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Genetic diversity of an entomopathogenic fungi collection from Ecuador using a modified AFLP approach

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Abstract

The genetic diversity of 41 Ecuadorian entomopathogenic fungal strains plus one isolate from the USA, from a collection maintained by INIAP (Instituto Nacional Autónomo de Investigaciones Agropecuarias) was determined using a modified AFLP (Amplified Fragment Length Polymorphism) approach. We found genetic similarity indexes lower than 0.50 and 121 polymorphic bands. The AMOVA analysis revealed that between-group and within-group variation contributed in similar amounts (59% and 41%, respectively) to the whole genetic variation detected. The dendrogram built using Jaccard's genetic coefficient shows twelve groups, where seven of them contain isolates clustered by genus. From these seven groups, five of them contain isolates clustered by the host from which they were recovered. The Bootstrap values show twelve reliable phylogenetic relations with values higher than 70% of confidence. The Principal Components Analysis (PCA) produced six clusters; four of them contain isolates associated by genus. The results suggest the existence of a considerable genetic diversity within the INIAP's entopathogenic fungi collection, and a clustering tendency related to the host from which they were isolated. We did not find common genomic regions among the most virulent entomopathogenic fungi strains. The high genetic diversity found within this collection represents a potential source of genotypes with potent bioinsecticide activity.

Keywords. Biocontrol, Genetic Diversity, AFLP, Entomopathogenic fungi, *Beauveria*, *Metarhizium*, *Verticillium*

Resumen

Se determinó la diversidad genética de 41 cepas ecuatorianas de hongos entomopatógenos y un aislamiento de los EEUU, de una colección mantenida por el INIAP (Instituto Nacional Autónomo de Investigaciones Agropecuarias) utilizando un protocolo modificado de AFLP (Amplified Fragment Length Polymorphism). Se encontraron índices de similitud genéticos inferiores a 0.50 y 121 bandas polimórficas. El análisis AMOVA reveló que la variación entre grupos y dentro de grupos contribuían en cantidades similares (59% y 41% respectivamente) a la variación genética total detectada. El dendograma construido a partir del coeficiente genético de Jaccard muestra doce grupos, de los cuales siete contienen aislamientos agrupados por género. De estos siete grupos, cinco contienen aislamientos agrupados en base al huésped de donde se recolectaron. Los valores de Bootstrap muestran doce relaciones filogenéticas confiables con valores de confianza mayores al 70%. El análisis de componentes principales (PCA) produjo seis grupos; cuatro de ellos contienen aislamientos asociados por género. Estos resultados sugieren la existencia de una diversidad genética considerable dentro de la colección de hongos entomopatógenos del INIAP, y una tendencia de agrupamiento relacionada con el huésped de donde fueron aislados. No se encontraron regiones genómicas comunes dentro de las cepas más virulentas de hongos entomopatógenos. La elevada diversidad genética dentro de esta colección representa una fuente potencial de genotipos con actividad bioinsecticida potente.

Palabras Clave. Biocontrol, Diversidad Genética, AFLP, Hongos entomopatógenos, *Beauveria*, *Metarhizium*, *Verticillium*

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Introduction

Entomopathogenic fungi are microorganisms capable of controlling pest insects that affect economically important crops worldwide. Spores of entomopathogenic fungi germinate and grow upon the cuticles of vulnerable hosts, and both physical and enzymatic activities produce the breaking of insect cuticles. This process allows the internal colonization of the insects by the fungus which ultimately causes the insects death [1].

Beauveria, *Metarhizium* and *Verticillium* are the most widely used entomopathogenic fungi genera due to their high effectiveness as biopesticides. The two main *Beauveria* species, *B. bassiana* and *B. brongniartii*, are frequently employed to control coleopteran and lepidopteran pests [2]; *Metarhizium anisopliae* is usually used to control members of the Isoptera, Orthoptera, Hemiptera and Coleoptera families [3]. Finally, *Verticillium lecanii* is an important fungal control agent of whiteflies and aphids [4]. In general, *Beauveria* and *Metarhizium* species have a wide range of possible hosts; however, particular strains or genotypes usually exhibit a more restricted host range [2, 3].

Benefits of entomopathogenic fungi usage include: little environmental impact, slight detrimental effects on plants and animals, minor effects on soil microbiota and little risk to human health [2, 3]. The economic potential of entomopathogenic fungi as biocontrol agents in agriculture has been evidenced by successful experiences on biological pest management since the 1970s. China, for instance, used *B. bassiana* in about 1.05 million hectares until the 1980s. Brazil also used this strategy on approximately one million hectares which were treated, in 2008, with *M. anisopliae* for controlling spittlebug [5].

The availability of indigenous virulent fungal isolates is a determining factor for the success of new biocontrol programs, and therefore the collection of indigenous strains and the determination of their virulence, are typically starting points for a biological pest management implementation [5].

Nowadays, the determination of the genetic diversity of fungal strains has also become a regular practice in biocontrol research, given that the natural variability is the main source of new genotypes with biopesticide potential. Molecular techniques have become frequent analysis methods, since it is now well-known that morphological characters such as conidia's size and shape are often not enough to reveal differences among species and/or strains [6]. Numerous studies have used molecular markers and DNA-sequence analysis to evaluate the polymorphisms in entomopathogenic fungi and to investigate possible correlations between genotype and geographical origin, pathogenicity or host range [1, 7–11].

Ecuador, a megadiverse country, could benefit from the use of entomopathogenic fungi in crop pest control. Consistent with this idea, INIAP (Instituto Nacional Autónomo de Investigaciones Agropecuarias) has established a collection of indigenous strains mostly isolated from coleopteran species in Ecuadorian highlands. Most of these isolates have already been morphologically classified and tested in terms of pathogenicity against *Premnotrypes vorax*, *Macrodactylus pulchripes*, and *Metamasius hemipterus*; frequent pests of potato, maize and sugarcane, respectively [12–16]. In this study, we employed a modified AFLP approach to evaluate the genetic diversity of an Ecuadorian entomopathogenic fungi collection mainly composed by Beauveria and Metarhizium isolates.

Materials and Methods

Fungal isolates

Forty-two monosporic entomopathogenic fungal isolates (Table 1) from a collection of INIAP were used in this study. These isolates represent four different genera (*Beauveria* (26), *Metarhizium* (9), *Verticilium* (2) and *Candida* (1)), plus four unidentified isolates. Forty one of these isolates came from different locations in Ecuador and were isolated from corresponding host insects, and one isolate (V037) came from the United States (Ohio University collection). All but four isolates (U058, U060, U061 and U062) were identified at the genus level using morphological descriptors by INIAP's researchers. Each isolate was assigned a code consisting in the initial letter of the genus, followed by an ordinal number.

DNA Extraction from fungal mycelium

To increase the amount of mycelium available for DNA extraction, all fungal isolates were grown for one week in PDA (Potato Dextrose Agar) medium supplemented with gentamicin (40 mg/L). Agar plugs of 5 mm diameter were taken from the edge of growing mycelium and transferred to a fresh Petri dish containing PDA medium. The plates were incubated at 28°C. Genomic DNA was isolated using a CTAB-based protocol [17] with the addition of Sand White Quartz (Sigma) during mycelium maceration (1:3 ratio) to efficiently break the fungal cell walls. One hundred and fifty milligrams of fungal mycelium from each isolate yielded 30-170 ng/μl DNA, estimated by using a Qubit™ fluorometer (Invitrogen) and a Quant-iT™ dsDNA BR Assay quantification kit (Invitrogen).

Modified AFLP analyses

A modified AFLP approach based on the AFLP Analysis System 1 (Invitrogen) was used to evaluate the genetic diversity of the fungal collection. Due to the reduced number of bands (3-8) generated by PCR amplification using several combinations of selective AFLP primers, which was attributed to the small fungal genome size (*Beauveria bassiana*: $\sim$40 Mbp, *Metarhizium anisopliae*: $\sim$30 Mbp) [18, 19], the analysis was based on a double PCR amplification approach using the preselective combination of EA/MC primers. This strategy produced clear and informative fingerprint patterns for all DNA samples analyzed (Fig. 1).

Isolate code	Fungus Species	Host	Origin	
			Locality	Province
M001	*Metarhizium sp.*	*Macrodactylus sp.* adult	Alance	Pichincha
M004	*Metarhizium sp.*	*Scarabaeidae pupa*	Alobuela	Pichincha
M005	*Metarhizium sp.*	*Macrodactylus sp.* adult	Alobuela	Pichincha
M007	*Metarhizium sp.*	*Macrodactylus sp.* adult	Alobuela	Pichincha
M010	*Metarhizium sp.*	*Scarabaeidae* larva	El Carmen	Bolívar
M032	*Metarhizium sp.*	----------	Hilsea (Floriculture Company)	Pichincha
M036	*Metarhizium sp.*	*Premnotrypes vorax* larva	Santa Marta de Cuba	Carchi
M100	*Metarhizium sp.*	*Scarabaeidae* larva	Tablas	Bolívar
M101	*Metarhizium sp.*	*Scarabaeidae* larva	San Franc. de Asapi	Bolívar
B012	*B.brongniarti*	*Macrodactylus sp.* adult	San José de Minas	Pichincha
B013	*Beauveria sp.*	*Macrodactylus sp.* adult	Alance	Pichincha
B014	*Beauveria sp.*	*Macrodactylus sp.* adult	El Carmen	Bolívar
B015	*Beauveria sp.*	Unidentified scarab	Alance	Pichincha
B016	*Beauveria sp.*	*Premnotrypes vorax* larva	Chanchaló	Cotopaxi
B017	*Beauveria sp.*	*Premnotrypes vorax* larva	San. José de Huaca	Carchi
B018	*Beauveria sp.*	*Premnotrypes vorax* larva	San Cristobal Island	Galápagos
B021	*Beauveria sp.*	*Premnotrypes vorax* larva	San Francisco	
B022	*Beauveria sp.*	*Premnotrypes vorax* larva	Trebon Guabug	Chimborazo
B023	*B.brongniarti*	*Premnotrypes vorax* larva	----------	----------
B024	*Beauveria sp.*	*Premnotrypes vorax* larva	E. E. Santa Catalina	Pichincha
B025	*Beauveria sp.*	*Premnotrypes vorax* larva	Yacubamba	Cotopaxi
B026	*Beauveria sp.*	*Premnotrypes vorax* larva	4 esquinas	Chimborazo
B028	*Beauveria sp.*	*Premnotrypes vorax* larva	Huacona San José	Chimborazo
B029	*Beauveria*	*Premnotrypes vorax* larva	Sablog	Chimborazo
B030	*Beauveria sp.*	*Coleop/Sthaphilidae*	Tumbaco	Pichincha
B031	*Beauveria sp.*	*Premnotrypes vorax* larva	Pull Chico	Pichincha
B043	*Possible Beauveria*	*Scarabaeidae* adult	Río Negro	
B102	*Beauveria sp.*	*Macrodactylus sp.* adult	Chontapamba	
B103	*Beauveria sp.*	*Premnotrypes vorax* adult	Cotojuan	Chimborazo
B104	*Beauveria sp.*	*Premnotrypes vorax* larva	----------	----------
B105	*Beauveria sp.*	*Scarabaeidae larva*	Jhashi	Bolívar
B106	*Beauveria sp.*	*Scarabaeidae larva*	S. F. Asapi	Bolívar
B107	*Beauveria sp.*	*Premnotrypes vorax* adult	Shaushi	Tungurahua
B109	*Beauveria sp.*	*Premnotrypes vorax* larva	Huacona San José	Chimborazo
B110	*Beauveria sp.*	Un-identified scarab	Alance	Pichincha
V037	*Verticilium lecannii*	----------	Ohio University	USA
V038	*Verticilium lecannii*	----------	Universidad Central del Ecuador	Pichincha
C040	*Candida sp.*	Soil Isolate	----------	Galápagos
U058	----------	*White Fly nimph*	Mascarilla V. Chota	Imbabura
U060	----------	*White Fly nimph*	Mascarilla V. Chota	Imbabura
U061	----------	*White Fly nimph*	Mascarilla V. Chota	Imbabura
U062	----------	*White Fly nimph*	EESC Cutuglagua	Pichincha

* Except for isolate V037, all strains used in the study were isolated in Ecuador by INIAP (Instituto Nacional Autónomo de Investigaciones Agropecuarias). Isolate code consists of the initial of the fungal genus followed by an ordinal number.

Table 1: Passport information of the INIAP's entopathogenic fungal isolates used in this study.

DNA digestion, adapter's ligation and preamplification reactions were performed according to the AFLP Kit manufacturer's instructions. A second amplification of the preamplified DNA samples using the preselective AFLP primer combination EA/MC was performed to increase banding intensity on the polyacrylamide gel. Products were resolved on a 6% polyacrylamide gel in 1X TBE and visualized by staining with $AgNO_3$ following the protocol described by Benbouza et al [20].

Statistical analyses

Banding patterns were treated as binary data (each band in an isolate was scored as 1 if present, or 0 if absent). A similarity matrix, an UPGMA dendrogram and a principal component analysis (PCA) comparison matrix were performed using Jaccard's coefficient in the NT-SYSpc (Ver. 2.0) software (Exeter Software, Setauket, NY). A bootstrap analysis, using the WINBOOT software (Ver, 2.0) (IRRI, Manila, Philippines), was also performed to evaluate the robustness of the phylogenetic relationships found. Finally, an analysis of molecular variance (AMOVA) was carried out to calculate genetic variation between and within groups of the UPGMA dendrogram by using the GENALEX 6 Software (School of Botany and Zoology, The Australian National University, Canberra, Australia).

Results

Genetic Diversity and Clustering

Double PCR amplification using the AFLP preselective primer combination EA/MC produced a total of 121 scorable bands which were 100% polymorphic for the whole fungal collection analyzed. The number of bands per genus was as follows: *Beauveria* (105 bands), *Metarhizium* (44 bands), *Verticilium* (20 bands), *Candida* (16 bands) and four unidentified fungal strains recovered from white flies (Aleyrodidae) (21 bands). All bands observed within each one of the four genera analyzed were also 100% polymorphic for all strains of the corresponding genus. For example, each one of the 44

Figure 1: Fingerprint patterns of 12 entomopathogenic fungal isolates, generated by using the preselective primer combination EA/MC, and visualized by silver staining. A considerable homogeneity among DNA profiles of Metarhizium isolates (lanes I = M001, J = M005, K = M007 and L = M010) and two of the Beauveria isolates (lanes A = B030 and B = B031), is shown. The rest of lanes correspond to C = B029, D = V037, E = B106 F = M101, G = B104, H = B103.

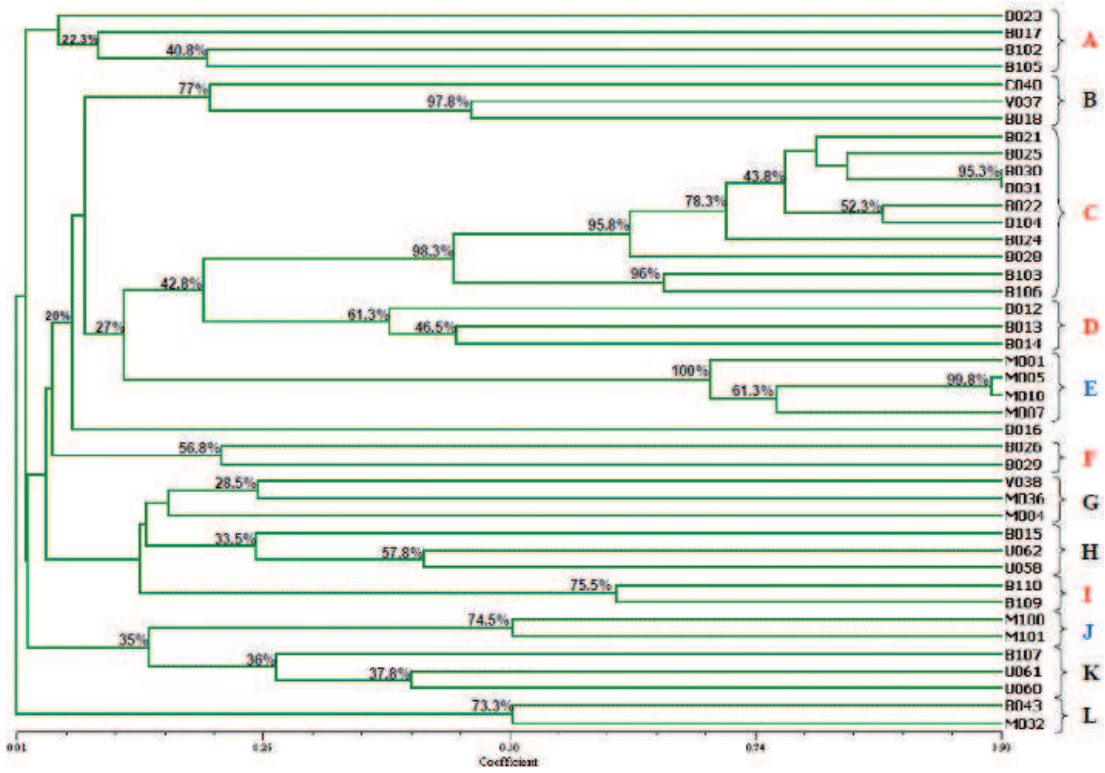

Figure 2: Dendrogram based on Jacccard's similarity coefficient for 41 Ecuadorian entomopathogenic fungal isolates and one isolate from the USA. UPGMA clustering produced twelve well-defined groups (A - L), seven of them associated by genera (A, C, D, E, F, I, J). Five groups, out of twelve, are associated both by genera and host preference (C, D, E, F, J). Numbers on branches indicate bootstrap (%) support for 400 replicates. The bootstrap analysis shows twelve trustworthy phylogenetic relations with values higher than 70% of confidence.

bands displayed by the *Metarhizium* isolates analyzed in this study was absent at least in one isolate from this genus. The genetic similarity indexes were lower than 0.50 for most of the phylogenetic relationships between strain pairs. Based on cluster analysis by the UPGMA method, twelve groups were obtained; seven of them contained isolates clustered by genus (Fig. 2). From these seven groups, five contained isolates also clustered by the host from which they were recovered. According to the AMOVA analysis, within-group differentiation and between-group differentiation accounted for 41% and 59% (prob.: 0.591) of the whole variation observed, respectively.

Five groups (A, C, D, F, I), out of twelve defined, are constituted exclusively by *Beauveria* isolates. Based on the number of isolates included, group C constitutes a major cluster (10 isolates) whereas groups A, D, F, & I are minor clusters (2-4 isolates). Group C contains isolates recovered from *Premnotrypes vorax* larvae (B021, B022, B024, B025, B028, B031, B103 & B104) with two exceptions, B030 and B106. The highest levels of genetic similarity (Jaccard's coefficient) among *Beauveria* isolates of the collection were showed by strain pairs within this cluster: B022 & B104 (0.86), B025 & B030 (0.84), B025 & B031 (0.82), and B030 & B031 (0.976). This last pair represents isolates from two different hosts in two distinct locations (Tumbaco and Pull Chico, Pichincha – Ecuador). Group A contains isolates obtained from *Premnotrypes vorax* larvae (B017, B023), a *Macrodactylus sp.* adult (B102) and a *Scarabaeidae* larva (B105). Group D is constituted by strains B012, B013 & B014; all of them recovered from *Macrodactylus sp* adults. Group F contains isolates B026 and B029, both of them recovered from *Premnotrypes vorax* larvae. Finally, Group I is constituted by two strains; B110, isolated from an unidentified beetle (Coleoptera), and B109, recovered from a *Premnotrypes vorax* larva. B016, also obtained from a *Premnotrypes vorax* larva, appears as an independent lineage not closely related to the rest of the *Beauveria* groups (Fig. 2).

Even though all *Metarhizium* isolates are not clustered together in a unique group, there are two well defined groups, E and J, constituted exclusively by *Metarhizium* strains. Group E enclosed four fungal isolates, three of them (M001, M005, M007) recovered from *Macrodactylus sp.* larvae, and one (M010) obtained from a *Scarabaeidae* larva. Interestingly, despite isolates M005 & M010 were recovered from different hosts in different locations (Alobuela and El Carmen, respectively), they presented the highest level of genetic similarity (0.97) among isolates of this cluster. Isolates M005 & M007 share the same site of collection (Alobuela, Pichincha – Ecuador) and both were isolated from *Macrodactylus sp.* larvae, but they exhibit lower genetic similarity (0.74) than isolates M005 & M010. Finally, Group J consists of two strains, M100 and M101, which were

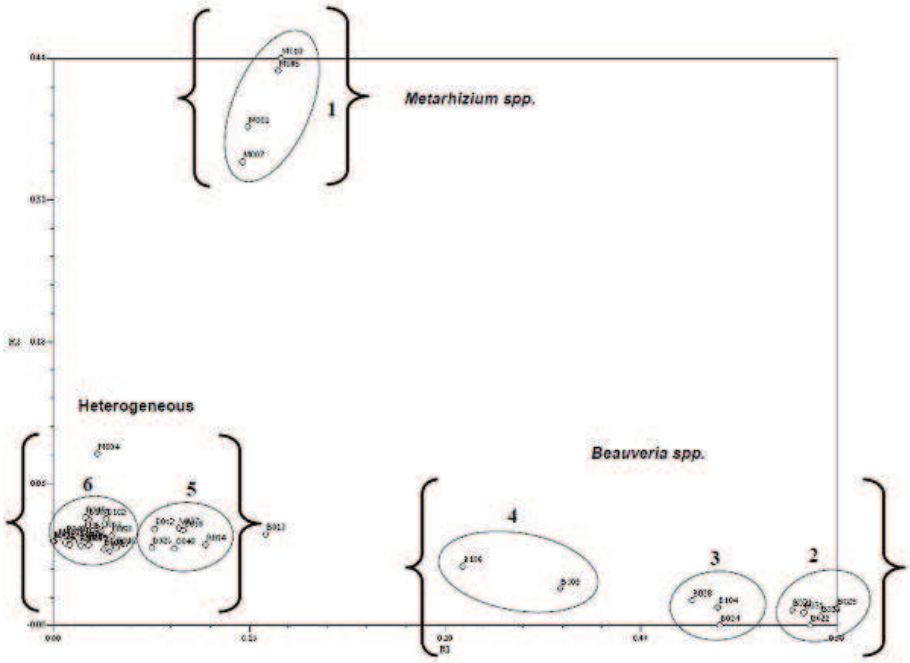

Figure 3: Principal component analysis on 41 Ecuadorian fungal isolates and one isolate from the USA based on preamplification AFLP data. Group 1 is composed exclusively by Metarhizium isolates, whereas groups 2, 3 and 4 include Beauveria isolates only. Groups 4 and 6 are heterogeneous sets which enclose different genera isolates.

isolated from *Scarabaeidae* larvae.

The two *Verticilium lecanii* strains included in this study have been assigned to separate groups (B and G) of the dendrogram. Group B is a heterogeneous cluster formed by three fungal isolates belonging to three different genera; V037 (*Verticilium lecanii*), C040 (Candida) and B018 (*Beauveria*). B018 and C040 share the same site of origin, the Galapagos Islands, but belong to different genera and are genetically dissimilar (0.21 similarity). Group G enclosed the second *Verticilium lecanii* strain (V038), and two *Metarhizium* isolates (M004, M036). M004 and M036 were recovered from different host species in Alobuela (Pichincha, Ecuador) and Santa Martha de Cuba (Carchi, Ecuador), respectively.

Fungal isolates which lack morphological identification are placed in groups H (U058, U062) and K (U060, U061), associated with two *Beauveria spp*. strains; B015 for Group H and B107 for Group K. The genetic similarity among isolates conforming clusters H and K is considerably low, in both cases lower than 0.31. Finally, different genera strains M032 (textitMetarhizium) and B043 (textitBeauveria) are grouped together in Group L.

It is important to observe that the geographical origin of the strains from the INIAP's collection is diverse; thus, UPGMA grouping does not suggest a correlation between the origin of the host insects and the phylogenetic relationships between the fungal strains isolated from them.

The bootstrap analysis performed showed twelve reliable phylogenetic relationships, seven of them with values higher than 90% of confidence, and the remaining five with values above 70% (Fig. 2).

PCA dispersion Analysis and Fungal Virulence

The two-dimensional PCA dispersion plot matches to a great extent the results produced by the dendrogram generated by the UPGMA cluster analysis. Four groups, out of six defined, can be easily related to the UPGMA dendrogram; Group 1 (formed exclusively by *Metarhizium* isolates) corresponds to Group E from the dendrogram, and collectively groups 2, 3 and 4 of the PCA dispersion plot (formed exclusively by *Beauveria* isolates) corresponds exactly to Group C from the dendrogram (Fig. 3). The remaining two groups of the PCA dispersion plot, 5 and 6, are heterogeneous sets formed by varied fungal isolates (*Beauveria*, *Metarhizium*, *Verticilium*, and *Candida*) (Fig. 3).

Although B018, M005 and M101 are typified by the INIAP researchers as the most virulent fungal isolates of the collection, they did not group together in the PCA dispersion plot. Also, we did not find common genomic regions (bands) exclusively present among these virulent entomopathogenic fungi strains.

Discussion

We found high genetic diversity within the entomopathogenic fungal collection analyzed (121 polymorphic bands). The AMOVA analysis established that between-group variation (59%) was only slightly higher than within-group variation (41%) of the strains. These data suggest that the majority of groups associated by genera are probably conformed by more than one species of

the same genus. Fungal strains of most of the groups in the dendrogram showed genetic similarity indexes lower than 0.50, except for group C (*Beauveria*), E (*Metarhizium*.) and I (*Beauveria*), where strains displayed indexes above 0.50 in most cases, and even over 0.70 for some strain pairs (Fig. 2). Group C, besides being a genetically homogeneous cluster, is almost entirely constituted by *Beauveria* strains recovered from *Premnotrypes vorax*. These results resemble to those of a PCR-RAPD genotyping study of *B. bassiana* [21], where 80% of 276 bands obtained were common to all strains recovered from *Diatraea saccharalis*. In contrast, while groups A, D and F are clusters completely comprised by *Beauveria* isolates, they exhibit low genetic similarity between strains (indexes below 0.50). Specifically, group A is a genetically dissimilar cluster which encloses isolates recovered from different coleopteran hosts (*Premnotrypes vorax*, *Macridactylus*, and an unidentified Scarabid), and groups D and F are clusters conformed by genetically dissimilar isolates which share the same host of origin. The data from group A resemble the results of a study carried out using PCR-RAPD and RFLP markers [22] where high genetic dissimilarity was revealed among *B. bassiana* strains derived from different coleopteran species. The heterogeneity shown by the rest of groups of the dendrogram (constituted by *Metarhizium*, *Verticillium*, *Candida* and four un-identified strains) might explain the low genetic similarity indexes found among their strains.

In the present study, five groups (C, D, E, F and J) were associated by genus and by their host of origin; however, the existence of a strict association between a particular entomopathogenic fungal strain and the host insect of which it came from seems to be improbable. Several publications [1–3, 23, 24] have pointed out the capability of entomopathogenic fungi to exhibit cross-infectivity. Our results show some correlation between genus and host of origin, suggesting a relative ability of particular fungal strains to more easily infect certain host insects. Indeed, B030 & B031 from groups C (*Beauveria sp.*) and M005 & M010 from Group E (*Metarhizium sp.*), which turned out to be highly genetically similar, were recovered from different host insects, evidencing exceptions to this general tendency even within host-associated groups. The high genetic similarity (0.97) between isolates M005 and M010, which were obtained from distinct locations besides of coming from different hosts, is also not a rare outcome as evidenced by other reports [7, 25, 26]. The opposite phenomenon was also evidenced in this study and has been previously documented as well by other studies [21, 27]; isolates M005 and M007 which came from the same host insect (*Macrodactylus sp.* adults) and location (Alobuela, Pichincha) showed less genetic similarity between them (0.74) than other strain pairs analyzed in this study.

Similarly to what was found in this study, Poprawski et al. [7] and Mugnai et al. [8] found apparent correlations between genotype and host preference using isoenzymatic markers. Other studies performed using RFLP markers and ITS region-analysis [9, 10] have also established correlations between molecular variation of *Beauveria* strains and their host range. Interestingly, Bidochka and Small (Bidochka and Small 2005 in Zimmermann 2007 [2]) concluded, based on population genetics studies, that an association of *M. anisopliae* genotypes occurs with host insect preference in tropical and subtropical regions and with habitat type in temperate and polar regions. Although almost all isolates from INIAP's collection came from Ecuadorian highlands, Ecuador is geographically located in the tropics; therefore, this conclusion seems to agree with our results. Despite these evidences, other studies have found no associations between genotype and host preference or geographic origin. For instance, *Bidochka et al*. [26] did not find evidence of clustering by host preference in *M. anisopliae* and *M. flavoviride* using PCR-RAPD markers. Analogously, Coates et al. [28] reported insufficient statistic evidence to correlate the BbMin1 minisatellite sequence, employed to analyze a *B. bassiana* collection, neither with host preference nor with geographic origin.

As stated earlier, the most virulent fungal strains of the collection (B018, M005 and M101) were genetically dissimilar. So far, a clear correlation between genotype and pathogenicity has not been established due to incongruous results. Several studies have found high genetic similarity among virulent isolates of the same species [9, 11, 29], whereas others have demonstrated considerable genetic variation among virulent fungal strains of the same species affecting a certain host insect [27, 30]. To explain the genetic homogeneity among certain virulent strains, some researchers have proposed virulence may be the result of minor variations of a common genotype [11]. Alternatively, others have interpreted these findings as the capacity of specific fungal genotypes to infect a particular host species [9]. In contrast, the genetic dissimilarity found among other virulent fungal isolates recovered from a particular host species has been explained as the presence of two or more infective strains living in sympatry [27]. The phylogenetic divergence found between B018 (*Beauveria*) and M005 & M101 (*Metarhizium*) is reasonably expectable because of the different taxa they belong to. However, the lack of grouping between virulent strains M005 and M101 suggests they are probably different *Metarhizium* species capable of infecting Scarabaeidae hosts, and therefore, their genomes differ considerably. Other studies [21] have reported dendrogram grouping of virulent entomopathogenic strains, hypothesizing the sharing of a genetic component related to pathogenicity among them. Our virulent isolates (B018, M005 and M101) did not share distinctive bands, and did not group together neither in the dendrogram nor in the PCA dispersion plot. These results may suggest virulence has different genetic components in each strain or maybe the technique used in our analysis could not reveal the common genomic region responsible for virulence among these strains.

A possible explanation for the grouping of B018 (*Beauveria sp.*) and C040 (*Candida sp.*) into the same cluster (Group B) is the sharing of homologous genomic regions related to their surviving capability in the Galapagos Islands' ecosystems with specific environmental conditions. Additionally, the grouping of unidentified isolates (U058, U060, U061 and U062) with two Beauveria strains (B015 and B107) in the dendrogram may suggest these isolates belong to the genus *Beauveria* or to another entomopathogenic genus closely related to it. Also, the genetic similarity index between M032 (*Metarhizium*) and B043 (*Beauveria*) was 0.50, suggesting a morphological identification error of B043, whose genus was suggested by INIAP to be *Beauveria*. This last observation is a possible explanation for the lack of grouping between the two *Verticillium lecanii* strains and the two *B. brongmiartii* strains present in our collection. Finally, it is important to observe that the lack of correspondence among some of the results from several studies regarding phylogenetics and correlations genotype-geographic origin, genotype-host preference and genotype-virulence is probably due to the using of different DNA-analysis techniques, which may screen different genomic regions, generating diverse outcomes [31].

The results from this study demonstrate that the modified AFLP technique described in this paper was effective to evaluate the genetic diversity of the INIAP's entomopathogenic fungi collection. The technique detected high genetic diversity among the isolates and a correlation between some isolates and their host preference. The data generated in this study encourages further research on Ecuadorian native entomopathogenic fungi, where virulent genotypes that could be used in biological pest management could be found.

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