Three new Curvularia species from clinical and environmental sources

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Abstract

Curvularia is a Pleosporalean monophyletic genus with a great diversity of species, including relevant phytopathogenic, animal and human pathogenic fungi. However, their microscopic identification is difficult due to overlapping morphological features amongst species. In recent years, multi-locus sequence analysis using the ITS region of the rDNA and fragments of the genes gapdh and tef1 revealed numerous cryptic species, especially in isolates that commonly produced 3-septate conidia. Therefore, based on sequence analysis of the above-mentioned DNA barcodes recommended for species delineation in Curvularia, we propose three novel species, C. paraverruculosa, C. suttoniae and C. vietnamensis, isolated from soil, human clinical specimens and plant material, respectively, collected in different countries. These new species are morphologically characterised and illustrated in the present study. Curvularia paraverruculosa differs from its counterparts, C. americana and C. verruculosa, mainly by its narrower conidia. Curvularia suttoniae and C. vietnamensis are closely related to C. petersonii, but the former two have larger conidia.

Keywords
Ascomycetes, Dematiaceous hyphomycetes, phylogeny, Pleosporaceae, taxonomy

Introduction

The genus Curvularia Boedijn (1933), typified by C. lunata (Wakker) Boedijn, belongs in Pleosporaceae, Pleosporales (Wijayawardene et al. 2018). Members of Curvularia show different life modes, i.e. saprophytic, endophytic and also pathogenic on plants and animals (Marin-Felix et al. 2017a). Phytopathogenic species can affect wild grasses...
and staple crops, such as rice, maize, wheat or sorghum and give rise to serious losses in agricultural production (Gautam et al. 2013, Manamgoda et al. 2015, Marin-Felix et al. 2017a, Tan et al. 2018). The endophytic species have garnered interest in recent years for their use in the production of bio-based products that are beneficial to living organisms and the environment (Bengyella et al. 2019). Since the first report of *Curvularia* as a human pathogen in a patient with mycetoma (Baylet et al. 1959), other clinical presentations have been reported, such as superficial and deep infections that mainly affect the respiratory tract but can even cause cerebral phaeohyphomycosis with an extremely poor prognosis (de Hoog et al. 2000).

The genus is morphologically distinguished mainly by its asexual morph, which shows sympodial conidiophores with mono- to polytretic conidiogenous cells and transversally septe conidia. Typically, the conidia in *Curvularia* are curved due to the hypertrophy of one of the intermediate cells and they are euseptate (Ellis 1971), although other authors opine that the conidia in *Curvularia* are distoseptate (Sivanesan 1987, Seifert et al. 2011, Madrid et al. 2014). The species of *Bipolaris* and *Exserohilum* have typically straight and distoseptate conidia; however, some of them have been transferred to *Curvularia*, based on their DNA sequence analyses (Manamgoda et al. 2012, Hernández-Restrepo et al. 2018, Tan et al. 2018). Furthermore, due to the overlapping of morphological characters amongst certain species of *Curvularia*, such as conidial size, shape and septation, an accurate identification at the species level is difficult without a DNA sequence analysis (da Cunha et al. 2013, Madrid et al. 2014, Manamgoda et al. 2015). Several cryptic species have been described recently using only multi-locus sequence analyses of the recommended DNA barcodes for species delimitation, i.e. the internal transcribed spacer (ITS) region of the rDNA and the protein-coding loci glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and translation elongation factor 1-a (*tef1*) (Marin-Felix et al. 2017a, Tan et al. 2018). Nearly 130 species have so far been accepted in *Curvularia*, including the species classified previously in the teleomorphic genera *Cochliobolus* and *Pseudocochliobolus* after applying the current criteria for fungal nomenclature (Manamgoda et al. 2012, 2015, Madrid et al. 2014, Hyde et al. 2017, Marin-Felix et al. 2017a, 2017b, Dehdari et al. 2018, Heidari et al. 2018, Hernández-Restrepo et al. 2018, Liang et al. 2018, Mehrabi-Koushki et al. 2018, Tan et al. 2018, Tibpromma et al. 2018, Kiss et al. 2019, Raza et al. 2019, Zhang et al. 2020).

Based on a polyphasic approach, combining morphological and phylogenetic analyses, three novel *Curvularia* species are proposed here, isolated from human clinical specimens in the USA, soil in Mexico and seed and plant debris in Vietnam and Indonesia, respectively.

**Material and methods**

**Origin of isolates**

Five unidentified *Curvularia* isolates, maintained in the fungal collection of the Medical School of the Rovira i Virgili University (FMR; Reus, Spain), were included in the
Novel species of *Curvularia*

Two of these (FMR 10992, FMR 11690) were isolated from human specimens in the USA by Deana A. Sutton of the Fungus Testing Laboratory at the University of Texas Health Sciences Center (UTHSC; San Antonio, USA) and the other three (FMR 11956, FMR 17656, FMR 17659) were isolated from environmental samples; the first from sorghum seeds collected in Indonesia, the second from soil collected in the Mexican region of Michoacán and the third from unidentified plant material collected in the north-east of Vietnam.

DNA extraction, PCR, sequencing and phylogenetic analysis

The fungal DNA was extracted from colonies growing on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) for 7 to 10 days at 25 °C in darkness and following the protocol of Müller et al. (1998). The ITS barcode, including the 5.8S gene and the genes *gapdh* and *tef1* were analysed following Marin-Felix et al. (2017a). Amplification was carried out using the primer pairs ITS5/ITS4 for the ITS region (White et al. 1990), gpd1/gpd2 for *gapdh* (Berbee et al. 1999) and EF983/2218R for *tef1* (Schoch et al. 2009). The PCR products were purified and stored at -20 °C until sequencing. The same pairs of primers used for the amplification were also used to obtain the DNA sequences, which were processed at Macrogen Europe (Macrogen Inc., Madrid, Spain). The sequences of each isolate were edited using SeqMan v. 7.0.0 (DNAStar Lasergene, Madison, WI, USA) to obtain the consensus sequences.

We made a preliminary comparison of *gapdh* sequences generated from our isolates with those of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLASTn) for their molecular identification. To establish the phylogenetic position of unidentified isolates with respect to the most accepted species in *Curvularia*, we carried out individual (data not shown) and combined alignments of the three loci complemented by all available sequences of the ex-type and reference strains of *Curvularia* species retrieved from NCBI (Table 1). Based on this first phylogeny of the genus, a more restricted multi-locus analysis was carried out, including only those *Curvularia* species most related to the isolates under study. The alignments were made in the MEGA (Molecular Evolutionary Genetics Analysis) software v.6.0. (Tamura et al. 2013), using ClustalW algorithm (Thompson et al. 1994), refined with MUSCLE (Edgar 2004) in the same platform and manually adjusted as necessary. Phylogenetic reconstructions were made using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches under RAxML-HPC2 on XSEDE v.8.2.12 (Stamatakis et al. 2014) in CIPRES Science gateway portal (Miller et al. 2010) and MrBayes v. 3.2.6 (Ronquist et al. 2012), respectively.

For the ML analysis, the best nucleotide substitution model for the combined analysis of ITS, *gapdh* and *tef1*, determined using the MEGA programme, was Kimura 2-parameters with Gamma distribution (K2+G); the combined analysis of these three phylogenetic markers was tested through Incongruence Length Difference (ILD) implemented in the Winclada programme (Farris et al. 1994). ML bootstrap values (bs) ≥ 70% were considered significant.
Table 1. Species included in this study, their substrate, origin and GenBank accession numbers.

| Species                  | Strain no | Substrate             | Country     | Genbank accession no. | ITS       | gapdh    | tefA    |
|--------------------------|-----------|-----------------------|-------------|-----------------------|-----------|----------|---------|
| *Bipolaris maydis*       | CBS 136.29 T | Zea mays               | USA         | AF071325              | KM034846  | KM093794 |
| *B. saccharicola*        | CBS 155.26 T | Unknown                | Unknown     | KY905674              | KY905686  | KY905694 |
| *Curvularia aeria*       | CBS 294.61 T | Air                    | Brazil      | HP934910              | HG779148  | –        |
| *C. affinis*             | CBS 154.34 T | Unknown                | Indonesia   | KJ909780              | KM230401  | KM196566 |
| *C. abvazensis*          | CBS 144673 T | Zinnia elegans         | Iran        | KX139029              | MG428693  | MG428686 |
| *C. akatii*              | CBS 317.86  | Themeda triandra        | Japan       | KJ909782              | KM230402  | KM196569 |
| *C. akatiensis*          | BRIP 16080 T | Unknown                | India       | KJ415539              | KJ415407  | KJ415453 |
| *C. alcornii*            | MFLUCC 10-0703 T | Z. mays               | Thailand    | JX256420              | JX276433  | JX266589 |
| *C. americana*           | UTHSC 08-3414 T | Human ankle            | USA         | HE861833              | HF565488  | –        |
|                         | UTHSC 08-278 | Human nasal sinus      | USA         | HE861834              | HF565486  | –        |
|                         | UTHSC 08-278 | Human peritoneal dialysis fluid | USA       | HE861832              | HF565487  | –        |
|                         | UTHSC 08-2697 | Human leg              | USA         | HG779016              | HG779117  | –        |
| *C. annelliconidiophori* | CGMCC3.19352 T | Roots of Saccharum officinarum | China       | MN215641              | MN264077  | MN263935 |
| *C. asiatica*            | MFLUCC 10-0711 T | Panicum sp.           | Thailand    | JX256424              | JX276436  | JX266593 |
| *C. australiensis*       | BRIP 12044 T | O. sativa seeds        | Australia   | KJ415540              | KJ415406  | KJ415452 |
|                         | CBS 172.57 | O. sativa seeds        | Vietnam     | JN601026              | JN601036  | JN601003 |
| *C. australis*           | BRIP 12521 T | Sporobolus caroli      | Australia   | KJ415554              | KJ415405  | KJ415451 |
| *C. bannonii*            | BRIP 16732 T | Jacobemontia tanenii    | USA         | KJ415542              | KJ415404  | KJ415450 |
| *C. beadleyi*            | BRIP 10972 T | Chloris geyana         | Australia   | MH414892              | MH433638  | MH433654 |
|                         | BRIP 15854 | Leersia hexandra       | Australia   | MH414893              | MH433639  | MH433655 |
| *C. beerburrumensis*     | BRIP 12942 T | Engronitis bahiensis    | Australia   | MH414895              | MH433634  | MH433657 |
| *C. boeremae*            | IMI 164633 T | Portulaca oleracea     | India       | MH414911              | MH433641  | –        |
| *C. borreri*             | CBS 859.73 | Volcanic ash soil      | Chile       | HE861848              | HF565453  | –        |
| *C. bothriochloae*       | BRIP 12522 T | Bothriochloa bladini    | Australia   | KJ415543              | KJ415403  | KJ415449 |
| *C. brachypora*          | CBS 186.50 | Soil                   | Indonesia   | KJ922372              | KM061784  | KM230405 |
| *C. buchloe*             | CBS 246.49 T | Buchloë dactyloides    | USA         | KJ909765              | KM061789  | KM196588 |
| *C. carica-papayae*      | CBS 135941 T | Carica papaya          | India       | HG778984              | HG779146  | –        |
| *C. chiangmatensis*      | CPC 28829 T | Z. mays                | Thailand    | MF490814              | MF490836  | MF490857 |
| *C. chlamydospora*       | UTHSC 07-2764 T | Human toe nail         | USA         | HG779021              | HG779151  | –        |
| *C. chonburiensis*       | MFLUCC 16-0375 T | Dead leaf of Pandanus sp. | Thailand    | MH275055              | MH412747  | –        |
| *C. clavata*             | BRIP 61680 | Oryza sp.              | Australia   | KU552205              | KU552167  | KU552159 |
| *C. cymbopogonis*        | CBS 419.78 | Yuca leaf spot         | Netherlands | HG778985              | HG779129  | –        |
| *C. coriacea*            | BRIP 24261 T | Litchi chinensis       | Australia   | MH414897              | MH433636  | MH433659 |
| *C. coicis*              | CBS 192.29 T | Coix lacryma-jobs      | Japan       | AF081447              | AF081410  | JN601006 |
| *C. coimbatorensis*      | SZMC 22225 T | Human corneal scraping | India       | MN628310              | MN628306  | MN628302 |
| *C. calibrani*           | BRIP 13066 T | Crinum zeylanicum      | Australia   | MH414898              | MH433642  | MH433660 |
| *C. comoriensis*         | CBS 110673 | Unknown                | Unknown     | LT631357              | LT715841  | –        |
| Species          | Strain no¹ | Substrate                  | Country     | Genbank accession no.² |
|------------------|------------|----------------------------|-------------|------------------------|
| *C. crassiseptum*| CBS 503.90T | Plant material             | Nigeria     | LT631310 LT715882 –   |
| *C. crustacea*   | BRIP 13524T | *Sporobolus* sp.          | Indonesia   | KJ415544 KJ415402 KJ415448 |
| *C. dactyloctenica* | CPC 28810T | *Dactyloctenium* *aegyptium* | Thailand    | MF490815 MF490837 MF490858 |
| *C. dactyloctenii* | BRIP 12846T | *Dactyloctenium* *radulans* | Australia   | KJ415545 KJ415401 KJ415447 |
| *C. deightonii*  | CBS 537.70T | Sorghum *vulgare*        | Denmark     | LT631356 LT715839 –   |
| *C. determinata* | CMGCC3.19340T | Leaves of *S.* *officinarum* | China     | MN215653 MN264088 MN263947 |
| *C. elliptiformis* | CMGCC3.19351T | Roots of *S.* *officinarum* | China     | MN215656 MN264091 MN263950 |
| *C. ellisii*     | CBS 193.62T | Air                        | Pakistan   | JN192375 JN600963 JN601007 |
| *C. engrosticola* | BRIP 12538T | *Eragrostis pilosa*       | Australia   | MH414899 MH433643 MH433661 |
| *C. engrostidis* | CBS 189.48 | Sorghum seed               | Indonesia   | HG778986 HG779154 –   |
| *C. falkumata*   | CMGCC3.19329T | Roots of *S.* *officinarum* | China     | MN215660 MN264093 MN263954 |
| *C. flexuosa*    | CMGCC3.19447T | Roots of *S.* *officinarum* | China     | MN215663 MN264096 MN263961 |
| *C. gladlioli*   | CBS 210.79T | *Gladiolus* leaf           | Romania     | HG778987 HG779123 –   |
| *C. geniculata*  | CBS 187.50 | *Andropogon* *sorghum* seed | Indonesia   | KJ909781 KM083609 KM230410 |
| *C. graminitola* | BRIP 23186T | *Arisitida* *ingusta*      | Australia   | JN192376 JN600964 JN601008 |
| *C. guangchiensi*| CMGCC3.19330T | Roots of *S.* *officinarum* | China     | MN215667 MN264100 MN263967 |
| *C. gulgusikasi* | DAOM 165085 | Unknown                    | Unknown     | AF071338 AF081393 –   |
| *C. harveyi*     | BRIP 57412T | *Triticum* *aestivum*     | Australia   | KJ415546 KJ415400 KJ415446 |
| *C. hauastenensis* | BRIP 11987T | *O. tata*                  | USA         | KJ415547 KJ415399 KJ415445 |
| *C. heteropogoncola* | BRIP 14579T | *Heteropogon* *contortus*  | India       | KJ415548 KJ415398 KJ415444 |
| *C. heteropogonis* | CBS 284.91T | *H. contortus*             | Australia   | KJ415549 JN600969 JN601013 |
| *C. hominis*     | CBS 136985T | Human cornea               | USA         | HG779011 HG779106 –   |
| *C. homomorpha*  | CBS 156.60T | Air                        | USA         | JN192380 JN600970 JN601014 |
| *C. inaequalis*  | CBS 102.42T | Soil                       | France      | KJ922375 KM061787 KM196574 |
| *C. intermedia*  | CBS 334.64 | *Avena versicolor*         | USA         | HG778991 HG779155 –   |
| *C. ishaeni*     | CBS 630.82T | *Ishaemum* *indicum*       | Solomon Islands | MH861533 JX276440 –   |
| *C. kenpeggi*    | BRIP 14530T | *Triticum* *aestivum*     | Australia   | MH414900 MH433644 MH433662 |
| *C. kusano*      | CBS 137.29 | *Eragrostis* *major*       | Japan       | JN192381 LT715862 JN601016 |
| *C. lamingtonensis* | BRIP 12259T | *Microtela* *stipoides*    | Australia   | MH414901 MH433645 MH433663 |
| *C. lunata*      | CBS 730.96T | Human lung biopsy         | USA         | JX256429 JX276441 JX266596 |
| *C. malina*      | CBS 131274T | *Zoysia* *matrellia*      | USA         | JF812154 KP153179 KR493095 |
| *C. manamgadai*  | CMGCC3.19446T | Roots of *S.* *officinarum* | China     | MN215677 MN264110 MN263971 |
| *C. mebaldii*    | BRIP 12900T | *Cynodon dactylon*         | Australia   | MH414902 MH433646 MH433664 |
| *C. micropus*    | BRIP 13983 | *Cynodon dactylon*         | Australia   | MH414903 MH433647 MH433665 |
| *C. micropus*    | CBS 127235ET | *Paspalum* *notatum*       | Georgia     | HE792934 LT715859 –   |
| Species          | Strain no\(^1\) | Substrate                     | Country       | Genbank accession no.\(^2\) |
|------------------|------------------|-------------------------------|---------------|------------------------------|
| C. microspora    | GUCC 6272 T      | Hippeastrum striatum          | China         | MFL19088                     |
|                  |                  |                               |               | MF139016                     |
|                  |                  |                               |               | MF139115                     |
| C. miyakei       | CBS 197.29 T     | Engrostis pilosa              | Japan         | KJ909770                     |
|                  |                  |                               |               | KM083611                     |
|                  |                  |                               |               | KM196568                     |
| C. mosaddoghi    | IRAN 3131 C.T    | Sparganium cunani            | Iran          | MG846737                     |
|                  |                  |                               |               | MH392155                     |
|                  |                  |                               |               | MH392152                     |
| C. muelheinekeci | CBS 144.63 T     | Sorgnum sp.                   | USA           | MH858242                     |
|                  |                  |                               |               | HG779108                     |
|                  |                  |                               |               | KM196578                     |
| C. neergaardii   | BRIP 1291 T      | O. sativa                     | Ghana         | KJ415550                     |
|                  |                  |                               |               | KJ415397                     |
|                  |                  |                               |               | KJ415443                     |
|                  | CBS 276.91       | Unknown                       | Australia     | LT631362                     |
|                  |                  |                               |               | LT715848                     |
| C. neopontica    | IMI 129790 T     | Brassica nigra                | India         | MH414910                     |
|                  |                  |                               |               | MH433649                     |
|                  |                  |                               |               | MH433667                     |
| C. neoroseae     | CBS 11983 T      | Soil                          | Algeria        | KJ415551                     |
|                  |                  |                               |               | KJ415396                     |
|                  |                  |                               |               | KJ415442                     |
| C. nodosa        | CPC 28800 T      | Digitaria ciliaris            | Thailand      | MF490816                     |
|                  |                  | Brachiaria repanda            | Thailand      | MF490817                     |
|                  |                  |                               |               | MF490839                     |
|                  |                  |                               |               | MF490860                     |
| C. nodosula      | CBS 160.58       | Elesine indica                | Unknown       | JN601033                     |
|                  |                  |                               |               | JN600975                     |
|                  |                  |                               |               | JN601019                     |
| C. noriizae      | CBS 169.53 T     | O. sativa                     | Vietnam       | KP400650                     |
|                  |                  |                               |               | KP645344                     |
|                  |                  |                               |               | KM196590                     |
| C. ovaricola     | CBS 470.90 T     | Engrostis interrupta          | Australia     | JN192384                     |
|                  |                  |                               |               | JN600976                     |
|                  |                  |                               |               | JN601020                     |
| C. pallescens    | CBS 156.35 T     | Air                           | Indonesia     | KJ922380                     |
|                  |                  |                               |               | KM083606                     |
|                  |                  |                               |               | KM196570                     |
| C. palmicola     | MFLUCC 14-0404 T | Dead branches of Acelorobaphe | Thailand      | MF621582                     |
|                  |                  | wrightii                      |               | –                            |
|                  |                  |                               |               | –                            |
| C. pandanicola   | MFLUCC 15-0746 T | Dead leaf of Pandanus sp.     | Thailand      | MH275056                     |
|                  |                  |                               |               | MH412748                     |
|                  |                  |                               |               | MH412763                     |
| C. papendorfii   | CBS 308.67 T     | Acacia karro                  | South Africa  | KJ909774                     |
|                  |                  |                               |               | KM083617                     |
|                  |                  |                               |               | KM196594                     |
| C. paraesculenta | FMR 17565 T      | Soil                          | Mexico        | LR736641                     |
|                  |                  |                               |               | LR736646                     |
|                  |                  |                               |               | LR736649                     |
| C. petersonii    | BRIP 14642 T     | D. aegyptium                  | Australia     | MH414905                     |
|                  |                  |                               |               | MH433650                     |
|                  |                  |                               |               | MH433668                     |
| C. perotidis     | CBS 350.90 T     | Perotis rara                  | Australia     | JN192385                     |
|                  |                  |                               |               | KJ415394                     |
|                  |                  |                               |               | KM230407                     |
| C. phaeospora    | CGMCC3.19448 T   | Roots of S. officinarum       | China         | MN215686                     |
|                  |                  |                               |               | MN264118                     |
|                  |                  |                               |               | MN263980                     |
| C. pisi          | CBS 190.48 T     | Pisum sativum                 | Canada        | KY905678                     |
|                  |                  |                               |               | KY905690                     |
|                  |                  |                               |               | KY909697                     |
| C. planfarum     | CGMCC3.19342 T   | Roots of S. officinarum       | China         | MN215688                     |
|                  |                  |                               |               | MN264120                     |
|                  |                  |                               |               | MN263982                     |
| C. platzi        | BRIP 27703b T    | Cenchrus clandestinum         | Australia     | MH414906                     |
|                  |                  |                               |               | MH433651                     |
|                  |                  |                               |               | MH433669                     |
| C. polytrata     | CGMCC3.19338 T   | Roots of S. officinarum       | China         | MN215691                     |
|                  |                  |                               |               | MN264123                     |
|                  |                  |                               |               | MN263984                     |
| C. portulaca     | BRIP 14541 T     | Portulaca oleracea            | USA           | KJ415553                     |
|                  |                  |                               |               | KJ415393                     |
|                  |                  |                               |               | KJ415440                     |
| C. pratadii      | CBS 143.64 T     | Jasmium sambac                | India         | KJ922373                     |
|                  |                  |                               |               | KM061785                     |
|                  |                  |                               |               | KM230408                     |
| C. prototheci    | CGMCC3.19360 T   | Leaves of S. officinarum      | China         | MN215693                     |
|                  |                  |                               |               | MN264125                     |
|                  |                  |                               |               | MN263986                     |
| C. protuberata   | CBS 376.65 T     | Deschampsia flexuosa          | UK            | KJ922376                     |
|                  |                  |                               |               | KM083605                     |
|                  |                  |                               |               | KM196576                     |
| C. pseudobrachyponsa | CPC 28808 T   | Elesine indica                | Thailand      | MF490819                     |
|                  |                  |                               |               | MF490841                     |
|                  |                  |                               |               | MF490862                     |
| C. pseudolunata  | UTHSC 09-2092 T  | Human nasal sinus             | USA           | HE861842                     |
|                  |                  |                               |               | HF565459                     |
|                  |                  |                               |               | –                            |
| C. pseudorobusta | UTHSC 08-3458 T  | Human nasal sinus             | USA           | HE861838                     |
|                  |                  |                               |               | HF565476                     |
|                  |                  |                               |               | –                            |
| C. radici-foliigens | CGMCC3.19328 T | Roots of S. officinarum       | China         | MN215695                     |
|                  |                  |                               |               | MN264127                     |
|                  |                  |                               |               | MN263988                     |
|                  |                  |                               |               | –                            |
| C. radiciola     | CGMCC3.19327 T   | Roots of S. officinarum       | China         | MN215699                     |
|                  |                  |                               |               | MN264131                     |
|                  |                  |                               |               | MN263992                     |
|                  |                  |                               |               | –                            |
| C. ravenelii     | BRIP 13165 T     | Sporobolus fertilis           | Australia     | JN192386                     |
|                  |                  |                               |               | JN600978                     |
|                  |                  |                               |               | JN601024                     |
| C. reesi         | BRIP 4358 T      | Air                           | Australia     | MFL14907                     |
|                  |                  |                               |               | MH433637                     |
|                  |                  |                               |               | MH433670                     |
| Species                  | Strain no¹ | Substrate                  | Country     | Genbank accession no.² |
|-------------------------|------------|----------------------------|-------------|------------------------|
| **C. richardiae**       | BRIP 4371 T| *Richardia brasiliensis*   | Australia   | KJ415555               |
|                         |            |                            |             | KJ415391               |
|                         |            |                            |             | KJ415438               |
| **C. robusta**          | CBS 624.68 T| * Dichanthium annulatum    | USA         | KJ909783               |
|                         |            |                            |             | KM083613               |
|                         |            |                            |             | KM196577               |
| **C. roubani**          | CBS 144674 T| *Syngonion velozianum*     | Iran        | KX139030               |
|                         |            |                            |             | MG428694               |
|                         |            |                            |             | MG428687               |
| **C. ryleyi**           | BRIP 12554 T| *Sporobolus creber*       | Australia   | KJ415556               |
|                         |            |                            |             | KJ415390               |
|                         |            |                            |             | KJ415437               |
| **C. saccharicola**     | CGMCC3.19344 T| Roots of S. officinarum | China       | MN215701               |
|                         |            |                            |             | MN264133               |
|                         |            |                            |             | MN263994               |
| **C. sacchari-officinarum** | CGMCC3.19331 T| Leaves of S. officinarum | China       | MN215705               |
|                         |            |                            |             | MN264137               |
|                         |            |                            |             | MN263998               |
| **C. senegalensis**    | CBS 149.71 | Unknown                    | Nigeria     | HG779001               |
|                         |            |                            |             | HG779128               |
| **C. shahidchamranensis** | IRAN 3133C T| Crude oil contaminated soil| Iran        | MH550084               |
|                         |            |                            |             | MH550083               |
| **C. soli**             | CBS 222.96 T| Soil                       | Papua New Guinea | KY905679               |
|                         |            |                            |             | KY905691               |
|                         |            |                            |             | KY905698               |
| **C. sorghina**         | BRIP 15900 T| *Sorghum bicolor*         | Australia   | KJ415558               |
|                         |            |                            |             | KJ415388               |
|                         |            |                            |             | KJ415435               |
| **C. spicifera**       | CBS 198.31 | *Capsicum annuum*         | Cyprus      | HP934916               |
|                         | CBS 274.52 | *Soil*                    | Spain       | JN192387               |
|                         |            |                            |             | JN600979               |
|                         |            |                            |             | JN601023               |
| **C. sporobolicola**   | BRIP 23040b T| *Sporobolus australicus*  | Australia   | MH414998               |
|                         |            |                            |             | MH433652               |
|                         |            |                            |             | MH433671               |
| **C. subpapendorfii**   | CBS 656.74 T| Soil                       | Egypt       | KJ909777               |
|                         |            |                            |             | KM061791               |
|                         |            |                            |             | KM196585               |
| **C. suttoniae**       | FMR 10992 T| Human leg wound           | USA         | HE861828               |
|                         |            |                            |             | HF65479                |
|                         |            |                            |             | LR736651               | FMR 11690
| **C. tamilnaduensis**  | SZMC 22226 T| Human cornal scraping     | India       | MN628311               |
|                         |            |                            |             | MN628307               |
|                         |            |                            |             | MN628303               |
|                         | SZMC 26758 | Human cornal scraping     | India       | MN628308               |
|                         |            |                            |             | MN628304               |
|                         |            |                            |             | MN628300               |
| **C. thaiailandicum**  | MFLUCC.15-0747 T| Decaying leaves of Pandanus sp. | Thailand | MH275057               |
|                         |            |                            |             | MH412749               |
|                         |            |                            |             | MH412764               |
| **C. trifoli**         | CBS 173.55 | *Trifolium repens*        | USA         | HG779023               |
|                         |            |                            |             | HG779124               |
| **C. tripogonis**      | BRIP 12375 T| *Tripogon latiliformis*   | Australia   | JN192388               |
|                         |            |                            |             | JN600980               |
|                         |            |                            |             | JN601025               |
| **C. tropicalis**      | BRIP 14834 T| *Coffea arabica*          | India       | KJ415559               |
|                         |            |                            |             | KJ415387               |
|                         |            |                            |             | KJ415434               |
| **C. tntdae**          | ATCC.44764 T| *Chloris gayana*         | Japan       | KC424596               |
|                         | BRIP 10967 | Leaf tip blight of C. gayana | Australia | KC424604               |
|                         |            |                            |             | KC424775               |
|                         |            |                            |             | KC503949               |
| **C. tuberculata**     | CBS 146.63 T| *Z. mays*                 | India       | JX256433               |
|                         |            |                            |             | JX276445               |
|                         |            |                            |             | JX266599               |
| **C. umbiliciformis**  | CGMCC3.19346 T| Roots of S. officinarum | China       | MN215711               |
|                         |            |                            |             | MN264142               |
|                         |            |                            |             | MN264004               |
| **C. vincenti**        | CBS 221.52 T| *O. sativa*               | Vietnam     | HG779024               |
|                         |            |                            |             | HG779134               |
| **C. variabilis**      | CPC 28815 T| *Chloris barbata*        | Thailand    | MF490822               |
|                         | CPC 28816 | *Imperata cylindrica*     | Thailand    | MF490823               |
|                         |            |                            |             | MF490845               |
|                         |            |                            |             | MF490865               |
| **C. verruciformis**   | CBS 537.75 | *Lobliix sp. feather*    | New Zealand | HG779026               |
|                         |            |                            |             | HG779133               |
| **C. verruculosa**     | CBS 149.63 | *Elaeis guineensis*      | Nigeria     | HP934909               |
|                         | CBS 150.63 | *Punica granatum leaf*   | India       | KP400652               |
|                         | CPC 28792 | *C. dactylon*            | Thailand    | MF490825               |
|                         | CPC 28809 | *E. indica*              | Thailand    | MF490824               |
|                         |            |                            |             | MF490846               |
|                         |            |                            |             | MF490867               |

¹ Strain no: Catalog number of the strain.
² Genbank accession no: Accession numbers for the ITS, gapdh, and tef genes.
| Species                     | Strain no¹ | Substrate                        | Country     | Genbank accession no.²  |
|----------------------------|------------|----------------------------------|-------------|-------------------------|
| C. vietnamensis            | FMR 17659 T| Unidentified dead leaves          | Vietnam     | LR736642 LR736644 LR736647 |
|                            | FMR 11956  | Sorghum seed                      | Indonesia   | LR736652 LR736643 LR736648 |
| C. warraberensis           | BRIP 14817 T| D. aegyptium                      | Australia   | MH414909 MH433653 MH433672 |
| C. xishuangbannaensis      | KUMCC 17-0185 T| Decaying leaves of Pandanus amarylifolia | China      | MH275058 MH412750 MH412765 |

¹ ATCC: American Type Culture Collection, Virginia, USA; BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CGMCC: China General Microbiological Culture Collection Center, China; CPC: Culture collection of Pedro Crous, housed at Westerdijk Fungal Biodiversity Institute; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; GUCC: Department of Plant Pathology, Agriculture College, Guizhou University, P.R. China; IMI: International Mycological Institute, Kew, UK; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; KUMCC: Culture Collection of Kunming Institute of Botany, Kunming, China; LC: Personal culture collection held in the laboratory of Prof. Lei Cai, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL: Mycothe`que de l’Universite` Catholique de Louvain, Louvain-la-Neuve, Belgium; SZMC: Szeged Microbiological Collection at the Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary; UTHSC: Fungus Testing Laboratory, Department of Pathology at the University of Texas Health Science Center, San Antonio, Texas, USA. T and ET indicate ex-type and ex-epitype strain.

² Sequences newly generated in this study and novel species proposed are indicated in bold.

For the BI phylogenetic analysis, the best nucleotide substitution model was determined using jModelTest (Posada 2008). For the ITS region, we used Kimura 2-parameter with Invariant sites (K80+I), for gapdh General Time Reversible with gamma distribution (GTR+G) and for tef1 General Time Reversible with invariant sites (GTR+I). The parameter settings used were two simultaneous runs of 5M generations, four Markov chains, sampled every 1000 generations. The 50% majority-rule consensus tree and posterior probability values were calculated after discarding the first 25% of the samples. A posterior probability (pp) value of ≥ 0.95 was considered significant.

Sequence data generated in the present study were deposited in GenBank (Table 1) and the alignments in TreeBASE (http://treebase.org).

Phenotypic study

Macroscopic characterisation of the colonies was made on PDA, oatmeal agar (OA; oatmeal 30 g, agar 13 g, distilled water 1 litre) and potato carrot agar (PCA; potato 20 g, carrot 20 g, agar 13 g, distilled water 1 litre), after 7 days at 25 °C in darkness. Colours of the colonies in descriptions were based on Kornerup & Wanscher (1978). Cardinal temperatures for growth were obtained on PDA after 7 days in darkness.

Microscopic features were studied from the specimens mounted in Shear’s solution growing on the same media (Madrid et al. 2014). At least 30 measurements were taken for the calculation of conidial and conidiophores length and width ranges, which are also reported as the mean plus or minus standard deviation in the descriptions.
Photomicrographs were taken using a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity X digital camera.

Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004). Ex-type cultures and holotypes, which were dried cultures, were deposited at the Westerdijk Fungal Biodiversity Institute from Utrecht (CBS, The Netherlands).

**Results**

BLASTn results with gapdh sequences showed that the isolate FMR 17656 was ≤ 97.6%, similar to C. verruculosa CPC 28792; FMR 11956 and FMR 17659 showed a similarity of 93.31% and 93.6%, respectively, with C. spicifera CBS 198.31; and isolates FMR 10992 and FMR 11690 both exhibited a similarity of 94.7% with the ex-type strain of C. petersonii (BRIP 14642). Sequence similarity with this marker between FMR 11956/17659 and FMR 10992/11690 was 97%. These values suggested that the unidentified isolates represented putative new species for the genus, which were then confirmed by multi-locus sequence analysis of ITS, gapdh and tef1 barcodes. The combined analysis included 128 sequences representing 126 taxa in the genus Curvularia and these were rooted with Bipolaris maydis (CBS 136.29) and B. saccharicola (CBS 155.26) (Suppl. material 1: Fig. S1). The alignment comprised a total of 1928 bp (ITS 432, gapdh 573 bp and tef1 923 bp), including 546 variable sites (ITS 119 bp, gapdh 253 bp and tef1 174 bp) and 445 phylogenetically informative (ITS 83 bp, gapdh 233 bp and tef1 129 bp). The unidentified isolates were allocated to three single lineages in the same clade (74/0.99) together with sequences of the ex-type strains of C. americana (UTHSC 08-3414), C. petersonii (BRIP 14642) and C. verruculosa (CBS 150.63), but with enough distance to be considered distinct species. The two clinical isolates (FMR 10992 and FMR 11690) formed a fully-supported clade closely related to isolates FMR 11956 and FMR 17659, which were collected in Indonesia and Vietnam, respectively and to C. petersonii. The fifth isolate (FMR 17656) was related to C. verruculosa and C. americana, but formed an independent and distant branch from the previously-mentioned species.

In order to evaluate possible intra- and inter-specific variability within the species and to confirm the novelty of these fungi, we performed a multi-locus analysis, including only those sequences of the species that were more related to the unidentified Curvularia isolates (Fig. 1). The alignment comprised a total of 1894 bp (ITS 409, gapdh 562 bp and tef1 923 bp), with 298 variable sites (ITS 66 bp, gapdh 135 bp and tef1 97 bp) and 240 being phylogenetically informative (ITS 51 bp, gapdh 117 bp and tef1 72 bp). The phylogenetic analyses show that these isolates indeed represent three new species, which are described and illustrated in the Taxonomy section. The species can be morphologically differentiated mainly by features of their conidia (Table 2).
Figure 1. Phylogenetic tree of the *Curvularia* species most related to the new taxa based on Maximum Likelihood analysis obtained by RAxML, using the combined analysis of ITS, *gapdh* and *tef1* and rooted with *Bipolaris maydis* CBS 136.29 and *Bipolaris saccharicola* CBS 155.26. Bootstrap values (bs) greater than 70% and Bayesian posterior probabilities (pp) greater than 0.95 are given at the nodes (bs/pp). Bold branches indicate bs/pp of 100/1. The novel species are highlighted in bold. Ex-type isolates are marked with a superscript T.
Novel species of *Curvularia*

**Taxonomy**

*Curvularia paraverruculosa* Iturrieta-González, Gené & Dania García, sp. nov.
Mycobank No: 833024

Fig. 2

**Etymology.** Name refers to the phylogenetic closeness to *Curvularia verruculosa*.

**Type.** Mexico, Michoacán, Villa Jiménez, from soil, Sept 2016, E. Rosas de Paz. (holotype CBS H-24293, culture ex-type FMR 17656, CBS 146220).

**Description** (PDA at 25 °C). *Mycelium* composed of branched, septate, subhyaline to pale brown, thin- and smooth-walled hyphae, 2–4 μm wide. *Conidiophores* semi- to macronematous, mononematous, septate, straight or flexuous, geniculate at upper part, unbranched or slightly branched, smooth-walled, yellowish-brown to brown, 19–85 (–145) × 3–6 μm (av. (±SD) 49.6 (±43.8) × 4.6 (±0.69)). *Conidiogenous cells* terminal or intercalary, polytretic, proliferating sympodially, yellowish-brown, with darkened scars, subcylindrical, 4–6 μm wide. *Conidia* 3(–4)-septate, mostly curved at the third cell from base which is usually larger than the others, sometimes apically bifurcate, verruculose to verrucose, apical and basal cells subhyaline to pale brown, middle cells brown, 11–37 × 8–12 μm (av. (±SD) 24 (±18.38) × 9.58 (±1.66)); hila slightly protuberant, thickened and darkened. Sexual morph not observed.

**Culture characteristics** (7 d at 25 °C). *Colonies* on PDA reaching 45 mm diam., dark green (30F8), final edge whitish, velvety, flat, margin regular and fimbriate; reverse dark green (30F8). On PCA and OA, reaching 58–60 mm diam., dark green (30F8), final edge whitish, slightly floccose, flat, margin regular and fimbriate; reverse dark green (30F8). Sporulation was abundant on the three media.

**Cardinal temperature for growth.** Optimum 30 °C, maximum 37 °C, minimum 15 °C.

**Distribution.** Mexico.

**Notes.** *Curvularia paraverruculosa* is allocated phylogenetically to a strongly-supported clade (100/1) with *C. verruculosa* and *C. americana* (Fig. 1). All three species commonly have 3-septate conidia, but these can be distinguished by their size and

### Table 2. Conidial features of the novel *Curvularia* species proposed here and of their closest relatives.

| Species          | Size (μm)          | Septum no. | Ornamentation                  | References                |
|------------------|--------------------|------------|--------------------------------|----------------------------|
| *C. americana*   | 13–28 × 7–15       | 3–4        | Smooth upper cells, verruculose basal cell | Madrid et al. (2014)       |
| *C. palmicola*   | 23.9–34.7 × 9.3–15.7 | 3          | Smooth                        | Hyde et al. (2017)         |
| *C. paraverruculosa* | 11–37 × 8–12     | 3(–4)     | Verruculose to verrucose       | Present study               |
| *C. petersonii*  | (15–)17–19(–21) × (5–)5.5–6(–7) | 3          | Smooth                        | Tan et al. (2018)          |
| *C. suttoniae*   | 8–22 × 5–9        | (2–)3      | Smooth upper cells, verruculose basal cell | Present study               |
| *C. verruculosa* | 20–40 × 12–17     | 3          | Rough to verrucose            | Sivanesan (1987)           |
| *C. vietnamensis*| 15–28 × 5–12      | (1–)3(–4) | Smooth                        | Present study               |
Figure 2. *Curvularia paraverruculosa* sp. nov. (ex-type FMR 17656). **A–C** Colonies on PDA, PCA and OA, respectively, at 25 °C after 7 d **D–H** conidiophores and conidia. Scale bars: 10 μm.

ornamentation. Although conidia in *C. verruculosa*, the closest phylogenetic species and *C. paraverruculosa* are entirely verruculose, they are larger in the former (20–40 × 12–17 μm) (Sivanesan 1987). Furthermore, *C. paraverruculosa* also produces apically bifurcate conidia (Fig. 2), which have not been described in *C. verruculosa*. The conidia of *C. americana* are smaller (13–28 × 7–15 μm) and smooth-walled with a slightly verruculose basal cell (Madrid et al. 2014). In addition, microconidiation, described in *C. americana*, has not been observed in *C. paraverruculosa*. 
Curvularia suttoniae Iturrieta-González, Wiederhold, Gené & Dania García, sp. nov.
Mycobank No: 833025
Fig. 3

Etymology. Named in honour of the American mycologist Deanna A. Sutton for her contribution to the body knowledge of microfungi.

Type. USA, Texas, from a human leg wound, 2009, D.A. Sutton (holotype CBS H-24294, culture ex-type UTHSC 09-3575, CBS 146221, FMR 10992).

Description (PDA at 25 °C). Mycelium consisting of branched, septate, pale brown, smooth-walled to verruculose hyphae, 1–4 μm wide. Conidiophores mononematous, semi-to macronematous, erect to slightly flexuous, geniculate at the apex, unbranched or branched, smooth-walled to verruculose, pale brown, 43–103 × 3–5 μm (av. (±SD) 80 (±32.35) × 3.7 (±0.67)). Conidiogenous cells terminal, subterminal or intercalary, polytretic, proliferating sympodially, pale brown, darkened scars, subcylindrical to slightly swollen, 3–5 μm wide. Conidia (2–)3-septate, straight or curved, with the third cell often larger than the rest, apical and middle cells smooth-walled, basal cell verruculose, pale brown to brown, apical and basal cells paler than the middle cells, 8–22 × 5–9 μm (av. (±SD) 15 (±9.89) × 6.88 (±1.18)); hila protuberant, thickened and darkened. Sexual morph not observed.

Culture characteristics (7 d at 25 °C). Colonies on PDA reaching 66–68 mm diam., yellowish-grey (4B2), velvety, flat, margin slightly irregular and fimbriate; reverse black to brownish-orange (5C4); soluble pigment brown (6E6) present in cultures between 30–37 °C. On PCA, reaching 67 mm diam., olive grey (3D2), slightly floccose at the centre, flat, margin regular and whitish; reverse olive grey (3D2), whitish towards periphery. On OA, reaching 64 mm diam., olive grey (3F2), slightly floccose at the centre, flat, margin regular and whitish; reverse olive grey (3F2). Scarce sporulation on the three media.

Cardinal temperature for growth. Optimum 25–30 °C, maximum 37 °C, minimum 5 °C.

Distribution. USA.

Additional specimen examined. USA, South Carolina, from human sphenoid sinus, 2008, D.A. Sutton (UTHSC 08-809, FMR 11690).

Notes. Curvularia suttoniae is included in a well-supported clade with C. petersonii and C. vietnamensis, the latter also described here. Although the three species are clearly differentiated phylogenetically (Fig. 1), they can be distinguished only by subtle morphological features. While the conidia of C. petersonii and C. vietnamensis are entirely smooth, those of C. suttoniae show verruculose basal cells. Furthermore, the conidia in C. petersonii are narrower (5–7 μm wide) (Tan et al. 2018) and, in C. vietnamensis, they are larger (15–28 × 5–12 μm) than those of C. suttoniae (8–22 × 5–9 μm). In addition to these morphological features, gapdh sequences easily distinguish the two latter species.
Figure 3. *Curvularia suttoniae* sp. nov. (ex-type FMR 10992). A–C Colonies on PDA, PCA and OA, respectively, at 25 °C after 7 d D–I conidiophores and conidia with verruculose basal cells (arrows). Scale bars: 10 μm.

*Curvularia vietnamensis* Iturrieta-González, Gené & Dania García, sp. nov.
MycoBank No: 833027
Fig. 4

**Etymology.** Name refers to the country where the species was collected.

**Type.** Vietnam, north-east region, on an unidentified dead leaf, Aug 2011, J. Guarro (holotype CBS H-24295, culture ex-type CBS 146222, FMR 17659).
**Description** (PDA at 25 °C). Mycelium composed of branched, septate, subhyaline to pale brown, thin and smooth-walled to verruculose hyphae, 2–4 μm wide. Conidiophores macronematous, mononematous, septate, straight or flexuous, sometimes slightly geniculate at upper part, unbranched to slightly branched, smooth to verruculose, pale brown to brown, 11–136 (–194) × 3–6 μm (av. (±SD) 92.2 (±72.86) × 4.21 (±0.85)). Conidiogenous cells terminal or intercalary, mono- or polytretic, proliferating sympodially, pale brown, with darkened scars, subcylindrical to swollen, 3–7 μm wide.
Conidia (1–)3(–4)-septate, curved, with the third cell from base unequally enlarged, some apically bifurcate, smooth-walled, apical and basal cells pale brown, middle cells brown, 15–28 × 5–12 μm (av. (±SD) 21.38 (± 3.44) × 9.34 (±1.83)); hila slightly protuberant, thickened and darkened. Sexual morph not observed.

**Culture characteristics** (7 d at 25 °C). Colonies on PDA reaching 62 mm diam., greenish-grey to dark green (28C2/29F8), final edge white, umbonate, densely floccose, margin regular; reverse grey (29F1), final edge pale grey (1B2). On PCA, reaching 58 mm diam., olive grey to grey (3F2/3B1), slightly floccose at the centre, margin regular, final edge whitish; reverse olive grey to grey (3F2/3B1). On OA, reaching 74 mm diam., olive (2F3) slightly floccose at the centre, margin regular, flat; reverse olive to greenish-grey (2F3/1C2). Sporulation abundant mainly on PCA and OA.

**Cardinal temperature for growth.** Optimum 30 °C, maximum 37 °C, minimum 15 °C.

**Distribution.** Indonesia and Vietnam.

**Additional specimen examined.** Indonesia, from Sorghum seed, 1948, J. van der Vecht (CBS 188.48 = FMR 11956).

**Notes.** See *C. suttoniae* described above.

**Discussion**

As in other Pleosporalean genera, *Curvularia* is currently a well-delineated genus on the basis of molecular data (Manamgoda et al. 2015, Marin-Felix et al. 2017a). However, morphological features and analyses of the ITS barcode are insufficient to accurately identify *Curvularia* species. Thus, the multi-locus sequence analysis of different gene markers (i.e. LSU, ITS, *gapdh*, *rpb2* and *tef1*) has been used to study the species diversity in *Curvularia* and phylogenetic relationships with other similar genera (Hernández-Restrepo et al. 2018, Manamgoda et al. 2012, 2015, Madrid et al. 2014, Marin-Felix et al. 2017a, 2017b, Tán et al. 2018). Marin-Felix et al. (2017a) regarded ITS, *gapdh* and *tef1* as the DNA barcodes for species delineation in the genus. During the last three years, numerous new *Curvularia* species have been introduced (Hyde et al. 2017, Marin-Felix et al. 2017a, 2017b, Dehdari et al. 2018, Heidari et al. 2018, Liang et al. 2018, Mehrabi-Koushi et al. 2018, Tan et al. 2018, Típbromma et al. 2018, Kiss et al. 2019, Raza et al. 2019, Zhang et al. 2020). Novel species are found, not only on fresh material collected in various geographical regions, but also in re-evaluation of *Curvularia* isolates deposited in fungal collections and earlier identified by morphological features or ITS sequence analysis.

The five isolates, studied here, showed morphological similarity with *C. americana* or *C. lunata* (Sivanesan 1987, Madrid et al. 2014), but they also showed subtle variations that did not match with these species. Multi-locus analysis of the recommended barcodes facilitated the delineation of the novel species *C. paraverruculosa*, *C. suttoniae* and *C. vietnamensis*, which were closely related to the known species *C. americana*, *C. petersonii* and *C. verruculosa* (Fig 1).
As in the case of *C. suttoniae*, other related species, such as *C. americana* and *C. verruculosa*, have also been associated with clinical specimens previously (da Cunha et al. 2013, Madrid et al. 2014). However, the role of all these fungi in human diseases has never been proven. Contrary to that, the recently described species *C. coimbatorensis* and *C. tamilnaduensis* were shown to be causal agents of fungal keratitis in India (Kiss et al. 2019). These two latter species, as with *C. suttoniae* and *C. vietnamensis* in our case, could only be molecularly differentiated by *gapdh* and *tef1* loci; ITS sequence similarity between *C. coimbatorensis* and *C. tamilnaduensis* was 99% (Kiss et al. 2019) and between *C. suttoniae* and *C. vietnamensis*, it was 100%. Therefore, considering clinical laboratories commonly use ITS barcode for fungal diagnosis, not only will the diversity of *Curvularia* species remain obscure in the clinical setting, but also, subsequently, the epidemiology of its species associated with human or animal diseases. Our results suggest that *gapdh* and *tef1* loci could be good alternatives as barcodes for *Curvularia* identification, since both have a high discriminatory power amongst species. However, *gapdh* would be the recommended locus because there are more sequences available for different species in the genus.

The ITS analysis revealed that *C. palmicola*, only known for its type specimen found on dead branches of *Acoelorrhaphe wrightii* in Thailand (Hyde et al. 2017), is also closely related to the novel species described here. However, this fungus was not included in our concatenate analysis since sequences of *gapdh* and *tef1* were not available for comparison. Nevertheless, *C. palmicola* can be distinguished morphologically from our species mainly by having conidia with constricted wall at the septum level. Furthermore, *C. palmicola* has longer conidia (23.9–34.7 μm) than *C. suttoniae* (8–22 μm) and *C. vietnamensis* (15–28 μm) and it differs from *C. paraverruculosa* by its smooth-walled conidia.

Despite the fact that DNA sequence analysis is currently mandatory for *Curvularia* identification, two species were recently characterised exclusively, based on morphological data and host association, i.e. *C. tremae* on living leaves of *Trema orientalis* (Haldar 2017) and *C. martyniicola* on *Martynia annua* (Kumar and Singh 2018), both from India. *Curvularia tremae* produces up to 4-septate and larger conidia (average length 152.21 μm and 67.75 μm wide at the broadest part) than those described here. Despite the conidia being mostly 3-septate, as in our species, *C. martyniicola* differs by having longer conidiophores (95–200 μm) than those of *C. paraverruculosa* (19–85(–145) μm) and *C. suttoniae* (43–103 μm) and by larger conidia (25–45 × 10–15 μm) than those observed in *C. vietnamensis* (15–28 × 5–12 μm).

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Supplementary material 1

Figure S1. Phylogenetic tree of the genus *Curvularia* based on Maximum Likelihood analysis obtained by RAxML, using the combined analysis of ITS, *gapdh* and *tef1* and rooted with *Bipolaris maydis* CBS 136.29 and *Bipolaris saccharicola* CBS 155.26

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Data type: phylogenetic tree

Explanation note: Bootstrap values (bs) greater than 70% and Bayesian posterior probabilities (pp) greater than 0.95 are given at the nodes (bs/pp). Bold branches indicate bs/pp of 100/1. The novel species are highlighted in bold. Ex-type and ex-epitype strain are marked with a superscript T and ET, respectively.

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