

ORIGINAL ARTICLE

Integrated morphological, CO1 and distributional analysis confirms many species in the *Iridomyrmex anceps* (Roger) complex of ants

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Abstract *Iridomyrmex* Mayr is Australia's most ecologically dominant ant genus and has been estimated to contain at least 350 species. However, a recent taxonomic revision of the genus recognized only 79 species. Such contrasting views have major implications for an understanding of not only the diversity of such an important genus, but also its biogeography and evolutionary history. The taxonomic revision considered *I. anceps* (Roger) to represent a single species, despite its marked morphological variation and range from Australia to India and China. Subsequently, a preliminary morphological analysis recognized six species within the complex. Here we describe results from an integrated morphological, CO1 and distributional analysis that assesses if the six morphologically based taxa are differentiated genetically, and if there are additional species in the complex that are yet to be recognized. We found the morphological differentiation within the complex to be matched by extensive genetic divergence, and that matched morphological and genetic differentiation frequently occurs in sympatry. We recognize up to 18 species among our sequenced specimens from the complex, with their centre of diversity occurring in Queensland. Our findings are consistent with results showing that other *Iridomyrmex* species recognized in the taxonomic revision represent several to many actual species. The recognition of only 79 species of *Iridomyrmex* does not reflect true diversity within this ecologically dominant genus, nor the complexity of its biogeography and evolutionary history.

Key words Australia, cryptic species, Indo-Pacific, South-East Asia, unrecognized diversity.

1 Introduction

Iridomyrmex Mayr is a highly diverse and exceptionally abundant ant genus that dominates the Australian environment to an extent that is unparalleled by ant genera on other continents (Greenslade, 1976; Andersen 1995, 2003). Despite such prominence, until recently the genus had received little taxonomic attention and most species were undescribed. Its species-level taxonomy is extremely challenging because morphology is highly generalized and conservative throughout, but a systematic overview of the genus estimated that it contained at least 350 species (Andersen, 2007). However, a subsequent taxonomic revision came to a quite different conclusion, recognizing only 79 species, most having very wide distributions, often occurring throughout almost all inland Australia (Heterick & Shattuck, 2011). Such contrasting views have major implications for an understanding of not only the diversity of such an important genus, but also its biogeography and

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evolutionary history.

The *Iridomyrmex anceps* (Roger) complex (Hymenoptera: Formicidae: Dolichoderinae; Fig. 1) is by far the most widespread of the genus. It is a dominant component of the savanna ant fauna throughout the Australian monsoonal tropics (Andersen, 2000), and it extends into the South Pacific region and through South-East Asia to India and southern China (Heterick & Shattuck, 2011). The taxa within the complex show considerable morphological variation relating to body size, head shape, relative scape length, and pilosity (Hoffmann *et al.*, 2011). The complex includes a substantial number of named taxa. In addition to *I. anceps sensu stricto* (described from Malaysia), three subspecies have been described: *I. anceps ignobilis* Mann (Fiji), *I. anceps sikkimensis* Forel (India) and *I. anceps watsonii* Forel (Myanmar). In addition, *I. discoidalis* (Donisthorpe) (New Guinea), *I. excisus* Mayr (Indonesia), *I. meinerti* Forel (New Guinea), *I. gracilis papuana* Emery (New Guinea), *I. bicknelli formosae* Forel (China: Taiwan) and *I. rufoniger metallescens* Emery (Indonesia) all belong to the complex (Heterick & Shattuck, 2011). However, in the taxonomic revision (Heterick & Shattuck, 2011) the *I. anceps* complex was described as a single species and all included taxa were synonymized under *I. anceps*. This was done without substantive analysis of morphological variation or the provision of genetic evidence.

Following the taxonomic revision, a preliminary morphological analysis of the *I. anceps* complex (Hoffmann *et al.*, 2011) distinguished six species (referred to as sp. A–F), including two each from Australia and East Timor, and others from Indonesia and China. Here we describe results from an integrated morphological, CO1 and distributional analysis of the complex, based on a very extensive collection of specimens from throughout its range, including representatives of all six species recognized by Hoffmann *et al.* (2011). We specifically address two questions. First, to what extent are the six morphologically based taxa recognized by Hoffmann *et al.* (2011) also differentiated genetically? Second, are there additional species that are yet to be recognized? Our analysis has important implications for not only an understanding of diversity and biogeography, but also for biosecurity given that the *I. anceps* complex occurs in many countries throughout the Indo-Pacific region. If it is a single species then any incursions of populations from elsewhere in its range would not trigger a biosecurity response. If, however, such populations are different species then they represent colonization by a potentially significant invader.



Figure 1. Head (A) and lateral (B) views of a typical member of the *Iridomyrmex anceps* complex (sp. A; specimen IRIDO217-16). Scale bars = 1 mm.

2 Materials and Methods

Our analysis was based on pinned specimens held in the ant collection located at CSIRO's Tropical Ecosystems Research Centre (TERC) in Darwin. TERC holds by far the largest collection of the *I. anceps* complex, with >3,000 pinned specimens. From 2016 to 2018, CO1 sequences were obtained from 82 specimens from the complex, including representatives of each of the six species recognized by Hoffmann *et al.* (2011). CO1 sequences were also obtained from a species of the related *I. minor* Forel complex as an outgroup.

DNA was extracted from foreleg tissue of all specimens. DNA extraction and sequencing were conducted through the

Barcode of Life Data (BOLD) System (for extraction details, see <http://ccdb.ca/resources>). Each sequenced specimen was assigned a unique BOLD identification code that combines the batch within which it was processed and its number within the batch (e.g. IRIDX088, IRIDO230, RHYIR079).

DNA sequences were checked and edited in MEGA 7 (Kumar *et al.*, 2016). Sequences were aligned using the UPGMB clustering method in MUSCLE (Edgar, 2004), and translated into (invertebrate) proteins to check for stop codons and nuclear paralogs. The aligned sequences were trimmed accordingly, resulting in 657 base pairs.

To explore overall CO1 diversity in the samples, the mean genetic pairwise distances between sequences were calculated in MEGA 7. This was done using the Kimura-2 parameter (K2P) model (Kimura, 1980) to ensure that results were comparable with those of most other studies of insect DNA barcoding, with 500 bootstrap replicates and the ‘pairwise deletion’ option of missing data (to remove all ambiguous positions for each sequences pair). Analysis involved all nucleotide sequences, excluding those of the outgroup. Codon positions included were 1st+2nd+3rd+Noncoding.

There is no specific level of CO1 divergence that can be used to define a species, but the level of CO1 variation within ant species is typically 1–3% (Smith *et al.*, 2005). However, some ant species can show substantially higher variation (e.g. Wild, 2009), and in other cases two clear species can show no CO1 differentiation (e.g. Schär *et al.*, 2018). We also note that some ant species from other genera are known to have workers that are virtually identical morphologically, and they can only be separated by detailed morphometric analysis or through reproductive castes (Wagner *et al.*, 2018). When delimiting species we focused on morphological differentiation between sister (i.e. most closely related) clades, considering all available samples from the same collections as sequenced specimens.

Tree inference by maximum likelihood was conducted through the IQTREE web server (<http://iqtree.cibiv.univie.ac.at/>; (Trifinopoulos *et al.*, 2016) using ultrafast bootstrap approximation (Minh *et al.*, 2013). IQTREE has been shown to be a robust algorithm for tree inference that compares favourably with other methods (Nguyen *et al.*, 2014). Model selection was inferred using a 3-codon partition file and linked branch lengths with the AutoMRE ‘ModelFinder’ function to find the best-fit model for tree inference (Chernomor *et al.*, 2016). Trees were viewed and edited in FigTree v1.4.3 (Rambaut, 2007) and annotated using Photoshop CS5.1®.

The following abbreviations are used in the main text:

WA— Western Australia;

NT—Northern Territory;

Qld—Queensland;

PNG—Papua New Guinea.

3 Results

The CO1 tree shows four primary clades, the first with six putative species and the second with eight, both with >70% bootstrap support (Fig. 2). The remaining sequenced specimens fall into primary clades of three and one putative species, respectively, both with >90% bootstrap support. These four primary clades show 6.7–8.7% inter-clade divergence. The six morphologically-based species (spp. A–F) of the *I. anceps* complex recognized by Hoffmann *et al.* (2011) are distributed across the four primary clades (Fig. 2). The first primary clade includes sp. A (Fig. 1; occurring throughout monsoonal Australia) and sp. F (from China), with 7.2% mean CO1 divergence between them; no specimens from SE Asia occur in this clade. The second primary clade contains sp. B (from far North Qld), the third primary clade sp. C and sp. D (both from Timor), and the final primary clade sp. E (Indonesia and Singapore).

In addition to the six morphologically based species outlined in Hoffmann *et al.* (2011), we recognize 12 other species among the sequenced specimens (spp. G–R, Fig. 2). The total of 18 species have a mean CO1 divergence among them of 6.0%. The first primary clade contains four additionally recognized Australian species (spp. G–J). Species I (from North Qld) and sp. J (Kimberley region of WA) are shown as forming a separate subclade, with mean divergence of 3.8% from sp. A. Compared with sp. A, they both have markedly shorter antennal scapes (Fig. 3A), and the head is more rectangular, broadest medially rather than posteriorly (Fig. 4E). There are no obvious morphological differences between sp. I and sp. J, but their CO1 divergence (2.4%) combined with their highly disjunct distributions (>1,000 km geographic separation; Fig. 5) suggests that they represent separate species. Species H (2.5% mean CO1 divergence from sp. A) is a relatively small (total length *ca.* 2 mm) and stout species with a more asymmetrical pronotal profile (Fig. 4A), and it also has shorter scapes (Fig. 3A). It can be further distinguished from sp. A (Fig. 1) by its gastric pubescence, which is denser and covers the lateral surfaces rather than occurring primarily on the dorsum. There is no obvious morphological differentiation between sp. G (Figs 3, 4D) and sp. A, and their CO1 divergence is only 1.8%; however, this divergence is maintained in sympatry throughout their

ranges across all monsoonal Australia (Fig. 5A), which suggests that they represent separate species.

The second primary clade comprises two subclades, one containing sp. B and two additional Australian species: sp. K (also from Qld) and sp. L (from the NT; Fig. 4B). The latter (3.0% mean CO1 divergence from sp. B) is a very small, less-gracile species, with shorter scapes and a more-prominently rounded promesonotum (Figs 3B, 4B). Species K has only 1.4% mean CO1 divergence from sp. B, but it has longer scapes (Fig. 3B) and its gastric pubescence is far longer and coarser (Figs 6A–B). The other subclade includes two Australian species, sp. M (from Qld) and sp. N (Qld and the NT), with 3.0% mean CO1 divergence between them. The former has a broadly rounded head with sparse setae anterolaterally (Fig. 4F), long and coarse gastric pubescence that covers the lateral as well as dorsal surfaces, and the gaster has numerous long, erect setae (Fig. 6C). The latter is smaller (Fig. 3B), has a rectangular head without lateral setae and with a convex occipital margin (Fig. 4G), the gastric pubescence is finer and absent laterally, and erect setae are mostly absent (Fig. 6D). The remaining specimens in the second primary clade show <1% CO1 differentiation among them, but they range from Qld to the Philippines and morphological variation is suggestive of three species (spp. O–Q). In sp. O and sp. P the hind femora have scattered setae throughout, whereas in sp. Q the setae are restricted to near the base or are absent, as is typical of members of the *I. anceps* complex. The former also have denser and longer pilosity on the mesosoma and head. Specimens of sp. P are larger than those of sp. O (total length 3.5 *cf.* 2.5 mm), have very broad heads, and have much longer and denser gastric pubescence (Figs 6E–F).

The third primary clade contains the two Timorese species (sp. C and sp. D), along with an additional species (sp. R) from WA. The latter has 6.2% and 5.9% mean CO1 divergence from sp. C and sp. D, respectively, and it has a broadly rounded head with sparse setae laterally (Fig. 4H). The two Timorese species were separated by Hoffmann *et al.* (2011) on the basis of head pilosity. However, glabrous and hairy specimens are interspersed on the CO1 tree, and there is only 1.0%

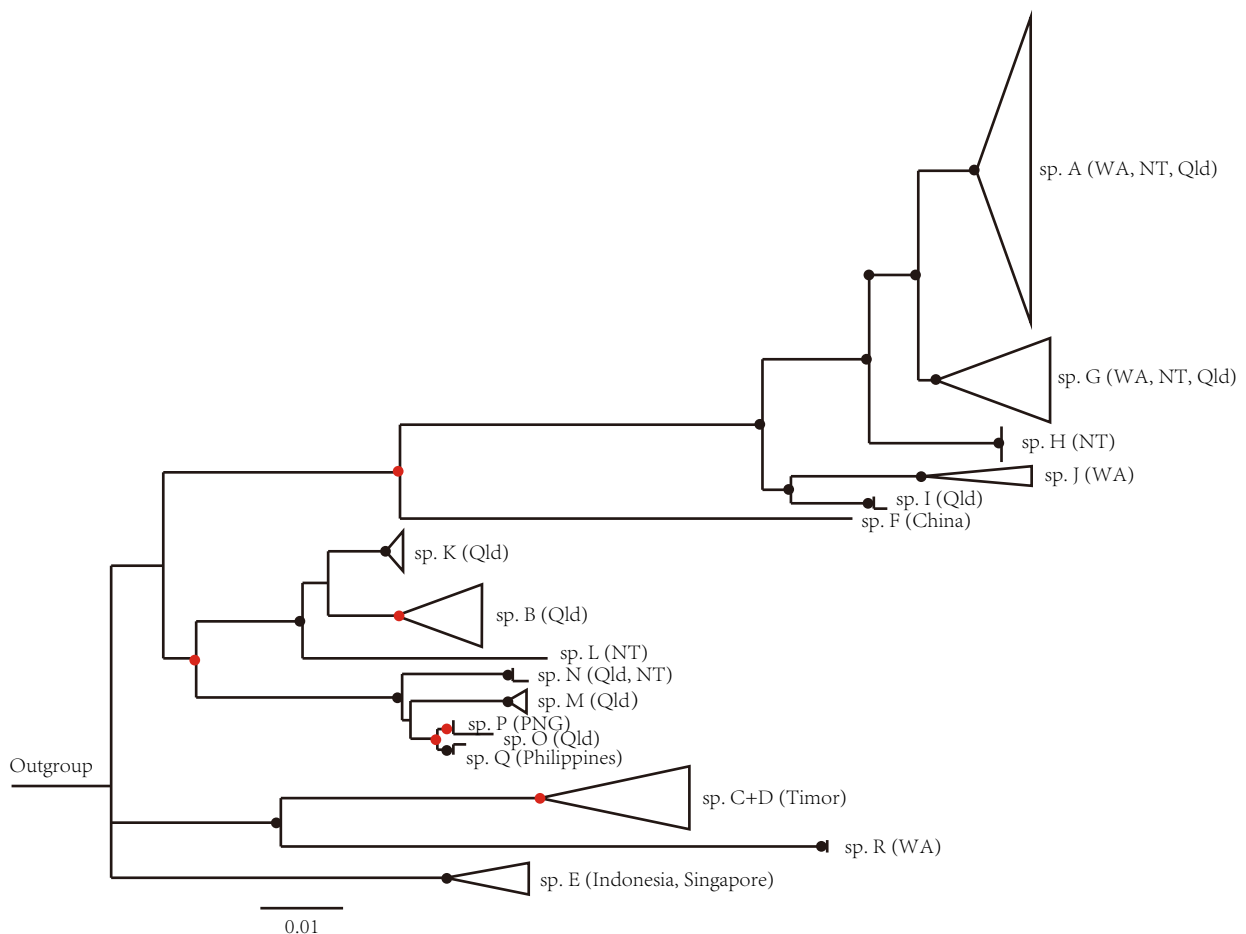


Figure 2. Summary CO1 tree of the 82 sequenced specimens of *Iridomyrmex anceps*. Maximum-likelihood phylogeny inferred using IQ-TREE. Black and red circles indicate bootstrap support values ≥ 90 and ≥ 70 , respectively. The full CO1 tree is shown in Supplementary Figure 1. Abbreviations: WA—Western Australia, NT—Northern Territory, Qld—Queensland, PNG—Papua New Guinea.

CO1 divergence among them.

The final primary clade consists of sp. E (having very short antennal scapes; Hoffmann *et al.*, 2011), with sequenced specimens occurring from Flores (Indonesia) to Singapore.

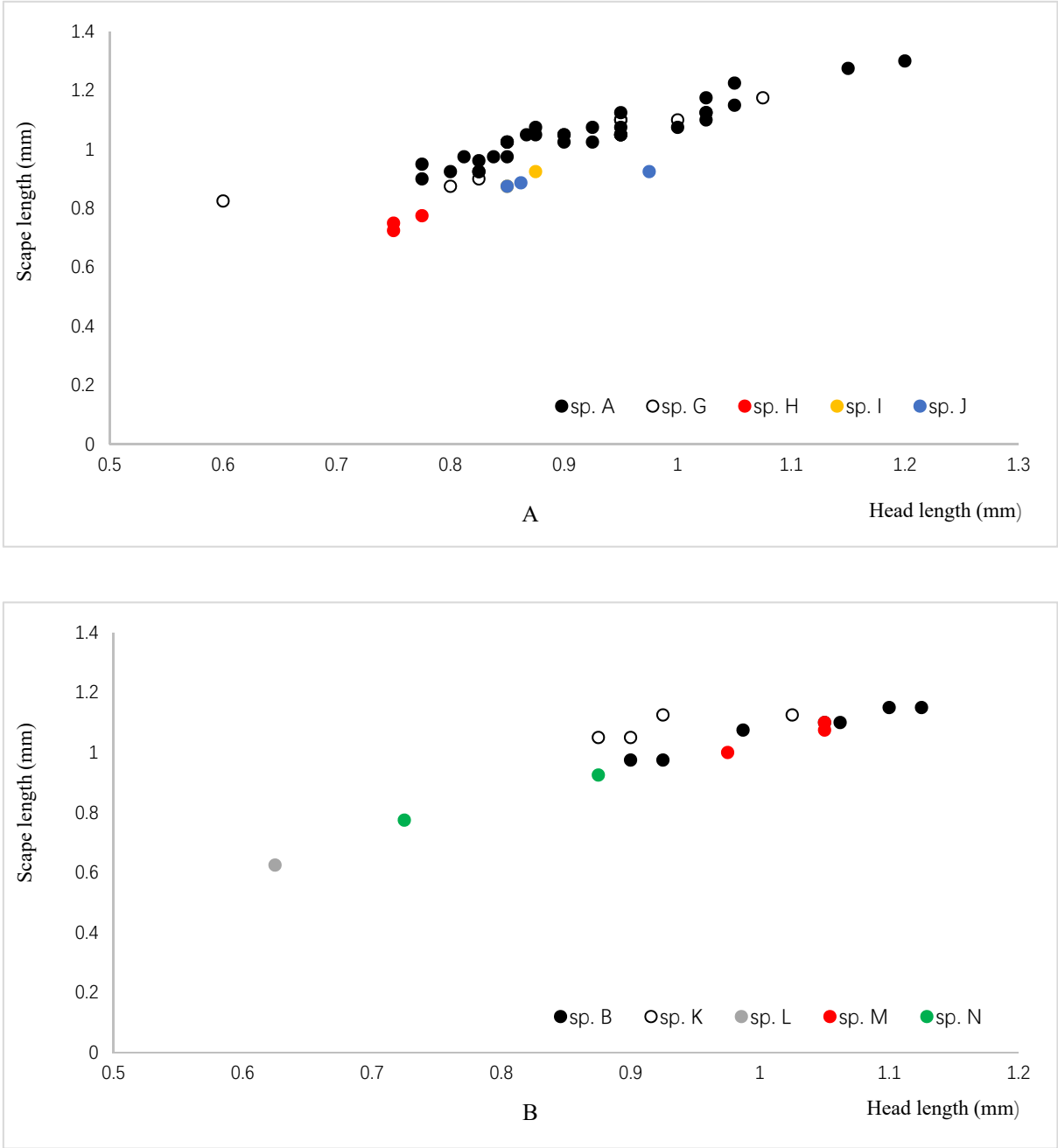


Figure 3. Scape length in relation to head length for selected species. A. Species A, G, H, I and J. B. Species B, K, L, M and N.

Key to species of *Iridomyrmex anceps* (Roger) complex.

1. Occurring in Australia..... 2
Occurring outside Australia..... 12
2. Antennal scapes very long (mean ratio of scape length to head length >1.1)..... 3
Antennal scapes not so long (mean ratio of scape length to head length <1.1) 4
3. Pubescence of first gastric tergite long and coarse, covering lateral as well as dorsal surfaces (Fig. 6B; Q1d)..... **sp. K**
Pubescence of first gastric tergite not so long and coarse, occurring primarily on the dorsal surface..... **sp. A and sp. G**

4.	Smaller species (mean total length approx. 2.0 mm) with relatively short antennal scapes (mean ratio of scape length to head length <1.02).....	5
	Larger species (mean total length ≥ 2.5 mm) with longer antennal scapes (mean ratio of scape length to head length >1.02)	6
5.	Promesonotal profile highly asymmetrical, more steeply rising anteriorly (Fig. 4A)	sp. H
	Promesonotal profile uniformly curved (Fig. 4B)	sp. L
6.	Gaster coarsely pubescent and with numerous erect hairs (Fig. 6C; Qld)	sp. M
	Gaster not so coarsely pubescent and without numerous erect hairs	7
7.	Head with sparse erect hairs laterally (WA)	sp. R
	Head without erect hairs laterally	8
8.	Hind femora with scattered erect hairs throughout (Qld)	sp. O
	Erect hairs on hind femora restricted to proximal third or absent	9
9.	Head broadest medially (Fig. 4E)	10
	Head broadest posteriorly	11
10.	Occurring in Qld	sp. I
	Occurring in WA	sp. J
11.	Occipital margin distinctly convex (Qld, NT)	sp. N
	Occipital margin flat, not distinctly convex (Qld)	sp. B
12.	Occurring in China	sp. F
	Occurring outside China	13
13.	Head with numerous erect hairs laterally (Timor)	sp. C
	Head lacking erect hairs laterally	14
14.	Hind femora with scattered erect hairs throughout (PNG)	sp. P
	Erect hairs on hind femora restricted to proximal third or absent	15
15.	Antennal scapes short, exceeding occipital margin by only one-quarter their total length (Indonesia, Singapore)	sp. E
	Antennal scapes longer, exceeding occipital margin by at least one-third their total length	16
16.	Occurring in Timor	sp. D
	Occurring in the Phillipines	sp. Q

4 Discussion

A recent revision of *Iridomyrmex* (Heterick & Shattuck, 2011) considered *I. anceps* to represent a single species despite its marked morphological variation (Hoffmann *et al.*, 2011) and range from Australia to India and China. We have shown that the morphological variation is matched by extensive genetic variation, and that matched morphological and genetic differentiation frequently occurs in sympatry. The *I. anceps* complex clearly consists of many species.

The six morphologically based taxa from the *I. anceps* complex recognized by Hoffmann *et al.* (2011) show up to 9% CO1 divergence from each other, and clearly represent multiple species. The only doubt concerns sp. C and sp. D from Timor, where CO1 divergence is low (1%) and there is no clear match between morphological and CO1 variation. They possibly represent a single species with variable pilosity on the head. However, we note that the two species from the *Iridomyrmex purpureus* (F. Smith) group in monsoonal Australia (*I. sanguineus* Forel and *I. reburrus* Viehmeyer) are most readily separated by head pilosity (Shattuck, 1993) and show no CO1 differentiation (A. N. Andersen, unpublished data).

Our analysis demonstrates the occurrence of many additional taxa within the *I. anceps* complex that are differentiated both genetically and morphologically. We have recognized 12 additional species among our sequenced specimens, mostly occurring in northern Australia. There are two cases where species from sister clades are not clearly differentiated morphologically, and so we have treated them as cryptic species. A more conservative assessment that considers them conspecific would still leave ten of our recognized additional species as being clearly differentiated morphologically from those in their sister clades, and would therefore not change our conclusion that the complex contains many valid species. Queensland appears to be the centre of diversity within the complex, with eight species recognized by us. This is higher than in either the Northern Territory (5) or Western Australia (4), despite Queensland having lower sampling effort. Our analyses indicate 15–18 species among our sequenced specimens, and we note that our sample is far from comprehensively representative of the *I. anceps* complex, given that many known locations outside Australia (Heterick & Shattuck, 2011) are not included, and that vast areas of northern Australia remain unsampled. It seems most likely that the *I. anceps* complex contains more than twenty species.

Although the *I. anceps* complex clearly contains many species, further sampling and analysis are required for a more-definitive estimate of total diversity. The sequencing of additional genes would also be informative, particularly for



Figure 4. Images of the *Iridomyrmex anceps* complex. A–C. Adult. A. Sp. H (specimen IRIDO 244-16); B. sp. L (specimen RHYIR 078-16); C. sp. M (specimen IRIDO 249-16). D–H. Head. D. Sp. G (specimen IRIDO 230-16); E. sp. J (specimen RHYIR 081-16); F. sp. M (specimen IRIDO 249-16); G. sp. N (specimen RHYIR 095-16); H. sp. R (specimen IRIDO 252-16). Scale bars=0.5 mm.

understanding phylogenetic relationships within the complex, including how closely phylogeny is linked to biogeography. Further analysis is also required to resolve the status of the various named taxa that have been synonymized under *I. anceps*. We suspect that our sp. E might be *I. anceps sensu stricto*, given that TERC holds morphologically identical specimens from peninsular Malaysia, the type locality of *I. anceps*. We also suspect that our sp. F from mainland China might be *I. bicknelli formosae* given the latter's type locality in Taiwan, China. One of our recognized species (sp. P) was recorded only from New Guinea, which suggests that at least one of the New Guinean *I. discoidalis*, *I. meinerti* and *I. gracilis papuana* is a valid taxon. A formal reconciliation of our recognized species with described species is beyond the scope of this study, and it might not even be possible given the loss of type specimens (Heterick & Shattuck, 2011).

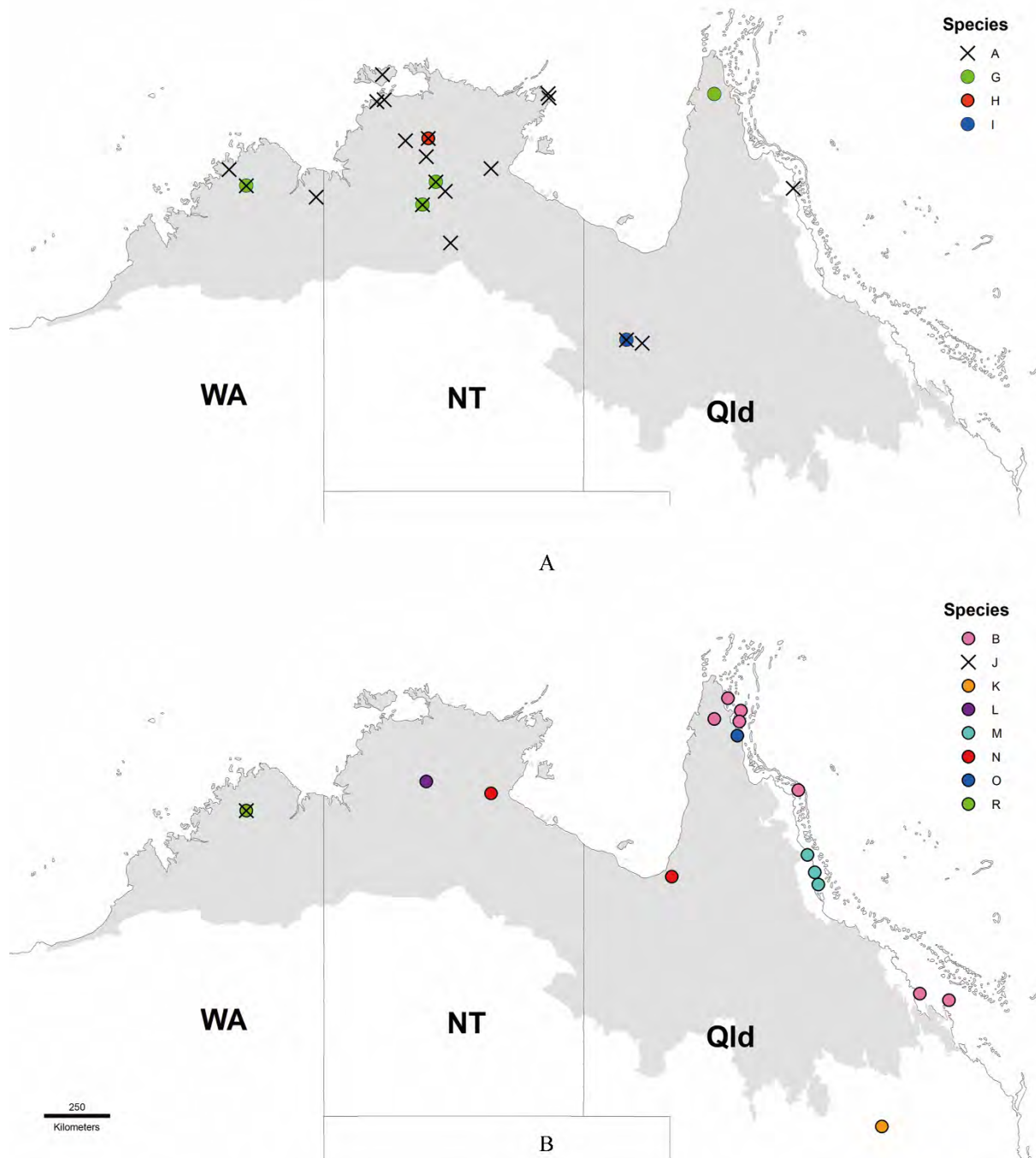


Figure 5. Distribution records of sequenced specimens from the *Iridomyrmex anceps* complex in Australia. Monsoonal tropics region shaded in grey. A. Species A, G, H and I. B. Species B, J, K, L, M, N, O and R. Abbreviations: WA—Western Australia, NT—Northern Territory, Qld—Queensland.

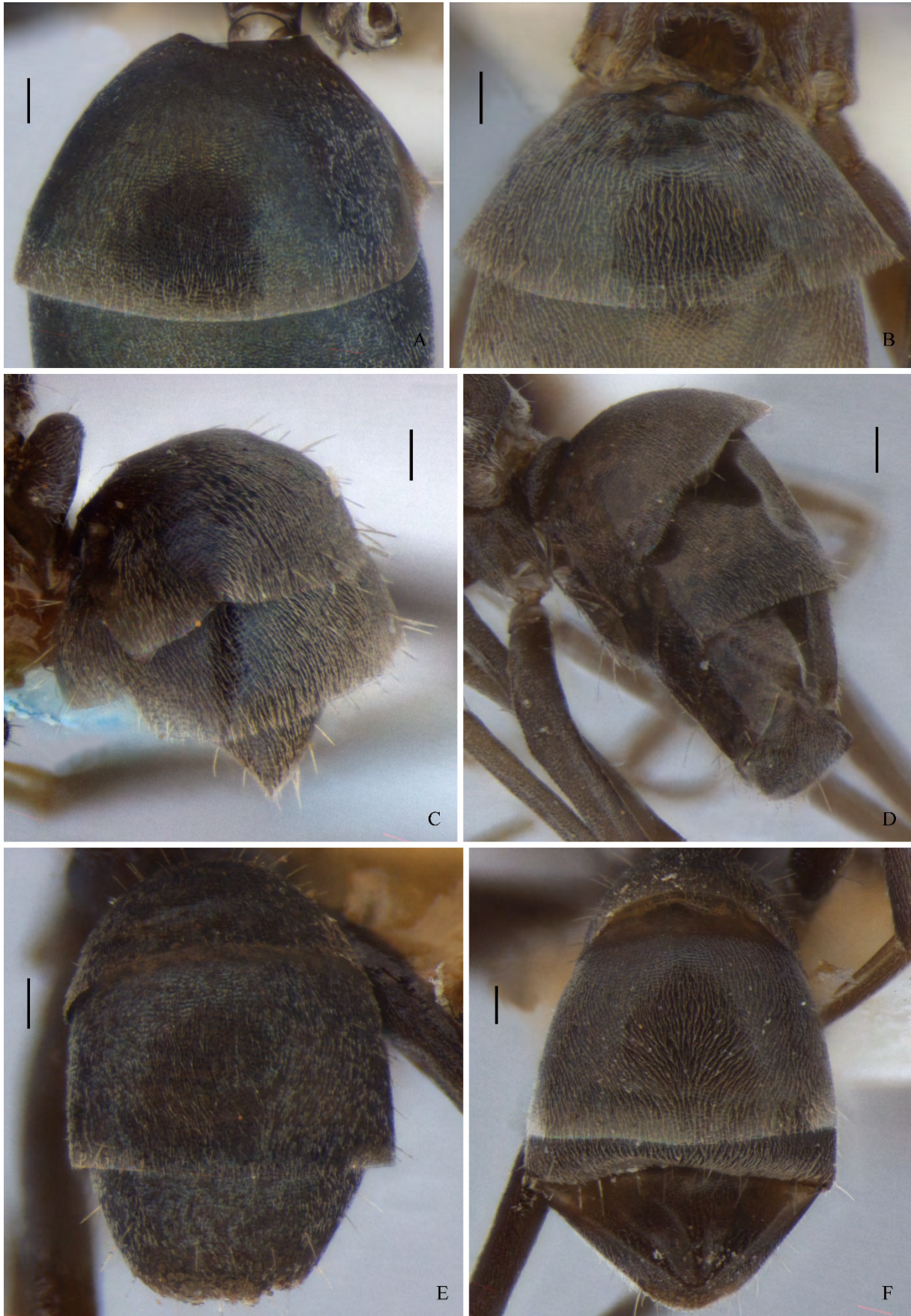


Figure 6. Variation in gastric pubescence among selected species of the *Iridomyrmex anceps* complex. A. Sp. B (specimen RHYIR 070), dorsal view. B. Sp. K (specimen RHYIR 076), dorsal view. C. Sp. M (specimen IRIDO 249), lateral view. D. Sp. N (specimen RHYIR 095), lateral view. E. Sp. O (specimen IRIDO 251-16), dorsal view. F. Sp. P (specimen IRIDO 250-16), dorsal view. Scale bars=0.1 mm.

The recognition of *I. anceps* as a single species (Heterick & Shattuck, 2011) does not reflect true diversity within this taxon nor the complexity of its evolutionary history, both within Australia and more broadly in the Indo-Pacific region. Many species occur in monsoonal Australia, some of which are widely distributed while others appear to be highly localized. The centre of diversity appears to be North Queensland. Its occurrence outside Australia does not reflect a simple range expansion as the Australasian (Sahul) continental plate converged with that (Sunda) of South-East Asia over recent geological time, but rather it represents a complex radiation that has produced many species with restricted distributions. Notably, the recognition of many species within the complex has important biosecurity implications. For example, any arrival of an overseas population into Australia should be recognized as an incursion by a non-native species and managed accordingly.

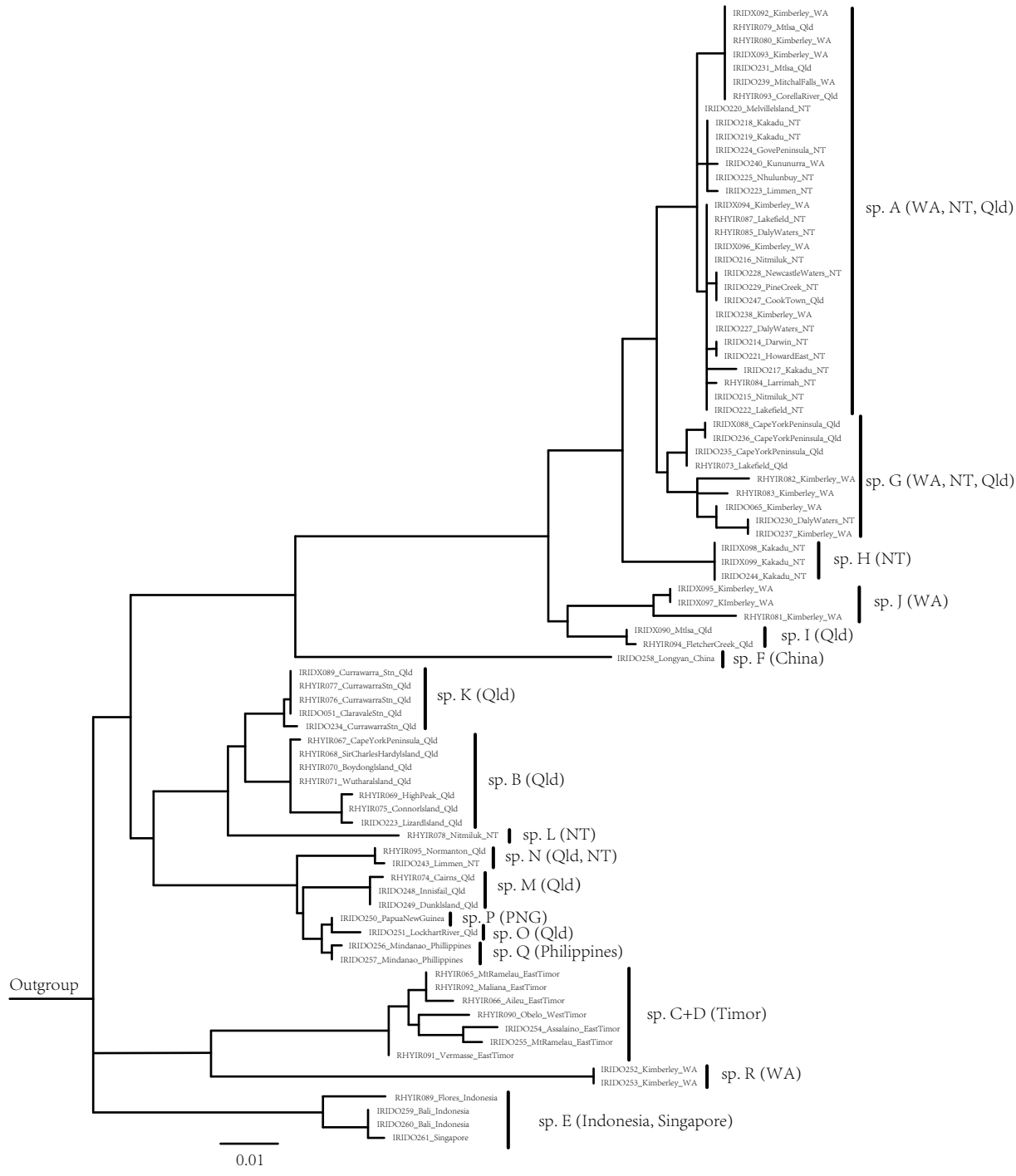
The conclusion that *I. anceps* represents many species has also been reached for other *Iridomyrmex* taxa recognized as single species in the taxonomic revision (Heterick & Shattuck, 2011): integrated morphological and genetic analysis shows that *I. coeruleus* Heterick & Shattuck represents at least five species that are clearly differentiated morphologically (Andersen *et al.*, 2013), and that *I. pallidus* Forel represents at least six species that vary in head shape, scape length and pilosity (Oberprieler *et al.*, 2018). Our unpublished CO1 data and morphological observations indicate that diversity is particularly high in taxa such as *I. mjobergi* Forel, *I. dromus* Clark and *I. minor* Forel. As of May 2020, the TERC collection has 329 morphologically based taxa of *Iridomyrmex*, and we believe that the genus contains at least 400 species. The recognition of only 79 species of *Iridomyrmex* (Heterick & Shattuck, 2011) does not reflect true diversity within this ecologically dominant genus, nor the complexity of its biogeography and evolutionary history.

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References

- Andersen, A.N. 1995. A classification of Australian ant communities, based on functional groups which parallel plant life-forms in relation to stress and disturbance. *Journal of Biogeography*, 22: 15–29.
- Andersen, A.N. 2000. *The Ants of Northern Australia: A Guide to the Monsoonal Fauna*. CSIRO Publishing, Collingwood, Australia. 106pp.
- Andersen, A.N. 2003. Ant biodiversity in arid Australia: productivity, species richness and community organization. *Records of the South Australian Museum Monograph Series*, 7: 79–92.
- Andersen, A.N. 2007. Ant diversity in arid Australia: a systematic overview. In: Snelling, R.R., Fisher, B.L., Ward, P.S. (eds.), *Advances in Ant (Hymenoptera, USA: Formicidae) Systematics: Homage to E. O. Wilson – 50 Years of Contributions*. American Entomological Institute, Gainesville, Florida. pp. 10–51.
- Andersen, A.N., Hoffmann, B.D., Berman, M. 2013. Diversity in the Australian ant genus *Iridomyrmex* Mayr, 1862 (Hymenoptera: Formicidae): A critique of Heterick & Shattuck (2011), with particular reference to *I. coeruleus* Heterick & Shattuck, 2011. *Myrmecological News*, 18: 103–111.
- Chernomor, O., von Haeseler, A., Minh, B.Q. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, 65: 997–1008.
- Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32: 1792–1797.
- Greenslade, P.J.M. 1976. The meat ant *Iridomyrmex purpureus* (Hymenoptera: Formicidae) as a dominant member of ant communities. *Journal of the Australian Entomological Society*, 15: 237–240.
- Heterick, B.E., Shattuck, S. 2011. Revision of the ant genus *Iridomyrmex* (Hymenoptera: Formicidae). *Zootaxa*, 2845: 1–175.
- Hoffmann, B.D., Andersen, A.N., Zhang, X. 2011. Taxonomic confusion of two tramp ant species: *Iridomyrmex anceps* and *Ochetellus glaber* are really species complexes. *Current Zoology*, 57: 662–667.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16: 111–120.
- Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33: 1870–1874.
- Minh, B.Q., Nguyen, M.A.T., von Haeseler, A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, 30: 188–195.
- Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q. 2014. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32: 268–274.
- Oberprieler, S.K., Andersen, A.N., Moritz, C.C. 2018. Ants in Australia's Monsoonal Tropics: CO1 barcoding reveals extensive unrecognised diversity. *Diversity*, 10: 36.

- Rambaut, A. 2007. FigTree: molecular evolution, phylogenetics and epidemiology. Available from <http://tree.bio.ed.ac.uk/software/figtree/> (accessed 20 May 2020).
- Schär, S., Talavera, G., Espadaler, X., Rana, J. D., Andersen A.A., Cover, S.P., Vila, R. 2018. Do Holarctic ant species exist? Trans-Beringian dispersal and homoplasy in the Formicidae. *Journal of Biogeography*, 45: 1917–1928.
- Smith, M.A., Fisher, B.L., Hebert, P.D. 2005. DNA barcoding for effective biodiversity assessment of a hyperdiversearthropod group: The ants of Madagascar. *Philosophical Transactions of the Royal Society London, B, Biological Sciences*, 360: 1825–1834.
- Shattuck, S.O. 1993. Revision of the *Iridomyrmex purpureus* species-group (Hymenoptera : Formicidae). *Invertebrate Taxonomy*, 7: 113–149.
- Trifinopoulos, J., Nguyen, L.-T., von Haeseler, A., Minh, B.Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, 44: W232–W235.
- Wagner, H. C., Gamisch, A., Arthofer, W., Moder, K., Steiner, F. M., Schlick-Steiner, B. C. 2018. Evolution of morphological crypsis in the *Tetramorium caespitum* ant species complex (Hymenoptera: Formicidae). *Scientific Reports*, 8: 12547.
- Wild, A. L. 2009. Evolution of the Neotropical ant genus *Linepithema*. *Systematic Entomology*, 34: 49–62.



Supplementary Figure 1. Detailed CO1 tree, showing Barcode of Life Data (BOLD) identification codes and collection locations of all sequenced specimens.