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Introducing *Hygrocybe ceracea* (Sowerby) P. Kumm: Parasite of *Funaria hygrometrica* Hedw. (Bryophyta) in the north of Iran

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ABSTRACT
Hygrocybe ceracea (Sowerby) P. Kumm, the small and macroscopic fungus, with a yellow, greasy, and fragile appearance is reported for the first time on *Funaria hygrometrica* in Tonekabon, west of Mazandaran province, North of Iran. This fungus appears among the stones and cobblestones with a little humic in high humidity of winter, after stopping rain. The stipe is white, 2 mm in average diameter and 1-2 cm in length. The cap diameter is 0.5 cm in average. Spores are lacrymoid, 4×2.35 µm in their dimensions. The average length of basidium is 10 µm. Tetraspores often remain attached together after separation from the basidium. The cap is initially convex, but it turns to the conic shape with passing time and aging. The middle part becomes turgid, the sugarloaf shape and the margins become flat, and its colour turns from yellow to brownish orange. Its companion species in Iran are *Funaria*, lichen and grass. The relationship between *H. ceracea* and *Funaria hygrometrica* is parasitic type. *Funaria* leaf spots are caused by infection with this fungus. It is not a poisonous fungus, hence domestic birds and crows feed on it with great interest. Regrowth and grazing resistance were evident in function of the fungus.

Keywords: Fungus, Hygrocybe *ceracea, Hygrophoraceae, Funaria hygrometrica,* Waxcaps.
Article type: Report.

INTRODUCTION
Bryophytes are pioneer of the land plants because they are the first plants to grow and colonize the barren rocks and lands. Several studies have described plant-pathogen interactions in the model mosses *Physcomitrella patens* and *Funaria hygrometrica.* Most of these studies describe interactions with necrotrophic fungi (*Alternaria*, *Atrididymella*, *Botrytis* and *Fusarium*), Oomycetes (*Pythium*) or bacteria (*Pectobacterium*), which colonize and degrade the leaves, stems and rhizoids of mosses (Carella & Schornack 2017). Waxcaps in Europe are generally found in unimproved pasture, old lawns and grassy churchyards, being more common in the wetter western areas, whereas in other parts of the world, e.g., North America, the same fungi are found in woodlands (Jordan 2004). In the UK alone there are over 40 *Hygrocybe* species (Griffith *et al.* 2004). *Hygrocybe ceracea* and *H. glutinipes*, two small species more tolerant of heavy grazing, were only recorded from here (Mclay 2018). The nutritional mode of waxcaps is uncertain (Halbwachs *et al*. 2013). *Hygrocybe* biology remains a mystery: while isotopic signatures indicate that waxcaps are neither mycorrhizal nor saprotrophic, they were recently observed in plant roots (as *Plantago lanceolata*) and molecularly detected in aboveground tissues (Tello *et al.* 2013). To date, they are mostly classified as saprotrophic fungi (e.g., Knudsen & Vesterholt 2012: 265), and more specifically being dependent on humic compounds in the soil (Arnolds 1981). However, as noted by Seitzman *et al.* (2011), many related taxa within the family *Hygrophoraceae* exhibit biotrophic nutritional strategies, including ectomycorrhizas (*Hygrophorus*) and lichenised taxa (Lichenomphalia) which is supported by the stable isotope

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signatures of waxcaps (Griffith *et al.* 2002; Seitzman *et al.* 2011). The latter authors have also suggested that waxcaps might be associated with bryophytes as hosts. All waxcaps share one clear-cut property. They cannot be grown under axenic laboratory conditions (Griffith *et al.* 2002; Halbwachs, unpublished data), which is a hallmark of most biotrophic fungi, e.g., obligate parasites (Cooke 1979: 39). *Hygrocybe* species can cope with a very wide range of habitats. They appear to prefer soils with low fertility, grassland over forests and temperate/boreal climates. Most significantly, waxcaps are found in forest habitats in the absence of grasses, and in grasslands where woody hosts are absent. Mosses are not obligatory associated. Their host associations are extremely flexible (Halbwachs *et al.* 2013).

The genus *Hygrocybe* from Hygrophoraceae was previously classified in the genus *Agaricus* that was separated subsequently. A list of Iranian *Agaricus* species was presented by Mohammadi Goltapeh *et al.* (2004) including *Agaricus campestris* L., *A. lalage* Berk., *A. nigrovinosus* Pegler., *A. nivescens* (F. H. Møller) F.H. Møller, *A. purpurellus* (F.H. Møller) F.H. Møller and *A. silvaticus* Schaeff. Seven other species viz, *A. comtulus* Fr., *A. osecanus* Pilát, *A. semotus* Fr., *A. pseudopratensis* (Bohus) Bohus, *A. subperonatus* (J.E. Lange) Singer, *A. xanthodermus*, and *A. xantholepis* (F.H. Møller) F.H. Møller were also reported from Iran (Saber & Esmaeili Taheri 2004; Saber & Zangeneh 2004). Seven other species viz, *A. silvicola* (Vitt.) Peck, *A. xanthodermus* (Genevier), *Hygrocybe laeta* (pers.: Fr.) Kummer, *Hygrocybe unguinosa* (Fr.) Karst. were also reported from Iran by Karim *et al.* (2013). Additional knowledge of the genus *Agaricus* in Iran were recorded by Asef (2014) Including *A. altipes* (F.H. Møller) F.H. Møller**,** A. *cupreobrunneus* (Jul. Schäff. & Steer) Pilát**,** *A. placomyces* Peck**,** *A. porphyrocephalus* F.H. Møller**.** Mahdizadeh *et al.* (2016) reported new records from Iran mycobiota. The aim of this study was to introduce fungus *Hygrocybe ceracea* (Sowerby) P. Kumm as a parasite for *Funaria hygrometrica* Hedw. which is in the first time in Iran.

MATERIALS AND METHODS

A macroscopic fungus was found interacting with *Funaria hygrometrica* Hedw. in Tonekabon City, west of Mazandaran Province (north of Iran), with longitude of 51°, 36´ and latitude of 36°, 43´ and 2 m a.s.l, in the first time from Iran. The fungal specimens were carefully monitored and photographed in their natural habitat, as it was likely that many features would change after the stabilization or transferring to the lab (Fig. 1a-f). Collected specimens were kept in Herbarium of Iranian Research Institute of Plant Protection under the accession number "IRAN 17602F". The fungal specimens were fixed in 40% and 60% formaldehyde solutions separately and kept in the laboratory. Microscopic observations were made of gills, basidium and basidiospore, and intracellular observations from moss. The diameter of the spores was measured. The cap diameter as well as the stipe height and diameter were measured and compared with other species for identification (Fig. 2a-f). Lactophenol cotton blue (Merck) 0.5% was used for staining the hyphae and determining the fungus life style (Fig. 3b-h). The identification keys suggested by Leonard 1997, Dilly 1981, and some authors such as Mohammadi Goltapeh *et al.* (2003) as well as mycobank, ohoubach and mykoweb sites were used for comparison and nomination.

RESULTS AND DISCUSSION

Hygrocybe ceracea (Sowerby) P. Kumm. 1871.

Syn: *Agaricus ceraceus* Wulfen (1781), *Agaricus cereus* J. F. Gmel. (1792), *Gymnopus ceraceus* Wulfen (1821), *Hygrophorus ceraceus* Wulfen (1838), *Hygrocybe vitellinoides* Bon (1979), *Hygrocybe citrina* (Rea) J.E. Lange, 1940.

Geographical distribution: Europe [Bulgaria (Melania *et al.* 2006), Germany, Britain and Ireland, Norway (Jordal *et al.* (2016)]; Asia [India (Senthilarasu 2014), China (Wang *et al.* 2018)]; North America (Gyosheva & Ganeva 2004). *H. ceracea* was observed with the *F. hygrometrica*, beside the lichen and grass, growing on a little humus which was between the rocks in northern Iran (Fig. 1a, b). They appeared at 6 °C in the winter, and also on the day after the rainfall in the sun and high humidity, sometimes after the rain stopped with no sunlight. Pet birds and crows were fond of them as food. This fungus is very fragile; it is sometimes isolated, and sometimes two or three stipes are connected together. The size of stipe and cap are not matched, so that, stipe is disproportionately high (Fig. 1d). Its high fat is squeezed out of the fungus, and it is well felt. The gills are thick, deep and small in number and are branched out at the edge of the cap twice or several times (Fig. 1c). The high depth of the gills and the clarity of the cap make the gills visible from the back of the cap. This characteristic will be more pronounced with aging of the fungus (Fig. 1d, e, f). Also, by aging the fungi and water loss, the peak of the cap becomes more prominent and its margins get more flat and also along the gills are grooved. However, while the fungi become older, the cap edges are screwed up, and the fungi also change colour, hence become brownish orange (Fig. 1f).

Fig. 1. *Hygrocybe ceracea*. a- Growing on the stones and cobblestones; b- The stipe length is approximately half of a knuckle; c- The broken cap and part of the gills are attached to the stipe (A=Adnate). The gills are thick, deep and in a small number under the cap; d- The mature fungus accompanied with *Funaria hygrometrica* Stipe length 1.5 cm; e - Aging the fungus and deformation of the cap from the curve to flat at the margins, the grooves resulting from the depth of the gills and the transparency of the pigments are observed; f-Then the edges are twisted and orange.

The curved form of the cap was rather retained in 40% formaldehyde and remained yellow, but the fungi were dehydrated in 60% formaldehyde, turned into aging, and its cap turned out orange-brown like a sugarloaf. The stipe was white, with an average diameter of 2 mm, and the length of 1 - 2 cm (Fig. 2a). Spores were lacrymoid with dorsal-ventral symmetry and the mean dimension was 4 × 2.35 µm (Fig. 2b, c, d). Tetraspores often remained attached together after separation from the basidium (Fig. 2d). The average length of basidium was 10 µm, embedded in the hymenium layer (Fig. 2e, f). Hyphae penetration into moss cells was started from the leaf lower surface. It was first entered the intercellular space and then into the cells (Figs. 3g, h). In the following, it formed the haustorium and evacuated the contents of moss cells. The moss apical growing region was also one of the areas under hyphae attack (Fig. 3c). These damages caused the moss length will be remained short or the leaves will be narrow and twisted (Fig.1a, f). *Hygrocybe chlorophana* (Fr.) Wünsche is very similar to *H. ceracea* with the difference that the stipe diameter in *H. chlorophana* (golden waxcap) is usually more than 5 mm and its cap never becomes conic until the end of growth; its stipe is opaque and it is a robust fungus. Another special feature of this fungus is its high fat content, which is easily felt by touching and squeezing and, thus, it is called butter waxcap (Table 1). Adhesion of tetraspores is also probably due to the fatty of fungus cap and can be of the characteristics of this genus (Fig. 2d).

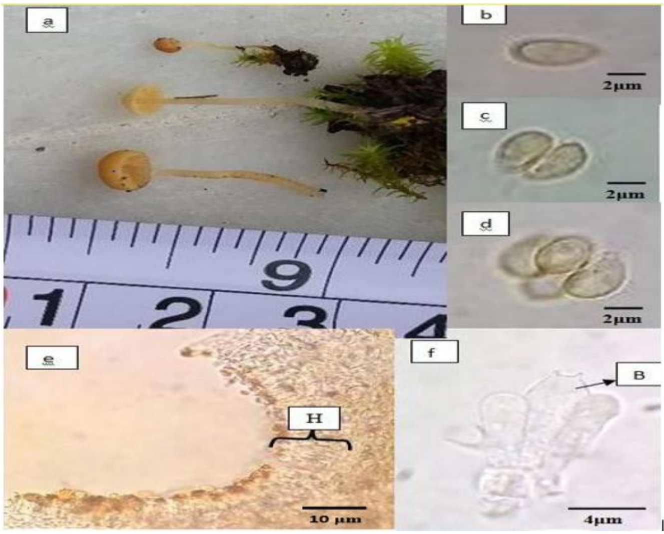

Fig. 2. *Hygrocybe ceracea*; a: measurement; b, c and d: spores; e: Hymenium layer (H); f: Basidium (B).

Table 1. Comparison of *Hygrocybe ceracea* in this study with *Hygrocybe chlorophana*.

Character \ Species	*H. ceracea*	*H. chlorophana*
Cap color	Yellow & greasy	Golden, orange, yellow, lemon & viscid
Cap diameter	0.5 cm	7 cm, 2-4 cm, average 4 cm
Stipe	White, very fragile	Opaque, robust
Stipe length	1-2 cm	6cm
Stipe diameter	2 mm	>5mm, 2-3mm
Spore	Lacrymoid	Ellipsoidal
Spores dimensions	4×2.35 µm	8.8-11×5.5-6.6 µm
Gills	Attached to the stipe, thick	Attached to the stipe or partly free, narrow or fairly thick

Note: Adopted from Leonard[1] 1997, Leonard[3] 1998; Arnolds 1986; Manjola 1983; Boertmann 2010; Zajac *et al.* 2016; https://www.plantlife.org.uk, https://www.wildfooduk.com.

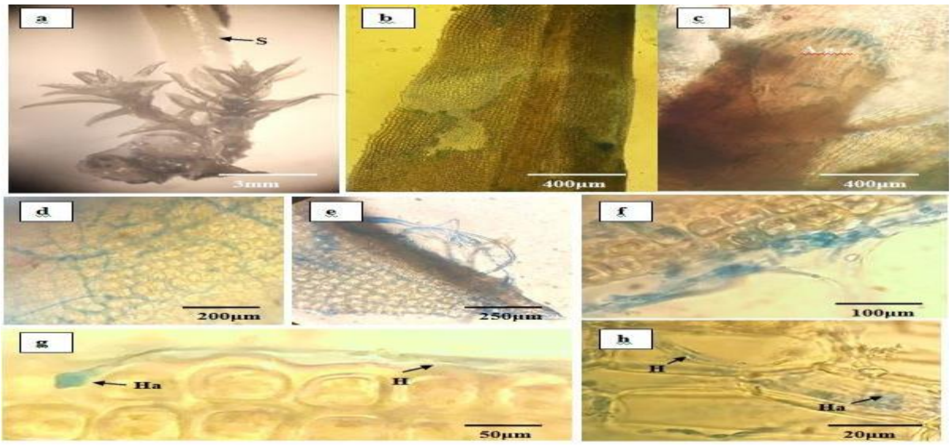

Fig. 3. The parasitic relationship between *H. ceracea* and *F. hygrometrica*. a: Connecting fungus stipe to the moss; b: *F. hygrometrica* leaf spots caused by Fungal contamination; c: The cells of moss apical growing region which are contaminated with fungus (blue sections); d, e and f: The growth of fungal hyphae (blue) in leaf; g and h- Hyphae penetration into moss cells and Haustorium production (blue sections).

A.g.r= **Apical growing region**, H= Hypha, Ha=Haustorium, S= Stipe

CONCLUSION

In this study, birds fed from *H. ceracea*. This means that the fungus is not toxic and by having the fat it can have a good nutritional value in the chicken ration. Grazing resistance with rapid growth showed that this application could be economical as well. Mahdizadeh *et al*. (2016) previously reported the medicinal and toxic roles of *Agaricus*. In the present study, it was found that the lifestyle of *H. ceracea* is parasitic, because twisting and foliage spots in the moss have been observed following fungal hyphae penetration (Figs. 3a-h; Figs. 1a and f). At the same time, previously researchers (Griffith *et al.* 2002; Tello *et al*. 2013) have described the life of *H. ceracea* as secretive and have referred to all saprophytic, parasitic and even symbiotic life forms. *H. ceracea* is a fungal pathogen of *F. hygrometrica* and since mosses are pioneer plants, it is important to recognize their parasites.

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