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RAPID AND EFFICIENT METHOD FOR DNA EXTRACTION FROM FUNGI OF THE GENUS *MORCHELLA* DILL. EX PERS.

The taxonomic features of true morels (genus Morchella, Ascomycota) have long been a difficult problem because the identification of species of such a taxonomically complex genus is impossible without the use of molecular phylogenetics methods. To date, there are a large number of DNA extraction methods, but the search for a fast and efficient method of DNA extraction from various organisms, including fungi, remains an urgent problem in modern biological research. ***Methods.*** *The study material consisted of the fruiting bodies of fungi belonging to the genus Morchella. Total DNA extraction was performed using the original DNA Microprep Isolation from Plants protocol (DNA Microprep method). For comparison, the modified CTAB method was also used to extract DNA from all samples. Extraction efficiency was determined by spectrophotometry. The ITS marker sequence of the ribosomal RNA nuclear gene cluster was amplified using primers ITS1 and ITS4, and the amplification products were separated by electrophoresis in an agarose gel.* ***Results.*** *DNA-containing pellets were formed in all samples extracted by the DNA Microprep method, as confirmed by spectrophotometric analysis. The extracted nucleic acid was of acceptable quantity and purity. The amplification of the ITS marker sequence was successful for all samples isolated by the DNA Microprep method, resulting in amplicons of different lengths, which may indicate a potential difference in the taxonomic affiliation of the studied fruiting bodies of morels. Regarding PCR products based on DNA extracted by the CTAB method, 80% of the samples tested were positive for PCR amplification.* ***Conclusions.*** *The study suggests that the DNA Microprep method is a convenient and effective way to extract DNA from morels' fruit bodies. The method offers advantages such as a short extraction process, availability of reagents, and environmental friendliness. Additionally, PCR products were successfully obtained from the extracted DNA. To accurately identify the species of the taxonomically complex genus Morchella, it is necessary to determine the nucleotide sequences of the obtained amplicons and perform a phylogenetic analysis.*

Keywords: *DNA extraction*, *fungi*, *Morchella*, *DNA amplification*, *PCR*.

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The taxonomic classification of the *Morchella* genus has been a subject of controversy due to the difficulty in morphologically distinguishing true morels. This is because of their phenotypic plasticity and lack of taxonomically useful characters (Kuo et al., 2012). Hawksworth et al. recognize 28 species within the genus *Morchella* (Hawksworth, 1995), while Kirk et al. note 36 species (Kirk et al., 2008). Some taxonomists cite up to 50 species, while others may be limited to 3—6 species (Weber & Weber, 1988). Currently, the genus *Morchella* has about 80 verified species, but the actual number could be closer to 100, considering the significant number of recently described new species (Clowez et al., 2022), as well as unpublished species in herbaria. Thus, there is considerable confusion in the literature regarding the number of authentic morel species. However, identifying species of such a taxonomically complex genus is currently impossible without molecular studies that utilize various molecular markers (the large subunit of the nuclear ribosomal RNA (LSU), ribosomal small subunit (SSU), the nuclear ribosomal internal transcribed spacer (ITS), RNA polymerase II largest subunit (RPB1) along with the RNA polymerase II second largest subunit (RPB2), and the translation elongation factor 1-alpha (EF1-α)) and implement molecular phylogenetic methods (Loizides et al., 2022; Sa et al., 2022; Cravero et al., 2023; Li et al., 2024).

The first step in any molecular phylogenetic study is DNA extraction. Therefore, a crucial aspect of modern molecular biological research on fungi is to identify the most efficient method of DNA extraction. This method should produce a high-quality product in a short time and with minimal use of low-toxic reagents.

Currently, there are a large number of methods for extracting DNA from fungi, which produce different yields of fungal DNA (Fredricks et al., 2005; Dörnte & Kües, 2013; Aamir et al., 2015; Mykchaylova et al., 2017; Clowez et al., 2022). There are also many commercially available DNA extraction kits (Sa et al., 2022; Snabl et al., 2023; Wani et al., 2023; Li et al., 2024), but their use is often limited by high cost.

The extraction of genomic DNA is usually done with cetyltrimethylammonium bromide (CTAB) extraction buffer followed by purification through phenol/chloroform extraction and precipitation with isopropanol or ethanol (Gardes & Bruns, 1993). The only limitations of this method may be the harmful nature of some solvents and the rather long isolation procedure. Currently, this method with various modifications is quite popular (Liu et al., 2014; Huang et al., 2018; Petrželová & Sochor, 2019).

Furthermore, common methods require sufficient amounts of fungal material, are time-consuming due to extensive handling and purification steps, involve costs for chemicals, and are an extra investment when applied in the form of commercial kits.

The DNA MicroPrep Isolation from Plants method (Dellaporta, 1994) is widely used for DNA extraction from various plants. However, there is no literature available on its effectiveness for extracting DNA from fungi.

The study **aimed** to evaluate the efficiency of the DNA MicroPrep Isolation from Plants method (DNA Microprep) for extracting DNA from fruiting bodies of *Morchella* and to analyze its suitability for species identification.

Materials and Methods. The sample material consisted of the fruiting bodies (ascocarps) collected during the spring season of 2023 from the western outskirts of the Kholodnyi Yar National Nature Park (Cherkaska oblast), as well as from household plot in Fastiv (Kyivska oblast) (Table 1). Macromorphological characteristics of the morels' ascocarps were recorded during field research, including information on their color, size, shape, and texture.

DNA was extracted from fresh and dried fruiting bodies using two methods — the DNA MicroPrep Isolation from Plants and CTAB method.

According to the DNA Microprep protocol, to homogenize the material, small samples of fruiting bodies (3—5 mm^3) were placed in microtubes and immersed in liquid nitrogen. Instead of using a mechanized homogenizer, a small amount of carborundum was added to the pieces of fungal fruiting bodies and crushed with a pestle. Next, 750 μL of extraction buffer (1% SDS, 100 mM NaCl, 10 mM EDTA, 50 mM Tris-HCl (pH 8.0), and 10 mM 2-mercaptoethanol (added immediately before use)) was added to each tube and mixed thoroughly. The tubes were incubated at 65 °C for 10 min.

Following the initial step, 200 μL of 3M sodium acetate solution was added to the samples and mixed thoroughly. The mixture was incubated on ice for 20 minutes. All further manipulations were carried out at room temperature. Subsequently, the material was centrifuged at maximum speed (18,000 g in our case) for 10 minutes. The supernatant was then transferred to new tubes and an equal volume of isopropanol was added and mixed thoroughly. Finally, the resulting mixture was centrifuged at maximum speed for 2 minutes. The supernatant was then removed, and 80% ethanol solution (450 μL) was added to the resulting DNA pellet. The mixture was centrifuged again at maximum speed for 2 minutes, after which the ethanol was poured off, and the tubes were dried until the ethanol evaporated completely. Finally, the resulting DNA was dissolved in a 50 μL TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA).

For comparison, the modified CTAB method, as described in the appropriate protocol (Tarieiev et al., 2011), was used to extract DNA from all samples. The DNA extraction was assessed visually by the formation of pellets at the final stage of extraction. The extraction efficiency was assessed using both electrophoresis and spectrophotometry with a DS-11 FX+ spectrophotometer (DeNovix, USA).

The amplification of the ITS marker sequence of the nuclear ribosomal RNA gene cluster was performed using primers ITS1(TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). The expected size of the amplification product is approximately 750 base pairs. PCR was carried out using MyTaq Reaction Buffer Colorless (Meridian Bioscience Inc., USA) and Pfu-X Polymerase.

Table 1. **Information about the fruiting bodies of *Morchella* spp. used for DNA extraction**

No.	Photo of the sample	Collection number and type of the sample	Place of finding
1		KY1872 (dried)	Kholodnyi Yar National Nature Park, oak and hornbeam forest edge
2		KY0901 (fresh)	Kholodnyi Yar National Nature Park, steppe area
3		KY0881 (fresh)	Kholodnyi Yar National Nature Park, steppe area
4		FS0082 (dried)	a private plot, Fastiv
5		KY1999 (fresh)	Kholodnyi Yar National Nature Park, oak and hornbeam forest edge

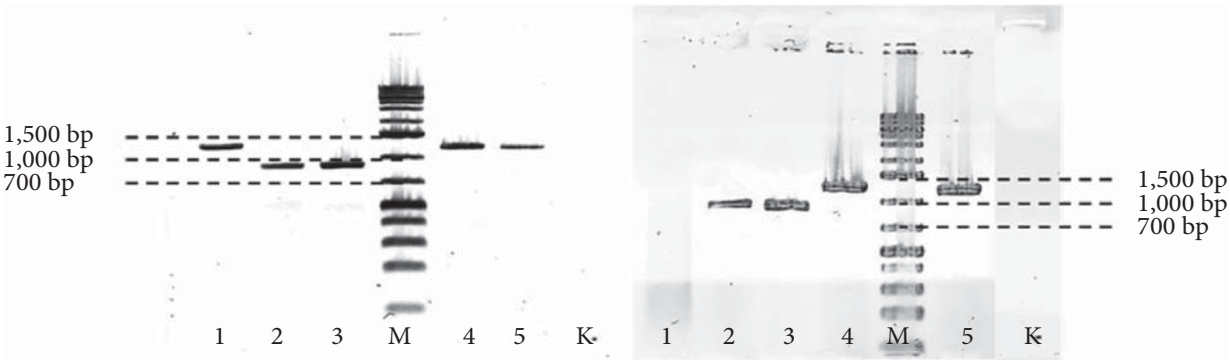

Fig. 1. Electrophoregrams of amplicons obtained by PCR: A — with DNA isolated by DNA Microprep method; B — with DNA isolated by CTAB; 1 — KY1872, 2 — KY0901, 3 — KY0881, 4 — FS0082, 5 — KY1999, M — 1 kb Plus DNA Ladder (Thermo Scientific), 75—20,000 bp; K — negative control

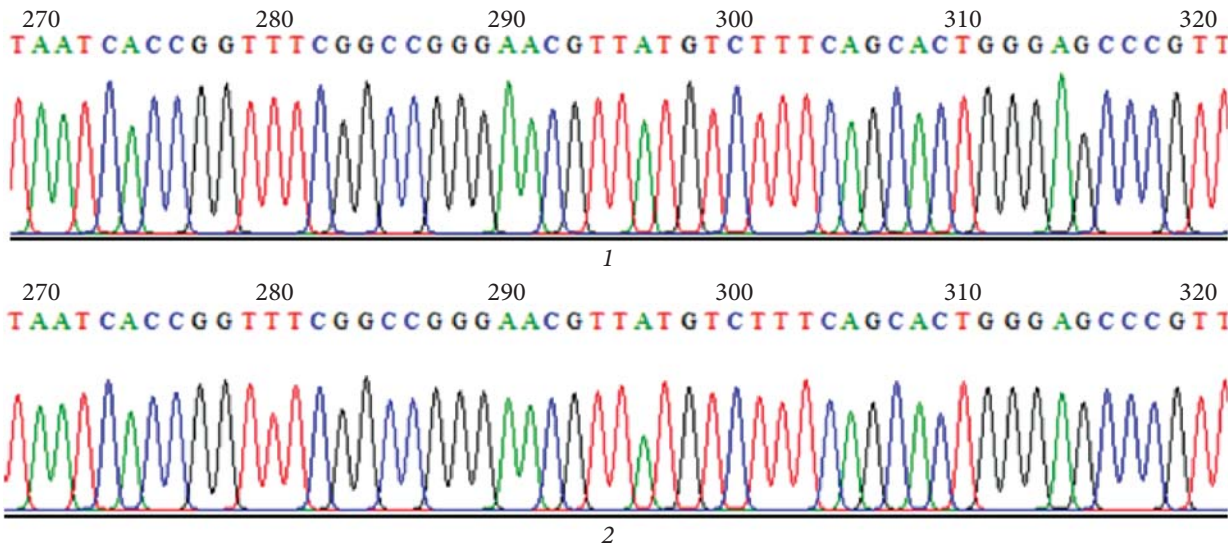

Fig. 2. Examples of successfully amplified ITS sequences chromatogram fragments from *Morchella* sp. KY1999: 1 — from DNA isolated by the DNA Microprep method; 2 — from DNA isolated by the CTAB method

Table 2. **The quantity of DNA extracted from the fruiting bodies of *Morchella* spp.**

Sample	DNA Microprep method			CTAB method		
	Concentration (ng/μL)	Purity		Concentration (ng/μL)	Purity	
		$A_{260/280}$	$A_{260/230}$		$A_{260/280}$	$A_{260/230}$
KY1872	621.382	1.95	1.92	26.412	1.82	2.02
KY0901	522.566	1.42	1.83	750.026	1.38	1.94
KY0881	199.687	2.29	2.04	221.692	1.44	1.88
FS0082	888.146	2.29	2.08	908.985	1.78	1.99
KY1999	890.448	2.06	2.02	382.800	1.53	1.88

Amplification was carried out on a Techne TC-3000X PCR thermocycler (Keison Products, UK). The initial DNA denaturation was performed at 98 °C for 3 minutes. Subsequently, 30 cycles were performed with the following parameters: denaturation at 98 °C for 30 seconds, primer annealing at 55 °C for 30 seconds, and elongation at 72 °C for 1 minute. The final extension was performed at 72 °C for 10 minutes. At the end of the amplification process, the temperature was lowered to 4 °C.

The amplification products were separated by electrophoresis in a 1% agarose gel.

Results. DNA-containing pellets were formed in all samples extracted by the DNA Microprep method. The DNA concentration was determined using spectrophotometry, as shown in Table 2.

As shown in Table 2, the yield of DNA extracted by the DNA Microprep method ranges from 199.687 to 890.448 ng/μL. The 260/280 ratio ranged from 1.42 to 2.29, and the 260/230 ratio — from 1.83 to 2.08. The purity of the isolated nucleic acid is acceptable, taking into account the accepted range of 260/280 (~1.8) and 260/230 (2.0—2.2) ratios (Thermo Scientific, 2011). The purity of DNA obtained by the CTAB method is slightly lower.

The amplification of the ITS marker sequence (≈ 800—1200 bp) was successful for all samples isolated by DNA Microprep method: 100% of all *Morchella* samples gave positive results in PCR (Fig. 1).

Regarding PCR products based on DNA extracted by the CTAB method, amplification was successful for 80% of samples (Fig. 1). This suggests that the DNA Microprep method is more effective for DNA extraction from morels, as it yields a sufficient amount of sufficiently pure DNA to be successfully amplified.

Since we obtained amplicons of varying lengths (≈ 800—1200 bp), potential differences in the taxonomic affiliation of the studied fruiting bodies could be inferred. To further analyze, the nucleotide sequences of the amplicons should be determined, and phylogenetic analysis should be performed. The amplicons were sent for sequencing to the Macrogen (Netherlands). The PCR products' purification was carried out on a commercial basis by Macrogen.

The resulting nucleotide sequences of ITS have been deposited in GenBank. Access codes: PP511294 (KY1872), PP582334 (KY0901), PP582336 (KY0881), PP582335 (FS0082), and PP582337 (KY1999).

Analysis of the chromatograms of the resulting nucleotide sequences showed that the quality and purity of the signal were high (Fig. 2).

Conclusions. In this study, we successfully tested the rapid and inexpensive method DNA Microprep Isolation from Plants, which could effectively reduce the time required for DNA sequencing-based molecular identification of fungi. Thus, based on the results of the study, the DNA Microprep method is generally suitable for use in DNA extraction from both fresh and herbarized morels fruit bodies. The method has a short extraction process, uses readily available reagents, is environmentally friendly, and produces fairly pure DNA. For comparison, we performed DNA extraction using a modified CTAB method. The DNA Microprep method was found to be the most successful. PCR products were obtained from the extracted DNA of the fungal samples. Subsequent sequencing of these products will enable accurate identification of species belonging to the taxonomically complex genus *Morchella*.

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Conflict of Interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ШВИДКИЙ ТА ЕФЕКТИВНИЙ МЕТОД ЕКСТРАКЦІЇ ДНК
З ГРИБІВ РОДУ *MORCHELLA* DILL. EX PERS.

Таксономія грибів роду *Morchella* вже тривалий час є складною проблемою, адже ідентифікація видів такого таксономічно складного роду неможлива без використання методів молекулярної філогенетики. На сьогоднішній день існує велика кількість методів екстракції ДНК, проте пошук швидкого та ефективного методу виділення ДНК з різних організмів, зокрема з грибів, залишається актуальною проблемою сучасних біологічних досліджень. **Методи.** Матеріалом для дослідження були плодові тіла грибів роду *Morchella*. Для екстракції тотальної ДНК використовували оригінальний протокол DNA Microprep Isolation from Plants (метод DNA Microprep). Для порівняння з усіх зразків було здійснено екстракцію ДНК з використанням модифікованого CTAB-методу. Ефективність виділення встановлювали за допомогою спектрофотометрії. Ампліфікацію маркерної послідовності ITS-ділянки кластеру ядерних генів рибосомальної РНК проводили з використанням праймерів ITS1 та ITS4. Продукти ампліфікації розділяли за допомогою електрофорезу в агарозному гелі. **Результати.** ДНК-вмісна пелета утворювалась у всіх зразках, виділених за допомогою методу DNA Microprep. Успішність виділення ДНК підтверджується спектрофотометричним методом. Кількість та чистота виділеної нуклеїнової кислоти є прийнятною. Ампліфікація маркерної послідовності ITS була успішною для всіх зразків, екстрагованих DNA Microprep методом, в результаті чого отримано амплікони різної довжини, що може вказувати на потенційні відмінності в таксономічній приналежності досліджуваних плодових тіл зморшок. Щодо продуктів ПЛР на основі ДНК, екстрагованої CTAB-методом, ампліфікація була успішною для 80% зразків. **Висновки.** Результати дослідження свідчать, що метод DNA Microprep Isolation from Plants можна вважати зручним та ефективним для екстракції ДНК з плодових тіл зморшків. Перевагами методу є коротка тривалість процесу екстракції, доступність реактивів, екологічність роботи тощо. На основі виділеної ДНК було отримано ПЛР-продукти. Для точної ідентифікації видів складного в таксономічному відношенні роду *Morchella* слід встановити нуклеотидні послідовності отриманих ампліконів та здійснити філогенетичний аналіз.

Ключові слова: *екстракція ДНК, гриби, Morchella, ампліфікація ДНК, ПЛР.*