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Research article

Campanula lactiflora – first report of a well-established neophyte in the Bulgarian flora, with some notes on *C. latifolia*

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Abstract: *Campanula lactiflora* (syn. *Gadellia lactiflora*) is a popular garden plant with natural range spanning through Türkiye, Caucasus, Transcaucasia and Iran. It is a well-documented alien species in some countries, but hitherto unknown in Southeastern Europe. The paper presents its first records as naturalised and propagating non-native species in Bulgaria and the Balkans. Hints for its occurrence in Bulgaria first emerged on the Facebook group “The plants in Bulgaria”, where it appeared on photographs from plant enthusiasts. The subsequent targeted search revealed that *C. lactiflora* is well-established and reproducing in two separate areas in Rila Mts. The origin of the plant was traced back to two estates of the former royal family of Bulgaria, where the species persists for over a century and from where it penetrated natural habitats, spreading up to 3 km from the source gardens. Data about the present state of the population of *C. lactiflora* are presented and a brief assessment of the reproductive capabilities of the species is given, including first observations on its seed germination. The occurrence of the rare *C. latifolia* in Rila Mts is tackled in relation to the finding of *C. lactiflora*. An overview is given of the hitherto known Bulgarian alien plants that are arguably linked to the Crown estates. The potential of the citizen science for documentation of non-native plants is also briefly discussed.

Keywords: alien plants, Balkan flora, Campanulaceae, garden escapes, ornamental plants

Introduction

Campanula L. is a species rich genus, represented by 34 native species in Bulgaria. Anchev (2012) recognised 33 species. Later, Marinov & Stoyanov (2017) reassessed one variety, elevating it to species rank. Stoyanov et al. (2022) retained the species accepted by those authors. Many of the members of the genus are popular ornamental plants and some are known for their ability to escape from gardening (Stace, 2010). The cultivation of various *Campanula* species in Bulgaria dates back over 100 years and was initially related to the establishments of the former Royal family of Bulgaria, in which estates, parks and botanic gardens about 70 representatives of the genus have been nursed (Buresch, 1939). This considerable diversity largely declined since and little remains from

these vast collections. The native species *C. persicifolia* L. and *C. trachelium* L. are known to us to occur in Vrana Park (the former represented by particularly large-flowered form). The exotic *C. barbata* L. persists on the rockery in Saragyoł Hunting Lodge near Borovets Resort (AP, unpublished observation) and *C. rotundifolia* L. was reported from the park of Ovnarsko Lodge (Anchev, 2012). The non-native for the area but otherwise native for the country *C. latifolia* L. was also documented from Ovnarsko Estate (Anchev, 2012). At present much less species are known in cultivation in this country, albeit some representatives of the genus are gradually introduced or reintroduced in the last decade (Stoyanov et al., 2022). By far only one presumably naturalised species of bellflower is known in Bulgaria, namely *C. rotundifolia* L. (Anchev, 2012).

Since year 2020 photographs of an unusual and remarkably robust *Campanula* appeared repeatedly on the Facebook group “Plants in Bulgaria”, including in what seemed to be natural habitats. In a period of three years four enthusiast botanists from the group shared their observations of the *Campanula* in question, all from Rila Mts, provoking lively discussions. The species was quickly recognised not to belong to any native Bulgarian *Campanula* taxa and was eventually identified as *C. lactiflora* M. Bieb., a popular ornamental plant, yet unknown as naturalised in this or any other Balkan country (Boršić et al., 2008; Stešević & Petrović 2010; Stešević 2013; Barina et al., 2018; Stojanović & Jovanović, 2018; Korakaki et al., 2021; Kadriaj et al., 2023; Vladimirov & Petrova, 2023). This intriguing bellflower prompted the authors to conduct a thorough survey and to document the extent of occurrence and the origins of this obvious garden escape, which are reported in this paper.

Materials and methods

Materials of the plant were collected from the localities of origin in Tsarska Bistritsa Manor and Ovnarsko Estate, as well as from those, that resulted from subsequent dispersal in their surroundings. Plants with flowers and fruits are both present among the voucher specimens. They are deposited in the herbaria of the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences in Sofia (SOM) and the Natural History Museum in Sofia (BNHM). The brief description below is based solely on studied Bulgarian specimens. The identity of the species was confirmed by the use of several relevant works on the genus *Campanula*, most notably those of Fedorov (1957), Fedorov & Kovanda (1976), Damboldt (1978) and Stace (2010).

Specimens of *C. lactiflora*, kept in the herbaria of the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences in Sofia (SOM) and the Agricultural University in Plovdiv (SOA) were also revisited. As *C. latifolia*, another robust species, co-occurs with *C. lactiflora* in the studied estates, and the latter is not presented in the contemporary Bulgarian treatments of the genus (Anchev, 2012; Stoyanov et al., 2022), for the purposes of comparison its specimens in the above herbaria were also studied. Further on, samples of

both species conserved in foreign herbaria were consulted online, with special reference to authentic specimens – the Linnaean Herbarium of the Linnaean Society of London – LINN (LSL, 2025), and the herbaria of the Botanic Garden Edinburgh – E (BGE, 2025), the Moscow State University – MW (MGU, 2025), and the Institute of Botany, Ilia State University of Georgia – TBI (ISU, 2025).

Seeds of *C. lactiflora* were collected for germination and preservation in the living collections of the Botanical Garden of the Bulgarian Academy of Sciences. Non-quantified germination test on the surface of substrate in a greenhouse was performed immediately after seed collection, in order to gain inceptive information related to the reproduction under conditions mimicking those in nature. Quantified tests followed after 16 months of storage at room temperature, without heating during winter, with the purpose to test if the species supports a soil seed bank with seeds remaining viable for longer period. The principal ‘top-of-paper’ method was employed (Rao et al., 2006) with some adjustments and modifications as described below. For the test, 100 randomly taken seeds were sown in triplicate on wetted with tap-water filter paper in petri dishes, which were then kept at room temperature (~20 °C during daytime and ~15 °C at night). The filter paper was delineated into numbered squares and up to three seeds were placed per square as to facilitate daily counts. Seeds were individually transferred onto the paper under a magnification glass with the aid of a sterilised preparatory needle, oft-wetted due course. Using mere low magnification helps to reduce sampling bias, such as a selection of ostensibly better developed seeds. No surface sterilisation was performed on the seeds, nor was any kind of treatment with germination enhancing agents. The design was thus devised as its aim was to test the germination rate in conditions not far too different from those in nature, where seed surface microbiota would interfere in the process. As far as it has been shown that *Campanula* seeds require light for their germination (Koutsovoulou et al., 2014), the petri dishes were placed in a moderately-lit place during day time (natural light), avoiding however direct sunlight. Seeds were monitored on a daily basis and those developing fungal infestation were promptly removed to prevent further contamination. Germination was observed and recorded, accounting for each square, in order to calculate both total germination ratio and

daily germination rate. The observations continued until the germination rate in all petri dishes fell to zero for three consecutive days.

Geographical coordinates, where cited in the text, always refer to the World Geodetic System (WGS84). The map of the localities of *C. lactiflora* was prepared in the open source software QGIS (QGIS, 2025). The base map is from Open Topo Map (OTM, 2026).

Results

Presentation of the species

Campanula lactiflora M. Bieb., Fl. Taur.-Caucas. 1: 153, 1808; $\equiv$ *Gadellia lactiflora* (M. Bieb.) Schulkina in Novosti Sist. Vyssh. Rast. 16: 178, 1979 (Figs 1 & 2)

Robust perennial plant with thick and long woody roots, producing numerous (sometimes over 30), densely clumped, erect or to different degree patent (particularly in shaded localities) flowering stems, often above 200 cm long, ribbed and with sparse setulose hairs pointing towards the base. Foliage is dense along the stem (basal rosette absent), of alternate, sessile, broadly lanceolate to ovate or oblong, serrate or doubly serrate leaves (the lowermost sometimes crenulate) with conspicuous short (ca 0.5 mm) whitish cartilaginous tips of the teeth, almost glabrous above and with scarce setulose hairs along the veins on the lower surface. Inflorescence is large, racemose, conical, lax to moderately compact, with 10 to 20 almost glabrous to sparsely hairy branches. Flowers are with pentamerous calyx with ovate to lanceolate, serrate, glabrous to sparsely setulose hairy, green or often blueish or purplish at the apex, erecto-patent or occasionally deflexed lobes up to 10 mm long, and with broadly campanulate, glabrous or sometimes with few ciliate hairs, deeply five-lobed (ca $\frac{1}{2}$ up to $\frac{3}{4}$ length; lobes patent), whitish blue, pale sky blue to blueish lavender or by exception whitish corolla up to 25 mm long, with the style included in the tube. The fruit is glabrous or rarely a strigose hairy, erect capsule (both morphotypes are present in Bulgarian localities), (8–)10 $\times$ (5–)8 mm, opening by 3 apical pores. Seeds light brown, 0.6–0.9 $\times$ 0.4–0.6 mm (on average 0.8 $\times$ 0.5 mm), average length/width ratio = 1.6.

General distribution. Described from Caucasus (Vladikavkaz and Mt. Kaishaur). The natural range of *C. lactiflora* encompasses Türkiye, Caucasus,

Transcaucasia and Iran, where it is said to grow in mountain meadows, scrub and forests (Fedorov, 1957; Damboldt, 1978). It is known as naturalised and successfully self-seeding in the British Isles, Finland, the NW of Russia and Latvia, and as casual in Belgium, the Czech Republic, and Sweden (Karlsson, 1998; Byalt & Vasilyev, 2009; Stace, 2010; Danihelka et al., 2012; Kravchenko et al., 2018; Nāburga & Evarts-Bunders, 2019; Verloove 2025).

Distribution in Bulgaria

Campanula lactiflora in this country is so far known only from Rila Mts, where it has been found during this study in numerous microlocalities (20 in total) split into two separate areas, namely Borovets Ski Resort and in Ovnarsko locality above Govedartsı Village (Sofia Province, Samokov Municipality). The coordinates and the elevation of all known microlocalities, along with some habitat characteristics, area of occupancy and the manner of growth of the plant there are presented in Table 1 and visualised on Fig. 3.

Bulgarian specimens examined. *Campanula lactiflora*: Sofia Province, Samokov Municipality, Ovnarsko locality above the village of Govedartsı, 42.235879, 23.439638, elev. ca 1355 m, by the road in an opening in a forest of *Picea abies* H. Karst and *Pinus sylvestris* L., 01.09.2023, leg. B. Assyov & R. Vassilev (SOM 179358; with flowers); idem, in the vicinity of Ovnarsko Hunting Lodge, 42.224855, 23.421968, elev. ca 1568 m, humid semi-natural habitats at the lodge, not far from a forest of *Picea abies* H. Karst and *Pinus sylvestris* L., 01.09.2023, leg. B. Assyov & R. Vassilev (SOM 179357 & BG-NMNHS-BOT-5159; with fruits); Sofia Province, Samokov Municipality, Borovets Resort, Tsarska Bistrıtsa Estate, a clump in the park, near the manor house, 42.258435, 23.595333, elev. ca 1370 m, 06.09.2023, leg. A. Petrova, B. Assyov & R. Vassilev (SOM 179369; with flowers & fruits); Sofia Province, Samokov Municipality, mountain slope above the road from Borovets Resort to Beli Iskar Village, on and around a ski track, 42.257090, 23.591613, elev. ca 1460 m, 06.09.2023, obs. A. Petrova, B. Assyov & R. Vassilev; *Campanula latifolia* (selected): Sofia Province, Samokov Municipality, Borovets Resort, Tsarska Bistrıtsa Estate, near the alpine garden at the manor house, 42.258435, 23.595333, elev. ca 1370 m, 13.08.2020, leg. A. Petrova (BG-NMNHS-BOT-



Fig. 1. Morphological features of *Campanula lactiflora*: (a, b, c) habitus; (d, e) mode of growth of stems and shape of the leaves; (f) stem indumentum and leaves attachment; (g) detail of the upper side of the leaves and the leaf margin; (h) detail of the indumentum on the lower side of the leaves. Photos a & b show plants with secondary inflorescences after cutting and photo c represents inflorescence in native state.



Fig. 2. Morphological features of *Campanula lactiflora*: (a, b) flowers; (c, d) capsules and variability of indumentum on capsules surface.

6583; with fruits); idem, 06.09.2023, leg. A. Petrova, B. Assyov & R. Vassilev (BG-NMNHS-BOT-6584; with fruits); Sofia Province, Samokov Municipality, at the lowermost, dilapidated building of the Ovnarsko Estate, 42.224556 23.420083, elev. ca 1555 m, 19.07.2025, leg. B. Assyov (BG-NMNHS-BOT-6585); Western Stara Planina Mts, Midzhur divide, shady places at Barza River, elev. 1300 m, 26.07.1995, leg. V. Vladimirov (SOM 154230); idem, opening in *Fagus* forest, 15.07.1998, leg. V. Vladimirov (SOM 155014); Central Stara Planina Mts, by the stream of Armankaya, 24.07.1902, leg. I. Neichev (SOM 74495).

Extralimital specimens examined for comparison (selected). *Campanula lactiflora*: Country unknown, Caucasus, s. d. (MW 0594918, syntypus ut notatum, photo); Russia, Ossetia, Caucasus, Vladikavkaz Urban Okrug, in the forests near Alagir Village, 12.06.1900.

leg. V.V. Markovich (MW 0722139, MW 0722143, photos); near Vladikavkaz, 07.1901, leg. Seidlitz, (TBI 1036867, photo); Georgia, Auadhari, elev. 2000 m, 15.08.1960, leg. V. Velchev & I. Bondev (SOM 108562); Transcaucasia, Georgia, prov. Tiflis, distr. Borzhom, near Bakuriani Village, 1500 m., at the banks of a river in a forest, 27.08.[19]25, leg. V. Kozlovsky (SOA 18832); United Kingdom, Scotland, grid 38/05, garden escape, 22.07.1966, leg. M. McCallum Welchen (E 01323009); Galashiels, above Tweed [River] at Sunderland Hall, 24.08.1986, leg. R.W.M. Corner (E 01323003, photo); Perthshire, Killiecrankie, 07.1995 (s. d. ex.), leg. M.E.D. Poore per J.G. Roger (E 1323004, photo); *Campanula latifolia*: s. d., loco et coll. (LINN 221.29, specim. authent., photo); United Kingdom, Yorkshire, Gordale, 10.08.1954, leg. M.W. Dick (SOM 134597); Sweden, Öland, Borgholm, in forests, 15.07.1911, leg.

Table 1. Localities of *Campanula lactiflora* in Rila Mts.

Coordinates (WGS84)	Elevation (approx.)	Habitat	Grouping	Area (approx.)
Ovnarsko locality above Govedarts				
42.224556, 23.423278	ca 1589 m	Above the lodge, clearing at forest road (PA)	Solitary	1 m ²
42.225306, 23.422667	ca 1566 m	Above the lodge, clearing at forest road (PA)	Solitary	1 m ²
42.224634° 23.422115°	ca 1573 m	Dilapidated lodge – origin spot	Scattered	15 m ²
42.224855° 23.421968°	ca 1568 m	Spring valley, surrounded by forest (PA) *	Groups and scattered	20 m ²
42.224518° 23.420377°	ca 1562 m	Roadside grasslands and forest margins (PA)	Scattered and small groups	10 m ²
42.224712° 23.420221°	ca 1555 m	Dilapidated building	Solitary	2 m ²
42.225336 23.418529	ca 1524 m	Not assessed (Mrs. Galya Veleva, pers. comm.)	Not assessed	Not assessed
42.224711° 23.419274°	ca 1546 m	Humid spot in a forest (PA, PS)	Large group	15 m ²
42.223995° 23.416233°	ca 1524 m	Ruderal grasslands among bungalows	Solitary	1 m ²
42.226380° 23.420315°	ca 1520 m	Roadside grasslands and forest margins (PA)	Solitary	1 m ²
42.227855° 23.422089°	ca 1497 m	Roadside grasslands and forest margins (PA)	Solitary	1 m ²
42.229171° 23.424365°	ca 1481 m	Roadside grasslands and forest margins (PA)	Solitary	1 m ²
42.235879° 23.439638°	ca 1359 m	Roadside grasslands and forest margins (PA) *	Solitary	1 m ²
42.239840° 23.444793°	ca 1318 m	Roadside grasslands and forest margins (PA, PS)	Solitary	1 m ²
42.240130° 23.444400°	ca 1314 m	Roadside grasslands and forest margins (PA, PS)	Solitary	1 m ²
42.241839° 23.447771°	ca 1304 m	Roadside grasslands and forest margins (PA)	Solitary	1 m ²
Borovets locality and Tsarska Bistritsa Estate				
42.258029° 23.595174°	ca 1411 m	Artificial (garden) – origin spot *	Solitary and large group	15 m ²
Not available	Not assessed	In the forest near the above place (2025, Dr V. Vladimirov, pers. comm.)	Solitary	Not assessed
42.25965° 23.59485° (approx.)	ca 1400 m	Along an alley near to the electric power station (2020, A. Petrova obs.)	Solitary	1 m ²
42.257048° 23.591870°	ca 1499 m	Humid spots along a ski track in a forest (PA, AA)	Large groups	>50 m ²

Abbreviations of tree species: AA – *Abies alba*, PA – *Picea abies*, PS – *Pinus sylvestris*. The asterisk in the third column denotes localities from which voucher specimens were collected.

F. Björkman (SOM 92869); Poland, Grodkow, Ulanowice Village, at the river in broadleaf forest, 24.07.1966, leg. K. Pender (SOM 118243).

Germination tests

Non-quantified germination test showed that seeds, sown immediately after collecting germinated en masse. The results of the quantified test after 16 months of storage are summarised in table 2. Germination first occurred on day 9, when the highest

rate was observed (34 seeds, 11.3%, n=300). On the next days the rate was lowered and uneven, varying between zero and 20 germinating seeds (6.7%, n=300). It ceased on day 16. Overall, most of the seeds germinated between day 9 and 11, the rate sharply falling after. The average germination was 26.3% ±10.1 (n=300), varying considerably between the repetitions. Throughout the experiment, 7% of the seeds were lost due to fungal infestation. The results of the tests imply that seeds of *C. lactiflora* are ready to germinate when dispersed from the capsules and are capable of retaining viability for prolonged period.

Table 2. Quantified germination test of *Campanula lactiflora* after 16 months of storage. Each repetition included 100 seeds.

Test	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Total
1	21	4	9	2	0	0	1	37
2	6	6	2	0	0	2	1	17
3	7	2	9	3	0	3	1	25
1+2+3	34	12	20	5	0	5	3	79
Average	11.3	4.0	6.7	1.7	0	1.7	1.0	26.3
St. dev.	8.4	2.0	4.0	1.5	0	1.5	0,0	10.1

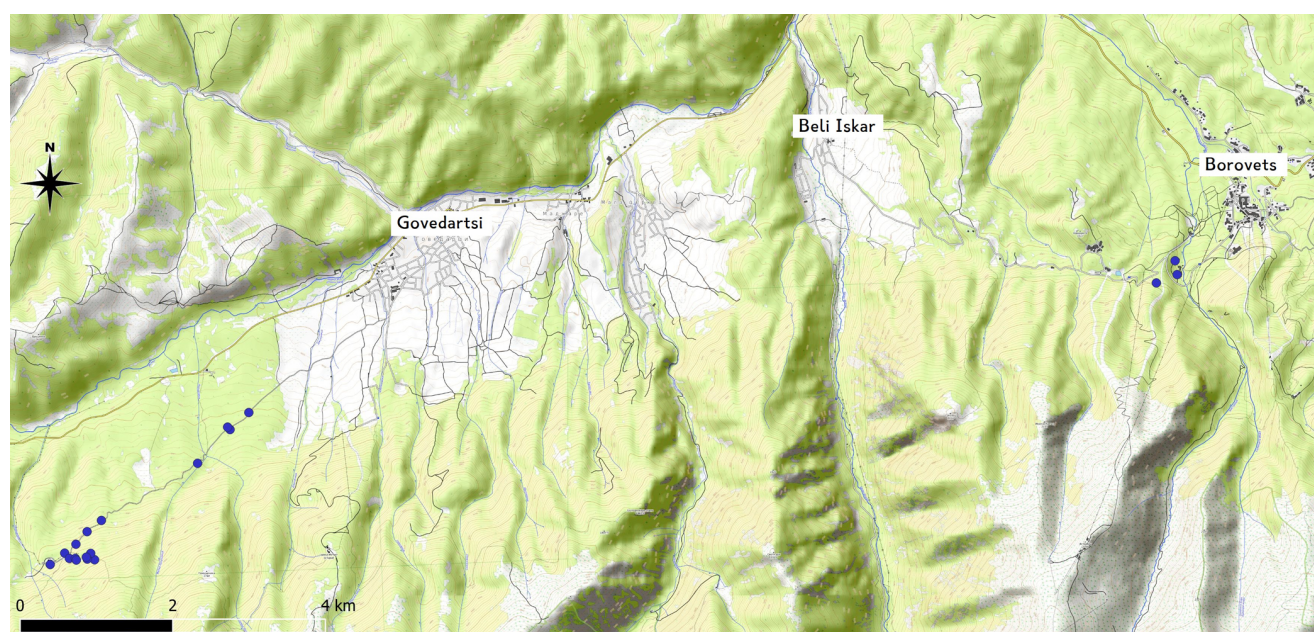


Fig. 3. Distribution of *Campanula lactiflora* in Bulgaria (Northern Rila Mts).

Discussion

Campanula lactiflora is fully naturalised and self-seeding freely in Rila Mts in Bulgaria, with considerable population in two geographically separated sites and 20 microlocalities known in total (including two source microlocalities) – Fig. 3. The species appears to be very well adapted to the conditions in both studied sites. The initial sources were identified in the two areas, where *C. lactiflora* was found as naturalised. In both cases the parental plants in cultivation are situated in different estates of the former royal family of Bulgaria. In the Tsarska Bistritsa Manor in Borovets a group of large plants at the edge of the alpine garden was the obvious source of seeds for expansion. At the now dilapidated former hunting lodge in the Ovnarsko locality the source was

apparently a plant or a clump, planted close to a fountain at the corner of the remains of the lodge. It could be reasonably assumed that in both places *C. lactiflora* obviously persists for over a century, considered the time of establishment of the two properties.

Different dispersal patterns were observed in the two localities. In the Ovnarsko area *C. lactiflora* apparently first spread in the close vicinity of the lodge, rigorously penetrating suitable habitats, which could be inferred from the large number of plants. The spread then has proceeded further down along the road with single or small number of individuals in those localities, reaching as far as 3 km from the source. However, dispersal in higher elevation was also noted here, although the species is present with solitary plants above the presumed source. In total 16

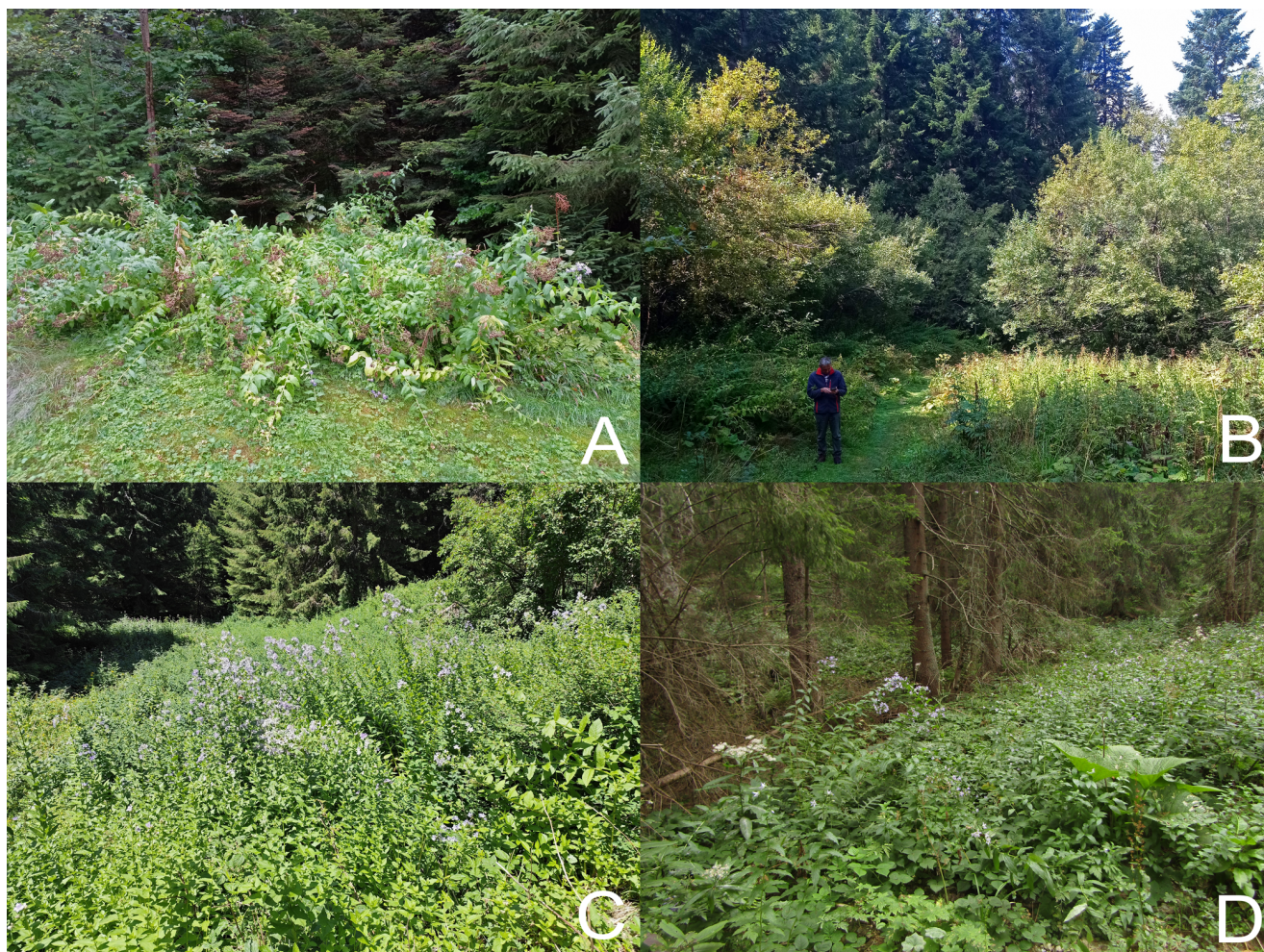


Fig. 4. Habitats of *Campanula lactiflora*: (a) plants in cultivation in Tsarska Bistritsa Estate; (b) naturalised plants in Borovets locality; (c, d) naturalised plants in Ovnarsko locality.

microlocalities were found in the area of Ovnarsko. Different pattern was observed in Borovets Ski Resort, where *C. lactiflora* does self-seed in the estate, but the most abundant escaped plants in this locality cover a large wet area situated in about 300 m from the source, but notably at higher elevation above the manor (approximately 100 m). The factors that facilitated this vertical migration, not that abundant in the Ovnarsko locality, are as of present unknown. Another foreign species naturalised in Rila was established there, with a large, numerous population. This is *Claytonia sibirica* L., which naturalisation is also associated with the Tsarska Bistritsa Estate (Petrova et al., 2024). In Rila Mts *C. lactiflora* preferentially inhabits stream-sides and wet areas at the edge of natural spruce, fir and Scots pine boreal forests, ski tracks in forested areas, and more rarely relatively humid grasslands along roads or ruderal communities next to buildings (see Fig. 3). The

species generally acts as a shade-tolerant mesophyte or hygrophite, albeit certainly not strictly limited to these conditions. This is generally consistent with the habitats in its natural range (Fedorov, 1957; Fedorov & Kovanda, 1976; Damboldt, 1978). Further on, while evidently preferring habitats with open ground, the species is apparently competitive and could occur also in communities having dense cover of other plants, as grasslands and tall-herb associations. The following accompanying plants are most often present in the Bulgarian localities: *Agrostis* spp., *Alchemilla* spp., *Anthriscus sylvestris* (L.) Hoffm., *Chaerophyllum hirsutum* L., *Cicerbita alpina* (L.) Wallr., *Cirsium appendiculatum* Griseb., *Dactylis glomerata* L., *Epilobium angustifolium* L., *Heracleum sibiricum* L., *Hypericum maculatum* Crantz, *Pastinaca hirsuta* Panč., *Prunella vulgaris* L., *Rumex obtusifolius* L., *Rubus* spp., *Senecio nemorensis* L., *Telekia speciosa* (Schreb.) Baumg.,

Tussilago farfara L., and *Urtica dioica* L. Several of these plants are commonly associated with tall herb plant communities. However, the species occurs in different forested and grassland stands and it will be interesting to examine in future in more detail the phytosociology of *C. lactiflora* in this country in order to get more detailed insights of its ecological preferences. Due to the mode of growth of *C. lactiflora*, the number of the individuals could not be accounted with certainty, moreover one of the microlocalities at Ovnarsko could only be surveyed from a distance. We estimate that at least 60 clumps are present in the area of Borovets and no less than 40 exist in the Ovnarsko locality.

Albeit not assessed exhaustively in this work, the reproductive capabilities of the species are apparently enormous. Mature plants produce between 5 and 10 flowering stalks, but in older rhizomes this number may exceed 30. The flowering stems bear numerous flowers, which are generally over 50 and may often exceed 80 per stem. In the Ovnarsko locality flowering stems are apparently often collected by passing-by tourists. Still, this seems not to hamper the flowering and the seed productivity whatsoever as cut stalks develop numerous lateral flowering branches and produce secondary inflorescences of a size that is often comparable to the primary ones (Fig. 1a, b). The flowering period is relatively long, extending from July to September. The seeds, similarly to other species of *Campanula* (Akcin et al., 2009; Liveri et al. 2020, and references therein), are relatively small (<1 mm in length) and are particularly abundant in the large capsules of *C. lactiflora*. Due to their small size they could be easily transported by air and water, but also by vehicles and pedestrians. Fresh seeds sown immediately after collecting, in substrate in a greenhouse germinated readily en masse. Their further development showed that they are sensitive to wilting. After transplanting into larger size pots (≥ 10 cm), they turned out to be preferred by snails and slugs, which seems worth mentioning as this could potentially limit the reproduction in nature. Young seedlings require higher air humidity and develop well when it is above 60%. Further on, the experiments on seed germination ascertained that about 26% of the seeds remain viable after two consequent winters. Those observations suggest that germination in nature likely occurs without dormancy period, immediately after seeds dispersal, provided that suitable conditions are available. This would be

consistent also with the small size of the seeds that apparently contain limited reserve of nutrients. Considered the mountain habitat of the plant part of the new generation would be almost certainly lost in the winter to follow. However, the retention of non-insignificant germination capacity after prolonged storage suggests that the species is capable also of building a seed bank in the soil. This may be consistent with the long-flowering period and it is possible that the seeds that ripen early in the reproductive term germinate in the same season, while those formed in the later months overwinter. All this points to significant reproductive potential of *C. lactiflora* under appropriate conditions and further focused studies on those capabilities in Bulgarian plants would be undoubtedly interesting. Such studies will also have implications for nature conservation in Rila Mts as all known spots of *C. lactiflora* in the vicinity of Ovnarsko are within the NATURA 2000 zone “Niska Rila” (BG0000636) and one of them is at the border of Rila National Park, while in Borovets locality the derivative subpopulation is situated within the NATURA 2000 zone “Rila buffer” (BG0002129). Given the capabilities for reproduction of *C. lactiflora*, it would be wise to avoid its further cultivation under mountain conditions, even more in places where natural habitats suitable for its growth are present in immediate vicinity.

Interestingly, Anchev (2012) in a note under *C. rotundifolia*, a species so far known in Bulgaria only from Ovnarsko, mentioned that it was found together with *C. latifolia* and assumed that both of them might have been introduced there as ornamental plants. The latter species is also mentioned for Rila Mts in Anchev (1992) and reiterated in Assyov et al. (2012) and Stoyanov et al. (2022). It is another robust bellflower, with general aspect that may somewhat remind *C. lactiflora*. This prompted us to assess the materials of *C. latifolia* in SOM, where no voucher specimen was found that would support its occurrence in Rila Mts. Whether this is the reason for the later exclusion of this locality by Goranova & Anchev (2015) or it was not mentioned because assumed to be introduced there is unknown. *Campanula latifolia* is well-known to the present authors from some of its natural localities in Stara Planina Mts and it was not encountered during the initial survey of *C. lactiflora* in Ovnarsko, although it was found to persist at the rockery in Tsarska Bistritsa Estate. This prompted us to undertake a purposeful

Table 3. Comparison of selected diagnostic characters of *Campanula lactiflora* and *C. latifolia*, after the specimens examined, supplemented with some data on *C. latifolia* from Damboldt (1978).

Characters	<i>C. lactiflora</i>	<i>C. latifolia</i>
Stem	Usually taller than 150 cm, often above 200 cm, glabrous or sparsely hairy, with straight or very slightly curved, pointing downwards hairs, 1–2 mm long	Usually above 80 cm, up to 150 cm, glabrous or hairy to different extent, with differently oriented, soft, curved hairs, mostly <0.5 mm long
Leaves	Basal ones when present ovate or ovate-oblong, sessile or shortly petiolate, middle and upper ones sessile, broadly lanceolate to ovate or oblong, glabrous or with sparse, 1–2 mm long, almost straight trichomes (rare and scattered on the upper surface and mostly along the veins on the lower)	Basal ones broadly ovate with a cordate base, long petiolate, middle ones ovate-lanceolate, shortly pedunculate, the uppermost sessile, with scattered, short (<0.5 mm long) trichomes on both surfaces, generally not clustered along the veins, sometimes notably scabrid
Leaf margin	Teeth with short (ca 0.5 mm) cartilaginous apiculus	Teeth without cartilaginous apiculus
Inflorescence	Broadly paniculate, with numerous, very often much over 30 flowers, disposed by several to many on branches	Spicate raceme, with relatively few, rarely more than 20 flowers, disposed by 1–3 in leaf axils
Calyx lobes	Ovate to lanceolate, acute, erect or erectopatent, often enclosing the corolla base in fully developed flowers, very rarely deflexed, serrate, usually with teeth with dark tips	Linear-lanceolate, long acuminate, spreading-erect, more or less deflexed from corolla in full bloom, serrate or entire, teeth usually without dark tips
Corolla	Broadly campanulate, <3 cm long, whitish, pale-blue to blue-violet; lobes glabrous or with very few ciliate hairs	Infundibular-campanulate, >3 cm long, deep blue or by exception white; lobes with ciliate margin
Style	Short, of approximately similar length to the stamens and the stigma	Visibly longer than the stamens and the stigma
Fruit capsule	Erect, obconical, glabrous to conspicuously strigose, opening with 3 apical pores; calycular lobes mostly pointing straight upwards or slightly patent	Nodding, ovoid, glabrous or hairy, opening with 3 basal pores; calycular lobes more or less notably deflexed

search in appropriate season in 2025 and a small population of *C. latifolia* was indeed found at the lowermost, now dilapidated building of the Ovnarsko Estate (coordinates 42.224556°, 23.420083°), which would have probably served as a gatehouse. It is noteworthy that the species is reproducing successfully as a few young individuals were found among the mature, flowering plants. A summary of the distinguishing characters of *C. lactiflora* and *C. latifolia* is presented in Table 2 and the latter is illustrated in Fig. 5, aiming to facilitate their recognition. Our attempts to confirm the existence of a population of *C. rotundifolia* at Ovnarsko remained so far futile.

The Bulgarian plants of *C. lactiflora* are well congruent with the modern descriptions of the

species, as well as to the representative specimens of *C. lactiflora* seen in Bulgarian and foreign herbaria (SOM, SOA, LINN, E, MW, TBI). It is notable that in Bulgaria the plants reach luxurious size of up to 250 cm, much larger than the mentioned in the literature (Fedorov, 1957; Damboldt, 1978). The stem and leaf indumentum in the population in Rila Mts varies considerably and differences are seen between the different plants in those microlocalities where a substantial number of individuals is present. The degree of hairs on the calyx and respectively on the fruit capsules also varies to a large extent (Fig. 2). While the vast majority of the plants are glabrous or nearly so, at the Ovnarsko Hunting Lodge the bulk of the individuals feature notably strigose calyces and capsules. The original description of *C. lactiflora*

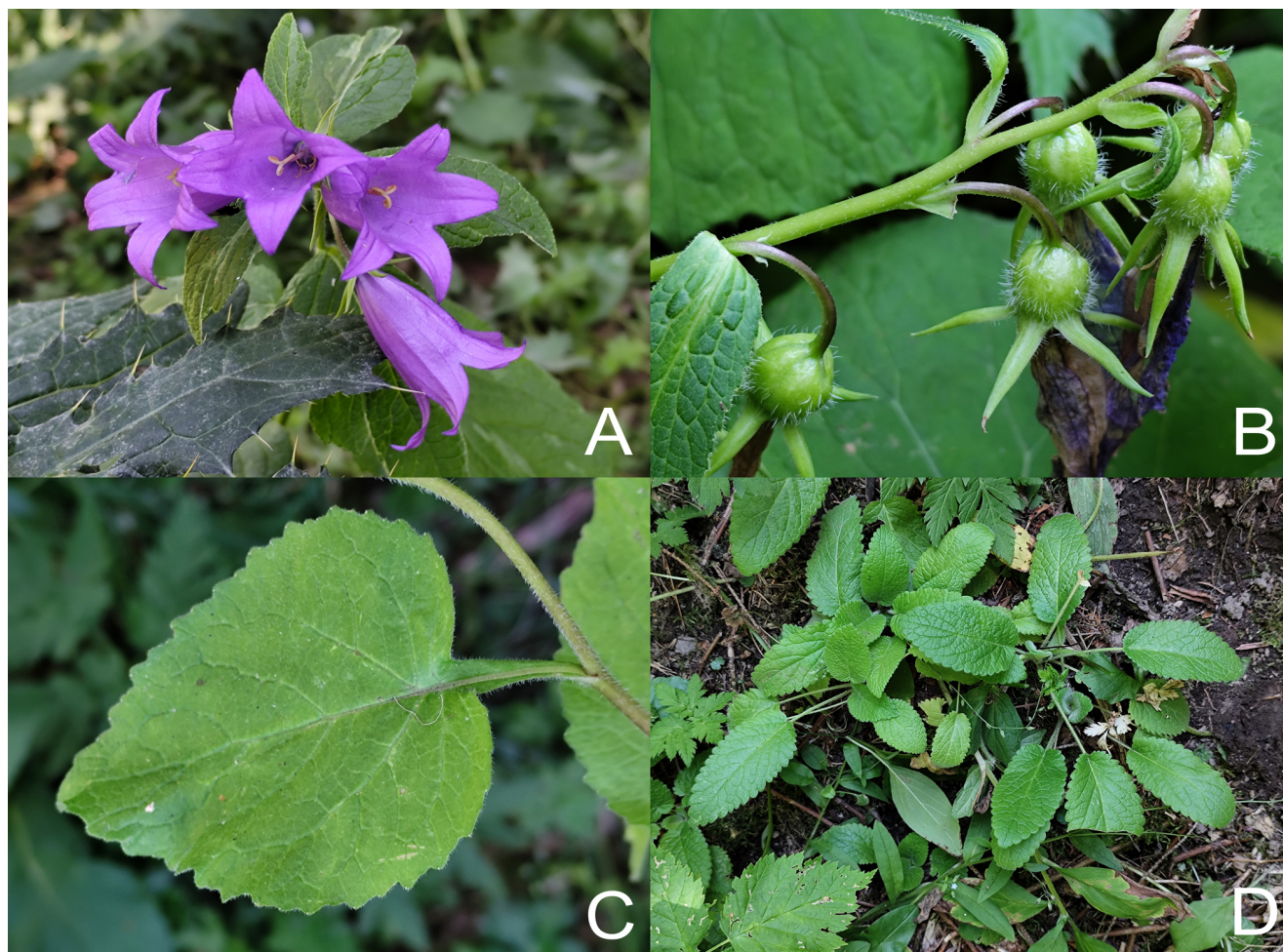


Fig. 5. Morphological features of *Campanula latifolia*: (a) flowering plant; (b) immature seed capsules; (c) lower cauline leaves; (d) basal leaves of seedlings.

(Bieberstein, 1808) emphasises on the calycular hairs, as also did Schulkina (1979), but this character is now known to be variable as this could be seen for example in the description in Damboldt (1978). Bieberstein (1808) described the flowers of the Caucasian plants as whitish or pale blue and similar colour variability is noted by other authors (Fedorov, 1957; Damboldt, 1978). White-flowering plants are uncommon in the Bulgarian population, albeit present in both Ovnarsko and Borovets areas.

Escape from gardening is recognised as a major pathway of introduction of alien plants in Europe (Arianoutsou et al., 2021). In this respect, the properties of the former royal family would hold a special place in Bulgaria, as far as both Tsar Ferdinand I and Tsar Boris III had a particular attitude towards gardening and landscaping, and an enormous diversity of both native and exotic plants was nursed

in their residences (Buresch, 1939; Fomina, 2003). For example, over 3000 species were cared for in the many rockeries of the Royal Botanic Gardens alone (Buresch, 1939). Although little addressed topic in the Bulgarian literature by far, the royal estates are already documented as sources of naturalised alien plants in Bulgaria, some of which readily show invasive behaviour. Several species are hitherto known to have their origins in the Crown estates (Table 4). The first plant to be reported as naturalised is apparently *Claytonia sibirica* from Tsarska Bistritsa manor by Stefanov (1951). In the few decades to follow the data are relatively sparse, but alien plants were generally sparingly recorded in - Bulgarian until late in the 20th century. The interest in these greatly increased since the early 2000s to become nowadays meticulously looked after (cf. Vladimirov & Petrova, 2023) and most species

Table 4. Alien plants, which occurrence in Bulgaria could be linked to naturalisation from royal estates.

Species and alien status	Estate(s) of origin	Reference(s)
<i>Campanula lactiflora</i> M. Bieb. Naturalised	Ovnarsko	This paper
<i>Campanula rotundifolia</i> L. Status not clarified	Ovnarsko	Anchev (2012)
<i>Claytonia sibirica</i> L. Naturalised	Tsarska Bistritsa	Stefanov (1951) Petrova et al. (2024)
<i>Hieracium petraeum</i> Bluff & Fingerh. Naturalised	Sitnyakovo	Vladimirov (2018)
<i>Sagittaria latifolia</i> Willd. Invasive	Vrana	Jordanov (1963) Vladimirov (2009) Petrova & Venkova (2017)
<i>Veronica filiformis</i> Sm. Naturalised	Vrana so far only found within the estate	Dimitrov & Assyov (2003) Assyov (pers. obs.)
<i>Veronica fruticans</i> Jacq. Status not clarified	Yastrebet	Peev (1995)
<i>Veronica fruticulosa</i> L. Status not clarified	Yastrebet	Peev (1995)

arguably traceable to royal estates were published only recently. In this respect, *C. lactiflora* is another representative example that these establishments are source of alien and invasive plants and that further studies around those grounds are worthy.

Groups in certain social networks were very recently recognised as valuable sources of diverse botanical data, including for early warning of potential new invasions (Marcenò et al., 2021). The occurrence of *C. lactiflora* in Bulgaria was first suggested by photographs of amateur botanists and plant lovers, distributed through groups in social media. Botanical data acquisition by plant enthusiasts is not unknown in Bulgaria. It has led to the recent findings of new species for the Bulgarian flora, usually published in a co-authorship with academic staff (Domozetski, 2024; Kunev et al., 2024; Stoyanov & Barzov, 2018, etc.) or new localities of some rare species (see e. g. Dimitrov & Veleva, 2023). The discovery of *C. lactiflora* is thus an evidence for the underexplored potential of the possible participation of citizen science initiatives for plant survey and mapping. It also shows that such involvement could be particularly productive in clarifying the distribution of species with restricted ranges. There could be little doubt that those opportunities are worth pursuing further.

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Conflicts of interest

No potential conflict of interest has been reported by the authors, reviewers, or subject editor.

Author's contributions

(using [CrediT](#))

Rossen Vassilev: data curation, investigation, methodology, visualisation, writing—original draft; Antoaneta Petrova: conceptualisation, data curation, investigation, methodology, writing—review and editing; Boris Assyov: conceptualisation, data curation, investigation, methodology, writing—original draft; Atanas Tanev: investigation, writing—review and editing.

References

- Akcin T.A. 2009 Seed coat morphology of some Turkish *Campanula* (Campanulaceae) species and its systematic implications. *Biologia* 64: 1089–1094.
<https://doi.org/10.2478/s11756-009-0177-5>
- Arianoutsou M., Bazos I., Christopoulou A., Kokkoris Y., Zikos A., Zervou S., Delipetrou P., Cardoso A.C., Deriu I., Gervasini E., Tsiamis K. 2021 Alien plants of Europe: introduction pathways, gateways and time trends. *PeerJ* 9: e11270.
<https://doi.org/10.7717/peerj.11270>
- Anchev M. 1992 Campanulaceae. In: Kozuharov S. (ed.) *Guidebook to the higher plants in Bulgaria*. Nauka i izkustvo, Sofia, 279–287.
- Anchev M. 2012 *Campanula* L. In: Kožuharov S., Anchev M. (eds) *Flora Reipublicae Popularis Bulgaricae*. Vol. 11. Editio Academica “Prof. Marin Drinov”, Sofia, 85–136 (In Bulgarian)
- Assyov B., Petrova A., Dimitrov D., Vassilev R. 2012 *Conspectus of the Bulgarian vascular flora*. Distribution maps and floristic elements. Ed. 4. Bulgarian Biodiversity Foundation, Sofia, 489 pp.
- Barina Z., Somogyi G., Pifkó D., Rakaj M. 2018 Checklist of vascular plants of Albania. *Phytotaxa* 378: 1–339.
<https://doi.org/10.11646/phytotaxa.378.1.1>
- BGE 2025 The Herbarium of the Botanic Garden Edinburgh online. <https://data.rbge.org.uk/search/herbarium/> (accessed 1 February 2025).
- von Bieberstein F.A.M. 1808 *Flora taurico-caucasica, exhibens stirpes phaenogamas in Chersoneso Taurica et regionibus caucasicis sponte crescentes*. Typis Academicis, Charkulæ, 428 pp.
<https://doi.org/10.5962/bhl.title.10825>
- Boršić I., Milović M., Dujmović I., Bogdanović S., Cigić P., Rešetnik I., Nikolić T., Mitić B. 2008 Preliminary check-list of invasive alien plant species (IAS) in Croatia. *Natura Croatica* 17 (2): 55–71.
- Buresch I. 1939 Johann Kellner und die Botanischen Gärten S. M. des Königs der Bulgaren (Anlässlich seines einjährigen Todestages). *Mitteilungen aus den Königlichen naturwissenschaftlichen Instituten in Sofia – Bulgarien* 12: 123–180. (In Bulgarian)
- Byalt V.V., Vassiljev N.P. 2009 *Gadellia lactiflora* (Campanulaceae) – the new adventive genus and species for flora of North Western Russia. *Turczaninowia* 12 (1–2): 54–57. (In Russian)
- Damboldt J. 1978 *Campanula* L. In: Davis P.H. (ed.) *Flora of Turkey*. Vol. 6. Edinburgh University Press, Edinburgh, 2–64.
- Danihelka J., Chrtek Jr. J., Kaplan Z. 2012 Checklist of vascular plants of the Czech Republic. *Preslia* 84: 647–811.
- Dimitrov D., Assyov B. 2003 New taxa for the Bulgarian flora. *Godishnik na Sofijska Universitet “St. Kliment Ohridski”* 95 (4): 269–272.
- Dimitrov P., Veleva G. 2023 Report 76. *Cypripedium calceolus* L. In: Vladimirov V., Aybeke M., Matevski V. et al. *New floristic records in the Balkans*: 52. *Phytologia Balcanica* 29 (3): 421–460.
<https://doi.org/10.7546/PhB.29.3.2023.11>
- Domozetski L. 2024 New data on the orchids (Orchidaceae) in Bulgaria. *Phytologia Balcanica* 30 (2): 203–224.
<https://doi.org/10.7546/PhB.30.2.2024.9>
- Fedorov A.A. 1957 Campanulaceae Juss. In: Shishkin B.K., Bobrov B.K. (eds) *Flora SSSR*. Vol. 24. Academy of Sciences Publishing House, Moscow–Leningrad, 126–453; 459–475. (In Russian)
- Fedorov A.A., Kovanda M. 1976 *Campanula* L. In: Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D.A. (eds) *Flora Europaea*. Vol. 4. Cambridge University Press, Cambridge, 74–93.
- Fomina L. 2003 *The Vrana Park – history and composition*. Zemizdat, Sofia, 135 pp. (In Bulgarian)

- Goranova V., Anchev M. 2015 *Campanula latifolia* L. In: Peev D., Petrova A., Anchev M., Temniskova D., Denchev C.M., Ganeva A., Gushev Ch., Vladimirov V. (eds) Red Data Book of the Republic of Bulgaria. Vol. 1 Plants and fungi. BAS and MOEW, Sofia, 426.
- ISU 2025 The Herbarium of the Institute of Botany – Ilia State University of Georgia online. <https://www.jacq.org/> (accessed 1 February 2025).
- Jordanov D. 1963 *Sagittaria* L. In: Jordanov D., Kitanov B., Valev S. (eds) Flora Reipublicae Popularis Bulgaricae. Vol. 1. Editio Academica “Prof. Marin Drinov”, Sofia, 220–221. (In Bulgarian)
- Korakaki E., Legakis A., Katsanevakis S., Koulelis P.P., Avramidou E.V., Soulioti N., Petrakis P.V. 2021 Invasive Alien Species of Greece. In: Pullaiah T., Ielmini M.R. (eds) Invasive Alien Species: Observations and Issues from Around the World. John Wiley & Sons Ltd., Hoboken, 124–189.
<https://doi.org/10.1002/9781119607045.ch29>
- Kadriaj B., Berisha N., Krasniqi E., Millaku F. 2023 Invasive alien plant species (IAPS) in the eastern region of Kosovo: a preliminary list. *Ecologia Balkanica* 15 (2): 22–31.
- Karlsson T. 1998 Förteckning över svenska kärlväxter. *Svensk Botanisk Tidskrift* 91 (5): 241–560.
- Kravchenko A.V., Sukhov A.V., Timofeeva V.V., Fadeeva M.A. 2018 New and rare for Karelia vascular plants. *Turczaninowia* 21 (2): 40–46. (In Russian)
<https://doi.org/10.14258/turczaninowia.21.2.5>
- Kunev G., Kostadinov I., Karakasheva D. 2024 *Dipsacus gmelinii* (Caprifoliaceae), a wetland species new to the Bulgarian flora. *Hacquetia* 23 (1): 145–150.
<https://doi.org/10.2478/hacq-2023-0007>
- Koutsovoulou K., Daws M.I., Thanos C.A. 2014 Campanulaceae: a family with small seeds that require light for germination. *Annals of Botany* 113 (1): 135–143.
<https://doi.org/10.1093/aob/mct250>
- Liveri E., Kalachanis D., Bareka P., Grammatikopoulos G., Kamari G. 2020 Contribution to the seed morphology of some Greek *Campanula* species of sect. *Quinqueloculares* (Campanulaceae). *Flora Mediterranea* 30: 347–363.
<https://doi.org/10.7320/FIMedit30.347>
- LSL 2025 The Linnaean Herbarium online. https://linnean-online.org/linnaean_herbarium.html (accessed 1 February 2025).
- Marcenò C., Padullés Cubino J., Chytrý M., Genduso E., Salemi D., La Rosa A., Gristina A.S., Agrillo E., Bonari G., Giusso del Galdo G., Ilardi V., Landucci F., Guarino R. 2021 Facebook groups as citizen science tools for plant species monitoring. *Journal of Applied Ecology* 58 (10): 2018–2028.
<https://doi.org/10.1111/1365-2664.13896>
- Marinov Y., Stoyanov S. 2017 Reappraisal of *Campanula rotundifolia* var. *bulgarica* Nežceff – a neglected taxon from Central Stara Planina Mountain, Bulgaria. *Phytotaxa* 329 (1): 69–79.
<https://doi.org/10.11646/phytotaxa.329.1.4>
- Maslo S., Wong L.J., Pagad S. 2020 GRIIS Checklist of introduced and invasive species – Bosnia and Herzegovina. v1.2. Invasive Species Specialist Group, ISSG.
<https://doi.org/10.15468/uuzhvt>
- MGU 2025 The Herbarium of the Moscow State University online. <https://plant.depo.msu.ru/open/public/search?collection=MW> (accessed 1 February 2025).
- Nāburga I., Evarts-Bunders P. 2019 Status of some escaped ornament perennials in the flora of Latvia. *Botanica* 25: 131–144.
- OTM 2026 Open Topo Map. <https://opentopomap.org/> (accessed 1 February 2026).
- Peev D. 1995 *Veronica* L. In: Jordanov D., Kitanov B., Valev S. (eds) Flora Reipublicae Popularis Bulgaricae. Vol. 10. Editio Academica “Prof. Marin Drinov”, Sofia, 142–189. (In Bulgarian)
- Petrova A., Venkova D. 2017 Reports 45–49. In: Vladimirov V., Aybeke M., Tan K. (eds) New floristic records in the Balkans: 32. *Phytologia Balcanica* 23 (1): 119–146.
- Petrova A., Venkova D., Assyov B., Vassilev R. 2024 On the current distribution and population size of non-native *Claytonia sibirica* in Bulgaria. *Phytologia Balcanica* 30 (1): 95–102.
<https://doi.org/10.7546/PhB.30.1.2024.9>
- QGis 2025 QGis. <https://qgis.org/> (accessed 1 February 2026).
- Rao N.K., Hanson J., Dulloo M.E., Ghosh K., Nowell D., Larinde M. 2006 Manual of seed handling in genebanks. Handbooks for Genebanks No. 8. Bioversity International, Rome, 147 pp.

- Schulkina T. 1979 De positione systematica *Campanula lactiflora* Bieb. Novosti Sistematiki Vysshikh Rastenii 16: 175–179. (In Russian)
- Stace C. 2010 New flora of the British Isles. Cambridge University Press, Cambridge, 1266 pp.
- Stefanov B. 1951 Floristic reports. Izvestiya na Botanicheskiya Institut (Sofia) 2: 270.
- Stešević D. 2013 Contribution to the alien flora of Montenegro and Supplementum to the Preliminary list of plant invaders. Biologica Nyssana 4 (1–2): 1–7.
- Stešević D., Petrović D. 2010 Preliminary list of plant invaders in Montenegro. Biologica Nyssana 1 (1–2): 35–42.
- Stoyanov K., Raycheva Ts., Cheschmedzhiev I. 2022 Key to the native and foreign vascular plants in Bulgaria. Interactive extended and supplemented edition. Academic Publishing House of the Agrarian University. <https://botanica.gallery/plantkeybg/app.html> . (In Bulgarian) <https://doi.org/10.13140/RG.2.2.36390.95047> 
- Stoyanov S., Barzov Z. 2018 *Cyperus eragrostis* – an addition to the Bulgarian alien flora. Phytologia Balcanica 24 (2): 239–242.
- Stojanović V., Jovanović I. 2018 The survey of invasive and potentially invasive plant species in Serbia and neighbouring countries for the purpose of determining their status at the national level. Nature Conservation 68 (1–2): 41–59.
- Verloove F. 2025 Manual of the alien plants of Belgium. Botanic Garden Meise, Belgium. <https://alienplantsbelgium.myspecies.info/>  (accessed 31 January 2025).
- Vladimirov V. 2009 Reports 83–91. In: Vladimirov V., Dane F., Stevanović V., Tan K. (eds) New floristic records in the Balkans: 20. Phytologia Balcanica 15 (3): 431–452.
- Vladimirov V. 2018 *Hieracium petraeum* (Asteraceae): a new casual record in the Bulgarian flora. Phytologia Balcanica 24 (3): 365–368.
- Vladimirov V., Petrova A. 2023 Alien species of vascular plants first reported for Bulgaria after 2000. Phytologia Balcanica 29 (3): 461–474. <https://doi.org/10.7546/PhB.29.3.2023.12> 

Research article

Parasitism in the armadillo *Zaedyus pichiy* (Cingulata: Chlamyphoridae) from San Juan Province, Argentina

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Abstract: The objective of this study was to describe parasitism in the piche armadillo *Zaedyus pichiy* (Desmarest, 1804) from San Juan Province, Argentina. An adult male *Z. pichiy* found run over on provincial route 510 (province of San Juan, Argentina) was analysed. The armadillo was examined externally in search of ectoparasites. Subsequently, the gastrointestinal contents were extracted and transported to the laboratory for endoparasite search. The presence of the tick *Amblyomma pseudoconcolor* (Acari: Ixodidae) and nematodes *Cyclobulura superinae* (Spirurida: Subuluridae), *Aspidodera scoleciformis*, and *Aspidodera fasciata* (Spirurida: Aspidoderidae) was determined. Our work provides new information on parasitism in this armadillo species in San Juan, Argentina.

Keywords: *Amblyomma pseudoconcolor*, *Aspidodera fasciata*, *Aspidodera scoleciformis*, *Cyclobulura superinae*, nematode, tick

Introduction

Xenarthrans are mammals almost exclusively distributed in the Neotropical Region, except for *Dasypus novemcinctus* Linnaeus, 1758, which ranges as far as the central United States (Nigro et al., 2021). In Argentina, two orders are represented, totalling 17 species: order Pilosa, with three species of terrestrial habits, arboreal and adapted to myrmecophagy, and order Cingulata, known as armadillos, equipped with a protective shell or bony armour, comprising 14 species (Nigro et al., 2021; Bauni et al., 2021). Within armadillos, there are two families, Dasypodidae with 4 species and Chlamyphoridae with 10 species (Bauni et al., 2021).

The genus *Zaedyus* Ameghino, 1889, is characterised by its relatively small body and ears (Nigro et al., 2021). The armadillo *Zaedyus pichiy* (Desmarest, 1804) (Chlamyphoridae) is distributed in Argentina and Chile (Superina & Abba, 2014). In Argentina, *Z. pichiy* is distributed across Buenos Aires, Chubut, Córdoba, La Pampa, La Rioja, Mendoza, Neuquén, Río Negro, San Juan, San Luis, and Santa Cruz provinces (Bauni et al., 2021). This species inhabits arid or semi-arid environments in firm and sandy soils. Is carnivorous-omnivorous, with a preference for carrion, but also consumes ants, beetles, scorpions, spiders, dipteran larvae, and vertebrates such as lizards and small mammals (Superina & Abba, 2014).

Information on parasite diversity in Xenarthra is represented in southern, western, and eastern Argentina (Daciuk et al., 1981; Ezquiaga et al., 2009; Navone et al., 2010; Monferrán & Silverio-Reyes, 2014; Ezquiaga et al., 2019; Ezquiaga et al., 2021; Gallo et al., 2021), mentioning nematodes of the Aspidoderidae and Subuluridae families (Ezquiaga et al., 2019) and ectoparasites, mainly Ixodidae ticks of the genus *Amblyomma* Koch, 1844 (Nava et al., 2017).

Nematodes of the Aspidoderidae family are parasites mainly of xenarthrans, didelphimorphs, hystriognathous and sigmodontine rodents (Santos et al., 1990; Anderson et al., 2009). The genus *Aspidodera* Railliet & Henry, 1912 includes 12 species: *Aspidodera scoleciformis* (Diesing, 1851); *Aspidodera subulata* (Molin, 1860); *Aspidodera fasciata* (Schneider, 1866); *Aspidodera binansata* Railliet & Henry, 1913; *Aspidodera raillieti* Travassos, 1913; *Aspidodera ansirupta* Proença, 1937; *Aspidodera vazi* Proença, 1937; *Aspidodera lacombeae* Vicente, 1964; *Aspidodera soganderesei* Jiménez-Ruiz, Gardner & Varela-Stokes, 2006; *Aspidodera kinsellai* Jiménez, Carreno, & Gardner, 2013; *Aspidodera lanfredi* Chagas-Moutinho, Sant'Anna, Oliveira-Mendes & De Souza, 2014 and *Aspidodera serrata* Cesário, Gomes, Maldonado, Olifiers, Jiménez & Bianchi, 2021 (Cesário et al., 2021).

The nematodes of the Subuluridae family include several genera, one of which is *Cyclobulura* Quentin, 1977, parasites of xenarthrans (Quentin, 1977; Anderson et al., 2009) with two species, *Cyclobulura lainsoni* Quentin, 1977, and *Cyclobulura superinae* Navone, Ezquiaga & Jiménez, 2010 (Anderson et al., 2009; Navone et al., 2010).

Our objective is to report the first record of parasitism by both ecto and endoparasites in the armadillo *Z. pichiy* from San Juan Province, Argentina.

Materials and methods

In April 2023, a male armadillo *Z. pichiy* was examined externally and internally. The specimen was found dead, run over on provincial route 510 (31°17'2.1"S; 67°13'45.7"W) 25 km from the town of Chucuma, Valle Fértil Department, San Juan Province, Argentina. An external examination was carried out; a male tick in the adult stage was collected and stored in

70° alcohol. The gastrointestinal contents were extracted and transported in containers with 70° alcohol to the laboratory to search for endoparasites. In the laboratory, the intestine and stomach were analysed for nematodes using a binocular stereoscopic microscope. The nematodes were preserved in 70° ethyl alcohol in Eppendorf tubes. For the observation of the nematodes, semi-permanent preparations were made using lactophenol solution. The identification was carried out using a light microscope (Arcano Xsp optical binocular). The tick was observed by direct observation under a magnifying glass. For tick identification, was followed Nava et al. (2017), and nematode identification followed Navone et al. (2010) and Cesário et al. (2021). Drawings were made using a camera lucida attached to a light microscope. Measurements are in μm , with mean ± 1 SD and range in parentheses unless otherwise noted.

The specimens were deposited in the Parasitological Collection of the Center for Wildlife Rehabilitation, Environmental Education and Responsible Recreation (Parque Faunístico) (*Amblyomma pseudoconcolor*: CRFSJ_59A; *Aspidodera scoleciformis*: CRFSJ_59B; *Aspidodera fasciata* CRFSJ_59C; *Cyclobulura superinae* CRFSJ_59D).

Ethical aspects

All applicable international, national, and/or institutional guidelines for the care and use of animals were strictly followed. All sample collection protocols complied with current Argentine laws. All animal procedures performed in this research were carried out in accordance with the ethical standards of the institution or organisation where the study was conducted.

Results

One adult male tick of *Amblyomma pseudoconcolor* Aragão, 1908 (Acari: Ixodidae) was recorded. The characteristics that allowed it to be identified are: body outline oval rounded, scapulae rounded; cervical grooves short, comma-shaped; marginal groove complete. Scutum ornate, with small pale spots adjacent to the marginal groove; Genital aperture located at level of coxa II, U-shaped. Legs:

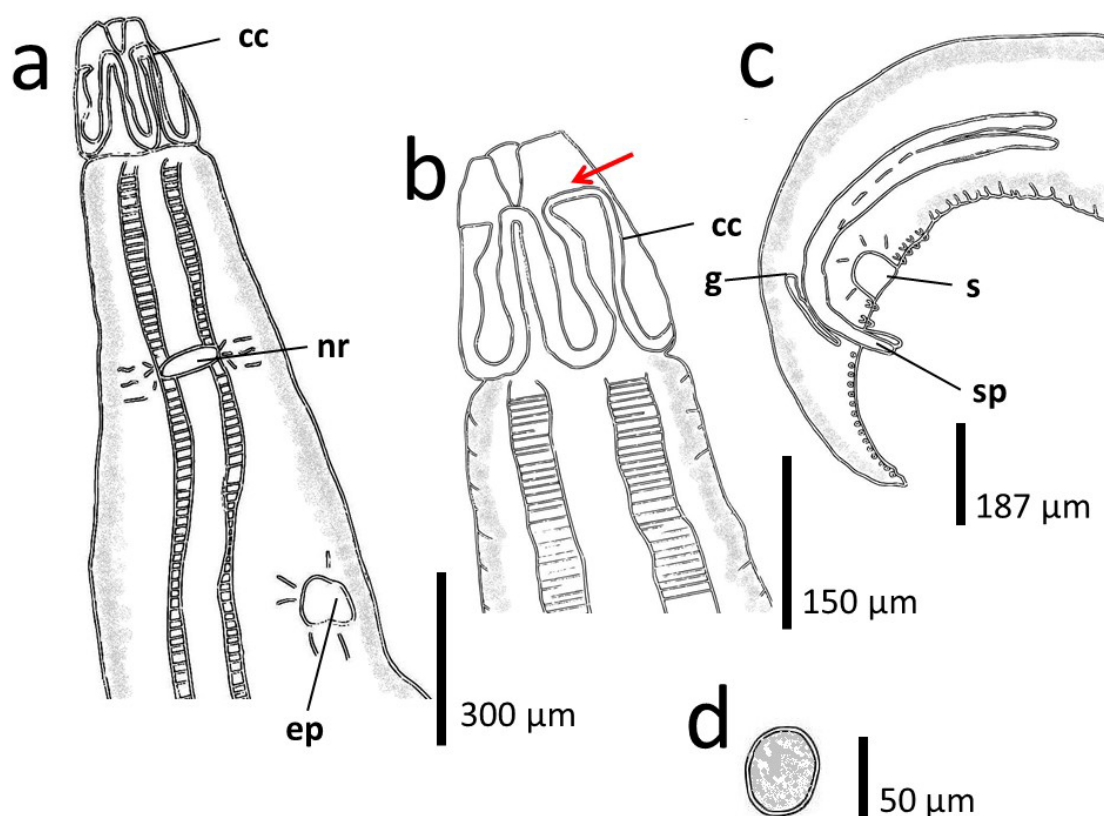


Fig. 1. *Aspidodera scoleciformis*. (a); female, anterior extremity, lateral view, (b); males, anterior extremity, lateral view, (c); males, posterior extremity, lateral view, (d); eggs. Cephalic cords = cc, nerve ring = nr, excretory pore = ep, sucker = s, spicule = sp, gubernaculum = g. Detail of the square-shaped cephalic cord at the labial root (red arrow).

coxa I with two distinct, short, triangular spurs, subequal in size; coxae II-IV with a triangular spur each; trochanters with spurs. Spiracular plates comma-shaped.

In the large intestine, 5 female and 2 male nematodes of *Aspidodera scoleciformis* and 6 females and 3 males of *Aspidodera fasciata* were recorded. In addition, 8 females and 2 males of *Cyclobulura superinae* in the small intestine were recorded.

Nematoda Rudolphi, 1808

Spirurida Railliet, 1915

Aspidoderidae Freitas, 1956

Aspidodera Railliet & Henry, 1912

Aspidodera scoleciformis (Diesing, 1851) (Fig. 1)

General: They present an expansion called cofia, which has cephalic cords with recurrent ramifications.

Cephalic cords present in a square shape at the labial root. Mouth with three lips. Esophagus with posterior bulb. The nerve ring and excretory pore are located in the middle of the esophagus. Females with the vulva located in the anterior third, approaching the middle of the body. Males without absent caudal alae.

Males (Based on 2 specimens) (μm): Total length 6693 ± 4.2 (6690–6696), maximum width 407 ± 1.4 (406–408), esophagus length (excluding bulb) 1473 ± 4.2 (1470–1476), esophagus width 76.5 ± 2.1 (75–78), bulb length 294 ± 1.4 (293–295), bulb width 316 ± 1.4 (315–317), cord length 61 ± 1.4 (60–62), distance to excretory pore from anterior region 831.5 ± 2.1 (830–833). Spicules of equal size, 752 ± 2.8 (750–754), distance to sucker from anterior region 6240 ± 2.1 (6239–6242), sucker length 86.5 ± 2.1 (85–88), sucker width 57.5 ± 0.7 (57–58), gubernaculum length 195.5 ± 0.7 (195–196). Presence of cloacal papillae: 4 precloacal before the sucker, 2 pairs posterior to the sucker and between 15 and 23 postcloacal pairs.

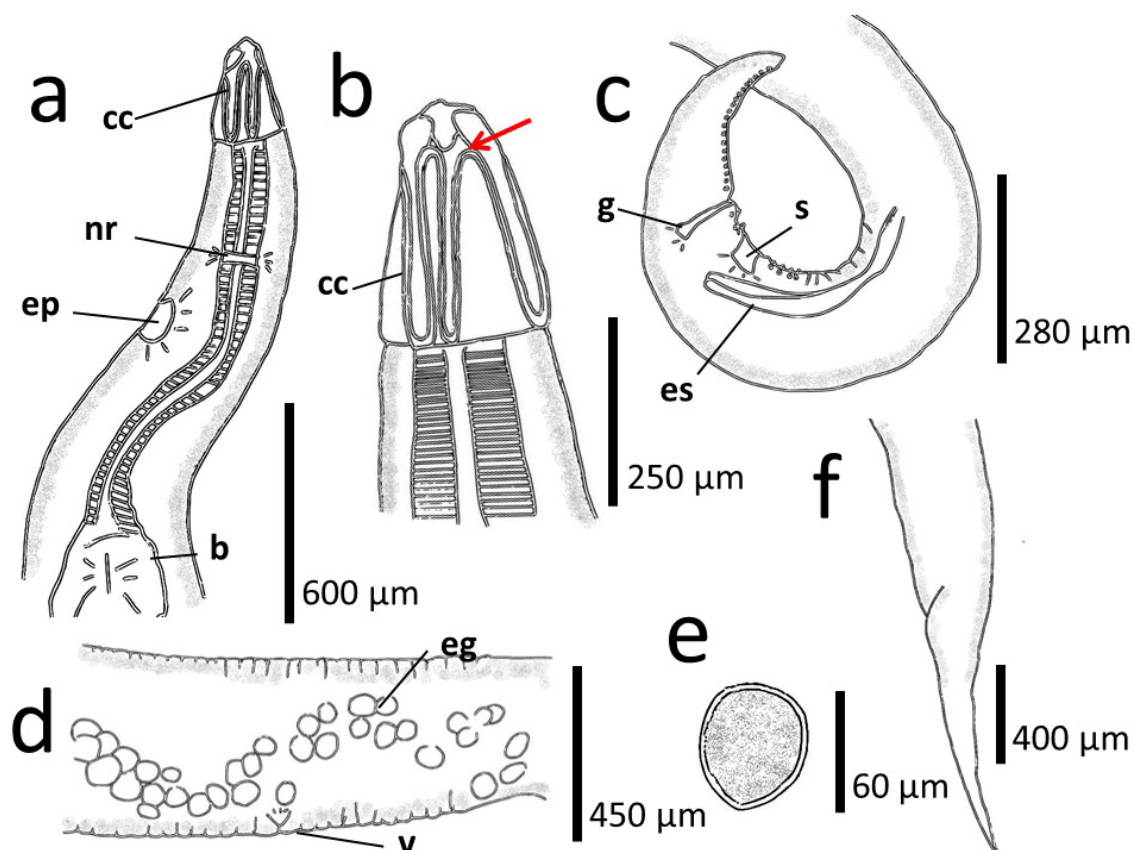


Fig. 2. *Aspidodera fasciata*. (a); female, anterior extremity, lateral view, (b); males, anterior extremity, lateral view, (c); males, posterior extremity, lateral view, (d); female with eggs, medium extremity, lateral view, (e); eggs, (f); female, posterior extremity, lateral view. The cephalic cords are observed = cc, nerve ring = nr, excretory pore = ep, bulb = b, vulva = v, eggs = eg, gubernaculum = g, sucker = s, spicule = sp. Detail of the round-shaped cephalic cord at the labial root (red arrow).

Females (Based on 2 specimens) (μm): Total length 6813 ± 4.2 (6810–6816), total width 521 ± 1.4 (520–522), esophagus length (excluding bulb) 1702 ± 2.8 (1700–1704), esophagus width 66.5 ± 2.1 (65–68), bulb length 339 ± 1.4 (338–340), bulb width 339 ± 1.4 (338–340), distance to nerve ring from anterior region 321.5 ± 2.1 (320–323), cord length 149.5 ± 0.7 (149–150), distance to vulva from anterior region 3292 ± 2.8 (3290–3294), egg width 50 (50) and egg length 50 (50).

Aspidodera fasciata (Schneider, 1866) (Fig. 2)

General: They present an expansion called cofia, which has cephalic cords with recurrent ramifications. Cephalic cords present in a round shape at the labial root. Mouth with three lips. Esophagus with posterior bulb. The nerve ring and excretory pore are located in

the middle of the esophagus. Females with the vulva located in the anterior third, approaching the middle of the body. Males with caudal wings absent.

Males (Based on 2 specimens) (μm): Total length 4882 ± 2.83 (4880–4884), total width 341 ± 2.1 (340–343), esophagus length (excluding bulb) 1178 ± 2.1 (1177–1180), esophagus width 48 ± 0.7 (48–49), bulb length 195.5 ± 0.7 (195–196), bulb width 213.5 ± 2.1 (212–215), distance to nerve ring from anterior region 732 ± 3.5 (730–735), cord length 212 (212), distance to excretory pore from anterior region 831 ± 2.1 (830–833). Spicules of equal size, 309 ± 1.4 (308–310), distance to sucker from anterior region 6012 ± 3.5 (6010–6015), sucker length 97 ± 0.7 (97–98), sucker width 48 ± 0.7 (48–49), gubernaculum size 136 ± 1.4 (135–137). Caudal papillae numerous and sessile. Presence of cloacal papillae: 4 precloacal before the sucker, 2 pairs posterior to the sucker and between 20 and 23 postcloacal pairs.

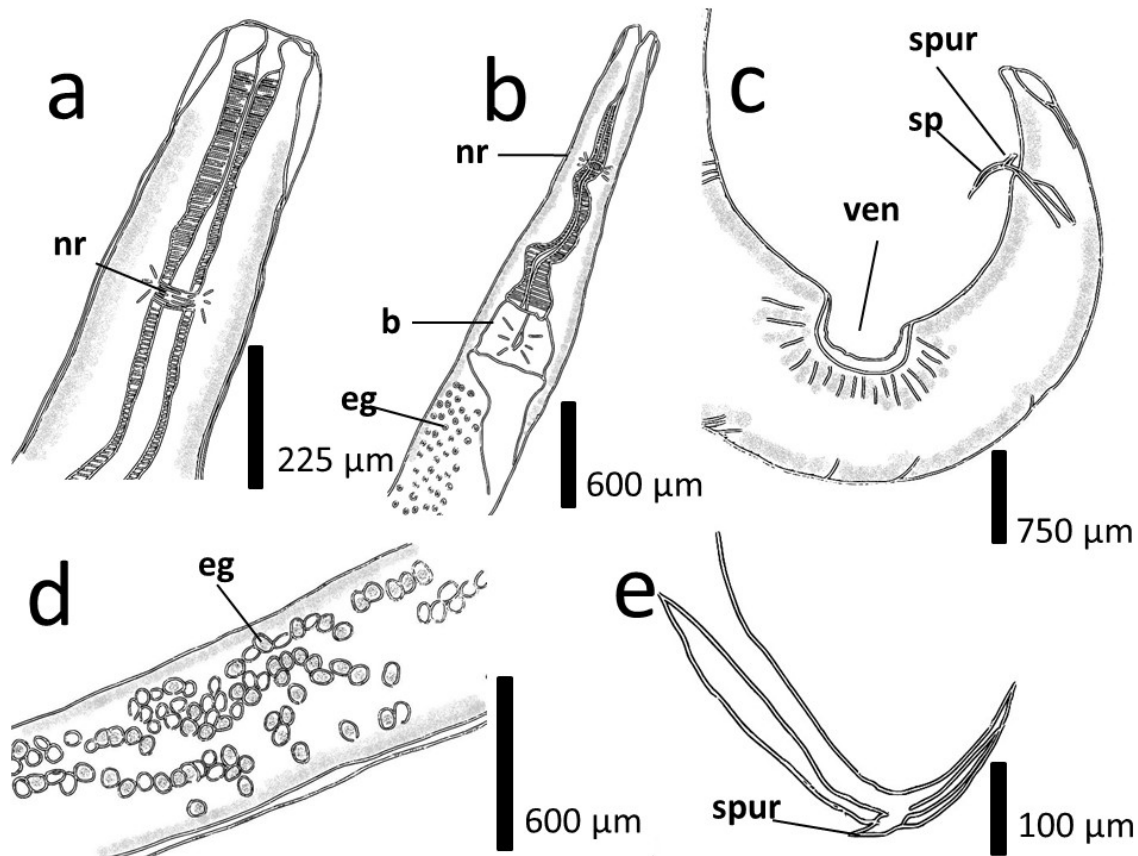


Fig. 3. *Cyclobulura superinae*. (a, b); female, anterior extremity, lateral view, (c); male, posterior extremity, lateral view, (d); female with eggs, medium extremity, (e); spicule and spur. Nerve ring = nr, bulb = b, eggs = eg. Sucker = s, spicule = sp.

Females (Based on 2 specimens) (μm): Total length 6701 ± 1.4 (6700–6702), total width 452 ± 2.8 (450–454), esophagus length (excluding bulb) 1244 ± 6.36 (1240–1249), esophagus width 44 ± 1.4 (43–45), bulb length 223 ± 4.9 (220–227), bulb width 271 ± 1.4 (270–272), cord length 249.5 ± 0.7 (249–250), distance to excretory pore from anterior region 732.5 ± 3.5 (730–735), distance to vulva from anterior region 2724 ± 2.1 (2723–2726), egg width 74.4 ± 0.7 (74–75) and egg length 61.4 ± 0.7 (61–62).

Subuluridae Yorke & Maplestone, 1926

Cyclobulura superinae Navone, Ezquiaga, Notarnicola & Jiménez, 2010 (Fig. 3)

General: White nematodes, buccal capsule divided into an anterior buccal part and a posterior pharyngeal part. Small buccal cavity, triangular in cross-section.

Simple oral opening without lip lobes. Present spurs on the spicules.

Males (Based on 2 specimens) (μm): Total length 11325 ± 35.3 (11300–11350), total width 534.5 ± 6.3 (530–539), esophagus length (excluding bulb) 1700 ± 1.4 (1700–1702), esophagus width 116 ± 1.4 (115–117), bulb length 339 ± 1.4 (338–340), bulb width 247 ± 2.8 (245–249), distance to nerve ring from anterior region 452 ± 2.8 (450–454), distance to excretory pore from anterior region 683 ± 4.2 (680–686). Spicules of equal size, spicule size 1073 ± 5.6 (1070–1078), spur size 49.4 ± 0.7 (49–50), distance to sucker from anterior region 10664 ± 6.3 (10660–10669), sucker width 97.4 ± 0.7 (97–98), sucker length 195 ± 0.7 (195–196). Presence of caudal papillae: 1 pair in the middle sector where the sucker is located, 3 precloacal and 4 postcloacal pairs.

Females (Based on 2 specimens) (μm): Total length 13823.5 ± 33.5 (13800–13847), total width 606.5 ± 9.1 (600–613), esophagus length (excluding bulb) 1915.5 ± 22 (1900–1931), esophagus width 87.5

± 3.5 (85–90), bulb length 337.5 ± 3.5 (335–340), bulb width 271 ± 1.4 (270–272), distance to nerve ring from anterior region 452 ± 2.8 (450–454), distance to vulva from anterior region 10318.5 ± 26 (10300–10337), egg width 54.5 ± 0.7 (54–55) and egg length 66 ± 1.4 (65–67).

Discussion

In the province of San Juan, 5 species of Cingulata armadillos are registered (Bauni et al., 2021): *Chaetophractus vellerosus* (Gray, 1865), *Chaetophractus villosus* (Desmarest, 1804), *Chlamyphorus truncatus* Harlan, 1825, *Tolypeutes matacus* (Desmarest, 1804), and *Z. pichiy*. So far, only the armadillo *C. vellerosus* has been studied in San Juan, reporting the nematodes *A. scoleciformis* and *C. superinae* (Ezquiaga et al., 2019).

In the present study, we analyse the parasitic fauna of *Z. pichiy* recorded in the locality of Valle Fértil. Our results from the analysis of this host indicated the presence of three species of nematodes: *C. superinae*, *A. scoleciformis* and *A. fasciata*. The analysis of a unique *Z. pichiy* specimen impedes the obtaining of conclusions on the ecological parasitic indexes.

The literature indicates that *C. villosus* has been studied in the provinces of Chubut, Santa Cruz, Tierra del Fuego, and La Rioja, where *C. superinae*, *A. fasciata*, and *A. scoleciformis* have been recorded (Navone et al., 2010; Gallo et al., 2021). *Chaetophractus vellerosus* has been studied in Buenos Aires, Córdoba, La Rioja, Mendoza, San Juan, and San Luis, with reports of *A. scoleciformis* and *C. superinae* (Ezquiaga et al., 2019). In *Z. pichiy*, *C. superinae*, *Aspidodera* sp., *A. fasciata*, and *A. scoleciformis* have been recorded in Mendoza, Chubut, Santa Cruz, and Tierra del Fuego provinces (Ezquiaga et al., 2009; Navone et al., 2010; Gallo et al., 2021). Lastly, in *T. matacus*, *A. scoleciformis* and *A. fasciata* were recorded in Catamarca (Monferrán & Silverio-Reyes, 2014).

These antecedents allow our work to be the first record on the parasitism of *Z. pichiy* in the province of San Juan, Argentina, recording the nematodes *C. superinae*, *A. scoleciformis*, and *A. fasciata*.

Regarding ectoparasites recorded in armadillos from Argentina, antecedents mention records in Patagonia, Argentina, reporting in *C. villosus* the ticks

A. pseudoconcolor and *Amblyomma auricularium* (Conil, 1878) (Daciuk et al., 1981; Guglielmone & Nava, 2006; Ezquiaga et al., 2021) and in *Z. pichiy* also from Patagonia, Argentina *A. pseudoconcolor* (Gallo et al., 2021). Due to this background, our results mention the first record of the tick *A. pseudoconcolor* in *Z. pichiy* for San Juan, Argentina.

Later studies in more armadillos will allow us to elucidate the parasitic ecological data and thus be able to compare with other regions of Argentina.

Conflicts of interest

No potential conflict of interest has been reported by the authors, reviewers, or subject editor.

Author's contributions

(using [CrediT](#) )

Gabriel N. Castillo: conceptualisation, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualisation, writing—original draft, writing—review and editing; Cynthia J. González-Rivas: data curation, formal analysis, investigation, supervision, validation, writing—original draft, writing—review and editing; Lucas C. Zuñiga: funding acquisition, writing—original draft; Ruben Fernandez-Reinoso: funding acquisition, writing—original draft; Leandro F. Castillo: funding acquisition, writing—original draft; Rodolfo O. Noriega: funding acquisition, writing—original draft.

References

- Anderson R.C., Chabaud A.G., Willmott S. 2009 Keys to the nematode parasites of vertebrates. CAB International, Cambridge, USA.
- Bauni V., Bertonatti C., Giacchino A. 2021 Inventario biológico argentino: vertebrados. Fundación de Historia Natural Félix de Azara, 1ª ed. – Ciudad Autónoma de Buenos Aires, Argentina.
- Cesário C.S., Gomes A.P.N., Maldonado A., Olifiers N., Jiménez F.A., Bianchi R.C. 2021 A New Species of *Aspidodera* (Nematoda: Heterakoidea) Parasitizing the Giant Anteater *Myrmecophaga tridactyla* (Pilosa: Myrmecophagidae) in Brazil

- and New Key to Species. Comparative Parasitology 88: 7–14.
<https://doi.org/10.1654/1525-2647-88.1.7>
- Daciuk J., Cicchino A.C., Mauri R., Capri J.J. 1981 Notas faunísticas y bioecológicas de Península Valdes y Patagonia. XXIV. Artrópodos ectoparásitos de mamíferos y aves colectados en la Península Valdés y alrededores (provincia de Chubut, Argentina). Physis 39: 41–48.
- Ezquiaga M.C., Abba A.M., Navone G.T. 2019 Helminth fauna of the screaming hairy armadillo *Chaetophractus vellerosus* from Argentina: the consequence of host isolation on parasite diversity. Heliyon 5: e01605.
- Ezquiaga M.C., Gallo J.A., D’Agostino R.L., Sauthier D.E.U., Abba A.M., Sanchez J. 2021 Fleas and ticks in armadillos from Argentinean Patagonia: Diversity, abundance and distribution. Acta Tropica 219: 105911.
- Ezquiaga M.C., Superina M., Navone G.T. 2009 Parásitos intestinales de *Zaedyus pichiy* (Xenarthra: Dasypodidae) de Mendoza, Argentina. Mastozoología Neotropical, 16: 309–319.
- Gallo J.A., Ezquiaga M.C., Fasola L., Abba A.M. 2021 Helminth-fauna of Patagonian armadillos: comparative analysis of parasites geographical variation. Anais da Academia Brasileira de Ciências 93: 1–12.
- Guglielmone A.A., Nava S. 2006 Las garrapatas argentinas del género *Amblyomma* (Acari: Ixodidae): distribución y hospedadores. Revista de Investigaciones Agropecuarias 35: 133–153.
- Monferrán M.C., Silverio-Reyes M.J. 2014 Parásitos gastrointestinales de *Tolypeutes matacus* (Xenarthra; Dasypodidae), de Catamarca. Biología en Agronomía 4: 87–94.
- Nava S., Venzal J.M., Acuña D.G., Martins T.F., Guglielmone A.A. 2017 Ticks of the Southern Cone of America: Diagnosis, Distribution, and Hosts with Taxonomy, Ecology and Sanitary Importance. Academic Press.
- Navone G.T., Ezquiaga M.C., Notarnicola J., Jiménez F.A. 2010 A new species of *Cyclobulura* (Nematoda: Subuluridae) from *Zaedyus pichiy* and *Chaetophractus vellerosus* (Xenarthra: Dasypodidae) in Argentina. Journal of Parasitology 96: 1191–1196.
- Nigro N.A., Gasparri B., Steger E.P. 2021 Xenartros argentinos: guía para su identificación. Fundación de Historia Natural Félix de Azara, 1ª ed. – Ciudad Autónoma de Buenos Aires, Argentina.
- Quentin J.C. 1977 *Cyclobulura lainsoni* n. gen. n. sp., nematode Subulure parasite d’un Xenarthre d’Amerique du Sud. Bulletin Muséum national d’histoire naturelle Série A Zoologie 326: 771–776.
- Santos C.P., Lent H., Gomes D.C.C. 1990 The genus *Aspidodera* Railliet and Henry, 1912 (Nematoda: Heterakoidea): revision, new synonyms and key for species. Revista Brasileira de Biologia 50: 1017–1031.
- Superina M., Abba A.M. 2014 *Zaedyus pichiy* (Cingulata: Dasypodidae). Mammalian Species 46: 1–10.

Research article

First records of *Stegonsporium pyriforme* (Hoffm.) Corda and *Massaria macra* (Vestergr.) Voglmayr & Jaklitsch on *Acer platanoides* L. and *A. campestre* L. in Bulgaria

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Abstract: In presents study, *Stegonsporium pyriforme* (Hoffm.) Corda and *Massaria macra* (Vestergr.) Voglmayr & Jaklitsch were recorded for the first time in Bulgaria. Symptomatic trees of *Acer platanoides* L. and *A. campestre* L. were noticed in 2024 in the field protective forest belts of the State Hunting Enterprise Balchik, Northeastern Bulgaria. Both pathogens were found on dying and dead trees heavily infected by the fungus *Cryptostroma corticale* (Ellis & Everh.) Greg. & Waller, the causative agent of sooty bark disease. Two micetophagous coleopteran species were found under the bark of dead maple trees: *Colobicus hirtus* (Rossi, 1790) and *Synchita undata* Guérin-Ménéville, 1844 (Coleoptera: Zopheridae).

Keywords: *Acer* spp., *Cryptostroma corticale*, field protective forest belts, *Massaria macra*, Southern Dobrudzha, *Stegonsporium pyriforme*

Introduction

The health status of maple species (*Acer pseudo-platanus* L., *A. platanoides* L. and *A. campestre* L.), planted in the field protective forest belts in Southern Dobrudzha (Northeastern Bulgaria), was assessed in 2024. During the study, the ascomycetous fungi *Stegonsporium pyriforme* (Hoffm.) Corda and *Massaria macra* (Vestergr.) Voglmayr & Jaklitsch were found on dying and dead trees, heavily infected by the fungal pathogen *Cryptostroma corticale* (Ellis & Everh.) Greg. & Waller (Ascomycota, Xylariaceae), caused sooty bark disease. *C. corticale* was first identified in an urban park in Bulgaria in 2014 (Bencheva, 2014). Since then, the pathogen has primarily spread throughout urban green spaces

nationwide. In 2023, it was detected causing significant damage and reducing maple trees vitality in the field protective forest belts in Southern Dobrudzha (Georgieva et al., 2024).

Stegonsporium pyriforme (Ascomycota, Pleo-massariaceae), with its sexual stage identified as *Prosthecium pyriforme* Jaklitsch & Voglmayr, is responsible for branch dieback in maple species (*Acer* spp.) across Europe, particularly affecting *A. pseudo-platanus* (Nageleisen, 1994; Tomiczek et al., 2005; Voglmayr & Jaklitsch, 2008). This fungus is mainly regarded as a weak parasite, predominantly occurring on dead trees, especially those previously infected by *C. corticale* (Lorenc, 2024). Voglmayr & Jaklitsch (2008) classify *S. pyriforme* as an opportunistic, moderately phytopathogenic coelomycete confined to

Table 1. Characteristics of sample plots observed in the State Hunting Enterprise Balchik.

Locality	Tree species	Date of observation	Latitude, °	Longitude, °	Altitude, m	Age, years
Hadzhi Dimitar	<i>A. pseudoplatanus</i>	15.05.2024	43.456667	28.427500	106	22
Kremena	<i>A. pseudoplatanus</i>	07.06.2024	43.560556	28.141111	210	71
Seltse	<i>A. platanoides</i>	27.05.2024	43.477055	28.305555	140	21
Trigortsi	<i>A. platanoides</i>	23.10.2024	43.522777	28.187222	200	41
Dropla	<i>A. platanoides</i>	24.10.2024	43.582777	28.079722	225	71
Dropla	<i>A. platanoides</i>	24.10.2024	43.592500	28.116666	215	71
Dropla	<i>A. platanoides</i>	24.10.2024	43.592500	28.126944	205	71
Dropla	<i>A. platanoides</i>	24.10.2024	43.585000	28.101944	215	71
Dropla	<i>A. campestre</i>	24.10.2024	43.572500	28.068055	215	51

Europe, with no presence in North America, while *S. acerinum* is limited to North America and absent from Europe. Tomiczek et al. (2005) reported that *S. pyriforme*-induced dieback of branches and twigs can result in the mortality of young plants.

Massaria macra (Ascomycota, Massariaceae) occurs on *A. campestre* and *A. platanoides* throughout Europe, especially in Sweden, Norway, Finland, Estonia, Austria, Italy, Germany, and France. While *M. macra* is predominantly found on recently cut or broken branches lying on the ground, it has also been observed on branches still attached to the tree (Voglmayr & Jaklitsch, 2011).

This note aims to announce the first record of two ascomycetous fungi, *Stegonsporium pyriforme* and *Massaria macra*, which were found on *Acer* spp. planted in field protective forest belts in Northeastern Bulgaria.

Materials and methods

Between May and October 2024, we observed the health status of *Acer* species (360 trees, aged 21 to 71 years) in nine field protective forest belts within the territory of the State Hunting Enterprise (SHE) Balchik (Table 1). Subsequently, sample trees underwent evaluation for defoliation and damage extent, following the ICP 'Forests' methodology (Eichhorn et al., 2020).

The materials for fungal infection observations were collected from trees with necrosis and fruit bodies on the trunks and branches, and transported to

the Forest Research Institute in Sofia. Samples were surface-washed with tap water, sterilised for 1 min in 96% ethanol, and then rinsed once in sterile distilled water. Sterilised parts were placed on moistened filter paper in Petri dishes and incubated at 22±2 °C under artificial light.

The fungal species were identified using microscopic methods. Morphological characteristics of the fruit bodies were observed at 40× magnification with a Zeiss Stemi 305 stereomicroscope, fitted with a DinoEye AM-423X digital camera and an ocular micrometre. The shape and size of 100 asci and ascospores were measured using an OPTIKA Microscope B810, also equipped with a digital camera. The identification of *S. pyriforme* was based on the key by Voglmayr & Jaklitsch (2008), while *M. macra* was identified using the key by Voglmayr & Jaklitsch (2011).

Results

An extensive decline from sooty bark disease affected nine field protective forest belts in the SHE Balchik (Table 1) among *A. pseudoplatanus* (22 and 71 years old), *A. platanoides* (21–71 years old), and *A. campestre* (51 years old). The fungal pathogen *Cryptostroma corticale* infected all maple species, causing the death of all trees in Hadzhi Dimitar and Kremena villages (Table 2) and defoliation of 70%–100% (average 91.4%) in other locations. Sooty bark disease led to tree and branch death in all observed maple trees.

Table 2. Fungal pathogens identified in the sample plots in the State Hunting Enterprise Balchik.

N	Locality	Tree species	Fungal pathogen	Defoliation, %
1	Hadzhi Dimitar	<i>A. pseudoplatanus</i>	<i>C. corticale</i>	100
2	Kremena	<i>A. pseudoplatanus</i>	<i>C. corticale</i>	100
3	Seltse	<i>A. platanoides</i>	<i>C. corticale</i> , <i>S. pyriforme</i> , <i>M. macra</i>	70–100
4	Trigortsi	<i>A. platanoides</i>	<i>C. corticale</i> , <i>S. pyriforme</i> , <i>M. macra</i> , <i>Fusarium</i> sp.	90–100
5	Dropla	<i>A. platanoides</i>	<i>C. corticale</i> , <i>S. pyriforme</i>	80–100
6	Dropla	<i>A. platanoides</i>	<i>C. corticale</i> , <i>S. pyriforme</i>	90–100
7	Dropla	<i>A. platanoides</i>	<i>C. corticale</i> , <i>S. pyriforme</i> , <i>M. macra</i>	80–100
8	Dropla	<i>A. platanoides</i>	<i>C. corticale</i> , <i>S. pyriforme</i> , <i>M. macra</i>	80–100
9	Dropla	<i>A. campestre</i>	<i>C. corticale</i> , <i>S. pyriforme</i> , <i>M. macra</i>	70–100

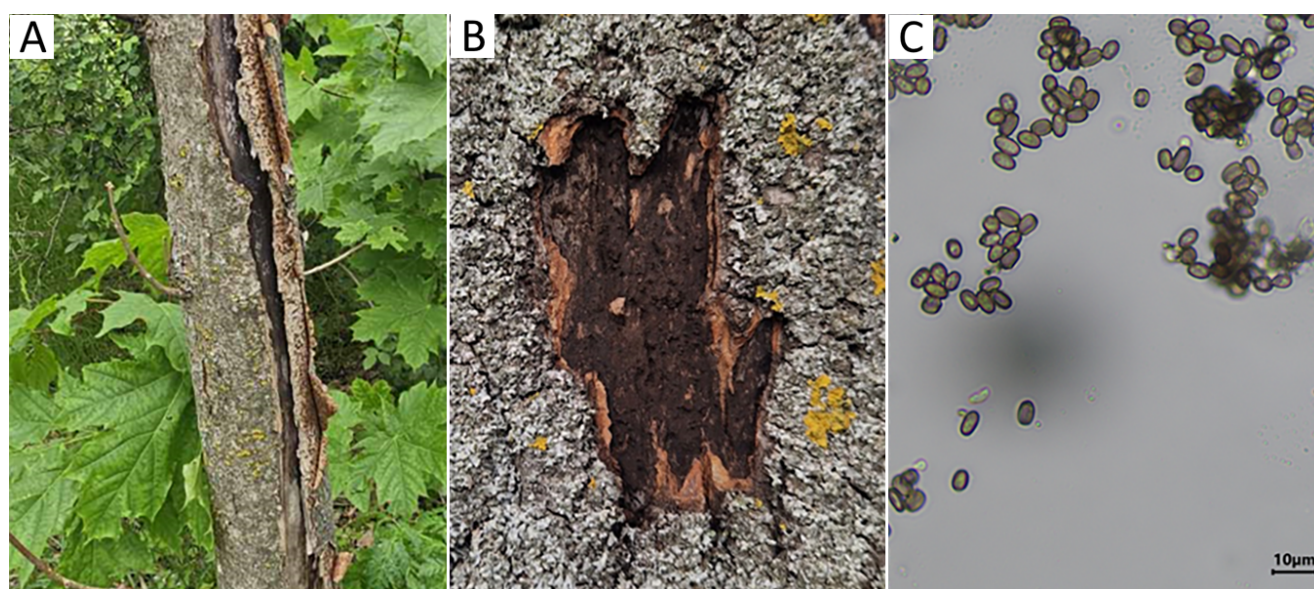


Fig. 1. *Cryptostroma corticale* infected trunk on *Acer pseudoplatanus* tree (Kremena Village, 7.06.2024): A) fungal growth beneath the bark; B) dense sooty fungal growth; C) conidia of fungus.

Symptoms of sooty bark disease appeared as stripes on infected parts, revealing black areas beneath the bark (Fig. 1A). The bark had long cracks and peeled easily, exposing dense masses of dark brown conidia (Fig. 1B). Ovoid, light to dark brown conidia ($4.5\text{--}6.5 \times 3.3\text{--}4.2 \mu\text{m}$) arose from the conidiomata (Fig. 1C).

Dead stems with lesions were found on infected *A. platanoides* trees in Seltse (Fig. 2A) and Trigortsi villages, and in five field protective belts in the vicinity of Dropla Village. The fruit bodies of ascomycetous fungus *Stegonsporium pyriforme* were

noticed on the stems killed by the fungus *C. corticale* (Fig. 2B). The findings revealed that the presence of *C. corticale* and *S. pyriforme* occurred significantly on dead trees. In Trigortsi Village, conidia of *Fusarium* sp. were observed together with conidia of *S. pyriforme*.

Stegonsporium pyriforme formed fruit body sized 1.1–3.2 mm ($n=30$), penetrated the bark from the inside, forming black spots of sporulation on the bark (Fig. 2C). A high percentage of infection was detected in the May 2024 in the field protective forest belts in Seltse, Trigortsi and Dropla villages. Orange, pear-

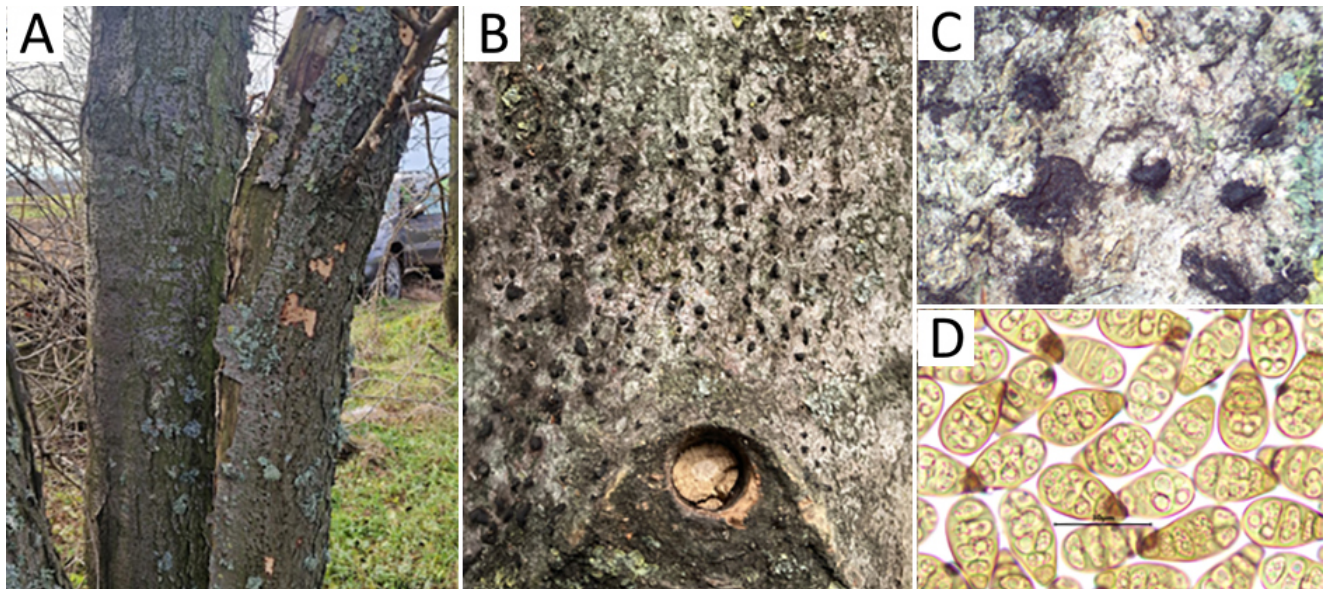


Fig. 2. *Acer platanoides* trees in the field protective forest belt (Seltse Village, 27.05.2024): A) Sooty bark disease; B, C) *Stegосporium pyriforme* fruit bodies; D) conidia.

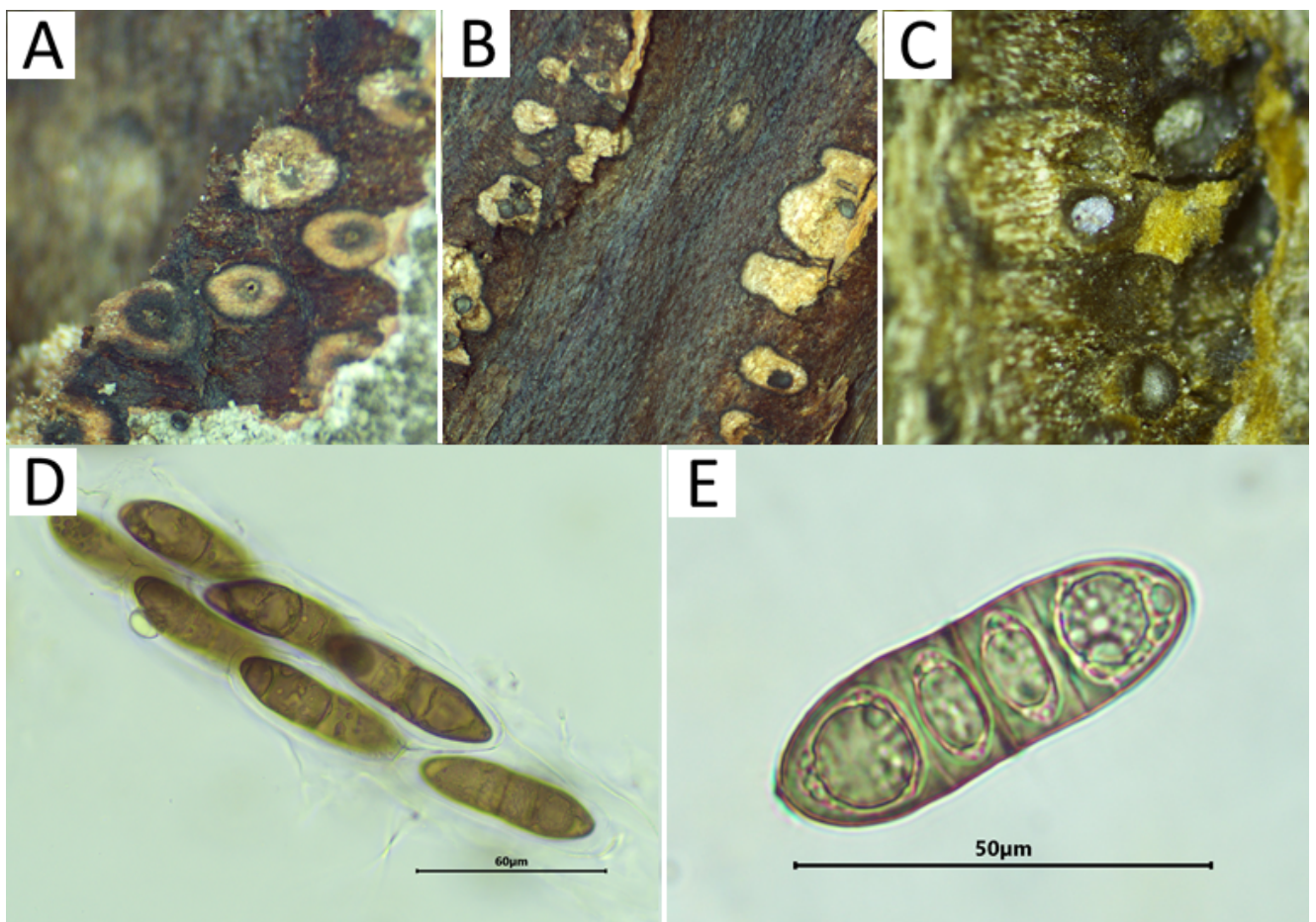


Fig. 3. *Massaria macra* on *Acer platanoides*: A) pseudostroma with perithecia; B–C) pseudothecia surrounded by pallid pseudostroma and black zone in transverse section surface of mycellium; D–E) ascospores.

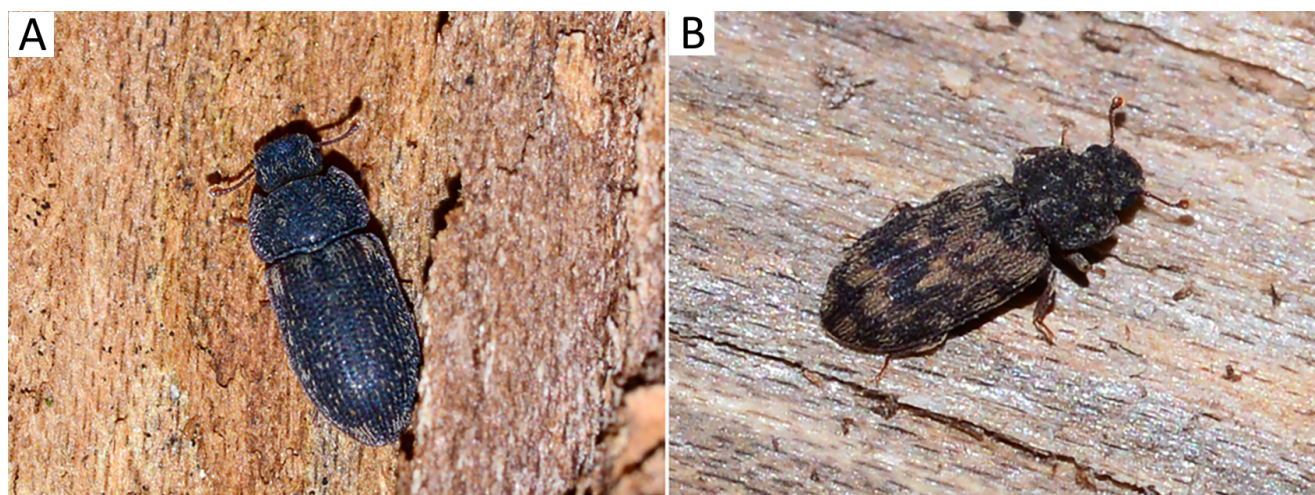


Fig. 4. Micetophagous insects found under the bark of dead maple trees: A) *Colobicus hirtus* on *Acer campestre* (Dropla Village, 22.11.2024); B) *Synchita undata* on *Acer platanoides* (Trigortsi Village, 29.10.2024).

shaped conidia measuring $28.5\text{--}48.3 \times 14.5\text{--}21.2\text{ }\mu\text{m}$ were formed inside the fruit bodies (Fig. 2D).

During the survey, fruit bodies of the ascomycete *Massaria macra* were observed on stems and branches of *A. platanoides* and *A. campestre* killed by the fungal pathogen *C. corticale* (Table 2). In areas where fruiting bodies developed, the wood exhibited yellow colouration (Fig. 4A–C). The oblong-cylindrical fungal asci, measuring $217.3\text{--}320.1 \times 33.5\text{--}48.5\text{ }\mu\text{m}$ ($n=100$), contained eight ascospores (Fig. 4D). These ascospores were elliptical, $49.09\text{--}70.7 \times 16.1\text{--}18.2\text{ }\mu\text{m}$ ($n=100$), and pale yellowish, becoming dark brown after separation from the asci. They possessed three septa with rounded terminal cells (Fig. 4E).

Two micetophagous coleopteran species were found under the bark of dead maple trees: *Colobicus hirtus* (Rossi, 1790) (Fig. 4A) and *Synchita undata* Guérin-Ménéville, 1844 (Coleoptera: Zopheridae) (Fig. 4B).

Discussion

Since *Cryptostroma corticale* was first reported in Bulgaria (Bencheva, 2014), it has spread throughout the country, causing damage to *A. pseudoplatanus* and *A. platanoides* trees mainly in urban green areas (Georgiev et al., 2017; Ivanov et al., 2026). It has also affected field protective forest belts in Northeastern Bulgaria (Georgieva et al. 2025). In the present study, for the first time, symptoms of the disease were

observed on *A. campestre* near Dropla Village. The intensive development of the pathogen in the branches and stems of infected trees was favoured by high temperatures and insufficient precipitation during recent growing seasons.

Stegonsporium pyriforme was confirmed for the first time on *A. platanoides* and *A. campestre* in field protective forest belts in Northeastern Bulgaria. This fungus is considered a weak pathogen of maple species (Voglmayr & Jaklitsch, 2008, 2014; Bußkamp et al., 2024) and typically attacks already weakened trees (Sinclair & Lyon, 2005). *Stegonsporium* species cause wilt symptoms, crown dieback, bark necrosis, and minor dieback of shoots, twigs, and branches (Voglmayr & Jaklitsch, 2008). Currently, the presence of *S. pyriforme* on dead trees in these forest belts in Northeastern Bulgaria is low.

The fungus *Massaria macra* was reported for the first time in Bulgaria in this study. It occurred on dead stems and branches of *A. platanoides* and *A. campestre* trees. Other representative species of the *Massaria* genus – *Massaria campestris* Voglmayr & Jaklitsch was recently recorded on *A. campestre* in Bulgaria (Sabovljević et al., 2022).

Colobicus hirtus and *Synchita undata* are relatively rare species in Bulgaria (Guéorguiev et al., 2026). For *C. hirtus*, records indicate its presence under the bark of dead *Quercus suber* L. trees in Southwestern Bulgaria (Bencheva & Doychev, 2022), as well as under the bark of *Tilia platyphyllos* Scopoli and *A. campestre* within field protective forest belts in South Dobrudzha (Guéorguiev et al., 2026). In

contrast, *S. undata* has been found under the bark of a dead *Pseudotsuga menziesii* tree in Belasitsa Mountain and under an *A. platanoides* tree in South Dobrudzha (Guéorguiev et al., 2026).

Conclusions

In the present study, the ascomycetous fungi *Stegonsporium pyriforme* and *Massaria macra* were recorded for the first time in Bulgaria. The appearance of weak pathogens *S. pyriforme* and *M. macra* on *Acer platanoides* and *A. campestre* in the field protective forest belts in northeastern Bulgaria is probably due to the severe dieback caused by the invasive pathogen *Cryptostroma corticale* and the physiological weakening of trees because of the long-term period of lack of precipitation during the last growing seasons.

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Conflicts of interest

No potential conflict of interest has been reported by the authors, reviewers, or subject editor.

Author’ contributions

(using [CrediT](#))

Veselin Ivanov: data curation, investigation, resources, visualisation, writing—original draft; Tsvetan Dimitrov: investigation, resources,

visualisation; Margarita Georgieva: data curation, investigation, methodology, resources, writing—original draft; Georgi Georgiev: data curation, investigation, methodology, resources, visualisation, writing—original draft; Denitsa Petrova: resources.

References

- Bencheva S. 2014 First report of *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller on *Acer platanoides* L. in Bulgaria. *Silva Balcanica* 15: 101–104.
https://silvabalcanica.wordpress.com/wp-content/uploads/2014/08/sb_152-2014_101-104.pdf
- Bencheva S., Doychev D. 2022 Distribution of *Biscogniauxia mediterranea* and its potential insect vectors on *Quercus suber* in Southwestern Bulgaria. *Silva Balcanica* 23 (1): 57–65.
<https://doi.org/10.3897/silvabalcanica.22.e89314>
- Bußkamp J., Bien S., Neumann L., Blumenstein K., Lange G.J. 2024 Endophytic community in juvenile *Acer pseudoplatanus* and pathogenicity of *Cryptostroma corticale* and other associated fungi under controlled conditions. *Journal of Plant Pathology* 106: 565–577.
<https://doi.org/10.1007/s42161-023-01575-y>
- Georgiev G., Georgieva M., Mirchev P., Zhiyanski M. 2017 Main insect pests and fungal pathogens on tree and shrub vegetation in urban ecosystems. Hlorind Ltd, Sofia, 54 pp.
- Georgieva M., Georgiev G., Mirchev P., Ivanov V., Hristova M., Belilov S., Dodev Y., Madzhov S., Georgieva L. 2024 Health status deterioration in field-protective forest belts in northeastern Bulgaria. *Silva Balcanica* 25 (3): 99–112.
<https://doi.org/10.3897/silvabalcanica.25.e139498>
- Georgieva M., Georgiev G., Ivanov V., Dodev Y., Belilov S., Kechev M., Mirchev P., Madzhov S., Georgieva L., Petrova D. 2025 Health status of *Acer* spp. in field protective forest belts in Southern Dobrudzha, Northeastern Bulgaria. In: *Proceeding 34 International Scientific Conference ‘Management and quality – Ecology and management of forest ecosystems’*, 5 June 2025, Sofia, Avangard Prima, 62–68.
- Guéorguiev B., Ivanov V., Georgieva M., Belilov S., Petrova D., Georgiev G. 2026 New records of

- arboreal beetles (Insecta: Coleoptera) from Bulgaria. *Historia naturalis bulgarica* 48 (1): 1–8.
<https://doi.org/10.48027/hnb.48.011> 
- Ivanov V., Georgieva M., Georgiev G., Hristova M., Georgieva L. 2026 Pests and pathogens threatening urban vegetation in ‘St. George’ City Park in Dobrich, northeastern Bulgaria. *Silva Balcanica* 27 (1) 21–36.
<https://doi.org/10.3897/silvabalcanica.27.e184700>
- Lorenc F. 2024 *Cryptostroma corticale* and its relationship to other pathogens and pests on *Acer pseudoplatanus*. *Journal of Forest Science* 70: 610–618.
<https://doi.org/10.17221/41/2024-JFS>
- Muller E., Dvořák M., Marçais B., Caeiro E., Clot B., Desprez-Loustau M.-L., Gedda B., Lundén K., Migliorini D., Oliver G., Ramos A.P., Rigling D., Rybníček O., Santini A., Schneider S., Stenlid J., Tedeschini E., Aguayo J., Gomez Gallego M. 2023 Conditions of emergence of the sooty bark disease and aerobiology of *Cryptostroma corticale* in Europe. In: Jactel H., Orazio C., Robinet C., Douma J.C., Santini A., Battisti A., Branco M., Seehausen L., Kenis M. (eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. *NeoBiota* 84: 319–347.
<https://doi.org/10.3897/neobiota.84.90549>
- Nageleisen L.-M. 1994 Le dépérissement Actuel De Feuillus divers: hêtre, merisier, alisier torminal, érable sycomore, peuplier, châtaignier, charme, aulne glutineux. *Revue Forestière Française* 554–562.
<https://doi.org/10.4267/2042/26583>
- Sabovljević M.S., Tomović G., Pantović J.P., Djurović S.Z., Buzurović U., Denchev T.T., Dencheva C.M., Boycheva P., Dimitrova T., Marković A., Sabovljević A.D., Ștefănuț S., Bîrsan C.C., Šabanović E., Djordjević V., Niketić M., Šovran S., Mašić E., Stoykov D., Papp B., Assyov B., Slavova M. 2022 New records and noteworthy data of plants, algae and fungi in SE Europe and adjacent regions. *Botanica Serbica* 46 (2): 311–320.
<https://doi.org/10.2298/BOTSERB2202311S>
- Voglmayr H., Jaklitsch W.M. 2008 *Prosthecium* species with *Stegonsporium* anamorphs on *Acer*. *Mycological Research* 112: 885–905.
<https://doi.org/10.1016/j.mycres.2008.01.020>
- Voglmayr H., Jaklitsch W.M. 2011 Molecular data reveal high host specificity in the phylogenetically isolated genus *Massaria* (Ascomycota, Massariaceae). *Fungal Diversity* 46: 133–170.
<https://doi.org/10.1007/s13225-010-0078-5>
