Egyptian Journal of Biological Pest Control

SCIENTIFIC (SHORT) NOTE

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Natural occurrence of *Beauveria bassiana* (Ascomycota: Hypocreales) infecting *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and earwig in eastern DR Congo

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Abstract

Background The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), poses a threat to the food security of populations in sub-Saharan Africa because of its damage to maize crops. As alternative to the use of hazardous pesticides, microbial control is one of the most promising sustainable approaches adopted to limit the damages caused by *S. frugiperda*. The sampling targeted mainly larvae of *S. frugiperda*; however, during the survey, cadavers of earwig found on the same sampling sites were also collected and involved in the study. Cadavers of targeted insects, with and without sign of fungal infection, were sampled from 3 localities in eastern DR Congo. Culture of fungal isolates was performed in selective Sabouraud dextrose agar media.

Results Morphological study of fungal features such as conidia (shape and size) and conidiophores showed that the isolates were from the genus *Beauveria*. Conidial measurements were highly variable and ranged from 2.4 to 3.6 µm in length and from 1.75 to 3.0 µm in width. Molecular characterization and phylogenetic analysis of the 2 *Beauveria* isolates based on DNA sequencing of ITS-5.8S region confirmed that both isolates belong to *Beauveria bassiana*. The 2 isolates of *B. bassiana* P5E (OP419735.1) and KA14 (OP419734.1) were isolated from cadavers of FAW and earwig, respectively. The alignment with different sequences of *B. bassiana* from different continent showed that P5E belonged to the same clade of previous isolates reported from Iran and Mexico, while KA14 was with the same clade as isolates from Kenya and China.

Conclusion To our knowledge, this is the first study reporting the occurrence of *B. bassiana* infecting FAW and earwig in eastern DR Congo and in Africa.

Keywords *Spodoptera frugiperda*, *Beauveria bassiana*, Epizooty, Earwig, Molecular characterization

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Background

Agriculture, food, fisheries, and forestry resources have been increasingly affected by invasive species such as the fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) throughout the world (Teem et al. 2020). The invasive behavior of a species depends on the characteristics related to its biology and the climatic conditions where it is introduced (Early et al. 2018). The FAW, *S. frugiperda* a lepidopteran insect pest, is well known as a devastating pest in North and South America where it originated and has become a major invasive pest at a global scale in the past decade (Tay et al. 2023). This pest was first reported in Africa in 2016 (Goergen et al. 2016) and has spread rapidly over the rest of the world excluding Europe. The polyphagous nature of FAW enables it to successfully establish in newly invaded areas with suitable climatic conditions for its survival (Cokola et al. 2021b).

Management of FAW is mainly based on the excessive use of pesticides to face with alarming damages observed among smallholder farmers in Africa (Hruska 2019). Studies reported losses of 26.5–58.8% when non-chemical applications are made to control FAW (Van den Berg et al. 2021). In the Democratic Republic of Congo, FAW losses in maize production are approximately 633.000 metric tons per year, with an estimated monetary value of US$ 74.5–185.5 million (Day et al. 2017). The environmental risks associated with pest-control measures have always urged scientists toward biological-based alternatives. Ecofriendly pest management approaches are nowadays of utmost importance to ensure a sustainable agriculture (Hruska 2019).

The FAW is naturally attacked by several microorganisms including entomopathogenic fungi (EPFs), nematodes, viruses and bacteria and larval mortalities were often found in infested corn fields (Withers et al. 2022). In its native area, e.g., Mexico, FAW has been found infected naturally by EPFs (Cruz-Avalos et al. 2019). In agroecosystems, EPFs, especially the anamorphic taxa *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales), are among the natural enemies of several insect pests that have been potential candidates for conservation biological control (Meyling and Eilenberg 2007).

Beauveria bassiana is widely distributed in nature and is the most common and ubiquitous EPF with the ability to infect a variety of insects belonging to various orders (Guo et al. 2020). In America, *B. bassiana* has been isolated from both FAW cadavers and from soil (Cruz-Avalos et al. 2019). However, *B. bassiana* has not been officially recorded in Africa on FAW cadavers. Most bioassays of *B. bassiana* to control FAW in Africa used existing strains from collections obtained either from soil or from other insects (Akutse et al. 2020). However, the fungal infections with *M. rileyi* have been reported in field populations at Africa (Withers et al. 2022) and Asia (Acharya et al. 2022). Data on the characterizations of EPFs to control insect pests of crops in Democratic Republic of Congo (DRC) are scarce. Therefore, no study has been conducted on FAW using *B. bassiana* in DRC. Existing information's on EPFs were provided by Akutse et al. (2020) from tests performed with *M. anisopliae* isolated from soil to determine their virulence against FAW. This study provides the first occurrence and characterization of a *B. bassiana* new isolates obtained from FAW and earwig cadavers under maize growing conditions in eastern DRC.

Methods

Sampling sites and collection of cadavers

A monitoring system has been developed in South Kivu province, Eastern DRC, during the period from December 2019 to June 2021. South Kivu is one of 26 provinces of the DRC with an area of approximately 65.070 Km^2. Three sites named Ruzizi plain (Kamanyola), Kabare and Kalehe were investigated, and characteristics are presented in Table 1. These sites are part of the corridor considered suitable for the FAW according to the existing bio-climatic zones in South Kivu (Cokola et al. 2020). Fields infested with FAW and in which the application of insecticides was not reported were targeted. Transect method was applied to select the studied fields. Accordingly, 14 fields were investigated in Kamanyola during the period between February and June 2021 versus 17 in Kabare during the period between December 2019 and May 2021 and 8 in Kalehe in December 2019. The W-sampling technique was used in each field to identify and collect cadavers from maize plants (Cokola et al. 2021a). Sampling was targeting FAW cadavers with and without symptoms of fungal infection. A total of 78 cadavers including 71 of FAW and 7 of earwig were collected. Fifty-four cadavers were collected in Kamanyola of which 46 were mycosed against 8 non-mycosed;

Table 1 Characteristics of the study sites

Characteristics	Sites		
	Kabare	Kalehe	Ruzizi plain (Kamanyola)
Latitude	2°18′57″ S	2°04′37″ S	2°46′21″ S
Longitude	28°47′20″ E	28°53′31″ E	29°00′5″ E
Elevation (m)	1637	1725	889
Mean annual temperature (°C)	22.06	24.01	25.06
Annual precipitations (mm)	1601	1527.3	1063.7

22 in Kabare of which 7 were from earwigs. Among the 15 cadavers from FAW, 9 were mycosed versus 6 non-mycosed and 2 mycosed cadavers in Kalehe. All collected specimens were placed in sterile 1.5-ml Eppendorf tubes and stored at 4 °C on the day of collection until isolation.

Fungal isolation

Collected samples were allocated into three groups: (1) freshly looking mycosed cadavers, (2) cadavers without visible mycelium, and (3) cadavers with very old state of mycosis. The latter group was excluded from the study. Same protocol of grouping was adopted for earwig's cadavers. Sabouraud dextrose agar (SDA) medium (Sigma-Aldrich®) supplemented with streptomycin (0.5 ml of 0.6 g ml^{-1}), tetracycline (0.5 ml of 0.05 g ml^{-1}) and cyclohexamide (1 ml of 0.05 g ml^{-1}) was used as media for fungal growth. Fungal isolation procedure from the collected cadavers was performed following two methods. From the first group, each cadaver was examined under binocular and a sterile inoculation needle was used to pick up a mycelia fragment and to inoculate the SDA media following a zigzag pattern. From the second group, the cadavers were surface-sterilized with 70% ethanol for a period of 10 s to be washed three times with demineralized sterile water and placed on filter paper to absorb the remaining water. Afterward, each cadaver was incubated in SDA. All the plates were incubated at 25 ± 1 °C in darkness. Plate with cadavers was checked for fungal growth up to 5–8 days. Cadavers showing a fungal outgrowth were subject to the protocol for fungal isolation as adopted for the first group. Plates inoculated with mycelia were checked up to 15 days for fungal growth.

Morphological identification

Beside the aspect and color of fungal colonies, morphological studies of the isolated fungi were mainly based on the shape and size of conidia. Fungal structures were mounted in lactophenol blue solution (Sigma-Aldrich®) and characterized using an Olympus microscope at 400× magnification. The fungi were identified using the key by Humber (2012). Microphotographs were taken with a DS-Qi2 camera (Nikon camera DSQi2, Nikon France, France). Intermediate internal magnification dial was set up to switch the magnification of the entire microscope between 1.0× and 1.5× with an exposure time of 300 ms and dial-illuminator's intensity of 30%. To determine the microscopic measurements of the conidia, the mean and standard deviation values were calculated from 50 randomly selected conidia using Fiji ImageJ 1.53t (National institute of health, USA). Parameters measured were length representing the diameter along major axis of conidia and width representing the diameter along minor axis of conidia (Talaei-Hassanloui et al. 2006). To characterize the *Beauveria* isolate, these parameters were compared to another *B. bassiana* isolate KA14 obtained in the same region (eastern DR Congo) on earwig cadaver. Conidial size differences between isolates were analyzed by Mann–Whitney U test at 5% significance level using RStudio 4.0.2 (R Core Team 2021).

DNA extraction, PCR amplification, and purification

Pure culture of mycelial was harvested with a sterile scalpel blade from the SDA plate and placed in sterile 2-ml Eppendorf tubes containing two sterile 3-mm-diameter Tungsten Carbide Beads (QIAGEN, Germany). The Eppendorf tubes were cooled in liquid nitrogen for 30 s, before being crushed using a Retsch Mixer Mill MM 400 for 1 min at 30 Hz. The freshly crushed material was used for ribosomal DNA extraction, using the Qiagen DNeasy® Plant Mini Kit following the manufacturer's protocol. The rDNA concentration was measured with the Nanodrop (Nanodrop One ISOGEN) and diluted to 10 ng/µl. Extracted genomic DNA was amplified by internal transcribed spacers (ITS-5.8S rDNA). Forward ITS5 (5'-TCCTCCGCTTATTGATATGC-3') and Reverse ITS4 (5'-GGAAGTAAAAGTCGTAACAAGG-3') primers were used to amplify the region (White et al. 1990). Amplification reactions were performed in a total volume of 50 µl consisting of a mixture of 25 µl of Q5® High-Fidelity PCR Kit (E0555L), 2.5 µl of reverse ITS4 primer, 2.5 µl of forward ITS5 primer, 15 µl of molecular grade H_2O and 5 µl of genomic DNA. PCRs were performed under the following conditions: an initial step at 98 °C for 3 min., followed by 35 cycles of 98 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min 30 s; a step at 72 °C for 10 min; and a final step at 4 °C infinitely. Amplicons were visualized by agarose gel electrophoresis (1%) in 100 ml of 1× Tris-Boric Acid-EDTA (TBE) and added 5 µl of ethidium bromide (EtBr 50 mg/ml). Electrophoresis was performed in 1× TBE buffer at 100 V for 45 min and recorded in a Bio Rad gel documentation system (Gel Doc EZ Imager).

Sequencing analysis

The purified amplicons were sequenced by Sanger sequencing Eurofins Genomics (Anzinger STR. 7A/D-85560 Ebersberg, Germany). The sequences were assembled and edited using BioEdit sequence alignment editor 7.2.5 (Hall 1999). The resulting contigs were processed through BLASTn analysis using the GenBank database (https://blast.ncbi.nlm.nih.gov/). The sequences of *B. bassiana* isolates were submitted to GenBank database and compared to those of the type strains previously reported in the literature to construct phylograms. The sequences of the *B. bassiana* isolate were grouped with other *Beauveria* sequences deposited in the GenBank

and were aligned by multiple sequence alignment (MUSCLE) using MEGA 11 software (Tamura et al. 2021). The ITS sequence of *Penicillium chrysogenum* was used as out-group. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa were clustered together in the bootstrap test with 1000 replicates (Felsenstein 1985). The evolutionary distances were computed using the p-distance method (Nei and Kumar 2000). This analysis involved 17 nucleotide sequences and conducted using MEGA11.

Results

Morphology of *Beauveria bassiana* isolates

Based on the morphological characteristics of the conidia of isolates P5E and KA14, preliminary results indicated that it was indeed *B. bassiana*. The morphological characteristics of the isolates are presented in Figs. 1 and 2. Isolates exhibited a cottony, powdery white mycelium without exudate drops on SDA medium. P5E refers to the location "Plaine de la Ruzizi" where the cadaver was collected, the number of the cadaver's sample in the batch collected, and the letter assigned to the replicate Petri dish according to the isolation method used. As with P5E, the name KA14 refers to the isolate obtained in Kabare territory from the 14 cadaver samples collected. The conidia of isolate P5E were generally ovoid to cylindrical and were white, gray to black in transparency compared to isolate KA14 whose conidia were cylindrical and white in transparency.

Conidia from *B. bassiana* isolate P5E were slightly larger than those from KA14 in size, on average. Conidial measurements were highly variable and ranged from 2.4 to 3.6 µm in length and from 1.75 to 3.0 µm in width. Conidial size between *B. bassiana* isolate P5E and KA14 was compared in terms of length and width using the Mann–Whitney *U* test (Fig. 3). Conidial length varied significantly between the two isolates ($W = 257$;

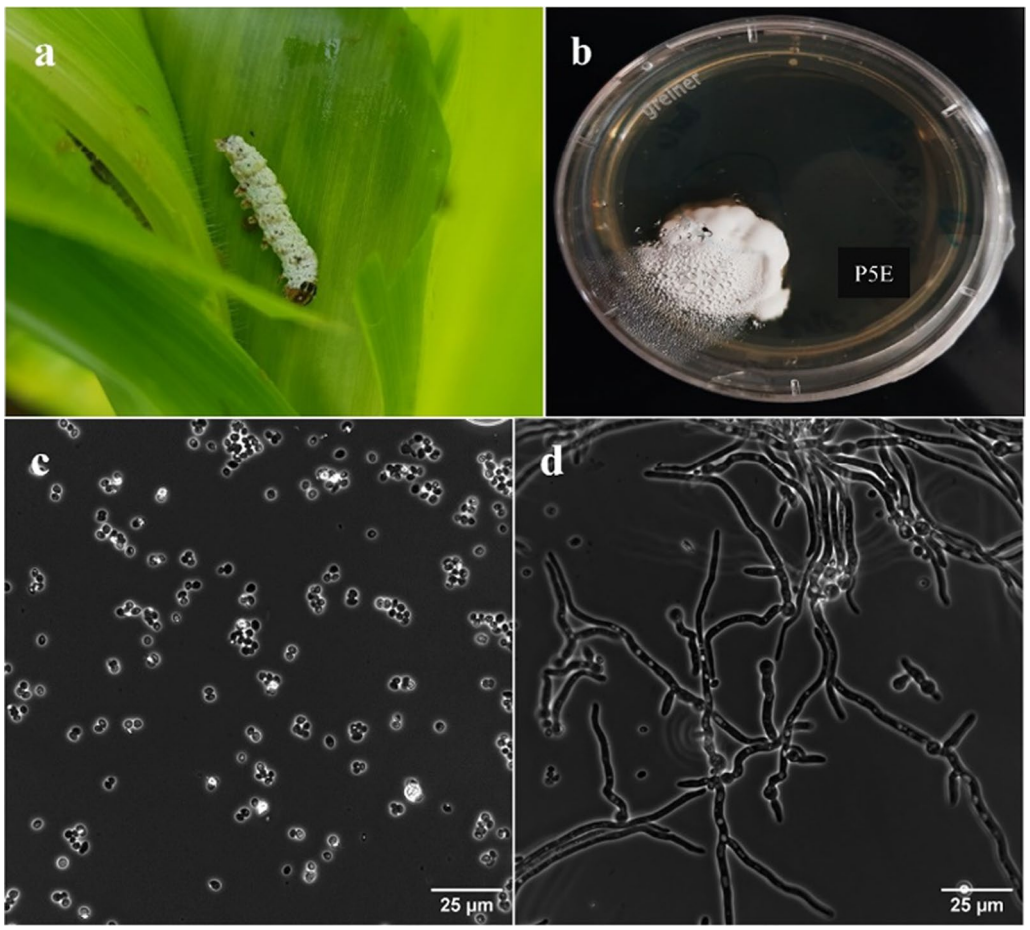

Fig. 1 Morphological characteristics of *Beauveria bassiana* infecting the fall armyworm in natural conditions of South Kivu, eastern DR Congo. **a** Mycosed fall armyworm cadaver found on maize leaves ©Marcellin C. Cokola; **b** Colony growth of *B. bassiana* isolate on SDA medium; **c** Conidia; **d** Conidiogenous cells. Pictures of conidia and conidiogenous cells were taken by Nikon Eclipse Ti2-E inverted automated microscope with a Nikon camera DSQi2

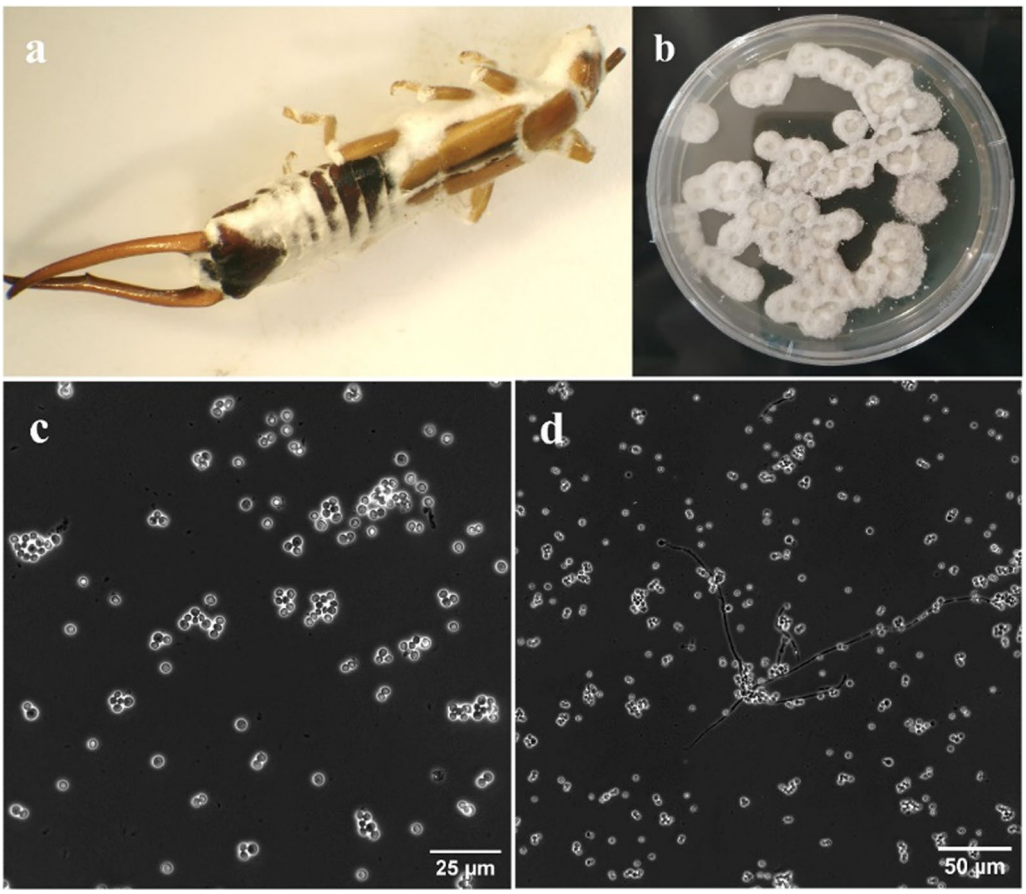

Fig. 2 Morphological characteristics of *Beauveria bassiana* infecting the earwig in natural conditions of South Kivu, eastern DR Congo. **a** mycosed earwig cadaver © Stereomicroscope Euromex DZ Serie; **b** Colony growth of *B. bassiana* isolate KA14 on SDA medium; **c** Conidia; **d** Conidiogenous cells. Pictures of conidia and conidiogenous cells were taken by Nikon Eclipse Ti2-E inverted automated microscope with a Nikon camera DSQi2

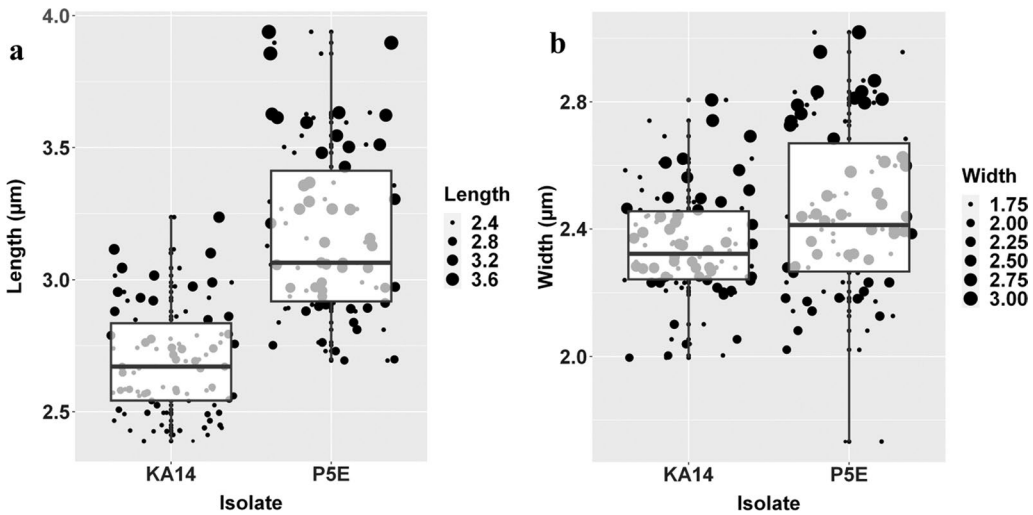

Fig. 3 Comparison of conidial size ($n = 50$) of *Beauveria bassiana* isolate P5E obtained from fall armyworm cadavers to isolate KA14. **a** Conidial length; **b** Conidial width

$p < 0.001$). The mean conidial length was 3.17 ± 0.32 µm for isolate P5E versus 2.69 ± 0.21 µm for isolate KA14. For conidial width, a significant difference was also obtained between the two isolates ($W = 965$; $p = 0.049$). The largest value of conidial width was recorded in isolate P5E (2.45 ± 0.27 µm) compared to isolate KA14 (2.34 ± 0.17 µm).

Molecular identification of *Beauveria bassiana* isolates

Sequencing of *B. bassiana* isolate ITS5-5.8S rDNA-ITS4 confirmed the identity of the species and corroborate with the morphological identification presented previously. Similarity of ITS amplicon of *B. bassiana* isolate P5E was checked with other sequences available in GenBank NCBI (Blastn). Furthermore, to illustrate differences between the amplicon sequences, phylograms were constructed. In this study, 17 sequences including the P5E isolate were used to build phylogenetic trees that were inferred from sequences of 10 isolates of *B. bassiana* and 6 other species belonging to the *Beauveria* genus. The sequence from *P. chrysogenum* was used as an out-group. The evolutionary history was inferred using the neighbor-joining method. The optimal tree was shown (Fig. 4). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown above the branches. The analyses clustered the *Beauveria* species into two major groups. The first cluster consisted of all *B. bassiana* isolates except for 1969 one. The P5E (OP419735.1) and KA14 (OP419734.1) isolates branched separately in this group. The P5E isolate was classified in the same clade as TS8 (KY515356.1) isolate from Iran and A30 (KC461101.1) from Mexico. Isolate KA14 was classified in the same clade as isolate 693 ICIPE (KM463112.1) from Kenya and isolate SY192 (OK482896.1) from China. The second group consisted of *B. bassiana* isolate 1969 (AY531998.1) and other *Beauveria* species, namely *B. brongniartii*, *B. pseudobassiana*, *B. varroae*, *B. australis*, *B. amorpha* and *B. caledonica*.

Discussion

Insecticide applications against FAW in maize crop are not more as effective due to the cryptic feeding behavior of FAW larvae (Hardke et al. 2011) and the application of insecticides when the larvae are too large and no longer susceptible, as well as incorrect application methods and timing (Van den Berg et al. 2021). In addition, pesticide application to control FAW poses a danger to

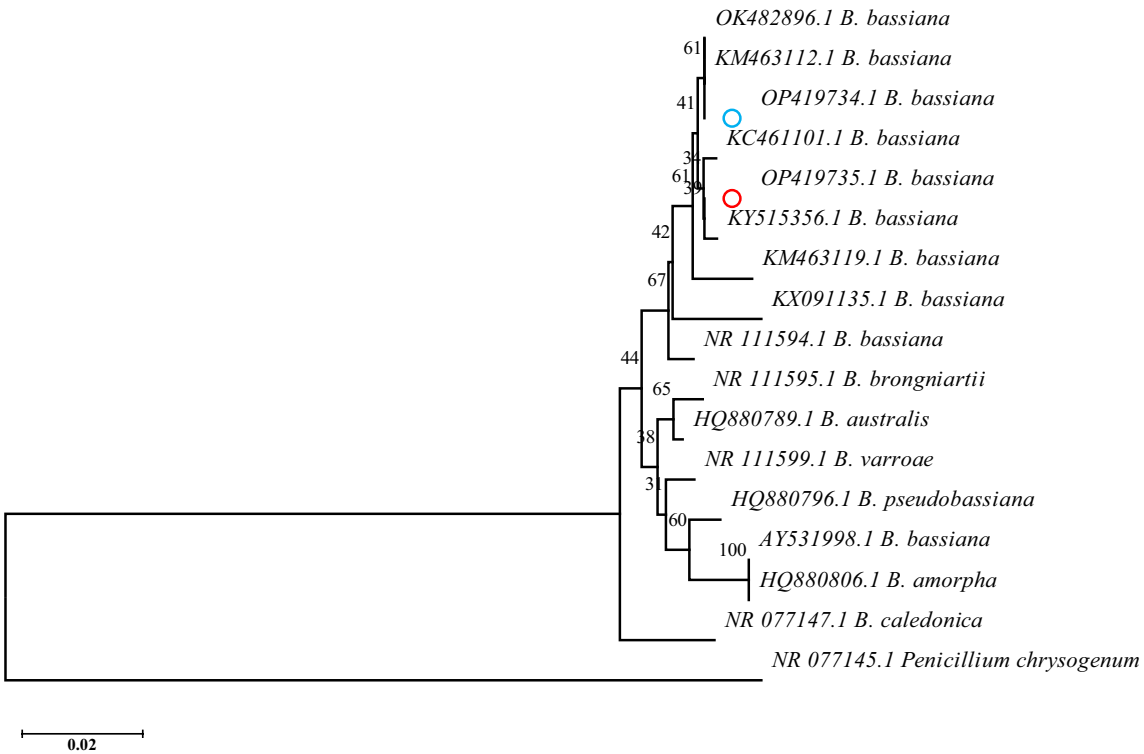

Fig. 4 Phylogenetic tree of *Beauveria bassiana* isolated from Eastern DR Congo based on ITS sequences by using the neighbor-joining method. The percentage of replicate trees in which the associated taxa were clustered together was shown next to the branches. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The red mark represents *B. bassiana* isolate P5E, whereas the blue mark represents isolate KA14

natural enemies' predators and parasitoids that regulate FAW populations in newly invaded areas (Abang et al. 2021). For the first time, this study describes 2 isolates of EPF *B. bassiana* from the DR Congo obtained from infecting FAW and earwig in maize crop as alternative biological control agents. In its native range, an invasive species such as FAW, has been found to be infected with entomopathogenic microorganisms (Cruz-Avalos et al. 2019). However, in newly invaded areas, new entomopathogenic agents have been reported and their presence indicated genotypes that potentially interact with FAW host populations, each other and their environment and are ideal candidates in the development of sustainable biological control (Withers et al. 2022). Since its invasion in Africa in 2016, no study has been reported about the presence of *B. bassiana* EPFs on FAW cadavers and beneficial insects such as earwig. At least, one study in Africa (Withers et al. 2022) and some others in Asia (Acharya et al. 2022) presented instead the occurrence of *M. rileyi* on FAW populations under natural conditions. However, this information remained unknown in DR Congo, where FAW was reported as a maize pest before being officially reported in Africa in 2016 (Cokola et al. 2021a).

Currently, it is relevant to analyze the multi-trophic interactions involving EPF, insect pest, maize plant and beneficial natural enemies. In this study, earwig known to be a predatory natural enemy of fall armyworm (Firake and Behere 2020) was found to be infected by EPF *B. bassiana* under natural maize growing conditions. This observation is not the first elucidated case where an entomopathogen infects a beneficial insect. Goettel et al. (1990) presented the effects or risk associated with the infection of beneficial insects by an entomopathogen and especially predators by giving some examples of mycosed insects under natural conditions. In the literature, infection of earwig by *B. bassiana* is rarely reported under natural conditions. This would have implications for biological control and understanding the epizootiology of this beneficial mycotic insect and the resulting effects in controlling FAW.

In the identification of *Beauveria* species, conidia are the primary morphological feature used, but they are not always sufficiently critical for classification and identification due to similarity with others (Zhang et al. 2022). The conidia of isolate P5E were generally ovoid to cylindrical and were white, gray to black in transparency. This corroborated the description of Rehner and Buckley (2005). Isolate P5E showed different morphological characteristics in terms of conidial size and shape compared to isolate KA14. Conidial measurements (length and width) were highly variable between the two isolates and fell within the range found by other authors (Talaei-Hassanloui et al. 2006). Although pathogenicity has not yet been determined for the P5E isolate, the morphological characteristics (conidial size) and the fungus isolation from FAW cadaver allowed to hypothesize the potentiality of this isolate as candidate for FAW biological control. Previous studies linked conidial size to virulence of EPF (Talaei-Hassanloui et al. 2006). For example, Talaei-Hassanloui et al. (2006) did not find a correlation between virulence and conidial size in *B. bassiana*. In contrast, Liu et al. (2003) found a positive correlation between conidial length and virulence of *B. bassiana* isolates. Additionally, a recent study (Ramírez-Ordorica et al. 2022) demonstrated a different chemical signature and higher virulence of *B. bassiana* isolates from mycosed insect cadavers than those obtained from soil.

The sequencing of the ITS5-5.8S rDNA-ITS4 of selected *B. bassiana* isolate confirmed the identity of the species and corroborated with the morphological identification. None of the sequences was 100% identical to each other, demonstrating the uniqueness and difference between the species considered. In most studies on the genetic diversity of *B. bassiana*, isolates from collections obtained either from infected insects or from soil were considered to build phylograms (Rehner et al. 2011). Phylogenetic analysis performed by Rehner and Buckley (2005) on *Beauveria* taxa showed that morphological species were paraphyletic and were classified into two unrelated clades, one of which was more related to *B. brongniartii* and the other to *B. bassiana*. This was observed in this study when building phylograms where the 1969 isolate of *B. bassiana* was found in the same group as the other species of the genus *Beauveria*. According to Meyling and Eilenberg (2007), the existence of two unrelated clades may partly explain the high genetic diversity within *B. bassiana*. This entomopathogenic species is not a specific host but an opportunist one capable of attacking a wide range of insects belonging to diverse taxa (Rehner and Buckley 2005). The minor genetic distances (as in this study) between *B. bassiana* isolates according to Fernandes et al. (2009) indicated a considerable correlation with their geographical origin. In this study, isolate P5E was classified in the same clade as isolates from Iran and Mexico, although the latter were isolated from soil. *B. bassiana* EPFs from infected insects are mostly classified in the same clade (Rehner and Buckley 2005).

Conclusions

This study provides the first information on the presence of EPF *B. bassiana* infecting FAW and earwig in the conditions of South Kivu, eastern DR Congo. Morphological and molecular characterization of the isolates confirmed the identity of the species and

represents a starting point in the development of alternative management methods against FAW in Africa. As data on EPFs are scarce in DR Congo, this study provides insight into the existence of a diversity of entomopathogenic microorganisms that have not yet been exploited and that could be ideal a biocontrol agent for sustainable management of FAW and other pests. However, other EPF species such as *M. rileyi* have been reported to infect FAW larvae in newly invaded areas, and it would be important to consider them in further investigations. The isolates reported in this study will be tested for their effectiveness in the management of FAW. Furthermore, this study has implications in understanding the interactions between entomopathogenic microorganisms' especially *B. bassiana*, FAW, earwig, and climatic conditions of the invaded region.

Abbreviations

DRC	Democratic Republic of Congo
FAW	Fall armyworm
SDA	Sabouraud dextrose agar
EPF	Entomopathogenic fungi
NCBI	National Center for Biotechnology Information
MEGA	Molecular Evolutionary Genetics Analysis
ITS	Internal transcribed spacers
MUSCLE	Multiple sequence alignment

Acknowledgements
The collection of cadavers in the fields would not be possible without the agreement of the farmers who allowed us access to their plantations. We would like to thank them immensely. Blaise Minani and David Mugula are acknowledged for their help during the collection of cadavers. The authors would like to thank Emilie Bera, for the technical assistance during laboratory investigations. Matheo Delvenne from TERRA/Gembloux Agro-Bio Tech is acknowledged for microscopic visualizations.

Author contributions
MCC and FF conceived and designed the project. MCC collected the data, performed analysis, and wrote the first draft of the manuscript; IBF designed the methodology of fungal isolation, cultivation and morphological and molecular identification; Supervision and funding of MCC were assured by EBB and FF. The first draft of the text was written by MCC and then edited by IBF, EBB, RCM, FD, and FF. All authors read and approved the final manuscript.

Funding
This study was partially funded by Brot für die Welt (Project A-COD-2018-0383).

Availability of data and materials
All data are available in the article. Sequences data are available on the NCBI website with the accession numbers OP419734.1 and OP419735.1.

Declarations

Ethics approval and consent to participate
The manuscript does not contain studies involving human participants, human data or human tissue.

Consent for publication
Not applicable.

Competing interests
The authors have no conflict of interest.

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Received: 17 February 2023 Accepted: 11 May 2023
Published online: 17 May 2023

References

Abang AF, Nanga SN, Fotso Kuate A, Kouebou C, Suh C, Masso C, Saethre M-G, Fiaboe KKM (2021) Natural enemies of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in different agro-ecologies. InSects 12:509. https://doi.org/10.3390/insects12060509

Acharya R, Lee J, Hwang H, Kim M, Lee S, Jung H, Park I, Lee K (2022) Identification of entomopathogenic fungus *Metarhizium rileyi* infested in fall armyworm in the cornfield of Korea, and evaluation of its virulence. Arch Insect Biochem Physiol 111:21965. https://doi.org/10.1002/arch.21965

Akutse KS, Khamis FM, Ambele FC, Kimemia JW, Ekesi S, Subramanian S (2020) Combining insect pathogenic fungi and a pheromone trap for sustainable management of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J Invertebr Pathol 177:107477. https://doi.org/10.1016/j.jip.2020.107477

Cokola MC, Mugumaarhahama Y, Noël G, Bisimwa EB, Bugeme DM, Chuma GB, Ndeko AB, Francis F (2020) Bioclimatic zonation and potential distribution of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in South Kivu Province, DR Congo. BMC Ecol 20:1–13. https://doi.org/10.1186/s12898-020-00335-1

Cokola MC, Mugumaarhahama Y, Noël G, Kazamwali LM, Bisimwa EB, Mugisho JZ, Aganze VM, Lubobo AK, Francis F (2021a) Fall Armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in South Kivu, DR Congo: understanding how season and environmental conditions influence field scale infestations. Neotrop Entomol 50:145–155. https://doi.org/10.1007/s13744-020-00833-3

Cokola MC, Ndjadi SS, Bisimwa EB, Ahoton LE, Francis F (2021b) First report of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on Onion (*Allium cepa* L.) in South Kivu, Eastern DR Congo. Rev Bras Entomol 65:e20200083. https://doi.org/10.1590/1806-9665-rbent-2020-0083

Cruz-Avalos AM, Bivián-Hernández MdlA, Ibarra JE, Del Rincón-Castro MC (2019) High virulence of mexican entomopathogenic fungi against fall armyworm, (Lepidoptera: Noctuidae). J Econ Entomol 112:99–107. https://doi.org/10.1093/jee/toy343

Day R, Abrahams P, Bateman M, Beale T, Clottey V, Cock M, Colmenarez Y, Corniani N, Early R, Godwin J, Gomez J, Moreno PG, Murphy ST, Oppong-Mensah B, Phiri N, Pratt C, Silvestri S, Witt A (2017) Fall armyworm: impacts and implications for Africa. Outlooks Pest Manag 28(5):196–201

Early R, González-Moreno P, Murphy ST, Day R (2018) Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. NeoBiota 40:25–50. https://doi.org/10.3897/neobiota.40.28165

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.2307/2408678

Fernandes ÉKK, Moraes ÁML, Pacheco RS, Rangel DEN, Miller MP, Bittencourt VREP, Roberts DW (2009) Genetic diversity among Brazilian isolates of *Beauveria bassiana*: comparisons with non-Brazilian isolates and other *Beauveria* species. J Appl Microbiol 107:760–774. https://doi.org/10.1111/j.1365-2672.2009.04258.x

Firake DM, Behere GT (2020) Natural mortality of invasive fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in maize agroecosystems of northeast India. Biol Control 148:104303. https://doi.org/10.1016/j.biocontrol.2020.104303

Goergen G, Kumar PL, Sankung SB, Togola A, Tamò M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in west and Central Africa. PLoS ONE 11(10):e0165632. https://doi.org/10.1371/journal.pone.0165632

Goettel MS, Poprawski TJ, Vandenberg JD, Li Z, Roberts DW (1990) Safety to nontarget invertebrates of fungal biocontrol agents. In: Laird M, Lacey LA, Davidson EW (eds) Safety of microbial insecticides. CRC, Boca Raton, pp 209–231

Guo J, Wu S, Zhang F, Huang C, He K, Babendreier D, Wang Z (2020) Prospects for microbial control of the fall armyworm *Spodoptera frugiperda*: a review. Biocontrol 65:647–662. https://doi.org/10.1007/s10526-020-10031-0

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Presented at the Nucleic acids symposium series, [London]: Information Retrieval Ltd, c1979-c2000, pp 95–98

Hardke JT, Temple JH, Leonard BR, Jackson RE (2011) Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). Fla Entomol 94:272–278. https://doi.org/10.1653/024.094.0221

Hruska A (2019) Fall armyworm (*Spodoptera frugiperda*) management by smallholders. CAB Rev 14:1–11. https://doi.org/10.1079/PAVSNNR201914043

Humber RA (2012) Identification of entomopathogenic fungi. In: Lacey LA (ed) Manual of techniques in invertebrate pathology, 2nd, edn. Academic Press imprint of Elsevier Science, Oxford, pp 151–187

Liu H, Skinner M, Brownbridge M, Parker BL (2003) Characterization of *Beauveria bassiana* and *Metarhizium anisopliae* isolates for management of tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae). J Invertebr Pathol 82:139–147. https://doi.org/10.1016/S0022-2011(03)00018-1

Meyling NV, Eilenberg J (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol Control 43:145–155. https://doi.org/10.1016/j.biocontrol.2007.07.007

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, New York, p 352

R Core Team (2021) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. https://www.R-project.org

Ramírez-Ordorica A, Contreras-Cornejo HA, Orduño-Cruz N, Luna-Cruz A, Winkler R, Macías-Rodríguez L (2022) Volatiles released by *Beauveria bassiana* induce oviposition behavior in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). FEMS Microbial Ecol 98:fiac114. https://doi.org/10.1093/femsec/fiac114

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84–98. https://doi.org/10.1080/15572536.2006.11832842

Rehner SA, Minnis AM, Sung G-H, Luangsa-ard JJ, Devotto L, Humber RA (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103:1055–1073. https://doi.org/10.3852/10-302

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Talaei-Hassanloui R, Kharazi-Pakdel A, Goettel M, Mozaffari J (2006) Variation in virulence of *Beauveria bassiana* isolates and its relatedness to some morphological characteristics. Biocontrol Sci Technol 16:525–534. https://doi.org/10.1080/09583150500532758

Tamura K, Stecher G, Kumar S (2021) MEGA 11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120

Tay WT, Meagher RL, Czepak C, Groot AT (2023) *Spodoptera frugiperda*: ecology, evolution, and management options of an invasive species. Annu Rev Entomol. https://doi.org/10.1146/annurev-ento-120220-102548

Teem JL, Alphey L, Descamps S, Edgington MP, Edwards O, Gemmell N, Harvey-Samuel T, Melnick RL, Oh KP, Piaggio AJ, Saah JR, Schill D, Thomas P, Smith T, Roberts A (2020) Genetic biocontrol for invasive species. Front Bioeng Biotechnol 8:452. https://doi.org/10.3389/fbioe.2020.00452

Van den Berg J, Britz C, du Plessis H (2021) Maize yield response to chemical control of *Spodoptera frugiperda* at different plant growth stages in South Africa. Agriculture 11:826. https://doi.org/10.3390/agriculture11090826

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

Withers AJ, Rice A, de Boer J, Donkersley P, Pearson AJ, Chipabika G, Karangwa P, Uzayisenga B, Mensah BA, Mensah SA, Nkunika POY, Kachigamba D, Smith JA, Jones CM, Wilson K (2022) The distribution of covert microbial natural enemies of a globally invasive crop pest, fall armyworm, in Africa: enemy release and spillover events. J Anim Ecol 91:826–1841. https://doi.org/10.1111/1365-2656.13760

Zhang Y, Yang X, Zhang J, Ma M, He P, Li Y, Wang Q, Tang X, Shen Z (2022) Isolation and identification of two *Beauveria bassiana* strains from silkworm, *Bombyx mori*. Folia Microbiol 67:891–898. https://doi.org/10.1007/s12223-022-00986-1

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