Research Article

Cribraria cancellata (Bastch) Nann.-Bremek. (Protista, Myxomycetes) in Yerba Mate Seedlings (*Ilex paraguariensis* A. St.-Hil. – Aquifoliaceae)

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Abstract

Myxomycetes are amoeboid protists often misidentified as fungi. They play important ecological roles in nutrient cycling and act as microbial predators in various ecosystems. *Ilex paraguariensis*, commonly known as yerba mate, is a culturally and economically significant tree species native to southern South America, widely consumed in traditional infusions such as "chimarrão" or "mate." Yerba mate cultivation is frequently impacted by phytopathological issues, primarily fungal diseases. Accurate identification of myxomycetes in association with economically important crops is essential to prevent the unnecessary use of fungicides, avoid confusion with true fungal pathogens, and reduce potential economic losses. In this study, individuals of *Cribraria cancellata* were observed growing on the substrate of yerba mate seedlings maintained in a greenhouse in southern Brazil. This is the first report of myxomycetes associated with yerba mate cultivation, with no symptoms or phytotoxic effects observed.

Introduction

Myxomycetes, known as mucilaginous molds, belong to the phylum Amoebozoa [1,2] but form spores contained in structures that resemble those of fungi, which has led to confusion in the observation of their presence in plants and to their erroneous identification as phytopathogens [3]. Just over 1000 species are known [4], of which approximately 25% have been recorded to Brazil [5]. Their life cycle is complex, involving mobile trophic stages, which include a flagellated amoeba and a multinucleated plasmodium, and a sporulating phase, when they attach themselves to the substrate [2,6-8]. Acting as predators of fungi and bacteria, these organisms play an important role in terrestrial nutrient cycling [2].

Some species of myxomycetes, almost all belonging to the order Physarales, have been treated as plant pathogens in agricultural crops in different countries [9-12]. Although the presence of these organisms does not pose a threat to crop health, this misunderstanding has led to the improper application of pesticides by producers, culminating in economic losses and hindering the adoption of more appropriate control methods [3].

Domingues et al. [13] documented for the first time in Brazil the presence of the myxomycete Diachea leucopodia (Bull.) Rostaf. in strawberry plants grown commercially in the interior of São Paulo; the authors found a great abundance of sporangia on the leaves, stolons and petioles of the plants in the cultivation area. Ribeiro and Brioso [14] described a similar episode in southern Minas Gerais, where the same species was detected in several organs of strawberry plants. In 1998, a new fungal disease called bean smut was identified affecting soybean and bean crops in Brazil, attributed to fungi of the genus *Ustilago* and, later, to *Microbotryum phaseoli* [15]. After analyzing the symptoms, Agra et al. [10] concluded that bean smut was not a fungal disease, since it consisted of extensive sporulation of Physarum cinereum (Batsch) Pers., a myxomycete that generates dark brown spores distributed on the surface of leaves, stems, and pods of beans and soybeans.

Silva and Bezerra [9] reported the presence of *Fuligo septica* (L.) Wiggers in lettuce (*Lactuca sativa* L.) and coriander (*Eryngium foetidum* L.), based on samples collected in Maranhão - Brazil; the authors highlighted that, although *F. septica* is not considered a parasite, its presence in lettuce and coriander caused significative losses, since the products were rejected by consumers in the market. In the municipality of São Gabriel, Rio Grande do Sul, Velloso et al. [16] documented the occurrence of *Physarum cinereum* in home-grown broccoli (*Brassica oleracea L. var. italica*) without causing damage to the plants.

Yerba mate (*Ilex paraguariensis* A.St.-Hil., Aquifoliaceae) is a very important crop culture in the southern of South America, whose area of occurrence encompasses the entire southern region of Brazil, northwestern Argentina and eastern Paraguay, and is recognized for its pharmacological, nutritional, therapeutic properties, and for its socioeconomic and ecological importance. Its leaves serve as raw material for a series of traditional drinks (mate tea, mate, chimarrão, tererê), consumed in the form of teas and infusions [17]. Considering the world production of yerba mate in 2012-2022, Argentina holds the title of the largest producer, with an average of 615 thousand tons produced, followed by Brazil, with 565 thousand tons and Paraguay, with 103 thousand tons [18]. However, it is important to consider that the average production value is highly variable, since it is directly related to the climate and crop management [19]. Given its importance, yerba mate was recognized in 2018 as a cultural heritage of Mercosul, and in 2023, as the 1st intangible cultural heritage of Rio Grande do Sul – Brazil [20,21]. Its cultivation plays an important socioeconomic role, since it is mainly produced by small and medium-sized producers, whose labor is family-based, distributed across more than 12,000 rural properties in southern Brazil [22].

The main challenges faced in the production of yerba mate are diseases caused by fungi, among which we can mention black spot, caused by the fungus *Calonectria pseudonaviculata* (Crous, JZ Groenew. & CF Hill) L. Lombard, MJ Wingf. & Crous, seedling damping-off and root rot, whose main associated fungi are species of *Fusarium, Rhizoctonia, Rhizopus* and oomycetes, such as *Pythium* and *Phytophthora* [23-25].

This paper reports the occurrence of *Cribraria cancellata* (Batsch) Nann.-Bremek. growing on substrate for yerba mate (*Ilex paraguariensis* A. St.-Hil.) seedling development in a greenhouse in Rio Grande do Sul, and describes and illustrates its sporangia for recognition in which its presence can be verified in cultivated areas.

Materials and Methods

The specimens were found sporulating on the soil, without contact with the yerba mate seedlings, in a culture prepared in Styrofoam trays containing a commercial substrate composed of pine bark, natural phosphate, carbonized rice husk, vermiculite and NPK chemical fertilizer, moistened by spraying water three times a day for a period of 30 minutes. The material was removed from the substrate, stored in a small cardboard box so that the fragile structures were not damaged, and sent to the Fungal Taxonomy Laboratory of the Universidade Federal do Pampa. For species identification, the sporangia were analyzed under an Olympus SZ2 ILST stereoscopic microscope and an optical microscope, using a potassium hydroxide solution (KOH 3%) to rehydrate the spores. Subsequently, the material was dehydrated in a DeLeo digital bacteriological culture oven and incorporated into the collection of the Bruno Edgar Irgang Herbarium (HBEI).

Results

The examined specimen presents the diagnostic characteristics of *Cribraria cancellata* (Batsch) Nann.- Bremek., as aggregated sporangia, long-pedicellate, 2.95-3.5 mm in total height, sporotheca hanging, globose, umbilicated at the base, 0.4-0.5 mm diam., rusty brown; cylindrical pedicel, tapering at the apex, about ¾ of the total height, dark brown, lighter in the twisted part of the apex; irregular, membranous, dark brown hypothallus; brown peridial network, formed by longitudinal ribs connected by thin transverse filaments; dark brown spores in mass; spores globose, light brown in transmitted light, warty, (5.10-) 5.21 x 6.035 (-6.10) µm diam (Figure 1).

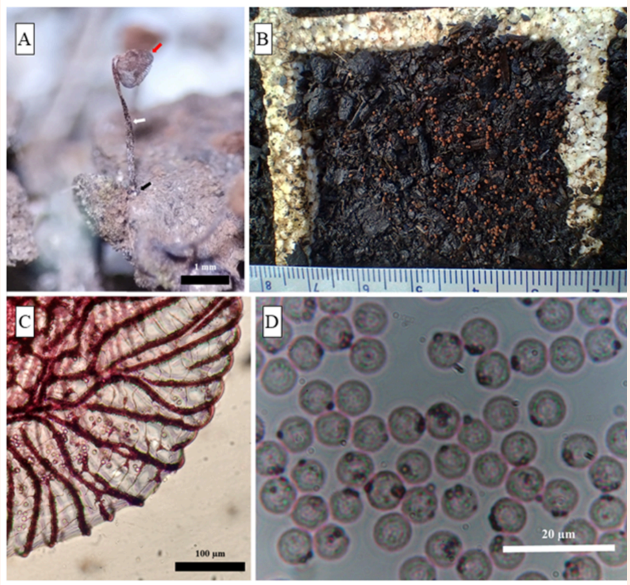

Figure 1: *Cribraria cancellata*. A. sporangia (red arrow: sporotheca; white arrow: Stalk; black arrow: hypothallus). B. Sporangia in situ. C. Details of the longitudinal ribs of the sporotheca. D. Spores.

Discussion

Cribraria cancellata is one of the most common species of myxomycetes, widely distributed in Brazil and worldwide [5,26]. It is also one of the most easily identified in the field, with the aid of a hand-held magnifying glass. Most reports of occurrence indicate that it is predominantly lignicolous, developing plasmodia and sporulating more frequently on dead trunks and branches, with no reports of causing damage to living plants.

Since the beginning of the second half of the 20th century, there has been discussion about the possible phytopathogenic potential of myxomycetes. Martin and Alexopoulos [27], in one of the best-known works on the group to date, mentioned that although myxomycetes had attracted attention, their phytopathogenic potential had not yet been proven at that time. Today it is known that, although plasmodium does not have the ability to penetrate the internal tissues of plants, it can cause a significant loss of the photosynthetic rate when covering the leaves and, depending on the extent of sporulation, even cause local lesions; when developing and sporulating on fruits, myxomycetes can reduce their commercial value [13]. After reviewing several studies that address the possible phytopathogenicity of myxomycetes, Nieves-Rivera [28] concluded that many studies were still necessary to understand whether important physiological relationships (photosynthesis, transpiration or gas exchange) actually occur in the "host" plants, discouraging the use of fungicides in their control.

On the other hand, it is important to emphasize that myxomycetes are predators of microorganisms, such as fungi and bacteria [2], which sheds light on a possible potential for biological control of true phytopathogens, opening gaps for future investigations on myxomycete-plant interactions. In conclusion, we recommend that, when observing myxomycetes in plants of economic interest, the most appropriate procedure is to remove the "infected" part of the substrate or plant and discard it in an environment with decomposing plant remains, such as the leaf litter of a forest.

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