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Tuberculation of *Macrhybopsis hyostoma* (Teleostei: Cyprinidae)

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The shoal chub *Macrhybopsis hyostoma* exhibits two different types of tubercle, including: (1) an unculiferous plate-like tubercle (present across the surface of the head and much of the body and fins in males and females); and (2) a conical tubercle (restricted to the pectoral fins of mature males). Stalk-like structures located along the anterior edge of the paired and median fins of *M. hyostoma* and referred to previously by some authors as tubercles are shown to be taste buds.

Introduction

Tubercles are multicellular keratinous structures of the epidermis that have been documented to date in members of four different orders of teleost fishes (Wiley & Collette, 1970; Collette, 1977). Tubercles often vary in size, shape and location between taxa. Characteristics of tubercles have long served as useful characters for distinguishing between closely related species (e. g. Hubbs, 1930; Hubbs & Black, 1947; Bailey & Suttkus, 1952; Kottelat, 1995; Ng & Kottelat, 2000; Ng & Ng, 2001; Ng, 2004; Birindelli et al., 2007; Freyhof & Kottelat, 2007; Pethiyagoda et al., 2008; Kottelat, 2008; Kottelat & Hui, 2009; Cashner et al., 2010; Lumbantobing, 2010; Conway et al., 2011) or as derived characters in support of putative monophyletic groups (e. g. Collette, 1965; Mayden, 1989; Coburn & Gaglione, 1992; Chen & Arratia, 1996; Hadiaty & Kottelat, 2009). At least in members of the order Cypriniformes, tubercles are typically more pronounced in males than in females and are commonly considered to play a role in reproduction (Reighard, 1903; Raney, 1947), especially when developed just prior to the spawning period (Ahnelt & Keckeis, 1994) and reduced or lost after the spawning period (Koehn, 1965). Low, oval tubercles forming loosely parallel ridges on the surface of the head and body in many benthic species of cypriniform living in high-flow environments have alternatively been hypothesized to serve a hydro-dynamic function (Hora, 1930; Wiley & Collette, 1970; Roberts, 1982).

The shoal chub *Macrhybopsis hyostoma* (Fig. 1a) is a benthic, pelagic broadcast spawning cyprinid (Platania & Altenbach, 1998) that inhabits larger creeks and rivers across the Great Plains region of the United States, from the Ohio River drainage in West Virginia to the Colorado and Brazos river drainages in Texas (Gilbert et al., 2017). *Macrhybopsis hyostoma* is typically found over sand or gravel substrates in areas with higher flow

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Fig. 1. *Macrhybopsis hyostoma*. **a,** TCWC 19738.01, female, 36.1 mm SL, Brazos River, Texas; **b, c, d**, scanning electron micrographs of lateral, dorsal, and ventral surface of head of male (TCWC 19732.01, 31.9 mm SL); **e,** close-up of box in c showing field of Type 1 tubercles; **f,** close-up of box in e showing single Type 1 tubercle; b–d share a scale bar.

(Cross, 1967; Becker, 1983; Eisenhour, 2004). Brief descriptions of the tuberculation of *M. hyostoma* have been provided by several authors working to document the diversity of freshwater fishes in different regions of the United States but the information in these accounts is largely conflicting. For example, Robison & Buchanan (1988: 182) and Pflieger (1997: 134) reported tubercles

on the head, breast and pectoral fins of "breeding males" of *M. hyostoma* whereas Boschung & Mayden (2004: 206) reported "nuptial" tubercles only on the pectoral fins. Eisenhour (2004: 13, 33) reported tubercles on the pectoral fins in "large, nuptial males" and "tiny tubercles rarely present on rays of dorsal and pelvic fins in large (>50 mm SL) nuptial males in peak condition." Etnier & Starnes (1993: 192–193) and Perkin (2014: 181) reported "nuptial" tubercles on the surface of pectoral-fin rays 2–10 and Etnier & Starnes (1993: 192–193) additionally reported smaller tubercles "near the tips of dorsal, anal, and pelvic fin-rays" but not elsewhere. Trautman (1981: 310) reported many "minute" tubercles on the head and breast of males and sometimes "large breeding females" and Becker (1983: 495) reported "fine" tubercles in both sexes across much of the body including tubercles on the breast "as thick as sandpaper [sic]".

This conflicting information encouraged us to investigate the tuberculation of *Macrhybopsis hyostoma* in more detail and during our investigation we discovered that two distinct types of tubercle are present in this species, including a type present in both sexes and a type present only in males. The purpose of this study is to describe these two different types of tubercle and attempt to clarify the confusion surrounding tuberculation of this common species of North American cyprinid.

Materials and methods

Specimens used in this study are housed in the Collection of Fishes at the Biodiversity Research and Teaching Collections (TCWC) at Texas A&M University, College Station, and the Biodiversity Collections (TNHC) at the University of Texas, Austin. Ethanol preserved specimens (n = 342, 18.2–54.4 mm SL) were examined using a ZEISS SteREO Discovery V20 stereomicroscope. Sex was verified for select individuals via examination of dissected gonads under the aforementioned microscope. The head, pectoral fin, and skin from the right side of the body anterior to the dorsal fin were dissected from three specimens, including two males (31.9, 40.0 mm standard length [SL]), and one female (35.9 mm SL). The head only was dissected from one juvenile specimen (18.2 mm SL). Dissected parts were chemically dried following the protocol of Ellis & Pendleton (2007), mounted on aluminum stubs and coated with gold or gold palladium using a Ted Pella Cressington 108 auto sputter coater. Coated specimens were examined using a TESCAN Vega 3 environmental Scanning Electron Microscope (SEM). The

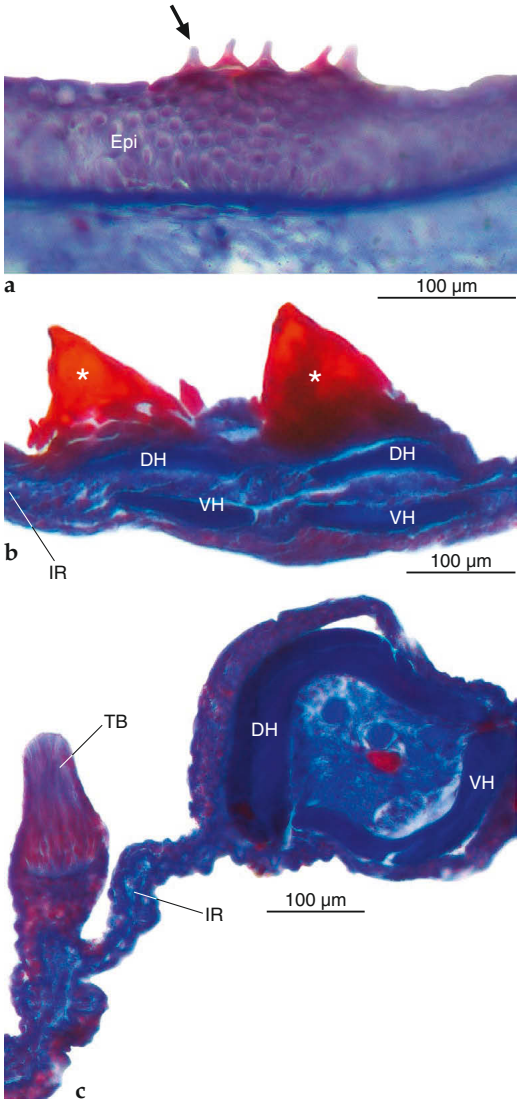


Fig. 2. Transverse sections through tubercles on the head and pectoral fin of *Macrhybopsis hyostoma*. **a,** Type 1 tubercle on dorsal surface of head of female (TCWC 19736.01, 34.0 mm SL), black arrow indicates a single unculus; **b,** Type 2 tubercles (*) on pectoral fin of male (TCWC 19733.01, 40.0 mm SL); **c,** taste bud on interradial membrane between first and second pectoral-fin ray of male (same specimen as in b). DH, dorsal hemitrichium; Epi, epidermis; IR, interradial membrane; TB, taste bud; VH, ventral hemitrichium.

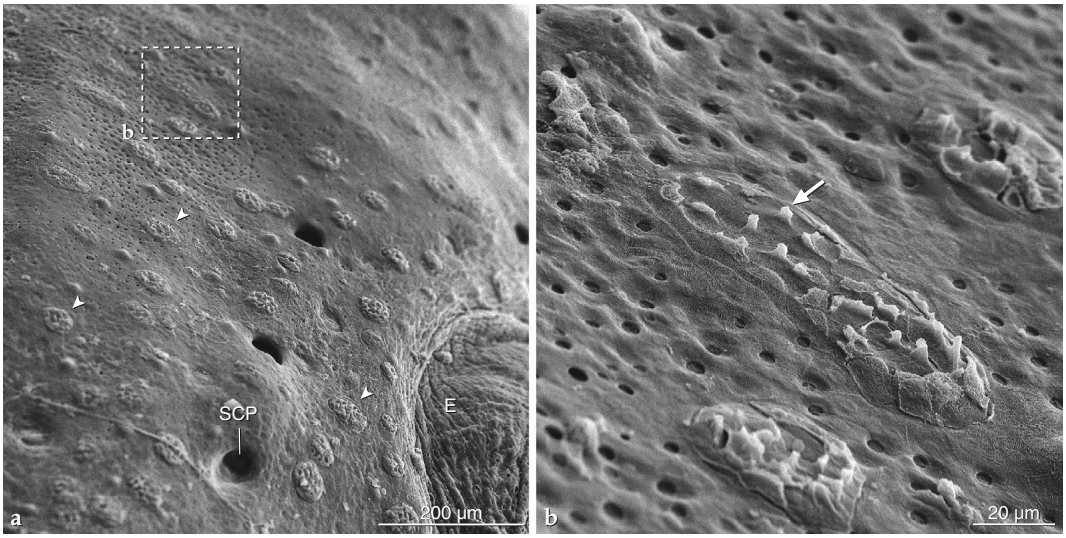


Fig. 3. Scanning electron micrographs of the dorsal surface of the head (oblique anterodorsal view) of *Macrhybopsis hyostoma,* TCWC 19342.06, 18.2 mm SL. **a,** field of type 1 tubercles dorsal to the eye, arrow heads indicate select Type 1 tubercles; **b,** close-up of box in a showing single Type 1 tubercle. Arrow indicates single unculus. E, eye; SCP, supraorbital canal pore.

dissected head of a female (34.0 mm SL) and the dissected pectoral fin of a male (40.0 mm SL) were rinsed for 1 hour in DI water, decalcified in RDO-GOLD (Apex Engineering Products Corp., Aurora, IL) for 3 hours, rinsed for 1 hour in DI water, and dehydrated through a graded series of ethanol (30 %, 50 %, 70 % and 95 %, for 1 hour each, then 100 %, two cycles for 30 minutes each). Specimens were then cleared in Toluene for two cycles of 20 minutes each and subsequently embedded in paraffin blocks for sectioning. The head and pectoral fin were sectioned in sagittal and transverse planes, respectively. Sections were cut at 9 µm thickness and fixed to slides using albumen, stained using the Ayoub-Shklar protocol for differentiating keratin (Ramulu et al., 2013) and examined using a Zeiss Primo Star compound microscope. Selected slides were photographed using a ZEISS axiocam MRc5 digital camera (Carl Zeiss, Jena, Germany) attached to the aforementioned scope. All digital images were processed using Adobe Photoshop CC and Adobe Illustrator CC.

Results

Tubercles on the surface of the head in both male and female individuals comprise low, roughly ovoid aggregations of ca. 25–50 unculiferous cells (sensu Roberts, 1982; Fig. 1b–f), each with an unculus ca. 5 µm tall (Fig. 2a). These tubercles are referred to herein as Type 1. There is no obvious sexual dimorphism in the size (ca. 150–200 µm diameter) or number of Type 1 tubercles (ranging from 59–70 per mm^2 on the dorsum of the head just posterior to the eye in three specimens examined using SEM). In both sexes, Type 1 tubercles are distributed over the entire dorsal surface of the head (Fig. 1b–d) from the occiput to the snout, including rostral cap, and even the skin covering the upper portion of the eye. On the lateral surface of the head, Type 1 tubercles are present over the entire surface, from the distal edge of the operculum to the anteriormost edge of the snout (Fig. 1b). On the ventral surface of the head, tubercles are absent other than a few sparsely distributed tubercles that encroach from the lateral surface (Fig. 1d). Type 1 tubercles are absent from the surface of the barbels and appear less dense on the skin adjacent to cephalic sensory pores. In juvenile specimens at least as small as 18.2 mm SL (e. g. TCWC 19342.06), Type

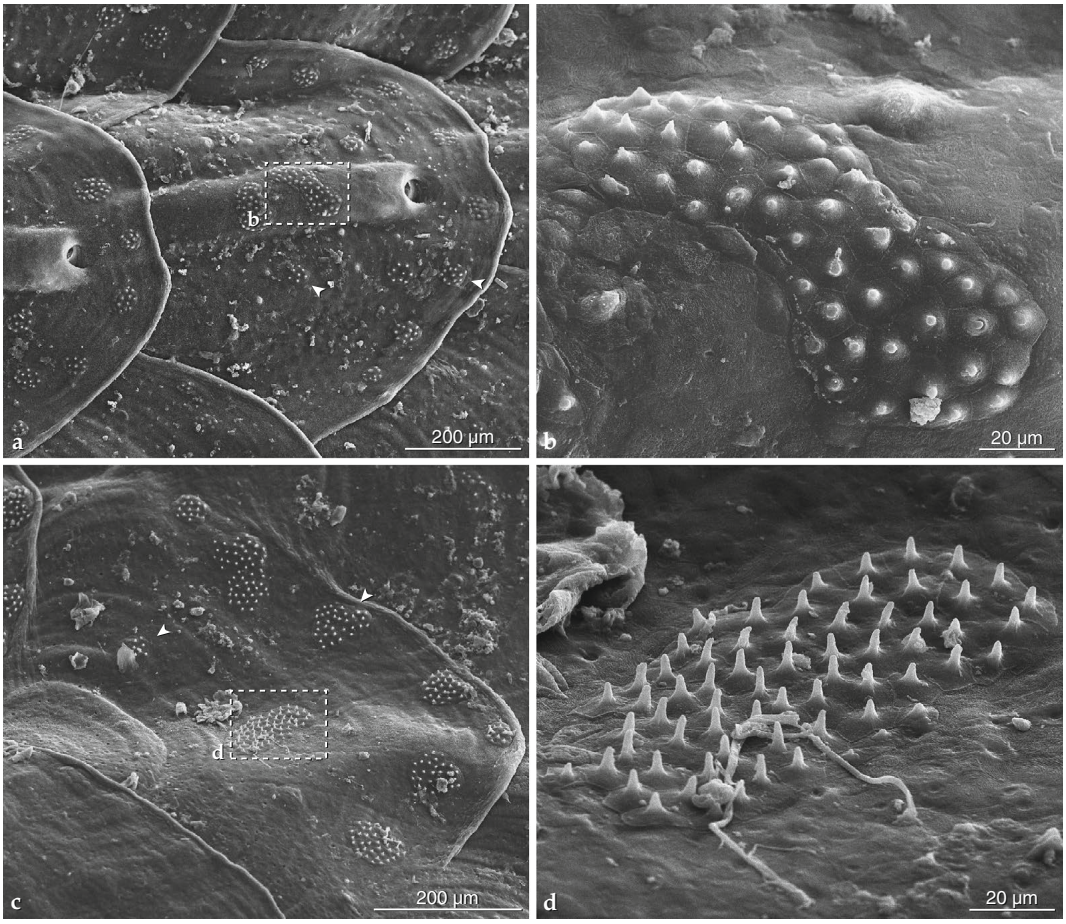


Fig. 4. Scanning electron micrographs of the scales of *Macrhybopsis hyostoma*. **a,** lateral line-bearing scale from anterior portion of left side of body of male (TCWC 19736.01, 40.0 mm SL); **b,** close-up of box in a; **c,** scale from anterior portion of left side of body of female (TCWC 19736.01, 34.0 mm SL); **d,** close-up of box in c. Type 1 tubercles indicated by arrowheads in a and c.

1 tubercles of the same form and a similar relative size as those of adults are scattered over the dorsal surface of the head between the orbits (Fig. 3a–b). In a juvenile specimen of 21.0 mm SL (e. g. TCWC 19342.0, not figured), tuberculation on the surface of the head is essentially that of the adult except that Type 1 tubercles are more numerous on the dorsal surface than on the lateral surface of the head. This could imply that Type 1 tubercles develop on the dorsal surface of the head earlier than on the lateral surface.

Much of the body and fins (excluding the caudal fin) of males and females are covered in Type 1 tubercles, though placement is highly variable between individuals. Larger individuals generally exhibit a greater abundance of Type 1 tubercles on the body and fins. In the majority of individuals, Type 1 tubercles are more numerous on scales located on the anterior half of the body (anterior to the dorsal fin) and are less numerous (or absent) on the posterior half. In a small number of individuals, Type 1 tubercles are present on caudal peduncle scales, including those located in the posteriormost circumpeduncular scale row at the base of the caudal fin. Scales located along the dorsal-midline and lateral side of the body anterior to the dorsal-fin exhibit an arc of 5–10 Type 1 tubercles along the posterior edge (Fig. 4a,c). The tubercles contributing to each arc are evenly spaced and this contrasts sharply

with the few irregularly placed Type 1 tubercles that are typically also present over the anterior part of each scale, including in some cases the surface of the lateral line canal on lateral line bearing scales (Fig. 3a–b). Type 1 tubercles are sparse and irregularly distributed on the fins and generally found only along the surface of fin rays (Fig. 4f,h,i) and absent from the interradial membranes. The chest is devoid of scales in the material examined and tubercles are absent from this region (though see Becker [1983], Robison & Buchanan [1988] and Pflieger [1997] for descriptions of scales and tubercles in this region).

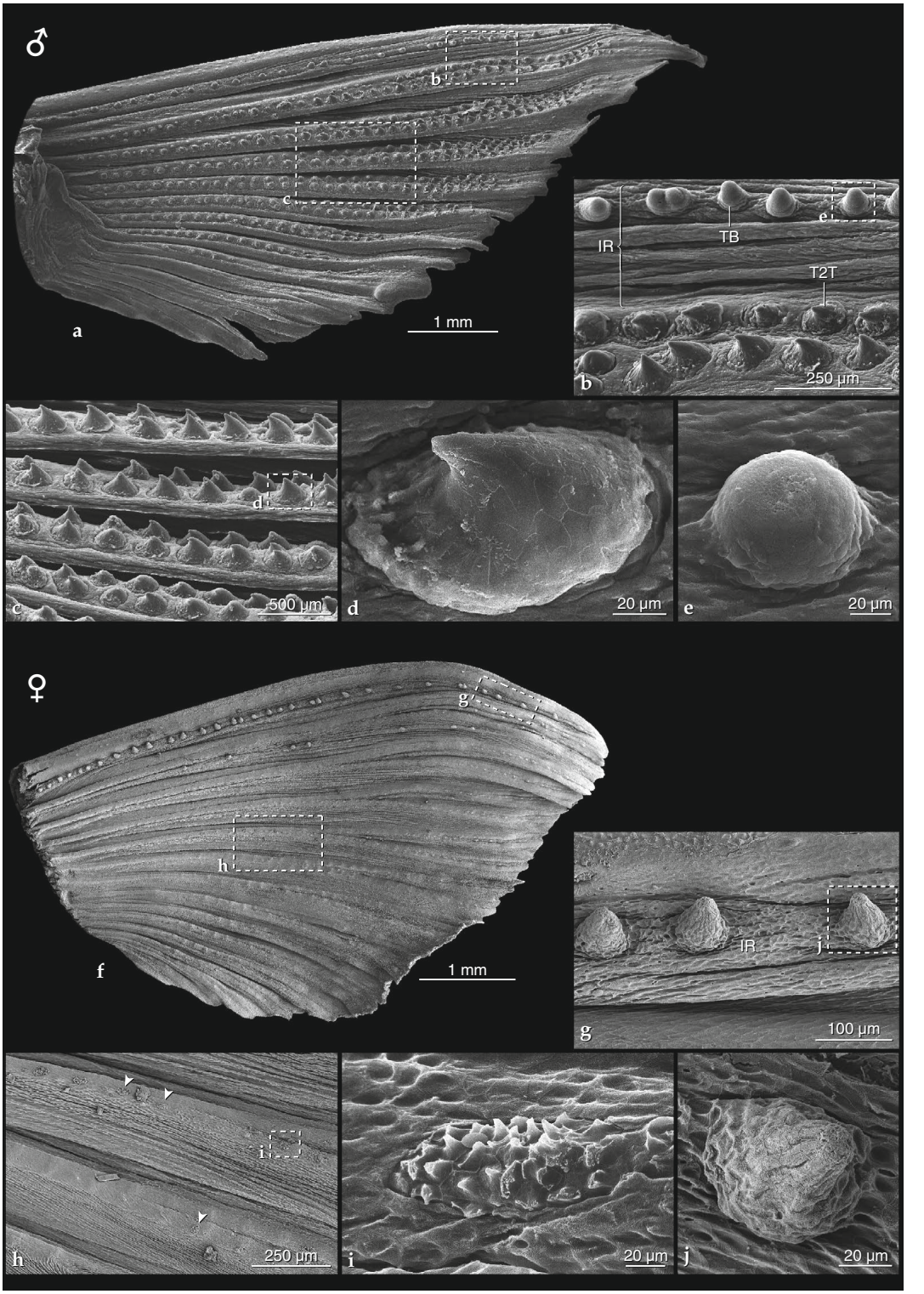
A second type of tubercle (referred to here as Type 2) is present only on the pectoral-fin rays of larger males (31–56 mm SL). Type 2 tubercles are conical in shape (Fig. 5a–d) and at ca. 100 µm in height are much taller than Type 1 tubercles. The tip of each Type 2 tubercle is slightly recurved (Fig. 5c–d) with the tip generally oriented toward the body axis. Type 2 tubercles are arranged in distinct rows along branched pectoral-fin rays 2–8/9 that mirror the pattern of fin-ray segmentation (i.e. a single tubercle is associated with each segment in the dorsal hemitrichium of each ray) and branching (i.e. the row of tubercles associated with each ray bifurcates at the point at which the ray branches). Rows of Type 2 tubercles appear to develop in a proximo-distal direction with smaller tubercles at an earlier stage of development located distally. Type 2 tubercles that are mound-like (rather than conical) and without a distinctly pointed tip are interpreted to represent tubercles that are not fully developed (e.g. see Fig. 6a–b). Fully developed Type 2 tubercles have a distinctly conical shape and pointed tip with cellular edges that are smooth and lack projections (Fig. 5d). Type 2 tubercles interpreted to be at an earlier stage of development at the time of preservation are present on a male of 31.9 mm SL (TCWC 19732.01) collected in March and were not observed in smaller individuals.

In both sexes, a row of highly modified, large (ca. 100 µm tall) stalk-like taste buds is present in the first (and occasionally second) interradial membrane of all paired and median fins (except the caudal fin) (Fig. 5a,b,f,g). Taste buds are present on both surfaces of the pectoral, dorsal, and anal fins. They are rarely present on the ventral surface of the pelvic fin (see below). In the dorsal and anal fins, the taste buds are roughly similar in number on each surface (left and right) with the dorsal fin bearing ca. 15 taste buds and the anal fin ca. 10. In the pectoral fin, taste buds are more numerous on the dorsal surface than the ventral surface (i.e. ca. 30–40 dorsally vs. ca. 15–20 ventrally). In the pelvic fin, there are generally 5–10 taste buds on the dorsal surface with the ventral surface of the pelvic fin most commonly without taste buds (taste buds on the ventral surface of the pelvic fin were observed only in a single specimen: TCWC 19733.01, female, 34 mm SL). The second interradial membrane of each fin occasionally bears taste buds, and in such cases they are fewer in number than on the first interradial membrane (e.g. TCWC 19737.01: one female of 41 mm SL exhibited 41 taste buds on the first interradial membrane and 8 on the second). In females and immature males, taste buds located on the fins are relatively slender with a greater length to diameter ratio (Fig. 5g,j) whereas in mature males they are rounded with an almost equal length to diameter ratio (Fig. 5b,e).

Discussion

We have documented two distinct types of tubercle in *Macrhybopsis hyostoma*. The first type, here referred to as Type 1, is present in both males and females. The report of "fine" or "minute" tubercles in members of both sexes of *M. hyostoma* by Becker (1983) and Trautman (1981), and "smaller tubercles" by Etnier & Starnes (1993), likely refers to the Type 1 tubercle described herein. Tubercles of this type were illustrated and described as "organs of unknown function" by Moore (1950) in *Hybopsis storerianus* (= *Macrhybopsis storeriana)* and *Extrarius aestivalis tetranemus* (= *Macrhybopsis tetranema*) suggesting that this tubercle type may be widespread across *Macrhybopsis*. The second type of tubercle, here referred to as Type 2, is

Fig. 5. Scanning electron micrographs of the pectoral fins of *Macrhybopsis hyostoma*. **a,** pectoral fin of male (TCWC 19736.01, 40.0 mm SL); **b,** close-up of box b in a; **c,** close-up of box c in a showing Type 2 tubercles along fin rays 2–8; **d,** close-up of box in c showing single Type 2 tubercle; **e,** close up of box in b showing single taste bud; **f,** pectoral fin of female (TCWC 19733.01, 35.9 mm SL); **g,** close-up of box g in f showing row of taste buds; **h,** close up of box h in f showing Type 1 tubercles, indicated by arrowheads, on fin rays 5 and 6; **i,** close up of box in h showing single Type 1 tubercle; **j,** close up of box in g showing single taste bud. IR, interradial membrane; T2T, Type 2 tubercle; TB, taste bud. ▷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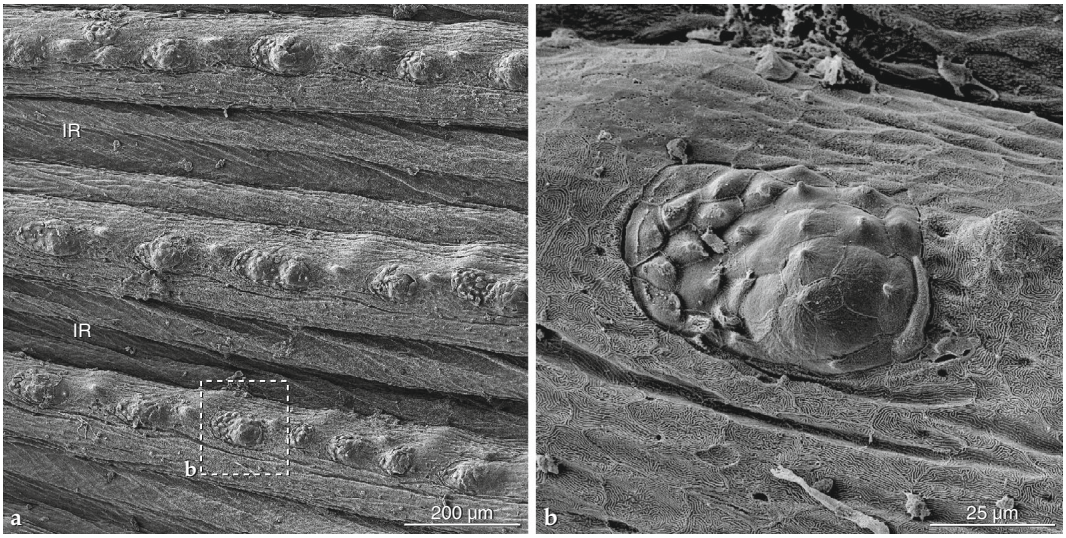

Fig. 6. Scanning electron micrographs of the pectoral fin of *Macrhybopsis hyostoma*, male (TCWC 19732.01, 31.9 mm SL. **a,** developing Type 2 tubercles on fin rays 3–5; **b,** close-up of box in a, showing single developing Type 2 tubercle. IR, interradial membrane.

present only on the pectoral fins of mature males and resembles the typical "nuptial" tubercle commonly reported for other cyprinids and for *M. hyostoma* by several authors (e.g., Robison & Buchanan, 1988; Etnier & Starnes, 1993; Boschung & Mayden, 2004; Eisenhour, 2004; Miller & Robison, 2004; Perkin, 2014; Gilbert et al. 2017). Though not as numerous as Type 1 tubercles, Type 2 tubercles are more conspicuous to the naked eye, which may explain why this type of tubercle is referenced more frequently in the literature on *M. hyostoma* than Type 1 tubercles.

Large and highly modified stalk-like taste buds arranged in a single row along the anteriormost interradial membrane of the paired fins and of the dorsal and anal fins were first reported from members of *Macrhybopsis* by Moore (1950). In addition to *Macrhybopsis*, Moore (1950) recorded these structures in members of *Erimystax*, *Hybopsis* and *Platygobio*, as did Dimmick (1988) who presented the first scanning electron micrograph of a single stalk-like taste bud on the surface of a pectoral fin of *P. gracilis* (Dimmick, 1988: 77, Fig. 4). Several authors (e.g. Trautman, 1981; Eisenhour, 2004; Gilbert et al., 2017) appear to have mistaken the large taste buds on the fins of *M. hyostoma* as "nuptial" or "breeding" tubercles. This is especially likely when authors have reported the presence of tubercles on dorsal or anal fins (e.g., Eisenhour, 2004; Gilbert et al., 2017), areas on which we have observed only a few scattered Type 1 tubercles. This confusion likely stems from the similarity in overall size between the stalk-like taste buds and Type 2 tubercles (both of which tower ca. 100 µm above the surface of the epidermis) and the arrangement of both in distinct rows (Fig. 5b). The presence of tubercles on the fins and bodies of North American cyprinids is commonly interpreted as an externally visible sign of maturity (e.g. Heins & Clemmer, 1976; Jenkins & Burkhead, 1984; Volkoff et al., 1999) and the misidentification of taste buds as tubercles on the fins of *M. hyostoma* and other members of *Macrhybopsis* could result in errors regarding reproductive state (i.e., immature individuals being classified incorrectly as mature) or even sex (i.e. females classified incorrectly as males). Those who use aspects of tuberculation to sex or estimate maturity of *M. hyostoma* based on external characters should note that the large taste buds located on the fins of *M. hyostoma* can be easily distinguished from Type 2 tubercles by shape (tip rounded in taste bud vs. pointed in Type 2 tubercle) and location (taste buds are located on the interradial membranes between fin rays vs. tubercles are located on the skin directly dorsal to the pectoral-fin rays only).

The Type 1 tubercles that we have reported for *Macrhybopsis hyostoma* are similar in general appearance to the unculiferous tubercles reported from the side of the body of the bunocephalid catfish *Bunocephalus verrucosus* by Roberts (1982: 72, Fig. 22f) or the tubercles reported from the surface of the body of the balitorid loach *Balitoropsis bartschi* and mochokid catfish *Chiloglanis brevibarbis* by Wiley & Collette (1970: 185, Fig. 9d; 191, Fig. 10d). The unculiferous Type 1 tubercle that we have described herein differs from the Type "H" unculiferous tubercle reported from the chest area of male members of the Eurasian minnow genus *Phoxinus* by Chen & Arratia (1996). This latter type of tubercle is conical in shape and though unculi are present across the surface, the overall structure of the Type "H" tubercle of Chen & Arratia (1996) is more similar to the Type 2 tubercle of *M. hyostoma*.

The exclusivity of Type 2 tubercles to mature males of *Macrhybopsis hyostoma* indicates a potential association between Type 2 tubercles and reproduction, as has been hypothesized for conical tubercles in other cyprinids (e. g. Koehn, 1965; Wiley & Collette, 1970; Kortet et al., 2004). In contrast, Type 1 tubercles are ubiquitous on the surface of the head and body of male and female individuals ≥18.2 mm SL. As such, we do not consider Type 1 tubercles to be associated with reproduction. Establishing whether a correlation exists (or not) between different aspects of tuberculation (e. g. presence of Type 1 tubercles or density of Type 2 tubercles) and a metric of reproductive condition (i. e. gonadosomatic index) could provide evidence to support (or refute) our hypotheses about the reproductive or non-reproductive function of the different types of tubercle present in *M. hyostoma* (e. g. Schönhofen Longoni et al., 2018). Wiley & Collette (1970) hypothesized that the unculiferous tubercles of the rheophilic *Balitoropsis bartschi* and *Chiloglanis brevibarbis* could have a hydrodynamic function and Roberts (1982) offered a similar hypothesis for the unculiferous plaques on the skin surface of *Bagarius*. It is possible that the Type 1 tubercle of *M. hyostoma* may have a similar hydrodynamic function.

Material examined. *Macrhybopsis hyostoma*. Texas, USA: Brazos River Drainage. TCWC 16916.05, 13, 36–54 mm SL; TCWC 15435.09, 20, 26–44 mm SL; TCWC 16942.02, 29, 36–48 mm SL; TCWC 16909.13, 63, 29–50 mm SL; TCWC 17154.03, 7, 19–25 mm SL; TCWC 19342.06, 4, 18–26 mm SL; TCWC 16910.11, 21, 32–54 mm SL; TCWC 19728.01, 20, 19–33 mm SL; TCWC 19729.01, 14, 24–34 mm SL; TCWC 19730.01, 6, 26–31 mm SL; TCWC 19731.01, 8, 31–42 mm SL; TCWC 19732.01, 27, 22–32 mm SL; TCWC 19733.01, 24, 24–37 mm SL; TCWC 19734.01, 3, 30–33 mm SL; TCWC 19735.01, 1, 32 mm SL; TCWC 19736.01, 9, 39–41 mm SL; and TCWC 19737.01, 34, 19–38 mm SL; Washington Co., Brazos River west of Navasota at highway 105. – TCWC 19682.11, 2, 28–32 mm SL; Brazos Co., Little Brazos River at highway 21. – TNHC 51984, 11, 25–50 mm SL; Falls Co., Brazos River downstream of highway 712. – TNHC 52970, 15, 45–50 mm SL; Falls Co., Brazos River at falls. Red River Drainage. TCWC 3910.07, 9, 43–48 mm SL; Wichita Co., Red River at highway 240. – TCWC 19688.06, 1, 36 mm SL; Wichita Co., Red River near Burkburnett. Neches River Drainage. TCWC 16206.01, 1, 33 mm SL; Jefferson Co., Neches River, beach on west shore, 2.4 river km north of saltwater barrier.

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