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Is the bioluminescence in many *Mycena* species overlooked? – A case study from *M. crocata* in Switzerland

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ABSTRACT

Fungal bioluminescence is mystifying humans since ancient times. Nevertheless, the biosynthetic pathway behind this phenomenon was only very recently resolved. Fungal bioluminescence occurs in five distantly related linages (*Omphalotaceae* lineage, *Armillaria* lineage, mycenoid lineage, Lucentipes lineage and *Eoscyphella* lineage) of the basidiomycete order *Agaricales*. Recent research suggests fungal bioluminescence has emerged 160 million years ago in the most common ancestor of the mycenoid and marasmioid clade and is maintained since then. Surprisingly, in the mycenoid linage, primarily represented by the genus *Mycena*, most species are considered non-luminescent, implying that many mycenoid species have lost their bioluminescent ability. Here, we report evidence for bioluminescence in *Mycena crocata* and show that the genome of this species is fully equipped with the genes associated with fungal bioluminescence. *Mycena crocata* is a long-known species frequently reported from Europe and Japan, which was considered non-luminescent until now. The low light emission intensity and the restriction of the luminescence to the vegetative mycelium and the base of the basidiome may be reasons why bioluminescence was not perceived earlier. We assume there might be other known *Mycena* species whose luminescent properties are not yet discovered, and that therefore the number of bioluminescent *Mycena* species is currently underestimated.

Keywords: bioluminescent mycelium, fungal bioluminescence, luciferase gene cluster, *Mycenaceae*, mycenoid lineage

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1. Introduction

Bioluminescence is the ability of a living organism to emit light resulting from oxidation of a substrate called luciferin usually catalyzed by an enzyme called luciferase (Shimomura, 2019). Fungal bioluminescence has been documented already in ancient times by the Greek philosopher Aristotle (384–322 BC) (Harvey, 1952). However, the biosynthetic cycle behind it was only recently resolved (Ke et al., 2020; Kotlobay et al., 2018) and experimental and phylogenetic evidence suggest that all known bioluminescent fungal lineages share the same bioluminescence pathway which is unique to fungi (Ke et al., 2020; Oliveira et al., 2012). The four key enzymes involved in fungal bioluminescence are: Hispidin synthase (Hisps) which converts caffeic acid to hispidin, hispidin-3-hydroxylase (H3h) which hydroxylates hispidin to yield 3-hydroxyhispidin (fungal luciferin), luciferase (Luz) which oxidizes luciferin to yield an unstable high-energy intermediate that emits light while it becomes oxyluciferin (caffeylpyruvate), which can be recycled by caffeylpyruvate hydrolase (Cph) into caffeic acid. Genome sequencing showed that in most bioluminescent fungi the *hisps*, *h3h*, and *luz* genes are organized in a cluster which may contain or not *cph* (Ke et al., 2020; Kotlobay et al., 2018). In the genera *Mycena* and *Armillaria* this gene cluster also consistently contains a cytochrome P450-like gene (*cyp450*), which function has not yet been elucidated (Ke et al., 2020).

To date, about 109 bioluminescent fungal species, all belonging to five distantly related lineages (*Omphalotaceae* lineage, *Armillaria* lineage, mycenoid lineage, Lucentipes lineage and *Eoscyphella* lineage) of the basidiomycete order *Agaricales*, are known (Ke & Tsai, 2022; Silva-Filho et al., 2023). Among them the mycenoid lineage with more than 600 described species (mostly *Mycena*) is the most diverse lineage. However, only a fraction, 85 species according to Ke and Tsai (2022), of those species is reported to be bioluminescent. As bioluminescence is considered to have evolved in the last common ancestor of the mycenoid and marasmioid clade (Ke et al., 2020; Kotlobay et al., 2018), this either means many mycenoid species have lost their ability to glow, or the luminous properties of many species may currently be undetected as e.g. suggested by Desjardin et al. (2008). Only in a few mycenoid species do the basidiome and mycelium, or very rarely only the basidiome, emit light. Most often only the mycelium of *Mycena* species is bioluminescent, and for many species the luminous properties of the mycelium are not documented (Desjardin et al., 2008). Also, the intensity of the emitted light may not be visible to dark-adapted

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human eyes and may only be captured by photomultipliers or digital cameras (Bermudes et al., 1992).

Mycena crocata (Schrad.) P. Kumm. is a long-known and widely distributed *Mycena* species in Europe that has also been recorded in Northern Africa, Asia and North America (Global Biodiversity Information Facility, 2023; Krieglsteiner et al., 2001; Maas Geesteranus, 1992). It is a saprotrophic species predominantly occurring on leaf litter and woody debris of beech (*Fagus*), and occasionally other hardwood trees like oaks (*Quercus*), ashes (*Fraxinus*), alders (*Alnus*) and birches (*Betula*) (Aronsen & Læssøe, 2016b; Krieglsteiner et al., 2001; Maas Geesteranus, 1992). Its basidiomes appear in late summer to late autumn and are easy to identify. They reach a height of 50–150 mm and have a slender stipe, which shows a characteristic color gradient ranging from bright orange-red at the base to a pale yellow or cream color at the top (Fig. 1 A1). In addition, yellow-red or orange-red stains may be present on the whitish gills. Most strikingly, however, the species exudates a distinctive saffron-red sap when cut, which is also reflected in its common name saffrondrop bonnet mushroom, as well in the Latin species epithet *crocata* which means saffron-colored (Krieglsteiner et al., 2001; Maas Geesteranus, 1992; Robich, 2003). *Mycena crocata* is the type species and the only representative species of the *Mycena* section *Crocatae* (Maas Geesteranus, 1992).

Although *M. crocata* is frequently reported from Europe and Japan (Global Biodiversity Information Facility, 2023), the species has not been described as bioluminescent to date (Aronsen & Læssøe, 2016a; Desjardin et al., 2008). Unexpectedly, we captured bioluminescent spots at the stipe base of a *M. crocata* basidiome, which led us to further explore the bioluminescence of this species.

Studying a larger collection of *M. crocata* from Switzerland, we investigated the bioluminescent properties of its basidiomes and mycelium using digital imaging and a photomultiplier (luminometer). In addition, we captured the bioluminescence spectrum emitted by *M. crocata* and confirmed the presence of bioluminescence-related genes in its genome.

2. Materials and methods

2.1. Collection and isolation of Mycena crocata

In 2017 one basidiome, and from 2022 to 2023 46 *M. crocata* basidiomes were collected in eight mixed forests with European beech (*Fagus sylvatica*) around the city of Zurich in Switzerland (Supplementary Table S1). In addition, 21 small wood fragments/branches with attached *M. crocata* basidiomes were collected on the forest floor of two sites in 2022 and 2023. Field identification of the species was based on basidiome morphology (size, stipe color, stains on gills and exudation of saffron-red sap when cut).

For 14 individual basidiomes, pure cultures were obtained as follows. The tip of the cap was cut off with a sterile scalpel, and small pieces of mycelium from inside the cap tip were plated on three different media: Maloy (1974) medium (15 g/L Plant Propagation Agar, PPA, Condalab, Madrid, Spain, 12 g/L Bacto Malt Extract, Thermo Fisher Scientific, Waltham, MA, USA, amended with 2 mg/L benomyl and 100 mg/L streptomycin), water agar (15 g/L PPA amended with 100 mg/L streptomycin) and malt extract agar (15 g/L PPA, 20 g/L Diamalt, Hefe Schweiz AG, Stettfurt, Switzerland). Isolation plates were incubated at room temperature in the dark and after 7–14 d outgrowing mycelium was transferred to fresh malt extract plates to obtain axenic cultures. From nine wood pieces with *M. crocata* basidiomes, infected tissue from inside of the wood fragment was plated on Maloy medium without and with surface sterilization (sodium hypochlorite solution with 0.5% (v/v) active chlorine for 20–30 s, washing with sterile water) and axenic cultures were obtained as described above.

2.2. Molecular identification of M. crocata

A small agar plug with mycelium was taken from pure cultures to extract DNA using the sbeadex Plant DNA Purification Kit (LGC, Teddington, UK) on the KingFisher Flex Purification System (Thermo Fisher Scientific) following the manufacturer's protocol. The ITS region was amplified with primers ITS5 and ITS4 (White et al., 1990) in reaction volumes of 25 µL with final concentrations of 1x GoTaq® G2 Hot Start Green Master Mix (Promega, Madison, WI, USA), 0.4 µM of each primer and 2 µL of 10-fold diluted DNA. The thermal cycling parameters were: 2 min 94 °C initial denaturation, 35 cycles of 30 s 94 °C, 30 s 54 °C, 1 min 72 °C, and 10 min at 72 °C final elongation. PCR products were prepared for Sanger sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and sequenced in both directions on an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific). The resulting sequences were assembled and edited with CLC Main Workbench 22 (www.clcbio.com) and consensus sequences were submitted to BLAST searches in the NCBI nucleotide collection (http://blast.ncbi.nlm.nih.gov) to confirm species identity.

2.3. Bioluminescence of M. crocata

First, the bioluminescence of *M. crocata* basidiomes was assessed. For this, the bioluminescence of 36 individual basidiomes was captured with a camera and/or luminometer (Supplementary Table S2). Photographs were taken in complete darkness with a Nikon D810 camera with a 24 mm (1:1.4) fixed focal length lens or a 60 mm Macro (1:2.8) fixed focal length lens, 240 s exposure, ISO 8000 and f/3.2. The color temperature was set at 4900 K / Tint +54. Luminometer measurements were conducted with a Lumat LB 9507 luminometer (Berthold Technologies, Bad Wildbad, Germany) which detects even weak light emission by the means of a photomultiplier tube. Fungal tissues assessed with the luminometer were: stipe base (section of 10–15 mm), middle part of the stipe (section of approx. 25 mm, split in three fragments), gills and cap surface (approx. 1/4 to 1/2 cap, depending on size). Samples were added to the bottom of the luminescence tube and inserted to the luminometer. Light emission intensity in relative light units per second (RLU/s) was consecutively measured for 2 s every 2 s and was read from the luminometer 20 s after sample insertion. To establish the background luminescence of the luminometer, the luminescence of 15 samples (approx. 10 × 15 mm tissue taken from the stipe or cap) of *Agaricus bisporus* (button mushroom, non-luminescent fungus of the order *Agaricales*), as well as 18 times of the empty luminescence tube were measured as described above.

Next the bioluminescence of *M. crocata* in colonized wood was assessed. For this, 21 wood sections with attached basidiomes were carefully split longitudinally with a knife. The bioluminescence of the cut surface was assessed 0, 4, 6 and 16 h after splitting with a camera as described above.

Finally, the bioluminescence of *M. crocata* mycelium on agar medium was assessed for 17 axenic cultures obtained from basidiomes (14) and infected wood (3). For each isolate 1 to 3 fresh malt extract agar plates (Ø 90 mm) were inoculated with a small agar plug from a stock culture. The plates were incubated in the dark at room temperature. Starting 12 d post inoculation (dpi), the bioluminescence of cultures was assessed weekly for 13 wk using a camera as described above. For cultures still emitting bioluminescence after 13 wk, the assessment was continued at a 2-to-3-wk in-

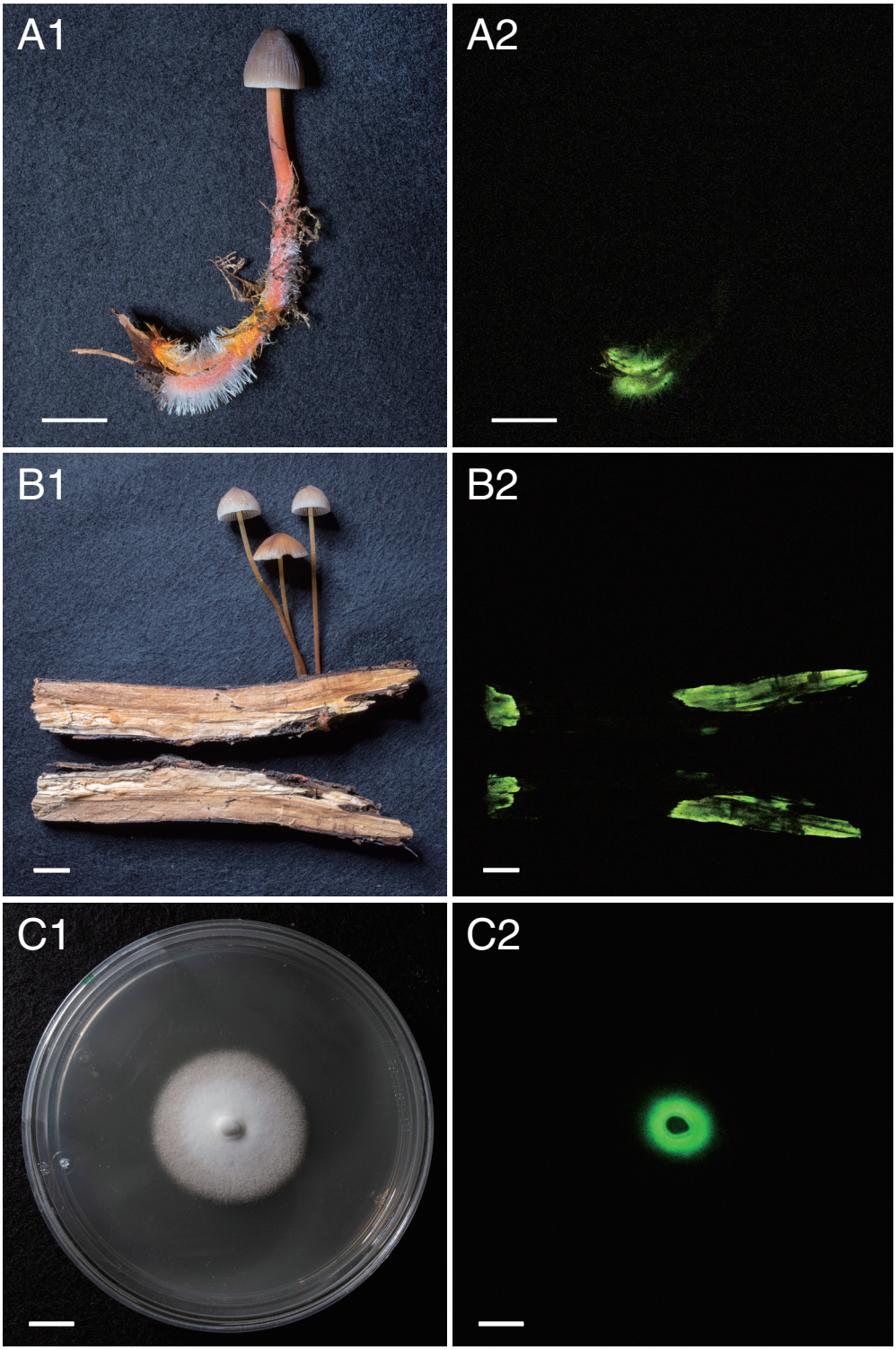

Fig. 1 - Bioluminescence of *Mycena crocata*. Basidiome (BR_1306) photographed in daylight (A1) and in complete darkness (A2) with time exposure (Nikon D810 with a 24 mm (1:1.4) fixed focal length lens, 240 s exposure, ISO 8000 and f/3.2. Infected wood (BR_1507) photographed 4 h after splitting in daylight (B1) and in complete darkness (B2). Axenic culture (BR_1102) on malt extract agar photographed 16 d post inoculation in daylight (C1) and complete darkness (C2). *Bars:* 10 mm. Photos: Baggenstos/Rudolf.

terval until the experiment was terminated in week 25 post inoculation.

2.4. Measurement of bioluminescent spectrum

Bioluminescent spectra of axenic cultures of two different isolates (BR_1003, BR_1201) were captured using a Multi-Mode Microplate Reader SpectraMax M2 (Molecular Devices, San Jose, CA,

USA). For the measurement, culture plates, covered with the lid, were positioned in the plate reader so that a highly bioluminescent spot of the mycelium was exposed. Light emission intensity was captured from 360 to 850 nm in 10 nm steps. For isolate BR_1003 one, and for isolate BR_1201 two measurements from two different spots of the culture were conducted. Cultures were 172 d (BR_1003) and 185 d (BR_1201) old.

2.5. Identification of the luciferase cluster

To identify putative orthologs of the genes associated with bioluminescence in the genome of *M. crocata*, we followed the reciprocal best hits approach. For this, first the protein sequences of the four genes of the luciferase cluster (*luz*, *h3h*, *cyp450*, *hisps*) and of caffeylpyruvate hydrolase (*cph*) of *M. kentingensis* (Ke et al., 2020) were used as queries to search for similar proteins among the (draft) proteins of the *Mycena crocata* CBHHK184 v1.0 genome (https://mycocosm.jgi.doe.gov/Myccro1/Myccro1.home.html). NCBI BALSTp searches restricted to *Mycena crocata* (taxid:220801) were used to query the non-redundant protein sequence collection of NCBI which includes the protein sequences of *M. crocata* CBHHK184 v1.0. Based on the JGIDB_ID the genomic location and orientation of the best matching genes in the *M. crocata* CBHHK184 v1.0 genome was deduced. In the next step, BLASTp searches restricted to *M. kentingensis* (taxid:1325293) were conducted using the best BLASTp hits from *M. crocata* as queries, to identify the reciprocal best hits.

3. Results

3.1. Bioluminescence of M. crocata basidiomes

The camera captured greenish bioluminescence in almost half (47.10%) of the assessed 17 *M. crocata* basidiomes (Supplementary Table S2). The recorded bioluminescence was restricted to the base of the stipe, to a zone where hyphae (fibrils) grew out of the stipe (Figs. 1 A1, 2; Supplementary Fig. S1). Congruent with this observation, light emission recorded with the luminometer exceeded the threshold of background luminescence (25 RLU/s corresponding to the maximum light emission intensity recorded for controls) for most stipe base samples and a few stipe samples, but not the other sample types (Fig. 2). Overall, 79.4% (27 out of 34) of the basidiomes showed light emission above background luminescence at the stipe base with the luminometer. Nevertheless, the light intensity emitted from the stipe base was highly variable among basidiomes (Fig. 2; Supplementary Table S2) and spanned the range from very dim to considerably brighter luminescence (26 to 233 358 RLU/s). Of stipe samples (middle part), only 14.3% (3 out of 21) showed light emission above background luminescence. However, the light emission of those stipe samples was dim and only ranged from 47 to 161 RLU/s (Fig. 2; Supplementary Table S2).

Bioluminescence detection with the camera was less sensitive than with the luminometer. Of 15 samples assessed with both devices, 12 samples (80.0%) showed light emission above background luminescence with the luminometer (stipe base), but only for 6 (40.0%) of them bioluminescence was also detected with the camera (Supplementary Table S2). Light emission intensity and detection with the camera were clearly correlated. No detection with the camera was observed for light intensities of 7 704 RLU/s or lower, whereas bioluminescence was always detected with the camera for light intensities of 11 460 RLU/s or more. Finally, the study authors needed to adapt their eyes to the darkness for at least 30 min to see the bioluminescence of basidiomes with a bright glowing stipe base.

3.2. Bioluminescence of wood infected by M. crocata

All 21 wood pieces with *M. crocata* basidiomes growing on them showed bioluminescence detectable with a camera on the cut surface (Figs. 1 B1, 2; Supplementary Fig. S2). In many cases, albeit weak, greenish bioluminescence could be already detected directly after splitting of the wood (Table 1; Supplementary Fig. S3). Generally, the bioluminescence became stronger some hours after splitting (Table 1; Supplementary Fig. S3). At the 4- and 6-h assessment, bioluminescence was detected for all but one wood fragment. Sixteen h after splitting, the bioluminescence of the cut surface was diminishing and in some cases not even detectable any more.

All nine attempts to isolate and identify *M. crocata* from bioluminescent spots of woody debris were successful (Table 1; Supplementary Table S1), confirming the species presence in bioluminescent wood.

3.3. Bioluminescence of M. crocata mycelium on malt extract agar

Eleven out of 17 axenic isolates (64.7%; 14 from basidiomes, 3 from infected wood, all confirmed as *M. crocata* based on ITS barcoding, see Supplementary Table S1) showed bioluminescence that

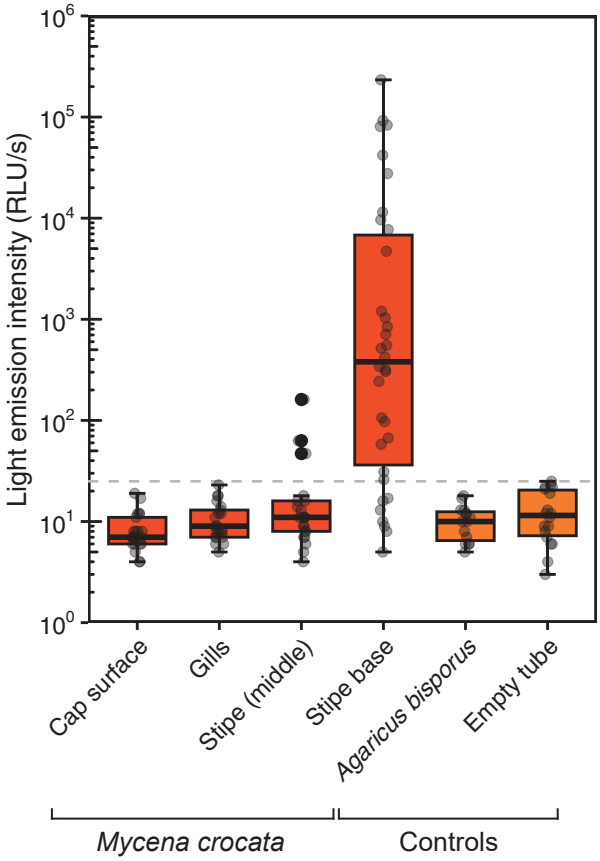

Fig. 2 - Light emission intensity (RLU/s) of different tissues of *Mycena crocata* (dark orange) and controls (light orange). In each boxplot, the thick central line represents the median. The colored boxes span from the first (25th percentile) to the third quartile (75th percentile) and the whiskers extend maximally 1.5 times the distance between the first and third quartile from the box edges. The grey horizontal dashed line indicates the threshold for background bioluminescence defined as the maximum light emission intensity measured for the controls (25 RLU/s). *Agaricus bisporus*: non-luminescent fungal control. Stipe base: N = 34; cap surface, gills, stipe (middle): N = 21, *A. bisporus*: N = 15; empty tube: N = 18.

Table 1. Bioluminescence of *Mycena crocata* in woody debris.

Sample Name	Light detection with camera[a]			
	0 h[b]	4 h	6 h	16 h
BR_1501[c]	+	++	+	–
BR_1502[c]	NA	+++	NA	+
BR_1503[c]	NA	+++	NA	+
BR_1504	NA	++	NA	–
BR_1505	NA	+	NA	–
BR_1506	–	+	NA	NA
BR_1507	–	+++	NA	NA
BR_1508	+	NA	+++	NA
BR_1509	+	NA	+++	NA
BR_1510	+	NA	+++	NA
BR_1511	+	NA	–	NA
BR_1512	+	NA	+	NA
BR_1513[c]	+	+++	++	+
BR_1514[c]	–	+++	+++	++
BR_1515[c]	+	+++	+++	+
BR_1516[c]	–	+++	++	+
BR_1517	+	++	+	–
BR_1518	–	+	+	–
BR_1604[c]	+	++	++	+
BR_1605[c]	–	+	+	+
BR_1606	+	+	+	–

[a] Photos were taken in complete darkness with a Nikon D810 equipped with a 24 mm (1:1.4) fixed focal length lens, 240 s exposure, ISO 8000 and f/3.2. + weak, ++ medium and +++ strong bioluminescence; – no bioluminescence detected; NA not assessed.
[b] Hours after splitting of the wood fragments.
[c] *Mycena crocata* was isolated from the substrate and the species identity confirmed by Sanger sequencing of the ITS-region.

could be captured with a camera and time exposure (Table 2). Most (81.8%) of the 11 replicated isolates were consistently luminous or not luminous. In most cases, bioluminescence could be already captured at the first (13 to 16 dpi) or second (20 to 22 dpi) assessment (Table 2). In two cases, the onset of bioluminescence was delayed to the third (29 dpi) or even seventh (59 dpi) assessment. Most cultures (83.3%, 15 out of 18) showed stable bioluminescence lasting 103 to 164 d (Table 2; Supplementary Fig. S4). The luminescence of cultures with a more transient glow lasted a few days (just detected at one assessment) to 28 d. Typically, cultures emitted a greenish light in the center (Fig. 1 C2; Supplementary Fig. S5). Older cultures also could be glowing at their edges and very old cultures (older than approx. 140 d) often showed discontinuous spots of luminescence scattered across the mycelium.

For two isolates (BR_1003, BR_1201) the bioluminescence spectrum was captured (Fig. 3). Light emission consistently reached a peak at a wavelength of 520 to 530 nm.

3.4. Detection of the luciferase cluster in the M. crocata genome

Using BLASTp search we identified putative homologs of the *M. kentingensis* genes associated with the luciferase cluster (*luz*, *h3h*, *cyp450*, *hisps*) and of caffeylpyruvate hydrolase (*cph*) in the *M. crocata* CBHHK184 v1.0 genome (Supplementary Table S3). The *M. crocata* top hits showed 48.6 to 78.4% amino acid identity to the query sequences and 93.0 to 100% coverage of them (Table 3). The top hits also fulfilled the criterium of reciprocal best BLASTp hits. In addition, the top hits for the four genes associated with the luciferase cluster were all located on the *M. crocata* scaffold 138 and were placed after each other and in the same order and orientation as in other bioluminescent *Mycena* species (e.g. *M. kentingensis*, *M. chlorophos*) or *Armillaria* species (Supplementary Fig. S6), providing further evidence we identified the correct orthologs. The top hit for *cph* was located on *M. crocata* scaffold 2 and did not co-locate with the genes of the luciferase cluster which matches with other *Mycena* species (Supplementary Fig. S6).

Table 2. Bioluminescence of *Mycena crocata* mycelium on malt extract agar.

Sample Name	Light detection with camera[a]		
	Replicate 1	Replicate 2	Replicate 3
BR_1001	–[b]	–	–
BR_1003	+ (29–177*)	+ (14–177*)	+ (22–177*)
BR_1005	+ (64)	–	–
BR_1007	–	–	–
BR_1008	–	–	–
BR_1101	–	–	NA
BR_1102	+ (16–119)	+ (16–119)	NA
BR_1103	–	–	+ (16–44)
BR_1201	+ (16–177*)	+ (16–177*)	+ (16–177*)
BR_1202	+ (16–177*)	NA	NA
BR_1203	+ (16–177*)	NA	NA
BR_1301	–	NA	NA
BR_1302	+ (13–41)	NA	NA
BR_1305	–	NA	NA
BR_1501	+ (22–177*)	+ (22–177*)	NA
BR_1502	+ (20–177*)	+ (20–177*)	NA
BR_1503	+ (13–177*)	NA	NA

[a] Photos were taken in complete darkness with a Nikon D810 equipped with a 24 mm (1:1.4) fixed focal length lens, 240 s exposure, ISO 8000 and f/3.2.
[b] – no bioluminescence detected; + bioluminescence detected, the interval in parentheses indicates the last and first day post inoculation bioluminescence was recorded, * indicates the last day of the experiment; NA not assessed.

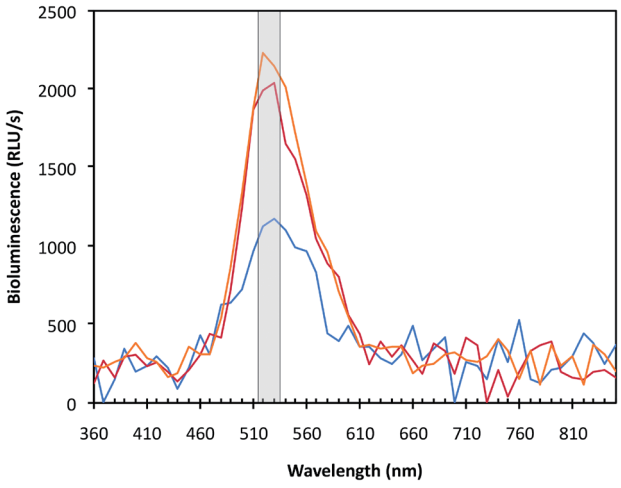

Fig. 3 - Bioluminescence spectrum of *Mycena crocata* mycelium growing on malt extract agar. Light emission intensity (RLU/s) was captured from 360 to 850 nm in 10 nm steps. Isolate BR_1003 (blue), 172 d post inoculation (dpi) and isolate BR_1201 (spot 1: red, spot 2: orange), 185 dpi. The grey area highlights the wavelengths with maximal light emission.

Table 3. Top hits of BLASTp search against the *Mycena crocata* CBHHK184 v1.0 genome using *Mycena kentingensis* proteins associated with the luciferase cluster and recycling of oxyluciferin as queries. Putative orthologs, based on reciprocal best BLASTp matches, are indicated in bold.

	M. kentingensis query	Top hit in *M. crocata* CBHHK184 v1.0 genome						
Gene	GenBank accession	GenBank accession	aa identity	Query cover	Protein length (aa)	JGIDB ID	Genomic position and orientation of gene	Reciprocal best hits
luciferase (*luz*)	**KAF7309053**	**KAJ7112865**	71.3%	93.0%	258	Myccro1_903514	scaffold_138:206790-207620 (-)	yes
hispidin-3-hydroxylase (*h3h*)	**KAF7309054**	**KAJ7112866**	66.7%	97.0%	432	Myccro1_134416	scaffold_138:207382-209599 (+)	yes
cytochrome P450 (*cyp450*)	**KAF7309055**	**KAJ7112867**	48.6%	98.0%	522	Myccro1_1113363	scaffold_138:209779-211653 (-)	yes
hispidin synthase (*hisps*)	**KAF7309056**	**KAJ7112868**	54.6%	99.0%	1676	Myccro1_1113364	scaffold_138:211972-218276 (+)	yes
caffeylpyruvate hydrolase (*cph*)	KAF7309057[a]	KAJ7181586	78.4%	98.0%	301	Myccro1_969572	scaffold_2:1027354-1029087 (+)	no
	KAF7332795	**KAJ7181586**	77.4%	100.0%	301	Myccro1_969572	scaffold_2:1027354-1029087 (+)	yes

[a]The gene for caffeylpyruvate hydrolase (*cph*) is duplicated in *M. kentingesis*.

4. Discussion

By examining several collections of *M. crocata* from Switzerland, this study has demonstrated the ability of this species to emit bioluminescence. In agreement with other studies on fungal bioluminescence (Kotlobay et al., 2018; Mihail, 2015; Niitsu et al., 2000; O'Kane et al., 1990; Oliveira & Stevani, 2009), the luminescence of *M. crocata* reached a peak at 520 to 530 nm, which corresponds to green light in the electromagnetic spectrum. In addition, we demonstrated that the genome of *M. crocata* possesses the luciferase gene cluster and the caffeylpyruvate hydrolase gene and is therefore fully equipped with the genes needed for fungal bioluminescence (Ke et al., 2020; Kotlobay et al., 2018).

Based on our findings, only the mycelium of *M. crocata* is bioluminescent, while the basidiome is non-luminescent, with the exception of the stipe base and in very rare cases the stipe itself (only very weak luminescence). This could be a reason why the bioluminescence of this species remained undiscovered for so long. Another reason could be the dim light that *M. crocata* emits. In some cases, the study authors were able to see the bioluminescence of *M. crocata* with their eyes, but only if they adapted them to the darkness long enough and if the glow was intense enough. With digital imaging the bioluminescence of *M. crocata* could be detected much more easily, even in cases where it was not perceived by human eyes. Nevertheless, we observed cases where the light emission was so weak that it could only be detected with the luminometer but not with the camera.

Mycelium of *M. crocata* naturally growing in wood substrate as well as on artificial culture media (malt extract agar) emitted bioluminescence that was intense enough to be detected with digital imaging. In infected wood bioluminescence was usually detectable within 0 to 4 h after splitting, but it was transient and lasted only a few hours. This, most likely, because the wood started to dry out and the growth conditions became unfavorable for *M. crocata*. In contrast, on malt extract agar, the mycelium had first to establish, and it always took days until bioluminescence became visible. Nonetheless, bioluminescence of many axenic cultures was long-lasting and could be observed over months, probably reflecting the sustained growing conditions. The non-detection of bioluminescence for about one third of the cultivated isolates could reflect a detection issue, i.e. cultures were only assessed with the camera, which may miss low emission intensities. Another possible explanation could be unfavorable culture conditions for the expression of luminescence (Weitz et al., 2001). Theoretically, it is also possible that some strains have lost their genetic ability for bioluminescence, even though this would need further confirmation.

The ecological role of bioluminescence in fungi is still a mystery (Ke et al., 2020). In some fungal species with basidiomes with intense luminescence, such as *Neonothopanus gardneri* from the *Omphalotaceae* lineage, bioluminescence attracts arthropods during night, which may promote spore dispersal (Oliveira et al., 2015). The basidiospores of *Mycena* species are usually dispersed by wind and in species that do not have luminous basidiomes, such as *M. crocata* shown here, bioluminescence likely plays a different, yet unknown role.

We conclude from our study that it is highly likely that there are other possibly well-known *Mycena* species whose bioluminescent properties have not yet been discovered. Consequently, existing studies on fungal bioluminescence may underestimate the frequency of bioluminescence in the mycenoid linage. Further studies on the evolution of luminescence within the mycenoid linage are recommended to verify the luminescent properties of putative "non-luminescent" species in the absence of clear evidence for their lack of bioluminescence.

Data availability

ITS-sequences of *M. crocata* isolates are available from the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/) with accession numbers PP130556–PP130578.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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