

***Tarnania fenestralis* (Meigen, 1818) (Diptera: Micetophilidae) associated with the fungus *Pleurotus eryngii* (DC.) Quél.**

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Abstract

Extensive natural mushroom cultivations of *Pleurotus eryngii* (DC.) Quél. were studied in their main productive area, the northwest of Segovia province, Spain, identifying a new parasite for this fungus, *Tarnania fenestralis* (Meigen, 1818) (Diptera: Micetophilidae). The prevalence of parasitization reaches 20.4% of the yield, the main damage being in the destruction of internal tissues of the carpophores, resulting in crop depreciation. Due to the high incidence of parasitization by the dipteran in the mushroom, as well as its abundance, the nematoceros fungivore *T. fenestralis* may be considered the main and most important parasite of extensive *P. eryngii* productions of all the zone in which the investigation is made.

Key words: Fungus gnats; Fungivoridae; Micetophilidae; Diptera; King Oyster mushrooms.

Resumen

***Tarnania fenestralis* (Meigen, 1818) (Diptera: Micetophilidae) asociado al hongo *Pleurotus eryngii* (DC.) Quél.**

En el estudio de los parásitos que afectan a las producciones extensivas naturales de seta de cardo *Pleurotus eryngii* (DC.) Quél. en su principal área productiva: noroeste de Segovia, España; se identifica un nuevo parásito para este hongo, *Tarnania fenestralis* (Meigen, 1818) (Diptera: Micetophilidae). La prevalencia de parasitación encontrada es un 20,4% de la producción y su principal daño radica en la destrucción de los tejidos internos de los carpóforos, depreciando este recurso. Debido a la alta incidencia de parasitaciones del díptero en la seta, así como su abundancia, se considera al nematócero fungívoro *T. fenestralis* como el principal y más importante parásito de las producciones extensivas de *P. eryngii* de toda la zona en la que se realiza la investigación.

Palabras clave: Micetophilidae; Diptera; seta de cardo; nematóceros parásitos; Fungivoridae.

Introduction

From a gastronomic point of view, wild mycological products are increasingly higher valued and more in demand. There is more and more demand for information about the mycological situation of our woodlands. Mycological resources are regarded as a key element in rural development, offering another source of profit from the forest, whether direct or indirect.

This work focuses on the taxonomic identification of the parasite found, *Tarnania fenestralis* (Meigen, 1818), this being the first sighting of this nematoceros fungivore in association with the edible mushroom *Pleurotus eryngii*, or king oyster mushroom. A study

—based on fieldwork and expressed in terms of crop loss per hectare of mushroom growing bed — was also made of the economic effects of damage in *P. eryngii* caused by *T. fenestralis*.

The plots studied were located in the district of Boceguillas, Segovia, Spain, and the fieldwork was carried out between 2006 and 2008. This area of study is one of the main King Oyster mushroom natural production areas. Plots of study are natural populations of *P. eryngii*, within a 2.68 ha surface; they are private and restricted to people and pasture.

The genus *Tarnania* (Diptera: Micetophilidae) acquired its own identity in 1966, having hitherto been grouped within the genus *Rymosia* (Diptera: Micetophilidae). Table 1 shows the divisions of *Rymosia* according to different authors, before becoming split into six separate genera (Kjaerandsen, 2006).

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Table 1. Division of the genus *Rymosia* Winnertz s. lat. Recorded by Kjaerandsen, 2006

Winnertz (1863) - Edwards (1925)	Tuomikoski (1966)	SØli et al. (2000)
Rymosia Winnertz, 1863	<i>Rymosia</i> s. str	<i>Rymosia</i> s. str.
—	<i>Pseudorymosia</i> Tuomikoski, 1966	<i>Pseudorymosia</i>
—	<i>Tarnania</i> Tuomikoski, 1966	<i>Tarnania</i>
—	<i>Allodiopsis</i> Tuomikoski, 1966	<i>Allodiopsis</i> s. str.
—	s.g. <i>Gymnogonia</i> Tuomikoski, 1966	<i>Synplasta</i> Skuse, 1890 ≈ <i>Gymnogonia</i>
—	s.g. <i>Myrosia</i> Tuomikoski, 1966	<i>Myrosia</i>
—	s.g. <i>Notolopha</i> Tuomikoski, 1966	<i>Notolopha</i>

Synonyms used for *T. fenestralis*, recorded by Kjaerandsen, 2006, with their respective authors, are:

— *Mycetophila fenestralis* (Meigen, 1818)-Meigen (1818).

— *Mycetophila apicalis* (Meigen, 1838)-Meigen (1838) renamed by Hackman and others (1988).

— *Rymosia fenestralis* (Meigen, 1818)-Winnertz (1863).

— *Rymosia fenestralis* (Meigen, 1818)-Edwards (1941).

— *Rymosia fenestralis* (Meigen, 1818)-Burghele (1965).

— *Tarnania fenestralis* (Meigen, 1818)-Tuomikoski (1966).

— *Tarnania bavaria* (Plasman, 1980)-Plasman (1980) renamed by Sevcik (2001).

— *Tarnania fenestralis* (Meigen, 1818)-Krivosheina and others (1986).

— *Tarnania fenestralis* (Meigen, 1818)-Zaitzev (2003).

Host fungi which have been associated with this parasite. Table 2 shows this host fungi associated with *T. fenestralis* at the present time.

In spite of the polyphagous nature of this parasite, all references to it are from abroad, not from Spain.

Of the same shape, the diptera currently associated with *P. eryngii* are those shown in the next table, Table 3.

Table 2. Host fungi associated with *Tarnania fenestralis* (Meigen, 1818)

Fungus	Reference
<i>Cortinarius armillatus</i>	(Rimsaité, 2000) Lithuania
<i>Hygrophorus</i> sp.	(Kurina, 1994) Estonia
<i>Clitocybe riburosa</i>	(Kurina, 1994) Estonia
<i>Clitocybe odora</i>	(Kurina, 1994) Estonia
<i>Cortinarius</i> sp.	(Kurina, 1994) Estonia
<i>Pleurotus ostreatus</i>	(Kurina, 1994) Estonia
<i>Agaricus</i> sp.	(Kurina, 1994) Estonia
<i>Pleurotus</i> sp.	(Zaitzev, 2003) Russia

The distribution area of the host and parasite are shown in the following maps, Figure 1.

Material and methods

The ideal climatic conditions of the fungus studied have been appropriated to with the conditions given in the period of study, being both of them the following ones:

— Ideal temperature for micelio growth: 23°C.

— Ideal temperature in the fruiting: 13°C.

— Very high humidity in the fruiting: close to 95%.

— Edafic humidity in the fruiting very high: close to the field capacity.

In order to gather and identify the parasite, we have mainly worked with immature specimens, taken directly from inside infested carpophores of in *P. eryngii*. This means of capture ensures that only these parasites are gathered from the carpophore of the fungus, and not other associated entomofauna. For natural breeding of the samples collected, the substrate used was the natural one for the larvae-the mushroom body, before the setting in of the decomposition process. The natural

Table 3. Diptera associated with *Pleurotus eryngii* (D.C.: Fr.) Quél.

Species	Reference
<i>Mycetophila flava</i>	Rimsaité, 2000 (Lithuania).
<i>Sciophila eryngii</i>	Chandler, 1994 (Israel).
<i>Mycetophila speyeri</i>	Ferri y Ciccadone, 1998 (Italy)
<i>Bradysia paupea</i>	Ferri y Ciccadone, 1998 (Italy)
<i>Lycoriella mali</i>	Ferri y Ciccadone, 1998 (Italy)
<i>Trichoderma</i> sp.	Ferri y Ciccadone, 1998 (Italy)
<i>Heteropeza pygmaea</i>	Ferri y Ciccadone, 1998 (Italy)
<i>Henria psalliotae</i>	Ferri y Ciccadone, 1998 (Italy)
<i>Mycophila speyeri</i>	Ferri y Ciccadone, 1998 (Italy)
<i>M. barnesi</i>	Ferri y Ciccadone, 1998 (Italy)
<i>Lestremia cinerea</i>	Ferri y Ciccadone, 1998 (Italy)

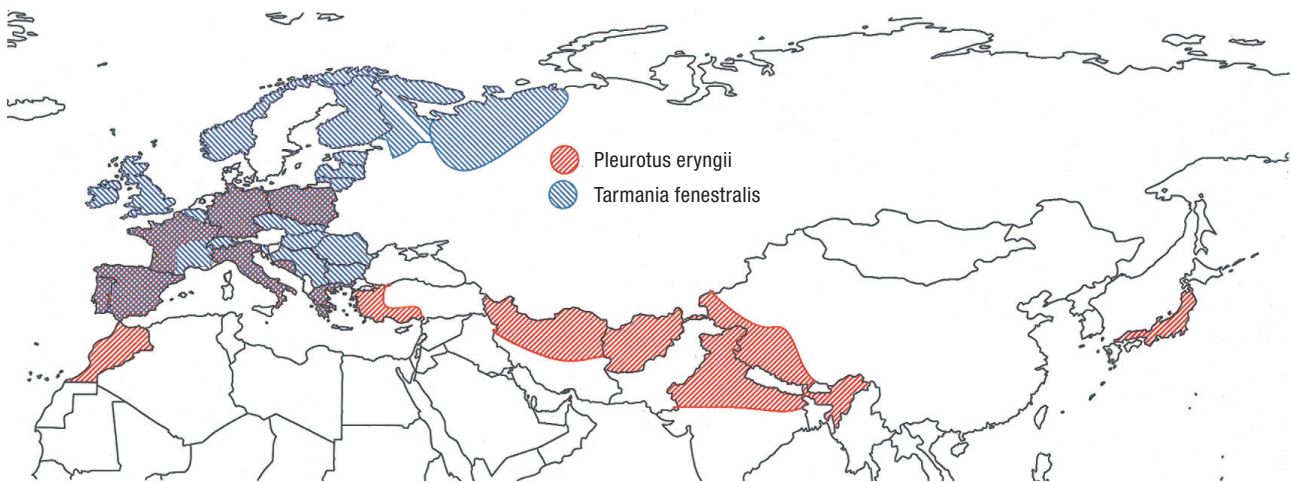


Figure 1. Distribution area of host and parasite. Sources: www.faunaeuropea.com and Kjaerandsen, 2006.

conditions of the fungus enable breeding to take place either *in situ*, protecting the parasitized sample in its natural place in the growing bed with 50 micron polyester net, in order to keep out new parasites or predators, or else by taking it to the laboratory with its original root-clod, and artificially reproducing natural growth conditions. Thus the joint development of the laid eggs and the carpophore is ensured, together with pupation and the capture of the adult Gnats. It shows two figures that represent the isolation methods of the fungus in the field and the laboratory respectively.

Another method of collecting adults for identification was by direct capture in the *P. eryngii* growing beds. This was achieved by attractants (9% hydrolyzed protein to 30% Smellfoll and 3% sodic tetraborato, both of the commercial house Manuel Riesgo, Madrid), placed in tramps of two models, Aesy-trap and Tephry-trap, both of the commercial house Sorygar, S.L.

Once collected, the adults were conserved by immersion in 70° ethyl alcohol in hermetic glass and polyethylene jars prior to their taxonomic identification.

In carrying out the taxonomic identification we must acknowledge the help provided by Dr Peter J Chandler (Slough, England), a specialist in taxonomy. The main taxonomic references were: Kjaerandsen (2005 and 2006), Tuomikoski (1966), Zaitzev (2003).

Material examined

ESPAÑA: Castilla y León, Segovia, Boceguillas, UTM: 447476X 4575010Y, 446650X 4576050Y, 446550X 4576000Y, Altitude 990 m.a.s.l., larvae bred and extracted from *Pleurotus eryngii*, 15-10-2006, 4♂ 8♀, P. Chandler leg., 25/10/2007, 5♂ 7♀, 6/11/2006, 3♀, 2/11/2007, 1♂ 1♀, 3/11/2007, 1♀, 8/11/2007, 1♀,



Figure 2. Laboratory breeding.



Figure 3. Outdoor breeding.

8/11/2007, 1 ♀, 11/11/2007, 2 ♀, 17/11/2007, 1 ♀, 17/11/2007, 8 ♂ 10 ♀, 23/11/2007, 1 ♀, V. Tobar leg. ESPAÑA: Castilla y León, Segovia, Boceguillas, UTM: 447476X 4575010Y, 446650X 4576050Y, 446550X 4576000Y, Altitude 990 m.a.s.l, capture by protein attractants, 28/10/2007, 2 ♂, 4/11/2006, 1 ♀, 11/11/2007, 1 ♀, 18/11/2007, 1 ♂ 1 ♀.

In order to assess the importance of parasitization by this nematoceros insect in king oyster mushroom natural cultivations, calculations were made in 2006 and 2007 of the prevalence of parasitization by means of samples of the weekly crop of this mushroom in the growing beds studied. It works in three plots of surfaces 0.3 ha, 1.445 ha, and 0.920 ha, with irregular shape located in the municipality of Boceguillas (Segovia) with the following U.T.M. coordinates:

- X-447476, Y-4575010.
- X-446650, Y-4576050.
- X-446550, Y-4576000.

Studied plots keep unchanged, without irrigations or reaping, fenced to personnel other people's to the investigation. Mushrooms harvest in the three grasslands is weekly and it picks all the adult mushrooms.

The taxonomic identification of the collected fungi is made by V. Tobar, expert in taxonomy of this species. The carpophores were catalogued into four grades of worm infestation and deterioration: Ga1-none, Ga2-little, Ga3-some, and Ga4-high, based on visual examination. Samples showing the two higher grades of worm infestation were considered unsuitable for consumption. All samples in the Ga1, parasite-free, category, were placed in quarantine to ensure that they contained no laid eggs. Any of these that developed larvae during the quarantine were reclassified Ga2. This sampling facilitated the calculation of the incidence of this dipteran in the fungus population (prevalence of parasitization).

Finally, the types of damage caused by this insect on the carpophores (the rest of the fungus is not involved in the parasitization process) were qualitatively classified, and calculations made of the depreciation caused to the crop. Depreciation caused by the parasites in the cultivations are quantified in euros per hectare thanks to King Oyster mushroom production data obtained in the study area. If we focus in the average price that a consumer pays, we obtain this data (the average price that a consumer pays obtained from a market study done in several shops that offer fresh *P. eryngii* from natural cultivation of communities of Madrid, Castilla y León y Castilla-La Mancha).

Results

The taxonomic identification of all the samples collected during the study, in the period 2006-2008, were: 40 ♂ 47 ♀ obtained by the controlled breeding of larvae, and 5 ♂ 6 ♀ obtained by capture using attractants, resulting in 63.2% of the cases being *T. fenestralis* (Meigen, 1818) (Fig. 4 to Fig. 11).

The prevalence of parasitization in the field of *T. fenestralis* in *Peryngii* was 20.4% as shown in the table, Figure 12.

The percentages of parasitization of season 2006 were 19.8 % and those of the season one corresponding to the 2007 were 21.0%. During season 2007 were registered an unusual period of drought, since 28th of October until 19th of November of 2007, with a precipitations register of 0.0 mm. During this period the production diminishes but the percentage of parasitization stayed.



Figure 4. *Tarnania fenestralis* ♂.



Figure 5. *Tarnania fenestralis* ♀.



Figure 6. Abdomen ♂. Ocular SWF15X.



Figure 7. Abdomen ♀. Ocular SWF15X.



Figure 8. Genitalia ♂. Ocular SWF50X.



Figure 9. Genitalia ♀. Ocular SWF50X.

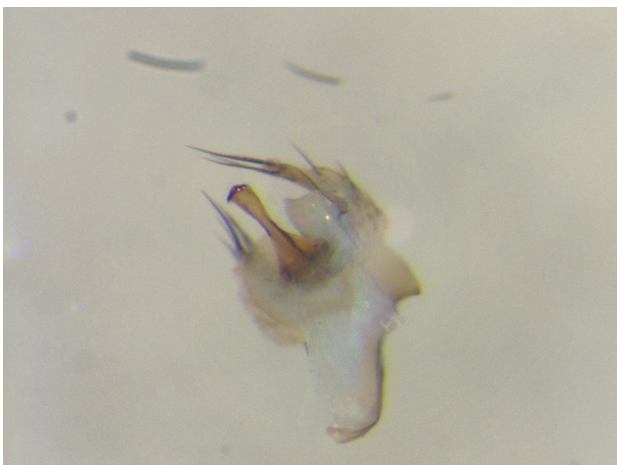


Figure 10. Genitalia ♂. Ocular SWF50X.



Figure 11. Larvae. Ocular SWF5.

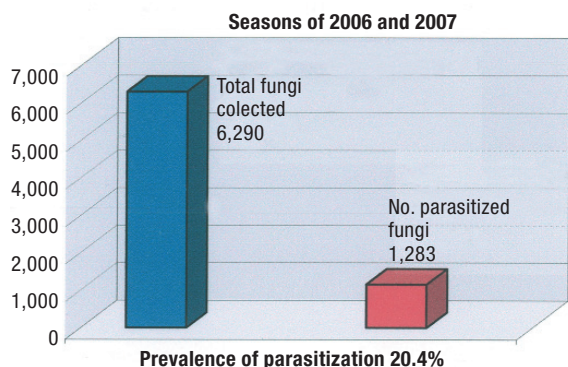


Figure 12. Prevalence of parasitization of *T. fenestralis* in to *P. eryngii* of all the zone in which the investigation is made.

In economic terms, the depreciation caused by *T. fenestralis* in crops (crops being understood as those gathered in extensive, uncontrolled cultivations—the main means of harvesting this resource), using the yield data for *P. eryngii* obtained by Tobar (2008), is as follows:

- Standard weight of king oyster mushroom: 23 g.
- Average commercial price of fresh product: 28.9 € · kg⁻¹.
- Area studied: 2.68 ha.
- Average king oyster mushroom crop:
 - 1,172.6 mushrooms · ha⁻¹ per autumn season.
 - 27 kg mushrooms · ha⁻¹ per autumn season.
 - 9 kg mushrooms · ha⁻¹ per month.
- Crop depreciation due to parasitization:
 - Mushrooms parasitized in the crop:
 - 239.21 parasitized mushrooms · ha⁻¹ per season.
 - 5.5 kg parasitized mushrooms · ha⁻¹ per season.
 - **≈ 160 € ha⁻¹ per month.**
 - Damage caused by *T. fenestralis* in the carpophores of *P. eryngii* includes:
 - Changes in mushroom flavour.
 - Indigestion and stomach pain.
 - Tissue degradation.
 - Accelerated decomposition.
 - Presence of worms can be provoking upset stomach on consumption.
 - Fostering of the dissemination of mites and nematodes.

Discussion

This is the first time that *T. fenestralis* has been associated with *P. eryngii*. The Iberian peninsula is within the species' distribution area: Kjaerandsen



Figure 13. Example of *Pleurotus eryngii* parasitized by *Tartania fenestralis*.

(2006) catalogues it in continental Spain, and Ribeiro (1991) in the central eastern part of Portugal, but the genus *Tarnania* had not hitherto been associated with the king oyster mushroom.

The nematoceros fungivore *T. fenestralis* is now established to be the main and most important parasite of the fungus *P. eryngii* of all the zone in which the investigation is made.

The prevalence of parasitization found with this dipteran is 20.4%, leading to economic damage in the mycological resource of up to 160 € · ha⁻¹ per month, during an average mushroom season calculated as 2.8 months. This latter includes only the autumn season, as spring growth of *P. eryngii* is irregular and sporadic, and has thus not been taken into account.

Acknowledgements

It is important to mention the help given by Dr Peter Chandler in the taxonomic identification of this dipteran, as well as the contributions provided by Dr Manuel González.

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