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Clastoderma confusum (Myxomycetes: Amoebozoa), a remarkable new species of slime mould from Western Australia

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SHORT COMMUNICATION

Myxomycetes (slime moulds) are a diverse group of *c*. 1,000 species of amoeboid eukaryotes (Lado 2005–2019). They are common in the soil and on decaying vegetable matter such as wood and leaf litter, although it may be difficult to detect these organisms due to their diminutive size (often less than 1 mm high) and dependence on conducive seasonal conditions for sporulation. Hence, while new species are sometimes discovered in the field, they are more often found through the use of what is termed the moist chamber culture. When conditions are unfavourable for continued growth, macroscopic fruiting bodies that contain spores are formed. Prior to this, myxomycetes exist in a vegetative phase as a plasmodium—a giant, multi-nucleate amoeba formed from the merging of two compatible free-living myxoflagellates or myxamoebae cells from germinating spores.

The remarkable new slime mould described herein was first discovered by Margaret H. Brims from a substrate that she collected from Mt Caroline, *c*. 170 km east of Perth in Western Australia. The material was collected in April 2003, placed in a moist chamber the following September, and then fruiting bodies harvested six days later. She was unable to confidently place the collection in a genus and so forwarded material to a specialist in the United Kingdom, who in early 2005 identified it as belonging to the genus *Cribraria* Pers. Its taxonomic status has remained unresolved due to a lack of adequate material.

New material from a substrate collected in the Little Sandy Desert in July 2018, placed in a moist chamber by one of us (KJK) in January 2019, and with fruiting bodies harvested eight days later, was also initially misidentified as a putative new species of *Cribraria* because of its beautiful, rigid, shiny net, lack of a columella, and an uncanny but superficial resemblance to *C. confusa* Nann.-Bremek. & Y.Yamam. (with the exception of its black colouring). However, upon further investigation it became apparent that this taxon belonged in the genus *Clastoderma* A.Blytt (Martin & Alexopoulos 1969; Poulain *et al.* 2011) since the net is formed by the capillitium rather than the peridium as in *Cribraria*.

Clastoderma is a small genus with a worldwide distribution (GBIF Secretariat 2017): *C. microcarpum* (Meyl.) Kowalski is confined to the northern hemisphere; *C. pachypus* Nann.-Bremek. also occurs in the northern hemisphere, with a single occurrence in New South Wales, Australia; and the most common species with a worldwide distribution, *C. debaryanum* A.Blytt, (Martin & Alexopolous

1969), is also found in the North Kimberley, Swan Coastal Plain and Jarrah Forest bioregions of Western Australia (Western Australian Herbarium 1998–). The new discovery described below is currently known only from three locations in Western Australia but is likely to be more widespread.

Clastoderma confusum K.J.Knight & Lado, sp. nov. (MB 832527).

Type: Eagle Highway, 450 km north-east of Wiluna, Little Sandy Desert, Western Australia, 24 July 2018, *K.J. Knight* MC 154 [from moist chamber culture of bark of prone dead *Acacia aneura*, January 2019] (*holo*: PERTH 09078509; *iso*: CANB, K, MA-Fungi 90498).

Clastoderma sp. Mungilli (K.J. Knight MC 154), Western Australian Herbarium, in *FloraBase*, https://florabase.dpaw.wa.gov.au/ [accessed 3 October 2019].

Sporocarps gregarious, stalked, 0.25–0.46 mm high. Hypothallus bulbous and/or disc-like, sometimes insignificant, black, white or light brownish. Stalks c. 75% total height of the sporocarp, curved, frequently nodding, cylindrical, gently tapering from base to near the apex, with a slightly flared apex subtended by a small, straight section c. $10-40 \times 5-10$ µm; surface longitudinally rugose, dull, black or dark brown, sometimes with a pale, almost colourless section below the apex; walls pale ochraceous to colourless by transmitted light, grading to jet black c. 25 µm below the apex, densely filled with dark, granular refuse basally which becomes lighter and less dense distally. Sporotheca globose, 80-130 µm diam., dark brown or black. Peridium membranous, shiny, fugacious except for a collar and numerous minute and irregular fragments persisting either directly on the external surface of the capillitial mesh or on minute capillitial stubs of variable length along the capillitium, fragments forming an interrupted patterning of a shiny mesh with copper-coloured reflections; brown or violaceous-brown by transmitted light; collar relatively large, c. 30 µm diam., irregularly stellatewebbed between the capillitial threads, attached to the capillitium at the base and for a short distance up the threads, minutely striate. Columella absent. Capillitium with 4-6 threads radiating at almost right angles from the stalk apex; threads branching and anastomosing, forming a globose, rigid, complete, many-meshed net (the net sometimes collapsed in at the nodes but quickly expanding to globose in Hoyer's medium); meshes mostly angular, (4–)5–7-sided, nodes not differentiated; threads straight, purple-brown or brown by transmitted light, hollow, thick-walled, flat, c. 2 µm wide throughout, without free ends, rarely with short, blunt branches within. Spores globose, (12–)13–16 µm diam., dull brown-black or black in mass, mid-brown, brown or pink-brown by transmitted light; surface verruculose, with minute, variably sized and unevenly distributed warts, sometimes arranged in lax, short lines at ×100 with patchy areas of smooth surface, inconspicuous darker patches of warts seen at $\times 40$; by SEM the spore surface is densely minutely ornamented with laxly sinuous ridges, the ridges decorated with struts from apex down to the spore wall and projections on the ridges forming undulating crests, ridges and struts are perforated in patches, warts rounded and occurring on the side of the ridges or between ridges in denser patches, with some thin projections emerging from the base of the wart forming fenestrations. (Figures 1, 2)

Diagnostic features. This species is readily distinguished from all other species of *Clastoderma* by the following combination of features: a distinctive, rigid, capillitial net that arises at almost right angles from the top of the stalk; a mostly angular mesh that is ornamented with abundant, highly reflective, peridial fragments that resemble a shiny, copper-coloured net; a stellate-webbed peridial collar situated in close proximity to the capillitium; the absence of a columella; and patchy, verruculose spore ornamentation.

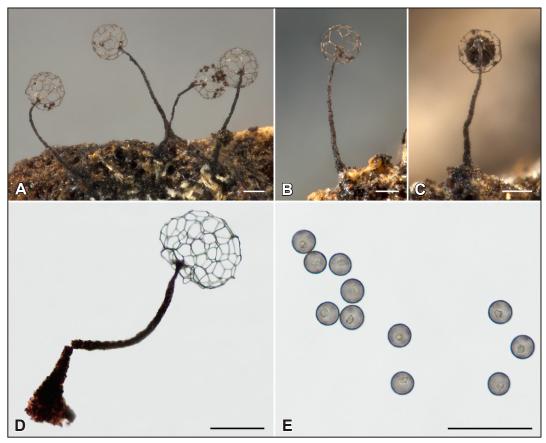


Figure 1. *Clastoderma confusum*. A – a group of sporocarps *in situ* showing the gregarious habit; B – sporocarp showing the globose sporotheca with wide-meshed angular capillitium appearing almost completely shiny; C – sporocarp showing the highly reflective large collar; D – sporocarp by transmitted light showing the dark, narrow straight section of stalk just prior to the flared apex; E – spores by transmitted light showing patches of denser warts on surface. Images from *K.J. Knight* MC 154. Photographs by C. de Mier (A–C) and J.M. Huisman (D, E). Scale bars = 0.1 mm (A–C); 100 μ m (D); 50 μ m (E).

Other specimens examined. WESTERN AUSTRALIA: Mt Caroline, 19 Apr. 2003, *M.H. Brims* 608 (PERTH); Giles Breakaway, 50 km NE of Laverton on Great Central Road, 1 Apr. 2019, *K.J. Knight* MC 169 (PERTH).

Ecology, distribution and habitat. Associated with acidic bark (bark of living *Callitris columellaris*, pH 4.4 – *K.J. Knight* MC 169, bark of prone dead *Acacia aneura*, pH 5.3 – *K.J. Knight* MC 154) and wood-based insect casings. The three known records are disjunct in Western Australia (Western Australian Herbarium 1998–), occurring in semi-arid to arid areas in the Little Sandy Desert, Murchison and Avon Wheatbelt bioregions (Department of the Environment 2013). The type collection and *K.J. Knight* MC 169 are from sparse mulga over spinifex rangeland with summer rainfall, while *M.H. Brims* 608 is from a region characterised by eucalypt woodland or proteaceous scrub with winter rainfall.

Etymology. From the Latin *confusus* (confused), in reference to its previous misidentification as a species of *Cribraria*.

Vernacular name. Copper-netted Clastoderma.

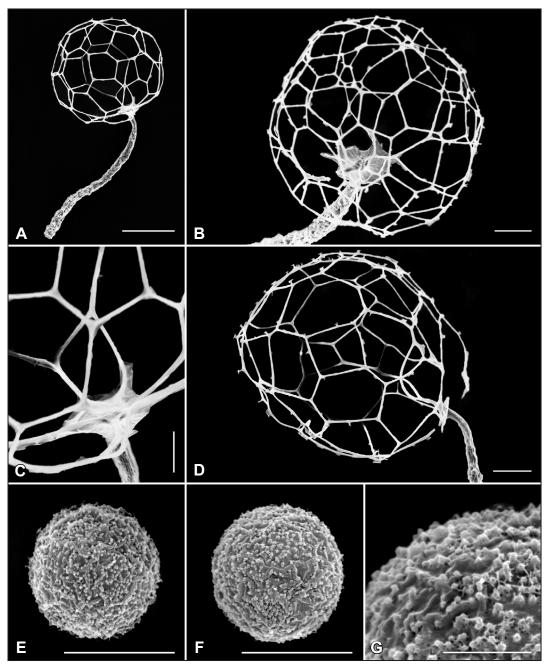


Figure 2. *Clastoderma confusum* by Scanning Electron Microscopy. A – sporocarp showing rugose striations on the stalk, which narrows toward the apex; B – collar showing striations, the web-stellate shape and attachment to the capillitium for a short distance up the threads; C – capillitium, with threads radiating at almost right angles from top of the stalk; D – capillitium, showing numerous peridial fragments on capillitial stubs or directly on capillitial net; E, F – different spores; G – spore surface, showing detail of ornamentation. Images from *K.J. Knight* MC 154. Photographs by K.A. Shepherd (A–D) and C. Lado and Y. Ruiz (E–G). Scale bars: = 50 μ m (A); 20 μ m (B, D); 10 μ m (C, E, F); 3 μ m (G).

Conservation status. Although *C. confusum* is known from only three disjunct collections, it is not considered to be under conservation threat as its occurrence is likely to be more common and widespread. Myxomycetes in Western Australia are poorly known and rarely collected.

Affinities. Clastoderma confusum appears to be most closely allied to C. microcarpum, a species that also has a complete, wide-meshed net and large spores, $13.5-15 \mu m$ diam. (Kowalski 1975). It can readily be distinguished from C. microcarpum by its dark brown or black sporocarps (vs ferruginous), black stalk apex (vs red-brown), patchy, verruculose spore ornamentation (vs evenly ornamented with numerous, scattered, minute papillae) and the absence of a columella. It also has a morphologically distinct capillitium with 4–6 threads that emerge at almost right angles from the stalk apex (vs branching from the columella apex into 2–4 main threads), and numerous persistent peridial fragments (vs peridial fragments entirely lacking or occasionally persisting as minute membranous expansions).

Clastoderma confusum does not adhere to the most recent diagnosis of the genus and new order Clastodermatales Leontyev, Schnittler, S.L. Stephenson, Novozhilov & Shchepin proposed by Leontyev *et al.* (2019). These authors describe the columella as always 'present, gradually turning into the capillitium' and the capillitial threads as 'branched and anastomosed, merging at the periphery to form plate-like swellings'. We note, however, that previous studies have observed that the columella may be lacking in *C. debaryanum* (Martin & Alexopolous 1969; Eliasson & Keller 1996; Poulain *et al.* 2011). Furthermore, the peridial fragments in *C. confusum* are shiny like the collar and irregularly shaped and thus appear to be of peridial rather than capillitial origin. As such, this contradicts what is indicated by Frederick *et al.* (1986) for *C. debaryanum*. The new species, therefore, seems to fit the broader concept of the genus *Clastoderma*, but an exhaustive review of their species is necessary to clarify its distinctive features.

Notes. The collection *M.H. Brims* 608 is in poor condition due to an attack by a filamentous fungus and depauperate since it was split to confirm identification in 2004. It is somewhat atypical in having sporotheca that have mostly collapsed inwards at the capillitial nodes (as some have in the other two collections), slightly darker and smaller spores (brown and *c*. 12 μ m diam. vs mid-brown or pinkbrown and 13–16 μ m diam.), and a stalk with a distinctly paler section distally rather than black or dark-brown as in the other collections where the paler section can only be seen by transmitted light. These differences are minor and not considered to be taxonomically significant.

The type substrate was not originally collected for use in a moist chamber but rather it was forwarded to the Western Australian Herbarium with the fungi *Gloeophyllum abietinum* growing on it. It is of interest to note that the substrate for the type species of the genus, *C. debaryanum*, is probably *G. odoratus* (Eliasson & Keller 1996), and although this new species was not growing directly on the hymenium of the fungi as per *G. odoratus*, there is a possibility of a relationship with this genus of fungus. For Herbarium quarantine purposes, the material was frozen at -18° C for seven consecutive days and accordingly the viability of spores was expected to be low, yet five species of slime mould were harvested, confirming that this procedure is a safe practice prior to moist chamber culture.

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References

- Department of the Environment (2013). Australia's bioregions (IBRA), IBRA7, Commonwealth of Australia. http://www.environment.gov.au/land/nrs/science/ibra#ibra [accessed 23 June 2019].
- Eliasson, U.H. & Keller, H.W. (1996). The genus Clastoderma: taxonomic evaluation and infraspecific variation of C. debaryanum. Mycological Research 100 (5): 601–609.
- Frederick, L., Roth, I.L. & Pendergrass, L. (1986). Observations on the 'Peridial Platelets' of Clastoderma debaryanum. Mycologia 78(2): 263–268.
- GBIF Secretariat (2017). *Clastoderma pachypus* Nann.-Bremek. GBIF Backbone Taxonomy. Checklist dataset https://doi. org/10.15468/39omei [accessed via GBIF.org on 7 September 2019].
- Kowalski, D.T. (1975). The Myxomycete taxa described by Charles Meylan. Mycologia 67(3): 448-494.
- Lado, C. (2005–2019). An online nomenclatural information system of Eumycetozoa. Real Jardín Botánico, CSIC, Madrid, Spain. http://www.nomen.eumycetozoa.com [accessed 23 June 2019].
- Leontyev, D.V., Schnittler, M., Stephenson, S.L., Novozhilov, Y.K. & Shchepin, O.N. (2019). Towards a phylogenetic classification of the Myxomycetes. *Phytotaxa* 399(3): 209–238.
- Martin, G.W. & Alexopoulos, C.J. (1969). The Myxomycetes. (University of Iowa Press: Iowa City.)
- Poulain, M., Meyer, M. & Bozonnet, J. (2011). Les Myxomycètes. (Fédération Mycologique et Botanique Dauphiné-Savoie: Delémont.)
- Western Australian Herbarium (1998–). FloraBase—the Western Australian Flora. Department of Biodiversity, Conservation and Attractions. https://florabase.dpaw.wa.gov.au/ [accessed 3 October 2019].