Light and Electron Microscope Studies of Species of Plant Pathogenic Basidiomycota Isolated from Plants in Kıbrıs Village Valley (Ankara, Turkey)

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Abstract: A search for basidiomycetous plant parasites present in Kıbrıs Village Valley (Ankara, Turkey) was carried out during the period 2009-2010. Twenty-two basidiomycetous plant parasites were identified from Kıbrıs Village Valley. Morphological data obtained by light and scanning electron microscopy of identified fungi are presented.

Key Words: Basidiomycota, Microstromatales, Uredinales, Ustilaginales, SEM.

Introduction

The order Microstromatales with the single family Microstromataceae was erected for species having simple-septate hyphae and local interaction zones without the formation of interaction apparatus. Haustoria or other intracellular fungal organs are lacking (Begerow et al. 2006).

The Microstoma Niessl, the single genus currently placed in the Microstromataceae, is represented by two species (Microstoma album (Desm.) Sacc. and Microstoma juglandis (Bérenger) Sacc.) in Turkey (Göbelez 1967).

Rust fungi (Uredinales) are one of the largest natural taxa within the kingdom Eumycota. More than 7000 species belonging to 100-125 genera and 14 families are accepted currently.
The largest genus, *Puccinia* Pers., contains ca. 4000 spp., 650 of which occur on Poaceae (Abbasi 1996). Recently, Bahcecioglu & Kabaktepe (2012) listed species of rust fungi and their hosts in Turkey. 351 species of rust fungi were registered on 778 species of high plants from 325 genera of 63 families.

The order *Ustilaginales* comprises the majority of smut fungi including the large genera *Ustilago* (Pers.) Roussel and *Sporisorium* Ehrenb. ex Link. Most species of this group sporulate in the reproductive parts of their hosts (Begerow et al. 2006). About 30 species, belonging to the genera *Anthracoeidea* Bref., *Sporisorium* Ehrenb. ex Link, *Tolyposporium* Woronin ex J. Schröt., *Tranzscheliella* Lavrov and *Ustilago* (Pers.) Roussel, have been reported from Turkey (Şahin and Tamer 1998; Kirbağ 2003; Bahçeciğlu et al. 2006; Kabaktepe and Bahçeciğlu 2006).

This research was carried out in valley of Kibris Village belonging to Mamak district which about 20 km southeast of Ankara province. Kibris Village is situated in the Irano–Turanian phytogeographic region and according to the grid square system adopted by Davis (1965–1985), it is located in the squares B4. The climate of the province is Mediterranean. Kibris Village Valley is 1st degree field of natural sites and its three area are 1st archaeological site.

As an aid in identification and classification of fungi, the scanning electron microscope (SEM) allowing the observation of surface structures of various organs is becoming increasingly available (Udagawa & Hoire 1973). The discovery of additional features with the SEM has provided useful support for identification when crucial characters are not clear with the light microscope (LM). The aim of this study is to investigate micromorphology of spores of basidiomycetous plant parasites present in Kibris Village Valley using SEM and LM.

**Materials and Methods**

Infected plant specimens were collected from Kibris Village Valley in Ankara province of Turkey. The host specimens were prepared according to established herbarium techniques. Host plants were identified using the Flora of Turkey and East Aegean Islands (Davis 1965–1985). The fungal specimens were isolated from the host plants by obtaining thin sections or scraping. For microscopic examination and microphotographs a Leica DM E light microscope was used. Spores were measured using a Leica DM E light microscope. Lenght and width of 20 spores were measured for each sample. Leica EZ4D stereo microscope was used for close-up photo of the uredinia and/or telia on leaf surface. The microfungi were identified using relevant literature (Azbukina 2005; Kuprevich and Ulijanishchev 1975; Ulijanishchev 1978; Ulijanishchev et al. 1985; Wilson and Henderson 1966). All specimens examined were deposited in the mycological collection of the Department of Biology, Faculty of Science, Gazi University, in Ankara province of Turkey.

For scanning electron microscopy (SEM), 8–10- mm-square pieces of infected leaves were mounted on the SEM stubs with double-sided adhesive tape. They were coated with gold using a Polaron SC 502 Sputter Coater and were examined with a Jeol JSM 6060 scanning electron microscope operated at 5-10 kV in the Electron Microscopy Unit, Faculty of Science, Gazi University (Turkey).

**Results**

Twenty-two microfungi were identified in the research area. Morphological data which was obtained by light and scanning electron microscopy of these fungi was provided. The author abbreviations of fungi are according to Kirk and Ansell (1992). The systematics of taxa were listed according to Index Fungorum (www.speciesfungorum.org, accessed 2013). Family and species names are listed in alphabetical order in the text.
List of Taxa

**Basidiomycota**

**Exobasidiomycetes**

**Microstromatales**

**Microstromataceae**

*Microstroma* Niessl (1861)

1- **Microstroma album** (Desm.) Sacc.
   Leaf spots circular to angular, small, speckled with yellow. Basidium hypophyllous, clavate, hyaline, 20-25 x 10 µm in size, bursting out through the ruptured cuticle. Basidiospores one celled, fusiform to ellipsoid, guttulate, hyaline, 7.5-12.5 x 2.5-3.5 µm in size, wall smooth (Fig. 1).

B4 Ankara: Kıbrıs Village, 39°52'7,7''N, 33°00'14,7''E, 1105-1115 m, roadside, stepe, on living leaves of *Quercus pubescens* Willd. *(Fagaceae)*, 24.09.2009, TE 1102.

**Pucciniomycetes**

**Pucciniales**

**Melampsoraceae**

*Melampsora* Castagne (1843)

2- **Melampsora salicis-albae** Kleb.
   Spermogonia: generally hypophyllous, rarely on the stems, lenticular, scattered. Uredinia: generally amphigenous, sometimes on the stems, scattered or confluent, causing yellow or orange spots on the leaves, paraphyses capitata, hyaline, 12-15 µm width. Urediniospores: yellow, globose, oblong or pyriform, 20-27.5 x (15-) 17.5-20 (25) µm in size, wall densely echinulate. Telia: amphigenous, subepidermal, brown. Teliospores: prismatic, rounded at both ends, brown, 25-55 x 10-13.5 µm in size (Fig. 2).

B4 Ankara: Kibris Village, 39°52'7,7''N, 33°00'14,7''E, 1050 m, steppe, on living leaves of *L. (Euphorbiaceae)*, 09.08.2009, TE 1088; A B4 Ankara: Kıbrıs Village, Akçadere location, 1062 m, riverside, on living leaves of *Euphorbia macroclada* L. *(Euphorbiaceae)*, 17.4.2009, TE 1049.
Phragmidiaceae

*Phragmidium* Link (1816)

**4-Phragmidium bulbosum** (Fr.) Schltdl.

Spermogonia: epiphyllous, in minute clusters, orange. Uredinia: hypophyllous, yellowish-brown, scattered or confluent, pulverulent. Urediniospores: yellow, globose, oblong or ovoid, 17.5-22.5 (25) x 12.5-15 (17.5) µm in size, wall echinulate. Telia: hypophyllous, black, scattered or in groups, rounded, pulvinate, pulverulent. Teliospores: black, broadly ellipsoid to cylindrical, 4- to 7 (mostly 5-6)-celled, rounded above with a hyaline apiculus 11-15 µm long, rounded at the base, not constricted or slightly constricted, 60-70 x 22.5-27.5 µm in size, wall verrucose, 2.5-5 µm thick at the side, pedicel hyaline, 100-110 µm long, clavate in lower half, persistent (Fig. 3).

B4 Ankara: Kıbrıs Village, 39°52’4,9”N, 33°00’18,7”E, 1050-1090 m, riverside, shady places, on living leaves of *Rubus sanctus* Schreb. (*Rosaceae*), 24.09.2009, TE 1096.
5-Phragmidium mucronatum (Pers.) Schltdl.

Uredinia: amphigenous, pale orange, scattered or in groups. Urediniospores: pale yellow, globose, ellipsoid, ovoid or angular, (20) 22.5-27.5 (-30) x 17.5-22.5 µm in size, wall echinulate, or verruculose. Telia: hypophyllous, scattered or in groups, rounded, black, 500 µm diam., pulverulent. Teliospores: blackish-brown, ellipsoid to cylindrical, 6- to 7-celled, not constricted, 85-100 x 32.5-37.5 µm in size, wall smooth or verrucose. The hyaline pedicel was swollen, clavate in lower half, 150-175 µm (Fig. 4).

B4 Ankara: Kıbrıs Village, 39°52'22″N, 33°00'01″E, 1027-1050 m, riverside, shady places, on living leaves of *Rosa canina* L. (Rosaceae), 24.09.2009, TE 1093.

**Pucciniaceae**

**Gymnosporangium** R. Hedw. ex DC. (1805)

6-Gymnosporangium confusum Dietel

Spermogonia: epiphyllous, orange, in small groups, subglobose, subepidermal in origin. Aecidia: hypophyllous, yellowish brown, 4-5 mm diam. Peridial cells in surface view lanceolate, in lateral view rhombic, elongate, obliquely arranged warts and ridges. Aecidiospores: cinnamon-brown, globose, ellipsoid, (20) 22.5-27.5 x (20) 22.5-27.5 µm in size, wall verruculose (Fig. 5).

B4 Ankara: Kıbrıs Village, 39°52'07.66″N, 33°00'12.43″E, 1128 m, roadside, steppe. on fruit and living leaves of *Crataegus monogyna* Jacq. var. monogyna (Rosaceae), 17.04.2009, TE 1036.

**Puccinia** Pers. (1801)

7-Puccinia acarna P. Syd. & Syd.

Uredinia: hypophyllous, chestnut-brown, roundish. Urediniospores: cinnamon-brown, globose, ellipsoid, 25-30 x (20) 22.5-27.5 µm in size, wall echinulate. Telia: amphigenous, blackish brown, rounded, pulverulent. Teliospores: chestnut-brown, ellipsoid, broadly ellipsoid, ovoid, oblong, 33.5-45 x 25-30 µm in size, constricted in septate, wall verruculose, pedicel hyaline, short, fragile (Fig. 6).

B4 Ankara: Kıbrıs Village, Cellinin kayası location, 1100m, steppe. on living leaves of *Picnomon acarna* (L.) Cass. (Asteraceae), 17.04.2009, TE 1031.
8- *Puccinia behenis* J. Schröt.

Uredinia: amphigenous, scattered, yellow, chestnut-brown, roundish, pulverulent. Urediniospores: subglobose to ellipsoid, 25-30 x (20) 22.5-27.5 μm in size, wall echinulate, with 3 or 4 pores. Teliospores not seen (Fig. 7).

B4 Ankara: Kıbrıs Village, Akçadere location, 1100-1150 m, riverside, on living leaves of *Silene pratensis* (Rafn) Godr. subsp. *eriocalycina* (Boiss.) McNeill & H.C.Prent. (Caryophyllaceae), 01.08.2010, TE 1187.
9- Puccinia calcitrapae DC.
Telia: amphigenous, generally epiphyllous, scattered or confluent, rounded, 400-1200 µm diam., black, pulverulent. Teliospores: ellipsoid, oblong, 32.5-40 x (20) 22.5-27.5 (30) µm in size, brown, rounded or attenuate both ends, slightly constricted in septate, wall 2.5-5 µm thick, verruculose, pedicel, filiform, hyaline, fragile (Fig. 8).

B4 Ankara: Kıbrıs Village, Kavakderesi location, 1000-1080 m, riverside, on living leaves of Carduus pynocephalus L. subsp. albidus (M.Bieb.) Kazmi (Asteraceae), 17.04.2009, TE 1025.

10- Puccinia cynodontis Lacroix ex Desm.

B4 Ankara: Kıbrıs Village, Dipsiz gölü location, 1200 m, riverside, shady places, on living leaves of Plantago major L. subsp. intermedia (Gilib.) Lange (Plantaginaceae), 24.09.2009, TE 1124.
11- Puccinia eryngii G. Winter
Uredinia: hypophyllous, scattered, yellow, chestnut-brown, roundish, pulverulent. Urediniospores: globose, ellipsoid, 25-32.5 x (20) 22.5-25 μm in size, wall echinulate. Telia: amphigenous, dark brown, scattered. Teliospores: chestnut-brown, broadly ellipsoid, ovoid, oblong, (32.5) 37.5-42.5 (45) x 22.5-27.5 μm in size, rounded at apex, attenuate at the base, slightly constricted, wall smooth, pedicel short, hyaline, fragile (Fig. 9).

B4 Ankara: Kıbrıs Village, Cellinin kayası location, 1105-1120 m, slopes, stepe, on living leaves of Eryngium campestre L. var. virens Link (Apiaceae), 17.04.2009, TE 1014.

12- Puccinia hieracii (Röhl.) H. Mart. f. hieracii
Uredinia: amphigenous, yellowish, small, scattered or in groups, ovate or irregular, 0.2-1 mm long, pulverulent. Urediniospores: cinnamon-brown, ellipsoid, globose, ovate, 22.5-30 (32.5) x (20) 22.5-25 μm in size, wall echinulate, 1-2 μm thick. Telia: amphigenous, blackish brown to black, scattered or rarely in groups, roundish, 0.3-0.8 mm diam., pulverulent. Teliospores: chestnut-brown, ovoid, ellipsoid, oblong, 27.5-32.5 x (17.5) 20-22.5 μm in size, rounded at both ends, sometimes attenuate at the base, wall verruculose, pedicel short, hyaline (Fig. 10).

B4 Ankara: Kıbrıs Village, Kaynar gölü location, 1140 m, riverside, on living leaves of Taraxacum officinale Weber (Asteraceae), 24.09.2009, TE 1098.

13- Puccinia jasmini DC.
Telia: amphigenous, generally hypophyllous, in dense groups on the stems and petioles, rounded or ovoid, gray or black. Teliospores: oblong, ellipsoid or ovoid, (40) 42.5-50 x 20-25 μm in size, brown, apex often tapering, conic or obtuse, narrowed below, constricted in septate, wall smooth, up to 3.5 μm thick at the side, 7.5 μm thick at the apex, pedicel up to 100 μm long, filiform, hyaline, strong (Fig. 11).

B4 Ankara: Kıbrıs Village, 39°52'09.73"N, 33°00'18.81"E, 1111 m, on living leaves of Jasminum fruticans L. (Oleaceae), 17.04.2009, TE 1038.
14- *Puccinia malvacearum* Bertero ex Mont.

Teliospores: hypophyllous and on the stems and petioles, scattered, rounded, pulvinate, compact, hard, 0.2-1 mm diam., often 2.5 mm diam. in groups, at first gray then black. Teliospores: subfusoid to ellipsoid, (22.5) 35.5-55 x 17.5-22.5 μm in size, brown, attenuate at both ends, slightly constricted in septate, wall smooth, up to 2-3 μm thick at the side, 8 μm thick at the apex, pedicel hyaline, strong (Fig. 12).

B4 Ankara: Kibris Village, Akçadere location, 1000-1200 m, riverside, on living leaves of *Malva sylvestris* L. (Malvaceae), 24.09.2009, TE 1106; B4 Ankara: Kibris Village, Akçadere location, 1079 m, riverside, on living leaves of *Alcea biennis* Winterl. (Malvaceae), 17.04.2009, TE 1058.

15- *Puccinia nevodovskii* Gamalizk.

Uredinia: amphigenous, yellowish, small, scattered or often confluent, ovate or irregular. Urediniospores: chestnut-brown, ellipsoid, globose, ovate, 17.5-25 x 17-22.5 μm in size, wall echinulate, pale chestnut-brown. Telia: amphigenous, blackish brown to black, scattered or rarely in groups, globose.
Teliospores: chestnut-brown, ovoid, broadly ellipsoid, oblong, 40-67.5 x 20-25 (30) \( \mu m \) in size, rounded or sometimes attenuate at the apex, attenuate at the base, constricted in septate, guttulate, wall smooth, 5-12.5 \( \mu m \) thick at the apex, pedicel persistent, hyaline (Fig. 13).

B4 Ankara: Kıbrıs Village, 39°52'01,71''N, 33°00'19,58''E, 1111 m, steppe, on stem and living leaves of *Galium floribundum* Sm. subsp. *floribundum* (Rubiceae), 17.04.2009, TE 1040.

![Fig. 12. *Puccinia malvacearum*: a-General appearance; b-Telia and teliospores(SEM); c-Teliospores(SEM); d-Teliospores](image1)

![Fig. 13. *Puccinia nevodovskii*: a-General appearance; b-Telia and teliospores(SEM); c-Teliospores(SEM); d-Teliospores](image2)

16-*Puccinia poarum* E. Nielsen

Spermogonia: epiphyllous, brownish-black, in the middle of thickened irregular spots on the leaf. Aecidia: hypophyllous, globose, 2-3 \( \mu m \) diam., orange. Aecidiospores: chestnut-brown, globose, ellipsoid, ovate, 25-30 x 15-27.5 \( \mu m \) in size, wall verruculose. Urediniospores and teliaspores on *Poa* L. species (Fig. 14).

B4 Ankara: Kibrıs Village, 39°52'7,7''N, 33°00'14,7''E, 1000-1100 m, slope, stony place, on living leaves of *Tussilago farfara* L. (Asteraceae), 24.09.2009, TE 1085.
17-Puccinia rubiae-tataricae Syd. & P. Syd.

Uredinia: amphigenous, chestnut-brown, globoid. Urediniospores: cinnamon-brown, ellipsoid, globose, ovate, 22.5-25 x 17.5-25 μm in size, wall echinulate, pale chestnut-brown. Telia: amphigenous, black, roundish, pulvinate. Teliospores: brown, ovoid, oblong, 37.5-50 x 15-22.5 μm in size, wall verruculose, pedicel short, hyaline, fragile (Fig. 15).

B4 Ankara: Kıbrıs Village, 39°52′11.40″N, 33°00′13.59″E, 1084 m, riverside, on living leaves of Rubia tinctorum L. (Rubiaceae), 24.09.2009, TE 1111.
18-**Puccinia striiformis** Westend.

Uredinia: amphigenous, chestnut-brown, small, scattered or often confluent, ovate or irregular. Urediniospores: chestnut-brown, ellipsoid, globoïd, oval, (17.5) 25-27.5 x 17.5-25 µm in size, wall echinulate, pale chestnut-brown. Telia: amphigenous, blackish brown to black, oblong. Teliospores: pale brown, cylindrical, broadly ellipsoid, oblong, 42.5-62.5 x 12.5-25 µm in size, wall smooth, rounded or sometimes attenuate at the apex, attenuate at the base, constricted in septate, pedicel hyaline, fragile (Fig. 16).


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**Uromyces (Link) Unger (1833)**

19-**Uromyces anthyllidis** (Grev.) J. Schröt.

Uredinia: amphigenous, chestnut-brown, small, scattered, in the concentric rings, roundish, pulverulent. Urediniospores: chestnut-brown, globoïd, 20-22.5 (28) x 20-22.5 µm in size, wall echinulate, 1.5-4 µm thick. Teliospores not seen. Spermogonia, aecidia on *Euphorbia* L. species (Fig. 17).

B4 Ankara: Kıbrıs Village, Dipsiz gölû location, 1100 m, steppe, on living leaves of *Onobrychis hypargyrea* Boiss. (Fabaceae), 01.08.2010, TE 1183.

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20-**Uromyces polygoni-avicularis** (Pers.) P. Karst.

Uredinia: amphigenous, cinnamon-brown, scattered, rounded, pulverulent. Urediniospores: pale-brown, globoïd, ellipsoid, (17.5) 22.5-27.5 x 17.5-20 µm in size, wall echinulate. Telia: hypophyllous, dark-brown, scattered or in the concentric rings, pulverulent. Teliospores: chestnut-brown, globoïd, obvoid, oblong, 22.5-25 x 17.5-22.5 µm in size, wall remotely echinulate, pedicels hyaline, short, persistent.

B4 Ankara: Kıbrıs Village, Akçadere location, 1000-1200 m, riverside, on living leaves of *Polygonum aviculae* L. (Polygonaceae), 24.09.2009, TE 1084.
21-Uromyces rumicis (Schumach.) G. Winter

Uredinia: amphigenous, sometimes in the concentric rings, cinnamon-brown, scattered, rounded, 200-1000 µm diam., pulverulent. Urediniospores: pale-brown, globose, ellipsoid, sometimes angular, 22.5-32.5 (35) x 20-25 µm in size, wall sparsely echinulate, 1.5-2 µm thick. Telia: hypophyllous, dark-brown, scattered or in the concentric rings, pulverulent. Teliospores: brown, ovate, ellipsoid, globose, obovoid, oblong, 30-37.5 x (17.5) 20-25 µm in size, wall sparsely echinulate, 2.5-3 µm thick, pedicels hyaline, short, fragile (Fig. 18).

B4 Ankara: Kıbrıs Village, Cellinin kayası location, 1128 m, rocky slope, on living leaves of Rumex scutatus L. (Polygonaceae), 24.09.2009, TE 1034.
Ustilaginomycetes
Ustilaginales
Ustilaginaceae
Ustilago (Pers.) Roussel (1806)
22-Ustilago bullata Berk.

Sori in the spikelets, usually in all spikelets on the inflorescence, replacing the floral parts and including the bases of the glumes, covered by a green to grey membrane of host tissue which at maturity ruptures to expose the spore mass. Spore mass at first firm then dusty, dark brown to purplish-black, consisting of spores only. Spores globose to subglobose or angular, dark chestnut-brown, 7.5-10 x 5-7.5 μm in size, wall densely verrucose (Fig. 19).

B4 Ankara: Kıbrıs Village, Cellinin kayası location, 1150 m, steppe, on spikes of Bromus sterilis L. (Poaceae), 17.04.2009, TE 1048.

Discussion
The Kıbrıs Village Valley was chosen as a research area, because its climatic conditions and plant distributions are suitable for the growth of microfungi. But the plants are completely covered by the dense dust mass caused from the activities of the stone quarries in the research area. This dust mass is a mechanical barrier for the penetration and distribution to the host plant of the fungi. This was detected as a decreasing factor on the fungi diversity and the rate of contamination.

As a result of field work carried out between 2009-2010 in Kıbrıs Village Valley (Ankara), 3 classes, 3 orders, 5 families, 7 genera and 22 species of the Basidiomycota divisio have been identified. As a classification of species within genera in class Pucciniomycetes: 12 species assigned to genus Puccinia, 3 species to Uromyces, two species to Melampsora, two species to Phragmidium and one species to Gymnosporangium.

Exobasidiomycetes is represented by Microstroma and Ustilaginomycetes by a single species of genus Ustilago. As a result of present study, Ustilago bromivora, a microfungus attributed to this divisio, is carpotroph in terms of trophic structure while the others are biophyllotroph. All of them consider as parasite.

In terms of ecological relationships of microfungi, their lifes on a substrate is also interesting in addition to the host. In this case, living together of species belonging to different systematic groups and genera, as well as different species classified in the same genus is the subject.
During the present investigation some fungi were recorded growing together on the same substratum. *Erysiphe buhrii* U. Braun was found developing together with *Uromyces polygoni-avicularis* (Pers.) P. Karst. on living leaves of *Polygonum aviculare* L., *Sporonema punctiforme* (Fuckel) Petr. together with *Puccinia rubiae-tataricae* Syd. & P. Syd. on living leaves of *Rubia tinctorum* L. In addition, some species of microfungi are known on different hosts. *Puccinia malvacearum* Bertero ex Mont. was found on living leaves of *Malva sylvestris* L. and *Alcea biennis* Winterl.

*Puccinia nevoddovskii* Gamalizk. on the leaves of *Galium floribundum* Sm. subsp. *floribundum* have only been given recently as new record (Ekici et al. 2010). We believe this study will contribute to mycoflora of Turkey which will be prepared in the future.

References


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