Contribution to the ecology of *Gyrophaena boleti* (Linnaeus, 1758) (Coleoptera, Staphylinidae) breeding in the pore layer of the fungus *Fomitopsis pinicola* (Fr.) Karst.

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The small staphylinid beetle *Gyrophaena boleti* (Linnaeus, 1758) can be found numerously on the pore layer (hymenium) of the perennial bracket fungus *Fomitopsis pinicola* (Fr.) Karst. Up to 400 beetles have been observed on a single fruiting body (sporocarp). The slender larvae live inside the narrow pores. In a spruce-dominated forest near Oslo, the phenology of *G. boleti* was studied during several years. A rapid colonisation of adult beetles in May was well synchronized with a short sporulation period of the fungus. Then followed a long egg-laying period, extending for weeks even after the sporulation had ceased, resulting in a mix of larval stages. Within a single sporocarp, all three stages could occur together, and different sporocarps could have different distribution patterns of larval stages. A few adults and larvae were observed as late as medio August. Earlier studies have assumed that adults and larvae are spore eaters, and this may be the case during 1–2 weeks of spore production. However, the main activity period of adults and larvae was after sporulation had ceased. Guts of adults and larvae in this period were free from both spores and hyphae, but contained a grey, fine-grained matter similar to that lining the inner part of the hymenium pores. Adults were too thick to enter the pores, but often put their head into them. The special, comb-like mouthparts of adults and larvae, which are assumed to sample spores during sporulation, are probably also useful to scrape this fine-grained matrix from the inner wall of the pores. Adults used only sporocarps with a water content of minimum 30–40 %, and summer drought was a threat to both adults and larvae. Even small sporocarps could harbour many beetles, if the water content was acceptable.

Key words: Coleoptera, Staphylinidae, *Gyrophaena boleti*, *Fomitopsis pinicola*, ecology, phenology.

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Introduction

Fomitopsis pinicola (Fr.) Karst. is a common and long-lived bracket fungus in Fennoscandia (Ryman & Holmåsen 1992). Its fruiting bodies (sporocarps) grow on weakened or dead wood, especially spruce (*Picea abies* (L.) Karst.) (Figure 1). A new pore layer is usually produced during spring and autumn when the forest environment is moist, and some sporocarps may live for at least 18 years (Hågvar 2008). Therefore, individual sporocarps represent a rather stable habitat over years for insects that may use the pore layer as habitat. Among the beetle family Staphylinidae, the Gyrophaenina is a subtribe within the large subfamily Aleocharinae. Members of this subtribe are obligate inhabitants of living mushrooms in both the larval and adult stage (Ashe 1984).

FIGURE 1. A fruiting body (sporocarp) of the fungus *Fomitopsis pinicola* (Fr.) Karst. on a dead stem of spruce (*Picea abies* (L.) Karst.), seen from below. About fifty adult *Gyrophaena boleti* beetles are sitting on the hymenium. Photo: S. Hågvar.

The Gyrophaenina have evolved mouthpart structures that allow them to graze on the pore layer (hymenium), including spores. In this way they avoid competition from the many insects that feed on the flesh of living mushrooms, or dead mushrooms (Ashe 1984). The staphylinid beetle *Gyrophaena boleti* (Linnaeus, 1758) is such a highly specialized species that can be found numerously on the pore layer of *F. pinicola* (Figure 2). The larvae are thin enough to live inside the pores (Figure 3), and according to Staniec *et al.* (2016) they are probably spore-eaters.

Earlier Norwegian studies have shown that the species has a high ability to localize living sporocarps of *F. pinicola* (Hågvar 1999). In trunk-window traps attached to sporocarps, *G. boleti* dominated the beetle catches, both in primeval forest, in managed forest, and on a clearcut area (Hågvar & Økland 1997). Adult *G. boleti* beetles colonise the hymenium of living sporocarps of *F. pinicola* during spring.

FIGURE 2. Adult beetles are small, slightly more than a mm long. They often put their head into the pores, but their body is just too wide to enter them. Photo: S. Hågvar.

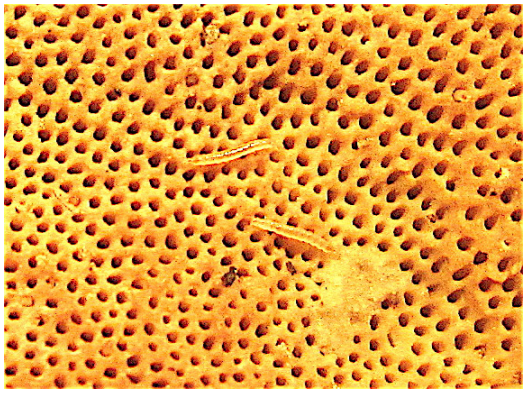

FIGURE 3. Two larvae on a short visit to the hymenium surface, to change pore. They always enter the pore with head first, and later have to back out. Photo: S. Hågvar.

The present paper gives more detailed information on the ecology of this specialized species. The phenology of adult numbers from snowmelt to autumn is described. The length of the egg-laying period is indicated, as well as the degree of synchronisation between larval stages. Food choice among adults and larvae was analysed by their gut content, to see whether the species really depends on spores or fungal hyphae. The relation between beetle numbers and the size of the sporcarps was studied, and further data were collected on the importance of sporocarp moisture.

Material and methods

The study was performed in an old spruce forest near Lake Tappenberg in Østmarka forest reserve (UTM: N 636100, E 148000), about 20 km east of Oslo (Økland & Hågvar 1994, Hågvar 1999). Within an area of about 30 x 30 m, containing much dead wood of spruce, one hundred sporocarps of *F. pinicola* were numbered individually 5 May 1993. During this year and the next, the number of adult *G. boleti* sitting on the hymenium of these sporocarps was counted repeatedly until autumn (August/September). During the following years, counting's were made only sporadically: In 1995 on 18 May and 25 June, in 1996 on 27 May, in 1997 on 25 May, and in 1998 on 21 May. Counting was facilitated by putting a mirror below the sporocarp. Sporulation tended to occur in May, when the forest was wet just after snow melt. Water content of the sporocarps was achieved by measuring the conductivity between two points in the hymenium with a "Protimeter III". In 1994, notes were made about the occurrence of larvae, but without identification to stage.

In 2018, a few sporocarps containing adults and larvae were collected at a shorter distance from Oslo (near the lake Eriksvann, 13 km east). The purpose was to study gut contents of adults and larvae, egg production in females, and the phenology of larval stages. Three suitable sporocarps were sampled on 25 May, two on 1 June, and six on 19 June. By enclosing removed sporocarps in tight plastic bags for some hours, larvae came out of the pores, more or less choked, and could be picked and preserved in alcohol. They were identified to stage I–III according to Staniec *et al.* (2016). An investigation of about 40 sporocarps 9 August 2018, after an unusually warm and dry summer, revealed no beetles and almost no growth of new hymenium.

Gut contents from seven adults and 27 larvae of various stages were analysed with a microscop at 400 x magnification with glycerol as medium. Substrate scraped from the inner wall of pores was also studied in microscope and compared with gut contents. Full-sized, or nearly full-sized, eggs, were counted in thirty-two females, covering all samplings.

Results

Phenology of fungus versus adult beetles. Yearly observations from 1992 to 2000 showed that *F. pinicola* typically sporulated 1–2 weeks in May, when the last snow patches melted. The timing varied according to the amount of snow and climate conditions.

In 1993 and 1994, beetles on hundred sporocarps were counted during several months (Figure 4). On 4 May 1994, the forest environment was wet and cold after a relatively late snow melt, and small patches of snow still remained. A few beetles had colonised sporocarps situated in south-faced sites, sitting in groups on the hymenium. No sporocarps had yet started sporulation. The next week had several very warm days, initiating both a rapid colonisation of beetles and the onset of sporulation. On 14 May, adults were numerous on the hymenium, the sporulation activity was heavy, and most sporocarps had a high water content. Very high number of adults were noted on 26 May, when sporulation had nearly ended, and on 8 June, when sporulation had ceased and a new hymenium layer had started to grow.

Both in 1993 and 1994, adult beetles were observed on the hymenium over a period of 3–4 months, with a maximum between medium May and medium June (Figure 4). High numbers per hundred sporocarps in late May were also observed in 1996 (1079 adults), in 1997 (763 adults), and especially in 1998 (2484 adults). In

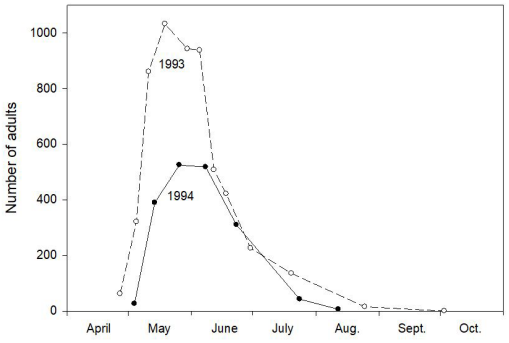

FIGURE 4. Phenology of adult *Gyrophaena boleti* in 1993 and 1994. Total numbers sitting on the hymenium of hundred *Fomitopsis pinicola* sporocarps, from spring to autumn.

1998, about four hundred beetles were observed on a single sporocarp.

Sporocarp moisture and beetle numbers. The effect of sporocarp moisture on the number of adult beetles was studied on 12 June 1993, in a situation when sporocarps showed great individual variation in water content. The highest number of *G. boleti* was found on sporocarps with a water content of 60 % or higher (Figure 5). The lowest acceptable water content was 40 %. From the same year, Figure 6 shows how two sporocarps harbouring a number of beetles became very dry in mid-June and lost their beetles. However, when a high-water content was regained shortly after, these sporocarps were recolonised.

Natural high stumps of spruce often hosted several sporocarps, at various heights. It was hypothesized that the lowest sporocarp had the best ability to retain a high-water percentage during dry periods, by extracting moisture from the ground. However, this was not always the case. During the snow-free season of 1993, water content was frequently measured in sporocarps growing on thirteen high stumps. In five of the stumps, the lowest sporocarp had the best ability to retain water, but in four other high stumps, the uppermost sporocarp retained water best. Still more remarkable was that in another four high stumps, the driest and the wettest sporocarp grew close to each other, at the same height (on case near the base, one case at the top, and in two cases in a medium position).

Effect of sporocarp size on beetle numbers. Sporocarp size, measured as horizontal length directly out from the stem, was related to the maximum number of *G. boleti* observed during 1993 (Figure 7). The four highest values, from 75 to 190 animals, were found on sporocarps larger than 8 cm. However, if we disregard these four, there was no effect of sporocarp size.

Egg production. In each of the three samplings in 2018, covering the period from 25 May to 19 June, 2–4 ripe, or nearly ripe eggs, were found in all dissected females. In this small beetle, two eggs filled the diameter of the abdomen.

Gut contents of adults. Fragments scraped from the inner wall of pores after sporulation contained no spores, but a characteristic, fine-

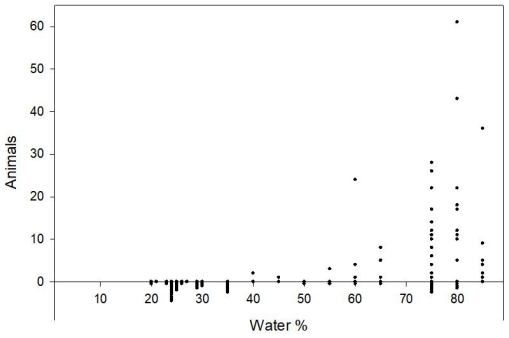

FIGURE 5. Relation between water percentage of individual sporocarps, and the number of adult beetles sitting on them. Beetles were not interested in sporocarps with a water content below 40 %. The data are from 12 June 1993, at a time when the water content varied greatly between the hundred sporocarps. A number of zero observations are indicated below the horizontal axis.

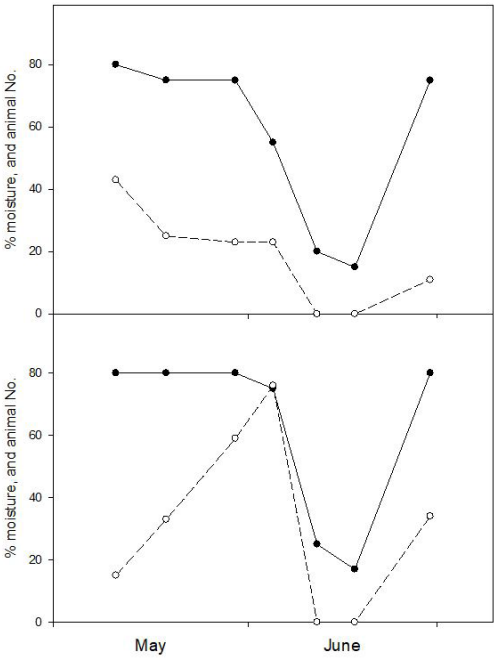

FIGURE 6. Two examples from 1993 illustrating that a sporocarp which loses its beetles during a drought period can be recolonised when the sporocarp regains a high moisture. Whole line: water content. Stippeled line: beetle number.

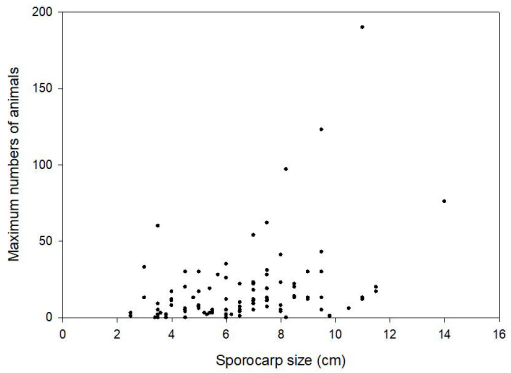

FIGURE 7. Relationship between sporocarp size (horizontal length) and maximum number of *Gyrophaena boleti* during 1993. Data are based on hundred sporocarps.

grained, grey matter in addition to some hyphae (Figure 8a). The gut contents of adult beetles varied somewhat in structure and colour, but neither spores nor hyphae were observed. A fine-grained matter, similar to that within pores, was often seen (Figure 8b).

Sex ratio. Sex ratio was close to 1:1 in all samplings from 2018. Among 103 adults, the male/female numbers were 23/20 on 25 May, 6/5 on 1 June, and 21/28 on 19 June.

Phenology of larvae. In 1994, larvae were observed over a long period, from 8 June to 12 August. At 8 June, larvae varied much in size, at 23 June and 24 July the observed larvae were perhaps fully grown, while later again, at 12 August, some larvae were not yet fully grown.

A more systematic sampling of larvae in 2018 confirmed a lack of synchronisation between stages (Table 1). At 25 May, stages I and II dominated, and stages II and III at 1 June. At 19 June, it was expected that stage III would dominate. Instead, stage I dominated the material, followed by stage II and then stage III. Unfortunately, the material from different sporocarp individuals were not kept apart, but it was observed that the distribution of stages could differ strongly between sporocarps. While one of the sporocarps contained mainly last instar larvae, other contained a mix of stages, and fist instar larvae could dominate. Also, the number of larvae in a given sporocarp varied greatly.

Both in 1994 and in 2018, larvae were present long after sporulation had ceased.

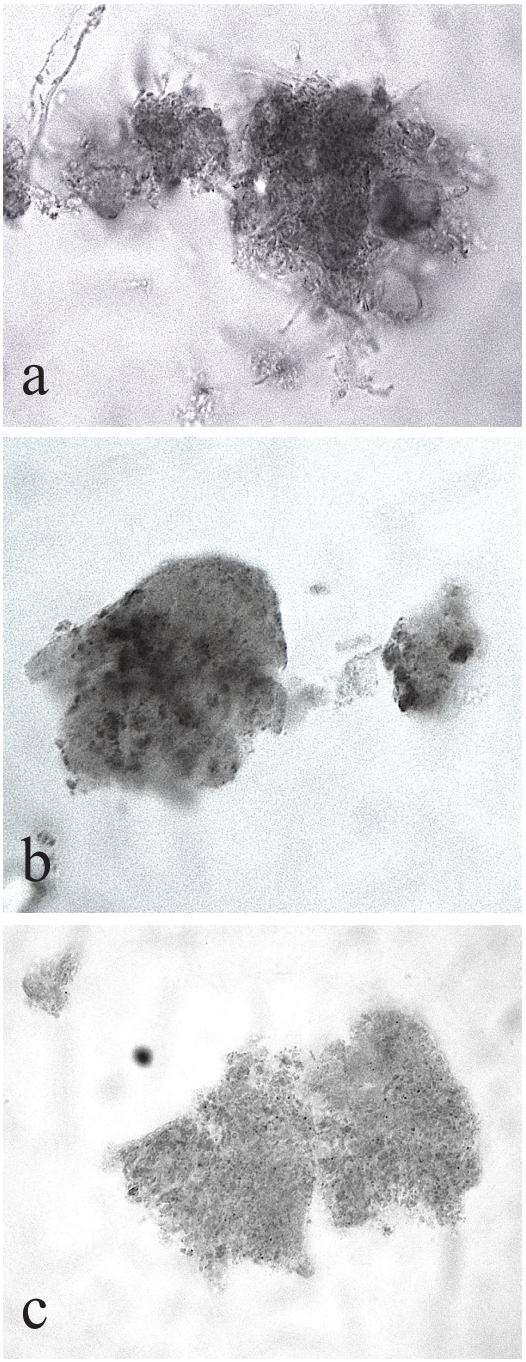

FIGURE 8. Microscope pictures of a grey, fine-grained matter that was isolated from a) the inner wall of a fungal pore, b) the gut of an adult female beetle, and c) the gut of a last instar larva.

TABLE 1. Number of *Gyrophaena* larvae in different stages, collected at different times in 2018.

Date	Stage I	Stage II	Stage III	Total
25 May 2018	31	34	3	68
1 June 2018	2	13	13	28
19 June 2018	73	40	17	130

Larval behaviour. The slender larva, which lives head up inside a pore, escaped deep into older pore layers if disturbed. Now and then the larva changed to another pore. Before it backed out, it hesitated for some seconds or minutes with the tail bristles just at the hymenium surface. In this position, the larva often released a drop of liquid, which was absorbed by the hymenium surface. Then it backed out rapidly, made a short migration on the pore layer, and escaped head first into another pore, usually only 1–4 pores away.

Gut content of larvae. Figure 8c shows a typical gut content of a larva (from19 June). The grey, fine-grained matter is visually similar to that that found in some adult guts and within pores.

Discussion

Observations during several years documented that colonisation of beetles on the hymenium was well synchronized with spring sporulation of the fungus. However, the sporulation period was short, and egg production lasted for weeks after sporulation had ceased. This had several implications. Firstly, there was a great overlap between larval stages. Secondly, the total larval period became long. Thirdly, a considerable part of the larvae had to develop without access to spores as food.

The present study sheds new light on feeding habits. Earlier authors have assumed that both larvae and adults of *G. boleti* are spore eaters (Ashe 1984, Økland & Hågvar 1994, Staniec *et al.* 2016). Spore eating is probably true during the sporulation period. However, neither spores nor hyphae were recognized in the guts from after sporulation time. Instead, guts of both larvae and adults often contained a grey, fine-grained matter which resembled the "matrix" found on the inside of the pores (Figure 8 a–c). Larvae and adults can probably use their specialized mouth parts not only to scrape the pores for spores, but also this fine-grained matrix. Adult beetles are unable to enter the pores completely, but they often put their narrow head inside them (Figure 2).

Regarding a possible grazing on hyphae, Stanic *et al.* (2016) reported small depressions in the hymenium layer, which they assumed were due to adult feeding. However, Økland & Hågvar (1994) could not see any such effect from adult feeding, and hyphae were not seen in the presently studied adult guts.

Sporocarp moisture is a critical factor for adults. Even small sporocarps, only 3–4 cm, can be attractive for adults and larvae, if only the water content is high enough. In some cases, these smaller sporocarps were relatively old, but slow-growing. Adults leave the sporocarp if it becomes too dry but may recolonise the same sporocarp if an acceptable moisture is regained (Figure 6). In 1993, the lowest water percentage of sporocarps with adult beetles was 30, and in 1994 it was 40. A somewhat lower acceptable value (28 %) was measured by Økland & Hågvar (1994).

Variations in the water content of sporocarps were often difficult to understand. For instance, during dry periods, sporocarps on the same stem or high stump could differ greatly in moisture. Sporocarps growing close to each other can perhaps belong to different mycelium systems, extracting moisture from different sources.

The moisture tolerance of larvae is unknown, but they cannot leave the sporocarp. In warm and dry summers, we may assume a high larval mortality. The 2018 summer was such an unusually warm and dry summer, and most sporocarps gradually went into a dry resting phase. A check of about 40 sporocarps on 9 August revealed mainly dry, hard sporocarps, no adult beetles, and a lacking or very thin new spore layer. Maybe only

the earliest laid eggs resulted in fully developed larvae and new adults that year.

Living within a pore is a very safe position for a larva. However, now and then it has to change pore, probably to find better food conditions. The rapid migration from one pore to another, as well as the drop secretion from the last abdominal segment just before it backs out could be antipredator behaviour. According to Ashe *et al.* (2016), both larvae and adults may release a drop of fluid from the so-called tergal gland. Ashe (1981) and Steidle & Dettner (1993) assumed a defensive role of the secretion of the tergal glands in gyrophaenines. However, Ashe (2016) suggested that the secretion had intraspecific functions as well: A recognition function between larva and adult, and an aggregation function between adults. Besides the great ability among the beetles to localize suitable sporocarps in the forest (Hågvar & Økland 1997), volatiles from tergal glands could perhaps explain the ability to aggregate effectively on certain sporocarps.

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References

Ashe, J.S. 1981. Studies of the life history and habits of *Phanerota fasciata* Say (Coleoptera: Staphylinidae: Aleocharinae) with notes on the mushroom as a habitat and descriptions of the immature stages. *Coleopterists Bulletin* 35, 183–196.

Ashe, J.S. 1984. *Major features in the evolution of relationship between gyrophaenine staphylinid beetles (Coleoptera: Staphylinidae: Aleocharinae) and fresh mushrooms.* Pp. 227–255 in Wheeler, Q. & Blackwell, M. (Eds.), Fungus-insect relationships. Columbia University Press, New York.

Hågvar, S. 1999. Saproxylic beetles visiting living sporocarps of *Fomitopsis pinicola* and *Fomes fomentarius. Norwegian Journal of Entomology* 46, 25–32.

Hågvar, S. 2008. How fast do the Tinder Polypore and the Red-banded Polypore grow? *Blyttia* 66, 225–230. (In Norwegian, English summary).

Hågvar, S. & Økland, B. 1997. Saproxylic beetle fauna associated with living sporocarps of *Fomitopsis pinicola* (Fr.) Karst. in four spruce forests with different management histories. *Fauna norv. Ser. B* 44, 95–105.

Økland, B. & Hågvar, S. 1994. The insect fauna associated with carpophores of the fungus *Fomitopsis pinicola* (Fr.) Karst. in a southern Norwegian spruce forest. *Fauna norv. Ser. B* 41, 29–42.

Ryman, S. & Holmåsen, I. 1992. Svampar. En fälthandbok. Interpublishing, Stockholm.

Staniec, B., Pietrykowska-Tudruj, E. & Czepiel-Mil, K. 2016. Larva of *Gyrophaena boleti* (Linnaeus, 1758) (Coleoptera: Staphylinidae) – an obligatory saproxylic and mycophagous species associated with *Fomitopsis pinicola*: Notes on tergal gland system and behaviour. *Annales Zoologici* 66 (1), 83–100.

Steidle, J.L.M. & Dettner, K. 1993. Chemistry and morphology of the tergal gland of free-living adult Aleocharinae (Coleoptera: Staphylinidae) and its phylogenetic significance. *Systematic Entomology* 18 (2), 149–168.

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