

A novel, sexually selected trait in poeciliid fishes: female preference for mustache-like, rostral filaments in male *Poecilia sphenops*

Ingo Schlupp · Rüdiger Riesch · Michael Tobler ·
Martin Plath · Jakob Parzefall · Manfred Schartl

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Abstract Female choice can often drive the elaboration of male traits, leading to the evolution of secondary sexual traits. In the Mexican molly, *Poecilia sphenops* (Poeciliidae), some males exhibit a mustache-like structure on the upper maxilla, the function of which has not been previously recognized. The mustache consists of epidermal outgrowths at the edge of the scales that appear to have no sensory function. Trait expression varies within as well as among populations of *P. sphenops*, but is not linked to male body size polymorphism. In mate choice experiments, female *P. sphenops* exhibited a visual mating preference

for males with mustaches, suggesting that the trait may be sexually selected. Since the mating behavior of *P. sphenops* involves contact of the male's snout and the female genital region prior to copulation, we hypothesize that the mustache may also convey tactile signals to the female.

Keywords Mate choice · Mating preference · Sexual selection · Structural ornaments

Introduction

Sexual selection is a powerful evolutionary force often leading to the elaboration of male traits and the evolution of sexual dimorphism (Andersson 1994; Andersson and Iwasa 1996). Numerous male traits, like conspicuous color patterns or structural ornaments, have evolved as a consequence of females preferring to mate with males bearing them. Live-bearing fishes of the family Poeciliidae have been particularly useful as model systems for studying sexual selection (Houde 1997; Magurran 2005) because of the diversity of male ornaments and the diversity of underlying female preferences found in this group (Endler 1983, 1992; Basolo 1998; Lindholm and Breden 2002).

In poeciliid fishes, there is a good understanding of how females respond to male traits ranging from distinct colors and color patterns (Schlüter et al. 1998; Kodric-Brown and Johnson 2002; Cummings et al. 2003) to structural ornaments (Ptacek 1998; Rosenthal and Evans 1998; MacLaren et al. 2004; Wong and Rosenthal 2006) and courtship patterns (Kodric-Brown 1993; Rosenthal and Evans 1998; Kodric-Brown and Nicoletto 2001). Even chemical communication and its role in mate choice has been studied to some extent (Shohet and Watt 2004; Fisher and Rosenthal 2006). In many of these cases, studies of the mechanisms

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I. Schlupp (✉) · R. Riesch
Department of Zoology, University of Oklahoma,
730 Van Vleet Oval,
Norman, OK 73019, USA
e-mail: schlupp@ou.edu

I. Schlupp · J. Parzefall
Biozentrum Grindel, University of Hamburg,
20146 Hamburg, Germany

M. Tobler
Departments of Biology and Wildlife and Fisheries Sciences,
Texas A&M University,
2258 TAMU,
College Station, TX 77840, USA

M. Plath
Department of Ecology and Evolution,
J.W. Goethe University of Frankfurt,
Siesmayerstr. 70a,
60054 Frankfurt a.M., Germany

M. Schartl
Physiological Chemistry I, University of Würzburg,
Am Hubland,
97074 Würzburg, Germany

underlying mate choice were combined with studies on the ultimate consequences of female choice.

In the present study, we investigated the form and function of an as yet unstudied structure found in some males of the Mexican molly, *Poecilia sphenops* Valenciennes, 1846. The structure consists of relatively long filaments found exclusively on the snout of males, resembling a mustache (Fig. 1a). The trait itself had been mentioned in a previous taxonomic study (Schultz and Miller 1971) and—already alluding to a potential function—was characterized as a “contact organ.” Contact organs typically are tiny hard bumps of keratin (sometimes with a core of bone) that grow on the fins, head, and body scales and have been described in at least 25 families of fishes (Wiley and Collette 1970; Collette 1977). They are predominantly found in males and usually expressed during the breeding season when they help males maintain contact with females during spawning. Best known are probably the breeding tubercles of cyprinid fishes in which high numbers of tubercles have been shown to increase male reproductive success (Jacob et al. 2009).

Contact organs have also been described in some genera of the family Poeciliidae, specifically in the genera *Poecilia*, *Poeciliopsis*, and *Phallichthys* (Wiley and Collette 1970). In these groups, contact organs are slender ossified extensions at the edge of the scales on the head posterior of the upper lip and are thought to play a role during mating as males of these genera touch females at the gonopore with

their snout prior to copulation (Rosen and Tucker 1961). Compared to the structures found in *P. sphenops*, however, the contact organs previously described in poeciliids are small, barely visible by eye, and unbranched. In the present study, we characterized male mustaches of *P. sphenops* using electron microscopy, surveyed the distribution of mustaches within and among populations, and studied their potential role in sexual selection by investigating female mate choice.

Materials and methods

Study system and material examined

P. sphenops is common along the Atlantic coast of Mexico from the Río de la Palma Sola on southward into Guatemala on the Pacific side (Miller 2005). They are also distributed inland along the Isthmus of Tehuantepec. Contact organs have previously been mentioned from several populations of *P. sphenops* and some other *Poecilia* species (Schultz and Miller 1971), but neither a thorough description of the structure nor systematic classification of populations with and without mustaches has been conducted so far. We surveyed the occurrence of mustaches in males of several populations of *P. sphenops* and museum holdings of the species (see Table 1 for material examined). Based on

Fig. 1 **a** Photograph of a male *P. sphenops*. The mustache-like structure is clearly visible on the male's rostrum. **b** Dorsal EM scan of the snout region of a *P. sphenops* male depicting the epidermal outgrowths that form the mustache. **c** EM scan of a single scale of the rostrum. The epidermis was removed in the posterior part of the scale. Epidermal outgrowths are present on the anterior edge of the scale

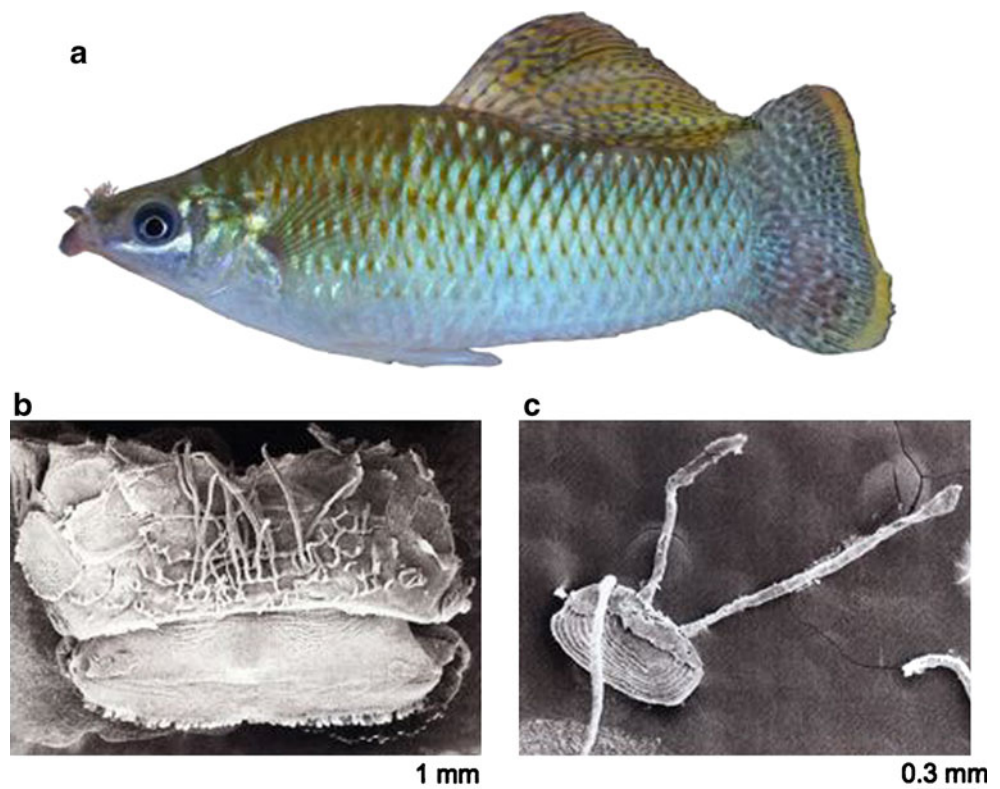


Table 1 Material examined in this study including field site/accession numbers, latitude and longitude, the presence of bearded males, and the number of individuals examined

	Latitude	Longitude	Mustached males	<i>N</i>
Collections by the authors				
IS 98/3	18.66	95.75	No	n.a. ^a
IS 98/4b	18.79	95.80	Yes	n.a.
IS 98/7	19.50	96.93	Yes	n.a.
IS VI/1	19.42	96.61	Yes	n.a.
IS VI/2	19.46	96.57	Yes	n.a.
IS VI/3	19.50	96.58	Yes	n.a.
IS VI/4	19.51	96.46	Yes	n.a.
IS VI/6	19.68	96.51	Yes	101
IS VI/8	19.25	96.37	No	n.a.
Museum collections examined				
CU64287	18.88	95.97	No	40
TNHC13876	16.27	94.15	No	34
TNHC13896	16.13	93.80	No	89
UMMZ157628	19.17	96.17	Yes	704
UMMZ164743	19.03	96.03	Yes	13
UMMZ181298	17.80	95.17	Yes	46
UMMZ184513	19.75	96.47	No	433
UMMZ184516	19.63	96.47	Yes	11
UMMZ184564	17.80	95.17	Yes	59
UMMZ187252	19.03	96.03	Yes	2
UMMZ187731	17.80	94.75	Yes	14
UMMZ187750	17.80	94.75	No	17
UMMZ187773	17.75	94.80	No	113
UMMZ187808	17.83	94.75	Yes	71
UMMZ192842	19.08	96.13	Yes	6
UMMZ194688	19.03	96.17	Yes	11
UMMZ194767	18.03	94.53	Yes	11

CU Cornell University Ichthyology Collection, Ithaca, NY, USA, TNHC Texas Natural History Collection, Austin, TX, USA, UMMZ University of Michigan Museum of Zoology, Ann Arbor, MI, USA

^aThis information is not available for this collection

topology of a molecular phylogeny (using cytochrome *b* and control region sequences of mitochondrial DNA), the presence or absence of this trait appears to represent within-species variation (data not shown).

Distribution patterns of mustached males within populations

To assess the occurrence of mustaches within a population, *P. sphenops* were caught in a small coastal stream north of Veracruz (El Limon, IS IV/6; Table 1) in April 2007. Males and females were examined for the presence of mustaches, and their standard length was measured to the closest

millimeter. Since sexual ornaments in poeciliids are often expressed in larger males only (Farr 1989), we compared the standard lengths of males with and without mustaches using an independent-samples *t* test.

Electron microscopy

In-depth examinations of mustaches and their significance in female mate choice were all performed on *P. sphenops* collected at the IS VI/6 site (Table 1). For the examination of the mustache structure, male *P. sphenops* were collected and fixed on site in 10% formalin. Samples were then studied using routine electron microscopy (EM) processing (Hansen and Finger 2000).

Mate choice experiments

In species like *P. sphenops*, males do not exhibit any courtship displays prior to gonopodial thrusting. Given that molly males constantly attempt to mate with females (e.g., even directly upon capture; Riesch et al. 2008), it is likely that time spent by a female associating with a given male (leading to physical proximity) facilitates male copulation attempts by that particular male. In fact, a recent study by Walling et al. (2010) experimentally demonstrated that association preferences actually do translate into male reproductive success in green swordtails, *Xiphophorus hellerii* (Poeciliidae). Hence, association preferences should translate into more copulations with the preferred male also in *P. sphenops*.

Female mate choice experiments with live stimulus fish were conducted in the field in Mexico in 1998 and 2006. We used a portable test tank (42.6×30×16.5 cm) built from UV-transparent Plexiglas. The tank was visually divided into three equal zones by black marks on the outside. The central zone was designated the neutral zone, the two lateral zones as preference zones. Two stimulus males were presented in two smaller tanks (19.5×30×14.5 cm) on either side of the test tank. Females were only tested once. The fish were collected using a seine and were acclimated in a 10-l bucket with creek water.

Video playback experiments were conducted in spring of 2009 at the University of Oklahoma in Norman. *P. sphenops* for this experiment originated from field collections in 2006 and were maintained as randomly outbred stocks in tanks (250–1,000 l) in a greenhouse. The tanks contained naturally growing algae and other submerged plants as well as a variety of naturally occurring invertebrates like chironomid larvae, copepods, and amphipods on which the fish could feed. In addition, the fish were fed ad libitum amounts of flake food every 2 days. All fish used in this study were sexually mature and had interacted with the opposite sex; thus, all females were most likely pregnant.

Generation of stimulus videos

To produce the video animations for our video playback experiment, digital images of two males were taken while a male was swimming in a small tank using a Nikon D70 digital camera. From each resulting picture, the image of the male was extracted from the background using the “magic extractor” function in Adobe® Photoshop Elements 6.0. In a first step, actual rostral filaments were virtually removed. Then, using the coloration of the snout region of each individual male, mustache-like rostral filaments were pasted onto the body of one male in each pair of images using the “brush tool.” Several pictures of mustached *P. sphenops* served as models to ensure visual accuracy (e.g., Fig. 1). Consequently, the pairs of animation showed two identical males differing only in the presence or absence of a mustache. Differences in the response to the animation by the tested females can therefore be attributed to the trait under study, rostral filaments.

The resulting images were then animated and converted to an AVI file (resolution, 800×600) using “Pencil v.0.4.4b” for Apple. A straight movement of the pictures from left to right and right to left was generated in front of a uniform white background. The animations were 14 s long: twice 6 s for the distance of 28.5 cm on the screen forth and back, each followed by an invisible turn of 1 s. Simultaneous playback was performed using two identical computer monitors (Belinea 10 30 40) with a Matrox Millennium G400 dual-head graphic card. The monitor refresh rate was 85 Hz, and the AVI films were run in infinite loops during the experiment using the “Monitor Plug-In” that is part of the Viewer software package (BIOBSERVE GmbH).

To ensure that male size was appropriate for the individual trials (i.e., resembled the approximate size of the original males photographed), sample videos were run and male sizes measured on the screen. If needed, male size was then digitally adjusted and new videos were created until male size was appropriate for each stimulus–video pair.

Experiment 1

We conducted three separate experiments. In the first experiment ($n=17$ replicates), we tested whether females (standard length; mean \pm SD, 62.9 \pm 6.7 mm) exhibit a preference for males with mustaches. To do so, pairs of stimulus males were matched for size [size difference < 2 mm standard length (SL)], and after cooling down the males on ice, the mustache was gently removed in one of two stimulus males using a scalpel. No mortality was associated with this procedure. We always removed the slightly larger male’s mustache, so female choice for large male size (see below) would not affect the results (standard lengths; males

with mustache, 59.4 \pm 4.3 mm; mustache removed, 60.4 \pm 5.4 mm; paired t test: $t_{16}=3.23$, $P=0.005$).

Before each trial, stimulus males were placed into the side tanks and given several minutes for acclimatization. Once the males were swimming calmly, a female was introduced into the neutral zone, and we measured the time the female subsequently spent in each preference zone during 5 min of observation. Note that females could exercise a “non-choice” by remaining in the neutral zone. To control for side biases, the stimuli were interchanged after the first trial and the measurement repeated. The actual choice test thus consisted of two trials and lasted for 10 min.

Experiment 2

In the first experiment, females may not exhibit a preference for mustached males, but they could actually be rejecting manipulated males, e.g., because handling during the physical removal of mustaches may have altered the males’ behavior. We thus performed a second experiment ($n=12$ replicates) where preferences of females (56.0 \pm 7.2 mm) for large manipulated (63.7 \pm 9.2 mm) as compared to smaller, unmanipulated males (39.7 \pm 5.2 mm) were assessed (difference in body size, paired t test: $t_{11}=8.63$, $P<0.001$). Preferences for large-sized males are common in female poeciliids (Ryan and Keddy-Hector 1992) and were confirmed by an experiment with lab-reared *P. sphenops* females from the same population (paired t test: $t_{16}=2.58$, $P=0.02$) using a standard experimental protocol. However, if females rejected manipulated males, they should prefer smaller males. The testing procedure was identical to that of the first experiment.

The females used in both experiments 1 and 2 were field-collected; hence, we had no knowledge of their reproductive status.

Experiment 3

To unequivocally test whether mustached males really are preferred by females and are not simply avoided after the manipulation (i.e., surgical removal of the mustache; experiments 1 and 2), we conducted a third experiment ($n=16$ replicates) using video stimuli rather than live fish. For this, monitors were placed on either side of a test tank (60×40×30 cm). Water level was maintained at 25 cm, which was also the height of the monitors, and the water temperature was 25°C. Using the “Zone Definition” function of the Bioobserve Viewer software, the tank was virtually divided into three equal-sized sections: a central neutral zone and two preference zones near the monitors. The behavior and movement of each focal female was monitored and tracked using a Sony camera (ExwaveHAD) placed above the setup, connected to the PC running the

Viewer program. To increase the contrast between fish and background (i.e., making it easier for the viewer software to track the focal fish), white opaque Plexiglas sheets covered the bottom and long sides of the tank. Pairs of videos were alternated between trials; hence, half of the females ($n=8$) were presented with the videos of male 1 and the other half with the videos of male 2.

A test female was placed into a transparent Plexiglas cylinder (8.5-cm diameter) in the middle of the neutral zone. The female was allowed to acclimate for 5 min, and then the cylinder was carefully removed and the experiment remotely started. The Viewer software now tracked the focal fish for an observation period of 5 min and recorded the time spent in each preference zone. To detect side biases, the female was immediately placed into the cylinder again, the video playbacks were switched, and the procedure was repeated. After the test, the SL of the female was measured to the closest millimeter. Females were then transferred into another tank so that each female was only tested once.

We decided a priori to exclude side biases (more than 80% of time during both parts of a trial in only one preference zone) and trials with low response (<50% of the time inside the preference zones). We assumed such females not to be motivated to choose (Landmann et al. 1999); however, neither side biases nor low response occurred during this experiment.

For all three experiments, we summed the time spent in the preference zone near each stimulus male for both trials. Association times with the two stimulus males were compared using paired t tests.

Results

Distribution of *P. sphenops* populations with mustaches

Males with mustaches were detected in 18 out of the 26 examined sites (Table 1 and Fig. 2). There was no clear geographical pattern, although males with mustaches were absent among the specimens examined from the two sites on the Pacific versant of Mexico.

Frequency and distribution of males with mustaches within populations

We examined 101 individuals from El Limon to assess the distribution of mustaches within a population. None of the 72 females (mean SL \pm SD, 60.2 ± 15.4 mm) exhibited a mustache. However, 17 of the 29 males (59%) had mustaches. There was no significant difference in the size of males with and without a mustache (SL of males with mustache, 62.0 ± 17.4 mm; males without mustache, $57.3 \pm$

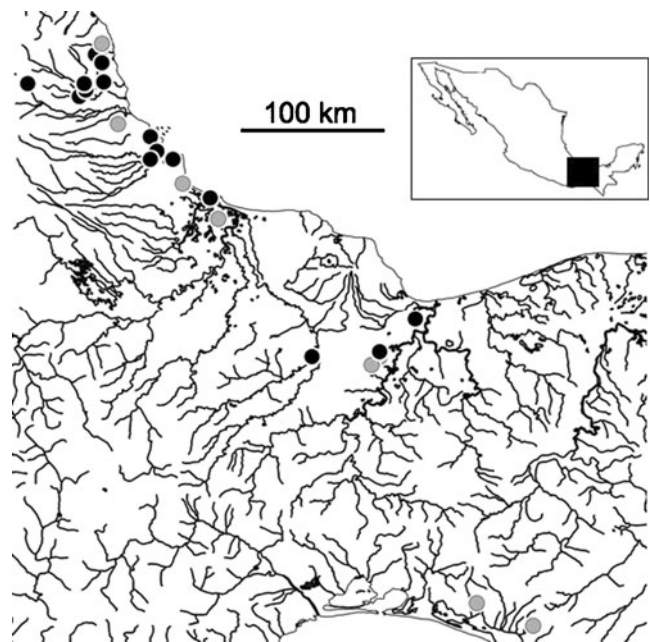


Fig. 2 Locations of *P. sphenops* examined for this study (see Table 1). Black circles indicate populations in which males with mustaches were found; gray circles are populations without mustaches. The inset highlights the location of the enhanced section within Mexico

9.3 mm; independent t test for samples with unequal variances: $t_{28} = -0.95$, $P = 0.35$).

Electron microscopy of mustaches

The EM examination of *P. sphenops* mustaches showed that they consist of epidermal filaments that emerge from the anterior edges of the scales located across the rostrum (Fig. 1). Most filaments appear to be unbranched, but some branched filaments were recorded. EM did not reveal any sensory structures in the filaments.

Mate choice experiments

Experiment 1

In the first behavioral experiment, focal females could choose between a male with a mustache and a male whose mustache had been removed. Females displayed a strong preference for males with mustaches: They spent 319.6 ± 78.5 s (mean \pm SD) near the mustached male vs. 200.4 ± 96.2 s near the non-mustached male (paired t test: $t_{16} = 2.74$, $P = 0.015$; Fig. 3a).

Experiment 2

In a second experiment, focal females could choose between a larger male with his mustache removed and a smaller mustached (i.e., non-manipulated) male. Females spent

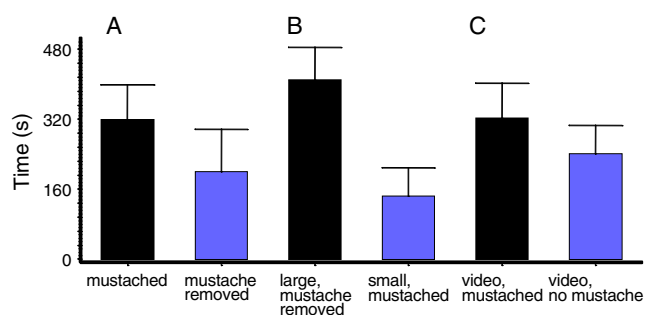


Fig. 3 Results of the behavioral experiments (mean \pm SD association times near the two types of males). **a** Female choice for males with and without mustaches. **b** Large males with mustaches removed and small unmanipulated stimulus males. **c** Video images of the same male with and without a mustache

significantly more time near the manipulated, larger male (410.3 ± 73.6 s) compared with the unmanipulated, smaller one (144.5 ± 64.8 ; paired t test: $t_{11} = 6.25$, $P < 0.001$; Fig. 3b).

Experiment 3

In a third experiment, focal females could choose between videos of the same male; however, one video-male sported a mustache, while the other one did not. Again, females spent significantly more time near the mustached male (323.5 ± 78.5 vs. 240.9 ± 64.7 s; paired t test: $t_{15} = 2.38$, $P = 0.031$; Fig. 3c).

Discussion

The mustache-like structures on the rostrum of *P. sphenops* consist of epidermal outgrowths on the edges of scales and seem to be an as yet unrecognized sexually selected trait in poeciliid fishes. Epidermal outgrowths on the snout have previously been reported for several other fishes (Collette 1977); however, compared to the structure in *P. sphenops*, epidermal contact organs are hard structures usually based on keratin or bone. Soft and fleshy filaments are otherwise only known from male armored suckermouth catfish of the genus *Ancistrus* (Sabaj et al. 1999). For these, it was speculated that male tentacles might mimic larvae, which may provide males with a mating advantage since females prefer spawning with males that already guard a clutch of hatched larvae (Sabaj et al. 1999).

The presence of the mustaches in *P. sphenops* seems to be facultative even within populations. Whether this reflects a genetic polymorphism or phenotypically plastic expression of the trait (which could be lost and regrown) remains to be examined. It also remains unclear whether populations in which we were not able to record mustached males indeed lack them or whether these just occur at very low frequencies. Unlike other ornaments in poeciliids, such as

the presence of swords in some *Xiphophorus* species (Zimmerer 1988), the presence of mustaches in *P. sphenops* is not restricted to large males as no differences in the mean size between mustached and non-mustached males were found.

The female choice experiments indicated a visually based mating preference for males exhibiting a mustache. At this time, we cannot assess the effect that the removal of the ornament per se had on the stimulus males, but our two follow-up experiments support the idea that males were not rejected simply because they were manipulated. It is also noteworthy that the widespread preference for larger males overrides the preference for the mustache.

Although the preference we documented is visual, the structure of the ornament, its position on the male's snout, and the mating behavior of *Poecilia* males make it likely that mustaches also provide tactile information for the females. Males of *P. sphenops* and several other poeciliids typically follow the female and touch their genital region with the snout prior to copulation attempts (a sexual behavior known as "nipping"; Rosen and Tucker 1961; Parzefall 1969). Given the size of the filaments, the structure likely will be felt by the females during nipping. In another species of the genus *Poecilia*, the cave molly (*Poecilia mexicana*) females have evolved an enlarged genital pad which—besides its function of secreting pheromones—is presumed to also have a sensory function in the perception of scale protuberances located on the head of the males (Parzefall 2001).

In fishes, tactile signals and stimulation as a means of sexual signaling have not been investigated in depth so far, and this phenomenon clearly requires additional investigation (Coleman 2009). If females indeed perceive tactile information by mustaches and integrate the information into the decision-making process during mate choice, mustaches in *P. sphenops*, along with male size, coloration, pheromones, and behavior, may contribute to a multimodal signal addressing visual, olfactory, and tactile sensory channels (Hölldobler 1999; Uetz and Roberts 2002; Candolin 2003; McLennan 2003; Partan and Marler 2005). Multimodal signals are especially interesting to study because both senders and receivers face trade-offs as these signals are hard to perfect for different sensory modalities. Many multimodal signals studied so far are bimodal, i.e., combine sensory input from two sensory modalities like vision and hearing (Cooper and Goller 2004) or vision and seismics (Hebets et al. 2006).

Based on the present study, we hypothesize that the "mustache" in *P. sphenops* could be such a multimodal signal, combining a visual and a tactile component. Future studies will have to elucidate the proposed tactile function, but also ask what maintains the interesting polymorphism for this trait.

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Ethical note The experiments comply with the current laws of the country in which they were performed.

The authors declare that they have no conflict of interest.

References

- Andersson M (1994) Sexual selection. Princeton University Press, Princeton
- Andersson M, Iwasa Y (1996) Sexual selection. Trends Ecol Evol 11:53–58
- Basolo AL (1998) Evolutionary change in a receiver bias: a comparison of female preference functions. Proc R Soc Lond B Biol Sci 265:2223–2228
- Candolin U (2003) The use of multiple cues in mate choice. Biol Rev 78:575–595
- Coleman S (2009) Taxonomic and sensory biases in the mate choice literature: there are far too few studies of chemical and multimodal communication. Acta Ethologica 12:45–48
- Collette BB (1977) Epidermal breeding tubercles and bony contact organs in fishes. Symp Zool Soc Lond 1977:225–168
- Cooper BG, Goller F (2004) Multimodal signals: enhancement and constraint of song motor patterns by visual display. Science 303:544–546
- Cummings ME, Rosenthal GG, Ryan MJ (2003) A private ultraviolet channel in visual communication. Proc R Soc Lond Series B: Biol Sci 270:897–904
- Endler JA (1983) Natural and sexual selection on color patterns in poeciliid fishes. Environ Biol Fish 9:173–190
- Endler JA (1992) Signals, signal conditions and the direction of evolution. Am Nat 139:S125–S153
- Farr JA (1989) Sexual selection and secondary sexual differentiation in poeciliids: determinants of male success and the evolution of female choice. In: Meffe GK, Snelson FF Jr (eds) Ecology and evolution of livebearing fishes (*Poeciliidae*). Prentice Hall, Englewood, pp 91–123
- Fisher HS, Rosenthal GG (2006) Female swordtail fish use chemical cues to select well-fed mates. Anim Behav 72:721–725
- Hansen A, Finger TE (2000) Phyletic distribution of crypt-type olfactory receptor neurons in fishes. Brain Behav Evol 55:100–110
- Hebets EA, Cuasay K, Rivlin PK (2006) The role of visual ornamentation in female choice of a multimodal male courtship display. Ethology 112:1062–1070
- Hölldobler B (1999) Multimodal signals in ant communication. J Comp Physiol A 184:129–141
- Houde AE (1997) Sex, color, and mate choice in guppies. Princeton University Press, Princeton
- Jacob A, Evanno G, Renai E, Sermier R, Wedekind C (2009) Male body size and breeding tubercles are both linked to intrasexual dominance and reproductive success. Anim Behav 77:823–829
- Kodric-Brown A (1993) Female choice of multiple male criteria in guppies: interacting effects dominance, coloration and courtship. Behav Ecol Sociobiol 32:415–420
- Kodric-Brown A, Johnson SC (2002) Ultraviolet reflectance patterns of male guppies enhance their attractiveness to females. Anim Behav 63:391–396
- Kodric-Brown A, Nicoletto PF (2001) Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. Behav Ecol Sociobiol 50:346–351
- Landmann K, Parzefall J, Schluppi I (1999) A sexual preference in the Amazon molly, *Poecilia formosa*. Environ Biol Fish 56:325–331
- Lindholm A, Breden F (2002) Sex chromosomes and sexual selection in poeciliid fishes. Am Nat 160:S214–S224
- MacLaren RD, Rowland WJ, Morgan N (2004) Female preferences for sailfin and body size in the sailfin molly, *Poecilia latipinna*. Ethology 110:363–379
- Magurran AE (2005) Evolutionary ecology: the Trinidadian guppy. Oxford University Press, Oxford
- McLennan DA (2003) The importance of olfactory signals in the gasterosteid mating system: sticklebacks go multimodal. Biol J Linn Soc 80:555–572
- Miller RR (2005) Freshwater fishes of Mexico. University of Chicago Press, Chicago
- Partan SR, Marler P (2005) Issues in the classification of multimodal communication signals. Am Nat 166:231–245
- Parzefall J (1969) Zur vergleichenden Ethologie verschiedener *Mollienesia*-Arten einschließlich einer Höhlenform von *M. sphenops*. Behaviour 33:1–37
- Parzefall J (2001) A review of morphological and behavioural changes in the cave molly, *Poecilia mexicana*, from Tabasco, Mexico. Environ Biol Fish 62:263–275
- Ptacek MB (1998) Interspecific mate choice in sailfin and shortfin species of mollies. Anim Behav 56:1145–1154
- Riesch R, Schlupp I, Plath M (2008) Female sperm-limitation in natural populations of a sexual/asexual mating-complex (*Poecilia latipinna*, *P. formosa*). Biol Lett 4:266–269
- Rosen DE, Tucker A (1961) Evolution of secondary sexual characters and sexual behavior patterns in a family of viviparous fishes (Cyprinodontiformes: Poeciliidae). Copeia 1961:201–212
- Rosenthal GG, Evans CS (1998) Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size. Proc Natl Acad Sci USA 95:4431–4436
- Ryan MJ, Keddy-Hector AC (1992) Directional patterns of female mate choice and the role of sensory biases. Amer Nat 139:4–35
- Sabaj MH, Armbruster JW, Page LM (1999) Spawning in *Ancistrus* (Siluriformes: Loricariidae) with comments on the evolution of snout tentacles as a novel reproductive strategy: larval mimicry. Ichthyol Explor Freshw 10:217–229
- Schlüter A, Parzefall J, Schlupp I (1998) Female preference for symmetrical vertical bars in male sailfin mollies. Anim Behav 56:147–153
- Schultz RJ, Miller RR (1971) Species of the *Poecilia sphenops* complex (Pisces: Poeciliidae) in Mexico. Copeia 1998:282–290
- Shohet AJ, Watt PJ (2004) Female association preferences based on olfactory cues in the guppy, *Poecilia reticulata*. Behav Ecol Sociobiol 55:363–369
- Uetz GW, Roberts JA (2002) Multisensory cues and multimodal communication in spiders: insights from video/audio playback studies. Brain Behav Evol 59:222–230
- Walling CA, Royle NJ, Lindström J, Metcalfe NB (2010) Do female association preferences predict the likelihood of reproduction? Behav Ecol Sociobiol 64:541–548
- Wiley B, Collette B (1970) Breeding tubercles and contact organs in fishes: their occurrence, structure, and significance. Bull Am Mus Nat Hist 143:145–216
- Wong BBM, Rosenthal GG (2006) Female disdain for swords in a swordtail fish. Am Nat 167:136–140
- Zimmerer EJKKD (1988) The inheritance of vertical barring (aggression and appeasement signals) in the pygmy swordtail *Xiphophorus nigrensis* (Poeciliidae: Teleostei). Copeia 1988: 299–307