A molecular phylogeny of *Dorylus* army ants provides evidence for multiple evolutionary transitions in foraging niche

Daniel JC Kronauer*1, Caspar Schöning1, Lars B Vilhelmsen2 and Jacobus J Boomsma1

Address: 1Institute of Biology, Department of Population Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark and 2Zoological Museum, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark

Email: Daniel JC Kronauer* - djckronauer@bi.ku.dk; Caspar Schöning - cschoning@bi.ku.dk; Lars B Vilhelmsen - lbvilhelmsen@zmuc.ku.dk; Jacobus J Boomsma - jjboomsma@bi.ku.dk

* Corresponding author

Abstract

**Background:** Army ants are the prime arthropod predators in tropical forests, with huge colonies and an evolutionary derived nomadic life style. Five of the six recognized subgenera of Old World *Dorylus* army ants forage in the soil, whereas some species of the sixth subgenus (*Anomma*) forage in the leaf-litter and some as conspicuous swarm raiders on the forest floor and in the lower vegetation (the infamous driver ants). Here we use a combination of nuclear and mitochondrial DNA sequences to reconstruct the phylogeny of the *Dorylus s.l.* army ants and to infer the evolutionary transitions in foraging niche and associated morphological adaptations.

**Results:** Underground foraging is basal and gave rise to leaf-litter foraging. Leaf-litter foraging in turn gave rise to two derived conditions: true surface foraging (the driver ants) and a reversal to subterranean foraging (a clade with most of the extant *Dorylus s.s.* species). This means that neither the subgenus *Anomma* nor *Dorylus s.s.* is monophyletic, and that one of the *Dorylus s.s.* lineages adopted subterranean foraging secondarily. We show that this latter group evolved a series of morphological adaptations to underground foraging that are remarkably convergent to the basal state.

**Conclusion:** The evolutionary transitions in foraging niche were more complex than previously thought, but our comparative analysis of worker morphology lends strong support to the contention that particular foraging niches have selected for very specific worker morphologies. The surprising reversal to underground foraging is therefore a striking example of convergent morphological evolution.

Background

Army ants are functionally defined by a suite of interrelated behavioural and morphological traits [1]: they are obligate group predators, they frequently emigrate to new nest sites, and their queens are permanently wingless and highly specialized egg-layers. Some species conduct spectacular swarm raids, in which hundreds of thousands of ants form a dense carpet that sweeps through areas of 1000 m² or more on a single day in search of prey. However, this remarkable foraging behaviour is found in
rather few species, as most army ants have an inconspicuous, completely underground lifestyle.

Army ants in the dorylomorph clade (sometimes referred to as the “true” army ants) form a monophyletic group that originated in Gondwana in the mid-Cretaceous, slightly over 100 million years ago [2,3]. With the subsequent break-up of this supercontinent, the clade split into the New World army ants (subfamily Ectitinae) and the Old World army ants. The latter comprises three monophyletic subfamilies, the Dorylinae (with a primarily Afro-tropical distribution), the Aenictinae (primarily distributed in Asia and the Oriental region with fewer African species), and the enigmatic subfamily Aenictogitoninae, which is only known from nocturnal males that have been sporadically collected at lights in sub-Saharan Africa, and which has been inferred to be the sister taxon to the Dorylinae [2,4,5]. Whether the Aenictogitoninae have all the defining army ant traits will thus not be known until colonies with workers and queens have been discovered.

The subfamily Dorylinae consists of the single genus Dorylus, which has been subdivided into six subgenera [6,7]. This subdivision has, however, not become generally accepted [8]. Five of these subgenera are entirely subterranean, but the sixth subgenus Anomma encompasses both species that hunt in the leaf-litter and species that forage on the forest floor and in the vegetation. The latter are the infamous “driver ants” [9], renown for their massive swarm raids that overwhelm most invertebrates that do not get out of their way and occasionally also small vertebrates [1].

A major reason for the confused taxonomy and classification of Dorylus s.l. is that most of the recognized species have only been described from a single caste or sex [7]. The large and distinctive males, which were originally described by Linné [10] as Vespa wasps and are known in Africa as “sausage flies”, are easily collected at light sources during the night, but have rarely been taken from nests together with workers or queens. The extreme worker polymorphism within colonies and the difficulty of finding the largest workers among the foragers of subterranean species poses an additional challenge to resolving the phylogenetic relationships between species and species-groups with morphological data, because valid comparisons of homologous character states can only be made between workers of the same size. Finally, similar worker morphologies in different species might reflect convergent adaptations to specific foraging niches and prey spectra rather than common ancestry.

In the present study we use DNA sequence data to overcome the problems mentioned in the previous paragraph and to obtain an accurate and unbiased phylogeny for the genus Dorylus. We use this phylogeny to reconstruct the evolution of the driver ants from their subterranean ancestors, and to infer the morphological changes that have occurred as putative adaptations to changes in foraging niche. The DNA sequences also allow us to establish new associations between males and workers for several species across the different subgenera, thus helping to clarify Dorylus taxonomy.

Results

Phylogenetic analyses
The maximum likelihood (ML) topology (likelihood score 17554.6) is given in Fig. 1 together with Bayesian posterior probabilities and ML and maximum parsimony (MP) bootstrap values for all nodes. All three methods recovered very similar topologies: disagreements were restricted to a few terminal taxa, mainly within the driver ant clade, and none of these affected the conclusions to be drawn.

The total evidence indicates that: 1. The Southeast Asian monotypic subgenus Dichthadia is sister to the remaining Dorylus s.l. species (although the respective node was collapsed in ML bootstrap analysis), confirming an earlier inference by Brady [2]. 2. Each of the subgenera Alaopone, Typhlopone, and Rhogmus is monophyletic according to the taxon sample obtained for the present study. 3. The species of the subgenus Anomma that forage in the leaf-litter are more closely related to subterranean species of the subgenus Dorylus s.s., than to the surface swarm foraging Anomma driver ants, which form a well supported clade. The close phylogenetic relationship between the leaf-litter Anomma species and the subgenus Dorylus s.s. (without D. spininodis – see below) also emerges from the position of the male of D. gribodoi, which clearly belongs to a clade of leaf-litter Anomma species, although it was originally described as belonging to the subgenus Dorylus s.s.. 4. While most species of the subgenus Dorylus s.s. form a clade nested within the leaf-litter Anomma species, D. (Dorylus) spininodis is more distantly related and may be the sister taxon to the remaining species of Dorylus s.s. plus Anomma (although this inferred relationship has low statistical support and D. spininodis may even be more distantly related; Fig. 1). This implies that the subgenus Anomma is paraphyletic and the subgenus Dorylus s.s. is polyphyletic.

Male-worker associations
Pair-wise genetic distances of mitochondrial sequences between closely related male-worker pairs were clearly bimodally distributed with five values ≤ 0.003 and the remaining five values ≥ 0.044 (Table 1). Using a 0.014 reference value for two distantly related specimens of Dorylus (Anomma) molestus from the same population, we concluded that the first five pairs represent workers and males
of the same species. Some of the latter pairs may eventually turn out to belong to the same species as well once additional data become available, because most of these worker and male specimens came from distant populations (Table 2) so that our threshold value for sympatric conspecifics is probably very conservative.

Reconstructing evolutionary transitions in foraging niche

MP and Bayesian analyses unambiguously reconstructed three transitions in foraging niche (Fig. 2). The first transition from subterranean to leaf-litter foraging took place between 34.1 and 29.9 million years ago (mya) (possible range: 47.9 – 19.8 mya) along the branch leading to the clade of Anomma plus Dorylus s.s. (without D. spininodis). The second transition, a reversal from leaf-litter foraging to subterranean foraging occurred between 22.9 and 16.8 mya (possible range: 32.2 – 11.3 mya) along the branch leading to the Dorylus s.s. clade (again without D. spininodis). Finally, a transition from leaf-litter foraging to surface swarm raiding occurred between 29.9 and 16.9 mya (possible range: 42.0 – 11.1 mya) along the branch leading to the driver ant clade.

Morphological analyses

The variation in maximum head width, antennal scape length, mandible length, and hind leg length was strongly correlated with foraging niche (Nested ANOVA, p < 0.025 for all traits). When comparing these traits between the
species that reversed to subterranean foraging from leaf-litter foraging and the extant leaf-litter species, we found that all measurements were significantly smaller in the former category (Fig. 3; Tukey's Studentized Range Test, p < 0.001 in all cases). In fact, maximum head width, antennal scape length and hind leg length were all intermediate between the extant leaf-litter foragers and the originally subterranean species, while mandible length was even smaller in the reversed than in the originally subterranean species. With the reversal to subterranean foraging, species of the subgenus Dorylus s.s. thus secondarily evolved a similar morphology in four key traits related to foraging performance and these convergent adaptations shifted trait values fully back to or even beyond the values that characterize the species that inherited subterranean foraging directly from the common ancestor of all Dorylus s.l. species.

**Discussion and Conclusion**

This study demonstrates that the currently recognized subgenera Anomma and Dorylus s.s. are not monophyletic. The driver ants of the subgenus Anomma clearly form a separate clade from the Anomma species that forage in the leaf-litter, which are more closely related to the subterranean species of the subgenus Dorylus s.s. (Fig. 1). The confusion about the subgenera Anomma and Dorylus s.s. can be traced back to the original descriptions of the leaf-litter Anomma species D. kohli, D. gerstaeckeri, and D. emeryi and of the subterranean D. (Dorylus) spininodis. Already Wasmann [11] pointed out that the workers of D. kohli seemed transitional between driver ants and Dorylus s.s. in both morphology and foraging habits. Because he assumed that the largest workers, which were not available to him for examination, would resemble those of driver ants, he assigned the species to Anomma [11]. Similarly, Emery [12] noted that workers of D. gerstaeckeri did not fit well with his diagnosis of Anomma and our present findings indicate that the male that he described as D. (Dorylus) gribodoi might in fact be the male of D. (Anomma) gerstaeckeri (Fig. 1, Table 1; although the genetic distance does not fall below our conservative cutoff value). Furthermore, Mayr [13] described Dorylus emeryi without reference to any subgenus, but the species was later assigned to Anomma without explicit justification [6,14]. Finally, males of D. kohli have been collected together with workers by J. van Boven and are now shown to be identical to males that have originally been described as a Dorylus s.s. species (W. Gotwald and CS, unpublished). It has thus always been acknowledged that males of leaf-litter Anomma species are morphologically more similar to males of the subgenus Dorylus s.s. than to the males of Anomma driver ants, but their correct associations were not established until the present study.

At the same time spurious assumptions and imprecise subgenus definitions have led to the incorrect assignment of the workers of leaf-litter species to the subgenus Anomma. While the workers of Dorylus spininodis (along with those of D. politus, which could not be included in this study) were recognized as clearly distinct from the Dorylus s.s. species of the "D. affinis species group" because they lack caudally projecting spines at the frontal carinae (a pair of longitudinal ridges between the antennal sockets and the anterior sclerite), the species was nevertheless assigned to the subgenus Dorylus s.s. [15]. We thus conclude that the subgenera Anomma and Dorylus s.s. are not monophyletic, that suspicions of this have existed for a long time but only make sense in light of our present data, and that the current subgenus classification cannot be upheld. We will formally propose the necessary taxonomic and nomenclature changes elsewhere.

Our results further show that the differences in life-style between Dorylus s.l. species are not merely the result of a simple unidirectional development from subterranean foraging to surface swarm raiding, as has been assumed so

| Worker          | Male                     | p-Distance |
|-----------------|--------------------------|------------|
| D. (An.) molestus 1 and 2 | -                       | 0.014      |
| D. (Rh.) sp. 1  | D. (Rh.) laevipodex      | 0.000*     |
| D. (An.) terrificus | D. (An.) sp. 1           | 0.000*     |
| D. (Al.) conradi | D. (Al.) sp. 2           | 0.002*     |
| D. (Ty.) fulvus  | D. (Ty.) sp. 3           | 0.002*     |
| D. (Do.) sp. 1  | D. (Do.) affinis         | 0.003*     |
| D. (Ty.) sp. 1  | D. (Ty.) sp. 2           | 0.044      |
| D. (An.) arcens | D. (An.) nigricans       | 0.048      |
| D. (An.) burmeisteri | D. (An.) nigricans     | 0.055      |
| D. (An.) gerstaeckeri | D. (Do.) gribodoi      | 0.063      |
| D. (Do.) sp. 2  | D. (Do.) sp. 5           | 0.073      |

Genetic distances were calculated from the mitochondrial DNA sequences. The distance between two worker specimens of Dorylus (Anomma) molestus from the same population at Mt. Kenya is included as a reference (in bold). Pair-wise distances below 0.014 were inferred to characterize pairs that belong to the same species and are marked with an asterisk.
analyses of litter foraging to subterranean foraging (Fig. 2). Previous far [16], but that there has also been a reversal from leaf-

Table 2: List of specimens used for DNA sequencing.

| Species/label | Sub-genus | Foraging niche | Specimen | Collection locality | GenBank accession numbers | Museum voucher no |
|---------------|-----------|----------------|----------|---------------------|--------------------------|------------------|
| wilerthi      | An.       | surface swarms worker | Kibale (Uganda) | EF413837 | EF413761 | EF413794 | EF413874 |
| terrificus    | An.       | surface swarms worker | Kibale (Uganda) | EF413842 | EF413767 | EF413804 | EF413880 |
| molestus 1    | An.       | surface swarms worker | Mt. Kenya (Kenya) | EF413809 | EF413732 | EF413772 | EF413848 |
| molestus 2    | An.       | surface swarms worker | Mt. Kenya (Kenya) | EF413836 | EF413760 | EF413797 | EF413873 |
| bermesteieri  | An.       | surface swarms worker | Comóe (Ivory Coast) | EF413808 | EF413731 | EF413771 | EF413847 |
| mayri         | An.       | surface swarms worker | Bossou (Guinea) | EF413844 | - | - | EF413882 |
| arcens        | An.       | surface swarms worker | Tai (Ivory Coast) | EF413829 | EF413753 | EF413791 | EF413867 |
| sjoestedi      | An.       | surface swarms worker | Ndoki (DR Congo) | EF413834 | EF413758 | EF413795 | EF413872 |
| rubellus      | An.       | surface swarms worker | Gashaka (Nigeria) | EF413833 | EF413757 | - | EF413871 |
| emeryi        | An.       | leaf-litter worker | Tai (Ivory Coast) | EF413810 | EF413733 | EF413773 | EF413849 |
| gerstaeckeri  | An.       | leaf-litter worker | Bossou (Guinea) | EF413812 | EF413736 | EF413776 | EF413852 |
| opacus        | An.       | leaf-litter worker | Kibale (Uganda) | EF413813 | EF413737 | EF413777 | EF413853 |
| kohli         | An.       | leaf-litter worker | Kibale (Uganda) | EF413814 | EF413738 | EF413778 | EF413854 |
| nigricans     | An. n. a. | male | Tai (Ivory Coast) | EF413841 | EF413766 | EF413803 | EF413879 |
| sp. 1         | An. n. a. | male | Kibale (Uganda) | EF413843 | EF413768 | EF413805 | EF413881 |
| sp. 2         | Do.       | subterranean worker | Mt. Kenya (Kenya) | EF413811 | EF413735 | EF413775 | EF413851 |
| sp. 3         | Do.       | subterranean worker | Bossou (Guinea) | EF413815 | EF413739 | EF413779 | EF413855 |
| helvolus      | Do.       | subterranean worker | Cullinan (South Africa) | EF413832 | EF413736 | EF413794 | EF413870 |
| braunsi       | Do.       | subterranean worker | Kakamega (Kenya) | EF413835 | EF413739 | EF413796 | EF413857 |
| spinodinis    | Do.       | subterranean worker | Gashaka (Nigeria) | EF413827 | EF413751 | EF413865 | EF413865 |
| gribboi       | Do. n. a. | male | Tai (Ivory Coast) | EF413817 | EF413741 | EF413781 | EF413867 |
| affinis       | Do. n. a. | male | Mt. Kenya (Kenya) | EF413846 | EF413770 | EF413807 | EF413884 |
| sp. 4         | Do. n. a. | male | Mt. Kenya (Kenya) | EF413816 | EF413740 | EF413780 | EF413856 |
| sp. 5         | Do. n. a. | male | Gashaka (Nigeria) | EF413845 | EF413769 | EF413806 | EF413883 |
| sp. 1         | Rh. n. a. | male | Mt. Kenya (Kenya) | EF413818 | EF413752 | EF413782 | EF413858 |
| laevipodex    | Rh. n. a. | male | Mt. Kenya (Kenya) | EF413840 | EF413764 | EF413801 | EF413877 |
| sp. 2         | Rh. n. a. | male | Gashaka (Nigeria) | EF413831 | EF413755 | EF413793 | EF413869 |
| laevigatus    | Di.       | subterranean worker | Poring (Malaysia) | EF413819 | EF413743 | EF413783 | AY233632 |
| fulvus        | Ty.       | subterranean worker | Mt. Kenya (Kenya) | EF413813 | EF413734 | EF413774 | EF413850 |
| sp. 1         | Ty.       | subterranean worker | Gashaka (Nigeria) | EF413828 | EF413752 | EF413790 | EF413866 |
| sp. 2         | Ty. n. a. | male | Ile-Ile (Nigeria) | EF413838 | EF413762 | EF413799 | EF413873 |
| sp. 3         | Ty. n. a. | male | Mt. Kenya (Kenya) | EF413836 | EF413765 | EF413802 | EF413878 |
| conradi       | Al.       | subterranean worker | Kakamega (Kenya) | EF413820 | EF413744 | EF413860 | EF413860 |
| cf. vishnui   | Al.       | subterranean worker | Poring (Malaysia) | EF413821 | EF413745 | EF413784 | EF413861 |
| orientalis    | Al.       | subterranean ? | ? | AY233706 | AY233706 | - | AY233631 |
| sp. 1         | Al. n. a. | male | Gashaka (Nigeria) | EF413820 | EF413754 | EF413792 | EF413868 |
| sp. 2         | Al. n. a. | male | Kakamega (Kenya) | EF413839 | EF413763 | EF413800 | EF413876 |
| Aenictus sp.  | n. a. n. a. | worker | Mt. Kenya (Kenya) | EF413822 | EF413746 | EF413785 | EF413862 |
| A. aratus     | n. a. n. a. | worker | Tawau Hills (Malaysia) | EF413824 | EF413748 | EF413787 | AY233628 |
| A. laeviceps  | n. a. n. a. | worker | Poring (Malaysia) | EF413823 | EF413747 | EF413786 | AY233627 |
| Eciton burchelli | n. a. n. a. | worker | Henri Pittier (Venezuela) | EF413825 | EF413749 | EF413788 | EF413863 |

Specimens have been deposited at the Zoological Museum in Copenhagen, numbered consecutively with a reference to this publication (Kronauer et al. 2007/1 – Kronauer et al. 2007/41). Abbreviations of subgenera are Anomma (An.), Dorylus s.s. (Do.), Rhogmus (Rh.), Dichthadia (Di.), Typhlopone (Ty.), and Aloepon (Al.). Foraging niches were not applicable (n. a.) for males and outgroup taxa.

far [16], but that there has also been a reversal from leaf-litter foraging to subterranean foraging (Fig. 2). Previous analyses of Dorylus s.l. worker morphology already showed that shifts in foraging niche are correlated with changes in several morphological adaptations [17]. Longer hind legs increase running speed on the soil surface and allow the ants to carry larger prey items [18], whereas shorter legs are probably adaptive in subterranean species which hunt in narrow tunnels [19]. Similarly, larger heads can accommodate larger mandibular muscles so that workers can bite more fiercely [20]. Large heads and mandibles are thus likely to be adaptative in driver ants, which have to defend their nests and foraging or migration columns against vertebrate predators (such as chimpanzees, gorillas, Jackson's mongooses and pangolins) [1,21] and that benefit from being able to cut up larger prey. Longer antennae, on the other hand, may allow workers to follow pheromone trails at higher speed while avoiding collisions [22], which seems adaptive on open trails with a high volume of traffic. While an earlier analysis [17] treated all species with subterranean foraging as a single monophyletic group, our present phylogenetic study shows that the subterranean Dorylus s.l. species consist of two clades and that one of them has secondarily re-evolved subterranean foraging and many of the morphological adaptations to an entirely subterranean life-
style (Fig. 2, Fig. 3). Our present findings thus give strong independent support for the inference of Schöning et al. [17] that many worker allometries of army ants represent adaptations to specific foraging niches, as we now know that the subterranean syndrome evolved twice convergently.

Our present study establishes that subterranean foraging is ancestral in Dorylus s.l. and that leaf-litter foraging and surface swarm raiding originated later. The imprecise age estimates resulting from the lack of Dorylus s.l. fossils make inferences regarding environmental conditions at the time of ecological niche shifts speculative. It is nevertheless interesting to note that the transition to surface swarm raiding and the reversal to subterranean foraging may have occurred at approximately the same time (Fig. 2). This raises the interesting question why one group of army ants reverted to the subterranean foraging niche, which was already occupied by distant relatives, roughly at the same time that a second group began to exploit the entirely new niche of surface swarm raiding. How foraging niches evolved in the other army ant lineages and whether the common ancestor of all army ants also hunted in the soil is as yet unknown. This information, combined with data on prey spectra and distributional ranges, will be crucial to infer under which conditions the army ant life-style

**Figure 2**

The evolution of foraging niche in *Dorylus s.l.* army ants. The most parsimonious reconstruction of the evolutionary transitions is illustrated by different branch colours, while the Bayesian reconstruction with the highest posterior probability is indicated with coloured rectangles at the relevant nodes. Age estimates (clocks) for key nodes are given in million years ago (mya), after fixing the most recent common ancestor of *Dorylus s.l.* at 45.6 mya [2]; see text for ranges. The phylogeny was obtained by Bayesian analysis of a dataset from which males had been excluded. Subgenera are abbreviated as in Fig. 1. Photographs to the right depict workers of representative species with different foraging niches and illustrate the differences in relative leg length, mandible length, and antennal scape length associated with the three foraging niches (all pictures are the same scale and all workers have a maximum head width of 2.27 mm; from top to bottom: 1. *D. (An.) arcens* (surface swarm raiding), 2. *D. (An.) gerstaeckeri* (leaf-litter), 3. *D. (Do.) affinis* (reversal subterranean) 4. *D. (Ty.) sp. 1* (original subterranean).
evolved, which general factors promoted the subsequent niche shifts in different lineages, and where these transitions took place. Finally, detailed comparative analyses of worker morphology in the other army ant clades are needed to establish whether they also show correlated shifts in foraging niche and morphological traits of workers.

Methods

Taxon sampling

Our sample for DNA sequencing consisted of a total of 38 Dorylus s.l. specimens, both males and workers, representing all six recognized subgenera (see Table 2). Eciton burchellii (Ecitoninae) and three species of Aenictus (Aenictinae) were used as outgroups, but no fresh material of Aenictogitoninae was available. All specimens were collected and stored in 96% ethanol until DNA extraction.

Molecular protocols

DNA was extracted from ant legs using the DNeasy® kit from QIAGEN®. Two fragments from the mitochondrial cytochrome oxidase I (COI) gene and one fragment from the mitochondrial cytochrome oxidase II (COII) gene were amplified in standard polymerase chain reaction (PCR), using primers LCO/HCO [23] and Jerry [24]/Ben [25] for COI and primers tRNALeu/Barbara [24] for COII. In addition, we amplified a fragment of the nuclear wingless (wg) gene using primers wg1/wg2 [26]. The tRNALeu primer
(5’ CAGATTAGCAATGAATTTAAGT 3’) was specifically designed for this study to replace primer George [24] to avoid the amplification of pseudogenes [27]. The annealing temperature was 45 °C for all mitochondrial primers and 58 °C for wg. We used a concentration of 2.5 mM MgCl₂ in all reactions. PCR products were purified using a MicroSpin™ kit from Omega Bio-Tek. Automated fluorescent dye sequencing was conducted on an Applied Biosystems 3130 × 1 Genetic Analyzer or reactions were sent to a sequencing facility (MWG-Biotech) using the forward primer and, in many cases, also the reverse primer. In several cases where clean sequences could not be obtained due to co-amplification of probable pseudogenes, PCR products were cloned using the TOPO TA Cloning® kit from Invitrogen® and sequences were obtained from one or several clones. We generated a final concatenated alignment of 1957 base pairs (bp) of sequence data, consisting of 1007 bp from COI, 547 bp from COII, and 403 bp from wg. Of these, 923 sites were variable and 740 sites were parsimony informative. Sequences have been deposited in GenBank and accession numbers are given in Table 2. All sequences used in this study are new, except for D. orientalis and the wg sequences for D. laevigatus, A. aratus, and A. laeviceps, which are from Brady [2].

**Phylogenetic analyses**

Several sequences with frame-shift mutations were discarded from the alignment as non-functional pseudogenes. The remaining DNA sequences were translated into amino acid sequences using the program MEGA 2.1 [28]. This did not reveal any stop codons that could have been indicative of additional pseudogenes, but made us exclude one more sequence from the alignment, because of an extremely high rate of unique amino acid substitutions (GenBank accession number [EF413886]). For several driver ant species, we obtained two alternative, potentially functional sequences for COII. One of them (obtained with primers George/Barbara) was identical across all species, indicating that it represented a nuclear pseudogene with a much lower mutation rate (GenBank accession number [EF413885]) [27], which we thus excluded. The second (obtained with primers tRNAleu/Barbara) was variable across species to an extent similar to what we observed for the other mitochondrial sequences and was thus assumed to be the true mitochondrial sequence.

Because all sequences are protein coding, alignment could be done unambiguously by eye in a text editor. To detect additional potential pseudogenes that would confound phylogenetic inference and to evaluate congruence among data sets, we conducted independent MP analyses of the single gene fragments with 1000 bootstrap replicates using heuristic searches, ACCTRAN character optimization and tree-bisection-reconnection branch swapping with steepest descent in PAUP*4.0b10 [29] with E. burchellii and three Aenictus species (Table 2) as outgroups. All well supported relationships were highly concordant across the different data partitions, except for two relationships within the Anomma driver ant clade (results not shown). This ambiguity was probably due to sequences for a number of driver ant taxa being unavailable in some of the data partitions as they had been excluded as probable pseudogenes (Table 2), which affected the resolution of relationships within that clade. However, some ambiguity in the precise relationships within the driver ants does not affect the overall conclusions of our study. We thus combined the data in a full evidence approach for all further analyses. The concatenated dataset was subjected to MP analysis in PAUP* as has been described for the single data partitions.

The best fitting model of DNA evolution for Bayesian analyses was selected for each codon position for both mitochondrial (COI + COII) and nuclear (wg) sequences with MrModeltest v2 [30], comparing 24 nested models with hierarchical likelihood ratio tests (hLRT). The best model to explain the data was a general time reversible model with an estimated proportion of invariable sites and gamma distributed rate heterogeneity (GTR + I + G) for first and second mitochondrial codon positions, a GTR + G model for third mitochondrial codon positions, a HKY model for first nuclear codon positions and a K80 model for second and third nuclear codon positions. Bayesian analyses were performed with MrBayes v3.1.2 [31]. To assure convergence of Markov Chain Monte Carlo runs we repeated the analysis three times beginning with independent starting trees. In each analysis, one cold and three heated chains were run in parallel for 1 × 10⁶ generations and trees were sampled every 100 generations. The first 1001 trees were discarded as burn-in and the consensus tree was calculated from the remaining 9000 trees in PAUP*4.0b10 [29]. E. burchellii was used as the single outgroup in MrBayes v3.1.2 [31], following [2-4].

For ML analyses, the best model of DNA evolution for the concatenated dataset was chosen among 56 nested models with Modeltest 3.7 [32]. The best model to explain the data, both according to hLRT and the Akaike information criterion, was a general time reversible model with an estimated proportion of invariable sites and gamma distributed rate heterogeneity (GTR + I + G). ML searches were performed in GARLI v0.942 [33], which implements the GTR + I + G model as default, using the same outgroups as in MP analyses. The run was repeated three times from random starting trees using default termination conditions. GARLI v0.942 [33] was also used to generate 1000 ML nonparametric bootstrap replicates which were used...
to calculate a majority rule consensus tree in PAUP*4.0b10 [29].

Male-worker associations
We computed uncorrected pairwise distances of mitochondrial DNA sequences between male and worker specimens in MEGA 2.1 [28] when the phylogenetic analyses indicated that they were closely related. We compared these distances to the genetic distance between two specimens of D. (Anomma) molestus collected from the same population at Mt. Kenya to identify a conservative cut-off point for inferring whether males and workers likely belonged to the same species.

Reconstructing evolutionary transitions in foraging niche
For the reconstruction of evolutionary transitions in foraging niche we excluded all males from the dataset, because the foraging niche of their associated workers was unknown (they had been light trapped) and in most cases the species was likely to be represented also by a worker specimen for which we had that ecological information. Data on foraging niche were based on direct observations or on information from the literature [17]. We investigated the evolution of foraging niche in Dorylus s.l. army ants under MP using the computer program MacClade 4.0 [34] on the Bayesian topology obtained from MrBayes v3.1.2 [31]. The latter procedure was also used to obtain Bayesian posterior probabilities of foraging niche at different nodes. Foraging niche was added as a standard character to the data matrix in a separate partition while constraining one node of interest in each individual run. We used the same model and chain parameters that have been described above. Ordered character states for foraging niche (subterranean – leaf-litter – surface swarm raiding) were assumed in all analyses, because it is most plausible that army ants adapt only gradually to more exposed foraging strata.

Morphological analyses
To evaluate the hypothesis that the relative dimensions of key morphological traits represent adaptations to different foraging niches, we examined the following traits in workers of thirteen species (Table 3): Maximum head width (the maximum measurable width across the head), mandible length (the distance between the apex of the left mandible and the proximal point of the ventral ridge when the mandibles are fully opened with forceps), antennal scape length (the maximum straight line scape length excluding the basal condyle), and hind leg length (the maximum length of the left hind leg from trochanter to tarsal tip in dorsal view with the leg fully extended).

For these morphometric analyses we selected about 100 workers (preserved in 70% ethanol) from at least three colonies (50 workers from a single colony if insufficient samples were available) from the same study site for each species to cover the entire worker size range as much as possible. Ideally, workers from more colonies per species should have been included in our analysis to better cover the total intraspecific variation of morphological traits, but this was not possible because most species are only rarely collected so that larger samples are not available. We assume, however, that our morphological analysis based on a few colonies is fairly representative for the species as a whole, because measurement data on driver ants show that variation within and between populations of the same species is much less than variation between species (C. Schöning, unpublished). All measurements were taken using a MS 5 Leica stereomicroscope fitted with an ocular micrometer, using methods recommended by Seifert [35] to minimise measurement errors (magnifications 10× – 64×). Dry mass (measured after drying specimens at 60°C for 48 h) was used as an indicator of overall body size. The statistical analysis followed Schöning et al. [17]. In order to compare the relative size of traits between workers of different species we first established the best fit model for raw linear data from all species combined over their common body size range (0.29 mg – 1.50 mg) as a function of dry mass1/3 by stepwise multiple regression. The relative residuals (absolute residuals divided by the predicted values) from this common regression model were then compared in a Nested ANOVA with foraging niche category (original subterranean – leaf-litter – surface swarm raiding – reversal subterranean) as a fixed factor and species as a random factor nested within foraging niche category. Tukey’s Studentized Range (HSD) Test was employed to examine differences between categories. Statistical analyses of morphometric data were performed with SAS (Version 9.1).

Divergence dating
The same dataset and topology that were used to reconstruct evolutionary transitions in foraging niche (see above) were also used for divergence dating analysis. Branch lengths were estimated in PAUP*4.0b10 [29] under maximum likelihood with a GTR + I + G model of DNA substitution. The model with a molecular clock enforced (-ln 15494.5) was compared with a model without a molecular clock (-ln 15433.9) using a likelihood ratio test. The null model of rate constancy (the molecular clock) was rejected at p < 0.001 (28 df). Prior to dating analyses, E. burchelli was pruned from the phylogram to avoid ambiguity about the placement of the root. Dating analyses were conducted with the program r8s v. 1.7 [36], using penalized likelihood [37], the TN algorithm and an additive penalty function. The smoothing parameter was chosen using cross-validation of parameter values as implemented in r8s v. 1.7 [36]. Solutions were checked with the “checkGradient” command. Because no Dorylus s.l. fossils are known that could have been used to cali-
Table 3: Samples used for morphometric measurements.

| Species       | Sub-genus | Foraging niche     | Number of specimens | Collection locality |
|---------------|-----------|--------------------|---------------------|--------------------|
| terrificus    | An.       | surface swarms     | 110                 | Kibale (Uganda)    |
| molestus      | An.       | surface swarms     | 102                 | Mt. Kenya (Kenya)  |
| mayri         | An.       | surface swarms     | 106                 | Bossou (Guinea)    |
| arcens        | An.       | surface swarms     | 106                 | Tai (Ivory Coast)  |
| emeryi        | An.       | leaf-litter        | 54                  | Tai (Ivory Coast)  |
| gerstaeckeri  | An.       | leaf-litter        | 104                 | Tai (Ivory Coast)  |
| kohli         | An.       | leaf-litter        | 102                 | Kibale (Uganda)    |
| braunsi       | Do.       | subterranean       | 50                  | Kakamega (Kenya)   |
| sp. 1         | Do.       | subterranean       | 102                 | Mt. Kenya (Kenya)  |
| sp. 2         | Rh.       | subterranean       | 102                 | Mt. Kenya (Kenya)  |
| sp. 1         | Ty.       | subterranean       | 102                 | Tai (Ivory Coast)  |
| cf. vishnui   | Al.       | subterranean       | 51                  | Gashaka (Nigeria)  |
|               |           |                    | 51                  | Poring (Malaysia)  |

Abbreviations are as in Table 2.

brate the analysis, we had to rely on earlier published dates that were calibrated with fossils in the frame of a large scale molecular phylogeny of army ants and their relatives [2]. To cover a range of reasonable dates for each node, we ran three analyses, using the mean, upper and lower bound of the 95% confidence interval for the most recent common ancestor of Dorylus s.l. (45.6, 64.0, 30.0 million years ago, respectively) [[2]. Brady pers. comm.] to fix the respective node.

Authors’ contributions

DJCK carried out the DNA sequencing work and the analyses of the molecular data, and drafted a first version of the manuscript. CS collected most samples, measured the morphological traits and analysed them. LBV participated in the analysis of morphological data. JJB coordinated the study and helped to design the format of the manuscript. All authors participated in designing and planning the study, in writing the various versions of the manuscript, and they all read and approved the final version.

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