

***Tetramorium pacificum* MAYR, 1870, *T. scabrum* MAYR, 1879 sp.rev., *T. manobo* (CALILUNG, 2000) (Hymenoptera: Formicidae) – three good species**

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Abstract

By combining morphological and molecular analyses we scrutinize the taxonomic status of selected ant species of the *Tetramorium bicarinatum* (NYLANDER, 1846) species group. We confirm *Apomyrmex manobo* CALILUNG, 2000 as a member of the genus *Tetramorium*, and evaluate whether *T. manobo* and *T. scabrum* MAYR, 1879, which currently is a junior synonym of *T. pacificum* MAYR, 1870, are specifically distinct from *T. pacificum*. Morphometry shows clear differences between workers of the three ants. Sequence comparison of 700 bp of the mitochondrial *COI* gene confirms that they constitute separate species, embedded in the *T. bicarinatum* species group. Thus, we confirm *T. manobo* as a valid species and revive *T. scabrum* sp.rev. from synonymy. Pronounced morphological variation between *T. scabrum* populations indicates the possible existence of more than one species. We discuss our findings in terms of plesiomorphy and / or convergent evolution of worker morphology. *Tetramorium manobo* appears to be a Philippine endemic restricted to the subregion "Greater Mindanao", where it inhabits forest habitats. In contrast, on the Philippines *T. pacificum* is found only in disturbed habitats. Additionally, we review the ants of the *T. bicarinatum* group currently known from the Philippines and add the first record of *T. obtusidens* VIEHMEYER, 1916.

Key words: *Tetramorium bicarinatum* group, *Tetramorium pacificum*, *Tetramorium scabrum*, *Tetramorium manobo*, *Tetramorium obtusidens*, Oriental region, Indo-Australian region, morphometry, mitochondrial DNA, taxonomy.

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Introduction

The myrmicine ant genus *Tetramorium* MAYR, 1855 comprises about 450 acknowledged species and subspecies worldwide (SHATTUCK & BARNETT 2001). The *T. bicarinatum* (NYLANDER, 1846) species group, as outlined by BOLTON (1977) based on worker morphology, encompasses some twenty species, half of which occur in the Oriental and Indo-Australian regions. The *T. bicarinatum* group has gained wide though dubious name recognition because several of the species have been introduced to non-native ranges around the globe, including green houses in temperate regions (e.g., MCGLYNN 1999).

The sad occasion of the memorial issue in honour of our friend Stefan Schödl prompted us to complete work on certain ants of the *T. bicarinatum* group, an effort that Stefan had envisaged and partly begun. Stefan's interest in *Tetramorium* had been stimulated by Barry Bolton, and he had focused on the *T. bicarinatum* group after travelling to the south-eastern Philippine islands with the senior author (HZ) in 2000. The work was further stimulated by the finding of an ant on Leyte which appeared to be similar to but not identical with *T. pacificum* MAYR, 1870. Together with HZ, Stefan had planned to describe the ant as a new species. It soon became clear, however, that CALILUNG (2000) had in the meantime presented the ant to science,

though under a newly established monotypic genus, proposing the name *Apomyrmex manobo* CALILUNG, 2000. In 2000, by courtesy of Augusto C. Sumalde, former Director of the Museum of Natural History, University of the Philippines Los Baños, Stefan was able to borrow the holotype of *A. manobo*, originating from Mount Apo in southern Mindanao, Philippines, and to compare it with material from Leyte. Conspecificity was confirmed by Stefan Schödl and HZ based on some of the morphological characteristics used in this paper.

BOLTON (2003: 227, 269) synonymized the genus *Apomyrmex* with *Tetramorium* and commented: "The name *Apomyrmex* should never have been published. From the description and figures its type species is obviously a *Tetramorium* of the *bicarinatum* group, close to or synonymous with *T. pacificum*. The author's motives for describing *Apomyrmex* remain a mystery but are probably best ascribed to deficient knowledge of ant taxonomy. For now, the combination of the type species of *Apomyrmex* is *Tetramorium manobo* comb. n., until its status can be properly assessed."

Stefan continued to believe that the ant was a good species distinct from *T. pacificum*. We started our follow-up work by looking through the ants compiled by Stefan and HZ and arrived at the subjective hypothesis that there are

three species among the ants morphologically identical with or close to *T. pacificum*. Our three-species-hypothesis was based on worker morphology, especially on overall body size, overall colour, head proportions, spine length and petiole shape. Based on qualitative comparison with type specimens, we tentatively name the three entities *T. pacificum*, *T. scabrum* MAYR, 1879, and *T. manobo*. *Tetramorium scabrum* has been synonymized with *T. pacificum* by BOLTON (1977).

High-precision morphometric analyses enable the discrimination of very similar species, also in ants (e.g., SEIFERT 2002). However, groups with small interspecific and high intraspecific variation are often poorly resolved by morphological methods alone (for review: WIENS 1999) and in such cases molecular genetic analyses may provide complementary information. Morphologically most similar species may differ markedly in mitochondrial DNA, as shown, among others, for *Tetramorium* ants (SCHLICK-STEINER & al. 2006b, STEINER & al. 2006c). Congruence of DNA and morphology data supports evolutionary hypotheses much more strongly than any of these approaches alone (WETTERER & al. 1998, CHIOTIS & al. 2000, WIENS & al. 2003, WARD & DOWNIE 2005, HASEGAWA & CROZIER 2006, STEINER & al. 2006c).

In this paper we test the above three-species-hypothesis. We conduct morphometric analyses and sequence analyses of the mitochondrial gene cytochrome c oxidase I, *COI*. Unfortunately, the type material of *T. manobo* was not available for re-examination in the course of the present study, but we present morphometric data on the holotype compiled by Stefan Schödl.

Material and methods

Prior to analyses conducted during this study, *Tetramorium* specimens were identified using the key and the species descriptions in BOLTON (1977). The correctness of synonymies given by BOLTON (1977) for *T. bicarinatum* and *T. insolens* was not questioned. Further taxonomic studies were limited to species which keyed out as *T. pacificum*.

Material examined

A total of 278 specimens safekept in the following collections was analysed:

| | |
|-------|--------------------------------------------------------------------------------------------------|
| CBFS | Coll. B.C. Schlick-Steiner & F.M. Steiner, Vienna, Austria |
| CMP | Coll. M. Pfeiffer, Ulm, Germany |
| CZW | Coll. H. & S.V. Zettel, Vienna, Austria |
| HNHM | Hungarian Natural History Museum, Budapest, Hungary |
| MHNG | Muséum d'Histoire Naturelle, Geneva, Switzerland |
| NHMW | Natural History Museum, Vienna, Austria |
| UPLB | Museum of Natural History, University of the Philippines, Los Baños, Laguna, The Philippines |
| USC | University of San Carlos, Cebu City, The Philippines |
| ViSCA | Leyte State University (formerly Visayan College of Agriculture), Baybay, Leyte, The Philippines |
| ZSBW | Zoologische Sammlung des Biozentrums der Universität Würzburg, Würzburg, Germany |

All analysed specimens are listed in the following, with geographical grouping below the level of countries according to physical rather than political entities in case of dis-

crepancies between the two. The sign # refers to collection numbers, but frequently does not represent nest series. The number of examined workers that were morphometrically analysed is given in [] and the number of examined workers *COI* sequenced is given in { }, the latter along with the code of voucher specimens, and the GenBank accession numbers.

Tetramorium pacificum MAYR, 1870

China: Hong Kong: eastern New Territories, Lower Wong Chuk Valley, Lungmei (22° 24' N, 114° 15' E), 1.XI.1996, leg. J. Fellows (# 2), 1 ♀ [1] (CBFS); Hainan Is.: Bawangling NNR (19° 04' N, 109° 04' E), 3.IV.1998, leg. J. Fellows (# 5), 2 ♀♀ [1] (CBFS).

Thailand: Surat Thani Prov.: Khao Sok NP (8° 54' N, 98° 31' E), 30.XI. - 1.XII.2002, leg. F. Seyfert (# 15), 1 ♀ [1] (CZW).

Singapore: Singapore Zoo, 12.I.2002, leg. H. Zettel (# 2), 1 ♀ [1] (CZW); National University of Singapore, campus, 26. - 28.I.2002, leg. H. Zettel (# 1), 1 ♀ [1] (CZW); *ibid.*, 21.X. - 7.XI.2003, leg. H. Zettel (# 1), 15 ♀♀ [3] (CZW).

Indonesia: Krakatoa, Sertung Is.: 16.VIII.2005, leg. S. Yamane (# RK05-SKY-88), 2 ♀♀ [1] {1, T391 = b&f15599, GenBank DQ523554} (CBFS); Krakatoa, Sebesi Is.: 13.VIII.2005, leg. S. Yamane (# RK05-SKY-53), 1 ♀ [1] {1, T397 = b&f15597, GenBank DQ523552} (CBFS); *ibid.*, 12.VIII.2005, leg. S. Yamane (# RK05-SKY-39), 1 ♀ [1] (CBFS); *ibid.*, 13.VIII.2005, leg. S. Yamane (# RK05-SKY-50), 2 ♀♀ [2] {1, T394 = b&f15598, GenBank DQ523553} (CBFS).

Malaysia: Borneo Is.: Sabah: Tawau, 11.X.2004, leg. M. Pfeiffer (# zo6), 2 ♀♀ [2] (CMP); *ibid.*, 5.X.2004, leg. M. Pfeiffer (# xa330), 1 ♀ [1] (CMP); *ibid.*, 1.X.2004, leg. M. Pfeiffer (# xa259), 2 ♀♀ [2] (CMP); *ibid.*, 16.X.2004, leg. M. Pfeiffer (# zo120), 2 ♀♀ [1] (CMP); Sarawak: Banting, 19.VIII.2005, leg. M. Pfeiffer, (# ba666), 2 ♀♀ [2] (CMP).

Philippines: Luzon Is.: Benguet Prov., W Baguio, Asin, Road km 7, 17.II.1999, leg. S. Schödl (# 11), 2 ♀♀ [1] (NHMW); Laguna Prov., Mt. Makiling, 13. - 18.XI.1992, leg. H. Zettel (# 1), 2 ♀♀ [2] (CZW); Camarines Sur Prov., Alanao, Bahi River, 14.II.1999, leg. H. Zettel (# 205), 17 ♀♀ [2] (CZW); Camarines Sur Prov., Lupi, Sooc, 1. - 9.IV.2000, var. collectors (# 252), 1 ♀ [1] (CZW); Albay Prov.: 40 km N Legaspi, 1 km W Malilipot, Busai Falls, 23. II.1998, leg. H. Zettel (# 143), 1 ♀ (CZW); Catanduanes Is.: W Bato, Maribini Falls, 6.III.1999, leg. H. Zettel (# 194), 1 ♀ (CZW); W Virac, Sto. Domingo, Pajo River area, 10.III.1999, leg. H. Zettel (# 199), 1 ♀ (CZW); Negros Is.: Negros Oriental Prov., W Dumaguete, Valencia, Apolong, Casaroro Falls, 26.II.1997, leg. H. Zettel (# 118), 1 ♀ (CZW); Cuernos de Negros, Valencia, Apolong, Casaroro Falls, 9. - 13.III.2005, leg. H. Zettel (# 420), 1 ♀ [1] {1, T386 = b&f15380, GenBank DQ523549} (CBFS); W Dumaguete, Valencia, Apolong, Banica River, 24.X.2004, leg. C. Pangantihon (# P399), 1 ♀ [1] (CZW); Cebu Is.: S Badian, Matutinao, Kawasan Falls, 2 - 50 m, 23. - 24.II.1997, leg. H. Zettel (# 116), 2 ♀♀ [2] (CZW); S Badian, Matutinao, Kawasan Falls, 1 - 30 m, 11.XI.2003, leg. C. Pangantihon (# P352a), 1 ♀ [1] (CZW); SE Moalboal, Balabagon, Busai Spring, 2.XII.1996, leg. H. Zettel (# 114), 1 ♀ [1] (CZW); Samar Is.: Western Samar Prov., SE Gandara, Blanca Aurora Falls, 26.I.2000, leg. H. Zettel (#

218), 21 ♀♀ [3] (CZW, UPLB, USC, NHMW); Eastern Samar Prov., Antipolo (# 991), 1 ♂ [1] (MHNG; = "Cotypus *T. subscabrum*", labelled "Cotypus", "Antipolo \ Philippines \ Samar", "Tetramorium \ pacificum \ subscabrum \ Philippines", "Coll. \ A. Forel", "v. subscabra Em.", "pacificum \ subscabrum", "# 991", "504"); Leyte Is.: Leyte Prov.: E Ormoc, Lake Danao, 13.II.2000, leg. H. Zettel (# 237), 1 ♂ (CZW); *ibid.*, 13.II.2000, leg. S. Schödl (# 237), 1 ♂ (NHMW); Baybay, Mt. Pangasugan, Calbiga-a River, 50 - 200 m, 12. II.2000, leg. H. Zettel (# 236), 1 ♂ (CZW); Baybay, ViSCA, Mt. Pangasugan, 31.I. - 1.II.2000, leg. H. Zettel (# 222), 8 ♀♀ (CZW, ViSCA); N Baybay, Mt. Pangasugan, 50 - 250 m, secondary forest along Lago-Lago River, 1.II. 2000, leg. S. Schödl (# 5), 14 ♀♀ [1] (NHMW); Baybay, LSU, 50 m, Lago-Lago River, 19.III.2005, leg. H. Zettel & C. Pangantihon (# 421), 18 ♀♀ [4] {1, T387 = b&f 15389, GenBank DQ523550} {CZW, CBFS, ViSCA}; N Baybay, ViSCA, Mt. Pangasugan, c. 250 m above Forestry Department, 11.II.2000, leg. S. Schödl (# 14), 2 ♀♀ [1] (NHMW); Hilusig, 14.II.2000, leg. H. Zettel (# 238), 1 ♂ [1] (CZW); Baybay, Mt. Pangasugan, Calbiga-a River, 50 - 200 m, 20. - 21.III.2005, leg. H. Zettel & C. Pangantihon (# 422), 1 ♂ [1] {1, T388 = b&f15391, GenBank DQ523551} (CBFS); Southern Leyte Prov.: E Sogod, large stream, 19. XI.2003, leg. H. Zettel & C. Pangantihon (# 361), 1 ♂ [1] (CZW); Bayagnan Is.: Surigao del Norte Prov., 15 km W Surigao San José, 7.II.2000, leg. S. Schödl (# 10), 17 ♀♀ [9], 2 ♀♀ (NHMW); Mindanao Is.: Zamboanga del Sur Prov., 12 km N Pagadian, Alegria Falls, 9.III.1997, leg. H. Zettel (# 126), 1 ♂ [1] (CZW); Zamboanga del Sur Prov., 8 km NE Pagadian, Manga Falls, 12.III.1997, leg. H. Zettel (# 129), 3 ♀♀ (CZW); Lanao del Norte Prov., W Iligan, Tinago Falls, 13.XI.1996, leg. H. Zettel (# 95), 1 ♂ [1] (CZW); Hikdop Is.: Surigao del Norte Prov., 10 km N Surigao, S & SE coast, 5.II.2000, leg. S. Schödl (# 8), 9 ♀♀ [1] (NHMW).

Samoa: no further locality data, dated 1876 (# 992), 1 ♂ [1] (NHMW).

French Polynesia: Moorea Is.: Mt. Rotui, c. 450 m, 29.VI. 2001, leg. P. Krushelnicky (# GPS2), 1 ♂ [1] {1, T390 = b&f15603, Genbank DQ523555} (CBFS); uptrail from Pte. Vaipahu, opposite Pao Pao at head of Cook's Bay, 240 m, 5.II.1991, leg. L. Morrison (# 62), 2 ♀♀ [2] (CBFS).

Tonga: Tongatabu Is. (# 993), 1 ♂ [1] (NHMW) = **type** of *T. pacificum* labelled "Tonga \ Coll. G. Mayr", "pacificum \ G. Mayr, type.", "SYNTYPE", "Tetramorium \ pacificum \ Mayr \ det. B. Bolton, 1976", "# 993", "T350".

***Tetramorium scabrum* MAYR, 1879**

China: Guangxi Prov. (NW), Cenwangaoshan NR (24° 19' N, 106° 34' E), 1.VIII.1999, leg. J. Fellows (# 10), 2 ♀♀ [2] {1, T402 = b&f15587, GenBank DQ523556} (CBFS).

Malaysia: Malay peninsula: Pahang Prov.: 30 km SE Ipoh, 1500 m, Banjaran Titi Wangsa, Tanah Rata, 14. - 15.III.2002, leg. P. Čechovský (# 999), 7 ♀♀ [6] (NHMW); Perak Prov.: 25 km NE Ipoh, 1200 m, Banjaran, Titi Wangsa Mountains, Korbu Mountain, 27.I. - 2.II.1999, leg. P. Čechovský (# 998), 1 ♂ [1] (NHMW); 40 km SE Ipoh, 900 m, Banjaran, Titi Wangsaringlett, 25.III. - 3.IV.2002, (# 997), 5 ♀♀ [4] (NHMW); 30 km SE Ipoh, 900 m, Cameron Highland, Ringlett, 25.IV. - 5.V.2001, leg. P. Čechovský (# 996), 4 ♀♀ [2] (NHMW); Borneo Is.: Sabah Prov.,

Kinabalu NP, Poring Hot Springs, East Ridge, 560 m, 11. - 20.VII.1995, leg. C. Brühl, (# AB 0), 1 ♂ [1] (CMP); *ibid.*, 10.VIII. - 25.IX.1995, leg. C. Brühl, (# AB 2), 1 ♂ [1] (CMP); Kinabalu NP, Poring Hot Springs, East Ridge, 1360 m, 3.VIII.1995, leg. C. Brühl, (# DW 1), 4 ♀♀ [4] (CMP); Poring Spring, Lower Montane, 650 m, mixed dipterocarp forest, 1.III.1992, leg. A. Floren (# Fog A51/F2), 1 ♂ [1] (ZSBUW); *ibid.*, 20.V.1992, leg. A. Floren (# Fog A8/F1), 1 ♂ [1] (ZSBUW); *ibid.*, 13.IV.1992, leg. A. Floren (# Fog A62/F1), 2 ♀♀ [2] (ZSBUW); Sarawak Prov.: Mulu, 3. - 5.III.1993, leg. H. Zettel (# 14), 1 ♂ [1] (CZW).

Malaysia or Indonesia: Borneo Is.: no further data, leg. Xanthus, 1 ♂ [1] (HNHM) = **type** of *T. scabrum* labelled "Borneo % \ leg. Xanthus", "305 \ 242", "Tetramorium \ scabrum Mayr i.l.", "Holotypus \ Tetramorium \ scabrum Mayr \ det. B. Bolton, 1976", "990", "T501".

Indonesia: Sumatra Is.: Balighe, X.1890 - III.1891, leg. E. Modigliani (# 995), 1 ♂ (NHMW); Si-Rambé, XII. 1890 - III.1891, leg. E. Modigliani (# 994), 1 ♂ [1] (NHMW); Krakatao, Sebesi Is.: 12.VIII.2005, S. Yamane (# RK05-SKY-39), 1 ♂ [1] (CBFS).

***Tetramorium manobo* (CALILUNG, 2000)**

Philippines: Bohol Is.: 3 km SW Bilar, Man Made Forest, 18.III.1997, leg. H. Zettel (# 131), 1 ♂ [1] (damaged) (CZW); Leyte Prov., Leyte Is.: Leyte Prov., E Ormoc, Lake Danao, 11.III.1998, leg. H. Zettel (# 158), 6 ♀♀ [6] (CZW); *ibid.*, 13.II.2000, leg. H. Zettel (# 237), 2 ♀♀ [2] (CZW, NHMW); *ibid.*, 13.II.2000, leg. S. Schödl (# 237), 6 ♀♀, 1 ♀ (NHMW); Leyte Prov., Baybay, ViSCA, Mt. Pangasugan, 31.I. - 1.II.2000, leg. H. Zettel (# 222), 11 ♀♀ [11] (CZW, NHMW, CMP, USC, UPLB, ViSCA); Baybay, ViSCA, 50 m, stream at "Forestry", 11.II.2000, leg. H. Zettel (# 235), 5 ♀♀ [5] (CZW); Baybay, Mt. Pangasugan, Calbiga-a River, 50 - 200 m, 12.II.2000, leg. H. Zettel (# 236), 1 ♂ [1] (CZW); *ibid.*, 20. - 21.III.2005, leg. H. Zettel & C. Pangantihon (# 422), 8 ♀♀ [8] {1, T64 = b&f 15396, GenBank DQ523557} (CZW, CBFS); N Baybay, Mt. Pangasugan, 50 - 250 m, secondary forest along Lago-Lago River, 1.II.2000, leg. S. Schödl (# 5), 14 ♀♀ [14] (NHMW); N Baybay, ViSCA, Mt. Pangasugan, c. 250 m above Forestry Department, 11.II.2000, leg. S. Schödl (# 14), 1 ♂ [1] (NHMW); Dinagat Is.: Surigao del Norte Prov., c. 6 km NE Dinagat, Bagumbayan, 3.II.2000, leg. H. Zettel (# 225), 2 ♀♀ [2] (CZW).

***Tetramorium cynicum* BOLTON, 1977**

Philippines: Negros Is., Negros oriental Prov., Cuernos de Negros, Valencia, Apolong, Casaroro Falls, 9. - 13. III.2005, leg. H. Zettel (# 420), 1 ♂ [1] {1, b&f15383, GenBank DQ523558} (CBFS).

***Tetramorium insolens* (SMITH, 1861)**

Philippines: Negros Is.: Negros Oriental Prov., Cuernos de Negros, Valencia, Apolong, Casaroro Falls, 9. - 13. III.2005, leg. H. Zettel (# 420), 1 ♂ {1, b&f 15381, GenBank DQ523560} (CBFS); Leyte Is.: Leyte Prov., ViSCA, bathroom, 20. - 21.III.2005, leg. H. Zettel, 1 ♂ {1, b&f 15376, GenBank DQ523559} (CBFS).

***Tetramorium obtusidens* VIEHMEYER, 1916**

Philippines: Luzon Is.: Laguna Prov., Los Baños, Mt. Makiling, Mud Spring, 23. - 24.I.1999, leg. H. Zettel (#

167), 1 ♀ (CZW); Camarines Sur Prov., Lupi, Sooc, dam area, 14.III.2004, leg. H. Zettel & C. Pangantihon (# 380), 1 ♀ (CZW); Camarines Sur Prov., Lupi, Sooc, 13. - 20. IX.1999, leg. H. Zettel (# 206), 1 ♀ (CZW); Leyte Is.: Leyte Prov., Baybay, ViSCA, 50 m, "Forestry", stream, 11.II.2000, leg. H. Zettel (# 235), 1 ♀ (NHMW); Baybay, LSU, 50 m, Lago-Lago River, 19.III.2005, leg. H. Zettel & C. Pangantihon (# 421), 1 ♀ (CZW).

Molecular analyses

The genetic analyses included 30 workers, but because of failure in PCR reactions, probably due to DNA degradation, the final sample comprised 12 workers from 12 colonies: 7 *T. pacificum*, 1 *T. scabrum*, 1 *T. manobo*, 1 *T. cynicum* and 2 *T. insolens*. For DNA extraction from the specimens of *T. pacificum*, *T. scabrum* and *T. manobo*, only the gaster was taken, allowing subsequent morphometric analyses. DNA extractions and PCR reactions using a touchdown program followed the standard procedures of STEINER & al. (2006c), except for annealing temperatures, which were set to 49 - 46 °C. The primers used for amplification of the approximately 950 bp of the *COI* gene segment were the newly developed "COI2f" (5' - AATACCTCATTGATCCATC - 3') with L2-N-3014r alias "Pat" (SIMON & al. 1994). PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany), subsequently sequenced in both directions using the Big Dye termination reaction chemistry (Applied Biosystems, Foster City, CA, USA) and analysed using an ABI 377 automatic sequencer (Applied Biosystems).

Nucleotide sequences were aligned with default settings of Clustal X (THOMPSON & al. 1997). 700 bp of *COI* were used in the phylogenetic analyses under the distance (Neighbour-Joining algorithm, NJ) criterion using PAUP* (test version 4.0b3a; SWOFFORD 1998) and by Bayesian inference using Markov Chain Monte Carlo sampling (MCMC), done in MrBayes 3.1 (RONQUIST & HUELSENBECK 2003). Uncorrected Distances and Tamura-Nei distances were calculated and the latter were used for the NJ tree. Support for the NJ tree was assessed with 1,000 bootstrap replicates. Prior to MCMC analysis the GTR+I+G model (TAVARÉ 1986, YANG 1993) was selected by MrModeltest 2.2. (NYLANDER 2004), using hierarchical likelihood ratio tests (LRT; HUELSENBECK & RANNALA 1997), which determine the concordance of data with competing substitution models. We defined three partitions according to codon positions. In the MCMC analysis 500,000 generations with a sampling frequency of 100 were run twice. After 250,000 generations, stationarity was achieved (average standard deviation of split frequencies constantly staying below 0.01, until the end of the run). The last 2,500 trees of each run were used to compute a majority rule consensus tree, assigning posterior probabilities of tree topology. In all phylogenetic reconstructions we added a homologous sequence of *T. bicarinatum* (AY 909379; sequenced for an earlier study by BCS and FMS; specimen identified using the key and the species descriptions in BOLTON (1977); the correctness of synonymies given by BOLTON (1977) was not questioned). Homologous sequences of the following Myrmicinae were used as outgroup: *T. caespitum* (LINNAEUS, 1758) (AY909170) and *Myrmica rubra* (LINNAEUS, 1758) (DQ074378).

Morphometric analyses

For worker morphometry, 150 workers were analysed: 62 *T. pacificum*, 29 *T. scabrum*, 59 *T. manobo*. Dry-mounted specimens were fixed on a pin-holding goniometer. A Nikon SMZ 1500 high-performance stereomicroscope with a 1.6 × planapochromatic lens and a cross-scaled ocular micrometer was used at magnifications of 50 - 320 ×. The following thirteen morphometric characters were measured:

- CL Maximum cephalic length in median line. The head must be carefully tilted to the position with the true maximum. Excavations of the posterior margin of the head and / or clypeus reduce CL. Surface irregularities due to sculpture, carinae in particular, are considered by averaging between peaks and valleys of sculpture.
- ClyNoD Maximum depth of clypeal notch; measured in same adjustment of ant as for CL, measured as orthogonal distance of caudalmost point of clypeal notch to a transversal reference line between frontal-most points of clypeus, to the left and to the right of the notch.
- CW Maximum cephalic width across eyes.
- FCHL Length of longest hair on frontal carina between deepest point of scape corner pit and posterior margin of the eye; arithmetic mean of both sides.
- MNH Distance of ventral reference line to dorsalmost point of mesonotum, measured in same adjustment and orthogonal to same ventral reference line as for PosSPI. Note that this point is, in the given adjustment, usually behind the median line of the mesonotum and can only be found after focusing movements. With the graduated scale of the ocular micrometer kept perpendicular in the visual field, focusing cannot induce a parallax error.
- MW Maximum mesosoma width.
- PEHL Length of longest hair laterally on petiole; arithmetic mean of both sides.
- PEW Maximum width of petiole.
- PosSPI Orthogonal distance of uppermost point of spine to a ventral reference line of the mesosoma. The ventral reference line is the line from the ventralmost point of the pronotum to the ventralmost point of the metapleuron. With the graduated scale of the ocular micrometer directed perpendicular in the visual field, this reference line is brought to coincidence with the horizontal line of the cross-scale at magnifications of about 100 ×. Then, at a magnification of ≥ 250 ×, the mesosoma is carefully tilted to a position in which the ventralmost point of the metapleuron and the tip of the spine are at the same focal level. Note that this character is not necessarily measured in lateral view, but frequently in a dorsolateral view.
- PPW Maximum width of postpetiole.
- PreOc Preocular distance in lateral view; measured as shortest distance between anterior eye margin and sharp frontal margin of gena; arithmetic mean of both sides.
- SPBA Smallest distance between outer margins of spines at their base, measured in dorsofrontal view. If the lateral margins of spines diverge continuously from the tip to the base, a smallest distance at base is not

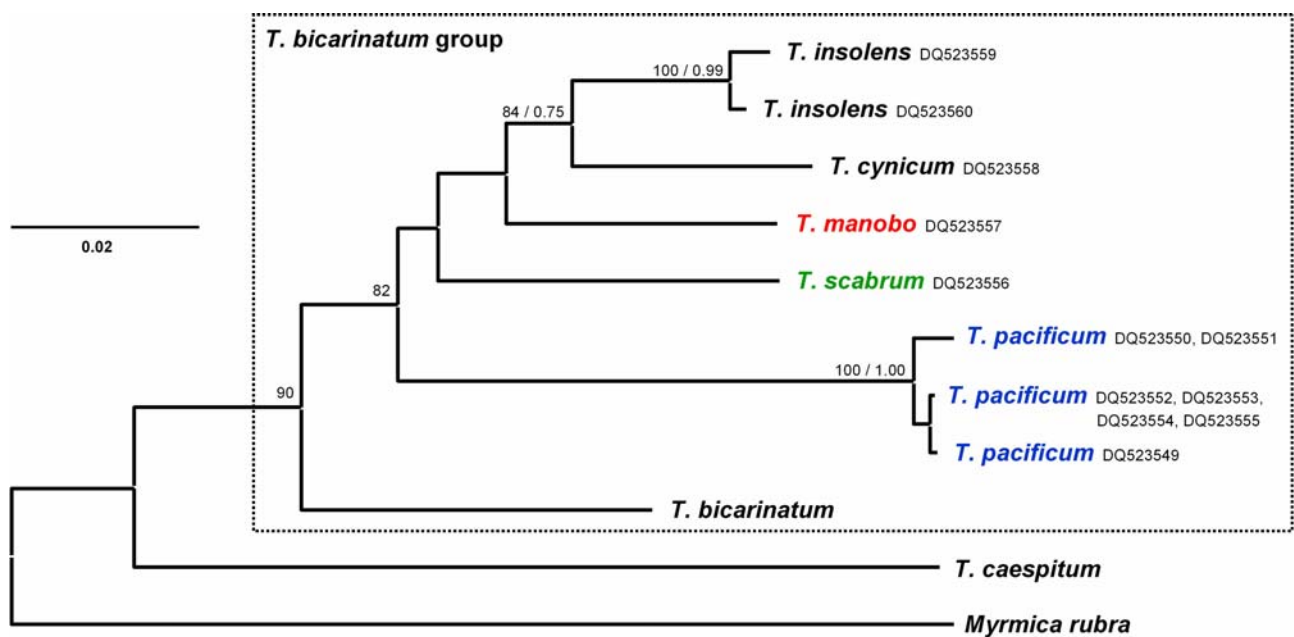


Fig. 1: Phylogeny of selected species of the *Tetramorium bicarinatum* group sensu BOLTON (1977). Neighbour Joining tree of nine haplotypes of *T. bicarinatum*, *T. pacificum*, *T. scabrum*, *T. manobo*, *T. insolens* and *T. cynicum* (GenBank accession numbers denoted for sequences obtained during this study), with *T. caespitum* and *Myrmica rubra* as outgroup, calculated with Tamura-Nei distances using 700 bp of the *COI* gene. The scale bar denotes 0.02 substitutions / site. Bootstrap values > 75 % are given above nodes, posterior probability values > 0.75 of the congruent MCMC branches after slashes.

defined. In this case, SPBA is measured at the level of the bottom of the interspinal meniscus.

SPWI Maximum distance between outer margins of spines; measured in same position as SPBA.

Single worker data were subjected to principal components analysis (PCA) using the software package Primer 5.2.9 (CLARKE & GORLEY 2001) and to discriminant analysis (DA) using SAS 9.1 (SAS INSTITUTE 2004). DA was based on pooled covariance matrices, according to the three-species-hypothesis. Optimal character combinations for DA were screened by using a combination procedure programmed as macro in SAS 9.1. The combination procedure allows an exhaustive search for the character combination that produces optimal discrimination (MODER & al. in press), i.e., discrimination with minimum classification error (and the fewest characters, if more than one combination produces minimum classification error).

The following morphometric data on the holotype of *T. manobo* were found in an electronic file of Stefan Schödl after his death, saved in .xls-format. No definitions were found along with the acronyms of characters, and we here give the original data. Brackets indicate formulas for index calculations as implemented in the .xls-spreadsheet.

"TL = 4.7, HL = 1.025, HW + eyes = 1.113, CI [(HW + eyes) * 100 / HL] = 108.5, SL = 0.825, SI [SL / 100 * (HW + eyes)] = 74.2, PW = 0.725, SPL = 0.375, SPB-Dist = 0.5, AL = 1.25, PTL = 0.6, PTH = 0.35, PTW = 0.4, PTI [PTL / PTH] = 171.4, PPL = 0.325, PPH = 0.338, PPW = 0.425, PPI [PPL / PPH] = 96.3, OMM = 12, EL = 0.221, REL EL = 0.216, EW = 0.171"

Most probably, the characters have been measured using an Olympus SZH 10 stereomicroscope with 1.0 × and 2.0 × achromatic lenses, and a cross-scaled ocular micrometer at magnifications of up to 140 ×. Data can be assumed to be given in mm.

Results and discussion

Integrating molecular and morphometric results

The 700 bp sequences of the *COI* gene of the 12 samples, representing nine haplotypes, were deposited in GenBank under accession numbers DQ523549 - DQ523560. No gaps arose in alignment. The phylogenetic analyses (Fig. 1) confirmed that *Tetramorium insolens*, *T. cynicum*, and *T. bicarinatum* are separate species and suggested that the samples hypothesized to be *T. pacificum*, *T. scabrum*, and *T. manobo* are embedded in the *T. bicarinatum* group. Furthermore, sequence divergence and node support suggested that these samples represent three species in accordance with our three-species-hypothesis. Minimum interspecific uncorrected sequence divergence between the analysed species of the *T. bicarinatum* group varied from 4.1 to 9.7 %, which is in the order of magnitude of the minimum interspecific divergences between congeners of *Cardiocondyla*, *Cataglyphis*, *Lasius*, *Messor*, *Myrmecina*, *Myrmica*, *Solenopsis* and *Tetramorium* ants (SAVOLAINEN & VEPSÄLÄINEN 2003, STEINER & al. 2004, HEINZE & al. 2005, KNADEN & al. 2005, ROSS & SHOEMAKER 2005, SCHLICK-STEINER & al. 2006a, b, STEINER & al. 2006a, b, c). Thus, the *COI* data are compatible with the three-species-hypothesis.

The values of the 13 morphometric characters for the 150 workers analysed are given in the Appendix (digital supplementary material to this article, at the journal's web pages); a synopsis is given in Table 1. PCA of the data suggested that the three ants hypothesized to represent three separate species were separated as a tendency, but partly overlapped (Fig. 2), the types of *T. pacificum* and *T. scabrum* being in regions of the species clouds well apart from the other species. *Tetramorium scabrum* appeared to be the most variable. Overall, PCA, an unsuper-

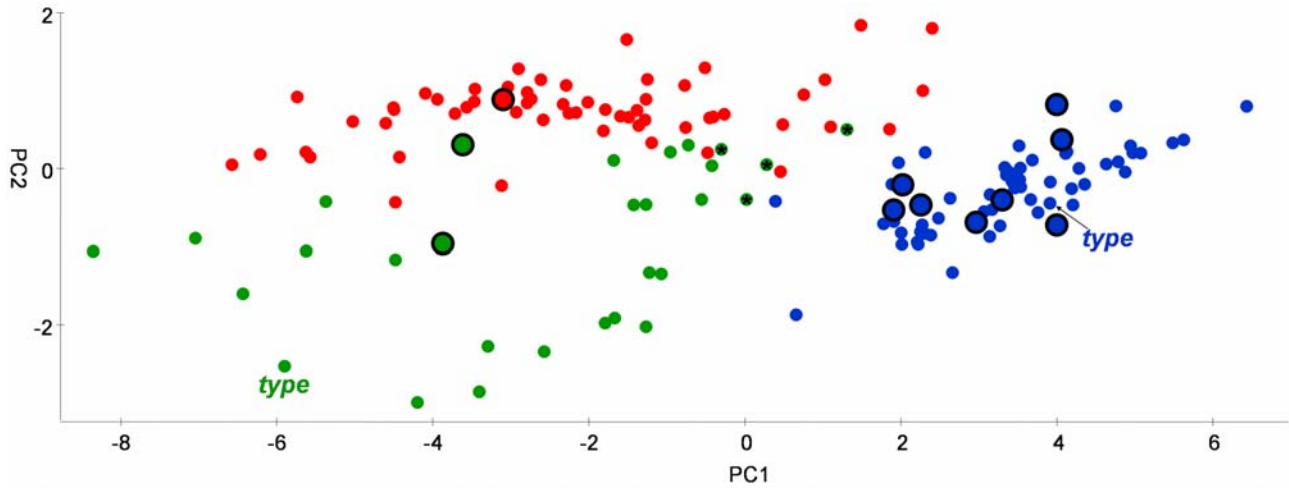


Fig. 2: Principal components analysis of the 13 worker morphometric characters of *Tetramorium pacificum* (blue), *T. scabrum* (green), *T. manobo* (red) ($n = 150$); specimens from nests of which *COI* sequences have been obtained are indicated by enlarged circles; types of *T. pacificum* and *T. scabrum* are indicated; deviating specimens of *T. scabrum* (# DW 1) are indicated by asterisks (for details see Results and discussion). PC1 explains 87.1 % of the total variation, PC2 6.3 %.

Tab. 1: Synopsis of morphometric data [μm] of the analysed *Tetramorium pacificum*, *T. scabrum* and *T. manobo* workers; n = number of analysed specimens; average = arithmetic mean, std = standard deviation, min - max = lower and upper extreme values; values of type workers indicated where available; for definition of morphometric characters see Material and methods.

| | <i>T. pacificum</i> (n = 62) | | | | <i>T. scabrum</i> (n = 29) | | | | <i>T. manobo</i> (n = 59) | | |
|---------|------------------------------|------|-----------|------|----------------------------|------|------------|------|---------------------------|------|------------|
| | average | std | min - max | type | average | std | min - max | type | average | std | min - max |
| CL | 850 | ± 33 | 745 - 933 | 832 | 985 | ± 68 | 866 - 1126 | 1032 | 977 | ± 67 | 818 - 1105 |
| ClyNoD | 12 | ± 2 | 9 - 18 | 13 | 17 | ± 5 | 10 - 28 | 17 | 21 | ± 3 | 16 - 26 |
| CW | 842 | ± 36 | 738 - 924 | 839 | 1019 | ± 87 | 893 - 1200 | 1103 | 1019 | ± 77 | 855 - 1184 |
| FCHL | 221 | ± 19 | 135 - 248 | 167 | 287 | ± 25 | 245 - 354 | 291 | 296 | ± 26 | 237 - 339 |
| MNH | 449 | ± 24 | 399 - 517 | 450 | 537 | ± 39 | 444 - 635 | 598 | 510 | ± 33 | 442 - 579 |
| MW | 566 | ± 28 | 508 - 638 | 567 | 674 | ± 46 | 590 - 776 | 715 | 640 | ± 38 | 550 - 727 |
| PEHL | 190 | ± 12 | 162 - 223 | 190 | 258 | ± 23 | 214 - 315 | 267 | 262 | ± 21 | 209 - 313 |
| PEW | 280 | ± 18 | 225 - 312 | 265 | 338 | ± 32 | 290 - 394 | 387 | 329 | ± 23 | 267 - 383 |
| PosSPI | 438 | ± 27 | 355 - 500 | 423 | 572 | ± 57 | 501 - 739 | 652 | 559 | ± 37 | 471 - 656 |
| PPW | 336 | ± 19 | 276 - 373 | 326 | 405 | ± 30 | 361 - 454 | 454 | 370 | ± 24 | 314 - 428 |
| PreOcLa | 226 | ± 12 | 196 - 257 | 224 | 273 | ± 25 | 233 - 317 | 278 | 293 | ± 29 | 241 - 346 |
| SPBA | 237 | ± 15 | 205 - 288 | 247 | 305 | ± 35 | 250 - 366 | 365 | 281 | ± 22 | 228 - 314 |
| SPWI | 296 | ± 24 | 245 - 375 | 296 | 392 | ± 54 | 328 - 506 | 506 | 389 | ± 27 | 322 - 451 |

vised method, illustrates the similarity of the analysed ants in worker morphology. However, PCA of morphometric data from other, very similar ant taxa of undoubted species status frequently result in much weaker separation of the species clouds (B.C. Schlick-Steiner & F.M. Steiner, unpubl.). In DA, a supervised method, from which the type specimens of *T. pacificum* and *T. scabrum* were excluded, the three species could be separated without error when all 13 characters were used. Using the 13 characters for classificatory DA, we classified the data of the *T. pacificum* and

T. scabrum types: with optimal probability values ($P = 0.9999$ and $P = 1.0$, respectively) our initial hypotheses on the identity of two of the entities were confirmed. In the current absence of the *T. manobo* type, we subsequently subjected the data of six characters of the *T. manobo* type measured by Stefan Schödl to a DA. Those six characters appear to coincide with characters of our analysis, partly based on comparison with the definitions in SCHÖDL (1999): "HL" = CL, "HW + eyes" = CW, "PW" = MW, "SPB-Dist" = SPWI, "PTW" = PEW, "PPW" = PPW. We

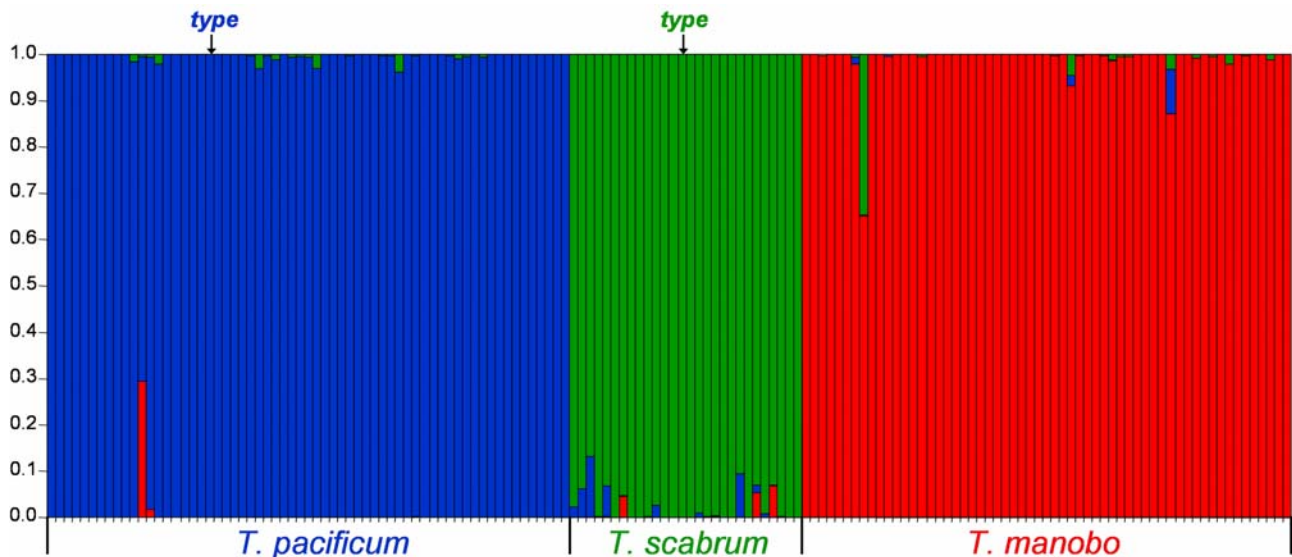


Fig. 3: Discriminant analysis (DA) of *Tetramorium pacificum* (blue), *T. scabrum* (green) and *T. manobo* (red) (n = 150), using eight selected worker morphometric characters (CL, CW, ClyNoD, FCHL, PEW, PPW, PosSPL, PreOcLa). Each worker is represented by a thin vertical bar, which is partitioned into shaded segments that represent the probabilities of its species membership as calculated by DA.

classified the *T. manobo* type. With a $P = 0.997$ our initial hypothesis was confirmed. Subsequently, the optimal character combination was sought using the combination procedure of MODER & al. (in press). Eight characters were selected, which resulted in discrimination without error: CL, CW, ClyNoD, FCHL, PEW, PPW, PosSPL, PreOcLa. Figure 3 illustrates the classification probabilities for the 150 analysed workers.

The molecular and the morphometric results thus confirm the existence of three entities among the analysed ant specimens. For taxonomic decisions, however, two sets of questions have to be solved: (1) are the three entities of species status and, if so, (2) what are their correct names?

Tackling the first set of questions requires biogeographical reasoning. The currently known distributions are: *T. pacificum* is very widespread over the Oriental and Indo-Australian regions (cf. BOLTON 1977; here samples analysed from southern China, Thailand, Singapore, Malaysia, Indonesia, Philippines, Samoa, and French Polynesia). However, it may not be possible to exactly delimit its native range due to frequent anthropogenic transfers (MCGLYNN 1999). *Tetramorium scabrum* is distributed on the south-east Asian mainland and on the islands of the Sunda Shelf, eastwards not crossing Wallace's and Dickerson's Lines. *Tetramorium manobo* is endemic to the southern parts of the Philippines. *Tetramorium pacificum* occurs in sympatry with *T. scabrum* as well as with *T. manobo* and hence can be regarded as reproductively isolated from the other two ants, under application of the biological species concept (reviewed by COYNES & ORR 2004). *Tetramorium scabrum* and *T. manobo*, however, are geographically vicariant, hindering evaluating whether the biological species concept really applies to these two ants. While we agree that the sympatry-argument is a very strong argument in favour of separated species status (cf. MALLETT 1995), it is clearly often not applicable to endemic taxa, especially on islands. We confide in the well-supported morphometric differentiation of the two ants (Tab. 1, Fig. 3) and in the

high value of uncorrected sequence divergence in *COI* of 6.3 % between *T. manobo* and *T. scabrum* (cf. Fig. 1), and therefore regard the two ants as separate species.

To answer the second set of questions, i.e., to allocate correct species names to the three separate species, we argue as follows. *Tetramorium pacificum* is the oldest name available for any of the considered ant species. Classification of morphometric data of the type of *T. pacificum* via DA confirmed the allocation of the name as initially hypothesized. *Tetramorium scabrum* is the next younger available name. DA of the type of *T. scabrum* also confirmed the allocation of this name as initially hypothesized. As the type of *T. manobo* could not be included in this study, caution should be exercised with this ant. Samples initially hypothesized to represent *Tetramorium manobo* key out as "*T. pacificum*" when applying the key of BOLTON (1977). In the frame of this study we explicitly accept the list of synonymies as given by BOLTON (1977). Hence, only the names "*scabrum*" and "*subscabrum*" have to be considered as potential senior synonyms of "*manobo*". *Tetramorium scabrum* has been confirmed as specifically separated. We could not confirm the status of *T. pacificum* var. *subscabrum* EMERY, 1893 by types. Unfortunately, the syntype workers examined by B. Bolton (BOLTON 1977) could not be found in MHNG at present (B. Mertz, pers. comm. 2006) and we were unable to receive specimens from the Museo Civico di Storia Naturale "G. Doria" in Genoa. However, we studied a non-type specimen from MHNG originating from Samar, Philippines (# 991) and identified by Emery, which bears a "Cotypus"-label. This worker was clearly classified as *T. pacificum* by DA, thus supporting the synonymy as established by BOLTON (1977). Overall, the theoretical possibility that *T. manobo* is a junior synonym of *T. pacificum* var. *subscabrum* is undermined by the current biogeographical picture, because *T. manobo* is endemic to the southern Philippines but the syntype workers of "*subscabrum*" originate from Sri Lanka (EMERY 1893).

Even if a future revisionary study might reveal that BOLTON's (1977) list of synonymies is erroneous, we do not expect that species described from other biogeographical regions could pertain to the same biological entity as "*manobo*". The validity of the name *T. manobo* thus does not appear to be endangered by any older name.

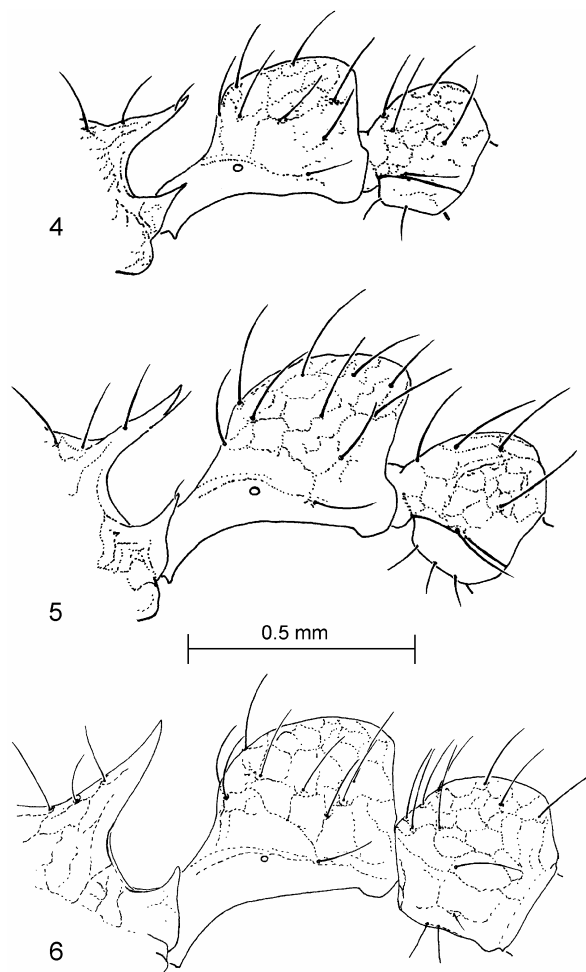
Combining all evidence, we thus confirm the valid species status of *T. manobo* and revive *T. scabrum* **sp.rev.** from synonymy with *T. pacificum*.

In-depth analysis of more material of *T. scabrum* might reveal the possible existence of several species presently summarized under the name *T. scabrum*. Especially the analysed workers from the high-altitude sample from Kinabalu NP, Sabah (# DW 1) appear to deviate (indicated by asterisks in Fig. 2), although they are smoothly classified as *T. scabrum* by DA. Considering further characters, especially surface sculpture, "typical" *Tetramorium scabrum* are known only from Borneo and West Malaysia.

From an evolutionary biology point of view the worker morphology states of the species of the *T. bicarinatum* group are interesting. First, as is evident from the NJ tree of *COI* (Fig. 1), the worker morphology of *T. pacificum*, *T. scabrum* and *T. manobo* could be the result of either convergent or plesiomorphic evolution. Note, however, that the branching order as depicted in Fig. 1 – namely that *T. cynicum* and *T. insolens*, morphologically well distinct from *T. pacificum*, *T. scabrum* and *T. manobo*, have emerged after speciation of the latter three species (Fig. 1) – is not supported by bootstrapping values > 75 % and can thus not be regarded resolved. Second, the phylogenetic reconstructions of the *COI* sequences indicate that the worker-morphology-based grouping of the *T. bicarinatum* group as suggested by BOLTON (1977) – namely into a species complex including, among others, *T. bicarinatum* and *T. insolens*, and another complex including, among others, *T. pacificum* and *T. cynicum* – may be blurred by plesiomorphy and / or convergence. The situation concerning worker morphology to some extent parallels that in the *Tetramorium caespitum/impurum* complex, where worker morphology likewise was speculated to be the result of convergent or plesiomorphic evolution (SCHLICK-STEINER & al. 2006b). However, the phylogeny presented here is based on a very small sample and is derived from a short stretch of a single gene. In recent times, incongruities between morphology and DNA were recognised to occur rather frequently, at a wide range of taxonomic levels (ant examples: JANDA & al. 2004, SEIFERT & GOROPASHNAYA 2004, KNADEN & al. 2005, ROSS & SHOEMAKER 2005). Among others, incongruities can be due to evolutionary processes affecting the markers of choice (see FUNK & OMLAND 2003 for a review). The presented phylogeny may not accurately reflect the species' history and thus more specimens per species, more comprehensive taxonomic sampling and more independent loci are needed to build a stronger inference of the branching history of these *Tetramorium* species.

Verbal characterizations

In the following we attempt verbal characterizations of *T. pacificum*, *T. scabrum* and *T. manobo*. Note, however, that several specimens pose exceptions to these characterizations and that the characterizations hence should only be used to obtain a first impression of the species identity of a sample. Important morphometric characters (μm) and re-



Figs. 4 - 6: Propodeal spines, petiole, and postpetiole, in lateral view (only setae on left body side drawn): (4) *T. pacificum* (Samar, Philippines); (5) *T. manobo* (Leyte, Philippines); (6) *T. scabrum* (Borneo; holotype).

lations of characters are highlighted here in the mode average ± 1 standard deviation, minimum - maximum.

***Tetramorium pacificum*:** Small sized (e.g., CW: 842 \pm 36, 738 - 924), dark brown to blackish-brown. Head longer than wide (CW / CL: 0.99 \pm 0.01, 0.96 - 1.03). Hairs on whole body short (e.g., FCHL: 221 \pm 19, 135 - 248). Postpetiole wider than maximum distance between tips of spines (SPWI / PPW: 0.88 \pm 0.05, 0.79 - 1.01), petiolar node in lateral view abruptly separated from peduncle (Fig. 4). Striae on base of first gaster tergite numerous, distinct, but short.

***Tetramorium scabrum*:** Large sized (e.g., CW: 1019 \pm 87, 893 - 1200), dark reddish-brown to blackish-brown. Head wider than long (CW / CL: 1.03 \pm 0.03, 0.98 - 1.09). Hairs on whole body frequently long (e.g., FCHL: 287 \pm 25, 245 - 354). Postpetiole wider than maximum distance between tips of spines (SPWI / PPW: 0.96 \pm 0.08, 0.87 - 1.12), petiolar node variable, in the type abruptly separated from peduncle (Fig. 5). Striae on base of first gaster tergite variable, very distinct and very long in typical specimens.

***Tetramorium manobo*:** Large sized (e.g., CW: 1019 \pm 77, 855 - 1184), light to middle reddish-brown. Head wider than long (CW / CL: 1.04 \pm 0.02, 1.00 - 1.08). Hairs on

whole body long (e.g., FCHL: 296 ± 26 , 237 - 339). Maximum distance between tips of spines wider than postpetiole (SPWI / PPW: 1.05 ± 0.03 , 0.97 - 1.10). Petiolar node in lateral view smoothly curved to peduncle (Fig. 6). Striae on base of first gaster tergite few, weak and short.

Habitats of *Tetramorium pacificum* and *T. manobo*

Collections in the Philippines by HZ yielded some information about differences in habitats of the two species. *Tetramorium pacificum* has never been collected in natural places but only in disturbed habitats, e.g., foraging on a small tree in a coconut groove along a road; feeding on food remnants on the posts of a shed and on the ground in a picnic area at a water fall. A nest of *T. pacificum* was found in a cut bamboo internode along a trail in an area which was recently converted from a degraded forest to a coconut plantation. In contrast, *T. manobo* has without exception been collected in near-natural or natural habitats: At Lake Danao Natural Park, Leyte, specimens were collected from lower vegetation along a path in an open area next to a degraded forest. In the Mt. Pangasugan area, Leyte, specimens were found foraging on lower vegetation near a stream and on the steep, wet banks of another small stream in forested areas. These observations allow the cautious conclusion that *T. manobo* is a native forest species, which is also important concerning its status as a true Philippine endemic and hence of taxonomic relevance as argued above. *T. pacificum* might either be a species of open habitats native to the Philippines, existing originally along seashores, river edges, landslides etc., or may have been introduced to the Philippines by humans.

Distribution of the species of the *T. bicarinatum* group in the Philippines

The Philippine Islands are divided into sixteen terrestrial biogeographical regions (ONG & al. 2000), but most of the land area belongs to only five regions, i.e., Greater Luzon, Greater Mindanao, Greater Negros-Panay, Greater Palawan, and Greater Mindoro, which are roughly based on the large Pleistocene islands during the time when the sea water level had dropped by about 120 metres (see, e.g., HEANEY 1991). The distribution of most land and freshwater organisms is related to those island complexes (ONG & al. 2000). Within the *Tetramorium bicarinatum* group, six described species are recorded from the Philippines (BOLTON 1977, and this paper): *Tetramorium bicarinatum*, *T. insolens*, and *T. pacificum* have an extraordinarily wide distribution and have been introduced to many countries by humans, so that the original distributions of these species remain unclear (e.g., BOLTON 1977, MCGLYNN 1999, RADCHENKO & al. 1999, STEINER & al. 2003). *Tetramorium obtusidens* (in the present wide sense, see BOLTON 1977) is widely distributed from south-east Asia to New Guinea; this paper presents the first records from the Philippines. On the other extreme, *T. cynicum* BOLTON, 1977 is only known from the island of Negros and is probably endemic to Greater Negros-Panay. It seems to be restricted to (semi-)natural places, as is *T. manobo*. *Tetramorium manobo* is probably restricted to Greater Mindanao and is presently known from the islands of Leyte, Bohol, Dinagat, Hikdop, and Mindanao.

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We dedicate this work to the late Stefan Schödl.

Zusammenfassung

Mit Hilfe morphologischer und molekularer Analysen untersuchen wir den taxonomischen Status ausgewählter Arten der *Tetramorium bicarinatum* (NYLANDER, 1846) Artengruppe. Wir bestätigen *Apomyrmex manobo* CALILUNG, 2000 als eine Art des Genus *Tetramorium* und bewerten, ob *T. manobo* und *T. scabrum* MAYR, 1879, welches derzeit als jüngeres Synonym von *T. pacificum* MAYR, 1870 gilt, von *T. pacificum* artverschieden sind. Morphometrie zeigt klare Unterschiede zwischen Arbeiterinnen der drei Arten. Sequenzvergleich von 700 bp des mitochondrialen *COI*-Gens bestätigt, dass sie unterschiedliche Arten in der *T. bicarinatum* Artengruppe sind. Daher bestätigen wir *T. manobo* als valide Art und heben *T. scabrum* sp.rev. aus seiner Synonymie. Auffällige morphologische Variabilität zwischen *T. scabrum* Populationen weist jedoch auf die mögliche Existenz von mehreren Arten hin. Wir diskutieren unsere Ergebnisse hinsichtlich Plesiomorphie und/oder konvergenter Entwicklung der Arbeiterinnen-Morphologie. *Tetramorium manobo* dürfte ein philippinischer Endemit sein, der auf die Subregion "Greater Mindanao" beschränkt ist, wo er Waldhabitate bewohnt. Im Gegensatz dazu findet man auf den Philippinen *T. pacificum* nur in gestörten Lebensräumen. Zusätzlich behandeln wir jene Arten der *T. bicarinatum* Gruppe, welche derzeit von den Philippinen bekannt sind, und weisen *T. obtusidens* VIEHMEYER, 1916 erstmals nach.

References

- BOLTON, B. 1977: The ant tribe Tetramoriini (Hymenoptera: Formicidae). The genus *Tetramorium* MAYR in the Oriental and Indo-Australian regions, and in Australia. – Bulletin of the British Museum (Natural History), Entomology series 36: 67-151.
- BOLTON, B. 2003: Synopsis and classification of Formicidae. – Memoirs of the American Entomological Institute 71: 1-369.
- CALILUNG, M.V.J. 2000: A new genus, two new species and a new subspecies of Philippine ants. – Philippine Entomologist 14: 65-73.
- CHIOTIS, M., JERMIIN, L.S. & CROZIER, R.H. 2000: A molecular framework for the phylogeny of the ant subfamily Dolichoderinae. – Molecular Phylogenetics and Evolution 17: 108-116.

- CLARKE, K.R. & GORLEY, R.N. 2001: PRIMER v5: User Manual/Tutorial. – Primer-E, Plymouth, 91 pp.
- COYNE, J.A. & ORR, H.A. 2004: Speciation. – Sinauer Associates, Sunderland, 545 pp.
- EMERY, C. 1893: Voyage de M. E. Simon à l'île de Ceylan (janvier-février 1892). Formicidés. – Annales de la Société entomologique de France 62: 239-258.
- FUNK, D.J. & OMLAND, K.E. 2003: Species-level parapatry and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. – Annual Reviews of Ecology, Evolution and Systematics 34: 397-423.
- HASEGAWA, E. & CROZIER, R.H. 2006: Phylogenetic relationships among species groups of the ant genus *Myrmecia*. – Molecular Phylogenetics and Evolution 38: 575-582.
- HEANEY, L.R. 1991: An analysis of patterns of distribution and species richness among Philippine fruit bats (Pteropodidae). – Bulletin of the American Museum of Natural History 206: 145-167.
- HEINZE, J., TRINDL, A., SEIFERT, B. & YAMAUCHI, K. 2005: Evolution of male morphology in the ant genus *Cardiocondyla*. – Molecular Phylogenetics and Evolution 37: 278-288.
- HUELSENBECK, J.P. & RANNALA, B. 1997: Phylogenetic methods come of age: testing hypotheses in an evolutionary context. – Science 276: 227-232.
- JANDA, M., FOLKOVA, D. & ZRZAVY, J. 2004: Phylogeny of *Lasius* ants based on mitochondrial DNA and morphology, and the evolution of social parasitism in the Lasiini (Hymenoptera: Formicidae). – Molecular Phylogenetics and Evolution 33: 595-614.
- KNADEN, M., TINAUT, A., CERDA, X., WEHNER, S. & WEHNER, R. 2005: Phylogeny of three parapatric species of desert ants, *Cataglyphis bicolor*, *C. viatica* and *C. savignyi*: a comparison of mitochondrial DNA, nuclear DNA, and morphological data. – Zoology 108: 169-177.
- MALLET, J. 1995: A species definition for the Modern Synthesis. – Trends in Ecology and Evolution 10: 294-298.
- MCGLYNN, T.P. 1999: The worldwide transfer of ants: geographical distribution and ecological invasions. – Journal of Biogeography 26: 535-548.
- MODER, K., SCHLICK-STEINER, B.C., STEINER, F.M., CREMER, S., CHRISTIAN, E. & SEIFERT, B. in press: Optimal species distinction by discriminant analysis: comparing established methods of character selection with a combination procedure using ant morphometrics as a case study. – Journal of Zoological Systematics and Evolutionary Research.
- NYLANDER, J.A.A. 2004: MrModeltest v2. – <<http://www.csit.fsu.edu/~nylander/>>, retrieved on 12 December 2005.
- ONG, P.S., AFUANG, L.E. & ROSELL-AMBAL, R.C. (Eds.) 2002: Philippine Biodiversity Conservation Priorities: A Second Iteration of the National Biodiversity Strategy and Action Plan. – Department of Environment and Natural Resources - Protected Areas and Wildlife Bureau, Conservation International Philippines, Biodiversity Conservation program - University of the Philippines Center for Integrative and Development Studies, and Foundation for the Philippine Environment, Quezon City, Philippines, 113 pp.
- RADCHENKO, A., CZECHOWSKI, W. & CZECHOWSKA, W. 1999: *Tetramorium insolens* (F. SM.) and *Tetramorium caldarium* (ROG.) (and not *T. guineense* (F.) and *T. simillimum* (F. SM.)) (Hymenoptera, Formicidae) in Poland. – Przegląd Zoologiczny 43: 105-106.
- RONQUIST, F. & HUELSENBECK, J.P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics 19: 1572-1574.
- ROSS, K.G. & SHOEMAKER, D.D. 2005: Species delimitation in native South American fire ants. – Molecular Ecology 14: 3419-3438.
- SAS INSTITUTE 2004: SAS/STAT User's Manual, Version 9. – SAS Institute Inc., Cary, NC.
- SAVOLAINEN, R. & VEPSÄLÄINEN, K. 2003: Sympatric speciation through intraspecific social parasitism. – Proceedings of the National Academy of the United States of America 100: 7169-7174.
- SCHLICK-STEINER, B.C., STEINER, F.M., KONRAD, H., MARKÓ, B., CSÓSZ, S., HELLER, G., FERENCZ, B., SIPOS, B., CHRISTIAN, E. & STAUFFER, C. 2006a: More than one species of *Messor* harvester ants (Hymenoptera: Formicidae) in Central Europe. – European Journal of Entomology 103: 469-476.
- SCHLICK-STEINER, B.C., STEINER, F.M., MODER, K., SEIFERT, B., SANETRA, M., DYRESON, E., STAUFFER, C. & CHRISTIAN, E. 2006b: A multidisciplinary approach reveals cryptic diversity in western Palaearctic *Tetramorium* ants (Hymenoptera: Formicidae). – Molecular Phylogenetics and Evolution 40: 259-273.
- SCHÖDL, S. 1999: Description of *Meranoplus birmanus* sp. nov. from Myanmar, and the first record of *M. bicolor* from Laos (Hymenoptera: Formicidae). – Entomological Problems 30: 61-65.
- SEIFERT, B. 2002: How to distinguish most similar insect species – improving the stereomicroscopic and mathematical evaluation of external characters by example of ants. – Journal of Applied Entomology 126: 1-9.
- SEIFERT, B. & GOROPASHNAYA, A.V. 2004: Ideal phenotypes and mismatching haplotypes - errors of mtDNA treeing in ants (Hymenoptera: Formicidae) detected by standardized morphometry. – Organisms, Diversity and Evolution 4: 295-305.
- SHATTUCK, S.O. & BARNETT, N.J. 2001: Australian ants online. – <<http://www.ento.csiro.au/science/ants/myrmicinae/tetramorium/tetramorium.htm>>, retrieved on 12 December 2005.
- SIMON, C., FRATI, F., BECKENBACH, A., CRESPI, B., LIU, H. & FLOOK, P. 1994: Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. – Annals of the entomological Society of America 87: 651-701.
- STEINER, F.M., SCHLICK-STEINER, B.C., KONRAD, H., LINKSVAYER, T.A., QUEK, S-P., CHRISTIAN, E., STAUFFER, C. & BUSCHINGER, A. 2006a: Phylogeny and evolutionary history of queen polymorphic *Myrmecina* ants (Hymenoptera, Formicidae). – European Journal of Entomology 103: 619-626.
- STEINER, F.M., SCHLICK-STEINER, B.C., KONRAD, H., MODER, K., CHRISTIAN, E., SEIFERT, B., CROZIER, R.H., STAUFFER, C. & BUSCHINGER, A. 2006b: No sympatric speciation here: Multiple data sources show that the ant *Myrmica microrubra* is not a separate species but an alternate reproductive morph of *Myrmica rubra*. – Journal of Evolutionary Biology 19: 777-787.
- STEINER, F.M., SCHLICK-STEINER, B.C., SCHÖDL, S., ESPADALER, X., SEIFERT, B., CHRISTIAN, E. & STAUFFER, C. 2004: Phylogeny and bionomics of *Lasius austriacus* (Hymenoptera, Formicidae). – Insectes Sociaux 51: 24-29.
- STEINER, F.M., SCHLICK-STEINER, B.C., SCHÖDL, S. & ZETTEL, H. 2003: Neues zur Kenntnis der Ameisen Wiens (Hymenoptera: Formicidae). – Myrmecologische Nachrichten 5: 31-35.
- STEINER, F.M., SCHLICK-STEINER, B.C., TRAGER, J.C., MODER, K., SANETRA, M., CHRISTIAN, E. & STAUFFER, C. 2006c: *Tetramorium tsushimae*, a new invasive ant in North America. – Biological Invasions 8: 117-123.
- SWOFFORD, D.L. 1998: PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b3. – Sinauer, Sunderland, MA.

- TAVARÉ, S. 1986: Some probabilistic and statistical problems on the analysis of DNA sequences. – *Lectures on Mathematics in the Life Sciences* 17: 57-86.
- THOMPSON, J.D., GIBSON, T.J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D.G. 1997: The Clustal-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. – *Nucleic Acids Research* 25: 4876-4882.
- WARD, P.S. & DOWNIE, D.A. 2005: The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. – *Systematic Entomology* 30: 310-335.
- WETTERER, J.K., SCHULTZ, T.R. & MEIER, R. 1998: Phylogeny of fungus-growing ants (Tribe Attini) based on mtDNA sequence and morphology. – *Molecular Phylogenetics and Evolution* 9: 42-47.
- WIENS, J.J. 1999: Polymorphism in systematics and comparative biology. – *Annual Review of Ecology and Systematics* 30: 327-362.
- WIENS, J.J., CHIPPINDALE, P.T. & HILLIS, D.M. 2003: When are phylogenetic analyses misled by convergence? A case study in Texas cave salamanders. – *Systematic Biology* 52: 501-514.
- YANG, Z. 1993: Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. – *Molecular Biology and Evolution* 10: 1396-1401.

Addendum

In early September 2006, when this article had already reached the stage of final layout, we received eight syntype workers of *T. pacificum* var. *subscabrum* EMERY, 1893 from the Museo Civico di Storia Naturale "G. Doria" in Genoa. We analysed three of the workers morphometrically according to the instructions given in this article (the other five workers are mounted in such way that the necessary measurements could not be completed).

The three specimens are labelled: "Colombo \ Ceylan \ Simon", "SYNTYPUS \ Tetram. pacificum \ var. subscabrum \ Emery, 1893", "MUSEO GENOVA \ coll. C. Emery \ (dono 1925)", "T505". "Colombo \ Ceylan \ Simon", "SYNTYPUS \ Tetram. pacificum \ var. subscabrum \ Emery, 1893", "MUSEO GENOVA \ coll. C. Emery \ (dono 1925)", "T506". "Kandy \ Ceylan \ Simon", "Tetramorium

\ pacificum \ Mayr \ [?] \ subscabrum \ Emery", "SYNTYPUS \ Tetram. pacificum \ var. subscabrum \ Emery, 1893", "MUSEO GENOVA \ coll. C. Emery \ (dono 1925)", "T509".

Subjecting the data of these specimens to a DA based on the eight selected characters CL, CW, ClyNoD, FCHL, PEW, PPW, PosSPl, and PreOcLa resulted in an unambiguous classification as *T. pacificum*, with *P* ranging from 0.999 to 1.0. We thus confirm the synonymy of *T. pacificum* var. *subscabrum* under *T. pacificum* MAYR, 1870 as established by BOLTON (1977), based on analysis of type material. This is in further support of our chain of arguments concerning the validity of the name "*manobo*", as offered on p. 187 of this article.

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