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A NEW RECORD OF *UROCYSTIS LEUCOJI* BUBÁK (UROCYSTIDALES) ON *GALANTHUS IKARIAE* SUBSP. *SNOGERUPII* KAMARI (AMARYLLIDACEAE) IN GREECE

Keywords: Biodiversity, snowdrop smut, *Urocystis galanthi*, snowdrop diseases, rare fungi

A NEW RECORD OF *UROCYSTIS LEUCOJI* BUBÁK (UROCYSTIDALES) ON *GALANTHUS IKARIAE* SUBSP. *SNOGERUPII* KAMARI (AMARYLLIDACEAE) IN GREECE. Ruslan I. Mishustin – A finding of the smut fungus *Urocystis leucoji* Bubák (Urocystidales, Ustilaginomycotina) in Greece is reported. The fungus was discovered on Skyros Island, on the leaves of *Galanthus ikariae* subsp. *snogerupii* Kamari. This represents the first documentation of the association between this host plant and the pathogen, and the first confirmed record of *U. leucoji* in Greece. The preliminary diagnosis was confirmed by morphological analysis of the teliospores. This finding substantially expands the knowledge regarding the distribution range and trophic relationships of *U. leucoji*.

НОВА ЗНАХІДКА *UROCYSTIS LEUCOJI* BUBÁK (UROCYSTIDALES) НА *GALANTHUS IKARIAE* SUBSP. *SNOGERUPII* KAMARI (AMARYLLIDACEAE) В ГРЕЦІЇ. Руслан І. Мішустін – Повідомляється про знахідку сажкового гриба *Urocystis leucoji* Bubák (Urocystidales, Ustilaginomycotina) у Греції. Гриб було виявлено на острові Скірос, на листках *Galanthus ikariae* subsp. *snogerupii* Kamari. Це перше документування зв'язку даної рослини-господаря і патогену та перше підтверджене виявлення *U. leucoji* у Греції. Попередній діагноз був підтверджений морфологічним аналізом теліоспор. Ця знахідка суттєво розширює уявлення про ареал та трофічні зв'язки *U. leucoji*.

Urocystis leucoji Bubák belongs to the large and taxonomically challenging genus *Urocystis* Rabenh. ex Fuckel within the phylum Basidiomycota. The genus *Urocystis* comprises about 175 species (Hyde et al., 2024), whose existence is closely associated with vascular plants.

Traditionally, the most extensively studied members of this genus are those of economic importance that affect crop yields. Such species include *Urocystis tritici* Koern. (the causal agent of stem smut of wheat) and *Urocystis cepulae* (the causal agent of onion smut). These species are of significant quarantine concern, and numerous studies have been devoted to their investigation, monitoring, and control measures (Chupp, 1960; Hoepting et al., 2004; Kashyap et al., 2020; Savchenko et al., 2011; Savchenko et al., 2017; Shivas et al., 2014).

Other species of the genus *Urocystis* have received considerably less attention. Many species share similar morphological features; therefore, the descriptions of new taxa were often based on the trophic pairs' "fungus–host plant". With the development of molecular research methods, several species were synonymized. Within such studies, J. Kruse conducted a comparative analysis of four DNA gene loci of *Urocystis galanthi* H. Pape and *Urocystis leucoji* Bubák, concluding that *U. galanthi* should be regarded as a synonym of *U. leucoji* (Kruse et al., 2017).

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Previously, *Galanthus nivalis* L. was considered the sole host plant of *U. galanthi*, whereas *Leucojum vernum* L. was associated with *U. leucoji*. Despite the wide distribution of these plants across Europe, *U. leucoji* remains rare and occurs locally, only in isolated populations of *G. nivalis* L. and *L. vernum* L. (Kruse et al., 2017; Thiel et al., 2023). Based on these data, *U. leucoji* has been classified as "very rare (sehr selten)" (Scholz & Scholz, 1988; Thiel et al., 2023).

Although the number of *Galanthus nivalis* populations infected by *Urocystis leucoji* is extremely low (potentially up to 24) (Denchev & Denchev, 2015), this fungal species should become a subject of monitoring for several reasons. The popularity of snowdrops as ornamental plants continues to grow every year, accompanied by active breeding efforts; hundreds of cultivars are either officially registered or have established names and are widely traded and exchanged among horticulturists (Cox, 2013, 2023; Repnow, 2024). Industrial propagation of several *Galanthus* species is currently underway, including numerous cultivars derived from *G. nivalis*. The reproduction and distribution of infected material may have economic consequences and pose a potential threat to nearby wild populations of snowdrops. All species of the genus *Galanthus* are considered vulnerable and possess some form of conservation status (Davis, 1999); thus, every natural population represents a valuable genetic and ecological resource requiring the highest level of protection.

Among European countries, Greece is the leader in the number of *Galanthus* species and subspecies. The taxonomic status of certain Greek snowdrop species remains debatable and requires further clarification, while the occurrence of some taxa within the country (for example, *G. nivalis*) has been questioned (Davis, 1999; Kamari, 1982; Tan et al., 2014). Overall, Greece is one of the most floristically diverse regions in Europe, harboring 5,752 species of vascular plants (Dimopoulos et al., 2013; Denchev & Denchev, 2016). At the same time, only 66 species of smut fungi (Ustilaginomycetes) parasitizing 77 host plant species have been reported from Greece (Denchev & Denchev, 2016), suggesting that this group remains poorly studied and incompletely documented in this highly distinctive biogeographical region.

Surveys of populations of various *Galanthus* L. species were conducted as part of studies on the trophic relationships between insects and plants of the genus *Galanthus* (Andrić et al., 2025; Popov & Mishustin, 2019; Popov et al., 2025). During investigations of the *Galanthus ikariae* subsp. *snogerupii* Kamari population on the island of Skyros, leaf infections caused by smut fungi (Ustilaginomycetes) were detected. In this regard, a decision was made to collect the specimens and carry out their examination.

Based on our research and consultations with specialised experts, we concluded that the collected fungal specimens belong to *Urocystis leucoji* Bubák. This species is reported here as new to the Mycota of Greece. Its description and ecological characteristics are presented below.

Materials and Methods

Macrophotography of leaves bearing sori was conducted in situ within the population (fig. 1) using a Nikon D5500 camera equipped with a Nikon DX 40 mm f/2.8G AF-S Micro-Nikkor lens.

Most samples were collected from leaves damaged by the passage of goat herds that repeatedly traversed the area occupied by this snowdrop population. No additional damage was inflicted on the plants′ leaf fragments containing sori (1.5–2 cm portions of the leaf blade) were placed in 15 ml plastic Falcon™ tubes filled with 96% ethanol. Each sample was assigned an identification code consisting of the collection year and the sequential number of the corresponding specimen photograph, e.g., MR-20-0965 (fig. 4: A). In cases where a specimen was photographed multiple times, the number of the first photograph was used. A total of 12 specimens were collected.

Permanent microscope slides were prepared using Aman′s basic solution (lactophenol) following De Kesel et al. (2020) and Rossi & Santamaria (2015). Nitrocellulose varnish was used to seal the slides. Each slide was labelled with an identification code derived from the sample number (a fragment of the leaf blade with sori) and the sequential number of the slide prepared from that sample, e.g., MR-20-0965-2 (fig. 4: B). Photographic documentation was performed using a MICROMED-2 microscope equipped with a Levenhuk C510 NG camera.

After two years of storage, the leaf fragments were removed from ethanol and air-dried for herbarium preservation. The specimens are deposited in the author′s personal collection.

Figure 1. Forest (*Acer sempervirens* L.) with a population of snowdrop *Galanthus ikariae* subsp. *snogerupii* on Skyros Island (Greece).

Рисунок 1. Ліс (*Acer sempervirens* L.) з популяцією підсніжників *Galanthus ikariae* subsp. *snogerupii* на острові Скірос (Греція).

Results and Discussion

Urocystis leucoji Bubák, *Annales Mycologici* **2**(5): 431 (1904).

= *Urocystis galanthi* Ellis & Everh.

Classification: Urocystidaceae, Urocystidales, Ustilaginomycotina, Basidiomycota, Fungi.

Type host: *Leucojum vernum* L.

New locality: Greece, Skyros Island, on leaves of *Galanthus ikariae* subsp. *snogerupii* Kamari.

Sori. In the areas of sorus formation, a distinct thickening of the leaf blade can be observed. Young sori occurring on etiolated portions of leaf blades that have recently emerged above the soil surface are yellowish-white and gradually darken during fungal development (fig. 2: A, B). Sori on photosynthetic leaves are oval and markedly elongated (3–14 × 1.5–6 mm), occasionally confluent. Their colouration is dark grey with a distinct pale-yellow margin. Sori located at the apical parts of the leaves are generally larger and more voluminous.

Numerous small sori were observed on the sheathing leaves (figs. 3: C, D; 4: D). They are markedly smaller, appearing as dark, minute tubercles 0.2–0.3 mm in diameter, which frequently coalesce into continuous strips along the upper margin of the sheathing leaf or form elongated zones up to 25 mm long along the leaf veins. Infection of the peduncle and bracts was rare and isolated. No sori were detected on the ovary or other floral parts.

Teliospores. Teliospores are brown to reddish-brown, measuring 18–22 × 12–15 µm; sterile cells surrounding them form aggregates 30–50 µm in diameter (fig. 4: B, D). Teliospores from sori of both leaf and sheathing blades were morphologically identical (fig. 4: B, D).

The morphological characteristics of the sori and teliospores, as well as their distribution on the host plant, correspond fully to the description of *Urocystis leucoji* Bubák (Kruse et al., 2017).

Despite the presence of numerous sori on the leaves (up to 20–25 per single leaf), the infected individuals did not exhibit any signs of depression or reduction in vigour and showed no apparent differences in size compared with the adjacent, externally healthy plants.

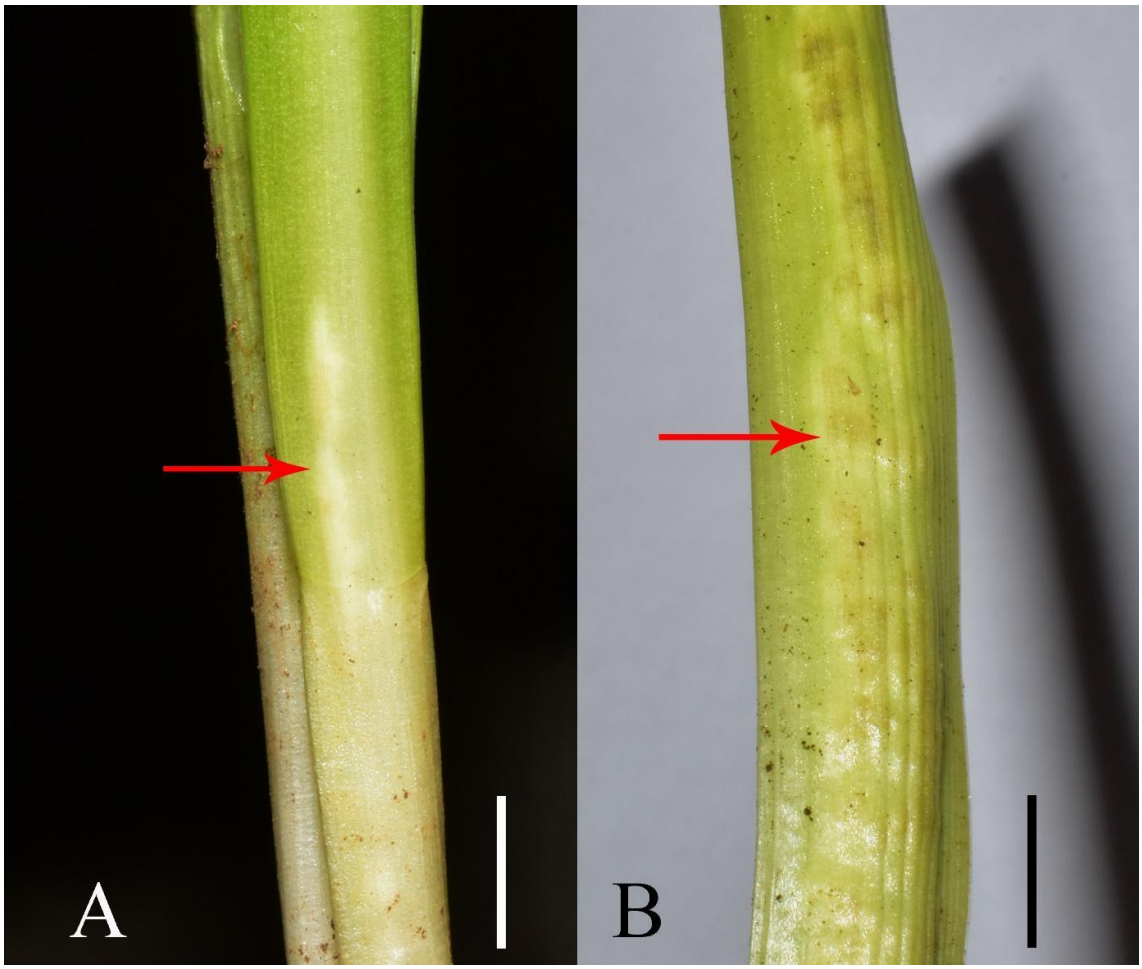

Figure 2. *Urocystis leucoji*: A, B – juvenile sori on leaves of *Galanthus ikariae* subsp. *snogerupii*. Scale bars: A, B – 5 mm.

Рисунок 2. *Urocystis leucoji*: A, B – молоді соруси на листі *Galanthus ikariae* subsp. *snogerupii*. Масштабні лінійки: A, B – 5 мм.

Location: Greece, Skyros Island, stream valley in the central part of the island, natural forest dominated by *Acer sempervirens* L., on *Galanthus ikariae* subsp. *snogerupii* Kamari, 38.815161° N, 24.643178° E, 9 February 2020 (fig. 1).

Of the three known populations of *Galanthus ikariae* subsp. *snogerupii* on Skyros Island, only one was surveyed. Within this population, infection of *G. ikariae* subsp. *snogerupii* individuals was extensive (10–12%). Considering that in many plants the sori were restricted to the sheathing leaves (fig. 3: C, D), the actual proportion of infected individuals was probably considerably higher.

The population is confined to a mountain depression within a stream valley. The lower slope and the bottom of the hollow are covered by natural forest dominated by *Acer sempervirens* L. The herbaceous layer in early February is composed primarily of *Erodium* sp., *Galium* sp., *Galanthus ikariae* subsp. *snogerupii*, *Stellaria* sp., and *Urtica* sp.

Three species of fungi belonging to the genus *Urocystis* are associated with hosts of the family Amaryllidaceae – *Urocystis cepulae* Frost, *U. leucoji* Bubák, and *U. narcissi* (Gonz. Frag.) Vánky.

Among these, the best known is *Urocystis cepulae* Frost, the causal agent of the economically important onion disease "onion smut". This species parasitises members of the subfamily Allioideae, particularly *Allium cepa* L., *A. porrum* L., *A. sativum* L., and *A. vineale* L., and is especially harmful to *A. cepa*. The fungus often causes severe plant mortality and complete crop loss (Mulder & Holliday, 1971).

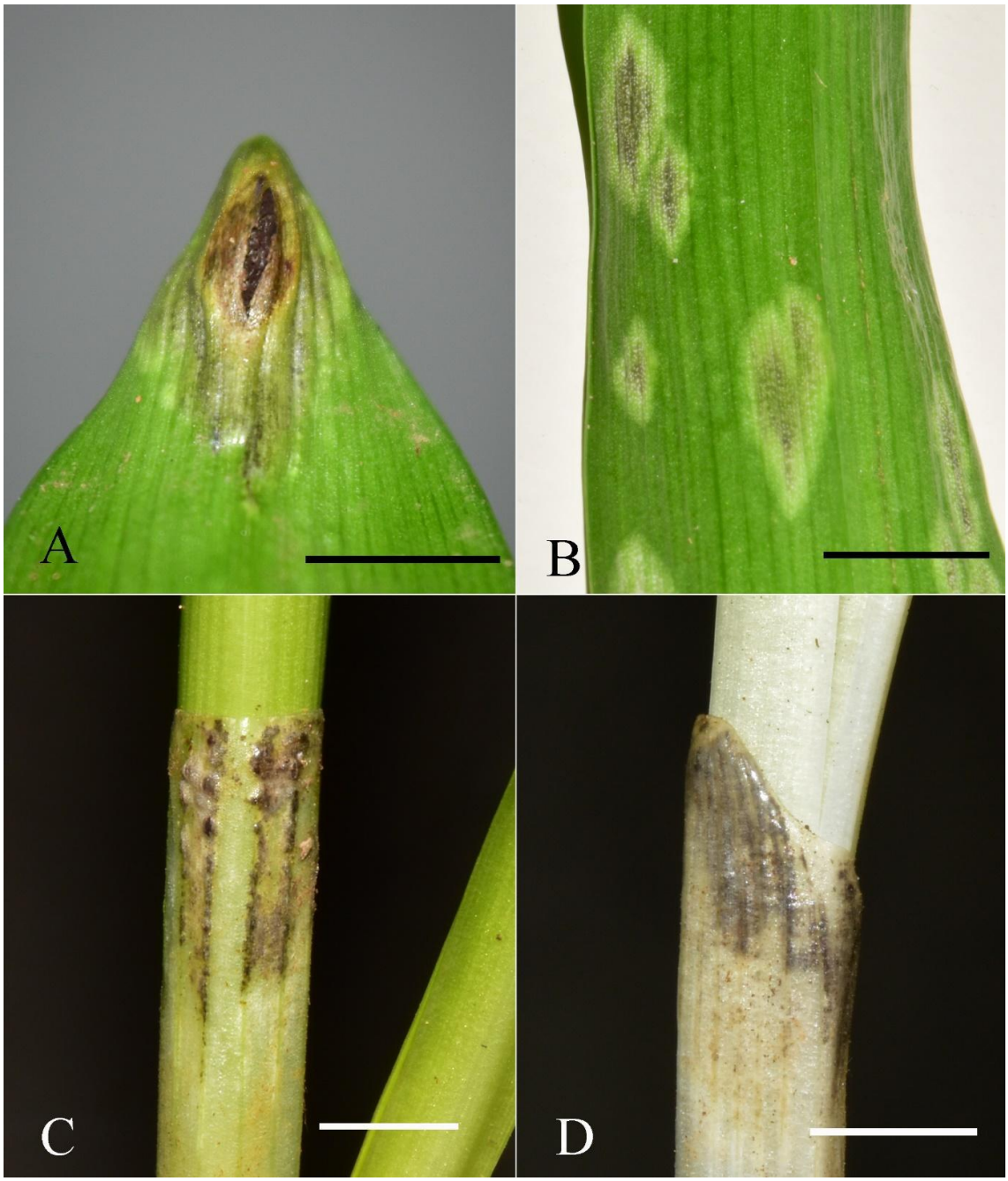

Figure 3. Sori of *Urocystis leucoji* on leaves of *Galanthus ikariae* subsp. *snogerupii*: A, B – sori on leaf blades, specimens: A – MR-20-0477 and B – MR-20-0618; C, D – sori on sheathing leaves, only photodocumentation was performed without collecting these specimens. Scale bars: A–D – 5 mm.

Рисунок 3. Соруси *Urocystis leucoji* на листі *Galanthus ikariae* subsp. *snogerupii*: A, B – соруси на листкових пластинах, зразки: A – MR-20-0477 і B – MR-20-0618; C, D – соруси на піхвових листках, було проведено лише фотодокументування без збору цих зразків. Масштабні лінійки: A–D – 5 мм.

Urocystis narcissi (Gonz. Frag.) Vánky is a much less well-known species. It was first discovered in Spain on the wild daffodil *Narcissus pallidulus* Graells and originally described as *Urocystis colchici* f. *narcissi* (González Fragoso, 1914). Following further examination of related material, Kálmán Vánky re-described it as an independent species (Vánky, 1993). In 2015, *U. narcissi* was reported from Pakistan on *Narcissus tazetta* L. in two districts (Shivas et al., 2017). Unfortunately, the authors did not specify whether these records originated from naturalised populations or cultivated plants.

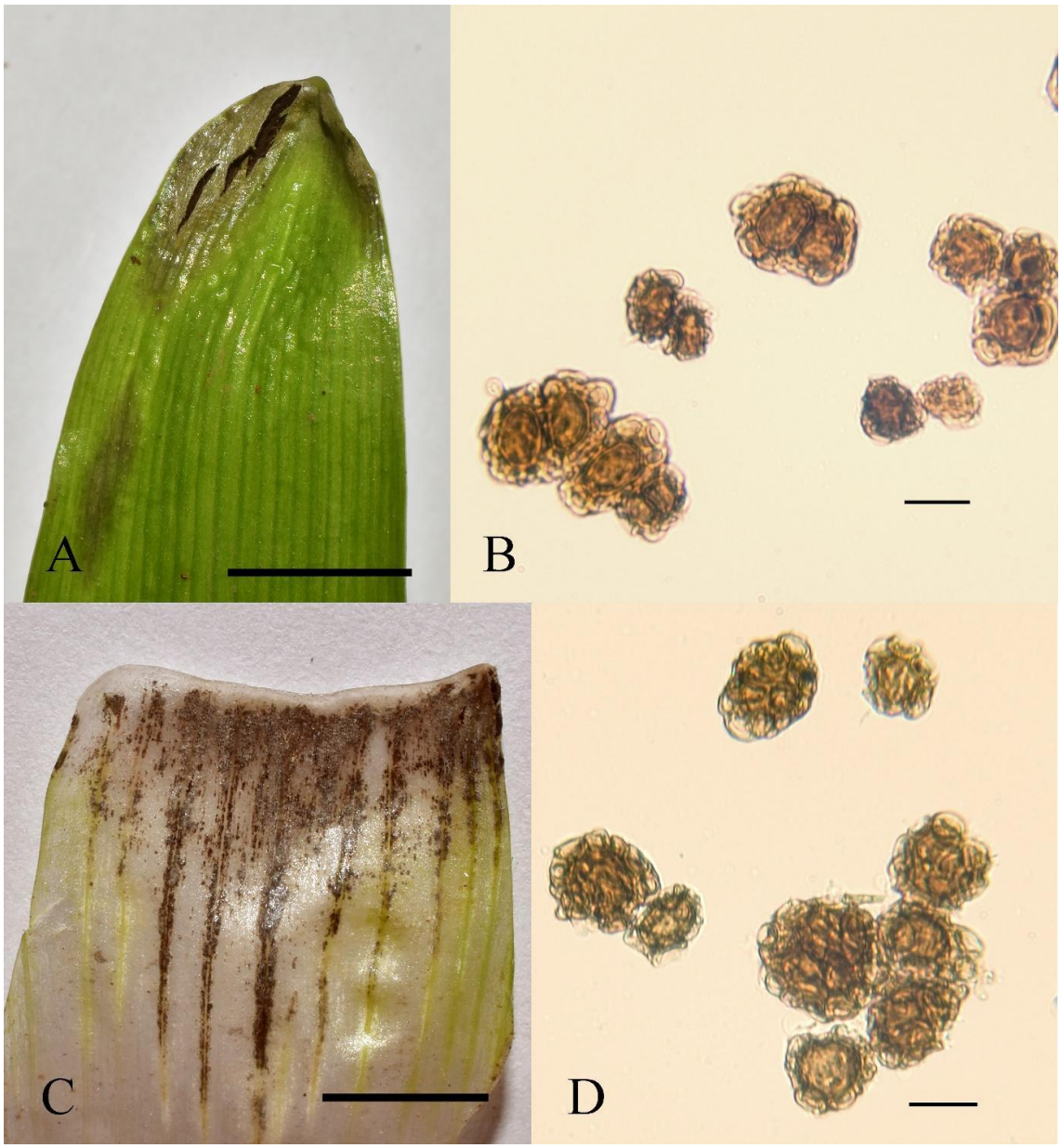

Figure 4. *Urocystis leucoji*: A, C – sori on leaves of *Galanthus ikariae* subsp. *snogerupii*, specimens: A – MR-20-0965 and C – MR-20-0962; B, D – teliospores., specimens: B – MR-20-0965-2 and D – MR-20-0962-1. Scale bars: A, C – 5 mm; B, D – 20 µm.

Рисунок 4. *Urocystis leucoji*: A, C – соруси на листі *Galanthus ikariae* subsp. *snogerupii*, зразки: A – MR-20-0965 і C – MR-20-0962; B, D – теліоспори, зразки: B – MR-20-0965-2 та D – MR-20-0962-1. Масштабні лінійки: A, C – 5 мм; B, D – 20 мкм.

To date, *Urocystis leucoji* has been recorded from Austria, Germany, Slovakia, Romania, the Czech Republic (?[1]), and Switzerland (Vánky, 1985; Scholz & Scholz, 1988; Zwetko & Blanz, 2004; Thiel et al., 2023). In all these countries, the species is considered rare, and its populations are extremely scarce (Denchev & Denchev, 2015; Scholz & Scholz, 1988; Thiel et al., 2023).

Scholz & Scholz (1988) expressed the opinion that "Germany should probably bear a greater responsibility for the conservation of this species". In 1996, Foitzik proposed to include *U. leucoji* in the Red List of Phytoparasitic Fungi of Germany (Rote Liste gefährdeter Pflanzen Deutschlands) as a rare species. Subsequently, it was listed in the updated Red List and General Species List of

[1] The species was originally reported from the territory of Czechoslovakia. Reliable records are available for Slovakia. The records from the Czech Republic require confirmation.

Phytoparasitic Micromycetes of Germany (Rote Liste und Gesamtartenliste der phytoparasitischen Kleinpilze Deutschlands), where it was assigned the conservation status "very rare" (sehr selten) (Thiel et al., 2023). In 2015, *U. leucoji* was included in the Global Fungal Red List Initiative (as *U. galanthi*) with the status VU C2a(i) – vulnerable species (Denchev & Denchev, 2015).

It has been noted that this species is considered vulnerable, primarily due to its close association with *Galanthus nivalis*, which is included in most European conservation lists, including the IUCN Red List of Threatened Species, where it is currently classified as Near Threatened (NT), with the possibility of an upgrade to Vulnerable (VU A3cd) in the near future (Crook & Davis, 2013). Secondly, only 12 confirmed populations of *Urocystis leucoji* (= *U. galanthi*) are known within the distribution range of *G. nivalis*, and such a small number itself highlights the need for additional conservation measures for the species. The authors of the Global Fungal Red List assessment expressed doubts that the number of populations will increase substantially with further research. The potential number of subpopulations is estimated at 24–30. At the same time, the limiting factors constraining the spread of this fungus within the range of its host species remain unknown.

Since *Urocystis leucoji* has been demonstrated to infect representatives of at least two genera of the family Amaryllidaceae J. St.-Hil. – namely *Leucojum* and *Galanthus* (Kruse et al., 2017) – the discovery of this fungus on additional European species of *Galanthus* beyond *G. nivalis* is not unexpected. At least four species of the genus *Galanthus* occur in Greece: *G. graecus* Boiss. ex Orph., *G. ikariae* Baker, *G. samothracicus* Kit Tan & Biel, and *G. reginae-olgae* Orph. (Davis, 1999; Kamari, 1982; Tan et al., 2014).

The distribution of *G. nivalis* L. in Greece remains uncertain. Some southeastern populations that were formerly referred to *G. nivalis* are now assigned to the recently described *G. samothracicus* (Tan et al., 2014), whereas populations from northern Greece (Pindus Mts.) are morphologically and ecologically more similar to *G. subalpinus* A. P. Davis & I. McEnery than to *G. nivalis* (Zubov, D., pers. comm., unpublished data).

Urocystis leucoji was recorded by the author only in a population of *Galanthus ikariae* subsp. *snogerupii* Kamari on Skyros Island. Other Greek snowdrop populations were also surveyed, both by the author and by volunteers. Examination of *G. ikariae* subsp. *snogerupii* populations on Andros Island revealed no infected plants. Three populations of the same taxon were also studied on Naxos Island, but *U. leucoji* was not detected there either. Likewise, none of the five examined populations of *G. ikariae* subsp. *ikariae* on Ikaria Island showed any infection. Similarly, two populations of *G. samothracicus* on Samothrace Island and two additional populations from the Halkidiki Peninsula (vicinity of Plana settlement) showed no evidence of infection. Surveys of *G. graecus* populations in eastern Greece, south of the Aisymi settlement, as well as two populations on Samos Island, also yielded no positive results.

Although these surveys covered only a fraction of the Greek populations of *Galanthus*, the preliminary observations suggest that *Urocystis leucoji* is not a widespread species within the country. The same pattern is evident across other European regions: despite the abundance of potential host populations, *U. leucoji* occurs only in a very small proportion of them.

Conclusions

Urocystis leucoji is reported here as a new species for the Mycota of Greece. Although this smut fungus is rare, its discovery enhances the conservation significance of the *Galanthus ikariae* subsp. *snogerupii* Kamari population on Skyros Island. At the same time, the occurrence of *U. leucoji* on a previously unrecorded host species of *Galanthus*, under environmental conditions that differ substantially from those documented for its Central European localities, suggests that the fungus possesses a greater degree of ecological plasticity than previously assumed.

Further field surveys of wild *Galanthus* populations across Europe are needed to detect additional natural occurrences of *U. leucoji* and to enable continued monitoring of its distribution beyond currently known localities.

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