

A new species of *Helicogermislita* from South Africa

Seonju Lee & Pedro W. Crous¹

Department of Plant Pathology, University of Stellenbosch, Private Bag X1,
Matieland 7602, South Africa

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A new xylariaceous ascomycete with ascospores having spiral germ slits and cellular appendages is described as *Helicogermislita diversa*. This species was isolated from twig litter of a *Leucadendron* sp. during the exploration of saprobic fungi occurring in the fynbos of the Cape Floral Kingdom. The fungus is illustrated, and described based on its morphological characters.

Keywords: Ascomycetes, Proteaceae, South African fynbos.

Helicogermislita was introduced by Hawksworth & Lodha (1983) to accommodate a single fungus, *H. celastri* (S. B. Kale & S. V. S. Kale) Lodha & D. Hawksw. (= *Amphisphaerella celastri* S. B. Kale & S. V. S. Kale), from India. The genus belongs to the Xylariaceae and is characterized by semi-immersed stromata, perithecia embedded in the host tissue, and by ascospores encircled more than once by spiral germ slits. Ascospores may have a cellular basal appendage or gelatinous sheaths. The genus remained monotypic until Læssøe & Spooner (1994) included three corticolous species described in other genera, namely *H. fleischhakii* (Auersw.) Læssøe & Spooner (= *Sordaria fleischhakii* Auersw.) from France and Germany, *H. gaudefroyi* (Fabre) Læssøe & Spooner (= *Rosellinia gaudefroyi* Fabre) from France, *H. valdiviensis* (Speg.) Læssøe & Spooner (= *Rosellinia valdiviensis* Speg.) from Chile. Læssøe & Spooner (1994) also considered *Anthostomella calligoni* Frolov to be a potential member of this genus.

As a part of the fungal biodiversity programme in the fynbos of the Cape Floral Kingdom, leaf or twig litters of Proteaceae were routinely collected over the years 2000–2001. During the course of this study, a strange ascomycete fungus was encountered at the Helderberg Nature Reserve in Western Cape province of South Africa, where it occurred on dead twigs of a *Leucadendron* sp. The fungus

¹ Present address: Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

was identified as a new member of the genus *Helicogermis*. The aim of the present paper is, therefore, to describe and illustrate the species from *Leucadendron*, and to contrast it to others occurring in the same genus.

Materials and methods

Dead twigs of *Leucadendron* sp. with intact tissue were about 5 mm diam., and their decomposition had not yet started. The samples were immediately inspected for fungal structures, and air-dried for further study. Fungal structures were initially mounted in water and later flooded in clear lactophenol for preservation. Measurements and photographs of structures were taken in clear lactophenol mounts when not stated otherwise. Nomarski contrast was employed throughout the whole examination, apart from the determination of colours, for which bright-field microscopy was adopted. Ascal apical structures were examined in Melzer's reagent. Whenever possible, 30 observations were made of each structure, from which the 95% confidence intervals were derived, with the extreme values given in parentheses. Ascospores were mounted in Jung tissue freezing mediumTM (Leica Instruments, Germany), and sections prepared with a Leica CM1100 Cryostat microtome. Photographic images were taken with a digital camera (Nikon DXM 1200) on a Nikon Eclipse E600 compound microscope or a Nikon SMZ800 stereomicroscope. The type specimen is deposited at the National Fungal Collection in Pretoria (PREM). Attempts to culture the fungus proved unsuccessful.

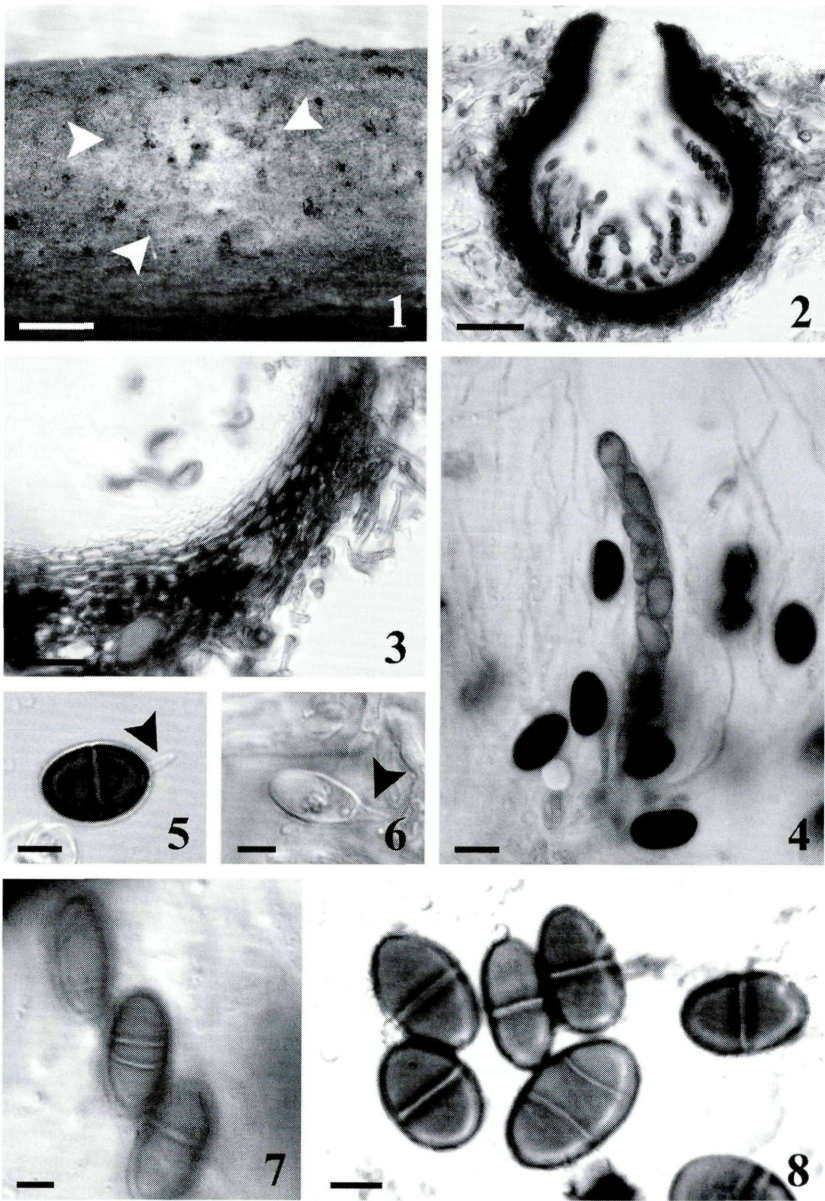
Taxonomy

***Helicogermis diversa* S. Lee & Crous, sp. nov.** – Figs. 1–8.

Stromata immersa, circum ventrem ascomatis, carbonacea, atra. Ascospores perithecialia, subglobosa vel obpyriformia, singula, usque ad 240 μm alta et ad 215 μm lata. Peridium pseudoparenchymatosum, textura angulari, 15–17.5 μm latum. Paraphyses persistentes, filiformes, non ramosae, (1.5–)2–2.5(–3.5) μm crassae. Asci 65–80 \times 10 8–10 μm , cylindrici, brevistipitati. Ascospores (11–)13.5–15(–17.5) \times 10 (6.5–)8–9(–11) μm , subglobosae vel ellipsoideae, late apicibus rotundatis, brunneae in maturitate, unicellularia, fissura germinali sporas circumdanti semel vel bis, plerumque aequatoriali, interdum de polo ad polum, sine tunica gelatinosa. Appendices (3–)3.5–4.5(–6) \times 10 1–2 μm , cellulares, basillares, plerumque centrales et obliquae, subulatae, hyalinae.

Etymology. – In reference to its variable germ slit on ascospores.

Holotype. – South Africa: Western Cape province, Helderberg Nature Reserve, on dead twigs of a *Leucadendron* sp. (Proteaceae), 14 Aug. 2000, S. Lee SL224, PREM 57481.



Figs. 1-8. *Helicogermisliia diversa* (holotype). - 1. Surface view of the lesion (arrowheads). - 2. Vertical section through ascoma showing the presence of stroma - 3. Peridium. - 4. Ascus and paraphyses (in lactophenol cotton blue). - 5. Mature ascospore with subulate appendage (arrowhead). - 6. Young ascospore with subulate appendage (arrowhead). - 7. Heterogeneity of germ slits on immature ascospores in a single ascus. - 8. Ascospores with equatorial and helicoid germ slits. - Bars 1 = 1 mm; 2 = 50 µm; 3, 4 = 10 µm; 5-8 = 5 µm.

Lesions white, mixed with decoloured wood, soft, tomentose (Fig. 1). – Stromata immersed, around the venter of the ascoma, uniloculate, carbonaceous, black, variable in thickness, up to 32.5 μm wide, consisting of 1 or 2 layers; when one layer, arachnoid, when two layers, inner layer thick, crust-like, outer layer arachnoid (Fig. 2). – Ascomata stromatic, perithecioid, subglobose to obpyriform, single, glabrous, up to 240 μm high, up to 215 μm wide, ostiole papillate, up to 75 μm wide, ostiolar canal lined by numerous simple, flexuose, hyaline, septate periphyses (Fig. 2). – Peridium pseudoparenchymatous, textura angularis, 15–17.5 μm wide, consisting of 2 layers, outer layer dark brown, thick-walled, inner layer hyaline, thin-walled (Fig. 3). – Paraphyses (1.5–)2–2.5(–3.5) μm thick, filiform, unbranched, flexuous, regularly septate, extending almost to the top of the cavity, abundant, persistent, lining the perithecial wall (Fig. 4). – Asci 65–80 \times 8–10 μm , cylindrical, short stipitate, unitunicate, octosporous, lining the perithecial wall, the apex initially thick-walled, becoming thinner with maturity, no apical apparatus observed, nonamyloid, (Fig. 4). – Ascospores (11–)13.5–15(–17.5) \times (6.5–)8–9(–11) μm (\bar{x} = 14.2 \times 8.5 μm), subglobose to ellipsoidal, broadly rounded at the apices, hyaline when young and becoming brown at maturity, unicellular, with a germ slit encircling the spores 1(–2)-times, mostly equatorial, occasionally from pole to pole, a gelatinous sheath absent, with one cellular appendage at the distal end, (3–)3.5–4.5(–6) μm long, 1–2 μm wide, mostly centric and oblique, subulate, hyaline, conspicuous when young, becoming inconspicuous with age (Figs. 5–8).

Known host. – *Leucadendron* sp. (Proteaceae).

Known distribution. – Western Cape province, South Africa.

The present fungus is placed in *Helicogermislita* owing to not only its helicoid ascospore germ slits, but also to its cellular ascospore appendage and stromatic structures. Barr (1990) and Cannon (1987) distinguished *Helicogermislita* from other xylariaceous fungi based on the fact that none of them had ascospore germ slits that encircle ascospores more than 1.5 times. The current species exhibits a mixture of ascospores having helicoid germ slits which spiral up to 2 times from pole to pole, as well as having equatorial germ slits which encircle the body of ascospores only once. This heterogeneity of germ slit morphology is present even within a single ascus (Fig. 7), a feature that has not been reported for any other species in this genus.

Among the known species of *Helicogermislita* cellular ascospore appendages are reported only in *H. celastri*, from which *H. diversa*

differs by smaller ascospores, less coiled germ slits, and subulate or triangular appendages. Furthermore, the three remaining species are distinct by all having apical amyloid ascus tips. Additional distinguishing features include gelatinous ascospore sheaths in *H. fleischhakii*, and larger ascospores in *H. gaudefroyi* ($29\text{--}44 \times 12\text{--}17 \mu\text{m}$) (Læssøe & Spooner, 1993), and *H. valdiviensis* ($17\text{--}21 \times 7.5\text{--}10 \mu\text{m}$) (Petrini, 1993).

In the original description of the type species, *H. celastri* (Hawksworth & Lodha, 1983), neither apical apparatus nor amyloid reaction were observed. This was later amended by Dargan & al. (1984), who reported a further collection having a discoid amyloid apical apparatus. The presence of an apical ascus apparatus and a positive amyloid reaction is considered to be typical of xylariaceous fungi. Rogers (1979), however, stated that there could be some variability in the iodine reaction, and even a total absence of the apical ascus apparatus. *Helicogermislita diversa* lacks an amyloid apical apparatus, which is present in all other species of the genus known to date. Furthermore, a basal cellular appendage was not originally described for the type species either, but was later observed by Dargan & al. (1984). In the present species, the basal appendages are more apparent when ascospores are immature.

During the observations of *H. diversa*, we observed that the surface lesions harbouring stromata are tomentose, softer and paler than other non-stromata-harbouring areas in the tissue. This may result from the presence of intermingled hyphae growing in the host tissue (Fig. 1). Læssøe & Spooner (1994) also recognized an extensive white surface around ascomata of *H. fleischhakii*, *H. gaudefroyi*, and *H. celastri* and regarded it as an ectostroma, and believed that it could be a constant and important feature for the genus. However, there is some discrepancy pertaining to the usage and value of this feature (L. Petrini, pers. comm.). As we did not examine any other species, we have been unable to resolve this issue, and it awaits further clarification.

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Autor(en)/Author(s): Lee Seonju, Crous Pedro W.

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