

## COLLECTING AND REARING FUNGIVOROUS COLEOPTERA

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RÉSUMÉ. — *La collecte et l'élevage de Coléoptères fungivores* — Le travail routinier d'étude sur le terrain et en laboratoire des Coléoptères associés aux fructifications fongiques est décrit depuis la récolte et l'élevage jusqu'à la conservation de collections. Cet article inclut des instructions pour la constitution à partir de fiches de terrain d'une documentation efficace, appuyée par des bases de données interactives, sur les interactions champignons – Coléoptères. Les facteurs environnementaux influençant les Coléoptères fungivores sont discutés. Le protocole d'élevage de Coléoptères adultes à partir de larves habitant les fructifications fongiques est décrit. Des méthodes de préparation et de gestion de collections de référence de Coléoptères et de champignons sont proposées.

*Mots-Clés:* Polypores, saproxylic, mycophagie, champignons du bois mort, base de données.

SUMMARY. — The field and lab work routine for study of Coleoptera associated with fungal fruit bodies is described from sampling and rearing to preservation of collections. This paper includes instructions for the efficient and database-friendly documenting the fungus-beetle interactions using the field forms. Environmental factors that influence the fungivorous beetles are discussed. The procedure of rearing adult Coleoptera from larvae, inhabiting fungal fruit bodies is described. Preparation and storage approaches of reference collections of beetles and fungi are outlined.

*Keywords:* Polypores, saproxylic, mycophagy, wood-rotting fungi, database.

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Fruit bodies of polypores, poroid non-bolete Basidiomycetes, serve as food substrate for the numerous larvae and adults of Coleoptera. Through decades of myco-entomological studies the overall knowledge of fungal substrates of Coleoptera remains limited to a few easily recognized species of host fungi.

Insects associated with fungi are essential components of forest ecosystems, being abundant and diverse. Many species are fungivorous, playing an important role in the destruction of fruit bodies of wood-decomposing fungi. Among these insects bio-, necro- and saprotrophs are found. The presence of fungivorous species in many phylogenetic branches of Coleoptera indicates the importance of fungivory (mycophagy) in the evolutionary history of Coleoptera.

Unlike many other groups of insects, fungivorous beetles are not always easy-to-spot in nature, and collecting them requires an arsenal of methods for the efficient documentation, rearing, and preservation. The goal of the present publication is to outline some of the environmental factors that influence fungivorous beetles, and to provide some advice for the fieldwork for both entomologists who plan to study fungi-associated beetles and mycologists, interested in beetles, utilizing fungi.

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In addition to Coleoptera and Diptera, the most diverse and abundant insect orders in fungi, there are other fungivorous arthropods on these substrates such as Lepidoptera, Hemiptera, Isoptera, Embioptera, Psocoptera, Homoptera, Collembola, Thysanoptera and Acarina.

Thousands of beetle species from many families of Coleoptera depend exclusively on fungal diet, utilizing hyphae, fruit bodies, or spores. Sometimes spores remain intact in beetle guts (Blackwell, 1984). Some fungi were never observed as insect substrates, and may contain repellents or poisons (Kukor & Martin, 1987). For many fungivorous insects the larval substrates are unknown, and many fungi were never studied for associated species.

The scientific interest to fungivorous beetles dates back many years. Most of this time, however, entomologists paid little attention to the host specificity of fungivorous Coleoptera, which can be seen from the labels in historical collections, stating in many cases just “on fungi”. References on polypore-associated Coleoptera provide ecological information of unequal quality. Generally, works published prior the middle of the XX<sup>th</sup> century give no indication on the collections where the original materials are preserved. Consequently, verification and comparison with modern data becomes practically impossible. Benick’s (1952) monograph accumulates information on 1116 species of Coleoptera from 57 families, including 202 obligate fungivorous species. This work, however, contains many uncertain literature references, and the data in general are difficult to interpret.

Paviour-Smith (1960) studied British fungivorous Coleoptera and focused on *Cis bilamelatus* and *Tetratoma fungorum*. She estimated the number of British fungivorous Coleoptera as much as 600 species. Several works were dedicated to beetle faunas of certain fungal species, such as *Polyporus squamosus* (Klimaszewski & Peck, 1989), *Cryptoporus volvatus* (Harrington, 1980), *Ganoderma applanatum* (Tuno, 1999), *Amylocystis lapponica* and *Fomitopsis rosea* (Komonen *et al.*, 2000, 2001). Two important works on Ciidae were published by Lawrence (1973) on North America and Reibnitz (1999) on southwestern Germany.

In 1980s two symposia on fungus–insect relationships, USA (Wheeler & Blackwell, 1984) and insect–fungus interactions, UK (Wilding *et al.*, 1989) summarized advances of the contemporary studies in the world. The latter volume includes sections on insects in wood decayed by fungi.

Lawrence (1989) described the role of fungi for various beetle taxa. Nitidulidae, Cerylonidae and Rhizophagidae contain species regularly collected on various, mostly decomposing, fungi. The majority of Erotylidae species depend on Basidiomycetes fruit bodies. Anamorphic fungi growing on dead wood and old fruit bodies of wood-rotting fungi attract Cryptophagidae, Sphaerosomatidae and Corylophidae. Fungi-feeding species are found among Coccinellidae (Psyloborinae), and Latridiidae, the latter also common on Myxomycetes. Larvae of Endomychidae feed on various fungi, while adults often visit wood-rotting macromycetes. Larvae of Tenebrionidae (Fig. 1), Mycetophagidae, Tetratomidae, Ciidae, and Melandryidae are most efficient decomposers of polypore fruit bodies.

A number of PhD theses is a valuable source of ecological information on fungivorous Coleoptera and bibliography: Speight (1989), Thunes (1993), Midtgaard (1996), Nilsson (1997), Jonsell (1999), Martikainen (2000), Rukke (2000), Sverdrup-Thygeson (2000), Similä (2002), and Komonen (2003).

## COLLECTING FUNGIVOROUS COLEOPTERA

The need for statistical approval of the ecological information made trap collecting a popular field technique. Such tools as Malaise trap and flight interception (window) trap provide large numbers of beetle individuals. However, even the traps designed to collect specific groups of Coleoptera (Kaila, 1993) provide limited data on the ecology of individual beetle species and the beetle community structure. Direct collecting on the fungus and rearing adults from the fruit bodies remains a reliable and sensible method of the field research.

While collecting the Coleoptera from a fungal fruit body, adult insects should be preserved separately from larvae in 70% alcohol. Boiling larvae in water for a few seconds before moving



Figure 1. — Adults of *Eledona agricola* (Herbst 1783) hatching from the dead fruit body of soft polypore *Laetiporus sulphureus* (Bull.: Fr.) Murrill. Løgnor, Lolland, Denmark, X-2007 (D.S. Schigel 5420).

them to alcohol will prevent the change of their colour. Detailed documentation should come along with the collected beetles, the herbarium specimen of the fungus, and the fruit bodies for rearing. Taking a photograph of fungus *in situ* may be of substantial help for identification of mushrooms, polypores, ascomycetes and slime moulds.

Eppendorf tubes (1.5 or 0.5 ml) are handy for both field collecting and long-term storage. Each tube should be labelled with the same number as herbarium and rearing specimens of the fungus. It is important to have a certain way to mark the tube with adults collected in nature differently from those reared in the lab. As the selection of permanent marker pens resistant to alcohol is limited at the moment of writing, it is recommended to develop a habit of double labelling every specimen, with an obligatory paper label signed by graphite pencil and placed inside the tube.

Sometimes researchers develop complicated codes to number their collections, with date, abbreviations etc., but the simple running numeration of the collections irrespectively of the collected object is the most simple, reliable, and database-friendly. It may be a good idea to forestall the number with your surname, e.g. Smith 2222.

Collecting days can be dissimilar, and in practice there is often no time for the detailed description in the notebook for each specimen. Weather conditions and many other factors may limit your time to document a promising specimen. The difficulty to remember tens of variables for each specimen is supplemented by high risks of wrong data input and rounding mistakes in numerical data.

The solution is to create a paper or digital field form with predefined characters and their states. Both methods have their pros and contras. Digital data input, e.g. with palmtop computer with Bluetooth-connected Global Positioning System (GPS) receiver, is fast and does not require further digitizing, but there are points of vulnerability, such as short battery life, sensitivity to weather conditions and virus attacks. Paper form does not suffer from the above-mentioned threats, but requires digitizing. It is necessary to use a pen with ink resistant to water or graphite pencil. Keeping the backup copy of the data is essential.

The paper form typically consists of boxes where both characters and their states are printed in pocket format in enough copies for the planned field study. All characters are present as corresponding data fields in the database. These characters include running specimen number, date (preferably in 01.IX.2000 format, with roman numbers for months), collector's name, detailed geographical label with GPS coordinates (grid should be indicated) and altitude above sea level, biotope (short description with dominant tree species), ground and light details, species of the fungus (often only provisional name in the field), type of rot, fungal host tree, and type of woody substrate (living, standing dead, snag, log, stump). For the variable characters simplified scales may be used, such as tree decay classes (Renvall, 1995) and consistency classes of fruit bodies (Schigel *et al.*, 2004).

Fungal fruit bodies are characterized by their altitude above ground or position on the log, diameter and thickness of the fruit body, decomposition stage, shape of the fruit body, and moisture. Water contents is a critical character, and it is worthy of note whether it is specific for the fungus of certain consistency, or is the result of the absorption of rain water.

If possible, adults and larvae are listed and counted in the field, with their locations within the fruit body. Sketch drawings are fast to make and they ease the following beetle species' name input after the rearing and identification. Marking the presence of anamorphic fungi and myxomycetes on the fruit body is important to trace food substrates of poorly-known beetle species.

The fungal fruit bodies are not uniform or homogeneous: simultaneous development of larvae of several species is optimized by separating ecological niches both temporarily and spatially. The location of beetle larvae and adults within a fruit body is worth documenting. Perennial fruit body provides the broadest scope of niches different in their microclimatic and consistency conditions: wood-fruit body transition, central core, crust, context, context-hymenophore transition, tube trama, interstitial spaces between seasonal trama layers, hymenophore surface, and released spores under the fruit body. In addition, some fungi make clusters of fruit bodies (*Trametes* spp.), or grow individually (*Inonotus hispidus*). Smaller species of polypores often develop mycelial layer under the bark (*Trichaptum* spp.). For such species the rearing material should be collected together with the bark and underlying hyphae.

Annual and perennial fruit bodies of various polypore fungi develop, stay on the substrate alive, sporulate, die, and remain dead for various periods of time. The duration of the larval stage in the life cycle of a fungivorous beetle is limited in flexibility, thus is limited the range of the substrates suitable for certain beetle species. Polypores at different stages of decomposition should be collected as separate samples to provide information on the succession of fungivorous beetles. The real-time succession study on beetles in polypores would need some patience, as the fruit bodies of some perennial species can persist on the tree for over 50 years (Niemelä, 2005).

## REARING

Rearing is the most technically sensitive part of the lab work, bringing the most exciting discoveries and the most disappointing fails. During the field day polypores for rearing can be kept in plastic bags. Mushrooms are easier to damage thus cloth-covered boxes are preferred. It may be recommended to keep the rearing chamber open for a few days to let extra moisture evaporate, and then close the lid for the remaining period of rearing.

Robust perennial and tough annual fruit bodies of polypores generally host species that are able to pupate inside the fruit bodies and thus rearing can be done in the plastic bag or box with no soil added. It is important for agarics and watery polypores to provide some material for the emerging larvae to pupate in, otherwise they die. Peat sold for gardening may be a good option, as it prevents the mould to develop in moistened boxes. However, particles of peat or sawdust are relatively coarse for many species to pupate comfortably – dry forest soil, sand or other materials of fine fraction may be considered. It is important to keep rearing chambers upright while moving them over longer distances, e.g. from the field to the lab.

Regular moistening by sprayer is recommended to prevent drying of the fruit bodies, but extreme moisture can cause mould growth and death of the larvae. Keeping some dry *Sphagnum* in the rearing chamber can balance the moisture more softly after spraying. Specimens

with green *Trichoderma* mould should not be kept for rearing. Green mould spreads fast to cover the set of specimens, which are then unsuitable for the beetle larvae.

There is usually no need to keep voluminous rearing specimens with tens of the fruit bodies: 1-litre containers are enough, but even 250 ml and 100 ml boxes serve well. For some species small bags made of washable cloth may be more suitable for rearing.

Rearing can be intensified by keeping material in outdoor temperature or in the fridge at +4° C for about 2–3 months and then for further 2–3 months in room temperature to let adults emerge. The tested practice in Finland is to keep the summer and autumn rearing specimens outdoors until January, then keep them indoors until April. Such schedule leaves enough time to sort and mount beetles on pins before late summer or early autumn — the peak collecting season, when most of fungi produce their fruit bodies. Polypore fruit bodies intended for beetle rearing can be collected throughout the year irrespective of the vegetation zone. Rearing at any stage should not be kept in the same building with the fungal herbarium specimens, as certain beetles species can be harmful pests of collections.

## PRESERVATION OF COLLECTIONS

Sometimes traps and large clusters of fungal fruit bodies produce thousand of beetle individuals. The mounting of all specimens on pins is time-consuming and costly. The recommended practice is to mount on pins only reference specimens, e.g. 3–5 individuals of both genders of each species, and keep the rest in 70% alcohol. It is preferred that specimens are still sorted by species for separate preservation in alcohol, and are guaranteed from drying.

The fresh-looking part of the fungal fruit body representing all its parts is to be stored as a herbarium specimen. Fungal specimens for the reference herbarium are to be dried in mushroom dryers with ventilated air at +40–45° C. In many cases the identification should be by performed from fruit body sections mounted in Cotton Blue or Melzer's reagent at ×1250 magnification and phase contrast illumination.

The fungal specimen is worth preserving even for the commonest species, which are usually poorly represented in herbaria. If the host species turns out to be a complex, the fungus specimen should be re-examined, otherwise the collecting, rearing, mounting and identification efforts would be spent in vain. One of the most important phytopathogen polypore species *Heterobasidion annosum* has been found in 1996 to make a complex together with *H. parviporum* in Europe, and more species are found in South-East Asia. In 2005, *Sarcoporia salmonicolor* has been found to consist of three species. All the beetle records from these species would have been of no value, if the original substrates were not preserved. Some groups of species are difficult to distinguish, such as *Trametes velutina* – *T. pubescens*, *Heterobasidion annosum* – *H. parviporum*; *Phellinus igniarius* – *P. nigricans* – *P. cinereus*. Irrespective of the preferred system of fungi, it is recommended to follow the splittest system: it is easy to merge ecological information of two provisional species, but it is nearly impossible to split information if the host species turns out to be a complex.

The taxonomy of fungi is now developing at high speed, and at present many taxa in Basidiomycetes above species level are unsettled. Many species in poorly known groups are revised and split. Making a reference to the specimen guarantees your publication from loosing its value when taxonomic novelties happen. The reference herbarium of the best specimens of fungi and the collection of beetles on pins are often necessary at the workplace. However, a good practice is to deposit the extra specimens to the scientific museums. Referring to the museum storage of the published materials provides possibility to verify the identification of specimens.

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