# Colletotrichum acericola sp. nov. from seeds of invasive alien Acer negundo in Poland: delayed pathogen introduction after its host appearance?

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**Article**

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## Abstract

The boxelder maple (Acer negundo, Sapindaceae) is one of the most common tree species of the genus Acer natively growing in North America. In the 17th century A. negundo was introduced to Europe as an ornamental plant, and from this point forward it started uncontrolled expansion on new territories, as an alien invasive species (IAS), threatening local ecosystems and human's safeness. Etiological symptoms of fungal development on boxelders' seeds and petioles, were observed in 2017 and 2021 in the municipality of Wrocław (SW Poland, Europe), outside of host native occurrence. Morphological analyses combined with multilocus phylogenetic analyses (using rDNA ITS, gapdh, act, tub2, chs-1, and his3 sequences), indicated that isolated strains belong to a new species of Colletotrichum, from the rare Colletotrichum agaves species complex. Name Colletotrichum acericola is proposed for this fungus, after its host on which it was found. It is the second species of the Colletotrichum agaves species complex described from the dicotyledonous host and outside host plants of the family Asparagaceae. Based on phylogenetic analyses and deposited in NCBI-GenBank environmental sequences (ex endophytes of Dalea purpurea and Panicum virgatum in prairies of USA) assigned here to this species, it is likely that Colletotrichum acericola is native to North America and introduced to Europe after its host plant appearance, maybe on nursery material. The possible future invasion of C. acericola on Acer negundo and its spillover to other (native) Acer species in Europe are discussed.

## Introduction

Biological invasions are one of the greatest challenges of the 21th century [1]. According to Hulme [2], up to 80% of alien taxa came to Europe as ornamental plants, due to their decorative features, which bring them to the centres of cities and gardens. It is estimated that up to 5–20% of them became invasive over time [3]. The uncontrolled movement of organisms beyond the natural ranges of their occurrence not only threatens biodiversity, but can also pose economic problems and threaten human health and life [4–9]. The negative impact of invasive alien species on local ecosystems may be direct, such as niche occupation and allelopathic impact, or indirect, e.g. through the transfer of pathogens and parasites to native organisms occurring in new areas [10, 11].

The phenomenon of increased competitiveness of alien invasive plants compared to native taxa is explained by many hypotheses, however, the most important of which, dealing with pathogen transfer, are the Enemy Release Hypothesis (ERH) and the Novel Weapons Hypothesis (NWH). According to the assumptions of ERH hypothesis, invasive alien species are under lower enemy pressure than native one, which gives them advantage in energy expending for sporulation, growth and expansion [12–15]. Alien species might also be a carrier of some alien, cryptic pathogens, that may be transferred unnoticed and be brought into new territories with the host plant, often as endophytes [10, 15–17]. In principle dragged alien pathogens are less harmful to the primary host with whom they share years of co-occurrence, but by spillovering to local plants, they can threaten organisms in native ecosystems [11, 18, 19]. This phenomenon indirectly is taken up by the NWH, which mainly explain IAS superiority as a result of allelopathic influence. Newly introduced host plants may pose a phytosanitary threat, by being a reservoir of native (for the host plant) pathogens attacking local species with double-barrelled strength (spillback mechanism), which may negatively influence native flora [16, 20, 21]. On the other hand, the introduction of alien pathogens often may happen long after introduction of alien host plants, what we often observed on example of powdery mildews [e.g., 22–26]; that shows that the threat posed by alien species is a long term issue that may affect local ecosystems for a long time.

The boxelder maple (Acer negundo, Sapindaceae) is the most common species of the genus Acer in North America. It was brought to Europe in 1688 to the Fulham Garden in England as an ornamental plant [27]. In Poland, the species joined the collection of the Kraków Botanical Garden (1808), the park's trees collection in Krzemieniec (1810) and the collection in Niedźwiedź in 1813. Based on information from neighboring countries [27], the first escape of A. negundo from cultivation in Central Europe was observed at the turn of the 19th and 20th centuries. At that time, it was also planted as a roadside plant [27]. However, its particularly high occurrence in the first stages of invasion was recorded along rivers where this species developed particularly well [27–29]. Currently, the boxelder is considered as an invasive alien species which occurs in natural and semi-natural habitats. The species invaded all European countries (except Iceland and Ireland) [30]. Due to its rapid growth, high resistance to drought and wide tolerance to soil and light conditions, A. negundo spreads the easiest in areas exposed to strong human pressure (e.g. heaps, post-industrial dumps or the surroundings of settlers). This ornamental plant is still used for greening poor, degraded soils, parks and alleys, where due to the above-mentioned features it is one of the few plants, capable to stand such harsh, urban conditions [28]. Importantly, the boxelder pose the greatest threat to phytocoenoses such as riparian forests and open ecosystems, e.g. xerothermic grasslands [30, 31], by displacing native species of woody and herbaceous plants. Moreover, overgrowing stems and twigs of A. negundo on the railway and hydrological infrastructure (e.g., fire embankments), makes it difficult to maintain them in a proper technical condition, indirectly threatening people's safety. Moreover, A. negundo may influence human health, as its pollen has allergenic properties.

Apart from the native area of the boxelder occurrence, only a few fungi causing plant diseases have been described from this alien tree in a secondary range of its occurrence. For example, in Europe the boxelder is a host of polyphagous fungus - Phymatotrichum omnivorum causing dangerous disease syndrome known as root rot [31]. Other diseases that were observed in Europe include: tar spot (caused by Rhytisma acerinum) [32], leaf spot (caused by Diplodina acerina), powdery mildew (caused by Sawadea bicornis) [33] and shoot dieback (caused by Fusarium lateritium) [34]. In 2015 the Slovenian forest service noted rarely recorded species Cristulariella depraedans, Schizophyllum commune, and oomycete pathogen Phytophthora sp. [35]. In turn, in Turkey and China, shoot dieback caused by Fusarium solani, Fusarium nirenbergiae and Fusarium lateritium were also observed from A. negundo [36–38].

Interestingly, among the genus Colletotrichum, only Colletotrichum gloeosporioides has been reported on Acer negundo, in the native range of boxelder occurrence: in New Jersey and Texas in the USA [39]. However, it is currently known that Colletotrichum gloeosporioides is a species complex [40, 41], and therefore, the identity of the Colletotrichum species on the boxelder in the USA is unclear. At the same time it is obvious that the genus Colletotrichum is one of the most important fungal genera, including saprobes, endophytes and plant pathogens causing anthracnoses on a wide range of angiosperm plants [42, 43]. In the past, the identification of Colletotrichum species was challenging due to simple and often overlapping morphological characteristics in many species complexes [44, 45]. The application of molecular phylogenetic methods, based on multi-locus DNA sequence analyses, provided a reliable tool to species identification [42, 46] leading to extensive studies on the genus Colletotrichum, especially in tropics and subtropics [43]. Recently, etiological symptoms of

fungal development on seeds and petioles of Acer negundo with conidiomata similar to Colletotrichum species were observed in south-western Poland. The aim of this study was to isolate the causative agent into cultures and to identify the isolated fungus to species based on morphological and multi-locus DNA sequences analyses. The possible geographical origin of the causative agent, its introduction to Europe after host appearance and its possible impact on the native plants are discussed.

## Material And Methods

# Isolates

Seeds and petioles of Acer negundo (Sapindaceae) with etiological symptoms of fungal development were collected in the municipality of Wrocław (SW Poland) in November 2017 and November 2021, from two localities in SE part of the city (det. & leg. K.Patejuk). On both study spots, plants were growing in small groups as a part of an urban green area, located in a close neighbourhood to the traffic route. Each locality, three individuals of A. negundo were surveyed. Fully matured seeds and rarely petioles were covered by brown smears that turned to the black, raised dots, breaking on the apical part with time. The use of plants in the present study complied with international, national and institutional guidelines. After one month of drying under room conditions seeds with symptoms of fungal development were disinfected with 1% sodium hypochlorite solution for 30 sec and placed on PDA medium (Biocorp). After 14 days from developed colonies, one representative strain of Colletotrichum species from three multispecies fungal cultures (representing three collections from two spots) was transferred to Petri dishes with PDA in order to obtain single-species cultures that were used for morphological and molecular analyses. Dried specimens (holotype, isotype and other specimens) are stored in the fungal collection of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (KRAM F). Ex-type culture and other culture are deposited in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS).

# Morphological analyses

Symptoms of infection were observed and photographed using dried seeds and petioles. Macroscopic characteristics and colony diameter of cultures were analyzed and measured on MEA (malt extract agar), OA (oatmeal agar), PDA (potato dextrose agar) and SNA (synthetic nutrient-poor agar) using one- and two-week-cultures incubated at 25°C. Microscopic characteristics were studied mostly on PDA, supplemented by some observations on OA, using cultures or slide cultures older than two weeks incubated at 25°C. Fungal structures taken from the cultures were placed in 80% lactic acid, heated to boiling point, cooled and examined under a Nikon Eclipse 80i light microscope at a magnification of ×1000. The microscopic structures were measured and photographed using NIS-Elements BR 3.0 imaging software.

# DNA isolation, amplification, and sequencing

Prior to molecular analyses, isolates were grown on PDB medium (Potato Dextrose Broth, BD Difco, USA) for 10 days at 23°C in the dark at Petri dishes protected with Parafilm.

DNA was extracted using Bead-Beat Micro AX Gravity Kit (A&A Biotechnology, Poland) from freeze dried (CoolSafe, Scanvac, Denmark) mycelium homogenized with quartz beads in a Magna Lyser homogenizer (Roche, Basel, Switzerland). Then, DNA was quantified fluorometrically on a Quantus device (Promega, WI, USA) and diluted in $ddH_2O$ to a concentration of 20 $ng \cdot L^{-1}$ for further analyses.

Amplification of ITS1-5.8S-ITS2 regions of ribosomal DNA (ITS) and fragments encoding β-tubulin (tub2), actin (act), glyceraldehyde-3-phosphate dehydrogenase (gadph) and chitin synthase (chs-1) genes was carried out in 37.5 µL of PCR Mix Plus (A&A Biotechnology, Poland). The final concentration of reagents was as follows: 1×PCR Mix Plus, 0.6 pM of each primer synthesized by IBB PAN, Poland (Table 1) and 4 ng of DNA template. Thermal cycling was performed on an Eppendorf EP Mastercycler using the following parameters: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation in 94°C for 1 min, annealing in temperature typical for each of the 5 primer set (Table 1) for 1 min and extension in 72°C for 2 min, followed by 10 min at 72°C for the final extension. The presence of PCR products was verified by electrophoretic separation of 2 µL of the post-reaction mixture in tris-borate-EDTA (TBE) buffered by a 1.2% agarose gel (Pronadisa, Spain) stained with SimplySafe (EURx, Poland).

Table 1
Annealing conditions of PCR assays, primers and their references

| Target | Primer name and sequence 5'→ 3' | Annealing conditions | References |
|---|---|---|---|
| ITS | ITS1: TCCGTAGGTGAACCTGCGG | 55°C/1 min | [68] |
| | ITS4: TCCTCCGCTTATTGATATGC | | |
| act | ACT-512F: ATGTGCAAGGCCGGTTTCGC | 60°C TD-PCR* | [69] |
| | ACT-783R: TACGAGTCCTTCTGGCCCAT | | |
| chs-1 | CHS-79F: TGGGGCAAGGATGCTTGGAAGAAG | 58°C/1 min | [69] |
| | CHS-354R: TGGAAGAACCATCTGTGAGAGTTG | | |
| gapdh | GDF1: GCCGTCAACGACCCCTTCATTGA | 58°C/1 min | [70] |
| | GDR1: GGGTGGAGTCGTACTTGAGCATGT | | |
| tub2 | Bt2a : GGTAACCAAATCGGTGCTGCTTTC | 58°C/1 min | [71] |
| | Bt2b: ACCCTCAGTGTAGTGACCCTTGGC | | |
| *65°C (cycle 1–5); 62°C (cycle 6–10); 60°C (cycle 11–35). | | | |

The amplification products were purified and sequenced by Genomed, Poland. Fluorograms were edited and consensus sequences were generated with FinchTV 1.4 (Geospiza Inc., Seattle, WA, USA). The Basic Local Alignment Search Tool (BLAST) [47] of the GenBank NCBI nucleotide sequence database (www.ncbi.nlm.nih.gov/genbank) was used to compare ITS, act, chs-1, gapdh and tub2 sequences of our isolates with sequences deposited in mentioned database.

## Phylogenetic analyses

Obtained sequence reads were trimmed and assembled in Geneious Prime® 2022.1.1. In order to resolve phylogenetic and systematic placement of obtained strains, a multilocus dataset, comprising rDNA ITS, gapdh, act, tub2, chs-1, his3 sequences, was assembled. Dataset comprised newly generated sequences, sequences from all sequenced representatives of the Colletotrichum agaves and Colletotrichum boninense species complexes and sequences of selected representatives of other related Colletotrichum species used by Talhinhas and Baroncelli [43] and Liu et al. [48] (with small modifications) (Table 2). Additionally, all rDNA ITS sequences present in GenBank [49, 50] that were identical to rDNA ITS sequences of our strains were included in phylogenetic reconstruction. Monilochaetes infuscans was used as an outgroup.

Table 2
List of species, with details of strain/voucher information, country of origin, host plant, and GenBank accession numbers (ITS, act, chs-1, gapdh, his3, tub2) used for phylogenetic analyses

| Species | Strain/ specimen voucher** | Country | Host plant | GenBank acc. no. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | ITS | act | chs-1 | gapdh | his3 | tub2 |
| Colletotrichum agaves | CBS 118190 | Mexico | Agave striata | DQ286221 | – | – | – | – | – |
| Colletotrichum acericola | CBS (= Col5B)* | Poland | Acer negundo | OP514720 | OP536817 | OP524187 | OP524190 | – | OP524184 |
| Colletotrichum acericola | CBS (= Col4B) | Poland | Acer negundo | OP514722 | OP536819 | OP524186 | OP524189 | – | OP524183 |
| Colletotrichum acericola | KRAM F-59696 (= KJN2) | Poland | Acer negundo | OP514721 | OP536818 | OP524188 | OP524191 | – | OP524185 |
| Colletotrichum agaves | LC0947 | Thailand | Agave sp. | MZ595831 | MZ664129 | MZ799266 | MZ664053 | MZ673842 | MZ673955 |
| Colletotrichum annellatum | CBS 129826* | Colombia | Hevea brasiliensis | JQ005222 | JQ005570 | JQ005396 | JQ005309 | JQ005483 | JQ005656 |
| Colletotrichum arecacearum | LC13850* | China | Arecaceae | MZ595867 | MZ664165 | MZ799262 | MZ664049 | MZ673887 | MZ673986 |
| Colletotrichum beeveri | CBS 128527* | New Zealand | Brachyglottis repanda | JQ005171 | JQ005519 | JQ005345 | JQ005258 | JQ005432 | JQ005605 |
| Colletotrichum boninense | CBS 123755* | Japan | Crinum asiaticum var. sinicum | JQ005153 | JQ005501 | JQ005327 | JQ005240 | JQ005414 | JQ005588 |
| Colletotrichum brasiliense | CBS 128501* | Brazil | Passiflora edulis | JQ005235 | JQ005583 | JQ005409 | JQ005322 | JQ005496 | JQ005669 |
| Colletotrichum brassicicola | CBS 101059* | New Zealand | Brassica oleracea var. gemmifera | JQ005172 | JQ005520 | JQ005346 | JQ005259 | JQ005433 | JQ005606 |
| Colletotrichum bromeliacearum | LC0951* | China | bromeliad | MZ595832 | MZ664130 | MZ799267 | MZ664077 | MZ673843 | MZ673956 |
| Colletotrichum camelliae | CGMCC 3.14925* | China | Camellia sinensis | KJ955081 | KJ954363 | MZ799255 | KJ954782 | MZ673847 | KJ955230 |
| Colletotrichum camelliae-japonicae | CGMCC 3.18118* | China | Camellia japonica | KX853165 | KX893576 | – | KX893584 | – | KX893580 |
| Colletotrichum catinaense | CBS 142417* | Italy | Citrus reticulata | KY856400 | KY855971 | KY856136 | KY856224 | KY856307 | KY856482 |
| Colletotrichum chamaedoreae | LC13868* | China | Chamaedorea erumpens | MZ595890 | MZ664188 | MZ799274 | MZ664084 | MZ673910 | MZ674008 |
| Colletotrichum citricola | CBS 134228* | China | Citrus unshiu | KC293576 | KC293616 | KC293792 | KC293736 | – | KC293656 |
| Colletotrichum citri-medicae | GUCC 1554* | China | Citrus medica | MN959910 | MT006325 | MT006328 | MT006331 | MT006334 | – |
| Colletotrichum colombiense | CBS 129818* | Colombia | Passiflora edulis | JQ005174 | JQ005522 | JQ005348 | JQ005261 | JQ005435 | JQ005608 |
| Colletotrichum condaoense | CBS 13429* | Vietnam | Ipomoea pes-caprae | MH229914 | – | MH229926 | MH229920 | MH229927 | MH229923 |
| Colletotrichum constrictum | CBS 128504* | New Zealand | Citrus limon | JQ005238 | JQ005586 | JQ005412 | JQ005325 | JQ005499 | JQ005672 |
| Colletotrichum cymbidiicola | IMI 347923* | Australia | Cymbidium sp. | JQ005166 | JQ005514 | JQ005340 | JQ005253 | JQ005427 | JQ005600 |
| Colletotrichum dacrycarpi | CBS 130241* | New Zealand | Dacrycarpus dacrydioides | JQ005236 | JQ005584 | JQ005410 | JQ005323 | JQ005497 | JQ005670 |

** Abbreviations: CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; KRAM F: Fungal collection of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland; LC: Working collection of Lei Cai, housed at Chinese Academy of Sciences, Beijing, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MBD: personal collection of Mara DeMers; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand.

* = ex-holotype or ex-epitype culture.

| Species | Strain/ specimen voucher** | Country | Host plant | GenBank acc. no. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | ITS | act | chs-1 | gapdh | his3 | tub2 |
| Colletotrichum diversum | LC11292 * | China | Thaumatophyllum bipinnatifidum | MZ595844 | MZ664142 | MZ799272 | MZ664081 | MZ673864 | MZ673965 |
| Colletotrichum doitungense | MFLUCC 14–0128* | Thailand | Dendrobium sp. | MF448524 | MH376385 | – | MH049480 | – | MH351277 |
| Colletotrichum euphorbiae | CBS 134725* | South Africa | Euphorbia sp. | KF777146 | KF777125 | KF777128 | KF777131 | KF777134 | KF777247 |
| Colletotrichum feijoicola | CBS 144633* | Portugal | Acca sellowiana | MK876413 | MK876466 | – | MK876475 | – | MK876507 |
| Colletotrichum gloeosporioides | IMI 356878* | Italy | Citrus sinensis | JX010152 | JX009531 | JX009818 | JX010056 | JQ005413 | JX010445 |
| Colletotrichum grevilleae | CBS 132879* | Italy | Grevillea sp. | KC297078 | KC296941 | KC296987 | KC297010 | KC297056 | KC297102 |
| Colletotrichum hippeastri | CBS 125376* | China | Hippeastrum vittatum | JQ005231 | JQ005579 | JQ005405 | JQ005318 | JQ005492 | JQ005665 |
| Colletotrichum karstii | CBS 132134* | China | Vanda sp. | HM585409 | HM581995 | HM582023 | HM585391 | – | HM585428 |
| Colletotrichum ledebouriae | CBS 141284* | South Africa | Ledebouria floridunda | KX228254 | KX228357 | – | – | KX228365 | – |
| Colletotrichum limonicola | CBS 142410* | Malta | Citrus limon | KY856472 | KY856045 | KY856213 | KY856296 | KY856388 | KY856554 |
| Colletotrichum nageiae | LC13866* | China | Nageia nagi | MZ595882 | MZ664180 | MZ799278 | MZ664087 | MZ673902 | MZ674000 |
| Colletotrichum neosansevieriae | CBS 139918* | South Africa | Sansevieria trifasciata | KR476747 | KR476790 | KF777128 | KR476791 | KR476792 | KR476797 |
| Colletotrichum novae-zelandiae | CBS 128505* | New Zealand | Capsicum annuum | JQ005228 | JQ005576 | JQ005402 | JQ005315 | JQ005489 | JQ005662 |
| Colletotrichum oncidii | CBS 129828* | Germany | Oncidium sp. | JQ005169 | JQ005517 | JQ005343 | JQ005256 | JQ005430 | JQ005603 |
| Colletotrichum parsonsiae | CBS 128525* | New Zealand | Parsonsia capsularis | JQ005233 | JQ005581 | JQ005407 | JQ005320 | JQ005494 | JQ005667 |
| Colletotrichum petchii | CBS 378.94* | Italy | Dracaena marginata | JQ005223 | JQ005571 | JQ005397 | JQ005310 | JQ005484 | JQ005657 |
| Colletotrichum phyllanthi | CBS 175.67* | India | Phyllanthus acidus | JQ005221 | JQ005569 | JQ005395 | JQ005308 | JQ005482 | JQ005655 |
| Colletotrichum pyrifoliae | CGMCC 3.18902* | China | Pyrus pyrifolia | MG748078 | MG747768 | MG747914 | MG747996 | – | MG748158 |
| Colletotrichum sansevieriae | MAFF 239721* | Japan | Sansevieria trifasciata | AB212991 | (Sequences available at http://www.gene.affrc.go.jp/databases-micro_search_en.php) | | | | |
| Colletotrichum “sansevieriae” | IUB: B.Whitaker: OTU58 | USA | Panicum virgatum | MH178727 | – | – | – | – | – |
| Colletotrichum sp. isolate MBD_190 | MBD_190 | USA | Dalea purpurea | MK595512 | – | – | – | – | – |
| Colletotrichum torulosum | CBS 128544* | New Zealand | Solanum melongena | JQ005164 | – | JQ005338 | JQ005251 | JQ005425 | JQ005598 |
| Colletotrichum watphraense | MFLUCC 14–0123* | Thailand | Dendrobium sp. | MF448523 | MH376384 | – | MH049479 | – | MH351276 |
| Monilochaetes infuscans | CBS 869.96* | South Africa | Ipomoea batatas | GU180626 | JQ005843 | JQ005801 | JX546612 | JQ005822 | JQ005864 |

** Abbreviations: CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; KRAM F: Fungal collection of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland; LC: Working collection of Lei Cai, housed at Chinese Academy of Sciences, Beijing, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MBD: personal collection of Mara DeMers; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand.

* = ex-holotype or ex-epitype culture.

Table 2
List of species, with details of strain information, country of origin, host plant, and GenBank accession numbers (ITS, *act*, *chs-1*, *gapdh*, *his3*, *tub2*) used for phylogenetic analyses

| Species | Strain** | Country | Host plant | GenBank acc. no. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | ITS | act | chs-1 | gapdh | his3 | tub2 |
| *Colletotrichum agaves* | CBS 118190 | Mexico | *Agave striata* | DQ286221 | – | – | – | – | – |
| *Colletotrichum acericola* | CBS XXX (= Col5B)* | Poland | *Acer negundo* | XXX | XXX | XXX | XXX | – | XXX |
| *Colletotrichum acericola* | CBS XXX (= Col4B) | Poland | *Acer negundo* | XXX | XXX | XXX | XXX | – | XXX |
| *Colletotrichum acericola* | KRAM F-59696 | Poland | *Acer negundo* | XXX | XXX | XXX | XXX | – | XXX |
| *Colletotrichum agaves* | LC0947 | Thailand | *Agave* sp. | MZ595831 | MZ664129 | MZ799266 | MZ664053 | MZ673842 | MZ673955 |
| *Colletotrichum annellatum* | CBS 129826* | Colombia | *Hevea brasiliensis* | JQ005222 | JQ005570 | JQ005396 | JQ005309 | JQ005483 | JQ005656 |
| *Colletotrichum arecacearum* | LC13850* | China | *Arecaceae* | MZ595867 | MZ664165 | MZ799262 | MZ664049 | MZ673887 | MZ673986 |
| *Colletotrichum beeveri* | CBS 128527* | New Zealand | *Brachyglottis repanda* | JQ005171 | JQ005519 | JQ005345 | JQ005258 | JQ005432 | JQ005605 |
| *Colletotrichum boninense* | CBS 123755* | Japan | *Crinum asiaticum* var. *sinicum* | JQ005153 | JQ005501 | JQ005327 | JQ005240 | JQ005414 | JQ005588 |
| *Colletotrichum brasiliense* | CBS 128501* | Brazil | *Passiflora edulis* | JQ005235 | JQ005583 | JQ005409 | JQ005322 | JQ005496 | JQ005669 |
| *Colletotrichum brassicicola* | CBS 101059* | New Zealand | *Brassica oleracea* var. *gemmifera* | JQ005172 | JQ005520 | JQ005346 | JQ005259 | JQ005433 | JQ005606 |
| *Colletotrichum bromeliacearum* | LC0951* | China | bromeliad | MZ595832 | MZ664130 | MZ799267 | MZ664077 | MZ673843 | MZ673956 |
| *Colletotrichum camelliae* | CGMCC 3.14925* | China | *Camellia sinensis* | KJ955081 | KJ954363 | MZ799255 | KJ954782 | MZ673847 | KJ955230 |
| *Colletotrichum camelliae-japonicae* | CGMCC 3.18118* | China | *Camellia japonica* | KX853165 | KX893576 | – | KX893584 | – | KX893580 |
| *Colletotrichum catinaense* | CBS 142417* | Italy | *Citrus reticulata* | KY856400 | KY855971 | KY856136 | KY856224 | KY856307 | KY856482 |
| *Colletotrichum chamaedoreae* | LC13868* | China | *Chamaedorea erumpens* | MZ595890 | MZ664188 | MZ799274 | MZ664084 | MZ673910 | MZ674008 |
| *Colletotrichum citricola* | CBS 134228* | China | *Citrus unshiu* | KC293576 | KC293616 | KC293792 | KC293736 | – | KC293656 |
| *Colletotrichum citri-medicae* | GUCC 1554* | China | *Citrus medica* | MN959910 | MT006325 | MT006328 | MT006331 | MT006334 | – |
| *Colletotrichum colombiense* | CBS 129818* | Colombia | *Passiflora edulis* | JQ005174 | JQ005522 | JQ005348 | JQ005261 | JQ005435 | JQ005608 |
| *Colletotrichum condaoense* | CBS 13429* | Vietnam | *Ipomoea pes-caprae* | MH229914 | – | MH229926 | MH229920 | MH229927 | MH229923 |
| *Colletotrichum constrictum* | CBS 128504* | New Zealand | *Citrus limon* | JQ005238 | JQ005586 | JQ005412 | JQ005325 | JQ005499 | JQ005672 |
| *Colletotrichum cymbidiicola* | IMI 347923* | Australia | *Cymbidium* sp. | JQ005166 | JQ005514 | JQ005340 | JQ005253 | JQ005427 | JQ005600 |
| *Colletotrichum dacrycarpi* | CBS 130241* | New Zealand | *Dacrycarpus dacrydioides* | JQ005236 | JQ005584 | JQ005410 | JQ005323 | JQ005497 | JQ005670 |
| *Colletotrichum diversum* | LC11292 * | China | *Thaumatophyllum bipinnatifidum* | MZ595844 | MZ664142 | MZ799272 | MZ664081 | MZ673864 | MZ673965 |

** Abbreviations: CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; KRAM F: Fungal collection of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland; LC: Working collection of Lei Cai, housed at Chinese Academy of Sciences, Beijing, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MBD: personal collection of Mara DeMers; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand.

* = ex-holotype or ex-epitype culture.

| Species | Strain** | Country | Host plant | GenBank acc. no. | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | ITS | act | chs-1 | gapdh | his3 | tub2 |
| *Colletotrichum doitungense* | MFLUCC 14–0128* | Thailand | *Dendrobium* sp. | MF448524 | MH376385 | – | MH049480 | – | MH351277 |
| *Colletotrichum euphorbiae* | CBS 134725* | South Africa | *Euphorbia* sp. | KF777146 | KF777125 | KF777128 | KF777131 | KF777134 | KF777247 |
| *Colletotrichum feijoicola* | CBS 144633* | Portugal | *Acca sellowiana* | MK876413 | MK876466 | – | MK876475 | – | MK876507 |
| *Colletotrichum gloeosporioides* | IMI 356878* | Italy | *Citrus sinensis* | JX010152 | JX009531 | JX009818 | JX010056 | JQ005413 | JX010445 |
| *Colletotrichum grevilleae* | CBS 132879* | Italy | *Grevillea* sp. | KC297078 | KC296941 | KC296987 | KC297010 | KC297056 | KC297102 |
| *Colletotrichum hippeastri* | CBS 125376* | China | *Hippeastrum vittatum* | JQ005231 | JQ005579 | JQ005405 | JQ005318 | JQ005492 | JQ005665 |
| *Colletotrichum karstii* | CBS 132134* | China | *Vanda* sp. | HM585409 | HM581995 | HM582023 | HM585391 | – | HM585428 |
| *Colletotrichum ledebouriae* | CBS 141284* | South Africa | *Ledebouria floridunda* | KX228254 | KX228357 | – | – | KX228365 | – |
| *Colletotrichum limonicola* | CBS 142410* | Malta | *Citrus limon* | KY856472 | KY856045 | KY856213 | KY856296 | KY856388 | KY856554 |
| *Colletotrichum nageiae* | LC13866* | China | *Nageia nagi* | MZ595882 | MZ664180 | MZ799278 | MZ664087 | MZ673902 | MZ674000 |
| *Colletotrichum neosansevieriae* | CBS 139918* | South Africa | *Sansevieria trifasciata* | KR476747 | KR476790 | KF777128 | KR476791 | KR476792 | KR476797 |
| *Colletotrichum novae-zelandiae* | CBS 128505* | New Zealand | *Capsicum annuum* | JQ005228 | JQ005576 | JQ005402 | JQ005315 | JQ005489 | JQ005662 |
| *Colletotrichum oncidii* | CBS 129828* | Germany | *Oncidium* sp. | JQ005169 | JQ005517 | JQ005343 | JQ005256 | JQ005430 | JQ005603 |
| *Colletotrichum parsonsiae* | CBS 128525* | New Zealand | *Parsonsia capsularis* | JQ005233 | JQ005581 | JQ005407 | JQ005320 | JQ005494 | JQ005667 |
| *Colletotrichum petchii* | CBS 378.94* | Italy | *Dracaena marginata* | JQ005223 | JQ005571 | JQ005397 | JQ005310 | JQ005484 | JQ005657 |
| *Colletotrichum phyllanthi* | CBS 175.67* | India | *Phyllanthus acidus* | JQ005221 | JQ005569 | JQ005395 | JQ005308 | JQ005482 | JQ005655 |
| *Colletotrichum pyrifoliae* | CGMCC 3.18902* | China | *Pyrus pyrifolia* | MG748078 | MG747768 | MG747914 | MG747996 | – | MG748158 |
| *Colletotrichum sansevieriae* | MAFF 239721* | Japan | *Sansevieria trifasciata* | AB212991 | (Sequences available at http://www.gene.affrc.go.jp/databases-micro_search_en.php) | | | | |
| *Colletotrichum* "*sansevieriae*" Whitaker OTU58 | IUB: B.Whitaker: OTU58 | USA | *Panicum virgatum* | MH178727 | – | – | – | – | – |
| *Colletotrichum* sp. isolate MBD_190 | MBD_190 | USA | *Dalea purpurea* | MK595512 | – | – | – | – | – |
| *Colletotrichum torulosum* | CBS 128544* | New Zealand | *Solanum melongena* | JQ005164 | JQ005598 | JQ005338 | JQ005251 | JQ005425 | JQ005598 |
| *Colletotrichum watphraense* | MFLUCC 14–0123* | Thailand | *Dendrobium* sp. | MF448523 | MH376384 | – | MH049479 | – | MH351276 |
| *Monilochaetes infuscans* | CBS 869.96* | South Africa | *Ipomoea batatas* | GU180626 | JQ005843 | JQ005801 | JX546612 | JQ005822 | JQ005864 |

** Abbreviations: CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; KRAM F: Fungal collection of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland; LC: Working collection of Lei Cai, housed at Chinese Academy of Sciences, Beijing, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MBD: personal collection of Mara DeMers; MFLUCC: Mae Fah Luang University Culture Collection, ChiangRai, Thailand.

* = ex-holotype or ex-epitype culture.

Multiple sequence alignments (MSA) were performed for each loci independently using the MAFFT algorithm [51] as implemented in Geneious Prime® 2022.1.1. and concatenated. The concatenated data block was used as an input file for the ModelTest-NG v. 0.2.0 in order to select the best-fit models of

nucleotide substitution for partitioned MSA. For maximum likelihood (ML) based phylogenetic inference we have used the RAxML-NG v. 1.0.3. and for the Bayesian inference (BI) - MrBayes 3.2.6 [52]. Final phylogenetic tree was prepared with FigTree 1.4.4.

## Results

# Phylogenetic analyses

For all sequenced samples high quality reads enabling assembly of reads were obtained. In case of gapdh sequences bidirectional reads have shared a limited number of nucleotides, but after comparison of sequences obtained in this study and in references from GenBank these were used with confidence.

For phylogenetic reconstruction a datablocks containing 47 ITS sequences (590 bp), 43 gapdh sequences (302), 43 act sequences (289 bp), 42 tub2 sequences (527 bp), 38 chs-1 sequences (280 bp) and 33 his3 sequences (396 bp) were used. The concatenated dataset consisted of 47 sequences of 2384 bp length. The best-fit substitution models were calculated separately for ML and BI templates and used in respective analyses based on Bayesian information criterion. ML analysis was performed with following models - TrN + I + G4 for ITS, TIM3ef + G4, K80 + G4 and HKY + G4 for gapdh codons, K80 + G4, K80 + G4 and TrNef + G4 for act codons, TPM2 + G4, TrNef + G4 and K80 + I + G4 for tub2 codons, TIM3ef + I + G4, HKY + G4 and JC for chs-1 codons, HKY + G4, TIM1 + I + G4 and TPM3 + I + G4 for his3 codons. The analysis was performed with 1000 bootstrap repetitions. The BI analysis used HKY + I + G4 substitution model for ITS, HKY + G4, K80 + G4 and HKY + G4 for gapdh codons, K80 + G4, K80 + G4 and HKY + G4 for act codons, K80 + G4, HKY + G4 and K80 + I + G4 for tub2 codons, JC + I + G4, HKY + G4 and JC for chs-1 codons, HKY + G4, GTR + I + G4 and K80 + G4 for his3 codons. The BI analyses were conducted using two concurrent analyses of four chains run for 165300 generations. First 25000 trees were eliminated as a burning.

Resulting trees from both analyses were congruent showing well-supported position of our strains within the Colletotrichum agaves species complex (Fig. 1). Sequences from studied strains, together with the two most similar rDNA ITS sequences recovered from GenBank [ex endophytes of Dalea purpurea (Fabaceae) and Panicum virgatum (Poaceae) in prairies of USA], are positioned in a sister position to sequences representing Colletotrichum agaves. The sequences of the studied Colletotrichum and C. agaves formed a sister group to subclade containing sequences of C. euphorbiae, C. ledebouriae, C. neosansevieriae and C. sansevieriae.

# Taxonomy

Colletotrichum acericola Patejuk, Piątek, Czachura, Baturo-Cieśniewska, sp. nov. (Figs. 2–4)

MycoBank no. MB …….

Etymology: Named after the host plant genus.

Description: Infection (in vivo) on seeds and seed petioles, at first forming orange patches, later encircled by darker borders and subsequently producing black rounded conidiomata that rupture and expose conidia. Mycelium (in vitro) consisting of hyaline or subhyaline, simple, septate, branched hyphae, sometimes constricted at septa, 1.5–6.0 µm. Conidiomata acervular, rounded, up to 500 µm in diam., black with setae and opening by rupture but sterile (on OA) or slimy orange consisting of conidial mass (on PDA and MEA), conidiophores formed on hyphae or on cushion-like, globose or subglobose cells, 4.0–13.5 × 4.0–6.5 µm. Setae numerous on OA but only one observed on PDA, brown, smooth, single or very rarely branched, 1–3-septate, 50–110 µm long, 3.0–5.5 µm wide at the base, tip acutely rounded. Conidiophores hyaline, smooth, straight, septate, single or rarely branched, 30.0–50.0 × 4.0–4.5 µm; conidiogenous cells hyaline, smooth, cylindrical, 18.0–31.0 × 3.5–6.0 µm. Conidia hyaline, smooth, with orange guttules, nonseptate, subcylindrical, straight or rarely slightly curved, apex obtuse, base sometimes indistinctly truncate, 17.5–22.5 × 5.0–7.5 µm. Appressoria brown, smooth, single, nonseptate, exceptionally 1-septate, rounded to clavate, margin sometimes undulate, 7.0–14.0 × 5.0– 9.5 µm.

Culture characteristics: Colony on MEA globose, flat, orange in the middle, covered with white aerial mycelium, which forms concentric zonation, and orange conidiomata, margin fimbriate, colony reaching 43 × 36 mm in diameter after 7 days and 68 × 47 mm after 14 days of growth at 25°C. Reverse orange with whitish margin. Colony on OA globose, flat, entirely orange, covered with white mycelium and grayish dots of emerging conidiomata (?) in the middle, with the entire edge, colony reaching 56 × 55 mm in diameter after 7 days and 85 × 85 mm after 14 days of growth at 25°C. Reverse orange. Colony on PDA undulate, flat, orange in the middle, partly covered with white aerial mycelium and orange conidiomata, margin white, colony reaching 31 × 24 mm in diameter after 7 days and 42 × 36 mm after 14 days of growth at 25°C. Reverse orange with whitish margin. Colony on SNA subglobose, flat, hyaline, with fimbriate margin, reaching 15 × 12 mm in diameter after 7 days and 39 × 29 mm after 14 days of growth at 25°C. Reverse white, margin fimbriate.

Type: Poland, Lower Silesia Province, Wrocław County: Wrocław city, Krakowska street, GPS: 51.08631, 17.06837, seeds and petioles of Acer negundo, 16 Oct. 2021, leg. K. Patejuk (KRAM F-59692 (dried culture) – holotype, KRAM F-59693 (material in planta) – isotype; culture ex type: col5b = CBS; GenBank sequences: ITS = OP514720, act = OP536817, chs-1 = OP524187, gapdh = OP524190, tub2 = OP524184).

Other specimens examined: Poland, Lower Silesia Province, Wrocław County: Wrocław city, Krakowska street, GPS: 51.08631, 17.06837, seeds of Acer negundo, 05 Dec. 2017, leg. K. Patejuk (KRAM F-59696 (dried culture); GenBank sequences: ITS = OP514721, act = OP536818, chs-1 = OP524188, gapdh = OP524191, tub2 = OP524185).

Poland, Lower Silesia Province, Wrocław County: Wrocław city, Bytomska street, GPS: 51.07698,17.08304, seeds and petioles of Acer negundo, 16 Oct. 2021, leg. K. Patejuk (KRAM F-59694 (dried culture), KRAM F-59695 (material in planta); culture ex type: col4b = CBS; GenBank sequences: ITS = OP514722, act = OP536819, chs-1 = OP524186, gapdh = OP524189, tub2 = OP524183).

## Discussion

In this study morphological and molecular phylogenetic analyses, based on sequences from multiple loci (rDNA ITS, gapdh, act, tub2, chs-1, his3), were used to resolve the identity of the Colletotrichum species inhabiting seeds and petioles of invasive alien Acer negundo surveyed in Poland. The analyses showed that causative agent represents a new species, which is described here as Colletotrichum acericola. Phylogenetically, this species is nested within the Colletotrichum agaves species complex.

The Colletotrichum agaves species complex is a relatively small complex established by Bhunjun et al. [53], comprising five species: C. agaves, C. euphorbiae, C. ledebouriae, C. neosansevieriae and C. sansevieriae [43, 48, 53, 54]. Colletotrichium acericola differs from C. agaves, which is the most closely related phylogenetic relative, by producing infection symptoms on seeds and petioles and by having shorter setae and smaller conidia. Colletotrichum agaves causes circular leaf spots with acervuli in concentric rings, its setae are 60–211 µm long and conidia are 17.5–33.0 µm long; additionally appressoria were not observed in cultures of this species [55]. The four remaining species of the C. agaves species complex are also morphologically different from C. acericola. First of all, they cause infections on leaves, either as leaf spots (C. ledebouriae, C. neosansevieriae) or water-soaked lesions and blight of the leaves (C. sansevieriae). In C. euphorbiae, the etiological symptoms on plants were not reported [56–59]. Morphologically, C. euphorbiae and C. sansevieriae differ in having longer conidia (17.0–28.5 µm long and 12.5–32.5 µm long, respectively) while C. ledebouriae and C. neosansevieriae differ in having more septa on setae that are also longer (setae 2–4-septate, 80–120 µm long and 3–7-septate, 120–200 µm long, respectively) [56–59].

In the genus Colletotrichum, there are different levels of host specificity. Generally, many species are generalists and could infect a wide range of host plants [41, 43, 48, 60, 61]. However, specialist species are also common in this genus - Jayawardena et al. [54] listed 153 host-specific species of Colletotrichum. Members of the Colletotrichum agaves species complex are considered as highly host specific and most of their host plants belong to monocot family Asparagaceae (C. agaves on Agave spp., C. ledebouriae on Ledebouria floridunda, C. sansevieriae on Sansevieria trifasciata, C. neosansevieriae on Sansevieria trifasciata) [43, 54, 62]. The only species that was previously described on plants from other host family is C. euphorbiae, found on Euphorbia sp. from the eudicot family Euphorbiaceae [57]. Colletotrichum agaves, that is genetically closely related to C. acericola, is reported from the biggest number of hosts as it was observed on numerous species of Agave and on Furcraea [55]; the reports on Furcraea are however not confirmed by molecular analyses. Based on our current knowledge, C. acericola is the second member of the Colletotrichum agaves species complex described from a dicotyledonous host (in the family Sapindaceae), outside of the family Asparagaceae. However, whether C. acericola is host-specific as the other members of its group is currently unclear and needs further studies. Two environmental sequences [ex endophytes of Dalea purpurea (Fabaceae) and Panicum virgatum (Poaceae) in prairies of USA] [49, 50] that were grouped with sequences of C. acericola suggest that this species may inhabit wider number of hosts, at least endophytically.

It is likely that the native occurrence of C. acericola might be in North America, possibly prairie in North-East USA. Sampling localisation of two identical to C. acericola rDNA ITS sequences (ex endophytes of Dalea purpurea and Panicum virgatum in prairies of Minnesota and Indiana/Illinois in the USA [49, 50]) provides us traces to support this hypothesis. The native occurrence of host plants of members of the Colletotrichum agaves complex species, which mainly contains warm, subtropical localisations in Southern Africa, Northern and Middle America, and the native range of host plants of C. acericola (Acer negundo, Dalea purpurea, Panicum virgatum) indirectly also spot American continent as a potential geographical origin of this species. Until now, besides C. agaves on introduced Agave species [55], none of the representatives of Colletotrichum agaves complex species was found in Europe. It is worth to mention that also other species in the Colletotrichum agaves species complex were introduced outside their possible native ranges, e.g. C. agaves or C. sansevieriae, which are currently known on alien ranges of respective host plants [55, 62–66].

The phenomenon of pathogens carried by IAS to the new areas is well known and documented [16]. It is likely that Colletotrichum acericola broke the geographic barrier, and was transferred to Europe, due to the transport of nursery material and seeds of A. negundo from the USA, to fulfill the need on the garden's market. The question remains whether Colletotrichum acericola was co-introduced to Europe together with its host plant Acer negundo already in the 17th or 19th century but remained unnoticed until now or whether it is a relatively recent introduction, long after host plant appearance. Still rare occurrence of C. acericola may suggest recent introduction. However, detailed searching for pathogen distribution is to be done. It is not excluded that if environmental conditions will be favorable, the abundance growth of C. acericola on the new localisations, leading to uncontrolled spillover of C. acericola to other (native) representatives of Acer spp. in Europe may be observed in the future. As many examples have shown, the consequences of alien pathogen adaptation to the new range of hosts in new areas might be disastrous for local environments [16]. The future development of invasion made by Colletotrichum acericola in Europe and its possible jump/spillover to native hosts should be monitored. However, because of the current cryptic life-style of the C. acericola it is hard to assess potential spread of this species in Europe and type of its interaction with european representatives of Acer spp. Nevertheless, as Blackburn and Even [15] point out, newly arrived pathogens are less harmful to invasive alien plants with which they have shared years of co-evolution. In turn, local taxa do not have any evolutionary adaptations against this pathogen. This approach is indicated by the Novel Weapons Hypothesis, proposed by Schmid-Hempel [67]. The potential threat that C. acericola might pose for a native european Acer species needs more studies, starting with pathogenicity tests in controlled conditions. This type of research will give us the opportunity for a rapid response, which may lead to reinforce one of the natural or anthropogenic barriers, which will cut-off or slow down the spillover mechanism [21]. From the practical point of view, information about new pathogens should be implemented during the planning of long-term forestry and municipal greenery projects. Monitoring of IAS's sanitary conditions seems like an indispensable action to avoid uncontrolled and rapid spread of new pathogens in a human-changed environment.

## Declarations


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### Data availability

Dried specimens (holotype, isotype and other specimens) are have been stored in the publicly available fungal collection of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (cal5b = KRAM F-59692 (dried culture), KRAM F-59693 (material in planta); cal4b = KRAM F-59694 (dried culture), KRAM F-59695 (material in planta); KJN2 = KRAM F-59696 (dried culture)). Sequences obtained in the present study have been deposited in the GenBank NCBI nucleotide sequence database (www.ncbi.nlm.nih.gov/genbank) in following accession numbers: col5b = CBS; GenBank sequences: ITS = OP514720, act = OP536817, chs-1 = OP524187, gapdh = OP524190, tub2 = OP524184; KJN2 = ITS = OP514721, act = OP536818, chs-1 = OP524188 , gapdh = OP524191, tub2 = OP524185; col4b = CBS; GenBank sequences: ITS = OP514722, act = OP536819, chs-1 = OP524186, gapdh = OP524189, tub2 = OP524183.

### Author contributions

KP: conceptualization, project administration, investigation - field work, fungal analysis, morphological analyses, writing; PC: investigation - morphological analyses, phylogenetic analyses, visualization, writing - review & editing; ABC: investigation - genetical analysis, writing; MOK: phylogenetic analyses, writing; WP: conceptualization, investigation - field work; KN: writing - review & editing; MP: conceptualization, investigation - morphological analyses, phylogenetic analyses, visualization, writing.

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## Figures

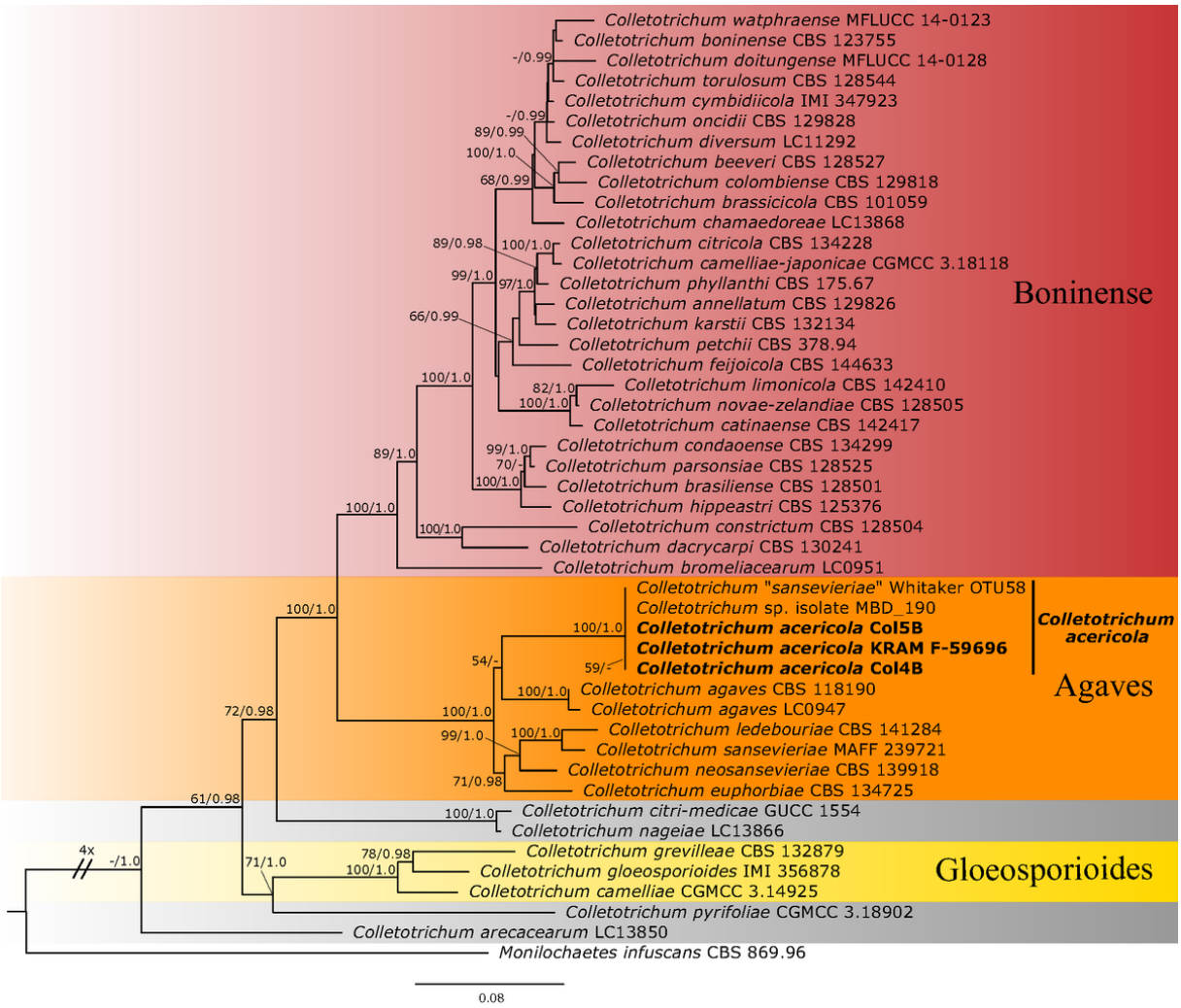


**Figure 1**

Maximum likelihood consensus tree inferred from a multilocus dataset (rDNA ITS, gapdh, act, tub2, chs-1, his3) of the sampled Colletotrichum species (species complexes are indicated with coloured boxes, with their names included at the right side). Newly sequenced strains of Colletotrichum acericola are indicated in bold. Numbers above branches indicate bootstrap support values (> 50 %) and posterior probabilities (PP > 0.90), respectively. The scale bar represents the expected changes per site. Monilochaetes infuscans (Chaetosphaeriales) is used as an outgroup

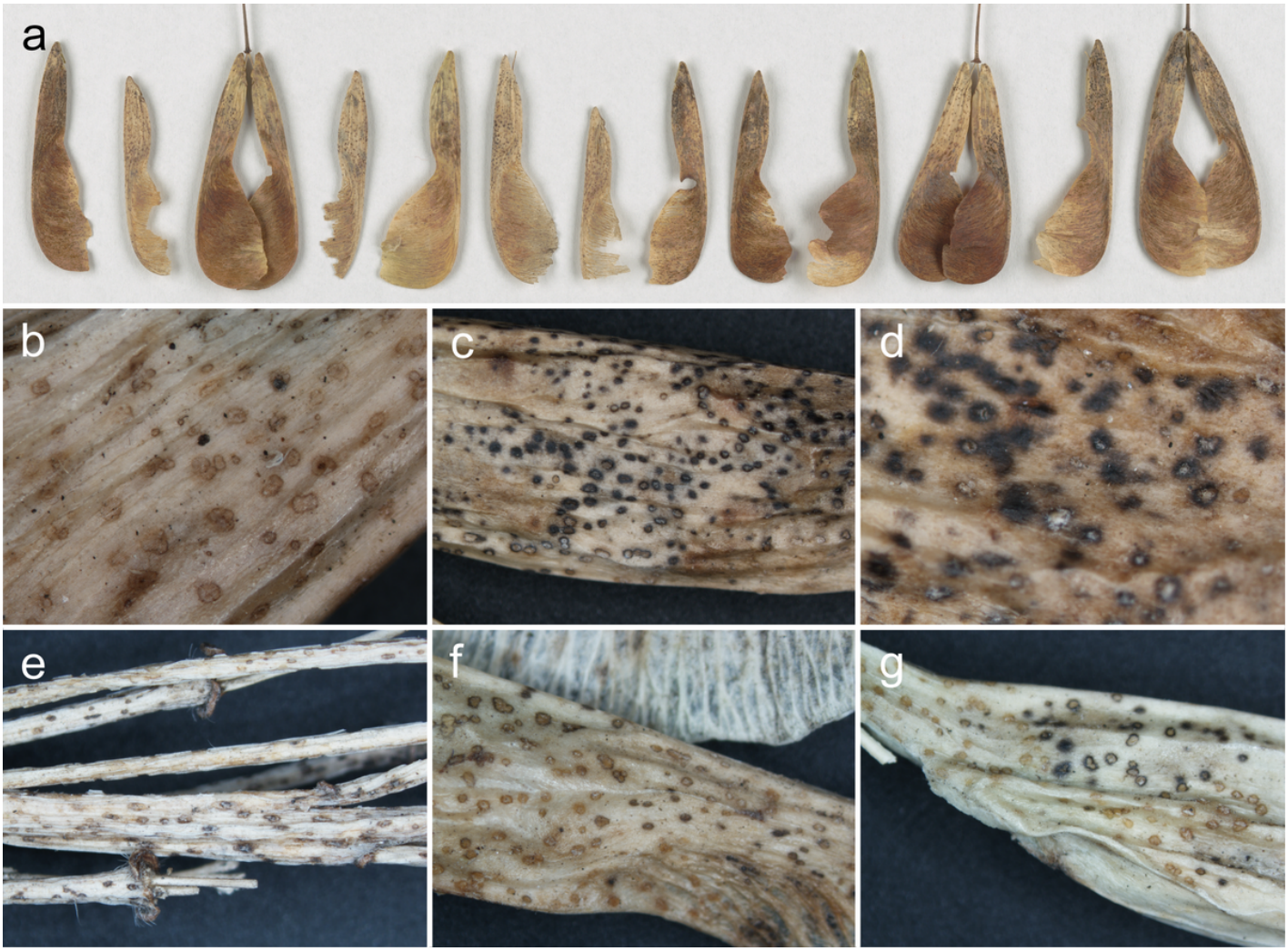


**Figure 2**

Symptoms of infection on seeds and petioles of Acer negundo caused by Colletotrichum acericola: (a) general view of infected seeds (KRAM F-59693); (b–d) details of infection on seeds, early to later stages of infection symptoms, respectively (KRAM F-59693); (e–g) details of infection symptoms on petioles and seeds (KRAM F-59695).

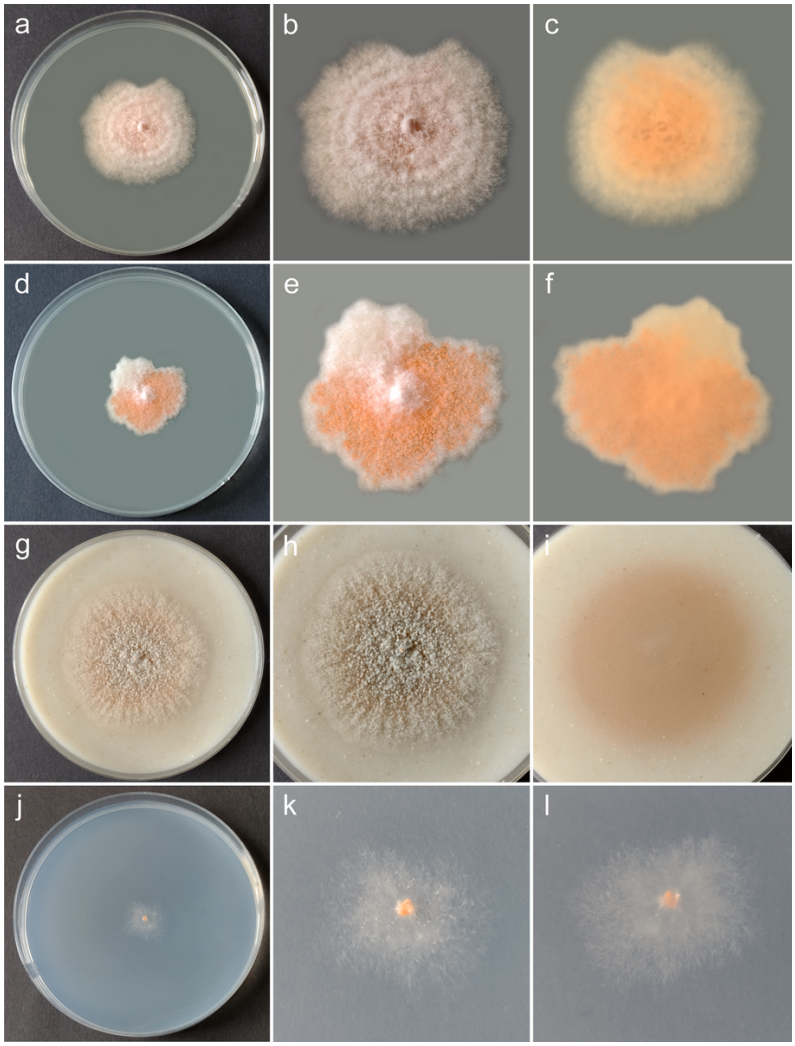


**Figure 3**

Morphology of cultures of Colletotrichum acericola (col5b): (a,d,g,j) general view on MEA, PDA, OA and SNA respectively after 7 days of growth at 25°C; (b,c) detailed view, upper side and reverse side of colony on MEA; (e,f) detailed view, upper side and reverse side of colony on PDA; (h,i) detailed view, upper side and reverse side of colony on OA; (k,l) detailed view, upper side and reverse side of colony on SNA.

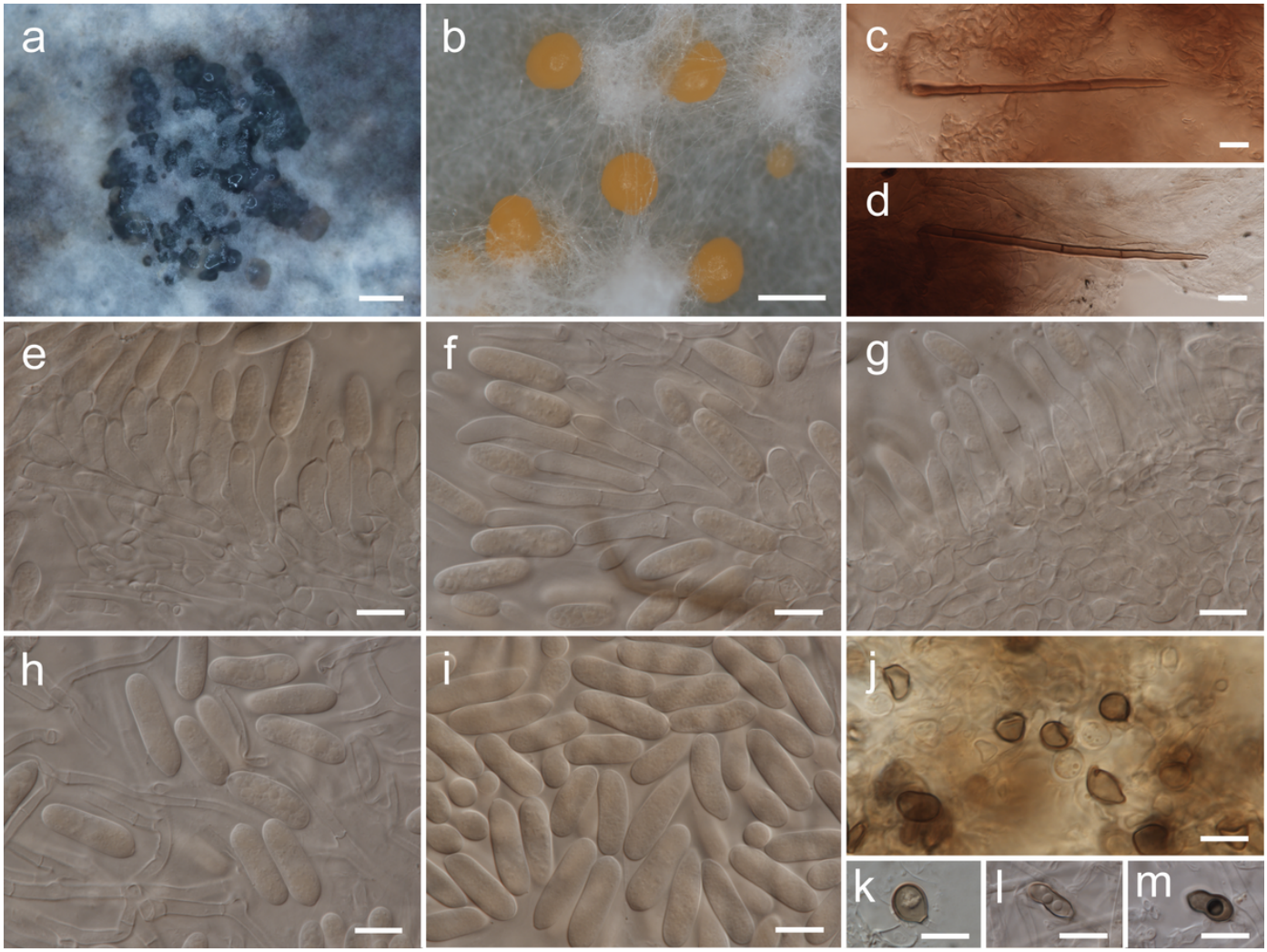


**Figure 4**

Morphology of Colletotrichum acericola (a,c,d from KRAM F-59694 on OA, j,k from KRAM F-59694 on PDA, b,e–i,l,m from KRAM F-59692 on PDA): (a) sterile conidiomata; (b) slimy orange conidiomata; (c,d) setae; (e–g) conidiophores with conidia, formed on hyphae and on cushion-like cells, respectively; (h,i) hyphae and conidia; (j-m) appressoria. Scale bars: a,b = 500 µm, d–m = 10 µm.