Scanning electron microscopy of scale and body morphology as taxonomic characteristics of two closely related cyprinid species of genus *Capoeta* Valenciennes, 1842 in southern Iran

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Comparative morphology and the microstructures of scales were studied in order to evaluate their possible contribution to taxonomic discrimination of two closely related cyprinid species, *Capoeta saadi* and *Capoeta mandica* in southern Iran. The results revealed certain features of fish body (mouth shape and the number and shape of gill rakers) relevant to taxonomic significance. Moreover, some characters related to scale morphology and microstructures (location of focus, inter-radial space, and number and type of radii, shape and size of lepidonts and tubercles) were considered important for discrimination of these species. Such a study will give necessary information for identification of fish species, particularly during field study and for the fossil materials, where no molecular data are available, and of specimen which are deposited in the museum.

Keywords: Body morphology, cyprinid species, scale microstructure, taxonomic discrimination.

SINCE the time of Agassiz[1] who was the first to use fish scales for taxonomy, the importance of scale morphology in the systematic studies of fish species has increased dramatically during the last decades owing to the introduction and development of scanning electron microscopy (SEM)[2–6]. It is currently being applied to evaluate population relationships in both freshwater and marine fishes[7,8].

Iranian freshwater fishes have remarkable diversity in comparison to their neighbours, in which cyprinid fishes are the most diverse group[9,10]. These fishes form one of the important links in the fish community structure in different water bodies. Due to considerable diversity seen in Iranian cyprinid fishes, as well as the lack of sufficient information concerning some taxa, it is difficult to distinguish them considering only their external morphology. Therefore, finding morphological features with taxonomic characteristics has always been important goal for ichthyologist in this region. A review of the literature indicates that few published reports are available on microscopy of scale structure of fishes in Iran[7,11–13]. Also, some taxa are completely ignored, and therefore, little information is available regarding the microscopic studies of scales, especially on native cyprinid fishes.

With this background, two closely related cyprinids of genus *Capoeta* Valenciennes, 1842 were studied employing comparative fish morphology and scale microstructures.

The members of this genus are usually characterized by a compressed and moderately elongated body, small scale (large in some taxa), lateral line scale count ranging from 37 to 100, and keratinous and transverse mouth[14]. Several species of the genus *Capoeta* occur within the Iranian water bodies, among which two, i.e. *Capoeta saadi* (Valenciennes, 1844), and *Capoeta mandica* Lortet, 1849 are close in their external body morphology. These similarities particularly in the habitats that are sympatric make them difficult to identify.

The aim of this study is to compare some characters related to fish body as well as scale microstructure, and consequently evaluate their taxonomic significance for discrimination of these two closely related cyprinids. The outcome of this study will give important information for the identification of these species particularly during field study, and of specimens deposited in museum.

Twenty-five (15 males and 10 females) specimens of *C. saadi* and 25 (13 males and 12 females) specimens of *C. mandica* were collected respectively, from Kohmarreh Sorkhi River (52°9′N 29°22′E), and Rudbal River (52°33′N 29°00′E) in southern Iran. Both sites belong to Mond Basin in Fars province.

To study scale microscopic structures, two scales were gently removed with fine forceps from the left side of the body between dorsal fin and lateral line (referred as key scales), and two scales from the lateral line (referred as lateral line scales). The scales were cleaned mechanically using fine brush, cleaned and washed with 1% potassium hydroxide solution and then rinsed with triple distilled water. The cleaned scales were dehydrated through an ascending ethanol series (30%, 50%, 70% and 90%) and dried on filter paper[4]. To avoid curling of the margins of scales, they were kept inside the filter paper and then between two microslides for 2–3 days[7]. The cleaned and dried scales were mounted on SEM stubs using double adhesive tape with dorsal surface upward and ventral surface sticking to the tape, and coated with a thick layer of gold in gold-coating unit. The images were captured with a LEO 1430VP (Zeiss Company) at 15 kV.

The meristic characters such as number of lateral line scales, gill rakers, pectoral fin rays and dorsal branched rays were counted using stereo-microscope. The data of meristic characters were analysed in IBM (SPSS 21) software. Student *t* test was applied to determine sex dimorphism in each species, and also show significant differences between two species. The morphological studies of fish include scale morphology, shape of gill rakers and mouth shape which were drawn with a camera lucida attached to the stereo-microscope (model ZEISS, Stemi SV6).

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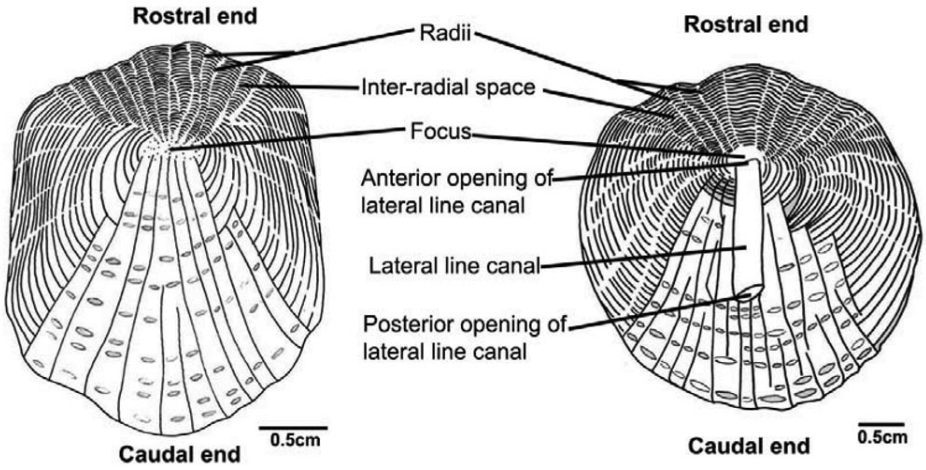

Figure 1. Surface view of body scales showing terminology used to define different parts. (Left) Lateral line scale. (Right) Key scale.

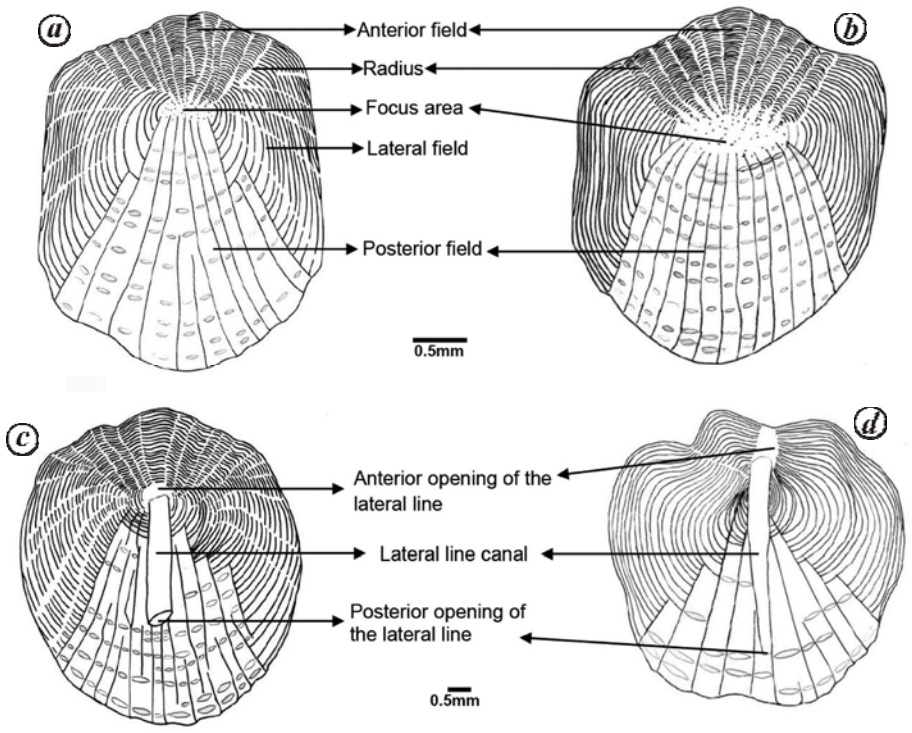

Figure 2. General morphological description. ***a***, ***b***, Key scales and ***c***, ***d***, Lateral line scales of *Capoeta saadi* and *Capoeta mandica* respectively.

Scales of the studied species show general morphology of scales in cyprinid fishes (Figure 1). The scales in these species can be divided into rostral (or anterior), caudal (or posterior), and lateral fields (Figure 1). There is no ctenus at the posterior part of the scales and is consequently called cycloid type. The anterior field is embedded in the skin and overlapped by posterior side of the next scale. The ventral part of scales is shiny and smooth, whereas dorsal part is rough, convex and has distinct structures, consisting of grooves and granules (tubercles). Each scale has a focus, which lies in the anterior part and divides scale into anterior, posterior and lateral fields (Figure 2). From the focus, lines of growth (the ridges) start appearing. These structures are called circuli (growth lines). Space between circuli is called inter-circular space. Circuli are distinct, overcrowded in anterior part and widely separated in lateral parts. This is because of the anterior location of focus on scale. Circuli are not found on posterior part of the scale. Inter-radial circuli in the anterior part of the scales are almost straight, but slightly convex or concave circuli also are observed. Three types of radii are present in the scales

Table 1. Microstructural details of scales and body morphology compared between two species

Characters	Status	*Capoeta saadi*	*Capoeta mandica*
Key scale			
General morphology	Lateral part	With radii	Without radii
Focus	Size	Small	Big
	Location	Anterior	Anterior (above center)
Radii	Location	Lateral and anterior parts	Only in anterior part
	Number in anterior part	Numerous	Few
	Inter-radial space in anterior part	Wide	Narrow
Posterior part	Size	Narrow	Wide
Lepidont	Number	Numerous	Few
Lateral line scales			
General shape	Anterior opening	Small	Big
	Length of canal	Short	Long
Body characters			
Mouth	Shape	Usually arced	Usually keratinous
Barbell	Length	Long and clubbing	Relatively short and slender
	Thickness	Thick	Narrow
Gill rakers	Number (in 25 specimens)	12–16	21–27
	Shape	Blunt	Serrated and pointed

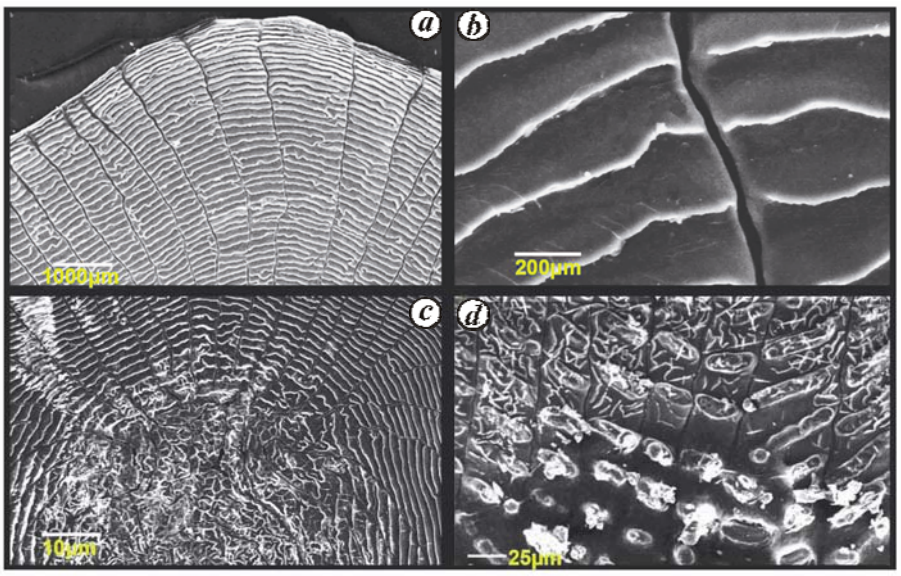

Figure 3. Microstructures of key scale in *C. saadi*. Fish standard length = 27.55 cm.

depending upon their point of origin on scale – primary radius (extends from focus to the margin of scale and interrupted continuity of circuli); secondary radius (fails to develop and does not extend all the way out to the margin of the scale, so circuli remain uninterrupted); and tertiary radius (extends between midway and margin) (Figure 2).

Lateral line scales have a canal, which lies almost along the anterior–posterior axis, and includes obvious anterior and posterior opening ends. Lateral line canal may vary in length (Figure 2 *c* and *d*).

Table 1 provides details of morphological characteristics of key scales.

Let us now consider microscopy of scale in *C. saadi* (Figure 3). Length of the studied scales in *C. saadi* is greater than its width; so it does not have a circular shape (see also Figure 2 *a*). Posterior part of the scale is wide and has no ctenus, ventral part is shiny and smooth, whereas dorsal part is rough, convex and has distinct structures which consists of ridges, grooves and granules (tubercles). Each scale has a focus, which is small and anteriorly positioned. Radii in the anterior part are primary and secondary type; 5–6 radii can be found in the lateral side of each scale.

In the anterior part, circuli are regularly positioned and divided mostly by primary radii (Figure 3 *a*). Circuli show developed crests, no obvious lepidont can be seen on them (Figure 3 *b*). The focus includes irregularly shaped structures (Figure 3 *c*). In the posterior part of the scales which is exposed, circuli lose their characteristic

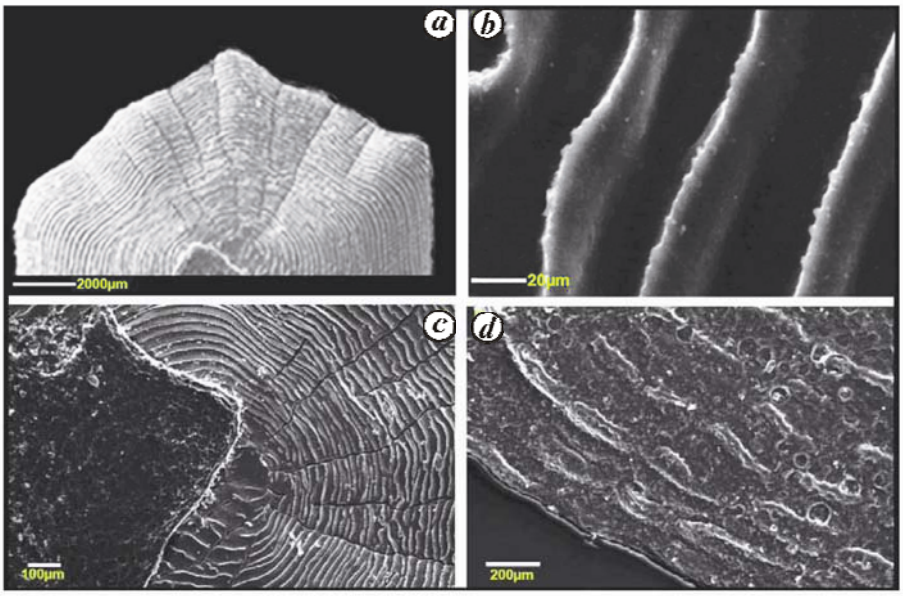

Figure 4. Microstructures of key scale in *C. mandica*. Fish standard length = 30.15 cm.

features and contain several rows of tubercles, which are not obviously concentrating. Shape of tubercles is often elongated, and some are rounded (Figure 3 *d*).

Now consider microscopy of scale in *C. mandica* (Figure 4). Scale is almost circular and posterior part is conical and relatively wide; scales are cycloid type (Figure 2 *b*). Each scale has an almost wide focus, which is situated in the anterior part (Figure 2 *b*). Radii in anterior part are primary and secondary type, and no radius occurs in the lateral side of the scale (Figure 4 *a*). Circuli contain obvious crest, including weak conical lepidonts, which are well spaced from each other (Figure 4 *b*). The focus area is smooth, with a complete circulus around it (Figure 4 *c*). In the posterior part of scales which is exposed, circuli lose their characteristic features and contain well-concentrated granules (tubercles). Shape of tubercles varies from round or oval and even elongated (Figure 4 *d*).

Table 1 also provides details of morphological characteristics of the lateral line scales.

Let us consider microscopy of lateral line scale in *C. saadi* (Figure 5 *a*). Figure 2 *c* shows the general morphology of the lateral line scale. Each circular scale has an anteriorly positioned focus, anterior end of lateral line canal opened just in focus area (Figure 2 *c*), number of radii 9–10, radii are of three types, i.e. primary, secondary and tertiary (Figure 2 *c*). Three to four radii are present in lateral sides of scale. The posterior part contains few rounded tubercles which are irregularly distributed (Figure 5 *a*). Circuli in rostral part of the scale are interrupted by anterior opening of lateral line canal. Posterior end of lateral line canal opened in posterior region.

Now consider microscopy of lateral line scale in *C. mandica* (Figure 5 *b* and *c*). Figure 2 *d* shows the general morphology of lateral line scale. Each circular scale has an anteriorly positioned focus, anterior end of the lateral line canal opens just in focus area (Figure 5 *b*); few radii occur in anterior part of scale, radii are only of primary type and no radius is present in lateral sides. Posterior part of scale has many tubercles, which are oval-shaped and irregularly distributed (Figure 5 *c*). Posterior end of lateral line canal opened in posterior region.

Figure 6 shows the general morphology of mouth shape and shape of gill raker. Mouth shape in *C. saadi* is horseshoe-like and arced with many papules in lower lip (Figure 6 *a*), whereas it is keratinous in *C. mandica* (Figure 6 *b*). Barbels in *C. saadi* are usually longer than in *C. mandica* (Figure 6 *a* and *b*), gill rakers are relatively short and thick in *C. saadi* (Figure 6 *c*), whereas they are pointed and almost villiform in *C. mandica* (Figure 6 *d*).

Moreover, Student *t*-test indicates no sexual dimorphism for the specimens of each species. It shows that two species are significantly different with regard to the number of lateral line scales and the number of gill rakers ($P < 0.05$).

A comparative descriptive analysis indicates that the total number of gill rakers is 13–19 (14.83 ± 1.5) in *C. saadi* and 21–27 (23.80 ± 1.8) in *C. mandica*; number of pectoral fin rays is 16–19 (18–19 ± 0.08) in *C. saadi*, and 15–19 (17.0 ± 0.05) in *C. mandica*; number of dorsal branched rays is 8 to 9 (mostly 9) in *C. saadi*, and 8–9 (mostly 8) in *C. mandica*.

The present study indicates that two closely related species of genus *Capoeta* in southern Iran (*C. saadi* and *C. mandica*) can be recognized by considering few morphological characters of fish body such as mouth shape as well as shape and number of gill rakers. In addition, some morphological features and microstructures of scales could contribute to discriminate the two species.

According to the present study, general architectural pattern of a cycloid cyprinid scale for the studied species

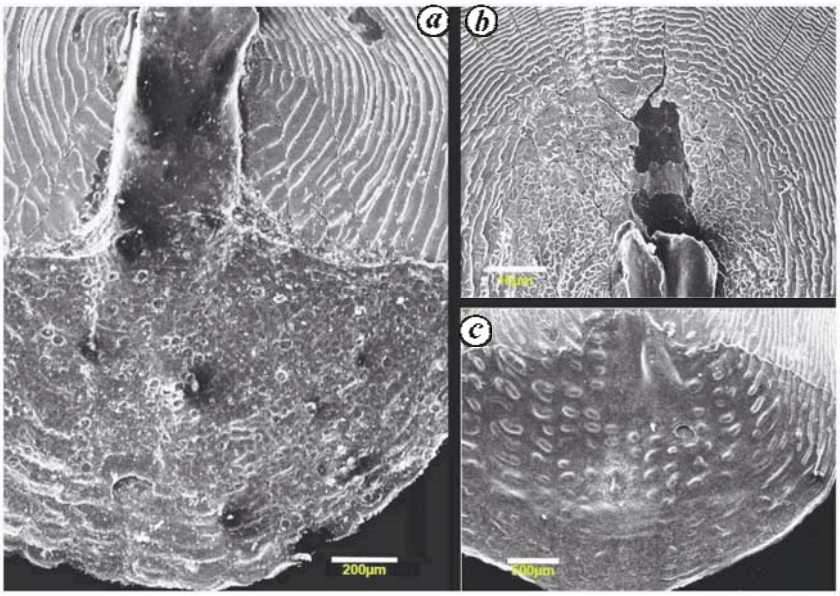

Figure 5. Microstructures of lateral line scale in (***a***) *C. saadi* (SL = 27.55 cm), and (***b***, ***c***) *C. mandica* (SL = 30.15 cm). SL, Fish standard length.

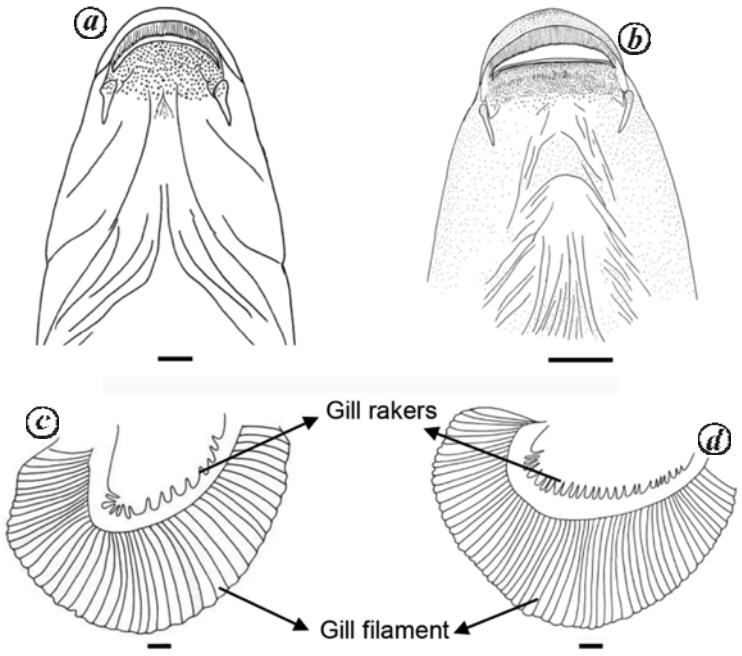

Figure 6. General morphology of mouth shape in (***a***) *C. saadi* and (***b***) *C. mandica*, as well as shape of gill raker in (***c***) *C. saadi*, (***d***) *C. mandica*. SL = 27.55 cm in *C. saadi* and 30.15 cm in *C. mandica*. Scale bar for (***a***) and (***b***) = 0.5 cm, and for (***c***) and (***d***) = 0.1 cm.

has been revealed which includes a focus, circuli and radii. Focus in scales of both species is clear and located in anterior field, and is the first part of scale to be formed during ontogenesis[15,16].

Further microscopic comparisons showed that in spite of general similarity seen in scales of two species, some microstructures are detected to be different between them. The most important discriminated microscopic characters are number of radii in the anterior and lateral parts of scale, type of radii, focus size as well as shape of tubercles in the posterior part. Some of these microscopic features have already been suggested to be important in cyprinids taxonomy[11,17,18], while some others such as types of radii are suggested to be growth phenomenon (such as nutritive conditions) and weakly influenced by genetic factors[4].

The use of scale morphology and microstructures in fish classification has a long history. However, this method has improved during recent decades owing to the development of SEM[4,5,6,19].

The use of scale morphology in taxonomy is particularly significant for taxa that are difficult to distinguish by considering their external body morphology[7,20]. Scale morphology is not only used to discriminate fish species, but also to study the ecological variation among fish species and even populations[7,21]. Therefore, it can be concluded that some characters related to scale morphology at least are partially genetically encoded at species level[6,22] and thus could be sufficiently applied to estimate phylogenetic relationships among the species[6,23] and even populations[7].

Moreover, the number and shape of lepidonts seem to be important microstructure characters for discrimination of the two studied species. Several previous studies have also documented the taxonomic relevance of lepidonts[7,20,21,24] and concluded that variation in their size and shape of lepidont made this feature as a good tool for species discrimination of fishes[4,7]. The other variations related to lepidonts are texture as well as attachment and orientation on crest of circuli[21], which are probably sufficient to discriminate species.

In addition, scale microstructure of a cyprinid species, *Alburnoides bipunctatus* from Iran has been recently studied[12]. It was found that some architectural specification of scales such as position and shape of focus, circuli, chromatophore in posterior part of scale, lepidonts and lateral line canal might be used as important taxonomic tools in cyprinids[12].

Based on evidences in the present study we can conclude that; (i) comparative study of scale morphology and its microstructure can be used to understand taxonomy of cyprinid fishes, (ii) scale morphology and microscopic structures of scale are particularly relevant for discrimination and identification of morphologically similar species; (iii) the results of further comparative studies on microstructures of scale could be important to understand taxonomic relations of Iranian native cyprinids, particularly endemic members, which form an important part of the icthyo-diversity in the country.

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