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Research article


Rediscovery and ITS-barcoding of *Entonaema cinnabarinum* (Xylariales, Ascomycota) from Bulgaria

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Abstract: *Entonaema* is a peculiar and morphologically easily recognised fungal genus with disjunct and predominantly tropical-subtropical distribution. *Entonaema cinnabarinum* is typified on material from Australia and is the sole known species in Europe. It was first reported on this continent three decades ago, based on a collection from Bulgaria and remains so far one of the utmost rare European fungi with merely five sites known on the continent. After diligent search, the Bulgarian *Entonaema* was rediscovered in the area where it was first collected, and new sites were also found. Two ITS rDNA sequences of Bulgarian collections were obtained, appearing to be the first verified accessions of European origin. In the phylogenetic inference they appear closely related to an accession from South Korea, dubbed *E. splendens*. The outcomes of the phylogenetic analysis confirm the self-standing status of *Entonaema*, but its precise affiliation within the order Xylariales remains to be assessed further. Morphological characterisation, ample macroscopic and microscopic illustrations, as well as SEM images of ascospores of the new Bulgarian findings are included.

Keywords: Balkan mycota, biogeography, DNA-barcoding, Hypoxylaceae, taxonomy, xylarialean fungi

Introduction

The genus *Entonaema* A. Möller is easily recognised in the field due to the azonate, filled with liquid and often conspicuously coloured ascostromata (Rogers, 1981; Stadler et al., 2008a). Its members have a mostly subtropical and tropical distribution (Rogers, 1981; Stadler et al., 2008a; Fedosova, 2012). The latest treatment, based on polyphasic approach, recognises six species across the globe (Stadler et al., 2008a).

Entonaema cinnabarinum (Cooke & Massee) Lloyd was described from Australia (Cooke, 1887) and is by far the only known European member of the genus (Rogers, 1981; Stadler et al., 2004, 2008a). It was first reported on this continent from Bulgaria by

Benkert (1993), who found it several years earlier at the estuary of Kamchia River. Shortly after, another finding from the same area was described and illustrated by Læssøe (1997). Apart of the Bulgarian site, merely four more findings are known in Europe, namely in France, Hungary, Russia and Spain (Stadler et al., 2004; Rubio Domínguez & Menéndez Valderey, 2011; Fedosova, 2012; Fintha et al., 2019). A sequence of this species was obtained by Triebel et al. (2005) from a culture of a French collection. It was however found later that the specimen from which this sequence was derived, was contaminated by a *Daldinia* species (Stadler et al., 2014). Two other sequences (AM292043, AM292044) initially released as *E. cinnabarinum* by Šrůtka et al. (2007) were also reassessed later to belong to a species of *Daldinia*

Ces. & De Not. (Pažoutová et al., 2013). Reference sequences of *E. cinnabarinum* are thus not available in public databases at present.

Being aware of the Bulgarian finds of Benkert and Læssøe, the first author searched continuously during his field trips to obtain fresh collections of this interesting species and indeed such were found in the autumn of 2014 at distant sites on the Bulgarian Black Sea coast. Additional observations were conducted in 2015, 2021 and 2022, and further specimens were collected in 2021 and 2022. Having in hands abundant material, we sought to obtain ITS rDNA sequences as to provide phylogenetic data and detailed morphological description of those rare collections. The results are communicated in this paper.

Materials and methods

The collections were photographed in-situ. Air-dried voucher specimens are deposited in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences (SOMF) or in the personal collection of M. Slavova (referred to as 'MSL'). The description is based solely upon the materials studied in this paper. Colours in the description refer to the chart of Kornerup & Wanscher (1978) as far as possible; colour names from this chart are followed by respective codes in parenthesis, beginning with the prefix 'K&W'. Colours lacking such codes are vernacular notations rather than chart entries.

Study of microscopic features was held with AmScope T360B light microscope, equipped with AmScope MU900 digital camera. All microscopic observations were done on dried material. Tap water was preferred as a mounting medium for slides preparation, but KOH (10%), IKI and Melzer's reagent were also used for testing colour reactions or to better visualise microscopic structures. Cotton blue in lactophenol was employed (with all due caution) to observe the germination slit of the spores. Ascospores were always measured in water on slides from spore deposits on stromatal surface. Measurements were taken with Piximetre v. 5.10 on digital images. Scanning electron microscopy (SEM) was performed with JEOL-JSM-5510. Samples for SEM were obtained from spore deposits on stromatal surface of specimen SOMF30878. These were transferred onto double-sided adhesive tape and sputter-coated with gold prior to observation.

Sequence of the rITS region of one of the specimens was obtained following the protocol presented in details in Bozok et al. (2020). Forward and reverse reads were produced. These were visualised, assembled into consensus sequences and edited upon necessity in Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI, USA). The sequencing of another specimen was outsourced to ALVALAB (Spain). The protocol communicated by the laboratory was already presented in another recently published work (Assyov 2022). The new sequences were deposited in GenBank. Their accession numbers are listed after the respective voucher specimens in the text below.

The obtained sequences were first subjected to BLAST search on GenBank (Altschul et al., 1990) and the resulting alignments were examined. The dataset for phylogenetic analysis was composed in BioEdit Sequence Alignment Editor v. 7.2.5 (Hall, 1999). The final dataset consisted of 3 sequences of *Annulohypoxylon* Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, 9 accessions of *Daldinia*, 1 of *Diatrype* Fr., 29 *Entonaema* sequences, 15 of *Hypoxylon* Bull., 3 of *Jackrogersella* L. Wendt, Kuhnert & M. Stadler and 1 accession of *Muscodor* Worapong, Strobel & W.M. Hess (see Supplementary table for details). The phylogenetic analysis was conducted, using the facilities of the 'Phylogeny.fr' platform (<http://www.phylogeny.fr/index.cgi>) in 'A la Carte' mode (Dereeper et al., 2008). It included multiple sequence alignment in MUSCLE 3.8.31 (Edgar, 2004) without Gblocks curation (Castresana, 2000), analysis in PhyML 3.0 with default substitution model (Guindon & Gascuel, 2003), employing the Shimodaira-Hasegawa-like aLRT-test (SH-aLRT) for calculation of branch support (Anisimova & Gascuel, 2006). Branch support values in the tree are considered significant, when ≥ 0.82 (Anisimova et al., 2011; Bellanger et al., 2015). The phylogenetic tree was rendered in TreeDyn 198.3 (Chevenet et al., 2006). Its technical formatting was done in InkScape v. 1.0.2-2.

Results

Analysis of rITS sequences

Two sequences were successfully obtained from specimens from distant Bulgarian localities. They are identical in pairwise comparison. The initial BLAST search retrieved as closest match a sequence from

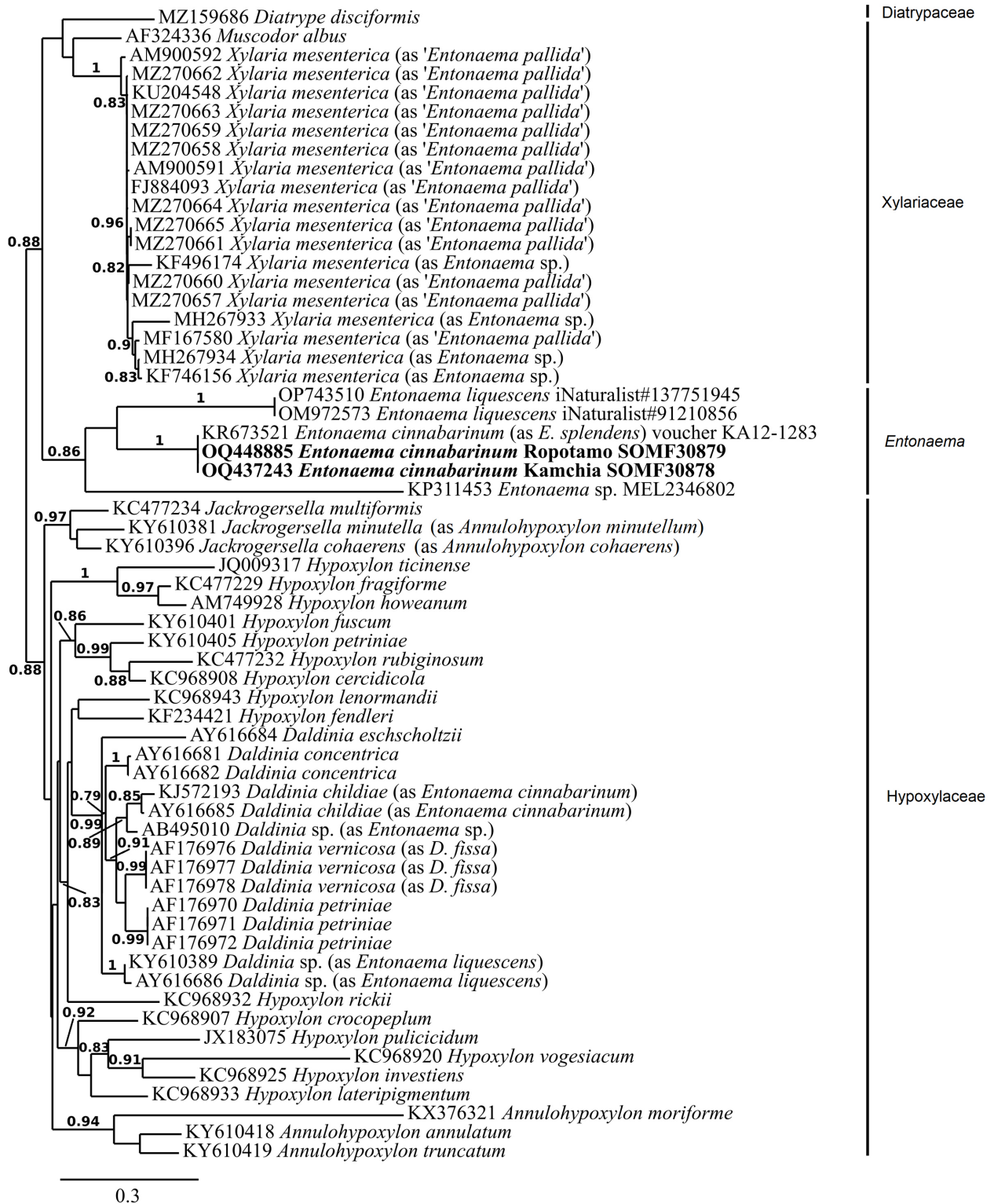


Fig. 1. PhyML phylogenetic tree of ITS rDNA sequences of the genus *Entonaema* and representatives of the families *Diatrypaceae*, *Hypoxylaceae* and *Xylariaceae*. Figures next to branches indicate branch support by SH-aLRT. Only values equal or above 0.75 are shown and 0.82 is accepted as cut-off value for significance. The sequences generated in this study appear in boldface. The taxonomic affiliation of the sequences as originally labelled on GenBank is shown in parenthesis where appropriate.

South Korea (KR673521), accessioned as *Entonaema splendens* (Berk. & M.A. Curtis) Lloyd. The phylogenetic analysis produced the tree presented in Fig. 1. The analysis separated the members of the families Diatrypaceae Nitschke, Hypoxylaceae DC. and Xylariaceae Tul. & C. Tul.. The two sequences from the Bulgarian specimens of *E. cinnabarinum* group together with the sequence KR673521, labelled as *E. splendens*, forming a fully supported clade, which is sister to similarly fully supported clade, containing two North American sequences, identified as *Entonaema liquescens* Möller. Those two lineages further cluster together with an accession of an unidentified Australian species of *Entonaema* into a statistically supported clade (branch support 0.86), which is related to the clades containing sequences of Diatrypaceae and Xylariaceae. Further on, the previously mentioned French sequence of culture of *E. cinnabarinum*, contaminated with *Daldinia childiae* J.D. Rogers & Y.M. Ju (cf. Stadler et al., 2014) groups with another sequence identified as *E. cinnabarinum* (KJ572193) and with a sequence labelled as *Entonaema* sp. (AB495010) forming a statistically supported clade (branch support 0.89), which nests deeply into a clade containing sequences of various species of *Daldinia*, itself placed into the Hypoxylaceae. This former clade also includes two sequences, originally labelled as *E. liquescens*. In respect of those two accessions, it must be noted that Stadler et al. (2020) and Wibberg et al. (2021) raised doubts about the identity of culture ATCC 46302 of *E. liquescens*, from which one of those two sequences (KY610389) was obtained. Eighteen sequences, designated as '*E. pallida*' [sic!] or as *Entonaema* sp. form a clade sister to a sequence of *Muscodor albus* Worapong, Strobel & W.M. Hess of Xylariaceae. These are reassessed as *Xylaria mesenterica* (Möller) M. Stadler, Læssøe & J. Fourn., of which *E. pallidum* G.W. Martin is a posterior synonym (Stadler et al., 2008a).

Morphological description

Entonaema cinnabarinum (Figs 2, 3)

Stromata up to 5 cm across and high (groups of stromata up to 17 cm across), at first soft, but turgid, later leathery to coriaceous, subglobose to confluent, irregularly convoluted or lobate, sometimes

depressed especially when overmature, constricted at the base, smooth and covered with pale yellow (K&W 3–4A3) or orange white or pale orange (K&W 5–6A2–3) pruina when young, bruising distinctly reddish orange or orange red (K&W 7–8A6–8) and often spotted pale red or pastel red (K&W 7A3–5), later usually finely rugulose, orange white, pale orange (K&W 5A2–3, 6A3), reddish white, pale red (K&W 7A2–4, 8A2–3), pastel red, red (K&W 9–10B4–6, 11E6–7), finally at extreme stages of overmaturity dingy, pale ochre, spotted dark violet (K&W 15F5–7). Stromatal surface with visible ostiolar papillae of perithecia, in state of maturation usually with abundant blackish spore deposits. Stromata in section consisting of up to 1.5 mm thick external crust, colouring in young state reddish orange or orange red (K&W 7–8A6–8) and turning blackish with maturation of perithecia. Internal context gelatinous, dull yellow, somewhat paler towards the perithecial layer, often in places greenish towards the base, sometimes liquefying, gradually drying and leaving a cavity in over-mature stromata; odour characteristic, spicy, reminding fenugreek, present in fresh and dried specimens. UV₃₆₅ on dry stromata produces blackish violaceous fluorescence on stromatal surface and dirty yellowish on internal tissues. External stromatal layer with abundant, dark orange-red to bright red pigment granules, KOH-extractable pigments orange. Internal layer (perithecial) of textura epidermoidea typica (cf. Hengstmengel, 2020), of hyphae 5.7–12.8 µm wide, with abundant extracellular deposits of black pigments; this carbonaceous layer intergrading inwards into extensive gelatinous stratum with irregularly, but more or less radially disposed hyphae, 5.5–14.5 µm wide, yellowish in KOH. Perithecia fully immersed into the stromatal crust, spherical, ovoid to flask-shaped, 0.75–0.90 × 0.50–0.65 mm; wall 30–50 µm thick, composed of several layers of subparallel hyphae with abundant extracellular deposits of blackish pigment granules; ostioles papillate. Asci 95.3–148.7 × 8.1–11.1 µm, narrowly clavate, with pedicel up to half length, and with disk-like, blueing in iodine solutions apical apparatus. Ascospores uniseriate in asci, broadly ellipsoid with rounded apices, sometimes one of the sides flattened, 8.2–10.9 × 5–6.4 µm, Q = 1.4–1.9; L_{av} = 9.3, W_{av} = 5.7–5.8 µm, Q_{av} = 1.6 (n=100), dark olivaceous in water and KOH, pale to dark olivaceous brown in Melzer's reagent, with two large distal guttules in



Fig. 2. Macromorphology and variability of *Entonaema cinnabarinum* from Bulgaria in different stages of maturation. Figures not to scale.

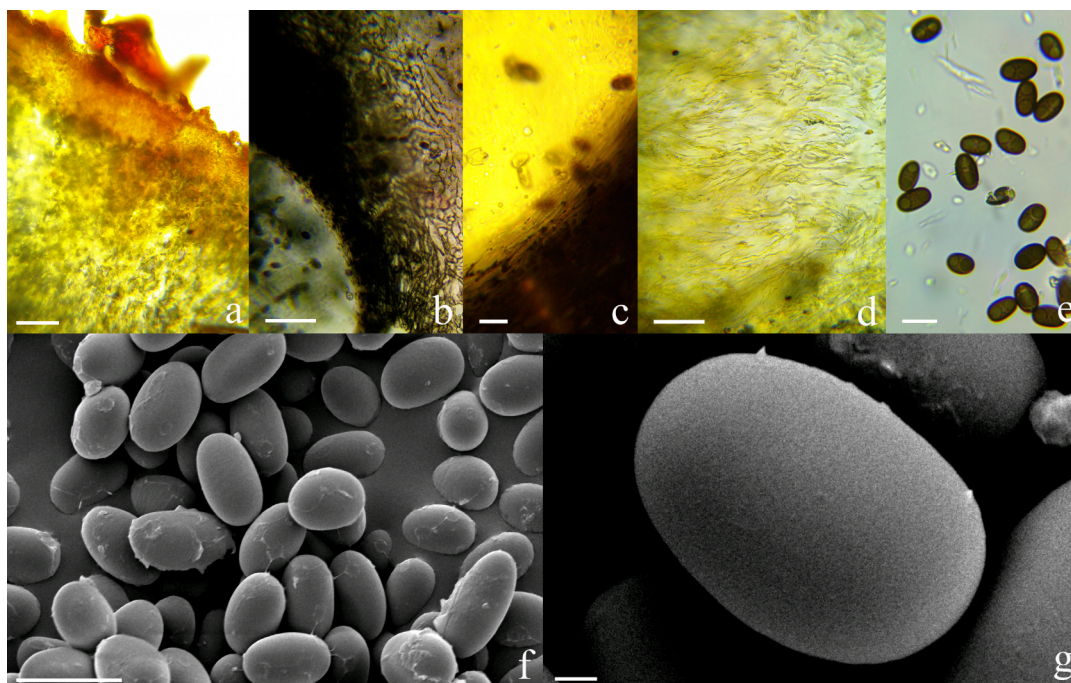


Fig. 3. Micromorphological features of Bulgarian collections of *Entonaema cinnabarinum*: (a) section of immature stroma with red pigment granules seen; (b) part of perithecium with surrounding perithecial layer with black pigment deposits; (c) structure of the perithecial wall with black pigment deposits; (d) inner, strongly gelatinised tramal layer; (e) ascospores in LM; (f) & (g) ascospores in SEM. Scale bars = 50 μm (a, b, d), 10 μm (c, e, f), 1 μm (g).



Fig. 4. Habitat of *Entonaema cinnabarinum* in the vicinity of Ropotamo Reserve. Arrows mark the occurrence of ascostromata of the fungus.

water and KOH, or one central guttule in Melzer's reagent and lactophenol, smooth under LM and SEM; wall ca 0.6 μm thick; germination slit straight, running almost through the spore length; perisporium indehiscent in 10%KOH.

Specimens examined. Varna District, between Bliznatsi and Staro Oryahovo villages, close to Kamchia River, 43°1'23.0"N, 27°48'47.4"E, elev. ca 12 m, on dead wood of *Fraxinus angustifolia* Vahl. in seasonally flooded riparian forest, 30.09.2014, leg. B. Assyov (SOMF30878; GenBank: OQ437243); Burgas District, close to Ropotamo Nature Reserve, 42°18'4.9"N, 27°43'45.7"E, elev. ca 15 m, on dead wood of *F. angustifolia* in seasonally flooded riparian forest, 12.10.2014, leg. B. Assyov (SOMF30879; GenBank: OQ448885); idem, 42°18'09.1"N, 27°43'14.5"E, elev. ca 23 m, on dead wood of *F. angustifolia* in mixed broadleaf forest, dominated by *F. angustifolia*, 06.09.2021, leg. M. Slavova

(MSL2850F6010); idem, 01.07.2022, leg. M. Slavova (SOMF30881, MSL2970F9146).

Observations. Burgas District, Velyov Vir Nature Reserve, 42°17'56.9"N, 27°42'53.0"E, elev. ca 18 m, on dead wood of *F. angustifolia* in mixed broadleaf forest, 05.11.2014, obs. B. Assyov; Burgas District, close to Ropotamo Nature Reserve, 42°18'15.3"N, 27°43'20.8"E, elev. ca 15 m, on dead wood of *F. angustifolia* in seasonally flooded riparian forest, 10.09.2015, obs. B. Assyov (Fig. 4).

Discussion

It was shown by the phylogenetic analysis that the sequences of the Bulgarian *Entonaema* collections cluster together in a well-supported clade with an Asian sequence, originally identified as *E. splendens*. The studied here specimens however, could not be

identified with *E. splendens*, which at present is believed to be conspecific with *E. liquescens*, based on HPLC profiles (Stadler et al., 2008a). A few characteristic traits of this latter species are not consistent with the morphology of our specimens, namely the dull yellow or olive yellow colouration at maturity, the greenish bruising on handling, and the green subsurface granules (Stadler et al., 2008a). The use of *E. splendens* for the sequence KR673521 (Kim et al., 2015) is thus very likely a misapplication, but its existence is important as it presents an additional evidence for the wide transcontinental distribution of *E. cinnabarinum*. Following the identification key in Stadler et al. (2008a) our specimens would key out confidently to *E. cinnabarinum* due to the orange extractable in KOH pigments and the orange to red subsurface granules. Further on, the outcomes of the analysis are congruent with the conclusion of Stadler et al. (2014) that the previously available sequences labelled as *E. cinnabarinum* in fact belong to a species of the genus *Daldinia*. As already mentioned above, Stadler et al. (2020) and Wibberg et al. (2021) also questioned the authenticity of culture ATCC 46302 of *E. liquescens*, based on genomic data, which failed to reveal presence of genes related to the assembly of mitorubrin-type compounds. The latter work also found that phylogenetic reconstructions place sequences of this culture into *Daldinia*. In our tree the reference ITS sequence KY610389, originally accessioned as *E. liquescens*, clusters similarly in a well-supported clade containing species of *Daldinia*. We thus provide the first genuine sequences of *E. cinnabarinum* of European origin. While the phylogenetic analysis in the present paper supports the self-standing status of the genus *Entonaema*, its phylogenetic position remains not precisely resolved as for the limitations of analysis of nrITS sequences and the relatively limited number of taxa, included in the dataset used for the present study. Our inference places the sequences of *Entonaema* somewhat close to some members of the family Xylariaceae, while they appear not that closely related to the species of Hypoxylaceae, included in the analysis. However, it was previously shown that *E. cinnabarinum*, *E. globosum* and *E. liquescens* contain pigments of mitorubrin/rubiginosin-type, not detected in studied species of Xylariaceae, while they lack xylarals that are present in a number of species of *Xylaria* (Stadler et al., 2004, 2008a). They are also known to lack binaphtalenes that are evidenced in

some studied members of Hypoxylaceae (Stadler et al., 2008a), but on the other hand, mitorubrin and rubiginosins are not unknown in some groups of *Hypoxylon* (Stadler et al., 2008b; Wibberg et al., 2021). This seems consistent with our phylogenetic inference, which resolved *Entonaema* as a well-separated lineage, but does not correspond to the inferred closer relation to the members of Xylariaceae. Studies employing other DNA-regions will hopefully establish the precise position of the genus *Entonaema* within the order Xylariales in future.

On account of the morphological features of the Bulgarian collections, they are essentially consistent with the contemporary descriptions in Stadler et al. (2008a) and Fedosova (2012). One difference, which attracted our attention, was the germ slit of the ascospores. This proved to be difficult to observe, but it was visualised in lactophenol cotton blue, Melzer's reagent, and occasionally in KOH and water. Where seen, it seemed to run almost through the entire spore length, while Stadler et al. (2008a) and Fedosova (2012) described it as 2/3 of the ascospore. We are at the moment uncertain as to how conclusive this difference may be. Further on, Fournier & Magni (2004) described the colours in the French collections as "sienna, rust, bay to dark brick", later affirmed also in Stadler et al. (2008a). The Bulgarian specimens deviate from this colour pattern, often featuring notable orange, pinkish and reddish tinges (Fig. 2). In addition, it seems that we report for the first time observations in SEM of the ascospores of an *Entonaema*-species, which appear to be smooth. It may be nevertheless worthy performing SEM-studies on other taxa of the genus, as it is now well-known that the ascospore ornamentation is a valuable taxonomic character in the related genus *Daldinia* (Stadler et al., 2014).

Previous research of pigments in *Entonaema* already pointed out the existence of some differences between the profiles of the holotype of *E. cinnabarinum* on one hand and the studied by the authors Bulgarian and French collections, on another (Stadler et al., 2004). Regrettably, for the moment we could not conduct an HPLC study of the Bulgarian specimens and a single sequence of an unidentified *Entonaema* from Australia exists on GenBank (KP311453). It is apparent that the lack of type-derived sequences is at present hampering the research in the genus *Entonaema*. Further DNA

studies, ideally of the holotype of *E. cinnabarinum*, or of representative, topotypic Australian collections, congruent with the type, are essential and could provide more conclusive answers about the taxonomic status of the European populations of *Entonaema*. There is no doubt that further effort to obtain sequences from more European collections will be also valuable.

The so far known habitats of *E. cinnabarinum* in Bulgaria are all situated in immediate vicinity to the coast of the Black Sea. The majority of the sites are in seasonally flooded riparian forest with exception of two, which are in mixed broadleaf non-flooded forests, but still not far from riparian habitats. The fungus is invariably confined to *F. angustifolia*, occurring on fallen trunks and large branches, almost always with bark still present on them. The findings of Benkert (1993) and Læssøe (1997) were also reported to have been related to this tree. Production of stromata apparently spans at least from July until November (Benkert, 1993; Læssøe, 1997; and the observations in this paper). The recent Hungarian finds were also documented as occurring on *F. angustifolia* (Fintha et al., 2019). The known French collections of *E. cinnabarinum*, were found on *F. excelsior* L., but also on *Acer negundo* L. and *Platanus* sp. (Fournier & Magni 2004). The recent Spanish findings, in contrast, were documented on *Corylus avellana* L. (Rubio Domínguez & Menéndez Valderey, 2011). The collection of Fedosova (2012) from the eastern coast of the Black Sea was found on a dead trunk of an unidentified broadleaf tree. Judging from the habitat requirements of *E. cinnabarinum* in Bulgaria, we anticipate that it may also occur in similar riparian habitats, which are present in coastal areas in the neighbouring Romania and Türkiye. The reporting of possible future well-documented findings in those two countries is of undoubted interest.





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<https://doi.org/10.1007/s13225-020-00447-5> 
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Supplementary table
List of sequences, used for the phylogenetic analysis

GenBank accession	Name as on GenBank	Revised name*	Identifier	Origin	References
KY610418	<i>Annulohypoxyylon annulatum</i>		culture CBS:140775	USA	Wendt et al. (2018)
KY610396	<i>Annulohypoxyylon cohaerens</i>	<i>Jackrogersella cohaerens</i>	culture CBS:119126	Germany	Wendt et al. (2018)
KX376321	<i>Annulohypoxyylon moriforme</i>		strain CBS123579	Martinique	Kuhnert et al. (2017)
KY610381	<i>Annulohypoxyylon minutellum</i>	<i>Jackrogersella minutella</i>	culture CBS:119015	Portugal	Wendt et al. (2018)
KY610419	<i>Annulohypoxyylon truncatum</i>		culture CBS:140778	USA	Wendt et al. (2018)
AY616681	<i>Daldinia concentrica</i>		voucher 0066225(M)	United Kingdom	Triebel et al. (2005)
AY616682	<i>Daldinia concentrica</i>		voucher 0066225(M)	United Kingdom	Triebel et al. (2005)
AY616684	<i>Daldinia eschscholtzii</i>		voucher 0066224(M)	Thailand	Triebel et al. (2005)
AF176976	<i>Daldinia fissa</i>	<i>Daldinia vernicosa</i>	unavailable	Denmark	Johaneson et al. (2000)
AF176977	<i>Daldinia fissa</i>	<i>Daldinia vernicosa</i>	B.J. Coppins 9033(E)	United Kingdom	Johaneson et al. (2000)
AF176978	<i>Daldinia fissa</i>	<i>Daldinia vernicosa</i>	Wat herb 20917(E)	United Kingdom	Johaneson et al. (2000)
AF176970	<i>Daldinia petriniae</i>		H. Knudsen s.n.(C)	Denmark	Johaneson et al. (2000)
AF176971	<i>Daldinia petriniae</i>		A. Strid 11656(S)	Sweden	Johaneson et al. (2000)
AF176972	<i>Daldinia petriniae</i>		J. Jeppson 1687(S)	Sweden	Johaneson et al. (2000)
MZ159686	<i>Diatrype disciformis</i>		voucher K(M):249991	United Kingdom	Gaya et al. (direct submission)
AY616685	<i>Entonaema cinnabarinum</i>	<i>Daldinia childiae</i>	isolate agtS377	France	Triebel et al. (2005); Stadler et al. (2014)
KJ572193	<i>Entonaema cinnabarinum</i>	<i>Daldinia childiae</i>	isolate SH22	Unknown	Min (direct submission)
OQ437243	<i>Entonaema cinnabarinum</i>		voucher SOMF30878	Bulgaria	this study
OQ448885	<i>Entonaema cinnabarinum</i>		voucher SOMF30879	Bulgaria	this study
AY616686	<i>Entonaema liquescens</i>	<i>Daldinia</i> sp.	isolate agtS279	USA	Triebel et al. (2005)
KY610389	<i>Entonaema liquescens</i>	<i>Daldinia</i> sp.	culture ATCC46302	USA	Wendt et al. (2018); Wibberg et al. (2021)
OM972573	<i>Entonaema liquescens</i>		iNaturalist#91210856	USA	Russell (direct submission)
OP743510	<i>Entonaema liquescens</i>		iNaturalist#137751945	USA	Russell (direct submission)
AM900591	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	strain CBS 121671	Mexico	Stadler et al. (2008)
AM900592	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	strain MUCL 49332	Panama	Stadler et al. (2008)
FJ884093	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	strain PP92a	Peru	Gazis & Chaverri (2010)
KU204548	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	voucher INBio204D	Costa Rica	Rojas-Jimenez & Tamayo-Castillo (2021)
MF167580	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate X01MQ	Brazil	Silva et al. (direct submission)

Supplementary table continued...

MZ270657	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 13i	Unknown	Carbajal-Valenzuela et al. (2022)
MZ270658	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 19i	Unknown	Carbajal-Valenzuela et al. (2022)
MZ270659	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 21i	Unknown	Carbajal-Valenzuela et al. (2022)
MZ270660	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 28i	Unknown	Carbajal-Valenzuela et al. (2022)
MZ270661	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 41i	Unknown	Carbajal-Valenzuela et al. (2022)
MZ270662	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 42i	Unknown	Carbajal-Valenzuela et al. (2022)
MZ270663	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 46i	Unknown	Carbajal-Valenzuela et al. (2022)
MZ270664	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 47i	Unknown	Carbajal-Valenzuela et al. (2022)
MZ270665	<i>Entonaema pallida</i>	<i>Xylaria mesenterica</i>	isolate 52i	Unknown	Carbajal-Valenzuela et al. (2022)
AB495010	<i>Entonaema</i> sp.	<i>Daldinia</i> sp.	TI070924	Unknown	Velmurugan et al. (direct submission)
KF496174	<i>Entonaema</i> sp.	<i>Xylaria mesenterica</i>	Fun83W1	Brazil	De Silva et al. (direct submission)
KF746156	<i>Entonaema</i> sp.	<i>Xylaria mesenterica</i>	F5071	Panama	Higginbotham et al. (2014)
KP311453	<i>Entonaema</i> sp.		MEL 2346802	Australia	Bonito & May (direct submission)
MH267933	<i>Entonaema</i> sp.	<i>Xylaria mesenterica</i>	strain JHGB08_1A	Peru	Skaltsas et al. (2019)
MH267934	<i>Entonaema</i> sp.	<i>Xylaria mesenterica</i>	strain AHB18_5B	Peru	Skaltsas et al. (2019)
KR673521	<i>Entonaema splendens</i>	<i>Entonaema cinnabarinum</i>	voucher KA12-1283	South Korea	Kim et al. (2015)
KC968908	<i>Hypoxylon cercidicola</i>		CBS 119009	France	Kuhnert et al. (2014)
KC968907	<i>Hypoxylon crocopleplum</i>		CBS 119004	France	Kuhnert et al. (2014)
KF234421	<i>Hypoxylon fendleri</i>		STMA 12152 MUCL 54792	French Guiana	Kuhnert et al. (2014)
KC477229	<i>Hypoxylon fragiforme</i>		MUCL 51264	Germany	Stadler et al. (2013)
KY610401	<i>Hypoxylon fuscum</i>		CBS 113049	France	Wendt et al. (2018)
AM749928	<i>Hypoxylon howeanum</i>		MUCL 47599 ex STMA 06012	Germany	Bitzer et al. (2008) Stadler et al. (2007)
KC968925	<i>Hypoxylon investiens</i>		CBS 118183	Malaysia	Kuhnert et al. (2014)

* Revised names are shown when applicable. In cases reference is available for such revision it is listed in the last column, after the work that generated the sequence.

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Tapinella phuketensis n. sp. – a new species from Thailand (Insecta: Psocoptera)

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<https://zoobank.org/D1508CA5-5DC7-41A3-8D57-233CE187AEA3> 

Abstract: A new species of *Tapinella* Enderlein, 1908 from Phuket Island, Thailand named *Tapinella phuketensis* n. sp. was described. It was found in a rain forest east of Karon Village at the west coast of the island. The species is unique in having blackish-brown wings with a white stripe in their middle part.

Keywords: biodiversity, equatorial, insects, rain forest, South-East Asia

Introduction

Faunistic and taxonomic works on the psocid fauna of Thailand are very scarce, especially compared to neighbouring regions like China and Indonesia (Thornton, 1984; Fasheng, 2002). Only 27 psocid species were known from this country (Lienhard, 2016). In this paper I describe a new species of *Tapinella* Enderlein, 1908 from the rain forests of the west coast of Phuket Island.

Material and methods

Psocoptera were collected from Phuket Island, Thailand by beating the vegetation on 26–27.02.2023. The specimens were stored in 96% ethanol. The photos (specimens in glycerin) were taken by a camera Canon PowerShot SX500IS through the eyepiece of a light microscope Optika. The type material was deposited at the National Museum of Natural History, Sofia, Bulgaria (NMNH). The species discussed in the paper were considered according to original descriptions. Measurements followed Lienhard (1998).

Measurements abbreviations (all in mm in the text): LC = body length; A = antenna length, P4:

fourth segment of maxillary palp, F+tr = hind femur and trochanter length; T = hind tibia length; t1, t2, t3 = tarsomeres of hindtarsus (lengths measured from condyle to condyle), FW = forewing, HW = hindwing, D = anteroposterior diameter of the compound eye, IO = shortest distance between compound eyes.

Results and discussion

Family Pachytroctidae Enderlein, 1904

Tapinella phuketensis n. sp.

urn:lsid:zoobank.org:act:

[FDCF63FB-D1A6-4B9D-BB80-7E374428C412](https://zoobank.org/FDCF63FB-D1A6-4B9D-BB80-7E374428C412) 

Material examined: Holotype ♀, 27.02.2023, Thailand, Phuket Island, East of Karon Village, near the road to Yuan Phueng Monastery, rain forest, from bushes, N07 50 51.1 E98 18 10.4, 80 m a.s.l., NMNH – Sofia, Bulgaria; Additional material: 1 ♀, 26.02.2023, East of Karon Village, rain forest, from dry leaves on a bush near a pond, N07 51 26.7 E98 17 49.0, 32 m a.s.l., coll. D. Georgiev.

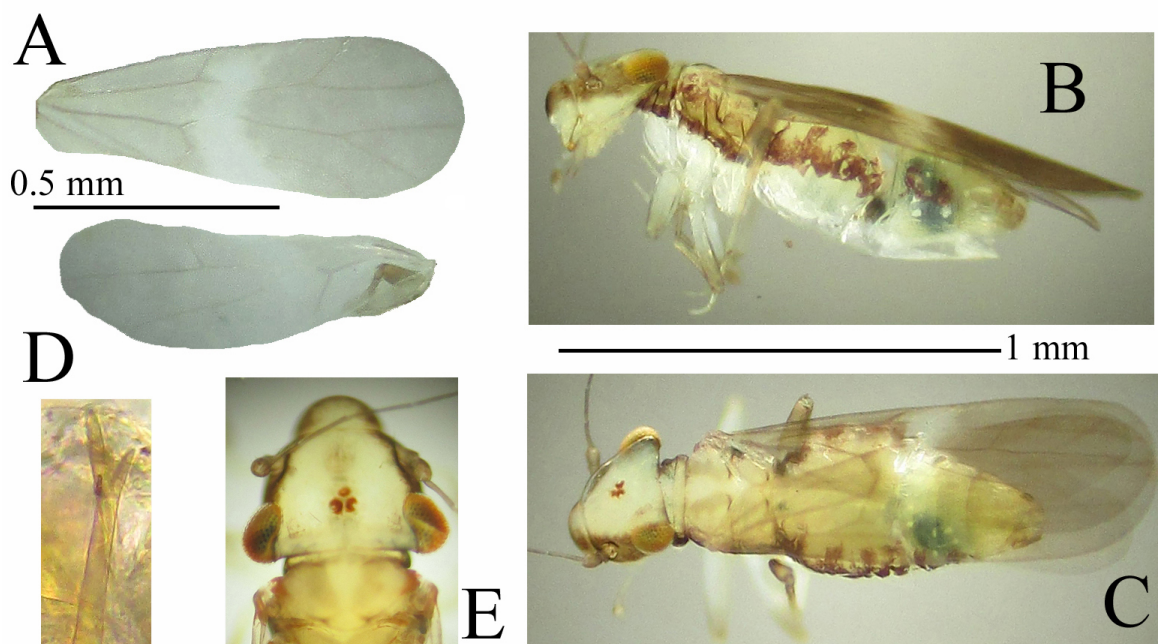


Fig. 1. *Tapinella phuketensis* n. sp.: female (holotype): A – forewing and hind wing, B – lateral view, C – dorsal view; female (additional material): D – apex of the lacinia, E – head and thorax (C and D not to scale).

Type locality: Thailand, Phuket Island, West coast, East of Karon Village, near the road to Yuan Phueng Monastery, rain forest, from bushes, N07 50 51.1 E98 18 10.4, 80 m a.s.l.

Description (after 25 days in 96% ethanol): Female: Colouration: Head creamy with some brown patches of pigment at its middle line and between the compound eyes. It has a thick dark brown lateral stripe at each side from the postclypeus to the antennal socket, continuing to the eye, the thorax and the abdomen. Viewed from above, the abdomen is also yellowish-creamy, with the lateral stripes on each side forming a spotted colouration on the lateral side of each tergite (Fig. 1: B, C, E). Apex of the abdomen darker. Ventrally it is pale yellowish. Antennae, palpi and legs yellowish-brown, darker at their distal parts. Compound eyes reddish-brown. Ocelli pale, surrounded by red-brown pigment at their inner side. Forewings and hind wings blackish-grey with a white transverse band at their middle area. Veins dark with an exception at the pale wing zones, where they are white (Fig. 1: A).

Morphology: Macropterous. Three ocelli present. Lacinia similar with this one of *T. curvata* Badonnel 1949 (see Lienhard, 2008), with smaller internal cusp and much longer lateral one, having a small cusp itself at its internal side (Fig. 1: D). Fore and hind

wings slender with venation typical for the genus. Subgenital plate with T-sclerite as typical for the genus, having a short stem and arms. Epiproct rounded, paraprocts elongated. Both with long setae. The setae of the epiproct are more numerous and denser but a little shorter than these of the paraprocts.

Measurements (in mm): Holotype (female): LC = 1.2; F+tr = 0.35; T = 0.40; t1 = 0.21, t2 = 0.04, t3 = 0.04, FW = 0.88, HW = 0.70, A = 0.92, D = 0.10, IO = 0.23, IO/D = 2.30.

Male: Unknown.

Diagnosis: *Tapinella phuketensis* n. sp. is unique in the genus with its transverse white stripe on the both wings. Having patterned wings, the new species is similar with *T. baliensis* Thornton, 1984 known from Indonesia but in this species (described on the base of a specimen without genital parts) the white areas of the wings are two in the forewing (at the base and the apex) and one in the hind wing (at the base) (Thornton, 1984). *T. fusca* Badonnel 1977 described from Angola also has white areas at the base of its forewing located mainly along R and A1 (Badonnel, 1977). The lacinia of this species is similar with this one of *T. phuketensis* n. sp. but the whole animal is darker: blackish-brown to reddish-brown. In *T. nebulosa* Vaughan, Thornton & New, 1991 from Indonesia the white areas are located along the wing

veins (Vaughan et al., 1991). The forewing colouration of *T. pictipenna* Thornton, Lee & Chui, 1972 (Indonesia) is opposite to this one of the new species: the wing is whitish with a dark stripe in its middle (Thornton et al., 1972).

Etymology: Named after the Phuket Island, Thailand, where the species was found.

Habitat: The species was collected from bushes in a rain forest.

Acknowledgements

I am grateful to Ramesh Gurusamy (Zoological Survey of India) for sending me the scanned descriptions of the Chinese *Tapinella* species.

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