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First Record of *Helvella capucina* in Türkiye: Morphological and Molecular Characterization

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Abstract: In the current study, *Helvella capucina* collected from Hakkari province in Türkiye is described as a new record using morphological and molecular characters. Ascocarp, hymenium, stipe, ascospores, ascus and paraphyses were used as diagnostic morphological characters. Images of microscopic characters, microphotographs of the ascospores, and colour pictures of the ascus in their natural habitats are provided. The species, which is placed in section *Elasticae*, was characterised by its short-stipitate apothecium and pure white when fresh, drying yellowish stipe. Molecular analysis was also carried out to characterize the new record reliably by using DNA sequences of the heat shock protein 90 (*HSP90*), RNA polymerase 2 (*RPB2*) and translation elongation factor 1-alpha (*TEF1-α*). Additionally, the analyses of the three regions were combined to create a concatenated tree. The multigene phylogenetic position and evolutionary relationships of *H. capucina* with other species of *Helvella* in *Elasticae* were inferred using Bayesian Inferences (BI) of the concatenated dataset. Both morphological and molecular data separated *Helvella capucina* from its close relatives. Detailed descriptions, colour images of fresh and dried ascomata, along with photographs of microscopic characters and the concatenated phylogenetic tree were provided.

Anahtar kelimeler: *Helvella*, *HSP90*, New record, *RPB2*, *TEF1-α*

Türkiye'den *Helvella capucina* Türünün İlk Kaydı ve Morfolojik ve Moleküler Karakterizasyonu

Öz: Bu çalışmada, Türkiye'nin Hakkari ilinden toplanan *Helvella capucina* türü, morfolojik ve moleküler özellikler kullanılarak yeni bir kayıt olarak tanımlanmaktadır. Belirleyici morfolojik karakterler arasında askokarp, himenyum, sap, askosporlar, askus ve parafiz yer almaktadır. Mikroskobik karakterlerin görselleri, askosporların mikrofotografik görüntüleri ve askusların doğal ortamlarındaki renkli resimleri sağlanmıştır. *Elasticae* seksiyonunda yer alan bu tür, kısa saplı apotezyum yapısı ve taze halde saf beyaz, kurudukça sarımsı renge dönüşen sap yapısıyla karakterizedir. Yeni kayıt türün güvenilir bir şekilde tanımlanması için ısı şok proteini 90 (*HSP90*), RNA polimeraz 2 (*RPB2*) ve translasyon uzatma faktörü 1-alfa (*TEF1-α*) DNA dizileri kullanılarak moleküler analizler de gerçekleştirilmiştir. Üç bölgenin analizleri birleştirilerek bir kombine ağaç oluşturulmuştur. *H. capucina* türünün *Elasticae* seksiyonunda yer alan diğer *Helvella* türleri ile çok

genli filogenetik pozisyonu ve evrimsel ilişkileri, birleştirilmiş veri setinin Bayes Çıkarım Yöntemi (BI) ile çıkarım yoluyla belirlenmiştir. Hem morfolojik hem de moleküler veriler, *Helvella capucina* türünü yakın akrabalarından ayırmıştır. Detaylı tanım, taze ve kurumuş askokarp yapısının renkli görüntüleri, mikroskobik karakterlerin fotoğrafları ve birleştirilmiş filogenetik ağaç sunulmuştur.

Keywords: *Helvella, HSP90, RPB2, TEF1-α,* yeni kayıt

Introduction

Helvella L. is a genus of apothecial *Ascomycetes* that occurs in both the terrestrial biomes of the Northern and Southern Hemispheres (Kirk et al., 2008). Species within the *Helvella* genus are characterized by their distinctive fruiting bodies, which may be attached directly or supported by a short stalk. The apothecia exhibit diverse morphologies, ranging from cup-shaped to saddle-shaped and curved to bell-shaped forms, often with caps that are wrinkled and lobed, supported by a single ribbed or grooved stipe (Skrede et al., 2017). Globally, the genus comprises approximately 600 species, of which 24 have been identified in Türkiye (Sesli et al., 2020; Uzun, 2023).

This research aims to contribute to the knowledge of Turkish mycobiota by further exploring and documenting the *Helvella* species present in the Türkiye.

Material and Method

Morphological studies

Macrofungal samples were collected from the Hakkari province in Türkiye and subsequently deposited in the Fungarium of Van Yüzüncü Yıl University (VANF). Microscopic characteristics of the samples were observed using distilled water, Melzer's reagent, and Congo red solutions (Fig. 1). Microscopic examinations were conducted with a Leica DM500 research microscope, and measurements were recorded using the Leica Application Suite software (version 3.2.0). Terminology used for describing morphological features adheres to the standards outlined by Van Vooren (2010).

Molecular studies

Genomic DNA was extracted from dried herbarium specimens using the cetyltrimethylammonium bromide (CTAB) method as described by Doyle and Doyle (1987). The concentration and purity of the extracted DNA were assessed using a NanoDrop 2000c UV-Vis Spectrophotometer (Thermo Scientific) and verified through 1% agarose gel electrophoresis. Polymerase chain reaction (PCR) was conducted on a Thermal Cycler (Thermo Scientific) using specific primer sets to amplify the *TEF1-α*, *RPB2*, and *HSP90* gene regions (Table 1).

Tablo 1. Primer sequences used in this study for three gene regions: *TEF1-α*, *RPB2*, and *HSP90*.

Region	Primers name	Nükleotit dizisi	References
TEF1-α	EF1-983F	5'ACHGTRCCRATACCACCRATCTT3'	(Rehner and Buckley, 2005)
	EF1-1567R	5'GCYCCYGGHCAYCGTGAY3'	
RPB2	6Fa	5'TGGGGATTRGTCTGCCCYGC3'	Liu et al., 2006
	7cR	5'CCCATRGCTTGYTTRCCCAT3'	
	7cF	5'ATGGGYAARCAAGCYATGGG3'	
HSP90	11aR	5'GCGTGGATCTTGTCRTCSACC3'	Johannesson et al., 2000
	hspf	5'CRGGCATCCGGGTGACGTAAT3'	
	hspr	5'AGGGKGTTGTCGACTCCGAGG3'	

PCR reactions were performed in a 25 µl total reaction volume containing genomic DNA (10 ng/µl), 10X PCR Buffer, $MgCl_2$ (25 mM), dNTP mix (10 mM), selected primer pair (10 µM), Taq polymerase (5u/µl) and sterile distilled water. The PCR conditions for three regions were optimised at 94°C for 4 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 45 sec, and elongation at 72°C for 1 min, and final extension at 72°C for 10 min to ensure complete primer-template extensions. The resulting PCR products were run on a 1.0% agarose gel and visualized by staining with Gelred dye. Successful amplifications were sequenced in both directions using the forward and reverse PCR primers using ABI 3730XL automated DNA sequencer (BM Labosis, Ankara, Türkiye).

Phylogenetic analysis

DNA sequences were assembled and edited using MAFFT version 7.311 (Katoh and Standley, 2013) within the Mesquite software package (version 3.7; Maddison and Maddison, 2009). For initial sequence comparison, the GenBank database was queried using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al., 1990). The sequences obtained from the current study were compared against homologous sequences in

GenBank to identify potential matches. A total of 30 sequences from the *TEF1-α* and *HSP90* regions and 26 sequences from the *RPB2* region, all corresponding to *Helvella* species within the *Elasticae* section, were included in the analysis. *Dissingia confusa* (OR192540, MK113843, and MK652180) was selected as the outgroup for the *TEF1-α*, *RPB2*, and *HSP90* regions, respectively (Table 2).

Tablo 2. The accession numbers of samples downloaded from GenBank

Species	GenBank Accession Number*			
	TEF1-α	***RPB2***	***HSP90***	**References**
H. alborava L. Fan, N. Mao and H. Zhou	OR359033	OR359155	OR366098	Mao et al.,2023
H. albopatella L. Fan, N. Mao & Y.Y. Xu	OR359014	OR359137	OR366079	Mao et al.,2023
H. bachu Q. Zhao, Zhu L. Yang & K.D. Hyde	OR358997	OR359121	OR366059	Mao et al.,2023
H. brunneogaleriformis L. Fan, N. Mao & Y.Y. Xu	OR358999	OR359123		Mao et al.,2023
H. caespitosa L. Fan, N. Mao & Y.Y. Xu	OR359022	-	OR366087	Mao et al.,2023
H. capucina Quél.	MK113878	MK113852	KY784216	
H. capucinoides Peck	OR358979	OR359106	OR366041	Mao et al.,2023
H. carnosa Skrede, T.A. Carlsen & T. Schumach	OR358969	-	OR366034	Mao et al.,2023
H. compressa (Snyder) N.S. Weber	DQ497604	KY772500	KY784250	Unpublished Skrede et al.,2017
H. corbierei (Malençon) Van Vooren & Frund	OR359007	OR359130	OR366069	Mao et al.,2023
H. danica Skrede, T.A. Carlsen & T. Schumach.	OR358993	OR359117	OR366055	Mao et al.,2023
H. deflexa L. Fan, N. Mao & Y.Y. Xu	OR359004	OR359127	OR366066	Mao et al.,2023
H. elastica Bull.	KY772858	KY772476	KY784230	Skrede et al., 2017
H. flavostipitata L. Fan, N. Mao & Y.Y. Xu	OR358977	OR359104	OR366039	Mao et al.,2023
H. guttata Q. Zhao & J.R. Lu	OQ863544	-	OQ863528	Mao et al.,2023
H. galeriformis B. Liu & J.Z. Cao	OR359001	OR359125	OR366063	Mao et al.,2023
H. macropus (Pers.) P. Karst.	MK113885	MK113859	MK179399	Hansen et al.,2019
H. nigrorava L. Fan, N. Mao & Y.Y. Xu	OR359032	OR359154	OR366097	Mao et al.,2023
H. nordlandica Skrede & T. Schumach.	OR359013	OR359136	OR366078	Mao et al.,2023
H. panormitana Inzenga	KY772856	KY772474	KY784228	Skrede et al., 2017
H. pezizoides Afzel.	KY772857	KY772475	KY784229	Skrede et al., 2017
H. pseudoelastica L. Fan, N. Mao & Y.Y. Xu	OR359083	OR359194	OR366149	Mao et al.,2023
H. pseudofallax L. Fan, N. Mao & Y.Y. Xu	OR359016	OR359139	OR366081	Mao et al.,2023
H. pseudopezizoides L. Fan, N. Mao & Y.Y. Xu	OR359020	OR359143	OR366085	Mao et al.,2023
H. rivularis Dissing & Sivertsen	MN689306	MN692295	MN692371	Loken et al.,2020
H. scyphoides Skrede, T.A. Carlsen & T. Schumach.	OR359008	OR359131	OR366070	Mao et al.,2023
H. stevensii Peck.	KU739855	KY772739	KY784491	Zhao et al., 2016 Skrede et al.,2017
H. subglabra N.S. Weber	KU739857	-	KY784394	
H. subglabroides L. Fan, N. Mao & Y.Y. Xu	OR359018	OR359141	OR366083	Mao et al.,2023
H. subspadicea	KU739848	-	OR220558	Zhao et al., 2016 Mao et al.,2023
Dissingia confusa (*Harmaja*) K. Hansen & X.H. Wang	OR192540	MK113843	MK652180	Terman et al., 2024; Wang et al., 2019

Phylogenetic inference was performed using the Bayesian Inference (BI) method. BI analyses were conducted with MrBayes version 3.2.6 (Ronquist and Huelsenbeck, 2003), employing four Markov Chain Monte Carlo (MCMC) chains run for 5 million generations under the GTR+G (General Time Reversible + Gamma) evolutionary model. The initial 25% of generations were discarded as burn-in, ensuring that the average standard deviation of split frequencies reached <0.01. Branch support was evaluated using Bayesian Posterior Probabilities (BPP). A consensus tree was generated using a majority-rule approach to combine the remaining

trees, displaying all compatible partitions to estimate BPP. The final phylogenetic trees were visualized using FigTree version 1.4.3 (Rambaut, 2010).

Results

***Helvella capucina* Quél., Bull. Soc. bot. Fr. 24: 319 (1878) [1877]**

Synonymy: *Leptopodia capucina* (Quél.) Boud., Hist. Class. Discom. Eur. (Paris): 37 (1907)

Ascocarp consists of a fertile cap and a sterile stipe (Fig 1a). **Cap** sub-hemispherical or with two lobes erect like a hood, measuring 7–11 mm in diameter, by 5–8 mm in height. Flesh fragile, elastic, and whitish. **Hymenium** pale brownish to black, darker when dried, external surface whitish, sometimes somewhat irregular, glabrous. **Stipe** 20–60 × 5–10 mm, solid, cylindrical, slightly furrowed, somewhat enlarged at base, entirely white, glabrous. **Asci** 280–340 × 17–23 µm, cylindrical, eight-spored, pleurorhynchal base (Fig 1b, c, d). **Ascospores** 21–25 × 13–16 µm, hyaline, broadly ellipsoidal, containing a large lipid droplet in the centre, surrounded by smaller ones (Fig 1e, f, g). **Paraphyses** cylindrical, septate, enlarged apex up to 7 µm, with brownish pigments.

Habitat features: Typically grows solitary or in groups under broad-leaved trees, particularly those of the *Salix* genus (willow), and is commonly found in alpine zones at elevations above 2000 meters (Skrede et al., 2017).

Molecular phylogeny

The phylogenetic analysis was conducted using a total of 32 sequences, including the outgroup and the studied specimen, for the *TEF1-α* and *HSP90* loci, and 28 sequences for the *RPB2* locus. The sequence lengths were 537 base pairs (bp) for *TEF1-α*, 245 bp for *HSP90*, and 350 bp for the *RPB2* region. All three gene regions exhibited distinct barcoding gaps, facilitating species-level differentiation. Even the phylogenetic resolution of the *TEF1-α* and *RPB2* trees was superior to that of the *HSP90* tree, each region produced a phylogenetic tree with well-supported clades (not given). To enhance the informativeness of the results, a concatenated phylogenetic tree was generated by merging the three gene regions (Fig. 2). *Helvella capucina* is classified within the *Elasticae* section. The sample investigated in this study was grouped with its representative retrieved from the database with high Posterior Probabilities (1.0).

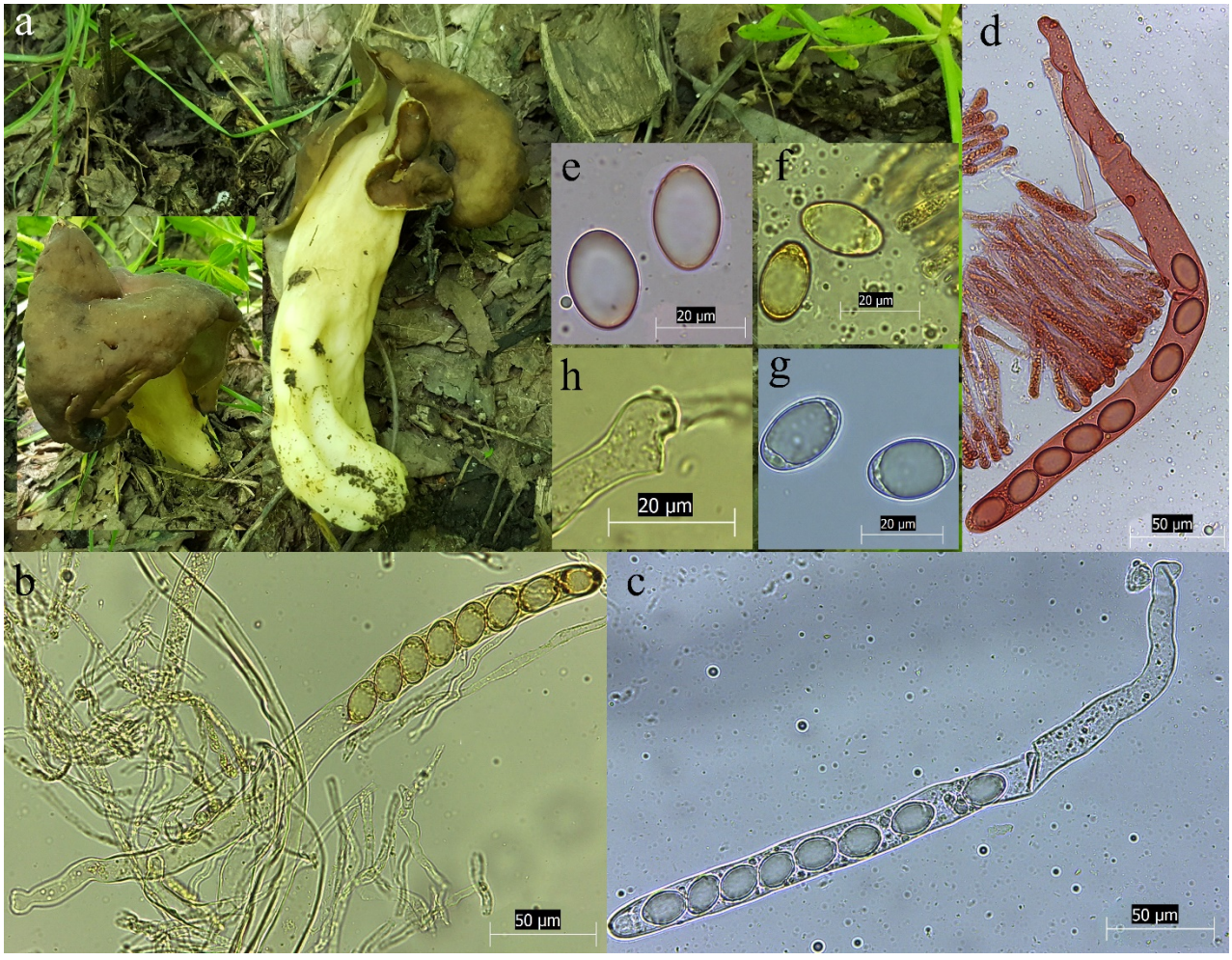

Figure 1. a. Fruiting body, b. asci (in Melzer's reagent), c. asci (in distilled water), d. asci (mounted with Congo red), e. ascospores (mounted with Congo red), f. ascospores (in Melzer'reagent), g. ascospores (in distilled water) and h. base of pleurorhynchous ascus (in Melzer's reagent) of *Helvella capucina*.

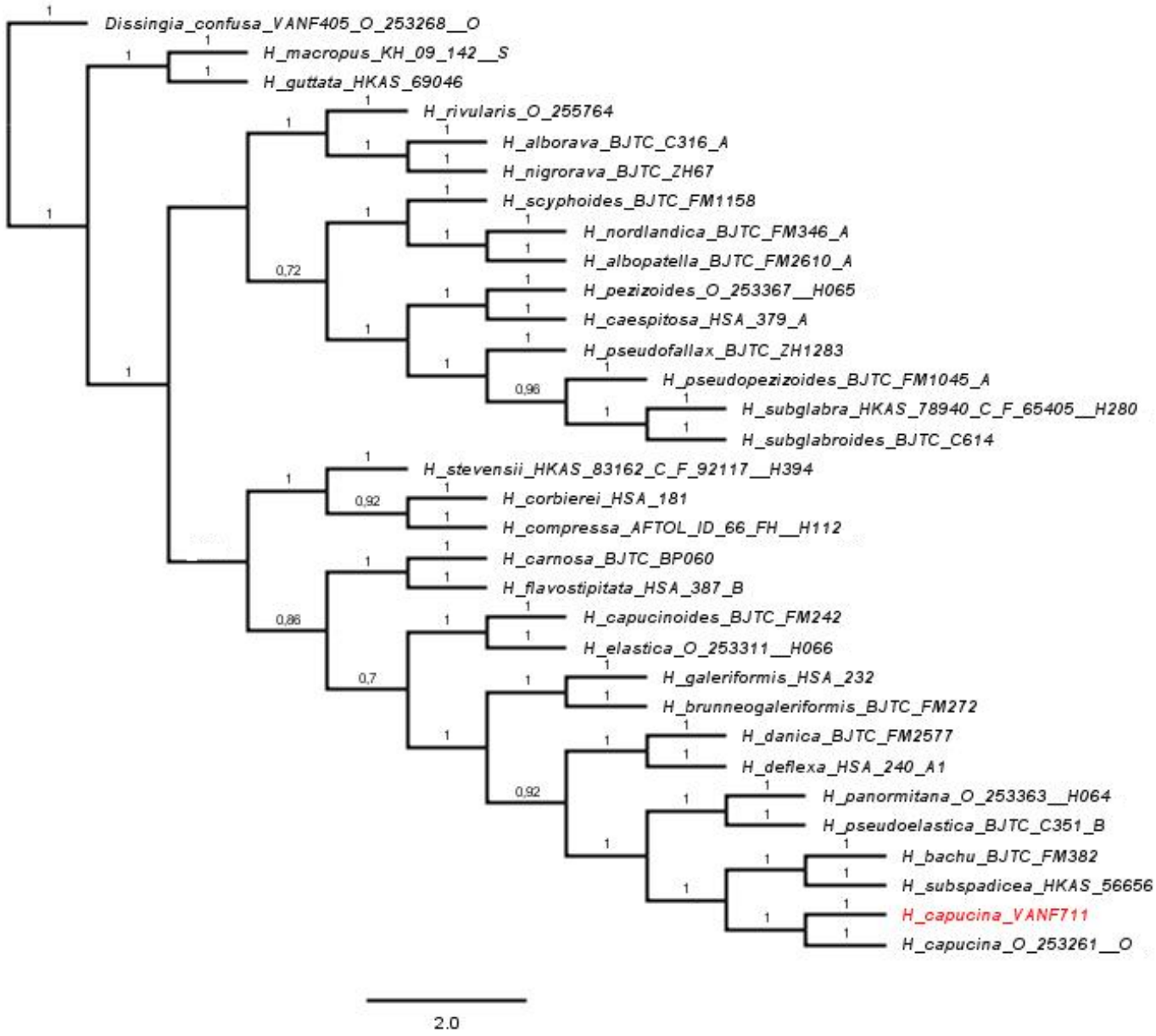

Figure 2. Phylogenetic tree of concatenated tree (*TEF1-α+RPB2+HSP90*) conducted by Bayesian analysis (Taxon marked in red indicates studied example).

Discussions

This study provides the first documented record of *Helvella capucina* in Türkiye, thereby contributing to the expansion of the region's mycobiota. The results provide significant insights into the phylogenetic relationships of *Helvella capucina* within the *Elasticae* section. The successful clustering of the investigated species with their corresponding database entries indicates that both morphological characteristics and molecular data are robust enough to facilitate accurate identification. The morphological characteristics observed in this study, including the unique apothecium shape, fragile white flesh, and distinct color changes in the stipe, are in consistent with descriptions, thereby confirming the identification of the species.

The phylogenetic analysis conducted on the concatenated sequences of the *TEF1-α*, *RPB2*, and *HSP90* regions revealed a well-supported tree structure, suggesting a strong resolution of evolutionary relationships among the examined taxa. The results significantly strengthens our understanding of *H. capucina*'s position within the *Helvella* genus, particularly in the *Elasticae* section. The phylogenetic analysis clearly demonstrates the separation of *Helvella capucina* from its close relatives. The superior resolution observed in the *TEF1-α* and *RPB2* regions compared to *HSP90* may be attributed to the variability of these loci, which provide more informative phylogenetic signals.

In conclusion, the identification of *Helvella capucina* significantly enhances the mycological knowledge of Türkiye and underscores the necessity for continued exploration and documentation of fungal diversity within the region.

Author contributions
The authors have equal contributions.

Conflicts of interest
The authors declare no competing interests.

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Şuheda ALDEMİR TERMAN, Mustafa Emre AKÇAY, Ayten DİZKIRICI)

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. *Journal Molecular Biology,* 215(3): 403–410.

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

Hansen, K., Schumacher, T., Skrede, I., Huhtinen, S. and Wang, X. H. (2019). *Pindara* revisited - evolution and generic limits in *Helvellaceae*. *Persoonia,* 42: 186–204.

Katoh, K. and Standley, M.D. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.,* 30(4): 772–80.

Kirk, P.M., Cannon, P.F., Minter, D.W. and Stalpers, J.A. (2008). *Ainsworth & Bisby's Dictionary of the Fungi*, 10th Edition. CABI Europe-UK.

Liu, Y.L., Hodson, M.C, Hall, B.D. (2006). Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of kingdom Fungi inferred from RNA polymerase II subunit genes. *BMC Evolutionary Biology,* 6: 74.

Loken, S.B., Skrede, I. and Schumacher, T. (2020). The *Helvella corium* species aggregate in Nordic countries - phylogeny and species delimitation *Fungal Systematic Evolotion,* 5: 169–186.

Johannesson, H.S., Johannesson, K.H.P. and Stenlid, J. (2000). Development of primer sets to amplify fragments of conserved genes for use in population studies of the fungus Daldinia loculata. *Molecular Ecology*, 9: 375–378.

Maddison, W. and Maddison, D. (2009). MESQUITE: a modular system for evolutionary analysis. *Evolution,* 11 (5).

Mao, N., Xu, Y.Y., Zhang, Y.X., Zhou, H., Huang, X.B., Hou, C.L., Fan, L. (2023). Phylogeny and species diversity of the genus *Helvella* with emphasis on eighteen new species from China. *Fungal Systematics and Evolution,* 12: 111–152.

Rambaut, A. (2010). *FigTree v1.3.1*. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

Rehner, S.A. and Buckley, E. (2005). A Beauveria phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. *Mycologia*, 97: 84–89.

Ronquist, F. and Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574.

Sesli, E., Asan, A., Selçuk, F. Abacı Günyar, Ö., Akata, I., Akgül, H., Aktaş, S., Alkan, S., Aydoğdu, H., Berikten, D., Demirel, K., Demirel, R., Doğan, H. H., Erdoğdu, M., Ergül, C. C., Eroğlu, G., Giray, G., Haliki Ustan, A., Keleş, A., Kırbağ, S., Kıvanç, M., Ocak, İ., Ökten, S., Özkale, E., Öztürk, C., Sevindik, M., Şen, B., Şen, İ., Türkekul, İ., Ulukapı, M., Uzun, Ya., Uzun, Yu. and Yoltaş, A. (2020). *Türkiye Mantarları Listesi* (The Checklist of Fungi of Turkey). Ali Nihat Gökyiğit Vakfı Yayını. 1177 sayfa, İstanbul.

Skrede, I., Carlsen, T. and Schumacher T. (2017). A synopsis of the saddle fungi (*Helvella*: *Ascomycota*) in Europe – species delimitation, taxonomy and typification. *Persoonia,* 39: 201–253.

Terman S. Ş., Dizkirici, A. and Akcay, M. E. (2024). Morphological and molecular identification of *Dissingia confusa* based on the first record of the species in Türkiye. *Trakya University Journal of Natural Sciences*, 25(1): 65–72.

Uzun, Y. (2023). '' The checklist of Turkish *Pezizales* species'', *Anatolian Journal of Botany*, 7(1): 1-20.

Van Vooren, N. (2010). Notes sur le genre *Helvella* L. (*Ascomycota*, *Pezizales*) Le sous-genre *Elasticae. Bulletin mycologique et botanique Dauphiné-Sa voie,* 199: 27–60.

Wang, X. C., Liu, T. Z., Chen, S. L., Li, Y. and Zhuang, W. Y. (2019). A four-locus phylogeny of rib-stiped cupulate species of *Helvella* (*Helvellaceae*, *Pezizales*) with discovery of three new species. *MycoKeys,* 60: 45–67.

Zhao, Q., Sulayman, M. and Zhu, X. (2016). Species clarification of the culinary Bachu mushroom in western China. *Mycologia,* 108: 828–836.