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Morphology and multigene phylogeny revealed *Peroneutypa aquilariae* sp. nov. (Diatrypaceae, Xylariales) from *Aquilaria sinensis* in Yunnan Province, China

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Abstract

Dead twigs of *Aquilaria sinensis* (Thymelaeaceae) with fungal fruiting bodies were collected from Xishuangbanna, Yunnan Province, China. After initial morphological observations, an interesting fungus whose morphologically resembled *Peroneutypa* was isolated. Molecular phylogeny of combined ITS and tub2 showed our fungal collection is phylogenetically closely related to *P. mackenziei*. However, in morphology, our fungal collection is distinct from *P. mackenziei* in having an ostiolar canal without periphyses and the absence of paraphyses. Based on unique morphological characteristics and multigene phylogenetic analyses results, our fungal isolate is described in this paper as *Peroneutypa aquilariae* sp. nov. In addition, this is the first report of the genus *Peroneutypa* from the host *A. sinensis*. Full description, illustrations, and a phylogenetic tree to show the placement of the new species are provided. A synoptic table of morphological characteristics in *Peroneutypa* reported worldwide is also provided.

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INTRODUCTION

Sordariomycetes O.E. Erikss. & Winka, is the second largest class in Ascomycota Caval.-Sm., characterized by unitunicate asci and are widely distributed in almost all ecosystems as endophytes, pathogens, or saprobes[1]. Hyde et al.[1], accepted 45 orders, 167 families, 1,499 genera and 308 genera *incertae sedis* in Sordariomycetes, while in the latest outline by Wijayawardene et al.[2], the numbers were increased to 46 orders, 184 families, 1,594 genera and 346 genera *incertae sedis*. In Sordariomycetes, there are seven subclasses viz. Diaporthomycetidae Senan., Maharachch. & K.D. Hyde, Hypocreomycetidae O.E. Erikss. & Winka, Lulworthiomycetidae Dayar., E.B.G. Jones & K.D. Hyde, Pisorisporiomycetidae Bundhun, Maharachch. & K.D. Hyde, Savoryellomycetidae Hongsanan, K.D. Hyde & Maharachch., Sordariomycetidae O.E. Erikss & Winka, and Xylariomycetidae O.E. Erikss & Winka[2].

Xylariales Nannf. is a large order and the only order in the subclass Xylariomycetidae which was accepted by Maharachchikumbura et al.[3] and later Amphispheriales and Xylariales were accepted in Xylariomycetidae by Samarakoon et al.[4] and Hongsanan et al.[5] based on phylogenetic analyses and divergence time estimations. Later, Delonicicolales R.H. Perera, Maharachch. & K.D. Hyde was introduced into Xylariomycetidae based on phylogenetic analyses by Perera et al.[6]. According to Wijayawardene et al.[2], currently, 20 families are listed in this order.

Diatrypaceae Nitschke is one important family of higher ascomycetes that belongs to Xylariales[3]. Diatrypaceae was introduced by Nitschke[7] and is typified by *Diatrype* Fr. Diatrypaceae is characterized by perithecial black stroma, ascomata usually embedded in stroma, cylindric-clavate to clavate asci and allantoid ascospores in its sexual morph[8–12]. The asexual morph is characterized by coelomycetous; acervuli conidiomata, erumpent, with branched conidiophores, conidiogenous cells in dense palisades, cylindrical with filiform conidia, curved, flattened base, blunt apex, and hyaline[1,3]. Zhu et al.[13] showed that Diatrypaceae has high diversity, and the members of this family are usually wood inhabiting fungi in China. According to Wijayawardene et al.[2], currently, 22 genera are listed in this family.

Peroneutypa Berl. was introduced by Berlese[14] to accommodate *P. bellula* (Desm.) Berl., *P. corniculata* (Ehrh.) Berl and *P. heteracantha* (Sacc.), without designating the type species until Rappaz[9] designated *P. bellula* as the type species and considered *Peroneutypa* as a synonym of *Eutypella* (Nitschke) Sacc. Later, based on morphological characteristics and phylogenetic analyses of Acero et al.[15], Carmarán et al.[16] resurrected *Peroneutypa* as an independent genus, and transferred eight species from *Eutypella* and *Echinomyces* Rappaz to *Peroneutypa* (viz: *P. alsophila* (Durieu & Mont.) Carmarán & A.I. Romero, *P. arecae* (Syd. & P. Syd.) Carmarán & A.I. Romero, *P. comosa* (Speg.) Carmarán & A.I. Romero, *P. curvispora* (Starbäck) Carmarán & A.I. Romero, *P. gliricidiae*

(Rehm) Carmarán & A.I. Romero, *P. kochiana* (Rehm) Carmarán & A.I. Romero, *P. scoparia* (Schwein.) Carmarán & A.I. Romero and *P. obesa* (Syd. & P. Syd.) Carmarán & A.I. Romero). Later, based on molecular phylogenetic analyses, several studies have clarified that *Peroneutypa* is a monophyletic group in Diatrypaceae[12,16–20]. *Peroneutypa* is characterized by poorly developed ascostromata, perithecia ascomata with long prominent necks, 8-spored, clavate asci with sessile to subsessile, and allantoid, hyaline or yellowish ascospores[11,12,16–24]. Recently, a new species (*P. polysporae* Devadatha, V.V. Sarma & E.B.G. Jones) isolated from decaying wood of *Suaeda monoica* in India was introduced by Dayarathne et al.[25]. This species has polysporous asci which differ from the generic description (8-spored asci), thus this information shows that *Peroneutypa* can accommodate taxa with polysporous asci[25]. The members of *Peroneutypa* are known as saprobes or pathogens and are widely distributed in terrestrial and marine habitats[12,25–28]. Currently, 35 species are recorded in Index Fungorum[29], with only 16 species having molecular data.

In this study, a new species, *Peroneutypa aquilariae* is introduced based on morphological characteristics and phylogenetic analyses of combined ITS and tub2 gene sequences. To our knowledge, this is the first *Peroneutypa* species from an *Aquilaria* host. In addition, a synoptic table of morphological characteristics in *Peroneutypa* is also provided.

MATERIALS AND METHODS

Sample collection, morphological study, and single spore isolation

The dead twigs of *Aquilaria sinensis* with fungal fruiting bodies were collected from Xishuangbanna, Yunnan Province, China in autumn. The specimens were placed in plastic bags and brought to the mycology laboratory. Morphological structures were examined under an OPTEC SZ650 dissecting stereomicroscope. Fruiting bodies were picked up with needles, hand sectioned by razor blades and mounted on a glass slide with water. OLYMPUS optical microscope (Japan) was used to observe microscopic fungal structures and an OLYMPUS DP74 (Japan) digital camera fitted to the microscope was used to take photographs. All micromorphological structures were measured with Tarosoft® Image Framework program and photo plates were made using Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA).

Senanayake et al.[30] was followed for the single spore isolation under sterile conditions. Once the spores were germinated, clear photographs were taken using the camera microscope and the germinated spores were transferred to a new potato dextrose agar (PDA) medium with a sterilized needle, and then incubated at 28 °C. Culture characteristics on PDA were observed after one month. The fresh mycelia of the fungus were used for DNA extraction.

The herbarium specimen was deposited in Kunming Institute of Botany Academia Sinica (HKAS), China, while the living cultures were deposited in Kunming Institute of Botany Culture Collection (KUNCC), China. Facesoffungi (FoF) was registered as described in Jayasiri et al.[31], and MycoBank number (MB) was registered as outlined in MycoBank (www.MycoBank.org).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fungal mycelium (from one-month old cultures) by using Biospin Fungus Genomic DNA Extraction Kit–BSC14S1 (BioFlux®, China) following the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify two gene regions using primers following Shang et al.[12] and Dayarathne et al.[25], with some modifications (Table 1). The total volume of PCR mixtures for amplifications following Du et al.[32], 25 μL containing 12.5 μL 2x Master Mix (mixture of Easy Taq TM DNA Polymerase, dNTPs, and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, China), 8.5 μL ddH_2O, 2 μL of DNA template, 1 μL of each forward and reverse primers (10 pM). Purification and sequencing of PCR products were carried out by Qinke Biotech Co., Yunnan, China.

Phylogenetic analyses

Dissanayake et al.[35] was followed for the phylogenetic analyses. The nucleotide BLAST search in GenBank (www.ncbi.nlm.nih.gov) was used to select the most closely related taxa. The sequences of the closely related taxa to the new taxon, which were used in the phylogenetic analyses (Table 2) were obtained from GenBank based on recently published data[19,20]. Multi-gene phylogenetic analyses were carried out based on combined ITS and tub2 genes, including 82 sequences and two outgroup taxa (*Xylaria atrosphaerica* (Cooke & Massee) Callan & J.D. Rogers (AFTOL-ID 51) and *Xylaria hypoxylon* (L.) Grev. (CBS 122620)). Multiple alignments of these sequences were automatically made with MAFFT v. 7 at the web server (http://mafft.cbrc.jp/alignment/server), using default settings[36]. Automatic cutting was done in trimAl.v1.2rev59, and manually combined the multiple sequences in BioEdit v. 7.0.5.2[37]. The multiple sequences were converted using ALTER (www.sing-group.org/ALTER/)[38].

Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian inference analyses (BI) were carried out in the CIPRES Science Gateway (www.phylo.org/portal2/login!input.action)[39]. The ML trees are performed via RAxML-HPC2 on XSEDE (8.2.12)[40,41] with GTR+I+G model of evolution and bootstrap supports were obtained by running 1000 pseudoreplicates. The BI tree was conducted by Markov Chain Monte Carlo (MCMC) in MrBayes (v3.2.a)[42] to evaluate posterior probabilities[43,44], and the best model was GTR+I+G. Six simultaneous Markov chains were run for one million generations, and trees were sampled every 100th generation. The first 20% of generated trees representing the burn-in phase of the analyses were discarded and the rest were used to calculate posterior probabilities (PP). The phylogenetic tree was visualized with FigTree v1.4.2 (http://tree.bio.ed.ac.uk/

Table 1. The primers and thermal cycles of PCR used in this study.

Gene/Locus	Primers (Forward/Reverse)	Thermal cycles	Reference
Internal transcribed spacers (ITS)	ITS5/ITS4	94 °C: 3 min, (94 °C: 30 s, 55 °C: 50 s, 72 °C: 90 s) × 35 cycles, 72 °C: 10 min, final 4 °C	[33]
β-tubulin (tub2)	T1/Bt2b		[34]

Table 2. Species, strain numbers, and GenBank accession numbers used in the phylogenetic analyses.

Species	Strain number	ITS	tub2	Reference
Allocryptovalsa castanea	CFCC 52427	MW632944	–	[13]
Allocryptovalsa castanea	CFCC 52428^{T}	MW632945	–	[13]
Allocryptovalsa castanea	CFCC 52429	MW632946	–	[13]
Allocryptovalsa castaneicola	CFCC 52432^{T}	MW632947	–	[13]
Allocryptovalsa cryptovalsoidea	HVFIG02^{T}	HQ692573	HQ692524	[34]
Allocryptovalsa cryptovalsoidea	HVFIG05	HQ692574	HQ692525	[34]
Allocryptovalsa elaeidis	MFLUCC 15-0707^{T}	MN308410	MN340296	[46]
Allocryptovalsa polyspora	MFLUCC 17-0364^{T}	MF959500	MG334556	[17]
Allocryptovalsa rabenhorstii	WA07CO	HQ692620	HQ692522	[34]
Allocryptovalsa rabenhorstii	WA08CB	HQ692619	HQ692523	[34]
Allocryptovalsa sichuanensis	HKAS 107017^{T}	MW240633	MW775592	[20]
Allocryptovalsa truncata	PUFNI 17639^{T}	MK990279	–	[1]
Allodiatrype arengae	MFLUCC 15-0713^{T}	MN308411	MN340297	[46]
Allodiatrype elaeidicola	MFLUCC 15-0737a^{T}	MN308415	MN340299	[46]
Anthostoma decipiens	JL567	JN975370	JN975407	[47]
Anthostoma decipiens	IPV-FW349	AM399021	AM920693	Unpublished
Cryptosphaeria eunomia	CBS 216.87^{T}	KT425230	KT425165	[48]
Cryptosphaeria eunomia	CBS 223.87	KT425231	KT425166	[48]
Cryptovalsa ampelina	A001	GQ293901	GQ293972	[49]
Cryptovalsa ampelina	DRO101	GQ293902	GQ293982	[49]
Diatrypasimilis australiensis	ATCC MYA-3540^{T}	NR111369	–	[50]
Diatrype disciformis	GNA14	KR605644	–	[23]
Diatrype disciformis	MFLU 17-1549	MW240629	–	[20]
Diatrypella elaeidis	MFLUCC 15-0279	MN308417	MN340300	[46]
Diatrypella verruciformis	UCROK1467	JX144793	JX174093	[51]
Eutypa lata	RGA01	HQ692614	HQ692497	[34]
Eutypa lata	EP18	HQ692611	HQ692501	[34]
Eutypa microasca	BAFC: 51550^{T}	KF964566	KF964572	[52]
Eutypa microasca	BAFC: 51551	KF964565	KF964570	[52]
Eutypa microasca	BAFC: 51556	KF964567	KF964571	[52]
Eutypella cerviculata	M68	JF340269	–	[53]
Eutypella semicircularis	MP4669	JQ517314	–	[54]
Halocryptovalsa salicorniae	MFLUCC 15-0185^{T}	MH304410	MH370274	[25]
Halodiatrype avicenniae	MFLUCC 15-0953	KX573916	KX573931	[55]
Halodiatrype salinicola	MFLUCC 15-1277	KX573915	KX573932	[55]
Monosporascus cannonballus	CMM3646	JX971617	–	[56]
Monosporascus cannonballus	ATCC 26931^{T}	FJ430598	–	Unpublished
Neoeutypella baoshanensis	HMAS:255436	MH822887	MH822888	[24]
Neoeutypella baoshanensis	GMBC0052	MW797106	MW814878	[57]
Pedumispora rhizophorae	BCC44877	KJ888853	–	[58]
Pedumispora rhizophorae	BCC44878	KJ888854	–	[58]
Peroneutypa alsophila	EL58C	AJ302467	–	[15]
Peroneutypa aquilariae	**KUNCC 22-10817^{T}**	**OP454038**	**OP572195**	**This study**
Peroneutypa aquilariae	**KUNCC 22-10818**	**OP482262**	**OP572196**	**This study**
Peroneutypa comosa	BAFC:393	KF964568	–	[52]
Peroneutypa curvispora	HUEFS 136877	KM396641	–	[11]
Peroneutypa diminutiasca	MFLUCC 17-2144^{T}	MG873479	MH316765	[18]
Peroneutypa diminutispora	HUEFS 192196^{T}	KM396647	–	[11]
Peroneutypa indica	NFCCI-4393	MN061368	MN431498	[25]
Peroneutypa kochiana	EL53M	AJ302462	–	[15]
Peroneutypa kunmingensis	KUMCC 21-0001^{T}	MZ475070	MZ490589	[19]
Peroneutypa leucaenae	MFLU 18-0816^{T}	MW240631	MW775591	[20]
Peroneutypa longiasca	MFLUCC 17-0371^{T}	MF959502	MG334558	[17]
Peroneutypa mackenziei	MFLUCC 16-0072^{T}	KY283083	KY706363	[12]
Peroneutypa mangrovei	PUFD526^{T}	MG844286	MH094409	[24]
Peroneutypa polysporae	NFCCI-4392	MN061367	MN431497	[25]
Peroneutypa scoparia	MFLUCC 11-0615	KU940152	–	[59]
Peroneutypa scoparia	MFLUCC 17-2143	MG873478	MH316764	[18]
Quaternaria quaternata	EL60C	AJ302469	–	[15]
Quaternaria quaternata	GNF13	KR605645	–	[23]
Xylaria atrosphaerica	AFTOL-ID 51	DQ491487	–	Unpublished
Xylaria hypoxylon	CBS 122620	AM993141	KX271279	[60]

The newly generated sequences are in black bold, superscripted ^{T} indicates ex-type, and '–' indicates sequence unavailable.

software/figtree/)[45], and bootstrap values were shown at the nodes, and edited by Microsoft Office PowerPoint 2010. The newly obtained alignments and phylogenetic trees were deposited in TreeBASE (https://treebase.org/treebase-web/user/submissionList.html, submission ID: 29758).

RESULTS

Phylogenetic analyses

The phylogenetic trees obtained from RAxML and BI gave similar topologies. The RAxML analyses of the combined dataset yielded the best scoring tree (Fig. 1), with a final ML

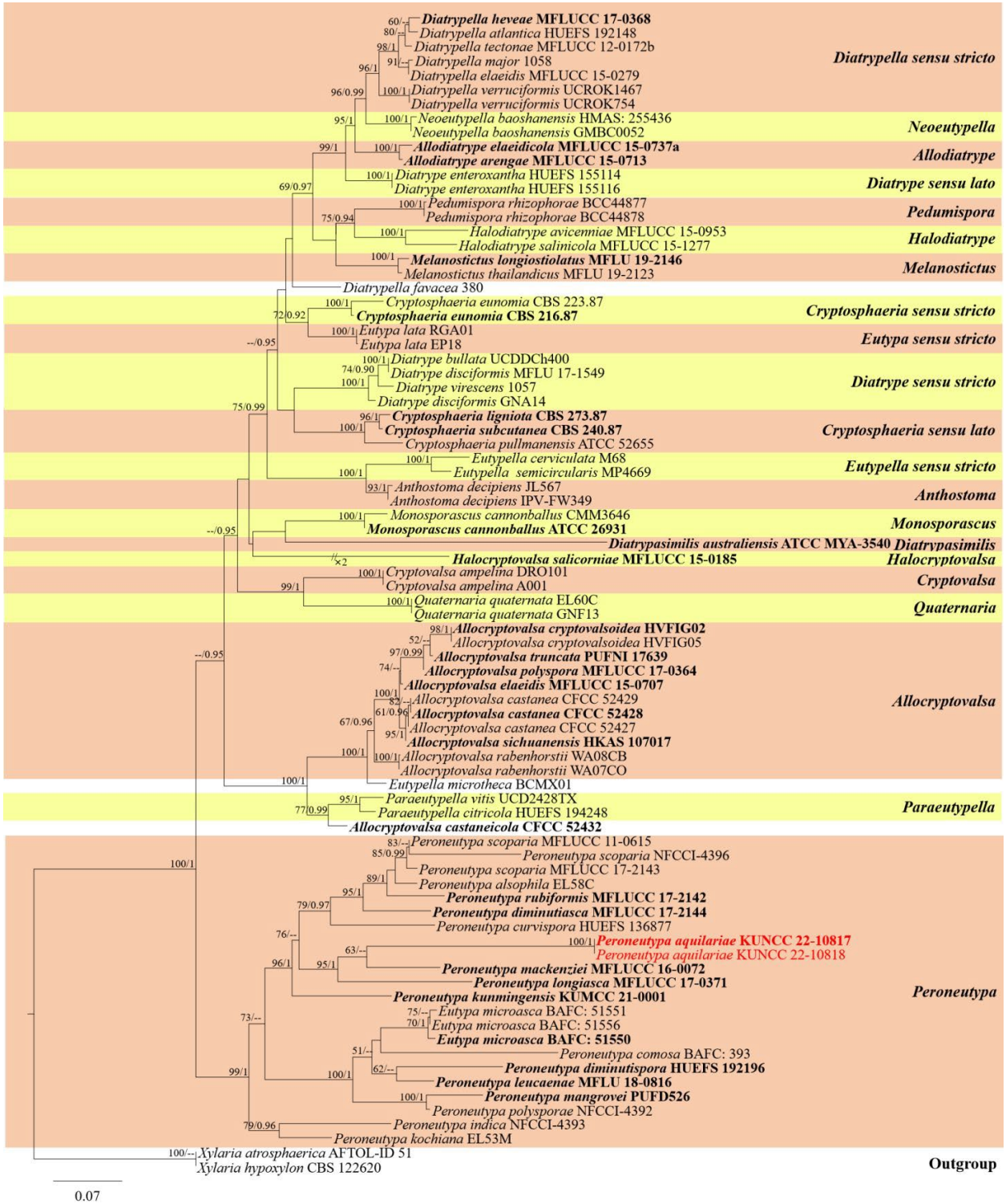

Fig. 1 RAxML tree of Diatrypaceae based on a combined dataset of ITS and tub2 partial sequences. Bootstrap support values for maximum likelihood (ML) equal to or higher than 50% and bayesian posterior probability (BYPP) equal to or higher than 0.90 are indicated above the branches. Newly generated sequences are shown in red, while the type species are shown in bold black and red.

optimization likelihood value of −20,817.822757. The matrix had 1,187 distinct alignment patterns, with 54.28% of undetermined characters or gaps. Parameters for the GTR+I+G model of the combined ITS and tub2 were as follows: estimated base frequencies A = 0.219035, C = 0.277925, G = 0.235904, T = 0.267137; substitution rates AC = 0.957294, AG = 2.875562, AT = 1.254222, CG = 0.889850, CT = 3.867387, GT = 1.000000; proportion of invariable sites I = 0.174656; and gamma distribution shape parameter α = 0.580597.

The final RAxML tree is shown in Fig. 1, which shows that *Peroneutypa* is a monophyletic group in Diatrypaceae, and *Peroneutypa aquilariae* is well separated from other strains in *Peroneutypa*. *Peroneutypa mackenziei* Q.J. Shang, Phook. & K.D. Hyde (MFLUCC 16-0072) is phylogenetically closely related to *P. aquilariae*. In addition, strains of *Eutypa microasca* E. Grassi & Carmarán are grouped within *Peroneutypa* (Fig. 1).

Taxonomy

Peroneutypa aquilariae T.Y. Du & Tibpromma, sp. nov. Fig. 2

MycoBank number: MB845438; Facesoffungi number: FoF12744

Etymology: named after the host genus, *Aquilaria*.

Saprobic on dead twigs of *Aquilaria sinensis* (Thymelaeaceae). Sexual morph: *Ascostromata* 0.5–1.5 mm wide, well-developed interior, solitary to gregarious, mostly solitary, immersed, long ostiolar canal raised through host tissue, black, irregular in shape, arranged irregularly, 1–6 locules. *Ascomata* (excluding necks) 300–570 μm diam., perithecial, immersed in ascostromata, subglobose to globose, dark brown to black. *Ostiolar canal* 20–40 μm wide, without periphysis, filled with hyaline cells, with 200–300 μm long, cylindrical, straight, dark-brown to black necks. *Peridium* 35–70 μm wide, composed of outer layer thick-walled, dark brown to pale brown cells of *textura angularis*, and inner layer thin-walled, hyaline cells of *textura prismatica*. *Paraphyses* absent. *Asci* 15–20 × 5–7 μm ($\bar{x}$ = 18 × 6 μm, n = 20), unitunicate, 8-spored, clavate to cylindrical, thin-walled, short pedicellate or non-pedicellate, apically rounded to truncate with indistinct J-apical ring. *Ascospores* (5–)5.5–7(–7.5) × (1.6–)1.8–2.2 μm ($\bar{x}$ = 6 × 2 μm, n = 30), overlapping 1–3-seriate, hyaline to pale yellow, oblong to allantoid, slightly curved, aseptate, smooth-walled, with granules, ascospores turn yellow after being stained by Melzer's reagent. Asexual morph: Undetermined.

Culture characteristics: Ascospores germinated on PDA within 24 h at a constant temperature incubator (28 °C). Colonies on PDA reaching 6 cm diam., after one week at 28 °C, mycelium white, flossy, circular with entire edge, with filiform margin. After one month, mycelium becomes white to light yellow from above and light brown to brown from below.

Material examined: China, Yunnan Province, Xishuangbanna, on dead twigs of *Aquilaria sinensis* (Thymelaeaceae), 15 September 2021, Tianye Du, YNA3 (holotype, HKAS 124185; ex-type cultures, KUNCC 22-10817 = KUNCC 22-10818).

Notes: The BLASTn search of ITS sequences of our strains is 87.67% similar to *P. mackenziei* (MFLUCC 16-0072, NR_154363). In the present phylogenetic analyses, our strains formed a sister branch with *P. mackenziei* (MFLUCC 16-0072) with a low bootstrap support (63% ML). However, they differ in morphological characteristics i.e. stromata of *P. aquilariae* have well-developed interior, ostiolar canal without periphyse, peridium inner layer comprises of 3–5 hyaline cell layers of *textura prismatica*, and

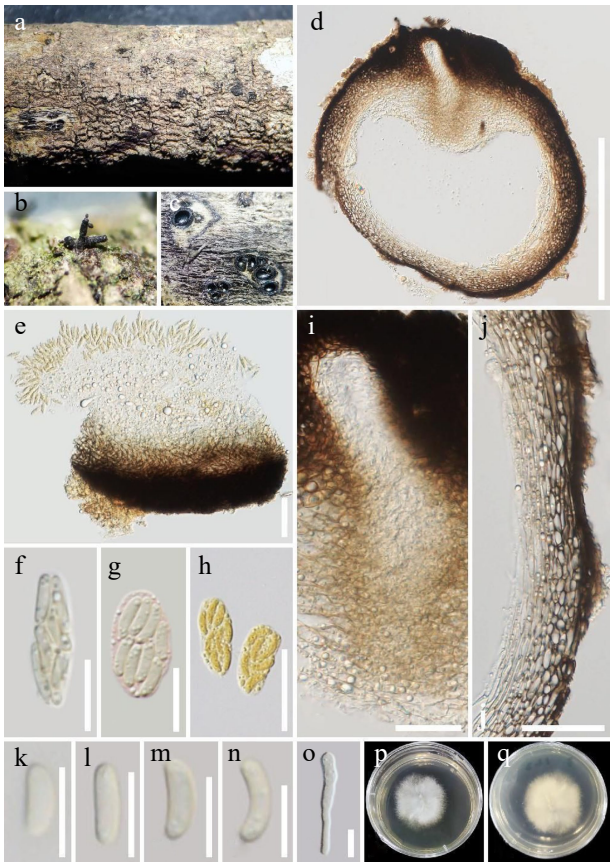

Fig. 2 *Peroneutypa aquilariae* (HKAS 124185, holotype). (a)–(c) Appearance of ascomata on the substrate. (d) Section through an ascoma. (e)–(h) Asci, (g) ascus stained with Congo red reagent, (h) asci stained with Melzer's reagent). (i) Ostiole. (j) Peridium. (k)–(n) Ascospores. (o) A germinating ascospore. (p), (q) Colony on PDA medium (after 7 d in culture). Scale bars: (d) = 300 μm, (e), (j) = 50 μm, (f), (g) = 10 μm, (h) = 20 μm, (i) = 30 μm, (k)–(o) = 5 μm.

paraphyses absent; while *P. mackenziei* has poorly developed interior stromata, ostiolar canal periphysate, peridium inner layer comprises 8–10 hyaline cell layers of *textura angularis*, and hamathecium is composed of paraphyses[12]. Furthermore, a comparison of ITS nucleotides between *P. aquilariae* and *P. mackenziei* (MFLUCC 16-0072) resulted in 13.8% differences (67/487 bp, without gaps), and 54.1% differences (173/320 bp, without gaps) respectively in tub2. Based on both morphological characteristics and multigene phylogenetic analyses results, we introduce *Peroneutypa aquilariae* as a distinct new species and this is the first report of *Peroneutypa* from *Aquilaria sinensis*. We also provide information about countries, hosts, and a comparison of morphological characteristics of *Peroneutypa* in Table 3.

DISCUSSION

The updated phylogenetic tree of this study shows that *Peroneutypa* is a monophyletic genus, with *E. microasca* strains clustered within *Peroneutypa*, and this also is consistent with the previous research results of de Almeida et al.[11], Senwanna et al.[17], and Phukhamsakda et al.[19]. Senwanna et al.[17] considered that *E. microasca* should be listed under *Peroneutypa*. Though we agree with them, fresh specimens of *E.*

Table 3. Morphological characteristics, host, and location information of *Peroneutypa* species.

Species	Ascostromata	Ascomata	Ostiolar canal	Peridium	Paraphyses	Asci	Ascospores	Countries	Hosts	References
P. alsophila	Slightly or well-developed, less than 1 mm long	N/A	Necks not prominent	N/A	N/A	Apical apparatus J+	N/A	Argelia, France	*Arthrocnemum fruticosum*	[15,16]
P. aquilariae	**Slightly or well-developed, 0.5–1.5 mm wide, 1–6 locules**	**300–570 μm (excluding necks)**	**With 200–300 μm long necks, 20–40 μm wide, without periphysis**	**35–70 μm wide**	Absent	**18.3 × 5.8 μm, short pedicellate or non-pedicellate, J- apical ring, apically rounded to truncate**	**6.3 × 2 μm, hyaline to pale yellow, oblong to allantoid**	**China**	***Aquilaria sinensis***	**This study**
P. bellula	Immersed, scattered	Globose	Long necks	N/A	N/A	Minimum, 5–6-spored, hyaline	Oblong, with granules	N/A	*Arundo donax*	[61]
P. coffeae	450–900 × 350–650 μm	N/A	700–1,500 × 80–100 μm	20–25 μm wide	N/A	23–25 × 4–5 μm, pedicellate	4.5–6 × 1–1.5 μm, allantoid	Central African Republic	*Coffea robusta*	[62]
P. comosa	Slightly or well-developed	N/A	Necks very long, over 1 mm, prominent	N/A	N/A	Apical apparatus J+ / J–	N/A	Argentina	*Celtis tala*	[16,52]
P. corniculata	Multi-ascoma	N/A	Prominent	N/A	N/A	N/A	N/A	America	*Prunus melanocarpa*	[9,63]
P. curvispora	Poorly-developed, 0.6–3 mm wide, 22 ascomata per stroma	300–700 μm wide	400–800 μm long, prominent	N/A	Absent	9–16.5 × 4–6 μm, long pedicellate, apex truncate	3–5 × 1–2 μm, allantoid, strongly curved	Brazil	Unidentified wood	[11]
P. cylindrica	3–4 mm, 8–10 locules	1 mm	1–2.5 mm long	N/A	Absent	10–20 × 4–5, clavate, pedicellate	4–5 × 2, hyaline, curved	South Africa	Unidentified wood	[64]
P. cyphelioides	1–3 mm	300 μm	1–2 mm long	N/A	N/A	15 × 4–5 μm	4 × 1 μm, allantoid	Philippines	*Streblus asper*	[65]
P. diminutiasca	With poorly-developed interior, 1.2–1.4 mm wide, 1–10 locules	147–218 μm (excluding necks)	193 × 48 μm wide, long neck, periphysate	15–32 μm wide	4–7 μm wide, dense, septate	22 × 4 μm, long pedicellate, with a J– subapical ring, apically rounded to truncate	4.2 × 1.7 μm, hyaline to pale yellowish, allantoid	Thailand	Unidentified wood	[18]
P. diminutispora	Absent or with poorly-developed, isolated or in groups of up to seven	400–700 μm	200–400 μm long	N/A	Absent	Long pedicellate, with a J– apical rings, apex truncate	Allantoid, slightly to moderately curved, yellowish in mass	Brazil	Unidentified wood	[11]
P. discriminis	1 mm	200 μm	1 mm long	N/A	N/A	12–14 × 4 μm	5–6 × 1.5–2 μm	India, Philippines	*Ficus racemosa*, *Macaranga tanarius*, *Streblus asper*	[65]
P. exigua	N/A	N/A	500–700 μm long	N/A	N/A	11–16 × 3–6 μm (sporiferous part)	3–3.5 × 1–1.5 μm	Brazil	*Citrus aurantium*	[66]
P. gliricidiae	Interior of the stroma not developed	N/A	Necks not prominent	N/A	N/A	10–16 μm, J+	Allantoid	Philippines	*Gliricidia sepium*	[16]
P. heteracanthoides	N/A	300 μm	N/A	N/A	Absent	16 × 3.5 μm	3–4 × 1 μm, allantoid, hyaline, with granules	Singapore	*Cassiae* sp., *Heveae brasiliensis*	[67]
P. indica	Well-developed	375 × 202 μm	100–250 μm wide, with moderate neck 100–350 μm long, periphysate	15–35 μm wide	1–2 μm	42 × 3.5 μm, short pedicellate, apically rounded to truncate, with a J– apical ring	5.5 × 1.3 μm, hyaline, straight to allantoid, light brown in mass	India	*Suaeda monoica*	[25]

(to be continued)

Table 3. (continued)

Species	Ascostromata	Ascomata	Ostiolar canal	Peridium	Paraphyses	Asci	Ascospores	Countries	Hosts	References
P. iranica	0.5–2 mm, 3–16 locules	300–500 μm	40–120 μm, periphysate	30–55 μm wide	Septate, hyaline	13.5 × 3.6 μm, with a J+ apical ring	3.6 × 1 μm, hyaline, allantoid	Iran	*Wisteria sinensis*	[68]
P. kochiana	Interior of the stroma not developed	N/A	Necks not prominent	N/A	N/A	18–28 μm long, J+	Allantoid	Russia, Spain	*Atriplex halimus*	[15,16]
P. komonoensis	N/A	250–450 × 200–350 μm	1–1.2 mm long	N/A	N/A	30–38 × 3.5–4 μm, long pedicellate	3–4 × 1–1.5 μm, allantoid	French Equatorial Africa	*Hevea brasiliensis*	[69]
P. kunmingensis	Absent or with poorly-developed interior	260 × 317 μm (excluding neck)	140 × 100 μm wide, long neck, periphysate	30–60 μm wide	Septate, hyaline	26 × 4.1 μm, straight or slight curved, hyaline, long pedicel up to 16 μm, with a J– apical ring	4.2 × 1.3 μm, hyaline to pale grey, oblong to allantoid	China	Unidentified wood	[19]
P. leucaenae	N/A	655 × 525 μm	275–350 μm long neck, periphysate	22–43 μm wide	3.2–7 μm wide, wider at the base, long, septate,	33 × 4.2 μm, pedicel 17–27 μm long, J+ apical ring	Yellowish-brown, ellipsoidal to cylindrical or elongate-allantoid	Thailand	*Leucaena leucocephala*	[20]
P. lignicola	609–685 μm high, 1,196–1,246 μm, 4–6 locules	448–662 × 355–445 μm	Long neck	62–92 μm wide	N/A	16 × 6 μm, sessile, hyaline	6 × 2 μm, elongate-allantoid, straight to slightly curved	Thailand	On decaying wood submerged in a freshwater stream	[28]
P. longiasca	N/A	180–450 × 170–390 μm	20–50 μm wide, 190–440 μm long	14–47 μm wide	N/A	Short to long pedicellate, apically rounded to truncate with indistinct J– subapical ring	5.8 × 2 μm, hyaline, oblong to allantoid	Thailand	*Hevea brasiliensis*	[17]
P. mackenziei	With poorly-developed interior, 1.2–2.2 mm wide, multi-ascoma	466 × 356 μm	265 × 100 μm, ostiolar canal with long neck, periphysate	45–65 μm wide	2.5–4.5 μm wide, dense, filamentous, aseptate, hyaline	17.7 × 4.2 μm, sessile, apically rounded to truncate, with a J– apical ring	5.6 × 1.6 μm, subhyaline to pale yellowish, elongate allantoid	Thailand	Unidentified wood	[12]
P. macroceras	600–1,500 × 500–650 μm	N/A	1,500–2,500 × 100–120 μm	N/A	N/A	30–35 × 4–4.5 μm, long pedicellate	4–5 × 1.2–1.5 μm, allantoid	Central African Republic	*Coffea robusta*	[62]
P. macrorostrata	1,500–2,000 × 350–500 μm	N/A	N/A	N/A	N/A	25–30 × 4–5 μm, pedicellate	4–6 × 1.5–2 μm, allantoid	French Equatorial Africa	*Hevea brasiliensis*	[69]
P. mangrovei	Absent or poorly-developed, up to four in groups	375 × 202 μm	50–85 μm wide, with moderate neck length 100–350 μm, periphysate	15–35 μm wide	1–2 μm wide, septate, longer than asci	17 × 3.5 μm, short pedicellate, apically rounded to truncate, with a J– apical ring	Hyaline to pale yellow, straight to allantoid	India	*Avicennia marina*	[24]
P. multistromata	400–900 × 200–700 μm	270–450 × 200–300 μm	N/A	N/A	Absent	30–40 × 3–5 μm, (sporiferous part: 14–18 μm), long pedicellate, 8-spored	3–4 × 0.75–1 μm, hyaline, allantoid	Cote d'Ivoire	*Coffea canephora*	[70]

(to be continued)

Table 3. (continued)

Species	Ascostromata	Ascomata	Ostiolar canal	Peridium	Paraphyses	Asci	Ascospores	Countries	Hosts	References
P. obesa	Slightly or well-developed, spiny or bristly appearance to the stromatic surface, black line present	N/A	Necks prominent	N/A	N/A	N/A	3–7 μm long, strongly curved	Argentina, Zaire	Unidentified wood	[16]
P. perseae	500–700 × 300–500 μm, 5–6 locules	300–600 × 150–200 μm	N/A	N/A	Absent	18–26 × 4–6.5 μm, 8-spored, hyaline	5–6 × 1.5–2 μm, hyaline	Morocco	*Persea americana*	[71]
P. philippinarum	2–3 locules	200 μm	300–1000 μm long	N/A	N/A	15 × 4–5 μm	4 × 1.5 μm, allantoid	Philippines	*Streblus asper*	[65]
P. polymorpha	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Cote d'Ivoire	*Manihot esculenta*	[72]
P. polysporae	N/A	286 × 223 μm	113 × 60 μm, periphysate	15–50 μm wide	Septate, hyaline	113 × 12 μm, polysporous	9 × 1.8 μm, hyaline to pale yellow, allantoid	India	*Suaeda monoica*	[25]
P. rubiformis	With poorly-developed interior, 1–1.4 mm wide, 1–10 locules	109 × 159 μm (excluding necks)	176 × 52 μm, roundish, prominent ostioles in the centre, rubus-like or star-like in shape, periphysate	20–25 μm wide	7–11 μm wide, septate, hyaline	50 × 5 μm, short pedicellate, apically rounded to truncate, with a J- subapical ring	4.3 × 1.6 μm, hyaline to pale yellowish, elongate-allantoid	Thailand	Unidentified wood	[18]
P. scoparia	Not developed or with poorly-developed interior,1.2–2 mm wide, 4–8 locules	256 × 266 μm	441 × 105 μm, long necks, prominent, periphysate	20–35 μm wide	Septate, hyaline	22 × 4.5 μm, long pedicellate, apically rounded to truncate, with a J– subapical ring	4.1 × 1.5 μm, oblong to allantoid, sometimes slightly curved	Argentina, Chile, Czech Republic, France, India, Iran, Italy, Paraguay, Sweden, Thailand	*Acer pseudoplatanus*, *Actinidia deliciosa*, *Avicennia marina*, bamboo culms, *Gleditsia sp.*, *Juglans regia*, *Robinia pseudoacacia*, *Vaccinium corymbosum*	[16,18,25,59]
P. variabilis	N/A	400–500 μm	700–2500 × 100–200 μm	N/A	N/A	12–18 × 3 μm (sporiferous part), tapering pedicel	3–5 × 1.5–2 μm, greenish-hyaline, cylindric, curved	Sri Lanka	Unidentified herbaccous	[73]

Host and location information of taxa extracted from the listed references and Farr & Rossman[74]. The symbol "N/A" denotes no information available or not mentioned in given papers, and the new species is in black bold.

microasca need to be recollected in the future and transferred to *Peroneutypa* based on the evidence of morphological comparison and phylogenetic analyses.

Previous studies have reported that it is difficult to distinguish the members of Diatrypaceae by morphology, and many taxa still lack molecular data, which undoubtedly make the classification of Diatrypaceae more difficult[11,17,18]. There are similar difficulties in *Peroneutypa*. At present, 35 species of *Peroneutypa* are listed, and only 16 of them have molecular data (Tables 2 & 3). In particular, *P. lignicola* Z.L. Luo, K.D. Hyde & H.Y. Su (MFLUCC 14-0040) was not included in the phylogenetic analyses of this study as this species has no ITS and tub2 gene sequences, and therefore, more fresh samples need to be collected in the future in order to supplement the known species with molecular data.

In *Peroneutypa*, currently, only ten species have tub2 gene sequences. The nucleotide fragments were compared and the results showed that the tub2 sequences of the ten species were quite different. Even among different strains (MFLUCC 17-2143 and NFCCI-4396) of the same species *P. scoparia*, 9% nucleotide differences (31/344 bp, without gaps) were observed. This may lead to the long branches of most species of this genus in the phylogenetic trees. The reason for the large difference in tub2 sequence might be due to the fact that different primers were selected in different strains, viz. using primers Bt2a/Bt2b: *P. indica* Devadatha, V.V. Sarma & E.B.G. Jones (NFCCI-4393), *P. mackenziei* (MFLUCC 16-0072), *P. mangrovei* Devadatha & V.V. Sarma (PUFD526), *P. polysporae* (NFCCI-4392), and *P. scoparia* (MFLUCC 17-2143); using primers T1/Bt2b: *P. diminutiasca* Q.J. Shang, Phukhamsak & K.D. Hyde (MFLUCC 17-2144), *P. kunmingensis* (KUMCC 21-0001), *P. longiasca* Senwanna, Phookamsak & K.D. Hyde (MFLUCC 17-0371), P. rubiformis Q.J. Shang, Phukhamsak & K.D. Hyde (MFLUCC 17-2142), and P. scoparia (NFCCI-4396); and using primers T1/T22: P. leucaenae (MFLU 18-0816). On the other hand, we suppose the difference of tub2 might be the unique characteristics of this genus, thus the tub2 gene can be used as a good marker in phylogeny. However, this study suggests that researchers working on this genus should use the same tub2 primers for PCR amplification.

In this study, no asexual morph of the new taxon was observed, but in Shang et al.[18], the asexual types of *P. rubiformis* and *P. scoparia* were made in photo plates and descriptions, with the main characteristics of pycnidial conidiomata, cylindrical conidiogenous cells, with holoblastic and hyaline conidia lunate to filiform, or fusiform, curved at the upper part, flattened at the base and blunt apex. The discovery of the asexual morph will be useful in the taxonomic identification of *Peroneutypa* in the future.

Our new species is associated with the genus *Aquilaria*, which belongs to Thymelaeaceae and is famous for producing agarwood[32,75,76]. Agarwood is very expensive and has a wide range of uses worldwide[76]. At present, the production mechanism of agarwood is not clear, but fungi are considered to be involved in producing agarwood[77,78]. Therefore, it is necessary to study the fungi associated with agarwood, including the saprophytic fungi. Saprophytic fungi associated with *Aquilaria* are poorly studied and this study reports the first *Peroneutypa* species from an *Aquilaria* host. In the future, more fresh samples need to be collected for in-depth research on the fungal diversity of *Aquilaria*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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