Biologia Serbica, 2025, 47: 00-00

DOI: 10.5281/zenodo.14738000

Original paper

Ontogeny and intraspecific variability of the tail shape in *Xiphinema montenegrinum* and *X. hyrcaniense* (Nematoda: Longidoridae)

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Accepted: 20 January 2025

Summary. The microscopic size of nematodes, their morphological similarity, limited number of distinguishable taxonomic characters and overlapping morphometric measurements, make them one of the most difficult organisms to identify. Mainly, various morphological and morphometrical features are used for their primary identification and characterization. Knowledge of interspecific and intraspecific variations of these features in different populations of different origins is essential and often crucial when dealing with species that are very similar to each other. *Xiphinema montenegrinum* from Montenegro and *X. hyrcaniense* from Iran represent two geographically distant but morphologically close species that can be separated by ontogeny of the tail shape and some morphometric indices. Females and males of *X. montenegrinum* have a slightly shorter tail, a shorter hyaline portion of the tail, and greater tail tip variation compared to females and males of *X. hyrcaniense*. The distance between the cloacal opening and the adanal pair of supplements in males is a good diagnostic character for separating these two species since there is no overlap in values.

Keywords: adanal pair of supplements, cloacal opening, geographically distant, Iran, Montenegro.

INTRODUCTION

Because of their microscopic size, morphological similarity, limited number of distinguishable taxonomic characters and overlapping morphometric measurements, nematodes are considered one of the most difficult organisms to identify (Oliveira et al. 2011). Their primary identification and characterization are mainly based on various morphological features (Bhat et al. 2022). Tail shape and length are some of these features, but they show considerable variation from long filiform to short bluntly rounded. Tail shapes in juveniles of various stages is a good character for the differentiation of *Xiphinema* species (Siddiqi 1997). The study of the variability of morpho-anatomical characters within and between different populations of different origins is essential. Intraspecific variability of morphology and morphometry is always present to a greater or lesser extent, even in parthenogenetic species. Knowing this variability is often crucial when dealing with species that are very similar to each other. Setting aside molecular analysis as an integral part of integrative taxonomy and focusing only on morphology, the morphological characteristics of an individual are what we first encounter during its observation. For the identification of the population of a species, it is always a lucky circumstance if, in addition to adults, juvenile stages are available in satisfactory number. However, in practice this is often not the case.

In Longidoridae, the number of juvenile developmental stages (JDS) before becoming adults is most often 4 (J1, J2, J3, J4), and less often 3 (JI, JII, JIII) (Robbins et al. 1995, 1996). Each subsequent JDS, starting from the first stage (J1/JI), shows a certain degree of difference in morphology and morphometry, compared to the previous stage. This is also the

Published online: 27 January 2025

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case with the development of the tail. Although knowledge of the ontogeny of the tail shape in one species is important for knowing the species itself; for comparison of two species that are similar in many ways, the ontogeny of the tail becomes even more important.

Xiphinema montenegrinum Barsi, Lamberti & Agostinelli, 1998 from Montenegro (Barsi et al. 1998) and *X. hyrcaniense* Fadakar, Pourjam, Barsi & Pedram, 2021 from Iran (Fadakar et al. 2021) represent two geographically distant but morphologically close species that can be separated by ontogenesis of tail shape and some morphometric indices. In the original description of *X. montenegrinum* (Barsi et al. 1998) tail shapes of females were illustrated with three drawings (fig. 4B-D) and one photomicrograph (fig. 5B), of males with one drawing (fig. 4G) and one photomicrograph (fig. 5C), and of four JDS with only one drawing for each stage (fig. 4H-K). In the original description of *X. hyrcaniense* (Fadakar et al. 2021) tail shapes of females, males and four JDS are well illustrated either with drawings (figs 1-2) or photomicrographs (figs 3-5).

The goals of the present study were: i) to show the intraspecific variability of tail shape in females, males and juveniles of *X. montenegrinum*; ii) to compare the characteristics of the tail region of females and males of *X. montenegrinum* and *X. hyrcaniense*; iii) to give a comparative account of the ontogenetic development of the tail shape in these two species.

MATERIAL AND METHODS

In this study for *X. montenegrinum*, permanent slides of the type population and unpublished material from three other populations found in different localities in Montenegro were used. These were: type material: rhizosphere of *Carpinus orientalis* Mill. at Morinj (Adriatic coast), 08-06-1994, 05-04-1997; unpublished material: rhizosphere of *C. orientalis*, Dobrčin, Canyon of Cijevna River, 16-06-2001; rhizosphere of *C. orientalis*, Kruševice, Orjen Mt., 24-09-2008; rhizosphere of *Acer campestre* L. and *Corylus avellana* L., Javljen (earlier Javljem), Golija Mt., 13-08-2010.

Photographs were taken using a Zeiss Axio Imager A1 compound microscope equipped with an AxioCam MRc 5 digital camera. Drawings were made based on original photographs of female tails and juvenile stages of *X. montenegrinum* and *X. hyrcaniense*.

RESULTS AND DISCUSSION

Intraspecific variability of tail shape in females, males and juvenile stages of *X. montenegrinum*

A comparative study of the tails of females and males in 4 populations revealed the existence of certain variability in shape (Figs 1 and 2). The position, shape and size of the peg is the most obvious expression of this variability. Pegless tails are extremely rare. Some mutually very similar or almost identical forms appear more often, and some forms less often in the populations. In the type population, each JDS has a characteristic tail shape, which shows the degree of variability within each stage (Fig. 3).

In females, tail short, rounded to dorsally more convex, with a sub-central peg of variable length and blind canal; exceptionally without peg and blind canal (Fig. 1).

In males, tail is similar to that of females, with a subcentral peg of variable length and blind canal; exceptionally without peg and blind canal (Fig. 2).

In juveniles, tail of J1 conical-subdigitate, of J2 dorsally convex conoid with subdigitate terminus, of J3 dorsally convex conoid, digitate with ventral peg, of J4 dorsally convex conoid, digitate with almost ventral or sub-central peg, rarely pegless (Fig. 3)

Comments on the tail regions of females and males of *X. montenegrinum* and *X. hyrcaniense*

At first glance, the tail shapes of females on the one hand, and males on the other hand, in these two species are very similar to each other (Fig. 4).

In females, tail short, rounded to dorsally more convex, with a sub-central peg and blind canal, exceptionally without peg and blind canal (Fig. 4A-B, C-D and Fig. 5). Females of *X. montenegrinum* have a slightly shorter tail, a shorter hyaline portion of the tail, and greater tail tip variation compared to females of *X. hyrcaniense* (Table 1, Fig. 5).

Males of *X. montenegrinum* have a slightly shorter tail, a shorter hyaline portion of the tail, and greater tail tip variation compared to males of *X. hyrcaniense* (Table 1, Fig. 4E-F, G-H). The most significant difference in males is the distance between adanal pair of supplements and cloacal opening. The distance between the cloacal opening and adanal pair of supplements in *X. montenegrinum* (n = 41) 14.7 (12.3-16.8) μm, and in *X. hyrcaniense* (n = 9) 25.3 (22.6-27.0) μm without overlapping. Spicule lengths mostly overlap, 80.7-92.9 μm in *X. montenegrinum* (n = 24), and 72-95 μm in *X. hyrcaniense* (n = 16).

The distance between the cloacal opening and the adanal pair of supplements is a good diagnostic character for separating these two species, because there is no overlap in values, whereas the length of the spicules is not suitable due to significant overlap.

Ontogeny of the tail shape in *X. montegrinum* and *X. hyrcaniense*

In females of *X. montegrinum* and *X. hyrcaniense*, the

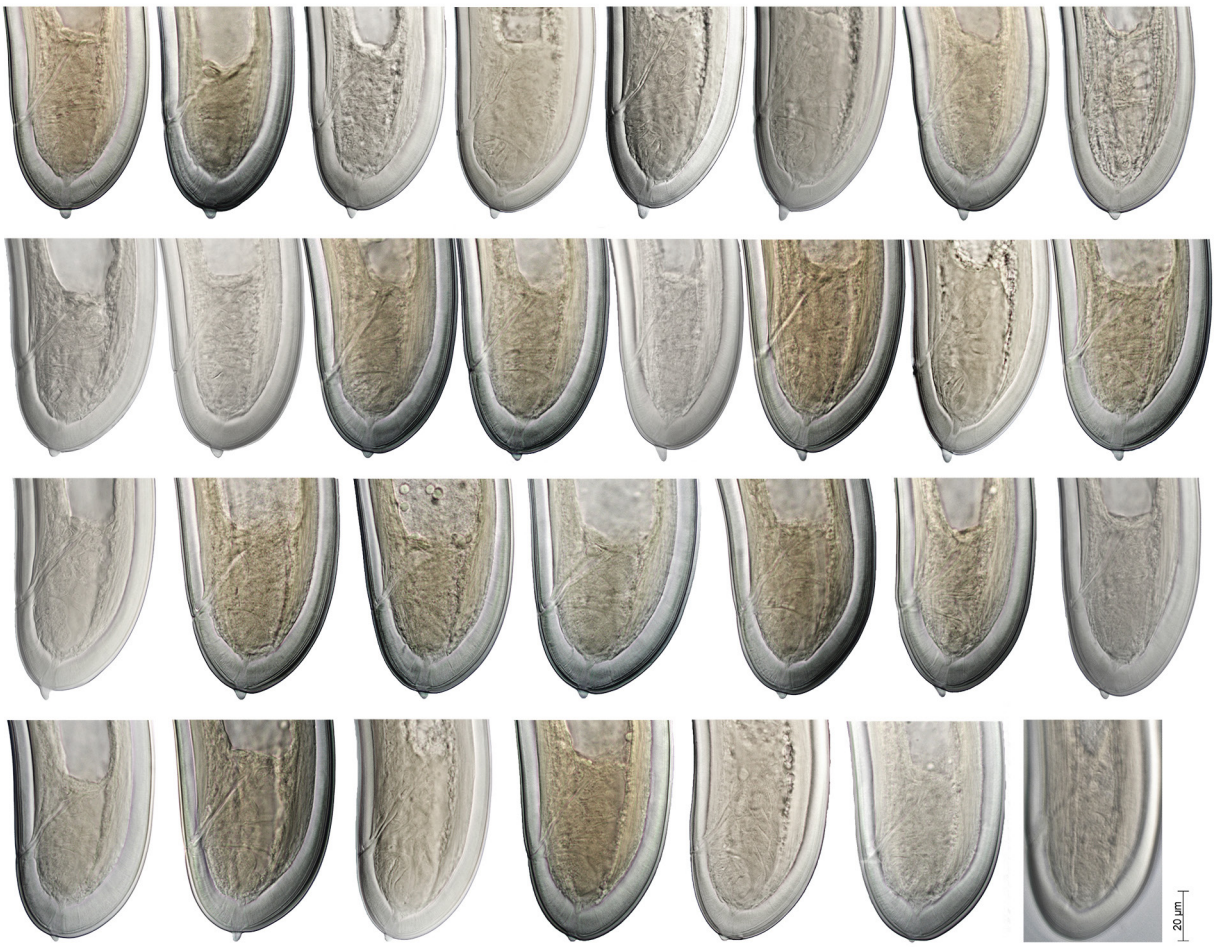

Fig. 1. Intraspecific variability of tail shape in females of *Xiphinema montenegrinum* from Montenegro. Based on type population from Morinj (Adriatic coast), and populations from Dobrčin (Canyon of Cijevna River), Kruševice (Orjen Mt.), and Javljen (Golija Mt.).

tails are relatively similar and to someone with less experience they may even seem the same (Fig. 5). When it comes to the first (J1), second (J2), third (J3) and fourth (J4) juvenile stages of these two species, the situation is somewhat different (Fig. 6).

Tail of J1 conical-subdigitate in *X. montegrinum* and elongate conoid, dorsally convex, ventrally concave, with bluntly rounded tip in *X. hyrcaniense* (Fig. 6).

Tail of J2 dorsally convex conoid with subdigitate terminus in *X. montegrinum* and dorsally convex and ventrally concave, ending to subdigitate tip in *X. hyrcaniense* (Fig. 6).

Tail of J3 dorsally convex conoid, digitate with ventral peg in *X. montegrinum* and conical dorsally convex subdigitate in *X. hyrcaniense* (Fig. 6).

Tail of J4 dorsally convex conoid, digitate with almost ventral or sub-central peg, rarely pegless in *X. montegrinum* and short dorsally more convex with a small mucro in *X. hyrcaniense* (Fig. 6).

Juvenile stages of *X. montegrinum* have somewhat shorter tail and shorter hyaline portion of the tail compared to juvenile stages of *X. hyrcaniense* (Table 1).

Coomans et al. (2001), in their capital work, highlighted that "The relations between the various tail types are complex and can be assessed through the study of the postembryonic development". After analyses of the tail morphology of juveniles and adults and tail development during embryogenesis in the genus *Xiphinema*, these authors classified the tail shapes in juveniles and adults into 13 types, ranging from filiform (Type 1) to broadly rounded (Type 13), and provided an illustration with some representatives of every type (Coomans et al. 2001, p. 31, fig. 11). They also defined the patterns of changes in the tail shape during postembryonic development and presented a scheme to illustrate which tail types could be derived from other types (Coomans et al.

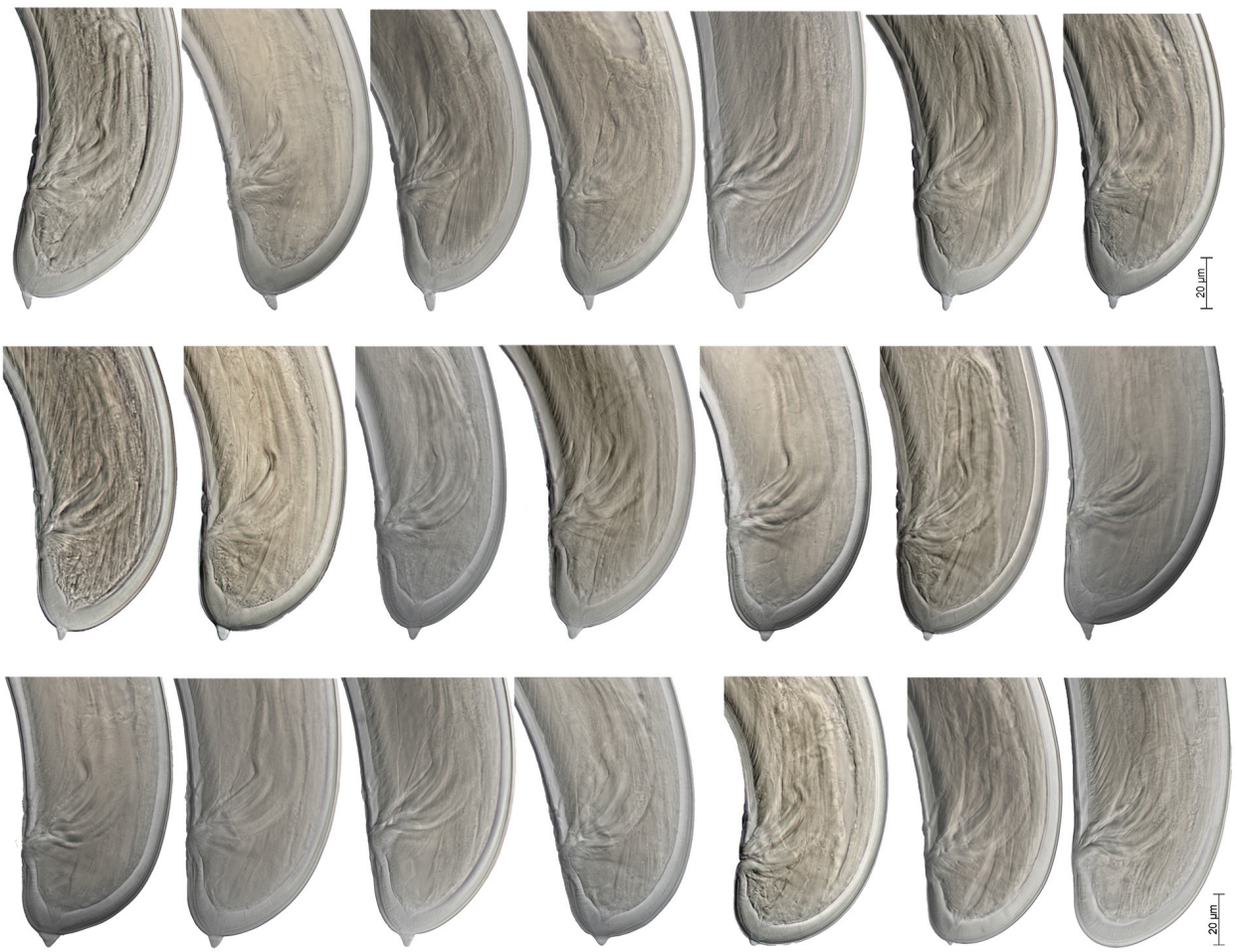

Fig. 2. Intraspecific variability of tail shape in males of *Xiphinema montenegrinum* from Montenegro. Based on type population from Morinj (Adriatic coast), and populations from Dobrčin (Canyon of Cijevna River), Kruševice (Orjen Mt.), and Javljen (Golija Mt.).

2001, p. 32, fig. 12). The whole pattern was based on published data for more than 80 species for which the tail shape of different juvenile stages was known at that time. One of these species was *X. montenegrinum* in which the tail shapes were illustrated with one drawing each for J1, J2, J3 and J4, and three drawings for the female. Among many others, these drawings were used for construction of tail shape scores of *Xiphinema* species for which morphological data of at least three juvenile stages were known (Coomans et al. 2001).

The tail shape score for *X. montenegrinum* was: J1 (7), J2 (8), J3 (9), J4 (9), female (10). The same tail shape score could apply to *X. hyrcaniense* as well. Based on the studied material from Montenegro and Iran, the intraspecific and interspecific variability of the tail shape of females and four juvenile stages in *X. montenegrinum* and *X. hyrcaniense* is shown here (Figs 5-6). It can be noted that females with pegless tails rarely appear in both species. Likewise, with *X. montenegrinum*,

Table 1. Comparison of tail length and hyaline portion of the tail of juveniles and adults in *Xiphinema montenegrinum* and *X. hyrcaniense*.

Tail length and hyaline portion of tail, min-max (µm)		
	X. montenegrinum	*X. hyrcaniense*
J1	47.1-57.8; 9.4-13.8	60-67.5; 16-20
J2	42.8-52.9; 11.9-17.3	55-67.5; 17.5-25
J3	37.5-50; 8.8-13.7	45-60; 15-21.3
J4	35.0-44.6; 7.5-11.9	42.5-55; 12.5-17.5
Female	31.4-42.7; 7.5-13.8	35-47.5; 12.5-21.3
Male	36.9-47.5; 6.9-14.4	41-55; 11.5-17.5

Sources: Barsi et al. 1998; Fadakar et al. 2021.

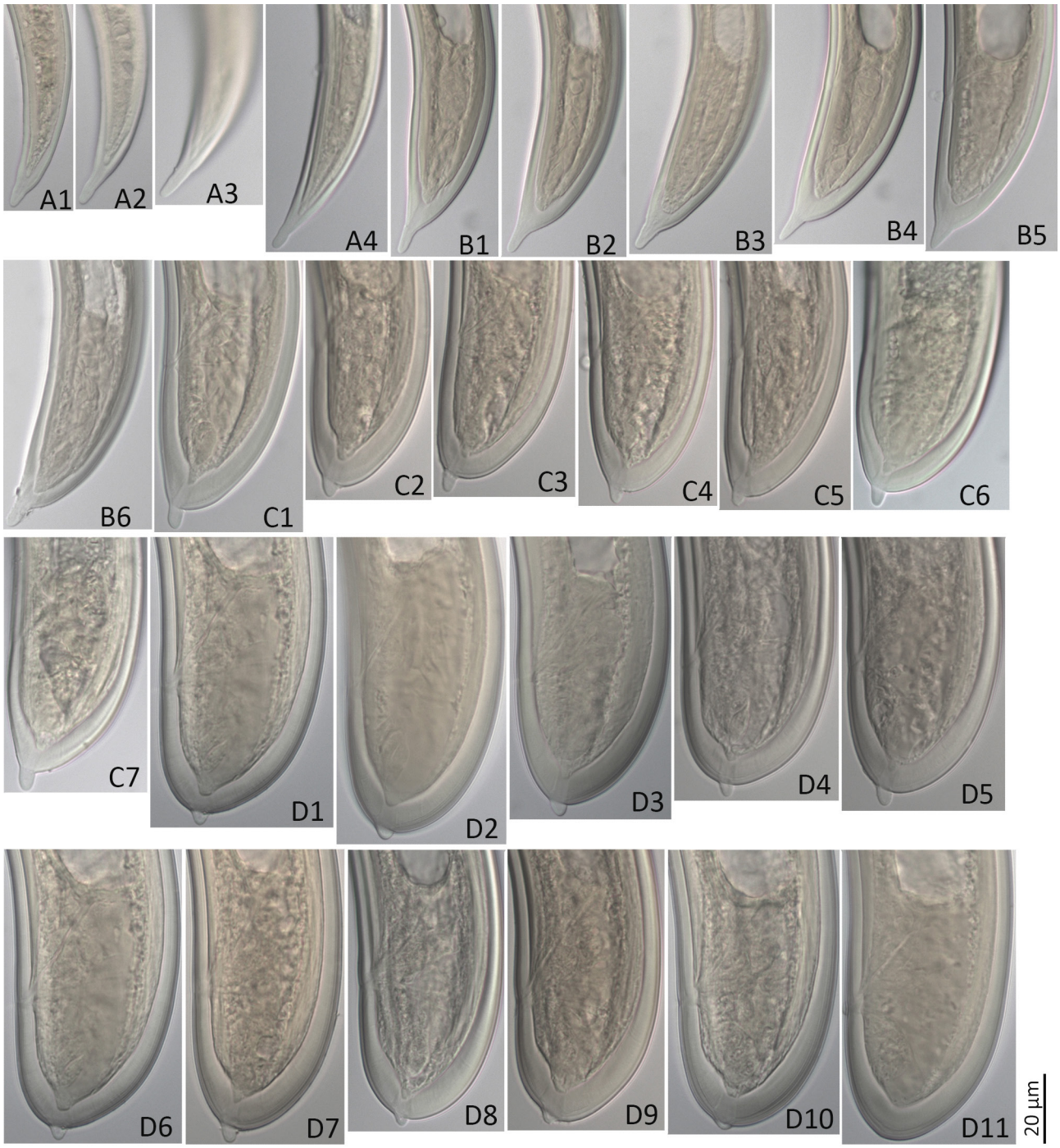

Fig. 3. Intraspecific variability of tail shape in four juvenile developmental stages of *Xiphinema montenegrinum*. **A1-A4** = J1; **B1-B6** = J2; **C1-C7** = J3; **D1-D11** = J4. Based on type population from Morinj (Adriatic coast), Montenegro.

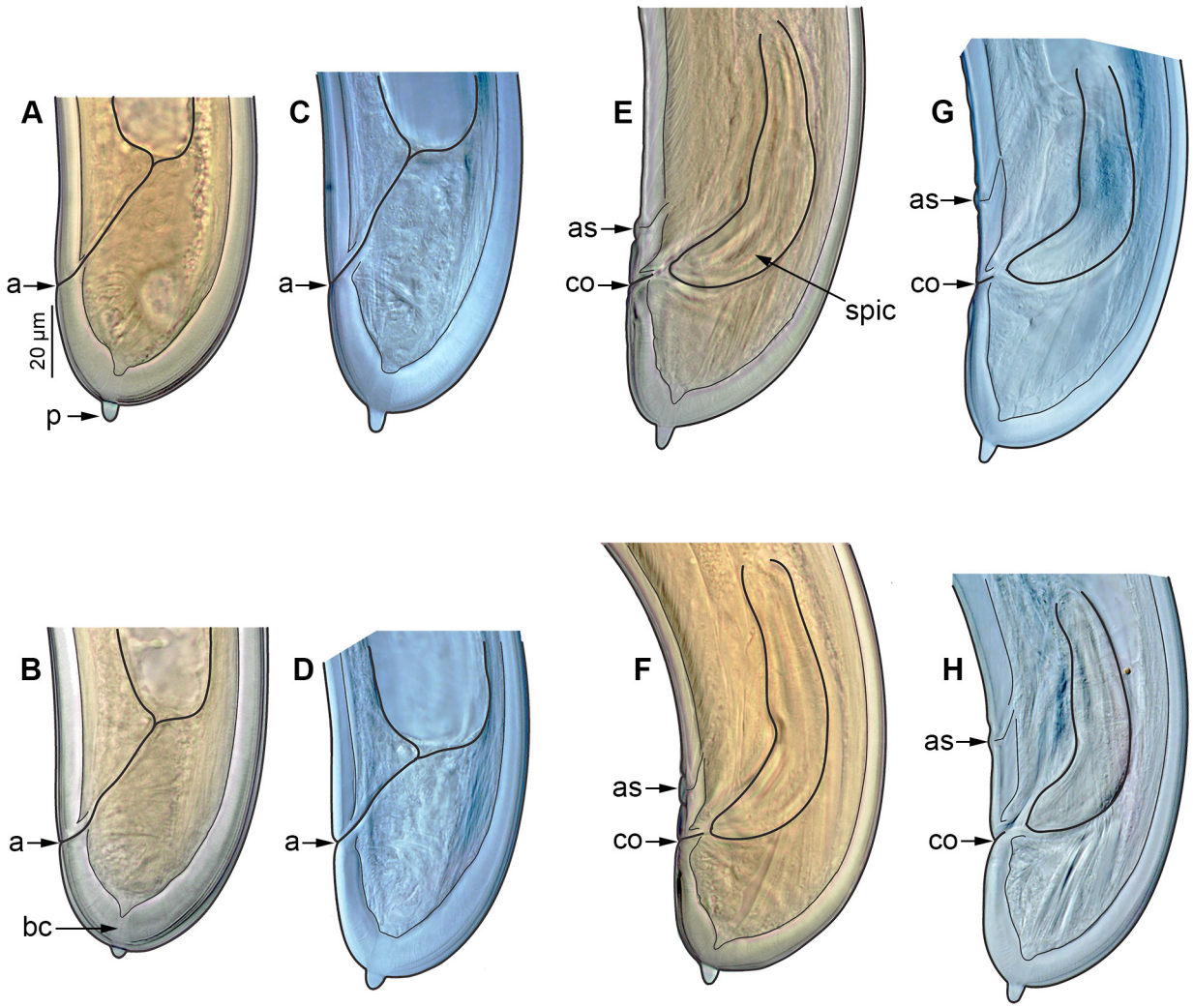

Fig. 4. Comparison of tail regions. *Xiphinema montenegrinum*: **A**-**B**, female tail region; **E**-**F**, mail tail region. *X. hyrcaniense*: **C**-**D**, female tail region; **G**-**H**, mail tail region. (Abbreviations: a = anal opening; as = adanal pair of supplements; bc = blind canal; co = cloacal opening; p = peg; spic = spicule.)

individuals of the J4 stage with a pegless tail rarely appear. These facts lead to the addition of the tail shape score in both species (Fig. 7). Additional tail shape scores for *X. montenegrinum* are: J1 (7), J2 (8), J3 (9), J4 (9), female (12); J1 (7), J2 (8), J3 (9), J4 (12), female (12) (Fig. 7). Additional tail shape score for *X. hyrcaniense* is: J1 (7), J2 (8), J3 (9), J4 (9), female (12) (Fig. 7).

The capital work of Coomans et al. (2001) on character analysis, phylogeny and biogeography of the genus *Xiphinema* is an indispensable source of information for researchers dealing with *Xiphinema* species. Over the past 23 years, a large number of new *Xiphinema* species have been described with detailed descriptions and illustrations of adult and juvenile stages, which represents an irreplaceable upgrade of the work of Coomans et al. (2001). The reality is that the work of Coomans et al. (2001) needs to be updated based on new descriptions, but the big question is whether anyone will ever do it.

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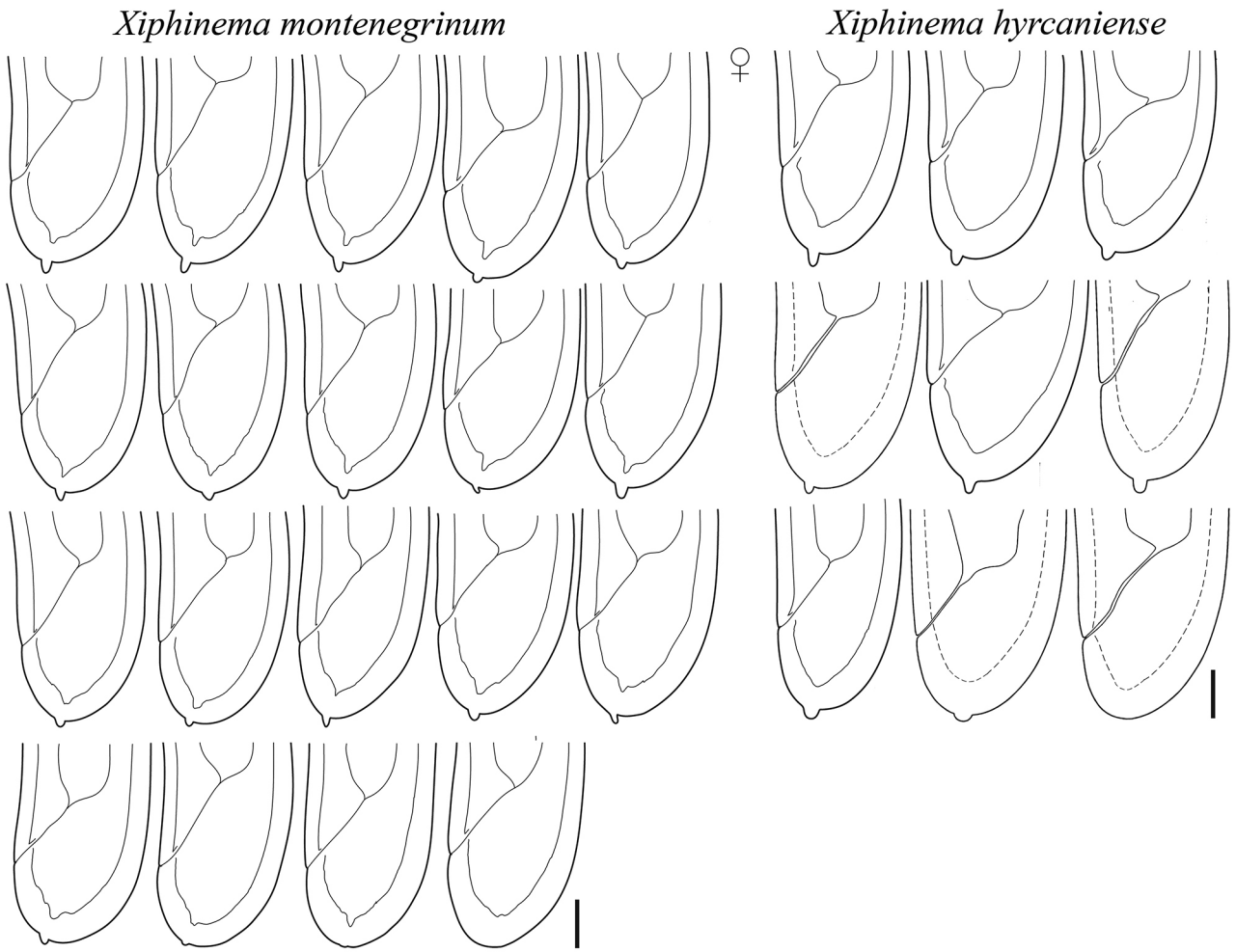

Fig. 5. Comparative presentation of intraspecific variability of tail shape in females of *Xiphinema montenegrinum* from Montenegro and *X. hyrcaniense* from Iran. (Scale bars = 20 µm.) (Drawings with dashed line redrawn from Fadakar et al. 2021, fig. 1h, i, j, n.)

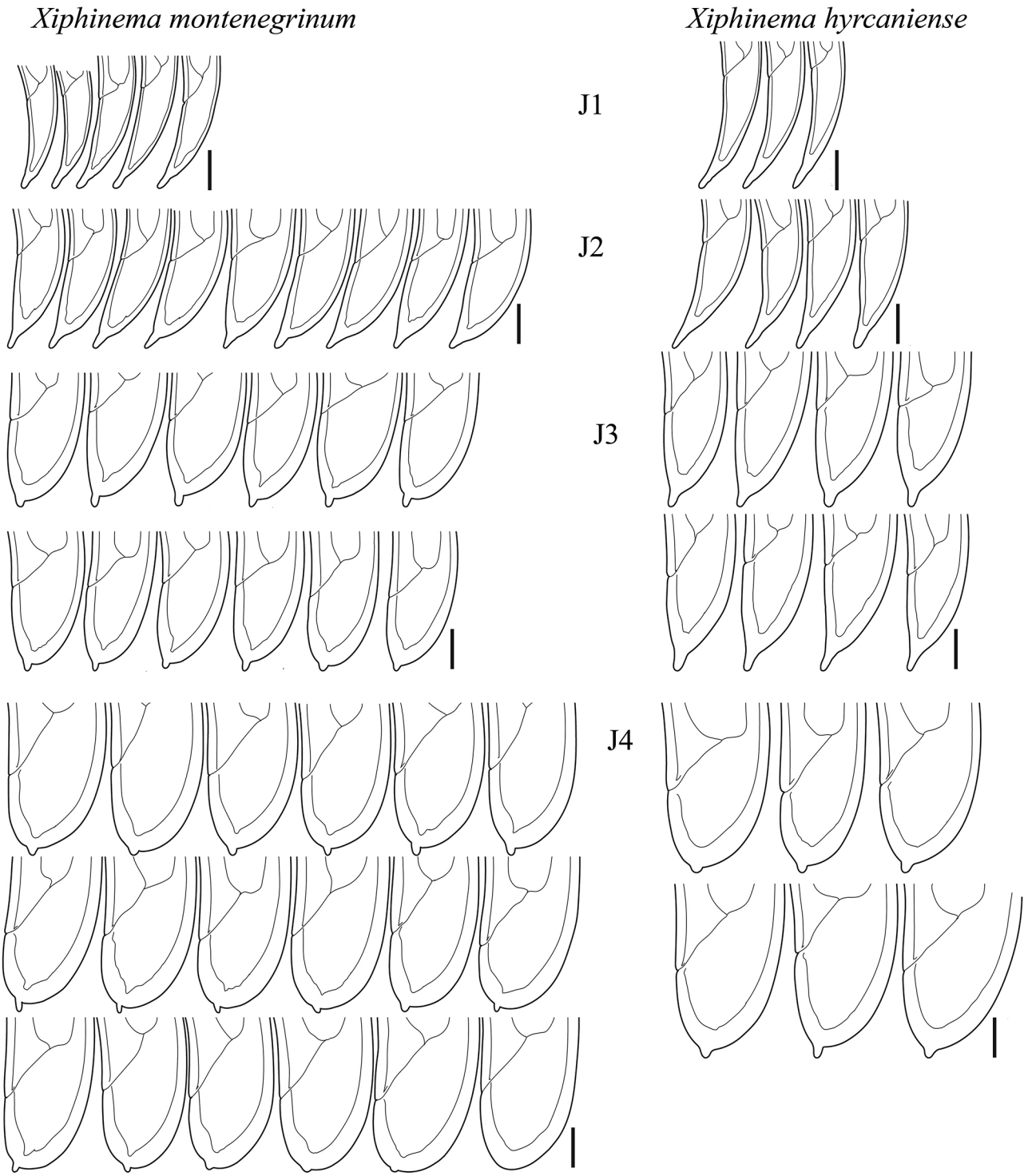

Fig. 6. Comparative presentation of intraspecific variability of tail shape in four juvenile developmental stages (J1, J2, J3, J4) of *Xiphinema montenegrinum* from Montenegro and *X. hyrcaniense* from Iran. (Scale bars = 20 μm.)

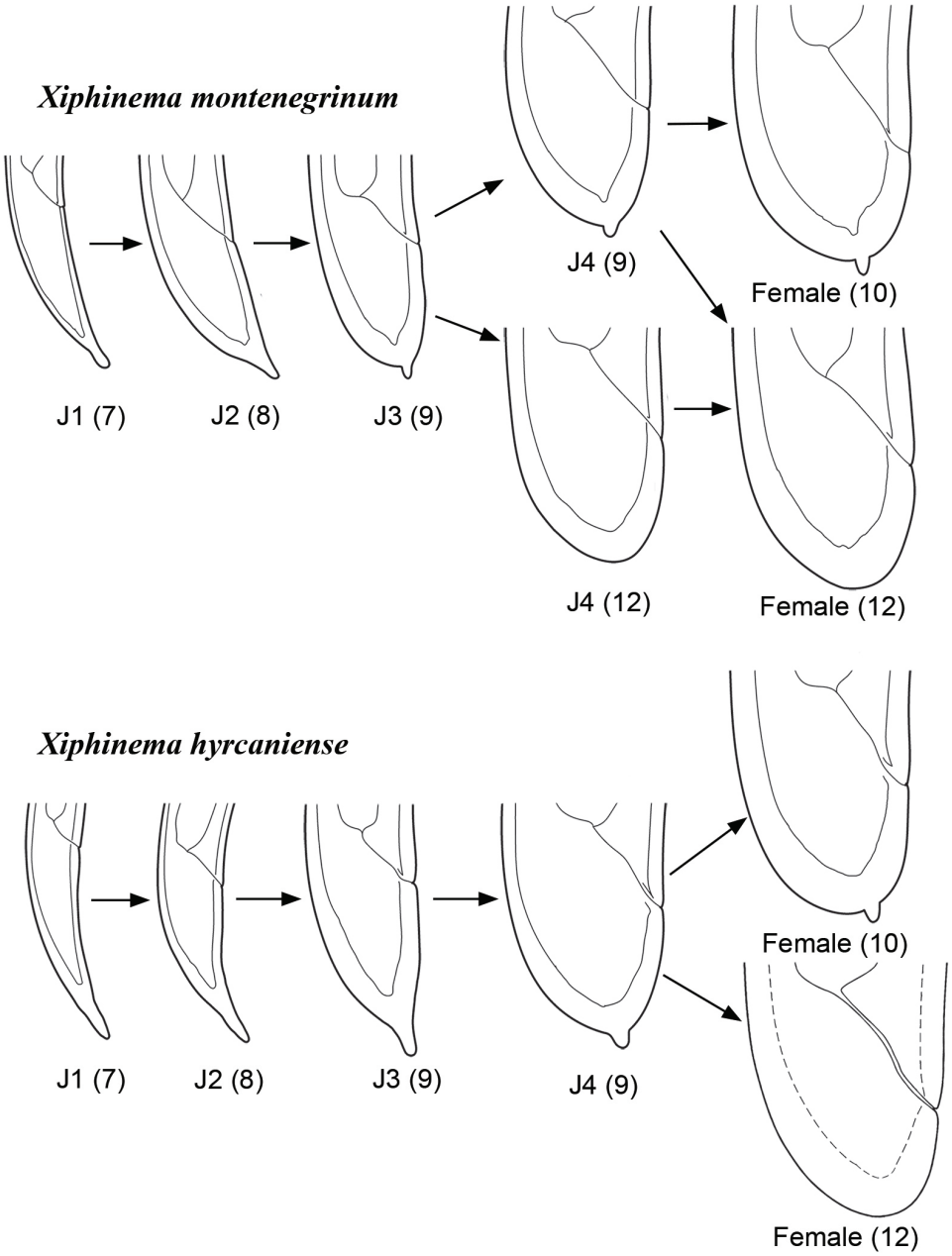

Fig. 7. Comparative presentation of the ontogeny of tail shape in *Xiphinema montenegrinum* from Montenegro and *X. hyrcaniense* from Iran. Types of the tail shapes after Coomans et al. 2001: 7 (conoid to dorsally convex conoid), 8 (dorsally convex conoid with subdigitate terminus), 9 (dorsally convex conoid, digitate with ventral peg), 10 (convex conoid, digitate with central [on the terminus] peg or bulge), 12 (conoid with broadly rounded tip).