

Survey of leaf litter ant species and assessment of invasive ants in the mining sites at Ambatovy, Madagascar

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Abstract

To validate the Biodiversity Action Plan (BAP) for the Ambatovy mining project, including forested zones destined for mining exploitation, an arthropod survey was conducted by the Madagascar Biodiversity Center. Two aims of this survey were to maximize the chance of collecting as many endemic litter ant species as possible for translocation to a receptor site and to provide baseline data on invasive ants in the region.

Two quantitative sampling methods, litter sifting and pit-fall traps, were used and evaluated within and across habitats at four sites within the Ambatovy-Analamay area. Slope exposure, elevation, and vegetation types were taken into account for the forest sites A-1 and B-C, whereas for two grassland sites, A-2 and A-3, the focus was on invasive ant species. Sites A-1 and B-C yielded 91 ant species and sites A-2 and A-3 a total of 46 species. Of these taxa, two are thought to be endemic to Ambatovy and three are considered introduced to Madagascar. In the forest habitats, litter sifting was the most effective technique, whereas pit-fall traps worked well in open habitats.

Although some of the most serious invasive ant species known from Madagascar and elsewhere in the world have not been identified at Ambatovy, expanding international travel and trade to Madagascar will increase their spread on the island. Hence, preventive monitoring of invasive ant species should be urgently initiated along with field studies that integrate new information on the taxonomy of ants.

Keywords: Litter ants, invasive species, quantitative collecting methods, mining exploitation, Ambatovy, Madagascar

Résumé détaillé

Dans le cadre du Plan d'Action Biodiversité d'Ambatovy, zone destinée à l'exploitation minière, le projet Ambatovy a dirigé une étude avec une équipe multidisciplinaire pour maximiser le maintien de la diversité biologique dans l'aire exploitée. Le projet Arthropodes, réalisé par le « Madagascar Biodiversity Center » a pour objectif d'échantillonner le maximum d'espèces de fourmis de la litière, afin de pouvoir assurer leur translocation vers un site récepteur et de fournir des données fondamentales sur les fourmis envahissantes dans la région.

L'échantillonnage a été réalisé en utilisant deux méthodes de collectes quantitatives de 25 stations chacune, le tamisage de litière et les pièges « pit-fall » dans deux sites forestiers : A-1 et B-C pour lesquels nous avons tenu compte de l'exposition au soleil, du gradient d'altitude et du type de végétation et aussi dans deux sites de savane A-2 et A-3 qui sont susceptibles de recéler des espèces de fourmis envahissantes. Par ailleurs, le site récepteur a été aussi prospecté pour estimer si toutes les conditions environnementales nécessaires pour la translocation des espèces endémiques sont réunies. L'efficacité des deux méthodes quantitatives et les menaces potentielles que les fourmis envahissantes pourraient causer dans la région d'Ambatovy et à Madagascar dans son ensemble ont été évaluées.

Cent douze espèces de fourmis appartenant à 29 genres ont été recensées. Vingt huit genres et 90 espèces ont été recensées dans les sites forestiers (A-1 et B-C), 18 genres et 46 espèces ont été trouvées dans les zones de savanes (A-2 et A-3). Parmi ces espèces, deux présentent une endémicité locale pour le moment et trois sont considérées comme étant introduites. Dans les habitats de forêt, le tamisage de litière est une méthode plus efficace que les pièges « pit-fall » pour collecter les fourmis. Par contre, l'emploi des pièges « pit-fall » est plus efficace dans les habitats ouverts. Il est intéressant de noter que le genre endémique *Pilotrochus*, localisé le long de la partie Est à une altitude de 900 m, est le plus abondant à Ambatovy. Les échantillons recueillis dans le cadre de ce projet ont contribué à enrichir les collections des musées à savoir le « California Academy of

Sciences » et le « Madagascar Biodiversity Center », ce qui encouragera des études ultérieures.

La systématique faisant appel à l'étude morphologique traditionnelle et au « barcode » de l'ADN favorisera la découverte d'autres espèces à Madagascar et la connaissance de cette classification facilitera les études écologiques et l'histoire naturelle de ces espèces. Ainsi, à partir de la compréhension de ces divers aspects, la translocation de fourmis et d'autres groupes d'arthropodes de l'île pourrait être entamée.

Les plus néfastes des espèces envahissantes n'ont pas été recensées à Ambatovy. Mais avec l'accroissement du commerce et des voyages internationaux, ces espèces qui passent souvent inaperçu, risquent de s'établir à Madagascar. Un contrôle strict devrait donc être mis en place. Les marchandises qui arrivent à Madagascar par fret aérien ou maritime doivent faire l'objet d'une attention particulière.

Mots clés : Fourmis de la litière, espèces envahissantes, méthodes de collecte quantitative, Exploitation minière, Ambatovy, Madagascar

Introduction

One of the primary goals of conservation biology is to protect biological diversity and the places where organisms are found, based on the principles of maximizing species diversity and complementarity (Van-Wright *et al.*, 1991; Pressey *et al.*, 1993). Conservation priority setting exercises require the design of reserve systems, which can be improved when extensive baseline data from biodiversity assessments are used. However, developing these reserve networks must take account of human activities such as land use and resource extraction, which have economic and social implications. An example of this is the exploitation of the forested areas of Madagascar for minerals in the Ambatovy region, where it is necessary to destroy natural habitat in order to access important nickel and cobalt deposits.

Research conducted over the past 15 years demonstrates that the Ambatovy region holds a considerable number of arthropod species, including ants (Fisher *et al.*, 2004). However, significant deforestation in the area has occurred due to the growing demand for firewood and destruction of forest for slash-and-burn agriculture. Further, this zone has considerable natural resources, specifically mineral deposits, the exploitation of which could financially benefit local residents and Madagascar as a whole. The destruction of natural forests associated

with mineral exploitation will certainly reduce regional forest cover and lead to the local decline or possible disappearance of terrestrial arthropods. Consequently, biological surveys of the region and the translocation of locally endemic or regionally rare taxa to a receptor site are important to help mitigate the impact of habitat disturbance associated with the mining project. It is critical to find the proper balance between resource extraction in the Ambatovy forests to benefit Madagascar's economic development and conservation strategies and actions that protect regional biodiversity.

In the face of continuous loss of biodiversity, animal translocations are considered increasingly important for maintaining the viability of certain populations. However, most translocation programs have focused on vertebrates (Fischer & Lindenmayer, 2000). Despite the known importance of arthropods in relation to species diversity and biomass within a given ecosystem, and the significant roles these animals play in ecosystem functioning (Kareiva *et al.*, 1993; Miller, 1993; Longino & Colwell, 1997), they are not the focus of conservation measures. The few exceptions involve certain butterfly species (Väisänen *et al.*, 1994; Witkowski *et al.*, 1997) and crickets (Pearce-Kelly *et al.*, 1998; Sherley, 1998; Berggren, 2005) that have been the subjects of translocation exercises. However, given that arthropods are small and have limited spatial requirements (Pearce-Kelly *et al.*, 1998), their translocations have the potential to be more successful than those for vertebrate taxa.

The proposed mining exploitation at Ambatovy will contribute to habitat fragmentation and perturbation, reduce biological diversity, and increase the chances of invasive species to colonize the zone (Suarez *et al.*, 1998; Holway *et al.*, 2002; Von Holle & Simberloff, 2005). The effect of these pressures may be greater on Madagascar due to the long evolutionary isolation of many of its endemic organisms. Ants have been unintentionally transported and transferred by humans across the world (Williams, 1994). In areas of such introduction, invasive ant species, once established, are difficult, if not impossible, to eradicate (Holway *et al.*, 2002). They pose major threats to native ecological assemblages and have been demonstrated to cause a wide range of drastic changes to these communities. This type of impact has been demonstrated for native ant communities and arthropod assemblages (Hölldobler & Wilson, 1990; Porter & Savignano, 1990; Holway, 1999; McGlynn, 1999; Underwood & Fisher, 2006). These impacts are also known to affect local vertebrate communities by altering available resources and habitat structures (Suarez *et al.*, 2000, 2005; O'Dowd

et al., 2003; Davis *et al.*, 2008). Hence, tracking the presence or absence of invasive ant species in the Ambatovy forests is one of the most effective methods of preventing the invasion of introduced ants to local habitats and microenvironments.

In 2007, to help in concordance with its Biodiversity Action Plan (BAP), the Ambatovy project conducted a multi-disciplinary study to maximize the preservation of the biological diversity in an area to be clear-cut for mining. One component of the project concerned terrestrial arthropods and the members of Madagascar Biodiversity Center carried out surveys. This work aimed to (1) establish an extensive and intensive inventory of the ground-dwelling ants of Ambatovy, focusing particularly on endemic taxa, to ensure their translocation to the reception site, and (2) provide baseline data on invasive ants from the area. Here, I present data associated with this project and compare the efficiency of the two quantitative sampling methods (mini-Winkler and pit-fall traps) within and among different habitats. Further, I utilize information on the current known distribution of introduced ant species in Madagascar (see www.antweb.org), consider their presence at Ambatovy, and use the recognized major effects of invasive ants elsewhere in the world to assess their potential risks to native community assemblages in the area and their threats to Malagasy biodiversity in general. One important aspect of this project is to increase ecological information on the ant fauna of the Ambatovy region, and provide insights to shape future translocation projects.

Materials and methods

Study sites

Ants were surveyed in the Ambatovy forest between 1 and 10 March 2007 in the areas delimited for clear-cutting in April 2007. Five sites were chosen to both maximize the chance to collect as many ant species endemic to the area as possible and consider habitats most likely to harbor invasive ants. The habitat factors we considered for collection sites were slope exposure, elevation, and vegetation types. Burnt forests or secondary grasslands adjacent to largely intact forests were selected for sampling to determine whether invasive ant species had colonized such zones.

Sampling sites within the Ambatovy forest, located 12.4 km NE of Moramanga, included the following sites: (1) area A-1: 18°50.978'S, 48°17.682'E, at 1010 m; (2) area B-C: 18°50.362'S, 48°18.505'E, at 1080 m. The local habitat in area B-C is a transition zone between typical montane humid forest and a more xeric habitat where many ant species, including

Pilotrochus besmerus, a monotypic genus endemic to Madagascar, were previously collected. Two open grassland habitat sites were also sampled: (3) area A-2, 18°50.864'S, 48°17.741'E, at 1000 m elevation and (4) area A-3, 18°51.488'S, 48°17.093'E, at 1040 m. The proposed translocation receptor site, located at 18°50.948'S, 48°18.093'E, at 1037 m, was also prospected to evaluate whether its local ecological conditions are similar to those required for endemic ant species to be translocated from future clearing areas.

Survey methods

We used two transects of mini-Winklers (MW) and four transects of pit-fall (PF) traps, as well as general hand collecting to document the ant fauna in the Ambatovy area. The MW collected ants and other invertebrates living in the leaf litter, and the PF traps captured ground foraging arthropods. Each method consisted of taking samples at 25 stations that are spaced 5 m apart along a linear transect line of 125 m. One transect of 25 samples of MW were used in the forest habitat at sites A-1 and B-C. Further, 25 stations of PF traps were established along the MW transect line in the identical areas. In the grassland habitat, at sites A-2 and A-3, 12 PF traps were installed at a distance of 5 m, and an additional 13 PF traps, separated by a distance of 5 m, were placed in the adjacent forest along a continuous transect.

The MW sampling at these two sites involved taking litter in three different plots around each station along the transect. These plots were marked with flags to facilitate (1) the search to determine whether endemic species are present in the MW and PF samples, and (2) the determination of suitable microhabitats for each endemic species as well as the georeferencing of their locations.

The MW method consists of extracting leaf litter, leaf mold, and rotten wood samples to obtain invertebrates. The material within each plot was finely diced with a machete to disturb ant nests occurring in small branches and decayed logs (Fisher, 1998; Fisher & Robertson, 2002). The chopped leaf litter from each plot was collected and sifted through a wire sieve with square holes of 1 x 1 cm. Ants and other arthropods were extracted from the sifted litter during a 48-hour period in Winkler sacks and conserved in 95% ethanol. The verification of MW samples using a binocular microscope was done one day after their extraction.

The PF trap method consisted of plastic cups of 65 mm internal diameter and 90 mm height, approximately half-filled with soapy water and a 5%

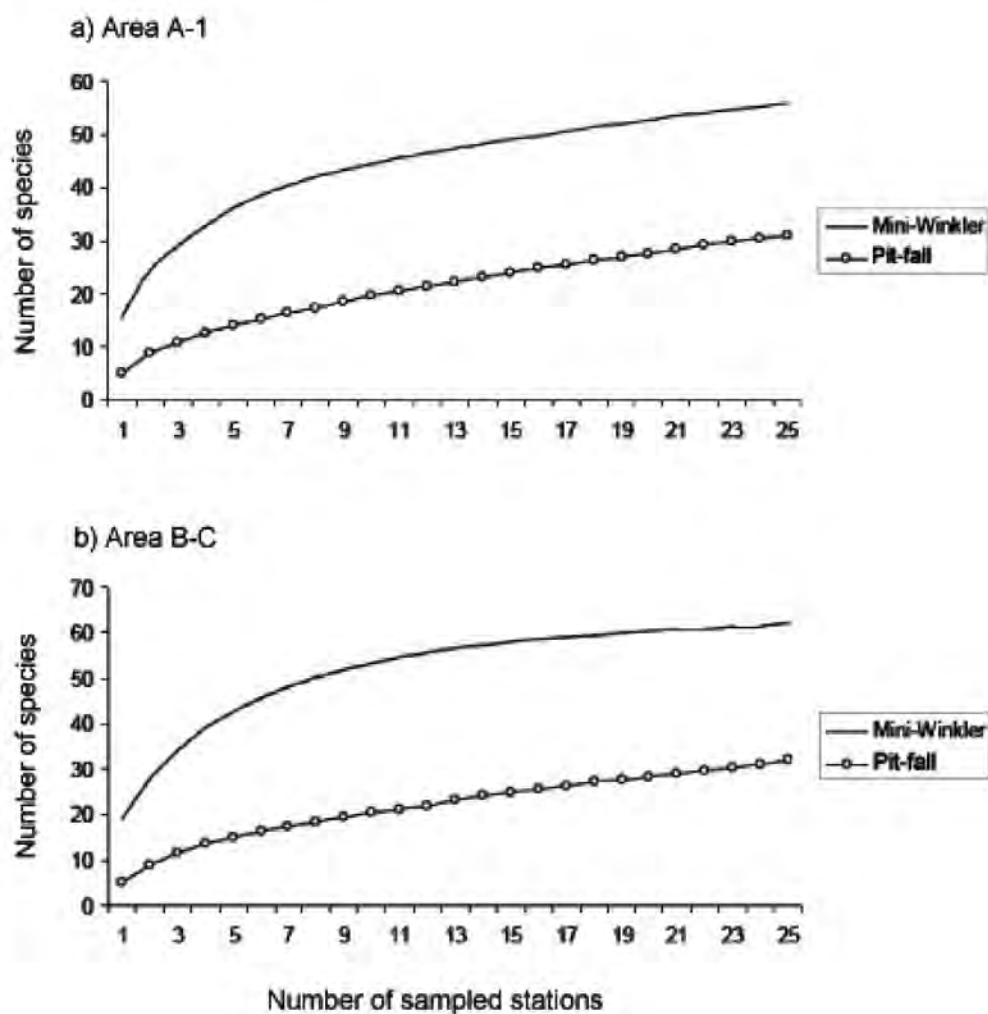


Figure 1. Comparison of the efficiency of mini-Winkler (MW) and pitfall (PF) trap methods and evaluation of ant species richness for the two Ambatovy forest sites: a) area A-1 and b) area B-C. The species accumulation curves in each chart plots the observed number of species as a function of the number of samples collected. Curves are plotted from the means of 100 randomizations of samples accumulation order.

solution of ethylene glycol, and then buried in the ground with the rim flush to the soil surface. Each trap was covered with a white plastic plate mounted above the trap leaving about 5 cm of space above the cup. Traps were left in place for four days.

General collecting is defined as randomly searching for ants in a variety of different microhabitats, including dead and live branches, low vegetation, rotten logs and stumps, under rocks, and in soil. This method helps to determine the likelihood of finding colonies of species potentially endemic to the area and important ecological information associated with their possible translocation to a receptor site.

The 2007 ant survey in the Ambatovy forest resulted in over 210 samples. All ant species collected during this survey were imaged using a digital camera and syncroscopy automontage software, and

can be accessed from Antweb (www.antweb.org). This web portal also includes distributional maps of different ant taxa, links to catalog information and original descriptions and re-descriptions of each named species. Pinned and alcohol preserved voucher specimens are stored at the Department of Entomology at the California Academy of Sciences, San Francisco. These samples were also used in different systematic and molecular studies concerning Malagasy ants and those from surrounding western Indian Ocean islands (Fisher & Smith, 2008).

Data analysis

Analyses were conducted in order to (1) to evaluate the efficacy of the quantitative methods, (2) to identify ants to species and morphospecies and determine whether local endemics occur at the study sites, and

Table 1. (cont.)

| Taxa | Area A-1 | | Area B-C | | | Area A-2 | | Area A-3 | |
|----------------------------------|----------|----|----------|----|----|----------|-----|----------|-----|
| | MW | PF | MW | PF | GC | PFg | PFf | PFg | PFf |
| <i>Paratrechina</i> sp01 | 1 | | 1 | 1 | | 1 | | | 1 |
| <i>Paratrechina</i> sp02 | 1 | 1 | 1 | | | | 1 | 1 | |
| <i>Paratrechina</i> sp03 | | | 1 | 1 | | | 1 | 1 | |
| <i>Paratrechina</i> sp04 | | | 1 | | | | | | |
| <i>Paratrechina</i> sp05 | | | 1 | | | | | | |
| <i>Plagiolepis alluaudi</i> | 1 | | 1 | | | | | 1 | 1 |
| Subfamily Myrmicinae | | | | | | | | | |
| <i>Aphaenogaster swammerdami</i> | | | | | | | 1 | 1 | |
| <i>Carebara</i> MG01 | 1 | | 1 | | | 1 | | | |
| <i>Cataulacus regularis</i> | 1 | | 1 | | | | | | |
| <i>Crematogaster</i> BBB13 | | 1 | | | | | | | |
| <i>Crematogaster</i> BBB14 | | | | | | 1 | | | |
| <i>Monomorium cryptobium</i> | 1 | | | | | | | | |
| <i>Monomorium fisheri</i> | 1 | 1 | | | | | | | |
| <i>Monomorium hanneli</i> | | | | | | | 1 | 1 | |
| <i>Monomorium hildebrandti</i> | 1 | | 1 | | | | | | |
| <i>Monomorium madecassum</i> | | | | | | 1 | 1 | 1 | |
| <i>Monomorium micrommaton</i> | 1 | | | | | | | | |
| <i>Monomorium termitobium</i> | 1 | | 1 | | | | | | |
| <i>Monomorium versicolor</i> | | | 1 | | | | | | |
| <i>Nesomyrmex retusispinosus</i> | | | 1 | | | | | | |
| <i>Pheidole</i> sp01 | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 |
| <i>Pheidole</i> sp02 | 1 | 1 | 1 | 1 | | | 1 | 1 | 1 |
| <i>Pheidole</i> sp03 | 1 | 1 | 1 | 1 | | | 1 | | 1 |
| <i>Pheidole</i> sp04 | 1 | 1 | 1 | 1 | | | 1 | | 1 |
| <i>Pheidole</i> sp05 | 1 | 1 | 1 | 1 | | 1 | 1 | | 1 |
| <i>Pheidole</i> sp06 | | | | | | 1 | 1 | | |
| <i>Pheidole</i> sp07 | 1 | | | | | 1 | | 1 | |
| <i>Pheidole</i> sp08 | | | | | | 1 | 1 | 1 | |
| <i>Pheidole</i> sp09 | | | 1 | | | | 1 | | |
| <i>Pheidole</i> sp10 | | | | | | | | | |
| <i>Pheidole</i> sp11 | | | 1 | 1 | | | | | |
| <i>Pheidole</i> sp12 | | | 1 | | | 1 | 1 | | |
| <i>Pheidole</i> sp13 | | 1 | | 1 | | | | | |
| <i>Pheidole</i> sp14 | | | | 1 | | | | | |
| <i>Pilotrochus besmerus</i> | | | 1 | | 1 | | | | |
| <i>Pyramica ambatrix</i> | 1 | | | | | | | | |
| <i>Pyramica ludovici</i> | | | | 1 | | | | | |
| <i>Strumigenys actis</i> | 1 | | 1 | | | | | | |
| <i>Strumigenys ampyx</i> | 1 | | | | | | | | |
| <i>Strumigenys chilo</i> | 1 | 1 | 1 | 1 | | 1 | 1 | | 1 |
| <i>Strumigenys dicomas</i> | 1 | | | | | | | | |
| <i>Strumigenys luca</i> | | | | | | | 1 | | |
| <i>Strumigenys lucomo</i> | 1 | | | | | | | | |
| <i>Strumigenys lura</i> | 1 | 1 | 1 | 1 | | 1 | 1 | | |
| <i>Strumigenys micrans</i> | | | 1 | | | | | | |
| <i>Strumigenys origo</i> | | | | | | | | | 1 |
| <i>Strumigenys rabesoni</i> | | | 1 | | | | | | |
| <i>Strumigenys sphaera</i> | | | 1 | | | | | | |
| <i>Strumigenys vazimba</i> | 1 | | 1 | | | | | | |
| <i>Strumigenys wardi</i> | 1 | | 1 | 1 | | | | | |
| <i>Tetramorium andrei</i> | | 1 | | | | | | | |
| <i>Tetramorium anodontion</i> | | | | | | 1 | | | |
| <i>Tetramorium bessonii</i> | | | | | | | 1 | 1 | 1 |
| <i>Tetramorium cognatum</i> | 1 | | 1 | | 1 | | | | |
| <i>Tetramorium coillum</i> | | 1 | | | | | | | |
| <i>Tetramorium degener</i> | | | | | | | 1 | | |
| <i>Tetramorium electrum</i> | 1 | 1 | 1 | 1 | | | 1 | | 1 |

Table 1. (cont.)

| Taxa | Area A-1 | | Area B-C | | | Area A-2 | | Area A-3 | |
|---------------------------------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|
| | MW | PF | MW | PF | GC | PFg | PFf | PFg | PFf |
| <i>Tetramorium marginatum</i> | 1 | 1 | 1 | 1 | | 1 | 1 | | 1 |
| <i>Tetramorium proximum</i> | 1 | | 1 | | | | 1 | | |
| <i>Tetramorium quasirum</i> | 1 | 1 | 1 | | | 1 | | | 1 |
| <i>Tetramorium ranarum</i> | 1 | | 1 | | | | | | |
| <i>Tetramorium robustior</i> | | | 1 | | | | 1 | | |
| <i>Tetramorium steinhelli</i> | 1 | 1 | 1 | 1 | | 1 | 1 | | 1 |
| Subfamily Ponerinae | | | | | | | | | |
| <i>Anochetus grandidieri</i> | 1 | 1 | 1 | | | 1 | | | |
| <i>Hypoponera</i> MG009 | | | 1 | | | | | | |
| <i>Hypoponera</i> MG014 | 1 | | 1 | | | | | | |
| <i>Hypoponera</i> MG026 | 1 | | | | | | | | |
| <i>Hypoponera</i> MG031 | 1 | | | | | | | | |
| <i>Hypoponera</i> MG038 | 1 | | 1 | | | | | | |
| <i>Hypoponera</i> MG039 | 1 | 1 | 1 | | | | | | |
| <i>Hypoponera</i> MG052 | 1 | | 1 | | | | | | |
| <i>Hypoponera</i> MG066 | 1 | | 1 | 1 | | | 1 | | |
| <i>Hypoponera</i> MG074 | 1 | 1 | 1 | | | | | | |
| <i>Hypoponera</i> MG104* | 1 | | 1 | | | | | | |
| <i>Leptogenys angusta</i> | | 1 | 1 | 1 | | | 1 | 1 | 1 |
| <i>Leptogenys</i> sp1 | 1 | | | | | | | | |
| <i>Pachycondyla cambouei</i> | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 |
| <i>Pachycondyla</i> JCR10 | | | 1 | | | | | | |
| <i>Platythyrea bicuspis</i> | 1 | 1 | | | | | | | 1 |
| <i>Platythyrea</i> MG02* | | 1 | | | | | | | |
| Subfamily Proceratiinae | | | | | | | | | |
| <i>Discothyrea</i> MG01 | 1 | | 1 | | 1 | | | | |
| <i>Proceratium</i> MG08 | 1 | | | | 1 | | | | |
| Subfamily Pseudomyrmecinae | | | | | | | | | |
| <i>Tetraoponera grandidieri</i> | 1 | | | | | | | | |
| Total number of species | 58 | 28 | 61 | 28 | 9 | 20 | 30 | 18 | 18 |

Results

Accumulation curves for ant species found on the transects of areas A-1 and B-C show a decrease in the rate at which species number accumulates as a function of increased sampling (Figure 1). However, these curves continued to increase slowly and did not reach an asymptote. Visual inspection of these species accumulation curves indicates that the litter sifting technique (mini-Winklers) yielded a higher rate of species accumulation than pit-fall traps. Further, data from the litter sifting method shows a slight increase in the curve after six samples, whereas curves for the pit-fall method increased linearly to the end of the sampling transect. The mini-Winkler method captured a greater number of ant species than the pit-fall traps: 58 versus 28 species in area A-1 and 61 versus 28 species in B-C.

Pit-fall trapping in forests sites A-1 and B-C (Figure 1) produced very different curves than in grassland sites A-2 and A-3 (Figure 2). The forest sites accumulated fewer species than the grassland sites. The grassland sites accumulated fewer additional

species with increased sampling and did not reach an asymptote, while at the forest sites, species number continued to increase in a nearly linear fashion across the different stations. Within grassland site A-2, the pit-fall method resulted in a larger number of ant species than in the forest habitats. In the case of grassland site A-3, the method recorded the same number of species in both open habitat and adjacent forest habitat (Figure 2).

In total, 112 species belonging to 29 ant genera were found at the four Ambatovy sites sampled in March 2007 (Table 1). Of these, three species (*Pyramica ludovici*, *Technomyrmex difficilis*, and *T. pallipes*) are thought to be introduced to Madagascar, and two species are considered as endemics to Ambatovy (*Hypoponera* MG104 and *Platythyrea* MG02). Forest sites A-1 and B-C yielded 28 genera and 91 species and grassland sites A-2 and A-3 contained 18 genera and 46 species. Of the 91 species found in the forest sites, 21 are shared with the grassland sites. According to the Madagascar Biodiversity Center database, the ant fauna of Ambatovy is largely similar

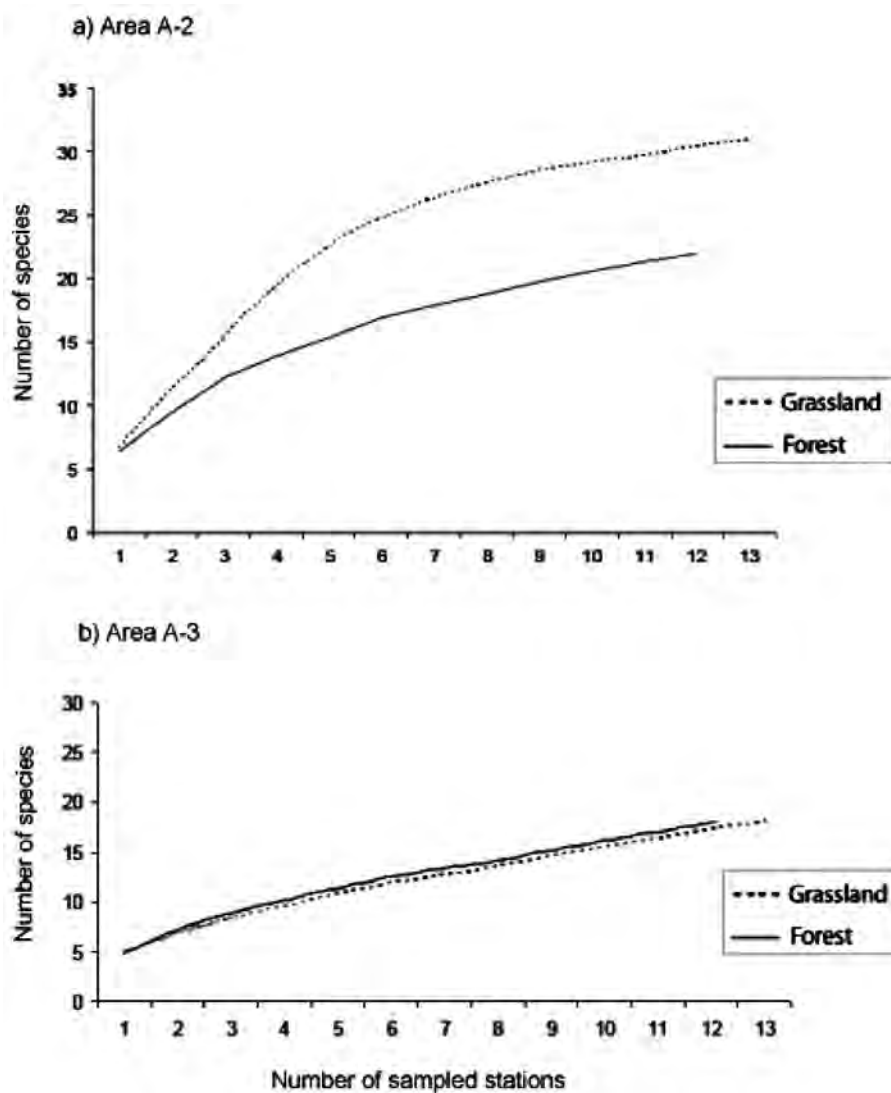


Figure 2. Assessment of the efficiency of pit-fall (PF) technique and comparison of ant species richness in the open grassland areas of Ambatovy, A-2 and A-3. Pit-fall traps were placed in a continuous line across the grassland and forest habitats. In each chart plot, solid lines indicate the results from the forest sites, and dashed lines show the results from grassland sites.

to that of other montane forests on the island located at comparable elevations.

The two grassland areas, A-2 and A-3, were chosen to study the presence and potential impact of invasive ants in anthropogenic habitats in close vicinity to forest ecosystems. Whereas the grassland site and the adjacent forest site sampled from area A-3 provided 18 species each, the grassland site in A-2 had notably less ant species diversity than its adjacent forest site: 20 versus 30 species (Table 1).

Discussion

Efficiency of the quantitative methods

Assessments of insect diversity can be achieved within a time frame relevant to conservation planning if we rely on a focal taxonomic group that is biogeographically informative, relatively well-known

taxonomically, and not difficult to capture or observe (Kremen, 1994). Efficient collecting methods (Longino & Colwell, 1997; Fisher, 1999) and a clear processing protocol (Fisher, 2005) have been developed for ants to obtain accurate, fine-scaled, and comparable data across sites. Because ants vary considerably in foraging behavior, nest sites, and interactions with the environment, various sampling methods yield different types of ant species (Longino & Colwell, 1997; Fisher, 1999). In this study, we evaluated the efficiency of mini-Winkler and pit-fall sampling methods to survey ground-dwelling ants in forest and grassland habitats in Madagascar.

Our results showed that litter sifting was more efficient than pit-fall traps in forest habitats. Indeed, the mini-Winkler and pit-fall traps method collected 87% and 85% of the total number of species recorded by both quantitative methods from forest sites A-1

Table 2. List of 23 ground-dwelling ant species not identified from the mini-Winkler and pit-fall samples collected in March 2007, but documented from the Ambatovy forests in 2004 or 2005 based on leaf litter sampling (litter) and general collecting (GC). The code for unknown species (e.g. BBB14, JCR06, MG01, MG042, L_MG02) are recognized undescribed taxa; *Cerapachys*, *Cerapachys L*, and *Cerapachys P* are three different genera.

| Subfamily | Species | Year for collection | Method |
|----------------------|-------------------------------|---------------------|------------|
| Amblyoponinae | <i>Adetomyrma</i> MG01 | 2005 | GC |
| | <i>Amblyopone</i> MG04 | 2004 | Litter |
| | <i>Cerapachys L_MG02</i> | 2004 | Litter |
| Cerapachyinae | <i>Cerapachys</i> MG03 | 2005 | GC, litter |
| | <i>Cerapachys P_MG06</i> | 2004 | GC |
| | <i>Cerapachys P_MG11</i> | 2005 | GC |
| | <i>Camponotus</i> MG010 | 2004 | GC |
| | <i>Camponotus</i> MG013 | 2004 | GC |
| Formicinae | <i>Camponotus</i> MG074 | 2004 | Litter |
| | <i>Camponotus</i> MG079 | 2004 | GC |
| | <i>Camponotus nasicus</i> | 2004 | GC |
| | <i>Paratrechina glabra</i> | 2004 | Litter |
| Myrmicinae | <i>Pyramica erynnes</i> | 2004 | Litter |
| | <i>Pyramica symmetrix</i> | 2004 | Litter |
| | <i>Tetramorium delagoense</i> | 2004 | GC |
| | <i>Hypoponera</i> MG048 | 2004 | Litter |
| | <i>Hypoponera</i> MG082 | 2004 | Litter |
| Ponerinae | <i>Hypoponera</i> MG084 | 2004 | Litter |
| | <i>Hypoponera</i> MG087 | 2004 | GC |
| | <i>Pachycondyla ambigua</i> | 2004 | GC |
| | <i>Pachycondyla</i> JCR06 | 2004 | GC |
| Proceratiinae | <i>Discothyrea</i> MG02 | 2004 | Litter |
| | <i>Proceratium</i> MG02 | 2005 | GC |
| Total species | 23 | | |

and B-C, respectively. This study underlines the importance of leaf litter sifting to survey ground-dwelling ants in forest habitats of Madagascar.

Although previous studies in Madagascar have demonstrated that pit-fall traps are relatively ineffective for collecting ants in humid forests (Fisher 1999; Fisher & Robertson, 2002), the present study contradicts those results. Pit-fall traps collected 43% and 40% of the total number of species sampled by mini-Winkler and pit-fall traps allowed for the recording of several new species (8 and 10 species) from areas A-1 and B-C not sampled via the MW method. Fisher (1999) ascribed his finding to the fact that large generalist scavenger ant species that forage on the forest floor and, therefore should be trapped by the pit-fall method, are either absent or of smaller size in the wet forests of Madagascar (but see Olson, 1991). However, our results show that pit-falls collected more species than expected in the Ambatovy forest sites. This can be explained by the fact that our approach in choosing transect sites was ecologically biased, which may have increased the number of species collected in the traps. Also, the collection of several species in pit-fall traps could be attributed to the disturbance of ant nests in litter, small twigs, rotten logs, under root mats, etc. made by ant collectors during the general collecting period

while the traps were still operating. This suggests that general hand collecting should be done cautiously if other traps in a transect line are still operating at the same site. However, further analyses are required to test these hypotheses.

It has been shown on Madagascar that pit-fall traps are more useful when sampling ants in grassland (Fisher & Robertson, 2002) and dry forest habitats (Fisher & Razafimandimby, 1997). Our study demonstrated that in the forest sites, species accumulation curves for the pit-fall method were nearly linear and increased steeply until the end of the transect sampling period. In contrast, in the grassland habitats the curves showed a decreased number of additional species as sampling increased. The number of ant species recorded within areas A-2 increased even though the sampling effort was slightly less (12 traps in the disturbed forests and 13 in grasslands) relative to that at sites A-1 and B-C (25 samples for each area). These results confirm that pit-fall traps are a useful method for collecting ants in open habitats.

Ants are considered one of the largest arthropod communities in arboreal microhabitats (Hölldobler & Wilson, 1990). Several studies have demonstrated their dominance in the canopy stratum (Erwin, 1983; Wilson, 1987; Stork, 1991). However, arboreal ants

were not sampled during our study at Ambatovy, although they have been documented to show associations with particular local plants (Dejean *et al.*, 2007) and may exhibit greater local endemism. This suggests that the number of endemic ant species within the Ambatovy region is most likely to increase if aboveground microhabitats are included in terrestrial arthropod surveys.

Comparison of the species richness at sites A-2 and A-3

At sites A-2 and A-3, the overall ant species richness collected in forest and grassland habitats were not the same. At site A-2, more species were collected in the forest (30 species) than in grassland (20 species), while sampling in each of these two habitats at site A-3 yielded the same number of species (18 species). These differences may be due to the condition of the forest at A-3, which is much more degraded and isolated from the forest core than at site A-2. Several studies have indicated that the degree of fragmentation and the distance to continuous forest significantly influence species richness (Fahrig & Jonsen, 1998; Suarez *et al.*, 1998; Collinge, 2000; Bruna *et al.*, 2005). The distance between site A-3 and the forest block may be large enough to hinder the dispersal of certain ant species. The results from site A-2 suggest that large continuous tracts of forest play important roles as population sources for isolated areas, as predicted from the theory of island biogeography (McArthur & Wilson, 1967).

The results of this study also support the idea that anthropogenic disturbances, such as habitat destruction and fragmentation, have drastically altered the ant community assemblages in the Ambatovy region. Given that ants are ecologically dominant and play an important role in ecosystem functioning, changes in their composition will affect biological diversity. Further studies are needed to understand the ecological effects in other disturbed areas and could be of great value for conservation planning in Madagascar.

Ecology of the genus *Pilotrochus*

Madagascar harbors great biological diversity (Lourenço & Goodman, 2000; Myers *et al.*, 2000). Ants are among the island's most diverse animal groups, occupying a wide range of terrestrial habitats, experiencing high species turnover, and include numerous microendemic taxa. In this study, we collected the endemic Malagasy ant genus

Pilotrochus at site B-C. *Pilotrochus besmerus* was collected only via the sifted litter method, but not in pit-fall traps. This implies that the worker caste does not travel on ground, but rather within litter. Thus, this ant appears to have an ecologically restricted niche. Neither live individuals nor colonies of the species have been collected during Madagascar Biodiversity Center surveys. The fieldwork at Ambatovy in 2007 allowed us to obtain several live individual workers of *P. besmerus*.

Though the genus has previously been sampled from leaf litter sifting in eastern rainforest habitats at 900 m, its microhabitat was previously unknown. Individual workers of the genus were found in a forest site that is characterized by a generally thin layer of leaf litter and other decaying matter, and is rich in soil containing many small roots. At this site, the vegetation structure consisted of varied herbaceous understory, and a widely open area of a few interspaced trees through which solar radiation can reach the forest floor. Taken together, this information suggests that *Pilotrochus* is one of the few Malagasy ants that forage in the leaf-litter and underground, preys on small invertebrates, and probably nests in the soil. The high abundance of the genus in Ambatovy region provides an ideal situation to study its biology.

Evaluation of the 25 sample transect

Although previous inventories of ants at 14 sites in the eastern rainforest of Madagascar used transects of 50 samples, it has been shown that transects of 25 samples were sufficient to compare both species richness and species turnover among sites (Fisher, 1999). As ants are among the most diverse fauna at a given site, obtaining more than 25 samples would most likely uncover additional species. Indeed, the comparison of the number of ant species collected in the March 2007 work at Ambatovy with those recorded in 2004 or 2005 in the same region showed that roughly 23 more native leaf litter species (Table 2) collected during the earlier fieldwork were not sampled in the 2007 fieldwork. Also, 42 more ground-dwelling species collected from fieldwork conducted in March 2007 were absent from the samples recorded in 2004 or 2005 (Table 3). This suggests that a transect of only 25 samples, though effective for checking samples in the field, is inadequate to survey the rare ant species in Madagascar.

Table 3. List of 42 ground-dwelling ant species sampled from Ambatovy forests in March 2007, but not collected in 2004 or 2005. The code for unknown species (e.g. sp01, sp02, etc) follows that of recognized morphospecies and those with a different code (e.g. BBB13, MG01, MG009, L_MG11) are undescribed taxa recognized by the Madagascar Biodiversity Center; *Cerapachys* and *Cerapachys L* are two different genera.

| Subfamily | Species | Methods |
|-------------------------------|--------------------------------|------------|
| Amblyoponinae | <i>Mystrium</i> MG01 | PF |
| Cerapachyinae | <i>Cerapachys L</i> MG11 | Litter |
| | <i>Cerapachys</i> MG05 | Litter |
| Dolichoderinae | <i>Technomyrmex difficilis</i> | PF, litter |
| | <i>Technomyrmex pallipes</i> | PF |
| | <i>Camponotus</i> MG024 | Litter |
| | <i>Camponotus</i> MG039 | PF |
| Formicinae | <i>Paratrechina</i> sp01 | PF, litter |
| | <i>Paratrechina</i> sp02 | PF, litter |
| | <i>Paratrechina</i> sp03 | PF, litter |
| | <i>Paratrechina</i> sp04 | Litter |
| | <i>Paratrechina</i> sp05 | Litter |
| | <i>Crematogaster</i> BBB13 | PF |
| | <i>Crematogaster</i> BBB14 | PF |
| | <i>Monomorium cryptobium</i> | Litter |
| | <i>Monomorium fisheri</i> | PF, litter |
| | <i>Monomorium hanneli</i> | PF |
| | <i>Monomorium vrsicolor</i> | Litter |
| | <i>Pheidole</i> MG03 | PF |
| | <i>Pyramica ambatrix</i> | Litter |
| | <i>Pyramica ludovici</i> | PF |
| Myrmicinae | <i>Strumigenys ampyx</i> | Litter |
| | <i>Strumigenys ection</i> | Litter |
| | <i>Strumigenys lucomo</i> | PF, litter |
| | <i>Strumigenys micrans</i> | Litter |
| | <i>Strumigenys origo</i> | PF |
| | <i>Strumigenys sphaera</i> | Litter |
| | <i>Strumigenys wardi</i> | Litter |
| | <i>Tetramorium andrei</i> | PF |
| | <i>Tetramorium anodontion</i> | PF |
| | <i>Tetramorium bessonii</i> | PF |
| | <i>Tetramorium cognatum</i> | Litter |
| | <i>Tetramorium coillum</i> | PF |
| | <i>Tetramorium degener</i> | PF |
| | <i>Tetramorium electrum</i> | PF |
| <i>Tetramorium marginatum</i> | PF | |
| Ponerinae | <i>Tetramorium proximum</i> | Litter |
| | <i>Hypoponera</i> MG009 | Litter |
| | <i>Leptogenys</i> MG01 | PF |
| Proceratiinae | <i>Platythyrea</i> MG02 | PF |
| | <i>Proceratium</i> MG08 | Litter |
| Pseudomyrmecinae | <i>Tetraponera grandidieri</i> | Litter |
| Total species | 42 | |

Native ants in grassland habitats

Most ant species collected from the grasslands during this survey are native to Madagascar (Table 1). Although tropical ant diversity correlates positively with plant diversity and structure (Perfecto & Snelling, 1995), and ants are susceptible to disturbance and forest fragmentation (Andersen *et al.*, 2002), the causal mechanisms that affect the high number of native ants in the grassland sites are unclear at the Ambatovy sites. It is possible that various types of

foraging behavior and nest site selection of these taxa may allow them to survive in a wide range of habitats, such as agricultural lands and other anthropogenic modified habitats. The ability of native ant species to colonize grasslands, a presumed anthropogenic habitat, could be attributed to the absence or the reduced number of introduced ant species in this habitat. Future studies should analyze how different types of landscapes influence ant assemblages and especially the invasiveness of introduced ant species.

The colonization of the disturbed ecosystems of Ambatovy by ants typically found in natural intact forest leads to several questions: (1) whether these species are indigenous and (2) whether they are competitively strong and able to limit the range expansion of aggressive introduced species. These are questions to be addressed by future research projects in the region. In addition, ecological studies of these native dominant and opportunistic species are needed in order to evaluate the threats they pose and inform future management plans.

Translocation overview

Intentional translocation of populations of certain species from one place to another may be essential for conservation planning in regions where natural habitats are destined for alteration or complete destruction. Recently, there has been increasing interest in translocation programs, most of which have focused on vertebrates (Griffith *et al.*, 1989; Dodd & Seigel, 1991; Wolf *et al.*, 1996; Lettink, 2007; Germano & Bishop, 2009). Attempts to address the translocation of arthropods have seldom been considered.

Translocation of locally endemics in the Ambatovy forests has been proposed as a mitigation measure associated with habitat loss caused by mining extraction. However, the effect of moving arthropods, for example ants, from one site to another may be of potential concern, as it seems difficult to determine if translocations have succeeded or not in the past. Some biologically based criteria have been suggested to evaluate the success of the translocation including stable, self-sustaining (Griffith *et al.*, 1989; Dodd & Seigel, 1991), and multiplying populations (Wolf *et al.*, 1996; Town & Ferreira, 2001). Thus, such factors should be taken into consideration to evaluate the success of translocation. As ants are social insects, their translocation necessitates moving the whole colony or the mated castes rather than only individual worker castes. In addition, the receptor site should be surveyed to evaluate its species diversity prior to the translocation of animals from another area.

Given their ecological diversity, they are an interesting group for translocation and monitoring experiments. However, given that numerous aspects of their systematics, life-history tactics, and basic natural history are largely lacking, decisions associated with conservation actions, including translocation, may be premature to properly advance certain programs. Ongoing projects employing traditional morphological taxonomy combined with

molecular sequence divergence (Hebert *et al.*, 2003a, 2003b; Fisher & Smith, 2008) will accelerate species diagnosis and the description of new species.

On the basis of current data, it is not possible to state how many ant species are restricted to the Ambatovy region, specifically at sites with disturbed faunas due to the mine and in possible need of translocation. In addition, there are indeed risks both for translocated colonies that fail to establish self-sustaining populations (Griffith *et al.*, 1989) and for the receptor ecological communities subjected to the effects of the translocated species (Winkler & Jenkins, 1991; Meltzer, 1993; Woodford & Rossiter, 1994; Wolf *et al.*, 1996). Therefore, until additional data on the ant fauna in the area are available and, for that matter other portions of Madagascar, moving an ant colony from one place to another, is risky.

Invasive ant species

Although other studies have demonstrated that habitat fragments and anthropogenically disturbed habitats contain a reduced ant fauna and that they are more prone to invasion by introduced ant species (Majer & Nichols, 1998; Suarez *et al.*, 1998), sampling at Ambatovy in the grassland sites recorded few exotic species and a relatively diverse native fauna. Three exotic ant species were documented, including *Technomyrmex difficilis* and *T. pallipes*, which are closely related to one of the notably detrimental ant invaders, *T. albipes*. Even though no data are currently available, these introduced species could have similar ecological impacts on the native fauna as *T. albipes*.

The 2007 fieldwork in the Ambatovy region did not document the local presence of *Pheidole megacephala* and *Technomyrmex albipes*, although the former species is known to occur in the nearby city of Moramanga and the later species is widely distributed in the lowland eastern portion of the island. In the littoral forest of Tampolo, north of Toamasina, *T. albipes* populations occurring in disturbed and fragmented forest have already been shown to be associated with reduced native ant populations (Fisher *et al.*, 1998; Ratsirarson *et al.*, 2005). Both *P. megacephala* and *T. albipes*, along with *Solenopsis geminata* recorded from northern Madagascar (Fisher, 1997), have been documented to be aggressive invaders and shown to have negative effects in other parts of the world (Lowe *et al.*, 2000). However, little is known about the degree to which they have invaded different portions of the island. For example, *T. albipes* is widely distributed along the eastern coastal portion of the island, below 200 m (Fisher *et al.*, 1998), and

it is unknown if its absence at Ambatovy is due to ecophysiological limitations associated with elevation or simply that it has not yet reached the area.

The species composition of introduced ant species on islands changes over time (Wilson & Taylor, 1967). Hence, other invasive species that are known elsewhere in the world (Lowe *et al.*, 2000) may be potential invaders in the Ambatovy area and other parts of Madagascar, at least in part associated with increasing international travel and trade and the risk of introduction. Such ants might include, for example, *Linepithema humile*, *Solenopsis invicta*, and *Wasmannia auropunctata* (Holway *et al.*, 2002). Accordingly, regular surveys of local ant species in the area surrounding the Ambatovy mining operation are needed to monitor and control any possible ant introduction. In addition, preventive monitoring should also be carried out by examining shipments that arrive via maritime vessels or air transport at ports of entry as international transport is an important source of introduced ants (Lockwood *et al.*, 2005; Suarez *et al.*, 2005). New research approaches to understanding the effects of invasive species in the Ambatovy area and across the island should be initiated and should be based on knowledge and ecological monitoring of individual species (Simberloff, 1997). In Madagascar, as in Australia (Lach & Thomas, 2008), introduced ants may negatively influence ecological aspects of the native ant species.

Conclusion

Due to limited resources and time, translocating locally endemic ant species in the Ambatovy region to non-threatened forest sites would most likely not be a viable conservation strategy. Further investigation on the behavior, ecology, and natural history of the species predicted to be displaced, as well as the habitat quality of the receptor site, are needed before this alternative can be properly considered. This current study provides new information on the ants and other ground-dwelling invertebrate groups of the Ambatovy region. These collections provide important archival information and should foster studies of these organisms in the future.

Discoveries of endemic ant species within a region are almost certain to increase when aboveground and canopy sampling are included. These microhabitats were not considered in the current study. The results from collaborative taxonomy in Madagascar, which applies traditional morphological taxonomy and molecular genetic data to accelerate species diagnosis and systematic advancements, will facilitate the

generation of methodologies for translocating ants and other arthropod groups on the island. Over the long term, introduced invasive ants on Madagascar could: (1) result in the extinction of a significant portion of the highly endemic and ecologically important native ant communities; and (2) increase the overall similarity of the region's ant diversity. Both regular surveys of the area surrounding the mining site, as well as across Madagascar, and preventive monitoring are needed to understand the ecological impacts of invasive species in the Ambatovy area and across the island.

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