

***Ligniera junci*, a plasmodiophorid re-discovered in roots of *Juncus* in Austria**

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Accepted 5. 10. 2009

Key words: Plasmodiophorids, *Ligniera junci*, *Polymyxa graminis*. – New record for Austria.

Abstract: *Ligniera junci*, a plasmodiophorid parasite of *Juncus* spp. was found at three locations in Tyrol. The morphologically similar plasmodiophorid of *Poaceae*, *Polymyxa graminis* was found to co-occur at two of these locations. This is the first record of these species in Austria. Colour photographs of resting spores of *Ligniera junci* in roots of *Juncus triglumis* and *Polymyxa graminis* in roots of *Poaceae* are given. A description of *Ligniera junci* supported by light micrographs of different stages of the life-cycle (sporosori, zoosporangia, zoospores) is presented.

Zusammenfassung: *Ligniera junci*, ein plasmodiophorider Parasit verschiedener *Juncus*-Arten, wurde an drei Standorten in Tirol gefunden. Auch die morphologisch ähnliche Art *Polymyxa graminis*, die sich vor allem durch das Wirtsspektrum (*Poaceae*) von *Ligniera junci* unterscheidet, konnte an drei Standorten nachgewiesen werden. Für beide Arten sind dies die ersten Nachweise in Österreich. Die Arten werden anhand von detaillierten Beschreibungen sowie Bildern der Dauersporen in den Wurzeln von *Juncus triglumis* (*Ligniera junci*) und *Poaceae* (*Polymyxa graminis*) vorgestellt. Die verschiedenen Stadien des Lebenszyklus von *Ligniera junci* (Dauersporen, Zoosporangien, Zoosporen) werden fotografisch dokumentiert.

Plasmodiophorids are an enigmatic group of obligate biotrophic pathogens of higher plants. They have long been treated as a basal group of fungi (BRASELTON 1995), but recent molecular phylogenies point to a close affiliation with the protozoan phylum Cercozoa (CAVALIER-SMITH & CHAO 2003). Plasmodiophorids are characterised by a multi-stage life cycle with zoosporic, plasmodial, and resting stages (BRASELTON 2001).

Only a small number of plasmodiophorids are quite well studied, especially plasmodiophorids which cause plant diseases (e. g., *Plasmodiophora brassicae* WORONIN) and/or have economic significance as vectors of plant viruses (e. g., *Polymyxa graminis* LEDINGHAM). Species without economic impact are reported rarely, probably because of their “hidden” life as obligate plant parasites. *Sorosphaera veronicae* J.

SCHRÖT., a plasmodiophorid recently reported to occur in *Veronica* spp. in Austria (NEUHAUSER & al. 2005), can be identified by the galls on the above ground plant parts of their host, but many plasmodiophorids can only be recognised by the presence of their resting spores in the roots (KARLING 1968, BRASELTON 2001, KIRCHMAIR & al. 2005, NEUHAUSER & al. 2009 a). This soil-borne and plant-associated nature of plasmodiophorids has hindered comprehensive research on the distribution of economically less important species. Some of them might be very common but reported records are rare.

During the vegetation periods in 2008 and 2009 sporosori of a plasmodiophorid parasite could be observed in root hairs of nearly every uprooted *Juncus triglumis* L. at the Rotmoosferner glacier foreland in the Central Alps (Ötztal, Tyrol, Austria). The species could be identified as *Ligniera junci* (SCHWARTZ) MAIRE & A. TISON, which is the first record for this species in Austria. Moreover, the occurrence of the *Poaceae*-infesting plasmodiophorid *Polymyxa graminis* in the rhizosphere of different *Poaceae* was indicated by DNA-sequences. The glacier foreland of the Rotmoosferner is a well-studied region in respect to vegetation succession patterns (RAFFL & al. 2006), mycorrhiza status of different plants (e.g., MÜHLMANN & PEINTNER 2008, FLEISCH 2009) or soil fungal communities (OBERKOFER & PEINTNER 2009). Despite all attempts in studying biodiversity at the glacier foreland of the Rotmoosferner there were no hints to the occurrence of plasmodiophorids, neither by direct observations nor by molecular soil analyses.

In autumn 2008 different *Juncus* spp. were sampled from different habitats in Tyrol. *Ligniera junci* was detected at two more sites (Brandenberg, Münster). *Polymyxa graminis* could be found at two sites in Brandenberg, Tyrol, where the occurrence of *S. veronicae* a plasmodiophorid of *Veronica* spp. was reported earlier (NEUHAUSER & al. 2005).

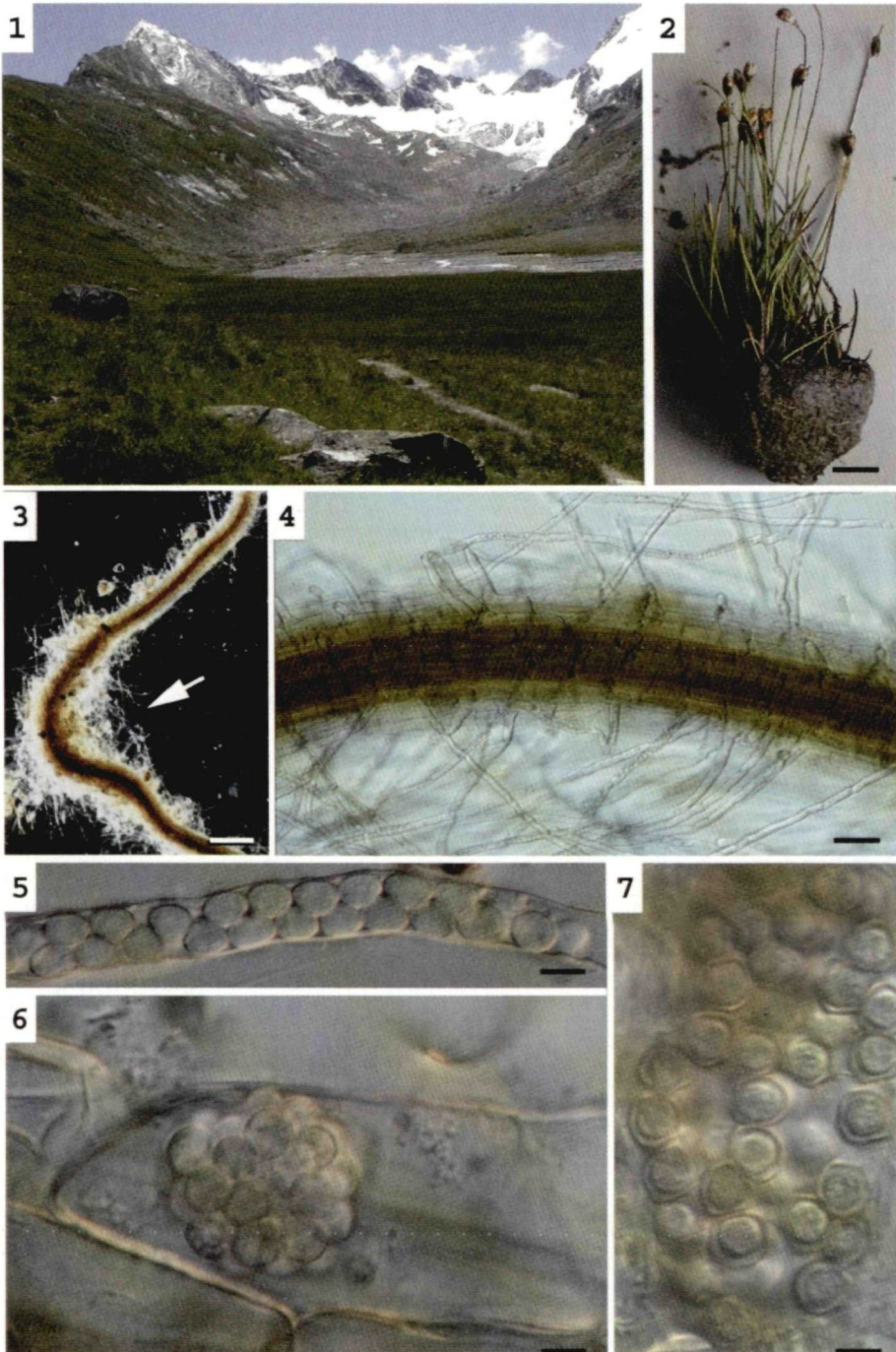
Material and methods

Light microscopic investigations. For light microscopy a Nikon Optiphot light microscope (DIC, 100 × oil) was used.

DNA extraction, PCR, sequence analyses. Root samples were rinsed with tap water and screened microscopically for the presence of plasmodiophorids. Roots with a proven infection were used for DNA extraction. DNA was extracted from roots as described previously (NEUHAUSER & al. 2009b). Plasmodiophorid specific primers Psvit F (ACG CGT TCC AAC TTC TTA GAG GGA) / Psvit R (CAT GCC TCT CTG AGT ATC GGT TTC) designed in our working group were used for the amplification of the 18S rDNA region (NEUHAUSER & al. 2010).

Material examined: *Ligniera junci*: Austria, Tyrol, Brandenberg, 47° 29' 20" N, 11° 54' 46" E, 989 m s. m., in roots of *Juncus* spec., 8. 9. 2008, leg. S. NEUHAUSER (IB 2008/0031); - Tyrol, Münster, 47° 24' 54" N, 11° 50' 19" E, 519 m s. m., in roots of *Juncus effusus*, 5. 10. 2008, leg. S. NEUHAUSER & M. KIRCHMAIR (IB 2008/0032); - Tyrol, Obergurgl, Rotmoostal, 46° 50' 46" N, 11° 01' 05" E, 2252 m s. m., in roots of *Juncus triglumis*, 16. 7. 2008, leg. S. NEUHAUSER & M. KIRCHMAIR (IB 2008/0030; GenBank GQ892043).

Polymyxa graminis: Austria, Tyrol, Brandenberg, 47° 29' 20" N, 11° 54' 46" E, 989 m s. m., 8. 9. 2008, in roots of *Poaceae*, leg. S. NEUHAUSER & M. KIRCHMAIR (IB 2008/33; GenBank GQ892044); - - 47° 29' 17" N, 11° 54' 09" E, 956 m s. m., 8. 9. 2008, in roots of *Poaceae*, leg. S. NEUHAUSER & M. KIRCHMAIR (IB 2008/35).



Figs. 1-6. *Ligniera junci*. – Fig. 1. Habitat at the Rotmoos Valley, Ötztal, Austria. – Fig. 2. Host species *Juncus triglumis*. – Figs. 3-4. Root of *J. triglumis* infected with *L. junci*. Resting spores can mainly be found in regions with numerous root hairs (arrow). – Figs. 5-6. Resting spores in a root hair (Fig. 5) and in a root cortical cell (Fig. 6). – Fig. 7. *Polymyxa graminis*. Resting spores in a root cortical cell of a *Poaceae*. – Bars: 1 cm (Fig. 2), 200 μ m (Fig. 3), 50 μ m (Fig. 4), 5 μ m (Figs. 6, 7).

***Ligniera junci* (SCHWARTZ) MAIRE & A. TISON, 1911.** C. R. Hebd. Seances. Acad. Sci. **152**: 206. (Figs. 4-6, 8-12)

≡ *Sorosphaera junci* SCHWARTZ 1910. Ann. Bot. London **24**: 514, pl. 40.

Resting spores of *L. junci* were observed in root hairs and root cortical cells of *Juncus triglumis* L. and *Juncus effusus* L. The resting spores are subspherical to obvoid: $(4.5-5.4 \pm 0.6(-6.8) \times (3.3-4.6 \pm 0.5(-5.6) \mu\text{m}$; $Q = (1.0-1.2 \pm 0.1(-1.6)$; $n = 49$. The resting spores are united into sporosori which are highly variable in size and shape, depending on the shape of the host cell: Linear series of resting spores were observed especially in root hairs (Figs. 4-5, 12), while in cortical cells globose or cylindrical aggregates were observed (Fig. 6).

Zoosporangia with delicate walls, lobed, forming loose aggregates filling up parts or the entire host cell (Fig. 8). Primary (?) zoospores biflagellate, amoeboid, $3-4 \mu\text{m}$ (Fig. 9). Primary plasmodia (Fig. 10) separate into resting spores (Fig. 11) with robust, smooth walls (Figs. 5, 6, 12).

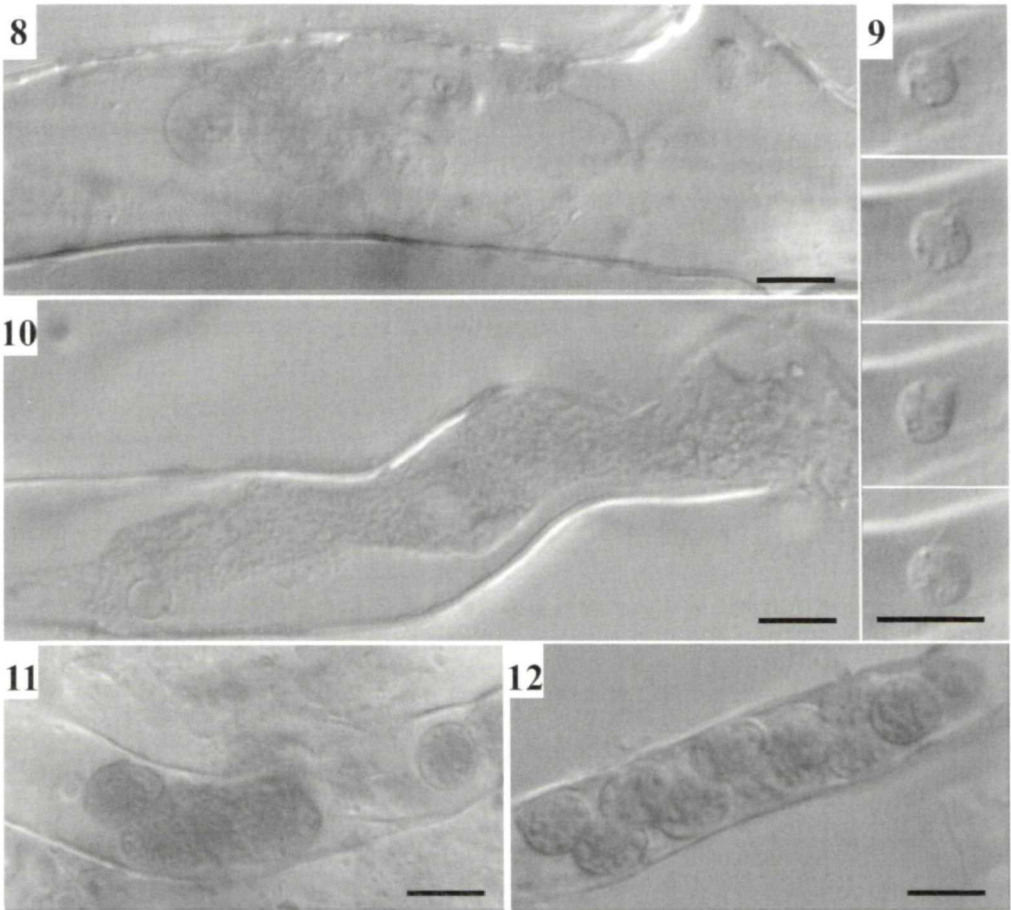
Resting spores can mainly be found in regions with numerous root hairs, but it remains unclear if such regions are preferably infected or if an infection with *L. junci* induces the formation of root hairs.

***Polymyxa graminis* LEDINGHAM, 1939.** Canad. J. Res. C., **17**: 50, figs. 1-3, pls. 1-4 (Fig. 7).

Resting spores of *P. graminis* were observed in root cortical cells of different *Poaceae*. The resting spores are subspherical to obvoid $(3.8-5.3 \pm 0.6(-7.4) \times (3.2-4.6 \pm 0.6(-6.3) \mu\text{m}$ in diam.; $Q = (1-1.2 \pm 0.1(-1.6)$; $n = 59$. The resting spores are united into loose sporosori which fill up the entire host cell (Fig. 7). The lobed irregular sporangia with exit tubes – which are the only morphologic character to discriminate *Polymyxa* from the genus from *Ligniera* (KARLING 1968) – could not be observed. Primary and secondary zoospores were not observed. The identity of the record was confirmed by molecular analyses.

Comments

SCHWARTZ (1910) found the resting spores of *Sorosphaera junci* united into spherical or ellipsoidal sporosori in root cortical cells or as loose aggregates filling the root cells of *Juncus bufonius* L. No measurements are given within the original description but interpreting SCHWARTZ'S (1910) drawings a diameter of the resting spores between 4.1 and $5.5 \mu\text{m}$ can be estimated. MAIRE & TISON (1911) established the genus *Ligniera* based on *L. junci* and the then new described two species *L. radicalis* MAIRE & A. TISON in roots of *Callitriche stagnalis* SCOP. and *L. verrucosa* MAIRE & A. TISON in roots of *Veronica arvensis* L. They specify the resting spore diameter of $5-7 \mu\text{m}$ for *L. junci* and $4-5 \mu\text{m}$ for *L. radicalis*. The same dimensions are given by COOK (1926). KARLING (1968) and DYLEWSKY (1990) followed the opinion of COOK (1933) who concluded from infection experiments that *L. radicalis* and *L. junci* are conspecific and referred to a resting spore diameter of $4-7 \mu\text{m}$.



Figs. 8-12. *Ligniera junci*. – Fig. 8. Thin-walled, lobed zoosporangia. – Fig. 9. Primary (?) zoospore in a root hair of *Juncus triglumis*. – Fig. 10. Plasmodium. – Figs. 11-12. Formation of resting spores. – Bars: 5 μ m.

Neither SCHWARTZ (1910) nor any other, later author reporting on *Ligniera junci* mentioned the high abundance of sporosori in root hairs – as observed in the present study. They reported the resting spores in root cortical cells of *Juncus* species.

FRON & GAILLANT (1925) described *Ligniera pilorum* in root hairs of different *Poaceae*. The diameter of the resting spores is given as 4-6 μ m and therefore identical with those of *L. junci*, but in contrast to the latter species all infected root hairs exhibited clear hypertrophies and became clavate.

In the years following his discovery of *L. junci* SCHWARTZ described a range of *Ligniera* species at the type locality of *L. junci*: *L. graminis* (SCHWARTZ 1911), *L. bel-lidis* and *L. menthae* (SCHWARTZ 1914). These species were differentiated mainly by their host plants. COOK (1926) collected different plants at SCHWARTZ' "*Ligniera* site". He found a range of plants infected with what he thought to be *L. junci* (*Poa an-*

nua L., *Plantago major* L., *Ranunculus aquatilis* L., *Mentha pulegium* L., *Callitriche stagnalis* SCOP., *Veronica beccabunga* L., *Juncus articulatus* L.). For testing the hypothesis of conspecificity COOK (1926) potted seedlings of each plant species into sterile soil and infected them with infested roots. All of them were successfully infected in different frequencies. The conspecificity of SCHWARTZ' (1911, 1914) later described *Ligniera* species and *L. radicalis* (parasitic in *Callitriche*; MAIRE & TISON 1911) seems convincing. But there is one drawback in the argumentation: *Polymyxa graminis* described by LEDINGHAM (1939) is hardly distinguishable from *Ligniera* at the base of resting spore morphology. There are records of *P. graminis* on many different hosts (KARLING 1968, LEGRÈVE & al. 2000). In the present study we could find *Ligniera junci* and *Polymyxa graminis* within a few square meters in a swamp and confirmed the species identity by molecular data. Although COOKS (1926) investigations were made very carefully, a mixing up with *P. graminis* in experiments seems at least imaginable as he did not use roots from the original host plant (*Juncus bufonius*). Many of the older records of *L. junci* might be mistakenly identified as has already been pointed out by BARR (1981).

Besides the fact that we found resting spores mainly in root hairs and only rarely in root cortical cells, our collections fit perfectly with the original description of *L. junci* by SCHWARTZ (1910) regarding resting spore size and form, size and form of sporosori in cortical cells, and of their host plant.

Thin-walled, lobed zoosporangia which discriminate *Ligniera* from *Polymyxa* as well as unusual flagellate zoospores within root hairs as described by COOK (1926) could be observed (Fig. 9). Moreover, sequence data confirmed the separation of the two taxa.

The authors wish to thank R. PÖDER, University of Innsbruck, Austria for passing on his knowledge and expertise. We are indebted to B. ERSCHBAMER for help with the determination of the *Juncus* species. SIGRID NEUHAUSER thanks the Austrian Science Fund for a Hertha-Firnberg research grant (FWF; project T379-B16).

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Zeitschrift/Journal: [Österreichische Zeitschrift für Pilzkunde](#)

Jahr/Year: 2009

Band/Volume: [18](#)

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Artikel/Article: [Ligniera junci, a plasmodiophorid re-discovered in roots of Juncus in Austria. 141-147](#)