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Supplementary material

Discovery of spontaneously fruiting Chinese black truffles in a hazelnut plantation in Bhutan

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Supplementary Table S1 - Climate data of Mongar district (Bhutan). Monthly Average Rainfall (mm) (a); Monthly Average Maximum Temperature (°C) (b); Monthly Average Minimum Temperature (°C) (c); Monthly Average Sunshine Hours. Data from National Center of Hydrology and Meteorology, Royal Government of Bhutan.

a) Monthly average rainfall (mm)

Month	2019	2020	2021	2022	2023	Mean
January	0.0	18.6	3.1	3.1	0.0	4.96
February	42.0	46.4	2.8	64.4	5.6	32.24
March	34.1	43.4	40.3	3.1	62.0	36.58
April	105.0	96.0	18.0	63.0	57.0	67.80
May	179.8	251.1	136.4	68.2	24.8	132.06
June	60.0	171	75.0	168	168.0	128.40
July	406.1	226.3	251.1	133.3	86.8	220.72
August	80.6	93.0	105.4	49.6	176.7	101.06
September	138.0	63.0	30.0	96.0	42.0	73.80
October	15.5	18.6	151.9	124.0	62.0	74.40
November	0.0	9.0	6.0	0.0	0.0	3.00
December	0.0	3.1	18.6	3.1	9.3	6.82

b) Monthly average maximum temperature (°C)

Month	2019	2020	2021	2022	2023	mean
January	17.5	14.6	15.4	15.5	16.2	15.84
February	16.9	17.2	19.6	15.1	18.4	17.44
March	20.2	20.6	21.3	24.4	20.2	21.34
April	22.9	21.6	25.7	23.1	22.9	23.24
May	23.0	23.0	24.1	24.7	24.2	23.80
June	26.8	24.5	25.6	23.7	25.2	25.16
July	24.7	23.5	25.5	27.0	25.6	25.26
August	28.3	26.9	24.6	27.0	24.7	26.30
September	24.3	24.4	27.1	25.1	27.1	25.60
October	22.4	24.4	24.3	22.5	23.7	23.46
November	20.4	20.2	19.6	20.3	21.8	20.46
December	15.5	16.1	16.6	17.1	17.7	16.60

c) Monthly average minimum temperature (°C)

Month	2019	2020	2021	2022	2023	Mean
January	5.8	5.4	7.1	5.4	6.9	6.12
February	7.4	7.2	8.2	5.2	9.6	7.52
March	9.4	10.4	11.2	12.6	11.0	10.92
April	13.5	12.3	13.0	14.8	13.5	13.42
May	15.9	15.0	15.9	16.4	15.1	15.66
June	18.1	18.0	18.5	18.0	18.4	18.20
July	18.4	18.7	18.7	19.2	18.9	18.78
August	19.0	19.3	18.8	19.1	19.0	19.04
September	17.7	18.4	18.0	17.9	18.7	18.14
October	14.2	15.9	15.5	14.9	14.6	15.02
November	12.0	10.3	10.1	10.2	10.9	10.70
December	6.3	7.6	7.2	8.4	8.5	7.60

d) Monthly average sunshine hours

Month	2019	2020	2021	2022	2023	Mean
January	8.0	5.4	3.9	5.7	5.9	5.78
February	5.2	5.0	6.1	5.0	4.5	5.16
March	5.0	5.5	4.5	5.9	4.6	5.10
April	5.1	4.5	6.6	3.7	6.2	5.22
May	2.6	3.8	2.9	4.4	4.4	3.62
June	5.0	3.1	3.1	1.6	3.3	3.22
July	3.0	1.2	2.3	5.2	2.4	2.82
August	5.7	4.5	2.0	5.0	2.2	3.88
September	2.9	2.5	6.0	4.2	5.2	4.16
October	5.5	6.4	5.6	5.4	6.8	5.94
November	5.7	7.1	6.7	8.9	8.4	7.36
December	6.0	4.1	5.9	5.7	5.9	5.52

Supplementary Table S2 - Soil characteristics of the hazel orchard where the truffles were collected (data from the Royal University of Bhutan).

Soil horizon	OM %	C %	pH H_2O	EC mS cm^{-1}	Texture (USDA)	Sand %	Silt %	Clay %	N %	P ppm	K ppm
Topsoil	8.10	13.97	6.82	0.05	Clay Loam	60	0	50	0.70	0.76	27
Sub-soil	4.84	8.34	6.98	0.03	Clay	30	2	68	0.42	0.98	25

Supplementary Table S3 - Oligonucleotide primers and PCR cycling conditions used in this study.

Primer	Sequence (5' – 3')	Target gene	PCR amplification conditions	Reference
ITS1F	CTTGGTCATTTAGAGGAAGTAA	ITS rRNA	95 °C 6 min; 34 x (94 °C 30 s, 56 °C 30 s, 72 °C 1 min), 72 °C 7 min	Gardes and Bruns (1993) White et al. (1990)
ITS4	TCCTCCGCTTATTGATATGC	ITS rRNA		
bRPB2-6F	TGGGGKWTGGTYTGYCCTGC	RPB2	94 °C 5 min; 35 x (94 °C 30 s, 52 °C 30 s, 72 °C 50 s), 72 °C 10 min	Denton et al. (1998) He and Yang (2021)
bRPB2-7R	CCCATWGCYTGCTTMCCCAT	RPB2		
EF1-983F	ACHGTRCCRATACCACCRATCTT	TEF1-α	94 °C 5 min; 35 x (94 °C 30 s, 52 °C 30 s, 72 °C 75 s), 72 °C 10 min	Rehner and Buckley (2005) He and Yang (2021)
EF1-1567R	GCYCCYGGHCAYCGTGAY	TEF1-α		

Supplementary Table S4 - Species, lineage voucher specimen, country, and corresponding GenBank accession numbers (ITS, TEF1-α, RPB2) of the *Tuber* specimens used for the phylogenetic analysis. Sequences generated in this study are in bold.

Taxon Name	Lineage	Voucher Specimen	Country	ITS	RPB2	TEF1-α
Tuber sp.	melanosporum	5501	Buthan	**PZ142840**	**PZ149757**	**PZ149755**
Tuber sp.	melanosporum	5503	Buthan	**PZ142841**	**PZ149758**	**PZ149756**
T. borchii	puberulum	BJTC FAN217	New Zealand	KT067681[a]	OM584229[b]	KT067717[a]
T. brumale	melanosporum	GB52	Italy	HM485345[c]	JQ954494[d]	–
T. brumale	melanosporum	GB53	Italy	FJ748900[e]	JQ954495[d]	–
T. formosanum	melanosporum	BJTC FAN107	Yunnan, China	MF621549[f]	OM584210[b]	OM649564[b]
T. formosanum	melanosporum	BJTC FAN356	Sichuan, China	MF627986[f]	OM584252[b]	OM649594[b]
T. longispinosum	melanosporum	K225	Japan	AB553414[g]	AB553558[g]	AB553538[g]
T. longispinosum	melanosporum	K447*	Japan	AB553423[g]	–	–
T. melanosporum	melanosporum	GB200	Italy	FJ748904[e]	JQ954515[d]	JX022594[d]
T. melanosporum	melanosporum	AQUI10152**	Italy	NR200421[h]	–	–
T. melanoumbilicatum	melanosporum	YNAU017*	Yunnan, China	OK625304[i]	OR832411[i]	OR832379[i]
T. melanoumbilicatum	melanosporum	YNAU018	Yunnan, China	OK625305[i]	OR832412[i]	OR832380[i]
T. microexcavatum	melanosporum	YNAU1263	Yunnan, China	OR250184[i]	OR832413[i]	OR832381[i]
T. microexcavatum	melanosporum	YNAU1264	Yunnan, China	OR250185[i]	OR832414[i]	OR832382[i]
T. pseudobrumale	melanosporum	YAASL3181*	Yunnan, China	KJ742703[l]	–	–
T. pseudobrumale	melanosporum	YNAU0228	Yunnan, China	OR665411[i]	OR832428[i]	OR832396[i]
T. pseudobrumale	melanosporum	YNAU0240	Yunnan, China	OR665415[i]	OR832432[i]	OR832400[i]
T. pseudohimalayense	melanosporum	YNAU1608	Yunnan, China	PP784763[i]	PP796861[i]	PP796851[i]
T. pseudohimalayense	melanosporum	YNAU1663	Yunnan, China	PP784764[i]	PP796862[i]	PP796852[i]
T. sinense	melanosporum	BJTC FAN108	China	MF627968[f]	OM584211[b]	OM649565[b]
T. sinense	melanosporum	BJTC FAN110	China	MF627970[f]	OM584212[b]	OM649566[b]
T. variabilisporum	melanosporum	BJTC FAN362*	Sichuan, China	OM287845[b]	OM584253[b]	OM649595[b]
T. variabilisporum	melanosporum	YNAU1670	Yunnan, China	PP784762[i]	PP796860[i]	PP796850[i]
T. yigongense	melanosporum	BJTC FAN729	Tibet, China	MF663716[f]	OM584277[b]	OM649623[b]
T. yigongense	melanosporum	BJTC FAN731*	Tibet, China	MF663714[f]	–	–
T. yunnanense	melanosporum	YNAU019*	Yunnan, China	OK625306[i]	OR832407[i]	OR813081[i]
T. yunnanense	melanosporum	YNAU020	Yunnan, China	OK625307[i]	OR832408[i]	OR813082[i]
T. yunnanense	melanosporum	YNAU0107	Yunnan, China	OR665397[i]	OR832409[i]	OR813083[i]
T. yunnanense	melanosporum	YNAU0491	Sichuan, China	OR250186[i]	OR832410[i]	OR813084[i]

*holotype; **epitype. References: [a]Fan et al. (2016); [b]Fan et al. (2022); [c]Bonito et al. (2010); [d]Bonito et al. (2013); [e]Bonito et al. (2011); [f]Fan et al. (2018); [g]Kinoshita et al. (2011); [h]Leonardi et al. (2021); [i]Wang et al. (2024); [l]Li et al. (2014)

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