Entomopathogenic fungi cause diseases in insects, but their phylogenies have not been systematically reviewed. We highlight the importance of evolutionary and ecological studies in coordination with their pathogenicity behaviors. However, accurate identification, nomenclature and classification of entomopathogenic fungi present serious taxonomic problems. Lack of holotypes, inappropriate typification and vague teleomorph–anamorph connections are considerable taxonomic limitations. Many fungal lineage comprise assemblage of several taxonomic species (species complex) that can't be resolved easily by the use of single gene marker such as the ITS (Rehner *et al.*, 2011). The ecological role of entomopathogenic fungi in the environment is not sufficiently understood and therefore is a limitation in their successful implementation in biocontrol strategies. Fungal DNA barcoding system based on multi-locus gene sequence-data has great potentials and importance in our efforts in accurate fungal species identification (Marshall and Berbee, 2013; Schoch *et al.*, 2014). Further, the growing number of entomopathogenic fungal genomes provides a remarkable opportunity to study the biology and evolution of fungal pathogenicity mechanisms (Bushley *et al.*, 2013; Gao *et al.*, 2011; Kubicek *et al.*, 2011; Xiao *et al.*, 2012). Therefore, in this thesis, we have emphasized on evolutionary, ecological and genome based studies to better understand the biology of hypocrealean entomogenous fungi. We have focused on molecular phylogenetics and genomic approaches to gain insights into their life-history traits and pathogen-host interactions that may add useful considerations to applied research on entomogenous fungi. In this thesis, we have framed eight objectives.

Objective-I was to reconstruct the phylogenetic relationships of *Beauveria* species based on ITS gene marker (**Chapter 2**). *Beauveria* is an insect-associated fungal genus and it represents a monophyletic lineage within the *Cordycipitaceae* (*Hypocreales*, *Sordariomycetes*).

In ITS-based phylogenetic analysis, 111 fungal isolates clustered within *Beauveria* clade (*Cordycipitaceae*), while 14 Beauveria-like isolates exhibited phylogenetic affinities with *Isaria* (*Cordycipitaceae*) and *Tolypocladium* (*Clavicipitaceae*) clades. It hints at the nomenclatural ambiguity of many isolates that were initially diagnosed based on morphological characters, reflecting the need of taxonomic revision of *Beauveria* cultures to avoid their ambiguous identification.

Objective-II was to reconstruct the phylogenetic relationships of *Beauveria sensu stricto* based on multi-gene phylogenetic analysis (**Chapter 3**). We employed the genealogical concordance phylogenetic species recognition (GCPSR) criterion to resolve the evolutionary relationships of 125 *Beauveria* and Beauveria-like isolates from India. In 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 as *Beauveria rudraprayagi* sp. nov.

Objective-III was to investigate the infra-specific diversity of 102 isolates of *B. bassiana sensu stricto* from India using DNA microsatellite markers (**Chapter 4**). We observed all the microsatellite markers to be easily amplifiable for all the isolates and provided a good genotype resolution. The microsatellite analysis indicated the presence of highly polymorphic, randomly distributed populations of *B. bassiana* with variable host range and apparently no host-specificity. We, however, observed region-wise clustering of the populations within *B. bassiana sensu stricto* from India.

Objective-IV was to investigate the evolutionary relationships of genus *Hirsutella* (**Chapter 5**). We presented the polyphyletic nature of this genus and its affiliations with *Bionectriaceae* and *Cordycipitaceae* families in addition to the *Ophiocordycipitaceae* family by

employing ITS and LSU gene markers. Chitinase and hirsutellin partial gene regions were also employed, but did not prove satisfactory, primarily due to the lack of reference sequences.

Objective-V was the phylogenetic analysis of chitinases from *Hirsutella thompsonii* (**Chapter 6**). Preliminary study resulted in isolation of two chitinases *Htchit1* and *Htchit2* with the use of RACE, TAIL PCR, Step-down PCR and primer-walk approaches. Further, the complete list of putative chitinases from *Hirsutella thompsonii* was retrieved through whole genome sequencing. The genome analysis identified 19 and 15 chitinases in two strains of *Hirsutella thompsonii* MTCC 3556 (*Ht3*) and MTCC 6686 (*Ht6*), respectively, based on the presence of conserved consensus motif [LIVMFY]-[DN]-G-[LIVMF]-[DN]-[LIVMF]-[DN]-x-E. We presented the phylogenetic division of chitinases into subgroups (A, B and C). Autonomous gene gain/loss events are suggested to be responsible for the observed difference in number of *Ht3* and *Ht6* chitinases. The classification of *Ht* chitinases into three subgroups was further supported by the modelled structures of *Ht* chitinases, represented by reported deep and narrow substrate binding cleft for subgroups (A and C) while shallow for subgroup-B. Moreover, high number of conserved aromatic amino acid residues were advocated in subgroups (A and C) than in subgroup-B that are crucial as binding hot spots in processive chitinases belonging to subgroups (A and C). In this study, we identified the presence of AMP+PP+NAD in subgroup-A *Chi-13* of *Ht*, which are known to be associated with secondary metabolites (non-ribosomal peptide synthases) production, virulence and fungal development (Molnar *et al.*, 2010). It postulates the role of subgroup-A chitinases in pathogenicity in addition to other routine functions (like growth and autolysis). Further, we compared the *Ht* chitinases with those from two closely related species: *Ophiocordyceps sinensis* (*Os*), teleomorph of *Hirsutella sinensis*

(*Ophiocordycipitaceae*) (Hu *et al.*, 2013; Liu *et al.*, 2002) and *Beauveria bassiana* (*Bb*) (*Cordycipitaceae*), an effective mycoinsecticide (Xiao *et al.*, 2012).

Objective-VI was the genome sequence of *Hirsutella thompsonii* and its comparative analysis with *Beauveria bassiana* (*Bb*) and *Ophiocordyceps sinensis* (*Os*) genomes (**Chapter 7**). We report the 34.6 Mb and 34.7 Mb draft genomes of *Ht3* and *Ht6*, respectively. Comparative analysis of carbohydrate active enzymes, pathogen-host interaction genes, metabolism-associated genes and genes involved in biosynthesis of secondary metabolites in the four genomes *Bb*, *Ht3*, *Ht6* and *Os* was carried out. Reduction in gene family sizes in *Ht3* and *Os* as compared to *Ht6* and *Bb* is revealed. Analysis of the mating type genes in *Ht* reveals the presence of MAT idiomorphs, suggesting cryptic sexual traits in *Ht*. The genome analysis displayed the presence of CYP55 family members of nitric oxide reductases in *Ht* genomes that plays crucial role in anaerobic denitrification suggesting an important target for bioremediation activities.

Objective-VII (**Chapter 8**) was the genome sequencing of *Aschersonia badia* (*Ab*: *Clavicipitaceae*, *Hypocreales*), specialist entomopathogenic fungus, and its comparative genomics with two clavicipitaceous fungi (*Metacordyceps* lineage), *Metarhizium roberstii* (*MR*: generalist entomopathogen) and *M. acridum* (*MAC*: acridid-specific entomopathogen), exhibiting variable host preferences. We report the 28.8 Mb long draft genome of *Ab*. Comparative analyses of virulence-associated genes, carbohydrate active enzymes and secondary metabolite biosynthesis genes display their reduction in sizes in *MAC* and *Ab*, whereas their expansion in *MR*. The present study supports the previous finding indicating the evolution of fungal host specificity in coordination with the presence of multi-modular NRPS gene cluster (*dtxS*) responsible for biosynthesis of destruxin secondary metabolite (Giuliano *et al.*, 2012; Wang *et al.*, 2012). Further, the domain survey of chitinases present the absence of CBM50 (LysM

domain) in *MAC*, and its presence in *Ab* and *MR*. However, apparent difference in frequency of CBM50 domains associated with chitinases of *Ab* and *MR* is identified, where *MR* chitinases display higher proportion of associated CBM50 domains than *Ab* chitinases. Based on this study, we speculate autonomous gene gain/ loss through domain distribution of *dtxS1* and chitinases. The association of higher number of CBMs with *MR* chitinases is expected to contribute to broader host affiliation in *MR*.

Objective-VIII (**Chapter 9**) was the genome sequencing of *Beauveria rudraprayagi* (*Br*) and *Isaria farinosa* (*If*) and their comparative analysis with *Beauveria bassiana* (*Bb*) and *Cordyceps militaris* (*Cm*). We report 36.6 Mb and 34.1 Mb genomes of *Br* and *If*, respectively. We observed 5% difference in *Br* and *Bb* genomes attributed to 11.8% unmapped *Br* reads. *Br* genome shows matches related to many bacterial-like genes and one bacteriocin. We observed the overall gene families contraction in *Br* and *Cm*, whereas their expansion in *Bb* and *If*. The genomic contraction observed in *Br* and *Cm* could be related to the variation in reproductive mode due to the RIP activity during sexual cycle. This study has identified 23, 19, 19 and 26 chitinases from *Bb*, *Br*, *Cm* and *If*, respectively. WD40 domain is exclusively seen in *Bb* and *Br* genome in association with sg-A classified chitinase.

In conclusion, the present study highlights the power of phylogenetic analysis tools in taxonomic resolution of two fungal entomopathogen genera: *Beauveria* and *Hirsutella*. Further studies with enhanced sample size are required to reveal the extent of cryptic diversity for the genus *Beauveria* from India, and to perform the efficient taxonomic revision of genus *Hirsutella*. The geography-centric population structure of *B. bassiana sensu stricto* from India, as revealed from this study, could have useful implications in future biocontrol research on entomopathogenic fungi from India. The genome sequencing of insect pathogenic fungi and its

comparative genome analysis sheds light on insect-fungal associations, evolution of entomopathogenicity, speciation, evolutionary insights into chitinases, and clues to improve the biocontrol efficacy of entomopathogenic fungi. NCBI-GenBank Genome portal lists sequencing information of about 50 hypocrealean fungi till the year 2014, hence demanding the sequencing of more members of hypocrealean fungal genomes. Therefore, the inclusion of four potential fungal entomopathogens belonging to families *Clavicipitaceae* (*Ab*: *Aschersonia badia*), *Cordycipitaceae* (*Br*: *Beauveria rudraprayagi* and *If*: *Isaria farinosa*) and *Ophiocordycipitaceae* (*Ht*: *Hirsutella thompsonii*) would support in further extension of genomic sequence data of hypocrealean fungi. These sequencing data will help in identification of various pathogen interacting genes, virulence associated genes, genes encoding biologically active substances, *etc.* that could raise the suitability of fungal entomopathogens for biocontrol measure.