Macedonian Journal of Ecology and Environment
Vol. 19, issue 1
pp. 28-35
Skopje (2017)
eISSN 1857 - 8330
Original scientific paper
Available online at www.mjee.org.mk

First record of the Black Bullhead *Ameiurus melas* (Pisces, Ictaluridae) in the Republic of Macedonia

Прв податок за присуство на црното американско сомче *Ameiurus melas* (Pisces, Ictaluridae) во Република Македонија

Milica RISTOVSKA[1,*], Julijana ARSOVSKA[1], Vasil KOSTOV[2], Aleksandra CVETKOVSKA GEORGIEVSKA[1], Dana PRELIK[1] and Kristina RADEVSKA[1]

[1] Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Skopje, Macedonia
[2] Institute of Animal Science, Department of Fishery, Boulevard Ilinden 92a, 1000 Skopje, Macedonia

The North American catfish – the black bullhead, *Ameiurus melas* (Rafinesque, 1820) is recorded for the first time in the Republic of Macedonia, in Pčinja River. Nine individuals of this ictalurid fish species were fished from the anglers in Pčinja River on September 2015 and another 8 samples on May 2016 were brought to the Institute of Biology, Faculty of Natural Science and Mathematics in Skopje. After a detailed observation of the external morphological features, osteology architecture, it was concluded that analysed individuals had all the distinguishing features of the species *A. melas.* The presence of different size class permitted the supposition that the black bullhead has established a self-sustaining population in this river.
Key words: black bullhead, catfish, invasive species, morphological features, osteology, Pčinja River.

Црното американско сомче *Ameiurus melas* (Rafinesque, 1820) за првпат е регистрирано во Република Македонија, во реката Пчиња. Девет единки од овој вид беа уловени од страна на риболовците во реката Пчиња во септември 2015 година, а други осум единки уловени во мај 2016 година беа донесени на Институтот за биологија, Природно-математички факултет во Скопје. По детална анализа на надворешните морфолошки карактеристики и остеолошката архитектура беше заклучено дека сите единки ги имаат сите карактери специфични за *A. melas*. Присуството на различни возрасни класи наведува на заклучокот дека црното американско сомче *A. melas* има воспоставено стабилна популација во реката Пчиња.
Клучни зборови: американско сомче, инвазивен вид, морфолошки карактеристики, остеологија, река Пчиња

Introduction

Invasive fish species constitute a major threat to biodiversity and ecosystem integrity and cause substantial economic damage (Pimentel et al. 2000; Copp et al. 2005). Environmental disturbance is known to greatly facilitate fish invasions. Habitat modification and fragmentation (e.g., water pollution, construction of dams and water diversions) threaten native fish faunas while favouring the invasion of more tolerant non-native species (Marchetti et al. 2004). It is the case for the brown bullhead *Ameiurus nebulosus* (Lesueur, 1819) and black bullhead *Ameiurus melas* (Rafinesque, 1820) which were introduced into many countries in Europe since the nineteenth century (Kottelat and Freyhof 2007). Both species have a negative impact on native populations of fish species (Rutkayová et al. 2013; Copp et al. 2016).

Concerning the Balkan Regions until now, *A. nebulosus* was recorded in Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Albania, Greece and Bulgaria and Macedonia, while *A. melas* was recorded in Slovenia, Croatia, Bosnia and Herzegovina and Serbia (Simonovic et al. 2013). Their impact in these countries (by FISK range) was calculated as a moderately high (*A. melas*) and high (*A. nebulosus*).

The reconstruction of the origin and paths of their distribution in Europe is very difficult due to the unclear taxonomic status of these species in the past, obstacles in the field identification and uncertainty of the literature data (Nowak et al. 2010). Moreover, the possibility of their hybridization (Scott and Crossman 1973) could also make additional problem in their identification. From the other side, osteological architecture, especially of the pectoral girdle and pectoral spine offer morphological clues for identification of this two species (Rutkayová et al. 2013).

Many years ago it was established that the brown bullhead, *A. nebulosus* and *Ictalurus punctatus*, were the only ictalurid species introduced into Macedonian waters (Naumovski 1995; Kostov et al. 2011). However, recent ichthyologic surveys brought to attention the introduction of another ictalurid species. In attempt to facilitate the identification of the newly introduced ictalurid species in Macedonia, this study presents a detail osteological analysis of *Ameiurus melas* from Pčinja River.

Submitted: 09.02.2017
Accepted: 12.04.2017

*Author for correspondence: milica.ristovska@yahoo.com

Material and Methods

In 2015, nine individuals of ictalurid fish were fished by the anglers in Pčinja River in September 2015 and another 8 specimens in May 2016 and all were brought to the laboratory for Comparative Anatomy in the Institute of Biology, Faculty of Natural Science and Mathematics in Skopje.

Pčinja River sources out from several streams on the western slopes of the Dukat Mountain which meet at the village of Radovnica (Serbia), and flows into the Vardar River in Taor Gorge, halfway between the cities of Skopje and Veles. The river's length is 135 km, of which 100 km are in Republic of Macedonia. Seventeen individuals of *A. melas* from Pčinja River were fished by the anglers and according to them individuals were fished in the parts of the river section named "Badar", characterised by a number of sand-extraction pits (Fig. 1).

Detailed morphometric analyses were performed according to Holčík (1998) (Fig. 2) as well as the meristic counts (Tab. 1 and 2). Seven specimens of *A. melas* were cleared and stained according to Taylor and Van Dyke's (1985) protocol for detailed of osteological analyses. A Camera Lucida attached to a Wild M3C microscope was used for examination of specimens and drawings (Fig 2; Fig 4-10). The anatomical structures in the figures are shaded and labelled a consistent manner (dark, medium, and clear) to facilitate the real position. The nomenclature of skeletal elements follows Rojo (2013) and Rutkayová et al. (2013).

Furthermore, already collected and stored samples of *A. nebulosus* from Tikveš Resirvoir were used for comparison. Dissection of some parts of the body (fins, branchial arches) from *A. nebulosus*, as well as technique of maceration with KOH solution of few skeletal structures was performed according to Green (1952). In addition, literature data about osteological characters of *A. nebulosus* was used for comparison with *A. melas* from Pčinja River (Rojo 2013; Rutkayová et al. 2013).

Figure 1. Location map showing the first record of *Ameiurus melas* in R. Macedonia

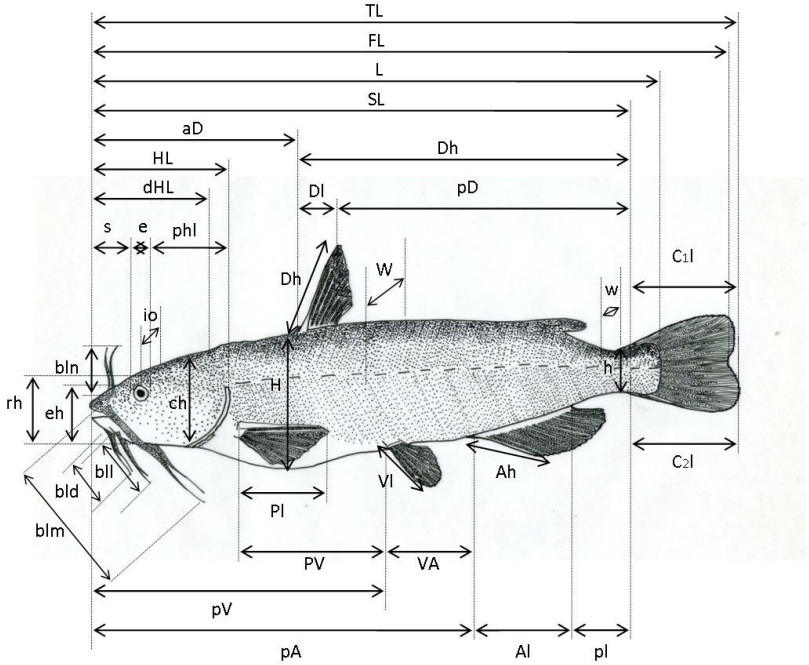

Figure 2. Morphometric measurements of *A. melas.* Abbreviations are given in Table 1.

Table 1. Morphometric measurements for *Ameiurus melas* from Pčinja River.

Measurement	min	max	mean	SD	
(TL) Total length	91.43	153.40	112.30	21.59	
(FL) Fork length	86.99	150.43	101.75	16.40	
(L) Body length	76.60	133.90	95.69	19.88	
(SL) Standard length	73.08	130.50	91.52	18.83	
					% of SL
(pD) Postdorsal length	40.97	70.82	50.78	10.60	55.48
(dh) Dorso-hypural distance	45.24	85.13	59.85	12.99	65.40
(pl) Caudal peduncle length	9.73	26.52	15.75	4.52	17.20
(C_1l) Caudal fin upper lobe length	17.35	27.79	21.33	3.57	23.30
(C_2l) Caudal fin lower lobe length	16.09	27.78	20.56	3.82	22.47
(aD) Predorsal length	28.26	52.23	36.60	8.10	40.00
(HL) Head length	21.92	41.54	28.52	6.00	31.16
(dHL) Dorsal head length	19.24	34.49	23.50	4.63	25.68
(pV) Prepelvic length	35.24	62.62	44.60	8.95	48.73
(pA) Preanal length	44.52	81.20	56.17	12.00	61.38
(PV) Pectoral to pelvic fin base	17.52	33.75	24.43	5.14	26.69
(VA) Pelvic to anal fin base	8.91	21.30	13.11	3.41	14.32
(Van) Pelvic fin base to anus	2.57	6.05	4.17	1.18	4.56
(anA) Anus to anterior most anal fin	2.56	3.88	3.36	0.47	3.67
(Dl) Length of dorsal fin	5.78	11.44	8.00	1.62	8.74
(Dh) Height of dorsal fin	9.87	24.28	18.10	4.01	19.78
(Al) length of the anal fin	15.17	29.88	20.18	3.98	22.05
(Ah) Height of the anal fin	14.53	31.80	17.94	4.58	19.60
(Pl) Length of the pectoral fin	3.40	22.60	9.19	6.81	10.04
(Vl) Length of the pelvic fin	3.33	19.38	8.80	6.24	9.62
(w) Caudal peduncle width	3.17	7.96	4.89	1.41	5.34
(W) Body width	11.36	22.83	16.08	3.44	17.57
(H) Body depth	15.63	27.50	20.26	2.97	22.14
(h) Caudal peduncle depth	7.72	14.60	10.49	2.47	11.46
(bld) Barbels length (medial mandibular).	10.72	20.12	16.04	3.44	17.53
(bll) Barbels length (lateral mandibular)	13.78	24.28	19.48	4.60	21.29
(bln) Barbels length (nasal)	9.99	19.86	15.32	3.82	16.74
(blm) Barbels length (maxillar)	21.94	32.90	28.15	4.87	30.75
					% of HL
(ch) Head depth at occiput	15.10	24.58	18.15	2.80	63.63
(eh) Head depth at eye	8.14	11.75	10.22	1.44	35.84
(io) Interorbital width	9.91	21.03	13.48	3.52	47.27
(s) Snout length	7.92	15.87	10.90	2.45	38.21
(e) Eye diameter	3.72	6.03	4.45	0.65	15.61
(phl) Postorbital length of the head	11.69	23.43	15.20	4.02	53.28
(lMx) Upper jaw length	5.88	14.01	8.48	2.98	29.72
(lMd) Mandible length	5.88	14.61	8.52	3.06	29.86
(p) Pupilla	1.21	2.72	1.97	0.42	6.91
(rh) Head depth at eye (from the roof)	8.68	18.93	12.08	3.39	42.35

Table 2. Meristic counts of *Ameiurus melas* from Pčinja River and *Ameiurus nebulosus* from Tikveš Resirvoir

Character	range	range
Number of caudal fin rays	17-20	17-20
Number of vestigal and dorsal spines in the dorsal fin	1+1	1+1
Number of branched dorsal fin rays	5-6	6-7
Number of anal fin rays	19-21	20-21
Number of pelvic fin rays	8	8
Number of branched pectoral fin rays	7-8	7-8
Number of spines in pectoral fin	1	1

Results and discussion

In spite of the great morphological similarity in many features, according to Movchan et al. (2014) *A. nebulosus* significantly differs from *A. melas* by the number of gill rakers, serration of the first spine of the pectoral fin and coloration of the fin membrane. However, according to Kottelat and Freyhof (2007) the colour and anterior serrae on pectoral spine are not reliable characters and have led to much confusion regarding the identification of these species in Europe.

In the case of *A. melas* (Pčinja River) and *A. nebulosus* (Tikveš Reservoir), the results show that these selected morphological characters vary. The base of the anal fin in *A. melas* from Pčinja River (Fig. 3A) is lightly coloured and thicker compared to the fin of *A. nebulosus* from Tikveš Reservoir (Fig. 3B). However, the membrane between the rays of the anal and caudal fins is black in both species contrary to the foundlings of Movchan et al. (2014).

The first spine of the pectoral fin in *A. melas* is covered with a few sharp denticles only in the middle part (Fig. 4B) while in *A. nebulosus* the denticles extend along and almost reach the end of the spine (Fig. 4A). Anterior serration is absent in *A. melas* unlike the samples of *A. nebuslosus* from Tikveš Reservoir. But it should be stressed out that this anterior serration in *A. nebulosus* specimens was not observed in all samples, thus making this character questionable for identification purposes.

The number of gill rakers at the first ceratobranchial in *A. melas* vary from 15 to 19 (Fig. 5B), while in *A. nebulosus* usually this number is 15 (Fig. 6). However, in one of the samples of *A. nebulosus* the number of the gill rakers was 17.

The plastic characteristics analysed in this study are in accordance with the data published about other European populations of these two species. The osteological features still play an important role in the systematics and identification of ictalurid fishes (Paruch 1986; Lundberg 1982; Traynor et al. 2010; Rojo 2013, Rutkayová et al. 2013) and thus we made a complete osteological analysis of *A. melas* in addition to the standard morphometric measurements. Morphometric measurements and meristic counts including osteological ones are presented in Tab. 1 and 2.

Within the presented osteological results and in order to distinguish *A. melas* in Pčinja River from *A. nebulosus* we propose an identification key regarding their skeletal structural differences:

Ameiurus melas	***Ameiurus nebulosus***
Possesses a secondary coracoid keel at the pectoral girdle (Fig. 8);	Secondary coracoid keel is always absent in *A. nebulosus* (Rutkayová et al. 2013);
Shorter posterior process of cleithrum;	Longer posterior process of cleithrum (Rojo 2013; Rutkayová et al. 2013);
Pectoral spine of *A. melas* possesses shorter and thinner teeth irregularly spaced (see Fig. 4);	Well developed longer and wider teeth regularly spaced in *A. nebulosus* from Tikveš Resirvoir and Rutkayová et al. (2013);
Median notch in anterior margin of supraethmoid bone at least twice wide as deep (Fig. 7);	The median notch in anterior margin of supraethmoid bone is as deep as wide;
Entopterygoid bone in *A. melas* is much wider and higher, reaching the entire surface of the anterior margin from the metapterygoid bone (Fig. 9).	Entopterygoid (=ectopterygoid) bone (Rojo 2013), is much smaller, with height about one third of the height of anterior margin of the metapterygoid bone.

It is well known that the number of vertebras and the number of branchiostegal rays are often used as taxonomic key characters (Kottelat and Frehof, 2007). Our results show that these characters offer useful information that can be considered for taxonomic discrimination between *A. melas* and *A. nebulosus*. Namely, in all examined specimens of *A. melas* from Pčinja River, the number of precaudal vertebra was 16, while caudal ones counted 25-26, corresponding with results for *A. melas* described by Lundberg (1982). In *A. nebulosus* described by Rojo (2013), the number of precaudal (abdominal) vertebra varies from 12 to 16, while the number of caudal ones from 22 to 29. In all examined specimens of *A. me-*

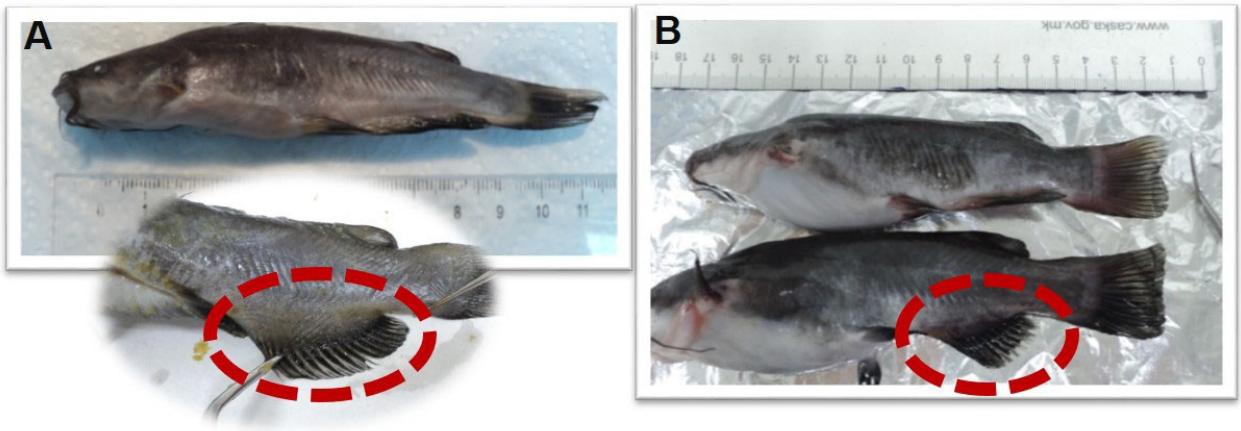

Figure 3. Anal fin in A. *Ameiurus melas* and B. *Ameiurus nebulosus*

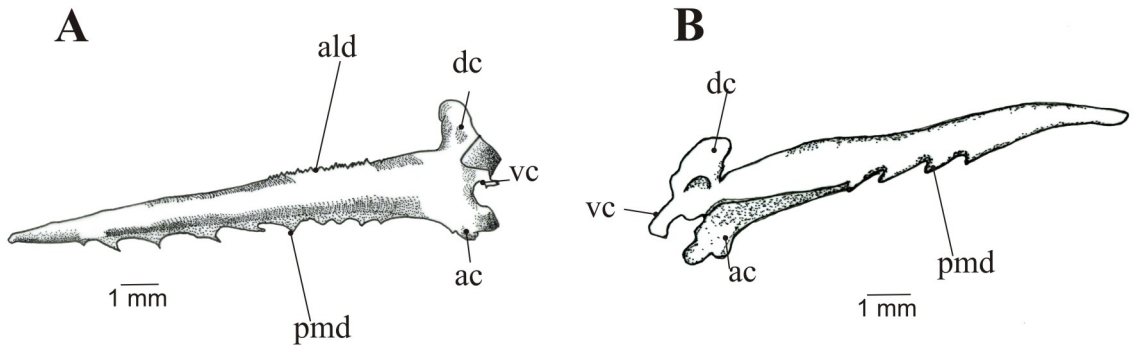

Figure 4. Pectoral spine in A. *Ameiurus nebulosus* and B. *Ameiurus melas* (ac – anterior condyle of pectoral spine; ald – antero-lateral dentitions of pectoral spine; dc – dorsal condyle of pectoral spine; pmd - postero-medial dentition of pectoral spine; vc - ventral condyle of pectoral spine).

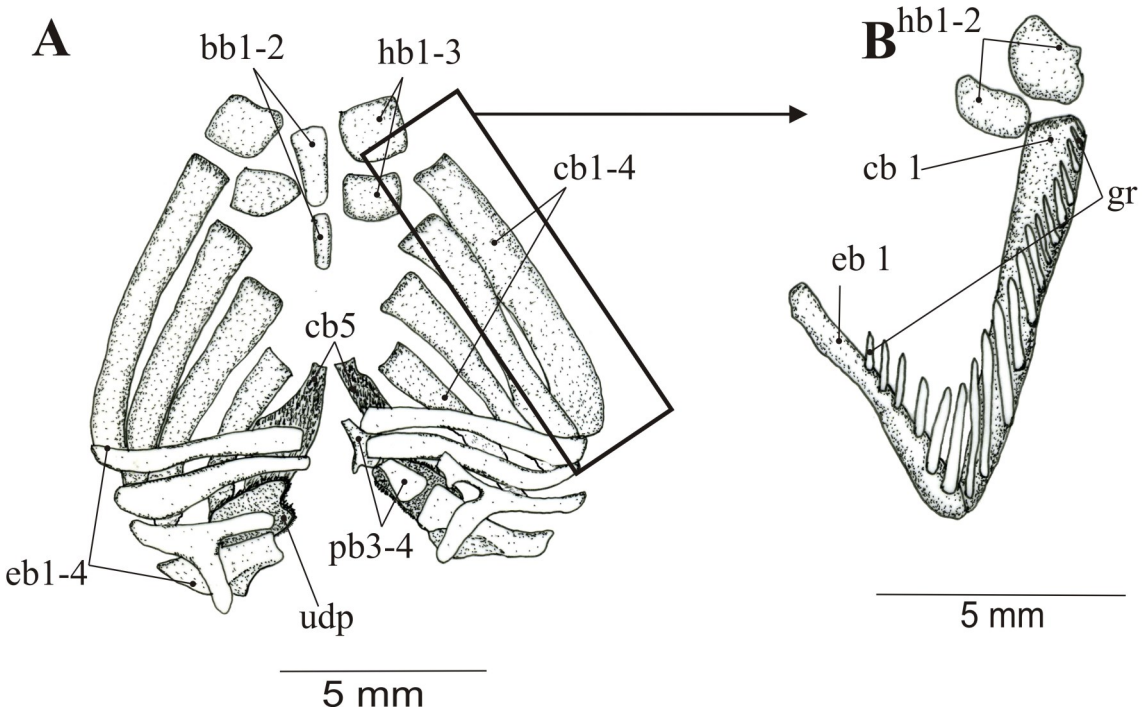

Figure 5. A. Branchial arches and B. gill rakers in *Ameiurus melas* (bb - basibranchials; cb - ceratobranchials; eb - epibranchials; gr - gill rakers; hb - hypobranchials; pb - pharyngobranchials; udp - upper dental plate).

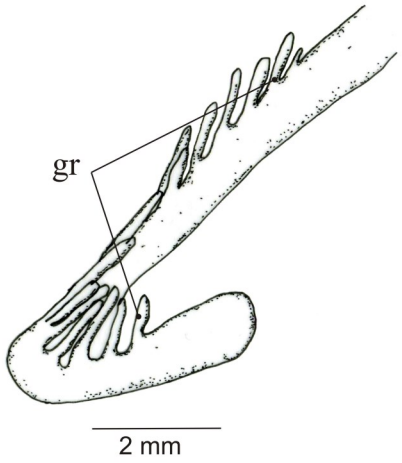

Figure 6. Gill rakers (gr) in *Ameiurus nebulosus.*

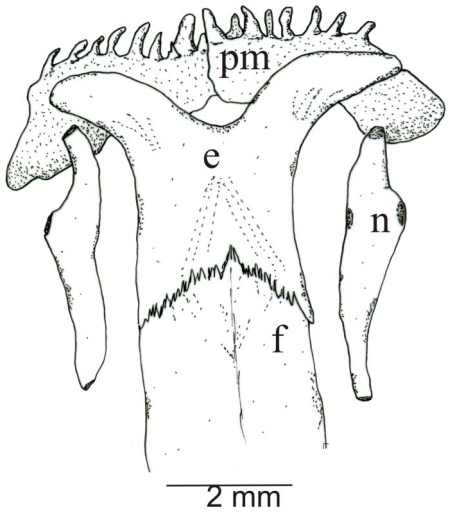

Figure 7. Ethmoid region in *Ameiurus melas* (e - ethmoid; f - frontal; n - nasal; pm - premaxillary).

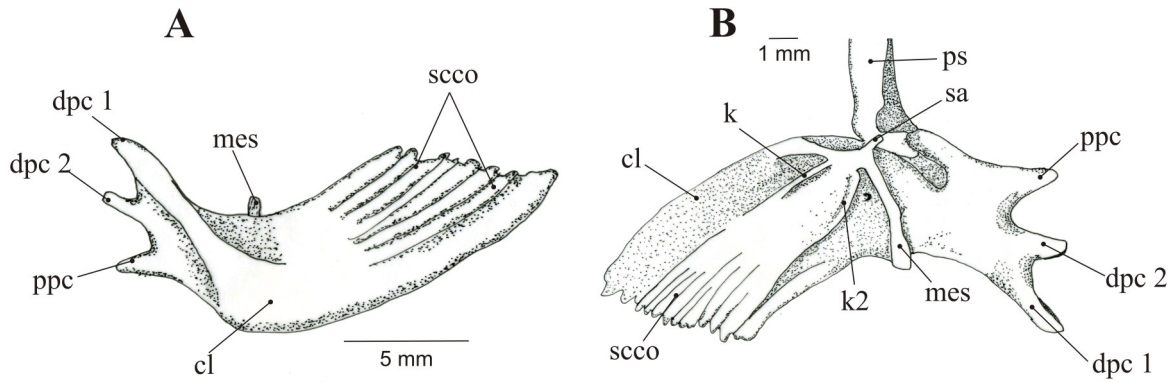

Figure 8. Pectoral girdle in *Ameiurus melas*. A. Dorsal view and B. ventral view (cl - cleithrum; dpc1 - first dorsal process of cleithrum; dpc2 – second dorsal process of cleithrum k1 - primary coracoid keel; k2 - secondary coracoid keel; mes - mesocoracoid; ppc - posterior process of cleithrum; ps - pectoral spine; sa – articulation groove of pectoral spine; ssco - scapulo-coracoid).

las from Pčinja River the number of the branchiostegal rays is 9 (Fig. 10), showing difference when compared with *A. nebulosus* described by Rojo (2013) where this number was 8 branchiostegal rays in 16 of 17 examined specimen, while 9 branchiostegal rays were detected in only one specimen.

The detailed analysis of the skeletal features of the ictalurid fish species from Pčinja River in comparison with samples from Tikveš Reservoir and the literature data, revealed the presence of *A. melas* in R. Macedonia. Osteological observations and results based on theme strongly support the importance of osteological markers in situation when external morphology is not conclusive. The presence of different size classes of *A. melas* in Pčinja River permits the supposition that the black bullhead has established a self-sustaining population in this river. The origin and introduction pathway of the black bullhead to the Pčinja River remains unclear, but knowing the fact that misidentification of *A. melas* occurred in many countries, it is reasonable to assume that the species might have been widely distributed throughout Macedonia for a long period. As many studies in Europe showed that distribution of *A. melas* in the water bodies is related to fading of *A. nebulosus,* it is necessary to undertake urgent surveys to ascertain the current distribution and the ecological impacts of this invasive species in Macedonian waters. The presence of this species in Macedonian waters should be considered in future management and conservation schemes.

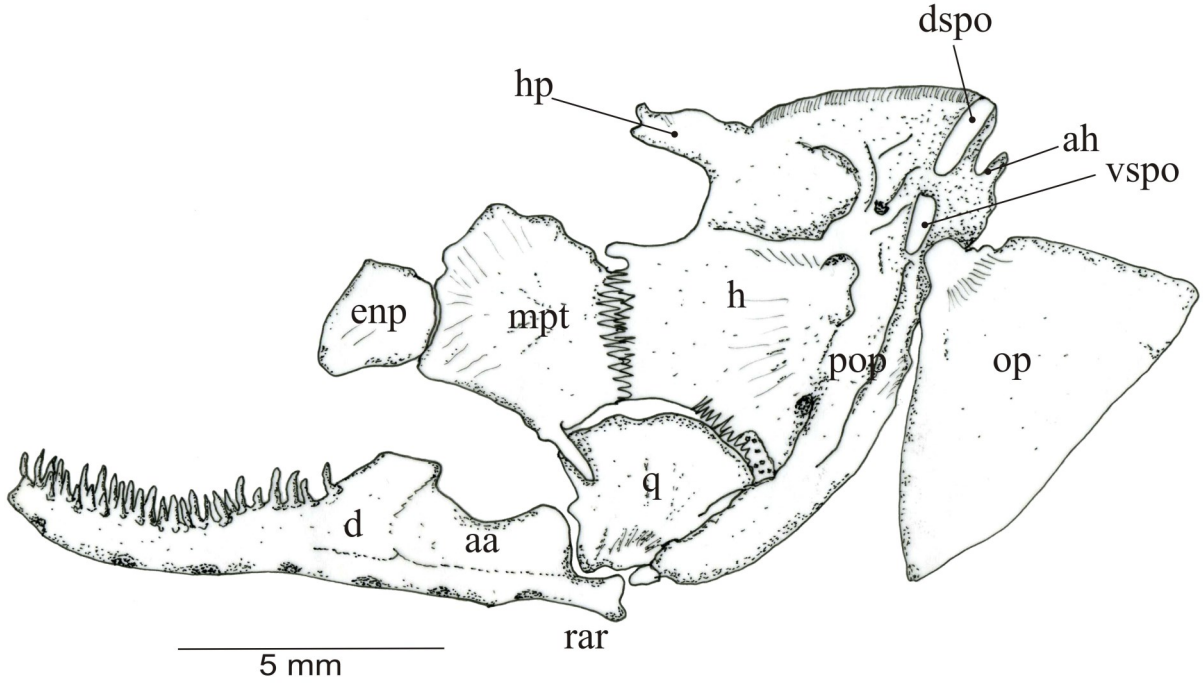

Figure 9. Suspensorium in *Ameiurus melas* (aa – angulo-articular; ah - attachment crest of adductor hyomandibularis muscle; d - dental; dspo – dorsal suprapreopercle; enp - entopterygoid; h - hyomandibular; hp - anterior process of hyomandibular; mpt -metapterygoid; op -opercular; pop -preopercular; rar - retroarticular; q - quadrate; vspo – ventral supraperoprecle).

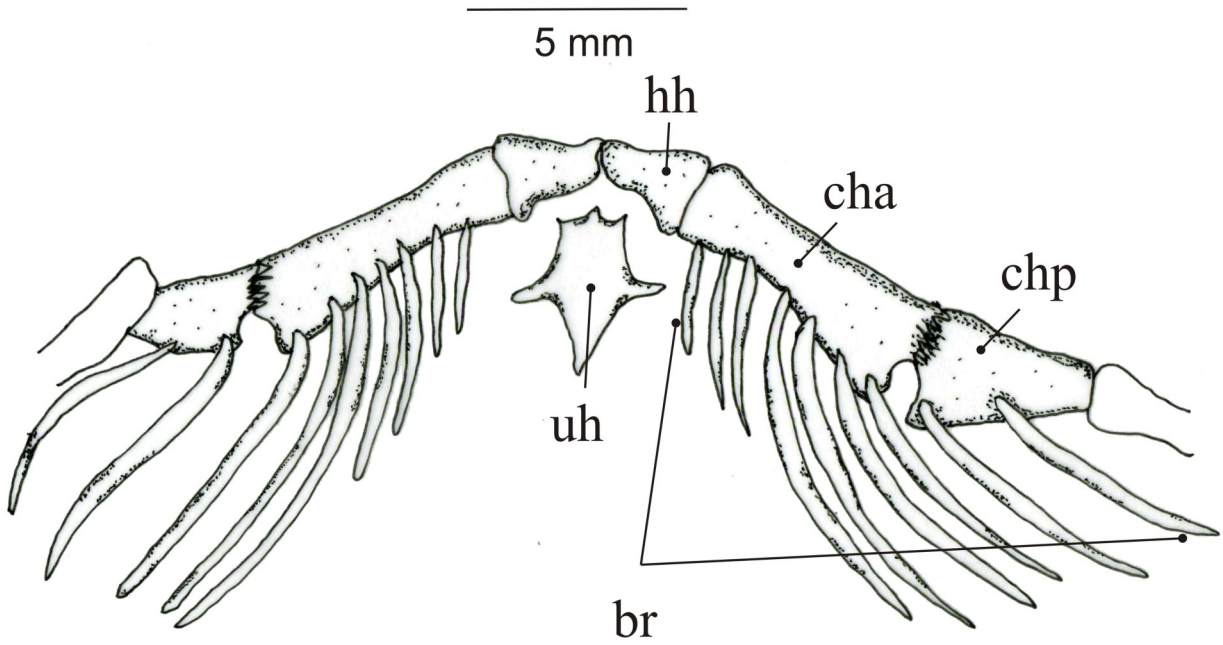

Figure 10. Hyoid arch with branchiostegal rays in *Ameiurus melas* (br – branchiostegal rays; cha - anterior ceratohyal; chp – posterior ceratohyal; hh - hypohyal; uh – urohyal).

References

Copp G. Tarkan A. S., Masson G., Godard M., Koščo J., Kováč V., Novomeská A., Miranda R., Cucherousset J., Pedicillo G., Brian & Blackwell G. (2016). A review of growth and life-history traits of native and non-native European populations of black bullhead *Ameiurus melas*. *Rev. Fish. Biol. Fisheries,* **26**:441–469.

Copp, G.H., Bianco, P.G., Bogutskaya, N.G., Erős, T., Falka, I., Ferreira, M.T., Fox, M.G., Freyhof, J., Gozlan, R.E., Grabowska, J. and Kováč, V. (2005). To be, or not to be, a non-native freshwater fish? *Journal of Applied Ichthyology* **21:**(4) 242-262.

Green, M. C. (1952). A rapid method for clearing and staining specimens for the demonstration of bone. *Ohio Journal of Science.* **52** (1): 31-33.

Holčík J. (1998). *Ichtyológia*. Bratislava, Príroda, 310 pp.

Kostov, V., Ristovska, M., Prelić, D. & Slavevska-Stamenković, V. (2011): Assessment of the Ecological Status of the Crna River Based on the Fish Fauna -Contribution of the Establishment of the Monitoring System of Rivers in R. Macedonia. Macedonian Journal of Animal Science, **1** (1): 261–270.

Kottelat M. & Freyhof J. (2007). *Handbook of European freshwater fishes*. Publications Kottelat, Cornol, Switzerland and Freyhof. Berlin, pp 646.

Lundberg G. (1982). The comparative anatomy of the toothless blindcat, *Trogloglanis pattersoni* Eigenmann, with a phylogenetic analysis of the ictalurid catfishes. *Misc. Publ. Mus. Zool. Univ. Mich.,* **163**:1–85.

Marchetti M. P., Moyle P. B., & Levine R. (2004). Invasive species profiling? Exploring the characteristics of non-native fishes across invasion stages in California. *Freshwater Biology*, **49**: 646–661.

Movchan Yu. V., Talabishka E. M. & Velikopolskiy I. J. (2014). Fishes of the Genus *Ameiurus* (Ictaluridae, Siluriformes) in the Transcarpathian Water Bodies. *Vestnik zoologii*, **48**(2): 149–156.

Naumovski, M. (1995). Fishes in Macedonia. *Zhaki, Skopje*.

Nowak, M., Koščo, J., Popek, W., & Epler, P. (2010). First record of the black bullhead Ameiurus melas (Teleostei: Ictaluridae) in Poland. *Journal of fish biology*, *76*(6), 1529-1532.

Paruch W. (1986). Identification of Wisconsin catfishes (Ictaluridae): a key based on pectoral fin spines. *Transactions of the Wisconsin Academy of Sciences, Arts and Letters,* **74**: 58-62.

Pimentel, D., Lach, L., Zuniga, R., & Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *BioScience*,**50:(**1), 53-65.

Rojo A. (2013). Osteological Atlas of the Brown Bullhead (*Ameiurus nebulosus*) from Nova Scotia Waters: A Morphological and Biometric Study. *Curatorial Report* Number 100, Nova Scotia Museum, Halifax.

Rutkayová J., Biskup R., Harant R., Šlechta V. & Koščo J. (2013). *Ameiurus melas* (black bullhead): morphological characteristics of new introduced species and its comparison with *Ameiurus nebulosus* (brown bullhead) Rev. Fish. Biol. Fisheries (2013) **23**:51–68.

Scott, B. & Crossman J. (1973). Freshwater fishes of Canada. *Bull. Fish. Res. Board* Canada **184**: 1-966.

Simonović P., Tošić A., Vassilev M., Apostolou A., Mrdak D., Ristovska M., Kostov V., Nikolić V., Škraba D., Vilizzi L. & Copp G.H. (2013). Risk assessment of non-native fishes in the Balkans Region using FISK, the invasiveness screening tool for non-native freshwater fishes. *Mediterr Mar Sci.* **14**:369–376.

Taylor W. R. & Van Dyke G. C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, **9**:107-119.

Traynor D., Moerke A. & Greil R. (2010). Identification of Michigan fishes using cleithra. Great Lakes Fish. *Comm. Misc. Publ.* 2010-02; pp. 53.