



LC-MS/MS profiling of the poisonous wild mushroom, *Entoloma mastoideum*, (Entolomataceae, Basidiomycota) in Sabah (Northern Borneo), Malaysia

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ABSTRACT

In Malaysia, mushroom poisoning poses a significant public health concern, particularly as local communities frequently consume wild mushrooms without fully recognizing the potential dangers they present. Ingestion of wild mushrooms, which resemble edible species but contain deadly chemicals, is the main cause of poisoning risk. The genus *Entoloma* (Fr.) P. Kumm is dispersed worldwide, with over 1500 species recorded. *Entoloma mastoideum*, a poisonous mushroom, was recorded for the first time at Kota Marudu district, Sabah, Malaysia, where ten cases of mushroom poisoning outbreaks were recorded during the rainy season of October 2019. The morphological characters of the specimens were documented and their microscopic features were analyzed using compound microscope and scanning electron microscope. A phylogenetic tree was constructed using Maximum likelihood and Bayesian analysis. Chemical profiling of the poisonous mushroom specimen was done via liquid chromatograph mass spectrometry (LC-MS/MS) to identify toxic metabolites. The phylogenetic analysis showed that the Bornean *E. mastoideum* is closely related to the Chinese isolates (100% BS/1.0 PP). LC-MS/MS profiling detected a total of 162 metabolites that were classified into ten general groups, where several toxic compounds were detected amongst the aromatics, essential amino acids, and fatty acid derivatives. The toxic compounds identified in the mushroom extract, including amino acid derivatives such as 3,4,5,6-tetrahydroxyynorleucine, valpromide, and betaine, have been reported to cause neurotoxicity, cardiotoxicity, liver damage, and gastrointestinal harm. The presence of these toxic compounds underscores the need for caution when consuming wild mushrooms. Further research on poisonous *Entoloma* species is vital for developing accurate identification methods and understanding their toxic potential. This knowledge is essential for enhancing public awareness, preventing mushroom poisoning incidents and safeguarding public health.

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1. Introduction

Globally mushroom poisoning remains a significant health concern, with fatalities reported annually, particularly in regions such as parts of Asia, Europe and North America where foraging is common (Saviuc and Danel, 2006; Diaz, 2005). The number of annual fatalities worldwide remains alarming with the estimation of 100 people die every year, where most deaths reported in Europe and China (White et al., 2019; Dadpour et al., 2017; Diaz, 2005). The global impact of mushroom poisoning is further complicated by the difficulty in diagnosing and treating it, as symptoms can range from mild gastrointestinal distress to life-threatening organ failure (Iseki et al., 2024). Globally, the most dangerous mushrooms include the *Amanita* species, such as *Amanita phalloides* (death cap), and *Amanita virosa* (destroying angel), both of which contain lethal toxins that cause organ failure. Other harmful species like *Gyromitra esculenta* (false morel) and various *Cortinarius* species also contribute to the global burden of mushroom poisoning (Wennig et al., 2020; Anantharam et al., 2016; Escudié et al., 2007). There are also several reports on severe gastrointestinal symptoms after consuming *Entoloma* species (Parnmen et al., 2024; Wennig et al., 2020; Govorushko et al., 2019; İşiloğlu et al., 2011). In Malaysia, mushroom poisoning is a significant public health concern, particularly as local communities frequently consume wild mushrooms without fully understanding the associated risks. The most common poisonous mushrooms in Malaysia are *Amanita* species and *Chlorophyllum molybdites* and to date, there have been no reported cases of *Entoloma* poisoning.

The genus *Entoloma* (Fr.) P. Kumm. is extremely species-rich and widely distributed across regions ranging from temperate to tropical climates (Gates and Noordeloos, 2007; Horak, 1980; Noordeloos, 2004). A small number of *Entoloma* species—pink-gilled mushrooms with tetrasporic basidia—have been shown to regularly produce two sterigmata on their basidia (Hesler, 1963, 1967; Hongo, 1957; Horak, 1980; Noordeloos, 1992; Romagnesi and Gilles, 1979). A significant portion of *Entoloma* species is ectomycorrhizal, while the majority are saprophytic (Co-David et al., 2009). Approximately 1500 species have been described for the genus globally (Kirk et al., 2008; Co-David et al., 2009), with 75 of those species having been reported from Malaysia (Lee et al., 2012). Records of *Entoloma* spp. in Sabah (Northern Borneo) were limited to the highland regions of Mount Kinabalu, Mesilau, and Kundasang (Horak, 1980; Lee et al., 2012). *Entoloma* mushrooms pose a challenge, as some species are toxic while others are safe for consumption. *Entoloma sarcopum*, for instance, is acknowledged as a suitable edible choice. However, food poisoning cases have occurred in Japan because of its similarities to deadly equivalents like *E. rhodopolium* and *E. sinuatum* (Aoki et al., 2020). On the other hand, historical reports of poisoning linked to *Entoloma* species, including *E. sericeum*, indicate that some *Entoloma* mushrooms may in fact cause gastrointestinal problems (Sai Latha et al., 2018). An effort has been made to establish reliable criteria for distinguishing between poisonous and edible *Entoloma* species by examining various aspects, including macroscopic and microscopic characteristics, as well as ecological factors like habitat preference (Morozova and Pham, 2023). Additionally, multi-locus phylogenetic analyses have been utilized to confirm species identification (Parnmen et al., 2024). Research has emphasized the significance of precise identification techniques by concentrating on genetic markers and phylogenetic studies to differentiate between them (Aoki et al., 2020). Furthermore, studies using toxicity evaluations have examined the potentially harmful properties of *Entoloma* mushrooms, emphasizing the need for caution while eating wild mushrooms. Reports of mushroom poisoning in some countries, such as Thailand and China, have included cases involving *Entoloma* species, highlighting the significance of understanding the difference between poisonous and edible mushrooms (Li et al., 2021). Wild mushrooms are highly valued in Malaysia for their unique taste and nutritional benefits. However, foraging wild mushrooms comes with significant risks, primarily due to the difficulty in distinguishing between edible and poisonous species. In Sabah, the

practice of mushroom foraging, or mycophilia, is less widespread among local communities compared to European or Western countries (Peintner et al., 2013; Foo et al., 2018). However, some rural communities continue to incorporate wild mushrooms into their daily meals when they find them, particularly in forested areas or near oil palm plantations close to their homes.

In 2019, an incident of mushroom poisoning was reported in the Kota Marudu district of Sabah. The cases occurred during the rainy season, specifically in October and November. Medical health officers in Sabah collected mushroom samples, which were subsequently analyzed by our team to identify the species involved. Morphological and genetic analyses confirmed the species as *Entoloma mastoideum*, a species first reported in China in 2013 (He et al., 2011). This study provides a comprehensive phylogenetic analysis and morphological description of the species, along with the detection of toxic metabolites using LC-MS/MS.

2. Materials & methods

2.1. Sample collections and morphological identification

Notification of a mushroom poisoning outbreak involving one family was received by the Kota Marudu District Health Office on October 15, 2019, from the closest district hospital. Cases were interviewed and Hospital Kota Marudu medical records were examined as part of the study. *Entoloma mastoideum* was collected by medical health inspectors at Kota Marudu, the samples were brought to the UMS Mycology laboratory. Fresh collections were photographed and described with color notations according to Kornerup and Wanscher (1978). The samples were dried using a food dehydrator (Primada Food Dehydrator MDP68) at 45 °C for 12 h. Macroscopic descriptions are based on the fresh basidiomata and original records of the collections. Spores, basidia and cystidia were observed in 5% KOH or 1% Congo Red (He et al., 2011). The pileipellis was examined in 5% KOH. Spore dimensions are based on the measurements of 20 basidiospores. Spore length to width ratios is reported as Q. The dried studied specimens (BORH(F)03187 and BORH(F)00453) were deposited in the Fungal Herbarium BORNEENSIS (BORH(F)), Institute for Tropical Biology and Conservation (UMS). A field photo with compound microscope and Scanning Electron Microscope (SEM) images were illustrated for the samples from Sabah.

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA (10–50 mg) was extracted from the dried specimens (N = 2) using E.Z.N.A Fungal DNA Kit (Omega Bio-Tek, USA) according to the manufacturer's protocol. The internal transcribed spacer regions (ITS) were amplified with fungal primer pair ITS1F/ITS4 (White et al., 1990) and the large subunit of nuclear ribosomal RNA gene (28S) was amplified with fungal primer pair LROR/LR7 (Vilgalys and Sun, 1994). Gene regions were amplified in 50 µL reactions containing 3 µL templated DNA, 27.75 µL ddH₂O, 4 µL of primers and 15.25 µL PCR mix were performed in a C 1000 thermal cycler (Bio-Rad, Hercules, CA). The PCR amplicons then sent to Apical Scientific (Seri Kembangan, Selangor) for Sanger sequencing.

2.3. Phylogenetic analysis

Four newly generated sequences (ITS and 28S) were subjected to Basic Local Alignment Search Tool (BLASTn). All sequences were determined in both directions to produce consensus sequences with BioEdit Sequence Alignment Editor Version 7.2.5 (Hall, 1999). A total of 33 sequences were retrieved from GenBank. The accession numbers of the ITS and 28S sequences used in the analysis are provided in Table 1. *Clitopilus hirneolus* and *Lyophyllum leucophaeum* were used as the out-group (Kondo et al., 2017). Sequences were manually edited and aligned using AliView (Larsson, 2014). Maximum likelihood (ML) was

Table 1

Details of the available voucher numbers and GenBank accession numbers for the sequences used in this study.

Species	Collection No.	Origin	GenBank Accessions		References
			ITS	28S (LSU)	
<i>Entoloma araneosum</i>	MEN200314	Belgium	KC710056	GQ289153	Morgado et al. (2013)
<i>E. araneosum</i>	GDGM28823	China	JQ320113	JQ410329	Unpublished
<i>E. azureosquamulosum</i>	GDGM27355	China	JQ410333	JQ410325	He et al. (2012)
<i>E. azureosquamulosum</i>	HKAS53408	China	JQ410334	JQ410326	He et al. (2012)
<i>E. caespitosum</i>	GDGM27564	China	JQ281477	JQ320133	He et al. (2012)
<i>E. caespitosum</i>	GDGM24025	China	JQ281490	JQ320130	He et al. (2012)
<i>E. caespitosum</i>	GDGM24026	China	JQ281491	JQ410327	He et al. (2012)
<i>E. chalybeum</i>	RBG Kew K (M) 90810	England	EU784215	–	Brock et al. (2009)
<i>E. coelestinum</i>	HMLD1659	China	KC257434	–	Unpublished
<i>E. cyanostipitum</i>	GDGM31294	China	KY972700	KY972693	He et al. (2017)
<i>E. cyanostipitum</i>	SAAS:2239	China	KY711238	KY972694	He et al. (2017)
<i>E. cyanostipitum</i>	GDGM31318	China	KY711237	KY972695	He et al. (2017)
<i>E. insidiosum</i>	L376	Norway	KC898443	–	Morozova et al. (2014)
<i>E. luteum</i>	GDGM27698	China	JQ281486	–	He et al. (2012)
<i>E. madidum</i>	985	Italy	JP907990	–	Osmundson et al. (2013)
<i>E. madidum</i>	MEN2004030	Netherlands	KC710127	–	Morgado et al. (2013)
<i>E. mastoideum</i>	GDGM26597	China	JQ291564	JQ410328	He et al. (2012)
<i>E. mastoideum</i>	GDGM28820	China	JQ281476	JQ320126	He et al. (2012)
<i>E. mastoideum</i>	BORHF03187	Sabah, Malaysia	PP506494	PP506546	This Study
<i>E. mastoideum</i>	BORHF00453	Sabah, Malaysia	PP506488	PP506497	This Study
<i>E. omiense</i>	GDGM27563	China	JQ281487	JQ410330	He et al. (2012)
<i>E. omiense</i>	GDGM27229	China	JQ291566	JQ320124	He et al. (2012)

Species	Collection No.	Origin	GenBank Accessions		References
			ITS	28S (LSU)	
<i>E. petchii</i>	HKAS56716	China	JQ281485	–	He et al. (2012)
<i>E. praegracile</i>	GDGM29251	China	JQ281482	JQ3210129	He et al. (2012)
<i>E. praegracile</i>	GDGM29256	China	JQ320107	–	Unpublished
<i>E. subclitocybooides</i>	D180	Thailand	OR066300	OR066328	Unpublished
<i>E. subclitocybooides</i>	D179	Thailand	OR066299	OR066327	Unpublished
<i>E. subclitocybooides</i>	D544	Thailand	OR066302	OR066329	Unpublished
<i>E. subclitocybooides</i>	GDGM26615	China	JQ320112	MT829099	Unpublished
<i>E. subclitocybooides</i>	GDGM16677	China	JX992850	JQ320135	Unpublished
<i>E. subtenuicystidiatum</i>	GDGM28459	China	JQ320109	JQ320132	Unpublished
<i>E. subtenuicystidiatum</i>	GDGM29246	China	JQ320114	JQ3210116	He et al. (2012)
<i>Clitopilus hirneolus</i>	MEN199956	Italy	KC710132	GQ289211	Morgado et al. (2013)
<i>Lyophyllum leucophaeatum</i>	Hae251.97	n/a	AF357032	AY2007228	Hofstetter et al. (2002)

Note: –=Not Available.

performed for both gene regions separately by using RAXML-HPC2 version 8.2.12 (Stamatakis, 2006) with GTR model and 1000 rapid bootstrap replicates. Bayesian inference analysis was performed using MrBayes 3.2 (Ronquist et al., 2012). The best fit model of nucleotide evolution was estimated using JModelTest2 in the CIPRESS portal based on AIC (Akaike Information Criterion) and the selected model was GTR+I+G. Bootstrap values (BS) ($\geq 70\%$) and posterior probability (PP) ($\geq 90\%$) were considered significantly supported. Phylogenetic trees were visualized using Figtree v1.4.4 and the tree was edited using Adobe Illustrator v27.6.1. Sequence alignment was deposited at TREEBASE (<http://purl.org/phylo/treebase>; submission ID 3107).

2.4. Chemical analysis

2.4.1. LC-MS/MS chemical profiling

The dried sample was macerated in methanol (100 %, v/v) for a week. The methanol solution was then filtered using Whatman No 1 filter paper, followed by *in-vacuo concentration* using a rotary evaporator. The resulting dried extract, at a concentration of 100 $\mu\text{g/mL}$, was analyzed using Liquid Chromatography (LC) on a Dionex UltiMate 3000 UHPLC system equipped with a Thermo Synchronis C18 column (1.7 μm particle size x 100 mm length x 2.1 mm diameter). The column temperature was set at 55 $^{\circ}\text{C}$, and a 2 μL injection volume was applied. Solvent A (water with 0.1% HCOOH) and solvent B (acetonitrile with 0.1% HCOOH) were used as the mobile phase at a flow rate of 450 $\mu\text{L}/\text{min}$. The LC program started at 0.5% solvent B for 1 min, then increased to 99.5% in 15 min and held for 4 min. The column was conditioned for

2 min before the next injection.

Data acquisition utilized Thermo Scientific Q Executive HF Orbitrap MS/MS in data-dependent acquisition mode as in the previous study (Venmathi Maran et al., 2023). Instrument calibration was performed, and data was processed using Thermo Scientific Compound Discoverer 3.0 software. Default settings included background subtraction, retention time alignment, feature detection, elemental composition analysis, library matching, and fragment ion search. Compound identification relied on MS/MS spectral matching against the mzCloud and mzVault databases, with unmatched signals reprocessed using the Chem Spider database and supported by FISh scoring above 50. (Venmathi Maran et al., 2021).

3. Results

3.1. Case reports

On October 15, 2019, a family from Koromoko Village in Kota Marudu, Sabah, was reported to have suffered from mushroom poisoning. The family, consisting of individuals aged between 4 and 21 years, collected wild mushrooms from the forest near their home, particularly from a site close to a termite mound. Mistaking the mushrooms for an edible variety, they undercooked and ingested them. Within 2–3 h of consumption, all family members developed symptoms consistent with food poisoning, including gastrointestinal disturbances. The patients presented with symptoms of nausea, vomiting, lethargy, headache, dizziness and drooling of saliva. The attack rate was 100%.

Two individuals were admitted to Hospital Kota Marudu for observation and were discharged the following day. Additionally, three members in the pediatric age group were referred to a tertiary hospital, where they were observed and discharged after two days. All patients received symptomatic treatment, and no fatalities were reported. Subsequent analysis identified the mushroom species responsible for the poisoning as *Entoloma mastoideum*. The mycotoxins present in *Entoloma mastoideum* are known to induce muscarinic effects upon ingestion. The case report was published by Mutthumanickam et al. (2019).

3.2. Phylogenetic analysis

Four sequences were generated (ITS and 28S) for the construction of phylogenetic trees. Single gene phylogenies from Maximum Likelihood analyses of the ITS, 28S and multigene datasets (ITS+28S) were constructed. Both the individual genes and multigene dataset were congruent. The Maximum Likelihood and Bayesian inference analysis trees generated from all analyses were largely congruent. Only the Maximum Likelihood of ITS tree topologies is illustrated as the main figure (Fig. 1) and the 28S (Fig. S1) and concatenated datasets of ITS+28S (Fig. S2) trees are shown in supplementary. The aligned dataset of ITS contains 489 total characters, of which 241 were constant, 489 variable characters were parsimony-uninformative and 357 were parsimony-informative. The phylogenetic analysis of ITS suggested that

the two Bornean specimens of *Entoloma* sp. from this study were closely related to the *Entoloma mastoideum* from China (100% BS; 1.0 PP) (Fig. 1).

3.3. Macroscopic and microscopic features of *E. mastoideum* (Fig. 2)

Pileus 2–9 cm broad, campanulate when young, expanding with age to umbonate pileus, never depressed, slightly translucent-striate, pinkish to pale pinkish (9A2–9A4, 10A2–10A4), slightly paler towards the margin, with membranous margin exceeding gills. **Lamellae** edge sterile, sub-free to adnexed, sub-ventricose, moderately crowded, white to pale pink, up to 7 mm deep, edge concolorous and even. **Lamellulae** present, 2–3 tiers. **Stipe** central, cylindrical, paler than pileus, white, ivory to yellowish (1A2–1A3), 3–10 × 0.5–0.8 cm, hollow, fragile. Context thin, whitish.

Basidiospores 9.3–11.5 (–13) × 7.5–9.5(–10.8) μm, 6–8- angled with irregular pronounced angles in side-view, thick-walled, Q = 1.5 μm, heterodiametrical. **Basidia** clavate, 4-spored, sometimes 2-spored, 35–40.2 × 9.3–12.0 μm. Lamellar trama regular. **Cheilocystidia** in clusters, terminal cells cylindrical to narrowly clavate, 16–40 × 3.5–8 μm, without pigment. **Pleurocystidia** absent. **Pileipellis** is a cutis of repent hyphae (5–15 μm diam.), with yellow internally encrusting pigment. Oleiferous hyphae are numerous. Clamp connections absent.

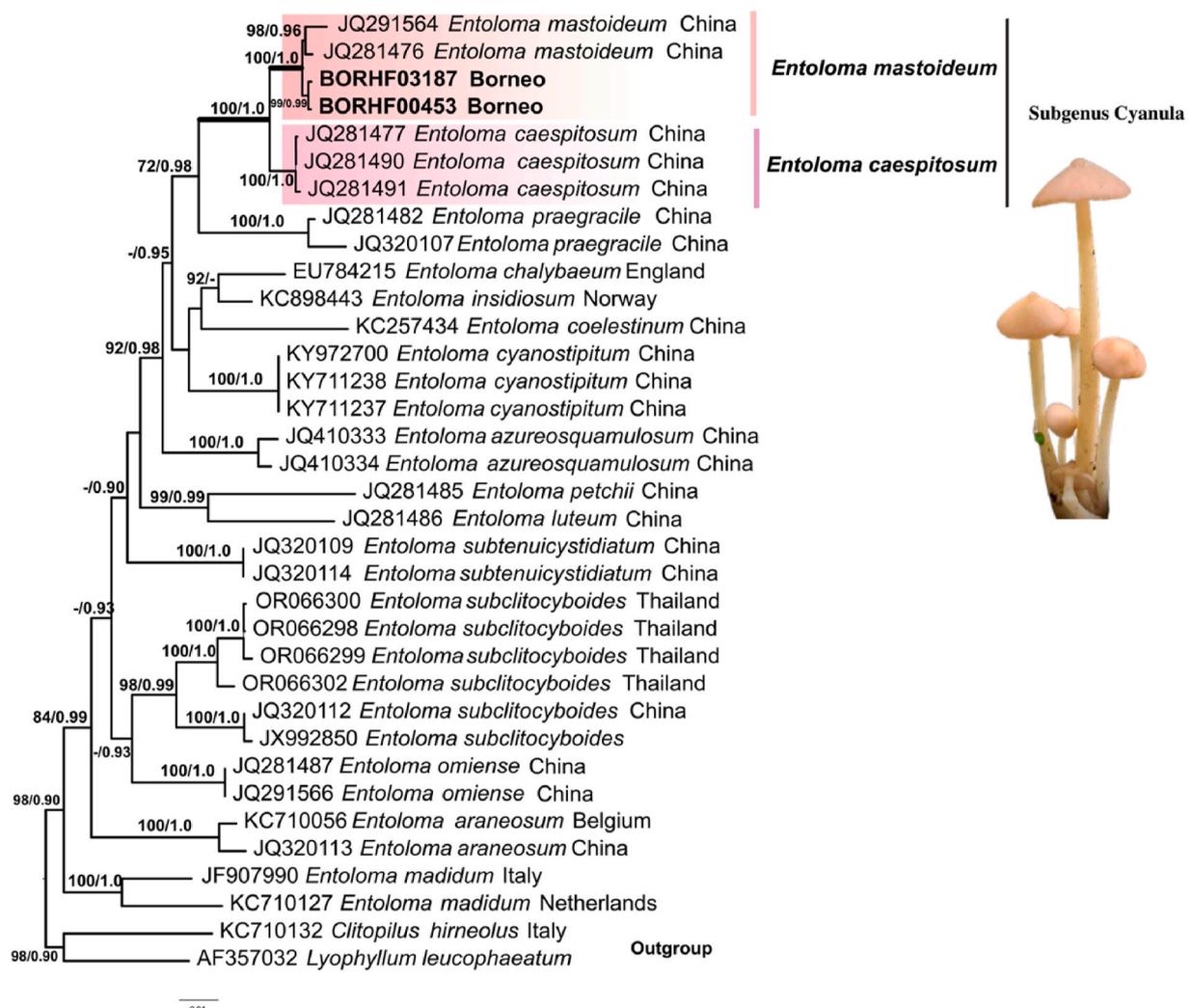


Fig. 1. Maximum Likelihood (ML) analysis of ITS of *Entoloma* spp. and related taxa. Support values are obtained from ML bootstrap values (BS ≥ 70) and Bayesian Posterior Probability (PP ≥ 0.90). Sequences from this study are indicated in bold.

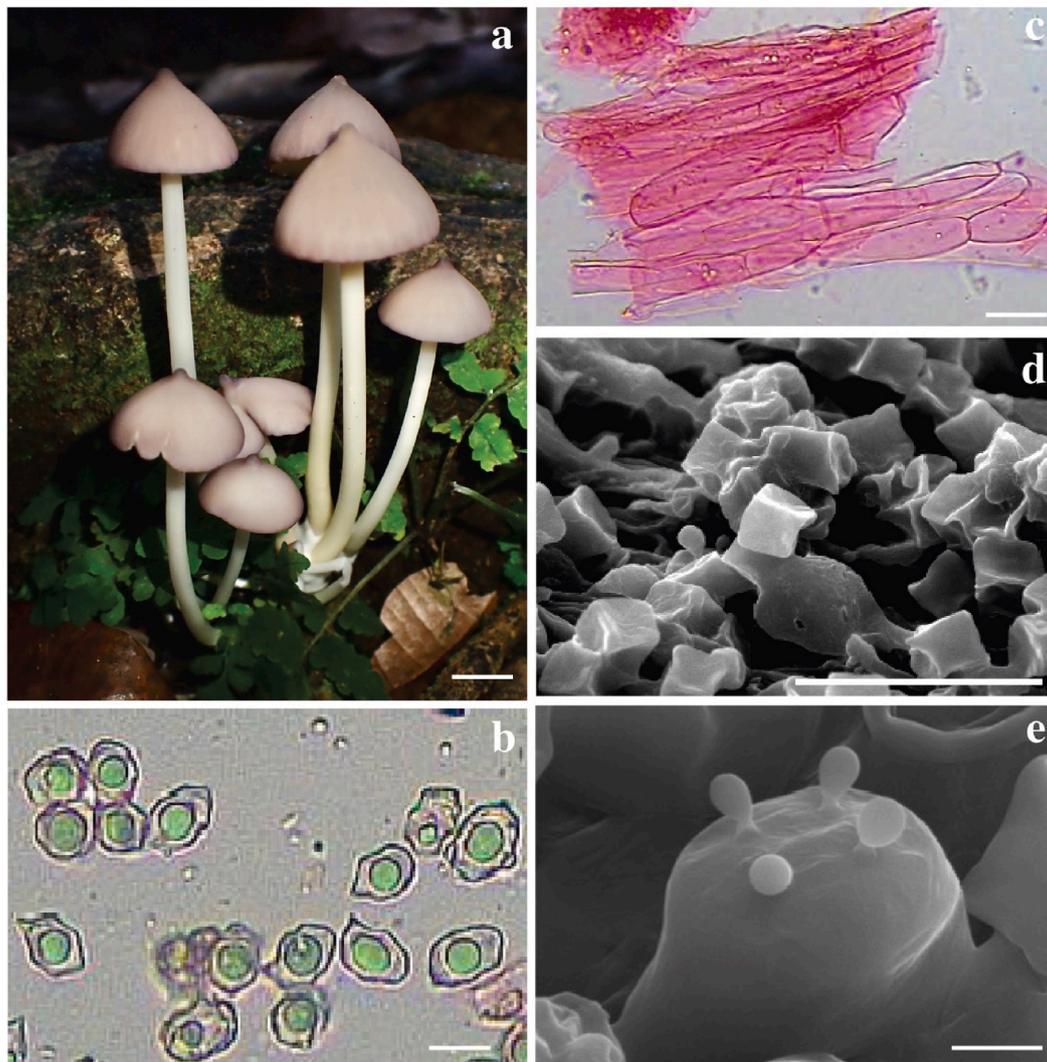


Fig. 2. (a) Basidiocarps of *Entoloma mastoideum*, (b, d) Basidiospores, (c) Pileipellis, (e) Basidia. Scale bar: (a, b, c) = 1 cm, (d) = 50 μm , and (e) = 5 μm .

3.3.1. Material examined

MALAYSIA. Sabah, Kota Belud, Kadamaian, Kadamaian Forest, 27 October 2019, Jaya Seelan, BORH(F) UMS-00453; 28 November 2019, JKNS, BORH(F) UMS-03187.

3.4. LC-MS/MS chemical profiling

Chemical profiling of the *E. mastoideum* methanolic extracts detected a total of 162 metabolites from 10 large chemical groups comprising amino acids, aromatic derivatives, fatty acids and their derivatives, heterocyclic compounds, oxo-fatty acids, peptides, phenolics, vitamin B3 and other hydrocarbons derivatives. The percentage substance detected for each chemical group is provided in Fig. 3. A list of the detected metabolites is provided in the supplementary materials (Tables S1–S4).

4. Discussion

This study provides a new record of *Entoloma mastoideum* from Northern Borneo (Sabah), in addition to the other species reported by Horak (1980) from Mount Kinabalu, Mesilau. Contrary to the previous study (Horak, 1980), the *Entoloma* species in Sabah were found in the mountainous region elevations above 1900 m whereas the *Entoloma* in this study were collected from Koromoko Village, Kota Marudu

elevations below 300 m. This suggests that the distribution of *Entoloma* species is not only confined to the mountainous region but also distributed at different altitudes in Sabah.

The new record of *E. mastoideum* is well supported by morphological evidence and phylogenetic analysis. The morphological characteristics of Bornean *E. mastoideum* in comparison to the Chinese *E. mastoideum* and the sister group *E. caespitosum* are presented in Table 2. The characteristics of the Bornean *Entoloma* species were indistinguishable from the Chinese *E. mastoideum* (He et al., 2011). Bornean *E. mastoideum* have similar basidiospores size but have a bigger size of four-spored basidia compared to the Chinese *E. mastoideum* with only two-spored basidia. *Entoloma mastoideum* from Borneo was found near termite mounds covered in sandy loamy habitat similar to the Chinese *E. mastoideum* which reported growing on sandy loamy soil (He et al., 2011). No morphological description or ecological information is available for the specimen from Thailand (Parmen et al., 2022).

Based on the phylogenetic analysis, the Bornean *E. mastoideum* were closely related to *E. mastoideum* from China with a high support value (100% BS/100% PP) under the subgenus *Cyanula*, one of the better-defined subgenera within *Entoloma* in terms of genetics, ecology, and morphology. Species in this subgenus lack clamp connections in their hyphae and the color of the pileus frequently have vivid and highly variable color (from blue and violaceous to brown, yellow, green, and pink), with squamulose-fibrillose pileus (Noordeloos, 2004; Noordeloos

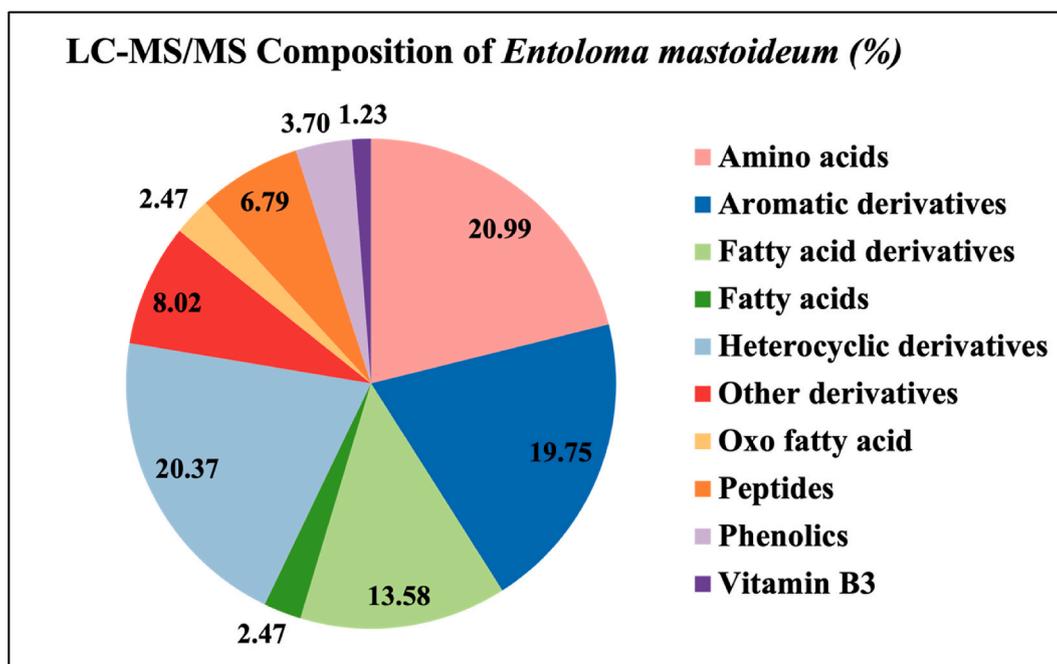


Fig. 3. Percentage of substance detected for each chemical group classification in the *E. mastoideum* methanolic extract.

Table 2

Comparison of microscopic characteristics between Bornean *Entoloma mastoideum* and Chinese *E. mastoideum* and closely related species of *E. caespitosum* from China.

Species	Voucher No.	Basidiospores	Basidia	Location	References
<i>Entoloma mastoideum</i>	BORHF00453	Angled with irregular pronounced angles in side-view, thick-walled 9.0–11(-12) × 7.7–9.8 (–11.0) μm	Clavate, 4 spored 37–41 × 11–12.1 μm	Kota Marudu, Sabah (Borneo)	This study
<i>E. mastoideum</i>	BORHF03187	Angled with irregular pronounced angles in side-view, thick-walled 9.3–11.5 (–13) × 7.5–9.5(-10.8) μm	Clavate, 4 spored 35–40.2 × 9.3 × 12.0 μm	Kota Marudu, Sabah (Borneo)	This study
<i>E. mastoideum</i>	GDGM26597	Angled with irregular pronounced angles in side-view, thick-walled 9.5–12(-13) × 7–8.0(-8.8) μm	Clavate, 2 spored 25–34 × 9–11.5 μm	China	He et al. (2011)
<i>E. caespitosum</i>	GDGM24026	Angled with blunt angles in profile view 8.5–10.5 × 6–7.5 μm	Clavate, 4 spored, sometimes 2 spored. 30–45 × 8–12 μm	China	He et al. (2012)

et al., 2022).

Entoloma mastoideum was first described by He et al. (2011) in China, although its toxicity was not established at the time. However, Parmen et al. (2022) later identified *E. mastoideum* as one of the toxic mushrooms in Thailand, associated with both gastrointestinal and neurotoxic effects. The specific neurotoxins present in *E. mastoideum* remain unidentified. In this study, chemical profiling of methanolic extracts from *E. mastoideum* revealed a total of 162 metabolites, classified into ten major chemical groups, including amino acids, aromatics, fatty acids, oxo-fatty acids, fatty alcohols, fatty amides, peptides, phenolics, heterocyclic compounds, and vitamin B3, as well as acetamide, carnitines, and choline.

The heterocyclic compounds (20.37%) and the amino acids (20.99%) made up the largest composition of the mushroom extract followed by the aromatics (19.75%) and fatty acid derivatives (13.58%). It is noteworthy that despite being regarded as a poisonous mushroom, the high percentage of aromatics in *E. mastoideum* which makes them fragrant may cause confusion and therefore be mistaken for safe consumption. Some of the notable aromatic metabolites are such as ectocarpene which is a sexual attractant, or pheromone that naturally has a fruity scent. The detection of benzamide probably contributes to a bitter taste in the mushroom and the waxy nature of the *Entoloma* spp. could be contributed by the presence of dihydrocapsaicin, a lipophilic colourless

compound normally associated with *Capsicum* and *Ganoderma lucidum* (Reyes-Escogido et al., 2011; Yang et al., 2019).

The toxic compounds identified in the mushroom extract have been associated with neurotoxicity, cardiotoxicity, hepatic damage, and gastrointestinal harm, particularly at moderate or high dosages. Among these, molecule 3,4,5,6-tetrahydroxynorleucine which is associated with toxicity in the poisonous mushroom *Amanita* sp. (Karimi and Razavi, 2014). Additionally betaine which was also identified is an amino acid previously isolated from the poisonous mushroom *Clitocybe acromelalga* (Konno et al., 1984). Valpromide has been reported to exhibit neurotoxicity at high doses (Payen et al., 2004). Similarly, linoleamide and oleamide have shown cytotoxic effects (Bertin et al., 2012). While 2,4-Diaminotoluene is known for its immunotoxicity (Bums et al., 1994). Agmatine, also previously reported from mushrooms, causes skin irritation, neurological dysfunction and severe bradycardia (Furer et al., 2011; Uzbay et al., 2017), antihyperglycemic effect through increased insulin secretion and glucose uptake in muscles, and preventing hepatic gluconeogenesis was also detected in the analyzed *Entoloma* extract (Hussein et al., 2023). The analyzed *E. mastoideum* interestingly contained tracers of nicotine such as nicotinic acid, nicotinamide and hydroxycotinine. For instance, hydroxycotinine is the main nicotine metabolite detected in smokers' urine. Such a finding is not the first as previously dried button mushrooms,

Agaricus bisporus were reported to contain nicotine as well. Nicotine is capable of increasing the blood pressure, heart rate, and flow of blood to the heart and nicotinic acid can result in nausea, diarrhoea dizziness (Van Elst et al., 2023). Mass spectrometry also identified validamycin A (Val-A) which is an effective antifungal agent widely used in Asian countries as a crop protectant. This is certainly an interesting finding since Val-A has only previously been reported from the fermentation broth of *Streptomyces hygroscopicus* and previously from the hydrolysate of antibiotic validamycin A with sulfuric acid (Zheng et al., 2012). This is the first report from *E. mastoideum*.

Contrary to the toxic nature of *E. mastoideum* as determined through the negative effect of human health, the mushroom is greatly aromatic, and high in essential amino acids, fatty acids and their derivatives. The list of metabolites provided highlights the variety of constituents detected in *E. mastoideum* but may not necessarily be the source of toxicity. For instance, betaine and agmatine can also present beneficial effect on human health (Arumugam et al., 2021; Valverde et al., 2021). For nicotine, it has been also reported in many edible mushrooms. Trace nicotine cannot damage human health (Gwenzi et al., 2021). Regardless, since consumption of this mushroom has been associated to poisoning, we recommended that *E. mastoideum* is not suitable as part of the diet. A bioassay guided assessment followed by isolation of causative chemicals along with toxicology evaluation is essential to definitively confirm the presence of toxic compounds in poisonous mushrooms, enabling better identification, treatment and prevention of mushroom poisoning incidents.

Entoloma mastoideum was previously identified as a poisonous mushroom in Thailand (Parmen et al., 2022), but this is the first report from Sabah, Northern Borneo, confirming the presence of toxins in this species. In addition to its toxic compounds, the Bornean *E. mastoideum* was found growing on termite mounds, offering new insights into its habitat preferences. These unique habitat characteristics have led to its misidentification as an edible species by local villagers.

In Malaysia, mushroom poisoning has been recognized as a public health problem that threatens human health, especially in Sabah. It is important to create awareness on poisonous mushrooms, especially on the importance of screening for the toxic components. Educating the community about the potential dangers of poisonous mushrooms is crucial to prevent mushroom poisoning incidents and strengthening health education on mushroom toxicity is particularly vital in rural areas. Ongoing public health education on the risks of consuming poisonous mushroom, along with the importance of maintaining personal and environmental health is needed to prevent any food poisoning outbreak in future.

5. Conclusion

This study confirmed the new record of *Entoloma mastoideum* from Sabah (Northern Borneo) based on morphological characteristics together with phylogenetic analyses. The metabolite presence also detected using LC-MS/MS analysis confirms the presence of toxic constituents that outweighs the vital metabolites detected in the mushroom making it unsuitable for consumption. Detecting and documenting poisonous mushrooms contribute to epidemiological surveillance, aiding in the monitoring and management of mushroom poisoning cases. Bornean *E. mastoideum* was identified as the first neurotoxic group in the list of poisonous mushrooms in Malaysia.

CRedit authorship contribution statement

Ily Azzedine Alaia M.H. Subari: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Gowri Muthumanickam:** Writing – review & editing. **Muhammad Hj. Jikal:** Writing – review & editing. **Muhammad Dawood Shah:** Investigation. **Jen Kit Tan:** Writing – original draft, Investigation. **Yee Shin Tan:**

Writing – review & editing, Methodology, Investigation. **Praneetha Palasubermaniam:** Writing – original draft, Investigation, Conceptualization. **Kishneth Palaniveloo:** Writing – original draft, Investigation, Conceptualization. **Jaya Seelan Sathiya Seelan:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Ethical statement

- 1) This material is the authors' own original work, which has not been previously published elsewhere.
- 2) The paper is not currently being considered for publication elsewhere.
- 3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
- 4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
- 5) The results are appropriately placed in the context of prior and existing research.
- 6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
- 7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

I agree with the above statements and declare that this submission follows the guidelines of Toxicon as outlined in the Guide for Authors and in the Ethics and policies.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jaya Seelan Sathiya Seelan reports financial support was provided by University of Malaysia Sabah. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.toxicon.2024.108133>.

Data availability

Data will be made available on request.

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