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DATA ABOUT THE PRESENCE OF SYGNATHUS NIGROLINEATUS EICHWALD 1831 IN THE DANUBE RIVER

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ZUSAMMENFASSUNG

In vorliegender Arbeit wird das Vorkommen der Seenadel (Syngnathus nigrolineatus Eichwald 1831) im ersten Abschnitt der Donau auf rumänischen Boden besprochen. Es erweist sich auf Grund meristischer Messungen und zugleich des genetischen Fingerabdrucks das zwischen den Küstenpopulationen des Schwarzen Meeres bei Agigea und derselben des künstlich endstandenen Ieşelniţa-Sees an der Donau ein wesentlicher Unterschied zu bemerken ist.

KEY WORDS: *black-striped pipefish, Danube, Black Sea, meristical analysis, electrophoresis, RFLP.*

Syngnathus nigrolineatus (the black-striped pipefish) is a common species in the Black Sea and Azov Sea. In the Romanian area this fish can be found along the entire coast of the Black Sea, in the Danube Delta, and the littoral lakes. In Danube, it can reach locations as far as Călăraşi (Buşniţă & Alexandrescu, 1963). *Bănărescu* (1964) considered this species as eurihaline: he found the black-striped pipefish in the Black Sea as well as in brackish or even fresh waters. However, the same author mentioned that *S. nigrolineatus* cannot swim against the river water currrents and because of this „*in Danube can be found only ocasionally."*

Since 1996, the black-striped pipefish was observed and hand collected in large numbers among the algae of Ieşelniţa gulf (southwest of Orşova town). This gulf was secondary formed by flooding of the terminal watershed of Ieşelniţa river after the Iron Gate dam was built.

We comparatively studied the diferences among the black-striped pipe fish populations from the Black Sea and Ieşelniţa gulf. These locations, almost 1000 km away from each other, are characterised by pregnant habitat differences. The specimens from the Black Sea were collected on the *Mytilus*-covered rocky substrata of Agigea and Eforie Nord.

METHOD

Collected material was 48 specimens from Danube river, and 36 specimens from the Black Sea. These specimens were meristically compared and the results tested for statistical significant differences. We performed classical meristical measurements in fish (Table 1). We also analysed the DNA restriction fragment length polymorphism (RFLP) [1] in the two populations. For this analysis we used pGEM DNA Markers (Promega, USA). This marker system is based on analysis of 15 DNA fragments (base pairs) of various sizes presented as following:

2645	*517*	*222*	*65*
1605	*460*	*179*	*51*
1198	*396*	*126*	*36*
676	*350*	*75*	

The extracted DNA was digested with EcoRI restriction enzyme.

RESULTS AND DISCUSSIONS

The meristic data were statisticaly tested (*t* test) for significance of differences between the two populations. We found statistical significant differences for the following measurements: ratio of the snouth length from head length (c), eye diameter in the interorbitary space (g), ratio between the predorsal space and the total body height (h), ratio between predorsal space and pectoral fin (i), and ratio between predorsal space and total body length (j).

Table 1

a – length of head from length of body without caudal fin	2.09
b – length of snouth from length of body	3.68
c – length of snouth from length of head	**1.24**
d – length of snouth from length of dorsal fin basis	2.47
e – mimimal height from the maximal body height	10.21
f – eye diameter from the minimal snouth height	5.67
g – eye diameter from the interorbitary space	**1.11**
h – predorsal space from the maximal body height	**1.76**
i – predorsal space from the length of pectoral fin	**0.37**
j – predorsal space from the body length	**0.86**
k – dorsal fin basis from body length	4.53
l – dorsal basis fin from head length	4.54

[1] We are grateful to prof. A. Anghel for using the facilities of the Biochemistry lab at UMF "Victor Babeş", Timişoara.

The analysis of the RFLP DNA fingerprinting presented us the following scenario: we observe in the figure 1a that fragments less than 222 bp (base pairs) (i.e. those from 179 to 36 bp) run out of gel.

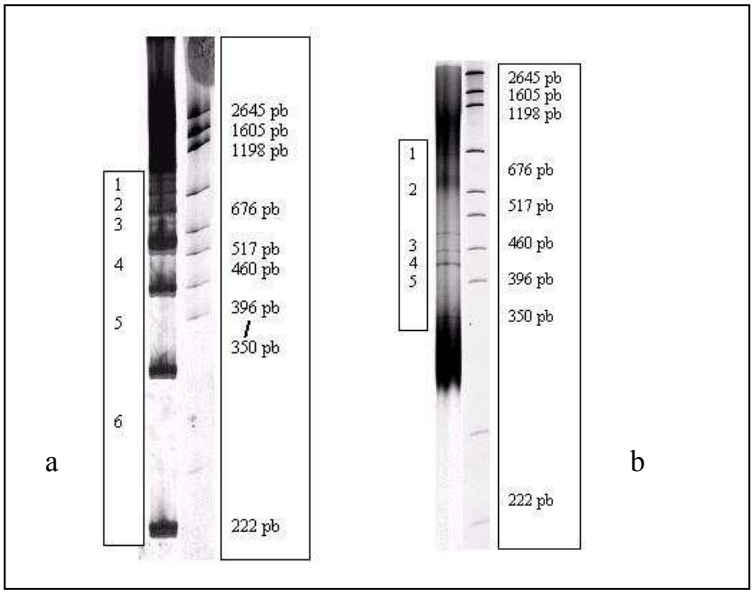

FIG. 1.
a RFLP electrophoresis gel of the specimen from Ieşelnița, Danube.
b RFLP electrophoresis gel of the specimen from Agigea, Black Sea.

The marker column contains seven restriction fragments (conventionally numbered from 1 to 7). Two of the fragments were of known length (2-676 bp and 5-396 bp). The rest were approximated: fragment 1 was between 676 and 1198 (aprox. 750 bp), fragment 3 migrated half of the distance between 517 and 676 (aprox. 596 bp), fragment 4 migrated half of the distance between 460 and 517 (aprox. 488 bp), fragment 6 was placed close to the inferior limit of superior third of distance between 222 and 350 (aprox. 307 bp), and fragment 7 is between 179 and 222 bp long.

From the figure 1b we conclude that in the marker column the fragments shorter than 179 bp (i.e. those from 126 to 36 bp) run out of gel.

The control column contains six restriction fragments conventionally numbered from 1 to 6. Two of the fragments were of known length (1-676 bp and 4-396 bp). The rest were approximated (for a better determination it is necessary to use a marker with different lengths): fragment 2 was placed at the superior limit of inferior fourth of the distance between 676 and 517 (aprox. 556 bp), fragment 3 migrated at half distance between 396 and 460 (aprox. 428 bp), fragment 5 migrated at half distance between 350 and 396 (aprox. 373 bp), and fragment 6 was placed close to the inferior limit of the superior fourth of distance between 222 and 350 (aprox. 318 bp).

Summarizing the data above we obtain the following results:

Fragment	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Danube	750	676	596	488	396	307	<222
Black Sea	676	556	428	396	373	318	-

From the table above we observe that there are significant differences between the two analyzed specimens. The first four restriction fragments had significant differences in length. Fragments 5 and 6 had very similar size and fragment 7 is missing in the specimen from the Black Sea.

We need to study more specimens in order to establish if the two populations have a common genealogy. We plan to investigate also the microsatellites in *Syngnathus nigrolineatus*. This approach should be more accurate for this kind of studies.

CONCLUSIONS

We found significant meristic and genetic differences between the specimens of *Syngnathus nigrolineatus* collected from the two habitats of the Blacks Sea and Danube River.

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