

Spectral data reveal unexpected cryptic colour polymorphism in female sailfin silverside fish from ancient Lake Matano

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Abstract Persistent colour polymorphisms can result from natural and/or sexual selection, and may occur in males, females, or both sexes. Contrary to conspicuous patterns frequently observed in courtship colouration, differences in cryptic colouration are not always perceived by the human sensory system. In sexually dimorphic sailfin silversides fishes, males show conspicuous colour polymorphisms whereas females appear monomorphic and cryptic. We measured the spectral composition of body, fin and peduncle colouration in male and female *Telmatherina*

antoniae ‘small’, a sailfin silverside species endemic to ancient Lake Matano, and found evidence for a colour polymorphism in both sexes. The three colour morphs distinguished by spectral data correspond to those commonly reported for males, and are also present in the visually (to a human eye) cryptic females. Females show hue value patterns similar to those present in males, but differ from males substantially in chroma and brightness. This is, to the best of our knowledge, the first example of a cryptic colour polymorphism in fishes; however, its significance for the mating system remains unknown. The present finding highlights the need for incorporating female spectral data into analyses of colour patterns, and suggests that colour analyses should include cryptic sexes.

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Introduction

Colour polymorphism, the maintenance of two or more genetically determined colour morphs within a single interbreeding population (Huxley, 1955), is a common phenomenon in various animals such as birds, butterflies and fishes (reviewed by Gray & McKinnon, 2007). Such polymorphisms play a role in visual signalling, including mate choice and territory defence (Andersson, 1994), and there is evidence that sexual as well as ecological selection may contribute to its persistence. Alternative colour morphs can likely facilitate population divergence and speciation (Chunco et al., 2007; Seehausen et al., 2008; Nosil et al., 2009; Hurtado-Gonzales & Uy, 2010); however, the processes maintaining within-population polymorphism remains only partially understood. Both theoretical work and case studies suggest that the persistence of alternative genetic colour morphs can be promoted through sensory drive, frequency-dependent selection, male–male competition, or heterogeneous environments (Chunco et al., 2007; Gray & McKinnon, 2007; Forsman et al., 2008; Gray et al., 2008a; Hurtado-Gonzales & Uy, 2010; Maan & Seehausen, 2010; Castillo Cajas et al., 2012; Hancox et al., 2013). Much of the work on colour polymorphism has focused on males with distinct courtship colouration, or on species with a persistent colour polymorphism in both sexes (Gray & McKinnon, 2007); a cryptic female colour polymorphism has not been reported from fishes thus far.

Conspicuous male colour polymorphisms occur in several species of the sailfin silversides (Atheriniformes: *Telmatherinidae*) species flock in Central Sulawesi, Indonesia (Herder et al., 2006a). Radiating in the clear waters of the ancient Malili Lakes and the surrounding watershed (Herder et al., 2006b; Pfaender et al., 2010, 2011), males of these predominantly small freshwater fishes show conspicuously elongated and coloured fins, as well as colourful bodies, whereas females appear cryptic: dusky grey to silvery or bronze-coloured body and short fins lacking conspicuous colours, depending on the species. Extraordinarily deep and clear Lake Matano harbours the small species flock of three endemic ‘roundfin’ sailfin silversides that most likely evolved following ecological selection pressure in sympatry (Herder et al., 2008). Clear population structure discriminates the three morphospecies, while in contrast there are no

restrictions to gene flow among blue, blue-yellow and yellow male colour morphs (Herder et al., 2008; Walter et al., 2009).

Here, we compare spectrometric data taken from both sexes of the roundfin species *Telmatherina antoniae* ‘small’ (Fig. 1). Our results clearly confirm the distinct colour polymorphism in males, which has so far only been reported based only on visual classification, but add the unexpected occurrence of a cryptic polymorphism in females, reflecting the conspicuous pattern in males.



Fig. 1 The three distinct *Telmatherina antoniae* ‘small’ male colour morphs (a; yellow; blue-yellow; blue) and a cryptic female (b) photographed in their natural environment

Materials and methods

Adult *T. antoniae* ‘small’ (♀ = 83; ♂ = 78) were caught with gill nets between May and August 2010 at 53 sampling locations distributed around Lake Matano’s shoreline. Directly after capture, fish were euthanized with an overdose of MS222 and reflectance spectra between 400 and 700 nm were immediately recorded at the mid-body, second dorsal fin and the peduncle (with fish placed on a black background; Fig. 2). No colour change was detectable during euthanization and the standardized measurement procedure took approximately 20 s in total for each individual. For measurements, we used a JAZ-EL200-XR1 (Ocean optics) with a tungsten halogen light source (JAZ-VIS-NIR, 300–2200 nm), a standard reflectance probe (QR-400-7-VIS-NIR, 400–2500 nm) with a 400 μm core diameter and a diffuse white-standard (WS-1-SL, Ocean Optics). To exclude environmental light and to obtain a standardized distance of 2.5 mm and a fixed angle of 90° to the specimen, a black tube with a diameter of 5 mm was mounted on the end of the probe and placed directly on the corresponding measurement point. The present study focused on the spectral analysis of colour morphs previously distinguished solely by human visual classification, and therefore did not incorporate UV. The reflectance probe chosen was hence sensitive to wavelengths between 400 and 700 nm, so that the UV part of the spectrum was not included. All body parts were measured once. Each spectrum is based on the average

of two scans, with an integration time of 100 ms. Measured spectra were directly visually inspected and directly repeated if they failed.

Since visual system characteristics of the study species are not available, we applied the segment classification method following Endler (1990). This method is independent of the specific properties of the observer’s visual system, and divides the spectrum range between 400 and 700 nm into four equal segments of 75 nm each (Endler, 1990). Respectively, these four segments correspond to the violet-blue (400–474 nm), blue-green (475–549 nm), green-orange (550–624 nm) and orange-red (625–699 nm) wavelengths (Endler, 1990). Each reflectance measurement was transformed into variables of brightness (intensity), chroma (saturation), and hue (function of the shape of the spectrum), holding visual sensitivity and environmental light constant (see Electronic Supplementary Material Table S1 for details; Hofmann et al., 2006).

A model-based clustering approach, implemented in the R package ‘mclust’ (Fraley et al., 2012; R Development Core Team, 2012), was used independently for males and females to determine the number of colour morphs in *T. antoniae* ‘small,’ independent of visual sensitivity or ambient light (e.g., Hofmann et al., 2006; Gumm et al., 2011); the best model and thus the number of clusters was chosen based on the Bayesian Information Criterion (BIC).

A one-way ANOVA and Tukey’s post hoc test in case of equal or Welch test with a Dunnett T3 post hoc

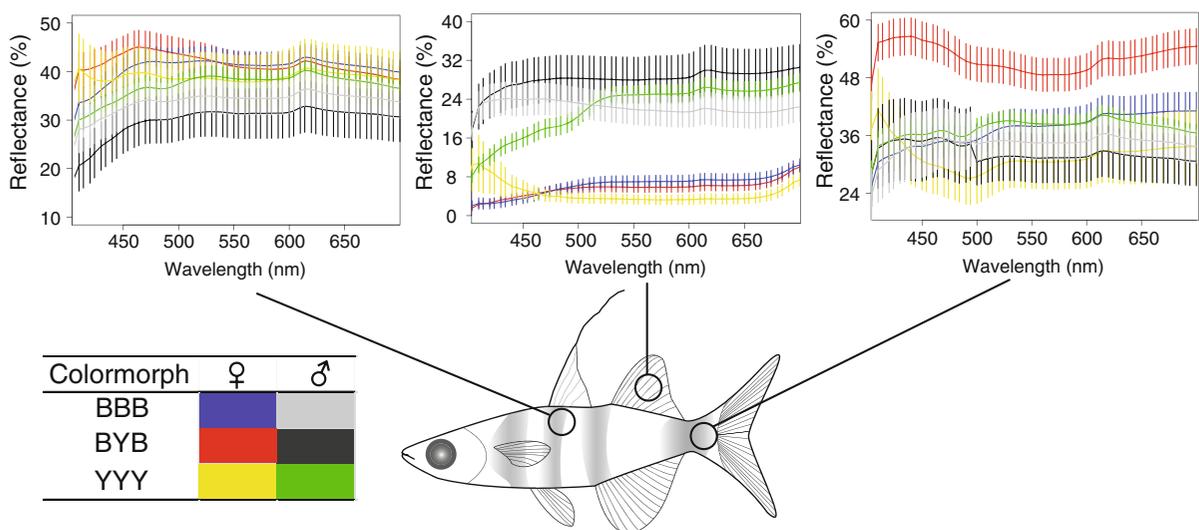


Fig. 2 Measured body parts and their corresponding reflectance spectra (mean values with standard deviation) for each colour morph

test in case of unequal variances was applied to each colour trait (hue, chroma and brightness) of each body part measured to test for differences among the six identified colour morphs (♀yyy, ♀byb, ♀bbb, ♂yyy, ♂byb and ♂bbb). Analyses were carried out using the software PAST (Hammer et al., 2001).

Results

Three colour morphs were identified among both females and males, with a reassignment probability of 98.24% for females and 98.10% for males. Blue (bbb; ♀ $n = 33$, ♂ $n = 20$) and yellow (yyy; ♀ $n = 39$, ♂ $n = 45$) morphs, respectively, show a blue or yellow body, caudal peduncle and second dorsal fin. The blue-yellow morph (byb; ♀ $n = 11$, ♂ $n = 13$) combines blue body and caudal peduncle with yellow fins. Multivariate analyses revealed significant differences in hue values for all body parts among the six colour morphs (Table 1). Differences in the hue values between female colour morphs widely match the male patterns as revealed by pairwise comparison (Fig. 3; Table 1). According to the chroma and/or brightness values of the fins, multivariate analyses revealed significant differences between males and females (see Electronic Supplementary Material Fig. S1; Table S2 for details). In opposite, as revealed by the multivariate analyses, males and females are neither distinguishable according to chroma values nor to brightness values in both, body and peduncle area (see Electronic Supplementary Material Fig. S1; Table S2 for details).

Discussion

We analysed spectrometric reflectance data in male and female *Telmatherina antoniae* 'small', a sailfin silverside species with a conspicuous male colour. The blue, yellow and blue-yellow male colour morphs (Fig. 1) had previously been distinguished by visual classification only. As expected, the spectrometric data clearly support the existence of these three male colour morphs (Fig. 3; Table 1), widely fitting patterns observed in the similarly colour polymorphic males of *T. sarasinorum* from the same lake. The unexpected finding of the present study is that females, which appear neither conspicuous nor polymorphic to

the human eye, show the same morph-specific pattern in hue as the males (Fig. 3; Table 1). Female sailfin silversides have short fins, and a body colouration usually considered dull brown to silvery, contrasted by males characterized by long unpaired fins and vivid courtship colouration (e.g. Kottelat, 1991; Herder et al., 2006a).

Male and female fin colouration, however, differs significantly in chroma (saturation) and brightness (see Electronic Supplementary Material Fig. S1; Table S2). A decrease in chroma and brightness leads to the visual impression of less intense colours (Endler, 1990); low levels of chroma and brightness in females compared to males accordingly provide a compelling explanation for the visual impression of female crypsis to the human eye. A pronounced sexual dichromatism, like in *T. antoniae* 'small', where female crypsis is most likely an adaptation to predation pressure, is indicative for a mating system with choosy females and male-male competition (Endler, 1980; Andersson, 1994). This is supported by field observations strongly suggesting that female *Telmatherina* choose mating partners among actively courting and competing males (Gray & McKinnon, 2006; Gray et al., 2007, 2008a, b).

Contrary to our expectations and to the data obtained from the fins, colour measurements did not support differences in brightness and chroma among male and female *T. antoniae* 'small' on the lateral body and on the caudal peduncle (see Electronic Supplementary Material Fig. S1; Table S2). The measured spot covered an area of 5 mm in diameter. At the lateral body and the caudal peduncle, the area of measurement was dominated by pronounced dark lateral bars of the male courtship colouration (illustrated in Fig. 2). These bars correspond in brightness and chroma to the rather uniform female colouration at body or peduncle, explaining the absence of significant differences in brightness and chroma between males and females at these spots.

In *T. antoniae* 'small,' the absence of restrictions to gene flow between the colour morphs (Herder et al., 2008) clearly contradicts the hypothesis that colour-assortative mating accounts for the existence of the polymorphism. Similarly, there is little indication for colour-assortative mating in the closely related *T. sarasinorum* (Gray et al., 2007), suggesting that mechanisms other than assortative mating might account for the existence of stable colour

Table 1 Results of one-way ANOVAs or Welch tests in case of unequal variances for the variation in hue values (a); and pairwise post hoc tests (b)

(a)																
Trait	Fin						Welch <i>F</i> -test									
							Body					Peduncle				
F 5,155	271.90						13.81					140.40				
<i>P</i>	<0.001						<0.001					<0.001				

(b)																
	♂									♀						
	bbb			byb			yyy			bbb			byb			
	Body	Fin	Peduncle	Body	Fin	Peduncle	Body	Fin	Peduncle	Body	Fin	Peduncle	Body	Fin	Peduncle	
♀ yyy	0.001	<0.001	<0.001	0.009	0.988	<0.001	0.529	0.202	1.000	0.033	<0.001	<0.001	0.033	0.985	<0.001	
♀ byb	0.942	<0.001	0.918	0.999	0.780	1.000	<0.001	0.038	<0.001	0.801	<0.001	<0.001				
♀ bbb	0.243	<0.001	<0.001	0.009	<0.001	<0.001	0.008	<0.001	<0.001							
♂ yyy	<0.001	<0.001	<0.001	<0.001	0.573	<0.001										
♂ byb	0.995	<0.001	0.797													

polymorphisms observed in many sailfin silverside species (see Herder et al., 2006a). Several hypotheses provide alternative possible explanations for this phenomenon, including environment contingent selection, sensory drive and frequency-dependent selection (Gray & McKinnon, 2007; Seehausen et al., 2008).

Environment contingent selection predicts that the conspicuousness of male colouration depends on the lighting conditions of the habitat (Gray & McKinnon, 2007; Gray et al., 2008a), and provides a plausible explanation for the case of an obviously stable colour polymorphism described here. In male *T. sarasinorum*, the conspicuousness of colour morphs has been demonstrated to vary spatially with light conditions in alternative habitats, translating into alternative levels of reproductive success (i.e., the most conspicuous morph in a given habitat has the highest probability of gaining reproductive success; Gray et al., 2008a). *Telmatherina antoniae* ‘small’ show very similar patterns in their male colour polymorphism, rendering a similar mechanism likely. Individual conspicuousness, correlated with correspondingly enhanced mating success in alternative light environments resulting from contrasting visual habitats (spatially and/or temporally), thus provides a suitable explanation for the persistence of colour polymorphism in male *T. antoniae* ‘small’. However, there are currently no indications of segregation along habitat gradients in *T. antoniae* ‘small’, and genetic differentiation is clearly absent (Herder et al., 2008). Thus, it appears unlikely that sensory drive, the spatial segregation of colour morphs along visual gradients, as observed for example with

increasing water depth (Seehausen et al., 2008), accounts for the persistence of *T. antoniae* ‘small’ colour polymorphism. Current data also do not indicate a role for frequency-dependent selection in this system (Herder et al., 2008).

The present analyses focus on the signaller role of colour traits, independent of visual sensitivity or ambient lighting conditions. Analyses targeting the proximate mechanisms maintaining this, and other spectacular polymorphisms in sailfin silversides clearly require the incorporation of colour perception, as well as detailed analyses of the photic environment, as exemplified by Gray et al. (2008a) for *T. sarasinorum*, or by Hancox et al. (2013) for the Australian ornate rainbowfish species *Rhadinocentrus ornatus*. In rainbowfishes (family Melanotaeniidae, closely related to the Telmatherinidae; see Dyer & Chernoff, 1996; Sparks & Smith, 2004; Unmack et al., 2013), intraspecific colour variation is common among allopatric populations of rainbow fishes, but the ornate rainbowfish is considered unique in having a red-blue within-population polymorphism in both males and females (Hancox et al., 2010). In that case, combined effects of visual background and illumination can explain the maintenance of the polymorphism (Hancox et al., 2013), similar to the habitat background scenario in *T. sarasinorum*. This raises the question if female *T. sarasinorum*, which have so far been considered cryptic and monomorphic, might in fact show a cryptic polymorphism as demonstrated here for *T. antoniae* ‘small’—a hypothesis requiring further investigation.

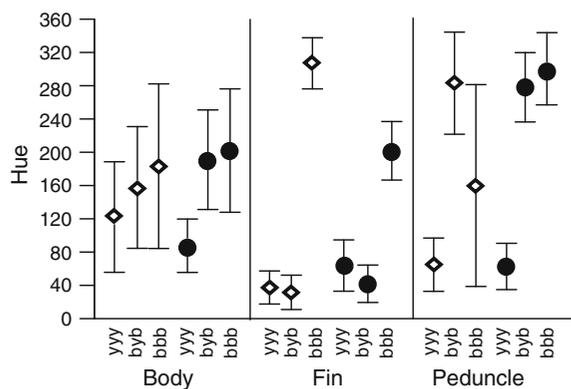


Fig. 3 Differences in hue values of *Telmatherina antoniae* 'small'. Hue values of the three body parts. Symbols (open diamond ♀; filled circle ♂) represent means with standard errors for each colour morph (yyy = yellow, byb = blue-yellow, bbb = blue)

Conclusions

Spectral data clearly confirm the existence of three male colour morphs in *T. antoniae* 'small'. The unexpected cryptic colour polymorphism uncovered in females is explained by similar patterns in hue, but lower brightness and saturation. The existence of colour polymorphic females corresponds to patterns in the ornate rainbowfish, a species where the female polymorphism is, however, not cryptic. This is, to the best of our knowledge, the first report of a cryptic female colour polymorphism in fishes, shedding light on the need to also incorporate the cryptic sex into analyses of colour patterns. The mechanisms maintaining the cryptic polymorphism, as well as its implications for the mating system, remain unknown, but spatial and temporal variations in the visual environment are promising candidates.

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