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Review paper

GEOMETRIC MORPHOMETRY IN ICHTHYOLOGICAL RESEARCH

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Summary

Classical morphometry has long been used as a standard method in the studies of morphometric characteristics of organisms. However, in the last two decades of 21 century, the application of geometric morphometry has increased rapidly because of advances in quantitative biological shape analysis. Its development is accompanied by the development of new technologies, primarily computer software. Geometric morphometry can be defined as a fusion of geometry, biology and computer science, and enables the study of biological forms in two-dimensional or three-dimensional space. Morphometric characters indicate the body proportions as well as the proportions of individual body parts that are measured using certain measurement units. The aim of this paper is to present geometric morphometry and explain its application in ichthyological research, and point out its advantages and possibilities, in a practical way.

Key words: geometric morphometry, ichthyology, bioinformatics, integrated taxonomic approaches

INTRODUCTION

Morphology is one of the oldest biological disciplines, which basically includes describing morphological units, their comparative study or comparison, understanding morphological units in relation to their function, then drawing conclusions, and determining the nature of connections between morphological units (Ivanović and Kalezić, 2009). In recent decades, great progress has been made in the field of morphometry, based on the application of geometric morphometry in various fields of research such as systematics and evolutionary biology (Webster and Sheets, 2010). The advantage and popularity of geometric morphometry is contained in the possibility of geometric representation of a large number of data that are easier to understand. This technique has a high statistical sensitivity, which means that it can detect small changes in morphological forms, which could not be determined using classical morphometry (Klingenberg, 2002).

Traditional morphometry experienced its full development between the 1960s and 1970s, and then provided a multitude of multivariate statistical tools to describe shape variations within and between different groups; however, there were still many shortcomings in these analyses (Adams *et al*., 2004). In this regard, there was a need to develop alternative methods for quantification and analysis of morphological forms, among which „truss network and geometric morphometry stands out.

The introduction of the "truss network" method in ichthyological research leads to much better results in the interpretation of biological differences. This method is based on measurements within the network that are obtained by setting certain landmarks. In this way, a system of adjacent cells or quadrangles is formed according to the shape of the body. In that way, a certain match in the morphology of different populations can be observed (Abhijit *et al*., 2020). Morphometric differences among the species, obtained in this way, have been recognized as an important tool for estimating population structure but also fish stocks (Rawat *et al*., 2017).

The geometric morphometric revolution has added to the sophistication of quantitative biological shape analysis, while at the same time making it easier to collect and analyse data to answer questions about shape of the phenotype (Laving and Polly, 2009). Geometric morphometrics presents the analysis of Cartesian geometric coordinates of morphological structures rather than linear, areal, or volumetric variables (Adams *et al.* 2004). A heterogeneous set of tools are combined under the term geometric morphometrics. Tools are available to analyse landmark points, curves, outlines (in either two- or three-dimensions), and three-dimensional surfaces (Laving and Polly, 2009). It enables the analysis of sizes and shapes using a combination of univariate and multivariate statistical methods and methods of direct graphical representation (Sekulić, 2013).

Organisms may be similar or differ in shape or size due to age, sex, geographic location, phylogenetic relationship, disease, or preservation. Geometric morphometrics can identify shape differences and help explore the causes of variation within and between individuals (Laving and Polly, 2009). In this respect, numerous ichthyologists around the world have used geometric morphometry for various studies purposes (to differentiate species, to describe intraspecific differences or sexual dimorphism and/or population level differentiation) (Martinez *et al*., 2013; Bower and Piller, 2015; Farré *et al*., 2016; Takács *et al*., 2018; Seth *et al*., 2019, Delariva and Neves, 2020; Sanchez-Gonzalez *et al*., 2021, etc.). The geometric morphometric can also be very useful for describing a new fish species as well (Chakrabarty *et al*., 2010).

Geometric morphometric can be integrated with other approaches of taxonomy to resolve systematic and phylogeny issues. In recent times, the molecular taxonomic approaches have gained momentum in numerous taxonomic studies. The standard mitochondrial DNA, COI barcode region (DNA bar coding) is very proficient for species identification. The use of multiple gene markers coupled with landmark-based morphometric analysis as integrated taxonomic approaches can be utilised in the future for the identification of species that are difficult to distinguish (Panda *et al*., 2021).

However, despite all the advantages offered by geometric morphometry in the study of fish populations, there is not much published research from the territory of Bosnia and

Herzegovina in which this technique is described or applied, so this paper can serve as a basis for future research (Bajrić, 2017).

The basic hypothesis of the paper was whether geometric morphometry has significant advantages over classical morphometry and whether its use is widespread among ichthyologists and other profiles of biologists in Bosnia and Herzegovina. Regarding the working hypothesis, the paper aimed to actualize geometric morphometry primarily among ichthyologists, to indicate ways of implementing methods of geometric morphometry as well as to present its possibilities through comprehensive literature data.

For the purposes of this paper, literature data dealing with this topic were used (Adams *et al*., 2004; Laving and Polly, 2009; Webster and Sheets, 2010; Rawat *et al*., 2017 etc.) as well as data from our previous research (Bajrić, 2017). This paper did not have an experimental character in the sense that geometric morphometry was applied to solve some specific ichthyological problem.

GEOMETRIC MORPHOMETRIC TECHNIQUES

To use geometric morphometry in ichthyology, it is necessary to take quality photographs of the studied fish. Precisely, all further research procedure is based on the manipulation of the obtained photographs, which are "passed" through various software packages (Table 1) in order to finally reach certain results on changing the shape and size of the studied fish species.

Table 1. Software packages most commonly used in geometric morphometry

Software	Purpose	Reference
Microsoft Office Excel 2007	- Range of variation -Value of variation, mean, standard deviation -Graphic representations	Microsoft Office
Statistica 10	- Descriptive statistics: -ANOVA- analysis of variance -Longitudinal-mass relations, -Graphic representations	StatSoft, Inc., (2011)
TPSDig	- Placing two-dimensional points	Rohlf (2010a)
TPSUtil	- Manipulation of tps files (merging documents and editing points)	Rohlf (2012)
TPSRelw	- Checking the accuracy of digitization	Rohlf (2010b)
CoordGen6f	- Procrustean analysis	Sheets (2000)
CVAGen6j	- Calculating values CS (Centroid Size) -CVA analysis	Sheets (2000)
MorphoJ	- Calculation of Procrustean distances -Visual display of shape change	Klingenberg (2011)
TwoGrup6h	- Calculation of distances between observed populations (Goodall's F test)	Sheets (2000)
Geomorph	-Manipulation and digitization of landmarks -Shape generation -Graphic description of shapes -Display shapes and variations	Adams *et al*., 2021

The process of gathering information, creating a database, and performing PCA[2] analysis of geometric morphometry has several stages.

The first step is certainly to collect the appropriate sample or catch fish. Then all individuals are photographed, with the obligatory use of a certain type of numeric system.

Photographing of individuals is carried out with the same camera and always from the same distance. The same side of the fish that is placed in the centre of focus is always photographed. It would be best to take a photo of fish immediately after the catch, but fish that have been previously frozen are most often used, which may ultimately have a disadvantage because thawed fish are difficult to place in their natural position, especially if they are small individuals. Photographs of fish for geometric morphometry should contain the designation of the locality or the number of the individual, which depends on the type of research.

The obtained photos need to be stored on a computer, thus creating the database needed to perform the analysis. Each photograph must be renamed as a code that will indicate the number of the individual, sex or name of the fish species, depending on the type of analysis. A separate folder is created for each population, for easier comparison.

The fact that many programs have improved since its introduction shows how current geometric morphometry is relevant in global research. In addition, some of the programs that we used in the research during 2016 and 2017 are no longer available (CordGen, CVA gne, Twogroup, etc.), but will certainly be mentioned in this paper.

One of the current and widely used packages in geometric morphometry is Geomorph (Adams *et al*., 2021). This package allows 2D and 3D manipulation of selected points.

The first in a series of programs for working with collected photos are programs from the tps group, which allow you to set landmarks, group them and save them for processing in the form of coordinates. These are the programs: TpsDIG, TpsUtil, TpsRelw.

TpsDIG allows you to define landmarks and save them in a format in which other programs can recognize them (Figure 1).

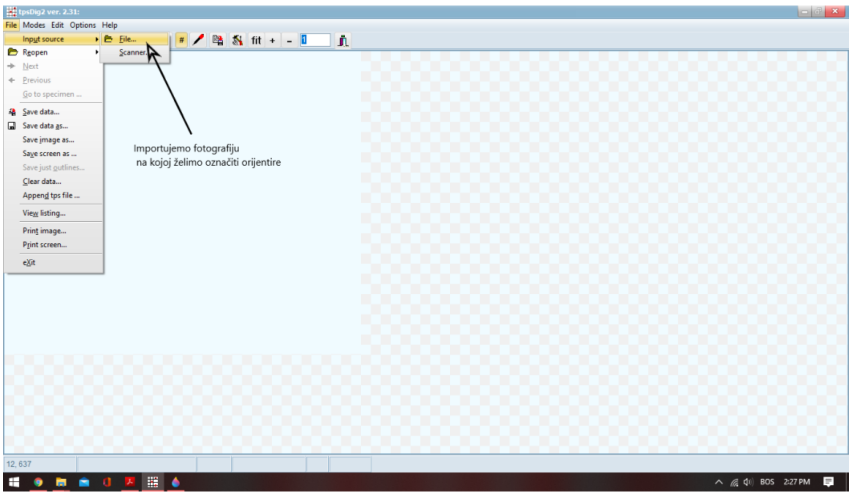


Figure 1. TpsDIG: photo import

Entering a photo into the program begins with marking the landmark using the icon on the toolbar that indicates "digitise landmarks". After marking the landmarks in the desired

[2] PCA analysis (principal component analysis) is the most common statistical procedure used in geometric morphometry.

places, the last two landmarks must be on a numerical system in order to be able to determine the scale on the basis of which the lengths are measured. This operation is performed on “Image tools” → “Measurements” (Figure 2).

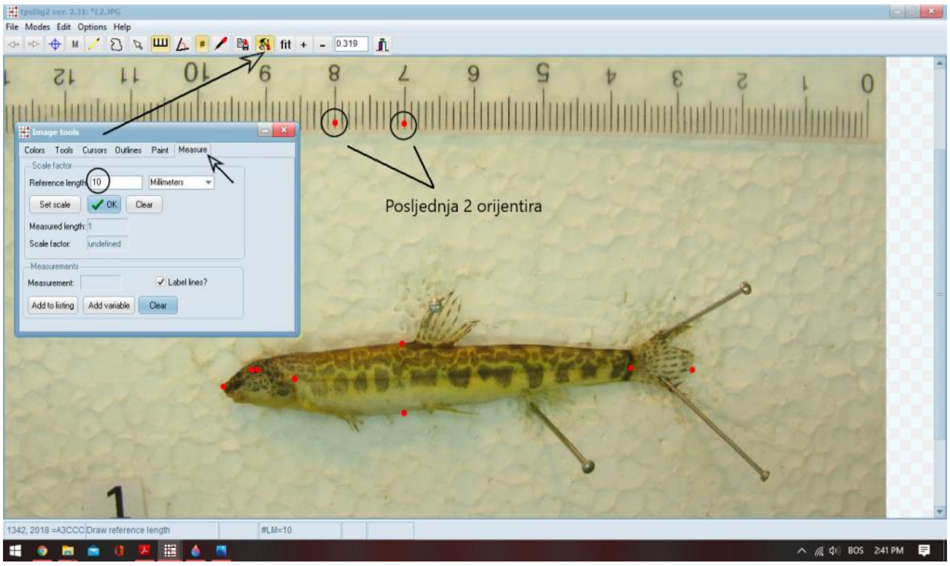


Figure 2. TpsDIG: entering landmarks

The next step in the process of processing photos for geometric morphometry is to merge all the separate photos into a single tps format, according to which it will be possible to check whether all landmarks, marked with the same numbers, are in the same positions. The merging of all individual tps formats is done using the TpsUtill program.

The last step in the tps group of programs is to check that all landmarks are set in the right place, which can be seen in the form of positioned points if the landmarks are set in the right positions or elongated vectors if an error has occurred (Figure 3). This part is done via tps relative wraps (TpsRelw).

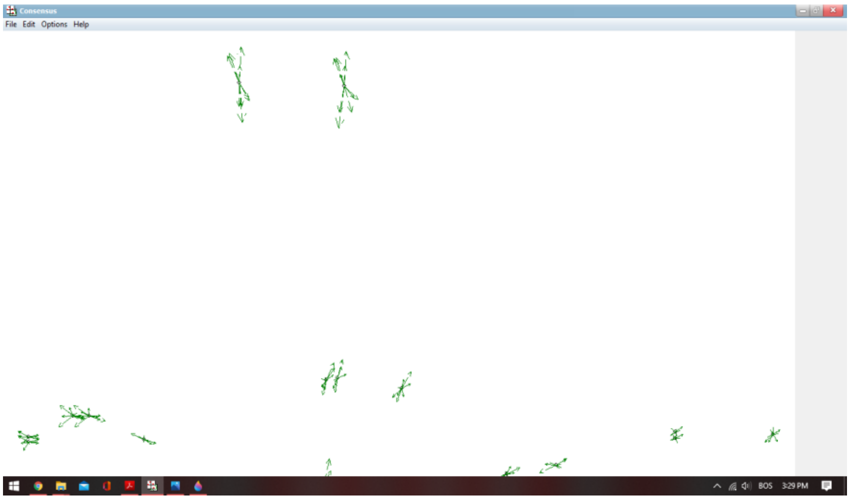


Figure 3. Vector overview and landmark deviations

Files created in the CoordGen8 program can also be used in Microsoft Excel, which is important for possible verification or some other calculations (Figure 4 and 5).

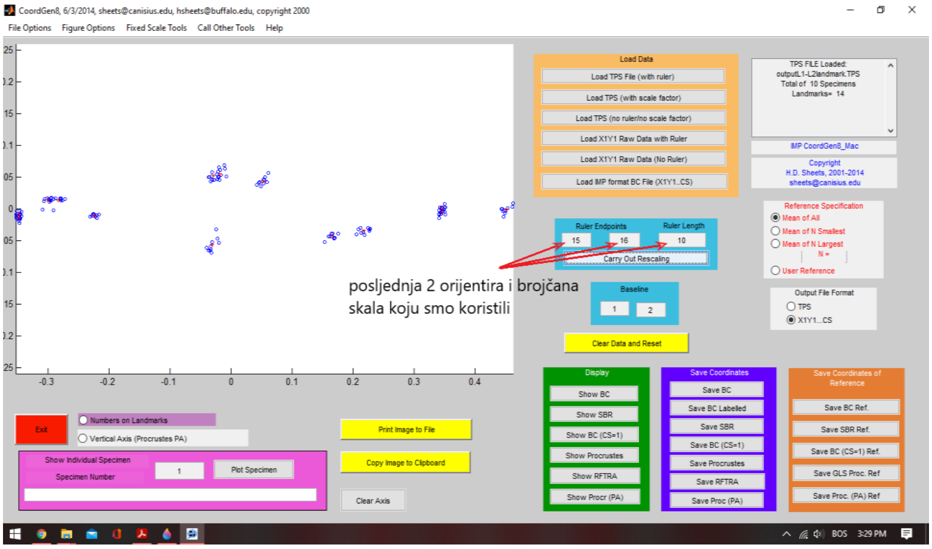
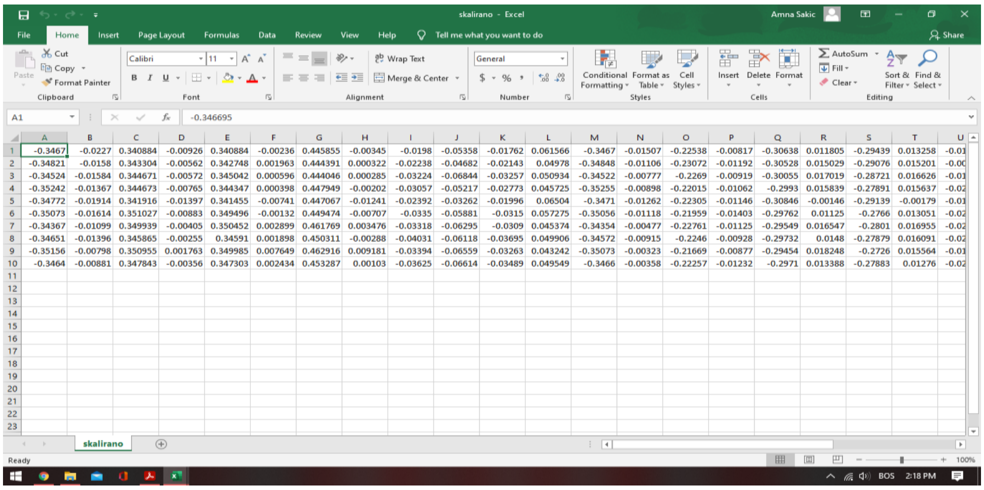


Figure 4. CoordGen8: landmark rescaling



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	-0.3467	-0.0227	0.340884	-0.00926	0.340884	-0.00236	0.445855	-0.00345	-0.0198	-0.05358	-0.01762	0.061566	-0.3467	-0.01507	-0.22538	-0.00817	-0.30638	0.011805	-0.29439	0.013258	-0.01
2	-0.34821	-0.0158	0.343304	-0.00562	0.342748	0.001963	0.444391	0.000322	-0.02238	-0.04682	-0.02143	0.04978	-0.34848	-0.01106	-0.23072	-0.01192	-0.30528	0.015029	-0.29076	0.015201	-0.00
3	-0.34524	-0.01584	0.344671	-0.00572	0.345042	0.000596	0.444046	0.000285	-0.03224	-0.06844	-0.03257	0.050934	-0.34522	-0.00777	-0.2269	-0.00919	-0.30055	0.017019	-0.28721	0.016626	-0.01
4	-0.35242	-0.01367	0.344673	-0.00765	0.344347	0.000398	0.447949	-0.00202	-0.03057	-0.05217	-0.02773	0.045725	-0.35255	-0.00898	-0.22015	-0.01062	-0.2993	0.015839	-0.27891	0.015637	-0.02
5	-0.34772	-0.01914	0.341916	-0.01397	0.341455	-0.00741	0.447067	-0.01241	-0.02392	-0.03262	-0.01996	0.06504	-0.3471	-0.01262	-0.22305	-0.01146	-0.30846	-0.00146	-0.29139	-0.00179	-0.01
6	-0.35073	-0.01614	0.351027	-0.00883	0.349496	-0.00132	0.449474	-0.00707	-0.0335	-0.05881	-0.0315	0.057275	-0.35056	-0.01118	-0.21959	-0.01403	-0.29762	0.01125	-0.2766	0.013051	-0.02
7	-0.34367	-0.01099	0.349939	-0.00405	0.350452	0.002899	0.461769	0.003476	-0.03318	-0.06295	-0.0309	0.045374	-0.34354	-0.00477	-0.22761	-0.01125	-0.29549	0.016547	-0.2801	0.016955	-0.02
8	-0.34651	-0.01396	0.345865	-0.00255	0.34591	0.001898	0.450311	-0.00288	-0.04031	-0.06118	-0.03695	0.049906	-0.34572	-0.00915	-0.2246	-0.00928	-0.29732	0.0148	-0.27879	0.016091	-0.02
9	-0.35156	-0.00798	0.350955	0.001763	0.349985	0.007649	0.462916	0.009181	-0.03394	-0.06559	-0.03263	0.043242	-0.35073	-0.00323	-0.21669	-0.00877	-0.29454	0.018248	-0.2726	0.015564	-0.01
10	-0.3464	-0.00881	0.347843	-0.00356	0.347303	0.002434	0.453287	0.00103	-0.03625	-0.06614	-0.03489	0.049549	-0.3466	-0.00358	-0.22257	-0.01232	-0.2971	0.013388	-0.27883	0.01276	-0.02

Figure 5. Data collected in CoordGene8 program and transferred to Microsoft Excel

The obtained changes in shape and size using geometric morphometry can be presented visually (Figure 6), and in this way to find out whether the differences between fish populations are statistically significant, all with the help of these computer programs. So that even the smallest change in the morphology of a certain part of the fish's body will be ascertained and visually presented. The obtained results can be used in interpopulation research of one species, determining morphometric differences between related species, but also researching the morphology of fish whose systematic position is not completely certain.

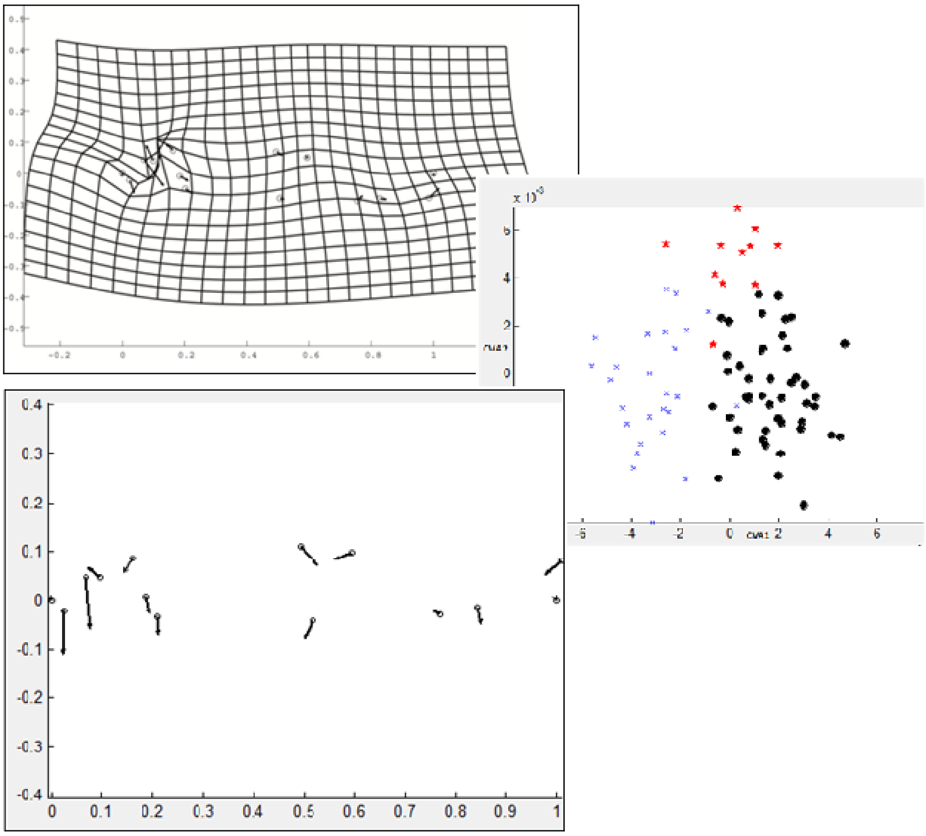


Figure 6. Visualisation of identified morphological changes

APPLICATION OF GEOMETRIC MORPHOMETRY IN ICHTHYOLOGY

Geometric morphometry has found great application in biological research which is indicated by the existence of a textbook written for biologists (Zelditch *et al*., 2004). This publication is the basis for the use of geometric morphometry among biology students with minimal mathematical education, given that many other publications dealing with this issue are intended for some other profiles of researchers. The special significance of this publication is that it also provides "links" for downloading free programs, as well as instructions for their use.

To date, geometric morphometry has proven to be an extremely popular set of tools for a wide range of research in biology. This popularity stems from years of scientific and practical interest in morphology as well as the essential strengths of the geometric approach to shape analysis. Over time, situations are emerging in which rapid, accurate, and geometrically comprehensive analysis of morphological variations are needed in many scientific, engineering, and applied mathematical fields, even in historical, cultural, and artistic contexts.

The study of shape change was initially interesting for biological anthropology; however, later interests included quantitative studies of natural variation and phylogenetic relationships between species (Richtsmeier *et al*., 2002).

Tulli *et al*. (2009) investigated the biometric characteristics of *Dicentrarchus labrax*. The effect of the cultivation system on biometric properties and yield calculation was investigated. The results showed that geometric morphometry is a valuable tool for describing changes in shape characteristics and assessing fish quality.

Fruciano *et al*. (2011) reveal the morphological variations of the lower pharyngeal jaw in *Coris julis* (Teleostei, Labridae) by geometric morphometry. Despite the close geographical distance and probably the large genetic flow between populations, statistically significant differences were found, both in size and shape.

Bravi *et al*. (2013) investigated the morphometric changes of several fish species in Italy. Ontogenetic variation of shape has been described by multivariate regression. Analyses showed proportional ontogenetic changes in several morphometric signs in all analysed populations, and that there were no variations of geographical shape for each species, but also certain interspecies differences.

Analysing ichthyological research in Bosnia and Herzegovina, it was found that geometric morphometry is used rarely, as evidenced by a very small number of published papers. Bajrić (2017) applied this method to morphological studies of *Sabanejewia balcanica* where the variability of size and shape of this species was indicated.

Buj *et al*., (2008) identified two cluster relationships among populations of *Sabanejewia balcanica* in Croatia. According to the mentioned authors, one cluster consists of individuals from the Drava and Petrinjčica rivers, and the other individuals from the Rijeka and Voćinska rivers. In this case, we are talking about clusters that include sites that are geographically closer and within which environmental factors are more similar.

By studying the differences in size, among the individuals of *Ameiurus melas* divided on the basis of the number of branchiospins and the number of rays in the anal fin, geometric morphometry determined the differences between localities but not "species" (Cvijanović, 2009).

Studying three populations of *Umbra krameri*, canonical analysis of variance found that there are separations according to body shape differences in certain populations (Sekulić, 2013).

The study of trout from the waters of the Danube basin of Croatia, based on geometric morphometry, showed that there is a significant difference in body shape between the Atlantic and Danube lineage as well as their hybrid. The most prominent differences were found in body depth, head length and eye size (Špelić *et al*., 2021).

Geometric analysis revealed the importance of the spatial distribution of spots on the flank of trout in distinguishing Mediterranean trout, which was used to determine the conservation status of wild trout populations (Lorenzoni *et al*., 2019).

It is also necessary to emphasise that geometric morphometry can be applied to other groups of organisms, and it is especially relevant in the study of insects. So are Zahiri *et al*. (2006), geometric morphometry investigated certain insect populations in Iran. The analysis showed a significant difference between the sexes and between the populations of the two provinces. A direct correlation between morphological and geographical distance was observed.

Aytekin *et al*. (2007) indicated the morphological characteristics of the wings of some insects by using geometric morphometry. They underline that this approach can help future studies in solving the problems of their systematics, in understanding the flight mechanisms of insects, and on the aerodynamics of different insect wing shapes.

CONCLUSION

From all the above, it can be concluded that geometric morphometry completely surpasses traditional morphometry in terms of theoretical strength, methodology and possibilities of explanation. Its use does not require special and extensive knowledge in the field of informatics, and does not require large financial expenditures, because the programs used are mostly free. For this purpose of application, it is necessary to have a camera and a computer with standard characteristics.

Advantages of geometric morphology in relation to classical morphometry:

• Ability to manipulate a very large amount of data;

• Statistical sensitivity;

• Digital visualisation of research results;

• Less time needed to get results;

• It is a more acceptable, i.e. less invasive method in relation to organisms, because for such analyses it is only necessary to take photographs of individuals. The sacrifice of individuals is avoided, which is invaluable when researching endangered fish species.

Geometric morphometry has found its application both in biological research and in many other scientific, engineering or applied mathematical fields. Also, her knowledge can be used even in a historical, cultural or artistic context. In connection with the above, the conclusion is that it is very important that researchers of all profiles are introduced to the presented technique.

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