absolute measures) significantly differed across species from different habitats for both sexes, with the highest present in the shrublands dwelling *B. ventrale*, and the lowest present in the fynbos dwelling species (Fig. 3; Table 5). However, there were no significant differences in FTs between species from different habitats for either sex when correcting for chameleon body or head size and accounting for phylogenetic relatedness (Table 6). Urban *B. damaranum* had greater numbers of FTs on average than natural counterparts (Fig. 3) from our sample when comparing absolute measures, but this difference was only significant for lateral FTs compared between females ($F_{1,43} = 4.757$, $P \leq 0.05$). Conversely, natural females of *B. ventrale* had significantly greater numbers of lateral FTs than urban counterparts ($F_{1,43} = 4.757$, $P \leq 0.05$; Fig. 3). In both cases, the group with more FTs was significantly larger than its counterpart (*B. damaranum* females: $F_{1,32} = 14.\ 875$, $P \leq 0.001$; *B. ventrale* females: $F_{1,50} = 5.127$, $P \leq 0.05$; Fig. 2). There were

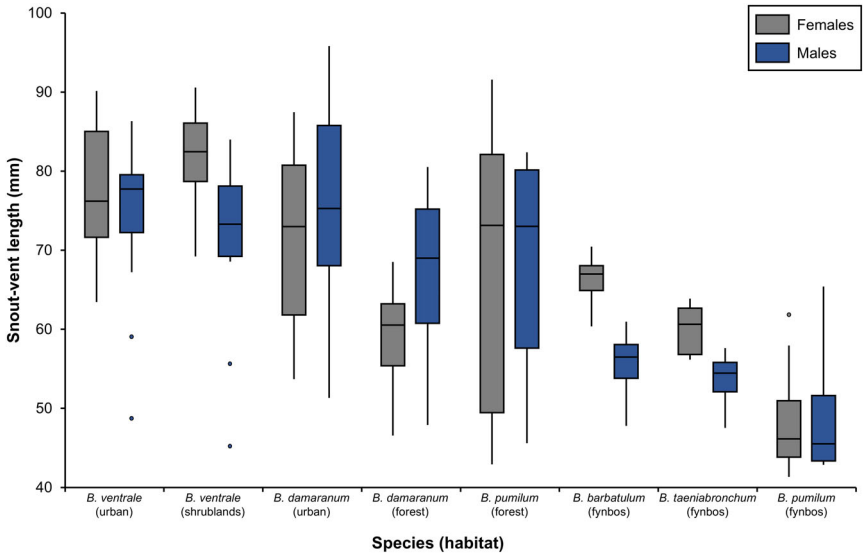

Figure 2 Box and whisker plots of snout-vent lengths (SVL) of females and males across five species of *Bradypodion* from natural and urban habitats. Species are ordered from left to right in descending order of SVL.

no significant differences in the numbers of lateral or dorsal FTs between sexes across natural and urban populations for both *B. damaranum* and *B. ventrale* when correcting for SVL (F = 0.010–0.885; $P > 0.05$ in all cases) or head size (F = 0.001–0.859; $P > 0.05$ in all cases).

Discussion

Biofluorescence of tubercles was present in all five species of *Bradypodion* investigated, adding to the growing body of work reporting on this trait in reptiles (Botelho et al., 2024; Gruber & Sparks, 2015; Paul & Mendyk, 2021; Prötzel et al., 2018, 2021) and other terrestrial animals (Douglas et al., 2021; Lamb & Davis, 2020). However, we found no support for this trait as a means of intraspecific communication relating to sexual selection via sensory drive. Our analyses suggest that there is no male-biased sexual dimorphism of this trait given that the number of FTs can be explained by body and head size rather than males being disproportionately more fluorescent. This finding does not support predictions regarding biofluorescence of cranial tubercles being linked to signaling for sexual selection in *Bradypodion*. Moreover, differences in FTs between species and between populations within species are also explained by body and head size and not by habitat type. This finding would not be expected should interspecific signaling via fluorescence to compensate for low light habitats (e.g. forest) be enhanced due to natural selection (Endler, 1993b).

Fluorescence in chameleons has been proposed as a trait that is male-biased and therefore used in signaling related to sexual selection, as suggested for the genus *Calumma* (Prötzel et al., 2018). Instead, we found that the number of tubercles in *Bradypodion* can be explained by body and head size and is greater for the larger sex, regardless of the direction of size dimorphism. This is because the absolute differences in head size between sexes allow for more tubercles to be accommodated on the heads of the larger group. Females are the larger sex in most species of *Bradypodion* (Tolley & Burger, 2007) whereas males are larger in most species of *Calumma* (Glaw & Vences, 2007; Raxworthy & Nussbaum, 2006), which explains why we did not observe the same trends in FTs for *Bradypodion* compared to *Calumma* species examined by Prötzel et al. (2018). In addition, we showed that males and females have similar numbers of FTs when correcting for chameleon body size or head size, further emphasizing a lack of male-bias. Similar comparisons that correct for chameleon body or head sizes have not yet been done for *Calumma*, and so it is unclear if the male-bias in FTs for these species would remain when accounting for those covariates. Indeed, the findings for *Calumma* do not provide explicit evidence to support the hypothesis that differences in the numbers of FTs are driven by sexual selection for increased signaling ability; whereas our findings for *Bradypodion* explicitly rule out the hypothesis that FTs are used for sexual signaling.

Regarding the hypothesis that FT fluorescence in chameleons is biased toward forest species due to sensory drive, our findings fail to provide support. When comparing across species from different habitats, females of the large-bodied shrubland species *B. ventrale* had more FTs than the two forest species *B. damaranum* and *B. pumilum*. Moreover, after correcting for body size and phylogeny, there was no significant difference in the number of FTs between groups of either sex despite the differences in their habitats. In addition, the lack of differences between chameleons from natural and urban populations further refutes the sensory drive hypothesis because urban habitats would likely not promote fluorescence due to the lack of vegetation cover (Petford et al., 2024). These findings suggest that variation in body and head sizes explains the differences in FTs among species and populations rather than

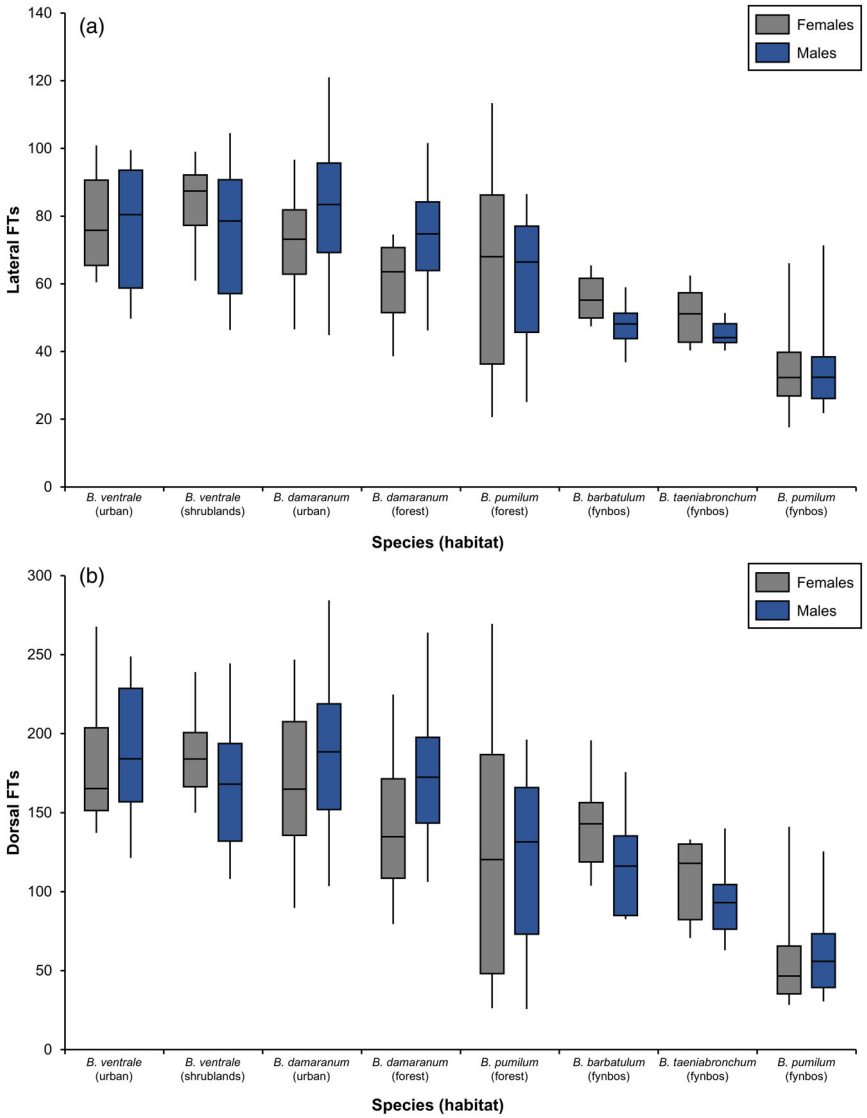

Figure 3 Box and whisker plots showing the numbers of (a) lateral and (b) dorsal fluorescent tubercles (FTs) of females and males across five species of *Bradypodion* from natural and urban habitats. Species are ordered from left to right in descending order of SVLs.

communication that is facilitated through biofluorescent tubercles under differing light levels (Endler, 1993b). The evolution of smaller body, head, and limb sizes in *Bradypodion* largely coincided with shifts toward utilizing open habitats such as fynbos due to adaptations to different vegetation (i.e. perch sizes and densities; da Silva et al., 2014; da Silva & Tolley, 2013; Hopkins & Tolley, 2011). Thus, the differences between species may simply be an indirect byproduct of natural selection for decreased conspicuousness rather than due to their habitats differing in suitability for fluorescent signaling.

Overall, our findings essentially provide no support for fluorescence of tubercles as having a signaling function in *Bradypodion*, and this also may be the case for other species and genera. In addition to the trait not showing characteristics typical of features used for signaling, the lifestyles of chameleons also do not support fluorescence as a communicative signal. Chameleons are diurnal lizards (Tolley & Herrel, 2013) and are active during a photoperiod when natural fluorescence would be at its most difficult to detect by conspecifics. During sunlight hours, fluorescence will almost certainly not be visible against the broad-spectrum light and reflectance produced by sunlight (Nicolaï et al., 2024). Although this has not been explicitly tested, measures of the quantum yields (i.e. the number of photons absorbed vs. those emitted) in FTs, and the spectral emission properties of the various environments in which chameleons occur could confirm if natural fluorescence is possible under full daylight conditions. If not used for signaling, the role of fluorescence in chameleons, if any, is

Table 4 Results of ANOVA and ANCOVA tests comparing the numbers of lateral and dorsal fluorescent tubercles (FTs) with *Bradypodion* sex as the fixed factor, and body and head sizes as covariate factors

				Lateral FTs				Dorsal FTs			
Species	Habitat	Statistical test	Covariate	Female means/ EMMs	Male means/ EMMs	*F*	*P*	Female means/ EMMs	Male means/ EMMs	*F*	*P*
B. barbatulum	Fynbos	ANOVA	–	**55.21**	**47.89**	**11.464**	**≤0.05**	**140.90**	**115.70**	**4.478**	**≤0.05**
B. damaranum	Forest	ANOVA	–	**60.21**	**73.38**	**7.248**	**≤0.05**	**139.86**	**170.24**	**4.628**	**≤0.05**
B. damaranum	Urban	ANOVA	–	**71.10**	**81.42**	**4.257**	**≤0.05**	166.85	183.68	1.638	0.207
B. pumilum	Forest	ANOVA		63.773	61.619	0.036	0.851	122.91	117.91	0.008	0.928
B. pumilum	Fynbos	ANOVA		35.47	36.55	0.034	0.855	57.24	60.01	0.232	0.634
B. taeniabronchum	Fynbos	ANOVA	–	50.01	45.01	3.082	0.098	107.60	91.46	1.695	0.211
B. ventrale	Shrublands	ANOVA	–	**84.13**	**75.79**	**5.511**	**≤0.05**	**186.47**	**170.53**	**4.193**	**≤0.05**
B. ventrale	Urban	ANOVA	–	76.67	76.39	0.047	0.830	179.60	185.28	0.191	0.666
B. barbatulum	Fynbos	ANCOVA	SVL	1.702[a]	1.706[a]	0.012	0.915	2.104[q]	2.088[q]	0.044	0.837
B. damaranum	Forest	ANCOVA	SVL	1.813[b]	1.829[b]	0.338	0.565	2.162[r]	2.195[r]	0.601	0.444
B. damaranum	Urban	ANCOVA	SVL	1.858[c]	1.891[c]	2.921	0.094	2.223[s]	2.241[s]	0.438	0.511
B. pumilum	Forest	ANCOVA	SVL	1.760[d]	1.761[d]	0.001	0.984	1.998[t]	1.989[t]	0.043	0.837
B. pumilum	Fynbos	ANCOVA	SVL	1.526[e]	1.527[e]	0.004	0.950	1.702[u]	1.730[u]	0.382	0.542
B. taeniabronchum	Fynbos	ANCOVA	SVL	1.667[f]	1.662[f]	0.021	0.886	1.993[v]	1.962[v]	0.172	0.684
B. ventrale	Shrublands	ANCOVA	SVL	1.897[g]	1.907[g]	0.216	0.644	2.243[w]	2.258[w]	0.474	0.495
B. ventrale	Urban	ANCOVA	SVL	1.868[h]	1.881[h]	0.311	0.581	2.236[x]	2.268[x]	1.842	0.185
B. barbatulum	Fynbos	ANCOVA	Head area	1.729[i]	1.686[i]	2.474	0.127	2.104[y]	2.089[y]	0.069	0.796
B. damaranum	Forest	ANCOVA	Head area	1.834[j]	1.815[j]	0.424	0.521	2.193[z]	2.175[z]	0.124	0.727
B. damaranum	Urban	ANCOVA	Head area	1.892[k]	1.867[k]	1.418	0.241	2.253[aa]	2.222[aa]	0.879	0.353
B. pumilum	Forest	ANCOVA	Head area	1.770[l]	1.750[l]	0.490	0.488	2.014[ab]	1.972[ab]	0.829	0.368
B. pumilum	Fynbos	ANCOVA	Head area	1.547[m]	1.495[m]	0.986	0.986	1.718[ac]	1.705[ac]	0.036	0.852
B. taeniabronchum	Fynbos	ANCOVA	Head area	1.693[n]	1.653[n]	4.079	0.062	1.999[ad]	1.961[ad]	0.299	0.593
B. ventrale	Shrublands	ANCOVA	Head area	1.908[o]	1.889[o]	0.673	0.416	2.261[ae]	2.229[ae]	1.947	0.171
B. ventrale	Urban	ANCOVA	Head area	1.881[p]	1.872[p]	0.107	0.745	2.242[af]	2.262[af]	0.494	0.487

Significant differences in boldface. Covariate values for calculation of EMMs from log-transformed SVL and head area sizes: [a]1.781; [b]1.810; [c]1.867; [d]1.826; [e]1.678; [f]1.745; [g]1.893; [h]1.882; [i]0.930; [j]1.032; [k]1.076; [l]1.024; [m]0.880; [n]0.906; [o]1.085; [p]1.071; [q]1.783; [r]1.810; [s]1.867; [t]1.826; [u]1.678; [v]1.745; [w]1.893; [x]1.882; [y]0.972; [z]1.057; [aa]1.107; [ab]1.046, [ac]0.925; [ad]0.936; [ae]1.125; [af]1.114.

EMMs, estimated marginal means.

Table 5 Results of ANOVA tests comparing the numbers of lateral and dorsal fluorescent tubercles (FTs) across five *Bradypodion* species with natural habitat as a fixed factor

		Lateral FTs					Dorsal FTs				
Comparison	Sex	Forest mean	Fynbos mean	Shrub mean	*F*	*P*	Forest mean	Fynbos mean	Shrub mean	*F*	*P*
All habitats	F	**62.389**	**45.167**	**84.133**	**32.715**	**≤0.001**	**129.500**	**91.250**	**186.467**	**21.717**	**≤0.001**
All habitats	M	**67.500**	**44.024**	**75.789**	**35.314**	**≤0.001**	**144.071**	**88.412**	**170.526**	**16.113**	**≤0.001**
Pairwise					T	*P*				T	*P*
Forest–Fynbos	F	**62.389**	**45.167**	–	**3.811**	**≤0.001**	**129.500**	**91.250**	–	**2.843**	**≤0.05**
Forest–Fynbos	M	**67.500**	**44.024**	–	**6.892**	**≤0.001**	**144.071**	**88.412**	–	**4.180**	**≤0.001**
Forest–Shrublands	F	**62.389**	–	**84.133**	**4.455**	**≤0.05**	**129.500**	–	**186.467**	**3.957**	**≤0.05**
Forest–Shrublands	M	67.500	–	75.789	1.700	0.208	144.071	–	170.526	2.003	0.116
Fynbos–Shrublands	F	–	**45.167**	**84.133**	**8.089**	**≤0.001**	–	**91.250**	**186.467**	**6.567**	**≤0.001**
Fynbos–Shrublands	M	–	**44.024**	**75.789**	**7.139**	**≤0.001**	–	**88.412**	**170.526**	**5.300**	**≤0.001**

Significant differences in boldface.

Table 6 Results of size-corrected phylogenetic ANOVAS tests comparing the numbers of lateral and dorsal fluorescent tubercles (FTs) across five *Bradypodion* species with natural habitat as fixed factor

		Lateral FTs		Dorsal FTs	
Sex	Size variable	*F*	*P*	*F*	*P*
F	SVL	2.018	0.147	0.293	0.739
M	SVL	0.244	0.754	0.034	0.954
F	Head area	0.133	0.843	0.281	0.706
M	Head area	0.249	0.742	0.076	0.891

Differences were not statistically significant in all cases.

unclear but is likely of little ecological importance, and this assumption may extend to other terrestrial species that exhibit the trait (Jeng, 2019; Nicolaï et al., 2024).

In conclusion, our findings show that body size rather than ecology best predicts fluorescence in *Bradypodion*. However, our study involved only five of the 20 currently recognized species of *Bradypodion* (Tolley et al., 2023) so our interpretations could be strengthened by additional data from other species in the genus. A key next step is to ascertain if other species of *Bradypodion* exhibit fluorescence and test if the trends observed here are similar across a larger sample of species and also other chameleon genera. We reiterate that hypotheses regarding the role of FTs in signaling, relating to both sexual and natural selection, must be evaluated with relevant covariates (e.g. body and head size). Here, we show that the inclusion of size covariates results in a lack of support for the original hypotheses regarding the role of FTs in chameleons. Spectral emission analyses of different species occupying environmentally diverse habitats could also provide important context toward clarifying the signaling potential of fluorescent tubercles or other fluorescent morphology. In addition to chameleons, fluorescence has been reported in a host of other reptiles such as geckos (Botelho et al., 2024; Burriel-Carranza et al., 2024), snakes (Paul & Mendyk, 2021) and turtles (Gruber & Sparks, 2015) and has been posited as having a role in signaling in these taxa. However, this has not been explicitly tested for any of those species. As more species are documented to exhibit fluorescence, we would recommend researchers employ caution before claiming that UV fluorescence functions for communication without explicit hypothesis testing.

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Author contributions

JMB, KAT, GJA and WKSJ conceptualized the study and collected data. JMB analysed the data and led the writing of the paper. All authors contributed critically to the text and approved the final submission.

Data availability statement

The data and R code used for analyses in this study are available at figshare: https://doi.org/10.6084/m9.figshare.29135747.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Linear regressions for the number lateral fluorescent tubercles (FTs) and snout-vent length of female and male (a) *Bradypodion barbatulum*, (b) *B. taeniabronchum*, (c) *B. damaranum* (natural populations), (d) *B. damaranum* (urban populations), (e) *B. pumilum* (forest populations), (f) B. *pumilum* (fynbos populations), *B. ventrale* (natural populations) and (g) *B. ventrale* (urban populations).

Figure S2. Linear regressions for the number dorsal fluorescent tubercles (FTs) and snout-vent length of female and male (a) *Bradypodion barbatulum*, (b) *B. taeniabronchum*, (c) *B. damaranum* (natural populations), (d) *B. damaranum* (urban populations), (e) *B. pumilum* (forest populations), (f) *B. pumilum* (fynbos populations), *B. ventrale* (natural populations) and (g) *B. ventrale* (urban populations).

Figure S3. Linear regressions for the number lateral fluorescent tubercles (FTs) head area of female and male (a) *Bradypodion barbatulum*, (b) *B. taeniabronchum*, (c) *B. damaranum* (natural populations), (d) *B. damaranum* (urban populations), (e) *B. pumilum* (forest populations), (f) *B. pumilum* (fynbos populations), *B. ventrale* (natural populations) and (g) *B. ventrale* (urban populations).

Figure S4. Linear regressions for the number dorsal fluorescent tubercles (FTs) head area of female and male (a) *Bradypodion barbatulum*, (b) *B. taeniabronchum*, (c) *B. damaranum* (natural populations), (d) *B. damaranum* (urban populations), (e) *B. pumilum* (forest populations), (f) *B. pumilum* (fynbos populations), *B. ventrale* (natural populations) and (g) *B. ventrale* (urban populations).