

The genus *Polycephalomyces* (*Hypocreales*) in the frame of monitoring Veternica cave (Croatia) with a new segregate genus *Perennicordyceps*

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Summary: During the regular biospeleological monitoring in the Veternica cave (Mt. Medvednica, Croatia), particular synnematosus entomogenous fungus parasitizing on an imago of the subtrogliphilic fly *Heteromyza* sp. have been found. Specimens found in the dark zone of the cave were sampled, microclimate data were recorded and photo documentation performed. The fungus was identified as *Polycephalomyces ramosus*. Detailed description, based on living material, supplemented with drawings and photographs is given. Microscopic study includes morphological, histo- and cyto-chemical data in order to improve future diagnostics on generic and species level. The new genus *Perennicordyceps* is described to accommodate four species of *Polycephalomyces* with superficial perithecia and hirsutella-like or acremonium-like anamorphs. A key to the species of *Perennicordyceps* gen. nov. and *Polycephalomyces* is provided. Remarks on life history and ecology on both genera are discussed.

Keywords: Anamorph, teleomorph, biospeleology, ecology, holistic key, taxonomy, entomogenous fungi.

Introduction

Mycology-speleological research in Croatian karstic underground began in 1988 by the first author and ascomycetous cave fungi have been intensively collected since 1990 (MATOČEĆ & OZIMEC, 2001). One of the most systematically explored sites for cave biota by the third author is the Veternica cave system situated in the vicinity of Zagreb. During the regular monitoring of cave biota in a touristic part of Veternica (the area under the influence of sporadic artificial light), *Polycephalomyces ramosus* (Peck) Mains was found to be the most frequent entomogenous fungus in this part of the cave system. This anamorphic fungus is reported to be often found in caves, living as a hyperparasite on entomogenous fungi viz. *Hirsutella guignardii* (Maheu) Samson, Rombach & Seifert, *Ophiocordyceps barnesii* (Thwaites) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora and *O. entomorrhiza* (Dicks.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (SEIFERT, 1985). Recent molecular studies of the genus *Polycephalomyces*, erected by KOBAYASI (1941), consider 12 species in the genus and clearly distinguish two sister clades (KEPLER *et al.*, 2013). Both clades are supported with multi-gene analysis as well as morphology, life history and ecological features.

Materials and methods

Specimens were collected in Veternica cave on Mt. Medvednica, within Medvednica Nature Park, Croatia, during the regular monitoring of cave biota in a period 2010-2014. In 2010 *Polycephalomyces ramosus* and *Hirsutella* spp. were found twice on imagines of the subtrogliphilic fly *Heteromyza* sp. (Heleomyzidae, Brachycera, Diptera) in the dark zone of the cave, an area called "Koncertna dvorana" ("Concert hall") (Fig. 1), which is sporadically subjected to artificial light. In 2014 *P. ramosus* was recollected but *Hirsutella* spp. were not found. Microclimatic data measured at the collection site on 5th Oct. 2014 were: air temp. 2 m above ground = 9.8°C, floor covering substrate temp. = 9.2°C, relative air humidity = 100 % and CO₂ concentration = 5078 ppm / 9.9°C. Synnemata were found at the temp. approx. 9.5°C. Highest temp. measured in earlier researches (performed by the first author during winter and summer season on seven occasions) in the "Koncertna dvorana" at 10 cm above ground was 11.9°C and floor covering substrate temp. was 10.8°C on 30th May 1990. Two lowest floor covering substrate temp. measured were 8.7°C on 11th Dec. 1988 and 6.5°C on 23th Jan. 1993 (random measurements). During these researches *P. ramosus* was not observed.

Macroscopic and microscopic characters based on living cells and tissues (*) were recorded using vital taxonomy methods (BARAL,

1992). The mounting media was tap water and the reagents used were Lugol's solution (IKI) and Brilliant Cresyl Blue solution (CRB). All described microscopic elements are observed in water unless otherwise stated. Drawings were made free hand to scale and microphotographs were taken with a camera mounted directly on the microscope. Species identification and determination of the variation range of qualitative and quantitative characters were based on synnemata anatomy and conidiogenous elements on a minimum of four synnemata in each collection. Values in braces {} indicate the number of collections examined and values in parentheses () indicate the number of species on corresponding substrate. Conidia measurements are given as minimal and maximal values together with Q (length/width ratio) and were taken directly using an ocular micrometer scale on bright-field compound microscopes. Variation range is based on samples of 20 fully mature normally developed and randomly selected conidia to an accuracy of 0.1 µm. Spore shapes are defined after KUŠAN *et al.* (2014). Dried material is deposited in the Croatian National Fungarium (CNF).

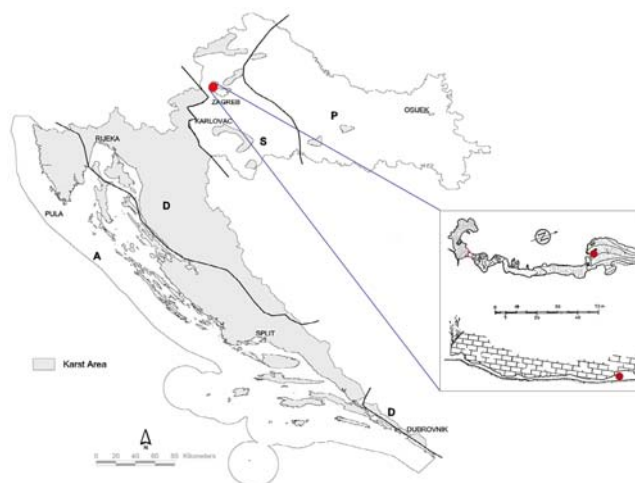


Fig. 1 – Simplified general tectonic map of Croatian part of the Dinarides with the distribution of the karst after KUŠTA (2002). Key to symbols: A – Adriatic carbonate platform (Adriaticum), D – carbonate platform (Dinaricum), S – Eudynamic area (Supradinaricum), P – Geological structures of the Pannonian basin. Red dots are showing the position of Veternica cave in Supradinaricum and sampling site of *Polycephalomyces ramosus* in the area called "Koncertna dvorana" in the part of the cave open to the public respectively (*del.* M. Čepelak).

Taxonomy

Polycephalomyces ramosus (Peck) Mains, *Mycologia*, 40 (4): 414 (1948).

Basionym: *Stilbum ramosum* Peck, *Bull. Buff. Soc. Nat. Sci.*, 1: 69 (1874).

≡ *Botryonipha ramosa* (Peck) Kuntze, *Revis. Gen. Pl.*, 2: 845 (1891).

≡ *Stilbella ramosa* (Peck) Petch, *Trans. Brit. Mycol. Soc.*, 21 (1-2): 53 (1937).

= *Polycephalomyces paludosus* Mains, *Mycologia*, 40 (4): 414 (1948).

= *Stilbum kervillei* Quél., *Bull. Soc. Amis Sci. Nat. Mus. Rouen*: 302 (1884).

= *Stilbella kervillei* (Quél.) Samson, Rombach & Seifert, *Persoonia*, 12 (2): 126 (1984).

Description of studied collections (Plates 1-3)

Anamorphic state: synnemata caespitose, arising from *Heteromyza* sp. corpse, mostly from thoracic part, also present on abdomen, unbranched to more often branched, branches simple, only of first order, 0–7 per synnema, branches *0.4–3.1 × 0.1 mm {1}, straight to curving upwards, cylindrical-capitate or rarely slightly subulate in sterile branches, apically straight, almost smooth to sub-

villose or pubescent, white or beige, bearing slimy heads when fresh, whole synnemata *3.2–8.4(–12.2) mm {3} tall, stipe *2.3–8.1 × 0.2–0.5 mm {3}.

Phialides of two types, A-phialides in the capitulum, B-phialides on stipe, producing A- and B-conidia respectively. Conidial mass forming globose to subglobose **capitulum** on almost every apex of synnematal branch, *0.4–0.9 × 0.2–0.8 mm {3}, opaque when fresh, pale yellow to pale ochraceous-yellow, becoming sulphur-yellow with age and clavate shaped, containing almost entirely only A-conidia. Conidiophores arranged in not tightly fasciculate spheroid-flared hymenioid structure, arising as verticillate branches on basal cells, producing acropleurogenously born phialides in (1)2–4 levels, *45–70 × 1.9–2.5 μm {1}; terminal phialides cylindrical to narrowly lageniform, *12.3–24.5 μm {2} long, *1.2–2.1 μm {2} wide at the base, intercalary phialides cylindrical, *5.2–18.1 × 1.0–1.9 μm {2}, with subterminal lateral conidiogenous extensions *4.7–8.2 × 0.6–1.4 μm {1}, collarettes not prominent, periclinal thickening not visible. A-conidia roughly ovoid to parabolic but slightly asymmetric, smooth, 1-celled, borne in large collective mucus, hyaline, heteropolar, without refractive content, *2.1–3 × 1.3–2 μm {2}, Q = 1.2–1.9. Marginal area contains few sterile paraphysis-like cell chains, *30–45 × 2.8–4.0 μm {1}.

Stipe in vertical section is composed of two regions; outer region of ± *textura prismatica*, hyphae *2.4–6.1 μm {2} wide; some sterile clavate surface cells filled with weakly refractive yellow sap, *3.6–

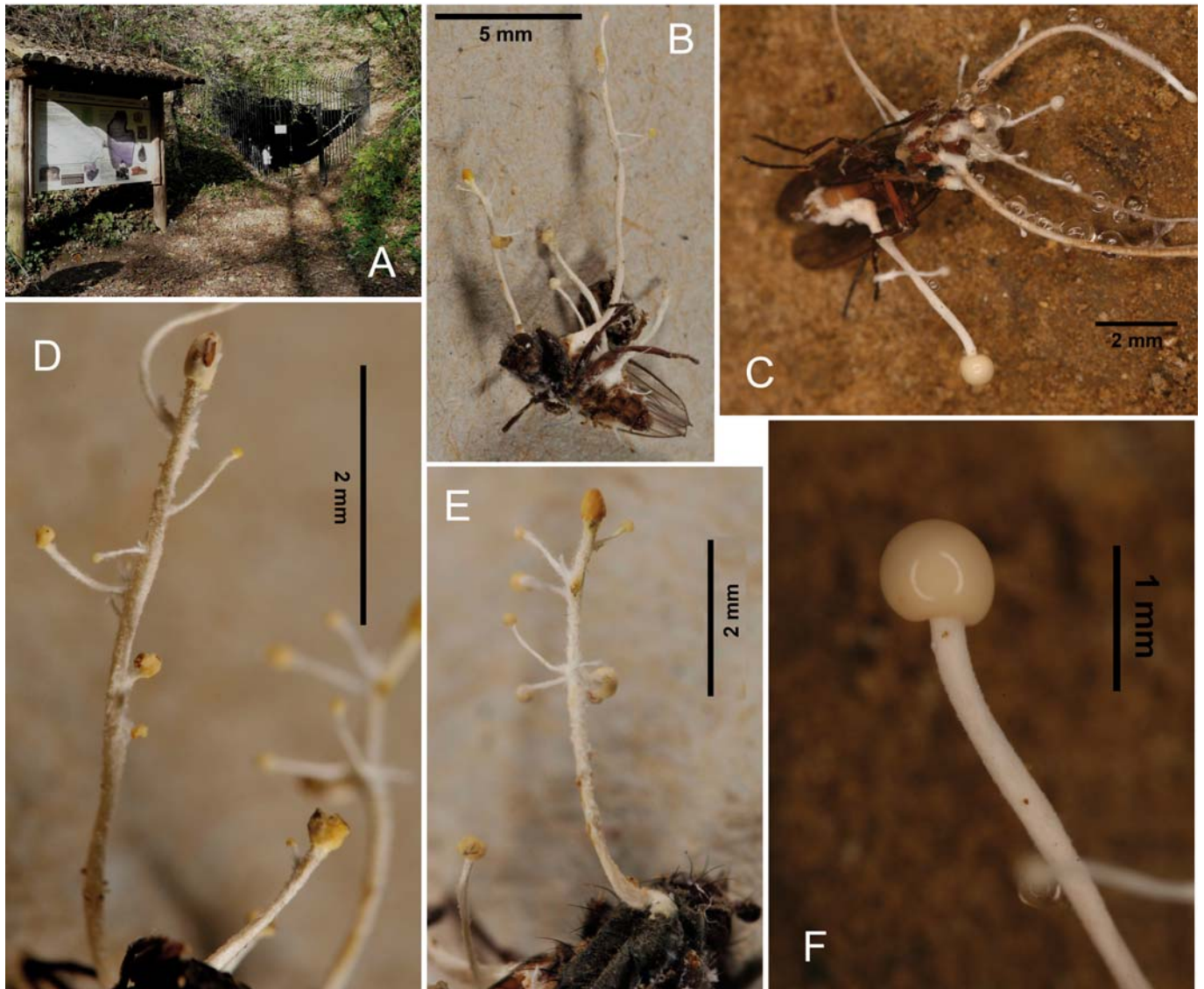


Plate 1 – A: Entrance to the Veternica cave. *Polycephalomyces ramosus* - B, C, D, E: synnemata on *Heteromyza* sp., F: Capitulum with a part of the stipe. C, F from CNF-2/8634; B, D, E from CNF-2/9600. Photos: A, C, F by R. Ozimec; B, D, E by N. Matočec.

4.8 μm {1} wide, other surface cells can be slightly verrucose {1}; inner region of \pm sinusoid *textura porrecta* with some obliquely directed hyphae, the latter *2.0–3.4 μm {1} wide. B-phialides arising along a synnematal stipe, singly lateral and sessile or 1-4 on terminal non-septate conidiophoral cells, *5–28 \times 2–5.6 μm {1}, phialides narrowly lageniform to ampullaceous or subulate, slightly curved or straight, sub-symmetrical to strongly asymmetrical, *6.0–16.7 μm {1} long, *1.8–5 μm {2} wide at the base, with a cylindrical to acicular neck, *2.2–6.1 \times 0.6–1.5 μm {2}, collarettes not prominent, periclinal

thickening not visible. B-conidia fusiform, smooth, 1-celled, dry, hyaline, homopolar, radially symmetric, without refractive content, *3–4.2 \times 1.1–1.9 μm {2}, Q = 2.1–2.8.

Chemical reactions: In *IKI overall structure inamyloid, conidia, phialides and texture without glycogens, single nucleus faintly discerned in both A- and B-conidia, basal part of B-phialides with yellow-greyish granules. In *CRB cytoplasm in lower cells of A-type conidiophores stained violet and partly also in stipal cortical cells but stipal medulla not stained, yellow sap in stipal surface cells re-

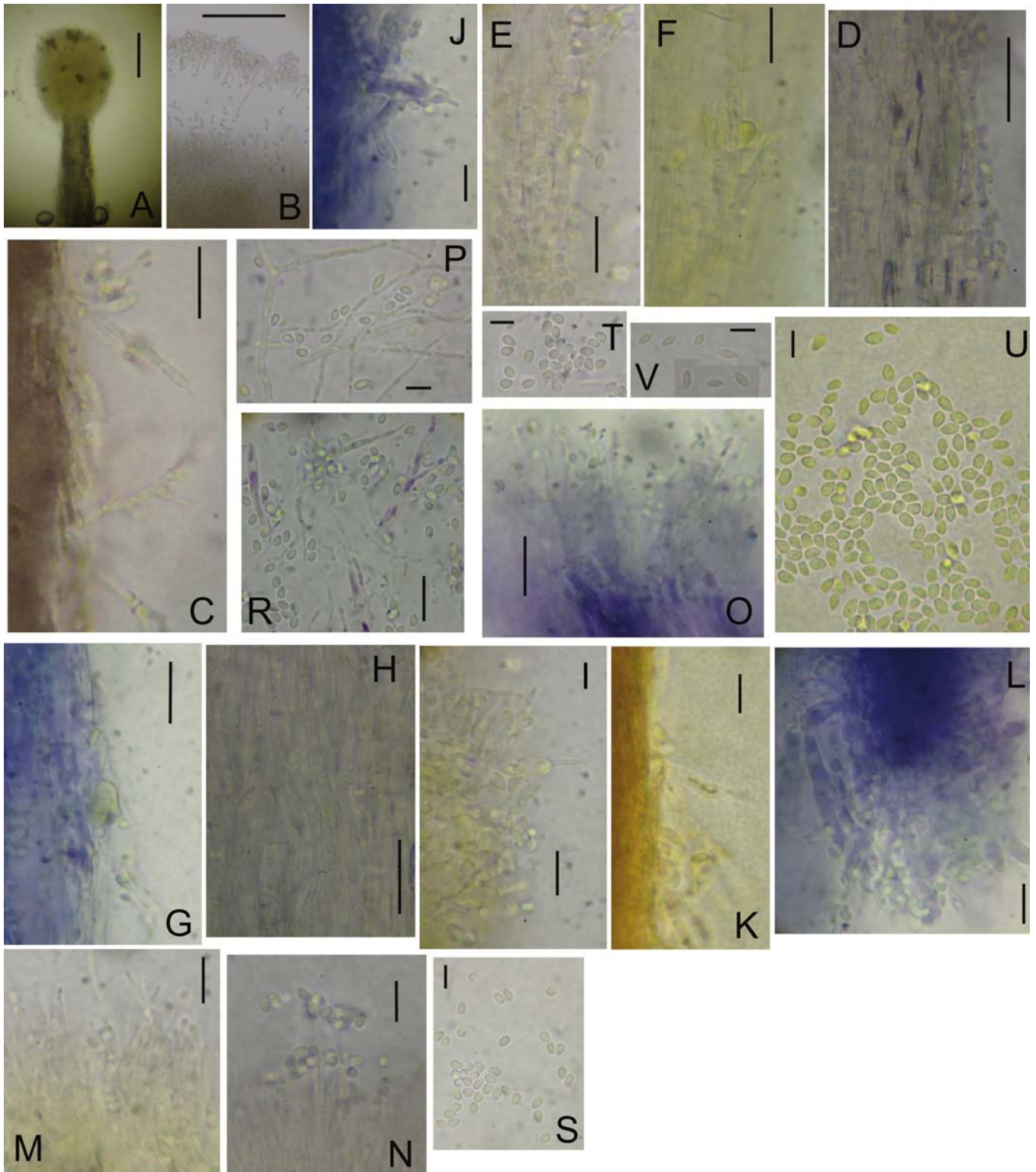


Plate 2 – *Polycephalomycetes ramosus*. Microscopic characters. A: Capitulum with mucus. B: A-conidia in mucus. C, D: Stipe surface. E, F, G: Yellow sap on the stipe surface. H: Stipe medulla. I, J, K: B-phialides. L: Marginal paraphysis-like cell chains of the capitulum. M, N, O: A-phialides forming A-conidia. P: A- and B-conidia. R: A-conidia and A-phialides. S, T, U: A-conidia. V: B-conidia. G, J, L, O, R in *CRB; K, U in *IKI; other in *H₂O. Scale bars: A = 0.5 mm, B = 50 μm , C, D = 20 μm , E-O, R = 10 μm , P, S-V = 5 μm . All photos from CNF-2/9600, by N. Matočec.

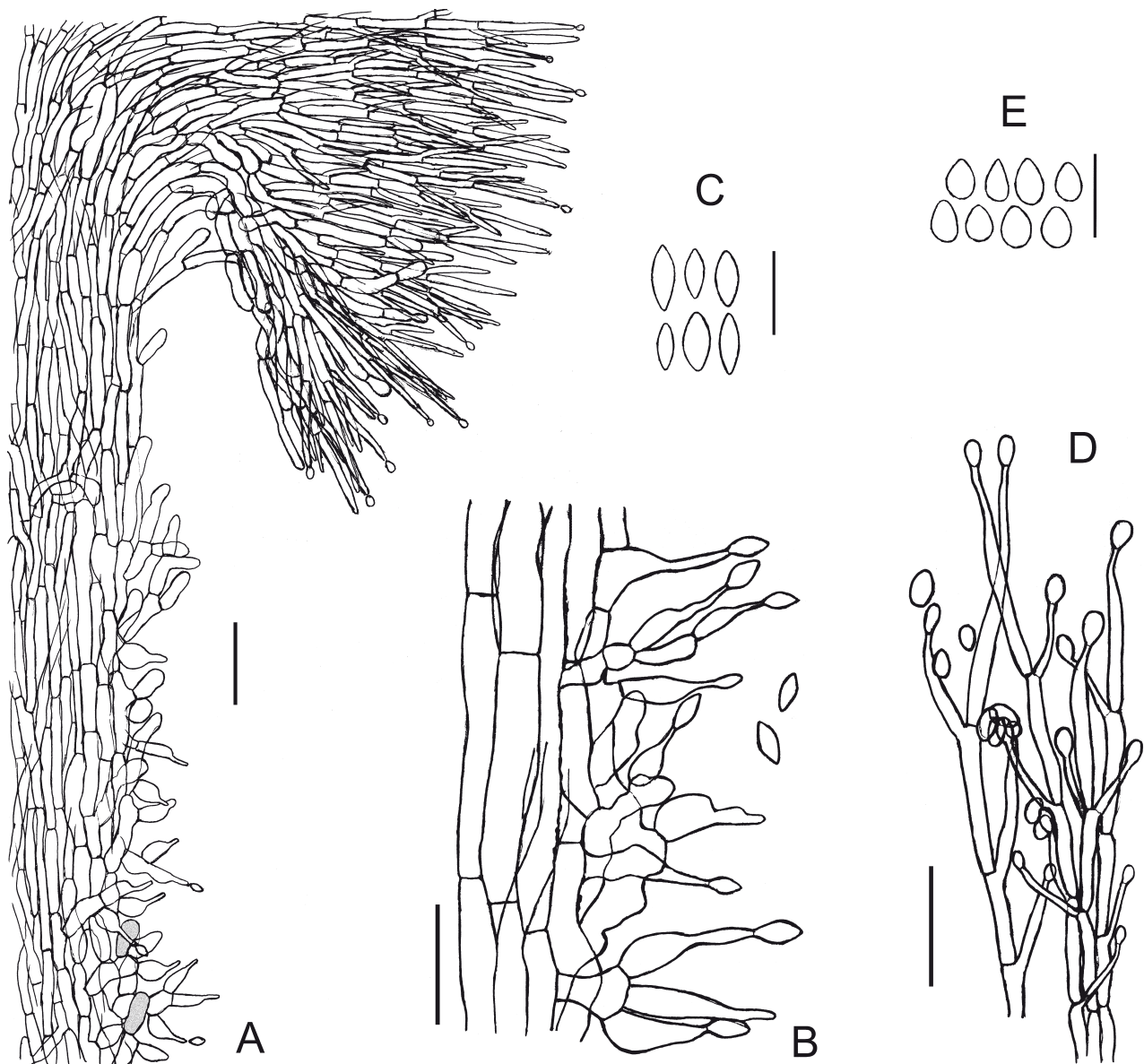


Plate 3 – *Polycephalomyces ramosus*. Microscopic characters. A: Vertical-median section through synnema (some clavate cells on stipe surface containing yellow sap are coloured grey). B: B-phialides on the surface of the stipe. C: B-conidia. D: A-phialides from the capitulum. E: A-conidia. Scale bars: A = 20 μm , B, D = 10 μm , C, E = 5 μm . A, C, E from CNF-2/9600; B, D from CNF-2/8780. Del. N. Matočec.

mains unstained, cytoplasm of marginal sterile paraphysis-like cell chains blue-violet, parts of B-phialides cytoplasm pale violet-grey when conidia are detached.

Teleomorphic state: not found.

No pure cultures from the collected material were drawn.

Specimens examined: CROATIA, Zagreb district, Mt. Medvednica, Veternica cave system, cave entrance at 320 m asl, habitat: dark zone in a karstic cave, on wet wall and floor communities, *legit* R. Ozimec: 7.7.2010 (CNF-2/8634), 21.11.2010 (CNF-2/8780), 5.10.2014 (CNF-2/9600).

Distribution: Asia (Japan, Sri Lanka), Europe (Austria, Croatia, France, Germany, Great Britain, Netherlands), North America (U.S.A.).

Habitat: Parasitic on entomogenous fungi, known from *Hirsutella guignardii*, *Ophiocordyceps barnesii* and *O. entomorrhiza*. Often found in caves.

NOTE: In Croatian collections *Polycephalomyces ramosus* was never found on the same substrate unit (host individual) together

with *Hirsutella* spp. and the synnemata were emerging directly from the insect corpses. However, in a single occasion (7th Jul. 2010) *Hirsutella* spp. synnemata were found on a number of separate *Heteromyza* sp. corpses in immediate vicinity of those occupied with *Polycephalomyces* synnemata.

Discussion

Recent molecular studies (KEPLER *et al.*, 2013), based on a concatenated five gene dataset (SSU, LSU, TEF, RPB1 and RPB2) showed that the monophyletic genus *Polycephalomyces* comprises: (a) pleomorphic species earlier placed in the genus *Ophiocordyceps* [*P. cuboideus* (Kobayasi & Shimizu) Kepler & Spatafora, *P. paracuboideus* (S. Ban, Sakane & Nakagiri) Kepler & Spatafora, *P. prolificus* (Kobayasi) Kepler & Spatafora and *P. ryogamiensis* (Kobayasi & Shimizu) Kepler & Spatafora] with hirsutella-like or acremonium-like anamorphs, (b) teleomorphic species earlier classified in the genus *Cordyceps* [*P. kanzashianus* (Kobayasi & Shimizu) Kepler & Spatafora, *P. nipponicus* (Kobayasi) Kepler & Spatafora and *P. ramosopulvinatus* (Kobayasi & Shimizu) Kepler & Spatafora] with currently unknown anamorphs and (c) anamorphic species with *Polycephalomyces* type synnemata [*P. cylindrosporus* Samson & H.C. Evans, *P. ditmari* Van

Vooren & Audibert, *P. formosus* Kobayasi, *P. ramosus* (Peck) Mains and *P. tomentosus* (Schrad.) Seifert] with currently unknown sexual state.

When non-molecular characters are superimposed to the five gene phylogeny given by KEPLER *et al.* (2013), also WANG *et al.* (2012), it is clearly visible that two sister clades (the one with type species *P. formosus* and the other containing four species: *P. cuboideus*, *P. paracuboideus*, *P. prolificus* and *P. ryogamiensis*) clearly differ from each other. The clade with type species is represented by taxa having either ramose synnemata with slimy globose or clavate conidial masses produced at the synnematal terminals (polycephalomyces-like) or by having ramose stromata with apical capitate inflations containing immersed perithecial aggregations. The sister clade (see also phylogenetic tree based on ITS region; BAN *et al.*, 2009), on the other hand, comprises the species with simple to branched stromata beset with scattered superficial perithecia over the aerial stromatal part leaving the stromatal apices naked, with hirsutella/acremonium-like anamorphic state.

Therefore, we feel the necessity to differentiate those two clades as separate genera, both retained their monophyly, and re-describe the genus *Polycephalomyces* in a new, more restricted sense using a polyphasic taxonomical approach for the reasons elaborated earlier (KUŠAN *et al.*, 2014). Accordingly, *Polycephalomyces* species in the sense of KEPLER *et al.* (2013) having hirsutella/acremonium-like anamorph should be segregated in a separate genus. Since there is no any applicable/available generic name for this clade known to the authors, a new genus *Perennicordyceps* is here described as a segregate from *Polycephalomyces* Kobayasi *emend.* Kepler & Spatafora. We currently consider *Ophiocordyceps ditmari* (Quél.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora separate from the genus *Polycephalomyces* *s. str.* until molecular research is done to clarify the taxonomic/phylogenetic relations between *O. ditmari* [also *O. sphecocephala* (Klotzsch ex Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora] and *Polycephalomyces ditmari* as well as the relations between *P. ditmari* and the rest of the genus *Polycephalomyces* *s. str.*

Perennicordyceps Matočec & I. Kušan *gen. nov.* – MB 810775

Teleomorph cordycepioid: Stroma cylindrical, apically tapered, pointed or lanceolate, aerial part whitish, orange, ochraceous to brown, single or bi- to oligo-furcate or with several simple stipes appearing during several cycles of growth, large part of the stroma (long-lived, perennial part) hypoxylous or hypogeous, stromatal cortex of prosenchymatic tissue. Perithecia orange-yellow, orange-brown or reddish, ovoid, 320–600 × 200–400 μm, Q ≈ 1.5–1.6, always superficial, perpendicular to rarely slightly oblique in relation to the surface, scattered or gregarious, not organized in a definite capitulum, concentrated almost over the whole aerial part of the stroma or on its subapical part, absent from naked, sterile, tapered to pointed tip. Asci filiform, containing filiform ascospores that disarticulate into many cuboid to narrowly prismatic partspores, 1–5 × 1–2 μm, Q ≈ 1–2.

Anamorph hirsutella-like (often accompanied with acremonium-like phialides): conidiogenous cells phialidic, phialides anemalous in natural conditions never found in synnematal form (synnema-like structures known only from pure culture in *P. prolifica*, see BAN *et al.*, 2009), produced on aerial mycelium, also on sporodochia (2) or directly on stroma (1). Phialides never sessile, 2–10 of them arising from irregularly branched or quasi-verticillate conidiophores, phialides produce up to 10 conidia enveloped in a common sheath. Conidiophores not arranged in hymenial structure. Conidia globose to ellipsoid or limoniform to fusiform, hyaline, smooth, 1-celled.

Ecology: entomogenous, on hypoxylous-xylophagous Coleoptera larvae (3), on hypogeous (soil dwelling) Cicadidae nymphs (1) or hyperparasitic on other cordycepioid species.

Etymology: The genus name refers to the deeply buried substromatal stromata that lasts for several years as well as the cordycepioid teleomorph.

Type species: *Perennicordyceps prolifica* (Kobayasi) Matočec & I. Kušan *comb. nov.* – MB 810776

Basionym: *Cordyceps prolifica* Kobayasi, *Bull. Nat. Sci. Mus., Tokyo, Bot.*, 6: 289 (1963).

≡ *Ophiocordyceps prolifica* (Kobayasi & Shimizu) S. Ban, Sakane & Nakagiri, *Mycoscience*, 50(4): 270 (2009).

≡ *Polycephalomyces prolificus* (Kobayasi) Kepler & Spatafora, *Fungal Biol.*, 117: 618 (2013).

Host: underground dwelling nymph of Cicadidae (Hemiptera).

Habitat: hypogeous.

Anamorph: hirsutella/acremonium-like.

The enumeration of other species in the genus is given below:

Perennicordyceps cuboidea (Kobayasi & Shimizu) Matočec & I. Kušan *comb. nov.* – MB 810778

Basionym: *Cordyceps cuboidea* Kobayasi & Shimizu, *Bull. Nat. Sci. Mus., Tokyo, Bot.*, 6(4): 131 (1980).

≡ *Ophiocordyceps cuboidea* (Kobayasi & Shimizu) S. Ban, Sakane & Nakagiri, *Mycoscience*, 50(4): 268 (2009).

≡ *Polycephalomyces cuboideus* (Kobayasi & Shimizu) Kepler & Spatafora, *Fungal Biol.*, 117: 618 (2013).

= *Cordyceps alboperitheciata* Kobayasi & Shimizu, *Bull. Nat. Sci. Mus., Ser. B.*, 8(3): 84 (1982).

Host: xylophagous larvae of Coleoptera.

Habitat: endoxylous, inside rotten wood.

Anamorph: hirsutella/acremonium-like.

Perennicordyceps paracuboidea (S. Ban, Sakane & Nakagiri) Matočec & I. Kušan *comb. nov.* – MB 810779

Basionym: *Ophiocordyceps paracuboidea* S. Ban, Sakane & Nakagiri, *Mycoscience*, 50(4): 268 (2009).

≡ *Polycephalomyces paracuboideus* (S. Ban, Sakane & Nakagiri) Kepler & Spatafora, *Fungal Biol.*, 117: 618 (2013).

Host: xylophagous larvae of Coleoptera.

Habitat: hypoxylous, inside rotten wood.

Anamorph: hirsutella/acremonium-like.

Perennicordyceps ryogamiensis (Kobayasi & Shimizu) Matočec & I. Kušan *comb. nov.* – MB 810780

Basionym: *Cordyceps ryogamiensis* Kobayasi & Shimizu, *Bull. Nat. Sci. Mus., Tokyo, Bot.*, 9(1): 4 (1983).

≡ *Ophiocordyceps ryogamiensis* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, *Stud. Mycol.*, 57: 45 (2007).

≡ *Polycephalomyces ryogamiensis* (Kobayasi & Shimizu) Kepler & Spatafora, *Fungal Biol.*, 117: 618 (2013).

Host: xylophagous larvae of Coleoptera.

Habitat: hypoxylous, inside rotten wood.

Anamorph: hirsutella/acremonium-like.

Polycephalomyces Kobayasi *emend.* Matočec & I. Kušan – MB 9494

Teleomorph cordycepioid: Stromata ± cylindrical, ochraceous, brown or reddish, more or less branched, branches ending up with fertile pulvinate, globose to tuberiform capitulum, 1.5–9 mm wide, containing immersed perithecia, branches produced by several cycles of growth; stromatal cortex of pseudoparenchymatic tissue (*textura globulosa* to *angularis*). Perithecia yellow to ochraceous, elongated pyriform, 750–1100 × 270–370 μm, Q ≈ 2–4, always immersed in stroma with ostioles that may protrude or not, perpendicular to rarely slightly oblique in relation to the surface. Asci filiform, containing filiform ascospores that disarticulate into truncate bacilliform partspores, 2.8–5.0 × 0.7–1.0 μm, Q ≈ 3–6.

Anamorph polycephalomyces-like: conidiomata synnematos, determinate, often branched, more rarely simple, white to brownish, not changing colour in KOH. Conidial mass very large, capitate, yellow or yellow-orange, slimy, opaque, terminally produced on synnematal branches. Stipe of subparallel, thin-walled, hyaline hyphae, often non-sporogenous (in three species with lateral B-phialides producing catenate fusiform conidia). Conidiophores concentrated on the top of synnematal branches, arranged in clavate to sphaerically flared hymenia. Conidiophore branched with one or two basal verticillate whorls, ending up with conidiogenous cells or composed of acropleurogenous chains of conidiogenous cells. Conidiogenous cells phialidic, terminal phialides awl-shaped, often with a long gradually narrowed neck, intercalary phialides often present. Conidia globose, subglobose, ellipsoid to oblong, also fusiform and dry when B-phialides are present, hyaline, smooth, 1-celled.

Ecology: entomogenous (on nymphs, larvae, pupae and imago), hyperparasitic on other entomogenous cordycepioid fungi or on Mycetozoa or on other myxomyceticolous fungi. On Coleoptera (2), Diptera (1), Hemiptera (4), Hymenoptera (2), Lepidoptera (2) and Mycetozoa (1).

Type species: *Polycephalomyces formosus* Kobayasi, *Sci. Rep. Tokyo Bunrika Daigaku*, 5: 245 (1941).

Notes on teleomorph – anamorph connections in *Polycephalomyces*

Byssostilbe stilbigera (Berk. & Broome) Petch is not considered at this point as the teleomorphic state of *Polycephalomyces tomentosus* until further molecular and/or cultural studies are done to clarify this supposed relationship. Teleomorphic *Polycephalomyces* species are quite different from *Byssostilbe stilbigera* in gross morphology and many important microscopic features. According to SEIFERT (1985) the connection between *Byssostilbe stilbigera* and *Polycephalomyces tomentosus* is only supposed because of similarity of perithecial tomentum vestiture composed of verrucose hyphae in *B. stilbigera*, similar to the ornamenting cells of the synnematal surface in *P. tomentosus* as well as because the same, myxomyceticolous hosts.

Ophiocordyceps ditmari is kept apart from *Polycephalomyces ditmari* until firm connection between those two is demonstrated, regardless of the fact that both parasitize closely related hosts

(Vespidae). *Polycephalomyces* has cordycepioid teleomorphs quite different in structure than *O. ditmari* (see note in the key for the genera) even though *P. ditmari* show some similarities in conidiogenous cell structure found in *Hymenostilbe sphecocephala* nom. nud., the anamorphic stage of *O. sphecocephala* (HYWEL-JONES, 1995). This species is apparently closely related to *O. ditmari* regarding their teleomorph morphology and ecology. There is another taxonomically related species living on Vespidae, i.e. *Hirsutella sphecocephala* (Ditmar) Van Vooren with unknown teleomorph (VAN VOOREN & AUDIBERT, 2005). In their molecular analysis SUNG *et al.* (2007), WEN *et al.* (2013) and QUANDT *et al.* (2014) demonstrated monophyly of the clade represented by *O. sphecocephala* with all taxa (when known) having a hymenostilbe-like anamorph.

Since it is already known that a single substrate unit (i.e. arthropod corpse) can host two or more different entomogenous and/or hyperparasitizing hypocrealean species at the same time, it is possible that CHEN *et al.* (1984) when isolating *Paecilomyces sinensis* Q.T. Chen, S.R. Xiao & Z.Y. Shi from larva of Hepialidae cultured a fungus that isn't *Ophiocordyceps sinensis* (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora. Because of that reason *Paecilomyces / Polycephalomyces sinensis* in WANG *et al.* (2012) is positioned in totally different clade than *O. sinensis*. LIANG *et al.* (2005) probably made an error claiming that *Paecilomyces sinensis* was obtained from a fresh specimen of *Ophiocordyceps sinensis* which is in contrary to the species protologue saying that the holotype is isolated from larva. Additionally, LIU *et al.* (2002) confirmed only *Hirsutella sinensis* X.J. Liu, Y.L. Guo, Y.X. Yu & W. Zeng to be a true anamorph of *Ophiocordyceps sinensis*.

World wide key

The following world wide holistic key to the genera *Polycephalomyces* and *Perrenicordyceps* summarise most important characters of both teleomorph and anamorph as well as the ecology. The key is based on the data given in this paper and the following references: KOBAYASI (1941), MAINS (1948), KOBAYASI & SHIMIZU (1963), KOBAYASI & SHIMIZU (1980), KOBAYASI & SHIMIZU (1982), KOBAYASI & SHIMIZU (1983), SEIFERT (1985), SAMSON *et al.* (1988), ROGERSON & STEPHENSON (1993), HYWEL-JONES (1995), BISCHOFF *et al.* (2003), SEPÚLVEDA-CHAVERA *et al.* (2004), VAN VOOREN & AUDIBERT (2005) and BAN *et al.* (2009). Included species belong only to phylogenetically researched taxa by WANG *et al.* (2012) and KEPLER *et al.* (2013) based on multi gene approach.

Table 1 – Differential characters between *Polycephalomyces* and *Perrenicordyceps*

	<i>Polycephalomyces</i>	<i>Perrenicordyceps</i>
Teleomorph	cordycepioid	cordycepioid
Perithecia in relation to the stromatal surface	immersed	superficial
Stromatal cortex	pseudoparenchymatic	prosenchymatic
Perithecia organized in capitulum	yes	no
Perithecia shape	elongated pyriform	ovoid
Perithecia height	750–1100	320–600
Perithecia Q value	2–4	1.5–1.6
Partspores shape	truncate bacilliform	cuboid to narrowly prismatic
Partspores width	0.7–1	1–2
Partspores Q value	3–6	1–2
Anamorph	when known polycephalomyces-like	hirsutella / acremonium-like
Synnemata occur in nature	yes	no

Key to the genera:

1) Stromata able to perform several cycles of growth with all newly produced parts with fertile, sexual structures (perithecia), therefore stromata branched or with several simple stipes on a common base and/or anamorphic synnemata of *Polycephalomyces* type, i.e. branched (in a single species mostly unbranched) with branches ending up with clavate to spherically flared hymeniform aggregations of conidiophores producing large mass of conidia united in collective globular mucus 2

1') Stromata unbranched, or if branched not able to perform several cycles of growth with sexual structures, at most able only to produce anamorphic state on a stroma; anamorph (when present) not polycephalomyces-like **Ophiocordyceps**¹ s.l.

¹ including *O. sphecocephala* and allies with hymenostilbe-like anamorph and *O. ditmari* with uncertain anamorph (suggested *Polycephalomyces ditmari*), both taxa with unbranched capitate stromata with obliquely immersed perithecia regarding both the stromatal surface and the stromatal main axis, arranged in determinate apical capitulum with cortex composed of short prismatic and clavate cell-chains oriented vertically to the surface.

2) Stromata cylindrical, apically tapered, pointed or lanceolate, aerial part loosely beset with superficial ovoid perithecia ($Q \approx 1.5-1.6; \leq 600 \mu\text{m}$ long) over the most of aerial surface, always with naked, sterile, tapered to pointed tip; stromatal cortex of prosenchymatic tissue; partspores cuboid to narrowly prismatic ($Q \approx 1-2; 1-5 \times 1-2 \mu\text{m}$) and/or anamorph anematus (in natural conditions

hirsutella/acremonium-like, with phialides produced on irregularly branched or quasi-verticillate conidiophores, not aggregated to form hymenial structures or aggregated in sporodochia, developing separately from the teleomorph or on stromata; conidia are produced singly or ≤ 10 enveloped in common sheath

..... **Perennicordyceps**
(on entomogenous, hypoxylous-xylophagous Coleoptera larvae or on hypogaeous, soil dwelling Cicadidae nymphs)

2') Stromata differentiated into cylindrical stipe and apical capitulum, fertile part containing immersed densely aggregated perithecia; stromatal cortex of pseudoparenchymatic tissue (*textura globulosa* to *angularis*); partspores truncate bacilliform ($Q \approx 3-6; 2.8-5.0 \times 0.7-1.0 \mu\text{m}$); anamorph (when present) polycephalomyces-like, i.e. synnematus, unbranched to most often branched, beset with viscous capitate apices consisting of numerous conidiophores (verticillate or acropleurogenous) arranged in spherical to clavate flared hymenia, terminated by phialides that produce numerous conidia aggregated in a collective mucus 3

3) Stromatal fertile capitulum elongated, containing semi-immersed ovoid perithecia ($Q \approx 1.5-1.8; \leq 650 \mu\text{m}$ long); anamorph unknown **"Cordyceps" pleuricapitata**

3') Stromatal fertile capitulum globose, tuberiform to pulvinate, containing immersed elongated pyriform perithecia ($Q \approx 2-4; \geq 750 \mu\text{m}$ long); anamorph polycephalomyces-like **Polycephalomyces**

Key to the species of the genus *Polycephalomyces*

- 1) Stroma differentiated into cylindrical stipe and apical capitulum, fertile part containing immersed densely aggregated perithecia; stromatal cortex of pseudoparenchymatic tissue (*textura globulosa* to *angularis*); part spores truncate bacilliform ($Q \approx 3-6$); anamorph unknown; on Cicadidae 2
- 1') Anamorph synnemata, polycephalomyces-like, unbranched to most often branched, beset with viscous capitate apices consisting of numerous conidiophores (verticillate or acropleurogenous) arranged in spherical to clavate flared hymenia, terminated by phialides that produce numerous conidia aggregated in collective mucus; teleomorph unknown; on other insects 4
- 2) Stromata very large, up to 240 mm tall, arising from long-lived sclerotia formed inside the host corpse; partspores $2.8-4.8 \times 0.7 \mu\text{m}$ **P. nipponicus**
- 2') Stromata smaller, 20-100 mm tall; partspores about $1 \mu\text{m}$ diam. 3
- 3) Stromatal capitulum 4.5-9 mm diam. with embedded perithecia partly protruding with their ostioles, $750-925 \mu\text{m}$ long; partspores about $3 \times 1 \mu\text{m}$ **P. ramosopolvinatus**
- 3') Stromatal capitulum 2-5 mm diam. with completely embedded perithecia, $900-1100 \mu\text{m}$ long; partspores $3-5 \times 1 \mu\text{m}$.. **P. kanzashianus**
- 4) Synnemata mostly unbranched and minute, 0.2-1.1 mm tall, $15-80 \mu\text{m}$ diam., ornamental cortical stipe cells subglobose to prismatic with wart-like projections; only A-conidia (those produced on hymeniform synnematal slimy capitulum) present, highly heteromorphous, globose, ellipsoid and cylindrical, $1-6 \times 0.5-2 \mu\text{m}$; on Mycetozoa or myxomyceticolous ascomycete *Byssostilbe stilbigera* **P. tomentosus**
- 4') Synnemata unbranched to ramose, much larger, 5-60 mm tall, $125-1000 \mu\text{m}$ diam., ornamental cortical stipe cells absent; if conidia heteromorphous, then A-conidia (ovoid to parabolic or cylindrical to bacilliform, aggregated in a collective mucus) differs from B-conidia (fusiform and dry) produced along the synnematal stipe surface and/or cultured mycelia; insecticolous, or hyperparasites on other cordycepioid fungi and their anamorphs 5
- 5) Synnemata comparatively large, (10)50-60 mm tall, clavate; fungus produce both ovoid to lacrimiform A-conidia, $1.7-2.6 \times 1.3-2.0 \mu\text{m}$ and arrowhead-shaped to lanceolate B-conidia (from stipe and mycelium), $3.3-6.8 \times 1.2-3.0 \mu\text{m}$; on *Hepialus* larvae (Lepidoptera) **P. sinensis**²
- 5') Synnemata medium, 5-25 mm tall, cylindrical-capitate; A-conidia exceed $2.6 \mu\text{m}$ in length, if B-conidia are produced, they are fusiform or naviform; on other substrata 6
- 6) Only parabolic A-conidia, $2.2-3.0 \times 1.3-1.6 \mu\text{m}$ are produced by 1-celled cylindrical-tapered phialides densely set in a palisade manner on synnematal capitulum, $20-37 \times 1.5-3.0 \mu\text{m}$; on Vespidae **P. ditmari**
- 6') A-conidia either cylindrical to bacilliform or ovoid to parabolic, produced by several celled conidiophores with phialides born on verticils and/or acropleurogenously forming loosely arranged flared hymenia; phialides $7-25 \mu\text{m}$ long; on other hosts 7
- 7) Synnemata slender, stipe $125-300 \mu\text{m}$ diam., branches $25-80 \mu\text{m}$ diam., only A-conidia present, cylindrical to bacilliform, $Q \approx 2.5-4$; on Coleoptera, Formicidae and Pentatomidae **P. cylindrosporus**
- 7') Synnemata stout, stipe $200-1000 \mu\text{m}$ diam., branches $100-300 \mu\text{m}$ diam., both A- and B-conidia are produced, at least in pure culture, A-conidia ovoid to parabolic, $Q \approx 1.2-2.0$; on Coleoptera, Diptera and Lepidoptera or hyperparasitic on entomopathogenic cordycepioid fungi and their anamorphs infecting the same hosts 8
- 8) Conidial branching biverticillate; A-phialides born in 3-4 terminal whorls; B-phialides occur only on cultured mycelia, never on synnematal stipe; B-conidia naviform, $Q \approx 3.0-3.5, 3.2-4.8 \times 0.8-1.6 \mu\text{m}$; on Coleoptera larvae or on cordycepioid fungi and their anamorphs **P. formosus**
- 8') A-phialides born acropleurogenously in 1-4 levels; B-phialides on both synnematal stipe and cultured mycelia; B-conidia fusiform, $Q \approx 2.1-2.8, 3.0-4.5 \times 1.5-2.0 \mu\text{m}$; on Diptera imago or Lepidoptera larvae/pupa or on cordycepioid fungi and their anamorphs **P. ramosus**

² *Polycephalomyces sinensis* (Q.T. Chen, S.R. Xiao & Z.Y. Shi) W.J. Wang, X.L. Wang, Y. Li, S.R. Xiao & Y.J. Yao. *Systematics and Biodiversity*, 10 (2): 228 (2012). MB 810781. Basionym: *Paecilomyces sinensis* Q.T. Chen, S.R. Xiao & Z.Y. Shi, *Acta Mycol. Sin.*, 3: 25 (1984).

Key to the species of the genus *Perennicordyceps*

- 1) Stromata very large, 60–350 mm tall, cylindrical-lanceolate; arising from soil dwelling Cicadidae nymphs; stromatal surface composed of *textura intricata*; synnema-like structure and anamorph occur only in the pure culture, composed of acremonium-like phialides produced on the mycelial colony edge, 20–30 µm long, while hirsutella-like phialides occur only in aged colonies, 11–16 µm long; conidia globose, oculiform to fusiform ***P. prolifica***
- 1') Stromata smaller, ≤ 50 mm tall, cylindrical tapered to cylindrical pointed; arising from endoxylous-xylophagous Coleoptera larvae; stromatal surface of different structure (*porrecta prismatica* or of elongated cell palisades); synnemata never formed, sporodochia may be present; if acremonium-like phialides are present, they are shorter (11–22 µm long), while hirsutella-like phialides exceed 16 µm in length 2
- 2) Part spores narrowly prismatical, $Q \approx 1.8\text{--}2.0$; $2.5\text{--}5.0 \times 1.5\text{--}2.0$ µm; only acremonium-like phialides are formed, either on stroma in nature or on sporodochia in pure culture, 17.5–55.2 µm long; conidia ellipsoid, $2.5\text{--}3.9 \times 1.0\text{--}1.4$ µm ***P. ryogamiensis***
- 2') Part spores cuboid to broadly prismatical, $Q \approx 1.0\text{--}1.7$; both acremonium- and hirsutella-like phialides occur but only in pure culture, hirsutella-like phialides 11–28 µm long; conidia globose to fusiform $1.3\text{--}3.7 \times 1.1\text{--}2.3$ µm 3
- 3) Stromata usually branched, perithecia distributed over upper half of the aerial part; conidiophores irregularly branched, beset with ≤ 10 phialides; acremonium-like phialides 19–22 µm long, hirsutella-like phialides 18–28 µm long; conidia globose to ellipsoid, $1.3\text{--}3.7 \times 1.1\text{--}2.3$ µm ***P. cuboidea***
- 3') Stromata unbranched, perithecia distributed almost on whole aerial part; conidiophores quasi-verticillate with 2-3 phialides; acremonium-like phialides 11–21 µm long, hirsutella-like phialides 11–22 µm long; conidia subglobose to oculiform, $1.3\text{--}1.9 \times 1.0\text{--}1.9$ µm ***P. paracuboidea***

Ecology and biogeography

Croatian collections of *Polycephalomyces ramosus* parasitizing on *Heteromyza* fly, gave us the opportunity to perform *in situ* morphological and ecological assessment and to evaluate its taxonomical status. Except *Heteromyza* fly, other dipterans and arthropods at the collection site were not infected by *P. ramosus*. Similarly to *Cordyceps riverae* treated in previous detailed ecological research (MATOČEC & OZIMEC, 2001), *P. ramosus* too is always found to produce fruitbodies (in this case synnemata) at extremely high air humidity (100%) and condensed water regularly appears on the surface of both synnemata and host bodies (Plate 1C). Air temperature (9–11°C) is also a fairly constant ecological factor (except for the winter when temp. drops below 9°C) occurring in such a large cave as Veternica, without daily fluctuations and negligible seasonal fluctuations (total amplitude measured for the floor covering substrate 6.5–11°C). This corresponds well with the ecological data given by SAMSON *et al.* (1984) for both *in situ* and pure culture monitored conditions. Both *P. ramosus* and *C. riverae* as well as *Hirsutella* spp. (here found on separate *Heteromyza* corpses nearby, those occupied by *P. ramosus*) are all members of the same order (*Hypocreales*) and are rather closely related, they share common subterranean and cavernicolous habitat where they parasitize or hyperparasitize on adult stages of arthropods and do not require sunlight for completion of their whole life cycle. They are therefore able to live constantly inside cave habitats. According to SAMSON *et al.* (1984), *P. ramosus* (published as *Stilbella kervillei* (Qué.) Samson, Rombach & Seifert, see also SAMSON *et al.* (1988), has been reported in Europe only from limestone caves. The species is also known from extracavernicolous habitats elsewhere (Japan, U.S.A. and Sri Lanka). Relying on data compiled as a preliminary checklist of Croatian cave fungi (MATOČEC, 2002), *P. ramosus* is considered as a first record for the Croatian mycobiota. It is so far known only from Veternica cave.

When we look at the *Polycephalomyces* biogeography in a new restricted generic sense it is obvious that all Japanese species exist in teleomorphic state and the anamorph is unknown. On the other hand, all species that occur in Europe (also China, U.S.A., etc.) occur only in anamorphic state and the teleomorph is unknown. Since much more energy is apparently required for production of sexual structures than in asexual ones, we can assume that species (or their phylogenetic ancestors) that might have been existed in the areas heavily influenced by the ice age(s) (viz. European mainland north of Pyrenees-Alpine-Dinaric-Pindos ranges) were forced to suppress their teleomorphic activity for a very long time. A new specific-phylogenetic lineage might have been evolved under such adverse eco-

logical pressure to lose the ability to form a sexual phase. Some species, such as *P. ramosus* might have occupied at that time karstic underground as the only available refuge area in the range of the species where temperatures still allowed its growth. The minimal temperature ascertained in pure cultures (cf. SEIFERT, 1985) was +9°C. Additionally, a species must have been adapted to new rather poor ecological conditions: temperature regime with faint oscillations, ranging near the minimal values during most of the year, no light, rare presence of new host individuals, etc. However, most of the Japanese territory (Honshu, Kyushu and oceanic islets) was not covered by the ice (e.g. GATES, 1976; PELTIER, 1994) and this may explain why Japanese *Polycephalomyces* species continued to exist in their teleomorphic stage, especially when habitats on the soil surface endured in some refuge areas (HARRISON *et al.*, 2001).

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