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Three new species and one new record of *Lobothallia* from China

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Abstract — A comprehensive analysis of morphological, anatomical, chemical and molecular data indicates the presence of three additional species in the genus *Lobothallia* (*Megasporaceae*, lichenized ascomycetes), which are described here as new to science under the names *L. crassimarginata*, *L. helanensis*, and *L. pruinosa*. *Lobothallia praeradiosa* is newly reported from China.

Key words — lichen, taxonomy

Introduction

The lichen genus *Lobothallia* (Clauzade & Cl. Roux) Hafellner was originally established for four species, *L. alphoplaca, L. melanaspis, L. praeradiosa,* and *L. radiosa* (Hafellner 1991), distinguished from *Aspicilia* by their lobate thallus, small spores, short conidia and chemistry. Recently, five additional species *L. farinosa*, *L. recedens* (Nordin et al. 2010), *L. cernohorskyana*, *L. chadefaudiana,* and *L. cheresina* (Roux 2012) have been included. As a consequence of this extension, *Lobothallia* is characterized by immersed to appressed or constricted-sessile apothecia, asci with an inamyloid tholus (*Aspicilia*-type), unbranched paraphyses, simple, hyaline spores and mainly bacilliform conidia. Lobes are distinct in some species, such as *L. alphoplaca*, *L. melanaspis*, *L. praeradiosa,* and *L. radiosa*, while other species have indistinct lobes, such as *L. farinosa* and *L. recedens*. In phylogenetic studies, *Lobothallia* is the sister group of the other genera within *Megasporaceae* (Nordin et al. 2010).

During our study on *Megasporaceae* of China, we discovered three new species of *Lobothallia,* described here as *L. crassimarginata*, *L. helanensis*, and *L. pruinosa*. *Lobothallia praeradiosa* is reported for the first time from China.

Materials & methods

The specimens studied were collected in Northern and Western China, and are preserved in SDNU (Lichen Section of the Botanical Herbarium, Shandong Normal

Table 1. Specimens used in the phylogenetic analyses. New sequences are in bold.

Species	Origin	Voucher	GenBank No.
Aspicilia cinerea	Sweden	UPS: Nordin 6213	JF703115
A. epiglypta	Sweden	UPS: Nordin 6105	HQ259262
Lobothallia alphoplaca	China, IM	SDNU: 20117646	**JX476025**
	China, IM	SDNU: 20117616	**JX499233**
L. crassimarginata	China, IM	SDNU: 20122565 (holotype)	**JX476026**
	China, IM	SDNU: 20122583	**KC007439**
L. helanensis	China, IM	SDNU: 20122791	**JX476031**
	China, IM	SDNU: 20122517 (holotype)	**JX476030**
L. melanaspis	Sweden	UPS: Nordin 6622	HQ259272
	Norway	UPS: Owe-Larsson 8943a	JF825524
L. praeradiosa	China, XJ	SDNU: 20126683	**JX499229**
	China, XJ	SDNU: 20126355	**JX499230**
	China, XJ	SDNU: 20126314	**JX499232**
	China, XJ	SDNU: 20126613	**JX499234**
L. pruinosa	China, IM	SDNU: 20123630	**JX476027**
	China, IM	SDNU: 20123278 (holotype)	**JX476028**
	China, IM	SDNU: 20123909	**JX499231**
L. radiosa	Sweden	UPS: Nordin 5889	JF703124
L. recedens	Sweden	UPS: Nordin 6035	HQ406807

*Province abbreviations: IM = Inner Mongolia; XJ = Xinjiang.

University). Specimen morphology and anatomy were examined using a dissecting microscope (Olympus SZ51) and a compact light microscope (Olympus CX41). Lichen substances were identified using standardized thin layer chromatography techniques (TLC) with solvent C (Orange et al. 2010). Photos of the thalli were taken using an Olympus SZX16 with DP72.

Sampling: Nuclear ITS1-5.8S-ITS2 rDNA sequences of 18 specimens representing 9 species were used in the molecular study. New sequences were produced from 13 specimens and six sequences were downloaded from GenBank (Tab. 1). *Aspicilia cinerea* and *A. epiglypta* were used as outgroup.

Extractions, PCR amplifications, and sequencing: Total DNA was extracted from the samples using the SanPrep Colum DNA Gel Extraction Kit. Lichen sample vouchers of the new sequences are listed in Table 1. To amplify the ITS1-5.8S-ITS2 regions, the primers ITS1-F (Gardes & Bruns 1993) combined with LR7 (Vilgalys & Hester 1990) or ITS4 combined with ITS5 (White et al. 1990) were used. The PCR ran for 37 cycles (1 min. at 95°C, 30 sec. at 53°C, 30 sec. at 72°C) using Tiangen Taq. Sequencing reactions were carried out by BGI (www.genomics.cn) with an ABI 3730 XL DNA Analyzer.

Phylogenetic analyses: The data set was processed with the Minimum Evolution method (Rzhetsky & Nei 1992). The percentages of replicate trees (1000 replicates) in which the associated taxa clustered together in the bootstrap test (Felsenstein 1985) are shown next to the branches (Fig. 1). The evolutionary distances were computed using

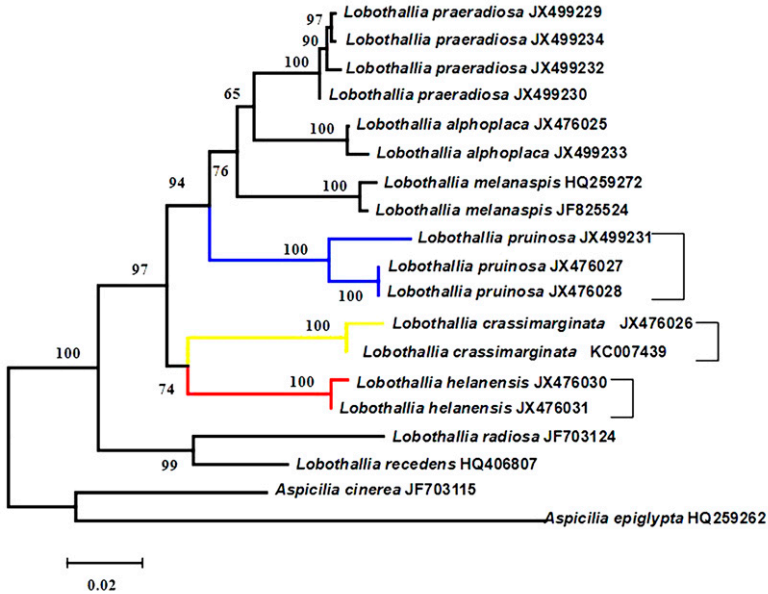

Figure 1. The ME tree inferred from ITS data. Bootstrap values greater than 60% (1000 replicates) are shown next to the branches.

the Maximum Composite Likelihood method (Tamura et al. 2004). There were a total of 571 positions in the final dataset. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Results & discussion

New Species

Lobothallia crassimarginata X.R. Kou & Q. Ren, **sp. nov.** Fig. 2

MycoBank MB 801753

Differs from all other *Lobothallia* spp. by its persistently prominent apothecial margin and thick thallus.

Type: China. Inner Mongolia, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, H.Y. Wang 20122565 (**Holotype**, SDNU; Genbank, JX476026).

Etymology: Latin *crassus* (= thick) + *marginatus* (= margined), referring to the apothecial margins.

Thallus placodioid, up to 2–5 mm thick centrally, tightly adnate, areolate; areoles 1–1.5 mm in diam., discrete, rounded, plane to somewhat convex; lobes 1–2 mm long, 0.5–0.8 mm across, 0.5–0.7 mm or more thick, usually distinctly elongated, radiating and separate, plane to more often strongly convex; upper surface ashy gray, sometimes tinted ochraceous or rosy,

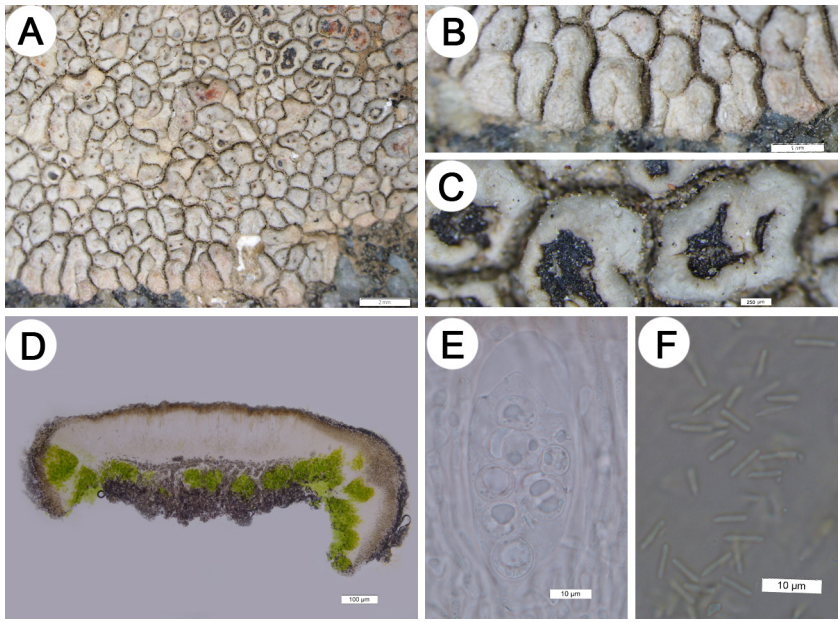

Figure 2. *Lobothallia crassimarginata* (Holotype). A. Thallus; B. Lobes; C. Apothecia; D. Apothecial anatomy; E. Ascus and spores; F. Conidia. Scale bars: A = 2 mm; B = 1 mm; C = 250 µm; D = 100 µm; E, F = 10 µm.

usually epruinose, smooth to somewhat wrinkled. Apothecia lecanorine, usually solitary, 0.6–1.5 mm in diam., appressed-sessile to distinctly sessile; disc black, plane or convex, epruinose; margin remaining strongly raised and inflexed, 0.2–0.5 mm wide, concolorous with the thallus; epihymenium brown, the pigment fading in K, N+ lightly green; hymenium hyaline, 70–90 µm tall; paraphyses separating in KOH, submoniliform; subhymenium and hypothecium hyaline, 50–70 µm tall together, with algae layer below; asci *Aspicilia*-type, 8-spored; ascospores hyaline, simple, subglobose to globose, 9–10 × 8–9 µm; conidia bacilliform, 5.4–7.6 × 1–1.2 µm.

Spot tests — medulla K+ yellow then red, C–, I–, P+ orange.

Secondary metabolites — norstictic, stictic and constictic acids.

Substrate — calciferous rock.

Additional specimens examined (all conserved in SDNU) — **China. Inner Mongolia**, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, H.Y. Wang 20122418B, 20123084B, 20123089C; D.B. Tong 20122513, 20122583 (GenBank, KC007439), 20122518.

Comments — *Lobothallia crassimarginata* differs from the other members of this genus by its persistently prominent apothecial margin and thick thallus. Both *Lobothallia alphoplaca* and *L. praeradiosa* have raised apothecial margins

only when young. Three other *Lobothallia* species with a thick thallus are *L. alphoplaca*, *L. helanensis*, and *L. recedens*. *Lobothallia alphoplaca* differs from *L. crassimarginata* by its intact removable thallus with longer lobes, *L. helanensis* by having a rough thallus, a thin apothecial margin, and lacking secondary metabolites, while *L. recedens* has a pruinose thallus and shorter conidia (3–5 × 1 μm; Smith et al. 2009). In the phylogenetic tree, these four species lie in different clades. Evolutionary distances between *L. crassimarginata* and other three species range from 0.074 to 0.111.

Lobothallia helanensis X.R. Kou & Q. Ren, **sp. nov.** Fig. 3

MycoBank MB 801755

Differs from *Lobothallia recedens* by its thallus having a gray to white-gray upper surface with a brown tinge, and by its larger conidia.

Type: China. Inner Mongolia, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, D.B. Tong 20122517 (**Holotype**, SDNU; Genbank, JX476030).

Etymology: Referring to the type locality.

Thallus crustose, rimose to areolate, gradually thicker towards the central part, sometimes up to 7 mm thick; areoles 0.7–1.4(–2) mm in diam., contiguous,

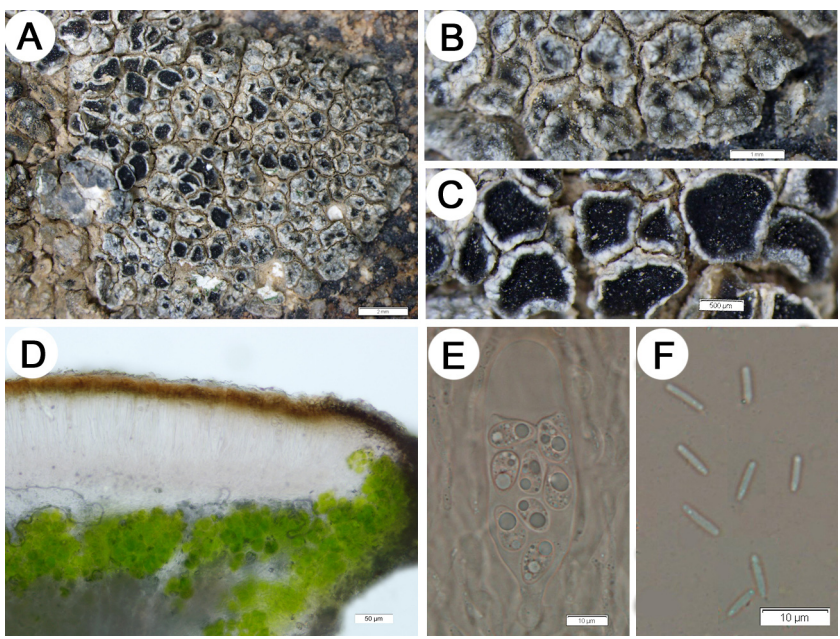

Figure 3. *Lobothallia helanensis* (Holotype). A. Thallus; B. Lobes; C. Apothecia; D. Apothecial anatomy; E. Ascus and spores; F. Conidia. Scale bars: A = 2 mm; B = 1 mm; C = 500 μm; D = 50 μm; E, F = 10 μm.

angular to irregular, ±convex; UPPER SURFACE gray to white-gray, with a brown tinge, distinctly rough. APOTHECIA 0.5–1.3(–2) mm in diam., aspicilioid, rounded or angular; thalline margin thin, slightly elevated, sometimes with slightly dentate incision, inner part brown, outer part concolorous with the thallus; DISC black, without pruina; EPIHYMENIUM brown, K+ brown, N+ weakly green; HYMENIUM hyaline, I+ blue, 70–80 µm tall; PARAPHYSES separating in KOH, submoniliform to moniliform; SUBHYMENIUM colourless to weakly brown, I+ blue, ±50 µm tall; HYPOTHECIUM colourless, I+ blue, 30 µm tall, algae continuous or grouped below the hypothecium. ASCI clavate, *Aspicilia*-type, 8-spored; ASCOSPORES hyaline, simple, ellipsoid, 10–12.5 × 5.5–6.5 µm; CONIDIA bacilliform, 5.5–6.4(–8) × 1.2–1.4 µm.

SPOT TESTS — medulla K–, C–, I–, P–.

SECONDARY METABOLITES — none detected by TLC.

SUBSTRATE — calciferous rock.

ADDITIONAL SPECIMENS EXAMINED (all conserved in SDNU) — **CHINA**. **INNER MONGOLIA**, Mt. Helan, on rock, alt. 1500 m, 17 Aug 2011, D.B. Tong 20122791 (GenBank, JX476031); 19 Aug 2011, H.Y. Wang 20122986, 20123043, 20122780A; P.M. Wang 20123301, 20123308A, 20123709.

COMMENTS — *Lobothallia helanensis* resembles most closely *L. recedens*, which shares a thick thallus and the absence of secondary metabolites but has a white sheen resembling pruina and smaller conidia. *Lobothallia alphoplaca*, another species with a thick thallus, differs by its loosely adnate thallus containing norstictic, constictic, or salazinic acids. In the phylogenetic tree, *Lobothallia helanensis* JX476030 and *Lobothallia helanensis* JX476031 form a clade supported by 99% bootstrap value. These three species lie in different clades. The evolutionary distances between *L. helanensis* and the other two species range from 0.091 to 0.132.

Lobothallia pruinosa X.R. Kou & Q. Ren, **sp. nov.** FIG. 4

MYCOBANK MB 801756

Differs from *Lobothallia recedens* by its thin and pruinose thallus, its more sparse apothecia, and its smaller ascospores.

TYPE: China. Inner Mongolia, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, H.Y. Wang 20123278 (**Holotype**, SDNU; Genbank, JX476028).

ETYMOLOGY: Referring to the pruinose thallus and apothecial discs.

THALLUS placodioid to squamulate, up to 0.5–1.0 mm thick, tightly attached, areolate, at the margin radiate; AREOLES 0.5–1 mm wide, contiguous, plane; LOBES plane, contiguous, confluent, tightly attached, 1–2 mm long, 0.7–1 mm wide, 0.15–0.2 mm thick; UPPER SURFACE usually whitish gray to brownish gray, often pruinose especially at the margins of the areoles. APOTHECIA solitary, round, 0.7–1.2 mm in diam., somewhat higher than the areoles; DISC

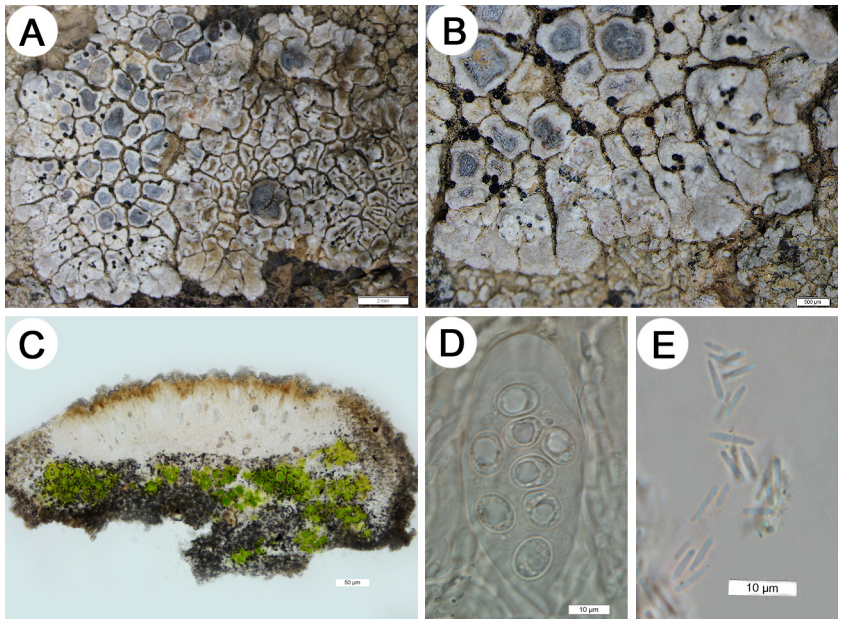

Figure 4. *Lobothallia pruinosa* (Holotype). A. Thallus with white pruina; B. Lobes and apothecia; C. Apothecial anatomy; D. Asci and spores; E. Conidia. Scale bars: A = 2 mm; B = 500 µm; C = 50 µm; D, E = 10 µm.

dark brown, usually pruinose; asci *Aspicilia*-type, 8-spored; epihymenium brown, the pigment fading in K, N+ weakly green; hymenium hyaline, I+ blue, 80–120 µm tall, paraphyses separating in KOH, submoniliform to moniliform; subhymenium and hypothecium colourless, I+ blue, 50–70 µm tall together, algae continuous or grouped below the hypothecium; ascospores broadly ellipsoid, 12.5–15 × 8.5–10 µm; conidia bacilliform, 5–7 × 1–1.3 µm.

Spot tests — medulla K+ yellow, C–, I–, P+ orange.

Secondary metabolites — norstictic and constictic acids.

Substrate — siliceous rock intermingled with calciferous granules.

Additional specimens examined (all conserved in SDNU) — **China**. **Inner Mongolia**, Mt. Helan, on rock, alt. 1500 m, 19 Aug 2011, H.Y. Wang 20123091, 20123630 (GenBank, JX476027); 1600 m, 19 Aug 2011, H.Y. Wang 20123575. Mt. Huhebashige, on rock, alt. 1600 m, 16 Aug 2011, H.Y. Wang 20123447B, 20123408; 17 Aug 2011, H.Y. Wang 20123909 (GenBank, JX499231); X.R. Kou 20123915, 20123950; P.M. Wang 20123678.

Comments — Another species with distinct pruina is *L. recedens*, which differs from *L. pruinosa* by its thick thallus, numerous apothecia and somewhat larger spores. The two species lie in different clades. The evolutionary distances between them range from 0.130 to 0.139.

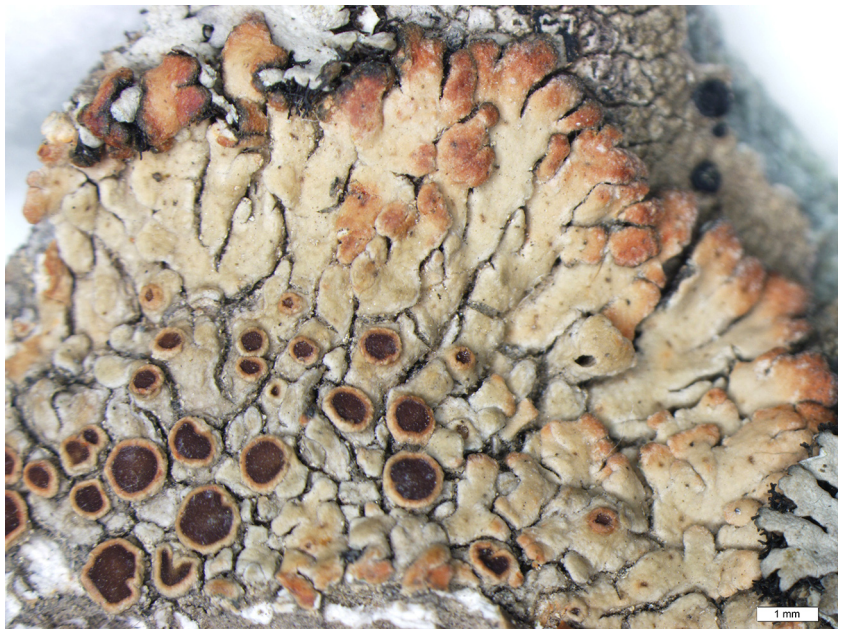

Figure 5. *Lobothallia praeradiosa*, 20126613 (SDNU). Scale = 1 mm.

New Record

Lobothallia praeradiosa (Nyl.) Hafellner, Acta Bot. Malac. 16: 138 (1991). Fig. 5

Thallus placodioid, up to 0.5–1.0 mm thick, tightly attached, areolate; areoles 0.5–1 mm wide, contiguous, elongate to irregular, plane to convex; lobes contiguous, confluent, plane, broad, sometimes imbricate, loosely attached, 3–6 mm long, 0.5–1.5 mm wide, 0.2–0.5 mm thick; upper surface usually green gray to orange brown, often radiate. Apothecia solitary to numerous, round, 0.5–1.5 mm in diam., adnate, somewhat higher than other areoles; disc: dark brown, usually epruinose, asci *Aspicilia*-type, 8-spored; epihymenium brown, K+ brown, N–; hymenium hyaline, I+ blue, 75–100 µm tall, paraphyses separating in KOH, submoniliform; subhymenium and hypothecium colourless, I+ blue, 40–50 µm tall together; algae continuous or grouped below the hypothecium; ascospores ellipsoid, 12.5–15 × 5–7.5 µm.

Spot tests — medulla K+ yellow, C–, I–, P+ orange or yellow.

Secondary metabolites — norstictic acid.

Substrate — siliceous rock.

Additional specimens examined (all conserved in SDNU) — **China. Xinjiang**, Mt. Tian, on rock, alt. 1910 m, 23 Aug 2011, Z.L. Huang 20126613 (GenBank, JX499234), Z.L. Huang 20126355 (GenBank, JX499230); L. Li 20126314 (GenBank, JX499232); Mt. Nan, on rock, alt. 1900 m, 28 Aug 2011, Z.L. Huang 20126683 (GenBank, JX499229).

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