

Study of the sexual dimorphism in the skeleton of guppy, *Poecilia reticulata* (Poeciliidae)

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Abstract. In this study, the sexual dimorphism in the skeleton of guppy, *Poecilia reticulata* was studied by comparing the skeletal structure of female and male individuals. For this purpose, ten specimens, 5 males and 5 females, were cleared and stained with alizarin red S and Alcian blue. Based on the results, the males of *P. reticulata* was osteologically different from the females due to having gonopodial suspensorium and modified 14th and 15th vertebra, owning a wide pelvic fin with postero-lateral pointed process and rounded anterior part, having a slightly narrow nasal bone, possessing a well-developed intro-ventral process of lateral ethmoid and, lacking a blade-shaped process at the antero-lateral margin of 5th ceratobranchial and an epiotic process that could be a different body shape in this sexes compared to that of the female.

Key Words: livebearer, skeletal structure, morphology, aquarium fish.

Introduction. The family Poeciliidae contains about 304 species in 37 genera and is widely distributed in the southeastern United States to north of Rio de la Plata, Argentina, and Africa (Nelson 2006). Poeciliids inhabit in fresh and brackish waters (Nelson 2006), and are characterized by viviparity and internal fertilization (Rosen 1964). In this family, the genus *Poecilia* with 40 recognized species (Nelson 2006) is native to fresh, brackish and salt water in the Americas.

The guppy, *Poecilia reticulata* Peters, 1859 is a popular aquarium fish and a highly adaptable fish and thrives in many different environmental and ecological conditions (Magurran 2005). *P. reticulata* occurs naturally in Antigua and Barbuda, Barbados, Brazil, Guyana, Jamaica, the Netherlands Antilles, Trinidad and Tobago, the U.S. Virgin Islands, and Venezuela (Rosen & Bailey 1963), but it has been introduced to many different countries on every continent except Antarctica (Froese & Pauly 2014) and even it has been observed from natural water bodies of Iran (Mousavi-Sabet & Eagderi 2014).

Guppy exhibits sexual dimorphism, a phenomena that the two sexes of the same species exhibit different characteristics beyond the differences in their sexual organs, in the body size and shape, and color patterns e.g. males have colorful spots and ornamentations while females are generally grey in color, and the males are typically 1.5-3.5 cm in total length, while females are 3-6 cm. Since, the differences in morphological characters are reflected in different muscle and skeletal structures (Ayala et al 2001). Hence, *P. reticulata* would be an interesting case study to understand its skeletal structure underlying the external morphology in both sexes i.e. the role of the skeleton in producing sexual dimorphism in the body shape. Therefore, the present study was conducted to study the nature of the sexual dimorphism in the skeleton of *P. reticulata* by providing a detailed osteological feature of this species.

Material and Method. Ten specimens, 5 males and 5 females of *P. reticulata* were anaesthetized using 1% clove solution and then fixed in 5% buffered formalin and stored in 70% ethanol after 24 hours for further examinations. For osteological examination, the specimens were cleared and stained with alizarin red S and alcian blue according to the protocol of Taylor & Van Dyke (1985). The stained specimens were studied using a

stereomicroscope (Leica MC5), and different skeletal elements were dissected and scanned by a scanner equipped with a glycerol bath (Epson V600). The obtained images were drawn using CorelDrawX6 software. Institutional abbreviations follow Rosen & Mendelson (1960), Leviton et al (1985) and Rojo (1991).

Results and Discussion. The neurocranium is short in the dorsal and ventral views, and shallow in lateral view (Figure 1a, b). The ethmoid region possesses four elements, including the pre-vomer, supraethmoid, lateral ethmoid and nasal. Pre-vomer is almost T-shaped and its posterior margin overlaps with the anterior portion of the parasphenoid (Figure 1b). The supra-ethmoid (equivalent to dermo-ethmoid in some references) is round and thin situated between two nasals. Two semi-circular nasal bones are located at the antero-lateral side of the frontal above the lateral ethmoids. This bone is slightly narrower and more fragile in males. In addition, the nasal bone is well-developed and crescent in shape in females. The lateral ethmoid is a perpendicular bony element with two intero-dorsal and intero-ventral processes; the latter one extended to the lateral side of the parasphenoid. This intro-ventral process is well-developed extending to the dorsal side of the pre-vomer in the males, whereas it is not well-developed and extended to the lateral side of the parasphenoid in the females. The lateral ethmoid also bears an internal depression between its two processes. The ventral edge of this bone is concave (Figure 1b).

The orbital region is composed of the frontal, parietal, parasphenoid, pterosphenoid and circumorbital series. The frontal is a large bone with three parts viz. supraorbital, interorbital, and sphenoidal. The dorsal margin of the frontal is rounded (Figure 1a). The parasphenoid has two middle wings bended dorsally; it has also a long ventral process extended from the end of the pre-vomer to its middle portion. The posterior half of the parasphenoid overlaps with the prootic and basioccipital. The pterosphenoid is enclosed by the frontal, sphenotic, ptootic and parasphenoid (Figure 1b). The lachrymal is the only bone of the circumorbital series; it is a dermal bone situating at the anterior side of the orbit connecting to the ventral edge of the nasal. The orbitosphenoid is absent (Figure 1b). There are two sclerotic bones and two sclerotic cartilages for supporting the eyes.

The otic region is formed by the sphenotic, dermo-sphenotic, epiotic, prootic and pterotic. Two small oval-shaped dermo-sphenotic are located between the frontal and sphenotic process. The sphenotic has a lateral, large pointed process. At the rear of the sphenotic, there is a semicircular pterotic; its anterior part bears a protuberance and its posterior part becomes shallower. The posterior part of the prootic also has a protuberance; there are some pores on its anterior margin. The epiotic possesses a posterior process extending to the first vertebra (Figure 1a). According to Ghedotti (2000), the epiotic processes could be short, long or absent in adults poeciliid fishes. In *P. reticulata*, those of the females was long extending beyond first vertebra, while it was short and not extending to the first vertebra in the males.

The occipital region has a supraoccipital bone with two long posterior processes extending beyond the first vertebra; it is longer than the epiotic process (Figure 1a). Two exoccipital are connected forming an exoccipital condyle. There is a pore at the ventral part of the exoccipitals. The anterior part of the basioccipital is V-shaped; there are two lateral triangular pointed process at its left and right edges. In addition, two bulge are seen at the lateral margin of the basioccipital (Figure 1b).

In the upper jaw, the premaxillae is a L-shaped bone with two posterior pointed process that the ventral one is longer than its dorsal one. There are two inner and outer rows of teeth on the anterior portion of the premaxillae. The inner dental plate bears very small teeth versus long ones of the outer row. There are many pores in the antero-medial part of the premaxillae. The maxillae has two dorsal processes, the longer one is narrower anteriorly. The medial part and the posterior margin of the maxillae are expanded (Figure 2a, b).

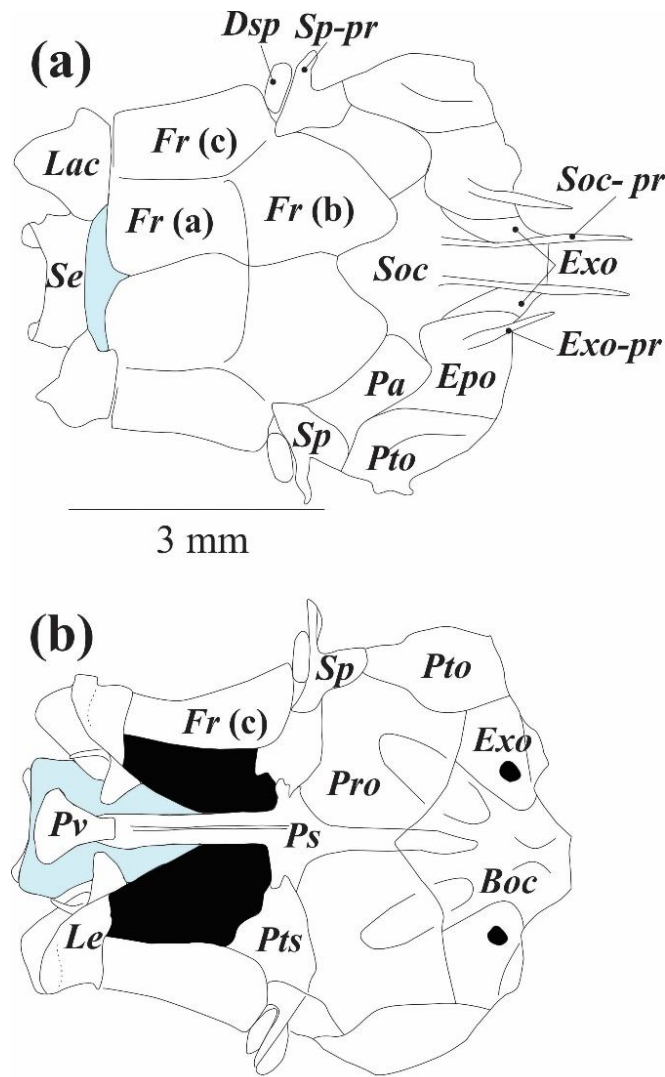


Figure 1. Dorsal (a) and ventral (b) views of the neurocranium in guppy, *Poecilia reticulata*. Abbreviation: Boc: basioccipital; Dsp: dermosphenotic; Epo: epiotic; Exo: exoccipital; Exo-pr: exoccipital process; Fr (a, b and c): frontal (a)- interorbital, (b)-sphenoidal, and (c)- supraorbital parts; Lac: lachrymal; Le: lateral ethmoid; Pa: parietal; Pro: prootic; Ps: parasphenoid; Pto: pterotic; Pts: pterosphenoid; Se: supra-ethmoid-ethmoid; Soc: supraoccipital; Soc- pr: supraoccipital process; Sp: sphenotic; Sp- pr: sphenotic process; Pv: pre-vomer.

In the lower jaw, the dentary bears an outer row of teeth and oval-shaped dental plate with many pores and small teeth. A lateral hollow in the medial side of the dentary is observed for connecting with the anterior process of the anguloarticular. The dentary also has a ventral process and a lateral notch. In the lateral wall of anguloarticular, there are two ventral and posterior hollow. A small retroarticular bone situated at the postero-ventral side of this anguloarticular. An oval-shaped coronomeckelian is observed at the mid-dorsal of the medial face of the anguloarticular. The Meckel's cartilage is long extending from the anterior part of the anguloarticular to the lateral face of the dentary (Figure 2c, d).

In the opercular series, the opercle almost has a triangular shape. The subopercle is crescent-shaped overlapping the ventral edge of the opercle. The interopercle has a knife-like shape widened posteriorly (Figure 3a). The preopercle is L-shaped (Figure 3b) and its vertical part has a dorsal spiral. It bears a depression postero-ventrally and is pointed antero-ventrally.

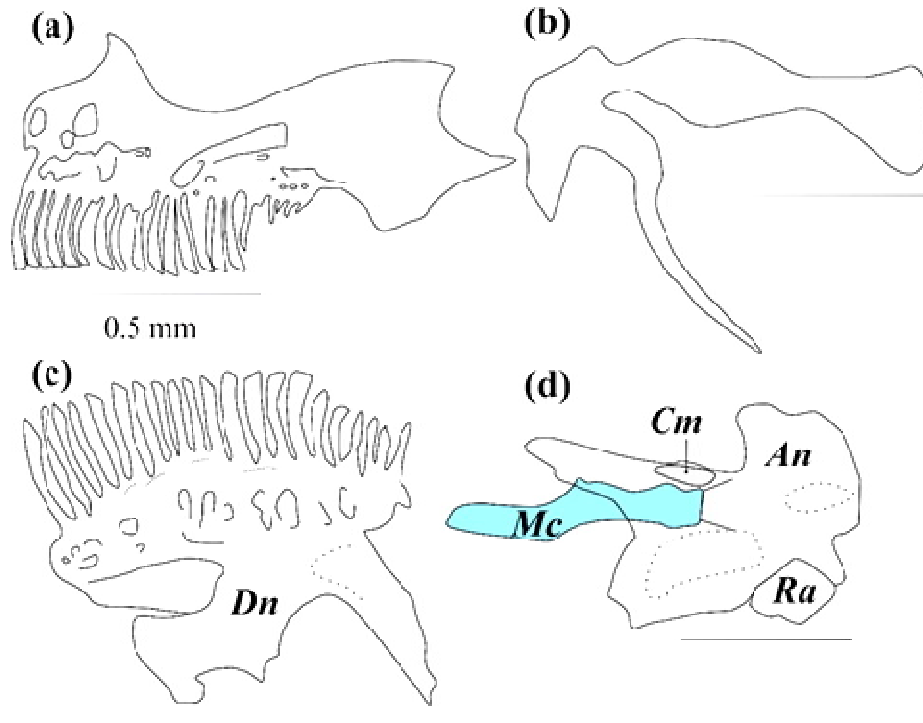


Figure 2. Lateral view of the premaxillae (a), maxillae (b), dentary (c) and other elements of lower jaw (d) in guppy, *Poecilia reticulata*. Abbreviation: An: anguloarticular; Cm: coronomeckelian; Dn: dentary; Mc: Meckel's cartilage; Ra: retroarticulare.

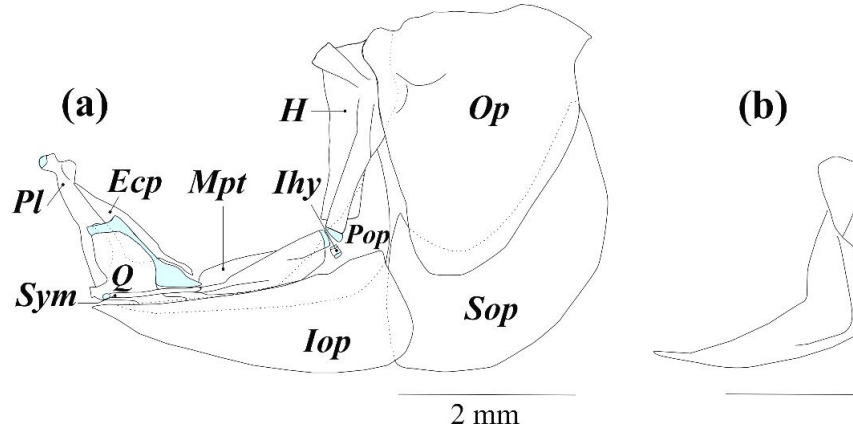


Figure 3. Lateral view of the suspensorium (a) and preoperculum bone (b) in guppy, *Poecilia reticulata*. Abbreviation: Ecp: ectopterygoid; H: hyomandibulare; Ihy: interhyal; Iop: interopercle; Mpt: metapterygoid; Op: opercle; Pl: palatine; Pop: preopercle; Q: quadrate; Sop: subopercle; Sym: symplectic.

In the suspensorium, the hyomandibular bears two antero-dorsal and lateral ridges; the lateral one extended from the middle part of the hyomandibular to its ventral edge. The ventral face of the hyomandibular is connected to the posterior edge of the metapterygoid. The quadrate bears two broadened dorsal and long ventral portions. The quadrate is not ossified posteriorly. The pterygoid arch has four elements, including the metapterygoid, ectopterygoid, palatine and dermo-palatine. The endopterygoid is absent. The metapterygoid has a long ridge and connected to the long and narrow symplectic bone. The ectopterygoid is elongated situating just near the dorso-posterior margin of the quadrate. The ectopterygoid is connected to the palatine antero-dorsally and widened

in its middle part. The palatine possesses a postero-dorsal circle-shaped ridge; its ventral side is fused to the dermo-palatine. The dermo-palatine is blade-shaped overlapping the anterior margin of the quadrate (Figure 3a).

The latero-external part of the ceratohyal is broadened. The anterior process of the fifth ceratobranchial could be narrower or wider than the anterior fourth ceratobranchial in poeciliids (Ghedotti 2000). In the males and females of *P. reticulata*, the anterior process of the fifth ceratobranchial was narrower than that of the fourth one, but there is a blade shape process at the antero-lateral margin of that of the females that it was not observed in the males. The hypohyal possesses two dorsal ossified and ventral cartilaginous parts. The two wide and slender shaped basihyals are reported in poeciliids (Ghedotti 2000), whereas, there is a slender shaped basihyal with an anterior cartilaginous part in both sexes of *P. reticulata*. The urohyal is an extremely elongated and blade shape bone with an antero-ventral process in the females; in the males, it is narrower. In poeciliid family, the number of the branchiostegal elements varies between 4 to 6 (Ghedotti 2000). There are a tiny cartilaginous urohyal extra at the anterior side of the urohual, and five branchiostegal bones in the hyoid arch of studied species. The third and fourth pharyngobranchial teeth plates are fused (Figure 4).

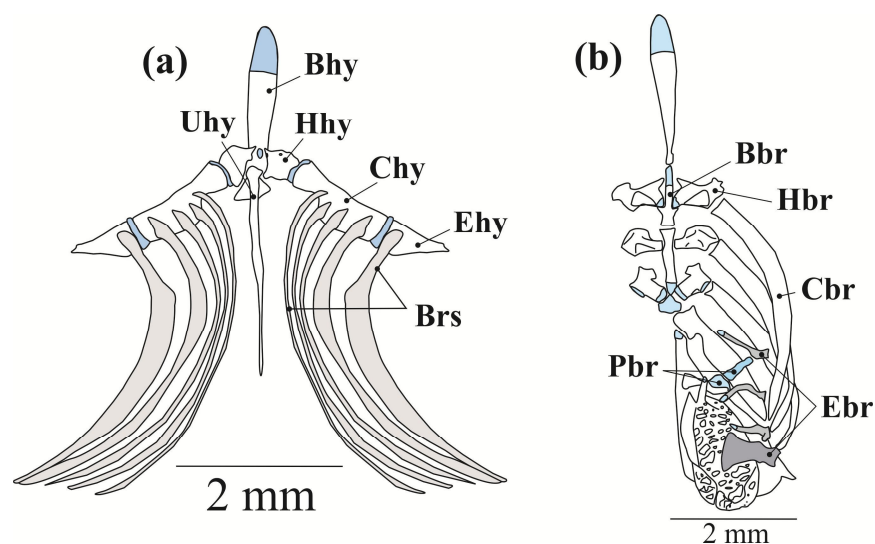


Figure 4. Dorsal view of (a) hyoid arch and (b) branch apparatus in guppy, *Poecilia reticulata*. Abbreviation: Bbr: basibranchial; Cbr: ceratobranchial; Ebr: epibranchial; Hbr: hypobranchial; Pbr: infrapharyngobranchial; Bhy: basihyal; Brs: branchiostegale; Chy: ceratohyal; Hhy: hypohyal; Epi: epihyal; Uhy: urohyal.

In the pectoral girdle, the cleithrum bears a blade shape part perpendicular to its posterior part. The dorsal part of the cleithrum is widened and its antero-dorsal part possesses a fossa for situating supracleithrum. The supracleithrum is small and broadened with a pointed dorsal edge. The dorsal part of the posttemporal is bifurcated and its outer process is longer. The coracoid is wide and its ventral part thin connecting to the ventral margin of the cleithrum. There is a notch at the postero-dorsal part of the coracoid for situating the lowest radial. A middle ridge is positioned on the lateral face of the coracoid. The scapula positions between the coracoid and cleithrum; this bone is wide with an anterior large foramen. There are four radials, two unbranched and 12 branched rays in the pectoral fin. The 1st unbranched ray is smaller than the 2nd one (Figure 5a).

In the pelvic girdle, the pelvic bone is almost triangular with a long ridge extending from its anterior point to its posterior portion in females, whereas it is rounded posteriorly in males (Figure 5b, c). In addition, the lateral margin of the pelvic bone is straight in the females versus concave one of the males. The medial process of the pelvic bone in the females is well-developed. The position of the pelvic girdle in adult males slightly anterior than females similar to *Girardinus metallicus*, *Poecilia sphenops*, *Phallichthys amates*, and *Poeciliopsis lutzi* (Ghedotti 2000). The pelvic bones are not in

contact with pleural ribs in two males and females. Two pelvic bones overlap in their middle process. This bone also has a postero-lateral process (Figure 5b) that it is sharper in males (Figure 5c). The number of the pelvic fin rays are 6, but they are longer in males.

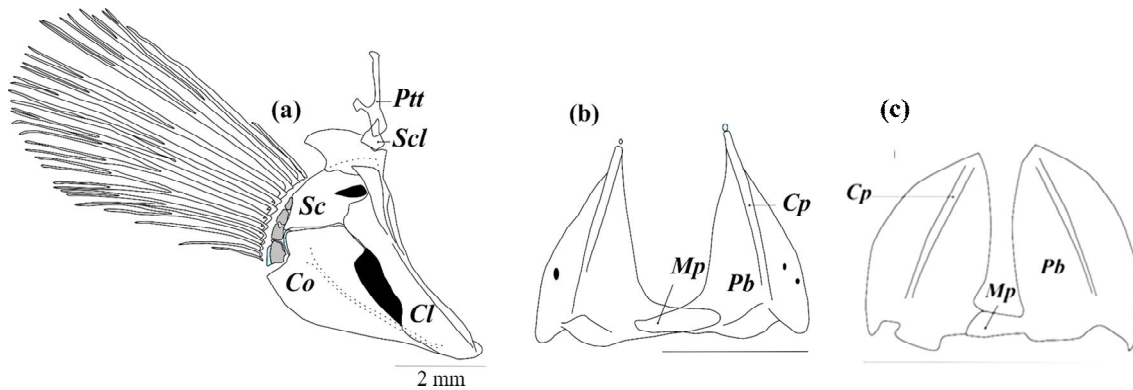


Figure 5. Lateral view of the pectoral girdle (a), and ventral view of the pelvic bone of the female (b) and male (c) in guppy, *Poecilia reticulata*. Abbreviation: Cl: cleithrum; Co: coracoid; Cp: central process; Mp: medial process; Pb: pelvic bone; Ptt: posttemporale; Sc: scapula; Scl: supracleithrum.

The dorsal fin has 1 unbranched and 6 branched rays supported by 8 actinost elements. The anal fin possesses 3 unbranched and 7 branched rays with 10 actinost elements. The position of the dorsal and anal fins origin is at the level of the 12th vertebra (Figure 6a, b).

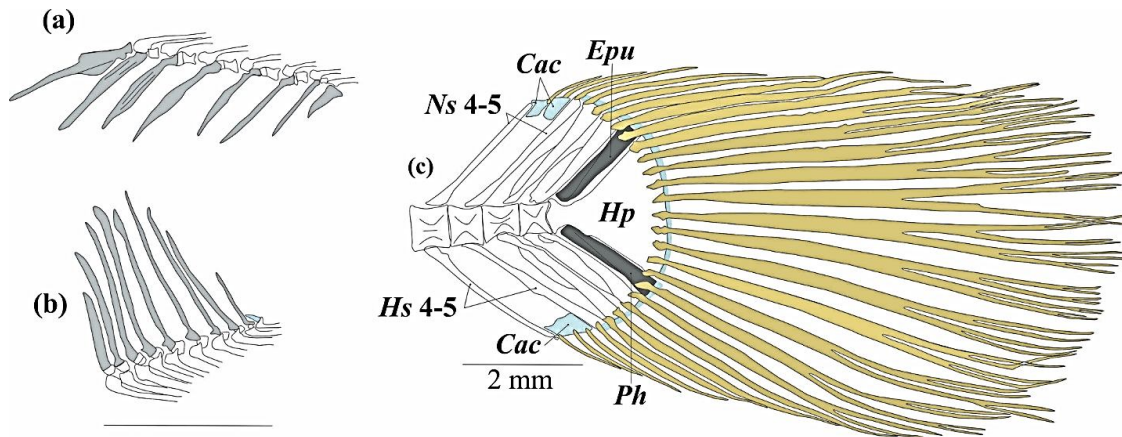


Figure 6. Dorsal (a), anal (b) and caudal (c) fins in Guppy, *Poecilia reticulata*.

Abbreviation: Cac: caudal accessory cartilage; Epu: epural; Hp: hypural; Hs 4-5: hemal spine of 4th and 5th vertebra; Ns 4-5: neural spine of 4th and 5th vertebra; Ph: parhypural.

Some caudal vertebra modified along with the anal fin elements to form gonopodial suspensorium in adult males (Rosen & Bailey 1963). This structure made up of four elements, including gonactinost (vs. actinost in female), gonopodium, basosteas and gonapophyses (Figure 7a and b). Neither of these structures are seen in the females. In the other members of poeciliids such as *Poeciliopsis infans*, the gonapophyses I, II, and III are attached to the caudal vertebrae 15, 16 and 17, respectively, by the hemal arches (Galindo-Villegas & Sosa-Lima 2002), while in *P. reticulata*, there are only two gonapophyses and respectively attached to the 14th and 15th caudal vertebra. The gonapophyses are bended forward. The posterior process of the first gonapophyses is longer than those of latter. The dorsal portion of the gonapophyses, at the junction of the vertebrae becomes very slim. In *P. reticulata*, there are 10 gonactinosts; the first one is

the shortest and supports two first rays of the anal fin; the 2nd to 7th gonactinosts fused together for creating a strong structure for supporting gonopodium that is a modification of the 3rd anal fin radius and fourth and fifth rays (Figure 7a). Unlike females, the hemal spines of 14th and 15th vertebrae modified in adult males as gonapophyses (Figure 8a).

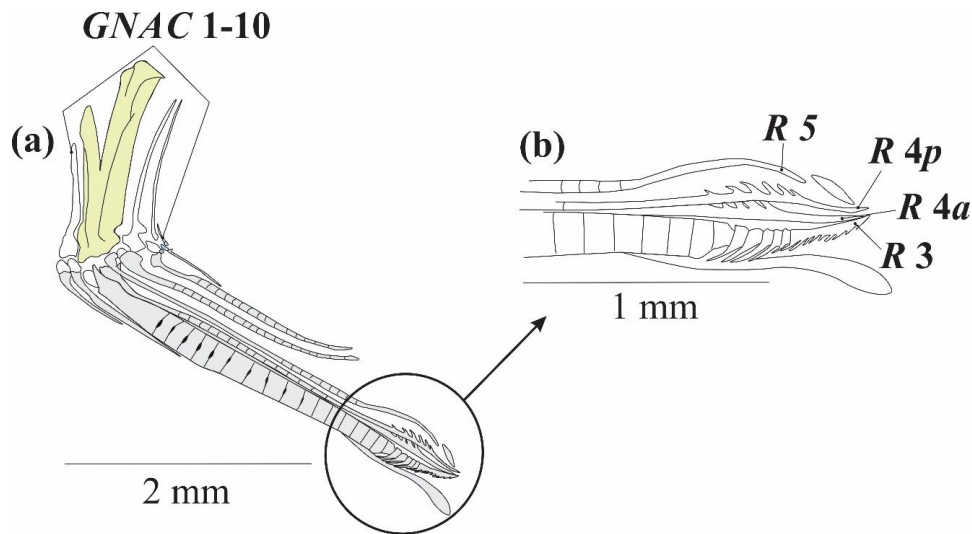


Figure 7. Lateral view of the anal fin skeleton (a) and gonopodium (b) of male in guppy, *Poecilia reticulata*. Abbreviation: GNAC 1-10: gonactinost complex; R: ray.

In the caudal skeleton, two broadened hyporals fused forming a single hypural plate (Figure 5c). Costa (1998) noted that a single hypural plate is present in all cyprinodontoids as *P. reticulata*, except some *Anablepids* (Ghedotti 2000). In some male specimens, we observed a long pore between two hyporals (Figure 8b). Other elements in this structure include one parhypural, one eipural and two cartilaginous caudal accessories between neural spine of 4th and 5th vertebra and one between hemal spine of 4th and 5th vertebra in females, whereas this cartilaginous caudal accessories present between the neural and hemal spines of 2nd, 3rd, 4th and 5th vertebrae in the males (Figure 8 b and c).

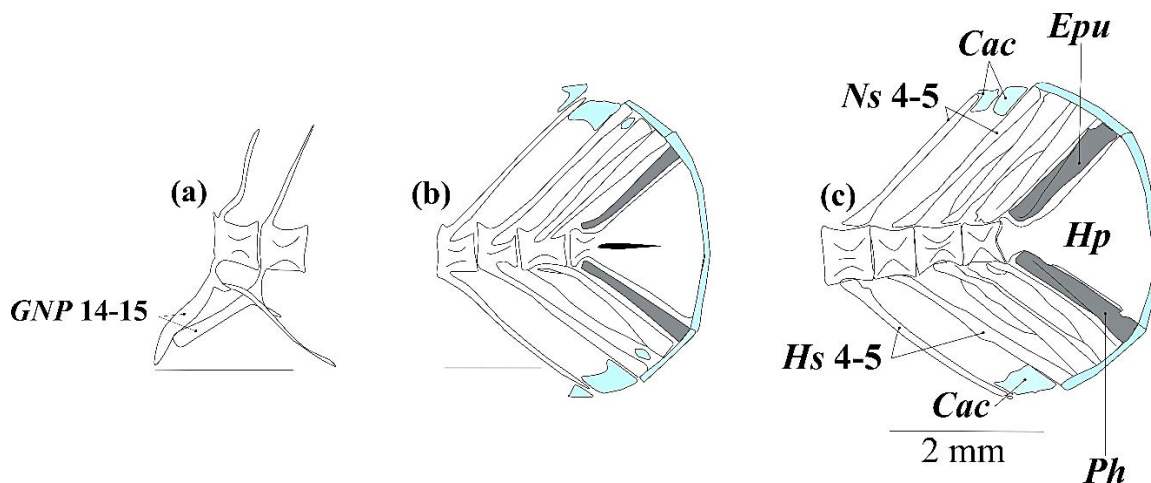


Figure 8. Lateral view of the gonapophyses (a) and caudal structure (b) of male and caudal structure of female (c) in guppy, *Poecilia reticulata*. Abbreviation: Cac: caudal accessory cartilage; Epu: epural; GNP 14-15: gonapophyses 14th and 15th vertebra; Hp: hypural; Hs 4-5: hemal spine of 4th and 5th vertebra; Ns 4-5: neural spine of 4th and 5th vertebra; Ph: parhypural.

In the Axial skeleton, the number of vertebra is 29 and the first vertebra pleural rib separated, during the maturity, named ligastyle. Neural spines of the fourth first vertebra are broadened. In the axial skeletons of the females, the neural spine of four first vertebra was more extensive than those of males that can support a longer body in the female.

Conclusions. Skeletal structure of *P. reticulata* showed numerous differences between males and females. Except the usual differences in the anal fin i.e. gonopodial suspensorium and related elements in the axial skeleton, a significant differences were observed in the caudal fin elements, pelvic girdle, branchial arch and neurocranium structure. Therefore based on the results, the males of *P. reticulata* is osteologically distinguished from females due to having gonopodial suspensorium and modified 14th and 15th vertebra, possessing a wide pelvic fin with postero-lateral pointed process and rounded anterior part, having a slightly narrow nasal bone, owning a well-developed intro-ventral process of the lateral ethmoid, and lacking a blade-shaped process at the antero-lateral margin of the 5th cetatobranchial and an epiotic process.

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