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# Functional Anatomy and Evolution of Male Genitalia in Poeciliid Fishes

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(Plates I-IV; Text-figures 1-47)

## I. INTRODUCTION

WITH the resurgence of interest in problems of systematics and the origin of species, the poeciliid fishes have been re-examined by systematists, geneticists and animal behaviorists. In connection with the work of the last group, the importance of reproductive behavior as an isolating mechanism has been emphasized. This, in turn, has opened up the subject of the mechanics of reproduction in these viviparous fishes, particularly the activities concerned with copulation and insemination. The viviparous mode of reproduction in poeciliid fishes is associated with internal fertilization which is made possible by the modification of the anterior rays of the anal fin of the male into a gonopodium, a complex, bony genitalium. The anatomical structure of this organ and of the others responsible for the various observed behaviors has not as yet been studied completely. The present paper is, in part, an attempt to identify the various mechanical adjustments involved in the male's sexual maneuvers and to correlate them with specific behavioral details.

A comparative survey of the functional anatomy of the gonopodia of all representative poeciliids has also been made in order to bring into focus the underlying mechanical types of gonopodia. By contrasting poeciliid anatomy and behavior with that of members of other cyprinodont families, material has been obtained for a consideration of the probable intermediate forms in the evolution of poeciliid gonopodia.

The fishes of the Family Poeciliidae of the Order Cyprinodontida (= Cyprinodontes, Cyprinodontiformes, or Microcyprini as recommended on page 326 of *Copeia*, 1950, and by Bailey, 1952) are found under natural conditions only in the Americas, ranging from Illinois and New Jersey to Argentina. Poeciliid fishes are generally small, the largest species not exceeding eight inches; the adult males of the smallest species, *Heterandria formosa*, rarely exceed three-quarters of an inch in total length (Pls. I-III, Figs. 1-8). Like other cyprinodonts, poeciliid fishes have the maxillae excluded from the gape of the mouth, and in all but two species the mouth parts are protrusible. In the giant of the family, *Belonesox belizanus*, and in a smaller, related species from Haiti, *Gambusia beebei*, the premaxillae and dentaries are strengthened and enlarged and have long, cardiform teeth. With one exception, *Tomeurus gracilis* from northern South America, poeciliids are viviparous, giving birth to as many as 315 young in a single brood. Some produce their relatively large broods of young at approximately monthly intervals; others have as few as one young at shorter intervals. In the latter or superfoetacious group, successive broods or individual embryos overlap each other in development.

Under the present taxonomic arrangement, poeciliid fishes are divided into six subfamilies, eight tribes and forty-five genera (Table 1). The members of each tribe or subfamily are distinguished to a greater or less degree by differences in their genitalia and by the manner in which they are distributed geographically. The most widely distributed poeciliids belong to the Tribes Gambusiini and Poeciliini, for they range from middle or eastern United States west to New Mexico and south through Mexico and Central America to the north and east coasts of South

TABLE 1. SYNOPTIC LIST OF GENERA IN THE FAMILY POECILIIDAE
WITH AN INVENTORY OF THEIR GONOPODIAL STRUCTURES[1]

| | Ray 3 | | | | Ray 4a | | | Ray 4p | | | | Ray 5a, 5p | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hooks | Spines | Denticulations | Extensions | Hooks | Serrae | Elbows | Retrorse elements | Distal serrae | Proximal serrae | Canoe supports | Retrorse elements | Denticulations | Serrae | Canoe-like form |
| **SUBFAMILY GAMBUSIINAE** | | | | | | | | | | | | | | | |
|   Tribe Gambusiini | | | | | | | | | | | | | | | |
|     *Heterophallus*[2] | · | + | · | · | · | · | + | + | · | + | + | + | · | * | + |
|     *Gambusia*[2,3] | · | + | * | · | · | · | + | + | · | + | + | + | · | * | + |
|     *Belonesox*[2] | · | + | · | · | · | · | + | + | · | + | + | + | · | · | + |
|   Tribe Heterandriini | | | | | | | | | | | | | | | |
|     *Brachyrhaphis* | · | + | · | · | · | · | · | · | · | + | + | — | · | · | + |
|     *Trigonophallus* | · | + | · | · | · | · | · | · | · | + | + | · | · | · | + |
|     *Priapichthys* | · | + | · | · | + | · | · | · | · | + | + | · | · | · | + |
|     *Panamichthys* | · | · | · | · | — | · | · | — | · | + | + | — | · | · | + |
|     *Pseudoxiphophorus*[2,3] | · | — | · | · | + | · | · | · | · | + | + | · | · | · | + |
|     *Heterandria*[2,3] | · | — | · | · | + | · | · | · | · | + | + | · | · | · | + |
|     *Priapella*[2] | + | + | · | · | · | · | · | · | · | + | + | · | · | · | + |
|     *Allogambusia*[2] | + | · | — | · | · | · | + | · | · | + | + | · | · | · | + |
|     *Alloheterandria* | · | + | · | · | + | · | · | — | · | + | + | · | · | · | + |
|     *Neoheterandria* | · | + | · | · | + | · | · | · | · | + | + | · | · | · | + |
|     *Pseudopoecilia* | · | + | · | · | — | · | · | · | · | + | + | · | · | · | + |
|   Tribe Quintanini | | | | | | | | | | | | | | | |
|     *Quintana*[2,3] | — | · | · | · | · | · | + | · | · | + | + | + | · | + | + |
|   Tribe Girardinini | | | | | | | | | | | | | | | |
|     *Girardinus*[2] | + | + | · | + | · | · | · | · | · | + | + | · | · | + | + |
|     *Toxus*[2] | + | + | · | + | · | · | · | · | · | + | + | · | · | + | + |
|     *Glaridichthys*[2] | + | + | · | + | · | · | · | · | · | + | + | · | · | + | + |
|     *Allodontium*[2] | + | + | · | + | · | · | · | · | · | + | + | · | · | + | + |
|     *Dactylophallus*[2] | + | + | · | + | · | · | · | · | · | + | + | · | · | + | + |
|   Tribe Cnesterodontini | | | | | | | | | | | | | | | |
|     *Cnesterodon*[2] | · | + | · | + | · | · | · | · | · | + | + | + | · | · | + |
|     *Darienichthys* | · | + | · | + | + | · | · | · | · | + | + | · | · | · | + |
|     *Diphyacantha* | · | + | + | + | · | · | · | · | · | + | + | · | · | · | + |
|     *Phallocerus*[2,3] | · | + | · | + | · | · | · | · | · | + | + | · | · | · | + |
|     *Phallotorynus* | · | — | · | + | · | · | · | · | · | + | — | · | · | · | — |
| **SUBFAMILY POECILIOPSINAE** | | | | | | | | | | | | | | | |
|     *Poeciliopsis*[2] | · | · | + | · | — | · | · | · | + | + | · | · | · | · | · |
|     *Poecilistes*[2,3] | · | · | · | · | — | · | · | · | + | + | · | · | · | · | · |
|     *Aulophallus*[2] | · | · | · | · | · | · | · | · | · | + | · | · | · | · | · |
|     *Phallichthys*[2,3] | · | + | · | · | · | · | · | · | · | + | · | · | · | · | · |
|     *Carlhubbsia*[2,4] | · | + | · | · | · | · | · | · | · | + | · | · | · | · | · |
|     *Phalloptychus*[2] | · | · | · | · | · | · | · | · | · | + | · | · | · | + | · |
|     *Xenophallus*[2] | · | · | · | — | · | · | · | — | · | · | · | · | · | · | · |
| **SUBFAMILY XENODEXIINAE** | | | | | | | | | | | | | | | |
|     *Xenodexia*[2] | + | + | · | · | · | · | · | · | · | · | + | · | · | · | · |

TABLE 1. Synoptic List of Genera in the Family Poeciliidae
with an Inventory of Their Gonopodial Structures[1]
(*Continued*)

| | Ray 3 | | | | Ray 4a | | | Ray 4p | | | | Ray 5a, 5p | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hooks | Spines | Denticulations | Extensions | Hooks | Serrae | Elbows | Retrorse elements | Distal serrae | Proximal serrae | Canoe supports | Retrorse elements | Denticulations | Serrae | Canoe-like form |
| **Subfamily Poeciliinae** | | | | | | | | | | | | | | | |
| Tribe Poeciliini | | | | | | | | | | | | | | | |
| *Poecilia*[2,3] | • | + | * | + | • | • | • | • | • | + | + | • | • | • | + |
| *Micropoecilia*[2] | • | + | • | + | • | • | • | • | • | + | + | • | + | • | + |
| *Limia*[2,3] | — | + | • | + | • | • | • | • | • | + | + | * | * | • | + |
| *Parapoecilia* | • | + | • | + | • | • | • | • | • | + | + | • | + | • | + |
| *Lebistes*[2,3] | • | + | • | + | • | + | • | • | • | + | + | + | • | • | + |
| *Allopoecilia* | — | + | • | + | • | • | • | • | • | + | + | + | • | • | + |
| *Mollienesia*[2,3] | — | + | + | + | • | • | • | • | • | + | + | * | + | • | + |
| Tribe Xiphophorini | | | | | | | | | | | | | | | |
| *Xiphophorus*[2,3] | + | + | • | — | — | • | • | • | + | + | + | + | • | * | + |
| Tribe Pamphorini | | | | | | | | | | | | | | | |
| *Pamphorichthys*[2] | • | + | • | + | • | • | • | • | • | + | + | • | • | • | + |
| *Pamphoria*[5] | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| **Subfamily Alfarinae** | | | | | | | | | | | | | | | |
| *Alfaro*[2] | • | + | + | + | • | • | • | • | • | * | + | • | • | • | + |
| **Subfamily Tomeurinae**[6] | | | | | | | | | | | | | | | |
| *Tomeurus*[2] | + | + | • | + | — | • | + | • | • | • | • | + | • | • | • |

[1]This list of genera is modified from Hubbs (1926). The presence or absence of terminal gonopodial structures listed is an inventory, *not* intended to indicate homologies. These are of importance in any discussion of sexual behavior, taxonomy and evolution.
[2]Representative specimens (preserved) were examined.
[3]Representative live specimens were maintained under laboratory conditions for study of their sexual behavior.
[4]*Carlhubbsia* Whitley (1952) is a replacement synonym of *Allophallus* Hubbs (1936), which is preoccupied.
[5]No data were available.
[6]Probably should be elevated to Family Tomeuridae.
+Structure, present.
—Structure, reduced.
*Structure present in some species only.

America; many species inhabit the islands of the Greater and Lesser Antilles and the British West Indies. Other poeciliids, the Cnesterodontini, are restricted, for the most part, to east-central South America around Brazil, Uruguay and Argentina, whereas the Girardinini are found only in Cuba. The xiphophorin species are found only in the freshwater streams of the Atlantic slope from Mexico to Honduras. The distributional areas of the different groups of poeciliids are illustrated in Text-figs. 1-11.

The structure and arrangement of the ter-minal bony elements of the gonopodium of poe-ciliid fishes are widely used in the systematic arrangement of the genera and species. Because of this, the new data obtained in the present study eventually will influence future system-atic accounts of these fishes. In numerous in-stances, the morphological relationships de-scribed in this paper reveal a need for at least a partial revision of the present classification. It is beyond the scope of this contribution, how-ever, to undertake such taxonomic reassessment. The taxonomic arrangement followed is essen-
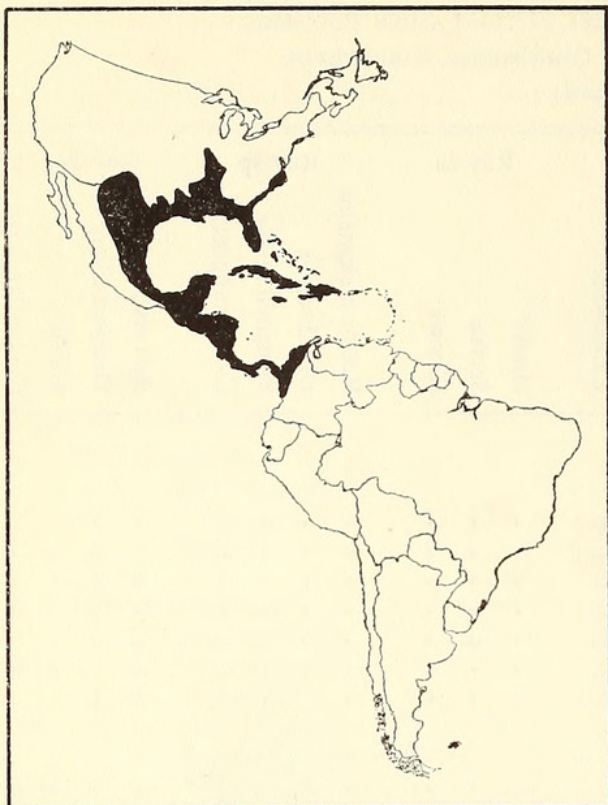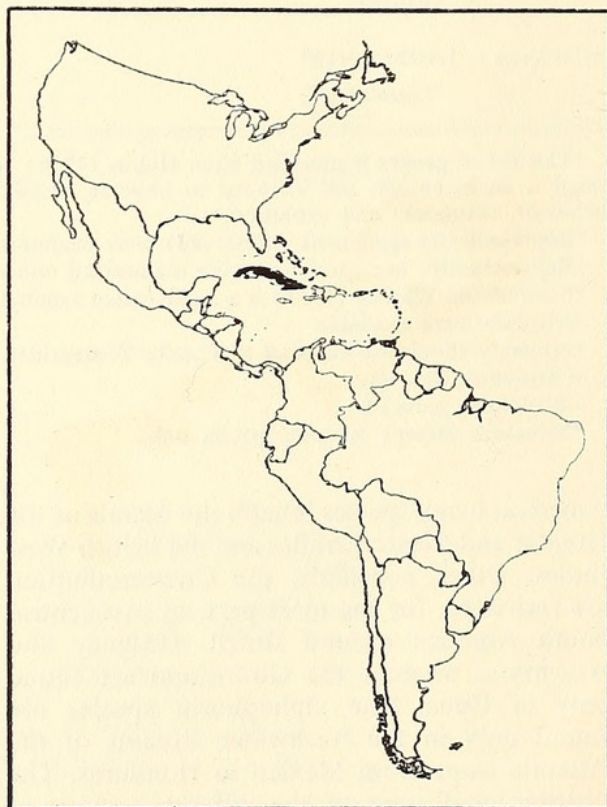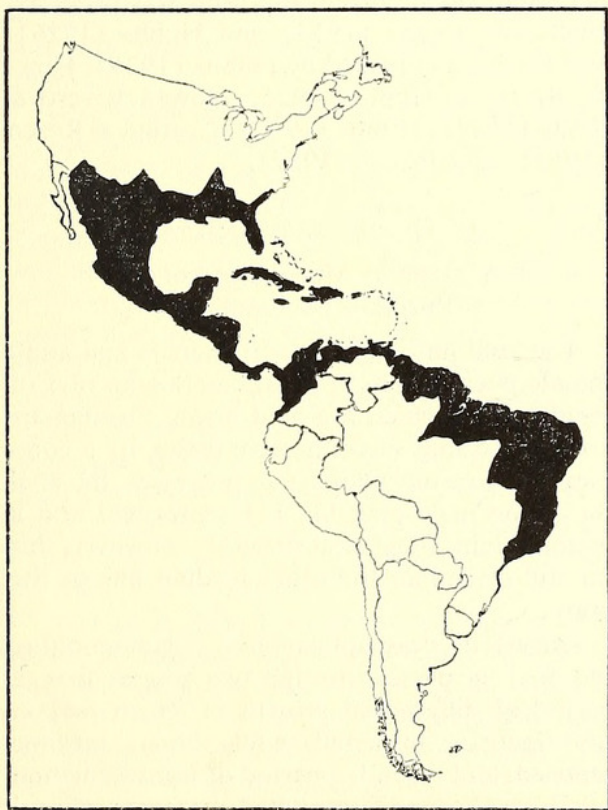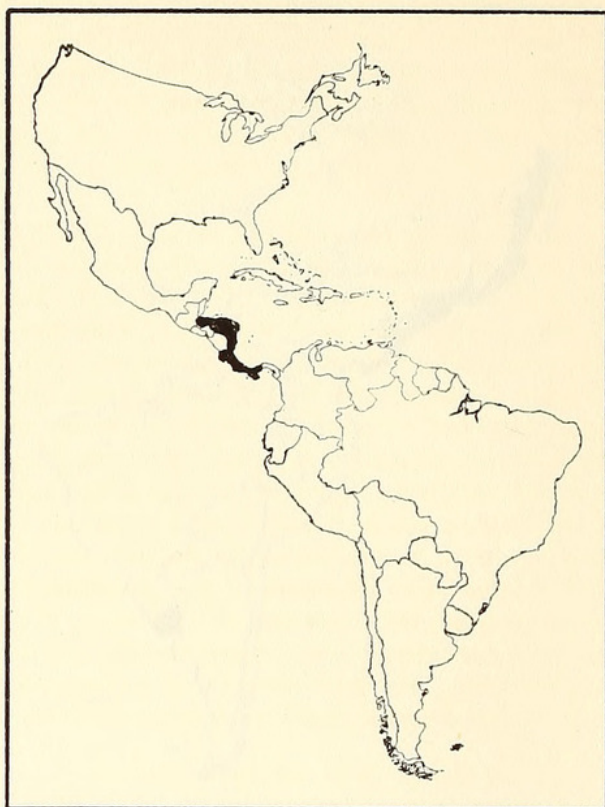
TEXT-FIG. 1. Approximate distributional area of the fishes of the Tribe Gambusiini.
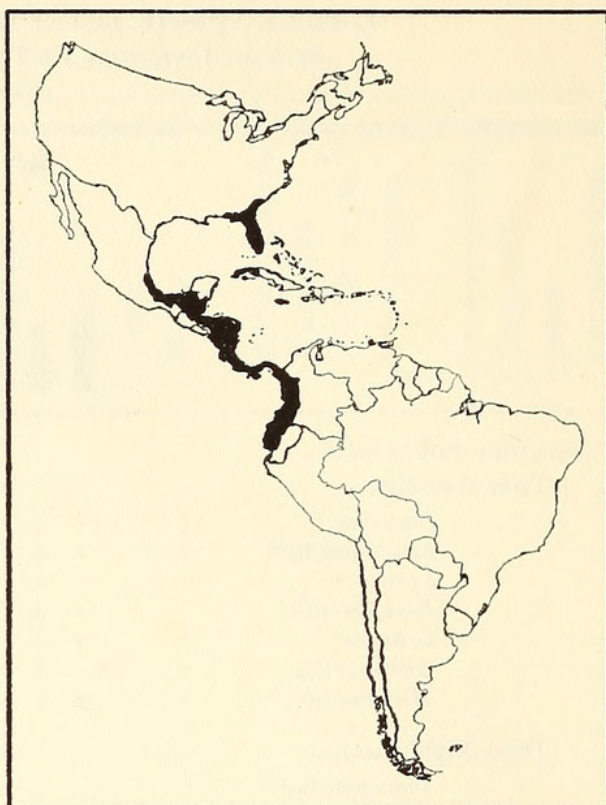
TEXT-FIG. 2. Approximate distributional area of the fishes of the Tribe Heterandriini. The distribution of the Quintanini is shown in Cuba and the Isle of Pines.

TEXT-FIG. 3. Approximate distributional area of the Tribe Cnesterodontini.

TEXT-FIG. 4. Approximate distributional area of the fishes of the Tribe Girardinini.

TEXT-FIG. 5. Approximate distributional area of the fishes of the Subfamily Poeciliopsinae.

TEXT-FIG. 6. Approximate distributional area of the fishes of the Subfamily Xenodexiinae.

TEXT-FIG. 7. Approximate distributional area of the fishes of the Tribe Poeciliini.

TEXT-FIG. 8. Approximate distributional area of the fishes of the Tribe Xiphophorini.

TEXT-FIG. 9. Approximate distributional area of the fishes of the Tribe Pamphorini.



TEXT-FIG. 10. Approximate distributional area of the fishes of the Subfamily Alfarinae.



TEXT-FIG. 11. Approximate distributional area of the fishes of the Subfamily Tomeurinae.

tially the one in current use. This was proposed chiefly by Regan (1913) and Hubbs (1926), and has been emended by Hubbs (1936), Howell Rivero & Hubbs (1936) Howell Rivero & Rivas (1944), Hubbs (1950), Gordon & Rosen (1951), and Rosen (1952).

## II. DESCRIPTIVE ANALYSIS

### A. General Morphology of the Poeciliid Gonopodium

The anal fin of the immature male and adult female poeciliid has approximately nine rays or lepidotrichia which are held firmly together by crossing strands of connective tissue. By a complex series of morphogenetic processes, the anal fin of the male poeciliid is transformed into a gonopodium. In general structure, however, this fin still resembles the other median fins of the body.

During its development into a gonopodium, the anal fin passes through two stages: first, a period of differential growth in which certain rays increase in length while others become reduced; and second, a period of transformation in which the undifferentiated segments of certain rays are changed into definite and specialized elements. Both stages overlap somewhat,

since differentiation begins prior to the cessation of differential growth. A rod-shaped organ is finally formed that consists of three enlarged and strengthened lepidotrichia (the 3rd, 4th and 5th), and six smaller ones which, for the most part, are small, gnarled, and lie close to the base of the fin.

Functionally, the enlarged rays, 3, 4 and 5, of the gonopodium are most important in the process of sperm transfer (Text-fig. 12). Ray 3 is the thickest and main supporting element of the gonopodium. Rays 4 and 5 are dorsal to ray 3 when the male holds its gonopodium in a resting position, i.e., pointed caudally. The dorsal and ventral edges of rays 3 and 5 flare out, while ray 4 is relatively thin and rod-like. The gonopodium as a whole is rigid and I-beam-like in cross-section. A detailed morphological description of the bony elements of the gonopodium of xiphophorins (Gordon & Rosen, 1951) may be considered fairly representative of the whole group. This may also serve as an introduction to the descriptive material which follows.

## B. Histology of the Gonopodium

A study of the histology of the gonopodium of a typical poeciliid fish, the swordtail (*Xiphophorus helleri*), was made. The fins were decalcified in nitric acid and phloroglucin and cut at 7 to 10 microns. They were stained with hematoxylin and eosin or Masson's modification of Mallory's trichrome stain. Comparable preparations were made of the anal fins of adult females.

In cross-sections cut through the distal region of the gonopodium at the level of the blade, the fin consists of compact, laterally compressed tubes, each consisting of paired lepidotrichial elements (Pl. IV, Fig. 9). In some cross-sections the claw of ray 5a is the dorsal element; in more proximal sections the subterminal segments and the beginning of the groove are in the dorsal position. The distal serrae of ray 4p lie directly ventral to the claw of ray 5a. Directly below the elements of 4p are a series of uniform segments of ray 4a which constitute the proximal portion of the distal ramus. The most ventral portion of the sections is occupied by the hook of ray 3 to which a gristly structure known as the blade is fused.

The blade, which is the last structure formed in the development of the gonopodium, is histologically different from the bony elements of ray 3. It is also the only part of the gonopodium in which true bone cells can be identified. The bone cells, which lie in the tough matrix of the blade, have large, deep-staining nuclei. These cells may have osteoclastic functions, for osteoblasts are generally found peripheral to bone.

At the distal tip of the fin, in cross-section, each of its rays contains a single, small centrally located blood vessel. The rays are connected by heavy collagenous tissue which forms the anchor membrane. Reticular connective tissue lies between the collagenous fibers and the epithelial membrane which, at this level, is only one cell thick. The reticular connective tissue contains capillaries which are enclosed by single cell layers of endothelium.

The central part of the tip of ray 4a contains blood vessels, for the most part, a few connective tissue cells which are revealed by their nuclei, and many fine collagenous fibers.

The inner part of the blade, near its proximal point, contains loose connective tissue only, but farther out it contains, in addition, small blood vessels surrounded by thin endothelial cells.

In the region near the posterior part of the blade and near the level of the spines on ray 3, large blood vessels are found. In this region the fin epithelium, together with the underlyiing collagenous membrane, begins to thicken.

Sections cut through the subterminal region of ray 5 (Pl. IV, Fig. 10) show large, thin-walled blood vessels within loose connective tissue which lies between the paired segments of ray 5a. Many erythrocytes are clumped within the meshes of the connective tissue, but this may be due to the effects of sectioning.

Four nerve trunks, circular in cross-section, lie within the core of ray 4. In the center of ray 3 there are two larger nerve trunks which, in cross-section, resemble opposing parenthesis marks. Nerve fibers from each trunk join, extend down between the segments of the fin ray, and are distributed through the loose connective tissue. The latter is surrounded by a basement membrane of collagenous tissue. The layer of loose connective tissue is thick and it is composed of a mixture of collagenous and reticular fibers, fibroblasts and various wandering cells.

Between fin ray and surface epithelium, strands of collagenous fibers cross between ray 3 and 4 and are attached to the periphery of the bone of ray 4. Collagenous fibers are also firmly attached to the inner surfaces of the two lateral grooves eccentrically placed on the ventral margin of ray 3. In some places the fibers continue up from ray 3 to join those of the membrane between rays 4 and 5. The general effect produced by the crossing of the collagenous fibers is the formation of a figure 8 in cross-section, the bony elements of ray 3 running through the ventral loop and the bony elements of rays 4a and 4p running through the dorsal loop (Pl. IV, Fig. 11).

In the region of the canoe-like depression of the posterior surface of ray 5 (Pl. IV, Fig. 12),
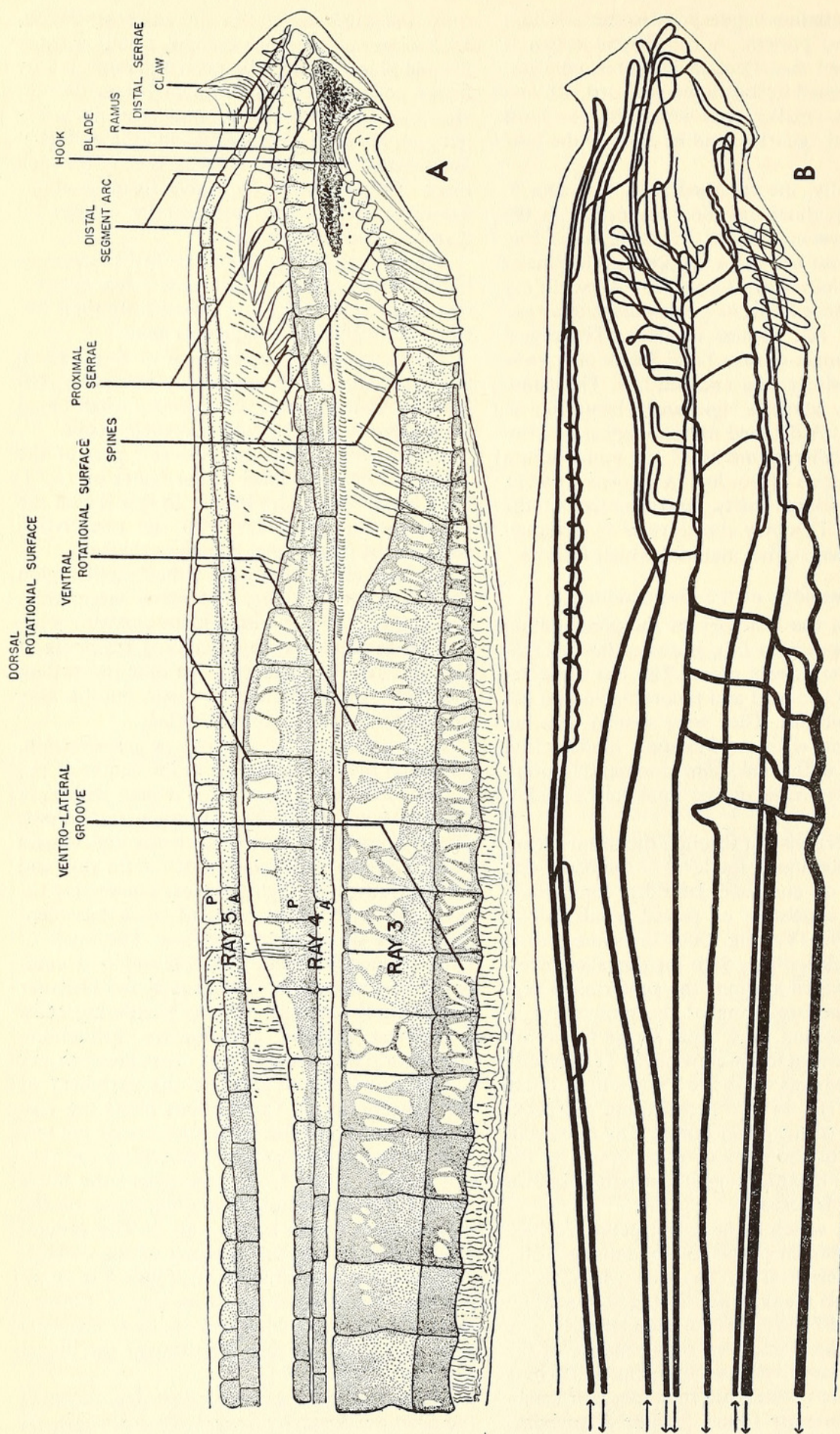
TEXT-FIG. 12. A. Topography of the bony elements of the gonopodium of *Xiphophorus helleri* Heckel. B. Distribution of the blood vessels in the fin as in A, with the bony elements not indicated. The direction taken by the blood in the major vessels is shown by arrows near the base of the fin.

one of the three blood vessels in the core of ray 4 has a heavier wall resembling that on an arteriole. At this point the large blood vessels in the connective tissue ventral to ray 3 are confluent, forming a large space or sinusoid, which is lined by endothelium. An arteriole is also present. In general, the blood vessels of all the rays in this region merge to some degree. Large vessels so produced may be considered veins but their walls are thinly lined with endothelial cells no thicker than those of the capillaries. Toward the base of the fin (Pl. IV, Fig. 13), the core of ray 3 contains one arteriole and three large sinusoides.

The integument over the gonopodium varies in thickness, being most prominent on the anterior margin of ray 3, slightly less so over the concavity of ray 5. Within the integumentary layer there are many mucus-secreting, goblet-shaped cells which are particularly dense in the thickened area of ray 3.

The development of the integumentary thickening along the anterior margin of ray 3 in the xiphophorins compared with some of the other poeciliids, is relatively moderate. In *Lebistes*, for example, the growth is quite prominent, like a tiny, oval wattle that is suspended from the subterminal region of the gonopodium by a short, slightly narrower pedicel. The growth is a solid, highly vascularized structure and tactile in function, according to the preliminary unpublished observations of sexual behavior in the guppy by Miss Patricia C. Kadow. Various terms have been used for these integumentary thickenings. Langer's (1913) term was *löffel*, apparently because it has a slight depression along the margin facing the 3rd ray and upon which it impinges when the gonopodium is erected. For the similar growth, Regan (1913) used the term *hood* in his text and *prepuce-like hood* in the legends for figures of it. Hubbs (1926), in a taxonomic key, used the "membraneous swelling along the anterior margin of gonopodium modified into a prepuce-like hood" to separate the Poeciliini from the Xiphophorini. In another part of the key he referred to a "knob-like enlargement" on the third ray in Alfarini. Recently Clark & Aronson (1951) and Rosen (1952) employed the term *gonopodial hood* for the thickening on ray 3 in *Lebistes* and *Alfaro*. Regardless of what the various integumentary modifications are called, they are probably homologous, as Figs. 14 and 15 of Pl. IV suggest in part.

Below the epithelium, broad sheets of collagenous fibers surround fin rays 3 and 4 in such a way that they describe a figure 8 around them. Two long fibrous tubes are thus formed along the long axis which extend from the subdistal to the subproximal limits of the fin. Tubular tissues are joined together along one edge by the crossing of the individual fibers. Within these tubes lie the fin rays, which are joined to the basement membrane by means of numerous strands which originate in the main sheet of the collagenous tissue and insert in the walls of the segment. Below the basement membrane are large masses and smaller islands of loose connective tissue in the matrix of which the blood vessels are embedded. Below the dermal layer of collagen and reticular tissue lie the fin rays.

Cross-sections through the gonopodium reveal clearly the bilateral arrangement of the paired mirror-image components of rays 3, 4 and 5. The opposing bony segments of the rays are not completely fused where they meet at the central plane of symmetry, for between the segments of rays 3 and 4 are large blood vessels, nerve trunks and loose connective tissue.

The bone of the fin rays is uniform in appearance; its texture is finely granular. The segments which form the supporting skeletal framework are hard and calcified. This is indicated by the rapidity with which they are stained by the dye Alizarin Red S, and by the manner in which they fracture when injured mechanically by the forceful application of a hard object. Interstitial canal systems or periosteal layers of bone have not been found. Sections cut at various points along the fin do not reveal any regional differentiation in the texture of the bone.

The anal fin of the adult female has morphological counterparts of most of the gonopodial structures. Some of the significant points of difference between the anal fin of male and female are as follows:

In the female's anal fin, rays 1 and 2 are quite small, ray 3 is the longest, and rays 4, 5, 6, 7, 8 and 9 are progressively shorter. The character of each ray is essentially the same. The first three rays are not bifurcate. The last six show primary, secondary, tertiary and sometimes quarternary bifurcations at their distal tips , the latter containing terminal connective tissue fibrils, or actinotrichia. On the other hand, in the gonopodium, rays 3, 4 and 5 become much elongated while the other rays are reduced; rays 4 and 5 have only primary bifurcations and these have no actinotrichia.

Like the male's gonopodium, the female's anal fin is surrounded by a layer of epithelium which is thickest at the anterior margin of ray 3. Beneath the epithelium is a basement membrane of collagenous fibers, and below this membrane are small islands of loose connective tissue.

### C. Vascular System of the Gonopodium

The pattern of distribution of the blood vessels in the gonopodium (Text-fig. 12) was determined both from an examination of serial cross-sections of fixed fins and from examination of the intact fins of anaesthetized *X. helleri* males which were studied under a compound microscope at a magnification of 200×, aided by brilliant transmitted light.

The main blood vessels in the gonopodium and in the undifferentiated anal fin of the swordtail are found generally within the central cores of the fin rays. Numerous smaller vessels are distributed along the surface of the rays and others are found within the loose connective tissue surrounding the bony layer.

### 1. The Afferent System

In the region of ray 5 there is a single median arteriole which runs undivided almost to the tip of the fin. Just posterior to the proximal serrae of ray 4p its numerous branches enter the tissues that form the trough. More distally, at the level of the proximal serrae of ray 4, a single large branch enters the extreme tip of the fin and runs to the distal serrae, comes back to the subterminal segments and then proceeds down to the ramus.

The center of ray 4 contains a single median arteriole. This vessel is slightly larger and has a thicker wall than its counterpart in ray 5. At the level of the proximal serrae it divides into two smaller vessels, one on the anterior and the other on the posterior margin of ray 4. The vessel on the anterior surface runs forward to the distal serrae and the ramus. The posterior vessel immediately branches into a series of tiny vessels of slightly greater than capillary size which arch upward on either side of the successive proximal serrae. At the same time it also sends off small vessels into the collagenous tissue between rays 3 and 4. It then proceeds forward to the distal serrae, the ramus and the blade.

The arteriole in the core of ray 3 proceeds unbranched towards the tip until it reaches the level of the spine. At this point it sends off unpaired median branches which loop ventrally between the shafts of the spines. From there it proceeds forward where it divides into a capillary network in the tissue postero-ventral to the hook.

### 2. The Efferent System

The arterial blood is supplied to the gonopodium by three vessels, each of which branches at the distal portion reaching the various specialized elements. The venous blood, however, takes a more devious path from the tip to the base of the fin, for there are approximately three times as many different routes along which the blood finally must pass in order to reach the proximal efferent trunk-lines.

Apart from its complex distribution among the different tissues, another characteristic feature of the venous blood is the manner in which it is picked up from the efferent vessels by anastomosing loops; these, in general, are found in the regions of greatest anatomical complexity. For example, anastomoses are particularly common in the regions of the proximal serrae, claw, distal serrae, ramus, spines and hook (Text-fig. 12). The anastomoses are so arranged that blood which enters the fin through the arteriole of ray 5 may be picked up through a network of terminal loops not only by the efferent vessels of ray 5 but also by the efferent vessels of rays 3 and 4.

The two vessels which run along the dorsal or exposed part of ray 5 pick up blood from the regions of the ramus, distal serrae, claw, and from the trough of ray 5. The arteriole of ray 5 supplies the blood to the regions of the claw and the trough. Blood draining from the distal serrae and the ramus is derived from arterioles in rays 4 and 5.

The two efferent vessels which course through the core of ray 4 drain almost the entire distal portion of that ray. The capillary networks and some larger vessels which surround the distal serrae, subterminal segments and proximal serrae empty into the two median efferents posterior or ventral to the proximal serrae. A single small capillary draws blood from the proximal portion of the blade and empties it into the ventral efferent within the core of the fourth ray.

The efferent vessels within the core of ray 3 are the largest of all gonopodial blood vessels. Their large size is associated with an increase in the amount of venous blood being drained from the fin tissues. The total capacity of the efferent system of ray 3 is augmented by the addition of another large vessel, so that three vessels constitute the ventral drainage system of this ray. Blood from the arterioles of rays 3, 4 and 5 empties into these vessels, but some of it from vessels in ray 4 is shunted off into the larger trunk-lines of ray 3 (Text-fig. 12).

Toward the base of ray 3 the three large efferent vessels become confluent and the blood empties into a single median vessel which fills almost the entire core of the ray.

From a general consideration of the vascularization of the gonopodium it may be said that the vessels comprising the efferent system are larger and more numerous than those of the afferent system. The blood enters the gonopo-

dium rapidly via three centrally located arterioles, and drains slowly into a complex network of peripherally located efferent vessels which merge successively to form a few centrally located vessels that empty into still larger basal sinusoides.

### D. Innervation of the Gonopodium

Histological sections and whole mount preparations were utilized in the analysis of the innervation of the xiphophorin gonopodium. The following technique was devised in order to study the distribution of the nerves in the intact fin:

An adult male xiphorphorin fish was lightly anaesthetized and then placed on a wad of moist cotton. The gonopodium was then grasped with a fine pair of forceps and the fin cut off at its base. The excised fin was immediately placed on a small plug of glass wool that had been moistened with a solution of 0.25% methylene blue and 0.6% NaCl in 100 cc of distilled water. By this procedure a thin film of the dye was kept in contact with the fin and at the same time all of the surface tissue was exposed to the oxygen of the air. The fin was removed from the methylene blue and washed with tap water at intervals of 15 minutes to observe the effects of the dye. A period of one hour was generally found sufficient for demonstrating paths of innervation. The nerves appeared as deep blue fibers, singly or in bundles.

There are three main routes along which the gonopodial nerves pass, one in the core of each ray. Ray 5 contains two nerve trunks which give off a few small bundles proximally in the fin. Our preparations do not reveal their relations distally. Ray 4, at the base of the fin, contains four main nerve trunks of which at least two extend out to the subdistal region of the fin where they break up into individual fibers. Some of these fibers extend up into the connective tissue between rays 4 and 5 at the level of the proximal serrae, while others extend ventrally into the tissue between rays 3 and 4 at the same level (Text-fig. 13).

The two large nerve trunks in the core of ray 3 give off small bundles of fibers en route to the tip of the fin. The bundles from the two main trunks extend ventrally in the core of the ray before passing into single larger bundles. These single median bundles pass between the paired segments of the ray to the outside where they enter the loose connective tissue ventral to ray 3 as individual fibers.

The two main nerve trunks of ray 3 become arborescent at the level of the spines. The principle branches arch ventrally and insert medially between the shafts of the spines (Text-fig. 13).

### E. Gonopodial Suspensorium
#### 1. Skeletal Supports

The gonopodial suspensorium and some of the adjacent vertebral elements form the supporting framework for the gonopodial muscles. The xiphophorin suspensorium, as described by Langer (1913) and by Gordon & Benzer (1945), consists of a series of sexually modified interhemal spines (called *gonactinosts* by Gordon & Benzer) which are associated ventrally with the rays of the gonopodium through a series of pivotal elements, the baseosts (pterygial elements of Gordon & Benzer). The gonactinosts are associated dorsally with the ventral processes of the vertebrae, the modified hemal spines or *gonapophyses*.

The most anterior hemal spine which originates from the 12th vertebra is highly modified. It lies embedded in a ligament connecting a vertebral centrum with the gonactinosts and has no articulation with the surrounding bony elements. In ontogeny this detached spine, called a *ligastyle* by Gordon & Benzer, moves anteriorly where it functions as a partial support for one of the gonopodial erector muscles.

The remaining three hemal spines or *gonapophyses*, which are radically modified in the gonopodial suspensorial system, stem usually
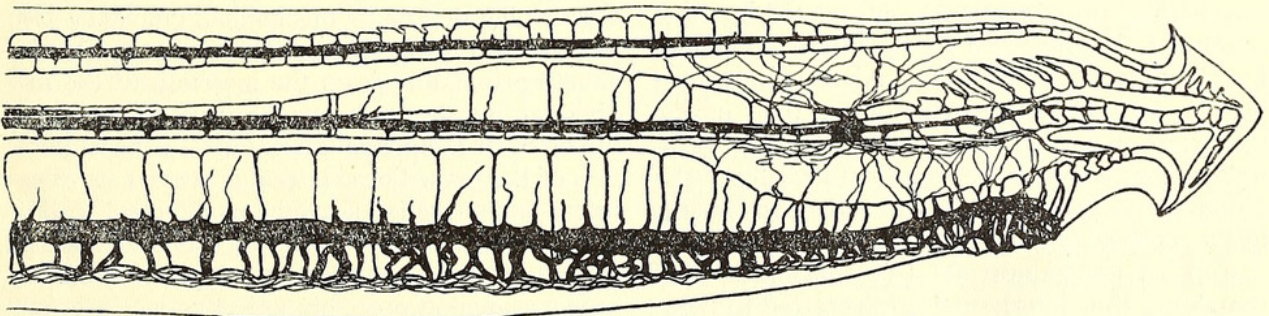


TEXT-FIG. 13. Distribution of paths of innervation as revealed by methylene blue preparations of whole intact fins of *Xiphophorus helleri* Heckel.
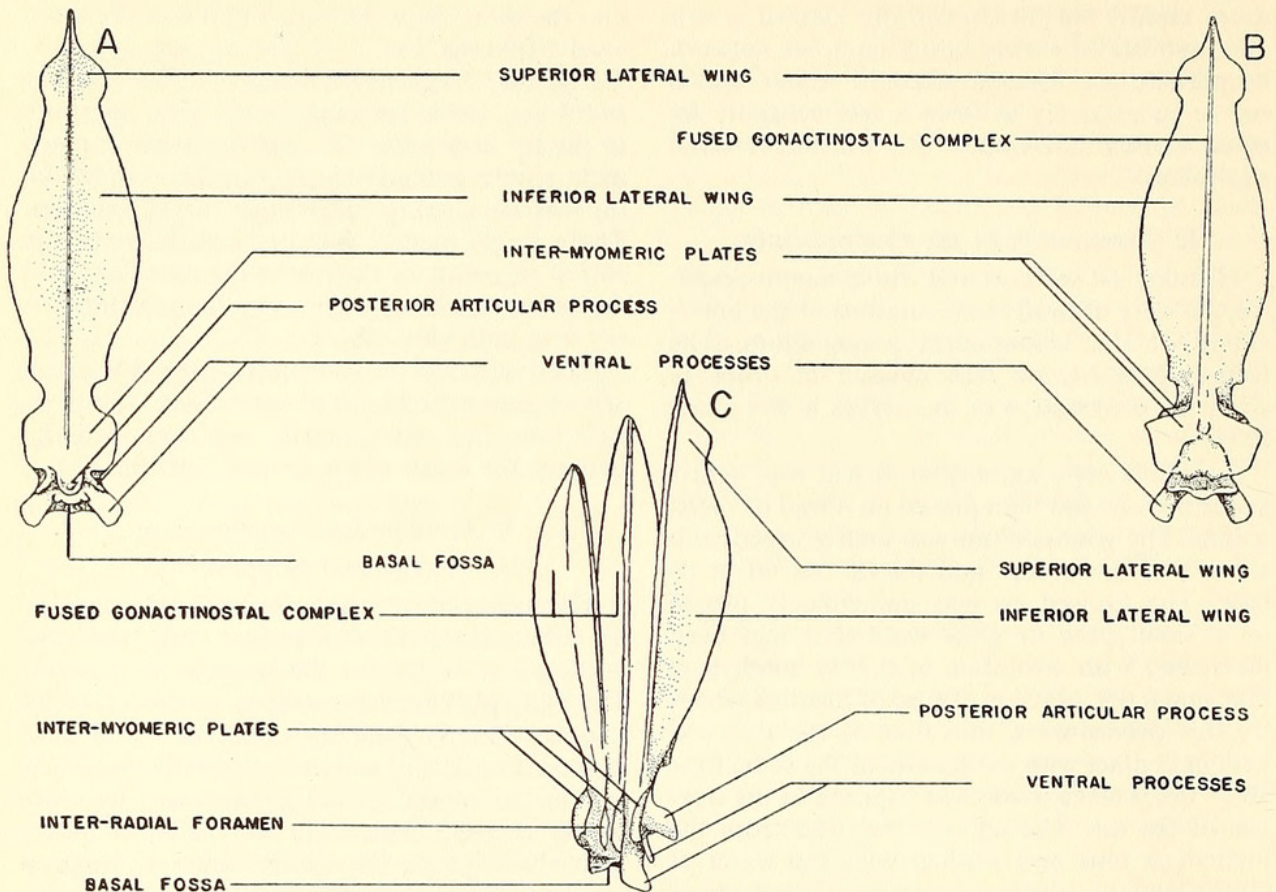
TEXT-FIG. 14. Three views of the primary gonactinostal complex of *Xiphophorus helleri* Heckel composed of the fused actinosts II, III and IV. A. Anterior; B. Posterior; C. Lateral.
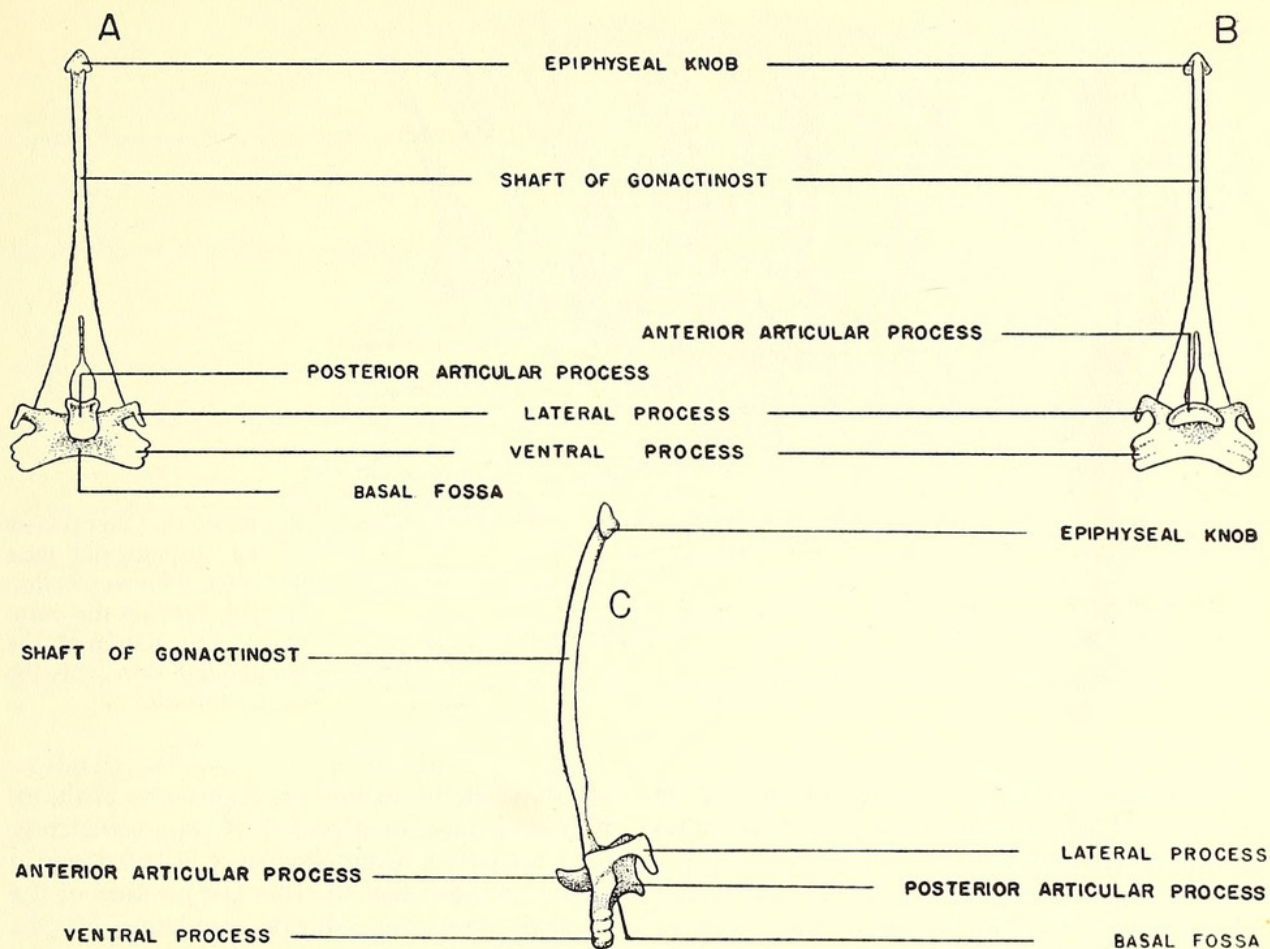
from the 13th, 14th and 15th vertebrae. Their ventral tips arch toward the projecting gonactinosts below and their posterior margins are supplied with tiny uncinus processes which serve as attachments for the tough suspensorial ligaments.

The modified interhemal spines form the basic anchorage for the muscles of the anal fin. In the adult male poeciliid fish they are variously modified and strengthened, which enables them to withstand the increased muscular tension that is produced during the forward movement of the gonopodium. The most anterior interhemal, or gonactinost, designated by the number I, is thick and its tip points cephalad in relation to the animal as a whole. Gonactinosts II, III and IV are fused along their edges to form a flat spatulate bone which acts as a unit in the movement of the fin (Text-fig. 14). Gonactinost V is a slender element with an anterior convexity that overlaps the posterior margin of gonactinost IV (Text-fig. 15). The remaining four interhemals are referred to here as gonactinosts VI, VII, VIII and IX because they, too, are definitely sexually modified and are involved in gonopodial movements.

The great freedom of movement that characterizes the anterior swing of the gonopodium is achieved, in part, by the rotation of the fin on tiny subspherical ossicles (the suspensorial baseosts) that are interposed between the bases of the gonactinosts and the bases of the rays of the gonopodium (Text-fig. 16).

All of the suspensorial elements, the gonopophyses, gonactinosts and baseosts, are held firmly together by a tough network of ligaments and fibrous connective tissue.

The origins and insertions of the gonopodial muscles are so arranged that they probably provide a high degree of mechanical efficiency for the forward swing of the gonopodium. This is gained principally from the insertion of the individual muscle heads over stout lateral bars on the bases of the gonactinosts onto the basal portion of the rays. These *lateral processes* serve as fulcra for muscular movements. Ventral to the *lateral processes* on gonactinost V and extending laterally in the same plane are a pair of *ventral processes* which probably are effective in bracing fin ray 5, preventing it from side-slipping its articulation during movement by providing a physical barrier against lateral shift.

A B

EPIPHYSEAL KNOB

SHAFT OF GONACTINOST

ANTERIOR ARTICULAR PROCESS

POSTERIOR ARTICULAR PROCESS

LATERAL PROCESS

VENTRAL PROCESS

BASAL FOSSA

C

EPIPHYSEAL KNOB

SHAFT OF GONACTINOST

ANTERIOR ARTICULAR PROCESS

LATERAL PROCESS

POSTERIOR ARTICULAR PROCESS

VENTRAL PROCESS

BASAL FOSSA

TEXT-FIG. 15. Three views of gonactinist V of *X. helleri* Heckel. A. Anterior; B. Posterior; C. Lateral.

The anterior gonactinosts, II, III and IV, differ from their serial homologs, for they are fused to form a broad plate provided with a winged edge posteriorly. The curved shaft of the fifth gonactinost fits snugly within the trough formed by the two *lateral wings*. Ventro-laterally on the fused gonactinostal plate are three flattened processes whose distribution corresponds to the arrangement of the fused shafts of gonactinosts II, III and IV. These small, flat processes are termed the *intermyomeric plates* and are probably homologous with the lateral processes of the more posterior elements. These plates separate individual muscles that arise from a fleshy origin on the face of the bone of the gonactinosts.

There are depressions medial to the transverse processes which open postero-ventrally. Within these depressions, or basal fossae, the heads of the baseosts lie. The baseosts, in turn, articulate loosely with the bases of the fin rays. The fin rays at their bases are split apart laterally. Their epiphyseal portions flare upward and away from the median plane. These latter processes are termed the *lateral epiphyseal*

*flanges,* and they represent points of insertion for the gonopodial muscles.

Each gonactinost, except the first and last of the series, has an *anterior* and *posterior articular process* by means of which the gonactinosts are joined together.

### 2. Musculature

There are two erector muscles attached to the gonopodial suspensorium (Text-fig. 17).

A large composite muscle, the *erector analis major,* originates on a terminal nubbin on the fused tips of gonactinosts II, III and IV and inserts by tendons on the lateral epiphyseal flanges of gonopodial rays 3 and 4. A few muscle fibers, taking their origin on the wall of the gonactinostal plate, also extend ventrally to the base of ray 3 where they insert by means of fine tendons. The belly of this muscle surrounds the plate of bone formed by the fused gonactinosts but it is limited posteriorly by the *superior* and *inferior lateral wings*.

A smaller muscle, the *erector analis minor,* is found antero-ventrally to the major erector. The analis minor has a fleshy origin on the stout
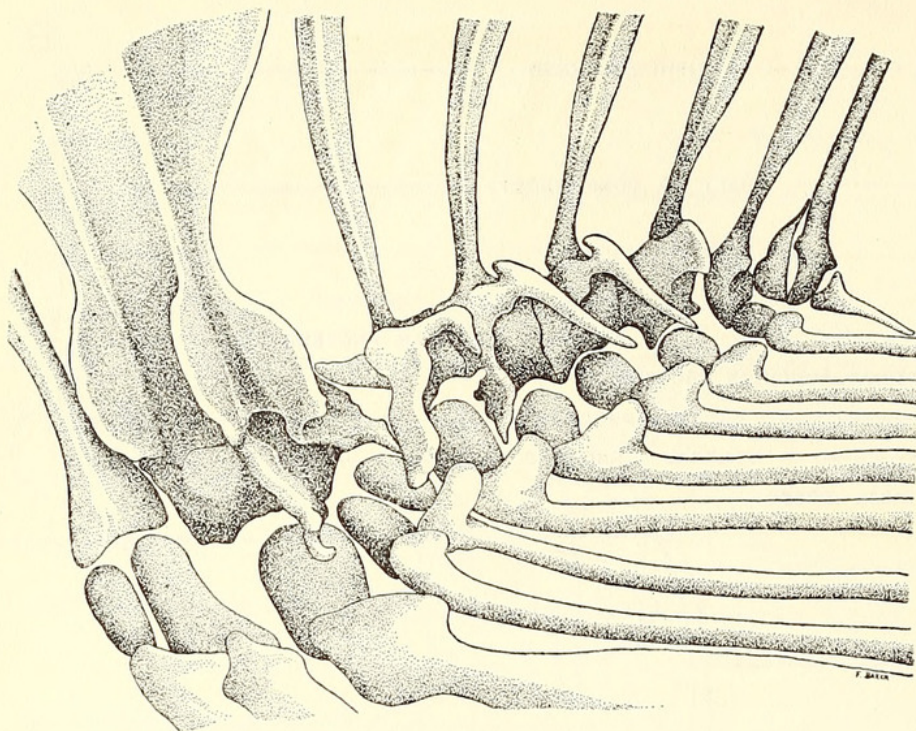
TEXT-FIG. 16. Lateral view of the suspensorial base in *Xiphophorus helleri* Heckel showing the manner of articulation of the gonopodial rays with the suspensorium.

shaft of the gonactinost I and it inserts on the overlapping lateral epiphyseal flanges of gonopodial rays 1 and 2.

In the gonopodial swing the erector analis major acts to bring the fin into forward rotation in a plane parallel with the long axis of the fish. On purely mechanical grounds, this muscle seems capable of rotating the fin through an arc of slightly greater than 90°. The erector analis minor, which is in the path of the forward rotation, would then be in a position to bring the fin still farther forward to complete an arc of about 145°. However, both muscles probably act together more or less simultaneously in bringing the fin forward.

Clark, Aronson & Gordon (1949) have shown that the gonopodium when swung forward is also brought out to one side. When in this position, the gonopodium forms an acute angle with the plane of the fish's long axis. The lateral movement of the gonopodium is accomplished by the action of the two lateral rotator muscles which lie on either side of the medial suspensorial musculature (Text-fig. 18). Their tendons insert on the lateral epiphyseal flanges of the fin rays. These are limbs of the *retractor ischii* muscle which Greene & Greene (1913) have shown, in the salmon, to act under certain conditions as a *protractor analis*. This rotator muscle has a composite structure in xiphophorin fishes, arising partly from the pelvic girdle musculature and partly from a ventro-lateral myomere.

When the gonopodium is swung forward an-

tero-laterally to assume the copulatory position, it is held rigid for a period of time sufficiently long for coitus to be effected. Clark & Kamrin (1951) suggest that the forward position of the erected gonopodium during copulation may be maintained, in part, by the buttressing action of a pelvic fin with which the gonopodium is swung forward. Our observations indicate that the maintenance of copulatory position is also dependent upon the action of a set of tensor muscles which affect the orientation of the main gonopodial support (Text-fig. 18).

Lateral to each erector analis muscle and just dorsal to the retractor ischii, the insertion of a heavy tendon may be traced posteriorly to the body of a stout muscle, the *retractor analis,* which continues posteriorly, ventral to the urosomal hypomeres.

If the two lateral slips of the retractor analis contract simultaneously, acting in the capacity of tensors, there is a two-fold effect on the fused gonactinosts and on the baseosts between them and the gonopodial rays. Contraction of the retractor analis draws the ventral margin of the gonactinosts posteriorly, while the rigid support of the ligastyle maintains the orientation of the gonactinosts' dorsal margin. The complete effect is a turning of the gonactinostal support around an imaginary axis located somewhere near its dorsal edge. The movement of the ventral edge posteriorly is associated with an equivalent posterior shift of the baseosts and of the gonopodial rays which articulate with them. Maintenance of this posterior position of the ventral margin

LIGASTYLE

ERECTOR ANALIS MAJOR

ERECTOR ANALIS MINOR

VENTRO-LATERAL HYPOMERE

PELVIC GIRDLE

ABDUCTOR VENTRALIS SUPERFICIALIS

RETRACTOR ISCHII (PROTRACTOR ANALIS)

DEPRESSOR ANALIS MUSCLES

RETRACTOR ANALIS

RAY 5

RAY 4

RAY 3
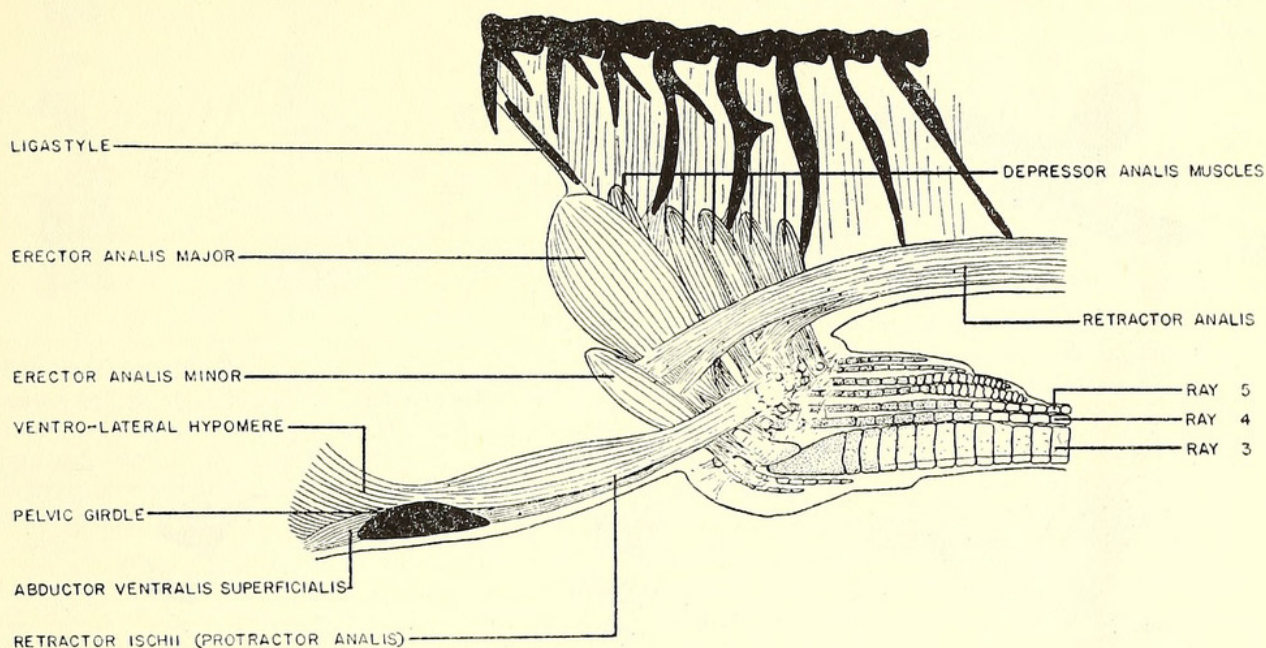
TEXT-FIG. 17. The medial muscles in the gonopodial mechanism in *Xiphophorus helleri* Heckel.

of the gonactinosts changes the direction of pull exerted by the erector analis muscles and by the lateral rotators. It in effect places the erectors and rotators in a more anterior position relative to the insertions of their tendons on the lateral epiphyseal flanges of the fin rays. In addition, a set of *inclinator analis* muscles is capable of exerting an upward pull on the fin rays at the base of the gonopodium (Text-figs. 19-21). This sharpening of the angle of insertion lends greater effectiveness to the operation of maintaining the full erection of the gonopodial rays by the inclinator analis muscles.

The release of the gonopodium from the copulatory position (Text-fig. 19) is effected by relaxation of the inclinator analis, retractor analis, retractor ischii and the erector analis muscles, and by the contraction of five relatively small *depressor analis* muscles. The depressor analis muscles arise from the shafts of gonactinosts V, VI, VII, VIII and IX, and they insert on the lateral epiphyseal flanges of their corresponding fin rays. In gross dissections the most anterior depressor analis is difficult to locate because it is embedded in the trough formed by the lateral wings of the primary gonactinostal complex.

VENTRO-LATERAL HYPOMERE

INCLINATOR ANALIS

RETRACTOR ANALIS

LINEA ALBA

RETRACTOR ISCHII
(PROTRACTOR ANALIS)

PELVIC FIN BASE

ANUS

UROGENITAL DUCT
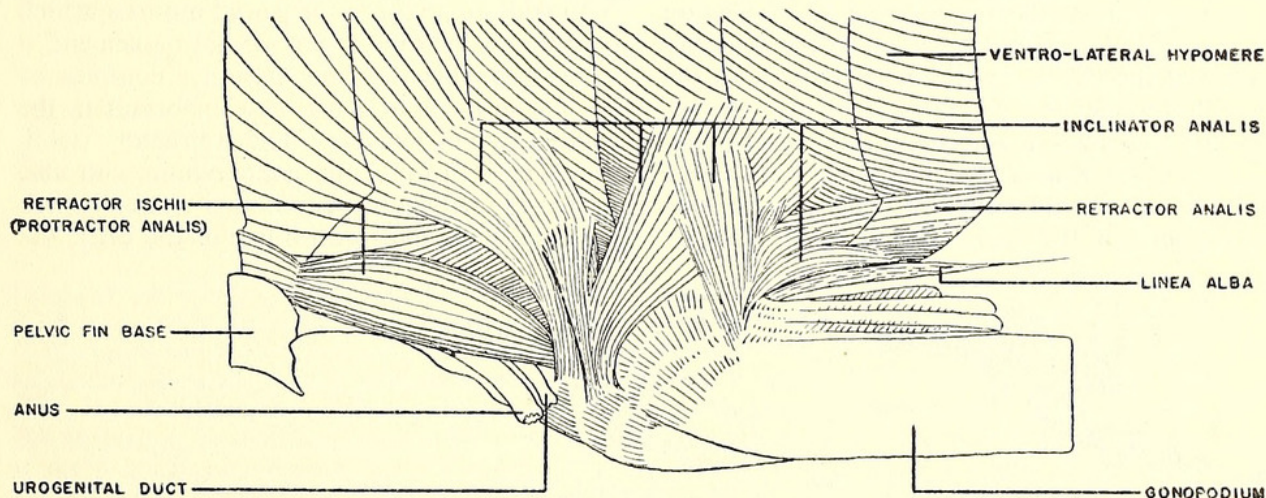
GONOPODIUM

TEXT-FIG. 18. The peripheral muscles in the gonopodial mechanism of *Xiphophorus helleri* Heckel. The difference in the slope of the retractor ischii muscle as figured here from its slope in Text-fig. 17 is attributed to a difference in the contour of the body of the two swordtails studied. The swordtail used in preparing Text-fig. 17 was a slender form from the Río Papaloapan in Veracruz, Mexico, while the one used in preparing this figure was a deep bodied form from Atlantida, Honduras.
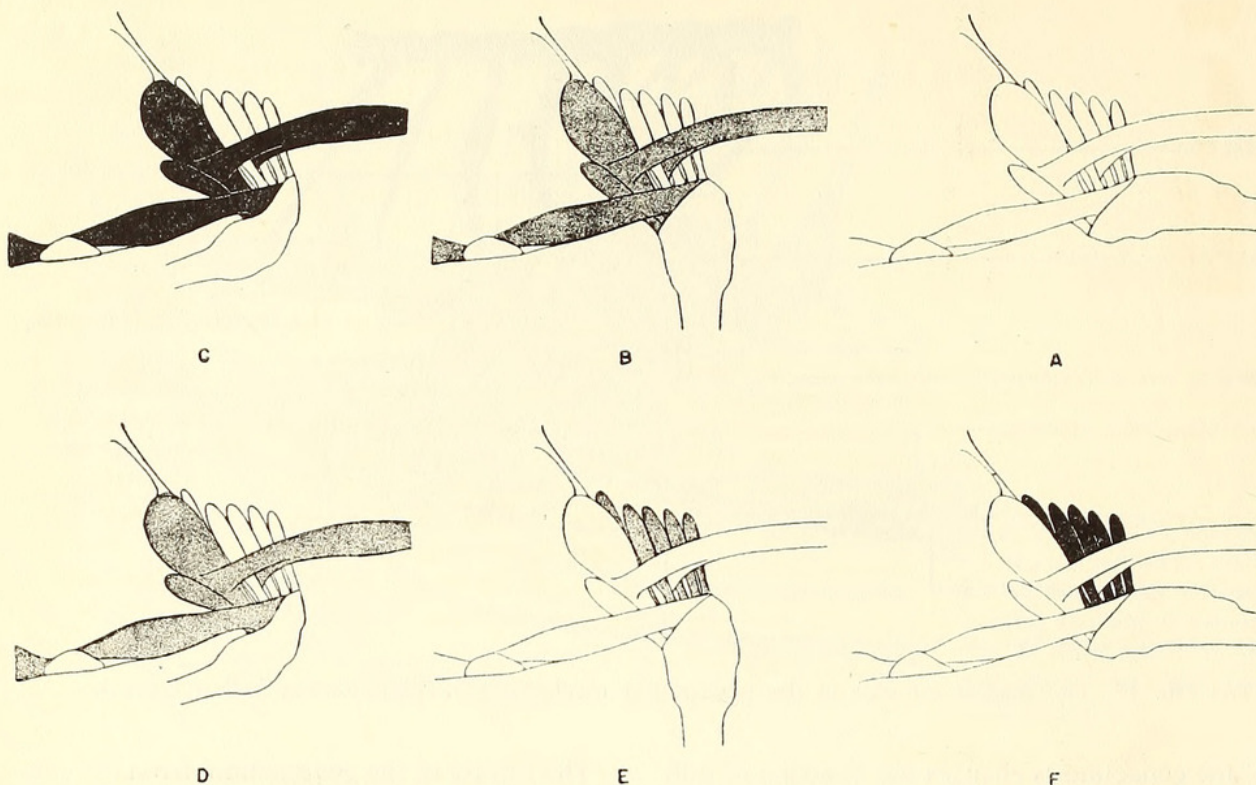
TEXT-FIG. 19. Diagram representing the actions of the medial muscles during fin movement. Partially contracted muscles are stippled, fully contracted muscles are blackened. A to C, erection (abduction); D to F, relaxation (adduction).

It functions, however, like the other depressors, and its skeletal attachments and other accommodations are also similar.

The distribution of the male's suspensorial musculature represents a profound reorganization of the basic female pattern. Each actinost of the unmodified anal fin of the female carries both erector and depressor muscles, and there appears to be no regional specialization or organizational predisposition to the development of the characteristic male pattern. In the female, neither the retractor ischii nor the retractor analis is imposed upon the lateral boundaries of the suspensorial elements; both insert basally on the fin supports. The side to side movement of the female fin is made possible by the well-developed inclinator analis muscles which originate in the integument of the body wall and insert laterally on the fin rays. The inclinator analis muscles are reduced in the male and this is not surprising since the side movement of the gonopodium may be accredited to the presence of the large, laterally inserted limbs of the retractor ischii.

In addition to the suspensorial muscles, which play the major role in gonopodial movement, it seems probable that the muscular complex of the pelvic fin is in some way important in the copulatory maneuvers. The retractor ischii, which may protract the gonopodium, can also retract the pelvic girdle. Another muscle which runs along the mid-ventral line of the belly, the
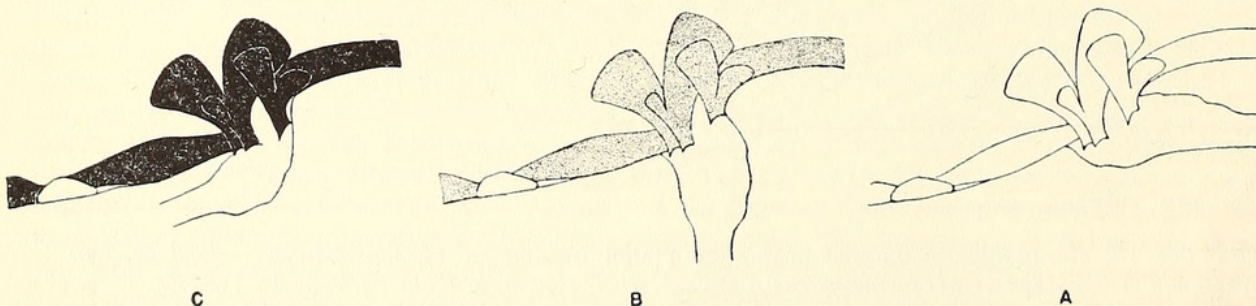


TEXT-FIG. 20. Diagram representing the action of the peripheral muscles during fin erection. Partially contracted muscles are stippled, fully contracted muscles are blackened.
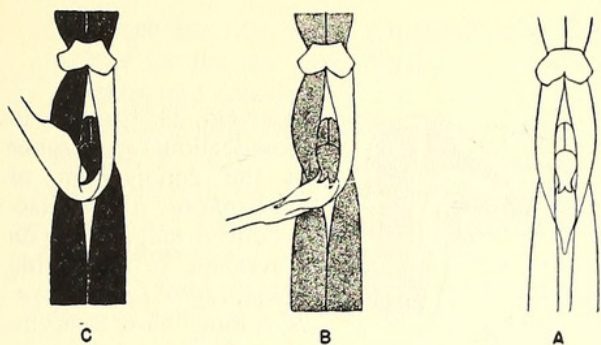
TEXT-FIG. 21. Diagram representing the unilateral action of the laterally inserted limbs of the retractor analis muscle in bringing the gonopodium out to one side during fin erection. Partially contracted muscles are stippled, fully contracted muscles are blackened.

*protractor ischii*, tends to protract the pelvic girdle. Greene & Greene (1913) state that in the salmon the protractor ischii may serve either to create a ventral flexion of the body or protraction of the girdle. The retractor ischii in the salmon may also produce a ventral flexion. It does this by fixing both the pelvic fins and the anal fin. When other muscles are relaxed, the fins are movable, and the action of the retractor ischii is to draw the ventral fins posteriorly. Complete retraction of the ventral fins makes it possible, in the salmon, for the retractor ischii to assume its secondary function of drawing the anal fin anteriorly. In male poeciliid fishes it is likely that the interactions of the muscles controlling the pelvic fins with the suspensorial muscles of the gonopodium are very elaborate; the correlated movements of a pelvic fin may be mechanically important for swinging the gonopodium forward.
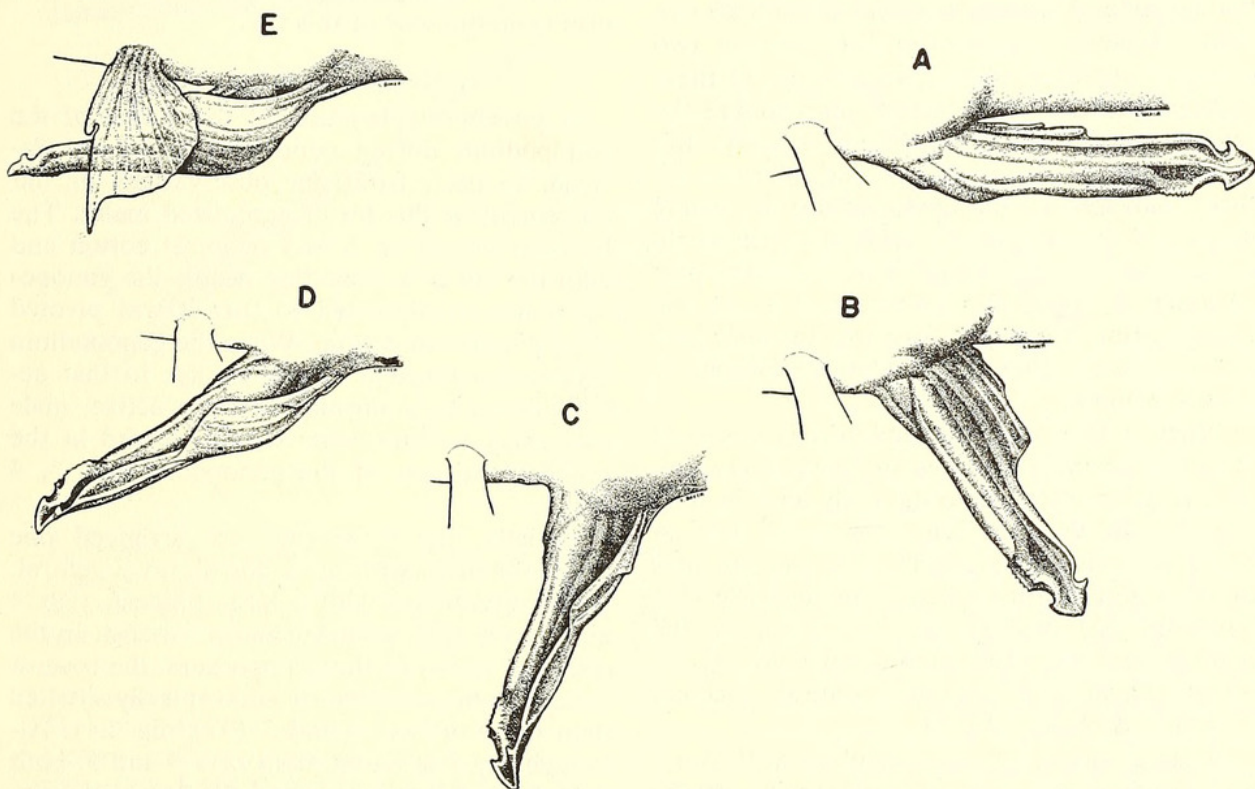


TEXT-FIG. 22. Changes in the external contours of the gonopodium of *Xiphophorus helleri* Heckel during erection, showing the manner in which a groove is formed along one side of the fin. In the resting phase, A, the much smaller rays 6, 7, 8 and 9 are depressed on the base of ray 5. As the gonopodium begins its swing downward and out to one side (toward the reader), B and C, rays 4 and 5 fall sideways and the tissue, which relates the smaller posterior rays, is distended. In C, the beginning of a groove along the side of the gonopodium facing the observer is revealed by the deeply shaded interior areas. In D, one edge of ray 5 is approaching the opposite edge of ray 3 preparatory to forming a closed channel, and the posterior rays are further separated in this region. Note position of spines on ray 3 in relation to the tip of the fin. Note also how both terminal holdfast elements are now facing in the same direction. In E, the gonopodium is fully erected and the opening at the base of the gonopodial groove is facing upward and slightly away from the reader. In their completely distended condition in E, the posterior rays together serve as a cup to receive discharged spermatophores. The position of the pelvic fin that is swung forward with the gonopodium is indicated in E. The base of this fin is indicated in outline in A to D,
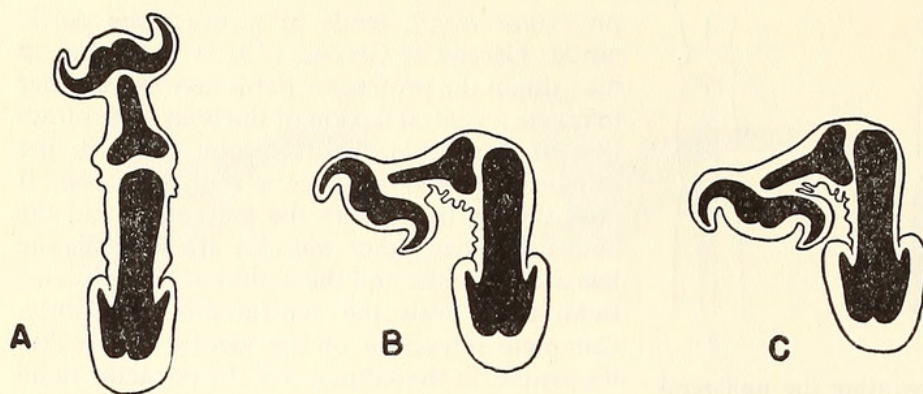
TEXT-FIG. 23. Changes in cross-sectional appearance of the gonopodium of *Xiphophorus helleri* Heckel during and prior to fin movement. In the resting phase, ray 5 is uppermost. A, resting phase; B, beginning of swinging; C, erection.

## F. Mechanical Specializations in the Gonopodium

Clark, Aronson & Gordon (1953) have shown that in courtship and reproductive behavior the male platyfish differs from the male swordtail in certain specific movements. According to them, the sum total of the differences in the behavioral activities serve as an effective sexual isolating mechanism between the two sympatric species. They observed that in these xiphophorins the male, while approaching the female, swings his gonopodium forward and thrusts the tip toward her genital aperture. In these movements the gonopodium is swung down and to one side in a wide arc until its tip is directed forward; then it is in a position preparatory to copulation (Text-fig. 22). As the gonopodium is brought forward, intrinsic mechanical forces change the relative positions of rays 3, 4 and 5.

When a live, male xiphophorin is observed at rest, its sexually modified anal fin points caudally. It is carried close to the body with its long axis parallel with the long axis of the fish. A broad surface runs along the 3rd and 5th rays of the gonopodium. Thus, in cross-section (Text-fig. 23) through the 3-4-5 complex, the gonopodium resembles an I-beam with slightly fluted dorsal and rounded ventral surfaces (Gordon & Rosen, 1951).

A large, strong baseost articulates with ray 3 and provides the main support for it, and indirectly for the other gonopodial rays. There is also a series of tiny baseostal elements between the other points of articulation of the suspensorium and the bases of the rays of the gonopodium. These provide the pivotal elements upon which the gonopodium swings (Text-fig. 16).

Various anatomic features of the suspensorium seem to indicate that not only are the 4th and 5th gonopodial rays mechanically supported by ray 3, but that the movement of these gonopodial rays is dependent in large part upon the muscular control of the 3rd ray. It would appear, then, that the ray 3 complex controls the abduction of the gonopodium, and that its baseost acts as the center of gonopodial movement during swinging. This opinion finds support in the fact that the most conspicuous musculature in the gonopodial suspensorium takes its origin on the gonactinosts articulated with ray 3 and inserts on the base of this ray.

### 1. Mechanics of Operation

A possible explanation of the action of the gonopodium during copulation has been derived, in part, from our observations of the movement of this fin in narcotized males. The fish was placed on a wad of moist cotton and with the aid of a dissecting needle the gonopodium was manipulated so that it was pivoted in all possible directions. When the gonopodium was swung through an arc similar to that accomplished by a normal, sexually active, male fish, certain adjustments were observed in the relative positions of the gonopodial rays 3, 4 and 5.

Initially, the three rays are arranged one above the other with ray 5 dorsal, ray 3 ventral. Ray 3 articulates with a large baseost, rays 4 and 5 each with a single smaller baseost. In the resting position of the gonopodium, the baseost of ray 3 is more anteriorly and ventrally situated than those of rays 4 and 5 (Text-fig. 24). Although ray 3 is longer than rays 4 and 5, both rays 4 and 5 extend beyond the 3rd at the tip.

All the rays are bound firmly together by broad sheets of collagenous fibers (see pages 7 & 8). The collagenous tissue sheets are anchored firmly in place by the proximal serrae of ray 4p. The tip of the shaft of each serra is inclined proximad and the pointed tip hooks into the fibrous sheet during fin movement. In addition, individual fibers or groups of fibers are continuous with the walls of the segments of ray 3, and the ventro-lateral portion of the entire sheet inserts in eccentrically placed grooves running along the lateral margin of this ray.

There are two major adjustments in the positions of the fin rays during fin movement: slipping and rotation.

As the gonopodium is swung downward (Text-fig. 24, B), ray 3 maintains the same relationship to the tip of the fin as in the resting condition. Rays 4 and 5, on the other hand, respond to the downward movement of the gonopodium and slip backward away from the tip. The position of ray 5 is behind ray 3, and ray 4 is adjacent to ray 3.

When the gonopodium reaches the half-way mark in its downward and forward swing (Text-fig. 24, C), the tips of rays 4 and 5 have slipped still farther behind the tip of ray 3. When the gonopodium is swung beyond the mid-point, ray 3 advances, maintaining approximately the same position relative to the tip of the gonopodium. At this point, rays 4 and 5 fall sideways along the distal half of the gonopodium. In doing this, the thin and flexible fifth ray rotates on its long axis (carrying ray 4 along with it) and its canoe-like outer surface is aligned with and makes contact with the broad, rounded outer surface of ray 3 (Text-figs. 23, 24). Thus, in the distal half, a transitory tube is formed along one side of the gonopodium through which spermatozoa may pass from male to female at the time of copulation. The proximal half of the gonopodium, which is not subject to folding for mechanical reasons (Text-figs. 22, 24), may be transformed into a closed chamber by the action of a pelvic fin; for, when the gonopodium is swung forward and to the right side
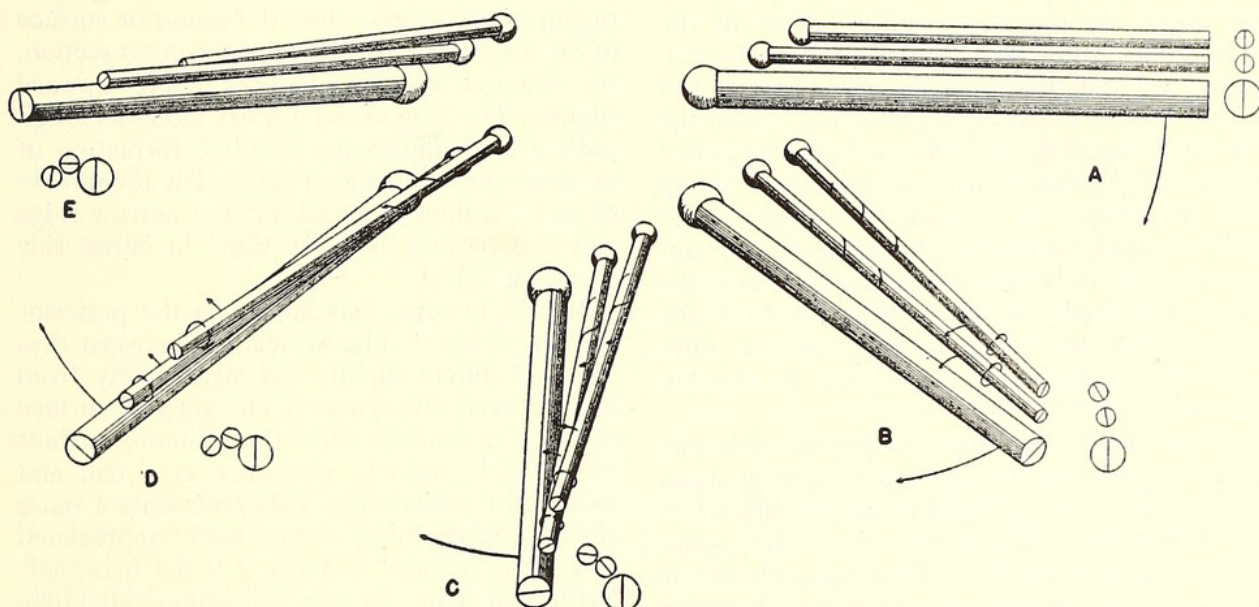


TEXT-FIG. 24. Diagram of the mechanics of erection of rays 3, 4 and 5 of the gonopodium by the aid of a model. Each ray is represented by a cylindrical rod, ray 3 being of greatest diameter and the most ventrally situated. The base of each rod is fused to a spherical pivot which permits free rotation of 180° ventrally or laterally along any plane which bisects the axis of rotation at the pivot's center. The circular cross-section of each rod at its free end in A is bisected by a vertical line. The deflection of this line from the vertical position in B to E represents an equivalent turning of each rod around its horizontal axis. The change in the relative positions of the three rods in relation to each other is revealed by the inset of a cross-sectional view near their free ends. The spiraled arrows encircling the bases of the slender, dorsal rods (rays 4 and 5) in positions B to D represent torsional movements which take place within the basal regions of these rods. Torsion, in turn, makes possible the turning of the extremity of each rod around its horizontal axis. Turning is indicated by the circular arrows which appear as open rings at the terminus of each rod. The subterminal, slightly arched arrows in positions B to D represent the folding movements of the two slender rods downward adjacent to the broad, lateral surface of the ventral rod. The long arrow originating initially on the ventral surface of the large, ventral rod (ray 3) represents the path of forward rotation for all three rods; thus, the large, ventral rod is the focus of directional changes during forward movement and may be said to guide the slender, dorsal rods along a prescribed path to their final positions in E. In position A, the free ends of the three rods are in line. In positions B to E, the two dorsal rods are sliping backward away from the tip, turning on their horizontal axes, and folding downward adjacent to the large ventral rod. Note that in the arc described from right to left the ventral rod does not turn around its horizontal axis; deflection poef its vertical bisector is made possible entirely by rotation on the basal pivot. In the final position, E, the mechanical adjustments have produced a triangular arrangement of the three rods in cross-section, as compared with their lineal arrangement in the initial position, A.

of the fish, the right pelvic fin moves forward and is applied to its base, and when the gonopodium is swung to the left, the left pelvic fin is applied to its base (Clark, Aronson & Gordon, 1948). The chamber so formed may serve as transitory sperm receptacle.

Theoretically it is possible to separate the accommodations for slipping and rotation, but in actual practice this is not possible because of the overlapping in function of the various mechanical specializations. For example, the broad sheets of collagenous fibers serve a dual purpose. They bind the rays together, creating a firm but flexible unit. They also provide a mechanism which controls rotation and prevents excess slipping.

When, for purely mechanical reasons, rays 4 and 5 have slipped backward away from the tip of the fin during the initial phase of the gonopodial swing, stress is placed upon the collagenous tissue that binds the rays together and a path of stress travels transversely across the fin. The idea that the tissue is being stretched is supported by study of the gonopodia of young and old males. The collagenous tissue in newly differentiated gonopodia is uniform and unbroken. The collagenous tissue in the gonopodia of older, sexually active males is wrinkled, the irregularities appearing as long parallel lines running perpendicular to the axis of the fin, i.e., from ray 3 to ray 5.

When the gonopodium reaches the half-way point in its swing forward, an additional stress is applied to the collagenous tissue. But when the gonopodium swings beyond the mid-point, the even greater stress placed on the collagenous tissue by the slipping of rays 4 and 5 causes these two rays to fall sideways. The strain on the collagenous tissue is distributed over the entire arc of swing by a movement down and to one side. An exclusively ventral movement of the gonopodium would probably damage the inter-radial membrane which relates the rays.

Many portions of the fin rays are specially adapted for radical changes in their positional relationships. An adjustment utilized principally to accommodate slipping is the distal *segment arc* on ray 5 (Text-fig. 12). This arc functions somewhat like a spring, by extending during fin movement and relaxing when the fin returns to the resting position. It effectively allows greater slipping of ray 5 by permitting the proximal segments of the ray to move proximad without seriously changing the relationships at the tip of the fin. Maintaining a stable arrangement of the distalmost segments is necessary because important gripping devices are located here.

The ramus on ray 4a is another device used to accommodate slipping. As ray 4 slips backward away from the tip during gonopodial swinging, the ramus slides over the posterior surface of the blade, straightening out as it does so. In *X. helleri* where the ramus is angular, fitting snugly around an angular blade, its function is largely to prevent excess slipping of the posterior rays. The swordtail, *X. helleri*, is the largest of the xiphophorins, possessing the largest gonopodium. Mechanical changes in its fin are more extensive and the special ramus may be a compensatory adjustment for increased size.

The folding of the gonopodium to produce a transitory tube for sperm passage is made possible by the rotational movements of rays 3, 4 and 5 along their long axes in the general region of the spines. Here the posterior surface of ray 5 is broad, low, U-shaped in cross-section. Its bilateral marginal flanges curve upward slightly. The base of the 5th ray along its longitudinal axis adjoins the keel-like formation of the posterior segments of ray 4. The broad base of ray 5 is thus balanced along a narrow ridge and this permits it to be tilted to either side (Text-fig. 23A).

Ray 4, in turn, may rotate on the posterior surface of ray 3. The association between rays 3 and 4 differs slightly but significantly from that between rays 4 and 5. The anterior surface of ray 4 is concave while the posterior surface of ray 3 is convex, appearing as a ball and socket in cross-section. This represents a more efficient arrangement, a fact easily appreciated when it is realized that ray 3 is the main axis of rotation. Thus, the transitory gonopodial tube is formed at the moment of copulation by the meeting of the lateral margin of one of the flanges of ray 5 with the lateral margin of the opposing flange of ray 3.

## 2. Copulatory Mechanisms

Special devices not directly related to the major mechanical adjustments are found at the tip of the fin. Clark, Aronson & Gordon (1949) indicated that in *X. maculatus* and *X. helleri* certain projecting spinous elements at the tip of the gonopodium are used by the male to grip the genital aperture of the female during copulation. Other structures, such as the spines of ray 3, which do not function as gripping devices, may also be effective in the holdfast mechanism.

### a. The Spines (Sensory Structures)

When the gonopodium is at rest, the posterior (dorsal) surface of the distal portion of ray 3 is slightly curved; the hook, which is distal, is
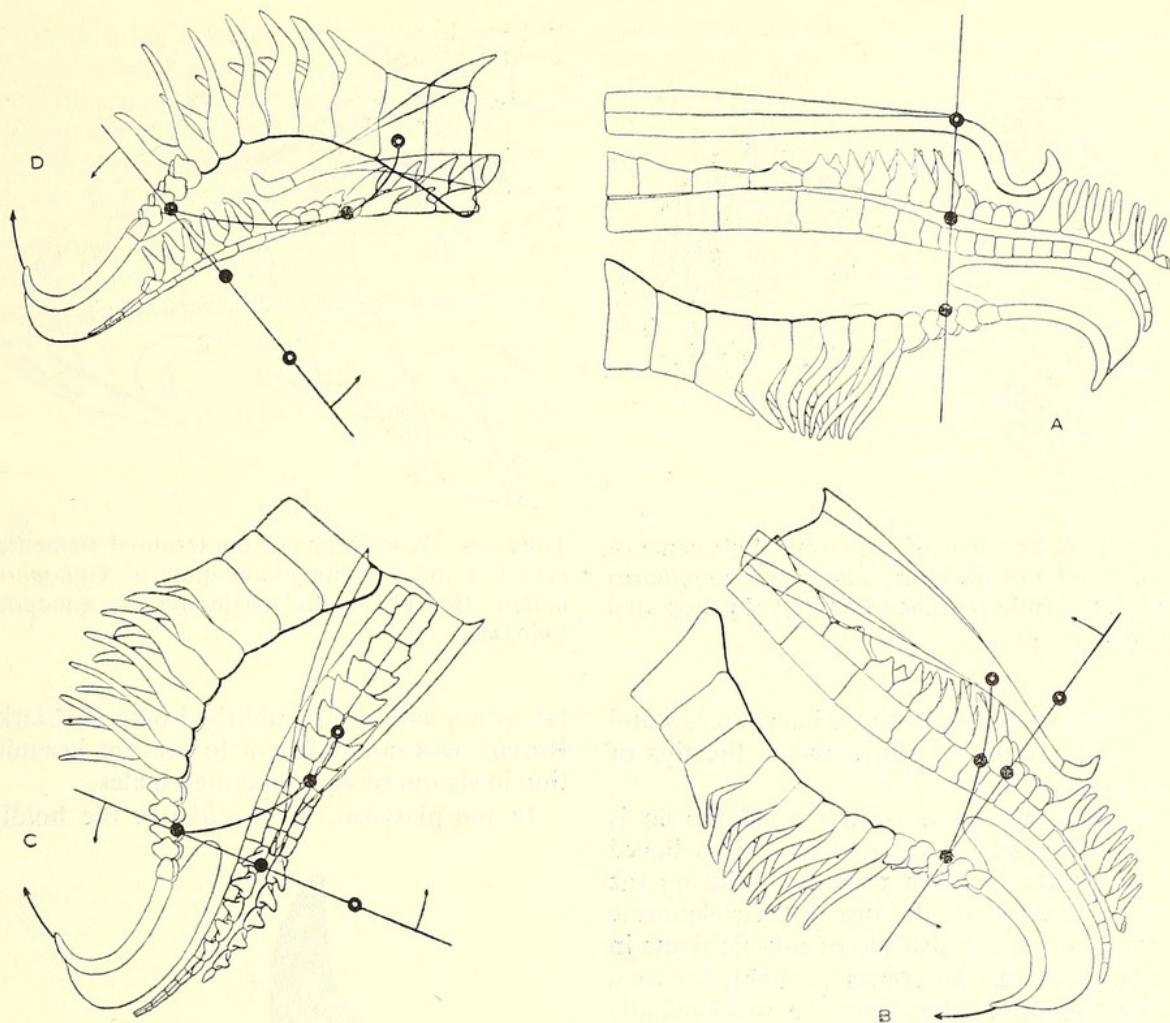
TEXT-FIG. 25. Changes in the relative positions of the terminal specialized elements of the gonopodium in *Xiphophorus pygmaeus* Hubbs and Gordon during fin movement. In A, a line which is perpendicular to the horizontal axis of ray 3 is drawn through the extreme tip of the fin. The positions along this line which make contact with rays 3, 4 and 5 are marked by circles on these positions. Each position on rays 3 and 4 is indicated by a solid black circle, while that on ray 5 is marked by an open circle. In B, C and D, the changes in the relative positions of the rays as 4 and 5 slip backward away from the tip of ray 3 are revealed by a distortion of the perpendicular line of reference in A. The initial line, however, is retained throughout in its original relation to the horizontal axis of ray 3 in order to bring the amount of displacement of the basal rays into sharp contrast. Note (1) that the amount of displacement is greatest in ray 5; (2) how the paired spines become further separated during movement in response to curving of the tip of ray 3; (3) how the dorsal, terminal holdfasts on rays 4 and 5 rotate over to a position adjacent to the ventral terminal holdfast of ray 3. Only the external contours of ray 5 have been indicated for clarity.

thus higher than the bases of the spines which are proximal to it (Text-fig. 25). The tips of the shafts of the spines arch anteriorly (ventrally) where they come together and form a compact unit. The bases of the paired sets of spines are close together while the tips of their shafts flare out to some degree. Two large nerve trunks enter the region of the spines from the core of the ray; they branch into the connective tissue between the tips of the laterally paired spine shafts (Text-fig. 13).

The tip of ray 3 (the hook) is drawn posteriorly by the connective tissue when rays 4 and 5 slip backward. In this way, a greater curvature is imparted to the posterior surface of ray 3, the degree of curvature increasing in proportion to the slippage of rays 4 and 5. The shafts of the spines respond to the curving by spreading apart like the fingers on a hand, while the laterally paired spines also become further separated. Thus, the connective tissue is being stretched both longitudinally between the tips
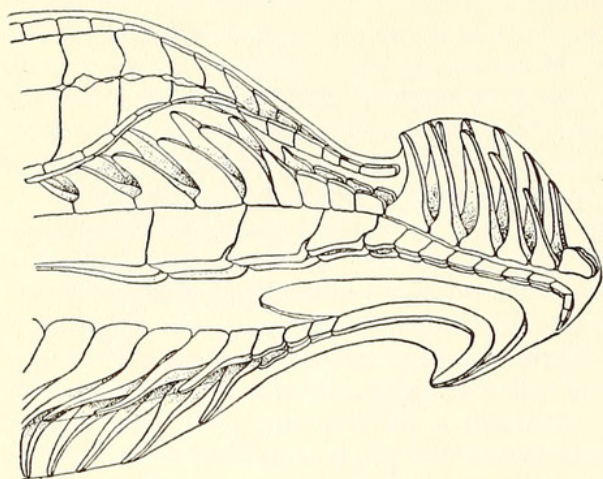
TEXT-FIG. 26. Positions of the terminal elements of rays 3, 4 and 5 of the gonopodium of *Xiphophorus maculatus* (Günther) at the beginning of gonopodial swinging.
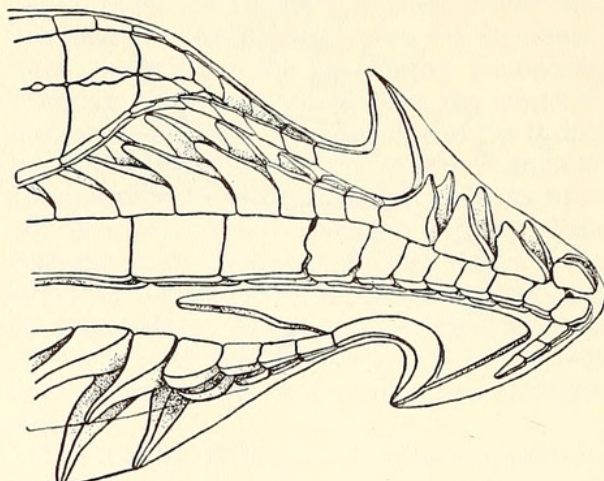


TEXT-FIG. 27. Positions of the terminal elements of rays 3, 4 and 5 of the gonopodium of *Xiphophorus helleri* Heckel at the beginning of gonopodial swinging.

of the shafts of the spines in a lateral series and transversely across the fin between the tips of paired spines.

Maximum spreading occurs when the fin is fully erected and for this reason it is believed that the spines function principally during the actual copulatory act. Because of their elaborate innervation and the absence of muscle tissue in the gonopodium, the spines probably serve a sensory function. They may be mechanically stimulated when the tip of the gonopodium is inserted into the genital aperture of the female and the impulse may stimulate the discharge of spermatophores.

b. The Hook, the Claw and the Distal Serrae, Holdfast Structures

There are two spinous elements in the swordtail, the hook (ray 3) and the claw (ray 5), which are utilized by the male to secure contact with the female. These spinous elements are inserted into the female's genital aperture. When the fin is at rest, the claw is above and in front of the hook, nearer the tip of the gonopodium. Its pointed tip is directed upward and proximally. As the gonopodium swings forward, ray 5 slips backward and rotates on its axis, carrying the claw with it. The slipping of ray 5 opens the segment arc with which the claw is articulated and draws it proximally. This causes a slight rotation of the claw, bringing its pointed tip into a vertical position. In the copulatory position the projecting tips of both the claw and the hook face in approximately the same direction, although the claw is now slightly behind the hook (Text-fig. 22). This might explain why removal of either the claw or the hook from a sword-

tail gonopodium (unpublished data of Clark & Rosen) was not sufficient to prevent insemination in six out of eight operated males.

In the platyfish, *X. maculatus,* the holdfast
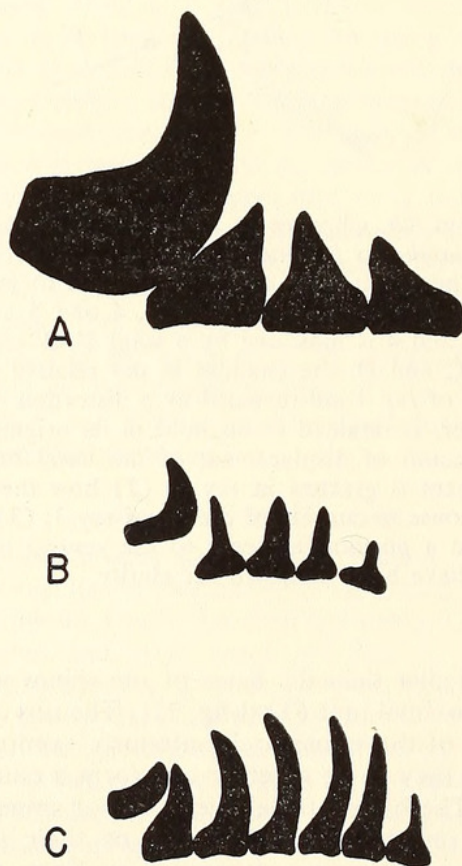


TEXT-FIG. 28. Dorsal terminal holdfast elements (claw and distal serrae) in the gonopodia of xiphophorin fishes. A. *Xiphophorus helleri* Heckel. B. *Xiphophorus pygmaeus* Hubbs & Gordon. C. *Xiphophorus montezumae* Jordan & Snyder.

device for securing contact between male and female is somewhat different from that of the swordtail. Functionally, the distal serrae of the platyfish replace the swordtail claw. When the gonopodium is moved forward to the copulatory position, ray 5 is rotated over adjacent to ray 3.
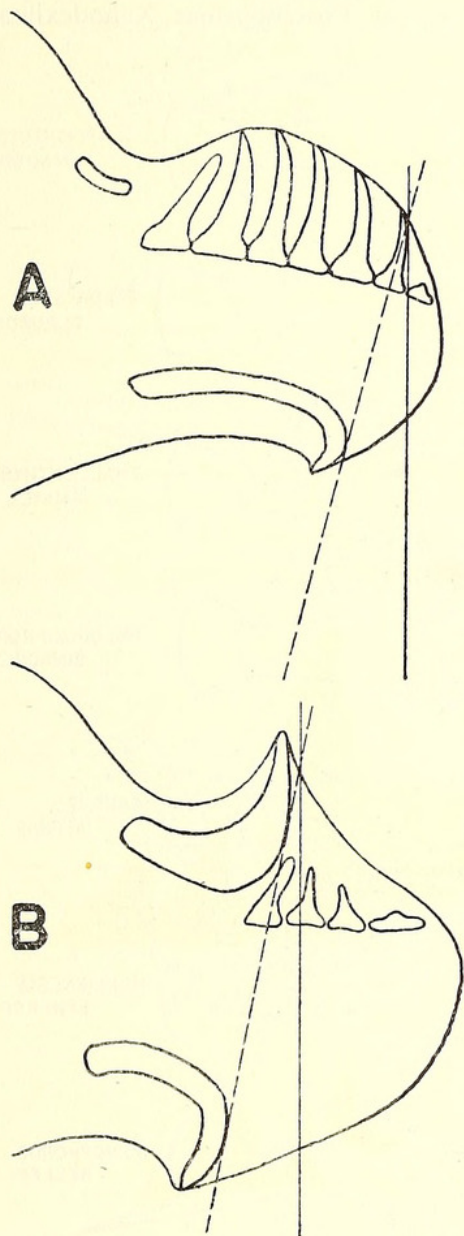


TEXT-FIG. 29. Profiles of the gonopodial tips of two xiphophorin species with the dorsal and ventral holdfast elements indicated. The more proximal position of the ventral element is measured by the angle between the dorsal and ventral series, determined by reference to a vertical dropped from the first projecting dorsal segment. A. *Xiphophorus maculatus* (Günther), B. *Xiphophorus helleri* Heckel. These angles are approximately equal in both species. Note the shorter stem and more proximal position of the ventral hook in *X. helleri* (B) when compared with the same elements in *X. maculatus* (A).

In addition, ray 4, carrying the distal serrae, is also forced to slip sideways, although not to the same extent, with the result that in the platyfish the hook and the distal serrae act together as the holdfast mechanism (Text-fig. 26). This relationship of the hook to the distal serrae is also found in *X. montezumae* and the other three species of platyfishes (*X. variatus, X. xiphidium* and *X. couchianus*).

In the gonopodium of the pygmy swordtail, *X. pygmaeus,* the distal serrae and the claw seem to be equally important as gripping devices (Text-fig. 25). The distal serrae in this species are relatively larger than those of *X. helleri* (Text-figs. 27 and 28), and the claw is smaller. However, the claw projects sufficiently to function as an effective holdfast.

The differences in the structure of the holdfast mechanism between *X. maculatus* and *X. helleri* are compensating differences in the sense that the serrae and the claw function similarly but develop from different fin rays, i.e., are probably not homologous.

In all xiphophorin species, with the exception of *X. helleri,* the distal serrae are probably functional in gripping the female. In the resting fin, the distal serrae extend out to the tip of the epidermal sheath. In these species the long hook is below and behind the serrae.

In *X. helleri* the claw, and not the distal serrae, is important in maintaining contact during copulation. The large claw in the swordtail, like the claw in the other species of xiphophorins, originates behind the distal serrae. Associated with the more proximal position of the dorsal spinous element in the swordtail gonopodium is a decrease in the length of the ventral hook on ray 3. This compensatory device in the swordtail permits the two projecting holdfast tips to maintain the same position relative to each other, both in the resting and active fin, that is observed in the gonopodia of the other xiphophorin species (Text-fig. 29).

## III. COMPARATIVE ANALYSIS

### A. Inter-relationships of Gonopodial Structures in the Poeciliidae

#### 1. Relationship of Gonopodial Length to Body Size

Although the interactions of the specialized gonopodial elements involve such dynamic processes as folding or rotation and the largely compensatory adjustment of slipping, features other than intrinsic mechanical arrangements are important in determining the effectiveness of the gonopodium. Among the additional extrinsic factors are (1) the overall length of the gonopodium in relation to the length of the fish,

and (2) the point of origin of this fin on the male's body (Text-fig. 30).

Regan (1913) thought that the relative length of the gonopodium in certain poeciliids is related to the approachability of the female during sexual activity. He implied that the females of species (like those of *Poecilia* and related genera) in which the male's gonopodium is short are more receptive than females of species (like those of *Gambusia* and *Phallocerus*) in which the gonopodium is long. Our recent studies do not bear this out. Female *Phallichthys amates* and *Poecilistes pleurospilus* apparently cooperate during the male's sexual advance in a manner somewhat comparable to that of female *Lebistes reticulatus*.

The diagram of eight species of representative poeciliid genera (Text-fig. 30) indicates that neither length nor position of the gonopodium in relation to the fish as a whole shows constant variations or any obvious structural correlatives. Nevertheless it may eventually be shown that the length of the gonopodium is closely related to special structural modifications not yet detected. The combined items of behavior and morphology are undoubtedly co-adapted in different ways and to varying degrees in the different species and genera, as illustrated by the analysis of seven xiphophorin species (discussed above on page 23). However, considering gonopodial length as a criterion for separating the poeciliid genera, two classes may be distinguished, one of which proves to be a natural grouping and the other chiefly an artificial one.

The first group includes the Tribes Xiphophorini and Pamphorini, the Subfamily Alfarinae and the Tribe Poeciliini, the latter containing seven genera, some of them perhaps only nominal (Text-figs. 37-40). In each of these groups, the gonopodium is quite short in relation to the standard length of the fish and the fin has an anterior insertion, almost between the pelvic fins, which are well developed. In *Xiphophorus* the gonopodium is about 25 per cent of the standard length, and its origin just posterior to the pelvic girdle is at the vertical from the origin of the dorsal fin. In both *Alfaro* and the poeciliin genera (*Poecilia, Micropoecilia, Limia, Parapoecilia, Lebistes, Allopoecilia* and *Mollienesia*), the gonopodium is conspicuously short, being slightly less than 25 per cent of the standard length and its origin is well anterior, midway between verticals from the origins of the dorsal and pectoral fins. On the basis of related gonopodial characters Hubbs (1926) grouped them in the Subfamily Poeciliinae. All of them, for example, have variously developed

integumentary growths on the anterior margin of the 3rd ray. In the xiphophorin species the growths of epidermal and dermal tissues are only moderately greater than in other regions, whereas in poeciliin species they are quite prominent and are of taxonomic value.

The second group includes the Subfamilies Gambusiinae, Poeciliopsinae, Xenodexiinae and
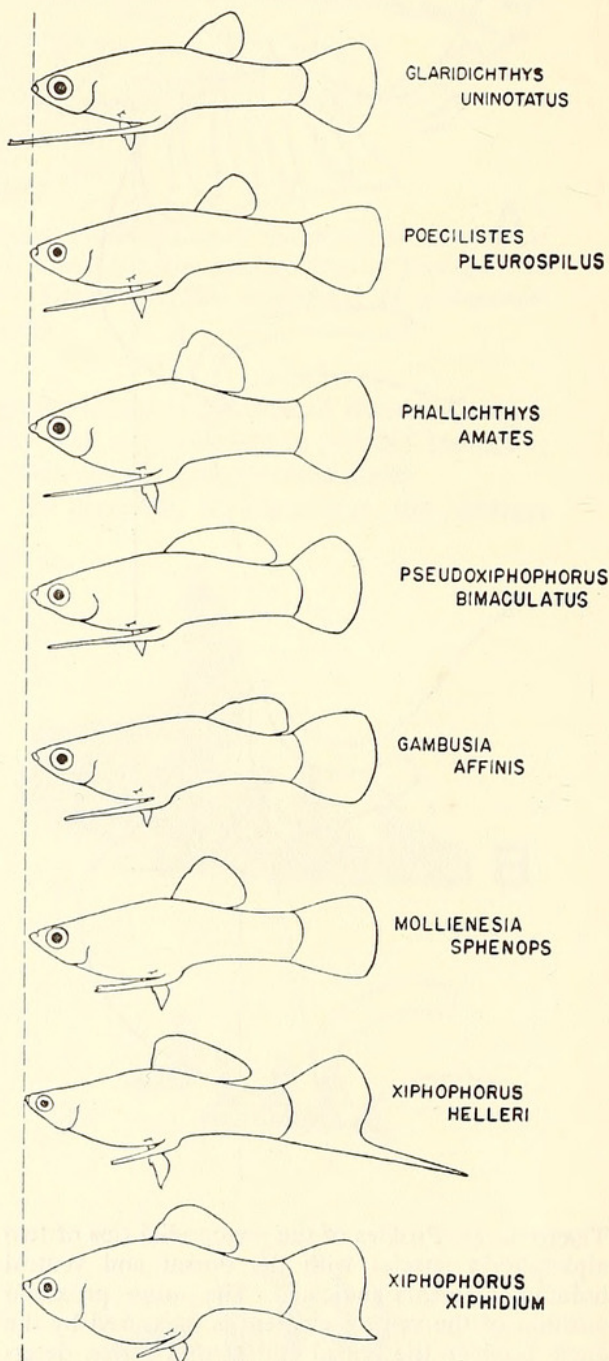


TEXT-FIG. 30. Comparison of the males of eight representative species of poeciliid fishes with their gonopodia swung forward into the erected position. The fish are drawn to the same size (standard length) to illustrate differences in the lengths and relative positions of their gonopodia. The left ventral fin is cut off at the base.
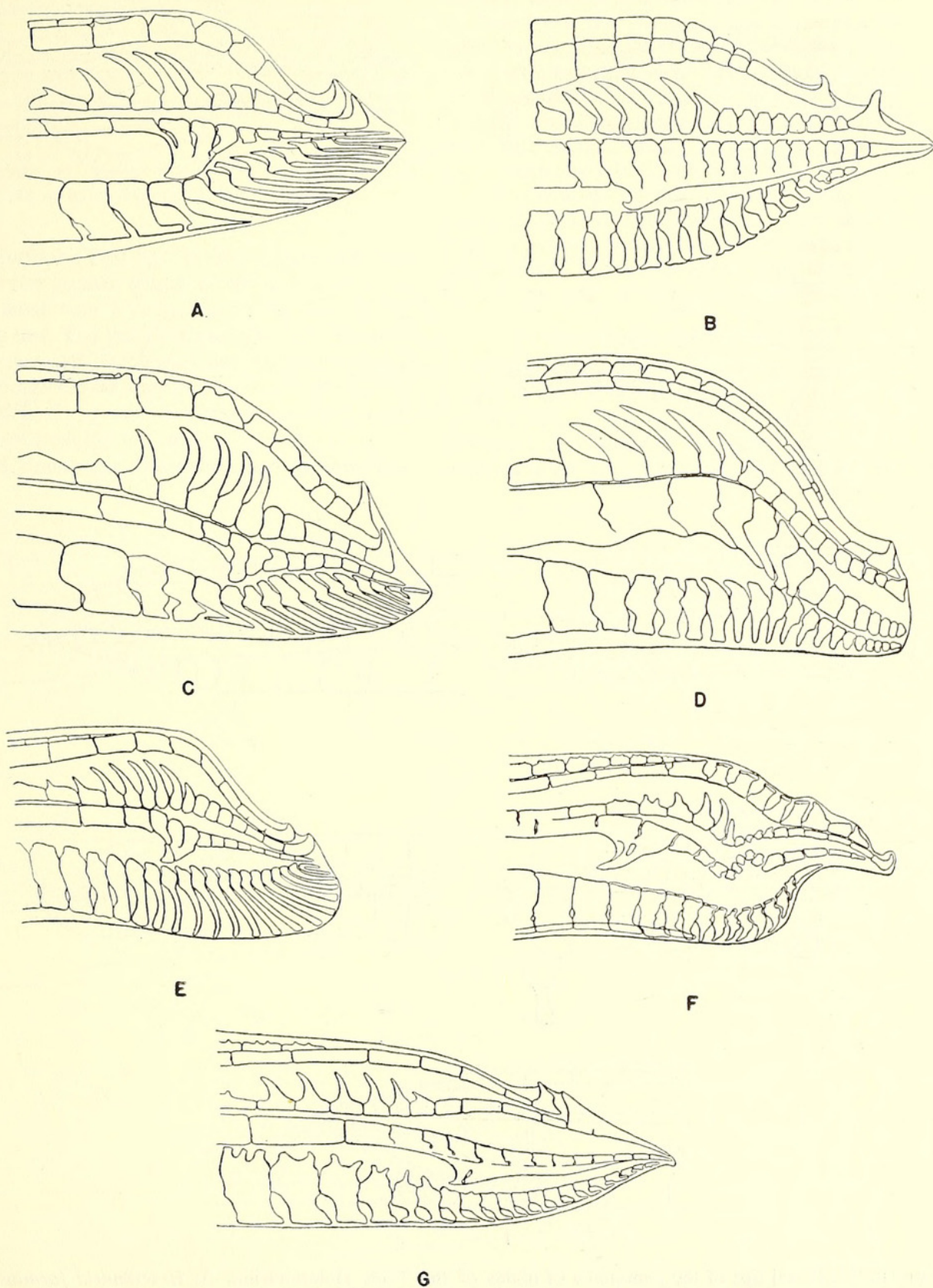
TEXT-FIG. 31. Distal tips of the gonopodia of males of the Tribe Gambusiini. A. *Gambusia nobilis* (Baird & Girard). B. *G. lemaitrei* Fowler. C. *G. nicaraguensis* Günther. D. *Belonesox belizanus* Kner, E. *G. panuco* Hubbs. F. *Heterophallus rachowi* (Regan) (=*Gambusia atzi* Rosen & Gordon). G. *G. affinis* (Baird & Girard).
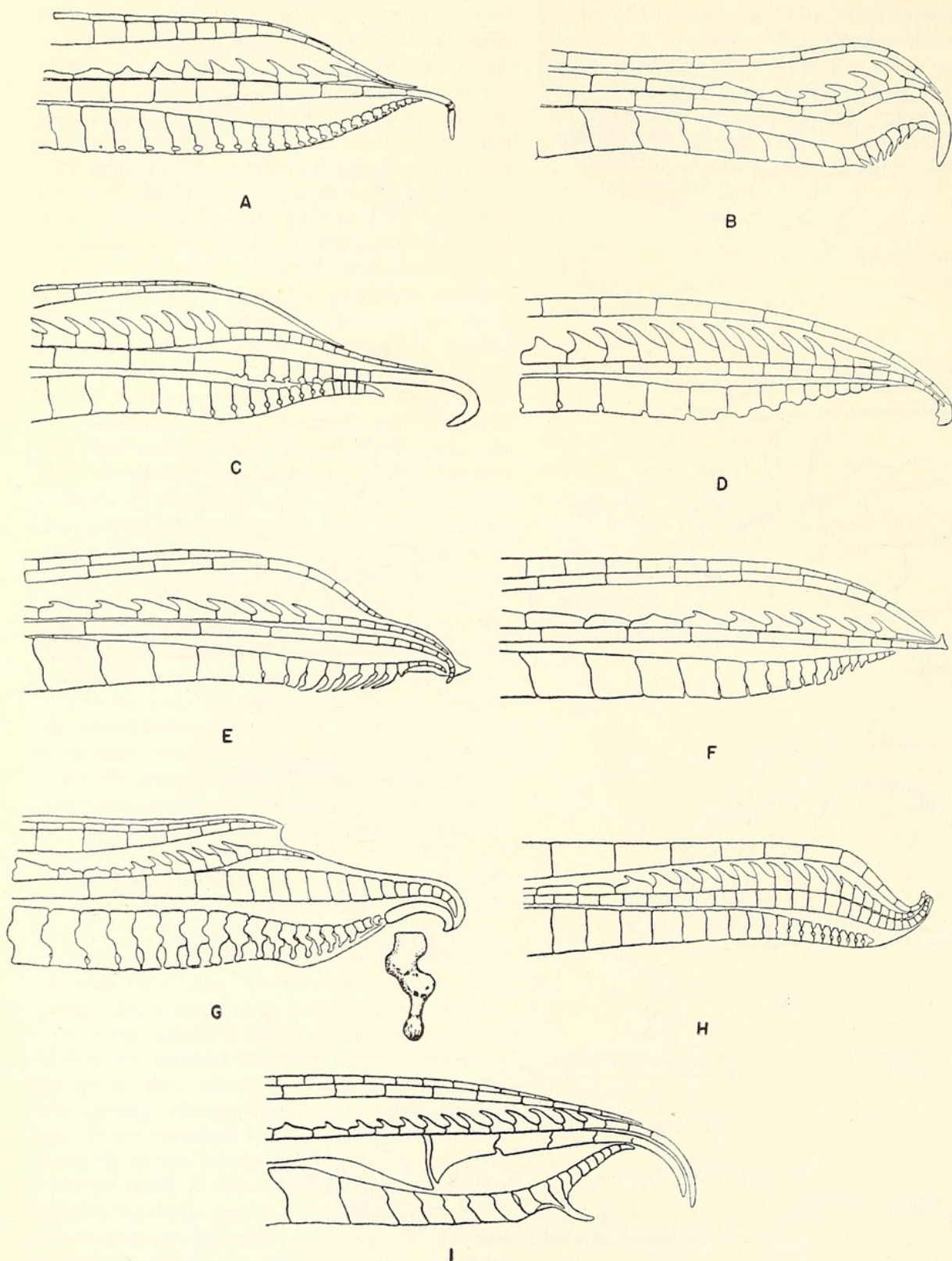
TEXT-FIG. 32. Distal tips of the gonopodia of males of the Tribe Heterandriini. A. *Heterandria formosa* Agassiz. B. *Neoheterandria elegans* Henn, after Henn, 1916. C. *Pseudoxiphophorus bimaculatus* Heckel. D. *Priapichthys annectans* Regan, after Regan, 1913. E. *Pseudopoecilia fria* (Eigenmann & Henn), after Henn, 1916. F. *Alloheterandria nigroventralis* (Eigenmann & Henn), after Henn. G. *Priapella bonita* Meek. Insert shows a spine enlarged. H. *Brachyrhaphis rhabdophora* (Regan), after Regan, 1913. I. *Allogambusia tridentiger* (Garman), after Meek & Hildebrand, 1916.
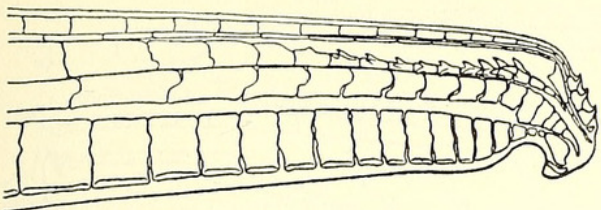
TEXT-FIG. 33. Distal tip of the gonopodium of *Quintana atrizona* Hubbs, Tribe Quintanini.

Tomeurinae (Text-figs. 31-36). In most of these fishes the greater length of the gonopodium is associated with a reduction in the size of the pelvic fins. The length of the gonopodium is 50 to 70 per cent of the standard length. The longest gonopodia in this group are found among the girardinins and poeciliopsines. The most anterior position of the gonopodium is to be found among the tomeurines and gambusiines,

namely, about 2 to 6 scale rows posterior to a vertical from the pectoral base; those of the other forms originate close to the vertical from the dorsal fin origin. With reference to individual gonopodial characters and those of the skull and dentition, these genera form a heterogeneous assemblage, having in common only the great elongation of gonopodial rays 3, 4 and 5. The members of this arbitrary group constitute the bulk of poeciliid fishes. They demonstrate a general trend toward elongation of the gonopodium and, at the same time, the reduction of size and effectiveness of the pelvic fins.

## 2. Symmetries and Asymmetries

Detailed descriptions have already been presented of the bilateral arrangement of the paired lepidotrichia of the gonopodium around a central plane of symmetry. Bilaterally symmetrical gonopodia are found not only among the males
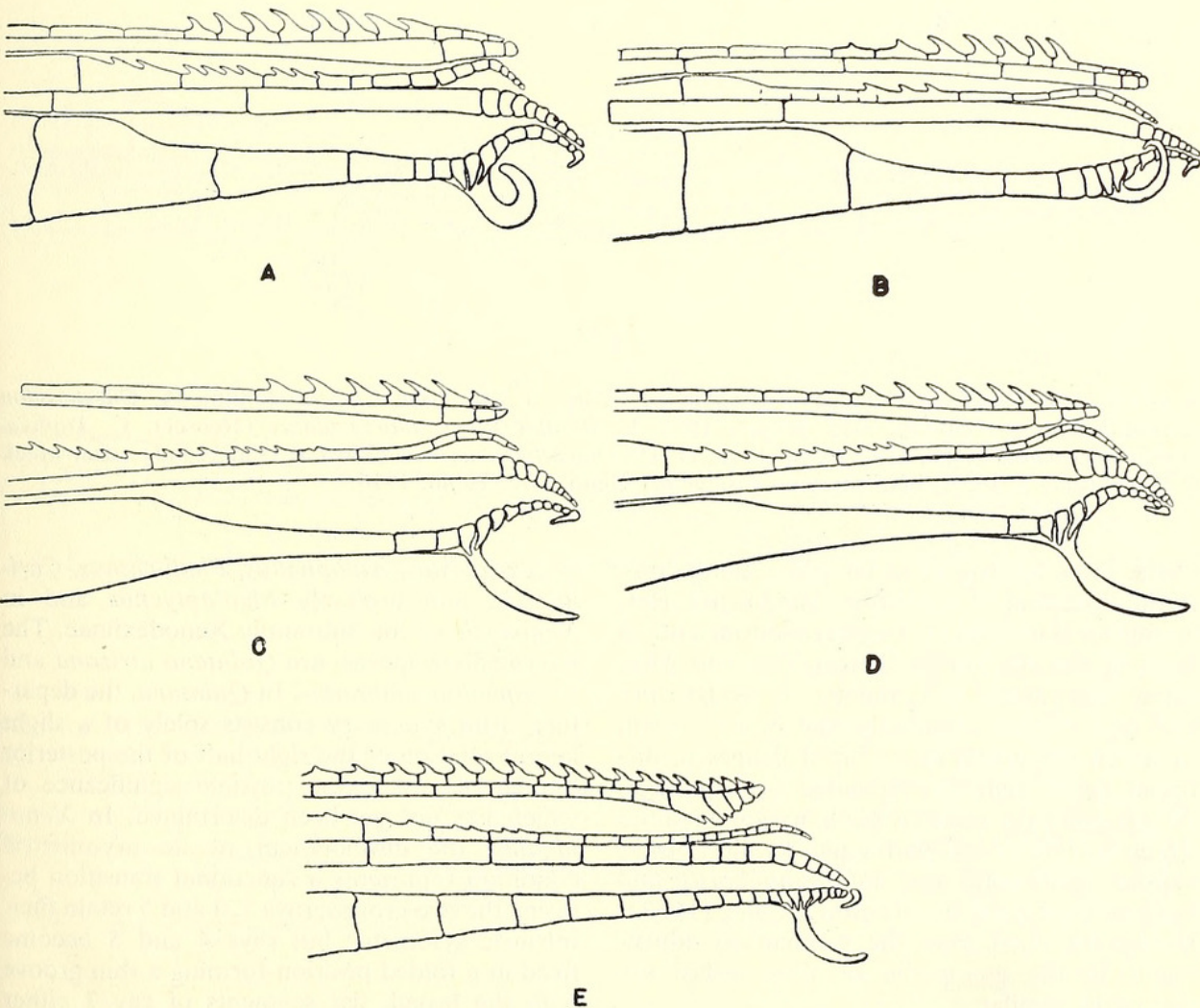


TEXT-FIG. 34. Distal tips of the gonopodia of males of the Tribe Girardinini. A. *Girardinus metallicus* Poey. B. *Glaridichthys uninotatus* (Poey), after Phillipi, 1908. C. *Dactylophallus denticulatus* (Garman), after Howell Rivero & Rivas, 1944. D. *Allodontium cubense* (Eigenmann), after Howell Rivero & Rivas; 1944. E. *Toxus creolus* Eigenmann, after Howell Rivero, 1946.
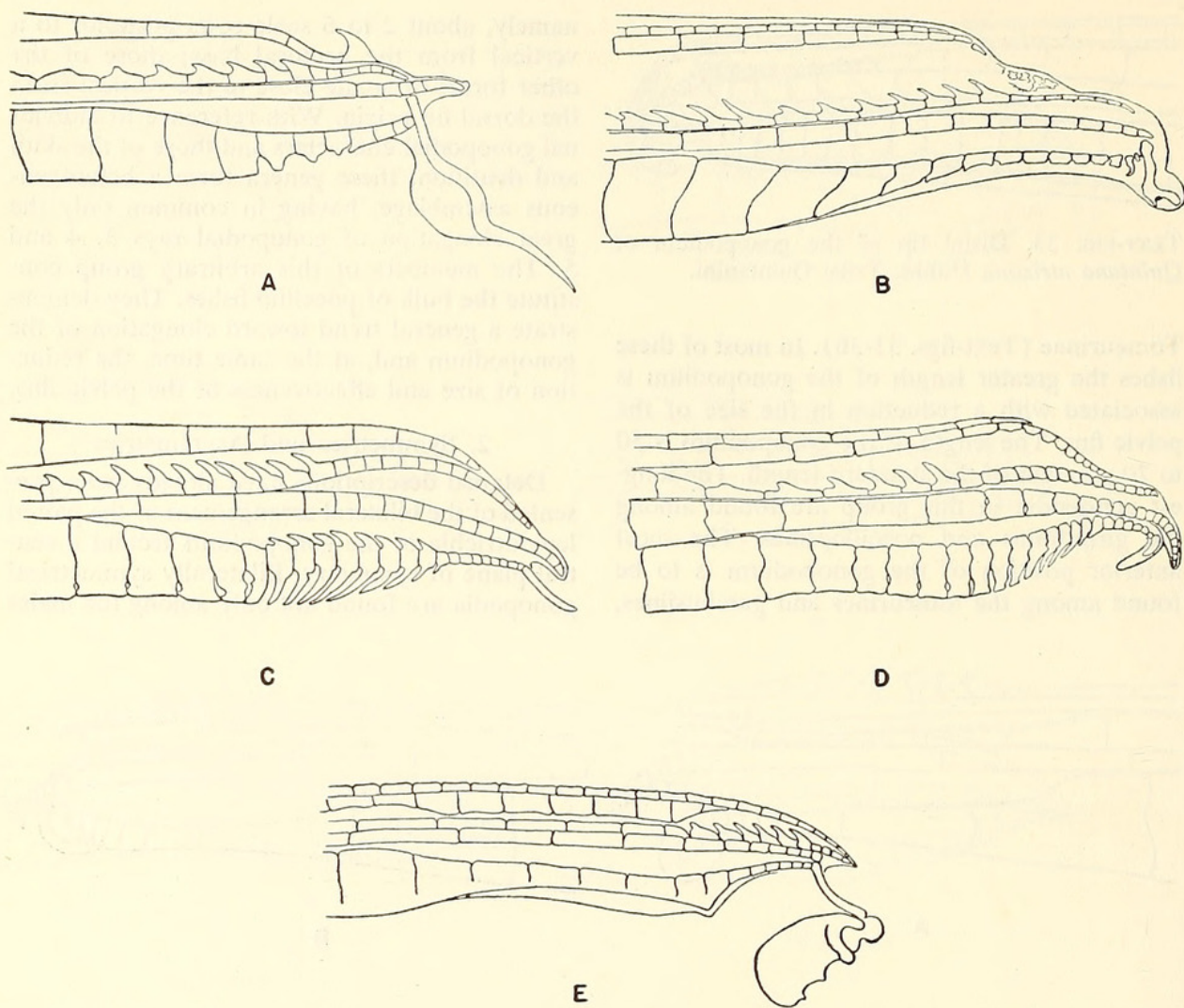
TEXT-FIG. 35. Distal tips of the gonopodia of males of the Tribe Cnesterodontini. A. *Cnesterodon decemmaculatus* (Jenyns), after Regan, 1913. B. *Phallocerus caudomaculatus* (Hensel). C. *Diphyacantha chocoënsis* Henn, after Henn, 1916. D. *Darienichthys dariensis* (Meek & Hildebrand), after Meek & Hildebrand, 1916. E. *Phallotorynus fasciolatus* Henn, after Henn, 1916.

of the Tribe Xiphophorini but also among those of the Poeciliini, Pamphorini, Gambusiini, Heterandriini, Girardinini, Cnesterodontini and in those of the Subfamilies Tomeurinae and Alfarinae. The pattern of symmetry of the gonopodial rays follows essentially that of an I-beam in which the dorsal and ventral flanges in different forms may be expanded or depressed. This enables the male to move its gonopodium either to right or left with equal facility; observations confirming this in *Xiphophorus* and *Lebistes* were made by Aronson & Clark (1952). It appears, then, that the mechanical adjustments in the gonopodia of these fishes are essentially similar.

Modifications of the symmetrical arrangement are found in different degrees in the other poeciliids. A climax of asymmetry is reached in the fishes of the poeciliopsine genera *Poeciliop-*

*sis, Poecilistes, Aulophallus, Phallichthys, Carlhubbsia* and probably *Phalloptychus* and in *Xenodexia* of the Subfamily Xenodexiinae. The intermediate species are *Quintana atrizona* and *Xenophallus umbratilis*. In *Quintana*, the departure from symmetry consists solely of a slight lateral rotation of the right half of the posterior branch of ray 4, the possible significance of which has not yet been determined. In *Xenophallus*, the development of an asymmetric condition represents a functional transition between the two groups; rays 3, 4 and 5 retain their intrinsic symmetry but rays 4 and 5 become fixed in a folded position forming a thin groove with the broad, flat segments of ray 3 either along the left or right sides of the gonopodium. Thus, when the gonopodium of *Xenophallus* is unfolded mechanically it reveals its bilateral symmetry; but in its normal permanently folded

RAY V

RAY IV

RAY III

A

B

C

D
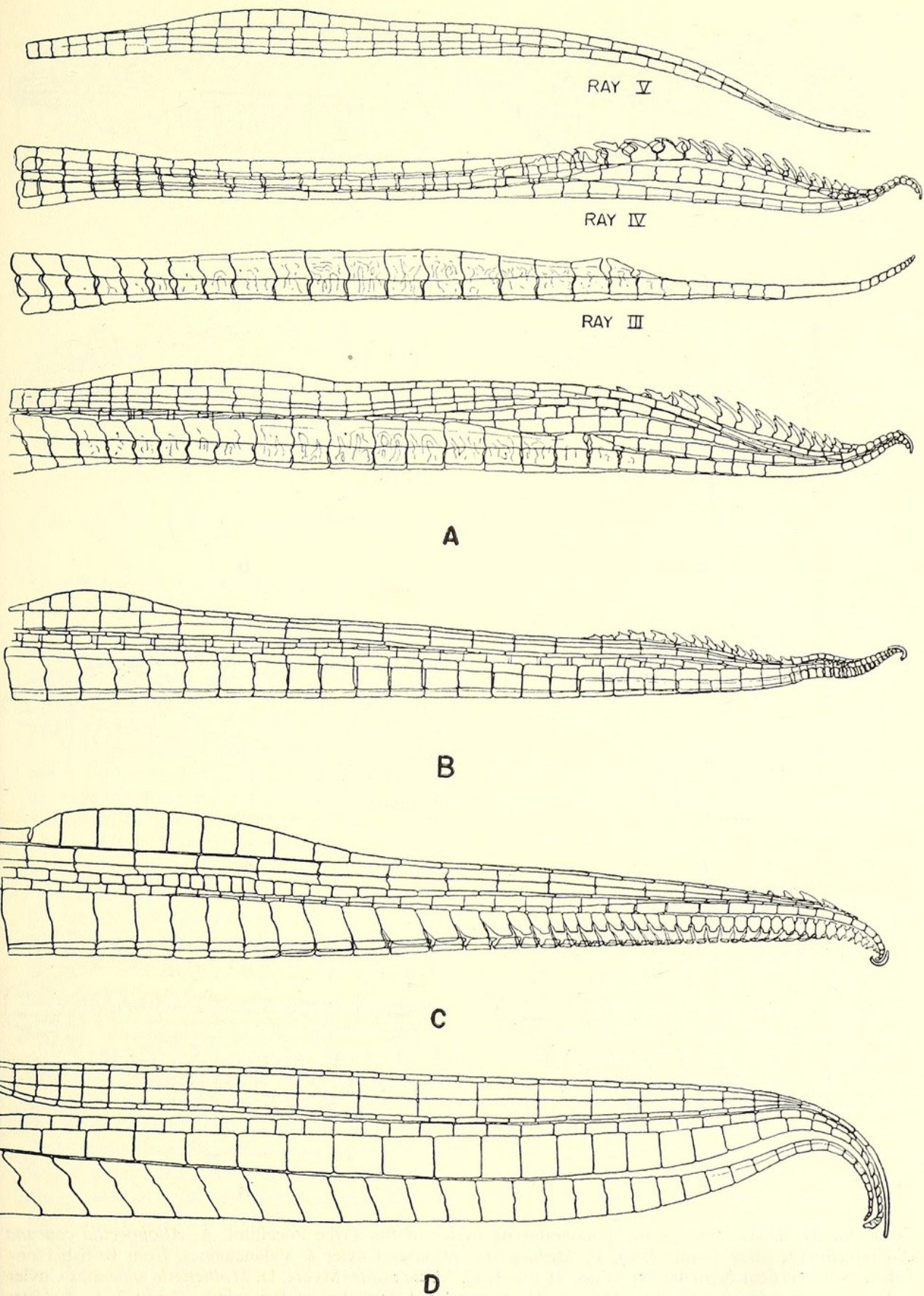
Text-fig. 36. Gonopodia of males of the Subfamily Poeciliopsinae. A. *Poeciliopsis presidionis* (Jordan & Culver). Rays 3, 4 and 5 are figured individually above. B. *Poecilistes pleurospilus* (Günther). C. *Phallichthys amates* (Miller). D. *Xenophallus umbratilis* (Meek).
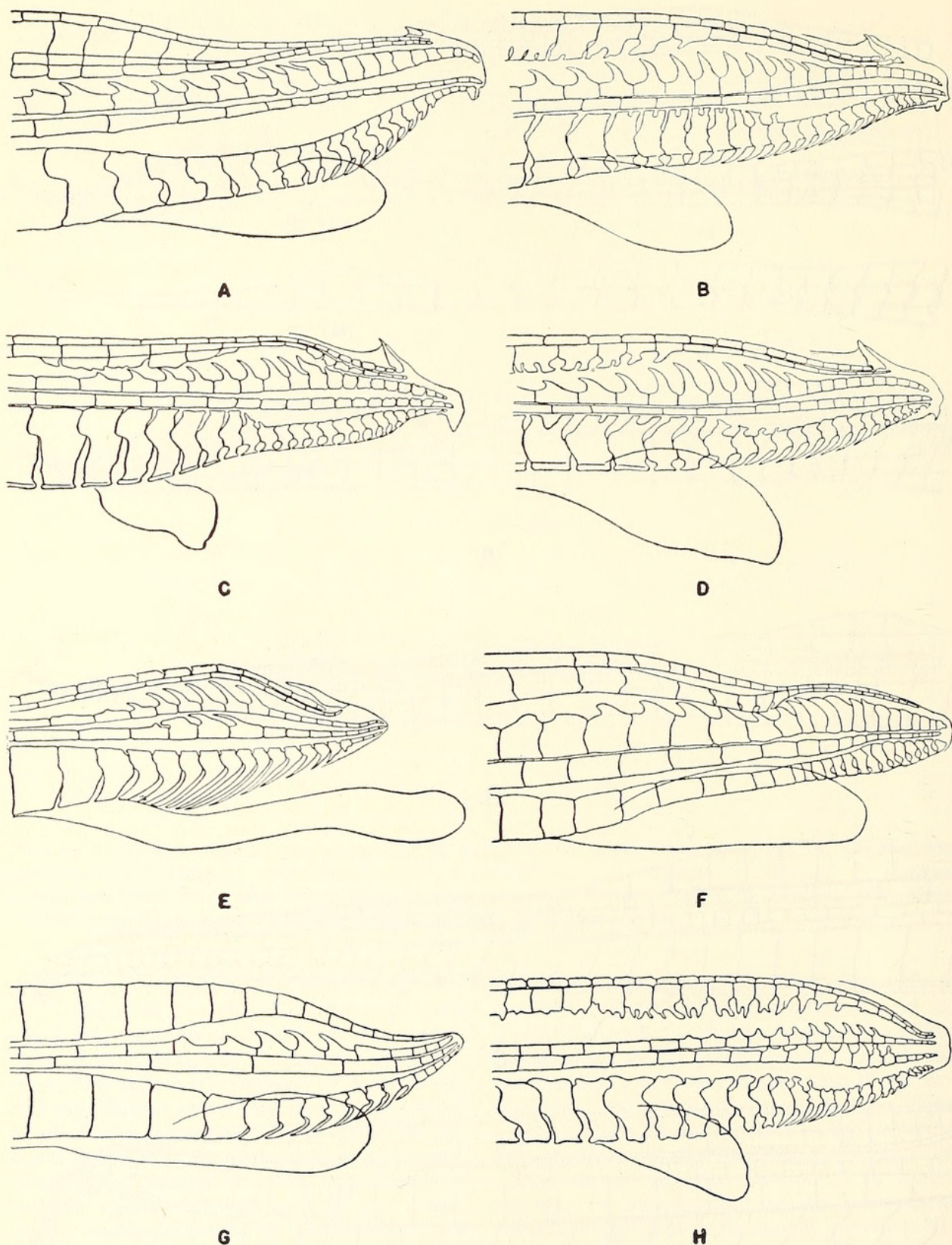
TEXT-FIG. 37. Distal tips of the gonopodia of males of the Tribe Poeciliini. A. *Allopoecilia caucana* (Steindachner), after Henn, 1916. B. *Mollienesia sphenops* Cuvier & Valenciennes, from British Honduras. Note vertical denticles on spines of ray 3. C. *Limia noblei* Myers. D. *Mollienesia sphenops* Cuvier & Valenciennes, from Veracruz, Mexico. Note angulated denticles on the spines of ray 3. E. *Lebistes reticulatus* (Peters). F. *Parapoecilia hollandi* (Henn), after Henn, 1916. G. *Micropoecilia parae* (Eigenmann), after Regan, 1913. H. *Poecilia vivipara* Bloch & Schneider, after Hubbs, 1924.
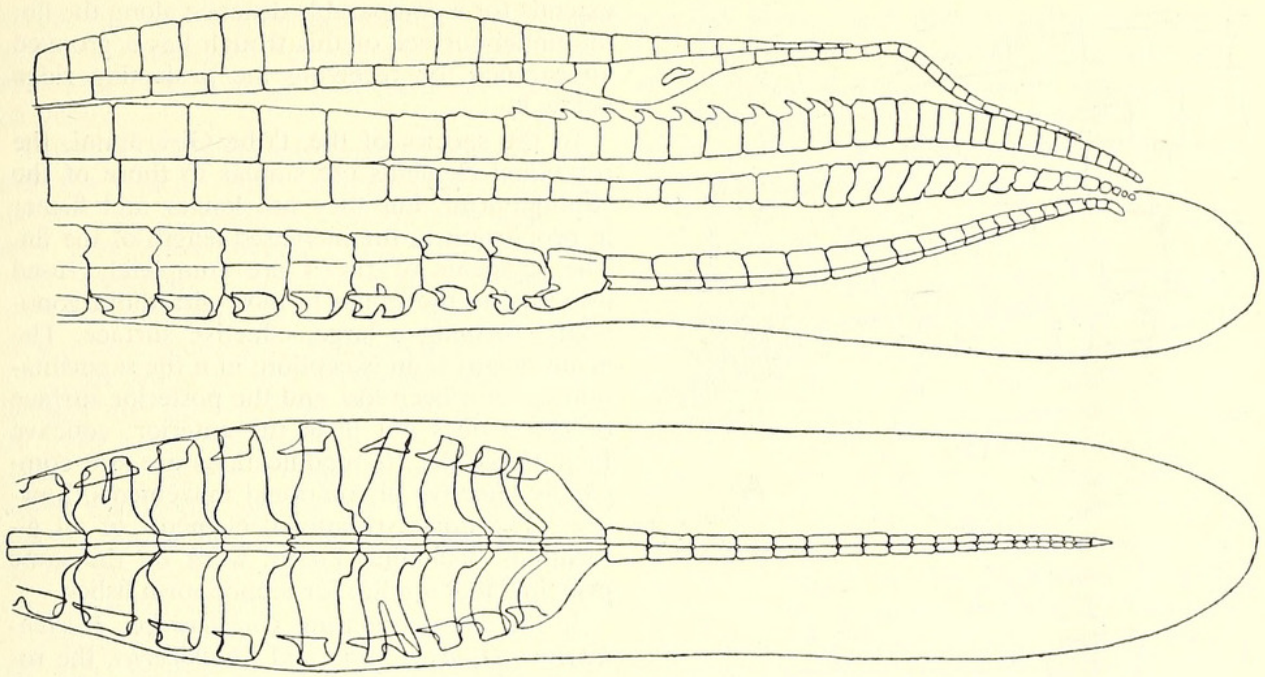
TEXT-FIG. 38. Gonopodium of *Limia vittata* Guichenot. Above, lateral view. Below, ventral view.

state it displays a sinistral or dextral asymmetry.

A *Xenophallus*-like asymmetry is characteristic of the other six poeciliopsine genera, but with the exception of the dextral *Carlhubbsia kidderi* their rays are deflected always to the sinistral position. In addition, the poeciliopsine genera possess other conspicuous modifications of their gonopodia which have so obliterated the basic internal symmetry patterns of individual rays that even the intrinsic symmetries of the *Xenophallus* gonopodium are lacking in these forms. These conspicuous changes which produce permanently, sinistrally of dextrally, folded rays, probably serve to increase the effectiveness of the gonopodial groove. Perhaps a permanently folded gonopodium is required to meet special demands as yet undetermined.

### 3. Rotational and Adjusting Mechanisms

The configuration of the groove that runs along one side of the erected symmetrical or asymmetrical gonopodium is dependent to some extent on the degree and kind of symmetry of the bony elements and their enveloping tissues. The mechanism that guides the rays into their folded positions consists of two units, a tongue and groove arrangement between rays 3 and 4, and a knife and beam relationship between rays 4 and 5 ("canoe" and "canoe support"). Both of these devices enable the rays to rotate in such a way that they form a trough when folded. It is along this trough, almost of capillary dimensions, that spermatophores undoubtedly pass from male to female during copulation.
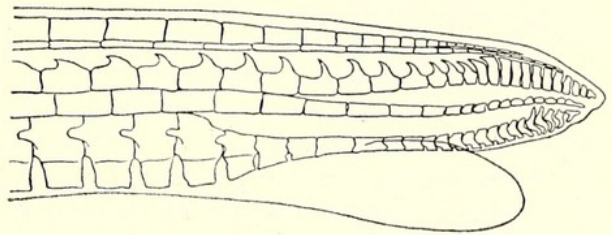


TEXT-FIG. 39. Distal tip of the gonopodium of *Pamphorichthys minor* (Garman). Our studies suggest that *Pamphorichthys* may be a synonym of *Parapoecilia,* see Text-fig. 37, F.

In those species possessing symmetrical gonopodia, the rotational elements make possible the formation of a trough on either side of the fin. In xiphophorin fishes, the anterior (ventral) center of rotation between rays 3 and 4 is located distally in the fin just proximal to the spines of ray 3. In lateral view the segments of ray 3 arch gently upward until their posterior, rounded portions are almost in contact with the concave surface formed by two halves of ray 4a (Text-fig. 12). The effective ventral rotational surface in these gonopodia extends nearly to the base of the fin. The posterior (dorsal) center of folding, which brings ray 5 down adjacent to ray 4 in the erected fin, originates about 5 segments proximal to the proximal serrae of ray 4p at the point where the elements of ray 5 broaden and flare out. The basal supporting knife-like portion of this system, upon which ray 5 rests, consists entirely of the raised, slender
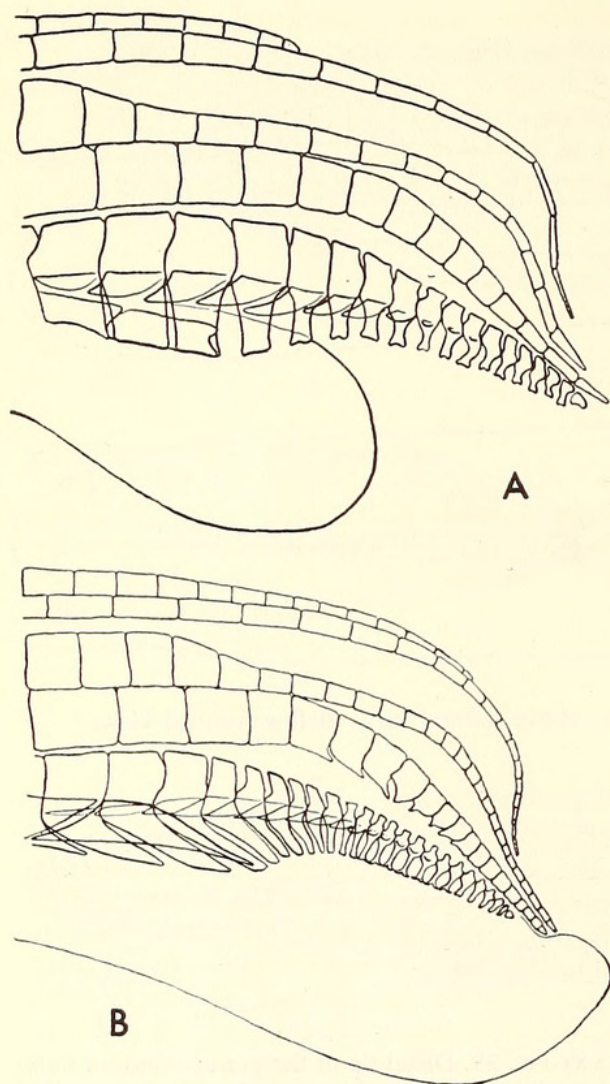
TEXT-FIG. 40. Distal tips of the gonopodia of males of the Subfamily Alfarinae. A. *Alfaro cultratus* (Regan). B. *Alfaro huberi* (Fowler).

segments of ray 4p. Viewed laterally, these segments together resemble a depressed bell-shaped curve, the raised but flattened region serving to support ray 5. This ridge extends proximally to within 10 to 15 segments from the base of the fin. The canoe-like outer surface of ray 5

extends for a comparable distance along the fin; the under-surface of this trough has a grooved appearance for receiving the projecting ridge below it.

In the species of the Tribe Girardinini, the rotational elements are similar to those of the Xiphophorini, but they are longer and firmer in proportion to the increased length of the fin. The segments of ray 3 are completely fused proximal to the spines in most girardinin gonopodia, forming a large, effective surface. The genus *Toxus* is an exception; in it the segmentation has not been lost and the posterior surface of ray 3 does not meet the anterior, concave face of ray 4a. Its modifications are still completely effective in rotational movements, however. The dorsal rotational elements of all girardinins, including *Toxus,* work on the same principle that applies for xiphophorin fishes.

In the cnesterodontins, *Cnesterodon, Darienichthys, Diphyacantha* and *Phallocerus,* the rotational centers are similar to those of the girardinins except that the initial distance between rays 3 and 4a at the tip of the fin is less. *Phallotorynus,* however, has a curious inversion in the ray 5 portion of the dorsal system. The supporting ridge of ray 4p in this genus has been replaced by an inverted ridge of comparable form which is composed of the segments of ray 5a that rest on the depressed 4p elements below. In the Tribes Gambusiini and Heterandriini, which are included in the Subfamily Gambusiinae, the rotational elements are depressed as in *Toxus* and the fins are long as in the Girardinini and Cnesterodontini. In the Poeciliini, a feature is present relating the fishes of this tribe functionally with *Phallotorynus.* In some members of this group, the elements of ray 5a project downward and terminate in denticulate processes that rest on the lightly raised platform in the same fashion as the inverted ridge of *Phallotorynus.* The ventral elements in the Poeciliini are composed chiefly of extensions on the posterior margin of ray 3 which offer support to ray 4a.
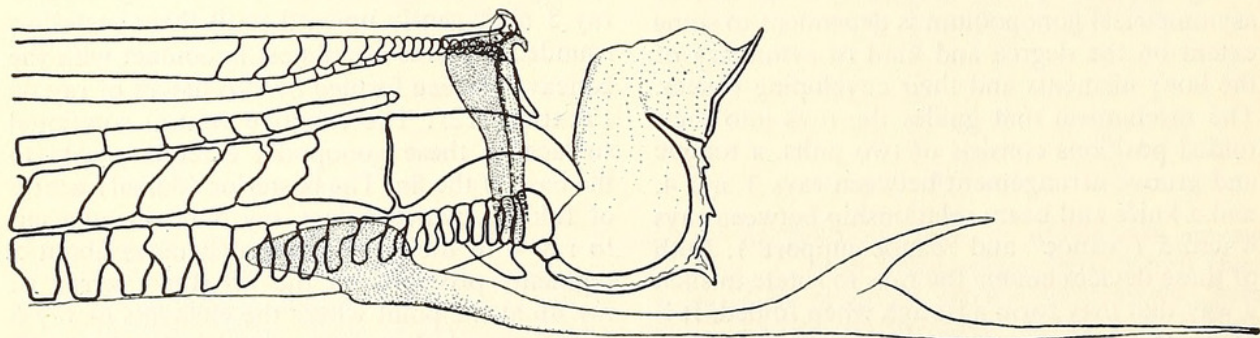


TEXT-FIG. 41. Distal tip of the gonopodium of *Tomeurus gracilis* Eigenmann, Subfamily Tomeurinae, probably Family Tomeuridae.

Frequently these extensions grade into the spines, producing the characteristic denticulations which are found in *Gambusia*—and also in *Mollienisia.*

Fishes which possess asymmetrical gonopodia lack the typical rotational elements described above. The permanently folded condition of their gonopodia suggests that rotational elements either are of specialized form or do not function as such. In *Xenophallus,* which retains an intrinsic gonopodial symmetry, the ventral rotational center is fully developed, possibly indicating that some further rotatory movements do occur here. The dorsal system, however, is absent and the ray 5 complex appears to be folded maximally against ray 4. In the other poeciliopsine genera, rudiments of both the dorsal and ventral systems are present; in *Poeciliopsis* and *Poecilistes* they are mainly vestigial.

Despite structural differences that appear in the fully mature and completely crystallized gonopodia of the symmetrical and asymmetrical groups, certain features of similarity are present in the gonopodial morphogenesis of both, which may indicate their possible mode of evolution. In both, the elaboration of a gonopodium begins with a perfectly symmetrical median fin. In the early stages of sexual differentiation, rays 3, 4 and 5 elongate. While differential growth is progressing, differentiation of specialized elements occurs to produce the individual segmentation patterns that characterize each species. The elements that appear first in their final state are located proximally in the fin. This is the region in which the rotational movements take place in fishes with symmetrical gonopodia.

In the latter fishes, the rotational centers are built up into a functional mechanism long before the terminal elements are differentiated. In fishes possessing asymmetrical gonopodia, the same sequence of developmental processes takes place, i.e., a symmetrically arranged rotational mechanism is formed first. In the asymmetrical types, however, all the gonopodial segments become further specialized. In this process, the 4th and 5th rays, together with elements of the rotational mechanism, produce a sinistral folding *during morphogenesis.* Only after this has been accomplished do the rays then lose their intrinsic symmetry by the addition of new structures and the realignment of the lepidotrichial elements. This circumstance suggests that forms ancestral to the poeciliopsines possessed symmetrical gonopodia; in evolution their gonopodia were probably further modified to produce the asymmetrical type with its permanent, left-sided gonopodial groove. Our numerous and repeated observations of the living poeciliopsines, *Phallichthys amates* and *Poecilistes pleurospilus,* indicate that permanent, sinistral folding of the gonopodium restricts copulation to the sinistral position. In contrast, so far as known, fishes with symmetrical gonopodia are able to copulate from either side.

### 4. Terminal Specializations

Whereas the subdistal specializations of the gonopodial segments are concerned primarily with mechanical adjustments in forward movements of this fin prior to copulation, the terminal elements appear to act during copulation. When classified according to function, the terminal specialized elements fall into at least five categories, discussed in sections "a" to "e" below. The most distal elements are simple in form, being trowel-shaped or merely pointed. In many species, the portion of the gonopodium that is inserted into the female's genital opening during copulation is supplemented by holdfast devices which serve to secure and maintain contact between the sexes. The holdfasts, which usually consist of serrae or barbs, mostly retrorse, are aided by two kinds of stabilizing mechanisms. The internal mechanisms assist in retaining the alignment of the rays, while the external kinds steady the gonopodium during insertion. The effectiveness of the terminal complex is probably mediated, in part, by a fifth series of specialized elements on the terminal portion of ray 3, the spines, which appear to serve a sensory function. When all of the necessary mechanical operations have been successfully fulfilled, these sensory structures may then stimulate the discharge of spermatophores.

### a. Organs of Contact and Insertion

Actual insertion of the gonopodial tip during copulation has been definitely observed in only three species of two genera of poeciliids, namely, *Xiphophorus* and *Lebistes.* In *Xiphophorus,* the inserted portion is shaped like a trowel and consists of the terminal elements of rays 4a and 4p. These segments project out at the tip and form a rigid platform that is pointed distally. In *Lebistes* and other poeciliin genera, and in the genera *Alfaro* and *Pamphorichthys,* rays 3, 4 and 5 participate in producing a pointed tip, with ray 4, the longest of the rays, forming the apex. In *Heterandria* and *Pseudoxiphophorus,* insertion is probably effected by a single fused element originating at the tip of ray 4a. Insertion of the gonopodial tip has not been established for any other poeciliid genera, and for some it appears uncertain that insertion is possible. In most gambusiines, the gonopodial tip is blunt and supplied with antrorse projections which would seem to

impede rather than aid insertion. In the cnes-
terodintins, girardinins and in *Tomeurus,* out-
growths of the 3rd ray probably prevent inser-
tion altogether. Insertion of the slender tip in the
poeciliopsine genera is mechanically possible,
but our observations on living *Phallichthys* sug-
gest that contact is, as a rule, not enduring and
that if insertion is effected, it is only in a very
small percentage of the total contacts, as in
*Xiphophorus.*

b. Holdfast Devices

In those genera in which insertion is regularly
achieved, contact is maintained by the presence
of holdfast devices that usually project from rays
3 and 4. In *Xiphophorus* the holdfast mechanism
is composed of dorsal and ventral segments: the
dorsal distal serrae (or claw on ray 5a) and the
ventral hook. In *Priapella,* only the ventral hook
is present, while in the Gambusiini only dor-
sal claws are found. In many poeciliins small
claws are found embedded in the tissue above
ray 5; these may play some role as holdfast
devices. On the other hand, many poeciliin spe-
cies normally lack claws. In this connection,
Sengün (1949) and Clark & Aronson (1951)
have shown that a claw in *Lebistes* is not essen-
tial for successful copulation. They amputated
the tips of gonopodia, which regenerated in time,
but some guppies lacked the claws. Neverthe-
less clawless male guppies could still inseminate
females. These experiments, of course, were
carried out under laboratory conditions. It is
highly probable, however, that *Lebistes,* living
under natural conditions, in rapid waters, as
they do in parts of Trinidad, require their claws
for successful sexual contacts.

c. Organs of Internal Stabilization

When the gonopodium is held erect prior to
and during copulation, stress is applied to the
connective tissue which binds the rays together.
During erection, in the absence of any compen-
satory adjustments, the alignment of the termi-
nal elements would probably be disturbed by
the tension so established. The proximal serrae
of ray 4p, however, anchor the binding tissues
and thus maintain the relationships of the ter-
minal features. This device is found almost uni-
versally in poeciliid gonopodia. Proximal serrae
are lacking in only three known species, in
*Alfaro cultratus, Tomeurus gracilis* and *Xeno-
phallus umbratilis.* In the last, with an asym-
metric gonopodium, at least partial folding is
already accomplished during gonopodial de-
velopment.

d. Organs of External Stabilization

In the gonopodium, rigidity of another sort

apparently is required in some species before
successful copulation may be effected. This is
provided by slender, hyaline processes that orig-
inate either from the membraneous tissue ven-
tral to ray 3 or from the terminal portion of ray
3 itself. In the girardinins these processes,
formed from extensions of the subradial tissues,
are paired and antler-like, rarely extending be-
yond the tip of the fin. Similar outgrowths or
papillae are found in the poeciliins, pamphorins
and alfarines, but in these groups the processes
are unpaired median structures that are cupped
around the ventral margin of the gonopodium.
Clark & Aronson (1951) found nerve fibres and
possibly some nerve endings in comparable tis-
sues of *Lebistes;* these structures may thus be
sensory in function. The ventral processes in
the cnesterodontins are unpaired like those
of the poeciliins, but they are formed from
sheathed bony elements originating at the ter-
minus of ray 3. Occasionally, as in *Cnesterodon,
Phallocerus* and *Phallotorynus,* the pedicular
portions of these outgrowths are loosely asso-
ciated with the elements of ray 4a. In *Phallo-
torynus,* the distal end of the pedicel supports
a complex, bulbous structure with a trough on
its posterior margin. This structure was first de-
scribed by Henn (1916) who thought that it
acted as a receptacle for spermatophores before
they were transferred to the genital pore of the
female. However, when the gonopodium is ro-
tated forward and erected, the originally ven-
tral ray 3 is brought into a dorsal position with
the exposed ventral surface and all its appen-
dages and papillae facing upward. When contact
is made with a female and the tip of the gono-
podium is directed toward the genital pore, the
flexible, hyaline processes would rest against the
soft tissue surrounding the female's genitalium.
Under these conditions the outgrowths of the
3rd ray might act as props or supports to steady
the gonopodium. In *Tomeurus gracilis,* bony
and subradial tissue supports are present, both
of which are well developed. In this form it
seems doubtful that genital insertion is effected;
it is more probable that the terminal specializa-
tions of its gonopodium serve as claspers during
the transfer of sperm (Text-fig. 41).

e. Sensory Structures

Our studies on the innervation of the gono-
podium suggest that in some fishes when
erection, insertion and fixation have been suc-
cessfully attained during the male's maneuvers,
the series of spines on ray 3 may act as sensory
receptors which stimulate the release of sperm-
atophores. The gonopodia of the seven species
of *Xiphophorus,* of *Mollienesia sphenops, Poe-*

cilia vivipara, Limia noblei, Limia versicolor, Limia melanotata and Lebistes reticulatus, possess an elaborate system of nerve fibers that extend down to the spine's shafts. The sensory nature of these elements has been deduced partly from changes in their relative positions during erection of the fin, when their shafts are spread apart and held rigid under a tension established by the taut connective tissue matrix. There are 8 to 12 long spines in the gonopodia of Xiphophorus and a slightly greater number in Lebistes. The spines are more numerous in other poeciliins but they are shorter. In the gambusiins of the West Indies and in some from the mainland, Gambusia nobilis, G. panuco and G. nicaraguensis, the spines are numerous and quite long. Spines occur in the gonopodia of representative species of every poeciliid group, although in some cases—i.e., Heterandria, Pseudoxiphophorus and some girardinins and cnesterodontins—they are so reduced as to suggest the absence of any specified function. In those species possessing long spines in their gonopodia, the effectiveness of these elements as possible sensory receptors depends (1) on the lengths of their shafts for leverage, (2) adequate hinge-joints at their bases permitting free rotation, (3) the number of spines in series, and (4) the flexibility of the unit as a whole. Possibly when the spines are numerous but reduced in size, as in Belonesox belizanus, Gambusia affinis and others, they may subserve a kinaesthetic sensory function, responding to change in internal pressure within the fin rather than to direct external tactile stimulation. In Limia vittata and L. nigrofasciata, the spines are quite long but their shafts flare out laterally to form a broad, oblanceolate platform, the edges of which support the base of the gonopodial hood (Text-fig. 38). In these two species the entire sensory function of the spines is probably assumed by their papillae. Similar arrangements are found in Alfaro (Rosen, 1952).

### B. Sexual Specializations of the Anal Fin in Other Members of the Order Cyprinodontida

Among the species of the Order Cyprinodontida, a wide variety of anal fin modifications have been described in the males, from the simple fins utilized principally in maintaining vertical stability in water in such oviparous genera as Cyprinodon and Rivulus to the elaborate, highly modified and specialized fins of the Poeciliidae.

The anal fin of Rivulus elegans, which may be taken as an example of a simple fin type, is rounded, and consists of 11 or 12 rays that bifurcate two or more times at their distal ends. The first few rays of the fin are strengthened by thickening and fusion or anchylosis of their basal segments, and they probably receive the main muscular support for the entire fin. The rays are simple, possessing no specializations other than the characteristic serial segmentation and the terminal, filamentous actinotrichia. This simple pattern is repeated in all members of the Rivulini as well as in such cyprinodontine genera as Cyprinodon and Floridichthys.

Fishes in the viviparous family Goodeidae, including, among others, the genera Goodea, Skiffia, Characodon and Lermichthys, have a more complex anal fin. In some species the anal fins are quite long, containing more than 25 individual rays. A notch separates the first few short but stiff rays from the rest of the fin. These specializations in males may aid in the processes of internal insemination.

In the males of the Asiatic aplocheilin Oryzias melastigma, a few of the anal fin rays, usually from the 8th to the 11th, are prolonged into filiform processes that extend beyond the fin membrane. No function has so far been assigned to these simple elongations but they may be secondary sexual characters used in display. The male Fundulus kansae possesses small, slender antrorse hooks and nuptial tubercles on its anal fin. The males of Fundulus heteroclitus, Fundulus majalis and others have similar finger-like spinules on the anal fins and sides during their breeding season. These spinous elements are utilized, according to Newman (1907), to help maintain close contact between male and female during spawning activities. Although the anal fins of the fundulines, as well as those of the goodeids and possibly the aplocheilins, have retained their fundamental stabilizing function, they are also used in a specialized manner in courtship and reproductive behavior.

In the Anablepidae, the anal fin of the male has mostly lost its stabilizing function and is used in the act of transmitting sperm. In the gonopodium of the male Anableps anableps, rays 2 to 8 are elongated and thickened. They form an arched bony enclosure that, according to Turner (1950), opens on one side except at the extreme apical end. In different males during morphogenesis rays 2 to 5 twist around each other and rotate either to the right or to the left on a common axis to produce a gentle spiral. This results in the formation of an asymmetric fin that is similar in its more conspicuous features to the gonopodium of the poeciliid Poeciliopsis. It differs, however, chiefly because it forms a tube which is continuous with the sperm duct; whereas in the Poeciliidae and in other

cyprinodonts the sperm duct is completely independent of the anal fin.

A still more complicated mechanism is found in the Indian cyprinodont, *Horaichthys setnai,* according to Kulkarni (1940). Rays 1 to 6 of the anal fin are incorporated into a slender but stiff rod. The elongate portion of this rod is composed of rays 3, 4 and 5 which are unsegmented except at their distal ends. The terminus of the 3rd ray is simple, while rays 4 and 5 are tipped by elaborate clavate and conical processes and by a single arced acicular bone. The last is enormous, equal to two-thirds of the longest fin ray in length. This anal fin apparently is not used in insemination but rather to brush a few tiny barbed spermatophores onto the genital pad of the female. It is of quite different form from both anablepid and poeciliid gonopodia.

Several modifications of the form and function of fins other than anal have arisen in conjunction with the sexual activity of the male. For example, during spawning, when a funduline male utilizes its barbed anal fin to grasp the female, the pelvic fin on the side toward the female is brought forward and hooked under the opposing pelvic fin of the female. In some male poeciliid fishes, such as the Xiphophorini, Poeciliini and Pamphorini, the first few pelvic fin rays are strengthened and enlarged. In *Lebistes* they are produced into a fleshy palp or lappet. The precise function of the modified pelvic fins in the Poeciliidae is uncertain but it has been variously suggested (1) that one of them, together with the gonopodium, forms an enclosed tube for the transmission of spermatophores, (2) that they may provide a prop for the erected gonopodium (Clark & Kamrin, 1951), and (3) that the correlated movement of a pelvic fin with the gonopodium may be a decisive factor in the mechanical efficiency of the system (page 17).

In the poeciliid subfamily Xenodexiinae, according to Hubbs (1950), a unilateral clasping organ has been developed together with an elaborate, spiraled gonopodium. In this instance, however, the right *pectoral* fin, and not the pelvics, has become specialized, for it has an assortment of hooks, pads and rod-like processes. Bailey (in Hubbs, 1950) suggested that certain of the complicated features of this fin may support the erected gonopodium just prior to and during copulation.

## C. Relationship between Morphology and Sexual Behavior in the Order Cyprinodontida

Although the fin structures of funduline and cyprinodontine fishes are comparatively simple, the several features that do appear as minor specializations are of great importance in a consideration of their sexual behavior. It was indicated above that not only the special morphological features of the fin, but also its position in relation to the distribution of trunk musculature and other appendages, are valuable in the analysis of the behavioral problems. Critical comparisons of the sexual behavior of the oviparous cyprinodontines and fundulines with that of the viviparous poeciliids reveal marked similarities of response among the three groups.

### 1. Spawning in Oviparous Cyprindonts

In most of the oviparous fundulines the trunk region is long, the caudal quite short. Consequently the pelvic and anal fins have a posterior insertion on the body, and the longest rays of the anal fin of males (as of *Fundulus heteroclitus* and *F. majalis*) extend slightly beyond the base of the caudal. In the males of both the fundulines and cyprinodontines, the anal, dorsal and pelvic fin rays are, as a rule, longer than those of the female, and the first few anterior rays are strengthened and thickened. The musculature is enlarged and strengthened in males of these fishes, particularly the appendicular portion which controls abduction and lateral inclination. During the breeding season, pearl-like contact organs develop on the sides of the body and on the anal and dorsal fin rays of some species, taking the form of small, finger-like processes. These organs disappear during the period of sexual quiescence.

Newman (1907) observed that the male of *Fundulus heteroclitus* in spawning assumes a position slightly below and behind the female. The anal and dorsal fins of the male are then brought forward and to one side, hooking under the homologous female fins which are raised to receive them. The pelvic fin is also brought forward and slips under the adjacent pelvic fin of the female. After clasping, the bodies of both sexes twist in a characteristic S-curve, in which the head of the male is bent inward toward the female and its tail projects upward and outward. In this juxtaposition (Text-fig. 42) the eggs and sperm are released, accompanied by a vibration of the posterior half of the female's body. In addition to its clasping function, the rigidly inclined anal fin of the male probably serves to deflect the sperm toward the falling eggs.

Although he had no direct evidence, Newman believed that the spawning behavior of *Fundulus majalis, F. diaphanus* and *Cyprinodon variegatus* are similar in principle to that described for *F. heteroclitus.* One of the present authors has observed adult males of *Cyprinodon baconi* assume an S-curve in aquaria with both

TEXT-FIG. 42. Ventral view of male and female of *Fundulus heteroclitus* (Linnaeus) during spawning. Note interlocked anal and pelvic fins. Modified after Newman, 1907.
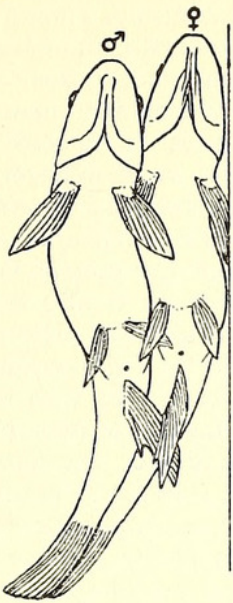
sexes of its species. Similar behavior patterns have also been described by aquarium hobbyists in *Nothobranchius taeniopygus* and related fishes, and by Gordon (1950) in *Epiplatys chaperi.*

### 2. Copulation in Viviparous Cyprinodonts

Most poeciliid fishes (as presently arranged systematically) are viviparous rather than oviparous and certain details of their sexual behavior have been modified to accomplish internal fertilization. These differences in behavior, of course, are dependent upon the anatomical relations that are associated with a new mode of reproduction. In most poeciliids the caudal region is longer than in oviparous cyprinodontids, resulting in the anterior displacement of the pelvic and anal fins. In males the trunk region is further abbreviated and the pelvic-anal complex is relocated further forward near the pectoral base. This anterior displacement of the ventral fins in the male poeciliid results in the intimate association of the pelvic girdle with the anterior
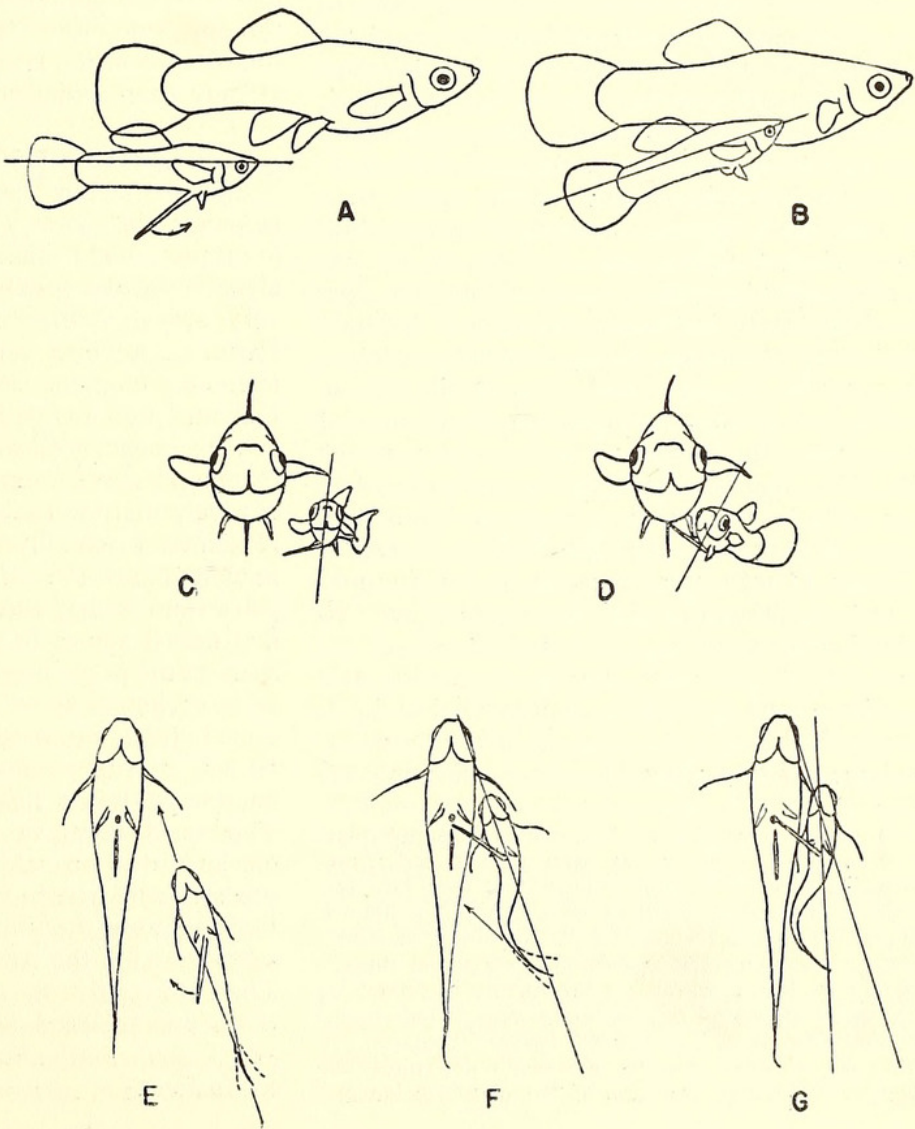


TEXT-FIG. 43. Copulation in male and female poeciliid fishes, showing changes in the orientation of the male in relation to the female. A and B. Increase in the angle of pitch. C and D. Roll on the horizontal axis. E to G. Approach and pivoting around an axis located just behind the head.

actinosts (gonactinosts) of the anal fin (gonopodium). The probable advantage of this reorganization lies in the greater effectiveness of the supporting hemal spines (gonapophyses) in anchoring the gonactinosts by connective tissue when the spines are arched cephalad. Were these hemal spines to retain their original vertical orientation, the effective supporting area would be limited to the tip of the spine. Their arching cephalad provides a bony arc along which supporting tissues originate for more than half of the spine's total length.

In many poeciliid fishes the male's maneuvers just prior to copulation involve the following shifts in position (Text-fig. 43): (1) an increase in the angle of pitch as determined by the higher position of the head in relation to the tail, which enables the male to perform an upward thrust with its gonopodium; (2) a roll on the horizontal axis which also makes possible an upward thrust, but which in addition, in those species with short gonopodia, provides a means of closer approach to the genital region of the female; (3) a pivoting of the body around a point located just behind the head, which tends to bring the posterior portion of the body and the erected gonopodium alongside the female's genital region. Thus, when copulating, the male poeciliid, like the cyprinodont male, approaches the female from behind and slightly below. If the male is not immediately driven away by a non-receptive female, it swings its gonopodium forward and to one side, rolling under the female, pitching upward and pivoting inward in a characteristic S-curve. The actual thrust of the gonopodium toward the female genitalium is probably accomplished by a stroke of the caudal fin when it is curved upward and outward away from the female in the S-curve posture[2] (Pl. III, Fig. 8). This type of posturing during and prior to copulation has been recorded by Clark & Aronson (1951), Clark, Aronson & Gordon (1953) and Rosen & Gordon (unpublished) in the males of *Lebistes reticulatus, Xiphophorus maculatus, X. helleri, Gambusia affinis* and *Heterandria formosa*. S-curving also has been observed without gonopodial and pelvic fin flexion in both sexes of *Xiphophorus maculatus* in establishing dominance-subordination

relationships among homosexual groupings and in sexually immature fishes as young as one month old. In this respect, this species is apparently similar to many of the oviparous cyprinodontids. In *Poecilistes* and *Phallichthys*, however, we have never observed S-curving in the characteristic manner.

In *L. reticulatus, X. maculatus* and *X. helleri*, when the gonopodium is brought forward, a pelvic fin is also brought forward and out to one side, as described by Clark & Kamrin (1951). This coordinated action of the fins may be compared with the homologous movements of the fins of male *Fundulus* when it is clasping the female. The forward and lateral position of the funduline anal fin may serve to deflect the sperm toward the extruded eggs. The poeciliid gonopodium also serves to deflect the sperm, in the form of spermatophores, by the formation of a cup consisting of the distended posterior portion of the fin containing rays 6, 7, 8 and 9. This cup presents an impasse to the spermatophores, which are expelled in an essentially downward path. They are thus forced into contact with the gonopodial groove, which is continuous with the basal cup. (The possible importance of the pelvic fin in these actions has already been indicated, p. 17).

### D. Evolution of Gonopodia

Species within three existing cyprinodont genera, *Cyprinodon, Fundulus* and *Aplocheilus*, are represented in the fossil record. The skeletal elements of the fossil cyprindonts are basically very similar to those of contemporary species. Careful study of a well-preserved fossil, *Proballostomus longulus* Cope, 1891, from South Dakota (probably Oligocene but possibly as late as Pleistocene, according to Dr. Bobb Schaeffer, from a personal communication) indicates conclusively that the basic skeletal architecture has remained essentially unchanged. The accompanying figure (Text-fig. 44) of its anal fin suspensorium clearly shows the presence of modified hemal spines that are arched cephalad to support the projecting actinosts below. The most anterior hemal spine in this complex is so reduced and so intimately associated with the enlarged anterior actinosts as to suggest its comparison with the ligastyle of some poeciliids. The exact form of the anterior actinosts is obscure, due probably to compression by the limestone matrix, but there appear to be plate-like outgrowths of their anterior and posterior surfaces, as in the Anablepidae and Poeciliidae. The anterior five fin rays which articulate with them are enlarged and strengthened, but the serial segmentation pattern has not been lost by fusion.

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[2]In their studies of sexual behavior in the guppy, platy and swordtail, Clark & Aronson (1951) and Clark, Aronson & Gordon (1953) show that there are two types of gonopodial contacts. One, in which contact is lasting, is termed "copulation." The other, in which contact is momentary, is termed "gonopodial thrust." In our studies, however, we are unable to relate any distinctive structural or mechanical adjustments in the gonopodium to each of the two types of contacts. For purposes of this paper we are combining these two terms under the generic term of "copulatory behavior."
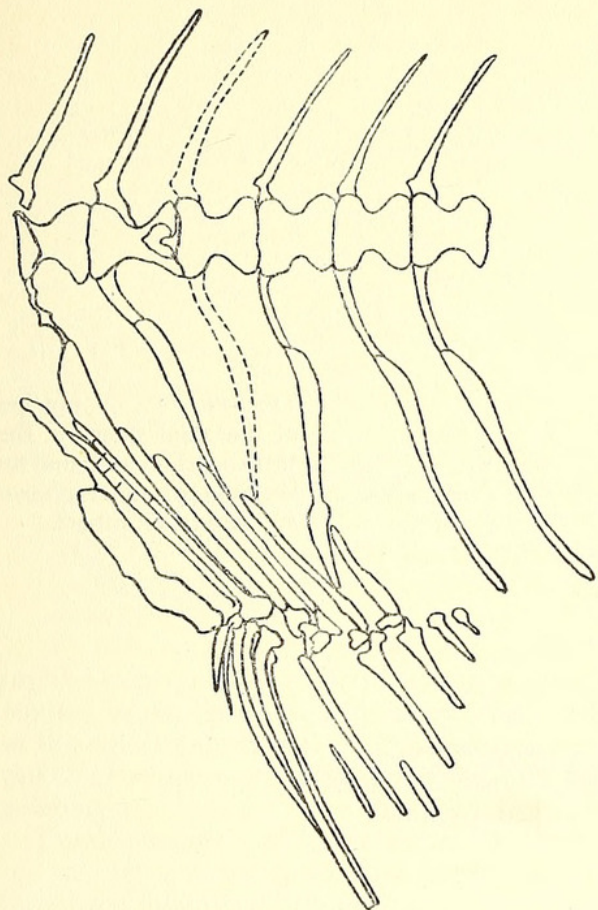
TEXT-FIG. 44. A portion of the axial skeleton, the suspensorium and anal fin skeleton of the fossil cyprinodontid fish, *Proballostomus longulus* Cope from Ree Hills, South Dakota. Drawn from a specimen in the collection of the American Museum of Natural History (A.M.N.H. 8090).

Of the structural details of the *Proballostomus* anal fin skeleton, probably the most important for a consideration of intermediate forms in the evolution of specialized reproductive organs are the supporting hemal spines which have enlarged and rotated forward. These structures may be compared with the comparable elements in the gonopodial suspensorium of the poeciliid, *Xiphophorus maculatus* (Text-fig. 45). In both forms, the spines are slightly expanded near the base and taper to a slender apical portion. The hemal spines of *X. maculatus* differ from those of *Proballostomus* chiefly in the addition of tiny uncini on their posterior margins near their attachment to the vertebral centra (Text-fig. 44). This contrasts with the comparatively unspecialized condition of the spines of *Fundulus,* where the characteristic caudal orientation is retained and where there is no thickening at the bases (Text-fig. 46). In general, the axial portion of the suspensorial skeleton in poeciliid fishes has been less conservative in its evolution

than is indicated by these structures in the genus *Xiphophorus,* for in the Gambusiinae the spines have rotated so far forward that their apical portions run almost parallel with the long axis of the fish. In addition, in *Gambusia* the shafts of the spines are greatly thickened and their long, slender uncini overlap the shaft of the next posterior spine (Text-fig. 47). The degree of specialization of the hemal spines may be measured, to some extent, by the amount of displacement anteriorly of the anal fin. Since the pelvic-anal complex is relocated anteriorly in most poeciliid fishes, the reorientation cephalad of the supporting elements would seem to be of great mechanical advantage. The mechanical principles involved in the morphological transformations are described above, on page 18.

In other respects, the anal fin skeleton of *Proballostomus* compares favorably with those of living cyprinodontids such as *Fundulus,* but it even appears to be slightly more specialized in the cephalo-caudal extension of its actinostal plates. In *Fundulus,* the actinosts have pointed tips dorsally, and both lateral and cephalo-caudal plates broaden gently toward the base but are never of any great width. Other differences are of a relatively minor character.

Utilizing a *Fundulus*-like suspensorium as a possible model of early skeletal structure in the group, it becomes apparent that the strengthening and enlargement of the anterior actinosts and anal fin rays, and their intimate association with the hemal spines, constitute a preadaptation for the development of the gonopodial mechanism. This interpretation may be further clarified by making a comparison between the homologous behavior patterns of the unspecialized fundulines and the specialized poeciliids, particularly with reference to fin action during spawning and copulation (p. 37).

Thus, probably one of the most important factors influencing the development of specialized external reproductive organs in the male is associated with his habit of clasping, in which increased strength and flexibility of the fins would be decisive. Strengthening of the system has been accomplished in several ways, but primarily in the growth of the hemal spines downward and forward so that they interdigitate with the actinosts of the anal fin. Enlargement of the parapophyses and the appearance of uncini on the posterior margins of the hemal spines provided additional anchorages for the supporting ligaments. Secondarily, the thin plates separating the paired erector and depressor muscles became enlarged and finally fused. The fusion was probably accompanied by a loss of the depressor function of the paired posterior muscle
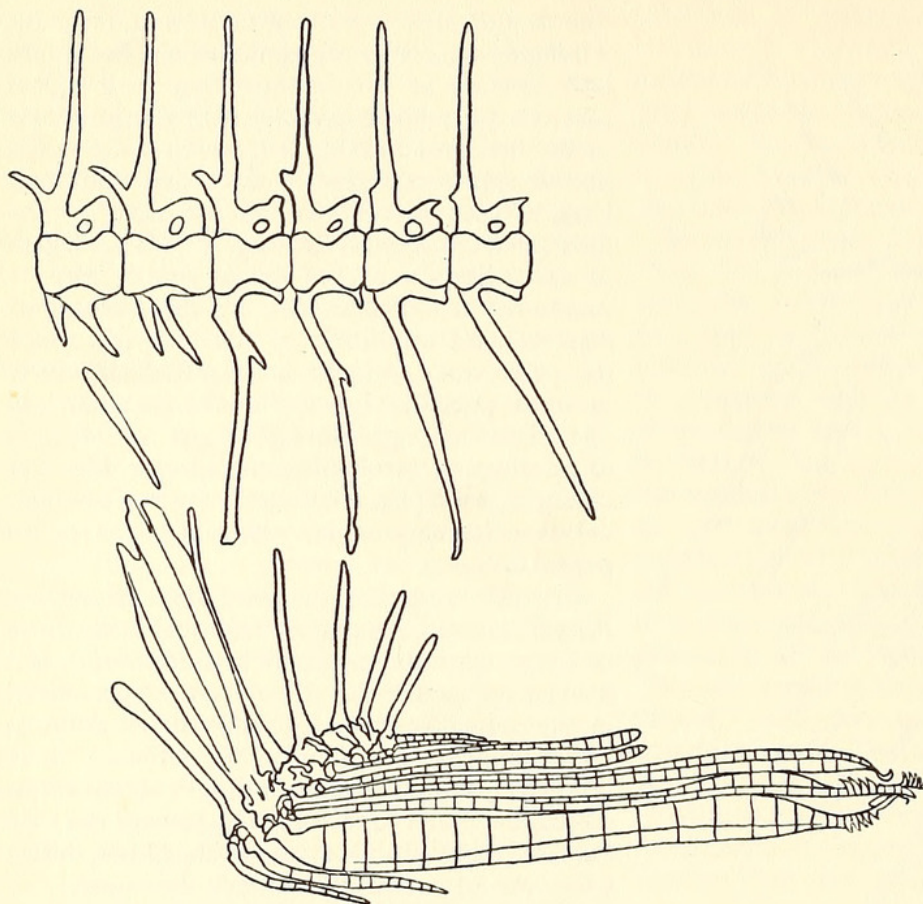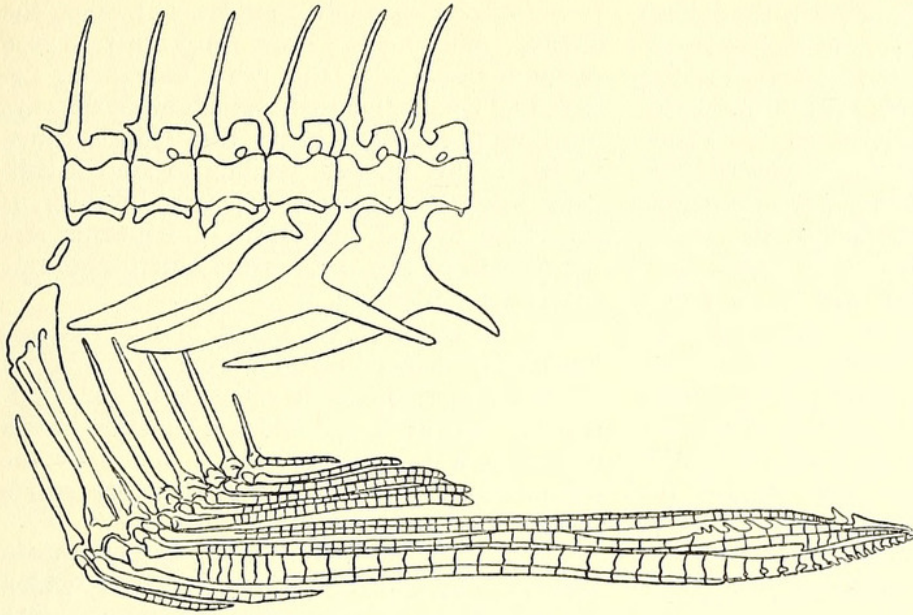
TEXT-FIG. 45. A portion of the axial skeleton, the suspensorium and anal fin skeleton of *Xiphophorus maculatus* (Günther).
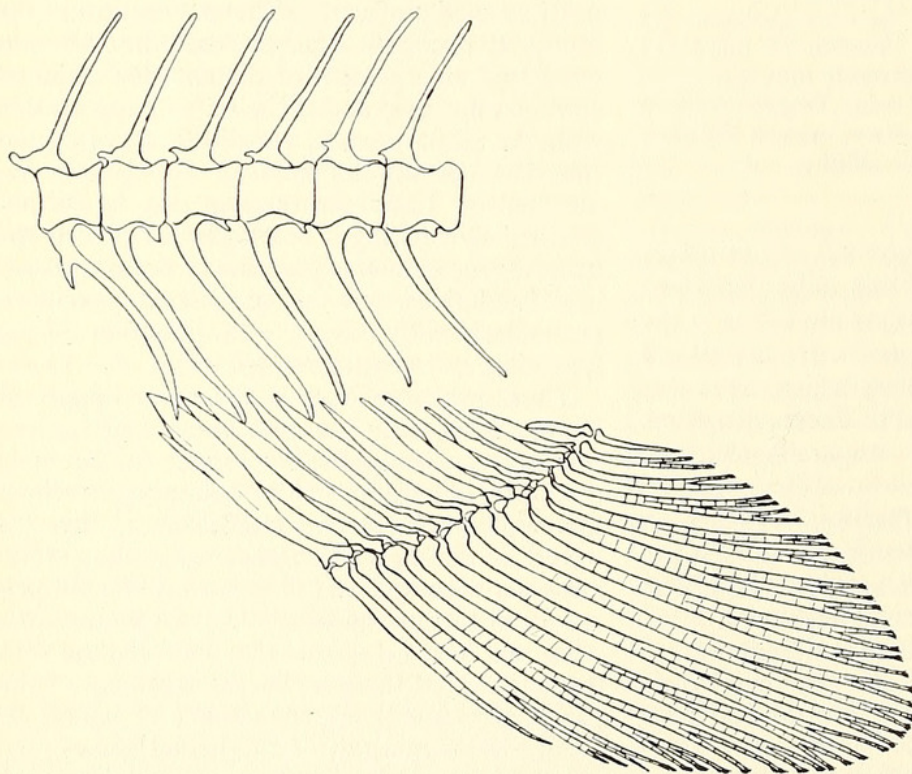
TEXT-FIG. 46. A portion of the axial skeleton, the suspensorium and anal fin skeleton of a male *Fundulus heteroclitus* (Linnaeus).

slips and their reorientation as erectors within the body of the composite muscle mass, the erector analis major. This change in the medial muscle system, coordinated with the displacement anteriorly of the retractor ischii for increased rotary movements, and the realignment of the inclinator analis muscles, made possible a greater flexibility of fin movement for grasp-

TEXT-FIG. 47. A portion of the axial skeleton, the suspensorium and anal fin skeleton of *Gambusia affinis* (Baird & Girard).

ing the underparts of the female during sexual contact. A strengthening and further elongation of the anterior fin rays that receive the greatest muscular support provided additional aids for grasping.

The development of specialized conditions of the anal fin in male cyprinodont fishes may also have been influenced to some degree by coordinated secondary sexual changes affecting both median elevated fins, according to Dr. Charles M. Breder, Jr. (personal communication). For example, the dorsal fins are highly colored and elongated in some males of the Family Cyprinodontidae and Tribes Poeciliini and Xiphophorini of the Poeciliidae, among others. Breder pointed out that locomotor demands established by an enlargement of the dorsal fin may have called for a compensatory elongation of the anal fin. Adjustments of this sort may have preadapted these fins for their specialized functions in reproductive activities. In the oviparous aplocheilin, *Oryzias latipes,* mating is facilitated by the interlocking of homologous male and female fins, as in the funduline fishes. Amemiya & Murayama (1931) found an exceptional female of this oviparous species in which developing embryos were contained within its body. Internal fertilization, which obviously must have taken place, was probably aided by the interlocking median fins, particularly by the anal fin of the male, which is long and flexible.

The progressive evolution of more complex anal fin control mechanisms, which presumably culminated in the development of gonopodia, is related also to the widespread habit among cyprinodonts of retaining the developing young within the body of the female. Nevertheless, viviparous cyprinodonts do exist, namely, the

Goodeidae and Adrianichthyidae, in which the anal fins of the males are not primarily specialized to effect internal fertilization and in which the absence of the complex pterygial modifications are compensated for by special behavior patterns and other devices for internal insemination, as in the exceptional *Oryzias* mentioned above. A contrasting situation occurs in *Horaichthys setanai* and *Tomeurus gracilis,* two species which are oviparous and in which the anal fins of the males are nevertheless transformed into gonopodia of elaborate form. The apparent contradiction of these examples is easily explained, however, since it may be seen from Kulkarni's (1940) excellent figure that the gonopodium of *Horaichthys* is not utilized as an intromittent organ as in anablepids and poeciliids, but rather as a contact organ for attaching barbed sperm capsules to the soft tissues that surround the exterior of the female's genital pore. Our studies of the gonopodial details of *Tomeurus* indicate that its functional relations also may be quite different from those of poeciliids. Myers (1947) was sufficiently impressed by the structural peculiarities of *Tomeurus* to suggest that it may have originated independently of the main line of poeciliid evolution—an interpretation which he feels should be, but is not, reflected in its present systematic position. The non-viviparous reproductive behavior of *Tomeurus* is one of the important factors which prompted Myers to propose that it be separated from the poeciliids in the new family Tomeuridae. This point has merit; a similar view is expressed by Dr. Hubbs (personal communication).

Since the comparatively unspecialized anal fins of some cyprinodontids appear to function,

in part, to deflect the sperm towards the falling eggs, it is not surprising that in several fishes special mechanisms have been evolved independently to meet this need. Not only do the anterior anal fin rays serve to deflect the sperm, but they may also hold sperm temporarily before transmitting them to the genitalium of the female. Probably the formation of the gonopodium itself was anticipated in its evolutionary development by an elongate anal fin in which the anterior and posterior regions of the fin could be folded to form an open trough. This hypothetical intermediate arrangement could have then been refined by (1) the advancement of the anterior rays for the formation of the transitory gonopodial tubes, and (2) the restriction of the posterior anal fin rays to serve as a receptacle for the freshly released spermatophores before they pass down the transitory gonopodial tube.

The elaboration of the rotational and adjusting mechanism was probably a natural outcome of the complete segregation of the anteriormost rays from their more posterior serial homologues. The morphological elements of an unspecialized cyprinodont anal fin being already preadapted for the development of a gonopodium, mechanical perfection was attained by a strengthening and elongation of these anterior rays. For example, the strengthening of the anterior rays by the addition of bone probably served originally to meet the demands for greater muscular action. The bone has been added mainly on the anterior and posterior edges; it is here that the interacting fin rays come into contact with each other during forward movement. It is only when lateral movements become important, for example, during the gonopodial swing, that protection against lateral fracture is necessary. Thus, on ray 5 of the poeciliid gonopodium, the two component elements of the ray together have undergone lateral expansion, probably in response to the inevitable rotary movements of this ray in an essentially ventro-lateral path during fin erection. Ray 3, on the other hand, while it received slight lateral support from the eccentric grooves, has become enlarged mainly in the vertical plane. The enlargement of ray 3 in the vertical plane may be accounted for by the following interpretations: (1) Ray 3, as the axial center of rotation for the gonopodium, must travel forward constantly in the same plane during erection in order to provide a stable structural surface in relation to which the other rays can orient themselves. Directional stability is provided by an increase in the height of its segments in the antero-posterior (vertical) dimension which is in a plane parallel with the applied force of the powerful erector analis muscles. (2) Since the erector analis muscles draw ray 3 forward in a plane parallel with the fish's long axis, the stretched connective tissue which binds the rays together produces a strain that travels transversely across the ray, tending to arch it caudally along its segments. Arching, however, is prevented by the anchylosis of segments and the growth of segments in the antero-posterior (vertical) dimension.

Ray 4, unlike rays 3 and 5, has retained much of its original form. During fin erection it is merely carried over to one side by the rotating force of ray 5, and its main function in this operation is to separate rays 3 and 5 by the formation of the bed of the transitory sperm trough.

The evolution of the terminal specializations such as spines, distal serrae and claws, which are regarded as of minor importance compared to the mechanical changes which have taken place, may be associated with differences in the behavior and ecology of the different species. For example, differences in the structure of the terminal holdfast in *Lebistes* and *Xiphophorus* have been shown by Clark, Aronson & Gordon (1953) to be intimately related to the behavioral pattern of the males during copulation. In *Xiphophorus*, where the holdfast consists of a large, pointed retrorse dorsal claw and a ventral retrorse hook, copulations are relatively long, i.e., five or six seconds. The male remains relatively passive during the act and terminates it in a sharp break. In *Lebistes*, in which the holdfast consists of a small retrorse claw embedded in the tissue above ray 5, copulations are less prolonged, lasting about two seconds. The male constantly swims against the female and thus maintains contact. This difference in copulatory behavior was explained by Clark & Aronson (1951), who suggested that the claw in *Lebistes* is not an essential factor in successful copulations. They pointed out that males lacking this element can still copulate successfully, whereas in *Xiphophorus* a holdfast is indispensable.

There is also a possibility that those fishes which live in turbulent waters may have stronger holdfast elements than those which live in slow waters (Rosen & Gordon, unpublished).

The earliest stages in the development of minor differences in the terminal elements of the gonopodium may be determined by study of these structures in the members of geographically isolated populations of a single species. For example, the *X. maculatus* population from one locality in the Río Coatzacoalcos in southern Mexico differs from all platyfish from surrounding areas by having a dorsal claw in the

gonopodium, a structure which is especially characteristic of *X. helleri* and *X. pygmaeus*. This peculiarity in one population of the platy-fish may be interpreted in genetic terms. The appearance of claws in some members of a normally clawless species may depend upon the chance recombination of complimentary genes for claws and their modifiers that had become separated in the speciation process. It has been indicated by Gordon & Rosen (1951) that the development of comparable structures in *Xiphophorus* is controlled by the action of a small number of independently assorting polygenes.

## E. General Discussion

The history of our knowledge of the structure of the gonopodium was presented in a previous paper by Gordon & Rosen (1951), and of the gonopodial suspensorium by Gordon & Benzer (1945). The earliest interpretation of the mechanics of transferring spermatophores from the male to female poeciliid fishes by means of the gonopodium was made by Ryder (1885). He indicated that the elongated rays of the gonopodium folded on each other, forming an open trough. He suggested that the rays might contain an extension of the sperm duct which communicates with the exterior at the tip of the fin. Kuntz (1915) noted also that the elongated rays of the gonopodium formed a groove, but he thought that the spermatophores travelled along the erected fin before being transferred to the genitalium of the female. Henn (1916) extended these observations, saying that the flexible, smaller rays at the base of the gonopodium together may serve as the initial receptor for the discharged spermatophores. Gordon (1947) pointed out the difficulties of explaining sperm transfer on the basis of the suggested mechanism.

An interpretation of the mechanics of gonopodial movements was made by Collier (1936) on *Gambusia a. affinis*. He described the erector analis major muscle and considered it alone to be responsible for the intrinsic movements of the gonopodium. This muscle, he claimed, is capable of swinging the gonopodium only to a maximum angle of 90° in relation to the horizontal axis of the caudal peduncle. Because of what he terms the "mechanical connections," erection of the gonopodium beyond that angle can not take place by action of the erector analis major. According to his view, an arching of the back enables the gonopodial suspensorium to slide forward and to rotate upward in such a manner that the gonopodium swings beyond 90° to 140° or 150°. His Figures 9 and 12 evidently were drawn with the assumption that the entire gonactinostal complex dissociates itself from the gonapophyses during the erection of the gonopodium. How this is accomplished was not made clear. Reference to Plate II, Figure 1, of Clark & Aronson (1951) shows that arching of the back does not take place when the gonopodium is swung forward. Our dissections reveal that the gonactinosts are firmly imbedded in surrounding tissues and that only a slight forward shift can take place at the base of the complex.

Fraser-Brunner (1947) thought that the gonopodium is effective in transferring spermatophores to the female only when its action is closely correlated with one of the pelvic fins. According to him, when both the anal and a pelvic fin are brought forward a closed tube is formed through which the male, at close range, "shoots" spermatophores toward the female genitalium; no physical contact is involved. This type of sperm transfer has been questioned by Gordon (1947). The observations and experiments by Clark & Aronson (1951), Clark & Kamrin (1951), Clark, Aronson & Gordon (1953), Miss Patricia C. Kadow (unpublished), and others provide the basis for the following statements concerning the probable method of insemination in some of the Poeciliinae, particularly in the Xiphophorini:

Spermatophores are ejaculated by the male when its gonopodium is in the erected position. At this time the base of the fully rotated gonopodium is directly below its genital orifice. Spermatophores pour into the trough formed by the short posterior gonopodial rays that are arranged around the base of its rays 3, 4 and 5. When the tip of the spermatophore-laden gonopodium is inserted into the female's genitalium, the female responds in such a way that fluid from its oviduct comes in contact with the distal end of the transitory, capillary-like tube formed by the folded gonopodium. The fluid from the oviduct has the property of changing the physical state of the spermatophores and their spermatic fluid. The ovarian secretion reduces the viscosity of the fluid and the substance that holds the sperms together within their spermatophores. The released spermatozoa, swimming freely in a more liquid medium, may then pass along the length of the gonopodial tube and enter the opening of the oviduct. It is possible that the spermatozoa are actually drawn up within the oviduct by capillary action.

## F. Résumé and Conclusions

The skeletal mechanisms which make possible certain specialized behavior patterns in poeciliid fishes during and prior to copulation

consist of two principle units: (1) the gonopodium or sexually modified anal fin of the male which serves to transfer spermatophores to the female genitalium, and (2) the gonopodial suspensorium, consisting of modified interhemals or gonactinosts, which supports the gonopodium by its ligamentous attachment to the hemal spines or gonapophyses of the axial skeleton.

The complex movements of the gonopodium which enable it to rotate forward and laterally to attain a copulatory position are made possible by the action of a series of enlarged erector, depressor, rotator, inclinator and tensor muscles. These originate, for the most part, on the gonactinosts. The muscles of the pelvic fins are also probably important in stabilizing the movements made by the male prior to and during copulation.

When the gonopodium is swung forward, the elongated bony fin rays 3, 4 and 5 and their enveloping sheets of collagenous fibers interact in such a way that a grooved surface is formed along one side of the fin. During the forward and lateral movement, the more posteriorly attached rays 4 and 5 slip backward away from the tip of the fin. This produces a strain in the collagenous fibers which bind the three rays together. Excess strain on this tissue is prevented by a folding of rays 4 and 5 against the broad, flat surface of ray 3. When one edge of the broad, fluted surface of ray 5 meets the expanded antero-ventral edge of ray 3, a partially enclosed channel is formed along which spermatophores may pass toward the tip of the fin. The tip of the gonopodium has various barbs and spinous elements which may serve as organs of contact and insertion, gripping or holdfast devices for securing and maintaining contact between the sexes, sensory receptors, and stabilizing mechanisms which aid in retaining the alignment of the rays and in steadying the gonopodium as a whole during copulation.

Comparisons among the forty-five recognized poeciliid genera with reference to the rotational and adjusting mechanisms and terminal specializations in their gonopodia show that most of them function according to almost identical mechanical principles. One difference may be mentioned. Male poeciliids with bilaterally symmetrical gonopodia may copulate with females from either side. Those poeciliids with asymmetrical gonopodia, like males of the Subfamily Poeciliopsinae, may copulate with females from one side only.

The results of comparing the modifications in anal fins of some oviparous cyprinodont fishes with those of the viviparous species also reveal a common trend toward the development of somewhat similar specialized structures which are associated with sexual activities.

The patterns of sexual behavior of the oviparous cyprinodontids and the viviparous poeciliids also show striking parallelisms. The conclusions reached as a result of these comparative studies, and from examination of a well-preserved cyprinodont fossil, is that the poeciliid gonopodium probably evolved from a simple, cyprinodontid-like anal fin. The original function of the anal fin was principally in maintaining vertical stability in water. The anatomical changes most important in the evolution of the gonopodium appear to be: (1) the elongation and modification of anal rays 3, 4 and 5; (2) the functional independence of these rays from the posterior rays; (3) the enlargement, strengthening and reorientation cephalad of the hemal and interhemal anal fin supports; (4) the development of powerful gonopodial muscles.

In the evolution of the viviparous from the oviparous type of reproduction there must have been, also, some modification in the physiological properties of the fluid substances associated with the primary sexual products. In the males of viviparous species, spermatozoa are ejaculated in the form of passive spermatophores surrounded by a viscous semen-like fluid. In the viviparous mature females an ovarian secretion is elaborated which has the property of lowering the viscosity of spermatic fluid and the substance which holds the spermatozoa together in their spermatophores.

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EXPLANATION OF THE PLATES

### PLATE I

FIG. 1. *Belonesox belizanus.* Female above. Male below. Note large gape of mouth in female.

FIG. 2. *Xiphophorus helleri* male, above. *Pseudo-xiphophorus bimaculatus* male, below.

FIG. 3. *Xiphophorus xiphidium.* Male, top, right. Female, bottom, left.

### PLATE II

FIG. 4. *Glaridichthys uninotatus.* Male above. Female below.

FIG. 5. *Mollienesia sphenops.* Male above. Female below.

### PLATE III

FIG. 6. *Poecilistes pleurospilus.*

FIG. 7. *Phallichthys amates.*

FIG. 8. *Lebistes reticulatus* male as seen from below in the S-curve posture with the gonopodium swung forward and out to the right side. The male is approaching the genital region of the female obliquely. The female is seen in rear quarter view while circling away from the male. The forward movements of the male while S-curving are caused chiefly by strokes of the caudal fin as revealed by the fin's undulatory appearance. (Photo by Dr. Eugenie Clark, Department of Animal Behavior, American Museum of Natural History).

### PLATE IV

FIG. 9. Cross-section through the distal tip of the gonopodium of *Xiphophorus helleri* at the level of the ramus and blade.

FIG. 10. Cross-section through the gonopodium of *Xiphophorus helleri* at the distal level of the spines on ray 3; ray 5 is dorsal, ray 3 ventral. The dorsal expanded ray marks the distal-most region of the canoe-shaped area of the 5th ray.

FIG. 11. Cross-section through the gonopodium of *Xiphophorus helleri* at the level of the rotational elements; ray 5 is dorsal, ray 3 ventral. Note rounded dorsal edge of ray 3 and pointed dorsal edge of ray 4p.

FIG. 12. Cross-section through the gonopodium of *Xiphophorus helleri* proximal to the rotational elements; ray 5 is dorsal, ray 3 ventral.

FIG. 13. Cross-section through the gonopodium of *Xiphophorus helleri* at its base; ray 3 is ventral.

FIG. 14. Cross-section through the integumentary thickening or incipient hood of the 3rd ray of the gonopodium of *Xiphophorus helleri.* Compare the similarities of tissues in Figs. 14 and 15.

FIG. 15. Cross-section through the mid-portion of gonopodial hood of the 3rd ray of *Lebistes reticulatus.*
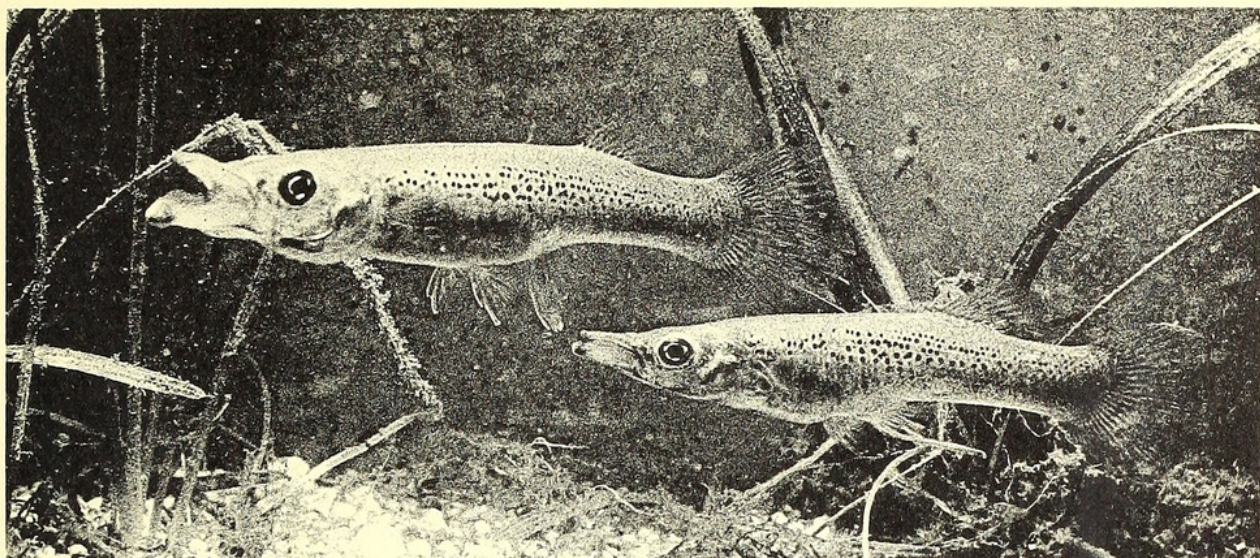
FIG. 1
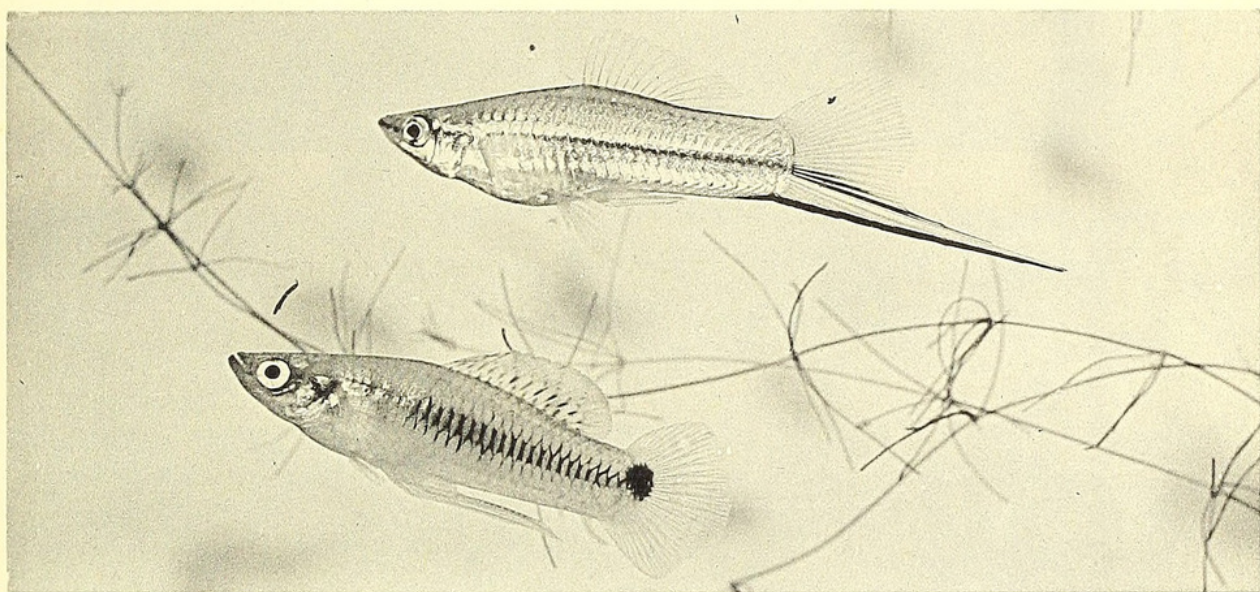


FIG. 2
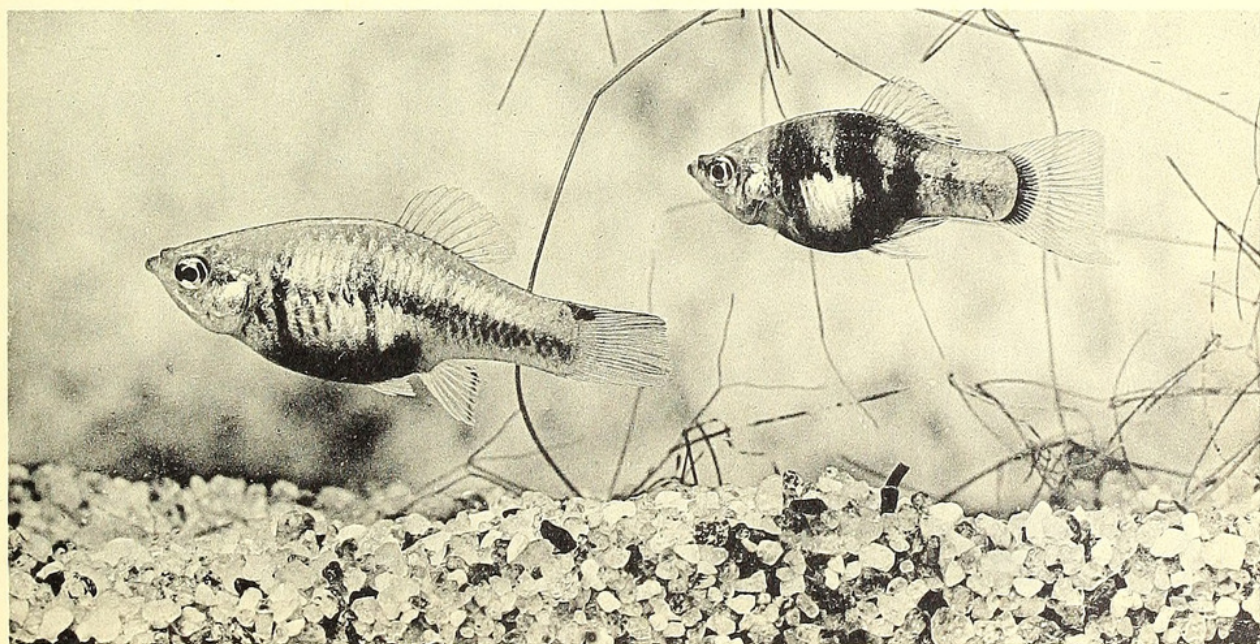


FIG. 3

FUNCTIONAL ANATOMY AND EVOLUTION OF MALE GENITALIA
IN POECILIID FISHES
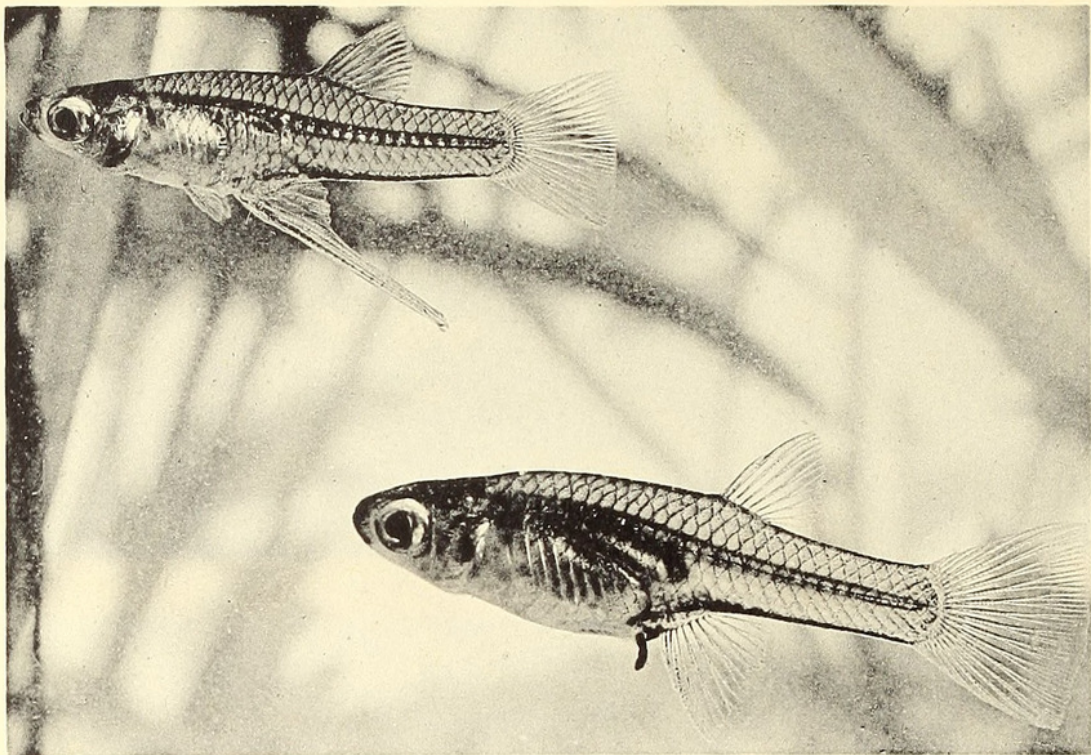
FIG. 4
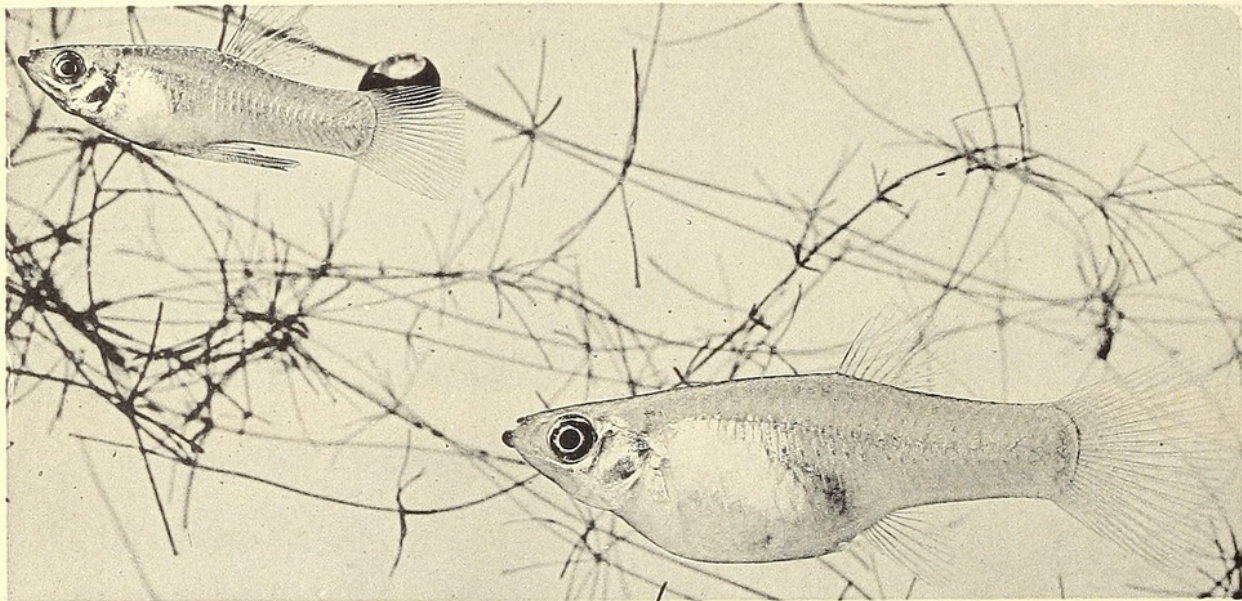


FIG. 5

FUNCTIONAL ANATOMY AND EVOLUTION OF MALE GENITALIA
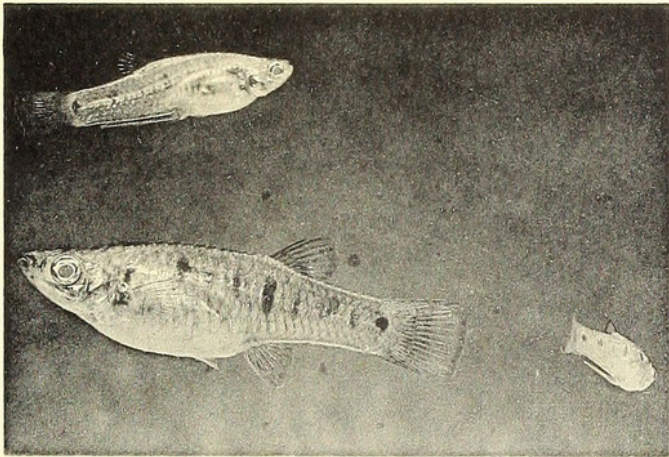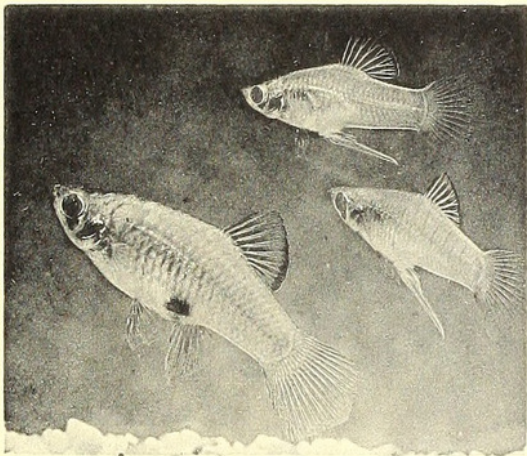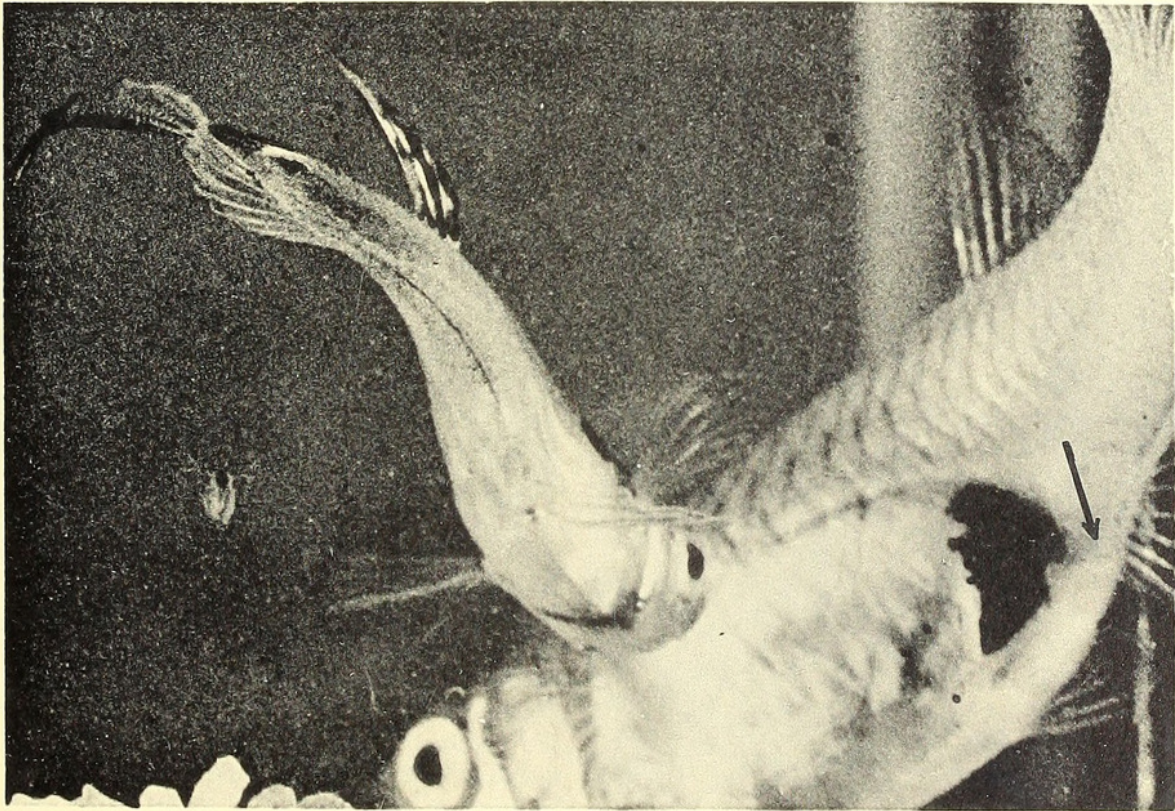IN POECILIID FISHES

FIG. 6



FIG. 7
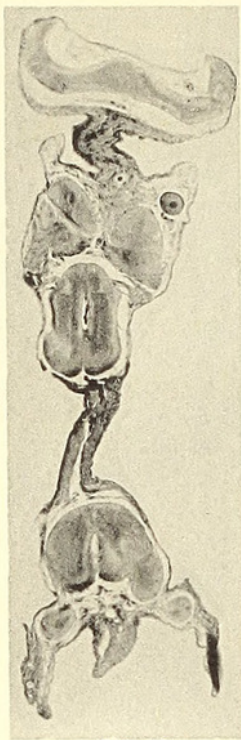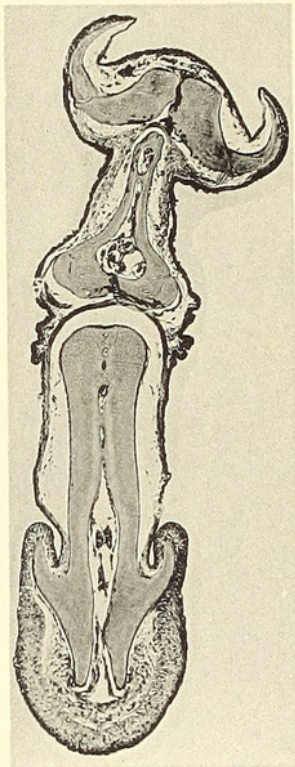


FIG. 8

FUNCTIONAL ANATOMY AND EVOLUTION OF MALE GENITALIA
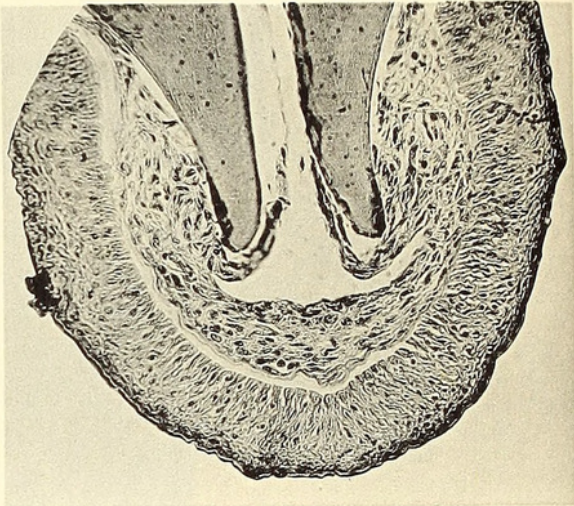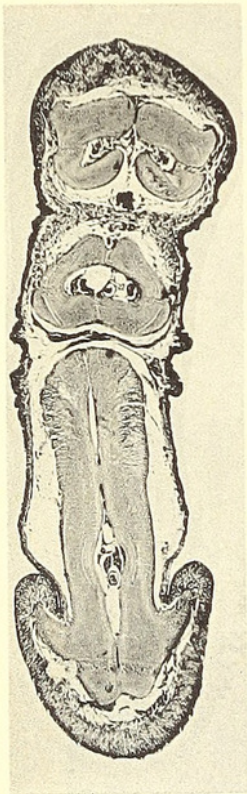IN POECILIID FISHES

FIG. 9

FIG. 10

FIG. 11

FIG. 14

FIG. 12

FIG. 13

FIG. 15

FUNCTIONAL ANATOMY AND EVOLUTION OF MALE GENITALIA
IN POECILIID FISHES