Understanding the life cycle of morels (*Morchella* spp.)

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REVISTA MEXICANA DE MICOLOGÍA 40: 47-50, 2014

NOTA CORTA

Entendiendo el ciclo de vida de las morillas (*Morchella* spp.)

Resumen. Se generó un ciclo de vida teórico de *Morchella* analizando los dos modelos existentes y complementándolos con información relacionada a su cultivo, observaciones experimentales y otras investigaciones. Se da especial atención a los diferentes estados celulares y a las condiciones ambientales para entender mejor su ciclo biológico.

Palabras clave: esclerocios, ascocarpos, domesticación, plasticidad genética.

Abstract. A theoretical life cycle of *Morchella* was generated, analyzing two existing models and complementing these with information relating to their cultivation, experimental observations and other research. Consideration was given to different stages, cellular states and environmental conditions in order to better understand its biological cycle.

Keywords: sclerotia, ascocarps, domestication, genetic plasticity.

Recibido 28 de mayo 2013; aceptado 20 de noviembre 2014.
Received 28 May 2013; accepted 20 November 2014.

Edible mushrooms of the genus *Morchella* (Ascomycota) are important for their ecological role and high commercial value at national and international level (Amir *et al.*, 1993; Masaphy, 2005; Greene *et al.*, 2010), for which reason numerous attempts have been made to cultivate them. However, the lack of knowledge regarding their biological processes, as well as the factors that trigger the differentiation and initiation of their fruiting bodies (Schmidt, 1983; Pilz *et al.*, 2007), their ecological interrelationships (Stamets, 2000) and especially their lifecycle, have limited their production. Furthermore, in Mexico and Latin America, few studies have been conducted on the genus *Morchella*.

Despite the scientific, ecological and commercial applications that represent the knowledge of the life cycle of *Morchella,* this has only been described by Volk and Leonard (1990) and Pilz *et al.* (2007). The former study proposed a general cycle, identifying the stages of vegetative mycelium, secondary mycelium (by the crossing of vegetative mycelium), formation of sclerotia (structures resistant to adverse conditions), "germination" of sclerotia, development of primordia and formation of fruiting bodies (ascocarps). The latter study is based on the previous model, but includes cellular stages of the phenological phases and some ecological conditions under which the cycle takes place.

In both cases, the life cycle is generic and may represent any class of *Morchella*, since knowledge regarding each individual species is scarce (Masaphy, 2010) and the challenge of their taxonomic identification is considerable given the brief fruiting season and diversity of phenotypic responses to different environmental conditions (Wurtz *et al.*, 2005). For example, morels divide into only two phenotypes: black (*M. angusticeps*, *M. elata* and *M. conica*) and yellow or white (*M. esculenta*, *M. crassipes* and *M. deliciosa*) (Barnes and Wilson, 1998), although reddish-brown morels have been characterized, represented by *M. rufobrunnea* (Guzmán and

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Tapia, 1998; Masaphy *et al.*, 2009), and these have been confirmed as being genetically distinct (Pilz *et al.*, 2007; Masaphy, 2010).

It is also possible that *M. esculenta*, *M. crassipes* and *M. deliciosa* may be ecotypes of the same species (Volk and Leonard, 1990). In this sense, recent molecular phylogenetic studies report at least 50 species worldwide, as well as high continental endemism, with 19 new species in existence in North America (Kuo *et al.*, 2012). However, there is a certain margin of error in the phylogeny of *Morchella*, since only 77% of the known species have been sequenced and it has been estimated that 66% of the sequences numbered in GenBank have been identified erroneously (Du *et al.*, 2012). Furthermore, *Morchella* can modify its interactions according to ecological circumstances; it can be saprophytic, mycorrhizal or facultative (Buscot, 1992; Dahlstrom *et al.*, 2000). Considering these characteristics, it is not possible to describe a life cycle for each species and, according to Masaphy *et al.* (2009), the same challenge exists for the different types of *Morchella*. This is probable because, in observations conducted during the process of sclerotia formation, no morphological differences were found between *M. esculenta* and *M. conica* (Alvarado-Castillo *et al.*, 2012). The objective of this study was therefore to contribute to the knowledge of *Morchella* through the generation of a theoretical life cycle (Figure 1) that integrates existing models, experimental observations and research related to this genus.

The cycle begins with the mature ascocarp, the asci of which contain eight ascospores (Miles and Chang, 1997) produced by the crossing of two haploid (n) nuclei, to form a diploid (2n) nucleus that, following meiosis, forms new haploid ascospores (Ower *et al.*, 1988; Pilz *et al.*, 2007) that are subsequently expulsed for dispersion. Under appropriate conditions (generally of temperature and humidity), the ascospores produce germinative tubes that thicken and

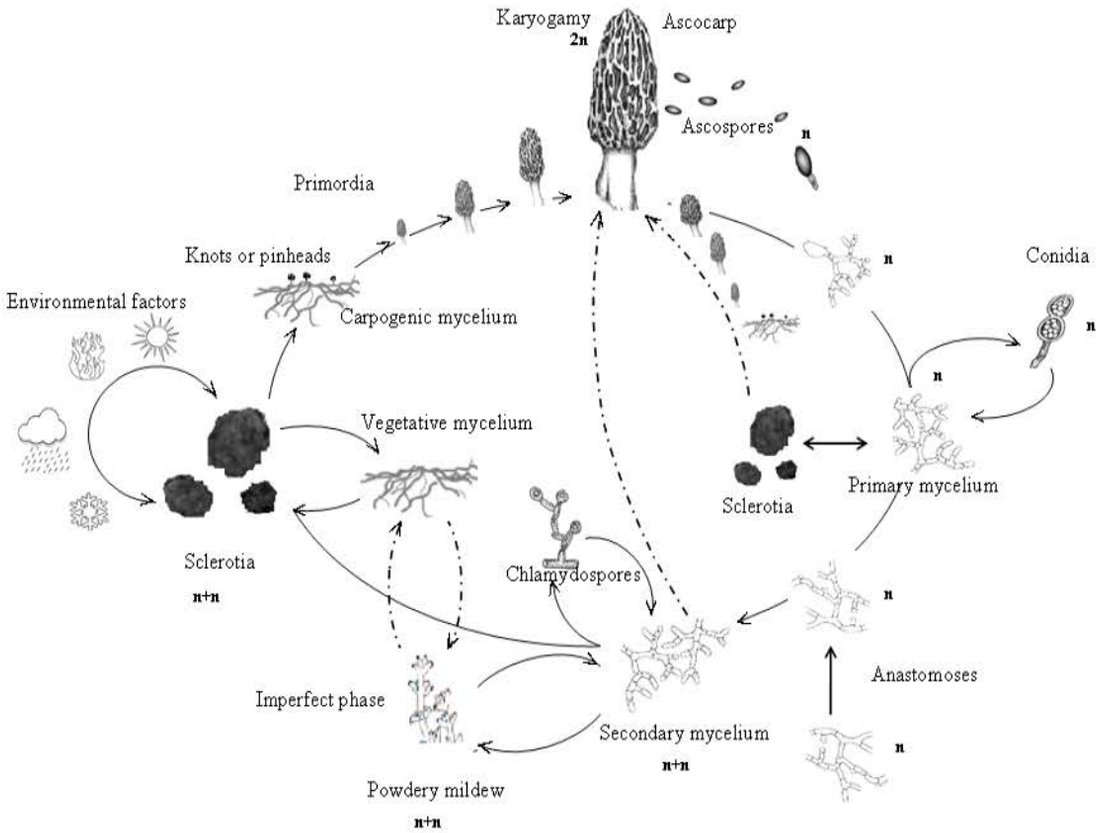

Figure 1. Theoretical life cycle of the genus *Morchella*. Dotted lines indicate possible routes are not proven and which have not been mentioned in the literature.

elongate to form a haploid hypha, giving rise to multikaryotic mycelium, where each cell is a multiple copy of a unique haploid nucleus formed by meiosis (Schmidt, 1983; Pilz *et al.*, 2007; Alvarado-Castillo *et al.*, 2012).

These hyphae grow and branch repeatedly to form an interconnected mass commonly known as primary mycelium (Ower *et al.*, 1986, 1988; Volk and Leonard, 1990) that continues in haploid form. In the same manner, all or part of this mycelium can form conidia, through an asexual process, (Ower *et al.*, 1988) and/or continue to grow, branching and intertwining to form compact masses that give rise to the sclerotia (Volk and Leonard, 1989a).

Through a process of anastomosis (hyphal intertwining) and plasmogamy (union of their cytoplasmic content), the primary mycelium can pair with another produced by the "germination" of spores of the same or another ascocarp, generating secondary or heterokaryotic (n+n) mycelium, the hyphae of which contain various haploid nuclei (Volk and Leonard, 1989a; Pilz *et al.*, 2007) that range from 40-50 (Pilz *et al.*, 2007) to 65 (Volk and Leonard, 1990) per septum, with an average of 10 a 15, conferring genetic, cytological and somatic stability (Volk and Leonard, 1989a). In this way, it is capable of producing totally fertile recombinant meiotic progeny (ascospores) (Pilz *et al.*, 2007). Moreover, this genetic diversity can confer adaptability to a wide range of ecological and environmental conditions (Volk and Leonard, 1989b; Buscot, 1992).

The secondary mycelium passes through repeated branching and plasmogamy of hyphae that compact to form masses that grow and mature to create sclerotia (Volk and Leonard, 1989b). During this process, they may form chlamydospores that give rise to and/or form part of the sclerotia (Alvarado-Castillo *et al.*, 2012). These asexual structures are a class of conidia (Ower, 1982; Ower *et al.*, 1986, 1988) produced by the modification of simple hyphae and represent a means of clonal propagation (Amir *et al.*, 1993; Pilz *et al.*, 2007). In the same way, the imperfect phase of *Morchella* has been identified and is similar to that utilized by fungi that produce "powdery mildews", representing another reproductive strategy (Pilz *et al.*, 2007). It is commonly presented in artificial cultivation, but is unlikely under normal conditions (Stamets, 2000).

The sclerotia represent an intermediate stage between mycelial growth and fructification, for which reason they are considered *conditio sine qua non* for the formation of ascocarps (Ower, 1982; Volk and Leonard, 1990; Masaphy, 2005). With appropriate stimulus, generally in the form of disturbances and adverse conditions (Ower, 1982; Volk and Leonard 1990) such as fire and drought (Wurtz *et al.*, 2005; Pilz *et al.*, 2007; Greene *et al.*, 2010), poor nutrition, lack of humidity, extreme temperatures (Volk and Leonard, 1990), intense rains, prolonged winters (Ower, 1982), flooding and snowfall (Stamets, 2000), the sclerotia can be distinguished by the production of vegetative (myceliogenic) or carpogenic mycelium in order to produce ascocarps (Ower *et al.*, 1986; Volk and Leonard, 1989a; Barnes and Wilson, 1998), the differentiation of which is determined by a combination of genetic and environmental factors, and which remains to be established in terms of the success of fructification.

It should be noted that a sclerotium does nor directly differentiate into ascocarps but instead follows one of the routes described. Similarly, it is not known whether the sclerotia produced by primary mycelium can produce ascocarps (Volk and Leonard, 1990). Pilz *et al.* (2007) indicate that this is impossible, since its haploid nature means that it produces sterile structures that are incapable of fruiting (although there are examples of haploid fructifications in basidiomycetes). Likewise, the possibility that the mycelium (primary or secondary) could differentiate directly into ascocarps, as in other fungi (Kües and Liu, 2000), remains to be explored.

The carpogenic mycelium produces structures similar to nodes or pinheads that give rise to primordia that continue growing and differentiating to form fructifications

(Masaphy, 2005). In turn, in the absence of appropriate conditions for growth and development, the primordia are prone to abortion (Ower, 1982; Volk and Leonard, 1990; Pilz *et al.*, 2007). As in other fungi, this indicates that the existence of triggers for fructification is very likely (Rodríguez, 2007), but that these are not clearly defined in the case of *Morchella* (Gessner, 1995; Pilz *et al.*, 2007).

In summary, *Morchella* presents a complex life cycle that includes the formation of conidia, chlamydospores, an imperfect phase and sclerotia, complemented by a genetic plasticity and the possible capacity for haploid meiosis (Pilz *et al.*, 2007). All of these factors suggest diverse strategies of reproduction and survival in the face of different environmental conditions (Alvarado-Castillo *et al.*, 2012). In fact, Ower *et al.* (1986, 1988) indicated that *Morchella* presents autogamous and heterogamous processes that can influence its fructification, since the fungi can reproduce both sexually and asexually (Miles and Chang, 1997).

While there have been advances in the understanding of the life cycle of this important genus, there are still gaps in the information regarding adaptations, modes of nutrition and reproductive strategies. It is therefore necessary to conduct further research in order to understand the dynamic of reproduction of the species of *Morchella*, not only for the purposes of its commercial production but also to better understand their role within ecosystems.

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