



Ant cuticle microsculpturing: diversity, classification, and evolution

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Abstract

Taxonomists have created over 150 unique terms to describe microsculpturing patterns in ants and other insects, but the evolution and function of these patterns has remained a mystery. To develop a better understanding of the evolution and function of cuticle microsculpturing, we developed a simplified classification system consisting of five broad categories: smooth, reticulate, striate, punctate, and tuberos. We used this system to classify microsculpturing patterns of 11,739 species and subspecies of ants, which we then mapped onto the current genus-level ant phylogeny. We found strong support that smooth was the ancestral state of microsculpturing in ants. All other microsculpturing patterns evolved independently multiple times, and there have been numerous instances of reversions and reacquisitions of similar patterns. In general, most ants were smooth, and rough textures tended to be associated with subfamilies whose members have a thicker cuticle, particularly Myrmicinae and Ectatomminae. Tuberos was the least common microsculpturing pattern and was largely confined to fungus-gardening ants in the tribe Attini. The function of cuticle microsculpturing is still unknown, but it may play a role in providing structural support, abrasion reduction, desiccation resistance, communication, and influence insect-microbe interactions. We review these proposed functions and discuss how ant microsculpturing may inspire future applications in bio-inspired design.

Key words: Functional morphology, insect cuticle, ants, review, Hymenoptera, Formicidae.

Received 5 May 2022; revision received 20 February 2023; accepted 21 February 2023

Subject Editor: Chris R. Smith

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Introduction

Ants are highly diverse and play important roles in terrestrial ecosystems (DEL TORO & al. 2012). But compared with other insects, ants are relatively small and lack flashy coloration or conspicuous ornamentation to distinguish one species from another. For this reason, ant taxonomists rely on more subtle features to distinguish species, including cuticle microsculpturing (BOLTON 1994). Microsculpturing refers to textures present on the surface of the cuticle that range from simple striations to complex netted patterns (Fig. 1). The definitive text on cuticle microsculpturing, “A glossary of surface sculpturing” by R.A. HARRIS (1979), includes over 150 terms to describe these patterns. When strung together, these terms create a complex language that can be nearly impenetrable for even the most ardent student of myrmecology (e.g., the ant *Stictoponera biroi* EMERY, 1901, whose workers are described as having a frons that is “densely foveolate with fine strigae frequently present on cuticle between foveolae,” a clypeus that is “longitudinally costulate, vertex

foveolate along anterior margin,” and a scape that “varies from mostly smooth to longitudinally strigulose” (LATTKE 2004)). Unsurprisingly, there can be substantial overlap among terms, and the sheer number and complexity of terms used to describe microsculpturing make it difficult to conduct broad comparisons required to understand the evolution and function of these patterns.

A growing interest in functional trait comparisons in ants and other insects has led to increased standardization in metrics used to assess morphology and life history characteristics (POFF & al. 2006). The Global Ants database has pulled together data on 26 morphological and life history traits for over 9000 ant species and subspecies to compare broad evolutionary and ecological patterns (PARR & al. 2017). Morphological trait databases like Global Ants typically include standardized measurements of body parts paired with common indices to assess more complex morphological traits, such as color, pilosity, or

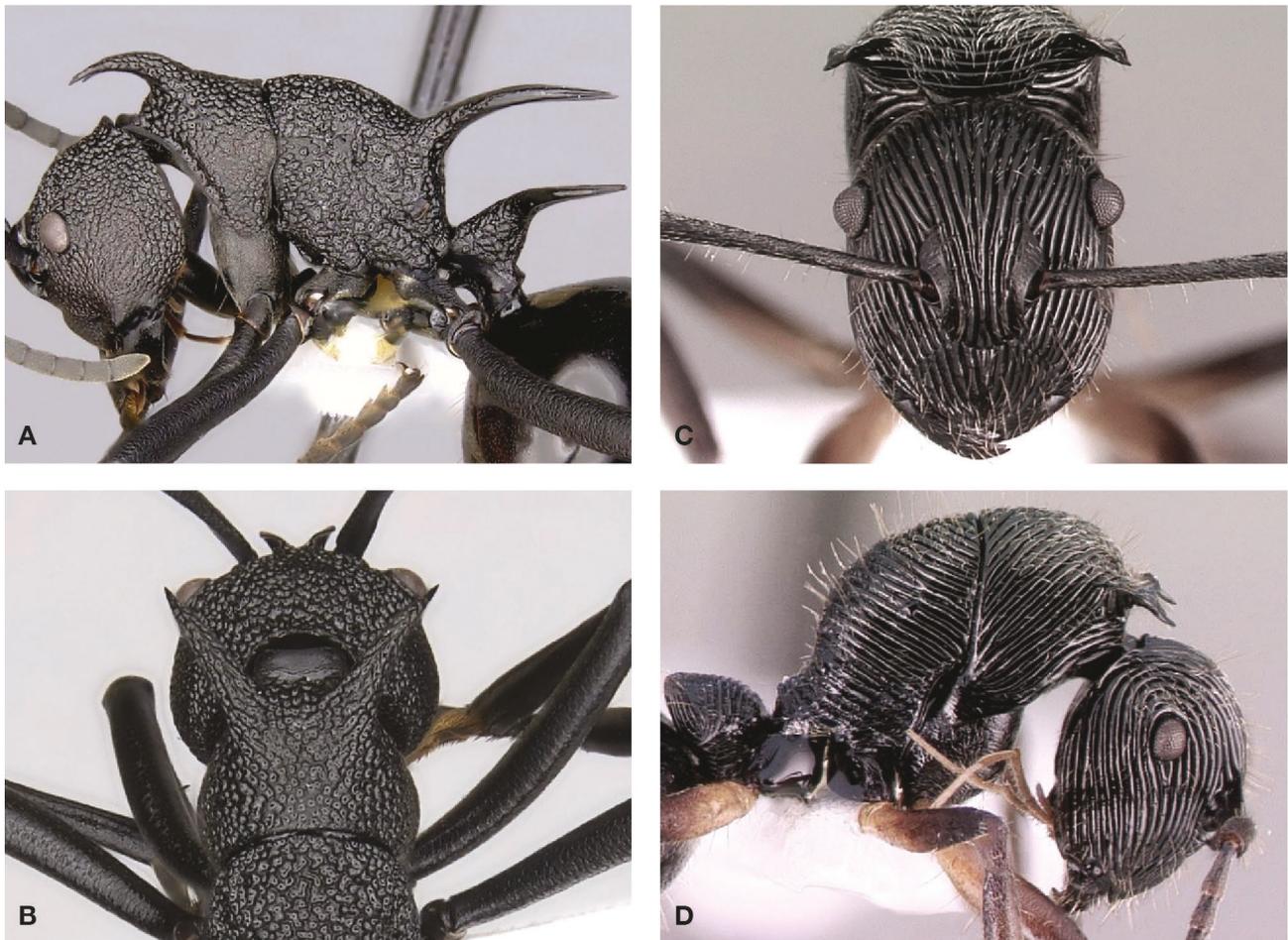


Fig. 1: Examples of cuticle microsculpturing; (A) side and (B) frontal view of *Polyrhachis maryatiaae* KOHOUT, 2007 featuring fine, punctate microsculpturing (CASENT0217434, photographer: Will Ericson); (C) frontal and (D) side view of *Polyrhachis kokoda* KOHOUT, 2007 featuring deeply striate microsculpturing (CASENT0009241, photographer: April Noble). Images modified from ANTWEB (2023).

microsculpturing. Regarding microsculpturing, current methods employ an ordinal ranking system to classify the severity of microsculpturing from “no markings, shiny” to “heavily textured with ridges, grooves, or pits” (PARR & al. 2017, BUXTON & al. 2021). The benefit of an ordinal ranking system is that it is simple and can be used to classify microsculpturing using online images, but it does not take into account qualitative differences in microsculpturing pattern. Given the diversity of microsculpturing patterns present in ants and other insects, it is likely that these patterns have evolved in response to unique evolutionary pressures and could serve equally diverse functions.

In the following sections, we review basic properties of the insect cuticle and develop a new framework to classify microsculpturing patterns into five broad categories. We then apply this framework to classify microsculpturing patterns of 11,739 ant species, which we map onto the current genus-level ant phylogeny. Based on this comparison, we discuss the evolutionary origins and diversification of microsculpturing patterns in ants, and we discuss how these traits vary among major subfamilies. Finally, we review major hypotheses for the function of

microsculpturing related to structural support, abrasion reduction, desiccation resistance, communication, and insect-microbe interactions.

Insect cuticle properties

The insect cuticle serves multiple functions and is recognized as a key factor in the ecological success of insects (KENNEDY 1927). The cuticle provides structural support, offers internal sites for muscle attachment, serves as a canvas for advertising visual and chemical signals, provides protection from predators, and restricts water loss (WIGGLESWORTH 1948, VINCENT & WEGST 2004). While the cuticle is relatively thin – typically 100 to 300 microns – it is composed of a complex series of layers that are permeated by glands, hairs, and setae (Fig. 2). The innermost layer, called the basement membrane, is a noncellular layer that provides a stable attachment site for the epidermis. The next layer is the epidermis, which is composed of a single layer of epidermal cells that secrete the outer layers of the cuticle. The outer layers of the cuticle include the procuticle, which is thick and provides the primary structural support (ANDERSEN 2010), and the

epicuticle, which is a relatively thin layer of waxes and lipids that coats the outer surface of an insect to prevent desiccation and provide olfactory cues (WIGGLESWORTH 1948, CARLSON & al. 1971). It is through the combination of these layers and their unique properties that the diverse functions of the cuticle arise.

The cuticle of ants can range from rigid and inflexible to soft and elastic. This depends on the thickness of the cuticle as well as its arrangement and composition. The cuticle derives its strength from hydrogen bonds between adjacent chains of chitin, an amino-sugar polysaccharide (MOUSSIAN 2019). Chains of chitin are arranged in sheets that lie on top of each other at a slight angle to produce a helicoid arrangement that increases flexural stiffness and strength (BOULIGAND 1972, GREENFELD & al. 2020). The cuticle hardens through the process of sclerotization (ANDERSEN 2010), which influences the mechanical properties of the cuticle (ANDERSEN 2012). Unsclerotized cuticle between articulated joints, for example, retains softness and elasticity to allow a free range of movement. In contrast, mandibles are typically the most sclerotized body parts and have increased hardness to resist abrasion (VINCENT & WEGST 2004). Ants may increase the hardness of the cuticle by increasing cuticle thickness (BUXTON & al. 2021), increasing nitrogen-to-carbon ratios (GIBB & al. 2015), or by incorporating metallic elements into the cuticle (SCHOFIELD & al. 2002, CRIBB & al. 2007). Metallic elements – including calcium, iron, manganese, and zinc – are found primarily within the mandibles and the surface of the exoskeleton, where they contribute to sharpness and cuticle durability (SCHOFIELD & al. 2021). To produce flexible and elastic cuticle, ants and other insects incorporate resilin, an elastomeric protein composed of coiled polypeptide chains that act like a spring when under compression (ANDERSEN & WEIS-FOGH 1964). Resilin is commonly found in insect wing ligaments and the tendons of jumping insects (WEIS-FOGH 1960, HEPBURN & CHANDLER 1976, VARMAN 1981), and it is also present in the arthrodistal membrane that joins gastral segments in ants. The arthrodistal membrane is highly-folded, unsclerotized cuticle that expands in size as it unfolds, which allows queen ants and replete workers to expand their gaster to accommodate increased egg production and an expanded crop (HÖLLDOBLER 1976).

Although ants are not known for exhibiting bright colors, ants do exhibit variation in pigmentation and structural color. Pigmentation is the most common type of color in ants and is produced via a combination of melanins and other chemical pigments. While the process of sclerotization refers to the hardening of the cuticle, melanization refers to the darkening of the cuticle. Melanins produce shades ranging from brown to black, and melanization can have diverse functions related to heat absorption, desiccation, UV damage, and disease resistance (TRUE 2003). Ants and other insects that live in cooler environments tend to have a darker cuticle that is thought to aid in heat absorption (CLUSELLA-TRULLAS & al. 2007, BISHOP & al. 2016), while ants that live in tree canopies also tend to have

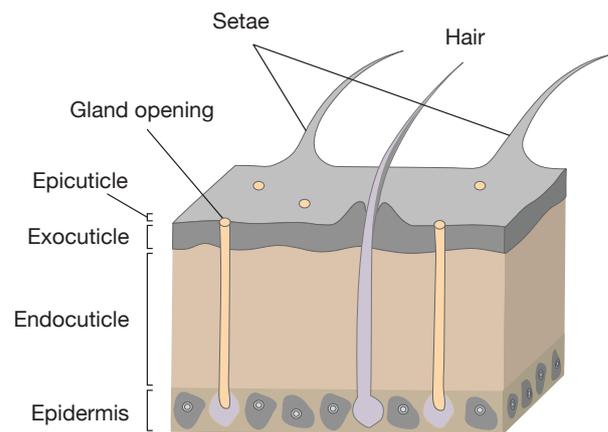


Fig. 2: Diagram of the insect cuticle in cross section showing four primary layers: epidermis, endocuticle, exocuticle, and epicuticle.

a darker cuticle due to increased melanization to help prevent UV damage (LAW & al. 2020). Melanin also plays a key role in the insect immune response by encapsulating foreign objects that enter the body as well as aiding in wound healing (SUGUMARAN 2002). In addition to melanin, ants produce hues of red, orange, and yellow through a combination of other pigments that include pterins, ommochromes, and carotenoids (BADEJO & al. 2020). Rarely, ants display structural colors that appear iridescent or metallic, which are produced by nanostructures that interfere with light reflection (SRINIVASARAO 1999). Structural coloration can produce a variety of hues that range from deep blue, as in the wings of *Morpho* butterflies, to vibrant gold and metallic green found in Buprestidae (jewel beetles) and the green-head ant *Rhytidoponera metallica* (SMITH, F., 1858) (see SHARMA & al. 2009, SMITH 2009).

Variation in cuticular traits among ants has been linked to a number of environmental and social factors. As described above, color appears to be under selection, with species inhabiting colder climates or those more exposed to UV radiation exhibiting darker coloration (LAW & al. 2020). Cuticle thickness is also expected to be under selection due to a tradeoff between investment in cuticle thickness and investment in greater colony size (WILLS & al. 2015). A thicker cuticle provides increased protection to individual workers, but at a cost – the biosynthesis of chitin and other cuticular proteins requires high amounts of nitrogen that can be limiting (DAVIDSON 2005). Thus, as colony size increases, cuticle thickness of individual workers is expected to decrease. This has been supported by a comparison of colony size and cuticle thickness across 42 ant species, which showed a negative relationship between colony size and cuticle thickness (PETERS & al. 2017). Ants with larger colonies also tend to produce workers with lower nitrogen levels, which provides further evidence that workers with a thinner cuticle are “less expensive” to produce (DAVIDSON 2005). Given evidence of selection on both color and cuticle thickness, it is likely that other cuticular traits are also under selection, including microsculpturing.

Classification of microsculpturing patterns

Taxonomists have opted for precision over economy of language when developing terms to classify microsculpturing patterns. As mentioned above, “A glossary of surface sculpturing” by R.A. HARRIS (1979) includes over 150 terms to describe microsculpturing patterns, of which 100 are indicated as “preferred.” Using the definitions for each of these terms provided by Harris, we found that they could be grouped into five broad categories: smooth, striate, punctate, reticulate, and tuberos (Tab. 1, Fig. 3). The number of categories and their definitions were honed through

multiple rounds of assessment and revision (Box 1), and the names were taken from previous terminology used to classify cuticle patterns by TORRE-BUENO (1962) and HARRIS (1979). While not every ant species could be classified into a single category based on microsculpturing present on the head, we found that 98% of species could be classified reliably.

The simplest category for describing cuticle microsculpturing is “smooth”, which includes species that are largely free of surface texture. Ants classified as smooth range from those that are nearly perfectly smooth and shiny, such as the fire ant *Solenopsis invicta* BUREN, 1972,

Tab. 1: Classification of ant sculpturing taxonomic terms. * Sculpturing terms sourced from HARRIS (1979) and includes only “preferred” terms; † includes terms that denote slight sculpturing that may appear dull, matte, or powdery.

Proposed Term	Taxonomic Terms*
Smooth†	coriarius, destitute, ecarinate, explicate, farinaeous, farinose, glabrate, glabrous, imbricate, immaculate, impunctate, inermis, irrorate, micans, mutic, nitid, nude, pruinose, pulverulent, rorulent
Reticulate	aciculate, alveolate, areolate, asperous, caelate, cariouse, catenate, catenulate, clathrate, consute, fatiscent, fissate, fossulate, muriculate, reticulate, rimose, rimulose, rivose, rivulose, scabriculous, scarified, scrobiculate, scutate, squarrose, tessellate
Striate	carinate, carinulate, costate, costulate, cristate, cristulate, institia, lineate, lineolate, plicate, porcate, rastrate, rugose, rugulose, scabrid, scabrous, striate, strigate, strigulate, striolate, sulcate, taeninate, undose, vermiculate
Punctate	foveate, foveolate, lacunose, porose, papillulate, punctate, puncticulate, punctulate, variolate
Tuberos	acinose, colliculate, echinate, echinulate, granulate, munite, nodulate, papillate, pustulate, spherulate, spinose, spinulate, torose, torulose, tuberculate, verrucose

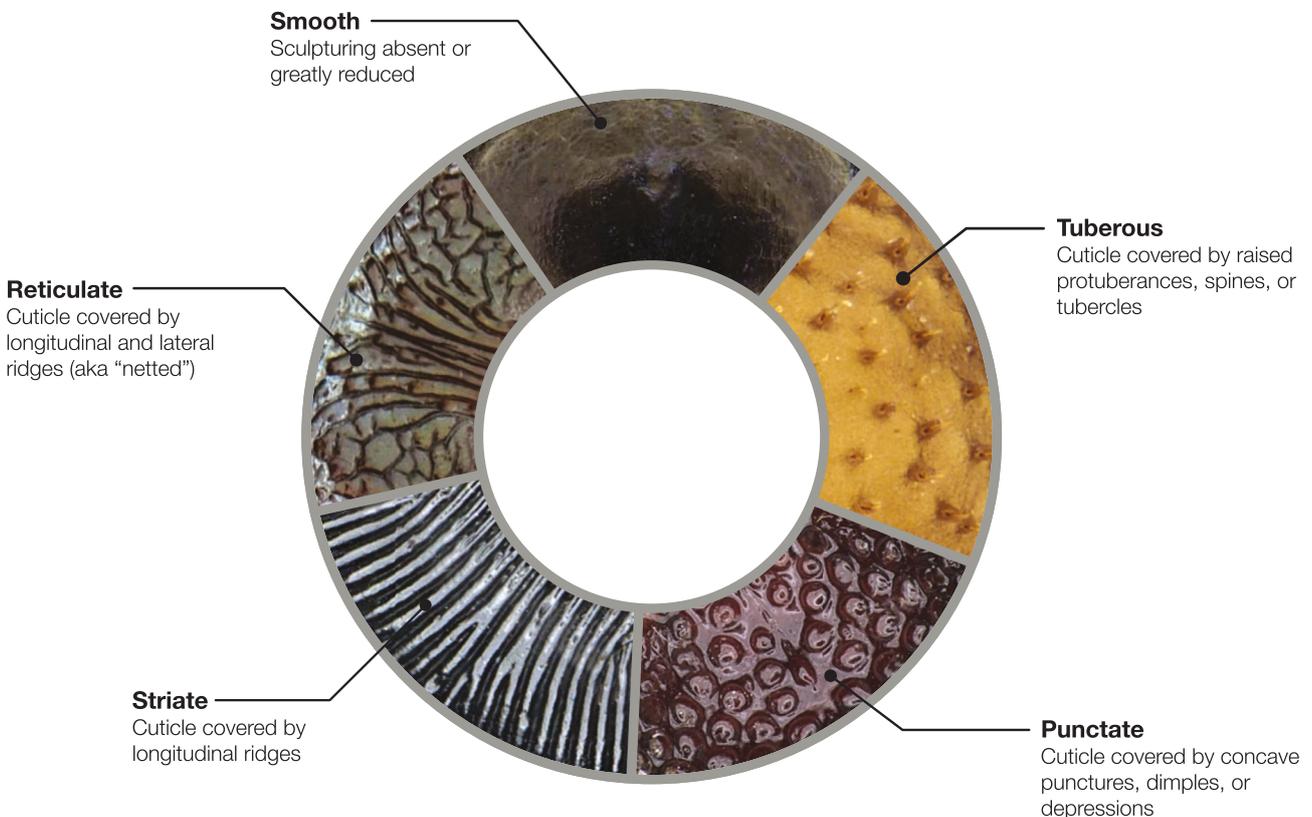


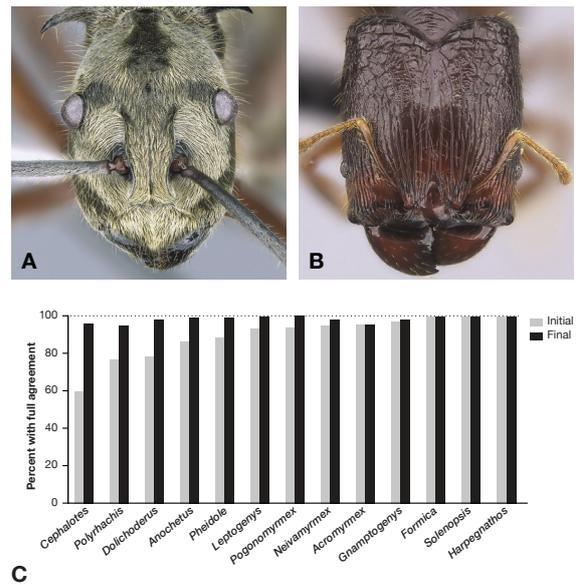
Fig. 3: Classification guide for ant microsculpturing patterns. Images modified from ANTWEB (2023).

Box 1: Classification and verification.

We classified microsculpturing present on the heads of 11,739 workers using images available on ANTWEB (2023; Data S1, as digital supplementary material to this article, at the journal's web pages). We focused on the region between the upper portion of the clypeus and the vertex of the head, and we ignored longitudinal carinae extending from the antennal sockets if they happened to occur in this area. We also excluded nano-scale textures present on the cuticle, sometimes referred to as “scaling”, which can be difficult to assess without access to physical specimens (WATSON & al. 2017). We chose to focus on the head for our classifications because microsculpturing on the head tends to be visible and consistent compared with microsculpturing present elsewhere on the body, such as the mesosoma. Microsculpturing patterns on the head tend to match patterns on the rest of the body, but differences in microsculpturing do occur among body regions, among distinct worker castes, and between worker and reproductive castes, though these were not included in our analyses. In addition, the gaster of most ants are free of microsculpturing with relatively few exceptions (e.g., *Echinopla* and some *Ectatomma* and *Polyrhachis*).

We initially tested a wider range of microsculpturing categories but ultimately landed on five that captured the majority of variation present in ants: smooth, reticulate, striate, punctate, and tuberos (Fig. 3). These categories were defined by qualitative differences in the patterns present on the cuticle and did not consider variation in size, depth, or severity of microsculpturing. A previous index of ant microsculpturing included severity as a factor (PARR & al. 2017), but severity can be difficult to assess without access to physical specimens or without taking scale into account. A detailed assessment of the relative size and severity of microsculpturing patterns would likely require direct measurements of the features associated with microsculpturing as well as histology to accurately measure the depth of microsculpturing in cross section. These measurements should also be normalized to body size to account for scaling effects.

To test the robustness of our classification system, we had three independent researchers classify a series of images from ANTWEB (2023) using our definitions. The initial test included images of 2,778 species and subspecies representing 13 genera (*Acromyrmex*, *Anochetus*, *Cephalotes*, *Dolichoderus*, *Formica*, *Gnamptogenys*, *Harpegnathos*, *Leptogenys*, *Neivamyrmex*, *Pheidole*, *Pogonomyrmex*, *Polyrhachis*, *Solenopsis*) from six subfamilies (Dolichoderinae, Dorylinae, Ectatomminae, Formicinae, Myrmicinae, and Ponerinae). We also tested our classifications using a machine learning approach that distinguished smooth vs. rough species (GARDNER & al. 2022). Using our initial definitions for the five microsculpturing categories, we had complete consensus for 87% of images. We then reviewed the remaining 13% of contested classifications to adjust our definitions to clarify previously ambiguous definitions and identify cases where microsculpturing could not be reliably assessed. For example, some ant species are covered by dense hairs that prevent observation of underlying microsculpturing (Box1 Fig. A), while others may display multiple types of microsculpturing on their head within the region we analyzed (Box1 Fig. B). The total number of ants we deemed unclassifiable using these methods was less than 2% of all classifications, and this number was likely inflated due to the high number of hair-covered ants present in *Polyrhachis*. After adjusting our classifications, we reached consensus for 99.99% of the images.



Box 1 Fig.: Examples of ant species whose microsculpturing could not be easily classified, including (A) *Polyrhachis aureovestita* DONISTHORPE, 1937 (CASENT0281395, photographer: Estella Ortega), which is covered by dense hairs that obscure potential microsculpturing, and (B) *Pheidole aelloea* SALATA & FISHER, 2020 (CASENT0076807, photographer: Will Ericson), which exhibits multiple microsculpturing types on the head (striate and reticulate); images modified from ANTWEB (2023). (C) Percent with full agreement among three researchers who classified 2,778 species and subspecies across each genus before and after adjusting microsculpturing definitions to their current form. Dotted line shows 100% agreement.

to those free of surface sculpturing but that have a dull or matte finish. A matte appearance typically results from presence of scaling, which arises from nanosculpturing that may appear as small scales, fine cracks, or nodules that give ants a dull appearance compared with ants that are completely smooth and shiny. Given its name, nanosculpturing is distinct from microsclpturing in that it is measured on the nanometer scale rather than the micrometer scale (WATSON & al. 2017). Because nanosculpturing can be difficult to see without access to physical specimens and may co-occur with different microsclpturing types, we did not include it in our classification scheme. Likewise, we did not include hairs in our classification of microsclpturing because they also vary independently of microsclpturing type. All hairs on ants are technically classified as setae, though depending on size and function, these can be referred to as appressed hairs, standing hairs, or macrochaetae (BOLTON 1994). Appressed hairs are short, fine hairs that lie flat and frequently appear in dense mats on the cuticle, creating a dull appearance. In contrast, standing hairs and macrochaetae are longer and thicker, often greater than 1 μm in length. Hairs range in density from sparse to incredibly dense (Box 1) and may function to increase reflectivity and dissipate heat (SHI & al. 2015).

“Striate” microsclpturing is defined by lines or ridges that extend longitudinally over the body surface. Striations tend to run parallel to each other, though striate lines may branch or rejoin to maintain consistent density across body regions that vary in size, similar to the pattern of rib bifurcation in columnar cacti (YEATON & al. 1980). Ridges are typically fine, but they can be prominent and severe in some species. For example, workers of *Crematogaster africana* MAYR, 1895 feature fine ridges only 1 μm wide, while workers of *Holcaponera porcata* (EMERY, 1896) feature prominent ridges that can be over 20 μm wide. In rare cases, striations may be oriented transversely rather than longitudinally, such as in *Chrysapace jacobsoni* CRAWLEY, 1924, *Chrysapace sauteri* (FOREL, 1913), *Leptogenys optica* VIEHMEYER, 1914, and *Leptogenys caeciliae* VIEHMEYER, 1912. We classified ants with transverse ridges as striate, though future studies may reveal that transverse ridges are functionally unique.

“Punctate” microsclpturing is defined by circular, concave depressions present on the cuticle. These depressions often give ants a dimpled look reminiscent of the texture on a golf ball. Punctate dimples can be present on the head and mesosoma, and they range in size from less than 1 μm in diameter in *Hypoponera punctiventris* (EMERY, 1901) to nearly 100 μm in diameter in *Stictoponera bicolor* (EMERY, 1889). Dimples are often associated with hairs, but when dimples were so small that they could not be easily distinguished from the base of hairs, we classified these species as smooth. As dimples increase in size and density at the other end of the spectrum, they may form a honeycomb-like pattern, which crosses into the reticulate category. What separates punctate microsclpturing from reticulate microsclpturing is that

punctate microsclpturing appears more circular, while reticulate microsclpturing is more angular. We took a conservative approach when classifying ants as punctate by only classifying species as punctate if the edges of each dimple were clearly defined and rounded. In some cases, ants with fine dimples appeared smooth, but we classified these as punctate if dimples were pronounced enough to give ants a textured appearance rather than a matte finish.

“Reticulate” microsclpturing is the most complex and includes ants that have intersecting longitudinal and transverse ridges that give the cuticle a netted appearance. These patterns can be difficult to distinguish from both striate and punctate textures, depending on the severity. To distinguish reticulate from striate microsclpturing, transverse ridges must be clearly defined in ants classified as reticulate. This is exemplified by workers of *Myrmica glacialis* EMERY, 1921, which feature prominent striations on their heads paired with fine but distinct transverse ridges. To distinguish reticulate from punctate microsclpturing, depressions had to be clearly angular rather than rounded. This is exemplified by workers of *Rhytidoponera metallica* (SMITH, F., 1858), which feature angular depressions on their heads that place them into the reticulate category. Similar to the punctate category, ants with fine reticulate microsclpturing may appear smooth, but we classified these as reticulate if sculpturing was pronounced enough to give a textured appearance rather than matte finish, which can be seen in workers of *Polyrhachis nudata* SMITH, F., 1860. Reticulate microsclpturing is by far the most diverse category, and for this reason it may be difficult to identify the function of reticulate microsclpturing unless studies focus on taxonomically similar groups or those with reticulate sculpturing at a similar scale. Reticulate patterns are also widely found on plant pollen (PRAGLOWSKI 1971) and seeds (BARTHLOTT 1981), for which the function is also unknown.

Finally, we defined “tuberous” microsclpturing as raised protuberances or blunt spines that cover the body surface. Tuberous microsclpturing was the least common category and is largely limited to fungus-gardening ants in the tribe Attini. While many ant species feature prominent spines on their propodeum, petiole, or head, ants classified as tuberous must have nodules or protuberances present on the general surface of the head and / or body to create a textured appearance. Typically, these protuberances are less than 100 μm in height and are found on the head and mesosoma. Ants classified as tuberous are typically rough and have a matte appearance, which may aid in the adhesion of symbiotic bacteria that form biofilms on the exoskeleton of some attine species (ANDERSEN & al. 2013). Similar protuberances also occur in ants of the genus *Echinopla*, where protuberances are associated with protruding hairs. Likewise, some ant larvae feature similar protuberances associated with upright hairs (WHEELER & WHEELER 1976, PENICK & al. 2012), though larvae are not considered in our comparisons.

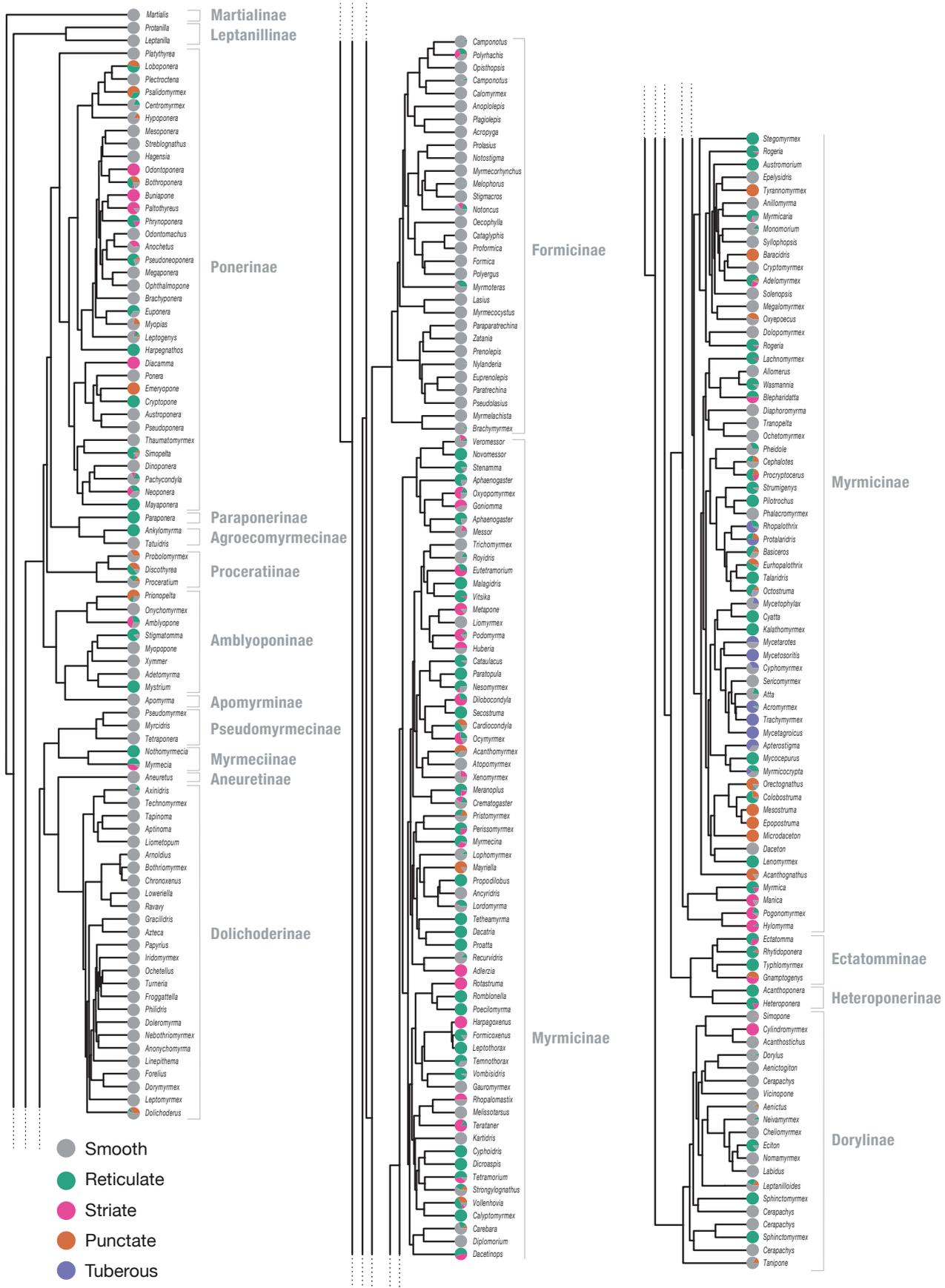


Fig. 4: Proportion of microsculpturing patterns found within genera mapped onto the ant phylogeny of BLANCHARD & MOREAU (2017). Note that the three basal lineages of Formicidae (*Martialis*, *Protanilla*, *Leptanilla*) are smooth and that cuticle microsculpturing likely evolved later in the ant phylogeny.

It is important to note that the simplified categories for classifying microsculpturing patterns do not assume homology, and there may be substantial variation in the form and severity of microsculpturing within each category. For example, we noted above that a limited number of striate species feature transverse rather than longitudinal ridges, which may be functionally distinct. We also did not include modifiers for severity of microsculpturing in our classification scheme, though this would be an interesting aspect of microsculpturing to consider using quantitative methods that capture the depth and scale of microsculpturing patterns. We opted for a more general classification scheme to make broad comparisons, but future studies on the evolution and function of microsculpturing at finer taxonomic scales may benefit from subdividing categories into smaller groups.

Evolution of microsculpturing patterns

To understand the evolution of cuticle microsculpturing patterns in ants, we classified microsculpturing on the heads of minor workers of 11,739 species and subspecies representing 342 genera and 18 subfamilies (for detailed methods, see Box 1). We then mapped the relative proportion of microsculpturing types within each genus onto the phylogeny by BLANCHARD & MOREAU (2017) to assess evolutionary trends (Fig. 4). Based on ancestral

trait reconstruction, we found strong support that smooth was the basal microsculpturing pattern in ants (Box 2). Members of the three most basal extant genera – *Martialis*, *Leptanilla*, and *Protanilla* – are smooth (Fig. 4), and representative members of two of the earliest extinct ant subfamilies – Sphecomyrmicinae and Haidomyrmecinae – are also smooth (Fig. 5). Therefore, whatever conditions selected for cuticle microsculpturing in ants must have occurred after early ants began living in colonies, hunting arthropod prey, and exploiting leaf-litter habitats. The subsequent evolution of microsculpturing in ants appears to be highly labile as each type evolved multiple times independently with repeated losses and reacquisitions. The evolutionary lability of microsculpturing in ants matches trends for other complex traits, including eye size, worker polymorphism, spines, and larval hairs (PENICK & al. 2012, BLANCHARD & MOREAU 2017, WILLS & al. 2018).

Smooth was by far the most common cuticle texture comprising 57% of species (Fig. 6a), though textured patterns evolved numerous times and in disparate ant lineages. To assess character state transitions, we focused on microsculpturing present in species of *Polyrhachis* and *Crematogaster*, which both have recently-constructed, species-level phylogenies and have among the highest diversity of cuticle microsculpturing patterns when compared with other genera (Box 2). Species of *Polyrhachis*

Box 2: Phylogenetic analyses and microsculpture lability.

We explored the evolution and lability of microsculpturing patterns in ants by mapping these traits onto the current genus-level phylogeny (Fig. 4) and conducting ancestral trait reconstruction using Mesquite v.3.70 (MADDISON & MADDISON 2011). We also examined the lability of microsculpturing traits in two genera with high variation in microsculpturing using species-level phylogenies of *Polyrhachis* and *Crematogaster*.

Ancestral-trait reconstruction

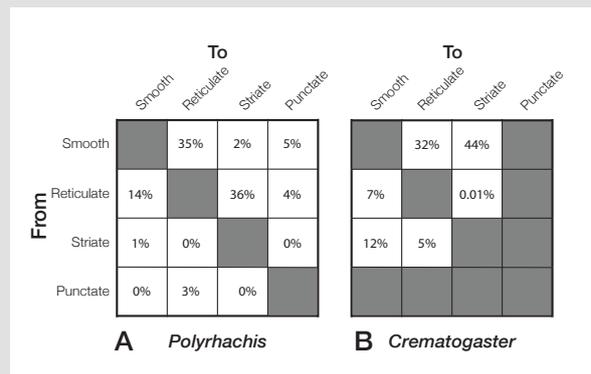
To estimate the ancestral character state for cuticle microsculpturing in ants, we used the default settings for the Markov k-state 1 parameter model (Mk1) likelihood reconstruction with the most recent ant phylogeny (BLANCHARD & MOREAU 2017). We assigned species to a binary and multi-state classification system (binary: smooth = 0, rough = 1; multi-state: smooth = 0, reticulate = 1, striate = 2, punctate = 3, and tuberosus = 4). Reconstruction was unordered to ensure that the transition from any one state to another was weighted equally. We inferred the ancestral state of cuticle microsculpturing across the maximum clade credibility tree with 291 tips using the function “Parsimony Ancestral States”. Our results show that smooth was likely the ancestral character state. This was supported further by the fact that the three most basal genera in the ant phylogeny are also smooth (*Leptanilla*, *Protanilla*, and *Martialis*). In addition, a comparison of fossil ants from the subfamilies Sphecomyrmicinae and Haidomyrmecinae show that all described individuals also display smooth sculpturing. Taken together, these findings provide strong support that the ant ancestor was smooth and that multiple microsculpturing patterns arose later in ant evolution.

To quantify the phylogenetic signal of microsculpturing traits mapped onto the phylogeny, we used Blomberg’s K statistic and Pagel’s λ statistic within the R package Phytools (REVELL 2012). We ran separate analyses for each coding scheme using a binary and multi-state classification system as described above, and we based our p-value estimates on 1000 randomizations. Across all analyses, we found evidence of significant phylogenetic signal for microsculpturing; however, K and λ values under 1 indicate that the phylogeny is unable to account for all variation in trait evolution (binary: K = 0.605, p = 0.001; λ = 0.506, p = 0.0001; multi-state: K = 0.551, p = 0.004; λ = 0.495, p = 0.001). Beyond phylogenetic relationships, the diversity of microsculpturing may, in part, be a product of convergent evolution across the ant phylogeny. Microsculpture is a phenotypically plastic trait that can vary greatly between closely related species and even within castes of the same species.

Microsculpture lability

To examine the lability of microsculpturing patterns and assess the likelihood that one microsculpturing type would evolve towards another, we focused on two genera that feature high variation in microsculpturing patterns among species and have well-supported phylogenies: *Polyrhachis* and *Crematogaster* (BLAIMER 2012, MEZGER & MOREAU 2016). For each genus, the same ancestral trait reconstruction as described above was conducted with an additional analysis of texture transition using the “Summarize Changes in Selected Clade Over Trees” function in Mesquite. We pruned trees to match our collected texture data (*Polyrhachis* = 112 tips, *Crematogaster* = 94 tips) and assigned the remaining species to the multi-state classification described above. We mapped state changes from one consensus tree and sampled mappings from the tree 50 times. We then summarized the average number of samples across the mappings as a percentage to illustrate which microsculpture transitions were most common.

From these analyses, we found that the ancestral state of each genus was smooth. Regarding *Polyrhachis*, we found that the most common cuticle pattern was smooth, and the most common transition was from reticulate to striate (Box2 Fig. A). The pattern we observed in *Crematogaster* was nearly identical, as the most common microsculpturing type was smooth, and the most common transition was from smooth to striate (Box2 Fig. B). Across both genera, we found evidence that smooth transitioned to striate, punctate, and reticulate (no ant in this dataset was tuberosus). Transitions from reticulate and striate to smooth were also common. Within *Polyrhachis*, striate was more likely to arise from reticulate than smooth, suggesting reticulate is a transitional state to striate in this genus. In *Crematogaster*, however, striate species evolved more commonly from smooth than reticulate, and there were almost zero instances of transitions from reticulate to striate. Most cuticle patterns showed that they could evolve from any other with two exceptions: There were no transitions from punctate to striate or striate to punctate. It appears that punctate and striate patterns are not closely related to each other, and both are more likely to evolve independently from a smooth or reticulate basal state.



Box 2 Fig.: State transitions from one microsculpturing type to another (represented as percent of total) for *Polyrhachis* (21 transitions) and *Crematogaster* (23 transitions). Note that neither genus included cases of tuberosus microsculpturing, and *Crematogaster* did not include cases of punctate microsculpturing.

exhibit all cuticle patterns except tuberosus, while species of *Crematogaster* can be either smooth, reticulate, or striate. The most common transition in both species was from smooth to any of the three rough categories. Likewise, reticulate and striate species commonly transitioned back to smooth. Regarding transitions among the three rough categories, patterns were relatively consistent between *Polyrhachis* and *Crematogaster*. In *Polyrhachis*, striate species were more likely to have evolved from reticulate species than from smooth, and punctate species were near equally likely to have evolved from reticulate or smooth species. In *Crematogaster*, reticulate and striate species both evolved from smooth species as well as from each other. From this small sample, it is difficult to say which transitions are most likely to occur in ants, though nearly all cuticle patterns showed that they could evolve from any other pattern with two exceptions – transitions from striate to punctate or punctate to striate. Given that we observed no transitions between these two categories, punctate-striate transitions may be the least likely to occur.

Across the phylogeny, there were major differences in the proportion of smooth versus textured species among subfamilies. Myrmicines and ectatommines exhibited the highest proportion of textured species, with 60% of Myrmicinae and all Ectatomminae exhibiting some form of microsculpturing (Fig. 6b). Microsculpturing was also relatively common among dorylines and ponerines, comprising 26% and 34% of all species, respectively. The two subfamilies where microsculpturing was uncommon were Dolichoderinae and Formicinae. Less than 20% of species in either subfamily exhibited textured microsculpturing, though there were some notable exceptions (e.g., 62% of *Polyrhachis* species were textured). The decreased presence of microsculpturing in dolichoderines and formicines may be explained, in part, by differences in cuticle thickness. PEETERS & al. (2017) found that dolichoderine and formicine ants tend to have a thinner cuticle than that found in other ant subfamilies, which suggests that a thick cuticle may be a necessary pre-condition for the evolution of microsculpturing. The one exception may be ponerines, which tend to have the thickest cuticle compared with

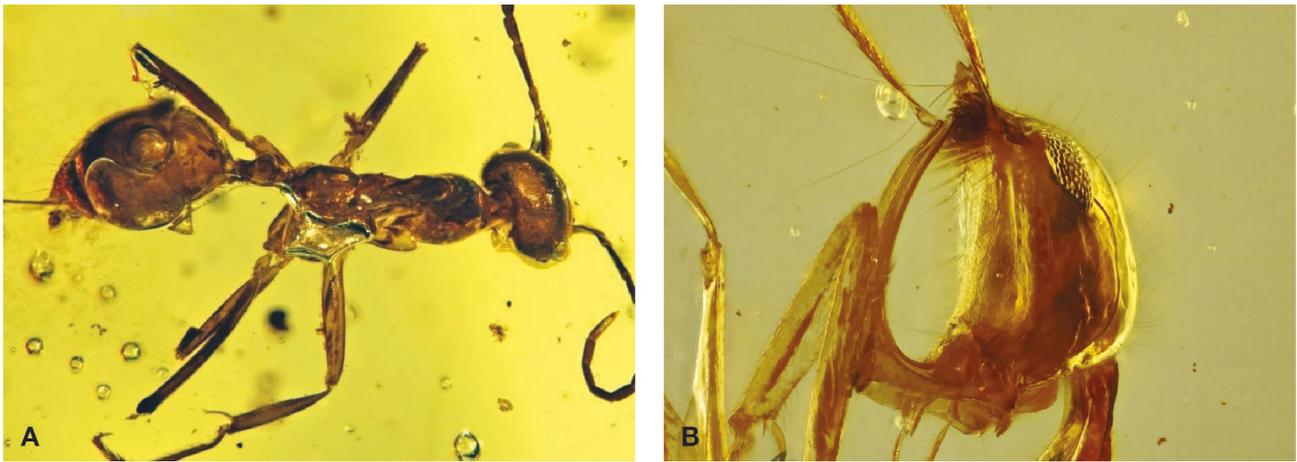


Fig. 5: Examples of smooth microsculpturing found on fossil ants in amber; **(A)** worker from the subfamily Sphecomyrminae, which dates to the Cretaceous; **(B)** worker from the genus *Haidomyrmex*, also known as “hell ants”, which dates to the late Cretaceous. Images modified from Burmese-amber.com under CC BY-SA 4.0 license.

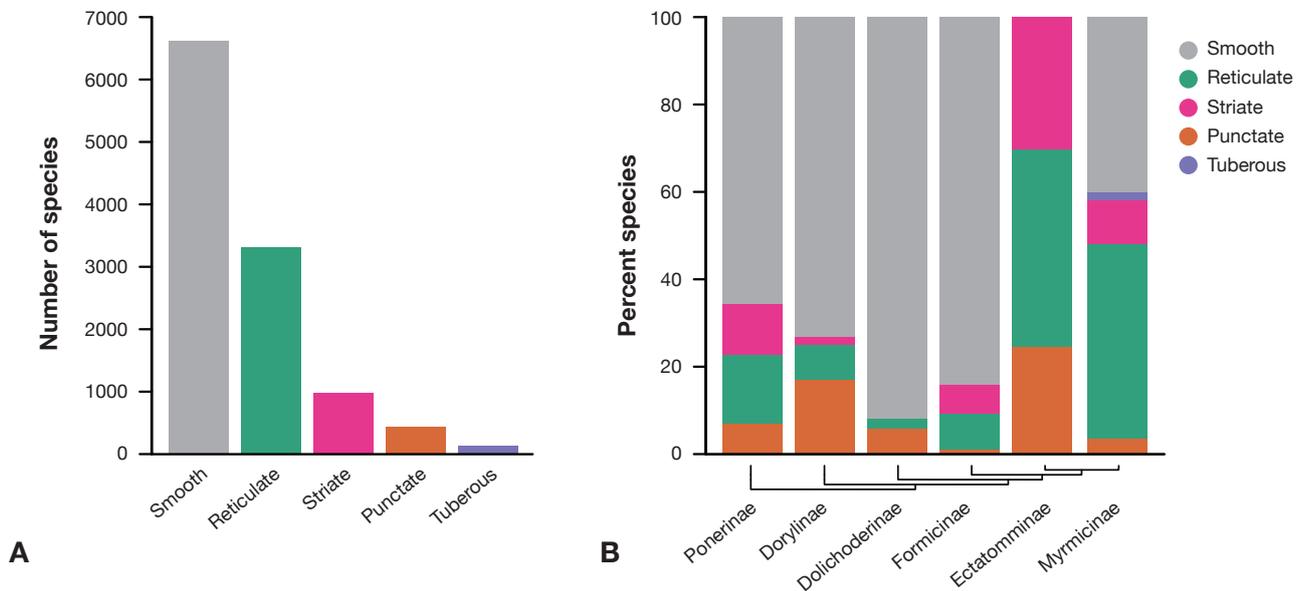


Fig. 6: **(A)** The number of ant species exhibiting each microsculpturing type out of 11,739 ant species and subspecies classified; **(B)** proportion of species featuring each microsculpturing type within the six largest ant subfamilies. Note that microsculpturing is most prevalent among members of Ectatomminae and Myrmicinae, whereas smooth dominates among the Dolichoderinae and Formicinae.

other ant lineages, but only 35% of ponerines are sculptured compared with 60% of myrmicines.

All microsculpturing patterns evolved numerous times across the ant phylogeny with the exception of tuberous, which was largely confined to fungus-gardening ants in the tribe Attini (Fig. 4). Tuberous microsculpturing differs from the other microsculpturing types in that it features raised protuberances that fall somewhere between true spines and surface texture. Indeed, workers in the genus *Atta* typically have smooth sculpturing on the frontal portion of the head but retain sharp spines at the rear margin. The reason why tuberous microsculpturing has evolved in attines is unclear, though the strong association could

simply be a case of phylogenetic inertia. Another potential explanation could be that increased surface roughness may facilitate the attachment of symbiotic bacteria that grow on the outside cuticle of some *Acromyrmex* and *Trachymyrmex* workers to help fight garden-infecting fungal pathogens (ANDERSEN & al. 2013). These two genera also happen to be among the most textured attines, which provides some support to this hypothesis. Although tuberous microsculpturing is predominately found in attines, it also appears in the formicine genus *Echinopla*. Unlike tuberous microsculpturing in attines, protuberances of *Echinopla* are associated with protruding hairs and cover the head, mesosoma, and first gastral segments (ZETTEL &

LACINY 2015). These “pedestals” and their accompanying hairs are thought to serve as a shield against attacks of other arthropods, especially ants (GNATZY & MASCHWITZ 2006), and their function may differ from that of tuberos microsculpturing in attines.

For our comparison, we focused specifically on microsculpturing present on the heads of minor workers, but it is important to note that microsculpturing can vary among body regions and among members of different castes. When present, microsculpturing is most often found on the head and mesosoma, though sculpturing can occur on the petiole, postpetiole, and gaster. For example, workers of the South American grooved ant, *Poneracantha triangularis* (MAYR, 1887), have longitudinal striations that run from the head to the tip of the first two gastral segments (CAMACHO & al. 2022). The reason that the gaster of most ants is smooth may be due to the fact that microsculpturing could interfere with the telescopic movement of gastral segments. Interestingly, many ants with microsculpturing present on the gaster also exhibit tergo-sternal fusion (e.g., members of Ectatomminae), which restricts the telescopic movement of the first two gastral segments. While we did not characterize microsculpturing in soldiers or reproductive castes, we did identify some instances where microsculpturing patterns clearly differed among castes. For example, minor workers of *Pheidole indica* MAYR, 1879 have perfectly smooth heads, while soldier heads feature prominent striations (WILSON 2003). Future studies on variation of cuticle microsculpturing among different body regions or among castes could help elucidate the function of these unique patterns.

Potential functions

The diversity of cuticle microsculpturing patterns in ants begs the question: What do these patterns actually do? Studies on the function of other well-known animal patterns have proven difficult. For example, there are multiple competing hypotheses to explain the function of zebra stripes, which include thermoregulation, predator confusion, social cohesion, and insect repellence (LARISON & al. 2015) – none of which are mutually exclusive. Similarly, the functions of microsculpturing patterns in ants are likely to be multifarious and complex. A recent study of ant microsculpturing and pilosity among 70 species found positive associations between increased microsculpturing and cuticle thickness, puncture resistance, body size, and decreased water-loss rates (BUXTON & al. 2021). Outside of research on ants, there have been studies on the function of microsculpturing patterns in other organisms, including other insects (RICHARDS & RICHARDS 1979, GORB 2000, BOEVE & al. 2004, WATSON & al. 2017), scorpions (HAN & al. 2017), sharks (WEN & al. 2015, DUNDAR ARISOY & al. 2018), snakes (GANS & BAIC 1977), and plants (BARTHLOTT 1981, RIGLET & al. 2021). The results from these studies suggest potential functions of microsculpturing related to structural support, abrasion reduction, desiccation resistance, communication, and insect-microbe interactions, which we review below.

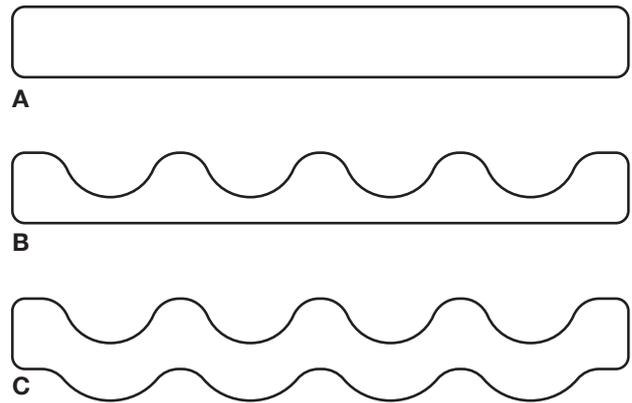


Fig. 7: Cuticle microsculpturing in potential cross section; (A) the cuticle may be smooth on the external and internal surfaces; (B) cuticle may be sculptured on the external surface but lack sculpturing on the internal surface; or (C) the cuticle may be sculptured on both the external and internal surfaces.

Structural support

The most consistent trait correlated with microsculpturing in ants is increased cuticle thickness (GIBB & al. 2015, BUXTON & al. 2021). Cuticle thickness in ants varies from 10 to 110 μm and tends to increase with body size (PEETERS & al. 2017). As mentioned above, a thick cuticle may be required for cuticle microsculpturing to evolve, though microsculpturing could also contribute to strength on its own. Striate and reticulate patterns resemble corrugation, which is used in manufacturing to reinforce metal sheeting and cardboard (MORNEMENT & HOLLOWAY 2007, KAUSHAL & al. 2015). Corrugation is also common in animal structures, from clam shells to insect wings, where it contributes to stiffness and increases strength-to-weight ratios (VOGEL 2000, LUO & SUN 2005). Our classifications focus on external microsculpturing, but it is possible that the internal surface of cuticle could be sculptured as well (Fig. 7). Few studies have published histological images showing ant cuticles in cross section, and those that have tend to focus on smooth ants. In smooth ants, the internal surface of the cuticle appears to be smooth as well (PEETERS & al. 2017), but this does not rule out the presence of microsculpturing on the inner surface of ants that exhibit microsculpturing. A cuticle with microsculpturing present on the inside could mirror external microsculpturing patterns similar to corrugated materials, and it could also provide increased surface area for muscle attachment, which may benefit species with large jaw muscles (Fig. 7c). Future histological studies are needed to determine if internal microsculpturing is present in ants, and if so, whether it has functional significance.

Abrasion reduction

Ants dominate soil habitats where they are exposed to abrasion from sand and other soil particles (JOHNSON 1998, 2000, JOHNSON & al. 2011). There is little evidence that insects can repair the cuticle once it is damaged

(WIGGLESWORTH 1945, JOHNSON & al. 2011, PARLE & al. 2017), so mechanisms that slow abrasion are required to stave off negative effects. The contribution of microsculpturing on abrasion reduction in ants has not been studied, but there is evidence from other organisms. Research on microsculpturing patterns in desert scorpions, for example, has inspired erosion-resistant materials featuring V-shaped grooves that reduced abrasion by 50 to 75% compared with smooth surfaces (ZHIWU & al. 2012, HAN & al. 2015). Likewise, sculpturing on the scales of lizards and the shells of molluscs also slow the effects of abrasion (TONG & al. 2005, ZHANG & al. 2020). If microsculpturing helps reduce abrasion in ants, then microsculpturing should be present on regions of the body where abrasion is most likely to occur. A study of the ant *Veromessor pergandei* (MAYR, 1886) found that the clypeus, occiput, and pronotum received significant abrasion (JOHNSON & al. 2011), and these, in general, are the same regions of the body most likely to have microsculpturing. Although correlative, these associations suggest that cuticle microsculpturing could play a role in abrasion reduction in ants.

Desiccation resistance

Because ants have small bodies with a high surface-area-to-volume ratio, they are at increased risk of desiccation. To prevent water loss across the cuticle, ants and other insects secrete a waxy layer of cuticular hydrocarbons to cover the body surface and provide a protective barrier against desiccation (LEES 1948, GREENE & GORDON 2003, GIBBS & RAJPUROHIT 2010). Any damage to this hydrocarbon layer through abrasion will increase water loss until the waxes can be restored (JOHNSON & al. 2011). Cuticular abrasion from digging and moving through tunnels increases water loss rates 2 to 3-fold compared with ants that have not been exposed to soil (JOHNSON 2000). Foundresses of new nests are particularly susceptible to desiccation, and increased water loss during this stage leads to higher colony mortality (LIGHTON & al. 1993, JOHNSON 1998, JOHNSON & al. 2011). While the cuticular hydrocarbon layer of smooth ants is completely exposed to abrasion, cuticular hydrocarbons of sculptured ants may be partially protected by raised sculpturing, which could reduce negative impacts of abrasion on water loss. BUXTON & al. (2021) found a weak, positive association between cuticle microsculpturing and reduced water-loss rates in ants, though the authors of this study suggested that other correlated traits could be more important, such as increased cuticle thickness. Further research on the impacts of microsculpturing on abrasion reduction and subsequent impacts on water loss are needed to confirm a potential role of microsculpturing in desiccation resistance.

Communication

Although cuticle microsculpturing patterns can be visually striking – ant cuticle patterns have even inspired art (ELLISON & al. 2018) – most ant communication is chemical or tactile. Cuticular hydrocarbons, in addition to providing a protective barrier against desiccation, may

serve as chemical signals for insect communication (HOWARD & BLOMQUIST 2005). In ants, cuticular hydrocarbons serve as signals for nestmate recognition, reproductive status, caste, life stage, and dominance relationships (VANDER MEER & MOREL 1998, PEETERS & al. 1999, PENICK & LIEBIG 2017, SMITH & LIEBIG 2017). An increase in cuticular surface area associated with microsculpturing would presumably amplify any chemical signal present on the cuticle. However, density of cuticular hydrocarbons on the cuticle has been found to decrease as surface area increases in some Hymenoptera (BRÜCKNER & al. 2017), so the relationship between hydrocarbon density and microsculpturing requires further testing.

While the primary mode of communication in ants is chemical, there is evidence that tactile communication may also be important for nestmate recognition. Indirect evidence for the role of tactile communication in ants comes from studies of myrmecophilous insects that mimic the physical appearance of ants as well as their body texture. A striking example occurs in rove beetles that are social parasites of army ants. These beetles physically resemble their hosts and often mimic the surface texture of host body parts (WASMANN 1895, PARKER 2016, VON BEEREN & al. 2018, FISCHER & al. 2020). Texture plays an even clearer role in social parasites of subterranean army ants, which tend to mimic the morphology and texture of their hosts but not the color (PARKER 2016). Parasites that attach directly to the bodies of ants also tend to mimic microsculpturing and pilosity of the parts to which they attach (VON BEEREN & TISHECHKIN 2017). Although the role of microsculpturing in tactile communication in ants has not been well studied, indirect evidence from ant parasites described above suggests that ants could use microsculpturing as a tactile cue.

Insect-microbe interactions

Ants evolved in soil and leaf-litter habitats, which are also home to rich microbial communities – a single gram of soil may contain up to 10 billion microbes representing thousands of different species (ROSSELLÓ-MORA 2001). Ants are thought to have developed a range of strategies to control or cooperate with microbes in their environment, including grooming behaviors (ZHUKOVSKAYA & al. 2013), the production of antimicrobials (MACKINTOSH & al. 1995, BRÜTSCH & al. 2017, PENICK & al. 2018), and the formation of symbiotic associations with beneficial microbes (MATOSO & al. 2012). The role of cuticle microsculpturing in disease defense is unclear, but it could have negative or positive effects. On the negative side, severe microsculpturing could make it more difficult for ants to effectively groom themselves compared with smooth species. On the positive side, however, surface roughness has been found to have antimicrobial properties in some organisms. Shark skin, for example, is composed by microscopic denticles that each feature a series of grooves that may disrupt the growth of biofilms (DUNDAR ARISOY & al. 2018). Engineered antimicrobial surfaces inspired by shark skin are coated with grooves 2 μm in width (CHUNG & al. 2007),

which is on the same scale as the finest striations found in ants.

Instead of inhibiting microbial growth, it is possible that microsculpturing could also aid in culturing beneficial microbes. Many organisms, including humans, are covered in symbiotic microbes that can outcompete pathogens or produce antimicrobials of their own that aid in host defense (BYRD & al. 2018). Fungus-gardening ants have developed a symbiotic relationship with bacteria that help them fight pathogens within their fungal gardens (CURRIE & al. 2003). Adult workers in *Acromyrmex subterraneus* (FOREL, 1893) develop a visible bacterial film on their cuticle within 12 to 15 days of eclosion. Symbiotic bacteria associated with *Acromyrmex* produce antimicrobials that have been found to inhibit infection in ants as well as their fungal gardens (FERNÁNDEZ-MARÍN & al. 2009, MATTOSO & al. 2012). Tuberos microsculpturing present on *Acromyrmex* and other attines could therefore provide surface roughness to help these symbiotic bacteria adhere. While microbes present on the surface of ants have rarely been studied, it is possible that microsculpturing could promote the growth of symbiotic bacteria in other ant species as well.

Conclusions

Ants exhibit a broad diversity of microsculpturing patterns represented by an equally diverse vocabulary to describe them. Ants likely began as smooth, but they evolved distinct microsculpturing patterns repeatedly with numerous instances of evolutionary convergence, losses, and reacquisitions. The ecological pressures that selected for microsculpturing remain a mystery, but this is also true of microsculpturing patterns found in other taxa. For example, the scales of snakes and lizards are often covered with microsculpturing, but broad phylogenetic comparisons have found little evidence of shared ecological selection pressures (GANS & BAIC 1977, PRICE 1982). Likewise, a study of seed surface textures across 5,000 plant species found that most seeds have microsculpturing (BARTHOLOTT 1981), but the presence of microsculpturing is not correlated with any specific ecological indicator (SCHENK & al. 2016). The lack of ecological explanations for the evolution of microsculpturing may be due to the fact that microsculpturing serves diverse functions. Future efforts to elucidate the functions of microsculpturing patterns in ants would benefit from species-level comparisons to disentangle phylogenetic constraints from ecological selection pressures. Species-level phylogenies for *Polyrhachis* and *Crematogaster* have already been useful for identifying transition rates among microsculpturing types as seen in Box 2, and these comparisons could be improved by integration with larger trait databases.

Ultimately, phylogenetic comparisons need to be paired with experimental tests to elucidate function. The impetus for such functional studies of insect morphological traits has often come from the field of bio-inspired design. Ants and other social insects have long served as models for bio-inspired design (HOLBROOK & al. 2010), and their high

diversity paired with an increasing number of quality phylogenies make them an ideal group for comparative methods (PENICK & al. 2022). Cuticle sculpturing on desert scorpions has already been used for bio-inspired applications for abrasion resistance (HAN & al. 2015), and ants likely face similar pressures. Ants are also being used as models to understand insect-microbe interactions (LITTLE & CURRIE 2008, CALDERA & al. 2009, KARLIK & al. 2016, LUCAS & al. 2019) as well as physiological traits that allow organisms to deal with climatic pressures (DIAMOND & al. 2013, PENICK & al. 2017, PARR & BISHOP 2022). It is likely that cuticle microsculpturing plays a role in many of the challenges faced by ants, and future studies on the functional role of microsculpturing would contribute to our understanding of ant biology and could lead to bio-inspired innovations.

Acknowledgments

We thank Rebecca Senft and Katy Chon for assisting in classifying ant microsculpturing as well as Dipali Aphale, Rachel Pollock, and Lisa Wong for early work on characterizing cuticle microsculpturing patterns. We also thank Adrian Smith, Aya Yanagawa, Margarita López-Urbe, and Robert Dunn for helpful discussions about the evolution and diversity of microsculpturing patterns in ants and other insects.

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