

## OBSERVATIONS ON *CORDYCEPS RIVERAE* (HYPOCREALES, ASCOMYCOTA) IN CROATIAN CAVES

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This paper appears to be the first contribution on cave macromycetes in Croatia. During the systematic biospeleological field work in the Dinaric karst area, eight more or less stable populations of the entomogenous species *Cordyceps riverae* Pacioni (Hypocreales, Ascomycota) have been located since 1990. Some 200 speleological sites have been visited so far in order to find both living and/or infected hosts, with 69 sites colonized by two subtrogliphilic lepidopteran species of the genus *Triphosa* (Geometridae) which are apparently the only surely known hosts of the fungus. *C. riverae* was collected from five localities (situated in Gorski kotar, Kordun and Dalmatia regions) out of eight speleological sites in which the species was recorded. A full description of *C. riverae*, a comparison with the holotype collection, detailed ecological observations, as well as the mapping of known distribution of both the species and host are given. *C. riverae* is recorded in Croatia for the first time.

**Key words:** Ascomycota, Hypocreales, *Cordyceps riverae*, Lepidoptera, *Triphosa dubitata*, *T. sabaudiata*, biospeleology, ecology, mapping, morphology, mycofloristics Croatia

Matočec, N. & Ozimec, R.: O vrsti *Cordyceps riverae* (Hypocreales, Ascomycota) u špiljama Hrvatske. *Nat. Croat.*, Vol. 10, No. 3, 197–206, 2001, Zagreb.

Ovaj rad predstavlja prvi prilog istraživanju špiljskih makromiceta Hrvatske. U sklopu sustavnog biospeleološkog istraživanja, koje se provodi na području Dinarida od 1990. godine, pronađeno je osam populacija entomopatogene vrste *Cordyceps riverae* Pacioni (Hypocreales, Ascomycota). Do sada je istraženo oko 200 speleoloških objekata, od čega je u 69 utvrđena prisutnost vrsta iz subtroglifilnog roda *Triphosa* (Geometridae) koje su jedini, do sada sigurno utvrđeni domadari vrste *C. riverae*. Od osam speleoloških objekata Gorskog kotara, Korduna, Like i Dalmacije, u kojima su pronađene populacije te entomopatogene gljive, uzorci su prikupljeni iz pet špilja sa područja Gorskog kotara, Korduna i Dalmacije. U radu dajemo detaljan opis vrste *C. riverae* uz usporedbu s holotipom te vrste, podrobnu ekološku analizu, te kartografski prikaz poznate raspro-

stranjenosti vrste i njenih domadara u Hrvatskoj. Vrsta *C. riverae* zabilježena je po prvi puta u mikoflori Hrvatske.

**Ključne riječi:** Ascomycota, Hypocreales, *Cordyceps riverae*, Lepidoptera, *Triphosa dubitata*, *T. sabaudiata*, biospeleologija, ekologija, kartiranje, morfologija, mikofloristika, Hrvatska

## INTRODUCTION

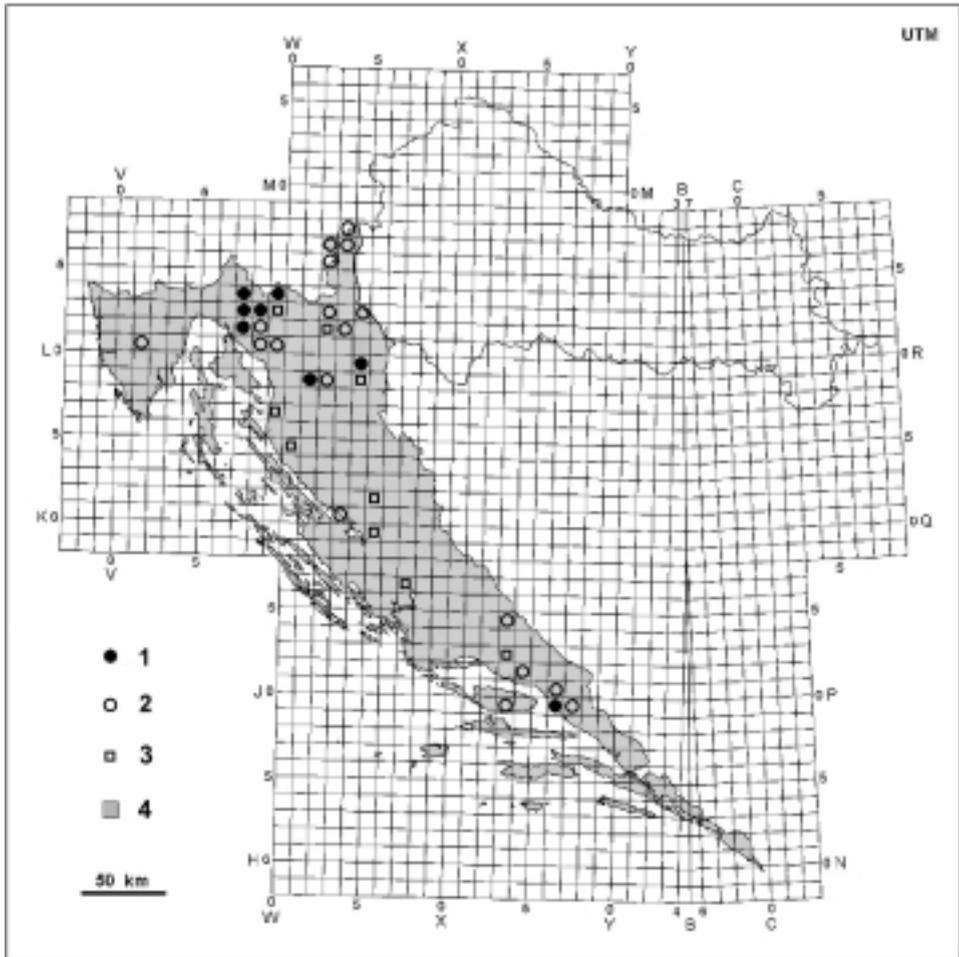
Apart from the Laboulbeniales, there is no existing literature relating to occurrence of fungi in Croatian caves. Thus, this paper appears to be the first contribution on cave Macromycetes in Croatia.

The entomogenous species *Cordyceps riverae* Pacioni was recently described from Italy (PACIONI, 1978) on the basis of the single stroma found on a dead moth (imago) in the cave Grotta Vaccamorta. The author also quotes an unidentified collection from a Belgian cave (THINÈS & TERCAFS, 1972) which might belong to the same species. Unfortunately, ecological data, especially on host, and data on characters *in statu vivo* are insufficient or absent. PACIONI (1978) wrote »..un *Lepidottero trogloxeno adulto, probabilmente un Noctuidae*..« and also states that the type material was preserved in 70% ethanol solution which made observation of the microscopic structures *in statu vivo* impossible (cfr. BARAL, 1992). Therefore, the aims of the present paper are to map known distribution and ecological patterns of the troglobitic species *C. riverae* in Croatia, whilst providing for the first time a description of its morphologic characters *in statu vivo* as well as details on its ecological behaviour. This species is recorded for the Croatian mycoflora for the first time.

## MATERIALS AND METHODS

Since 1990, some 200 speleological sites have been visited so far during systematic biospeleological field work on the Dinaric karst area in order to find both living and/or infected hosts. Of these, 69 sites were inhabited by two subtroglophilic lepidopteran species of the genus *Triphosa* (Geometridae) which are apparently the only surely known hosts of *C. riverae*. The fungus itself was recorded in only eight sites (all settled with *Triphosa* species) situated in Gorski kotar, Kordun Lika and Dalmatia regions (Fig. 1). Of these, *C. riverae* was collected from five such objects. The other three records were identified with certainty on the basis of macrophotographs taken of the living stromata according to our present knowledge of the species.

This systematic and comprehensive biospeleological field work was made possible thanks to our colleagues and to diligent efforts and observations of many people from local speleological societies all over the country. In the next stage of the research, the second author with his colleagues were guided in field work according to the large quantity of previously gathered data on *Triphosa* spp. distribution and continued with detailed biospeleological work on selected caves proved to be settled by the *Triphosa*. They collected the lepidopteran material from ca. 100 sites



**Fig. 1.** Known distribution of *Cordyceps riverae* Pacioni and its hosts *Triphosa* spp. in Croatia; 1 – localities with records and collections of *C. riverae*, 2 – localities with records and collections of the *Triphosa* spp. not colonized by *C. riverae*, 3 – additional data on records of *Triphosa* spp. without being monitored for the presence of *C. riverae*, and 4 – Dinaric system area with Adriatic plate – predominantly highly karstified region.

(KUČINIĆ *et al.*, 1999). In all caves with *Triphosa* species parasitised by the fungus macrophotographs were taken. The second author then made collections with basic ecological measurements (e.g. air temperature) and additional observations *in situ*. The material was collected in humid plastic boxes in order to keep it *in statu vivo*. The first author was able then to examine the fungus both in the living and dried state with procedures described and explained elsewhere (BARAL, 1992; MATOČEC *et al.*, 1995) and to take closer macrophotographs of the stromata. The characters of

the dried material are in agreement with PACIONI's data (1978). We concentrate therefore on vital taxonomy research and on ecology of the *C. riverae*. All collections with photographs, descriptions and drawings were deposited in private fungarium Matočec (N.M.).

## MATERIAL STUDIED

### *Cordyceps riverae* Pacioni

Giorn. Bot. Ital. 112(5–6): 395, 1978.

Illustrations: Pacioni (1978, Fig. 1.), this paper Figs. 2–4.

**Stromata** gregarious to somewhat basally confluent, white, cylindric to cylindric-conical (figs. 2, 4), 0.6 to 2.8 mm in height, 0.4–0.8 mm in diam., fleshy, surface finely felty, apically beset with (1)2–4(5) perithecia in a crown-like or crest-like formation (fig. 2). **Perithecia** prominent, superficial, attached to the stroma only with its basal part, long pyriform shaped to cylindric-conical, pale sulphur-yellow, ca. 0.5 mm in height (figs. 2, 3, 4). Ostiole papillate, yellow in living material, becoming brownish on drying. Perithecia dark brown to almost black in completely dried material.

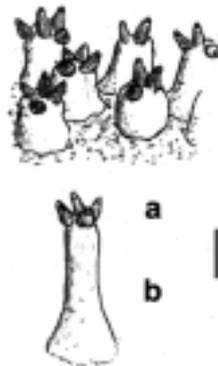
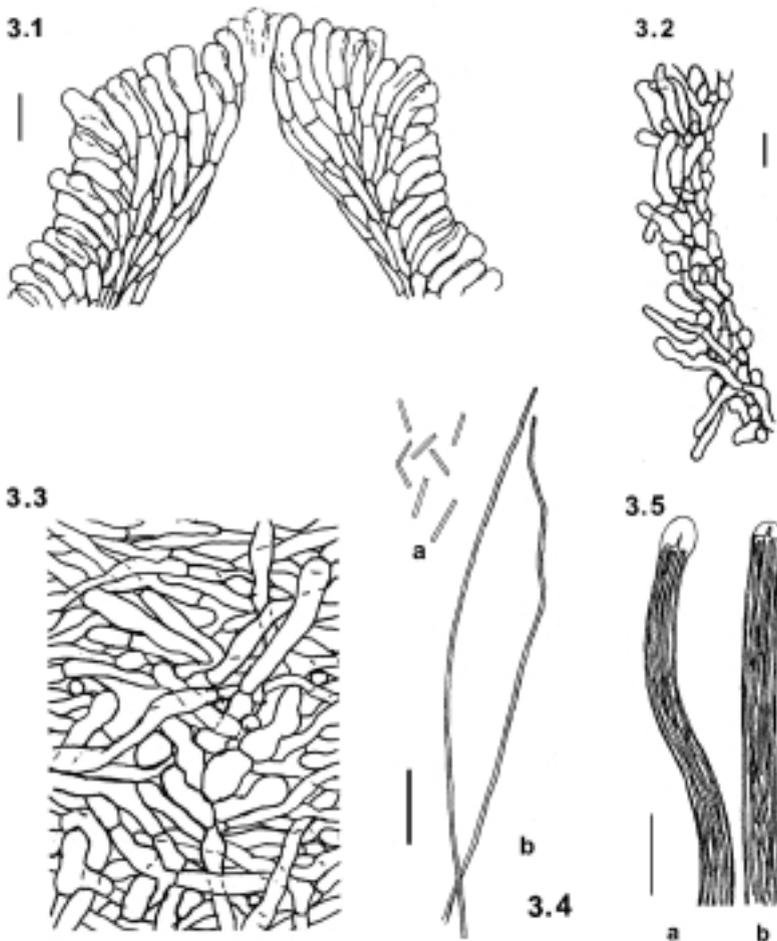


Fig. 2. Macromorphology of the ascomata of *C. riverae*; a – »apical crown« of the perithecia, b – stroma, bar = 1 mm

**Perithecia** (\*) long pyriform to lemon-shaped, 550–715 × 242–364 μm. **Ostiole** (\*) (fig. 3.1) low papillate, 108–125 × 111–182 μm, composed of palisade-like, cylindric-clavate to cylindric-truncate terminal cells, 3.8–5.7 μm in diam., with bright yellow to golden-yellow walls. **Perithecial wall** (\*) (figs. 3.2 and 3.3) 23–33 μm thick, broadened to 48 μm in ostiole, composed of intricately glued cylindric to slightly swollen cells (bird's nest-like configuration of the hyphae), 3.2–7.2 μm in diam., some cells irregularly projecting on the surface as short terminals in various directions. **Paraphyses** absent. **Asci** (fig. 3.5) filiform, 347–446 × 4.0–4.6 μm (♣ but in fresh material) and 342–445 × 4.0–4.5 μm (♣ from rehydrated exsiccata), with

aporphynchous tapered base (hardly visible in dried material), hyaline, 8-spored, with inoperculate, capitate apex, ( $\ddagger$ )  $3.2\text{--}3.8 \times 3.8\text{--}4.2 \mu\text{m}$  in immature (fig. 3.5a) and thickened-hemisphaerical apex, (\*)  $1.6\text{--}2.0 \times 3.8\text{--}4.2 \mu\text{m}$  and ( $\ddagger$ )  $1.8\text{--}2.6 \times 3.8\text{--}4.2 \mu\text{m}$  in mature asci (fig. 3.5b). **Ascospores** (fig. 3.4b) hyaline, aseptate in immature state, eguttulate, with homogenous refractive cytoplasm, filiform, slightly tapered at both ends, smooth walled, in complete maturity fragmented in numerous part-spores. **Part-spores** (fig. 3.4a) hyaline, eguttulate, aseptate, rod shaped with truncate ends, smooth walled, (\*)  $5.0\text{--}7.1 \times 0.4\text{--}0.8 \mu\text{m}$ , ( $\ddagger$ )  $4.3\text{--}7.0 \times 0.4\text{--}0.7 \mu\text{m}$ .

Symbols used in description are: (\*) for characters of the living cell and ( $\ddagger$ ) for characters of the dead cell.



**Fig. 3.** Micromorphology of the perithecia of *C. riverae*: 3.1 ostiole, 3.2 perithecial wall-vertical section, 3.3 perithecial wall, surface view, 3.4 ascospores (a – immature ascospores and b – part-spores), 3.5 asci (a – immature ascus and b – mature ascus). Bar = 10  $\mu\text{m}$  (same scale for Fig. 3.2 and 3.3); all in living state.



**Fig. 4.** *Cordyceps riverae* Pacioni. A colony of the living stromata on *Triphosa sabaudiata* Duponchel 1830; fungarium N.M. 4217; from Tučepska vilenjača cave above Tučepi, Mt. Biokovo, UTM XH 69; 9. Apr. 1999; leg. S. Gottstein & R. Ozimec. Photo by Neven Matočec.

**Collections examined:** N.M. 4217 (private fungarium Matočec); Tučepska vilenjača cave above Tučepi, Mt. Biokovo, Dalmatia, UTM 33T XH 69; 9. Apr. 1999; host: *Triphosa sabaudiata* leg. S. Gottstein & R. Ozimec; N.M. 4277; cave system Matešićeva spilja-Popovačka spilja near Slunj, Kordun region, UTM 33T WK 49, 15. May 1999; leg. R. Ozimec.; N.M. 4168; Muževa hižica cave in Vražji prolaz canyon near Skrad, UTM 33T VL 93, 24. Feb. 1991; leg. R. Ozimec; N.M. 4309; Bukovac cave near Fužine, UTM 33T VL 71, 20. Feb. 1999; leg. R. Ozimec; N.M. 4291; Ledenica cave in Golubinjak area near Lokve, all from Gorski kotar region, UTM 33T VL 82, 14. June 1999; leg. S. Gottstein, all hosts *Triphosa dubitata*. All collections originated from humid wall communities in aphotic zone but always near entrance of the sites. Type not seen (data taken from PACIONI, 1978).

**Additional records:** (1) Siničića cave near Brinje, Lika region, UTM 33T WK 18, 7. Oct. 1995.; (2) Trlica hole, Mt. Risnjak, Gorski kotar region, UTM 33T VL 72, 31. Aug. 1996. and (3) Prva jama hole, Mt. Risnjak, Gorski kotar region, UTM 33T VL 73, 30. July 1997. Host: *Triphosa dubitata*. All obs. by R. Ozimec.

## DISCUSSION

### Morphological observations

We always found at least several, but frequently even numerous crowded to basally confluent stromata on a single host specimen, while PACIONI (1978) described the holotype with only a single stroma. The author reports the lack of exact data on perithecial colour due to dead state of the material (conserved in 70% ethanol) he worked on. He quotes the collector's data as follows: »..l'esemplare era non pigmentato.«, but he did not include this data in the species diagnosis. The author also speculates that perithecia could be without pigment according to the black & white photograph of cave *Cordyceps* sp. from Belgium published by Thinès & Tercafs. The author concludes that the Belgian and Italian material are conspecific. We do not follow this conclusion since as we consider that black and white macromorphology data alone are insufficient for identification to species in the genus *Cordyceps*, and consider the Belgian material dubious. A microscopical analysis of the voucher specimen (now apparently lacking) would be necessary to determine about its identity. We found the perithecial colour taxonomically important, and observed its dramatic change. Perithecial colour in both young and mature living specimens is pale sulphur-yellow, due to pigments (perhaps carotenoid) deposited in the perithecial cell walls. Perithecial colour turns to dark brown rapidly through the process of dehydration and becomes almost black in completely dried material.

Since we were unable to isolate the individual asci in whole length from hymenium *in statu vivo*, it was possible to measure their length and breadth *in statu emortuo* in both fresh material and rehydrated exsiccata. Our ascus measurements are therefore in agreement with those from PACIONI's paper (300–550 × 4.0–4.5 µm) obtained from dead material (1978). The apical wall appeared significantly thicker in dead state. However, Pacioni found apical wall height to be 1.6–1.8 µm, which is thinner than in our material (both measured in dead state, see description above). Part spores in vital state are not significantly different from those measured in dead state in our material nor from data given by Pacioni. We ascribe all observed differences between our and the Italian material (including possible differences of perithecial colour) to the insufficient knowledge of species variation due to small number of studied collections or to geographic and ecological isolation between Croatian and Italian populations. Consequently, we consider the Croatian and Italian material to be conspecific and the present species to be distinct from others in genus.

### Hosts

*Triphosa dubitata* (L., 1758) and *T. sabaudiata* (Duponchel, 1831). (Geometridae) are apparently the only surely known hosts of the entomogenous fungus *Cordyceps riverae*. Although another lepidopteran species occurs together with *Triphosa* species, viz. *Scoliopteryx libatrix* (L., 1758). (Noctuidae) in almost all investigated caves, it is never seen to be infected by *C. riverae*. Equally, this fungus is never found on any other lepidopteran or arthropod species inside or outside the caves. This suggests that *C. riverae* is a highly specialised troglobiont parasite confined most proba-

bly to the genus *Triphosa* or to the Geometridae family. In Continental Croatian caves the only potential host is *T. dubitata* which is most frequent subtroglophilic moth in the Croatian caves (KUČINIĆ, 1990). In Mediterranean part of the Croatia both *Triphosa* species occur but we found the fungus only on *T. sabaudiata* (collection from the Tučepska Vilenjača cave). We suppose however, that *C. riverae* can infect also *T. dubitata* in that area as well. According to the ecological observations *in situ*, we are certain that those moths can be infected by the fungus only inside the caves in late autumn and/or in winter where these subtroglophilic moths (imago) enter for hibernation.

### Fungal dynamics and habitat conditions

Since all records of the fungus originate from caves with permanent water, or at least with highly increased air humidity during the winter, and since in all cases stromata bearing moths were always beset with numerous water drops, it can be assumed that spore germination and host infection take place in conditions of increased cave air humidity. This coincides with late autumnal arrival of the adults of *Triphosa* moths inside the caves. A massive condensation of water drops occurs on the cave walls and on resting animals at that time. Production of the stromata takes place as early as February and may continue to October if humidity remains sufficiently high. The species can be considered therefore as strongly xero-intolerant. The moths that survived fungal attacks leave the caves during the spring after hibernation.

Since this species is obviously restricted to *Triphosa* moths – a species that has been recorded in all karstic regions of Croatian Dinaric area (Fig. 1), we can expect much more localities with *C. riverae* in wet or humid caves from Istria to South Dalmatia. It is possible however that the species is more frequent in perhumid Gorski kotar region than, for example, in more arid Dalmatia region. Several visits to the caves situated outside the Dinaric hyperkarst area resulted in no *Triphosa* records. The fungus has a widely distributed vertical range, with the lowest cave being situated at 215 m a. s. l. and highest at 1300 m a. s. l. We have measured the air temperature during every cave visit that revealed the air temperature range at which stromata were produced. The air temperature ranged as low as 5.2 °C in highest known cave up to 14 °C in the only locality known so far in the Mediterranean area (Dalmatia).

### ACKNOWLEDGEMENTS

The present paper is a result of the systematic and comprehensive biospeleological field work which was undertaken largely thanks to diligent efforts and observations of many people gathered in local speleological societies all over the country. They made this scientific progress possible. We thank Sanja Gottstein from Department of Zoology, Faculty of Science, University of Zagreb; SD »Dinaridi« from Zagreb, especially N. Buzjak; SD »Pauk« from Fužine, especially G. Polić; SD »Karlovac«, especially H. Cvitanović; SO HPD »Dubovac«, (both organizations from

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## SAŽETAK

### O vrsti *Cordyceps riverae* (*Hypocreales*, *Ascomycota*) u špiljama Hrvatske

N. Matočec & R. Ozimec

Izuzevši red Laboulbeniales, u literaturi poznatoj autorima nije pronađen niti jedan podatak o prisutnosti gljiva u speleološkim objektima Hrvatske. Ovaj rad predstavlja tako prvi prilog istraživanju špiljskih makromiceta Hrvatske. U sklopu sustavnog biospeleološkog istraživanja, koje se provodi na području Dinarida od 1990. godine, pronađeno je osam populacija entomopatogene vrste *Cordyceps riverae* Pacioni (*Hypocreales*, *Ascomycota*). Do sada je istraženo oko 200 speleoloških objekata, pri čemu je u 69 utvrđena prisutnost vrsta iz subtroglofilnog roda *Triphosa* (*Geometridae*) koje su, čini se, jedini sigurno utvrđeni domadar vrste *C. riverae*. Gotovo na svim lokalitetima u kojima je ova gljiva pronađena, uz pripadnike roda *Triphosa*, pronađeni su i leptiri vrste *Scoliopteryx libatrix*. Međutim, vrsta *C. riverae* nije dosad pronađena na jedinkama te vrste, kao ni na bilo kojem drugom člankonošcu bilo unutar, bilo izvan speleoloških objekata. Na temelju toga zaključujemo da je vrsta *C. riverae* troglobiont, specijalizirani parazit vrsta roda *Triphosa* ili porodice *Geometridae*. Prema terenskim istraživanjima, do infekcije domadara dolazi u kasnu jesen ili zimi, kada dolazak ovih subtroglofilnih leptira u špilje zbog hibernacije

koincidira s porastom zračne vlage i snažnom kondenzacijom vodenih kapljica na pridošlim leptirima unutar tih špiljskih prostora. Od osam speleoloških objekata Gorskog kotara, Korduna, Like i Dalmacije, u kojima su pronađene populacije te entomopatogene gljive, uzorci su prikupljeni iz pet špilja sa područja Gorskog kotara, Korduna i Dalmacije. U radu dajemo detaljan opis vrste *C. riverae* uz usporedbu s holotipom te vrste, detaljnu ekološku analizu, te kartografski prikaz poznate rasprostranjenosti vrste i njenog domadara u Hrvatskoj. Budući da je ova vrsta do sada bila nedovoljno poznata, u radu dajemo po prvi put opis njenih vitalnih taksonomskih obilježja, kao i prve podatke o ekološkim osobitostima vrste. Neke razlike, npr. u pogledu debljine apikalne stijenke askusa, mogu se pripisati slaboj istraženosti vrste (jedini sigurni publicirani materijal je holotip, koji se sastoji od samo jedne strome s peritecijima) kao i mogućoj zemljopisnoj i ekološkoj izoliranosti naših i talijanskih populacija. Vrsta *C. riverae* zabilježena je po prvi puta u mikoflori Hrvatske, a njeno prisustvo može se očekivati u svim speleološkim objektima dinarskog područja koji imaju stalnu vodu ili barem potpuno zasićenje vlažnosti zraka zimi, a koji su istovremeno naseljeni i vrstama roda *Triphosa*.