TAXONOMIC REVIEW OF THE GENUS *PUNGITIUS*, NINESPINE STICKLEBACKS (GASTEROSTEIDAE)

by

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ABSTRACT. - The number of valid species in the genus *Pungitius* is uncertain due to the wide geographic variation in morphology and plasticity of many characters. Currently, two to eight species are recognized. Keivany *et al*. (1997) confirmed the validity of *P. hellenicus* and recognized three valid species in *Pungitius*, namely, *P. hellenicus*, *P. platygaster*, and *P. pungitius*. In order to assess the taxonomic status of various nominal taxa, we examined 266 specimens of the five nominal species often referred to as *P. pungitius* complex; *P. laevis*, *P. pungitius*, *P. tymensis*, *P. sinensis*, and *P. occidentalis*. Osteology and some meristic features of the nominal taxa were studied and morphometric characters are discussed. We find no evidence to justify recognizing four of the nominal species as valid, however, we recognize five valid subspecies in *P. pungitius*; *P. p. laevis*, *P. p. pungitius*, *P. p. tymensis*, *P. p. sinensis*, and *P. p. occidentalis*. The three species and five subspecies are described, a key to the species and subspecies is provided, and the taxonomy of the taxa is discussed.

RÉSUMÉ. - Révision taxinomique du genre *Pungitius*, épinochettes (Gasterosteidae).

Le nombre d'espèces valides dans le genre *Pungitius* est incertain du fait de la grande variation géographique de leur morphologie et de la plasticité de beaucoup de caractères. Actuellement, deux à huit espèces sont reconnues. Keivany *et al*. (1997) ont confirmé la validité de *P. hellenicus* et ont identifié trois espèces valides de *Pungitius*, à savoir, *P. hellenicus*, *P. platygaster* et *P. pungitius*. Afin d'évaluer le statut taxinomique de diverses espèces nominales, nous avons examiné 266 spécimens des cinq espèces nominales souvent désignées sous le nom de complexe *P. pungitius: P. laevis*, *P. pungitius*, *P. tymensis*, *P. sinensis*, et *P. occidentalis*. L'ostéologie et quelques caractères méristiques ont été étudiés; les caractères morphométriques sont discutés. Nous ne trouvons aucune preuve pour justifier l'identification de quatre des espèces nominales comme valide. Cependant, nous identifions cinq sous-espèces valides dans le complexe *P. pungitius: P. p. laevis*, *P. p. pungitius*, *P. p. tymensis*, *P. p. sinensis*, et *P. p. occidentalis*. Les trois espèces et les cinq sous-espèces sont décrites, une clé d'identification d'espèces et de sous-espèces est fournie, et leur taxinomie est discutée.

Key words. - Gasterosteidae - *Pungitius* - Sticklebacks - Osteology - Taxonomy.

The family Gasterosteidae of the suborder Gasterosteoidei, order Gasterosteiformes (Nelson, 1994) contains five genera, three of which, *Apeltes*, *Culaea* and *Spinachia*, are monotypic. However, the number of valid species in the two remaining circumpolar and highly variable genera, *Gasterosteus* and *Pungitius*, is uncertain.

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. Of the 32 nominal species (McAllister, 1987; Eschmeyer, 1998), recent workers recognize two to eight species as valid. For example, Haglund *et al*. (1992) who

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discussed the taxonomic history of *Pungitius*, suggested on the basis of allozyme variations, that the *P. pungitius* complex contains three clades: *P. pungitius* in Europe, *P. occidentalis* in North America, and *P. sinensis* in Asia. Berg (1949), based on the absence of the scutes, recognized *P. laevis* as a valid species, but Gross (1979) and Wootton (1984) recognized it as a subspecies. Takata *et al*. (1984), using morphometric and meristic characters, suggested that *P. tymensis* from Japan and Sakhalin Island is a valid species. The eight nominal species recognized in the recent literature are *P. hellenicus*, *P. platygaster*, *P. pungitius*, *P. laevis*, *P. sinensis*, *P. kaibarae*, *P. tymensis*, and *P. occidentalis*. Since *P. platygaster* is generally regarded as a valid species (Nelson, 1971a; Wootton, 1984; Haglund *et al*., 1992; Keivany, 1996) and validity of *P. hellenicus* was confirmed by Keivany *et al*. (1997), our objectives here are to examine the validity of the other nominal species and summarize the taxonomy of the genus on the basis of known information.

MATERIALS AND METHODS

Meristic characters were counted on 266 specimens of *Pungitius* from throughout much of the range of the genus, namely Canada, United States, Ireland, Germany, Poland, Baltic Sea, Russia and Japan. Osteology of the specimens was studied using Taylor and Van Dyke's (1985) method for clearing and staining bone and cartilage.

All specimens listed below are housed in the University of Alberta Museum of Zoology (UAMZ). Reference is made to the nominal species, our taxonomic conclusions are not anticipated.

P. laevis (Cuvier, 1829) ($n = 43$, $\overline{SL} = 33$ mm, $r = 24$-45, $SD = 5$): UAMZ 4759 (River Brosna, Ireland), UAMZ 6556, UAMZ 6557, UAMZ 6558, UAMZ 6559, UAMZ 6560, UAMZ 6561, UAMZ 6562 (River Suck, Ireland).

P. pungitius (Linnaeus, 1758) ($n = 109$, $\overline{SL} = 39$ mm, $r = 22$-57, $SD = 7$): UAMZ 4739 (Baltic Sea), UAMZ 4740 (Bzuva River, Poland), UAMZ 4741 (Sylt, Frisian Islands, Germany), UAMZ 4742 (Hunna Brook, Germany), UAMZ 4743 (Neva River, Russia), UAMZ 4746 (Aomori Prefecture, Japan), UAMZ 4748 (Toro Lake, Hokkaido, Japan), UAMZ 5460 (Konuma Lake, Hokkaido, Japan), UAMZ 5462 (Hakodate, Hokkaido, Japan), UAMZ 5463 (Shinjo, Yamagata, Japan), UAMZ 5465 (Tomakomai, Hokkaido, Japan), UAMZ 5461 (Tendo, Yamagata, Japan), UAMZ 5464 (Tsutanuma Pond, Aomori, Japan).

P. tymensis (Nikolskii, 1889) ($n = 10$, $\overline{SL} = 54$ mm, $r = 42$-62, $SD = 7$): UAMZ 4744, UAMZ 4745 (Sea of Okhotsk, Sakhalin Island, Russia).

P. sinensis (Guichenot, 1869) ($n = 20$, $\overline{SL} = 38$ mm, $r = 24$-62, $SD = 9$): UAMZ 4749, UAMZ 5466 (Tedori Fan, Ishikawa, Japan).

Table 1. Mean number, range, and standard deviation of some meristic characters in *Pungitius* spp.

	Dorsal spines			Pelvic spines			Pelvic rays			Total scutes			
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	n
P. laevis	9.1	5-10	0.9	0.8	0-2	1.0	0.8	0-2	1.0	4.3	2-8	1.1	43
P. pungitius	9.2	8-11	0.7	2.0	2-2	0.0	2.4	1-4	0.8	10.6	3-33	7.1	109
P. tymensis	11.8	11-13	0.6	2.0	2-2	0.0	2.0	2-2	0.0	10.5	8-12	1.3	10
P. sinensis	9.1	8-10	0.5	2.0	2-2	0.0	3.9	3-5	0.4	33.0	31-34	0.8	20
P. occidentalis	9.2	8-11	0.7	2.0	0-2	0.3	1.7	0-4	0.8	9.7	6-34	3.1	84

P. occidentalis (Cuvier, 1829) ($n = 34$, $\overline{SL} = 36$ mm, $r = 12\text{-}59$, $SD = 10$): UAMZ 3218 (Pine Lake, Wood Bufflo National Park, Alberta), UAMZ 3319 (Firth River, Yukon, Canada), UAMZ 5439 (Cold Lake, Alberta, Canada), UAMZ 4755 (Crooked Lake, Indiana, USA).

RESULTS

There is overlap in most of the meristic characters of the nominal species. Statistics of four meristic characters that show less overlap than other characters examined is presented in table 1. *Pungitius tymensis* has the highest number of the dorsal spines with a mean of 11.8 and a range of 11-13. *Pungitius sinensis* has the highest mean number of pelvic soft rays (3.9 of the total for both sides) 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 from Japan have 3 or 4 pelvic soft rays (mean 2.4). *Pungitius tymensis* usually has 2 pelvic soft rays, one associated with each of the pelvic spines. Some specimens of *P. laevis* and *P. occidentalis* with a reduced pelvic girdle have one spine and one or no soft ray. The total number of scutes is highly variable in *P. pungitius* and *P. occidentalis*, but relatively constant in the other nominal taxa. The lowest mean number is in *P. laevis* (4.3) and the highest mean number is in *P. sinensis* (33). The large lateral scutes (relatively large and vertically ovoid shape scutes on the midlateral sides of the body) are present in *P. sinensis* but absent in other nominal species.

There are relatively few osteological differences among the nominal species of *Pungitius* (Keivany and Nelson, 1998). The basals of the dorsal spines are reduced in *P. laevis* (Fig. 1). They are usually roundish, but in some specimens are ovoid; they do not overlap each other. In the other taxa they are somewhat rectangular and overlap each other. In some specimens of *P. occidentalis* from Crooked Lake, Indiana, unlike from other areas, the dorsal basals are reduced and ovoid in shape (Fig. 1E).

In some specimens of *P. laevis* the anal spine is short and when depressed it does not reach the base of the second anal soft ray. In other taxa, the anal spine is relatively long and always passes the second soft ray. Haemal and neural spines of preurals 4 and 5 in *P. occidentalis* and *P. sinensis* are very short and almost horizontal (Fig. 2). Very small specimens (smaller than 15 mm) of a population of *P. occidentalis* from the Firth River, Yukon, have oblique and long haemal and neural spines on preurals 4 and 5.

Pungitius laevis has only a few keel scutes forming a thin keel on the caudal peduncle that cannot be seen in unstained specimens (Fig. 3), resulting in some authors describing *P. laevis* as a keelless species. A variety of patterns can be seen in *P. pungitius*, but all specimens bear a keel on the caudal region. *Pungitius tymensis* has a caudal peduncle keel and usually a few small scutes behind the head. Most *P. sinensis* specimens have a full row of scutes forming a keel on the caudal peduncle and it is the only form among the five with large lateral scutes on the anterior part of the body. *Pungitius occidentalis* shows the same pattern as *P. pungitius*.

In some specimens of *P. laevis* the ectocoracoid is reduced (Fig. 4), but not as much as in *P. hellenicus*. In other species it meets the cleithral joint and is heavily sculptured. Specimens of *P. laevis* from Ireland and *P. occidentalis* from Wood Buffalo National Park may have a complete or a reduced pelvic girdle or may lack the entire pelvic girdle. *Pungitius tymensis* usually has a reduced pelvic girdle. In species with a fully developed pelvic girdle, the pelvic bones are overlapped by the ectocoracoids. We correct an error in Keivany *et al.* (1997, fig. 4) in referring to a postcleithrum; this bone is absent in sticklebacks.

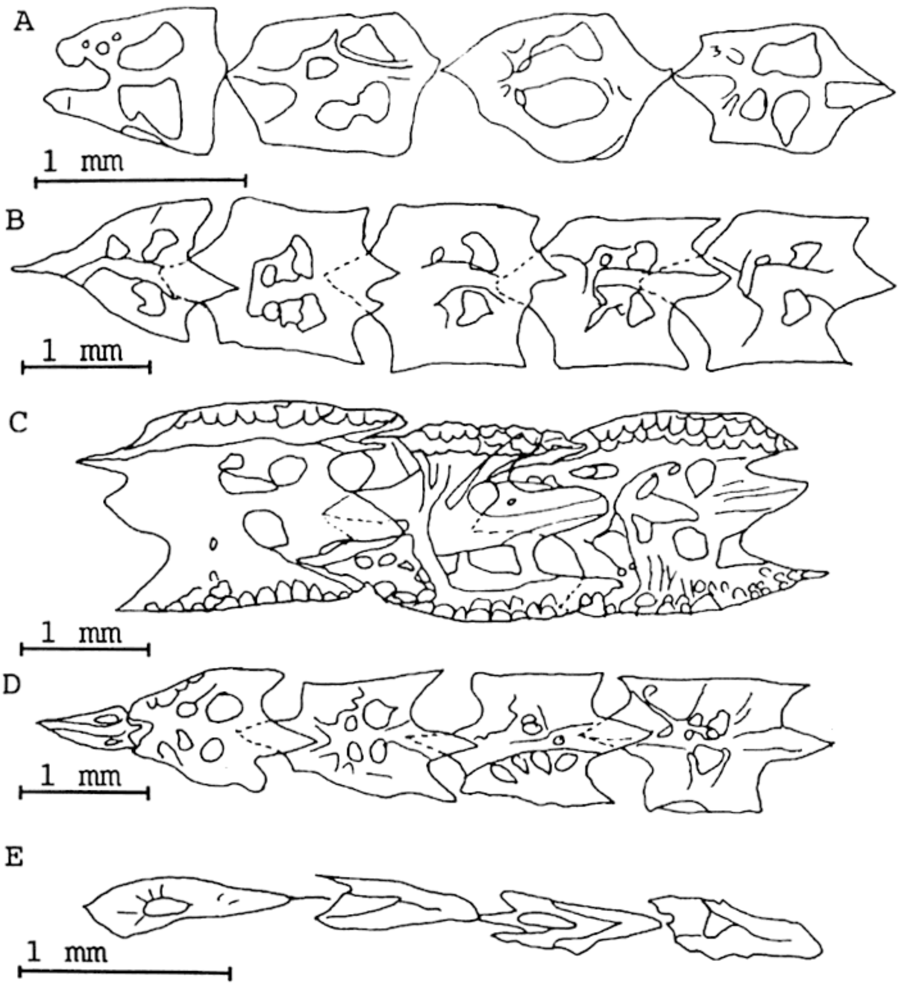

Fig. 1. Lateral view of basals of the dorsal spines in *Pungitius* spp. **A**: *P. laevis*; **B**: *P. tymensis*; **C**: *P. sinensis*; **D**: *P. occidentalis*; **E**: *P. occidentalis* from Crooked Lake.

DISCUSSION

Morphometrics and meristics

Morphometric, meristic, and molecular characters have been used by past authors to establish nominal species of *Pungitius*; however, many did not consider geographic variation in their studies. For example, Kim *et al.* (1989) established the species *P. kaibarae* based on differences with *P. sinensis* in mean number of the dorsal fin spines and soft rays and vertebrae. Haglund *et al.* (1992) used samples from only nine localities; one from Canada, two from England, one from Sweden, one from Korea, and four from Japan to recognize *P. occidentalis* and *P. sinensis* as separate species from *P. pungitius*. *Pungitius*

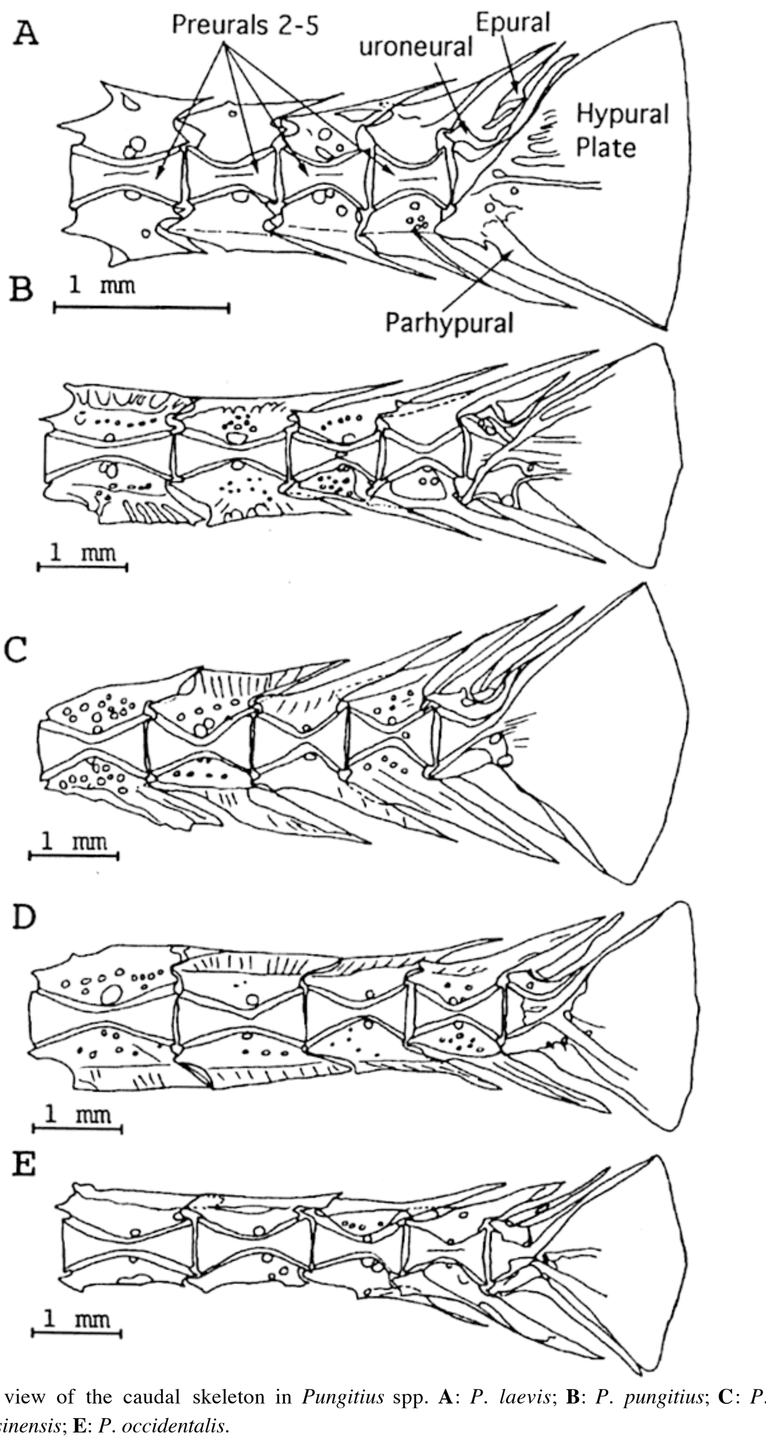

Fig. [illegible]. Lateral view of the caudal skeleton in *Pungitius* spp. **A**: *P. laevis*; **B**: *P. pungitius*; **C**: *P. tymensis*; **D**: *P. sinensis*; **E**: *P. occidentalis*.

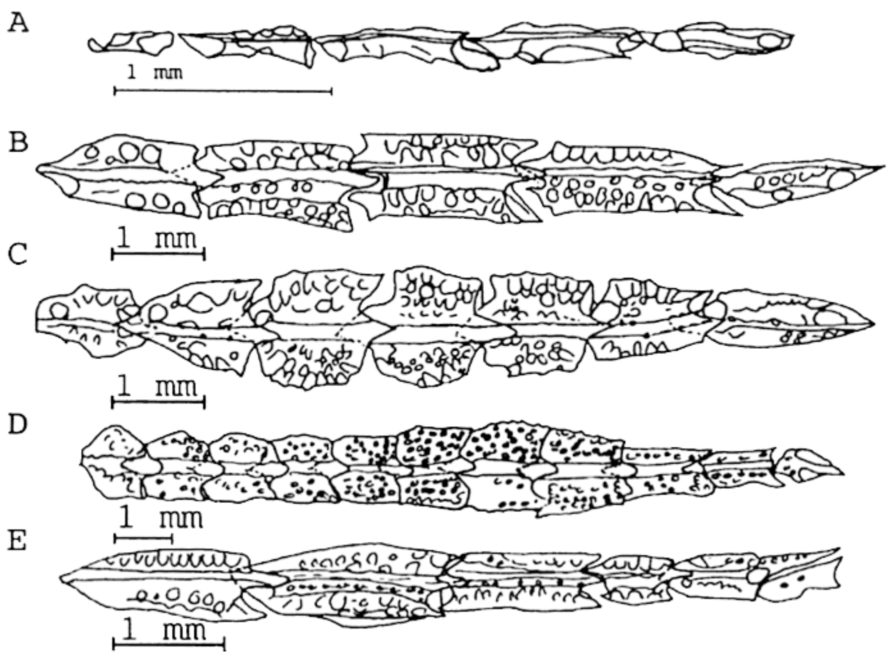

Fig. 3. Lateral view of the keel scutes in *Pungitius* spp. **A**: *P. laevis*; **B**: *P. pungitius*; **C**: *P. tymensis*; **D**: *P. sinensis*; **E**: *P. occidentalis*.

tymensis, *P. laevis*, *P. platygaster*, and *P. hellenicus* were not included in their analysis for comparison of the allozyme variation.

Morphometric and most meristic characters are influenced by environmental conditions (Lindsey, 1962) and may exhibit geographic variation that may or may not be genetically based (McPhail, 1963; Gross, 1979; Tanaka, 1982; Takata *et al.*, 1984, 1987). It is thus difficult to know, with available studies, the taxonomic importance of observed differences. In addition, Baumgartner (1992) suggested that in threespine sticklebacks many body-shape differences among localities, such as spine and fin length and body depth, are accounted for by size-related allometric variation. The same phenomena may be responsible for some of the variations in the nominal species of *Pungitius*. Therefore, because of allometry, ratios must be used with extreme caution in comparative studies. Based on studies of Wimberger (1993) and Day *et al.* (1994), different diets may also cause non-genetic variation in morphometric characters, thereby making their use in taxonomic studies suspect. If we can interpolate again from threespine sticklebacks, morphological similarities in such features as lateral scute number, dorsal and anal fin ray number, body-shape, and pectoral fin length may under certain situations be in response to similar selective regimes (Taylor and McPhail, 1986; Baumgartner, 1992; Walker, 1997) and not necessarily warrant taxonomic recognition. In any event, morphometric and most of the meristic characters overlap in *Pungitius* and cannot be used to differentiate the species (Keivany *et al.*, 1997).

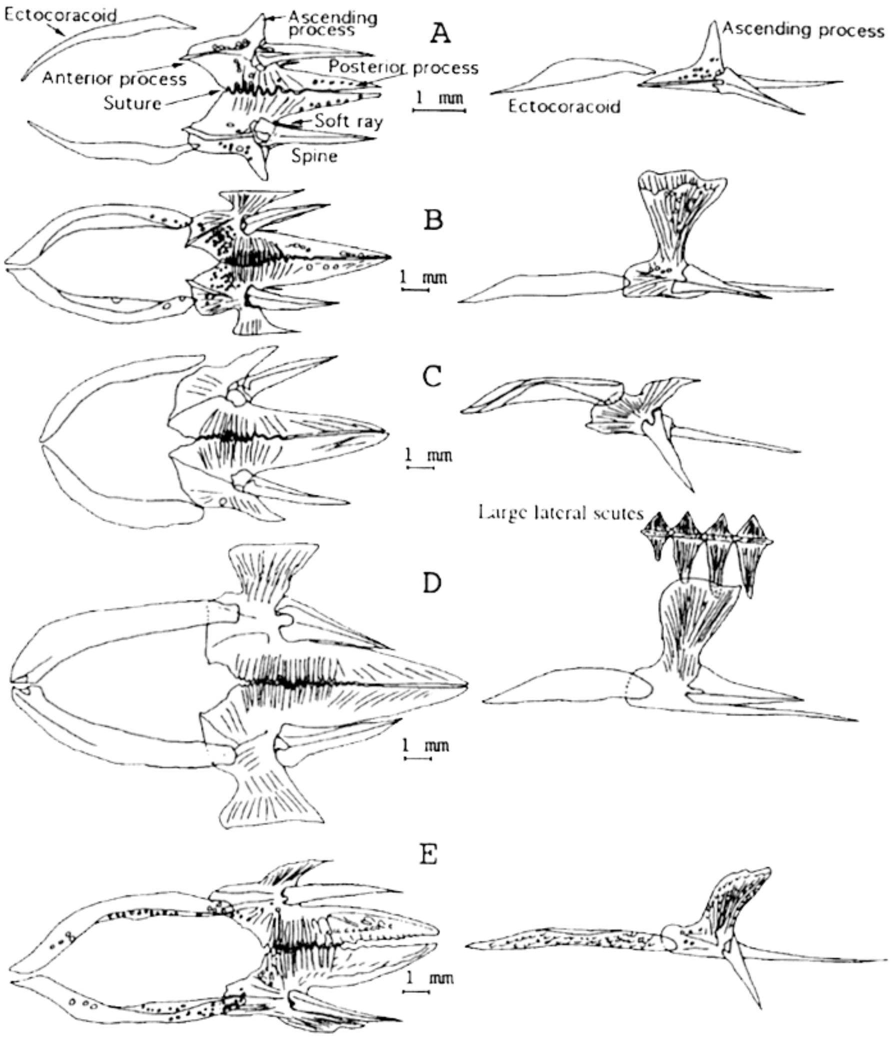

Fig. 4. Lateral (right) and ventral (left) views of the pelvic girdle and ectocoracoids in *Pungitius* spp. **A**: *P. laevis*; **B**: *P. pungitius*; **C**: *P. tymensis*; **D**: *P. sinensis*; **E**: *P. occidentalis*.

Osteology

Although there are some diagnostic osteological characters for *Pungitius* spp., many of the bones show interspecific and intraspecific variation. For example, Sroivattana (1972) described the 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 anterolateral foramina on the dentary as a synapomorphy for *Pungitius*, but it occurs in many specimens, although it is small in some. Symplectic dorsal flanges vary in size and shape in specimens of the same species. The nasal process is also variable in shape. Although some specimens of

Gasterosteus aculeatus and species of *Pungitius* have a posterodorsal extension on the third infraorbital, its size is very variable.

The number, size, and arrangement of the dorsal spines show much variation within the nominal species of *Pungitius*. Shadrin (1994) studied the ontogeny of *P. pungitius* in Russia, and observed that dorsal fin formation starts in the midpoint and spine formation proceeds craniad and soft ray proceeds caudad. In a developing fish, the posterior spine is the longest and the first spine is the shortest. The low number of dorsal spines in *P. hellenicus* and their small size and arrangement may be the result of neoteny. In a *P. occidentalis* population from Firth River, 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 centrum and are not fused to the basals. They do not bear a caudal keel and the caudal peduncle is deeper than wide and the epural plate is relatively large. 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 supports the possibility of neoteny in *P. hellenicus*.

Reduction or loss of the pelvic girdle, a derived condition in many actinopterygians (Nelson, 1993), occurs in some populations of ninespine sticklebacks, brook stickleback, and threespine stickleback. Various studies have suggested an advantage to pelvic loss in sticklebacks related to predator avoidance or escape (e.g., Nelson, 1969; Reimchen, 1980, 1983; Reist, 1980) or to reduced calcium demand (Giles, 1983; Bell and Orti, 1994); there is some evidence for the absence having a genetic basis (Nelson, 1977; Bell, 1987; Blouw and Boyd, 1992). *Pungitius occidentalis* from Crooked lake is less ossified and this might be in part the result of calcium deficiency; however, the absence of the pelvic skeleton in some specimens of *P. occidentalis* from Pine lake, Wood Buffalo National Park, is unlikely to be related to calcium deficiency because normal specimens are found in the same population. *Pungitius hellenicus* shows both weak ossification and pelvic girdle loss, but we think that this is not related to calcium deficiency because a sympatric population of *Gasterosteus aculeatus* shows the normal condition. Liu and Wang (1974) reported a ninespine stickleback fossil fish from lacustrine formations of late Pliocene from northern 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*. In considering variation in *Pungitius* spp., we believe that *P. nihowanensis* is a junior synonym of *P. tymensis* based on its reduced pelvic spines, overall shape of the pelvic bones and shape of the scutes. Pelvic reduction appears to have a genetic basis and may occur independently in response to local selective pressures. As concluded by Nelson (1971b) in noting ninespine sticklebacks in some localities in Ireland and Canada to lack a complete pelvic skeleton, such absence by itself does not seem to warrant taxonomic recognition.

Shape and position of scutes are another feature that exhibits much variation in sticklebacks. Igarashi (1962, 1963) observed similar scute ontogeny in *P. pungitius* and *P. sinensis*, but different scute ontogeny in *P. kaibarae* (Igarashi, 1969) and *P. tymensis* (Igarashi, 1970). Based on these studies, he concluded that *P. pungitius* and *P. sinensis* are close to each other, but that *P. kaibarae* and *P. tymensis* should be recognized as valid species. Ayvazian and Krueger (1992) found the same development in the ontogeny of scutes in *P. occidentalis* as in *P. pungitius* in Japan and concluded that the partial phenotype of *P. pungitius* evolved from the complete phenotype through neoteny and postdisplacement and that the caudal phenotype was derived by progenesis and postdisplacement. The large lateral scutes on the threespine stickleback provide a structural base for the spine supports and a greater surface area over which the forces on the spines are distributed, and the principal function of the large lateral scutes appears to stabilize the long erected spines during manipulation by predators (Reimchen, 1983). However, in *P. platygaster* and *P. sinensis* with short dorsal spines, the function of the large lateral scutes is uncertain and the loss in other species might be a response to a loss of function.

Taxonomy

One of the problems in studying this group is the large amount of variation in many characters and uncertainty in distinguishing between genetic and non-genetic characters. Münzing (1966, 1969) linked divergence in *Pungitius* to Pleistocene glacial events. He hypothesized the existence of three refugia in Eurasia: southern France, Ponto-Caspian region and eastern Asia and felt that all refugia were originally populated by a morph resembling *P. sinensis*. He suggested that divergence occurred independently in these three refugia resulting in the four taxa recognized respectively as *P. laevis*, *P. platygaster*, *P. pungitius*, and *P. tymensis*. Gross (1979) suggested that poorly armoured populations may arise under relaxed predation, irrespective of their postglacial origin. He concluded that variation in *Pungitius* may be the result of recent or long-standing environmental selection, rather than a relict of historical events. He questioned the taxonomic status of *P. laevis* because the caudal keel in *P. laevis* is at one end of an apparent east-west cline in scute number. Our data is consistent with his analysis. However, there are differences in the caudal region and shape of the dorsal basals and ectocoracoid that suggest a genetic divergence in this form, and we recognize it as a valid taxon at subspecies level.

Ziuganov and Gomeluk (1985) believed that the differences between *P. platygaster* and *P. pungitius* are adaptations to a greater number of predators on *P. platygaster* at low latitudes and that the difference in sexual behavior between them are clearly insufficient for ethological reproductive isolation. Although we believe that the biological species concept cannot be applied to any of the forms of *Pungitius*, we recognize *P. platygaster* as a separate species because all specimens can be distinguished from other species. Based on differences in the serration on the pelvic spines, Berg (1949) and Wootton (1984) recognized two subspecies in *P. platygaster*: *P. platygaster platygaster* in the Black Sea, Sea of Azov and Caspian Sea basins, and *P. platygaster aralensis* in the Aral Sea basin. However, our specimens from the Sea of Azov show weak serration similar to that described for the latter subspecies. Therefore, we do not recognize subspecies in *P. platygaster*. Also, 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 is based on meristic, morphometric, and isozyme characters that exhibit much variation and are not considered to be taxonomically significant in sticklebacks.

Decisions on the validity of taxa of *Pungitius* are difficult due to the variation in meristics and morphometric characters and the influence of environmental factors; however, some of the meristic and osteological characters are taxonomically useful. Although molecular studies will continue to provide valuable evidence, either supporting the recognition of various taxa (e.g., Haglund *et al*., 1992) or not (e.g., Niwa, 1987), we believe that taxonomic changes should be made, as is true in morphological studies, only after a good understanding of geographic variation. In the absence of sympatric taxa and our resulting reliance of analyzing differences between allopatric populations without the benefit of a detailed cladistic study, we believe that species should be recognized only if demonstrated to be separated from other such species by the 100% guideline of Bailey *et al*. (1954). We suggest that subspecies should be recognized for geographically distinct units that can be separated in approximately 90% of the specimens. Based on these criteria, we believe that the species recognition of *P. tymensis*, *P. sinensis*, *P. kaibarae*, *P. laevis*, and *P. occidentalis* is doubtful and only three species should be recognized in *Pungitius*, namely *P. hellenicus*, *P. platygaster* and *P. pungitius*. However, the divergence and percentage of specimen identification in *P. laevis*, *P. tymensis*, *P. sinensis*, and *P. occidentalis* is high enough to grant them a subspecific recognition. The shape and development of dorsal spines, pectoral and pelvic girdles, caudal skeleton and scutes in *P. hellenicus* compared to those of juvenile specimens of *P. pungitius* and *P. platygaster*, and its geographic distribution, suggests that *P. hellenicus* is a neotenic form of *P. platygaster*.

DESCRIPTION AND CLASSIFICATION

1. *Pungitius hellenicus* Stephanidis, 1971

Synonym.—*Pungitius pungitius hellenicus* Stephanidis, 1971.

Common names.—Greek ninespine stickleback; ellinopygosteos.

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 colour pale olive, sides of the body pigmented with dark bars or blotches. Body moderately compressed. Head conical and interorbital area flattened. Bones weakly ossified and sculpturing poorly developed on the cranial bones. Mouth slightly supraterminal, oblique, and a continuous groove separates upper lip from the maxillary. Numerous small sharp teeth confined to the upper and lower jaws, absent on roof of the mouth. Gill membranes extending forward, broadly joined to each other, and posteriorly free from isthmus. Gill rakers 7-10. Opercular opening extends above pectoral fin base. Pectoral fin base is vertical and consists of 10 (rarely 11) soft rays. Dorsal fin spines 2-6, isolated (with small triangular fin membrane), directed posteriorly and not inclined from the middorsal line and depressible in a shallow groove. Dorsal spine length increases progressively from first to last. Dorsal fin soft rays 8-11. Anal fin with one spine and 6-10 soft rays. Lateral line inconspicuous with 28-38 (usually 32) small round scutes. Number of vertebrae 29-30 (usually 30) with 12-13 precaudal vertebrae. Caudal fin with 12 soft rays, rounded, and deeper than wide. The longest specimens reported by Stephanidis (1971) were 50 mm.

Distribution.—Confined to three localities in the Sperchios River Basin in central Greece: the Aghia Paraskevi Spring and associated channels extending over the areas Diplosoudi and Bourdara, a large system of connected drainage and irrigation channels and natural wells extending over the areas Lycochoria and Kaikia near the village of Moschohori, and a small number of natural wells near the village of Kompotades (Keivany *et al*., 1999).

2. *Pungitius platygaster* (Kessler, 1859)

Synonyms.—*Gasterosteus platygaster* Kessler, 1859; *Gasterosteus pungitius var. kessleri* Yakovlev, 1870; *Gasterosteus pungitius var. niger* Yakovlev, 1870; *Gasterosteus platygaster var. caucasicus* Kessler, 1877; *Gasterosteus platygaster var.*

aralensis Kessler, 1877; *Gasterosteus platygaster var. danubica* Steindachner, 1899; *Pygosteus platygaster var. nuda* Berg, 1905; *Pygosteus nudus* Berg, 1916.

Common names.—Caspian ninespine stickleback; southern ninespine stickleback; Aral ninespine stickleback.

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* (juvenile specimens from Iran show the same color as *P. hellenicus*). All the bones well ossified, cranial bones and pelvic bones highly sculptured. Pelvic girdle present with one spine and one small soft ray on each side. Dorsal spines 8-11, inclined alternatively 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; bony scutes 29-32 with 7-12 large lateral scutes; total vertebrae 29-31; precaudal vertebrae usually 13. Caudal fin truncated.

Distribution.—Black Sea, Sea of Azov, Aral Sea and Caspian Sea basins. It is distributed in the Caspian Sea basin, but is rarely reported from the southern coast. Armantrout (1980) reported this species from a single specimen caught in Bandar Anzali, Iran. However, recently, it has been seen frequently in disjunct lakes and ponds in northern Iran (e.g., samples from Gomeishan wetland, UAMZ 8059, and observations of A. Abdoli and B.H. Kiabi, pers. comm., 1996).

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

Synonyms.—See subspecies.

Common name.—Ninespine stickleback.

Diagnosis.—Presence of a caudal peduncle keel.

Description.—Generally similar to *P. hellenicus* with the following differences. Body quite variable in colour, but generally darker than *P. hellenicus*. Bones well ossified with relatively strong sculpturing on the cranial and pelvic bones. Pelvic spines usually two, each associated with one pelvic soft ray, and some specimens completely lack the pelvic girdle. Dorsal spines 5-13; dorsal soft rays 8-12; anal soft rays 7-13; gill rakers 8-14; scutes 2-34; total vertebrae 30-35 with 12-16 precaudal vertebrae. Caudal fin polymorphic, in some rounded and in others truncated.

Distribution.—Circumpolar; see subspecies.

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

Synonyms.—*Gasterosteus pungitius* Linnaeus, 1758; *Pygosteus pungitius* Gill, 1861; *Gasterosteus pungitia* Sauvage, 1874 (Fossil).

Common names.—Ninespine stickleback; Eurasian ninespine stickleback.

Diagnosis.—Lack of large lateral scutes, long and oblique haemal and neural spines on preural 4.

Description.—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 side, and some specimens totally lack the pelvic girdle (Zyuganov, 1989). Dorsal spines 7-11; dorsal soft rays 9-12; anal soft rays 7-11; gill rakers 9-13; scutes 3-33; total vertebrae 30-34 (usually 32 or 33) with 12 -16 precaudal vertebrae (usually 14 or 15).

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 vulgaris* Mauduyt, 1849-51; *Gasterosteus lotharingus* Blanchard, 1866; *Gasterosteus burgundianus* Blanchard, 1866; *Gasterosteus breviceps* Blanchard, 1866.

Common names.—Irish ninespine stickleback; western ninespine stickleback.

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

Description.—Some specimens (or populations) lack the pelvic girdle and have a reduced ectocoracoid. Dorsal spines 8-10, sometimes fewer than 8 and rarely one, two or three (Ure, 1962); dorsal soft rays 9-12; anal soft rays 8-10; gill rakers 9-12; scutes on the caudal peduncle 2-8, probably no caudal peduncle scutes in some specimens; no lateral scutes; total vertebrae 31-34 with 14 or 15 precaudal vertebrae. Caudal fin rounded.

Distribution.—Ireland, southern England, and southern France.

3.3. *Pungitius pungitius occidentalis* (Cuvier, 1829)

Synonyms.—*Gasterosteus occidentalis* Cuvier, 1829; *Gasterosteus concinnus* Richardson, 1836; *Gasterosteus mainensis* Storer, 1837; *Gasterosteus dekayi* Agassiz, 1850; *Gasterosteus nebulosus* Agassiz, 1850; *Gasterosteus blanchardi* Sauvage, 1874; *Gasterosteus globiceps* Sauvage, 1874; *Gasterosteus brachypoda* Bean, 1879.

Note.—The descriptions of *Gasterosteus occidentalis* by Linnaeus in 1758 and Gmelin in 1789 do not match with this subspecies and may refer to a perciform fish, so we recognize Cuvier as the author of this subspecies, following Haglund *et al.* (1992).

Common name.—North American ninespine stickleback.

Diagnosis.—Short and horizontal haemal and neural spines on the preural 4. Caudal fin usually truncated.

Description.—Colour variable, usually silvery on the ventral side. Some specimens from Pine Lake, Wood Buffalo National Park, lack the pelvic girdle or have a reduced one. Dorsal spines 8-11; dorsal soft rays 8-12; anal soft rays 7-13; gill rakers 8-16; scutes 6-17; no large lateral scutes; total vertebrae 32-35 with 13-16 (usually 14 or 15) precaudal vertebrae.

Distribution.—North America, along the northern coastline from Cook Inlet, east of Aleutian Islands, Alaska (Morrow, 1980) 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).

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

Synonyms.—*Gasterosteus sinensis* Guichenot, 1869; *Pygosteus stenurus* Kessler, 1876; *Gasterosteus wosnesenjenskyi* Kessler, 1876; *Gasterosteus bussei* Warpakchow, 1887; *Pygosteus steindachneri* Jordan & Snyder, 1901 (replacement for *Gasterosteus japonicus* Steindachner, 1881); *Pungitius brevispinosus* Otaki, 1908; *Pygosteus kaibarae* Tanaka, 1915.

Common name.—Chinese ninespine stickleback.

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

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

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

3.5. *Pungitius pungitius tymensis* (Nikolskii, 1889)

Synonyms.—*Gasterosteus tymensis* Nikolskii, 1889; *Pygosteus undecimalis* Jordan & Starks, 1902; *Pungitius nihowanensis* Liu & Wang, 1974 (Fossil).

Common name.—Sakhalin ninespine stickleback.

Diagnosis.—Usually 11 or more dorsal spines.

Description.—Pelvic girdle reduced, some lack the pelvic spines and soft rays. Dorsal spines 8-13 (usually 11); dorsal soft rays 10-11; anal soft rays 8-12; gill rakers 8-11; scutes on the caudal peduncle 5-8; no large lateral scutes; a few scutes behind the head; total vertebrae 33-35 with 14-16 precaudal vertebrae. Caudal fin rounded.

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

A key to the species and subspecies

1 a		Caudal peduncle keel present (*P. pungitius*)	**2**
1 b		Caudal peduncle keel absent	**6**
2 a	(1a)	A full row of scutes with large lateral scutes present. Usually two soft rays with each pelvic spine. Japan, Korea	*P. p. sinensis*
2 b	(1a)	Incomplete row of scutes, no large lateral scutes. Usually one soft ray with each pelvic spine	**3**
3 a	(2b)	Dorsal spines usually 11 or more. Sakhalin Island, Japan	*P. p. tymensis*
3 b	(2b)	Dorsal spines fewer than 11	**4**
4 a	(3b)	Caudal peduncle deeper than wide. Reduced basals. Caudal peduncle keel not apparent in unstained specimens. Southern Europe, Ireland	*P. p. laevis*
4 b	(3b)	Caudal peduncle wider than deep. Developed basals. Caudal peduncle keel apparent in unstained specimens	**5**
5 a	(4b)	Haemal and neural spines of preural 4 oblique and longer than the centrum. Eurasia	*P. p. pungitius*
5 b	(4b)	Haemal and neural spines of preural 4 horizontal and shorter than the centrum. North America	*P. p. occidentalis*
6 a	(1b)	Pelvic girdle and large lateral scutes present. Dorsal spines seven or more. Aral Sea, Sea of Azov, Black Sea and Caspian Sea basins	*P. platygaster*
6 b	(1b)	Pelvic girdle absent or very reduced, large lateral scutes absent. Dorsal spines fewer than seven. Greece	*P. hellenicus*

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