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Meristics of the Etruscan goby *Padogobius nigricans*

Caracteres merísticos del góbido etrusco *Padogobius nigricans*

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ABSTRACT

The Etruscan goby *Padogobius nigricans* is a running water dwelling vulnerable species inhabiting the Tuscano-Latium district. Since many aspects of the biology of this species are still unknown, our research was aimed at describing its meristic features to observe how ontogeny and sexual dimorphism affect the studied traits, and thus provide useful characters for preservation purposes. Significant intra- and inter-population differences were detected for some meristic traits. In particular most of the studied parameters were affected by ontogeny but not sexual dimorphism except for the caudal fin, for which different values were registered for both sexes in two of the three studied populations. This suggests that the meristic pattern may change from one population to another. Considering the short geographic distance and the similar environmental conditions among the analysed locations, this differentiation was not expected. From the results of this research, there seems to be a considerable scope for further studies on *P. nigricans* due to the paucity of data regarding the morphology and meristics of this species.

Key words: Central Italy, etruscan goby, meristics, *Padogobius nigricans*, phenotype.

RESUMEN

El góbido etrusco *Padogobius nigricans* es una especie vulnerable de aguas corrientes del distrito Tuscano-Lacio. Dado que muchos aspectos de la biología de esta especie son todavía desconocidas, nuestra investigación tuvo como objetivo describir las características merísticas de los individuos de la misma, para observar cómo el dimorfismo sexual afecta la ontogenia de las características estudiadas, y con ello obtener caracteres útiles para fines de conservación. La existencia de diferencias significativas intra- e inter-poblacionales se detectaron para algunos caracteres merísticos. En particular, la mayoría de los parámetros estudiados fueron afectados por la ontogenia y no por el dimorfismo sexual, a excepción de la aleta caudal, para la cual se registraron valores diferentes para ambos sexos en dos de las tres poblaciones estudiadas. Este resultado sugiere que el patrón merístico puede cambiar de una población a otra. Teniendo en cuenta la corta distancia geográfica y las condiciones ambientales similares entre las localidades analizadas, esta diferenciación no se esperaba. A partir de los resultados de esta investigación, parece que hay un margen considerable para nuevos estudios sobre *P. nigricans*, debido a la escasez de datos sobre la morfología y la merística de esta especie.

Palabras clave: Fenotipo, góbido etrusco, Italia central, merística, *Podagobius nigricans.*

INTRODUCTION

Gobiidae is a perciform group represented by several thousand species (about 2500), which adaptative radiation allowed to colonize marine, brackish, and inland water habitats of tropical and temperate regions (Gandolfi *et al.,* 1991). They are characterized by small body size, pelvic fin fusion (forming a sucker like-structure), and acoustic communication (Lugli *et al.,* 1997). Among the forty-four western Mediterranean goby species (belonging to 18 genera), only 10 of them occur within Italian inland waters, and only the Orsini goby *Knipowitschia punctatissima* (Canestrini, 1864), the Martens goby *Padogobius bonelli* (Bonaparte, 1846), and the Etruscan goby *Padogobius nigricans* (Canestrini, 1867) show strictly freshwater habit (Gandolfi *et al.,* 1991), probably acquired after a long isolation during the Messinian salinity crisis from the euryhaline Ponto-Caspian group of species (Bianco, 1987).

Phylogenetic relationships among Italian gobies, including the three freshwater species, were controversial and represented a long-standing problem for this group. Given this conflicting background, the systematic position of *P. bonelli* and *P. nigricans* was investigated by Penzo *et al.* (1998) which provided an evolutionary scenario where the genus *Gobius* was seemingly paraphyletic. They clustered the two *Padogobius* species together with *Gobius paganellus*. These three species constitute a sister group to other *Gobius* species. Similar results have been described by Huyse *et al.* (2004). So far, since the still controversial systematic, in this study we adopted the classification of Miller (2004) ongoing to insert the Etruscan goby within the genus *Padogobius*.

The three strictly freshwater gobies show different habitat preferences: *Knipowitschia punctatissima* inhabits northeastern Italy spring waters with a sandy or muddy bottom covered by a rich vegetation (Gandolfi *et al.,* 1991); *Padogobius bonelli* which prefers stony substrates within the River Po basin (Gandolfi *et al.,* 1991), and *P. nigricans* which is endemic of the Tuscano-Latium ichthyogeographic district (Bianco, 1995) and inhabits brooks characterized by good water quality (Zerunian, 2002). Within its restricted geographical range, *P. nigricans* seems to display a certain degree of morphological (Gandolfi & Tongiorgi, 1974) and genetic (Cervelli *et al.,* 2007) variation, suggesting that environmental or stochastic events directly affected the life history traits of isolated populations (Zerunian & Gandolfi, 1986; Scalici & Gibertini, 2009).

Because of the transfaunation (Gherardi *et al.,* 2008) and river habitat deterioration, the Etruscan goby is becoming a more and more imperilled species, listed as vulnerable by the IUCN, in Appendix III of the Bern Convention, and in Annex II and V of the Directive 92/43/ECC. Notwithstanding its threatened status, little is known about many aspects of its biology. Conservation actions proposed until today have been based on little field evidence upon *Padogobius nigricans*. Within this context, morphology is to be considered a crucial tool for the evaluation of management unit and strategy formulation (Cadrin *et al.,* 2005). In particular, understanding morphological aspects appear critical for the conservation of goby populations living in fragmented habitats (Zerunian *et al.,* 1988).

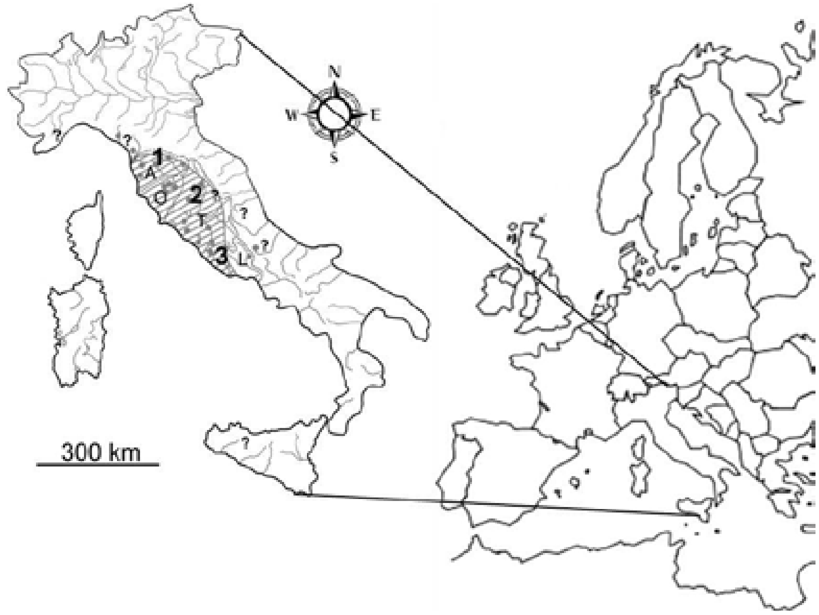

Figure 1. Actual distribution of *P. nigricans* in Italy (area with bars, from Zerunian 2002 modified). Spots and question marks represent historical records (from Bruno 1987 modified). Sampling sites: 1) River Carsa (Prov. Florence); 2) River Farfa (Prov. Rieti); 3) River Amaseno (Prov. Latina). Dotted lines delimit the main river basins: A = Arno: O = Ombrone; T = Tiber; L = Liri-Garigliano.

Main aims of this work are (I) to describe meristic parameters of *Padogobius nigricans* affected by ontogeny and sexual dimorphism, (II) to compare our results with those reported by other authors, in order to clarify their morphological variability, and (III) to propose useful characters for further preservation purposes.

MATERIAL AND METHODS

In total 108 individuals were collected by electrofishing from the same populations studied by Cervelli *et al.* (2007) (Fig. 1), and subsequently were used for other genetic and population studies: 1) 18 adult specimens (8 females + 10 males) from River Carsa (CA – Prov. Florence) were collected in the summer 2007; 2) 28 juveniles and 40 adults (20 + 20) from River Farfa (FA – Prov. Rieti) in the summer 2004-2008; 3) 22 adults (10 + 12) from River Amaseno (AM – Prov. Latina) in the summer 2007. The sex was recognized by the head shape and urogenital papillae. Small individuals with a standard length (SL) < 3.5 cm whose sex could not be identified were classified as juveniles (25). Then we counted both spines (with Roman numbers) and the segmented fin rays (indicated with Arabic numbers) of the first dorsal (D1), second dorsal (D2), pectoral (P), pelvic (V), anal (A), and caudal (C) fins, and the number of lateral line (LL) and predorsal (PD) scales, the latter being observed with a digital microscope Konus 5820 Set Micro-Eye with a serial USB port. The last two branched rays of D2 and A articulating on a single pterygiophore were counted as 1. We used only FA individuals to observe how ontogeny affected the analysed meristic parameters. Subsequently data-sets of the studied populations were compared with data reported in literature (see Table 1).

Intra- and inter-populations differences were analysed with the non-parametric Kruskal-Wallies because data-sets did not meet Gaussian distributions after their normality evaluation by the Kolmogorov-Smirnoff test. Both tests were performed using the STATISTICA Statsoft software version 6.0, setting always $\alpha = 0.05$.

RESULTS

Among all the analysed parameters, only D1 and P did not show variability, being always VI and I+5-5+I rays, respectively, in all studied populations. All the remaining parameters showed differences for age, sex, and site (Table 1 and Fig. 2).

No differences between genders were found in neither of the three sampling locations (after H test, with p always >0.05), with the exception for C both in CA (H (2, N = 16) = 12.44, $p < 0.01$) and in FA (H (2, N = 123) = 7.89, $p < 0.05$).

In the case of the FA data, the statistical analyses were performed to

compare juveniles and adults in order to observe potential differences attributed to ontogeny. Adults were not divided per sex since no sexual dimorphism emerged, with the exception of C. Only for the latter case, juveniles were compared with the two sexes separately. All the parameters showed significant differences (after H test, with p always <0.01, with the exception of the anal fin that was not significant). Regarding C, juveniles showed always median values lower than those of both females (H (2, N = 97) = 8.87, $p < 0.01$) and males (H (2, N = 94) = 11.53, $p < 0.01$).

In the case of adults data, significant differences were only evident among the studied populations for D2 (H (3, N = 145) = 9.61, $p < 0.05$; median hierarchy: FA = AM > CA) and A (H (3, N = 145) = 16.33, $p < 0.01$; median hierarchy: AM > FA > CA). Since the sexual dimorphism was evident for the caudal fin (C), females and males were analysed separately. Females differences were not significant whereas significant differences among all the three sites were evident for males (H (3, N = 84) = 8.43, $p < 0.05$; median hierarchy: FA > CA > AM). Although no differences were detected regarding the median values for LL, it was possible to observe that the CA range was smaller than those of the two other sites.

DISCUSSION

Although meristic traits provide limited evidences for differentiation (Hermida *et al.,* 2005), analyses of meristic features have been widely used by ichthyologists (Waldman 2005) for differentiating populations (e.g. Vidalis *et al.,* 1997; Tudela, 1999; Murta, 2000) and species (Kullander & Ferreira, 2006), monitoring ontogenesis (Beacham *et al.,* 1983; Tolan & Newstead, 2004), assessing morphological status of a species (Favaloro & Mazzola, 2000), and performing palaeontological studies (Carnevale & Bannikov, 2006). Differences in meristic characters are less pronounced than in morphometric ones, but they can be successfully used to discriminate samples or stocks within wide geographic regions (Hermida *et al.,* 2005; Hulen *et al.,* 2005). In fact, the consistency between morphometric and meristics indicates that they should be considered as complementary and not necessarily alternative approaches to the same problem (Cortés *et al.,* 1998).

Our study demonstrated that populations of the Etruscan goby significantly differed among them for some meristic traits. In particular, a great deal of intra- and inter- populations variations was observed for the fin rays. The results showed that morphological analyses concerning meristic parameters need to take into account the variability due to ontogeny, sexual dimorphism and geographic locality. In particular, it was found that the studied parameters were affected by (I) ontogeny (see the FA population) and (II) in a lesser extent by sexual dimorphism, which was found to be significant only in the case of the caudal fin in the populations of River Carsa (CA) and River Farfa (FA). Overall, these results suggest that the meristic parameters can vary from one population to another.

Considering the geographic distance and the similar environmental conditions among the studied sites (personal obser-

Table 1. Minimum (min), maximum (max), and mode (the latter provided only for the branched rays) of the variable meristic traits of the analysed population and those ones reported in literature. In chronological order: 1) Gandolfi & Tongiorgi, 1974; 2) Bianco, 1977; 3) Pirisinu & Natali, 1980; 4) Zerunian & Gandolfi, 1986 (in this case, authors described also V-VI rays for D1); 5) Nocita & Vanni, 2001; 6) present study. Abbreviations (in alphabetical order): A = River Arno; L = River Liri-Garigliano; T = River Tiber.

					second dorsal fin			pectoral fin			anal fin			caudal fin			lateral line scales			predorsal scales		
	sex/age	n	river	basin	min	max	mode	min	max	mode	min	max	mode	min	max	mode	min	max	mode	min	max	mode
1	—	27	Turbone	A	I-11	I-13	—	14	15	—	I-6	I-8	—	—	—	—	44	49	—	—	—	—
2	—	10	Fomello	T	I-12	I-13	—	14	15	—	I-9	I-9	—	—	—	—	45	47	—	—	—	—
	—	6	Tevere	T	I-12	I-13	—	14	16	—	I-9	I-10	—	16	18	—	46	49	—	—	—	—
3	—	5	Chiascio	T	I-12	I-13	—	14	16	—	I-8	I-9	—	16	18	—	44	50	—	—	—	—
	—	9	Topino	T	I-11	I-13	—	15	17	—	I-8	I-10	—	15	18	—	45	49	—	—	—	—
4	—	28	Amaseno	L	I-12	I-14	—	14	16	—	I-9	I-11	—	III-12-III	V-15-V	—	43	51	—	—	—	—
5	—	49*	Arno	A	I-12$^{1}/_{2}$	I-12$^{1}/_{2}$	—	15	16	—	I-8$^{1}/_{2}$	I-9$^{1}/_{2}$	—	—	—	—	44	49	—	3	16	—
	females	8	Carsa	A	I-11	I-13	12	13	15	14	I-6	I-8	6	IV-13-IV	V-15-V	15	46	48	47	9	13	10
	males	8	Carsa	A	I-11	I-13	12	13	15	14	I-6	I-8	7	IV-14-IV	V-17-V	16	47	48	47	8	13	11
	juveniles	32	Farfa	T	I-11	I-13	12	12	13	13	I-8	I-10	9	V-11-IV	V-13-V	12	40	45	43	3	10	7
6	females	65	Farfa	T	I-11	I-13	13	13	15	14	I-8	I-10	9	V-12-IV	V-16-V	14	44	50	47	8	13	11
	males	62	Farfa	T	I-12	I-14	13	13	15	14	I-8	I-10	9	V-13-IV	V-18-V	15	44	51	47	8	13	10
	females	13	Amaseno	L	I-12	I-14	13	13	16	14	I-9	I-11	11	III-12-III	V-15-V	15	44	49	47	9	13	11
	males	11	Amaseno	L	I-13	I-14	13	13	15	14	I-9	I-11	11	III-12-III	V-15-V	14	44	49	47	9	13	11

° = Nocita & Vanni (2001) counted the branched rays of D2 and A articulating on a single pterygiophore as 1$^{1}/_{2}$ according to Berti & Ercolini (1991); * = specimens analyzed by Nocita & Vanni (2001) ranged from 15 to 49 depending on the meristic parameter.

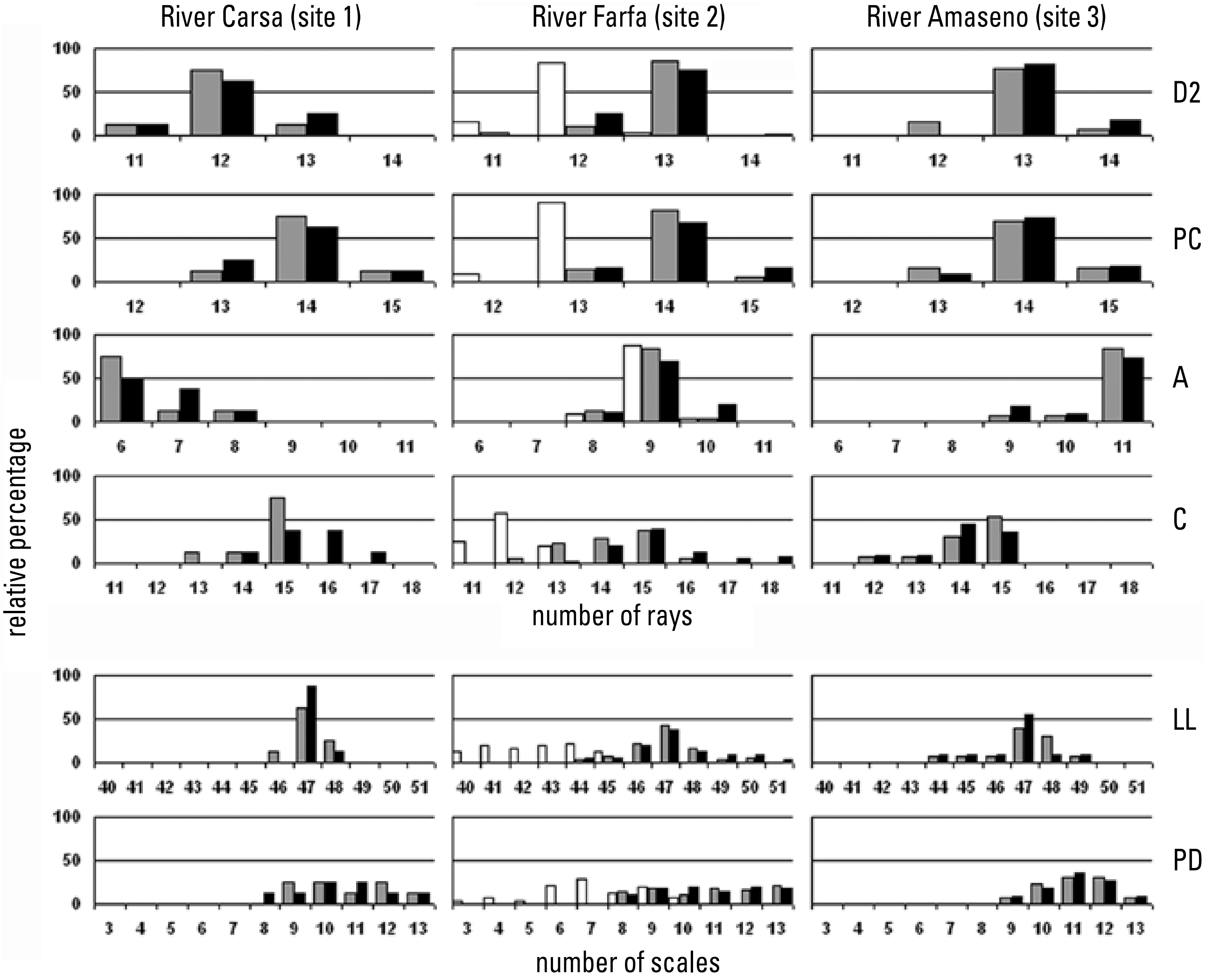

Figure 2. Frequency distribution of the branched rays, and lateral line and predorsal scales of the populations analysed in the present study, divided per sex and age in the case of the River Farfa. □ juveniles, ▨ adult females, ■ adult males. Abbreviations (in alphabetical order): A = anal fin; C = caudal fin; D2 = second dorsal fin; LL = lateral line scales; P = pectoral fin; PD = predorsal scales.

vations), these differences would not be expected, mostly when taking the restricted geographic range of *Padogobius nigricans* into account. However, the body shape variation patterns were in accordance with our expectative (see Cervelli *et al.,* 2007).

Morphological variability among populations may arise from interpopulation differences in genetic structure and/or environmental conditions (e.g., Vidalis *et al.*, 1997; Walsh *et al.,* 2001). From an evolutionary standpoint, the existence of a genetic basis for such variability is important because this is the prerequisite for selection driven divergence of populations, which may in turn eventually result in speciation (Penzo *et al.,* 1998). Our results are in agreement with those of Cervelli *et al.* (2007), which analysed the mtDNA control region as a potential molecular marker to assess individual genetic differences. While the FA and AM populations from Latium showed a very high sequence homology, the case of the CA population from Tuscany was unique in terms of its primary structure, repeatedness, and organization, suggesting that this population was genetically isolated from the remnant two after a bottleneck event.

Considering the amount of genetic variation from some of these traits in the Etruscan goby and related species (Penzo *et al.,* 1998; Dillon & Stepien, 2001; Huyse *et al.,* 2004), it may be possible that interpopulation differences could be attributed to the actions of natural selection (Smith *et al.,* 2006). In relation to this, it has been stated that environmental factors, such as salinity, calcium concentration and temperature, could also play a role in the development of different interpopulation traits (Swain & Foote, 1999). However, selective pressures that act on morphology features may be multiple, complex, and sometimes synergistic or antagonistic, hence generating an adaptative plasticity at the species level (Gottahard & Nylin, 1995). In this study many factors, among which environmental fluctuations, could have affected the direction and intensity of the meristic interpopulation differences. In fact, environmental fluctuations may affect the growth rate and osteogenesis (Cadrin *et al.,* 2005), further complicating the situation, such as in this study.

Additionally, this study shows features that can vary according to the different locations within the basin, suggesting the existence of three different management units (see Moritz, 1994; Paetkau, 1999). In fact, each population of *Padogobius nigricans* seems to have an own 'meristic-print'. However, this need to be further confirmed. There is considerable scope for further work on the *P. nigricans* meristics because information on the morphological variation from different localities, and an assessment of the factors affecting it are still insufficient. Filling this lack of information can improve preservation actions on the Etruscan goby.

The *P. nigricans* populations can be reliably differentiated based upon morphological traits from northern to southern distribution range, providing additional support for the decision to manage and preserve the Etruscan goby as imperilled taxon. In the near future, several morphological data could be analysed in order to shed more light on its biology and contribute to set up protocols to improve its monitoring, conservation and managing.

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