https://doi.org/10.54500/2790-1203-2025-1-125-59-66

Original article

Isolation of new strains of Agaricales and medicinal fungi distributed in Burabay and Kokshetau national parks

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Abstract

In Kazakhstan, agaric mushrooms remain an understudied group despite their ecological and nutritional significance. This study is the first to investigate the species composition of edible agaric mushrooms in the Burabay and Kokshetau National Parks, their morphometric characteristics, and the creation of a strain collection.

The purpose of this research: to determine the species diversity of edible agaric mushrooms, analyze their morphometric characteristics, and establish a strain collection by isolating new samples for further biological studies and potential applications.

Methods. Macromycetes were collected using a route method, sorted, dried (45–50°C, 30–40 min), and stored. Morphological characteristics were examined visually, and microscopic structures were analyzed using a Mikmed-1 microscope and cameras. Young specimens were cultivated on nutrient media (Czapek-Dox, Murashige-Skoog, potato-glucose agar) for strain isolation and further study.

Results. Over 80 mushroom samples were collected, identifying 71 species from two classes (Basidiomycetes and Ascomycetes), four orders (Aphyllophorales, Agaricales, Lycoperdales, Pezizales), 11 families, and 26 genera. The isolated strains hold potential for further ecological research and artificial cultivation.

Conclusion. The current sample size is limited. Further collection and analysis using additional methods are planned to refine knowledge of agaric mushroom diversity in Kazakhstan.

Keywords: mushroom, macromycetes, strains, cap, stipe, spore, agaric mushrooms.

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2025; 1 (125): 59-66
Recieved: 04-01-2025
Accepted: 27-01-2025

Introduction

In Kazakhstan, agaric fungi remain an understudied group of macrofungi despite their significant ecological role and nutritional value. Systematic studies of their species diversity in the country's national parks have been conducted only rarely. Edible agaric fungi play an important role in forest ecosystems by participating in the decomposition of organic matter and forming symbiotic relationships that contribute to nutrient cycling and soil structure improvement. Furthermore, many species have economic significance due to their nutritional and potential medicinal properties [1].

Until now, comprehensive studies of edible agaric fungi in Kazakhstan's national parks have not been conducted. This study is the first to focus on identifying the species composition of edible agaric fungi growing in the Burabay and Kokshetau National Parks, their morphometric characteristics, and the creation of a strain collection for further research and possible practical applications. The aim of this study is to determine the species diversity of edible agaric fungi, provide their morphometric characterization, and isolate new strains for the creation of a collection. This will allow for the study of their biological characteristics and potential applications in biotechnology, agriculture, and medicine [2].

Mushrooms are a highly valuable food product. They are rich in proteins, various vitamins essential for the human body, and trace elements such as iron, calcium, zinc, iodine, potassium, and phosphorus. Due to their nutritional properties, mushrooms are considered a gerontological product. They do not contain harmful substances such as cholesterol, nitrates, or nitrites. Currently, mushrooms are consumed fried, boiled, salted, pickled, as well as in the form of powder, paste, and as a seasoning for various dishes. The protein content in mushrooms, in terms of dry matter, ranges from 21% to 30%, which is significantly higher than that of meat or wheat. One hectare of land can yield 110 tons of mushrooms containing approximately 330 kg of protein. For comparison, one hectare of land can produce 30 tons of potatoes with only 3 kg of protein and an average of 3 tons of grain crops containing only 5 kg of protein [3].

The Burabay State National Nature Park, under the jurisdiction of the Administration of the President of the Republic of Kazakhstan, was established on August 12, 2000. The total area of the park is 129,299 hectares, of which 47.4 thousand hectares are covered by forests. Water bodies occupy 8,493.5 hectares. The flora of the park includes 754 plant species, while the fauna comprises 469 animal species. The national park encompasses the Burabay mountain-forest massif, located in the eastern part of the Kokshetau Upland in the northwestern part of the Kazakh Uplands (Saryarka). The park includes eutrophic lakes such as Burabay, Zerendi, Ulken and Kishi Shabakty, and Shchuchye. The lakes in this region mainly contain freshwater. Additionally, the park features small rivers and streams. The Burabay forest massif has preserved coniferous and mixed deciduous forests dating back to the Pleistocene epoch [1-3].

Currently, the national park is a state organization and is part of the system of specially protected natural areas of republican significance. The Burabay State National Nature Park consists of seven forest management units: Akylbai, Burabay, Katar-Kol, Zolotobor, Barmash, Mirnoye, and Priozyornoye. The park also includes facilities that support operational activities. The entire park is divided into 15 economic units and 56 protected zones. The climate of the region where the national park is located is continental, with cold, long winters and short, hot summers. The average temperature in January is -16.7°C, while in July, it is +18.6°C [4].

The national park has preserved relict plant and animal species characteristic of northern Kazakhstan. The park contains 757 plant species, 95 of which are rare or endangered. Among them are the large-flowered lady's slipper orchid, black alder, reindeer lichens, Fuchs' sedge, and others. The dominant tree species in the forested areas is pine, while aspen, willow, honeysuckle, spirea, wild rose, hawthorn, black currant, and other species are also present. Relict species include ferns, horsetails, clubmosses, sedges, cranberries, and other plants. The flora of the park also includes medicinal, edible, and ornamental plant species. Some small lakes have preserved relict peat bogs [5].

The fauna of the national park is also diverse, with 305 species of vertebrates, 87 of which are rare. The park is home to noble deer and their hybrids from the Askania-Nova reserve. Lynxes, ermines, white-backed woodpeckers, and forest martens inhabit the forests. The park's water bodies host six species of fish, three species of amphibians, six species of reptiles, and more than 200 bird species, including those listed in Kazakhstan's Red Book, such as the white-tailed eagle, black kite, and Eurasian eagle-owl. The invertebrate fauna is not fully studied, but protected insect species such as the beautiful horsefly, Serville's grasshopper, steppe scolia wasp, and carmine Polish beetle can be found in the park [6].

Local residents and tourists admire the picturesque natural landscapes of the park, often referring to it as the "Kazakh Switzerland." As a result of weathering and wind erosion, unique rock formations have been shaped here, including "Okzhetpes," "Sleeping Warrior," "Eagle," "Camel," "Kudyr," and "Sphinx" [6].

The objective of the study is to determine the species composition of edible agaric fungi identified in the Burabay and Kokshetau State National Natural Parks (SNNP), provide a morphometric description, and create a strain collection by obtaining new strains.

Materials and methods

The object of the study is cap fungi in the Burabay State National Natural Park (SNNP) in the Akmola region, including edible mushrooms, as well as the creation of a valuable strain collection from natural populations. During the expedition, the territories of the Burabay and Kokshetau State National Natural Parks were studied from July 15 to July 28.

A total of three groups of mycologists and algologists participated in the comprehensive expedition:

- Scientists from L.N. Gumilyov Eurasian National University (M.A. Ashikbayeva, S. Abiev, R.Z. Asylkhanova).

- Scientists from the Institute of Botany and Phytointroduction of the Ministry of Education and Science (two mycologists and two algologists).

All seven forest districts of the national park were studied: Akylbay, Burabay, Katarkol, Zolotobor, Barmashyn, Mirny, and Priozerny. The weather conditions were highly favorable for the mass emergence of macromycetes.

Macromycetes were collected using the route method during an expedition to the Burabay and Kokshetau

SNNP. The collection, sorting, drying, packaging, and transportation of the gathered material were carried out in accordance with mycological and botanical methodologies. For disinfection, the collected samples were dried and heated in a hot-air oven at a temperature of 45–50°C for 30–40 minutes. Each processed specimen was stored in a specially designed box, labeled with the date, location, time of collection, and herbarium number.

The external morphological characteristics of the fungal fruiting bodies were determined through visual observation. The microscopic structure and spore characteristics were analyzed using a Mikmed-1 microscope, along with Exilim-S880, SAMSUNG-ES65, and Canon-PC1474 cameras for documentation. For tissue isolation from the fruiting bodies, only fresh, nutrient-rich, and non-darkened specimens were used. Pre-cleaned fruiting bodies were cut into cube-shaped or triangular pyramid-shaped tissue samples using a scalpel. These tissue samples were then inoculated onto a nutrient medium (slant agar) using an inoculation needle. The isolation process was conducted on different parts of the fruiting body: the cap, the stipe, and the transition zone between the cap and the stipe.

The growth and development of the isolated pure culture depended on the composition of the nutrient medium. Various nutrient media were used, including Czapek-Dox, Murashige-Skoog, and potato-glucose agar [7-9].

During the expedition, more than 130 mushroom samples were collected. Morphometric analysis of these collected fungi revealed 71 species of cap fungi, classified into two classes (Basidiomycetes and Ascomycetes), four orders (Aphyllophorales, Agaricales, Lycoperdales, Pezizales), 11 families, and 26 genera (Table 1).

Results

Macromycetes belonging to one family (Polyporaceae), four genera, and six species from the order Aphyllophorales were identified. The edibility of one species (Coriolus zonatus) remains unknown, while the remaining species are edible.

Table 1 – Fungal Species Identified in Burabay and Kokshetau SNNP

№	Latin Name of the Fungus	Herbarium No.	Edibility
Order: Aphyllophorales			
Family: Polyporacea			
1.	Coriolus vercicolor (Fr.) Quel.	19	Edibility unknown
	Coriolus zonatus (Fr.) Quel	28	Edibility unknown
2.	(Trametes) Hizschioporus pergamentus Fr.	38	Inedible
3.	Fomitopsis rosea (Fr.) Karst.	51	Inedible
4.	Fomitopsis penicola (Sw.ex. Fr.), Karst.	71	Inedible
5.	Fomes fomentarius Fr.	65	Inedible
Order: Agaricales			
Family: Agaricaceae			
1.	Agaricus arvensis Schaeff.ex. Secr.	1	Edible
2	Agaricus bisporus Lange	3	Edible
3.	Agaricus silvaticus Schaeff.ex. Secr.	8	Edible
4.	Agaricus silvicolus (Vitt.) Sacc., Syll.	2	Edible
5.	Lepiota alba (Bres.) Sacc.	14	Edible
6.	Macrolepiota campestris Samg.	72	Edibility unknown
Family: Russulaceae			
1.	Russula aeruginea Lindbl.	37	Edible
2.	Russula flava Rom. in Lonnegren.	55	Edible
3.	Russula integra (Fr.) Epicr.	21	Edible
4	Russula rosacea (Fr.) Epicr.	4	Edible
5	Russula pulchella Borszczow	35	Edible
6	Russula sardonia (Fr.) Epicr.	24	Edible
7	Russula verescens (Schaeff. ex Zanedschi) Fr.	12	Edible
8	Russula xerampelina (Schaeff. ex Secr.)	5	Edible
9	Lactarius necator (Fr.) Karst.	49	Edible
10	Lactarius scrobiculatus (Fr.) Epicr.	31	Edible
11.	Lactarius trivialis (Fr.) Epicr.	41	Edible
Family: Tricholomataceae			
1	Armilariella mellea (Fr.) Karst.	53	Edible
2	Clitocybe gibba (Fr.) Kumm.	6	Edible
3	Clitocybe gilva (Fr.) Kumm.	50	Edibility unknown
4	Clitocybe inversa (Fr.), Quel.	13	Edible

Table 1 (Continuation) – Fungal Species Identified in Burabay and Kokshetau SNNP

5	Clitocybe odora (Fr.), Kumm.	10	Edible
6	Clitocybe vibecina (Fr.), Quel.	58	Edible
7	Marasmius oreades (Fr.) Epicr.	36	Edible
8	Mycena poliadelpha (Lasch.), Kuhner.	60	Edibility unknown
9	Omphalina ericetorum (Fr.), M. Lge.	7	Inedible
Family: Strophariaceae			
1	Hhypholoma sullaterium (Fr.)Quel., Champ., Jura Vosg.	54	Poisonous
2	Pholiota squarrosa (Fr.) Kumm.	16	Edible
Family : Comphidaceae			
1	Chroogomphus rutilis (Fr.), O. K. Miller, Canad., J. Bot.	69	Edible
Family: Amanitaceae			
1	Amanita fulva (Schaeff. Ex Pers.) Fr.	20	Edible
2	Amanita muscaria (Fr.) Hook.	17	Inedible
3	Pluteus pellitus (Fr.) Kumm.	42	Edible
Family: Boletaceae			
1	Leccinum scabrum (Fr.) S.F. Gray	40	Edible
2	Suillus granulatus (Fr.) Kuntze	39	Edible
3	Suillus luteus (Fr.) S.F.Gray	18	Edible
Family: Cortinariaceae			
1	Cortinarius cinnamomeoluteus P. D. Orton	27	Inedible
2	Cortinarius mucosus (Fr.), Kickx.	46	Edible
3	Cortinarius varius (Fr), Fr., Epicr.	9	Edibility unknown
4	Hebeloma mesophaeum (Fr.) Quel.	45	Inedible
5	Hebeloma strophosum (Fr.) Sacc.	29	Inedible
Family: Paxillaceae			
1	Paxillus involutus (Fr.) Epicr.	23	Edible
2	Paxillus pannuoides (Fr.), Fr., Epicr.	61	Inedible
Order: Lycoperdales			
Family: Lycoperdaceae			
1	Langermania gigantea (Pers.), Rostk.	30	A young specimen Edible
2	Lycoperdon perlatum Pers. Syn. . Fung.	15	A young specimen Edible
3	Lycoperdon piriforme (Pers). Schaef.	25	A young specimen Edible
4	Lycoperdon pusillum Pers.	26	Edibility unknown
Class: Ascomycetes			
Order: Pezizales			
Family: Helvellaceae			
1	Helvella lacunose Fr.	57	Edibility unknown

From the order Agaricales, macromycetes belonging to eight families, 18 genera, and 40 species were identified. Among them, six species from three genera of the Agaricaceae family and 11 species from two genera of the Russulaceae family were found to be edible.

In the Tricholomataceae family, one species (Omphalina ericetorum) from five genera and nine species was found to be inedible, while the edibility of another species (Mycena poliadelpha) remains uncertain. The remaining seven species from three genera are edible.

None of the macromycetes belonging to the Cortinariaceae family (two genera, five species) were found to be edible.

All identified species from the Boletaceae family (three species) and Gomphidaceae family (one species) were edible.

Two species were identified from the Strophariaceae family, of which one (Pholiota squarrosa) was edible, while the other (Hypholoma sublateritium) was toxic.

In the Amanitaceae family, one species (Amanita muscaria) was toxic, while the remaining two species (Amanita fulva and Pluteus pellitus) were edible.

Two species from the Paxillaceae family were described: one (Paxillus involutus) was edible, while the other (Paxillus pannuoides) was inedible.

From the order Lycoperdales, macromycetes belonging to one family, two genera, and four species were identified. The edibility of one species (Lycoperdon pusillum) is uncertain, while the remaining three species were found to be edible.

Macromycetes are widely distributed in Burabay National Park. Research was conducted to identify their species composition, provide morphometric descriptions, and obtain new strains. As a result of the study, a total of 26 macromycete species belonging to four orders and 11 families were identified.

Edible mushrooms were collected during their peak fruiting period. New strains were isolated on the same day of collection or within one to three days, stored in polyethylene bags in a refrigerator.

For isolation, fresh, firm, and non-infected carpophores were selected. Before isolation, the fruiting bodies were cleaned of adhered plant debris and soil, washed briefly with running and sterilized water (to prevent excessive absorption), and dried with filter paper. The dried fruiting bodies were wiped with 96% ethanol.

Using these preliminary cleaning methods, small tissue fragments in the shape of cubes or triangular pyramids were cut from the fruiting bodies with a scalpel and inoculated onto nutrient media (slant agar) using an inoculation needle. The samples were taken from different parts of the fruiting body: the cap, stipe, and the transition zone between the cap and stipe (Figure 1).

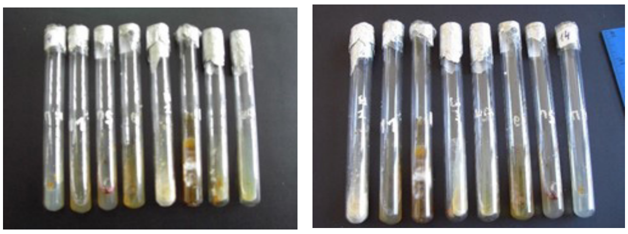

Figure 1 - Tissue fragments of the mushroom placed on slant agar

To evaluate the morphological and cultural characteristics of the mycelium and growth parameters, we used 25 strains of saprotrophic and xylotrophic basidiomycetes isolated through the tissue culture method. These included Suillus granulatus (Fr.) Kuntze, Lyophyllum connatum (Fr.) Sing., Pholiota squarrosa (Fr.) Kumm., and Kuehneromyces mutabilis (Fr.) Sing.

Table 2 presents the growth dynamics and colony formation characteristics of 11 actively growing mycelial strains on solid agar medium in Petri dishes (Figure 2).

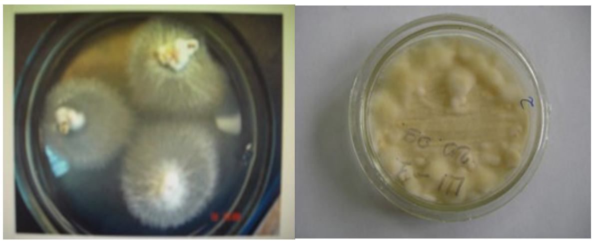

Figure 2 - Growth of strain colonies in Petri dishes

Table 2 - Growth characteristics and colony formation dynamics of different mycelial strains

Strains	Cultivation Time, Days				
	Mycelial Network Formation	Mycelial Density	Formation of Dense Nodules in Mycelium	Pigmentation of Mycelial Colony	Mycelial Colony Covered with a Membrane
Sg-1	8	23	29	33	45
Sg-23	10	22	27	35	43
Sg-8	8	16	22	34	39
Lc-1	11	20	25	43	52
Lc-15	11	22	24	37	50
Lc-18	9	16	20	33	48
Phs-3	18	25	31	39	57
Phs-15	14	24	33	39	54
Phs-10	17	28	35	42	58
Km-25	10	23	33	35	45
Km-34	12	23	30	34	45

Note Sg - Suillus granulatus, Lc - Lyophyllum connatum, Phs – Pholiota squarrosa, Km - Kuehneromyces mutabilis; numbers indicate strain identifiers*

According to A.S. Bukhalo's classification, the growth rate of basidiomycete mycelial colonies is divided into three groups: Group I – Fast-growing (Growth Coefficient, GC* > 100); Group II – Moderately growing (GC = 50-100); Group III – Slow-growing (GC < 50).

All of the strains studied in our research had a growth coefficient below 50, categorizing them as slow-growing mycelial colonies. Their mycelium was initially reticulated and powdery, later forming a felt-like structure spreading over the substrate surface. On average, complete colonization of the nutrient medium in Petri dishes took about one month.

Mycelial densification occurred between the 16th and 23rd days, while fully compacted cotton-like mycelial nodules formed approximately one month after inoculation.

The fastest-growing strains were Sg-1, Sg-8, Lc-18, Sg-23, and Km-25, reaching notable growth within 8-10 days. In contrast, Phs-3 and Phs-10 exhibited the slowest growth, requiring 17-18 days to develop. Full mycelial densification was observed 4 to 4.5 weeks after the start of cultivation.

Discussion

The conducted study provided valuable insights into the diversity and morphological characteristics of Agaricales mushrooms in Burabay and Kokshetau National Parks. The identification of 42 species from 26 genera and 11 families highlights the rich fungal biodiversity in these regions. The compiled species catalog serves as an important reference for future research and conservation efforts [10].

One of the key findings of this study is the differentiation of mushroom species based on their edibility and toxicity. The discovery of one toxic species underlines the importance of accurate identification to prevent potential health hazards. Meanwhile, the presence of 31 edible species suggests significant potential for their sustainable use in local communities, while the 10 inedible species contribute to the overall ecological balance of the forest ecosystem [11].

Compared to previous studies in similar temperate forest regions, our findings align with existing fungal biodiversity assessments but also expand the known distribution of certain species. The use of morphometric characteristics and specialized identification keys allowed for precise species classification, reinforcing the reliability of traditional taxonomic approaches in mycological research.

Despite the comprehensive nature of this study, certain limitations should be acknowledged. Environmental factors such as seasonal variations and microhabitat specificity may have influenced species diversity. Additionally, some cryptic species might require molecular analysis for more accurate identification. Future research could integrate DNA-based methods to complement morphological descriptions and provide deeper insights into species phylogeny.

The findings of this study contribute not only to mycology but also to conservation biology. Understanding the fungal diversity in protected areas aids in the development of sustainable resource management strategies. Given the ecological role of fungi in nutrient cycling and forest health, further monitoring of Agaricales species could help assess the impact of environmental changes on fungal populations [12,13].

In conclusion, this study enhances the knowledge of Agaricales diversity in Burabay and Kokshetau National Parks, providing a foundation for further ecological and conservation studies. Future research should aim at exploring additional regions, incorporating molecular techniques, and assessing the potential socio-economic applications of edible species.

Conclusion

Based on the conducted research, the following conclusions were made:

1. Morphological descriptions of mushroom structures were provided;

2. Spores were isolated, their micrometric characteristics were analyzed, and photographic documentation was performed;

3. Using the obtained morphometric characteristics and specialized identification keys, the species of the mushrooms were determined.

The study of all collected mushrooms in Burabay and Kokshetau National Parks identified 42 species from 26 genera and 11 families of the order Agaricales. Among them:1 species was toxic; 31 species were edible; 10 species were inedible.

4. A species catalog of Agaricales mushrooms from Burabay and Kokshetau National Parks was compiled;

5. The collected data and findings hold significant value for the sustainable use and conservation of natural resources.

Conflict of interests. The authors declare no conflict of interest.

Funding. There are no funding sources for this work.

Authors' Contributions.

Conceptualization: A.S. and A.M.A.; Draft writing: A.M.A.; Writing and editing: A.S., A.M.A., A.N.Z.; Data collection and analysis: A.M.A., S.A.

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Бурабай және Көкшетау ұлттық парктерінде таралған Agaricales саңырауқұлақтарының жаңа штаммдарын алу

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Түйіндеме

Қазақстанда агарикті саңырауқұлақтар экологиялық және тағамдық маңыздылығына қарамастан аз зерттелген. Бұл зерттеу Бурабай және Көкшетау ұлттық парктерінде кездесетін жеуге жарамды агарикті саңырауқұлақтардың түрлік құрамын, морфометриялық сипаттамаларын және штаммдар коллекциясын жасауды алғаш рет қарастырады.

Зерттеу мақсаты: жеуге жарамды агарикті саңырауқұлақтардың түрлік әртүрлілігін анықтау, олардың морфометриялық сипаттамаларын зерттеу және жаңа үлгілерді бөліп алу арқылы штаммдар коллекциясын құру. Бұл олардың биологиялық ерекшеліктерін әрі қарай зерттеуге және қолдану мүмкіндіктерін айқындауға мүмкіндік береді.

*Әдістері. Макромицеттер маршруттық әдіспен жиналып, сұрыпталып, кептіріліп (45–50°C, 30–40 мин) сақталды. Морфологиялық сипаттамалар визуалды түрде, микроскопиялық құрылымы *Микмед-1* микроскопы және фотокамералар көмегімен зерттелді. Жас үлгілер қоректік орталарға (*Чапек-Докс, Мурасиге-Скуг, картоп-глюкоза агары*) көшіріліп, штаммдар бөліп алынды.*

*Нәтижелері. 80-ден астам саңырауқұлақ үлгісі жиналып, олардың 71 түрі анықталды. Бұл түрлер екі класқа (базидиомицеттер және аскомицеттер), төрт қатарға (*Aphyllophorales, Agaricales, Lycoperdales, Pezizales*), 11 тұқымдасқа және 26 туысқа жатады. Бөлініп алынған штаммдар экологиялық зерттеулер мен жасанды өсіру үшін перспективалы.*

Қорытынды. Үлгі көлемі әлі де шектеулі. Агарикті саңырауқұлақтардың түрлік әртүрлілігін нақтылау үшін қосымша әдістерді қолдана отырып, әрі қарай үлгілер жинау және талдау жоспарлануда.

Түйін сөздер: саңырауқұлақ, макромицеттер, штаммдар, қалпақша, сабақ, спора, агарикті саңырауқұлақтар.

Получение новых штаммов грибов порядка Agaricales, распространенных в национальных парках Бурабай и Кокшетау

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Резюме

В Казахстане агариковые грибы остаются малоизученными, несмотря на их экологическую и пищевую значимость. Настоящая работа – первое исследование видового состава съедобных агариковых грибов в национальных парках Бурабай и Кокшетау, их морфометрической характеристики и создание коллекции штаммов.

Целью данного исследования является определение видового разнообразия съедобных агариковых грибов, их морфометрическая характеристика и создание коллекции штаммов за счет выделения новых образцов, что позволит в дальнейшем изучить их биологические особенности и потенциальное применение.

Методы. Сбор макромицетов осуществлялся маршрутным методом, образцы сортировали, высушивали и хранили. Морфологические характеристики изучались визуально и микроскопически. Молодые экземпляры культивировали на питательных средах для выделения штаммов.

Результаты. Собрано более 80 образцов, идентифицирован 71 вид, относящийся к двум классам, четырем порядкам, 11 семействам и 26 родам. Выделенные штаммы могут быть использованы для дальнейшего изучения и культивирования.

Выводы. Выборка пока ограничена, планируется дальнейший сбор и анализ образцов с применением дополнительных методов для уточнения видового разнообразия агариковых грибов Казахстана.

Ключевые слова: гриб, макромицеты, штаммы, шляпка, ножка, спора, агариковые грибы.