Uromyces plumbarius **first reported on** ***Oenothera lindheimeri*** **in Europe with widespread distribution and association with the rust hyperparasite** ***Sphaerellopsis*** **sp.**

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Summary

The rust fungus *Uromyces plumbarius* Peck was reported in Europe for the first time on *Oenothera lindheimeri* (Engelm. & A. Gray) W.L. Wagner & Hoch (Lindheimer's beeblossom), a highly popular ornamental plant widely cultivated since the 2000s. The fungus was found in 11 locations across France and two locations in the Netherlands. The identification was based on an analysis of morphological characteristics and ITS rDNA sequences. The pathogenicity was confirmed through reinoculation using fresh urediniospores. This represents the first record of *U. plumbarius* outside its native range in North America. Its widespread distribution in France appears to result from rapid dissemination, likely facilitated by the plant trade between nursery gardeners. The discovery of *U. plumbarius* was accompanied by the detection of the hyperparasitic fungus *Sphaerellopsis* sp. (teleomorph *Eudarluca* sp.), which may play a role in limiting the impact of rust infections.

Main text

Oenothera lindheimeri (Engelm. & A. Gray) W.L. Wagner & Hoch (syn. *Gaura lindheimeri*), commonly known as Lindheimer's beeblossom or Butterfly bush (Wagner et al., 2007), is a nearly shrubby herbaceous perennial plant in the family Onagraceae, growing to 70–150 cm tall, with densely clustered branched stems. The leaves are lanceolate, finely hairy, and the stems produce clusters of small flowers opening sequentially along the open terminal panicles. *O. lindheimeri* is native to southern Louisiana and Texas (USA), where it grows as a perennial plant. In Europe, it is generally treated as a perennial, but in other regions it may also be grown as an annual ornamental plant. It has been intensively propagated by nursery gardeners across many countries, particularly in Europe, including France and its neighboring countries such as Spain, Portugal, Italy, Greece, the Netherlands, and the United Kingdom. This popular ornamental plant is admired for its graceful, airy form, prolonged blooming period, and resilience to drought conditions. Several cultivars have been selected for varying flower colour, from nearly pure white to darker pink. Since the 2000s *O. lindheimeri* is frequently offered for sale in many garden centers and its presence has increased significantly in urban areas, public parks and private gardens. More recently, *O. lindheimeri* has become a common garden escape in urban areas across Europe, on waste

ground or levelled soil, often in places where garden waste has been deposited (GBIF.org, 2025). In the Netherlands, for instance, it has been observed in dry grasslands within the Vlissingen port area near the railway station, where it persists in small number since its initial discovery in 2009 (Rostański & Verloove, 2015). *O. lindheimeri* was also introduced in Australia some years ago and was declared an emerging, noxious weed after it escaped from cultivation.

A total of 18 samples of *O. lindheimeri* exhibiting rust were collected between 2020 and 2024 from different locations in France and in the Netherlands (Table 1; Figure 1 and 2). The infected leaves displayed uredinia on their abaxial surfaces, with several samples also showing reddish spots on the adaxial sides. Most of the uredinia, 1-2 mm in diameter, were amphigenous, scattered, at first covered by the epidermis and of a peculiar shining leaden hue, sometimes turning a darker metallic blackish brown (Figure 3). These observations align with Peck's (1879) description of the “beautiful metallic hue of the epidermis covering the uredinia”. On young leaves—at the end of autumn on newly formed stems near the ground—uredinia initially appear as small orange blisters, 0.5–1 mm in diameter, that turn light brown and quickly rupture, exposing a mass of powdery urediniospores while remnants of the epidermis remain visible along the edges (Figure 3). On older leaves, the uredinia transition to a red-brown color and are surrounded by a reddish halo caused by the pigmentation of the plant cells. A few uredinia also develop on the stems, accompanied by reddish spots.

Urediniospores, present in all of the samples, are globose to slightly elongated, finely echinulate, and measure 19–24 µm by 16–19 µm on average (Table 2). Their walls are 1.5–2.0 µm thick, and they possess a short pedicel that is often absent, with germ pores arranged supraequatorially (Figure 4). In some samples, a mixture of hyaline immature and darker mature urediniospores was observed.

Teliospores were found in the first sample from Les Clayes-sous-Bois in mid-November 2020 (from *O. lindheimeri*, which was mistakenly named *Oenothera biennis* in Suffert & Suffert, 2021), in Lunéville in mid-October 2021, and in Wageningen in mid-November 2021. Teliospores are elliptical, inconspicuously echinulate, and measure 24-28 µm x 17-22 µm on average (Table 2). Their walls are 1.0-1.5 µm thick, and they possess a long (40-45 µm) pedicel and a thickened (4-6 µm) cell wall at the apex, with germ pores arranged mostly equatorially (Figure 4).

This description of symptoms and spore morphology aligns with previous reports on *U. plumbarius* (Peck, 1879; Saccardo, 1888; Sydow & Sydow, 1904; Bisby, 1916; Arthur & Cummins, 1962; Blomquist et al., 2015). Given the current state of knowledge, since the aecial form has never been observed on *Oenothera* sp. or any other host, *U. plumbarius* should be considered an autoecious hemicyclic rust. In our samples, uredinia and telia were indistinguishable, leaving it uncertain whether urediniospores and teliospores are produced within the same uredinia, possibly at different times. This question has not been explored in previous studies, and in retrospect, it remains unclear whether the uredinia described by Peck (1879) were considered telia, uredinia, or both.

A leaf specimen collected in Clayes-sous-Bois (26-09-2021), exhibiting uredinia, was deposited in the fungal herbarium of the Muséum National d’Histoire Naturelle (Paris,

France) under voucher number PC0798793. From this leaf, two samples were taken: one consisting of the entire content of an excised uredinium and the other containing only a few spores from a nearby uredinium. The DNA of both samples was extracted at INRAE BIOGER (Palaiseau) using the DNeasy Plant Mini Kit, following the manufacturer's instructions (Qiagen). The rust fungus was identified using two pairs of rust-specific primers as follows, confirming it as *U. plumbarius* Peck. A region of the internal transcribed spacer region 1 (ITS-1) was amplified with primers ITS1rustF10d (5′-TGAACCTGCAGAAGGATCATTA-3′; Barnes & Szabo, 2007) and ITS1rustR3c (5′-TGAGAGCCTAGAGATCCATTGTTA-3′; Barnes & Szabo, 2007). A region of the ribosomal repeat spanning the 5.8S subunit, the internal transcribed spacer region 2 (ITS2), and the large subunit (28S), was amplified with primer Rust2inv (5′-GATGAAGAACACAGTGAAA-3′; Aime, 2006) and LR6 (5′-CGCCAGTTCTGCTTACC-3′; Vilgalys & Hester, 1990). PCR were performed in a 25 µl reaction volume containing DNA from each sample, 0.5 µM of each primer, 1.5 mM MgCl2, 0.2 mM of each dNTP, buffer 1X and 2 U of GoTaq G2 Flexi DNA polymerase (PROMEGA, USA). Thermocycling parameters were set as follows: 4 min at 95°C followed by 35 cycles of 30 sec at 95°C, 30 sec annealing at 56°C, 1 min at 72°C, and a final extension step of 5 min à 72°C. PCR products were visualized on a 1 % agarose gel. Successfully amplified nuclear rDNA fragments obtained were forward and reverse sequenced (Sanger Sequencing Service, Eurofins, France) using each PCR primer pairs. Forward and reverse sequences were assembled using Unipro UGENE software v51.0.0.0, followed by alignment and trimming. Sequence alignments of each ITS1 and 5.8S-ITS2-28S region showed 100% identity between the two samples, which was expected due to their close origin and the clonal reproduction typical of rust fungi. A consensus sequence was subsequently generated for each region and compared using the Basic Local Alignment Search Tool (BLASTN) to find similarities in GenBank (http://www.ncbi.nlm.nih.gov/). No match was found for the 168-bp ITS1 sequence, deposited in GenBank under accession no. PV151527 (Supplementary Material 1A) due to the absence of a reference sequence. The 1097-bp 5.8S-ITS2-28S sequence (PV151527; Supplementary Material 1B), was found to share 100% identity with two sequences from samples of *U. plumbarius* colllected from *O. lindheimeri* in the USA: one collected in Louisiana (JQ312670; Kaur et al., 2012) and the other in California (KP313731; Blomquist et al., 2016). The sequence also exhibited a close relationship, with 99.8% identity to sequence GU058019, from a rust fungus sample found on *Oenothera fruticosa*, which was identified by Dixon et al. (2010) as *Puccinia dioicae*. Although *Puccinia* and *Uromyces* have been shown to be highly polyphyletic and to have evolved in close association (Maier et al., 2007; van der Merwe et al., 2007), it remains possible that this is a case of morphological misidentification, perpetuated by Kaur et al. (2012). On one hand, *P. dioicae*, known to be a heteroecious rust species with Asteraceae as aecial hosts and Cyperaceae as uredinial/telial hosts, has never been reported on Onagraceae apart from this single reference. On the other hand, the current French specimen of *U. plumbarius* shares a lower sequence identity with the sequences of other specimens of *P. dioicae* available (from 94.0% for EF635897 to 98.4% for MK518754). It is worth noting that the use of the fungal-specific primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3'; Gardes and Bruns, 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White et al., 1990) on DNA extracted from the same two samples was unsuccessful. Moreover, amplifications performed on extracts from other leaf fragments covered with several uredinia (a few mm²) using the primer pairs Rust2inv / LR6 and ITS1rustF10d / ITS1rustR3c also failed, likely due to the presence of PCR inhibitors.

Pathogenicity tests were conducted at INRAE IAM (Champenoux) by inoculating detached *O. lindheimeri* leaves with a fresh urediniospore suspension derived from a symptomatic potted plant purchased in 2021 at the Nancy garden center (Table 1), following an inoculation protocol routinely used for poplar rusts and other rust fungi (Gérard et al., 2006). A 10-µl droplet of water agar (0.1 $g.l^{-1}$) was deposited onto each *U. plumbarius* uredinium with a micropipette and urediniospores were dispersed within the droplet with the micropipette. The resulting urediniospore suspension was applied as 1-µl droplets on the abaxial surface of fresh leaves of *O. lindheimeri*. The inoculated leaves were incubated on wet filter paper in 24 x 24 cm Petri dishes, at 19±1°C under continuous LED illumination (50 $mmol.s^{-1}.m^{-2}$) in an incubation room. New uredinia appeared between 12 and 20 days post-inoculation.

The hyperparasite of rust fungi, *Sphaerellopsis* sp. (teleomorph *Eudarluca* sp.), was found in two *U. plumbarius* samples: one associated with uredinia and telia collected from Wageningen (06-11-2021) and the other associated with uredinia from Les Clayes-sous-Bois (27-11-2021) (Figure 5). The conidiomata of *Sphaerellopsis* sp., which pierced the uredinia, were globose, measuring 150–250 µm in diameter, dark brown, and featured a central ostiole exuding numerous conidia aggregated into a white, filamentous cirrhus of varying thickness. The conidia were hyaline, 1-septate, fusiform, measuring 13.5–17.5 µm × 3.5–4.5 µm, slightly constricted at the septa, and possessed funnel-shaped mucoid appendages at both ends. These observations align with the description by Trakunyingcharoen et al. (2014) and one might assume it to be *S. filum* (teleomorph *E. caricis*), previously recorded in association with 369 rust species from 30 genera across more than 50 countries (Kranz & Brandenburger, 1981). However, without molecular analysis, it was not possible to determine the species given the recently highlighted diversity within the genus *Sphaerellopsis* (Gómez-Zapata et al., 2024). To our knowledge, this is the first report of *Sphaerellopsis* sp. infecting *U. plumbarius*.

This report documents the presence of *U. plumbarius* across a dozen locations, spanning more than 1,500 km between the most distant sites, with symptomatic plants consistently observed. To our knowledge, *U. plumbarius* has not been previously recorded outside the USA and Mexico (GBIF.org, 2024), making this its first report in Europe. Furthermore, this constitutes the first detection of a rust fungus on the genus *Oenothera* in Europe. These preliminary data suggest that *U. plumbarius* might be widely distributed across Western Europe, wherever *O. lindheimeri* is cultivated, therefore considered newly endemic to the area.

It can be assumed that the pathogen was introduced from North America with one of its host plants—most likely *O. lindheimeri*—and subsequently spread through the propagation and distribution of plants by gardener nurseries and garden centers. This hypothesis of a recent introduction aligns with the fact that no rust species has yet been described on this genus in Europe (Klenke & Scholler, 2015). According to Wagner et al. (2007) the centre of diversity of Onagraceae is in southwestern North America, and the family is now widely distributed in temperate to subtropical regions of the Americas (Rostański et al., 1994; Rostański & Verloove, 2015). Several *Oenothera* species have become naturalized nearly worldwide, most likely in post-Columbian times. Many of these species are now found in environments heavily disturbed by human activity, such as railway grounds, gravel pits, dredge dumping areas, sand and mineral depots, and ruderalized sand dunes. Whether several species are

endemic to or originated in Europe is still debated. The consensus, however, is that *Oenothera biennis* has been naturalized in Europe, particularly in France, since the late 18th century. Many other taxa are considered either newcomers that arrived during the 19th century or neotaxa that evolved from the original species.

The synthesis of *U. plumbarius* records in the GBIF.org database (GBIF.org, 2024), reports 391 occurrences between 1819 and 1994, with a distribution across the entire United States and a presence in Mexico. Numerous specimens of this rust species are preserved in the fungal herbariums of university laboratories, museums and USDA fungal collections. *U. plumbarius* was reported on different Onagraceae taxa by Bisby (1916): *Gayophytum* sp., *Chamanirion* sp., *Epilobium* sp., *Taraxia* sp., *Boiduvalia* sp., *Chylisma* sp., *Eulobus* sp., *Clarkia* sp., *Godelia* sp., *Phaestoma* sp., *Sphaerostigma* sp., *Zauchneria* sp., *Gaura* sp., *Kneiffia* sp., *Oenothera* sp., *Luxavia* sp., *Pachylopus* sp., *Onagra* sp., *Megapterum* sp., *Meriolix* sp., *Circaea* sp., *Anogra* sp., *Jussiaea* sp., *Ludwigia* sp., *Fuchsia* sp. Concerning *Oenothera* sp., *U. plumbarius* was notably reported on *O. linderheimeri* in Ohio (Ellett, 1955), Iowa (Tiffany & Knaphus, 2004), Louisiana (Kaur et al., 2012; Ferrin, 2012) and California (Blomquist et al., 2015); on *O. caespitosa* in Colorado (Peck, 1879); on *O. biennis* in Illinois (Manaaki Whenua, 2002), Ohio (Ellett, 1955) and Iowa (Tiffany & Knaphus, 2004); on *O. suffrutescens* (syn. *Gaura coccinea*) in Nebraska (Manaaki Whenua, 2002), Texas (Manaaki Whenua, 2002), Mississippi (Presley, 1947), Colorado (Manaaki Whenua, 2002), and in Mexico (Hennen & Cummins, 1967); on *O. simulans* (syn. *Gaura angustifolia*) and *O. laciniate* in Mississippi (Presley, 1947); on *O. sinuosa* in Texas (Manaaki Whenua, 2002).

While the impact of *U. plumbarius* on the growth of *O. lindheimeri* seems currently limited, gardeners may have to contend with this rust in the future, as it could diminish the ornamental value of the plants due to the highly visible reddish discoloration it induces on the leaves and stems. A concern is that plants sold in garden centers in France (in Nancy and Champenoux; Table 1) and in the Netherlands (Ochten; Erik Slootweg, pers. comm.) were found to be infected. It is thus therefore that gardener nurseries, along with potentially several others, have unintentionally contributed to the spread of this rust in Europe through the trade of *O. lindheimeri*. As this species is still gaining in popularity as a garden plant, *U. plumbarius* will probably remain well established in Europe. A subsequent concern to address is the risk of a potential host jump by *U. plumbarius* to other European wild *Oenothera* species (27 recorded since 1950; Dietrich et al., 1997; Rostański et al., 1994), for instance *O. biennis*, *O. erythrosepala*, *O. silesiaca*, *O. fallax*, *O. glazioviana*, *O. deflexa*, which are quite common in France and Belgium. In Europe, only few species are successful in terms of increasing abundance while the majority of the species remain rare and local, constituting a wealth in terms of biodiversity. This issue is all the more preoccupying since *U. plumbarius* was identified on some other *Oenothera* species in the United States (see above).

The report of *E. caricis* associated with *U. plumbarius* in Les Clayes-sous-Bois (France) and Wageningen (The Netherlands) suggests that a natural balance may already be establishing between the pathogen and hyperparasite populations, potentially contributing to the limitation of rust attacks. While complete natural regulation can be ruled out, it has been suggested that *E. caricis* could help reduce losses caused by certain rusts (e.g., Yuan et al., 1999; Gordon & Pfender, 2012; Kajamuhan et al., 2015). This requires that the local population of *E. caricis* be sufficiently present at the start of the season, enabling it to

increase rapidly enough to slow rust development before the disease becomes damaging. This can occur if a population of another rust species on a weed preexists, creating a reservoir from which *E. caricis* can spread to *U. plumbarius*. This was the case in Les Clayes-sous-Bois, where infected *O. lindheimeri* were found along a grassy embankment on the edge of a road, in direct contact with grasses infected by *Puccinia* sp. hyperparasitized by *E. caricis*. The results of Kajamuhan et al. (2015) on several rusts of grasses demonstrate substantial and wide variation in naturally occurring populations of *E. caricis*, with some evidence of specialization within sympatric populations. Understanding such complex patterns would be valuable in developing effective strategies for promoting natural regulation or biological control.

To conclude, the discovery in Europe of a new species of rust fungus infected by a nonspecific hyperparasite fungal species on a recently introduced ornamental plant host is a noteworthy epidemiological situation. This *O. lindheimeri - U. plumbarius - E. caricis* system might correspond to what would happen if, in order to control a disease, one plans to introduce a biocontrol hyperparasite species whose populations/strains have not yet been in contact with the pathogen population. That makes think to invasions by exotic pathogenic fungi characterized by an absence of coevolution between the non-native fungus and its (new) hosts, referred to as “new encounter” (Desprez Loustau et al., 2007), but also to interactions with native hyperparasites which can be viewed as another type of “new encounter”. The host range of individual hyperparasite genotypes is a question of practical interest and holds theoretical significance for testing concepts related to the functioning of co-evolutionary pathosystems (Thompson, 1999).

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Table 1. Origin of *Oenothera lindheimeri* samples infected by *Uromyces plumbarius* collected from 2020 to 2024 in France (FR) and in The Netherlands (NL)

Origin	Ornamental plant status	Observer
Portet-sur-Garonne, FR 11-07-2021	garden beds in a shopping centre area	Suffert, 2021
Agen, FR 24-10-2021	garden beds in an urban area	Suffert, 2021
Villard-de-Lans, FR 23-09-2021	garden beds in an urban area	Suffert & Marcel, 2021
Les Clayes-sous-Bois, FR 20-11-2020	garden beds in an urban area	Suffert, 2020
Les Clayes-sous-Bois, FR 26-09-2021; 25-11-2022; 14-12-2024	grassy embankment on the edge of a road in an urban area	Suffert, 2021, 2022, 2024
Nancy and Champenoux, FR 09-09-2021	pots offered for sale in garden centers, originating from two gardener nurseries in Western France	Frey, 2021
Lunéville, FR 18-09-2021; 17-10-2021	garden beds in an urban area	Frey, 2021
Vincennes, FR 26-09-2021	garden beds in a public park	Marcel, 2021
Castets, FR, 29-09-2021	garden beds in an urban area	Frey, 2021
Le Vésinet, FR 19-11-2022	garden beds in an urban area	Suffert, 2022
Wageningen, NL 28-08-2021; 06-11-2021	garden beds in a public park	Slootweg, 2021
Utrecht, NL 17-09-2021; 18-10-2022	garden beds in a public park	Verhoeven & Slootweg, 2021, 2022

Table 2. Characteristics of *Uromyces plumbarius* urediniospores and teliospores.

Stage	Length	Width	Sample origin	Observer
Urediniospore	19-24 µm	16-19 µm	Les Clayes-sous-Bois, FR 25-09-2021	Suffert, 2021
	20-24 µm	17-19 µm	Wageningen, NL 28-08-2021	Slootweg, 2021
	22-25 µm	16-19 µm	Utrecht, NL 18-10-2022	Verhoeven & Slootweg, 2022
Teliospore	24-28 µm	17-21 µm	Les Clayes-sous-Bois, FR 20-11-2020	Suffert, 2020
	25-28 µm	18-22 µm	Wageningen, NL 06-11-2021	Slootweg, 2021

Figure 1. Origin of *Oenothera lindheimeri* samples infected by *Uromyces plumbarius* collected from 2020 to 2024 in France (FR; 1. Les Clayes-sous-Bois; 2. Vincennes; 3. Nancy and Champenoux; 4. Lunéville; 5. Castets; 6. Agen; 7. Portet-sur-Garonne, 8. Villard-de-Lans) and in The Netherlands (NL; 9. Utrecht; 10. Wageningen).

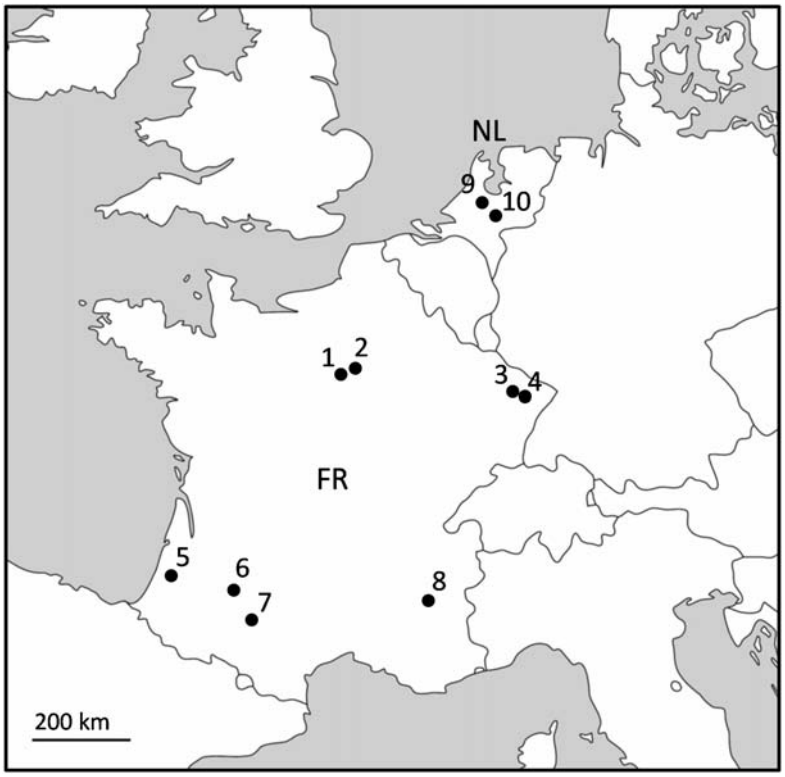


Figure 2. *Oenothera lindheimeri* infected by *Uromyces plumbarius.* **A-C.** Grassy embankment on the edge of a road, Les Clayes-sous-Bois, FR, 26-09-2021. **D.** Garden beds in a public park, Utrecht, NL, 17-09-2021. **E.** Garden beds of a shopping centre area, Portet-sur-Garonne, FR, 11-07-2021. **F.** Along the Canal du Midi, Agen, FR, 24-10-2021. **G.** Pots in a garden center, Nancy, FR, 09-09-2021. **H.** Garden beds, Lunéville, FR, 18-09-2021.

Figure 3. *Uromyces plumbarius* on *Oenothera lindheimeri* (Les Clayes-sous-Bois, FR). **A-B.** Immature light orange uredinia on the abaxial face of young leaves. **C.** Mature red uredinia surrounded by reddish spots on the abaxial face of leaves. **D.** Mature uredinia and *Mycodiplosis* sp. larva. **E.** Broken off uredinial epidermis revealing a mass of powdery urediniospores. **F.** Uredinia on a stem. **G-H.** Uredinia still protected under epidermis turning a darker metallic hue on old leaves.

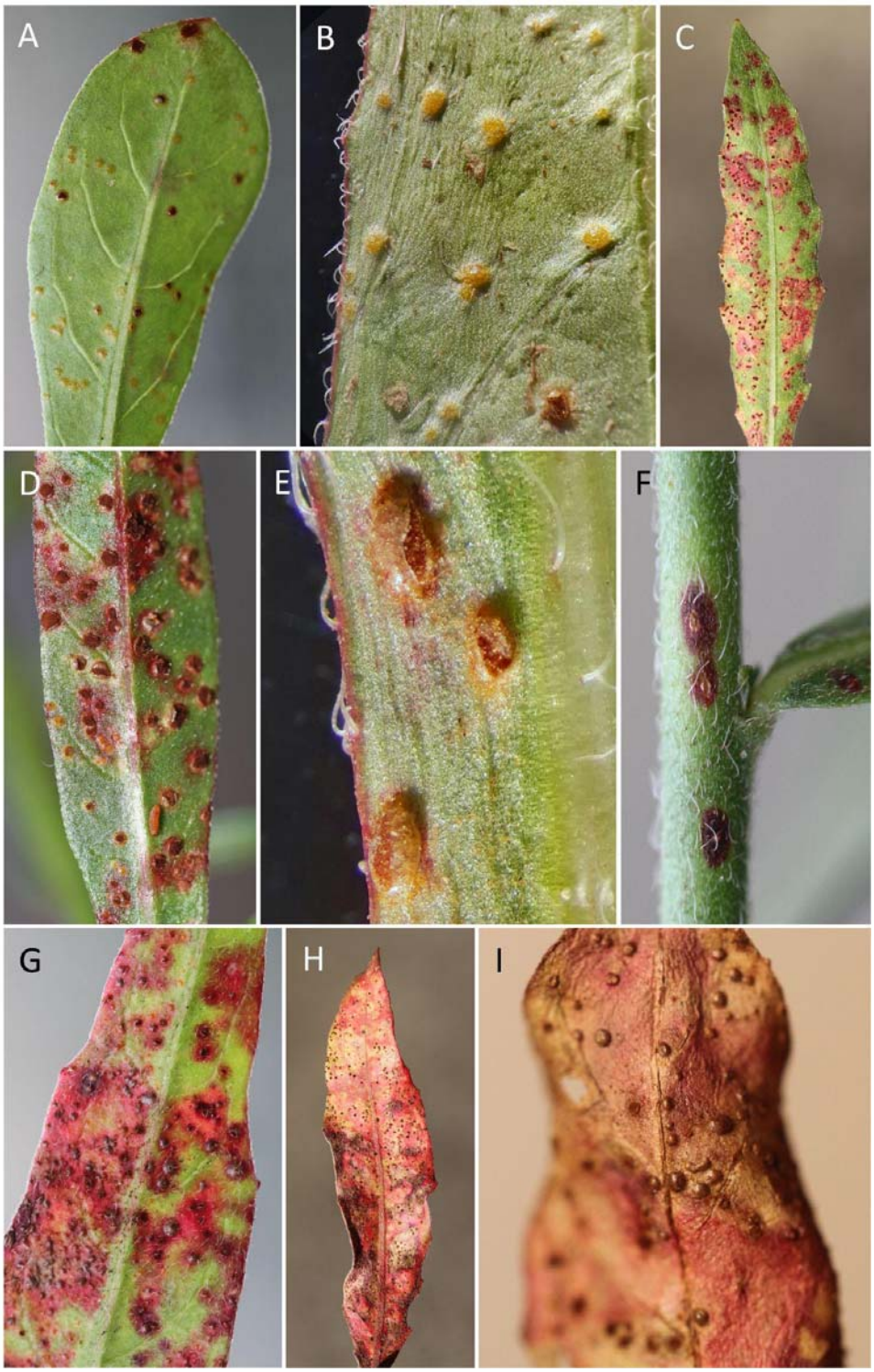


Figure 4. Urediniospores and teliospores of *Uromyces plumbarius.* **A.** Uredinia section. **B-D.** Urediniospores. **E-F.** Teliospores.

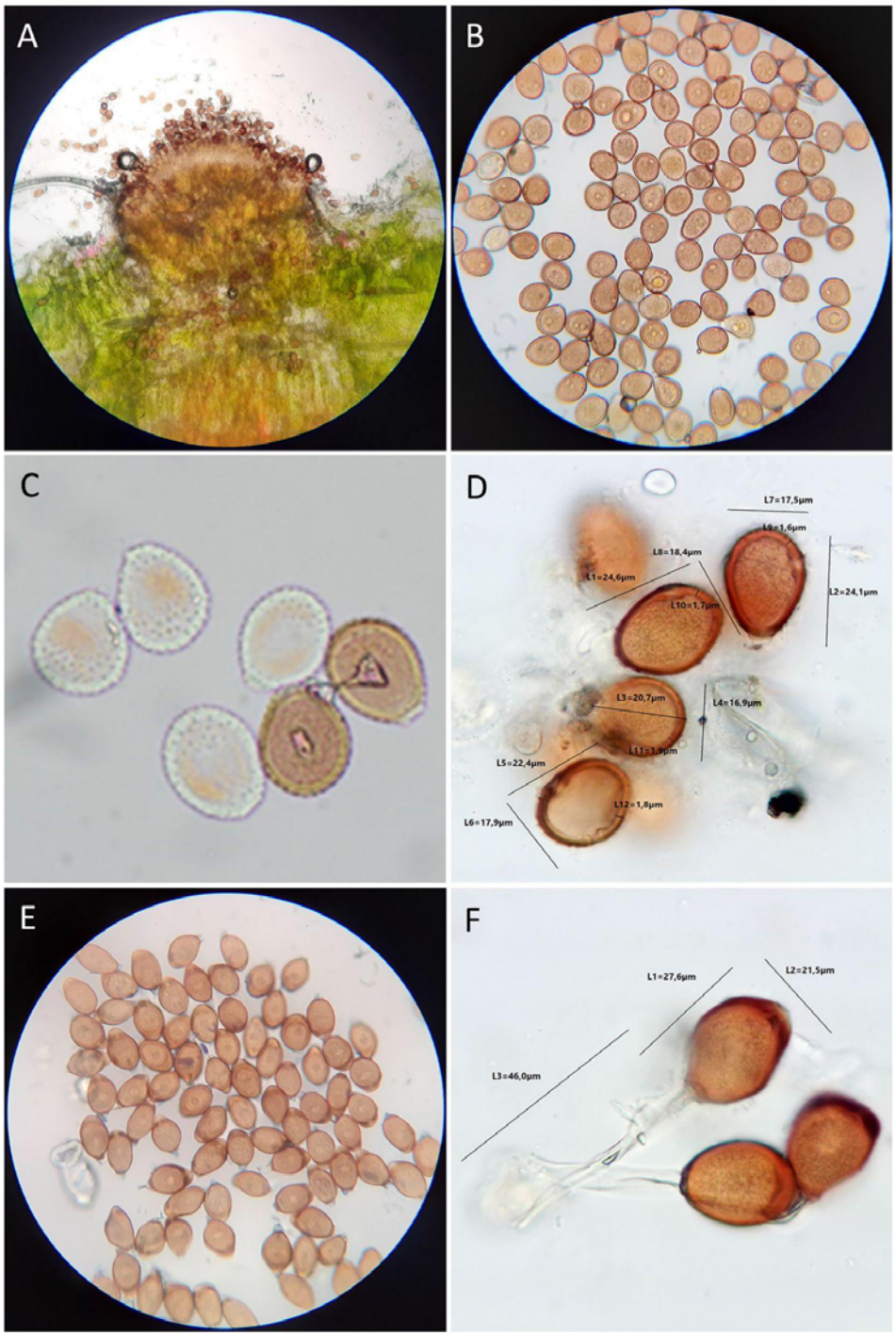
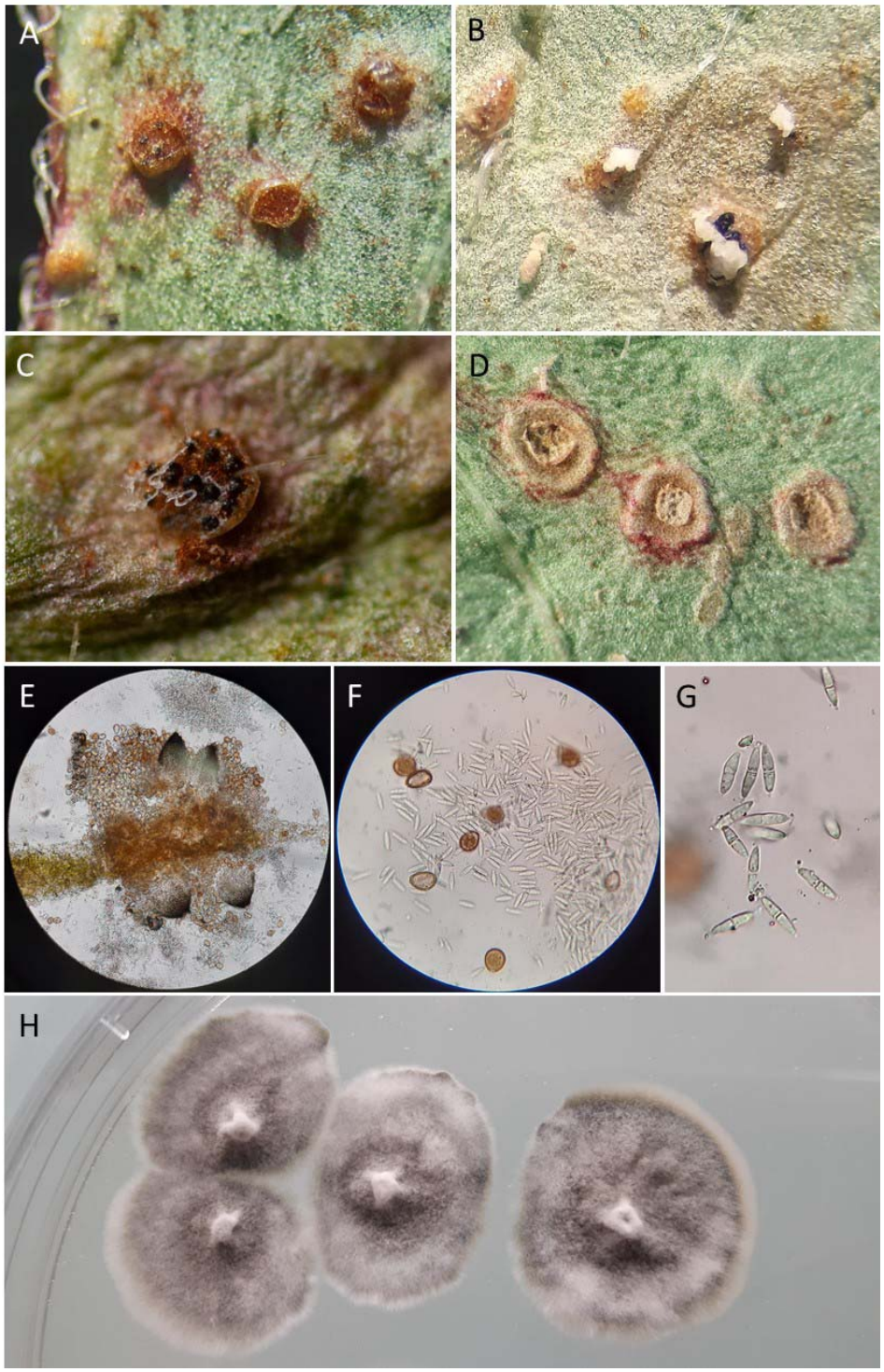


Figure 5. *Sphaerellopsis* sp. (teleomorph *Eudarluca* sp.) parasiting *Uromyces plumbarius* on *Oenothera lindheimeri.* **A.** Mature *U. plumbarius* uredinia parasitized by *Sphaerellopsis* sp., with one pierced by three conidiomata. **B-C.** Cirrhi (white filaments) extruded from conidiomata (black). **D.** Insertion scars from old *U. plumbarius* uredinia and *Sphaerellopsis* sp. conidiomata. **E.** Section of *U. plumbarius* uredinium and *Sphaerellopsis* sp. conidiomata. **F-G.** *Sphaerellopsis* sp. conidia. **H.** Colonies of *Sphaerellopsis* sp. growing on PDA medium in a Petri dish.



Supplementary Material 1. Consensus sequences of the specimen *Uromyces plumbarius* obtained with the pairs of primers (**A**) ITS1F10D / ITSRustR3C (GeneBank accession no. PV153707) and (**B**) Rust2inv / Lr6 (GeneBank accession no. PV151527)

A.

> ITS1_PC0798793

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B.

> ITS2-28S_PC0798793

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