Egypt. J. Bot. **Vol. 62**, No. 1, pp. 149-158 (2022)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

Ex-situ Conservation of the Micro and Macro Flora of Omayed Biosphere Reserve (OBR): A Survey Report

S.I.A. El-Sakaty[(1)], M. Magdy[(2)#], S.M. Rizk[(2)], A. Hashim[(1)], M.F. Abu-Elhamd[(1)], S.Y. Elateek[(2)]

[(1)]Botany Department, Faculty of Science, Ain Shams University, Cairo,11566, Egypt; [(2)]Genetic Department, Faculty of Agriculture, Ain Shams University, Cairo, 11241, Egypt.

OMAYED Biosphere Reserve (OBR) is the only biosphere on the northwestern coast of Egypt, representing the Mediterranean ecosystem. It has characteristic fauna and flora, as it comprises 12% and 17% of Egypt's flora and bryoflora, respectively, aside from the numbers of endemic and endangered animals. In the past decade, OBR was subjected to extensive human activities—the building of coastal touristic villages, irrigation canals, plantation, and some military construction—resulting in a massive decrease in its flora from 253 to 145 taxa and an increase in the number of invasive plants. This study aimed to survey the microflora and macroflora of OBR. Results recorded three new invasive flowering plants—*Herniaria hemistemon*, *Reseda decursiva*, and *Trigonella corniculata*—and *Barbula unguiculata* Hedw. and *Fissidens arnoldenii* R. Ruthe for the first time from the Mariotic sector of Egypt. The survey for the microflora was performed using metagenomic analysis, revealing 72 identified fungal species belonging to 10 phyla, 24 classes, 42 orders, 72 families, and 72 genera, 257 identified bacterial/archaeal species belong to 2 kingdoms, 51 phyla, 61 classes, 119 orders, and 221 families, and 490 genera identified from the plant rhizosphere. Based on the Kyoto Encyclopedia of Genes and Genomes pathway annotations, the highest number of bacterial species was counted for membrane transport pathways, carbohydrates, and amino acid metabolism, reflecting the abundance of symbiotic and beneficial microbiota. The most abundant fungi found associated in the plant rhizosphere were mostly pathotrophs-symbiotrophs.

Keywords: Bryoflora, Invasive plants, Mediterranean ecosystem, Microflora, Omayed Biosphere Reserve.

Introduction

Omayed Biosphere Reserve (OBR) is the only protectorate in Egypt representing the Mediterranean ecosystem in the northwestern coastal belt of Egypt between Marsa Matrouh and Alexandria. It represents an essential hotspot for the biodiversity of the Egyptian flora in the Mediterranean coastal belt. Its flora comprises 12% of Egypt's flowering plants with 253 taxa (Kassas et al., 2002) and ~17% of the bryoflora with 33 taxa (Khalil & Farag, 2018; El-Saadawi et al., 2013). OBR was declared a managed resource-protected area in 1986 in the Man and Biosphere program of the United Nations Educational, Scientific and Cultural Organization. It is supposed to serve as a site for a balanced man-nature relationship by rationalizing tourism, rangeland management, plantation of different woody species, and encouraging local industries (Ahmed et al., 2015). In the past few years, OBR, as a part of the western Egyptian coast, suffered from the pressure of human activities (construction of irrigation canals, paved roads, touristic resorts on the coastal ridge, and extensive agriculture activity accompanied by plantation of a large number of windbreak trees) aside from environmental changes that led to a severe stage of destruction for natural habitats. As a result, there was a catastrophic decrease in

#Corresponding author email: m.elmosallamy@agr.asu.edu.eg
Received 19/10/ 2020; Accepted 18 /08/ 2021
DOI: 10.21608/ejbo.2021.46939.1573
Edited by: Prof. Dr. Monier M. Abd El-Ghani, Faculty of Science, Cairo University, Giza 12613, Egypt.

the flora of flowering plants to 145 taxa and an increased number of invasive plants, where 22 alien plants have been reported (Ahmed et al., 2015). Nowadays, the direct approach to survey and record plants is the newly emerged approach known as DNA barcoding, which was previously applied to wild and endangered plants from the Egyptian environment (e.g., El-Atroush et al., 2015; Werner et al., 2016; Magdy et al., 2019; Fouad et al., 2019; Shamso & Fouad, 2019; El-Sherif & Ibrahim, 2020).

The destruction of natural habitats also affect the microorganism's diversity naturally associated with different plant parts, such as the phyllosphere, rhizosphere, and endosphere (Turner et al., 2013; Haney & Ausubel, 2015; Rodriguez et al., 2019). They play a role in disease control, improve supplement acquisition, and influence plants' stress tolerance (Andreote et al., 2014). Microbes in the plant rhizosphere (the soil region around the root where its impact appears) have been found to produce regulatory compounds. Metagenomics is the most advanced tool to study, profile, and build this picture (Singh et al., 2019). Metagenomics is considered responsible for the advances in microbial ecology, evolution, and diversity over the past decade. It gives a broad description of microbial communities by providing data on their functional genes (Wilmes & Bond, 2006; Gilbert et al., 2008) and provides genetic information on potentially novel biocatalysts or enzymes, genomic linkages between function and phylogeny for uncultured organisms, and evolutionary profiles of community function and structure (Beja et al., 2000; Nicol & Schleper, 2006).

This study aimed to survey the flowering flora and bryoflora of OBR, aside from recognizing their rhizosphere microbiome, via metagenomic analysis to enrich the floral and nucleotide databases (*in silico* conservation) as a part of the *ex situ* conservation of those accessions for the present and future tracking of critical records from the OBR threatened area.

Materials and Methods

Sampling area

OBR is located in the western Mediterranean coastal region of Egypt, at 80 km west of Alexandria (29°00′–29°18′E and 30°52′–20°38′N). It extends ~30 km along the Mediterranean coast from west El-Hammam to El-Alamein, with a width of 23.5km to the south (Fig. 1). OBR is characterized by a subdesert climate where rainfall is concentrated in autumn and winter. Water resources vary between rainfall and wells (Ayyad, 1977–1979). It has three central physiographic systems parallel to each other: Coastal system at the sea level, ridges, and depressions—the central part of the territory, and the inland plateau (Khashm El-Aish; Ayyad & Le Floc'h, 1983). Twenty moss samples were collected from the inland plateau of Khashm El-Aish, and 31 flowering plants were collected from nine localities (Fig. 2). Forty soil samples were collected from the rhizosphere of gathered plant materials (31 flowering and 9 moss samples).

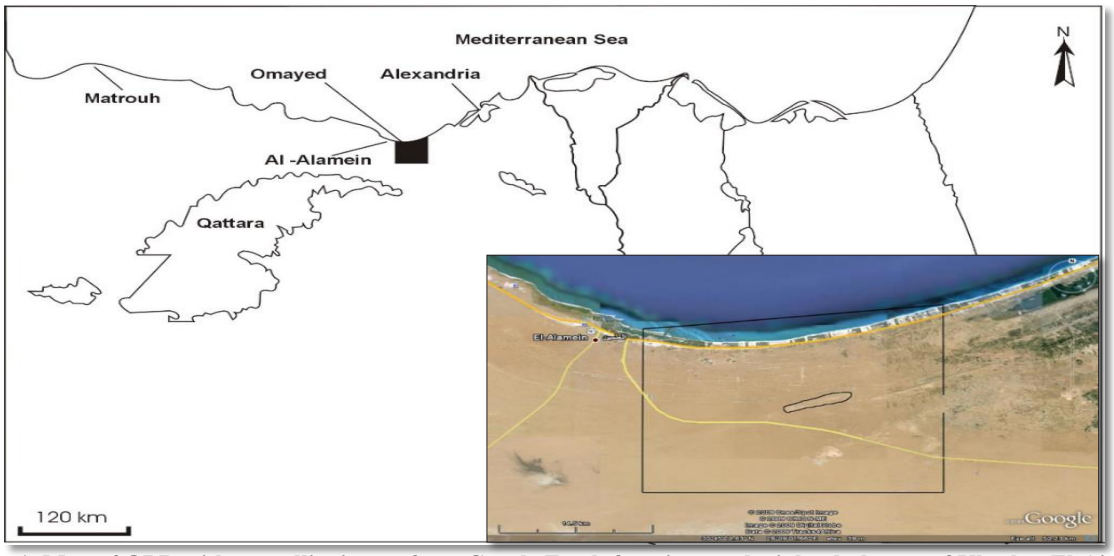

Fig. 1. Map of OBR with a satellite image from Google Earth focusing on the inland plateau of Khashm El-Aish (site of moss collection)

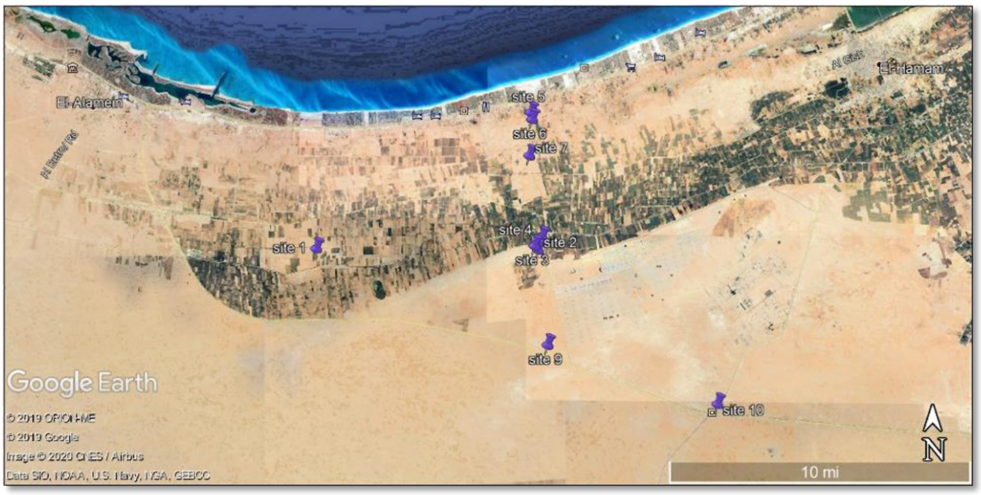

Fig. 2. A satellite image from Google Earth showing the site of flowering plant collection

Methods

All moss samples and flowering plants were examined morphologically and anatomically for identification and conservation inside the CAIA herbarium. Only samples of doubtful identification (two moss samples and three flowering plants) were chosen for molecular identification via the DNA barcoding technique using the *rbc*L gene.

DNA barcoding

DNA was extracted from the plant material by the cetyltrimethylammonium bromide protocol of Doyle & Doyle (1987) modified as in Shaw (2000). Extracted DNA concentration was determined by measuring the optical density (OD) at 260nm. Polymerase chain reaction (PCR) was performed to obtain the *rbc*L region using Thermo master mix and the primers *rbc*La-F and *rbc*La-R3 (Kress & Erikson, 2007). The PCR product (5µL) was examined by 1.5× agarose gel, purified by PCR purification kit, and sent for sequencing.

Metagenomic analysis

DNA was obtained from 40 soil samples following the MoBio Powersoil DNA extraction kit protocol (MoBio Laboratories, Inc. Carlsbad, CA, USA). DNA quality and quantity were measured using a NanoDrop device (Thermo Scientific, Wilmington, DE, USA) or other similar equipment and electrophoresis (0.8% agarose gel, including a 1kb plus ladder). 16S and ITS2 amplicon and metagenomic library preparation and sequencing were performed according to the manufacturer's protocol at BGI-Shenzhen (China). For amplicon library preparation, 16S and ITS2 DNA fragments were amplified using the standard amplified primers and the prokaryotic 16S rDNA V4 region (515F and 806R; Caporaso et al., 2011) and fungal ITS2 (Tedersoo et al., 2015). Metagenomic DNA fragments were amplified using Illumina sequencing adapter-specific primers.

Data analysis

The obtained rbc*L* sequences were aligned using BioEdit version 3 (Hall, 1999) to confirm the correct sequences and blasted on GenBank, and all closely related species were downloaded for further analysis. MEGA X (Kumar et al., 2018) was used to align sequences. Trees constructed at the sample level were inferred using maximum likelihood using MEGA X with an evolution model test estimated by jModelTest version 0.11 (Posada, 2008).

Metagenomic DNA with high-quality paired-end reads of the 16S V4 region and ITS2 were merged using FLASH software with the default settings (Magoč & Salzberg, 2011). Operational taxonomic units (OTUs) were obtained using the UPARSE pipeline (Edgar, 2013) based on the merged sequences. To obtain the taxonomic information of the OTU, representative sequences of each OTU were generated and aligned against the SILVA and UNITE (Abarenkov et al., 2010; Quast et al., 2012) databases using the RDP classifier (Wang et al., 2007) for 16S and ITS2, respectively. The OTUs and merged sequences defined as unknown, chloroplasts, mitochondria, or plants were removed.

Raw reads from metagenomic sequencing were used to generate clean reads by removing adaptor sequences, trimming, and removing low-quality reads at BGI-Shenzhen. The clean reads were prepared to generate taxonomic information for the soil rhizosphere. Sequences were aligned against the National Center for Biotechnology Information microbial nr database, including bacteria, archaea, fungi, viruses, protozoa, algae, and plants, using DIAMOND (Buchfink et al., 2014) software with an E value cutoff of 1e-5. Based on the MEGAN LCA algorithm (Huson et al., 2007), the unigenes' taxonomic annotation was assigned.

The functional potential of the identified fungal species was determined by FunGuild analysis version 1.1 (Nguyen et al., 2016), whereas that of bacterial species was performed by PICRUSt analysis (Zaneveld et al., 2013) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations.

Results and Discussion

Mosses survey

Twenty-three moss taxa were identified: 17 taxa belong to family Pottiaceae [*Aloina ambigua* (Bruch. et Schimp.) Limpr., *Aloina brevirostris* (Hook. et Grev.) Kindb., *Aloina rigida* (Hedw.) Limpr., *Barbula unguiculata* Hedw., *Crossidium laevipilum* Thér. Et Trab., *Crossidium laxefilamentosum* W. Frey et Kürschner, *Didymodon fallax* (Hedw.) R. H. Zander, *Didymodon luridus* Hornsch., *Didymodon rigidulus* Hedw., *Didymodon tophaceus* (Brid.) Lisa, *Didymodon vinealis* (Brid.) R. H. Zander, *Gymnostomum viridulum* Brid., *Tortella flavovirens* (Bruch.) Broth., *Tortella nitida* (Lindb.) Broth., *Tortula brevissima* Schiffn., *Tortula muralis* Hedw., and *Trichostomum crispulum* Bruch.], 4 taxa belong to family Bryaceae [*Bryum argenteum* Hedw., *Bryum dichotomum* Hedw., *Bryum gemmiparum* De Not., and *Bryum radiculosum* Brid.], and 2 taxa belong to family Fissidentaceae [*Fissidens arnoldenii* R. Ruthe and *Fissidens bryoides* Hedw.]. The two moss samples tested by DNA barcoding were shown to be *B. unguiculata* (Fig. 3).

This survey revealed a noticeable decrease in moss number by 12 species (Khalil & Farag, 2018) and recorded *B. unguiculata* for the first time from the Mariotic sector of Egypt, which was recorded only from arable fields (El-Saadawi et al., 2015).

This decrease could be attributed directly or indirectly to the increase in human activities at the protectorate (Hernández-Hernández et al., 2019). Despite the importance of bryophytes as a fundamental ecosystem element due to their role in carbon fixation and regulation of water humidity and water retention capacity (Bortoluzzi et al., 2006), investigations considering its diversity versus land use are rare (Müller et al., 2012). All recent studies (Müller et al., 2012, Hernández-Hernández et al., 2019) agreed with the survey results those extensive human activities are accompanied by a major decrease in species diversity related to degradation in habitat heterogeneity, water and nutrient balance, soil physiochemical characters, and flowering plant composition (Ren et al., 2021).

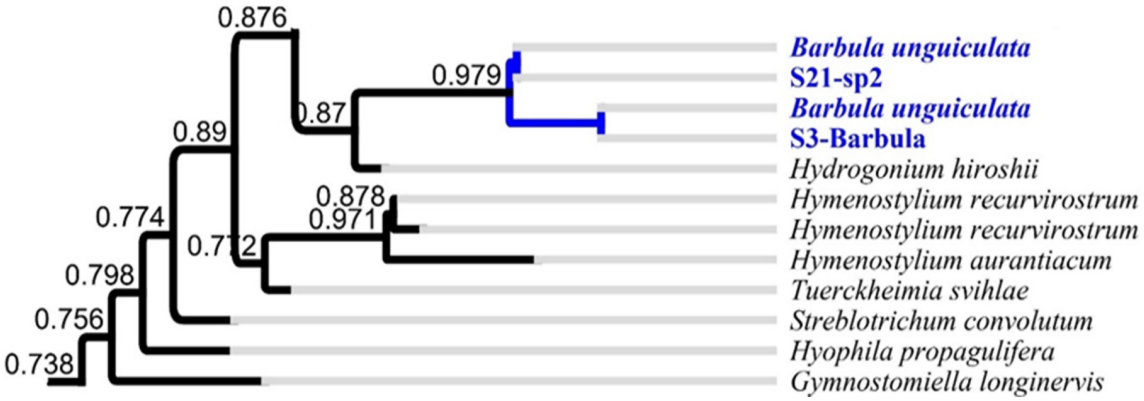

Fig. 3. Phylogenetic tree of the *rbcL* gene of *B. unguiculata*

Survey of flowering plants

Twenty-nine samples were identified morphologically from the 32 collected samples: *Allium roseum* L. var. *tourneuxii* Boiss., *Ammophila arenaria* (L.) Link, *Anacyclus monanthos* Thell. subsp. *monanthos*, *Anthemis microsperma* Boiss. & Kotschy, *Artemisia monosperma* Delile, *Asphodelus aestivus* Brot., *Astragalus spinosus* Muschl., *Atractylis carduus* (Forssk.) C. Chr., *Atriplex halimus* L., *Bassia indica* (Wight) A. J. Scott, *Bassia muricata* (L.) Asch., *Brassica tournefortii* Gouan, *Centaurea calcitrapa* L., *Chenopodium murale* L., *Convolvulus lanatus* Vahl, *Deverra tortuosa* (Desf.) DC., *Echiochilon fruticosum* Desf., *Gymnocarpos decandrus* Forssk., *Haloxylon salicornicum* (Moq.) Bunge ex Boiss, *Helianthemum kahiricum* Delile, *Limonium pruinosum* (L.) Chaz. var. *pruinosum*, *Lygeum spartum* Loefl. Ex L., *Malva parviflora* L., *Noaea mucronata* (Forssk.) Asch. & Schweinf., *Rumex pictus* Forssk., *Salsola kali* L., *Salvia lanigera* Poir., *Thymelaea hirsuta* (L.) Endl., and *Zygophyllum album* L. Three samples were identified at the generic level as *Herniaria*, *Reseda*, and *Trigonella.* The species were identified using the *rbc*L-based phylogenetic tree as *Herniaria hemistemon*, *Reseda decursiva*, and *Trigonella corniculata.* They were recorded for the first time from OBR (Fig. 4). This survey showed an increase in the number of invasive plants recorded in OBR (Ahmed et al., 2015) from 22 to 25 species. Extensive human activities might cause the introduction of these plants (e.g., summer resorts, overgrazing, woodcutting, construction of irrigation canal, and agriculture; Shaltout & Al-Sodany, 2002).

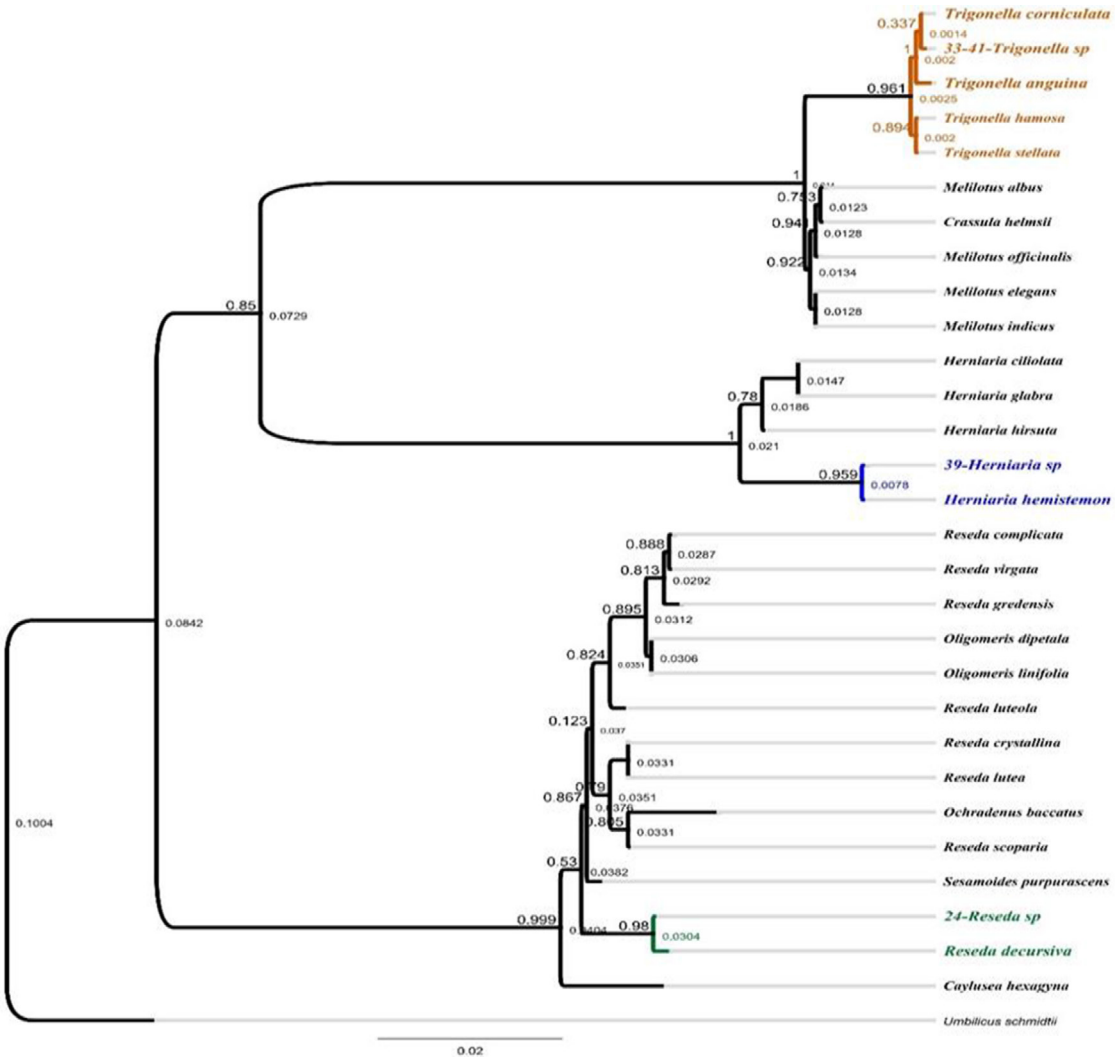

Fig. 4. Phylogenetic tree of the *rbcL* gene partial sequences for three flowering plants collected from OBR [Colored clades are aside from other related taxa from GenBank. Bootstrap values are shown at each node]

Metagenomic analysis

The metagenomic analysis gave detailed information about microbial diversity, composition, and microbiota associated with the sampling sites' plant rhizosphere. Based on the metagenomic analysis, 325 ITS-based OUT (72 identified fungal species belonging to 10 phyla, 24 classes, 42 orders, 72 families, and 72 genera) were identified from the plant rhizosphere. The remaining unidentified ITS OTUs belong to nonfungal species (e.g., Plants and Metazoa), and 2563 16S-based OTU (257 identified bacterial/archaeal species belong to 2 kingdoms, 51 phyla, 61 classes, 119 orders, 221 families, and 490 genera) were identified from the plant rhizosphere. Their evolutionary relationship and abundance are shown (Figs. 5 and 6).

At the fungal level, species belonging to *Issatchenkia* yeast, *Candida* yeast, *Alternaria*, *Aureobasidium*, and *Cladosporium* (Ascomycota) and *Vishniacozyma* (Basidiomycota) were abundant among many plant rhizospheres (Fig. 5). At the bacterial level, species belonging to Cyanobacteria, *Flavobacterium* (Bacteroidetes), and *Caenimonas* (Proteobacteria) were abundant among many plant rhizospheres, whereas species belonging to *Micrococcus* (Actinobacteria), *Bacillus* (Firmicutes), and *Brevundimonas* (Proteobacteria) were abundant but limited to one or few samples (Fig. 6).

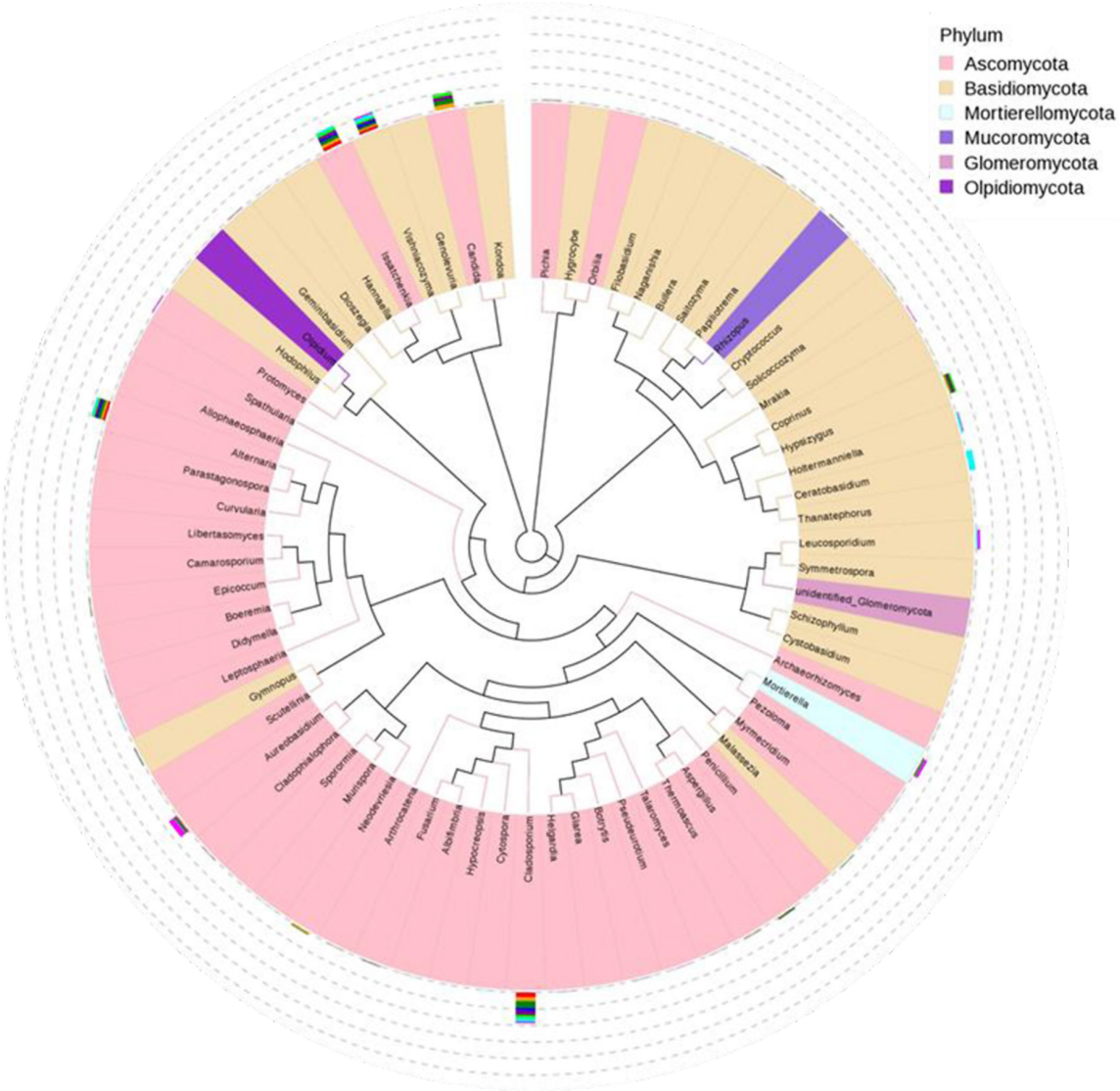

Fig. 5. Evolutionary tree and abundance of the identified fungal species [Clades are colored by their phylum ID]

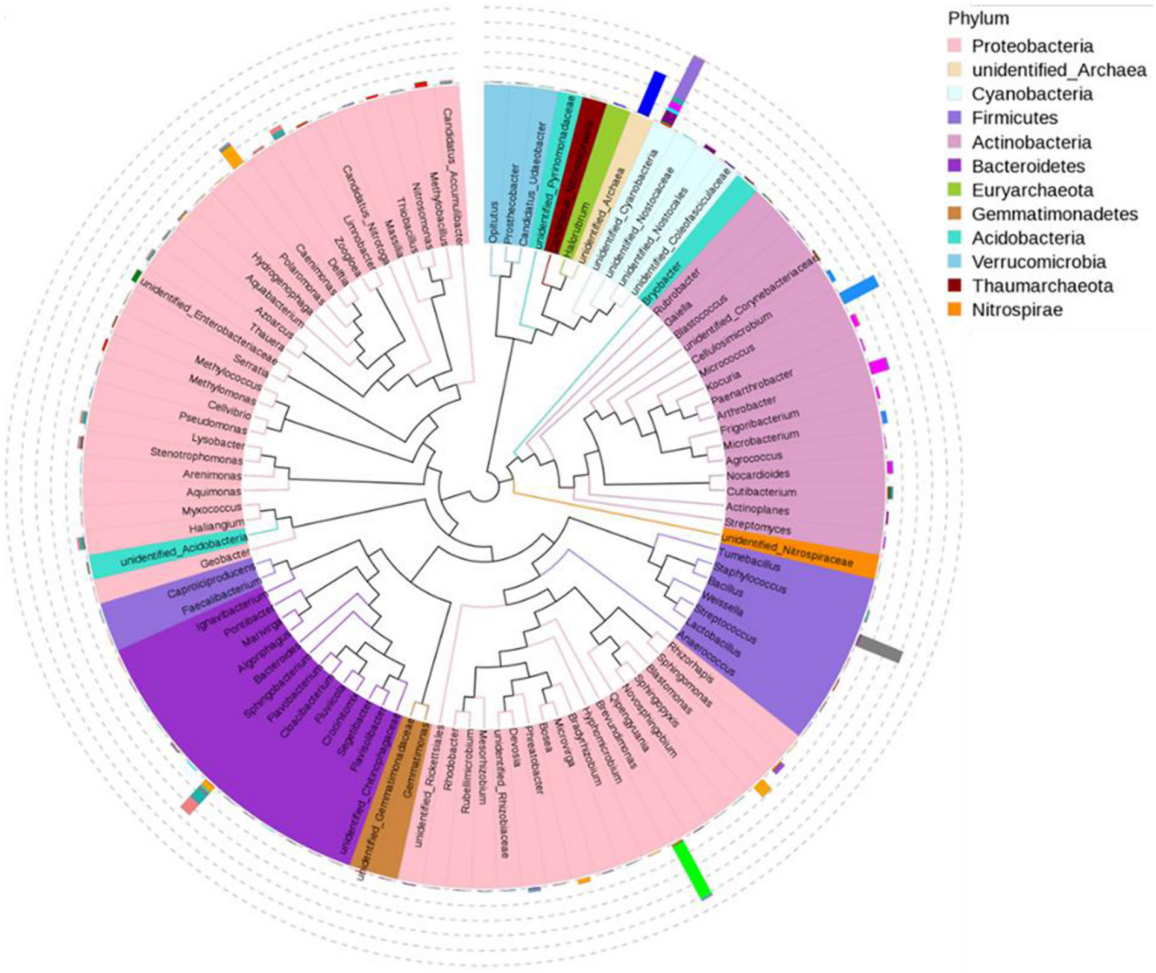

Fig. 6. Evolutionary tree and abundance of the identified bacterial species [Clades are colored by their phylum ID]

By analyzing the identified species' functional potential, FunGuild analysis for fungal species and PICRUSt analysis for bacterial species were performed. For fungal species, the most abundant fungi were related to wood and soil saprotrophs, plant endophyte pathogens, and a mixed feature of all previous species in addition to the identification of some animal pathogens and arbuscular mycorrhiza (Fig. 7a). Mostly, pathotrophs-symbiotrophs are the most abundant fungi found associated with the plant rhizosphere (Fig. 7b). For bacterial species, and based on the KEGG pathway annotations, the highest number of species was counted for pathways of membrane transport (Environmental information process pathway group), carbohydrates, and amino acid metabolism (Metabolism pathway group), followed by replication and repair (Genetic information process) and cell motility (Cellular process pathway group), all reflecting the abundance of symbiotic and beneficial microbiota in the plant rhizosphere (Fig. 8).

Acknowledgments: This research was funded by Ain Shams University under its strategic plan for supporting young researchers. The authors are grateful for the support provided by the Faculty of Science, Ain Shams University.

Conflicts of interest: No conflicts of interest have been declared.

Author contribution: All authors contributed equally. All authors read and approved the final manuscript.

Ethical approval: Not applicable

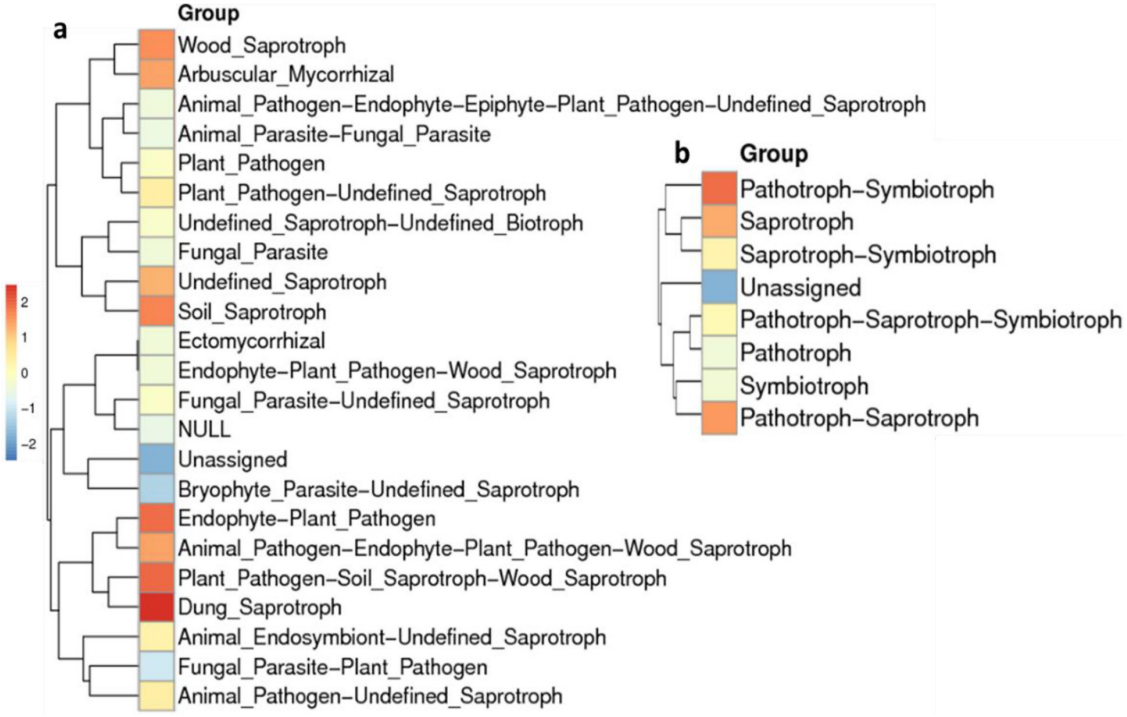

Fig. 7. FunGuild-based analysis of the identified fungal species counts per each functional group based on (a) Host type and (b) Trophy group

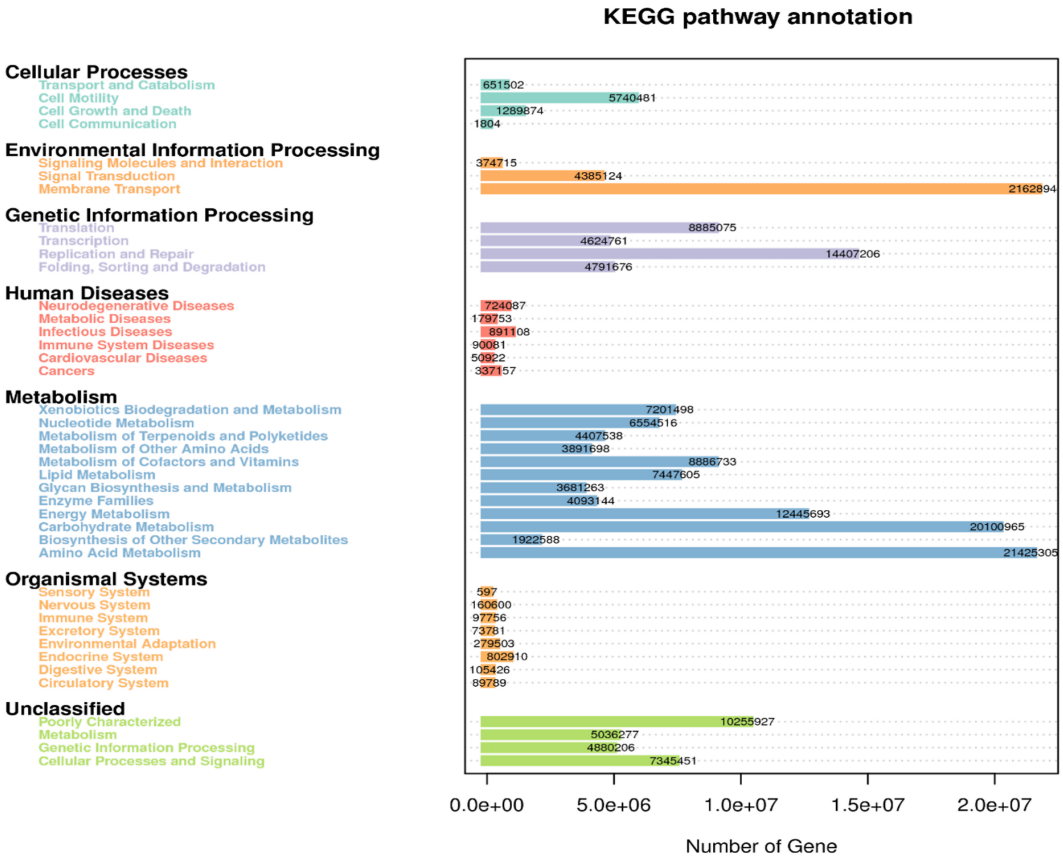

Fig. 8. KEGG pathway annotation based on the identified bacterial species using PICRUSt analysis

References

Abarenkov, K., Nilsson, R.H., Larsson, K., Alexander, I.J., Eberhardt, U., Erland, S., et al. (2010) The UNITE database for molecular identification of fungi—recent updates and future perspectives. *New Phytologist,* **186**, 281–285.

Ahmed, D.A., Fawzy, M., Saeed, N.M. Awad, M.A. (2015) Effect of the recent land use on the plant diversity and community structure of Omayed Biosphere Reserve, Egypt. *Global EcolConserv,* **4**, 26–37.

Andreote, F.D., Gumiere, T., Durrer, A. (2014) Exploring interactions of plant microbiomes. *Scientia Agricola*, **71**, 528-539.

Ayyad, M.A. (1977-1979) Systems Analysis of Mediterranean Desert Ecosystem of Northern Egypt (SaMDENE). progress reports nos. 1-5.

Ayyad, M.A., Le Floc'h, E. (1983) An ecological assessment of renewable resources for rural agricultural development in the western Mediterranean coastal region of Egypt. (Case study: El Omayed Test-area). Alexandria, Academy of Scientific Research and Technology, REMDENE Project. Alexandria University, Egypt, p. 104.

Beja, O., Aravind, L., Koonin, E.V., Suzuki, M.T., Hadd, A., Nguyen, L.P., Jovanovich, S.B., Gates, C.M., Feldman, R.A., Spudich, J.L., Spudich, E.N., DeLong, E.F. (2000) Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science*, **289**, 1902-1906.

Bortoluzzi, E., Epron, D., Siegenthaler, A., Gilbert, D., Buttler, A. (2006) Carbon balance of a European mountain bog at contrasting stages of regeneration. *New Phytologist*, **172**, 708–718.

Buchfink, B., Xie, C., Huson, D.H. (2014) Fast and sensitive protein alignment using DIAMOND. *Nature Methods,* **12**, 59–60.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, USA, **108**(Suppl 1), 4516–4522.

Doyle, J.J. Doyle, J.L. (1987) A rapid DNA isolation for small quantities of fresh tissue. *Phytochemical Bulletin,* **19**, 11–15.

Edgar, R.C. (2013) UPARSE: Highly Iurate OTU sequences from microbial amplicon reads. *Nature Methods,* **10**, 996–998.

El-Atroush, H., Magdy, M., Werner, O. (2015) DNA Barcoding of two endangered medicinal Plants from Abou Galoom protectorate. *Life Science Journal,* **12**, 101–109.

El-Saadawi, W.E., Shabbara, H.M., El Sakaty, S. (2013) Mosses of the Egyptian conservation areas: II. Omayed Protected Area. *Cryptogamie. Bryologie*, **34**, 61–71.

El-Saadawi, W.E., Shabbara, H.M., Khalil, M.I., Taha, M.A. (2015) An annotated checklist of Egyptian mosses. *Taeckholmia,* **35**, 1-23.

El-Sherif, N., Ibrahim, M. (2020) Implications of *rbc*L and *rpo*C1 DNA Barcoding in Phylogenetic Relationships of some Egyptian *Medicago sativa* L. Cultivars. *Egyptian Journal of Botany,* **60**(2), 451-460.

Fouad, A., Hafez, R., Hosni, H. (2019) Authentication of three endemic species of the Caryophyllaceae from Sinai Peninsula using DNA barcoding. *Egyptian Journal of Botany*, **59**(2), 483-491.

Gilbert, J.A., Field, D., Huang, Y., Edwards, R., Li, W., Gilna, P., Joint, I. (2008) Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities. *PLoS One*, **3**, e3042.

Hall, T.A. (1999) BioEdit. A user-friendly biological sequence alignment editor and analysis program for windows 95/98/N.T. *Nucleic Acids Symposium,* **41**S, 95–98.

Haney, C.H., Ausubel, F.M. (2015) Plant microbiome blueprints. *Science,* **349**, 788–789.

Hernández-Hernández, R., Kluge, J., Ah-Peng, C., Gonza´lez-Mancebo, J.M. (2019) Natural and human-impacted diversity of bryophytes along an elevational gradient on an oceanic island (La Palma, canarias). *PLoS ONE*, **14**, e0213823.

Huson, D.H., Auch, A.F., Qi, J., Schuster, S.C. (2007) MEGAN analysis of metagenomic data. *Genome*

Research, **17**, 377–386.

Kassas, M.A., Abdallah, A., Abul-Dahab, T., Atta, G.M., Esawi, M., Farouk, H. (2002) Management plan for Omayed protected area. Township Med Wet Coast, Global Environment Facility & Egyptian Environment Affairs Agency, 130p.

Khalil, M.I., Farag, M. (2018) Additional pottiaceae records from Omayed protected area, Egypt. *Egyptian Journal of Experimental Biology (Botany),* **14**, 339–345.

Kress W.J., Erickson D.L. (2007) A two -locus global DNA for land plants. The coding *rbcL*gene complements the non-coding *trnh-psbA* spacer region. *PLos ONE,* **2**, e508.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018) MEGA X: Molecular evolutionary Genetics analysis across computing platforms. *Molecular Biology and Evolution,* **35**, 1547–1549.

Magdy, M., Ou, L., Yu, H., Chen, R., Zhou, Y., Isan, H., Li, F. (2019) Pan-plastome approach empowers the assessment of genetic variation in cultivated Capsicum species. *Horticulture Research*, **6***,* 1–15.

Magoč, T., Salzberg, S.L. (2011) FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics,* **27**, 2957–2963.

Müller, J., Klaus, V.H., Kleinebecker, T., Prati, D., Hölzel, N., Fischer, M. (2012) Impact of land-use intensity and productivity on bryophyte diversity in agricultural grasslands. *PLoS ONE,* **7**, e51520.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., et al. (2016) Funguild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology,* **20**, 241–248.

Nicol, G.W., Schleper, C. (2006) Ammonia-oxidisingCrenarchaeota: important players in the nitrogen cycle? *Trends in Microbiology,* **14**, 207-212.

Posada, D. (2008) JModeltest. Phylogenetic model averaging. *Molecular Biology and Evolution,* **25**, 1253–1256.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2012) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research,* **41**, D590–D596.

Ren, H., Wang, F., Ye, W., Zhang, Q., Han, T., Huang, Y., et al. (2021) Bryophyte diversity is related to vascular plant diversity and microhabitat under disturbance in karst caves. *Ecological Indicators,* **120**, 106947.

Rodriguez, P.A., Rothballer, M., Chowdhury, S.P., Nussbaumer, T., Gutjahr, C., Falter- Braun, P. (2019) Systems biology of plant microbiome interactions. *Molecular Plant,* **12**, 804–821.

Shaltout, K.H., Al-Sodany, Y.M. (2002) Phytoecology of Omayed Site. Mediterranean West Coast Project. Egyptian Environmental Affairs Agency, Cairo, 89p.

Shamso, E., Fouad, A. (2019) *Dicliptera aegyptiaca* (Acanthaceae), A New Species from Egypt Supported by Morphological Characters and *rbc*l-based DNA Barcoding. *Egyptian Journal of Botany*, **59**(2), 475-482.

Shaw, A.J. (2000) Phylogeny of the Sphagnopsida based on nuclear and chloroplast DNA sequences. *Bryologist,* **103**, 277–306.

Singh, D., Rainaa, T.K., Kumara, A., Singha, J., Prasadb, R. (2019) Plant microbiome: A reservoir of novel genes and metabolites. *Plant Gene,* **18**, 100–177.

Tedersoo, L., Anslan, S., Bahram, M., Põlme, S., Riit, T., Liiv, I., et al. (2015) Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MycoKeys*, **10**, 1–43.

Turner, T.R., James, E.K., Poole, P.S. (2013) The plant microbiome. *Genome Biology,* **14**, 209–215.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology,* **73**, 5261–5267.

Werner, O., Magdy, M., Ros, R.M. (2016) Molecular systematics of *Abelmoschus* (Malvaceae) and genetic diversity within the cultivated species of this genus based on nuclear ITS and chloroplast

rpL16 sequence data. *Genetic Resources and Crop Evolution,* **63**, 429–445.

Wilmes, P., Bond, P.L. (2006) Metaproteomics: studying functional gene expression in microbial ecosystems. *Trends in Microbiology,* **14**, 92–97.

Zaneveld, J., Langille, M.G.I., Caporaso, J.G., McDonald, D., Knights, D., Reyes J.A., et al. (2013) Predictive functional profiling of microbial communities using 16s rRNA marker gene sequences. *Nature Biotechnology,* **8**, 1–8.

الحفظ خارج-الموقع للفلورا الدقيقة والكليّة: تقرير مسح للمحيط الحيوي لمحمية العميد

سحر ابراهيم علي السقطي[1]، محمود مجدي[2]، سماح محمد رزق[2]، أحمد هاشم[1]، محمد فرج أبو الحمد[1]، سوسن يونس العتيق[2]

[1]قسم النبات - كلية العلوم - جامعة عين شمس - العباسية - القاهرة 11566 - مصر، [2]قسم الوراثة - كلية الزراعة - جامعة عين شمس - القاهرة -11241 - مصر.

محمية المحيط الحيوي العميد (OBR) هي المحيط الحيوي الوحيد على الساحل الشمالي الغربي لمصر، والتي تمثل نوع النظام البيئي للبحر الأبيض المتوسط. تتميز بخصائصها الحيوانية والنباتية حيث تشكل 12% و 17% من فلورة النباتات وفلورة الحزازيات في مصر على التوالي، إلى جانب عدد من الحيوانات المتوطنة والمعرضة للانقراض. في العقد الماضي، تعرضت المحمية لعديد من الأنشطة البشرية واسعة النطاق - كبناء القرى السياحية الساحلية، وقنوات الري، والمزارع الواسعة، وبعض الإنشاءات العسكرية – والتى أدت إلى انخفاض هائل في نباتاتها من 253 إلى 145 نوعًا وزيادة في عدد النباتات الغازية. هدفت الدراسة الحالية إلى عمل مسح للنباتات الدقيقة والكلية في المحمية. وأشارت النتائج إلى تسجيل ثلاث نباتات مزهرة غازية جديدة - *Herniaria hemistemon* و *Reseda decursiva* و *Trigonella corniculata* – وسجلت نوعين من الحزازيات القائمة *Barbula unguiculata* Hedw. و *Fissidens arnoldenii* R. Ruthe لأول مرة من القطاع المريوطي في مصر. على التوازي تم إجراء مسح للفلورة الدقيقة بالتربة حول جذور النباتات بالمحمية عن طريق التحليل الميتاجينومي، والذي أظهر 72 نوعًا فطريًا ينتموا إلى 72 عائلة و 72 جنسًا، كما أظهر257 نوعًا من البكتيريا البدائية تنتمي إلى 221 عائلة و 490 جنساً. استنادًا إلى موسوعة كيوتو للجينات والجينوم (KEGG)، تم حساب أكبر عدد من الأنواع البكتيرية لمسارات نقل الأغشية والكربوهيدرات وأيض الأحماض الأمينية، والتي تعكس وفرة الكائنات الحية الدقيقة التكافلية والمفيدة. وكانت الفطريات الأكثر وفرة المرتبطة بجذور النباتات هي في الغالب من النوع التكافلي.