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The composition of intestine content of *Orchesella cincta* (Linné) (Insecta: Collembola)

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Abstract. The composition of intestine content of the collembolan species *Orchesella cincta* (L.) was studied. The specimens were collected from soil covering moss, *Tortella tortuosa* (Hedw.) Limp. cushions at two sites. The composition of cultivable mycobiota of this Collembola was compared with that of mosses to show, whether collembolans feed selectively on fungi occurring on mosses. The intestine content was composed of detritus, moss particles, and fungal propagules. Difference in diversity of mycobiota found both in moss and in Collembola was similar at the two collecting sites. Comparing the mycobiota of the moss as diet and that of the intestine content, clear evidences of selective feeding were observed. The preference of Collembola for particular fungi differed between the collecting sites.

The examinations of the intestine content of Collembola species collected from the original habitat and the experiments made in laboratory can answer for the question: what kind of food can be eaten by Collembola species and which food resources do they prefer?

It is generally known from the literature that collembolans feed with little algal cells, musci, detritus.

Fungi which are found in bryophyte cushions play important role in the decomposition of organic material. The examination of the feeding Collembola species and other invertebrates have importance because these animals may influence the rate of decomposition, which has an effect in an indirect way for the feeding with mycobiota. The composition of mycobiota association may change if these animals feed selectively with fungi in bryophyte, and therefore the intensity of decomposition processes will change, too.

According to feeding studies in connection with Collembola species during Hungarian and international examinations, it can be seen that one part of them eat saprophytic and phytopathogenic fungi (Lartey et al., 1989; Wittaker, 1981; Bengston et al., 1983; Hedlund et al., 1991; Leonard & Anderson, 1991), other species eat living and dead bryophyte fragments, decayed leaf materials and mycelia (Ulber, 1980; Sadaka & Poinsot-Balaguer, 1989; Bakonyi, 1998). By means of feeding with mycobiota and of their motility, collembolans may have an important role in the dispersion of heterotrophyc mycobiota (Christen, 1975; Petersen & Luxton, 1982).

Walsh and Bogler (1990, 1993) studied the preference of Collembola species for fungi. According to their results, the food preferences can be frequently detected in connection with the offered mycobiota, but the rate of it can be different. It is also known that the preferred and eaten mycobiota may influence the population dynamics, growth and reproduction of the Collembola species.

The common soil fungal taxa (e.g. *Alternaria, Fusarium, Trichoderma*) can be found in bryophytes. Mycobiota settling in bryophytes are important factors in the life of animal associations as it is supported by feeding preference examinations (Bakonyi, 1989; Bakonyi et al., 1995; Walsh & Bolger, 1993). The mycobiota living in bryophytes can be served as food for the animals occurring in bryophytes. For all of these facts, it is an im-

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portant study to examine the composition of mycobiota occurring in bryophytes.

In the first step of our examination, we isolated and identified the fungi occurring in bryophyte *Tortella tortuosa*. In the second step, we surveyed the alimentary content of *Orchesella cincta*, and separated the food fragments. We examined the food structure and the ratio of different food types. After all, in order to get an overall picture about the composition of the eaten mycobiota, we isolated and identified the fungi occurring in the alimentary of *Orchesella cincta*.

MATERIAL AND METHODS

Sampling area and collecting sites

The collecting sites were chosen in different plant communities at the village Szarvaskő (Bükk National Park) in the Northern Mountain Range of Hungary. Collecting Site I: A rocky habitat with south exposition, Puls. Fest. rupicolae. Collecting Site II: A dry habitat divide with big rocks, Potentillo Festucetum. It has a south-west exposition.

Collecting the bryophyte samples

For the examination of the alimentary, collections were taken by aspects (1997–98) and the samples were analysed in the chosen communities during the investigation. Five bryophyte cushions of 10 × 10 cm size were taken from both sites according to the statements of Cohran (1963): in order to know the animal associations of bryophytes, the size of the bryophytes cushions must be at least 20 times bigger than that of the animals collected in them. The collection of Collembola species was made by prespan funnel (Berlese System) in room temperature. Berlese–Balogh's Salting Method was applied to separate invertebrates from debris. The lactic acid clearing of Collembola species were made in Gisin's Fluid (10 cm^3 lactic acid, 2 cm^3 glycerol, 40 % formalin), then they were picked up and identified in open preparations.

Examination of mycobiota in the bryophyte cushion

Five pieces (10 g) of each examined bryophyte sample were separated and washed through in 100 ml of sterile distilled water. The mycobiota were cultured from the inoculum washed from the surface of the bryophyte. The washed byrophyte fragments were ground in a tissue homogenizer to culture the fungi living in bryophyte. Aliquots of both suspensions (washed or suspended) were spread on Martin-agar or glucose-pepton agar plates. Inoculated plates were cultured at 27–28° C in thermostat. Each developing fungal colony was picked up and transferred on glucose-pepton agar slants. Isolates were identified microscopically in lactophenol-anilin blue stained preparations.

Microscopic examinations of the intestine content

The whole intestine of collembolans (10 individuals/species per collection site) was prepared with needles in three replicates.

The intestine contents were homogenized in 5 cm^3 of distilled water, dropped with lactophenol-aniline blue stain and spread on microscopic slides.

At the separation of the food types we rely on the microscopic morphological features. During the analyses of the intestinal content, we used the method of Hodkinson et al. (1994) by which the particles (bryophytes, mycelia, bryophyte spores, detritus) are counted on the examined surface along linear transects. Percent value data were logarithmically transformed before statistical analyses.

The assessment of mycobiota of collembolan intestine and the bryophyte cushion

Collembolans collected alive from the moss samples were washed 10 times with sterile distilled water to clean them from soil fragments and fungal spores. During the exploration of the mycobiota occurring in the intestine, they were homogenized in 5 cm^3 of sterile water with tissue homogenizer. The suspensions were spread on agar plates as it was applied for bryophytes.

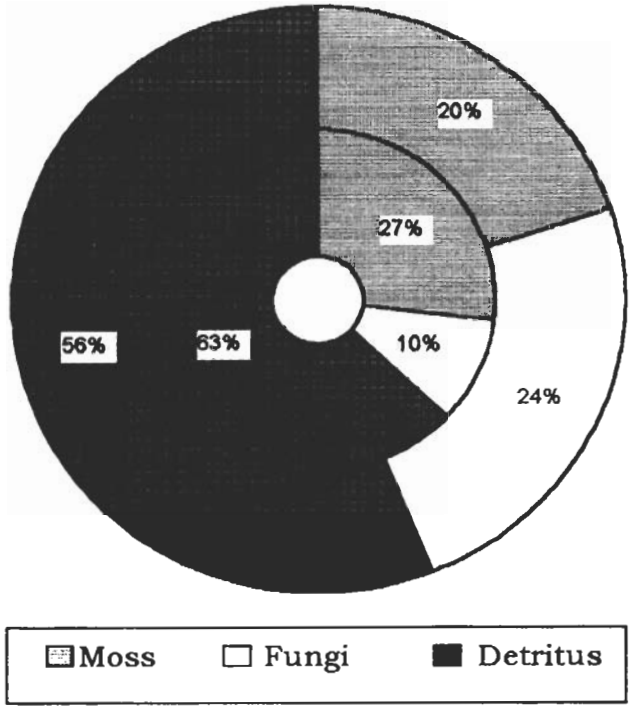

Figure 1. The composition of the bowel content at the Collecting Site I (inner ring) and II (outer ring)

The evaluation of the preference of collembolans for fungi

Fungi occurring in bryophytes mean a kind of food for Collembolan species. The preference can be calculated with Ivlev's (1961) formula:

$$E_i = \frac{r_i - n_i}{r_i + n_i}$$

where E_i = Ivlev's Electivity Index measure for species, r_i = percentage of species i in the diet, n_i = percentage of species i in the environment.

The electivity index shows the relative density of food types exactly, including many of them. The electivity variations extend from –1 to +1. The values from 0 to +1 refer to the degree of preference while the values between 0 and –1 indicate avoidance.

During the evaluation of Ivlev's Electivity Index, it became clear that fungi are not consumed in equal rates, so there may be a difference between the supply and demand. It can be seen in the supply of the two collecting sites and it can be extended for the choose of the analysed Collembola species, too. The Ivlev's Index allows to make consequences for the preference. Because of the characteristics of the index, avoidance does not mean exactly that the species does not feed with the given food type, even if there was a little quantity of it in the food of the species.

In order to evaluate the preference of mycobiota, the Manly's Alfa Index was calculated as well. Using the index, the lack of selectivity in feeding can be estimated and in the case of selective feeding one could estimate the degree of preference for each food type. Manly's Alfa Preference Index was calculated with the formula:

$$\alpha_i = \frac{r_i}{n_i} \quad \frac{1}{\sum r_j / n_j}$$

Table 1. *Fungi recovered from the intestine of Orchesella cincta*

Fungal taxa	Collecting sites	
	I.	II.
Absidia spinosa Lendner	1	6
Aspergillus sp. Micheli ex Fries	0	5
Gliocladium sp. Corda	1	0
G. roseum Bain.	0	6
Isaria arachnophila Ditmar	0	5
Papulaspora sp. Preuss	1	0
Paecilomyces sp. Bainier	0	2
Penicillium sp. Link	10	13
Stachybotris alternans Bonorden	2	6
S. lobulata Berkeley	2	25
Trichoderma atroviride Bisett	1	0
T. harzianum Rifai	3	0
T. koningii Oud.	0	7
T. longipilis Bissett	0	3
Verticillium lateritium Berkeley	2	7
Diversity (Shannon)	1.95	2.13
Total colony number	26	85

where a_i = Manly's Alfa (Preference) Index, r_i and r_j = proportion of food types i and j in the diet (i and j = 1, 2, 3...m), n_i and n_j = proportion of food type i and j in the environment, m = number of the possible food types.

When selective feeding doesn't exist then a_i = 1/m. When a_i > 1/m, then food type i is preferred in the diet. So, when a_i <1/m then food type i is avoided and it is not preferred in the diet of the examined species. The alfa values measure the probability of the selection of a food type (in our case a fungal species) from the main food type of all the species in the supply when all of the presented food types are choosable food sources. It must be noted that there is no general agreement in the literature for the best index to measure preference, but many researchers drafted that Manly's Alfa is one of the most frequently used preference indices that can be used in most situations (Ellis, 1976; Stephens & Krebs, 1986; Rogers, 1984).

RESULTS

The mycobiota of the bryophyte species

Ten genera and species of fungi were identified from the bryophyte species *Tortella tortuosa*. The mycobiota of the bryophyte cushion collected from Collecting Site II had a wider spectrum. This might

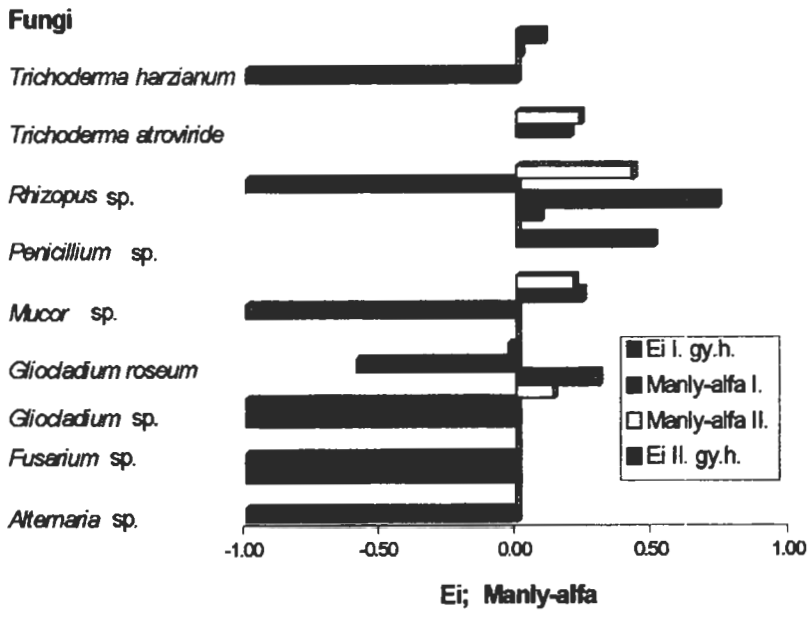

Figure 2. Electivity (E_i) and preference (Manley's Alfa) index of *Orchesella cincta* at the Collecting Site I and II

be the result of the microclimatic differences in the two different types of plant communities. We did not find *Aspergillus* sp., *Mucor* sp. and *Trichoderma atroviride* on the bryophyte cushion collected from Collecting Site I.

The intestine content and its mycobiota

During the microscopic examination of the intestine content, it was observed that *O. cincta* utilized various food sources. There was no individual with only one type of food in its intestine. The foregut was extremely green due to the presence of algae, bryophyte fragments or both of them. Some members of mycobiota with characteristic features could be recognized directly, e.g. an *Alternaria* colony on a consumed bryophyte fragment. There were indigested food fragments of animal origins (legs, wings, etc.) in the detritus.

The percentage composition of intestine content is shown in Fig. 1. The rate of bryophyte fragments was higher on Collecting Site I than on Site II. It was the reverse in the case of fungal propagules. Collembolans eaten two times more fungi on Collecting Site II than on the Site I. There were no essential differences in the rate of detritus. These differences in the rate of bryophyte fragments in fungal propagules might be the consequence of the different developing digestive process because the individuals were collected in different feeding phase.

In all, fifteen fungal taxa were identified from the intestine content with cultivation-isolation method (Table 1). Nine and eleven taxa were observed on Collecting Site I and II, respectively. Five out of them occurred on both sites. The intestine mycobiota on Collecting Site II was both more diverse and abundant. Nine fungal species or genera occurring in the intestine were not found in the bryophyte samples. We think that these fungi were eaten by collembolans when they grazed out of the bryophyte cushion. It is presumable that these fungi are preferred by *O. cincta* because their rate in the culturable mycobiota overcame 58 % and 28 % on Collecting Site I and II, respectively.

Preference

During the preference examinations, we analyzed the degree of utilization of the bryophyte's mycobiota by *O. cincta* as a food-base and its preference for members of mycobiota. The rest of the intestine content (detritus and mycelia) could not be analyzed because of lack of distinguishable features that would inform us about their origin.

As the preference indices show on Fig. 2, *O. cincta* searched and feed with *Penicillium* species but it did not prefer *Alternaria* and *Fusarium*; it avoided these fungi and did not feed with them. In some cases, individuals avoided some fungal taxa on one of the collecting sites, but they eat them on the other one. They avoided *Gliocladium* spp. on both sites, although they did eat a less quantity of them. *Rhizopus* sp. was eaten by *O. cincta*, but it occurred only on Collecting Site II.

The preference for six fungi common in bryophyte and intestine content at both sites is represented in Fig. 3 with rate of Manly's Alfa values. It can be stated that the preference of *O. cincta* greatly varied by sites. *Gliocladium* sp. was the only fungus, of which values were similar; the other ones were eaten only at one of the sites.

DISCUSSION

The aim of our study was to detect whether *O. cincta* feed with bryophyte fragments of its habitat and the fungi in that, and to determine the rate of the eaten fungi in the feed of *O. cincta*. The exploration of intestine and the analysis of its content supported the observation that the individuals of this Collembola species, like those of other ones, are feeding continuously (Anderson & Healey, 1972). We observed that the food consisted of fragments of different origin in the most individuals. The different colour and structure of the fragments showed that animal fed with one kind of food first and then changed for another food source (in the bryophyte or out of it) and fed with it. The recognization and separation of the food types became harder along the intestine as the digestive processes were going on.

More examinations show that Collembola species prefer a kind of food source to the other ones provided them in laboratory experiments (e.g. Lartey et al., 1989; Leonard & Anderson, 1991; Walsh & Bolger, 1993). However, the animals do not distinct food types in that manner in the nature, where a more wide and complex food-base is available. We found *O. cincta* to be an opportunist species considering its feeding habits. It has a wide taste and flexibility, thus it can explore the variably consisted food-base at different microhabitats. For instance, the rate of fungal propagules in the intestine content was considerably higher on Collecting Site II, where the bryophyte mycobiota was more diverse and abundant. However, the major part of components of fungal origin was changed to bryophyte fragments at Collecting Site I, where a more sparse mycobiota was available. Furthermore, the preference rank for particular fungi differed at the sites giving a reason to doubt the reliability of laboratory preference examinations. We expect that the different microclimatic conditions resulted in a different physiological status of fungi leading to changed taste for *O. cincta*.

There are examinations under laboratory conditions, which emphasize the importance of *Trichoderma* spp. for the collembolans. During a population dynamics study of collembolans, Walsh and Bolger (1993) noted that these animals select among the provided fungi, even if they were in mixed culture. Collembolans feeding with not liked *Trichoderma viride* produced the fastest growth rates and the highest population size. This fact leads to the consequence that the preference for some food types and their physiological importance are not cover each other. In fact, the less preferred food may have a fundamental importance for the animal. It may occur that fungi having a good effect for the reproductive processes contain some not liked compounds. Thus, the animals eat less quantities of this food type, but continuously, so it seems to be less preferred food type during the preference examinations.

Also our examinations supported that collembolans hold high quantities of fungal spores, thus it can be stated that collembolans may play an important role in the distribution of the fungi living in or out of the bryophyte cushion (Varga & Naár, 2002). These animals are not tied with the bryophyte species; they go from the one kind to the other, delivering the spores with their faeces.

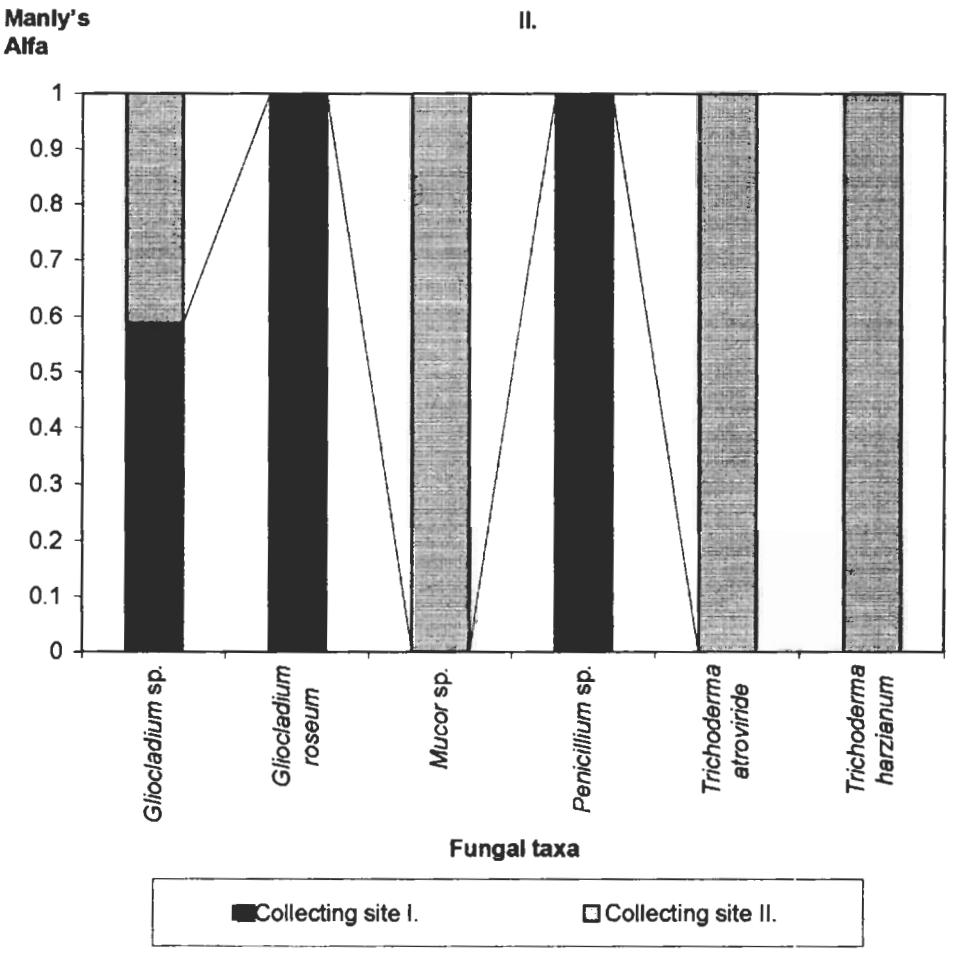

Figure 3. The diet of *Orchesella cincta* on the basis of preference index (Manly's Alfa) at the Collecting Site I and II

The cultivation of fungi from the intestine of Collembola species can be used not only as a control examination, but it may contribute to the mycologists with selective isolation of such fungi that are preferred by collembolans and are hard to culture because of their low rate in the mycobiota.

The cultivation of fungal content of intestine gives a good mean to identify the fungi that are actually eaten under natural conditions.

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