

An investigation of lemma micromorphology in Thai Oryzeae (Poaceae)

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ABSTRACT: Nine taxa of Oryzeae that occur naturally in Thailand were studied for their lemma micromorphology using scanning electron microscopy. Common features found in most taxa were microhairs, prickles, silica bodies, and papillae. Here, features of the lemma surface are described, classified, and discussed in terms of their taxonomic significance. In addition, a key to species based on the shape of silica bodies, type of papillae and tubercles, and the arrangement of tubercles is presented. However, lemma micromorphology alone cannot be used to distinguish three species in the *Oryza sativa* group, namely, *O. sativa*, *O. rufipogon*, and *O. officinalis*.

KEYWORDS: *Oryza*, *Leersia*, *Hygroryza*, *Zizania*

INTRODUCTION

Oryzeae Dumort. is a tribe in the grass family (Poaceae) consisting of twelve genera and around seventy species distributed in tropical and warm temperate regions of the world¹. Notably, the tribe contains cultivated rice (*Oryza sativa* L.) and its relatives, making it an attractive group for systematic studies. Accordingly, various investigations into the morphology², anatomy³, cytology⁴, and molecular phylogenetics^{5–7} of the tribe have been published. The consensus of these and other studies is that tribe Oryzeae belongs in subfamily Ehrhartoideae, one of three subfamilies of the BEP (Bambusoideae, Ehrhartoideae, and Pooideae) clade^{8,9}.

In Thailand, four genera of Oryzeae are found¹⁰: *Oryza* L., *Leersia* Sw., *Hygroryza* Nees, and *Zizania* L. The genus *Oryza* contains five species and two varieties, while *Hygroryza* is a monotypic genus containing only *H. aristata* Nees. The genus *Leersia* contains *L. hexandra* Sw., a common weed of rice fields, and *L. stipitata* Bor, which has been recorded only in Chanthaburi province in 1961. Whereas these nine taxa are native to Thailand, *Z. latifolia* Turcz. ex Stapf, also known as wild rice, was introduced to Thailand for cultivation.

In general, taxonomic classification of grasses has relied on spikelet structure and the number of florets, as well as lemma and palea characters. However, the use of this classification has some limitations because of similarity in morphology. Barthlott¹¹ emphasised the value of epidermal and seed surface characters

in a systematic analysis for classification at both the specific and familial levels, since these characters are little affected by the growing environment. Scanning electron microscope (SEM) observations of achene morphology in *Polygonum s.l.*¹² and of fruit and seed structure in Scrophulariaceae¹³ have underscored the systematic importance of these characters in a taxonomic context.

Numerous studies have investigated micromorphology in various groups within the Poaceae, and overall these have helped establish the usefulness of this field of study in the family. For example, SEM was used to investigate surface characters of both vegetative and reproductive parts of the subfamily Danthonioideae¹⁴, and to identify five lemma micromorphological characters (namely, long cells, cork cells, stomata, papillae, and microprickles) that optimize a molecular cladogram of Chloridoideae grasses¹⁵. Hence micromorphology of lemma surfaces is expected to aid taxonomic classification of grasses.

In tribe Oryzeae, SEM has been used for many diverse applications. It was used for ultrastructural description of leaf blades, ligules, auricles and spikelets among *Oryza latifolia*, *O. glumaepatula*, and *O. grandiglumis*, wild rice species of Tropical America^{16–18}. In addition, Hoagland and Paul¹⁹ used SEM to compare the surfaces of seeds and coleoptiles of red rice and other commercial varieties, while Thammawongsa et al²⁰ found that the lemma surface is a taxonomically valuable source of information when used in combination with other morphological

Table 1 List of voucher specimens of Thai Oryzeae used in this study. Herbarium acronyms are given in parentheses.

Taxon	Voucher specimen
<i>Hygroryza aristata</i> Nees	Thailand: Bangkok, A.F.G. Kerr 3883 (BK) Thailand: Songkhla, A.F.G. Kerr 15119 (BK)
<i>Leersia hexandra</i> Sw.	Thailand: Kamphaeng Phet, P. Sumanon & P. Traiperms 5 (BKF) Thailand: Khon Kaen, C. Jaroenchai 31 (KKU) Thailand: Sakon Nakhon, Piyarat & Monthon 1 (KKU)
<i>Oryza meyeriana</i> (Zoll. & Moritzi) Baill. var. <i>meyeriana</i>	Thailand: Phatthalung, P. Sumanon & P. Traiperms 1 (BKF)
<i>Oryza meyeriana</i> (Zoll. & Moritzi) Baill. var. <i>granulata</i> (Nees & Arn. ex G. Watt) Duist.	Thailand: Phetchabun, P. Sumanon & P. Traiperms 3 (BKF) Thailand: Phetchabun, P. Sumanon & P. Traiperms 4 (BKF) Thailand: Chon Buri, J.F. Maxwell 75-399 (BK) Thailand: Loei, C. Jaroenchai 187 (KKU)
<i>Oryza officinalis</i> Wall.	Thailand: Saraburi, J.F. Maxwell 74-595 (BK) Thailand: Saraburi, C. Chermisiriwattana 620 (BK) Thailand: Nonthaburi, Umpai s.n. (BK)
<i>Oryza ridleyi</i> Hook. f.	Thailand: A.F.G. Kerr 21662 (BK) Thailand: Chachoengsao, A.F.G. Kerr 19780 (BK) Thailand: Sa Kaeo, Put 3103 (BK)
<i>Oryza rufipogon</i> Griff.	Thailand: Kamphaeng Phet, P. Sumanon & P. Traiperms 6 (BKF) Thailand: Kamphaeng Phet, P. Sumanon & P. Traiperms 8 (BKF) Thailand: Phetchaburi, A.F.G. Kerr 11088 (BK) Thailand: Chachaengsao, Yingyong P. S2-20-79 (BK)
<i>Oryza sativa</i> L.	Thailand: Kamphaeng Phet, P. Sumanon & P. Traiperms 10 (BKF) Thailand: Chiang Mai, Yingyong Paisooksantivatana s.n. (KKU) Thailand: Khon Kaen, P. Chantaranonthai & J. Parnell 90/675 (KKU)
<i>Zizania latifolia</i> Turcz. ex Stapf	Thailand: Bangkok, Umpai 518A (BK)

and anatomical characters and were able to construct a key for the identification of 26 lowland black rice specimens.

Even though epidermal features and spikelet micromorphology in *Oryza* and related genera including *Leersia*, *Zizania*, *Rhynchoryza*, and *Porteresia* have been examined using SEM method in previous studies^{21–23}, not all taxa found in Thailand have been investigated. In particular, *Hygroryza* has never been studied in this way before, and no key for species-level identification based on micromorphological characters has been provided. Hence the present study sought to use SEM in a micromorphological survey of the Oryzeae of Thailand in an attempt to describe and compare lemma surface details upon which a key to the composite species could be constructed.

MATERIALS AND METHODS

Plant materials

Plant materials were collected from the field and from herbarium specimens (Table 1).

SEM investigation

The spikelets of plants were cleaned by immersion in 70% (v/v) ethanol and sonicated for 10 min to remove contaminants. Then the samples were soaked in 95% ethanol, dried, and mounted on stubs using carbon tape. After that, they were coated with platinum-palladium in a sputter coater (HITACHI E-102 ION SPUTTER) and photographed using an HITACHI S-2500 scanning electron microscope. The terminology used in this study mainly follows Metcalfe²⁴, Terrell et al²¹, Terrell and Wergin²³, and Terrell et al²².

RESULTS

Common features found on lemma surfaces of the Oryzeae species studied were microhairs, prickles, silica bodies, and papillae. For nine taxa of Oryzeae examined in this study, SEM photographs showed clear differences in lemma surfaces, as summarised below.

Hygroryza aristata (Fig. 1). Lemma and palea were 5–5.5 mm long and 1–1.25 mm wide. Prickles

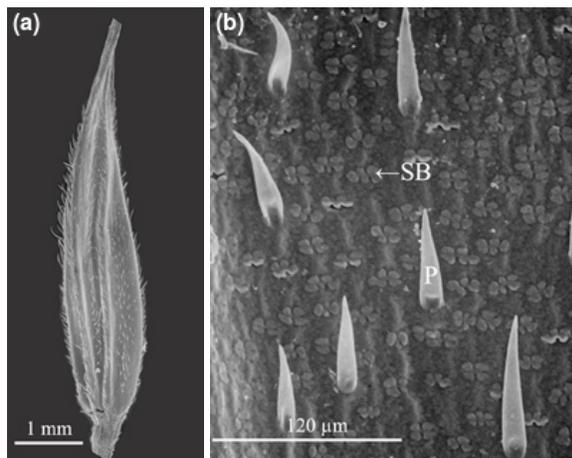


Fig. 1 SEM micrographs of the lemma of *H. aristata*, (a) whole lemma, (b) details on lemma surface (P = prickle; SB = silica body).

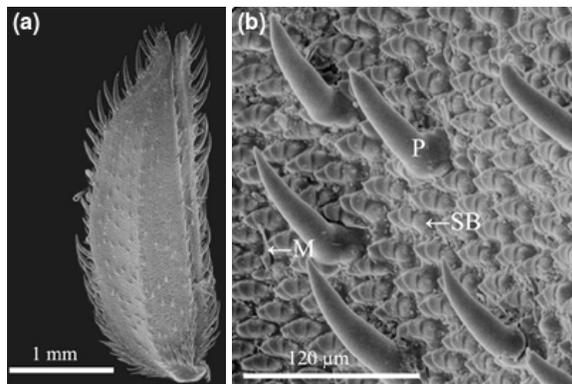


Fig. 2 SEM micrographs of the lemma of *L. hexandra*, (a) whole lemma, (b) details on lemma surface (P = prickle; M = microhair; SB = silica body).

were one-celled, pointed, 60–90 μm long, mostly arranged in rows. Silica bodies were four lobed, scattered over the lemma surface. No papillae were found.

Leersia hexandra (Fig. 2). Lemma and palea were 3–4 mm long and 1–1.2 mm wide. Microhairs were two-celled, panicoid type, with a short basal cell and a longer distal cell, 18–27 μm long, scattered, and arranged in furrows between siliceous triads. Silica bodies were triads formed of tubercles, with terminal pores, scattered over the lemma surface. Prickles were one-celled, pointed, 75–120 μm long, arranged in rows. No papillae were found.

Zizania latifolia (Fig. 3). Since this taxon produced unisexual florets, both female and male florets were examined. These two floret types differed in

length, but not in lemma surface sculpturing. In female florets, lemma and palea were 1–1.2 cm long and 1–1.5 mm wide, while lemma and palea of male florets were 1.5–2 cm long and 1.2–1.5 mm wide. Pistillate and staminate lemmas were sculptured with silica bodies, papillae, pitted papillae, prickles, and microhairs. Microhairs were two-celled, panicoid type, with a short basal cell and a longer distal cell, 30–45 μm long, and arranged in rows with prickles between cells. Silica bodies were bi-lobed to orbicular, placed in furrows between cells, scattered over lemma surface. Prickles were one-celled, pointed, and variable in length from 30–120 μm . Two types of papillae were found - rounded siliceous papillae and pitted siliceous papillae. Each cell contained 1 or 2 papillae.

Species belonging to the genus *Oryza* could be divided into three groups based on their surface sculpturing, especially with respect to tubercle characters. The first group included the two varieties of *O. meyeriana*, var. *granulata* and var. *meyeriana*, the second group consisted of *O. sativa*, *O. rufipogon*, and *O. officinalis* (the '*O. sativa* group'), and the last group contained only one species, *O. ridleyi*.

The two varieties of *O. meyeriana* exhibited unique tubercle characters (Fig. 4). The tubercles consisted of rounded papillae and bent prickles. These large, complex tubercles were conspicuous, positioned over the furrows and distributed over the entire surface of the lemma in both varieties.

O. meyeriana var. *meyeriana* (Fig. 4a,b). Lemma and palea were 5–6.5 mm long and 1.5–2 mm wide. Microhairs were two-celled, panicoid type, very small (approx. 21 μm in length) and occurred in furrows. Tubercles were 50–70 μm wide, composed of rounded papillae and one short (23–34 μm in length), sharp pointed to bent prickle at the centre of tubercles, located over the furrows, and scattered over the surface of the lemma. Numerous papillae were found over the surface of the lemma. Many papillae were on the same cell. All papillae were rounded.

O. meyeriana var. *granulata* (Fig. 4c,d). Lemma and palea were 4.5–5.5 mm long and 2.2–3 mm wide. Tubercles were 40–65 μm wide, composed of rounded papillae and one short (34–49 μm in length), bent prickle at the centre of tubercles, positioned over the furrows, and densely scattered over the surface of lemma. Numerous papillae, each cell had many papillae. All papillae were rounded.

O. ridleyi (Fig. 5a,b) also had tubercles over the furrows as in the *O. meyeriana* group; but, it was separated from the two varieties of that group because of its simple tubercles. Lemma and palea were 0.7–

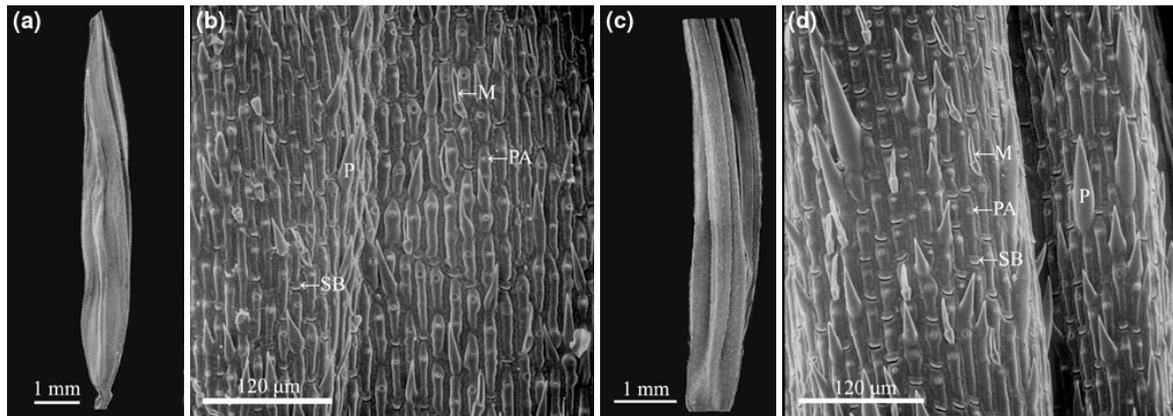


Fig. 3 SEM micrographs of the lemma of *Z. latifolia*, (a)–(b) female floret and (c)–(d) male floret (P = prickle; M = microhair; SB = silica body; PA = papillae).

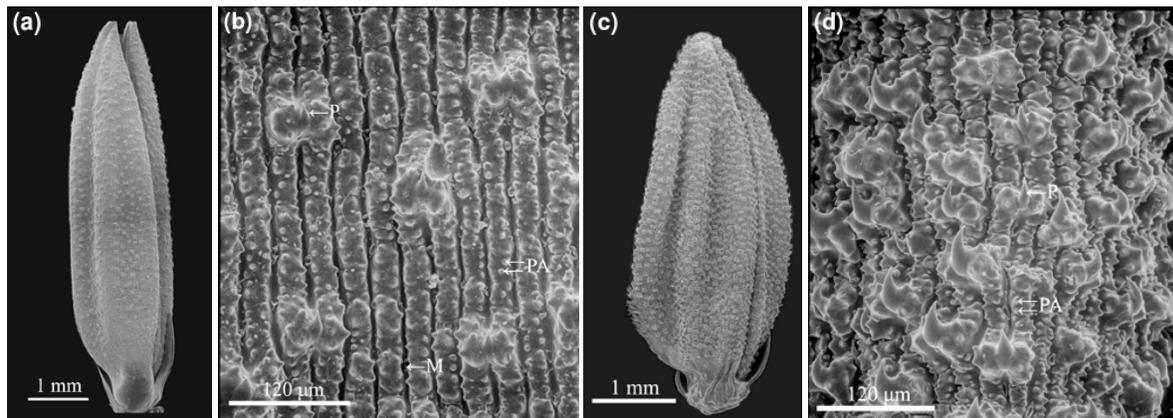


Fig. 4 SEM micrographs of the lemma of (a)–(b) *O. meyeriana* var. *meyeriana* and (c)–(d) *O. meyeriana* var. *granulata* (P = prickle; PA = papillae; M = microhair).

1 cm long and 1.5–2 mm wide. Tubercles were 38–47 μm wide, simple, nearly arranged in rows and tiers. Many tubercles were assembled with a short pointed prickle in the centre. Microhairs were two-celled, panicoid type, with short basal cell and longer distal cell, 41–47 μm long, located in the furrows. Papillae were rounded, numerous.

All members of the *O. sativa* group had simple tubercles, without prickles on the centre, positioned on the ridges. The tubercles were arranged in order and formed into rows and tiers. Microhairs and prickles located in furrows between tubercles. Microhairs were two-celled, panicoid type. Prickles were long, large and pointed. Papillae scattered.

O. sativa (Fig. 5c,d). Lemma and palea were 8.5–9 mm long and 2.4–3 mm wide. Tubercles were 61–77 μm wide, with 2–4 sharp points, arranged on the ridges in orderly rows and tiers. Microhairs were two-celled, panicoid type, 40–52 μm in length, located

in the furrows between tubercles, scattered. Prickles were one-celled, pointed, 245–310 μm in length, in the furrows between tubercles, scattered. Papillae rounded, scattered.

O. rufipogon (Fig. 6a,b). Lemma and palea were 7–8 mm long and 2.2–2.5 mm wide. Tubercles were 51–57 μm wide, with 3 or 4 sharp points, arranged on the ridges in orderly rows and tiers. Microhairs were two-celled, panicoid type, 34–40 μm length, located in the furrows between tubercles. Prickles were one-celled, pointed, 170–220 μm in length, arranged in the furrows between tubercles, scattered. Papillae rounded, scattered.

O. officinalis (Fig. 6c,d). Lemma and palea were 4–5 mm long and 2–2.4 mm wide. Tubercles were 77–86 μm wide, with 2–4 sharp points, arranged on the ridges in orderly rows and tiers. Microhairs were two-celled, located in the furrows between tubercles. Prickles were one-celled, pointed, about 350 μm in

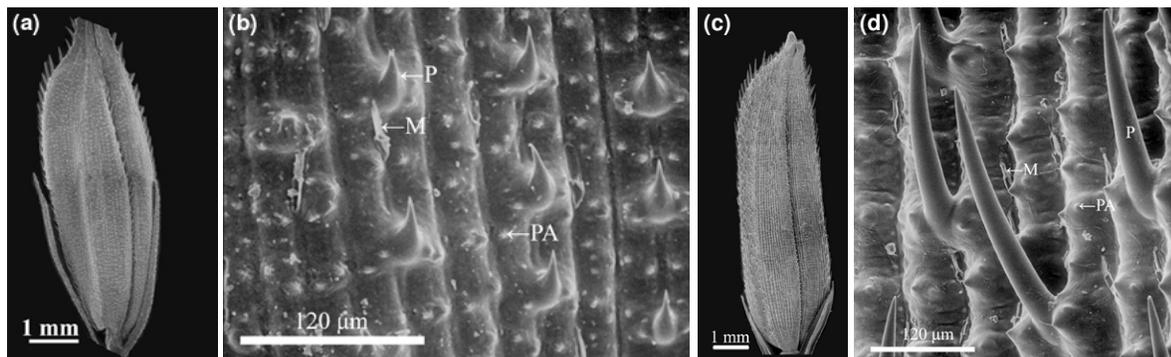


Fig. 5 SEM micrographs of the lemma of (a)–(b) *O. ridleyi* and (c)–(d) *O. sativa* (P = prickle; M = microhair; PA = papillae).

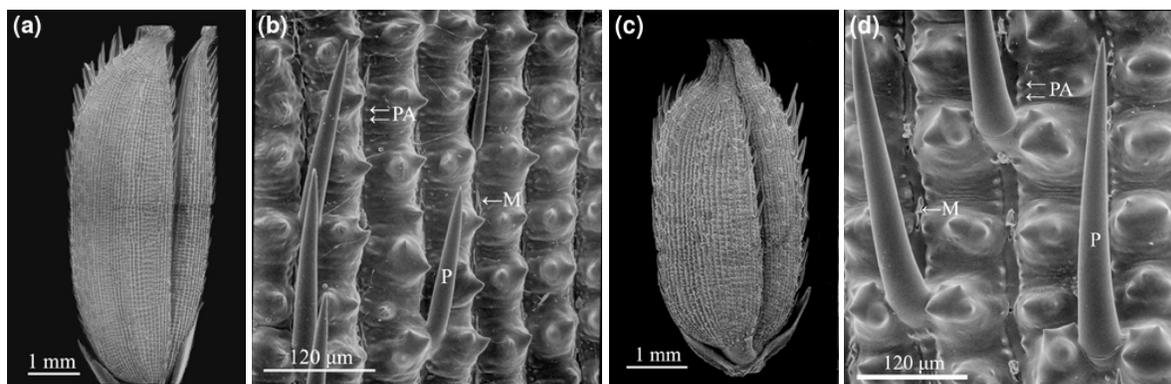


Fig. 6 SEM micrographs of the lemma of (a)–(b) *O. rufipogon* and (c)–(d) *O. officinalis* (P = prickle; M = microhair; PA = papillae).

length, scattered over the surface of lemma. Papillae rounded, scattered.

These SEM observations revealed micromorphological characters of lemma surfaces which can be used to construct a key to species for Oryzae in Thailand (Table 2). However, these characters are insufficient to separate the species of the *O. sativa* group (*O. sativa*, *O. rufipogon*, and *O. officinalis*).

DISCUSSION

Epidermal characters provide valuable information for classification and identification at the species and family levels, since these characters often exhibit highly specific forms that are unaffected by the environment¹¹. In general, epidermal features of paleas are similar to those of lemmas^{20,21}. The micromorphology of lemma corresponds to that of palea. The basic features on epidermal structures are microhairs, prickles, papillae, and silica bodies.

All Oryzae species examined in this study contained bicellular microhairs (panicoid type: a short basal cell and a longer apical cell). Microhairs in most

Table 2 Identification key based on lemma surfaces.

1a. Silica bodies four-lobed	<i>H. aristata</i>
1b. Silica bodies not as above	2
2a. Silica bodies triads with terminal pores	<i>L. hexandra</i>
2b. Silica bodies not as above	3
3a. Papillae of two types: rounded and pitted	<i>Z. latifolia</i>
3b. Papillae of one type: rounded	4
4a. Tubercles on the ridges, arranged in orderly rows and tiers	<i>O. sativa</i> group
4b. Tubercles over the furrows	5
5a. Simple tubercles	<i>O. ridleyi</i>
5b. Complex tubercles	6
6a. Tubercles scattered densely	<i>O. meyeriana</i> var. <i>granulata</i>
6b. Tubercles scattered almost orderly	<i>O. meyeriana</i> var. <i>meyeriana</i>

of our materials were collapsed and broken off due to air-drying as part of the herbarium specimen preparation and SEM fixation process. Other researchers

also faced this problem^{20,22}. To solve this problem, fresh material should be used and prepared through chemical fixation and critical point drying.

Prickles are unicellular and sharp pointed, except in two varieties of *O. meyeriana* which have slightly bent prickles. Papillae are rounded, except in *Z. latifolia* which has two types of papillae (rounded and pitted). This observation of *Z. latifolia* corroborates the findings of Terrell and Wergin²². The pitted siliceous papillae are unique to *Z. latifolia*, readily distinguishing it from other *Zizania* species. Terrell and Wergin²² suggested that pits developed from the collapse of the top of papillae.

Silica bodies are valuable for identification of Oryzae species. The four-lobed silica bodies and siliceous triads can separate *H. aristata* and *L. hexandra* from the others, respectively. The siliceous triads consist of a silica body and two large tubercles with terminal pores on each side of silica body. This result agrees with a previous study of *Leersia*²¹.

Terrell et al²⁰ supposed that tubercles are unique to genus *Oryza*. However, we found a different form of tubercle in *L. hexandra*. A phylogenetic tree based on chloroplast DNA fragment data showed a sister relationship between *Oryza* and *Leersia* in the subtribe Oryzinae⁶, whereas *Hygroryza* and *Zizania*, which do not have tubercles, were included in the subtribe Zizaniinae. Tubercles might therefore be an important feature for the circumscription of taxa in this subtribe. Micromorphological studies of the remaining genera are required to confirm the value of this feature in distinguishing between the two subtribes.

Although *L. hexandra* has tubercles formed of triads with silica bodies, the tubercles of *Oryza* are more specialised, siliceous, 1- to 4- pronged structures. This structure is significant for identification and classification of the taxa in *Oryza*. Based on our findings, we can divide the six taxa of Oryzae in Thailand into three groups: the *O. sativa*, *O. ridleyi*, and *O. meyeriana* groups, respectively. These are consistent with the groupings identified by Terrell et al²⁰. However, we cannot separate the three species in the *O. sativa* group by using micromorphology of lemma surface alone. These three species have similar characters in terms of lemma surfaces, so other characters are needed to identify them. For instance, they can be distinguished by awn and spikelet size.

Furthermore, the surface patterns of cereal grains can be used in archaeobotanical studies, for instance in wheat and rye²⁵. Rice is one of the crops that archaeologists were interested in its domestication²⁶ and rice grains were found in many archaeological sites^{26–28}. Hence we can use lemma micromorpholog-

ical information to identify species of plant remains which were collected from the sites.

In conclusion, the SEM study of lemma surfaces in Thai Oryzae reveals several informative features, especially with respect to silica bodies, tubercles, and papillae useful for classification and identification. A key based on these characters clearly separates each taxon, except those of the *O. sativa* group, which require additional characters from other parts of the spikelet in combination with other morphological characters for accurate discrimination.

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