

# 中国落叶松八齿小蠹伴生菌的鉴定和系统发育分析

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**摘要:** 真菌与小蠹虫伴生关系十分复杂, 尽管对这种伴生关系的研究已超过了一个世纪, 但对它的认识及其机制的理解仍然十分有限。长喙壳类真菌能够引起木材蓝变, 而且其中一部分真菌也是很强的病原菌, 但在中国, 这些病原菌中只有很少的一部分得以深入细致的研究。落叶松八齿小蠹 (*Ips subelongatus*) 是我国为害落叶松 (*Larix* spp.) 的主要害虫。作为次期性害虫的先锋种, 除了侵害新伐倒木、濒死木, 在发生盛期还可以危害健康木和衰弱活立木。2003 年上半年大兴安岭过火区内森林大面积发生落叶松八齿小蠹, 达 39.78 万 ha。在个别林分平均被害株率曾达到 52%, 株平均虫口密度达 5 头/1 000 cm<sup>2</sup>, 造成我国落叶松人工林的大面积死亡和衰退。落叶松八齿小蠹在我国东北和华北 (黑龙江、吉林、内蒙古、辽宁、山西和河北) 等地发生。其主要危害 3 种落叶松: 兴安落叶松 (*L.gmelini*)、长白落叶松 (*L.olgensis*) 和华北落叶松 (*L.prineipis-rupprechtii*)。与落叶松八齿小蠹伴生的长喙壳类真菌是小蠹虫伴生菌种类中最主要的类群, 其与小蠹虫的伴生关系得到了广泛的研究。在欧洲目前发现 11 种长喙壳类真菌与落叶松八齿小蠹伴生, 其中与强致病性病原菌 *C.laricicola* 形成稳定的伴生关系。日本也较早的开展了落叶松八齿小蠹伴生菌的研究, 到目前报道与 12 个长喙壳真菌的种类伴生, 其中包括强致病菌 *Ceratocystis fujiiensis* 能够在人工接种条件下致使健康树木死亡。日本研究表明, *Ceratocystis fujiiensis* 是与亚洲落叶松八齿小蠹伴生的主要真菌, 也是伴生菌区系中的先锋种和致病力最强的病原菌, 人工接种条件下可以将 30 年的树龄的日本落叶松 (*Larix kaempferi*) 致死。本研究针对三种异域分布的落叶松林, 为比较种特异性是否存在区域差异。通过采集落叶松八齿小蠹成虫, 幼虫以及母坑道、子坑道的韧皮组织和蓝变组织进行伴生真菌分离培养。标本采集地主要集中在寄主落叶松分布区, 主要为东北三省以及内蒙古, 河北省等地, 目前在根河等 11 个地方进行了标本采集工作, 共采集到坑道标本 587 份, 虫体标本 140 份。采用 2% 水琼脂和 PDA 综合培养基对采集到的标本进行分离纯化培养, 共分离到菌株 278 株。对分离纯化后得到的菌株, 通过形态学和分子生物学相结合的方法进行鉴定。广泛使用 ITS&LSU、 $\beta$ -tubulin、EF-1 $\alpha$  基因 3 个基因位点作为 DNA 序列的测定对象, 采用 MP 以及 Mrbayers 方法分析落叶松八齿小蠹发生

区长喙壳真菌种的系统发育关系。分析结果表明,从落叶松八齿小蠹体表、坑道以及木材蓝变部位共分离到落叶松八齿小蠹伴生菌 70 株,为 6 个种,其中 2 种属于小囊菌目

(Microascales) 分别为: *Ceratocystis fujiensis*, *Graphium laricis*。; 另有 4 种为蛇口壳目

(Ophiostomatales), 分别是: *Ophiostoma brunneociliatum*, *Ophiostoma kryptum*, *Ophiostoma floccosum* 和 *Ophiostoma bicolor*。目前结果表明, *Ophiostoma brunneociliatum* 为小蠹虫稳定伴生菌,在多数标本采集地均有大量分离到。分离纯化获得的菌株中, *Ophiostoma bicolor* 为中国的新纪录种,而 *Ophiostoma brunneociliatum* 具有较多的进化分枝,可见该种在中国具有较长时间的发展历史。本实验中,在寄主兴安落叶松、华北落叶松上均有分离到 *Ceratocystis fujiensis*, 致病性有待进一步测定。

关键词: 长喙壳类真菌; 落叶松; 系统发育; *Ceratocystis fujiensis*

## The phylogenetic analysis of *Ips subelongatus* associated fungi from China

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**Abstract:** *Ips subelongatus* was the main pest which infected the *Larix* spp. in our country as the pioneer species. The bark-beetles can infect the felled trees, the dying trees, and also can lead the healthy wood to die. It had been reported that *Ips subelongatus* had happened in northeast and north China, including Heilongjiang, Jilin, Inner Mongolia, Shanxi, Liaoning and Hebei province. Mainly, the *Ips subelongatus* infected the three larches, *L.gmelini*, *L.olgensis* and *L.prineipis-rupprechtii*. The ophiostomatoid fungi were the main bark-beetles associated fungi and it had been extensive researched. The associated relationships between fungi and bark-beetles was complicated, although the study had been a long time, the understanding of the mechanism was still limit. Ophiostomatoid fungi infecting larch logs are typically barkbeetle associates that cause sapstain in timber and some are pathogens. Only very little ophiostomatoid fungi is known which associated with bark beetles in China. In Europe, 11 kinds of isolates were found associate with bark-beetles and *C.lariciloca* was constant bark-beetle associates. Similarly, Japanese found 12 kinds of ophiostomatoid fungi which associated with *Ips subelongatus*, and had studied on the *Ips subelongatus* associated fungi earlier. The study in Japan had confirmed that *Ceratocystis fujiensis* was the most associated fungi with *Ips subelongatus* and it was the pioneer species and

strongest pathogenic which could lead 30 years' larch to dead under artificial infection. Collections were made between July and August in 2011 in four provinces, including Inner Mongolia, Hei Long Jiang, Jilin and Liaoning province. The samples collected from the host of the *Ips subelongatus* and that hosts sampled included *Larix principis-rupprechtii*, *L. gmelini*, and *L. olgensis*. In total, 587 gallery samples and 140 insect body samples were collected in eleven places including Genhe. The fungi were isolated from the surface of the *Ips subelongatus* adult and larvae, the blue-stained tissue and galleries by PDA medium or 2% water agar. 278 isolates were achieved, including 57 *Ips subelongatus* associated fungi. The identification of the strain use the morphology and molecular biology. As usual, DNA sequence of the internal transcribed spacer regions(ITS), the nuclear large subunit(LSU), the partial  $\beta$ -tubulin gene and the EF-1  $\alpha$  gene were used for identifications. DNA sequence comparisons and phylogenetic analysis confirmed that 70 isolates had acquired and these isolated fungi groups represented six species, four in the Ophiostomatales (Sordariomycetiade) and two in the Microascales (Hypocreomycetidae). The majority of these isolated ophiostomatoid fungi were *O.brunneociliatum*, *Ceratocystis fujiensis*. In addition, there were also some other bark beetle-associated fungi, such as *Graphium laricis*, *Ophiostoma kryptum*, *Ophiostoma floccosum* and *Ophiostoma bicolor*. *Ceratocystis*, *Graphium*, and *Ophiostoma* species were isolated from *Ips subelongatus* and beetles-infested larch logs collected at several areas in northern China. *Ceratocystis fujiensis*, *Graphium laricis*, and five species of *Ophiostoma* were isolated. Of the fungi isolated in the present study, *O.brunneociliatum* were constant associates of the bark beetles. The preliminary study result confirmed that the *Ophiostoma bicolor* was a new record in China, and *Ophiostoma brunneociliatum* had a long history in our country. Moreover, the *Ceratocystis fujiensis* was isolated from *Larix gmelini* and *Larix principis-rupprechtii* which had strong pathogenic.

**Key words:** Ophiostomatoid fungi, *Larix* spp., Phylogeny, *Ceratocystis fujiensis*