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A record of *Ceratiomyxa hemisphaerica* from Germany

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Abstract: *Ceratiomyxa hemisphaerica* was described by Olive and Stoianovitch in 1979 from material collected in the United States and has since been reported from Canada and New Zealand. Specimens which appear to represent the species were cultivated several times in a moist chamber on pieces of decayed wood collected in southern Germany and are described, illustrated, and discussed herein.

Keywords: biogeography, Ceratiomyxomycetes, distribution, ecology

Introduction

Species with visible fructifications within the eukaryotic protists of the supergroup Amoebozoa (Ruggiero et al. 2015; Adl et al. 2019) have attracted the interest of researchers for more than 200 years due to their ability to form macroscopic fructifications releasing airborne spores (Stephenson et al. 2008). Within the Amoebozoa, several groups independently evolved the ability to fruit (Kang et al. 2017), with the plasmodial slime molds (class Myxomycetes [Leontyev et al. 2019]) as the most diverse and most conspicuous group.

However, one sister group, the class Ceratiomyxomycetes, develops as well macroscopic fructifications. In contrast to the myxomycetes, the spores are not formed internally in a sporocarp but externally on separate stalks. Whereas the genera *Clastostelium* and *Protosporangium* (formerly regarded as members of the protostelids) remain microscopically small, fructifications of *Ceratiomyxa* develop multiple fruit bodies, each consisting of one spore on a stalk. They share a common layer of extracellular slime, called the hypothallus, which gives them macroscopic size. For this reason, the genus is considered in nearly all field studies of myxomycetes (Schnittler et al. 2021) and in nomenclatorial data bases such as Lado (2005-2023).

According to this data base, ten taxa are described within *Ceratiomyxa*, of which only four are known from numerous observations, with the very variable *C. fruticulosa* (O.F. Müll.) T. Macbr. as the taxon best known, since it is, together with the varieties *arbuscula* and *poroides*, widely distributed in

temperate and tropical zones. As well locally abundant, but virtually restricted to the tropics, are *C. morchella* A.L. Welden and *C. sphaerosperma* Boedijn, which differ remarkedly in their ecological niches (Rojas et al. 2008). *Ceratiomyxa hemisphaerica* Olive & Stoianovitch (1979) has rarely been reported and is currently known from only a few locations in the United States (incl. Hawaii), Canada, and New Zealand.

During his activities as a macro photographer, Karsten Buch began cultivating myxomycetes in moist chambers, a technique widely applied in diversity research on myxomycetes (e.g., Härkönen 1981). Located in Berlin, Germany, he collected several pieces of wood in southern Germany for cultivation. By the time of collection in July 2021, some old fruit bodies of *Arcyria* cf. *affinis* were found on a pile of logs, which he intended to cultivate.

Materials and methods

On July 18th, 2021, Karsten collected several pieces of decaying wood on an excursion in the area of the rural community of Kusel in southern Germany in a mixed forest. Some old fruit bodies of *Arcyria* cf. *affinis* and *Physarum album* were present, which he intended to cultivate. One of the pieces was taken from a pile of logs of presumably *Picea abies*. The wood was in a state of decay where it was possible to break off pieces by hand, but it still had considerable stability.

Back in Berlin the next day, he started the well-known moist chamber cultivation (i.e., watered the wood overnight, poured the excess water the next day, and put it on kitchen paper in a plastic box with a closed lid). He stored it indoors in a room away from direct sunlight. The temperature in the room presumably did not drop below 22° Celsius during the night and was likely to reach over 25° by day. The substrate was kept very moist. Tap water was added when it started to dry.

Karsten took pictures with a reversed 24-70 mm wide angle lens at 24 mm focal length mounted on a 136 mm stack of extension rings on a Nikon D750 DSLR, resulting in a 9x magnification. Between 60 and 90 images were stacked to achieve a suitable depth-of-field, using Adobe Lightroom and Photoshop for pre- and post-processing and Helicon Focus for stacking. A basic microscopic study was conducted with a Bresser Erudit microscope before the fruit bodies became degraded about one week later.

From another fructification in 2022, images were taken with Mitutoyo 10x and 20x microscope objectives adapted to the DSLR, which required 300 to 400 photos for stacking, and allowed for more detailed measuring, and counting of fruit bodies per hypothallus ‘head’. Spores were analyzed at the Univ. of Greifswald with a Keyence stereo microscope.

Results

Location: Germany, Rhineland-Palatinate, near Kaiserslautern, Nordpfälzer Bergland region: Potzberg (Föckelberg), mixed forest, at a pile of logs, presumably *Picea abies*, 7.479° E, 49.522° N, 540 m asl.

Culture: First fruit bodies appeared after three weeks in moist chamber culture (July 18-August 8, 2021) on decayed, but still solid wood kept very moist. On August 10, 13, and September 9 more fruit bodies appeared. Some showed up between moss that was growing on the wood. In mid-June 2022,

Karsten started the cultivation again, and on the 5th of July – again in warm summer indoor conditions – a large group of over 40 individuals appeared. In comparison to the ones from 2021, their hypothallus did not form a noticeable stalk-like structure.

Figure 1. Fructifications. A, hypothallus forming pronounced stalks below the hemispherical head that carries the fruit bodies (9x, 2021). B, group between moss on wood (9x, 2021). C, nearly lacking the hypothallus stalk (20x, 2022). D, extended hypothallus carrying two spherical heads with fruit bodies (20x, 2022). E (detail of C), fruit bodies with flexuous stalk and spore. F, group from above, showing the spherical hypothallus heads with fruit bodies.

Microscopic observations:

Hypothallus transparent, gelatinous (nearly liquid), colorless, forming pedicel-like elevated structures with ‘heads’ that carry colonies of sporophores. Sometimes a ‘head’ is supported by a ‘stalk’. The ‘pedicels’ are gregarious, in groups of sometimes reaching or exceeding 40 individuals (Fig. 1A–C, F), sometimes coalescing into a gelatinous base with up to three heads (Fig. 1D). Note: the ‚pedicel’ heads and stalks are part of the hypothallus and must not be confused with those of myxomycetes which are part of the fruit body.

Hypothallus stalk absent or transparent, gelatinous, colorless, up to 0.15 mm tall (Fig. 2A), sometimes clearly separated from the head, which was very prominent in the individuals cultivated in 2021 (Fig. 1A).

Hypothallus head hemispherical to nearly (when supported by a hypothallus stalk) spherical, transparent, gelatinous, colorless, 0.07–0.135 mm of diameter (Fig. 2B), carrying around 400 radially arranged fruit bodies.

Fruit bodies consist of a long, thin, stalk-like sporophore and a spore.

Sporophores 0.03–0.26 mm in length and 2.4–3.0 µm in diameter, more or less flexuous, colorless, sometimes twisted, each carrying one transparent spore on the nodding end (Fig. 1E).

Spores colorless, spherical, 8.5–12.0 x 8.0–10.5 µm (Fig. 2C–D).

Discussion

In contrast to fructifications of the slime molds of the class myxomycetes, where fructifications may last all winter at sheltered lower sides of logs, the fruit bodies of *Ceratiomyxa* are extremely ephemeral, hardly lasting more than a week. Specimens sent to the working group of Martin Schnittler were hardly visible when arriving. However, spores and/or plasmodia must be persistent: Karsten interrupted his cultivation attempts between October and May with an intermediate short period of two weeks in February, and the wood dried out in the meantime. A second cultivation attempt from the same piece of wood a year later (2022) once again produced fruit bodies. Another part of the material was sent to D. Leontyev (currently Greifswald), who succeeded in extracting and analyzing the spores. Fruit bodies of *Cribraria microcarpa* were present simultaneously.

Generally speaking, successful cultivation seems to need a warm (over 20° Celsius during day and night, likely higher temperatures), very moist environment.

After several attempts to find experts who could identify the specimen, Karsten posted the photos in the 'Slime Mold Identification & Appreciation’ group in Facebook, where Frederick W. Spiegel suggested that it might be a *Ceratiomyxa* sp., and, judging from the photo, potentially a new species.

Via Marion Geib, who published a field guide on German myxomycetes, Karsten got in contact with Martin Schnittler at the Univ. of Greifswald, Germany, who was interested in analyzing the material. As with other members of the Ceratiomyxales, the fruit bodies decayed within a few days, and did not occur again until July 2022. A specimen was then sent to Dmytro Leontyev at the University of Greifswald for analysis and sequencing, but arrived in a very poor condition. Sequencing failed but a microscopic

analysis of the spores suggested that it was *C. hemisphaerica*. To our knowledge, this is the first record of the species in Germany and Europe. A search of records at GBIF (April 2023) resulted in 101 records (but many from the same locations, probably the same records uploaded from multiple sources), coming from the Canada and the US (5 locations), Hawaii (1) and New Zealand (1); records from Europe, but as well from equatorial zones, are absent.

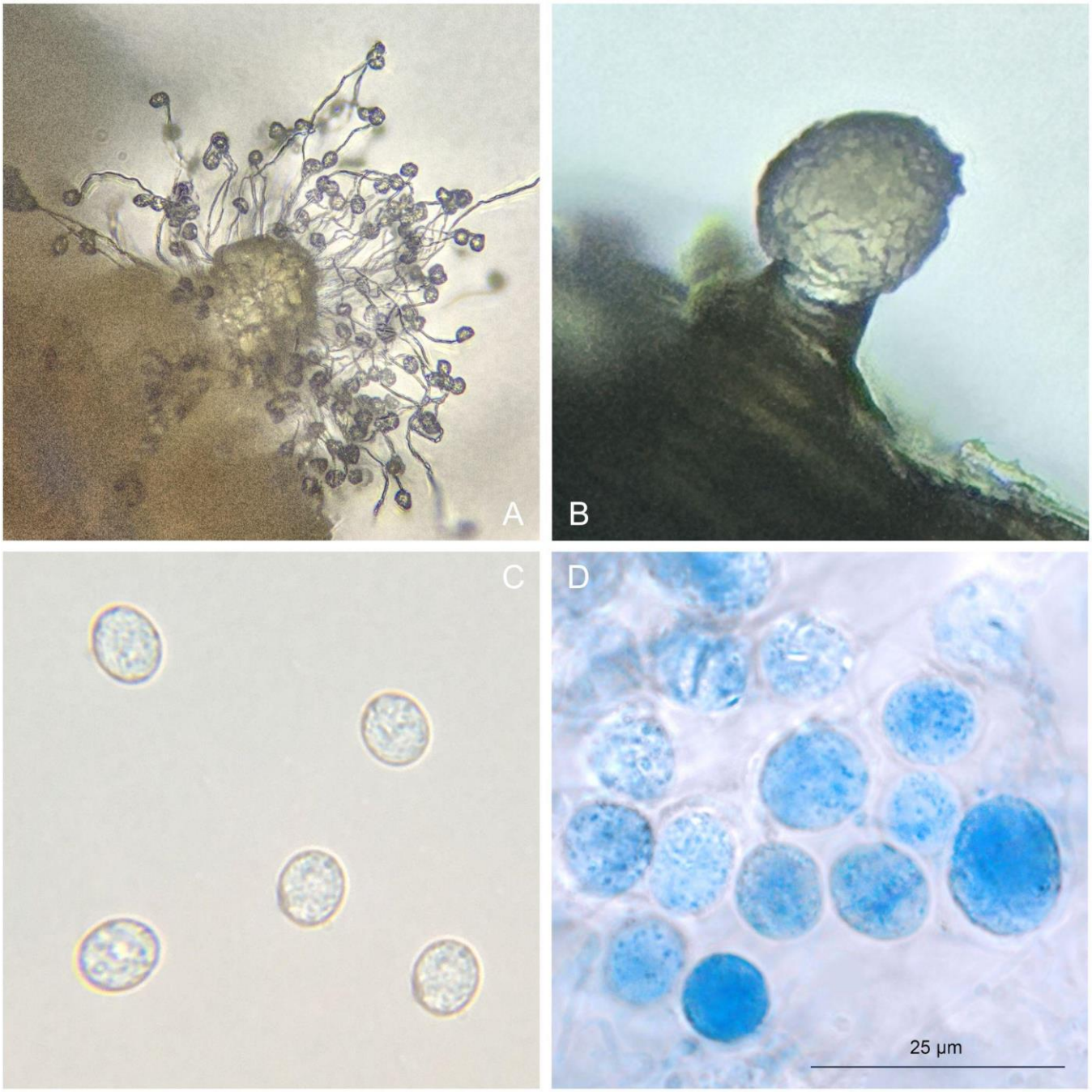

Figure 2. Microscopic analysis. A, fruit body (200x, 2021). B, fruit body after spores are shed (200x, 2021). C, spores (1000x, 2021). D, spores in water (1000x, 2021), stained with methyl blue in lactic acid (image D. Leontyev).

The morphology of the fruit bodies seems to be rather plastic. While the stalks that were present on the fruit bodies in 2021 seem to suggest a new species, all other properties – and the nearly stalk-less appearance in 2022 – are in line with the description of *C. hemisphaerica*. Due to the failure of the sequencing attempt – and the lack of reference sequences in Genbank – it seems not to be justified to describe a new species based on the presence of a stalk. It should also be taken into account that myxomycetes may develop aberrant forms under moist chamber conditions. We therefore suggest amending the description of the species with the possibility of developing a stalk.

Further attempts should be undertaken to confirm the species in situ. Unfortunately, the original pile of logs was removed in the meantime but the location is publicly accessible, and decaying wood is always present there.

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