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FULL PAPER

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Establishment of the case of *Hebeloma radicosum* growth on the latrine of the wood mouse

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Abstract Three cases of *Hebeloma radicosum* growth on the wood-mouse latrine near a wood-mouse nest are here described from Switzerland. This study establishes that this fungus may associate with wood mice, besides associating with moles as has been previously known. The nest occupant and the latrine maker were independently identified to ensure that the two were not different. For this identification, the characteristics of fallen hairs found from the nest and of those found from the deserted latrines now colonized by the fungus were taken as the most important information. In addition to these case studies, information about the occurrence of *H. radicosum* in northern Europe and northern Japan (Hokkaido) where moles do not occur is presented, which explicitly indicates the possibility of customer animals other than moles.

Key words Agaricales · *Apodemus* sp. · Excrement · Nest · Talpidae

Introduction

Moles (Mammalia, Talpidae; including shrew moles) make latrines (middens) near their nests below ground (Sagara 1999). *Hebeloma radicosum* (Bull.: Fr.) Ricken (Agaricales) colonize and fruit on deserted mole latrines, as, except those 4 (1 plus 3) cases discussed in this article, 70 cases of *H. radicosum* growth from Japan and Europe have all been determined to have occurred on mole latrines (Sagara 1999, unpublished data). In one report from Switzerland, the same fungus was found to have grown on the presumed latrines near a nest of the wood mouse *Apodemus sylvaticus* L. or *A. flavicollis* Melchior (Muridae) (Sagara et al. 1988). This report seemed not sufficient to establish the case of *H. radicosum* growth by the wood mouse because it had failed to precisely eliminate the possibility that the latrine maker (excrement depositor), i.e., the causal animal for the fungal growth (the customer for the fungus) and the nest occupant might have been different. This problem arose from the confusing case subsequently found again in Switzerland, a case in which the nest seemed to have been occupied by a mouse or mice (perhaps after a mole) whereas the latrine colonized by the fungus seemed to have been made by a mole (Sagara 1995b).

To resolve this problem and to establish the wood-mouse case, we studied ten cases of *H. radicosum* growth in Europe, nine from Switzerland and one from Sweden. As a result, we found that three of the ten, all from Switzerland, had certainly been caused by the mouse dwelling as well as excreting, and that the rest were by the mole *Talpa europaea* L. Those three mouse cases are described here. Furthermore, we collected information about the occurrence of *H. radicosum* in northern areas where moles do not occur, which should indicate the involvement of animals other than moles as the customer for this fungus as well.

Methods

Each case of *H. radicosum* growth was investigated after Sagara (1998) in general, as follows.

As soon as *H. radicosum* fruit-bodies were found, the fruiting points were marked out with pegs by the finders (Figs. 1–4, M). The fruit-body specimens from case 1 have been deposited at the personal herbarium of the second author (B. S.-I.) at WSL as nos. 97/261 and 97/263 but will

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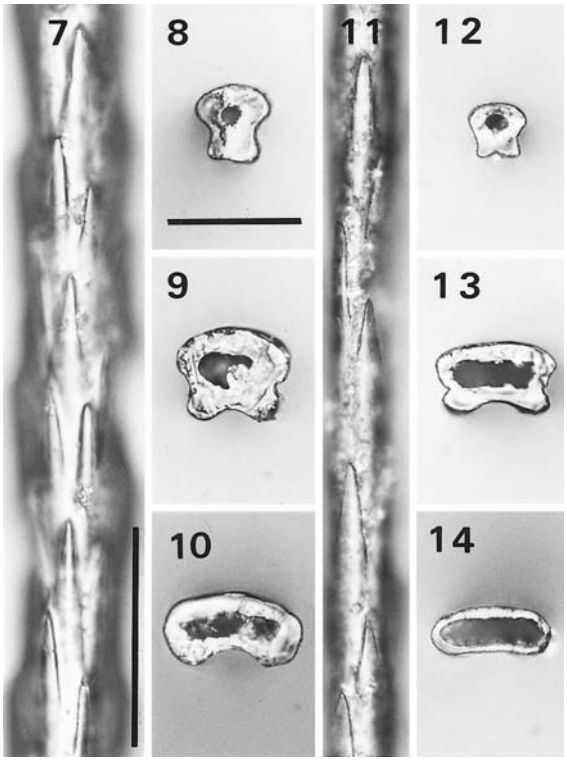
Figs. 1–6. *Hebeloma radicosum* and wood-mouse nests in Switzerland. **1** Case 1. Soil profile showing *H. radicosum* (*H*) fruiting on the mouse latrine (*L*) near the mouse nest (*N*). The film container (*M*) marks the previous fruiting on October 12, 1997. The *thick lines* on the folding scale are at 10-cm intervals; the same scale is seen in **3** and **4**. Photograph on November 21, 1997. **2, 3** Case 2. **2** Point of *H. radicosum* fruiting (*M*) and the beech forest as a habitat of the fungus and the wood mouse. **3** Soil profile under the fruiting point (*M*), showing the mouse nest (*N*) and a part of the accumulated food remains (*F*). Photographs on November 22, 1977. **4–6** Case 3. **4** Soil profile under the point of *H. radicosum* fruiting (*M*), showing the mouse nest (*N*) and a part of the accumulated food remains (*F*). **5** Nest collected from this profile, with its cavity (*C*; the resting and sleeping place) unveiled by removal of the ceiling leaves. **6** A part of the food remains collected from the same profile, showing beechnut shells therein. Photographs **4** and **5,** November 23, 1997; **6**, April 26, 1998. *Bars* **5** 50 mm; **6** 10 mm

go to ZT (ETH Zürich) in the future. The fruit-bodies from cases 2 and 3 have not been preserved as specimens.

Later in the same year, we studied geographic, topographic, and vegetational characteristics of the sites that held those points (Table 1, entries 1–7), and made excavations to investigate characteristics of the nesting (Table 1, entries 8–19) and to collect materials for identification of the animal(s) involved. The collected materials include the nest (see Fig. 5), the deserted latrines near the nest, and the remains of stored food (see Fig. 6). The "deserted latrine" was recognized as the part of the soil that had been colonized by the *H. radicosum* mycelium and fine tree roots. These items have been deposited at Kyoto University Museum, Kyoto, as nos. 62–64 (for cases 1–3, respectively).

Small mammals to be considered in that identification were mole, shrew, vole, rat, wood mouse, and red squirrel, as they nest and excrete under ground (Bang and Dahlstrøm 2001). We made the identification primarily independently for the nest user and for the excrement depositor; we determined the former from the observations on the nest and the latter from those on the latrine. In this identification, the characteristics of the hairs collected from the nests and latrines (Figs. 7–14, Table 1, entries 20–24) were critically important, hairs that had naturally fallen out, e.g., by molting and grooming (Sagara et al. 1988; Sagara 1989).

Collection of the information on *H. radicosum* occurrence from the areas devoid of moles (Abe 1967; Mitchell-Jones et al. 1999) covered Norway, Ireland, and Hokkaido (northern Japan), accessing the Norwegian Mycology Database, surveying literature, or corresponding with mycologists.



Figs. 7–14. Photomicrographs of two guard hairs, one collected from the nest (**7–10**) and the other from the deserted latrine near the nest (**11–14**), showing the nest occupant and the latrine maker were the same animal (material from case 2, incident light microscopy). Both hairs represent the wood mouse *Apodemus* with a combination of lanceolate scale pattern and triconcave to kidney-shaped cross sections. **7, 11** Scales at the central part in the shaft region of the guard hair. **8–10, 12–14** Cross sections of the guard hair. **8, 12** Proximal part of the tip region, having three concave sides. **9, 13** Distal part of the shield region, still having three concave sides. **10, 14** Central part of the shield region, being kidney-shaped with one concave side. *Bars* **7, 8** 50 μm (**7, 11** same magnification; **8–10, 12–14** same magnification)

Results

The three mouse cases from Switzerland

Data are shown in Figs. 1–14 and Table 1. First, the presence of *Fagus sylvatica* trees at those sites (Table 1, entry 7) satisfies the condition that certain species of Fagaceae, Betulaceae, or Salicaceae must coexist with *H. radicosum* to form ectomycorrhizas in the tripartite association among plant, animal, and fungus (Sagara 1995a, 1999).

The hair characteristics (Figs. 7–14; Table 1, entries 20–23) are almost enough to conclude that, in all three cases, the wood mouse (*Apodemus* sp.) caused the fungal growth by occupying the nest as well as making the latrine. Namely, the combination of the lanceolate scale pattern and the triconcave to kidney-shaped cross sections in the guard hairs distinguishes *Apodemus* from any other small mammals (Day 1966; Teerink 1991), and this observation applies to materials both from the nest and from the deserted latrine. The absence of other mammalian hair, particularly of mole hair (entry 24), is also important.

Additional features support this conclusion. (1) All the sites are located in the woodland on the dry slope of the hill (entries 6, 7), reducing possible use by voles and rats (Mitchell-Jones et al. 1999). (2) The wood-mouse nest and the mole nest resemble each other in material (unshredded fallen leaves; entry 9), but differ from the vole nest, which is constructed with finely shredded plant material (review by Sagara 1999). This difference excludes the possibility of voles as the nest user. (3) The wood-mouse tunnel (ca. 30–40 mm diameter; Montgomery and Gurnell 1985; Bang and Dahlstrøm 2001) is usually smaller than the mole tunnel (ca. 50 × 40 mm; Bang and Dahlstrøm 2001), and the present data (entry 14) rather fit the former. (4) The observed nest chambers were not those of moles (entry 15) but were similar to those of wood mice (Sagara et al. 1988, 1993), those that do not have any hole at their bottoms (Sagara 1999). (5) The absence of the mole-type latrine (entry 16) eliminates the possibility of the mole as the excrement depositor. (6) Wood mice and voles store food that consists of plant material (Hansson 1985; Montgomery and Gurnell 1985), and food remains are found in huge quantities near their used nests (Bang and Dahlstrøm 2001; Sagara et al. 1988). In contrast, such remains are not found near moles' nests (Sagara 1989, 1999). The present observations (entry 18) agree with the former, and the toothmarks along the

Table 1. Observations on each case of *Hebeloma radicosum* growth at the nesting site of the wood mouse in Switzerland

Entries	Case 1	Case 2	Case 3
1. Locality	Tschädigen, Meggen, Luzern; 47°4′10″ N, 8°23′25″ E; 620 m alt.	Gümligental, Muri, Bern; 46°56′40″ N, 7°30′39″ E; 650 m alt.	Gümligenberg, Muri, Bern; 46°56′27″ N, 7°30′42″ E; 640 m alt.
2. Date of finding the fungus	October 12 and November 21, 1997	July 12 and 22, 1997	September 25, 1997
3. Number of fruit-bodies found	2 on each day (Fig. 1, M, H)	1 on each day (Figs. 2, 3, M)	1 (Fig. 4, M)
4. Finder of the fungus	Rolf Mürner	Jakob Fahrni	Jakob Fahrni
5. Date of the field study	November 21, 1997	November 22, 1997	November 23, 1997
6. Topography	Hill slope, 10° SSW	Hill slope, 35° WNW	Hill slope, 28° WSW
7. Vegetation (dominant plants)	*Picea abies*, *Fagus sylvatica*	*Fagus sylvatica*	*Fagus sylvatica*, *Picea abies*
8. Location of the nest chamber[a]	17–30 cm in the ground (Fig. 1, N)	25–35 cm in the ground (Fig. 3, N)	25–35 cm in the ground (Fig. 4, N)
9. Material of the nest	Fallen broad-leaves, unshredded	Fallen broad-leaves, unshredded	Fallen broad-leaves, unshredded
10. Shape of the nest	Subspherical	Shape lost by deterioration	Subspherical (Fig. 5)
11. Conditions of the nest	Slightly collapsed, not decayed but wet	Totally collapsed, decayed, and wet	Intact, not wet (Fig. 5, see text)
12. Location of the deserted latrine[a]	25–35 cm in the ground (Fig. 1, L)	30–35 cm in the ground	30–35 cm in the ground
13. Latrines in current use	Not found	Not found	Not found
14. Tunnel diameter measurements	47 × 40, 50 × 42, 43 × 26, 48 × 30 mm	35 × 30, 39 × 32 mm (see text)	34 × 37, 31 × 31 mm
15. Type of the nest chamber[b]	Not the mole type	Not the mole type	Not the mole type
16. Type of the latrines[c]	Not the mole type	Not the mole type	Not the mole type
17. Droppings of small rodents	Found in the nest chamber	Not found (see text)	Found in the nest cavity (see text)
18. Remains of stored food	Present: beechnuts, rosehip (?)	Present: beechnuts, hazelnuts, etc. (Fig. 3, F)	Present: beechnuts (Fig. 4, F; Fig. 6)
19. Toothmarks on the food remains	Wood-mouse type	Wood-mouse type	Wood-mouse type
20. Scale pattern on the hair from N[d]	Lanceolate	Lanceolate (Fig. 7)	Lanceolate
21. Scale pattern on the hair from L[e]	Lanceolate	Lanceolate (Fig. 11)	Lanceolate
22. Cross sections of the hair from N[d]	Triconcave to kidney-shaped	Triconcave to kidney-shaped (Figs. 8–10)	Triconcave to kidney-shaped
23. Cross sections of the hair from L[e]	Triconcave to kidney-shaped	Triconcave to kidney-shaped (Figs. 12–14)	Triconcave to kidney-shaped
24. Hair of other mammals	Not found	Not found	Not found

[a] Location is shown by the vertical depth from top to bottom (range) of the factor

[b] The nest chamber of moles has a downward hole at its bottom, a hole that is not connected to the nest cavity (for "nest cavity," see Fig. 5, C) but is connected to a tunnel running beneath that bottom (Sagara 1989, 1999; see text)

[c] The latrine of moles contains a huge quantity of undigested fragments of arthropod exoskeletons (Sagara et al. 1981; Sagara 1989)

[d,e] The guard hair (GH 1, sensu Teerink 1991) found from the nest (N) or from the deserted latrine (L)

gnawed edge of nuts (entry 19) indicate the responsible animal exclusively as the wood mouse (Bang and Dahlstrøm 2001). We did not attempt to distinguish between the wood-mouse species *A. sylvaticus* and *A. flavicollis*, as this distinction is known to be difficult (Day 1966; Teerink 1991; Bang and Dahlstrøm 2001).

In all the cases, the nest seemed to have been abandoned or unused for some time, based on the nest conditions (entry 11) and on the absence of currently used latrines (entry 13). Further notes on each case are added next.

Case 1. Even on the day of the field study in very late autumn, we found fresh fruit-bodies, one of which remained under leaf litter (Fig. 1, H). The observed tunnel ran through the cavity of a decaying tree root, and this may explain its larger and irregular size (entry 14) compared to the normal mouse tunnels.

Case 2. A huge amount of unidentified plant material and the remains of various fruits were found accumulated along the tunnel beside the nest chamber to a depth of 50 cm (Fig. 3, F). This tunnel measured 67 × 48, 59 × 46, 66 × 42, and 54 × 39 mm at the different cross sections, being larger than the normal mouse tunnels. The nature of the unidentified material and the larger size of the tunnel remain problems. However, a second tunnel on the other side of the nest chamber (entry 14), which was connected to the aforementioned tunnel, seemed to be a normal mouse tunnel. The whole mass of material collected here was too large to bring to the laboratory, so that only some parts of it were examined for droppings. No droppings were observed in these portions (entry 17), but this does not mean that droppings were absent from other parts of the material.

Case 3. The nest was not wet and still held its shape, with its ceiling and nest cavity being intact (entry 11). The nest cavity (Fig. 5, C), being about 50 mm in diameter, contained some small-rodent droppings that had not deteriorated. However, these did not seem to indicate that the nest was currently used, as the surrounding soil appeared to be dry enough to preserve these items in a good condition.

Information from the northern areas devoid of moles

From Norway, there have been seven records of *H. radicosum*: (1) collected at Himle, Bordalen, Voss, Hordaland, by Jens Stordal on September 10, 1951; (2) collected between Drøbak and Husvik, Frogn, Akershus, by Jens Stordal on September 12, 1953; (3) collected at Kajalunden, Rygge, Østfold, by Ø. Weholt in 1985; (4) collected at Hella, Nøtterøy, Vestfold, by Steinar Aase on September 4, 1985; (5) collected at the coast of Lake Farris, Larvik, Vestfold, by Erik Blomdal in 1988; (6) collected near Kråkerøy Ungdomsskole, Fredrikstad, Østfold, by Inger-Johanne Seem in September 2001; and (7) collected at Ramsøya, Hisøy, Arendal, by Inger-Lise Fonneland on September 25, 2004. We inspected collections 4 and 5 and found no differences in fruit-body appearance from collections in other areas.

From Ireland also, *H. radicosum* has been collected (Svrček 1983; Gerald P. Shannon, personal communication), although details are not available. From Hokkaido, there have been two records of *H. radicosum*, one from Sapporo (Imai 1938) and another from Iwamizawa (collection on September 20, 2003; Hokkaido Kinoko-no-kai, personal communication).

This information as well indicates that other animals than moles are involved as the customer in the association with *H. radicosum*.

Discussion

The case of *H. radicosum* growth on the latrine of the wood mouse *Apodemus* has now been established. We reexamined the material used for the previous report of a case of *H. radicosum* growth on a wood-mouse nest in Switzerland (Sagara et al. 1988), referring to the problem mentioned in the Introduction and comparing with the present cases. As a result, we found that that case had certainly been a wood-mouse case and that that report had provided almost enough data. A few comments are to be added on that case. The nonspherical shape of the nest should have been the result of deterioration and collapse but not the nest-making habit of the animal concerned; that is, the nest could have originally been spherical as in case 3 of the present report. The breakage of the nest material could have been due to long-term use but not to shredding as done by voles. Counting that case, the number of cases of the *H. radicosum*–wood mouse association become 4 among 12 cases studied so far in Europe; the rest are the association with the mole (Sagara 1989, unpublished data). Such a confusing case as mentioned in the Introduction can now be considered an exception.

At the earliest stage of studies on the relationship between *H. radicosum* and small mammals, Sagara (1978) published an erroneous report that this fungus had grown on the deserted latrines of wood mice in Japan, misidentifying moles' nests as those of mice (Sagara 1980). It has turned out that, under certain conditions, the *H. radicosum*–wood mouse association really does occur.

In the herbarium of Professor Dr. Meinhard Moser at the Institute of Microbiology, University of Innsbruck, Austria, we found in 1986 the following notes attached to *H. radicosum* specimens (translation and boldface typing by N.S.): (1) no. 82/279: place of collection, Molinatico above Baselica, Val di Taro, Parma, Italy; habitat, under *Castanea sativa* (**above mouse nest!**); collector, M. Moser; date, 1982-09-17; (2) no. 85/224: place of collection, Dullaberget, Femsjo, Smaland, Sweden; habitat, beech forest (**mouse runway**); collector, M. Moser; date, 1985-09-18. From these notes, one might think that the association between *H. radicosum* and the wood mouse had already been known before our studies. Actually, however, the "mouse nest" or "mouse runway" noted by Moser was not identified by scientific procedures (Reinhold Pöder, personal communication). Also, Sagara's erroneous report might have affected Moser's judgment. Therefore, we do not take Moser's notes as evidence of earlier recognition of the *H. radicosum*–wood mouse association.

In those mouse cases described in this article, the nests were not being used, at least at the time of excavation. However, in mole cases, the nests are often being used at the time of *H. radicosum* fruiting or of excavation (Sagara 1999).

Strangely, no case of *H. radicosum*–wood mouse association has been found among the 62 cases studied so far in Honshu, Japan, although related *Apodemus* species occur there; all cases were with moles (Sagara 1999, unpublished data). It may be that the *H. radicosum*–wood mouse association occurs in colder regions instead. Further survey and identification of the customer animals in northern areas and at higher altitudes is urged.

The establishment of the *H. radicosum*–wood mouse association implies that the relationship between the customer animal species and this fungus is not very specific, because moles and wood mice are quite different in their phylogeny, food habits, and excrement characteristics. The only common aspect expected from their excrement may be the liberation of ammonia upon its decomposition. However, this fungus cannot be grown experimentally in the field by the addition of ammonia or urea to soil, in contrast to the ammonia fungi such as *Hebeloma radicosoides* Sagara, Hongo et Y. Murak. and *H. spoliatum* (Fr.) P. Karsten (syn. *H. danicum* Gröger) (Sagara 1995a; Sagara et al. 2000), whereas it can be cultured easily in the laboratory (Ohta 1998; Kaneko and Sagara 2002). Thus, the field ecology of this fungus is still peculiar.

The identity between *H. radicosum* specimens from mole latrines and those from wood-mouse latrines, and between specimens from Europe and those from Japan, may need to be studied carefully.

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