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Genetic Analysis of Invasive Conehead Termites (Blattodea: Termitidae) Reveals a Single Origin for Two Populations in Florida

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Abstract

In 2001, *Nasutitermes corniger* (Motschulsky), common name conehead termite, were discovered near a marina in Dania Beach, FL, where the invasive species was probably transported from its native range in Central and South America or the Caribbean. In January 2016, an infestation was found in Pompano Beach, Florida, approximately 21 km north of the Dania Beach population. This study compares variants in seven microsatellite loci across specimens from 11 nests in Dania Beach and 8 nests in Pompano Beach. Results are consistent with all *N. corniger* in both locations being descendants of a single introduced colony, spreading within Broward County, FL through human transport of infested materials. No more than four alleles were found at any of the seven microsatellite loci analyzed, inferring that a single Queen and King, or multiple sibling reproductives descended from a monogamous pair, headed the colony that arrived in Florida. The potential economic and environmental impacts of this invasive termite are enormous due to its broad diet, including agricultural crops and orchards, native and ornamental plants, natural landscapes, and structures. Conspicuous tunnels and aboveground nests are the key aspects of *N. corniger* biology that render colonies vulnerable to discovery and control. The now proven ability of *N. corniger* to establish breeding populations in the United States, to cause extensive property and landscape destruction, and to spread by human transport underscores the need for continued aggressive efforts toward eradication of known infestations as well as quick operational actions the next time invasive *N. corniger* are discovered.

Key words: eradication program, *Nasutitermes corniger*, microsatellites, polygyny, population genetics

The only populations of conehead termites, *Nasutitermes corniger* (Motschulsky), ever established in the United States are 21 km (13 miles) apart in Dania Beach and Pompano Beach, Broward County, in south Florida. This report analyzes variable genetic markers to determine whether those two infestations are the result of spread via human transport following a single invasion, or due to independent introductions of invasive colonies from location(s) in the New World tropics.

Nasutitermes corniger is an ecologically flexible species that nests in or on a wide variety of substrates and consumes a broad diet. Trees, shrubs, stumps, roots, structures, fences, wooden pallets and furniture, scrap wood, paper products, and probably many other items made of cellulose have been recorded as nest locations and food sources.

Within its native range in the Neotropics, *N. corniger* (including *N. costalis* now synonymized as *N. corniger*; Scheffrahn et al. 2005a) is an economically important agricultural, structural, and forest (impacting silviculture and natural areas) pest. *Nasutitermes corniger* is considered a 'major', 'important', or 'common' pest of fruit trees and crops such as sugar cane (Snyder and Zetek 1934, Tucker 1939, Harris 1961, Araujo 1970, Jutsum et al. 1981, Medina-Gaud et al. 1989, Mill 1992, Constantino 2002; see Dietz and Snyder 1923 reinfestations of cacao and soursop). The species is also a highly destructive structural pest, 'the single most important' and 'voracious' in parts of Brazil and Argentina (Bandeira et al. 1989, Torales 1998, Constantino 2002) and as well as in Panama (Dietz and Snyder 1923; Snyder and Zetek 1924, 1934), and Puerto Rico (Brooks et al. 1941). *Nasutitermes corniger* is active and abundant in tropical forests, where—as in its other native habitats such as savannahs and second growth—these termites have an essential ecological role in nutrient recycling. The species' consumption of a wide variety of tree species, however, also renders it a pest of silviculture (Martorell 1945, Wolcott 1948, Harris 1961, Mill 1992, Constantino 2002) and of urban trees in parks and streets (Torales 2002). *Nasutitermes corniger* consumes dead wood, including heartwood, in live (or dead) trees, and dead portions of other plants (Wolcott 1948; Thorne 2013, 2015). Scheffrahn et al. (2005b, p. 28) noted that in locations where *N. corniger* occurs, it is often a 'dominant species'.

Risk of invasion into the United States by this pest has long been of concern given the species' adaptability, broad diet, rapid growth rate, and proficiency in dispersing—often by human transport—to colonize diverse habitats and expand its range. *Nasutitermes corniger* has the broadest distribution of any termite in the Neotropics: documented from 13 countries in Central and South America as well as 42 Caribbean islands (reviewed in Scheffrahn et al. 2005a,b), rendering *N. corniger* '… arguably the most common, least cryptic, and widespread (termite) species in the Neotropics, …' (Scheffrahn 2005a, p. 279; also see Araujo 1970, Constantino 2002).

Nasutitermes corniger is well documented as a capable 'hitchhiker' and colonist. The species is broadly established in New Guinea where it was transported from the Neotropics, likely through the Dutch sugar trade over 100 yr ago (Roisin and Pasteels 1986, Evans et al. 2013; see notes on synonomy of *N. polygynous* and *N. corniger* in Miura et al. 2000). Live *N. corniger* reached a commercial building in Scotland by traveling in plant material shipped from the tropics (Scheffrahn et al. 2002), and the species is considered a nonindigenous pest in the Bahamas as well as Turks and Caicos Islands (Scheffrahn et al. 2006). Thompson and Hebert (1998) infer that 'recent' *N. corniger* (reported as *N. costalis*) colonization across Jamaica by small number of initial founders may explain the lack of genetic variation in samples they analyzed from the island.

For decades the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) has listed the termite genus *Nasutitermes*, and the conehead termite species *N. corniger* (along with synonym *N. costalis*), as living plant pests of quarantine significance. APHIS inspectors have found live *N. corniger* in, on, or with plants, plant products, carriers, and container vans, entering into the continental United States and Hawaii. Between 1984 and 2011, APHIS intercepted *Nasutitermes* termites 118 times in 19 states. Twenty-one of those interceptions were specifically identified as the species *N. corniger* (or as *N. costalis*), with interception ports ranging from California to Texas to Miami to New York. The Florida infestations, proving capability of *N. corniger* to colonize, reproduce, and thrive in the United States, highlight vulnerability of infestation from off-shore introductions as well as importance of protocols for a rapid response to future conehead termite discoveries to prevent establishment elsewhere in the United States.

As with most termites, winged female and male *N. corniger* fly *en masse* during their swarm season, typically beginning around twilight following the first heavy rain of the spring wet season (Dudley and Beaumont 1889a; Barreto 1923; Dietz and Snyder 1923; Becker 1953; Thorne 1983, 2015; Clarke 1993). Large colonies can produce over 20,000 winged alates to disperse in one flight season (Thorne 1983, Roisin and Pasteels 1986, Adams and Atkinson 2007).

Following their dispersal flight, finding a mate, and locating a nest site, the new Queen and King (or, in this species, multiple Queens [polygyny] and Kings [polyandry] are common (Dudley and Beaumont 1889a,b, 1890; Dietz and Snyder 1923; Thorne 1982, 1984; Atkinson and Adams 1997; Adams et al. 2007) and sequester themselves to initiate a new colony. All Queens in polygynous groups produce eggs. Young *N. corniger* colonies remain completely hidden, feeding and traveling concealed within wood while they increase population size. During this cryptic stage of early colony development, coneheads evade visual inspection, but successful colonies eventually grow to a size empowering their 'big reveal', first by building conspicuous foraging tunnels, and ultimately a carton nest (Becker 1953; Thorne 1984, 2013, 2015; Thorne and Haverty 2000).

The initial phase of nest construction creates a hub 7–10 cm in diameter, containing approximately 10,000–16,000 termites (Thorne 1984, Thorne and Haverty 2000). Thriving colonies can grow rapidly, expanding their nest to 25 cm height or larger within a few months, and the same year may produce alates (Thorne and Haverty 2000; B. L. Thorne, S. Alspach, K. Tenn, personal observation). Nests may be on tree trunks or branches, or on shrubs, stumps, logs, grass clumps, structures, or on open ground (epigeal) where usually anchored—at least when young—to a piece of wood, stump, or root. Coneheads are not classic subterranean termites, but their nests (and foraging galleries) can extend underground (Snyder and Zetek 1934).

Nasutitermes corniger nests comprise the 'heart' of a colony, typically housing the Queen cell and reproductives, the nursery of eggs and young brood, the majority of nymphs, and mature alates prior to flight. Mature nests also contain hundreds of thousands of workers and soldiers busy inside while others of their castes are foraging throughout the extensive tunnel network (Thorne 1984, 1985, 2013). *Nasutitermes corniger* is unique among termites in the United States in that colonies produce conspicuous tunnels and nests once they have emerged from their young cryptic phase. All other native and introduced termite species in the United States remain largely hidden underground or in wood for their entire life cycle, making it impractical to find and target treat each colony with liquid termiticide (Thorne 2013, 2015).

Also uniquely among all termites in the United States, *N. corniger* exploits a wide variety of exceptional reproductive capabilities. In addition to multiple primary (alate-derived, not neotenic) Queens and Kings cofounding colonies, polygyny and polyandry can result from replacement or budding. Multiple offspring alates can drop their wings within a nest to replace senescing or dead parents, or reproductives can move from an established colony into one or more satellite nests. This habit, polydomy, means that a single intermingling colony may construct and occupy more than one nest; Dietz and Snyder 1923, Thorne 1982, 1984; Levings and Adams 1984; Roisin and Pasteels 1986; Adams and Levings 1987; Clarke 1993; Atkinson and Adams 1997; Thorne and Haverty 2000; Adams and Atkinson 2007). Multiple primary reproductives in *N. corniger* may be siblings or unrelated, depending on how the colony was formed, and whether the current reproductives are replacements in the original nest or moved into a satellite nest (Thorne 1982, 1984; Atkinson and Adams 1997; Adams and Atkinson 2007; Adams et al. 2007). In native habitats where *N. corniger* has been studied polygyny is facultative, with a mix of monogynous and polygynous nests found in the field. Thus far in the Florida populations, since B.L.T. joined the project in 2012, however, multiple Queens (up to 52) have been found in all of the more than 70 nests in which we have seen a reproductive; Cabrera et al. (2004) note discovering 13 Queens in one nest in Dania Beach, FL.

Multiple Queens and Kings fuel *N. corniger*'s potential for exceptionally rapid population growth and thus earlier age of first production of alates (Thorne 1984). Queen(s) and King(s) are able to walk to a new nest site, even traveling vertically, if the colony relocates or builds satellite nests (Emerson 1929; Thorne 1982, 1984; Thorne and Haverty 2000). Extraordinary reproductive, nesting, and diet flexibility make *N. corniger* highly adaptable, resilient, and a successful colonist.

In May 2001, *N. corniger* colonies were identified in Dania Beach, southeastern Florida, first recognized as different, and important, by pest management professionals. The termites were originally determined as *N. costalis*, but *N. costalis* was later synonymized as *N. corniger* (Scheffrahn et al. 2005a). This invasive termite is assumed to have arrived in wooden materials on a boat that traveled through the termite's native range in the Caribbean islands or Central or South America before docking at a private marina in Dania Beach (Scheffrahn et al. 2002, 2014). Alate flights were recorded several weeks after discovery of the infestation (Scheffrahn et al. 2002). Applying the first termiticide treatments in April 2003, the Florida Department of Agriculture and Consumer Services (FDACS) has led a force of government officials, research scientists, pest management professionals, trade associations, and manufacturers to work collectively and productively toward eradication of this invasive pest species. From 2004 through 2010, small colonies were occasionally found and treated. Visible *N. corniger* activity was substantially reduced, with some claims of eradication or close (Hickman 2006). In 2011, however, a significant population resurgence was discovered (Scheffrahn et al. 2014; also see Hochmair et al. 2013 p. 750 noting infestations discovered in 2012). During the years when there were reports of 'eradication' of the conehead termite population in Dania Beach, healthy young colonies were evidently still present, but they evaded detection because they were in the early stages of growth and development, concealed within wood. By 2011, when the infestation resurgence was noticed, next-generation conehead termite colonies had reached sufficient population size to 'reveal themselves' by building visible foraging tunnels and nests (Thorne 2015).

The conehead termite infestation in Dania Beach spread to approximately 105 properties (Fig. 1A; 'approximately' because some property boundaries shift over time), including residential and commercial structures; landscapes; 'back yard' fruit trees including mangoes, avocado, citrus, and coconut palms; native trees, shrubs, and grasses; and natural areas. FDACS's aggressive containment, control, and eradication program was rebooted in fall 2012 (Thorne 2013, 2015; Alspach and Thorne 2015). As of this writing, the Dania Beach *N. corniger* population is largely suppressed, but diligent monitoring continues (Alspach and Thorne 2015, Thorne 2015).

In January 2016, *N. corniger* colonies were discovered in Pompano Beach, Florida, 13 miles north of the infested zone in Dania Beach, FL, and approximately 7.3 km (4.5 miles) inland and landlocked from the Atlantic coast. As with the Dania Beach invasion, the Pompano Beach population was also first recognized by an alert pest management professional. Before recent suppression, the Pompano Beach infestation involved 13 properties, including commercial structures and landscapes as well as densely overgrown

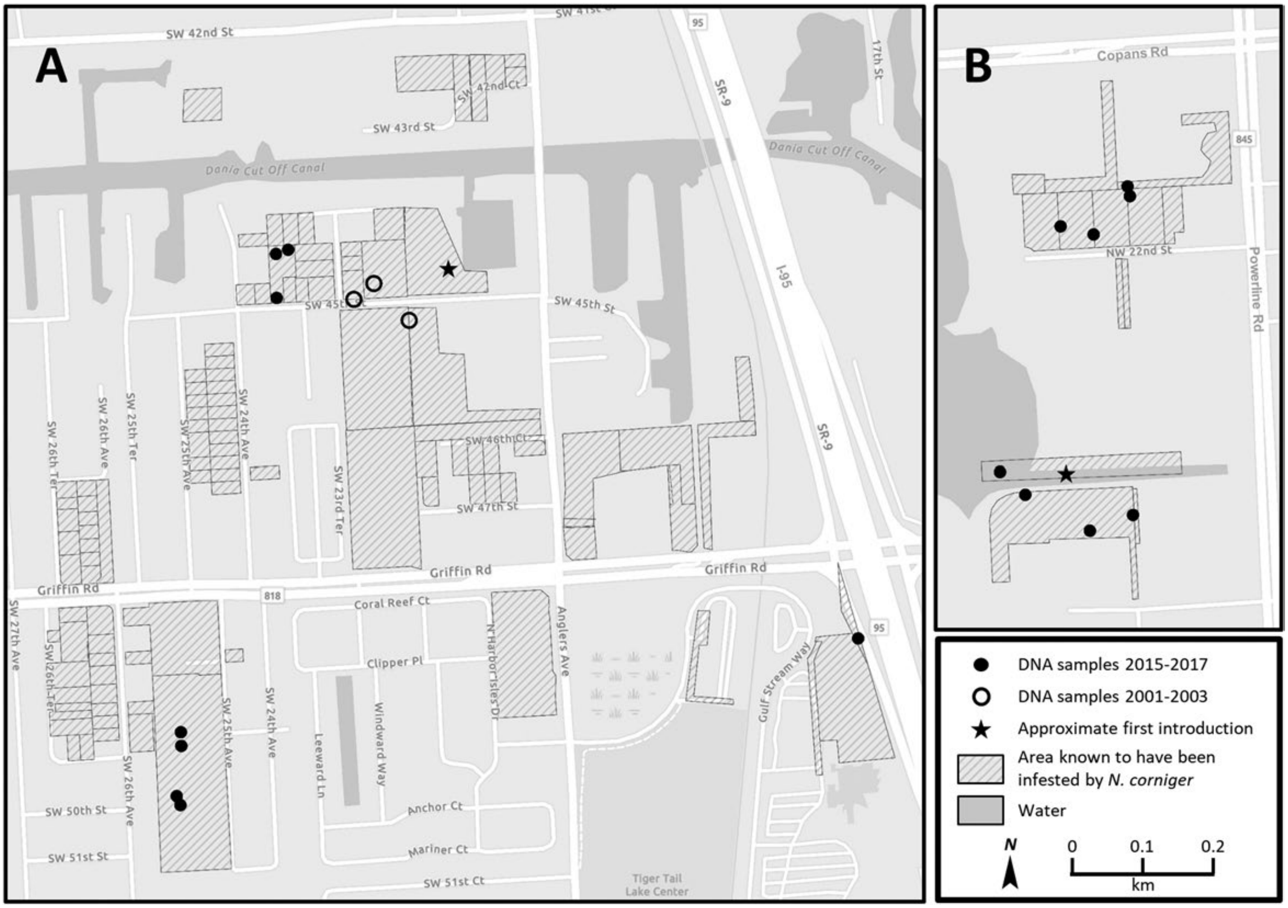

Fig. 1. Maps of known *Nasutitermes corniger* infestations in south Florida, shown at identical scale. (**A**) Dania Beach, FL properties with *N. corniger* discovered 2001–2018. (B) Pompano Beach, FL properties discovered 2016–2018. Sites of genetic samples analyzed in this article are mapped, as are locations of 'approximate first introduction' in each city inferred based on known data. Total number of properties known to have been infested is 105 in Dania Beach and 13 in Pompano Beach. Shapes of infested areas on maps do not necessarily follow property boundaries. The maximum linear distance across known infestations in Dania Beach is 1.30 km; maximum linear distance across known infestations in Pompano Beach is 0.77 km. Specimens collected in Dania Beach 2001–2003 courtesy of R. H. Scheffrahn. Maps created by the Florida Department of Agriculture and Consumer Services.

Table 1. Collection locations for Florida *Nasutitermes corniger* nests sampled for DNA analyses

Sample ID	City in Florida	Collection fate	Latitude	Longitude	Queens/Kings found during swift nest sampling in field (all primary, alate-derived reproductives)
1	Pompano Beach	2 Feb. 2016	26.25773	−80.15418	Eggs, brood; no reproductives found
2	Dania Beach	27 April 2015	26.06635	−80.17253	Eggs, brood; no reproductives found
3	Pompano Beach	5 April 2016	26.25383	−80.15437	Eggs, brood; no reproductives found
4	Pompano Beach	5 April 2016	26.25464	−80.15566	Eggs, brood; no reproductives found
5	Pompano Beach	5 April 2016	26.25402	−80.15373	Eggs, brood; no reproductives found
6	Pompano Beach	21 Jan. 2016	26.25835	−80.15366	Eggs, brood, 38 Queens, 49 Kings
7	Dania Beach	27 April 2015	26.06693	−80.17252	Eggs, brood, 21 Queens, 26 Kings
8	Dania Beach	27 April 2015	26.06698	−80.17234	Eggs, brood; no reproductives found
9	Pompano Beach	6 April 2016	26.25433	−80.1553	Eggs, brood; no reproductives found
10	Pompano Beach	2 Feb. 2016	26.25822	−80.15363	Eggs, brood; no reproductives found
11	Pompano Beach	2 Feb. 2016	26.25785	−80.15466	Eggs, brood, 18 Queens, 11 Kings
12	Dania Beach	22 April 2015	26.06163	−80.16417	Foraging center; workers and soldiers only
13	Dania Beach	19 April 2017	26.06067	−80.17413	Eggs, brood, 29 Queens, 2 Kings
14	Dania Beach	19 April 2017	26.06049	−80.17413	Eggs, brood, 49 Queens, 10 Kings
15	Dania Beach	20 April 2017	26.05983	−80.17422	Eggs, brood; no reproductives found
16	Dania Beach	20 April 2017	26.05971	−80.17417	Eggs, brood; no reproductives found
17	Dania Beach	23 April 2003	26.066	−80.1706	Unknown
18	Dania Beach	10 May 2001	26.0665	−80.1711	Unknown
19	Dania Beach	18 July 2001	26.0663	−80.1714	Unknown

natural areas (Fig. 1B). Modes of transit of potentially infested wood and plant materials (wooden pallets; downed tree trunks, branches, yard waste; railroad ties; discarded wooden furniture) are known between infested properties in the two locations (at least from Dania Beach to one of the infested sites in Pompano Beach, a waste disposal and recycling transfer station).

The objective of this study was to use genetic markers to determine whether one of the two invasive conehead termite populations in Broward County, FL, is the result of human transport of infested materials from the other location (termite 'hitchhiking' to disperse), or alternatively if coneheads in Dania Beach and Pompano Beach originated independently by introduction of separate colonies into Florida. Given concurrent timing of the infestations, transit opportunities for 'hitchhiking' between sites, and well-established ability and agility of *N. corniger* to travel within wood to successfully colonize new locations, our hypothesis was that DNA analysis would conclusively establish that the Dania Beach and Pompano Beach conehead termite populations are descendants of a single colony introduced into Florida.

Materials and Methods

Collection Sites and Samples

Specimens from samples 1–16 were collected in 2015–2017 from eight nests spanning three different areas of the Dania Beach and, similarly, eight nests from three areas of the Pompano Beach conehead termite infestation (Fig. 1A and B maps; Table 1 nest numbers, collection dates, and GPS coordinates). The termites were preserved in 100% EtOH, decanted, and refilled after 24 h, then refrigerated. The first series of samples 1–11, from both cities, was randomly numbered and sent, blind with regard to collection site, to the Vargo Lab for analysis. The second series of samples 12–16, representing broader geography across the infested zone in Dania Beach, was sent later.

Samples 1–11 and 13–16 came from nests containing numerous eggs and brood, indicating active reproduction. Often Queens and King(s) were collected (Table 1). Because samples were taken swiftly during the course of high priority termiticide treatment programs, nests were not completely or systematically dissected; thus, reproductives even if present would not always have been seen. Table 1 notes numbers of collected Queens and King(s); however, in many of those nests, we observed additional reproductives dart into the host wood. Recorded numbers of Queens and King(s) represent collected specimens, definitely not the full reproductive entourage. Sample 12 from Dania Beach was collected from a 'foraging center'—a nest-like, thin carton structure built by *N. corniger* but containing only workers and soldiers: no eggs, brood, reproductives, or distinguishing infrastructure within the carton galleries. Foraging centers are nodes of activity connected to thriving colonies, but in this case, control efforts had - as far as we could tell - killed the parent colony and left the Sample 12 bud isolated but active.

Following analysis of samples 1–16, collected in 2015–2017, we were pleased to receive specimens from Dania Beach preserved during the early years following identification of the *N. corniger* population (collected and provided, along with GPS coordinates, by Dr. Rudolf H. Scheffrahn). Those three samples, #17–19, were collected in 2001 (sample 18 collected on 10 May 2001, the date the invasive infestation was first reported; Scheffrahn et al. 2002) and 2003 from sites near the original discovery location (Fig. 1A, Table 1). Specimens were stored in 85% EtOH at room temperature.

DNA Extraction, Polymerase Chain Reaction, and Microsatellite Markers

Ten worker termites from each sample were genotyped at seven of the eight microsatellite loci identified as highly variable for *N. corniger* by Atkinson et al. (2007). DNA was extracted from individual termites using a modification of the PureGene (QIAGEN, Redwood City, CA) extraction method. We ran *Ncor2*, *Ncor3*, *Ncor4*, *Ncor5*, *Ncor6*, *Ncor7*, and *Ncor8* following the polymerase chain reaction (PCR) protocols of Atkinson et al. (2007). We fluorescently labeled the forward primers using dyes FAM, NED, PET, or VIC (Applied Biosystems, Foster City, CA) in the PCR, yielding labeled products which we detected using an ABI 3500 Genetic Analyzer (Applied Biosystems). We used the program Geneious ver. 9.1.6 (Newark, NJ) to score peaks and determine genotypes from the resulting chromatograms.

Table 2. Genotypes present in sampled nests of *Nasutitermes corniger* in Florida

	Nest no.																		
	Dania Beach											Pompano Beach							
	2015–2017								2001–2003			2015–2017							
Genotype	2	7	8	12	13	14	15	16	17	18	19	4	1	6	10	11	3	5	9
Ncor2																			
133/133				2	7	4	7	9	6	6	4	5	7	6	5	5	4	8	2
133/135				7	2	5	3	1	4	4	6	3	3	4	2	2	5	2	4
135/135	10	10	10		1	1						1			2				
Ncor3																			
158/158										1							1		
158/164										1						1	1		
158/170										1									
158/176									1		1		2		2	4	2		1
164/164				2												1	1	2	2
164/170	1	2	1							2	3								
164/176	4	2	1							3	1	5	3	2	5	3	2	2	4
170/170		1		1			3		1	1	1								
170/176	1	3	6						2		1								
176/176	4	2	2		2	3	2	3	5	1		4	5	7	2	1		6	2
Ncor4																			
156/156	9	9	10	9	10	8	10	10	4	10	8	9		8	3	2	8	10	10
156/189									3				2		3	7			
189/189									2				1	1	3	1	2		
Ncor5																			
169/169						2	1	2	1	1	4				1				8
169/181				3	4	7	6	2	1	5	3		6	4	2	3		2	
181/181	9	10	10	4	6	1	3		5	4	1	10	2	6	6	6	10	8	2
Ncor6																			
166/166				2		2	2	1	4	4	3			2	2	2		3	2
166/168				1	3	3	1	1	2					2			1	1	
166/193												1							
166/195				5	3	2	2	2	3	4	5	1	8	3	5	5	2	2	5
168/168														1			1	1	
168/193																			
168/195					2	1	3	2						1			4	1	1
193/193	9	9	9									3							
193/195				1		1			1	1		3							
195/195				1	2	1	2	3		1		2	1	1	2	3	2		
Ncor7																			
250/250				1	1	4	1	1				3		4			2	3	
250/252	1	3	5	4	4	3	4	2		1		5	1	5				5	1
250/256												1							
250/258									5	4							4	1	
252/252	8	6	4	4	2	2	5	1				1	8		9	10			1
252/256																			
252/258									3	2									6
256/256																			
256/258																			
258/258									1		10			1			3	1	1
Ncor8																			
116/116	10	9	10	10	10	10	10	10	10	9	7	10	10	10	9	10	9	10	10

For each nest, 10 individuals were analyzed, but in some cases, fewer than 10 genotypes were obtained. The order of sample numbers (columns) organized by city, collection dates, and geographic location amidst broader infestation delineated by lines between columns (see Fig. 1).

Data Analysis

We compared genotypes from the different sample sites manually to determine the numbers of alleles shared in common. We also used GenePop on the Web (Raymond and Rousset 1995; Rousset 2008) to test for genotypic differentiation among samples using a G test. Finally, we performed principal component analysis using the FactorMiner Package in R based on the genotypic array for each individual.

Results

Six of the seven microsatellite loci were polymorphic with between two and four alleles per locus (Table 2). Locus *Ncor8* was fixed for allele 116, despite having 34 alleles present in a population of 1,429 individuals from 140 colonies in Panama (Atkinson et al. 2007). In fact, the seven microsatellite loci genotyped in this study had only 2.7 alleles per locus compared with a native population in Panama where they had an average of 16.9 alleles per locus (Atkinson et al. 2007), although a much larger number of samples were analyzed in Panama. Importantly, there were no more than four alleles at a locus across all samples analyzed, a finding consistent with all of the invasive colonies being descendants of monogamous founding by a Queen and King.

In all samples except nest 1, workers had more than four genotypes present at a locus or had genotypic combinations not possible from single Queen and King parents (Table 2). The inference that many nests contained multiple reproductives (polygyny/polyandry) is consistent with field observations; of the four nests where reproductives were found, all had multiple Queens and Kings (Table 1).

Examination of alleles present in the 2001 and 2003 specimens (samples 17, 18, 19) lends further support to Florida's *N. corniger* populations arising from a single colony headed by a royal pair (1 Queen, 1 King). All alleles found in the more recent (2015–2017) samples from Dania Beach and Pompano Beach were present in the earlier Dania Beach samples, with the exception of allele 256 at *Ncor7*, found uniquely in a single individual in nest 4. This sole occurrence of allele 256 could be due to a mutation or the allele may be present in the population at a very low frequency. Nest 4 was also the only sample from Pompano Beach that contained allele 193 at *Ncor6*, an allele present in 7 of the 11 nests in Dania Beach. The 2015–2017 Dania Beach and Pompano Beach samples differed in alleles at *Ncor3*: allele 158 occurred only in Pompano Beach (in five of eight nests), whereas allele 170 occurred only in Dania Beach, where it was also found in five of eight nests. Allele 189 at *Ncor4* was present in the 2015–2017 Pompano Beach samples (in four of eight nests), but not in the 2015–2017 Dania Beach nests (Table 3).

For nearly all sampled nests, there were no more than three alleles per locus (Table 3). The exceptions were four alleles at a single locus in all three 2001–2003 Dania Beach samples: nests 18 and 19 at locus *Ncor3* and nest 17 at locus *Ncor6*. More recently (2015–2017) sampled nests 12 and 14 from Dania Beach also contained four alleles at *Ncor6*.

Results of the principal component analysis (Fig. 2) and tests of genotypic differentiation (Table 4) showed clear genetic differences among some nests but in most cases gave unclear results. Among the early year samples from Dania Beach, nest 17 differed significantly from 19 but not 18, whereas 18 did not differ significantly from either 17 or 19 (Fig. 2, Table 4). Among the 2015–2017 samples, nests 2, 7, and 8, all located at the northwest end of the Dania Beach infestation (SW 45th St.), were not significantly different from each other; they clustered into a single group (Fig. 2, Table 4) and differed significantly from all other samples. Those three samples were collected from nests on the same 0.25-ha parcel, within 84 m (275 ft) of one another. Given geographic proximity and identical allele representation at each locus across those three nests (Table 3), it is possible, if not likely, that the collections represent samples of satellite nests that were part of the same polydomous colony. More than 30

Table 3. List of allele copy numbers present in sampled nests of *Nasutitermes corniger* in Florida

	Nest no.																			
	Dania Beach, FL											Pompano Beach, FL								
	2015–2017								2001–2003			2015–2016								
Genotype	2	7	8	12	13	14	15	16	17	18	19	4	1	6	10	11	3	5	9	Overall
Ncor2																				
133	0	0	0	11	16	13	17	19	16	16	14	13	17	16	12	12	13	18	8	
135	20	20	20	7	4	7	3	1	4	4	6	5	3	4	6	2	5	2	4	
Ncor3																				
158	0	0	0	0	0	0	0	0	1	4	1	0	2	0	2	5	5	0	1	
164	5	4	2	4	0	0	0	0	0	6	4	5	3	2	5	6	5	6	8	
170	2	7	7	2	0	0	6	0	4	5	6	0	0	0	0	0	0	0	0	
176	13	9	11	0	4	6	4	6	13	5	3	13	15	16	11	9	4	14	9	
Ncor4																				
156	18	18	20	18	20	16	20	20	11	20	16	18	2	16	9	11	16	20	20	
189	0	0	0	0	0	0	0	0	7	0	0	0	4	2	9	9	4	0	0	
Ncor5																				
169	0	0	0	3	4	11	8	6	3	7	11	0	6	4	4	3	0	2	16	
181	18	20	20	11	16	9	12	2	11	13	5	20	10	16	14	15	20	18	4	
Ncor6																				
166	0	0	0	10	6	9	7	5	13	12	11	2	8	9	9	9	3	9	9	
168	0	0	0	1	5	4	4	3	2	0	0	0	0	5	0	0	7	4	1	
193	18	18	18	1	0	1	0	0	1	1	0	10	0	0	0	0	0	0	0	
195	0	0	0	8	9	6	9	10	4	7	5	8	10	6	9	11	10	3	6	
Ncor7																				
250	1	3	5	6	6	11	6	4	5	5	0	12	1	13	0	0	8	12	1	
252	17	15	13	12	8	7	14	4	3	3	0	7	17	5	18	20	0	5	9	
256	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
258	0	0	0	0	0	0	0	0	10	6	20	0	0	2	0	0	10	3	8	
Ncor8																				
116	20	18	20	20	20	20	20	20	20	18	14	20	20	20	18	20	18	20	20	
No. alleles	11	10	10	14	12	13	13	12	17	16	13	13	14	15	13	13	14	14	15	19
Mean. no. alleles	1.6	1.4	1.4	2.0	1.7	1.9	1.9	1.7	2.3	2.3	1.9	1.9	2.0	2.1	1.9	1.9	2.0	2.0	2.1	2.7

For each nest, 10 individuals were analyzed, but in some cases, fewer than 10 genotypes were obtained. The order of sample numbers (columns) organized by city, collection dates, and geographic location amidst broader infestation delineated by lines between columns (see Fig. 1).

nests were found in that general location, nearly all relatively small (≤30 cm diameter) and featuring thin, recently constructed carton. This geographic and genetic cluster may also represent colonies derived from alate swarm(s) that colonized the area, perhaps dispersing from a single parent colony or a group of very closely related colonies and landing in the same area due to wind conditions or other environmental influences.

In contrast, nests were typically not cleanly differentiated into groups based on genotypes (Fig. 2, Table 4). Some groups partially overlapped with others such that some nests belonged to two or more groups according to the test of genetic differentiation. For example, nests 13, 14, 15, and 16, located close together south of Griffin Road/Route 818 in Dania Beach, were not significantly different from each other. Three of those, nests 13, 14, and 16, did not differ significantly from Pompano Beach nests 1, 9, and 10, but nest 15 differed significantly from 9 to 10. Examining allele variants, all five Dania Beach samples collected south of Griffin Road, sample 12 near Rt. I-95 and nests 13–16 from a property approximately 1 km to the west, cluster tightly with respect to allele composition (although not identically as do samples 2, 7, 8). Unique among the five, sample 12 contains allele 164 at *Ncor3*.

Samples collected from proximate nest locations in Pompano Beach are somewhat more genetically diverse than the above clusters within Dania Beach (Fig. 2, Tables 2–4), although the grouping north of NW 22nd Street (samples 1, 6, 10, 11) shares nearly the same alleles at each locus, with the few exceptions potentially due to only 10 individuals analyzed from each nest.

Lack of distinct group membership exhibited by many samples is attributed to low genetic diversity overall and high relatedness of all nests from Dania Beach and Pompano Beach, each of which almost certainly descended from the same founder population. Due to low genetic variation in the populations and the fact that *N. corniger* colonies can contain many nests separated by as much as 120 linear meters (Levings and Adams 1984), in most cases, we cannot distinguish nests collected within that distance from one another as being part of a polydomous association or belonging to different colonies.

Discussion

DNA comparisons of invasive *N. corniger* from Dania Beach and Pompano Beach are entirely consistent with all conehead termites in both locations being descendants from a single 'source' colony introduced into south Florida. Our results indicate that one of the populations resulted from human transport, or 'hitchhiking', from the other, supporting our hypothesis. No more than four alleles were found at any locus, consistent with a single Queen and King, or multiple sibling reproductives descended from a monogamous pair, arriving in Florida. The only *N. corniger* population within the species' native range that has been similarly analyzed contained 9–34 alleles at the same microsatellite loci in a sample of 140 colonies from seven sites in Panama (Atkinson et al. 2007). Unfortunately, there is no comparative genetic database sampled from *N. corniger* populations across their broad range to determine the Neotropical location of origin of Florida's invasive *N. corniger*.

In all but one of the Florida nest samples (no. 1), genotypes of the workers rule out the possibility of a monogamous royal pair: the nests contain multiple Queens, multiple Kings, or both. This result corroborates recent field nest dissections showing that multiple alate-derived reproductives are nearly ubiquitous. The pervasiveness of polygyny/polyandry in the south Florida populations differs from monogamy as well as polygyny present in all other well-studied *N. corniger* locations (Dudley and Beaumont 1889a,b, 1890; Thorne 1982, 1984; Roisin and Pasteels 1986; Atkinson and Adams 1997; Adams and Atkinson 2007). Perhaps this is because in Florida so many colonies are young at the time of discovery/sampling, and all colonies are very closely related. Because all conehead termites in south Florida are descendants from a single colony, it is impossible to distinguish between colonies founded by multiple Queens and King(s) and nests that contain multiple reproductives due to replacement of parent(s), sibling dealates moving into a satellite nest, or reproductives joining a young or established colony through adoption/fusion.

Invasive *N. corniger* Introduction, Population Expansion, and Transport

Sampled *N. corniger* from Florida are too closely related to tell with certainty whether Dania Beach or Pompano Beach was the 'epicenter' site of the original, founding colony introduction. However, several lines of evidence, strengthened by these DNA analyses, support the hypothesis that conehead termites arrived first in Dania Beach and were subsequently transported from there to Pompano Beach.

Nasutitermes corniger colonies were first reported in Florida on 10 May 2001, near a marina in Dania Beach (Scheffrahn et al. 2002). Proximity of that discovery to the marina's docks and waterway access connecting to the Atlantic ocean supports Scheffrahn et al.'s (2002, 2014) conclusion that the original colony arrived at the marina, transported by an infested boat or its cargo traveling from somewhere in the Neotropics. The Dania Beach infestation thrived until the first termiticide treatments applied on 23 April 2003, nearly 2 yr after discovery (Scheffrahn et al. 2002, 2014). The comprehensive pesticide treatments, inspections, along with additional applications later in 2003 substantially suppressed the known Dania Beach population (Scheffrahn et al. 2004, 2014; Cabrera et al. 2004; Hickman 2006, Tonini et al. 2013).

Although our results confirm no more than four alleles at any one locus, and therefore very little overall genetic variation, analysis of the 2015–2017 samples (nests 1–16) identified four alleles found in Pompano Beach that were not present in the Dania Beach samples (*Ncor3*: Allele 158; *Ncor4*: Allele 189; *Ncor7*: Allele 258 and Allele 256, the latter found in a single individual in nest 4), and one allele identified in every sampled Dania Beach nest but not found in Pompano Beach (*Ncor3*: Allele 170).

To gain more perspective on genetic variation in Florida's *N. corniger* when the introduction was first discovered and 2 yr later, Dr. Scheffrahn's specimens collected in Dania Beach in 2001 (nests 18 and 19) and 2003 (nest 17) were helpful. Genetic diversity within single nests was highest in those 'early year' specimens. Across all 19 nest samples analyzed, four alleles were found at one locus only in the 2001 and 2003 Dania Beach (samples 17, 18, 19) and in Dania Beach nests 12 and 14 (Tables 2 and 3). Furthermore, three of the four alleles found in Pompano Beach samples collected in 2016, but absent in Dania Beach nests sampled 2015–2017 (*Ncor3*: allele 158; *Ncor4*: Allele 189, and *Ncor7*: Allele 258) were identified multiple times in the 2001 and 2003 Dania Beach samples. Only two unique-location alleles remain among the Florida samples analyzed: a single individual in Pompano Beach nest 4 contained *Ncor7*: 256, a variant not revealed in Dania Beach, and 8 of 11 nests sampled from Dania Beach, 2001–2017, contain *Ncor3*: 170, an allele absent among all sampled nests in Pompano Beach. A difficulty with using genotypes to infer the history of transport is that both the Dania Beach and Pompano Beach samples contained at least one allele not found at the other site. One might expect the source population to contain

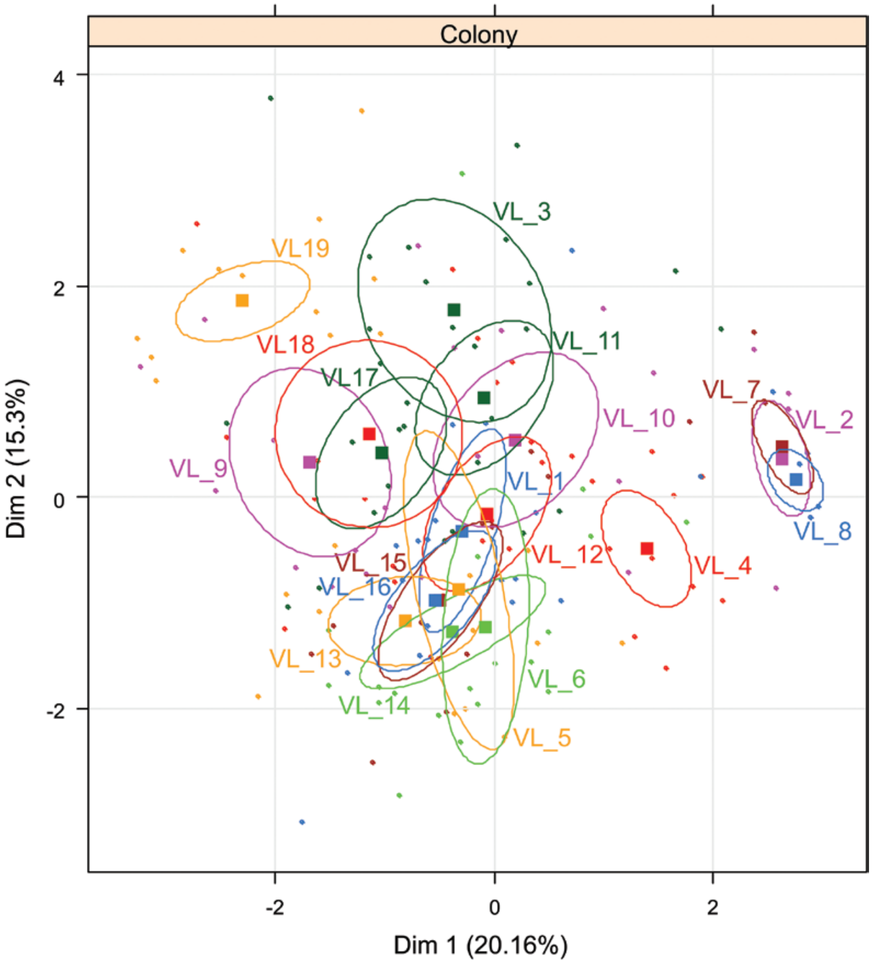

Fig. 2. Principal component analysis (PCA) of genetic differentiation based on microsatellite genotypes for individuals of *Nasutitermes corniger*. Samples from each nest are color coded, and the ovals show the 95% confidence interval around the centroid for each nest.

all alleles present at any descendant population, but alleles can be missing due to the modest number of samples or to historical losses driven by aggressive attempts at eradication.

The enigma of unique alleles in the Dania Beach and Pompano Beach 2015–2017 samples despite same single-colony ancestor of each infestation is enlightened by presence of all but one of those alleles identified in the Dania Beach samples collected in 2001 and 2003. All genetic data, as well as known history, size, and expanse of each of the two *N. corniger* populations in south Florida, are consistent with the following most parsimonious interpretation of events. As suggested by Scheffrahn et al. (2002, 2004, 2014; Cabrera et al. 2004; Tonini et al. 2013), the original introduction into Florida occurred when an *N. corniger* colony arrived on a boat that docked at a marina in Dania Beach. Human transport of this invasive termite to Pompano Beach occurred relatively early, before the Dania Beach *N. corniger* population was reduced following extensive termiticide treatments that commenced on 23 April 2003 (Scheffrahn et al. 2004, 2014), days after sample 17 was collected. The population size reduction resulting from pesticide applications created a genetic 'bottleneck', and loss of alleles simply due to chance, in the relatively small population of hidden young colonies that survived in Dania Beach.

In addition to allele distribution patterns in space and time, four additional, compelling lines of evidence support the scenario of transport of conehead termites from Dania Beach to Pompano Beach prior to 23 April 2003.

First, the Pompano Beach infestation had been there for many years before it was discovered in January 2016 with over 100 nests extending a maximum linear distance of nearly 0.7 km. Based on current evidence, 1951 Powerline Road is the most likely 'ground zero' site of first introduction of conehead termites to Pompano Beach (near nest sample 4; Fig. 1B). The property served as a waste disposal and recycling facility since the mid-1990s. Nests found along the wooded canal bank on the south side of the property were the largest nests seen thus far in Pompano Beach, several ≥75 cm diameter, with hard, densely reinforced carton typical of older nests (Dudley and Beaumont 1889a, Thorne 1980; all vegetation, including large trees along the south bank of that property, were eliminated in 2018 due to site development). Thankfully most of the large nests were dead at the time of discovery, with the exception of a young (thin carton) nest on the west end of trees along the canal—sample4. Significantly, no. 4 was the only genetic sample from Pompano Beach containing allele *Ncor6*: 193 (otherwise found only in Dania Beach) and *Ncor7*: 256, present in a single individual of the 10 sampled, and unique among all other Florida samples. The distinctive allele composition of individuals within sample 4 is consistent with that colony being in the same site as the ancestor that colonized Pompano.

Second, transport opportunities from Dania Beach to the municipal waste receiving and processing property in Pompano Beach were common. A truck carrying wood, plant debris, or other cellulose products infested with a visible or hidden *N. corniger* colony,

Table 4. Pairwise comparisons of genetic differentiation

	Nest No.																	
	Dania Beach, FL										Pompano Beach, FL							
	2015-2017							2001-2003			2015-2016							
Nest No.	**7**	**8**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**4**	**1**	**6**	**10**	**11**	**3**	**5**	**9**
2	□	□	■	■	■	■	■	□	□	■	■	■	■	■	■	■	■	■
7		□	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
8			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
12				□	□	□	□	■	□	■	■	□	□	□	■	■	□	■
13					□	□	□	■	■	■	■	□	□	■	■	■	□	■
14						□	□	□	□	■	■	□	□	■	■	■	□	■
15							□	■	□	■	■	□	□	■	■	■	□	■
16								■	□	■	■	□	□	■	■	■	□	■
17									□	■	■	■	□	■	■	□	□	■
18										□	■	■	□	■	■	□	□	■
19											■	■	■	■	■	■	■	□
4												■	□	■	■	■	□	■
1													□	□	□	■	■	□
6														■	■	□	□	□
10															□	■	■	□
11																■	■	■
3																	□	■
5																		□

Dark gray cells indicate pairs of nests that were significantly differentiated according to an exact test of genotypic differentiation ($P < 0.05$ with Bonferroni correction); light gray cells indicate pairs that were not significantly differentiated.

or developing or mature alates from Dania Beach, could have 'sparked' the Pompano Beach infestation if dumped materials were left undisturbed for even one night. Conehead termites, including mature Queens and Kings, are agile colonists, often exploring and relocating overnight. The heavily wooded area along the south border of the property was replete with food and harborage, adjacent to year-round water, largely isolated from human activity, and within termite walking distance from trucked debris dump sites. *Nasutitermes corniger* could have grown, reproduced, and dispersed from that 'epicenter' to expand across the broader Pompano Beach infested zone. Although refuse truck traffic from Dania Beach to the center of the Pompano Beach *N. corniger* population was frequent, we are unaware of any municipal or commercial reason for reverse transport of cellulose materials from any of the known infested properties in Pompano Beach to the earliest 'epicenter' (marina area) of the Dania Beach *N. corniger* invasion.

Third, in contrast to the Dania Beach marina, the area infested by *N. corniger* in Pompano Beach is landlocked. If Pompano Beach was the site of first establishment of conehead termites in Florida, the historical account must address transport of the original colony from where it entered the United States by sea or air, then moved to the infested area in inland Pompano Beach. There are indeed possible events, such as an infested boat dismantled somewhere along the coast and trucked to the disposal site at 1951 Powerline Road in Pompano Beach. However, had such an infestation been known, causing damage sufficiently severe to motivate destruction of the boat, the responsible standard protocol would have been fumigation of the vessel before it was moved.

Finally, the maximum linear distance spanning the infestation as well as total infested area in Dania Beach greatly exceeds geographic spread in Pompano Beach, as does the number of separate 'disjunct' infested zones that would have had to be colonized by alate flights or transport of infested materials (rather than termites walking to expand their spread; Fig. 1). The larger, broader 'footprint' of the invasive population in Dania Beach suggests, although not irrefutably, that *N. corniger* has been established and expanding for a longer time period in Dania Beach relative to Pompano Beach. This piece of evidence would be equivocal to interpret on its own as perhaps, for reasons unknown to us, Dania Beach is a more hospitable habitat for this species. However, invasive population size and spread of the same species in similar environments typically increase with time since establishment, so in combination with the other lines of evidence and reasoning, known distribution of conehead termites in the two cities supports first colonization in Dania Beach.

Other explanations for the ontogeny of the Dania Beach and Pompano Beach *N. corniger* infestations are possible, but in our view, the plausibility of alternative chronologies is slim. Regardless of which location was the site of establishment of the first *N. corniger* colony introduced into Florida, the two relatively small populations have been isolated for quite a while, likely longer than a decade before samples 1–16 were collected, although we will never know exactly. Thus with all information consistent with both populations derived from the same single colony introduction but each remaining small and reproductively isolated for quite a while, genetic drift could readily explain allele distribution and frequency across samples.

How Long Has *N. corniger* Been in Florida?

DNA data do not directly inform the time of *N. corniger*'s invasion into Dania Beach or Pompano Beach, Florida. Scheffrahn et al.

(2002 (and Cabrera et al. 2004, Hickman 2006, Tonini et al. 2013, Scheffrahn et al. 2014) estimate that the Dania Beach infestation reached shore via an alate flight from an infested boat or cargo 8–10 yr prior to discovery near a marina on 10 May 2001. As far as is known, *N. corniger* alates fly from the surface of their nest or from substrates (typically vegetation or openings in soil) close to the nest (Barreto 1923, Thorne 2013, 2015). Flight directly from the boat would require a carton nest amidst a network of foraging tunnels. An active nest and associated tunnels may have evaded notice by people in and around the boat by remaining relatively hidden under the deck or within cargo, or perhaps signs of the infestation were ignored as harmless 'ants'.

Alternatively or in addition to alates flying from the infested boat that carried *N. corniger* to Florida from the Neotropics, the entire colony, including reproductives, may have relocated to land near the Dania Beach marina by walking, a known capability of this termite (Emerson 1929, Thorne and Haverty 2000). Possibly the colony moved over mooring ropes from the boat onto docks then terrain. More likely, infested items were offloaded onto land or the vessel itself may have been lifted to dry dock for maintenance, storage, trailer transport, etc. If materials harboring the invasive termites had access to or remained on land even for one night, the swift, opportunistic *N. corniger*, including reproductives, could relocate enough of their colony to succeed. For the 'relocation by walking' scenario the original colony may have arrived in a mature nest, or still in its young 'hidden phase', cryptically riding in the boat or its cargo prior to building foraging tunnels or a carton nest. Regardless of its stage of development, movement of a colony 'endows' the reproductives pair with a support staff of workers and soldiers, possibly even developing alate nymphs. Workers often transport eggs and/or brood in their mouths. Colonizing a new habitat by relocating an already established family speeds population growth rate, acquisition of food and water resources, and defensive capabilities, thereby increasing the probability of success compared with the vulnerabilities and time required for alates to initiate colonies that survive and thrive.

Once they invaded Florida, in a semitropical environment replete with resources and lacking their native range competitors, predators, and pathogens (although Florida biota exploit some of those roles), all indications suggest that the invasive *N. corniger* population grew, spread (naturally and by transport), and reproduced quickly until the aggressive mitigation program launched in 2003. Although the most parsimonious interpretation from the DNA data is that the original colony igniting the Florida infestations was headed by a single Queen and King, polygyny/polyandry has since proven nearly ubiquitous in the invasive populations (Thorne 2013, 2015; also see Cabrera et al. 2004). The genetic results are also consistent with polydomy, a life-history option known in its native range (Dietz and Snyder 1923; Thorne 1982, 1984; Levings and Adams 1984; Roisin and Pasteels 1986; Adams and Levings 1987; Clarke 1993; Atkinson and Adams 1997; Thorne and Haverty 2000; Adams and Atkinson 2007), occurring in Florida (e.g., Area 5). Multiple egg-laying Queens and satellite nest expansions boost population growth and reproductive flexibility of this invasive species, escalating its risk as a consequential invader.

There is no way to definitively reconstruct when, where, or how the *N. corniger* colony that sparked the Dania Beach and Pompano Beach populations first arrived in Florida. Earliest colonization on land could have been more recent than 1993 or earlier than 1991 (1993 and 1991 being 8–10 yr before discovery in May 2001, the invasion timeline suggested by Scheffrahn et al.). Another critical question is how early and for how many years did *N. corniger* alates fly in south Florida? Flights in Dania Beach in May 2001 and 2012 were documented (Scheffrahn et al. 2002; R. Hickman, personal communication and video); they likely occurred at least in 2002 also. Regardless of invasion chronology and years of maturity, this species is an adept, agile hitchhiker and colonist. A crucial priority and first step if, more likely when, *N. corniger* establishes again in the United States, all nests should be destroyed immediately (even without pesticide treatment as described below) to minimize risk of dispersal flights.

Containment: Immediate and Sustained Actions to Prevent Dispersal Flights and Minimize Risk of Transport

The now proven ability of *N. corniger* to establish breeding populations in the United States, cause extensive property and landscape destruction, and the confirmed risk of human transport spreading this invasive termite underscore the need for quick operational actions the next time *N. corniger* is discovered in Florida or beyond (Thorne 2013, 2015).

Even if regulatory constraints (such as decisions regarding pesticide labels, applications for special circumstance exemptions or local permissions), funding, or any other logistics delay termiticide application to a newly discovered conehead infestation, the immediate action of manual destruction of nests is an effective, simple, quick first step for rapid mitigation of colonies. Quickly extracting a nest from its perch, laying it on the ground, and crushing the carton (using as basic a process as stomping on the nest) will kill tens to hundreds of thousands of termites, usually including the reproductive center of the colony (Queen(s), King(s), eggs, nursery brood) as well as developing or mature alates. Physical destruction of a nest, even without pesticide application, has immense impact in suppressing a colony and preventing alate flights, which is a first principle of containment. The ability to find and destroy the reproductive 'heart' (i.e., nest) of a conehead colony enables a targeted, rapid control approach unavailable in most insect invasive species situations (Thorne 2013, 2015; Alspach and Thorne 2015). Pesticide treatment of residual activity should occur as soon as possible to prevent resprouting by colony members left behind (Thorne and Haverty 2000), but immediate growth and dispersal disruption via nest destruction is an essential step to be taken as immediately as possible following discovery of a new infestation location, always, but even more critically within weeks or months of alate flight season at the beginning of the wet season (Dudley and Beaumont 1889a; Barreto 1923; Dietz and Snyder 1923; Becker 1953; Thorne 1983, 2015; Clarke 1993). If alate flights are prevented, this termite can expand its range only by walking or transport.

Nearly all *N. corniger* nests in Florida have been small to modest sized (nearly all 40 cm or less in diameter) compared with sizes achieved by older nests in their native range (Dietz and Snyder 1923, Wolcott 1948, Thorne 1984, Clarke 1993). Almost all Florida nests have had carton thin and fragile enough to crush or smash with little effort and requiring no special equipment. This is typical early in an infestation, when nests are relatively young and not yet densely reinforced. Another huge advantage in addressing the Florida infestations has been that, to date, we have discovered no nests above 20 ft high (off the ground), with most built much lower or on the ground. Thus nests in Florida have been accessible in contrast with those constructed high in forest canopies as occurs in much of the species' range (Emerson 1938, Thorne 2013).

In addition to delivering severe injury and disruption to a colony, nest removal and destruction before applying liquid termiticide is a best practice. *Nasutitermes corniger* lives in tropical rainforests (among other habitats) so must cope with heavy, sustained rain. Exterior nest carton absorbs liquid, but to prevent persistent drenching, interior, denser carton can stay dry even after a substantial tropical storm (Dietz and Snyder 1923, Thorne et al. 1996). Thus liquid pesticide does not readily or uniformly penetrate mature nest carton and is more appropriately and efficiently applied to address residual activity following removal and destruction of conehead nests (Thorne 2013, 2015).

A critical aspect of the conehead containment program is preventing the termite from 'hitchhiking' via human transport to colonize another part of Florida (or beyond), as this study demonstrates occurred between Dania Beach and Pompano Beach. That possibility must be foiled by preventing relocation of trees and shrubs, cut branches and wood debris, railroad ties, and wooden furniture or pallets out of the infested and surrounding locations. Young hidden colonies may lurk concealed within even small pieces of wood. The risk of coneheads expanding their range can be substantially constrained by not moving wood items out of currently infested and neighboring areas (Thorne 2013, 2015). People in Florida and other southern states also need to immediately recognize, report, and treat any new infestations. Florida's Department of Agriculture and Consumer Services, leader of the invasive conehead termite containment, control, and eradication program, continues to enhance these operational priorities in collaboration with local constituencies. Improved mechanisms to intercept potential *N. corniger* introductions at ports and marinas are also a priority.

How Far Could Coneheads Spread?

Since 2012, conehead termites have been discovered infesting across an area covering less than 2 km^2 of residential, commercial, and natural landscape areas in Dania Beach and Pompano Beach, Florida. Expanse and impacts of the invasion, including higher risk of transport to additional locations within or beyond Florida, would have been far worse if not for aggressive mitigation actions in 2003–2010 and since 2012 (Cabrera et al. 2004; Scheffrahn et al. 2004, 2014; Tonini et al. 2013; Thorne 2013, 2015; Alspach and Thorne 2015). Continued inspections, treatments, interventions, and outreach now can halt the species before it spreads further and becomes irreversibly established in the United States as a powerfully damaging, expensive, obnoxious, and permanent pest to agriculture, landscapes, natural areas, and structures.

Because the breeding population of conehead termites in the United States has been known only since 2001 within limited areas, we must rely on literature and experiences abroad to inform projections regarding potential range of this invasive species. *Nasutitermes corniger* is one of the most widely distributed, adaptable, termite species known, capable of exploiting a wide variety of food sources and thriving in diverse habitats including dry grassy savannas, mixed second growth, tropical rain forests, cultivated trees and crops, and urban landscapes and structures. In contrast to termite-resistant cement block and metal roof construction practices prevalent in *N. corniger's* native range, buildings in Florida and the southern United States often involve substantial wood in framing and roofs, and are heated when temperatures fall (Thorne 2013, 2015). *Nasutitermes corniger*'s dark nests can also retain heat (Emerson 1938, Cabrera et al. 2004). Year-round water availability from standing sources or sprinkler/irrigation systems also differentiate many areas of the United States from true 'dry season' habitats in the tropics, rendering sites hospitable to coneheads that might otherwise be stressed by droughts, also potentially enabling tunnel and nest expansion year-round (Thorne and Haverty 2000, Thorne 2013).

Even if the United States does not offer optimal habitat for *N. corniger*, potentially resulting in slower growth rate, adjusted seasonality, or modified behaviors and life histories of conehead colonies in the United States compared with those in the Neotropics, this adaptable species has proven that it can flourish in south Florida. Most invasive species surprise scientists who attempt to forecast their potential distribution by colonizing and succeeding more broadly than projected. There is no way to accurately predict how far *N. corniger* could spread further in Florida or the United States; hopefully, conehead termites will be stopped before we find out (Thorne 2013, 2015).

Containment/Control/Eradication Program Status

Recent inspections document that of all Dania Beach and Pompano Beach properties known to have ever been infested with *N. corniger* since 2001, live coneheads were found on only one within the past year. The dramatic suppression of the invasive termite demonstrates that inspection and treatment protocols are effective. However, adaptability of this pest, the fact that incipient colonies can remain hidden within wood for years before building visible foraging tunnels or nests, and the risk of human transport motivate continued diligence to mitigate against conehead population resurgence or spread (Thorne 2013, 2015; Alspach and Thorne 2015).

Coneheads are remarkably flexible in adapting to a wide variety of habitats, nest sites, and acceptable foods, so they should not be underestimated. They are a formidable pest, with potential economic and ecological impacts influencing nearly every constituency—including property owners, growers, businesses, institutions, and natural areas such as the Everglades.

The goal of eradicating *N. corniger* in Florida is challenging, but based on known facts and analyses, we are confident that extermination is possible and absolutely worth intensive efforts and investments toward that objective. There is urgency to act now because if conehead termites spread further and become irreversibly established in the United States, they could become damaging, expensive, permanent pests.

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