ORIGINAL ARTICLE

3D morphology of pharyngeal dentition of the genus *Capoeta* (Cyprinidae): Implications for taxonomy and phylogeny

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Abstract

Capoeta is a herbivorous cyprinid fish genus, widely distributed in water bodies of Western Asia. Recent species show a distinct biogeographic pattern with endemic distribution in large fluvial drainage basins. As other cyprinids, the species of this genus are characterized by the presence of the pharyngeal bone with pharyngeal teeth. Despite this, the detailed morphology of the pharyngeal teeth, its interspecific and topologic variations, and the importance for taxonomy and phylogeny of the genus *Capoeta* are still not established. For the first time, a detailed comprehensive study of the pharyngeal dentition of 10 *Capoeta* species has been provided. The morphologic study of the pharyngeal dentition bases on the 3D microtomography and follows the purpose to evaluate the potential taxonomic and phylogenetic signals of these elements, as well as to study interspecific and topologic variations of the pharyngeal teeth. In this study, we propose a new methodology to categorize the studied pharyngeal teeth in 18 shape classes. The results of this study show that the detailed 3D morphology of the pharyngeal teeth is a useful tool for the identification of isolated teeth at the generic and/or specific level and that in certain cases, the tooth position in the teeth rows can be identified. Additionally, the preliminary analysis shows that the morphology of the pharyngeal teeth provides a potential phylogenetic signal. Both these patterns are very important for the taxonomy of cyprinid fishes and especially can be applied to fossil records.

KEYWORDS
3D microtomography, *Capoeta*, Cyprinidae, pharyngeal teeth

1 | INTRODUCTION

Extant cyprinid fishes are known with more than 2,000 species and represent the most diverse family of bony fishes in Eurasia and Africa (Nelson, 2006). In fresh water bodies, they build the main part of the biodiversity of the fish community. The family includes several large clades (subfamilies), that is, Cyprininae and Leuciscinae. In Western Asia among cyprinids, one of the widely distributed genera is the cyprinine *Capoeta*, which is considered as endemic to the region. The monophyletic genus *Capoeta* includes herbivorous species, feeding mainly on algae and periphyton, which they scrap from the substrate by the horny sheath on their lower lip (Banarescu, 1999; Karaman, 1969; Türkmen, Erdoğan, Yıldırım, & Akyurt, 2002).

Currently, more than 20 *Capoeta* species are described based on genetic studies and morphologic and meristic characters (Levin, Rubenyan, & Salnikov, 2005; Levin et al., 2012; Turan, Kottelat, & Ekmekçi, 2008). The earlier taxonomical studies of the genus *Capoeta* are based mainly on morphometrics and meristic characters (Karaman, 1969; Krupp & Schneider, 1989), whereas the recent studies mostly rely on genetic analyses (Alwan, Esmaeili, & Krupp, 2016; Levin et al., 2012; Turan, 2008).

Levin et al. (2012) studied the phylogenetic relationships of the genus *Capoeta* based on the complete mitochondrial gene for

cytochrome *b* sequences obtained from 20 species. According to the study (Levin et al., 2012), three main groups are recognized: the Mesopotamian, the Anatolian–Iranian, and the Aralo-Caspian. Later, Zareian, Esmaeili, Heuídari, Khoshkholgh, and Mousavi-Sabet (2016) based on mitochondrial cytochrome *b* gene sequences distinguished three main groups: the *Capoeta trutta* group (the Mesopotamian *Capoeta* group), the *Capoeta damascina* complex group (the Anatolian–Iranian group), and the *Capoeta capoeta* complex group (the Aralo-Caspian group) (Zareian et al., 2016).

A diagnostic character of all cyprinid fishes is the presence of the pharyngeal bone with pharyngeal teeth located in up to three rows (Howes, 1991). It builds as a result of ossification of the right and left fifth ceratobranchials and forms tooth-bearing pharyngeal jaw, which is specialized for food processing. The morphology of the pharyngeal jaw and pharyngeal tooth shape and configuration also have taxonomic significance for cyprinids (Howes, 1991) and can be represented by a formula, for example, 4.3.2-2.3.4; these numbers indicate the amount of the teeth on the left and right jaws from the first to the third and the third to the first row correspondingly. The number of tooth rows and the amount of teeth in the each row are mentioned as one of the significant taxonomic characters for the genus *Capoeta* (Banarescu, 1999; Karaman, 1969; Krupp & Schneider, 1989). Besides this, several studies have shown that the pharyngeal dentition can also be considered as an essential character complex for the study of cyprinid evolution (Ahnelt, Herdina, & Metscher, 2015; Böhme, 2002; Pasco-Viel et al., 2010; Zardoya & Ignacio, 1999; Zeng & Liu, 2011). However, little is known about the morphology of pharyngeal bones and teeth of the genus *Capoeta*, as well as its significance for taxonomy and phylogeny.

Heckel (1843) described the pharyngeal teeth of cyprinid fishes for the first time. He classified them according to the grinding surfaces in four main groups and 13 subgroups. One of the subgroups described by him with "shovel-shaped teeth" includes the genus *Capoeta*. According to Heckel (1843), the teeth formula of the genus *Capoeta* is 2.3.4-4.3.2 (respectively from the third to the first and from the first to the third row). By examining different species of the genus, later studies (Banarescu, 1999; Karaman, 1969; Krupp & Schneider, 1989) found four or five teeth to be present in the main row, two to four in the second row, and two in the third row. Banarescu (1999) gave a rough morphologic description of the pharyngeal teeth and mentioned that the teeth in the main row are compressed and have irregular shape and those in the second and third rows are more or less cylindrical in shape (Banarescu, 1999). However, the detailed morphology, interspecific and topologic variations, and the importance of the tooth morphology for taxonomy and phylogeny of this genus are still not established.

Taking this into account, the main goals of this publication were (i) to provide a detailed morphologic description of pharyngeal teeth in 10 *Capoeta* species by applying 3D approaches; (ii) to check the interspecific and topologic variations of pharyngeal teeth; and (iii) to test the possible phylogenetic signal embedded in the tooth morphology.

2 | MATERIALS AND METHODS

2.1 | Sampling

Pharyngeal dentitions of 10 *Capoeta* species from different water basins of Anatolia, Iraq, Iran, Armenia, Georgia, and Syria are studied (Table 1). The comparative material of pharyngeal bones is stored at the Bavarian State Collection for Anthropology and Palaeoanatomy, Munich (SNSB); the National Museum of Natural Sciences of Madrid (MNCN); the Palaeontological Collection of Tübingen University (GPIT); and Senckenberg Naturmuseum Frankfurt (SMF). The sampled information about studied specimens and locations is listed in Table 1 and Figures 1 and 2. The osteologic and morphologic description of the pharyngeal bones and teeth follows the nomenclature introduced in Figure 3.

The left pharyngeal bones of adult individuals are used in the study (except for *Capoeta umbla* and *C. damascina*). Each pharyngeal bone possesses 9 to 10 teeth (pharyngeal teeth a3, a5, c1, and c2 in *Capoeta saadii* and b2, c1, and c2 in *Capoeta buhsei* are missing). So, in total, the morphologic characters of 84 teeth are examined and analyzed. The other samples of the same species were examined according to an established morphologic set of characters.

2.2 | Species identification

The studied species were collected and identified by different scholars. *C. saadii* and *C. buhsei* are collected by Ignacio Doadrio in 2015, *C. umbla* by Angela Van den Driesch, *C. trutta* and *Capoeta* sp. by Eva Maria Cornelssen in 1978, *Capoeta baliki* and *Capoeta sieboldii* by Madelaine Böhme in 2010, *C. capoeta* by Samvel Pipoyan in 2012, *Capoeta sevangi* by Anna Ayvazyan in 2014, and *C. damascina* by Nisreen Alwan in 2008. All species are identified by the collectors based on external morphology and meristic characters.

Capoeta sp. from the Dokan Reservoir, Iraq, was collected by Cornelssen and stored as dried skeleton in SNSB as *Barbus belayewi*. According to our results, this specimen is closely related but not identical to the species *C. trutta*, which is also supported by the detailed study and comparison of the morphology of the last unbranched ray of the dorsal fin (unpublished results).

2.3 | X-ray microtomography

The pharyngeal bones were prepared in small polystyrene boxes for scanning.

The pharyngeal bones of the extant *Capoeta* species were scanned using X-ray computed tomography (μCT). MicroCT images were taken using the microtomography system Phoenix v|tome|x s at the Tübingen University and Erlangen University, as well as NIKON XT H 160 at the Scanning electron microscopy and analytic laboratories of MNCN. The pharyngeal bones were scanned with the following settings: 0.025 mm resolution, 100 to 150 mA, and 83 to 150 kV depending on the size of the bones and teeth (the bigger the bone, the higher the voltage due to the increased thickness of

TABLE 1 *Capoeta* species included in the present study

Scientific name	Locality	Number of samples (n)	Depository
Capoeta sieboldii	Kizilirmak River, town of Avanos, Turkey	1	GPIT-OS-00858
Capoeta baliki	Kizilirmak River, Avanos, Turkey	1	GPIT-OS-00859
Capoeta trutta	Assad Sea, Syria	2	SAPM-PI-02908, SNSB SAPM-PI-02910, SNSB
Capoeta capoeta	Saghamo Lake, Georgia	13	GPIT-OS-00860[a]
Capoeta umbla	Khata River, Adiyaman, eastern Turkey	1	SAPM-PI-00718, SNSB
Capoeta sevangi	Sevan Lake, Armenia	9	GPIT-OS-00861[a]
Capoeta sp.	Dokan Reservoir, Iraq	2	SAPM-PI-00719, SNSB SAPM-PI-00721, SNSB
Capoeta buhsei	Soleghan River, Namak Lake, Tehran, Iran	1	AT241586, MNCN
Capoeta saadii	Shahpur River, Dalaki River, Bishapur, Iran	1	IR3, MNCN
Capoeta damascina	Homs or Qattinah Lake, Orontes River drainage, Syria	1	SYR08/25, SMF

SNSB, Bavarian State Collection for Anthropology and Palaeoanatomy, Munich; MNCN, National Museum of Natural Sciences of Madrid; GPIT, Palaeontological Collection of Tübingen University; SMF, Senckenberg Naturmuseum Frankfurt.
[a]Collection numbers of scanned samples.

the element the X-rays must traverse). The tomographic reconstruction was performed using the following software: Phoenix datos|x CT in Tübingen, VGStudio 3.0 in Erlangen, and Amira 8.0 in Madrid.

2.4 | Morphological analyses

The virtual sections and 3D volume renderings from the reconstructed volume images were evaluated in the Avizo package (version 8.0). The digitalization of the bones allows observing models from different sides and recording the microstructures of bones and teeth which are difficult to observe under a light microscope. The teeth were further edited in the Geomagic professional engineering (version 15.3.0) and Freeform Plus (2014.3.0. 172) software packages. Besides these, the pharyngeal bones were examined under the Leica DVM5000 digital microscope and Leica M50 stereomicroscope available at the University of Tübingen.

To study the morphology of each pharyngeal tooth, we reconstructed 3D models of pharyngeal bones and virtually separated each tooth as an isolated model (in Avizo and Geomagic). The isolated 3D tooth models allow for an examination of the tooth from different sides by rotating the models, as in the tooth rows the teeth are covered sidewise by others and it is difficult to observe all morphologic features of the teeth. Based on these 3D models, the set of teeth for each species is generated, which makes it easy to categorize teeth and record the intraspecific variation (Figure S1).

We established a set of shape characters: lateral outline (α) and transverse cross section (β). Based on them, the teeth were described and categorized into shape classes. To record the lateral outline of each tooth, we used the images of isolated 3D tooth models and marked the outlines using Adobe Illustrator. The lateral outlines were taken for each tooth in dorsal view, from the top of the tooth until its foot basis. The transverse cross section is performed using the tool "Slice" from the Avizo package. To record the cross sections, the tooth surfaces of every sample were virtually cut at the

same anatomical position where the surfaces of all teeth appear on the slice plate. To describe the shape characters, the coding used in phytolith (silicified plant particles) nomenclature is applied (Wautier, van der Heyden, & Huysseune, 2001). The same "Slice" tool is used to apply a virtual experiment to understand the robustness of the transverse cross section (β). For this experiment, the teeth surfaces of *C. sieboldii* were cut together in one slice and a4 of *C. buhsei* was cut separately from the other teeth to get the section at the uppermost part of the surface.

To describe and categorize the pharyngeal teeth of *Capoeta* species based on 3D models of 84 pharyngeal teeth, we used basic terminology (Wautier et al., 2001). In addition, to better formulize tooth morphology, we introduced shape classes defined by character stages α and β. To check the intraspecific variation of tooth morphology and left–right asymmetry among studied species, two control groups, *C. sevangi* (n = 13) and *C. capoeta* (n = 9), as well as all other species represented with two or more samples were examined.

To test the phylogenetic information of shape classes, the dendrogram has been performed using the morphologic characters by applying the Euclidean similarity index in the PAST (Paleontological Statistics, version 3) software.

3 | RESULTS

3.1 | General aspects of the pharyngeal tooth morphology of the genus *Capoeta*

Figure 3a and b shows the localization of the pharyngeal dentition in the fish body. The pharyngeal bones of the genus *Capoeta* can be distinguished by the well-developed dorsal and curved ventral limbs as well as the relatively large tooth-bearing area (Figure 3c and d). Each tooth consists of a tooth foot, a crown, a foot–crown border, a grinding surface, and an edge of the grinding surface (Figure 3e and f). The pharyngeal teeth of studied species are arranged at the
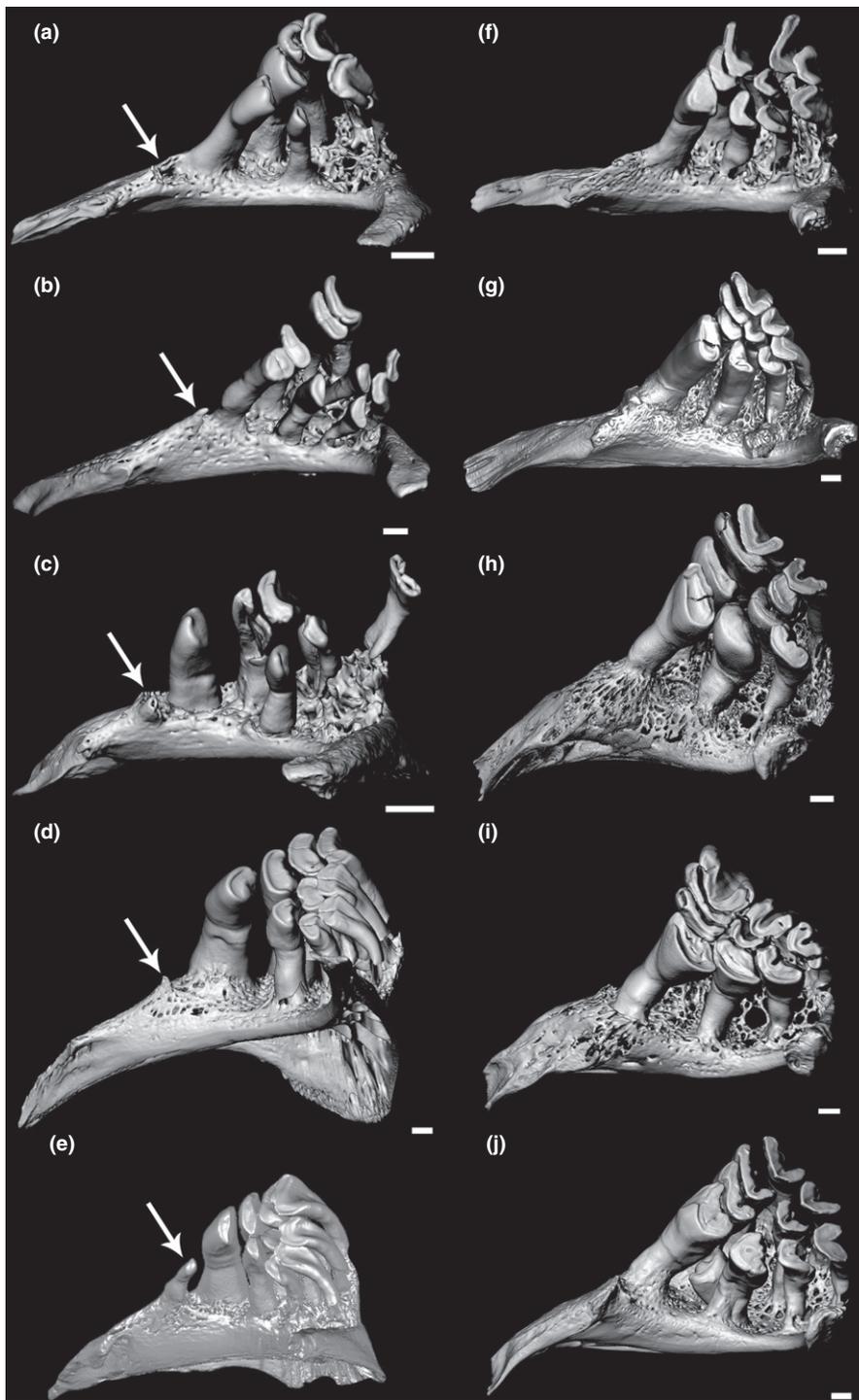
FIGURE 1 (a–j) Pharyngeal bones with teeth of the studied nine extant species of the genus *Capoeta*. (a) *Capoeta buhsei*; (b) *Capoeta umbla* (mirrored); (c) *Capoeta saadii*; (d) *Capoeta baliki*; (e) *Capoeta damascina* (mirrored); (f) *Capoeta capoeta*; (g) *Capoeta sevangi*; (h) *Capoeta* sp.; (i) *Capoeta trutta*; and (j) *Capoeta sieboldii*. The white arrows show a1 or presence of its bases. The scales are equal to 1 mm

pharyngeal bone in three rows. Each of them has different tooth count. The main row possesses five or four (a1, a2, a3, a4, a5), the second row three (b1, b2, b3), and the third row two (c1, c2) teeth (Figure 3c). The pharyngeal tooth formula is (i) 4.3.2-2.3.4 in *C. capoeta, C. sevangi, C. sieboldii, C. trutta,* and *Capoeta* sp; or (ii) 5.3.2-2.3.5 in *C. damascina, C. umbla, C. buhsei, C. saadii,* and *C. baliki,* which have a1 (*C. damascina*) or the tooth base at the a1 position.

The pharyngeal bone of *Capoeta* shows heterodont dentition. The teeth of the main row are larger than those of the second and third rows. The first tooth of the main row can be absent (*C. capoeta, C. sevangi, C. sieboldii, C. trutta,* and *Capoeta* sp.), strongly reduced (*C. umbla*), or less reduced as in *C. damascina*. A1 is a small accessorial tooth and can be easily broken. In the case of *C. saadii, C. buhsei,* and *C. baliki,* it is broken and only the tooth basis is visible.

As a rule, the second tooth of the main row (a2) within all studied species is robust and relatively large with a wide tooth base and grinding surface. The other teeth of the main row (a3, a4, a5) as well as the teeth of two other rows (b2, b3, c1, c2) compared to
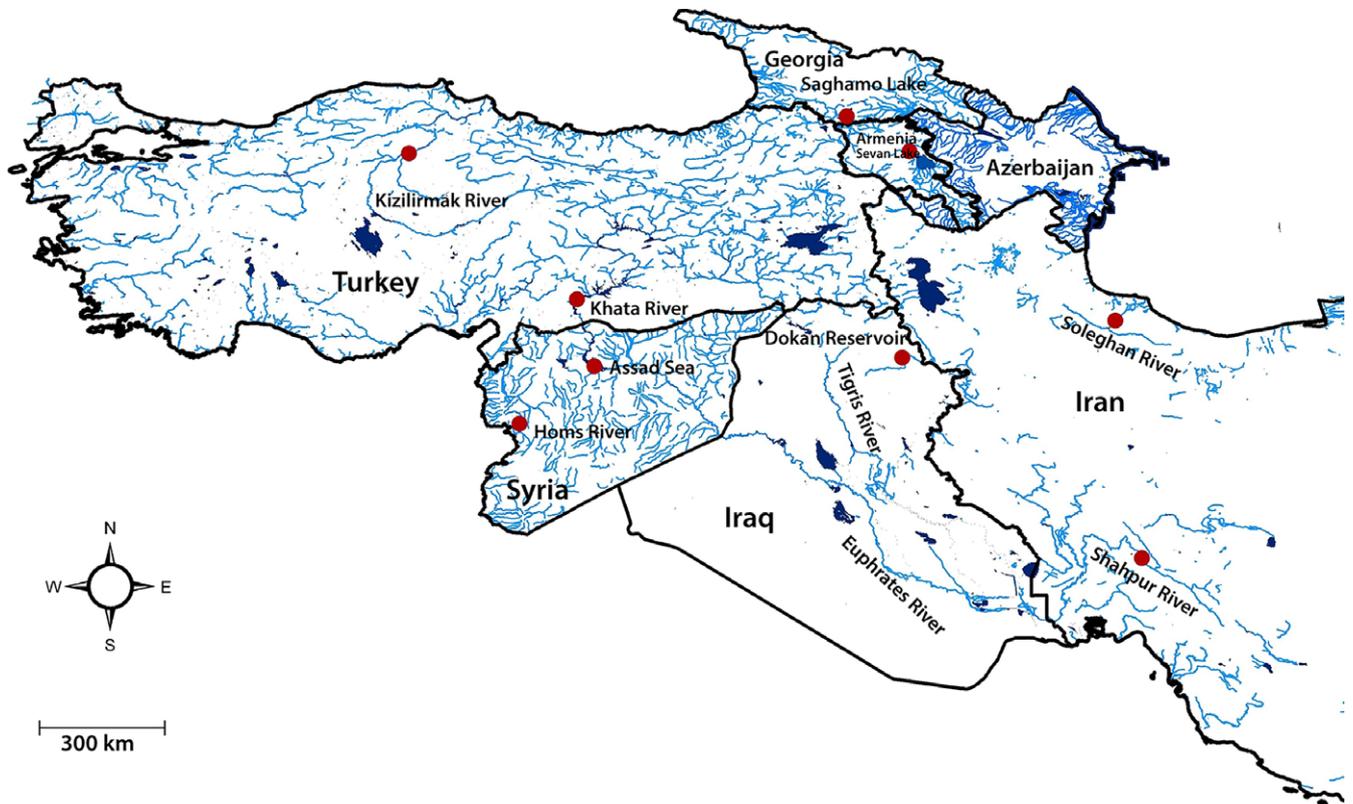
FIGURE 2 Drainage system of Western Asia (Turkey, Iraq, Iran, Armenia, Georgia, and Syria). The sampled (circles) localities of the studied *Capoeta* species
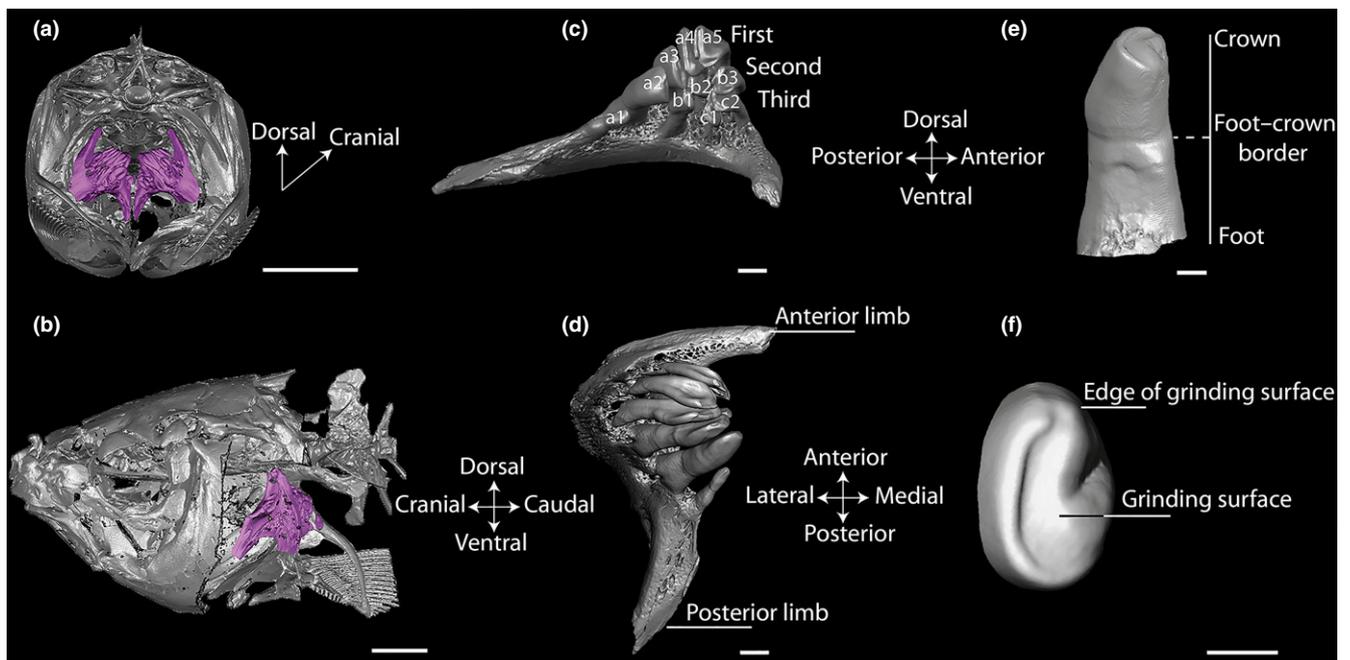
FIGURE 3 The terminology of the pharyngeal bones and teeth used in the present work: Skull of *Capoeta sevangi* in (a) posterior and (b) lateral views, showing the position of pharyngeal bones. Pharyngeal bone with teeth in (c) anterior and (d) medial views. The pharyngeal tooth (e) and grinding surface (f). The scale bars are equal to 1 cm (a, b) and 1 mm (c–f)

a2 are slender. They widen distally and are bent laterally. These characters are more pronounced ventrodorsally along the main row and well expressed in the most dorsal tooth (a5). The first tooth of

the second row (b1) is usually similar to the a2 with its morphology, but it is more slender. The other teeth of the second row are slender and bent laterally. Two teeth of the third row (c1, c2) are

usually the smallest. The grinding surfaces in all three rows narrow ventrodorsally.

The intraspecific variation and left–right asymmetry among both studied control groups (*C. sevangi* and *C. capoeta*) were not recorded.

3.2 | Pharyngeal tooth characterization and classification

On the basis of the 3D models and images of pharyngeal teeth, we describe *Capoeta* tooth morphology using two sets of the shape characters: lateral outline (α) and transverse cross section (β, measured at the distal tooth crown). According to the lateral outline, we define 14 character stages (α1–α14; Figure 4; Table S1). Among the studied species, the most frequently occurring lateral outline has spatulate form. It occurs mainly in the a3–a5, b2–b3, and c1–c2 tooth positions. As a rule, nearly all a2 teeth are molariform with a few differences.

The outline of the transverse cross section is variable among the studied teeth, and overall, eleven character stages (β1–β11; Figure 5, Table S1) can be defined for them. The variability of the outline of the transverse cross section of the grinding surfaces is a result of the morphological diversity of the masticatory surface in the studied 10 species.

We applied the (virtual) artificial wear experiment (for details, see Materials and methods) to understand the robustness of the

transverse cross section (β). Different layers/slices from the top of the grinding surface were cut to follow the variability, that is, development of these characters during the wearing process. In this experiment, the pharyngeal teeth of *C. sieboldii* were examined as the folded edge of grinding surface is characteristic of them and the applied experiment allows to test the development of the crenated grinding surface during the wearing process. Therefore, three different height sections from the top of the grinding surface (0.57 mm, 0.87 mm, and 1.42 mm) were processed. The heights of the cut slices are the points after which the form of the examined characters (crenated edge of the grinding surface) was changed. As shown in Figure S2, there are no any significant changes of transverse cross section (β) and it stays stable during applied wearing process, while folds of the grinding surface can change during the wearing process: They deepen, enlarge, or disappear (Figure S2 A1–A3). Therefore, the number or deepening of these folds cannot be used to describe the tooth as they are not applicable for the comparison if the samples have different degree of tooth wearing. The other example is the serrated posterior edge of the grinding surface, which is well expressed in the a4 tooth of *C. buhsei* (Figure S2 B). Its presence can be considered as a character of an unworn or less worn tooth. The application of virtual wearing by applying four different height sections of grinding surface (0.42 mm, 0.78 mm, 1.31 mm, and 1.87 mm) allows to observe the development of the serration during the wearing process. As shown in Figure S2, the serration of the surface is disappearing after a few layers were cut which can be identified during the wearing process (Figure S2 C1–C4).
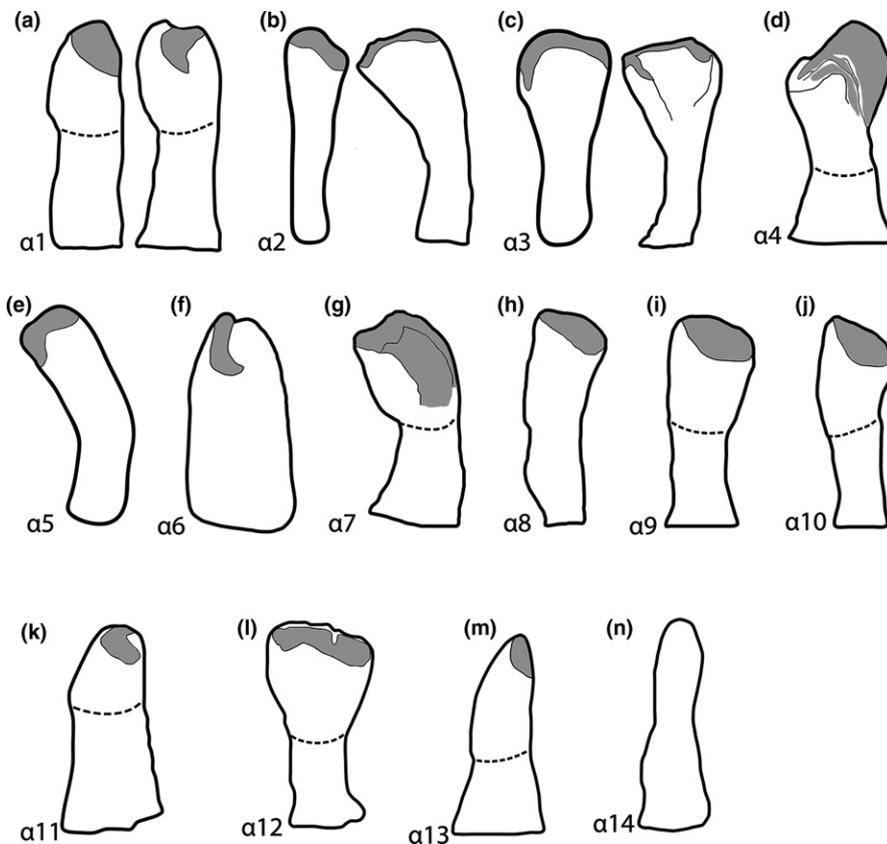
FIGURE 4 Lateral outlines of pharyngeal teeth in the studied *Capoeta* species. Illustrations (a–n) of the 14 character stages (α1–α14) for the tooth lateral outline. The presence of the groove on the grinding surface is indicated in gray color
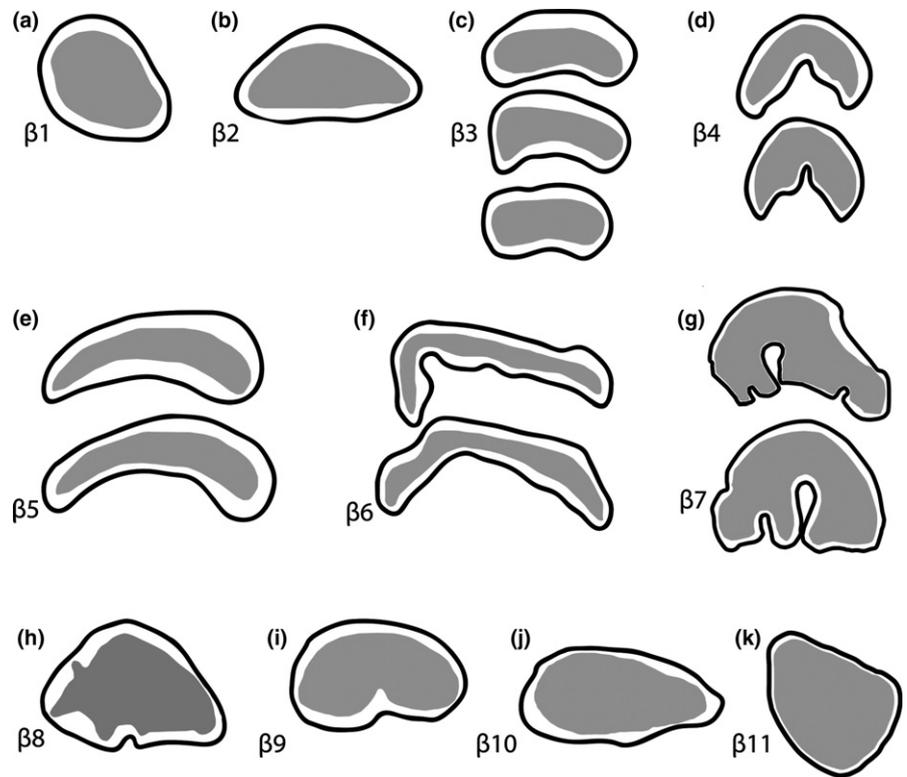
FIGURE 5 Eleven character stages (β1–β11) (a–f) of the transverse cross sections of pharyngeal teeth of the studied *Capoeta* species. The gray color indicates the presence of the groove on the grinding surface, and the white color corresponds to the tooth "enamel"

The grinding surface of some studied dorsal teeth has sloped edges. This character appears in teeth of different rows and possibly points out the tooth's movement direction during the grinding or which part of the grinding surface is actively participating in the grinding process (Figure S2 D, E).

So two main groups of characters of the pharyngeal teeth were distinguished: (i) applicable for the teeth description as the lateral outline (α) and transverse cross section (β); and (ii) variable during the ontogeny as folded, serrated, and sloped edge of the grinding surface.

The lateral outline (α) and the outline of the transverse cross section (β) were used to categorize the pharyngeal teeth of the studied 10 *Capoeta* species into 18 shape classes (Figure 6a–r; Table S2). Within the described shape classes, the most frequent one is shape class "C," which is common to all studied species (Figures S3 and S4).

The detailed description of all the shape classes can be found in the Supporting Information (Tables S1 and S2).

3.3 | Dendrogram based on the tooth shape classes

To test the potential taxonomic and phylogenetic signal of the pharyngeal tooth morphology, we performed a simple dendrogram based on the distribution (presence/absence) of the described shape classes within the studied species (Figure 7; Table S3). The dendrogram divided the studied species into four phenotypic clades: Clade I (*C. saadii*, *C. buhsei*, *C. damascina*, *C. umbla*, and *C. baliki*), Clade II (*C. sieboldii*), Clade III (*C. capoeta* and *C. sevangi*), and Clade IV (*C. trutta* and *Capoeta* sp.).

3.4 | Distribution of shape classes across species

The distribution of the studied species on the dendrogram is based on the morphological characters of these elements. The clustering of a few species inside one clade not only indicates that these species have similar (but not identical) tooth morphology, but also points out their close phylogenetic relationship.

According to the dendrogram, each clade is described with shape classes, and certain species on the dendrogram have own characteristic shape classes (Figure 7). Therefore, the described 18 shape classes are divided into three groups: diagnostic for the genus, clade, and species. The shape class "C" appears in all 10 studied *Capoeta* species, and it is the characteristic shape class of the genus *Capoeta*. The clade diagnostic shape classes are characteristic of a group of species which belong to the same clade, for example, shape classes "B, E, F, H, I, K, and M." The other shape classes, "D, G, J, L, N, O, P, Q, and R," are characteristic of certain species (Figure 7). Besides this, the described tooth shape classes are characteristic of certain tooth positions as well; for example, the shape class "C" is characteristic of teeth belonging to the main row (besides a1 and a2). To test the frequency of the occurrences of shape classes in different teeth positions, the graph was drawn (Figure S5). It shows that the teeth in a2 and b1 positions are the most heteromorph and the ones in position a5 are homomorph or less heteromorph. So the second tooth of the main row (a2) of each studied species (expect *C. buhsei*) has a distinct shape class found only in one species; thus, a2 can be used for the identification at species level. The identification key of the pharyngeal teeth within the studied species was established based on the shape classes (Figure S6).
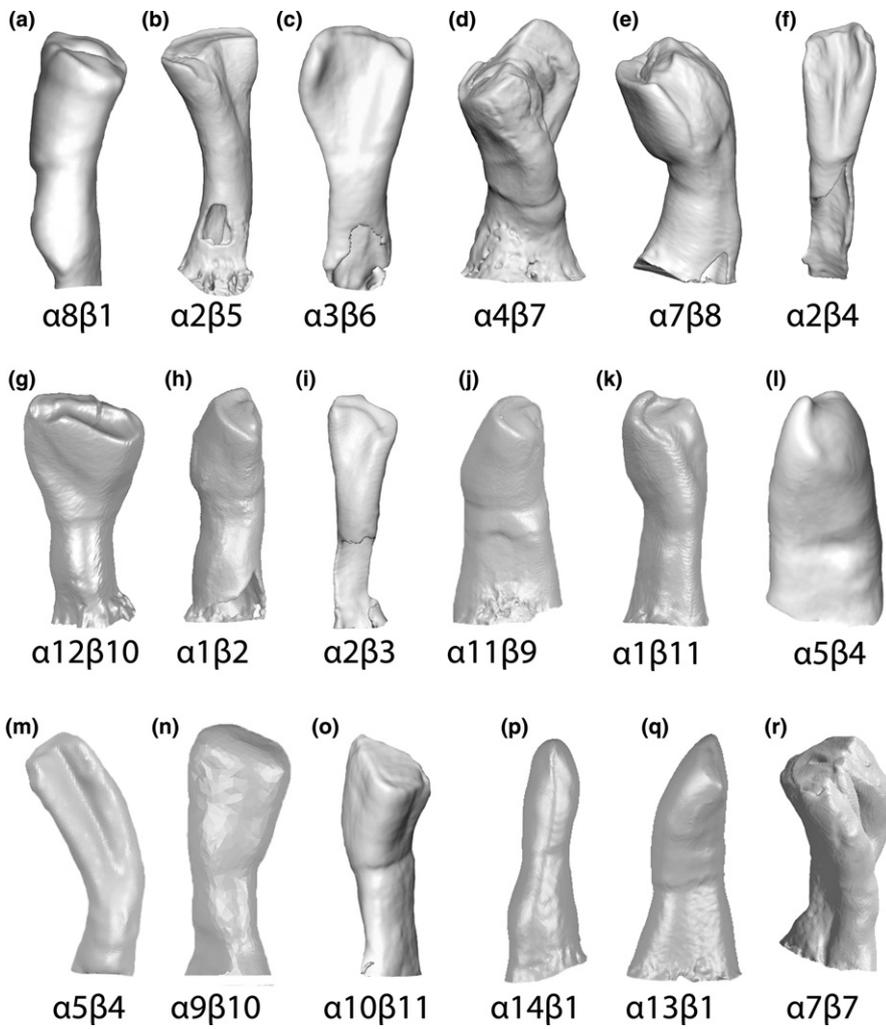
FIGURE 6 3D images of the recorded shape classes of the pharyngeal tooth of the genus *Capoeta*. (a–r) Shape classes proposed in the present work; for the descriptions, Tables S1 and S2. The scales are not given to avoid scaling up of the figures
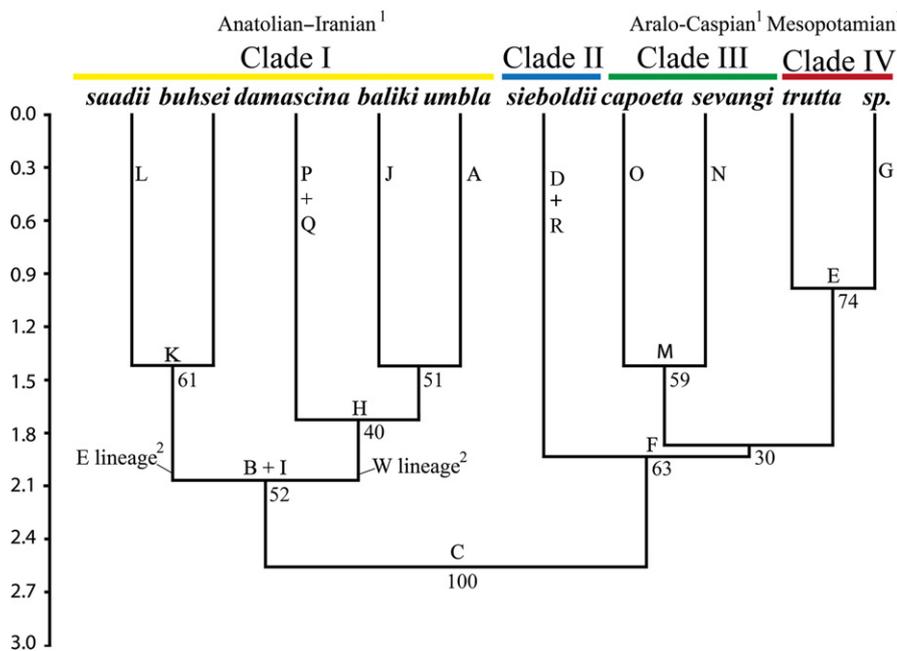
FIGURE 7 Phenotypic dendrogram generated based on the pharyngeal tooth shape classes of the *Capoeta* species. The letters (a–n) indicate the characteristic shape classes of nodes or branches. Numbers indicate the bootstrap support (branch support). [1]Distinguished clades of the genus *Capoeta* following Levin et al. (2012). [2]Eastern (E lineage) and Western (W lineage) lineages within the *Capoeta damascina* complex established by Alwan, Esmaeili, & Krupp, 2016; Alwan, Zareian, & Esmaeili, 2016

4 | DISCUSSION

4.1 | Presence of a1 as a diagnostic character for Clade I

The presence of the a1 within 10 studied *Capoeta* species is characteristic of five of them, which are all clustered in Clade I: *C. saadi*, *C. buhsei*, *C. umbla*, *C. damascina*, and *C. baliki*. The tooth is well developed in *C. damascina*, and in the other above-mentioned species, it is reduced, missing, or broken, but the tooth base is well visible (Figures 1 and 8).

4.2 | Potential phylogenetic signal of the pharyngeal tooth morphology

The performed dendrogram shows not only the morphologic similarity of the species, which belong to the same clade, but also the potential phylogenetic relationship of these species.

C. damascina is considered as a complex of nearly related species with two distinguished lineages: the eastern represented by *C. buhsei*, *Capoeta coadi*, and *C. saadii* and the western represented by *Capoeta caelestis*, *C. damascina*, and *C. umbla*. In this study, the members of both lineages are included: *C. buhsei*, *C. saadii*, *C. damascina*, and *C. umbla*. As the dendrogram shows, these species are clustered (based on their pharyngeal tooth morphology as well as the presence of the a1) in one group and form the *damascina* complex clade (Clade I, Anatolian–Iranian group), respectively with the

western and eastern lineages as it has been shown based on genetic analyses (Alwan, Zareian, & Esmaeili, 2016; Alwan, Esmaeili, & Krupp, 2016).

To check the correspondence between morphological and genetic results, we simplify already existing phylogenetic trees based on genetic analyses to show how the studied species cluster within phylogenetic trees based on genetic and morphologic analyses (Figure 9). Therefore, phylogenetic trees from three recent studies were used (Bektas et al., 2017; Levin et al., 2012; Zareian et al., 2016). The comparison of dendrograms (Figure 9) shows that the species of Anatolian–Iranian or *C. damascina* complex group (*saadi*, *buhsei*, *damascina*, *umbla*, and *baliki*) cluster within one clade (indicated by yellow color). The Aralo-Caspian or *C. capoeta* complex group (*C. sevangi* and *C. capoeta*) cluster together in the same clade and are indicated in green. *C. trutta* in all three dendrograms as well as in our results clusters as the distinct clade Mesopotamian *Capoeta* or *C. trutta* group and is indicated in red.

According to the dendrogram (Figure 7), *C.* sp. from Dokan Reservoir clusters within the *trutta* clade, and we suppose it is one of the closely related species of the *trutta* complex.

The studies of Levin et al. (2012) and Zareian et al. (2016) have shown that *C. sieboldii* clusters as a sister lineage to the *damascina* complex. According to Bektas et al. (2017), *C. sieboldii* is easily distinguishable from all *Capoeta* species distributed in Anatolian rivers by its pleated lips and single-paired barbels (the other *Capoeta* species distributed in Anatolian rivers are characterized by double-paired barbels) and represented as a separate clade. This
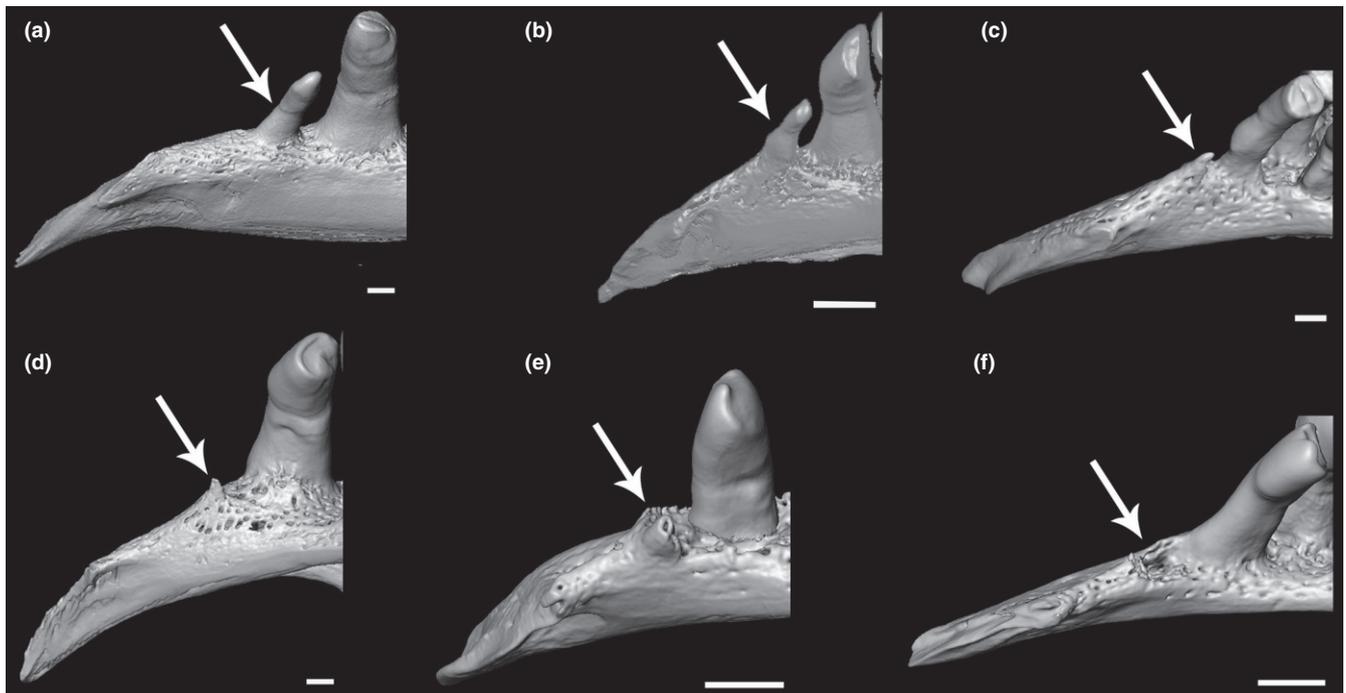
FIGURE 8 Reduction of the a1 tooth in the genus *Capoeta* in comparison with *Barbus barbus*. (a) *B. barbus*, (b) *Capoeta damascina*, (c) *Capoeta umbla* (strongly reduced), (d) *Capoeta baliki* (tooth broken), (e) *Capoeta saadii* (tooth broken), and (f) *Capoeta buhsei* (resorption pit visible). The white arrows show the a1 tooth or the position of its tooth basis. The scale bars are equal to 1 mm
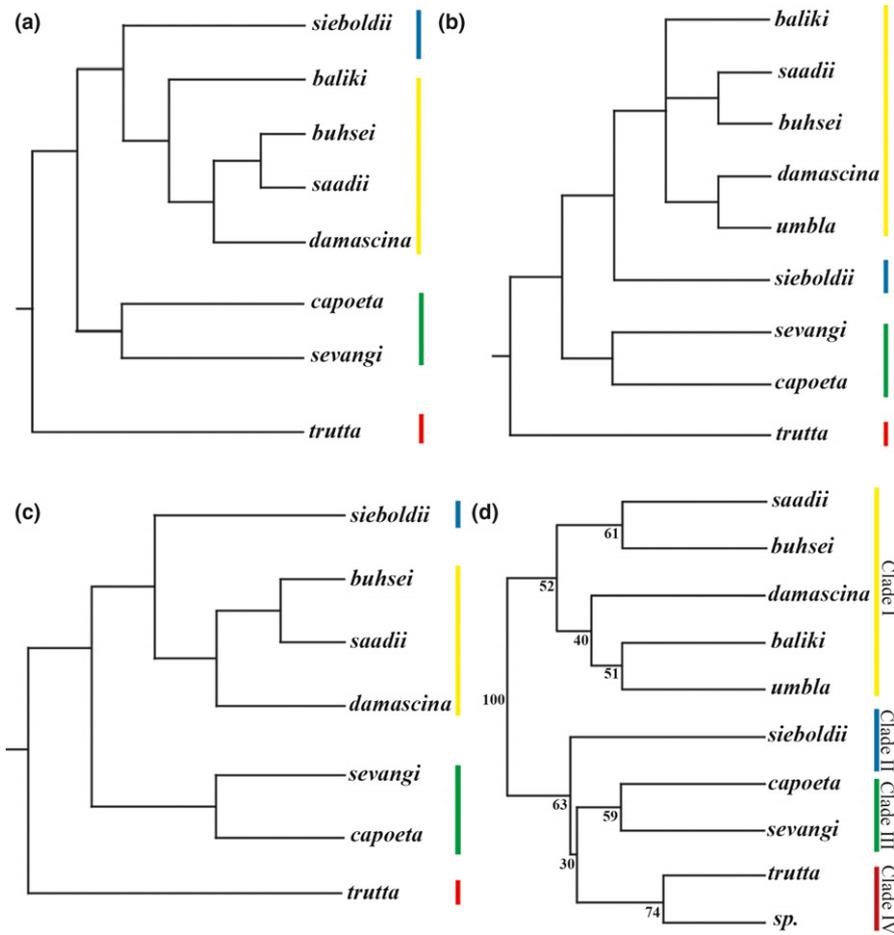
FIGURE 9 Simplified phylogenetic trees show the distribution of the studied *Capoeta* species within phylogenetic trees, based on genetic analyses of (a) Levin et al. (2012), (b) Bektas et al. (2017), (c) Zareian et al. (2016), and (d) this study

pattern is also supported by our results. According to our data, *C. sieboldii* is represented as a distinct clade (Figure 7, in blue color, Clade II). However, our analysis shows only one difference from genetic results (Bektas et al., 2017): *C. sieboldii* is placed as a sister clade to Aralo-Caspian (Clade III) and Mesopotamian (Clade IV) clades (Figure 7), whereas the genetic data cluster it as a sister clade to *C. damascina* (Clade I, small scale; Bektas et al., 2017).

4.3 | Is the reduction of a1 plesiomorphic or apomorphic for the genus *Capoeta*?

The phylogenetic tree based on molecular analyses of the genus *Capoeta* published by Levin et al. (2012) was simplified to show the presence of the a1 in different clades within this genus and its sister groups (Figure 10). The pharyngeal bones most of species of *Barbus* and *Luciobarbus* clades were available to us and the presence/absence of a1 was recorded first-hand, and the information about missing species was taken from the existing literature. This dendrogram shows that a1 tooth or its basis is present in representatives of clade *Barbus* and *clade B (Capoeta* clade), but absent in the other two sister groups (*Luciobarbus* and *L. subquincunciatus*). We assume that the absence of a1 is plesiomorphic for the genus *Capoeta*, which means it was lost among the species of clades A and C and reappeared or was regained in the species of
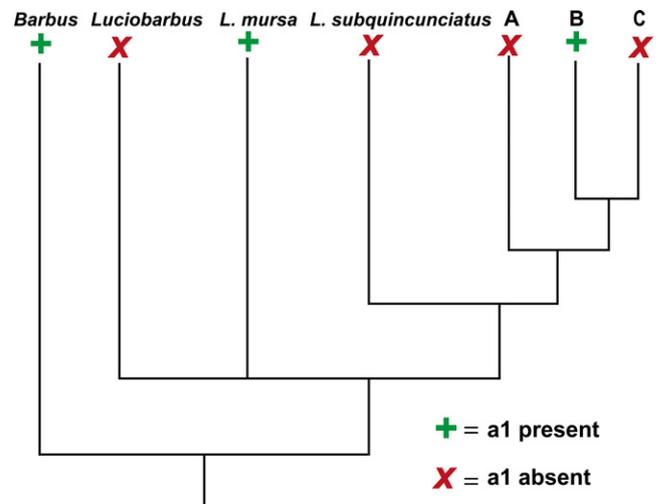
FIGURE 10 Presence/absence of a1 tooth shown on the phylogenetic tree based on mitochondrial gene for cytochrome b sequences (Levin et al., 2012). The clades are respectively corresponding to the clades mentioned in the work. A, B, and C the Mesopotamian group (A), the Anatolian–Iranian group (B), and the Aralo-Caspian group (C), are included in the clade *Capoeta*

clade B. The other possibility is that the presence of a1 tooth or its basis is a derived character that distinguishes the *damascina* clade.

4.4 | Potential ecological signal of the pharyngeal tooth

The preliminary interpretation of the possible ecological signal or the connection between feeding habits and pharyngeal tooth morphology of the studied species is provided based on literature data (Coad, 2010; Karaman, 1969; Krupp & Schneider, 1989).

The studies regarding the feeding habits of the genus *Capoeta* are unanimous and suggest that these species are herbivorous and feeding mainly on algae and periphyton, which they scrape from the substrate using the horny sheath on their lower lip (Banarescu, 1999; Karaman, 1969; Krupp & Schneider, 1989; Türkmen et al., 2002). The similar feeding habits should indicate that the tooth morphology is identical, in other words that the studied species should have homodont dentitions if the main driven factor is ecology, which has not been supported by our study. According to our results, the studied *Capoeta* species have heterodont dentitions and there is an interspecific variation of tooth morphology and tooth numbers within the studied species. Besides this, the dense packaging of the tooth arrangement in the tooth rows on the tooth-bearing area differs as well.

On the other hand, in case the a1 tooth is an apomorphic character of the *C. damascina* clade, a more omnivorous diet of the species of this complex could be suggested, as in *L. subquincunciatus* having a specialized dentition for feeding on algae or benthos. Thus, the a1 tooth could not be considered to provide selective advantage. This indicates the possible trophic segregation within these species.

The mouth and the lower lip covered by horny sheath are used mainly to scrap the algae; therefore, their morphology could also be an important trait to understand the trophic variation of the species and its reflection in tooth morphology.

Within the genus *Capoeta*, two types of mouth forms have been described: horseshoe-shaped and transverse (Karaman, 1969). The horseshoe-shaped is the basal form and can develop into the highly specialized transverse form. In the study by Karaman (1969), it has also been mentioned that all studied populations, during their development, first have the horseshoe mouth form without horny sheath. So we can assume that the horseshoe form of the mouth is a plesiomorphic and the transverse form is an apomorphic character. The mouth form has been described in different studies (Banarescu, 1999; Coad, 2010; Krupp & Schneider, 1989), but we could not find any significant difference between the given morphological descriptions.

So additional morphologic and ecologic studies are necessary to understand whether there is indeed trophic segregation between the *Capoeta* clades and whether there is a possible relation of the tooth morphology and feeding habits.

5 | CONCLUSION

5.1 | Pharyngeal tooth characterization and classification

For the first time, the detailed comprehensive study of pharyngeal dentition of 10 species of the genus *Capoeta* has been provided. The morphology of the pharyngeal dentition has been studied using the 3D microtomography to test its potential relevance for answering to taxonomic and phylogenetic questions. Special tools in the 3D software Avizo 8.0 allow to perform different effects (wearing process) and to test the stability of the morphological characters. These can be applied for the characterization and identification of pharyngeal teeth.

In this study, the set of morphological characters ($\alpha\beta$) were established to categorize the studied pharyngeal teeth into 18 shape classes. The results of different analyses based on the described shape classes show that based on the detailed morphology of these elements, the isolated pharyngeal teeth can be identified at the generic or specific level. Besides this, it is also possible to determine the relative or even the exact position of the isolated tooth in the tooth rows.

The identification key of the pharyngeal teeth of the studied species could be used for the identification of the isolated pharyngeal teeth, which is important not only for the taxonomy of recent species but also for the fossil record, as mainly the isolated pharyngeal teeth are found in the fossil record.

5.2 | Correspondence between morphological and molecular results

The comparison of the results of morphology and genetic analyses shows significant similarities of the generated trees. This supports our assumption that the pharyngeal tooth morphology of this genus has not only taxonomic but also phylogenetic relevance. The morphological results strongly support the presence of four clades: (i) *C. damascina* clade; (ii) *C. sieboldii* clade; (iii) *C. capoeta* clade; and (iv) *C. trutta* clade.

Summing up our results, we conclude that:

1. the detailed morphology using the 3D microtomography of pharyngeal teeth is a useful tool for the identification of the isolated pharyngeal teeth at the generic and specific levels, as well as in certain cases the tooth position in tooth rows;
2. the morphology of the pharyngeal teeth provides an obvious phylogenetic signal, supporting results derived from molecular genetic analyses;
3. both these patterns are important for the taxonomy of the genus and can be applied for the fossil records as well;
4. the a1 tooth is an apomorphic character for the *C. damascina* complex;
5. there is possible trophic segregation (the species of the *C. damascina* complex are more omnivorous/less dietary specialized); further studies are necessary to confirm this.

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SUPPORTING INFORMATION

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