SYNONYMY OF TWO ARBOREAL TERMITES (ISOPTERA: TERMITIDAE: NASUTITERMITINAE): *NASUTITERMES CORNIGER* FROM THE NEOTROPICS AND *N. POLYGYNUS* FROM NEW GUINEA

RUDOLF H. SCHEFFRAHN[1], JAN KRECEK[1], ALLEN L. SZALANSKI[2], JAMES W. AUSTIN[2] AND YVES ROISIN[3]
[1]Fort Lauderdale Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences
3205 College Ave., Fort Lauderdale, FL 33314

[2]Department of Entomology, University of Arkansas, Insect Genetics Research Laboratory, Fayetteville, AR 72701

[3]Behavioral and Evolutionary Ecology, CP 160/12, Université Libre de Bruxelles
Av. F.D. Roosevelt 50, B-1050 Brussels, Belgium

ABSTRACT

Morphological examination of soldiers and imagos assigned to *Nasutitermes polygynus* from New Guinea were determined to be conspecific with the neotropical species, *N. corniger*. A portion of the mtDNA 16S rRNA gene was sequenced from nine *N. corniger* samples and found to be congruent with that reported for *N. polygynus*. Complementary biological, behavioral, chemical, and reproductive ecology data further support this synonymy. *Nasutitermes corniger* was likely introduced to New Guinea as a result of accidental human transport.

Key Words: arboreal termites, taxonomy, distribution

RESUMEN

Se determinó por medio de una examinación morfológica de los soldados e imagos de *Nasutitermes polygynus* de Nueva Guinea que esta especie es conespecifica con la especie Neotropical, *N. corniger*. Se determinó que una porción de ADNmt 16S ARNr que fue secuenciada de nueve muestras de *N. corniger* fue congruente con la porción de ADN conocida para *N. polygynus*. Los datos biologicos complementarios, el comportamiento, además de la ecologia química y reproductiva apoyan esta sinonomia. Es probable que, *Nasutitermes corniger* fue introducida al Nueva Guinea como resultado accidental del transporte humano.

Nasutitermes corniger (Motschulsky 1855) has the broadest distribution of any neotropical termite species and is capable of establishment in non-endemic localities (Scheffrahn et al. 2002). In many places where *N. corniger* occurs, it is a dominant species. *Nasutitermes polygynus* Roisin and Pasteels 1985, is broadly distributed on the island of New Guinea but is less common than other arboreal nasutes from there (Roisin & Pasteels 1996).

In a molecular genetic analysis of *Nasutitermes* from the tropical Pacific, Miura et al. (2000) determined that *N. polygynus* and *N. corniger* are sister species based on single mtDNA COII and 16S rRNA sequences from each species. Miura et al. (2000) did not make morphological comparisons but noted remarkable similarities between the two species. Because of the widespread range of *N. polygynus* in New Guinea, Miura et al. (2000) hypothesized that *N. polygynus* evolved from an ancestral arrival of *N. corniger* from the New World. It was difficult, however, for the authors to reconcile a natural trans-Pacific crossing, thus inferring introduction by humans and a synonymy of *N. polygynus* and *N. corniger*.

In this paper we provide morphological, genetic, behavioral, and chemical evidence that *N. polygynus* is a synonym of *N. corniger*.

MATERIALS AND METHODS

Morphological examinations are based on an extensive collection of *N. corniger* from the New World (Scheffrahn et al. 2005) and nine samples from New Guinea. A synopsis of synonymy of *N. corniger* is presented in Scheffrahn et al. (2005) with the following additions:

Nasutitermes corniger (Motschulsky)

Nasutitermes polygynus Roisin and Pasteels 1985: [imago, Fig. 1; soldier Fig. 2. Type loc.: Papua New Guinea, Nubia, 3 km on road to Bunapas (Bogia District)]; Roisin & Pasteels 1996: 546-551 [imago, Fig. 40; soldier Fig. 41; large worker, Figs. 42, 43; distribution, Fig. 44]; Roisin & Pasteels 1986: 149-167 [polycaly, polygyny] Roisin & Pasteels (1985) described *N. polygynus* from specimens collected in northeastern Papua New Guinea. Additional soldiers from

southeastern and southwestern Papua New Guinea were measured in their redescription (Roisin & Pasteels 1996) that also included photographs of the soldier, large worker mandible, and large worker enteric valve armature.

Material Examined

All specimens are from Island of New Guinea and were fixed in Bouin or FAA. TYPE COLONY of *N. polygynus*, Nubia, Hansa Bay, Bogia District, 3 km on road to Bunapas, 16-XI-1978; J. M. Pasteels (PNGT 4). Nubia, Hansa Bay, Bogia District, Sakula River bridge; Y. Roisin; 2-I-1984 (PNGT 508). Bunapas, Ramu River, Bogia District, behind airstrip; Y. Roisin; 23-VII-1984 (PNGT 751). Sisimangum, Hansa Bay, Bogia District; Y. Roisin; 8-IX-1984 (PNGT 827). Bogia, 12 km on road to Josephstaal, Bogia District; Y. Roisin and J. M. Pasteels; 25-II-1985 (PNGT 900). Gogol River valley, S. of Madang, 35 km from main (coastal) road; Y. Roisin; 16-IX-1988 (PNGT 1274). Lake Murray, Western Province; Y. Roisin and M. Leponce; 23-V-1990 (PNGT 1566). Nabire (Irian Jaya); Y. Roisin; 12-XI-1995 (IRJT 3). Kaimana (Irian Jaya), near airstrip; Y. Roisin; 21-XI-1995 (IRJT 118).

Genetic Analysis

DNA was extracted from four *Nasutitermes ephratae* (Holmgren), one *N. guayanae* (Holmgren), one *N. nigriceps* (Haldeman), one *N. rippertii* (Rambur), and nine *N. corniger* samples from the Dominican Republic, Dominica, Nevis, Guadeloupe, Puerto Rico, Mexico, Ecuador, Suriname, and Jamaica per Szalanski et al. (2004). Polymerase chain reaction (PCR) was conducted with the primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al., 1994). These PCR primers amplify an approximately 428-bp region of the mtDNA 16S rRNA gene. PCR reactions were conducted with 1 µl of the extracted DNA per Szalanski et al. (2000), with a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 45 s. Amplified DNA from individual termites was purified and concentrated on Microcon-PCR Filter Units (Millipore, Bedford, MA). Samples were sent to University of Arkansas Medical Sciences DNA Sequencing Core Facility (Little Rock, AR) for direct sequencing in both directions with an ABI Prism 377 DNA sequencer (Foster City, CA). GenBank accession numbers for the *Nasutitermes* termites subjected to DNA sequencing in this study are AY623085 to AY623100. Consensus sequences for each sample were obtained by using BioEdit 5.09 (Hall 1999). The position of variable nucleotide sites among the DNA sequences was obtained with MacClade v4 (Sinauer Associates, Sunderland, MA).

The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model (Kimura 1980) of sequence evolution. Mitochondrial DNA sequence of *N. acajutlae* (Holmgren) (Kambhampati et al. 1996) was included for phylogenetic analysis, along with mtDNA 16S sequences for *N. polygynus*, *N. triodiae* (Froggatt), *N. magnus* (Froggatt), *N. walkeri* (Hill), *N. exitiosus* (Hill), *N. princeps* (Desneux), *N. bikpelanus* Roisin and Pasteels and *N. pinocchio* Roisin and Pasteels from Miura et al. (2000). *Longipeditermes longipes* (Haviland) and *Hospitalitermes medioflavus* (Holmgren) (Termitidae: Nasutitermitinae) sequences from Miura et al. (2000) were used as the outgroup taxa for the *Nasutitermes* dataset. DNA sequences were aligned with CLUSTAL W (Thompson et al. 1994) and adjusted manually. Maximum likelihood and unweighted parsimony analysis on the alignments were conducted by using PAUP* 4.0b10 (Swofford 2001). Gaps were treated as a fifth character state. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings with the Branch and Bound algorithm of PAUP*. For maximum likelihood analysis, the default likelihood parameter settings were used (HKY85 6-parameter model of nucleotide substitution, empirical base frequencies) with the exception of the transition/transversion ratio, which was set to 1.357845:1. These parameters were used to carry out a bootstrap analysis by either step-wise addition or the maximum parsimony tree as the starting tree.

Results

Geographical Distribution

Nasutitermes corniger occurs over a north-south distance of more than 6,000 km from southern Mexico to northern Argentina, including the West Indies, and much of the region except Chile, Uruguay, and the Bahamas (Scheffrahn et al. 2005). There is one introduced population in southeastern Florida (Scheffrahn et al. 2002) currently under an eradication program. The distribution of *N. polygynus* is given in Figure 1.

Morphology

Roisin & Pasteels (1985, 1996) reported some variability in measurements as observed by Scheffrahn et al. (2005), but character dimensions of *N. corniger* (Scheffrahn et al. 2005) from the Neotropics and *N. polygynus* from New Guinea (Roisin & Pasteels 1996) overlap for all 12 comparable measurements (7 imagos, 5 soldiers). Coloration, pilosity, and fine structure for both groups are also congruent (Fig. 2).

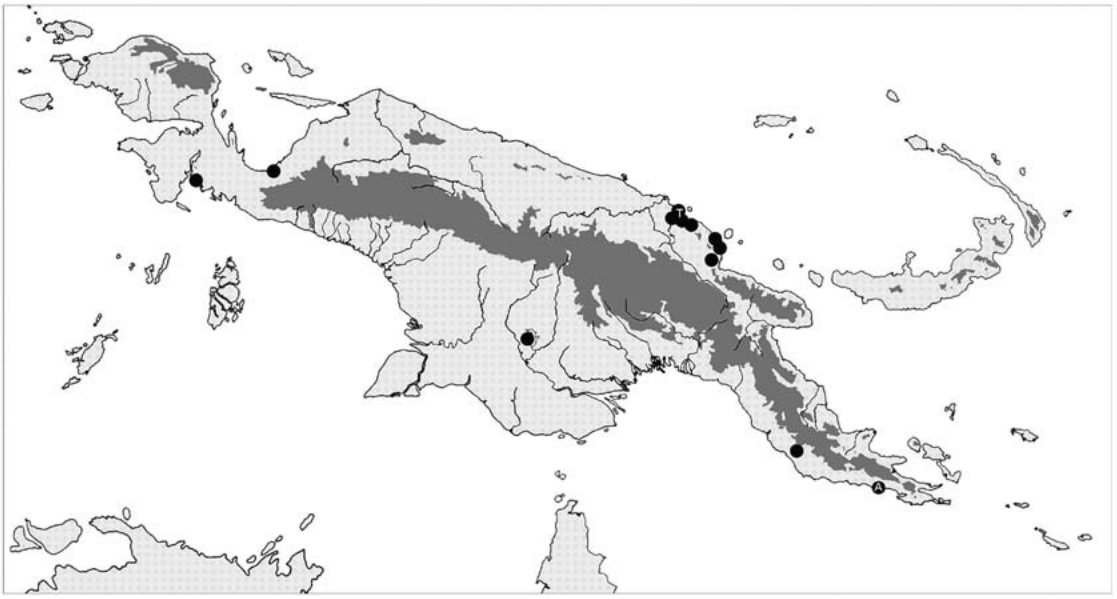

Fig. 1. Collection sites (dark circles) of *Nasutitermes corniger* in New Guinea. Dark grey: elevation above 1000 m.

Genetic Analysis

The 428-bp region of the mtDNA 16S rRNA gene was subjected to DNA sequencing from *Nasutitermes corniger* and 13 other *Nasutitermes* taxa (Fig. 3). Among the nine *N. corniger* DNA sequences, 13 nucleotides were variable and genetic diversity ranged from 0.0% between the Guadeloupe and Nevis samples to 1.8% between the Jamaica and Nevis samples. To facilitate analysis with the DNA sequences from Miura et al. (2000) 17 base pairs at the 5' end of our DNA sequences were excluded for phylogenetic analysis. The aligned DNA data matrix, which included 14 *Nasutitermes* taxa as well as the two outgroup taxa, resulted in a total of 421 characters. Of these characters, 111 (26%) were variable and 63 (15%) were phylogenetically informative. This dataset had only one most parsimonious tree (Fig. 4), (length = 272, CI = 0.577), as documented using the Branch and Bound search algorithm of PAUP*. Bootstrap analysis of the aligned *Nasutitermes* taxa revealed that *N. corniger* and *N. ephratae* are monophyletic. Based on genetic distance data, the *N. polygynus* DNA sequence from Miura et al. (2000) collected from New Guinea was most similar to *N. corniger* from Mexico and Ecuador. The consensus tree from the maximum likelihood analysis (-ln L = 1672.20365) was identical to the maximum parsimony analysis.

Discussion

The synonymy of *N. polygynus* and *N. corniger* is supported by morphological and genetic congruency. Furthermore, Roisin & Pasteels (1986) reported biological similarities for *N. corniger* and *N. polygynus* by virtue that both species are polygynic and build polycalic (satellite) nests. Also like *N. corniger*, Roisin & Pasteels (1996) report crepuscular dispersal flights for *N. polygynus* following the first rains of the wet season.

Vrkoc et al. (1973) identified six monoterpenes in the defensive secretion of *N. costalis* (=*corniger*) from Cuba including the two major components, terpinolene and limonene, found in *N. polygynus* (Everaerts et al. 1988). The major diterpenic components identified from the defensive secretion of *N. polygynus* are trinervita-1(15),8(19)-dien-2β,3α-diol and trinervita-1(15),8(19)-dien-2β-ol (Dupont et al. 1981: *Nasutitermes* sp. B). Vrkoc et at. (1978) identified the diol as the major diterpene component in the defensive secretion of *N. corniger* from Cuba. In the four populations of *N. corniger* from Central America analyzed by Gush et al. (1985), the diol is also dominant, although sometimes partially replaced by its 2α,3α and 2α,3β-diol isomers, whereas the latter constitutes 0.4-21.4% of the diterpenic fraction.

We hypothesize that *N. corniger* was introduced to New Guinea as a result of unintentional human transport. This species was actually intercepted several times in the U.S. and the U.K. in plants from Central America or the West Indies (Gay 1967). Established populations have been introduced to Florida and Scotland (Scheffrahn et al. 2002) and recent interceptions from Columbia and Puerto Rico have been recorded, respectively, in Clearwater and Jacksonville, Florida. We, like

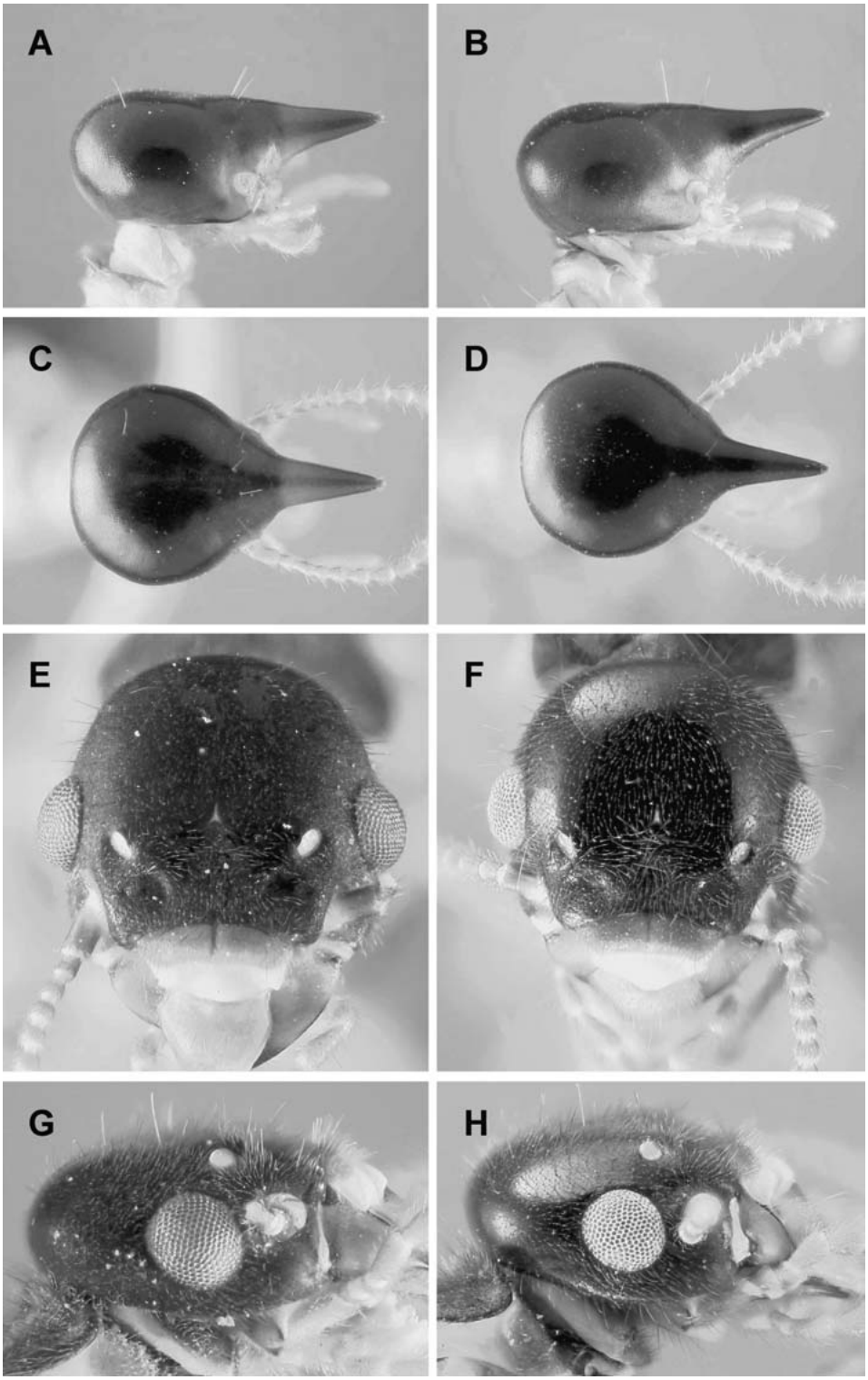

Fig. 2. Photomicrographs of *Nasutitermes corniger*. Lateral (A) and dorsal (C) views of soldier head capsule from Irian Jaya, New Guinea. Lateral (B) and dorsal (D) views of soldier head capsule from Honduras. Dorsal (E) and lateral (G) views of imago head capsule from Irian Jaya, New Guinea. Dorsal (F) and lateral (H) views of imago head capsule from Venezuela.

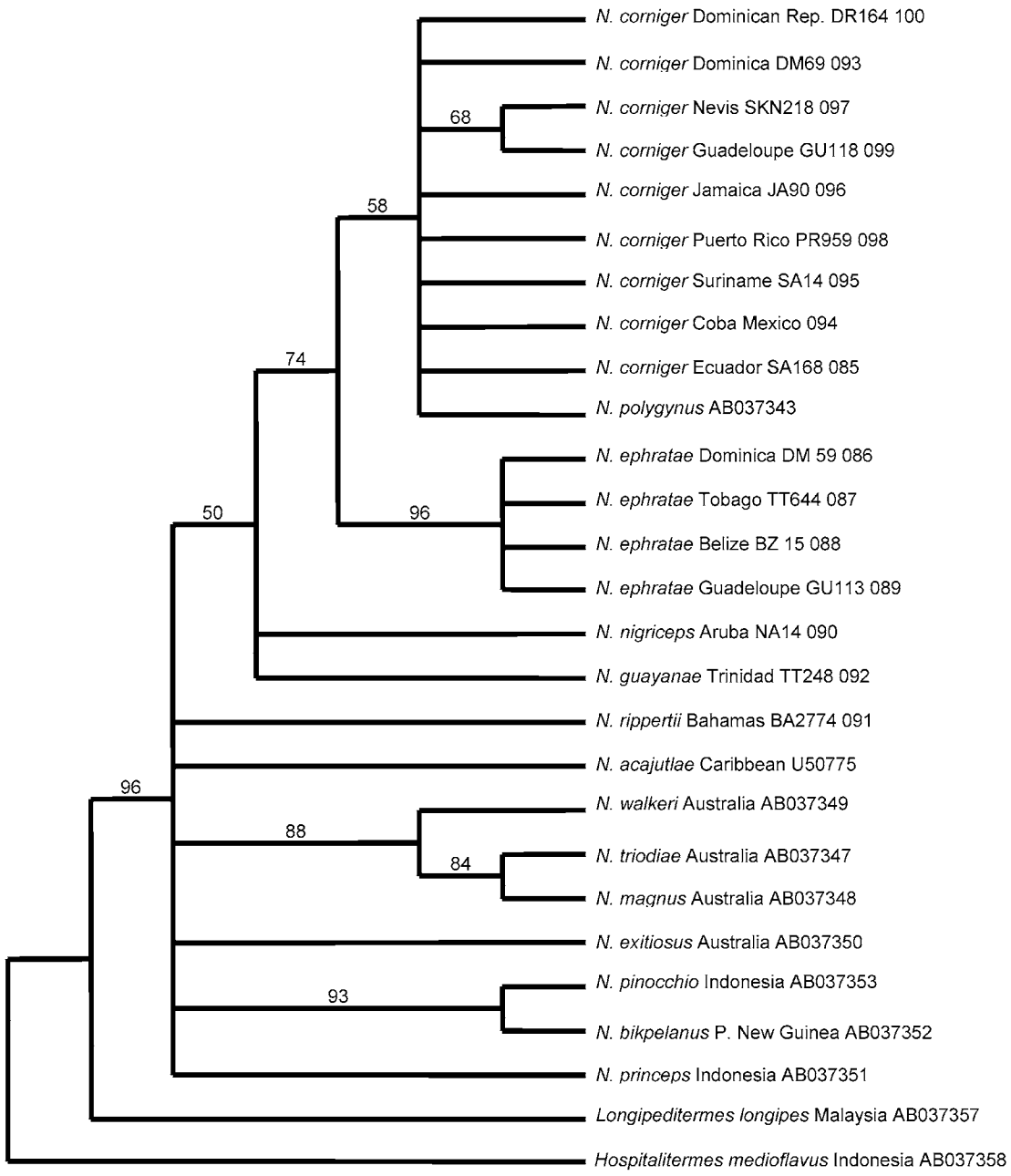

Fig. 3. Single most parsimonious tree during a branch and bound search from PAUP* (Swofford 2001). Bootstrap values for 1,000 replicates are listed above the branches supported at ≥50%. GenBank accession numbers for samples not sequenced in this study also are provided.

Miura et al. (2000), find it difficult to explain the widely separated localities (≤1900 km, Fig. 2) of *N. corniger* on the island of New Guinea. We speculate that the New Guinea distribution is the result of (1) a single early maritime introduction centuries ago from which the termites dispersed around New Guinea, possibly helped by human transportation, (2) multiple recent introductions by ship or aircraft, or (3) a combination of both. The fact that *N. corniger* has not been reported from other islands in the southwestern Pacific region suggests that introductions into this region have been few, making the first hypothesis more likely.

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