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## *Chamaegastrodia reiekensis*: A new holomycotrophic orchid from Mizoram, Northeast India

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**Abstract.** *Chamaegastrodia reiekensis*, a holomycotrophic orchid from Northeast India (Mizoram) is described and illustrated as a new species. It is compared with its closely allied species *Chamaegastrodia guidongensis*, which although similar in morphology and mycoheterotrophic nature can be distinguished by the presence of slender rhizome, compact sheathing scales, floral bracts equalling or exceeding the ovary in length, larger sepals and petals with more dilated hypochile and distinctly sinuate mesochile margin. A key to species for the genus *Chamaegastrodia* is also incorporated. The conservation status of the newly described species is also provisionally assessed here as Critically Endangered (CR) according to International Union for Conservation of Nature (IUCN) Red List Categories and Criteria.

**Keywords:** *Chamaegastrodia reiekensis*, *Chamaegastrodia guidongensis*, mycoheterotrophic, Indo-Burma Hotspot, taxonomy.

### INTRODUCTION

The genus *Chamaegastrodia* Makino & F.Maek. (Maekawa 1935) comprises terrestrial holomycotrophic orchids which are easily overlooked due to their inconspicuous size and lack of leaves. They are distributed from the Eastern Himalayas to South Central China, Japan, Korea, Myanmar, Nepal, Thailand and Vietnam (Govaerts et al. 2022). There are seven species reported worldwide namely, *Chamaegastrodia guidongensis* L.Wu, H.Z.Tian & C.Z.Huang (Qu et al. 2022), *C. asraoa* (J.Joseph & Abbar.) Seidenf. & A.N.Rao (Seidenfaden 1994), *C. inverta* (W.W.Sm.) Seidenf. (Seidenfaden 1994), *C. nanlingensis* H.Z.Tian & F.W.Xing (Tian and Xing 2008), *C. poilanei* (Gagnep.) Seidenf. & A.N.Rao (Seidenfaden 1994), *C. shikokiana* Makino & F.Maek. (Maekawa 1935) and *C. vaginata* (Hook.f.) Seidenf. (Seidenfaden 1994). The genus shares a close relationship with *Odontochilus* as per molecular studies by Li et al. (2016), but differs primarily in the shape and posi-

tion of its column wings. Several researchers have previously classified certain *Chamaegastrodia* species under *Odontochilus* (Ormerod 2002; Ormerod and Cribb 2003; Chen et al. 2009 a, b; Hsu and Chung 2009; Govaerts et al. 2022). A precise demarcation between the genus *Chamaegastrodia* and *Odontochilus* is still lacking and a comprehensive molecular study to confirm the taxonomic position of these taxa has been recommended (Tiwari et al. 2021). In the present study, we confirm seven species under *Chamaegastrodia* recorded worldwide after critically analysing the literature (Liu 2018; Tiwari et al. 2021, Qu et al. 2022). Among them, four species, namely, *C. asraoa*, *C. poilanei*, *C. shikokiana* and *C. vaginata* have been documented from India. They are predominantly found in northeastern states of India, particularly in Arunachal Pradesh and Meghalaya. The majority of these plants occur at elevations ranging from 500 to 1500 meters, with *C. shikokiana* being found at the lowest elevation (Jalal and Jayanthi 2013). With the discovery of the newly described taxa, the distribution of the genus *Chamaegastrodia* is expanded to Mizoram, which is another Northeast Indian state.

Mizoram possesses a distinctive topography and climatic conditions, with largely unexplored terrain that supports a rich diversity of orchid species. About 74 genera of orchids, with around 273 species have been identified so far from the state (Singh et al. 1990; Rao 2007; Odyuo and Tham 2008; Zote et al. 2009, 2011; Kumar and Singh 2012; Roy et al. 2012). However, the presence of *Chamaegastrodia* has not been reported until now and is here documented for the first time from Mizoram. In the ongoing exploration of Mizoram's flora, recent expeditions to Reiek Peak have revealed a previously unknown member of the *Chamaegastrodia* genus. This discovery not only enhances our comprehension of *Chamaegastrodia* as a whole but also prompts a re-evaluation of our broader understanding of biodiversity patterns and ecological interactions within the Reiek Peak, as the entire state is a part of Indo-Burma biodiversity hotspot. Despite the morphological resemblance of the collected specimen to other members of the genus, a critical analysis of morphological characters, relevant literature, and herbarium specimens from various collections indicates that it represents a new species of *Chamaegastrodia*, which is described here for the first time.

#### MATERIALS AND METHODS

Morphological characters and measurements were recorded from living samples gathered at the type locality of Mizoram during September 2023 to October 2024.

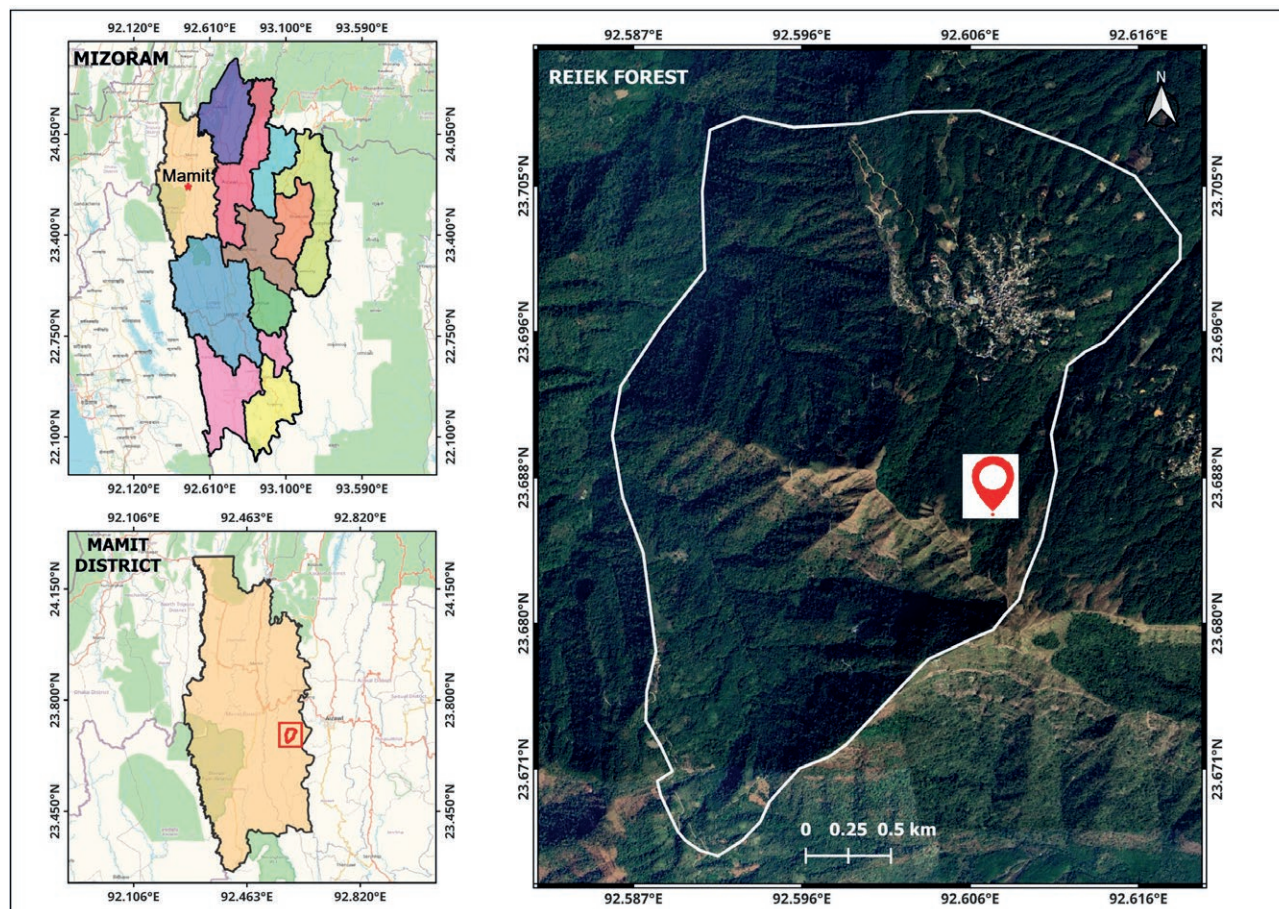
To ensure the novelty of the species, relevant literature (Chen et al. 2009 a,b; Jalal and Jayanthi 2013; Bhattacharjee and Chowdhery 2018; Tiwari et al. 2021; Govaerts et al. 2022; Qu et al. 2022) was critically analysed along with consultation of several herbaria and virtual repositories (ARUN, ASSAM, CAL, K). The description of the new species adheres to the terminology provided by Beentje (2016), ensuring consistency and accuracy in the taxonomic description. The assessment of the conservation status was conducted following the IUCN Red List Categories and Criteria (2022), ensuring a standardized approach to evaluate the conservation needs of the species. Images of different parts of the plants were captured using a digital camera (Sony DSC-W610, Tokyo, Japan) and a BTE digital microscope (Cilika, Thane, India).

Genomic DNA was extracted from the fresh tissue sample of the newly described *Chamaegastrodia* using the CTAB method (Doyle and Doyle 1987). PCR amplification of *matK* gene was performed using universal pair of primer (F- CGTACAGTACTTTTGTGTTTACGAG; R- ACCCAGTCCATCTGGAAATCTTGGTTC). The PCR product was purified and sequencing was done using Sanger sequencing method. The consensus and annotated *matK* sequence was deposited in the GenBank of National Centre for Biotechnology Information (NCBI) with accession No. PP873640. Phylogenetic tree was constructed in MEGA 12 (Kumar et al. 2024) to reveal evolutionary relationship of the newly described species, *Chamaegastrodia reiekensis* with other members of Orchidaceae. Twenty five *matK* sequences of eight genera, *Anoectochilus* (2), *Chamaegastrodia* (6), *Dossinia* (3), *Goodyera* (4), *Hetaeria* (3), *Ludicia* (3), *Macodes* (1) and *Odontochilus* (3) were obtained from GenBank database and used in the phylogenetic analyses. *Vanilla ptilifera* was included as an outgroup species to properly root the tree and determine the evolutionary relationships among these Orchidaceae members. *C. guidongensis* was not included in the phylogenetic analysis since *matK* sequence of the species was not available in the GenBank (NCBI) database.

#### TAXONOMIC TREATMENT

***Chamaegastrodia reiekensis*** Tlanhlui, S.D.Khomdram & S.D.Yumkham, **sp. nov.** (Figures 1, 2, 3)

Type: India, Mizoram, Mamit District, Reiek Tlang, 23.687007 N 92.606352 E, 1390 m, 05 Sept 2023, *Lal Tlanhlui 100030* (holotype ASSAM; isotypes MZUH, MUMP).



**Figure 1.** Location map of Mizoram and Mamit District showing the type location of *Chamaegastrodia reiekensis* Tlanhlui, S.D.Khomdram & S.D.Yumkham.

LSID.: urn:lsid:ipni.org:names:77355021-1

### Diagnosis

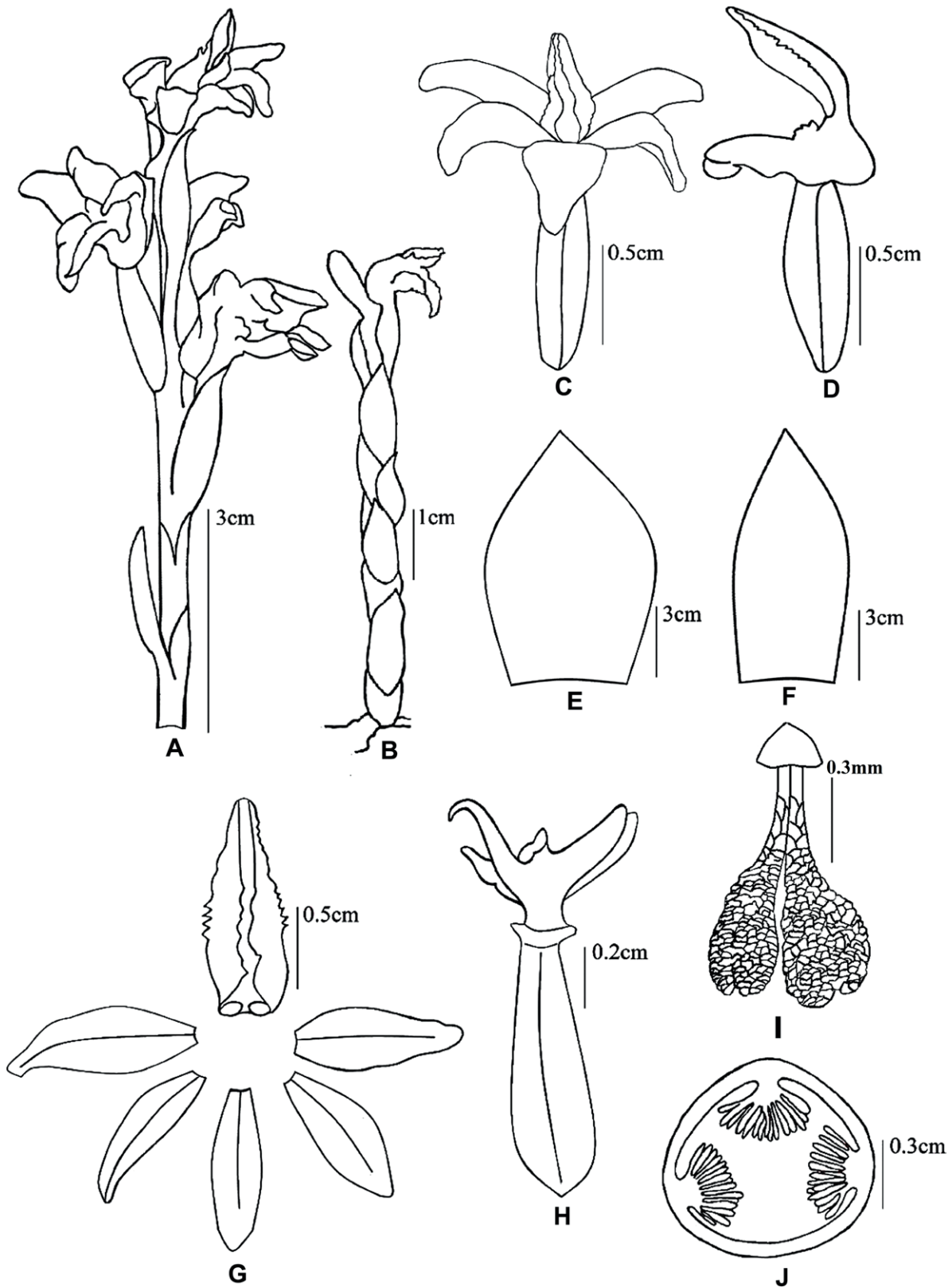
*Chamaegastrodia reiekensis* differs morphologically from *C. guidongensis* in having slender rhizome with 2–3 mm diameter (vs. stout rhizome, 3–8 mm diameter), compact, non-adpressed sheathing scales, (vs. lax, adpressed sheathing scales), floral bracts equalling or exceeding the ovary (vs. 2/3 the length of ovary), glossy red flower (vs. pale hazel to orange yellow flower), larger dorsal sepals 7–9 × 4–5 mm, lateral sepal 9–12 × 3–4 mm (vs. dorsal sepal 1.7 × 4.5 mm, lateral sepal 6.4 × 3.1 mm), petals 9–10 × 3–4 mm (vs. 4.8 × 2.3 mm), lip with hypochile more dilated, sharp and more prominently dentate flanges confined to the mesochile.

### Description

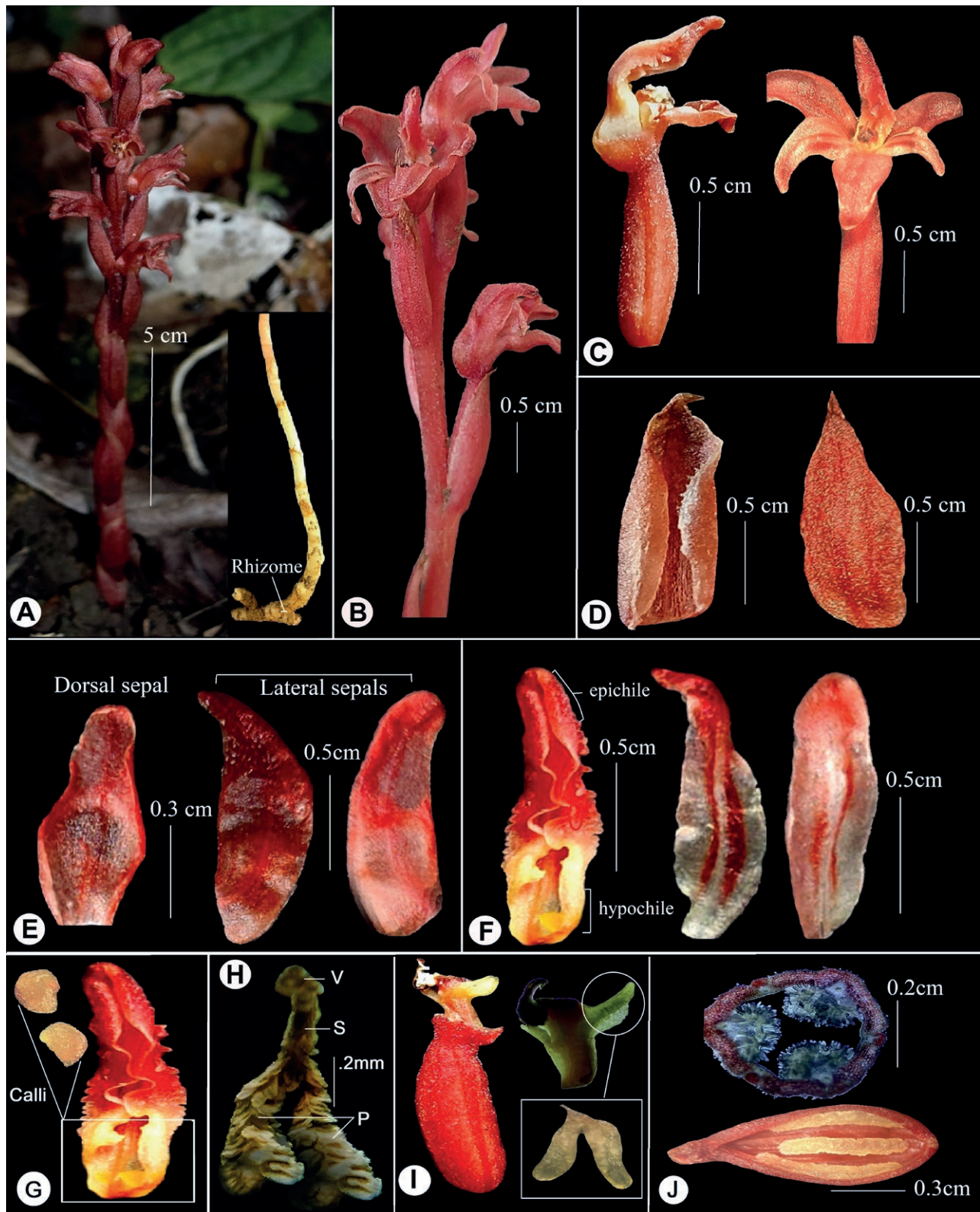
Terrestrial achlorophyllous herbs, rhizome slender, 2–3 mm in diameter, 9–14.5 cm tall, erect, covered

with overlapping non-adpressed sheathing scales. Scales 1.3–1.5 × 0.8–1.2 cm, decurrent at base, apex acute, red, alternate, margin sparsely hairy. Inflorescence with 2–9 flowers, rachis 1.5–3 cm. Floral bracts red, 1.2–1.4 × 0.5 cm, glabrous, equalling or exceeding the ovary in length, lanceolate, sessile. Flowers non-resupinate. Sepals bright red, glossy, sparse glandular hair at margin, cuticular ridges present. Dorsal sepal 7–9 × 4–5 mm, ovate, apex acute. Lateral sepals 9–12 × 3–4 mm, falcate, apex acute. Lateral petals 9–10 × 3–4 mm, red parallel stripes present on either side of the mid-rib throughout the length, apex acute to obtuse, lanceolate, falcate. Lip 11–13 × 4–6 mm, canoe-shaped, mesochile margin distinctly sinuate, incurved, hypochile broader than epichile, concave-saccate, wrapping the column, yellowish red, basally two yellow triangular shaped calli at each side, mesochile with sharply dentate flanges, epichile folded inward, almost touching the margin. Column short, stout, 5–8 mm, two triangular falcate yellow wings attached to apex vertically, 1 mm, operculum ovate, apex acuminate in





**Figure 2.** *Chamaegastrodia reiekensis* Tlanhlui, S.D. Khomdram & S.D. Yumkham A. Inflorescence B. Habit C–D. flower (front & side view) E. Sheathing scales F. Floral bract G. Calyx & corolla opened H. Column I. Pollinia J. Transverse section of ovary.



**Figure 3.** *Chamaegastrodia reiekensis* Tlanhlui, S.D. Khomdram & S.D. Yumkham A. Habit (inset showing rhizome) B. Inflorescence C. Flower D. Sheathing scale and floral bract E. Calyx F. Corolla G. Lip with calli H. Pollinia (P: pollinarium, S: Stipe, V: Viscidium) I. column showing wings J. transverse section and longitudinal section of ovary.

front, attached to column by a short linear filament, 2 pollinia, 0.8–1 mm, sectile, massulately, solitary viscidium, slender stalk, rostellum short, dark brown. Ovary fusiform, 9–10 mm long, 4 mm in diameter, glandular hairs present, placentation parietal, 3-loculed, ovules numerous. Fruit a long cylindrical capsule, 10–11 × 2–3 mm, pubescent, reddish brown at maturity with 3 longitudinal ridges, seeds numerous.

#### Etymology

The species takes its name from the type locality, Reiek, a well-known mountainous tourist destination in Mizoram state, Northeast India.

#### Suggested Common name

Reiek nauban par (Mizo).

#### Flowering & Fruiting

Flowering from August to September and fruiting from September to October.

#### Habitat and Ecology

Reiek forest exhibits a complex canopy structure with tall emergent trees, a dense understorey, and various intermediary layers providing habitats for many unique plant species. With the temperatures fluctuating between 20 to 28°C, Reiek forest sustains a conducive environment for tropical vegetation throughout the year. The annual rainfall ranges from 200 to 250 cm. *Chamaegastrodia reiekensis* grows in moist humus rich areas near bamboo clumps at an elevation of 1500 m above sea level. The recent discovery of *Aeschynanthus reiekensis* M. Lalhlupuii, S.D. Khomdram and S.D. Yumkham of Gesneriaceae family (Lalhlupuii et al. 2023) from this locality also highlights the biodiversity richness of the region.

#### Distribution

Only known from the type locality at Reiek Tlang, located in the state of Mizoram, India, which is part of the Indo-Burma biodiversity hotspot.

#### Preliminary Conservation status

The newly described species was first collected in September 2023 and again during the end of August to October, 2024 from the type locality of Reiek Tlang within the Reiek forest, which is a community-protected area in Mizoram. Despite extensive exploration in other parts of Mizoram, this newly described orchid has not been found outside of Reiek Tlang, suggesting that its distribution is restricted within the type locality only.

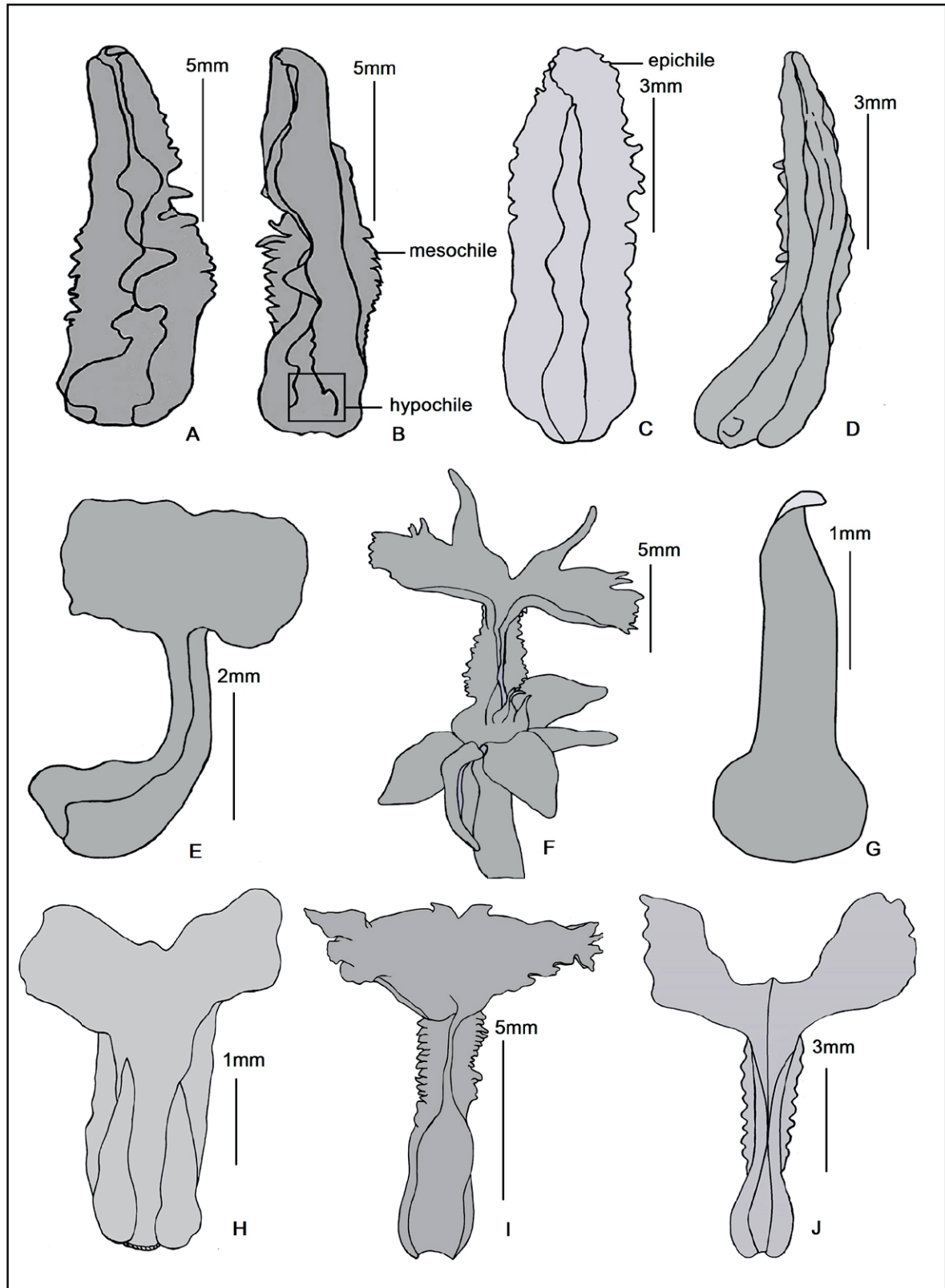
Reiek forest is significant as a prominent tourist destination in the state. The species was found solely within the type locality, where it forms a single population with an area of occupancy less than 10 square kilometre (<10 km<sup>2</sup>), occurring in a single location. Additionally, the population is sparse with fewer than 50 mature individuals (<50). Based on these findings, the species has been provisionally categorized here as Critically Endangered (CR) according to the criteria B2ab (ii, iii) c (ii, iii); D of IUCN Red List Categories and Criteria (IUCN 2022).

#### COMMENTS

The generic placement of *Chamaegastrodia* has long been debated among taxonomists and orchidologists (Tiwari et al. 2021). The currently discovered new species is placed under *Chamaegastrodia* along with already known taxa - *C. asraoa*, *C. guidongensis*, *C. inverta*, *C. nanlingensis*, *C. poilanei*, *C. shikokiana* and *C. vaginata*. In the present study, we recognized *C. asraoa*, *C. nanlingensis* and *C. poilanei* as a part of the genus instead of *Odon-tochilus* based on monophyly (Liu 2018; Qu et al. 2022). During the detailed studies done on *Chamaegastrodia*, all the species exhibit distinct morphological differences in the lip which varies from a seemingly peloric form in *C. vaginata* to Y-shaped (*C. nanlingensis* and *C. poilanei*) to T-shaped (*C. asraoa* and *C. shikokiana*) as well as canoe-shaped in *C. guidongensis* (Fig. 5). Although *C. guidongensis* and the newly described *C. reiekensis* share a similar lip shape, *C. reiekensis* differs in having a wider hypochile and presence of sharp and more prominent dentate flanges mainly confined to mesochile and a distinctly sinuate mesochile margin (Tab. 1, Fig. 4). The stability of the characteristics of *C. reiekensis* was confirmed by multiple field visits during 2023 to 2024 confirming its status as a new species. The discovery of this new holomycotrophic species underscores the unique characteristics of the habitats of Northeast India, from where all other Indian taxa of the genus have been reported so far, emphasizing the urgent need for their conservation due to their sensitivity and risk of habitat loss.

#### Molecular characteristics of *Chamaegastrodia reiekensis*

The *matK* sequence from *Chamaegastrodia reiekensis* was analyzed and submitted to GenBank under the accession number PP873640. The consensus length of the *matK* sequence is 503 bases with 30.1% G:C content. A Maximum Likelihood (ML) tree was constructed using *matK* sequences in MEGA 12 to analyze the placement of *Chamaegastrodia reiekensis* among different



**Figure 4.** Variation of Lips in *Chamaegastrodia* A –B. *C. reiekensis* C –D. *C. guidongensis* E. *C. inverta* F. *C. poilanei* G. *C. vaginata* H. *C. shikokiana* I. *C. asraoa* J. *C. nanlingensis* (Seidenfaden 1994; Tian and Xing 2008; Qu et al. 2022).



**Table 1.** Morphological comparison of *Chamaegastrodia reiekensis* and *C. guidongensis*

Characters	<i>C. reiekensis</i>	<i>C. guidongensis</i>
Rhizome	Slender, 2–3 mm in diameter	Stout, 3–8 mm in diameter
Sheathing Scales	Compact, non-adpressed to stem, 1.3–1.5 cm long, glossy red, non-translucent, non-membranous	Lax, adpressed to stem, 0.7–1.5 cm long, pale hazel, translucent, membranous
Floral bracts	1.2–1.4 cm long, equalling or exceeding the ovary, glossy red	0.7 cm long, 2/3 the length of ovary, orange yellow-pale brown
Placement of floral whorls	Obliquely placed above ovary	Strongly incurved towards ovary
Sepals	Dorsal sepal 7–9 × 4–5 mm; lateral sepal 9–12 × 3–4 mm, sparse glandular hair at margin, non-translucent	Dorsal sepal 1.7 × 4.5 mm; lateral sepal 6.4 × 3.1 mm, densely puberulous, translucent
Petals	9–10 × 3–4 mm, reddish white at base, red parallel stripes present on either side of the mid-rib throughout the length	4.8 × 2.3 mm, pale yellow-orange at base, parallel veins only at apex
Lip	1.1–1.3 cm long, hypochile more dilated, glossy red colour, mesochile margin prominently wavy, 2 triangular yellow calli at base	Upto 0.93 cm long, yellow orange colour, hypochile less dilated, mesochile margin slightly wavy, 2 sarcoma-shaped orange calli at base
External Flanges	Dentation prominent and mainly confined in mesochile	Dentation less prominent and confined to mesochile and epichile
Column	5–8 mm long	Upto 5 mm long
Distribution	Mizoram (India)	Guidong County, Hunan, China

Source for *C. guidongensis*: Qu et al. (2022).

members of Orchidaceae with *Vanilla ptilifera* as an out-group species (Fig. 5). The phylogram suggests a close relationship between *C. reiekensis* and *C. inverta* since they are clustered in Clade II(b) as compared to *C. shikokiana* in the Clade II (a). The tree is consistent with monophyly of *Chamaegastrodia*.

#### Key to the species of *Chamaegastrodia* Makino & F.Maek.

1. Lip bilobulate ..... 2
  - Lip not bilobulate ..... 6
2. Lip T-shaped ..... 3
  - Lip Y-shaped ..... 5
3. Lobules of lip epichile entire or with slightly erose edges 4
  - Lobules on lip epichile coarsely fringed ..... *C. asraoa*
4. Lip mesochile with external flanges, 2 sub-globose calli at base ..... *C. shikokiana*
  - Lip mesochile lacking external flanges, 2 domed-shaped calli at base ..... *C. inverta*
5. Lip with a pair of elongate introrse lobules at the epichile lobes ..... *C. poilanei*
  - Lip without a pair of elongate introrse lobule at the epichile lobes ..... *C. nanlingensis*

6. Lip canoe-shaped ..... 7
  - Lip narrowly ovate to oblong ..... *C. vaginata*
7. Lip light yellow to pale hazel red, floral bracts 2/3<sup>rd</sup> length of ovary, mesochile margin weakly sinuate ..... *C. guidongensis*
  - Lip bright red, floral bracts equalling or exceeding the ovary, mesochile margin distinctly sinuate ..... *C. reiekensis*

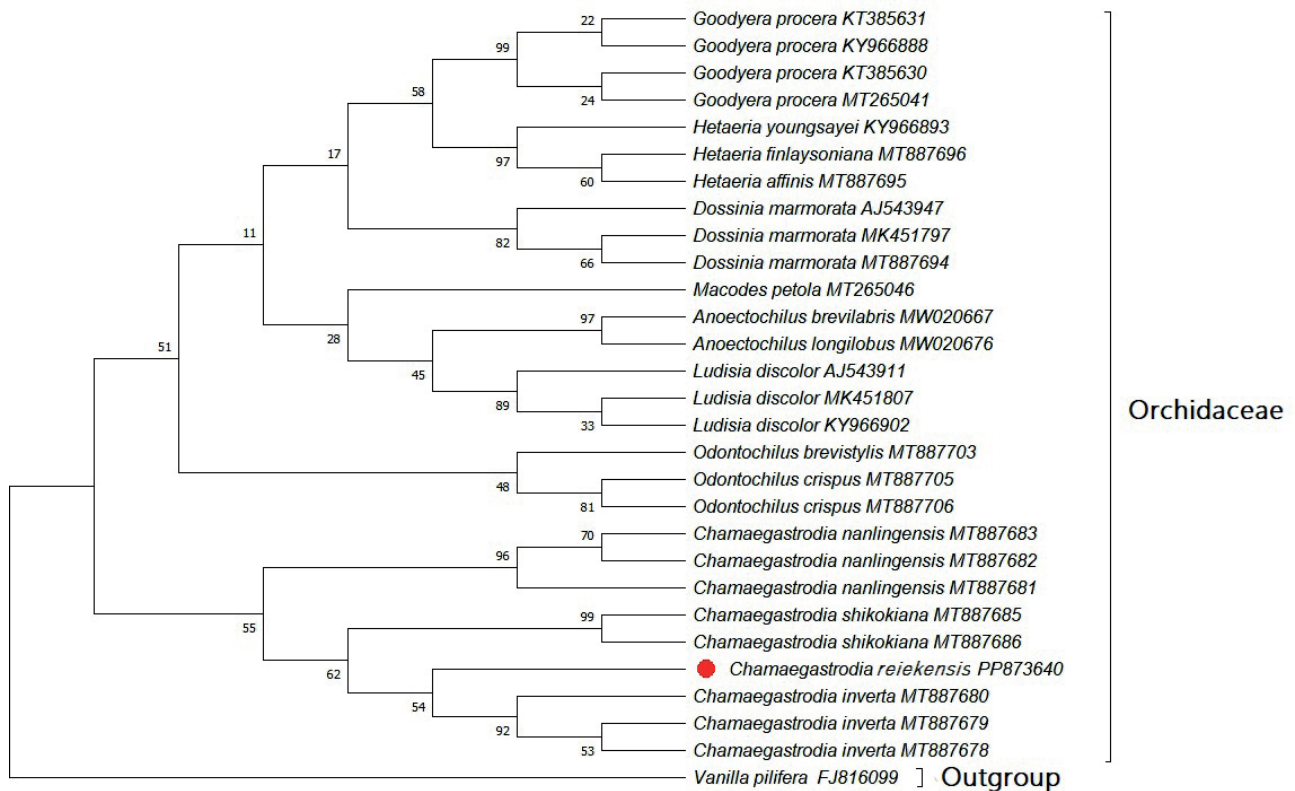
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**Figure 5.** Phylogenetic tree of *Chamaegastrodia reiekensis* reconstructed based on Maximum Likelihood (ML) as a cladistic method comprising of 27 *matK* nucleotide sequences using MEGA 12. Red solid circle indicates the placement of *C. reiekensis* between *C. inverta* and *C. shikokiana* in the phylogenetic tree.

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