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Par

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Diversité Cryptique, Bioacoustique et Interactions Intra et Interspécifiques dans le Complexe d'Espèces Primitives Néotropicales *Pachycondyla apicalis* (Hymenoptera : Formicidae : Ponerinae)

Soutenue le 10 Décembre 2010 devant le jury composé de :

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“Conheci uma guerreira que distribuía flores... mas ela partiu.”

A la mémoire de ma mamie Cecília Maria das Dores (Vó Filinha)

je dédie cette thèse.

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INTRODUCTION

''Quoique dans l'immense série des êtres, la fourmi ne soit qu'un point qui sans sa mobilité échapperait presque à nos regards, il n'en est pas moins vrai que cet atome animé est digne d'être l'objet de nos méditations. C'est ici qu'il convient de dire que l'Auteur de la nature n'est jamais plus lui même que dans ce qu'il y a de plus petit.''

Latreille, 1802

Le succès écologique des fourmis

Si par la taille de ses individus elle peut paraître discrète et négligeable, la famille des Formicidae étonne par son succès écologique incomparable. Depuis leur origine il y a environ 120-140 millions d'années, les fourmis ont évolué de manière extraordinaire pour devenir le groupe d'insectes sociaux le plus diversifié morphologiquement et écologiquement (Grimaldi & Engel 2005, Hölldobler & Wilson 1990). En effet, avec plus de 12.500 espèces décrites (Bolton et al. 2006) et regroupées actuellement en 21 sous-familles (Bolton 2003, Grimaldi & Agosti 2000, Ward & Brady 2003), on estime que les Formicidae pourraient compter probablement plus de 20.000 espèces (Agosti com. pers.). Les fourmis ont subi, au cours de leur évolution, un rayonnement adaptatif leur permettant d'occuper quasiment tous les écosystèmes de la planète. La seule région Néotropicale abrite ainsi 14 de ces sous-familles, représentées par plus de 3016 espèces valides (Fernandez & Sendoya 2004), ce qui en fait l'une des régions les plus riches au monde en termes d'espèces.

De par leur succès écologique, l'impact écologique des fourmis est aussi considérable. Dans les forêts tropicales de la planète, les fourmis dominent tous les autres groupes d'animaux métazoaires par leur abondance, la biomasse totale qu'elles représentent, la gamme de leurs interactions écologiques et leur influence sur les processus de fonctionnement des écosystèmes (Hölldobler & Wilson 1990, Agosti et al. 2000, Kaspari et al. 2000, Wilson & Hölldobler 2005). Elles sont les canalisateurs principaux de l'énergie et de la matière organique, des aérateurs importants du sol et leur activité de réaménagement des sols est égale ou supérieure à celles des vers de terre (Hölldobler & Wilson 1990, Passera & Aron 2005). Elles sont parmi les prédateurs principaux des invertébrés dans la plupart des écosystèmes et participent aussi à la dissémination de plus d'un tiers des espèces végétales (Handel et al. 1981). Elles sont parfois considérées comme des ravageurs (*Atta* et *Acromyrmex*, par

exemple), des agents de lutte biologique (Delabie & Mariano 2001, Sujji et al. 2004), ou encore des bioindicateurs de la diversité de l'environnement (Agosti et al. 2000).

Une grande partie du succès écologique des fourmis peut être attribuée au haut niveau de coopération qu'on observe au sein de ses colonies parce qu'il permet une plus grande efficacité et l'exploitation de niches autrement indisponibles (Wilson 1971, Hölldobler & Wilson 1990). La complexité du fonctionnement des colonies a mené les biologistes à les considérer comme eusociales, ce qui représente le stade de coopération le plus élaboré entre les animaux vivants en groupe. L'eusocialité est définie par trois caractéristiques biologiques strictes: la coopération des adultes dans le soin aux jeunes, le chevauchement d'au moins deux générations d'individus à un moment donné de la vie coloniale, et la division du travail au niveau reproducteur, c'est-à-dire l'existence d'individus spécialisés dans la reproduction (Wilson 1971). Ces caractéristiques leur permettent de développer des sociétés incomparables en taille dont la coordination par un système de communication complexe est indispensable à leur cohésion et au maintien de la colonie comme une unité adaptative (O'Donnell & Bulova 2007, Hölldobler &Wilson 2009).

La communication chez les fourmis

La communication animale est un phénomène très diversifié, et ses fonctions et ses effets dépendent tellement du contexte environnemental et social que certains auteurs refusent de donner une définition précise à ce concept (Hölldobler 1984). Tous s'accordent cependant sur un schéma général des systèmes de communication. Ce dernier repose toujours sur l'existence d'un couple émetteur(s)/récepteur(s) ayant une interaction à valeur informative. Toutefois, un simple transfert d'informations ne suffit pas à définir une communication. Car elle doit inclure un échange réciproque, où le comportement de l'émetteur est conçu pour influencer le récepteur de telle façon qu'il change son comportement (McFarland 2001).

Comme la plupart des insectes, les fourmis peuvent utiliser de nombreux canaux de communication : chimique, visuel, tactile et acoustique (Hölldobler & Wilson 1990, Virant-Doberlet & Cokl 2004). Ce dernier est le moins bien compris car l'anthropomorphisme y est omniprésent, à commencer par les définitions du son et de l'ouïe qui sont implicitement basées sur nos paramètres humains. Pourtant, le monde « auditif » des insectes est bien plus complexe que le nôtre. Il en va de même des modes de production du son qui sont beaucoup plus variés. Les sons perçus par l'oreille humaine sont plus limités que chez les insectes, parce qu'ils correspondent uniquement à des ondes de pression véhiculées par l'air. Chez de nombreuses espèces animales, dont plusieurs groupes d'insectes, la « fonction auditive »

intègre une dimension sensorielle bien plus vaste, incluant par exemple les infra et ultrasons ainsi que la perception des vibrations transmises par les substrats (Claridge 2006).

Chez les fourmis, la communication chimique est considérée comme largement prépondérante par rapport aux autres moyens (Billen 2006, Le Conte & Hefetz 2008, Monnin 2006). Comme chez la plupart des insectes, il existe chez les fourmis de nombreuses glandes exocrines réparties sur l'ensemble du corps qui permettent de produire, séquestrer et sécréter divers composés chimiques qui sont utilisés comme signaux dans les divers contextes comportementaux (Billen & Morgan 1998). Ces composés constituent les phéromones qui garantissent la cohésion et la coordination des tâches, comme par exemple dans le cas de l'approvisionnement où différents modes de recrutements par piste peuvent indiquer la présence ou la localisation d'une source de nourriture (Jackson & Ratnieks 2006) ou dans la défense des colonies où les phéromones d'alarme conditionnent une réponse groupée (Van der Meer & Alonso 1998). Les signaux portés par les fourmis véhiculent des informations diverses pouvant porter à la fois sur l'espèce (Errard et al 2006, Delattre et al. données non publiées), la colonie (Lahav et al. 1999), la caste (Wheeler & Nijhout 1984) et même le stade de développement des individus (Tschinkel 1995), comme c'est le cas des hydrocarbures cuticulaires (Blomquist & Bagnères 2010). Ces bouquets de molécules permettent les phénomènes de reconnaissance entre colonies (Martin et al. 2008), individus (d'Ettorre & Heinze 2005) ; individus de différents statuts et états physiologiques (Blacher et al. 2010, Dietemann et al. 2003), ou encore du territoire (Tanner & Adler 2009). Au cours des 30 dernières années, des progrès énormes ont été réalisés dans la compréhension des phéromones d'insectes, de régulation de leur biosynthèse à leurs modes de perception en passant par leurs modes de sécrétion (Vargo & Laurel 1994).

Outre la machinerie glandulaire pour produire les phéromones, de nombreuses espèces de fourmis sont aussi dotées d'un organe spécialisé dans la production de sons et de vibrations (Markl 1973, Taylor 1978). Ces signaux acoustiques, les stridulations, ont de manière similaire aux signaux chimiques différentes rôles dans le fonctionnement des colonies de certaines espèces, en agissant en particulier comme modulateurs de comportements sociaux (Hölldobler et al. 1978, Markl & Hölldobler 1978, Baroni-Urbani et al. 1988). Les stridulations sont donc des événements couramment observés dans les sociétés de fourmis.

Pourtant, les premières mentions de la présence de cette faculté chez les fourmis datent à peine de la fin du 19ème siècle (Landois 1874, Lubbock 1877, Janet 1893, 1894, Sharp 1893). Il a en effet fallu très longtemps avant de commencer à comprendre comment les insectes utilisaient les sons. Ce n'est que depuis 60 ans que la recherche des associations entre

les signaux acoustiques des insectes et leur comportement est devenue un important sujet d'étude scientifique (Claridge 2006). En conséquence, les signaux acoustiques n'ont été étudiés chez les fourmis, à l'inverse des signaux chimiques, que dans un nombre très limité de recherches jusqu'à présent (Figure 1), et la plupart des travaux abordent uniquement la sous-famille des Myrmicinae. Des études détaillées sur les caractéristiques acoustiques de ces signaux sont beaucoup plus rares chez les fourmis « primitives » comme les Ponerinae. De ce fait, la communication acoustique demeure un des moyens de communication le moins bien compris chez les fourmis (Ferreira & Fresneau 2009).

A cause de cet intérêt disproportionné pour un canal de communication par rapport aux autres, Hölldobler (1999) a attiré l'attention sur le fait que la complexité de la communication chez ces insectes peut avoir été sous-estimée et sur la nécessité d'étendre désormais ces études à d'autres modalités, ainsi qu'à la combinaison des signaux provenant de différentes sources.

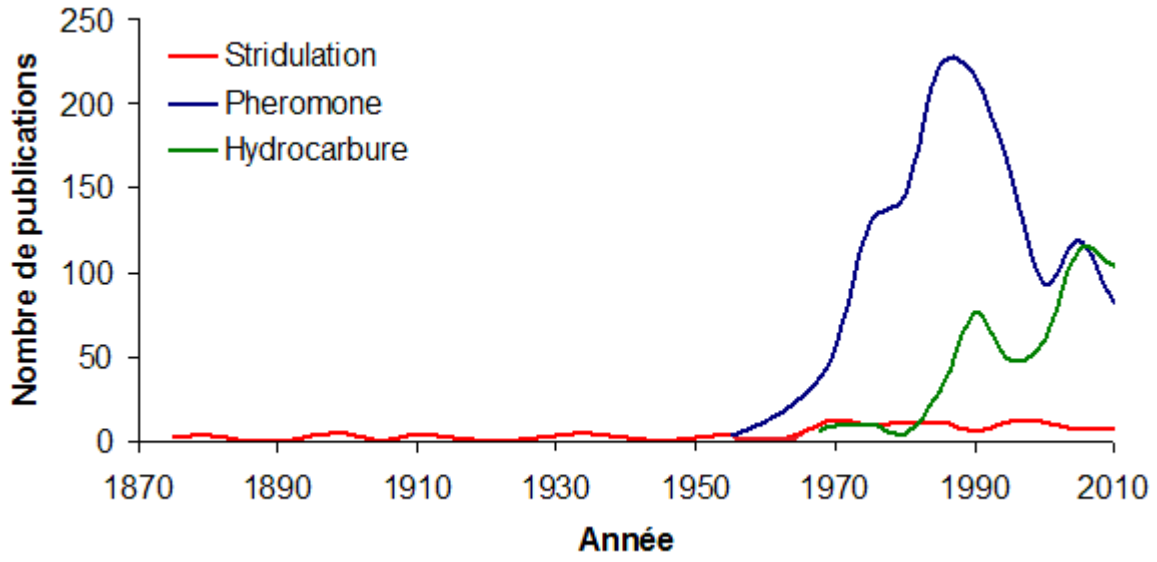


Figure 1. Effort de recherche sur la communication chez les fourmis depuis la découverte de l'organe stridulatoire (Landois 1874), la définition du terme phéromone (Karlson & Lüscher 1959) et premières évidences de l'implication des hydrocarbures cuticulaires dans le transfert d'informations (Bergström & Löfqvist 1968). Nombre de publications dans la base de données FORMIS 2009 (Wojcik & Porter 2009) contenant les mots 'stridulation', 'pheromone' ou 'hydrocarbure', sur un total de plus de 45000 références.

La communication acoustique chez les fourmis

Les premiers chercheurs ayant testé la capacité des fourmis à percevoir des sons l'ont fait avec leur propre voix et en jouant devant les fourmilières d'instruments acoustiques comme des pianos, violons, flûtes, sifflets, diapasons, etc. (Lubbock 1882, Fielde & Parker 1904). Malgré tous leurs efforts, ils n'ont pas observé la moindre réaction chez leurs fourmis et en ont donc tous arrivés à la conclusion que les fourmis étaient sourdes. Contrairement à cette idée, Hickling & Brown (2000) ont suggéré que, bien qu'elles soient dépourvues d'organes tympaniques et qu'elles soient incapables de répondre aux sons distants, les fourmis possèdent des poils sur leurs antennes capables de percevoir le son véhiculé par l'air dans un champ proche, à une échelle d'environ 10 cm. Pourtant, d'après Roces & Tautz (2001) les seuls mécanorécepteurs que les fourmis possèdent sont portés par *l'organe de Johnston*, situé à la base des antennes. Cet organe impose plusieurs contraintes sur les caractéristiques des sons à produire par les fourmis pour leur utilisation : il faudrait un son d'une intensité de 100 décibels à moins de 1 cm de distance pour les stimuler, ce qu'aucune fourmi n'est capable de produire. Ainsi, contrairement aux abeilles qui sont capables de détecter et d'utiliser ces signaux (Kirchner et al. 1991, Dreller & Kirchner 1993), il n'y a actuellement aucune preuve que les sons transmis par l'air possèdent une fonction intraspécifique chez les fourmis.

En revanche, les fourmis sont extrêmement sensibles aux vibrations du substrat – suffisamment sensibles pour détecter les sons véhiculés à travers les feuilles, leurs nids ou même le sol (Hölldobler & Wilson 1990). En fait, les fourmis possèdent dans leurs pattes des organes sensibles aux vibrations. Si l'homme est capable de percevoir tactilement les vibrations via un substrat, il est cependant peu apte à les décoder finement les informations transmises de cette façon. Il nous est donc difficile d'imaginer comment se déroule la vie dans un « monde vibratoire » (Hrncir et al. 2006), ce qui explique pourquoi la communication vibratoire a longtemps été considérée comme mineure. Pourtant, avec le développement de nouvelles techniques d'enregistrement et d'analyse, notamment numérique, il est apparu que l'utilisation des vibrations du substrat dans la communication chez les insectes est plus répandue et plus importante qu'on ne l'avait initialement imaginé (Virant-Doberlet & Cokl 2004).

Chez les fourmis, deux formes de productions vibratoires ont été identifiées: la percussion ou « drumming » et la stridulation. Le premier mécanisme d'émission a été décrit chez les fourmis arboricoles appartenant aux genres *Camponotus* et *Polyrhachis*. Ces fourmis génèrent des vibrations en frappant le substrat à l'aide de leurs mandibules, leur gastre ou

leurs pattes. Ces vibrations servent de signaux modulateurs altérant les probabilités de transition entre les différents comportements, et également de signal d'alarme pour la colonie (Kirchner 1997). La stridulation est un mécanisme d'émission vibratoire plus sophistiqué car il met en jeu un organe spécialisé : l'appareil stridulatoire. Cet organe particulier n'est pas présent chez toutes les fourmis mais il a été observé chez un grand nombre d'espèces appartenant à cinq sous-familles de fourmis : *Ponerinae, Ectatomminae, Nothomyrmeciinae, Pseudomyrmecinae* et *Myrmicinae* (Markl 1973, Taylor 1978).

Ici, nous nous intéresserons au deuxième mécanisme de productions vibratoires : les stridulations, ainsi qu'aux « messages » délivrés par ses signaux, c'est-à-dire, leur signification biologique chez les fourmis.

L'appareil stridulatoire

Sauf rares exceptions (Dumortier 1963, Taylor 1978, Hölldobler & Wilson 1990), l'organe stridulatoire typique des fourmis est composé d'un grattoir, ou *plectrum,* dans la partie post-dorsale du troisième segment abdominal et d'une plaque stridulatoire, ou *pars stridens,* qui correspond à une partie différenciée sur la région dorsale antérieure du segment suivant (Figure 2). La plaque stridulatoire est constituée d'une série de fines stries parallèles d'une très grande régularité. Le grattoir peut être formé par un épaississement ou un prolongement de l'arrière du segment où il se trouve et formera alors une crête dont la largeur est voisine de celle de la plaque stridulatoire. Mais le grattoir peut aussi ne pas avoir de structure modifiée. Dans ce cas, c'est la propre extrémité du segment qui gratte la plaque stridulatoire (Raignier 1933, Marchand et al. 2004) (Figure 2).

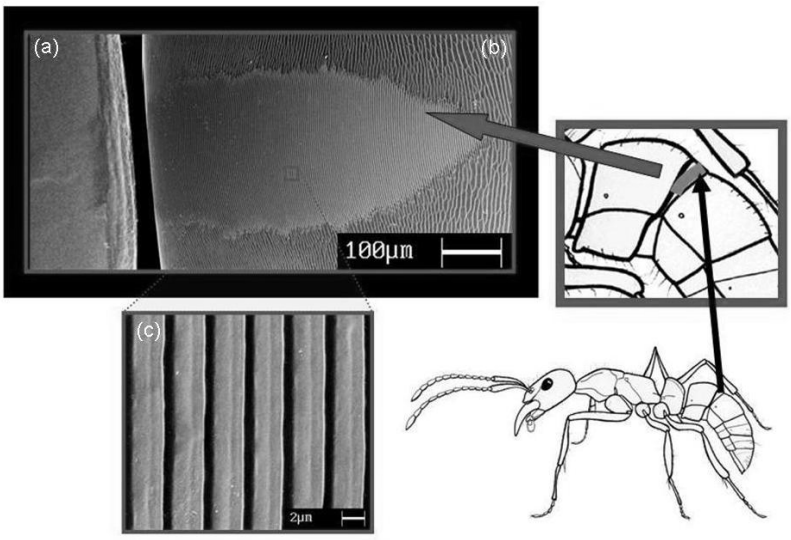


Figure 2. Appareil stridulatoire d'une fourmi *Pachycondyla* : localisation et morphologie. Grattoir (a), plaque stridulatoire (b), détail des stries de la région médiane de la plaque (c).

Production des signaux

Les stridulations sont produites lorsque l'animal effectue des mouvements dorso-ventraux du gastre (Spangler 1967, Kermarrec et al. 1976). Elles consistent en des séries ou trains d'impulsions très courts que peuvent être transmises par le substrat, par l'air ou lors de contacts tactiles directs (Hölldobler 1984) (Figure 3a). Chaque impulsion correspond au frottement du grattoir contre une strie de la plaque stridulatoire (Hölldobler & Wilson 1990) (Figure 3b).

Ces signaux ont habituellement une amplitude trop basse pour être entendus à de grandes distances sans amplification (Markl 1973, Hickling & Brown 2000), mais certaines espèces de Ponerinae comme quelques *Pachycondyla* ou *Dinoponera* peuvent produire des sons audibles pour l'oreille humaine même à plus d'un mètre de distance (observations personnelles). La fréquence des stridulations peut varier de quelques centaines de hertz, comme chez *Solenopsis richteri* (Hickling & Brown 2000), à l'ultrasonique, comme chez *Pachycondyla apicalis* (Pavan et al. 1997).

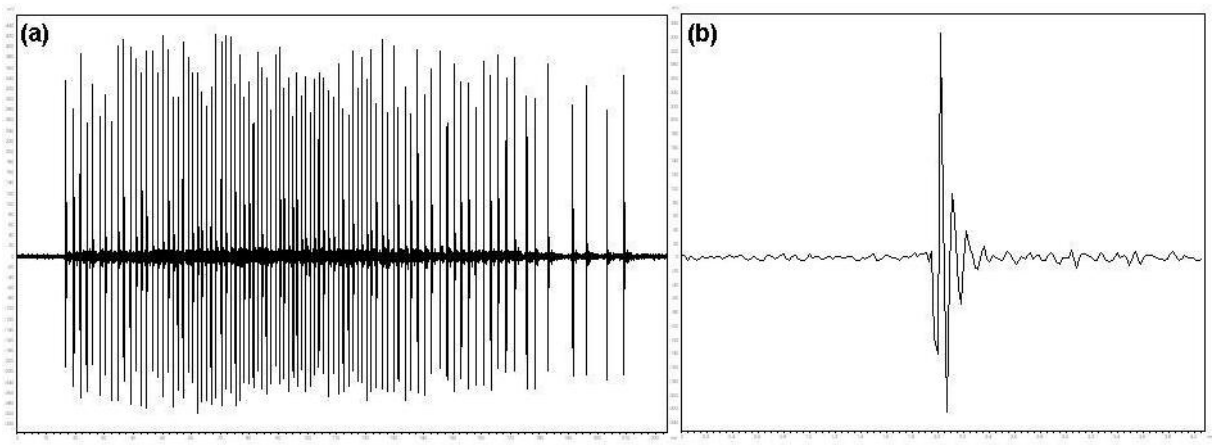


Figure 3. Oscillogrammes d'une stridulation de fourmi *Pachycondyla* : (a) train d'impulsions (durée : 200 ms) et (b) détail d'une impulsion (durée : 0,5ms).

L'origine évolutive

Plusieurs auteurs se sont interrogés sur la fonction primordiale des stridulations chez les fourmis. Ainsi, Rauth & Vinson (2006) se demandent si l'utilisation des stridulations relève bien de la communication, comme cela était suggéré par la plupart des auteurs, ou si les organes stridulatoires correspondent plutôt à un caractère ancestral hérité de vespidés solitaires nichant dans le sol. L'appareil stridulatoire pourrait alors servir d'aide mécanique pour l'excavation et/ou la construction du nid. Dans ce contexte d'excavation, les vibrations générées par les stridulations, transmises via le corps aux mandibules des fourmis, joueraient

un rôle dans l'enlèvement et le déplacement des particules. Ces auteurs n'excluent toutefois pas la possibilité de que ces signaux puissent aussi être impliquées dans la communication de plusieurs façons.

Chez les fourmis champignonnistes *Atta cephalotes*, Tautz et al. (1995) ont de même observé que le pattern temporel des stridulations est corrélé avec les mouvements mandibulaires des fourmis pendant le découpage des feuilles. Les vibrations produites par les stridulations sont transmises de la mandibule à la feuille, et permettraient de faciliter la coupe des feuilles tendres et minces, à l'image du vibratome des histologistes qui rigidifie les tissus pour permettre leur découpage. Comme les stridulations jouent aussi un rôle dans le recrutement chez ces fourmis (voir ci-dessous), ces auteurs ont spéculé sur l'évolution fonctionnelle de ces vibrations stridulatoires. Auraient-elles d'abord évolué comme aide mécanique pour faciliter la découpe des feuilles, avant d'être utilisées dans le recrutement ? Les diverses expériences de Roces & Hölldobler (1996) destinées à tester cette hypothèse aboutissent cependant à la conclusion que la facilitation mécanique engendrée par les stridulations est en fait un épiphénomène de la communication de recrutement.

Les stridulations peuvent donc servir à des tâches mécaniques, mais d'après Hölldobler & Wilson (1990), il n'y a pas de doute : l'appareil stridulatoire des fourmis a clairement évolué dans un but de communication même si l'usage des signaux stridulatoires reste moins développé que la communication phéromonale. Certains auteurs vont même encore plus loin dans l'interprétation du rôle joué par les stridulations dans la communication chez les fourmis puisqu'ils estiment qu'elles peuvent constituer une sorte de langage rudimentaire (Hickling & Brown 2001).

Les significations biologiques des stridulations:

Modulateurs des comportements sociaux

La communication dans les systèmes sociaux complexes est rarement caractérisée par une réponse directe, du type « tout ou rien ». Les signaux n'entraînent pas toujours de réponses comportementales d'un type particulier, mais ils semblent plutôt réorienter le comportement des congénères d'une manière appropriée au milieu environnant. Ces signaux influencent donc les comportements des individus récepteurs, non en les engageant dans un comportement strictement défini, mais en modulant la probabilité de leur transition vers d'autres actes comportementaux (Hölldobler & Wilson 1990).

C'est ainsi le cas des stridulations émises par les fourrageuses des fourmis du désert *Aphaenogaster cockerelli* et *A. albisetosus.* Lors de la découverte d'une proie impossible à

transporter seule, la fourmi stridule, ce qui a pour effet d'attirer ses congénères. Simultanément, elle libère aussi un signal chimique de recrutement. Cumulés, les deux signaux optimisent l'attraction des ouvrières environnantes vers la source de nourriture, permettant le transport de la proie au nid. L'effet de facilitation des stridulations sur le recrutement chimique diminue de 1 à 2 minutes le délai du recrutement et de récolte de la nourriture (Hölldobler et al. 1978, Markl & Hölldobler 1978). Le même phénomène est à l'œuvre chez la fourmi moissonneuse *Messor capitatus* qui stridule quand elle trouve des graines. Dans ce cas, si les stridulations ne permettent pas de recruter un nombre plus important de fourmis, elles favorisent cependant une mobilisation plus rapide. (Baroni-Urbani et al. 1988). Même si elles ne sont pas indispensables au recrutement des congénères, les stridulations ont donc pour effet d'optimiser le processus en l'accélérant. Pour ces fourmis qui vivent dans des environnements où règne une forte compétition alimentaire, l'exploitation rapide d'une nouvelle source de nourriture constitue un avantage considérable sur les espèces concurrentes (Passera & Aron 2005).

Signaux de stress et d'alarme

Une fourmi stridule quand elle est dérangée, privée de ses mouvements ou attaquée. Les stridulations peuvent être interprétées tout d'abord comme un signal de stress individuel. La fourmi moissonneuse *Pogonomyrmex badius* ainsi que de nombreuses autres espèces stridulent très souvent quand elles sont maintenues par des pinces, pressées sous un objet ou placées dans un espace restreint (Wilson 1971). Les ouvrières de plusieurs espèces du genre *Messor* stridulent aussi quand elles se battent avec des ouvrières étrangères (Grasso et al. 2000).

Ces stridulations peuvent également servir de signal d'alarme pour alerter la colonie d'un danger potentiel, par exemple lorsque le nid a été attaqué ou a souffert d'un effondrement (Masters 1980, Markl 1965, 1967).

Signal aposématique

Ces signaux peuvent avoir une fonction aposématique, c'est-à-dire qu'ils informent ou « avertissent » les prédateurs potentiels que leurs émetteurs peuvent représenter pour eux un danger à éviter (Eisner 1981). Par exemple, ils risquent de se faire piquer, puisque la plupart des fourmis qui possèdent un organe stridulatoire sont pourvues également d'un aiguillon (Markl 1973), ou de subir la morsure douloureuse que certaines fourmis peuvent infliger (Masters 1979, 1980). Quelques fourmis stridulent aussi en réponse au dioxyde de carbone ce

qui suggère un rôle de défense contre d'éventuels prédateurs vertébrés tels que certains oiseaux, reptiles ou mammifères (Hölldobler et al. 1994, Ware 1994). De plus, le fait que la colonie entière se mette à striduler quand elle est dérangée, comme c'est le cas pour certaines espèces de *Pachycondyla,* pourrait permettre d'amplifier l'effet de dissuasion sur le prédateur. Dans ce cas, on peut suggérer que c'est la composante audible des stridulations, c'est-à-dire le son véhiculé par l'air, qui est susceptible de transmettre le message d'avertissement.

Appel au secours

Les stridulations d'alarme peuvent aussi être utilisées comme un « appel au secours » chez les fourmis champignonnistes *Atta cephalotes* (Markl 1965, 1967). Lors d'un éboulement du nid, les fourmis enterrées se mettent à striduler, ce qui attire les ouvrières vers la source de vibration. Les ouvrières attirées creusent alors là où l'intensité des vibrations est la plus forte. Ainsi, les stridulations d'un soldat enterré jusqu'à 5 cm de profondeur attirent des ouvrières, mais le sauvetage ne sera effectif que si la fourmi a été enfouie sous moins de 3 cm de substrat.

Chez cette espèce dont les nids peuvent atteindre plusieurs mètres de profondeur, les affaissements de galeries peuvent piéger une partie considérable de la population ou l'unique reine du nid. Par conséquent, la localisation exacte et rapide de ces individus peut être d'ordre vital pour la colonie. Dans un tel contexte, la communication souterraine de secours par signalisation vibratoire s'avère nettement plus efficace que par voie olfactive (Markl 1965).

Recrutement alimentaire

Roces et al. (1993) ont démontré une nouvelle fonction des stridulations qui illustre le sens des réponses contexte-spécifiques aux signaux de communication. Les ouvrières des fourmis champignonnistes *Atta cephalotes* stridulent quand elles découpent des feuilles. Les vibrations stridulatoires traversent le corps et la tête de la fourmi engagée dans le découpage et se répandent dans le substrat via ses mandibules. Les ouvrières voisines répondent à ces signaux propagées par la plante en s'orientant vers la source vibratoire et se joignent alors au découpage de la feuille. Dans ce contexte, les stridulations servent, de signal de recrutement à courte distance. En plus d'orienter les fourmis vers la source de nourriture, ces signaux informent aussi sur la qualité de la source. En offrant aux fourmis des feuilles de différentes qualités (tendres ou coriacées, imbibées ou non de sirop de sucre), ces auteurs ont démontré que meilleure est la qualité de la feuille, plus les fourmis stridulent en la découpant, attirant ainsi un plus grand nombre de fourmis vers cette feuille. En résumé, les signaux stridulatoires

produits par les ouvrières lors du découpage des feuilles semblent avoir évolué afin de concentrer les efforts de récolte au niveau des parcelles de haute qualité.

Déménagement de la société

La fourmi ponerine orientale *Leptogenis chinensis*, spécialisée dans la chasse aux termites, forme des colonies de taille réduite (environ 200 à 300 individus). Cette fourmi vit dans des cavités naturelles et déménage fréquemment (environ toutes les deux semaines, voire plus). Chez cette espèce, les stridulations, combinées à de signaux chimiques, sont utilisées pendant les déménagements de nid. Quand les fourmis éclaireuses trouvent un endroit approprié pour déménager, elles retournent au nid en déposant une piste chimique. Elles stridulent alors à leur retour au nid, ce qui incite les autres fourmis de la colonie à initier le déménagement. Les signaux stridulatoires sont ici nécessaires pour la stimulation et la synchronisation des membres de la colonie lors du déménagement (Maschwitz & Schönegge 1983).

Accouplement

Chez les fourmis, le comportement sexuel consiste en une séquence de comportements simples qui comprend généralement la localisation, l'approche du partenaire, une reconnaissance antennaire entre les protagonistes et enfin quelques secondes d'accouplement. En revanche, les mâles des fourmis *Cardiocondyla* ont une vie sexuelle considérablement plus « passionnante » : l'accouplement de ces fourmis est régulièrement précédé par une parade nuptiale prolongée et stéréotypée. Les stridulations semblent être une des composantes comportementales prédominantes dans la parade sexuelle et l'accouplement des mâles non ailés des *Cardiocondyla spp* pour exprimer leur niveau de stimulation. Chez *Cardiocondyla elegans*, la stridulation et la boxe antennaire sont des étapes obligatoires avant la copulation, et les transitions entre stridulation et boxe antennaire jusqu'à la copulation sont fortement ritualisées (Mercier et al. 2007). Même si elles n'ont pas encore été enregistrées ou analysées, les stridulations des mâles de *Cardiocondyla* bouleversent l'état actuel des connaissances sur la communication acoustique et vibratoire des fourmis, et leur étude pourrait permettre de répondre à plusieurs questions quant à leur évolution chez les fourmis. Par exemple, pourquoi les mâles possèdent-ils aussi un appareil stridulatoire développé alors qu'ils ne participent jamais aux tâches de la colonie ? Les fourmis sont-elles capables de produire des signaux acoustiques différents en fonction du contexte d'émission? Ces questions demeurent encore sans réponse.

Le seul autre cas de stridulation rapporté chez des individus reproducteurs est celui des femelles de fourmis moissonneuses *Pogonomyrmex spp*. (Markl et al. 1977). Chez ces fourmis, mâles et femelles se rassemblent pour l'accouplement après le début des pluies d'été dans des arènes nuptiales ou aires de parade. Les femelles s'accouplent en général plusieurs fois et l'accès aux femelles entraîne une compétition importante entre mâles. Après plusieurs accouplements successifs, les femelles ne sont plus réceptives et tentent de quitter l'arène. Assaillies de mâles qui tentent encore de s'accoupler, elles stridulent alors régulièrement. Dans ce contexte, les stridulations peuvent être considérées comme un « signal de libération de la femelle ». Celui-ci pourrait présenter des avantages pour les deux sexes. Ainsi, ce signal indiquerait aux mâles que les femelles ne sont plus réceptives et les inciteraient donc à en « courtiser » d'autres. Par ailleurs, il permettrait aux femelles de réduire les risques de mutilation par morsures quand les mâles les retiennent, ainsi que de quitter rapidement l'arène afin de limiter les dangers liés à la prédation. Une fois l'arène quitée, ces jeunes reines partent en quête d'un endroit sûr où fonder leur colonie. Les reines fondatrices se retrouvent alors en compétition pour l'accès aux rares sites disponibles, et stridulent là encore lorsqu'elles s'engagent dans un combat pour un terrier. Enfin, comme les ouvrières de ces espèces stridulent aussi quand elles se battent avec d'autres fourmis, aucune fonction de communication n'a pu être clairement attribuée à ces sons (Markl et al. 1977).

Mutualisme et parasitisme

Un autre fait fascinant est la capacité des fourmis à « écouter » les stridulations d'autres espèces d'insectes et de répondre à leur signal. Bien que les signaux stridulatoires interspécifiques des fourmis soient généralement émis dans un contexte de défense, il existe des cas de stridulations émises par d'autres insectes en direction de fourmis dans un but mutuellement bénéfique. DeVries (1990, 1991) a en effet montré que les chenilles de lépidoptères des familles Riodinidae et Lycaenidae produisent des vibrations propagées par le substrat afin d'attirer les fourmis avec lesquelles elles vivent en association. En échange de sécrétions nutritives et sucrées produites par les chenilles, les fourmis protègent activement ces dernières contre prédateurs et parasites. Ainsi l'émission de stridulations permet le maintien d'une protection par les fourmis : les chenilles qui stridulent le plus sont aussi les mieux protégées. Quant aux espèces qui ne stridulent pas, elles ne sont jamais gardées par des fourmis (Travassos & Pierce 2000).

Les membracides *Publilia concava* partagent une association semblable avec les fourmis du groupe *Formica fusca* comme l'ont démontré Morales et al. (2008). Ces

membracides produisent des vibrations en réponse aux menaces de leur prédateur, une coccinelle. Ces signaux augmentent l'activité des fourmis autour des membracides ainsi que la probabilité de détection des prédateurs par les fourmis. De plus, ces auteurs ont prouvé que ces fourmis ne répondent pas à n'importe quel signal vibratoire émis par l'espèce associée. En effet, des expériences ont montré que la diffusion de signaux de parade nuptiale des mâles de membracides n'a aucun effet sur les fourmis.

Une particularité de ces deux exemples de mutualisme est qu'ils mettent souvent en jeu des espèces de fourmis ne possédant pas d'appareil stridulatoire développé. Ces fourmis ne produisent donc pas de signaux vibratoires mais sont cependant capables de percevoir des vibrations hétérospécifiques et d'en intégrer le signal. La communication acoustique interspécifique entre les fourmis et d'autres insectes peut atteindre des niveaux encore plus élaborés, comme le montre l'exemple du lépidoptère *Maculinea rebeli* qui parasite les nids de la fourmi *Myrmica schencki*, et qui dépend de celle-ci pour le développement de ses larves et nymphes. Chez cette espèce de fourmi, les reines produisent des sons distincts de ceux des ouvrières et renforcent ainsi son contrôle sur la colonie. Les sons produits par les larves et les pupes du papillon parasite miment les stridulations de la reine, trompant les ouvrières qui ainsi les prootègent de manière particulière (Barbero et al. 2009a, 2009b).

La biodiversité cryptique

La région Néotropicale abrite une extraordinaire diversité biologique, dont de nombreuses espèces encore non identifiées. En outre, parmi les espèces déjà inventoriées, plusieurs groupes sont soumis à des classifications imparfaites (Folgarait 1998). Ceci est particulièrement dû à l'existence d'espèces cryptiques (i.e. deux ou plusieurs espèces distinctes qui sont classés à tort (et donc cachées) sous un seul nom d'espèce en raison de leur morphologie très similaire) (Encadré 1). Ces ensembles forment des complexes d'espèces qui empêchent l'évaluation correcte de la biodiversité (Bickford et al. 2007).

La biodiversité peut être évaluée et subdivisée en fonction de différents niveaux, allant de la diversité des gènes à celle des écosystèmes, mais c'est le niveau de l'espèce qui reste le plus important et étudié en biologie, en particulier pour les problèmes liés a l'écologie et la conservation (May 1990, Mace 2004, de Queiroz 2005, Tobias et al. 2010). Cependant, le concept d'espèce est un concept flou dont il existe de nombreuses définitions dans la littérature scientifique (Encadré 2). Malgré les difficultés de définir objectivement une espèce, caractériser la diversité du vivant de manière opérationnelle nécessite de décrire les entités biologiques et de les regrouper de façon à permettre leur identification et inventaire.

L'étude de la diversité cryptique est donc primordiale pour permettre la compréhension des mécanismes de spéciation et de maintien de la diversité au sein des écosystèmes. De plus, l'étude et la description des espèces cryptiques et de leur processus de spéciation peuvent avoir des implications importantes pour la santé humaine, l'agriculture, ainsi que pour la compréhension des mécanismes de coévolution et des interactions interspécifiques (Bickford et al. 2007).

S'il n'existe pas de groupe taxonomique sans espèces cryptiques, le fait que leur occurrence soit plus fréquente chez certains groupes ou dans certains écosystèmes est plus débattu. Cependant, le biais cognitif humain envers les informations visuelles entraîne une attention plus particulière sur la morphologie, alors que les paramètres chimiques ou acoustiques sont moins utilisés (Bickford et al. 2007, Seifert 2009).

Les espèces cryptiques sont donc probablement plus fréquentes chez des organismes ayant des systèmes de communication et reconnaissance basés principalement sur des caractéristiques non-visuelles (e.g. les sons, les hydrocarbures ou d'autres phéromones), comme par example chez un grande nombre d'insectes (Broza et al. 1998, Cade & Otte 2000, Claridge et al. 1997, Henry 1994, Lucas et al. 2002, Noor & Aquadro 1998, Ritchie & Gleason 1995, Walker et al. 2003), amphibiens (Angulo & Reichle 2008) et certains oiseaux (Alström et al. 2008), ou dans des écosystèmes extrêmes, parce que ces habitats imposent des sélections stabilisatrices sur la morphologie, en réduisant ou éliminant les changements morphologiques qui peuvent accompagner la spéciation (Nevo 2001, Rothschild & Mancinelli 2001), ainsi que dans les forêts tropicales (Willig et al. 2003) ou les habitats marins (Knowlton 1993, 2000), parce qu'ils sont les habitats plus riches de la planète et parce qu'une grande partie de ses organismes sont impliqués dans des interactions interspécifiques spécialisées.

ENCADRE 1 : La terminologie des espèces cryptiques

La littérature taxonomique est remplie de termes pour désigner les espèces cryptiques (« cryptic species ») ou espèces cachées (« hidden species »), comme par exemple espèces sœurs («sister species ») ou espèces jumelles ou parentes (« sibling species »). De plus, pour ajouter à la confusion, ces termes sont souvent utilisés de manière aléatoire et interchangeable.

D'après Bickford et al. (2007) and Seifert (2009), il est possible de clarifier ces différentes nomenclatures afin de réduire les confusions :

- *Les espèces cryptiques* (cryptic species) sont des groupes de deux ou plusieurs espèces qui ne sont pas séparables facilement par un expert au premier examen par leur morphologie ou leurs productions sonores. Cette définition reflète le sens immédiat du mot et son utilisation devrait donc être restreinte aux cas d'espèces effectivement cryptiques, c'est à dire aux espèces pour lesquelles l'identification reste problématique malgré un apprentissage soutenu des possibles différences. En conséquence, l'identification ne peut faire de manière fiable qu'en utilisant des méthodes élaborées comme des enregistrements numériques, une analyse de caractères morphologiques précise, de l'ADN, de la biochimie ou de sonogrammes. Un seuil de 10% d'erreurs peut être défini comme raisonnable pour définir des espèces cryptiques. Les espèces cryptiques ne sont pas nécessairement les taxons les plus apparentés, mais une proximité élevée est généralement observé.

- *Les espèces sœurs* (sister species) sont deux taxons qui ont évolué immédiatement à partir du même ancêtre commun. Le terme se réfère donc uniquement à la proximité phylogénétique et ne fait aucune référence implicite à des similarités phénotypiques. Cependant, des ressemblances sont souvent observées entre espèces sœurs.

- *Les espèces parentes ou jumelles* (sibling species) représentent le croisement entre les deux premiers cas. Elles sont donc des espèces issues immédiatement du même ancêtre commun et qui ne sont pas séparables sans l'utilisation de méthodes spécifiques.

Par ailleurs, hors du contexte taxonomique, certains auteurs utilisent aussi le terme « cryptique » pour désigner des espèces capables de se camoufler ou se dissimuler dans leur environnement, notamment pour échapper à la prédation (les proies) ou s'approcher de leurs proies (les prédateurs).

Dans ce travail où nous nous intéressons à la diversité cryptique, nous ne connaissons pas a priori les relations phylogénétiques entre les taxons étudiés et nous parlerons donc de complexe d'espèces cryptiques pour faire référence à la difficulté de catégoriser les individus observés facilement. Il est également important de réaliser une série d'études multidisciplinaires pour comprendre comment cette diversité se distribue, entre variations au sein de l'espèce ou proximité morphologique d'espèces ne représentant pas forcément des espèces sœurs (Schlick-Steiner et al 2010, Seifert 2009).

ENCADRE 2 : Qu'est- ce qu'une espèce ?

Dans son sens le plus simpliste, le concept de l'espèce permet de distinguer les différents types d'organismes vivants. Cependant, la complexité des mécanismes à l'origine de l'évolution des différentes espèces et de leur séparation rendent une telle définition inutile. Avant de caractériser et répertorier la diversité spécifique des différents taxons, il est donc nécessaire de définir de manière claire le sens que l'on donne à l'entité spécifique. En conséquence, différentes définitions permettent d'identifier plus précisément les critères distinctifs de l'espèce et celles-ci vont permettre d'établir des éléments pertinents pour l'étude de la diversité biologique.

Le *concept morphologique* de l'espèce est le concept le plus communément utilisé en pratique par les taxonomistes. C'est aussi le premier concept utilisé historiquement en raison des techniques disponibles aux premiers naturalistes. Il consiste à identifier une espèce d'après ses caractéristiques structurales ou morphologiques distinctives (Raven et al. 2003). L'avantage de ce concept est qu'il est applicable aussi bien aux organismes sexués qu'asexués et qu'il ne nécessite pas de connaître l'étendue des flux géniques entre populations. Néanmoins, l'inconvénient majeur de ce concept réside dans la subjectivité de sa définition de l'espèce, qui peut aboutir à des désaccords quant aux critères retenus pour définir une espèce (Mayr, 1942).

Pour cette raison, la définition la plus couramment adoptée est celle du *concept biologique* de l'espèce énoncé ainsi par Ernst Mayr (1942) : « les espèces sont des groupes de populations naturelles, effectivement ou potentiellement interfécondes, qui sont génétiquement isolées d'autres groupes similaires ». L'espèce représente donc le plus grand groupe d'individus au sein duquel le flux génique est observé ou possible dans des conditions naturelles, les individus d'une même espèce étant génétiquement isolés d'autres (cont.)

ENCADRE 2. Continuation

ensembles équivalents du point de vue reproductif. Cette définition, si elle est la plupart du temps valide pour les animaux, n'est pas cependant aussi pertinente pour la taxonomie des plantes puisque des croisements fertiles surviennent souvent entre des plantes de genres très différents. Cette distinction s'applique aussi aux organismes qui ne se reproduisent pas sexuellement car ici chaque lignée pourrait être considérée comme une espèce distincte et isolée malgré la similarité entre lignées clonales.

Ces limites ont amené les chercheurs à essayer de trouver de nouvelles définitions, qui s'appuient grandement sur les données génétiques et phylogénétiques récemment obtenues (Tobias et al. 2010). Ainsi, on peut parler du *concept d'espèce phylogénétique* dont la base est l'existence de critères génétiques diagnostiques clairs (Cracraft 1989) et du *concept d'espèce monophylétique* qui s'appuie sur l'approche cladistique et repose donc sur la monophylie (Mishler & Donoghue 1982). D'autres définitions sont encore disponibles, comme par exemple, le *concept écologique* en rapport avec la notion de niche écologique (voir la révision en de Queiroz 2005, 2007).

Ces évolutions dans la définition de l'espèce entraîne une incertitude même sur la validité de ces concepts pour caractériser les espèces, d'autant plus que la disponibilité croissante de données moléculaires, si elle a permis de mieux comprendre l'histoire des différentes lignées, a aussi entraîné une prise de conscience accrue de l'absence de correspondance entre éloignement génétique et limite entre les espèces (Joseph & Omland 2009, Winker 2009).

Les différents concepts d'espèces ne s'opposent donc pas clairement et diffèrent en fait plus dans l'approche et le point de vue utilisés. De plus, aucun d'entre eux n'est assez objectif pour se passer de la nécessité de décisions basées sur le bon sens et la connaissance des organismes étudiés et au final, il est probable que la complexité du vivant rende impossible une définition objective et que les biologistes doivent se contenter d'utiliser la définition la plus opérationnelle (Tobias et al. 2010). Dès lors, l'utilisation de l'une ou l'autre des définitions dépendra plus de la question posée et des organismes étudiés en fonction de critères plus pragmatiques.

La sous-famille Ponerinae

Parmi les 21 sous-familles de fourmis, certaines sont considérées comme « primitives » car elles sont morphologiquement proches de la forme ancestrale, *Sphecomyrma freyi*, une fourmi avec une mosaïque de caractéristiques de fourmis modernes et de guêpes aculéates, datant d'environ 92 millions d'années (Hölldobler & Wilson 1990, Agosti et al. 1997). Ceci est le cas, par exemple, de la sous-famille des Ponerinae. Cette sous-famille serait aussi contemporaine de nombreux autres groupes de fourmis déjà éteints et sa divergence au sein des Formicidae est l'une des plus anciennes (Schmidt 2009). Engel & Grimaldi (2005) énumère deux genres fossiles du Crétacé appartenant à cette sous-famille: *Afropone* et *Canapone*, découverts respectivement au Botswana et au Canada. Bolton (2003) cite trois autres genres fossiles du tertiaire.

Dans sa définition moderne, la sous-famille Ponerinae (Hymenoptera: Formicidae) constitue la plus grande sous-famille de fourmis dites « primitives ». Elle compte 1.053 espèces valides, avec 260 espèces recensées dans la région Néotropicale (Fernandez & Sendoya 2004, Agosti & Johnson 2005).

Contrairement à d'autres lignées de fourmis d'âge et de diversité comparables, les fourmis de la sous-famille Ponerinae affichent généralement une série de traits comportementaux et écologiques qui sont considérés comme ancestraux, comme la petite taille des colonies (généralement environ une centaine d'individus), le peu de différenciation morphologique entre les ouvrières et les reines, une caste ouvrière monomorphe et un fourragement en général solitaire (Peeters 1997, Wilson & Hölldobler 2005). Cependant, si on a longtemps considéré que leur structure sociale était elle-même primitive, il apparaît maintenant que la diversité des ponérines se retrouve autant dans les adaptations écologiques et comportementales que dans le cycle de vie et la reproduction. La combinaison de ces variations rend difficile l'estimation a priori de leur caractère primitif ou dérivé (Fresneau 1994). Ainsi la famille des Ponerinae constitue un modèle sans parallèle pour étudier l'évolution de nombreux traits apparus de manière répétée chez les fourmis et pour apporter un éclairage nouveau sur leur écologie et leur évolution sociale (Schmidt 2009).

Le genre *Pachycondyla*

Le genre *Pachycondyla sensu* Brown est l'un des plus anciens genres de fourmis connu et toujours existant. Une espèce fossile, *Pachycondyla rebekkae* Rust & Andersen, décrite dans les formations calcaires du début du Tertiaire (± 55 millions d'années) (Rust & Andersen 1999) représente le plus ancien membre du genre. Six autres espèces fossiles de l'Oligocène

européen ont également été décrites (Bolton 1995). Actuellement, *Pachycondyla sensu* Brown a une distribution pantropicale avec plus de 270 espèces valides (Bolton 1995, Wild 2005). Avec 63 espèces décrites dans la seule région Néotropicale (Fernández & Sendoya 2004), c'est le genre possédant la plus grande diversité parmi les Ponerinae dans cette région. Les *Pachycondyla* Néotropicales sont particulièrement abondantes dans les forêts humides, mais on les trouve aussi dans d'autres types de végétation possédant une structure d'habitat semblable à celui d'une forêt (Lattke 2003). Ces espèces de taille moyenne à grande (jusqu'à 28,4 mm) forment des colonies matures de taille modérée allant de 10 à 5000 individus quand elles sont matures. Beaucoup ont une structure monogyne simple (avec une seule reine) mais certaines, comme *Pachycondyla verenae* et d'autres espèces du groupe *P. villosa* sont facultativement polygynes. La nidification se fait dans des cavités souvent préexistantes en raison de l'absence de comportement de construction élaboré et cette nécessité d'opportunisme fait qu'on les trouve suivant l'espèce sous terre, dans du bois creux ou pourri sur le sol ou dans les cavités des arbres. Certaines espèces sont pourtant, obligatoirement associées à des épiphytes dont elles peuvent même contrôler le développement (e.g. *Pachycondyla goeldii* et ses jardins de fourmis (Orivel et al. 1998, Orivel 2000, Orivel et al. 2001).

La plupart des espèces sont des prédatrices généralistes mais on peut observer des régimes alimentaires plus spécialisés comme par exemple la prédation exclusive de termites par des raids élaborés (par exemple pour les espèces de l'ancien genre *Termitopone)* ou des adaptations particulières dévéloppée par plusieurs espèces arboricoles du groupe *Pachycondyla villosa* (Delabie 2001) comme la capacité d'exploiter les excrétions sucrées (miellat) des insectes suceurs, en particulier des Membracidae.

En outre, les fourmis du genre *Pachycondyla,* sont considérées suite à des études inventaires écologiques comme un indicateur important de la biodiversité myrmécologique et de l'équilibre des écosystèmes Néotropicaux (Delabie et al. 2000, Majer & Delabie 1993, 1999).

La biodiversité cryptique chez les *Pachycondyla* Néotropicales

Des études récentes (Lucas et al. 2002, Mariano 2004, Mariano et al. 2006, Delabie et al. 2008) montrent que de nombreuses *Pachycondyla* néotropicales sont en fait regroupées dans des complexes d'espèces dans lesquels de nombreux taxons connexes ont dans un premier temps été insérés, et où peuvent coexister plusieurs taxons cryptiques qui, du fait de l'impossibilité de les distinguer seulement sur une base morphologique, posent d'énormes

problèmes aux taxonomistes. Les complexes de *Pachycondyla* les plus remarquables de la région Néotropicale, et plus spécifiquement du Brésil sont: '*apicalis*', '*crenata*', '*harpax*', '*magnifica*', '*stigma*', '*venusta*' et '*villosa*', toutes représentées par plusieurs taxons. Ces complexes peuvent inclure des taxons sympatriques (cas des complexes '*apicalis*', '*villosa*' et '*crenata*') ou allopatriques (complexe '*venusta*'), de grande étendue géographique (complexes '*apicalis*' et '*villosa*') ou de distribution restreinte (complexes '*magnifica*' et '*venusta*') (Delabie et al. non publié).

Le complexe *Pachycondyla apicalis*

Les fourmis de ce groupe sont des insectes grands et très visibles que l'on rencontre dans les forêts Néotropicales, du sud du Mexique au Paraguay. Outre une morphologie similaire, ces espèces ont généralement les mêmes caractéristiques biologiques et écologiques, ce qui rend encore plus difficile leur identification correcte (Wild 2005, Fresneau 1994). Leur morphologie et coloration générale commune (morph '*apicalis*') sont probablement dues à l'effet d'une sélection stabilisatrice, puisque d'autres arthropodes, parmi lesquels des araignées les partagent également (Reiskind 1977, Cushing 1997, McIver & Stonedahl 1993). Ces espèces largement sympatriques pourrait ainsi former un anneau de mimétisme massif, ce qui représenterait une avantage contre de potentiels prédateurs (Delabie et al. 2008, Hebert et al. 2004, McIver & Stonedahl 1993).

Wild (2005) a révisé la taxonomie de ce groupe en se basant sur plusieurs critères morphologiques et biométriques et a pu reconnaitre trois espèces largement sympatriques: *P. apicalis* (Latreille 1802), *P. obscuricornis* (Emery 1890) et *P. verenae* (Forel 1922) (Figures 4 et 5) au lieu des deux reconnues auparavant (Brown 1957). Il montre que le nom *P. obscuricornis* a constamment été attribué par erreur dans la littérature à l'espèce répandue *P. verenae* depuis une vintaigne d'années. En outre, il a aussi observé que *P. verenae* et *P. apicalis* montrent des variations considérables pour plusieurs caractères morphologiques à travers leur zone de distribution, comme notamment pour la tête et la forme du pétiole, la longueur du scape, la taille des yeux et la pilosité du corps. Selon lui cette variation, localisée ou largement allopatrique sur un gradient nord-sud, ne justifiait pas en dépit de son importance globale d'autres divisions taxonomiques dans le complexe.

Plus tard, dans une étude morphologique combinant des données cytogénétiques et écologiques lorsqu'elles étaient disponibles, Delabie et al. (2008) ont démontré que compte tenu de la stabilité de ces différences dans de multiples cas de sympatrie les variations soulignées par Wild (2005) pourraient en fait correspondre à une mosaïque d'espèces au lieu

d'un cline biogéographique. Ces auteurs ont reconnu sept taxons distincts au sein de ce groupe: quatre pour *P. apicalis*, deux pour *P. verenae* et un pour *P. obscuricornis* (Encadré 3). Cependant, même si l'existence d'une diversité intriquée dans le complexe d'espèces *P. apicalis* a été suggérée, ces auteurs n'ont pas pu conclure sur la validité et le statut taxonomique (*e.g.* bonnes espèces, simple populations ou encore écotypes) des différentes taxons sans d'autres études plus poussées.

Dans la présente thèse nous utiliserons, à priori, la classification proposée par Delabie et al. (2008), qui considère les différents taxons comme étant des morpho-espèces au sein des espèces déjà décrites et reconnues pour le complexe (voir Wild 2005).

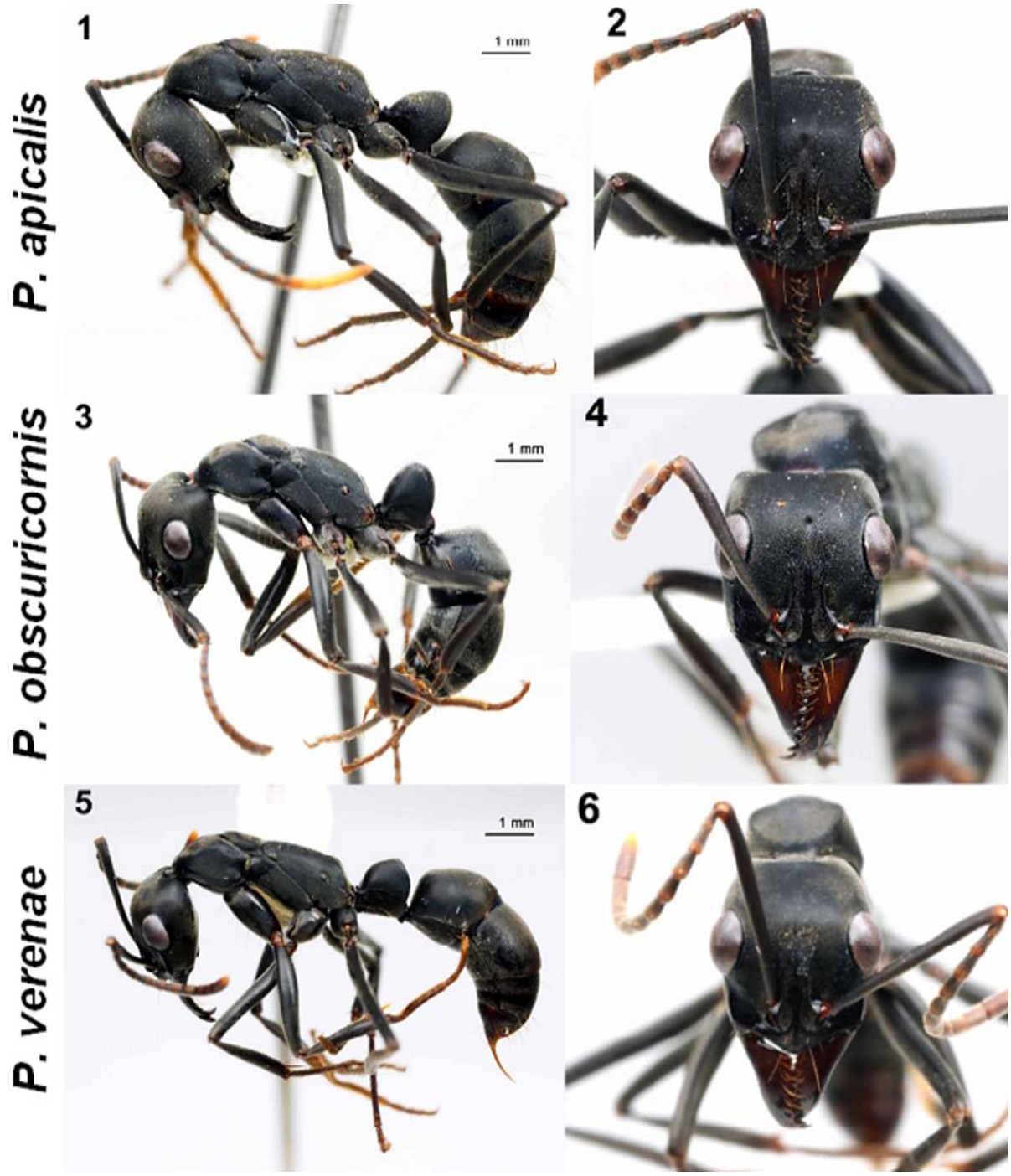


Figure 4. Les trois espèces du complexe *Pachycondyla apicalis* reconnues par Wild (2005). (Photos extraites de Wild, A. (2005), Zootaxa 834: 1–25).

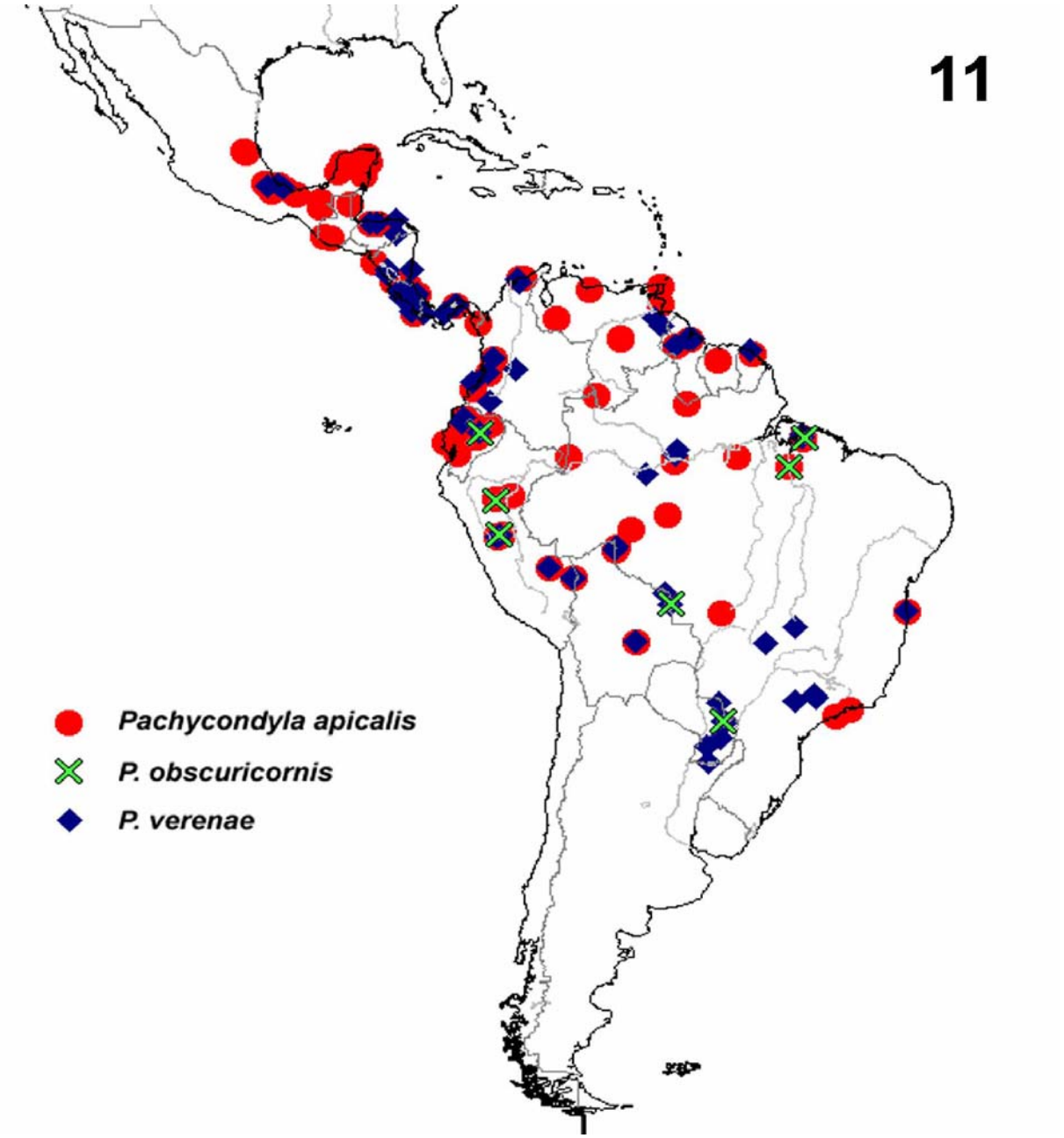


Figure 5. Distribution des trois espèces décrites du complexe *Pachycondyla apicalis*, selon Wild (2005). (Extrait de Wild, A. Zootaxa 834: 1–25, 2005).

ENCADRE 3: Clé de détermination des taxons du complexe d'espèces *Pachycondyla apicalis* (selon Delabie et al. 2008) :

1 - Antennomère apical distinctement jaune ..... **2 (groupe *'apicalis'*)**

1'- Antennomère apical foncé (habituellement de couleur brune avec l'extrémité un peu plus claire) nœud du pétiole, et tergites abdominaux III et IV glabres…..... **5**

2 - Intersections de la face postérieure du pétiole avec les bords arrondis …..... **3**

2'- Intersections de la face postérieure du pétiole avec les côtés formant un angle arrondi discrète ..... ***'apicalis'* Morpho-espèce IV**

3 - Pétiole en vue dorsale nettement trapézoïdale : largeur de la face postérieure près de deux fois celle de l'antérieure et de 0,8 à 0,9 fois sa longueur, le nœud du pétiole glabre, 2-4 paires de poils dressés sur tergite abdominal III, 2-6 paires de poils dressés sur tergite abdominal IV, 7 antennomères apicales couleur jaune……..... ***'apicalis'* Morpho-espèce III**

3'- Pétiole en vue dorsale: largeur de sa face postérieure un peu plus grande que l'antérieure et environ la moitié de sa longueur, 4-5 antennomères apicales jaunes..... **4**

4 - Nœud du pétiole glabre (rarement avec une paire de poils), 1-2 paires de poils dressés sur tergite abdominal III, <10 paires de poils dressés sur le tergite abdominal IV ……..… ***'apicalis'* Morpho-espèce I**

4'- Nœud du pétiole avec 0-4 paires de poils, >5 paires de poils dressés sur le tergite abdominal III, >6 paires de poils dressés (généralement plus) sur le tergite abdominal IV ……... ***'apicalis'* Morpho-espèce II**

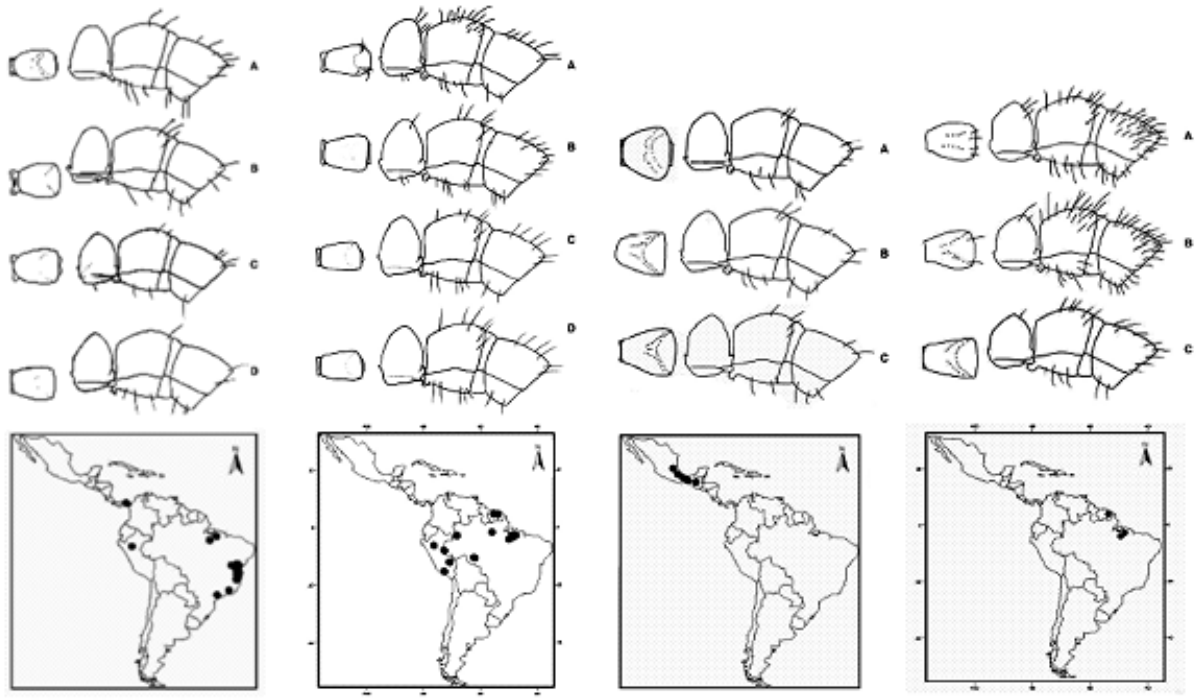
5 - Intersections sur la face postérieure du pétiole avec les côtés nettement arrondis, ne formant pas d'angle, hypogydium densément pubescent dans la région près de l'aiguillon (voir Wild, 2005) ..... ***obscuricornis***

5'- Intersections sur la face postérieure du pétiole avec les côtés formant un angle aigu ….. **6 (groupe *'verenae'*)**

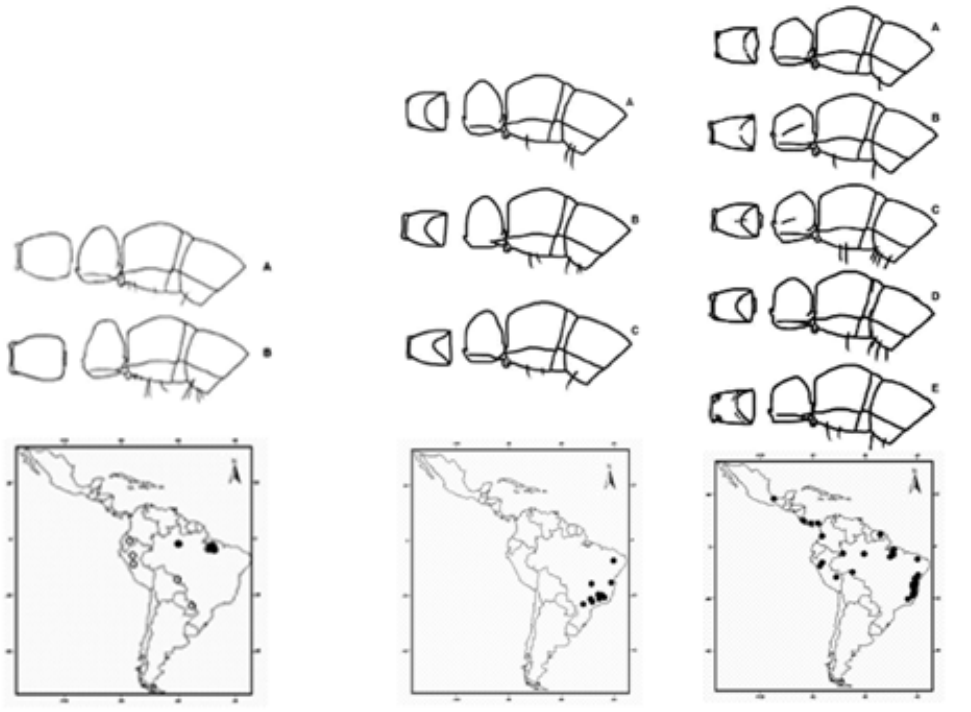
6 – Pétiole, en vue latérale, presque aussi haut que long ..... ***'verenae'* Morpho-espèce I**

6'- Pétiole, en vue latérale, 1,2 à 1,4 plus haut que long ..... ***'verenae'* Morpho-espèce II**

ENCADRE 3. Continuation



P. apicalis Morpho-espèce I | *P. apicalis* Morpho-espèce II | *P. apicalis* Morpho-espèce III | *P. apicalis* Morpho-espèce IV



P. obscuricornis | *P. verenae* Morpho-espèce I | *P. verenae* Morpho-espèce II

La bioacoustique comme un outil taxonomique chez les insectes

Bien que les différences morphologiques aient traditionnellement été utilisées pour définir et décrire de nouvelles espèces pour presque tous les groupes taxonomiques, parfois l'étude des caractéristiques morphologiques seule ne suffit pas à identifier et définir de nouvelles espèces (Angulo & Reichle 2008). Il existe donc un besoin urgent de lui adjoindre des outils efficaces pour permettre de résoudre ces problèmes taxonomiques afin d'estimer la biodiversité réelle dans ces groupes d'espèces. De plus, dans les cas où la morphologie suffirait, l'intégration d'autres approches pourrait aider à accélérer significativement le processus de délimitation des espèces (Schlick-Steiner et al. 2010).

Ainsi, l'étude des signaux sonores et vibratoires comme critère diagnostique de l'identification des espèces est devenue une pratique courante dans plusieurs groupes taxonomiques qui communiquent acoustiquement (Otte 1989, Angulo & Reichle 2008).

Les signaux acoustiques ont souvent été impliqués dans l'étude des espèces cryptiques et ils ont parfois été la première indication d'une diversité cachée dans de nombreux groupes d'insectes comme les homoptères (Claridge et al. 1997), les chrysopes (Henry 1994), les orthoptères (Walker et al. 2003, Cade & Otte 2000, Broza et al. 1998) et les diptères (Ritchie & Gleason 1995, Noor & Aquadro 1998). Ces travaux combinés à de nombreux autres ont permis ainsi d'établir la bioacoustique comme un outil taxonomique important pour un grand nombre de taxons cryptiques (Sueur 2006, Angulo & Reichle 2008). La bioacoustique est une science multidisciplinaire qui englobe l'étude de la production sonore, de sa propagation dans un milieu et de sa réception par les animaux. Elle implique la connaissance des bases neurophysiologiques et anatomiques de la production et de la détection du son et du rapport entre les signaux acoustiques et les moyens par lesquels ils sont dispersés. Les résultats de ces études nous donnent des indications sur l'évolution des mécanismes de la communication acoustique et de là sur l'évolution des animaux qui les utilisent (Miller et al 2010).

Chez les fourmis, le peu de groupes d'espèces étudiés à ce jour n'a pas permis d'observer de différences interspécifiques importantes ni dans les structures productrices de son ni encore dans les caractéristiques du signal (Markl et al. 1977, Grasso et al. 1998, 2000, Pavan et al. 1997, Barbero et al. 2009 a,b). Ainsi, jusqu'à présent, les stridulations ont été considérées comme un simple signal généraliste qui ne présente pas d'avantage sélectif à être spécifique de l'espèce et qui n'est que rarement spécifique, même au niveau du genre (Markl 1973). Cependant, ces généralisations se basent sur un nombre très limité d'espèces et sur de rares études ayant porté sur les caractéristiques acoustiques (Figure 1) et sont loin d'être

représentatives des Formicidae, étant donné le grande nombre d'espèces dotées d'un organe spécialisé dans la production de signaux acoustiques (Markl 1973, Taylor 1978).

Objectifs de l'étude et organisation de la thèse

Comme on l'a vu précédemment, la taxonomie du complexe d'espèces *Pachycondyla apicalis* est loin d'être consensuelle (Wild 2005, Delabie et al. 2008). Etant donné que l'identification exacte des espèces est essentielle à la fois à la recherche dans tous les domaines de la biologie et à la conservation de la biodiversité (Balakrishnan 2005), l'un des objectifs premiers de ce travail est la détermination de la diversité cryptique au sein de ce complexe d'espèces. Bien qu'indiscernables pour l'œil humain, les espèces cryptiques sont évidemment dissemblables les unes des autres, et elles présentent normalement des caractéristiques biologiques, écologiques et comportementales stables et particulières à chacune (Bickford et al. 2007). De ce fait, nous avons cherché au travers d'études bioacoustiques, génétiques, chimiques, écologiques et comportementales à caractériser des éléments de la biologie de ces espèces pouvant être considérés comme indicateurs de l'espèce, et ceci également dans le but de mieux comprendre les interactions intra- et interspécifiques dans ce groupe d'espèces phylogénétiquement proches.

Ce travail s'organise en quatre chapitres. Dans le premier, nous commençons par examiner en détail les organes producteurs de son et les signaux acoustiques produits par les fourmis du groupe *Pachycondyla apicalis* afin de comprendre si l'évolution des signaux acoustiques est liée au processus de spéciation dans ce complexe d'espèces. Pour estimer le potentiel de la bioacoustique comme outil de discrimination des espèces cryptiques, les relations phylogénétiques entre les taxons du complexe ont été évaluées par une analyse moléculaire, ce qui nous permettra aussi d'améliorer la cohérence de nos conclusions. Dans ce chapitre, la variabilité intra-spécifique des signaux acoustiques a également été étudiée pour les différentes castes des deux espèces les plus amplement sympatriques du complexe à savoir : *P. apicalis* et *P. verenae*.

Au deuxième chapitre, l'étude des contextes de production des stridulations, de la réponse de fourmis à ces signaux et de leurs possibles fonctions dans le complexe d'espèces *P. apicalis* seront considerés. Comme on l'a vu précédemment, ce groupe d'espèces appartient à l'un des plus anciens genres de la sous-famille « primitive » des Ponerinae, et présente donc un grand intérêt pour comprendre la position de ce mode de communication dans le cours de l'évolution de ces insectes.

Par ailleurs, étant donné que la spéciation est un processus très complexe influencé par différents facteurs (génétique, écologique, développemental, environnemental, etc.) qui interagissent de manière non linéaire (Gavrilets 2003), nous étudions ensuite le comportement reproducteur des fourmis du complexe *P. apicalis*. En effet, dans le cas de spéciations sympatriques ou de remise en contact d'espèces ayant divergé en allopatrie, la question de l'établissement d'un isolement reproductif au sein des populations est importante (Coyne & Orr 1998). En outre, comme les sons spécifiques sont généralement produits dans des situations où il existe un intérêt adaptatif à discriminer les espèces étroitement apparentées (Sueur 2006), l'occurrence de productions sonores au cours de l'ensemble des comportements sexuels a aussi été considérée pour ces espèces largement sympatriques et écologiquement comparables.

Au troisième chapitre, une étude des hydrocarbures cuticulaires, de la biométrie détaillée et des préférences écologiques des différentes espèces du complexe *P. apicalis* provenant de plusieurs populations de Guyane Française et du Brésil a été réalisée afin de mieux comprendre les caractéristiques biologiques représentant des idiosyncrasies et celles qui sont communes aux espèces du complexe, ainsi que l'évolution de ces caractéristiques selon les conditions biogéographiques.

Enfin, au quatrième chapitre, nous nous intéressons particulièrement aux interactions intra-spécifiques chez *P. verenae* dans une population de forte densité, où les aires de fourragement de nids distincts peuvent se superposer et des fourmis allo-coloniales peuvent souvent interagir. Ici, nous avons étudié l'effet de la distance entre les nids sur l'agression intra-spécifique à une échelle fine et si et comment les divergences chimiques et génétiques entre les colonies sont corrélées aux règles de décision sous-tendant ce comportement. Nous examinerons l'importance relative de ces différents facteurs dans la mise en place des seuils de tolérance chez cette espèce facultativement polygyne.

Au cours des expérimentations, en disséquant les fourmis pour les analyses morphométriques de l'organe stridulatoire, nous avons détecté dans l'abdomen d'une fourmi *P. verenae* de Guyane Française la présence d'un insecte de l'ordre Strepsiptera, qui s'est avéré être un parasite spécialisé des Formicidae. En inspectant les colonies de fourmis récoltées dans ce site, d'autres individus immatures et/ou adultes de cette espèce ont pu être retrouvés chez *P. verenae* et *P. apicalis*. Cette découverte nous a permis, en collaboration avec le Department of Zoology de l'Oxford University, UK et le Laboratório de Entomologia, de l'Universidade Federal do Rio Grande do Norte, Brésil, de re-décrire le mâle de cette espèce assez particulier, où les sexes exhibent un dimorphisme extrême, non seulement dans

leur morphologie, mais aussi dans leurs hôtes respectifs, et de retrouver par des analyses moléculaires, la femelle qui lui correspond, ainsi que de décrire pour la première fois, le comportement des fourmis parasités. Ce travail est présenté dans les annexes de cette thèse.

CHAPITRE 1 :

Etude des signaux acoustiques dans le complexe d'espèces *Pachycondyla apicalis*

Résumé

Bien que peu étudiée, la communication acoustique est largement répandue chez les fourmis. En effet, nombre d'espèces possèdent un organe stridulatoire et elles en font usage dans des contextes aussi variés que la défense coloniale, le recrutement ou encore l'accouplement. Toutefois, les caractéristiques acoustiques et fonctionnelles de ces signaux n'ont été étudiées que de façon préliminaire et chez un nombre d'espèces très restreint, montrant la nécessité d'investigations plus approfondies.

Les signaux acoustiques ainsi que les relations phylogénétiques des différents morphes du complexe d'espèces cryptiques *Pachycondyla apicalis* ont été étudiés afin de déterminer s'il existe des variations inter-morphes dans ces signaux et si celles-ci peuvent être utilisées pour estimer la diversité spécifique réelle de ce complexe, et ainsi tester la validité de cette approche en tant qu'outil taxonomique. Par la suite, ces signaux ont également été analysés chez *P. apicalis* et une espèce sympatrique et phylogénétiquement apparentée, *P. verenae*, du niveau intracolonial (castes) au niveau interspécifique.

Les résultats montrent des différences morphologiques dans l'organe stridulatoire associées à des différences dans les stridulations produites pour chacun des morphes de *P. apicalis*, ces variations étant en outre corroborées par les analyses génétiques. Les signaux stridulatoires présentent des caractéristiques inter-castes distinctes chez les deux espèces *P. apicalis* et *P. verenae*, les stridulations des mâles divergeant à un degré plus important que celles des femelles. De plus, l'analyse révèle l'existence de caractéristiques temporelles et fréquentielles spécifiques de l'espèce pour chacune des castes, avec des niveaux de divergence plus marqués chez les femelles.

Cette étude est la première démonstration de telles spécificités d'émissions acoustiques au sein des castes et des espèces chez les fourmis. Un tel degré de spécialisation de l'organe acoustique ainsi que du signal produit suggère que les stridulations pourraient faire partie des signaux intervenant dans les relations intra et interspécifiques. Cette étude confirme par ailleurs l'intérêt d'utiliser les signaux acoustiques pour mettre en lumière les frontières entre les espèces. Nous proposons ainsi que chaque morphe étudié soit considéré dorénavant comme une nouvelle espèce valide, suggérant que le complexe *P. apicalis* comprenne en réalité entre six et neuf espèces distinctes.

Article 1: Stridulations reveal cryptic speciation in Neotropical sympatric ants

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Stridulations reveal cryptic speciation in Neotropical sympatric ants

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Abstract

The taxonomic challenge posed by cryptic species underlines the importance of using multiple criteria in species delimitation. In the current paper we tested the use of acoustic analysis as a tool to assess the real diversity in a cryptic species complex of Neotropical ants. In order to understand the potential of acoustics and to improve consistency in the conclusions by comparing different approaches, phylogenetic relationships of all the morphs considered were assessed by the analysis of a fragment of the mitochondrial DNA cytochrome b. We observed that each of the cryptic morph studied presents a morphologically distinct stridulatory organ and that all sympatric morphs produce distinctive stridulations. This is the first evidence of such a degree of specialization in the acoustic organ and signals in ants, which suggests that stridulations may be among the cues used by these ants during inter-specific interactions. Mitochondrial DNA variation corroborated the acoustic differences observed, confirming acoustics as a helpful tool to determine cryptic species in this group of ants, and possibly in stridulating ants in general. Congruent morphological, acoustic and genetic results constitute sufficient evidence to propose each morph studied here as a valid new species, suggesting that *P. apicalis* is a complex of at least 6 to 9 species, even if they present different levels of divergence. Finally, our results highlight that ant stridulations may be much more informative than hitherto thought, as much for ant communication as for integrative taxonomists.

Introduction

The Tropics are home to nearly two-thirds of the World's known biodiversity, but also to a large amount of species that have remained unnoticed (Willig et al. 2003), partly due to the occurrence of cryptic species, i.e. of two or more distinct species that are erroneously classified (and hidden) under a single species name, due to their very similar morphology (Bickford et al. 2007). Such species cause great problems for taxonomists as they cannot be readily or reliably distinguished only on a morphological basis, and the taxonomic challenge they pose underlines the importance of using multiple criteria in species delimitation. Indeed, concordant changes in several characteristics of an organism, and corroboration from independent data constitute better evidence for separating species (Bickford et al. 2007, Schlick-Steiner et al. 2010). As accurate species identification is crucial both to research in all areas of biology and to biodiversity conservation (Balakrishnan 2007, Bickford et al. 2007) there is an urgent need to coalesce effective tools which allow the clarification of these taxonomic issues in order to estimate the real biodiversity in cryptic species groups.

Acoustic signals may differ between cryptic species and were often the first clue of a hidden diversity in many insect groups, like hemipterans (Claridge et al. 1997), lacewings (Henry 1994), orthopterans (Broza et al. 1998, Cade & Otte 2000, Walker et al. 2003) and flies (Ritchie & Gleason 1995, Noor & Aquadro 1998). By delimitating species phenotypic variability, acoustic analyses have revealed many unsuspected species and solved several confusing taxonomic problems. Thus, acoustic descriptions should be systematically included in species diagnosis (Sueur 2006). The use of DNA sequences ("DNA barcoding") in extensive phylogenetic studies has also revealed an effectively high level of hidden biodiversity (Wilcox et al. 1997, Berkov 2002, Hebert et al. 2004, Smith et al. 2008), suggesting that molecular data should be incorporated by taxonomists as a matter of routine (Bickford et al. 2007).

In ants, stridulatory sound production is known since the late nineteenth century (Landois 1874, Lubbock 1877, Janet 1893, 1894, Sharp 1893). This faculty seems to have evolved several times independently and can be found in a great number of species from five subfamilies (Myrmicinae, Pseudomyrmecinae, Ponerinae, Ectatomminae, Nothomyrmecinae) (Markl 1973, Taylor 1978). Stridulations are produced during dorso-ventral movements of the gaster by the rubbing of the distal border of the 3rd segment of the abdomen that acts like a scraper on the *stridulatory file*, made up of perfectly parallel and rectilinear tegument ridges on the mid-dorsal edge of the fourth abdominal segment (Spangler 1967, Kermarrec et al. 1976). They consist in *chirps* made by train of pulses, in which each pulse corresponds to the

rubbing movement of the scraper on one ridge on the stridulatory file (Hölldobler & Wilson 1990). These signals are essentially transmitted by the substrate (Roces et al. 1993, Roces & Tautz 2001) but evidence for a perception of air-transmitted sounds, at least over distances of a few centimetres, is also available (Hickling & Brown 2000). These signals are generally barely audible without amplification (Markl 1973, Hickling & Brown 2000) but in some Ponerinae species, like *Pachycondyla apicalis*, the intensity of the airborne sound emitted by a single ant at a distance of 1 cm can reach more than 93 dB (Pavan et al. 1997). The frequency of the signal can vary from a few hertz, like in *Solenopsis richteri* (Hickling & Brown 2000) and *Myrmica spp* (Barbero et al. 2009a, 2009b) to up to 84 kHz in *P. apicalis* (Pavan et al. 1997). Thus far, stridulations have been shown to be produced in several behavioural contexts depending on the species examined, like food recruitment (Roces et al. 1993, Hölldobler et al. 1978, Baroni Urbani et al. 1988), trophallaxis (Stuart & Bell 1980), nest emigration Maschwitz & Schönegge 1983), intra- and inter-specific conflicts (Markl 1965, Grasso et al. 2000), and mating (Markl et al. 1977, Mercier et al. 2007). Some ants can also respond to stridulations produced by their mutualists (DeVries 1990, 1991, Travassos & Pierce 2000, Morales et al. 2008) or even their parasites (Barbero et al. 2009a, 2009b).

However, even if stridulations are common events in ant societies, it is still probably the least understood mode of communication and detailed studies on the acoustic characteristics of these signals are scarce (Ferreira & Fresneau 2009). Furthermore, up to now, most work on ant stridulations refers only to the subfamily Myrmicinae, and almost nothing is known about primitive ants like the Ponerinae. In this ant subfamily, the genus *Pachycondyla* is one of the most ancient still living (Hölldobler & Wilson 1990, Bolton 1995). It presents over 60 described species just for the Neotropics (Fernández & Sendoya 2004) and after some assessment studies (Majer & Delabie 1993, 1999, Delabie et al. 2000), it appears to be a good bioindicator of the myrmecological diversity and quality of Neotropical ecosystems. Recent studies (Mariano et al. 2000, 2006, Lucas et al. 2002) demonstrated that many Neotropical *Pachycondyla* actually consist of cryptic species complexes, indicating that the diversity in this genus can be really underestimated.

The *Pachycondyla apicalis* species complex is a good example of this problem and its taxonomy remains unsettled. Ants in this group are large, conspicuous insects found in Neotropical forests from southern Mexico to Paraguay, overall presenting similar ecological and biological features (Wild 2005, Fresneau 1994). Furthermore, ants in this complex also share the same general morphology and coloration (the "morph *apicalis*") which is probably under stabilizing selection as it is also copied by other arthropods like spiders (Reiskind 1977,

McIver & Stonedahl 1993, Cushing 1997). These sympatric species could thus form a massive mimicry ring which would represent an advantage against predators (Hebert et al. 2004, McIver & Stonedahl 1993, Delabie et al. 2008). Wild (2005) revised the taxonomy of this group using several morphological and biometrical criteria and recognised three broadly sympatric species: *P. apicalis* (Latreille 1802), *P. obscuricornis* (Emery 1890) and *P. verenae* (Forel 1922) instead of only two as thought before (Brown 1957). Although he observed a considerable morphological variation for *P. verenae* and *P. apicalis* across their distribution ranges he did not consider further division of the complex. Later, in a morphological study combining cytogenetical and ecological data when available, Delabie et al. (2008) demonstrated that, given the stability of differences through multiple cases of sympatry, the variability pointed by Wild (2005) could in fact refer to a species mosaic rather than a geographic cline. They recognized seven distinct taxa within this group: four for *P. apicalis*, two for *P. verenae* and one for *P. obscuricornis*, and despite supporting a really intricate diversity inside the *P. apicalis* species complex, the authors were not able to conclude about the validity and the taxonomic status (e.g. species, subspecies, ecotypes) of the different morphs within each species without further investigations.

Here for the first time, we test acoustics as a tool to assess the real diversity in a cryptic species complex of Neotropical ants. We measured the overall structure of the stridulatory file and performed a detailed acoustic analysis of the stridulations produced in five morphs of the *P. apicalis* species complex. To improve the consistency in conclusions by comparing different approaches, we also analysed a fragment of the mitochondrial DNA cytochrome b for all morphs considered.

Methods

Ants

Colonies of the *P. apicalis* species complex were collected at Petit Saut, French Guiana (n=10) and Los Tuxlas, Mexico (n=1). Colonies were reared in the laboratory in artificial plastered nests. The nests were maintained at 25 ± 1°C, with approximately 65 ± 10 % relative humidity, and a 12L:12D photoperiod. All colonies were provided with an identical diet (honey/apple mixture and crickets) twice a week. Ant collection, husbandry and experimental procedures used in this study fulfilled all the legal requirements concerning insect experimentation of France.

Ants were classified into morphs within the currently named species according to Delabie et al. (2008) classification. Three different morphs could be identified, one for *P.*

verenae (PVE) and two for *P. apicalis* (PAP), respectively: PVE Morph 1 (5 colonies), PAP Morph 3 (1 colony) and PAP Morph 4 (2 colonies) (Figures S1.1-S1.3). Among our collected colonies, we did not find the *P. verenae* Morph 2 or *P. apicalis* Morphs 1 or 2. Moreover, three colonies of *P. apicalis* from Petit Saut did not fit any described morph in Delabie et al. (2008). We treated them here as 2 new morphs: PAP Morph 5 (2 colonies) and PAP Morph 6 (1 colony), as they also presented subtle distinctive morphological traits. PAP Morph 5 is moderate sized, with an emarginated petiole and a very finely striated cuticle and hairy head and body (Figure S1.4). PAP Morph 6 presents a rounder petiole and is clearly bigger than all other *P. apicalis* examined (Figure S1.5). PVE Morph 1 and PAP Morphs 4, 5, and 6 were found in sympatry in the same site at Petit Saut, French Guyana. PAP Morph 3 is allopatric to all other morphs and occurs only in Mexico (2008). Vouchers of each morph were deposited in the CPDC collection of the Laboratório de Mirmecologia, Cocoa Research Center at Itabuna (Bahia, Brazil).

Morphometric Study of the Stridulatory Organ

A total of 41 workers from 8 colonies (Table 1) were dissected and the segments of the gaster containing the stridulatory file were cleaned in an ultrasonic-wave bath, air-dried and then placed on aluminium stubs. The samples were coated with a mixture of 80% gold / 20% palladium and examined with a Leica Stereoscan 440 scanning electron microscope (SEM). Measurements of the stridulatory organ were obtained from the digitalized SEM images. Eight variables were measured from each stridulatory file: Length, maximal width, 1st, 2nd and 3rd quartile widths, number of ridges and inter-ridge distance in the medial and distal portions of the stridulatory file (Figure 1). For these two last variables, 5 measures were taken from each worker and the mean value was computed. Also, as an estimate of the ants' size, the thorax length of 42 other workers was measured with a Zeiss Stereo Microscope at a magnification of 10x.

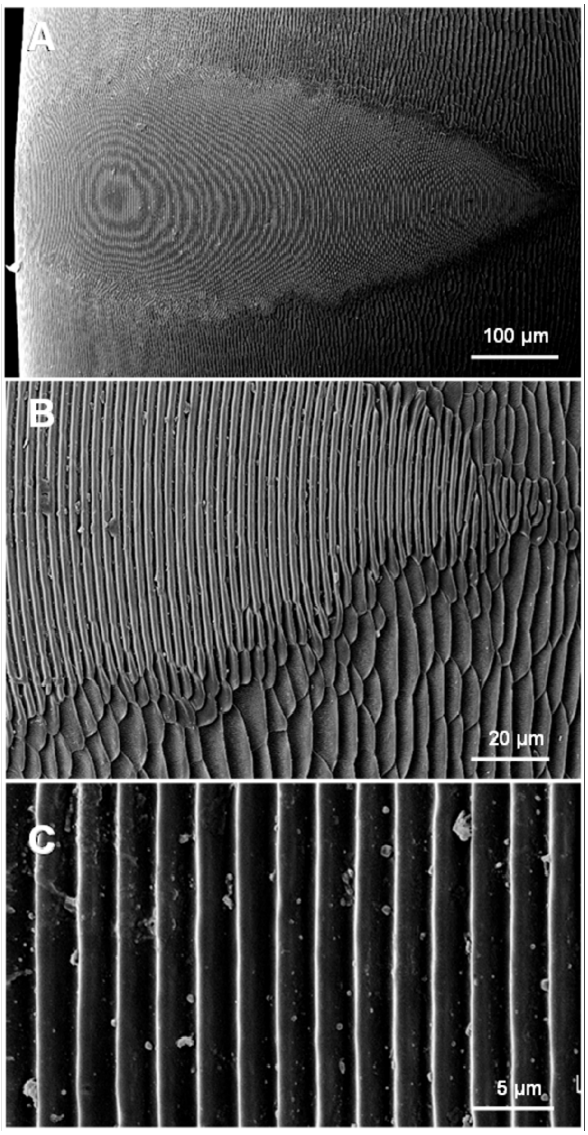

Figure 1. Scanning electron micrographs of the stridulatory file of a *Pachycondyla apicalis* species complex worker. (A) General view of the stridulatory file, (B) detail of the ridges showing the interface between the fine structure of the file and the surrounding cuticle and (C) detail in the medial portion of the stridulatory file, showing the inter-ridge distances.

Stridulation recording and analysis

Ants from the *P. apicalis* species complex produce stridulations which result in both airborne sound and substrate vibrations. Airborne sound presents audible and ultrasound components, and in the following we refer to the audible component only. A total of 40 workers from 5 colonies were recorded. All recordings were carried out in a low-noise room where the ambient temperature was kept at 25 ± 1 °C and the relative humidity at 65 ± 10 %. The recording setup consisted of an omnidirectional Sennheiser K6 microphone (frequency response: 30 to 20000 Hz ± 1 dB) connected to a Marantz PMD 671 digital recorder with sampling frequency at 48 kHz. We did not consider frequencies superior to 20 KHz, due to technical limitations of the microphone. Ants were held with forceps 1 cm from the microphone during recording. The following *temporal parameters* were analysed using the software Avisoft-SASLab Pro, version 4.40 (Specht 2008): the chirp duration, the inter-chirp interval, and for each chirp we measured the number of pulses, the pulse repetition rate, the

mean inter-pulse interval as well as the inter-pulse interval in the 1st, 2nd and 3rd thirds of the chirp. The *frequency parameters* considered for each chirp were: the dominant frequency, the frequencies at 25, 50 and 75% of the signal energy, and the percentage of energy below 14 kHz. The maximal and minimal intra-pulse frequencies were also calculated for each individual, by the zero-crossing method (Mbu Nyamsi et al. 1994). For each chirp analysed, we calculated the maximal and minimal intra-pulse frequencies for 10 pulses and the mean value was computed (Figure 2). A series of ten chirps was analyzed for each ant, and the mean value was computed.

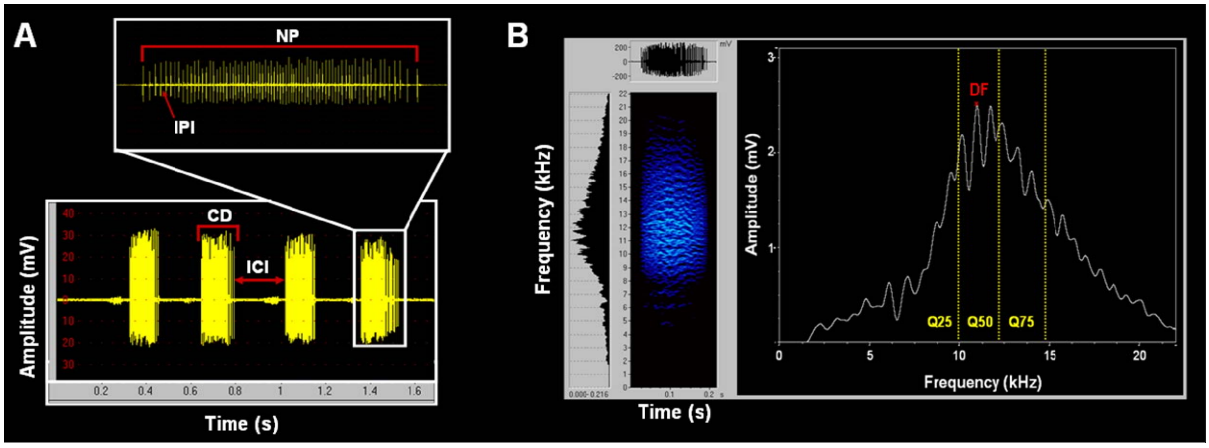

Figure 2. Stridulatory signal of a *Pachycondyla apicalis* species complex worker. (A) Oscillogram of a series of chirps, showing the chirp duration (CD), the inter-chirp interval (ICI), the number of pulses (NP) in a chirp and the inter-pulse interval (IPI). (B) Spectrogram of a chirp, showing the dominant frequency (DM) and the quartiles of frequencies at 25, 50 and 75% of the signal energy (Q25, Q50 and Q75 respectively).

DNA extraction, amplification and sequencing

The DNA of 22 workers from 11 colonies was extracted from ethanol-preserved tissues (head and thorax) using a DNeasy Blood & Tissue kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocol. Mitochondrial DNA variation was assayed by the amplification of a portion of the mtDNA cytochrome b (cyt b, ~700bp) using primers CB1 (5'-TAT-GTA-CTA-CCA-TGA-GGA-CAA-ATA-TC-3') and tRS (5'-TAT-TTC-TTT-ATT-ATG-TTT-TCA-AAA-C-3') from Simon et al. (1994). Each PCR was carried out in a 50-µL volume according to a standard protocol using a T1 thermal cycler (Biometra). The thermal cycle profile was as follows: 2 min at 94°C; 35 cycles at [30 s at 94°C/ 60 s at 50°C/ 60 s at 72°C]; 5 min at 72°C. Amplified products were sequenced using the same primers as used for

the amplification by Genoscreen (Lille, France) using an ABI 3730XL automatic sequencer (Applied Biosystems).

Sequences analyses were edited and aligned using the default settings of Clustal X (Thompson et al. 1997) and checked by eye. To generate phylogenetic trees, we used pairwise distances (Neighbor Joining algorithm, NJ). As *Pachycondyla* is a paraphyletic genus (C. Schmidt, pers. com.), we used two other divergent *Pachycondyla* species as outgroups: *P. villosa* and *P. goeldi*, respectively. Average intermorph genetic divergence was calculated using the Kimura 2-parameter model (Kimura 1980) using the MEGA4 program (Tamura et al. 2007). Other models (Jukes–Cantor or Tamura-Nei distance) when applied, resulted in similar results. NJ tree was constructed with the MEGA4 program. The robustness of the tree was tested with 1000 bootstrap replications. The equality of evolutionary rate between all sequences was tested using Tajima' relative rate test (Tajima 1993) in MEGA4.

Statistical analysis

Workers used for the morphometric measurements came from two different colonies for PVE Morph 1 and PAP Morphs 4 and 5. As these colonies did not differ for the structure of the stridulatory organ (MANOVA "colonies nested within morphs", Wilk's $\lambda = 0.249$, $F_{24,47} = 1.205$, $p = 0{,}2863$), their data were pooled for further comparisons between morphs. Discriminant function analyses (DFA) were performed to identify potential differences of the stridulatory file morphometry and the acoustic characteristics of stridulations between morphs and to calculate the success rate of individual discrimination (correct classification rate) using Mahalanobis distances. As the temporal parameter of stridulations "inter-chirp interval" showed a very high coefficient of variation within morphs (ranging from 13.31 to 45.50%), this parameter was not included in the DFA. Additionally, we compared each morphometric and acoustic parameter using one-way ANOVA followed by Unequal N HSD (Honest Significance Difference) post hoc tests (Spjotvol & Stoline 1973) to understand how these parameters varied between morphs. Paired Student's t-tests were used to establish the differences between the medial and distal inter-ridge distances within groups. When necessary, Box-Cox transformation was applied to achieve normality on some parameters (Box & Cox 1964). All results are stated as mean±SE. The significance level was taken at $\alpha \leq 0.05$ to assess differences. All analyses were conducted using Statistica v8.0 (StatSoft France 2007).

Results

Morphometry of the stridulatory organ

The discriminant function analysis of all morphometric parameters considered for the stridulatory file clearly separate all studied morphs (Wilk's λ= 0. 005, $F_{32,108}$= 10.712, $p<0.001$; Figure 3, Table 1).

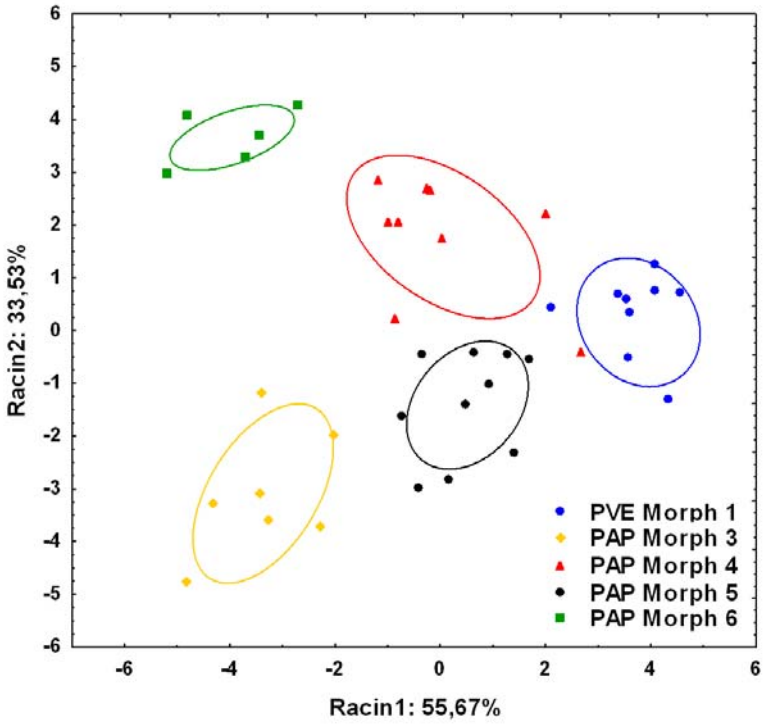

Figure 3. Discriminant function analysis of the stridulatory file morphometry of the *P. apicalis* species complex. PVE: *P. verenae*, PAP: *P. apicalis.* Ellipses are 95% confidence intervals around centroids.

Table 1. Morphometric characteristics (mean±SE) of the stridulatory file in five morphs of the *Pachycondyla apicalis* species complex.

FILE OF RIDGES	*P. verenae* Morph 1	*P. apicalis* Morph 3	*P. apicalis* Morph 4	*P. apicalis* Morph 5	*P. apicalis* Morph 6
Number of ridges	245,10 ± 2,86	212,71 ± 4,76	242,78 ± 5,03	242,60 ± 4,32	258,40 ± 8,97
Length (µm)	574,22 ± 8,41	618,88 ± 10,63	624,43 ± 16,84	617,38 ± 8,06	693,72 ± 18,42
Maximal width (µm)	282,12 ± 7,43	234,63 ± 5,14	239,60 ± 4,71	279,24 ± 3,90	214,48 ± 4,14
1st quartile width (µm)	272,46 ± 6,67	209,09 ± 7,42	232,80 ± 4,64	260,86 ± 6,46	214,48 ± 4,14
2nd quartile width (µm)	216,73 ± 10,34	232,33 ± 6,22	177,84 ± 6,85	230,91 ± 5,08	143,64 ± 3,75
3rd quartile width (µm)	161,04 ± 5,63	143,19 ± 9,03	135,63 ± 4,82	146,23 ± 3,23	73,44 ± 5,14
Inter-ridge distance medial region of the file (µm)	2,51 ± 0,04	2,89 ± 0,03	2,86 ± 0,07	2,58 ± 0,06	3,07 ± 0,08
Inter-ridge distance distal region of the file (µm)	2,35 ± 0,04	3,02 ± 0,03	2,55 ± 0,07	2,81 ± 0,06	2,80 ± 0,08
# Workers (# Colonies)	10 (2)	7 (1)	9 (2)	10 (2)	5 (1)

The file (Figure S2) can vary in length (Figures 4 and 5.a), maximal and quartile widths (Figure 4), medial and distal inter-ridge distances (Figure 6) and number of ridges between morphs (Figure 5.b). These differences are combined in a distinctive way within each morph, which allows a 97,5% correct classification rate of all individuals based on the overall pattern of the stridulatory file.

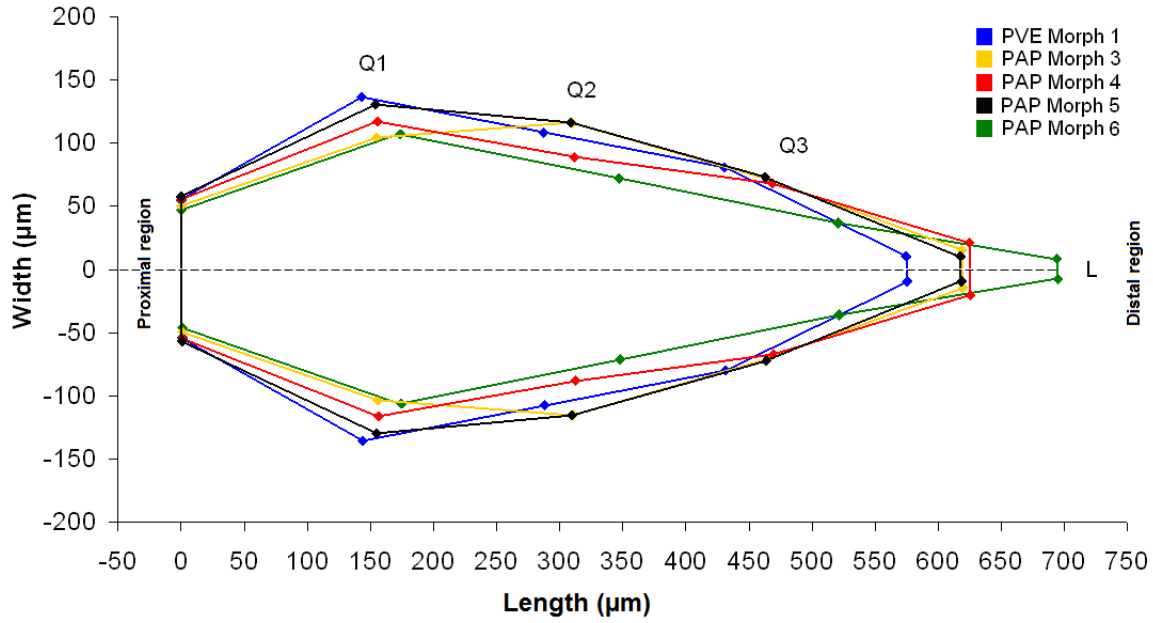

Figure 4. Pattern of the stridulatory file of five morphs from the *P. apicalis* species complex. First (Q1), second (Q2) and third (Q3) quartile widths of the file of ridges in relation with the length (L). $ANOVA_{Q1}$, $F_{4,36}$=18.74, $ANOVA_{Q2}$, $F_{4,36}$=20.25, $ANOVA_{Q3}$, $F_{4,36}$=25.88 and $ANOVA_{Length}$, $F_{4,36}$=9.82. All $p<0.001$. PVE: *P. verenae*, PAP: *P. apicalis.*

Differences observed in the pattern of the stridulatory file are not due only to allometric differences between individuals. Morphs with different body size present the same length for the stridulatory file (Figure 5.a). Moreover, stridulatory files of the same length can comprise different number of ridges (Figure 5.b), which directly affect the inter-ridges distance. This latter parameter presents complex variation patterns along the stridulatory file depending on the morph. It can vary between the medial (ANOVA, $F_{4,36}$=15.17, $p<0.001$) and distal (ANOVA, $F_{4,36}$=21.61, $p<0.001$) regions of the file and the pattern is not the same for all morphs: PVE Morph 1 and PAP Morphs 4 and 6 present ridges more spaced in the middle than in the distal region of the file, while PAP Morph 3 and 5 present the opposite pattern (Figure 6).

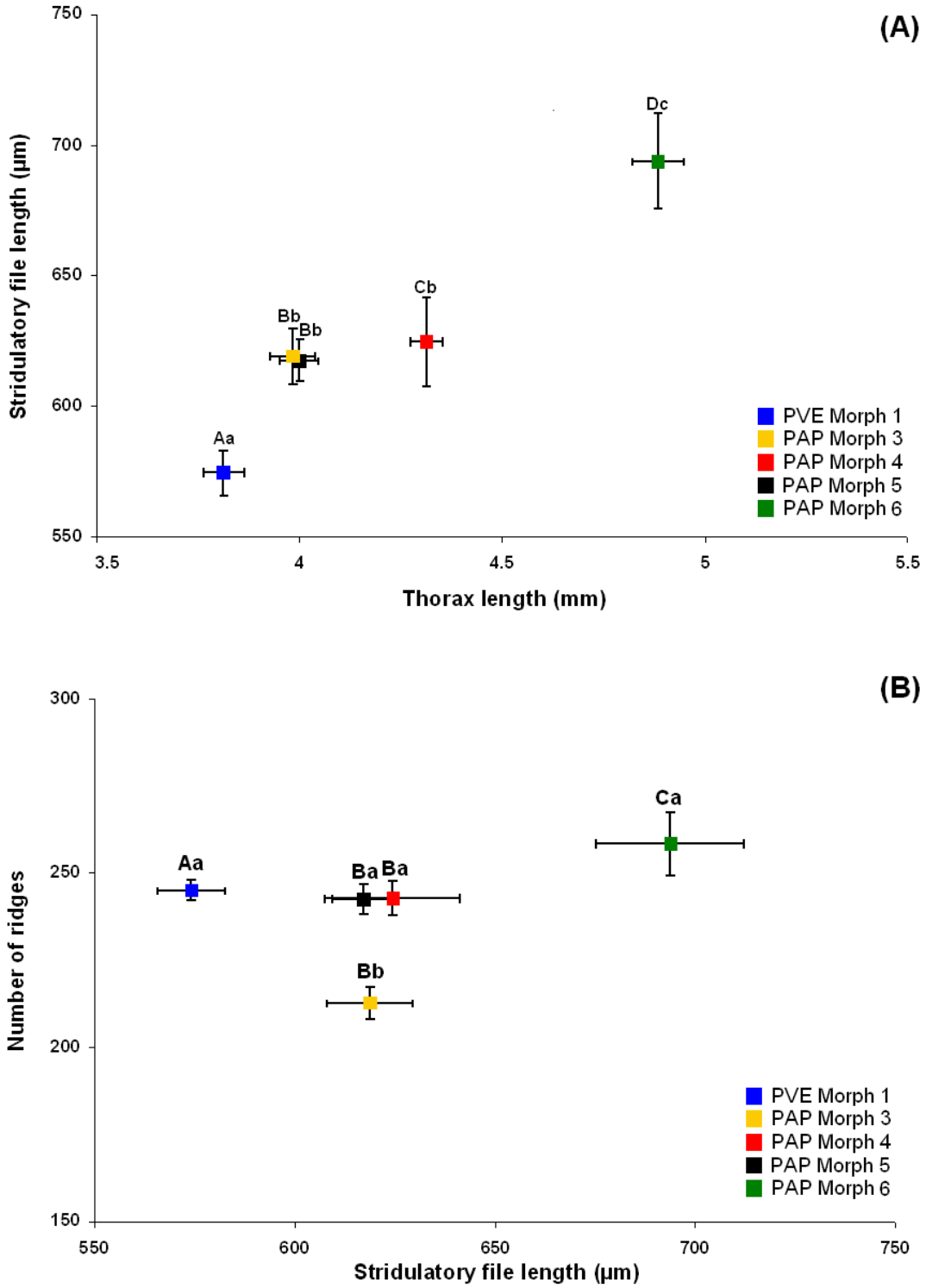

Figure 5. Ant size and stridulatory file features. Relationship between the stridulatory file length and (A) the ant size (represented by thorax length) and (B) the number of rigdes of five morphs from the *P. apicalis* species complex. ($ANOVA_{Thorax}$, $F_{4,37}$=57.82, p<0.001, $ANOVA_{File}$, $F_{4,36}$=9.83, p<0.001 and $ANOVA_{Ridges}$, $F_{4,36}$=9.66, p<0.001). When different, capital letters indicate significant differences for parameters on the X axes, and small letters indicate significant differences b for parameters on the Y axes tests, p<0.05. PVE: *P. verenae*, PAP: *P. apicalis.*

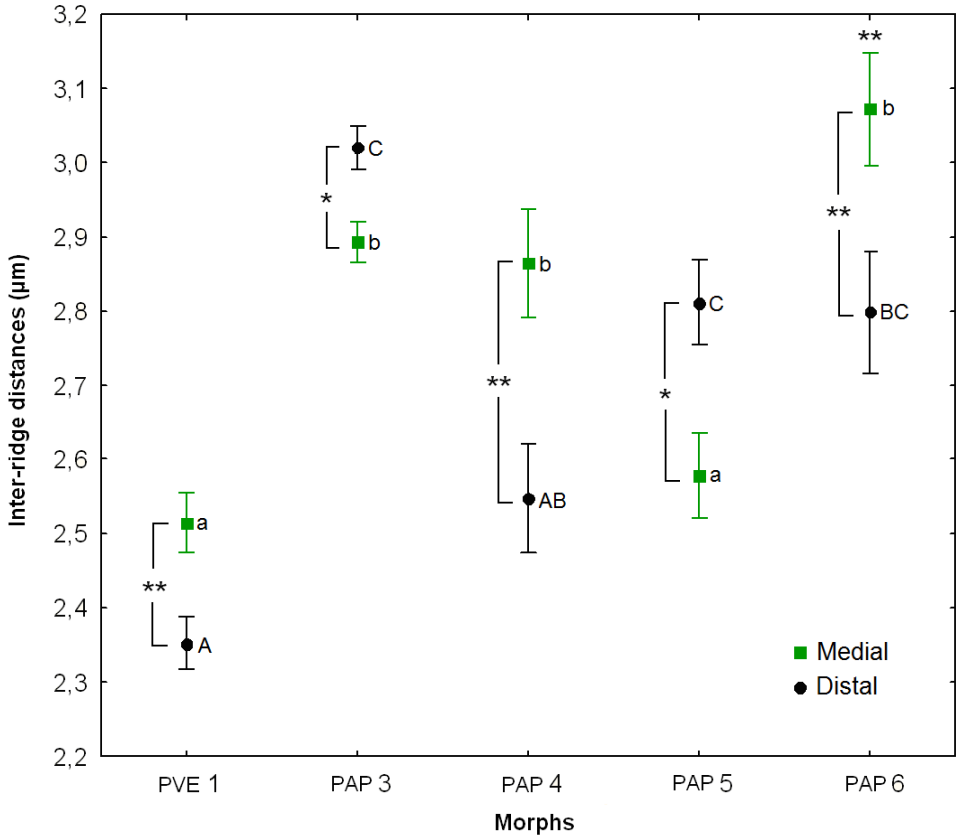

Figure 6. Inter-ridge distances of the stridulatory file in the *P. apicalis* species complex. Comparison of the inter-ridge distances in the medial and distal portions of the stridulatory file within morphs (Paired Student's t-tests within morphs, $t_{PVE\ Morph\ 1}$=4.28, $t_{PAP\ Morph\ 3}$=-3.11, $t_{PAP\ Morph\ 4}$=3.86, $t_{PAP\ Morph\ 5}$=-2.93, $t_{PAP\ Morph\ 6}$=7.18; * $p<0.05$ and ** $p<0.01$) and between morphs ($ANOVA_{Medial}$, $F_{4,36}$=15.17, $p<0.001$, $ANOVA_{Distal}$, $F_{4,36}$=21.61, $p<0.001$. When different, small letters indicate significant differences of inter-ridge distances on the medial portion of the stridulatory file and capital letters indicate significant differences of inter-ridge distances on the distal portion of the stridulatory file (Unequal N HSD tests, $p<0.05$)). PVE: *P. verenae*, PAP: *P. apicalis.*

Acoustic analyses of stridulations

The DFA of all temporal and frequency parameters (except the inter-chirp interval) reveals that each sympatric morph from French Guiana produce distinctive sounds (Wilk's λ: 0.01987, $F_{56,87}$: 2.724, p< 0,0001; Figure 7, supporting information S3). Here, the differences in the acoustic characteristics of stridulations allowed a correct classification rate of 95% of all individuals.

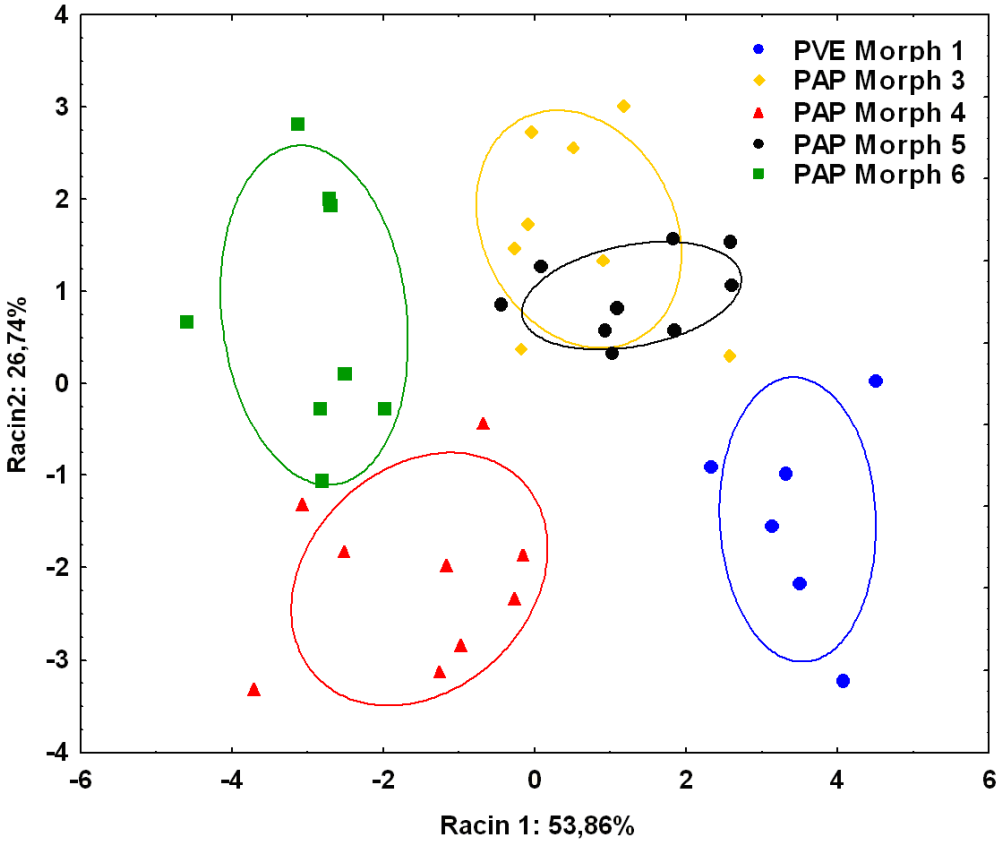

Figure 7. Discriminant function analysis of the stridulations of the *P. apicalis* species complex. All temporal and frequency parameters measured (except the inter-chirp interval) are considered in the model. PVE: *P. verenae*, PAP: *P. apicalis*. Ellipses are 95% confidence intervals around centroids.

PVE Morph 1 produces shorter chirps than PAP Morphs 4 and 6 ($ANOVA_{Chirps}$, $F_{4,35}$=5.24, p<0.01, Unequal N HSD , p<0.01 and p<0.05 respectively, Table 2), and with fewer pulses than PAP Morph 6 ($ANOVA_{NPulses}$, $F_{4,35}$=4.18, p<0.01, Unequal N HSD , p<0.01, Table 2), notwithstanding the similarity in the number of ridges in the stridulatory file (Figure 5.b). This result is due to the smaller inter-ridge distances presented by PVE Morph 1 (Figure 6), but also to the smaller number of ridges that are rubbed during stridulatory movements (Table 2). In spite of presenting a high inter-individual variation, the inter-chirp interval in PAP Morph 6 is bigger than all the other PAP morphs ($ANOVA_{ICI}$, $F_{4,35}$=4.84, p<0.01, Unequal N HSD , p<0.05 for PAP Morph 3 and p<0.01 for PAP Morphs 4 and 5, Table 2), which means this morph produces fewer stridulations in a given time.

Table 2. Acoustic characteristics (mean±SE) of the stridulatory signals in five morphs of the *Pachycondyla apicalis* species complex.

STRIDULATIONS	*P. verenae* Morph 1	*P. apicalis* Morph 3	*P. apicalis* Morph 4	*P. apicalis* Morph 5	*P. apicalis* Morph 6
Chirp duration (ms)	105,83 ± 3,67	141,25 ± 7,97	162,87 ± 8,76	128,89 ± 7,07	150,17 ± 13,18
Inter-chirp interval (ms)	286,82 ± 40,94	245,46 ± 20,80	225,59 ± 9,49	229,86 ± 9,53	436,65 ± 77,02
Number of pulses	77,68 ± 7,83	95,70 ± 7,90	96,78 ± 5,28	98,69 ± 8,08	121,59 ± 6,64
Pulse repetition rate (Hz)	695,85 ± 79,01	682,70 ± 85,83	605,17 ± 37,15	771,81 ± 62,89	856,87 ± 106,73
Mean Inter-pulse interval (ms)	1,38 ± 0,17	1,55 ± 0,22	1,61 ± 0,12	1,26 ± 0,11	1,20 ± 0,16
Inter-pulse interval 1st third (ms)	1,24 ± 0,16	1,52 ± 0,18	1,39 ± 0,15	1,14 ± 0,10	1,12 ± 0,10
Inter-pulse interval 2nd third (ms)	1,05 ± 0,14	1,18 ± 0,16	1,25 ± 0,10	0,96 ± 0,09	0,98 ± 0,11
Inter-pulse interval 3rd third (ms)	1,84 ± 0,23	1,95 ± 0,33	2,18 ± 0,13	1,67 ± 0,14	1,50 ± 0,29
Dominant frequency (kHz)	10,56 ± 0,65	11,10 ± 0,27	12,26 ± 0,41	10,77 ± 0,25	11,85 ± 0,15
Frequency at 25% energy (kHz)	8,90 ± 0,14	9,31 ± 0,11	9,70 ± 0,25	9,18 ± 0,11	9,40 ± 0,15
Frequency at 50% energy (kHz)	11,40 ± 0,21	11,79 ± 0,08	12,24 ± 0,20	11,72 ± 0,13	12,06 ± 0,12
Frequency at 75% energy (kHz)	14,55 ± 0,17	14,71 ± 0,07	14,64 ± 0,13	14,67 ± 0,13	14,87 ± 0,08
Proportion of energy below 14Khz	0,71 ± 0,01	0,69 ± 0,01	0,68 ± 0,01	0,70 ± 0,01	0,68 ± 0,01
Minimal intra-pulse frequency (kHz)	9,13 ± 0,07	9,12 ± 0,20	11,53 ± 0,43	9,31 ± 0,22	9,47 ± 0,41
Maximal intra-pulse frequency (kHz)	10,82 ± 0,33	11,54 ± 0,12	12,22 ± 0,27	11,57 ± 0,12	11,93 ± 0,17
# Workers	6	8	9	9	8

Nevertheless, this morph seems to rub the stridulatory file faster during stridulation production, as suggested by the tendencies for a higher pulse repetition rate and a lower mean inter-pulse interval together with larger inter-ridge distances (Figure 6, Tables 1 and 2).

Concerning frequency parameters, PAP Morph 4 stridulations have higher dominant frequency than PVE Morph 1 and PAP Morph 5 ($ANOVA_{DF}$, $F_{4,35}$=4.21, p<0.01, Unequal N HSD, p<0.05 for both morphs, Table 2), higher frequencies at 25% and 50% of the signal energy than PVE Morph 1 ($ANOVA_{F25\%}$, $F_{4,35}$=2.82, p<0.05, Unequal N HSD, p<0.05 and $ANOVA_{F50\%}$, $F_{4,35}$=4.05, p<0.01, Unequal N HSD, p<0.05, Table 2), and higher intra-pulse minimal frequency than all other morphs ($ANOVA_{MFP}$, $F_{4,35}$=8.17, p<0.01, Unequal N HSD, p<0.01 for all morphs). The intra-pulse maximal frequency is also significantly lower in PVE Morph 1 compared to PAP Morphs 4 and 6 ($ANOVA_{LFP}$, $F_{4,35}$=5.62, p<0.01, Unequal N HSD, p<0.01 for PAP Morph 4 and p<0.05 for PAP Morph 6). Last, the stridulations of the allopatric PAP Morph 3 from Mexico did not significantly differ only from those of PAP Morph 5 from French Guiana (Figure 7) even if these morphs present distinctive stridulatory organs (Figures 3-6).

Nucleotide composition and sequence variation

A total of 690 base pairs were analysed for cyt b. Accession numbers range from HM770106 to HM770124 in Genbank. No pseudogenes, no insertions, deletions nor any rearrangements were detected. As in other hymenopteran mitochondrial genome (Crozier & Crozier 1993), there is an A-T bias in the base composition of cyt b: we obtained on average 33.9% of A, 44.1% of T, 14.7% of C, and 7.3% of G. Over the 690 base pairs analysed, the number of variable characters was 259 among which 156 were found to be parsimony-informative. The transition/transversion rate ratios are k_1 = 1.207 (purines) and k_2 = 2.974 (pyrimidines), with an overall transition/transversion bias of 0.584. NJ tree of haplotypes obtained for the different morphs is presented in Figure 8, rooted with *P. villosa* and *P. goeldi* as outgroups. Tajima's relative tests are significant (X^2= 4.25, p=0.038 to X^2= 8, p=0.005, df=1) when morphs of the clade constituted by *P. apicalis* are compared to *P. verenae* or PAP Morph 5, rejecting as a consequence the molecular clock hypothesis for this set of sequences.

We could observe 3 main groups, in which PAP Morph 5 clearly differs from all other morphs (about 15%), and presents a basal position to the *P. apicalis* and *P. verenae* groups (Figure 8).

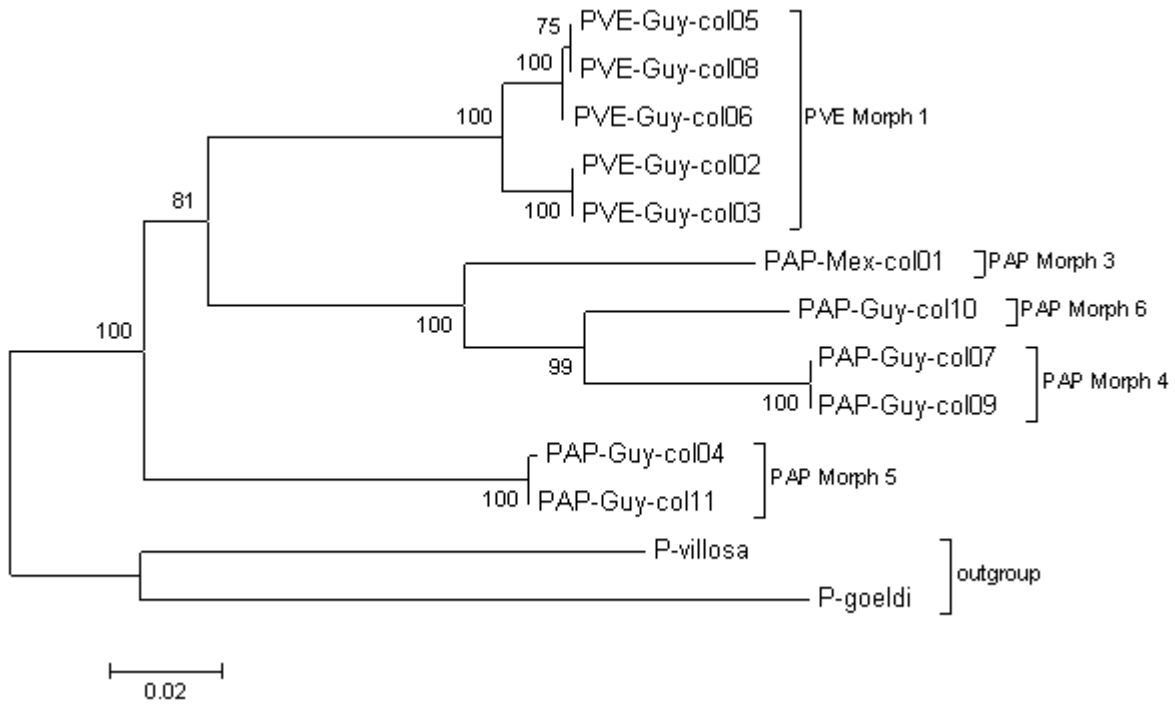

Figure 8. Neighbour-joining phylogenetic tree of mtDNA haplotypes of the *Pachycondyla apicalis* species complex. The tree is rooted using *P. villosa* and *P. goeldi* sequences as outgroups 1 and 2, respectively. Numbers indicate bootsrap values. PVE: *P. verenae*, PAP: *P. apicalis.*

Within *P. verenae* Morph 1, we obtain two clades but the level of divergence is low (mean distance of 1.5 ± 0.3%). Within the group *P. apicalis*, the genetic distance between Morphs 3, 4 and 6 is considerable. When compared to the other morphs of *P. apicalis*, PAP Morph 3 varies in 10% of the base pairs considered, which nearly corresponds of the level of divergence between *P. verenae* and the clade *P. apicalis*. Between the sympatric *P. apicalis* Morphs 4 and Morph 6 the genetic variation is about 7% (Figure 8).

Discussion

Our results demonstrate that each studied cryptic morph of the *P. apicalis* complex presents a morphologically distinct stridulatory organ, and that all sympatric morphs make distinctive sounds. The differentiation observed for the stridulatory organs is not only due to allometric differences between individuals but also to intrinsic morph features. The distinct acoustic signals produced, in their turn, are the result of this morphological specificity together with ant behaviour for stridulation production. Indeed, inter-specific competitive interactions in sympatry may have led to divergent selection acting in contrasting directions between morphs. In contrast, the similarity observed between the acoustic signals produced

by PAP Morphs 3 and 5 in spite of the distinctiveness of their stridulatory files, can be due to the fact that the allopatric PAP Morph 3 is not subjected to the same selective pressure as the sympatric morphs.

To our knowledge, we provide the first record of such a degree of acoustic specialization in closely related ants, both at the level of the production organ and of the produced signal. For a long time, stridulations have been considered as a mere generalist alarm signal that has no selective advantage to be species-specific and that could rarely be found specific even at the genus level (Markl 1973). In the few groups of sympatric and/or related species studied to date (three sympatric species of *Pogonomyrmex* ssp (Markl et al. 1977), four sympatric *Messor* species (Grasso et al. 1998, 2000), four Neotropical *Ectatomma* (Pavan et al. 1997) and five *Myrmica spp.* (Barbero et al. 2009a, 2009b) no prominent inter-specific differences could be observed in the structures of the stridulatory file and in the characteristics of the signal. The only specificity already demonstrated in ant stridulations was at the intra-specific level, between different castes: for major and minor workers in *Atta cephalotes* (Markl 1968) and for gynes and males or workers in four sympatric *Messor* species (Grasso et al. 2000). Recently, Barbero et al (2009a, 2009b) demonstrated that acoustic signals carry information about the caste and the status of a colony member in *Myrmica schencki* and trigger distinct behavioural responses by workers as a function of the identity of the emitter.

In ants, the most important channel for communication involves chemical and, to a smaller degree, tactile cues (Hölldobler & Wilson 1990, 2009). However, the clear differentiation and specificity in the stridulatory file and signals observed in this study in a group of species with considerable morphological stasis suggests that acoustic communication may have a more important role than generally thought during interspecific relationships in these ants. We can also expect that, in this group of cryptic ants, acoustic signals might modulate chemical and tactile cues in different ways (Barbero et al. 2009a), and that a synergy between distinct-source information might improve communication in different behavioural contexts.

The variations observed here for some acoustic parameters would have likely been decreased and the differences between morphs would have likely been built up by studying individuals coming from different colonies for each morph. Nevertheless, mitochondrial DNA variation estimated for cytochrome b well supports the acoustic differences observed for all five morphs and confirms the taxonomic potential of acoustics for this group of cryptic ants and possibly for other stridulating ant species. We thus found that each studied morph in the

P. apicalis species presents a real genetic identity. The most surprising result is the basal position of PAP Morph 5 and its high level of genetic divergence compared to other sympatric *P. apicalis.* The genetic isolation of PAP Morph 5 is mirrored by some morphological characters that could have been neglected, leaving this morph hidden until now. Moreover, despite the non-divergence of PAP Morph 5 stridulations from those of the allopatric PAP Morph 3, the corroboration of the distinct morphometry of the stridulatory file, genetics and morphology are enough evidence to separate these two taxa. Similarly, the genetic distances observed between the other two morphs of *P. apicalis,* even if lower than between PAP Morphs 3 and 5, are all well above the usual inter-specific values found for some cryptic insects groups, i.e. the 3% sequence divergence threshold typically used in the barcoding studies (Song et al. 2008).

The congruence of the genetic data with acoustic and morphological information leads us to propose each morph studied here as a valid new species. In fact, the subtle morphological variations observed for these ants confirm not to be random, and what Wild (2005) previously thought to be only intra-specific morphological variation, is verified by acoustic analysis and genetics to be distinct inter-specific traits, as supposed by Delabie et al (2008). Given that acoustics matched the mitochondrial DNA divergence in the *P. apicalis* species complex and showed a high potential for species diagnosis in this group of cryptic species, further studies should consider including this tool to investigate cryptic diversity in stridulating ants. Indeed, with all the specific acoustic and morphological traits evidenced by the present study, the cryptic species in the *P. apicalis* complex can be considered from now on as pseudo-cryptic species, i.e. cryptic species for which after detailed comparisons of morphological and non-morphological features key characters can be established for their identification (Schlick-Steiner et al. 2010, Saez & Lozano 2005). In addition, further work should try to compare the type specimens of the described species, seeking to verify which morphs are the true *P. apicalis* (Latreille 1802) and *P. verenae* (Forel 1922), and even *P. flavicornis* (Latreille 1802), a related *P. apicalis* species described by Latreille at the same time as *P. apicalis*, but synonymised later by Brown (1957).

Speciation is a very complex process which is affected by many different factors (genetic, ecological, developmental, environmental, etc.) interacting in nonlinear ways (Gavrilets 2003). The integrative approach undertaken in this paper was thus essential for the recognition of the real biodiversity in our sample of morphs of the *P. apicalis* species complex. Yet, the biodiversity inside this complex could even be more important than shown here. In this study, we had access to only three of seven morphs described in Delabie et al.

(2008:*P. verenae* Morph 1 and *P. apicalis* Morphs 3 and 4) and to two new sympatric morphs of *P. apicalis* that we identified for French Guiana (*P. apicalis* Morphs 5 and 6). When taking into consideration that within the morphs non studied here there are still the 'rare' *P. obscuricornis*, a second morph of *P. verenae* and two additional *P. apicalis* morphs (Delabie et al. 2008), the diversity inside the *P. apicalis* complex is expected to reach nine cryptic species, and a complete survey over the whole distribution range of the complex might uncover many more species. Additional research on the biogeography, ecology and behaviour of these ants could also reveal species idiosyncrasies, and help us to better understand the speciation process within this Neotropical species complex. For example, one might uncover the selective forces that have driven this high diversification in the *P. apicalis* clade and not in the *P. verenae*, or elucidate if this group diverged in sympatry or if their actual distribution is derived. Finally, as evidenced in skipper butterflies (Hebert et al. 2004), fig-pollinating wasps (Molbo et al. 2003), cerambycid beetles (Berkov 2002), pseudoscorpions (Wilcox et al. 1997) and ants (Mariano et al. 2000, 2006, Lucas et al. 2002), our results add support to the hypothesis of a higher incidence of cryptic species in the tropics. They thus highlight the importance of large-scale studies and the necessity of testing new complementary conclusive tools to correctly quantify Neotropical biological diversity. Such research endeavours are certainly overwhelming, but they are essential for the understanding of the world's true diversity of life and the first step to assure its conservation.

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Supplemental information

S1. Studied morphs of the *Pachycondyla apicalis* species complex. Lateral view (A), Full-face view (B) and petiole, oblique lateral view. PVE Morph 1 and PAP Morphs 4, 5, and 6 from Petit Saut, French Guiana and PAP Morph 3 from Los Tuxlas, Mexico.

S1.1. Worker specimen of *Pachycondyla verenae* (PVE) Morph 1

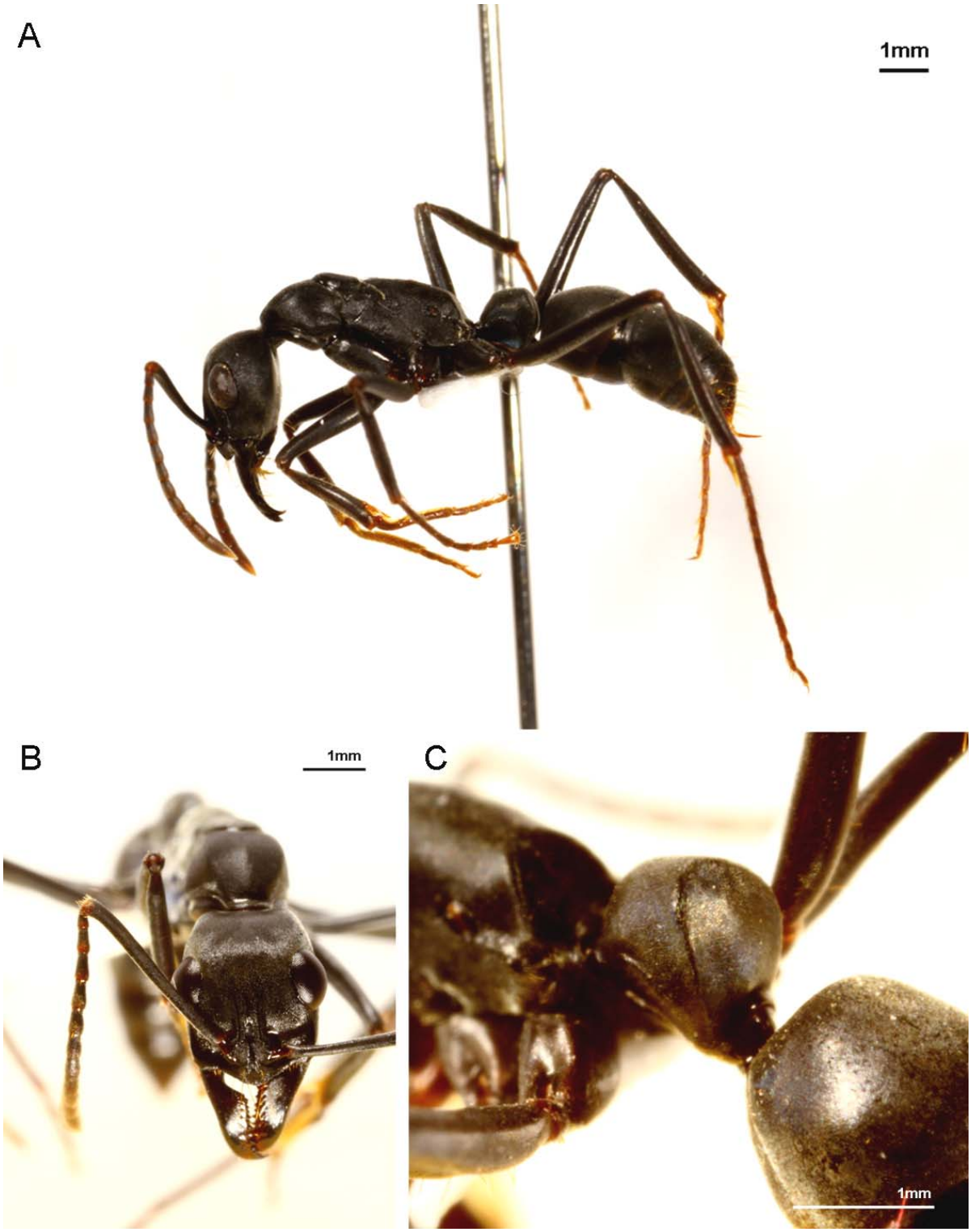
S1.2. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 3

S1.3. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 4

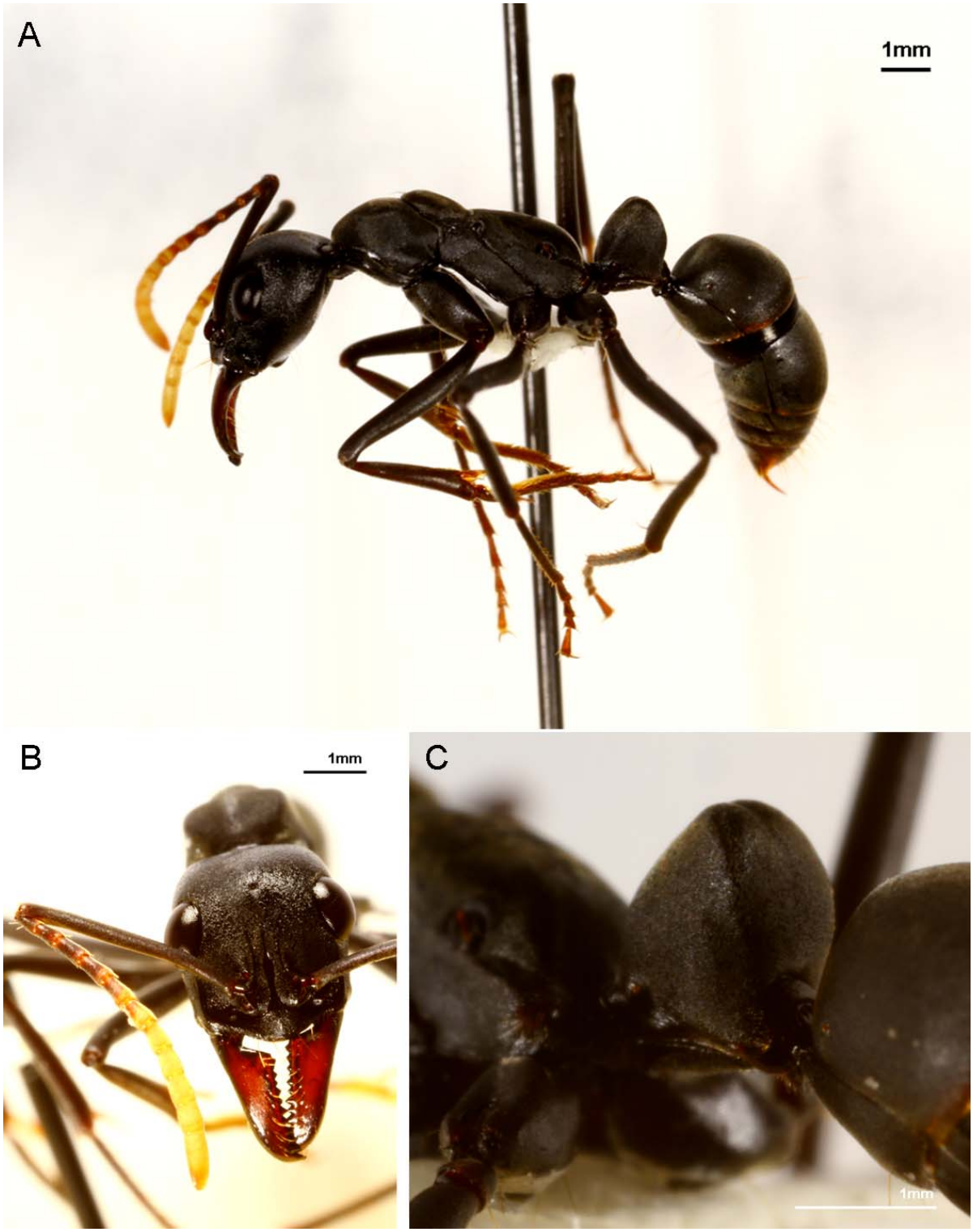
S1.4. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 5

S1.5. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 6

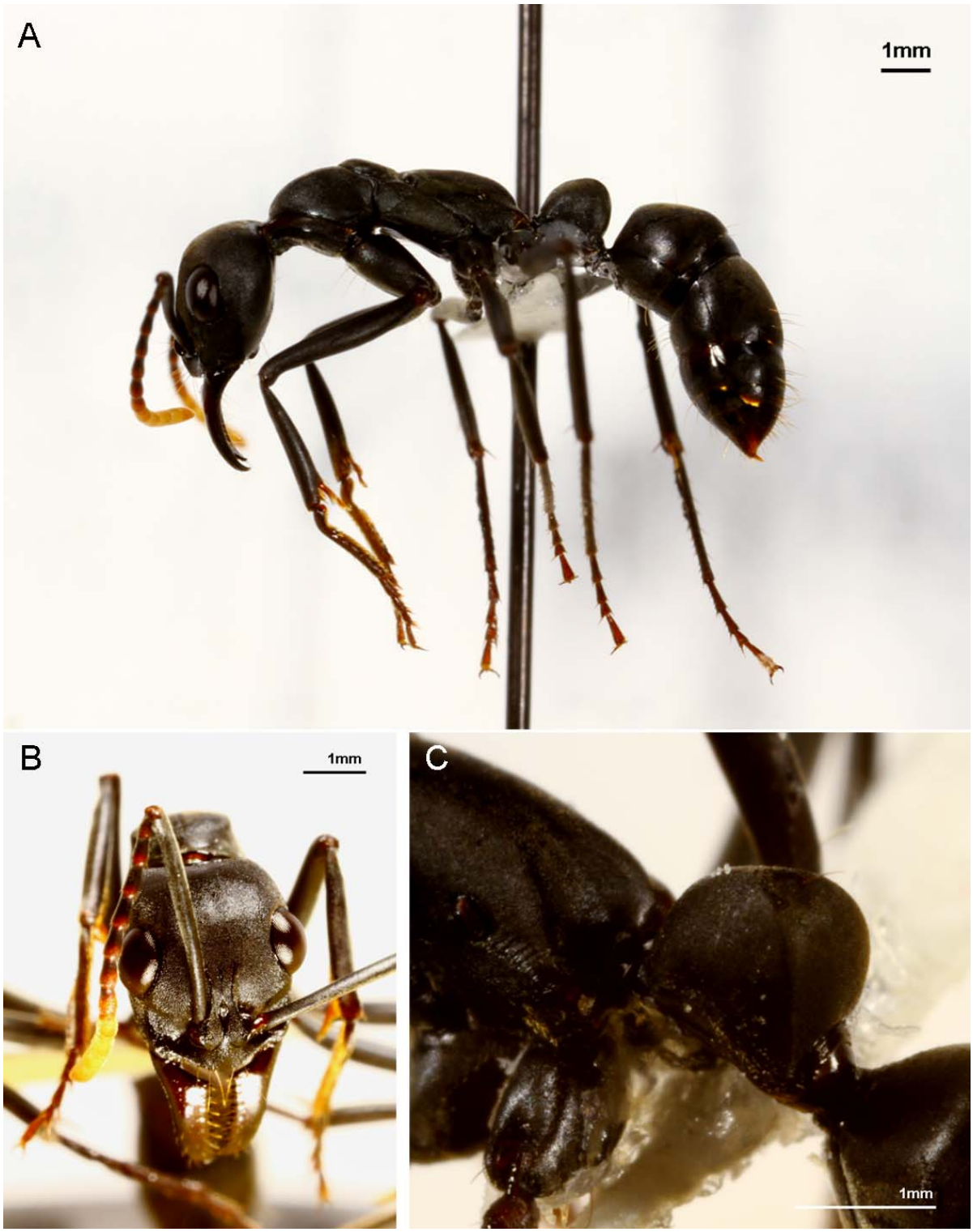
S2. SEM (Scanning Electron Microscopy) of the stridulatory file of five morphs from the *Pachycondyla apicalis* species complex. PVE Morph 1 (A), PAP Morphs 3 (B), 4 (C), 5 (D) and 6 (E). PVE Morph 1 and PAP Morphs 4, 5, and 6 from Petit Saut, French Guiana and PAP Morph 3 from Los Tuxlas, Mexico. PVE: *P. verenae*, PAP: *P. apicalis.*



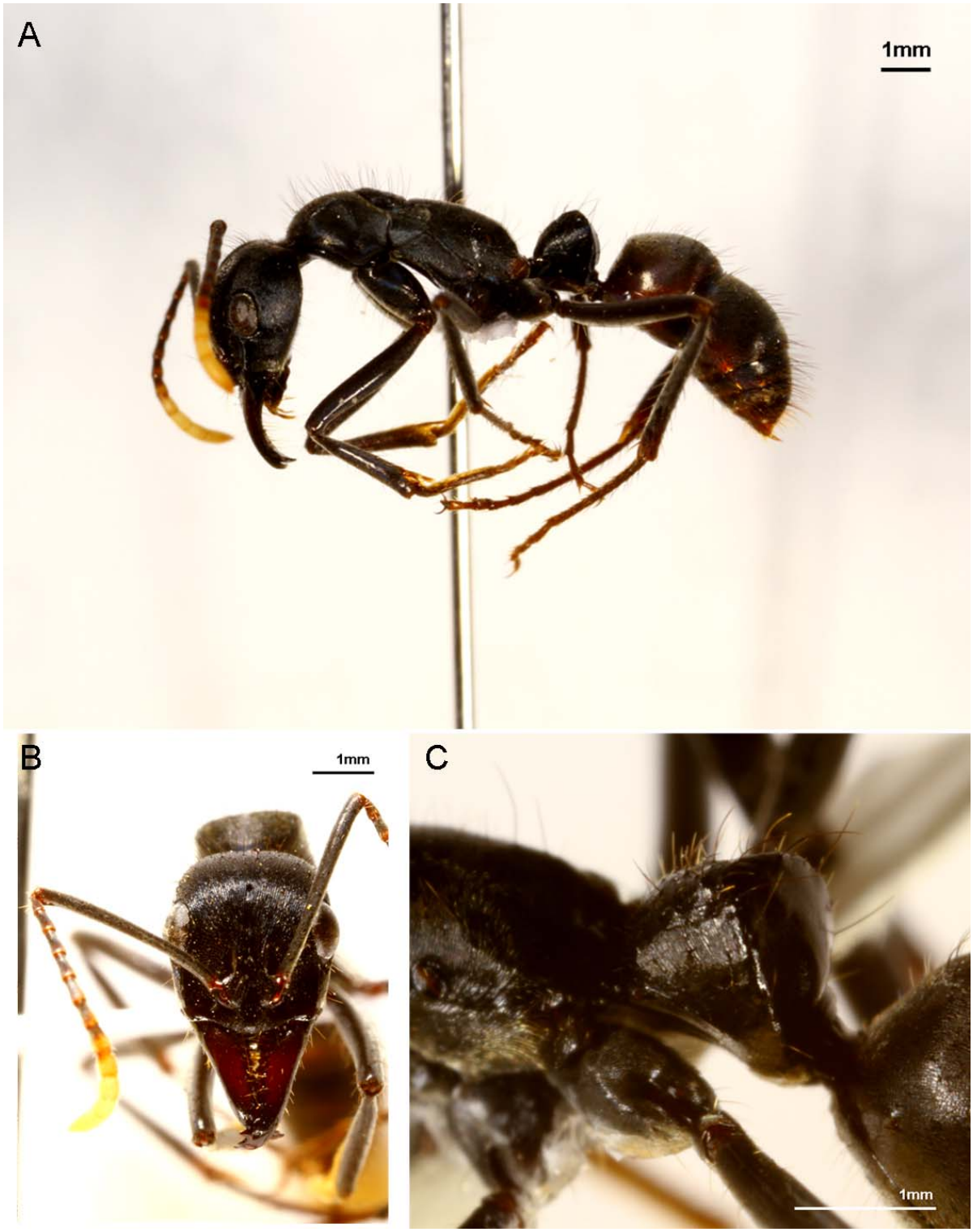
S1.1. Worker specimen of *Pachycondyla verenae* (PVE) Morph 1



S1.2. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 3



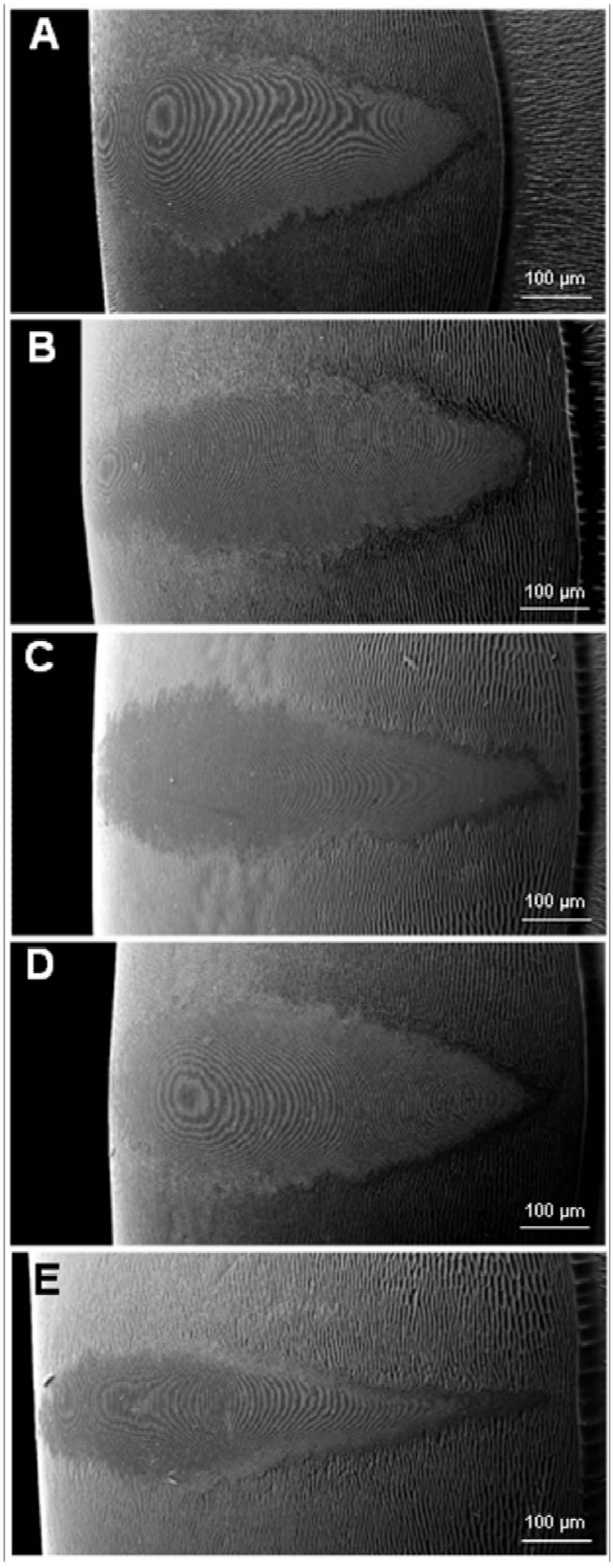
S1.3. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 4



S1.4. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 5

S1.5. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 6

S2



Article 2: Acoustical caste-specificity in the stridulatory signals of two Neotropical primitive ants (Ponerinae: *Pachycondyla*)

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En préparation

Acoustical caste-specificity in the stridulatory signals of two Neotropical primitive ants (Ponerinae: *Pachycondyla*)

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Abstract

Stridulation is a widespread phenomenon in ants. It occurs during several behavioural contexts such as alarm, recruitment, trophallaxis, nest immigration, intra- or inter-specific conflicts or even during mating for a few species. However, the acoustic characteristics and functions of these signals have been studied in only a very small number of species, and almost always taking into consideration only the stridulations of the worker caste. In this perspective, we studied the acoustic signals emitted by males, gynes and workers of two broadly sympatric and phylogenetically related species, *P. verenae* and *P. apicalis*, in which sexuals are likely to co-occur during periods of reproduction and workers to interact during foraging or nest emigrations, to determine whether there is a specific stridulatory signal for the different castes within or between species. In both species, stridulations have audible and ultrasound components and are composed of chirps of numerous pulses, usually emitted in series. Our acoustic analysis demonstrated that the stridulatory signals present distinctive characteristics between all three castes within both species, with males' stridulations diverging to a greater degree from the two female castes. Furthermore, the comparison of the signals of each caste between species revealed species-specific temporal and/or frequency characteristics for all three castes, with higher levels of divergence observed in gynes and workers, and at an even greater level for workers than previously reported in the literature. This is the first record of such caste and species-specificities in the sounds produced by sexual castes of closely related ant species. Finally, our results reopen some statements about the significance of stridulations in ants' life, and draw attention to the need for further research on acoustic communication in ants.

Key-words: *Pachycondyla apicalis, Pachycondyla verenae,* Acoustic analysis, Castes, Stridulation.

Introduction

Ant social organization is based on an efficient communication system, in which multimodal signals of chemical, visual, acoustic and tactile nature can be distinguished (Hölldobler & Wilson 2009). When compared with pheromones, the use of acoustic signals is considered undeveloped and irrelevant for these insects (Hickling & Brown 2000). However, if examined narrowly, the lack of emphasis on acoustic communication seems to be in part caused by the scarceness of studies on the subject (Ferreira & Fresneau 2009).

The ability of ants to produce sounds and vibrations using a specialized organ located on the dorsal surface of the gaster has been known for almost a century and a half (Landois 1874). Since then, this particular organ has been observed in a large number of species belonging to the subfamilies Ponerinae, Ectatomminae, Pseudomyrmecinae, Myrmicinae and Nothomyrmeciinae (Markl 1973, Taylor 1978). It has been demonstrated for some species that stridulations can be produced in several behavioural contexts such as alarm (Masters 1980, Markl 1965, 1967), food recruitment (Markl & Hölldobler 1978, Baroni-Urbani et al. 1988, Roces et al. 1993), trophallaxis (Stuart & Bell 1980) nest emigration (Maschwitz & Schönegge 1983), intra- and inter-specific conflicts (Markl 1965, Grasso et al. 2000) and mating (Markl et al. 1977, Mercier et al. 2007). However, the acoustic characteristics and functions of these signals have been studied in only a very small number of species, and almost always only taking into consideration the stridulations of the worker caste.

Indeed, only a few studies have considered the acoustic signals of the sexual castes. Markl et al. (1977) studied the acoustic characteristics of the stridulations of gynes and males and the sound production during sexual behaviour in *Pogonomyrmex* harvester ants. Despite they found no prominent interspecies and inter-caste differences in the stridulatory signals, these authors demonstrated that in these polyandrous species, after several copulations, gynes stridulate regularly to avoid further mating attempts from males, and they therefore considered stridulations in this context as a "sign of female liberation", indicating that gynes were not receptive anymore. In *Cardiocondyla* ants which have a sexual behaviour much more elaborate than usual for ants, stridulations seem to be a predominant component during sexual behaviour in non-winged males and could serve to express their level of excitation (Mercier et al. 2007) but the exact function of the acoustic signals has not been tested nor the signals recorded and analysed. Grasso et al. (2000) studied the acoustic signals of different castes in four *Messor* species and, besides finding no difference in the stridulations of gynes from different species, only observed some temporal differences between castes. In particular the values of chirp duration and inter-chirp interval were twice as high as those of males and

workers. Consequently, gynes chirp rate was about half that of the other castes. Recently, Barbero et al. (2009a,b) reported morphological and acoustic differences for *Myrmica spp.* queens and workers, in which the former presented a longer stridulatory file, with greater inter-ridges interval and a lower dominant frequency. These authors demonstrated that for *Myrmica* ants, acoustical communication alone can trigger appropriate behavioural responses towards it by the workers. Until these work, there was no direct evidence that ants from different castes could produce distinctive caste-specific sounds that induce appropriate patterns of behaviour in nestmates.

Up to now, studies on the stridulations of sexual castes have focused only on Myrmicinae, a "derived" subfamily of ants. The Ponerinae subfamily is considered a primitive subfamily based on morphological criteria and also on the social organization of the colonies (Hölldobler & Wilson 1990). The species *Pachycondyla apicalis* and *P. verenae* belongs to one of the oldest genera of this subfamily, which make them of great importance for the understanding the development of the acoustical channel of communication during ant evolution. Moreover, these species are particularly interesting because they belong to the same cryptic species complex, the "*P. apicalis* species complex" (Wild 2005, Delabie et al. 2008). Ferreira et al. (*in press*) demonstrated in a multidisciplinary study of this group that workers of these two morphologically similar species, as well as the other sympatric cryptic species in the *P. apicalis* complex, present morphologically distinct stridulatory organs and produce distinctive stridulations. This was the first evidence of such degree of specialization in the organ and acoustic signals in ants, which suggests that stridulations may be among the cues used by ants during inter-specific discrimination. Moreover this acoustic specificity also suggests the production of species-specific signals in other behavioural contexts such as mating, as known in many other insects such as orthopterans, cicadas, stink bugs and lacewings that use acoustic signals for sexual communication, (reviews in Drosopoulous & Claridge 2006 and Gerhardt & Huber 2002).

In this work, we provide a detailed acoustic analysis of the stridulations produced by individuals of different castes from *P. verenae* and *P. apicalis*, in which sexual individuals are likely to co-occur during periods of reproduction and workers to interact during foraging or nest emigrations, aiming to determine whether signals differ according to caste within and between species. If present, such specific acoustic signals could play a role in the regulation of intra- and inter-specific interactions between castes and species. Indeed, life in complex hierarchical societies in which different castes or individuals perform different activities requires a rapid, effective communication system (Hölldobler & Wilson 2009). The chemical

component is highly developed in ant communication and its predominant role is well established (Blomquist & Bagnères 2010). However, Barbero et al. (2009) observed a differential behavioural response from *Myrmica* workers towards queen-specific stridulations which induced increased "on guard" behaviour, as typically observed when they perform queen attendance. This suggests that acoustic communication can also participate in colony functioning, by signalling the caste and the status of a colony member.

Thus, specific acoustic signals containing information about the caste, sex, species, or even hierarchical status could favour the cohesion and coordination of colony members and influence the decision rules of colony defence as well as mate discrimination, as is the case for chemical signals (Hölldobler & Wilson 1990, Blomquist & Bagnères 2010). In addition they may complement chemical signalling synergistically (Hölldobler 1999) or even provide a versatile and rapid alternative in situations where chemical signalling is not flexible enough (Hickling & Brown 2000).

Methods

Ants

For this study, we used individuals of different castes (workers, gynes and males) from two species of the Neotropical *Pachycondyla apicalis* species complex: *P. apicalis* Morph 4 and *P. verenae* Morph 1. As species in the *P. apicalis* complex await formal taxonomic description, we refer to them by the names of their morphs according to Delabie et al. (2008) and Ferreira et al. (*in press*). These species are broadly sympatric through their whole distribution range (Wild 2005, Delabie et al. 2008). However, in the present study, our individuals came from different populations (all *P. apicalis* are from Petit Saut, French Guyana and *P. verenae* from Itabuna, Brazil). The term 'gyne' is here used to indicate a potential queen, since 'queen' refers more appropriately only to reigning fertilized 'queens' (Michener 1974, Grasso et al. 2000). Ants were reared in the laboratory in artificial plastered nests, which were maintained at 25±1°C, with approximately 65±10 % relative humidity, a 12L:12D photoperiod and provided with an identical diet (honey/apple mixture and crickets) twice a week.

Stridulation recording and analysis

Recordings were carried out in a low-noise room where the ambient temperature was kept at 24±1 °C and the relative humidity at 30±10 %. The recording setup consisted of an

omnidirectional Sennheiser K6 microphone (frequency response: 30 to 20000 Hz ± 1 dB) and an Avisoft Bioacoustics ultrasound microphone connected to a Marantz PMD 671 digital recorder with sampling frequency at 96 kHz. Ants were held with forceps 1 cm from the microphone during recording.

Audible and ultrasound recording could be extracted in a single file using the software Goldwave v5.23 (Goldwave Inc.) and analysed using the software Avisoft-SASLab Pro, version 4.40 (Specht 2008). The following *temporal parameters* were analysed: the chirp duration, the inter-chirp interval, and for each chirp we measured the number of pulses, the mean inter-pulse interval and the pulse repetition rate. The *frequency parameters* considered for each chirp were: the dominant frequency, the frequencies at 25, 50 and 75% of the signal energy, and the percentage of energy below 20 kHz. The maximal and minimal intra-pulse frequencies were also calculated for one pulse in each chirp, by the zero-crossing method (Mbu Nyamsi et al 1994) which consists in directly measuring the subsequent periods (T) of the signal by counting the number of passage at amplitude zero. The frequency is then given by the relation f=1/T. We recorded 55 individuals (21 males, 12 gynes and 22 workers) of *P. apicalis* and 32 individuals (10 males, 12 gynes and 10 workers) of *P. verenae*. For each individual, a series of 10 chirps was analyzed.

Also, as an estimate of the ants' size, the thorax length of 60 individuals (10 individuals from each caste and species) was measured with a Zeiss Stereo Microscope at a magnification of 16x.

Statistical analysis

To test whether stridulations differed between castes within each species, we analyse each acoustic parameter with Linear Mixed Models (LMM) fitted with Restricted Estimate Maximum Likelihood (REML) to account for data hierarchical structure using 'Individual' as random factor and 'Caste' as fixed factor. To test whether stridulations from the same caste differed between species, similar analyses were performed, with 'Species' as fixed factor. Pairwise differences among castes were established using Least Significant Difference (LSD) post hoc tests. Additionally, we compared the thorax length of each caste within both species using one-way ANOVA followed by Tukey post hoc tests and between species using Student's t-tests. Residuals were inspected to ensure normality of errors. Statistical analyses were carried out using Statistica 8.0 (StatSoft Inc France). All results are stated as mean±SE. The significance level was taken at $\alpha \leq 0.05$.

Results

General characteristics of the signals

Our results confirm the previous descriptions by Pavan (1997) and Ferreira et al. (*in press*) of the stridulatory signals of *P. apicalis* and *P. verenae.* The monosyllabic pattern of the chirp, made of a single train of pulses, shows that the plectrum is rubbed on the stridulatory file in only one of the two movements involved in the production of sound in both species (Figure 1).

Comparison of the stridulations from different castes within each species

The comparison of the acoustic signals from males, gynes and workers of *P. apicalis* showed significant differences for all temporal parameters studied (Figure 2). Males presented shorter chirps than gynes and workers (LMM, $F_{2,29}$=10.297, p=0.00015; mean±SE, males=122.08±1.9 ms, gynes=160.21±3.31 ms, workers=134.30±2.29 ms, Figure 2.a) and larger inter-chirp intervals than workers (LMM, $F_{2,29}$=5.256, p=0.00834; mean±SE, males=267.44±6.28 ms, workers=205.42±5.22 ms, Figure 2.b). Both sexual castes had chirps with more pulses than workers (LMM, $F_{2,29}$=4.940, p=0,01; mean±SE, males=97.69±2.0, gynes=104.01±3.12, workers=79.69±1.74, Figure 2.c). Furthermore, males showed smaller inter-pulse intervals than gynes and workers (LMM, $F_{2,29}$=5.997, p=0.00453; mean±SE, males=1.12±0.03 ms; gynes=1.37±0.04 ms, workers=1.46±0.03 ms, Figure 2.d), as well as a greater pulse repetition rate (LMM, $F_{2,29}$=7.129, p=0.00184; mean±SE, males=910.61±19.83 Hz; gynes=736.56±23.70 Hz, workers=665.03±10.69 Hz, Figure 2.e). No frequency differences between castes could be observed for this species (Figure 3).

In *P. verenae*, as in *P. apicalis*, the comparison of the acoustic signals from distinct castes revealed significant differences for several temporal parameters. Moreover, in this species, some frequency caste-specificities could also be observed. Males presented shorter chirps than gynes (LMM, $F_{2,29}$=6.266, p=0.005; mean±SE, males=110.66±1.48 ms; gynes=136.16±2.34 ms, Figure 2.a) and larger inter-chirp intervals than both female castes (LMM, $F_{2,29}$=26.50, p<0.0001; mean±SE, males=354.50±14.76 ms, gynes=191.43±6.43 ms, workers=175.22±6.27 ms, Figure 2.b).

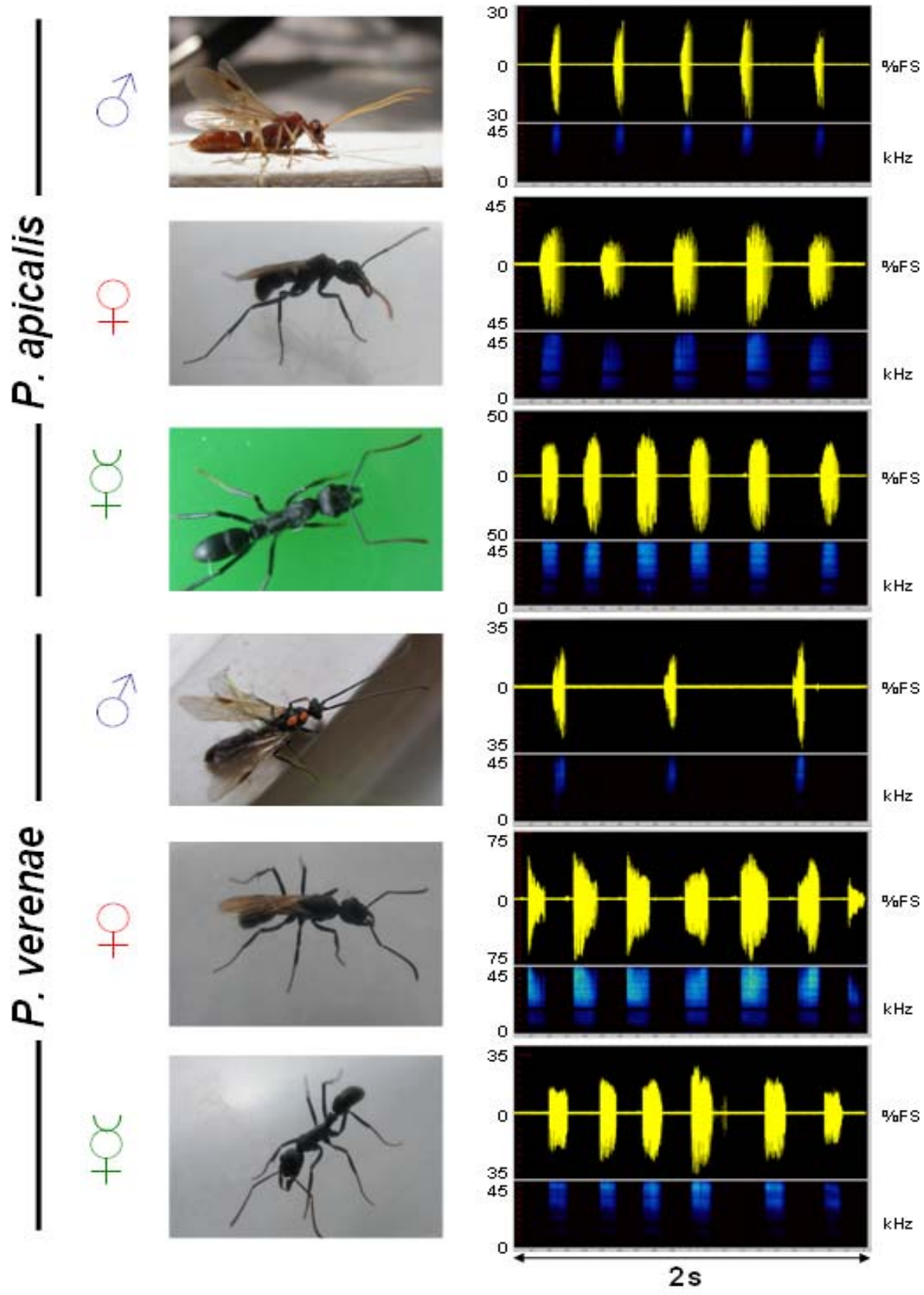

Figure 1. Stridulations of *Pachycondyla apicalis* and *Pachycondyla verenae* ants. Oscillograms (above) and spectrograms (below) of the three castes (♂ males, ♀ gynes and ☿ workers) of each species.

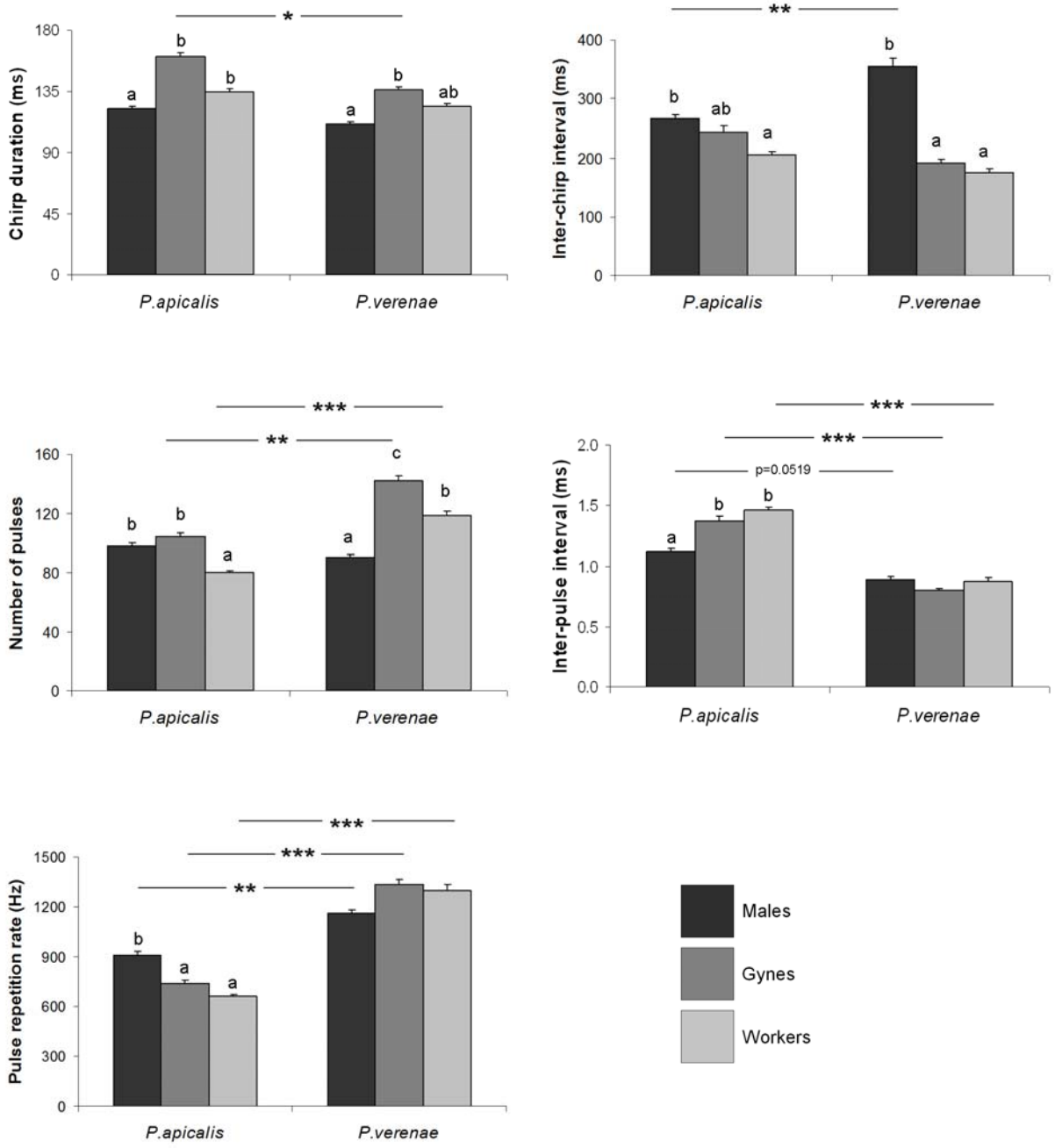

Figure 2. Temporal parameters of *Pachycondyla apicalis* and *Pachycondyla verenae* stridulations. When different, small letters indicate significant differences between castes for each species (LSD Post Hoc tests, $p \leq 0.05$). Significant differences of castes between species are stated as * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$, LSD Post Hoc tests).

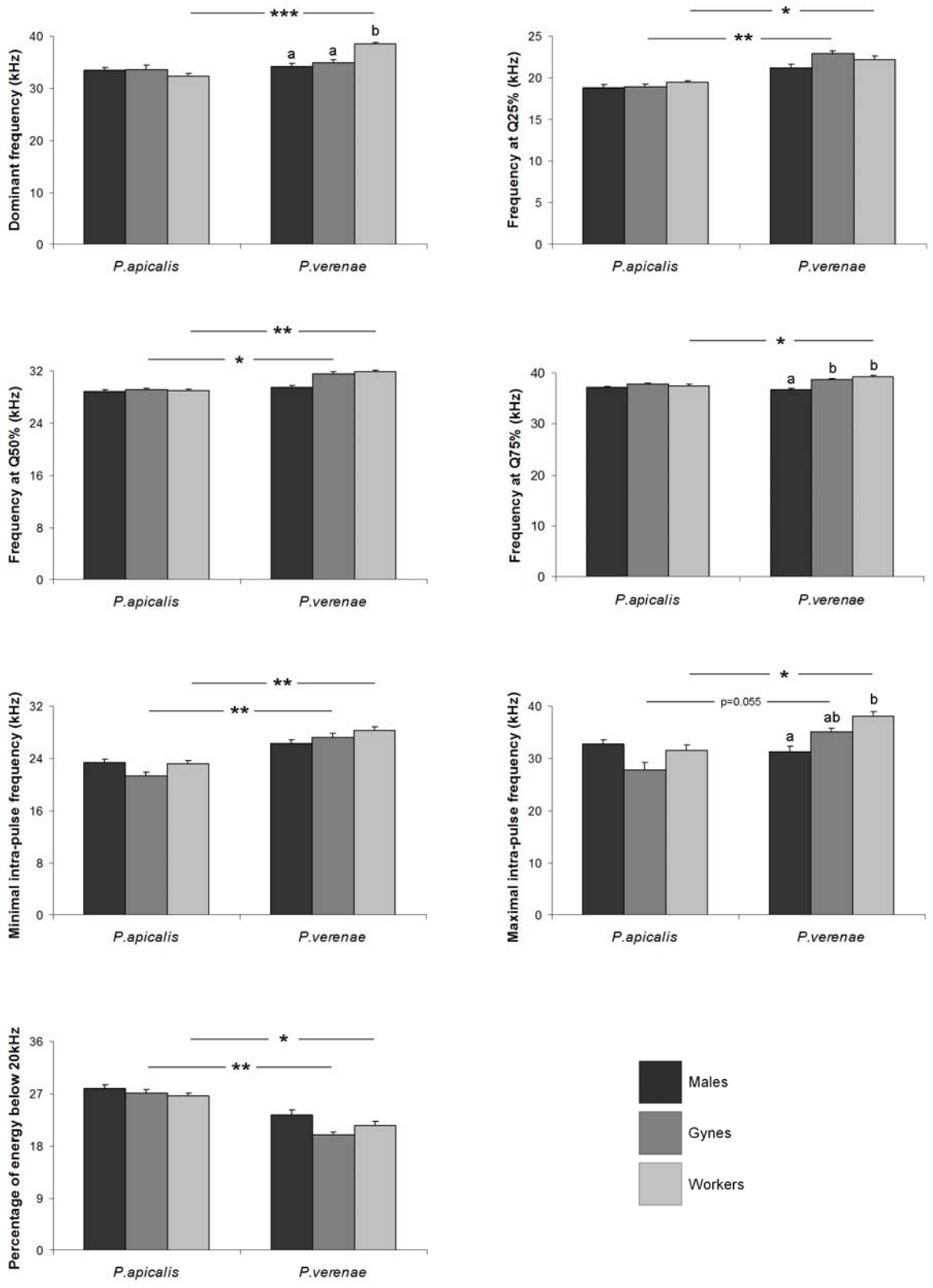

Figure 3. Frequency parameters of *Pachycondyla apicalis* and *Pachycondyla verenae* stridulations. When different, small letters indicate significant differences between castes for each species (LSD Post Hoc tests, $p \leq 0.05$). Significant differences of castes between species are stated as * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$, LSD Post Hoc tests).

The three castes differed in the number of pulses, in which gynes showed chirps with more pulses and males with fewer pulses than workers (LMM, $F_{2,29}$=13.093, p<0.0001; mean±SE, gynes=142.36±3.23, workers=118.26±3.25, males=90.06±2.19, Figure 2.c). In the frequency domain, workers presented higher dominant frequencies than the sexual castes (LMM, $F_{2,29}$=3.367, p=0.048; mean±SE, workers=38.49±0.39 kHz, males=34.12±0.63 kHz, gynes=34.83±0.59 kHz, Figure 3.a). Males showed lower frequencies at 75% of the signal energy than gynes and workers (LMM, $F_{2,29}$=3.763, p=0.036; mean±SE, males=36.65±0.33 kHz, gynes=38.71±0.21 kHz, workers=39.23±0.23 kHz, Figure 3.d) and at last, they also showed a lower maximal intra-pulse frequency than workers (LMM, $F_{2,29}$=5.361, p=0.01; mean±SE, males=31.25±1.02 kHz, gynes=35.09±0.73 kHz, workers=38.12±0.91 kHz, Figure 3.f). In sum, for both *Pachycondyla* species, we observed acoustical caste-specificities for all three castes.

Comparison of the stridulations from the same caste between species

Acoustic signals of males differed between species in the temporal pattern. *P. verenae* presented a slower rhythm of sound production, i.e. greater inter-chirp intervals than *P. apicalis* (LMM, $F_{1,29}$=9.771, p=0.004; mean±SE, PVE=354.50±14.76 ms, PAP=267.44±6.28 ms, Figure 2.b). They also presented a greater pulse repetition rate in the chirps (LMM, $F_{1,29}$=9.771, p=0.01; mean±SE, PVE=1162.01±22.32 Hz, PAP=910.61±19.83 Hz, Figure 2.e), which is related to the tendency observed for a smaller inter-pulse interval (LMM, $F_{1,29}$=9.771, p=0.052; mean±SE, PVE=0.89±0.02 ms, PAP=1.12±0.03 ms, Figure 2.d). All frequency parameters showed similar values between males.

In the female sexual caste, an even greater specificity could be observed between species. *P. apicalis* gynes showed longer chirps than *P. verenae* (LMM, $F_{1,22}$=6.393, p=0.019; mean±SE, PAP=160.21±3.31 ms, PVE=136.16±2.37 ms, Figure 2.a) but with fewer pulses (LMM, $F_{1,22}$=12.246, p=0.002; mean±SE, PAP=104.01±3.12, PVE=142.36±3.23, Figure 2.c). The latter, however, presented smaller inter-pulse intervals (LMM, $F_{1,22}$=18.624, p=0.0002; mean±SE, PVE=0.80±0.02 ms, PAP=1.37±0.04 ms, Figure 2.d) and consequently, a higher pulse repetition rate than *P. apicalis* (LMM, $F_{1,22}$=30.808, p<0.0001; mean±SE, PVE=1334.93±28.93 Hz, PAP=736.56±23,70 Hz, Figure 2.e). Gynes also differed in the frequencies at 25% and 50% of the signal energy, in which *P. apicalis* had lower frequencies than *P. verenae* ($LMM_{25\%}$, $F_{1,22}$=13.416, p=0.001; mean±SE, PAP=18.88±0.33 kHz,

PVE=22.90±0.35 kHz, Figure 3.b and $LMM_{50\%}$, $F_{1,22}$=6.89, p<0.015; mean±SE, PAP=29.03±0.30 kHz, PVE=31.58±0.29 kHz, Figure 3.c, respectively) as well as a smaller minimal intra-pulse frequency (LMM, $F_{1,22}$=10.56, p=0.004; mean±SE, PAP=21.28±0.62 kHz, PVE=27.23±0.52 kHz). Therefore, *P. verenae* gynes presented a smaller percentage of signal energy in the human audible spectrum, i.e. below 20 kHz (LMM, $F_{1,22}$=12.763, p=0.0017; mean±SE, PVE=19.79±0.59 kHz, PAP=27.04±0.67 kHz, Figure 3.e).

In workers stridulations, we observed a similar pattern of temporal distinctiveness as observed for gynes in the number of pulses (LMM, $F_{1,30}$=19.965, p=0.001; mean±SE, PVE=118.26±3.25, PAP=79.69±1.73, Figure 2.c), inter-pulse interval (LMM, $F_{1,30}$=24.349, p<0.0001; mean±SE, PVE=0.87±0.03 ms, PAP=1.46±0.03 ms, Figure 2.d) and pulse repetition rate (LMM, $F_{1,30}$=49.289, p<0.0001; mean±SE, PVE=1297.27±39.90 Hz, PAP=665.03±10.69 Hz, Figure 2.e). Furthermore, workers of the two *Pachycondyla* species differed for all frequency parameters considered, with *P. verenae* showing higher levels than *P. apicalis* for dominant frequency (LMM, $F_{1,30}$=14.645, p=0.0006; mean±SE, PVE=38.49±0.39 kHz, PAP=32.21±0.61 kHz, Figure 3.a), frequencies at 25, 50 and 75% of the signal energy ($LMM_{25\%}$, $F_{1,30}$=4.979, p=0.0333; mean±SE, PVE=22.14±0.45 kHz, PAP=19.40±0.25 kHz; $LMM_{50\%}$, $F_{1,30}$=7.480, p=0.0104; mean±SE, PVE=31.85±0.32 kHz, PAP=29.00±0.24 kHz and $LMM_{75\%}$, $F_{1,30}$=5.247, p=0.0292; mean±SE, PVE=39.23±0.23 kHz, PAP=37.53±0.18 kHz, Figures 3.b-d, respectively) and minimal and maximal intra-pulse frequency ($LMM_{Minimal}$, $F_{1,30}$=10.119, p=0.0034; mean±SE, PVE=28.22±0.58 kHz, PAP=23.10±0.45 kHz and $LMM_{Maximal}$, $F_{1,30}$=4.950, p=0.0338; mean±SE, PVE=38.12±0.91 kHz, PAP=31.55±1.00 kHz, Figure 3.f and g, respectively).

Comparison of the size of ants from different castes and species

The thorax length, used here as an indicative of ants' size, was significantly different between males and both females castes in *P. apicalis,* with males being smaller than both gynes and workers (ANOVA, $F_{2,27}$=185.68, p<0.00001; mean±SE, males=5.43±0.04 µm, gynes=6.93±0.8 µm, workers=6.75±0.05 µm, Figure 4). In *P. verenae*, each caste presented a different thorax length (ANOVA, $F_{2,27}$=64.44, p<0.00001; mean±SE, males=5.69±0.05 µm, gynes=6.67±0.07 µm, workers=6.18±0.05 µm, Figure 4). At last, the comparison of the thorax length of each caste between both species revealed significant differences for all three castes, in which *P. verenae* presented bigger males and smaller gynes and workers than *P.*

apicalis (Student t-tests, t_{males}=3.77, p=0.0014, PVE=5.69±0.05 µm and PAP=5.43±0.04 µm; t_{gynes}=-2.44, p=0.0253, PVE=6.67±0.07 µm and PAP=6.93±0.8 µm and $t_{workers}$=-7.49, p<0.00001, PVE=6.18±0.05 µm and PAP=6.75±0.05 µm, Figure 4).

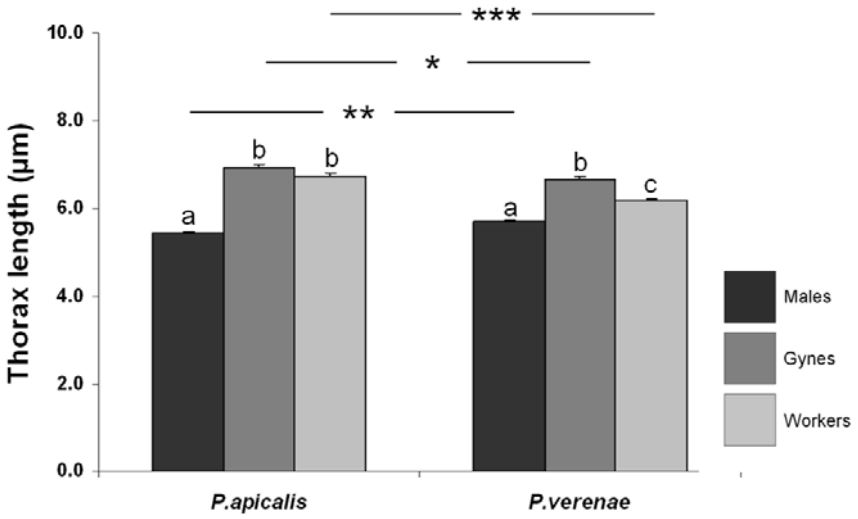

Figure 4. Thorax length of *Pachycondyla apicalis* and *Pachycondyla verenae* ants. When different, small letters indicate significant differences between castes within each species (Tukey tests, p≤0.05). Significant differences of castes between species are stated as * p≤0.05, ** p≤0.01 and *** p≤0.001, Student's t-tests).

Discussion

The present study is the first description of sounds produced by the sexual castes of the Neotropical primitive ants *Pachycondyla apicalis* and *P. verenae*, respectively *P. apicalis* Morph 4 and *P. verenae* Morph 1 according to Delabie et al. (2008). We also obtained further information on the sounds produced by the worker caste of both species, first described by Pavan et al. (1997) for *P. apicalis*, and by Ferreira et al. *(in press),* for the audible component of signals of both species. We investigated if individuals of different castes could produce distinctive stridulations according to their caste or species.

Our results obtained by comparing the acoustic parameters showed that the stridulations of *P. verenae* and *P. apicalis,* besides presenting the same monosyllabic structure have great specific distinctiveness (Figure 1). Given the differences in the thorax

length observed for castes within and between species, one could imagine that these acoustic specificities were mere effects of the individuals' size. However, Ferreira et al. (*in press*) observed that differences in the pattern of the stridulatory file are not due only to allometric differences between workers in cryptic species of the *P. apicalis* complex. Ants with different body sizes present the same length for the stridulatory file. Moreover, stridulatory files of the same length can comprise different number of ridges, which directly affect the inter-ridge distances, and consequently, the signal produced. Furthermore, these authors also observed that the distinct acoustic signals produced by some of the different cryptic species, were not only the result of the morphology of the stridulatory organ, but also of ant behaviour during stridulation production. Thus, it seems that the relationships between ants' size, stridulatory file morphology and signals features are more complex than simple linear correlations. We can then expect the same complexity to apply to the sexual castes of different species or also to different castes within a species.

Acoustic caste differentiation was mainly observed for temporal parameters in both species. In *P. apicalis*, the shorter inter-pulse interval presented by males, in the presence of a high number of pulses, resulted in a shorter chirp and a higher pulse repetition rate than gynes and workers. This could be the consequence either of a faster rubbing of the file against the scraper or of the arrangement of the ridges along the file. Grasso et al. (2000) observed similar results for the stridulations of males in *Messor* spp., in which males had the ridges of their stridulatory organ more narrowly spaced (Grasso et al. 1998). A negative correlation between the inter-ridge distance and the pulse repetition rate has already been demonstrated in several dung beetles of the genus *Trycopris* (Carisio et al. 2004). However, Ferreira et al. *(in press)* showed also some influence of the stridulatory behaviour, in which tendencies for a higher pulse repetition rate and a lower mean inter-pulse interval together with larger inter-ridge distances could be observed for workers of *P. apicalis* Morph 6, another cryptic species of the *P. apicalis* species complex. We could also observe a different behaviour for *P. apicalis* males during stridulation production concerning the rhythm of the chirps, in which they present a slower rhythm than workers. The same pattern is observed for males of *P. verenae*, and in the latter, males present a slower rhythm than both female castes. Similarly to stink bugs, species specificity of male acoustic signals was found mainly in temporal characteristics such as duration of the signals and their repetition time (Virant-Doberlet & Cokl 2004). In *P. verenae*, each caste rubs a different number of ridges on the stridulatory file, and besides other temporal differences, we also observed some frequency distinctiveness in castes' stridulations. Reproductive castes stridulate in lower dominant frequencies than

workers. Thus, these caste-specificities suggest that the acoustic properties of the stridulations could have evolved as a function of intra-specific interactions, for example during mating behaviour, where they could help in finding and attracting a partner, play a role in mate choice and acceptance or in the competition among males for females as well as indicate motivation or receptivity, like in many other species that communicate acoustically during mating interactions (Drosopoulous & Claridge 2006, Gerhardt & Huber 2002).

Ferreira et al. *(in press)* showed that it was possible to discriminate different species from the *Pachycondyla apicalis* species complex through the stridulations of workers, which have each their own acoustic signature. We have shown here that for *P. apicalis* and *P. verenae* this specificity is not restricted to the caste of workers, but that it also exists in sexuals. Differences in the rhythm of chirp production, the intervals between pulses and the pulse repetition rate for males and distinctiveness in four out of the five temporal parameters and four out of the seven frequency parameters for gynes of the two species reinforce the usefulness of acoustics as a helpful taxonomic tool for the *Pachycondyla apicalis* species complex. Indeed, the acoustic specificity observed for sexual castes within these species provide additional information to separate species with more confidence. Furthermore, differences in worker stridulations were more clear-cut than we previously reported in Ferreira et al. *(in press)*. Despite having a similar number of ridges in the stridulatory file (Ferreira et al. *in press), P. verenae* workers rub more ridges and in a faster way than *P. apicalis,* due to the smaller distance between ridges in the file, which is also reflected by the small inter-pulse intervals observed for this species. Therefore, we observed acoustic differences for all frequency parameters between workers of both species, in which *P. verenae* workers always showed higher frequencies.

In the previous studies considering the stridulations of castes other than workers, no significant difference could be observed between species. Besides differing from the workers', queen stridulations from the three *Myrmica* species studied by Barbero et al. (2009a,b) were indistinguishable, as were worker stridulations in two of the three species studied. Similar results were obtained by Grasso et al. (2000) which recorded inter-caste specificities despite an absence of differences in the stridulations of gynes from four *Messor* species. And in Markl et al. (1977), as described above, no unambiguous species or caste-specific signal characteristics could be recognized for *Pogonomyrmex* harvester ants. The great specific distinctiveness observed in the stridulatory signals in this study suggests that acoustic communication may have a more important role not only in intraspecific but also in interspecific relationships these ants.

In stark contrast, we observed in our study an acoustic divergence both at the intra and interspecific levels. The signals thus had temporal and/or frequency features typical of each caste or species. Gynes and workers differed more greatly between species than within the same species (Figure 2 and 3). Conversely, the signals of males differed more from their conspecific gynes and workers than between the two species (Figure 2 and 3).

The main function attributed to stridulations in several species is alarm (Ferreira & Fresneau 2009). However, if the acoustic signals of *P. apicalis* and *P. verenae* have alarm as their sole function, these broadly sympatric species, subjected to the same environmental pressures, have no evolutionary interest in producing signals specific to the species (Masters 1979). Also, as reproductive individuals do not participate in the protection tasks of the colony, our results reinforce the idea that stridulations could present a more important role in different aspects of the biology of these species. One of the most important roles of species and sex-specific acoustic signals used in insect communication is to enable mate recognition and reproductive isolation (Virant-Doberlet & Cokl 2004). This suggests that one or more other functions of these signals remain to be discovered in *P. apicalis* and *P. verenae*. Therefore the study of the stridulatory organ of the sexual individuals could help us to better understand the whole phenomenon, for example how much acoustic characteristics are related to the morphological distinctiveness of the file and also how different the behaviour during stridulation production is. In addition, it remains to be tested whether and how all these differences are indeed perceived or acted upon by the different castes and species. Further studies, including laboratory and field investigations are needed to clarify the causes of this acoustic specificity in these Neotropical ants.

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CHAPITRE 2:

Contextes de production et fonctions biologiques des signaux acoustiques dans le complexe d'espèces *Pachycondyla apicalis*

Résumé

Les signaux acoustiques chez le complexe d'espèces *P. apicalis* présentent des spécificités allant des castes aux espèces. Ceci pose la question des contextes d'émission de ces stridulations, ainsi que de leurs fonctions biologiques et donc de leur implication dans les comportements sociaux de ces fourmis.

Ces signaux ont donc fait l'objet d'une investigation dans des contextes variés tels que des perturbations du nid, un déménagement ou la présence de prédateurs. Le comportement reproducteur a ensuite été étudié de façon approfondie afin de déterminer l'implication de la communication acoustique dans la reconnaissance des partenaires et l'isolement des espèces puisque chaque sexe et chaque espèce produit des sons distincts. Enfin les réponses des ouvrières à des signaux homo ou hétérospécifiques ont été envisagées afin d'appréhender si ces signaux ont pu se diversifier en réponse à la compétition entre ces espèces sympatriques.

Les études réalisées montrent que si les stridulations ne sont pas produites lors des activités journalières ni des processus de déménagement, elles le sont en revanche dans des situations de perturbations du nid et de présence de prédateurs. L'analyse de l'accouplement au sein de ce complexe indique qu'il met en jeu des comportements ritualisés. Les femelles ne se sont jamais accouplées plus d'une fois, alors que les mâles essaient de multiplier les copulations. Aucun signal acoustique n'est émis lors de la reconnaissance des partenaires ou des comportements copulatoires. En revanche les femelles nouvellement inséminées produisent des stridulations lorsque les mâles sollicitent l'accouplement. Par ailleurs des croisements entre deux différents morphes de *P. apicalis* ont été observés mais n'ont engendré aucune descendance. Enfin les études de diffusion de stridulations homo ou hétérospécifiques n'ont pas révélé de réponses comportementales dirigées chez ces fourmis.

Ces résultats illustrent ainsi le rôle des signaux acoustiques en tant que signal d'alarme, bien qu'ils revêtent aussi probablement une fonction aposématique. Ceux-ci semblent par contre n'avoir aucun rôle dans l'isolement des espèces, et d'autres mécanismes écologiques et/ou chromosomiques peuvent être impliqués. Les stridulations post-copulatoires des gynes pourraient agir comme « signal de libération de la femelle » et ainsi indiquer leur non-réceptivité. Notre étude souligne ainsi la signification contexte-dépendante de ces signaux acoustiques mais laisse ouverte la question de la spécificité des stridulations ainsi que de leur fonctions.

Article 3: Behavioural contexts of stridulation production in two Neotropical primitive ants (Formicidae: Ponerinae)

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En préparation

SHORT COMMUNICATION

Behavioural contexts of stridulation production in two Neotropical primitive ants (Formicidae: Ponerinae)

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Keywords: nest disturbance, nest emigration, distress signal, alarm

Stridulations are of common occurrence in a great number of ant species (Ferreira & Fresneau 2009). These signals have been observed in many behavioural contexts such as nest disturbance (Markl 1965, 1967, Masters 1980), food recruitment (Markl & Hölldobler 1978, Baroni-Urbani et al. 1988, Roces et al. 1993), trophallaxis (Stuart & Bell 1980), nest emigration (Maschwitz & Schönegge 1983), intra- and inter-specific conflicts (Markl 1965, Grasso et al. 2000) and even mating (Markl et al. 1977, Mercier et al. 2007). Stridulations are also produced during nest maintenance and in this context the vibrations generated could help ants to displace soil particles during nest construction or excavation (Rauth & Vinson 2006). Tautz et al (1995) also observed a facilitating effect of stridulations vibrations during leaf cutting activities. Nevertheless these contexts are extremely variable according to the species and the social organization of the colonies (Hölldobler & Wilson 1990, Ferreira & Fresneau 2009). Furthermore, given that among the stridulating ants the only well studied subfamily are the Myrmicinae (with leaf-cutting ants the most studied example), almost all generalisations about the functions of these acoustic signals are up to now based on them. Therefore, more detailed investigations of other subfamilies will probably reveal an even more extensive and important use of these signals in ants.

Among the stridulating subfamilies (Markl 1973), the primitive subfamily Ponerinae presents a particular social organization, strongly different from the derived subfamily Myrmicinae. Apart from a few exceptions, Ponerine ants usually form moderate size colonies,

forage solitary and never recruit (Peeters 1997, Wilson & Hölldobler 2005), do not exchange food by trophallaxis (Peeters 1997) and nest in pre-existing cavities or dead wood without any building behaviour (Peeters et al. 1994, Fresneau 1994). Despite the ability of a great number of species to produce stridulations (Markl 1973), the potential use of these signals is barely known for this group of ants. Here we study some possible contexts of stridulation production and the possible functions of these signals in two primitive ponerine ants from the *P. apicalis* species complex. Similar sized colonies (~60 individuals) or groups of workers (30 individuals) of *P. apicalis* and *P. verenae* [PAP Morph 4 and PVE Morph 1 respectively, according to Delabie et al. (2008)] were placed in different situations for which their behavior was analyzed:

(a) *Intra colonial spontaneous stridulation*: To test whether stridulations occur spontaneously within the nest, as observed in the fire ant *Solenopsis richteri* (Hickling & Brown 2000), or while performing daily tasks such as brood and queen care, nest maintenance, etc., a microphone (bandwidth: 20 Hz - 20 kHz) was placed on the top of colonies of *P. apicalis* (n=5) and the activity of ants was recorded continuously during 24 hours.

(b) *Nest disturbance:* In this experiment we tested the hypothesis that the stridulations serve as a mean to alert nestmates in response to nest disturbance. To verify this, six colonies of *P. apicalis* and five of *P. verenae* were tested. As a form of disturbance, we tested a sudden movement of the nest. In addition, by removing the dark cover and the glass plate covering the nest, we exposed the ants to light and open-air conditions. For each colony, the ants' stridulatory activity, number of stridulations produced and stridulations repetition rate was recorded during the whole period of ant activity after disturbance. Before each test the same parameters were measured for 60 s to assert normal activity in the tested colonies.

(c) *Nest emigration:* We also tested if in *P. apicalis* stridulations could be used for the rapid mobilization of individuals during emigration. This has already been demonstrated for *Leptogenys chinensis*, an Asian species from the same primitive subfamily as *P. apicalis* and *P. verenae* (Maschwitz & Schönegge 1983). To force ants to emigrate from their nests (n=5), an halogen lamp was placed over the occupied nests at a height of 1 m. The original nests were connected to a 1.5 m long arena which was connected on the opposite side to another nest with the same dimensions but with optimum conditions of darkness, temperature and humidity. The whole process of emigration was recorded until the arrival of the last ant in the new nest. Stridulatory activity of ants inside the nest and during the emigration was examined

using a mini-microphone installed inside the nest (bandwidth: 20 Hz - 20 kHz) and a mini-amplifier placed in the foraging area.

(d) *Mammalian breath:* Some species of ants stridulate when exposed to carbon dioxide, as is the case for the ponerines *Streblognathus aethiopicus* (Ware 1994) and *Megaponera foetens* (Holldobler et al. 1994), suggesting a defensive role against potential predators. Six groups of 30 workers of *P. apicalis* and five of *P. verenae* were exposed to both a flow of compressed air and a breath of a mammal (in this case the experimenter). The duration of the stridulatory response, the number of stridulations produced and the stridulation repetition rate were recorded in both cases for each colony, and the means compared within and between species. When not significantly different, data from both species were pooled in order to analyse the general response of ants from the *P. apicalis* species complex.

Results

Our results showed no audible stridulatory activity in the 24 hours that *P. apicalis* undisturbed nests (n=5) were recorded nor during the whole process of nest emigrations (n=5). However, nest disturbance caused an immediate stridulatory response in ants from the *P. apicalis* species complex, and a significant effect of the type of disturbance could be observed (Figure 1.a). A longer stridulatory activity was shown by *P. apicalis* when nests were opened and ants exposed to the light and open-air conditions than when nest were only shaken (36.33±7.58 s and 12.00±3.10 s respectively, Permutation tests for paired samples, n=6, $p<0.05$, Figure 1.a) and a similar tendency was also observed for *P. verenae* (28.40±2.09 s and 3.80±2.48 s respectively, Permutation tests for paired samples, n=5, $p=0.0625$, Figure 1.a). As the stridulatory activity did not differ between *P. apicalis* and *P. verenae* nests for both types of disturbance (Permutation tests, n=11, $p>0.05$), the general response of *P. apicalis* and *P. verenae* was then compared together, and we could observe the same effect of the type of disturbance, in which the duration of stridulation production was greater during nest opening than nest shaking (32.73±4.24 s and 8.27±2.32 s respectively, Permutation tests, n=11, $p<0.001$). The number of stridulations produced during nest shaking was significantly higher in *P. apicalis* than *P. verenae* (27.17±8.37 and 5.80±4.61 respectively, Permutation tests, n=11, $p<0.05$, Figures 1.b). As the pattern observed for the number of stridulations produced in different types of nest disturbance was similar between species, we also compared the general response of *P. apicalis* and *P. verenae* together.

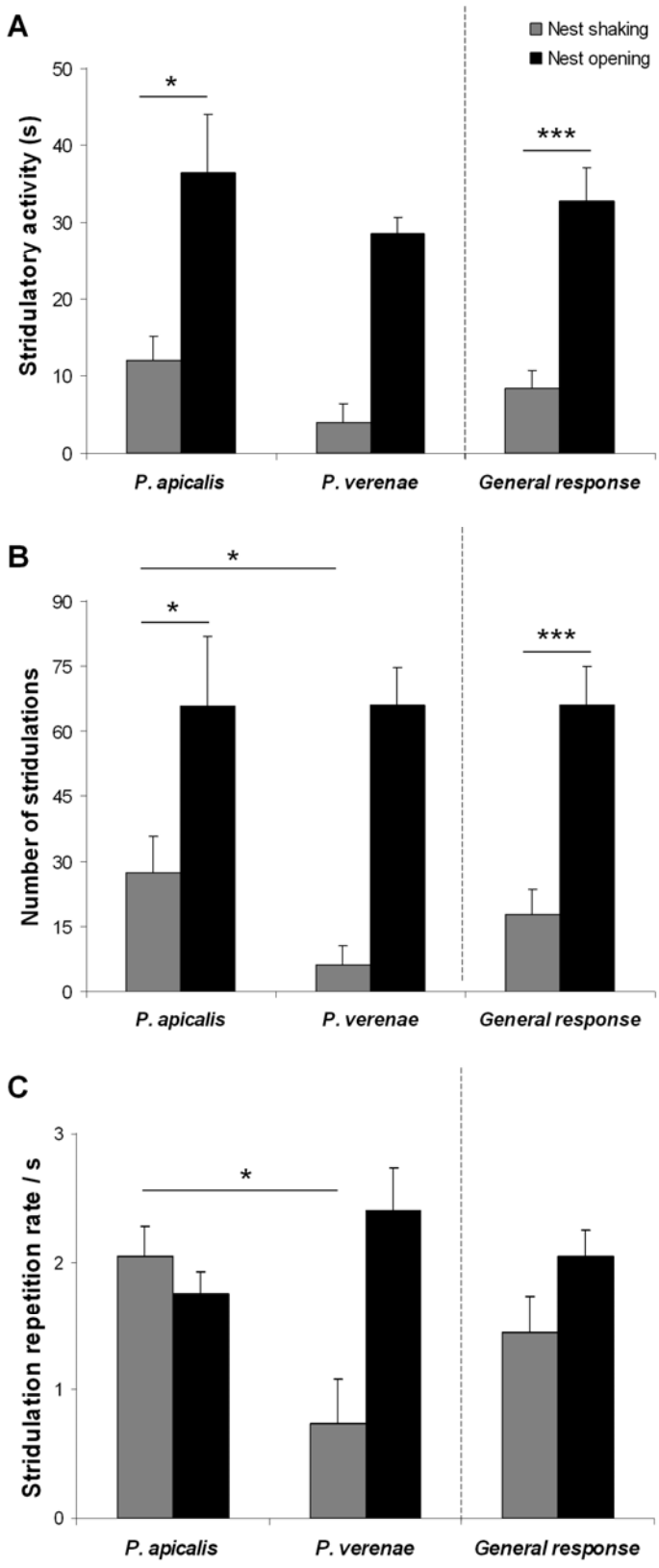

Figure 1. Stridulatory response of *P. apicalis* species complex ants to nest disturbance. Nest shaking (A) and Nest opening (B). The means ant standard errors are given. Exact permutations tests (between species) and exact permutation tests for paired samples (for different treatments within species) were used. $p<0.05$ and $p<0.001$.

We observed an increased number of stridulations during nest opening compared with nest shaking (65.82±9.09 and 17.45±5.86 respectively, Permutation tests for paired samples, n=11, $p<0.001$, Figure 1.b). The ants responded to these stimuli by strong group stridulation, and therefore repeated analysis of the audio recordings had to be done to access the number of signals produced. However, in spite of being significantly higher in *P. apicalis* than *P. verenae* during nest shaking (2.04±0.23 and 0.73±0.35 respectively, Permutation tests, n=11, $p<0.05$, Figures 1.c), the stridulation repetition rate was not significantly different between both types of disturbance for *P. apicalis* (Permutation tests for paired samples, n=6, $p>0.05$, Figure 1.c) and only a tendency for a higher stridulation repetition rate during nest opening could be observed for *P. verenae* (nest opening: 2.39±0.34 and nest shaking: 0.73±0.35, Permutation tests for paired samples, n=5, p=0.0625, Figure 1.a).

At last, the stridulation-eliciting effect of the carbon dioxide from the human breath could be confirmed in undisturbed ants from the *P. apicalis* species complex. *P. apicalis* and *P. verenae* did not differ in any of the parameters considered for the stridulatory response (Permutations tests, $p>0.05$, Figure 2). When species were considered together, we could observe a longer stridulatory activity (human breath: 13.18±3.61 s, air: 4.09±1.83 s, Permutation tests for paired samples, n=11, $p<0.001$, Figure 2.a), with more stridulations produced (human breath: 31.82±9.61, air: 6.91±3.55, Permutation tests for paired samples, n=11, $p<0.001$, Figure 2.b) and in a higher repetition rate (human breath: 2.25±0.17, air: 0.67±0.25, Permutation tests for paired samples, n=11, $p<0.001$, Figure 2.c) when ants where exposed to a human breath than to pure air flow. Similar results were observed in *P. apicalis* for the number of stridulations (human breath: 33.00±12.35, air: 8.17±5.10, Permutation tests for paired samples, n=6, $p<0.05$, Figure 2.b) and the repetition rate (human breath: 2.47±0.14, air: 0.96±0.36, Permutation tests for paired samples, n=6, $p<0.05$, Figure 2.c) and only a tendency to the same pattern was observed for the stridulation repetition rate in *P. verenae* (human breath: 1.99±0.32, air: 0.32±0.32, Permutation tests for paired samples, n=5, p=0.0625, Figure 2.c), which might be due to the smaller sample size.

Discussion

During previous observations of undisturbed nests of *P. apicalis* ants, we noted that some of the ants (workers and even the queens) frequently displayed conspicuous dorsoventral movements of the gaster, with an identical pattern to that of restrained stridulating ants. However, one-day recordings showed no audible intra-colonial stridulatory activity.

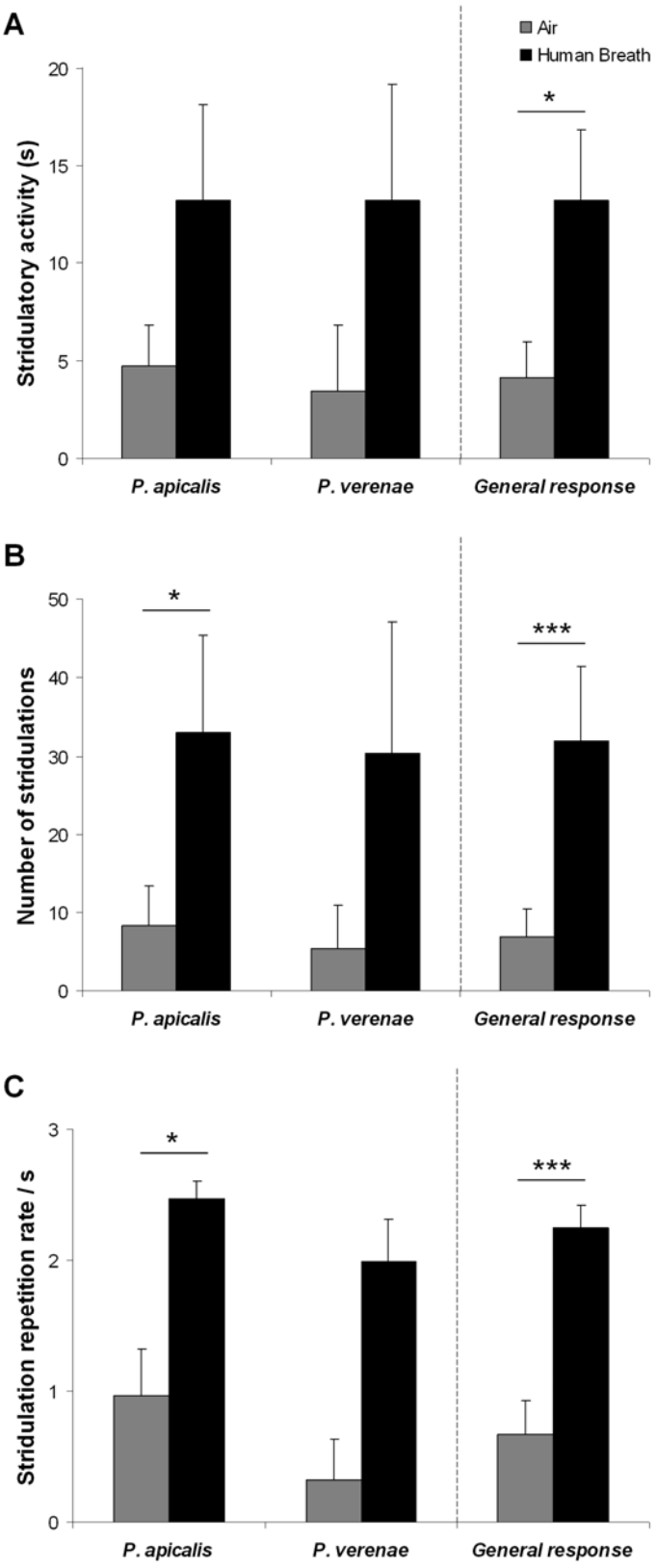

Figure 2. Stridulatory response of *P. apicalis* species complex ants to disturbance by air (A) or human breath (B). The means ant standard errors are given. Exact permutations tests (between species) and exact permutation tests for paired samples (for different treatments within species) were used. $p<0.05$ and $p<0.001$.

These results lead us to ask whether *P. apicalis* ants are able to produce stridulatory vibrations without audible components or alternately what could be the functions of such clearly recognizable noiseless gaster movements. In *Solenopsis* ants, Obin & Vander Meer (1985) suggested that such gaster movements represent a way of aerosolizing antibiotic venom on the brood. Unfortunately, these questions could not be investigated in the present study.

Stridulations play an important role during nest emigrations of the ponerine *L. chinensis*. These signals are necessary for stimulation and synchronization of colony members, and are always associated with chemical signals (Maschwitz & Schönegge 1983). In *P. apicalis* ants, on the contrary, nest emigrations lack any acoustic productions. In this species, emigrations are regulated by scouts' tandem running bouts, in which scout ants lead other workers (one at a time) from the ancient nest to the new nest (Fresneau 1994, Pezon 2004). They also perform social transport behaviour, in which scouts carry other workers and brood to the new nest. As this species do not lay chemical trails (Fresneau 1985, Fresneau 1994), only workers that have visited the new nest after a tandem run and thus have learnt visual landmarks will afterwards participate actively to the emigration process, for example, by recruiting other workers to the new nest through tandem runs or transporting the brood. In the context of nest emigration, stridulations appear to be correlated with the mass recruitment observed for *L. chinensis* (Maschwitz & Schönegge 1983), as a mean to inform and mobilize the whole colony at the same time. The individual recruitments performed by *P. apicalis* ants during nest emigration, could thus explain the lack of need to employ stridulatory signals in this context.

The behavioural contexts in which we observed increased stridulation production in ants from the *P. apicalis* species complex were during nest opening, or as a response to mammal breath. These results confirm for *P. apicalis* and *P. verenae* the main function already demonstrated for acoustic signals in ants, i.e. alarm signal. Stridulations could thus inform the colony of a potential danger, for example when it suffers an attack (Markl 1965, 1967, Masters 1980). Hickling & Brown (2000) suggest that acoustic signals can be propagated inside the nest faster than pheromones, therefore causing a rapid response in ants, which could improve the defence of the nest. Moreover, our results strongly supports the hypothesis that stridulations can also serves as an aposematic signal, warning potential predators such as lizards, birds and mammals of the powerful sting these ants can inflict. This phenomenon is also suggested for *Megaponera foetens* (Hölldobler et al. 1994), and *Streblognathus aethiopicus* (Ware 1994) in which sound production is observed for the same

contexts as *P. apicalis* and *P. verenae* ants. Furthermore, it is also well known from other hymenopterans, such as mutillids (Masters 1979, 1980). As in *M. foetens*, this hypothesis is supported by the behavioural shifts observed during stridulatory activity, in particular the increased walking speed and dispersal of the ants, which are also considered as adaptations to predation by vertebrates, by generating a dilution effect and the confusion of the predator (Hölldobler et al. 1994).

Here we reported some contexts which elicit stridulation production in the ponerine ants *P. apicalis* and *P. verenae* and the possible functions of these acoustic signals at the colony level. Given the species and caste-specificities observed in the stridulatory signals of these broadly sympatric species (Ferreira et al. *in press*), and their particular social organization, further investigations at different levels such as intraspecific heterocolonial and heterospecific interactions are currently being carried out in order to better understand the behavioural contexts in which acoustic communication have been selected for.

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Article 4: Mating behaviour and sound production in the *Pachycondyla apicalis* species complex

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En préparation

Mating behaviour and sound production in the *Pachycondyla apicalis* species complex (Hymenoptera: Ponerinae)

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Abstract

The detailed mating behaviour of the ants from the *Pachycondyla apicalis* species complex in laboratory conditions is described here for the first time. To assess inbreeding and interfecondity between the different cryptic species, several combinations of crossings were tested. Behavioural acts such as antennal boxing, stridulation, mounting and copulation were scored. In laboratory conditions, the mating behaviour of *P. apicalis* can be described as a sequence of relatively stereotyped behaviour: (1) localization and approach (performed mostly by males); (2) intense antennal stroking of the male on the gyne's head and body; (3) mounting, where male climbs onto the gyne's thorax and tries to copulate; and (4) copulation. Antennal boxing, performed during the whole process, is an essential part of male behavior. Only single matings were observed. However, mated males tried to copulate with other gynes and even with already mated females. Mated gynes, on the contrary, refused all further male mating attempts. Most mating attempts did not lead to copulation. Only four successful matings were observed: two between individuals from the same morphs and population and two between different morphs and populations. Stridulatory communication is not involved in mate recognition or discrimination during mating behavior in the *P. apicalis* species complex. However, stridulatory activity was recorded in newly inseminated gynes right after mating, when further males tried to copulate with them.

Key-words: ant, cryptic species, reproductive isolation, interbreeding, stridulation

Introduction

The *Pachycondyla apicalis* species complex is a widespread group of broadly sympatric ant species in Neotropical forests. This complex comprises a small monophyletic assemblage of morphologically very similar species that also present similar overall biological and ecological characteristics (Wild 2005, Fresneau 1994). All these resemblances make it difficult to correctly identify the cryptic biodiversity within this group and for a long time the subtle differences observed between species had been thought to only represent mere intra-specific variability (Wild 2005, Ferreira et al., *in press*). Nevertheless, Ferreira et al. (*in press*) demonstrated that in this complex morphologically similar species have clear species-specific differences with regards to their respective acoustic communication systems. These authors showed that it is possible to discriminate sympatric cryptic species from the *P. apicalis* species complex through the analysis of acoustic parameters in the temporal and the frequency domains of their stridulations. They also showed that each species presents a distinct stridulatory organ that can vary in length, width and inter-ridge distance according to the species. This is the first evidence of such a degree of specialization in the acoustic organ and signals in ants, which suggests that stridulations may be among the cues used by these ants during inter-specific interactions.

Furthermore, Ferreira et al. (*unpublished data)* recently showed that for *P. apicalis* (Morph 4) and *P. verenae* (Morph 1) this acoustic specificity is not restricted to the worker caste, but that it also exists in sexuals. Acoustic analyses demonstrated that the stridulatory signals present distinctive characteristics for all three castes within both species, with males' stridulations diverging to a greater degree from the two female castes. Moreover, the comparison of the signals of each caste between species revealed species-specific temporal and/or frequency characteristics for all three castes, with higher levels of divergence observed between gynes and workers.

Because species-specific sounds are usually produced in situations where there is an adaptive advantage for them to be different (Sueur, 2006), and given that one of the most important roles of species and sex-specific acoustic signals used in insect communication is to enable mate recognition and reproductive isolation (Virant-Doberlet & Cokl, 2004), we expect that for these closely related sympatric species, acoustic signals could play a role during mate recognition and discrimination. Indeed, in the case of sympatric speciation or of secondary contact of species that diverged in allopatry, the question of the establishment of reproductive isolation among populations is an important issue, especially when the ecological differences between these species seem low. The relative importance of pre-

copulatory isolating mechanisms based on behavioral differences or preferences of females, and post-copulatory leading to reduced fitness of hybrids or absence of viable offspring are important issues (Coyne & Orr 1998). Therefore, studies of mating behaviour and copulatory mechanisms could help us to understand the evolution of mating systems (Thornhill & Alcock 1983), including the evolution of the signals and information used during such interactions.

Indeed, the reproductive behaviour in the *P. apicalis* species complex is barely known, and nuptial flights have never been recorded in the field. Fresneau (1994) demonstrated for Mexican populations that only one period of the year favors matings, when colonies invest in the production of sexuals individuals. Males and females are then released daily in small numbers, and the mating season can extend for up to 3 weeks.

Hence, in the present work we studied the mating behaviour of ants from the *P. apicalis* species complex in laboratory conditions to find out whether stridulation signals are involved during reproductive activities in this complex of cryptic ants and to test the reproductive isolation of such cryptic species.

Methods

Ants

We used individuals of the sexual castes (gynes and males) from 11 colonies and four distinct taxa of the Neotropical *Pachycondyla apicalis* species complex (*P. apicalis* Morphs 2, 3 and 4 and *P. verenae* Morph 1) from Petit Saut, French Guyana, Itabuna, Brazil and Los Tuxlas, Mexico. As these taxa present different taxonomic statuses at the present time, i.e. *P. verenae* is a described species, *P. apicalis* Morphs 3 and 4 are new valid species that await formal taxonomic description (Ferreira et al. *in press*) and *P. apicalis* Morph 2 does not have its taxonomic status confirmed yet, we decided to refer to these taxa using the classification proposed by Delabie et al. (2008), with ants being classified into morphs within their currently named species.

Ant colonies were reared in the laboratory in artificial plastered nests, which were maintained at 25±1°C, with approximately 65±10 % relative humidity, a 12L:12D photoperiod. They were fed with an identical diet (honey/apple mixture and crickets) twice a week.

Experimental groups

Six experimental groups of sexuals (i.e. gynes and males) were constituted. For each group, 5-8 gynes from a single colony were paired with a group of 10-12 males from 5-6 distinct colonies (2 males/colony). In four groups, gynes belonged to *P. apicalis* Morph 4, and in the two remaining groups, they were from *P. apicalis* Morphs 2 and *P. apicalis* Morph 3 respectively (Table 1). In these groups, besides being from different colonies, males were also from distinct morphs or species (*P. apicalis* Morphs 2 and 4 or *P. verenae* Morph 1), in order to allow interactions between gynes and males of different categories, i.e. homocolonial (between related males and gynes from the same colony), heterocolonial but of the same morph, different morph/species of *P. apicalis* or heterospecific (designing for convenience couples formed with *P. verenae* males) and from sympatric or allopatric populations (Table 1). Homocolonial couples constitute one control since we expect, as in most ant species, some kind of incest avoidance during mate choice. This is supported by the fact that no intranidal fertilization has ever been observed in our laboratory colonies (Ferreira and Fresneau, pers. observations). Interactions between *P. apicalis* gynes and *P. verenae* males are considered as another control because this species is more genetically distant from the other *P. apicalis* taxa (Ferreira et al. *in press*) and thus likely to be completely reproductively isolated from them.

All individuals were marked for individual monitoring and used in only one experimental group. The sexual receptivity of all individuals, aged from 3 to 8 weeks, was evaluated before the tests. Gynes that are not receptive to males become aggressive towards them, so we selected those tolerant to hetero-colonial males. Males when mature leave the nest and are capable of flying, hence only the most active males found outside the nest in the foraging arena were chosen.

Behavioural analyses

Each group of sexuals (Table 1) was tested during two sessions of 3h each in a Perspex cage (44 x 23 x 25 cm). Behavioural acts such as number and duration of antennation, mounting and copulation were recorded by direct observations and with 2 Sony DCR-SR72 digital cameras. Stridulation production during all phases of the mating behaviour was also monitored with a micro sound amplifier and the same cameras.

Table 1. Groups of *Pachycondyla apicalis* species complex sexuals used in the experiment. Cryptic species and morphs are classified according to Delabie et al. 2008.

	Males									
Gynes	**COL07 M2-FG**	**COL24 M4-FG**	**COL27 M4-FG**	**COL05 M4-FG**	**COL2R M4-FG**	**COL16 M4-FG**	**COL26 M2-BR**	**COL15 M2-BR**	**COL32 M2-FG**	**PVE COL02 PVE M1-FG**
COL07 M4-FG	HoC	HeC	HeC	HeC			DifM			HeSp
COL24 M4-FG	HeC	HoC	HeC	HeC						HeSp
COL2R M4-FG	HeC	HeC	HeC		HoC		DifM			HeSp
COL16 M4-FG	HeC	HeC	HeC			HoC				HeSp
COL26 M2-BR	DifM	DifM	DifM				HoC		HeC	
COL M3-MEX	DifM	DifM	DifM					DifM		HeSp

*Couple type: **HoC:** Homocolonial; **HeC:** Heterocolonial, same morph; **DifM:** Different morphs; **HeSp:** Heterospecific

** Taxon: **M2:** *P. apicalis* Morph 2, **M3:** Morph 3, **M4:** Morph 4 and **PVE M1:** *P. verenae* Morph 1

***Colony origin: **FG:** French Guiana, **BR:** Brazil and **MEX:** Mexico

Rearing of possibly mated gynes and dissections

All gynes identified as mated or having one or more possible penetrations were reared to test for effective insemination. If inseminated, founding gynes could lay fertilized (diploid) eggs that would develop in workers. If not, they could only lay unfertilized (haploid) eggs, which would develop in males. Moreover, two mated gynes (one mated with a male from the same morph and the other with a male from a distinct morph) were dissected right after mating under a stereo microscope to confirm for presence or absence of sperm in the spermathecae. This organ was then dissected to confirm the viability of the spermatozoids stored inside. All the other reared gynes were also dissected after two months of foundation.

Results

Only four successful matings, confirmed by diploid offspring production or dissections (see below), were observed: two between individuals of the same morph and population (gynes and males from *P. apicalis* Morph 4, from French Guiana) and two between individuals of different morphs and populations (gynes of *P. apicalis* Morph 4, from French Guiana and males of *P. apicalis* Morph 2 from Brazil).

Mating sequence

Mating behaviour in the *P. apicalis* complex can be described as a relatively stereotyped sequence of behaviours: (1) *Location* and approach of the partner, mostly initiated by males; (2) *Antennal boxing*, intense antennal strokes by the male on the female's body and head as soon as he comes into contact with her (Figure 1.a); (3) *Mounting*, the male mounts the gyne, grasps her prothorax with his front legs, extrude his copulatory organ and try to insert it into her genitalia (Figures 1.b,c). Antennal boxing is also performed during this entire phase; (4) *Copulation*, after successfully inserting his copulatory organ, the male stops the antennal boxing and releases the gyne's thorax during the few seconds of sperm transfer (Figure 1.d). Copulation could only occur when the female had protruded her sting to allow male penetration; (5) *Dealation,* soon after copulation, mated gynes take their wings off.

All mating behaviour observed took place on the ground or on the walls of the experimental cage. Males fly well and seem to be able to locate gynes while in flight, as several landed straight in contact with them. Gynes do not fly actively like males and seem to use their wings only for gliding.

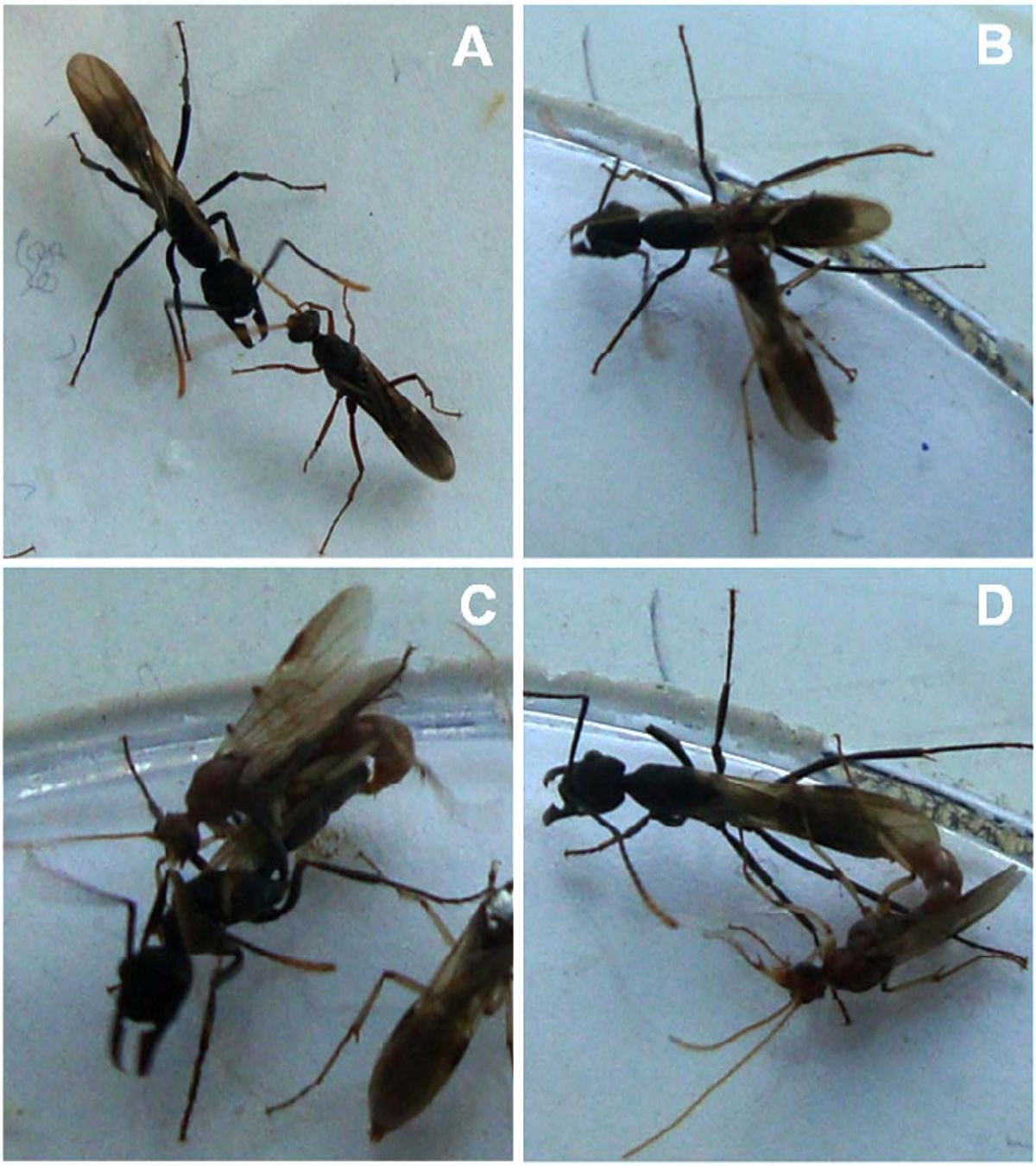

Figure 1. Mating sequence in ants from the *Pachycondyla apicalis* species complex. (A) Approaching and antennal boxing, (B) mounting attempt with antennal boxing (C) mounting with penetration attempts and (D) copulation, when the male stops the antennal boxing and releases the gyne's thorax during sperm transfer.

Only single matings were observed. However, mated males tried to copulate with other gynes, and even with already mated females. Mated gynes, on the contrary, refused all further male attempts.

Stridulation production

No stridulatory activity could be observed during any part of the pre-copulatory behaviour or during copulation. However, series of audible stridulations were recorded from inseminated gynes (n=3) right after mating, when further males tried to copulate with them. The confirmation that the stridulatory signals were really originating from the gynes came from observations in which the movements of the gaster could clearly be correlated with the sound heard. Male stridulation was not observed in any part of the mating behaviour.

Sexual interactions between the different morphs and species

Most of the interactions observed between male and female sexuals did not lead to copulation. These interactions could reach different steps of the mating sequence, depending on the type of couple or even the different couples within categories (Table 2). Therefore, in addition to the behavioural elements described during the mating sequence, we defined two intermediate ones: *mounting attempt*, when the male tries to mount the gyne's thorax without success; and *pseudo-penetrations*, when the male manages to clasp the gyne's genitalia for a few seconds, but without successful copulation, as shown by only male offspring production by gynes having had this kind of interaction and later dissection.

Table 2. The different behavioural sequences observed in each couple category in the *P. apicalis* species complex during sexual interactions.

Type of couple	Behavioural sequences
Heterospecific	Antennal boxing, Mounting attempt
Homocolonial	Antennal boxing, Mounting attempt
	Antennal boxing, Mounting
Same morphs	Antennal boxing, Mounting attempt
	Antennal boxing, Mounting
	Antennal boxing, Mounting, Pseudo-penetration
	Antennal boxing, Mounting, Copulation
Different morphs	Antennal boxing, Mounting attempt
	Antennal boxing, Mounting
	Antennal boxing, Mounting, Copulation

Mating attempts were mostly performed between couples of the same morphs (Figure 2). We also observed some interactions between males and gynes of other categories (e.g; homocolonial and different morphs or species) (Figures 2), but these also varied according to the type of couples. *P. verenae* males, for example, almost never interacted with *P. apicalis* gynes of any morph. Only one case of a brief antennal boxing with mounting attempt could be recorded (Figures 2 and 3). Some antennal boxing, mounting attempts and even mountings were observed between homocolonial males and gynes (Figure 2), but, lasted less time than interactions between heterocolonial individuals of the same morphs (Figure 3).

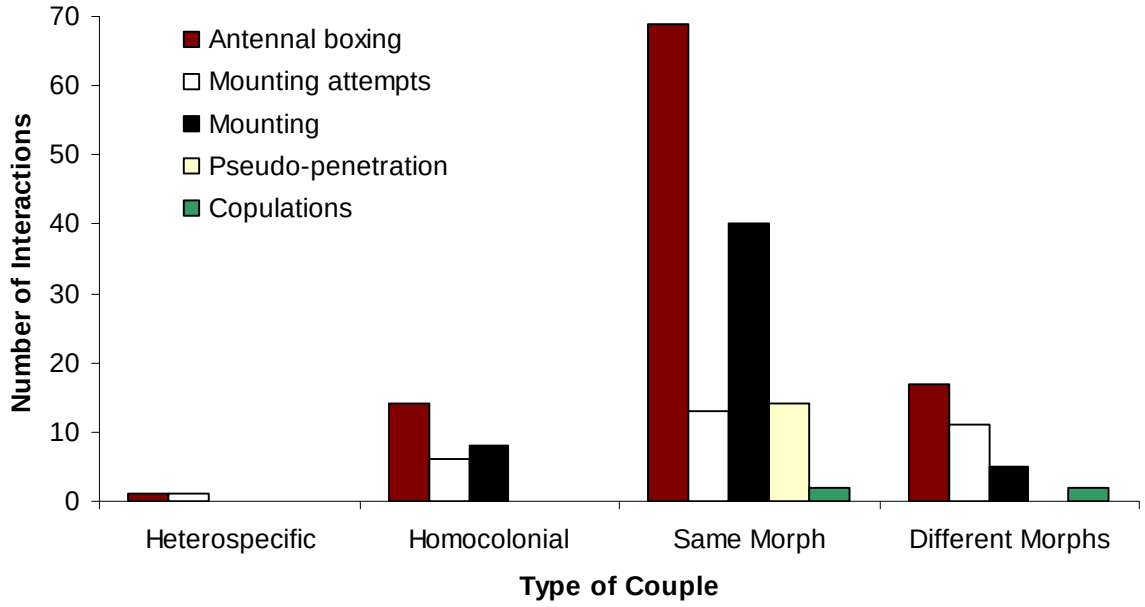

Figure 2. Total number of interactions observed for different types of couples

We also observed some pseudo-penetrations as well as two successful copulations between heterocolonial gynes and males of Morph 4 from French Guiana. The dissection of one of these mated gynes showed a spermatheca inseminated with live spermatozoids. The other mated gyne was reared and could produce workers and found a new colony. Interactions between males and females from distinct morphs, despite in smaller numbers, also led to two successful copulations between Morph 4 gynes from French Guyana and Morph 2 males from Brazil (Figure 2). These copulations were similar in duration to the ones observed between individuals of the same morphs (Figure 3).

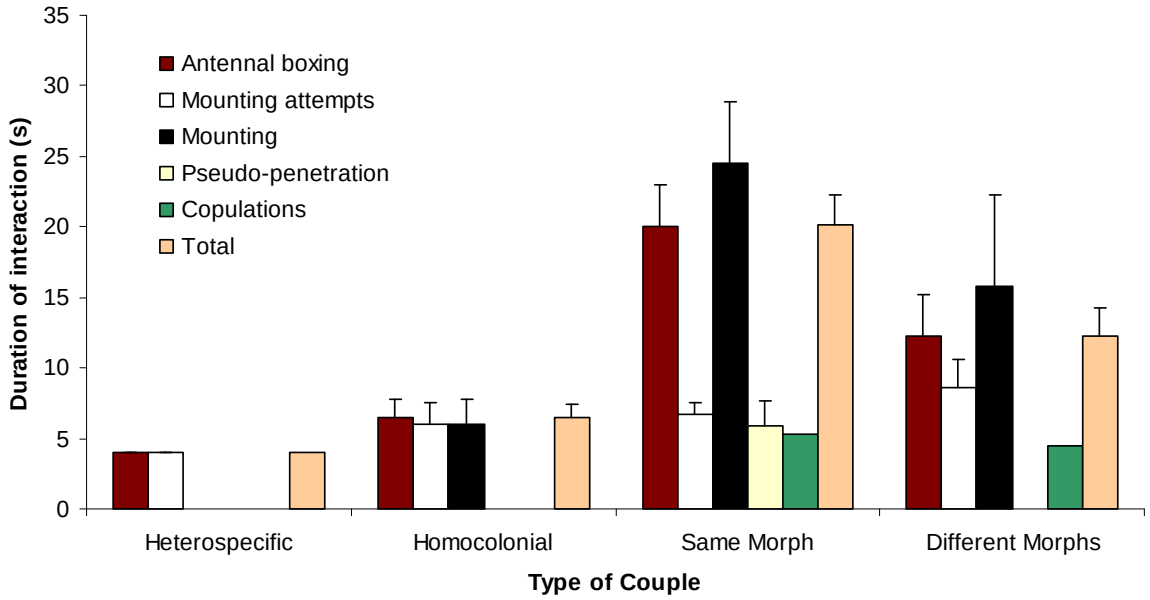

Figure 3. Duration of interactions observed for different types of couples (mean ± SE).

The dissection of a gyne mated with a male from a different morph also showed a spermatheca filled with live spermatozoids (Figure 4). However, the other mated gyne reared to confirm the viability of the interbreeding, despite having laid eggs, died before producing offspring. Later dissection revealed that she was also inseminated.

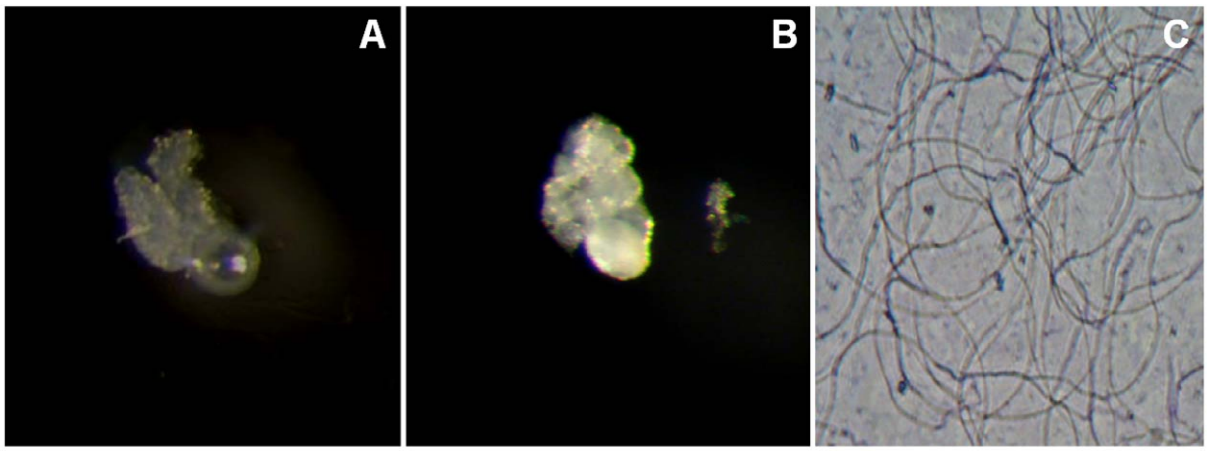

Figure 4. Spermathecae and spermatozoids of ants from the *Pachycondyla apicalis* species complex. (A) Non-inseminated, transparent and (B) inseminated, pale spermathecae. (C) Spermatozoids dissected from a spermatheca of a *P. apicalis* Morph 4 gyne mated with a *P. apicalis* Morph 2 male.

Discussion

In ants, sexual interactions generally consist merely of locating and approaching the partner followed by several short, investigative antennation strokes and a few seconds of sperm transfer (Hölldobler & Wilson, 1990). In our study we showed that the mating behaviour of ants from the *Pachycondyla apicalis* species complex can be described as a relatively stereotyped sequence of behaviours, as in a few species of myrmicine ants of the genus *Cardiocondyla,* although not as elaborated. Antennal boxing plays an important role in this courtship: it is a highly repeated antennal contact, which goes over the primary function of odour discrimination to a ritualized behaviour in *Cardiocondyla elegans* (Mercier et al. 2007) and may thus help males to keep females under control and maybe stimulate gynes receptivity.

During mating behaviour of ants from the *P. apicalis* species complex, at least under laboratory conditions, stridulatory signals were used neither for species recognition nor for sex recognition during the mate selection process, since no stridulations occurred during this phase. Stridulations therefore probably do not function as a reproductive isolation signal in these closely related species, despite the fact they are able to produce species-specific signals (Ferreira et al. *in press*).

Here, stridulations were observed from gynes only after they had mated once and did not accept further male mating attempts. This corroborates the "female liberation signal" hypothesis proposed by Markl et al. (1977) for *Pogonomyrmex* ants, in so far as they may communicate gynes non-receptivity to approaching males. The fact that gynes reject males after one copulation suggest that *P. apicalis* Morph 4 is a monoandrous species, but male polygyny is expected in this species and also in *P. apicalis* Morph 2, as males still tried to copulate with other gynes after having mated.

The absence of stridulations before copulation indicates that discrimination and mate recognition within and between the different taxa in the *P. apicalis* complex are thus probably regulated by chemical signals, as in most ant species (Hölldobler & Wilson 1990). Even if males may make errors in female identification after approach, they do not persist long with an inappropriate partner. The mistaken interactions observed in males of the *P. apicalis* species complex (e.g. homocolonial males that tried to or mounted their sisters, or the *P. verenae* male that tried to mount a *P. apicalis* gyne), lasted just a few seconds, whereas interactions with gynes of the same morph lasted three times more (Figure 2). Nevertheless,

interactions between different morphs of *P. apicalis* had comparable durations to those observed for couples of individuals belonging to the same morph. Interactions between different morphs were mostly performed by individuals of *P. apicalis* Morphs 2 and 4 from distinct populations (Brazil and French Guiana, respectively), with two successful copulations observed. Despite the fact that *P. apicalis* Morph 2 does not have its taxonomic status confirmed (e.g. whether it is a good species, subspecies or an ecotype) and that gynes present low morphological differences (i.e. petiole shape and pilosity) with those of *P. apicalis* Morph 4, males, in their turn, show more conspicuous phenotypic divergence that could act as sexual barriers. In *P. apicalis* Morph 2 males are always dark brown to almost black and have opaque wings while in *P. apicalis* Morph 4 they are always reddish with transparent wings (Ferreira et al., *unpublished data*). Such differences could be taken as mere biogeographic variation if they were not also found in sympatry, as recorded for French Guiana populations (see Chapter 3). However, these differences were not sufficient to prevent interbreeding between such morphs. In addition, under laboratory conditions, other pre-copulatory isolation mechanisms supposed to occur in the field, like difference in time of sexuals maturation and activity (i.e. temporal isolation), as well as subtle differences in microhabitats and niches (i.e. ecological isolation) (reviews in Coyne & Orr 1998, Nosil et al. 2009, Futuyma 1998) are excluded, thus allowing the interbreeding of close related species (Skarżyński 2004, Kuwajima et al. 2010).

Moreover, even if *P. apicalis* Morphs 2 and 4 were able to copulate, no offspring could be observed. On the one hand it could be due to simply natural complexity for colony foundation in non-claustral species, but on the other hand it could be caused by a more complex mechanism of post-copulatory isolation, which would prevent such progeny to be produced. This type of mechanisms include the existence of divergent numbers and morphologies of the chromosomes in the parent species, which could result in the production of hybrids that can experience a certain loss of fertility, and therefore a poor adaptation, because of irregular meiosis, or even the inability to produce hybrid offspring (Noor et al. 2001, Rieseberg 2001). Indeed, Mariano (2004) and Delabie et al. (2008), studying several morphs of the *P. apicalis* species complex (*P. apicalis* Morph 1 and *P. verenae* Morphs 1 and 2) from different populations, observed an extreme chromosomal variation between and even within these morphs, as also observed by Imai et al. (1994) for the Australian ants of the *Myrmecia pilosula* species complex.

At last, as the copulations observed between *P. apicalis* Morphs 2 and 4 were between sexuals from distant populations, and if selection reinforces the degree of reproductive isolation that exists between two species due to the poor adaptive value of the hybrids, it is expected that the populations of two species located in the same area will show a greater reproductive isolation than populations that are geographically separated (Noor 1999, Nosil et al. 2003, 2009). Therefore, a possible smaller divergence between allopatric populations of such cryptic morphs could have thus allowed their interbreeding.

Given the limited data obtained in our study, i.e. only a few cryptic morphs/species of the *P. apicalis* complex considered, and the small number of successful matings obtained, one could deem the above hypotheses as at most speculative. However, due the difficulty to observe copulation in natural habitats or to rear individuals in the laboratory and correctly control for their physiological status, our results constitute a first step in order to elucidate the evolution of mating systems and mechanisms of reproductive isolation in this group of close related species of primitive ants.

Acknowledgements

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Article 5: Responsiveness of the primitive ant *Pachycondyla apicalis* (Formicidae: Ponerinae) towards homospecific and heterospecific stridulations: a laboratory diffusion test

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En préparation

SHORT COMMUNICATION

Responsiveness of the primitive ant *Pachycondyla apicalis* (Formicidae: Ponerinae) towards homospecific and heterospecific stridulations: a laboratory diffusion test

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As observed for other ant species (reviewed in Ferreira & Fresneau 2009), the effect of stridulatory signals in the primitive Neotropical ant *Pachycondyla apicalis* seems to depend on the behavioural context. For example, *P. apicalis* workers stridulate when they are prevented from moving freely, and also in response to nest disturbance or mammal breath. This suggests that this behaviour, besides being an alarm signal of individual distress, may also be a response to intruders or potential predators and may have a defence role (Ferreira et al. *unpublished data*). In this species, stridulatory activity has also been recorded in newly inseminated gynes right after mating, similarly to what Markl et al. (1977) described for the harvester ants *Pogonomyrmex* spp. In this last context, stridulations might act as a post-copulatory "female liberation signal" to inform males that gynes are not receptive anymore (Ferreira et al. *unpublished data*). This suggests that in stridulatory communication, the message signalled by the sender and the meaning of the signal to the receiver can vary according to the situation it is produced (Hölldobler 1999), thus raising the question of the determinism of ants behavioural decisions in response to stridulations.

Here we investigate whether and how acoustic signals alone can influence the behaviour of the receiver ants. We performed a free-choice diffusion test in which *P. apicalis* workers were exposed to two types of acoustic signals (one at a time): the stridulations of their own colony (homospecific homocolonial signal) and the stridulations of the closely

related species *P. verenae* (heterospecific signal). A control situation consisted in the same procedure but with no signal being diffused. We predict that if homospecific homocolonial stridulations alone carry a distress signal, they should attract nestmates. Such alarm function has been observed in leaf-cutting ants, in which substrate-borne vibrations generated by stridulating trapped ants attracted nestmates and induced digging behaviour until the stridulating ant has been freed (Markl 1965, 1967). A converse prediction for homospecific stridulations is that if they carry the information of an alarm signal, they can indicate a potential danger and may create a repellent behaviour in the receiver. *P. apicalis* and *P. verenae* are broadly sympatric over their whole distribution area (Wild 2005) and present relatively similar ecological and behavioural characteristics (Fresneau 1994, Delabie et al. 2008). A recent study by Ferreira et al. (*in press*) showed that these species produce distinctive stridulations. Such species-specific signals could have evolved in response to competitive interactions between these sympatric species. Thus, a similar avoidance response can be expected from *P. apicalis* workers to the heterospecific stridulations of *P. verenae*, as these especies share the same niche and thus are likely to compete for resources and nesting sites (Fresneau 1994, Pezon 2004).

To test these hypothesis, we developed a wood and acrylic T-maze in which ants could freely choose between a "stridulating" and a "mute" arm. Each arm was attached to a loudspeaker which acted also as a vibrator, as illustrated in Figure 1. Stridulations were produced with a computer playing loops of the original recordings, with playback volume adjusted to the natural level reached during recordings. The transmission of vibrations on the substrate in the stridulating arm was confirmed using an accelerometer. The T-maze was positioned inside a 1 m tall cardboard cylinder with open top so that tested ants had no access to, and thus could not be disturbed by, external visual stimuli. The maze was illuminated from above by equidistant fluorescent light tubes on the ceiling.

Twenty-five workers of *P. apicalis* were tested with each type of signals (homospecific and heterospecific) and 30 workers were tested in the control situation (both arms mute). The "stridulating" and "mute" arms were randomly changed between trials to avoid any effects of potential side preferences. A single worker was placed in an introduction chamber connected to the maze where it was able to settle and enter the pre-choice arena by itself (Figure 1), and the trial started when the ant reached the pre-choice arena. The arm where the ant entered as its first choice was scored as behavioural response. We expected ants to show preference for, or avoidance of, one of the arms depending upon the differential stimuli provided. Ants that did not choose after 3 minutes were excluded from the analysis.

After each trial, the filter paper in the pre-choice arena was changed and both arms of the set-up were thoroughly cleaned so that no odours remained from other ants.

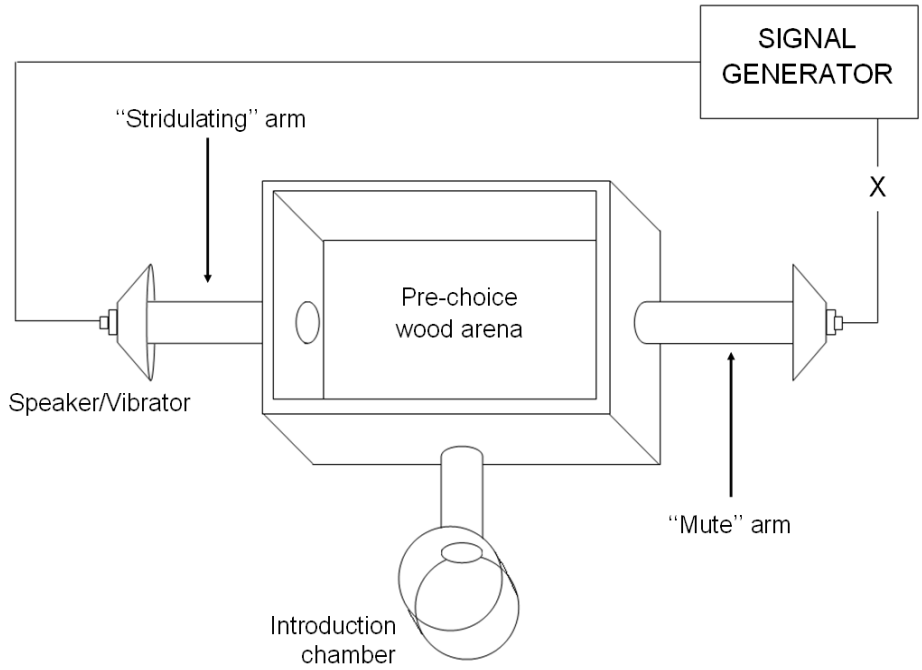

Figure 1. Experimental T-maze used during the choice experiments. Single workers were placed in the introduction chamber, where they were able to settle and enter the pre-choice wood arena by themselves and choose between a "stridulating" and a "mute" arm (randomly changed between trials). Each arm was attached to a loudspeaker which acted also as a vibrator, and the "stridulating" arm diffused one at a time homospecific homocolonial or heterospecific stridulations. Control experiments were performed with two "mute" arms. We expected ants to show preference for, or avoidance of, one of the arms depending upon the differential stimuli provided.

The great majority of tested ants made a choice in all three experiments ($n_{tested}/n_{analysed}$, control: 26/30, homospecific: 23/25 and heterospecific: 24/25), confirming the feasibility of the task. Moreover, in control experiments where both arms were "mute", no significant preference for any arm could be observed (X^2=0.32, df=1, n=26, p=0.3356, Table 1). The results of these control trials exclude the possibility of any influence of the maze itself in the ants' choice, and therefore validated the experimental set-up.

Table 1. Number of workers of the primitive ant *Pachycondyla apicalis* that selected a "stridulating" or a "mute" (control) arm in the choice experiments. Values in parentheses are expressed as percentage of the total number of ants tested. Control experiments were performed with two "mute" arms.

Played-back signal	n	"Stridulating" arm	"Mute" arm	X^2 Exact	*P*
Control	26	14 (53.85%)	12 (46.15%)	0.32	0.3356
Homospecific (homocolonial)	23	12 (52.17%)	11 (47.83%)	0.08	0.4438
Heterospecific (*P. verenae*)	24	13 (54.17%)	11 (45.83%)	0.32	0.3356

Homospecific as well as heterospecific stridulations did not influence the behaviour of the tested ants in any way. The distribution of the ants' choices did not differ significantly between the "stridulating" and the "mute" arms for both types of signals (Homospecific: X^2=0.08, df=1, n=23, p=0.4438 and Heterospecific: X^2=0.32, df=1, n=24, p=0.3356, Table 1). Moreover, the percentage of ants that choose the "stridulating" arm in the three experiments did not differ statistically (X^2=0.08, df=2, p=0.96, Figure 2).

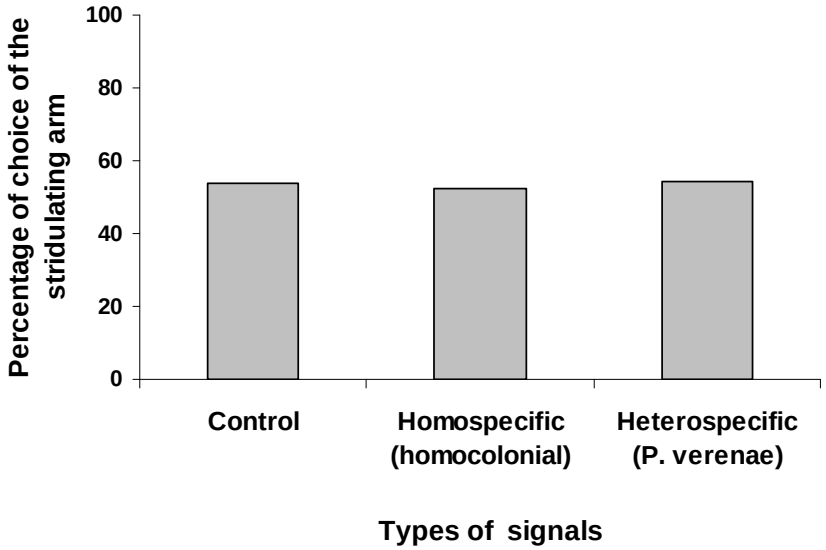

Figure 2. Percentage of choice of the "stridulating" arm by workers of the primitive ant *Pachycondyla apicalis* exposed to different types of stridulations in the choice experiments. Control series were performed with two "mute" arms (X^2=0.08, df=2, p=0.96).

In the current study, we tried to assess with a T-maze paradigm whether acoustic signals could influence the behaviour of *P. apicalis* ants in the absence of social and behavioural contexts as well as any other visual or chemical stimuli. Despite the fact that we've demonstrated the general feasibility of the maze task, the ants' choices seemed to be random and thus no effect of any stridulatory signals could be observed.

The lack of responsiveness to stridulatory stimuli observed has to be analysed with care because of methodological reasons. First, although we confirmed the transmission of both components of the signals played-back (sound and vibrations) through the "stridulatory" arm, some stridulatory signals may also have reached the pre-choice wood arena and may thus have confused the ants about the source of the stridulations. In addition we considered only the first choice performed by the ants in the maze as the behavioural response. However, other parameters about the ants' movements, such as the number of visits and time spent in each arm as well as the velocity and the latency for choice, could also be important indicators of attractiveness or repulsiveness to the stimuli. Furthermore, other behaviours such as antennation or "on guard" attendance (see Barbero et al. 2009) could also have been considered.

Nonetheless, Markl & Hölldobler (1978) suggested that ant stridulations belong to a form of modulatory communication, and that these signals do not release any specific response, but merely modulates the responses of ants to other stimuli. This could explain why ants failed to show an oriented behaviour toward or away from the stimuli. However several authors demonstrated that ant acoustic communication is much more complex and that stridulatory signals, in addition to being modulators of many social behaviours, are also active actors in some instances of multimodal communication (Markl 1965, 1967, Roces et al. 1993, Hölldobler 1999, Barbero et al. 2009, Hölldobler & Wilson 2009). Markl (1965, 1967) observed that *Atta* workers responded to caved in stridulating nestmates even when the latter were unable to release alarm pheromones. Roces et al (1993) demonstrated that *Atta* workers respond to stridulatory vibrations produced by other workers during the cutting of leaf fragments by orientating towards the source of the vibrations, even in the absence of chemical signals. Recently, Barbero et al (2009) demonstrated that for *Myrmica* ants' acoustical signals alone can inform about the caste and the status of a colony member, and trigger appropriate behavioural responses toward it by the workers.

Nevertheless, in all these experiments, diffusion tests reproduced the social and behavioural contexts in which stridulations were previously observed in the studied species. Following this view, our results seem to confirm for *P. apicalis* the context dependency for

stridulatory signals to cause behavioural responses in the recipient ants. However, in this species some behavioural tasks such as foraging require single ants to deal with unfamiliar situations and competitors. We expected here, even by excluding the social context as well as any other visual or chemical stimuli, that *P. apicalis* workers could respond to *P. verenae* stridulations, as these close sympatric species are an integral part of each other's ecology. Further playback experiments in different set-ups are thus needed to fully understand how and in which contexts stridulations induce behavioural responses in *P. apicalis*.

Acknowledgements

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CHAPITRE 3:

Biosystématique du complexe d'espèces *P. apicalis*: Spécificités biométriques, chimiques, biologiques et écologiques de fourmis cryptiques

Résumé

L'étude de la bioacoustique et les données génétiques sur les différents taxons du complexe d'espèces *P. apicalis* nous ont permis de démontrer l'existence de six à neuf espèces valides là où seules trois étaient décrites précédemment. Les caractéristiques écologiques et comportementales de chaque espèce ont donc pu être mélangées dans les études précédentes et il est possible que les espèces/morphes puissent posséder des particularités jusqu'alors ignorées. L'étude biosystématique de ces particularités peut permettre non seulement de consolider la taxonomie du complexe en apportant de nouveaux éléments diagnostiques mais aussi de révéler des différences potentiellement importantes pour le maintien des populations sympatriques.

Nous avons donc tenté dans ce chapitre de rassembler un certain nombre d'informations supplémentaires et présentons de nouvelles données morphologiques, biologiques et écologiques des différents morphes/espèces du complexe à partir de récoltes dans plusieurs sites. La communication chimique étant importante dans la plupart des relations inter et intra spécifiques chez les fourmis, nous avons également réalisé une étude de la différentiation au niveau des hydrocarbures cuticulaires des différents taxons.

Nous avons confirmé l'existence de caractéristiques propres à chaque morphe à tous les niveaux d'études. La morphologie des mâles de chaque morphe des groupes *'apicalis'* et *'verenae'* est décrite pour la première fois. Cette étude confirme et prolonge nos données sur l'existence de neuf espèces potentielles dans le complexe d'espèces *P. apicalis*. Nous avons également des indications de préférences écologiques qui devront être approfondies pour comprendre les assemblages et la distribution de ces espèces proches. A partir de l'étude de différents sites plus ou moins éloignés allant du Mexique au Brésil, nous avons également découvert qu'il pouvait exister une diversité supplémentaire à l'intérieur des taxons définis ici, en particulier pour les profils chimiques, et il serait intéressant de pouvoir réaliser un relevé plus exhaustif de cette variation sur l'ensemble de l'aire de distribution du complexe pour comprendre sa diversification et essayer de reconstituer son histoire évolutive.

Introduction

Biosystematics is the sum of biological knowledge which can be used in separating species morphologically difficult to distinguish (Drosopoulous 2006). It can be compared to a jigsaw puzzle in which each piece has a small part of the picture on it, that means not much when considered alone. However, as they are put together, we start to realize which scenario is hidden behind it. In the first chapter of the present thesis, we could confirm by the corroboration of acoustic, morphological and genetic data the taxonomic status of several morphs in the Neotropical *Pachycondyla apicalis* species complex as valid new species. Our results also suggested that the diversity inside this complex can reach up to nine cryptic species with varying levels of divergence (Ferreira et al. *in press*).

Given that these cryptic species were until recently deemed as only three distinct species (Wild 2005), their biological and ecological characteristics were always mixed together. Thus the two most widespread and studied species, *P. apicalis* and *P. verenae* were considered to present high intraspecific levels of variation in such aspects (Fresneau et al. 1994, Wild 2005, Delabie et al. 2008).

However, studies on cryptic complexes of diverse groups of insects, especially herbivores and parasites, have shown that presumed generalist species were indeed groups of specialists (Hebert et al. 2004, Blair et al. 2005, Stireman et al. 2005, Smith et al. 2006, Kankare et al. 2005) and that some phenotypic traits, sometimes of other life stages than adults, were not only intraspecific variation but cryptic species-specificities also clearly correlated to ecological, ethological and genetic traits (Hebert et al. 2004).

In social insects like ants, such variations and specializations can encompass the social organization of the colonies as well as the different castes of individuals composing them (Hölldobler & Wilson 2009). Moreover, intra and interspecific relationship in ants are much more diverse than in most non-social species, and this leads to increased complexity in the signals involved, which can provide additional cues of species diversification.

For all these reasons, we explored further aspects of the biology and ecology of the *P. apicalis* species complex searching for new evidence to facilitate the identification of these cryptic species and better understand the characteristics that can represent idiosyncrasies of each taxon. We performed a biometric study of the ants, a chemical analysis of their cuticular hydrocarbons and also a comparative study of the reproductive systems, male phenotypes as well as ecological preferences for nesting sites in ants from sympatric and allopatric populations in Brazil, French Guiana and Mexico.

Methods

Ant collection and geographical distribution

Colonies of the *P. apicalis* species complex (*P. apicalis* and *P. verenae* groups) were collected from several sympatric and allopatric populations in Brazil, French Guiana and Mexico (Table 1, Figure 1). Ants were classified according to the classification proposed by Delabie et al. (2008) and Ferreira et al. (*in press*), with ants being classified into morphs within their currently named species. *P. obscuricornis* (POB) workers used in this study were from the entomological collection of the Museu Paraense Emilio Goeldi, Pará, Brazil.

Table 1: Origin and distribution of the colonies of the *P. apicalis* species complex used in the present study.

Collecting site*	Population	PAP Morph 1	PAP Morph 2	PAP Morph 3	PAP Morph 4	PAP Morph 5	PAP Morph 6	POB **	PVE Morph 1	PVE Morph 2
BR	Belém				X		X	X	X	
BR	Ilhéus	X								
BR	Porto Seguro								X	
BR	Una		X						X	
BR	Viçosa									X
FG	Petit Saut		X		X	X	X		X	
FG	Rt Petit Saut				X					
FG	Rt Saint Elis								X	
FG	Camp Patawa				X				X	
MEX	Los Tuxlas			X						

* BR: Brazil, FG: French Guiana, MEX: Mexico.

** Other *P. obscuricornis* individuals were also from northern and north-western Brazil (Melgaço-Caxiuanã, Benevides and Ouro Preto do Oeste).

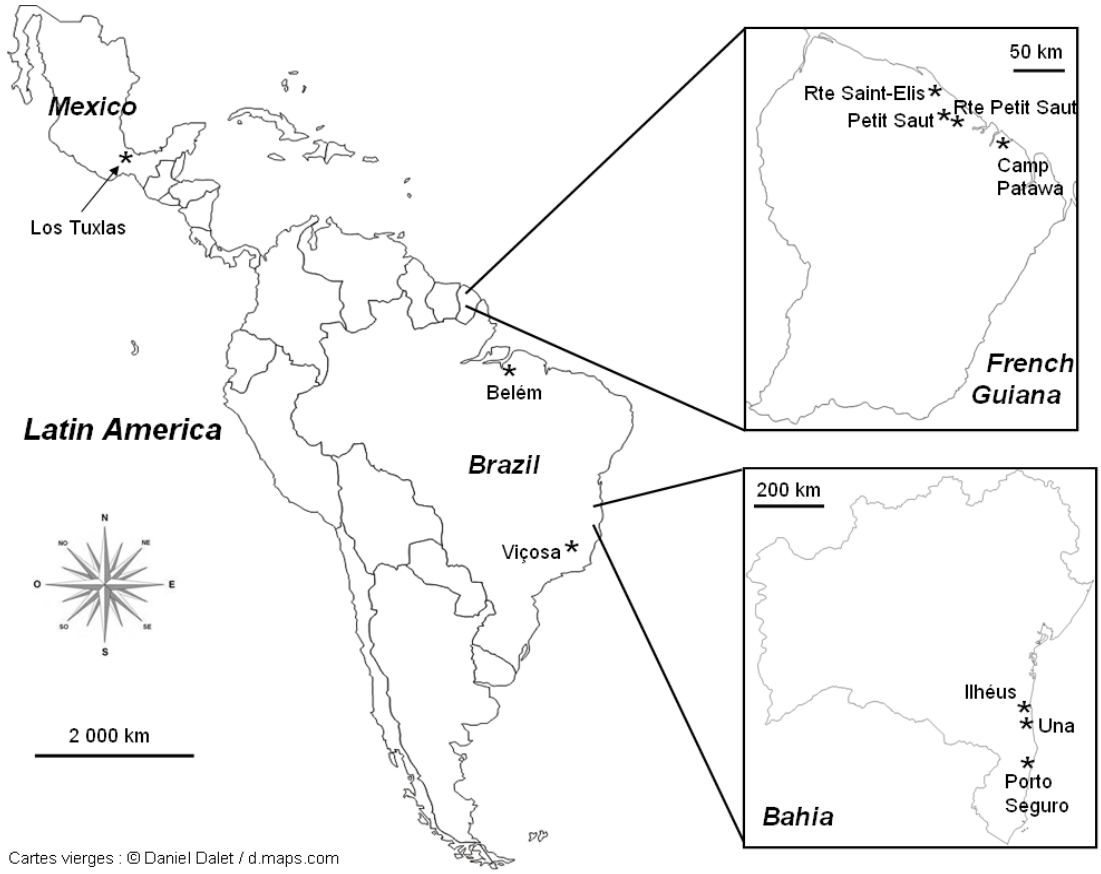

Figure 1. Study populations of the *P. apicalis* species complex from Brazil, French Guiana and Mexico.

Biometric study

Biometric measurements were conducted on 98 individual workers from 8 distinct taxa of the *Pachycondyla apicalis* species complex. Individuals from the *'apicalis'* and *'verenae'* groups came from 13 distinct colonies (7 individuals/colony) from several populations in Brazil (Belém, Una, Viçosa), French Guiana (Petit Saut, Route de Saint Elis) and Mexico (Los Tuxlas) (Table 1). We measured the same morphological characters as Wild described and employed in his 2005 study (below). All observations were made at 10-20x magnification with a Zeiss stereo microscope.

(1) *Head length.* In full-face view, the midline distance from the level of the maximum posterior projection of the posterior margin of the head to the level of the most anterior projection of the anterior clypeal margin.

(2) *Head width.* In full-face view, the maximum width of the head posterior to the compound eyes.

(3) *Antennal scape length.* Measured from the apex of the first antennal segment to the base, exclusive of the radicle.
(4) *Fore femur length*. In posterior view, measured along the longitudinal axis from the apex to the junction with the trochanter.
(5) *Hind tibia length.* In dorsal view, measured along the longitudinal axis from the apex to the level of the lateral condyles, excluding the medial proximal condyle.
(6) *Petiole height.* In lateral view, the distance from the ventrum of the petiolar sternite to the apex of the petiolar tergite, taken as a vertical measurement perpendicular to the longitudinal axis of the petiole.
(7) *Petiole length.* In lateral view, the maximum longitudinal distance between the anterior and posterior extentions of the petiolar node, excluding the anterior and posterior condyles.
(8) *Weber's length.* In lateral view, the distance between the anterior margin of the pronotum exclusive of the collar to the posterior margin of the metapleural bulla.
(9) *Scape index.* Scape length / Head width.
(10) *Cephalic index.* Head width / Head length.
(11) *Petiole index*. Petiole height / Petiole length.

Chemical study

Individual ants (3-5 workers/colony, 16 colonies, total=71) from 8 distinct morphs of the *Pachycondyla apicalis* species complex from diverse sites in Brazil, French Guiana and Mexico (Tables 1 and 2, Figure 1) were placed into glass vials and immersed into 1ml of pentane to extract cuticular hydrocarbons (CHC). After one hour, the ants were removed and the samples stored at 5° C. Samples were analysed using Gas Chromatography and Mass Spectometry (GC-MS). CHC compounds were identified by their mass fragmentation pattern and retention time comparisons. The peak area for each compound was calculated for each sample and then standardized by calculating its proportion and percentage abundance. The proportions were then arcsine transformed prior to analyses.

To compare the cuticular hydrocarbons profile between morphs and populations we performed a principal component analyses (PCA) and then used the individuals coordinates from the first 12 factors, explaining 95% of the variance; in subsequent descriptive discriminant analyses (DFA).

This chemical study was developed in collaboration with Prof. Abraham Hefetz, Tel Aviv University.

Ecological and biological data

Observations on populations, sympatry levels, nesting preferences, foraging activities, reproductive systems and male phenotypes in the *P. apicalis* complex were made at different sites in Brazil, French Guiana and Mexico (Table 1, Figure 1).

Results and discussion

Biometric study

The biometric analyses of the ants from the *P. apicalis* species complex provided further useful information about the morphology of these ants, especially for the two new taxa revealed in Ferreira et al. (*sous presse*). We confirmed the stability of several size differences as well as allometric ones between the different morphs and species.

P. apicalis Morph 6 stood out by its size. Bigger than all the other taxa in the complex, it is clearly differentiated in all the variables of absolute measurements such as lengths and widths of the body, head and appendages despite presenting similar cephalic, scape and petiole indexes to other morphs of the group *'apicalis'*, i.e. the morphs with yellow antennomeres (Figure 2).

P. apicalis Morph 5 is a moderate size species, with a head as broad as all other morphs of the group *'apicalis'* (except for *P. apicalis* Morph 6) but with the smallest scape length for the group, thus allowing it to be distinguished with its scape index (Figure 2). In addition, the abundant body pilosity, the striated cuticle and the marginated petiole of this morph (Ferreira et al. *sous presse*) are also useful characteristics for its identification and make it a quite distinct taxon. Indeed, in a just published revision of the new world ants of the genus *Pachycondyla* (Mackay & Mackay 2010), the authors have described a new species for the *P. apicalis* species complex, which they named *Pachycondyla cooki* Mackay & Mackay and which presents all the above mentioned characteristics. Further comparisons of our *P. apicalis* Morph 5 with the type specimens of *P. cooki* will confirm if they are the same species.

The other three morphs studied in the group *'apicalis'* present less biometric divergence. For *P. apicalis* morph 4, biometric differences with *P. apicalis* Morphs 2 and 3 were observed mainly for the petiole shape, as it is broader than in both other species, but with a marginated condition close to that of *P. verenae* (Delabie et al. 2008). This morph also presents leg segments that can be longer than other *'apicalis'* morphs (except Morph 6) (Figure 2). *P. apicalis* Morphs 2 (Supporting information 1) and 3, on the contrary, do not

present clear-cut biometric differences, apart from a tendency for a higher cephalic index in *P. apicalis* Morph 2 (Figure 2). However, the two morphs can be discriminated thanks to male phenotypes (see below) and distribution ranges (Delabie et al. 2008).

Unfortunately, *P. apicalis* Morph 1 (Supporting information 1) could not be considered in our biometric analysis. Nevertheless, at least some individuals of this morph could be analysed in other analyses of the present work (i.e. chemical, biological and ecological).

P. obscuricornis is a somewhat smaller species, with a very tall rounded petiole and very short antennal scape. It also presents the smallest scape index in the complex (Figure 2, Supporting information 1).

At last, the two morphs in the group *'verenae'* are also smaller species compared with other *P. apicalis* morphs, but *P. verenae* Morph 1 can sometimes present sizes similar to *P. obscuricornis* and *P. apicalis* Morph 5. The two *P. verenae* morphs present almost no overlap of their scape index, as *P. verenae* Morph 1 generally has a long scape, sometimes longer than the head length and *P. verenae* Morph 2 (Supporting information 1) usually presents a shorter scape and a broader head (Figure 2). They also present completely different petiole indexes (Figure 2), in which *P. verenae* morph 1 has a petiole node as broad as tall, while in *P. verenae* Morph 2 it is clearly taller than broad (Delabie at al. 2008).

Chemical study

The cuticular hydrocarbons (CHC) present in the *P. apicalis* species complex ranged in chain length from C20 to C36 and comprised saturated, unsaturated and branched compounds. Fifty-one distinct hydrocarbons could be identified for this complex (Table 2).

The comparison of the relative abundance of the different CHC categories between morphs showed that the chemical profiles of *P. apicalis* Morphs 1 and 6 were essentially represented by linear saturated hydrocarbons (alkanes), with a small proportion of alkenes and alkadienes in Morph 6. *P. apicalis* Morphs 2 and 3 had similar percentages of saturated and mono-unsaturated hydrocarbons (alkenes), but distinct percentages of alkadienes. This was due to the fact that *P. apicalis* Morph 2 also had a considerable percentage of methyl branched compounds. The *P. apicalis* Morph 4 profile was in its turn mainly constituted of alkanes and alkenes (Figure 3).

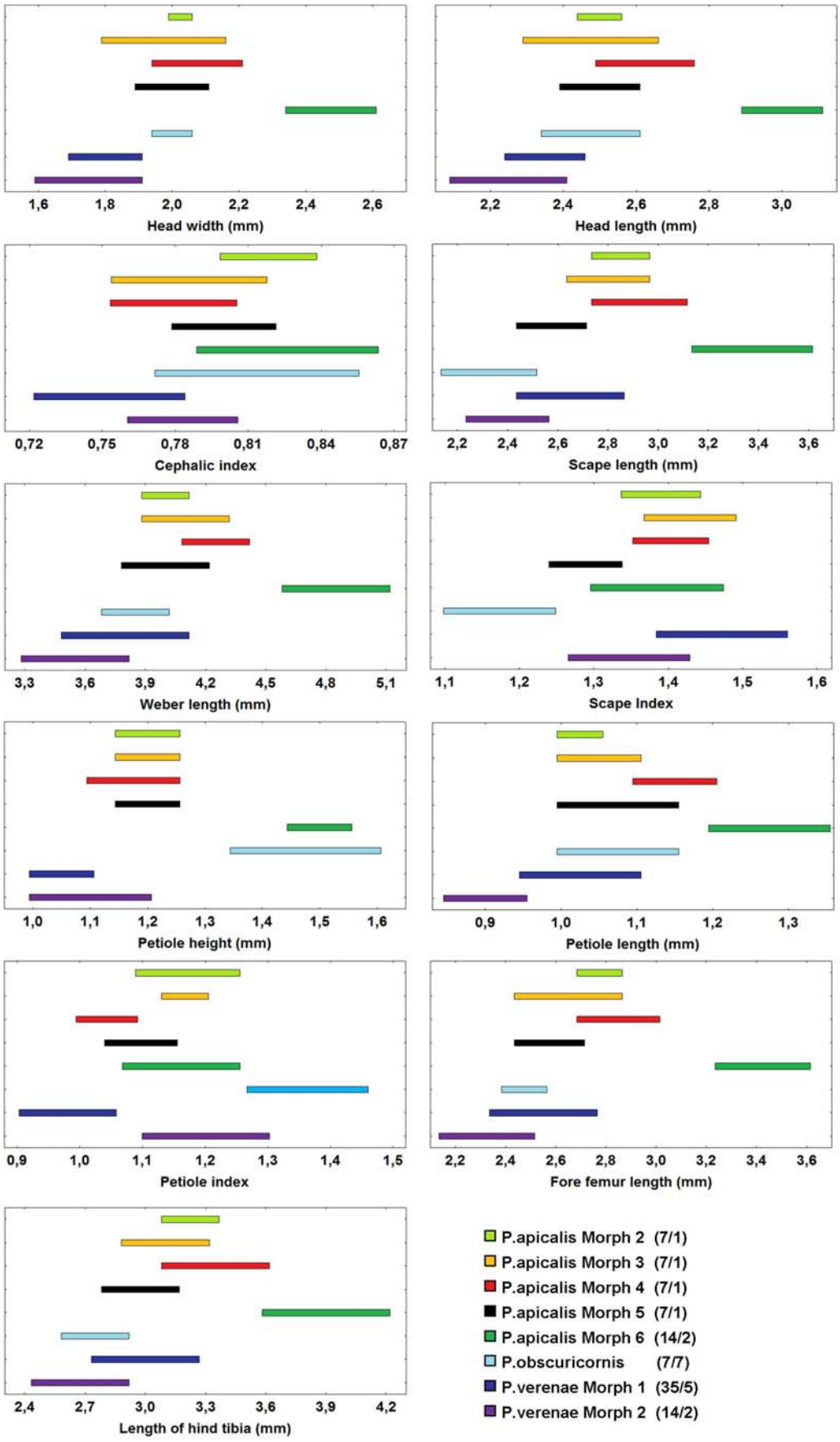

Figure 2. Biometric analysis of the *P. apicalis* species complex. Intervals correspond to the range of intramorph variation. Numbers in parenthesis indicate n individuals / n colonies sampled.

Table 2. Percentages of CHC compounds in different morphs of the *P. apicalis* species complex (mean±S.D.) from several sympatric and allopatric populations in Brazil, French Guiana and Mexico.

		PAP Morph 1	PAP Morph 2			PAP Morph 3	PAP Morph 4					PAP Morph 5	PAP Morph 6
		Brazil	Brazil	French Guiana	Mean	Mexico	Brazil		French Guiana		Mean	French Guiana	French Guiana
		Ilhéus	Una	Petit Saut		Los Tuxlas	Belém	Camp Patawa	Petit Saut	Rte Petit Saut		Petit Saut	Petit Saut
Peak	Compound	n=3	n=5	n=3	n=8	n=5	n=5	n=3	n=5	n=5	n=18	n=4	n=5
1	nC20	0.05 ± 0.01	0.04 ± 0.02	0.00 ± 0.00	0.027 ± 0.02	0.09 ± 0.02	0.24 ± 0.08	0.07 ± 0.00	0.07 ± 0.03	0.17 ± 0.08	0.14 ± 0.09		0.03 ± 0.02
2	nC21:1	0.06 ± 0.03	0.02 ± 0.01	0.01 ± 0.01	0.013 ± 0.01	0.93 ± 0.28	0.05 ± 0.01	3.06 ± 0.45	0.15 ± 0.10	0.30 ± 0.29	0.69 ± 1.16		0.67 ± 0.21
3	nC21	0.40 ± 0.14	0.96 ± 0.98	2.37 ± 2.73	1.489 ± 1.79	14.60 ± 1.60	36.57 ± 9.42	10.42 ± 0.91	14.29 ± 5.73	34.69 ± 4.02	25.50 ± 13.06		3.22 ± 0.68
4	nC22:1	0.05 ± 0.08	0.20 ± 0.15	0.04 ± 0.06	0.143 ± 0.14	0.98 ± 0.10	0.03 ± 0.03	1.20 ± 0.17	0.76 ± 0.27		0.58 ± 0.51		0.06 ± 0.04
5	nC22	0.57 ± 0.18	0.69 ± 0.67	0.76 ± 0.38	0.713 ± 0.55	1.62 ± 0.52	0.02 ± 0.03	1.06 ± 0.11	1.84 ± 0.44	5.48 ± 0.48	2.22 ± 2.22		0.51 ± 0.21
6	14-MeC22												
7	nC23:2	0.13 ± 0.22	5.77 ± 10.27		4.126 ± 8.84	48.85 ± 7.57	1.38 ± 0.31	0.02 ± 0.02	0.01 ± 0.01		0.54 ± 0.72		0.05 ± 0.06
8	nC23:1	0.44 ± 0.66		3.11 ± 4.68	3.106 ± 4.68	0.24 ± 0.19	2.71 ± 2.08	56.91 ± 3.35	56.25 ± 10.06		35.81 ± 27.91		3.08 ± 1.03
9	nC23	58.39 ± 6.07	23.85 ± 10.82	33.50 ± 4.72	27.466 ± 9.91	14.89 ± 4.40	34.33 ± 2.45	17.60 ± 1.81	20.87 ± 5.62	48.35 ± 2.87	31.70 ± 12.82		45.68 ± 6.46
10	11-MeC23			0.43 ± 0.18	0.433 ± 0.18								
11	3-MeC23			0.21 ± 0.15	0.212 ± 0.15								
12	nC24:1												
13	nC24	1.71 ± 0.17	3.20 ± 2.09	0.64 ± 0.36	2.239 ± 2.07	0.25 ± 0.07	0.33 ± 0.12	0.13 ± 0.03	0.10 ± 0.05	0.31 ± 0.02	0.23 ± 0.13		0.82 ± 0.11
14	16-MeC24												
15	nC25:2	0.09 ± 0.07	14.90 ± 9.35		14.895 ± 9.35	0.60 ± 0.14	0.03 ± 0.03	0.80 ± 0.17	1.80 ± 0.32		0.81 ± 0.82		2.87 ± 1.01
16	nC25:1			8.17 ± 10.33	8.170 ± 10.33								
17	nC25	27.02 ± 2.79	34.74 ± 22.64	8.84 ± 5.29	25.028 ± 21.92	5.68 ± 1.59	9.35 ± 3.23	2.45 ± 0.93	1.12 ± 0.71	3.47 ± 0.81	4.28 ± 3.76		23.61 ± 3.22
18	11-MeC25			1.45 ± 0.09	1.447 ± 0.09								
19	3-MeC25												
20	nC26	0.84 ± 0.12	2.41 ± 0.86	0.42 ± 0.08	1.665 ± 1.22	0.57 ± 0.19	0.42 ± 0.19	0.19 ± 0.07	0.05 ± 0.04	0.20 ± 0.05	0.21 ± 0.18		2.06 ± 1.65
21	X-MeC26												
22	2-MeC26												
23	nC27:1			9.48 ± 8.88	9.481 ± 8.88								
24	nC27	7.47 ± 2.55	7.89 ± 0.52	5.17 ± 1.48	6.866 ± 1.66	7.03 ± 2.54	9.26 ± 3.83	2.29 ± 0.87	0.94 ± 0.75	2.40 ± 1.56	3.88 ± 4.05	0.52 ± 0.19	7.57 ± 1.41
25	11-MeC27			13.45 ± 8.78	13.448 ± 8.78								
26	nC28	0.19 ± 0.30	1.88 ± 0.42	0.69 ± 0.21	1.435 ± 0.71	0.37 ± 0.14	0.54 ± 0.25	0.27 ± 0.14	0.08 ± 0.04	0.20 ± 0.04	0.27 ± 0.23	0.33 ± 0.10	1.67 ± 0.93
27	2-MeC28		0.53 ± 0.36	2.32 ± 0.65	1.202 ± 1.03	0.53 ± 0.34	0.33 ± 0.25	0.59 ± 0.51	0.07 ± 0.07	0.13 ± 0.11	0.24 ± 0.29	1.83 ± 0.70	1.27 ± 0.45
28	nC29:1			1.83 ± 1.00	1.831 ± 1.00							0.14 ± 0.04	
29	nC29	2.36 ± 0.71	2.47 ± 1.16	2.83 ± 1.41	2.602 ± 1.17	2.66 ± 0.81	4.16 ± 1.81	2.33 ± 1.09	1.33 ± 0.61	2.30 ± 1.35	2.55 ± 1.63	2.55 ± 0.94	3.91 ± 0.90
30	11-MeC29			5.58 ± 3.99	5.582 ± 3.99							2.70 ± 0.33	
31	7-, 17-diMeC29											0.39 ± 0.20	
32	nC30	0.02 ± 0.02	0.29 ± 0.16	0.06 ± 0.04	0.204 ± 0.18		0.03 ± 0.01	0.05 ± 0.03	0.04 ± 0.02	0.11 ± 0.02	0.06 ± 0.04	0.27 ± 0.07	0.14 ± 0.05
33	10+12 -MeC30											1.83 ± 0.33	
34	4-MeC30											1.48 ± 0.39	
35	nC31:1											2.98 ± 0.65	
36	nC31	0.18 ± 0.15	0.16 ± 0.24	0.32 ± 0.08	0.220 ± 0.20		0.17 ± 0.08	0.47 ± 0.32	0.49 ± 0.35	1.35 ± 0.34	0.64 ± 0.54	1.46 ± 0.63	1.85 ± 0.52
37	11+13+15-MeC31											8.15 ± 1.24	
38	11-,19-diMeC31											2.96 ± 0.65	
39	nC32	0.00 ± 0.00		0.06 ± 0.08	0.057 ± 0.08		0.06 ± 0.06	0.06 ± 0.01	0.04 ± 0.04	0.29 ± 0.11	0.12 ± 0.13	0.34 ± 0.09	0.32 ± 0.15
40	12+14-MeC32											2.12 ± 0.30	
41	nC33:1											2.18 ± 0.65	
42	nC33	0.03 ± 0.02	0.01 ± 0.02	0.11 ± 0.13	0.048 ± 0.09		0.01 ± 0.00	0.03 ± 0.02	0.07 ± 0.07	0.26 ± 0.10	0.10 ± 0.12	2.35 ± 0.70	0.61 ± 0.20
43	13+15-MeC31											11.05 ± 1.20	
44	11-,19- + 13-,19-diMeC33											6.68 ± 0.76	
45	12+14-MeC34											3.11 ± 0.15	
46	nC35:1											10.39 ± 2.71	
47	nC35											1.88 ± 1.52	
48	13+15-MeC35											18.62 ± 1.83	
49	11-,19+13-,19-diMeC35											9.91 ± 1.28	
50	nC36											0.83 ± 0.31	
51	14-MeC36											2.97 ± 0.54	

Table 2. (continued) Percentages of CHC compounds in different morphs of the *P. apicalis* species complex (mean±S.D.) from several sympatric and allopatric populations in Brazil, French Guiana and Mexico.

		PVE Morph 1						
		Brazil				French Guiana		Mean
		Porto Seguro	Una	Belém	Camp Patawa	Petit Saut	Rte Ste Elise	
Peak	Compound	n=5	n=5	n=3	n=5	n=5	n=5	n=28
1	nC20	0.02 ± 0.01	0.02 ± 0.01	0.12 ± 0.03	0.03 ± 0.01	0.16 ± 0.06	0.08 ± 0.04	0.07 ± 0.06
2	nC21:1	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.05 ± 0.03	0.06 ± 0.03	0.05 ± 0.05	0.03 ± 0.03
3	nC21	1.60 ± 0.23	1.60 ± 0.23	1.64 ± 1.08	0.73 ± 0.20	0.26 ± 0.14	4.70 ± 5.22	1.76 ± 2.52
4	nC22:1	0.07 ± 0.02	0.07 ± 0.02	0.29 ± 0.05	0.20 ± 0.03	0.06 ± 0.03	0.99 ± 0.67	0.28 ± 0.43
5	nC22	1.22 ± 0.13	1.22 ± 0.13	1.29 ± 0.14	0.44 ± 0.11	0.10 ± 0.06	3.46 ± 2.29	1.29 ± 1.43
6	14-MeC22	0.77 ± 0.17	0.77 ± 0.17					0.77 ± 0.16
7	nC23:2		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.03	0.34 ± 0.13	0.11 ± 0.15
8	nC23:1	1.30 ± 0.48	1.30 ± 0.48	2.06 ± 0.29	0.91 ± 1.05	0.04 ± 0.04	10.10 ± 6.89	2.72 ± 4.55
9	nC23	40.07 ± 5.04	40.07 ± 5.04	43.84 ± 6.49	15.65 ± 2.83	3.34 ± 3.10	31.42 ± 6.67	28.01 ± 15.64
10	11-MeC23	44.55 ± 6.01	44.55 ± 6.01		0.99 ± 0.31			30.03 ± 21.73
11	3-MeC23	4.20 ± 0.46	4.20 ± 0.46		1.08 ± 0.22			3.16 ± 1.57
12	nC24:1	0.34 ± 0.06	0.34 ± 0.06					0.34 ± 0.06
13	nC24	1.02 ± 0.21	1.02 ± 0.21	2.15 ± 0.78	0.54 ± 0.10	0.56 ± 0.19	4.10 ± 1.26	1.52 ± 1.42
14	16-MeC24	1.71 ± 0.15	1.71 ± 0.15					1.71 ± 0.14
15	nC25:2	3.11 ± 1.30	3.11 ± 1.30		0.98 ± 0.24			2.40 ± 1.44
16	nC25:1							
17	nC25	7.56 ± 2.54	7.56 ± 2.54	25.51 ± 3.94	5.72 ± 0.87	25.59 ± 5.81	29.53 ± 13.88	16.30 ± 11.98
18	11-MeC25	3.62 ± 2.20	4.28 ± 1.05		3.47 ± 1.18	15.50 ± 8.35		6.72 ± 6.59
19	3-MeC25	0.27 ± 0.51	1.35 ± 0.34		45.63 ± 5.49			15.75 ± 22.07
20	nC26	1.16 ± 0.61	0.25 ± 0.08	2.39 ± 0.58	3.94 ± 1.01	3.50 ± 0.79	4.93 ± 1.48	2.72 ± 1.88
21	X-MeC26	0.22 ± 0.11		0.11 ± 0.06				0.18 ± 0.11
22	2-MeC26				2.91 ± 0.97			2.91 ± 0.97
23	nC27:1	0.03 ± 0.04		1.05 ± 0.09				0.41 ± 0.53
24	nC27	2.41 ± 1.09	2.41 ± 1.09	18.01 ± 2.35	5.03 ± 0.46	29.61 ± 11.40	7.57 ± 2.96	10.33 ± 11.25
25	11-MeC27				9.59 ± 1.08			9.59 ± 1.08
26	nC28	0.22 ± 0.06	0.22 ± 0.06	0.08 ± 0.05	0.23 ± 0.06	16.75 ± 7.27	0.96 ± 0.50	3.29 ± 6.99
27	2-MeC28	0.02 ± 0.00	0.08 ± 0.03	0.05 ± 0.05		1.45 ± 0.52	0.13 ± 0.16	0.37 ± 0.63
28	nC29:1							
29	nC29	0.73 ± 0.30	0.73 ± 0.30	1.27 ± 0.05	1.10 ± 0.21	0.87 ± 0.31	1.32 ± 0.62	0.98 ± 0.41
30	11-MeC29							
31	7-, 17-diMeC29							
32	nC30	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	1.61 ± 0.67	0.04 ± 0.03	0.31 ± 0.67
33	10+12 -MeC30							
34	4-MeC30							
35	nC31:1							
36	nC31	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.03	0.13 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.04
37	11+13+15-MeC31							
38	11-,19-diMeC31							
39	nC32	0.12 ± 0.07	0.12 ± 0.07	0.04 ± 0.02	0.56 ± 0.18	0.21 ± 0.41	0.25 ± 0.17	0.23 ± 0.25
40	12+14-MeC32							
41	nC33:1							
42	nC33	0.01 ± 0.01		0.04 ± 0.03	0.27 ± 0.07	0.13 ± 0.09	0.01 ± 0.00	0.10 ± 0.12
43	13+15-MeC31							
44	11-,19- + 13-,19-diMeC33							
45	12+14-MeC34							
46	nC35:1							
47	nC35							
48	13+15-MeC35							
49	11-,19+13-,19-diMeC35							
50	nC36							
51	14-MeC36							

P. apicalis Morph 5 presented the most complex and most distinct profile of the complex, for which the most abundant categories were methyl and dimethyl branched hydrocarbons. It is the only species in which the latter type of compounds was present. In addition, *P. apicalis* Morph 5 profile started at C27 and ended at C36, while all other morphs in the complex started in C20 and ended in C33 (Table 2). The degree of chemical divergence observed for *P. apicalis* Morph 5 is the same as in our phylogenetic study (Ferreira et al. *in press*), in which this morph branched outside the *P. apicalis* and *P. verenae* clades. For all these qualitative and quantitative chemical dissimilarities, *P. apicalis* Morph 5 does not need to be included in our descriptive discriminant analyses.

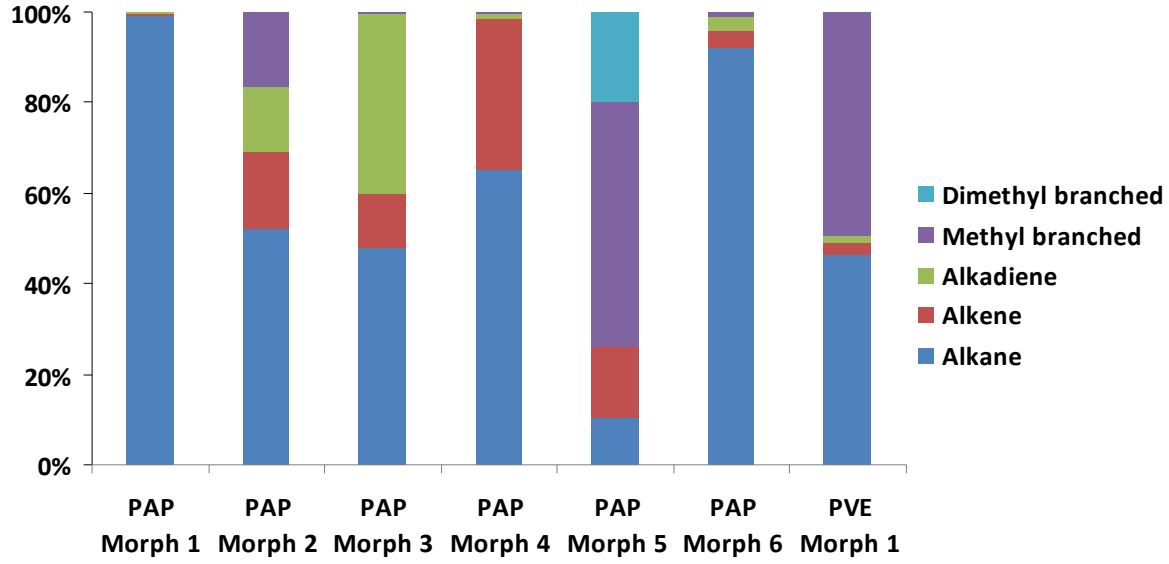

Figure 3. Percentages of each CHC category in the chemical profile of different morphs of the *P. apicalis* species complex.

At last, even if its profile presented an alkane percentage similar to *P. apicalis* Morph 2 and 3 and a methyl branched compounds percentage comparable to *P. apicalis* Morph 5, *P. verenae* Morph 1 was chemically different from all other *P. apicalis* Morphs (Figure 3).

This great quantitative and qualitative variability among the different morphs of the *P. apicalis* complex is further evidenced by our chemical analyses which confirm that each morph presents a distinct CHC profile (Figure 4).

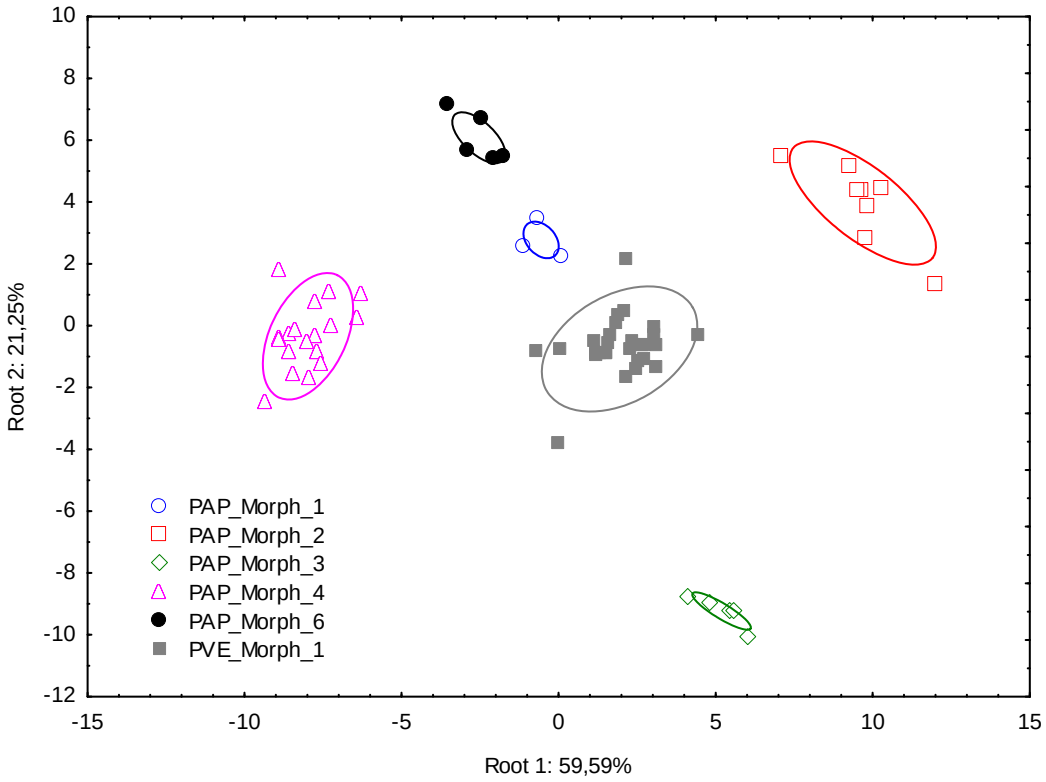

Figure 4. Descriptive DFA of the CHC profiles of different morphs in the *P. apicalis* species complex.

Furthermore, we could also observe different chemical phenotypes in different populations of the same morph from Brazil and French Guiana (Figure 5). For example in *P. apicalis* Morph 4, the most abundant '*apicalis*' morph in French Guiana (*pers. obs.*), nearby populations such as Petit Saut and Route de Petit Saut are more differentiated than distant populations such as Petit Saut and Camp Patawa (Figures 1 and 5.A). However, in *P. verenae* Morph 1, populations from south north-eastern Brazil (Una and Porto Seguro) are closely chemically related, despite these habitats having highly differentiated ecological characteristics, i.e. the colony from Una came from a cocoa and palm plantation, and the colony from Porto Seguro came from a fragment of Atlantic Forest. On the contrary, the distant northern Brazil (Belém) and French Guiana populations (Route de Saint Elis and Petit Saut), both representative of Amazonian forest habitat, cluster together (Figures 1 and 5.B).

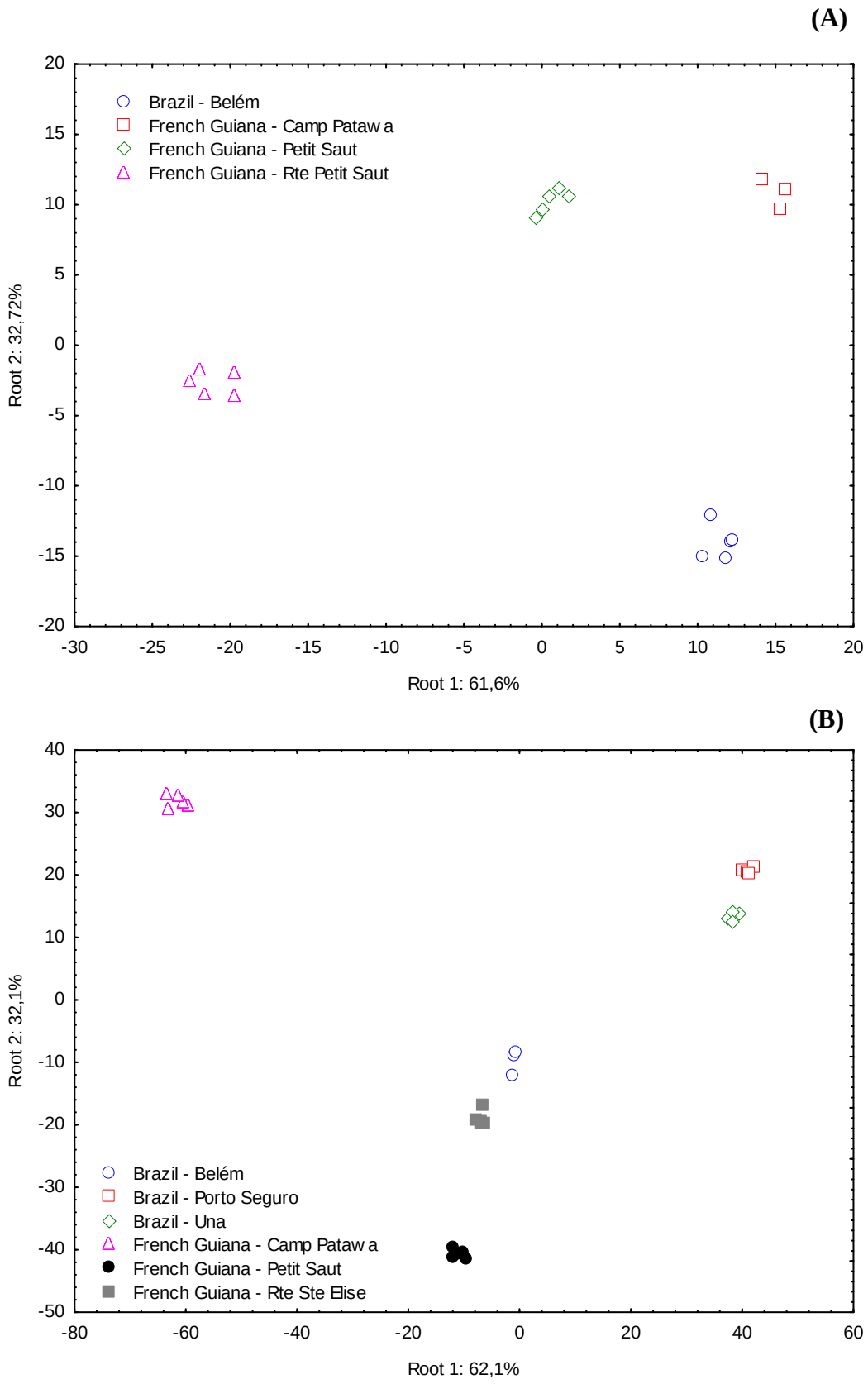

Figure 5. Descriptive DFA of the CHC profiles of *P. apicalis* Morph 4 (A) and *P. verenae* Morph 1 (B) from different populations in French Guiana and Brasil.

Such chemical differentiation within the different morphs of the *P. apicalis* complex suggests that further divergence could also exist, as cytogenetic results also suggest (Mariano et al. 2004, Delabie et al. 2008). Thus our chemical data confirm the clear divergence between morphs but probably call for further studies of intramorphs chemical diversity and this could also allow to characterize the various factors, phylogenetic, geographic and ecological which have led to the evolution of CHC and chemical signature in this widely distributed monophyletic clade.

Biological and ecological data

Despite presenting overall similar biological and ecological features (Fresneau 1994), ants from the *P. apicalis* species complex also vary in their reproductive systems. According to Fresneau (1994), colonies of the group *'apicalis'* are strictly monogynous and those of the group *'verenae'* are facultatively polygynous (Fresneau 1984, Pezon 2004). Our field observations suggest that inside the group *'apicalis'* we can also expect a facultative polygyny for some morphs in adult colonies (Table 3) or at least during colony foundation, since two polygyne foundations (with two queens) were recorded for *P. apicalis* Morph 4 in French Guiana. In addition, we observed for *P. verenae* Morphs 1 and 2, from northern and south-eastern Brazil a very high level of polygyny (colonies had 16-18 queens when collected). Nevertheless, our sample is not sufficient and as we did not dissect those gynes to confirm if they were inseminated and could lay eggs, such hypotheses need further investigations.

Up to now, no conclusive information is available concerning males of the *P. apicalis* species complex. Wild (2005), in his revision of the complex, examined six specimens of all three described species. However, after morphological analyses he concluded that males were too variable within species to provide any robust estimates of species-specific characters. In Delabie et al. (2008) the morphology of males could not be studied in detail because part of the material was not associated with their respective workers, and also due to the lack of material for all morphs in the complex. Regardless, these authors could report some differences in the petiole shape and thorax pilosity in males from the *P. apicalis* Morph 1, 2, and 4. However, no conclusions could be done for males of *P. verenae* Morphs 1 and 2, from different populations, as their morphology were extremely variable.

In our study, we could examine males from all distinct morphs of the *P. apicalis* species complex, except for *P. obscuricornis*. As already observed for workers, the high

variation pointed by Wild (2005) refers, at least in part, to specific characteristics of each morph. Indeed, males produced by the colonies of the different morphs reared in our laboratory (during 4 years) always presented the same phenotype.

We observed that males from different morphs varied in body and wings colour (Table 3) as well as in petiole shape (Supporting information 2). Males from the group *'apicalis'* always have yellow antennae, even when they are black, and those from the group *'verenae'* are always black, as in workers (Table 3). As a result, males of different morphs present, in general, clearer morphological and phenotypic divergence than workers, and thus, constitute important information for the correct identification of this group of ants.

Table 3. Reproductive systems and male phenotypes in the *P. apicalis* species complex.

Taxon	Number of Queens	Male Phenotype (Body colour)	Male Phenotype (Wings colour)	Number of Colonies
PAP Morph 1	1	black	brown, opaque	1
PAP Morph 2	1	dark brown-black	brown, transparent	1
PAP Morph 3	1	reddish brown	gray, transparent	1
PAP Morph 4	1-2	reddish	transparent	24
PAP Morph 5	?-2	red	transparent	2
PAP Morph 6	1-?	reddish	transparent	3
PVE Morph 1	1-16	bright black	brown, transparent	25
PVE Morph 2	1-18	bright black	brown, transparent	9

? No queen found (incomplete colonies collected).

PAP: *P. apicalis*, PVE: *P. verenae*

Finally, during this work we were also interested in how all these largely sympatric and closely related morphs and species are able to coexist. We tried to obtain further information about nest preferences in this complex, especially for the new morphs described by Ferreira et al. (*in press*). Our data confirmed most of the nest preferences described by Delabie et al. (2008), for example for *P. apicalis* Morphs 3, 4 and *P. verenae* Morphs 1 and 2 (Table 4). Concerning *P. apicalis* Morphs 1 and 2, our small samples do not allow further conclusions. However, Delabie et al. (2008) described a considerable differentiation for *P. apicalis* Morph 1 nesting sites. From the 15 colonies of this morph they studied in Ilhéus, Brazil, 10 were found in the suspended soil at the base of the roots of *Aechmea lingulata*, a giant bromeliad widespread in the region, at 5 to 10 meters above the ground. Surprisingly,

the large *P. apicalis* Morph 6, from northern Brazil (Belém) showed also an arboreal nest preference. However, in this latter, nests were not associated to epiphytes but completely inside the hollow trunks of living trees, with the nest entrance at 8 to 10 meters from the ground. Noteworthy is the fact that in both morphs, the solitary foraging strategy in the litter on the ground (Fresneau 1985) was preserved.

Table 4. Nesting preferences in the different morphs of the *P. apicalis* species complex.

Taxon	Nesting sites	Number of colonies
PAP Morph 1	Rotting wood on the ground.	1
PAP Morph 2	Rotting liana and wood on the ground.	2
PAP Morph 3	Rotting wood on the ground.	1
PAP Morph 4	Mostly in large rotting trunks near the ground (mean circumference: 77 cm, mean distance from the ground: 23 cm), but also on rooting wood on the ground. Rarely in the ground.	24
PAP Morph 5	Large rotting trunks (mean circumference: 67 cm) on or near the ground.	2
PAP Morph 6	Large rotting trunk near the ground (circumference: 154 cm) and inside the hollow trunk of living trees at ± 8-10 m high.	3
PVE Morph 1	Mostly in small rotting trunks, bamboo, lianas (mean circumference: 23 cm) or tough fruits on the ground. Rarely in the ground.	25
PVE Morph 2	Always in the ground, occupying abandoned nests of other insect species, in open habitats.	9

In sum, this study resulted in important discoveries about cryptic speciation in the complex *P. apicalis*. The biological, ecological, and chemical differentiation observed among these cryptic taxa represents another important step in the understanding of the biodiversity in this complex. It reinforces our previous findings (i.e. Ferreira et al. *in press*) and also provides new pieces of evidence towards the resolution of the taxonomic puzzle of the other morphs as yet not studied here.

Supporting information

S1. M**orphs of the *Pachycondyla apicalis* species complex not considered in Ferreira et al (*in press*).** Lateral view (A), Full-face view (B) and petiole, oblique lateral view (C).

Figure S1.1. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 1. Ilhéus, Brazil.

Figure S1.2. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 2. Una, Brazil.

Figure S1.3. Worker specimen of *Pachycondyla obscuricornis* (POB). Belém, Brazil.

Figure S1.4. Worker specimen of *Pachycondyla verenae* (PVE) Morph 2.Viçosa,Brazil.

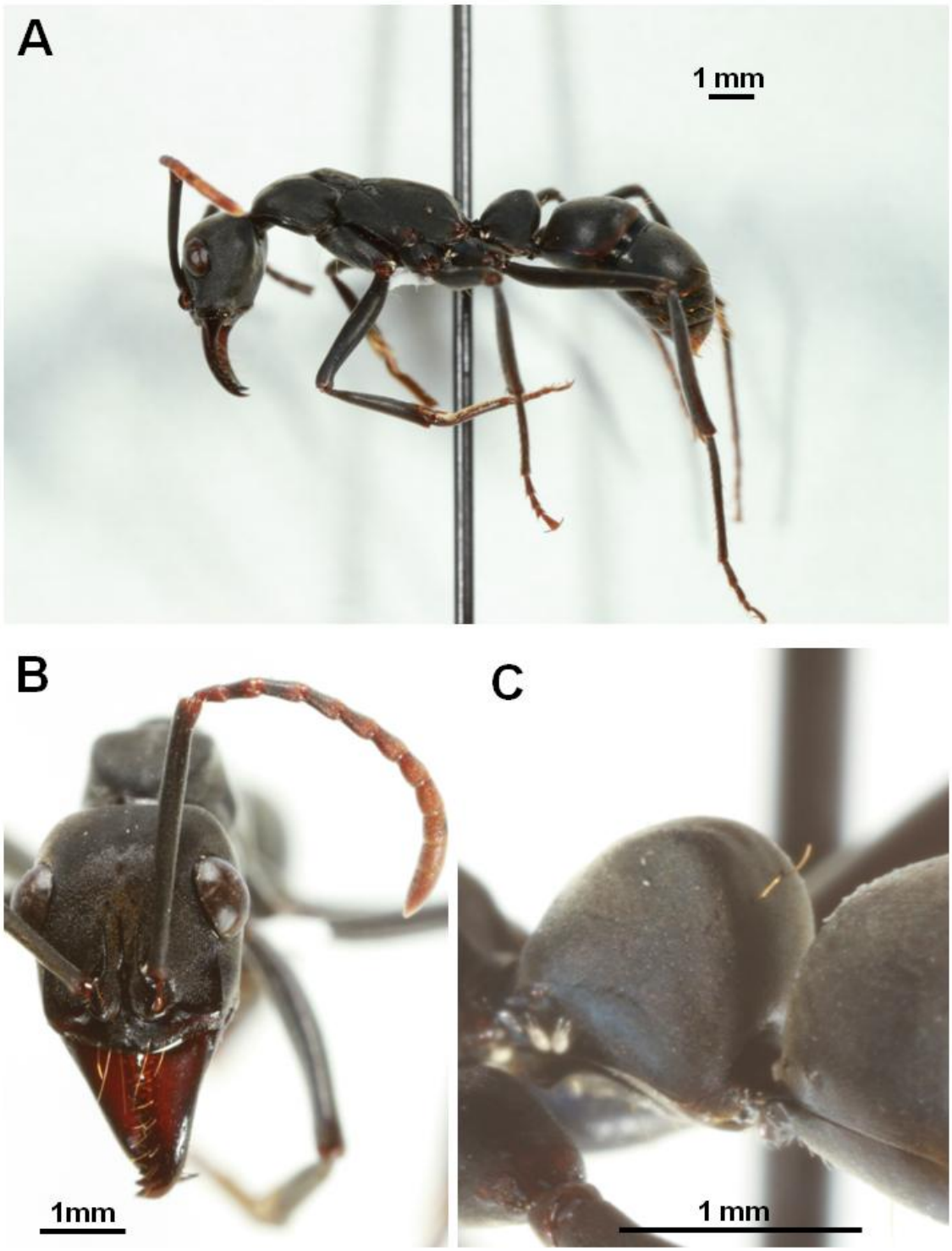

Figure S1.1. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 1. Ilhéus, Brazil.

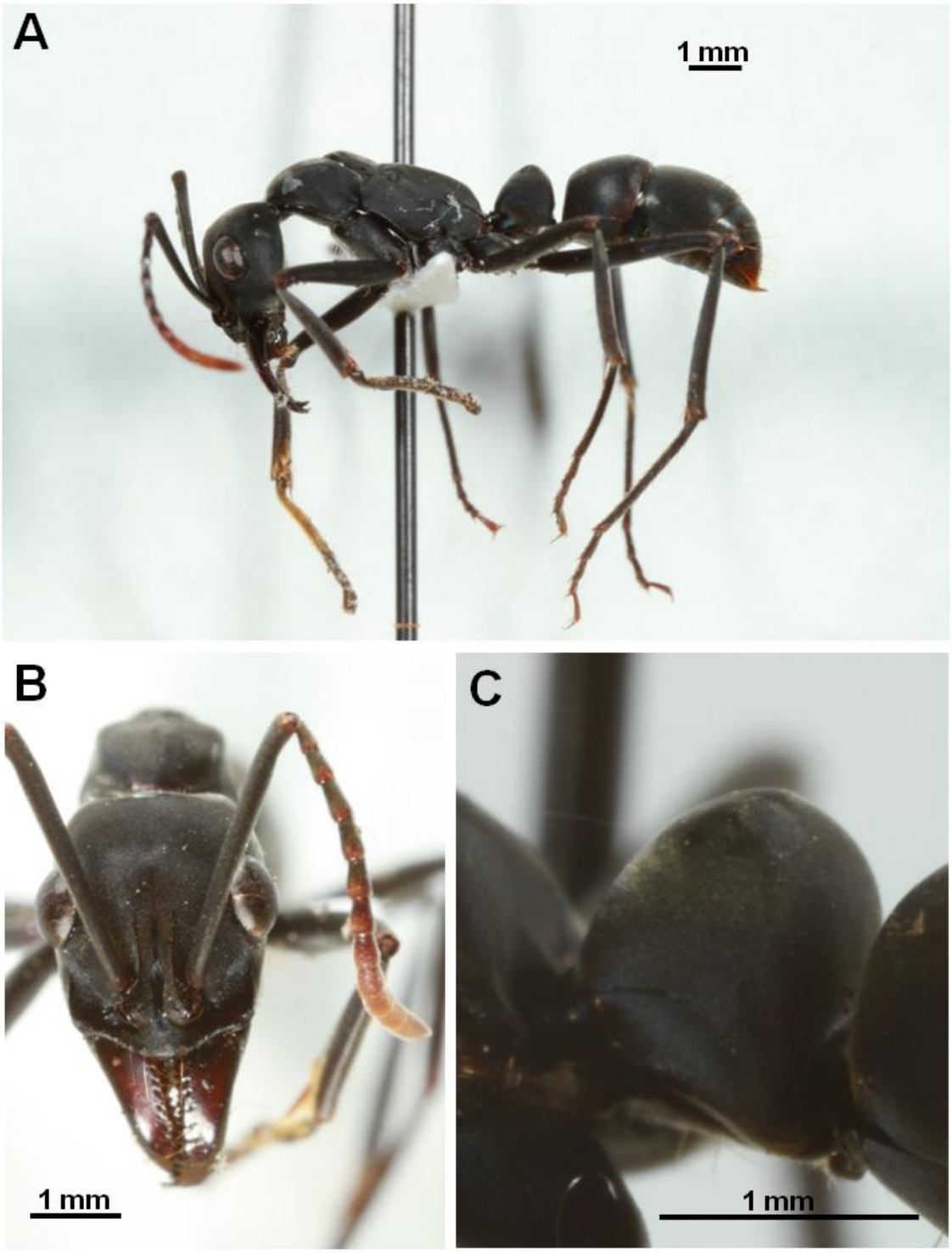

Figure S1.2. Worker specimen of *Pachycondyla apicalis* (PAP) Morph 2. Una, Brazil.

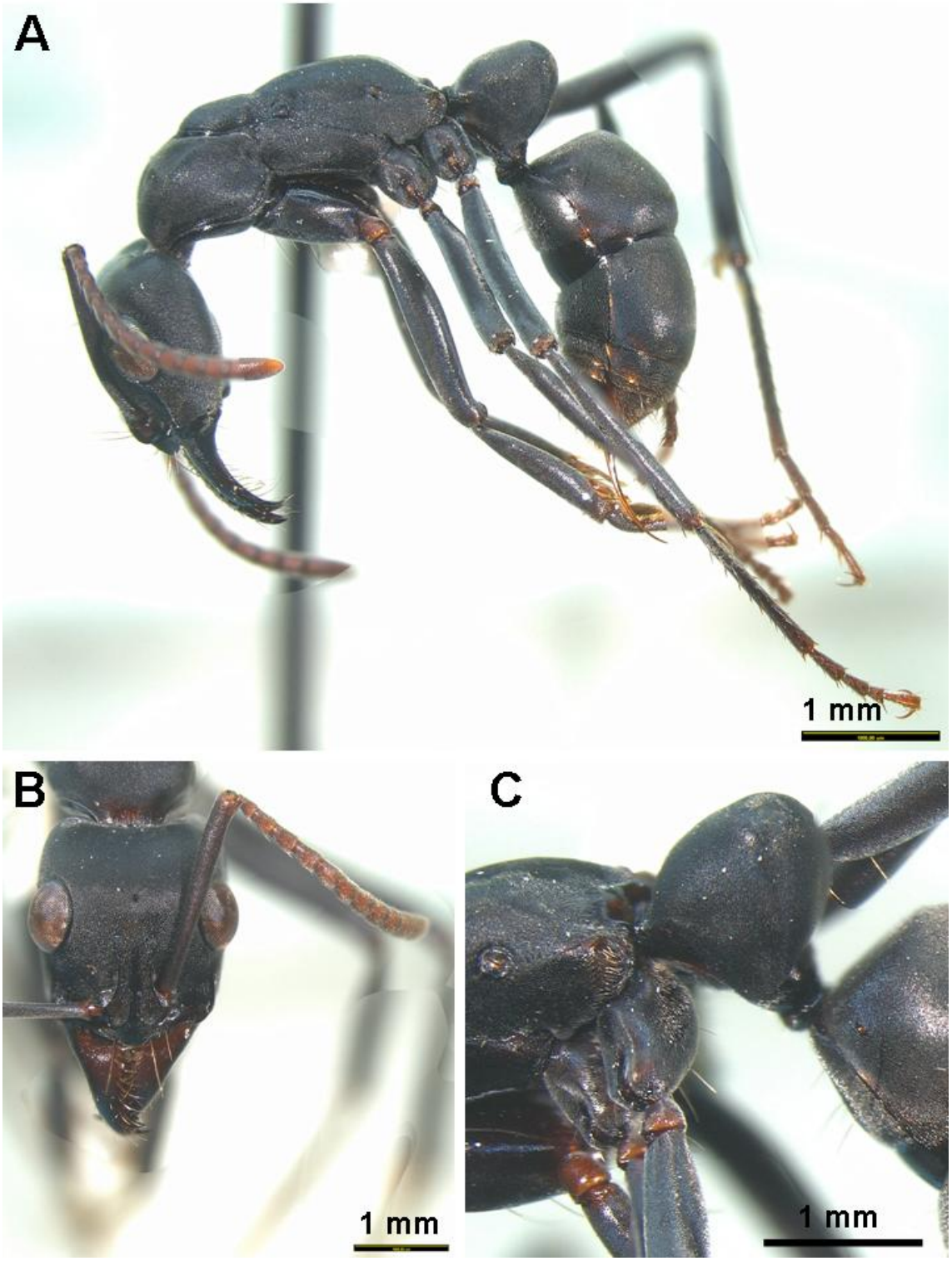

Figure S1.3. Worker specimen of *Pachycondyla obscuricornis* (POB). Belém, Brazil.

Figure S1.4. Worker specimen of *Pachycondyla verenae* (PVE) Morph 2. Viçosa, Brazil.

S2. Males of the *Pachycondyla apicalis* species complex. Male lateral view (A), full-face view (B), dorsal view (C) and petiole, lateral view (D).

Figure S2.1. Male specimen of *Pachycondyla apicalis* (PAP) Morph 1. Ilhéus, Brazil.

Figure S2.2. Male specimen of *Pachycondyla apicalis* (PAP) Morph 2. Petit Saut, French Guiana.

Figure S2.3. Male specimen of *Pachycondyla apicalis* (PAP) Morph 3. Los Tuxlas, Mexico.

Figure S2.4. Male specimen of *Pachycondyla apicalis* (PAP) Morph 4. Petit Saut, French Guiana.

Figure S2.5. Male specimen of *Pachycondyla apicalis* (PAP) Morph 5. Petit Saut, French Guiana.

Figure S2.6. Male specimen of *Pachycondyla apicalis* (PAP) Morph 6. Petit Saut, French Guiana.

Figure S2.7. Male specimen of *Pachycondyla verenae* (PVE) Morph 1. Camp Patawa, French Guiana.

Figure S2.8. Male specimen of *Pachycondyla verenae* (PVE) Morph 2. Viçosa, Brazil.

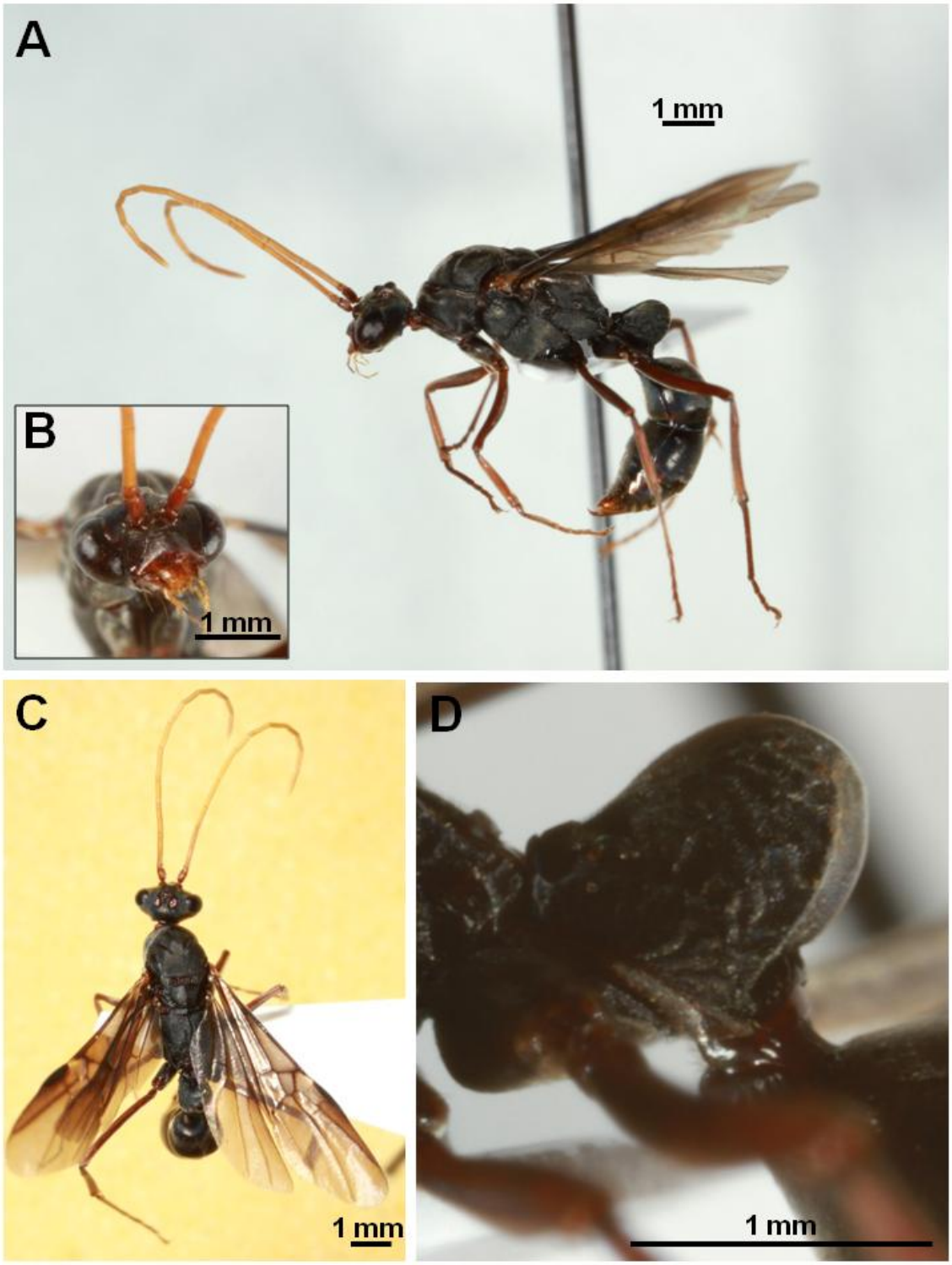

Figure S2.1. Male specimen of *Pachycondyla apicalis* (PAP) Morph 1. Ilhéus, Brazil.

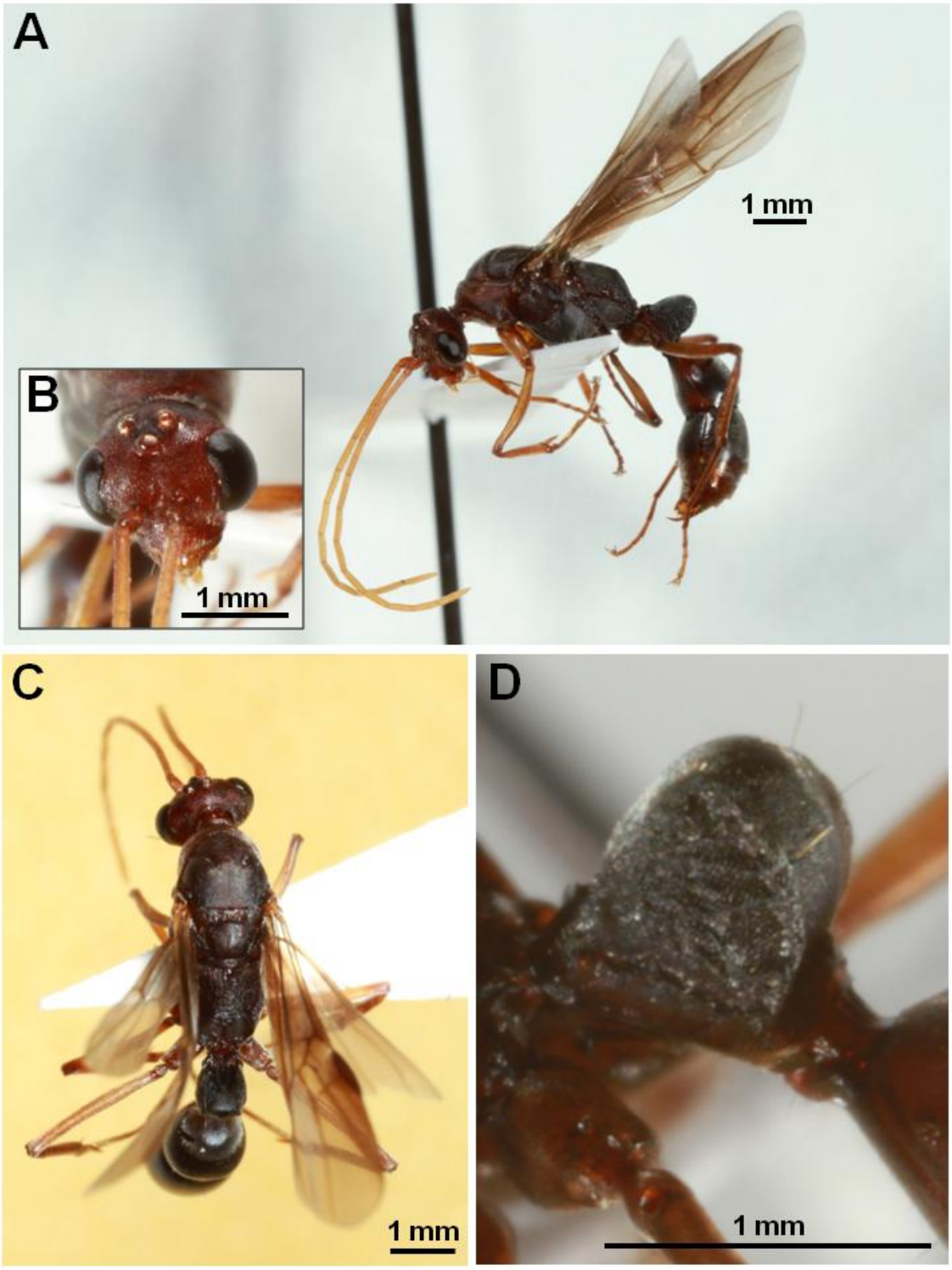

Figure S2.2. Male specimen of *Pachycondyla apicalis* (PAP) Morph 2. Petit Saut, French Guiana.

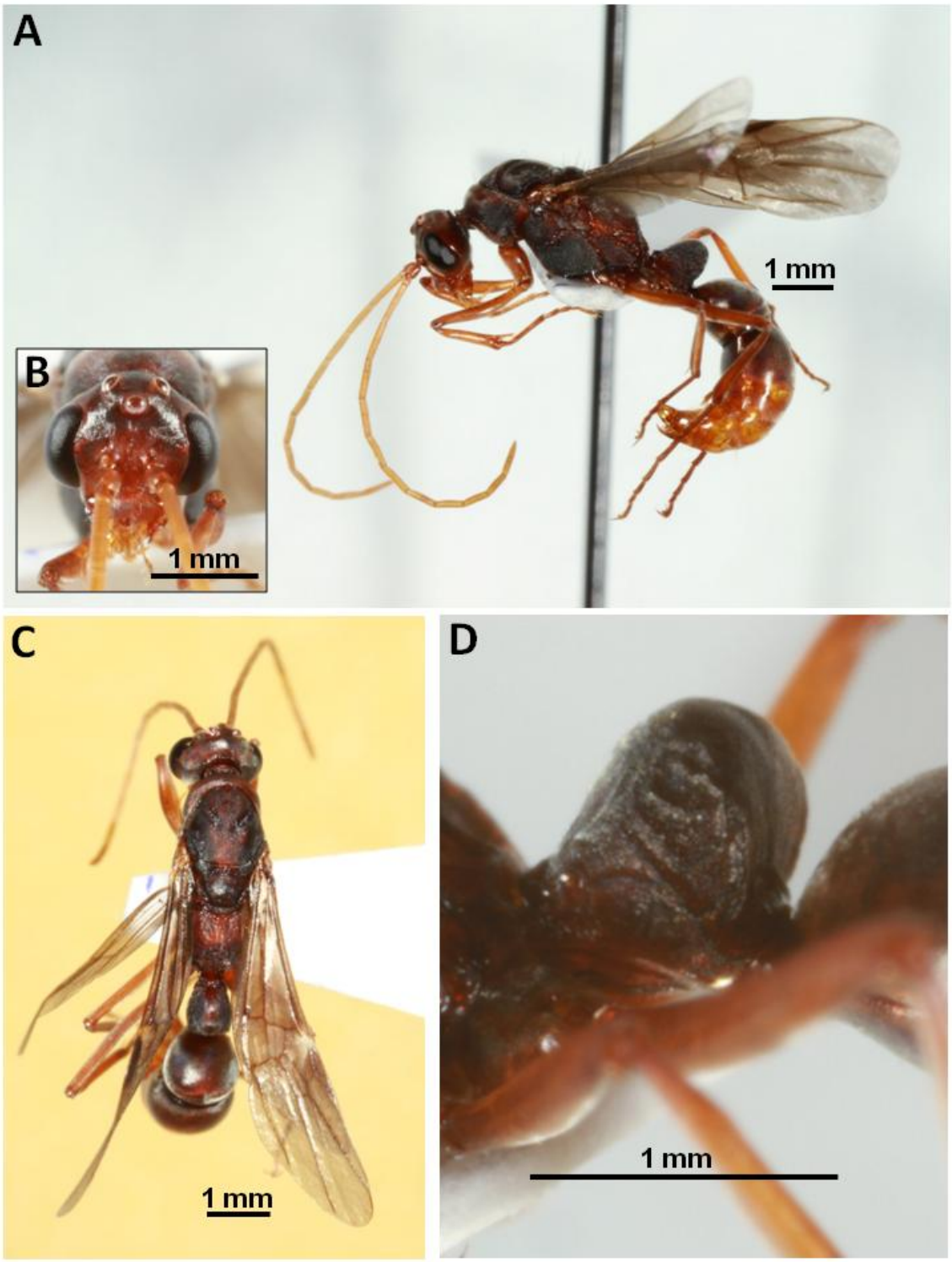

Figure S2.3. Male specimen of *Pachycondyla apicalis* (PAP) Morph 3. Los Tuxlas, Mexico.

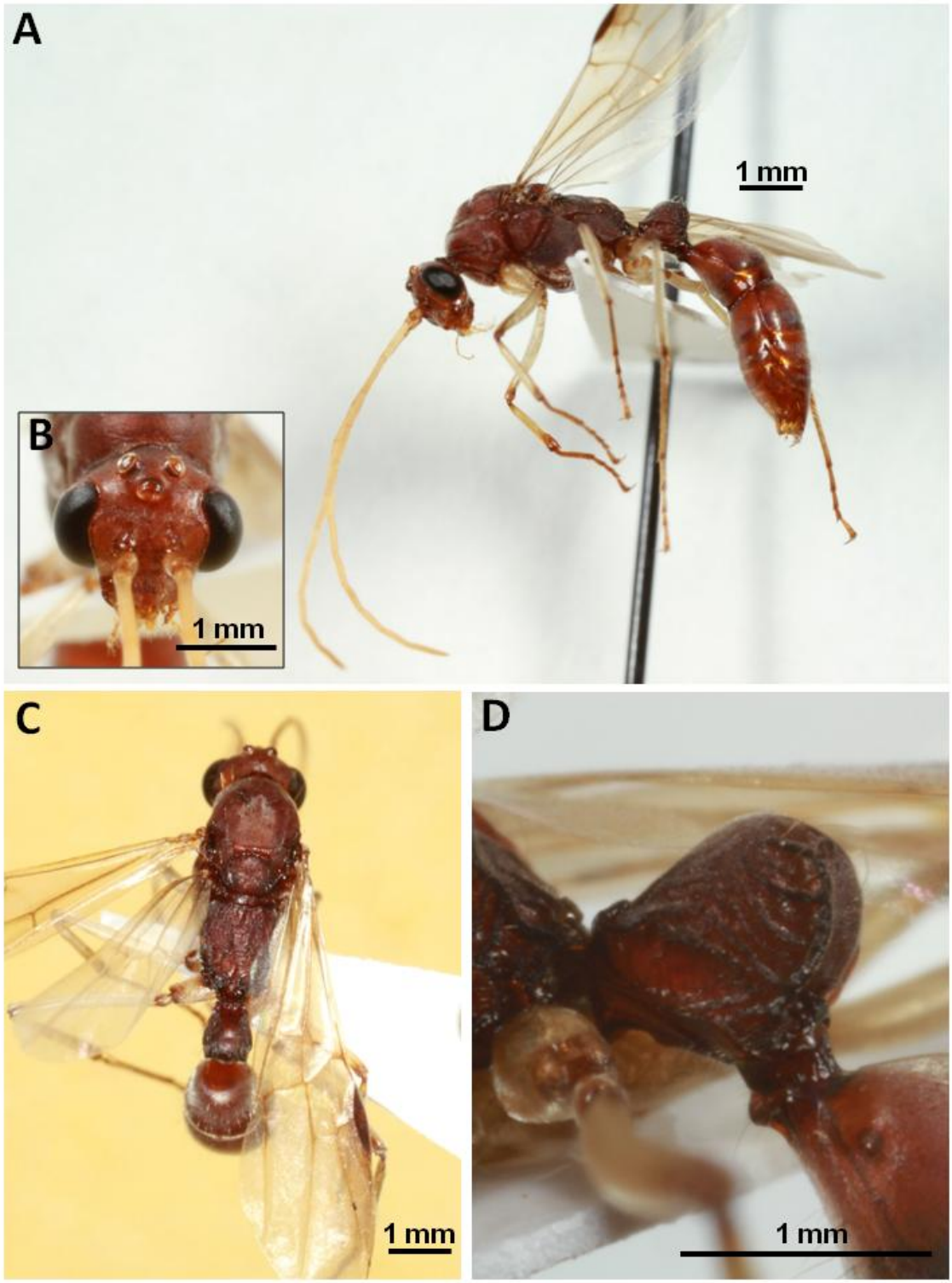

Figure S2.4. Male specimen of *Pachycondyla apicalis* (PAP) Morph 4. Petit Saut, French Guiana.

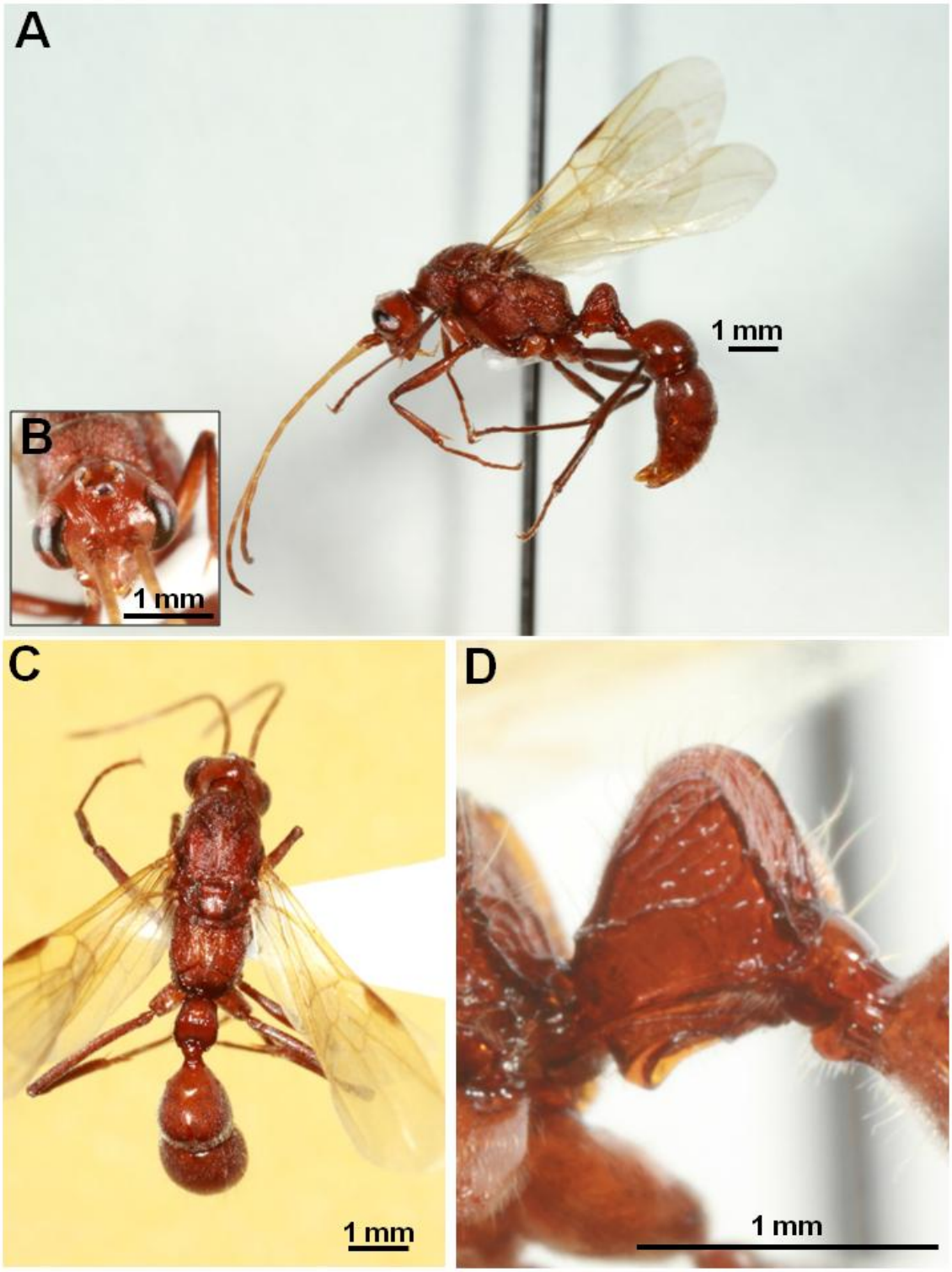

Figure S2.5. Male specimen of *Pachycondyla apicalis* (PAP) Morph 5. Petit Saut, French Guiana.

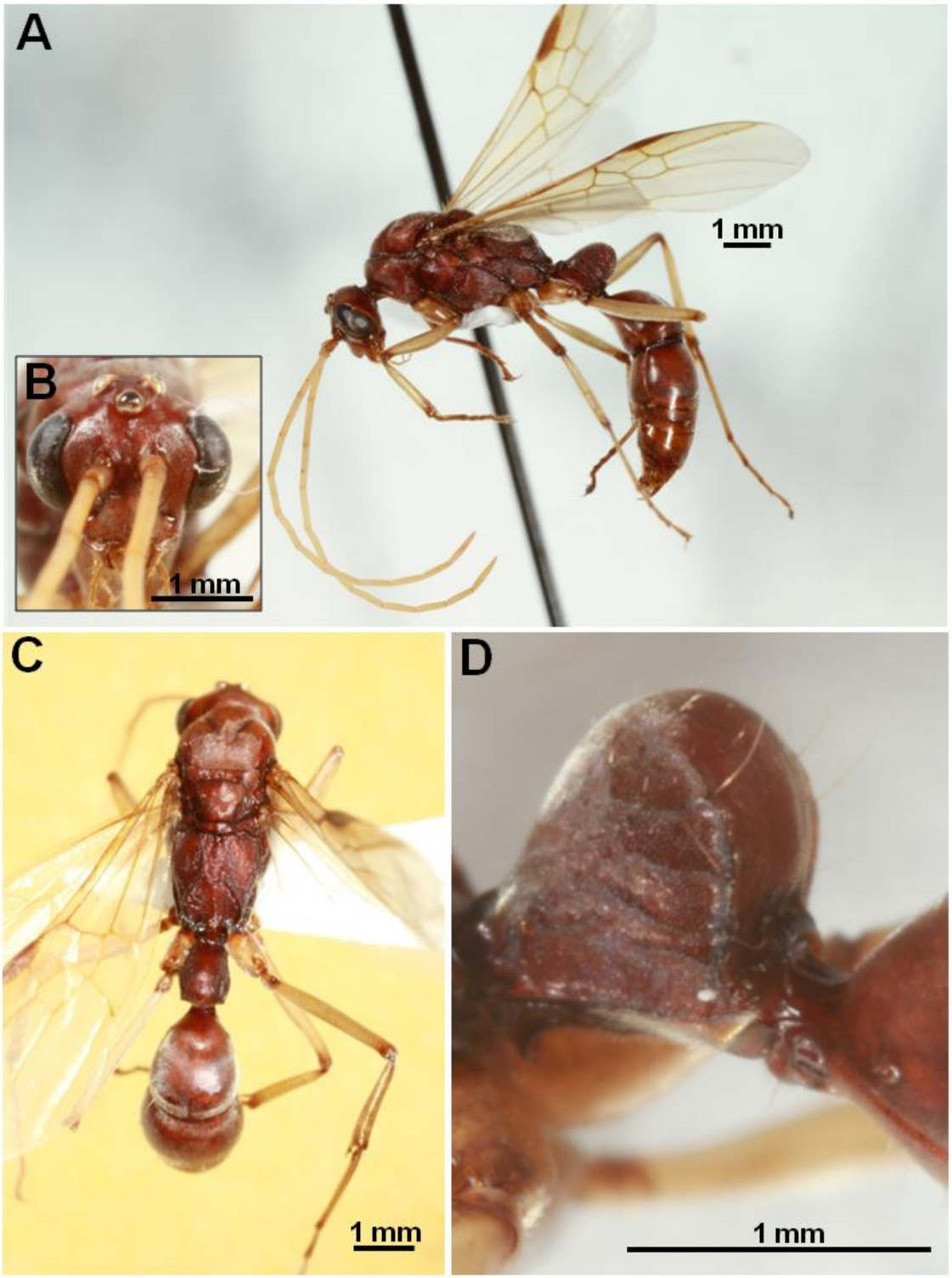

Figure S2.6. Male specimen of *Pachycondyla apicalis* (PAP) Morph 6. Petit Saut, French Guiana.

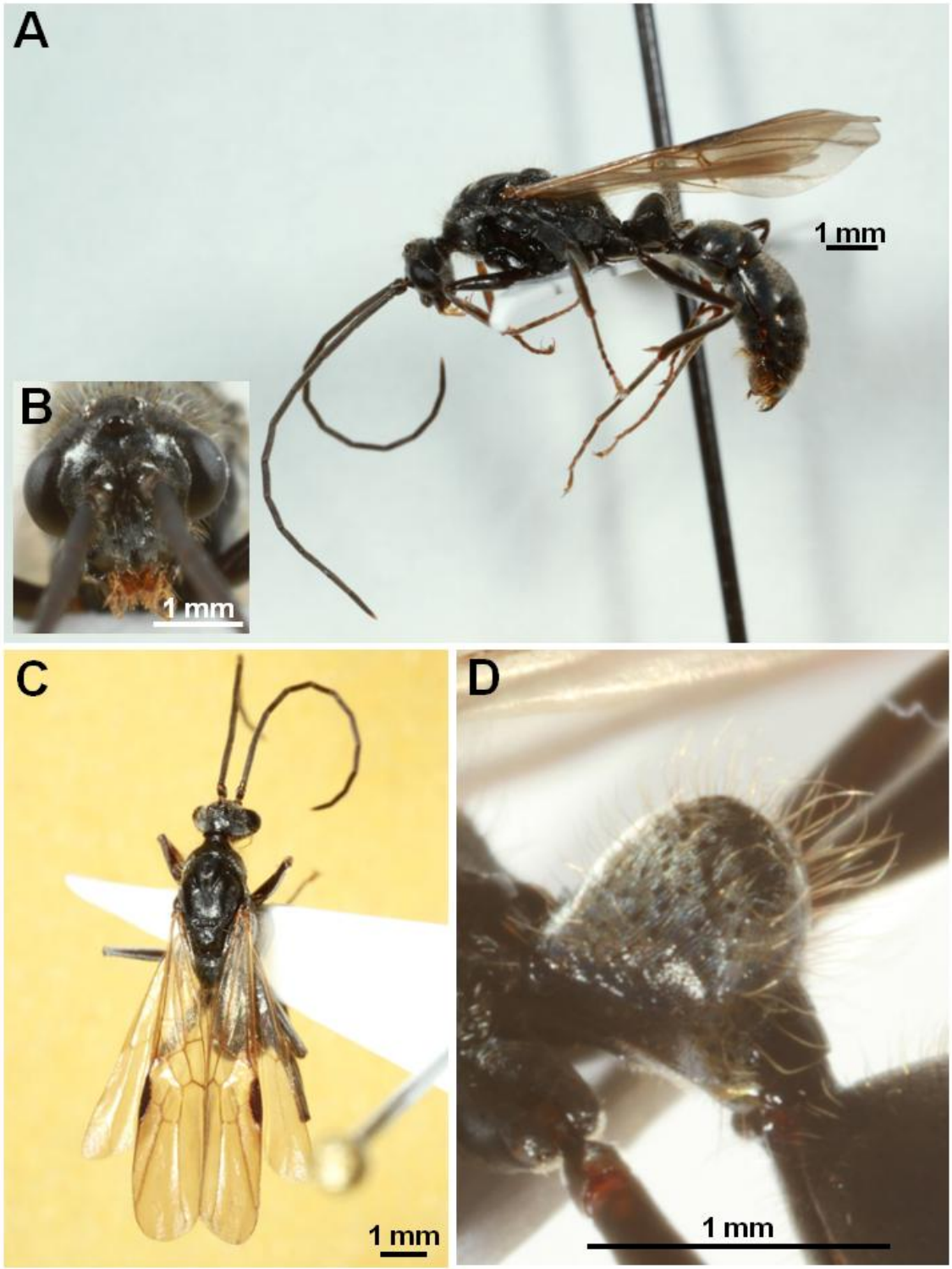

Figure S2.7. Male specimen of *Pachycondyla verenae* (PVE) Morph 1. Camp Patawa, French Guiana.

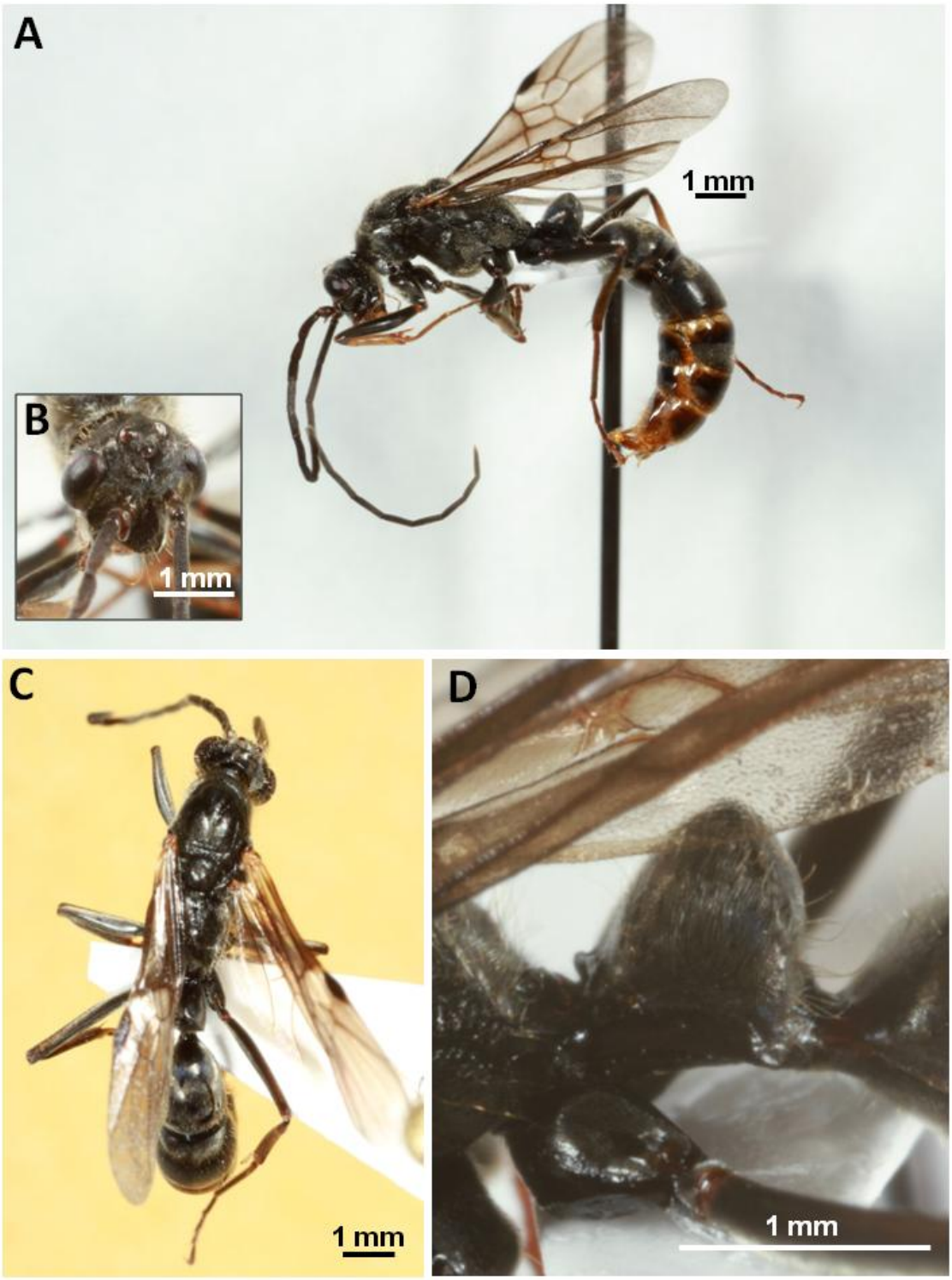

Figure S2.8. Male specimen of *Pachycondyla verenae* (PVE) Morph 2. Viçosa, Brazil.

CHAPITRE 4:

Interactions intra-spécifiques chez la fourmi primitive *Pachycondyla verenae* (Ponerinae)

Résumé

De nombreuses espèces de fourmis ont des niveaux d'agressivité variables envers les colonies étrangères. Ceci suggère une capacité des fourmis à discriminer différents types de voisins. De plus, les mécanismes de reconnaissance des individus hétérocoloniaux peuvent varier grandement suivant l'organisation sociale et les caractéristiques écologiques des espèces. Chez la fourmi ponérine *Pachycondyla verenae*, les ouvrières s'approvisionnent seules à des distances du nid pouvant aller jusqu'à 20m. A cette distance, les aires de fourragement des différents nids peuvent se chevaucher et en conséquence les fourmis hétérocoloniales se rencontrer. Chez cette espèce, on observe un comportement territorial, même si seul le pourtour proche du nid est défendu ardemment.

Dans cette étude, le niveau d'agressivité intraspécifique est étudié pour une population dense où les nids pouvaient être distants de moins de 3m. Nous avons testé l'hypothèse que la proximité topographique des colonies influe sur l'expression des comportements agressifs corrélativement aux distances chimiques et génétiques. Des rencontres dyadiques ont été mises en place entre des ouvrières provenant de nids distribués à des gammes de distances différentes qui correspondaient à des colonies voisines et sympatriques, des colonies sympatriques non voisines et des colonies allopatriques. La variabilité dans la composition en hydrocarbures cuticulaires ainsi que la structure génétique des colonies utilisées dans les tests comportementaux ont été décrites en utilisant une analyse CG-SM MEPS et des marqueurs microsatellites développés spécifiquement pour cette espèce.

Les distances chimique et génétique n'expliquent pas à elle seules la complexité des différentes réponses observées, ce qui suggère l'importance d'autres facteurs comme l'apprentissage. La fonction potentielle de l'existence de tels comportements diversifiés envers les individus hétérocoloniaux est discutée.

Article 6: Isolation and characterisation of eight microsatellite loci in the Ponerine ant *Pachycondyla verenae* (Hymenoptera: Formicidae)

EVISON, S. E. F.; FERREIRA, R. S.; FRESNEAU, D.; POTEAUX, C.

Molecular Ecology Resources, Sous presse

Isolation and characterisation of eight microsatellite loci in the Ponerine ant *Pachycondyla verenae* (Hymenoptera, Formicidae)

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Keywords: Hymenoptera, microsatellites, *Pachycondyla*, Ponerinae

Running title: Eight new microsatellite loci for *P. verenae*

Abstract

We characterized eight polymorphic DNA microsatellite loci for the neotropical ponerine ant *Pachycondyla verenae*. The variability was tested in 48 workers from six colonies from a Brazilian population with varying levels of polygyny. Polymorphism ranged from 2 to 11 alleles per locus (mean = 5.5) and observed and expected heterozygosities ranged from 0.146 to 0.958 (mean = 0.529), and from 0.172 to 0.778 (mean = 0.497), respectively. The allele size ranged from 90 to 330 bp. These markers will promote studies of population and colony structure in this facultatively polygynous ant.

Although social groups are characterized by cooperation, they are also often the scene of conflict. If social insect colonies are polygynous (multiple queens) or polyandrous (multiple mating by queens), their genetic composition will be heterogeneous. Asymmetrical relatedness among nestmates leads to selfish behaviour and to conflicts over partitioning of reproduction and the production of males (Trivers & Hare 1976; Ratnieks & Reeve 1992). In order to understand where these conflicts arise, it is necessary to determine effective levels of polygyny, polyandry, and nestmate relatedness through genetic analyses.

The neotropical Ponerine ant *Pachycondyla verenae* exhibits facultative polygyny. Moreover, we have collected mature nests exhibiting a high level of polygyny for a species of this genus (up to 18 queens per nest), possibly favoured by ecological constraints. High numbers of queens per colony may reflect primary polygyny, resulting from colony foundations established by unrelated queens, who stay together throughout the lifetime of the colony. On the other hand, the occurrence of several queens may reflect secondary polygyny through readoption of daughter queens. Preliminary results from behavioural observations showed that reproduction seemed not to be equally shared among queens (Evison et al. in prep). In order to investigate the genetic structure of these nests we characterised eight new polymorphic microsatellite loci. Entire nests of *P. verenae* were collected from Viçosa in southern Brazil and kept under standard conditions in the laboratory in France.

A microsatellite enriched library of *P. verenae* was built using biotin-labelled microsatellite oligoprobes (TG and TC) and streptavidin-coated magnetic beads following modifications as in Giraud et al. (2002). A total of 1295 clones were screened: 510 gave a positive response and 180 of them were of interesting size (300-600 bp). From the 96 sequenced clones, Polymerase Chain Reaction (PCR) primers were designed for 20 microsatellite loci (mainly 11 to 30 dinucleotide repeats), using the web-based computer program Primer3 (http://frodo.wi.mit.edu/primer3) and subsequently tested for polymorphism.

PCR conditions were first determined on a set of seven workers from three different colonies, then microsatellite loci polymorphism was investigated in 48 workers from six different colonies. DNA was extracted from the head and thorax in 500µL 10% Chelex® solution and 20µL proteinase K (10mg/ml), and diluted 5-fold. Each locus was screened for variation in the sample of *P. verenae* workers. Out of the 20 primers tested, eight showed interesting variation and were selected for expanded analysis. For primers 1015, 1048, 1078, 2056, 2096, and 4049 the PCR was carried out in a final volume of 10µL containing 1 µl of diluted DNA (about 50 ng/µl), 0.1µL *Taq* polymerase (5U/µL; Promega®), a final

concentration of 1μM each of forward and reverse primer, 2μl of 5X polymerase buffer, 1.5mM $MgCl_2$ and 5mM per dNTP (Promega®). For primers 2111 and 4053 the forward and reverse primer concentration was 0.5μM. Primers were labelled with NED, VIC or FAM at the 5'-end (Table 1).

The DNA was amplified in a Biometra T1 thermocycler using an initial denaturation step of 94 ºC for 4 min followed by 35 cycles of denaturation at 94 ºC for 30 s, the locus-specific annealing temperature (Table 1) for 30 s and extension at 72 ºC for 30 s, followed by a final extension at 72 ºC for 10 min. For primer 2056, a touchdown program was used. This had an initial denaturation step of 94 ºC for 3 min followed by 2 cycles of denaturation at 94 ºC for 30 s, annealing at 62 ºC for 30 s and extension at 72 ºC for 30 s, following this were 9 cycles of 94 ºC for 15 s, 62 ºC for 15 s, and 72 ºC for 15 s, with the annealing temperature reducing by 0.5 ºC each cycle till it reached 58 ºC. This final cycle was repeated 21 times followed by a final extension at 72 ºC for 3 min. The labelled products were mixed with internal size marker GeneScan™ Liz500 (Applied Biosystems, Foster City, CA, USA) and run on an automated ABi 3100 Sequencer (Applied Biosystems). Fragment length was scored using the freeware application Peak Scanner™ v1.0 (Applied Biosystems).

All eight loci showed polymorphism and alleles could be scored easily. The number of alleles per locus ranged from 2 to 11 with a mean of 5.5 alleles per locus (Table 1). The observed and expected heterozygosities were calculated by Fstat 2.9.3 (Goudet 1995; 2001) and ranged from 0.146 to 0.958 (mean = 0.529), and from 0.172 to 0.778 (mean = 0.497), respectively (Table 1). Tests for deviation from Hardy-Weinberg proportions, gametic disequilibrium and the presence of null alleles were calculated in Genepop (Raymond & Rousset 2004; Rousset 2008). Some significant deviations from Hardy-Weinberg proportions (loci 1048, 1078, 2056 and 4049), gametic disequilibrium (all loci), and null alleles (all loci) were detected in one of the colonies tested, however, this colony has an unusual structure due to extremely high queen number (up to 18 queens) and will require higher sample sizes to fully analyse the colony genetic structure.

Acknowledgements

We thank P. Devienne for ant collection laboratory care, M. Solignac and P. Capy for providing the laboratory for carrying out primer design, and D. Vautrin and C. Capdevielle-Dulac for technical assistance. This work was supported by Foundation Fyssen and the Laboratoire d'Ethologie Expérimentale et Comparée.

Table 1. Characteristics of microsatellite loci in *Pachycondyla verenae*. T_a, optimized annealing temperature; n, number of individuals genotyped; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

Locus	Primer sequence (5'-3')	Label	Repeat motif	T_a (ºC)	Allele size range (bp)	n	N_A	H_O	H_E	GenBank Accession no.
Pv1015	F: ATGACGGCGGAGAATACATC	NED	(GA)23	60	185-202	48	3	0.5417	0.5063	HQ263030
	R: GTGACGCGAAGTTTACGTTG									
Pv1048	F: GCGAGAGAAAGATGAGCATA	NED	(CA)9 (GC)4 (CA)11	56	130-183	48	10	0.9583	0.7778	HQ263031
	R: GCCATTGCCTGTATAAAAGA									
Pv1078	F: TGTTTGTTGCCTCTCGAAAA	FAM	(GT)19	60	201-216	48	3	0.5833	0.436	HQ263032
	R: AGTCGCAACGAGGAATATGG									
Pv2056	F: GTAACGCTGACATCCATCAC	FAM	(GA)26	62-58	116-184	48	11	0.6875	0.7151	HQ263033
	R: GCGCGTAAAAGAAAAGCTAA									
Pv2096	F: GGCGTATTTCCTCAAAGGTT	NED	(CT)18	58	317-330	48	2	0.1458	0.2029	HQ263034
	R: AAGACTACGCACAGCAGGAG									
Pv2111	F: GCGGAGACAGAGGTGCTATT	VIC	(GT)15	60	175-197	48	3	0.4167	0.421	HQ263035
	R: AAGGAATCTCGGCCTGCTA									
Pv4049	F: AATCAAGCTCGACGCTCTTT	FAM	(CT)15	58	100-145	48	9	0.7083	0.7411	HQ263036
	R: CAGGCGTATCCTCTTTACCG									
Pv4053	F: CGAAAGTGGTGTAGGAGGAA	VIC	(GA)11	58	90-100	48	3	0.1875	0.1717	HQ263037
	R: GCGACGTAGCAGACTATACGA									

Article 7: Non-nestmate recognition leads to differentiated colony-specific behaviour in the facultative polygynous ant *Pachycondyla verenae* (Hymenoptera: Ponerinae)

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En préparation

Non-nestmate recognition leads to differentiated colony-specific behaviour in the facultative polygynous ant *Pachycondyla verenae* (Hymenoptera: Ponerinae)

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Abstract

The fact that many ant species vary their level of aggressiveness towards foreign colonies suggests an ability of ants to discriminate between different types of neighbors. Moreover, non-nestmate recognition mechanisms can greatly vary according to the social system and ecological characteristics of each species. In the ponerine ant *Pachycondyla verenae* workers forage solitarily at distances of up to 20 meters. At these distances, foraging areas of distinct nests can overlap and allocolonial ants may often interact. This species presents territorial behaviour, but only the vicinity of the nest is highly defended. Here, we studied the intraspecific level of aggression in a dense population where nests could be found less than three meters apart. We investigated the effect of colony proximity on aggressive behaviour on a fine scale and whether and how chemical and genetic divergences among nests are correlated to it. Dyadic encounters were conducted between individuals from colonies distributed over different distance ranges, which corresponded to sympatric neighbours, sympatric non-neighbours, and allopatric colonies. The variability in the composition of the cuticular hydrocarbons as well as the population structure of the colonies used in the behavioural tests was investigated thanks to GC-MS SPME analysis and species-specific microsatellites markers. Genetic or chemical distances were not sufficient to explain the full complexity of the responses, suggesting the importance of other factors such as learning. We discuss the potential function for the establishment of such different non-nestmate directed behaviour in *P. verenae*.

Key-words: Discrimination, Formicidae, *Pachycondyla verenae*, ponerine, cuticular hydrocarbons, nestmate recognition, relatenedness, dear enemy phenomenon, learning, transport.

Introduction

The ability to recognise self from non-self extends, in almost all species, to recognition of species, kin, sex, and colony member. Recognition systems in social insects have been extensively studied and are typically based on chemical cues (reviewed by d'Ettorre & Lenoir 2010, Howard & Blomquist 2005). Social insects are characterized by a colonial closure that relies on their ability to discriminate between conspecific nestmates and alien non-nestmates. This mechanism of nestmate recognition acts to guarantee that resources are directed to colony-members only, and not to intruders. It has been shown repeatedly that cuticular hydrocarbons are the main cues used in social insect nestmate recognition (Akino et al. 2004, Bonavita-Cougourdan et al. 1987, Châline et al. 2005, Dani et al. 2005; Guerrieri et al. 2009, Lahav et al. 1999, Martin et al. 2008). They also play a role in processes of within-colony recognition, such as of queen fertility (d'Ettorre et al. 2004, Dietemann et al. 2003, Greene & Gordon 2003, Smith et al. 2009, Holman et al. 2010). The individual cuticular hydrocarbon (CHC) profiles of ants have a genetic basis (Lockey 1991, Nehring et al. 2010) and CHC profiles have been used as indicators of genetic isolation or distance between populations (for example Akino et al. 2002, Martin et al. 2008, Rouault et al. 2001, Symonds & Elgar 2004).

In ants, recognition mechanisms involve the matching of perceived chemical cues to an internal template of colony odour, which is usually acquired during early adult life (Errard et al. 2006, Lenoir et al. 1999). The ant's behavioural response depends on the result of this analysis and its tolerance threshold: below this level, the response tends to be affiliative behaviours, and above the threshold, responses tend to be agonistic behaviours (Liebert & Starks 2004, Reeve 1989). In a more precise way, inter-colonial relationships can also be ruled by the "dear-enemy effect", which consists of a differential treatment of familiar non-nestmates (neighbours) compared to non-familiar non-nestmates (non-neighbours, Langen et al. 2000, Dimarco et al. 2010, Tanner & Adler 2009). Such discrimination can arise either from differential olfactory distance between close neighbours and distant strangers, or from a true learning process of neighbours' colonial label (Temeles 1994, Knaden & Wehner 2003, Sanada-Morimura et al. 2003). Therefore, according to the species life-history traits, ecologies and to the structure of the community, non-nestmate discrimination can greatly vary in its expression (Dimarco et al. 2010, Muller & Manser 2007, Newey et al. 2010, Temeles 1994).

Here we studied the Neo-tropical ponerine ant *Pachycondyla verenae*, a primitive species that is facultatively polygynous. Colonies of this species are small, with about 40 (Gobin et al. 2003) to 200 workers (Fresneau 1994). In addition, this species presents genetic sympatric morphs (Delabie et al. 2008) and belongs to a complex of closely related sympatric species (the *Pachycondyla apicalis* species complex, Ferreira et al. in press), and is a good candidate to test the links between behaviour, experience, chemical and genetic distance. Workers forage solitarily at mean distances of 10 to 15 metres, but sometimes up to 20 metres from the nest. At these distances, foraging areas of distinct nests can overlap and allocolonial ants may often interact. It is thought that this species presents the same territorial behaviour as that observed in *P. apicalis*, in which only the vicinity of the nest is highly defended (Fresneau 1984). During fieldwork we observed a novel behaviour, in which an ant clearly transported an allocolonial worker for several metres before 'throwing it away' in the opposite direction of the carrier ant's nest. All these idiosyncrasies led us to ask the following two questions:

Q1: Does the distance between nests of origin influence behavioural responses towards non-nestmates in a population with high nest density?

Q2: Can chemical and genetic divergence alone explain the observed behaviour?

Materials and Methods

Study species

P. verenae is a common Neotropical species with huge flexibility in its habitat, which ranges from rainforests to natural open habitats such as "campo cerrado " or even anthropized fields (Delabie *et al.* 2008; Wild 2005), as in the present study. The particular population studied here belongs to Morph 2 described by Delabie et al. (2008) which has many distinctive features, the most significant here perhaps being the habit of nesting in underground hollows rather than in rotten branches, as Morph 1 does.

We collected eight *P. verenae* colonies from a dense population where nests could be found less than 3 metres apart in the fruit growing experimental fields of the Universidade Federal de Viçosa – UFV, Minas Gerais, southestern Brazil (S 20°45'22,4" W 42°52'01,5"). Four other colonies, used as allopatric controls in our experiments were collected in northern Brazil, at the Centro de Pesquisas do Cacau, CEPLAC - ERJOH, Belém, Pará (S 01°22'43,7" W 48°17'24,5").

Ant colonies were reared in the laboratory in a controlled environment (temperature 25±2°C; relative humidity 60±10%; light/dark cycle 12 h/12 h) and housed in artificial plaster nests (18x14 cm) connected to a foraging arena of the same size. Colonies were provided with an identical diet (honey/apple mixture and crickets) twice a week.

Effect of between-nest distance on behaviour

To study the influence of nest distance on the behaviour of *P. verenae* ants, we assigned the collected nests to seven distinct distance ranges prior to the behavioural experiments (Table 1).

Table 1. Name and definition of the different behavioural classes according to the distance between the nests of origin of the workers used in the dyadic encounters

NEST DISTANCE	BEHAVIOURAL CLASS
0 m	**Nestmates:** Ants from the same nest
<10 m	**Close Neighbours:** Nests that share the same foraging area and workers probably encounter each other frequently
11-20 m	**Distant Neighbours**: Nests that can still share the same foraging area, but workers probably encounter each other rarely, if ever
21-40 m **41-70 m** **71-90 m**	**Sympatric Non-Neighbours:** Nests in the same site but with non-overlapping foraging areas
> 2800 km	**Allopatric:** Nests from distinct distant populations

Discrimination and aggression tests

The bioassay consisted in dyadic encounters performed between pairs of ants from the seven nest distance ranges. Encounters lasted 3 min and were conducted in a neutral arena (an acrylic tube, 25 mm diameter and 100 mm height, with Fluon® coated sides to prevent escape, and a filter paper substrate. The filter paper was systematically changed between tests so that no odour contamination persisted). Before each encounter, the tested ants were allowed to become accustomed to the device without interaction by keeping them apart with a

microscope slide for 30 s in the apparatus. Tests began when the microscope slide was removed and the behaviour of ants towards each other was video-recorded. The number of replicate experiments was 10-20 per nest distance with a total of 130 encounters. Individuals were only tested once in a given encounter to avoid possible effects of familiarization.

For each encounter, we quantified the time ants spent performing five types of behaviour: *antennation* (antennal contact and inspection), *transport* (a stereotypical behaviour that includes manipulation with the mandibles and legs until the other ant assumes a nymphal posture to be transported), *threat* (indicated by mandibular opening), *biting,* and *stinging* (curling of the abdomen in stinging attempts). The analysis of the videos was performed in a random order and under 'blind' conditions for the analyst.

We considered the behavioural categories to represent an escalating level of aggressiveness, where the first level (*antennation*) was a neutral behaviour in which ants investigate and demonstrate maximal acceptance of each other. The second level (*transport*) is an intermediate behaviour in which ants are not aggressive but are not accepting each other either. This behaviour resembles typical transport behaviour during nest emigration (Möglich & Hölldobler 1974) and is initiated after much antennal and more notably front-leg boxing, another ritualised behaviour observed during territotial fights in other species (Ettershank & Ettershank 1982, Pfeiffer & Linsenmair 2001). Although this behaviour is non violent and more akin to a non violent ejection of an intruder, we gave it an intermediate score as it is not either an acceptance. In the other categories (*threat, biting* and *stinging*) ants perform overt aggressive behaviours. We calculated an aggression index (AI) by adapting the scores proposed by Hefetz et al. (1996) and Errard & Hefetz (1997) and used the following values for the respective behaviour: 0, *antennation*; 1, *transport*; 2, *threat*; 3, *biting* ; 4, *stinging*. The frequencies and duration of each of the behaviours were recorded, and the overall aggression exhibited during each encounter was calculated using the same formula as in Hefetz et al. (1996) and Errard & Hefetz (1997),

$$\frac{\sum_{i=1}^{n} AI_i * t_i}{T}$$

where AI_i represents the index of aggression, t_i, the duration of each act and T, the total interaction time defined as the sum of durations in which the ants were in physical contact.

Since the duration of antennations is commonly used to quantify the interest shown by an ant towards a social stimulus (Boulay et al 2000, Blacher et al. 2010), we considered it, together with the total time ants spent interacting, as indicative of ants' discrimination and recognition towards the intruder. Indeed perceiving and evaluating differences between an internal template and the opponent's signature depends on profile similarities and antennation levels thus illustrate this.

Genetic analysis and population structure

DNA was extracted from the head and thorax using a standard 10% Chelex protocol with a set of eight microsatellite loci specifically designed for this species (Evison et al.2010). PCR's were performed individually in 10 µl volumes containing 1 µl of DNA (about 50 ng/µl); 2 µl of 5X polymerase buffer; 1 µl of dNTP mix (5 mM); 0.5 µl of each primer (10 µM); 0.1 µl of Taq DNA polymerase (5 U/µl) (Promega); 0.6 µl of $MgCl_2$ (25 mM). For two of loci, the forward and reverse primer concentration was 0.25µl.

PCR products were mixed in two sets: mix1 (Pv1078, Pv2096, Pv4049 and Pv4053) and mix2 (Pv1015, Pv1048, Pv2056 and Pv2111) with 9µl of Highly deionized formamide (Hi-Di™ Formamide, Applied Biosystems). Fragment length was analyzed with the internal size marker GeneScan™ 500 LIZ™ (Applied Biosystems) by an automated Applied Biosystems Prism 3100 Sequencer (Applied Biosystems, Foster City, CA, USA) and scored using the freeware application Peak Scanner™ v1.0 (Applied Biosystems). We genotyped 16 and 24 workers in monogynous and polygynous colonies respectively from two sites: Viçosa (8 colonies, n = 157 individuals) and Belém (4 colonies, n = 74 individuals).

Linkage disequilibrium for all pairs of loci in all populations were calculated to assess independence of loci. The amount of genetic variation at each locus was quantified by calculating the number of alleles (N_A) and, because of difference in sample size between both populations, we also calculated the allelic richness (*R*S; El Mousadik & Petit, 1996) and the unbiased expected heterozygosities with FSTAT 2.9.3 (Goudet, 2001). The mating structure of the population was estimated as the inbreeding coefficient, *F* (Weir and Cockerham, 1984).

To assess the spatial population structure, we first estimated the genetic divergence between populations by estimating *F*ST between both populations (Weir & Cockerham 1984) using FSTAT 2.9.3 (Goudet, 2001). A pattern of isolation-by distance (IBD) between pairs of colonies of Viçosa population was assessed by plotting the genetic differentiation [$F_{ST}/(1 - F_{ST})$] coefficients estimated using Genepop on the web (Raymond and Rousset 1995; Rousset 2008) against the matrix of transformed geographical distances to estimate the Pearson

correlation coefficient (Rousset 1997). IBD was tested using a Mantel test with 10 000 permutations to estimate the level of significance of the obtained Spearman rank correlation coefficients using Genepop On The Web (Raymond and Rousset 1995; Rousset 2008).

Chemical distance

To study the influence of chemical distance on ant behaviour, the variability in the composition of the cuticular hydrocarbons between the colonies used for behavioural tests was investigated using Gas Chromatography and Mass Spectometry (GC-MS). Six to seven ants from each colony (82 individuals in total) were analysed. Cuticular hydrocarbon (CHC) samples were collected by Solid Phase Microextration (SPME) on live ants by rubbing the first and second segments of the ant gaster with a Supelco 7-um polydimethylsiloxane coated fibre for 3 min, which was then desorbed in the injection port of a gas-chromatograph (Agilent 7890A gas-chromatograph, equipped with a HP-5MS capillary column (30 m x 250 µm, 0.25 µm thickness), a split-splitless injector and coupled with a 5975 Agilent Mass Spectrometer with 70 eV electron impact ionization. The carrier gas was helium at 1 ml min^{-1}). After an initial hold of 1 min at 70°C, the temperature rose to 260°C at a rate of 30°C min^{-1}, then to 280°C at a rate of 1°C min^{-1}, and then to 320 °C at 10°C min^{-1} where it was held for 5 minutes. The areas of 50 peaks common to all ant cuticular extracts were integrated for further analysis. The substances were identified on the basis of their mass spectra and their retention time as compared to standard linear hydrocarbons.

Statistics

We performed one-way ANOVAs followed by Unequal N HSD post hoc tests to compare the mean duration of antennations and the percentage of time ants spent interacting during dyadic encounters, as well as the mean aggression index obtained for each nest distance ranges (i.e. 0 m, <10 m, 11-20 m, 21-40 m, 41-70 m, 71-90 m and > 2800 km) using Statistica v8.0 (Statsoft, 2007). The mean percentages of transports were compared two-by-two between the different distance ranges with non-parametric permutation tests, and a sequential Bonferroni correction was applied to the obtained P-value using StatXact-8 (Cytel Software Corporation, Cambridge, MA, USA). Pearson's correlation coefficient was calculated between the mean score of aggression found for each pairs of nests and the geographical distance using Statistica v8.0 (Statsoft, 2007).

To compare the cuticular hydrocarbon profiles of all studied colonies, a discriminant analysis and a cluster analysis (Euclidian distances and Ward's method) were performed to

test for significant differences and to establish the relationships between the colonies. In addition, the Euclidean chemical distance was then compared between the different nest distances ranges by one-way ANOVA followed by Unequal N HSD post hoc tests using Statistica v8.0 (Statsoft, 2007). The correlation between chemical Mahalanobis distances and behavioural variables was tested for sympatric colonies, as well as with genetic distance.

Results

Effect of nest distance in the behaviour

Discrimination tests

We found a significant difference between the percentage of time *P. verenae* ants spent interacting with nestmates compared to non-nestmates (ANOVA, $F_{6,123}$=18.56, p<0,001), with nestmates interacting for less time than all other classes of non-nestmates (Unequal N HSD, p<0,05, Figure 1). Among non-nestmates, ants interacted more with distant sympatric neighbours and allopatric nests than with near neighbours or other sympatric non-neighbours (Figure 1).

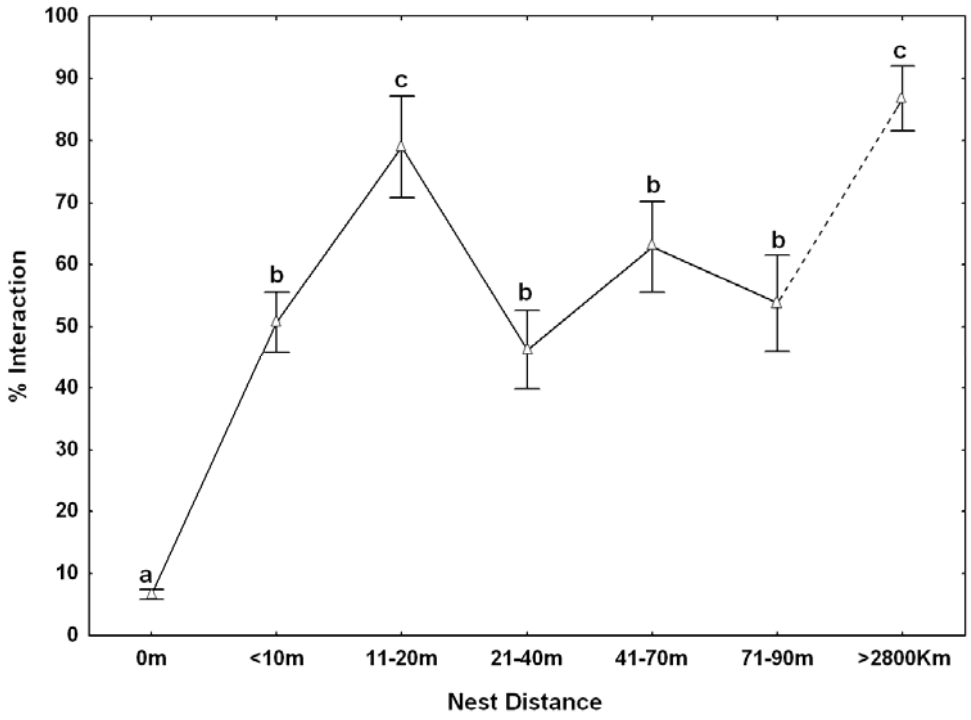

Figure 1. Mean (± s.e.) proportion of interactions between workers during dyadic encounters according to the distance between their nests. ANOVA, $F_{6,123}$=18.56, p<0,001. Different letters represents significant differences between distance range after Unequal N HSD (p<0,05).

We also found a significant difference in the duration of antennation between ants from different nest distances ranges (ANOVA, $F_{6,123}$= 18.8, P < 0,001). Despite presenting the higher levels of interaction (Figure 1), ants from allopatric nests performed almost no antennal contact, with a mean statistically similar only to the one observed between nestmates. The highest level of antennation was observed between close neighbours (Figure 2).

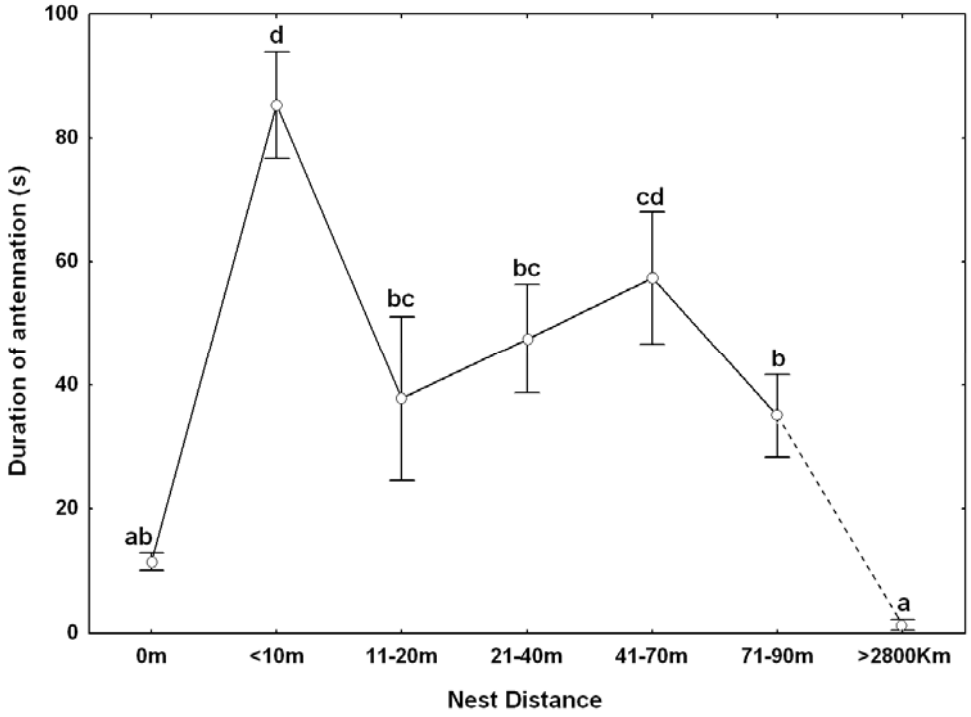

Figure 2. Mean (± s.e.) of the duration of antennations between workers during dyadic encounters according to the distance between their nests. ANOVA, $F_{6,123}$= 18.8, P < 0,001. Different letters represents significant differences between distance range after Unequal N HSD (p<0,05).

Aggression index

The level of aggression was not significantly correlated with nest distance between sympatric non-nestmates (Pearson's r = -0.1329; P = 0.1316) but significant differences in the level of aggression were found depending on the geographical distance between nests (ANOVA, $F_{6,123}$ = 53.36, P < 0,001, Unequal N HSD, P < 0,05, Figure 3). Furthermore, the intraspecific level of aggression was found to be relatively low in *P. verenae* sympatric colonies, in which the mean level of aggression was as low as 1 over a scale of 4, compared

with allopatric colonies in which the aggression score reaches almost 3 (Figure 3). Our results also show that *P. verenae* ants discriminate between close and distant neighbours.

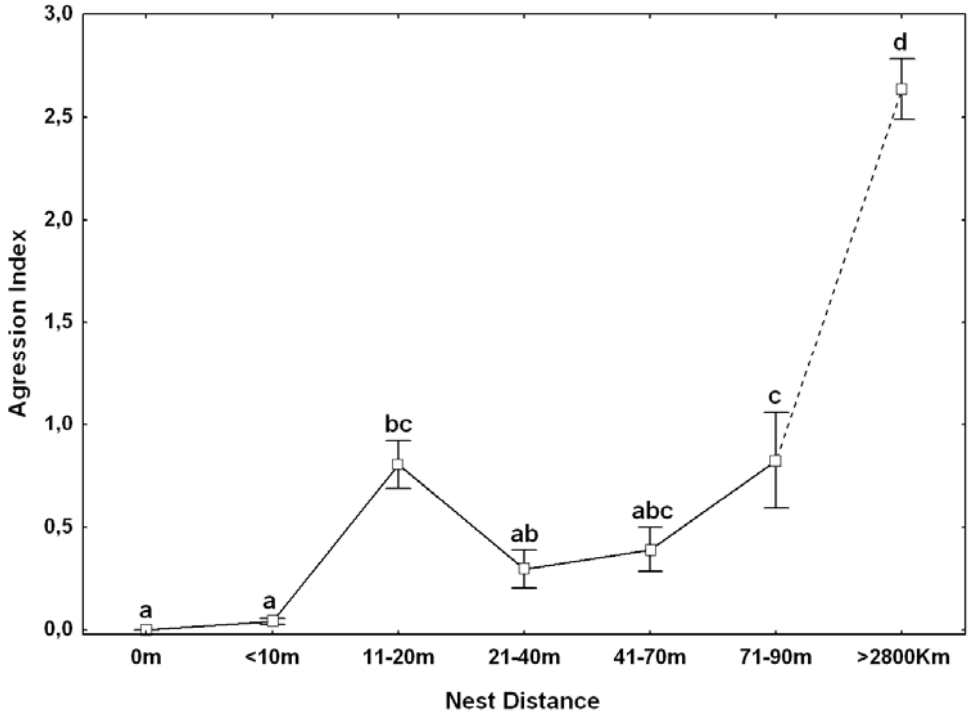

Figure 3. Mean (± s.e.) aggression index between workers during dyadic encounters according to the distance between their nests. ANOVA, $F_{6,123} = 53.36$, $P < 0{,}001$. Different letters represents significant differences between distance range after Unequal N HSD ($p<0{,}05$).

The level of aggression was not significantly different between workers from nests at a distance of <10 m and between nestmates, while it was significantly higher between workers from nests 11-20 m apart than between nestmates. Therefore, their acceptance of direct neighbours is total, with a level of aggression of 0, which corresponds to antennal contact only, and a different behaviour is performed towards indirect neighbours, with an aggression score of about 1, which corresponds to the *transport* level. When the percentage of time spent performing each behavioural item was compared, we found that transport is the main behavioural component in this group (Permutation Paired Tests, $P < 0{,}05$, Figure 4). Ants perform a ritualized behaviour towards distant neighbours which, as stated before, corresponds to a stereotypical behaviour that includes manipulation with the mandibles and legs until the other ant assumes a nymphal posture to be transported, without any overt aggressive behaviour.

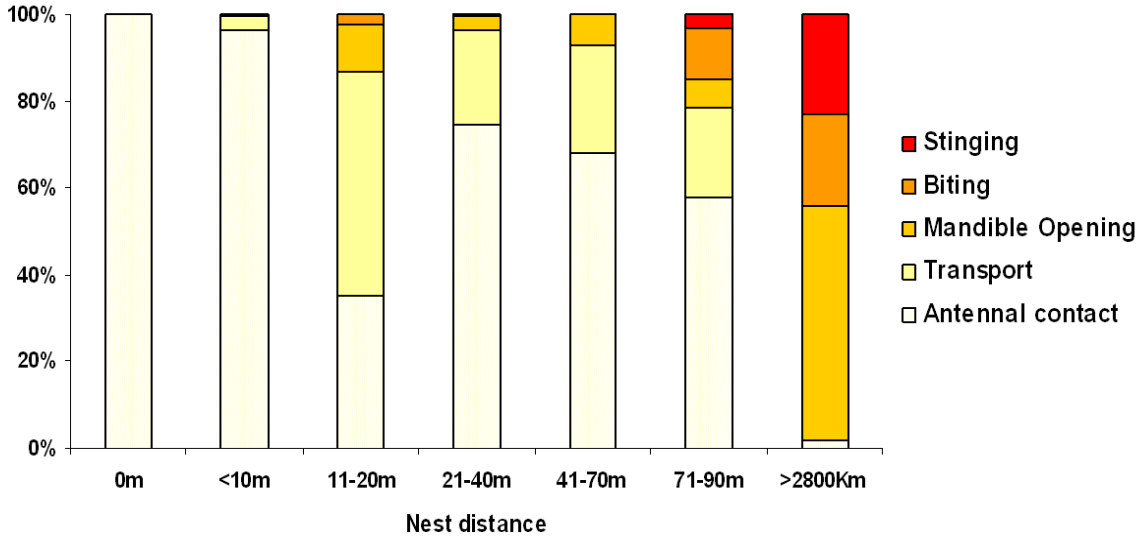

Figure 4. Mean percentage of each of the behavioural items recorded during dyadic encounters between workers coming from nests differently separated

Genetic distance

After correcting for multiple samples, none of the locus pairs showed significant linkage disequilibrium in any of the two populations. Both populations had similar amounts of genetic variation but they differed a little in their patterns of microsatellite variation (Table 2).

Table 2. Genetic variation in the eight microsatellite loci studied. *N*, number of individuals analyzed; N_A, number of alleles in each locus, *Rs*, allelic richness; H_E, unbiased expected heterozygosity.

		Viçosa 8 colonies (N=157)			Belém 4 colonies (*N*=74)		
	Total	N_A	***Rs***	H_E	N_A	***Rs***	H_E
1078	8	5	4,41	0,56	5	4,93	0,58
4049	10	10	8,32	0,79	2	2	0,48
2096	10	7	4,72	0,36	6	5,85	0,42
4053	4	4	3,38	0,23	4	4	0,59
1048	17	14	11,88	0,83	7	6,91	0,26
1015	12	7	5,17	0,47	10	9,66	0,81
2056	27	17	13,65	0,75	13	13	0,86
2111	8	6	4,52	0,33	4	4	0,65
Mean	12	8,75	7,01	0,54	6,38	6,29	0,58

The genetic divergence between both populations is high and significant, with a value of F_{ST} of 0.35. At the intra-population level, in Viçosa, we did not observe a pattern of isolation-by-distance (IBD) between pairs of colonies (Figure 5 and Spearman rank correlation: N=28, rs=0.169, p=0.24).

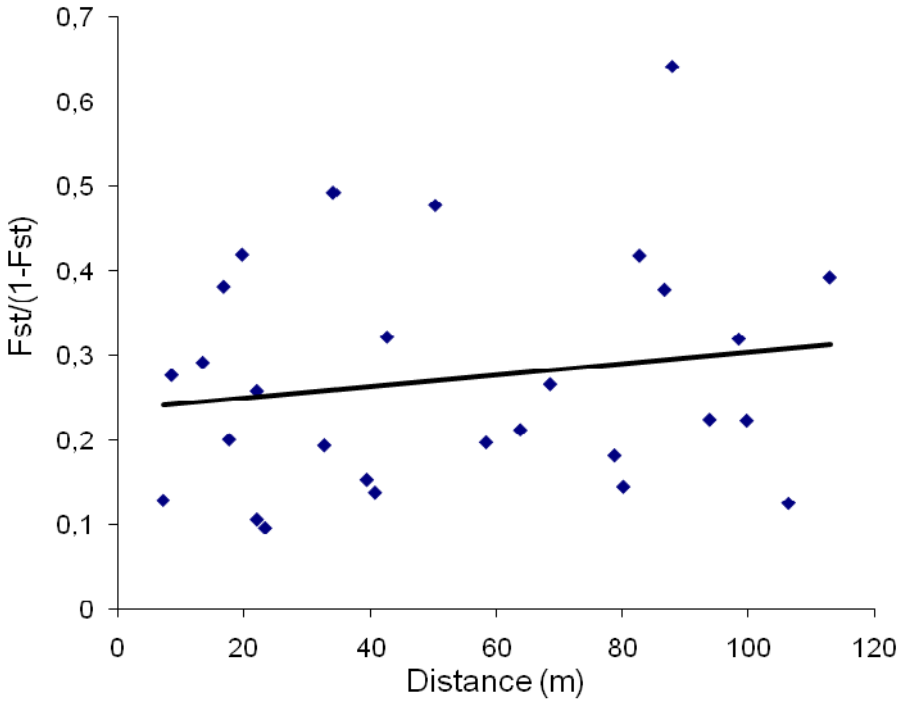

Figure 5. Isolation by distance showed distance showed as $F_{ST}/(1- F_{ST})$ according to distance between each pair of nest (Rousset 1997)

Chemical distance

The discriminant function analysis of all 48 cuticular hydrocarbons identified shows that each nest presents a distinct chemical label, with small intra-colony variability (Wilk's $\lambda = <1^{-E5}$, $F_{296,260}=10,73$ $p<0,001$, Figure 6). Furthermore, the cluster analysis shows that the closest nests (i.e. UFV01 x UFV02 or UFV08 x UFV10) are not the most chemically related (Figure 7). Significant differences in the cuticular hydrocarbon profiles were found depending on the geographical distance between nests (ANOVA, $F_{6,155}=19.76$, $p<0,001$, Unequal N HSD, $p<0,05$, Figure 8). Non-nestmates presented a higher chemical distance than nestmates, with distant neighbours presenting the most distinct visa for sympatric colonies. As expected, allopatric colonies present an even higher level of chemical distinctiveness than all sympatric colonies.

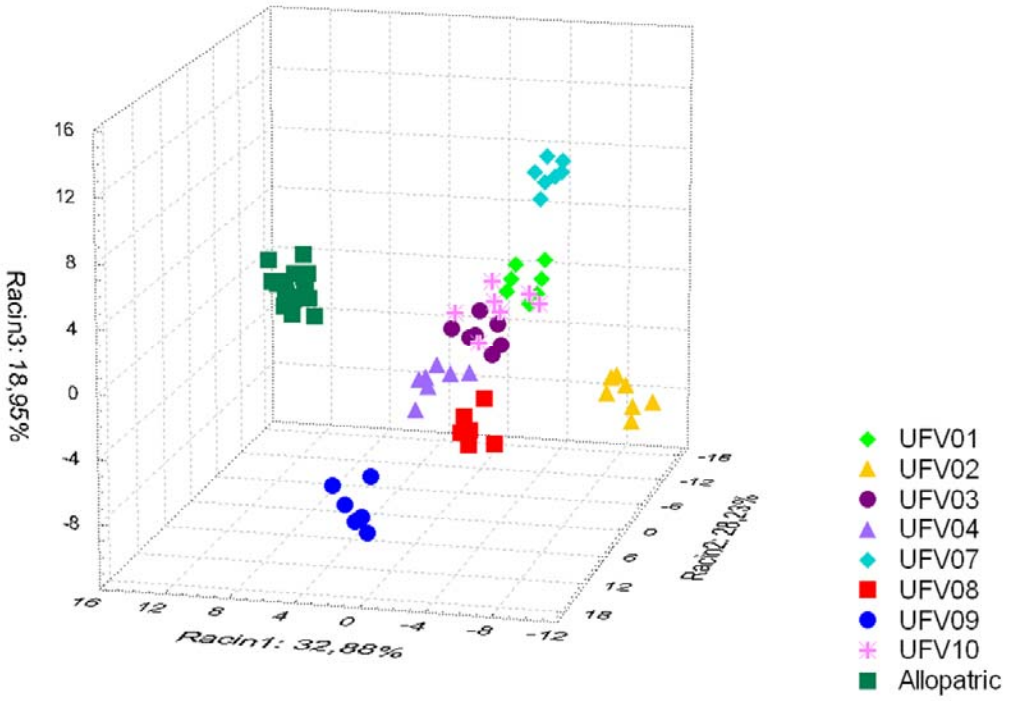

Figure 6. Representation of the coordinates of the chemical profiles of individual workers from the experimental colonies on the first three axes of the discriminant function analysis performed using 48 identified hydrocarbons (10 colonies, n=82, Wilk's $\lambda = <1^{-E5}$, $F_{296,260}=10,73$ $p<0,001$)

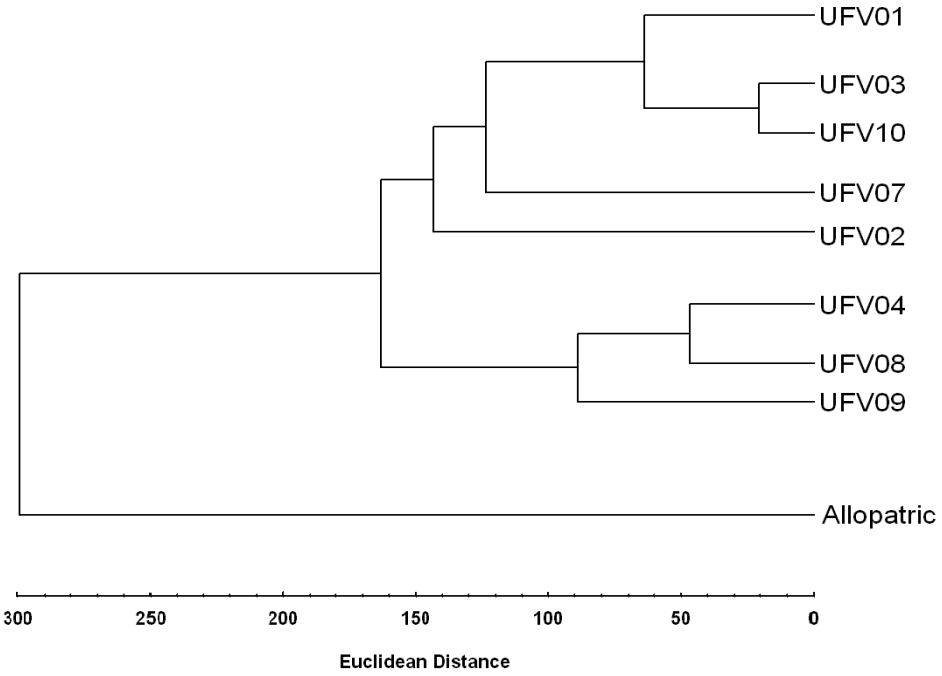

Figure 7. Hierarchical cluster analysis of the chemical distance between experimental colonies using Euclidean distances with Ward's method

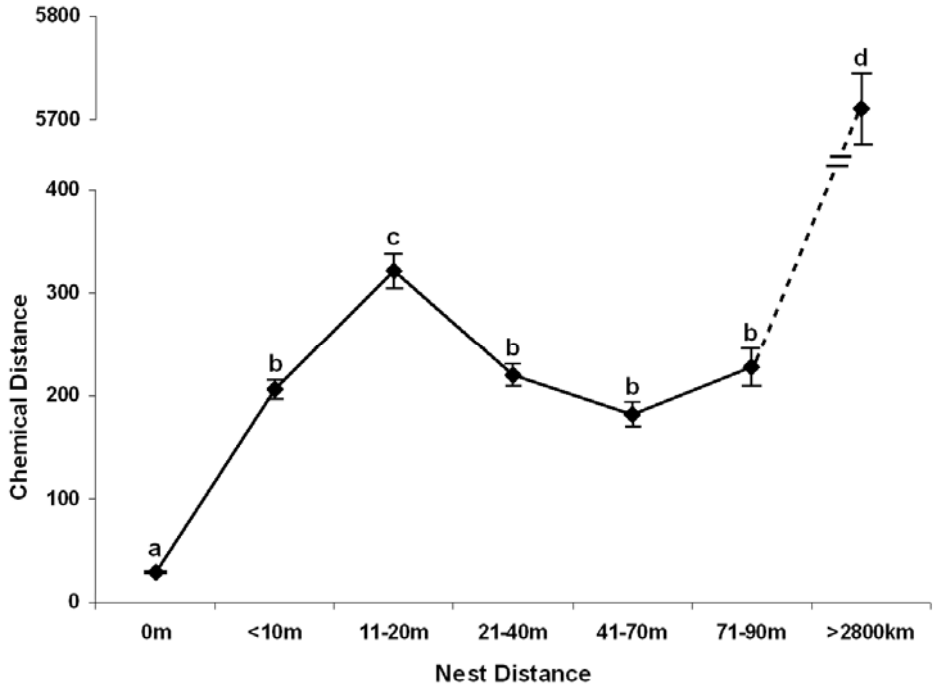

Figure 8. Mean (± s.e.) chemical distance between the chemical profiles of workers from the colonies used during dyadic encounters according to the distance between their nests. ANOVA, $F_{6,155}$=19.76, p<0,001. Different letters represents significant differences between distance range after Unequal N HSD (p<0,05).

Chemical Mahalanobis distance and genetic distance (Fst/1-Fst) were weakly but significantly correlated with aggression index (r^2=0,186, t=4,24, n=81, p<0,001; r^2=0,26, t=5,27, n=81, p<0,001), and they were also positively correlated to each other (r^2=0,626; t=7,14, n=81, p<0,001).

Discussion

Our results clearly show that, in addition to being able to fully discriminate nestmates from non-nestmates, *P. verenae* ants from the studied population exhibit different responses when confronted with workers from different foreign colonies. Even more interestingly, neighboring colonies appear to trigger drastically different behavior, with near-neighbours being completely tolerated, although they were clearly recognized or at least perceived as different, and distant neighbours ritually "ejected" though intense transport behaviour, thus allowing non violent conflict resolution. Differential expression of aggressive behavior towards neighbours is a well-known phenomenon in territorial species (Temeles 1994, Muller & Manser 2007) and in ants in particular these differences range from higher tolerance

(known as the dear-enemy phenomenon Dimarco et al. 2010, Langen et al. 2000, Tanner & Adler 2009) to escalated aggression (or "nasty neighbour" effect, Gordon 1989, Knaden & Wehner 2003, Newey et al. 2010). However this is the first time to our knowledge that two different behavioural adjustment towards neighbours are observed in individuals from the same colonies of the same species.

Different mechanisms can explain such phenomena, among whom genetic distance, chemical distance, learning and social factors (Dimarco et al. 2010, Gordon 1989, Langen et al. 2000, Temeles 1994, Thomas et al. 2007), with chemical distance possibly being dependent on both genetic and environmental factors (Dimarco et al. 2010, Heinze et al. 1996). We first hypothesized that nest distances would produce a graded variation in the behavior of ants. This proved to be inexact as nest distance is not related linearly to any other dependent or independent variable in our study. The genetic analysis of isolation by distance also suggests that population structure is not involved here either. Closer nests do not appear to be more related and at this fine scale, no genetic structure was evident in the population. It is less clear whether chemistry could be responsible for this particular discrimination since some behavioural variables seems to correlate with it. Since genetic distance is also correlated to aggression and hence to chemical distance, we confirm here an often hypothesized combined (or linked) influence of genetic and chemical divergence on the expression of aggressive behaviour, exemplified at another level by the highest aggression exhibited towards allopatric ants. However, colonies are as distant chemically from near-neighbours as from sympatric non-neighbours while exhibiting totally different responses towards them. It is also important to note that the higher aggression towards distant neighbours is qualitatively very different from the response towards all other types of colonies, in particular because of the high proportion of transports. The higher chemical distance of distant neighbour could thus be an artefact. It is thus probable that familiarity and learning are also important factors in the differential treatment of neighbouring colonies in this population of *P. verenae*. The very distinct responses to the two types of neighbours could stem from a combination of different chemical distances with familiarity, duration of coexistence, and/or frequency of contacts.

Other factors could influence individual ant motivation. Indeed, the perceived location of ants when encountering strangers greatly modifies their response. For example, aggression can increase close to the nest or to protect valuable resources (Knaden & Wehner 2003, 2004, Tanner & Adler 2009) and also be dependent on the competitor's behaviour (Tanner & Adler 2009). Our experiments were conducted in laboratory conditions using a neutral arena which

introduced potential confounding factors. However we used recently collected colonies (3-6 months) and ants were kept in the foraging arena close to their colonies prior to the encounters. In these ants that forage solitarily it is possible that location as well as spatial landmarks, rather than chemical environment influence context-dependent decisions, as in *Cataglyphis fortis* (Fresneau 1994, Knaden & Wehner 2003, 2004). In addition, we observed during encounters staged in larger arenas that transport behaviour could last for more than 10 min and that transporting ants could travel more than 10 m with its load, similar to what had been observed in the field (Ferreira, personal observation). As a consequence, the data presented here probably represent a reliable picture of colony relationships in the field. It is also noteworthy that highly aggressive responses were only exhibited towards colonies from distant populations and that encounters rarely went further than mandible opening. The absence of any aggression towards near neighbours suggests a dear enemy phenomenon that is based on familiarity and possible environmental cues. The existence of intense interactions and particularly of an increase in transport behaviour towards distant neighbours is harder to comprehend. In our experiments, reciprocal transport attempts were observed and the ritualized sequences by which one worker submits to the other in order to be transported also suggest that a very specific combination of factors triggers these interactions.

Transport behaviour has been described in the context of nest emigration in several species (Abraham et al. 1980; Möglich & Holldöbler 1994, Pratt et al. 2002), including *P. verenae* (Pezon et al. 2005). In the context of nestmate recognition, it has also been demonstrated in a few species, but the ritualized aspect of it is not always present (Salzemann & Jaffe 1990, Dimarco et al. 2010) and it mostly consists only in excluding the intruder from a resource and not in a prolonged and probably costly behavior which can last several minutes. Because of its duration and potential cost, it is likely that this behaviour is involved in the regulation of territorial dispute and foraging range determination in closely associated colonies. *P. verenae* ants forage solitarily and are susceptible to scramble competition, which is dependent on population density. Hence their highly territorial behaviour is limited to the vicinity of the nest (Pezon 2004) and opportunities of escalated aggressions are probably few and far between. However, an efficient partitioning of available resources is necessary to sustain high densities of colonies such as the ones encountered here. In trail laying species, trunk trails are used to partition foraging resources between nearby colonies and encounters are thus less frequent and thus less costly. These can settle dominance between colonies of the same or other species (Dimarco et al 2010, Gordon & Kulig 1996, Langen et al. 2000). Another possibility of encounter is during scouting trips to find new nests. Indeed, P. verenae

does not exhibit any building behaviour and its colonies occupy already dug cavities. Therefore, nest longevity can be limited and emigrations quite common (Pezon et al. 2005). This could cause another type of competition for nesting sites between colonies. However, there is no reason that familiar but distant intruders would cause a higher threat than more remote ones. One hypothesis is that competition for resources modifies relationships between colonies and that these encounters allow the partitioning of foraging areas between this solitary foraging species in which workers most probably uses visual landmarks and adopt preferred foraging directions (Fresneau 1995). The difference between close and distant neighbours could stem from differences in the time since which the different colonies have been founded or established in their current locations, however learning processes are usually rapid and long-lasting (Sanada-Murimura et al. 2003) and thus this is quite unlikely. Dear-enemy and other familiarity based phenomena can rely on the learning of a heterocolonial template and this learning could be influenced by the dynamics of encounters or their context or location. For example, near neighbours could be encountered mostly further from the nest during their foraging trip than more distant neighbours and thus higher or lower acceptance levels could rise through familiarity combined with the context of the encounters. More simply, this could also stem from different encounter rates between the different neighbours linked with different distances of the nests of origin. Even if it is difficult to predict whether workers from closer or more distant nests would encounter more frequently during foraging trips, one can hypothesize that very close nests have higher rates of worker encounter, so much so that complete tolerance is finally observed. For more distant neighbours, less frequent encounters would lead to ritualised aggression and ejection from the foraging range through directed transports. For other sympatric colonies, unlikely previous meeting causes mild aggression to be expressed. It is interesting that this tolerance of an unrelated nest could open opportunities of parasitism such as kleptobiosis.

Learning based differential discrimination has seldom been documented in primitive ponerine ants but instances of heterospecific colonial template learning exist in ant garden parabiotic associations involving species of this subfamily (Orivel et al. 1997). In this study, we show that *P. verenae* workers seem to adjust their behaviour to nature of the foreign ants they meet, possibly through learning of the template of nearby colonies. This could allow them to settle disputes less costly through more ritualized behaviour towards or complete tolerance of frequently encountered neighbours and ultimately a more optimal partitioning of hunting grounds through a peaceful coexistence with potential competitors. Further field experiments are thus needed to confirm this laboratory study and to fully understand the

complex relationships between the colonies of *P. verenae* living in high densities in this disturbed habitat. This type of study could also allow the precise understanding of the set-up of flexible behavioural decisions according to external factors such as between nest distances in natural conditions.

Acknowledgements

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CONCLUSIONS GENERALES ET PERSPECTIVES

Les résultats obtenus à partir des différents travaux et observations qui constituent cette thèse apportent des informations essentielles quant à la compréhension de la biodiversité cryptique au sein du complexe d'espèces Néotropicales *P. apicalis*.

Le défi taxonomique posé par ce groupe d'espèces morphologiquement semblables nous a amené à tester de nouveaux outils pour aider à les identifier. Ainsi, nous avons cherché au travers d'études bioacoustiques, génétiques, chimiques, écologiques et comportementales à caractériser des éléments de la biologie de ces espèces pouvant être considérés comme indicateurs de l'espèce, et ceci également dans le but de mieux comprendre les interactions intra- et interspécifiques dans ce groupe d'espèces phylogénétiquement proches.

L'utilisation de la bioacoustique pour distinguer des espèces cryptiques, bien que courante pour plusieurs ordres d'insectes chanteurs (Claridge et al. 1997, Henry 1994, Walker et al. 2003, Cade & Otte 2000, Broza et al. 1998, Ritchie & Gleason, 1995, Noor & Aquadro, 1998), constitue une première dans les recherches sur les Formicidae. En effet, même si les productions acoustiques sont connues chez les fourmis depuis longtemps, elles n'ont fait l'objet que de très peu d'études, rendant ainsi ce domaine l'un des moins bien compris dans la biologie de cette famille (Ferreira & Fresneau 2009). En outre, il est très souvent considéré comme anodin par rapport aux autres moyens de communication employés par ces insectes, notamment la signalisation chimique (Hölldobler & Wilson, 1990). Cependant nos travaux ont démontré qu'une différenciation acoustique jamais observée auparavant est présente au sein du complexe *P. apicalis* et que, grâce à l'appui d'analyses phylogénétiques, cette évolution des signaux acoustiques et des organes producteurs de son est liée au processus de spéciation dans ce complexe d'espèces. Ceci nous a ainsi permis d'inférer sur la validité taxonomique des divers morphes étudiés, comme étant de vraies espèces. Une autre caractéristique notable de cette spécificité acoustique est le fait qu'elle semble découler d'une sélection divergente entre les morphes présents en sympatrie, possiblement à cause de leurs interactions interspécifiques compétitives suggerant ainsi que les stridulations pourraient être beaucoup plus informatives pour ces insectes que ce qui était courrament admis jusqu'à présent.

De plus, dans le complexe d'espèces *P. apicalis*, les spécificités acoustiques ne sont pas restreintes au niveau interspécifique. Une étude des stridulations des différentes castes des deux espèces largement sympatriques *P. apicalis* (Morphe 4) et *P. verenae* (Morphe 1) a démontré que cette spécificité acoustique existe aussi au niveau intra-spécifique. Les signaux stridulatoires au sein de chaque espèce présentent donc des caractéristiques distinctes pour les trois castes, où les signaux des mâles divergent à un degré plus élevé de ceux des deux castes

femelles. En outre, la comparaison des signaux de chaque caste entre ces deux espèces a révélé des caractéristiques temporelles et/ou fréquentielles spécifiques pour les trois castes, avec des niveaux de divergence plus élevés entre les gynes et les ouvrières. Ceci correspond à la première description d'une telle spécificité dans les signaux acoustiques des individus reproducteurs d'espèces de fourmis phylogénétiquement proches.

L'ensemble de ces résultats pose la question des fonctions et des contextes d'utilisation de ces signaux chez ce groupe d'espèces. Chez les autres fourmis déjà répertoriées, les contextes d'émission des stridulations sont extrêmement variés selon l'espèce et la structure sociale des colonies (Markl 1965, 1967, Hölldobler et al. 1994, Roces et al. 1993, Barbero et al. 2009a, Maschwitz & Schönegge 1983, Markl et al. 1977, Mercier et al. 2007).

Chez le complexe *P. apicalis*, les stridulations ne se sont pas avérées être utilisées au niveau intra-colonial (en colonies non manipulés) pendant les activités journalières des fourmis. De même ces signaux n'ont pas étés non plus observés pendant les activités de déménagement des sociétés, qui correspondent à une importante étape de la vie des ces espèces primitives qui, parce qu'elles ne construisent pas leurs propres nids, doivent déménager régulièrement quand le nid occupé est détruit ou quand il ne convient plus à la démographie de la colonie.

Les contextes comportementaux où les stridulations ont pu être observées, lors des perturbations du nid ou en réponse au souffle d'un mammifère, indiquent que pour le complexe *P. apicalis,* comme chez la plupart des fourmis, la fonction principale des signaux acoustiques est d'être un signal d'alarme. Les stridulations pourraient ainsi informer la colonie d'un danger potentiel, par exemple lors d'une attaque. En outre, nos résultats appuient fortement l'hypothèse que les stridulations peuvent servir également de signal aposématique mettant en garde d'éventuels prédateurs comme les lézards, les oiseaux et les mammifères de la piqûre douloureuse que ces fourmis peuvent infliger.

Cependant, si ces signaux acoustiques ont seulement l'alarme comme fonction, ces espèces largement sympatriques, soumises aux mêmes pressions de l'environnement, n'ont aucun intérêt évolutif à produire des signaux spécifiques à l'espèce (Masters 1979). En outre, comme les individus reproducteurs ne participent pas aux tâches de protection de la colonie, ces résultats ont renforcé l'hypothèse que les stridulations pourraient présenter un rôle plus important dans les différents aspects de la biologie de ces espèces. En effet, l'existence d'une telle spécificité acoustique au sein de ce groupe d'espèces pourrait indiquer une utilisation de ces signaux dans des contextes où il y a un intérêt évolutif à ce qu'ils soient différents. Etant

donné qu'une des fonctions les plus importantes des signaux spécifiques de l'espèce chez les insectes est de permettre la reconnaissance des partenaires et l'isolement reproducteur (Sueur 2006, Virant-Doberlet & Cokl 2004), nous nous attendions à ce que les signaux acoustiques jouent un rôle dans la discrimination et la reconnaissance des partenaires chez le complexe d'espèces *P. apicalis*. De plus, dans le cas de ces espèces cryptiques phylogénétiquement proches et largement sympatriques, l'existence de mécanismes d'isolement pré- et/ou post-copulatoire ne peut être négligé (Coyne & Orr 1998).

Cependant, lors de l'étude du comportement d'accouplement de plusieurs morphes du complexe *P. apicalis* en conditions de laboratoire, les signaux stridulatoires n'ont été utilisés ni pour la reconnaissance des espèces ni du sexe lors du processus de sélection de partenaire, et aucune stridulation n'a eu lieu ni au cours de cette phase ni pendant tout l'accouplement. Les stridulations semblent donc ne pas servir de signal permettant l'isolement reproducteur entre ces espèces étroitement apparentées, en dépit du fait qu'elles sont capables de produire des signaux spécifiques à l'espèce et à la caste. Néanmoins, des stridulations ont été observées chez des femelles déjà accouplées une fois et qui n'acceptaient plus d'autres tentatives d'accouplement de la part des mâles. Ceci corrobore l'hypothèse que les stridulations dans ce contexte pourraient servir de « signal de libération de la femelle », comme proposé par Markl et al. (1977) pour les fourmis *Pogonomyrmex*, et présenter ainsi des avantages pour les deux sexes, dans la mesure où elles pourraient communiquer la non réceptivité des femelles aux mâles s'approchant, ce qui leur éviterait de perdre du temps et les inciterait à courtiser d'autres femelles, en plus de permettre aux femelles récemment fécondées de partir rapidement à la recherche d'un endroit où fonder leur colonie afin de réduire les dangers liés à la prédation.

L'autre fait intriguant observé durant l'étude des accouplements chez différents morphes du complexe *P. apicalis*, est la capacité de croisement observée entre *P. apicalis* Morphe 4 et *P. apicalis* Morphe 2, répresentant de plus des populations allopatriques. L'absence d'isolement pré-copulatoire entre ces deux morphes pourrait indiquer que les différences morphologiques observées entre elles ne seraient que des variations biogéographiques. Cependant, le fait que ces deux morphes (apparentés) peuvent aussi vivre en sympatrie pourrait suggérer que les conditions de laboratoire où ces croisements ont été observés n'ont pas permis à d'autres mécanismes potentiels d'isolement pré-copulatoires d'opérer (par exemple la désynchronisation des temps de maturation, les périodes de sorties des individus sexuées, etc). Ces facteurs sont en effet susceptibles d'effectuer un filtrage des rencontres dans les conditions naturelles alors que les conditions artificielles du laboratoire ne

le permettent pas, ouvrant la possibilité à des accouplements n'ayant donné aucune descendance. En dehors de ces facteurs écologiques, l'absence de descendance peut aussi s'expliquer par d'autres mécanismes plus complexes d'isolement post-copulatoires. Ainsi l'existence d'une grande divergence entre le nombre et la morphologie des chromosomes, comme cela a déjà été observé pour d'autres complexes d'espèces de fourmis (Imai 1994, Mariano 2004).

Si l'utilisation des signaux acoustiques spécifiques de l'espèce chez le complexe *P. apicalis* n'a pas été démontrée dans les interactions interspécifiques de ces fourmis, les travaux de diffusion des stridulations n'ont pas non plus révélé de réponses comportementales des fourmis aux signaux homospécifiques ou aux signaux heterospécifiques testés. Il reste donc à vérifier si et comment toutes ces différences sont effectivement perçues ou sollicitées par les différentes castes et espèces de ce complexe. D'autres études, comme l'électrophysiologie des organes récepteurs, des tests comportementaux en situations plus contrôlées au laboratoire et des tests complémentaires sur le terrain sont nécessaires pour clarifier les causes de cette spécificité acoustique dans ce groupe de fourmis Néotropicales.

L'étude de la bioacoustique et les données génétique sur les différents taxons du complexe d'espèces *P. apicalis* nous ont permis de démontrer l'existence de six à neuf espèces valides là où seules trois étaient décrites précédemment. Les caractéristiques écologiques et comportementales de chaque espèce ont probablement été mélangées dans les études précédentes et il est possible que les espèces/morphes puissent posséder des particularités jusqu'alors ignorées. Ainsi, par une étude des hydrocarbures cuticulaires, de la biométrie détaillée et des préférences écologiques des différentes espèces du complexe *P. apicalis* provenant de plusieurs populations de Guyane Française, du Mexique et du Brésil, nous avons confirmé l'existence de caractéristiques propres à chaque morphe à tous les niveaux d'études. La spécificité du phénotype des mâles de chaque morphe a été décrite pour la première fois. Cette étude confirme et prolonge nos données sur l'existence de neuf espèces potentielles dans le complexe d'espèces *P. apicalis*, ainsi que peut-être même une diversité supplémentaire à l'intérieur de certains taxons définis ici, en particulier pour les profils chimiques, comme déjà indiqué par des études cytogénétiques précédentes (Delabie et al. 2008).

Les singularités biologiques et écologiques révélées par notre étude biosystématique influencent probablement grandement les traits d'histoire de vie de ces espèces, de leur organisation sociale jusqu'au niveau de la communauté. Par exemple, un contraste très important existe entre les deux morphes de *P. verenae* dont le Morphe 1 est présent en milieu

forestier plutôt fermé et qui niche dans des branches en décomposition de la litière, alors que le Morphe 2 se retrouve en milieu ouvert et semble avoir une habitude de nidification hypogée (Delabie et al. 2008). Cela nous a amené à nous intéresser aux propriétés des relations intraspécifiques d'une population très dense dans une zone de culture. Les relations entre les colonies de *P. verenae* Morphe 2 se sont avérées très inhabituelles, puisqu'il semble que des phénomènes d'apprentissage permettent de réguler les conflits territoriaux par des comportements ritualisés entre les colonies voisines. Le transport orienté semble un comportement très important dans ces rencontres. La coexistence de colonies extrêmement proches est même apparemment accompagnée d'une complète tolérance dont la cause ne semble être ni l'apparentement ni la proximité chimique. Notre étude en laboratoire mérite d'être confirmée sur le terrain, surtout que ce modèle peut offrir une très grande opportunité d'analyser plus finement les mécanismes liés au phénomène du « cher ennemi » en nature. Elle montre aussi l'intérêt de réaliser d'autres études de ce type, en particulier dans les zones de sympatrie pour mieux comprendre les relations écologiques entre les espèces du complexe.

Le travail accompli au cours de cette thèse a fait émerger de nombreuses questions, questions beaucoup plus nombreuses que lorsque ce projet a été initié. En effet, l'ensemble des travaux ici réalisés a démontré que le processus de spéciation cryptique et les relations interspécifiques au sein du complexe *P. apicalis* s'avèrent encore plus complexes que ce que l'on croyait il y a quatre ans à l'initiation de ce travail. Il serait donc intéressant de pouvoir réaliser un relevé plus exhaustif de ces espèces sur l'ensemble de l'aire de distribution du complexe pour comprendre sa diversification et essayer de reconstituer son histoire évolutive. L'approche multidisciplinaire choisie ici sera sûrement dans cette optique un atout majeur.

En complétant nos données sur la bioacoustique et la morphologie des organes stridulatoires de ces espèces dans différentes conditions écologiques (sympatrie, allopatrie, etc.), nous pourrons grandement éclaircir les connaissances sur ce complexe tout en comprenant la façon dont ces caractéristiques ont évolué. D'autres complexes d'espèces de Ponerinae pourraient également être étudiés par ce type d'approches et je suis convaincue qu'elles permettraient de mettre à jour une diversité insoupçonnée chez les espèces Néotropicales. Cela s'avère un champ d'étude extrêmement large pour l'écologie, la biogéographie et le comportement social des fourmis.

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ANNEXE

Article 8: Conspecifics of a heterotrophic heteronomous species of Strepsiptera (Insecta) are matched by molecular characterization

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Conspecifics of a heterotrophic heteronomous species of Strepsiptera (Insecta) are matched by molecular characterization

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Abstract. The family Myrmecolacidae (Strepsiptera) exhibit the unusual phenomenon of sexually dimorphic host relationships known as heterotrophic heteronomy, whereby males parasitize ants and females parasitize grasshoppers, crickets and mantids. It has therefore been impossible phenotypically to match male Myrmecolacidae to their conspecific females: the male and female of only one species have so far been unequivocally matched, and this was by molecular characterization. Here we report another match of a male and its conspecific female: by comparison of the CO1 and 18S genes of male *Myrmecolax incautus* Oliveira and Kogan, which parasitizes a ponerine ant from French Guyana, and a female strepsipteran, which parasitizes a mantid from Brazil. The male *M. incautus* is redescribed, and the first descriptions of the neotenic female, the male cephalotheca and the first instar larva are given. We also report for the first time dimorphic hosts of the male and the female *M. incautus*, and describe for the first time, the behaviour of stylopized ants.

Introduction

One of the most extraordinary features of the family Myrmecolacidae (Strepsiptera) is that the males and females exhibit extreme sexual dimorphism not only in their morphology but also in their host relationships. The two sexes parasitize hosts belonging both to different species and to different orders of Insecta. The adult free-living males parasitize ants (Formicidae); the neotenic, totally endoparasitic females, which are devoid of all external insect morphological characters, parasitize grasshoppers, crickets and mantids (Orthoptera: Tettigoniidae, Grillidae; and Mantidea) (Ogloblin, 1939; Kathirithamby, 1991, 2009; Kathirithamby & Hamilton, 1992; Kathirithamby & Johnston, 2004). This unusual, complex and extreme form of behaviour was classified by Walter (1983) as heterotrophic heteronomy, and occurs in only one other lineage of sexually dimorphic parasitic insects: two species of *Encarsia* of the hymenopteran family Aphelinidae, in which the males develop in eggs of Lepidoptera and the females in two closely related species of whiteflies (Flanders, 1924, 1925, 1926, 1936a, b, 1959; Walter, 1983; Hunter & Woolley, 2001).

Of the 110 described species of extant Myrmecolacidae, only nine species of females are known (Table 1). The rest of the described species are free-living males that have come into traps. Of these, the hosts of only 13 males are known, seven of which were obtained when whole nests of ants were collected and the strepsipteran males dissected from the stylopized ants within the nests (Table 2). Wandering stylopized ants

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*RSF, RA, HTAA & DF collected the material; AH & DM performed the molecular analysis, RSF & DF provided the data for the nest collection of the ants, and JK carried out the identification and wrote the paper.

Table 1. Female Myrmecolacidae and their hosts.

Species	Host of female (Orthoptera, Mantodea)	Distribution
1. *Stichotrema dallatorreanum* Hofeneder, 1910, 1920	*Segestes decoratus* Redtenbacher	Papua New Guinea (West New Britain Province)
	Sexava nublia (Stål)	Papua New Guinea (Pak Island)
	Segestes novaeguineae (Brancsik)	Papua New Guinea (Mainland)
	Segestes defoliaria (Uvarov)	Papua New Guinea (West New Britain Province)
	(Orthoptera: Tettigoniidae)	
2. *Myrmecolax ogloblini* Luna de Carvalho, 1973 *Mantidoxenos argentinus* (Ogloblin, 1939)	*Acanthosis (Mantis) coninna* (Party) (Mantodea)	Argentina (Province de Misiones. Loreto)
3. *Stichotrema vilhenai* Luna de Carvalho, 1972 *Caenocholax vilhenai* (Luna de Carvalho, 1956)	*Sphodromantis lineola pinguis* La Greca (Mantodea)	Angola (Dundo; Cuango)
4. *Stichhotrema asahinai* Hirashima & Kifune, 1974	*Mecopoda elongata* L. (Orthoptera: Tettigoniidae)	Japan (Okinawa Island)
5. *Stichotrema yasumatsui* Kifune, 1983	*Euscyrtus* sp. (Orthoptera: Gryllidae)	Thailand (Chiang Mai; Bangkhang)
6. *Stichotrema jeyasothie* Kathirithamby 2001 in Kathirithamby *et al*., 2001	*Phyllophorella subinermis* Karny (Orthoptera: Tettigoniidae)	Papua New Guinea (West New Britain Province)
7. *Stichotrema waterhousi* Kathirithamby 2001 in Kathirithamby *et al*., 2001	*Panacaedicia* sp. (Orthoptera: Tettigoniidae)	Papua New Guinea (West New Britain Province)
8. *Caenocholax fenyesi waloffi* Kathirithamby & Johnston, 2004	*Macroanaxipha macilenta* (Saussure) (Orthoptera: Gryllidae)	Mexico (Los Tuxtlas)
9. *Caenocholax fenyesi* sensu lato Kathirithamby, 2009	*Pteronemobius* sp. (Orthoptera: Gryllidae)	Mexico (Tapachula)
10. *Myrmecolax incautus* Oliveira & Kogan, 1959	*Stagmatoptera* sp. (Mantodea) (new record)	Brazil (Parnamirim, Rio Grande do Norte Province)

are rarely encountered in nature, and it is hypothesized that stylopized ants neither leave the nest to forage nor abandon the nest when the strepsipteran male puparium extrudes through the host cuticle. Stylopized ants leave the nest only just before the emergence of the free-living male strepsipteran from the partially endoparasitic puparium, and this might be to avoid predation (Kathirithamby & Hamilton, 1992; Kathirithamby, 2005, 2009). Since female Myrmecolacidae (like all Strepsiptera, except the family Mengenillidae) are endoparasitoids, the hosts of all nine described females are known (Table 1). Because of their extreme sexual dimorphism and heterotrophic heteronomy, only one species of male and female Myrmecolacidae (Kathirithamby & Johnston, 2004) (and a second in this study) have been unequivocally matched by molecular characterization so far (Table 3).

Heteronomy in Aphelinidae is fairly well studied (see the review by Hunter & Woolley, 2001), but it is less well known in the strepsipteran family Myrmecolacidae. This may be because hosts stylopized by female myrmecolacids are elusive (Kathirithamby & Hamilton, 1992). Aphelinidae are haplodiploid, and the host-seeking females are able to control the sex of their offspring by selectively fertilizing the eggs as they pass through the oviduct (Hunter & Woolley, 2001). Strepsiptera are not haplodiploid (Ferreira *et al*., 1984; Johnston *et al*., 2004) and it is the first instar larvae, rather than the mother, that represent the host-seeking stage. The sex-determining mechanism in this family is still not confirmed, and we do not know whether it is genetic or environmental (i.e. occurring after host entry).

This is the first report of the match of a male myrmecolacid from the genus *Myrmecolax* with its conspecific female, and only the second so far for any Myrmecolacidae. We also report the host of the male *Myrmecolax incautus* Oliveira & Kogan, which is a primitive ponerine ant from French Guyana, and that of its conspecific female, which is a praying mantis (Mantodea) from Brazil. A redescription of the male *M. incautus*, and the first description of the first instar larvae of *M. incautus* (which were present in the viviparous female), the cephalotheca of the male pupa and the cephalothorax of the female are also provided. The behaviour of stylopized ants is described for the first time.

Family Myrmecolacidae, Saunders, 1872

Myrmecolacides Saunders, 1872
Myrmecolacidae Pierce, 1908
Stichotrematoidea Hofeneder, 1910
Stichotrematidae Hofeneder, 1910

Myrmecolax incautus Oliveira & Kogan, 1959

Holotype ♂, BRAZIL: no. 10, Carmo do Rio Claro, Estado de Minas Gerais, light trap, 16.i.1958 (*J. Becker*) Instituto Oswaldo Cruz.

♂ *adult.* (Fig. 1). Total length 1.80 mm., 20 ommatidia. Palpi of maxilla 0.34 mm. III antennomere plus flabellum = 1.16 mm, antennomere V = 0.70 mm, VI = 0.36 mm, VII =

Table 2. Male Myrmecolacidae and their hosts (Formicidae).

Species	Host of male	Distribution
1. *Myrmecolax nietneri* Westwood, 1861	*Camponotus maculatus* F. (Formicinae: Wasmann ap. Hofeneder, 1927)	Sri Lanka (Rombola), Malaysia
2. *Myrmecolax ogloblini* Luna de Carvalho, 1973 *Mantidoxenos argentinus* (Ogloblin, 1939)	*Camponotus punctulatus* Mayr (Formicinae)	Argentina (Misiones)
3. *Myrmecolax borgmeieri* Hofeneder, 1949	*Eciton dulcius* Forel (Ecitoninae)	Argentina (Cordoba)
4. *Stichotrema vilhenai* Luna de Carvalho, 1972 *Caenocholax vilhenai* (Luna de Carvalho, 1956)	*Crematogaster* sp. (Myrmicinae)	Angola (Dundo, Cuango: Luzemba)
5. *Stiochotrema robertsoni* Kathirithamby, 1991	*Pheidole* sp. (Myrmicinae)	South Africa (Natal)
6. *Caenocholax fenyesi* sensu lato Kathirithamby & Hughes, 2002	*Camponotus planatus* Roger (Formicinae)	Mexico, (Los Tuxtlas)
7. *Caenocholax fenyesi waloffi* Kathirithamby & Johnston, 2004	*Dolichoderus bispinosus* Olivier (Dolichoderinae)	Mexico (Los Tuxtlas)
8. *Caenocholax fenyesi texensis* Kathirithamby & Johnston, 1992 Cook *et al*., 2004	*Solenopsis invicta* Bruen (Myrmicinae)	U.S.A. (Texas) Argentina (Formosa Province)
9. *Caenocholax fenyesi* sensu lato Kathirithamby, 2008	*Camponotus atriaps* (Fr. Smith) (Formicinae)	Mexico (Tapachula)
10. *Caenocholax fenyesi sensu lato* Kathirithamby, 2008	*Pheidole* sp. (Myrmicinae)	Mexico (Tapachula)
11. *Caenocholax fenyesi* sensu lato Kathirithamby *et al*., 2009	*Myrmelachista zeledoni* Emery (Formicinae)	Costa Rica (Puntarenas Province)
12. *Caenocholax fenyesi* sensu lato Cook, 2009	*Crematogaster laeviuscula* Mayr (Myrmicinae)	U.S.A. (Texas)
13. *Myrmecolax incautus* Oliveira & Kogan, 1959	*Pachycondyla verenae* (Forel) *Pachycondyla apicalis* Latreille (Ponerinae) (new record)	French Guyana (Camp Patawa Kerrenroch, Petit Saut)

Table 3. Hosts of male and female Myrmecolacidae.

Species	Host of male (Hymenoptera: Formicidae)	Host of female (Orthoptera, Mantodea)	Distribution
1. *Myrmecolax ogloblini* Luna de Carvalho, 1973[a] *Mantidoxenos argentinus* (Ogloblin, 1939)	*Camponotus punctulatus* Mayr (Myrmicinae)	*Acontista* (Mantis) *coninna* (Party) (Mantodea)	Argentina (Misiones)
2. *Stichotrema vilhenai* Luna de Carvalho, 1972[b] *Caenocholax vilhenai* (Luna de Carvalho, 1956)	*Crematogaster* sp. (Myrmicinae)	*Sphodromantis lineola pinguis* La Greca (Mantodea)	Angola (Dundo, Cuango: Luzemba)
3. *Caenocholax fenyesi waloffi* Kathirithamby & Johnston, 2004[c]	*Dolichoderus bispinosus* Olivier (Dolichoderinae)	*Macroanaxipha macilenta* (Saussure) (Orthoptera: Gryllidae)	Mexico (Los Tuxtlas)
4. *Myrmecolax incautus* Oliveira & Kogan, 1959 (new record)[c]	*Pachycondyla verenae* (Forel) *P. apicalis* Latreille (Ponerinae)	*Stagmatoptera sp.* (Mantodea)	Brazil (Parnamirim) ♂ French Guyana (Camp Patawa Kerrenroch, Petit Saut) ♀ Brazil (Parnamirim, Rio Grande do Norte Province)

[a]Owing to the similarity of the exuviae of the first instar larva found in an ant and a mantid host, the male parasitic in the ant and the female parasitic in the mantid were hypothesized to be conspecific.
[b]Owing to the discovery of the ant and mantid hosts in the same location, the Strepsiptera parasitoids were hypothesized to be conspecific.
[c]Unequivocally matched by molecular characterization.

Fig. 1. Male *Myrmecolax incautus* Oliveira & Kogan, dorsal view of head and thorax (×25).

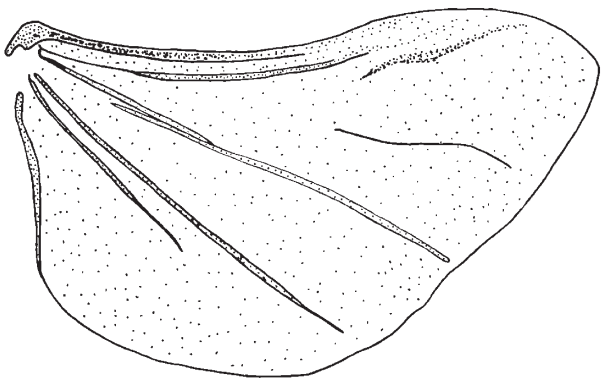

Fig. 2. Male *Myrmecolax incautus* Oliveira & Kogan, wing (×32).

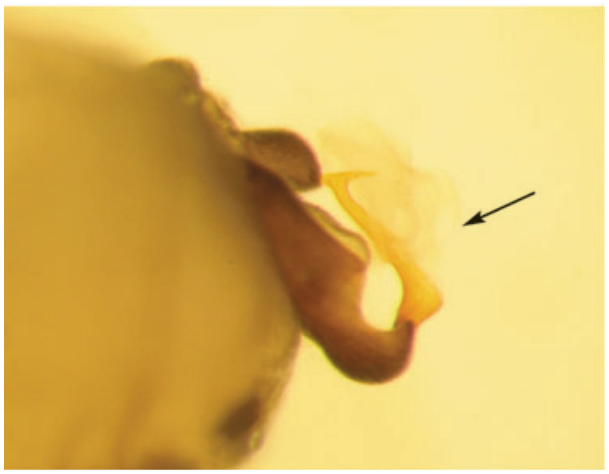

Fig. 3. Male *Myrmecolax incautus* Oliveira & Kogan, aedeagus lateral view (×120).

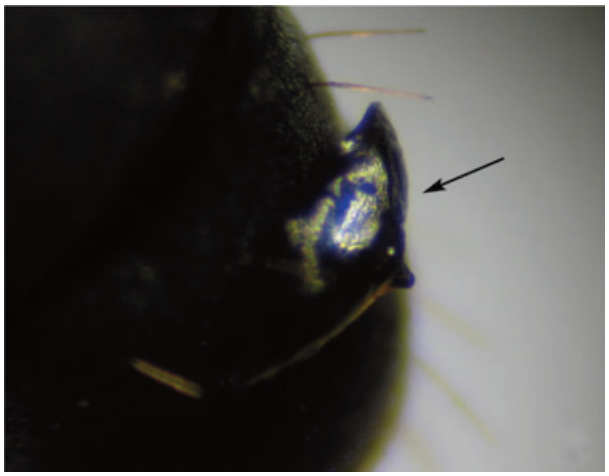

Fig. 4. Pupa of male *Myrmecolax incautus* Oliveira & Kogan, cephalotheca (×60).

0.42 mm. Prescutum length 0.29 mm, scutellum as long as wide 0.21 mm, postlumbium length 0.13 mm and width 0.39 mm, postnotum length 0.44 mm and width 0.26 mm; Xth abdominal segment 0.07 mm. Wing length = 2.5 mm. R_2 almost as long as R_3 R_5 reaching wing margin, CuA two-thirds as long as MA, and as long as CuP (Fig. 2). Aedeagus total length 0.2 mm and dorsally with anterior and posterior projections (Fig. 3).

Diagnosis. Only two species of the male of *Myrmecolax* have been described from South America, *M. incautus* Oliveira & Kogan and *M. borgmeieri* Hofeneder 1949. *Myrmecolax incautus* differs from *M. borgmeirei* by an R_2vein, which ends in a fork in *M. borgmeieri*.

Cephalotheca of the male pupa. (Figs 4–6) width = 0.78 mm, length = 0.65 mm. As in other Myrmecolacidae, after the emergence of the free-living male from the partially endoparasitic puparium the cap of the puparium remains partly attached, by its hinge, to the host ant (Fig. 5). Rudiments of ommatidia are represented by 30 small thickenings of the sclerite on either side of the cephalotheca. Antennal rudiments represent the scapus and pedicellus. Paired mandibles and maxilla appear just below the eyes, with a mouth-opening in between. The clypeus is the raised region in the centre of the cephalotheca. Vertex and occipital sutures are found on the dorsal region of the cap (Fig. 6).

The above description of the cephalotheca of *M. incautus* is the first recorded for a male *Myrmecolax*, as no male pupa of this genus has been found in its host before.

Host of male. Pachycondyla verenae (Forel, 1922) and *P. apicalis* (Latreille, 1802) (Hymenoptera: Ponerinae). These are large, dark brown to black, conspicuous ants found in Neotropical forests from southern Mexico to Paraguay (Wild, 2005). They are generalist predators and scavengers. Foragers

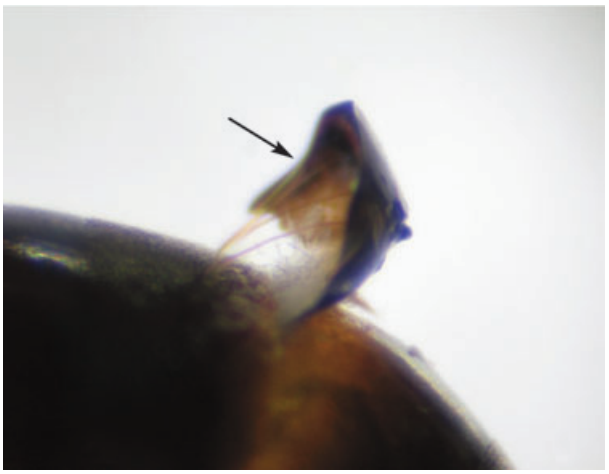

Fig. 5. Cephalotheca of *Myrmecolax incautus* Oliveira & Kogan, partly attached to ant host *Pachycondyla* (arrow) (×60).

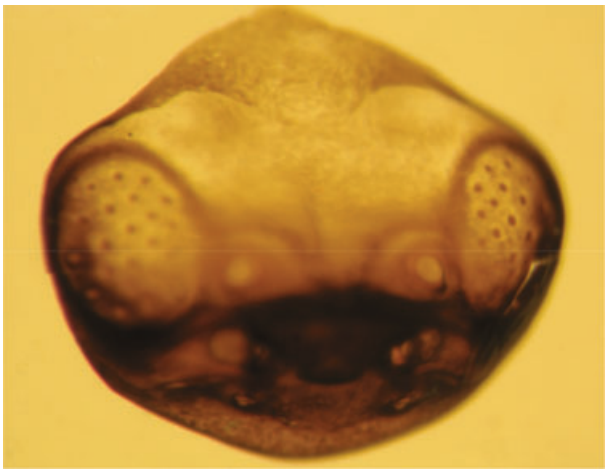

Fig. 6. Cephalotheca of male *Myrmecolax incautus* Oliveira & Kogan (arrow) (×60).

are diurnal and solitary, and individual foragers show strong fidelity over time to a particular area (Fresneau, 1985). The nests are in dead wood or in hollow twigs (Delabie *et al*., 2008). Colonies are usually small, with fewer than 200 workers (Fresneau, 1985; Goss *et al*., 1989; Dietemann & Peeters, 2000). This is the first record of the host of a *Myrmecolax.*

Collection of nests. The nests of *P. verenae* and *P. apicalis* were located in the field by following solitary foraging workers. In order to facilitate the monitoring of the foragers, bait consisting of small pieces of apple (with honey) that contrast with the dark coloration of the ants and litter was employed (Fresneau, 1985, 1994). When provided with bait, the ants return to the nest quickly. Most nests of *Pachycondyla* are in rotten logs (Delabie *et al*., 2008). When the nests were accessible, the whole stem was taken back for examination, or the log was opened up and the contents placed in a plastic bag. After about half an hour, near the nest location, the foraging workers (which were absent at the time of the nest collection) were found returning to the nest, and were collected.

In the laboratory, the colonies were reared in artificial, plastered nests fitted out with a foraging area, at 25° ± 2°C, with about 65 ± 10% relative humidity and a 12L : 12D photoperiod. The stylopized ants were found outside the nest, in the foraging arena, around 1 month after being collected in the field. Furthermore, an adult male *M. incautus* was found in the foraging area of a *P. apicalis* nest.

Behaviour of stylopized hosts. Although ants form the largest number of invertebrates in most habitats, single stylopized ants have seldom been encountered or collected in the field, unlike stylopized bees, wasps, and leaf/planthoppers (Kathirithamby & Hamilton, 1992). It is hypothesized that stylopized ants do not abandon and wander away from the nest (Kathirithamby, 2005, 2009). However, just before the emergence of the free-living male strepsipteran from the partially endoparasitic puparium, the ant leaves the nest, and it is its behaviour during this period that was observed by Ogloblin (1939). Ogloblin stated that, 'when ants are stylopized, they change their nocturnal habits, acquire positive phototropism, and lose their social instincts, abandoning their nests and rambling singly, often climbing high on grass and bush'. He said that this change in behaviour was why stylopized ants were never found when whole nests were examined by myrmecologists.

In recent studies of the collection and dissection of whole nests of ants, however, a large proportion of ants with extruded cephalotheca were found within the nest (Kathirithamby, 1991; Kathirithamby & Johnston, 1992, 2004; Kathirithamby & Hughes, 2002; Hughes *et al*., 2003). It is hypothesized that the stylopized ants went unnoticed by myrmecologists because the extruded male cephalotheca is cryptic, and is the same colour as the cuticle of the host ant. If a stylopized ant were to desert the nest soon after the extrusion of its cephalotheca, it would need to wander around in the vegetation for a long while, until completion of the (extended) pupal stage of the male myrmecolacid. During this period, the host ant might be vulnerable to predation (Kathirithamby & Hamilton, 1992). Stylopized ants are therefore thought to remain in the nest until just before the emergence of the male strepsipteran, and this is the reason why wandering stylopized ants are seldom encountered in the field.

On extrusion of the cephalotheca of the male *M. incautus*, the host ant remained in/on the nest, stopped working, and was stationary for most of the time. Prior to the emergence of the male *M. incautus* the stylopized ant was active, running along the foliage of a plant in order to reach a high point (Info S1). This is the first recording of the behaviour of a stylopized ant directly before the emergence of a male strepsipteran.

Collection of taxa. FRENCH GUYANA: Camp Patawa, N4°32′40.5″, W 52°09′08.4″, 14.iii.07; Forêt Kerrenroch, Petit Saut, N5°04′09.7″, 18.ii.2007 (*R. S. Ferreira*, *D. Fresneau*, *P. Devienne).*

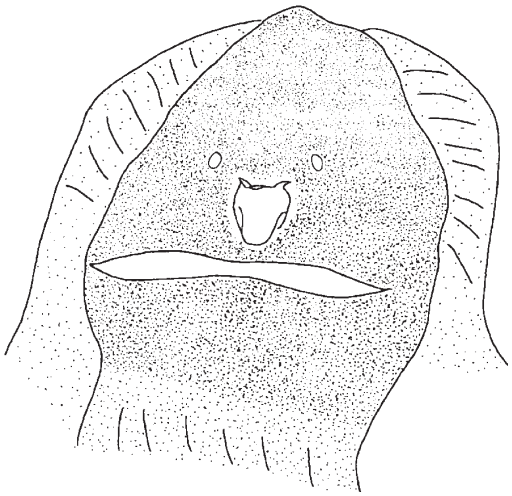

Fig. 7. Female *Myrmecolax incautus* Oliveira & Kogan, cephalothorax (×70).

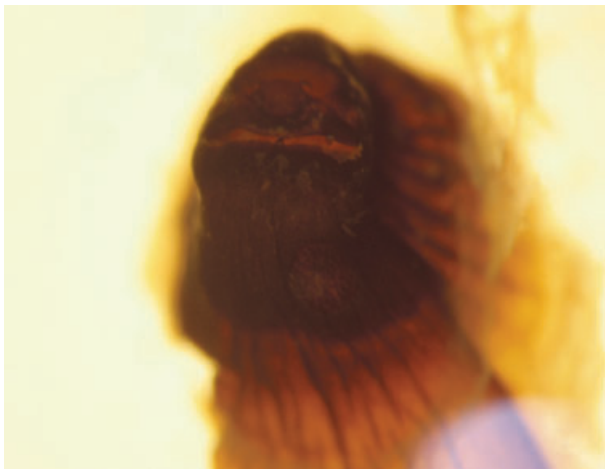

Fig. 8. Female *Myrmecolax incautus* Oliveira & Kogan, cephalothorax (×43).

Neotenic ♀ cephalothorax (Figs 7, 8). (i) The cephalothorax is as long as wide (width/length = 0.7 mm). The extruded cephalothorax is raised from the host abdomen and is dark brown in colour with a conical anterior region. There is a pair of mandibles, and, in the central region, the hypopharynx; below that, an elongated brood canal opening (length = 0.75 mm; width at mid-point 70 mm). This is the first description of a female cephalothorax of a *Myrmecolax*, and the second unequivocal match of a female to its conspecific male (Table 3).

Diagnosis of host of female. Stagmatoptera (Mantodea: Mantidae: Stagmatopterinae) (Reinhard Ehrmann, personal communication). Total length 7 cm. Wings light green in colour, with a brown body. Head without projections and eyes rounded. Pronotum elongated, with dilation rounded, anterior

Fig. 9. *Stagmatoptera* sp. (Mantodea: Mantidae), host of female *Myrmecolax incautus* Oliveira & Kogan (total length = 7 cm).

edge not broader than posterior, finely toothed along the edge for about two-thirds of length. Coxae unarmed and anterior coxa without distal expansion. Anterior femora with no deep pit, posterior femur not lobed and internal face of femur plain. Anterior tibia not compressed, external margin with 8 spines, and internal margin with 15. Cerci cylindrical.

The closest sequence match identified by Genbank was 92% similarity to *Stagmatoptera* sp. (Mantodea: Mantidae) (Fig. 9) over 594 bp; the gene examined was for the mitochondrial cytochrome oxidase (Svenson, G. J. & Whiting, M. F. MN117, unpublished).

Collection of taxa. BRAZIL: EMPARN-Jiqui–city of Parnamirim, state of Rio Grande do Norte, 5°55′36″S, 35°11′33″W, Malaise trap, 25.vi.2004 (*R. Andreazze, H. T. A. Andrade & M. G. Pinheiro*).

First instar larva (Figs 10–12). Length 130 μm. One of the smallest strepsipteran first instar larvae known: Pohl & Beutel (2005) say that the average size of a first instar larva is 230 μm; the smallest is *Caenocholax fenyesi* sensu lato (c. 80 μm). Medinally the maxilla is fused with a pair of maxillary palps, and mandibles are teeth-like. Maxilla and labium, and maxilla and head capsule are not fused. A pair of setae is present on the labium. Microtrichia are reduced on both the dorsal and ventral surfaces of the head, thorax and abdomen. Large plates (sternites) separate the coxa of each leg. There are three short stout spines on the coxa and one on the trochanterofemur. Tarsi of the pro- and mesothoracic legs are enlarged, with the prothoracic tarsus being round and the mesothoracic triangular, whereas the metathoracic tarsi are elongated with a terminal lobe. A pair of setae is present on the lateral abdominal tergites and sternites, and the caudal margin of the abdominal sternite has short spinulae. The IXth abdominal tergite and sternites are equipped with a pair of setae that are positioned latero-medinally and extend as far as the posterior margin of the

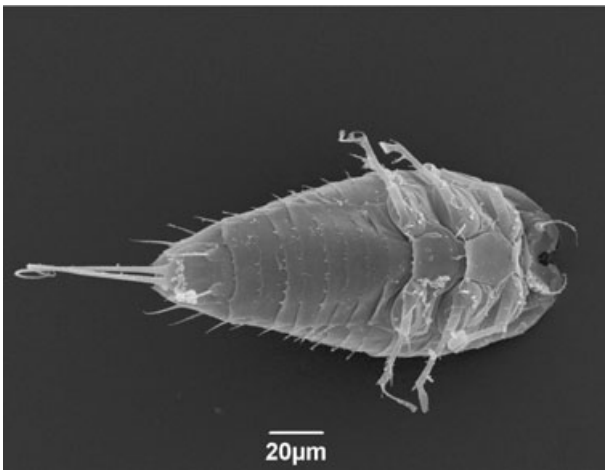

Fig. 10. SEM of first instar larvae of *Myrmecolax incautus* Oliveira & Kogan, ventral view (scale bar = 20 µm).

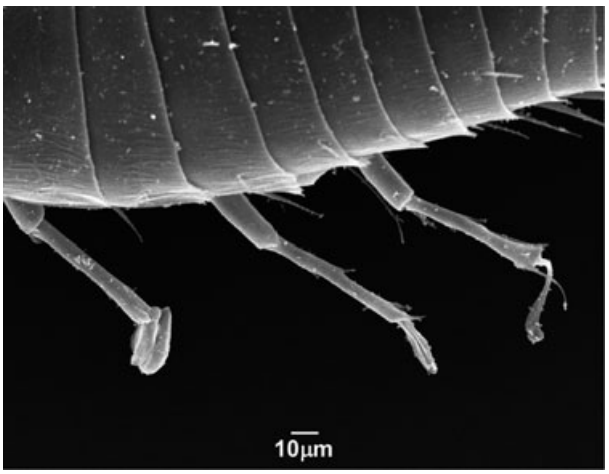

Fig. 11. SEM of first instar of *Myrmecolax incautus* Oliveira & Kogan, lateral view of legs (scale bar = 10 µm).

XIth segment. Abdominal sternites IX + X are fused and have no segmental border. A pair of long medial caudal setae is present on the XIth sternite, but lateral caudal setae are absent. The base of the Xth abdominal tergite is equipped with a pair of short, pointed projections that are lateral in position. This is the first description of the first instar larva of a *Myrmecolax.*

DNA analysis. Two overlapping partial gene sequences for the mitochondrial cytochrome c oxidase subunit I gene (COI) were amplified for the male strepsipteran parasitic in the ants *Pachycondyla verenae* and *P. apicalis*, and for the female strepsipteran parasitic in the mantid *Stagmatoptera* sp. The first fragment was amplified, using degenerate primers based on the primer combination LCO-1490 (Folmer *et al*., 1994) and C1-N-2191 (alias Nancy) (Simon *et al*., 1994), and the second using degenerate primers based on C1-J-2183 (alias Jerry) (Simon *et al*., 1994) and C1-J-2792 (alias George) (Bogdanowicz *et al*., 1993).

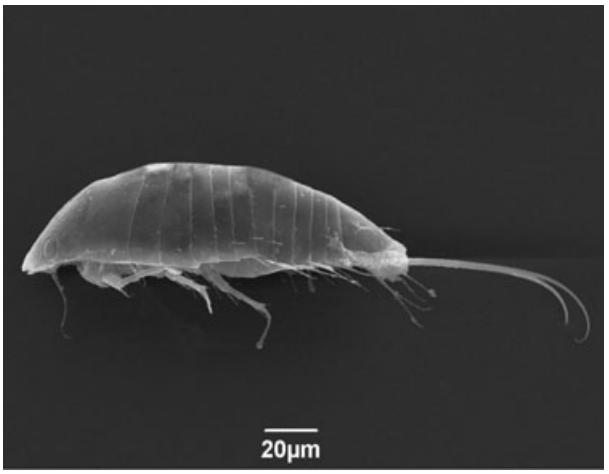

Fig. 12. SEM of first instar of *Myrmecolax incautus* Oliveira & Kogan, lateral view (scale bar = 20 µm).

Over the 1202 total base pairs sequenced for COI, 15 single nucleotide polymorphisms were observed (13 polymorphisms at third codon positions, and two at first positions), which equates to 1.2% sequence divergence. Sequences from other myrmecolacid taxa so far studied display divergences in the range of 0 to 3.2% within phylogenetically determined sequence clusters, and greater than 8.1% between clusters, in some cases with distinct morphological differences accompanying specimens belonging to different clusters (in preparation). Thus, 1.2% divergence places the sequences observed in this study within the range of what we consider intraspecific variation for other myrmecolacid taxa.

Partial gene sequences were also amplified for the (18S) rRNA gene, in two non-overlapping fragments. The first fragment was amplified using the primer combination 18S-23 and 18S-615; and the second using 18S-849 and 18S-1574, corresponding roughly to regions V1-2 and V3-4. All rDNA primers were designed from pre-existing strepsipteran 18S data, and information from secondary structural conservation. Primer sequences were as follows:

18S-23–GGATCCTGGCAGTAGTTATATG
18S-615–GTAGRCATGTAAYCTACCATCG
18S-849–CGGTAATTCCAGCTCCATTAG
18S-1574–GTACGAATGCCCCCATCCG

The resultant sequences of 656 and 629 bp were identical between the two specimens. A number of nucleotide ambiguities were also strongly supported in both specimens, suggesting that polymorphic positions across rDNA genes were also shared. Phylogenetically determined sequence clusters from other myrmecolacid taxa display similar levels of sequence identity (always less than 0.5%). Between clusters, however, RNA fragments vary greatly in length, as well as in composition (in preparation). Length variation between sequence clusters can be as great as 118 and 449 bp across first and second fragments respectively, with levels of sequence divergence being no lower than 10.9% (first fragment). In a similar fashion, therefore, identitiy between 18S rRNA fragments

places the sequences observed in this study within the range of what we consider intraspecific variation for other myrmecolacid taxa.

Voucher specimens. (i) FRENCH GUYANA: 2 ♂ cephalotheca of *Myrmecolax incautus* Oliveira & Kogan Camp Patawa (in alcohol), N4°32′40.5″, W52º09′08.4″, 14.iii.2007; Forêt Kerrenroch, Petit Saut, N5°0409.7, 18.iii.2007 (*R. S. Ferreira, D. Fresneau, P. Devienne*) (Hope Entomological Collections, Oxford University Museum of Natural History, Oxford). (ii) FRENCH GUYANA: 2 adult ♂ *Myrmecolax incautus* Oliveira & Kogan, Camp Patawa (in alcohol), N4°32′40.5″, W52º09′08.4″, 14.iii.2007; Forêt Kerrenroch, Petit Saut, N5°0409.7, 18.iii.2007 (*R. S. Ferreira, D. Fresneau, P. Devienne*); Hope Entomological Collections, Oxford University Museum of Natural History, Oxford). (iii) BRAZIL: 1 ♀ cephalothorax of *Myrmecolax incautus* Oliveira & Kogan, EMPARN-Jiqui–city of Parnamirim, state of Rio Grande do Norte (in alcohol), S5°55′36″S, W35°11′33″W, Malaise trap, 25.vi.2004 (*R. Andreazze, H. T. A. Andrade, M.G. Pinheiro*) (Hope Entomological Collections, Oxford University Museum of Natural History, Oxford).

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-3113.2009.00507.x

Info S1. A stylopized *Pachycondyla* active just before emergence of male *Myrmecolax incautus* (taken by R. S. Ferreira).

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Résumé : La région Néotropicale abrite une extraordinaire diversité biologique, dont une grande partie encore non identifiée. En outre, parmi les espèces déjà inventoriées, plusieurs groupes sont soumis à des classifications imparfaites. Ceci est particulièrement dû à l'existence d'espèces cryptiques formant des complexes d'espèces qui empêchent l'évaluation correcte de la biodiversité. Les fourmis primitives du complexe *Pachycondyla apicalis* sont un bon exemple de ce fait et leur taxonomie n'est pas consensuelle. Etant donné que l'identification exacte des espèces est essentielle à la fois à la recherche dans tous les domaines de la biologie et à la conservation de la biodiversité, l'un des objectifs premiers de ce travail a été la détermination de la diversité cryptique au sein de ce complexe d'espèces. L'étude des organes producteurs de son et des signaux stridulatoires ont démontré que ces espèces morphologiquement semblables possèdent des différences spécifiques nettes dans leurs systèmes de communication acoustique respectifs. Les résultats acoustiques ont été corroborés par des analyses moléculaires, confirmant ainsi que la bioacoustique est un outil potentiel pour la détermination des espèces cryptiques dans ce groupe de fourmis. Ce résultat est la première indication d'un tel degré de spécialisation dans les signaux stridulatoires des fourmis, ce qui suggère que les stridulations peuvent présenter un rôle encore plus important dans la vie de ces espèces. Bien qu'indiscernables pour l'œil humain, ces espèces cryptiques sont évidemment dissemblables les unes des autres. De ce fait, nous avons pu au travers d'études bioacoustiques, génétiques, chimiques, écologiques et comportementales caractériser des éléments de la biologie de ces espèces pouvant être considérés comme indicateurs de l'espèce, et ceci également dans le but de mieux comprendre les interactions intra- et interspécifiques dans ce groupe d'espèces phylogénétiquement proches.

Cryptic diversity, bioacoustics and intra and interspecific interactions in the primitive Neotropical species complex *Pachycondyla apicalis* (Hymenoptera: Formicidae: Ponerinae)

Abstract: The Neotropical region is home to an extraordinary biological diversity, much of which still unidentified. In addition, among the species already described, several groups are subject to imperfect classifications. This is particularly due to the existence of cryptic species which form species complex that prevent the correct assessment of biodiversity. The primitive ants from the Neotropical *Pachycondyla apicalis* complex are a good example of this fact and their taxonomy is still unsolved. Since the correct identification of species is essential both for research in all fields of biology and the conservation of biodiversity, one of the primary objectives of this work was the determination of cryptic diversity within this species complex. The study of the stridulatory organs and signals showed that these morphologically similar species have clear specific differences in their respective acoustic communication systems. The acoustic results were corroborated by molecular analysis, confirming bioacoustics as a potential tool for the determination of cryptic species in this group of ants. This is the first indication of such a degree of specialization in the stridulatory signals of ants, suggesting that stridulations may have an even more important role in the lives of these species. Even if indistinguishable to the human eye, these cryptic species are obviously dissimilar from each other. Thus we were able through acoustic, genetic, chemical, ecological and behavioural studies to characterize biological elements of these species that can be considered as indicators of the species, and this also in order to better understand intra and interspecific interactions in this group of phylogenetically related species.

Mots-clés: *Pachycondyla apicalis*, *P. verenae*, *P. obscuricornis*, insectes sociaux, fourmis, espèces cryptiques, bioacoustique, stridulations, phylogénie moléculaire, morphométrie, hydrocarbures cuticulaires, écologie, spéciation, comportement, discrimination, cher ennemi, interactions.

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