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Chrysosporium leigongshanense sp. nov. from Guizhou Province, China

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Abstract

A new species of the genus *Chrysosporium*, *C. leigongshanense*, was isolated from the soils in Leigong Mountain, Guizhou Province, China. The isolate was morphologically characterized and subjected to molecular analysis based on ITS rDNA sequence data. Diagnostic characters are as follows: racquet hyphae present; terminal and lateral conidia produced on protrusions, the stalk or side branches; conidia smooth, single- to double- celled, cylindrical to clavate, 5.4-19.4 µm × 1-3.2 µm; Basal scars were 0.5-2 µm wide; Intercalary conidia and chlamydospores absent. Holotype was deposited as HMAS 255243.

Key words – filamentous fungi – morphological character – phylogeny, taxonomy

Introduction

The genus *Chrysosporium* Corda was established by Corda (Sturm, 1833), who designated *C. corii* Corda as the type species (Sturm, 1833). Sexual morphs of this genus belong to Onygenaceae and Arthrodermataceae (Onygenales, Eurotiomycetes, Ascomycota) (Kirk et al. 2001; Qorschot 1980). According to the most recent Index Fungorum (2016), 98 species have been reported. Excluding synonyms and invalid species, a total of 73 species are currently accepted (Zhang et al. 2016b). *Chrysosporium* species have a wide variety of potential applications. Members of the genus produce many enzymes, such as keratinase, cellulase, lipase, inulinase and galactosidase (Kushwaha, 2000). *Chrysosporium* species are often keratinolytic, suggesting their use for the industrial production of medicine, feed, fertilizer, leather and other materials (Zeng et al. 2004; Liang et al. 2007). They also produce some useful secondary metabolites and antibiotics. For example, Hiramo et al. (2001) isolated the antibiotics TMC-69 from the fermentation liquid of *Chrysosporium* sp. TC 1068. Its hydrogenated derivative, TMC-69-6H, had strong antitumor activity against P388 leukemia and B16 melanoma in the mice and was especially suppressive effect on Cdc25A and B phosphatases. Prakash et al. (2001) and Priyanka & Prakash (2003) found that *C. tropicum* J.W. Carmich. could produce several metabolites which are toxic to the larva of *Anopheles stephensi* and *Culex quinquefasciatus*. In addition, *C. tropicum* is pathogenic under certain conditions (Chabasse et al. 1989). Species of *Chrysosporium* are widely distributed in various environments such as soils, sludge, sandbeach and the skin of some animals (Liang et al. 2007). One such habitat is Leigongshan Mountain, Guizhou Provinces, China. Located in a national nature reserve, this mountain is rich in the natural resources. GZUIFR-EB2702H was isolated from the soils collected at 1700m. The isolate

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was identified as a new *Chrysosporium* species on the basis of morphological characteristics and phylogenetic relationships and was named as *C. leigongshanense* according to the collection sites. The activity of enzymes produced by this species will be identified and studied in the further.

Materials & Methods

Sample collection and strain isolation

Strain EB2702H was isolated from soil samples collected at an altitude of 1700m from Leigongshan Mountain, Guizhou Province (N25° 30′ , E107° 40′), China. Soil samples were added to sterilized hair powder and kept moist at 40°C for approximately 1 month. When fungal growth was observed, the hair powder was mixed with the sterilized water in an Erlenmeyer flask, and 1mL suspensions were evenly spread on Martin's medium and incubated at 40 °C. The pure cultures were then transferred to potato dextrose agar (PDA) slants stored at –70 °C. Holotype and ex-type specimens are deposited in the Institute of Microbiology, Chinese Academy of Sciences, Beijing; at the same time, in the Institute of Fungus Resources, Guizhou University (GZAC) (Zhang et al. 2016a, b).

Morphological identification

Isolates were transferred to PDA and Czapek agar, incubated at 40 °C for14 days, and subjected to macroscopic examination. Fungal micro-characteristics were examined with Serial B1 Motic microscope (Motic, Guangzhou, China) and photographed. Diagnostic features were then defined on the basis of these observations. Finally, the isolate was morphologically identified according to colony characteristics and conidiogenous structures (Oorschot 1980; Han et al. 2013; Zhang et al. 2013, 2016a, b).

DNA extraction, PCR amplification and nucleotide sequencing

Total genomic DNA was extracted from fresh cultures after seven days of incubation at 40 °C using a Fungal DNA mini kit (Omega Biotech, Doraville, GA, USA) according to the manufacturer's protocol and then stored at –20°C. The ITS rDNA region was amplified with primers ITS5 (5′-GGTGAGAGATTTCTGTGC-3′) and ITS4 (5′-TCCTCCGCTTAT TGA TATGC-3′) (Luo et al. 2016). The Amplifications was carried out in a 25 μL volumes consisting of 12.5 μm 2 × Master Mix, 2μm template DNA, 1μm of each primer and 8.5 μm distilled deionized water. Amplification conditions were as follows: 5 min at 94 °C, followed by 35 cycles of 94 °C for 40 s, 50 °C for 40 s and 72 °C for 1 min, and final extension step of 72 °C for 10 min. The resulting PCR product was sequenced by Sangon Biotech (Shanghai, China) using the same primers. The generated ITS rDNA sequence was submitted to GenBank (KX668869).

Molecular phylogenetic analysis

ITS sequences of 38 accessions representing 35 *Chrysosporium* species were downloaded from GenBank. ITS rDNA sequence of *Taifanglania hechuanensis* Z.Q. Liang, Y.F. Han, H.L. Chu & R.T.V. Fox was used as an outgroup (Figure 2). Alignment of the ITS rDNA region of the 39 downloaded sequences and the sequences generated in this study was carried using MAFFT v. 7.037b (Katoh et al. 2013), with subsequent manual adjustment conducted to maximize sequence similarity. Sequence alignment editing was performed in BioEdit (Hall 1999).

Phylogenetic trees were constructed from the aligned sequences using three different methods: Bayesian analysis (Huelsenbeck & Ronquist 2001), maximum parsimony (MP) and maximum likelihood (ML) (Felsenstein 1981). The Bayesian analysis was carried out using the GTR+I+G nucleotide substitution model by Modeltest 3.7 (Posada & Crandall 1998). Posterior probabilities were determined by Markov chain Monte Carlo sampling in MrBayes v. 3.2 (Ronquist et al. 2012) using the estimated model of evolution. Six simultaneous Markov chains were run for 1,000,000 generations, with trees sampled every 100th generations (resulting in 10,000 total trees). The first 2,000 trees, which represented the burn-in phase of the analysis, were discarded; the remaining

8,000 trees were used to calculate posterior probabilities in the majority rule consensus tree. Each run at completion was examined with the program Tracer v. 1.5 (Drummond & Rambaut 2007) after the analysis finished, to determine burn-in and to confirm that the runs had converged.

The MP and ML analyses were conducted in MEGA 6 (Tamura et al. 2013), with gaps treated as missing data and all other parameters following the default condition. Bootstrap support for nodes in the resulting trees was assessed using 1,000 replications per analysis. The final aligned data set and the phylogenetic tree are available in TreeBASE under the submission ID 20422 (http://purl.org/phylo/treebase/phylows/study/TB2:S20422?x-access-code=29dcd3c1f3579363a59e8adefda91e58&format=html) and information on the new species has been deposited in MycoBank (www.MycoBank.org).

Results

Taxonomy

Chrysosporium leigongshanense Z. Li, G.P. Zeng & Y.F. Han. sp. nov. Fig. 1

MycoBank no.: MB 819532, FOF02875

Holotype: HMAS 255243, dried culture.

Etymology – *leigongshanense* (Latin), referring to leigongshan mountain where the type locality is situated.

Colonies on PDA attaining 37–42mm in 14d at 40 °C, white, densely fluffy, uplifted in the middle, with a ring radius, regular in the margins. Reverse yellow to yellowish. Hyphae hyaline, septate, smooth, 0.5–1.5 µm wide. Racquet hyphae present, 5.4–10.8 × 2–4.5 µm. Terminal and more lateral conidia on short protrusions, the short stalk or the side branches, solitary, 1– to 2–celled, sometimes forming short conidial chains, hyaline, smooth, mostly cylindrical to clavate, 5.4–19.4 × 1–3.2 µm, sometimes curved, a few obovate, 5.4–6.5 × 2.2–3.2 µm. Basal scars 0.5–2 µm wide. Intercalary conidia and chlamydospores absent.

Material examined – Holotype HMAS 255243 (= GZUIFR-EB2702H), dried culture, isolated from soil collected in Leigongshan Mountain, Guizhou Province, China, N25°30′, E107°40′, at an altitude of 1,700m, September 2013, by X. Zou, and its ex-type EB2702H deposited in China General Microbiological Culture Collection Center (CGMCC3.18621).

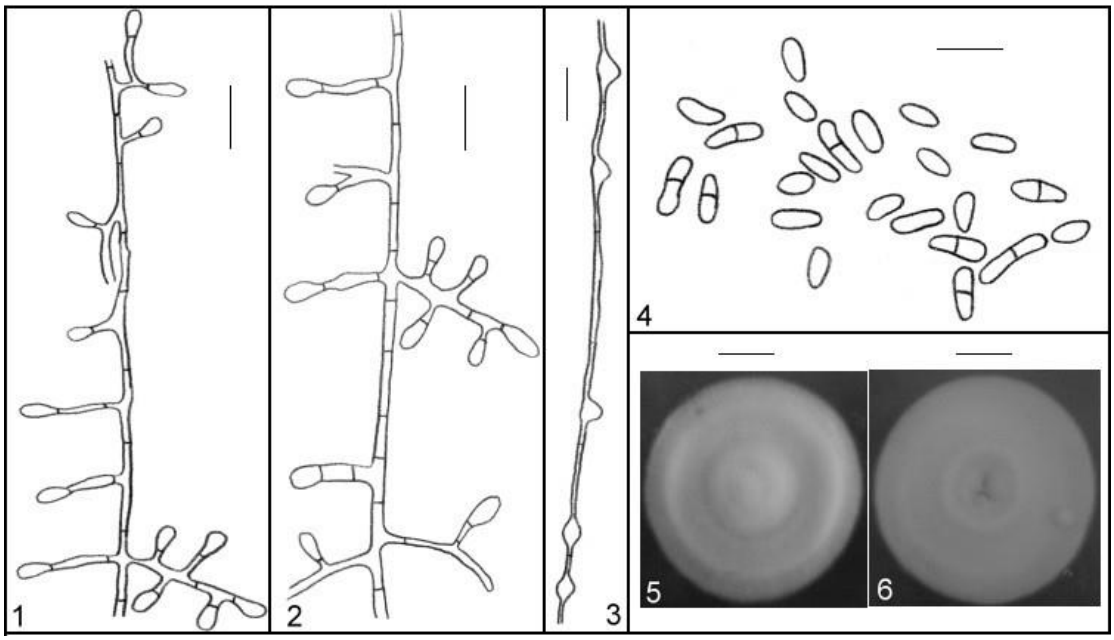

Fig. 1 – *Chrysosporium leigongshanense* (holotype). 1, 2. Conidiogenous structures.3 Racquet hyphae. 4 Conidia. 5-6 Colony (Front and reverse) on PDA Scale bars 1–4 = 10 µm; 5–6 = 10 mm.

Notes – We compared *C. leigongshanense* with similar species of this genus. *C. georgii* (Varsavsky & Ajello) Oorschot, *C. lucknowense* Garg, *C.* anamorph of *Rollandina vriesii* Apinis, *C.* anamorph of *Arthroderma curreyi* Berk., *C.* anamorph of *Arthroderma cuniculi* Dawson, *C.* anamorph of *Pectinotrichum llanense* Varsavsky & Orr and *C. hubeiense* Y.W. Zhang, Y.F. Han & Z.Q. Liang. However, *C. leigongshanense* can easily be distinguished by its morphological characteristics (see Table 1).

Phylogenetic analyses

Three methods (Bayesian inference, MP and ML) were used to phylogenetically analyze the 705bp aligned ITS rDNA sequence data set of 39 *Chrysosporium* accessions and one outgroup (Fig. 2). Phylogenies obtained using the three different methods were mostly congruent, with most branches supported in all three trees. Consequently, the tree with the statistical support values from the three methods is shown in Fig.2. The phylogenetic tree comprised two major clades, one consisting of *C. filiforme* Sigler, J.W. Carmich. & H.S. Whitney, *C. pseudomerdarium* Oorschot, *C. sanyaense* Y.W. Zhang, Y.F. Han, J.D. Liang & Z.Q. Liang and *C. leigongshanense* GZUIFR-EB2702H, and the other composed of the remaining analyzed *Chrysosporium* species. Our presumptive new species (GZUIFR-EB2702H) was strongly supported (Bayesian posterior probability = 0.99; MP bootstrap = 99%; ML bootstrap = 99%) as sister to *C. sanyaense* Y.W. Zhang, Y.F. Han, J.D. Liang & Z.Q. Liang.

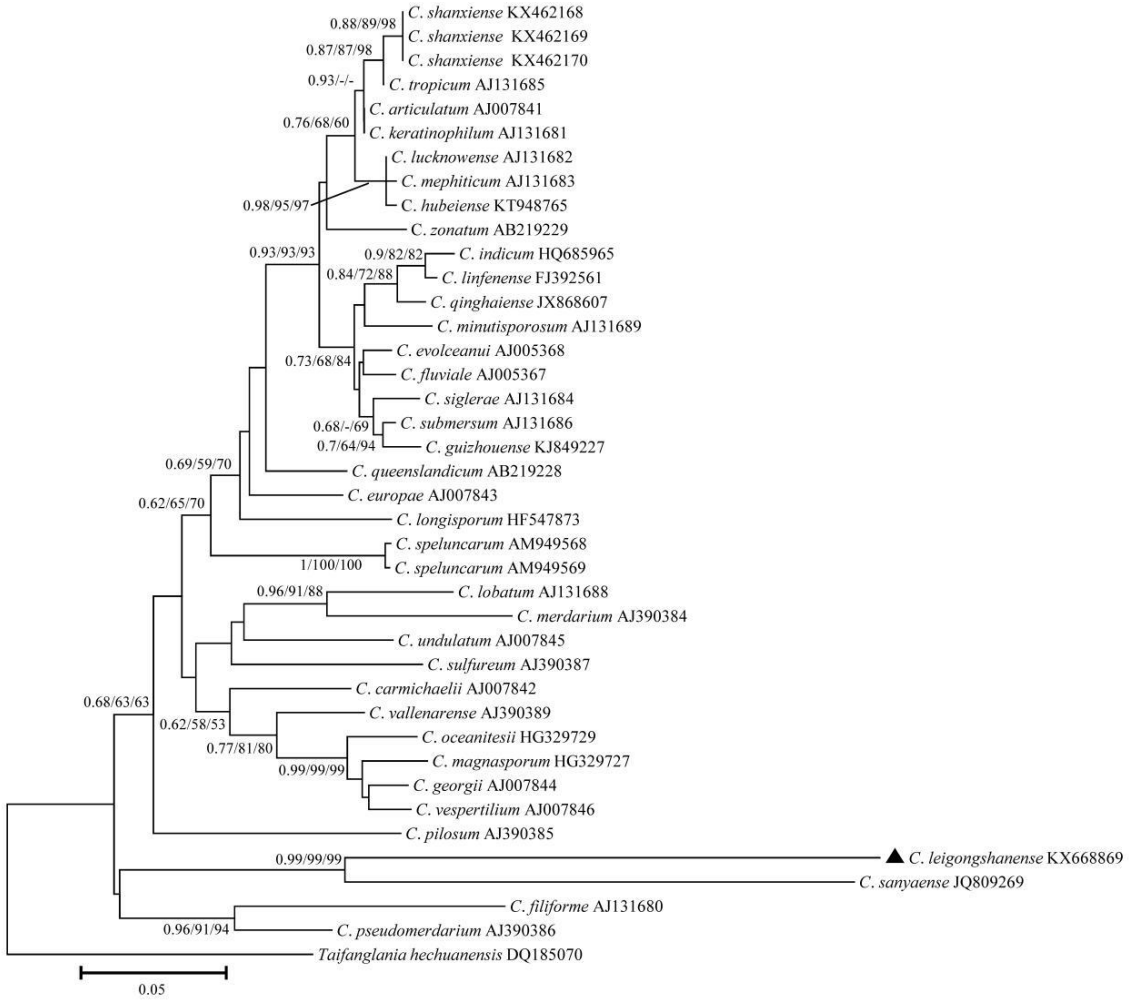

Fig. 2– Phylogenetic tree of *Chrysosporium* spp. constructed from ITS rDNA sequences from BI/MP/ML. Statistical support values (Bayesian posterior probability/maximum parsimony percentage/maximum likelihood bootstrap percentage) are shown at nodes. The tree was rooted by using *Taifanglania hechuanensis* as outgroup.

Table 1 Morphological comparison between GZUIFR-EB2702H and the related *Chrysosporium* species

Species	Color of colony	Texture of colony	Generated structures of conidia	Shape of Conidia	conidia (μm)	Surface of Conidia
C. lucknowense	Creamy	Densely fluffy	On short protrusions or on side branch; Single-celled	Subglobose to oval to clavate	2.5-11 ×1.5-6	Smooth
C. georgii	White, yellowish to pink	Felty, powdery to fluffy	On short protrusions; Single-or multi-celled	Subglobose to obovate to clavate	3-8×2-3	Rough-smooth
C. anamorph of *Arthroderma curreyi*	White	Felty to fluffy	On short protrusions or on side branch; Single- to double-celled	Oval to clavate	2-3×1-2	Rough-smooth
C. anamorph of *Arthroderma cuniculi*	White	Felty to densely fluffy	On short protrusions or on side branch; Single-, double- to multi-celled	Oval to clavate	2-3×1-2	Smooth
C. anamorph of *Pectinotrichum llanense*	White	Fluffy to powdery	On short protrusions or on side branch; Single-celled	Oval to clavate	5-19.5×1-3	Smooth
C. anamorph of *Rollandina vriesii*	White	Felty to powdery	On short protrusions or on side branch; Single-celled	Oval, pyriform to clavate	3-6×2-3	Smooth
C. hubeiense	White	Powdery	On short protrusions or on side branch; Single-celled	Oval to ellipsoidal	1.5-3×2-3	Smooth
C. leigongshanense GZUIFR-EB2702H (this study)	White	Densely fluffy	On short protrusions, on short stalk or on side branch; Single- to double-celled	Cylindrical to clavate	5.4-19.4 ×1-3.2	Smooth

Discussion

The following *Chrysosporium* species are morphologically similar to *C. leigongshanense*: *C. Georgii* (Varsavsky & Ajello) Oorschot, *C. Lucknowense* Garg, *C.* anamorph of *Rollandina vriesii* Apinis, *C.* anamorphof *Arthroderma curreyi* Berk., *C.* anamorph of *Arthroderma cuniculi* Dawson, *C.* anamorph of *Pectinotrichum llanense* Varsavsky & Orr and *C. hubeiense* Y.W. Zhang, Y.F. Han & Z.Q. Liang. Details of their distinguishing characteristics relative to *C. leigongshanense* are given in table 2. Briefly, *C. georgii* and *C.* anamorph of *Arthroderma curreyi* bear the rough conidia on the surface (Oorschot 1980); *C.* anamorph of *Arthroderma cuniculi*, *C.* anamorph of *Pectinotrichum llanens* and *C. lucknowense* produce the smaller conidia (Oorschot 1980); *C.* anamorph of *Rollandina vriesii* Apinis have the oval, pyriform to clavate conidia (Oorschot 1980); and *C. hubeiense* has powdery colonies, obovate to ellipsoidal conidia and does not grow at 40 °C (Zhang et al. 2016a).

In the phylogenetic tree, *C. leigongshanense* GZUIFR-EB2702H and *C. sanyaense* clustered together with strong support value (0.99/99%/99%). These two species can be distinguished as follows: *C. sanyaense* produces subglobose to obovate conidia, abundant intercalary conidia and single-celled conidia, whereas *C. leigongshanense* has cylindrical to clavate conidia and lacks intercalary conidia.

In conclusion, the holotype GZUIFR-EB2702H proposed as a new taxon on the basis of morphological characteristics and phylogenetic analysis, is named as *C. leigongshanense* according to its collection sites. Its diagnostic characteristics are as follows: colonies densely fluffy; the racquet hyphae present, 5.4–10.8 × 2–4.5 μm; terminal or lateral conidia were on the protrusions, short stalks or the side branches, solitary, single- or double-celled, mostly clavate to cylindrical, some curved, 5.4–19.4 × 1–3.2 μm; basal scars 0.5–2 μm wide; intercalary conidia and chlamydospores absent.

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References

Chabasse D, De Gentile L, Bouchara JP. 1989 – Pathogenicity of some *Chrysosporium* speciesisolated in France. Mycopathologia 106, 171–177.

HanYF, Liang ZQ, Liang JD. 2006 – Two new records of *Chrysosporium Corda* in China. Journal of Fungal Research 4, 53–56.

Hirano N, Kohno J, Tsunoda S, Nishio M et al. 2001 – A new antitumor antibiotic with Cdc25A inhibitory activity, produced by *Chrysosporium* sp. TC1068. Taxonomy, fermentation and biological activities. Journal of Antibiotics 54, 421–427.

Huelsenbeck JP, Ronquist F. 2001 – MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Katoh K, Standley DM. 2013 – Mafft multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30, 772–780.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001 – Ainsworth & Bisby's Dictionary of the Fungi (9th ed). CAB Internation, Wallingford 106–107.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008 – Dictionary of the fungi. 10th edition. CAB International, Oxon, 1–771.

Kushwaha RKS. 1980 – The genus *Chrysosporium*, its physiology and biotechnological potential. Studies in Mycology 20, 66–76.

Liang JD, Han YF, Du W, Liang ZQ, Li ZZ. 2009 – *Chrysosporium linfenense*: a new *Chrysosporium* species with keratinolytic activity. Mycotaxon 110, 65–71.

Luo Y, Chen WH, Wang Y, Han YF, Liang ZQ. 2016 – A new *Geomyces* species producing melanin in the medium. Mycosystema 35, 123–130.

Posada D, Crandall KA. 1998 – Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Prakash P, Prakash S. 2003 – Laboratory efficacy tests for fungal metabolites of *Chrysosporium tropicum* against *Culex quinquefasciatus*. Journal of the American Mosquito Control Association 19, 404–407.

Prakash P, Srivastava JN, Prakash S. 2001 – *Chrysosporium tropicum* efficacy against *Anopheles stephensi* larvae in the laboratory. Journal of the American Mosquito Control Association 17, 127–130.

Qorschot CAN Van. 1980 – A revision of *Chrysosporium* and alliedgenera. Studies in Mycology 20, 1–89.

Ronquist F, Teslenko M, van der Mark P, Ayres DL et al. 2012 – MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61, 539–542.

Sturm J. 1833 – Deutschlands Flora 3, Nurnberg.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 – Mega 6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30, 2725–2729.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997 – The Clustsal X windows interface: flexible strategies for multiple sequence alignment aided by quality 1 analysis tools. Nucleic Acids Research 24, 4876–4882.

Zeng Y, Li ZQ, Xue XP. 2004 – Advances in the study on keratinase. Strait Pharmaceutical Journal 16, 10–13.

Zhang YE, Han YF, Liang JD, Liang ZQ. 2013 – A new species of the genus *Chrysosporium* from the rhizosphere soil of palm. Mycosystema 32, 612–616.
Zhang YW, Chen WH, Zeng GP, Wang YR et al. 2016a – Two new *Chrysosporium* (Onygenaceae, Onygenales) from China. Phytotaxa 270, 210–216.
Zhang YW, Chen WH, Zou X, Han YF, Liang ZQ. 2016b – A new *Chrysosporium* species from sparrow's nest in Shanxi Province. Mycosystema 35, 1337–1343.