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# Differentiation of visual spectra and nuptial colorations of two *Paratanakia himantegus* subspecies (Cyprinoidea: Acheilognathidae) in response to the distinct photic conditions of their habitats

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## Abstract

**Background:** Vision, an important sensory modality of many animals, exhibits plasticity in that it adapts to environmental conditions to maintain its sensory efficiency. Nuptial coloration is used to attract mates and hence should be tightly coupled to vision. In Taiwan, two closely related bitterlings (*Paratanakia himantegus himantegus* and *Paratanakia himantegus chii*) with different male nuptial colorations reside in different habitats. We compared the visual spectral sensitivities of these subspecies with the ambient light spectra of their habitats to determine whether their visual abilities correspond with photic parameters and correlate with nuptial colorations.

**Results:** The electroretinogram (ERG) results revealed that the relative spectral sensitivity of *P. h. himantegus* was higher at 670 nm, but lower at 370 nm, than the sensitivity of *P. h. chii*. Both bitterlings could perceive and reflect UV light, but the UV reflection patterns differed between genders. Furthermore, the relative irradiance intensity of the light spectra in the habitat of *P. h. himantegus* was higher at long wavelengths (480–700 nm), but lower at short wavelengths (350–450 nm), than the light spectra in the habitats of *P. h. chii*.

**Conclusions:** Two phylogenetically closely related bitterlings, *P. h. himantegus* and *P. h. chii*, dwell in different waters and exhibit different nuptial colorations and spectral sensitivities, which may be the results of speciation by sensory drive. Sensory ability and signal diversity accommodating photic environment may promote diversity of bitterling fishes. UV light was demonstrated to be a possible component of bitterling visual communication. The UV cue may assist bitterlings in gender identification.

**Keywords:** Bitterling; Electroretinogram; Sensory drive; UV reflection pattern

## Background

Vision is an important sensory modality in fish, with the exception of some fishes, such as cave-dwelling species without eyes. Almost all fishes, from those inhabiting shallow and clear waters to those in the aphotic zone in the deep ocean, can perceive visual signals (Pitcher 1986) in order to communicate, find mates, forage for food, avoid predators, camouflage, or navigate (Hawryshyn 1992; Losey et al. 1999).

Light spectra in aquatic environments are distinct from those on land. Not just water itself, but also suspended materials (e.g., mineral particles, detritus, and planktons), can selectively absorb or scatter different wavelengths of light (Litjens et al. 1999). Since the underwater photic environments may be affected by depth and suspended materials as well as unique features of the habitats, fish under different photic conditions should physiologically adjust its spectral sensitivity in order to enhance detection ability by maximizing the visual contrast (Endler 1992; Lythgoe

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1984). For example, the spectral sensitivity of milkfish (*Chanos chanos*), humpback salmon (*Oncorhynchus gorbuscha*), and yellowfin tuna (*Thunnus albacares*) ontogenetically change to fit the photic environment during each developmental stage (Chang et al. 2009a; Cheng and Flamarique 2004; Loew et al. 2002). Moreover, it has been reported that spectral sensitivity has evolved to adapt to environmental light parameters in cichlids, seabreams, and moray eels (Seehausen et al. 2008; Wang et al. 2011; Wang et al. 2009), especially, the adaptations of RH1 and LWS opsins to light environments which are documented in cichlid fishes (Sugawara et al. 2005; Terai et al. 2006), as well as in different populations of three-spined stickleback (*Gasterosteus aculeatus*) and guppy (*Poecilia reticulata*) (McDonald and Hawryshyn 1995; Tezuka et al. 2014). The retinal photoreceptor cells of fishes utilize visual pigments, which contain a chromophore (11-*cis*-retinal, A1 retinal, or 11-*cis*-3,4-dehydroretinal, A2 retinal) and opsin, to capture photons. Variations in spectral sensitivity mainly result from the alternative usage of different opsin genes (Flamarique 2013; Johnson et al. 2013; Kasagi et al. 2015; Kondrashev et al. 2013; Valen et al. 2013). Furthermore, sexual selection favors individuals that can display visual signals that are readily distinguishable from the background (Hurtado-Gonzales et al. 2014; Miyagi et al. 2012; Morrongiello et al. 2010; Selz et al. 2014). Therefore, in addition to their link with visual properties, photic environments may also influence overall body coloration of fish.

A good example of the interactions among the photic environment, spectral sensitivity, and nuptial coloration is observed in two cichlids inhabiting Lake Victoria, *Pundamilia nyererei* and *Pundamilia pundamilia*. These two cichlids are closely related and *P. nyererei* is sympatric with *P. pundamilia* over its distribution range; moreover, they are morphologically and anatomically similar. Females of these two species are hard to tell from each other, but males of them are obviously different with distinct coloration: male *P. pundamilia* is metallic blue-grey and male *P. nyererei* is bright red dorsally and yellow laterally (Maan et al. 2004). Also, *P. nyererei* lives in deeper water than *P. pundamilia* (Seehausen and Bouton 1997), and so the former is relatively exposed to more red light with a proportionally greater wavelength (Maan et al. 2006). In response to variations in ambient light environments, these two species have evolved distinct spectral sensitivities and nuptial colorations (Maan et al. 2006; Miyagi and Terai 2013).

Bitterling (Acheilognathidae, Cyprinoidea) are cyprinids, distributed over East and Southeast Asia and Europe. They inhabit various lowland freshwater habitats, such as lakes, rivers, and ditches (Kottelat and Freyhof 2007), and have evolved a symbiotic relationship with freshwater mussels (family Unionidae) for reproduction. The reproductive success of male bitterlings (which establish a territory and guard mussels from rivals) is mainly dependent on body size (Casalini et al. 2009; Reichard et al. 2008). However, a recent study demonstrated that nuptial coloration is a critical factor for female mate choice and male–male competition (Smith et al. 2014).

In Taiwan, two native *Paratanakia* bitterlings, *Paratanakia himantegus himantegus* and *Paratanakia himantegus chii* (Fig. 1), are phylogenetically related (Chang et al. 2014; Chang et al. 2009b), and their distributions largely overlap on a geographical scale; however, they are rarely sympatric. Usually, the two bitterlings are found in separate microhabitats: *P. h. chii* mainly dwells in shallow floodplain habitats, such as irrigation channels of rice paddy fields, whereas *P. h. himantegus* is commonly found in lakes and ponds (Chen and Chang 2005; Chen et al. 2012). The most obvious morphological difference between the two bitterlings is the nuptial coloration (mating signals). Breeding males of *P. h. himantegus* possess a red stripe on their dorsal fin, red patches on the operculum and pectoral fin, and red irises, whereas *P. h. chii* breeding males have a yellow-white stripe on the dorsal fin, and their irises are not red (Chen and Chang 2005).

The perception of visual signals is determined by two components: the physical characteristics of a habitat and the sensory properties of a receiver (Endler 1990); therefore, a change in one of these two components tends to drive the development of substitute signals in response to variable habitats (Chunco et al. 2007; Endler 1980), which has been demonstrated by several studies (Dalton et al. 2010; Fuller et al. 2005; Gray et al. 2008; Hoffmann et al. 2007). Based on the observation that the two aforementioned and closely related bitterlings exhibit distinct nuptial colorations, it is hypothesized that the differences in their nuptial colorations may be related to variations in photic properties of its habitats and/or spectral sensitivities of the fish. To test this hypothesis, the spectral sensitivities of these two bitterlings were recorded with electrophysiological method and the light spectra of their habitats were measured and compared.

## Methods

### Subjects

Fish were caught from Chinlung Lake, Hsichih, New Taipei City (*P. h. himantegus*) and Dashi, Taoyuan County (*P. h. chii*). These two sites belong to the Tamsui River basin. All fish were collected during the spawning season (July 2013). The males exhibited nuptial coloration and the females had long ovipositors. Fish were 25 to 40 mm in standard length. Fish were kept in the laboratory in two 36-L aquaria (separated by taxa) under a natural photoperiod, with water temperature maintained at 25 ~ 28 °C. They were fed ad libitum with artificial fish feed or frozen *Artemia* twice a day. Specimen collection and experiments
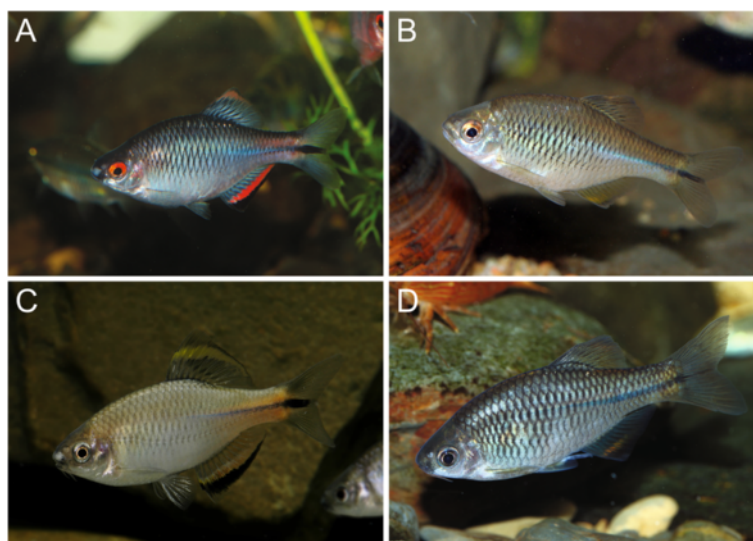
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**Fig. 1** Photos of studied species taken in an indoor aquarium. **a** Male *P. himantegus himantegus*; **b** female *P. h. himantegus*; **c** male *Paratanakia himantegus chii*; **d** female *P. h. chii*. [Photos were taken by Dr. Yun-Ching Chang and Mr. Ta-Ching Chang]

were performed with permission (RFiZOOHY20060701) from the Institutional Animal Care and Use Committee (IACUC) of Academia Sinica.

### Electroretinogram recording

Electroretinogram (ERG) recordings were mainly modified from Shao et al. (2014). Fish were anesthetized with 0.025 % buffered MS-222 (ethyl 3-aminobenzoate, methane-sulfonic acid salt), and then immobilized through an injection of gallamine triethiodide (Flaxedil; Sigma, St. Louis, MO, USA.), a neuromuscular junction blocking agent, into the dorsal muscle (0.4–0.6 μg per gram of body weight). Each fish was wrapped in wet KimWipes (Kimberley-Clark Taiwan, Taipei) to prevent skin from scraping or drying, and a small tube was inserted into the mouth to deliver well-oxygenated water to the gills. Immobilized fish were kept under dark conditions for at least 1.5 h for dark adaptation before the ERG recording. ERG tests were performed as described previously (Morita et al. 1997; Seeliger et al. 2002; Shao et al. 2014). A 100-W halogen lamp (Osram 64637 100 W/12 V) in a lamp housing device (Nikon C-FI 115, Japan) was used as the light source for tests of visible spectra (400–670 nm). For the shorter wavelengths (≤370 nm), a custom-made UV curing device (Great Lighting Corp. 100 W/365 nm) was used as the light source. A series of neutral density (ND) filters (Andover, NH, USA) were used to reduce the light intensity in 0.3 log unit steps, from −0.3 to −4.8 log units. Peak photon fluxes at each wavelength were determined using a USB 2000 spectrophotometer (Ocean Optics, USA), calibrated by LC-1-cal (Ocean Optics, USA) with OODBase32 software (Ocean Optics, USA), at the fish eye surface. A shutter (Sutter Instrument Lambda SC, USA)

was used to provide fixed durations of light stimuli: 10 stimuli, each of 20 ms in duration, with an inter-stimuli interval of 5 s. The ERG tests started from the lowest light intensity, and ND filters were adjusted step-by-step to increase stimuli intensity (16 steps to reach the maximum). ERG signals were recorded using Teflon-coated insulated silver electrodes (63.5 μm in diameter), and the Telfon on the tip end (5 mm) was removed and chlorinated to enhance picking up of evoked potentials. The recording electrode was positioned on the cornea, and the reference electrode was placed on the head. The signals from both electrodes were amplified 20 k-fold and filtered with a high–low band pass filter set between 1 and 3000 Hz (ER-1, Cygnus Technology Inc. USA). The signals were digitized at a 2-kHz sampling rate with PowerLab 4/25 (ADInstruments, Inc. ML845, Australia) and recorded with Scope 3 data acquisition software (ADInstruments, Inc. Australia). The recorded signals were analyzed using custom-made software (programmed by K. Anraku). Signals recorded at each step (100 to 700 ms after stimuli) were compared to the model ERG trace obtained at maximum light stimuli using the correlation method. The replicates of waveforms from each test were compared using Spearman's correlation test. Our pilot study revealed that when a correlation coefficient ($r$) between two replicates was less than 0.7, the two ERG traces showed little resemblance. Hence, ERG traces were considered valid when $r$ was greater than 0.7 and the $b$-wave latency time delay was less than 100 ms (Seeliger et al. 2002). Deflection of the ERG trace was taken as an indication of the light intensity threshold of the particular wavelength (Morita et al. 1997). Sensitivity was defined as − log10 of the threshold for each tested wavelength. To remove

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variations between trials, values for each individual were standardized to a 0–100 % scale, where 100 % indicated the maximum sensitivity spectrum (Lisney et al. 2010). After standardization, the sensitivity values of the two bitterlings for each light wavelength stimulus were compared using Tukey's pairwise comparisons (one-way ANOVA). In this study, eight *P. h. himantegus* individuals (four males and four females) and nine *P. h. chii* individuals (six males and three females) were used for recordings.

### Body UV reflection pattern

Fish were anesthetized with buffered MS-222 before being photographed. The body UV reflection pattern was recorded using a Nikon D70 camera equipped with a Nikon 105-mm micro lens. The whole process was completed inside a dark room, where fish bodies were illuminated using two UV lamps (UVGL-58 P/N 95-0007-05, long wave mode, 367 nm). A UV passing filter (B + W 403 52 mm) and an IR cut filter (B + W 489 52 mm) were mounted on the lens to block any possible ambient light leakage. The spectral transmission ranges of these two filters are available online (http://diglloyd.com/articles/Filters/spectral-B+W-403.html). The UV reflection patterns of one male and one female of each subspecies were photographed.
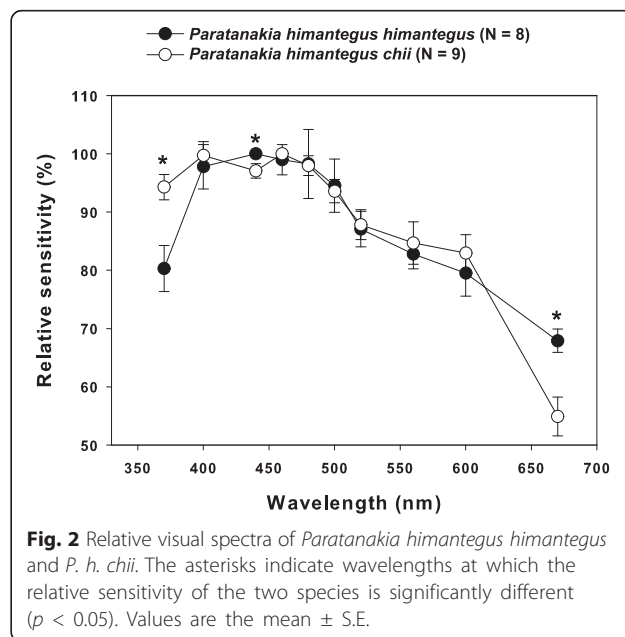
### Ambient light spectra

The ambient light spectra of four habitats, including the only two known habitats of *P. h. chii* in Taiwan (Dashi Town, Taoyuan County and Longtan Township, Taoyuan County), and two typical habitats of *P. h. himantegus* (Chinlung Lake, Hsichih, New Taipei City and Jiaoxi Township, Yilan County), were measured using a USB-2000 spectrometer with a waterproof fiber optic cable (QP1000-2-UV–VIS) (Ocean Optics, Dunedin, FL, USA), 1000 μm in diameter and 2 m in length; absolute spectral irradiance was measured at three to five sites in each sampling location. Before each measurement, USB-2000 was calibrated with calibration light sources, LS-1-cal (Ocean Optics, Dunedin, FL, USA), with OODBase32 software (Ocean Optics, Dunedin, FL, USA). The light in air was measured upward above the water surface, and the light underwater was measured both upward and sideway at a depth of 25 cm from the water surface at all locations. The underwater sideway light spectrum was the arithmetic mean of two scans in the sun and anti-sun directions (Flamarique et al. 2013). Habitat photic parameters were measured between 10:00 and 14:00 in October, 2014. The 25-cm depth is the averaged depths of the trenches or the near-shore area of the pond, where bitterling breeding pairs and hosting mussels were found. The relative spectral irradiance was smoothed by a factor of 15 using a simple moving average (McLean et al. 2007), and then normalized to a 0–100 % scale, where 100 % indicated the

maximum intensity spectrum. Light spectra of the typical habitats of *P. h. chii* and *P. h. himantegus* were the geometric means of the relative spectral irradiance measured in the respective locations. Moreover, the light spectra curves of the typical habitats were compared by Spearman's correlation coefficients with two-tailed tests, which were performed with SPSS v.14 (SPSS Inc., Chicago, IL, USA).

### Results

The relative visual spectra of these two *Paratanakia* subspecies measured with ERG recording is shown in Fig. 2, which showed that bitterlings of both subspecies are able to detect light within a range of 370 nm to 670 nm under dark adaptation conditions. The highest sensitivity (lowest threshold) was found at the wavelength of 440 nm for *P. h. himantegus* and 460 nm for *P. h. chii*. Both fishes were less sensitive (higher threshold) to light at a wavelength of 670 nm as compared with other tested wavelengths. No differences in thresholds of spectral sensitivity were observed between gender for either species. To obtain relative spectral sensitivity values, the sensitivity of *P. h. himantegus* was normalized to the stimulation intensity of the threshold at 440 nm (which was set as 100 %), and the sensitivity of *P. h. chii* was normalized to the stimulation intensity of the threshold at 460 nm. The relative visual spectra of the two subspecies are of inverted U shapes, with some variations (Fig. 2). The relative spectral sensitivity curve of *P. h. himantegus* displayed a single peak at 440 nm, but that of *P. h. chii* exhibited two peaks; a maximum sensitivity peak at 460 nm and a secondary peak at 400 nm (99.73 %). Compared to the most sensitive wavelength for the respective fish, the



**Fig. 2** Relative visual spectra of *Paratanakia himantegus himantegus* and *P. h. chii*. The asterisks indicate wavelengths at which the relative sensitivity of the two species is significantly different ($p < 0.05$). Values are the mean ± S.E.

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relative sensitivity of the least sensitive wavelengths (670 nm) was only 70 % in *P. h. himantegus* and 55 % in *P. h. chii*. Examining the two ends of the visual spectra revealed that the relative sensitivity at 670 nm is roughly the same as that at 370 nm for *P. h. himantegus*, but in *P. h. chii*, the relative sensitivity at 670 nm is much lower than that at 370 nm (Fig. 2). Tukey's pairwise (one-way ANOVA) tests were performed to demonstrate that *P. h. himantegus* was more sensitive at 670 and 440 nm but less sensitive at 370 nm ($p < 0.05$) than *P. h. chii* (Fig. 2).

Examination of body UV reflection patterns showed that both species reflect UV light (Fig. 3). The dorsal and anal fins of both *P. h. himantegus* and *P. h. himantegus* males under visible light were more vividly colored (Fig. 3(A-VIS
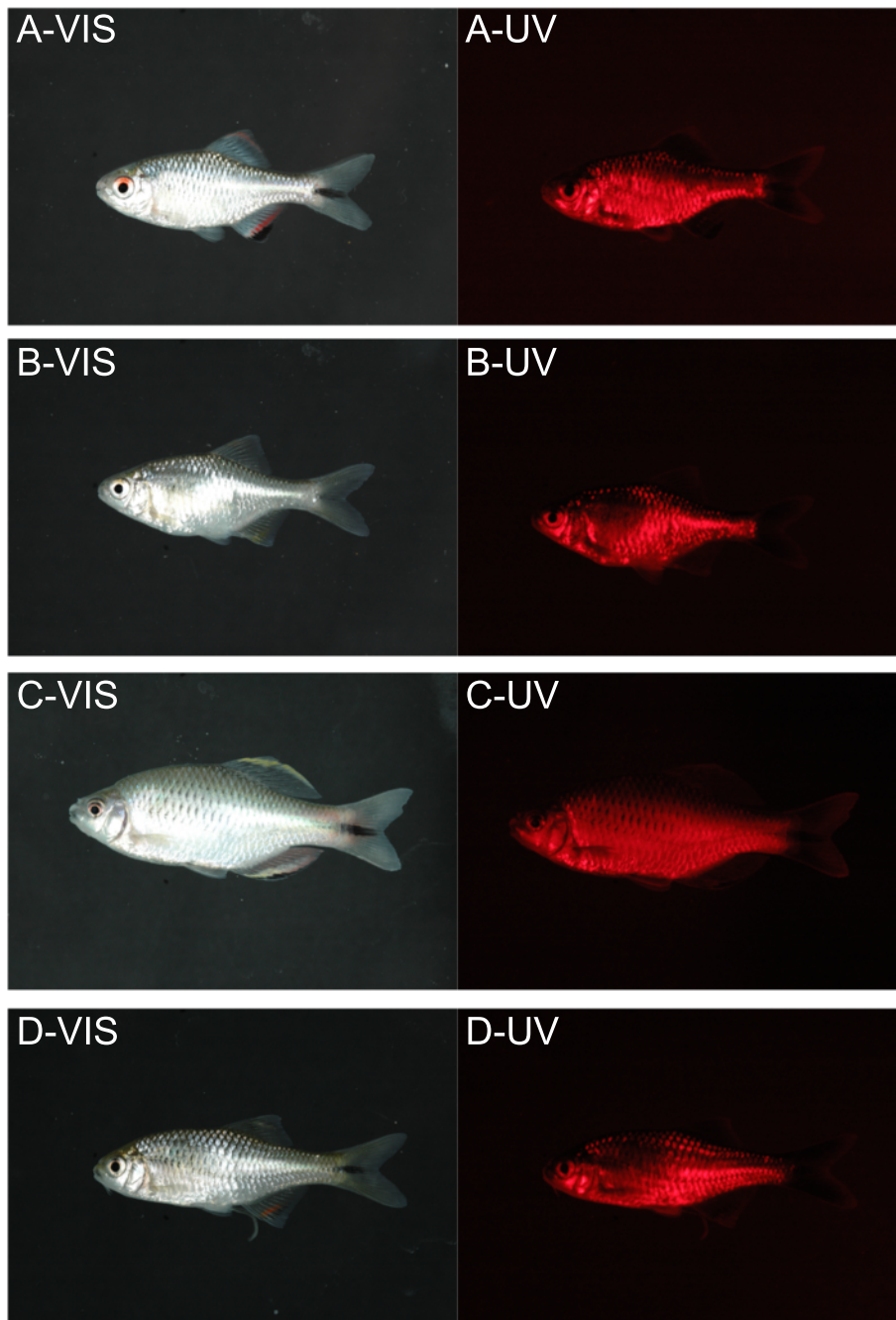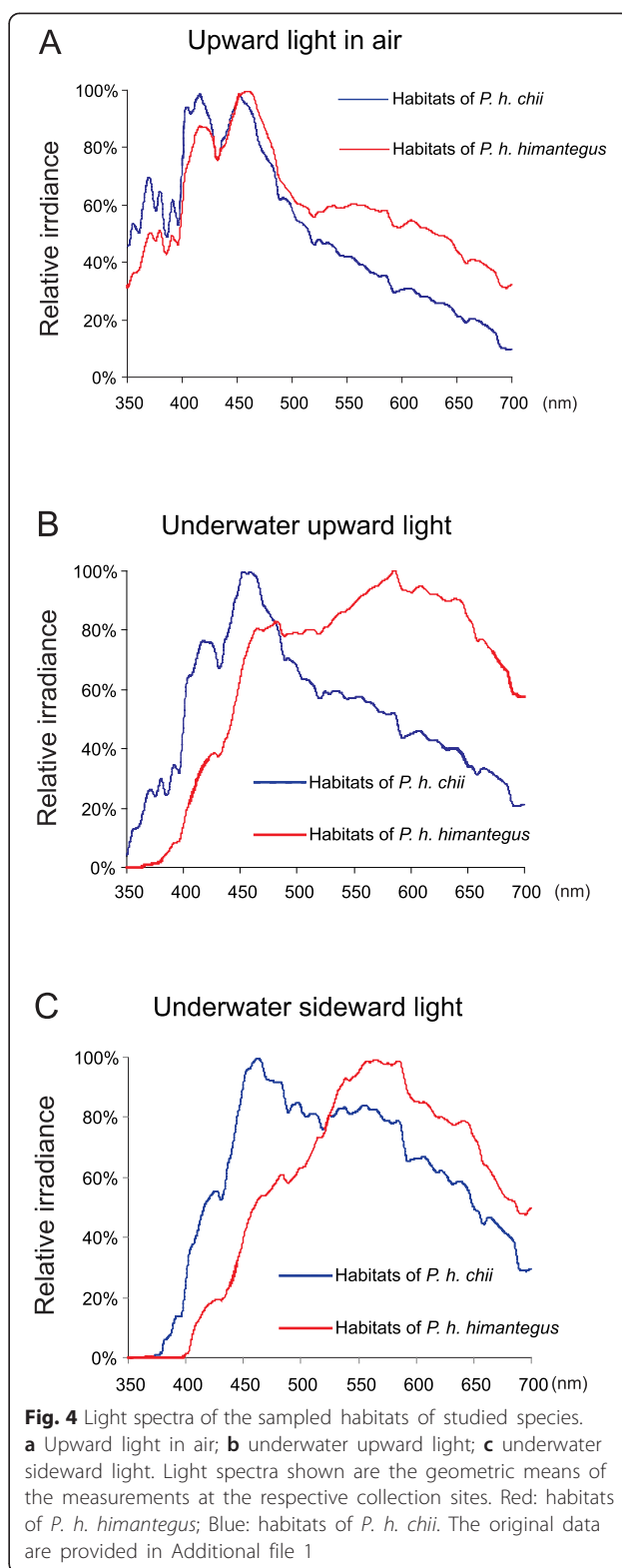


**Fig. 3** Photos of body reflection patterns of *Paratanakia himantegus himantegus* and *P. h. chii* under UV light (*UV*) and visual light (*VIS*). (*A-UV*) Male *P. himantegus himantegus*; (*A-VIS*) male *P. h. himantegus*; (*B-UV*) female *P. h. himantegus*; (*B-VIS*) female *P. h. himantegus*; (*C-UV*) male *P. h. chii*; (*C-VIS*) male *P. h. chii*; (*D-UV*) female *P. h. chii*; (*D-VIS*) female *P. h. chii*

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and C-VIS)) than those of females (Fig. 3(B-VIS and D-VIS)). Nevertheless, the fins of neither males nor females reflected UV (Fig. 3(A-UV, B-UV, C-UV and D-UV)). There is no clear difference in UV reflection pattern between subspecies. Males of both fishes reflected UV throughout the body (Fig. 3(A-UV, and C-UV)), whereas females had a non-reflecting region above the pelvic fin. UV reflection by the ventral part was stronger than reflection by the dorsal part of the body (Fig. 3(B-UV and D-UV)).

The original data of relative ambient spectra gathered from four habitats were shown in Additional file 1. The light spectra in the air in the sampled habitats were largely consistent (Spearman's rho $r = 0.781$, $p < 0.001$) (Fig. 4a); however, significant differences of light transmission underwater were observed upon comparing spectral irradiance from various habitats. The irradiance of upward light in the habitat of *P. h. himantegus* was higher at relatively long wavelengths (480–700 nm), but lower at shorter wavelengths (350–450 nm), than in the habitats of *P. h. chii* (Fig. 4b). Furthermore, Spearman's correlation coefficients were used to show that the upward underwater spectral curve in the habitats of *P. h. himantegus* and *P. h. chii* were not correlative ($r = 0.161$, $p < 0.001$). At 670 nm, i.e., red light, the relative irradiance in the habitat of *P. h. himantegus* was more than double that in the habitat of *P. h. chii*. However, the relative irradiance in the UV light spectrum, i.e., 370 nm, was far higher in the *P. h. chii* habitat than that in *P. h. himantegus* habitat (Fig. 4b). The sideways light spectra of the *P. h. himantegus* habitat were shifted toward longer wavelengths as compared to the spectra of the habitat of *P. h. chii* (Spearman's rho $r = 0.559$, $p < 0.001$) (Fig. 4c). The highest relative irradiance in the *P. h. himantegus* habitat was observed at 560 nm, whereas the relative irradiance in the *P. h. chii* habitat peaked at a shorter wavelength, 455 nm (Fig. 4c).

## Discussion

According to the surveys carried out by Taiwan Conservation Association of Native Fishes (TCANF), the two bitterlings examined here live in different habitats. *P. h. chii* prefers shallow habitats, such as irrigation channels of rice paddy fields, whereas *P. h. himantegus* prefers deeper water, including lakes and ponds (TCANF unpublished data). Therefore, although these two subspecies can co-exist in one large drainage system, they are seldom sympatric in distribution. These two typical habitats, ditch and pond, not only differ in terms of depth and current but may also differ in their photic environments. Due to phytoplankton, the ambient light spectra of turbid or eutrophic waters may have higher relative irradiance at longer wavelengths (Bowling et al. 1986; Jerlov 1976). As compared with the habitats of *P. h. himantegus*, the habitats of *P. h.*



**Fig. 4** Light spectra of the sampled habitats of studied species. **a** Upward light in air; **b** underwater upward light; **c** underwater sideward light. Light spectra shown are the geometric means of the measurements at the respective collection sites. Red: habitats of *P. h. himantegus*; Blue: habitats of *P. h. chii*. The original data are provided in Additional file 1

*chii* usually have a lower density of planktonic algae and clear/running water. Consequently, it has been suggested that *P. h. himantegus* dwells in locations where longer

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wavelength light predominates to a greater extent than that in the habitats of *P. h. chii* (Fig. 4).

The visual system is responsible not only for recognizing color signals but also for foraging for food or detecting predators. The light environment is a factor known to drive both the reception and production of visual signals of fishes (Hornsby et al. 2013; Hurtado-Gonzales et al. 2014; Shin and Choi 2014; Terai et al. 2006; Tezuka et al. 2014). Indeed, the present study showed that the spectral sensitivities of *P. h. himantegus* and *P. h. chii* are distinct: *P. h. himantegus* is more sensitive than *P. h. chii* to longer wavelength light, while it is less sensitive than *P. h. chii* at shorter wavelengths. As such, the spectral sensitivities of these subspecies are suitable for their respective ambient light conditions. Furthermore, the diverse nuptial colorations of these two bitterling subspecies may also result from the variation in the photic environments and/or the distinction of sensory properties.

Fishes commonly use UV signals in communication (Losey et al. 1999; Witzany 2014). In this study, ERG data demonstrated that both subspecies can perceive UV light, i.e., 370 nm, and *P. h. chii* was more sensitive to UV light than *P. h. himantegus*, which is consistent with the light spectra of their respective habitats. However, since the body UV reflection patterns cannot be quantitatively compared between species, it is not possible to conclude whether the differences in UV sensitivity between the species are related to their body UV reflection patterns. However, the UV reflection patterns of the bitterlings were sexually dimorphic, suggesting that the UV pattern may be a secondary sexual characteristic in these fishes. Furthermore, the nuptial coloration of the males is a crucial signal for the mate choice of females, and the UV cue has been demonstrated to be a factor for nuptial coloration in many fishes (Garcia and Perera 2002; Kodric-Brown and Johnson 2002; Palmer and Hankison 2014; Rick et al. 2006; Smith et al. 2002). Our results imply that the UV reflection pattern of bitterlings may be an important characteristic for consideration in future taxonomic or ecological studies.

The diversification of *P. nyererei* and *P. pundamilia* cichlids is considered to be a good example of speciation caused by sensory drives (Miyagi and Terai 2013). A hypothesis posits that closely related and morphologically similar species should differ in mating signals, which is mainly engendered by three processes: habitat transmission, perceptual tuning, and signal matching. Habitat transmission is important when the habitat physically degrades the intensity of the signal during transmission; therefore, it is advantageous for signals to have a long transmitting distance (Bradury and Vehrencamp 2011; Morrill et al. 2013). Perceptual tuning is the phenomenon whereby some individuals may be inherently more sensitive to certain stimuli, e.g., a specific wavelength of light or frequency of sound, than other organisms, which may be a consequence of

adaptation to their habitat (Boughman 2002). Signal matching refers to intimate coupling between the evolution of sexual display in the signal sender and the development of sensory capabilities in the signal receiver (Fisher 1915; Kirkpatrick 1982). Usually, mating signals are compatible with the receiver's perception capabilities, because a more detectable mating signal can increase the reproductive opportunities of the sender and reduce the search cost of the receiver (partner) (Dawkins and Guilford 1996), thereby improving reproductive success. Sensory drive speciation suggests that the gene flow between two sibling species is constrained during contact (Boughman 2002). Habitat transmission, perceptual tuning, and signal matching are all apparent in the two closely related *Paratanakia* subspecies examined in this study. Moreover, earlier genetic studies suggested that there is no gene flow between *P. h. himantegus* and *P. h. chii* (Chang et al. 2014; Chang et al. 2009b), and therefore, it is possible that speciation by sensory drive promotes differentiation of these two bitterlings.

Matsumoto et al. (2014) suggested that speciation by sensory drive most likely occurs when the sensory trait is determined by a small number of loci. Indeed, the visual spectral sensitivity is related to the opsin genes, e.g., long-wavelength-sensitive opsin gene (*lws*) in cichlids, zebrafish (*Danio rerio*), and three-spined stickleback (Seehausen et al. 2008; Shao et al. 2014), or short-wavelength-sensitive opsin gene 2a (*sws2a*) in cichlids (Seehausen et al. 2008). Moreover, body coloration is also likely to be determined by a limited number of genes. For example, the male body coloration of East African cichlid, i.e., *Pseudotropheus saulosi*, may be controlled by only five genes (Gunter et al. 2011). Allopatric speciation (Bohlen et al. 2006) and switches in mussel host preference (Kitamura et al. 2012) have been suggested to be the main factors of bitterling diversification. Future studies of the genes that affect spectral sensitivity and nuptial coloration of these two *Paratanakia* bitterlings are needed to clarify the interactions between natural selection and sexual selection (Maan and Seehausen 2011). Such studies promise to clarify whether speciation by sensory drive promoted the speciation of these two *Paratanakia* bitterling subspecies.

## Conclusions

We report that two morphologically similar and phylogenetically close bitterlings, *P. h. himantegus* and *P. h. chii*, have different nuptial colorations and spectral sensitivities and reside in habitats with different environmental photic conditions; these findings support the hypothesis that differences in nuptial coloration may be a consequence of variations in photic properties and spectral sensitivities and imply that speciation could be driven by the sensory system and related environmental

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conditions (Endler and McLella 1988), thereby promoting bitterling diversity. The spectral sensitivity and body UV reflection pattern data also support the hypothesis that the UV cue is a major component of the visual signals of bitterlings. While the UV cue may not be utilized in species recognition, it may assist bitterlings in gender identification.

## Additional file

**Additional file 1: The original data of relative ambient light spectra gathered from each of two habitats of *Paratanakia himantegus himantegus* and *Paratanakia himantegus chii*.** In each sampling location, the relative spectral irradiance was smoothed by a factor of 15 using a simple moving average (McLean et al. 2007), and then normalized to a 0–100 % scale, where 100 % indicated the maximum intensity spectrum.

**Competing interests**
The authors declare that they have no competing interests.

**Authors' contributions**
CHC and YTS carried out the ERG measurements and UV reflection photography experiments, and wrote the first draft of the manuscript. KA designed and wrote the codes for the ERG recording and data analysis systems. WCF assisted in ERG recordings. YSL made comments on the manuscript. HYY provided logistical and financial support during the study, and revised the manuscript. All authors read and approved the final manuscript.

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