Mitochondrial evolution in the entomopathogenic fungal genus *Beauveria*

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Abstract
Species in the fungal genus *Beauveria* are pathogens of invertebrates and have been commonly used as the active agent in biopesticides. After many decades with few species described, recent molecular approaches to classification have led to over 25 species now delimited. Little attention has been given to the mitochondrial genomes of *Beauveria* but better understanding may lead to insights into the nature of species and evolution in this important genus. In this study, we sequenced the mitochondrial genomes of four new strains belonging to *Beauveria bassiana*, *Beauveria caledonica* and *Beauveria malawiensis*, and compared them to existing mitochondrial sequences of related fungi. The mitochondrial genomes of *Beauveria* ranged widely from 28,806 to 44,135 base pairs, with intron insertions accounting for most size variation and up to 39% (*B. malawiensis*) of the mitochondrial length due to introns in genes. Gene order of the common mitochondrial genes did not vary among the *Beauveria* sequences, but variation was observed in the number of transfer ribonucleic acid genes. Although phylogenetic analysis using whole mitochondrial genomes showed, unsurprisingly, that *B. bassiana* isolates were the most closely related to each other, mitochondrial codon usage suggested that some *B. bassiana*
isolates were more similar to *B. malawiensis* and *B. caledonica* than the other *B. bassiana* isolates analyzed.

**KEYWORDS**

*Beauveria*, biological control, entomopathogenic fungi, evolution, mitochondrial genome

1 | INTRODUCTION

*Beauveria* is a genus of anamorphic entomopathogenic fungi that occur over most of the world. Isolates have been recovered from many invertebrate species, especially from many orders of insects. The first described species was *Beauveria bassiana*, named after Agostino Bassi who used the fungus and the host silkworms to provide the first demonstration of germ theory in 1834 (Ainsworth, 1956). The broad host range of the entomopathogenic nature of *Beauveria* has led to a significant amount of research into the species, due to their potential for use as biological control agents (Adane et al., 1996; Fancelli et al., 2013; Nankinga & Moore, 2000).

Classification of *Beauveria* species has been problematic over the years, with the name *B. bassiana* applied to most occurrences. Originally, classification of the different species was based on the morphology of *Beauveria*, which was complicated by the lack of observable morphological differences. Various methods have since been used to define the described species. Morphological and biochemical analysis successfully distinguished six species, *Beauveria amorpha*, *Baptisia alba*, *Beauveria bassiana*, *Beauveria brongniartii*, *Brazieria velata* and *Beauveria vermiconia* (Mugnai et al., 1989). A further species, *Beauveria caledonica* was then described (Bissett & Widden, 1988), followed by the addition of *Beauveria malawiensis* in 2005 (Rehner & Buckley, 2005).

The advent of molecular approaches allowed for the application of phylogenetic methods in the taxonomy of *Beauveria*. The definitive taxonomy of 12 species (*Beauveria bassiana*, *Beauveria varroroae*, *Beauveria kipukae*, *Beauveria brongniartii*, *Beauveria australis*, *Beauveria asiatic*, *Beauveria amorpha*, *Beauveria pseudobassiana*, *Beauveria sungii*, *Beauveria caledonica*, *Beauveria vermiconia* and *Beauveria malawiensis*) was developed using a phylogenetic approach based on five genomic regions (Rehner et al., 2011). This study also redescribed *B. bassiana* to restrict the definition of the species. Further species have been added in recent years, with up to 26 species now recognized (Kepler et al., 2017, Khonsanit et al., 2020).

From a biological perspective, the *Beauveria* constitute a very interesting group. They have been well characterized as effective and widespread invertebrate pathogens, including applications as biological control agents (Meyling & Eilenberg, 2007; Scholte et al., 2004). As insect pathogens, *Beauveria* has a complex disease cycle. Invasion of the host insect is achieved by penetration of the cuticle by direct pressure from the germ tube of a germinated conidium (Pekrul & Grula, 1979). The insect cuticle is further disrupted by the production of enzymes by the fungus, such as extracellular proteases. Such enzymes allow *Beauveria* to enter the hemocoel as well as providing a nutrient source for proliferation (Hepburn, 2013). Successful colonization of the host insect is only achieved by successful evasion of host immune defense systems, such as by the production of hyphal bodies, in vivo, which lack a fully intact cell wall and, therefore, prevent recognition as “non-self” by the host (Bidochka & Khachatourians, 1987; Götz, 1991; Pendland et al., 1993). Insect death has been observed to occur following only minimal growth of the fungus. Therefore, it is the production of toxic secondary metabolites by *Beauveria* that are responsible for at least some insect mortality (Quesada-Moraga & Vey, 2003; Roberts, 1981).

*Beauveria bassiana* has also been described as an endophyte of a range of plants, including numerous crop species (McKinnon et al., 2017). Currently, the identified strains found in plants have been restricted to *B. bassiana sensu stricto* and a single report of *B. brongniartii* (Jaber & Enkerli, 2017), but whether the ability to colonise plants is restricted to this species is unclear. Strains recovered from plants remain directly pathogenic to insects (Vega et al., 2009) and
may, in some cases, have negatively impacts on insects while in a plant (McKinnon et al., 2017; Quesada-Moraga et al., 2009). Beauveria are also known to produce many active secondary metabolites, some of which are reported to have potential medical applications (Patocka, 2016).

The very different biological characteristics, such as range of hosts, occurrence, and endophytic ability, make the task of understanding genotypes and intraspecies variation particularly challenging, but also appealing. Very few studies have examined the mitochondria genomes of Beauveria species and the relationship between mitochondrial variation and genome variation is largely unknown. It is well known that the evolutionary rate of fungal mitochondrial DNA (mtDNA) is close to that of plants, which have low nucleotide substitution rates (Aguileta et al., 2014; Fonseca et al., 2020). The rate of evolution is based on the percentage of base substitutions observed in conserved genetic regions. Clark-Walker (1992) demonstrated that the percentage of substitution in mitochondrial genes was lower than that of nuclear genes, indicating that fungal mitogenomes may evolve more slowly compared to their respective nuclear genomes. Insights from recent genomic studies of other species demonstrate how fungal mitochondrial genomes can mutate at a different rate than nuclear genomes (Fonseca et al., 2020; Li et al., 2019; Ying et al. 2020). Fonseca et al. (2020) observed that among 34 mitochondrial genomes of fungal species compared within the order Hypocreales, there was significant variation in both structure and length. The number and size of noncoding DNA, such as Groups I and II introns and homing endonucleases genes (HEGs), were thought to be the main contributors to these differences. The authors concluded from their study that the mitochondrial genomes that were longer in length and contained longer noncoding region, were those that evolved more quickly.

Although not used often for phylogenetic studies in Beauveria, the mitochondrial genome (mtDNA) has some advantages for taxonomic and phylogenetic typing, such as intraspecific diversity, a small genome, lack of methylated bases, and high copy number (Uribe & Khachatourians, 2004). Early studies on a range of B. bassiana mtDNA showed only two mitochondrial genotypes (mitotypes), which they suggested indicated a highly conserved mitochondrial genome (Hegedus & Khachatourians, 1993). Subsequently, restriction digestion and probing with a single mtDNA region (BbmtE2) of a more diverse group of Beauveria, Uribe and Khachatourians (2004) identified a further three mitotypes. Whole mtDNA enzyme digestion profiles revealed nine mitotypes among the strains of Beauveria.

In this study, we describe the newly sequenced mitochondrial genomes of four Beauveria species. By comparing genomic markers, transfer RNA (tRNA) order and codon usage, a greater understanding of the relationship between mtDNA diversity and ecological niche in this genus can be gained.

2 | METHODS

2.1 | DNA preparation and sequencing

DNA was extracted from 1 to 2-day-old cultures grown in Sabourauds broth (Oxoid, UK) using the Plant Maxi Kit (Qiagen). Whole genomic DNA was sequenced with 100 base pairs (bp) paired end Illumina MiSeq technology by New Zealand Genomics Ltd (NZGL) and, in the case of B. caledonica, also using long reads from PacBio. Genomes were assembled into contigs using ABySS version 2 (Simpson et al., 2009). Four Beauveria strains, from three species, were sequenced (B. bassiana e17, B. bassiana k4, B. caledonica frh4, and B. malawiensis Bwetak89). All isolates are held in the Lincoln University culture collection (New Zealand; Table 1).

2.2 | Quality control and assembly

Low-quality sequences and sections of sequences of raw data for each sample were trimmed with SolexaQA++ version 3.0 using the dynamictrim option (Cox et al., 2010). Subsequently, reads less than 50 bp were removed with
SolexaQA++. To remove nuclear DNA sequences, cleaned reads were filtered by mapping with Bowtie2 version 2.2.5 against the *B. bassiana* genome assembly ARSEF2860 (GCA_000280675.1; Xiao et al., 2012). Unmapped reads were removed from the resulting sequence alignment/map (sam) file with SAMtools version 0.1.19 (Li et al., 2009; Li, 2010). The clean and filtered reads were pooled and normalized with a k-mer based approach targeting 40× coverage with BBnorm from BBTools version 15 (Bushnell 2015) and Illumina adapter sequences were removed with Trimmomatic version 0.32 (Bolger et al., 2014). Normalized reads were then assembled with Velvet version 1.2.10 (Zerbino & Birney, 2008). Each sample was assembled at varying kmers (the length of subsequences used in assembly) until a fully circular assembly was made. As a check, additional assemblies were made with kmers greater and smaller than the first kmer found to give a circular assembly, and the resulting mitochondrial genomes were checked against each other for convergence on size and composition.

### 2.3 Genome alignment and phylogeny

We downloaded all completely assembled mitochondrial genome sequences available from the Cordycipitaceae from GenBank. To root our tree, we downloaded outgroups from the Clavicipitaceae and Hypocreaceae (Table 1). Newly determined and existing mitochondrial genomes were aligned with Mauve version 2015 using the progressive alignment method and varying seeds (Darling et al., 2004). The alignment of mitochondrial genomes that was consistent between seeds was retained. An alignment of mitochondrial genomes was exported from Mauve and trimmed with Block Mapping and Gathering Entropy (BMGE) version 1.12 (Criscuolo & Gribaldo, 2010). Each sample was assembled at varying kmers (the length of subsequences used in assembly) until a fully circular assembly was made. As a check, additional assemblies were made with kmers greater and smaller than the first kmer found to give a circular assembly, and the resulting mitochondrial genomes were checked against each other for convergence on size and composition.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Strain</th>
<th>Accession</th>
</tr>
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<tr>
<td>Cordycipitaceae</td>
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<td>KT201149*</td>
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<td>k4</td>
<td>KT201148*</td>
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<td>Bb147</td>
<td>EU100742</td>
</tr>
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<td>BwetaK89</td>
<td>KT201147*</td>
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Note: Newly determined sequences are indicated with an asterisk (*).
2.4 | Annotation and comparative mitochondrial genomics

All genomes, including previously published ones, were annotated with MFannot (https://megasun.bch.umontreal.ca/RNAweasel/) with the fungal mitochondrial code specified. Where there was a difference between a published annotation and the results of MFannot, files were edited to correspond to the MFannot annotation. We compared preexisting Beauveria mitochondrial genome annotations to de novo annotations produced by MFannot to check the thoroughness of the MFannot webserver. Uncharacterized open reading frames detected by MFannot were annotated with Blast2GO version 3.0.10 (Conesa & Götz, 2008) by comparison to the NCBI protein reference sequence database with an expect value of $1 \times 10^{-3}$.

All tRNAs were confirmed using tRNAscan-SE version 2.0 (Lowe & Chan, 2016) and the Rfam RNA database (Griffiths-Jones et al., 2003). Comparative nucleotide alignments and codon usage were determined using Geneious version 6.1.8 (Biomatters Ltd).

2.5 | Genomic markers

Five genomic regions were used, based on the approach of Rehner et al. (2011), and the data for three type isolates (ARSEF 1565, 2567, and IMI228343) were retrieved from GenBank (Table S1). These sequences were used to find homologs in genomes sequences of Frh1, Bwetak89, K4, and e17. All new sequences have been submitted to GenBank (Table S1).

3 | RESULTS

3.1 | Annotation and comparative mitochondrial genomics

Assemblies of all four samples were determined to be completely circular and are 28.8–44.1 thousand bp in length (Table 2). All genes found in the newly sequenced genomes were also found in the previously described Beauveria genomes; the coverage and annotation of the eight Beauveria sequences are summarised in Figure 1. All Beauveria species contain 15 protein-coding genes, with variation in gene length and number of introns across species. Groups I and II introns and numerous unidentified open-reading frames were present in Beauveria mitochondrial genomes. Most of the open reading frames present in Beauveria mitochondrial genomes can be identified as GIY-YIG or LAGLIDADG endonucleases. Comparative analysis of the Beauveria mitochondrial genomes used in this study showed that they have 70% pairwise identity.

The low G–C content of the newly sequenced Beauveria (K4 = 27.1%, e17 = 27.1%, Bwetak89 = 26.7%, and Frh1 = 26.3%) is comparable to the low values of other Hypocreales (Table 3). The standard composition and gene

<table>
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<th>Length</th>
<th>Coverage</th>
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<td>Hylastes ater (Coleoptera), NZ</td>
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</table>

Abbreviation: NZ, New Zealand.
order of *Beauveria* mitochondrial genomes is shown in Figure 2, with *B. bassiana* Bb147 as an example. The genes encoded within the mitochondrial genomes are an apocytochrome b (*cob*), three ATP synthase subunits (*atp6*, *atp8*, and *atp9*), three c-oxidase subunits (*cox1*, *cox 2*, and *cox3*), seven NADH dehydrogenases (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), as well as the large and small ribosomal RNA (rRNA) subunits (*rnl* and *rns*; Figure 1). The newly sequenced mitochondrial genomes of *B. bassiana* e17 and K4, as well as *B. malawiensis* Bwetak89 and *B. caledonica* Fhr1 conform to the previously described gene order of *Beauveria* mitochondrial genomes (Figure 1). All of the genes of *B. bassiana* Bb13, Bb147, and *B. caledonica* Fhr1 are encoded on the positive strand, but *B. bassiana* e17, K4 and *B. malawiensis* BwetaK89 encode one open reading frame (ORF) each on the negative strand.

### 3.2 Genome alignment and phylogeny

Full-length alignment of the mitochondrial genomes produced an alignment of base pairs largely composed of two locally collinear blocks (LCBs) that occur in the same order. A third LCB was present in *B. caledonica* and *B. bassiana*.
<table>
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<th>Mitochondrial genome length</th>
<th>Number of introns</th>
<th>Combined length of introns</th>
<th>% Introns</th>
<th>Mitochondrial genome size without introns</th>
<th>%GC</th>
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<sup>a</sup>Sequenced during this study.
Bb147, which occurs as the last LCB in *B. caledonica* and between the first and second LCBs in *B. bassiana* Bb147. Trimming of the alignment with BMGE reduced the alignment to 20,544 characters from 84,484. The ML phylogeny of the mitochondrial genomes used in this study was calculated with bootstrap values (Figure 3). The separation of mitochondrial sequences of the genus *Beauveria* compared to the other Cordyciptaceae was clear and supported by a high bootstrap value. *Beauveria bassiana* isolates formed a strongly supported clade, compared to the other species of *Beauveria*. The other *Beauveria* spp. were less clearly separated using ML comparisons, with bootstrap values ranging from 57 to 87.

### 3.3 Intron relationships

Although the genes in the mitochondrial genome are conserved, the introns vary across the species in both occurrence and size. The size variation in mitochondrial genomes is largely dependent on intron number and length (Table 3). In all of the mitochondrial genomes considered here, the large subunit rRNA gene (*rnl*) contains an intron-encoded S3 ribosomal protein (Figures 1 and 2). In *Lecanicillium muscarium* and *Metarhizium anisopliae*, this is the only intron present. In comparison, *B. malawiensis* has a total of 13 introns making up 35% of the mitochondrial genome size. All *Beauveria* examined had at least two introns and a total of nine protein coding genes had at least one intron in at least one mitochondrial genome.
3.4 | Five chromosomal genes versus mitochondrial phylogenetics

Using five conversed genomic regions (Table S1), a phylogeny was generated, similar to that reported by Rehner et al. (2011; Figure 4). In comparison to the phylogenetic relationships found using the whole mitochondrial genome (Figure 3), some Beauveria species were aligned differentially. Chromosomally, B. bassiana, B. brongniartii and B. pseudobassiana were clustered, whereas on the basis of the mitochondrial genome, B. malawiensis was closer to B. bassiana than B. pseudobassiana. With genomic regions, B. malawiensis and B. caledonica clustered apart. With the mitochondrial genomes, B. caledonica was the outlying isolate of the Beauveria, whereas with genomic regions, B. malawiensis was more basal than B. caledonica.

3.5 | tRNA order

The tRNA gene order between the four new Beauveria strains was invariant, except for B. caledonica which had two additional tRNAs (G) between cox3 and NADH dehydrogenase subunit 6 before VISWP (Table 4).

The occurrence of tRNA genes within the mitochondrial genomes analysed showed that trnM, trnL, and trnR are present as multiple genes in all genomes (apart for M. chlamydosporia which contains a single trnL, which is absent completely from L. muscarium). The other tRNAs within the genomes (trnT, trnE, trnA, trnF, trnK, trnQ, trnH, trnY, trnD, trnN, trnG, trnV, trnI, trnS, trnW, and trnP) are only present as single copies, the exception being B. caledonica.
Fhr1 which contains three copies of trnG. Two tRNAs were absent in all of the mitochondrial genomes, trnB and trnZ.

The distribution of tRNAs was also analysed as some tRNAs group together within the genomes; four groups were identified. First, the TEM group is present in all of the genomes. The second group, LAFKLQHM, is also

**FIGURE 4** Five chromosomal gene phylogeny of *Beauveria* and related species. Numbers at nodes indicate bootstrap values generated from 1000 bootstrap replicates

**TABLE 4** tRNA gene order of *Beauveria* and other Hypocreales mitochondrial genome sequences

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<tr>
<th>Species</th>
<th>Strain</th>
<th>tRNA order</th>
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<tr>
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<td>TEMML AFKLQHMR CRYN SN G VISWP</td>
</tr>
<tr>
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</tr>
<tr>
<td><em>Lecanisillium muscarium</em></td>
<td></td>
<td>TEMML AFKLQHMR CRY DSN GL ISW</td>
</tr>
<tr>
<td><em>Metacordyceps chlamydospora</em></td>
<td>170</td>
<td>TEMML AFKLQHMR CRY DSN G VISWP</td>
</tr>
<tr>
<td><em>Trichoderma reesei</em></td>
<td></td>
<td>TEMML AFKLQHMR CRY DSN G VISWP</td>
</tr>
</tbody>
</table>

Abbreviation: tRNA, transfer RNA.
present within all Beauveria genomes apart from in B. caledonica. Thirdly, YDSN was identified in all of the studied isolates apart from B. bassiana Bb13 (YNSN), Bb147 (YDSA), and M. chlamydosporia. The final group, VISWP is the final five tRNAs in all species except M. chlamydosporia (VISP) and M. anisopliae (LISW). In all of the mitochondrial genomes, it was found that trnR was never present within a cluster of tRNAs, instead being present as singular tRNAs.

Between the four newly sequenced and other Beauveria mitochondrial genomes, there is great similarity in the nucleotide alignments of the trn genes. There was 100% similarity between the trnF, trnA, trnW, trnN, trnI, trnQ, and trnS genes of the five Beauveria species. The greatest difference was observed between the trnG genes. The trnG genes of B. bassiana e17 and K4 showed 100% similarity to one another, 98.6% with Bwetak89, whereas B. caledonica Fhr1 is only 77.5% similar. The trnL genes also showed variation.

3.6 | Codon usage

The codon usage of the protein encoding genes of all mitochondrial genomes were determined using translation Table 4 (Fox, 1987). Of the newly sequenced Beauveria, 72 codons were identified (Figure S3). The codons TGA (W), TAT (Y), AGA (R), CAA (Q), TTA (L), AAA (K), GAA (E) and Gat (D), and the start codon ATG are used at a high frequency in all of the mitochondrial genomes. As with many other fungal species, the codon usage in all species showed a strong bias to codons with A or T in the third position (Ghikas et al., 2006; Kouvelis et al., 2004; Lin et al., 2015).

As has been previously found, codon usage bias did not relate closely to species definition in this group of fungi. Generally, B. bassiana K4, B malawiensis Bwetak89 and B. caelondica Fhr1 showed similar patterns of codon usage, as seen for the amino acids alanine (A), glutamic acid (E), isoleucine (I), proline (P), arginine (R) and valine (V) (Figure 5), rather than the three B. bassiana isolates, although this was not pronounced. The codon usage within the mitochondrial genomes of all the species are summarised in Figure S2.

Stop codon usage was also variable between species. Beauveria bassiana K4, B. pseudobassiana, L. muscarium, C. militaris, and M. anisopliae ME1 use exclusively the preferred mitochondrial stop codon TAA (Paquin & Lang, 1996). The other genomes use mostly TAA but also use the alternative codon TAG. Conversely, in all mitochondrial genomes analysed, the start codons for the encoded proteins generally used the standard start codon ATG. The rnl and rns genes used alternative start codons in all cases.

4 | DISCUSSION

4.1 | Annotation and comparative mitochondrial genomics

Fungal mitochondrial genomes can be very large, up to 157 kb, and protein-coding genes can have multiple introns formed by homing endonucleases (Dean et al., 2014). These mobile elements are commonplace in fungal mitochondrial genomes (Hausner, 2012). Homing endonucleases are self-splicing (Mullineux et al., 2010) and may be intronic or free-standing (Saguez et al., 2000). Our results demonstrate the dynamic nature of these elements in fungal mitogenomes. The number and locations of introns vary substantially across our mitochondrial genomes. Moreover, there is little phylogenetic signal in the distribution of these elements, with the different B. bassiana strains having between two and six introns compared to 13 introns in the sister species B. malawiensis. These results suggest that the presence or absence of mitochondrial introns cannot serve as a taxonomic marker in themselves, and that care should be taken to remove these highly dynamic sequences from phylogenetic analyses. However, a recent study by Fonseca et al. (2020) estimated that there may be a correlation between the divergence time of each Hypocrealean fungal species they investigated and the quantity of coding and noncoding elements in the
mtDNA, in addition to the effect these elements have on the length of mitochondrial genomes between species. They showed that introns and HEGs are key components on mitochondrial genomes in that the presence of these elements indicates more rapidly evolving mtDNA, which could be partially explained by species divergence time, despite that the intron pattern shared between species suggests the involvement of other mechanisms of evolution, such as horizontal transference. Since in our study, we observed some differences in intron number between strains of *B. bassiana*, it may be inferred that, even within species, whole mitochondrial evolution can happen quite rapidly when noncoding elements are considered. Future studies could therefore address whether there is a correlation between mitochondrial introns, HEGs and mitogenome length, and within species evolutionary rates. This would likely require substantially more mitochondrial genomes for each species of interest to be sequenced and compared than is currently available.

The large number of introns observed in some of these mitochondrial genomes is consistent with other studies of these elements. The gene with the most introns in the *Beauveria* mitochondrial genomes, *cox1*, had as many as six introns. Up to nineteen introns have been reported in a fungal *cox1* gene (Férandon et al., 2010), with investigation of closely related species indicating substantial variability in mitochondrial genomes in terms of the number and placement of mobile open reading frames (Beaudet et al., 2013). For some of the currently analysed mitochondrial genomes, only a single intron was found, but in a study of six isolates of *Annulohypoxylon stygium* (Xylariaceae, Ascomycota) each mitochondrial genome contained 45 or more introns (Deng et al., 2018), potentially demonstrating the diversity of mitochondrial mutation rates that can occur in fungal species mtDNA.

Aguileta et al. (2014) examined 38 mitochondrial genomes across the fungi and found significant changes in gene order. As would be expected from our more limited comparisons, there were no differences in the order of the main mitochondrial genes. Aguileta et al. (2014) also found that the Sorariomycetes have the most conserved in gene order of all the fungi they examined. They found a correlation between gene order variability and more variation in the scattering of tRNAs, whereas tRNA distribution was fairly consistent in the current mitochondrial genomes examined.

### 4.2 Relationship between endophytic and other ecological niches

*Beauveria bassiana* (Brownbridge et al., 2012; McKinnon et al., 2017; Vega, 2008) and a single reported instance of *B. brongniartii* (Jaber and Enerki 2017) have been isolated as plant endophytes as well as insect pathogens. Phylogenetic analysis of the whole mitochondrial genome sequences did not group these species together, with *B. malawiensis* closer to *B. bassiana* than *B. brongniartii* (Figure 1). However, a phylogeny determined using five conserved genomic regions within the chromosomes grouped the *B. bassiana* strains with *B. brongniartii* 617, independently from the *B. malawiensis*, and *B. caledonica* strains. Previous phylogenetic analyses of *Beauveria* based on nuclear genes also place *B. brongniartii* closer to *B. bassiana* than the other species studied here (Khonsanit et al., 2020, Rehner et al., 2011). These conserved regions may provide insight into the evolution of the endophytic *Beauveria* species, and the insect pathogenic (but not endophytic) species *B. malawiensis* and *B. caledonica*. Such regions may therefore be utilised to determine the ecological niche of isolated *Beauveria* strains. In contrast, mitochondrial evolution does not appear to relate to niche or host.

**FIGURE 5** Codon usage for selected amino acids. (B_bas_E17 = *Beauveria bassiana*_E17; B_bas_EU100742 = *Beauveria bassiana*_EU100742; B_bas_EU371503 = *Beauveria bassiana*_EU371503; B_bas_K4 = *Beauveria bassiana*_K4; B_bro = *Beauveria brongniartii*; B_cae_frh1 = *Beauveria caelondica*_frh1; B_mal = *Beauveria malawiensis*; B_pseudobas = *Beauveria pseudobassiana*; C_mil = *Cordyceps militaris*; L_mus = *Lecanicillium muscarium*; M_ani = *Metarhizium anisopliae*; P_chl = *Pochonia chlamydosporia*; T_ree = *Trichoderma reesei*);
4.3 | Codon usage

It has been previously described that mtDNA has a stronger codon bias than genomic DNA (Wei et al., 2014). For all of the mitochondrial genomes used in this study, there was a strong codon bias to A and T, particularly at the third position of the codon. This may be expected due to the relatively high AT content of the mitochondrial genomes. The AT, or GC, content of a mitochondrial genome is known to influence codon bias and is known as a “mutational affect” (Bulmer, 1991; Sharp & Li, 1986). However, it has been demonstrated that natural selection effects, such as gene length and function, have greater influence in the codon bias of fungal mitochondrial DNA (Wei et al., 2014). Therefore, it may be that the codon usage of *Beauveria* species offers a more interesting insight into evolution than merely reflecting the GC content.

Interestingly, codon usage did not always reflect the species relationships, with strains of *B. bassiana* displaying patterns that differed from each other. *B. bassiana* K4, *B. malawiensis* Bwetak89 and *B. caledonica* Frh1 showed more similarity in codon use than the other *B. bassiana* to K4. Based on the data in this study, we could not conclude much based on the codon usage in evolutionary terms. A further, more detailed analysis, of codon usage within *Beauveria* will be required to gain greater evolutionary insight (Wei et al., 2014).

5 | CONCLUSIONS

We sequenced four *Beauveria* mitochondria genomes and compared the sequences to previously sequenced *Beauveria*, *Leccanicillium*, *Metarhizium*, *Cordyceps*, *Metacordyceps*, and *Trichoderma* mitochondria genomes. Gene arrangement was conserved among the *Beauveria*, but codon usage varied, as well as mt length. Intron occurrence was the main variant, accounting for most of the variations in length of the mitochondria. Although our dataset was limited to a few *Beauveria* spp., we observed a notable difference between phylogenetic trees conducted with the conventional multigene method, that uses nuclear genes, and the whole mitochondrial analysis. Future phylogenetic studies should, therefore, include nuclear and some mtDNA coding regions to reconcile these differences observed or to clarify species delimitation within the *Beauveria* genera.

AUTHOR CONTRIBUTION
Travis Glare: Conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (lead); investigation (lead); methodology (equal); project administration (lead); resources (equal); software (equal); supervision (equal); visualization (equal); writing-original draft (lead); writing-review & editing (lead). Matt Campbell: Data curation (equal); formal analysis (equal); investigation (equal); resources (equal); software (equal); visualization (equal). Patrick Biggs: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal). David Winter: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); validation (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal). Abigail Durrant: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); visualization (equal); writing-review & editing (equal). Aimee McKinnon: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); validation (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal). Murray Cox: Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing-original draft (equal); writing-review & editing (equal).

DATA AVAILABILITY STATEMENT
Supplementary material has been provided and sequences uploaded to GenBank.
REFERENCES


**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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