

Original paper

Two anomalies in a population of *Longidorus* sp. (Nematoda: Longidoridae) from Serbia

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Summary. Two anomalies in a population of *Longidorus* sp. from Serbia have been described and illustrated. Several females and males had extra odontostyles, and one female had extra odontostyle and two vulvae.

Keywords: anomaly, Cerova, extra odontostyle, genital tract, replacement odontostyle, Serbia, two vulvae.

INTRODUCTION

Postembryonic development in *Longidorus* species includes four, rarely three juvenile stages (labelled J1, J2, J3, J4, and JI, JII, JIII, respectively) and the adult stage. The transition from one juvenile stage to another is a complex process, during which there is a significant increase in body size, and among other processes, there are changes in the feeding apparatus too. A needle-like odontostyle, as a part of the feeding apparatus, has its own developmental history. Adults have one functional odontostyle, while each of the four, rarely three juvenile developmental stages (JDS) have one functional odontostyle and one replacement odontostyle. The first juvenile stage (J1 / JI) hatched from egg, possesses a functional odontostyle and a replacement odontostyle embedded within the wall of the odontophore. In species with

four JDS starting from J1 during the transition to J2 stage during the complex process of replacement odontostyle in J1 will be a functional odontostyle in J2. At the same time, a replacement odontostyle is formed in J2. This whole process also happens during the transition from J2 to J3 and J3 to J4. When switching from J4 to adult form, no new replacement odontostyle is created. In species with three JDS starting from JI during the transition to JII stage during the complex process of replacement odontostyle in JI will be a functional odontostyle in JII. At the same time, a replacement odontostyle is formed in JII. This whole process also happens during the transition from JII to JIII. When switching from JIII to adult form, no new replacement odontostyle is created. But there are cases when, in addition to functional odontostyle, another so-called extra odontostyle appears in adults. It is an anomaly and this phenomenon in adults of *Longidorus*

spp. has been documented by several authors (Zheng et al. 2000; Krnjaić S and Krnjaić Đ 2006; Ferris et al. 2012; Groza et al. 2017).

In females of *Longidorus* spp. the genital system comprises two functional ovaries with their corresponding genital tracts, forming two genital branches that merge and lead to a common vagina opening on the body surface via the vulva. The two branches extending in opposite directions from an almost equatorial vulva. Anomaly in genital system of females such as presence of two vulvae rarely occur and it has been documented in several papers (Lamberti et al. 1987; Barsi 1994; Robbins and Rubtsova 1996; Rubtsova et al. 1999; Širca et al. 2007; Kornobis and Peneva 2011; Kornobis 2012).

A preliminary study of a population of *Longidorus* sp. from Serbia revealed presence of several specimens with one or two anomalies. Some females and males had extra odontostyle, and one female had two vulvae and an extra odontostyle.

The aim of this paper was to present morphological and morphometrical data of selected characteristics, and provide illustrations of selected females and males of *Longidorus* sp. with extra odontostyle, as well as a female specimen with extra odontostyle and two vulvae from Cerova (Serbia).

MATERIALS AND METHODS

A soil sample with specimens of *Longidorus* sp. was collected in 10th May 2007 in the rhizosphere of *Carpinus betulus* (common hornbeam) near Kovačevića cave at Cerova (Serbia). Nematodes were extracted by Cobb's wet sieving technique, killed and fixed with hot FP 4:1, transferred to glycerin by the slow evaporation method and mounted on permanent microscope slides. Selected characteristics used in this study were measured using Digimizer Version 4.6.1 software for digital measurements. Body length and vulva position in individual specimens were measured from scanned drawings made using a drawing tube mounted on an Olympus CX31 microscope. Odontostyle and extra

odontostyle lengths were measured from photographs of individual specimens, taken using a Zeiss Axio Imager A1 compound microscope equipped with the digital camera AxioCam MRc 5. Helicon Focus 7 software was used to align most of the images with the extra odontostyle.

RESULTS

A female of *Longidorus* sp. with two anomalies (presence of an extra odontostyle, and two vulvae, respectively), and a female without any anomaly are illustrated in Fig. 1. Microphotographs of morphological details of extra odontostyle in females and males are presented in Figs 2 and 3, while details of genital system of a female with two vulvae and a normal female with one vulva in Figs 4 and 5.

Extra odontostyle was present in 22 out of 66 females and 7 out of 65 males in the population studied (Table 1 and Fig. 6). In three females the extra odontostyles were shorter than the functional ones (Table 1).

One female out of 22 females with extra odontostyle (Table 2 and Fig. 7) and 44 females without that, had two vulvae instead of one, which is a normal condition in females. It is morphologically similar to other adult females of the same population except for the presence of the two vulvae. The two vulvae are at about regular position for this population (49.6-56.9%) and are close to one another, 99 µm apart (Fig. 1A, C). The vulvae are connected by a common simple, short proximal part of uterus (Fig. 1A, C, Fig. 4A-C); the distal gonads are normally developed and both seem to be functional (Fig. 1A, Fig. 4A).

DISCUSSION

Presence of extra odontostyle in *Longidorus* spp. has been reported by Zheng et al. (2000), Krnjaić S and Krnjaić Đ (2006/2009), Ferris et al. (2012), Groza et al. (2017), and in the present study (Table 3). The reported numeric values for extra odontostyle were higher than the values for functional

Table 1. Body length and measurements of functional and extra odontostyles in selected females and males of *Longidorus* sp. from Cerova (Serbia) in the form average (minimum-maximum).

| | Female (n = 19) | Female (n = 3) | Male (n = 7) |
|------------------------|---------------------|---------------------|---------------------|
| Body length (µm) | 4905 (4239-5728) | 5056 (4592-5372) | 4923 (4482-5426) |
| Odontostyle (µm) | 109.0 (100.4-120.5) | 106.0 (104.6-107.8) | 109.2 (99.1-115.3) |
| Extra odontostyle (µm) | 120.3 (113.0-128.6) | 88.6 (76.3-97.3) | 123.5 (114.4-132.5) |
| Longer (µm) | 11.3 (0.3-19.3) | – | 14.3 (10.3-20.1) |
| Longer (%) | 10.5 (0.3-18.5) | – | 13.1 (9.7-17.9) |
| Shorter (µ) | – | 17.3 (7.3-31.5) | – |
| Shorter (%) | – | 16.2 (7.0-29.2) | – |

odontostyle with two exceptions. In one female of *L. piceicola* from Romania (Groza et al. 2017) the extra odontostyle had the same value as the functional one (Table 3). In three females of *Longidorus* sp. from Serbia, extra odontostyles were shorter than functional ones (Table 3), and as far as I know this is the first finding of this type for some species of *Longidorus*.

Bivulval females among *Longidorus* spp. have been reported by Jairajpuri and Ahmad (1969), Lamberti et al.

(1987), Barsi (1994), Robbins and Rubtsova (1996), Rubtsova et al. (1999), Širca et al. (2007), Kornobis and Peneva (2011), and Kornobis (2012) (Table 4).

More details on atypical developmental and anatomical characteristics in dorylaims can be found in Ferris et al. (2012), as well as a review of malformations observed in the female genital system of dorylaims in Peña-Santiago (2019).

Table 2. Body length and vulva position in females of *Longidorus* sp. with extra odontostyle from Cerova (Serbia).

| Females | Body length (µm) | V (%) | V1 (%) | V2 (%) |
|---------|------------------|------------------|--------|--------|
| n = 1 | 5245 | – | 53.8 | 55.7 |
| n = 21 | 4911 (4239-5728) | 53.5 (49.6-56.9) | – | – |

Table 3. Review of available data for the presence of extra odontostyle in *Longidorus* spp.

| <i>Longidorus</i> spp. / Country | Odontostyle (µm) | Extra odontostyle (µm) | Status | Reference |
|--|---------------------|------------------------|----------|-------------------------------------|
| <i>L. camelliae</i> 1 f / China | 87 | 97 | Increase | Zheng et al. 2000 |
| <i>L. iranicus</i> ¹ 5 f / Serbia | 114.8 (112-118) | 123.6 (118-135) | Increase | Krnjaić S and Krnjaić Đ 2006 (2009) |
| <i>L. iranicus</i> ¹ 3 m / Serbia | 112.3 (108-117) | 117 (1 m) | Increase | Krnjaić S and Krnjaić Đ 2006 (2009) |
| <i>L. paravineacola</i> 1 f / ? | – | – | ? | Ferris et al. 2012, p. 2, fig. 2 |
| <i>L. piceicola</i> 1 f / Romania | 165 | 175 | Increase | Groza et al. 2017 |
| <i>L. piceicola</i> 1 f / Romania | 158 | 158 | Same | Groza et al. 2017 |
| <i>Longidorus</i> sp. 19 f / Serbia | 109.0 (100.4-120.5) | 120.3 (113.0-128.6) | Increase | Original |
| <i>Longidorus</i> sp. 3 f / Serbia | 106.0 (104.6-107.8) | 88.6 (76.3-97.3) | Decrease | Original |
| <i>Longidorus</i> sp. 7 m / Serbia | 109.2 (99.1-115.3) | 123.5 (114.4-132.5) | Increase | Original |

Legends: f = female; m = male.

¹ In 2002 Krnjaić et al. described *Paraongidorus serbicus* sp. n. from Serbia; in 2006 Roca based on the study of paratypes (with light microscope and SEM) transferred it to the genus *Longidorus* as *L. serbicus* n. comb. and concluded that it was morphologically identical with *L. moesicus*, therefore he proposed it as a junior synonym of *L. moesicus*; in 2015 Tanha Maafi et al. in 2015 suggested the synonymy of *L. iranicus* and *L. moesicus* based on the morphology and molecular similarities between the two species. Both species were described in 1983, but the description of *L. iranicus* appeared in the journal issue earlier in that year in comparison with the description of *L. moesicus*. Consequently, the name *L. iranicus* has priority over *L. moesicus*, the latter being a junior synonym of the former.

Table 4. Review of available data for the presence of two vulvae in females of *Longidorus* spp.

| <i>Longidorus</i> spp. | Country | Reference |
|-----------------------------------|----------------------------------|---------------------------|
| <i>Longidorus</i> sp. | ? | Jairajpuri and Ahmad 1969 |
| <i>L. laeovicapitatus</i> | São Tomé and Príncipe | Lamberti et al. 1987 |
| <i>L. euonymus</i> | Serbia | Barsi 1994 |
| <i>L. artemisiae</i> ¹ | Rostov region of European Russia | Rubtsova et al. 1999 |
| <i>L. juvenilis</i> | Slovenia | Širca et al. 2007 |
| <i>L. poessneckensis</i> | Poland | Kornobis and Peneva 2011 |
| <i>L. danuvii</i> | Poland | Kornobis 2012 |
| <i>Longidorus</i> sp. | Serbia | Original |

¹ Robbins and Rubtsova in 1996 reported this female as *Longidorus elongatus*.

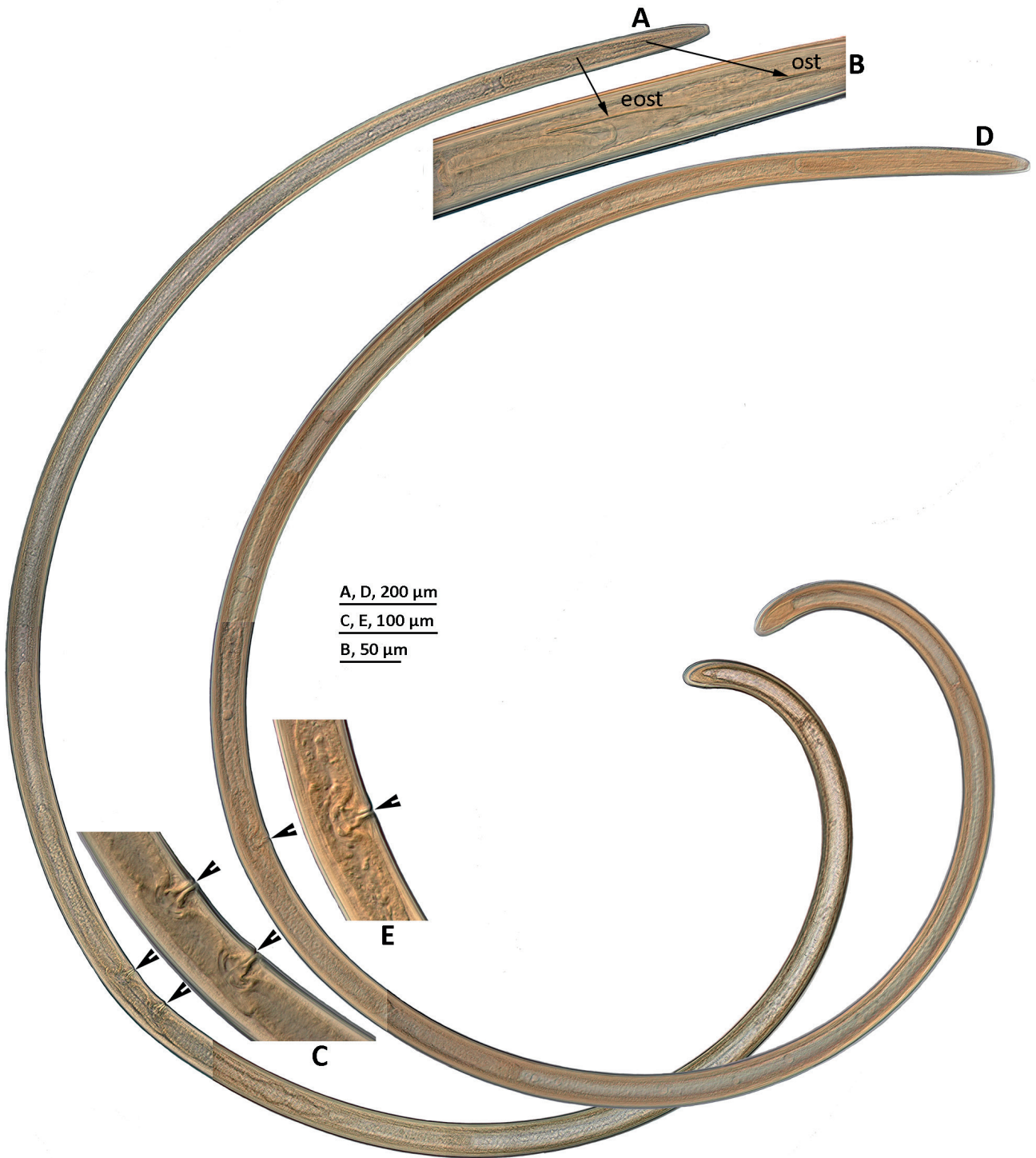


Fig. 1. *Longidorus* sp. from Cerova, Serbia. **A**, female with extra odontostyle and two vulvae, entire body (black arrowhead denotes vulva in **A**, **C**, **D**, and **E**, respectively); **B**, enlarged details of anterior region of female with extra odontostyle (ost = odontostyle, eost = extra odontostyle); **C**, enlarged details of vulval region of female with two vulvae; **D**, female without any anomaly, entire body; **E**, enlarged detail of vulval region of female without any anomaly.

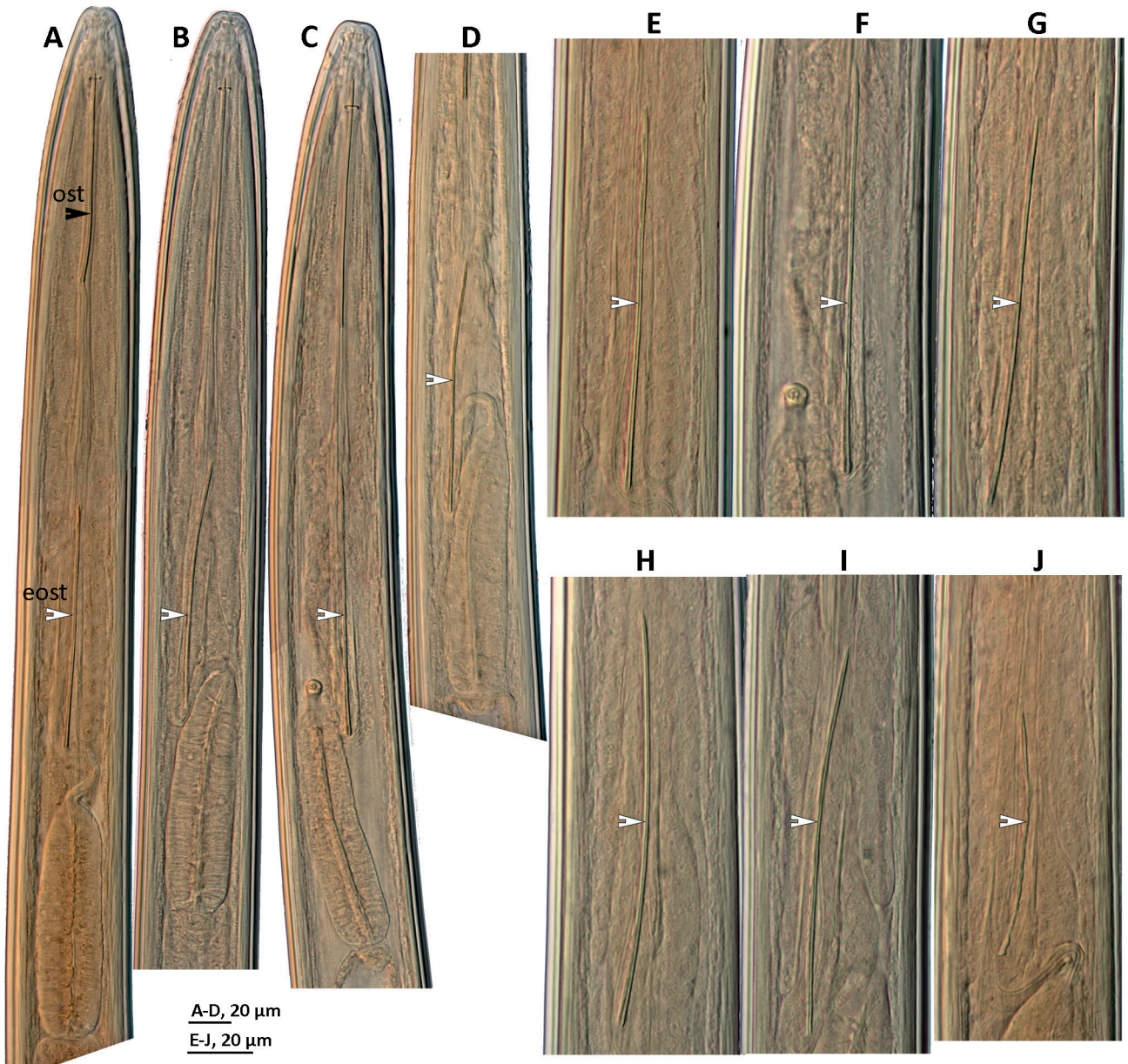


Fig. 2. *Longidorus* sp. from Cerova, Serbia. A-C, female anterior region showing odotostyle (ost) and odontophore (oph) denoted by black arrowhead (A), and extra odotostyle (eost) denoted by white arrowhead (A-C); D-J, details of anterior region with extra odotostyle.



Fig. 3. *Longidorus* sp. from Cerova, Serbia. **A-B**, male anterior region showing odotostyle (ost) and odontohore (oph) denoted by black arrowhead (A), and extra odotostyle (eost) denoted by white arrowhead (A-B); **C**, detail of anterior region with extra odotostyle.

REFERENCES

- Barsi L. 1994. Bivulval females of *Longidorus euonymus*, *Xiphinema diversicaudatum* and *X. vuittenezi* (Nematoda: Dorylaimida). *Nematologia Mediterranea*. 17:97–108.
- Ferris H, Robbins R, Yeates G. 2012. Atypical development in plant and soil nematodes. *Journal of Nematology*. 44(1):1–6.
- Groza M, Lazarova S, De Luca F, Fanelli E, Elshishka M, Radoslavov G, Hristov P, Coman M, Peneva V. 2017. The morphological and molecular identity of *Longidorus piceicola* Lišková, Robbins & Brown, 1997 from Romania (Nematoda, Dorylaimida). *ZooKeys* 667: 1–19. doi: 10.3897/zookeys.667.12011.
- Jairajpuri MS, Ahmad S. 1969. Record of bivulval specimens of *Longidorus* sp. and *Nygolaimus* sp. *Science and Culture* 35. 492.
- Kornobis FW. 2012. First record of *Longidorus danuvii* Barsi, Lamberti & De Luca, 2007 (Nematoda: Longidoridae) from Poland with description of pathologies of the lip region and reproductive system. *Helminthologia*. 49:104–107.
- Kornobis FW, Peneva V. 2011. *Longidorus poessneckensis* Altherr, 1974 and *L. piceicola* Lišková, Robbins & Brown, 1997 (Nematoda: Longidoridae): new records from Poland and the first description of the *Longidorus poessneckensis* male and a bivulval female. *Systematic Parasitology*. 80:205–216. DOI 10.1007/s11230-011-9325-8
- Krnjaić Dj, Lamberti F, Krnjaić S, Agostinelli A, Radicci V. 2002. Longidoridae (Nematoda) occurring in the Topchider park of Belgrade, Serbia, with the description of *Paralongidorus serbicus* sp. n. *Nematologia Mediterranea*. 30:185–200.
- Krnjaić S, Krnjaić Đ. 2006(2009). Novi nalazi kopljaste nematode *Paralongidorus serbicus* (Nematoda: Longidoridae), prvi nalaz mužjaka i imaga sa zamenom odotostileta. [New records of *Paralongidorus serbicus*, Krnjaic et al. (Nematoda: Longidoridae) from Serbia, with description of the male and adults with odotostyle replacement]. *Zaštita bilja*. 57(255-258):99–114.
- Lamberti F, Agostinelli A, Neto ESS. 1987. A bivulval *Longidorus laevicapitatus* from Sao Tomé. *Nematologia Mediterranea*. 15:379–381.
- Peña-Santiago R. 2019. A very rare malformation affecting the female genital system of one *Labronema* specimen (Dorylaimida, Dorylaimidae). *Journal of Nematology*. 51:e2019-17.
- Robbins RT, Rubtsova TV. 1996. A specimen of *Longidorus elongatus* with two vulvas from Russia. *Nematropica*. 26:305.
- Roca F. 2006. On the identity of two *Paralongidorus* species (Nematoda: Longidoridae) described from Montenegro and Serbia. *Nematology*. 8(5):781–785.
- Rubtsova TV, Chizov VN, Subbotin SA. 1999. *Longidorus artemisiae* sp. n. (Nematoda: Longidoridae) from roots of *Artemisia* sp., Rostov region, Russia. *Russian Journal of Nematology*. 7(1):33–38.
- Širca S, Stare BG, Pleško IM, Marn MV, Urek G. 2007. First record of *Longidorus juvenilis* and *L. leptcephalus* (Nematoda: Dorylaimida) in Slovenia and their morphometrical and ribosomal DNA sequence analysis. *Russian Journal of Nematology*. 15:1–8.
- Tanha Maafi Z, Subbotin SA, Sturhan D, Barooti S, Majd Taheri Z. 2015. Characterisation of *Longidorus iranicus* Sturhan & Barooti, 1983 (Nematoda: Longidoridae) from Iran and synonymisation of *L. moesicus* Lamberti, Choleva & Agostinelli, 1983. *Russian Journal of Nematology*. 23(1):21–28.
- Zheng J, Peneva V, Brown DJF. 2000. *Longidorus camelliae* n. sp. (Nematoda: Longidoridae) associated with ornamental cultivars of *Camellia japonica* L. growing in a nursery at Fuyang, Zhejiang Province, eastern China. *Systematic Parasitology*. 47:119–125.

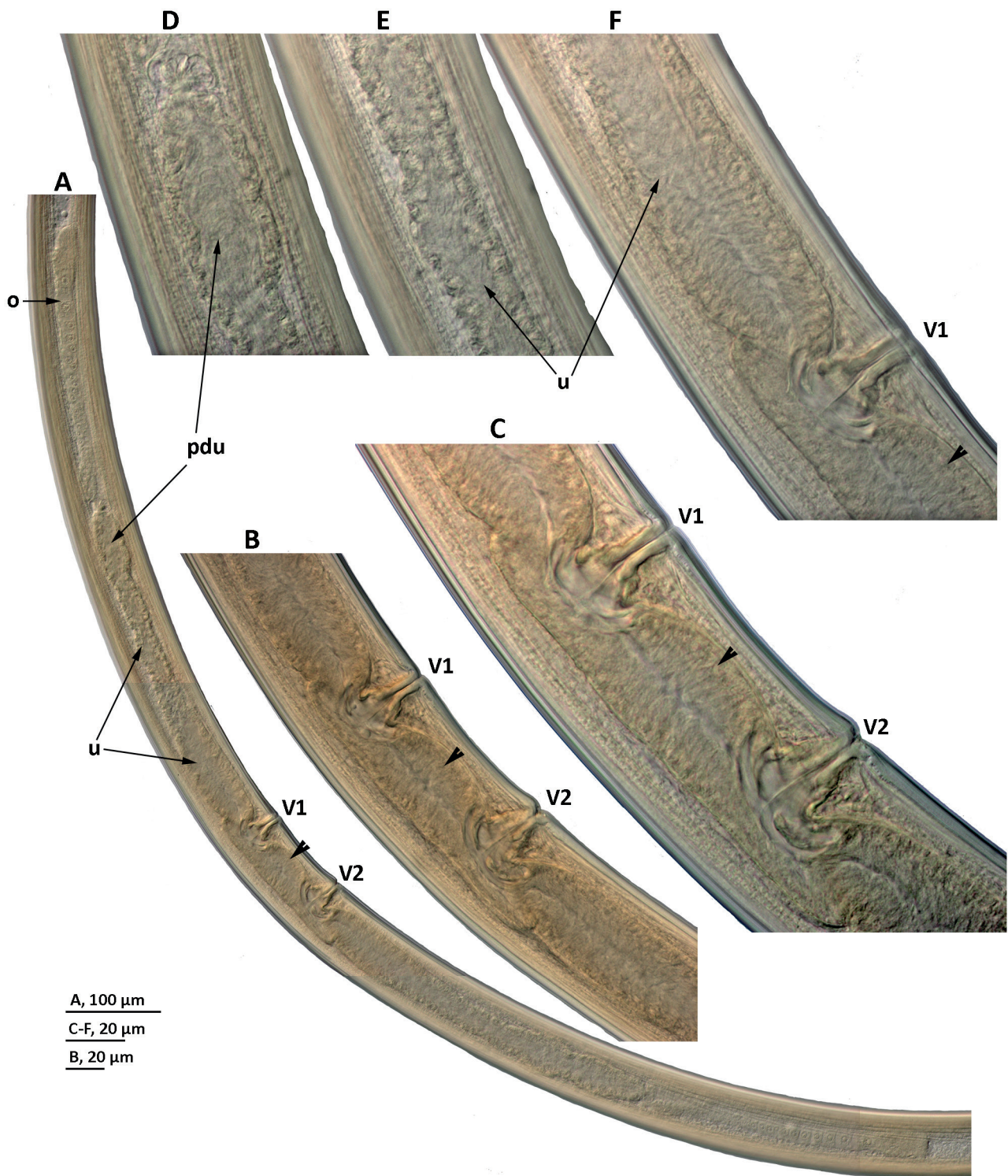


Fig. 4. *Longidorus* sp. from Cerova, Serbia. **A**, a part of the female body depicting the reproductive system with two vulvae (o = ovary; pdu = *pars dilatata uteri*, u = uterus, V1 = first vulva, V2 = second vulva; black arrowhead denotes connection between V1 and V2); **B-C**, enlarged portion with two vulvae in different magnification; **D-F**, enlarged parts of the body showing details of *pars dilatata uteri*, uterus, V1, and part of the connection between V1 and V2.

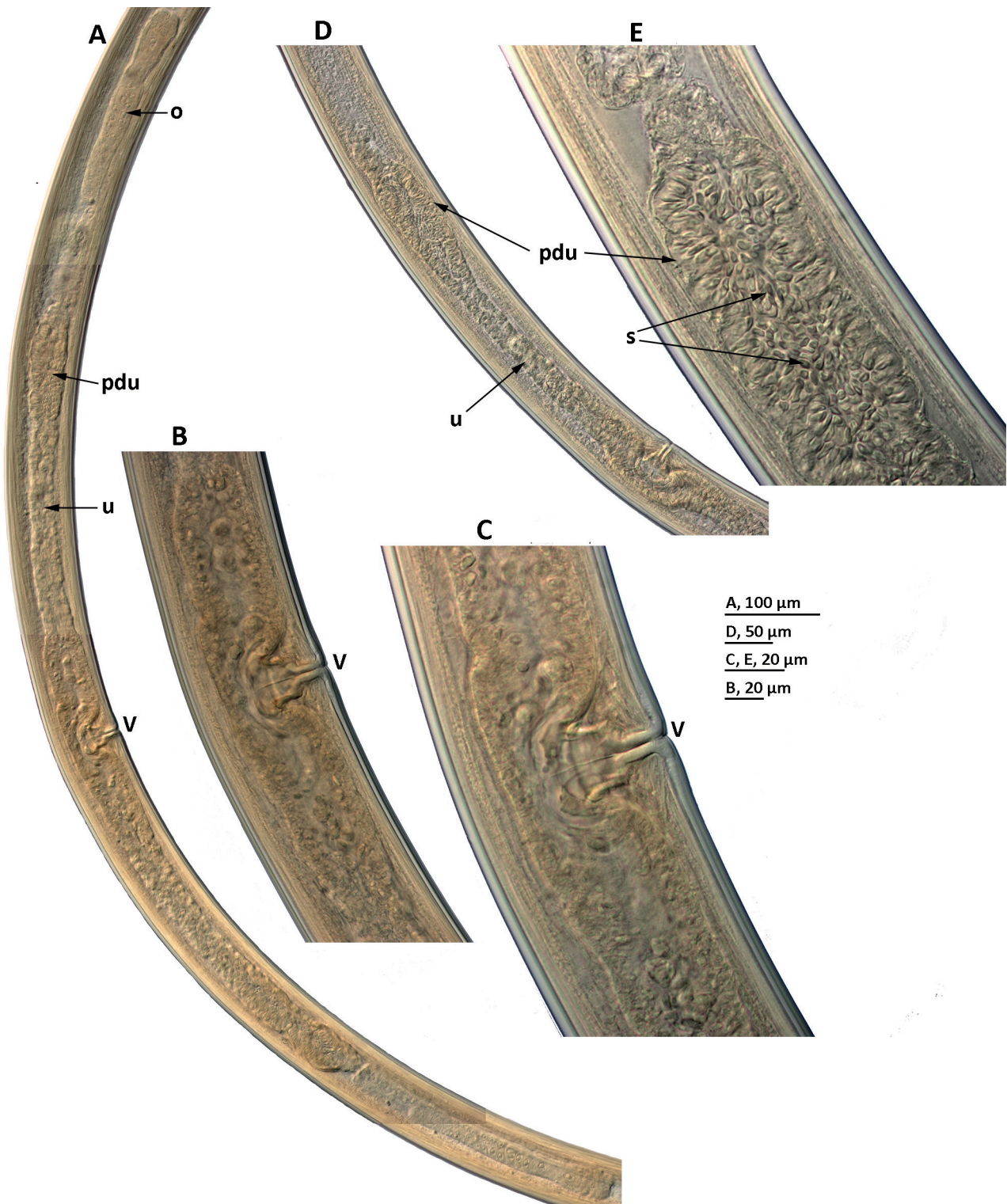


Fig. 5. *Longidorus* sp. from Cerova, Serbia. **A**, a part of the female body depicting the reproductive system with one vulva (o = ovary; pdu = *pars dilatata uteri*, u = uterus, V = vulva); **B-C**, vulval region enlarged in two different magnification; **D**, details of anterior genital tract; **E**, enlarged portion showing part of *pars dilatata uteri* filled with sperm.

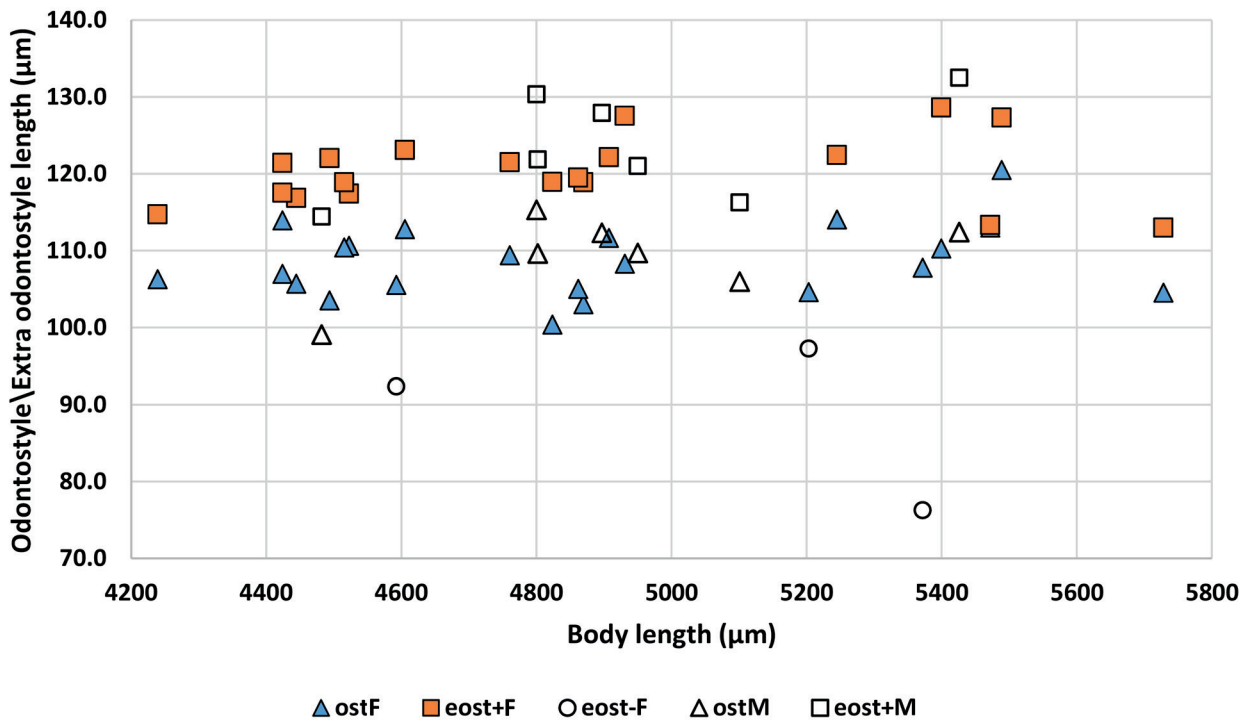


Fig. 6. *Longidorus* sp. from Cerova, Serbia. Scatter diagram showing odontostyle and extra odontostyle lengths in females (ostF, east+F, respectively) and males (ostM, east+M, respectively). The extra odontostyles in three females were shorter than the functional odontostyles and there are denoted with east-F.

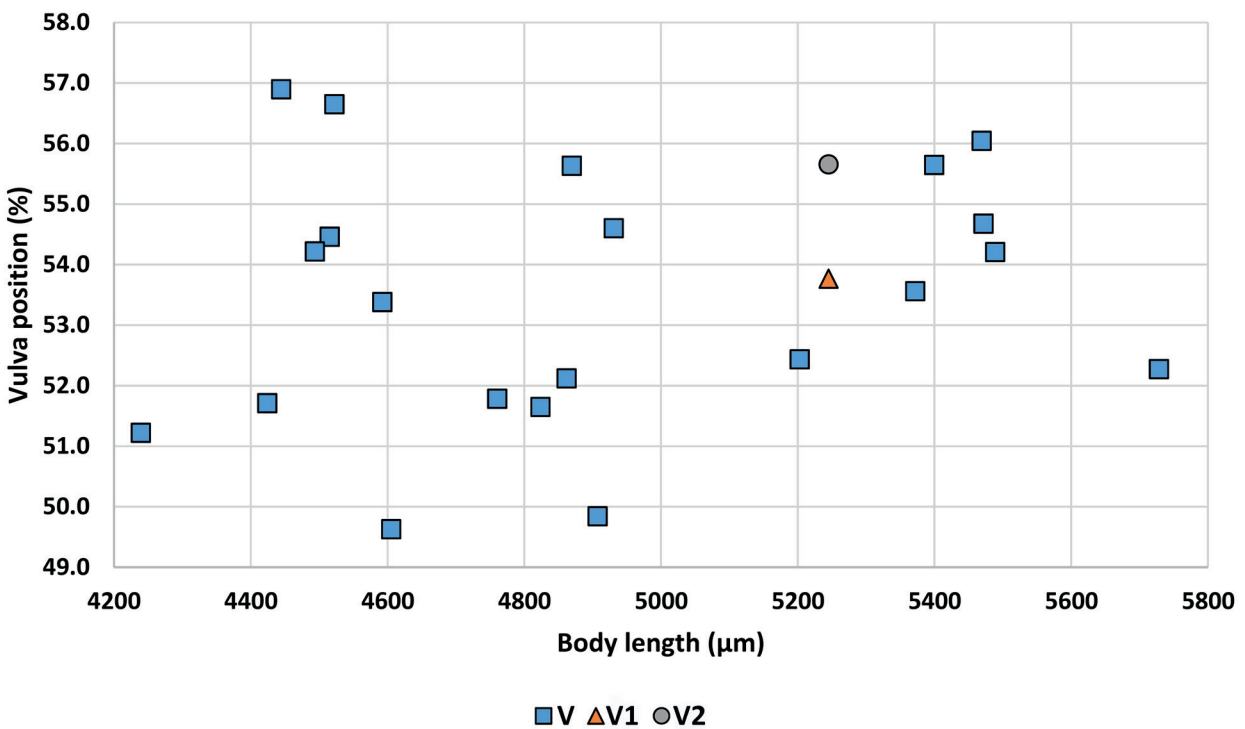


Fig. 7. *Longidorus* sp. from Cerova, Serbia. Scatter diagram showing vulva position in 21 females with one vulva (V), and one female with two vulvae (V1, V2).