Different on the inside: extreme swimbladder sexual dimorphism in the South Asian torrent minnows

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The swimbladder plays an important role in buoyancy regulation but is typically reduced or even absent in benthic freshwater fishes that inhabit fast flowing water. Here, we document, for the first time, a remarkable example of swimbladder sexual dimorphism in the highly rheophilic South Asian torrent minnows (Psilorhynchus). The male swimbladder is not only much larger than that of the female (up to five times the diameter and up to 98 times the volume in some cases), but is also structurally more complex, with multiple internal septa dividing it into smaller chambers. Males also exhibit a strange organ of unknown function or homology in association with the swimbladder that is absent in females. Extreme sexual dimorphism of non-gonadal internal organs is rare among vertebrates and the swimbladder sexual dimorphisms that we describe for Psilorhynchus are unique among fishes.

1. Introduction

The swimbladder is a visceral organ derived embryologically from a dorsal outgrowth of the gut and is an evolutionary novelty of the actinopteran fishes (Actinopterygii minus Polypteriformes) [1]. Homologous with the lungs of sarcopterygian and polypteriform fishes [1 and references therein], the swimbladder is hypothesized to have originally functioned as a respiratory organ [2], but serves predominantly as a hydrostatic organ in modern ray-finned fishes, involved in the maintenance of neutral buoyancy [3]. Although great diversity exists in the structure of the swimbladder across actinopteran fishes [4], especially within the highly diverse Teleostei (approx. 33 500 species), a number of general trends between swimbladder morphology and ecology are well established. Perhaps the earliest trend in swimbladder morphology to be identified is the reduction of this organ in fishes that live close to, or in direct contact with, the substrate [5]. In numerous groups of benthic teleosts, the swimbladder is reduced to a small vestige or is absent [6], which is hypothesized to enhance fish–substrate interaction through the attainment of negative buoyancy [3].

Members of the South Asian freshwater fish genus Psilorhynchus (torrent minnows; figure 1a) are obligate benthic rheophiles in foothill areas of the Himalayan, Indo-Burman and Western Ghats mountain ranges [7]. The swimbladder of Psilorhynchus has been considered vestigial since it was first described in the late-nineteenth century [8]. During continuing investigations of Psilorhynchus, we encountered a strikingly different anatomical structure of the swimbladder among members of the same species, including an anticipated vestigial type (figure 1b(iii)) and a second type, much larger than the first that is often divided internally and associated with large accessory structures (figure 1b(ii)). Upon further investigation, we discovered that these radical differences in members of the same species are the result of sexual dimorphism, with males exhibiting a larger and structurally more complex swimbladder than females. Extreme sexual dimorphism of the swimbladder is rare among...
Figure 1. Extreme sexual dimorphism in Psilorhynchus. (a) P. tenura, in life: (i) male; (ii) female. (b) Eviscerated abdominal cavity (ventral view) of P. robustus, CMK 17941: (i) male; (ii) female (swimbladder circled). (c) Dissected c&s specimens (lateral view) of P. balitora, KU 40386, showing swimbladder in relation to surrounding sexually dimorphic skeleton: (i) male; (ii) female. (d) Sagittal section through eviscerated trunk of P. balitora, KU 40386: (i) male; (ii) female. (e) Transverse section through posterior swimbladder chamber of P. sucatio, KU 40664: (i) male; (ii) female. (f) Transverse section through posterior swimbladder of P. balitora, KU 40386: (i) male; (ii) female; (g) Anterior trunk musculature (lateral view) of P. sucatio, KU 40664, showing sexually dimorphic hypaxial musculature (muscle segments exhibiting sexual dimorphism are indicated with asterisks (*, **, ***)): (i) male; (ii) female. Scale bar shared in (e) and (f). AC, anterior chamber; Ep, epineural; EpM, epaxial musculature; HpM, hypaxial musculature; LLN, lateral line nerve; MaPl, masticatory plate; NeuCo, neural complex; Ns, neural spine; PC, posterior chamber; PT, pseudo-tympanum; PWP, post-Weberian process; R, rib; Sh, sheath surrounding anterior chamber; TE, tunica externa; TI, tunica interna; TFO, tuning fork-shaped organ; WeCa, Weberian capsule.
teleost fishes [9] and has never been reported for any of the roughly 10 000 species of the Otophysi, the teleost group to which Psilorhynchos belongs. Herein, we describe the remarkably sexually dimorphic swimbladder of Psilorhynchos, which also provides a rare example of swimbladder enlargement in a benthic lineage of freshwater fishes.

2. Material and methods

(a) Gross morphological investigation
Specimens of Psilorhynchos (see the electronic supplementary material) were examined and photographed using a Zeiss SteReo Discovery V20 microscope equipped with an Axiocam MRc5 digital camera. Prior to examination, specimens were eviscerated to expose the swimbladder, and the skin from the right side of the body anterior to the dorsal fin was also removed to expose body musculature adjacent to the swimbladder. Selected specimens were also cleared and stained (c&s [10]) to enable observation of the skeleton surrounding the swimbladder.

(b) Histological investigation
The eviscerated trunk of one male and one female each of Psilorhynchos balitora and Psilorhynchos sucato was embedded in paraplast, sectioned serially in the transverse or sagittal plane and stained with haematoxylin and eosin. We also sectioned the posterior swimbladder chamber (ex situ) of male and/or female individuals of multiple species (see the electronic supplementary material) using a plastic embedding technique. All slides were examined with an Olympus BX40 compound microscope equipped with a Canon Tsi digital camera. An in-depth overview of histological methods is included in the electronic supplementary material.

3. Results and discussion
As is typical in otophysans, the physostomous swimbladder of Psilorhynchos is divided into an anterior and posterior chamber, connected by a constriction close to the origin of the pneumatic duct [11]. Sexual size dimorphism is evident in both chambers in all species examined (excluding Psilorhynchos pseudocheneis), but is most pronounced in the posterior chamber (figures 1b–f and electronic supplementary material, figure S1). Assuming each chamber is representative of a simple sphere, we estimate the average volume of the anterior chamber of males to be approximately two times greater (range 0.0–7.4; electronic supplementary material, table S1) than that of females (data pooled across all species; see the electronic supplementary material, table S1). The sexual size dimorphism exhibited by the swimbladder of Psilorhynchos is further emphasized by the physical space that the organ occupies inside of the abdominal cavity. In females, the swimbladder is restricted to the anterodorsal most part of the abdominal cavity (figure 1b(ii),d(iii)), and terminates directly below the fifth or sixth centrum of the vertebral column (figure 1c(iii)). In males of all species (excluding P. pseudocheneis), the swimbladder extends much further posteriorly into the abdominal cavity (figure 1b(i),d(ii)), terminating directly below the ninth or 10th centrum (figure 1c(i)).

In addition to pronounced sexual size dimorphism, there is also dimorphism in the shape and arrangement of the lumen inside of the posterior chamber in several of the examined species. In females of all species, the lumen is a simple sphere (figure 1d(ii)–f(ii)), and this is also the case in males of four of the species examined (figure 2). In males of Psilorhynchos sucato and those species belonging to the P. balitora species group [8], the posterior chamber is divided internally by septa that originate from the internal layer of smooth muscle that encircles the inner surface of the chamber. In males of P. sucato, the septa are restricted to the caudal end of the posterior chamber, forming two to three large cavities (figure 1e(i)) that are connected anteriorly with the main lumen. In members of the P. balitora species group (as exemplified by P. balitora; figure 1d(i),f(i)), septa are present throughout the entire posterior chamber, forming an extensive alveolar-like network of small vesicles that encircle, and are often connected to, a restricted central lumen.

Undoubtedly, the most striking sexually dimorphic feature associated with the swimbladder of Psilorhynchos is the tuning fork-shaped organ that straddles the dorsal surface of the swimbladder (figure 1c(i)) in males of all species (excluding P. pseudocheneis). Our histological examination has revealed this organ to comprise predominantly connective tissue, including a fibrous sheath of irregularly arranged collagen fibres surrounding an inner core of regularly arranged fibres intertwined with undifferentiated mesenchymal tissue and small blood vessels. The fibrous sheath surrounding the paired anterior arms of this organ is in direct contact (medially) with the tunica externa of the anterior chamber and is attached (laterally) to the adjacent skeleton (Weberian capsule and fifth rib; figure 1c(i)). Posteriorly, the organ rests along the dorsal surface of the posterior chamber and may extend beyond the swimbladder, along the dorsal surface of the abdominal cavity (figure 1b(i)). Our detailed anatomical investigation of females failed to identify the tuning fork-shaped organ, and we are currently unaware of a homologue for this organ in other teleosts.

**Figure 2.** Phylogenetic relationships among species of Psilorhynchos [7] illustrating evolution of size dimorphism (character 1) and compartmentalization (character 2) in the posterior swimbladder chamber. Hyphens denote no information.
Based on available phylogenetic hypotheses for *Psilorhynchus* (figure 2), sexual dimorphism of the swimbladder is interpreted as the result of a single evolutionary event, with the absence of sexual dimorphism in *P. pseudecheneis* the result of secondary loss. Swimbladder sexual dimorphism in *Psilorhynchus* has evolved in concert with a suite of other dimorphic features, including elements of the skeleton [7]; figure 1c] and associated hypaxial musculature (figure 1g), which probably work together as a single functional unit. Sexual dimorphism of the muscles and skeletal elements associated with or adjacent to the swimbladder are frequently involved with sound production in other teleosts [9,12] and we predict a similar function for the sexual dimorphisms that we have identified in *Psilorhynchus*. If we are correct, then it will be interesting to learn how *Psilorhynchus* has overcome the problems of sound transmission and reception in the inherently ‘noisy’ conditions characteristic of shallow, fast-flowing rivers and streams [13].

Sexual dimorphism in fishes most commonly involves external features that play a role during reproductive behaviour and that range from variably sized fins [14,15], teeth and/or jaws [16,17] in males to extreme differences in body size [18] and/or shape [19] between the sexes. Sexual dimorphism of internal organs other than the gonads is rare, not only among fishes, but also in other groups of vertebrates, and is limited to a few examples, including (but not restricted to) the sexually dimorphic kidney of sticklebacks [20], salamanders [21] and squamates [22] and the larynx in vocal tetrapods [23].

Our study has introduced a remarkable example of sexual dimorphism of internal organs unparalleled in the fish world. Surprisingly, the swimbladder dimorphism in *Psilorhynchus* involves enlargement and elaboration of an organ system that is generally simplified in benthic freshwater teleosts. Given the multiple detailed studies of *Psilorhynchus* anatomy in the 170 years since the genus was described [8,24–26], it is remarkable that such obvious sexual dimorphism has gone unnoticed. Our study thus offers a compelling argument for further detailed anatomical investigation of even relatively well-studied groups of vertebrates.

Acknowledgements. We thank J. Shrestha, N. Sood and A. Ali for facilitating fieldwork and M. Taylor, I. Kaatz and A. Summers for helpful comments and discussions.

Funding statement. Funding was provided by Texas Agrilife Research (TEX09452 to K.W.C.) and the Natural History Museum Collection Enhancement Grant (to R.B.). This is publication number 1472 of the Biodiversity Research and Teaching Collections of Texas A&M University.

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