

The first discovery of the “*Pheidole quadricuspis* group” in the Indo-Chinese Peninsula (Insecta: Hymenoptera: Formicidae: Myrmicinae)

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Abstract

Pheidole leloi is described as a new species based on a colony series collected in an evergreen forest on the Da Lat Plateau's eastern edge (Hon Ba Nature Reserve, Khanh Hoa Province, Vietnam). It is the first discovery of the *Pheidole quadricuspis* group in the Indo-Chinese Peninsula. The p-distance between COI sequences of *P. leloi* and its putative named allies of the species group is 13.4-15.8%. This may indicate that *P. leloi* has been genetically isolated for several million years. An ancestor of *P. leloi* probably expanded its distribution into the Indo-Chinese peninsula during the Miocene expansions of rainforests, and survived into rainforest patches (refugia) during the Quaternary glacial age.

Key words: *Pheidole leloi* - new species - Vietnam - DNA barcoding - biogeography.

INTRODUCTION

The genus *Pheidolacanthinus* was established by F. Smith (1865) for *Pheidolacanthinus armatus* (junior synonym of *Pheidole quadrispinosa*) and was later demoted to a subgenus of *Pheidole* by Forel (1900). Emery (1921) subdivided “*Pheidolacanthinus*” into three species groups, i.e., *P. quadricuspis* Emery group (Indo-Malayan), *P. quadrispinosa* F. Smith group (Austro-Malayan and Australian), and *P. cervicornis* Emery group (New Guinean). Finally *Pheidolacanthinus* was synonymized with *Pheidole* by D. R. Smith (1979), and his treatment has been widely accepted (see Bolton, 2013). Furthermore, Moreau (2008) and Economo *et al.* (2014) proposed a molecular phylogeny of *Pheidole* of the world and showed the polyphyly of *Pheidolacanthinus* sensu Emery (1921).

Based on morphological similarity Eguchi (2001) inferred that the following named species and several other undescribed species are members of a single lineage equivalent to the *P. quadricuspis* group sensu Emery (1921): *P. acantha* Eguchi, *P. lokitae* Forel, *P. quadrensis* Forel, *P. quadricuspis*, *P. sperata* Forel and *P. spinicornis* Eguchi. They share the following morphological characteristics: (1) promesonotal dome of the major and

minor armed with a pair of long and pointed spines, (2) hypostoma of the major bearing a pair of stout submedian processes; (3) frontal carina of the major and minor inconspicuous or almost absent.

Until recently, the members of the *P. quadricuspis* group had been known from lowland and/or hill rainforests of the Indo-Malayan, the Austro-Malayan and/or the Australian subregions (Eguchi, 2001, 2008, 2011; Eguchi & Yamane, pers. observ.). However, in the course of the authors' last field survey in an evergreen forest on the Da Lat Plateau's eastern edge (Hon Ba Nature Reserve, Khanh Hoa Province, Vietnam), an unknown species having the above-mentioned characteristics was collected. In the present paper, it is described as a new species, and brief biogeographical remarks are provided.

MATERIALS AND METHODS

Abbreviations of the specimen depositories are: VNMN, Vietnam National Museum of Nature, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam; ACEG, Ant Collection of Katsuyuki Eguchi (see his contact address given under the title of this article); BMNH, the Natural History Museum, Cromwell Road, London, England; MBD, Mu-

seum Brunei, Bandar Seri Begawan, Brunei Darussalam; MCZC, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA; MHNG, Muséum d'Histoire Naturelle, Geneva, Switzerland; MSNG, Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy; NHMW, Naturhistorisches Museum Wien, Austria; UMS, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia.

Multi-focused, montage images were produced using Helicon Focus Pro 5.3.10 from a series of source images (5184x3456 pixels) taken by a Canon Eos Kiss X5 digital camera attached to a Nikon SMZ-1270 microscope. Fine hairs and other features that were not recognized automatically were copied from the focused parts from the source images on to the montage image using the retouching function of Helicon Focus. Artifacts (ghost images) and unnecessary parts (unfocused appendages, etc.) surrounding or covering target objects were erased and cleaned up using the retouching function of Helicon Focus. Finally, the background was cleaned up, and the color balance, contrast and sharpness were adjusted using Adobe Photoshop CS6.

Photographs (5184 x 3456 pixels) for measuring were taken using a Canon Eos Kiss X5 digital camera attached to the Nikon SMZ-1270 microscope under suitable magnifications, and the following parts of bodies were measured using ImageJ 1.49m (National Institute of Mental Health, USA, available at <http://imagej.nih.gov/ij/>) and then indices calculated: HL, maximal length of head capsule (in cases where anterior margin of clypeus and/or posterior margin of head are concave, the landmarks for measuring are set at the mid-point of a transverse line spanning the anteriormost points and/or posteriormost points); HW, maximal width of head capsule excluding eyes; SL, length of antennal scape excluding the basal condylar bulb; PW, maximal width of promesonotum measured at the bottom in anterodorsal view (major) or in dorsal view (minor); MSW, maximal width of mesoscutum in dorsal view (queen); ML, mesosomal length measured from the mid-point of anterior margin

of promesontal dome to the mid-point of a transverse line spanning the posteriormost points of metapleuron; HFL, length of hind femur; PtL, petiolar length measured from the anterodorsalmost point of petiolar peduncle to the posterodorsal corner of the petiole in lateral view; PtW, maximal width of petiolar node in dorsal view; PPtL, length of postpetiolar tergite (excluding helcium) measured from the anteroventral corner to the posterodorsal corner of the tergite in lateral view; PPtW, maximal width of postpetiole in dorsal view; CI=HW/HLx100; SI=SL/HWx100; MI=ML/PWx100 (major and minor), or MI=ML/MSWx100 (queen); PtI1=PtL/PPtLx100; PtI2=PtW/PPtWx100; HFI=FL/HWx100.

Five dry-mounted specimens were used for DNA barcoding (see under "Description" and "Recognition"). The body was washed with ca. 500 µL TE (pH 8.0) in a sterilized disposable dish and the gaster was partly broken by sterilized forceps. The whole body was then transferred into 105 µL of extraction buffer (100 µL of 10% Chelex-TE solution and 5 µL Qiagen Proteinase K) and incubated at 56°C for 24-48h, and then heated at 99°C for 10 minutes for inactivating Qiagen Proteinase K in the extraction buffer.

The 658-base standard DNA barcoding region (Folmer region) near the 5' terminus of the CO1 gene was amplified using the primer set LCO-EG/HCO-EG (Table 1). Each PCR contained 5 µL of 2xPCR buffer, 2 µL of dNTPs (final 0.4 mM), 0.3 µL of 10 pmol/µL forward and reverse primers (final 0.3 µM), 0.2 µL of 1.0 U/µL DNA polymerase KOD FX Neo (TOYOBO KFX-2015), and 0.5 µL of DNA template. In cases where the target region was not successfully amplified, three shorter overlapping segments that allowed the creation of a composite sequence of the region were amplified using primer sets LCO-EG/CO1-286R, CI-13-EG/CO1-441R and CO1-362F/CI-14-EG. The PCR thermal regime consisted of one cycle of 2 min at 94 °C; five cycles of 10 sec at 98 °C, 30 sec at 45 °C and 45 sec at 68 °C; 40 cycles of 10 sec at 98 °C, 30 sec at 48.5 °C and 45 sec at 68 °C; and a final cycle of 7 min at 68 °C. Further-

Table 1. Primers used in the present study. Nucleotide positions referring to the mitochondrion complete genome of *Solenopsis invicta* pop-variant PMS (Accession No. HQ215538) are shown.

| Name | Sequence | Position | Reference |
|----------|----------------------------|----------|---|
| LCO-EG | TTTCAACAAATCACAAAGAYATYGG | 17-41 | Modified from Folmer <i>et al.</i> (1994) |
| CI-13-EG | ATAATTTTTTTTATAGTWATRCC | 187-209 | Modified from Hasegawa <i>et al.</i> (2002) |
| CO1-286R | TTATTCGTGGRTADGCTATRTC | 286-265 | Eguchi <i>et al.</i> (2013) |
| CO1-362F | GAACAGGWTGAACWRHTAYCC | 362-383 | Eguchi <i>et al.</i> (2013) |
| CO1-441R | TTAATAGCTCCTADRATNGADGATA | 482-458 | Eguchi <i>et al.</i> (2013) |
| CO1-479R | ATTGTCYCTAAAATWGADGAYAT | 479-457 | Eguchi original |
| HCO-EG | TAAACTTCAGGRTGACCRAAAAATCA | 725-700 | Modified from Folmer <i>et al.</i> (1994) |

more, in cases where these two methods did not work well, semi-nested PCR were employed. Primer sets were LCO-EG/HCO-EG (outer), LCO-EG/CO1-479R (inner), and CO1-362F/HCO-EG (inner). The PCR thermal regime for both the first and second runs consisted of one cycle of 2 min at 94 °C; 35 cycles of 10 sec at 98 °C, 30 sec at 50 °C and 45 sec at 68 °C; and a final cycle of 7 min at 68 °C. After confirming the PCR amplification on a 2.0% agarose gel, the amplified products were incubated at 37 °C for 30 min and 80 °C for 20 min with Illustra™ ExoStar (GE Healthcare, Buckinghamshire, UK) to remove any excess primers and nucleotides. The cycle sequencing reactions were run with ABI PRISM BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems). The sequencing reaction products were purified, concentrated by ethanol precipitation with sodium acetate, and their nucleotide sequences were determined using an automated sequencer (ABI PRISM 3100, Applied Biosystems). The sequences obtained were submitted to the DDBJ database.

These sequences, in addition to the homologue sequences of *Pheidole quadrensis* (Accession No. EF518392.1, Borneo), *P. quadricuspis* (EF518393.1, Borneo) and *P. sexspinosa* (EF518404.1, Palau), were then aligned using Clustal W (Larkin *et al.*, 2007) built in MEGA 6.06 (Tamura *et al.*, 2013). *Pheidole sexspinosa* was included as a representative of the *Pheidolacanthinus quadrispinosa* group sensu Emery (1921). The extensions beyond the Folmer region of *P. sexspinosa*, *P. quadrensis* and *P. quadricuspis* were trimmed, and then pairwise divergences were calculated using the p-distance (obtained by dividing the number of nucleotide differences by the total number of nucleotides compared) and K2P distance model (Kimura, 1980).

DESCRIPTION

Pheidole leloi Eguchi & Bui, sp. n.

Figs 1-13

Type material examined: VNMN; holotype (major); Vietnam, Khanh Hoa, Hon Ba Nature Reserve, 12°07'24"-38"N, 108°58'24"-28"E, ca. 1030-1050 m alt.; K. Eguchi leg.; 16/iii/2013 [colony: Eg16iii13-24]. – VNMN, MCZC, MHNG, ACEG; paratypes; 8 majors, 9 minors, 1 queen from the same colony as holotype; Accession No. LC020537; voucher specimen No. Ext20130410-1 (paratype) from the same colony as holotype (ACEG).

Diagnosis: In the major, vertex as well as dorsal, lateral and ventrolateral faces of vertexal lobe strongly and coarsely reticulate; promesonotal dome with an inconspicuous transverse ridge on its posterior slope; pronotal spine almost straight; posterior slope and lateral face of the dome, mesopleuron and metapleuron irregularly rugoso-reticulate; propodeal spine somewhat

digitiform, with blunt apex; ventral face of petiole with a conspicuous longitudinal carina; anterior half to two thirds of first gastral tergite finely rugoso-punctate. In the minor, posterior part of frons and vertex reticulate; mesosoma relatively densely covered with standing hairs; dorsal face of promesonotal dome weakly punctured, overlain with several rugae, and lateral face of the dome, mesopleuron, metapleuron and dorsal face of propodeum punctate; propodeal spine in lateral view elongate-triangular; petiolar node in lateral view acute at apex, and in posterior view widely and shallowly emarginate at apex.

Description

Major (Figs 1-5): Body dark reddish brown with darker gaster, relatively densely covered with standing hairs (Figs 1, 2, 4, 5). Head in full-face view subrectangular, with its posterior margin narrowly and shallowly emarginate medially, in lateral view relatively thick, faintly impressed on vertex; frons longitudinally rugoso-reticulate; vertex, and dorsal, lateral and ventrolateral faces of vertexal lobe strongly and coarsely reticulate; gena longitudinally rugose; frontal carina almost absent or present just as rugula(e); antennal scrobe absent; median portion of clypeus almost smooth or faintly rugose longitudinally, with a few longitudinal rugae laterally; median longitudinal carina of clypeus relatively distinct; hypostoma with a pair of stout submedian processes ("SM" in Fig. 3) but without median process; lateral processes of hypostoma present but tiny ("L" in Fig. 3); masticatory margin of mandible with apical and preapical teeth, and a tooth in front of basal angle; outer surface of mandible smooth except its base, scattering relatively long decumbent/appressed hairs; antenna with 3-segmented club; maximal diameter of eye longer than or almost as long as antennal segment X. Promesonotal dome with an inconspicuous transverse ridge on its posterior slope; pronotal spine long, slender, almost straight, with pointed apex; anterior slope of promesonotal dome largely smooth with several transverse rugae; posterior slope and lateral face of the dome, mesopleuron and metapleuron irregularly rugoso-reticulate; propodeum irregularly rugoso-reticulate dorsally, longitudinally rugose laterally, and transversely rugose posteriorly; propodeal spine somewhat digitiform, with blunt apex. Ventral surface of midcoxa and hindcoxa smooth. Petiole longer than postpetiole (excluding helcium); petiolar node in lateral view blunt at apex, in posterior view widely and shallowly emarginate at apex, in dorsal view laterally with a narrow flange; ventral face of petiole with a conspicuous longitudinal carina; postpetiole in dorsal view much broader than long, somewhat spindle-shaped; its anteroventral part forming a sharp transverse ridge. Anterior half to two thirds of first gastral tergite finely rugoso-punctate.

Holotype (major): HL, 2.36 mm; HW, 2.62 mm; SL, 1.22 mm; ML, 2.27 mm; PW, 1.18 mm; HFL 1.76 mm; PtL 0.58 mm; PtW 0.47 mm; PPtL 0.45 mm; PPtW



Figs 1-5. *Pheidole leloi* sp. nov., major. (1) Head in full-face view. (2) Head in lateral view. (3) Hypostoma in ventral view: (SM) submedian processes; (L) lateral processes. (4) Mesosoma and waist in lateral view. (5) Body in dorsal view.

0.77 mm; CI, 111; SI, 46; MI, 193; HFI, 67; Ptl1, 130; Ptl2, 62. Paratype majors (n=8): HL, 2.39-2.49 mm, HW, 2.55-2.71 mm; SL, 1.18-1.24 mm; ML, 2.23-2.35 mm; PW, 1.16-1.20 mm; HFL, 1.74-1.80 mm; PtL, 0.61-0.67 mm; PtW, 0.46-0.52 mm; PPtL, 0.45-0.49 mm; PPtW, 0.77-0.86 mm; CI, 107-110; SI, 45-47; MI, 188-198; HFI, 65-68; Ptl1, 128-145; Ptl2, 60-63.

Minor (Figs 6-9): Body dark reddish brown, relatively densely covered with standing hairs (Figs 6, 8, 9). Anteromedian part of frons largely smooth; posterior part of frons and vertex reticulate; area between antennal insertion and eye, and gena rugose; preoccipital carina conspicuous dorsally and laterally; median portion of clypeus slightly punctate, with a median longitudinal carina; masticatory margin of mandible with apical and preapical teeth followed by several small teeth; 1 or 2 small denticles between the preapical tooth and 3rd tooth; outer surface of mandible longitudinally rugose, scattered with long decumbent hairs; antenna with 3-segmented club; scape extending far beyond posterolateral margin of head; maximal diameter of eye shorter than antennal segment X. Promesonotal dome with a pair of spines that are long, slender and pointed apically; posterior slope of the dome with an inconspicuous transverse ridge; dorsal face of the dome weakly punctate, overlain with several rugae; lateral face of the dome, mesopleuron, metapleuron and dorsal face of propodeum punctate; propodeal spine in lateral view elongate-triangular. Petiole a little longer than postpetiole (excluding helcium); petiolar node in lateral view acute at apex, and in posterior view widely and shallowly emarginate at apex; ventral face of petiole without any process and longitudinal carina.

HL, 0.97-1.01 mm, HW, 0.89-0.93 mm; SL, 1.21-1.27 mm; ML, 1.42-1.50 mm; PW, 0.61-0.65 mm; HFL, 1.36-1.43 mm; PtL, 0.31-0.34 mm; PtW, 0.14-0.16 mm; PPtL, 0.28-0.29 mm; PPtW, 0.31-0.34 mm; CI, 91-93; SI, 134-138; MI, 227-235; HFI, 152-155; Ptl1, 111-119; Ptl2, 45-49.

Queen (Figs 10-13): Body dark reddish brown with darker gaster, relatively densely covered with standing hairs (Figs 10, 12, 13). Head in full-face view subtrapezoidal, with its posterior margin broadly and shallowly concave, in lateral view relatively thick, not impressed on vertex, with its posterior margin narrowly and shallowly emarginated medially; frons longitudinally rugoso-reticulate; vertex, and dorsal, lateral and ventrolateral faces of vertexal lobe strongly and coarsely reticulate; gena longitudinally rugose; frontal carina present just as rugula(e); antennal scrobe absent; median portion of clypeus faintly rugose longitudinally, with a few longitudinal rugae laterally; median longitudinal carina of clypeus relatively conspicuous; hypostoma with a pair of stout submedian processes but without median process; lateral processes of hypostoma present but tiny; masticatory margin of mandible with apical and preapical teeth, and a tooth in front of basal angle; outer surface of mandible smooth except its base, scattered with relatively long decum-

bent to appressed hairs; antenna with 3-segmented club; maximal diameter of eye longer than or almost as long as antennal segment X; median ocellus in full-face view located a little behind the level of the posterior margin of compound eye; maximum diameter of median ocellus a little longer than the distance between the median and lateral ocelli (Fig. 11). Mesosoma fully segmented; pronotum almost smooth anteriorly and reticulate laterally; pronotal spine stout and straight, directing anterolaterad; mesoscutum longitudinally rugose medially and irregularly rugoso-reticulate laterally; parapsidal line weakly recognized; scuto-scutellar suture present as a deep and broad impression; mesoscutellum largely smooth dorsally; mesopleuron irregularly rugoso-reticulate, subdivided into anepisternum and katapisternum; metapleuron irregularly rugoso-reticulate; propodeum longitudinally rugose laterally, and transversely rugose posteriorly; propodeal spine somewhat digitiform, with blunt apex. Ventral surface of midcoxa and hindcoxa smooth. Petiole longer than postpetiole (excluding helcium); petiolar node in lateral view blunt at apex, in posterior view widely and shallowly emarginate at apex, in dorsal view laterally with a narrow flange; ventral face of petiole with a conspicuous longitudinal carina; postpetiole in dorsal view much broader than long, somewhat spindle-shaped; its anteroventral part forming a sharp transverse ridge. Anterior three fifths of first gastral tergite finely rugoso-punctate.

HL, 2.10 mm, HW, 2.50 mm; SL, 1.21 mm; ML, 3.19 mm; MSW, 2.03 mm; HFL, 1.90 mm; PtL, 0.82 mm; PtW, 0.72 mm; PPtL, 0.60 mm; PPtW, 0.91 mm; CI, 119; SI, 48; MI, 157; HFI, 76; Ptl1, 136; Ptl2, 79.

Bionomics: The type series (a single colony) was collected from rotten wood on the forest floor of a relatively disturbed patch of an evergreen forest at an altitude of ca. 1,000 m.

SIMILAR SPECIES

Pheidole leloi can be morphologically well distinguished from *P. quadricuspis* and its named allies as follows (see also "Diagnosis" of *P. leloi*).

Pheidole acantha Eguchi, 2001

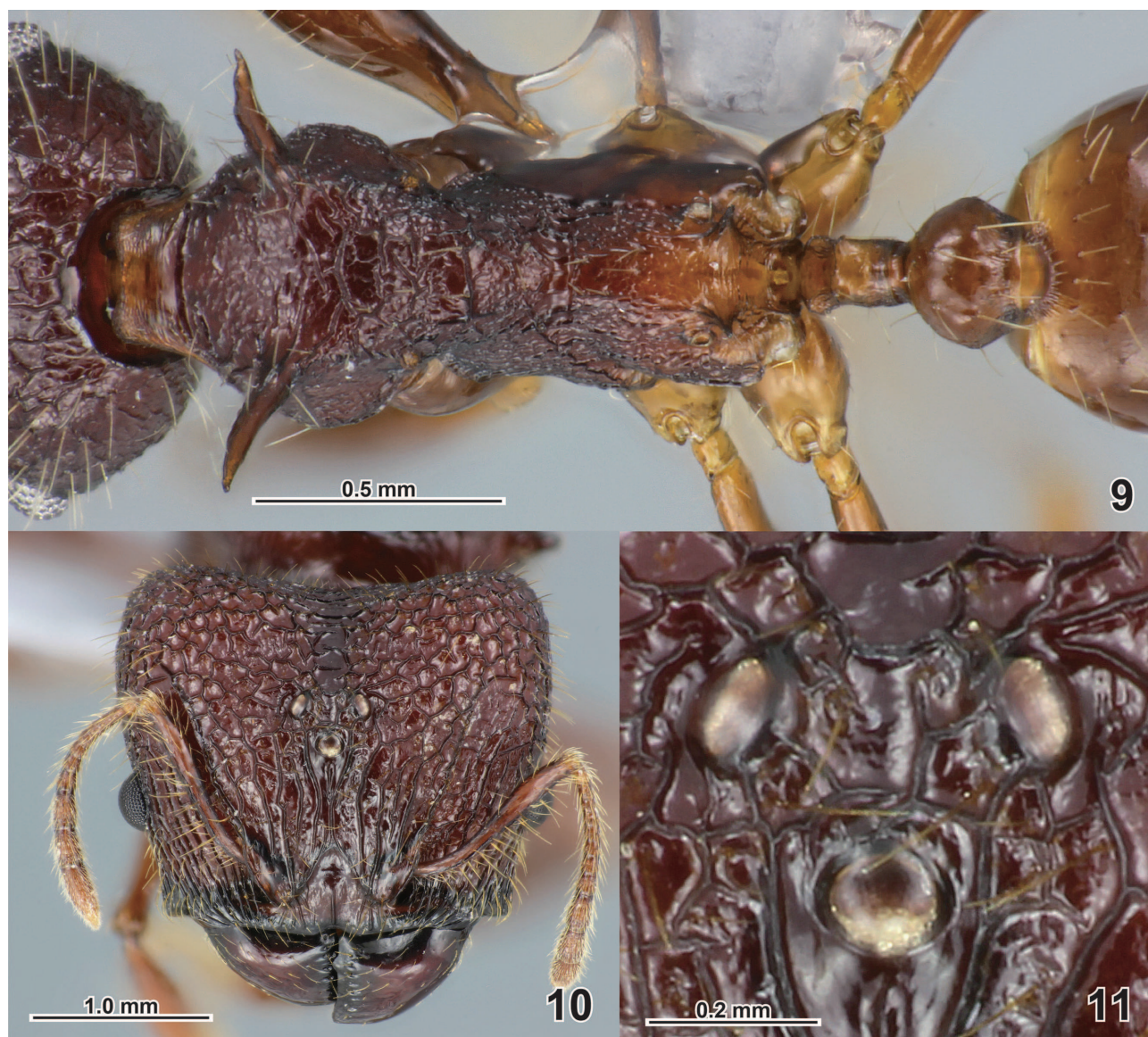
Pheidole acantha Eguchi, 2001: 25-27.

Type material examined: BMNH, MCZC, MSNG, NHMW, UMS; Malaysia, Sabah, Taman Kinabalu (KPHQ); K. Eguchi leg. [colony No.: Eg97-BOR-404, type images: CASENT0901618 and CASENT0901619 of AntWeb (<http://www.antweb.org/>)].

Nontype material examined: ACEG; Malaysia, Sabah, Taman Kinabalu (KPHQ); K. Eguchi leg. [Eg97-BOR-377 (Accession No. LC020538: voucher specimen No. Ext20131019-10), -386], Mt. Kinabalu, ca. 1500 m



Figs 6-8. *Pheidole leloi* sp. nov., minor. (6) Head in full-face view. (7) Right antenna. (8) Mesosoma and waist in lateral view.



Figs 9-11. *Pheidole leloi* sp. nov. (9) Minor, mesosoma and waist in dorsal view. (10) Queen, head in full-face view. (11) Queen, ocelli.

alt., T. Kikuta leg. [7IV0714-1-B1; 15Q12S3, 15Q22B5; 118AC, 177A, 179A, 185A], Mt. Kinabalu, ca. 1800 m alt., T. Kikuta leg. [593A, 604A, 607A, 617A, 626A, 664A, 668A, 873A].

Distinction from *P. leloi*: In the major, promesonotal dome with a conspicuous transverse ridge on its posterior slope; ventral face of petiole without a longitudinal carina. In the minor, dorsum of mesosoma in profile bearing less than 10 standing hairs.

Pheidole lokitae Forel, 1913

Pheidole (*Pheidolacanthinus*) *lokitae* Forel, 1913: 46.
Pheidole lokitae. – Smith, D.R., 1979: 1365.

Type material examined: MNHG; Indonesia, Central Sumatra, Bandar Baroe; v. Buttel-Reepen leg. [9 syntypes (3 majors, 3 minors, 3 queens), type images: CASENT0907766 and CASENT0907767 of AntWeb (<http://www.antweb.org/>)].

Nontype material examined: ACEG; Malaysia, Sabah, Mahua Waterfall area, ca. 1000 m alt., Crocker Range N. P., K. Eguchi leg. [Eg00-BOR-102]. – ACEG; Indonesia: North Sumatra, Parapat, 900 m alt., Danau Toba, Sk. Yamane leg. [SU02-SKY-95, -96 (Accession No. LC020541: voucher specimen No. Ext20130415-2)]; W. Sumatra, Padang, Sukarami, F. Ito leg. [FI92-78, FI96-153, -180].

Distinction from *P. leloi*: In the major, promesonotal dome with a conspicuous transverse ridge on its



Figs 12-13. *Pheidole leloi* sp. nov., queen. (12) Mesosoma and waist in lateral view. (13) Mesosoma and waist in dorsal view.

posterior slope; ventral face of petiole without a longitudinal carina. In the minor, propodeal spine extremely long; petiolar node in lateral view blunt at apex.

***Pheidole quadrensis* Forel, 1900 complex**

Pheidole quadrensis Forel, 1900: 25.

Pheidole (*Pheidolacanthinus*) *quadrensis*. – Forel, 1913: 45.

Type material examined: MHNG; Indonesia, Sumatra, Kajactonam, Sumatra; M. Weber leg.; 1888 [holotype (major), type images: CASENT0907768 of AntWeb (<http://www.antweb.org/>)].

Nontype material examined: ACEG; Malaysia, E. Malaysia, Sabah, Mahua Waterfall area, ca. 1000 m alt.,

Crocker Range N. P., K. Eguchi leg. [Eg00-BOR-129 (Accession No.: EF518392.1)], Poring, 450-500 m alt., Kinabalu Park; K. Eguchi leg. [Eg96-BOR-279], Poring, 600 m alt., T. Kikuta leg. [06Q36B5], Poring, 900 m alt., T. Kikuta leg. [512-A; 71V0510-7-1-a]. – ACEG; Sepilok Forest, K. Eguchi [Eg97-BOR-471; Eg98-BOR-870, -876], Tawau Hills N. P., K. Eguchi leg. [Eg96-BOR-034]; Tawau, Gunong Rara, K. Eguchi [Eg96-BOR-328; Eg97-BOR-542, -535, -571], Sarawak, Miri, Lambir N. P., K. Eguchi [Eg98-BOR-806, -822], Bako, F. Ito leg. [FI96-535], Bt. Entimau, 390 m alt., Mahmud leg., 20/iv/1994. – ACEG; Indonesia, W. Sumatra, Padang, Ulu Gaduk, F. Ito leg. [FI97-440], 28/iii/1997 [FI97-499], Lubuk Gadang, Sk. Yamane leg., 21-23/viii/1985; Sitiung, F. Ito leg. [FI93-255]. – ACEG; Brunei, Tutong, Tasek Merimbun, K. Eguchi leg. [Eg99-BOR-052, -535;

Eg00-BOR-070], Temburong, Kuala Belalong Field Studies Centre, K. Eguchi [Eg99-BOR-217].

Distinction from *P. leloi*: In the major, propodeal spine in lateral view broadly based, pointed apically. In the minor, petiolar node in lateral view blunt at apex, and in posterior view not emarginate at apex.

Remarks: *Pheidole quadrensis* sensu Eguchi (2001) is highly heterogenous in worker morphology and undoubtedly constitutes a species complex. Thus, it needs to be revised based on future intensive sampling in Sumatra and the Malay Peninsula. However, *Pheidole leloi* is morphologically distinguishable from the complex as mentioned above.

Two minors collected by v. Buttel-Reepen from Maxwell's Hill, Taiping, Malacca, and labeled as types (CASENT0904266 and CASENT0907769) (see Forel, 1913) were treated as "syntypes" of *P. quadrensis* by the AntWeb. However, the original description (Forel, 1900) was based on a single major collected by M. Weber from Kajactonam, Sumatra (CASENT0907768). Actually, the two minors seem to be related to *P. aristotelis* Forel, 1911 (see Eguchi, 2001 for diagnosis of *P. aristotelis*).

Pheidole quadricuspis Emery, 1900

Pheidole quadricuspis Emery, 1900: 683.

Pheidole (Pheidolacanthinus) quadricuspis. – Emery, 1921: 683.

Type material examined: MSNG; Indonesia, Sumatra, Si-Rambé; E. Modigliani leg.; xii/1890-iii/1891 [3 syntypes (1 major, 2 minor), type images: CASENT0904267 and CASENT0905766 of AntWeb (<http://www.antweb.org/>)].

Nontype material examined: ACEG; Malaysia, Selangor, Ulu Gombak, F. Ito leg. [FI96- 604, -605; FI98-113, -114, -130, -188, -197], Pahang, Cameron Highlands, ca. 1000 m alt., F. Ito leg. [FI92MCH-16, -32], Malaysia, Sabah, Gunong Rara, K. Eguchi leg. [Eg96-BOR-315, -319, -320, -343A, -343B, -353, -370, -372; Eg97-BOR-530, -557, -558], Sepilok forest, K. Eguchi leg. [Eg97-BOR-495]. – ACEG; Brunei, Tutong, Tasek Merimbun, K. Eguchi leg. [Eg99- BOR-039, 111, 112, 522, 585. – ACEG; Eg00-BOR-037 (Accession No.: EF518393.1), 048, -057, -058], Temburong, Kuala Belalong Field Studies Centre, K. Eguchi leg. [Eg99-BOR-222]. – ACEG; Indonesia, East Kalimantan, Kutai N. P.; Sk. Yamane leg., 13/ ix/1993, North Sumatra, Pulau Nias (Lotu), Sk. Yamane leg. [SU02-SKY-126, -134, -137], West Sumatra, Lubuk Gadang; Sk. Yamane leg., 21-23/viii/1985. – ACEG; Philippines, Camarines Sur, Naga City, Panicason Vill., D. General *et al.* leg., 7/iii/2003.

Distinction from *P. leloi*: In the major, anterior part of vertex longitudinally rugose. In the minor, head almost

completely smooth, with a few standing hairs; dorsal face of promesonotal dome smooth dorsomedially, and weakly punctate anterodorsally, laterally and posterodorsally; mesosoma lacking standing hairs; petiolar node in lateral view blunt at apex, and in posterior view not emarginate at apex.

Pheidole sperata Forel, 1915

Pheidole (Pheidolacanthinus) sperata Forel, 1915: 29-31.

Type material examined: MHNG; Indonesia, Sumatra, Simalur, Sinabang; E. Jacobson leg.; ii/1913 [4 syntypes (2 majors, 2 minors), type images: CASENT0907770 and CASENT0907771 of AntWeb (<http://www.antweb.org/>)].

Nontype material examined: ACEG; Indonesia, West Java, Mt. Halimon, F. Ito leg. [FI96-259, -297, -283, -302; FI98-309], M. Kawamura leg. [No. 161; No. 10/16a (Accession No. LC020540: voucher specimen No. Ext20130524-3)].

Distinction from *P. leloi*: In the major, ventrolateral face of vertexal lobe weakly and finely rugoso-punctate; lateral face of promesonotal dome, mesopleuron, metapleuron and dorsal face of propodeum punctate; first gastral tergite finely punctate over the surface or in anterior half to two thirds. In the minor, dorsum of mesosoma sparsely with relatively short standing hairs; dorsal faces of head and promesonotal dome punctate but not overlain with rugae; petiolar node in lateral view blunt at apex, and in posterior view not emarginate at apex.

Pheidole spinicornis Eguchi, 2001

Pheidole spinicornis Eguchi, 2001: 116-117.

Type material examined: BMNH, MBD, MCZC, MSNG, NHMW, UMS; Malaysia, Sabah, Sepilok Forest; K. Eguchi leg. [Eg98-BOR-880 (Accession No. LC020539: voucher specimen No. Ext20131029-1), type images: CASENT0901620 and CASENT0901621 of AntWeb (<http://www.antweb.org/>)].

Nontype material examined: ACEG; Malaysia, Sabah, Poring, 500 m alt., Kinabalu Park, H. Hirose leg., 20/ vii/1997, Poring, 600 m alt., T. Kikuta leg. [6x2906-5-Bd], Sayap Kinabalu, ca. 1000 m alt., K. Eguchi leg., 15/vii/1996.

Distinction from *P. leloi*: In the major, promesonotal dome with a pair of spines which slightly or weakly curve backward, with a distinct transverse ridge on its posterior slope; first gastral tergite finely punctate over the surface. In the minor, propodeal spine horn-like, weakly downcurved; petiolar node in lateral view blunt at apex, and in posterior view not emarginate at apex.

BIOGEOGRAPHICAL REMARKS

Pheidole leloi has so far been collected just once from an evergreen forest located in the Da Lat Plateau's eastern edge despite our long-term intensive surveys in various localities of Vietnam (Eguchi, 2008, 2011). In contrast with southern Vietnam under the subtropical climate with relatively distinct dry season, along the Da Lat Plateau's eastern edge annual rainfall reaches 3,850 mm and there is essentially no dry season (Sterling *et al.*, 2006). This suggests that *P. leloi*, as well as *Pheidole quadricuspis* and its allies (Eguchi, 2001), are adapted to humid wood-ed environments.

The p-distance between COI sequences of *P. leloi* and its putative allies, i.e., *P. acantha*, *P. lokitae*, *P. quadrensis* complex, *P. quadricuspis*, *P. sperata*, *P. spinicornis*, is 13.4–15.8% (Table 2). Previous studies (Brower, 1994; Quek *et al.*, 2004) estimated that nucleotide substitution rate of COI is around 1.3–2.3% per million years in several arthropod groups including Insecta. When extrapolating this value to our case, *P. leloi* may have been genetically isolated for several million years.

In the middle and late Miocene rainforests periodically extended from tropical Asia northward to southern China or even to Japan (Morley, 1998). Although, in the Quaternary fluctuations between wetter and drier climates became more pronounced, rainforest refugia persisted in north Sumatra, the Mentawai Islands, north Borneo, west Java, northeast Indochina and southern India, and some of these refugia are located at 1,000 meters or more in altitude (Brandon-Jones, 1998). Such paleoclimatic phe-

nomena seem to explain the present isolation of *P. leloi* from its possible relatives, i.e., an ancestor of *P. leloi* probably expanded its distribution into the Indo-Chinese peninsula during the Miocene expansions of rainforests, and survived into rainforest patches (refugia) during the Quaternary glacial age.

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Table 2. Pairwise divergences calculated using the p-distance (upper right) the K2P distance model (lower left). The p-distance is shown indicated by percentage. Nucleotide positions referring to the mitochondrion complete genome of *Solenopsis invicta* pop-variant PMS (Accession No. HQ215538) are shown.

| | Species name Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|-------|-------|-------|-------|-------|-------|-------|------|
| 1 | <i>P. leloi</i> sp. n. 42-699 | | 15.7 | 15.8 | 14.6 | 15.2 | 13.4 | 14.0 | 15.4 |
| 2 | <i>P. acanthi</i> 42-665 | 0.179 | | 17.4 | 17.3 | 15.9 | 15.5 | 15.1 | 17.6 |
| 3 | <i>P. lokitae</i> 46-687 | 0.178 | 0.203 | | 15.9 | 16.7 | 15.6 | 15.8 | 15.6 |
| 4 | <i>P. quadrensis</i> complex 42-699 | 0.165 | 0.202 | 0.182 | | 13.4 | 13.5 | 13.1 | 15.7 |
| 5 | <i>P. quadricuspis</i> 42-699 | 0.172 | 0.182 | 0.192 | 0.150 | | 10.5 | 11.2 | 15.9 |
| 6 | <i>P. sperata</i> 77-699 | 0.149 | 0.176 | 0.177 | 0.152 | 0.115 | | 10.6 | 14.8 |
| 7 | <i>P. spinicornis</i> 42-699 | 0.157 | 0.171 | 0.179 | 0.146 | 0.123 | 0.116 | | 14.2 |
| 8 | <i>P. sexspinosa</i> 58-699 | 0.174 | 0.205 | 0.176 | 0.179 | 0.181 | 0.166 | 0.158 | |

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