First records of *trichomycetes* from Collserola Park

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Introduction and objectives

Trichomycetes are an **ecological group** of endosymbiotic arthropods with a **polyphyletic origin** (Benny & O'Donnell 2000, Cafaro 2005) that consists of 7 families, 56 genera and 340 species known today (Lichtwardt et al. 2001).

In this study, the *trichomycetes* found can be placed inside the eukaryotes: in the **order Harpellales** in the Kingdom Fungi and in the **order Amoebidiales** in the Kingdom Protista (Figure 1). **Hosts** of *trichomycetes* are **jawed** and **detritivorous**, mostly consumers of algae and plant remains. Most live in freshwater, although there have also been described *trichomycetes* in marine habitats for crustaceans that inhabit from the surface of the sea to depths of 2600 m. (Lichtwardt et al, 2001).

The foregut and the hindgut of the larvae of the host arthropods are a part of the exoskeleton, made largely of **chitin**. In the middle intestine there is a thin covering layer of chitin called peritrophic layer. T**he thalli of *trichomycetes*** will attach to these cuticle layers(Lichtwardt, 2001).

The aim of this study is to make a first approach to the existing diversity of *trichomycetes* at the Collserola Park (Barcelona) to later discuss the results obtained and their possible causes.

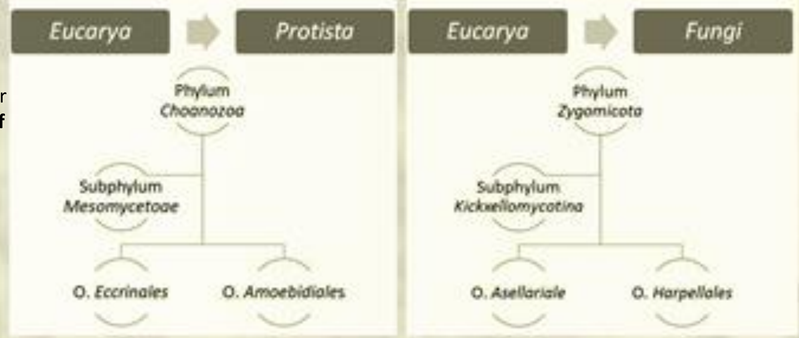

Figura 1. Polyphyletic origin of *trichomycetes*

Methods and materials

Our area of study, the **Collserola massif**, is 17 km long and 6 km wide, bordered to the North by the Valley of Bessós, to the South by the Llobregat, in the East with the pla of Barcelona and in the West it is flanked by a depression. Thus, the Natural Park of Collserola (declared so in 2010 by the Generalitat of Catalunya) is located **in the middle of one of the densest urban areas of the Mediterranean basin**.

Within this area **seven locations were sampled** (Figure 2) with **limnology nets** of 0,5 mm. It is important to do it against the direction of flow of the water and stirring the substrate gently with your feet in order to collect both swimming hosts and those that live in the interstitial area. Then we emptied the contents of the net in a white tray previously filled with water from the same sampling site and with Pasteur pipettes we captured arthropods and put them in airtight containers. There were hosts that live under the stones, in this case we had to raise them and pick them up carefully with the needle nose pliers. Before we concluded the sampling we measured the **Tª and pH of the sampled site**. The airtight containers were transported to the laboratory for examination inside a portable refrigerator.

The dissections were made under a **binocular microscope under 60 x** magnification with a cold light source and focused on a dark surface where we placed each of the guests to a microscope slide with a drop of water. The tools used for the handling and dissection were ultrafine needle and with entomological needles in clamps which separate the head and the anal segment and bowel were extract.

Once we had the bowel cleaned, we put it on top of a slide with a drop of water in order to perform the **observation under the microscope**. If the host contained *trichomycetes*, in order **to preserve the sample**, we added with **lactophenol cotton blue** and seller with a transparent nail lacquer.

Results and Discussions

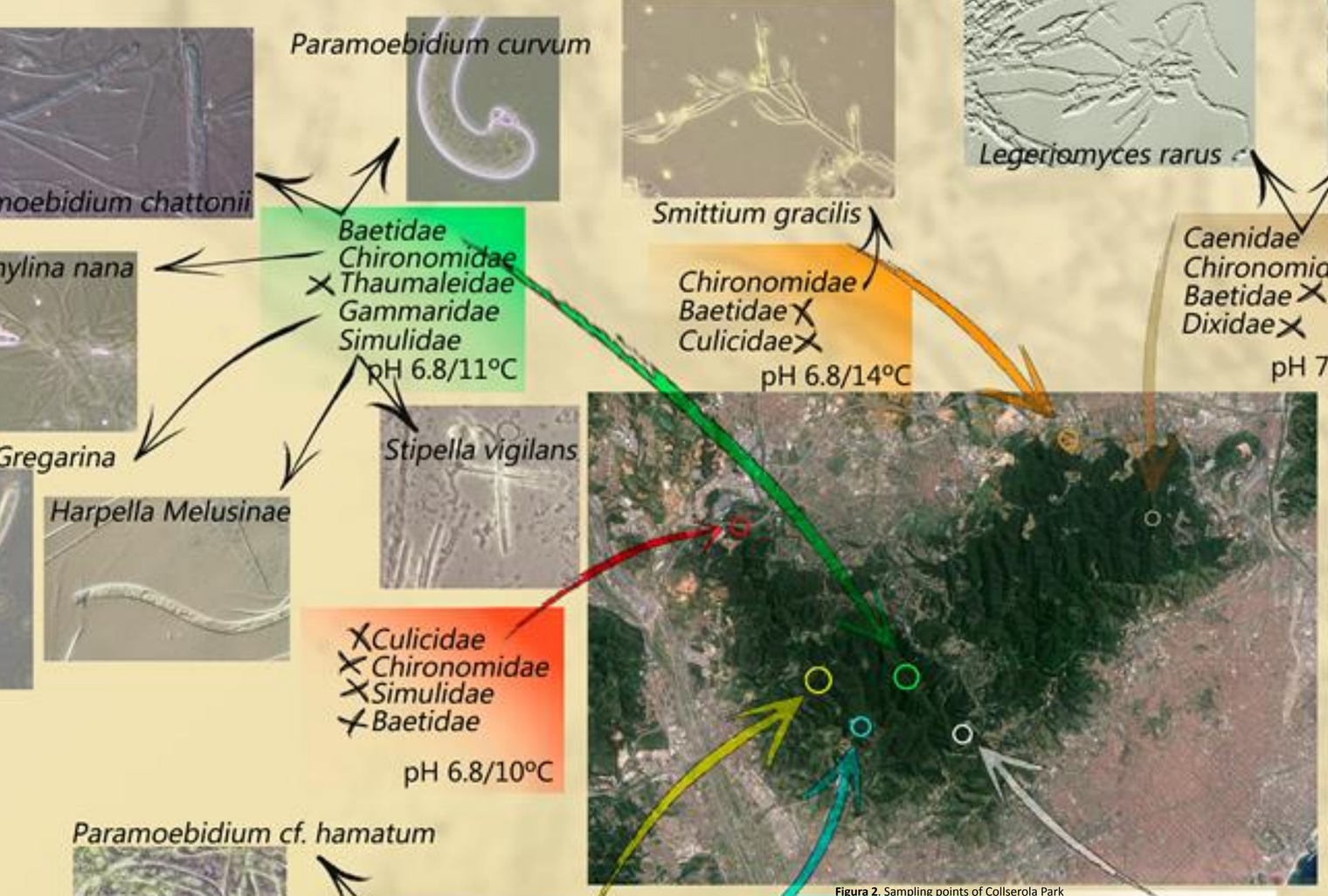

Figura 2. Sampling points of Collserola Park

Paramoebidium cf. Hamatum. Their morphology and size is similar to previous descriptions (Bench & White, 2009). Its rate of infection is remarkable compared to the rest of the *trichomycetes* found in the same area. It was found in three hosts and at different stages of development of the thalli.

Harpella melusinae. The sampling carried out in Collserola represents 21% of the species found, being one of the most fructiferous. It is considered a subcosmopolitan species and its distribution is one of the most extensive within the Harpellales, which, as we can see, is consistent with the results obtained.

Bojamyces transfuga has been found associated with the presence of *Legeriomyces rarus*; the same was observed the first time this species was described in Spain. (L.G. Valle & Santamaría, 2004)

*** Gregarina** does not belong to the group of *trichomycetes*, but we wanted to include it because it is protist that parasitizes potential hosts of *trichomycetes* and therefore they share host and habitat. Unlike *trichomycetes, Gregarinas* are pathogenic, although their levels of pathogenicity are very low.

Stachylina nana. The specimens found in Collserola have in common with the reference specimen a cumbeiform thalli, as well as a production of between 2 to 4 trichospores with a small holdfast. The trichospores described by Lichtwardt are 25-40 x 7-10 µm; on the other hand, the ones recollected in Collserola have a size range between 20-24 x 7-8 µm.

Stipella vigilans. The trichospores, from the specimens previously described in Spain match the characteristics of the specimens described in France (L.G. Valle, 2004) with a size of 41 – 58.5 x 2.5-4 µm. These results do not coincide with the Collserola observations with sizes of 57.8-64,9 x 3.7-4.5 µm.

Smittium gracilis was described by Valle & Santamaría in 2004 in this same family of hosts. Currently, the known distribution is limited to Spain and Canada. The size of trichospores described are 18-29 x 2-3.5 µm. These data differ with the meetings in Collserola, 35-41 x 5-6.5 µm. These differences are attributable to interspecific variation, since the morphology, and other defining characteristics of the species include the thalli with 2-6 branches arising directly from the holdfast, also, the secondary and tertiary branches arranged vertically are preserved.

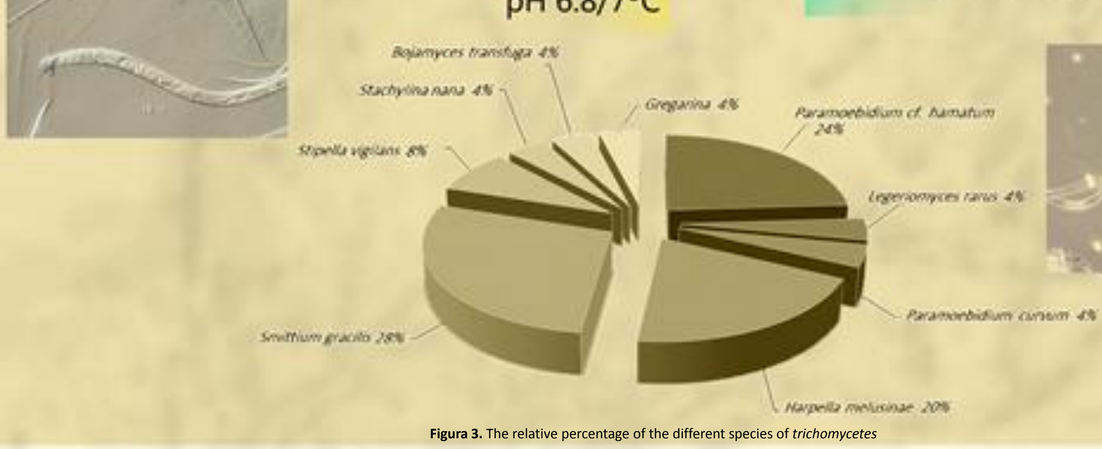

Figura 3. The relative percentage of the different species of *trichomycetes*

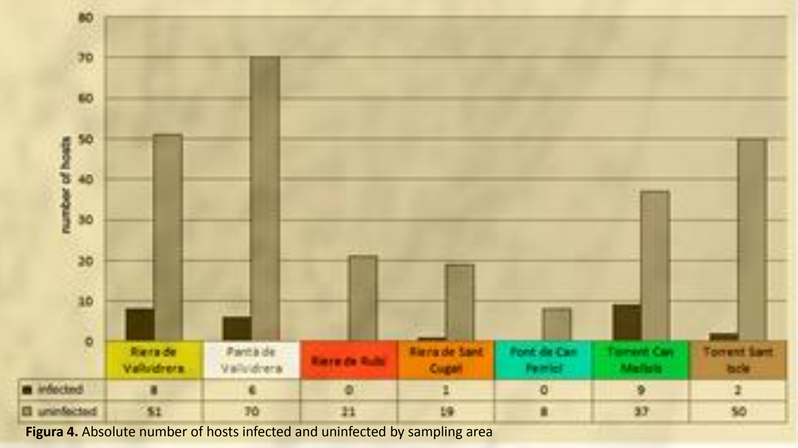

Figura 4. Absolute number of hosts infected and uninfected by sampling area

Conclusions

Because this Park had not been sampled before, all data obtained and described are new.

One of the facts that is worth mentioning is that, due to the time limitation for the realization of this work, the sampling done at the Collserola Park could not be carried out throughout all the seasons of the year, and bearing in mind that this is a temperate zone, temperature differences and of pluvial regime are very significant, therefore the results might have been limited. Making a brief comparison with the amount and diversity of both hosts and *trichomycetes* found in a relatively near Natural Park, belonging also to the area of Barcelona, we find that the differences are very significant: in Montseny 21 species have been described belonging to the Harpellales, whereas in Collserola only 6 have been described. The host species are bio-indicators of the level of pollution of the water and the majority have been collected in a level of low quality. In addition, Montseny, in terms of biogeography, could be considered as a Siberian island in the Mediterranean (Valle LG, 2010) where the differences of humidity, rainfall and temperature lead to a stratified vegetation formation, characteristic of the Mediterranean in the lower parts of the mountain, whereas higher up the mountain slope, Central European environments are found, even subalpine environments on the highest peaks. This variety of habitats makes it possible to find a wide range of hosts with the consequent variability of trichomycetes. These differences, in addition to the differences in amplitude temporal sampling, may be due to both biotic abiotic causes, such as high pressure or anthropological found the Collserola Natural Park, with a high rate of pollution and alteration of natural ecosystems. In 1975, the Diputació of Barcelona, he proposed declaring the Natural Park of Montseny. Finally, it was granted in 1978, the same year that UNESCO declared biosphere reserve, therefore, the Country for many more years than is protected versus the human pressure. The infestation rate in the Collserola Park is only 9,22%, whereas in the Natural Park of Montseny is never below 20% (Urgell IA. 2012).

Also, we must emphasize the need to isolate the axenic crops in order to obtain conclusive new data about the trichomycetes' life cycle, the power of infestation, the tolerance to pollutant substances, their specificity in terms of guests and many other parameters.

References

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