



HAL
open science

Molecular phylogenetic analysis and taxonomic reconsideration of *Ceropegia hirsuta* (Apocynaceae, Asclepiadoideae) reveal a novelty in Thailand, *Ceropegia citrina* sp. nov., with notes on its pollination ecology

Aroonrat Kidyoo, Manit Kidyoo, Rumsais Blatrix, Gwenaëlle Deconninck, Doyle Mckey, Paweena Ekkaphan, Magali Proffit

► **To cite this version:**

Aroonrat Kidyoo, Manit Kidyoo, Rumsais Blatrix, Gwenaëlle Deconninck, Doyle Mckey, et al.. Molecular phylogenetic analysis and taxonomic reconsideration of *Ceropegia hirsuta* (Apocynaceae, Asclepiadoideae) reveal a novelty in Thailand, *Ceropegia citrina* sp. nov., with notes on its pollination ecology. *Plant Systematics and Evolution*, 2021, 307 (2), 10.1007/s00606-020-01723-2 . hal-03357839

HAL Id: hal-03357839

<https://hal.science/hal-03357839v1>

Submitted on 29 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Molecular phylogenetic analysis and taxonomic reconsideration of *Ceropegia hirsuta* (Apocynaceae, Asclepiadoideae) reveals a novelty in Thailand, *Ceropegia citrina* sp. nov., with notes on its pollination ecology

Aroonrat Kidyoo¹ · Manit Kidyoo¹ · Rumsais Blatrix² · Gwenaëlle Deconninck² · Doyle McKey² · Paweena Ekkaphan³ · Magali Proffit²

¹ Plants of Thailand Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand

² CEFÉ, University of Montpellier, CNRS, EPHE, IRD, University Paul Valéry Montpellier 3, Montpellier, France

³ Scientific and Technological Research Equipment Centre, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand

Corresponding author:

Aroonrat Kidyoo E-mail: aroonratm@hotmail.com

ORCID of the authors:

Aroonrat Kidyoo 0000-0003-2590-2441

Manit Kidyoo 0000-0003-1740-9999

Rumsais Blatrix 0000-0003-1662-7791

Doyle McKey 0000-0002-7271-901X

Magali Proffit 0000-0002-2227-0410

Abstract

The identity of *Ceropegia hirsuta* in Thailand has been questioned. Although this plant is quite similar to the original *C. hirsuta* from India, being entirely hirsute, with yellowish-shaded flowers, a curved, funnel-shaped corolla tube, and broad corolla lobes shorter than the corolla tube, there are several clear differences in morphological traits between the materials collected in Thailand and the type and further specimens collected in India. Moreover, *C. hirsuta* s.s. is restricted to Peninsular India. In this study, using molecular phylogenetic analysis based on DNA sequence data for ITS (nuclear) and *trnT-L*, *trnL*, and *trnL-F* (all cpDNA) markers, and classical morphology-based delineation, we demonstrate that the plant from Thailand represents a species on its own clearly separated from *C. hirsuta* s.s. as well as from all other known congeners. Therefore, we here describe it as a new species, *C. citrina*. A detailed description, illustrations, and photographs are provided, together with a comparison of morphological traits of *C. citrina*, *C. hirsuta* and another Thai species, *C. foetidiflora*. We also provide characterization of floral volatile compounds and pollinators. Our study emphasizes that morphological convergence may lead to errors in taxonomic treatment within the genus *Ceropegia*, and that phylogenetic relationships are largely congruent with geographic distribution.

Keywords Ceropegieae · eastern Thailand · floral scent · fly pollination · phylogeny · sect. *Indopegia* ·

Declarations

Funding

This research was supported by ‘Franco-Thai Mobility Programme (PHC SIAM, France) 2018–2019’ (project number 40533RA), by the programme 'International Emerging Actions' of the CNRS, France (project FOOLFLY 2020-2021) and by ‘Grant for Research, Rachadaphiseksomphot Endowment Fund 2019 (CU-GR_62_58_23_21) and 2020 (CU-GR_63_62_23_06) of Chulalongkorn University.’

Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All DNA sequences that support the findings of this study are publicly available in GenBank at <http://www.ncbi.nlm.nih.gov/genbank/>.

Code availability

Not applicable.

Running title

Ceropegia citrina sp. nov. with notes on its pollination.

Introduction

Traditionally, the genus *Ceropegia* L. is characterized by trap flowers with a basally dilated corolla tube topped by five corolla lobes connate at their apices, forming a cage-like structure (Huber 1957; Li et al. 1995; Meve 2002). This morphologically clearly circumscribed group encompasses more than 200 species naturally occurring in Southeast Asia, China, India, Madagascar, tropical Arabia, the Canary Islands, Africa, New Guinea and northern Australia (Huber 1957; Li et al. 1995; Meve 2002; Thaithong et al. 2018). However, after several molecular phylogenetic analyses and taxonomic revisions, the current generic circumscription is considerably enlarged with the incorporation of *Brachystelma* species and the stem-succulent stapeliads (Surveswaran et al. 2009; Bruyns et al. 2015, 2017). A more recent study (Endress et al. 2018), which takes into consideration growth habits and the appearance of multiple switches between open and pitfall flowers, accepts the incorporation of the former group into *Ceropegia*, but not that of the latter group.

Ceropegia hirsuta Wight & Arn. belongs to the sect. *Indopegia* H. Huber (Huber 1957; Bruyns et al. 2017). It is a variable species native to peninsular India, characterized by a twining growth habit with stems arising from a tuber, and all plant parts except the corolla hirsute, a few-flowered, pedunculate cyme, linear-subulate sepals, basally inflated, funnel-shaped corolla tube topped by a dome-shaped structure formed by apically connate corolla lobes that are shorter than the corolla tube, and linear-lanceolate staminal corona with curved or hooked tips. Owing to a great range of variation in the shape of leaves, as well as in the shape and color of the corolla lobes, this species was formerly divided into five varieties. One of these, *C. hirsuta* var. *vincifolia* (Hook.) Hook. f. (glabrous branches and oblong corolla lobes) (Hooker 1883), is now treated as a distinct species, *C. vincifolia* Hook. (Kambale and

Yadav 2019; Pullaiah et al. 2019). The other four, *C. hirsuta* var. *hirsuta*, *C. hirsuta* var. *jacquemontiana* (Decne.) Hook. f. (entirely green corolla lobes), *C. hirsuta* var. *ophiocephala* (Dalzell) Hook. f. (oblong corolla lobes with purple-colored proximal part, yellow-colored middle part and green-colored distal part), and *C. hirsuta* var. *stenophylla* Hook. f. (narrowly linear leaves), are considered synonyms of *C. hirsuta* (Kambale and Yadav 2019; Pullaiah et al. 2019).

Molecular phylogenetic study based on ITS and cpDNA markers of the genus *Ceropegia* (Surveswaran et al. 2009) showed that all *Ceropegia* species from India's Western Ghats, including *C. hirsuta*, are all placed in the same clade. In turn, almost all members of the sect. *Indopegia*, including *C. hirsuta*, formed a subclade characterized by broad leaves. A more recent phylogenetic reconstruction found similar results (Bruyns et al. 2017).

Among the 20 known *Ceropegia* species in Thailand (Thaithong et al. 2018; Suwannakote and Kidyoo 2020), one occurring in Pha Taem National Park (Ubon Ratchathani province) has been reported as *C. hirsuta* (Boonjaras and Thaithong 2003), and is hereinafter referred to as "*C. hirsuta* sensu Boonjaras & Thaithong". It was first discovered more than seventeen years ago. Typically, the plants are found growing in sandy soil in relatively open areas (often under bamboo) between patches of dry deciduous dipterocarp forest. It is a hirsute climber bearing broad ovate leaves and yellow flowers with broadly ovate lobes, thus resembling to some extent *C. hirsuta* s.s. However, as noted by Thaithong et al. (2018), several morphological characters of the material collected from Thailand are quite different from those of the type specimen collected from India. The former has very short peduncles and a corolla tube that is hairy on the outside, while the latter has long peduncles

and a glabrous corolla tube. Furthermore, if the Thai population were shown to belong to *C. hirsuta* s.s., this species would show a great disjunction, i.e. between peninsular India and eastern Thailand, as *C. hirsuta* s.s. is restricted to Peninsular India and has not been found elsewhere.

Hence, the purpose of this paper is to clarify the identity of *C. hirsuta* sensu Boonjaras & Thaithong in Thailand with the recent collection of material and detailed examination of its morphology and its systematic position based on ITS and *trnT-L*, *trnL*, and *trnL-F* DNA sequence data. In addition, we studied its pollination ecology. Although pollination ecology of *Ceropegia* species has been increasingly studied over the past few decades in a wide range of areas covering most of the major regions where the genus is found, including the Indian subcontinent, the Arabian Peninsula, and especially Africa (e.g. Masinde 2004; Coombs et al. 2011; Heiduk et al. 2017; Ollerton et al. 2019), only a few studies have been carried out in Southeast Asia (Auttama et al. 2018). We thus also report here information on pollination of *C. hirsuta* sensu Boonjaras & Thaithong, including the characterization of floral scent, obtained from a preliminary study during field surveys.

Materials and methods

Molecular phylogenetic analysis

To ascertain the identity of *C. hirsuta* sensu Boonjaras & Thaithong, evaluate its taxonomic status, and understand its phylogenetic relationships with congeners, we included new DNA sequences of one accession of this plant (voucher: M. Kidyoo 1651, BCU) and one of *C. foetidiflora* (voucher: M. Kidyoo 1646, BCU) from Thailand in a phylogenetic analysis of the

genus based on combined ITS (nuclear marker) and cpDNA datasets. Species of three other genera of tribe Ceropegieae, *Riocreuxia*, *Anisotoma*, and *Sisyranthus*, were used as outgroups. The homologous sequences of 60 other *Ceropegia* species and of outgroup taxa were downloaded from GenBank for comparative analysis (Meve and Liede-Schumann 2007, Surveswaran et al. 2009). The accession of *C. hirsuta* used as a voucher by Surveswaran et al. (2009) was Kamble 2107 deposited at SUK (India). This specimen was re-examined and its identity authenticated by Pullaiah et al. (2019) in their revision of the genus in India. Moreover, this voucher was also cited in a phylogenetic study of two *Ceropegia* species from northeastern India (Kumar et al. 2018)

DNA extraction and sequencing: Genomic DNA was extracted from silica-dried leaves using a Plant Genomic DNA Mini Kit (Geneaid). DNA amplification and sequencing of the entire ITS region (ITS1, 5.8S and ITS2) were performed using the ITS4 and ITS5m primers (White et al. 1990, Sang et al. 1995). Chloroplast *trnT-L*, *trnL*, and *trnL-F* regions were amplified using primer pairs a/b, c/d, and e/f (Taberlet et al. 1991), respectively. Amplifications by polymerase chain reaction (PCR) were carried out on a PTC-100 thermal cycler (MJ Research, Inc.) in a 25 µl volume containing 10 ng genomic DNA template, 1 µl (10 pM/µl) of both forward and reverse primers and 12.5 µl of Green PCR Master Mix (Biotechrabbit). The PCR programme consisted of an initial cycle at 94 °C for 4 min, followed by 35 cycles of 45 sec at 94 °C, 45 sec at 57–61 °C, 1–2 min at 72 °C, and a final extension for 5 min at 72 °C. Purification of PCR products and DNA sequencing of both strands were performed by Macrogen Inc. (Seoul, Korea). Sequences assembly and contig editing were done using CodonCode Aligner v.3.7.1 (CodonCode, Dedham, MA). All sequences have been deposited in GenBank (Table 3).

Phylogenetic analyses: MUSCLE sequence alignments were performed with Mesquite v.3.51 (Maddison and Maddison 2019). A partition scheme was defined with PartitionFinder v.2.0.0 (Lanfear et al. 2016) using the Akaike information criterion for nucleotide substitution model selection. Prior data blocks were defined by marker and codon position. Following the best scheme defined by PartitionFinder, we used the substitution models SYM+G for the ITS marker and GTR+I+G for cpDNA markers. Phylogenetic reconstructions based on the concatenated ITS and cpDNA datasets were performed using Bayesian inference with MrBayes v. 3 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Two analyses of four chains were run for 1,000,000 generations (after which split deviation frequency value was lower than 0.01), sampling trees every 500 generations with 25 % burn-in samples discarded for each run.

The remaining trees were used to build a majority-rule consensus tree and to calculate Bayesian posterior probabilities. All trees were visualized in Mesquite v.3.61 (Maddison and Maddison 2019).

Taxonomic study

Plant materials were collected from the plant's natural habitats during 2012 to 2017. Voucher specimens were prepared and deposited at BKF and BCU. Vegetative and reproductive structures of the freshly collected specimens and of specimens preserved in eight herbaria (BK, BKF, QBG, BCU, K, L, BM and P) were examined and compared. Moreover, a similar species, *C. foetidiflora* Kidyoo, was also included in comparisons. Morphological characters were mostly observed under light microscopy (LM).

Preliminary study of pollination ecology

Observation of floral visitors: During collection of plant specimens in May 2017, to obtain data on floral visitors of *C. hirsuta* sensu Boonjaras & Thaithong, continuous video recording was made on *in situ* flowers at anthesis as well as pre- and post-anthesis stages, for 2-3 consecutive days using an NV-GS75 digital camera (Panasonic, Matsushita Electric Industrial Co., Ltd., Japan). Moreover, the insects trapped inside the flowers collected in the field in 2016 and 2019 were examined under a stereomicroscope. Diptera were identified using the identification keys in Marshall (2012) (family level) and the website <http://milichiidae.info/> (genus level) curated by the world specialist of the Milichiidae, Irina Brake (Natural History Museum, London). Specimens are stored in the collections of A. Kidyoo at Chulalongkorn University, Bangkok, Thailand, and Rumsaïs Blatrix at CEFÉ, Montpellier, France.

Analysis of floral volatile organic compounds (VOCs): Floral volatiles of four first-day flowers were collected separately by dynamic headspace technique (Dötterl et al. 2005; Souto-Vilarós et al. 2018). Flowers were placed in odorless airtight polyethylene terephthalate (PET) bags (Nalophane®, Kalle Nalo GmbH, Wiesbaden, Germany) for 30 min, then the air containing VOCs was pulled from the bag using a membrane pump (NMP850KNDC; KNF, Switzerland) for 5 min at a flow rate of 200 mL·min⁻¹ through a small adsorbent tube made of quartz (ChromatoProbe®, Varian Inc., CA, USA; length: 15 mm; internal diameter: 2 mm) and filled with 3 mg of the mixture of 1:1 Tenax-TA and Carbotrap® (mesh size 60–80 and 20–40 respectively; Sigma Aldrich, Munich, Germany) encrusted with glass wool. To maintain integrity of samples during transport and storage, and

for calibration in gas chromatography–mass spectrometry (GC-MS) analyses, 1 μl of internal standard solution (n-nonane and n-dodecane, 110 $\text{ng}\cdot\mu\text{l}^{-1}$ for each) was added in each adsorbent tube before extraction of volatiles. In parallel, control extractions were performed using empty bags, to control for ambient contaminant compounds.

The volatile extracts were subsequently analyzed by GC-MS at the “Platform for Chemical Analyses in Ecology” (PACE, Montpellier) technical facilities of the LabEx CeMEB (“Centre Méditerranéen pour l’Environnement et la Biodiversité”, Montpellier, France) following the method of Souto-Vilarós et al. (2018). Data processing was done using MZmine™ version 2.18 (Pluskal et al. 2010) adapted to GC data processing (customized software available on demand), using the same automated protocol ensuring the consistency of peak integration. By comparing samples to the controls collected on the same day, potential contaminant compounds were subtracted from the samples. The retention times of a series of n-alkanes (standard solution of alkanes, 04070, Sigma Aldrich®, Munich, Germany) were used for converting retention times to retention indices. Identity of volatile compounds was determined by comparing the mass spectra with those in the NIST database (NIST 2007 library; Wiley, 9th edition), and by comparing the calculated retention indices with those reported in the literature (Adams, 2009). Moreover, when possible, the identity of VOCs was confirmed by comparison with mass spectra and retention time of authentic standards, using the same equipment and method.

Results

Molecular phylogenetic analysis

GenBank accession numbers for the new sequences generated are MT731612 to MT731619 (Table 1). Alignments used to produce phylogenies were submitted to TreeBase (ID 27051). Bayesian analyses of combined ITS and cpDNA sequences revealed the two analysed Thai *Ceropegia* species to group together (Fig. 1). *Ceropegia hirsuta* sensu Boonjaras & Thaithong is placed as sister to *C. foetidiflora* with high support (PP = 1.0). This pair is sister to *C. cumingiana* (a species occurring in the Philippines but also extending south to Australia), although with moderate support (PP = 0.79). This group of three species is in turn sister to *C. pubescens* from Nepal (PP = 0.59), forming a subclade of an unresolved terminal branch distinct from another subclade consisting wholly of species from India's Western Ghats (PP = 0.99). Within the latter clade, *C. hirsuta* and almost all other species in the sect. *Indopegia* (sole exception was *C. odorata*) (PP = 0.8) are grouped together. Hence, the phylogenetic relationships revealed in this study showed that *C. hirsuta* sensu Boonjaras & Thaithong from Thailand and *C. hirsuta* from India are not closely related.

Taxonomic study

Ceropegia hirsuta sensu Boonjaras & Thaithong has several morphological characters that are quite similar to those of *C. hirsuta* from India. Both are hirsute on nearly all parts, have yellowish-shaded flowers, a long, curved, funnel-shaped corolla tube with a basal inflated portion, and broad corolla lobes that are shorter than the corolla tube. Because of these similarities, this Thai species has long been confused with Indian *C. hirsuta*. Nevertheless, our studies reveal significant differences in morphological characteristics between these two entities (Table 2). Further comparison with another Thai species, *C. foetidiflora*, as well as other known *Ceropegia* species, allowed us to conclude that, although sharing certain traits with *C. foetidiflora* (Table 2), both the classical morphology-based delineation and molecular

phylogenetic relationships indicate that *C. hirsuta* sensu Boonjaras & Thaithong from Thailand represents a species on its own clearly distinct from *C. hirsuta* from India, and from all other known *Ceropegia* species. We therefore describe it as a new species, naming it *Ceropegia citrina*.

Notes on pollination ecology

Anthesis of *Ceropegia citrina* began soon after dawn. Flowers began to split at the proximal parts of the corolla lobes, gradually widening until the window-like orifices were fully opened. Anthesis generally lasted one day. The next morning, most flowers were in post-anthesis stage, diagnosed by wilting of the corolla lobes and by a change in the orientation of flowers from vertical to horizontal (Fig. 4). In some flowers, anthesis was completed by late afternoon on the first day.

Floral visitors: Flowers were visited during daytime by two morphospecies of milichiid flies of the genus *Neophyllomyza* (Diptera, Schizophora, Milichiidae). Out of 23 individual flies found in flowers, the first and second morphospecies were represented respectively by 11 individuals (of which one carried a pollinarium on its mouthparts) and 12 individuals (of which two individuals each carried a pollinarium on its mouthparts). As individuals of both fly species carried pollinaria (Fig. 4), these species were confirmed as pollinators. Only females of these two species were found in the flowers. Visitation started soon after flower opening and appeared to peak during late morning and mid-afternoon.

Analysis of VOCs: To the human nose, flowers of *C. citrina* have a very faint sweet and musty odour. Actually, more than 50 VOCs constituted the floral scent bouquet. However, only those twenty-four compounds with relative amounts of more than 0.1%, mainly fatty acid derivatives, were present in Table 3, of which four were major constituents (each with relative amount more than 5 %) that together accounted for more than 85% of the total amount of VOCs emitted: isoamyl acetate, 9-decenyl acetate, hexyl butyrate and (E)-2-octenyl acetate.

Discussion

Phylogenetic analysis and taxonomic reconsideration

Both molecular phylogenetic analysis and morphological comparison clearly revealed that *Ceropegia citrina* is a distinct and well-defined species, clearly separated from the morphologically similar *C. hirsuta* and *C. foetidiflora*. *Ceropegia hirsuta* belongs to the sect. *Indopegia*, which, as originally circumscribed, is endemic to the Western Ghats of peninsular India. Apart from *C. hirsuta*, about 21 other Indian species are assigned to this section (Huber 1957; Bruyns et al. 2017; Kambale and Yadav 2019).

Morphological traits of the two Thai species, *C. citrina* and *C. foetidiflora*, may suggest close relationships to species of the sect. *Indopegia*. Like species of sect. *Indopegia*, both Thai species produce subglobose tubers, flowers with slender corolla tubes that are basally inflated and corolla lobes joined at their tips. Moreover, these two species resemble each other in having hairy stems and leaves, yellow flowers, and basal dilated portions of corolla tubes that are hairy inside and apically widen into a funnel-shaped tube. However, *C.*

citrina is clearly distinguished by its broadly ovate corolla lobes that are shorter than the corolla tube and connate at their tips, forming a subglobose-shaped structure. *Ceropegia foetidiflora*, on the contrary, has lanceolate corolla lobes that are as long as the corolla tube and connate at their tips, forming a conical-shaped structure (Kidyoo 2018).

Furthermore, phylogenetic analysis showed that *C. citrina* and *C. foetidiflora* are evolutionarily more closely related to *C. cumingiana* from Philippines, whose morphology is quite dissimilar (with glabrous plant and fibrous roots, features characterizing the section *Janthina* H. Huber), than to *C. hirsuta* and other Indian species in the same section. Thus, morphological similarity between *C. hirsuta* and the two Thai species studied here appears to be explained by convergence. Our study established that the distribution of *C. hirsuta* is not disjunct, as the easternmost population formerly attributed to this species belongs to a distinct species (Fig. 5), *C. citrina*. Thus, *C. hirsuta* is endemic to India. As already shown by previous work, phylogenetic relationships within the genus *Ceropegia* are largely congruent with geographic distribution (Bruyns et al. 2014, 2015; Meve and Liede-Schumann 2007; Meve et al. 2017). Our results add a new example of morphological convergence between two phylogenetically and geographically distant taxa.

Pollination ecology

Flowers of *C. citrina*, like those of other *Ceropegia* species, are trap flowers, possessing a complicated floral construction formed by their curved corolla tube with an inflated basal portion topped by a cage-like structure comprised of apically connate corolla lobes that aid in temporarily confining the plant's fly pollinators inside, promoting pollination success (Vogel 1961; Faegri and van der Pijl 1979; Endress 1994). Anthesis of *Ceropegia* flowers is usually

completed in 1-2 days (Vogel 1961, Heiduk et al. 2015), but this period can be shorter, depending on the successful removal or deposition of pollinia (Auttama et al. 2018). Wilting of the corolla lobes and of the hairs inside the corolla tube, as well as the re-orientation of the post-anthesis flowers into a horizontal position, allow the trapped insects to escape (Masinde 2004; Coombs et al. 2011).

Flowers of *C. citrina* at anthesis emitted a quite simple floral scent blend, with only 24 compounds occurring at frequencies of more than 0.1%, compared to blends containing more than 50 compounds in *C. sandersonii*, *C. denticulata* and *C. dolichophylla* (Heiduk 2017). In another Thai species reported earlier, *C. thaithongiae* (Auttama et al. 2018), flowers released a faint scent composed of only two volatile compounds, but still detectable by the human nose. Floral scent of *C. citrina*, on the other hand, though having a larger number of compounds, had practically no smell detectable by the human nose.

The most abundant constituent of the floral bouquet of *C. citrina*, isoamyl acetate, has never been reported in other *Ceropegia* species. However, this VOC was revealed to be an important active component of the sting pheromone involving defensive behavior in honeybee (*Apis mellifera*) (Boch et al. 1962). Hexyl butyrate and (E)-2-octenyl acetate, two other major VOCs of *C. citrina*, also detected earlier in *C. sandersonii* (Heiduk et al. 2016) and *C. cyniflora* (Heiduk et al. 2017), are well-known sex pheromones of plant bugs (Heteroptera: Miridae) (Zhang and Aldrich 2008) and defensive volatile compounds released by various other true bugs (Oelschlägel et al. 2015). In addition, these VOCs are known to be kairomones used as cues to find food by kleptoparasitic milichiid and chloropid flies that regularly exploit the prey of other predatory invertebrates (Zhang and Aldrich 2004). This suggests a possible food-deceptive pollination mechanism in *C. citrina*. Such a system was reported in *Ceropegia* for the first time in *C. sandersonii*, whose flowers emit volatiles linked

to honeybees attacked by xxx(?), which are the food source of its *Desmometopa* fly pollinators (Heiduk et al. 2016). Later, this mechanism was also documented in several other *Ceropegia* species (Heiduk et al. 2017; Ollerton et al. 2017).

In other plant groups pollinated by dipterans, several studies have revealed that floral volatiles contribute to the attraction of unrewarded pollinators to flowers by mimicking food or brood sources, such as carrion and dung (Raguso 2020). Some of the VOCs emitted in their volatile blends are the same as those emitted by *C. citrina*, e.g. isoamyl acetate, hexyl acetate and hexyl butyrate in fetid floral scents of carrion flowers (Stapeliinae, Ceropegieae) (Jürgens et al. 2006), 2-ethyl-1-hexanol in the floral scents of *Ferraria crispa* (Iridaceae), whose flowers were found to be visited by insects associated with decaying organic matter (Johnson and Jürgens 2010), and hexyl butyrate in the floral odors of a deceitful *Aristolochia rotunda* that smell like freshly killed mirid bugs, the food that its chloropid pollinators usually thief from arthropod predators (Oelschlägel et al. 2015).

As in previous studies on *Ceropegia* (Vogel 1961; Ollerton and Forster 1995; Masinde 2004; Karuppusamy and Pullaiah 2009; Ollerton et al. 2009; Heiduk et al. 2010; Coombs et al. 2011; Heiduk et al. 2015, 2016, 2017; Ollerton et al. 2017; Auttama et al. 2018), the flies found in the flowers of *C. citrina* are mostly female dipterans. Fly species of the genus *Neophyllomyza*, found to be pollinators in this study, have also been reported as visitors of flowers of *C. nilotica* in southern Africa (Ollerton et al. 2009) and of *C. dolichophylla* in China (Heiduk et al. 2010). *Ceropegia* flowers are known to interact with Diptera belonging to 25 different families, of which 16 have been proven to be pollinators (Ollerton et al. 2017). Many studies (Heiduk et al. 2015, 2016, 2017; Ollerton et al. 2009,

2017; Coombs et al. 2011; Auttama et al. 2018) have shown that *Ceropegia* flowers attract their fly pollinators by emitting volatile floral cues that mimic odours of food sources and oviposition sites that attract these insects to flowers, in combination with complex floral constructions that trap the insects inside for a short period of time, promoting pollination success.

Furthermore, our preliminary results revealed that, although the floral volatiles collected in the morning and in the afternoon clearly differed in chemical composition, they did not differ in total amount of volatiles emitted. Further study with larger sample sizes comparing VOCs emitted during different periods of the day, and more detailed observations of fly visitation, will allow a better understanding activities of pollinating flies in relation to variation of floral volatiles.

Our understanding of the deceptive pollination system of *Ceropegia* spp., and of how associations between *Ceropegia* and flies vary over the geographic range of the genus, remains incomplete. Our observations serve as a basis for more detailed studies in Southeast Asia, a region in which *Ceropegia* has been little studied, which will allow comparison over the entire geographic range of the genus.

Taxonomic treatment

Ceropegia citrina Kidyoo & A. Kidyoo **sp. nov.** —HOLOTYPE: Thailand, Ubon Ratchathani, Pha Taem National Park, 240 m a. s. l., 13 July 2016, *M. Kidyoo 1644* (BKF!), ISOTYPE (BCU!) (Figs. 2 & 3)

Diagnosis: *Ceropegia citrina* differs from the similar *C. hirsuta* in having the outside surface of the corolla tube pubescent throughout, the interior surface of the inflated basal portion of the corolla tube covered with long white hairs, corolla lobes glabrous adaxially except on the midrib and along the distal half of margins that are covered with long white hairs, and the interstaminal corona lobes only shallowly bifid. In contrast, *C. hirsuta* has the corolla tube glabrous outside. The interior surface of the inflated basal portion of the corolla tube is also glabrous but distal to this portion is covered with long white hairs. Corolla lobes are with white puberulence on the entire distal half of their adaxial surface, also with long white hairs on midribs and long white to purplish-white hairs along margins, and the interstaminal corona lobes are markedly bifid with triangular segments.

Etymology: The specific epithet 'citrina' alludes to its largely bright yellow-colored flowers.

Description: Decumbent or twining herb, hirsute in nearly all parts. *Rootstock* tuberous, tuber subglobose, smooth, light brown, 2–3 cm in diam. *Stem* terete, 30–50 cm long, 2.5–3 mm in diam., green to greenish brown, usually unbranched. *Leaves:* petiole grooved above, 1–2 cm long, 1–1.2 mm in diam., hirsute; blade ovate, ovate-elliptic or elliptic, chartaceous to coriaceous, 5–6.5 × 2.5–3 cm, base rounded or subcordate, apex acute to acuminate; margins finely ciliated; adaxial surface green, abaxial surface pale green, hirsute on both surfaces; midrib prominent abaxially; lateral veins 4–5 pairs. *Inflorescences* 1–2-flowered, extra-axillary; peduncle green to reddish brown, 0.5–1 cm long, 1–1.3 mm in diam.; pedicel pale green or reddish brown, pubescent, 1–1.5 cm long, ca. 1 mm in diam.; bracts solitary, minute, ovate-triangular, ca. 1 mm long, pale green, apex acute. *Calyx lobes* pale green or reddish

brown, linear-lanceolate, $5-8 \times 1-1.5$ mm, apex sharply acute, outside hirsute. *Corolla* tubular-infundibuliform, 4–5 cm long, upright, curved, yellowish white or yellow with scattered reddish brown or purple blotches; tube 3–3.5 cm long, outside pubescent, inflated at base, 0.6–0.7 cm in diam., interior of this portion maroon, purple brown to dark purple and covered with long white hairs 1.5–2 mm long, tube becoming straight above the inflated portion and then gradually widening upwards; mouth of tube 1–1.2 cm in diam., with long white hairs inside; lobes yellow, connate at tips, each lobe broadly ovate-triangular, $0.7-0.8 \times 0.4-0.5$ cm, apex acute to obtuse, reduplicate from near the base, margins slightly revolute particularly when in full bloom, glabrous adaxially except on the midrib, which is covered with long white hairs, and on the margins, with white hairs at the upper half. *Gynostegium* sessile. Corona double, interstaminal corona formed by five lobes, joined to form a shallow cup, 4.5–4.7 mm in diam., lobes yellowish white with purple-brown apices, apex shallowly bifid or retuse, margins and adaxial surface with long white hairs; staminal corona formed by five lobes, lobes linear-lanceolate or subulate, 3–3.3 mm long, 0.2–0.25 mm in diam., proximal part purple-white, distal part usually long, connivent-erect, creamy white, recurved. *Pollinaria*: pollinium broadly ovoid, yellow, $0.36-0.4 \times 0.25-0.28$ mm; corpusculum spatulate, reddish brown, $0.37-0.38 \times 0.18-0.19$ mm. *Follicles* usually 2, slightly diverging, each linear-lanceolate in outline, green when young and turning reddish brown with age, 7–8 cm long, 0.25–0.35 cm in diam. *Seeds* oblong-elliptic, $4-6 \times 2.5-3$ mm; coma 1.5–2 cm long, silky white.

Phenology: Flowering occurs from June to August.

Habitat: This plant grows in sandy soil among small bamboos in open areas within dry deciduous dipterocarp forest.

Distribution: *Ceropegia citrina* occurs at about 150–240 m elevation in Pha Taem National Park, Ubon Ratchathani Province, eastern Thailand.

Additional specimens examined:

Ceropegia citrina Kidyoo & A. Kidyoo: Thailand, Ubon Ratchathani, Pha Taem National Park, 200 m, 9 September 2012, *M. Kidyoo 1566* (paratype: BCU!)

Ceropegia hirsuta Wight & Arn.: INDIA, Tamil Nadu, Nilgiris, s.d., R. Wight Cat. n. 1510 (holotype, K!, isotype E photo!); Maharashtra, Dalzell s.n. (K!); 1836, Wight, R. 2204 (K!), Shiggegudda, 23 Aug 1969, C.J. Saldanha 14687 (K!)

Conservation Status: *Ceropegia citrina* is endemic to Thailand, apparently very rare, being known only from a single locality, Pha Taem National Park, where three populations were found at distances ranging from 0.5–10 km from each other. In each of the three populations, about 5–10 individuals occurred within an area of about 100 m². These sites have suffered from severe soil erosion by water and by human exploitation (modification of land to install buildings for tourism in the national park). Over a 10-year observation period, two of the populations have disappeared and the number of plants in the last remaining population has been reduced from more than 15 to fewer than 10 individuals. Following IUCN (2019), a conservation assessment of Critically Endangered (CR) should be assigned to this species.

Acknowledgments

We highly appreciate the effort and time devoted by the editors and the two reviewers in evaluating our manuscript. Thanks to their instructive comments and suggestions, we could significantly improve the original manuscript. We express our deepest gratitude to Assoc. Prof. Dr. Obchant Thaithong for her continued support. We also thank the staff and curators of the herbaria BKF, BK, QBG, BM, K, L, and P for their permission to study herbarium specimens, and Nicolas Barthes and Bruno Buatois for help during the chemical analysis conducted at the LabEx CeMEB (Centre Méditerranéen Environnement et Biodiversité, Montpellier), an ANR "Investissements d'avenir" program (ANR-10-LABX-04-01). This research was supported by 'Franco-Thai Mobility Programme (PHC SIAM, France) 2018–2019' (project number 40533RA), by the programme 'International Emerging Actions' of the CNRS, France (project FOOLFLY 2020-2021) and by 'Grant for Research, Rachadaphiseksomphot Endowment Fund 2019 (CU-GR_62_58_23_21) and 2020 (CU-GR_63_62_23_06) of Chulalongkorn University.'

References

- Adams, RP (2009) Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th ed. Allured Publishing Corporation, Carol Stream, Illinois
- Auttama P, McKey D, Kidyoo A (2018) Flowering phenology and trap pollination of the rare endemic plant *Ceropegia thaithongiae* in montane forest of northern Thailand. Botany 96:601-620. <https://doi.org/10.1139/cjb-2018-0045>
- Boch R, Shearer DA, Stone BC (1962) Identification of isoamyl acetate as an active component in the sting pheromone of the honey bee. Nature 195:1018–1020. <https://doi.org/10.1038/1951018b0>

- Boonjaras T, Thaithong O (2003) *Ceropegia hirsuta* (Asclepiadaceae), a new record for Thailand. Thai Forest Bull Bot 31:1–6
- Bruyns PV, Klak C, Hanáček P (2014) Evolution of the stapeliads (Apocynaceae-Asclepiadoideae) – repeated major radiation across Africa in an Old World group. Molec Phylogen Evol 77:251–263. <https://doi.org/10.1016/j.ympev.2014.03.022>
- Bruyns PV, Klak C, Hanáček P (2015) Recent radiation of *Brachystelma* and *Ceropegia* (Apocynaceae) across the Old World against a background of climatic change. Molec Phylogen Evol 90:49–66. <https://doi.org/10.1016/j.ympev.2015.04.015>
- Bruyns PV, Klak C, Hanáček P (2017) A revised, phylogenetically-based concept of *Ceropegia* (Apocynaceae). S African J Bot 112:399–436. <https://doi.org/10.1016/j.sajb.2017.06.021>
- Coombs G, Dold AP, Peter CI (2011) Generalized fly pollination in *Ceropegia ampliata* (Apocynaceae–Asclepiadoideae): the role of trapping hairs in pollen export and receipt. Pl Syst Evol 296:137–148. <https://doi.org/10.1007/s00606-011-0483-6>
- Dötterl S, Wolfe LM, et Jürgens A (2005) Qualitative and quantitative analyses of flower scent in *Silene latifolia*. Phytochemistry 66:203–213. <https://doi.org/10.1016/j.phytochem.2004.12.002>
- Endress ME, Meve U, Middleton DJ, Liede-Schumann S (2018) Apocynaceae. In: Kadereit JW, Bittrich V (eds) The Families and Genera of Vascular Plants 15. Springer International Publishing, Cham, pp 207–411
- Endress PK (1994) Diversity and evolutionary biology of tropical flowers. University Press, Cambridge

- Faegri K, van der Pijl L (1979) *The Principles of Pollination Ecology*, 3rd edn. Pergamon Press, Oxford
- Heiduk A, Brake I, von Tschirnhaus M, Göhl M, Jürgens A, Johnson SD, Meve U, Dötterl S (2016) *Ceropegia sandersonii* mimics attacked honeybees to attract kleptoparasitic flies for pollination. *Curr Biol* 26:2787–2793. <https://doi.org/10.1016/j.cub.2016.07.085>
- Heiduk A, Brake I, Tolasch T, Frank J, Jürgens A, Meve U, Dötterl S (2010) Scent chemistry and pollinator attraction in the deceptive trap flowers of *Ceropegia dolichophylla*. *S African J Bot* 76:762–769. <https://doi.org/10.1016/j.sajb.2010.07.022>
- Heiduk A, Brake I, von Tschirnhaus M, Haenni JP, Miller R, Hash J, et al. (2017) Floral scent and pollinators of *Ceropegia* trap flowers. *Flora* 232:169–182. <https://doi.org/10.1016/j.flora.2017.02.001>
- Heiduk A, Kong H, Brake I, von Tschirnhaus M, Tolasch T, Tröger AG, et al. (2015) Deceptive *Ceropegia dolichophylla* fools its kleptoparasitic fly pollinators with exceptional floral scent. *Front Ecol Evol* 3: e66. <https://doi.org/10.3389/fevo.2015.00066>
- Huber H (1957) Revision der Gattung *Ceropegia*. *Mem Soc Brot* 12:1–203
- IUCN Standards and Petitions Committee (2019) *Guidelines for Using the IUCN Red List Categories and Criteria*. Version 14. Prepared by the Standards and Petitions Committee. Available at: [www.iucnredlist.org/documents/RedList Guidelines.pdf](http://www.iucnredlist.org/documents/RedList%20Guidelines.pdf). Accessed 11 June 2020
- Johnson SD, Jürgens A (2010) Convergent evolution of carrion and faecal scent mimicry in fly-pollinated angiosperm flowers and a stinkhorn fungus. *S African J Bot* 76:796–807. <https://doi.org/10.1016/j.sajb.2010.07.012>

- Jürgens A, Dötterl S, Meve U (2006) The chemical nature of fetid floral odors in stapeliads (Apocynaceae-Asclepiadoideae-Ceropegieae). *New Phytol* 172:452–468.
<https://doi.org/10.1111/j.1469-8137.2006.01845.x>
- Kambale SS, Yadav SR (2019) Taxonomic revision of *Ceropegia* (Apocynaceae: Ceropegieae) in India. *Rheedea* 29:1–115. <https://doi.org/10.22244/rheedea.2019.29.1.01>
- Kidyoo M (2018) *Ceropegia foetidiflora* sp. nov. (Asclepiadoideae, Apocynaceae), a new species from northeastern Thailand. *Taiwania* 63:327–332.
<https://doi.org/10.6165/tai.2018.63.327>
- Kumar et al. (2018). *Ceropegia mizoramensis* and *C. murlensis* (Asclepiadaceae) - Two new species from Northeast India with phylogenetic and morphological evidence support. *Taiwania* 63:163–170. <https://doi.org/10.6165/tai.2018.63.163>
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molec Biol Evol* 34:772–773.
<https://doi.org/10.1093/molbev/msw260>.
- Li PT, Gilbert MG, Stevens WD (1995) Asclepiadaceae. In: Wu ZY, Raven PH (eds) *Flora of China*, Vol. 16. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, pp 189–270
- Maddison WP, Maddison DR (2019) Mesquite: a modular system for evolutionary analysis, Version 3.61. Available at: www.mesquiteproject.org. Accessed 1 June 2020
- Marshall SA (2012) *Flies - The Natural History and Diversity of Diptera*. Firefly Books Ltd, Ontario, Canada

- Masinde PS (2004) Trap-flower fly pollination in East African *Ceropegia* L. (Apocynaceae). *Int. J. Trop Insect Sci* 24:55–72. <https://doi.org/10.1079/IJT20044>
- Meve U (2002) *Ceropegia* L. In: Albers F, Meve U (eds) *Illustrated handbook of succulent plants: Asclepiadaceae*. Springer, Berlin, pp 63–108
- Meve U, Liede-Schumann S (2007) *Ceropegia* (Apocynaceae, Ceropegieae, Stapeliinae): paraphyletic but still taxonomically sound. *Ann Missouri Bot Gard* 94:392–406. [https://doi.org/10.3417/0026-6493\(2007\)94\[392:CACSPB\]2.0.CO;2](https://doi.org/10.3417/0026-6493(2007)94[392:CACSPB]2.0.CO;2)
- Meve U, Heiduk A, Liede-Schumann S (2017) Origin and early evolution of Ceropegieae (Apocynaceae-Asclepiadoideae). *Syst Biodivers* 15:143–155. <https://doi.org/10.1080/14772000.2016.1238019>
- Oelschlägel B, Nuss M, von Tschirnhaus M et al (2015) The betrayed thief – the extraordinary strategy of *Aristolochia rotunda* to deceive its pollinators. *New Phytol* 206:342-351. <https://doi.org/10.1111/nph.13210>
- Ollerton J, Forster P (1995) Diptera associated with flowers of *Ceropegia cumingiana* in Australia, *Asklepios* 66:21–22
- Ollerton J, Dötterl S, Ghorpadé K, Heiduk A, Liede-Schumann S, Masinde S, et al. (2017) Diversity of Diptera families that pollinate *Ceropegia* (Apocynaceae) trap flowers: an update in light of new data and phylogenetic analyses. *Flora* 234:233–244. <https://doi.org/10.1016/j.flora.2017.07.013>
- Ollerton J, Liede-Schumann S, Endress ME, Meve U, Rech AR, Shuttleworth A, et al. (2019) The diversity and evolution of pollination systems in large plant clades: Apocynaceae as a case study. *Ann Bot (Oxford)* 123:311–325. <https://doi.org/10.1093/aob/mcy127>

- Ollerton J, Masinde S, Meve U, Picker M, Whittington A (2009) Fly pollination in *Ceropegia* (Apocynaceae: Asclepiadoideae): biogeographic and phylogenetic perspectives. *Ann Bot (Oxford)* 103:1501–1514. <https://doi.org/10.1093/aob/mcp072>. PMID: 19339298
- Pluskal T, Castillo S, Villar-Briones A, and Orešič M (2010) MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *B M C Bioinf* 11:395. <https://doi.org/10.1186/1471-2105-11-395>
- Pullaiah T, Karuppusamy S, Rama Murthy KS (2019) Monograph on *Brachystelma* and *Ceropegia* in India. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA
- Raguso R A (2020) Don't forget the flies: dipteran diversity and its consequences for floral ecology and evolution. *Appl Entomol Zool* 55:1–7. <https://doi.org/10.1007/s13355-020-00668-9>
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Syst Biol* 61:539–542. <https://doi.org/10.1093/sysbio/sys029>
- Sang T, Crawford DJ, Stuessy TF (1995) Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and concerted evolution. *Proc Natl Acad Sci USA* 92:6813–6817. <https://doi.org/10.1073/pnas.92.15.6813>

- Souto-Vilarós D, Proffit M, Buatois B, Rindos M, Sisol M, Kuyaiva T, et al. (2018) Pollination along an elevational gradient mediated both by floral scent and pollinator compatibility in the fig and fig-wasp mutualism. *J Ecol* 106:2256–2273.
<https://doi.org/10.1111/1365-2745.12995>
- Surveswaran S, Kamble MY, Yadav SR, Sun M (2009) Molecular phylogeny of *Ceropegia* (Asclepiadoideae, Apocynaceae) from Indian Western Ghats. *Pl Syst Evol* 281:51–63.
<https://doi.org/10.1007/s00606-009-0182-8>
- Suwannakote K, Kidyoo M (2020) *Ceropegia graminea* (Apocynaceae: Asclepiadoideae), a new species from eastern Thailand. *Kew Bull* 75. <https://doi.org/10.1007/S12225-020-9869-3>
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of the three non-coding regions of chloroplast DNA. *Pl Molec Biol* 17:1105–1109.
<https://doi.org/10.1007/BF00037152>
- Thaithong O, Kidyoo A, Kidyoo M (2018) Handbook of Asclepiads of Thailand. Amarin Printing and Publishing Public Company Limited, Bangkok
- Vogel S (1961) Die Bestäubung der Kesselfallenblüten von *Ceropegia*. *Beitr Biol Pflanzen* 36:159–237
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols-a guide to methods and applications. Academic Press, San Diego, Calif, pp 315–322

Zhang Q, Aldrich J (2004) Attraction of scavenging chloropid and milichiid flies (Diptera) to metathoracic scent gland compounds of plant bugs (Heteroptera: Miridae). *Environ Entomol* 33:12–20. <https://doi.org/10.1603/0046-225X-33.1.12>

Zhang Q, Aldrich J (2008) Sex pheromone of the plant bug, *Phytocoris calli* Knight. *J Chem Ecol* 34:719–724. <https://doi.org/10.1007/s10886-008-9479-2>

Figure captions

Fig. 2 *Ceropegia citrina* sp. nov. **a** habit; **b** flower; **c** longitudinal section of flower showing corona and gynostegium; **d** side view of gynostegium; **e** top view of gynostegium; **f** calyx; **g** pollinarium; **h** fruit; **i** seed; a-g from *M. Kidyoo 1644*, h-i from *M. Kidyoo 1566*. Drawn by *M. Kidyoo*

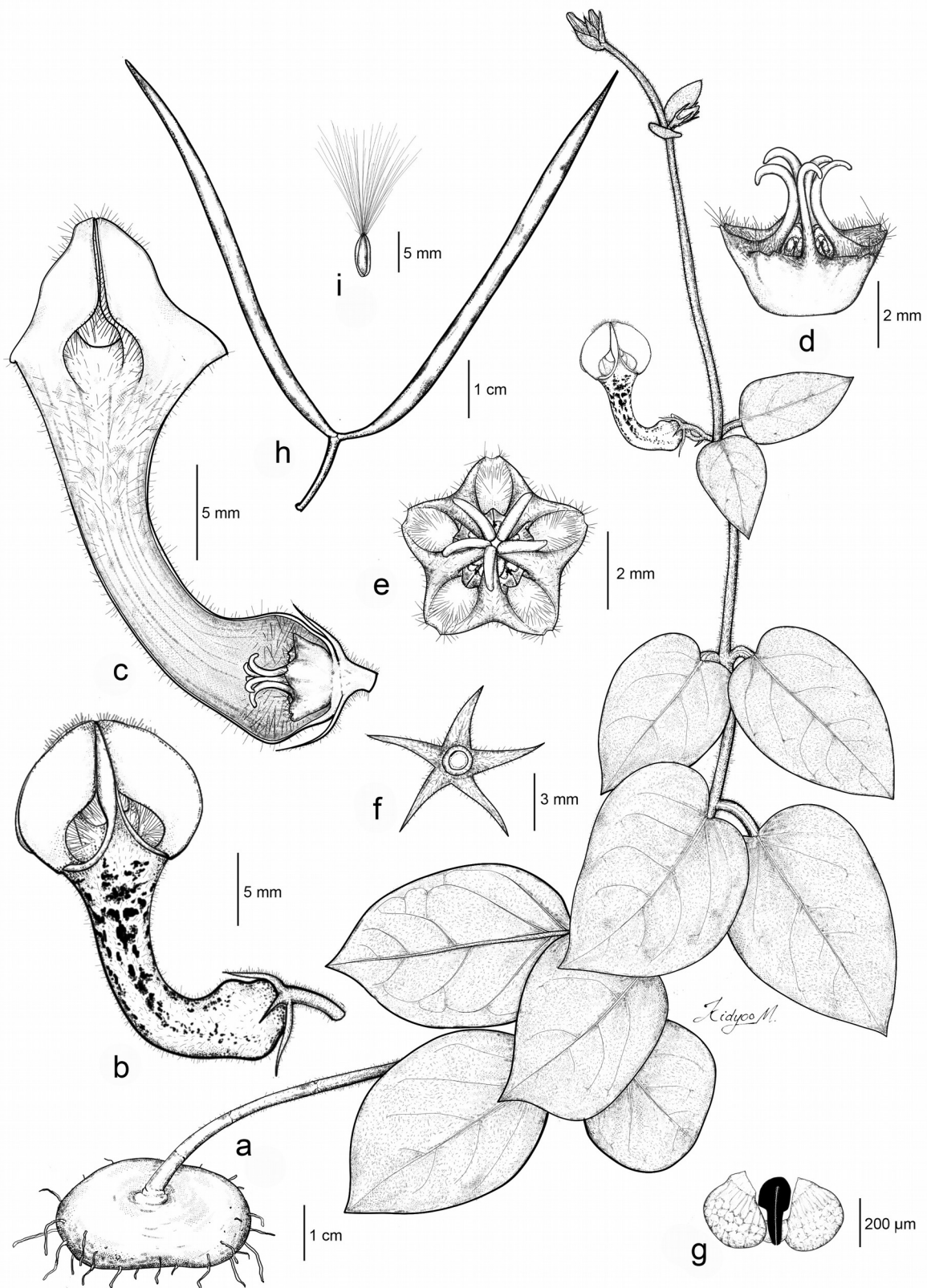


Fig. 3 *Ceropegia citrina* sp. nov. **a** habitat in Pha Taem National Park, Ubon Ratchathani Province, taken on 14 July 2019 ; **b, c** habit; **d** inflorescence with a flower in side view; **e** flower in top view. Photos taken by A. Kidyoo



Fig. 4 Fly visits of flowers of *Ceropegia citrina*. **a, b** a milichiid fly, with pollinarium attached to its mouthparts, landing near a window-like orifice of the flower (red arrowhead); **b** magnification of **a**; **c** the same fly entering inside the mouth of corolla tube; **d** a second-day flower that is already bent downward into horizontal position. Note that a fly previously trapped in the flower is getting out; **e** *Neophyllomyza* flies (Milichiidae), all found trapped inside one single flower of *Ceropegia citrina*, one of which carried a pollinarium on its mouthparts (blue arrowhead). Photos taken by A. Kidyoo

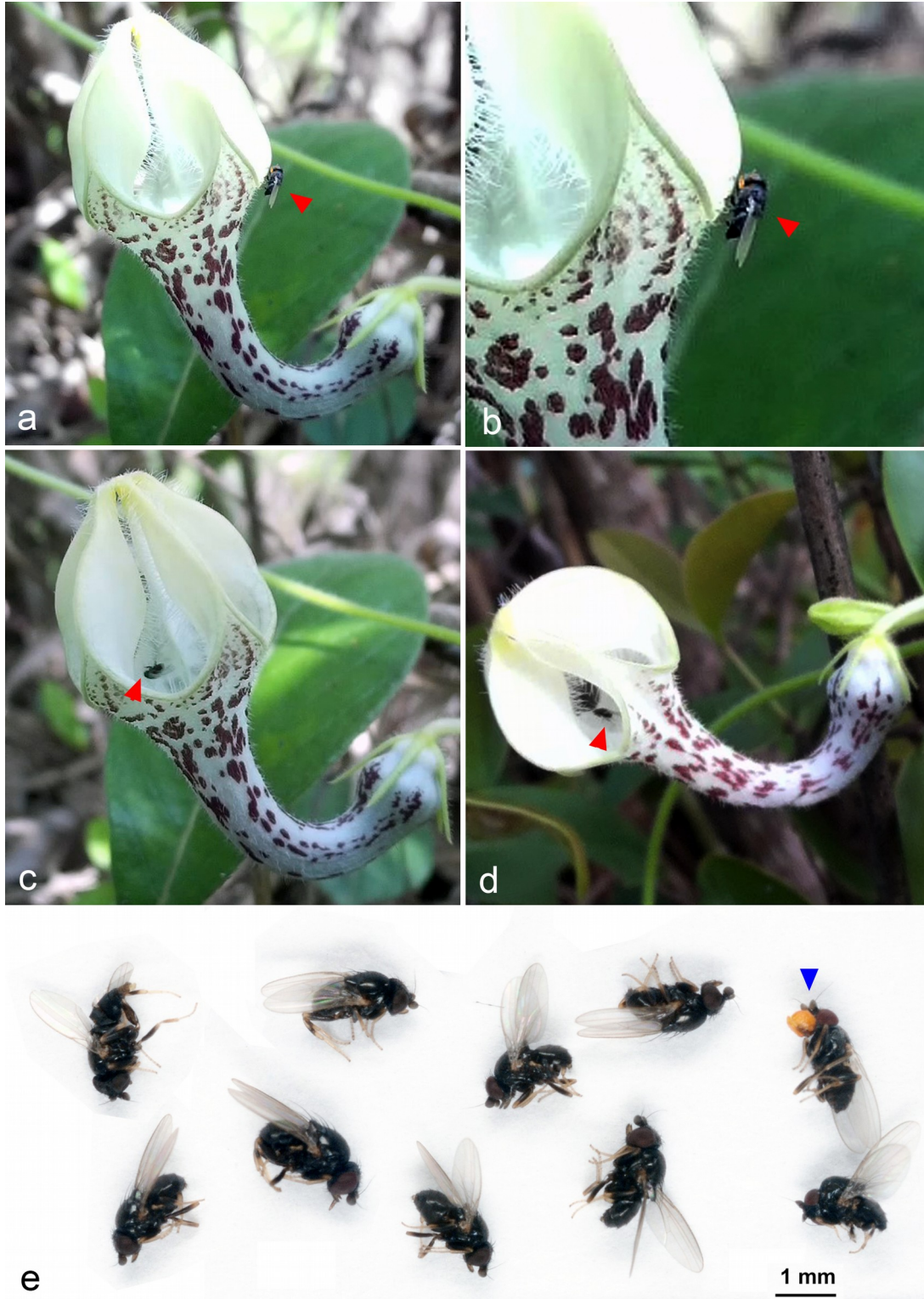


Fig. 5 Map showing the distributions of *Ceropegia citrina* (star) and two morphologically similar species, *C. hirsuta* (squares; data from Pullaiah et al. (2019)). and *C. foetidiflora* (circle). The map was created using SimpleMappr (www.simplemappr.net)

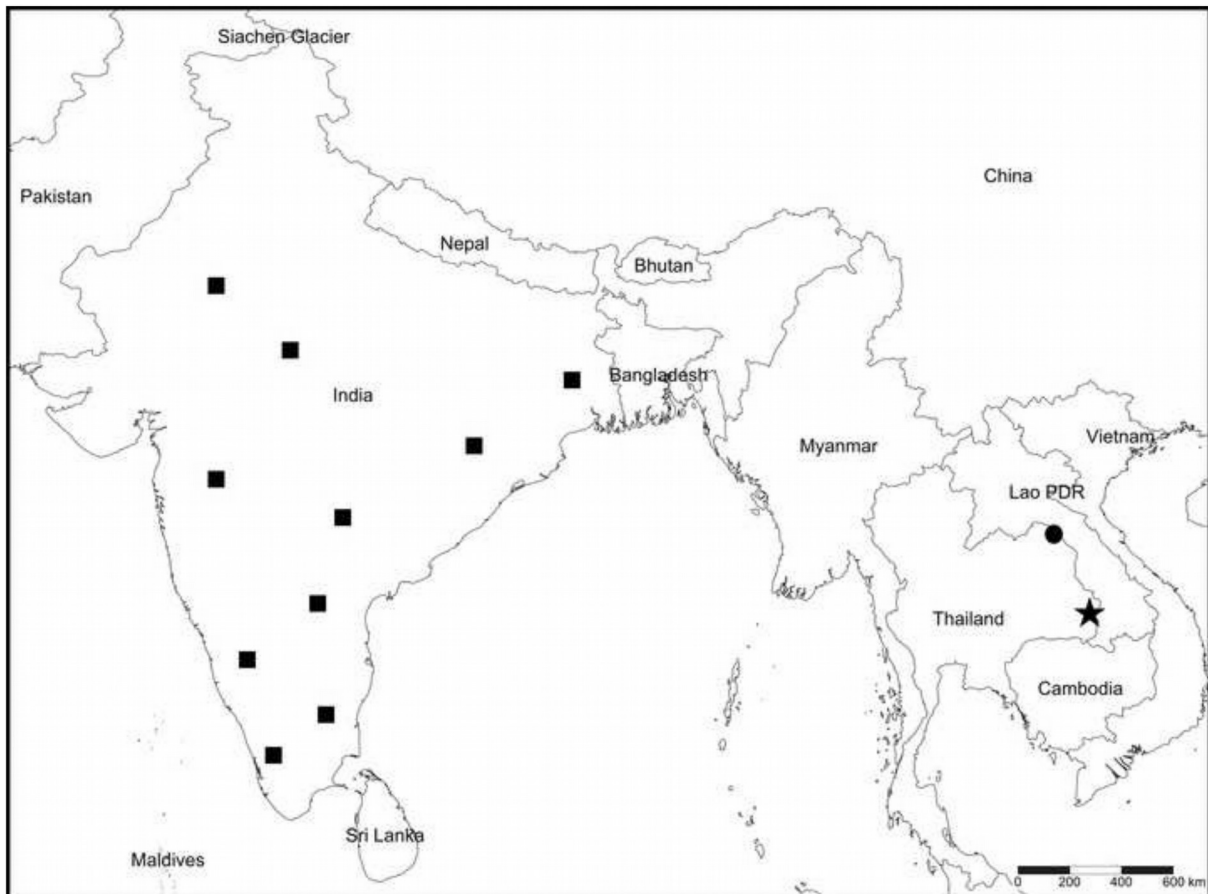


Table 1 Accession numbers of DNA sequences of *Ceropegia foetidiflora* and *C. citrina*

No.	Name	ITS	<i>TrnT-L</i>	<i>TrnL</i>	<i>TrnL-F</i>
1	<i>Ceropegia foetidiflora</i>	MT731612	MT731618	MT731616	MT731614
2	<i>Ceropegia citrina</i>	MT731613	MT731619	MT731617	MT731615

Table 2 Morphological comparison of *Ceropegia citrina* and morphologically similar species.

Characters	<i>C. citrina</i>	<i>C. hirsuta</i>	<i>C. foetidiflora</i>
Stem length	30–50 cm	30–200 cm	15–40 cm
leaves			
petiole length	1.0–2.0 cm	0.5–2.8 cm	0.4–0.7 cm
blade	ovate, ovate-elliptic or elliptic	linear, lanceolate to ovate	ovate to elliptic
base	rounded to subcordate	rounded to rounded-cordate	acute, obtuse to rounded
apex	acute to acuminate	acute	acute
inflorescence	1–2-flowered cyme, peduncle 0.5–1.0 cm long	3–5-flowered cyme, peduncle 0.6–2.8 cm long	1–2-flowered cyme, extra-axillary; peduncle sessile to subsessile, up to 0.5 cm long
flowers			
total length of corolla	3.7–4.3 cm	2.8–7.2 cm	6.9–7.2 cm
corolla tube	pubescent outside, inside of inflated basal portion covered with long white hairs	glabrous outside, inside of inflated basal portion glabrous, but above this portion being covered with long white hairs	glabrous outside, inside of inflated basal portion covered with long white hairs
corolla lobes	broadly ovate-triangular, abaxially pubescent, glabrous adaxially except on the midrib and along the distal half of margins that are covered with long white hairs	broadly oblong or oblong obovate, abaxially glabrous, but puberulent on the entire distal half of adaxial surface of lobes, also with long white hairs on midrib and long white to purplish-white hairs along margins	lanceolate-oblong, abaxially glabrous, the proximal one third of the adaxial midrib covered with long white hairs, and the entire distal two third of the adaxial surface covered with dense purplish hairs
interstaminal corona lobes	slightly shorter than or as high as gynostegium, shallowly bifid or retuse at apex	higher than gynostegium, apically bifid with triangular-shaped segments that are acute at apices	higher than gynostegium, apically bifid with triangular-shaped segments that are acute at apices
staminal corona lobes	linear- lanceolate, with acute-obtuse and recurved apex	linear-lanceolate, with acute-obtuse and recurved apex	lanceolate, with acute and slightly recurved apex

Table 3 Relative amounts of volatile organic compounds (VOCs) (mean \pm SE) identified in floral scents of *Ceropegia citrina* collected from four flowers at first day of anthesis. Sum of minor compounds: sum of relative proportion for VOCs representing $< 0.1\%$. RI = Retention index; SE = standard error; values in bold = main constituents of the floral bouquet (relative amount $> 5\%$). ^a : compound identity verified through authentic standard.

VOCs	RI	Mean \pm SE
Aliphatic compounds		
3-methylbutanal	654	0.11 \pm 0.07
2,4-dimethyl-1-heptene	841	0.18 \pm 0.12
Isoamyl acetate ^a	878	43.02 \pm 15.27
6-methyl-5-heptene-2-one	987	0.51 \pm 0.28
Butyl butyrate ^a	996	1.33 \pm 0.71
2-ethyl-1-hexanol	1031	1.78 \pm 0.64
Hexyl acetate ^a	1007	0.23 \pm 0.20
2-Nonanone ^a	1091	0.13 \pm 0.05
Nonanal ^a	1103	0.88 \pm 0.47
Hexyl butyrate^a	1191	28.25 \pm 18.29
(E)-2-octenyl acetate^a	1217	5.35 \pm 2.25
Tridecane ^a	1300	0.37 \pm 0.22
9-Decenyl acetate	1395	10.89 \pm 5.26
(E)-2-decenyl acetate	1407	0.77 \pm 0.36
Hexadecanoic acid	1869	1.26 \pm 0.11
Terpenoids		
Linalool ^a	1101	0.59 \pm 0.59
(E)-linalool oxide (pyranoid) ^a	1177	0.19 \pm 0.06
Other compounds		
m/z: 59, 123	1073	0.18 \pm 0.10
m/z: 43, 69, 107, 136, 151	1453	0.36 \pm 0.19
m/z: 99, 44, 57, 191, 155	1517	0.16 \pm 0.07
m/z: 44, 67, 91, 134, 202, 161	1556	0.14 \pm 0.11
m/z: 43, 57, 73, 91, 105, 218, 189	1771	0.16 \pm 0.07
m/z: 55, 43, 69, 83, 97, 218, 189	1782	0.14 \pm 0.07
m/z: 69, 43, 138, 109, 218	1799	0.16 \pm 0.13
Sum of minor compounds		2.89 \pm 0.81