

***Ramariopsis robusta* (Basidiomycota, Clavariaceae),
a new European species similar to *R. kunzei***

JAN MATOUŠ^{1,2*}, JAN HOLEC², ONDŘEJ KOUKOL¹

¹ Charles University, Faculty of Science, Department of Botany, Benátská 2, CZ-128 01 Praha 2, Czech Republic

² Mycological Department, National Museum, Cirkusová 1740, CZ-193 00 Praha 9, Czech Republic
*corresponding author; j.p.matous@seznam.cz

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The new species *Ramariopsis robusta* Matouš & Holec is described based on collections from the Czech Republic and Slovakia. The species is highly supported in a phylogenetic tree based on the 28S rDNA gene. Morphologically, it is distinguished by its robust and densely branched white to cream basidiomata often growing in fascicles, with wide, often flattened branches, and distinctly ornamented spores with up to 1.5 µm high spines. The most similar species *R. kunzei* differs by subtler, more sparsely branched basidiomata, lower spore ornamentation, smaller Q value and shorter basidia. The species is described in detail and figures showing its macro- and microcharacters are provided including SEM photographs of spores. Differences with the similar taxa *R. kunzei* (including its varieties), *R. atlantica*, *R. bispora*, *R. tenuiramosa*, *R. biformis*, *R. rufipes* and *Clavaria lentofragilis* are outlined. *Ramariopsis atlantica* is newly documented from Panama.

Key words: ramarioid fungi, clavarioid fungi, taxonomy, phylogeny, 28S rDNA, Central Europe.

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Nový druh *Ramariopsis robusta* Matouš & Holec je popsán na základě sběrů z České republiky a Slovenska. Tento druh je silně podpořen ve fylogenetickém stromu na základě genu 28S rDNA. Morfologicky je význačný robustními a hustě větvenými, bílými až krémovými plodnicemi, často rostoucími ve shlucích, se širokými, často zploštělými větvemi a výrazně ornamentovanými spory s výskytem ostnů dlouhých až 1,5 µm. Nejpodobnější druh *R. kunzei* se odlišuje subtilnějšími, řídkěji větvenými plodnicemi, méně výraznou ornamentikou spor, menší hodnotou Q a kratšími bazidiemi. Druh je podrobně popsán a jeho makro- a mikroznaky jsou ukázány na fotografiích a kresbách, včetně SEM fotografií spor. Jsou uvedeny rozdíly od podobných druhů *R. kunzei* (včetně jeho variet), *R. atlantica*, *R. bispora*, *R. tenuiramosa*, *R. biformis*, *R. rufipes* a *Clavaria lentofragilis*. Druh *Ramariopsis atlantica* je nově doložen z Panamy.

INTRODUCTION

Ramariopsis (Donk) Corner is a genus of saprotrophic fungi and potentially biotrophic plant associates (Birkebak et al. 2013) with more or less branched, rarely simple basidiomata (Corner 1950). *Ramariopsis* species mostly inhabit grassland or shrub communities, rarely forests. Seventy-two legitimate *Ramariopsis* names are registered (www.mycobank.org).

However, some of the species treated as *Ramariopsis* probably belong to the related genus *Clavulinopsis* Overeem 1923 (Petersen 1978, Birkebak et al. 2013). The number of really existing species is unknown, as some of the described taxa seem to be synonyms and others are poorly known. Some species are considered cosmopolitan (e.g. Corner 1950, 1970, Petersen 1988) but it is highly probable that detailed studies reveal the existence of vicariant populations or cryptic species within them (Kautmanová et al. 2012, Birkebak et al. 2013).

Molecular studies dealing with *Ramariopsis* species have primarily been devoted to relations between ramarioid and clavarioid genera (Dentinger & McLaughlin 2006). However, the phylogenetic relations within these groups are still insufficiently known. At the species level, the most valuable works are those by Kautmanová et al. (2012) and Birkebak et al. (2013).

In Europe, about 15 species of *Ramariopsis* are known based on more or less reliable taxonomic literature (Corner 1950, 1970, Jülich 1984, Olariaga 2009, Knudsen et al. 2012). The best known species is *R. kunzei* (Fr.) Corner, the type species of *Ramariopsis* (Donk 1933). It is distinguished by its white, rather large, multi-branched basidiomata and echinulate spores (Corner 1950, Olariaga 2009, Knudsen et al. 2012). Its basidiomata are rather variable in size, way of branching and colour tinges, which is reflected in the existence of seven varieties (www.indexfungorum.org). One of them, *R. kunzei* var. *bispora* Schild (Schild 1971), has recently been transferred to the rank of species as *R. bispora* (Schild) Olariaga (Olariaga & Salcedo 2012). Other morphologically similar species are *R. atlantica* Araujo-Neta, G.A. Silva & Gibertoni, *R. tenuiramosa* Corner, *R. biformis* (G.F. Atk.) R.H. Petersen, and *R. rufipes* (G.F. Atk.) R.H. Petersen.

During field work in the Czech Republic and Slovakia in 2013–2014, the first author repeatedly found unusual basidiomata of a species tentatively identified as *R. kunzei*, although possessing a large and robust stature and more prominent spines on the spore surface. DNA sequence data showed that these collections form a highly supported clade which stands apart from other collections labelled as *R. kunzei*. Based on these findings we decided to describe the robust collections as a new species.

MATERIAL AND METHODS

Collections. Fresh material of the studied *Ramariopsis* species was collected in the Czech Republic and Slovakia in 2013–2014 as part of the diploma thesis of the first author (JM). We also used collections from the PRM (National Museum, Prague, Czech Republic) and BRA (Slovak National Museum, Bratislava, Slovakia) herbaria. Voucher specimens are deposited in PRM, BRA and PRC (Charles University, Prague, Czech Republic). Duplicates of some collections are kept in the private herbarium of JM.

Morphology. The description of macrocharacters is based on fresh basidiomata collected by JM and dried basidiomata and photographs of other collectors. For the microscopic study, samples were put into 5% KOH for 15 min., then transferred to aqueous Congo Red and finally mounted in Melzer's solution. In each collection, we measured 30 spores, 5–15 basidia (depending on the fertility of the material), 20 hyphae (from several parts of the basidiomata) and about 10 replications of the width of hymenium and subhymenium. Microcharacters were observed under oil immersion at a magnification of 1000× using an Olympus BX51 light microscope equipped with an Olympus C50-50 digital camera and measured by means of the QuickPhoto micro 3.0 software (Olympus, Japan). The length/width quotient (Q) was calculated for each spore and the mean value (Q_{av}) was calculated from all spores measured. Basidiospore size is given as the prevailing values with extremes (5 and 95 percentiles) in brackets. Immature and extraordinary large spores were not measured.

Scanning Electron Microscopy (SEM). Dry, small, up to 10 mm long pieces of branches were used for SEM. Samples were attached to an aluminium plate using a self-adhesive carbon disc and inserted into a vacuum chamber, where they were coated with ions of gold. The plate with samples was afterwards studied under a Hitachi S-3700N scanning electron microscope. Photographs were taken using a digital camera.

DNA extraction, PCR and analyses (for details on used specimens, see Tab. 1). For DNA extraction, several pieces of dried basidioma branches (1–2 cm in length) were taken. DNA extraction was performed using a commercial kit (ZR Plant/Seed DNA MiniPrep, Zymo Research, Orange, USA) and according to the manufacturer's instructions. The last step involving the elution of extracted material was adapted. The elution buffer was preheated to 65° C, and a rather small volume (22 µl) was used. The 28S rDNA region was amplified with primer pairs NL1/NL4 (O'Donnell 1993). PCR purification was carried out using the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., Bade City, Taiwan). Both PCR fragments were sequenced in the Sequencing Laboratory of the Faculty of

Science, Charles University in Prague, Czech Republic using the same primers. Consensus sequences were constructed in the Geneious 6.1.5 software (Biomatters, Auckland, New Zealand).

The final alignment dataset comprised 26 sequences of 619 characters, of which 91 were parsimony informative and 173 were variable. Alignment was performed using the MAFFT algorithm implemented in the Geneious 6.1.5 software and manually edited in the same software. Phylogenetic analysis was performed by means of Bayesian inference using MrBayes version 3.2 (Ronquist et al. 2012) and by means of Maximum likelihood analysis using the RAxML Web Server version 7.7.1 (Stamatakis et al. 2008). For the Bayesian analysis, the best-fit model TrN+G was determined using jModeltest version 2.1.5 (Darriba et al. 2012). Two independent runs of 5,000,000 generations were run with sampling every 1000th generation, with the first 25% of samples discarded as burn-in. Posterior probabilities (PP) were used as Bayesian branch support for the consensus trees. The average standard deviation of split frequencies estimating convergence reached the level of 0.002 at the end of the analysis. *Mucronella pendula* (Masse) R.H. Petersen (Acc. Nr. HQ829921) was used as the outgroup. Maximum likelihood analysis employing GTRCAT approximation was conducted on CIPRES Science Gateway (Miller et al. 2010). Support for branching was calculated using a bootstrap test with 1,000 replicates.

RESULTS AND DISCUSSION

Ramariopsis robusta Matouš & Holec, **sp. nov.**

Figs. 1–3

(Mycobank MB 821227)

H o l o t y p e. Slovakia, Biele Karpaty Mts., municipality of Nová Bošáca, settlement named Grúň, 48°53'42.860" N, 17°47'53.157" E, alt. 460 m, sloping extensively pastured meadow with scattered fruit trees, 23 Oct. 2014, leg. & det. J. Matouš (PRM 945410).

I s o t y p e. PRC 3986.

P a r a t y p e s. See Collections studied.

E t y m o l o g y. The species name “*robusta*” (from *robustus*) refers to the large and thick stature of the basidiomata.

D i a g n o s i s. *Ramariopsis robusta* is characterised by robust, dense and repeatedly branched white to cream basidiomata up to 95 mm high and 50 mm wide, often growing in fascicles, with branches reaching a diameter of up to 10 mm and axils up to 15 mm, often flattened, with usually ellipsoid to subglobose, verruculose to echinulate spores typical by irregularly distributed, up to 1.5 µm high spines. The most similar species *R. kunzei* differs by more subtle, smaller, usually sparsely branched basidiomata, narrower branches, spore ornamentation up to 1.0 µm high but usually less, more globose spores and shorter usually up to 30 µm high basidia.

D e s c r i p t i o n. Basidiomata growing in small groups or gregariously in fascicles of up to 150 mm wide, single basidiomata up to 95 mm high and 50 mm wide,

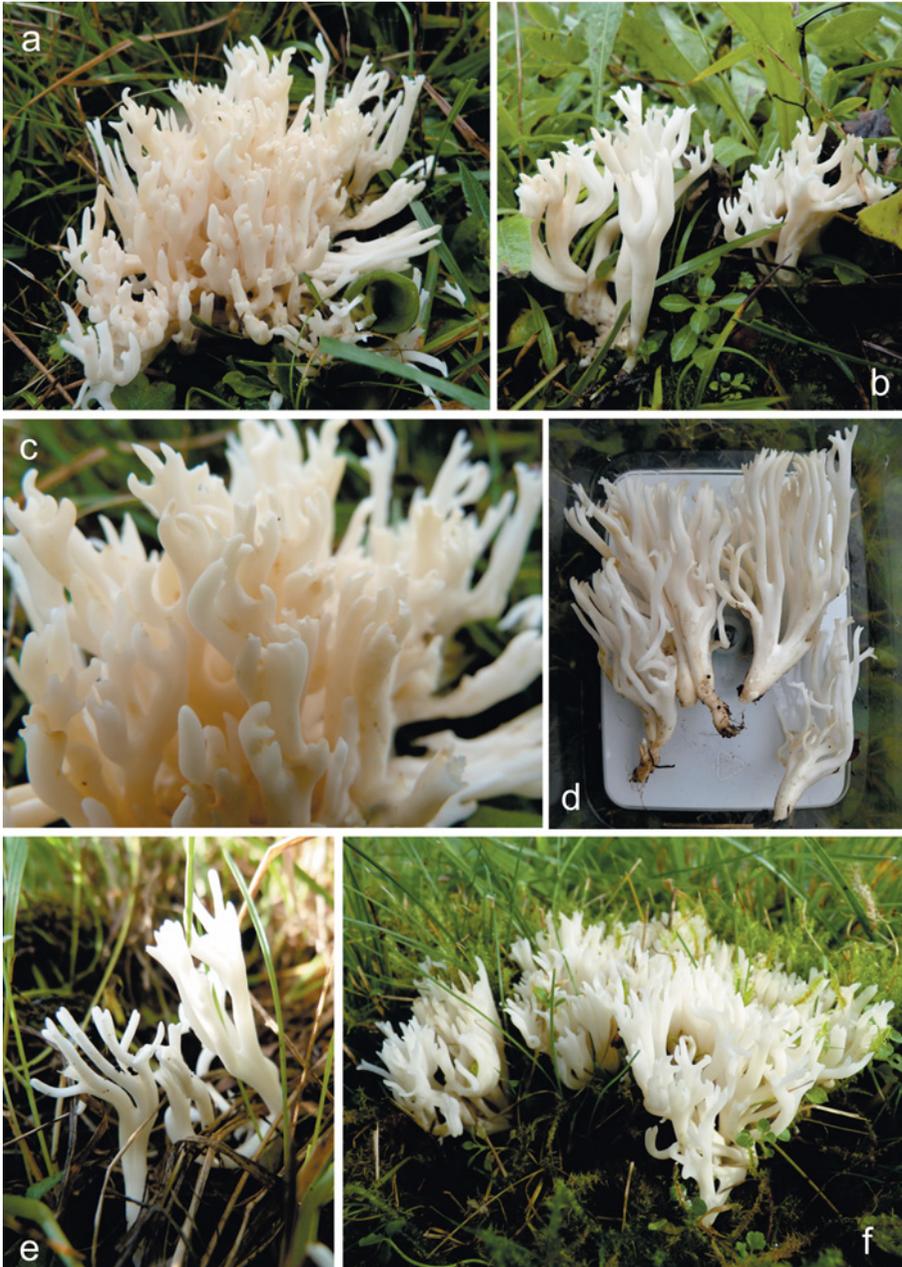


Fig. 1. *Ramariopsis robusta*, fresh basidiomata. **a–c** – Grúň, Nová Bošáca, Slovakia (PRM 945410, holotype); **d** – Mariánské Lázně, Czech Republic (PRM 945424); **e** – Bříšejov, Czech Republic (PRM 945411); **f** – Panská Habrová, Czech Republic (PRM 933826). For details, see Collections studied. Photo J. Matouš (a–c, e), M. Hamadák (d), S. Fleková (f).

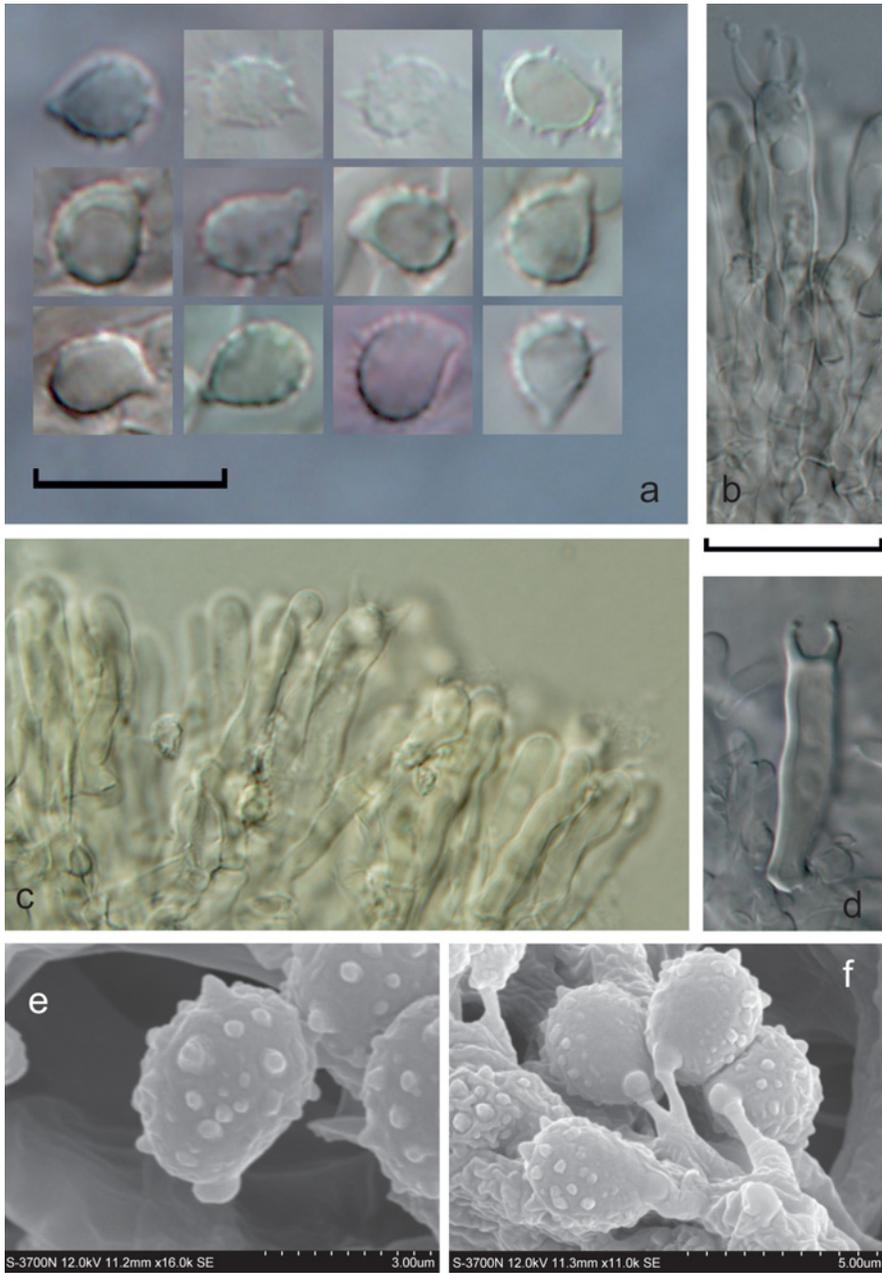


Fig. 2. *Ramariopsis robusta*, photographs of microcharacters. **a** – basidiospores (spores originate from all collections studied); **b** – basidia; **c** – hymenium with basidia; **d** – thick-walled basidium; **e**, **f** – basidiospores in SEM. Scale bar for basidiospores = 10 µm. Scale bar for other structures = 20 µm. Photo J. Matouš.

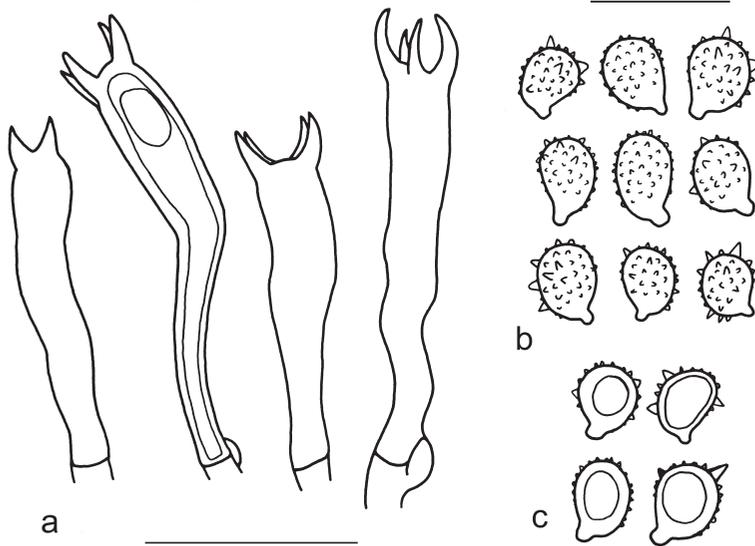


Fig. 3. *Ramariopsis robusta*, line drawings of microcharacters. **a** – basidia; **b** – basidiospores (surface); **c** – basidiospores (outline). Scale bar for basidia = 20 μm . Scale bar for basidiospores = 10 μm . Del. J. Matouš.

richly branched, usually white to pale creamy white or creamy, locally with yellowish to ochraceous spots occurring mainly on injuries, axils of branches or in older parts; dry basidiomata pale creamy, creamy or pale ochre, sometimes darker to ochre towards base or apices of branches. Branches up to 10 mm wide, 3–6 \times dichotomously, trichotomously or polytomously branched, in axils up to 15 mm wide, cylindrical, often slightly to strongly flattened, axils U-shaped or V-shaped, apices of branches rounded to subacute, often bifurcate. Sterile part up to 15 mm wide, constituting 1/5–1/3 of the total basidioma height, and concolorous, or vaguely delimited and slightly darker than branches, creamy to creamy-ochraceous. Flesh usually fragile, white, sometimes creamy. Taste mild, smell indistinct or slightly fungoid. Macrochemical reactions not tested.

Spores (3.6)4.0–5.2(5.8) \times (2.7)3.1–4.3(4.9) μm (av. 4.63 \times 3.68 μm), $Q = 1.02$ –1.55, $Q_{av} = 1.26$, broadly ellipsoid to ellipsoid, rarely subglobose or drop-shaped, sometimes with distinct suprahilar depression, often containing one big drop, slightly thick-walled, hyaline, non-amyloid, non-dextrinoid, strongly to very strongly ornamented with small and large ornaments present on one spore at the same time, verruculose to echinulate with verrucae to obtuse spines 0.4–0.7 μm high and up to 0.7 μm wide, typically with even higher, irregularly distributed spines 1.0–1.5 μm long and up to 1.6 μm wide (very rarely up to 2.0 μm wide) which lack in some spores; hilar appendix very distinct, 0.5–1.2 μm long (av. 0.87). Basidia 22.5–39.5 \times 4.5–6.5 μm (av. 34 \times 5.7 μm), hyaline, thin-walled, rarely thick-walled and up to

46 µm long, narrowly clavate to clavate, rarely almost cylindrical, tetrasporic, exceptionally bisporic, clamped, sterigmata 1.5–5.6 µm (av. 3.62 µm), up to 6 µm long in bisporic basidia. Hymenium 27–40 µm thick. Subhymenium 12–22 µm thick, formed by interwoven hyphae 1.9–3.9 µm (av. 3.03 µm) in diameter, clamped. Hyphae of the branches parallel, with cells 1.8–8.4 µm wide (av. 4.69 µm), rarely inflated up to 8.0–17.5 µm, clamped. Hyphae of the stem interwoven, with cells 2–8 µm wide (av. 4.17 µm), often with inflated cells 8–18 µm wide, clamped.

Tab. 1. Sequences used for constructing the phylogenetic tree.

Abbreviations: H – holotype, P – paratype, T – type (epitype or neotype).

Species	Country	Voucher	28S rDNA GenBank Accession Nr.	References
<i>Mucronella pendula</i>	Tasmania	PBM 3437	HQ829921	Birkebak et al. (2013)
<i>Ramariopsis atlantica</i>	Brazil	URM 6985	KX227748	Araujo-Neta et al. (in Hyde et al. 2016)
<i>Ramariopsis atlantica</i> ^H	Brazil	URM 84210	KX227747	Araujo-Neta et al. (in Hyde et al. 2016)
<i>Ramariopsis atlantica</i>	Brazil	URM 84213	KX227746	Araujo-Neta et al. (in Hyde et al. 2016)
<i>Ramariopsis atlantica</i>	Panama	PRC 3980	LT837965	This paper
<i>Ramariopsis atlantica</i>	Panama	PRM 945425	LT837964	This paper
<i>Ramariopsis bififormis</i>	USA	JMB 10061006	HQ877712	Birkebak et al. (2013)
<i>Ramariopsis cremicolor</i> ^H	New Zealand	RHP 55785	HQ877714	Birkebak et al. (2013)
<i>Ramariopsis crocea</i>	Slovakia	SAV F1255	GU299492	Kautmanová et al. (2012)
<i>Ramariopsis crocea</i>	USA	JMB 10071001	HQ877715	Birkebak et al. (2013)
<i>Ramariopsis crocea</i> f. <i>conspicua</i> ^H	USA	RHP 3595	HQ877716	Birkebak et al. (2013)
<i>Ramariopsis kunzei</i>	Czech Republic	BRA CR17281	LT837934	This paper
<i>Ramariopsis kunzei</i>	Czech Republic	PRM 935145	LT837931	This paper
<i>Ramariopsis kunzei</i>	Czech Republic	PRM 945415	LT837933	This paper
<i>Ramariopsis kunzei</i>	Slovakia	BRA CR15077	LT837935	This paper
<i>Ramariopsis kunzei</i>	Slovakia	BRA CR15092	LT837930	This paper
<i>Ramariopsis kunzei</i> var. <i>bispora</i>	Slovakia	BRA CR25735	LT837932	This paper
<i>Ramariopsis pulchella</i> ^T	Slovakia	BRA CR12765	GU299497	Kautmanová et al. (2012)
<i>Ramariopsis pulchella</i>	Slovakia	BRA CR12766	GU299496	Kautmanová et al. (2012)
<i>Ramariopsis robusta</i> ^P	Czech Republic	PRM 933826	LT837961	This paper
<i>Ramariopsis robusta</i> ^P	Czech Republic	PRM 945411	LT837958	This paper
<i>Ramariopsis robusta</i> ^P	Czech Republic	PRM 945424	LT837960	This paper
<i>Ramariopsis robusta</i> ^P	Slovakia	BRA CR3395	LT837963	This paper
<i>Ramariopsis robusta</i> ^P	Slovakia	BRA CR25525	LT837962	This paper
<i>Ramariopsis robusta</i> ^H	Slovakia	PRM 945410	LT837959	This paper
<i>Ramariopsis tenuiramosa</i>	Wales	GG 061104	EF535269	Unpublished

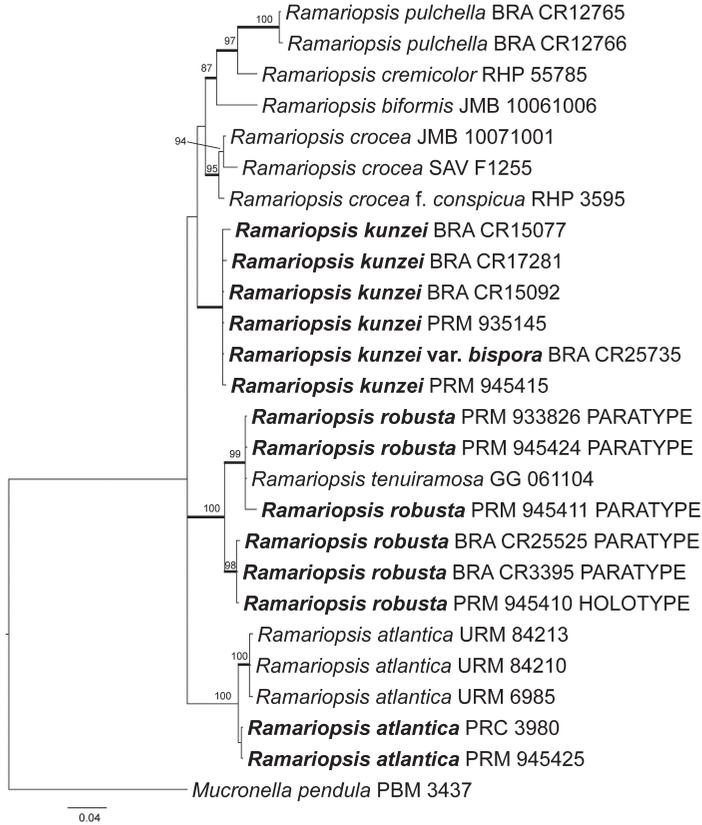


Fig. 4. Phylogenetic tree from Bayesian analysis of the rDNA region of *Ramariopsis* species. Codes following names represent vouchers, types are indicated. Thick branches indicate posterior probabilities > 0.95. Values above branches represent ML bootstrap values (only values > 85 are shown). Sequences obtained in this study are in bold.

Ecology. Probably saprotrophic terricolous species of semi-natural, oligotrophic, extensively mowed or pastured, non-manured meadows and grasslands, growing among grasses, herbs or mosses, at altitudes of 350–770 m.

Distribution. To date, the species has been well documented from seven localities in Central Europe, 4 of them located in the Czech Republic and 3 in Slovakia. There is also one molecularly documented record (see next chapter) from the United Kingdom (Wales).

Systematic position. The phylogenetic tree (Fig. 4) based on a large ribosomal subunit (28S rDNA) shows several well-supported lineages. *Ramariopsis atlantica* and the newly described *R. robusta* form distinct, well-supported clades basal to the whole genus.

The sequence EF535269 derived from a collection labelled as *R. tenuiramosa* in GenBank is positioned in our *R. robusta* clade. It obviously represents a misidentification and provides evidence for the occurrence of *R. robusta* also in the United Kingdom (Wales).

Within the *R. robusta* clade, two subclades can be distinguished. This fact demonstrates certain genetic variability, which is however not expressed morphologically. Nevertheless, there is a correlation with the geographic distribution of the collections, as those from the Czech Republic and Wales are in one clade, while those from Slovakia in the other one.

Notes. The most important characters of *Ramariopsis robusta* are the robust, white to creamy, densely branched basidiomata with unusually broad branches and spores with conspicuous ornamentation. The longest spines reach 1.5 μm . Such spines are not present on all spores and their distribution on one particular spore is irregular. However, spores bearing them are regularly present in all collections studied. The longest spines mostly occur in the upper spore part (opposite to the hilar appendix; Figs. 2, 3).

As follows from our revision of herbarium material, the collections of *R. robusta* have been identified as *Ramariopsis kunzei* (Fr.) Corner in the past. The morphologically most similar species *R. kunzei* usually possesses white, smaller, subtler, less densely branched basidiomata with narrower branches, and spores with lower ornamentation usually reaching a height of up to 0.7 μm and smaller Q values (1.02–1.35, $Q_{\text{av}} = 1.15$). This means that spores of *R. kunzei* are more globose than those of *R. robusta*. Rarely, some collections of *R. kunzei* exhibit an ornamentation reaching up to 1 μm , which is however visible only on a minor part of the spores. Basidia of *R. kunzei* are shorter, usually 19–30 μm long.

The currently recognised varieties of *R. kunzei* are not supported by molecular data, i.e. only their morphological differences can be discussed. The European *Ramariopsis kunzei* var. *deformis* Corner resembles *R. robusta* by its growth in dense fascicles. However, it possesses smaller basidiomata (30–60 mm high and broad) which are less densely branched and the branches are “deformed” (Corner 1950). Its spores do not differ from *R. kunzei* var. *kunzei* sensu Corner (1950). The same facts relate to *Ramariopsis kunzei* var. *favreae* Corner described from France, whose branches are distinctly “flattened, spatulate-flabelliform or laminate (as a rudimentary *Sparassis*), often curved, the ultimate branches horn-like with dentate or subcristate tips” (Corner 1950). *Ramariopsis kunzei* var. *megaspora* Corner reported from Tibet, Borneo and the Solomon Islands (Corner 1967, 1970) resembles *R. robusta* in having large, up to 120 mm high basidiomata of a similar colour. However, the basidiomata become darker (“alutaceous to dull fawn ochraceous”) when old and its spores, allegedly amyloid, are distinctly larger (5–6.5 \times 4.3–5.7 μm), subglobose, smooth, asperulate to

echinulate with shorter spines (0.3–0.7 μm). Its basidia are distinctly larger (48–75 \times 7–9.5 μm) with longer sterigmata (4.5–7 μm). *Ramariopsis kunzei* var. *subasperata* Corner described from France (Corner 1950) clearly differs from *R. robusta* by its very small (10–20 mm high), subtle, sparsely branched basidiomata, finely ornamented spores (“very laxly and vaguely asperulate”) and distinctly shorter basidia (18–24 \times 4–4.5 μm). *Ramariopsis kunzei* var. *sublaevispora* S.S. Rattan & Khurana, described from a mixed forest in the Sikkim Himalayas, India (Rattan & Khurana 1978), has rather large (up to 70 mm high and 50 mm broad), white basidiomata with rather robust stems, usually occurring singly and rarely in caespitose clusters. However, its branches are narrower (up to 3 mm) than in *R. robusta* and its spores are shorter [3.2–4.3(5) μm], subglobose to globose with “somewhat angular outline” and finely ornamented (“point-like markings on the surface”).

Finally, the European *Ramariopsis bispora* (Schild) Olariaga is a species formerly considered a variety of *R. kunzei*, from which it differs by bisporic basidia and clampless hyphae (Olariaga 2009). *Ramariopsis bispora* differs from *R. robusta* by these two characters and most of other characters typical for *R. kunzei*.

Interestingly, another species placed into close relationship of *R. robusta*, *Ramariopsis atlantica* Araujo-Neta, G.A. Silva & Gibertoni, is a Neotropic species with white basidiomata (Araujo-Neta et al. in Hyde et al. 2016). It is distinctly smaller (up to 50 mm high and 32 mm wide) than *R. robusta*, without flattened branches, straw-yellow after drying, with distinctly echinulate but smaller spores (4 \times 3 μm). This species was described from Atlantic rain forests in Brazil and most recently documented from Panama. Two *Ramariopsis* specimens collected by O. Koukol and P. Zehnález in Chiriquí Province, Panama (see Collections studied) clearly represent this species, based on molecular and phenotypic data.

Ramariopsis robusta may be also misidentified as *Clavaria lentofragilis* G.F. Atk., a name synonymised with *R. kunzei* by Corner (1950). The original description of *C. lentofragilis* (Atkinson 1908) nevertheless shows a fungus superficially resembling *R. robusta* by its large basidiomata (up to 150 mm high, tufts up to 120 mm broad), white branches with fragile tips and “oboval to subglobose” spores measuring 4–6 μm . However, *C. lentofragilis* clearly differs from *R. robusta* by less distinct spore ornamentation (asperulate only) and growth on rotten wood. It has to be mentioned that Petersen (1969) used the combination *Ramariopsis lentofragilis*, although it has never been validly published.

In view of the overall morphological plasticity of *Ramariopsis* species and great variability of their basidiomata (even in the holotype specimens), *R. robusta* is compared also with some unrelated species. Some collections of *Ramariopsis tenuiramosa* Corner can superficially resemble *R. robusta* by the white to cream colour of some basidiomata (Corner 1950, 1970). However, *R. tenuiramosa* clearly differs from *R. robusta* by distinctly smaller (up to 35 mm

high), sparsely branched, slightly tough and pliant basidiomata which may be darker (pale ochraceous, pale straw to yellowish drab) and possess narrow branches (0.5–1 mm) and slightly smaller spores ($3.5\text{--}4.5 \times 3\text{--}3.5 \mu\text{m}$) with finer ornamentation (minutely verruculose or subechinulate, Corner 1950; distinctly echinulate, Corner 1970). This species is reported from several continents (Corner 1950, 1970, Petersen 1964, Jülich 1984, Knudsen et al. 2012).

Ramariopsis bififormis (G.F. Atk.) R.H. Petersen (Petersen 1964) is very similar to *R. tenuiramosa* (Corner 1970: 81, 82). It differs from *R. robusta* by distinctly smaller basidiomata (up to 20 mm high) which are simple to sparingly branched and somewhat darker in colour (with grey tinges), and by its slightly smaller spores ($3.3\text{--}5.0 \times 2.6\text{--}3.8 \mu\text{m}$) with fine ornamentation (“very delicately verruculose to echinulate”) and shorter basidia (15–25 μm) (Petersen 1964, based on type study).

Ramariopsis rufipes (G.F. Atk.) R.H. Petersen is a white to cream species having delicately verruculose to echinulate spores. It differs from *R. robusta* by its smaller (up to 60 mm high and 30 mm wide), sparsely branched basidiomata, sometimes with reddish spots when old and usually with a darker stipe coloured brownish ochre (Olariaga 2009) or reddish (Petersen 1964). Moreover, its spores are slightly longer [up to $6.2(7) \mu\text{m}$], and therefore have a higher Q value ($Q_{\text{av}} = 1.37\text{--}1.52$, Olariaga 2009; in *R. robusta*, Q_{av} is 1.26). *Ramariopsis rufipes* was originally reported from North America (Corner 1950, 1970, Petersen 1964) but has recently been found in southern Europe, too (Olariaga 2009).

Except for *R. robusta* records included here and studied in detail, there are two potential records published online which could represent this species. The collections by J. Gaisler (2010, 2012) from the vicinity of Liberec (northern Bohemia, Czech Republic) look like a typical *R. robusta*. However, they should be studied microscopically to verify this hypothesis. Similarly, all robust collections of *R. kunzei* kept in herbaria should be revised, as some of them could represent *R. robusta*.

Collections studied

Ramariopsis robusta

Czech Republic. West Bohemia, town of Mariánské Lázně, in park near Ferdinand Spring Colonnade, $49^{\circ}57'47.295'' \text{N}$, $12^{\circ}42'24.370'' \text{E}$, alt. 580 m, mowed grassland with scattered deciduous trees, 26 Oct. 2016, leg. M. Hamadák, det. J. Matouš (PRM 945424). – Central Bohemia, village of Bříšejev, ca 270 m NNW of bus stop, upper part of sloping, dry, occasionally mowed meadow, $49^{\circ}42'21.865'' \text{N}$, $14^{\circ}29'07.772'' \text{E}$, alt. ca 400 m, on the ground in grass, 23 Sept. 2014, leg. & det. J. Matouš (PRM 945411). – North Bohemia, Liberec-Machnín, Hamrštejn Nature Reserve, 9 Aug. 2011, leg. V. Kautman, det. J. Matouš (BRA CR17115 as *R. kunzei*). – East Bohemia, village of Panská Habrová, urban area, garden, $50^{\circ}10'58'' \text{N}$, $16^{\circ}17'55'' \text{E}$, alt. 350 m, on grassy and mossy ground, under *Malus domestica*, 15 Nov. 2014, leg. T. Tejkllová, det. J. Matouš (PRM 933826 as *R. kunzei*).

Slovakia. Biele Karpaty Mts., village of Nová Bošáca, settlement of Grúň, $48^{\circ}53'42.860'' \text{N}$, $17^{\circ}47'53.157'' \text{E}$, alt. 460 m, sloping, extensively pastured meadow with scattered fruit trees, 23 Oct.

2014, leg. & det. J. Matouš (PRM 945410, holotype). – Nízke Tatry Mts., village of Malužiná, Michalovo valley, alt. 770 m, mountain meadow, 24 Aug. 2002, leg. I. Kautmanová, det. J. Matouš (BRA CR3395 as *R. kunzei*, rev. as *R. cf. lentofragilis*). – Laborecká vrchovina Highlands, village of Vyšná Jablonka, in pasture, 5 Oct. 2014, leg. V. Kautman, det. J. Matouš (BRA CR25525).

Ramariopsis atlantica

Panama. Chiriquí Province, Boquete, Sendero Culebra, on the ground along pathway, 9 Jul. 2015, leg. O. Koukol & P. Zehnálek, det. J. Matouš (PRM 945425). – Ibid., 15 Jul. 2015, leg. O. Koukol & P. Zehnálek, det. J. Matouš (PRC 3980).

Ramariopsis kunzei (sensu Olariaga 2009)

Czech Republic. Central Bohemia, Bohemian Karst, village of Liteň, at Obora Pond, under maples and ashes, on ground, 21 Aug. 2011, leg. & det. M. Kříž (PRM 935145). – North Bohemia, town of Kosmonosy, Baba hill, deciduous forest, 8 Aug. 2011, leg. J. Gaisler, det. I. Kautmanová (BRA CR17281). – East Moravia, Bílé Karpaty Mts., village of Vyškovec, Vlčí Nature Reserve, non-mowed grassland with scattered fruit trees, 48°55'42.137" N, 17°51'22.034" E, alt. 673 m, on ground amongst grass and moss, 24 Oct. 2014, leg. & det. J. Matouš (PRM 945415).

Slovakia. Malé Karpaty Mts., village of Chtelnica, Plešivá hora hill, 48°34'22" N, 17°35'44" E, alt. 350 m, 12 Sept. 2010, leg. I. Kautmanová & V. Kautman, det. I. Kautmanová (BRA CR15077). – Považský Inovec Mts., village of Banka near the town of Piešťany, Vápenišťia, in pasture and shrubs, 48°34'29" N, 17°51'46" E, alt. 220 m, 29 Sept. 2010, leg. V. Kautman & V. Kučera, det. I. Kautmanová (BRA CR15092).

Ramariopsis kunzei* var. *bispora

Slovakia. Stolické vrchy Mts., village of Muránska Huta, Predná Hora recreation area, old ski slope, 3 Oct. 2014, leg. V. Kautman, det. J. Matouš (BRA CR25735 as *R. kunzei*).

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