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Article

First finding of *Greeffiella* Cobb, 1922 (Nematoda, Desmoscolecida) in the Black Sea

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Abstract
The first finding of the genus *Greeffiella* Cobb 1922 (Greeffiellinae, Desmoscolecidae) in the Black Sea is presented. Two mature females were collected in Northwestern Shelf of Crimea in strongly silted fine sand with detritus at a water depth of 56 m. *Greeffiella* sp. is described and illustrated. The absence of males in the collections does not allow the authors to present it as a new species for science or to identify it as one of the known species of the genus *Greeffiella*. Black sea specimen is distinguished from the other known species of the genus *Greeffiella* with the presence of 8 pairs of thicker specific setae along the body, the basis of which looks like a small lamina, but without hairs, which was previously described for *G. pierri* Schrage & Gerlach, 1975 and *G. australis* Schrage & Gerlach, 1975. The short esophagus at the base has two salivary glands and a cardia. Cardia has not been mentioned before for the known species of the genus *Greeffiella*.

Key words: *Greeffiella*, Desmoscolecida, Crimean Shelf, Black Sea.

Introduction

Representatives of Desmoscolecida are ordinary inhabitants of coastal and deep-water zones in the Black Sea. Some of them are eurybiontic, having a wide spatial and bathymetric distribution in the Black Sea (Sergeeva 2003, 2004). Most of the representatives of Desmoscolecidae are confined to the conditions of normoxia, but some species live in hydrogen sulfide environment under the conditions of hypoxia and anoxia (Sergeeva 2004; Kosheleva 2012; Sergeeva & Mazlumyan, 2015; Sergeeva & Ürkmez, 2017).

13 valid species are known in the Black Sea representing three genera as *Desmoscolex, Quadricoma*, and *Tricoma* from the family Desmoscolecidae (Bezerra *et al.* 2021). However, the fauna of Desmoscolecidae is much more diverse than the recorded data in the Black Sea, therefore would need further study for identification of other species. More than 20 species (*incertae sedis*) of desmoscolecids have been listed in the publications and indicated as sp. or cf. of known species for the Black Sea.

The present study provides a contribution to the nematode biodiversity of the Black Sea with a new genus record. The genus *Greeffiella* was described by Cobb (1922) and later accepted as the family Greeffiellidae by Filipjev (1929). Following the modern taxonomy of *Greeffiella* Lorenzen, 1969, *Calligyrus* Lorenzen, 1969, *Greeffiellopsis* Schrage et Gerlach, 1975, *Hapalomus* Lorenzen, 1969 and *Progreeffiella*

Timm, 1970 are united under the tribe Greeffiellini Filipjev, 1929 as part of the subfamily Desmoscolecinae. In contrast to the listed genera represented only by 1–2 species, the genus *Greeffiella* includes 13 valid species (Bezerra *et al*. 2021).

Specimens of *Greeffiella* are unusual marine free-living nematodes whose entire bodies are covered with spinous hairs. It is known that species of this genus are found in sea sand – on beaches, coral reefs, and even at great depths (in the Pacific, Atlantic, Southern, Indian Oceans (southern Australia), Mediterranean Sea) (Timm 1970; Da Silva 2012; Bezerra *et al*. 2021).

Material and methods

In this work, representatives of the genus *Greeffiella* are reported for the first time from the Black Sea. Samples of bottom sediments were obtained on 15.12.2001 at the Crimean Shelf, during cruise 56 of the R/V "Professor Vodyanitsky" in the framework of the National Program of Ukraine.
Collected samples of bottom sediments were fixed in 75% alcohol. In the laboratory, sediment samples from the study area were washed through two sieves, the upper one with a mesh size of 1 mm, the lower one with a mesh size of 63 μm, and stained with Rose Bengal solution targeting the "live" (i. e., stained) meiobenthic fauna before being sorted under a stereo-microscope using a Bogorov chamber. For taxonomic analysis, nematodes were mounted on permanent gelatin- glycerine slides (Tsalolikhin 1980)

A new representative is described here based solely on morphology since only two specimens are available and they are clearly different from the known species of this genus. It should also be noted that there is no DNA sequence information for none of the species of the tribe Greeffiellini. Figures and measurements were taken using an Olympus E-410 camera attached to an upright biological microscope Olympus CX41. Type specimens are kept in the collection of A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (Sevastopol, Russia). In this work, the classification of the World Database of Nematodes (NEMYS) is followed (Bezerra *et al*. 2021).

Abbreviations: a – body length divided by maximum body diameter; b – body length divided by esophageal length; c – body length divided by tail length; L – body length (μm), V% – vulva distance from anterior end of body.

Results

The systematic position of the genus is defined as follows:
Class Nematoda
Order Desmoscolecida Filipjev, 1929
Family Desmoscolecidae Shipley, 1896
Tribe Greeffiellini Filipjev, 1929

Genus *Greeffiella* Cobb, 1922
Diagnosis (modified by Decraemer & Rho, 2014): Body cuticle annulated, annules with long hairy spines; posterior spines with thickened base; numerous hairy spines arranged either in circlets or dorso- or ventrolateral groups. Four cephalic setae in between hairy-like ornamentation. Somatic setae with desmoscolecoid arrangement; subventral setae absent in female. Ocelli may be present. Male monorchic. Paired spicules without gubernaculum. Males with or without subventral pre- and postanal papilla described as transformed somatic setae inserted on a teat-like base. The last ring elongated with or without elongated end tube in some species.

Type species: *G. oxycaudata* (Greeff, 1869) Cobb 1922.

***Greeffiella* sp.**
(Figs. 1, 2; Table 1)

Type material: Two females with accession numbers Meib.1.N.1v and Meib.2.N.2v were fixed in gelatin-glycerine and deposited in the collection of the Institute of Biology of the Southern Seas of RAS, Sevastopol, Russia.

Type locality: NW of the Black Sea, (lat. $45^0$11 N, long. $32^0$12 E) Crimean Shelf, Russia. Station No. 5549, water depth 56 m, strongly silted fine sand with detritus; No.56 cruise of the R/V "Professor Vodyanitsky" (15.12.2001).

Description. The total length of the female body (Lt) 380–400 μm (Table 1; Fig. 1 A, D; Fig. 2 A, H). Body composed of 67–70 annules, (annuli 3–6 μm wide). Cuticle along the entire length of the body transparent, with large numbers of setae of different length. The setae allocated strictly in transverse rows from the head to the base of the tail. Head short, rounded, narrower than the first annulus, bearing large, elliptical, thick-rimmed amphids, much broader than the head. About 80–90 long and thin cephalic sensory setae anterior to amphids. Pharynx short, slightly expanding to the basis, without a bulb. A pair of salivary glands at its base (Fig. 1 B, D). A special elongated area at the very posterior edge of the esophagus, by the location of the salivary glands, which is the cardia (valve) that includes the beginning of the intestine. Eyes not observed (problematically). The body smoothly widening from the head to the middle and then gradually narrowing to the tail. Each annule of the body bearing a row of thin hair-like setae and spinous hairs, their length increasing from anterior towards the middle and the posterior parts of the body. Eight pairs of specific setae (15 μm) on a small basic cell without hairs along the body. These specific setae with diverse lengths and with a slightly thicker appearance than the rest of the setae. Reproductive system didelphic, amphidelphic with equally developed genital branches. Vulva located between annules 41 and 43 in holotype, 42 and 44 in paratype. Mature egg 30×32 μm. The tail composed of 6–8 annules. The last ring (18–19×23–32 μm) of the tail large and smooth in the form of a triangle with a pointed end and rows of short setae, three long terminal tubes (13 μm) on the end of the tail.

Table 1. Morphometric data of *Greeffiella* sp. (in μm except a, b, c, c′ and V%).

Characters	Specimens	
	Holotype female 1	Paratype female 2
L	378	398
a	5,1	5,3
b	15,7	16,6
c	6,5	9,3
V %	52	49,5
Number of annules	67–68	69–70
First ring, L x W	18×3	17×4
Head, L x W	7×10	7×10
Pharynx, L x W	24×10	24×10
Cardia, length	11	12
Cephalic setae, length	12–17	12–15
Maximal body diameter	74	75
Number of annules on the tail	7–8	6–8
Tail, length	58	43
Anal body diameter	34	31

Discussion

Representatives of *Greeffiella* were recorded for the first time in the Black Sea, however, only two females were found. In this work, descriptions of the specimens are given based on two mature females from one habitat. In our opinion, these samples are distinctly different from the known species of the genus *Greeffiella* found in other marine waters, which allows us to describe this new specimen more in detail. Perhaps representatives of this genus are more numerous and widespread in the Black Sea. But, obviously, due to their small size and very delicate body, it is difficult to detect them in bottom sediment samples when using accepted methods for processing bottom sediments to study meiobenthos. Timm (1978), for example, mentioned that most specimens of *Greeffiella antarctica* collapsed, even with slow dehydration to glycerin during their study.

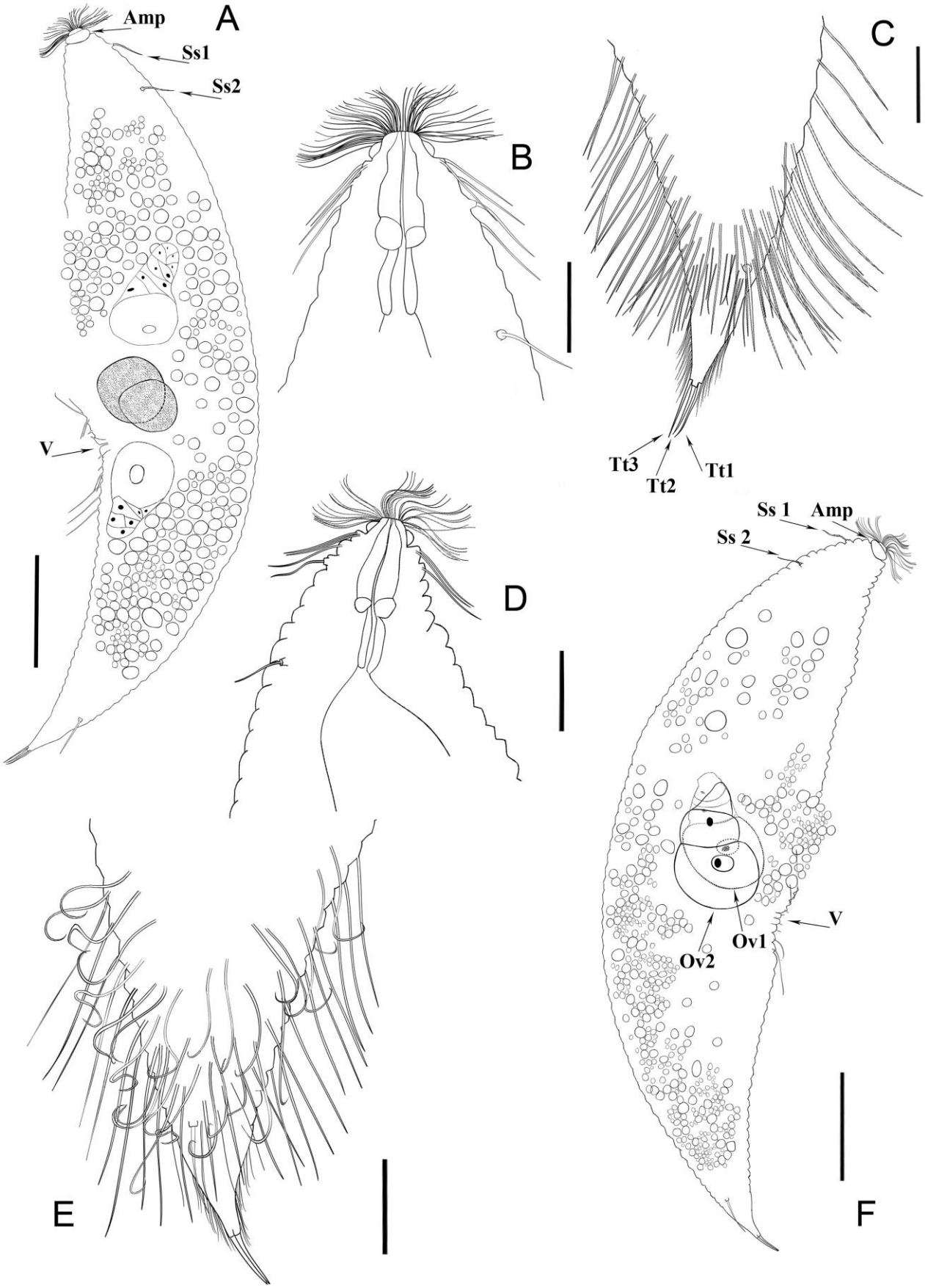
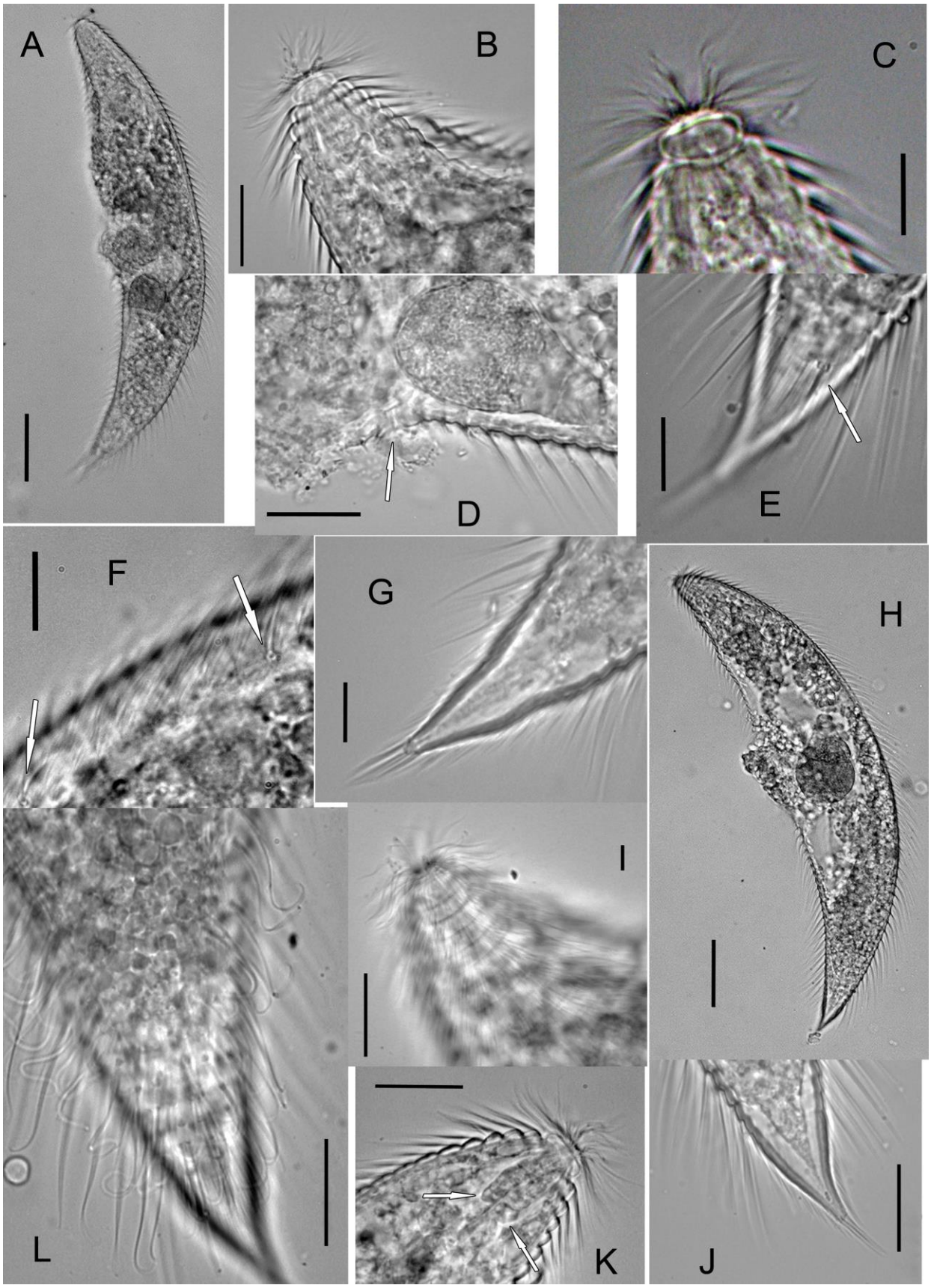


Figure 1. *Greeffiella* sp.: A–C – specimen 1. A – overall view, B – head region, C – tail region; D–F – specimen 2. D – head region, E – tail region, F – overall view. Scale bars: A, F = 50 μm; B, C, D, E = 20 μm.



Figures 2. *Greeffiella* sp.: A–G – specimen 1. A – overall view, B – head region, C – amphid, D – vulva, E – specific setae at the tail region, F – specific setae in the middle part of the body; G – tail with terminal tubes; H–L – specimen 2, H – overall view of the specimen, I – head, J – tail with terminal tubes, K – pharynx, salivary glands and cardia, L – long hair-like and spike–like setae on tail part. Scale bars: A, H = 50 μm; B, D, I, J, K, L = 20 μm; C, E, F, G = 10 μm.

Black Sea specimens are related to the order Desmoscolecida, due to their annulated shape of the body, the form of tail, the large size of amphid located on the head, and several other morphological characteristics. Their body is equipped with numerous lengths of somatic setae from head to tail, which create the appearance of a completely pubescent animal. Somatic setae have diverse appearances, some in the form of long hairs on all their length, often curled (in the form of curls), others spike-like, but by the end, appearing like hair. All setae are distributed horizontally together on the rings. Along with these setae, there are two vertical rows with 8 pairs of thicker specific setae along the body, the basis of which looks like a small lamina, but without hairs, which was described for *G. pierri* Schrage & Gerlach, 1975 and *G. australis* Schrage & Gerlach, 1975. Such specific setae and their location along the body distinguishes *Greeffiella* sp. from the other species with the same specific setae. The short esophagus at the base has two salivary glands and a cardia. Cardia has not been mentioned before for the known species of the genus *Greeffiella*. The Black Sea representative is also distinguished from the other known species of the genus by the presence of three terminal tubes on the tail.

Unfortunately, the absence of males in our collections does not allow us to certainly identify this as one of the known species of the genus *Greeffiella* and give not the possibility to describe it as a new species for science. The present study provides a contribution to the nematode biodiversity of the Black Sea with a new genus record.

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