



Multi-gene genealogies reveal cryptic species *Beauveria rudraprayagi* sp. nov. from India

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Abstract

Beauveria is an insect-associated fungal genus and it represents a monophyletic lineage within the Cordycipitaceae (Hypocreales, Sordariomycetes). *Beauveria* species are economically important as mycoinsecticides and a good source of secondary metabolites. In this study, we employed the genealogical concordance phylogenetic species recognition criterion to resolve the evolutionary relationships of 125 *Beauveria* and *Beauveria*-like isolates from India. In ITS-based phylogenetic analysis, 111 fungal isolates clustered within *Beauveria* clade (Cordycipitaceae), while 14 *Beauveria*-like isolates showed phylogenetic affinities with *Isaria* (Cordycipitaceae) and *Tolypocladium* (Clavicipitaceae) clades. In a multi-gene phylogenetic analysis involving the partial *Bloc*, *EF1α*, *RPB1* and *RPB2* gene sequence-data, a novel *Beauveria* lineage was recovered that has been described here as *Beauveria rudraprayagi* sp. nov. This is the first study from India on the molecular taxonomy of *Beauveria* using GCPSR criterion.

Key words – Ascomycetes – diversity – Entomopathogenic fungi – GCPSR – taxonomy

Introduction

Beauveria (Balsamo-Crivelli) Vuill. (Cordycipitaceae, Hypocreales) includes ecologically and economically important insect-pathogenic fungal species (Posada & Vega 2005, Ownley et al. 2008, Roy et al. 2010). *Beauveria* species have been extensively studied for their role as mycoinsecticide (e.g., Thomas & Read 2007) and as sources of secondary metabolites such as bassianolide, beauvericin, non-ribosomal peptides, polyketides, terpenoids etc. (Xu et al. 2007, Ames & Walsh 2010, Molnar et al. 2010). The taxonomy of the genus, however, is in transition. In recent years, identification techniques and delimitation of *Beauveria* species have seen major improvements because of incorporation of multigene sequence-data and sophisticated phylogenetic analysis tools (Rehner et al. 2011).

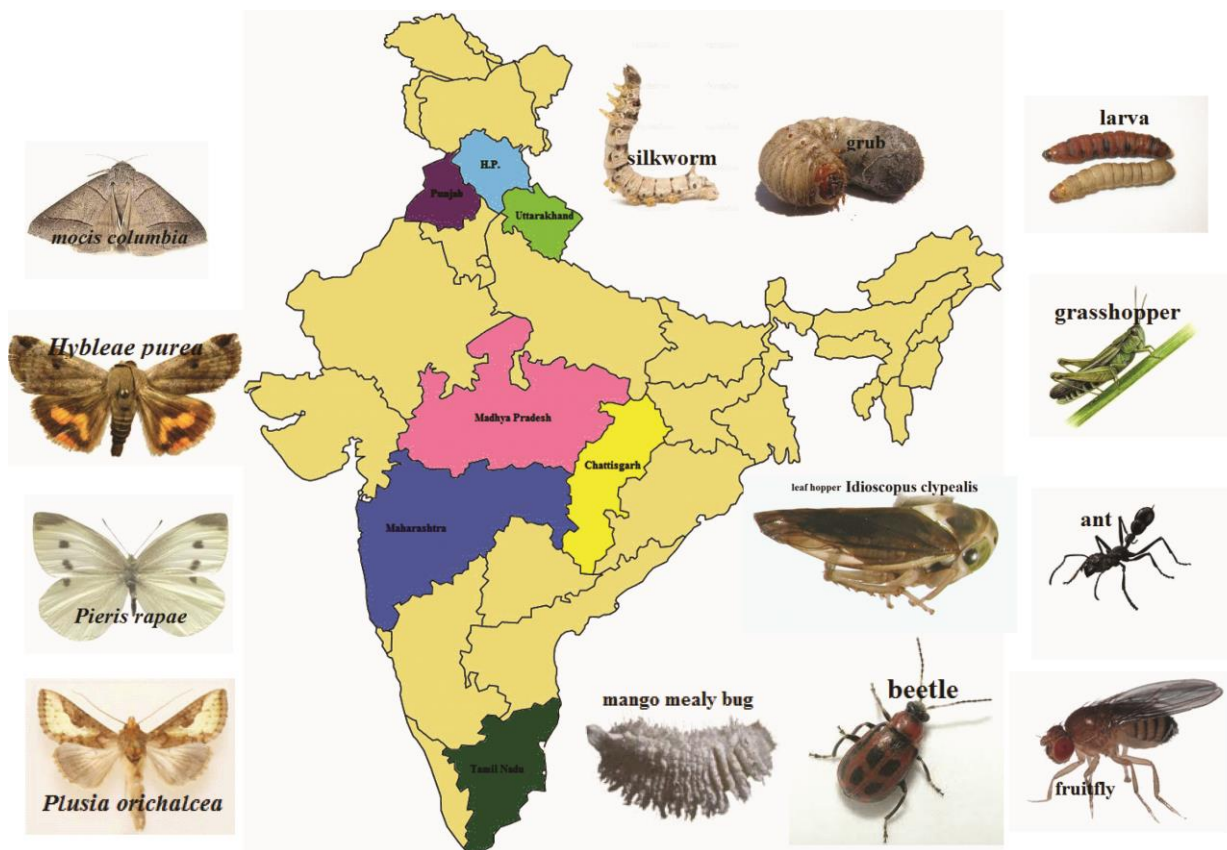


Fig. 1 – Site locations and associated hosts of *Beauveria* isolates under study from India

Deviating from traditional morphotaxonomic approach, Rehner and group (Rehner et al. 2011) have adopted multigene phylogenetic approach to revise the taxonomy of the genus and recognized 6 new species (*B. asiatica*, *B. australis*, *B. kipukae*, *B. pseudobassiana*, *B. sungii* and *B. varroae*) within monophyletic *Beauveria*. Following this approach, *Beauveria lii* sp. nov. have recently been described from China (Zhang et al. 2012). There have been, however, few studies from India on molecular taxonomy of this genus. This study, therefore, revisited the taxonomy of *Beauveria* and aimed to resolve the phylogenetic relationships of 125 *Beauveria* and *Beauveria*-like isolates of Indian origin based on the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) criterion (Taylor et al. 2000).

Materials & methods

Fungal isolates and DNA isolation

A collection of 127 *Beauveria* isolates, including 125 isolates of Indian origin (Table 1) was included in this study. The site locations of these isolates have been mapped in Fig. 1. Isolates were grown on suitable growth media as mentioned in Table 1, and incubated at 25°C for 5–8 days. The glycerol stocks were prepared from fresh mycelia or harvested conidia in 10% glycerol and stored at -70°C. The genomic DNA was extracted from fresh mycelia using ZR Fungal/ Bacterial DNA isolation kit (Zymo Research, Irvine, USA, Catalogue number D6005) following manufacturer's protocol. The concentration and quality of DNA extracted was assessed by 0.8% agarose gel electrophoresis and Nanodrop Spectrophotometer ND-1000 (Thermo Scientific, Wilmington, USA).

Table 1 Fungal isolates used in the phylogenetic analysis, including 13 ex-type strains, with their origin, host and GenBank number (ITS)

S. No.	Strain no.	Species	Culture location	Collected by	Host/ substrate	Host order	Growth condition	GenBank number: ITS
1	MTCC 557	<i>B. nivea</i>	ATCC, USA	NA	NA	NA	PDA, 25°C	JQ266196
2	MTCC 984	<i>B. bassiana</i>	ATCC, USA	NA	NA	NA	SDA, 25°C	JQ266094
3	MTCC 2028	<i>B. bassiana</i>	IMTECH, Chandigarh	D. Ananthapadmanaban, CSIR-IMTECH, Chandigarh	NA	NA	PCA, 25°C	JQ266095
4	MTCC 2499	<i>B. felina</i>	Pune	M.C. Srinivasan, CSIR-NCL, Pune, India	Mouse dung	NA	MYA, 25°C	JQ266096
5	MTCC 3653	<i>B. bassiana</i>	Jogendra Nagar, Dist. Mandi, HP	Rajaram, CTRATI Research Extension Center, C.S.B, Himachal Pradesh	Oak-tasar silkworm pupae	Lepidoptera	SDA, 25°C	JQ266097
6	MTCC 4105	<i>B. bassiana</i>	Maneri, Mandla, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	<i>Hyblaea puera</i>	Lepidoptera	OMA, 25°C	JQ266167
7	MTCC 4106	<i>B. bassiana</i>	Gosalpur, Jabalpur, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	Grasshopper	Orthoptera	OMA, 25°C	JQ266197
8	MTCC 4107	<i>B. bassiana</i>	Gosalpur, Jabalpur, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	Grasshopper	Orthoptera	OMA, 25°C	JQ266098
9	MTCC 4108	<i>B. bassiana</i>	Gosalpur, Jabalpur, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	Crop beetle	Coleoptera	OMA, 25°C	JQ266099
10	MTCC 4109	<i>B. bassiana</i>	Kanger, Bastar, Chhattisgarh	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	Black ant	Hymenoptera	OMA, 25°C	JQ266198
11	MTCC 4110	<i>B. bassiana</i>	Mandla, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	<i>Eutectona machaeralis</i>	Lepidoptera	OMA, 25°C	JQ266100
12	MTCC 4111	<i>B. bassiana</i>	Bijadandi, Jabalpur, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	small black ant	Hymenoptera	OMA, 25°C	JQ266101
13	MTCC 4119	<i>B. bassiana</i>	Tiratgarh, Bastar, Chhattisgarh	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	<i>Mocis columbia</i>		OMA, 25°C	JQ266175
14	MTCC 4120	<i>B. bassiana</i>	Pariyat, Jabalpur, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	<i>Hyblaea puera</i>	Lepidoptera	OMA, 25°C	JQ266102
15	MTCC 4121	<i>B. bassiana</i>	Udaipur, Mandla, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	<i>Pieris rapae</i>	Lepidoptera	OMA, 25°C	JQ266176
16	MTCC 4122	<i>B. bassiana</i>	Tikariya, Mandla, MP	R.C. Rajak and R. Thakur, R.D. University, Jabalpur	Beetle	Coleoptera	OMA, 25°C	JQ266103
17	MTCC 4492	<i>B. bassiana</i>	Katni, MP	R.G. Rajak, R.D. University, Jabalpur	<i>Pieris rapae</i>	Lepidoptera	PDA, 25°C	JQ266216
18	MTCC 4495	<i>B. bassiana</i>	Katni, MP	R.G. Rajak, R.D. University,	<i>Hyblaea puera</i>	Lepidoptera	PDA, 25°C	JQ266104

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19	MTCC 4496	<i>B. bassiana</i>	Katni, MP	Jabalpur R.G. Rajak, R.D. University,	Grasshopper	Orthoptera	PDA, 25°C	JQ266199
20	MTCC 4497	<i>B. bassiana</i>	Katni, MP	Jabalpur R.G. Rajak, R.D. University,	Crop beetle	Coleoptera	PDA, 25°C	JQ266217
21	MTCC 4498	<i>B. bassiana</i>	Balaghat, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i> <i>machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266177
22	MTCC 4499	<i>B. bassiana</i>	Balaghat, MP	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266105
23	MTCC 4500	<i>B. bassiana</i>	Balaghat, MP	Jabalpur R.G. Rajak, R.D. University,	Black ant	Hymenoptera	PDA, 25°C	JQ266200
24	MTCC 4501	<i>B. bassiana</i>	Balaghat, MP	Jabalpur R.G. Rajak, R.D. University,	Grub	Coleoptera	PDA, 25°C	JQ266106
25	MTCC 4502	<i>Beauveria</i> sp.	Dantewada, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266178
26	MTCC 4503	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266107
27	MTCC 4504	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	NA	NA	PDA, 25°C	JQ266179
28	MTCC 4505	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	NA	NA	PDA, 25°C	JQ266108
29	MTCC 4506	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266220
30	MTCC 4507	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266109
31	MTCC 4508	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266180
32	MTCC 4509	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i> <i>machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266201
33	MTCC 4510	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i> <i>machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266168
34	MTCC 4511	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i> <i>machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266218
35	MTCC 4512	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i> <i>machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266110
36	MTCC 4513	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Hyblaea puera</i>	Lepidoptera	PDA, 25°C	JQ266181
37	MTCC 4514	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Hyblaea puera</i>	Lepidoptera	PDA, 25°C	JQ266182

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38	MTCC 4515	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Hyblaea puera</i>	Lepidoptera	PDA, 25°C	JQ266183
39	MTCC 4516	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266184
40	MTCC 4517	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Hyblaea puera</i>	Lepidoptera	PDA, 25°C	JQ266185
41	MTCC 4526	<i>Beauveria</i> sp.	Dantewada, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266129
42	MTCC 4527	<i>Beauveria</i> sp.	Dantewada, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266202
43	MTCC 4528	<i>B. bassiana</i>	Chhindwara, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266140
44	MTCC 4529	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266186
45	MTCC 4530	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266195
46	MTCC 4531	<i>B. bassiana</i>	Betul, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266141
47	MTCC 4532	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Hyblaea puera</i>	Lepidoptera	PDA, 25°C	JQ266142
48	MTCC 4533	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Hyblaea puera</i>	Lepidoptera	PDA, 25°C	JQ266143
49	MTCC 4534	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Hyblaea puera</i>	Lepidoptera	PDA, 25°C	JQ266111
50	MTCC 4535	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266144
51	MTCC 4536	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	Black ant	Hymenoptera	PDA, 25°C	JQ266112
52	MTCC 4537	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266113
53	MTCC 4538	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266114
54	MTCC 4539	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266187
55	MTCC 4540	<i>B. bassiana</i>	Hoshangabad, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266115
56	MTCC 4542	<i>B. bassiana</i>	Chhindwara, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266116

S. No.	Strain no.	Species	Culture location	Collected by	Host/ substrate	Host order	Growth condition	GenBank number: ITS
57	MTCC 4543	<i>B. bassiana</i>	Chhindwara, MP	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> <i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266219
58	MTCC 4544	<i>B. bassiana</i>	Chhindwara, MP	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> <i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266117
59	MTCC 4546	<i>B. bassiana</i>	Chhindwara, MP	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> <i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266188
60	MTCC 4547	<i>B. bassiana</i>	Chhindwara, MP	Jabalpur R.G. Rajak, R.D. University,	Fruit fly	Diptera	PDA, 25°C	JQ266118
61	MTCC 4548	<i>B. bassiana</i>	Chhindwara, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266119
62	MTCC 4549	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	Black ant	Hymenoptera	PDA, 25°C	JQ266189
63	MTCC 4550	<i>B. bassiana</i>	Seoni, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266120
64	MTCC 4551	<i>B. bassiana</i>	Seoni, MP	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> <i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266121
65	MTCC 4552	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> Beetle	Coleoptera	PDA, 25°C	JQ266122
66	MTCC 4553	<i>B. bassiana</i>	Seoni, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266130
67	MTCC 4554	<i>B. bassiana</i>	Seoni, MP	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> <i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266131
68	MTCC 4557	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> <i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266132
69	MTCC 4559	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> <i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266190
70	MTCC 4560	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> <i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266123
71	MTCC 4562	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>machaeralis</i> Fly	Diptera	PDA, 25°C	JQ266133
72	MTCC 4563	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	unidentified insect	NA	PDA, 25°C	JQ266191
73	MTCC 4564	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266134
74	MTCC 4565	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266124
75	MTCC 4566	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266154

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76	MTCC 4567	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266192
77	MTCC 4568	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266169
78	MTCC 4569	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266125
79	MTCC 4571	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266126
80	MTCC 4572	<i>B. bassiana</i>	Mandla, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266145
81	MTCC 4575	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266193
82	MTCC 4576	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266155
83	MTCC 4577	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266203
84	MTCC 4578	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266135
85	MTCC 4579	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona machaeralis</i>	Lepidoptera	PDA, 25°C	JQ266156
86	MTCC 4580	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	Insect	NA	PDA, 25°C	JQ266127
87	MTCC 4581	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266157
88	MTCC 4582	<i>B. bassiana</i>	Jabalpur, MP	Jabalpur R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266158
89	MTCC 4583	<i>Beauveria</i> sp.	Ambikapur, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266159
90	MTCC 4584	<i>Beauveria</i> sp.	Ambikapur, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266204
91	MTCC 4585	<i>Beauveria</i> sp.	Ambikapur, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266160
92	MTCC 4587	<i>Beauveria</i> sp.	Ambikapur, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266170
93	MTCC 4589	<i>Beauveria</i> sp.	Ambikapur, Chhattisgarh	Jabalpur R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266213
94	MTCC 4591	<i>Beauveria</i>	Dantewada,	Jabalpur R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266146

S. No.	Strain no.	Species	Culture location	Collected by	Host/ substrate	Host order	Growth condition	GenBank number: ITS
		sp.	Chhattisgarh	Jabalpur	<i>machaeralis</i>			
95	MTCC 4593	<i>Beauveria</i>	Dantewada,	R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266147
		sp.	Chhattisgarh	Jabalpur	<i>machaeralis</i>			
96	MTCC 4594	<i>Beauveria</i>	Bastar,	R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266194
		sp.	Chhattisgarh	Jabalpur	<i>machaeralis</i>			
97	MTCC 4595	<i>Beauveria</i>	Bastar,	R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266148
		sp.	Chhattisgarh	Jabalpur	<i>machaeralis</i>			
98	MTCC 4596	<i>Beauveria</i>	Bastar,	R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266149
		sp.	Chhattisgarh	Jabalpur	<i>machaeralis</i>			
99	MTCC 4597	<i>Beauveria</i>	Bastar,	R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266171
		sp.	Chhattisgarh	Jabalpur	<i>machaeralis</i>			
100	MTCC 4598	<i>B. bassiana</i>	Bastar,	R.G. Rajak, R.D. University,	<i>Eutectona</i>	Lepidoptera	PDA, 25°C	JQ266205
			Chhattisgarh	Jabalpur	<i>machaeralis</i>			
101	MTCC 4599	<i>B. bassiana</i>	Shahdol, MP	R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266206
				Jabalpur				
102	MTCC 4600	<i>B. bassiana</i>	Shahdol, MP	R.G. Rajak, R.D. University,	<i>Plusia orichalcea</i>	Lepidoptera	PDA, 25°C	JQ266128
				Jabalpur				
103	MTCC 4605	<i>B. bassiana</i>	Shahdol, MP	R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266207
				Jabalpur				
104	MTCC 4606	<i>Beauveria</i>	Shahdol, MP	R.G. Rajak, R.D. University,	Beetle	Coleoptera	PDA, 25°C	JQ266136
		sp.		Jabalpur				
105	MTCC 6097	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu,	<i>Eutectona</i>	Lepidoptera	PCA, 25°C	JQ266161
				R.D. University, Jabalpur	<i>machaeralis</i>			
106	MTCC 6098	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu,	<i>Eutectona</i>	Lepidoptera	PCA, 25°C	JQ266162
				R.D. University, Jabalpur	<i>machaeralis</i>			
107	MTCC 6099	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu,	<i>Eutectona</i>	Lepidoptera	PCA, 25°C	JQ266163
				R.D. University, Jabalpur	<i>machaeralis</i>			
108	MTCC 6100	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu,	<i>Eutectona</i>	Lepidoptera	PCA, 25°C	JQ266164
				R.D. University, Jabalpur	<i>machaeralis</i>			
109	MTCC 6286	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu,	<i>Eutectona</i>	Lepidoptera	PCA, 25°C	JQ266208
				R.D. University, Jabalpur	<i>machaeralis</i>			
110	MTCC 6287	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu,	<i>Eutectona</i>	Lepidoptera	PCA, 25°C	JQ266209
				R.D. University, Jabalpur	<i>machaeralis</i>			
111	MTCC 6288	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu,	<i>Eutectona</i>	Lepidoptera	PCA, 25°C	JQ266210
				R.D. University, Jabalpur	<i>machaeralis</i>			
112	MTCC 6289	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu,	<i>Eutectona</i>	Lepidoptera	PCA, 25°C	JQ266137
				R.D. University, Jabalpur	<i>machaeralis</i>			
113	MTCC 6291	<i>B. bassiana</i>	Dantewada,	S.S. Sandhu,	<i>Hyblaea puera</i>	Lepidoptera	PCA, 25°C	JQ266211

S. No.	Strain no.	Species	Culture location	Collected by	Host/ substrate	Host order	Growth condition	GenBank number: ITS
114	MTCC 6294	<i>B. felina</i>	Chhattisgarh Hoshangabad, MP	R.D. University, Jabalpur S.S. Sandhu, R.D. University, Jabalpur	Beetle	Coleoptera	PCA, 25°C	JQ266212
115	MTCC 6297	<i>B. bassiana</i>	Dantewada, Chhattisgarh	S.S. Sandhu, R.D. University, Jabalpur	<i>Hyblaea puera</i>	Lepidoptera	PCA, 25°C	JQ266174
116	MTCC 6298	<i>B. bassiana</i>	Bastar, Chhattisgarh	S.S. Sandhu, R.D. University, Jabalpur	<i>Eutectona machaeralis</i>	Lepidoptera	PCA, 25°C	JQ266138
117	MTCC 6341	<i>B. bassiana</i>	Mandla, MP	S.S. Sandhu, R.D. University, Jabalpur	<i>Eutectona machaeralis</i>	Lepidoptera	PCA, 25°C	JQ266139
118	MTCC 6685	<i>B. bassiana</i>	Dindigul, Tamil Nadu	A. Balu, IFGTB,Coimbatore	Soil	NA	PDA, 25°C	JQ266165
119	MTCC 6779	<i>B. bassiana</i>	Dindigul, Tamil Nadu	A. Balu, IFGTB,Coimbatore	Field infected larva	NA	PCA, 25°C	JQ266166
120	MTCC 7689	<i>B. bassiana</i>	Dindigul, Tamil Nadu	A. Balu, IFGTB,Coimbatore	Field infected larva	NA	PDA, 25°C	JQ266172
121	MTCC 7690	<i>B. bassiana</i>	Dindigul, Tamil Nadu	A. Balu, IFGTB,Coimbatore	Soil	NA	PDA, 25°C	JQ266150
122	MTCC 8017	<i>B. bassiana</i>	Ukhimath, Rudraprayag, Uttarakhand	D Ananthapadmanaban, CSIR-IMTECH, Chandigarh	Silkworm	Lepidoptera	PDA, 25°C	JQ266173
123	MTCC 9348	<i>B. bassiana</i>	US Nagar, Uttarakhand	J. Kumar, College of Agricult ure, G.B. Pant University , U.S. Nagar	Infected <i>Ideoscopus clypealis</i>	Hemiptera	PCA, 25°C	JQ266151
124	MTCC 9967	<i>B. bassiana</i>	Pant Nagar, Uttarakhand	NA	Mango mealy bug	Hemiptera	PDA, 25°C	JQ266152
125	MTCC 9968	<i>B. bassiana</i>	Pant Nagar, Uttarakhand	NA	Mango mealy bug	Hemiptera	PDA, 25°C	JQ266153
126	NFCCI-1	<i>B. bassiana</i>	NA	NA	NA	NA	PDA, 25°C	JQ266214
127	NFCCI- 2133	<i>B. bassiana</i>	NA	NA	NA	NA	PDA, 25°C	JQ266215
128	ARSEF 2567*	<i>B. caledonica</i>	Scotland	Bissett and Widden, 1988	Soil	NA		AY532003
129	ARSEF 4850*	<i>B. asiatica</i>	Republic of Korea	JM Sung, 1994	Coleoptera: Cerambycidae	Coleoptera		AY531936
130	ARSEF 2641*	<i>B. amorpha</i>	Brazil	SB Alves, 1988	Hymenoptera: Formicidae	Hymenopter a		AY532008

S. No.	Strain no.	Species	Culture location	Collected by	Host/ substrate	Host order	Growth condition	GenBank number: ITS
131	ARSEF 1564*	<i>B. bassiana</i>	Italy	KV Deseo, 1984	Lepidoptera: Arctiidae	Lepidoptera		HQ880761
132	ARSEF 3405*	<i>B. pseudobassiana</i>	USA, Kentucky	AE Hajek, 1991	Lepidoptera: Tortricidae	Lepidoptera		AY532022
133	ARSEF 4598*	<i>B. australis</i>	Australia	AC Rath, 1988	Soil	NA		HQ880789
134	ARSEF 7032*	<i>B. kipukae</i>	USA, Hawaii	RG Hollingsworth, 2002	Homoptera: Delphacidae	Homoptera		HQ880803
135	ARSEF 8257*	<i>B. varroae</i>	France	WG Meikle and G Mercadier, 2005	Acari: Varroidae	Acari		HQ880800
136	ARSEF 617*	<i>B. brongniartii</i>	France	P. Ferron, 1981	Coleoptera: Scarabaeidae	Coleoptera		HQ880782
137	ARSEF 7760*	<i>B. malawiensis</i>	Malawi	Rehner, 2006b	Coleoptera: Cerambycidae	Coleoptera		DQ376247
138	ARSEF 1685*	<i>B. sungii</i>	Japan	M. Shimazu, 1984	Coleoptera: Scarabaeidae	Coleoptera		AY531990
139	ARSEF 2922*	<i>B. vermiconia</i>	Chile	de Hoog and V. Rao, 1975	Soil	NA		AY532012
140	RCEF5500* / ARSEF 11741*	<i>B. lii</i>	China	Ling-ming He, 2010	<i>Henosepilachna vigintioctopunctata</i>	Coleoptera		JN689372

Species names included are as provided by culture collection centers (primarily based on morphological characteristics).

Ex-type strains are indicated with '*'.

Abbreviations:

- MTCC: Microbial Type Culture Collection, Chandigarh, India
- IMTECH: Institute of Microbial Technology, Chandigarh, India
- ATCC: American Type Culture Collection, Manassas, USA
- ARSEF: The Agricultural Research Service Collection of Entomopathogenic Fungi, USDA-ARS, Ithaca, NY, USA
- NFCCI: National Fungal Culture Collection of India, ARI, Pune, India
- HP: Himachal Pradesh, MP: Madhya Pradesh
- PDA: Potato dextrose agar, PCA: Potato carrot agar, SDA: Sabouraud dextrose agar, MYA: Malt yeast agar, OMA: Oatmeal agar
- NA: (information) not available

PCR amplification and sequencing of the ITS region from 127 Beauveria isolates

To start with, the 5.8 S ribosomal RNA and the flanking internal transcribed spacer gene region (ITS) was amplified from 127 *Beauveria* isolates using polymerase chain reaction (PCR). PCR was done in a 50 µl reaction volume. Each reaction tube contained 100 ng of total genomic DNA, 1x Taq Buffer (NEB, Ipswich, MA), 0.5 µM each of forward primer ITS5 and reverse primer ITS4 (White et al. 1990), 1.5 mM MgCl₂, 0.2 mM each dNTPs, 1.5 units Taq Polymerase (NEB, Ipswich, MA) and remaining sterile water. The PCR reactions were carried out in Eppendorf Mastercycler with cycling parameters as previously described (Rehner & Buckley 2005). The PCR products were sequenced using respective primers with ABI Big dye v3.1 terminator ready reaction cycle sequencing kit (Applied Biosystems, Carlsbad, California) using manufacturer's protocol. The samples were purified to remove excess salt, denatured with HiDi-Formamide at 95°C for 3 minutes and analyzed using 3730 DNA Analyzer (Applied Biosystems, Carlsbad, California) at the central DNA sequencing facility at the Institute of Microbial Technology, Chandigarh, India.

ITS-based phylogenetic analysis

Sequences obtained from the respective primers (ITS5 and ITS4) were aligned and consensus sequences were generated using Sequencher 4.10.1 (Gene codes Corp., Ann Arbor, MI, USA) and were deposited in NCBI-GenBank (Table 1). Sequences were assembled and aligned using the web-interface of MAFFT (Kato et al. 2005) and MEGA version 5.0 (Tamura et al. 2011). The alignment was manually adjusted to allow maximum alignment and minimize gaps. The 127 newly generated sequences were aligned with 43 sequences including the sequence of *B.lii* (Zhang et al. 2012), 7 *Tolypocladium* sequences and 36 sequences used in the analysis of Rehner et al. 2011 (33 *Beauveria* sequences and 3 *Isaria* sequences).

Molecular phylogenetic reconstruction was performed using MEGA/ PAUP version 4.0 (Swofford 2003). Ambiguously aligned regions were excluded from the analysis. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura & Nei 1993). Gaps were treated as missing data. Trees were inferred using the heuristic search option with 1000 random sequence additions. Max trees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics such as tree length (TL), consistency index (CI), retention index (RI), related consistency index (RC), homoplasy index (HI) and log likelihood (-lnL) were calculated for the trees generated. Clade stability was assessed in bootstrap analysis with 1000 replicates each with 10 replicates of random step-wise addition of taxa. Kishino-Hasegawa (KH) tests (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Trees were figured in TreeView (Page 1996) and FigTree version 1.4.0 (<http://tree.bio.ed.ac.uk/>).

Multi-gene sequence analysis

On the basis of the phylogenetic tree retrieved from the ITS-based analysis (Fig. 2), sixteen *Beauveria* isolates were selected for multi-gene phylogenetic analysis (Table 2). PCR amplification of selected genes (nuclear transgenic factor *Bloc*, the translation elongation factor 1α (*EF-1α*) and partial sequences of largest subunits of ribosomal polymerase gene *RPB1*, *RPB2*) was done using primer-set and PCR conditions as already described (Rehner et al. 2011).

For each of the sixteen isolates, the consensus sequences for *Bloc*, *EF-1α*, *RPB1*, and *RPB2* were generated using Sequencher. A multi-gene dataset was prepared using the sequences from this study and selected sequences from the dataset of Rehner et al. 2011 and *B.lii* (Zhang et al. 2012) using MEGA and Sequence Matrix 1.7.8 (Vaidya et al. 2011). The combined dataset comprised of 85 *Beauveria* isolates and *Cordyceps militaris* as outgroup. Maximum parsimony analysis using the multi-gene dataset was performed in PAUP using search parameters as described in the ITS-based analysis. Maximum likelihood analysis was performed using RAxML (Stamatakis et al. 2008). All the datasets have been submitted to TreeBASE (www.treebase.org) and the sequences have been deposited in NCBI-GenBank with accession number detailed in Table 2.

Table 2 Selected strains for multi-gene phylogeny and their GenBank numbers.

Strain	Culture location	Host/ Substrate	Bloc	<i>EF-1α</i>	<i>RPB1</i>	<i>RPB2</i>
MTCC 984	ATCC, USA	NA	JQ990832	JQ990898	JQ990876	JQ990854
MTCC 4507	Betul, MP	Beetle	JQ990836	JQ990902	JQ990880	JQ990858
MTCC 4508	Betul, MP	Beetle	JQ990837	JQ990903	JQ990881	JQ990859
MTCC 4509	Betul, MP	<i>Eutectona machaeralis</i>	JQ990838	JQ990904	JQ990882	JQ990860
MTCC 4553	Seoni, MP	<i>Eutectona machaeralis</i>	JQ990840	JQ990906	JQ990884	JQ990862
MTCC 4585	Ambikapur, Chhattisgarh	Beetle	JQ990841	JQ990907	JQ990885	JQ990863
MTCC 4599	Shahdol, MP	Beetle	JQ990843	JQ990909	JQ990887	JQ990865
MTCC 6098	Mandla, MP	<i>Eutectona machaeralis</i>	JQ990844	JQ990910	JQ990888	JQ990866
MTCC 6294	Hoshangabad, MP	Beetle	JQ990845	JQ990911	JQ990889	JQ990867
MTCC 6685	Tamil Nadu	Soil	JQ990847	JQ990913	JQ990891	JQ990869
MTCC 8017	Ukhimath, Rudraprayag, Uttarakhand	Silkworm	JQ990848	JQ990914	JQ990892	JQ990870
MTCC 9967	Uttarakhand	Mango mealy bug	JQ990849	JQ990915	JQ990893	JQ990871
MTCC 9968	Uttarakhand	Mango mealy bug	JQ990850	JQ990916	JQ990894	JQ990872
NFCCI-1	NA	NA	JQ990851	JQ990917	JQ990895	JQ990873
NFCCI-2133	NA	NA	JQ990852	JQ990918	JQ990896	JQ990874

Morphological study

Colony morphology was recorded for the recovered novel species from this study (*B. rudraprayagi* sp. nov. MTCC 8017). The isolate was grown on Sabouraud's dextrose agar (SDA) and potato dextrose agar (PDA) at 25°C for 7 days. Microscopic measurements of conidiogenous cells and conidia were taken from slide cultures at 7–10 days. Images were captured with Olympus XC10 digital camera attached with Olympus BX51 microscope, and analyzed using CellB image analysis software (Olympus, Tokyo, Japan).

Results

ITS phylogeny

The ITS sequence dataset included 170 sequences, including 127 newly generated from this study. *Cordyceps militaris* ARSEF 5050 was the designated outgroup. The ITS dataset comprised 583 characters. In un-weighted parsimony (UP) analysis, when gaps were treated as missing data, there were 381 constant, 71 parsimony-uninformative and 131 parsimony-informative characters. This analysis resulted in 10000 trees. Based on KH test, the resultant UP trees was not significantly different (details not shown). One of the 10000 UP trees with the sum of branch length 0.74823850 is shown in Fig. 2. Bootstrap values equal to or more than 50% (for 1000 replicates) are shown next to the branches.

The 127 *Beauveria* isolates clustered in 5 distinct clades (Fig. 2). Clade-I included the ex-type strain of *B. bassiana* ARSEF 1564, 102 non ex-type strains of *B. bassiana* from India, 8 *Beauveria* isolates with undesignated species name (MTCC 4526, MTCC 4583, MTCC 4584, MTCC 4585, MTCC 4587, MTCC 4589, MTCC 4597, MTCC 4606) and one isolate identified as '*B. felina*' MTCC 6294. Clade-II included the ex-type strain of *B. varroae* ARSEF 8257 and an isolate identified as '*B. bassiana*' MTCC 9968. Clade-III included the ex-type strain of *B. pseudobassiana* ARSEF 3405 and an isolate identified as '*B. bassiana*' MTCC 8017. Clade IV included 5 isolates identified as '*B. bassiana*' (MTCC 4111, MTCC 4550, MTCC 6291, MTCC 6297, MTCC 6298), 7 *Beauveria* isolates with undesignated species name (MTCC 4502, MTCC 4527, MTCC 4591, MTCC 4593, MTCC 4594, MTCC 4595, MTCC 4596) and the ex-type strain of *Isaria cicadae* ARSEF 4029. Clade-V included 2 isolates; one identified as '*B. felina*' MTCC 2499 and another as '*B. nivea*' MTCC 557 and 3 sequences of *Tolypocladium*.

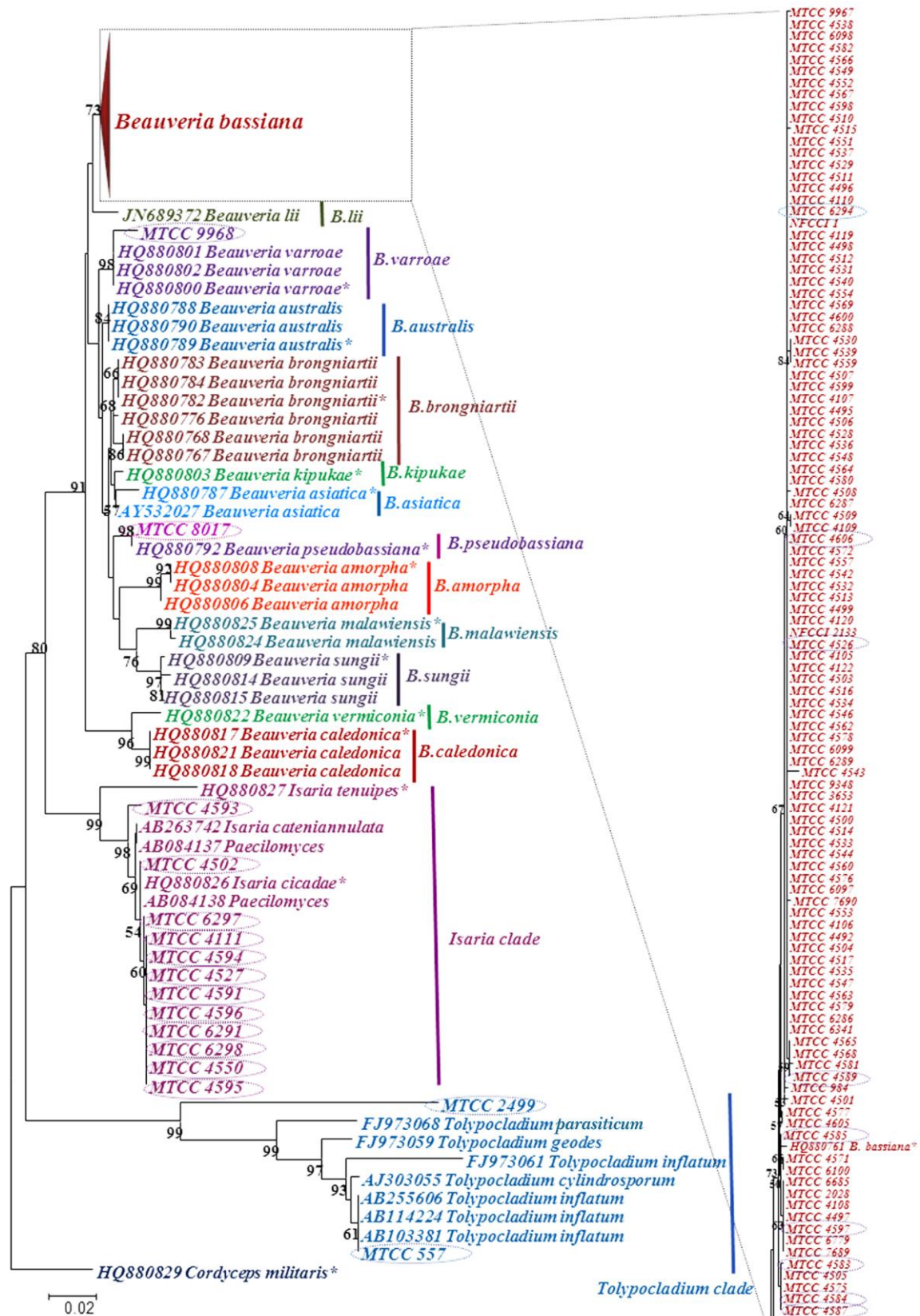


Fig. 2 – Phylogenetic relationships of *Beauveria* and *Beauveria*-like species based on the ITS/ 5.8 rRNA gene sequence-data. Bootstrap values greater than 50% are shown. The tree is rooted with *Cordyceps militaris*. Type strain is indicated by the mark ‘*’. Vertical bars represent the species clades

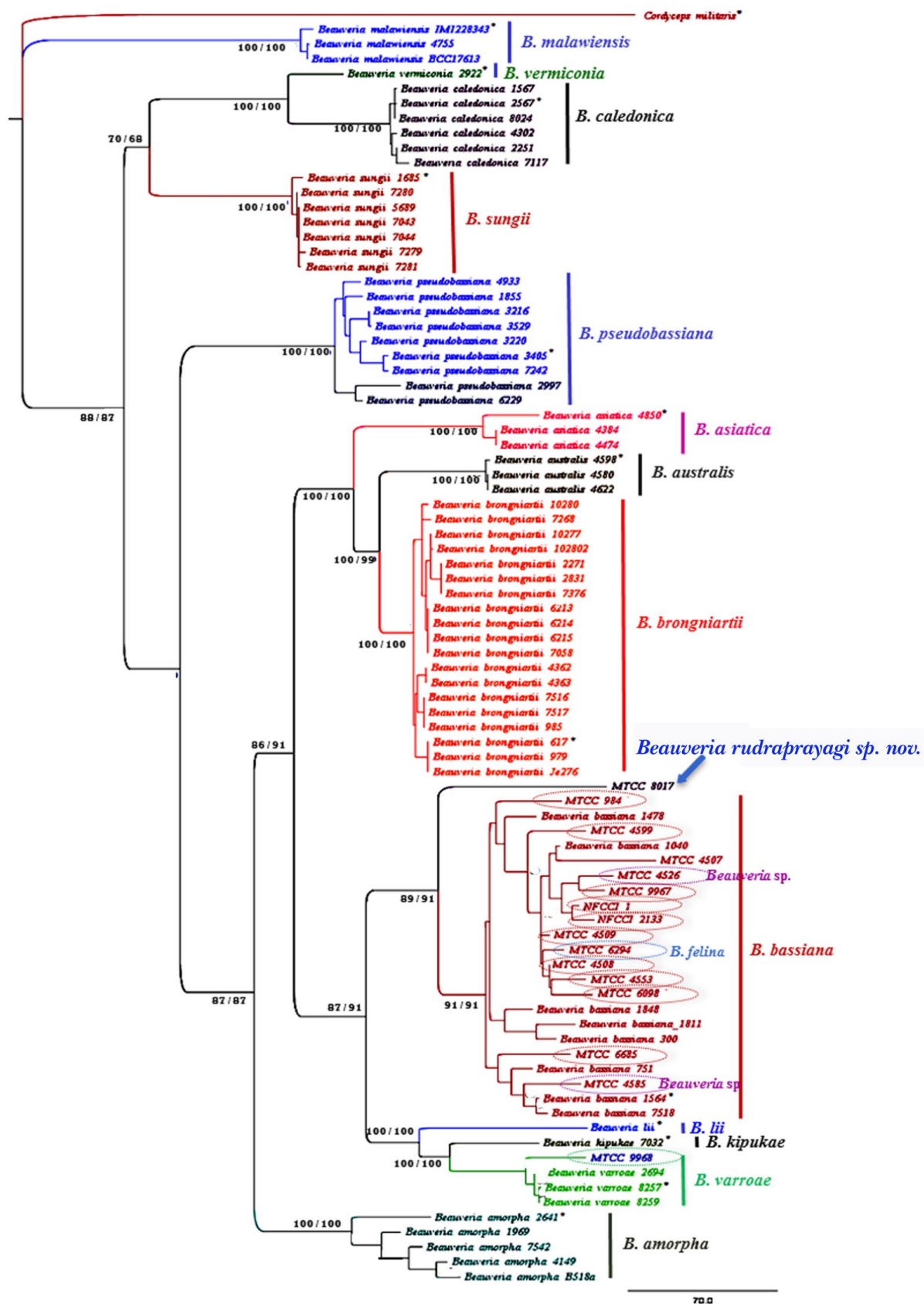


Fig. 3 – Phylogenetic relationships of *Beauveria* species from India based on the concatenated *RPB1*, *RPB2*, *EF-1a* and *Bloc* gene sequence-data. The series of values over the branches corresponds to MP Bootstrap values (>60%) and ML Bootstrap values (>60%), respectively. The tree is rooted with *Cordyceps militaris*. * indicates the type strain. Vertical bars represent the species clades.

Multigene phylogeny

From the above ITS analysis, a subset of sixteen isolates was selected for multigene phylogeny. The concatenated dataset included 5933 positions, out of which 249 ambiguously-aligned characters were excluded from the analysis. In un-weighted parsimony analysis of the remaining 5684 characters, there were 4204 constant, 601 parsimony-uninformative and 879 parsimony-informative characters. The gaps were treated as missing data. This analysis resulted in 1764 trees. One of the 1764 UP trees with *Cordyceps militaris* as outgroup is shown in Fig. 3. Bootstrap values (equal to or above 60%) based on 1000 replicates are shown on the upper side of branches. Both MP and ML analysis generated similar tree topology and comparable bootstrap values (Fig. 3). *B. rudraprayagi* type strain was found to have 92%, 99%, 96%, 98%, and 99% sequence identities with the *Bloc*, *EF-1 α* , ITS, *RPB1*, and *RBP2* gene regions, respectively of the type strain of *B. bassiana sensu stricto* ARSEF 1564.

Taxonomy

Beauveria rudraprayagi Y. Agrawal, Mual P. & Shenoy B.D., sp. nov. MycoBank MB 804029.

Fig. 4

Differs from all the accepted *Beauveria* species based on multigene sequence-data using GCPSR criterion.

Type. India, Uttaranchal, Rudraprayag, Ukhimath, Isolated from silkworm by Dr. D. Ananthapadmanaban, Scientist, Microbial Type Culture Collection, CSIR-Institute of Microbial Technology, Chandigarh, India, identified as '*B. bassiana*' and deposited as a preserved dried specimen in MTCC with accession number MTCC 8017 (H).

Etymology. *rudraprayagi* named after the place, Rudraprayag from where the fungus was collected.

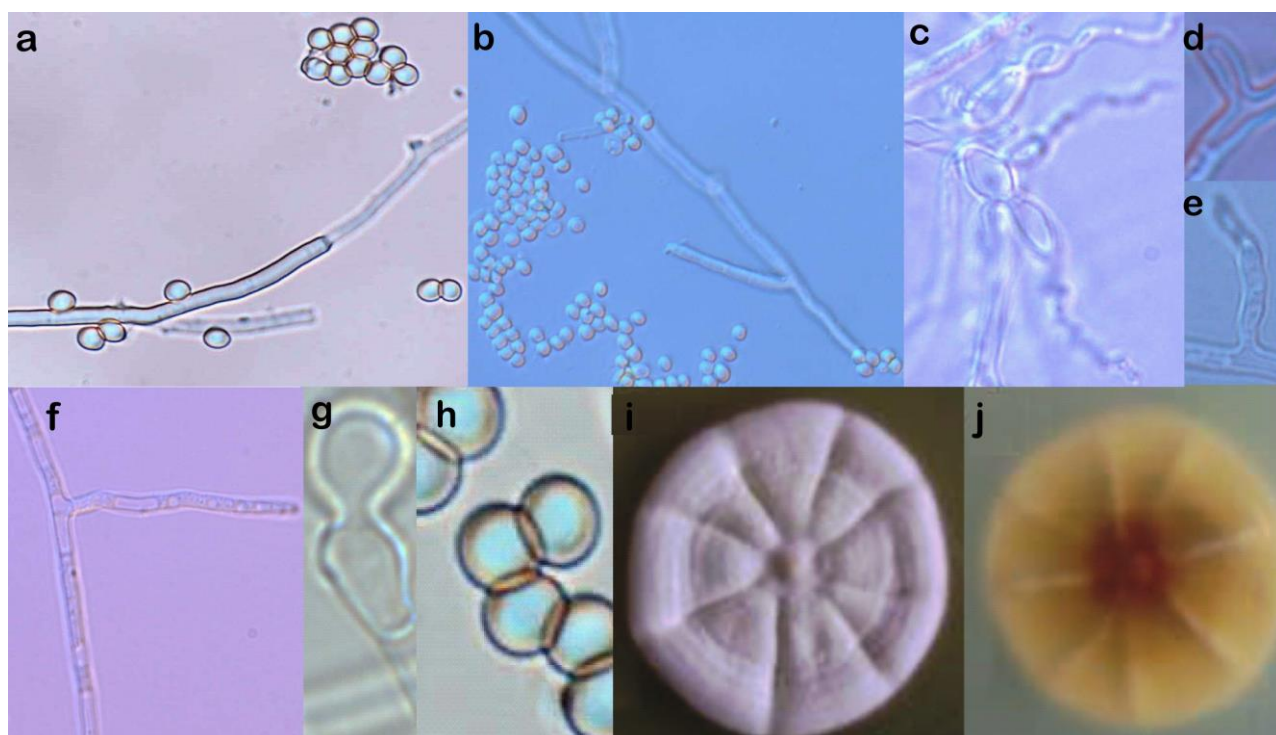


Fig. 4 – Morphological characterization of *Beauveria rudraprayagi* sp. nov. MTCC 8017. Architecture of conidia and conidiogenous cell (a–h) and plate morphology (i, front; j, reverse; on PDA, 25°C, 7 days). (a: hypha and conidia; b, f: septate hypha and denticulate rachis; c–e, g: solitary, sub-spherical to ampulliform conidiogenous cells; h: globose conidia)

Colony diameter reaches 26–30 mm after 10 days inoculation at 25°C on PDA and SDA. Colony cottony and white to light yellow, becoming farinaceous during sporulation. Reverse pale yellow with white margin. Vegetative hyphae septate, branched, hyaline, smooth walled, 1–2 µm wide. Conidiogenous cells solitary but usually in dense clusters of four or more, base sub-spherical to ampulliform and 3.5–6 µm wide, apex with an indeterminate denticulate rachis, produced laterally on aerial hyphae or from subtending cells mostly. Conidia globose, subglobose with 2.5–4.0 × 2.5–4.0 µm size, smooth walled, scattered or gregarious in clusters, sessile on aerial hyphae. Teleomorph not observed in culture.

Discussion

Fungal taxonomy has been transformed with the use of DNA-based approach in species delimitation (Shenoy et al. 2007). Though the ITS region, which is the universal barcode for Fungi, is widely used in taxonomic analysis (Seifert 2009, Schoch et al. 2012), application of multiple gene-regions in phylogenetic analysis has enhanced the taxonomic resolution at inter-species or inter-genus level (Meyling 2008). Thus, the GCPSR criterion (Taylor et al. 2000) has been applied to detect the cryptic species within a species complex, thereby overcoming the challenges posed by morphotaxonomic characters traditionally used in fungal taxonomy (Bickford et al. 2006, Samuels et al. 2010, Cai et al. 2011, Marshall & Berbee 2013).

The ITS-based phylogeny excluded 14 *Beauveria*-like fungi from the *Beauveria* clade and grouped them within *Tolypocladium* (Hypocreales, Sordariomycetes, Clavicipitaceae) and *Isaria* (Hypocreales, Sordariomycetes, Cordycipitaceae) clades (Fig. 2). This highlights the limitations of phylogenetically-informative morphotaxonomic characters. The taxonomy of these fungi is dealt in a separate manuscript. The ITS region precisely placed MTCC 6294, MTCC 4526, MTCC 4583, MTCC 4584, MTCC 4585, MTCC 4587, MTCC 4589, MTCC 4597 and MTCC 4606 as *B. bassiana sensu stricto* and MTCC 9968 as *B. varroae*, it could not resolve MTCC 8017 as a novel clade, incorrectly grouping it with *B. pseudobassiana* clade (Fig. 2).

The multi-gene phylogenetic analysis was successful in recovering a novel lineage that has been described as *Beauveria rudraprayagi* sp. nov. (Fig. 3). The currently accepted species names under *Beauveria* include *B. amorphia*, *B. asiatica*, *B. australis*, *B. bassiana*, *B. brongniartii*, *B. caledonica*, *B. kipukae*, *B. lii*, *B. malawiensis*, *B. pseudobassiana*, *B. sungii*, *B. varroae* and *B. vermiconia* (Rehner et al. 2011, Zhang et al. 2012). Interestingly, there is a morphological overlap between *B. rudraprayagi* sp. nov. and *B. bassiana*, that hints at the efficiency of GCPSR approach in resolving *Beauveria* species complex from India. Likewise earlier descriptions of *B. vermiconia*, *B. kipukae* (Rehner et al. 2011) and *B. lii* (Zhang et al. 2012), the novel species is described by means of single isolate, in the absence of multiple isolates.

This study highlights the power of phylogenetic analysis tools in efficient taxonomic resolution of *Beauveria* species from India. Further studies with enhanced sample size are required to reveal the extent of cryptic diversity for the genus *Beauveria* from India.

Acknowledgments

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