Isolation of the most common species of *Myxomycetes* in Iran

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Abstract/Summary: For collection and identification of *Myxomycetes*, numerous travels were during different seasons in geographical areas of the country. In any case, for all the samples on decaying wood and plant parts water surface and lateral margins, the magnifying glass samples by hand and then in the laboratory studies were microscopic and macroscopic. In order to study photography and drawing pictures, microscope, Olympus BH2, stereomicroscope Zeiss SV8, Nikon Coolpix 3000 digital camera was used. From different geographic regions of Iran, the southern shores of the Caspian Sea, including the final standard of Gilan, Mazandaran and golestan. The forest cover was dense and moist; we were able to isolate strains *Myxomycetes*. Of the 50 samples collected from samples of different substrates, nine species *Myxomycetes*: *Arcyriadenudata-Arcyrianutans-Fuligoseptica-LycogalaepidendrumPhysarumnutans-Stemonitisaxifera-Stemonitisfusca-Stemonitissplendens-Trichiadecipiens* were identified**.** However these finding are significant because this is one of the few surveys of *Myxomycetes* from Iran.
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Key words*:* *Myxomycetes*, geographical areas, Iran.

Introduction:

Different studies have shown that very important compoundshave been extracted from *Myxomycetes*. Some of these compounds are biologically active and they have been recognized as antibiotic and antimicrobial and have been proved to be cytotoxic for cancerous cells (1). Polycefin, a drug used for treating brain and breast tumors, has been extracted from physarum polycephalum. Polycefin is purified from poly –L-β malic acid and is then modified for the production of morpholino, which is antisense oligonucleotide (2). The drug is used for silence gene therapy and is non-toxic and non-immunogenic. It is capable of biologically analyzing the nano-conjugated drug delivery (3). More than 100 secondary metabolites have been made of *Myxomycetes*. They include lipid amides of fatty acid and their derivatives, alkaloids, amino acid, peptides, naphthoquinone, aromatic compounds, carbohydrates, and trepenoides(4). An experiment has been conducted on the inhibitory activity of Arcyriacyanin A isolated from Arcyrianutans against some cancerous cells (39 cancerous cell lines in human being) including lung, stomach, colon, ovary, central nervous system, kidney, skin and prostate. It is proved that this substance helps inhibit C protein kinase and tyrosine kinase (5).

Materials and Methods:

To collect and identify Myxomycetes, we traveled to different parts of Iran in different seasons. The geographical locations where sampling was conducted were as follows: Gilan Province: Siahkal Forest, Fouman, Shalma Forest and Chesli; Golestan Province: Rangou Forest and Golestan National Forest; Mazandaran Province: Flord Forest, Sangdeh and Nour; the surrounding areas of ZayandehRoud lagoon; the banks of Amir Kabir Dam, Anzali lagoon, and Kiv Lake in Lorestan Province. In each place, we were looking for samples on wood and decomposed parts of plants on the ground and along bodies of water. First, we studied the samples with a magnifier. Then, we selected each sample in a healthy, complete and proper manner for identification based on morphologic features. Samples were placed in paper bags and allowed to air dry to inhibit further decomposition. Dried samples were taken back to the lab and plated for *Myxomycetes*. Substrates in these samples included decaying plant matter that had fallen into the water, dead parts of living emergent or submerged plants, pieces of driftwood, leaves and seeds. To better record the features, we took pictures of the samples. The samples were then taken to the laboratory where they were kept in the freezer for at least five days so that they will be cleared of fungal, insect and tick contaminations. All the samples were studied microscopically and macroscopically at the laboratory. To study the samples and take photos, we

used Olympus BH2 microscope, binocular stereomicroscope Zeiss SV8 and Nicon Coolpix 300 digital camera. During macroscopic studies, we focused on the color and form of the mushroom, presence or absence of the base, presence or absence of lime coating in different parts of sporophores, peridium form, color and overall form of capilitium, and many other morphologic features. In microscopic studies, it is necessary to observe and measure spores, capitilium and other features. For microscopic observation, we selected the sample and placed it in a cover slip which was placed in sterilizeddistilled water or cotton blue. After the cover slip was placed in water, the sample was observed using different magnifications. In case the sample lacked fruiting body for identification or sporophores was not collected properly, we used the humidity chamber technique. The remainder of each sample was used to set up moist chambers to observe *Myxomycetes* (6). Moist chambers were prepared by placing a 90 mm diam filter paper inside a sterile 100×15mm polystyrene petri plate. Substrates were placed so that plates were as full as possible without overlapping pieces of substrate. Samples then were moistened with deionized water. The sample pH was recorded approximately 24h later. Plates then were observed under a dissecting microscope every 2 or 3 d for approximately 2 mo. mature fruiting bodies were observed, collected, identified, placed in small boxes for permanent storage and deposited in the *Myxomycete* herbarium at the university.

Results:

The results showed that from among different geographical locations in Iran, we managed to collect *Myxomycetes* only in the southern coastal areas of the Caspian Sea including Gilan, Mazandaran and Golestan Provinces, which have thick and humid forests. From among 50 substrate samples, 9 *Myxomycetes* were identified as follows:

Arcyria cinerea(Bull.) Pers.**(figA,a)**

Arcyriaalbida Pers., Neues Mag. Bot. 1: 90 (1794)
Stemonitiscinerea (Bull.) J.F. Gmel., Syst. Nat. 2(2): 1467 (1792)
Trichiacinerea Bull., Herb. Fr. 10: tab. 477, fig. 3 (1790)

Sporocarps dispersed or in small groups, erect stalked 1-4 mm tall, rarely almost spherical, but usually cylindrical, 0.5-0.8 mm diam., almost white, pale grey, beige, or rarely ochraceous. Stalk concolorous with the sporotheca or darker.Calyculus smooth or minutely reticulate on the inside. Capillitial net usually small-meshed, expanding slightly upwards to 1.5 x the original height, firmly connected to the cup, tubules in the centre and base of the sporotheca 4-6 µm diam. and strongly warted or spinulose, the basal threads smooth or nearly so, at the periphery covered with spines and half rings, 2-4 µm diam. Spores almost colorless, covered with very fine warts (oil-immersion) and a few larger warts, (6-) 7-8 µm diam. Materials examined: Gilan, Siahkal, on rotten wood in humid forest.

Arcyria denudata(L.) Wettst**(fig B, b)**

Arcyriaclathroides (Scop.) Weber ex F.H. Wigg., *Prim. Fl. holsat.* (Kiliae): 109 (1780)
Arcyriapunicea Pers., *Neues Mag. Bot.* 1: 90 (1794)
Clathrusdenudatus L., *Sp. pl.* 2: 1179 (1753)
*Mucorclathroides*Scop.,*Fl. carniol.*, Edn 2 (Wien) 2: 493 (1772)
Stemonitisdenudata (L.)Relhan, *Fl. Cantab.*, Edn 3: 574 (1820)
Trichia denudata (L.) Vill.,*Hist. pl. Dauphiné* 3(2): 1060 (1789)

Sporocarps erect, often in large colonies, deep red, fading to red-brown, crowded or gregarious, and 1-2 mm total height. Sporothecae subglobose, ovoid or cylindrical, erect, 0.5-1 mm diam., expanding to 1(-2) x the original size.Stalk dark or red-brown, 0.5-1.5 mm long, cysts mostly 12-15 µm diam. Capillitium net rather dense, the tubules slender, 3-4 µm diam., usually with coarse half rings arranged in a spiral, elsewhere smooth or minutely warted or with fine subreticulate ridges. Calyculus plicate, usually rather coarsely papillate-reticulate, usually rather small and shallow, funnel-shaped. Spores 6-8 µm diam. minutely, pale-warted and with scattered larger wartlets.

Materials examined: Gilan, Fooman, Shalma, on little wood segments in humid forest.

Arcyrianutans(Bull.) Grev **(fig C,c)**

Arcyrellanutans (Bull.) Racib.,Hedwigia 24: 170 (1885)
Arcyriaflava Pers., Neues Mag. Bot. 1: 90 (1794)
Stemonitisnutans (Bull.) J.F. Gmel., Syst. Nat. 2(2): 1467 (1792)
Trichianutans Bull., Herb. Fr. 11: tab. 502, fig. 3 (1791)

Sporocarps crowded, cylindric, 1.5-2 mm total height before expansion. Sporothecae 0.3-0.5 mm diam., expanding to 4-12 mm and then lax and drooping, at first bright yellow, but soon changing to pale ochraceous or buff,Stalk short-stipitate or sessile. Hypothallus extensive, membranous.Peridium fugacious, is leaving a shallow, translucent yellowish calyculus, spinulose-reticulate within. Capillitiumconcolorous, extremely elastic, scarcely

attached at the base, the tubules 3-4 µm diam., marked with spines, half-rings and irregular reticulations. Spores nearly colourless, with a few indistinct, scattered warts, 7-8 µm diam. Spore-mass buff or ochraceous. Plasmodium watery-white.

Materials examined: Golestan, Rango, on wood segments in humid forest.

Fuligoseptica(L.) F.H. Wigg**(Fig D,d).**
Aethaliumsepticum (L.) Fr., Syst. mycol. (Lundae) 3(1): 93 (1829)
Fuligoseptica (L.) F.H. Wigg., Prim. Fl. holsat. (Kiliae): 112 (1780)
*Fuligovarians*Sommerf. Suppl. Fl. lapp. (Oslo): 239 (1826)
Mucorsepticus L., Sp. pl. 2: 1656 (1763)
Reticulariaseptica (L.) With, Bot. Arr. Brit. Pl., Edn 2 (London) 3: 470 (1792)

Aethalia irregular, pulvinate, 2-13 cm across and 0.5-3 cm thick. Cortex lemon-yellow, green-yellow or ochraceous, spongy, brittle, rough on the outside and fragile, crumbling away (when the aethalia develop in a very humid atmosphere the wall may be absent or thin membranous). Hypothallus membranous, often consisting of several perforated layers, white and containing a little coloured lime, usually protruding somewhat outside the aethalium. Peridia inside the aethalia white or colourless, often encrusted with globular lime and in that case brittle, usually fragmentary, sometimes the tubes in places with small spaces between them. Capillitium colourless, abundant or sparse, tubules with many or few anastomoses, if abundant then protruding elastically, the tubules with few or many small fusiform or branched white lime nodes. Spores pale lilac-grey or lilac- brown, almost spherical, 7-9 µm or c. 7 x 9 µm diam., verruculose. Spore-mass dark brown. Plasmodium yellow. Ecology: on dead wood.
Materials examined: Mazandaran, Floord, on wood in humid forest.

Lycogala epidendrum(J.C. Buxb. ex L.) Fr **(Fig E,e).**
Galeperdonepidendron (L.) Weber ex F.H. Wigg., Prim. fl. holsat. (Kiliae): 108 (1780)
Lycogalaminiatum Pers., Neues Mag. Bot. 1: 87 (1794)
Lycoperdonepidendron Bull., Hist. Champ.France (Paris) 1: 145, tab. 503 (1791)
Lycoperdon epidendrum J.C. Buxb. ex L., Sp. pl. 2: 1184 (1753)
Reticularia miniata (Pers.) Poir. In Lamarck, Encyclop. Mycol. 6: 184 (1804)

Aethalia usually dispersed or crowded, pulvinate, never taller than wide, 3-10 mm diam., dark grey; covered with (sometimes sunken into the wall) scales. Hypothallus inconspicuous. Cortex sturdy, persistent and consisting of several layers, the surface covered with sunken or superficial, irregularly shaped vesicles, filled with yellow fluid, drying into ± rounded scales, 0.05-0.3 mm diam. Dehiscence starts with an apical pore or crack which tears further later. Pseudocapillitium of tubules varying between 6 and 25 µm diam. with wrinkled surfaces which are smooth or minutely warted or spinulose. Spores almost colourless, 6.0-7.5 µm diam., covered with a fine reticulum of thin, low ridges, interrupted at the site of germination. Spore-mass when fresh grey, later fading to beige. Plasmodium carmine-red or carmine-pink developing into a grey-brown aethalium with grey spores.
Materials examined: Gilan, Siahkal, on wood; Mazandaran, Sangdeh, on rotten wood; Mazandaran, Floord, on rotten wood in humid forest.

PhysarumnutansPers**(Fig F,f).**
Tilmadochenutans (Pers.) Rostaf. Śluzowcemonogr. (Paryz): 127 (1875) [1874]

Sporangia gregarious, total height 1 to 1.5 mm., erect or nodding, subglobose or lenticular, more or less flattened or concave beneath, 0.4 to 0.7 mm. broad, stalked, white, grayish white, or iridescent from absence of lime; sporangial wall membranous, with included white lime-granules in more or less dense clusters. Stalk subulate, longitudinally wrinkled, and gray, yellowish, olivaceous, or black, translucent above; sometimes opaque and white from deposits of lime in the wall, the tube of the stalk containing refuse matter. Capillitium of colorless slender threads, branching at acute angles and anastomosing, with few flat expansions at the axils, and few small, white lime nodes.

Spores Brownish violet, minutely spinulose or nearly smooth, 8-10 µm diam. Plasmodium: Watery white or yellowish gray.

Materials examined: Golestan, Rango, on rotten wood in humid forest.

Stemonitis axifera(Bull.) T. Macbr((**Fig G ,g).**
Trichiaaxifera Bull., Hist. Champ. France (Paris) 1: 118 (1791)

Sporocarps in tufts, 7-20 mm tall.Hypothallus membranous, brown, continuous under the tuft.

Stalk 1.5-6 mm long, shining black, opaque. Sporotheca cylindrical, tapered around the apex and base, rust-coloured when fresh. Columella gradually tapered towards the apex of the sporotheca and flexuose apically and there merging into the

capillitium. Capillitial internal net with c. 3 meshes over the radius, usually with expansions in the axils, pale brown, the surface net consisting of thin threads with angular meshes which are usually ca. 5-34 µm diam., almost without free ends.

Spores pale rosy-brown, 5.0-7.5 µm diam., with very fine pale warts seen by oil-immersion. Plasmodium white.

Materials examined: Gilan, Siahkal, on wood in humid forest.

***Stemonitisfusca*Roth(Fig H, h).**

Sporocarps tufted on a brown, membranous hypothallus, 6-20 mm tall. Stalk black, shining, 20-45% total height. Sporotheca deep fuscous varying to dark red-brown, becoming paler as the spores are shed.Columella dark brown or blackish, reaching nearly or quite to the apex.Capillitium arising from all parts of the columella, freely branched and anastomosed, the ultimate branchlets united into a close-meshed surface net.

Spores violet-brown, prominently to delicately verrucose-reticulate or rarely spinulose or papillate, 7.5-9 µm diam. Spore-mass fuscous. Plasmodium white.

Materials examined: Mazandaran, Floord, on rotten wood in humid forest.

Trichiadecipiens (Pers.) T. Macbr(Fig M,m).

*Arcyriadecipiens*Berk., Ann. Mag. nat. Hist., Ser. 1 1: 447 (1842)

Trichiacerina Ditmar, in Sturm, Deutschl. Fl., 3 Abt. (PilzeDeutschl.) 1(2): 51 (1814)

Trichia fallax Pers., Observ. mycol. (Lipsiae) 1: 59 (1796)

Trichia fallax f. *cerina* (Ditmar) Rostaf., Śluzowce monogr. (Paryz): 245 (1875) [1874]

Trichia fallax f. *minor* Rostaf., Śluzowce monogr. (Paryz): 245 (1875) [1874]

Trichia fallax var. *cerina* (Ditmar) Berl., in Saccardo, Syll. fung. (Abellini) 7: 440 (1888)

Trichiafallax var. *minor* (Rostaf.) Berl., in Saccardo, Syll. fung. (Abellini) 7: 440 (1888)

Sporothecaobovate or pyriform, ochraceous, very shiny.

Stalk gradually merging into the sporotheca, plicate, brown, filled with spore-like bodies. Peridium yellow or ochraceous-yellow by TL, smooth, pleated towards the stalk, dehiscing at the apex and remaining as a basal cup. Elaters usually unbranched, ochraceous-yellow by TL, 4.5-5.5 µm diam. in the middle, gradually attenuate into the very long points (75-150 µm), with 4-5 smooth, rather thick spirals, without longitudinal striae. Spore-mass, including the capillitium, ochraceous or ochraceous-brown.

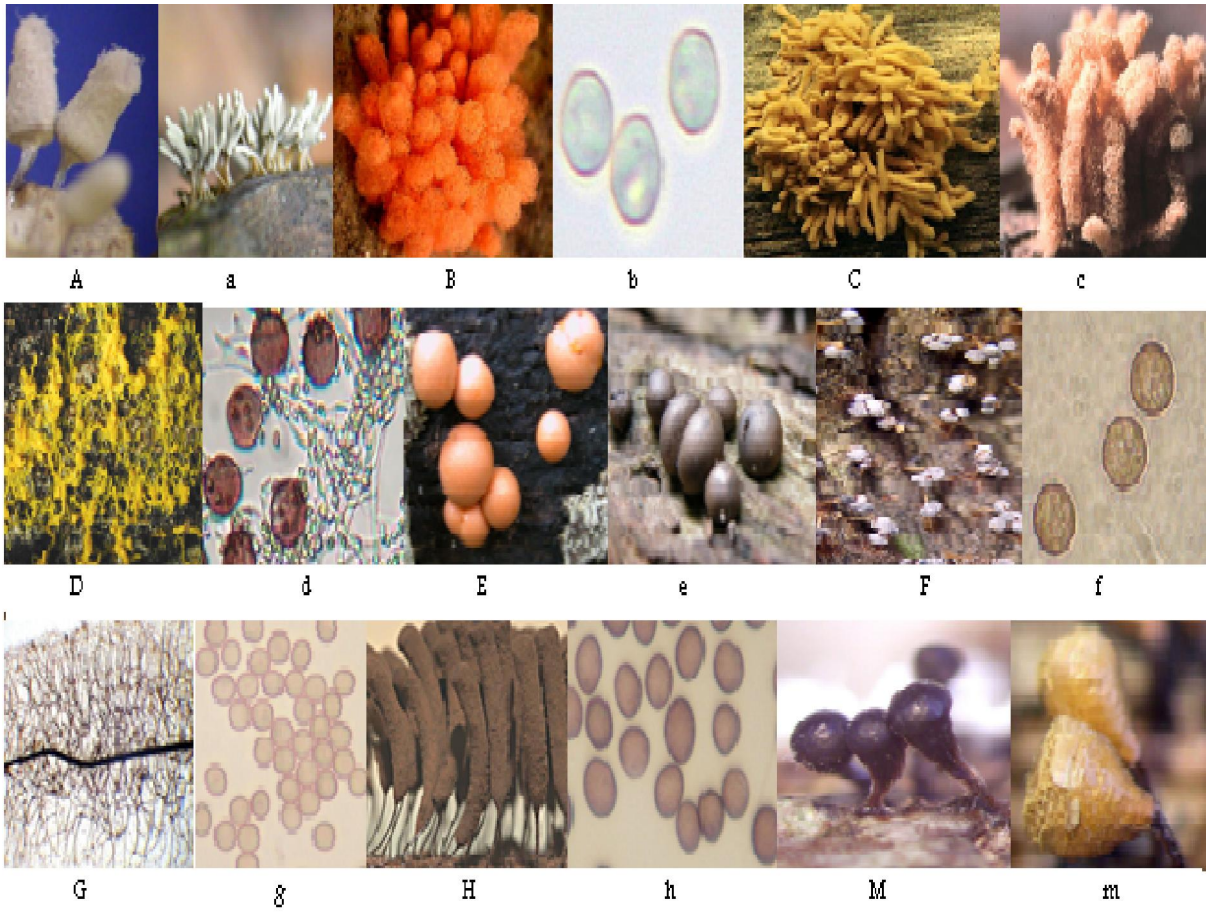
Spores pale yellow, 10-13 µm diam., decorated with short irregular ridges and a broken small-meshed reticulum of bands up to 1 µm high, showing as a border in optical section. Plasmodium white, pink or orange.

Materials examined: Gilan, Siahkal, on wood in humid forest.

Discussion:

Generally, little is known as the geographical distribution, taxonomy, identification, biodiversity, ecological diversity, biochemistry, metabolites and other features of *Myxomycetes* in the world(7). Only in limited and special areas, thorough studies have been conducted on different biological aspects of these living things. In many other regions across the world, no comprehensive research has been conducted. In light of the geographical vastness and ecological diversity of Iran, any study on the *Myxomycetes*is of high significance.mycology and related disciplines are fledgling academic courses. Therefore, limited information exists about the *Myxomycetes* (8). We managed to collect *Myxomycetes* only in the southern coastal areas of the Caspian Sea including Gilan, Mazandaran and Golestan Provinces, which have thick and humid forests. From among 50 substrate samples, 9 *Myxomycetes* were identified. The present study confirms that the forests of northern Iran are among the geographical habitats of *Myxomycetes*in the country and the species exist diversely in the areas. The study shows that *Myxomycetes* can be found in the humid and semi-humid forests of Iran just as some samples of the organisms have been identified in most forest areas where research has been conducted including the forests of Gilan, Mazandaran and Golestan Provinces. The present research aimed to study the importance of the organisms for modern science.

شکل3 : اندام بارده *Arcyrianutans*



شکل5 : اندام بارده نابالغ (سمت راست) و بالغ *Lycogala epidendrum* (سمت چپ)

A: Sporocarps *Arcyria cinerea*
B: Sporophore *Arcyria denudate*
C: Sporophore *Arcyria nutans*
D: Plasmodium *Fuligo septica*
E: Immature fruiting body *Lycogala epidendrum*
F: mature fruiting body *Physarum nutans*
G: Columella and capillitium *Stemonitis axifera*
H: fruiting body *Stemonitis fusca*
M: Immature fruiting body *Trichia decipiens*

a: sporophore *Arcyria cinerea*
b: spore *Arcyria denudate*
c: Capillitium *Arcyria nutans*
d: spore *Fuligo septic*
e: mature fruiting body *Lycogala epidendrum*
f: spore *Physarum nutans*
g: spore *Stemonitis axifera*
h: spore *Stemonitis fusca*
m: mature fruiting body *Trichia decipiens*

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