



HAL
open science

The integrative taxonomy of *Beauveria asiatica* and *B. bassiana* species complexes with whole-genome sequencing, morphometric and chemical analyses

Noppol Kobmoo, Nuntanat Arnarnart, Wirulda Pootakham, Chutima Sonthirod, Artit Khonsanit, Wilawan Kuephadungphan, Rinrada Suntivich, Tatiana E Giraud, Janet Jennifer Luangsa-Ard

► To cite this version:

Noppol Kobmoo, Nuntanat Arnarnart, Wirulda Pootakham, Chutima Sonthirod, Artit Khonsanit, et al.. The integrative taxonomy of *Beauveria asiatica* and *B. bassiana* species complexes with whole-genome sequencing, morphometric and chemical analyses. *Persoonia*, 2021, 47 (15), pp.136-150. 10.3767/persoonia.2021.47.04 . hal-03357214

HAL Id: hal-03357214

<https://hal.science/hal-03357214>

Submitted on 28 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 **The integrative taxonomy of *Beauveria asiatica* and *B. bassiana* species**
2 **complexes with whole-genome sequencing, morphometric and chemical**
3 **analyses.**

4

5 Noppol Kobmoo¹, Nuntanat Arnarnart¹, Wirulda Pootakham², Chutima Sonthirod²,
6 Artit Khonsanit¹, Wilawan Kuephadungphan¹, Rinrada Suntivich¹, Tatiana Giraud³,
7 Janet Jennifer Luangsa-ard¹

8

9 ¹ National Center for Genetic Engineering and Biotechnology (BIOTEC), National
10 Science and Technology Development Agency (NSTDA), 113 Thailand Science Park,
11 Phahonyothin Rd., Khlong Nueng, Khlong Luang, Pathum Thani 12120, Thailand.

12 ² National Omics Center, National Science and Technology Development Agency
13 (NSTDA), Pathum Thani, Thailand

14 ³ Ecologie Systématique Evolution, CNRS, AgroParisTech, Université Paris-Saclay,
15 Orsay, France.

16

17

18 **Abstract**

19 Fungi are rich in complexes of sibling species that need combinations of approaches
20 to be delimited, including with genomic information. *Beauveria* (Cordycipitaceae,
21 Hypocreales) is a well-known genus of entomopathogenic fungi, used as a biocontrol
22 agent. We present in this study a polyphasic taxonomy regarding two widely
23 distributed complexes of *Beauveria*: *B. asiatica* and *B. bassiana* sensu lato. Some of
24 the genetic groups as previously detected within both taxa were either confirmed or
25 fused using population genomics. High levels of divergence were found between two
26 clades in *B. asiatica* and between three clades in *B. bassiana*, supporting their
27 subdivision as distinct species. Morphological examination focusing on the width and
28 the length of phialides and conidia showed no difference between the clades within *B.*
29 *bassiana* while the length of conidia was significantly different between the clades
30 within *B. asiatica*. The secondary metabolites profiles obtained by liquid
31 chromatography-mass spectrometry (LC-MS) allowed a distinction between *B.*
32 *asiatica* and *B. bassiana*, but not between the clades therein. The maximum-likelihood
33 reconstruction of ancestral host association by mapping information of the hosts'
34 taxonomic order onto the whole-genome phylogeny established that the common

35 ancestor to *Beauveria* was predominantly associated with *Coleoptera* while the
36 common ancestors to one clade of *B. bassiana* was predominantly associated with
37 *Orthoptera*, and that of another clade in *B. asiatica* was partially associated with
38 *Lepidoptera*. Based on these genomic, morphological, chemical and ecological data,
39 we proposed a clade of *B. asiatica* as a new species, named *B. thailandica*, and two
40 clades of *B. bassiana* to be respectively *B. namnaoensis* and *B. neobassiana*. Such
41 closely related but divergent species with different host ranges have potential to
42 elucidate the evolution of host specificity with application in biocontrol.

43

44

45 **Introduction**

46 Fungi are rich in complexes of sibling species, i.e. morphologically similar but
47 genetically isolated. In pathogenic fungi, sibling species are often specialized on
48 different hosts (Le Gac et al. 2007; Giraud et al. 2010; Kobmoo et al. 2012), so that it
49 is essential to elucidate their genetic subdivision and the species limits. Taxonomy is
50 therefore an essential tool for understanding the evolution of host specificity in
51 pathogens and to evaluate the risk of attacking untargeted species for biocontrol
52 agents.

53 *Beauveria* (*Cordycipitaceae*, *Hypocreales*) is a well-known genus of
54 entomopathogenic fungi first discovered in the 18th century (Basalmo-Crivelli 1835)
55 with *B. bassiana* as the type species (Vuillemin 1912). It is widely distributed worldwide
56 (Rehner & Buckley 2005; Imoulan et al. 2017). Being recognized as a rich source of
57 efficient mycoinsecticides, in particular *B. bassiana* and *B. brongniartii* (Zimmermann
58 2007), *Beauveria* fungi have received a lot of attention as potential biological control
59 agents (García-Estrada et al. 2016). The discovery of new species and their precise
60 delimitation contributes to enlarge potential for finding suitable agents and to their safe
61 use. The genus is characterized by the formation of short, sympodial, globose to flask-
62 shaped phialides with holoblastic conidia (Khonsanit et al. 2020). The size and the
63 shape of conidia are variable within the genus and historically constituted discriminant
64 characters for delimitating and identifying species (Rehner & Buckley 2005; Imoulan
65 et al. 2017; Abdessamad 2019). However, these morphological characters can exhibit
66 overlapping values between species, even between relatively distant ones (Khonsanit
67 et al. 2020). Furthermore, many new species have been proposed these recent years
68 in *Beauveria*, mainly based on the monophyly of clades in molecular phylogenies, but

69 sometimes with only a few samples, without clear morphological distinction (Chen et
70 al. 2018). *Beauveria* is thus a genus with potential cryptic species to be discovered.
71 However, there has never been a thorough morphometric analysis with solid statistical
72 tests.

73 Although *Beauveria* fungi are known to produce an array of secondary
74 metabolites (Xu et al. 2008, 2009; Rohlf & Churchill 2011; Udompaisarn et al. 2020),
75 chemical compounds produced have been rarely used for taxonomic purpose in this
76 genus; the potential of using chemical diversity as markers for classification and
77 species identification was explored a long time ago for *Beauveria* (see Mugnai et al.
78 1989; Bridge et al. 1990) without any recent update for recently discovered species.
79 Chemical compounds allowed the distinction between populations within *B. bassiana*
80 (Bridge et al. 1990) but this finding was not compared with genetic data.

81 The abundant genomic resources available now for *Beauveria* (Valero-Jiménez
82 et al. 2016; Toopaang et al. 2017; Mei et al. 2020), combined with decreasing cost for
83 whole-genome sequencing (WGS), make it possible to access novel genetic
84 information on a large number of samples with reasonable cost and time. Next
85 generation sequencing (NGS) has in fact been used to reveal cryptic species with
86 success these recent years (Sepúlveda et al. 2017; Kobmoo et al. 2019; Matute &
87 Sepúlveda 2019).

88 In the present study, we aimed at revising the taxonomy of two important
89 *Beauveria* taxa, *B. bassiana* and *B. asiatica*. In the past, populations of *B. bassiana*
90 were studied using limited numbers of markers (Wang et al. 2003, 2005; Mitina et al.
91 2011; Meyling et al. 2012), some of which are now considered unreliable, such as
92 Amplified Fragment Length Polymorphism (AFLP) (Aquino De Muro et al. 2005; Trissi
93 et al. 2013). A recent population genomics study of this species showed clear
94 intraspecific genetic groups but their taxonomic status was not evaluated with
95 appropriate phylogenetic frameworks or detailed examination of their morphology and
96 chemistry (Mei et al. 2020).

97 In our previous taxonomic revision of the genus, we found poorly supported
98 monophyletic clades based on multigene phylogenies within *B. asiatica* (four clades
99 named A, B, C and D) and *B. bassiana* (three clades named A, B and C) (Khonsanit
100 et al. 2020). The poor node supports however did not allow the recognition of these
101 clades as distinct species and morphological traits appeared overlapping. The poor
102 node support may have been due to the limited number of markers. In the current

103 study, our objectives were to evaluate the taxonomic status of the genetic groups
104 within *B. asiatica* and *B. bassiana* by combining various data including whole-
105 genome sequencing (WGS), as well as statistical analyses of morphological
106 characters (the width and the length of conidia and phialides) and chemical profiles
107 determined by liquid chromatography-mass spectrometry (LC-MS). A solid taxonomic
108 revision should indeed be based on a combined dataset including a sufficient
109 amount of genetic information, as well as morphological and chemical traits on a
110 high number of individuals. This constitutes the foundation of integrative (or holistic)
111 taxonomy of fungi.

112

113

114 **Materials and Methods.**

115 ***Fungal culture and DNA extraction.***

116 Seventy-eight strains of presumably 12 *Beauveria* species from BIOTEC culture
117 collection (BCC) and ARS collection of entomopathogenic fungal cultures (ARSEF),
118 including the type strains of known species and strains previously identified as *B.*
119 *bassiana* or *B. asiatica* as well as some unidentified strains following Khonsanit et al.
120 (2020), were used in this study (Table S1). The strains were cultured in 50 ml of potato
121 dextrose broth and incubated at 25 °C for a week. Fungal mycelia were harvested by
122 filtering with a sterilized nylon mesh and washed with ethylene diamine tetraacetic acid
123 (EDTA) and distilled water. DNA extraction was done using a cetrimonium bromide
124 (CTAB)-based method following Kobmoo et al. (2018); the DNAs were purified using
125 the high pure PCR template preparation kit (Roche). The quality and quantity of DNA
126 were verified using Nanodrop™ One Microvolume UV-Vis Spectrophotometry
127 (Thermo Fisher) and an electrophoresis on 0.8% agarose gel at 100V for 1/2h.

128

129 ***DNA library construction and sequencing***

130 For whole genome shotgun sequencing of 78 strains, approximately 300 ng of each
131 DNA sample was used for a library construction following the protocol in the MGIEazy
132 FS library prep kit (MGI Tech, Shenzhen, China). The samples were pooled and se-
133 quenced in a single lane. Paired-end (150 bp) sequencing was performed on the
134 MGISEQ-2000RS (MGI Tech, Shenzhen, China) according to the manufacturer's in-
135 structions.

136

137 **Data processing and detection of single nucleotide polymorphisms (SNPs)**

138 Using the MegaBOLT V1.5.6.11 software package, raw reads were de-
139 multiplexed according to their barcodes and the adapter/barcode sequences were
140 removed. After removing the low-quality regions, clean reads were mapped to the *B.*
141 *bassiana* reference genome ARSEF8028 (Valero-Jiménez et al. 2016) using the
142 MegaBOLT V1.5.6.11 alignment software (Minimap2 version 2.11-r797-v03) and the
143 variants were called using GATK HaplotypeCaller version 3.8. SNP markers with poor
144 quality data were filtered out using the following criteria: (a) a minor allele frequency
145 <0.1; (b) depth coverage less than 10x; (c) more than 20% missing data. The ploidy
146 was set as haploid as all the strains were collected from asexual mycelia, i.e. putatively
147 haploid for this ascomycete fungus. The raw reads data were deposited at NCBI
148 Sequence Read Archive (SRA....) associated to the BioProject accession.... The
149 SNPs data were deposited at.....

150

151 **Population structures, genetic diversity and linkage disequilibrium**

152 The selected SNPs (729,549 SNPs) using RaxML v.8 (Stamatakis 2014), by
153 specifying GTRCAT model with Lewis biases ascertainment correction. The reliability
154 of the resulting tree was evaluated with 1000 bootstraps. Bayesian clustering analyses
155 were conducted with FastStructure (Raj et al. 2014) and a principal component
156 analysis (PCA) was achieved using the package *adegenet* in R (Jombart & Collins
157 2015). These analyses were intended to give an initial insight into interspecific
158 divergence and intraspecific population structures, in order to assess the taxonomic
159 status of the samples included as well and to reveal any genetic group that can
160 potentially be identified as new species. We calculated the fixation index (F_{st}) and
161 metrics of absolute divergence (D_{xy}) between *B. asiatica* and *B. bassiana*, as well as
162 between intraspecific genetic clusters as revealed by previous analyses. The
163 nucleotide diversity (π) within species and genetic clusters were also calculated using
164 the PopGenome package in R (Pfeifer et al. 2014). The r^2 (square of correlation
165 coefficient representing statistical association between pairwise SNPs) among
166 isolates belonging to *B. asiatica*, *B. bassiana* and their respective intraspecific genetic
167 clusters were calculated between pairs of SNPs using VCFtools (Danecek et al. 2011).
168 The decay of linkage disequilibrium was visualized using R. Neighbor-net phylogenetic
169 network based on p-distance was constructed using the software SplitsTree v4.14.18
170 (Huson & Bryant 2006).

171

172 Species Tree and Ancestral Host Association Reconstruction

173 Gene sequences were obtained using *FasterAlternateReferenceMaker* tool
174 from GATK (McKenna et al. 2010) which altered the reference sequences with SNPs
175 and simple indels; all complex substitutions were masked as Ns to reduce ambiguous
176 alignment. The sequences were then aligned using the software MAFFT (Kato &
177 Standley 2013). A total of 1,132 genes were first selected based on 1) their size (1 Kbp
178 minimum and 10 Kbp maximum) to ensure an absence of recombination within genes,
179 on 2) their physical distance on the genome; only genes with at least 20 Kbp between
180 the start and the end of consecutive genes were selected to avoid the linkage
181 disequilibrium, on 3) the number of SNPs per bp (> 0.02) to provide sufficient
182 phylogenetic signal. The alignments of the 1,132 genes were subjected to Bayesian
183 analyses with MrBayes (Ronquist et al. 2012), each with 2 Markov chain Monte Carlo
184 (MCMC) runs for 1 million generations with a 20% burn-in phase, allowed to sample
185 across the substitution model space. Only the final gene trees with < 0.02 split
186 frequencies were retained for 1034 genes. The 1034 genes sequences were also
187 concatenated and subjected to MrBayes with the model GTRGAMMAI, as chosen by
188 ModelTest-NG (Darriba et al. 2020), for 5 millions of MCMC generations. The final tree
189 was obtained after a 25% burn-in phase. The taxonomic classification corresponding
190 to the order of hosts was mapped onto the fungal species tree based on concatenated
191 genes, and the ancestral host associations were reconstructed based on maximum
192 likelihood reconstruct module in MESQUITE (Maddison & Maddison 2019).

193

194 Bayesian Concordance Analyses (BCA)

195 To evaluate whether the genetic clusters within species were consistently
196 recovered by different markers throughout the genome, Bayesian concordance
197 analyses (BCA) were conducted for the three clusters of *B. bassiana* with ARSEF7032
198 (*B. kipukae*) as an outgroup, and for the two clusters of *B. asiatica* with ARSEF617 (*B.*
199 *brongniartii*) as an outgroup. By focusing on each taxon of interest with its respective
200 outgroup, the number of genes with variable SNPs naturally reduced. The BCA were
201 thus done by selecting, among the 1034 concatenated genes, only those which had
202 at least two SNPs with no missing data and the number of SNPs per bp > 0.001 ,
203 resulting in 887 genes for *B. asiatica* and 100 genes for *B. bassiana*. The single-gene
204 trees obtained from Bayesian inferences were processed into BUCKy (Larget et al.

205 2010) which evaluated the concordance between the selected loci for the clades by
206 giving genome-wide concordance factors with 95% interval which can be 0 (absence
207 of concordance) to 1 (total concordance).

208

209 **Secondary Metabolites Profiling**

210 Strains of *Beauveria*, selected to represent the various genetic groups within *B.*
211 *asiatica* and *B. bassiana* as well as type strains of other species (Table S1), were
212 grown in 200 mL of yeast with malt extract and glucose (YMG) medium (10 g of malt
213 extract, 4 g of D-glucose, 4 g of yeast extract and 1,000 ml of distilled water, pH 6.3),
214 incubated at 25 °C under shaking condition (140 rpm). Since growth rate varies among
215 strains, the prolonged fermentation period for an individual strain was set to be half
216 the time required for that particular strain to reach glucose depletion. The glucose
217 content of each fermented broth was estimated using urine glucose test strips (DIRUI®,
218 Jilin, China). The mycelia were then separated from the broth either by filtration or
219 centrifugation.

220 The extraction of fungal secondary metabolites was performed according to
221 Phainuphong et al. (2017) with culture filtrates passing through successive extraction
222 using acetone, ethyl acetate and methanol, resulting in a final methanol-based cell
223 extract (CE). The experiment of liquid chromatography-mass spectrometry (LC-MS)
224 was done using ultra-high performance liquid chromatography (UHPLC) - Orbitrap
225 Fusion™ Tribrid™ mass spectrometer (Thermo Scientific, Massachusetts, USA),
226 equipped with electrospray ionization (ESI) source. The separation of compounds was
227 done on an Acquity UPLC® HSS T3 C18 column (1.8 µm diameter, 2.1 x 100 mm)
228 maintained at 40 °C with flow rate of 0.4 ml/min. A mobile phase system was solvent
229 A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). The
230 optimized gradient for the best separation was 0.0-12.0 min, 12%-95%B; 12.0-14.0min,
231 95%-95%B; 14.0-16.0 min, 12%-12%B. The mass spectrometer was operated in ESI
232 positive which covered more than 80% of metabolites present in the tested pool
233 sample. Each sample was injected in triplicate. The MS calibration was conducted
234 using Pierce LTQ Velos ESI Positive Ion Calibration (PSP3A 88323) and Pierce ESI
235 Negative Ion Calibration (PSP3A 88324) according to manufacturer's protocol.

236 The software Compound Discoverer 3.1 was used for data-preprocessing steps.
237 The MS raw files were subjected to peak alignment, peak picking, adduct grouping
238 and normalization with parameters adjusted to fit to the chromatographic data obtained

239 from this experiment. The area under curve (AUC) of each metabolite was determined
240 and normalized with that of pooled samples. The features (m/z at specific retention
241 time (rt)) with relative standard deviations over 30% and average group area in non-
242 inoculated YMG media over 5×10^5 were filtered to ensure good-quality peaks and to
243 cover peaks apart from culture media. Top 200 highest abundant features in the CE
244 pool sample were selected for statistical analyses including a Euclidean distance-
245 based neighbor-joining (NJ) tree and principal component analysis (PCA) using
246 respectively the packages “ape” (Paradis & Schliep 2019) and “FactoMineR” (Lê et al.
247 2008) in R (R Core Team 2020).

248

249 ***Morphological examination and species description***

250 A thorough morphological investigation was conducted following the techniques
251 described in Khonsanit et al. (2020). The samples and culture plates were
252 photographed by using a digital Nikon D5100 camera. Colony characteristics and
253 microscopic measurements of phialides and conidia (length and width) were done after
254 growth on potato dextrose agar medium (PDA, 20 g Difco potato dextrose agar, 1 L
255 distilled water) and incubation under white light/dark cycles at room temperature; 30
256 phialides and conidia were measured for each strain. To statistically test the difference
257 between the genetic groups within each of *B. asiatica* and *B. bassiana*, five strains for
258 each genetic group were examined for the width and the length of conidia and
259 phialides (Table S2). The data were analyzed with one-factor ANOVA for testing
260 difference between species, as well as between the genetic groups within each
261 species.

262

263

264 **Results**

265

266 ***Population Structure and Molecular Diversity***

267 The maximum-likelihood tree inferred from all the 729,549 SNPs showed clear
268 differentiation between *B. asiatica* and *B. bassiana* as expected, and also intraspecific
269 genetic subdivision (Fig.1a). Two and three clades could be distinguished within *B.*
270 *asiatica* and *B. bassiana* respectively. One of the two clades from *B. asiatica*
271 corresponds to the Clade C as found in Khonsanit et al. (2020), including the type
272 strain ARSEF 4850; the other clade comprised a mix of individuals from the Clades A,

273 B, C and D from that previous study, therefore named here as “Clade Mixed”. The
 274 three clades from *B. bassiana* corresponded to the Clades A, B and C established in
 275 this species by Khonsanit et al. (2020); the Clade C includes the type strain ARSEF
 276 1564. The principal component analysis (PCA) also showed clear difference as
 277 expected between *B. asiatica* and *B. bassiana* as well as between these two species
 278 and the others included (Fig.1b). Not enough individuals had been analysed from the
 279 other species to assess their differentiation one from each other. The Bayesian
 280 clustering within each of the two species recovered the same genetic clusters as
 281 above (Fig.1c and d) but, for *B. asiatica*, an additional subdivision was observed within
 282 Clade Mixed (Fig.1c). The PCA also confirmed the strong differentiation between the
 283 clades identified in the analyses above (Fig.1e-f) as well as the subdivision within *B.*
 284 *asiatica* Clade Mixed (clusters hereafter called mix1 and mix2: Fig.1f). These sub-
 285 clusters included mixes of strains from various genetic groups from Khonsanit et al.
 286 (2020). The various analyses thus consistently indicated the existence of intraspecific
 287 subdivision. The only exception was the strain NHJ10436 which clustered with *B.*
 288 *bassiana* Clade B in the Bayesian clustering and the tree, but had intermediate
 289 coordinates on the PCA (Fig.1b and 1e). This is probably due to missing data as this
 290 strain only carried 14,427 SNPs (1.97 % from total SNPs). This strain was though kept
 291 in our analyses as it personally interested us for use in biocontrol.

292 The F_{ST} between the two species was high (0.875) as expected for interspecific
 293 differentiation. The nucleotide diversity (P_i) was higher in *B. bassiana* (0.107) than *B.*
 294 *asiatica* (0.057); this may be due to the use of *B. bassiana* genome as reference to
 295 call SNPs. The differentiation levels between clades within species were also very high,
 296 with F_{ST} reaching more than 0.7 (Table 1), but the F_{ST} between the sub-clusters mix1
 297 and mix2 within Clade Mixed of *B. asiatica* was relatively weak (0.262). The divergence
 298 estimated by D_{xy} was also relatively high between clades, close to or higher than 0.1
 299 (Table.1), but that between the sub-clusters mix1 and mix2 was much lower (0.041).
 300 The nucleotide diversity (P_i) was higher in *B. asiatica* Clade Mixed (0.024 and 0.036
 301 for the sub-clusters mix1 and mix2 respectively) than in the Clade C (0.009). In *B.*
 302 *bassiana*, Clade B had the highest P_i (0.031), followed by Clade C (0.018) and Clade
 303 A (0.003).

304 **Table 1.** Genetic differentiation (F_{ST} : below the diagonal), genetic divergence (D_{xy} :
 305 above the diagonal) between clades of *B. asiatica* and *B. bassiana*, and nucleotide
 306 diversity (P_i : the diagonal) within clades.

307

		<i>B. asiatica</i>		<i>B. bassiana</i>		
		Clade C	Clade Mixed	Clade A	Clade B	Clade C
<i>B. asiatica</i>	Clade C	0.009	0.0875	0.651	0.675	0.669
	Clade Mixed	0.742	0.036	0.630	0.654	0.648
<i>B. bassiana</i>	Clade A	0.991	0.969	0.003	0.157	0.119
	Clade B	0.970	0.949	0.893	0.031	0.161
	Clade C	0.980	0.958	0.913	0.848	0.018

308

309

310 **Phylogenetic Network and Linkage Disequilibrium**

311 The Neighbor-Net network inferred from p-distances between the strains
 312 showed clear separation between clades within each species, with minimal reticulation
 313 between them, confirming lack of gene flow among clades (Fig.S1). The sub-cluster
 314 mix1 was nested within the sub-cluster mix2 for *B. asiatica* Clade Mixed. Reticulations
 315 were observed within *B. asiatica* Clade C and Clade Mixed. For *B. bassiana*,
 316 reticulation events could be observed only within Clade B. In all the clades within
 317 species, the r^2 dropped to rapidly reach a plateau except *B. bassiana* Clade A which
 318 sustained a longer LD with r^2 dropping under half the maximum around 300 Kbp
 319 (Fig.S1). These results are consistent with the absence of reticulation in the *B.*
 320 *bassiana* Clade A, for which the nucleotide diversity was also the lowest (0.002: Table
 321 1).

322

323 **Phylogenetics Species Recognition**

324 The species tree inferred from 1034 concatenated genes gave strongly
 325 supported monophyletic clades corresponding to the genetic subdivisions within *B.*
 326 *asiatica* (Clade C and Clade Mixed) and *B. bassiana* (Clades A, B, C) (Fig.2). The
 327 subcluster mix 1 within *B. asiatica* Clade Mixed was recovered as a well-supported
 328 monophyletic clade but not the subcluster mix 2, further supporting the view that mix1
 329 is a lineage having originated from mix2. To evaluate whether these genetic
 330 subdivisions could be considered as distinct species using phylogenetics species
 331 criteria, we conducted Bayesian concordance analyses between genes having no
 332 missing data for *B. asiatica* with its outgroup *B. brongniartii* (ARSEF617) (887 genes),

333 and for *B. bassiana* with its outgroup *B. kipukae* (ARSEF7032) (100 genes). We found
334 that the Clades A, B, C within *B. bassiana* were highly concordant throughout the
335 genome with high Bayesian concordance factors (BCF), NHJ10436 being well
336 anchored in Clade B. For *B. asiatica*, Clade C had a high BCF (> 0.8) but Clade Mixed
337 had a low BCF (0.201) (Fig.2).

338

339 **Morphological variation**

340 The average among measures per strain of the length and the width of phialides
341 and conidia are reported in Table S2. The length of phialides and conidia were
342 significantly different between *B. asiatica* and *B. bassiana* (phialide length: $F = 16.858$,
343 $p\text{-value} = 4.324e^{-04}$; conidia length: $F = 79.566$, $p\text{-value} = 6.309e^{-09}$) but the other traits
344 were not significantly different between the two species (phialide width: $F = 0.199$, $p\text{-}$
345 $\text{value} = 0.659$; conidia width: $F = 79.566$, $p\text{-value} = 6.309e^{-09}$) (Fig.3a). Between Clade
346 C and Clade Mixed of *B. asiatica*, only the length of conidia was significantly different
347 ($F = 7.219$, $p\text{-value} = 0.02$, Fig.3b). For *B. bassiana*, none of the examined traits was
348 significantly different between clades (Table S3) (Fig.3b).

349

350 **Chemotaxonomy**

351 Metabolite profiles obtained with LC-MS are shown in Fig.S2-S3. Principal
352 component analyses (PCA) and the Euclidean distance-based tree showed a relatively
353 clear separation between *B. asiatica* and *B. bassiana* with some exceptions (Fig.4);
354 the two strains MY11641 and NHJ13734 of *B. asiatica* showed a chemical similarity to
355 *B. bassiana*. The other species included in the chemical analyses were not all well
356 discriminated from *B. asiatica* and *B. bassiana*. On the NJ tree, *B. caledonica*, *B.*
357 *pseudobassiana*, *B. sungii* and *B. vermiconia* clustered within *B. asiatica* (Fig.4a).
358 Based on the PCA, *B. amorpha*, *B. malawiensis*, *B. pseudobassiana*, *B. varrae* could
359 be well distinguished from both *B. asiatica* and *B. bassiana*. In contrast, *B.*
360 *gryllotalpidicola* and *B. kipukae* appeared nested within *B. bassiana* while *B.*
361 *caledonica*, *B. sungii*, *B. vermiconia* were found very close to *B. asiatica* (Fig.4b, top
362 panel). The intraspecific clades were not well separated based on these methods.

363

364 **Ancestral host association**

365 The ancestral reconstruction of host association showed that *Beauveria* is
366 definitely a genus of generalists, with a predominance for *Coleoptera* as the insect

367 host order associated with its common ancestor. (Fig.5). The ancestral hosts of *B.*
 368 *asiatica* and *B. bassiana* were inferred to be also most likely coleopteran insects.
 369 However, *B. asiatica* Clade Mixed had a notable likelihood for *Lepidoptera* while the
 370 ancestral host association of *B. bassiana* Clade A was most likely *Orthoptera*.

371

372 **Taxonomy**

373 Based on genomic, morphological and ecological results above, *B. asiatica*
 374 Clade Mixed is proposed as *B. thailandica*; *B. bassiana* Clade A and Clade B are
 375 proposed as *B. namnaoensis* and *B. neobassiana*, respectively.

376

377 ***Beauveria namnaoensis*** Khons., Kobmoo & Luangsa-ard, *sp. nov.* — MycoBank
 378 MB No. 838940; Fig. 6

379

380 *Etymology.* The name derived from the place where the type specimen was
 381 found, Nam Nao National Park, Nam Nao District.

382

383 *Holotypus.* THAILAND, Phetchabun Province, Nam Nao District, Nam Nao
 384 National Park, Headquarter Nature Trail, 16.74°N, 101.57°E, on adult of *Xylocopa*
 385 *latipes* (*Hymenoptera:Apidae*), 4 Jul. 2012, A. Khonsanit, K. Tasanathai, P.
 386 Srikitikulchai, S. Mongkolsamrit, W. Noisripoom (holotype BBH36158; ex-holotype
 387 strain BCC64218).

388

389 *Sexual morph* — Unknown.

390

391 *Asexual morph* — Specimens found on blue milkweed beetle, grasshopper, moth,
 392 lepidopteran pupa, stink bug and wasp. Hosts covered with white mycelium, powdery
 393 when sporulating. *Phialides* hyaline, solitary, smooth-walled, base subspherical to
 394 lageniform, ampulliform, globose, subglobose, pyriform, clavate, mucronate,
 395 (2)2–3.2(5) × (1.5)1.9–2.7(3.5) μm (*n* = 150). *Conidia* hyaline, smooth-walled,
 396 globose to subglobose, occasionally obovoid, (1.5)1.9–2.8(4) × (1.5)1.7–2.3(3) μm (*n*
 397 = 150).

398

399 *Colony characteristics* — Colonies growing at room temperature attaining a diam of
 400 29–31 mm in 10 d, and 42–43 mm in 20 d. Surface mycelium dense, floccose,
 401 sporulation starts at 4 d after inoculation, white with green-yellow (1D), becoming to
 402 green-yellow (1C) and powdery white sporulating. Colony reverse green-yellow (1A),
 403 white at the margin.

404

405 *Additional specimens examined.* THAILAND, Chanthaburi Province, Khao Soi Dao
 406 District, Khao Soi Dao Wildlife Sanctuary, 13.10°N, 102.19°E, on stink bug, 1 Jan.
 407 2000, *B. Thongnuch*, *J.J. Luangsa-ard*, *K. Tasanathai*, *P. Srikitikulchai*, *S.*
 408 *Mongkolsamrit*, *W. Chaygate* (BBH14343:BCC18114); Kanchanaburi Province,
 409 Thong Pha Phum District, Thung Yai Naresuan Wildlife Sanctuary, Krathon Ruesi
 410 Nature Trail, 15.33°N, 98.92°E, on moth adult, 2 Dec. 2005, *B. Thongnuch*, *K.*
 411 *Tasanathai*, *P. Srikitikulchai*, *W. Chaygate* (BBH15191:BCC19745); Phetchaburi
 412 Province, Kaeng Krachan District, Kaeng Krachan National Park, Ban Krang Camp
 413 Nature Trail, 12.88°N, 99.63°E, on blue milkweed beetle, 26 Apr. 2006, *B.*
 414 *Thongnuch*, *K. Tasanathai*, *R. Ridkaew*, *W. Chaygate* (BBH18375:BCC21293);
 415 Nakhon Ratchasima Province, Pak Chong District, Khao Yai National Park, Bueng
 416 Phai Nature Trail, 14.44°N, 101.37°E, on wasp, 29 Nov. 2006, *B. Thongnuch*, *P.*
 417 *Puyngain*; *T. Keokene*, *W. Chaygate* (BBH23082:BCC23823); Chiang Mai Province,
 418 Doi Inthanon National Park, Mae Chaem District, Mae Chaem Junction (KM.38)
 419 Nature Trail, 18.54°N, 98.52°E, on a grasshopper, 27 Nov. 2008, *A. Khonsanit*, *K.*
 420 *Tasanathai*, *P. Srikitikulchai* (BBH25297:BCC34350).

421

422 *Notes* — *Beauveria namnaoensis* is hardly distinguishable morphologically from
 423 other closely related species such as, *B. bassiana* and *B. neobassiana* (Table 2 and
 424 refer to the results on morphological variation). However, this new species is based
 425 on phylogenetic studies.

426

427 ***Beauveria neobassiana*** Khons., Kobmoo & Luangsa-ard, *sp. nov.* — MycoBank
 428 MB No. 838939; Fig. 6

429

430 *Etymology.* The name is derived from the morphological similarity to *Beauveria*
 431 *bassiana*

432

433 *Holotypus*. THAILAND, Nakhon Ratchasima Province, Pak Chong District, Khao
 434 Yai National Park, Trail from Heo Sawat to Heo Sai Waterfall Nature Trail, 14.44°N,
 435 101.37°E, on cicada adult, 20 May. 1996, *K. Tasanathai*, *N.L. Hywel-Jones*, *S.*
 436 *Sivichai*, *S. Thienhirun* (holotype BBH5054: ex-holotype strain BCC1848);

437

438 *Sexual morph* — Unknown.

439

440 *Asexual morph* — Specimens found on ant, adult beetles, adult cicadas, lepidopteran
 441 larvae, queen ants, moth and weevils. Hosts covered with white mycelium, powdery
 442 while sporulating. *Phialides* hyaline, solitary, smooth-walled, base subspherical,
 443 ovoid, (2)2.3–3.1(3.5) × (2)2.1–2.9(3) μm (*n* = 150). *Conidia* hyaline, smooth-walled,
 444 globose, occasionally subglobose, (2)2–2.6(3) × (1.5)1.8–2.2(2.5) μm (*n* = 150).

445

446 *Colony characteristics* — Colonies on PDA at room temperature attaining a diam of
 447 20–21 mm in 10 d, 40–41 mm in 20 d, surface mycelium dense, floccose. Sporulation
 448 starts at 5 d after inoculation, white with green-yellow (2D), powdery when sporulating.
 449 Colony reverse yellow (6C), becoming to yellow-orange (20A) and white at the
 450 margin.

451

452 *Additional specimens examined*. THAILAND, Kanchanaburi Province, Sangkhla Buri
 453 District, Khao Laem National Park, Sum Nuk Buhd To, 15.02°N, 98.60°E, on beetle
 454 adult (*Coleoptera*), 21 Jun. 1995, *N.L. Hywel-Jones*, *R. Nasit*, *S. Sivichai*
 455 (BBH4614:BCC1446); Nakhon Ratchasima Province, Pak Chong District, Khao Yai
 456 National Park, Trail from Heo Sawat to Heo Sai Waterfall Nature Trail, 14.44°N,
 457 101.37°E, on adult cicada, 20 May. 1996, *K. Tasanathai*, *N.L. Hywel-Jones*, *S.*
 458 *Sivichai*, *S. Thienhirun* (BBH5054:BCC1848); Phetchaburi Province, Kaeng Krachan
 459 District, Kaeng Krachan National Park, Khlong 1 Nature Trail, 12.88°N, 99.63°E, on
 460 moth, *J.J. Luangsa-ard*, *K. Tasanathai*, *N.L. Hywel-Jones*, *P. Srikitikulchai*, *R. Nasit*,
 461 *S. Mongkolsamrit*, *W. Chaygate* (BBH14301), Chiang Mai Province, Mae Chaem
 462 District, Doi Inthanon National Park, 18.54°N, 98.52°E; on ant, 1 Jan. 2000, *B.*
 463 *Thongnuch*, *K. Tasanathai*, *P. Srikitikulchai*, *R. Ridkaew*, *S. Mongkolsamrit*, *W.*
 464 *Chaygate* (BBH14373:BCC18124, BBH14374:BCC18171); Phetchabun Province,

465 Nam Nao District, Nam Nao National Park, Trail to nature study area, 16.74°N,
 466 101.57°E, on weevil (*Coleoptera:Curculionoidea*), 23 May. 2000, K. *Tasanathai*, P.
 467 *Lutthisungneon*, R. *Nasit*, S. *Sivichai* (BBH7666:BCC2660); Chiang Mai Province,
 468 Fang District, Doi Phahompok National Park, Doi Phahompok Nature Trail, 20.00°N,
 469 99.14°E, on queen ant, 5 Feb. 2006, B. *Thongnuch*, K. *Tasanathai*, S. *Mongkolsamrit*,
 470 W. *Chaygate* (BBH16599:BCC20197); Nakhon Ratchasima Province, Pak Chong
 471 District, Khao Yai National Park, Kong Kaeo Waterfall Nature Trail, 14.44°N,
 472 101.37°E, on adult beetle, 12 Jun. 2007, C. *Chuaseeharonnachai*, S. *Sivichai*, S.
 473 *Mongkolsamrit* (BBH24543:BCC25950); Chanthaburi Province, Soi Dao District,
 474 Khao Soi Dao Wildlife Sanctuary, Headquater Nature Trail, 13.10°N, 102.19°E, on
 475 adult cicada, 22 Jul. 2008, A. *Khonsanit*, J.J. *Luangsa-ard*, K. *Tasanathai*, P.
 476 *Srikitikulchai*, S. *Mongkolsamrit* (BBH24402:BCC30545), on adult beetle
 477 (*Coleoptera*), 22 Jul. 2008, A. *Khonsanit*, J.J. *Luangsa-ard*, K. *Tasanathai*, P.
 478 *Srikitikulchai*, S. *Mongkolsamrit* (BBH23856:BCC31604); Tak Province, Umphang
 479 District, Umphang Wildlife Sanctuary, Pi Tu Kro Waterfall Nature Trail, 15.92°N,
 480 98.76°E, on queen ant, 26 Jun. 2001, A. *Khonsanit*, B. *Thongnuch*, J.J. *Luangsa-ard*,
 481 K. *Tasanathai*, P. *Srikitikulchai*, S. *Mongkolsamrit*, W. *Chaygate*
 482 (BBH23919:BCC31619); Chiang Rai Province, Chiang Khong District, Phlu Kaeng
 483 Waterfall Nature Trail, 20.26°N, 100.39°E, on lepidopteran larva, 17 Jan. 2009, A.
 484 *Khonsanit*, K. *Tasanathai*, P. *Srikitikulchai*, S. *Mongkolsamrit* (BBH26635:BCC35935);
 485 Nakhon Ratchasima Province, Pak Chong District, Khao Yai National Park, Mo Sing
 486 To Nature Trail, 14.44°N, 101.37°E, on adult beetle (*Coleoptera*), 20 Jul. 2009, K.
 487 *Tasanathai*, P. *Srikitikulchai*, R. *Ridkaew*, S. *Mongkolsamrit*, T. *Chohmee*
 488 (BBH26358:BCC37372); *ibid.*, 16 Aug. 2009, K. *Tasanathai*, P. *Srikitikulchai*, R.
 489 *Ridkaew*, S. *Mongkolsamrit*, T. *Chohmee* (BBH26797:BCC37938); Chanthaburi
 490 Province, Pong Nam Ron District, Suchin's Orchard, 12.92°N, 102.39°E, on adult
 491 beetle (*Coleoptera*), 16 Dec. 2014, A. *Khonsanit*, D. *Thanakitpipattana*, N.
 492 *Wiriyathanawudhiwong*, W. *Noisriboom* (BBH39718:BCC76579).

493

494 Notes — *Beauveria neobassiana* is hardly distinguishable morphologically from other
 495 closely related species, such as *B. bassiana* and *B. namnaoensis* (Table 2 and refer
 496 to the results on morphological variation). However, this new species is based on
 497 phylogenetic studies.

498

499 ***Beauveria thailandica*** Khons., Kobmoo & Luangsa-ard, *sp. nov.* —MycoBank MB
500 838941; Fig. 6

501

502 *Etymology.* The epithet refers to the locality where the type specimen was found,
503 Thailand.

504

505 *Holotype.* THAILAND, Chanthaburi Province, Soi Dao District, Khao Soi Dao
506 Wildlife Sanctuary, 13.10°N, 102.19°E, on *Coleoptera* larva, 1 Jan. 2000, N. L.
507 Hywel-Jones (holotype BBH13831; ex-type culture BCC16585, TBRC8350).

508

509 *Sexual morph* — *Stromata* arising from the posterior part of coleopteran larva and
510 between head and thorax of click beetle, cylindrical with rounded apices, yellow
511 (8B–8A) when fresh and yellow-orange (19B–19A) when dry, 38–50 × 0.8–2.5 mm.

512 *Perithecia* semi-immersed, ovoid, (420)469.6–539(580) × (150)182.2–258.5(290) μm
513 ($n = 30$). *Asci* hyaline, cylindrical, capitate, (180)–215.4–299.5(335) × (3)3.2–4.7(6) ×
514 μm ($n = 50$). *Asci-caps* hyaline, hemispherical, (2)2.2–3.1(3) × (3.5)3.6–4.1(4) μm (n
515 = 30). *Ascospores* hyaline, filiform, multiseptate, 241–320 × 1 μm ($n = 8$). *Part-spores*
516 hyaline, cylindrical with truncated end, (4)–5.2–12.9(23) × 1 μm ($n = 50$).

517

518 *Asexual morph* — Specimens found on adult cicada, earwigs, ladybugs, leafhoppers,
519 longhorn beetle, queen ant, stink bug and wasps. Hosts covered with white mycelium,
520 powdery when sporulating. *Phialides* hyaline, solitary, smooth-walled, base
521 subspherical to lageniform, ampulliform, mucronate, rostrate, (2)2.5–3.7(5) ×
522 (1.5)2–3(4) μm ($n = 150$). *Conidia* hyaline, smooth-walled, obovoid, ovoid,
523 occasionally globose to subglobose, (2.5)3.1–4.3(6) × (1.5)1.6–2.3(3) μm ($n = 150$).

524

525 *Colony characteristics* — Colony growth at room temperature attaining a diam of
526 21–22 mm 10 d, 30–32 mm in 20 d. Surface mycelium dense, convex to the agar
527 surface, floccose, cottony, sporulation starts at 10 d after inoculation, white to green-
528 yellow (1C). Colony reverse green-yellow (1C), white at the margin.

529

530 *Additional specimens examined.* THAILAND, Phetchabun Province, Nam Nao
531 District, Nam Nao National Park, Lum Nam Cheun Nature Trail, 16.74°N, 101.57°E,
532 on stink bug (*Hemiptera: Podopidae*), 11 Oct. 1994. *N.L. Hywel-Jones, R. Nasit, S.*
533 *Sivichai* (BBH4362:BCC1442); Phetchaburi Province, Kaeng Krachan District, Kaeng
534 Krachan National Park, KM. 15 on road to Tor Tip Waterfall, 12.88°N, 99.63°E, on
535 adult cicada (*Hemiptera: Cicadidae*), 23 May 1995, *N.L. Hywel-Jones*
536 (BBH4541:BCC1654); *ibid.*, on adult beetle (*Coleoptera*), 23 May 1995, *N.L. Hywel-*
537 *Jones* (BBH4542:BCC1655); *ibid.*, on earwigs (*Dermaptera: Chelisochidae*), 25 May
538 1995, *N.L. Hywel-Jones* (BBH4563:BCC2044); Kanchanaburi Province, Sangkhla
539 Buri District, Khao Laem National Park, Sum Nuk Buhd To, 15.02°N, 98.60°E, on
540 adult beetle (*Coleoptera*), 21 Jun. 1995, *N.L. Hywel-Jones, R. Nasit, S. Sivichai*
541 (BBH4561:BCC1665); Chanthaburi Province, Khao Soi Dao District, Khao Soi
542 Dao Wildlife Sanctuary, Behind office Nature Trail, 13.10°N, 102.19°E, on *Lepidoptera*
543 larva, 20 Jun. 1996, *K. Tasanathai, R. Nasit, S. Sivichai* (BBH5182:BCC1906);
544 Phetchabun Province, Nam Nao District, Nam Nao National Park, behind office to
545 bungalow 1 Nature Trail, 16.74°N, 101.57°E, on *Coleoptera* larva, 20 Aug. 1996, *K.*
546 *Tasanathai, R. Nasit, S. Sivichai* (BBH5227:BCC2086); Surat Thani Province,
547 Phanom District, Khao Sok National Park, Sip Et Shin Waterfall Nature Trail, 8.99°N,
548 98.63°E, on adult beetle (*Coleoptera*), *K. Tasanathai, N.L. Hywel-Jones, S. Sivichai*
549 (BBH5353:BCC2120); Phetchaburi Province, Kaeng Krachan District, Kaeng
550 Krachan National Park, KM. 15 on road to Tor Tip Waterfall, 12.88°N, 99.63°E, on
551 weevil bug (*Coleoptera: Curculionidae*), 2 Jun. 2000, *N.L. Hywel-Jones*
552 (BBH7769:BCC2676); Mae Hong Son Province, Mae Hong Son Road marker KM.
553 5.7, 19.30°N, 97.97°E, on Earwigs (*Dermaptera: Chelisochidae*), 30 Jun. 2002, *N.L.*
554 *Hywel-Jones, R. Nasit*, (BBH16766:BCC12907); Chanthaburi Province, Khao Soi
555 Dao District, Khao Soi Dao Wildlife Sanctuary, 13.10°N, 102.19°E, on longhorn beetle
556 (*Coleoptera*), 21 Aug. 2009, *B. Thongnuch, J.J. Luangsa-ard, K. Tasanathai, P.*
557 *Srikitikulchai, S. Mongkolsamrit, W. Chaygate* (BBH14344:BCC18115); Phetchabun
558 Province, Nam Nao District, Nam Nao National Park, Headquarter Nature Trail,
559 16.74°N, 101.57°E, on adult leafhoppers (*Hemiptera, Cicadellidae*), 30 Jun. 2004, *B.*
560 *Thongnuch, K. Tasanathai, W. Chaygate* (BBH10140:BCC16183); Tak Province,
561 Umphang District, Umphang Wildlife Sanctuary, Pi Tu Kro Waterfall (Preto Lo Su),
562 15.92°N, 98.76°E, on adult longhorn beetle (*Coleoptera: Cerambycidae*), 26 June
563 2008, *A. Khonsanit, J.J. Luangsa-ard, K. Tasanathai, P. Srikitikulchai, S.*

564 *Mongkolsamrit* (BBH23918:BCC31618); Nakhon Ratchasima Province, Pak Chong
 565 District, Khao Yai National Park, Mo Sing To Nature Trail, 14.7116666666667 N,
 566 101.421666666667 E, on adult click beetle (*Coleoptera:Elateridae*), 18 Jun. 2009,
 567 N.L. Hywel-Jones, K. Tسانathai, R. Ridkaew, S. Mongkolsamrit, T. Chohmee
 568 (BBH38825:BCC36657); Nakhon Ratchasima Province, Pak Chong District, Khao
 569 Yai National Park, Mo Sing To Nature Trail, 14.44°N, 101.37°E, on *Coleoptera* larva,
 570 27 Sep. 2011, A. Khonsanit, K. Sansatchanon, K. Tسانathai, S. Mongkolsamrit, W.
 571 Noisriboom (BBH32175: BCC49762); Chiang Mai Province, Chiang Dao District,
 572 Chiang Dao Wildlife Sanctuary, Chiang Dao Wildlife Research Station, 19.39°N,
 573 98.84°E, on *Lepidoptera* larva, 5 Oct. 2012, A. Khonsanit, K. Tسانathai, P.
 574 Srikitikulchai, R. Promharn, W. Noisriboom (BBH38847:BCC56283); Chiang Mai
 575 Province, Chiang Dao District, Ban Hua Thung Community Forest, 19.39°N, 98.84°E,
 576 on adult wasp, 31 Oct. 2014, A. Khonsanit, D. Thanakitpipattana, K. Tسانathai, P.
 577 Srikitikulchai, S. Wongkanoun, W. Noisriboom (BBH40622:BCC76509).

578

579 Notes — Phylogenetically, *B. thailandica* is closely related to *B. asiatica*. It occurs on
 580 a wide range of insect hosts. Morphologically, *B. thailandica* occasionally has smaller
 581 perithecia, shorter asci and ascospores as well as longer part-spores than *B. asiatica*
 582 (Table 3). Detailed statistical analyses showed a significant difference in terms of
 583 conidia length and a notable higher variation for the length and the width of phialides
 584 and conidia.

585 **Table 2** Morphological comparisons of *Beauveria bassiana* species complex

Species	Host/substrate	Phialide (μm)	Conidia (μm)	Distribution	References
<i>B. bassiana</i> s.s.	Beetle adults, cucurbit beetle, mantid, maple leafcutter moth larva, moth adult, mulberry moth pupa, spittle bug, weevils	2.5–6 × 3–6	2–3 × 2–3	Brazil, Canada, Hungary, Morocco, Republic of Korea, Thailand, United States of America	Rehner et al. (2011), Khonsanit et al. 2020, This study
<i>B. namnaoensis</i> sp. nov.	Blue milkweed beetle, grasshopper, moth, lepidopteran pupa, stink bug, wasp	2–5 × 1.5–3.5	1.5–4 × 1.5–3	Thailand	This study
<i>B. neobassiana</i> sp. nov.	Ant, beetle adults, cicada adults, lepidopteran larva, queen ants, moth, weevils	2–3.5 × 2–3	2–3 × 1.5–2.5	Thailand	This study

586

587

588

589

590

591

592

593

594

595

596

597 **Table 3** Morphological comparisons of *Beauveria asiatica* species complex

Species	Host	Stromata (mm)	Perithecia (μm)	Asci (μm)	Asci-caps (μm)	Ascospores (μm)	Part-spores (μm)	Phialide (μm)	Conidia (μm)	Distribution	References
<i>B. asiatica</i> s.s.	Beetle adult, cicada adult, click beetle, dark black chafer beetle, grasshopper eggs, longhorn beetles	27–50 × 1.5–5	300–630 × 130–330	268–513 × 3–5	2–3 × 3–5	228–455 × 1	3–12 × 1	2.5–6.3 × 3–6	2.5–4.3 × 2–3	China, Republic of Korea, Thailand	Khonsanit et al. 2020, Rehner et al. 2011
<i>B. thailandica</i> sp. nov.	Cicada adult, click beetle, earwigs, ladybugs, leafhoppers, longhorn beetle, queen ant, stink bug, wasps	38–46 × 0.8–2.5	420–580 × 150–290	180–335 × 3–6	2–3 × 3.5–4	241–320 × 1	4–23 × 1	2–5 × 1.5–4	2.5–6 × 1.5–3	Thailand	This study

598

599

600 Discussion

601 *Beauveria* is a genus with many cryptic species. There has been no comprehensive
602 with genetic, morphological and chemical data. In this study, our objectives were to assess
603 the species status of genetic groups found within *B. asiatica* sensu lato and *B. bassiana* s.l.
604 based on population genomics, morphological and chemical data.

605

606 **Phylogenetics Species Criterion**

607 The intraspecific genetic groups as found in these two species complexes by
608 Khonsanit et al. (2020) were recovered by population genomics data in this study. The main
609 question is whether they should be considered as distinct species. The evolutionary species
610 concept (ESC) defined a species as “a single lineage of ancestor-descendant populations
611 which maintain its identity from other such lineages, and which has its own evolutionary
612 tendencies and historical fate” (Wiley 1978). This concept is not debated but is not an
613 operational species delimitation criterion (De Queiroz 2007; Giraud et al. 2008). For fungi,
614 the so-called phylogenetic species concept (PSC) is an operational concept that has been
615 predominant and proposed as the most consistent to ESC (Taylor et al. 2000). According to
616 the theoretical concept related to the most common ancestor and descending progenies,
617 PSC is naturally associated to the monophyly and consistency of clades across genes,
618 which became the basis to phylogenetic species recognition (PSR) (Cracraft 1983; Mishler
619 & Brandon 1987; Mishler & Theriot 2000). According to this criterion, *B. asiatica* Clade C,
620 Clade Mixed, and *B. bassiana* Clade A, Clade B and Clade C should be considered as
621 distinct species. However, monophyly-based species recognition received also some
622 criticisms; monophyly-based species recognition cannot apply to asexual species because,
623 without recombination, all lineages are monophyletic and with consistent gene genealogies.
624 *Beauveria* had actually long been thought to be an asexual genus (Basalmo-Crivelli 1835).
625 Our analyses of LD decay suggested substantial within some of these clades, which is
626 consistent to observations of teleomorphs for several *Beauveria* species, including *B.*
627 *bassiana* (Khonsanit et al. 2020; Rehner et al. 2011). The phylogenetic networks which take
628 into account reticulated evolution still showed clear separation between them.

629 However, PSC can be arbitrary in regard of the limit of where to put species
630 boundaries as any kind of molecular markers with polymorphisms would allow grouping
631 individuals into monophyletic clades based on their respective alleles. PSC was thus
632 proposed to rely on the concordance between distinct gene genealogies (Avis & Ball; Baum
633 & Shaw 1995), resulting in the genealogical concordance phylogenetic species recognition
634 (GCPSR) criterion for which the limit of species is placed where there is a transition from the

635 concordance among branches connecting different species to the conflict between branches
636 within species due to intraspecific recombination (Taylor et al. 2000). Some fungal species
637 were in fact erroneously defined based on only concatenated analysis of multi-gene
638 phylogenies without concordance between markers and any corroboration from
639 morphological, chemical and ecological data (Liu et al. 2016). Our results showed that the
640 different genetic clades had actually high concordance factors except for *B. asiatica* Clade
641 Mixed. Except for this latest case, the clades as revealed by whole-genome data are thus
642 supported as distinct phylogenetic species.

643

644 ***Insights from the morphology, chemistry and host association***

645 Previous studies have shown that the size and the shape of conidia can be used, to
646 some extent, to discriminate between species but these characters were largely overlapping
647 across species in *Beauveria* (Rehner & Buckley 2005; Imoulan et al. 2017; Khonsanit et al.
648 2020). Our study revealed that the morphological differences between clades within *B.*
649 *asiatica* and *B. bassiana* were much less evident than the genomic data. Nevertheless, the
650 morphology was significantly different between *B. asiatica* Clade C and Clade Mixed (the
651 length of conidia). The chemotaxonomy of *Beauveria* has not been updated from a few
652 studies conducted more than three decades ago (Mugnai et al. 1989; Bridge et al. 1990),
653 which revealed that chemical profiles corresponded more or less to species based on
654 morphological criteria but were heterogenous and overlapping between populations within
655 species (e.g. *B. bassiana*). In our study, we found a discriminant pattern of secondary
656 metabolites distribution between *B. asiatica* and *B. bassiana*, but not between the clades
657 within each species. Altogether, our findings support the idea that the two *Beauveria* species
658 are composed of sibling species with similar phenotypes, i.e. cryptic species.

659 Our study also suggested ecological differentiation between these clades. *Beauveria*
660 is known to display a very broad host range as a whole (Imoulan et al. 2017) but the host
661 ranges of the various species had not been well established. By reconstructing from the
662 information on the hosts' taxonomic order associated to our specimens, the most recent
663 common ancestor (MRCA) of *Beauveria* seemed to be predominantly associated to
664 *Coleoptera*. The MRCA of *B. asiatica* Clade Mixed had actually broader host range than
665 Clade C, and the MRCA of *B. bassiana* Clade A had a wider host range than Clade B and
666 C. These two particular clades seemed to have gained the ability to exploit other insect
667 orders than *Coleoptera*. This might be accompanied by specific selection, that may have
668 leaved genomic signatures which could be investigated in future studies. There are many

669 examples of pathogenic fungi showing notable genomic modification related to host range
670 and specificity (Wang & Wang 2017; Borah et al. 2018).

671

672 **Taxonomy**

673 Given the evidence discussed above, we propose *B. bassiana* Clade A and Clade B
674 as new species, named as *B. namnaoensis* and *B. neobassiana*, while Clade C which
675 includes the type strain ARSEF1564 can be considered as *B. bassiana* sensu stricto.
676 Regarding *B. asiatica* sensu lato, the Clade Mixed is proposed here as a new species, *B.*
677 *thailandica*, and the Clade C containing the type strain ARSEF4850 remains *B. asiatica* s. s.
678 The description and photographic materials for the novel species are given above in the
679 results. These species are largely overlapping in their distribution, e.g. sympatric species,
680 (Fig.S4) but are genetically well isolated with limited recombination between them. Despite
681 lack of distinctive distribution pattern of secondary metabolites, the genomic, morphological
682 and ecological data supported that they are distinct species.

683

684 **Future investigation**

685 Investigation regarding the sexual compatibility between the closely related species
686 within each of the two species complexes should be conducted in the future to assess
687 whether they could fit the criterion of reproductive isolation for biological species (De Queiroz
688 2005). Reproduction mode and mating systems are extremely variable in fungi, from pure
689 clonality to outcrossing sexual reproduction, with significant implications in genetic diversity
690 and adaptability (Billiard et al. 2011; Taylor et al. 2015). Some studies in fungal pathogens
691 have already shown that closely related cryptic species could be intersterile (e.g. *Calonectria*
692 *pauciramosa*: Lombard et al. 2010) or sexually compatible with post-mating isolation (e.g.
693 *Microbotryum* spp.: de Vienne et al. 2009). *Beauveria* was initially proposed to be an
694 anamorphic genus, i.e. being present naturally only under asexual form (Basalmo-Crivelli
695 1835). Sexual reproduction structures could however occasionally be observed in natural
696 habitats (Sanjuan et al. 2014; Khonsanit et al. 2020). Our study furthermore suggested some
697 extent of genetic recombination and sexual reproduction in all the newly proposed species.

698

699

700 **Conclusion**

701 Our work showed that whole-genome data could provide strong support for intra-
702 species genetic groups and be powerful for delimiting species. During the last decade,
703 genomics data have greatly contributed to the elucidation of species complexes in many

704 organisms (Chan et al. 2017; Cerca et al. 2021) including fungi (Sepúlveda et al. 2017;
 705 Kobmoo et al. 2019; Matute & Sepúlveda 2019). For Hypocrealean entomopathogenic fungi,
 706 such approaches should be particularly useful as there are likely many cryptic species in
 707 different genera (*Blackwellomyces* and *Cordyceps*: Mongkolsamrit et al. 2020b), *Isaria*:
 708 Mongkolsamrit et al. 2018, *Metarhizium*: Mongkolsamrit et al. 2020). Many taxonomic works
 709 were based on a few samples with a limited number of markers which has likely led to the
 710 underestimation of the diversity in this fungal group. Combining whole-genome data,
 711 thorough morphological examination with statistical analyses and chemical profiling will
 712 contribute to establish a solid basis for species discovery in fungi.

713

714

715 **References**

- 716 Abdessamad I (2019) Transitions from single-to multi-locus approach in determining
 717 cryptic and describing new species within beuveria genus (Cordycipitaceae,
 718 hypocreales): A review. *Phytotaxa* 413:257–273.
 719 <https://doi.org/10.11646/phytotaxa.413.4.1>
- 720 Aquino De Muro M, Elliott S, Moore D, et al (2005) Molecular characterisation of *Beauveria*
 721 *bassiana* isolates obtained from overwintering sites of Sunn Pests (*Eurygaster* and
 722 *Aelia* species). *Mycol Res* 109:294–306. <https://doi.org/10.1017/S0953756204001832>
- 723 Avis JC, Ball RMJ Principles of genealogical concordance in species concepts and
 724 biological taxonomy. In: Futuyma D, Antonovics J (eds) *Oxford Surveys in*
 725 *Evolutionary Biology*. Oxford Univ. Press, Oxford
- 726 Basalmo-Crivelli G (1835) Aufstellung von zwei neuen Arten Mucedineen, *Botrytis*
 727 *bassiana* und *Mucor radicans*, etc. *Linnaea* 10:609–618
- 728 Baum DA, Shaw KL (1995) Genealogical perspectives on the species problem. In: Hoch
 729 PC, Stephenson AG (eds) *Experimental and Molecular Approaches to Plant*
 730 *Biosystematics*. Missouri Botanical Garden, St. Louis, pp 289–303
- 731 Billiard S, López-Villavicencio M, Devier B, et al (2011) Having sex, yes, but with whom?
 732 Inferences from fungi on the evolution of anisogamy and mating types. *Biol. Rev.*
 733 86:421–442
- 734 Borah N, Albarouki E, Schirawski J (2018) Comparative Methods for Molecular
 735 Determination of Host-Specificity Factors in Plant-Pathogenic Fungi. *Int J Mol Sci*
 736 19:863. <https://doi.org/10.3390/ijms19030863>
- 737 Bridge PD, Abraham YJ, Cornish MC, et al (1990) The chemotaxonomy of *Beauveria*
 738 *bassiana* (Deuteromycotina: Hyphomycetes) isolates from the coffee berry borer

- 739 Hypothenemus hampei (Coleoptera: Scolytidae). *Mycopathologia* 111:85–90.
740 <https://doi.org/10.1007/BF02277310>
- 741 Cerca J, Rivera-Colón AG, Ferreira MS, et al (2021) Incomplete lineage sorting and
742 ancient admixture, and speciation without morphological change in ghost-worm cryptic
743 species. *PeerJ* 9:e10896. <https://doi.org/10.7717/peerj.10896>
- 744 Chan KO, Alexander AM, Grismer LL, et al (2017) Species delimitation with gene flow: A
745 methodological comparison and population genomics approach to elucidate cryptic
746 species boundaries in Malaysian Torrent Frogs. *Mol Ecol* 26:5435–5450.
747 <https://doi.org/10.1111/mec.14296>
- 748 Chen WH, Liu M, Huang ZX, et al (2018) *Beauveria majiangensis*, a new
749 entomopathogenic fungus from Guizhou, China. *Phytotaxa* 333:243–250.
750 <https://doi.org/10.11646/phytotaxa.333.2.8>
- 751 Cracraft J (1983) *Species Concept and Speciation Analysis*. Plenum Press, New York
- 752 Danecek P, Auton A, Abecasis G, et al (2011) The variant call format and VCFtools.
753 *Bioinformatics* 27:2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>
- 754 Darriba Di, Posada D, Kozlov AM, et al (2020) ModelTest-NG: A New and Scalable Tool for
755 the Selection of DNA and Protein Evolutionary Models. *Mol Biol Evol* 37:291–294.
756 <https://doi.org/10.1093/molbev/msz189>
- 757 De Queiroz K (2007) Species concepts and species delimitation. *Syst Biol* 56:879–886.
758 <https://doi.org/10.1080/10635150701701083>
- 759 De Queiroz K (2005) Ernst Mayr and the modern concept of species. *Syst Orig Species*
760 Ernst Mayr's 100th Anniv 243–263. <https://doi.org/10.17226/11310>
- 761 De Vienne DM, Refrégier G, Hood ME, et al (2009) Hybrid sterility and inviability in the
762 parasitic fungal species complex *Microbotryum*. *J Evol Biol*.
763 <https://doi.org/10.1111/j.1420-9101.2009.01702.x>
- 764 García-Estrada C, Cat E, Santamarta I (2016) *Beauveria bassiana* as Biocontrol Agent:
765 Formulation and Commercialization for Pest Management. In: Singh HB, Sarma BK,
766 Keswani C (eds) *Agriculturally Important Microorganisms: Commercialization and*
767 *Regulatory Requirements in Asia*. Springer Singapore, Singapore, pp 81–96
- 768 Giraud T, Gladieux P, Gavrillets S (2010) Linking the emergence of fungal plant diseases
769 with ecological speciation. *Trends Ecol Evol* 25:387–395.
770 <https://doi.org/10.1016/j.tree.2010.03.006>
- 771 Giraud T, Refrégier G, Le Gac M, et al (2008) Speciation in fungi. *Fungal Genet. Biol.*
772 45:791–802
- 773 Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies.

- 774 Mol Biol Evol 23:254–267. <https://doi.org/10.1093/molbev/msj030>
- 775 Imoulan A, Hussain M, Kirk PM, et al (2017) Entomopathogenic fungus *Beauveria*: Host
776 specificity, ecology and significance of morpho-molecular characterization in accurate
777 taxonomic classification. *J Asia Pac Entomol* 20:1204–1212.
778 <https://doi.org/10.1016/j.aspen.2017.08.015>
- 779 Jombart T, Collins C (2015) Analysing genome-wide SNP data using adegenet 2.0.0
- 780 Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7:
781 Improvements in Performance and Usability. *Mol Biol Evol* 30:772–780.
782 <https://doi.org/10.1093/molbev/mst010>
- 783 Khonsanit A, Luangsa-ard JJ, Thanakitpipattana D, et al (2020) Cryptic diversity of the
784 genus *Beauveria* with a new species from Thailand. *Mycol Prog* 19:291–315.
785 <https://doi.org/10.1007/s11557-020-01557-9>
- 786 Kobmoo N, Mongkolsamrit S, Arnarnart N, et al (2019) Population genomics revealed
787 cryptic species within host-specific zombie-ant fungi (*Ophiocordyceps unilateralis*).
788 *Mol Phylogenet Evol* 140:106580. <https://doi.org/10.1016/j.ympev.2019.106580>
- 789 Kobmoo N, Mongkolsamrit S, Tasanathai K, et al (2012) Molecular phylogenies reveal
790 host-specific divergence of *Ophiocordyceps unilateralis sensu lato* following its host
791 ants. *Mol Ecol* 21:3022–3031. <https://doi.org/10.1111/j.1365-294X.2012.05574.x>
- 792 Larget BR, Kotha SK, Dewey CN, Ané C (2010) BUCKy: Gene tree/species tree
793 reconciliation with Bayesian concordance analysis *Bret. Bioinformatics* 26:2910–2911.
794 <https://doi.org/10.1007/s00285-010-0355-7>
- 795 Le Gac M, Hood ME, Fournier E, Giraud T (2007) Phylogenetic evidence of host-specific
796 cryptic species in the anther smut fungus. *Evolution (N Y)* 61:15–26.
797 <https://doi.org/10.1111/j.1558-5646.2007.00002.x>
- 798 Lê S, Josse J, Husson F (2008) FactoMineR : An R Package for Multivariate Analysis. *J*
799 *Stat Softw* 25:253–258. <https://doi.org/10.18637/jss.v025.i01>
- 800 Liu F, Wang M, Damm U, et al (2016) Species boundaries in plant pathogenic fungi: A
801 *Colletotrichum* case study. *BMC Evol Biol* 16:1–14. [https://doi.org/10.1186/s12862-](https://doi.org/10.1186/s12862-016-0649-5)
802 [016-0649-5](https://doi.org/10.1186/s12862-016-0649-5)
- 803 Lombard L, Crous PW, Wingfield BD, Wingfield MJ (2010) Multigene phylogeny and
804 mating tests reveal three cryptic species related to *Calonectria pauciramosa*. *Stud*
805 *Mycol* 66:15–30. <https://doi.org/10.3114/sim.2010.66.02>
- 806 Maddison WP, Maddison DR (2019) Mesquite: a modular system for evolutionary analysis.
807 Version 3.61. In: <http://www.mesquiteproject.org>
- 808 Matute DR, Sepúlveda VE (2019) Fungal species boundaries in the genomics era. *Fungal*

- 809 Genet Biol 131:103249. <https://doi.org/10.1016/j.fgb.2019.103249>
- 810 McKenna A, Hanna M, Banks E, et al (2010) The Genome Analysis Toolkit: a MapReduce
811 framework for analyzing next-generation DNA sequencing data. *Genome Res*
812 20:1297–1303. <https://doi.org/10.1101/gr.107524.110>
- 813 Mei L, Chen M, Shang Y, et al (2020) Population genomics and evolution of a fungal
814 pathogen after releasing exotic strains to control insect pests for 20 years. *ISME J*.
815 <https://doi.org/10.1038/s41396-020-0620-8>
- 816 Meyling N V., Pilz C, Keller S, et al (2012) Diversity of *Beauveria* spp. isolates from pollen
817 beetles *Meligethes aeneus* in Switzerland. *J Invertebr Pathol* 109:76–82.
818 <https://doi.org/10.1016/j.jip.2011.10.001>
- 819 Mishler BD, Brandon RN (1987) Individuality, pluralism, and the phylogenetic species
820 concept. *Biol Philos* 2:397–414. <https://doi.org/10.1007/BF00127698>
- 821 Mishler BD, Theriot EC (2000) The phylogenetic species concept (sensu Mishler and
822 Theriot): Monophyly, apomorphy, and phylogenetic species concepts. In: *Species*
823 *Concepts and Phylogenetic Theory: A Debate*. Columbia University Press, New York,
824 pp 44–54
- 825 Mitina G V., Tokarev YS, Movila AA, Yli-Mattila T (2011) Polymorphism of *Beauveria*
826 *bassiana* (Deuteromycota: Hyphomycetes) strains isolated from *Ixodes ricinus* (Acari:
827 Ixodidae) in Moldova. *Ticks Tick Borne Dis* 2:50–54.
828 <https://doi.org/10.1016/j.ttbdis.2010.10.005>
- 829 Mongkolsamrit S, Khonsanit A, Thanakitpipattana D, et al (2020a) Revisiting *Metarhizium*
830 and the description of new species from Thailand. *Stud Mycol* 95:171–251.
831 <https://doi.org/10.1016/j.simyco.2020.04.001>
- 832 Mongkolsamrit S, Noisripoom W, Tasanathai K, et al (2020b) Molecular phylogeny and
833 morphology reveal cryptic species in *Blackwellomyces* and *Cordyceps*
834 (*Cordycipitaceae*) from Thailand. *Mycol Prog* 19:957–983.
835 <https://doi.org/10.1007/s11557-020-01615-2>
- 836 Mongkolsamrit S, Noisripoom W, Thanakitpipattana D, et al (2018) Disentangling cryptic
837 species with isaria-like morphs in *Cordycipitaceae*. *Mycologia* 110:230–257.
838 <https://doi.org/10.1080/00275514.2018.1446651>
- 839 Mugnai L, Bridge PD, Evans HC (1989) A chemotaxonomic evaluation of the genus
840 *Beauveria*. *Mycol Res* 92:199–209. [https://doi.org/10.1016/S0953-7562\(89\)80012-7](https://doi.org/10.1016/S0953-7562(89)80012-7)
- 841 Paradis E, Schliep K (2019) Ape 5.0: An environment for modern phylogenetics and
842 evolutionary analyses in R. *Bioinformatics* 35:526–528.
843 <https://doi.org/10.1093/bioinformatics/bty633>

- 844 Pfeifer B, Wittelsburger U, Ramos-Onsins SE, Lercher MJ (2014) PopGenome: An
845 Efficient Swiss Army Knife for Population Genomic Analyses in R. *Mol Biol Evol*
846 31:1929–1936. <https://doi.org/10.1093/molbev/msu136>
- 847 Phainuphong P, Rukachaisirikul V, Phongpaichit S, et al (2017) Diphenyl ethers and
848 indanones from the soil-derived fungus *Aspergillus unguis* PSU-RSPG204.
849 *Tetrahedron* 73:5920–5925. <https://doi.org/10.1016/j.tet.2017.08.039>
- 850 R Core Team (2020) R: A language and environment for statistical computing.
- 851 Raj A, Stephens M, Pritchard JK (2014) fastSTRUCTURE: Variational Inference of
852 Population Structure in Large SNP Data Sets. *Genetics* 197:573–589.
853 <https://doi.org/10.1534/genetics.114.164350>
- 854 Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-
855 sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs.
856 *Mycologia* 97:84–98. <https://doi.org/10.3852/mycologia.97.1.84>
- 857 Rohlf M, Churchill ACL (2011) Fungal secondary metabolites as modulators of
858 interactions with insects and other arthropods. *Fungal Genet Biol* 48:23–34.
859 <https://doi.org/10.1016/j.fgb.2010.08.008>
- 860 Ronquist F, Teslenko M, van der Mark P, et al (2012) MrBayes 3.2: Efficient Bayesian
861 Phylogenetic Inference and Model Choice Across a Large Model Space. *Syst Biol*
862 61:539–542. <https://doi.org/10.1093/sysbio/sys029>
- 863 Sanjuan T, Tabima J, Restrepo S, et al (2014) Entomopathogens of Amazonian stick
864 insects and locusts are members of the *Beauveria* species complex (*Cordyceps*
865 *sensu stricto*). *Mycologia* 106:260–275. <https://doi.org/10.3852/106.2.260>
- 866 Sepulveda VE, Marquez R, Turissini DA, et al (2017) Genome Sequences Reveal Cryptic
867 Speciation in the Human Pathogen *Histoplasma capsulatum*. *MBio* 8:e01339-17
- 868 Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis
869 of large phylogenies. *Bioinformatics* 30:1312–1313.
870 <https://doi.org/10.1093/bioinformatics/btu033>
- 871 Taylor JW, Hann-Soden C, Branco S, et al (2015) Clonal reproduction in fungi. *Proc Natl*
872 *Acad Sci* 112:8901–8908. <https://doi.org/10.1073/pnas.1503159112>
- 873 Taylor JW, Jacobson DJ, Kroken S, et al (2000) Phylogenetic Species Recognition and
874 Species Concepts in Fungi. *Fungal Genet Biol* 31:21–32.
875 <https://doi.org/10.1006/fgbi.2000.1228>
- 876 Toopaang W, Phonghanpot S, Punya J, et al (2017) Targeted disruption of the polyketide
877 synthase gene *pks15* affects virulence against insects and phagocytic survival in the
878 fungus *Beauveria bassiana*. *Fungal Biol* 121:664–675.

- 879 <https://doi.org/10.1016/j.funbio.2017.04.007>
- 880 Trissi AN, El Bouhsini M, Alsalti MN, et al (2013) Genetic diversity among summer and
881 winter *Beauveria bassiana* populations as revealed by AFLP analysis. *J Asia Pac*
882 *Entomol* 16:269–273. <https://doi.org/10.1016/j.aspen.2013.03.006>
- 883 Udompaisarn S, Toopaang W, Sae-Ueng U, et al (2020) The polyketide synthase PKS15
884 has a crucial role in cell wall formation in *Beauveria bassiana*. *Sci Rep* 10:1–16.
885 <https://doi.org/10.1038/s41598-020-69417-w>
- 886 Valero-Jiménez CA, Faino L, Spring in't Veld D, et al (2016) Comparative genomics of
887 *Beauveria bassiana*: Uncovering signatures of virulence against mosquitoes. *BMC*
888 *Genomics* 17:1–11. <https://doi.org/10.1186/s12864-016-3339-1>
- 889 Vuillemin P (1912) *Beauveria*-nouveau genre de Verticilliacies. *Bull la Société Bot Fr*
890 59:34–40
- 891 Wang C, Shah FA, Patel N, et al (2003) Molecular investigation on strain genetic
892 relatedness and population structure of *Beauveria bassiana*. *Environ Microbiol* 5:908–
893 915. <https://doi.org/10.1046/j.1462-2920.2003.00485.x>
- 894 Wang C, Wang S (2017) Insect Pathogenic Fungi: Genomics, Molecular Interactions, and
895 Genetic Improvements. *Annu Rev Entomol* 62:73–90. <https://doi.org/10.1146/annurev-ento-031616-035509>
- 897 Wang S, Miao X, Zhao W, et al (2005) Genetic diversity and population structure among
898 strains of the entomopathogenic fungus, *Beauveria bassiana*, as revealed by inter-
899 simple sequence repeats (ISSR). *Mycol Res* 109:1364–1372.
900 <https://doi.org/10.1017/S0953756205003709>
- 901 Wiley EO (1978) The Evolutionary Species Concept Reconsidered. *Syst Zool* 27:17–26
- 902 Xu Y, Orozco R, Kithsiri Wijeratne EM, et al (2009) Biosynthesis of the cyclooligomer
903 depsipeptide bassianolide, an insecticidal virulence factor of *Beauveria bassiana*.
904 *Fungal Genet Biol* 46:353–364. <https://doi.org/10.1016/j.fgb.2009.03.001>
- 905 Xu Y, Orozco R, Wijeratne EMK, et al (2008) Biosynthesis of the Cyclooligomer
906 Depsipeptide Beauvericin, a Virulence Factor of the Entomopathogenic Fungus
907 *Beauveria bassiana*. *Chem Biol* 15:898–907.
908 <https://doi.org/10.1016/j.chembiol.2008.07.011>
- 909 Zimmermann G (2007) Review on safety of the entomopathogenic fungi *Beauveria*
910 *bassiana* and *Beauveria brongniartii*. *Biocontrol Sci Technol* 17:553–596.
911 <https://doi.org/10.1080/09583150701309006>
- 912
- 913

914 **Figures Captions**

915

916 **Fig.1** Population genomics analyses: a. A circular NJ tree based on whole-genome SNPs,
 917 the red asterisks represent the type strains of *Beauveria asiatica* (ARSEF4850) and *B.*
 918 *bassiana* (ARSEF1564), thick branches correspond to those supporting 100%
 919 bootstrapp; b. Principal component analysis (PCA) for total sampling; c. Bayesian
 920 clustering for total sampling; d. PCA for *B. asiatica*; e. PCA for *B. bassiana*; f. PCA for
 921 *B. asiatica*.

922

923 **Fig.2A** Bayesian phylogenetic tree based on concatenated 1034 genes in *Beauveria* species.
 924 The red dots represent nodes supported by 1.00 posterior probability. The highlighted
 925 clades are supported by Bayesian concordance factors with 95% confidence interval.

926

927 **Fig.3** Morphometric analysis: a. Boxplots representing the distribution of the length and the
 928 width of phialides and conidia between *Beauveria asiatica* and *B. bassiana*. The red
 929 asterisks denote significant difference between the two species; b. Boxplots
 930 representing the distribution of the length and the width of phialides and conidia
 931 between the clades within *B. asiatica* (left panel) and within *B. bassiana* (right panel).

932

933 **Fig.4** Statistical analyses of secondary metabolite profiles resulting from liquid
 934 chromatography-mass spectrometry (LC-MS): a. a Euclidean distance-based NJ tree,
 935 b. Principal component analysis (PCA) for total sampling (top panel) and for the clades
 936 within *Beauveria asiatica* and *B. bassiana* (bottom panel).

937

938 **Fig.5** Maximum-likelihood reconstruction of ancestral host association.

939

940 **Fig.6** *Beauveria namnaoensis* (a–g). a–c. Fungus on the hosts; d. colony obverse on PDA
 941 after 10 d; e. colony obverse on PDA after 20 d; f. phialides and conidia; g. conidia.
 942 *Beauveria neobassiana* (h–o). h–k. Fungus on the hosts; l. colony obverse on PDA
 943 after 10 d; m. obverse on PDA after 20 d; n. phialides and conidia; o. conidia. *Beauveria*
 944 *thailandica* (a–zc). p–t. Fungus on the hosts; u. fertile head; v. perithecia; w. asci with
 945 asci caps; x. ascospore; y. part-spores; z. colony obverse on PDA after 10 d; za.
 946 obverse on PDA after 20 d; zb. phialides and conidia; zc. conidia. — Scale bars: a, d,
 947 e, i, l, m, p, z, za = 10 mm; b, c, h, i, j, k, q, r, s, t = 5 mm; u = 1 mm; v = 200 μ m; x = 20
 948 μ m; f, n, w, y, zb = 10 μ m; g, o, zc = 5 μ m.

949 **Supplementary Data**

950

951 **Fig.S1** Analyses of recombination footprint for *Beauveria asiatica* (a.) and *B. bassiana* (b.).952 The left panel represents phylogenetic networks based on SNPs from respective
953 species. The right panel represents the analyses of linkage disequilibrium (LD) decay.

954

955 **Fig.S2** Liquid chromatography-mass spectrometry (LC-MS) profiles of mycelia extracts from
956 *Beauveria asiatica* and *B. bassiana* species complexes.

957

958 **Fig.S3** Liquid chromatography-mass spectrometry (LC-MS) profiles of mycelia extracts from
959 *Beauveria spp.* included for comparison with *B. asiatica* and *B. bassiana*.

960

961 **Fig.S4** Distribution map of *Beauveria asiatica* sensu lato (left panel) and *B. bassiana* s.l.
962 (right panel).

963

964 **Table S1** List of *Beauveria* strains used in the study. The species were attributed according
965 to previous studies or routine identification in our laboratory. Specimens of *B. asiatica* were
966 proposed to be a novel species, *B. thailandica*, while some of *B. bassiana* were proposed
967 as new species, *B. neobassiana* or *B. namnaoensis* sp. nov., based on phylogenomics
968 results.

969 Note 1 : s.s. = sensu stricto, sp. nov. = species nouveau.

970 Note 2: ^a = used in analyses of secondary metabolites profiling

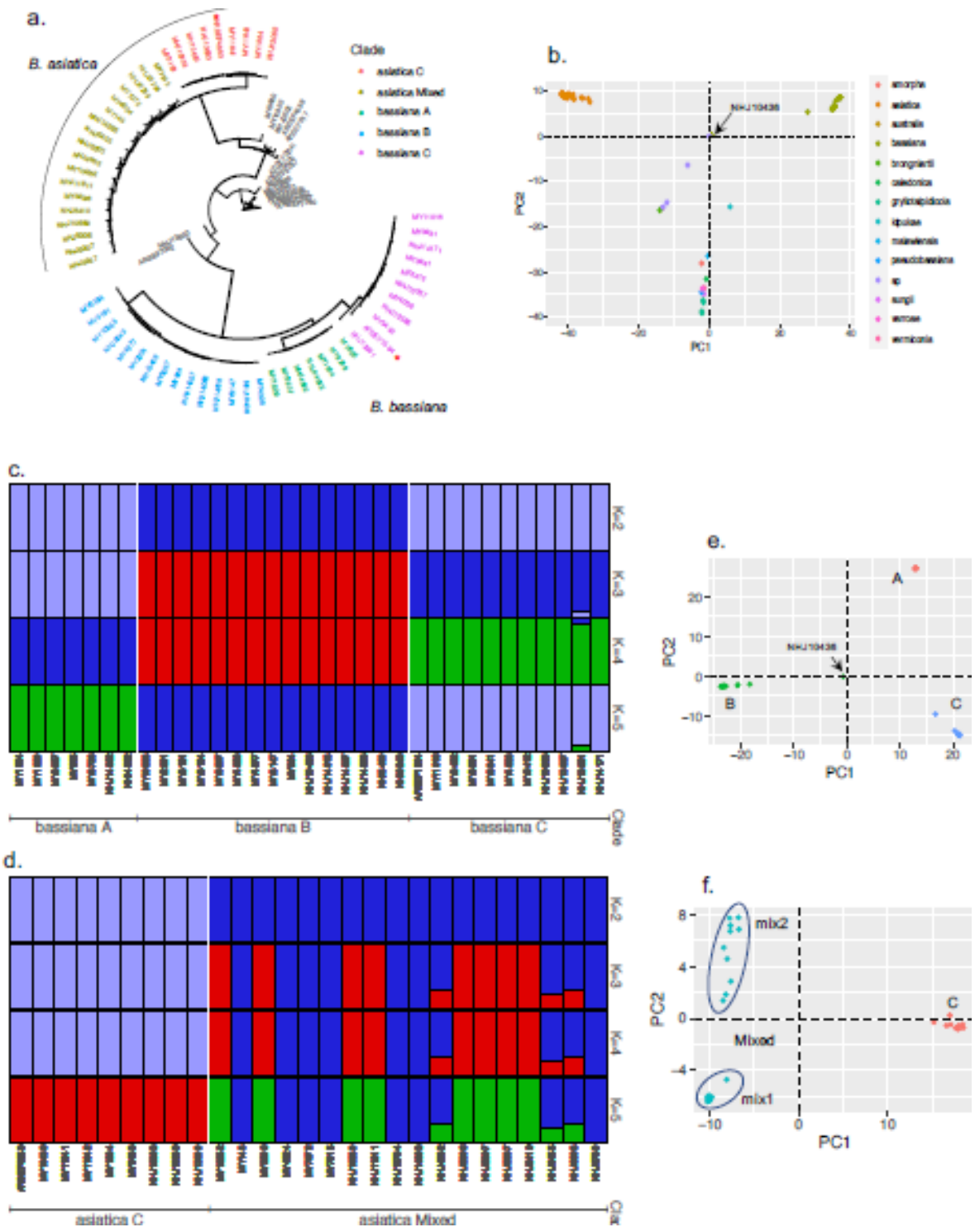
971

972 **Table S2** Average width and length (μm) of conidia and phialides per strain for the strains
973 used in statistical analyses of morphological traits.

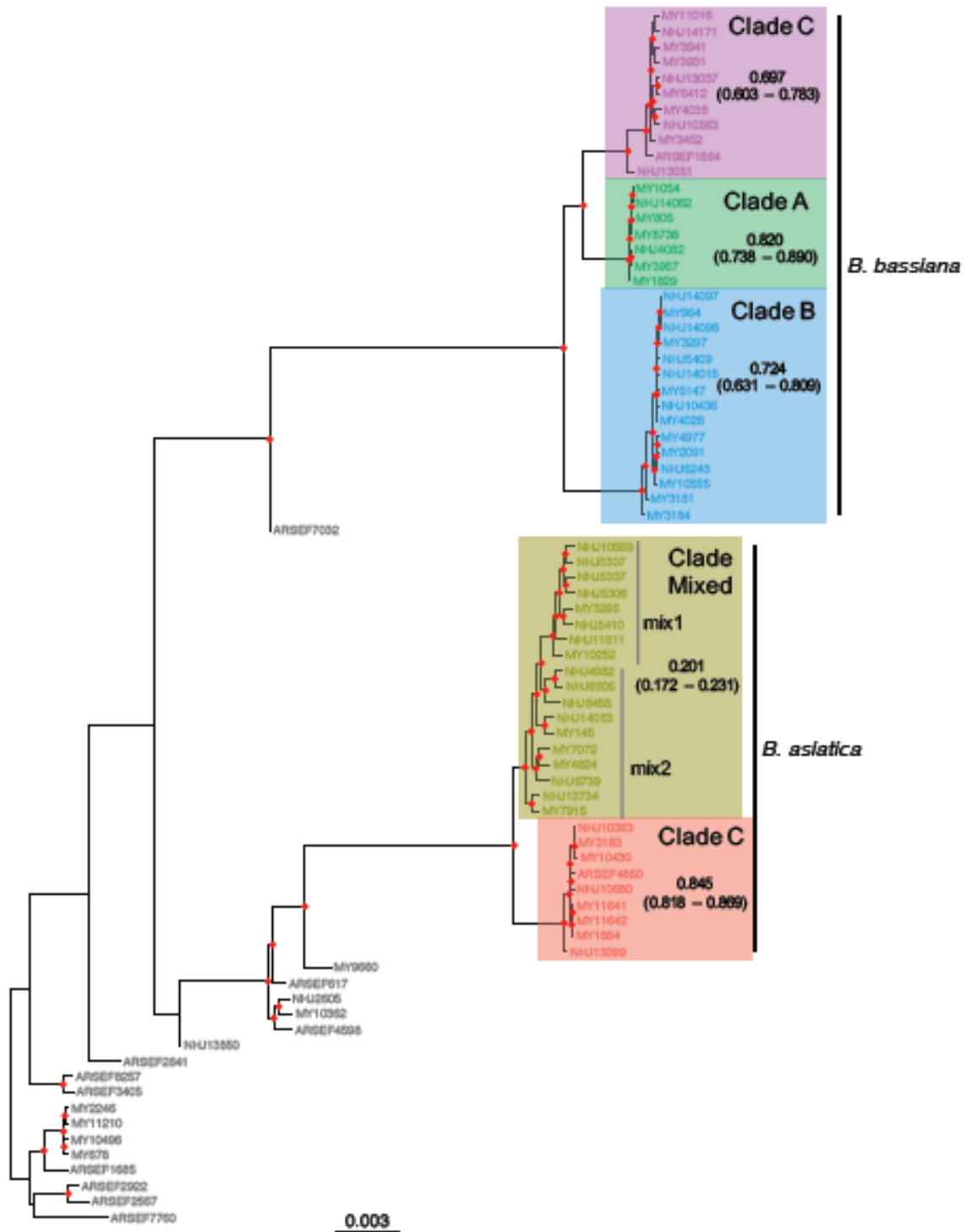
974

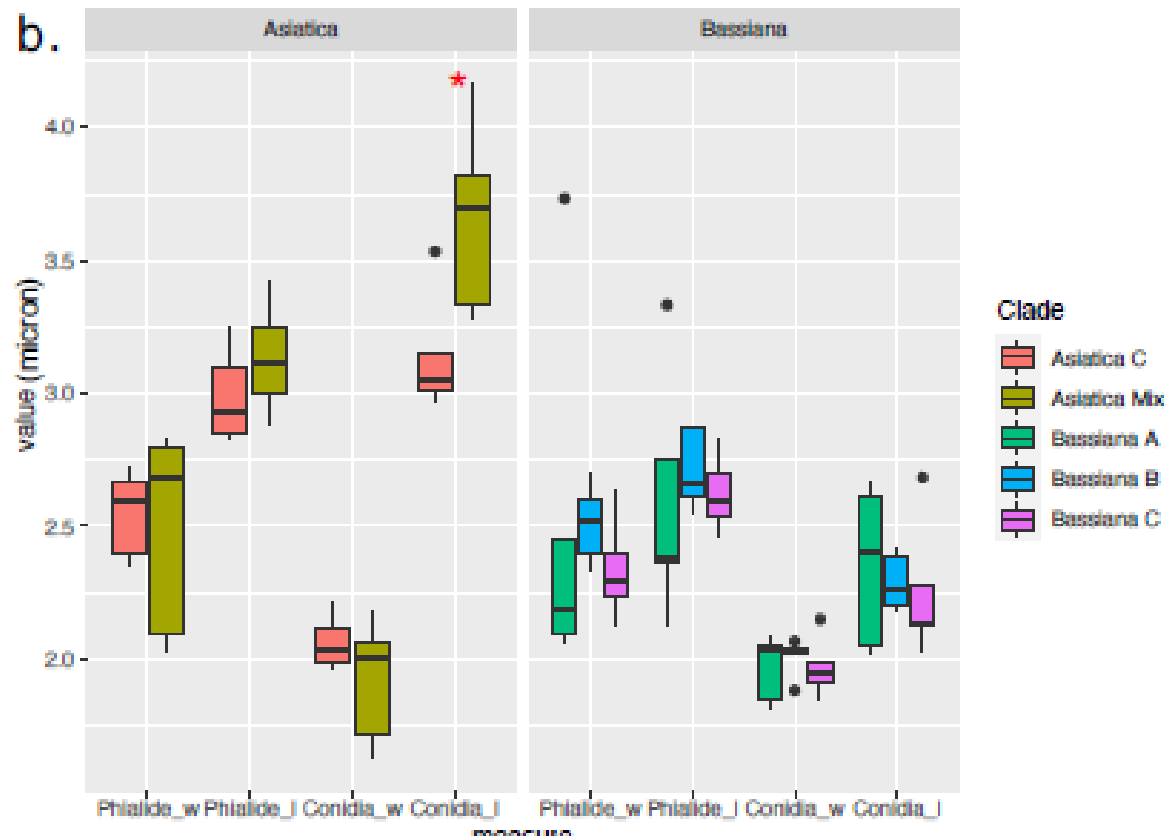
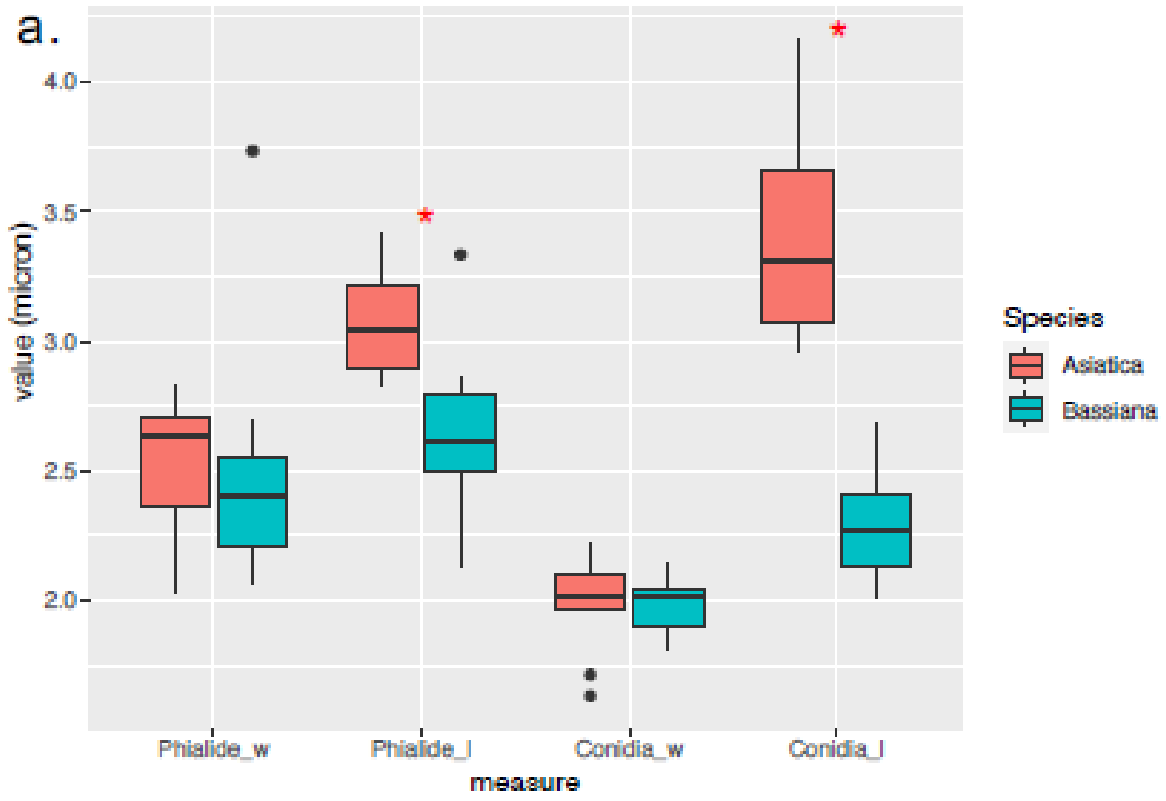
975 **Table S3** The results of statistical analyses to test difference between clades within *B.*
976 *asiatica* and *B. bassiana*. * denotes a statistical significance ($p < 0.05$).

977



978
979
980

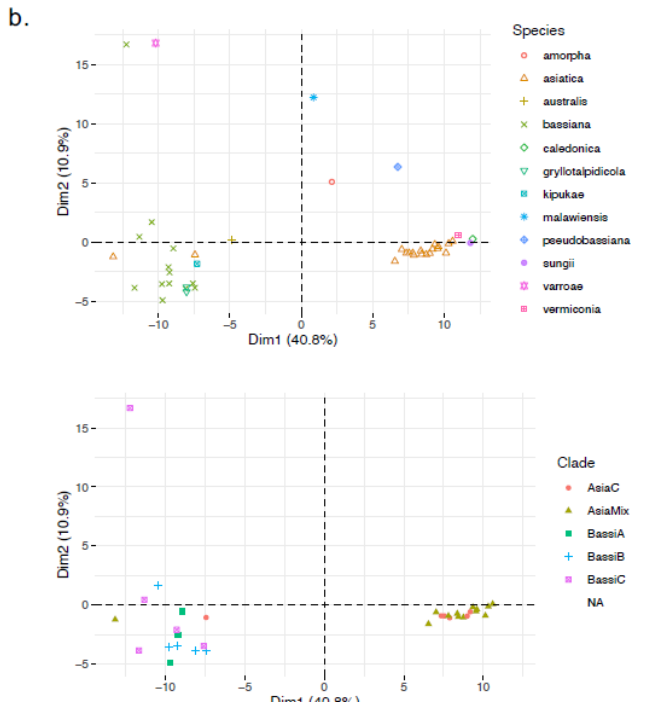
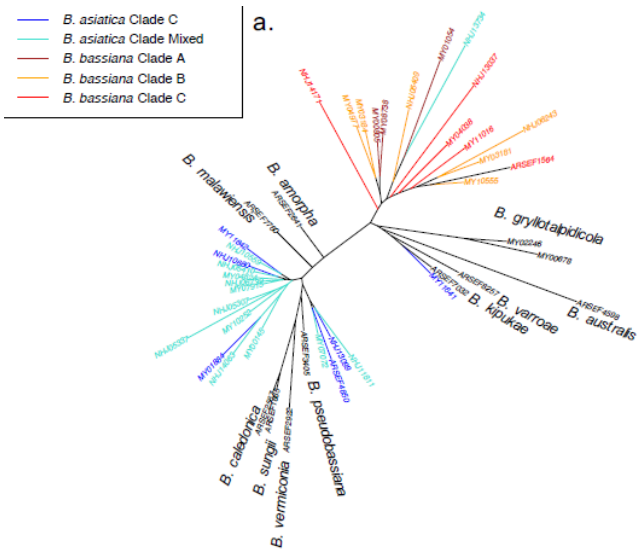


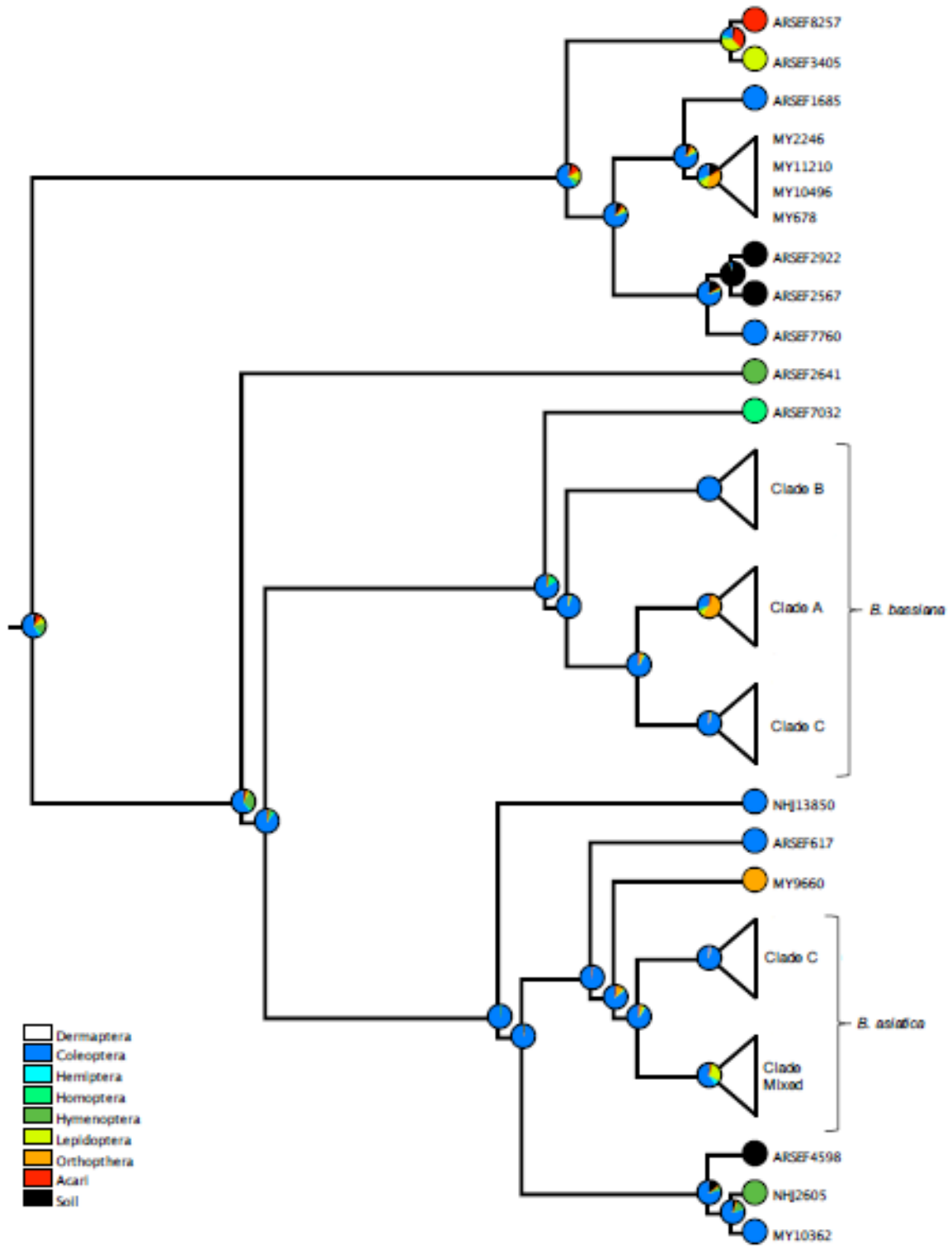


982

983

984





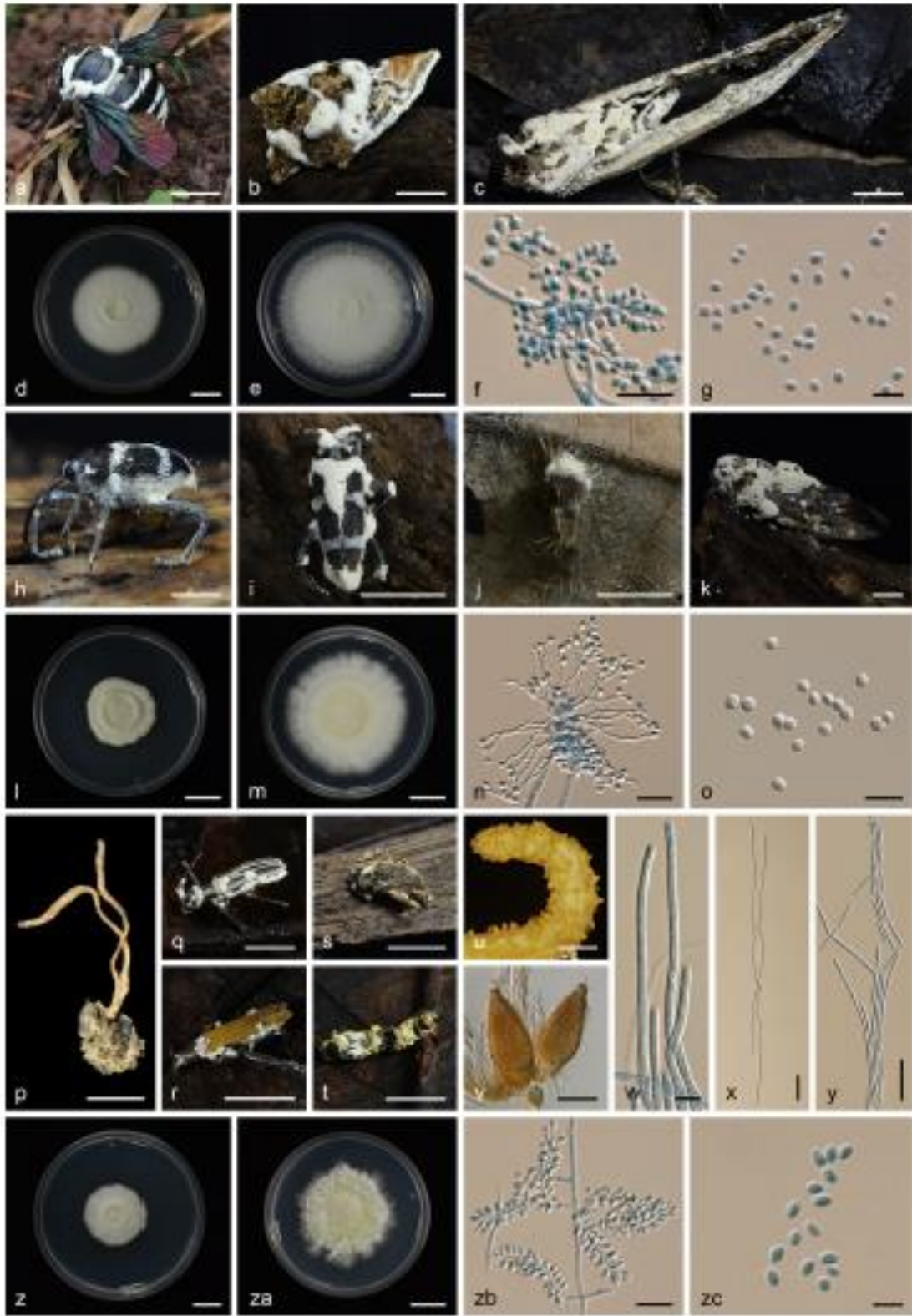
986

987

988

989

990



991

992

993