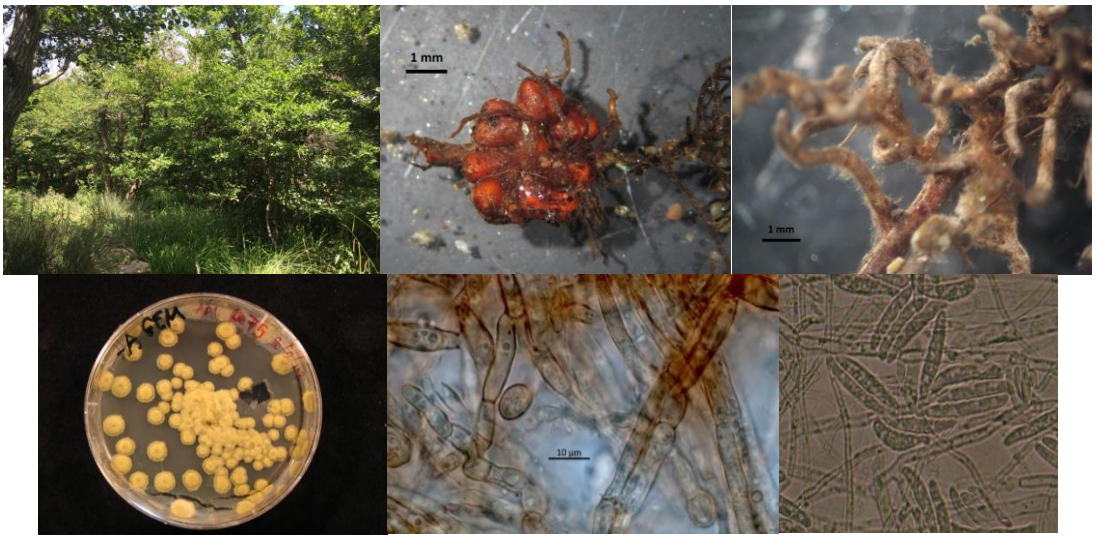
ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS

LIFE ANDROS PARK

LIFE ANDROS PARK

"Conservation of priority species and habitats of Andros Island protected area integrating socioeconomic considerations"



ACTION A.1

Pure cultures of several ectomycorrhizal fungi growing in association with *A. glutinosa* for preparing inocula, which will be used in producing alder seedlings in the nursery

Vassileios Daskalopoulos, Io Kefalogianni and Georgios I. Zervakis

with contributions from E. Polemis, A. Venieraki and V. Fryssouli

AGRICULTURAL UNIVERSITY OF ATHENS

March 2019
(updated version of the previously submitted Deliverable)

Abstract

Alluvial forests with *Alnus glutinosa* constitute a habitat of Community interest (91E0*). For this reason, restoration of the alluvial stands with *A. glutinosa* is one of the primary objectives of the LIFE Andros Park project. Alder trees show a remarkably high degree of host specificity compared with other tree species. In the frame of Action A.1, collection, isolation in pure cultures and identification of alder-associated microorganisms (i.e. those growing either in plant roots or rhizosphere) was carried out in conjunction with evaluation of inoculation withectomycorrhizal fungi (ECM) to enhance growth and/or adaptability of young alder seedlings during restoration/regeneration of deteriorated *A. glutinosa* stands.

Field-trips were performed in selected sites of Andros island during 2017-2018. ECM mushrooms, alder roots and rhizosphere soil samples were obtained and further processed in the lab to isolate microorganisms in pure culture (e.g. ectomycorrhizas, other endophytic and soil-inhabiting fungi and actinobacteria) through the use of various techniques, and then to identify them by morphological and molecular approaches. Several hundred fungi were isolated in pure cultures, studied and grouped to provide 91 representative types of colonies which were finally selected for further examination, separated according to their morphology (colony appearance, microscopical features) and subjected to molecular analysis (DNA sequencing). Finally, the identity of 25 isolates was determined; 12 of them were assigned to the following species: *Alternaria alternata, Apiognomonia lasiopetali, Chaetomium murorum, Fusarium solani, Ilyonectria radicicola, Lambertella tubulosa, Metacordyceps chlamydosporia, Neurospora reticulata, Penicillium chrysogenum, Phialocephala fortinii, Pleurotus ostreatus* and *Talaromyces ruber*. In addition, 13 isolates were identified to genus level, i.e. *Botrytis, Fusarium, Knufia, Penicillium, Phomopsis, Trichoderma* and *Umbellopsis*. The majority of the materials identified are soil-borne ascomycetes which generally possess an opportunistic ecology, maneuvering between different trophic habits depending on environmental conditions.

Περίληψη

Τα αλλουβιακά δάση με *Alnus glutinosa* αποτελούν οικότοπο προτεραιότητας για την Ε.Ε. (91Ε0*). Για τον λόγο αυτόν η αποκατάσταση των αλλουβιακών συστάδων με σκλήθρα της Άνδρου αποτελεί σημαντική προτεραιότητα στα πλαίσια της υλοποίησης του έργου LIFE Andros Park. Τα σκλήθρα εμφανίζουν ιδιαίτερα υψηλό βαθμό εξειδίκευσης στις συμβιωτικές τους σχέσεις σε σύγκριση με άλλα είδη. Κύριοι στόχοι της Δράσης Α.1 του έργου ήταν η συλλογή, απομόνωση και ταυτοποίηση μικροοργανισμών που σχετίζονται με το σκλήθρο (όσων αναπτύσσονται μέσα στο ριζικό σύστημα ή στην περιοχή της ριζόσφαιρας), όπως επίσης και η εξέταση της δυνατότητας εμβολιασμού νεαρών δενδρυλίων με εκτομυκορριζικούς μύκητες ώστε να αυξηθεί το ποσοστό επιβίωσης και να ενισχυθεί η προσαρμοστικότητα τους μετά τη μεταφύτευση στο πεδίο.

Κατά την διάρκεια 2017-2018 πραγματοποιήθηκαν εξορμήσεις για αυτόν τον σκοπό σε επιλεγμένες περιοχές της Άνδρου. Συλλέχθηκαν εκτομυκορριζικά μανιτάρια, τμήματα από το ριζικό σύστημα των σκλήθρων και δείγματα ριζοσφαιρικού εδάφους και εξετάστηκαν περαιτέρω με διάφορες μεθόδους στο εργαστήριο με σκοπό να απομονωθούν όλοι οι συμβιωτικοί μικροοργανισμοί (ενδοφυτικοί, εκτομυκοριζικοί, ριζοσφαιρικοί μύκητες και ακτινοβακτήρια) σε καθαρές καλλιέργειες και να ταυτοποιηθούν μορφοανατομικά και μοριακά. Πραγματοποιήθηκαν μερικές εκατοντάδες απομονώσεις μυκήτων σε καθαρές καλλιέργειες, οι οποίες αφού εξετάστηκαν, ομαδοποιήθηκαν σε 91 μορφοτύπους που επιλέχθηκαν προς περαιτέρω διερεύνηση όσον αφορά μορφοανατομικά χαρακτηριστικά (αποικία, μικροσκοπία υφών και καρποφοριών) ώστε να επιλεγούν αντιπροσωπευτικές απομονώσεις προς μοριακή ανάλυση (αλληλούχηση DNA). Συνολικά ταυτοποιήθηκαν 25 απομονώσεις, 12 εκ των οποίων μέχρι το επίπεδο του είδους, ήτοι: *Alternaria alternata, Apiognomonia lasiopetali, Chaetomium murorum, Fusarium solani, Ilyonectria radicicola, Lambertella tubulosa, Metacordyceps chlamydosporia, Neurospora reticulata, Penicillium chrysogenum, Phialocephala fortinii, Pleurotus ostreatus* και *Talaromyces ruber*, ενώ 13 ταυτοποιήθηκαν μέχρι το επίπεδο του γένους: *Botrytis, Fusarium, Knufia, Penicillium, Phomopsis, Trichoderma* και *Umbellopsis*. Η πλειονότητα των απομονωμένων μικροοργανισμών αποδείχθηκε πως είναι εδαφογενείς Ασκομύκητες οι οποίοι εμφανίζουν ιδιαίτερα οικολογικά χαρακτηριστικά με ποικίλες τροφικές προσαρμογές ανάλογα με το περιβάλλον-συνθήκες ανάπτυξης.

Action A.1

Deliverable titled: "Pure cultures of several ectomycorrhizal fungi growing in association with *A. glutinosa* for preparing inocula, which will be used in producing alder seedlings in the nursery"

Introduction

Alluvial forests with *Alnus glutinosa* constitute a habitat of Community interest (91E0*) found on low-lying damp and riparian localities of alluvial and marshland ecosystems. Black alder (*A. glutinosa*) tolerates waterlogged soil conditions and a high groundwater table, and the respective stands occur as a stable component within transitions to surrounding dry-ground forest, sometimes including other Annex I woodland types. Such transitions from wet to drier woodland and from open to more closed communities provide an important facet of ecological variation.

Andros island lies at the southernmost limit of priority habitat 91E0* distribution in the Balkan Peninsula. The alluvial *A. glutinosa* forests of Andros present a patchy distribution along the main streams of the site GR4220001. The main alluvial deposit of the site is located at Vori area, while several other smaller alluvial stands exist along the rivers of GR4220001. The deep alluvial soils of 91E0* are flooded, especially during the spring, while during the warm months, plants are often faced with extended period of drought.

Trees of the genus *Alnus* are known to form symbiotic relationships with various basidiomycetes and ascomycetes (Harley and Smith, 1983); approx. 1000 fungal species were described from ecologically diverse alder stands in Europe, and an estimated 120 species are considered to be symbiotic (Boyle 1996, Dimou et al. 2002, Polemis et al. 2012). In addition, mycocoenological studies from Europe and North America evidenced that ectomycorrhizal (ECM) fungi of *Alnus* show a remarkably high degree of host specificity compared with other tree species (Griesser, 1992; Arnolds et al., 1995, Pritsch et al., 1997).

It has been suggested that the abundant occurrence of symbiotic fungi in wet forests, such as the alder forests, is presumably an adaptation to survive adverse environmental conditions, e.g. those in water-saturated soil under oxygen poor conditions (Baar et al. 2000). Therefore, both partners (plant and fungus) depend heavily on one another for their survival and growth.

For this reason, the assessment of the diversity of *A. glutinosa* associated mycobiota was considered to be of primary importance for the successful implementation of Actions A1, C2 and C3 of the LIFE Andros Park project. The primary objectives were to collect, isolate and identify as many as possible of the alder-associated fungi (i.e. those growing either in plant roots or rhizosphere), as well as to evaluate the potential use of selected strains to serve as inocula for enhancing growth and/or adaptability of young alder seedlings in restoration/regeneration of deteriorated *A. glutinosa* stands. Moreover, a large battery of strains will be suitably prepared for long-term ex-situ conservation, which will permit the development of future pertinent applications.

Study approach – Methodology adopted

The assessment of the diversity of fungi associated to *A. glutinosa* was combined with the establishment of their occurrence/distribution within the target habitat. For this purpose, several field-trips were performed in selected sites of Andros island during autumn, winter and early summer (11-15/11/2017, 23-28/11/2017, 22-24/2/2018 and 2-5/6/2018). Sampling took place in three mountainous (>500 m a.s.l.; Evrousies, Vourkoti, Katakaleoi), and three coastal and low-altitude (0-250 m a.s.l.; Achla, Vori and Lefka) *A. glutinosa* stands (Fig. 1); the relatively high elevational range of the sampling sites is anticipated to facilitate monitoring of possible altitude effects on alder-associated species richness. Mushrooms as well as alder roots and rhizosphere soil samples were obtained and further processed in the lab to isolate microorganisms in pure culture (e.g. ectomycorrhizas, other endophytic and soil-inhabiting fungi and actinobacteria) through the use of various techniques, and then to identify them by morphological and molecular approaches.

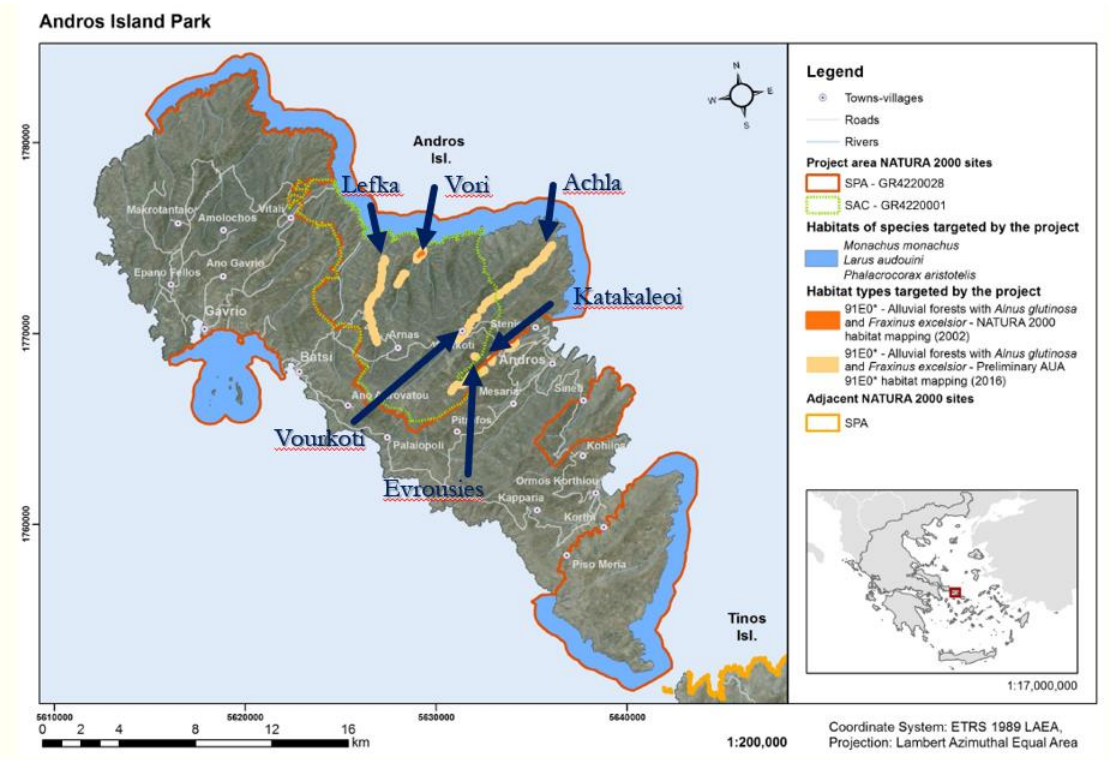


Fig. 1. Map of Andros depicting the sites (indicated by arrows and their respective names) from where sampling of alder-associated material was performed (Action A1).

Mushrooms were collected from the priority habitat, while environmental samples (alder rhizosphere soil and root fragments) were obtained from positions adjacent to *Alnus glutinosa* trees and/or from where the presence of ectomycorrhizal mushrooms was detected. Then, sampled material was placed in bags, kept in a portable fridge and transferred to AUA (Laboratory of General and Agricultural Microbiology) facilities for further processing.

In the lab, different processes were implemented depending on the type of sample:

1. For obtaining pure cultures from basidiomes of ectomycorrhizal fungi, small fragments of fresh samples were transferred under aseptic conditions to petri dishes with suitable nutrient media (MMN, Hagem). In addition, spores (obtained from spore-prints) and mushroom-tissue suspensions in sterile water were used to inoculate nutrient media in petri dishes. After this process was completed, mushrooms specimens were dried at 50 °C,

frozen at -80 °C for 24 hours, and further stored as dried specimens prior to their examination (for identification purposes).

2. For isolation of alder symbiotic (endophytic) microorganisms, the following procedure was adopted: Each soil sample was passed through sieves of different diameter, and then root tips and nodule-like structures were harvested and submerged into distilled water for 12 h. Each sample was then examined with a stereoscope. Different ECM morphotypes and nodule-like structures were separated, recorded and photographed. Then they were provisionally stored at 4 °C prior to further microscopic examination and isolation of microorganisms in pure culture.

2.a. Root tips from each distinct ECM morphotype was surface-sterilized (solutions of either 30% chlorine or 70% ethanol with 3-5% chlorine, v/v) fragmented into parts of 1-2 mm and placed into petri dishes with suitable nutrient media (MMN, Hagem).

2.b. Root-fragments including nodule-like structures were thoroughly washed to remove soil, transferred to flasks containing 50 ml of CTAB and agitated at 150 rpm for 30 min. After rinsing with sterilized water, they were surface sterilized with $HgCl_2$ 0.1% (w/v) or 3% H_2O_2 or with Na-hypochlorite solution, and then rinsed five times with sterilized water. The nodules were cut with a razor blade, transferred in sterile Eppendorf-tubes with 1 ml sterilized water and crushed with a sterile glass or metallic pestle. The resulting slurry was placed into petri dishes with nutrient medium (PDA).

3. For isolation of culturable fungi from the rhizosphere of *A. glutinosa*, root-fragments were gently shaken off, and the excess soil was discarded. Then the root-fragment with the closely adhering soil was placed into a flask containing sterilized tap water and stirred for 2 hours at 100 rpm. Serial soil dilutions were prepared and shaken vigorously. This method yielded dilutions of 10^{-1} to 10^{-4}, which were spread (1 ml) onto petri dishes with PDA. All plates were placed for incubation and inspected at regular intervals. The rhizosphere fungal population ranged from 1.5 X 10^5 to 3.0 x 10^6 propagules/root.

Irrespectively of whether cultures derived directly from mushrooms, ECM root tips, root nodule-like structures or rhizosphere soil, when two or more different colonies appeared, they were individually transferred to new nutrient media in petri dishes until pure

cultures were established for each isolate. Then colonies and their microscopic features were studied, and photographs were obtained. Pure cultures of symbiotic microorganisms were further maintained either in slants at 4 °C or in cryovials at -80 °C.

Fungal isolates were identified by molecular analysis of the internal transcribed spacer (ITS, rDNA). For each selected sample, total genomic DNA extraction was performed from fungal mycelium using the protocol described by Izumitsu et al. (2012). DNA concentration and purity were assessed by Nanodrop ND-1000 spectrophotometer. The primer set ITS1 and ITS4 targeting ITS1 and ITS2 fragments of approximately 600bp was used for polymerase chain reaction (PCR) amplification. PCR was performed by using Platinum Taq DNA Polymerase Kit (Invitrogen). PCR reactions were cycled in a BIORAD thermocycler (MJ mini) with a hot start step at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, annealing at 54°C for 1 min, and 72°C for 1 min, with a final extension step at 72°C for 10 min. PCR product were purified by NucleoSpin Gel and PCR clean-up (Macherey-Nagel). The ITS sequence was determined by direct sequencing of the PCR product and was performed by CeMIA SA, Greece. Analysis of sequence was performed with the basic sequence alignment BLAST Program run against the database provided in the website of National Center for Biotechnology Information (web site: http://www.ncbi.nlm.nih.gov/BLAST).

Results

In the frame of Action A1, a few hundred fungal isolates were obtained in pure culture. However, a large part of them was rejected due to similar/identical morphology or because they were not related to target fungi (or not linked to symbiotic actinobacteria of alder trees). Initial identification was based on morphological features of ECM root tips (Fig. 2) and fungal cultures, e.g. characteristics of colonies formed on nutrient media, structure of hyphae, type of sexual and asexual fructifications, microscopic characters, etc. (Fig. 3 and Fig. 4), and led to the classification of fungi up to the genus level.

A total of 91 isolates associated to *A. glutinosa* were finally selected for further examination, they were grouped according to their morphology, and were subjected to molecular analysis. Results of ITS sequencing revealed the existence of 12 species (12

genera) as follows: *Alternaria alternata*, *Apiognomonia lasiopetali*, *Chaetomium murorum*, *Fusarium solani*, *Ilyonectria radicicola*, *Lambertella tubulosa*, *Metacordyceps chlamydosporia*, *Neurospora reticulata*, *Penicillium chrysogenum*, *Phialocephala fortinii*, *Pleurotus ostreatus*, and *Talaromyces ruber*. Other 13 isolates were identified to genus (6 genera, i.e. *Botrytis*, *Fusarium*, *Knufia*, *Penicillium*, *Phomopsis*, *Trichoderma and Umbellopsis*), while for 55 isolates the identification process is under progress (Table 1).

Among the fungi identified, *Phialocephala fortinii* (culture code: AAL 101 D) is a dark septate endophyte, which is considered as a symbiotic fungus that can promote plant growth (Narisawa 2017). *Knufia* sp. (AAL 83) is also a dark septate endophyte with a potentially beneficial effect on plants. *Metacordyceps chlamydosporia* (48-b2, 47-11b, 47-1D) is a nematoctonous fungus used in Sustainable Nematode Management (Manzanilla-López and Lopez-Llorca 2017). *Pleurotus ostreatus* (B2, B3), the well-known oyster mushroom was found on alder trees in Andros island during site visits for the LIFE Andros Park project, is also a nematoctonous species. *F. solani* (EnAg1, EnAg2 FEnPl11, FEnPl13, FEnPl14, FEnPl16, FEnPl34, FEnPl37) is a soil fungus with a wide distribution; it often causes root rots in plants but some strains were demonstrated to exert a beneficial effect to plants and are exploited as biocontrol agents against soil-borne pathogens. *Trichoderma* sp. (AgTF1) colonizes the roots of plants (penetrating into the epidermis) and is reported to promote plant growth. Fungi in the genus *Umbelopsis* belong to the subphylum Mucoromycotina (phylum Mucoromycota). These fungi are industrially important given their unique lipid and fatty acid metabolism, and they are common and abundant in soil and plant roots. However, their biology and ecology is still not well understood.

Conclusions:

The majority of the fungi identified are soil ascomycetes that generally possess an opportunistic ecology, maneuvering between different trophic habits depending on environmental conditions. Many of them also inhabit plant tissues and often exert a beneficial effect on plant growth and/or its resistance to adverse conditions and pests/pathogens. Therefore, some isolates obtained from the target habitat (associated to *A. glutinosa*) could be further examined in this respect. Work is under progress to finalize

molecular identification of the biological material collected in order to properly develop and maintain a battery of fully-identified/characterized pure cultures for (a) use as fungal inocula in alder seedlings prior to their planting at the selected restoration sites (Action C2), and (b) ex-situ conservation purposes (Action C3). The most interesting findings associated with this Deliverable were recently presented in the Congress of the Scientific Society "Mikrobiokosmos" and relevant material is attached hereby.

Table 1. Molecular and morphological identification of pure cultures of fungi associated with *Alnus glutinosa*.

s/n	Culture code	Identity (BLAST query cover and DNA sequence identity, %) [closest phylogenetic relative, (when appropriate)]	Isolation source (alder-associated)	Geographic origin (Andros)	Additional information – Comments
1	EnFAg 1	*Fusarium solani* (100, 98)	Root-fragments incl. nodule-like structures	Katakalaioi	*F. solani* is a cosmopolitan species with opportunistic biology and it can be found as a plant pathogen, as endophyte or in rhizosphere.
2	EnFAg 2	*Fusarium solani* (97, 97)	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
3	FEnPl 4	*Fusarium solani* (75, 99)	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
4	FEnPl 5bact	Under examination	Root-fragments incl. nodule-like structures	Katakalaioi	
5	FEnPl 6	*Fusarium solani* (99, 98)	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
6	FEnPl 7	*Ilyonectria radicicola* (98, 99)	Root-fragments incl. nodule-like structures	Katakalaioi	*I. radicicola* is associated with root and decay of woody and herbaceous plants (root rot)

7	FEnPl 8	*Chaetomium murorum* (syn. *Botryotrichum murorum*) (96, 99)	Root-fragments incl. nodule-like structures	Katakalaioi	*Chaetomium* fungi colonize various substrates and are well-known for their ability to degrade cellulose and to produce a variety of bioactive metabolites.
8	FEnPl 11bact1	*Fusarium solani* (79, 96)	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
9	FEnPl 12	Under examination	Root-fragments incl. nodule-like structures	Katakalaioi	
10	FEnpl 13	*Fusarium solani* (99, 99)	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
11	FEnPl 14	Under examination	Root-fragments incl. nodule-like structures	Achla	
12	FEnPl 17	*Fusarium* sp. [*F. solani* (59, 95)]	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
13	FEnPl 19	Under examination	Root-fragments incl. nodule-like structures	Achla	
14	FEnPl 25	*Neurospora reticulata* (60, 97)	Root-fragments incl. nodule-like structures	Achla	*N. reticulata* is a root pathogen of some plants (e.g. tobacco). *Neurospora* spp. are coprophilous, soilborne or grow on plant debris
15	FEnPl 31	*Ilyonectria radicicola* (93, 96)	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 6
16	FEnPl 33	Under examination	Root-fragments incl. nodule-like structures	Achla	

17	FEnPl 34	*Fusarium solani* (76, 99)	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
18	FEnPl 35	*Fusarium* sp. [*F. solani* (83, 91)]	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
19	FEnPl 37	*Fusarium solani* (72, 100)	Root-fragments incl. nodule-like structures	Katakalaioi	See s/n 1
20	FEnPl 38	*Alternaria alternata* (90, 95)	Root-fragments incl. nodule-like structures	Katakalaioi	*Alternaria alternata* is a saprotroph or plant pathogen. *Alternaria* spp. are saprotrophs, involved in the decomposition of organic matter, and producers of several toxic metabolites (some species are endophytic and pathogenic).
21	FEnPl 39	*Phomopsis* sp. (40, 92) [teleomorph: *Diaporthe*]	Root-fragments incl. nodule-like structures	Katakalaioi	*Phomopsis* fungi are saprotrophs, plant pathogens or endophytes (sapwood of angiosperms) with potential role in protecting plants from fungal diseases. Black alder (*A. glutinosa*) is attacked by *Phomopsis alnea* which causes perennial stem cankers and dieback.

22	AgPF 4	Under examination	Rhizosphere soil under *Paxillus olivellus* /241131.	Vori	
23	AgIF 1	Under examination	Rhizosphere soil under *Inocybe* sp, /251135.	Vori	
24	AgIF 2	Under examination	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
25	AgIF 3	Under examination	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
26	AgTF 1	*Trichoderma* sp. [*T. harzianum* (86, 80)]	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	*T. harzianum* colonizes the roots of plants (penetrating into the epidermis) and promotes plant growth. *Trichoderma* spp. are used as biocontrol and plant growth promoting agents, while they increase plant tolerance against abiotic stresses.
27	AgTF 4	*Phomopsis* sp. [*P. columnaris* (78, 96)]	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	*Phomopsis* spp. are usually found as endophytes. *P. columnaris* is causing twig dieback of lingonberry. AgtF4 morphocharacters are different from those of FEnPl 39.
28	AgTF 5	Under examination	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	

29	AgTF 9	*Penicillium* sp. [*P. toxicarium* (81, 94)]	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	*P. toxicarium* is an endophyte usually associated with pine trees.
30	AgTF 11	Under examination	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	
31	AgTF 12	Under examination	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	
32	AgN26F 3	Under examination	Rhizosphere soil under *Naucoria* sp. /241126.	Vori	
33	AgN26F 6	Under examination	Rhizosphere soil under *Naucoria* sp. /241126.	Vori	
34	AgN26F 10	Under examination	Rhizosphere soil under *Naucoria* sp. /241126.	Vori	
35	AgN26F 11	*Penicillium chrysogenum* (92, 99)	Rhizosphere soil under *Naucoria* sp. /241126.	Vori	*P. chrysogenum* is the source of several b-lactam antibiotics (penicillin), or other secondary metabolites.
36	AgIF 10	Under examination	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
37	AgIF 13	*Talaromyces ruber*	Rhizosphere soil under *Inocybe*sp. /251135.	Vori	*T. ruber* produces pigments and other metabolites (antibiotics).
38	AgIF 15	*Botrytis* sp. [*Botrytis cinerea* (83, 85)]	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	*B. cinerea* is a cosmopolitan plant pathogen which causes grey molds.
39	AgIF 16	Under examination	Rhizosphere soil under *Inocybe*s sp. /251135.	Vori	

40	AgIF 19	*Penicillium* sp. [*P. brevicompactum* (24, 100)]	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	*P. brevicompactum* produces mycotoxins and is contaminant for a large range of foods
41	AgIF 21	Under examination	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
42	AgIF 23	Under examination	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
43	AgIF 26	basidiomycete [*Pholiota adiposa* (48,77)]	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
44	AgIF 27	*Penicillium* sp. [*P. expansum* (46, 85)]	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	*P. expansum* is a common postharvest pathogen of several products, producer of mycotoxin
45	AgIF 28	Under examination	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
46	AgIF 30	Under examination	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
47	AgIF 32	Under examination	Rhizosphere soil under *Inocybe* sp. /251135.	Vori	
48	AgPF 5	Under examination	Rhizosphere soil under *P. olivellus* /241131.	Vori	
49	AgTF 8	Under examination	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	
50	AgTF 10	Under examination	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	

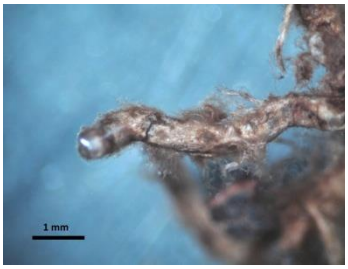
51	AgTF 13	Under examination	Rhizosphere soil under *Tomentella* sp. /251142.	Vori	
52	AgN25F 6	Under examination	Rhizosphere soil under *Naucoria* sp. /241125.	Vori	
53	AgN25F 2	Under examination	Rhizosphere soil under *Naucoria* sp. /241125.	Vori	
54	AgN25F 1	Under examination	Rhizosphere soil under *Naucoria* sp. /241125.	Vori	
55	B2	*Pleurotus ostreatus* (98, 99)	Rhizosphere soil	Vori	See s/n 15
56	B3	*Pleurotus ostreatus* (97, 99)	Rhizosphere soil	Vori	See s/n 15
57	47-1 C,D	*Metacordyceps chlamydosporia* (98, 99)	ECM root tip	Katakalaioi.	*M. chlamydosporia* is a nematoctonous fungus used in sustainable management of nematodes
58	47-11 A,B	*Metacordyceps chlamydosporia* (95, 99)	ECM root tip	Katakalaioi	See s/n 59
59	47-31,32 (A,B)	*Phomopsis sp.* (*Phomopsis columnaris* 64, 94)	ECM root tip	Katakalaioi	Possible dark septate endophyte according to colony features.
60	48-b1 A,B	Under examination	ECM root tip	Katakalaioi	Similar morphology like 47-1 (C-D), 47-11 (A-B) , 47-31,32 (A,B).
61	48-b2 A,B (48-b A,B)	*Metacordyceps chlamydosporia* (97, 99)	ECM root tip	Katakalaioi	See s/n 59
62	48-b3 A,B	Under examination	ECM root tip	Katakalaioi	

63	AAL 101 A	Under examination	ECM root tip	Achla	
64	AAL 101 B	Under examination	ECM root tip	Achla	
65	AAL 101 C, 102 B	Under examination	ECM root tip	Achla	
66	AAL 101 D	*Phialocephala fortinii* (96, 99)	ECM root tip	Achla	Dark septate endophyte in plant roots.
67	AAL 102 A	Under examination	ECM root tip	Achla	Possible dark septate endophyte according to colony features.
68	AAL 102 C	Under examination	ECM root tip	Achla	Possible dark septate endophyte according to colony features.
69	AAL 102 D	Under examination	ECM root tip	Achla	
70	AAL 103	Under examination	ECM root tip	Evrousies	
71	AAL 114	Under examination	ECM root tip	Katakalaioi	
72	AAL 142 A	*Lambertella tubulosa*, (*Helicodendrum tubulosum*) (91, 99)	ECM root tip	Vourkoti	*Helicodendron tubulosum* is the anamorph name of *L. tubulosa*, i.e. a fungus usually found in aquatic environments.
73	AAL 142 B	*Umbelopsis* sp. (93,95)	ECM root tip	Vourkoti	Zygomycete *Umbelopsis* sp. are industrially important given their unique lipid and fatty acid metabolism and they are common and abundant inhabitants of soil and plant roots. However, their biology and ecology is still not fully understood.

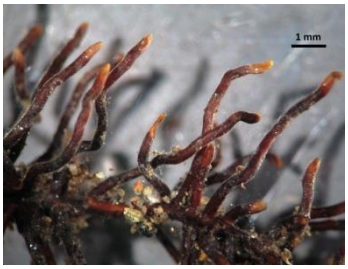
74	AAL 142 C	Under examination	ECM root tip	Vourkoti	
75	AAL 144	*Apiognomonia lasiopetali, Gnomoniaceae* sp.	ECM root tip	Vourkoti	Possible plant pathogen.
76	AAL 147 A	Under examination	ECM root tip	Lefka	
77	AAL 147 B	Under examination	ECM root tip	Lefka	Possible dark saptate endophyte according to colony features.
78	AAL 54 A	Under examination	ECM root tip (under *Cortinarius* sp. /121131)	Katakalaioi	
79	AAL 55	Under examination	ECM root tip (under *Naucoria* sp. /121133)	Katakalaioi	
80	AAL 56 A	*Cylindrocarpon* sp. (*Ilyonectria radicicola* 88, 98)	ECM root tip (under *Naucoria* sp. /121133)	Katakalaioi	See n6
81	AAL 58 A, 60 B	Under examination	ECM root tip (under *Naucoria* sp. /121133)	Katakalaioi	Possible dark septate endophyte according to colony features.
82	AAL 60 A	Under examination	ECM root tip (under *Naucoria* sp. /121133)	Katakalaioi	
83	AAL 75	Under examination	ECM root tip (under *Inocybe* sp. /251135)	Vori	
84	AAL 83	*Knufia* sp. (93, 96) [*Knufia tsunedae* (91, 95)]	ECM root tip (under *Naucoria* sp. /251125)	Vori	Dark septate endophyte *K. tsuneade* was isolated from soil. *Knufia* spp. are often found inside plants.
85	AAL 90 A	Under examination	ECM root tip (under *Naucoria* sp. /251126)	Vori	Possible dark septate endophyte according to colony features.
86	AAL 91	Under examination	ECM root tip (under *Naucoria* sp. /251126)	Vori	

87	AAL 98	Under establishment	Ectomycorrhizal tip	Achla	
88	Nau1	Under establishment	Sporocarp suspension from *Naucoria* sp. mushroom	Vori	
89	Nau2	Under establishment	Sprocarp tissue from *Naucoria* sp. /251126.	Vori	
90	Pax1	Under establishment	Sporocarp tissue from *P. olivellus* /11115.	Katakalaioi	
91	Pax2	Under establishment	Sporocarp tissue from *P. olivellus* /220216.	Vori	

AAL 42

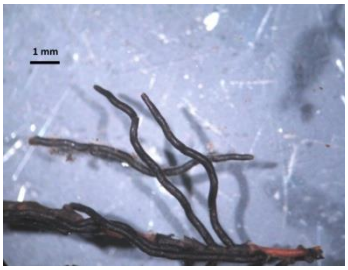


AAL 49

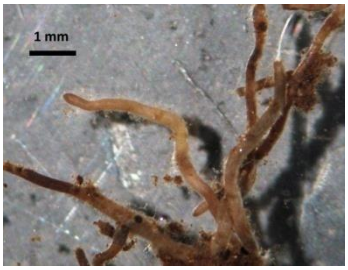


AAL 54

AAL 55



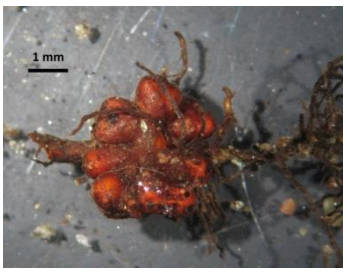
AAL 56



AAL 57

AAL 58

AAL 60

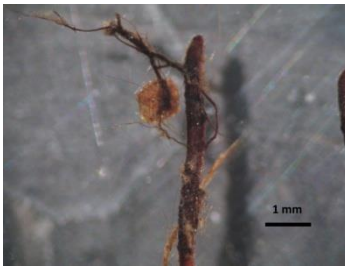


AAL 66

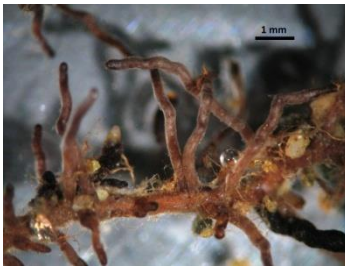
AAL 67

AAL 68

AAL 70



AAL 71

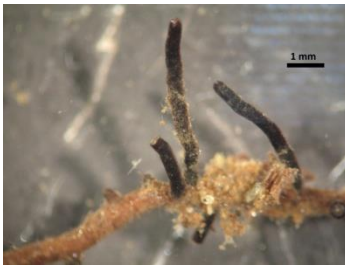


AAL 72

AAL 77

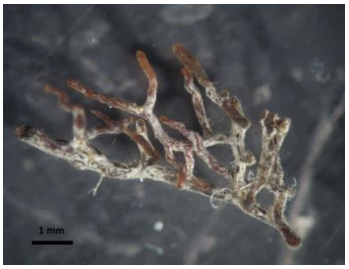
AAL 78

AAL 81

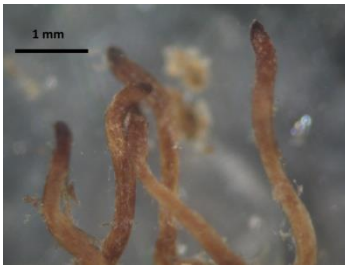


AAL 91

AAL 94



AAL 99



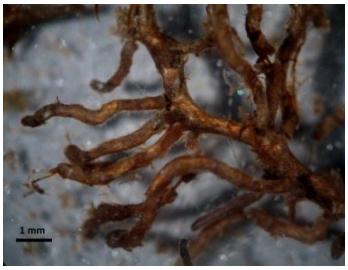
AAL 101


AAL 102

AAL 103

AAL 107

AAL 118



AAL 122

AAL 125

AAL 135

AAL 141

AAL 144

AAL 145

AAL 147

AAL 151

AAL 154

AAL 155

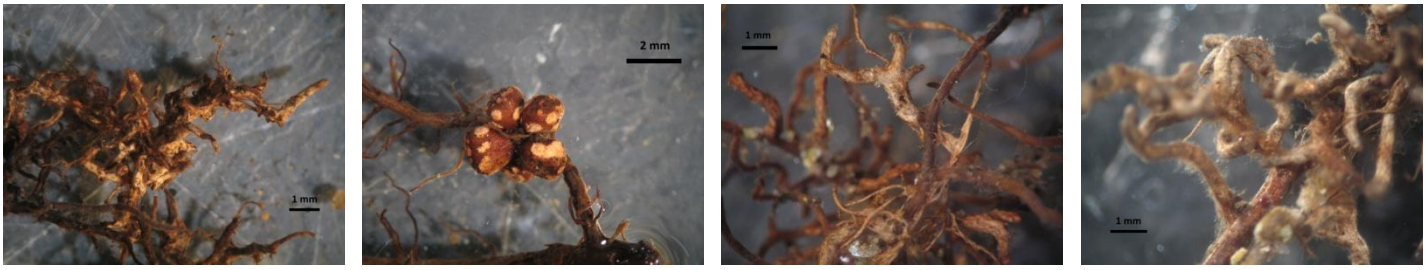
AAL 161

AAL 163

AAL 164

AAL 165

AAL 167



AAL 169 AAL 172 AAL 173 AAL 98

Composite Fig. 2. Various morphotypes of ECM root tips and nodule-like structures formed on alder roots (collected and studied in the frame of Action A1).

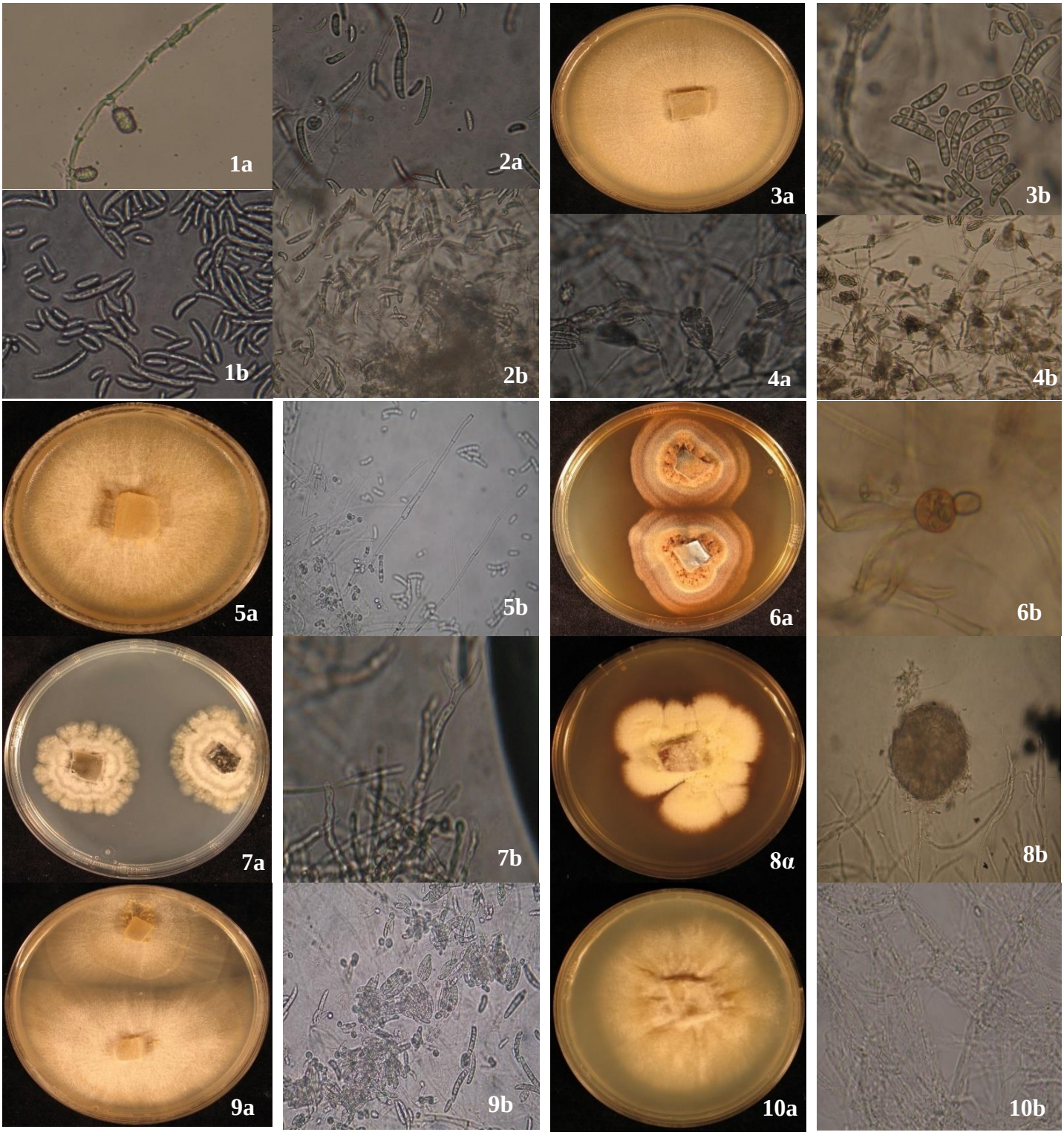


Fig. 3a: 1a) EnFAg1 chlamydospores intercalary, solitary; 1b) EnFAg1 macroconidia elongate to slightly curved; 2a) EnFAg2 macroconidia 4-celled, elongate to slightly curved; 2b) conidiophore 3a) FEnPl4 colony; 3b) FEnPl4 macroconidia 2-celled, elongate; 4a) FEnPl5bact conidiophore; 4b) FEnPl5bact conidiophore, conidia elongate; 5a) FEnPl6 colony 5b) microconidia, ellipsoidal, straight or slightly curved, hyaline, microconidiation; 6a) FEnPl7colony; 6b) FEnPl7 chlamydospore; 7a) FEnPl8 colony; 7b) FEnPl8 lateral or terminal conidiogenous cells (phialides); 8a) FEnPl12 colony; 8b) FEnPl12 pycnidia with conidiophores inside; 9a) FEnPl13 colony; 9b) FEnPl13 conidiophore with elongate conidia, 2-celled, straight or slightly curved; 10a) FEnPl14 colony; 10b) FEnPl14 hyphae septate.

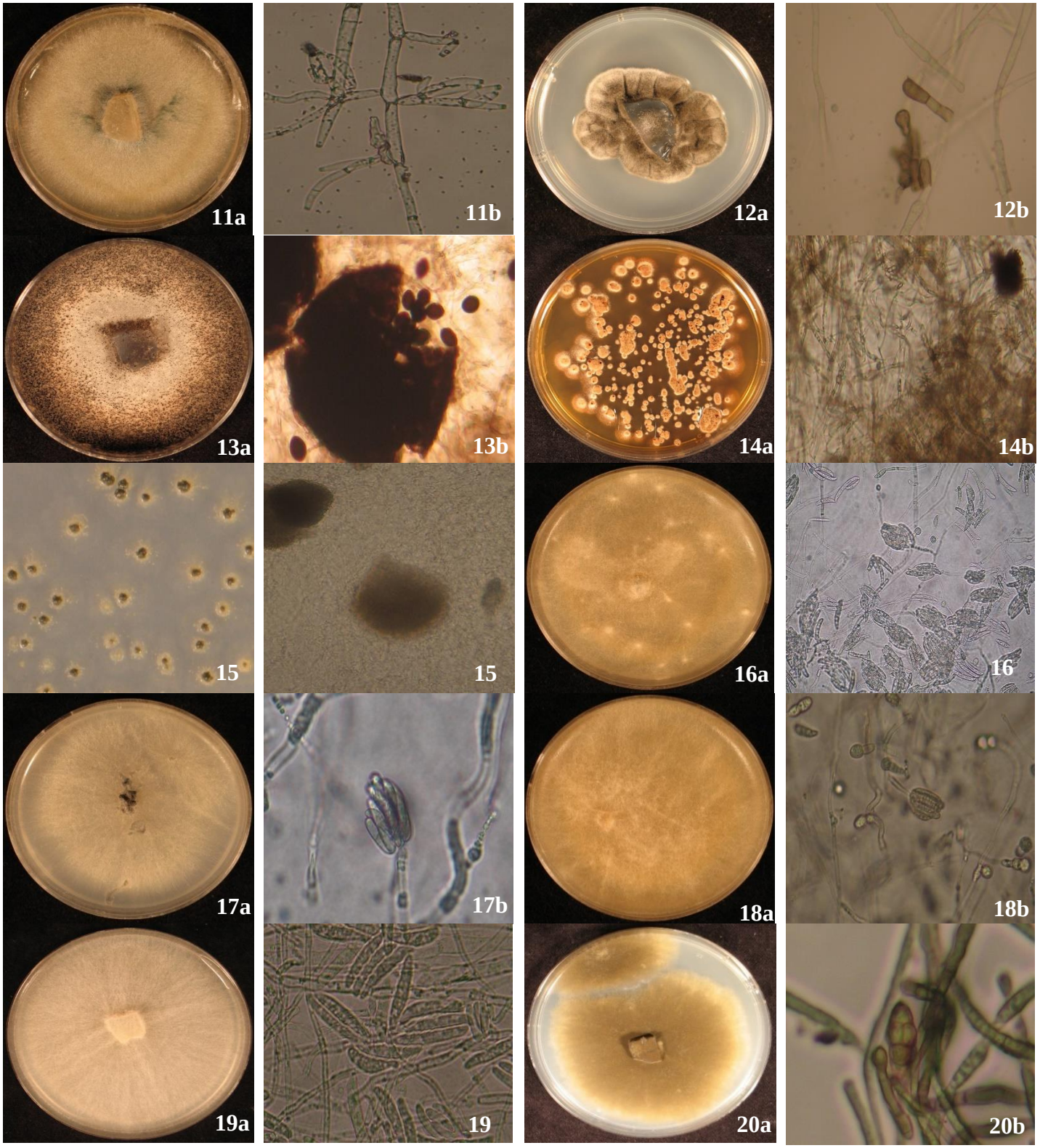


Fig. 3b: 11a) FEnPl17 colony 11b) FEnPl17 conidiophore with phialides lateral, intercalary or terminal, conidia elongate, 2-celled, straight or slightly curved; 12a) FEnPl19 colony; 12b) FEnPl19 conidia brown, hyphae septate; 13a) FEnPl25 colony; 13b) FEnPl25 perithecium asci and dark ascospores; 14a) FEnPl31 colony; 14b) FEnPl31 sporodochial conidiophore, chlamydospores; 15a) FEnPl33 colonies; 15b) FEnPl33 pycnidia, conidia hyaline (colorless); 16a) FEnPl34 colony; 16b) FEnPl34 conidiophore, macroconidia elongate, straight or slightly curved and microconidia ellipsoidal, straight or round; 17a) FEnPl35 colony 17b) FEnPl35 conidiophore, conidia elongate, straight or slightly curved; 18a) FEnPl11bact1 colony; 18b) FEnPl11bact1 conidiophore, conidia, chlamydospores; 19a) FEnPl37 colony; 19b) FEnPl37 macroconidia elongate, straight or slightly curved; 20a) FEnPl38 colony; 20b) FEnPl38 conidia cylindrical, multi-septate, brown colored;

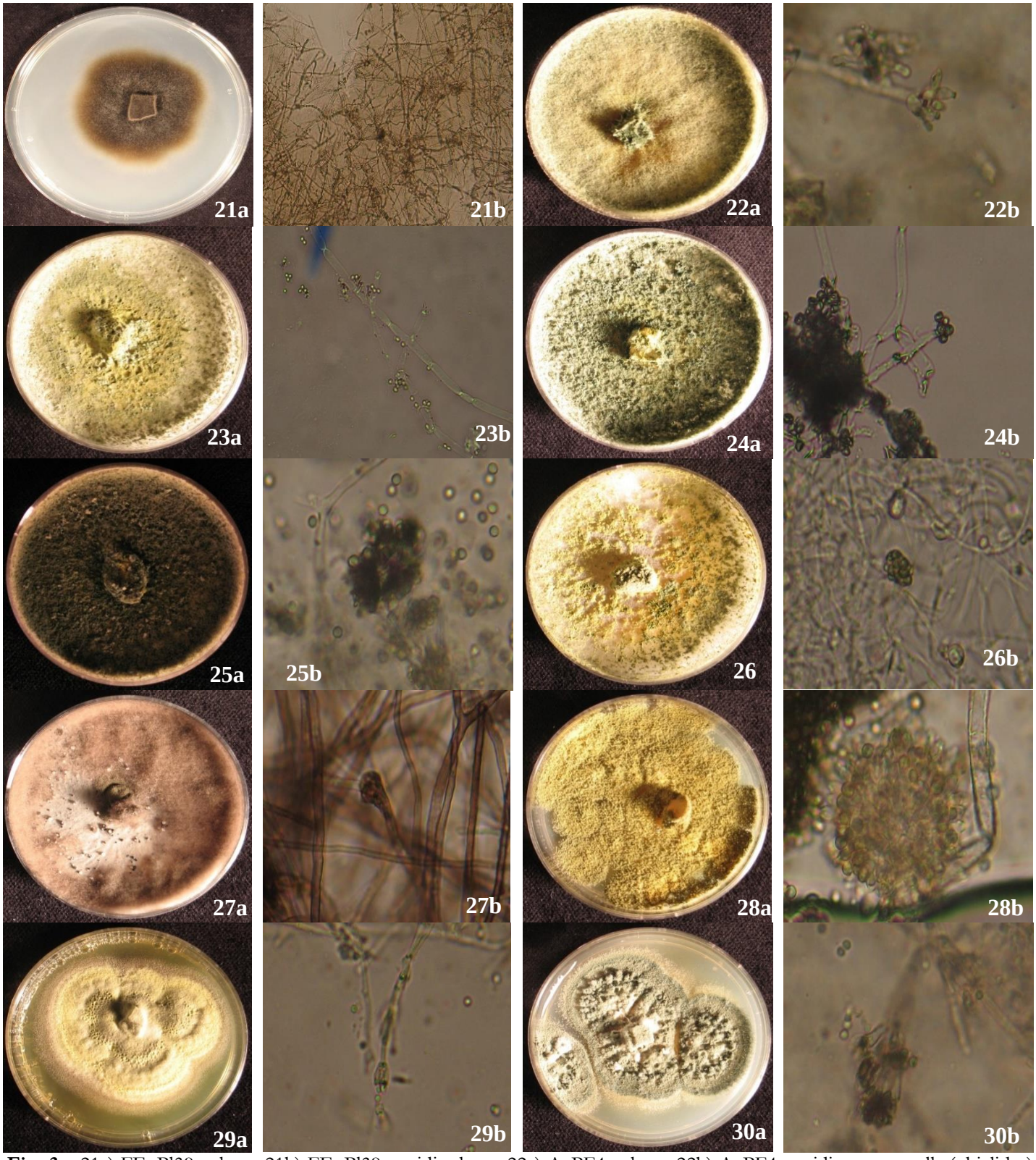


Fig. 3c: 21a) FEnPl39 colony; 21b) FEnPl39 conidiophore; 22a) AgPF4 colony; 22b) AgPF4 conidiogenous cells (phialides) with swollen bases and prominent necks, conidia spherical hyaline; 23a) AgIF1 colony; 23b) AgIF1 conidiophore, conidia round hyaline; 24a) AgIF2 colony; 24b) AgIF2 conidiophore, cluster of conidia spherical and colored; 25a) AgIF3 colony; 25b) AgIF3 conidiophore, bear cluster of conidia spherical and dark; 26a) AgTF1 colony; 26b) AgTF1 conidiophore, cluster of spherical, colored conidia, chlamydospores; 27a) AgTF4 colony; 27b) AgTF4 conidia, medium brown hyphae forming hyphae coils; 28a) AgTF5 colony; 28b) AgTF5 conidiophore, globose covered with flask shaped phialides, conidia spherical colored; 29a) AgTF9 colony; 29b) AgTF9 conidiophore with phialides and spherical, colored conidia; 30a) AgTF11 colony; 30b) AgTF11 branched conidiophores with phialides and chains of spherical, colored conidia;

Fig. 3d: 31a) AgTF12 colony; 31b) AgTF12 sporangium with sporangiospores; 32a) AgN26F3 colony; 32b) AgN26F3 intercalary phialides, 1-septate conidia; 33a) AgN26F6 colony; 33b) AgN26F6 phialides, conidia, and sporodochium; 34a) AgN26F10 colony; 34b) AgN26F10 conidiophores spherical colored conidia; 35a) AgN26F11 colony; 35b) AgN26F11 branched conidiophores with phialides and chains of spherical, colored conidia; 36a) AgIF10 colony; 36b) AgIF10 hyphae septate, clamp connections; 37a) AgIF13 colony; 37b) AgIF13 conidiophore phialidia, conidia spherical, colored; 38a) AgIF15 colony; 38b) AgIF15 conidiophores, spherical, colored conidia; 39a) AgIF16 colony; 39b) AgIF16 conidiophores, phialides, spherical, colored conidia; 40a) AgIF19 colony; 40b) AgIF19 conidiophores mostly not branched, phialides, spherical, colored conidia

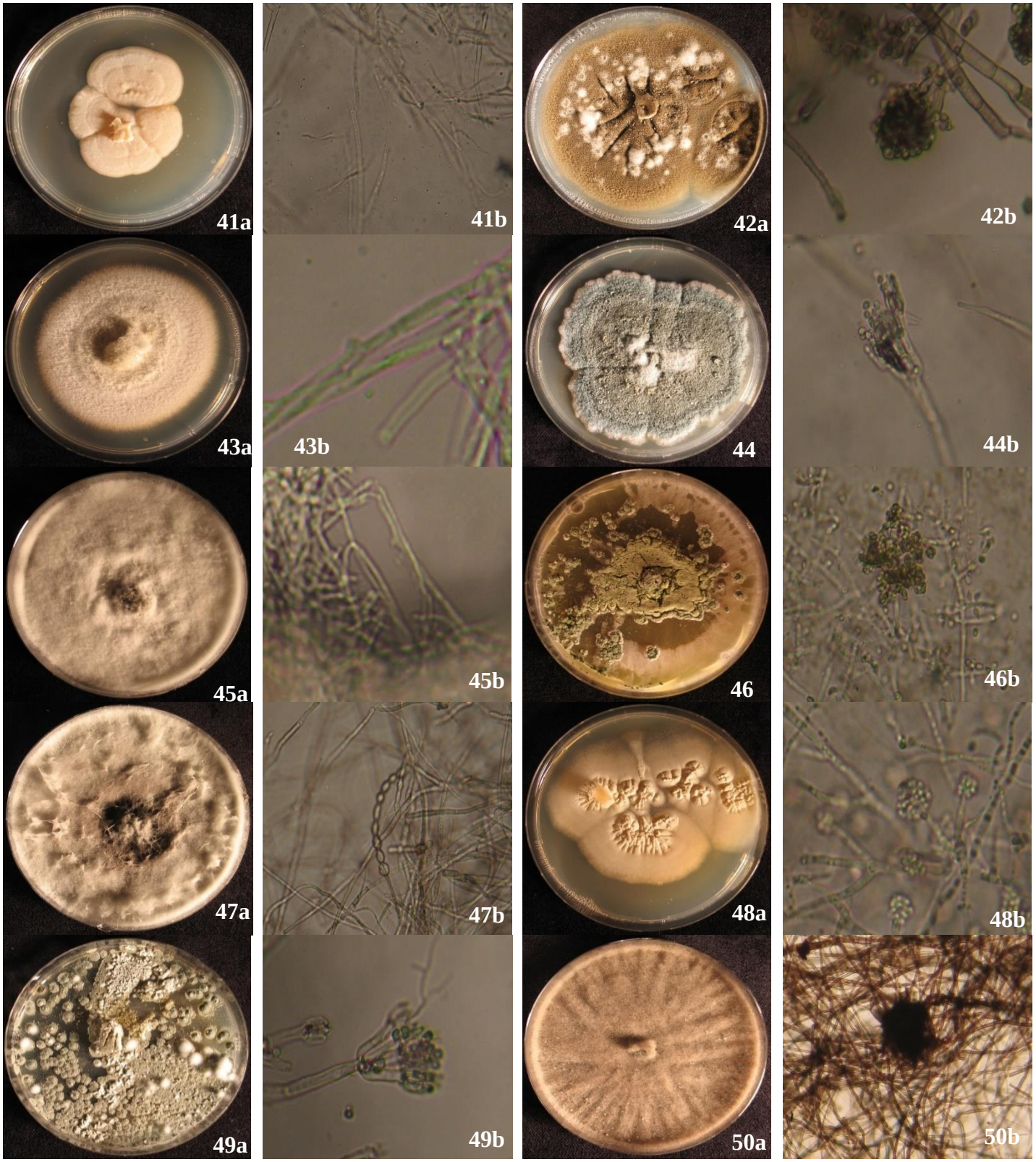


Fig. 3e: 41a) AgIF21 colony; 41b) AgIF21 hyphae with septa; 42a) AgIF23 colony; 42b) AgIF23 conidiophore, cluster of spherical, dark conidia; 43a) AgIF26 colony; 43b) AgIF26 hyphae septate, anastomized 44a) AgIF27 colony 44b) AgIF27 conidiophore, phialides, chains of spherical, dark conidia; 45a) AgIF28 colony; 45b) AgIF28 hyphae septate; 46a) AgIF30 colony; 46b) AgIF30 conidiophore with cluster of spherical, dark conidia; 47a) AgIF32 colony; 47b) AgIF32 conidiophore, conidia produced in single chain; 48a) AgPF5 colony; 48b) AgPF5 conidiophore bearing clusters of spherical conidia; 49a) AgTF8 colony; 49b) AgTF8 conidiophores branched, phialides, spherical, colored conidia; 50a) AgTF10 colony; 50b) AgTF10 chlamydospore, medium brown hyphae forming coils.

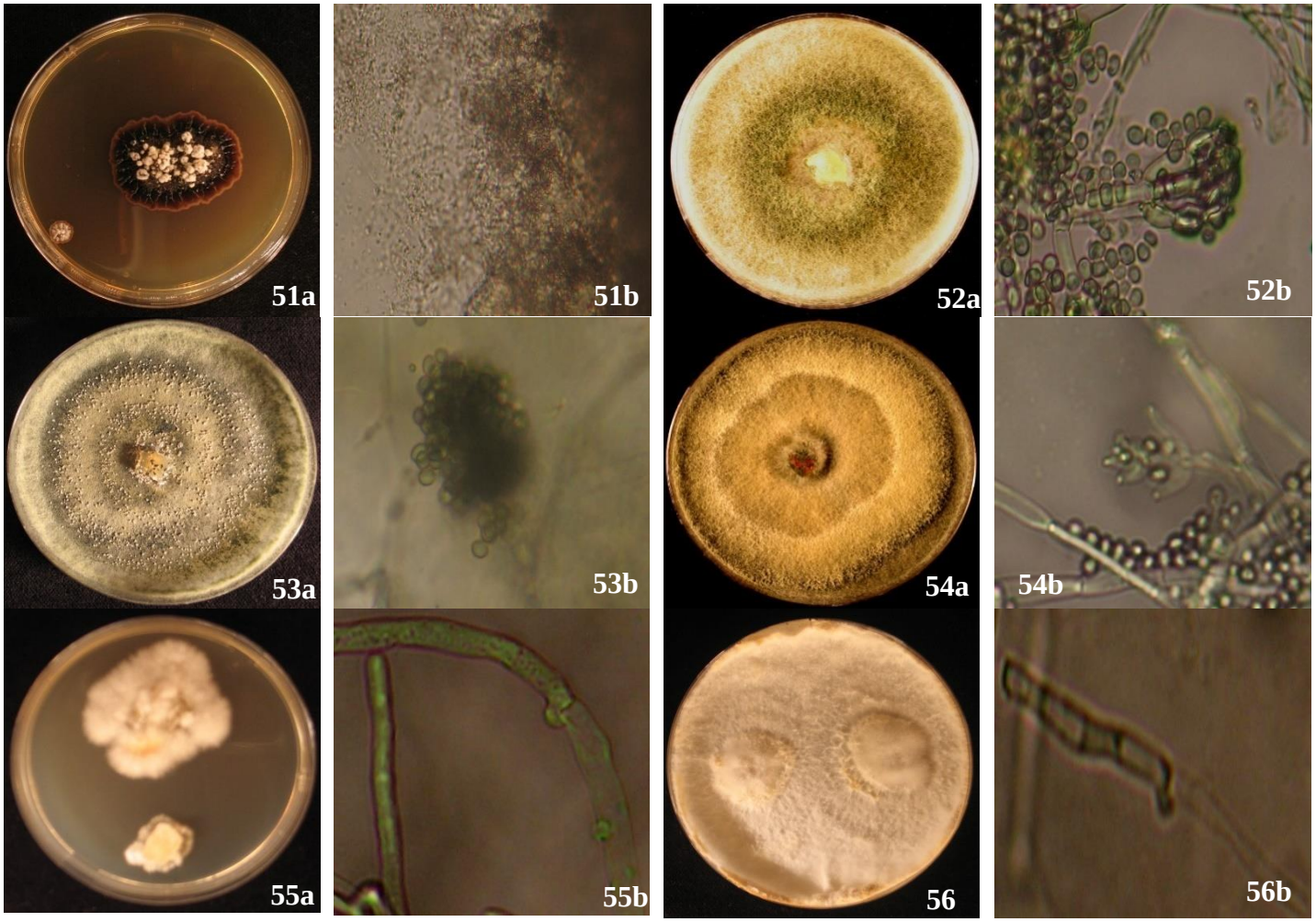


Fig. 3f: 51a) AgTF13 colony; 51b) AgTF13 fine hyphae with spores; 52a) AgN25F6 colony; 52b) AgN25F6 conidiophore, phialides with narrow neck, spherical, colored conidia; 53a) AgN25F2 colony; 53b) AgN25F2 conidiophore with spherical, dark conidia and chlamydospores; 54a) AgN25F1 colony; 54b) AgN25F1 immature conidiophore, phialides with narrow neck, spherical, colored conidia; 55a) B_2 colony; 55b) B_2 hyphae with clamp connections; 56a) B_3 colony; 56b)) B_3 hyphae with clamp connections.

Composite Fig. 3. Colonies and microscopic features of alder-associated fungi isolated from rhizosphere soil and root-fragments incl. nodule-like structures (collected and studied in the frame of Action A1).

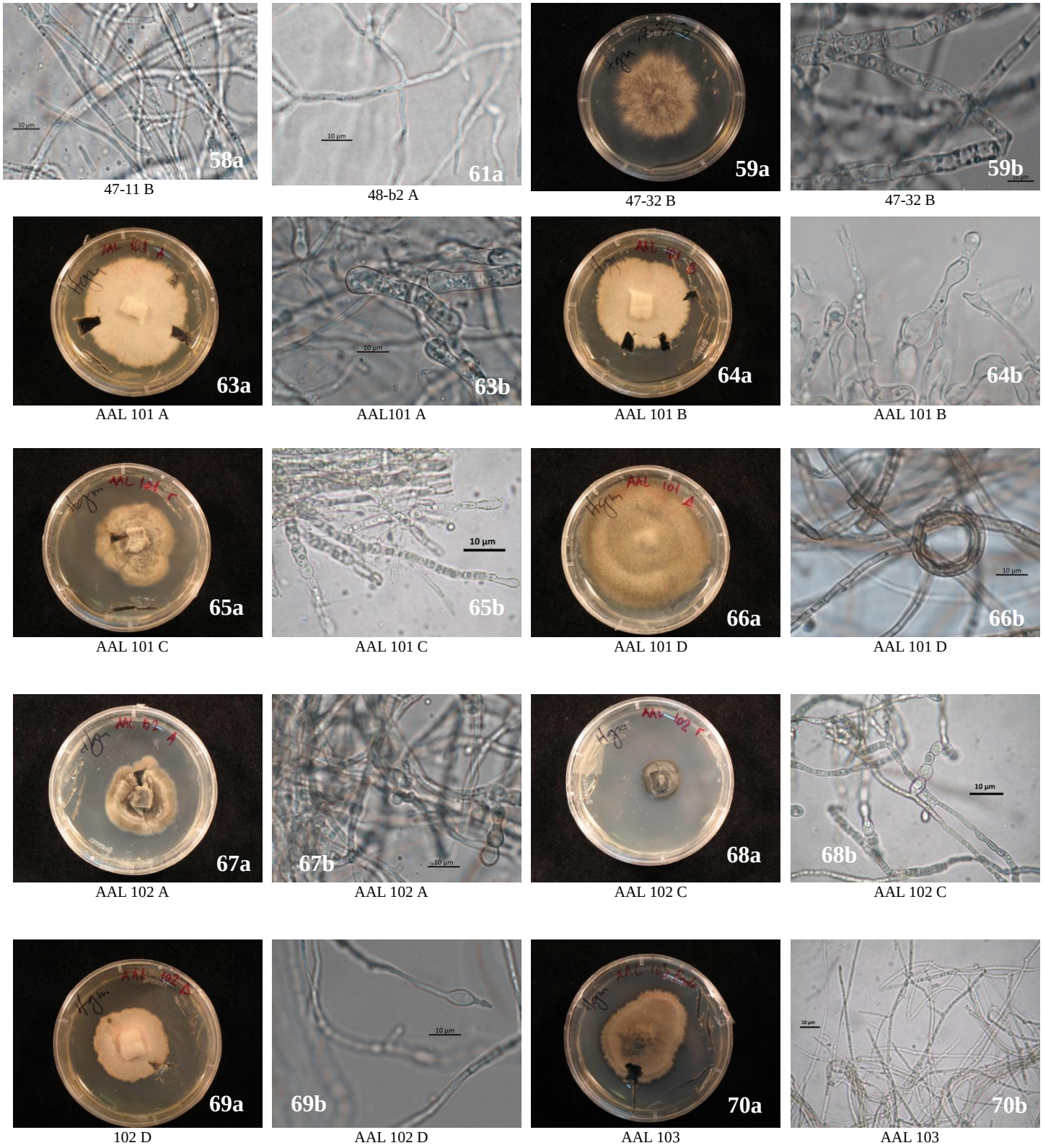
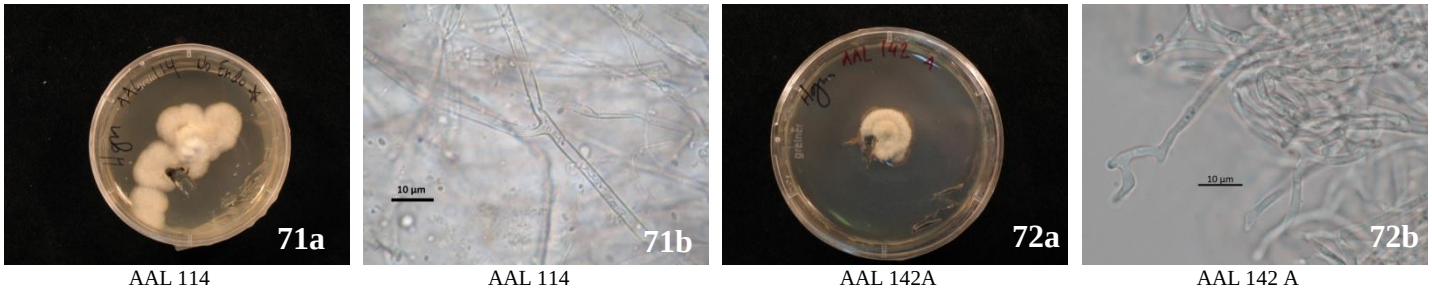
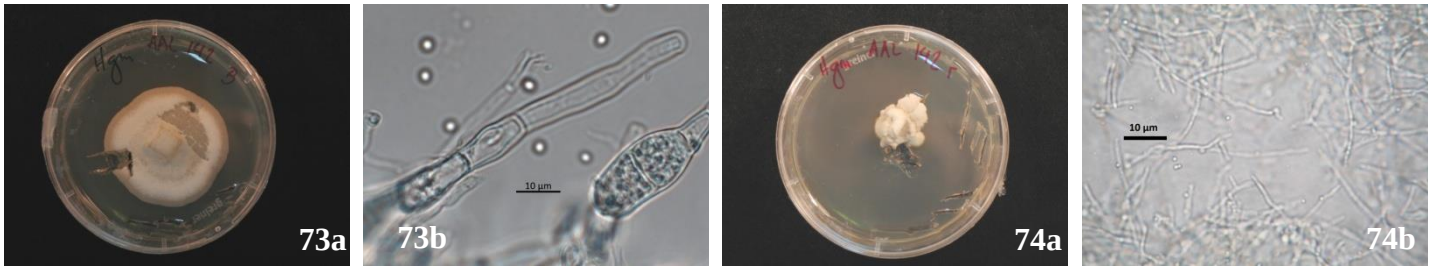


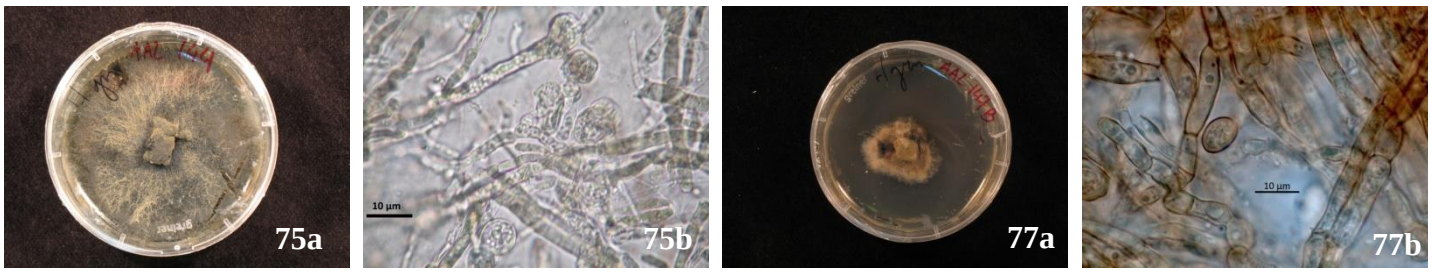
Fig. 4a: 58a) hyphae of 47 -11 B; 61a) hyphae with trifurcate ramification.; 59a,b) colony and wide hyphae of 47-32 B; 63a,b) colony and macroconidia; 64a,b) colony and inflated, phialidic hyphal cells and probably endoconidia; 65a,b) colony and endoconidia; 66a,b) dark colony and circling pigmented hyphae; 67a,b) dark colony and hyphae with lateral conidia; 68a,b) dark colony and hyphae with lateral conidia in chains; 69a,b) colony and hyphae with apical phialidic conidia; 70a,b) colony and hyphae with microconidia.



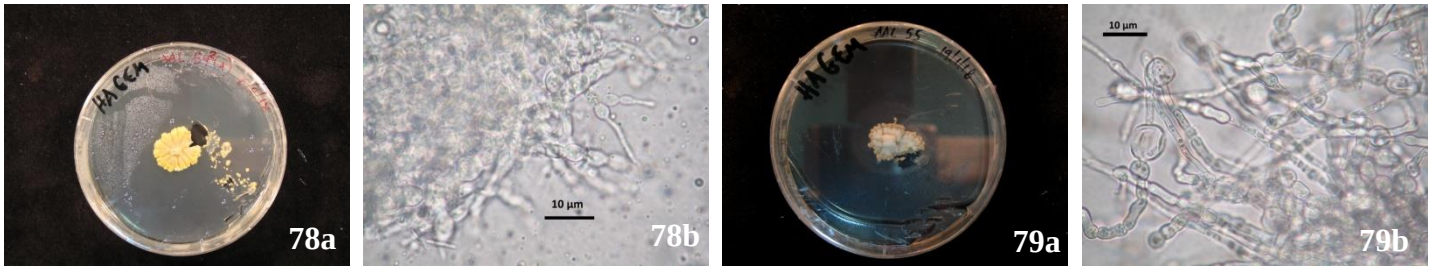
AAL 114 | AAL 114 | AAL 142A | AAL 142 A



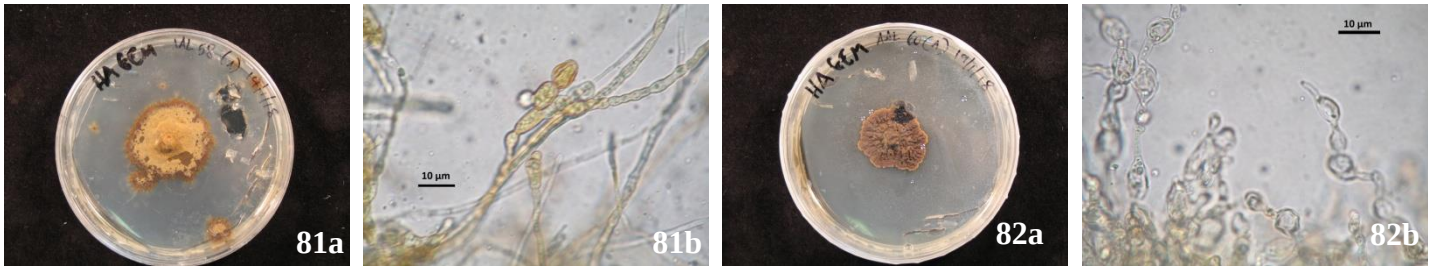
AAL 142 B | AAL 142 B | AAL 142 C | AAL 142 C



AAL 144 | AAL 144 | AAL 147 B | AAL 147 B

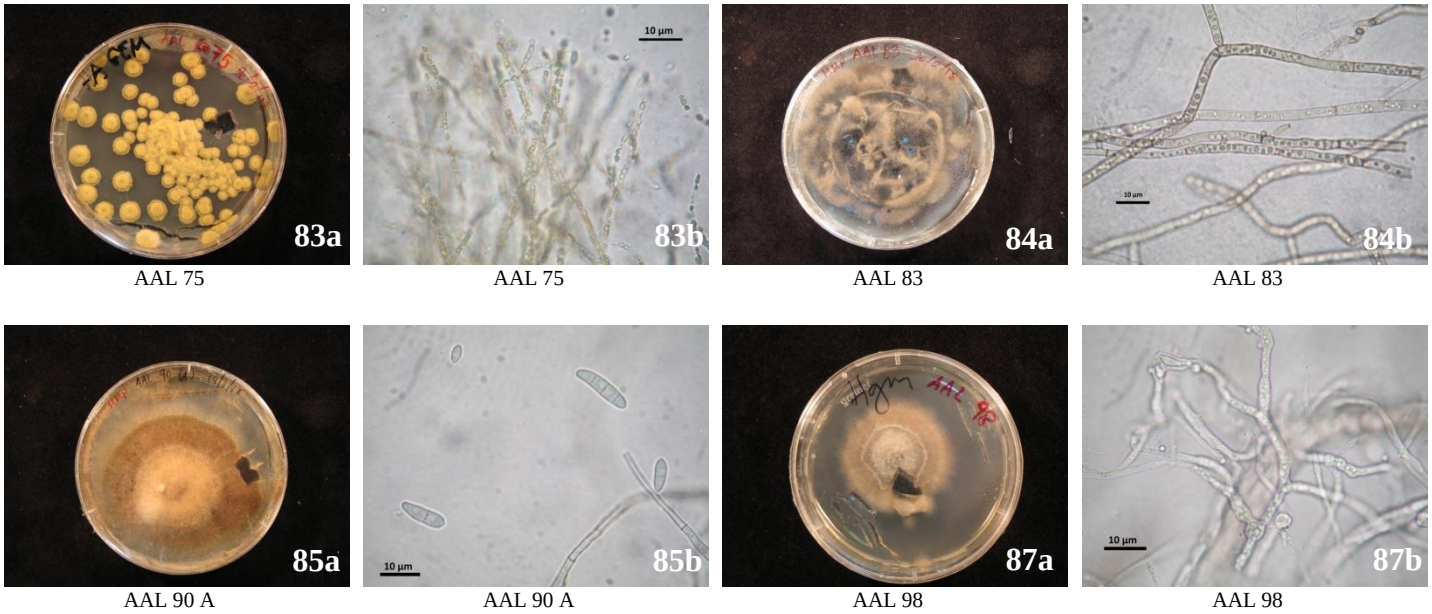


AAL 54 A | AAL 54 A | AAL 55 | AAL 55



AAL 58 A | AAL 58 A | AAL 60 A | 60 A

Fig. 4b: 71a,b) colony and hyphae; 72a,b) colony and hyphae; 73a,b) colony and wide, phialidic hyphal cells with endoconidia; 74a,b) colony and hyphae; 75a,b) dark colony and septate hyphae with conidia; 77a,b) dark colony and wide pigmented hyphae; 78a,b) colony and hyphae in yeast-like chains; 79a,b) colony and hyphae in chains with endoconidia; 81a,b) dark colony and pigmented conidiophores with conidia; 82a,b) colony and hyphae in yeast-like chains.



AAL 75 | AAL 75 | AAL 83 | AAL 83

AAL 90 A | AAL 90 A | AAL 98 | AAL 98

Fig. 4c: 83 a,b) colony and conidiophores; 84a,b) dark colony and pigmented, septate hyphae; 85a,b) dark colony and diploconidia; 87a,b) colony and hyphae.

Composite Fig. 4. Colonies and microscopic features of fungal isolates deriving from ECM root tips and mushrooms (studied in the frame of Action A1).