

## ***Sorosphaera viticola* sp. nov. (plasmodiophorids), an intracellular parasite in roots of grape vine**

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The plasmodiophorid *Sorosphaera viticola* sp. nov. has been found in roots of *Vitis riparia* × *V. berlandieri* rootstocks in the German Rheingau. The species is described on the basis of light- and electron-micrographs. *Sorosphaera viticola* is distinguished from other species of this genus by its host, by form and size of its resting spores and that it does not cause hypertrophies in the host.

Keywords: Cercozoa, parasitic slimemolds, taxonomy, *Vitis*.

Plasmodiophorids are obligate intercellular parasites of plants and straminopiles. Their distribution follows that of their hosts; no member conclusively has been shown to complete a life cycle in the absence of host cells (Dylewski 1990, Braselton 2001). As the taxonomic status of this group has been controversially discussed, the informal term “plasmodiophorid”, as suggested by Braselton (1995), is used in this paper. When Woronin established the genus *Plasmodiophora*, he proposed an affiliation to the protists in the sense of Haeckel and considered them as the simplest group of the Myxomycetes (Woronin 1878). Historically, Myxomycetes and therefore the plasmodiophorids were considered as fungi. Cavalier-Smith (1998) included the plasmodiophorids within the newly proposed protozoan phylum Cercozoa, which is one of the eight major groups of eukaryotes according to Baldauf (2003). The inclusion of the plasmodiophorids in the Cercozoa was subsequently confirmed by Bulman *et al.* (2001), Cavalier-Smith and Chao (2003) and Archibald and Keeling (2004).

The plasmodiophorid life cycle is characterised by a sequence of primary zoospores, cysts, sporangial plasmodia, zoosporangia, secondary zoospores, plasmodia and resting spores. Due to their propagation with zoospores they require moisture to move and

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therefore prefer aquatic habitats or marshy grounds. Nevertheless, plasmodiophorids can be found in numerous terrestrial plants and are very common in typical agricultural environments.

Plasmodiophorids comprise well known plant parasites of economically important crops: *Plasmodiophora brassicae* Woronin is causing the club root disease of cruciferes, *Spongospora subterrannea* Walr. ssp. *subterrannea* J.A. Toml. is known as the causal agent of the powdery scab of potatoes. *Polymyxa betae* Keskin causes rhizomania in sugar beets by transmitting the *Beet Necrotic Yellow Vein Virus* (BNYVV) and *Polymyxa graminis* Ledingham is known as vector of several cereal viruses. Recently, the discovery of a new plasmodiophorid of the genus *Sorosphaera* was reported (Huber *et al.* 2004). The species is parasiting grape vine, one of the oldest cultivated plants. A description and differential diagnosis of this grape vine *Sorosphaera* is presented within this paper.

## Materials and Methods

### Light microscopy (LM)

Fresh vine roots and rootlets with visible necrosis were fixed in AFA (aqueous ethanol (70 % v/v): formol (40 % aqueous formaldehyde v/v): glacial acetic acid = 90:5:5), dehydrated in an ascending ethanol series and embedded in paraffin.

Spores and sporosori from dried roots were mounted in 2.5 % KOH and measured using a Nikon Optiphot light microscope (Nomarski interference contrast; epifluorescence, oil immersion objective 100x).

### Scanning electron microscopy (SEM)

Rootlets were fixed for 30 minutes in 2.5 % watery glutaraldehyde (v/v). After washing in 30% ethanol, samples were dehydrated in ascending concentrations of ethanol and transferred into 100% acetone. Specimens were then critical-point dried, mounted on aluminium stubs, and subsequently sputtered with gold.

For zoospore preparation, infected roots were ground with a ceramic mortar and pistil and incubated in tap water at 23 °C in darkness. When swimming zoospores could be observed by LM (about ten days after inoculation) 1 mL of the suspension was mixed with 100 µL of 25 % watery glutaraldehyde (v/v) for 30 minutes. With the help of a syringe, the fixed suspension was carefully pressed through a sterile cellulose acetate filter (pore size 0.22 µm). The filter was subsequently dehydrated by an ascending ethanol series, transferred into 100 % acetone, critical point dried, mounted on an aluminium stub and sputtered with gold.

Micrographs were taken at 30 kV acceleration voltage using a PHILIPS ESEM scanning electron microscope.

Transmission electron microscopy (TEM):

For transmission electron microscopy, root samples were prefixed in 2.5% glutaraldehyde (v/v) and 0.05 M sodium cacodylate (pH 7.2) overnight and washed with 0.05 M sodium cacodylate before being incubated in 1% osmium tetroxide for 1 h. After rinsing in distilled water and dehydration in an ascending ethanol series, the samples were embedded in Spurr. Thin sections (0.1–0.2  $\mu\text{m}$ ) were stained with 2% (w/v) uranyl acetate and 1% (w/v) lead citrate. Samples were viewed with a FEI tecnai transmission electron microscope.

### Taxonomy

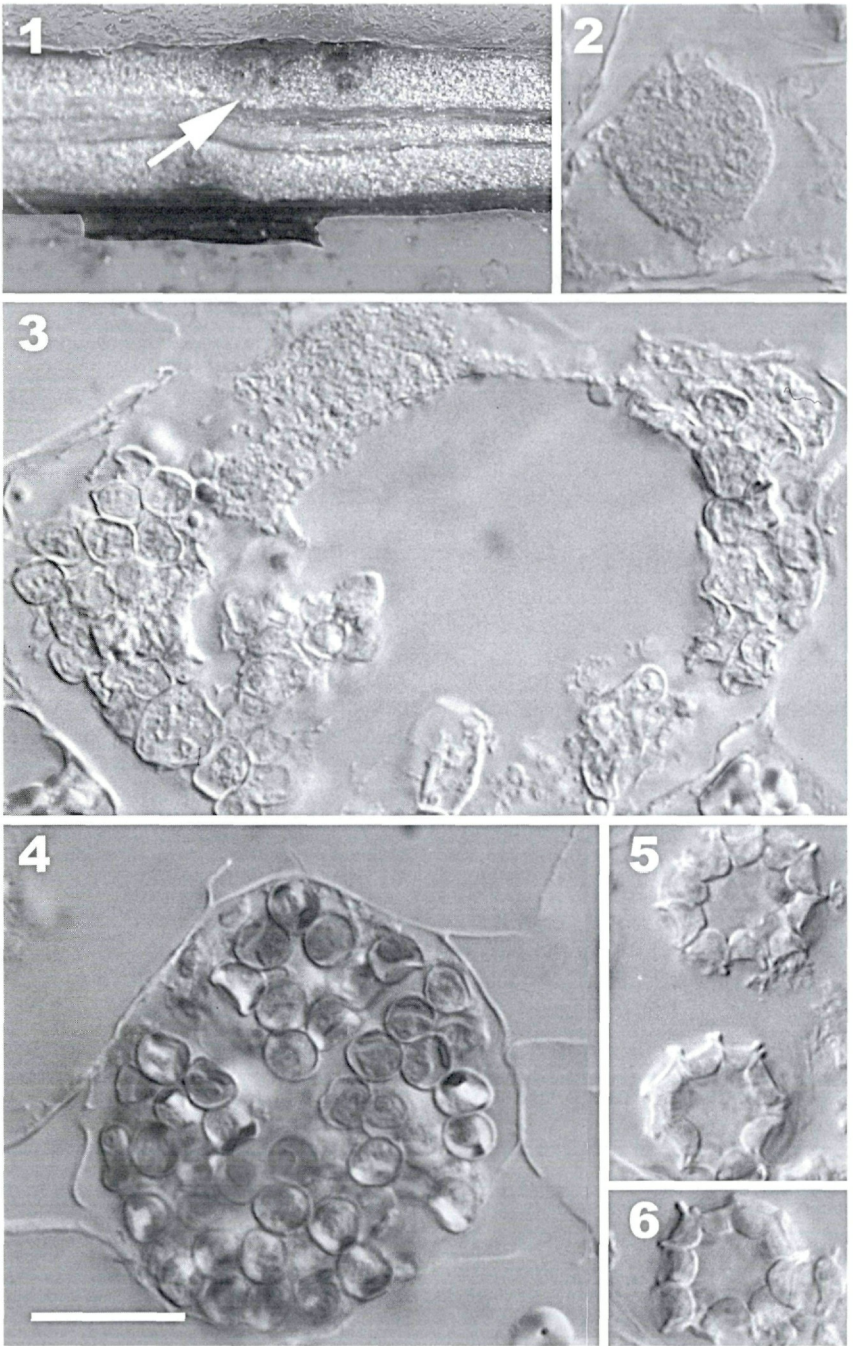
***Sorosphaera viticola* sp. nov.** – Figs. 1–14.

Sporae breves ellipsoideo-cuneatae, apice depresso. 4–5  $\mu\text{m}$  lato, 2.5–3  $\mu\text{m}$  alto, plerumque parietibus lateralibus in soros cavos arcte conjunctae. Soris sporarum globosis vel ellipsoideis, 9–17  $\mu\text{m}$  latis. In cellulis corticis radicum, valde incrassatis, numerose nidulantibus.

Planta hospitalis: in radicibus *Vitis ripariae*  $\times$  *V. berlandieri*.

Holotypus. – GERMANY, Rheingau (Kiedrich), 6 May 2003, in roots of *Vitis berlandieri*  $\times$  *V. riparia* SO4, leg. et det. Huber, Hammes, Kirchmair (IB 2003/0001).

Root necrosis of different dimensions are restricted to the cortex, the central stele is not affected (Fig. 1). – Sporogenic plasmodia are multinucleate and often fill out the entire infected cortex cell; cleavage into uninucleate cells leads to the formation of resting spores (Figs. 2–6). – Resting spores: Primarily disordered, thin-walled cells (Fig. 4) mature to thick-walled spores which are saucer shaped, roundish to somewhat polygonal in plan view, measuring  $4.4 \pm 0.3 \mu\text{m}$  in diameter and  $2.7 \pm 0.2 \mu\text{m}$  in total height (side view;  $n = 31$ ). TEM micrographs (Figs. 9–12) show a three-layered cell wall with a total thickness of 200–250 nm. A thin, electron-dense outer layer ( $W_1$ ) passes into the interlacing matrix between the single spores of the sporosorus and can be seen as remnant of plasmodium. The middle, more or less electron-opaque  $W_2$  layer is distinctly thickened in the region of the germ pore. The innermost, electron lucent  $W_3$  layer is interspersed by electron dense rootlets. This layer forms a germ pore plug ( $W_3'$ ) which becomes thicker and more electron lucent during the ripening of the resting spore (wall layer terminology according to Yukawa & Tanaka 1979). Fifteen to thirty mature resting spores arrange to hollow, spherical to elliptic sporosori of  $13.5 \pm 1.9 \mu\text{m} \times 12.2 \pm 1.9 \mu\text{m}$  ( $Q = 1.1 \pm 0.1$ ;  $n = 31$ )



with a hollow centre (Figs. 5–6 and Figs. 7–8). Using epifluorescence microscopy, the major part of the sporosori develops a characteristic yellow to yellowish-green fluorescence at an acceleration of 450–490 nm. – Primary zoospores [ $4.0\text{--}5.2 \times 2.7\text{--}3.3 \mu\text{m}$  ( $n = 5$ )] are biflagellate with two unequal long flagella: a longer flagellum trailed behind ( $10.5\text{--}11.9 \mu\text{m}$  long) and a shorter pulling flagellum of  $6.0\text{--}8.6 \mu\text{m}$  length (Figs. 13–14). – Sporogenic plasmodia and zoosporangia were not observed.

**Etymology.** – The species epithet refers to the host genus (Lat.: *viticulus*, vine-inhabiting).

**Host plant.** – The parasite can be found in roots and rootlets of vines. Small necrotic areas but no hypertrophies around the infected plant tissue are developed.

**Distribution.** – Until now infected roots were only found in two vineyards in the German Rheingau.

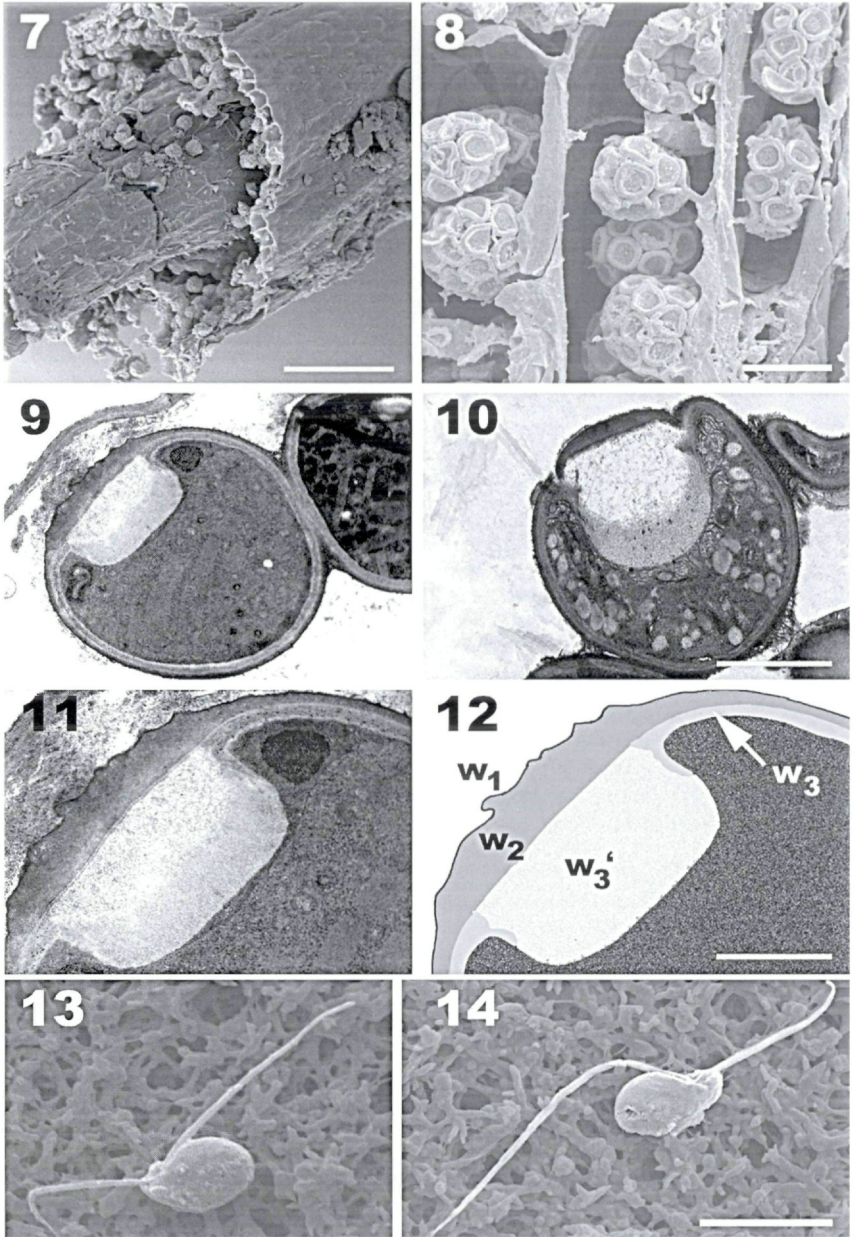
**Material examined.** – *Sorosphaera viticola*: GERMANY, Rheingau (Kiedrich), 6 May 2003, in roots of *Vitis berlandieri*  $\times$  *V. riparia* SO4, *leg. et det.* Huber, Hammes, Kirchmair (IB 2003/0001 – holotypus), Rheingau (Geisenheim) 14 Sep 2004 in roots of *Vitis berlandieri*  $\times$  *V. riparia* 5C, *leg. et det.* Huber, Scholz (IB 2004/0300). – *Plasmodiophora vitis* Viala & Sauv.: ITALY, 1892, *leg. et det.* Briosi and Cavara, (BR 026042.46; M 0049029). – *Sorosphaera veronicae* J. Schröt.: AUSTRIA, Tyrol, Brandenburg, in shoot galls of *Veronica persica* Poirlet, 22 May 2005, *leg. et det.* S. Neuhauser (IB 2005/0100); Tyrol, Brandenburg, in shoot galls of *Veronica persica*, 4 Jun 2005, *leg. et det.* S. Neuhauser (IB 2005/0102); Tyrol, Schwaz, in shoot galls of *Veronica chamaedris* L. 29 May 2005, *leg. et det.* M. Kirchmair (IB 2005/0101); GERMANY, Rheinland, Kempten, in roots, shoots and petioles of *Veronica chamaedris*, 17 Nov – 20 Nov 1912 and 27 Jun – 4 May 1913, *leg.* A. V. Grevillius (BR 026091.95); SWEDEN, Ölandia, Borgholm, in shoots of *Veronicae hederifoliae* L., Jun 1900, *leg.* G. v. Lagerheim (BR 026090.94); Ölandia, Borgholm, in caulibus *Veronica hederifolia* Jun 1900, *leg.* G. v. Lagerheim (BR 026089.93); POLAND, Silesia, Liegnitz, 1879, on the way from Katzbachbrücke to Panten in *Veronica agr.* and *triphyllos*, *leg.* Gerhard und Lehrer (BR 026088.92); Silesia, Liegnitz, 1879, in shoots of *Veronica hederifoliae*, *leg.* Gerhard (BR 026087.91). CZECHIA, Mähr.-Weisskirchen (Hrabuvka), 20 May 1922, on *Veronica hederifolia*, *leg.* Petrak F. (BR 26092.96).

## Discussion

In general, the delimitation of the different plasmodiophorid genera is based on the morphologies of the sporosori (Braselton 2001). The genus *Sorosphaera* was originally described with elliptic-cuneate

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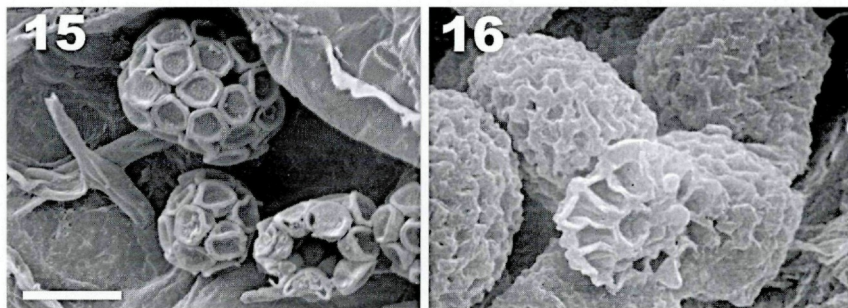
**Figs. 1–6.** Light micrographs of *Sorosphaera viticola*. **1.** Necrosis in a fine root of *Vitis berlandieri*  $\times$  *V. riparia* SO4 (arrow). **2.** Young plasmodium. **3.** Plasmodium cleaving into resting spores. **4.** Un-mature resting spores. **5–6.** Mature resting spores aggregated into hollow sporosori (bar = 10  $\mu\text{m}$ ).



**Figs. 7–14.** Electron micrographs of *Sorospaera viticola*. **7–8.** Sporosori in a fine root of *Vitis berlandieri* × *V. riparia* SO4 (fig. 7: bar = 100  $\mu$ m; fig. 8: bar = 10  $\mu$ m). **9–10.** TEM micrographs of resting spores (bar = 2  $\mu$ m). **11–12.** Higher magnification of fig. 9, showing the assembly of the cell wall: a relatively thin W<sub>1</sub> layer; the W<sub>2</sub> layer with a distinct thickening at the germ pore region; an electron opaque W<sub>3</sub> layer forming a germ pore plug (W<sub>3</sub>’; bar = 1  $\mu$ m). **13–14.** SEM micrographs of the primary zoospores (bar = 5  $\mu$ m).

resting spores, which are aggregated to hollow spheres (Schröter, 1889). In the original diagnosis of *S. veronicae*, Schröter (1889) described the sporosori enveloped by an additional 'cuticula'. This feature was included to the genus diagnosis by Cook (1933). The presence of a common envelope could not be observed by subsequent studies on the development of the sporosori of *S. veronicae* (Miller 1958).

Dylewski (1990) named *S. veronicae* as the only species of the genus. In Cook (1933), Karling (1968) and Dick (2001) two species (*S. veronicae* and *S. radicalis* Cook & Schwartz) are described. The grapevine parasite is distinct from both species by morphological features and by its host (Tab. 1): *Sorosphaera veronicae* is known from different speedwell species only. Single resting spores are significantly longer and the entire sporosori are larger than that of *S. viticola* (Figs 15–16). The shape of the resting spores is different as well: A length/width quotient of about 1.4 was calculated for *S. veronicae* whereas a value of 0.6 was found for *S. viticola*. *Sorosphaera radicalis* occurs in root-hairs of different Poaceae. Beside of the hosts this species differs from *S. viticola* by the size and shape of resting spores as well (length/width quotient: 1.5). As no material of *S. radicalis* was available, measurements are based on figures from Karling (1968).



**Figs. 15–16.** Sporosori of *Sorosphaera viticola* (15) and *Sorosphaera veronicae* (16). Scanning electron micrographs (bar =10 µm).

At the end of the 19<sup>th</sup> century two plasmodiophorids parasiting grapevine have been described: *Plasmodiophora vitis* Viala and Sauvageau and *Plasmodiophora californicae* Viala and Sauvageau (Viala & Sauvageau 1882 a, b). *Plasmodiophora vitis* was described as causative agent for the so called “brunnisure”, an early browning of vine leaves (Viala & Sauvageau 1882 a). In 1893 Massee came to the conclusion that the plasmodia and amoebae figured by Viala and Sauvageau (1892 a, b) were nothing but vacuolated tannin vesicles.

This view was confirmed by Ducomet (1903, 1907) and Ravaz (1904). During this study we carried out another careful microscopical investigation (LM and SEM) of *Plasmodiophora vitis* specimens from Italy studied by Briosi and Cavarra (1892). No organisms or structures reminding a plasmodiophorid could be observed in these specimens. *Plasmodiophora californicae* was thought to cause the Californian grapevine disease (Viala & Sauvageau 1892b), today better known as Pierce’s disease. The Pierce’s disease is caused by *Xylella fastidiosa* Wells *et al.*, a gram negative bacterium in the xylem of grapevine leaves (Wells *et al.* 1987). The structures observed by Viala and Sauvageau (1892b) could have been bacteria or as proposed by Masee (1893) vacuolated tannin vesicles. Both species have been excluded as “doubtful species” from the plasmodiophorids in the monographs of Cook (1933), Karling (1968) and Dick (2001).

The type locality of *S. viticola* was planted with “Pinot Noir” on SO4, a hybrid of *V. berlandieri* Planch × *V. riparia* Michx. In a second stand *S. viticola* was found in roots of 5C, another hybrid of *V. berlandieri* × *V. riparia*. One can speculate that this plasmodiophorid was introduced to Europe when it became necessary to graft American rootstocks to vinifera plants after the phyloxera epidemic of the 1870s. To detect the original host of *S. viticola* it will be necessary to screen American *V. riparia* or *V. berlandieri* stands as well as “wild” European *V. vinifera* populations. Some plasmodiophorid species cause economic losses by transmission of a range of plant viruses (Rochon *et al.* 2004). Recently, grapevines have been shown to suffer from several viruses for which the vectors remain unknown (Bovey *et al.* 1980; Frison & Ikin 1991). The new plasmodiophorid could be such a potential vector of grapevine viruses. Molecular characterisation as well as its distribution, abundance and host range are subject of future research.

Table 1: Discriminating characters in the genus *Sorosphaera*.

	<i>S. veronicae</i>	<i>S. radicalis</i> *	<i>S. viticola</i>
Host	<i>Veronica spp.</i>	Different Poaceae	<i>Vitis spp.</i>
Hypertrophies	Shoot galls	Enlargement of root hairs	none
Sporosori	Spherical, ellipsoid	Ovoid, ellipsoid,	Spherical, ellipsoid
Resting spores	Cuneate	elongate	Saucer shaped
	Exterior diameter:	Exterior diameter:	Exterior diameter:
	4–5 µm	3 µm	4–5 µm
	Length:	Length:	Length:
	5,5–6,5 µm	4 µm	2.5–3 µm

\* specifications after Cook (1933).



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