# Three Newly Recorded Species of the Lichen Genus Podosticta from China (Post-print)

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## Abstract

To investigate the species diversity of the lichen genus Malmidea in China and further clarify its species composition and distribution, this study conducted field surveys of lichen resources and specimen collection, integrating morphological, anatomical, and chemical research methods to classify Malmidea specimens from Yunnan Province. A total of three new records for China were identified in the genus Malmidea: M. indica (D. D. Awasthi & M. R. Agarwal) Hafellner & T. Sprib, M. reunionis Kalb, and M. hechicerae Kalb. This paper provides detailed descriptions, morphological and anatomical figures for each new record, with comparisons and discussions to similar species; additionally, a taxonomic key to Malmidea species in China is provided. These findings enrich the data on the genus Malmidea in China and hold significance for the conservation and research of lichen diversity in the country.

## Full Text

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## Three New Records of Lichen Genus *Malmidea* from China

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### Abstract

This study investigates the species diversity of *Malmidea* in China to clarify its species composition and distribution. Through field surveys and specimen

collection in Yunnan Province, combined with morphological, anatomical, and chemical analyses, we identified three new national records of *Malmidea*: *M. indica* (D. D. Awasthi & M. R. Agarwal) Hafellner & T. Sprib, *M. reunionis* Kalb, and *M. hechicerae* Kalb. Detailed descriptions, morphological and anatomical illustrations, and comparisons with similar species are provided for each taxon. Additionally, a taxonomic key to all known Chinese *Malmidea* species is presented. These findings enrich the knowledge of *Malmidea* diversity in China and contribute to the conservation and study of the country's lichen biodiversity.

**Key words:** lichenized fungi, morphology, taxonomy, Malmideaceae, crustose lichens

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Lichens represent extracellular symbiotic associations between lichenized fungi and corresponding algae or cyanobacteria, constituting an important component of biodiversity in the biosphere. Currently, approximately 19,500 lichen species are known globally (Lücking et al., 2017), with about 3,050 species recorded in China (Wei, 2020), representing roughly 15.7% of the world's known species. Although China possesses rich lichen resources, taxonomic research started relatively late and remains inadequately developed, with numerous species yet to be discovered and described.

The genus *Malmidea* was established by Kalb et al. in 2011 and belongs to the family Malmideaceae, order Lecanorales, class Lecanoromycetes, phylum Ascomycota, kingdom Fungi (Gothamie et al., 2016). Species of this genus primarily inhabit tropical rainforests, particularly in South America and Southeast Asia (Breuss & Lücking, 2015), often growing in moist, shaded locations. The genus is characterized by crustose thalli (rarely fruticose), corticolous or foliicolous habit; presence of wart-like structures ranging from granular to pustulose, occasionally smooth; pigmented medulla in the thallus or warts (Cáceres et al., 2017); photobionts being green algae, typically spherical or flattened; presence of prothallus; sessile, circular apothecia of two types: piperis-type with excipula composed of radiating hyphae, and granifera-type with excipula consisting of external hyphae and internal medulla (Kalb et al., 2009); hymenium colorless to dark brown; asci clavate with indistinct apical apparatus, containing 1–8 spores; ascospores hyaline, ellipsoid to fusiform, single-celled, with uniformly or terminally thickened walls and a halo; pycnidia rare, conidia filiform (Sodamuk et al., 2017).

Currently, 72 *Malmidea* species have been reported worldwide (Kalb, 2021), with only four species recorded from China: *Malmidea hypomelaena* (Nyl.) Kalb & Lücking, *M. vinosa* (Eschw.) Kalb, Rivas Plata & Lumbsch, *M. aurigera* (Fée) Kalb, Rivas Plata & Lumbsch, and *M. granifera* (Ach.) Kalb, Rivas Plata & Lumbsch. Previous investigations of lichen diversity in Yunnan Province revealed abundant *Malmidea* resources (Feng & Yang, 2018), prompting a systematic study of the genus in this region. This research uncovered three new

national records: *M. indica* (D. D. Awasthi & M. R. Agarwal) Hafellner & T. Sprib, *M. reunionis* Kalb, and *M. hechicerae* Kalb.

Our preliminary surveys indicate that *Malmidea* research in China remains limited to sporadic reports of individual species, lacking systematic and comprehensive studies. This deficiency arises partly because *Malmidea* apothecia closely resemble those of lecanoroid lichens, leading to frequent misidentification, and partly due to inconsistent generic and specific delimitations that complicate specimen identification. Consequently, systematic research on Chinese *Malmidea* is essential. Yunnan Province, located in southwestern China at low latitudes with dramatically varied topography and altitude, exhibits diverse climate types. These unique geographical and climatic conditions provide important habitats for many specialized organisms, serving as a center for species origin and differentiation with extremely rich biodiversity. This study focuses on tropical and subtropical regions of Yunnan, utilizing fresh field collections and specimens housed at the Herbarium of Shandong Normal University (SDNU), Lichen Species and Gene Resource Biology Research Center of Liaocheng University (LCUF), and Cryptogamic Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Through field surveys and laboratory taxonomic research, we aim to: (1) clarify the species composition of *Malmidea* in Yunnan to establish a foundation for Chinese *Malmidea* research, and (2) accumulate resource data for Yunnan species and Chinese lichens to provide a basis for future floristic and phylogenetic studies.

### 1.1 Study Materials

The experimental materials consisted of *Malmidea* specimens collected from Yunnan Province, China, and deposited in SDNU, LCUF, and KUN herbaria.

#### 1.2.1 Morphological and Anatomical Methods

Specimens were first examined under a stereomicroscope (Olympus SZ51) to observe thallus color, presence of warts, medulla color within thalli and warts, presence and size of soredia and isidia, apothecial color, size, and shape, and presence of prothallus, with relevant data measured under an optical microscope (Zhao et al., 2021). External morphology was photographed using an Olympus SZX16 optical microscope equipped with an Olympus DP72 camera. Hand sections of thalli and apothecia prepared under the stereomicroscope were examined under an Olympus CX21 optical microscope to record anatomical details, including colors and heights of the epihymenium, hymenium, hypothecium, and hamathecium; ascus shape and type; ascospore number, size, color, morphology (shape and presence of halo); presence, location, and color of calcium oxalate crystals; and presence of pycnidia. Anatomical structures were photographed using an Olympus BX61 optical microscope with an Olympus DP72 camera.

### 1.2.2 Chemical Methods

Cortical and medullary spot tests were performed on each specimen using 5% *p*-phenylenediamine in ethanol (Pd), 3% Lugol's solution (I), 10% aqueous potassium hydroxide (K), saturated aqueous calcium hypochlorite (C), and 10% KOH followed by saturated calcium hypochlorite (KC) (Joseph et al., 2018). Lichen substances were identified using thin-layer chromatography (TLC) (Elix et al., 2003).

### 2.1.1 *Malmidea indica* (Fig. 1)

**Malmidea indica** (D. D. Awasthi & M. R. Agarwal) Hafellner & T. Sprib., Lichenologist 46: 463 (2011).

The thallus is crustose and corticolous, with a rough surface that is white to gray and bears wart-like structures 0.1–0.35 mm in diameter; soredia and isidia are absent. The medulla within warts is white to pale yellow, K+ lemon yellow. Apothecia are adnate, circular, up to 1.6 mm in diameter, with a flat to slightly convex disc that is dark brown to black and a thin margin that is gray-white to gray. The excipulum is granifera-type, with a pale outer layer and an inner medulla containing opaque, hydrophobic granules. The epihymenium is pale brown to brown; the hymenium measures 80–125  m and is colorless; the hamathecium is 100–110  m thick at the center, tapering laterally, and dark brown. Asci are clavate and 2–4-spored. Ascospores are hyaline, single-celled, with a halo, ellipsoid, thick-walled, measuring 30–34 × 14–18 (–22) µm.

**Chemistry:** K+ yellow, C–, P–; TLC: trace atranorin or no secondary metabolites.
**Substrate:** Bark.
**Specimens examined:** Yunnan, Pu'er National Forest Park, corticolous, 1,596 m, 22°35 53.68  N, 101°06 31.71  E, 18 Dec 2013, Xinyu Wang & Dong Liu, 13-41448, 13-41461 (KUN); near Yakou, Heping Township, Zhenyuan to Xinping, corticolous, 2,300 m, 23°56 22.43  N, 101°29 05.14  E, 10 Jun 2013, Lisong Wang, Xinyu Wang & Jianwen Li, 13-38158 (KUN).

**Discussion:** This species is distinguished by 2–4-spored asci and large spores (length >30  m) with thick walls, which separate it from other *Malmidea* species. Initially described as *Mycoblastus indicum*, it was transferred to *Malmidea* by Spribille et al. because *Mycoblastus* differs in having reduced apothecial margins, blue-green epihymenium, and Biatora-type ascus apical apparatus (Flakus et al., 2019). Kalb et al. (2011) reported slightly longer spores (30–40 × 12–18 (–22) µm) in Indian specimens compared to our Yunnan material.

A. Thallus (scale: 1 mm); B. Apothecia (scale: 500  m); C. Ascospores (scale: 10  m); D. Excipulum K+ yellow (scale: 100  m); E. Hydrophobic granules (scale: 100  m); F. Section through apothecium (scale: 100  m).

[Figure 1: see original paper] *Malmidea indica* (Herb. No. 13-38158)

### 2.1.2 *Malmidea reunionis* (Fig. 2)

**Malmidea reunionis** Kalb., Phytotaxa 42: 42 (2012).

The thallus is crustose and corticolous, with a rough, gray-white surface bearing wart-like structures 0.1–0.25 mm in diameter; soredia and isidia are absent. The medulla within warts and thallus is off-white, K+ yellow to pale orange. Apothecia are adnate, circular, 0.75–1.35 mm in diameter, with a flat or slightly convex brown disc and a thin margin that is off-white to pale yellow. The excipulum is granifera-type, with a colorless outer layer and an inner medulla containing ochre-yellow hydrophobic granules that are K+ lemon yellow. The epihymenium is indistinct; the hymenium measures 120–170 m and is colorless; the hamathecium is 120–150 m thick and pale brown to brown. Asci are predominantly 4-spored, occasionally 8-spored. Ascospores are hyaline, single-celled, with a halo, ellipsoid to fusiform, with unevenly thickened walls that are notably thicker at the ends, measuring (21–)22–30 × (9–)12–15 (–16) m.

**Chemistry:** K+ yellow to pale orange, C–, P–; TLC: lacks atranorin but contains at least six unknown lichen secondary metabolites (Chen, 2022).
**Substrate:** Bark.
**Specimens examined:** Yunnan, western slope of Gaoligong Mountains, Tengchong County, corticolous, 2,300 m, 25°17 38.70 N, 98°42 06.65 E, 18 Mar 2014, Xinyu Wang, 14-42867 (KUN); Linjiapuzi, Gaoligong Mountains, Tengchong County, corticolous, 2,190 m, 25°17 42.39 N, 98°42 01.82 E, 17 Mar 2014, Xinyu Wang, 14-42844 (KUN); Linjiapuzi, Gaoligong Mountains, Tengchong County, corticolous, 2,190 m, 25°17 42.39 N, 98°42 01.82 E, 17 Mar 2014, Xinyu Wang, 14-42848 (KUN).

**Discussion:** *Malmidea reunionis* is characterized by large spores with distinctly thickened apical walls. While *M. chrysostigma* and *M. incrassata* also have unevenly thickened spore walls, *M. chrysostigma* has a golden to orange-red medulla with emodin as the main secondary metabolite, and *M. incrassata* possesses a piperis-type excipulum, lacks warts, and has smaller spores. Kalb reported higher hymenium (160–200 m) and hamathecium (180–250 m) in Indian Ocean island specimens (Cáceres et al., 2012) compared to our Yunnan material.

A. Thallus (scale: 1 mm); B. Apothecia (scale: 500 m); C. Section through apothecium (scale: 100 m); D. Hydrophobic granules (scale: 100 m); E. Excipulum K+ yellow (scale: 100 m); F. Ascospores (scale: 10 m).

[Figure 2: see original paper] *Malmidea reunionis* (Herb. No. 14-42867)

### 2.1.3 *Malmidea hechicerae* (Fig. 3)

**Malmidea hechicerae** Kalb., Archive for Lichenology 27: 18 (2021).

The thallus is crustose and corticolous, with a rough, gray-white surface bearing wart-like structures 0.1–0.2 mm in diameter; soredia and isidia are absent.

The medulla of both thallus and warts is white, K+ lemon yellow, P+ orange. Apothecia are adnate, circular, 0.6–1.3 mm in diameter, with a flat or slightly convex brown to dark brown disc. The excipulum is granifera-type, with a pale outer layer and an inner medulla containing opaque hydrophobic granules that dissolve in KOH (K+ yellow-green). The epihymenium is pale brown; the hymenium measures 70–80  m and is colorless; the hypothecium is 20  m and pale brown; the hamathecium is 50–70  m thick at the center, tapering laterally, dark brown, K–. Asci are 6–8-spored. Ascospores are hyaline, single-celled, with a halo, ellipsoid, with uniformly thickened walls, measuring 12–16 × 7–9  m.

**Chemistry:** K+ lemon yellow, C–, P+ orange; TLC: lacks atranorin but contains 4–5 xantholepinones.
**Substrate:** Bark.
**Specimens examined:** Yunnan, Laojun Mountain, Malipo County, corticolous, 1,200 m, 22°55 34.66  N, 104°36 28.44  E, 2 Oct 1995, Lisong Wang, 95-15732 (KUN); Xishuangbanna Tropical Botanical Garden, Menglun County, corticolous, 560 m, 21°55 07.13  N, 101°16 07.69  E, 19 Dec 2013, Xinyu Wang, 13-41542 (KUN); Xishuangbanna Primeval Forest Park, Jinghong City, corticolous, 710 m, 22°01 54  N, 100°52 41  E, 3 Jul 2021, Xiuzhi Zhang, YN211041 (LCUF); Tropical Rainforest Valley, Jinghong City, corticolous, 640 m, 21°54 52.26  N, 101°11 33.04  E, 3 Mar 2023, Linlin Liu, Lei Wang & Chunjiao Zhong, 20230483 (SDNU).

**Discussion:** *Malmidea hechicerae* is characterized by white medulla in both thallus and warts (K+ lemon yellow) and small spores. It resembles *M. coralliformis* in morphology and also lacks atranorin, but the two species differ in their xantholepinone content (Elix, 2014). Kalb et al. (2021) reported slightly longer spores (13–17 × 7–9  m) in northern South American specimens compared to our Yunnan material.

A. Thallus (scale: 1 mm); B. Apothecia (scale: 500  m); C. Asci (scale: 10  m); D. Ascospores (scale: 10  m); E. Excipulum K+ yellow (scale: 100  m); F. Hydrophobic granules (scale: 100  m).

[Figure 3: see original paper] *Malmidea hechicerae* (Herb. No. 95-15732)

### 2.2 Key to *Malmidea* Species in China

1a Spores 30–40 × 12–18  m, asci 2–4-spored; apothecia dark brown to black……. *M. indica*
1b Spores <30  m……
2
2a Spore walls distinctly thickened at both ends, medulla K+ yellow to pale orange; excipulum with medulla and granular material (K+ lemon yellow); spores (21–)22–30 × (9–)12–15(–16)  m…… *M. reunionis*
2b Spore walls uniformly thickened……
3
3a Spores 20–25 × 10–14  m; apothecia gray-brown to dark brown, margins pale

to dark brown……
*M. hypomelaena*
3b Spores smaller, <20 m……
4
4a Medulla of thallus and warts white……
5
4b Medulla of thallus and/or warts yellow to orange or red……
6
5a Medulla K–; spores 10–17 × 6–8 m……
*M. vinosa*
5b Medulla K+ lemon yellow, rarely orange-yellow; spores 13–17 × 7–9 m…… *M. hechicerae*
6a Medulla of warts yellow to peach-colored; asci 8-spored, spores 12–18 × 7–10 m…… *M. granifera*
6b Medulla of warts bright yellow; asci 6–8-spored, spores (9–)10–13 × 6–8(–9) m…… *M. aurigera*

## 3 Discussion and Conclusions

We conducted preliminary taxonomic identification of 233 specimens from Yunnan, recognizing six *Malmidea* species. To date, seven *Malmidea* species have been discovered in China, six of which occur in Yunnan Province (all except *Malmidea hypomelaena*). Our study reveals that *Malmidea* species mainly grow on tree trunks in primary forests below 2,000 m elevation, though *M. indica* can be found at 2,300 m and *M. granifera* at 3,400 m. *Malmidea granifera* exhibits the widest distribution in China, with records from Sichuan, Yunnan, Fujian, Taiwan, and Hong Kong.

During our research, we encountered numerous problematic and similar taxa that were difficult to identify accurately. The primary challenge is that *Malmidea*, as micro-crustose lichens, offers limited phenotypic characters for taxonomic identification, typically restricted to medulla color, presence of warts, excipulum type, crystal color, and spore shape and size. Furthermore, morphological characteristics of the same species can vary considerably under different environmental conditions, inevitably leading to interspecific overlap or synonyms. Additionally, each specimen's taxonomic traits fall within certain ranges of variation, introducing subjective elements into identification.

Although molecular methods have been applied to lichen phylogenetics in recent years, research data on *Malmidea* remain extremely scarce, with only 19 gene sequences from 11 species available in GenBank. In recent years, we have conducted extensive field surveys and collections of *Malmidea* in China. Based on traditional taxonomy combined with molecular approaches, we have obtained gene sequences for some species, though specimens older than five years rarely yield successful DNA extractions. Future work will involve more comprehensive and systematic surveys, collection of fresh specimens, and improved DNA extraction methods to conduct in-depth phylogenetic analyses and reevaluate the

taxonomic criteria and framework of the genus.

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*Note: Figure translations are in progress. See original paper for figures.*