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GENETIC VARIABILITY OF TWO EVOLUTIONARY DISTINCT CLASSES OF THE ACANTHOCEPHALA ISOLATED FROM EUROPEAN CHUB FROM RIVERS OF THE ADRIATIC AND BLACK SEA BASINS IN CROATIA

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ABSTRACT

The aim of our study was to investigate the genetic variability of two members of two evolutionary different classes of the Acanthocephala, Eoacanthocephala (*Neoechinirhynchus* sp.) and Palaeacanthocephala (*Acanthocephalus* sp.) from the same host, *Squalius cephalus*, from rivers of the Adriatic (rivers in Lika region: Lika, Novčica, Bogdanica and Jadova) and the Black Sea basins (Medsave location on the Sava River) in Croatia. The samples were collected from May 2021 to July 2022. To put the genetic position of the analyzed acanthocephalans in a broader context, the COI sequences of other specimens from the NCBI GenBank were used. BLAST analysis of partial COI sequences of acanthocephalans showed the presence of two different species: *Acanthocephalus* sp. and *Neoechinorhynchus* sp. *Acanthocephalus* sp. specimens did not show different grouping related to geographical origin (Bogdanica, Lika, Jadova) or Black Sea basin (Medsave-Sava River). *Acanthocephalus* cluster was subdivided into five main subclusters and 13 haplotypes were determined. When we compared our haplotypes with those from the NCBI GenBank, our haplotypes were closer to the *A. anguillae* specimens from the Kupa River and Dobra River in Croatia, and also to the haplotypes from Austria. For the species *Neoechinorhynchus* sp. from the Lika River and the specimens from the NCBI GenBank, four haplotypes were found out of five specimens. This genus was recorded for the first time in the Lika River area and for the first time in European chub in Croatia. The closest species *Neoechinorhynchus* sp. from Austria and Finland were grouped separately. Due to the lack of NCBI GenBank data for this species from other geographical regions, our data could not be compared. This shows that the taxonomy of the genus *Neoechinorhynchus* from Europe remains a challenge and underlines the need for further DNA sequence data and in-depth morphological study.

Keywords:
Eoacanthocephala
Neoechinirhynchus sp.
Palaeacanthocephala
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Lika Region
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INTRODUCTION

The Acanthocephala, thorny-headed worms, are a small monophyletic taxon of endoparasites with about 1,200 species presently known (Smales, 2015). They are ubiquitous obligatory intestinal worms encountered in a range of taxonomically diverse host species, from aquatic to terrestrial habitats. These intestinal parasites are commonly transmitted to vertebrate definitive hosts by aquatic crustacean prey serving as intermediate hosts (Kennedy, 2006). Adult acanthocephalans attach themselves to the intestinal wall of the final host with their hooks and take nutrients through their body surface (Starling, 1985). Although most intestinal parasites of fish do not cause significant damage to the intestine due to shallow surface contact with the tissue, acanthocephalans can have a pathogenic effect and cause significant damage to the intestinal wall as a result of the proboscis with which they penetrate deeply through the intestinal wall and due to the high density of individuals (Dezfuli et al., 2002; Lewisch et al., 2020). After mating, an acanthor larva develops from the fertilized egg in the body cavity of the female (Parshad and Crompton, 1982). Acanthors are released into the host's digestive tract and exit the aquatic environment via feces. Rarely, the entire female is released in the digestive tract and then comes out with feces. Its decomposition releases mature acanthors into the water environment. The life cycle continues when they are eaten by a suitable intermediate host (Grabner et al., 2023).

One of the main problems in studying the species distribution of the Acanthocephala is their complex taxonomy, which can lead and/or has led to misidentifications in the past (Perrot-Minnot et al., 2023). The interpretation of the evolutionary relationships of the Acanthocephala began in the confusion that has only recently begun to be clarified (Smales, 2015). This complexity is due to their sparse morphological characters and high intraspecific variation (Kennedy, 2006). To address these issues, molecular markers such as ribosomal DNA (small 18S and large 28S subunits and internal transcribed spacers 1 and 2, ITS) and mitochondrial (mt) cytochrome c oxidase subunit 1 (COI) are used (Perrot-Minnot, 2004; Steinauer et al., 2007; Wayland et al., 2015; Perrot-Minnot et al., 2018; Pinacho-Pinacho et al., 2018; Lewisch et al., 2020; Reier et al., 2020; Rosas-Valdez et al., 2020; García-Varela et al., 2023; Vardić Smrzlić et al., 2023).

In the river basins of continental Europe, the most common fish-parasitizing acanthocephalans belong to the class Palaeacanthocephala, order Echinorhynchida, members of the family Echinorhynchidae and Pomphorhynchidae, with 22 recognised species (Gibson et al., 2014; Reier et al., 2020). Only one member of the class Eoacanthocephala, order Neoechinorhynchida, family Neoechinorhynchidae, namely *Neoechinorhynchus rutili* (Müller, 1780), is also widely distributed throughout Europe (Moravec, 2004). Croatia is located on the Balkan Peninsula and its continental biogeographical region is bounded by the rivers of the Black Sea basin (Danube drainage, e.g. Sava), while the rivers of the Mediterranean biogeographical region have direct (e.g. Krka) or underground (e.g. Lika) connections to the Adriatic Sea (Žganec et al., 2020). The Balkan Peninsula is considered a hotspot in the evolution of many European species (Žganec et al., 2016), and this is also reflected in the diversity of intermediate and final fish hosts of the Acanthocephala. However, the increase of alien and invasive species that can serve as intermediate (Kralj et al.,2022) and final hosts (Ćaleta et al., 2019) of the Acanthocephala in Croatian freshwaters could change species diversity in Croatian freshwater systems. Members of the families Echinorhynchidae, Pomphorhynchidae, Neoechinorhynchidae and Illiosentidae have been identified morphologically or by using molecular methods with at least 5 genera since their first record in 1935 (Babić, 1935): Pomphorhynchus, Acanthocephalus, Echinorhynchus, Neoechinorhynchus and Dentitruncus (Šinžar, 1955; Topić-Popović et al., 1999; Mladineo et al., 2009; Filipović Marijić et al., 2013, 2014; Vardić Smrzlić et al., 2013, 2015, 2023; Mijošek et al., 2022). Although the genus *Acanthocephalus* is widespread in Europe and has recently been the subject of intensive research, it has hardly been studied at the level of genetic variability (O`Mahony et al., 2004; Benesh et al., 2006; Amin et al., 2019; Reier et al., 2020; Vardic Smrzlić; 2023).

The aim of our study was to investigate the genetic variability of two members of two evolutionary different classes of the Acanthocephala: Eoacanthocephala (*Neoechinirhynchus* sp.) and Palaeacanthocephala (*Acanthocephalus* sp.) from the same host, *Squalius cephalus*, from rivers of the Adriatic and Black Sea basins in Croatia.

MATERIALS AND METHODS

Study area

Sava River

The one location on the Sava River tributary of the Danube River basin was chosen to capture fish species from the native habitat as a control site. The Medsave (45°50'04.0”N, 15°46'28.3"L) was the site on the Sava River (Fig. 1). At a stretch between the town of Krško (Slovenia) and the city of Zagreb (Croatia), the Sava River is characterized by fast-flowing water, a mean annual water temperature of 14 °C, a rip-rap riverbank and a river bed covered with gravel (Piria et al., 2019).

Lika region

In the Lika region, the focus was on the following four rivers: Lika, Novčica, Bogdanica and Jadova (intermittent river). The Bogdanica River is a left tributary of the Novčica, while the Novčica is one of the main tributaries of the Lika (left) and the Jadova (right). The River Lika flows into the Kruščica reservoir. The dam of the Kruščica reservoir was built in the Lika Canyon (Ličko Polje). Lake Kruščica was created by the construction of a dam on the Lika River in 1971 for the need of Senj HPP (https://sru-lika-gospic.hr/ribolovna-podrucja).

The Lika River covers an approximate area of 1570 km^2 and a length of 78 km, and rises at the foot of the Velebit Mountains in the southern part of Ličko Polje (Bonacci and Andrić, 2008; Jelić et al., 2016). The study location on the Lika River was in the small village of Bilaj (44°51'45.1″N, 15°42'29.0"E). The second river is the Novčica and the sampling location was in the town of Gospić (44°54'28.8″N, 15°37'19.3 "E). The source of the Jadove River is around Gornja Ploča. The total length of the River Jadova is 41.2 km (https://sru-lika-gospic.hr/ribolovna-podrucja). Most of the course of the Jadova lies within Natura 2000 ecological network and the name of the area is HR2001272 Jadova (NN 80/2019). The study locations on the Jadova River were in the small villages of Barlete (44°53'24.6″N, 15°46'90.7"E) and Vrebac (44°52'07.21″N, 15°51'22.84"E). In early spring and late autumn, the river swells considerably, while in the summer months it dries up completely. Many streams flow into the Jadova River. The Bogdanica River is 8.4 km long and is formed by the confluence of mountain streams from Velebit (https://sru-lika-gospic.hr/ribolovna-podrucja). The study location on the Bogdanica River was near the small village of Bogdanić (44°55'16.3″N, 15°32'24.8"E).

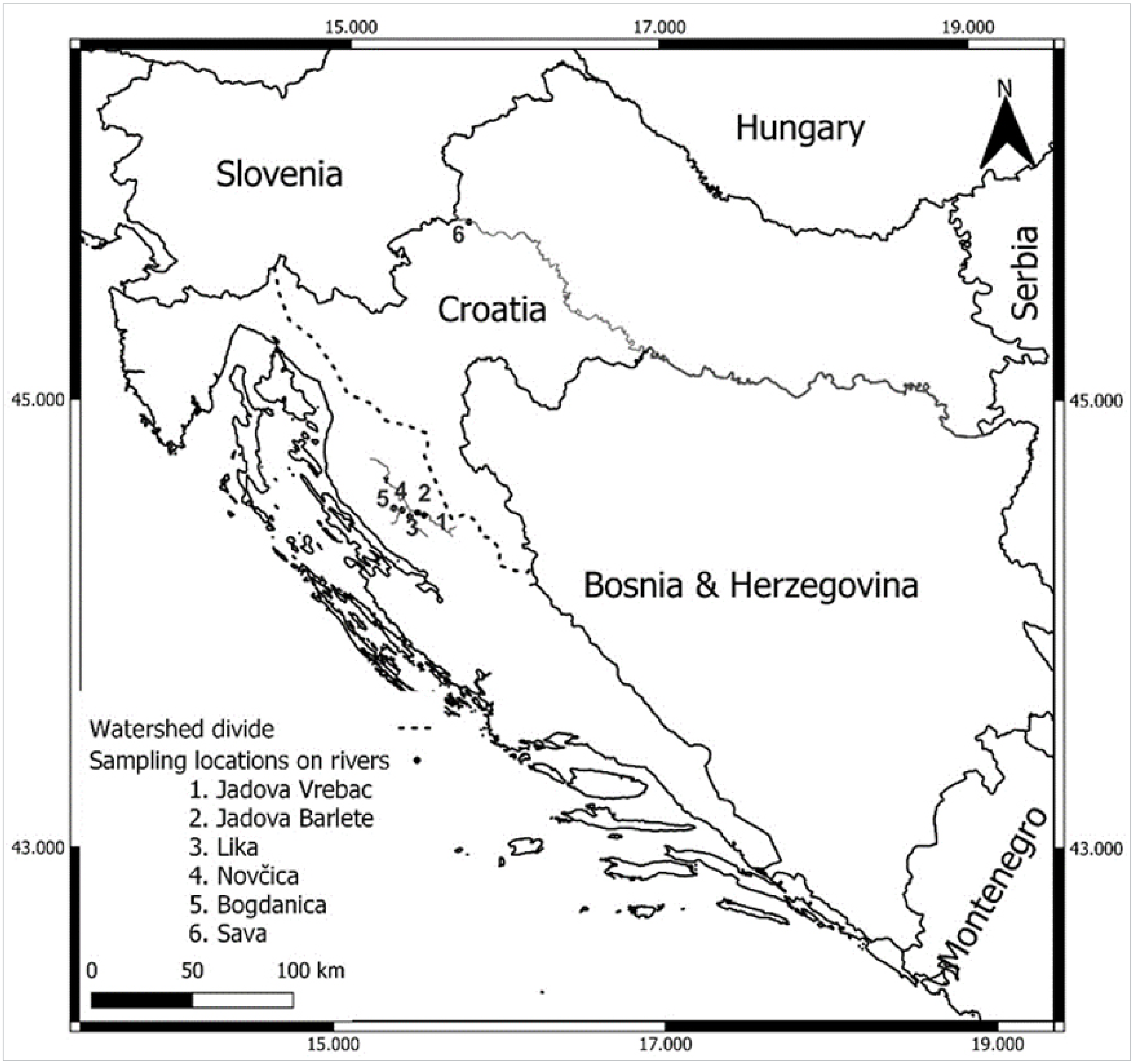

Fig 1. Map of the research area with the sampling locations

Fish sampling

From May 2021 to July 2022, monthly samples were collected from the Sava and the rivers Lika, Novčica, Bogdanica and Jadova. Sampling was performed by electrofishing (Hans Grassl EL 63 II, 220/440 V, 17.8/8.9 A) with a rounded Ø50 cm stainless steel anode and a net with a mesh size of 10 mm for the sampling in accordance with HRN EN 14011:2005. Each sampled fish stunned by electroanesthesia was euthanized and transported to the laboratory for further processing. Following dissection, parasite specimens found in the gut were collected and fixed in 96% ethanol for morphological identification (Bauer, 1985) and molecular analyses (Vardić Smrzlić et al., 2015). This study included all isolated acanthocephalans except the species *Pomphorhynchus laevis*, which was described separately (Radočaj et al., 2024).

Molecular method analysis

For molecular analysis, the acanthocephalans were separated from fish host guts and washed in phosphate buffer. The proboscis was removed under a magnifying glass to prevent contamination with fish material. DNA isolation was performed using the Quick-DNA Microprep Kit (Zymo) from each parasite, which had previously been homogenised using a TissueLyser LT homogeniser (Qiagen). The isolated DNA from the parasites served as a template in the PCR reaction to amplify a portion of the cytochrome oxidase subunit I gene (COI). The reaction mixture had the following composition: 21 µl sterile water, 25 µl PCR buffer, 1 ql of each primer (20 µM) and 50 ng template DNA. The reaction conditions were: initial denaturation (95 °C for 10 min), 35 cycles of 30 s at 94 °C (denaturation), 45 s at 46 °C or (annealing) and 1 min at 72 °C (extension), and a final extension of 10 min at 72 °C. The PCR products visualised on a 1.5% agarose gel (Sigma) were then sent to Macrogen Europe Inc. for commercial nucleotide sequencing.

The sequences obtained were compared with similar sequences from the NCBI GenBank using NCBI BLAST software. They were then aligned using the Clustal W programme and aligned sequences were used for phylogenetic tree construction in MEGA 11 software (Tamura et al., 2021). The evolutionary history was inferred by using the Maximum Likelihood methods and HKY model. The median-joining (MJ) haplotype network for *Acanthocephalus* sp. and *Neoechinorhynchus* sp. were created using the PopART 1.7 software (http:// www. popart. otago. ac. nz, Leigh & Bryant, 2015) (Bandelt et al., 1999). To compare our sequences with the dataset from the NCBI GenBank, we used sequences under the following accession numbers: MT682931, MT682932, MT682934; MN780911-MN780919 for *Acanthocephalus* sp. and MN780975, OR832864; KF156883-KF156885, KF156888 and KF156890 for *Neoechinorhynchus*. For rooting in the phylogenetic tree, we used *Moniliformis moniliformis*, a member of the different class of the Acanthocephala.

RESULTS

BLAST analysis of partial COI sequences of acanthocephalans found in the intestine of *S. cephalus* from examined rivers showed the presence of two different species, *Acanthocephalus* sp. and *Neoechinorhynchus* sp.. Phylogenetic analysis based on partial COI sequences confirmed the separate grouping of *Acanthocephalus* sp. and *Neoechinorhynchus* sp. isolated from different rivers (Figure 2). *Neoechinorhynchus* sp. was found only in the River Lika and was most closely grouped with an Austrian isolate (Figure 2). Isolates from Russia, including *N. simansularis*, were grouped separately (Figure 2). *Acanthocephalus* sp. specimens did not show different grouping related to geographical origin (rivers of the Adriatic basin: Bogdanica, Lika, Jadova; or the Black Sea basin: Medsave-Sava River) (Figure 2). The *Acanthocephalus* cluster was subdivided into five main subclusters. The first consisted only of *A. anguillae* species from Austria and Germany. Specimens of *Acanthocephalus* sp. from the Croatian rivers Sava, Bogdanica, Jadova and Lika were these isolates distributed within 5 subgroups (marked with asterisks), of which they independently form 3 subgroups, in one they are grouped with isolates from Austria, and in another there is only one isolate from Bogdanica, which was grouped with *A. anguillae* from the Kupa and Dobra rivers in Croatia.

MJ network analysis of *Acanthocephalus* sp. from our study, together with those from NCBI GenBank based on COI sequences, showed the presence of 24 haplotypes (out of 43 specimens) and is roughly divided into five clusters that do not reflect a clear geographical structuring (Fig. 3a). Out of 23 specimens analysed in our study, 13 haplotypes were determined (Fig. 3a). One main haplotype (Hap 1 in Fig. 3a) included specimens found in all four rivers (Sava, Bogdanica, Jadova and Lika). The specimens from the Bogdanica River showed the greatest diversity, as they were distributed in several subgroups together with geographically distinct isolates: the largest with the Sava River specimens, the second with Austria and the Jadova River, and one with *A. anguillae* from the Kupa River. When we compared our haplotypes to others from the NCBI GenBank (Fig. 2 and 3), we found our haplotypes were most closely related to the *A. anguillae* specimens from the Kupa River and Dobra River in Croatia, and also to haplotypes from Austria (Figure 2 and 3a).

The haplotype network of *Acanthocephalus* sp. based on COI dataset showed low nucleotide diversity and higher haplotype diversity, similar to the other species studied. A value of nucleotide diversity $\pi = 0.01019$ is partly due to missing haplotypes connecting clusters. A value of haplotype diversity $Hd = 0.941$ is due to the large number of haplotypes shared by only one or two individuals and sometimes separated by more mutational steps (Fig. 3a).

For the species *Neoechinorhynchus* sp. from the River Lika and others from the NCBI GenBank, 4 haplotypes were found out of 5 specimens (Fig. 3b).

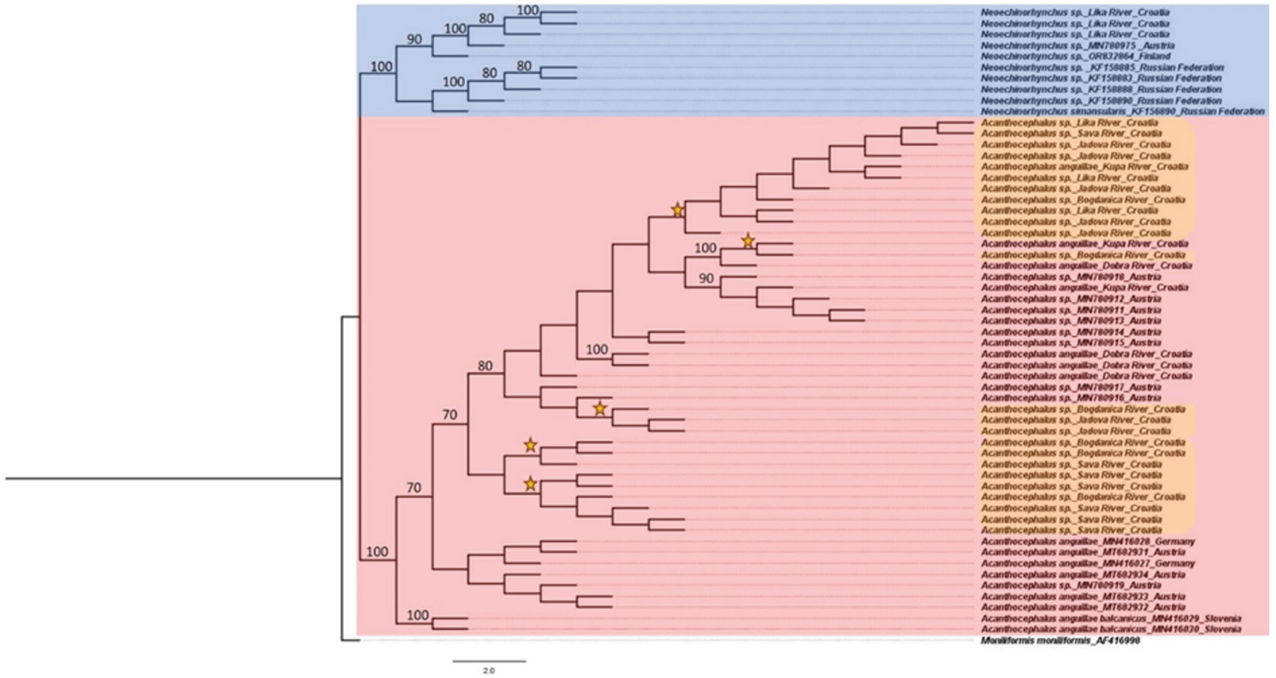

Fig 2. Phylogenetic analysis of the members of two Acanthocephala genera from Croatia based on the partial COI marker sequence (610 bp). The evolutionary history was inferred using the maximum likelihood method and the Hasegawa-Kishino-Yano model in MEGA11. *Neoechinorhynchus* specimens were marked by blue and *Acanthocephalus* by red. Asterisks show clusters with specimens from our study.

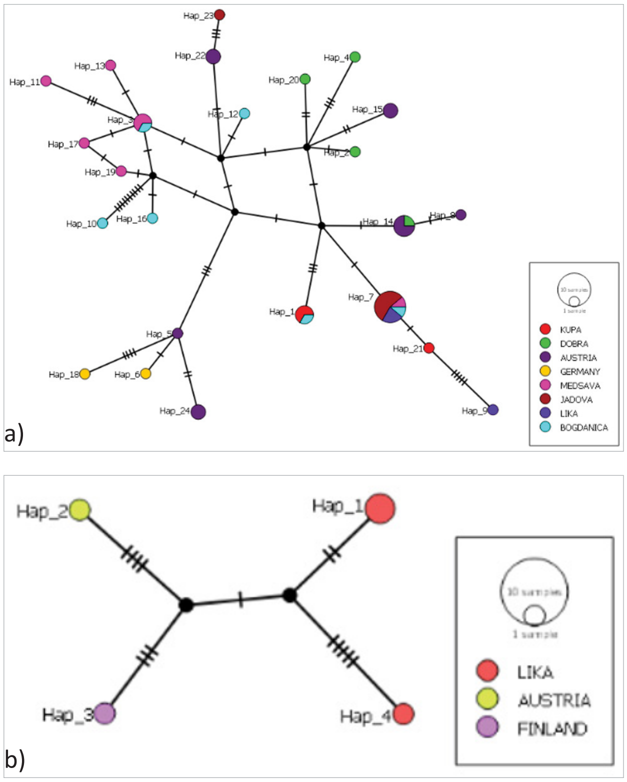

Fig 3. Median-joining networks of *Acanthocephalus* (a) and *Neoechinorhynchus* (b) specimens were performed in PopART 1.7. Mutation steps are indicated by vertical lines. Black dots represent haplotypes missing in the study sample. Coloured dots represent haplotypes from different sites, whereas the size of the dot indicates the number of haplotypes.

In our study, three specimens from the Lika River formed 2 haplotypes, which were separated by missing haplotype and more mutational steps (Fig. 3b). However, the closest species, *Neoechinorhynchus* sp. from Austria and Finland which originate from the NCBI GenBank database, were grouped separately, also by one missing haplotype and more mutational steps (Figure 3b). Due to missing NCBI GenBank data for this species from other geographical regions, we could not compare our data to those from the NCBI GenBank. For such a small dataset, nucleotide diversity was small (π = 0.01073) and haplotype diversity was higher (Hd = 0.900), due to missing haplotypes and one individual – one haplotype relationship.

DISCUSSION

During the study of intestinal parasitofauna of *S. cephalus* from different Croatian rivers of the Black Sea (Medsave – Sava River) and the Adriatic Sea basins (Lika, Jadova and Bogdanica), two different acanthocephalan species were found, one from *Acanthocephalus* and another from the genus *Neoechinorhynchus*. As specimens from both species had their proboscis invaginated or damaged, their complete morphological identification was impossible, and the species remained undetermined. In our research, the species of the genus *Neoechinorhynchus* was recorded for the first time in the area of the Lika River, and for the first time in European chub in Croatia.

The genus *Acanthocephalus* has long been studied in the countries of former Yugoslavia (Babić, 1935; Šinžar, 1955; Vardić Smrzlić et al., 2023), and two main species have been described: *Acanthocephalus anguillae* and *Acanthocephalus lucii*. In the previous study on the genus *Acanthocephalus* from the Dobra and Kupa rivers in Croatia, the species *A. anguillae* was determined. It was closely related to the *Acanthocephalus* sp. from Austria which were also undetermined and closely related to the specimens from our study. However, without morphological analysis and due to the results of Reier et al. (2020) who found that a COI sequence of several Austrian specimens of the genus *Acanthocephalus* differed by a high genetic distance from *A. anguillae* and *A. lucii*, we prefer to leave our species undetermined. It is possible that there are unknown *Acanthocephalus* species or subspecies of *A. anguillae*.

As well as the genus *Acanthocephalus*, the genus *Neoechinorhynchus* has also long been studied in the southeastern European region (Babić, 1935; Šinžar, 1956; Brglez, 1962; Ćorić, 1963; Vardić Smrzlić et al., 2010). *Neoechinorhynchus rutili* was predominantly recorded (Babić, 1935; Šinžar, 1956; Vardić Smrzlić et al., 2010), while Ćorić (1963) found *N. agilis* in Bosnia and Herzegovina. *N. rutili* is widely distributed in Central Europe (Moravec, 2004), but recent investigation showed high biodiversity and cryptic speciation of the genus *Neoechinorhynchus* in Central America (Pinacho-Pinacho et al., 2018). The taxonomy of the genus *Neoechinorhynchus* from Europe remains challenging and highlights the need for more DNA sequence data and in-depth morphological examination.

Some species of the Acanthocephala have been analyzed at the DNA level, but few such analyses have been carried out on fish in central Europe. In Croatia, until now, fish from the Krka River (Vardić Smrzlić et al. 2013) and the Sava (Filipović Marijić et al., 2013; Smrzlić et al., 2015; 2023) have been analyzed in detail using molecular markers. The results published by Mujakić (2014) indicated the existence of great biodiversity of the Acanthocephala in the rivers of Lika-Senj County, as at least two different species of the Acanthocephala were found in each river: *E. salmonis* and *N. rutili* in the Gacka and Matica rivers, *A. lucii* and *A. anguillae* in the Novčica River, *P. laevis*, *P. tereticollis* and *D. truttae* in the River Una. The hookworm species was characteristic of the host fish; *E. salmonis*, *N. rutili* and *D. truttae* were found in trout (brown and/or rainbow), while *A. lucii* and *A. anguillae* were identified in pike (northern), *P. laevis* in chub, common carp, bream, gibel carp, European perch and ide, and *P. tereticollis* in catfish (wels). The authors interpret the great biodiversity of the Acanthocephala in the rivers of Lika-Senj County as a probable consequence of the quality and cleanliness of water, which are necessary for the settlement of different species of crustaceans that are their intermediate hosts.

Non-native parasitic freshwater fish fauna can be introduced into the new area together with the translocated fish. Fish translocations between the Danube and Adriatic basins in Croatia are not well documented, and the origin, path or reason for translocation for many species is still unclear (Pofuk et al., 2017). The limited basin of the Adriatic - the Lika plateau, especially the Lika River, was stocked with carp *Cyprinus carpio* between the 1930s and 1950s (Piria et al., 2016). The repeated stocking of carp resulted in the accidental translocation of several mainly cyprinid fish species that are autochthonous in the Danube basin but not in the Adriatic basin, among which was chub *Squalius cephalus* (Linnaeus 1758) (Piria, 2016; Plančić, 1946). The introduction of non-native parasite species is often associated with negative implications for aquaculture production and wild native fish species (Pofuk, 2021). For example, for the two investigated European chubs infected with *Neoechinorhynchus* sp. in Austria, where the infection intensity was high (>50 parasites in one) and medium (10-50 parasites per fish in another), Lewisch et al. (2020) reported that main pathological findings were granulomas in serosa and anal prolapse. This threat to the health of native fish species in Croatia has not yet been extensively studied, despite a long history of introducing and moving foreign fish species into inland waters. Nowadays, these translocations mostly occur for recreational fishing and as such the introduction of parasites poses a serious threat. Anal prolapse was only found in the sample with high infection intensity (Lewisch et al., 2020). The fact that *Neoechinorhynchus* sp. was found in European chub only in the Lika River with four haplotypes out of five specimens but not in its tributaries can probably be attributed to the recent introduction of this parasite to the area. Future research should include a larger number of samples both from tributaries and other geographical areas in order to obtain more detailed data on *Neoechinorhynchus* sp. In addition to the molecular analysis, a detailed morphological description of this species is recommended. Furthermore, the inclusion of ecological data from sampling sites would help understand the impact of the environment on its genetic variability.

Despite the aforementioned fact that the taxonomy of the genus *Neoechinorhynchus* from Europe still presents a challenge and that this genus should be studied in detail due to the lack of DNA sequences, these findings emphasize that stocking protocols that include fish health controls and biosecurity measures should be developed and properly monitored.

CONCLUSION

During the study of intestinal parasitofauna of *S. cephalus* from Croatian rivers of the Black Sea (Medsave – Sava River) and Adriatic Sea basins (Lika, Jadova and Bogdanica), two different acanthocephalan species were found, one from the genus *Acanthocephalus* and another from *Neoechinorhynchus*. Phylogenetic analysis based on partial COI sequences confirmed the separate grouping of *Acanthocephalus* sp. and *Neoechinorhynchus* sp. isolated from different rivers. *Acanthocephalus* sp. specimens did not show different grouping related to geographical origin (Bogdanica, Lika, Jadova) or Black Sea basin (Medsave-Sava River). The cluster of these acanthocephalans was subdivided into five main subclusters, and 13 haplotypes were determined. Comparison of these haplotypes with others from the NCBI GenBank showed that haplotypes found in this study were most closely related to the *A. anguillae* specimens from the Kupa River and Dobra River in Croatia, and haplotypes from Austria.

For *Neoechinorhynchus* sp. from the River Lika and those from the NCBI GenBank four haplotypes were found out of five specimens. Three specimens from the Lika River in this study formed two haplotypes separated by the lack of a haplotype and more mutational steps. Due to the lack of NCBI GenBank data for this species from other geographical regions, we could not compare our data to those of the NCBI GenBank. These results show that the taxonomy of the genus *Neoechinorhynchus* from Europe remains a challenge and underlines the need for further DNA sequence data and in-depth morphological examination.

The genus *Neoechinorhynchus* was first recorded in the Lika River, and for the first time in European chub in Croatia. The fact that *Neoechinorhynchus* sp. was found in European chub only in the Lika River but not in its tributaries could most likely be attributed to the recent introduction of this parasite to the Lika area. Despite the aforementioned fact that the taxonomy of the genus *Neoechinorhynchus* from Europe still presents a challenge and that this genus should be studied in detail due to the lack of DNA sequences, this probable recent introduction of *Neoechinorhynchus* sp. as a consequence of fish stocking from other areas emphasized that stocking protocols that include fish health controls and biosecurity measures should be developed and properly monitored. The closest species *Neoechinorhynchus* sp. from Austria and Finland obtained from the NCBI GenBank database were grouped separately.

GENETSKA VARIJABILNOST DVA EVOLUCIJSKI RAZLIČITA RAZREDA ACANTHOCEPHALA IZOLIRANA IZ KLENA IZ RIJEKA JADRANSKOG I CRNOMORSKOG SLIJEVA U HRVATSKOJ

SAŽETAK

Cilj našeg rada bio je istražiti genetsku varijabilnost dvaju pripadnika evolucijski različitih klasa Acanthocephala, Eoacanthocephala (*Neoechinirhynchus* sp.) i Palaeacanthocephala (*Acanthocephalus* sp.), iz istog domaćina, *Squalius cephalus*, iz rijeka koje pripadaju jadranskom (rijeke u regiji Lika: Lika, Novčica, Bogdanica i Jadova) i crnomorskom slivu (lokacija Medsave na rijeci Savi) u Hrvatskoj. Uzorci su prikupljani od svibnja 2021. do srpnja 2022. Kako bi se genetski položaj analiziranih akantocefala stavio u širi kontekst, korištene su COI sekvence drugih uzoraka iz NCBI banke gena. BLAST analiza djelomičnih COI sekvenci acanthocephla pokazala je prisutnost dvije različite vrste: *Acanthocephalus* sp. i *Neoechinorhynchus* sp. *Acanthocephalus* sp. primjerci nisu pokazali različito grupiranje vezano uz geografsko podrijetlo (Bogdanica, Lika, Jadova) ili crnomorski sliv (rijeka Medsave-Sava). Klaster *Acantocephalus* sp. je podijeljen u pet glavnih podklastera, te je utvrđeno 13 haplotipova. Kada smo usporedili naše haplotipove s onima iz NCBI banke gena, naši haplotipovi bili su slični primjercima *A. anguillae* iz rijeke Kupe i Dobre iz Hrvatske, a također i haplotipovima iz Austrije. Za vrstu *Neoechinorhynchus* sp. iz rijeke Like i one iz NCBI banke gena, od pet primjeraka pronađena su četiri haplotipa. Ovaj rod je prvi put zabilježen na području rijeke Like, i prvi put kod klena u Hrvatskoj. Najsličnije vrste *Neoechinorhynchus* sp. iz Austrije i Finske grupirane su zasebno. Zbog nedostatka podataka u NCBI banci gena za ovu vrstu podaci iz drugih geografskih regija nisu se mogli usporediti s našim podacima. To pokazuje da taksonomija roda *Neoechinorhynchus* iz Europe ostaje izazov i naglašava potrebu za više podataka o sekvencama DNK i dubinskim morfološkim ispitivanjem.

Ključne riječi: endoparaziti, Lika, Rijeka Sava

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