



## RESEARCH ARTICLE - ANTS

## Second Record and DNA Barcode of the Ant *Tyrannomyrmex rex* Fernández (Hymenoptera: Formicidae: Myrmicinae)

J JACQUEMIN<sup>1</sup>, G SONET<sup>1</sup>, T BOURGUIGNON<sup>2,3</sup>, TA EVANS<sup>2</sup>, T DELSINNE<sup>1,4</sup>

1 - Royal Belgian Institute of Natural Sciences, Brussels, Belgium

2 - Department of Biological Sciences, National University of Singapore

3 - Czech University of Life Sciences, Prague, Czech Republic

4 - Universidad Técnica Particular de Loja, Loja, Ecuador

### Article History

#### Edited by

Rodrigo M. Feitosa, UFPR, Brazil.

Received 26 December 2014

Initial acceptance 30 January 2015

Final acceptance 12 February 2015

#### Keywords

Solenopsidini, Singapore, primary forest, leaf-litter ant, DNA barcoding, scanning electron microscopy.

#### Corresponding author

Justine Jacquemin

OD Nature (ATECO), Royal Belgian Institute of Natural Sciences, 29 Rue Vautier, B-1000 Brussels, Belgium

E-Mail: [jjacquemin@naturalsciences.be](mailto:jjacquemin@naturalsciences.be)

### Abstract

*Tyrannomyrmex* is a rarely collected ant genus from Old World tropical forests comprising only three described species, all of them known from a single worker. Here we report the discovery of a second worker of *Tyrannomyrmex rex* from a selectively logged primary forest of Singapore, increasing the known distribution range of the species to nearly 250 km South-East. We also provide a DNA barcode for the species and a partial sequence of the wingless gene. Although insufficient evidence prevents us to draw any firm conclusion, the genus seems to be restricted to pristine or relatively undisturbed forests and, as a result, could be highly sensitive to habitat degradation.

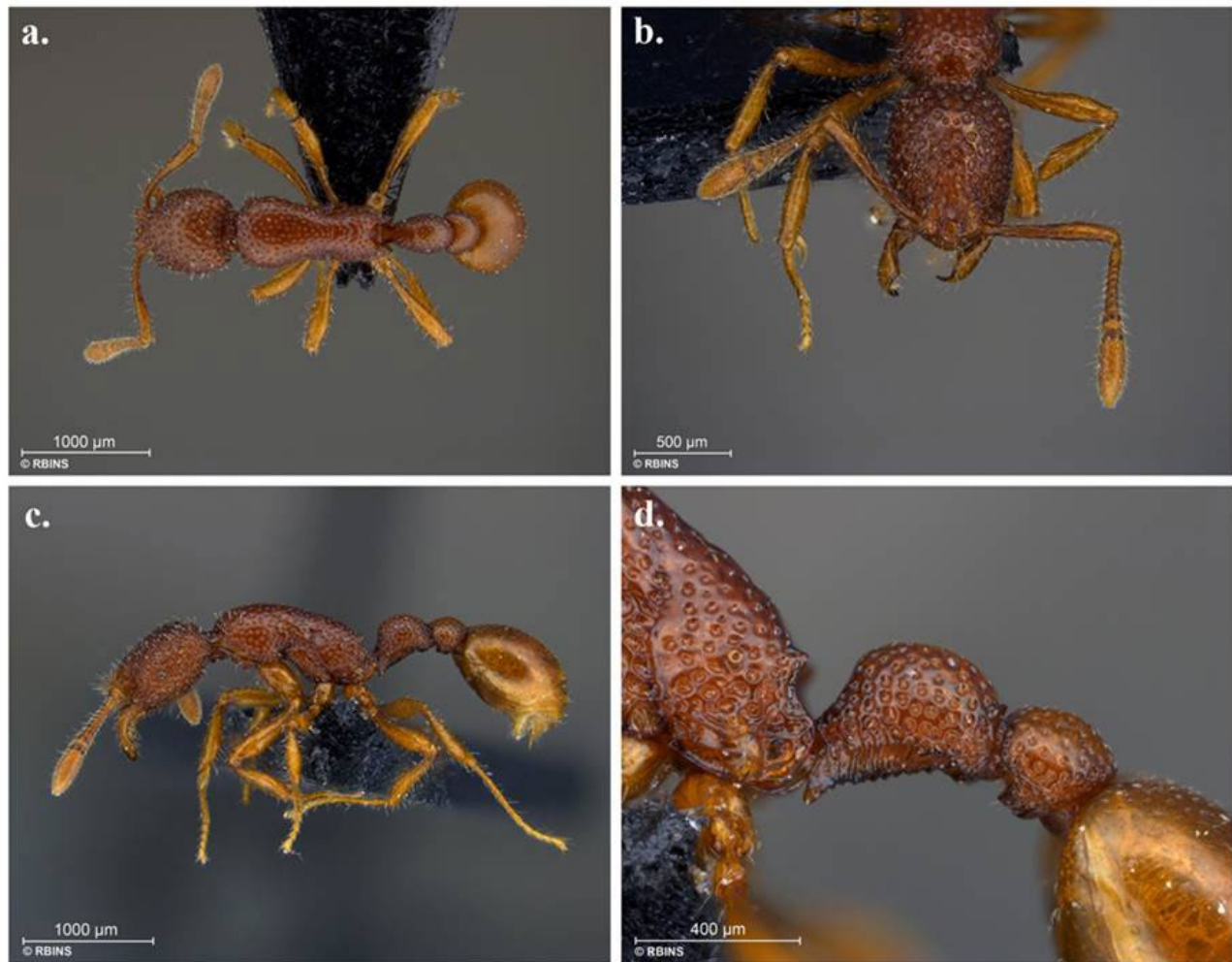
### Introduction

The ant genus *Tyrannomyrmex* (Fig 1) is relatively small and endemic to tropical broadleaf wet forests of South and Southeast Asia (Indomalaya biogeographic region, Udvardy, 1975; Olson et al., 2001). The genus was described from peninsular Malaysia based on a single worker, which was designated as *T. rex* (Fernández, 2003). Since then, two new species have been described: *T. dux*, from India (Borowiec, 2007), and *T. legatus*, from Sri Lanka (Alpert, 2013), both known from a single worker. The genus has been recently collected in Borneo (Fisher et al., in press), and it is possible that it has been found in the Philippines as well. Indeed, a single male collected in 1965 in the Philippines has been putatively placed in the genus based on the similarity of petiole shape and mandibular teeth with the worker described by Fernández (2003), but this placement needs to be confirmed (General & Alpert, 2012; Alpert, 2013).

*Tyrannomyrmex* is placed in the Myrmicinae. The principal traits of this rarely collected and documented genus are mandibles with only two apical teeth, and inner margin of the edentate masticatory border of the mandibles with setae. These setae are modified in *T. rex* (i.e. thick and cylindrical) (Fig 2c., d.) but simple and similar to other setae of the body in *T. dux* and *T. legatus*. The three species have small eyes reduced to a few ommatidia (Fig 2a.), 11-segmented antennae with an ill-defined 3-segmented club (Fig 1a., b., c.) and an apparent palpal formula 2,2 (the formula could not be reliably determined in *T. dux*). Also, the mesosoma does not present any groove or suture, forming a single broad and continuous convexity in side view (Fig 1c) and the metapleural gland seems to be lacking (Fernández, 2003; Borowiec, 2007; Fig 1d., 2e., f.).

The reliable placement of *Tyrannomyrmex* among the Myrmicinae was tentative based on morphological data only.





**Fig 1.** High resolution pictures of the *Tyrannomyrmex rex* worker collected in Singapore. Dorsal (a.), frontal (b.) and lateral (c.) views, and a close-up lateral view of the propodeum, petiole and postpetiole (d.).

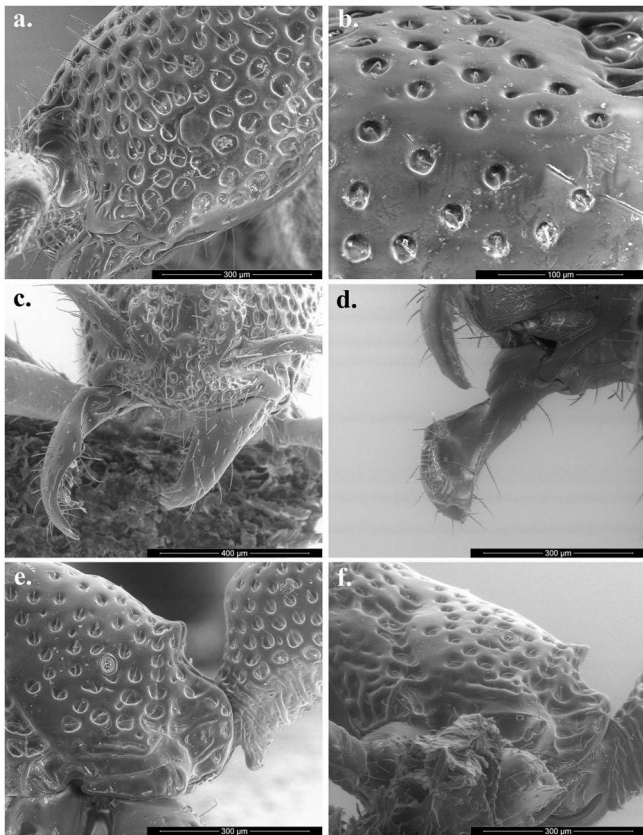
Based on morphological characters (i.e. presence of transformed setae along internal border of mandibles), Fernández (2003) suggested that the genus was close to the Adelomyrmecini and to the Solenopsidini tribes (according to the taxonomy of that time). Alpert (2013) suggested that *Tyrannomyrmex* was close to *Monomorium*, a member of the Solenopsidini, by using the comprehensive morphology-based treatment of the Formicidae described by Bolton (2003). A recent phylogeny of the Myrmicinae, based on 11 nuclear gene fragments, confirmed that *Tyrannomyrmex* belongs to the Solenopsidini, albeit this clade was somehow redefined (Ward et al., 2015). According to this work, *Tyrannomyrmex legatus* is the sister taxon to *Monomorium latinode* + *M. brocha*, two species distributed mostly in tropical Asia, similar to *Tyrannomyrmex*. It should be noted that *M. brocha* has been transferred to the genus *Epelysidris* and a new genus under description by Barry Bolton will be erected for *M. latinode* (Ward et al., 2015).

Here, we report the discovery of a second worker of *T. rex*, which becomes therefore the first duplicate (species known from two individuals) of the genus. The specimen is documented by high resolution pictures and SEM photographs and its DNA barcode is provided.

## Materials and methods

We collected the ant specimen in December 2012 in MacRitchie Reservoir, Singapore (N 01°20'30", E 103°49'43"), during a biodiversity survey of the ants from Singapore. It was found in a selectively logged primary forest not exploited for decades, comprising primary forest species at an intermediate stage of regeneration. While the plant species composition was that of a primary forest, it lacked large emergent trees present in adjacent unlogged primary forest. The site was located near an old growth primary forest.

Altogether, we collected 5525 ant specimens in six sites and 877 specimens in the site in which *T. rex* was collected. The sampling consisted of 25 samples of leaf-litter (1 m<sup>2</sup> each) collected in each site and placed in Berlese extractors. The extractors consisted of a plastic funnel heated with a 100 Watt light bulb which we kept running for two to five days, until the leaf litter was completely dried out. We preserved all specimens in 80% ethanol until identification. The *T. rex* individual was mounted for photography and a hind leg was dissected immediately afterwards for DNA analysis.



**Fig 2.** Close-up SEM pictures of *Tyrannomyrmex rex* illustrating the eye composed of 6 ommatidia (a.), the very short appressed hairs located within foveae on the pronotum (b.), the modified stout and cylindrical setae on the inner margin of the masticatory border of the mandible (dorsal view c., lateroventral view d.), the large and round propodeal lobe (e.), and an oblique view of the metapleuron (f.). Note the absence of metapleural gland orifice on (e.) and (f.).

We took high resolution digital images using a Leica MC170 camera attached to a Leica S8APO stereomicroscope. A series of images was taken by focusing the sharpness on different levels of the specimen, using the Leica Application Suite v38 (2003-2011) and combined with the stacking software Combine ZP (Hadley, 2010). Final editing of the images was done in Adobe Photoshop CS5. SEM photographs of the specimen were taken using a scanning electron microscope FEI Quanta 200. The specimen is deposited at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium (SPM\_ID 4904401; RBINS general inventory 33045).

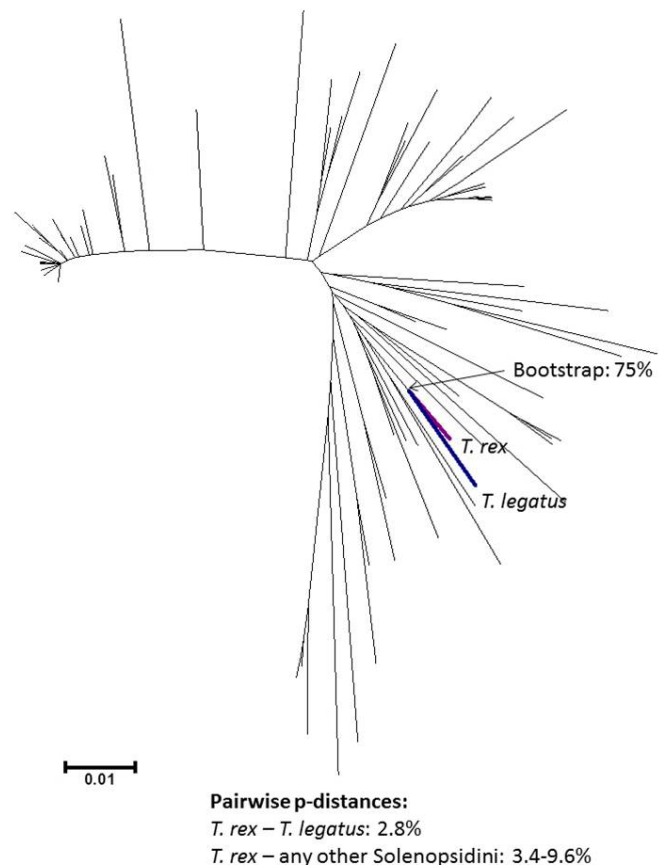
We extracted DNA from one hind leg using the commercial NucleoSpin Tissue Kit (Macherey-Nagel, Germany) and performed the PCR amplification of the 5' end of the mitochondrial cytochrome *c* oxidase subunit I (COI) marker (standard DNA barcode region) and a fragment of the wingless gene (Wg). PCR amplification and bidirectional DNA sequencing were executed according to the protocol described by Delsinne et al. (2012) and using the primer pairs LCO1490 / HCO2198 (Folmer et al., 1994) for COI, and Wg578F (Ward & Downie, 2005) / Wg1032R (Abouheif & Wray, 2002) for Wg. Using the BLAST algorithm (Altschul et al., 1990), we searched through GenBank and

The Barcode of Life Data System (Ratnasingham & Hebert, 2007) for best matches with the two sequences obtained here. Finally, the same two sequences were compared to all COI and Wg sequences available for the tribe Solenopsidini in GenBank. Alignments, pairwise uncorrected p-distances (proportion of nucleotide sites at which two sequences differ) and a neighbor-joining tree were calculated using MEGA 6.06 (Tamura et al., 2013).

## Results and discussion

The COI barcode of *T. rex* (GenBank accession number: KP294328) has never been sequenced before and is markedly different from all COI sequences available in GenBank and BOLD (< 86% similarity). The best matches involve species of the genera *Pheidole* and *Monomorium*.

The GenBank sequence that matches the best with our *T. rex* Wg (GenBank accession number: KP294329) is the single Wg sequence already available for the genus *Tyrannomyrmex* (accession number: KJ861941; species: *Tyrannomyrmex legatus*). On the basis of this marker, the distance between *T. rex* and *T. legatus* is 2.8% whereas the inter-generic distances between *T. rex* and any other Solenopsidini sequence range from 3.4 to 9.6% (Fig 3).



**Fig 3.** Unrooted neighbor-joining tree based on pairwise p-distances among all Solenopsidini wingless sequences available in GenBank.

The discovery of a second specimen of *T. rex* expands the known range of the species to nearly 250 km to the South-East of the only other known location in Malaysia. It also expands the known altitudinal range of the genus, from sea level (24 m; this study) to 1300-1500 m (*T. dux*) (*T. legatus* was discovered at intermediate elevation, between 432 and 571 m).

It is worth mentioning that *Tyrannomyrmex rex* seems to be rare at the scale of the country. Indeed, the specimen mentioned is the single one discovered despite our recent efforts to document leaf-litter ant assemblages from other Singaporean forested sites, representative of both degraded and pristine habitats (six sites; 25 Berlese samples per site). However, this apparent rarity could result from a methodological artifact since the morphology of the genus suggests subterranean habits and the Berlese extraction of leaf-litter fauna is not the more efficient method for documenting hypogean ants.

Interestingly and as anteriorly noted (Fernández, 2003; Borowiec, 2007), the genus seems to lack a functional metapleural gland (Fig 1d., Fig 2e., f.), a structure unique to ants and playing a key role in sanitation and chemical defense (Yek & Mueller, 2011). This gland has been secondarily lost in some ant lineages, mostly in social parasites and arboreal formicines, probably because their life histories render them less exposed to pathogens than ground-dwelling species (Yek & Mueller, 2011 and references therein). Its absence in *Tyrannomyrmex*, a probable hypogean genus, suggests either that these ants could be social parasites or that they possess other protections against pathogens as observed for instance in some *Camponotus* (Walker & Hughes, 2011) and *Polyrhachis* species (Graystock & Hughes, 2011). Additional material and anatomical studies are obviously required to test these hypotheses.

The unique specimen of *T. legatus* was found in an undisturbed and unlogged lowland dipterocarp forest in Sri Lanka, and the *T. rex* individual studied by Fernández (2003) was collected in a forest reserve. We found the second *T. rex* worker in a selectively logged primary forest unexploited for decades, very similar in terms of vegetation composition to the primary forest nearby. Although information about the habitat where *T. dux* was collected is lacking, available data suggest that this rarely collected genus occurs in pristine or little disturbed tropical forests of Asia. Singapore has lost more than 95% of its original forest over the last two centuries leading to a massive extinction of its biodiversity (Brook et al., 2003). Fortunately, efforts were recently made to preserve the remaining pristine areas of the island, to improve the biodiversity value of secondary forests and urban areas, and to increase the connectivity among green patches (Fam et al., 2014; Tan & Hamid, 2014). The presence of *Tyrannomyrmex rex*, a species suspected to be restricted to well-preserved tropical forests, points out that efforts for maintaining Singaporean biodiversity are worth being pursued.

## Acknowledgments

We warmly thank Julien Cillis (RBINS) for SEM photographs and Lee Gang for field assistance. This work was supported by the LHK fund of the National University of Singapore; by the Singapore-MIT Alliance for Research and Technology; by the Belgian Federal Science Policy Office (BELSPO) through an Action 1 Impulse for Research and by the SENESCYT through its Prometeo grant to TD (<http://prometeo.educacionsuperior.gob.ec/>); and by the Internal Grant Agency of the Faculty of Forestry and Wood Sciences, Czech University of Life Sciences (registration number A08/14).

## References

- Abouheif, E. & Wray, G.A. (2002). Evolution of the gene network underlying wing polyphenism in ants. *Science*, 297: 249-252. DOI: 10.1126/science.1071468
- Alpert, G.D. (2013). A new species of *Tyrannomyrmex* (Hymenoptera: Formicidae) from Sri Lanka. *Zootaxa*, 3721: 286-290. DOI: 10.11646/zootaxa.3721.3.5
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215: 403-410. doi: 10.1016/S0022-2836(05)80360-2
- Bolton, B. (2003). Synopsis and Classification of Formicidae. *Memoirs of the American Entomological Institute*, 71: 1-370
- Borowiec, M.L. (2007). A new species of *Tyrannomyrmex* (Hymenoptera: Formicidae: Myrmicinae) from India. *Zootaxa*, 1642: 65-68
- Brook, B.W., Sodhi, N.S. & Ng, P.K.L. (2003). Catastrophic extinctions follow deforestation in Singapore. *Nature*, 424: 420-423. doi: 10.1038/nature01795
- Delsinne, T., Sonet, G., Nagy, Z.T., Wauters, N., Jacquemin, J. & Leponce, M. (2012). High species turnover of the ant genus *Solenopsis* (Hymenoptera : Formicidae) along an altitudinal gradient in the Ecuadorian Andes, indicated by a combined DNA sequencing and morphological approach. *Invertebrate Systematics*, 26: 457-469. doi: 10.1071/IS12030
- Fam, S.D., Lee, B.P.Y-H. & Shekelle, M. (2014). The conservation status of slow lorises *Nycticebus* spp. in Singapore. *Endangered Species Research*, 25: 69-77. doi: 10.3354/esr00599
- Fernández, F. (2003). A new myrmicine ant genus from Malaysia with uncertain affinities (Hymenoptera: Formicidae). *Zootaxa*, 341: 1-6
- Fisher, B.L., Guénard, B. & Robson, S. Borneo, fANTastique! Asian Myrmecology (in press)
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan

invertebrates. *Mol Mar Biol Biotech*, 3: 294-299

General, D.M. & Alpert, G.D. (2012). A synoptic review of the ant genera (Hymenoptera, Formicidae) of the Philippines. *ZooKeys*, 200: 1-111. DOI: 10.3897/zookeys.200.2447

Graystock, P. & Hughes, W.O.H. (2011). Disease resistance in a weaver ant, *Polyrhachis dives*, and the role of antibiotic-producing glands. *Behavioral Ecology and Sociobiology*, 65: 2319-2327

Hadley, A. (2010). *CombineZP*, Available from: <http://www.hadleyweb.pwp.blueyonder.co.uk/CZP/News.htm> [6.vi.2010]

Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P. & Kassem, K.R. (2001). Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience*, 51: 933-938

Ratnasingham, S. & Hebert, P.D.N. (2007). BOLD: The Barcode of Life Data System ([www.barcodinglife.org](http://www.barcodinglife.org)). *Molecular Ecology Notes*, 7: 355-364. doi: 10.1111/j.1471-8286.2006.01678.x

Tamura, K., Stecher, G., Peterson, D., Filipowski, A. & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30: 2725-2729. doi: 10.1093/molbev/mst197

Tan, P.Y. & Hamid, A.R.b.A. (2014). Urban ecological research in Singapore and its relevance to the advancement of urban ecology and sustainability. *Landscape Urban Plan*, 125: 271-289. doi: 10.1016/j.landurbplan.2014.01.019

Udvardy, M.D.F. (1975). A classification of the biogeographical provinces of the world. Morges (Switzerland): International Union of Conservation of Nature and Natural Resources. IUCN Occasional Paper no. 18

Walker, T.N. & Hughes, W.O.H. (2011). Arboreality and the evolution of disease resistance in ants. *Ecological Entomology*, 36: 588-595. doi: 10.1111/j.1365-2311.2011.01312.x

Ward, P.S. & Downie, D.A. (2005). The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): Phylogeny and evolution of big-eyed arboreal ants. *Systematic Entomology*, 30: 310-335. doi: 10.1111/j.1365-3113.2004.00281.x

Ward, P.S., Brady, S.G., Fisher, B.L. & Schultz, T.R. (2015). The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Systematic Entomology*, 40: 61-80. doi: 10.1111/syen.12090

Yek, S.H. & Mueller, U.G. (2011). The metapleural gland of ants. *Biological Review*, 86: 774-791. doi: 10.1111/j.1469-185X.2010.00170.x

