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Taxonomic Revision of the Genus *Pungitius* with Emphasis on *P. hellenicus*

by

Yazdan Keivany ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Systematics and Evolution

Department of Biological Sciences

Edmonton, Alberta

Spring 1996

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Dr. J. S. Nelson, supervisor

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Dr. W. M. Tonn, committee member

Date: 29 January 1996

"**Study** from your **crib** to your **grave.**"

"People are three categories: **teachers**; **learners**; and **nothing.**"

" **Studying** is a **necessity** for any Muslim **man** and **woman.**"

****** Quotes from Mohammad, the great prophet of Islam (570-630 A. D.) *****

This work is dedicated to my parents, who raised me,

and to my teachers, who taught me.

Abstract

Variation in morphology of species of the genus *Pungitius* is enormous. Due to this variation, the taxonomic status of many taxa is uncertain; while some workers recognize two species, *P. pungitius* and *P. platygaster*, others recognize more. I examined the seven nominal species (*P. pungitius*, *P. occidentalis*, *P. sinensis*, *P. tymensis*, *P. platygaster*, *P. laevis* and *P. hellenicus*) to determine their taxonomic status, especially the validity of *P. hellenicus* from Greece, and to hypothesize their systematic relationships within the genus.

Twenty four traditional morphometric characters and 21 truss distances were measured and fifteen meristic characters were counted on 310 specimens from much of the range of the species. The osteology of *P. hellenicus* was compared to that of the other species. Morphometric and meristic characters were of little value in differentiating among the species because of the variation and overlap of their values. However, I recognize three valid species in the genus, namely, *P. hellenicus*, *P. platygaster*, and *P. pungitius* with five subspecies (*P. p. pungitius*, *P. p. laevis*, *P. p. tymensis*, *P. p. sinensis*, and *P. p. occidentalis*). *Pungitius hellenicus* can be recognized by a combination of five characters: fewer than seven dorsal spines, reduced ectocoracoid, absence of a keel on the caudal peduncle, and lack of the pelvic girdle and large lateral scutes.

The phylogenetic relationship of the species is uncertain, but it seems that *P. hellenicus* is the most primitive sister group of the other species. However, *P. hellenicus* may be a neoteric form of *P. platygaster*. The phylogenetic relationships of *Gasterosteus*, *Culaea*, and *Pungitius* are also uncertain; however, this study suggests that *Culaea inconstans* is a member of the genus *Pungitius*.

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Abbreviations

ACT: Actinosts
ANG: Angular
ASP: Anal spine
ART: Articular
BBR: Basibranchial
BHY: Basihyal
BOC: Basioccipital
BSR: Branchiostegal rays
CBR: Ceratobranchial
CHY: Ceratohyal
CLT: Cleithrum
COR: Coracoid
DHH: Dorsal hypohyal
DNT: Dentary
EBR: Epibranchial
ECT: Ectocoracoid
ENP: Endopterygoid
EPH: Epihyal
EPO: Epiotic
EPR: Epural
EPT: Ectopterygoid
EOC: Exoccipital
ETM: Ethmoid
FRT: Frontal
HBR: Hypobranchial
HYM: Hyomandibula
HYP: Hypural
IHY: Interhyal
INC: Intercalary
IOP: Interoperculum
IOR: Infraorbitals
LAC: Lachrymal
LET: Lateral ethmoid
MAX: Maxilla
MPT: Metapterygoid
NAS: Nasal
OPR: Operculum
PAL: Palatine
PAR: Parietal
PBN: Pelvic bones
PBR: Pharyngobranchial
PCL: Postcleithrum
PMX: Premaxilla
POP: Preoperculum
PRO: Prootic
PSN: Pelvic spine
PSP: Parasphenoid
PTR: Pterotic
PTT: Posttemporal
PU2: Preural 2
PU3: Preural 3
QUD: Quadrate
SCL: Supracleithrum
SYM: Symplectic
SAR: Sesamoid articular
SCP: Scapula
SOP: Suboperculum
SOC: Supraoccipital
SPH: Sphenoid
UHY: Urohyal
URN: Uroneural
URS: Urostyle
VHH: Ventral hypohyal
VOM: Vomer

1. Introduction

The family Gasterosteidae of the suborder Gasterosteoidei, order Gasterostiformes (Nelson, 1994) contains five genera, three of which, *Apeltes, Culaea* and *Spinachia*, are monotypic. However, there is much uncertainty about the number of species that should be recognized in the two remaining genera, *Gasterosteus* and *Pungitius*; a conservative view being that each genus contains two species (Wootton, 1976).

After *Gasterosteus, Pungitius,* the ninespine sticklebacks and specifically *P. pungitius* (Linnaeus, 1758), has the widest distribution and the greatest variability of sticklebacks. Species of *Pungitius* are widely distributed in freshwater and brackishwater habitats of Eurasia and North America and on the Pacific, Atlantic, and Arctic coasts of both areas.

There is much uncertainty concerning the number of species of *Pungitius* that should be recognized. Of the more than 30 nominal species (McAllister, 1987), recent workers recognize from two to eight species. For example, Haglund et al. (1992) suggested that the *P. pungitius* complex contains three clades: *P. pungitius* in Europe, *P. occidentalis* (Gmelin, 1789) in North America, and *P. sinensis* (Guichenot, 1869) in Asia. *Pungitius platygaster* (Kessler, 1859), is generally regarded as a valid species (Nelson, 1971a; Wootton, 1984; Haglund et al., 1992). *Pungitius laevis* (Cuvier, 1829) was recognized as a valid species by Berg (1949), but some others recognized it as a subspecies (Gross, 1979; Wootton, 1984). Takata et al. (1984) provided evidence that *P. tymensis* from Japan and Sakhalin Island is a valid species. Stephanidis (1971) described *P. hellenicus* from the Sperchios Valley in Greece based on the absence of pelvic spines, differences in the number of dorsal spines (fewer dorsal spines) and differences in the size and shape of the pelvic bones between *P. hellenicus* and *P. pungitius*. Some authors recognized *P. kaibarae* (Tanaka, 1915) as a subspecies of *P. sinensis* (Kim et al., 1989) and some as a valid species (Yang and Min, 1990), but their recognition was based on meristic and morphometric characters that exhibit much variation and are not considered to be taxonomically significant. For this thesis I recognize there being seven nominal species recognized in the recent literature.

Reasonable doubt might be cast on the validity of *P. hellenicus* given that other authors have found variation in the number of dorsal spines and in the presence and absence of the pelvic girdle without it appearing to be taxonomically significant. Ure (1962) reported a marked reduction in the number of dorsal spines in *P. pungitius* without a loss of the pelvic complex. McPhail (1963) observed a tendency for there to be more dorsal spines in the tidal form of *Pungitius* in North America than in the inland form. Nelson (1971b) reported the absence of the pelvic complex and the intermediate condition in ninespine stickleback in Ireland and Wood Buffalo National Park region. In some locations he found individuals with and without the pelvic skeleton to be sympatric and seemingly being the part of the same population. Zyuganov (1989) reported reproductive isolation in parapatric populations of the ninespine stickleback with and without a pelvic girdle. However, none of these authors gave species status to their different forms of *Pungitius*.

The main objectives of this study are to revise the taxonomy of the genus *Pungitius* and to determine the validity of *P. hellenicus*. These are achieved through the following subobjectives:

1. To revise and provide diagnoses and descriptions with information on synonymy and distribution of each species.
2. To provide a detailed osteological description of *P. hellenicus* and to discuss the osteological differences in other species.

3. To count meristic characters and to measure morphometric characters on the specimens of the seven nominal species and to estimate their variation in the different species.
4. To test the hypothesis that the genus *Pungitius* is monophyletic and to prepare a phylogenetic hypothesis of interrelationships of the seven nominal species.

2. Methods and Materials

Taxonomic methods

Speciation and the species concept have been debated for years and various criteria have been used to define species. It is therefore important in the study of phylogenetic relationships to state what criteria are used to define a species (Mayr and Ashlock, 1991). The biological species concept was rejected by Frost and Kluge (1994), but it is still perhaps the one most commonly followed in fish systematics and is the most accepted. However, in practice, the biological species concept cannot be used in many taxonomic works (e.g., in those dealing with allopatric populations). Thus morphological characters must often be employed to infer reproductive isolation (Mayr and Ashlock, 1991), and some of these characters and others may also be used to study interrelationships among species. I used the one hundred percent classical morphological rule (Bailey et al., 1954) to infer species status of the species I recognize as valid. According to this rule, a species can be considered as valid if one hundred percent of its specimens can be distinguished from specimens of other related species in allopatry. In order for this condition to be met, the allopatric species must bear at least one unique character or a combination of characters that separate it from other species.

I used 45 morphometric (including 21 truss distances), and 15 meristic characters to study these species. Good descriptions of the fish are available and some workers have used many of these characters as efficient discriminants of the genus. Truss characters were used because some recent workers (e.g., Strauss and Bookstein, 1982) have criticized the use of traditional morphometrics for describing form in systematic studies because they tend to concentrate along the horizontal axis of the body. In contrast, truss character sets using landmark points are less biased in their directional description of the body and produce a more systematic geometric characterization of shape (Strauss and Bookstein, 1982).

For the osteological study, I stained and cleared specimens of the seven nominal species of *Pungitius* and of three species of the outgroups, *Culaea inconstans* (Kirtland, 1841), *Gasterosteus aculeatus* Linnaeus, 1758, and *Aulorhynchus flavidus* Gill, 1861. I used Taylor and van Dyke's (1985) method for staining and clearing bone and cartilage and made radiographs of all specimens available of ninespine sticklebacks.

I measured morphometric characters using a Limnoterra digital caliper connected to an APCO Turbo PC computer and a basic computer program developed by Limnoterra and modified by Keith Jackson, Department of Biological Sciences, University of Alberta. I viewed meristic and morphometric characters on specimens under magnification with a WILD M5 stereomicroscope and prepared drawings using a camera lucida.

Principal component analysis (PCA) was used to analyze variation among specimens of different species based on their morphological characters. PCA is a data transformation technique. If for a series of objects a number of variables is measured, then each variable will have a variance and usually the variables will be associated with each other. The data set as a whole will have a total variance which is the sum of the individual variances. Each variable measured is an axis of variability. In PCA data are transformed to describe the same amount of variability and total variance with the same number of axes and variables. The new axes are uncorrelated with each other and are weighted according to the amount of the total variance that they describe. Normally this results in there being a few large axes accounting for most of the total variance and many small axes accounting for very small amount of the total variance. These small axes are normally discounted from further consideration, so that the analyst has transformed his correlated variables to an uncorrelated data set with few principal components. PCA was calculated from the correlation matrix of characters using StatView SE + Graphics™ statistical program on a Macintosh computer. In this program dissimilar groups can be identified by the separation of clusters in plots of principal component scores. In multigroup analysis of morphology, the first principal component primarily accounts for size variation, the second and subsequent principal components are generally interpreted as shape contrasts, however, they contain size information not accounted for by the first component (Baumgartner, 1991).

Systematic methods

For systematic analysis, I adopted a cladistic approach using the computer program MacClade (Maddison and Maddison, 1992). The goal of this analysis is to discover and describe monophyletic groups by identifying shared, derived character states or synapomorphies (Wiley et al., 1991). To determine polarity of characters, the most controversial part of any cladistic analysis, there are several methods among which I used the out-group comparison method (Watrous and Wheeler, 1981). In this method derived characters can be distinguished from ancestral states by being absent in the outgroups. Some workers consider *Culaea* to be the sister group of *Pungitius* or of *Gasterosteus* or of both (McLennan, 1993) and Aulorhynchidae of Gasterosteidae (Bowne, 1985; McLennan, 1993). The principle of parsimony, which minimizes the number of assumptions needed to explain character transformations, was used to distinguish the relationship of species within the genus. When a cladistic analysis allows for the construction of several possible cladograms, that one which is the shortest, that is, has the fewest changes in character states is considered to be the best. This is called the principle of parsimony (Mayr and Ashlock, 1991).

Materials

There is a good collection of sticklebacks in the University of Alberta Museum of Zoology (UAMZ). This collection contains representatives of all genera of Gasterosteidae and of all the species of *Pungitius* from much of their range (Japan, Russia, Romania, Germany, Greece, Poland, Ireland, Baltic Sea, and North America). Some 310 specimens from different localities were examined and measured (table 2.1)

The following materials were examined:

P. hellenicus: UAMZ 7590, UAMZ 7734.

P. platygaster: UAMZ 4734, UAMZ 4736.

P. laevis: UAMZ 4758, UAMZ 4759, UAMZ 4760, UAMZ 6556, UAMZ 6557, UAMZ 6558, UAMZ 6559, UAMZ 6560, UAMZ 6561, UAMZ 6562.

P. pungitius: UAMZ 4739, UAMZ 4740, UAMZ 4741, UAMZ 4742, UAMZ 4743, UAMZ 4746, UAMZ 4748, UAMZ 5460, UAMZ 5461, UAMZ 5462, UAMZ 5463, UAMZ 5464, UAMZ 5465.

P. tymensis: UAMZ 4744, UAMZ 4745.

P. sinensis: UAMZ 4749, UAMZ 5466.

P. occidentalis: UAMZ 3049, UAMZ 3318, UAMZ 3319, UAMZ 3794, UAMZ 4437, UAMZ 4747, UAMZ 4755, UAMZ 4756, UAMZ 4757, UAMZ 5427, UAMZ 5439, UAMZ 5440, UAMZ 5474, UAMZ 5474, UAMZ 6355, UAMZ 5475, UAMZ 7017, UAMZ 7100, UAMZ 7018, UAMZ 7021.

Gasterosteus aculeatus: UAMZ 3894, UAMZ 7735.

Culaea inconstans: UAMZ 3797.

Aulorhynchus flavidus: UAMZ 3783.

Table 2.1. List of the specimens counted, measured, and used for statistical analyses.

Nominal species	Country	Locality	Number of specimens
P. hellenicus	Greece	Sperchios River	20
P. platygaster	Romania	Lake Caraorman	18
	Russia	Sea of Azov	06
P. laevis	Ireland	Brosna River	22
		Suck River	21
P. pungitius	Russia	Neva River	03
		Baltic Sea	06
	Japan	Hakkodate, Hokkaido	10
		Tomakomai, Hokkaido	10
		Konuma Lake, Hokkaido	12
		Toro Lake, Hokkaido	05
		Tendo, Yamagata	10
		Shinto, Yamagata	10
		Aomori	05
		Tsutanuma Pond, Aomori	09
	Poland	Bzura River	09
	Germany	Hunnan Brook, Abrensburg	10
		Sylt, Northern Frisian Island	10
P. tymensis	Russia	Sakhalin Island	10
P. sinensis	Japan	Tedori Fan, Ishikawa	20
P. occidentalis	Canada	Firth River, Yukon	23
		Pine Lake, Wood Buffalo National Park	20
		Cold Lake, Alberta	20
	U. S. A.	Crooked Lake, Indiana	21
Total			310

3. A brief history of the genus *Pungitius*

Ecology

Ninespine sticklebacks are small, mainly freshwater fish, feeding on crustaceans and other small invertebrates. Many workers have studied the ecology and biology of *P. pungitius* (Munzing, 1966; Wilz, 1971; Griswold and Smith, 1973; Goto et al., 1979; Maksimenko and Tokranov, 1995) and a few of *P. sinensis* (Tanaka and Hoshino, 1979; Chae and Yang, 1993), but there is not much information on the biology of other nominal species and subspecies.

The typical habitat of *P. hellenicus* is clean, well oxygenated and cool springs, reaching no more than 12 °C in summer, with rich vegetation (Economidis, 1995). There is no other information on the ecology or biology of the species. From the specimens I examined, it seems that none of the spawned fish survives, so, the maximum life span of *P. hellenicus* is only one year and they reach the sexual maturity during the first year. This phenomenon has been reported for other species of *Pungitius* and for other genera of sticklebacks (Tanaka and Hoshino, 1979).

Taxonomy

Originally, Linnaeus (1758) recognized *P. pungitius* as a species of *Gasterosteus* (threespine stickleback) from Europe, but later, other authors recognized it in a separate genus and gave various names to it. Coste (1848) erected the genus *Pungitius*. Among its synonyms are *Pygosteus* Gill, 1861, *Gasterostea* Sauvage, 1874, and *Gasterosteops* Schtylko, 1934. Due to variation in the morphology of these fish, many forms were recognized in this genus and given species status. North America and Japan were the two problematic areas and Japan still is.

Gmelin (1789) distinguished between *P. pungitius* and the North American form naming the latter *G. occidentalis*. Cuvier (1829) recognized *P. laevis* from southern France as a separate species. Guichenot (1869) recognized *P. sinensis* as a separate species and Tanaka (1915) recognized another subspecies in this species, *P. s. kaibarae*. *Pungitius platygaster* was recognized as a separate species from *P. pungitius* by Kessler (1859). Nikolsky (1889) named *P. tymensis* from Sakhalin island as a valid species. Berg (1907) recognized three species of *Pungitius* in Japan: *Pygosteus pungitius*, *P. tymensis*, and *P. sinensis* with two subspecies; *P. s. stenurus* Kessler and *P. s. wossnessenskyi* Kessler. Otaki (1907) recognized three species in Japan: *Pygosteus steindacheri*, *P. brevispinosus*, and *P. undecimalis* and Kobayashi (1932) recognized the species: *Pungitius brevispinosus* Otaki, and *P. sinensis* with one variety, *P. s. kaibarae*, and one subspecies, *P. p. tymensis*, in Japan.

Berg (1949) recognized four species within *Pungitius*: *P. pungitius* with two subspecies (*P. p. pungitius* and *P. p. sinensis*), *P. tymensis*, *P. platygaster* with two subspecies (*P. platygaster platygaster* and *P. platygaster aralensis*) and *P. laevis*. Many other species were recognized in North America, Europe, and Japan. McAllister (1987) listed more than 30 species of *Pungitius* recognized during 1789-1918. Bertin (1925) lumped all species of *Pungitius* into one species, *Pungitius pungitius*. However, nowadays some workers recognize two species in the genus, *P. pungitius* and *P. platygaster*, and some recognize more than two.

In 1939 A. Stephanidis found a specimen of a new form of *Pungitius* in a freshwater spring of the Sperchios valley in Greece and recognized it as *Pungitius pungitius*. In spite of his continuing efforts to collect more specimens, he was only successful in 1970, when he collected 40 specimens, 20-50 mm long from the same valley.

Due to structural differences between these specimens and those of *P. pungitius* (e.g., loss of pelvic girdle, and lower number of dorsal spines) he named them as a new species, *Pungitius hellenicus* (Stephanidis, 1971).

McPhail (1963) observed geographic variation in inland and tidal populations of *P. pungitius* in North America and recognized two forms of *Pungitius*, the Mississippi and Bering forms, but believed the degree of differentiation between them not to be taxonomically significant. Narita (1970), in studying physiological and ecological characteristics of the two forms of *Pungitius* in the Churchill area, claimed that the inland and tidal forms of *Pungitius* in the Churchill area fulfill the requirements of subspecies recognition and they may deserve to be recognized as subspecies if studies in other areas confirm findings in the Churchill area.

Nelson (1971b) found that a high proportion of *P. pungitius* from Ireland and Pine Lake, Wood Buffalo National Park, failed to develop a complete pelvic girdle. Zyuganov and Rozanov (1987) discovered a population of *P. pungitius* without a pelvic girdle in the Loukhi district of Russia. Zyuganov (1989) discovered reproductively isolated parapatric forms of the ninespine stickleback, with and without a pelvic girdle.

Tanaka (1982) found morphological differences between the sympatric populations of *P. pungitius* and *P. sinensis* in Honshu, Japan with intermediate forms that could have resulted from natural hybridization and introgression. Takata et al. (1984), by comparing morphological characters and distribution patterns of *Pungitius* in Hokkaido, suggested that *P. tymensis* is a separate species from *P. pungitius* and *P. sinensis*. Even though their comparative study did not show *P. pungitius* and *P. sinensis* to differ from each other at the species level, neither did it show them to be identical. They suggested that *P. sinensis* should be recognized from *P. pungitius* at a lower taxonomic level than species.

Ziuganov and Gomeluk (1985) observed hybridization between *P. pungitius* and *P. platygaster* in aquaria and ponds. There were no ethological isolating mechanisms and F1, F2, and F3 hybrids were fertile. They believed that although *P. pungitius* and *P. platygaster* have diverged significantly, ecologically and morphologically, they seem to have diverged only slightly ethologically and genetically and the differences between them being not enough to exceed the subspecies or at the most, the semispecies rank.

Takata and Goto (1986) reported a brackishwater type of *P. pungitius* from Japan. They examined genetic differences based on 13 loci of six enzymes and muscle proteins. Even though their distributions partially overlapped in the river, the brackishwater type differed from the freshwater form in having a higher number of dorsal spines and gill rakers and a lower number of vertebrae. Segregation in spawning sites and differences in nuptual colors of the male were also found. They concluded that the brackish water type is reproductively isolated from the freshwater type, and should be regarded as separate species.

Takata et al. (1987) observed significant differences in average number of meristic characters in sympatric populations of *P. pungitius* and *P. sinensis* in the Omono River. Genetic distances between the two species showed a value similar to congeneric species in other fishes, suggesting that they could be regarded as separate species. They also suggested that *P. pungitius* and *P. sinensis* in the Omono River belong to different species than those of Hokkaido.

Niwa (1987) in estimating gene frequencies by electrophoretical analysis, detected significant differences between *P. tymensis* and other species and suggested that *P. tymensis* is reproductively isolated from other species even when they coexist. However, a few hybrids were found between *P. tymensis* and *P. pungitius*. No significant differences between *P. pungitius* and *P. sinensis* were detected.

Kim et al. (1989) observed differences between the mean number of dorsal spines, dorsal soft rays and vertebrae of *P. sinensis* and *P. s. kaibarae* in Korea, but no karyotypic differences. Yang and Min (1990) in studying the electrophoretic variations in isozymes and proteins encoded by 25 presumptive loci among Korean sticklebacks, believed the value of genetic similarity coefficents between *P. sinensis* and *P. kaibarae* to be low enough to consider them as separate species. However, moderate hybridization between them in sympatry indicates that isolating mechanisms are incomplete.

Haglund et al. (1992) provided evidence to suggest that what was conventionally regarded as *P. pungitius* consists of three clades and recommended that the name *P. pungitius* be applied to the European form, *P. sinensis* to the Asian form and *P. occidentalis* to the North American form.

Paleontology

Despite the present wide distribution of *Pungitius spp.*, few fossils have been reported. The first was reported from eastern Siberia by Schtylko (1934). David (1945) reported a new species of *Pungitius, P. haynesi* from California, but Bell (1973) provided evidence to reject her identification and claimed that *P. hayensi* is a junior synonym of *Gasterosteus aculeatus*. Liu and Wang (1974) reported a ninespine stickleback fossil fish from lacustrine formations of late Pliocene from North China and named their specimen *P. nihowanensis* due to differences in shape of the pelvic bones and scutes of the fossil relative to those of extant *P. pungitius*. However, they claimed it to be similar to *P. sinensis*. It seems they were not aware of the variation in the pelvic girdle of *Pungitius spp.*, so I believe that *P. nihowanensis* is a junior synonym of *P. pungitius* and probably of *P. p. tymensis*. The reduced pelvic spines, overall shape of the pelvic bones and shape of the scutes in *P. nihowanensis* more closely resemble those in *P. p. tymensis* than in other species or subspecies of *Pungitius*. Rawlinson and Bell (1982) reported another specimen of *Pungitius* from a Miocene formation of Alaska, but the description of the fossils is almost the same as that of the modern *P. pungitius*.

4. Morphometrics

Traditional morphometric characters

Twenty four traditional morphometric characters were measured on the specimens (figure 4.1). These characters are: total length, standard length, interorbital width, head width, caudal peduncle width, predorsal length, preanal length, orbit diameter, snout length, dorsal spine base length, head length, pectoral length, upper prepectoral length, occiput-pectoral distance, pectoral base length, last dorsal spine length, anal spine length, orbit-preoperculum distance, orbit-articular distance, body depth, length of the longest gill raker on the first arch, lachrymal width, maxilla length, and caudal peduncle depth. All measurements were obtained from the left side of the specimens. Table (4.1) shows the mean, range and standard deviation of the standard length of the examined specimens.

After doing PCA analyses on combinations of paired species, I plotted the factor scores of PCA against each other. *Pungitius hellenicus* showed different clusters from the other species (figures 4.2-4.4); however, its difference from *P. tymensis* is uncertain, because of the size differences (no overlap in size of specimens) and the small sample size of *P. tymensis*. *Pungitius platygaster* showed different clusters from *P. sinensis,* and *P. occidentalis* (figures 4.5-4.6), but because of its limited size range, it is difficult to estimate the size trend in this species, so, these differences might be size related differences. Two populations of *P. occidentalis* from Pine Lake and Crooked Lake also differ from each other in traditional morphometric characters (figure 4.7). There is no apparent difference between *P. sinensis* and *P. pungitius.* Principal component factor scores also do not show separate clusters in comparisons of *P. laevis, P. pungitius,* and *P. occidentalis.*

Truss distances

I analyzed a box-truss scheme consisting of twenty one truss distances (figure 4.8) using the following points:

1) snout tip, 2) occiput, 3 and 4) origin and end of dorsal soft fin, 5 and 6) hypural plate, 7 and 8) end and origin of anal fin, 9) cleithrum joint, and 10) articular.

Distances between the following points comprise the 21 truss distances:

Truss 1 (1-2), truss 2 (2-3), truss 3 (3-4), truss 4 (4-5), truss 5 (5-6), truss 6 (6-7), truss 7 (7-8), truss 8 (8-9), truss 9 (9-10), truss 10 (10-1), truss 11 (2-9), truss 12 (3-8), truss 13 (4-7), truss 14 (1-9), truss 15 (2-10), truss 16 (2-8), truss 17 (3-9), truss 18 (3-7), truss 19 (4-8), truss 20 (4-6), truss 21 (5-7).

Similar to the traditional morphometric characters, after doing a PCA analysis for each set of paired species, the factor scores were plotted against each other. *Pungitius hellenicus* showed differences from all the other species except *P. laevis* (figures 4.9-4.12). *Pungitius platygaster* showed differences from *P. pungitius* and *P. sinensis* (figure 4.12). *Pungitius laevis* differed from *P. sinensis*. *Pungitius tymensis* showed differences from all the other species, however, these differences might be size related factors. Two populations of the North American inland form show differences in their body form (figure 4.13).

To specifically distinguish among the more variable parts in the nominal species, I did a separate PCA analysis for the head and tail regions. In the head region *P. hellenicus* and *P. tymensis* differ distinctively from each other and from the other species except *P. pungitius* and *P. occidentalis*. PCA shows a difference in the tail region of *P. hellenicus* from those of the other species and it seems that the major variable parts are the head and the tail regions.

Ratios

Twelve ratios were calculated from the traditional morphometric characters and truss distances (Table 4.2). Those ratios are body depth to standard length, head length to standard length, truss 11 to truss 12, interorbital width to head width, caudal peduncle width to caudal peduncle depth, truss six to standard length, truss five to standard length, caudal peduncle depth to standard length, snout length to truss one, last dorsal spine length to truss 12, anal spine length to truss 12, and head length to standard length. Correlation matrices were calculated between all ratios and the standard length; none showed an apparent correlation with the size.

Pungitius hellenicus and *P. platygaster* have a deeper body than the other species while *P. occidentalis* shows the shallowest body among the nominal species. The longest head is in *P. hellenicus* and the shortest one in *P. tymensis*. *Pungitius hellenicus, P. platygaster, P. laevis* and *P. occidentalis* have a relatively deeper head than the other species. The interorbital width in *P. hellenicus* is shorter than in the others. *Pungitius hellenicus* has the narrowest caudal peduncle and *P. occidentalis* the widest one. The longest caudal peduncle (represented by truss 6) is in *P. occidentalis*, *P. sinensis* and *P. platygaster* and the shortest one in *P. hellenicus*. *Pungitius hellenicus* has the deepest hypural plate (represented by truss 5) among the nominal species.

The deepest caudal peduncle is found in *P. hellenicus* and the shallowest one in *P. occidentalis*. There is an enormous variation in snout length of the species, but *P. hellenicus* has the shortest one. The length of the dorsal spine and anal spine in *P. hellenicus* is distinctively shorter than those of the other species, however, they overlap with those of *P. laevis*.

Pungitius hellenicus has the longest head and *P. tymensis* the shortest head.

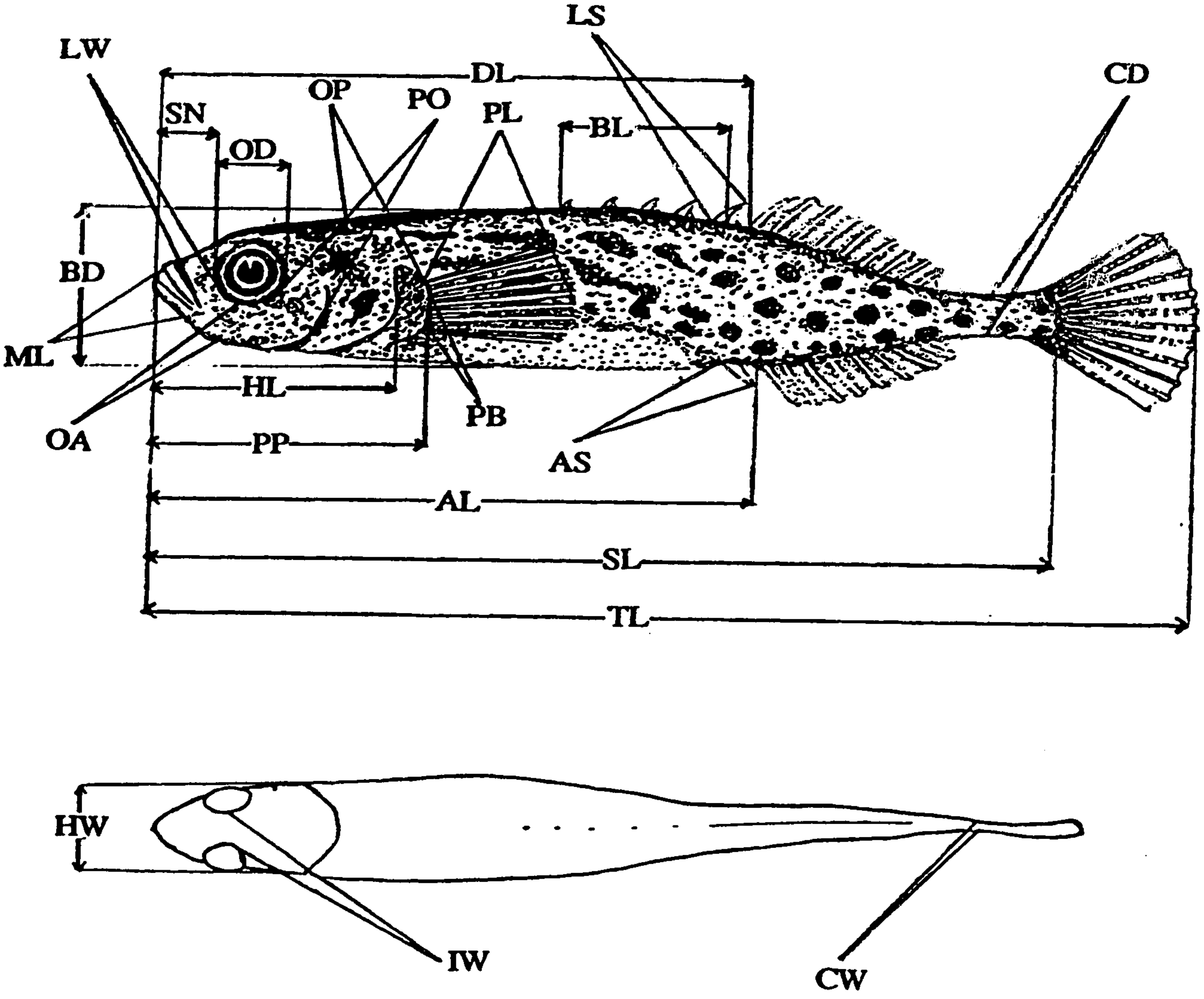


Figure 4.1. Traditional morphometric characters measured on the specimens. Top, lateral view; bottom, dorsal view.

Preanal length (AL), anal spine length (AS), body depth (BD), dorsal spine base length (BL), caudal peduncle depth (CD), caudal peduncle width (CW), predorsal length (DL), head length (HL), head width (HW), interorbital width (IW), last dorsal spine length (LS), lachrymal width (LW), maxilla length (ML), orbit-articular distance (OA), orbit diameter (OD), occiput-pectoral distance (OP), pectoral base length (PB), pectoral length (PL), orbit-preoperculum distance (PO), upper prepectoral length (PP), standard length (SL), snout length (SN), and total length (TL).

Table 4.1. Mean, range, and standard deviation of the examined specimens.

Species	Mean (mm)	Range (mm)	Sd
P. hellenicus	25	20-33	03
P. platygaster	37	33-42	02
P. laevis	33	24-45	05
P. pungitius	39	22-57	07
P. tymensis	54	42-62	07
P. sinensis	38	24-62	09
P. occidentalis	36	12-59	10

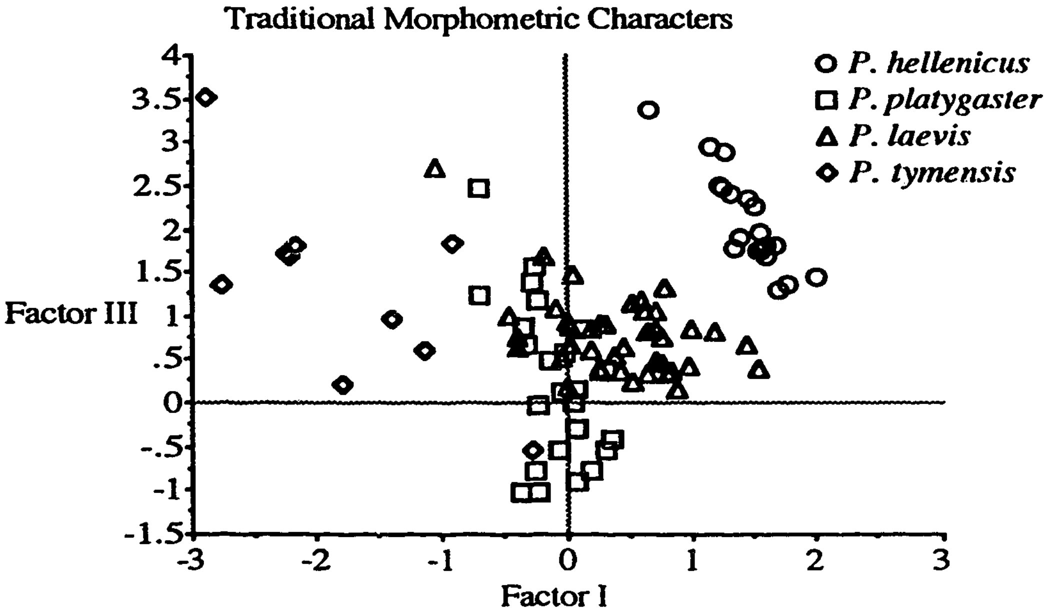


Figure 4.2. Factors I and III plotted against each other for *P. hellenicus*, *P. platygaster*, *P. laevis*, and *P. tymensis*.

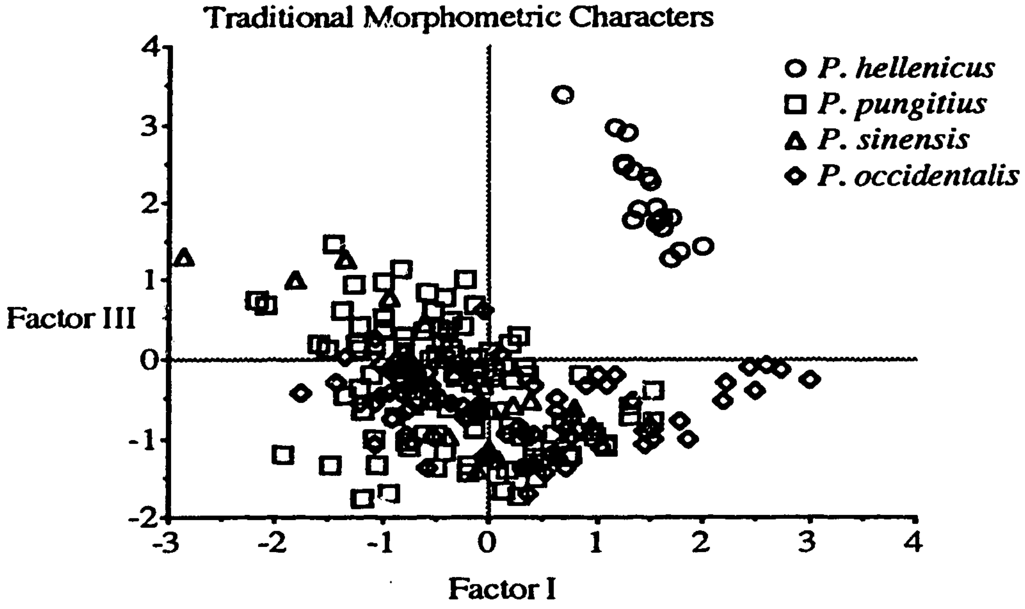


Figure 4.3. Factors I and III plotted against each other for *P. hellenicus*, *P. pungitius*, *P. sinensis*, and *P. occidentalis*.

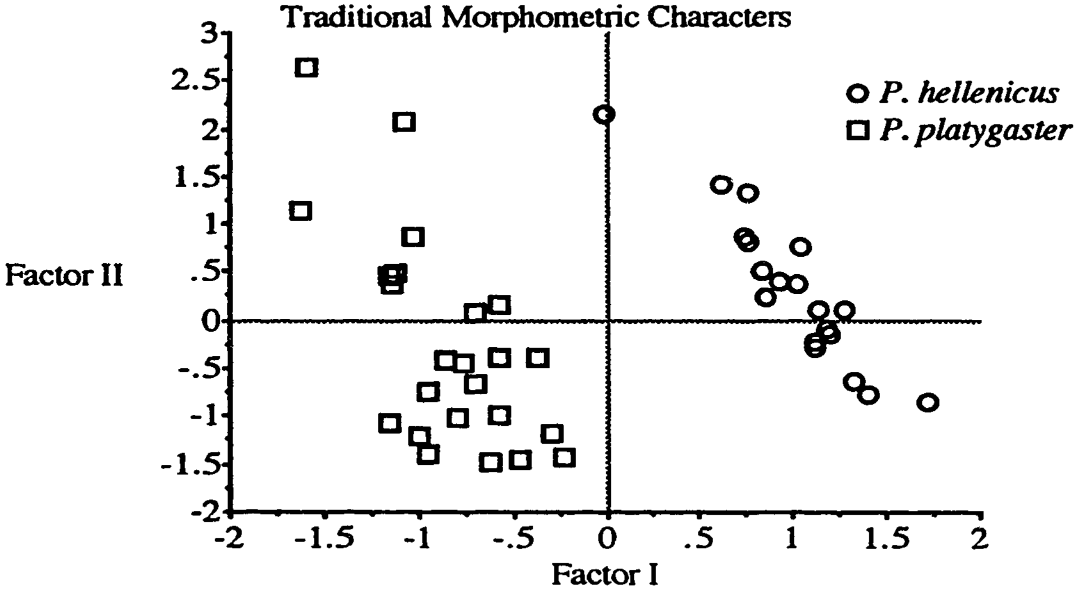


Figure 4.4. Factors I and II plotted against each other for *P. hellenicus* and *P. platygaster*.

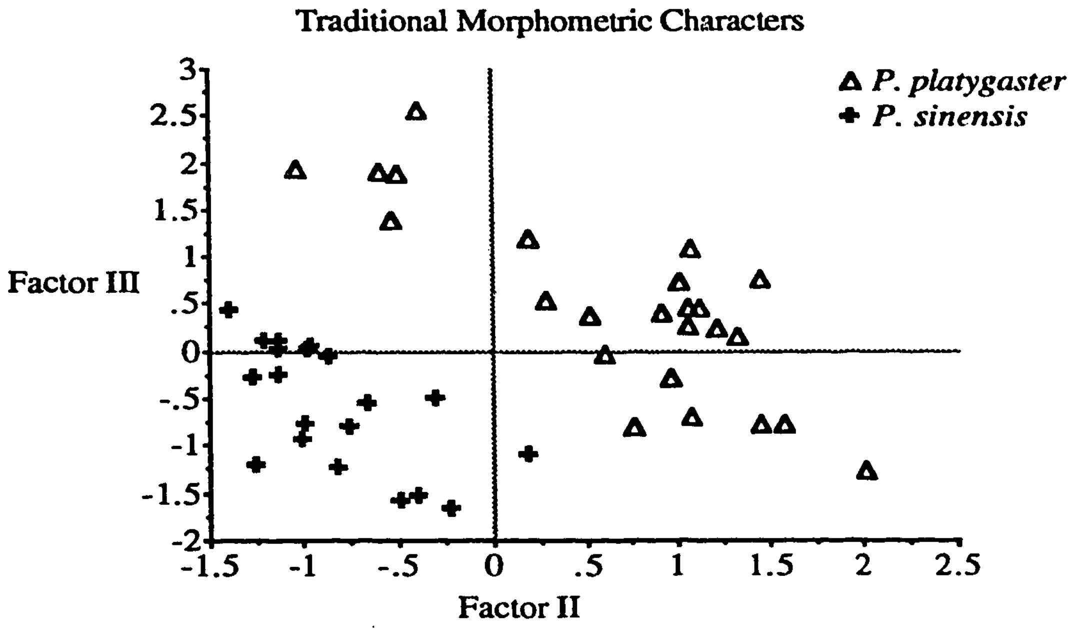


Figure 4.5. Factors II and III plotted against each other for *P. platygaster* and *P. sinensis.*

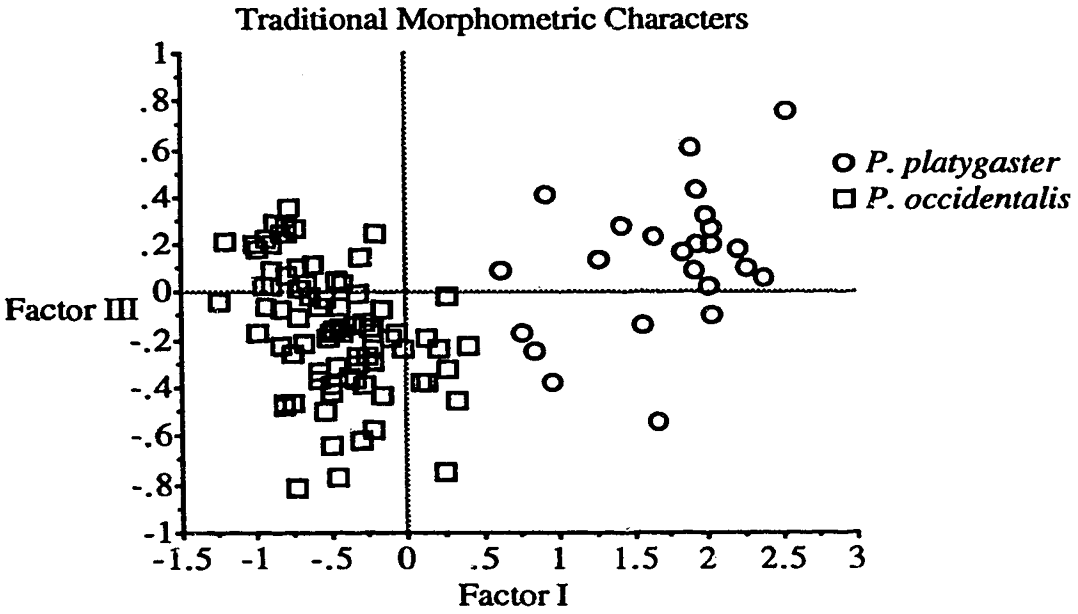


Figure 4.6. Factors I and III plotted against each other for *P. platygaster* and

P. occidentalis.

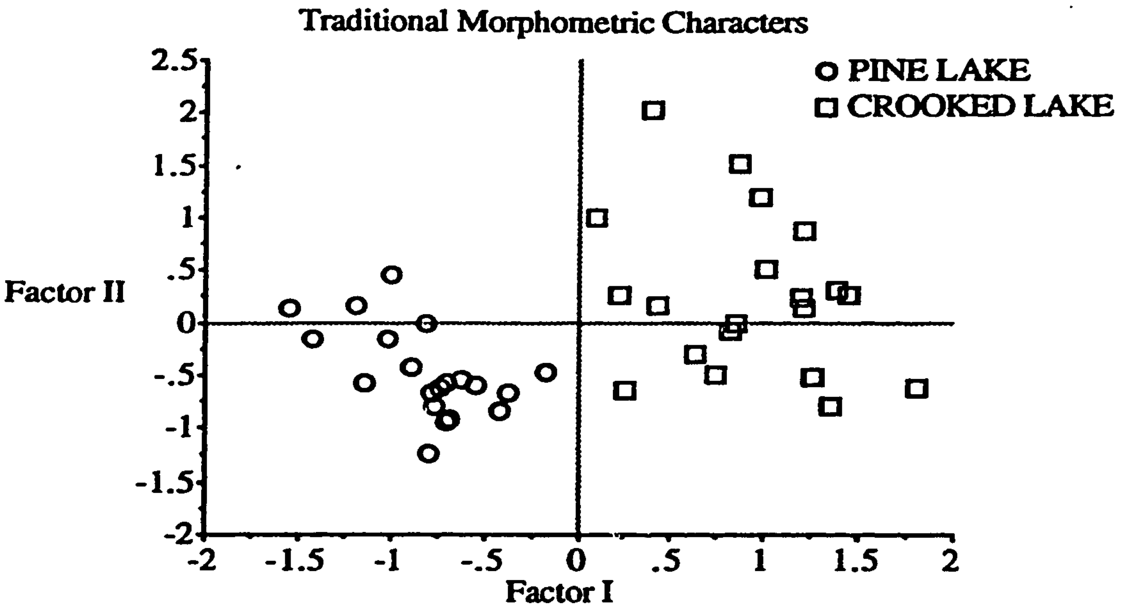


Figure 4.7. Factors I and II plotted against each other for two populations of *P. occidentalis* in North America.

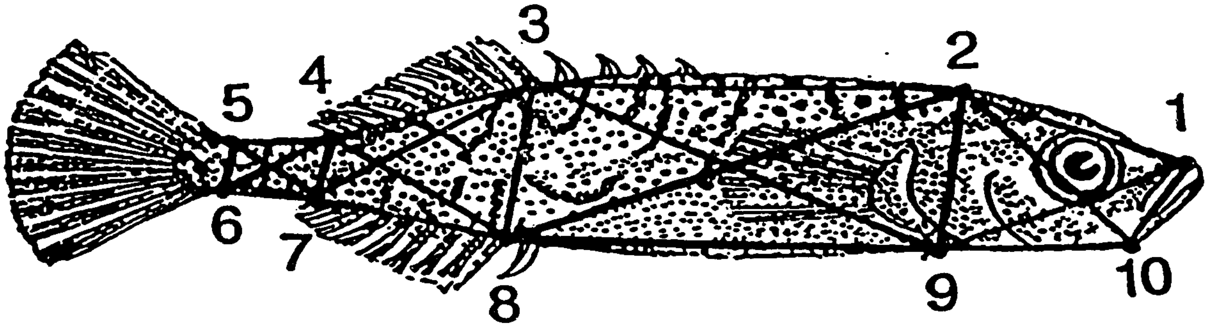


Figure 4.8. A truss box showing the 10 selected landmarks and the 21 truss distances on a specimen of *P. hellenicus*.

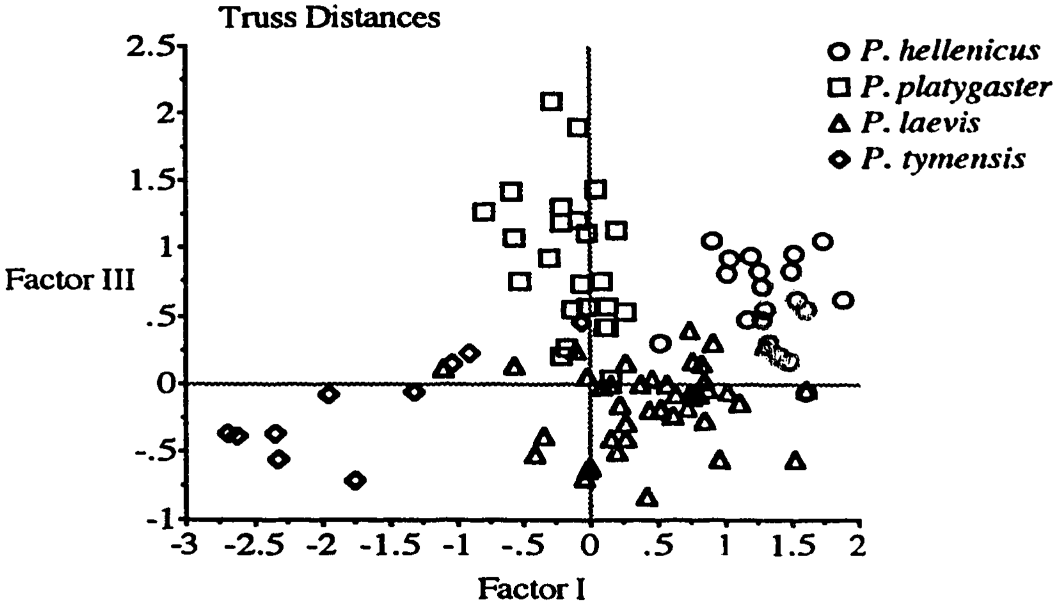


Figure 4.9. Factors I and III plotted against each other for *P. hellenicus*, *P. platygaster*, *P. laevis*, and *P. tymensis*.

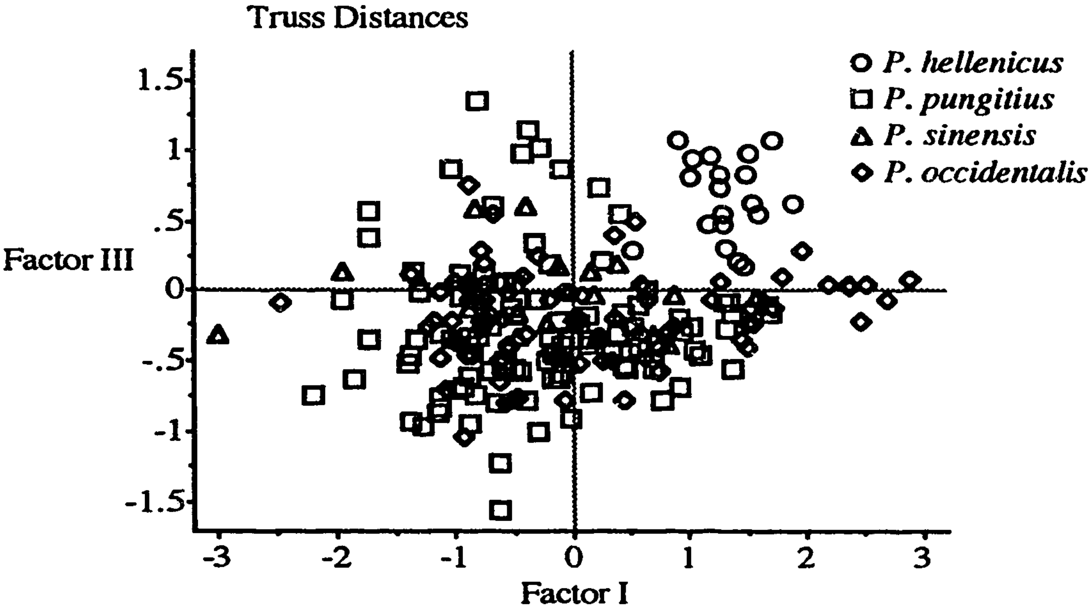


Figure 4.10. Factors I and III plotted against each other for *P. hellenicus*, *P. pungitius*, *P. sinensis*, and *P. occidentalis*.

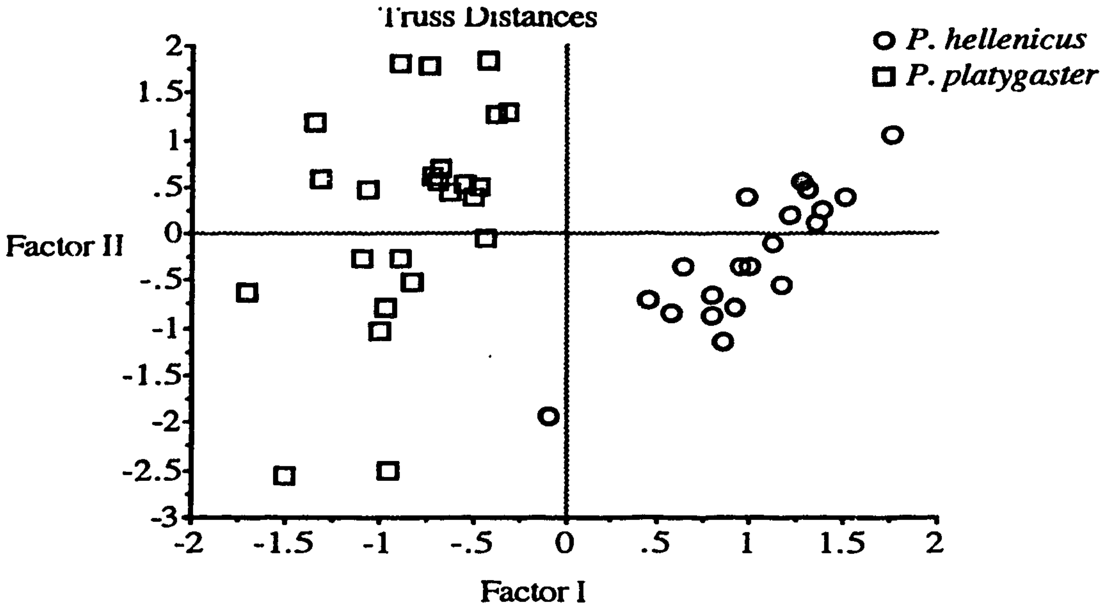


Figure 4.11. Factors I and II plotted against each other for *P. hellenicus* and *P. platygaster.*

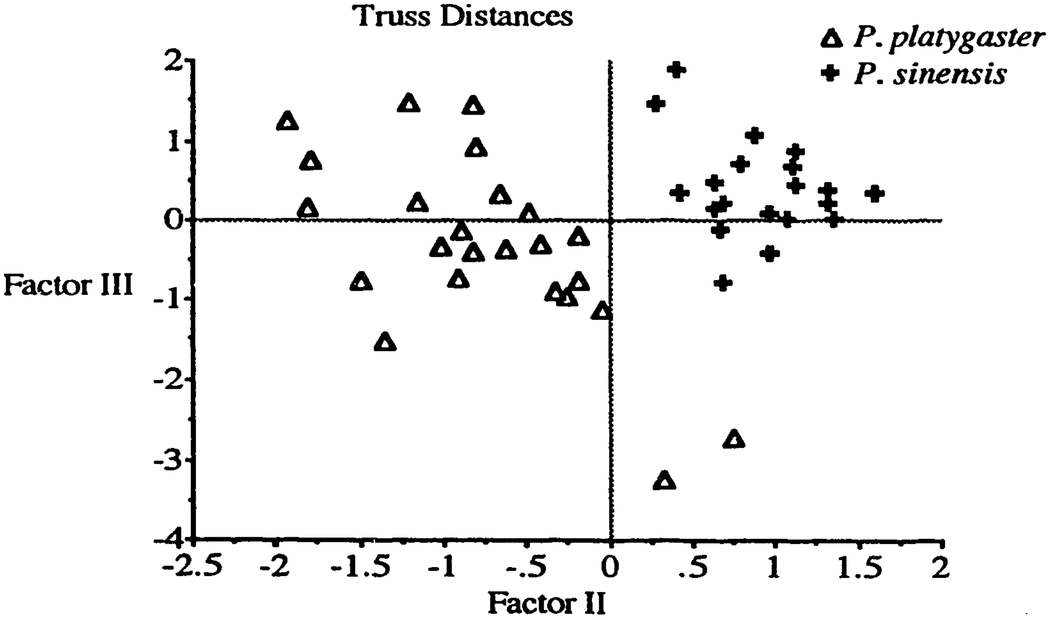


Figure 4.12. Factors II and III plotted against each other for *P. platygaster* and *P. sinensis.*

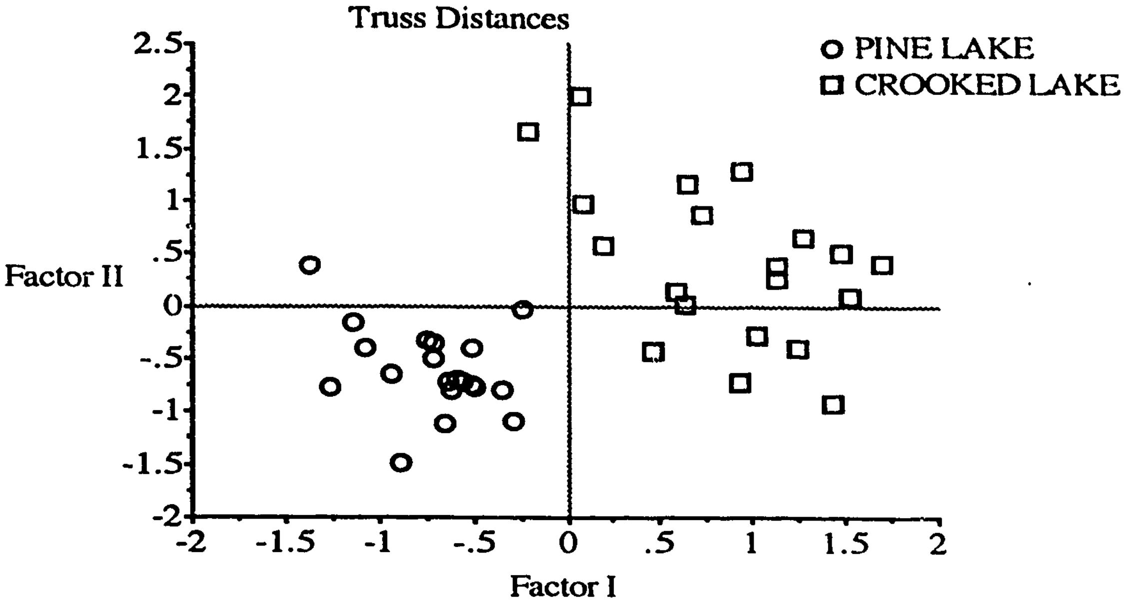


Figure 4.13. Factors I and II plotted against each other for two popuiations of *P. occidentalis* in North America.

Table 4.2. Mean, range, and standard deviation of the twelve ratios.

	Body Depth / S. L.			Head Length / S. L.			Truss 11 / Truss 12		
	Mean	Range	Sd	Mean	Range	Sd	Mean	Range	Sd
P. hellenicus	0.21	0.19-0.24	0.02	0.31	0.29-0.33	0.01	1.02	0.94-1.10	0.04
P. platygaster	0.21	0.19-0.24	0.01	0.29	0.16-0.33	0.03	1.04	0.89-1.18	0.08
P. laevis	0.20	0.17-0.23	0.01	0.29	0.26-0.31	0.01	1.07	0.91-1.27	0.08
P. pungitius	0.20	0.17-0.25	0.02	0.29	0.24-0.32	0.02	0.98	0.51-1.15	0.09
P. tymensis	0.19	0.16-0.21	0.02	0.26	0.25-0.28	0.01	0.98	0.87-1.09	0.07
P. sinensis	0.20	0.18-0.21	0.01	0.29	0.26-0.31	0.01	0.99	0.92-1.07	0.05
P. occidentalis	0.18	0.14-0.24	0.02	0.28	0.23-0.32	0.02	1.07	0.51-1.24	0.09
	Interorbital Width Head Width			Caudal Peduncle Width Caudal Peduncle Depth			Truss 6 / S. L.		
P. hellenicus	0.40	0.35-0.45	0.03	0.39	0.24-0.47	0.07	0.11	0.09-0.14	0.01
P. platygaster	0.50	0.41-0.83	0.08	0.55	0.25-0.94	0.15	0.16	0.10-0.20	0.02
P. laevis	0.45	0.38-0.53	0.03	0.78	0.45-1.25	0.19	0.14	0.10-0.17	0.02
P. pungitius	0.48	0.36-0.58	0.05	1.24	0.67-1.92	0.26	0.14	0.10-0.19	0.09
P. tymensis	0.43	0.34-0.48	0.05	1.01	0.83-1.32	0.15	0.12	0.09-0.14	0.02
P. sinensis	0.48	0.43-0.54	0.03	1.40	0.89-1.89	0.23	0.16	0.14-0.20	0.01
P. occidentalis	0.47	0.35-0.63	0.04	1.58	0.29-2.38	0.48	0.16	0.05-0.23	0.03
	Truss 5 / S. L.			Caudal Peduncle Depth S. L.			Snout L. / Truss 1		
P. hellenicus	0.07	0.05-0.09	0.01	0.06	0.05-0.07	0.01	0.29	0.24-0.33	0.02
P. platygaster	0.06	0.05-0.08	0.01	0.04	0.03-0.06	0.01	0.31	0.21-0.38	0.04
P. laevis	0.05	0.04-0.07	0.01	0.03	0.02-0.04	0.00	0.31	0.25-0.36	0.02
P. pungitius	0.06	0.04-0.08	0.01	0.03	0.02-0.04	0.01	0.31	0.26-0.37	0.02
P. tymensis	0.06	0.05-0.07	0.01	0.03	0.03-0.04	0.00	0.32	0.27-0.35	0.02
P. sinensis	0.05	0.05-0.06	0.00	0.03	0.02-0.04	0.00	0.32	0.29-0.35	0.02
P. occidentalis	0.05	0.03-0.15	0.01	0.02	0.02-0.06	0.01	0.31	0.13-0.35	0.03
	Last Dorsal Spine Length Truss 12			Anal Spine Length Truss 12			Head Length / S. L.		
P. hellenicus	0.18	0.09-0.23	0.04	0.22	0.12-0.31	0.05	0.31	0.29-0.33	0.01
P. platygaster	0.37	0.23-0.48	0.08	0.42	0.29-0.57	0.08	0.29	0.16-0.33	0.03
P. laevis	0.26	0.11-0.37	0.05	0.28	0.21-0.36	0.04	0.29	0.26-0.31	0.01
P. pungitius	0.35	0.21-0.57	0.09	0.38	0.13-0.61	0.10	0.29	0.24-0.32	0.02
P. tymensis	0.33	0.27-0.44	0.06	0.40	0.27-0.54	0.07	0.26	0.25-0.28	0.01
P. sinensis	0.34	0.23-0.44	0.07	0.36	0.23-0.51	0.08	0.29	0.26-0.31	0.01
P. occidentalis	0.31	0.19-0.50	0.06	0.36	0.23-0.55	0.07	0.28	0.23-0.32	0.02

5. Meristics

Fifteen meristic characters were counted on the specimens (Table 5.1). The left sides of the specimens were counted for paired structures such as gill rakers. Number of the pelvic spines and soft rays were counted on both sides due to the asymmetry in these characters. None of the meristic characters shows an obvious correlation with standard length.

Dorsal and anal spines

Pungitius hellenicus has the lowest number of the dorsal spines with a mean of 4.1, and *P. tymensis* the highest number with a mean of 11.8. Other species are intermediate, *P. platygaster* (8.8), *P. laevis* (9.1), *P. pungitius* (9.2), *P. sinensis* (9.1), and *P. occidentalis* (9.2). The range for the dorsal spines is 2-6 in *P. hellenicus*, 8-10 in *P. platygaster*, 5-10 in *P. laevis*, 8-11 in *P. pungitius*, 11-13 in *P. tymensis*, 8-10 in *P. sinensis*, and 8-11 in *P. occidentalis*. Among the nominal species only the dorsal spines of *P. hellenicus* are all vertical, those of the other species are mostly inclined alternately to left and right. All species of *Pungitius* have one anal spine preceding the anal soft rays.

Dorsal and anal fin soft rays

Dorsal and anal soft rays vary greatly in *Pungitius*. Dorsal soft rays vary from 6-12. *Pungitius platygaster* has the lowest mean number of the dorsal soft rays (8.0) and *P. tymensis* has the highest number (10.6). *Pungitius hellenicus* has the second lowest number (9.2).

The number of anal soft rays varies from 6-13 in *Pungitius*; however, the mean number of the anal soft rays is less than the mean number of the dorsal soft rays. The lowest mean number of the anal soft rays is also in *P. platygaster* (7.0). *Pungitius* *hellenicus* has the next lowest mean number (8.8). *Pungitius tymensis* has the highest mean number of the anal soft rays (10.1).

Dorsal and anal basals

Pungitius has fewer dorsal basals than its adjacent vertebrae and usually two more dorsal basals precede the dorsal spines. *Pungitius hellenicus* and *P. platygaster* have the lowest mean number of dorsal basals (22.8 and 22.4) and anal basals (12.2 and 11.2). The highest mean number of the dorsal basals occurs in *P. tymensis* (26.4) and the highest mean number of the anal basals in *P. occidentalis* (14.0).

Caudal and pectoral fin rays

There is very little variation in number of the caudal and pectoral fin soft rays. Usually there are 10 soft rays in the pectoral fin (rarely 9 or 11) and 12 soft hypural rays in the caudal fin of all species of *Pungitius*, two of which are unbranched. However, the mean number of rays on the epural differs slightly in the nominal species. *Pungitius hellenicus* and *P. tymensis* have the highest mean number of the epural rays (5.3), and *P. sinensis* has the lowest number (4.1).

Pelvic spines and soft rays

The presence or absence of the pelvic spines and soft rays are related to the developmental status of the pelvic girdle. In species such as *P. hellenicus* with no developed pelvic girdle there are no pelvic spines or soft rays. In *P. platygaster*, usually there are two pelvic spines (one on each side), each one associated with one soft ray (rarely the soft ray is absent on one side). Some specimens of *P. laevis* (0-2) and *P. occidentalis* (0-2) with reduced pelvic girdle, have one spine and one or no soft ray. *Pungitius sinensis* has the highest mean number of the pelvic soft rays (3.9) with a range of 3-5 (usually 4) on the two sides. *Pungitius pungitius* in Europe usually has two pelvic soft rays, but many specimens sympatric with ***P. sinensis*** in Japan, have 3 or 4 pelvic soft rays (2.4). This may result from hybridization with ***P. sinensis***. ***Pungitius tymensis*** usually has 2 pelvic soft rays, each associated with one of the pelvic spines.

Gill rakers

The number of the gill rakers on the first left branchial arch varies from seven to sixteen in the specimens with the lowest mean number in ***P. hellenicus*** and ***P. tymensis*** (9.1) and the highest mean number in ***P. occidentalis*** (12.3). The range is 7-10 in ***P. hellenicus***, 9-11 (10.2) in ***P. platygaster***, 9-12 (10.7) in ***P. laevis***, 9-13 (10.9) in ***P. pungitius***, 8-11 in ***P. tymensis***, 10-14 (11.6) in ***P. sinensis***, and 8-16 in ***P. occidentalis***.

Vertebrae

Precaudal vertebrae were counted from the first vertebra to the beginning of the anal spine pterygiophore insertion. ***Pungitius hellenicus*** has the lowest mean number of the precaudal vertebrae (12.9). ***Pungitius platygaster*** has the second lowest precaudal vertebrae (13.4). The highest mean number belongs to ***P. tymensis*** (15.5).The precaudal vertebrae ranges from 12-13 in ***P. hellenicus***, 13-15 in ***P. platygaster***, 14-15 in ***P. laevis***, 12-16 in ***P. pungitius***, 14-16 in ***P. tymensis***, 14-15 in ***P. sinensis***, and 13-16 in ***P. occidentalis***.

Variation in the total number of the vertebrae is almost the same as in the precaudal vertebrae. The lowest mean number is in ***P. hellenicus*** (29.7) and the highest mean number is in ***P. tymensis*** (34.7). The total mean number of the vertebrae is 30.2 in ***P. platygaster***, 32.4 in ***P. laevis***, 32.5 in ***P. pungitius***, 32 in ***P. sinensis***, and 33.6 in ***P. occidentalis***. This character varies from 29 to 35 in the specimens.

Scutes

The total number of scutes is highly variable in *P. pungitius* and *P. occidentalis* but relatively constant in other species. The lowest mean number is in *P. laevis* (4.3). The highest mean number is in *P. sinensis* (33). The range in *P. hellenicus* is 28-38 (33), in *P. platygaster* is 29-32 (31), in *P. laevis* is 2-8, in *P. pungitius* is 3-33 (10.6), in *P. tymensis* is 8-12 (10.5), in *P. sinensis* is 31-34, and in *P. occidentalis* is 6-17 (9.7). Small specimens of *P. occidentalis* (smaller than 18 mm in standard length) from Firth River, were too small to have scutes and were excluded from the analysis.

The large lateral scutes (relatively large and vertically ovoid shape scutes on the midlateral sides of the body (figure 6.11)) are present in *P. platygaster* (7.7) and *P. sinensis* (6.7) but are absent in other species. The range in *P. platygaster* is 4-12, and in *P. sinensis* is 6-8. One specimen of *P. occidentalis* from Cold Lake had a full row of the scutes and the large lateral scutes. This specimen was excluded from the analysis.

The keel scutes are absent in *P. hellenicus* and *P. platygaster* and range 2-8 (4.3) in *P. laevis*, 3-16 (8.1) in *P. pungitius*, 5-8 (6.7) in *P. tymensis*, and 6-17 (9.1) in *P. occidentalis*. In *P. sinensis* because the keel scutes are continuous with anterior scutes, it is difficult to separate them from each other, so, I gave the constant number eight to make the analysis possible.

Meristic characters in principal component analysis

Principal component analysis (PCA) was used for all meristic characters and indicated separate clusters for *P. hellenicus, P. platygaster, P. laevis,* and *P. sinensis* (figure 5.1). To provide more details, a PCA was conducted on a separate set consisting of *P. pungitius, P. tymensis,* and *P. occidentalis*, but no separate clusters were demonstrated.

Table 5.1. Mean number, range, and standard deviation of the fifteen meristic characters.

	Dorsal spines			Vertical spines (%)			Dorsal rays		
	Mean	Range	Sd	Mean	Range	Sd	Mean	Range	Sd
P. hellenicus	4.1	2-6	0.9	100	100	0	9.2	8-11	0.8
P. platygaster	8.8	8-10	0.6	27	0-60	15	8.0	6-10	0.8
P. laevis	9.1	5-10	0.9	60	33-100	20	10.0	9-12	0.8
P. pungitius	9.2	8-11	0.7	32	0-75	15	10.5	9-12	0.8
P. tymensis	11.8	11-13	0.6	57	36-82	15	10.6	10-11	0.5
p. sinensis	9.1	8-10	0.5	29	11-56	13	10.1	9-11	0.6
P. occidentalis	9.2	8-11	0.7	41	11-100	17	10.3	8-12	0.8
	Anal rays			Dorsal basals			Anal basals		
P. hellenicus	8.8	6-10	1	22.8	21-25	0.9	12.2	10-13	0.8
P. platygaster	7.0	6-8	0.7	22.4	20-25	1.1	11.2	10-13	0.9
P. laevis	8.9	8-10	0.7	24.0	22-27	1.1	12.6	11-16	1.2
P. pungitius	9.5	7-11	0.8	24.2	21-26	1.1	13.2	11-15	0.9
P. tymensis	10.1	9-11	0.6	26.4	25-27	0.8	13.2	12-14	0.6
p. sinensis	8.9	8-10	0.6	24.9	23-27	1.1	13.3	12-15	0.9
P. occidentalis	9.4	7-13	0.8	25.0	20-28	1.4	14.0	11-18	1.4
	Epural rays			Pelvic spines			Pelvic rays		
P. hellenicus	5.3	4-6	0.6	0.0	0	0.0	0	0	0.0
P. platygaster	4.8	4-6	0.6	2.0	2	0.0	2.0	1-2	0.2
P. laevis	4.5	3-6	0.7	0.8	0-2	1.0	0.8	0-2	1.0
P. pungitius	4.9	2-7	0.8	2.0	2	0.0	2.4	1-4	0.8
P. tymensis	5.3	4-6	0.7	2.0	2	0.0	2.0	2	0.0
p. sinensis	4.1	3-5	0.6	2.0	2	0.0	3.9	3-5	0.4
P. occidentalis	4.5	3-6	0.6	2.0	0-2	0.3	1.7	0-4	0.8
	Gill rakers			Precaudal vertebrae			Caudal vertebrae		
P. hellenicus	9.1	7-10	0.9	12.9	12-13	0.2	29.7	29-30	0.5
P, platygaster	10.2	9-11	0.7	13.4	13-15	0.6	30.2	29-31	0.5
P. laevis	10.7	9-12	1.0	14.5	14-15	0.5	32.4	31-34	0.6
P. pungitius	10.9	9-13	0.9	14.6	12-16	0.6	32.5	30-34	0.9
P. tymensis	9.1	8-11	0.9	15.5	14-16	0.7	34.7	33-35	0.7
p. sinensis	11.6	10-14	0.8	14.6	14-15	0.5	32.0	33-35	0.9
P. occidentalis	12.3	8-16	1.6	14.6	13-16	0.6	33.6	32-35	0.7
	Large lateral scutes			Keel scutes			Total scutes		
P. hellenicus	0	0	0.0	0	0	0.0	31.0	28-38	1.9
P. platygaster	7.7	4-12	2.4	0	0	0.0	30.5	29-32	0.9
P. laevis	0	0	0.0	4.3	2-8	1.1	4.3	2-8	1.1
P. pungitius	0	0	0.0	8.1	3-16	2.5	10.6	3-33	7.1
P. tymensis	0	0	0.0	6.7	5-8	0.8	10.5	8-12	1.3
p. sinensis	6.7	6-8	0.9	8.0	8	0.0	33.0	31-34	0.8
P. occidentalis	0	0	0.0	9.1	6-17	2.8	9.7	6-34	3.1

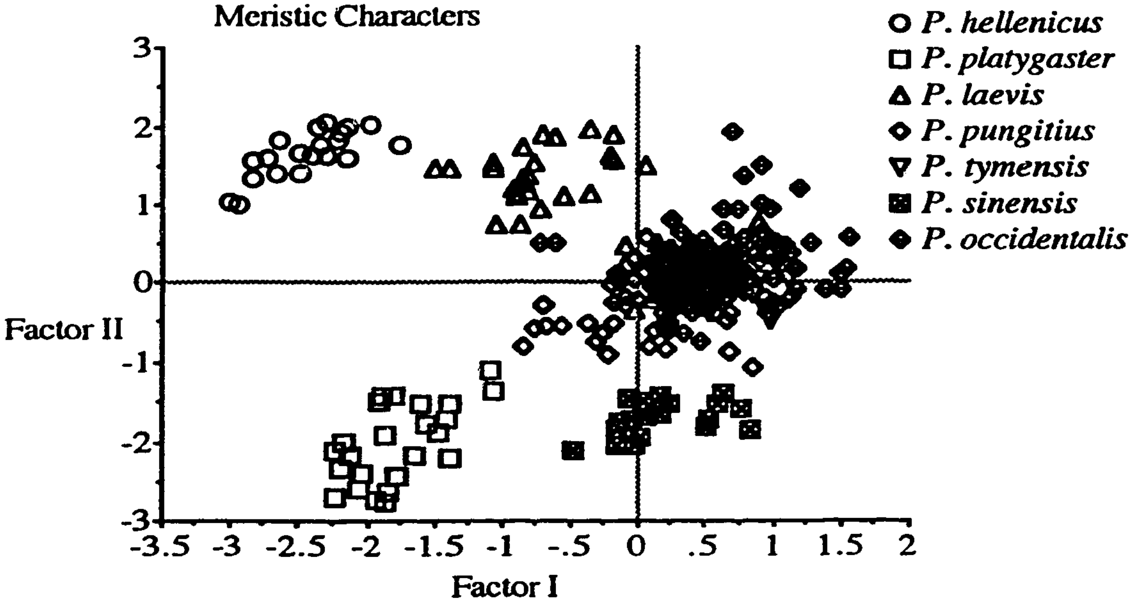


Figure 5.1. Factors I and II plotted against each other for the meristic characters of the seven nominal species.

6. Osteology

The osteology of the fishes examined is discussed in this section. Each anatomical section consists of a brief description of *Pungitius hellenicus* and of differences observed among members of the ingroup. In general, if differences with other species are not mentioned, then the description applies to the genus as well. Bowne (1985 and 1994) gave a detailed description of the osteology of the Gasterosteiformes and some of her characters were used in this study.

I. Axial skeleton

a)Neurocranium

1. The olfactory region (figure 6.1)

The ethmoid region consists of the single ethmoid, lateral ethmoid, nasal, and unpaired vomer. The nasal is relatively narrow and does not meet the midline. A closed canal runs the full length of each nasal, which is loosely attached to the frontal. A laminae extends ventrally from the lateral edge of the nasal, bending medially toward the vomer and touching the lateral ethmoid. This laminae is reduced in some specimens of *P. laevis* from Brosna River, Ireland. The vomer is toothless and its head not strongly attached to the surrounding bones. Its shaft extends past the posterior end of the lateral ethmoid.

The lateral ethmoid is a vertical, cylinderlike bone placed between the frontal and the lachrymal. The ethmoid is flat and it has an anterior flange forming a horizontal medial septum. The anterior flange is absent in some specimens of *P. laevis*.

2. The orbital region (figure 6.2)

The orbital region is composed of the frontal, lachrymal, and the infraorbitals. The frontal bears a lateral postorbital process which contacts the lateral process of the parasphenoid and excludes the sphenotic from the orbit. The infraorbital series is incomplete posteriorly, consisting of a lachrymal, a small second infraorbital, and a large third infraorbital contacting the horizontal branch of the preopercle. All the elements bear the infraorbital canal.

3. The cranium floor (figure 6.3)

The bones forming the cranium floor are the parasphenoid, basioccipital, prootic, exoccipital, intercalary, and the ventral face of the sphenotic and pterotic. The basioccipital is roughly pentagonal and reaches the exoccipital. The exoccipital of ***Pungitius*** has two ventral foramina. The sphenotic is covered posteriorly by the pterotic and its anterior border is almost excluded from the orbit by the lateral postorbital process of the frontal.

4. The cranium roof (figure 6.4)

The bones of the cranium roof are the supraoccipital, parietal, pterotic, epiotic, and the dorsal face of the sphenotic and pterotic. The supraoccipital is relatively large with a wedge-shaped anterior process which lies under the frontal. The supraoccipital is roughly diamond shaped, its anterior margin bordered by the frontal and its lateral and posterior margins by the parietal and epiotic. The parietal is relatively large and flat, its posterior border meeting the epiotic and its lateral boarder meeting the pterotic.

b) Branchiocranium

5. The upper jaw (figure 6.5)

The upper jaw is composed of the premaxilla and maxilla. The premaxilla has a long ascending process and the maxilla articulates with the dorsal and ventral sides of the ascending process. Usually a small process is present on the posterior margin of the premaxilla (postmaxillary process).

6. The lower jaw (figure 6.6)

The lower jaw is formed of the dentary, articular, angular, and the sesamoid articular. The dentary is toothed and well developed. The angular consists of a vertical cone shape bone and anteroventral processes. The posterior tips of the flanges connecting the dorsal and ventral articular heads are developed into distinct posterior processes.

7. Suspensorium (figure 6.7)

The suspensorium contains the palatine, quadrate, symplectic, endopterygoid, ectopterygoid, metapterygoid, and hyomandibula. The palatine is an elongate cylindrical bone with a conical head which gives rise to a lateral process. Posterioventrally, the palatine is attached to a trivadiate bone consisting of the ectopterygoid and endopterygoid which runs down the dorsal margin of the quadrate. The metapterygoids are reduced to small splints of bone over the symplectic hyomandibular connections.

The quadrate is expanded into a fan with a posterioventral process. The symplectic is cylindrical with an anteriodorsal flange lying along the border of the pterygoids. A small ventral flange is present at the mid points of the symplectics. The hyomandibular cranial condyles are separate. Their shafts bear anterior laminae and medial foramina.

8. The opercular series (figure 6.8)

The opercular region contains the preopercula, subopercula, interopercula, and the opercula. The preopercula is L-shaped and bears the preoperculomandibular canal. The horizontal bar is serrated and sutured with the third infraorbital in *P. sinensis* and in some specimens of *P. pungitius* and *P. occidentalis.* The interopercula is slender and its posterior end is enlarged. The opercula is roughly triangular with its posterior margin almost horizontal and its anterior margin vertical. The subopercula has ascending and posterior processes. The anterior ends of the posterior process closely follows the opercular margin.

The length of the posterior limb of the subopercula is variable in all the species; in some specimens it reaches the posterior margin of the opercula and in some others it does not.

9. The hyoid arch (figure 6.9)

The hyoid arch is composed of the unpaired urohyal and basihyal, one pair of hypohyals, ceratohyals, epihyals, interhyals and six branchoistegals. The basihyal is a simple rod, slightly flattened posteriorly. One pair of hypohyals is present, the dorsal and the ventral, overlapping the ceratohyal. The ceratohyal has a narrow shaft and the wide head bears a dorsal strut extending to the epihyal. The epihyal is triangular and the interhyal hourglass-shaped. The urohyal is divided into a ventral and dorsal blade and has ventral and dorsolateral flanges which are posteriorly incised in some specimens of ***Pungitius***. The branchiostegals do not articulate with the hypohyals.

10. The branchial arches (figure 6.10)

The branchial arches consist of three unpaired basibranchials, hypobranchials, ceratobranhials, epibranchials, pharyngobranchials, and associated teeth and tooth plates. ***Pungitius*** has three toothless, cylindrical basibranchials. The three hypobranchials are flattened structures. They lie beside the basibranchials and do not extend ventrally beneath them. The four ceratobranchials are cylindrical and none bear tooth plates; the fifth one expanded and toothed. The first two bear long gill rakers. Four cylindrical epibranchials are present. The unicate processes on the first and second epibranchial are small. The first pharyngobranchial is absent. The second and the third ones are both toothed. The third one extends anteriorly but does not exclude the second one from the dorsal midline.

c) Vertebral column (figures 6.16, 6.20 and 6.21)

The vertebral column is composed of 29-30 vertebrae with about 13 precaudal and 17 caudal in ***P. hellenicus***. The associated pleural and epipleural ribs are present in the precaudal part. The first vertebra lacks the pleural rib. Small nonarticular postneurapophyses are present.

The haemal spines arise from the centers or posterior borders of the haemal arches in the caudal region. The prehaemapophyses are absent or very small in some specimens of *P. hellenicus*. Posthaemapophyses are present in *Pungitius*. Pleural ribs are borne on most precaudal vertebrae. Haemal and neural spines of preurals 2 and 3 in *P. hellenicus* and *P. platygaster* are wide and long, while in *P. occidentalis* they are very narrow. Members of other species show an intermediate condition. Haemal and neural spines of preurals 4 and 5 in *P. hellenicus* are usually narrow, and long and make a wide angle (about 45°) relative to the axis of vertebral column. Haemal and neural spines of preurals 4 and 5 in *P. occidentalis* are very short and almost horizontal. Other species show an intermediate condition. Length of the other haemal and neural spines of the caudal peduncle are also different; in *P. hellenicus* they are long and in *P. occidentalis* they are very short. Other species show an intermediate condition. Very small specimens (smaller than 15 mm) of a population of *P. occidentalis* from the Firth River, Yukon, show the same pattern of vertebral spines as in *P. hellenicus*.

d) scutes (figure 6.11)

All the scutes in *P. hellenicus* are similar in size and roughly round and do not form a keel in the caudal peduncle region. All bear a trace of a canal or foramina, but there is no canal. In *P. platygaster* the anterior lateral scutes are ovoid shape and larger than the others. *Pungitius laevis* has a few scutes making a weak keel on the caudal peduncle. The keel is thin and cannot be seen in unstained specimens. This confused some authors into describing *P. laevis* as a keelless species. A variety of different patterns can be seen in *P. pungitius*, but all specimens bear a keel on the caudal region. Some specimens have a full row of scutes on the midlateral line of the body, some have a few small scutes behind the head, and some do not have any of the anterior scutes. It seems that in *P. hellenicus* scutes appear earlier in development than in the other species, because specimens 17 mm in standard length have a full row of scutes. In some specimens of *P. occidentalis* from the Firth River, scutes do not appear until fish reach 18 mm in standard length.

Pungitius tymensis has a caudal peduncle keel and usually a few small scutes behind the head. A typical *P. sinensis* has a full row of scutes making a keel on the caudal peduncle and the large lateral scutes on the anterior part of the body. *Pungitius occidentalis* shows the same pattern as *P. pungitius*, but rarely is the body fully covered with scutes. One specimen of *P. occidentalis* from Cold Lake was fully covered by scutes and had the large lateral scutes (similar to those in *P. sinensis*).

II. Apendicular skeleton

e) Paired fins

11. The pectoral girdle (figures 6.12 and 6.13)

The bones of the pectoral girdle are the posttemporal, supracleithra, cleithra, coracoid, ectocoracoid, scapula, postcleithra, pectoral actinosts and fin rays. The posttemporal bears a medial strut, a developed canal, the anteriodorsal projections, and an anterioventral process. The supracleithra lies nearly vertically and is usually completely included in the outline of the cleithra. The cleithra has a distinct head with an anterior process. Its shaft bears lateral and medial posterior laminae between which the scapula and coracoid fit. The coracoid and ectocoracoid are united with a plain suture line. In *P. hellenicus*, the ectocoracoid is very reduced. In some specimens of *P. laevis* the ectocoracoid is also partially reduced. In other species it meets the cleithra joint and heavily is sculptured. The coracoid is a flat bone extending dorsally from the ectocoracoid and sutured to it for its full length. The scapula lies along the ventral border of the cleithra head and bears the scapula foramina. The scapula is posteriorly notched to receive the actinosts. The actinosts are separated from each other and the surrounding bones.

12. The pelvic girdle (figures 6.14 and 6.15)

The pelvic bones are single bones supporting the pelvic rays. In *P. hellenicus* they are usually absent, but when present they consist of small round plates on the ventral surface of the body. In some specimens of *P. occidentalis*, *P. laevis*, and *P. pungitius*, the pelvic girdle is reduced or completely absent (Nelson, 1971b; Zyuganov & Rozanov, 1987). *Pungitius tymensis* usually has a reduced pelvic girdle. In species with a fully developed pelvic girdle, the pelvic bones are overlapped by the ectocoracoids, but not strongly attached to them. They are attached to one another by medial processes which meet in complex sutures. Long and pointed posteriomedial extensions and ascending processes are present. Nelson (1971b) provided terminology for different parts of the pelvic girdle in sticklebacks.

f) Unpaired fins

The unpaired fins are the dorsal, anal, and caudal fins. All have internal supports consisting of proximal, medial, and distal pterygiophores in the dorsal and anal fins, and hypural, epural, uroneural, and parhypural in the caudal fin.

13. The dorsal fin (figures 6.16-6.19)

There is a row of isolated spines in front of the dorsal fin with the largest spine usually being at the leading edge of the fin. The origin of the dorsal fin lies over the precaudal vertebrae. In *P. hellenicus* almost all the spines lie on the middorsal line and do not diverge from that. In other species they zigzag and diverge from the midline. Dorsal spines in *P. hellenicus* are short, the last one is the longest one and the first one is the shortest. In other species all the dorsal spines are the same size except the very last one which is a little longer.

The basals of the dorsal spines are reduced in *P. hellenicus* and *P. laevis* . They are usually roundish, but in some specimens are ovoid; they do not overlap each other. In the other species they are somewhat rectangular and overlap each other. In a population of *P. occidentalis* from Crooked Lake, Indiana the dorsal basals are relatively reduced and ovoid in shape, but overlapping.

14. The anal fin (figure 6.16)

The anal fin bears an anterior spine and lies below the caudal vertebrae. In *P. hellenicus* the anal spine is very short and when depressed, usually does not reach the base of the second anal soft ray, but in other species, the anal spine is relatively long and always passes the second soft ray. In some specimens of *P. laevis,* the anal spine is short as in *P. hellenicus.*

15. The caudal fin (figures 6.20 and 6.21)

The caudal skeleton is highly fused and made up of the posterior preural vertebrae, the ural centra, and the bones supported by or arising from them (hypural, epurals, uroneural and parhyporal), and the rays supported by these bones. The hypural plate is a single element fused with the uroneural at the upper and the parhypural at the lower edge. In one specimen of *P. platygaster*, the preural centrum 2 had a complete neural spine and lacked the epural (figure 6.21b).

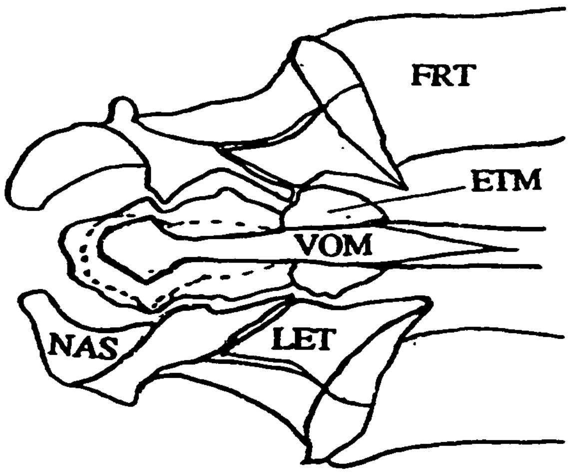


Figure 6.1. Ventral view of the olfactory region of *P. hellenicus* (X 12).

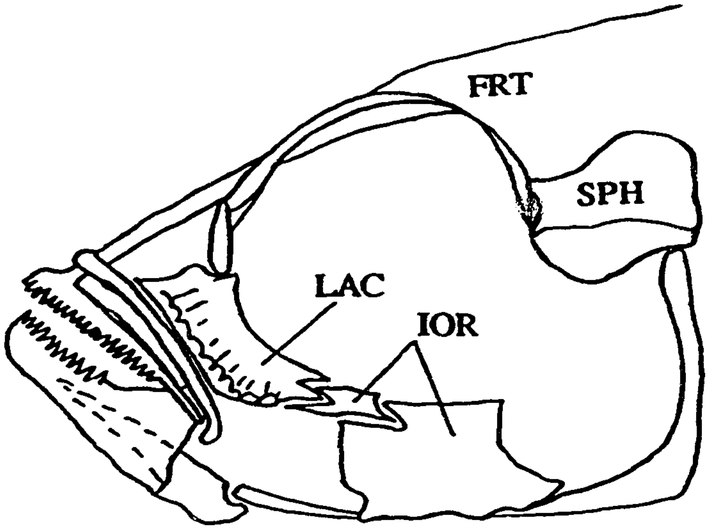


Figure 6.2. Lateral view of the orbital region of *P. hellenicus* (X 12).

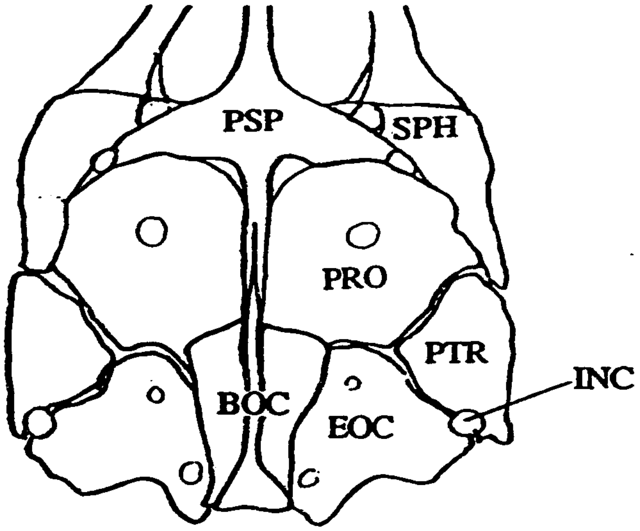


Figure 6.3. Ventral view of the cranium floor of *P. hellenicus* (X 12).

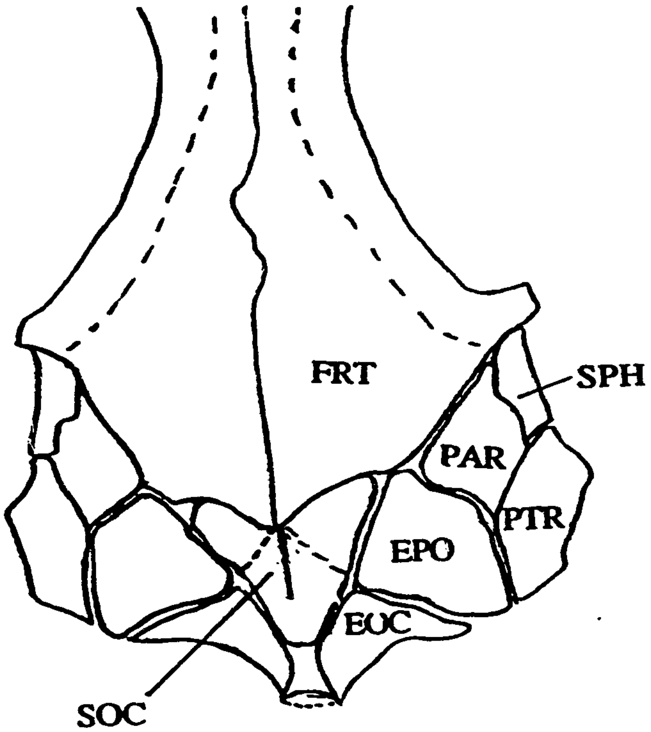


Figure 6.4. Dorsal view of the cranium roof of *P. hellenicus* (X 12).

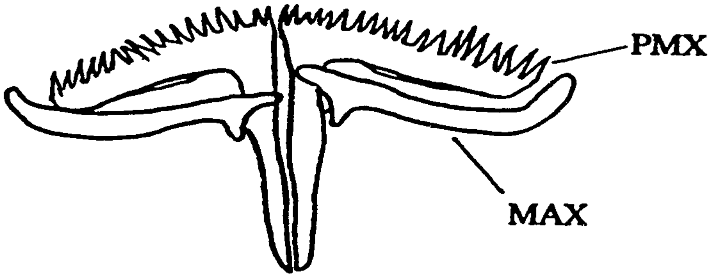


Figure 6.5. Dorsal view of the upper jaw of *P. hellenicus* (X 25).

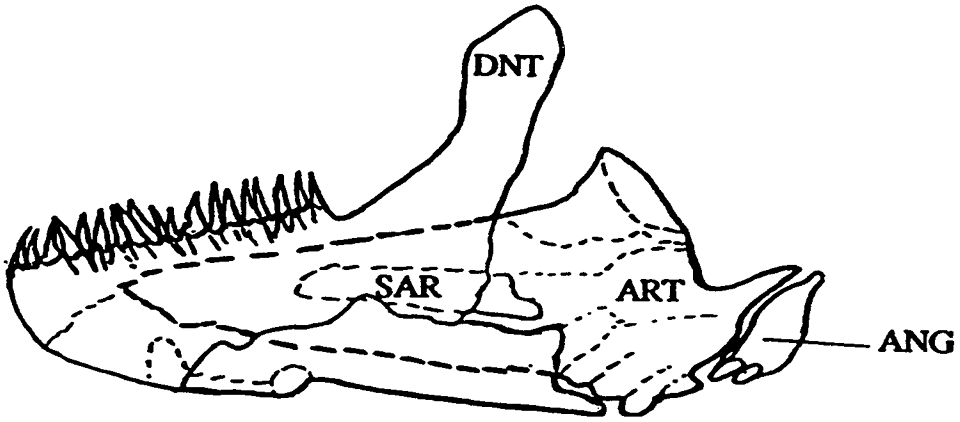


Figure 6.6. Lateral view of the lower jaw of *P. hellenicus* (X 25).

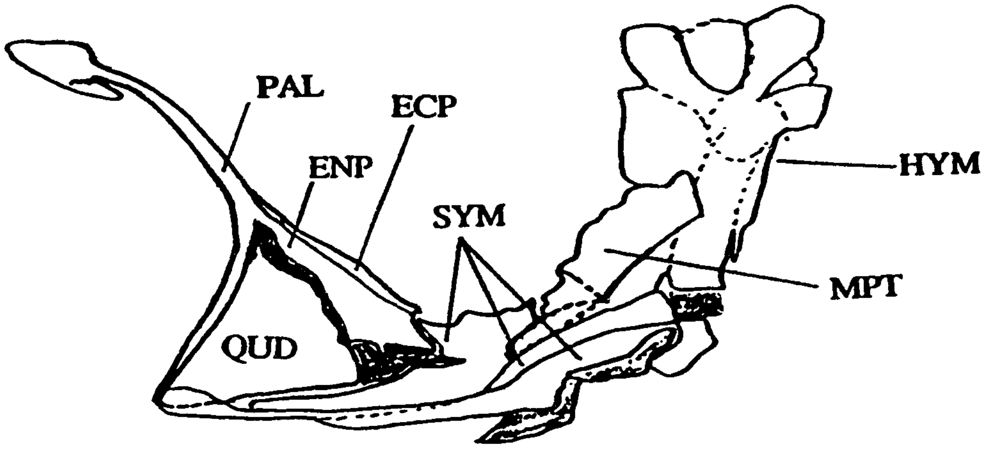


Figure 6.7. The suspensorium of *P. hellenicus* (X 25). Shaded areas are the unossified regions.

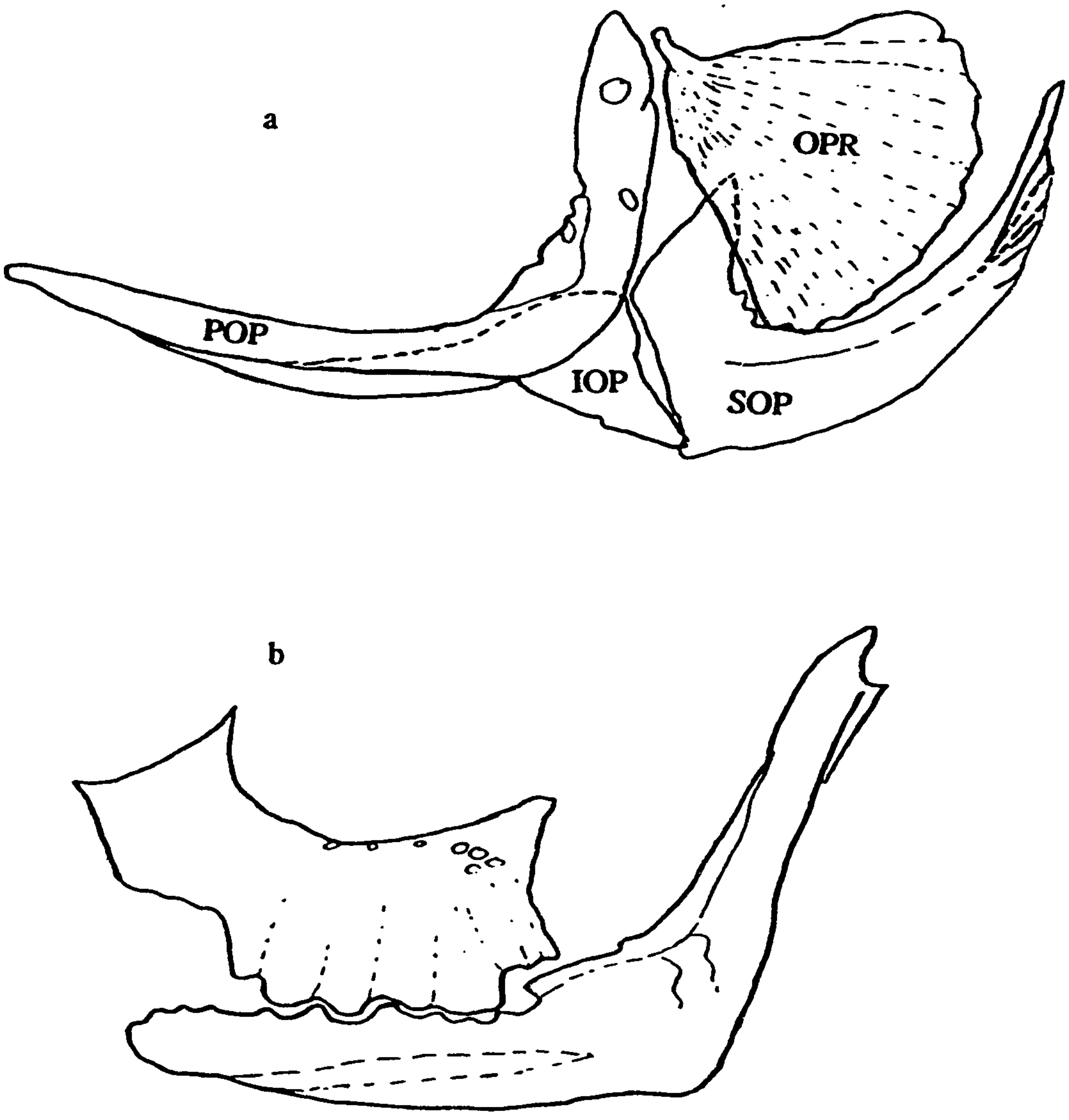


Figure 6.8. a) Lateral view of the opercular series of *P. hellenicus* (X 25).

b) Lateral view of a serrated preopercula in *P. sinensis*.

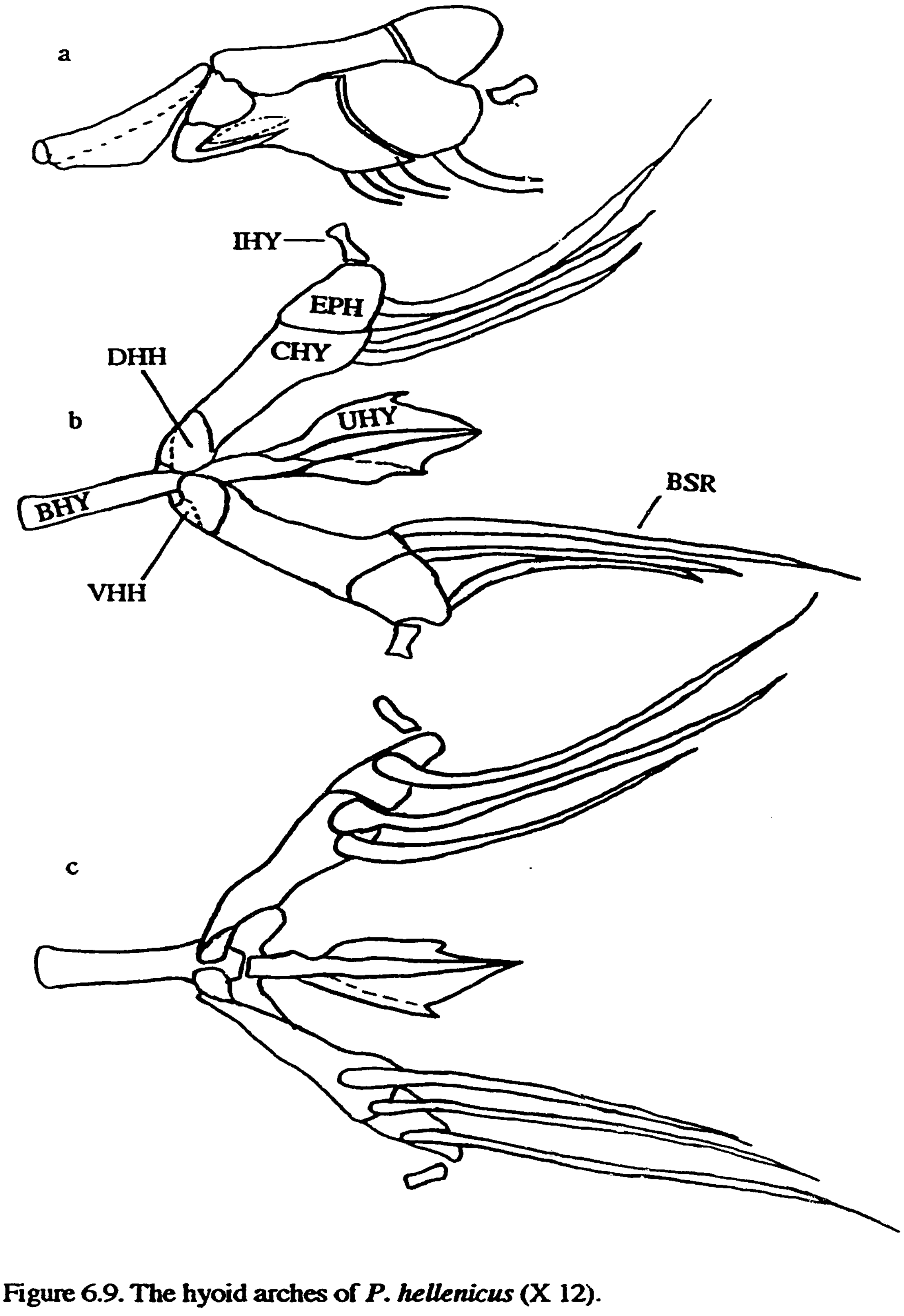


Figure 6.9. The hyoid arches of *P. hellenicus* (X 12).

a) Lateral view b) Ventral view c) Dorsal view

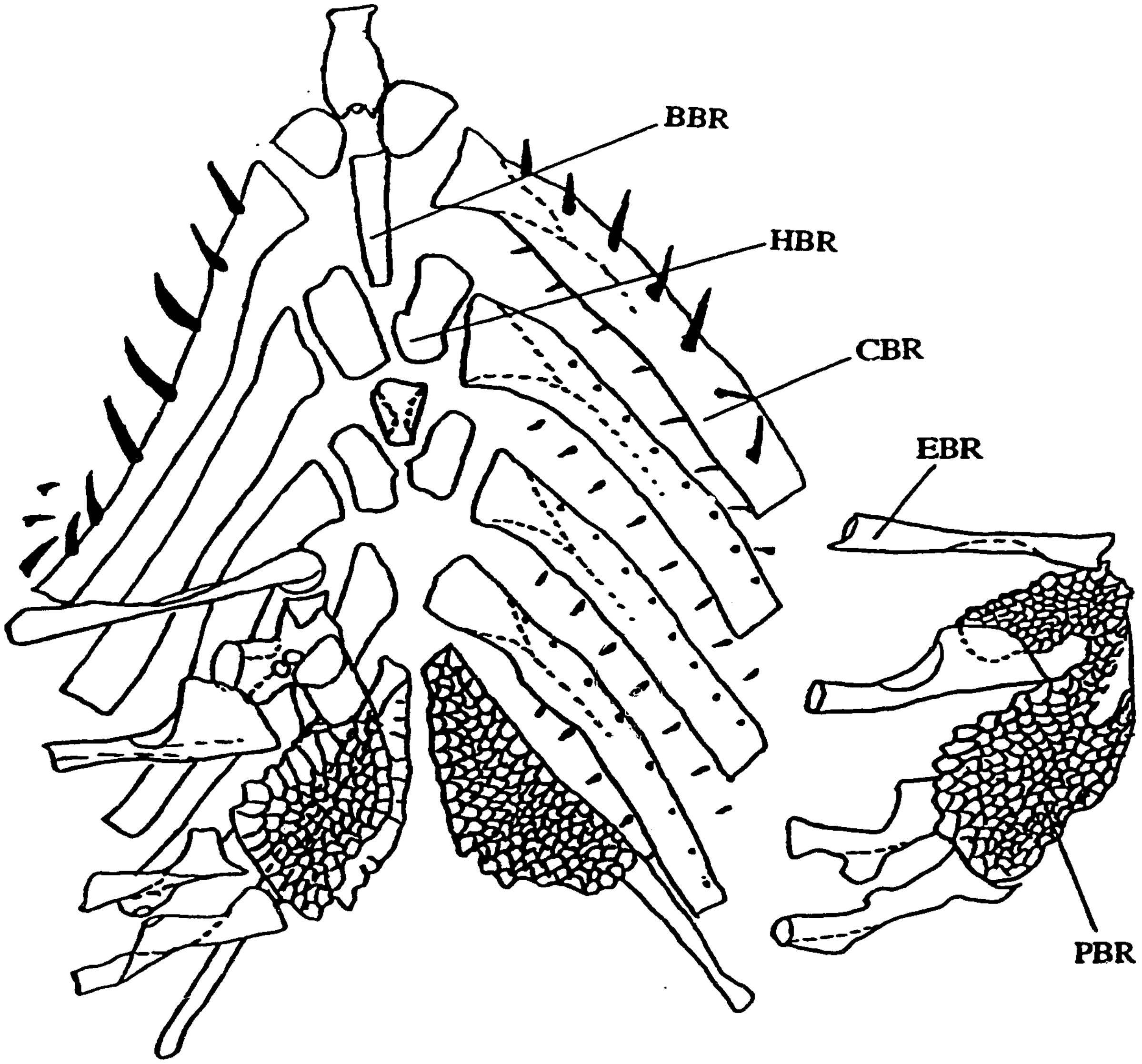


Figure 6.10. Dorsal view of the branchial arches of *P. hellenicus* (X 25).

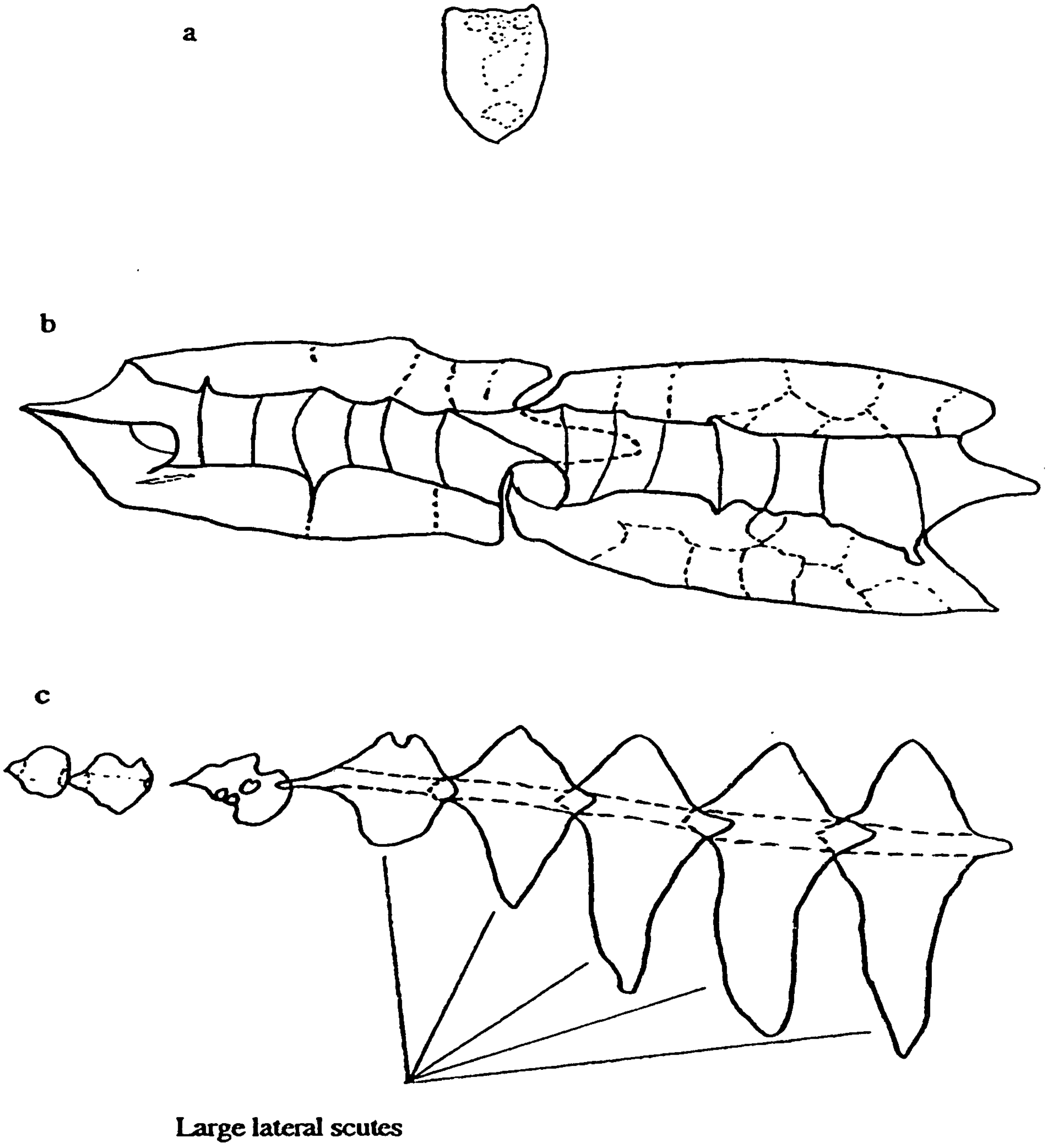


Figure 6.11. Different shapes of scutes in *Pungitius*.

a) Lateral scutes of *P. hellenicus* (X 50).

b) Keel scutes of *P. occidentalis* (X 50).

c) Large lateral scutes of *P. platygaster* (X 12).

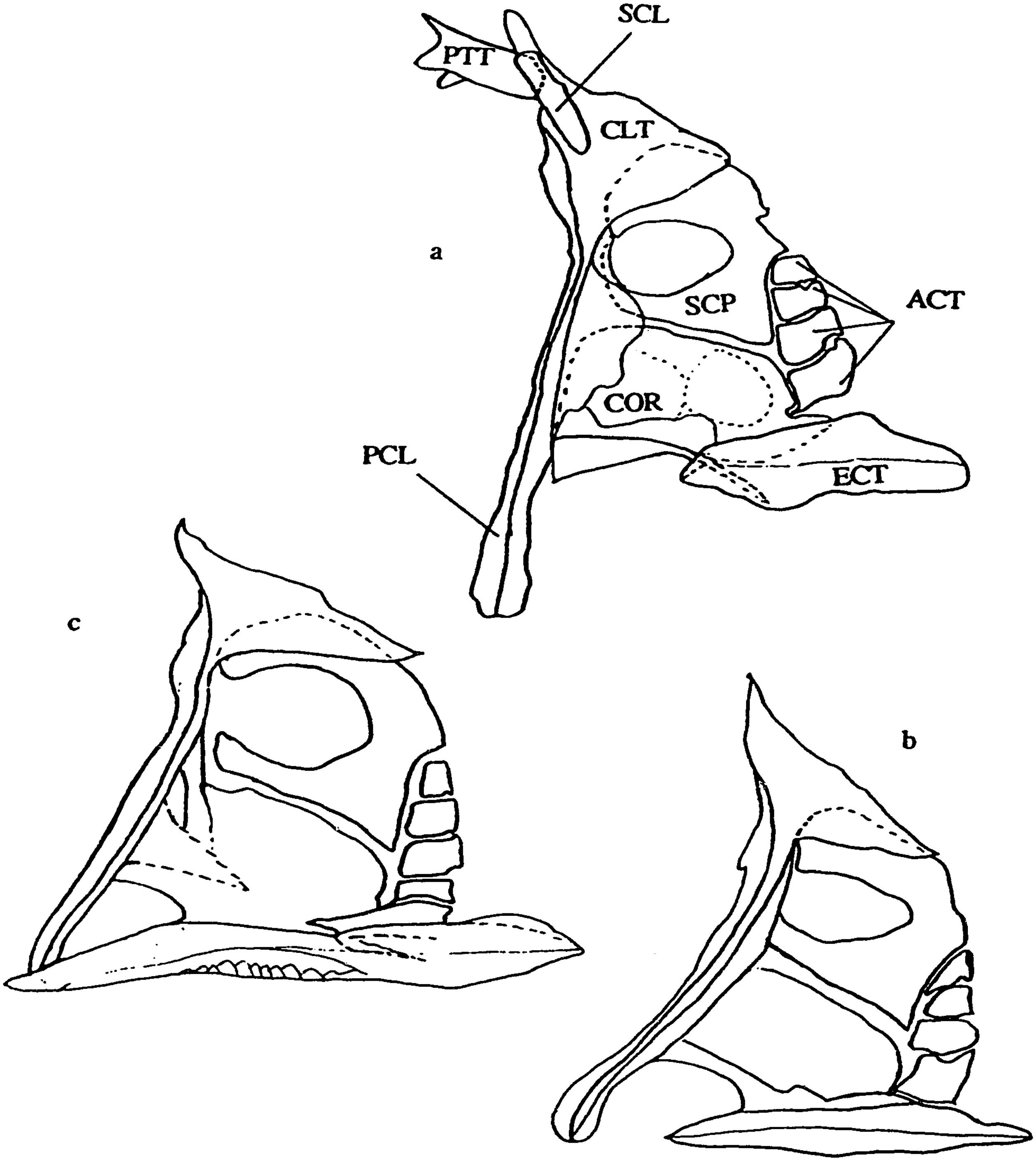


Figure 6.12. Lateral view of the pectoral girdle (X 12).

a) *P. hellenicus* b) *P. laevis* c) *P. occidentalis*

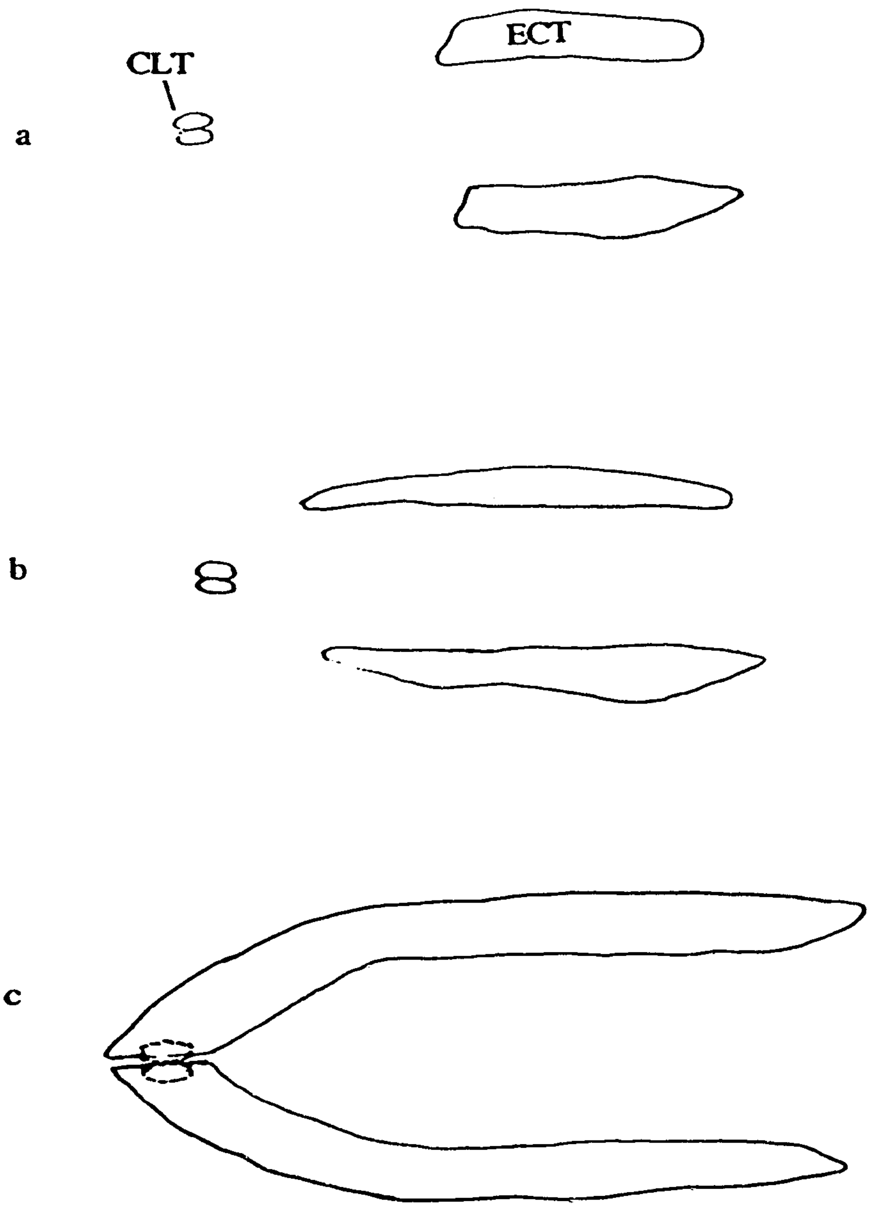


Figure 6.13. Ventral view of the pectoral girdle (X 12).

a). *P. hellenicus* b) *P. laevis* c) *P. occidentalis*

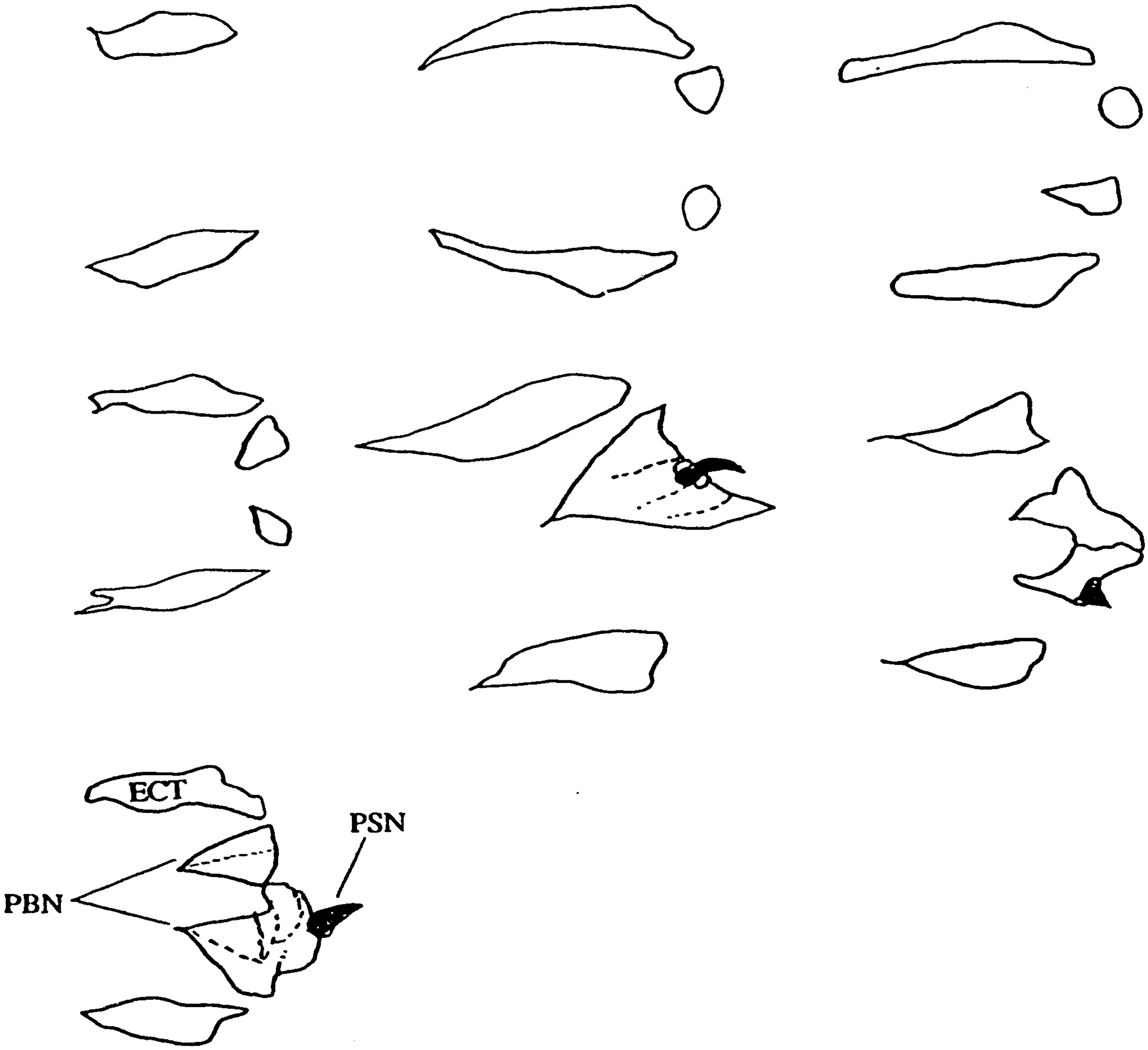


Figure 6.14. Ventral view of the pelvic bones in different specimens of *P. hellenicus* (X 12).

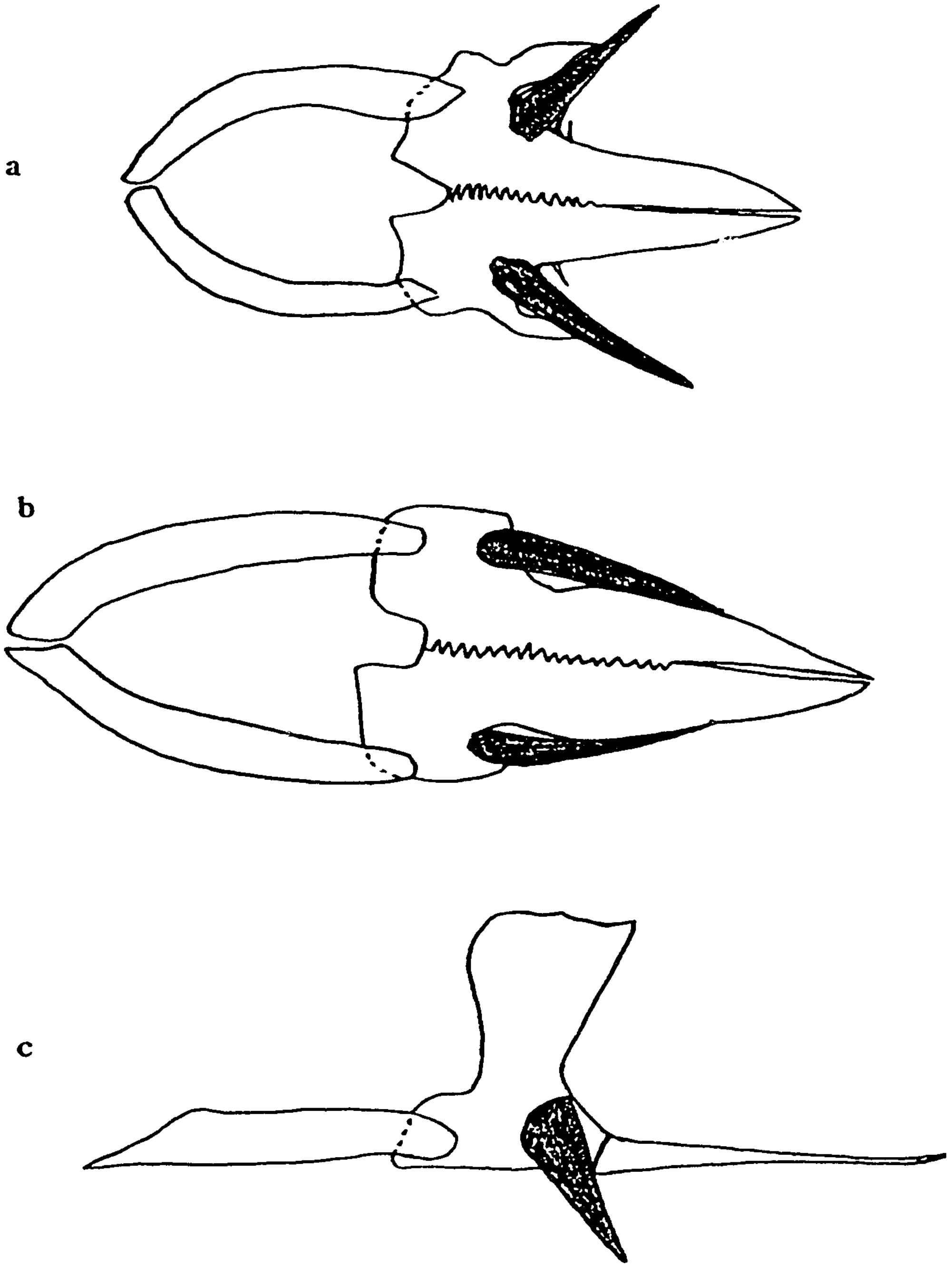


Figure 6.15. a) Ventral view of the pelvic girdle in *P. tymensis* (X 12).

b) Ventral view of the pelvic girdle in *P. sinensis*.

c) Lateral view of the pelvic girdle in *P. sinensis*.

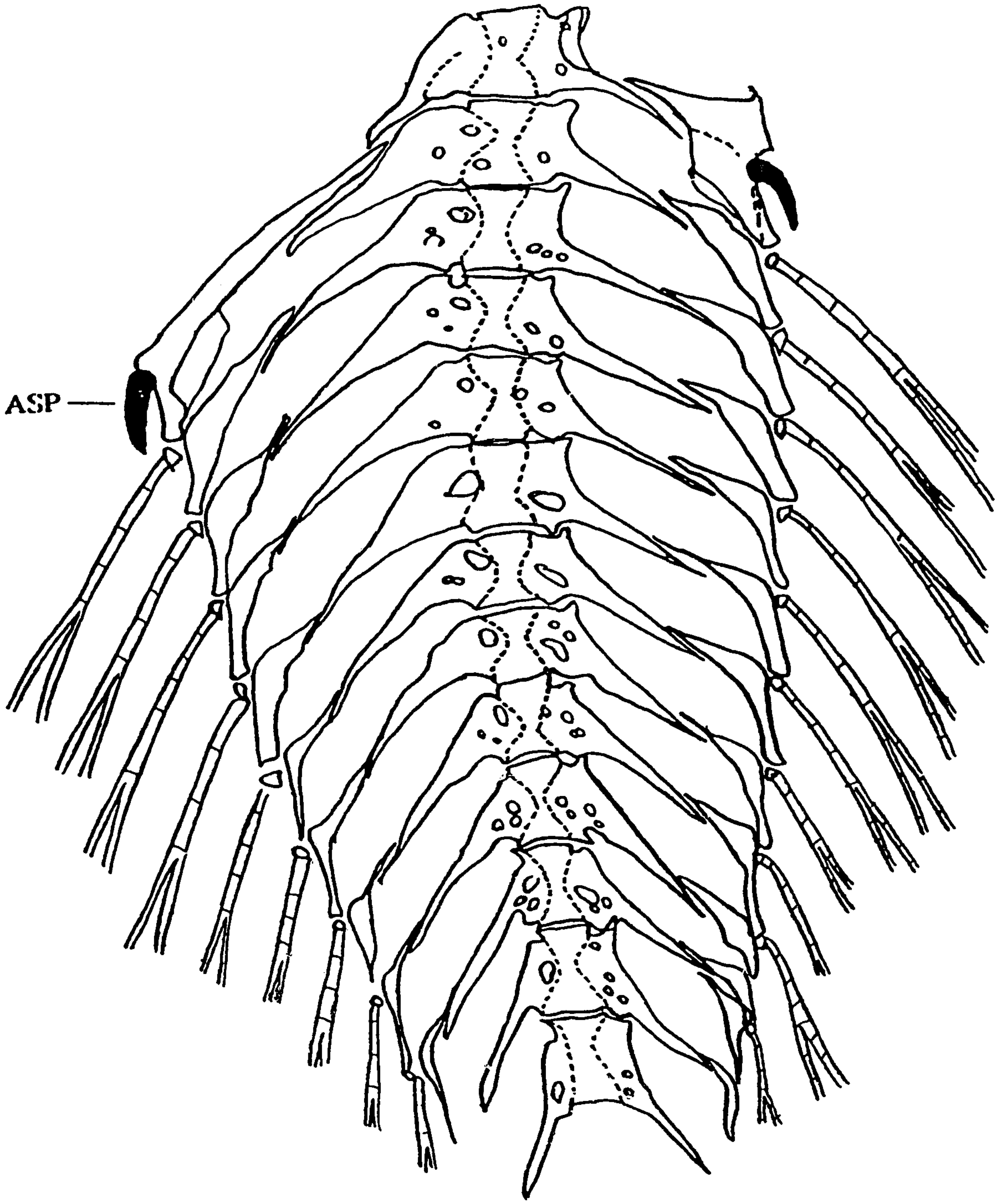


Figure 6.16. Dorsal and anal soft fins, and caudal vertebrae of *P. hellenicus* (X 12).

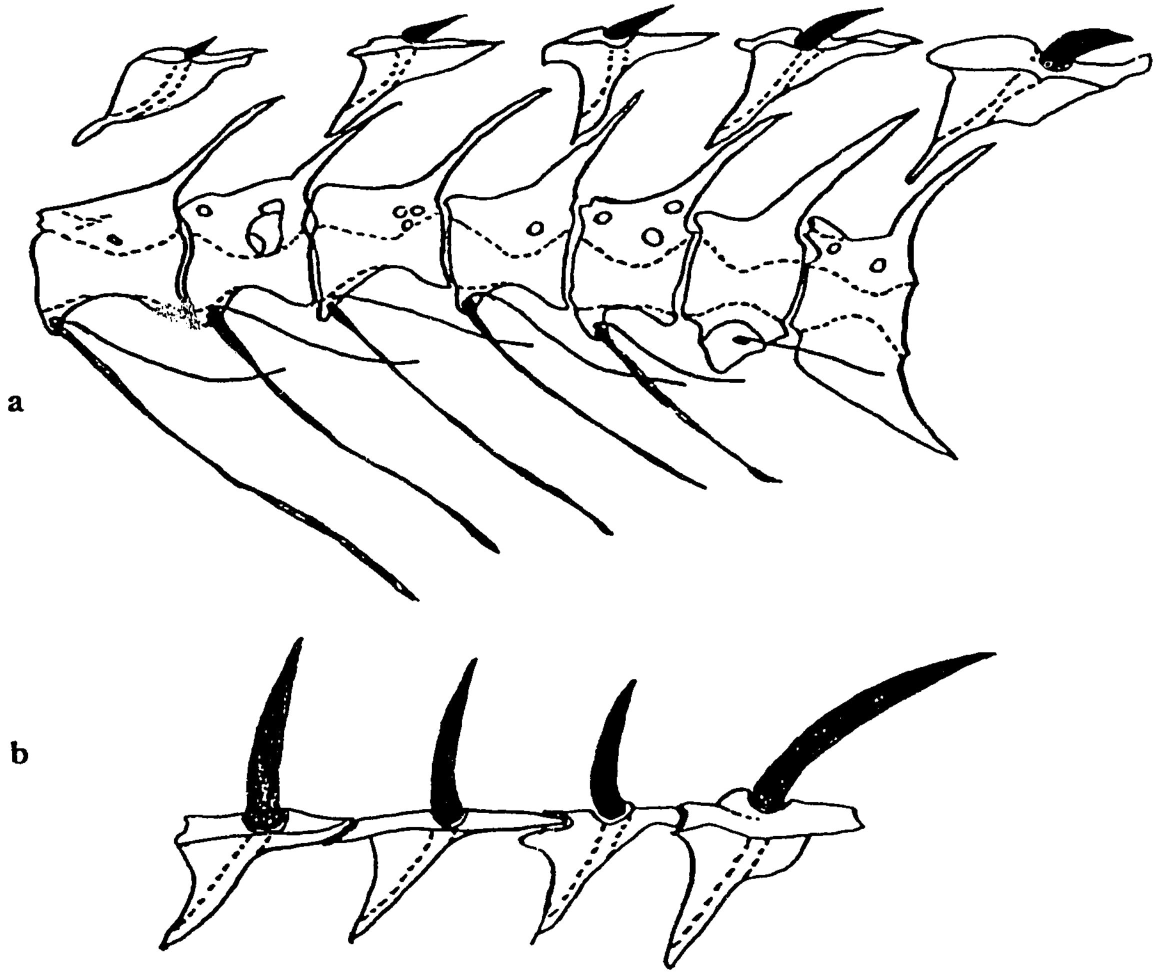


Figure 6.17. a) Dorsal spines and precaudal vertebrae of *P. hellenicus* (X 12).

b) Dorsal spines of *P. occidentalis*.

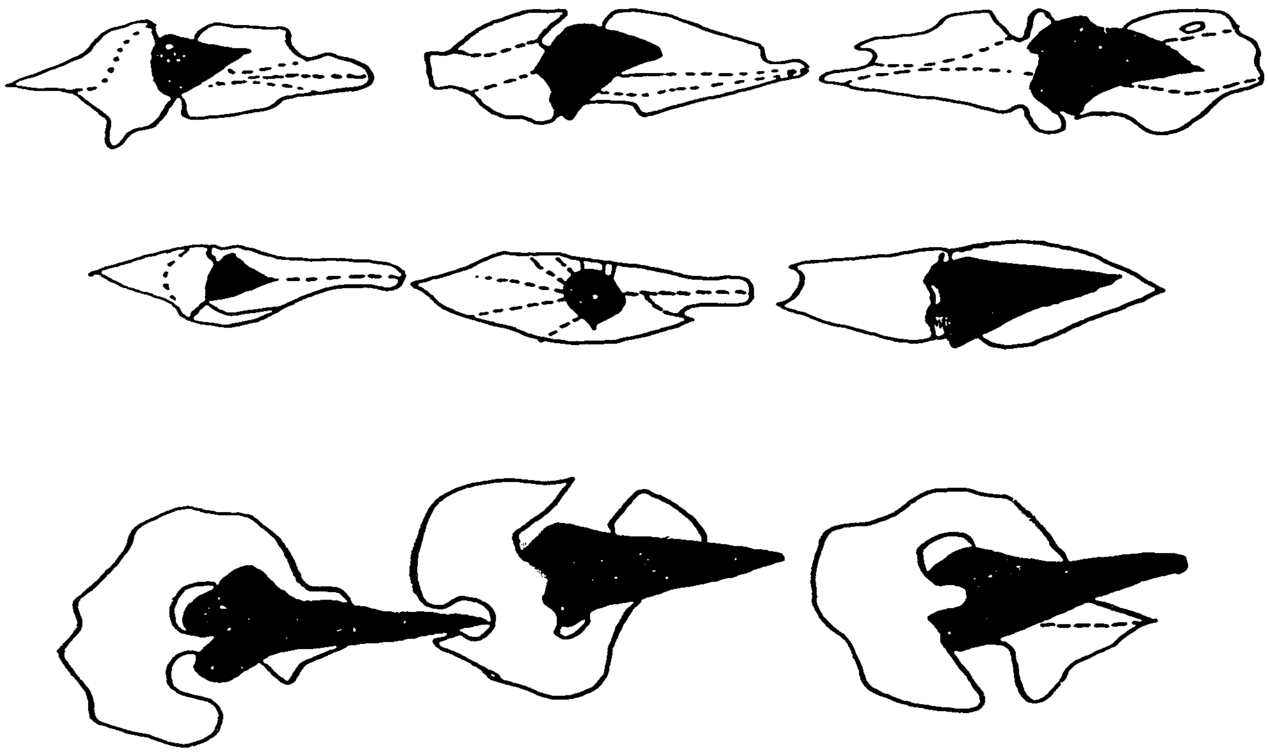


Figure 6.18. Different shapes of the dorsal basals that found in both *P. hellenicus* and *P. laevis* (X 25).

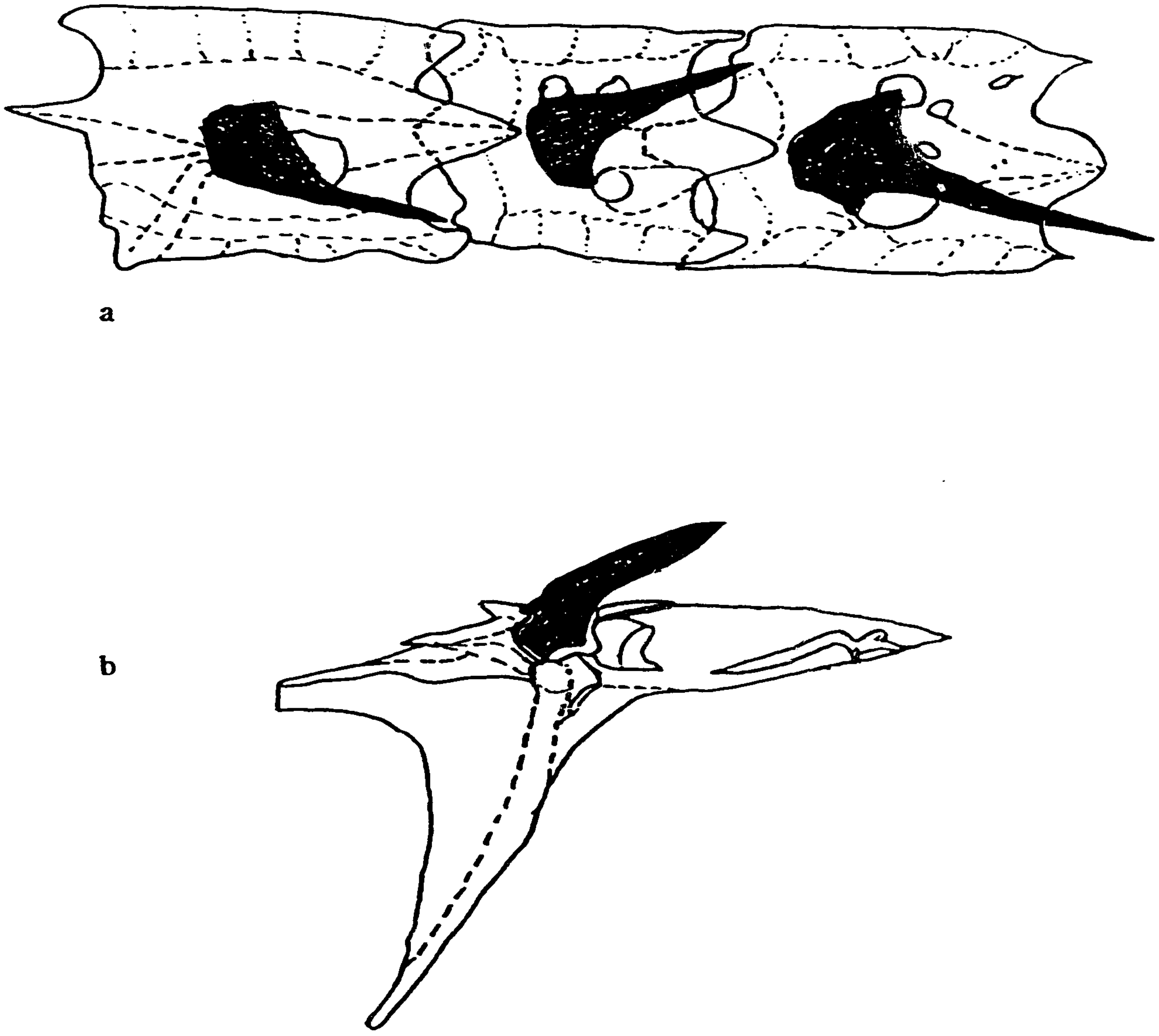
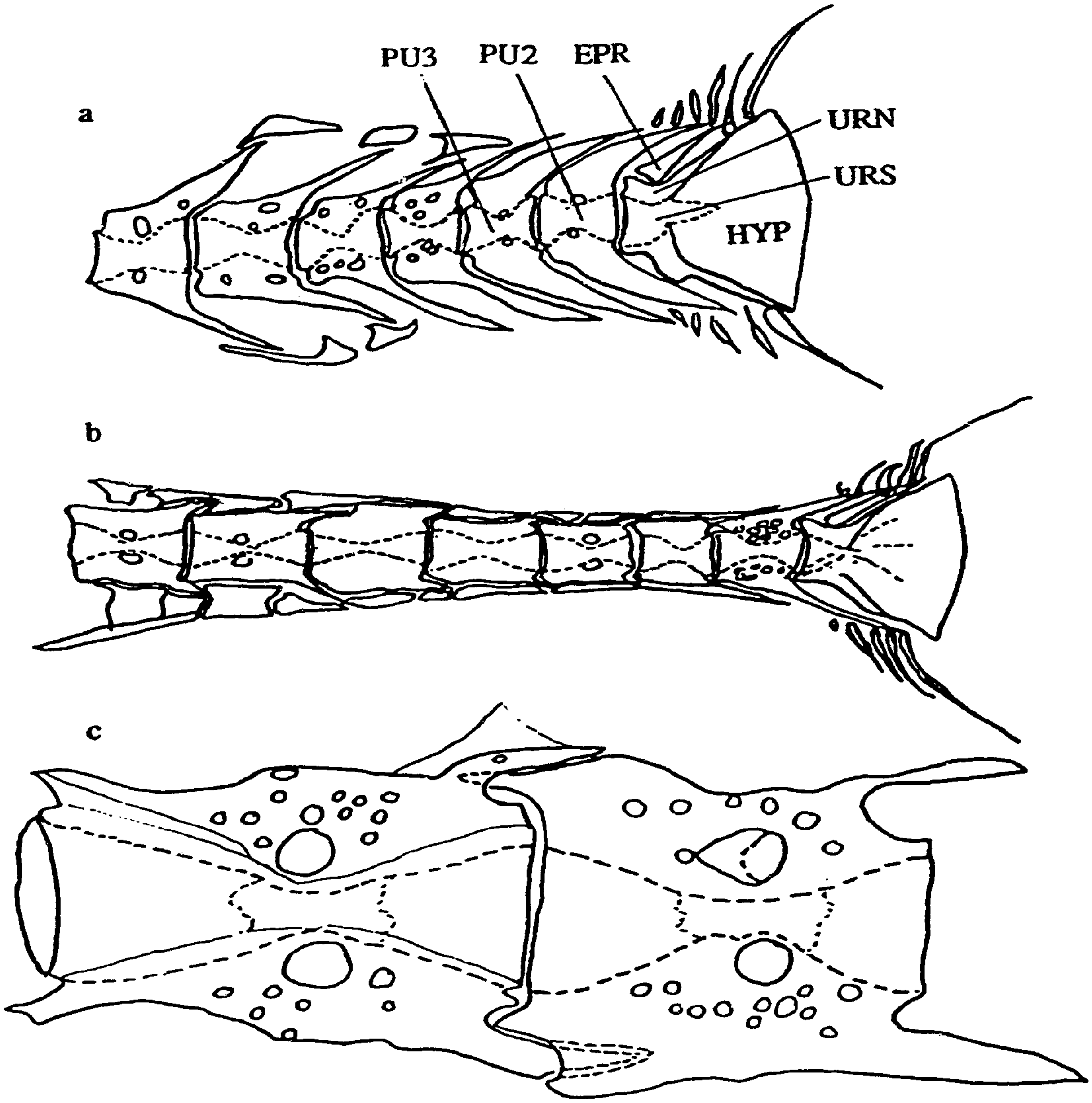


Figure 6.19. a) Dorsal view of the dorsal basals of *P. occidentalis* (X 25).

b) Lateral view of the dorsal basals of *P. occidentalis*.



Figures 6.20. a) Caudal fin of *P. hellenicus* (X 12).

b) Caudal fin of *P. occidentalis* (X 12).

c) Preurals 4 and 5 of *P. occidentalis* (X 50).

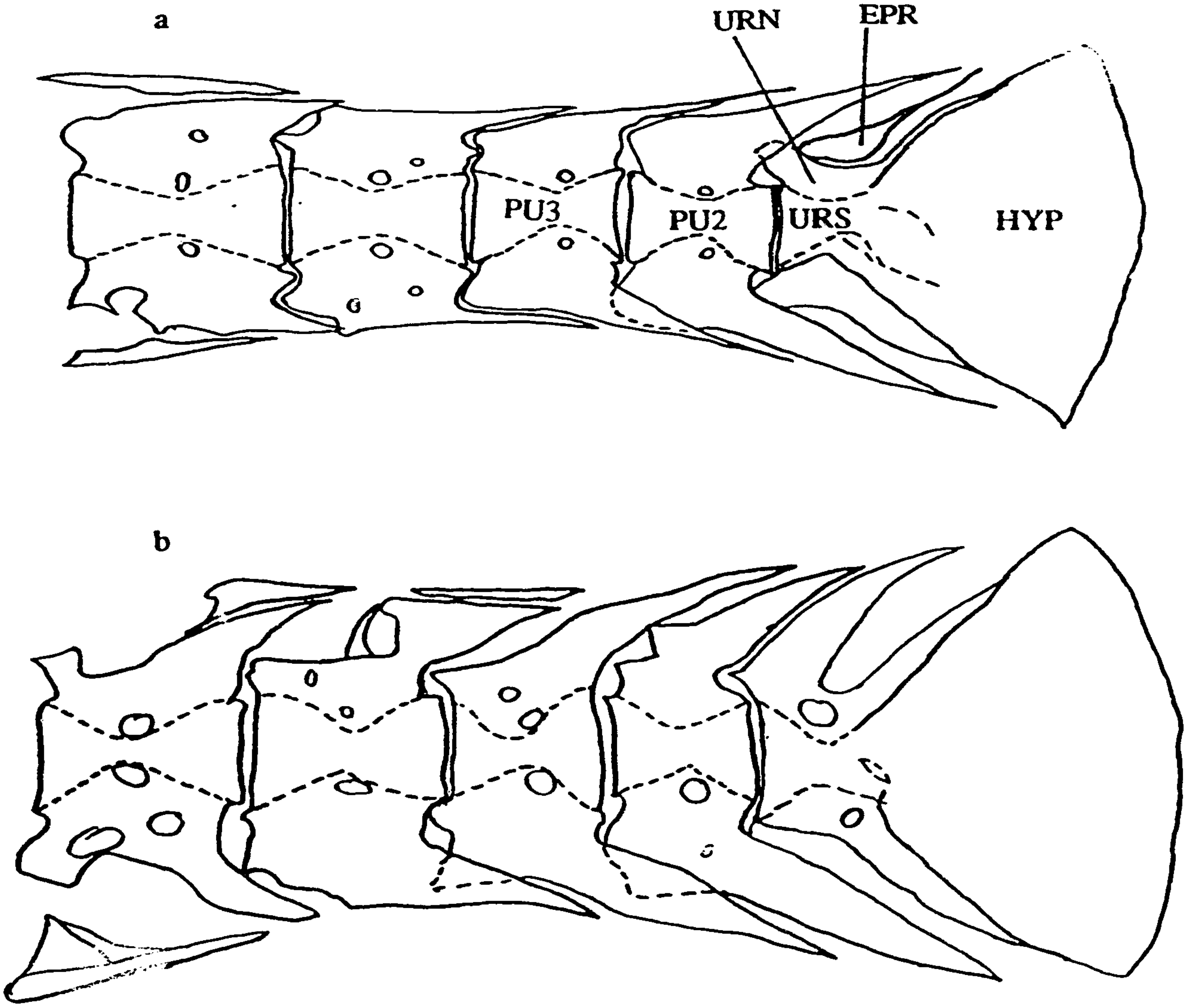


Figure 6.21. a) Caudal fin of *P. laevis* (X 25).

b) Caudal fin of *P. platygaster*.

7. Systematic Analysis

Taxonomy

The main objectives of this study were to determine the validity of *Pungitius hellenicus*, to taxonomically revise the genus *Pungitius*, and to hypothesize the phylogenetic relationships of the species of *Pungitius*. The first two objectives are achieved in this subsection and the last in the following subsection. In using the one hundred percent classical morphological rule (Bailey et al., 1954) for species recognition, I recognize three valid species in the genus: *P. hellenicus*, *P. platygaster*, and *P. pungitius* with five subspecies, *P. p. pungitius*, *P. p. laevis*, *P. p. tymensis*, *P. p. sinensis*, and *P. p. occidentalis*. *Pungitius hellenicus* differs from *P. pungitius* in lacking a caudal peduncle keel, from *P. platygaster* in lacking a pelvic girdle and lateral large scutes, and from both species in bearing fewer dorsal spines and a reduced ectocoracoid. The main diagnostic difference between *P. pungitius* and *P. platygaster* is the presence of a caudal peduncle keel in *P. pungitius* and its absence in *P. platygaster*. The following subsection gives the synonymy, diagnoses, descriptions and distributions of the species and subspecies.

Description of the species

1. *Pungitius hellenicus* Stephanidis, 1971.

Diagnosis: A combination of five characters are diagnostic for this species: lack of caudal peduncle keel, reduced ectocoracoid, fewer than seven dorsal spines, and lack of the pelvic girdle and large lateral scutes.

Description: Background color pale olive, sides of the body pigmented with dark bars or blotches. Body moderately compressed. Head conical and flattened dorsally, mouth slightly supraterminal and oblique. A continuous groove separating upper lip from the maxillary. Numerous small sharp teeth confined to the upper and lower jaws, absent on inside of the upper mouth. Gill membranes extending forward and joined to each other. Opercular opening extends above upper end of pectoral fin. Bones not well ossified and sculptures poorly developed on the cranial bones. Pectoral fin base vertical and consisting of 10 (rarely 11) soft rays. Lateral line inconspicuous with 28-38 (usually 32) small, round scutes. Caudal peduncle deeper than wide, keel absent. Dorsal spines 2-6, isolated, directed backward and vertically (not inclined from the middorsal line) with triangular fin membrane and depressible in shallow groove. First spine shortest and the last one longest. Dorsal soft rays 8-11; anal soft rays 6-10; gill rakers 7-10. Caudal fin rounded and consisting of 12 soft rays. Number of vertebrae 29-30 (usually 30) with 12-13 precaudal vertebrae. Pelvic girdle absent or rudimentary. The longest specimens reported by Stephanidis (1971) were 50 mm.

Distribution: Sperchios River basin and probably nearby basins in central Greece.

2. *Pungitius platygaster* (Kessler, 1859)

Synonyms: *Gasterosteus platygaster* Kessler, 1859; *Gasterosteus kessleri* Yakovlev, 1870; *Gasterosteus niger* Yakovlev, 1870; *Gasterosteus caucasicus* Kessler, 1877; *Gasterosteus aralensis* Kessler, 1877; *Gasterosteus danubica* Steindachner, 1899; *Pygosteus nudus* Berg, 1916.

Diagnosis: A combination of two characters: lack of caudal peduncle keel, presence of large lateral scutes.

Description: Similar to *P. hellenicus* with the following differences. Body darker in color than *P. hellenicus*. Pelvic girdle present with one spine and one small soft ray on each side. All the bones well ossified, cranial and pelvic bones highly sculptured. Caudal peduncle keel absent as in *P. hellenicus*. Bony scutes 29-32 with 7-12 large lateral scutes. Dorsal spines 8-11, inclined alternately to left and right. Last spine slightly longer than the others which are relatively uniform. Dorsal soft rays 6-10; anal soft rays 6-9; gill rakers 9-11; total vertebrae 29-31; precaudal vertebrae usually 13. Caudal fin truncated.

Based on differences in the serration of the pelvic spines, Berg (1949) and Wootton (1984) recognized two subspecies: *P. platygaster platygaster* in the Black Sea, Sea of Azov and Caspian Sea basins, and *P. platygaster aralensis* in the Aral Sea basin. However, my specimens from the Sea of Azov show weak serration similar to that described for the latter subspecies. This observation and the fact that the pelvic girdle shows much variation in sticklebacks suggest that *P. platygaster* is monotypic.

Distribution: Black Sea, Sea of Azov, Aral Sea and Caspian Sea basins. It is distributed in the Caspian Sea basin, but is rarely found on the southern coast. Armantrout (1980) reported this species from a single specimen caught in Bandar Anzali, Iran.

3. *Pungitius pungitius* (Linnaeus, 1758).

3.1. *Pungitius pungitius pungitius* (Linnaeus, 1758).

Synonyms: *Gasterosteus pungitius* L., 1758; *Pygosteus pungitius* Gill, 1861; *Gasterosteus burgundianus* Blanchard, 1866; *Gasterosteus pungitia* Sauvage, 1874.

Diagnosis: A combination of four characters: conspicuous caudal peduncle keel, lack of large lateral scutes, Long haemal and neural spines on the preurals 4 and 5, and usually fewer than 11 dorsal spines.

Description: Generally similar to *P. hellenicus* with the following differences. Bones well ossified with relatively strong sculptures on the cranial and pelvic bones. Body quite variable in color, but generally darker than in *P. hellenicus*. Dorsal spines 7-11; dorsal soft rays 9-12; anal soft rays 7-11; Scutes 3-33; gill rakers 9-13; total vertebrae 30-34 (usually 32 or 33) with 12 -16 precaudal vertebrae (usually 14 or 15). Pelvic spines usually two, each associated with one pelvic soft ray, some with only one soft ray on one side, some with one soft ray on one side and two on the other, and some specimens totally lack the pelvic girdle (Ziuganov and Rozanov 1987). Caudal fin polymorphic, in some rounded, in others truncated.

Distribution: Atlantic, Arctic and Pacific coasts, and inland waters of Eurasia, and Japan.

3.2. *Pungitius pungitius laevis* (Cuvier, 1829)

Synonyms: *Gasterosteus laevis* Cuvier, 1829; *Gasterosteus lotharingus* Blanchard, 1866; *Gasterosteus breviceps* Blanchard, 1866.

Diagnosis: Caudal peduncle keel not apparent in unstained specimens and caudal peduncle relatively deep.

Description: Mainly similar to *P. p. pungitius* with the following differences. Scutes on the caudal peduncle 2-8, probably absent in some specimens; no lateral scutes; dorsal spines 8-10, sometimes less than 8 and rarely one, two or three (Ure 1962); dorsal soft rays 9-12; anal soft rays 8-10; gill rakers 9-12; total vertebrae 31-34. Pelvic spines usually two and each associated with a pelvic soft ray, some specimens (or populations) lack the pelvic girdle and have a reduced ectocoracoid. Caudal fin rounded.

Distribution: Ireland, Southern England, and southern France.

3.3. *Pungitius pungitius tymensis* (Nikolsky, 1889).

Synonyms: *Gasterosteus tymensis* Nikolsky, 1889; *Pygosteus undecimalis* Jordan and Stark, 1902.

Diagnosis: Usually 11 or more dorsal spines.

Description: As *P. p. pungitius* with the following differences. Darker in color. Dorsal spines 8-13 (usually 11); dorsal soft rays 10-11; anal soft rays 8-12; scutes on the caudal peduncle 5-8; no large lateral scutes; a few scutes behind the head; gill rakers 8-11; total vertebrae 33-35 with 14-16 precaudal vertebrae. Pelvic girdle reduced with one spine and one pelvic soft ray on each side. Some lack the pelvic spines and soft rays. Caudal fin rounded.

Distribution: Sakhalin Island, Russia and Hokkaido Island, Japan.

3.4. *Pungitius pungitius sinensis* (Guichenot, 1869).

Synonyms: *Pungitius sinensis sinensis* (Guichenot, 1869); *Pygosteus stenurus* Kessler, 1876; *Gasterosteus wosnessenkyi* Kessler, 1876; *Gasterosteus japonicus* Steindachner, 1880; *Gasterosteus bussei* Warpakchow, 1887; *Pygosteus steindachneri* Jordan and Snyder, 1901; *Pungitius sinensis* kaibarae (Tanaka, 1915); *Pygosteus kaibarae* Tanaka, 1918.

Diagnosis: Presence of large lateral scutes and usually two pelvic soft rays on each side.

Description: As *P. p. pungitius* with the following differences. Dorsal spines 8-11; dorsal soft rays 8-12; scutes 31-33 with 6-8 large lateral scutes; gill rakers 10-14; total vertebrae 33-35 with 14-15 precaudal vertebrae. Pelvic girdle well developed and usually each spine associated with two pelvic soft rays. Caudal fin usually rounded.

Distribution: Northern Honshu Island and Hokkaido Island in Japan, and Korea.

3.5. *Pungitius pungitius occidentalis* (Gmelin, 1789).

Synonyms: *Gasterosteus occidentalis* Gmelin, 1789; *Gasterosteus concinnus* Richardson, 1836; *Gasterosteus mainensis* Storer, 1843; *Gasterosteus dekayi* Agassiz, 1850; *Gasterosteus blanchardi* Sauvage, 1874; *Gasterosteus globiceps* Sauvage, 1874; *Gasterosteus brachypoda* Bean, 1879.

Diagnosis: Short haemal and neural spines on the preurals 4 and 5. Caudal fin usually truncated.

Description: As *P. p. pungitius* with the following differences. Color variable, usually silvery on the ventral side. Dorsal soft rays 8-12; anal soft rays 7-13; scutes 6-17; no large lateral scutes; gill rakers 8-16; total vertebrae 32-35 with 13-16 (usually 14 or 15) precaudal vertebrae. Some specimens from Pine Lake, Wood Buffalo National Park, lack the pelvic girdle or have a reduced one. Caudal fin polymorphic, but usually truncated.

Distribution: North America, along the Northern coastline from Aleutian Islands, Alaska, to New Jersey, penetrates inland from Fort Nelson, British Columbia to western Quebec and extends south to Minnesota and northern Indiana (Nelson and Paetz, 1992).

A key to the species and subspecies

1. a) Caudal peduncle keel present (*P. pungitius*) 3

1. b) Caudal peduncle keel absent 2

2. a) Pevic girdle and large lateral scutes present. Dorsal spines seven or more. Aral Sea, Sea of Azov, Black Sea and Caspian Sea basins .... *P. platygaster*

2. b) Pelvic girdle absent or very reduced, large lateral scutes absent. Dorsal spines fewer than seven. Greece .... *P. hellenicus*

3. a) A full row of scutes with large lateral scutes present. Usually two soft rays with each pelvic spine. Japan, Korea .... *P. p. sinensis*

3. b) Incomplete row of scutes, no large lateral scutes. Usually one soft ray with each pelvic spine 4

4. a) Dorsal spines usually 11 or more. Sakhalin Island, Japan .... *P. p. tymensis*

4. b) Dorsal spines fewer than 11 5

5. a) Deep caudal peduncle. Reduced basals. Caudal peduncle keel not apparent in unstained specimens. Southern Europe, Ireland .... *P. p. laevis*

5. b) Wide caudal peduncle. Developed basals. Caudal peduncle keel apparent in unstained specimens 6

6. a) Long haemal and neural spines on the preurals 4 and 5. Caudal peduncle relatively short and deep. Eurasia .... *P. p. pungitius*

6. b) Short haemal and neural spines on the preurals 4 and 5. Caudal peduncle relatively long and narrow. North America .... *P. p. occidentalis*

Systematics

Phylogenetic analysis

Thirty four characters were entered into the MacClade 3.04 computer program (Maddison and Maddison 1992) and analyzed in Paup 3.1.1 (Swofford 1993) to demonstrate the phylogenetic relationships of the three species. Sixteen characters were uninformative and excluded from the analysis, however, they are explained herein because they may be of interest to researchers investigating the evolution of this group. Table 7.1 shows the data matrix for the characters. All the characters were weighted equally and all were treated as unordered, while all the multistate characters were treated as polymorphic characters.

Aulorhynchus flavidus, *Gasterosteus aculeatus* and *Culaea inconstans* were used as outgroups and the seven nominal species as the ingroup. Two methods were used for the character polarization. In the first method those states most common in the outgroup were considered the primitive state (0) and in the second *A. flavidus* was considered as the most primitive outgroup and all the states in that species were regarded as the primitive states (0). The results for both sets were the same. In all the analyses trees were rooted on

A. flavidus. Any combination of options (Acctran or Deltran optimization, Heuristic or Branch and Bound algorithms, all characters ordered, all characters unordered or only multistate characters ordered) produced the same tree. However, this tree showed a polychotomy for all the taxa and was not informative. Using the constraint tree option, I forced the program to avoid mixing the outgroup with the ingroup and I asked it to make the outgroups paraphyletic relative to the ingroup. Figure 7.1 shows the consensus of the six most parsimonious trees that resulted from strict rule in Paup (38 steps, C.I. 0.66, H.I. 0.50, R.I. 0.52). The strict consensus tree made a trichotomy for *Culaea, Gasterosteus*, and *Pungitius* and suggested that either *G. aculaetus* or *C. inconstans* is the sister group of the ingroup. *Pungitius hellenicus* is the primitive sister group of the other species and subspecies. However, there is only one synapomorphy for the genus *Pungitius* (character 28, presence of the postmaxillary process). Members of the clade above branch 12, consisting of the ingroup, *C. inconstans* and *G. aculaetus* share five primitive character states (characters 1, 3, 4, 8, 12 and 33). No osteological synapomorphic character supports the clade above branch 12. One synapomorphic character (24) supports the clade above branch 14. This may suggest that either the genus *Pungitius* is not a monophyletic group or that additional good characters need be found to study the phylogenetic relationships in Gasterosteidae.

To solve this problem, seven informative behavioral characters (out of 51) from the literature (McLennan, 1993) were added to the data matrix. The result was almost the same (59 steps, C.I. 0.64, H.I. 0.46, R. I. 0.46), with the exception that the polychotomy between *G. aculeatus* and *C. inconstans* was solved and *C. inconstans* was shown as the sister group to *Pungitius* (figure 7.2). In this case seven synapomorphies (9,10,35,38,39,40, and 41) support the branch number 13. The uncertainty in the relationship of the ingroup and outgroups is due to the presence of ambiguous characters (15 characters) and a high degree of homoplasy.

Without using the constraint tree option (multistate characters ordered), Paup produced a polychotomy for all the taxa of *Pungitius* and made *Culaea* the sister group to *P. hellenicus*. When all characters are unordered, Paup produced the strict consensus tree in figure (7.3) for the osteological and behavioral characters (43 steps, C.I. 0.72, H.I. 0.42, R.I. 0.64). This tree shows *C. inconstans* as the sister group of *P. hellenicus* and both as the sister group of all other species of *Pungitius*. Two synapomorphic osteological characters (15 and 20) and three homoplasic characters (14, 17, 19) support the clade consisting of *P. hellenicus* and *C. inconstans*. Only one synapomorphic character supports the genus *Pungitius*, excluding *P. hellenicus*. This character (24) is the inclined dorsal spines. Also, one reversed or homoplasic character supports the clade above branch 16, the depressed haemal and neural spines of preurals four and five (character 22). One homoplastic or reversed character supports the clade consisting of *P. p. sinensis* and *P. p. occidentalis* (21). None of the clades supports the monophyly of *P. pungitius* or *P. platygaster*. Figures (7.1) and (7.2) show a polytomy for *P. platygaster* and all the subspecies of *P. pungitius*. Figure (7.3) shows a polytomy for *P. platygaster*, *P. p. tymensis* and the clade consisting of other subspecies of *P. pungitius*. This shows the close relationship of *P. platygaster* to *P. pungitius*.

The evolution of eight osteological characters (out of eighteen informative characters) is shown on the tree (characters 9, 10, 14, 15, 18, 19, 20 and 25). Absence of postneurohypophyses and posthaemapophyses (characters 9 and 10) is primitive and they were gained between nodes B and C. The expanded shape of the basals (character 14) is primitive and the reduced shape was gained independently in branch 14 and in *P. p. laevis*. The absence of the large lateral scutes (character 18) is primitive and they were gained independently in *P. platygaster* and *P. p. sinensis*; the condition in *G. aculeatus* is uncertain. Overlapping in the dorsal basals (character 19) is primitive and branches 14 and

7 gained the advanced state independently. Bearing uniform dorsal spines (character 25) is the primitive state and those states in *G. aculaetus* and *P. hellenicus* were gained independently.

Ten characters, 6, 7, 17, 21, 22, 23, 24, 28, 30 and 33, are not resolved and may have a different evolutionary trend from what was estimated by the character optimization algorithm in Paup (figure 7.3). Although interpreting the evolution of these characters is difficult due to their ambiguity and the polychotomy in the tree, I comment on other hypotheses of evolutionary trends of these characters. Character six (shape of the coracoid fan), state 1, in *Pungitius* and the outgroup *G. aculeatus* could either be an independent gain in this group (homoplasy) or an independent gain of state 0 in the two other outgroups. The third possibility for this character is a reversal in *C. inconstans*. Character seven, shape of the scapula foramina, also shows the same evolutionary trend. For character 17 (absence of keel on the caudal peduncle) the most parsimonious pathway is that there were two independent losses, one in node C and one in *P. platygaster.* For characters 21 (length of the haemal and neural spines of preurals 4 and 5) there are two possibilities, two independent gains in *A. flavidus* and branch 17 or a reversal in branch 17. For character 22 (the angle between the haemal and neural spines of preurals 4 and 5), the wide angle between the spines is primitive and there are two parsimonious possibilities: two independent gains in *A. flavidus* and branch 16 or a reversal in branch 16. The most parsimonious evolution of character 23 (shape of the caudal fin) is that the round caudal fin is primitive with three independent gains (state 1 in *P. platygaster* and *P. p. occidentalis* and state 2 in *A. flavidus*). The condition in *P. p. pungitius* is uncertain. For character 24, arrangement of dorsal spines, Paup shows an equivocal condition between nodes C and D, but there is only one parsimonious way in the evolution of this trait; one gain between nodes C and D. For character 30, character states 0 or 1 (bearing fewer than seven or 7-10 spines) could be primitive based on this tree. However, I believe that bearing more spines is the more primitive condition. There are two parsimonious evolutionary trends in character 33. One possibility is that bearing four pelvic soft rays on each side of the pelvic girdle is primitive, and in one step, Gasterosteidae lost three or all of them and in another step, *P. p. sinensis* gained two pelvic soft rays. The other possibility is that bearing one pelvic soft ray on each side is primitive and *A. flavidus* and *P. p. sinensis* independently gained four and two pelvic soft rays, respectively.

Character description

The 41 characters used in the phylogenetic analysis are briefly described here. For more detail refer to sections 5 and 6 (also see Bowne, 1985), and for behavioral characters see McLennan (1993).

1. Lateral ethmoids without (0) / with anteriolateral extensions (1).
2. Frontals lack (0) / bear interorbital commisure (1).
3. Infraorbital rings incomplete (0) / complete (1).
4. Exoccipital condyles absent (0) / present (1).
5. Posttemporal bear (0) / lack lateral canals (1).
6. Coracoid fans incised (0) / entire (1).
7. Scapula foramina triangular or ovoid (0) / circular (1).
8. Preural ribs present (0) / absent (1).
9. Postneurohypophyses absent (0) / present (1).
10. Posthaemapophyses absent (0) / present (1).
11. Prehaemapophyses present (0) / absent (1).
12. Vertebrae lack (0) / bear Lateral processes to scutes (1).
13. Hypural plate fused (0) / divided (1).
14. Basals expanded (0) / reduced (1).
15. Ectocoracoid complete (0) / partially reduced (1) / reduced (2).
16. Pelvic spines present (0) / absent (1).
17. Keel on the caudal peduncle present (0) / absent (1).

18. Lateral large scutes absent (0) / present (1).
19. Dorsal spine basals overlap (0) / separated (1).
20. Scutes bear a canal (0) / lack the canal (1).
21. Haemal and neural spines of preurals 4 and 5 long (0) / short (1).
22. Haemal and neural spines of preurals 4 and 5 make a wide angle with the vertebral column (0) / are almost horizontal (1).
23. Caudal fin rounded (0) / truncated (1) / forked (2).
24. Almost all the dorsal spines vertical (0) / inclined (1).
25. All the dorsal spines, except the last one, the same size (0) / the first spine is the shortest (1) / the second spine is the longest (2).
26. Number of the dorsal spine basals almost equal to (0) / as twice the number of precaudal vertebrae (1).
27. Posttemporal anterioventral process absent (0) / short (1) / long (2).
28. Postmaxillary process absent (0) / present (1).
29. Gill membrane united (0) / attached to isthmus.
30. Fewer than 7 (0) / 7-10 (1) / more than 11 (2) / more than 20 (3) dorsal spines.
31. Mean number of the dorsal soft rays more than nine (0) / less than nine (1).
32. Mean number of the anal soft rays more than eight (0) / less than eight (1).
33. Number of the pelvic rays is less than three (0) / usually four (1), more than four (2).
34. Number of the vertebrae less than 40 (0) / more than 50 (1).
35. Insertion gluing absent (0) / present (1).
36. Male creeps (0) / does not creep through nest upon completion of building (1).
37. Zigzag male courtship dance, absent (0) / present (1).
38. Nest showing display, hover (0) / snout in nest (1) / snout above nest (2).
39. Fan during the nest showing, absent (0) / present (1).
40. Male dances toward nest, present (0) / absent (1).
41. Male moves nursery, present (0) / absent (1).

Table 7.1. Data matrix for the 41 osteological and behavioral characters.

Ph (*P. hellenicus*), Pp (*P. platygaster*), Ppl (*P. p. laevis*), Ppg (*P. p. pungitius*), Ppt (*P. p. tymensis*), Pps (*P. p. sinensis*), Ppo (*P. p. occidentalis*), Ci (*Culaea inconstans*), Ga (*G. aculeatus*), Af (*A. flavidus*).

Characters	Ph	Pp	Ppl	Ppg	Ppt	Pps	Ppo	Ci	Ga	Af
01.	0	0	0	0	0	0	0	0	0	1
02.	0	0	0	0	0	0	0	0	1	0
03.	0	0	0	0	0	0	0	0	0	1
04	0	0	0	0	0	0	0	0	0	1
05.	0	0	0	0	0	0	0	0	1	0
06.	1	1	1	1	1	1	1	0	1	0
07.	1	1	1	1	1	1	1	0	1	0
08.	0	0	0	0	0	0	0	0	0	1
09.	1	1	1	1	1	1	1	1	0	0
10.	1	1	1	1	1	1	1	1	0	0
11.	0	0	0	0	0	0	0	0	1	0
12.	0	0	0	0	0	0	0	0	0	1
13.	0	0	0	0	0	0	0	0	1	0
14.	1	0	1	0	0	0	0	1	0	0
15.	2	0	0/1	0	0	0	0	2	0	0
16.	1	0	0/1	0/1	0/1	0	0/1	0/1	0/1	0
17.	1	1	0	0	0	0	0	1	0/1	0
18.	0	1	0	0	0	1	0	0	0/1	0
19.	1	0	1	0	0	0	0	1	0	0
20.	1	0	0	0	0	0	0	1	0	0
21.	0	0	0	0	0	1	1	0	0	1
22.	0	0	1	1	0	1	1	0	0	1
23.	0	1	0	0/1	0	0	1	0/1	0/1	2
24.	0	1	1	1	1	1	1	0	0	0
25.	1	0	0	0	0	0	0	0	2	0
26.	0	0	0	0	0	0	0	1	0	0
27.	2	2	2	2	2	2	2	1	2	0
28.	1	1	1	1	1	1	1	0	0	0
29.	0	0	0	0	0	0	0	0	1	0
30.	0	1	1	1	2	1	1	0	0	3
31.	0	1	0	0	0	0	0	0	0	0
32.	0	1	0	0	0	0	0	0	0	0
33.	0	0	0	0	0	1	0	0	0	2
34.	0	0	0	0	0	0	0	0	0	1
35.	?	?	?	?	?	?	1	1	0	0
36.	?	?	?	?	?	?	1	0	1	0
37.	?	?	?	?	?	?	1	0	1	0
38.	?	?	?	?	?	?	2	2	1	0
39.	?	?	?	?	?	?	1	1	0	0
40.	?	?	?	?	?	?	1	1	0	0
41.	?	?	?	?	?	?	1	1	0	0

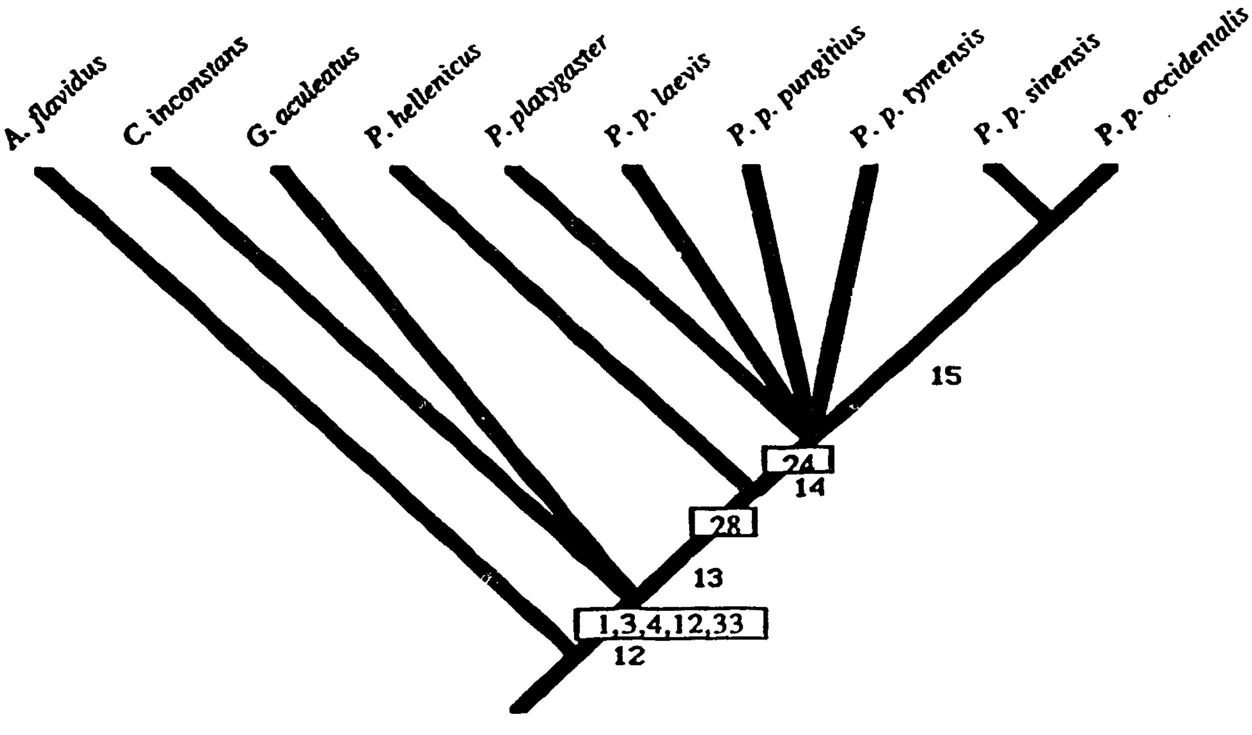


Figure 7.1. A consensus tree obtained from analysis of 34 osteological characters.

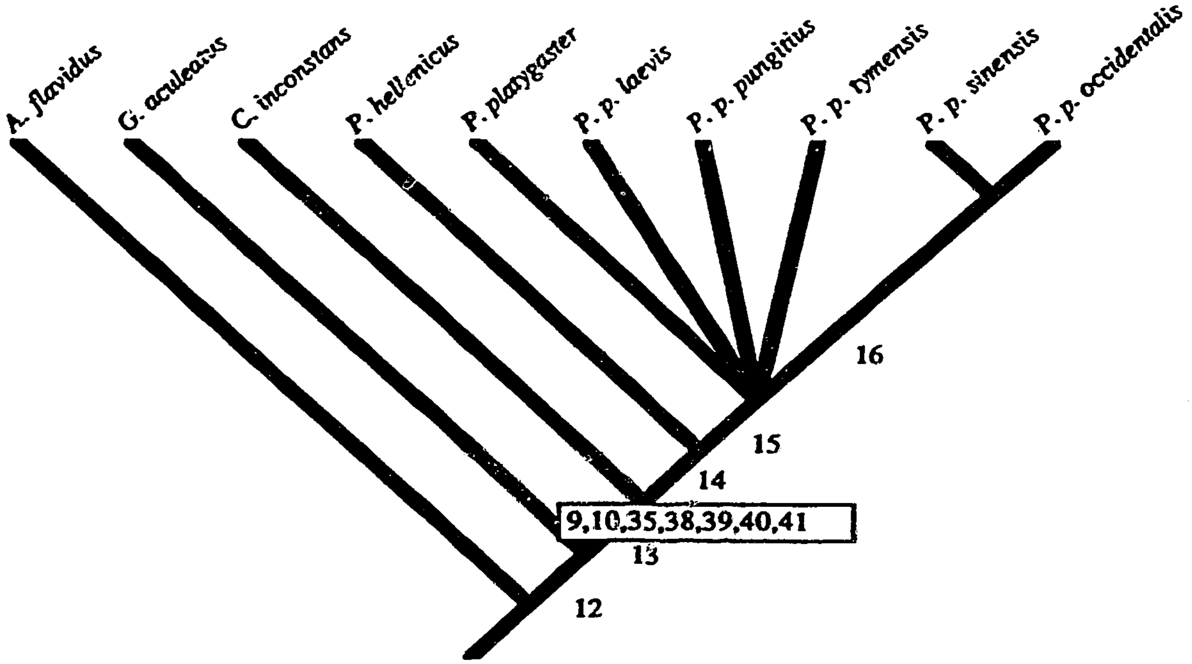


Figure 7.2. A consensus tree obtained from analysis of 41 osteological and behavioral characters.

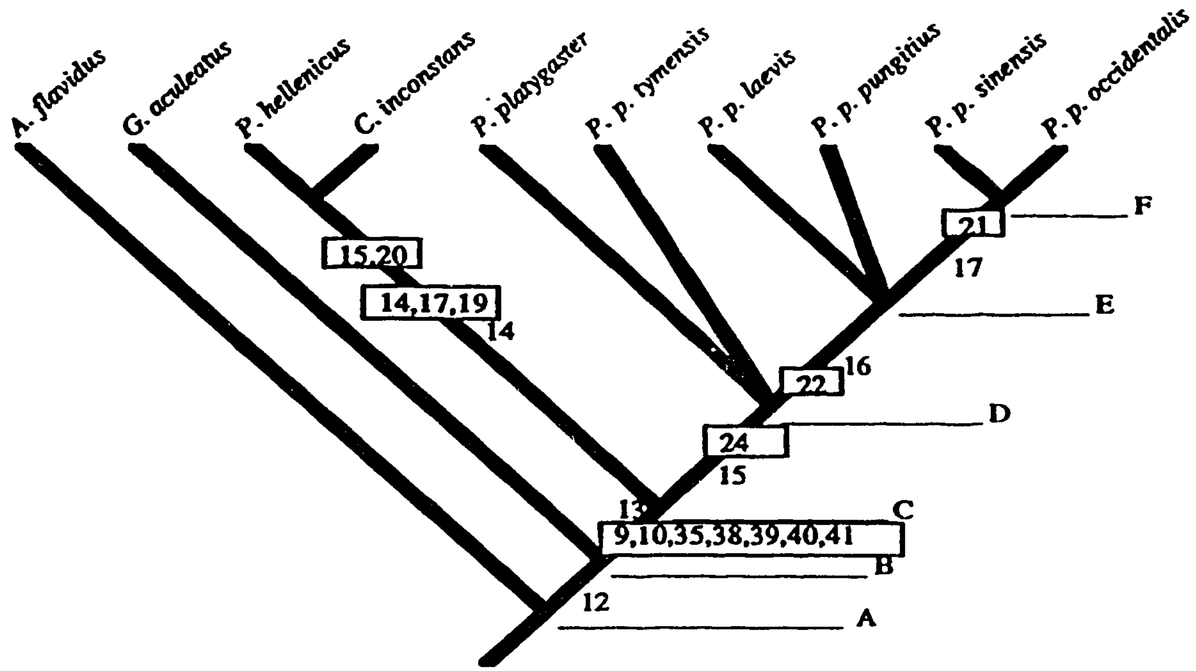


Figure 7.3. A consensus tree obtained from analysis of 41 osteological and behavioral characters, without using the constraint tree option.

8. Discussion and Conclusions

Discussion

Many characters, mainly morphological, have been used by past authors to differentiate among species of *Pungitius*. For example, Stephanidis (1971) recognized ***P. hellenicus*** as new because of differences in number of dorsal spines, absence of pelvic spines, shape of the pelvic bones and color. However, some characters may show much variation due to environmental factors. For example, sticklebacks show much variation in color. Although none of the species and subspecies of *Pungitius* is as light in color as ***P. hellenicus***, I believe that this pattern is an ecological factor, probably resulting from a different diet or absence of predators, because a sympatric population of threespine stickleback, *Gasterosteus aculeatus*, also has the same color. In the following subsections I will discuss morphometrics, meristics, osteology, and phylogenetic relationships among the species.

Morphometrics

McPhail (1963) observed that the mean pelvic spine length in *P. p. occidentalis* decreases from tidal to inland forms (1- 0.5 of pectoral fin length). Gross (1979) studied three forms of *P. p. pungitius*: marine, lake, and stream forms and observed body depth of specimens to increase from marine to stream forms (20%, 22%, and 23% of the standard length respectively). *Pungitius pungitius occidentalis* in North America contradicts this trend (20 % in tidal form versus 17 % in the inland form). He also found that lentic populations have a longer and narrower caudal peduncle than the others and stream populations have a humpbacked body form. I found a stream population of *P. p. occidentalis* from Firth River to have a relatively short caudal peduncle (14 % versus 17 % in lentic populations from inland lakes). Specimens of *P. p. sinensis* tend to be deeper at the middle part of the body than the other species and subspecies. This phenomenon can be seen even in small specimens. In general, larger specimens of all the species are deeper in the middle. Furthermore, Gross (1979) believed that *P. p. laevis* would show the same patterns of variation, but that *P. platygaster* would never be as shallow bodied as *P. pungitius*. My data confirm his conclusion for *P. p. laevis* but not for *P. platygaster* (*P. p. pungitius* 17-25 % (mean 20 %), *P. p. laevis* 17-23 % (20 %)). and *P. platygaster* 19-24 % (22 %). His study also revealed a correlation between length of the dorsal and pelvic spines with presence of predators.

Tanaka (1982) studied allopatric populations of *P. p. pungitius* and *P. p. sinensis* in Honshu, Japan and observed geographic and clinal variation in length of the last dorsal spine, anal and pelvic spines (longer near coast and shorter toward inland). In an exception to McPhail's (1963) general finding in North America, in a population of *P. p. occidentalis* from Firth River, dorsal spine length in the tidal form was found to be shorter than that in the inland populations (1.2 mm in specimens from Firth River versus 1.7, 1.8 and 1.7 mm in specimens from Pine Lake, Crooked Lake and Cold Lake, respectively). Takata et al. (1984) calculated percentage of pelvic spine length to standard length in *P. p. tymensis* (3.5-9.5%), *P. p. pungitius* (6-15%), and *P. p. sinensis* (7-16%). Kim et al. (1989) calculated the ratio of standard length to anal spine length in *P. p. sinensis* (5.5-19.5) and *P. kaibarae* (4-20.5). My calculations show a ratio of 13-25 for *P. p. sinensis*.

Baumgartner (1992) found in a study of body form variation in the threespine stickleback that most body-shape differences among localities were accounted for by size-related allometric variation. Small specimens had long spines and fins whereas larger individuals tended to be deep bodied and have shorter fins and spines. Large fish had shorter spines but wider pelvic girdles and were larger in the mid body than small fish. Significant size dependent differentiation with respect to the defensive complex and overall body form was also observed. Completely plated individuals were more elongated and had longer fins, shorter spines and a greater proportion of the abdomen covered by the pelvic girdle. Furthermore, Baumgartner (1992) found the association of completely plated elongate sticklebacks with the high gradient habitats and low plated, robust bodied sticklebacks with low gradient or standing water habitats. He concluded that various aspects of stickleback morphology may respond independently and rapidly to different evolutionary forces and be functionally related to hydrodynamics. The same phenomena may be seen in the species of *Pungitius*. For example, *Pungitius hellenicus* with a lower mean standard length has a longer pectoral fin and shorter spines than the other species. This negative allometric growth prevents using the ratios in comparative studies.

Wimberger (1993) in studying effects of vitamin C on the body shape and skull osteology in a cichlid fish found a trend toward shortening of all the skull and jaw measurements in fish fed a vitamin C deficient diet. The ascending process of the maxilla, lower jaw, eye diameter and the distance from the interopercle to the hyomandibular-opercular joint were shorter, but the premaxilla was a little longer. The caudal peduncle length, the distance between snout and pelvic girdle and the caudal peduncle depth were longer and the mid body depth shallower in fish fed a vitamin C deficient diet. Do different diets affect sticklebacks in the same way and cause the variation in their shape? For example, is the shorter snout in *P. hellenicus* caused by its diet? Day et al. (1994) raised fish from two recently diverged species of threespine sticklebacks on two different diets representing the natural prey of the two species. Both species exhibited morphological plasticity in an adaptive direction; individuals of each species more closely resembled those of the other when raised on the other's diet (wider gape, deeper head, shorter gill rakers, and shorter snout in the bentic than in the limnetic form).

Despite the abundant work on traditional morphometric characters, no truss analysis has been conducted on members of this genus. The principal component scores show separate clusters for some of the nominal species (section 4), but the size related factors jeopardize the validity of this method. Bookstein et al. (1985) suggested the shear size free method to eliminate the size differences among populations with different sizes. Due to lack of software necessary for this analysis, this could not be conducted. However, most of the plots show different trends for each species with increase in size. Besides, there is no overlap in some of the plots, while their size range somehow overlap. Also to solve the problem caused by allometric growth, I used only specimens with a size close to that of *P. hellenicus* specimens, but I lacked such specimens for *P. platygaster* and *P. p. tymensis* and had only a few of *P. p. laevis* and *P. p. sinensis*. A comparison of all the species was therefore impossible. Figures 8.1 and 8.2 show the result of this analysis for traditional morphometric characters and truss distances. These figures show separate clusters for *P. hellenicus* and *P. p. occidentalis* but weaken the previous conclusion that *P. hellenicus, P. p. laevis* and *P. p. pungitius* differ in their body form.

Meristics

Lindsey (1962) studied meristic differences in *P. pungitius* reared at different temperatures (16 °C, 22 °C, 24 °C, and 26 °C). In higher temperatures specimens had a higher mean number of dorsal spine basals (11-12) and dorsal ray basals (12-13.4) and lower numbers of anal ray basals (10.33-10), anal rays (9.4 - 7.4), pectoral rays (9.55-9), and dorsal spines (8-7.4). McPhail (1963) observed variation between the tidal and inland forms of *P. p. occidentalis* in North America. The tidal form had a higher number of lateral plates (3 - 5 in tidal form versus 0 - 2 in inland form) and dorsal spines (9.7 - 10.7 versus 8.7 -9.4) and lower numbers of gill rakers (10.8 - 12.5 versus 12.8 - 13.6). He believed that there is a north-south cline for number of dorsal spines, but no cline for the other meristic characters. My data show a higher mean number in the tidal form, but no cline for the dorsal spines. Mean number of the dorsal spines is 9.0 in specimens from Crooked lake, 8.5 from Pine lake, and 9.3 from Cold lake, versus 9.9 from Firth River. Mean number of gill rakers in the specimens from Crooked Lake is 12.4, Pine Lake 14, and Cold Lake 11.9 versus Firth River 11.2. Mean number of the scutes on the caudal peduncle keel is lower for the tidal form from Firth River, 4.5 versus 12.8, 8.3 and 8.4 in Crooked Lake, Pine Lake and Cold Lake, respectively. Mean number of the dorsal and anal basals is also lower in the tidal form.

Gross (1979) observed clinal trends in all meristic traits of *P. p. pungitius* in Europe. Marine populations had more gill rakers than freshwater populations (this contradicts data from North America in *P. p. occidentalis*). The highest number of total vertebrae, precaudal vertebrae, caudal vertebrae, and anal and dorsal basals was on the 65° latitude and the lowest number on 40 ° and 70 °. There was a north-south cline for number of dorsal spines (9.8-8.3). However, I believe that some exceptions may be expected to this general finding as in North America. Tanaka (1982) observed geographic but not clinal variation in the number of dorsal spines, dorsal rays, pelvic, and anal soft rays in *P. p. pungitius*, *P. p. sinensis* and their hybrids (scutes: 11.3, 33.2, and 25.7; dorsal spines: 8.5, 9.1, and 8.9; dorsal soft rays: 9.2, 11.6, and 10; anal soft rays: 8.8, 9.7, and 8.9; pelvic soft rays: 1, 1.4, and 1.4; and gill rakers: 8, 10.1, and 9, respectively). The range for different meristics was 4-36 for scutes, 7-11 for dorsal spines, 8-13 for dorsal soft rays, 6-12 for anal soft rays, 0-3 for pelvic soft rays, and 6-12 for gill rakers.

Takata et al. (1984) reported the following range of meristic characters in *P. p. pungitius, P. p. sinensis and P. p. tymensis*. Number of dorsal spines 8-12, 7-10, and 9-13 respectively, number of lateral plates 6-20, 31-37, and 3-12, number of gill rakers 9-15, 10-15, and 8-12, number of vertebrae 31-36, 31-36, and 30-35. They believed there to be a north-south cline in number of vertebrae on the Japan sea slope. My data show a cline in number of vertebrae and dorsal spines from Sakhalin Island toward Yamagata. Takata et al. (1987) observed higher numbers of lateral plates, dorsal spines, and gill rakers and lower numbers of vertebrae in the brackish form of *P. p. pungitius* in Hokkaido, Japan. Kim et al. (1989) observed variation in meristic characters of *P. p. sinensis* in Korea (because I do not accept *P. kaibarae* even as a subspecies, I combined the meristic characters he reported for *P. sinensis* and *P. kaibarae*); dorsal spines 6-10 (mean 7.7-8.7), dorsal rays 9-12, pelvic soft rays 1-2 (mean 1.2-1.8), anal rays 8-11, scutes 31- 35 (32.1-33.4), gill rakers 9-14 (10.5-12.3), and vertebrae 28-35 (mean 32.1-34.4).

The significance of different numbers of meristic characters is uncertain. Swain (1992) observed selective predation for number of precaudal and total vertebrae and found it to depend on the larval length (as length increases optimal ratios of precaudal to caudal vertebrae decrease). He suggested that selection may associate with differential reproduction because the ratio of precaudal vertebrae is related to egg carrying capacity. Reimchen and Nelson (1987) present evidence that vertebral number of sticklebacks may also be subject to mortality selection at later life history stages.

Osteology

Although there is a little major intraspecific variation in the osteology of the sticklebacks (excluding *Spinachia spinachia*), there is much minor variation in that of populations. Many of the characters used by Bowne (1985) and Sroivattana (1972) as synapomorphies for *Pungitius* or *Culaea* are variable and unreliable. For example, Sroivattana (1972) described shape of the posterior margin of the interopercle as vertical in *Culaea* and oblique in *Pungitius*, but both conditions can be seen in the same species and population. Bowne (1985) described the absence of the anteriolateral foramina on the dentary as a synapomorphy for *Pungitius*, but it can be seen on many specimens, even though it is small in some. She described the lateral flanges of the ethmoid as being absent in *Pungitius*, but in some specimens a shallow flange can be seen. Symplectic dorsal flanges vary widely in different specimens of the same species. The nasal process also varies in shape. Although some specimens of *Gasterosteus aculeatus* and species of *Pungitius* have the posteriodorsal extension on the third infraorbital, many specimens lack that process.

The number, size, and arrangement of the dorsal spines show much variation in the species and subspecies of *Pungitius*. Shadrin (1994) studied the ontogeny of *P. p. pungitius* in Russia, and observed that, on the middorsal line, dorsal fin formation starts at the midpoint, spine formation occurs craniad and soft ray occurs caudad. In a developing fish, the last spine is the longest and the first the shortest. The fewer numbers of dorsal spines in specimens of *P. hellenicus* and their different size and arrangement may result from neoteny; spine growth stops during the larval stages and adults retain the condition before the spines reach their full size and ultimate arrangement. In the population from Firth River, small specimens (smaller than 16 mm in standard length) show the same arrangement of dorsal spines as in *P. hellenicus*. The first spine is the shortest and the last spine the longest; dorsal basals are also reduced and do not overlap. There is no trace of the ectocoracoid and pelvic girdle. The neural and haemal spines of all the caudal vertebrae make a wide angle relative to the vertebral column and are not fused to the basals. They do not bear a caudal keel, the caudal peduncle is deeper than wide, and the epural plate is relatively larger. However, these specimens have longer dorsal and anal spines than *P. hellenicus* and all the dorsal basals bear dorsal spines (10 dorsal spines). This observation confirms the possibility of neoteny in *P. hellenicus*.

Stephanidis (1971) described the absence of the pelvic girdle in *P. hellenicus* and Nelson (1971b) studied its absence in some populations of *P. p. occidentalis* in North America and *P. p. laevis* in Ireland. Takata et al. (1985) reported pelvic spine deficiency in

P. p. tymensis from Japan. Zyuganov and Rozanov (1987) discovered loss of the pelvic girdle in a population of *P. p. pungitius* from Russia. However, the significance of this loss and its inheritance is uncertain. Nelson (1969) in a discussion of pelvic deficiency in *Culaea inconstans*, suggested an advantage of reduced weight and streamlining for efficient escape responses in dense vegetation. Nelson (1971b and 1993) wrote that loss of part or all of the pelvic complex in actinopterygians is a derived condition. The fact that several localities are known in each of the only two widely separated regions where the pelvic complex is lost suggests the existence of common genetic or environmental components restricted to each region. He also noted a correlation between pelvic girdle deficiency and pelvic spine length and dorsal spine length. Coad (1973) failed to detect an interpopulation correlation between the frequency of reduced pelvic structures and mean pelvic spine length. Nelson (1971b) also found that the distance between the pelvic spine bases is small in specimens with reduced pelvic spines. Bell (1984) suggested that this difference and the difference between spine length combine to produce a small body cross sectional area, but the correlation between dorsal spines and pelvic reduction is small. *Pungitius hellenicus* not only loses the pelvic girdle and reduces the spine length, but also reduces the ectocoracoid for a much smaller cross sectional area, probably to achieve a lighter body and a more hydrodynamic shape for living in current water. Absence of fish predators provides the opportunity to lose weight without risk of being captured by predators. Reimchen (1980) suggested that reduction of the spines reduces frictional contact for grappling predators. Reimchen (1983) studied the relationship between condition of dorsal and pelvic spines of *Gasterosteus aculeatus* and their predators and suggested that deficiency of dorsal and pelvic spines was advantageous to juvenile fish when they escaped from grappling predators such as Odonate nymphs. Reist (1980) also suggested that polymorphism of pelvic spines of *Culaea inconstans* was developed and maintained by of predators.

Giles (1983) made crosses between the normal and pelvis reduced *G. aculeatus* and concluded that reduction has some inheritable basis. Nelson (1977) in an experiment on *Culaea inconstans*, scored the pelvic phenotypes in three classes (absent, incomplete and complete) and concluded that pelvis loss and reduction have some heritable basis. Giles (1983) noticed that *G. aculeatus* populations while exhibiting pelvis reduction exist in water with low calcium concentration in Scotland and suggested that pelvis reduction in these populations is in response to selection for phenotypes with reduced calcium demand. Bell (1987) argued that vestiges are non functional and the target of selection is not their form, but perhaps skeletal reduction generally or absence of the spines specifically. ***Pungitius pungitius occidentalis*** from Crooked lake is less ossified and this might in part result from calcium deficiency, but loss of the spines in *P. p. occidentalis* from Pine lake, Wood Buffalo National Park, is unlikely to relate to calcium deficiency because normal specimens are found in the same population. *Pungitius hellenicus* shows both conditions (weak ossification and pelvic girdle loss), but I think that it is not related to calcium deficiency because a sympatric population of *Gasterosteus aculeatus* shows the normal condition. Nelson (1977) considered absence of the pelvis in *P. p. occidentalis* and *Culaea inconstans* as a parallel evolution in these species in North America. Bell (1988) also believed that pelvis reduction has evolved independently in widely separated populations within each of the three genera, *Gasterosteus*, *Culaea*, and *Pungitius*, and it also occurred in the Miocene *Gasterosteus doryssus*.

Blouw and Boyd (1992) believed that directional selection for pelvis reduction by invertebrate predators should have different consequences from that of selection to reduce calcium demand. The target of selection by grasping invertebrate predators is a structure with more frictional contact, whereas the target with low calcium concentrations is any structure which requires calcium uptake against a steep concentration gradient from the environment or any physiological attribute which enhances the ability to sequester calcium.

They also suggested that pelvic reduction is heritable and is consistent with a model of polygenic inheritance with two thresholds. The upper threshold is for loss of spines and the lower for absence of the pelvis. Above the upper threshold and between the thresholds, the size of the pelvis behaves as a simple quantitative trait. Given polygenic inheritance with thresholds, the appropriate selective environments must be coupled with restricted gene flow for pelvis reduction to evolve in natural populations. This may explain why reduction tends to occur in isolated populations and in headwater lakes, where opportunity for gene flow with individuals from normal populations is limited. In general I believe that pelvic reduction has a genetic basis and is heritable, but that it is also affected by environmental factors such as calcium deficiency or predators.

Shape and position of scutes are other features exhibiting much variation in the sticklebacks. Solving the inheritance pattern of scutes may explain their polymorphism and resolve the taxonomy of sticklebacks. Igarashi (1962 and 1963) observed scutes not to arise until *P. p. pungitius* grow to 15 mm and *P. p. sinensis* to 20 mm in total length. Then, about four small scutes appear on the lateral line in the posterior region of caudal peduncle and from there scute formation runs mainly in an anterior direction. When *P. p. pungitius* and *P. p. sinensis* grow to 30 mm two or three small scutes resembling scale-like particles, form in the anterior region of the lateral line. Due to this similarity, he concluded that they are phylogenetically close to each other. However, there is no scute formation in the middle part of *P. p. pungitius*. In *P. p. tymensis* the earliest development of the scutes starts with four foci on the caudal peduncle. When the fish reach 23 mm, anterior scutes appear (Igarashi, 1970). Study of lateral scutes convinced him that *P. p. tymensis* is a species rather than a subspecies of *P. pungitius*, because the completed scutes on the caudal peduncle show a few branched ridges but lack the network ridges present in those of *P. kaibarae* and *P. p. pungitius*. Development of the scutes is suspended earlier in *P. p. tymensis* than in other species of *Pungitius* and he attributed this to neoteny. He also believed that *P. p. tymensis* must originally have had a full armor like *P. p. sinensis* and more primitive than *P. p. pungitius* and *P. p. sinensis*. If we accept that absence of network ridges is the primitive condition, then scutes of *P. hellenicus* must be regarded as most primitive because they lack both the network ridges and branched ridges.

Igarashi (1969), in studying the ontogeny of scutes in *P. kaibarae*, realized that scutes first develop in juveniles at most 18 mm in total length (it could be earlier, because his smallest specimen was 18 mm in total length). The first scutes appear on the caudal peduncle followed by those on the dorsolateral part of body above the pectoral fin and then each group grow forward and backward respectively and eventually form the trachuran series of scutes (similar to those in *Gasterosteus aculeatus*). In this form, scutes do not overlap and sometimes are completely isolated. This phenomenon lead him to conclude that *P. kaibarae* should be recognized as a species.

Ayvazian and Krueger (1992) found the same scute ontogeny in *P. p. occidentalis* to *P. p. pungitius* in Japan and concluded that the partial phenotype of *P. p. pungitius* evolved from the complete phenotype through neoteny and postdisplacement and that the caudal phenotype was derived by progenesis and postdisplacement. Specimens of *P. hellenicus* 17 mm in standard length have a full row of the scutes. This means that scute formation starts earlier in *P. hellenicus* and probably resembles the neotenic form of *P. platygaster* in scute condition.

Reimchen (1983) in studying the significance of large lateral scutes on the threespine stickleback, wrote that the lateral plates provide a structural base for the spine supports and a greater surface area over which forces on the spines are distributed and proposed that their principal function is to stabilize the long erected spines during manipulation by predators. However, in *P. platygaster* and *P. p. sinensis* with no long spines, the function of these scutes is uncertain and their loss in other species might be a response to loss of function. Taylor and McPhail (1986) believed streamlining, longer pectoral fins and presence of a caudal peduncle keel are important in the reduction of swimming drag, the ability to hold a position within the stream and increased prolonged swimming ability. The overall burst-swimming performance of low plated sticklebacks is better than that of completely plated sticklebacks, apparently because the former curve their bodies during the initial phase of burst swimming.

Honkanen (1993) compared canals of the lateral line system in *P. p. pungitius* and *G. aculeatus* and observed them to be absent from the head of threespine stickleback but well developed on that of ninespine stickleback. The preopercular and infraorbital lines of free neuromasts on the head of ninespine stickleback are continuous with neuromasts enclosed in canals and, accordingly, can be defined as replacement line. Threespine stickleback has a more specialized lateral line system than ninespine stickleback because there are two lines of free neuromasts in the former, but only one on the latter. From these differences he concluded that these two specie are well separated from each other. Studying the lateral line system of species of *Pungitius* may help in resolving their taxonomy.

Phylogeny

The evolution of the characters were traced on the tree in figure (7.3); however, this tree contradicts the classical view of taxonomy of the Gasterosteidae where *Culaea* is recognized as a separate genus and probably the primitive sister taxon of *Pungitius*. In this analysis, it is shown as the sister taxon of *P. hellenicus*. This result may suggest either that the genus *Pungitius* is not monophyletic without including *C. inconstans* or that the osteological characters are not enough to resolve the phylogenetic relationships within Gasterosteidae. Nelson (1971) believed that osteological characters can not clearly resolve the phylogenetic relationships within Gasterosteidae. My data and Bowne's (1994) analysis strongly support his conclusion. Sroivattana (1972) also could not find any significant difference in the osteology of *P. pungitius occidentalis*, *Culaea inconstans* and *Apeltes quadracus*, except in ratio of some cranial bones that could be variant in the same species.

One of the problems in studying this group is the enormous variation in all the characters that makes it difficult to decide which character should be used. Only one character in this study seems to be synapomorphic for the genus *Pungitius*, but by including *Spinachia spinachia* in the analysis, this synapomorphy is also lost. So, I believe that in studying the sticklebacks, all the species must be included to reach relatively reliable results. Munzing (1966 and 1969) attributed divergences in *Pungitius* to divergence during glacial events in the Pleistocene. He hypothesized the existence of three refugia in Eurasia; southern France, the Ponto-Caspian region and eastern Asia and that all were originally populated by a morph resembling *P. p. sinensis*. It was suggested that divergence occurred independently in each refugium into the phenotypes now recognized respectively as *P. p. laevis, P. platygaster, P. p. pungitius*, and *P. p. tymensis*. Gross (1979) suggested that poorly armored populations may arise under relaxed predation, irrespective of their postglacial origin. He concluded that variation in *Pungitius* may result from recent or longstanding environmental selection, rather than be a relict of historical events. He doubted the taxonomic status of *P. p. laevis* because he believed the caudal keel in *P. p. laevis* to be at one end of an apparent east-west cline in scute number. My data is consistent with his analysis (figure 8.3). However, there are differences in the caudal region, shape of the dorsal basals and ectocoracoid that show genetic divergence in this taxon and I think it deserves the subspecific recognition.

Ziuganov and Gomeluk (1985) believed the differences between *P. platygaster* and *P. pungitius* to be adaptations to a greater number of various predators at low latitudes and the differences in sexual behavior between them are clearly insufficient for ethological reproductive isolation. Although I believe that the biological species concept cannot be applied to any species of *Pungitius*, and that none of the trees support the separation of *P. platygaster* from *P. pungitius*, I recognize *P. platygaster* as a separate species because 100 % of its specimens can be distinguished from those of other species.

Chen and Reisman (1970) studied chromosomes of North American sticklebacks and found that *Apeltes quadracus* and *Culaea inconstans* to have 2n=46 chromosomes and *Gasterosteus aculeatus*, *G. wheatlandi*, and *P. p. occidentalis* 2n=42. They suggested that common occurrence of a large submetacentric pair of chromosomes in the chromosome sets of all five species shows a monophyletic origin for these characters and concluded that *Apeltes* is perhaps related to their common ancestor. However, they did not include *Spinachia* in their study.

Bowne (1994) presented a cladogram for the family Gasterosteidae which is consistent with the osteological, behavioral, electrophoretic and karyotypic data. The position of *Culaea inconstans* remains unresolved because karyotypic and behavioral characters disagree, *Pungitius*, *Culaea*, and *Gasterosteus*, making a polytomy. She argued that it is prudent to regard the group as unresolved because the behavioral characters conflict with the karyotypic data and because *Culaea inconstans* was not included in the only allozyme study conducted to date (Hudon and Guderley, 1984). McLennan (1993) argued that behavioral characters are not only useful for systematic analysis but that they resolve the trichotomy. My data with using only the osteological characters and ordering all or multistate characters show a polychotomy for all the taxa. The polychotomy is resolved when behavioral characters are added and all the characters are analyzed as unordered, but shows *P. hellenicus* as the sister group of *Culaea inconstans* (figure 7.3). Although *Culaea inconstans* has been assigned to its own genus, it shares seven synapomorphic osteological and behavioral characters with the species of *Pungitius* and two synapomorphies with *P.*

hellenicus. This study suggests that the genus *Culaea* should be lumped with the genus *Pungitius* (i. e., that *C. inconstans* should be called *P. inconstans*). However, all similarities between *C. inconstans* and *P. hellenicus* could be the result of parallel evolution caused by similar impacts of environmental factors. For this reason, I do not propose to formally make a change in generic placement of *C. inconstans* at this time. Using the constraint tree option and only the osteological characters in the analysis gives a trichotomy for *Gasterosteus*, *Culaea*, and *Pungitius* (figure 7.1) and adding the behavioral characters resolves the trichotomy. Nevertheless, there is variation in behavioral characters. For example, *P. p. sinensis* in Japan and *P. p. pungitius* in Lake Huron do not build a nursery but straying young are returned to the nest (Kobayashi, 1933; McKenzie and Keenlyside, 1970). Males of *P. p. pungitius* in Hokkaido neither make a nursery nor retrieve the young (Goto et al., 1979). In Korea male *P. p. sinensis* makes two nurseries (Chae and Yang, 1993). Male *P. p. sinensis (kaibarae*) in Japan, builds the nest away from the stems of aquatic plants near the surface of the water and the nest has two openings, one in front and one in the back (Kobayashi, 1933). Males of *P. p. pungitius* nest near roots of the aquatic rooted vegetation (Goto et al., 1979). Those of *P. p. sinensis* do not do the insertion gluing in Korea (Chae and Yang, 1993) while those of *P. p. pungitius* and *P. p. occidentalis* do both the superficial and insertion gluing. If McLennan (1993) had included all the variations in behavioral characters in her analysis, she might have obtained an unresolved trichotomy for the aforementioned genera.

Recently, molecular characters (isozyme and allozyme variations) have been widely used to resolve the relationships in the sticklebacks (Niwa, 1987; Takata et al., 1987; Yang and Min, 1990; Haglund et al., 1992), but all of them suffer from disregarding the variation in different populations and most of them are based on few specimens from few populations.

Conclusions

Considering the enormous variation in the morphometric and meristic characters and the impact of ecological factors, using merely meristics, morphometrics or body shape in recognizing new species is invalid for sticklebacks. An integrated, comprehensive study including behavioral, osteological, karyological, electrophoretical and specifically ontogenetical traits of all the species of Gasterosteidae may resolve the taxonomy of this group. This study convinced me that not more than three species can be recognized in *Pungitius*, namely *P. hellenicus, P. platygaster* and *P. pungitius*. Based on the classical morphological rule, *P. hellenicus*, regardless of its phylogenetic relationships with other species, deserves to be recognized as a valid species, because 100 % of its specimens can be recognized from other species of *Pungitius*.

The phylogenetic relationships of *Pungitius*, *Culaea*, and *Gasterosteus* remain uncertain, but this study suggests that *Culaea inconstans* be regarded as a member of the genus *Pungitius* (figure 7.3). A formal proposal for such a change in generic placement is not made here pending further study. Within *Pungitius,* without information on the behavioral and ecological aspects of the species and specifically of *P. hellenicus*, it is difficult to reconstruct a good phylogenetic tree for them, but it seems that *P. hellenicus* is the most primitive species and *P. p. sinensis* and *P. p. occidentalis* are the most advanced taxa. However, *P. hellenicus* may be a neotenic form of *P. platygaster*.

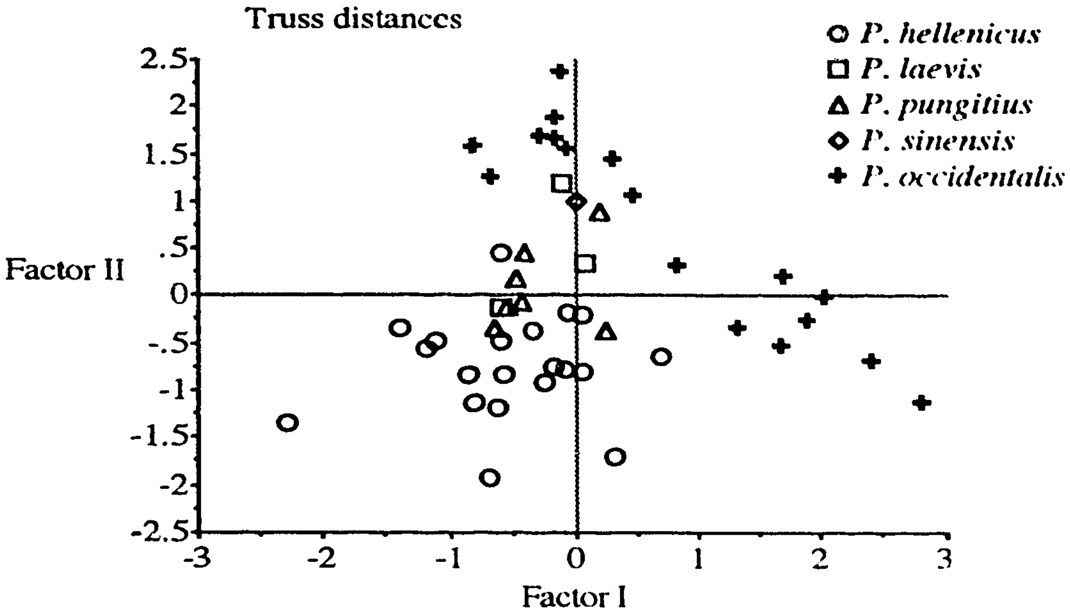


Figure 8.1. Factor I plotted against factor II for specimens smaller than 27 mm in standard length.

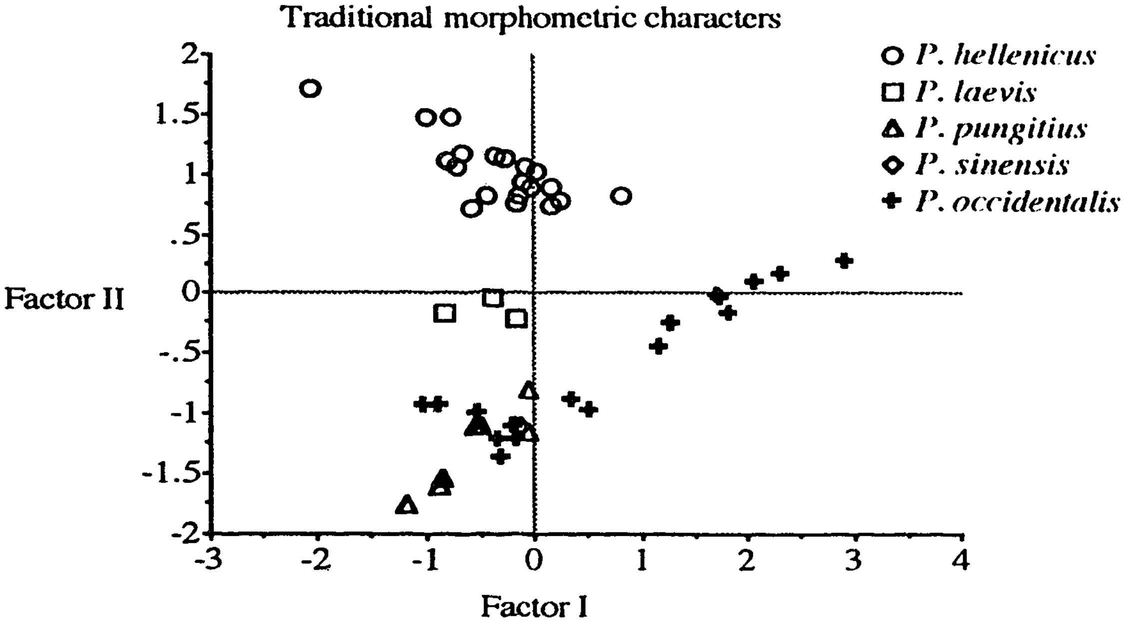


Figure 8.2. Factor I plotted against factor II for specimens smaller than 27 mm in standard length.

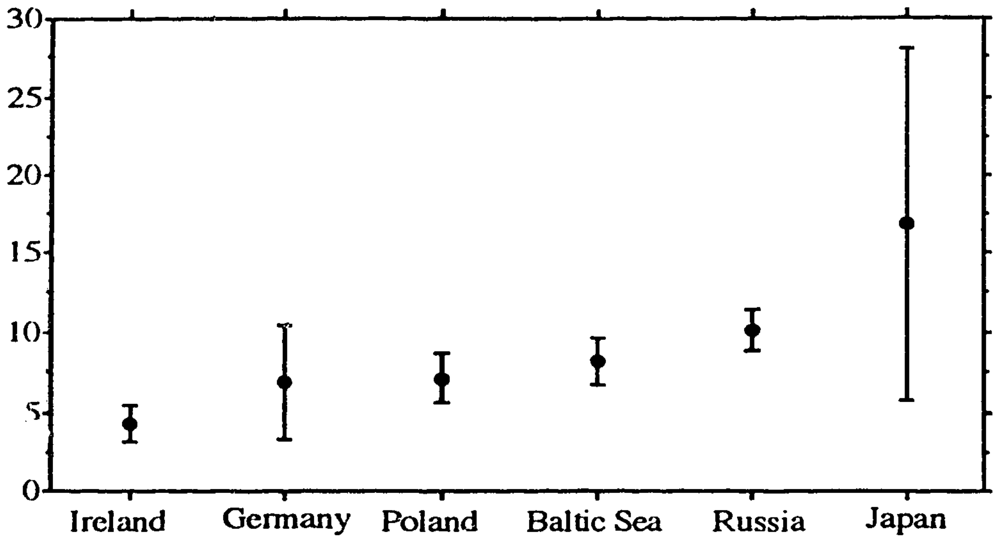


Figure 8.3. Geographic distribution of the number of scutes in *P. pungitius*.

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