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Cuticular microstructure of Australian ant mandibles confirms common appendage construction

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Abstract

Exoskeletons characterise Arthropoda and have allowed the morphological and taxonomic diversity of the phylum. Exoskeletal sclerotisation occurs in genetically designated regions, and mandibles represent one such area of high sclerotisation. Mandible morphology reflects dietary preferences and niche partitioning and has therefore been well documented. However, mandibular cuticular microstructure has been under-documented. Here we use scanning electron microscopy to explore mandible microstructure in four disparate Australian Formicidae taxa (ants) with different life modes and diets: *Camponotus nigriceps*, *Iridomyrmex purpureus*, *Odontomachus simillimus* and *Rhytidoponera aciculata*. We test the hypothesis that mandible construction is highly conserved across these species, as would be expected for arthropod cuticle. We show broadly similar mandible microstructure but report that pore canals and cuticular indentations are not ubiquitous among all studied taxa. Our preliminary results demonstrate that ant taxa have morphologically plastic mandibles with a highly conserved construction, potentially reflecting an interesting record of evolutionary stasis.

KEYWORDS

ants, arthropoda, Australia, *Camponotus nigriceps*, cuticular microstructure, *Iridomyrmex purpureus*, mandibles, *Odontomachus simillimus*, *Rhytidoponera aciculata*

1 | INTRODUCTION

Arthropods represent one of the most species-rich groups with between 2.9–12.7 million extant species, accounting for ca. 80% of animal biodiversity (Hamilton et al., 2013; Rota-Stabelli et al., 2011). The arthropod exoskeleton provides shape, rigidity and protection against predators and allowed the group to diversify across the Phanerozoic. The exoskeletal cuticle is secreted from epidermal cells during ecdysis and consists of a thin (1–4 µm) epicuticle layer (Mani, 1973) and the thicker (10–100 µm) procuticle (Chen, Peng, Wang, Zhang, & Zhang, 2002; Muzzarelli, 2011). Procuticle is divided into a sclerotised exocuticle—hardened and darkened cuticle (Richards & Davies, 1977)—and a softer, more elastic endocuticle (Chen et al., 2002; Muzzarelli, 2011).

The degree of sclerotisation varies across the exoskeleton, and mandibles—modified anterior appendages that characterise Mandibulata—are often highly sclerotised regions (Edgecombe, Richter, & Wilson, 2003; Vincent & Wegst, 2004). The highly sclerotised nature of mandibles means that they are used in fighting, food manipulation, and larvae nesting (Fabritius et al., 2011; Patterson, 1984; Wheeler, 1926). These are all applications that produce variable intra- and inter-taxon mandible morphology (Fabritius et al., 2011; Manting, Torres, & Demayo, 2015).

Hymenoptera is a large and diverse insect group with taxa exhibiting a range of mandible morphologies (Keller, 2011). External morphology of ant (Hymenoptera: Formicidae) mandibles is thoroughly documented (Cribb et al., 2008; Da Silva Camargo, Hastenreiter, Forti, Lopes, & Floriano, 2015;

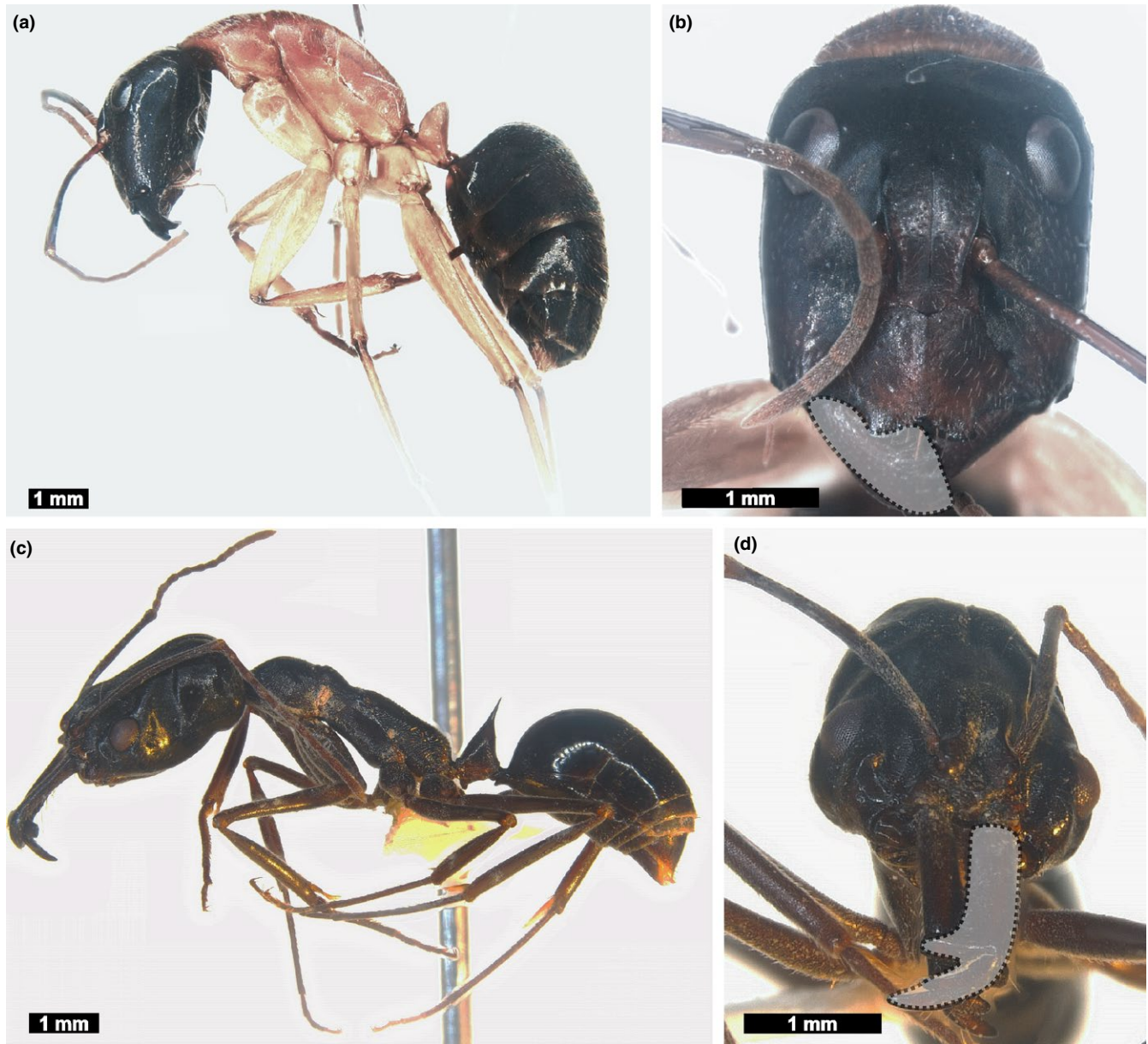


FIGURE 1 Complete specimens of *Camponotus nigriceps* and *Odontomachus simillimus* showing areas of sectioned mandible. (a, b) *C. nigriceps* specimen (NENH Pinned Insect Collection #1143). (a) Left lateral view. (b) Anterior view of head, showing where mandible was sectioned (area in white). (c, d) *O. simillimus* specimen (NENH Pinned Insect Collection #1145). (c) Left lateral view. (d) Anterior view of head, showing where mandible was sectioned (area in white) [Colour figure can be viewed at wileyonlinelibrary.com]

Manting et al., 2015; Manton, 1964). However, the internal microstructure appears somewhat under-explored (e.g., Brito et al., 2017; Joshua Gibson, pers. comms, 2018), despite the possible use of cuticle microstructure in taxonomy (Wheeler, 1926). To expand this knowledge, we have explored the mandible cuticular microstructure of four Australian Formicidae: *Camponotus nigriceps*, *Iridomyrmex purpureus*, *Odontomachus simillimus* and *Rhytidoponera aciculata* (Smith, 1853) (Figures 1 and 2) using scanning electron microscopy. These four species were chosen as they have disparate ecological roles (predator, scavenger and myrmecochorous), distributions and different mandible morphologies

(Shattuck, 2000). Furthermore, these taxa are commonly assessed in ecological, morphological and physiological studies across environmental gradients (Andrew, Miller, Hall, Hemmings, & Oliver, 2019; Gibb et al., 2015; Oliver, Dorrough, Doherty, & Andrew, 2016; Yates & Andrew, 2011). Important anatomical information is presented herein to augment the use of these taxa in such studies.

C. nigriceps (Figure 1a)—the sugar ant (Andersen, 2002)—is widespread along the east, south and western sections of Australia. This species consumes nectar, plant secretions and honeydew (Andersen, 2002; Shattuck, 2000). The mandibles are short and rounded with a blunt gnathal edge



FIGURE 2 Complete specimens of *Iridomyrmex purpureus* and *Rhytidoponera aciculata* showing areas of sectioned mandible. (a, b) *I. purpureus* specimen (NENH Pinned Insect Collection #1144). (a) Left lateral view, showing where mandible was sectioned (area in white). Note that this mandible was sectioned in an oblique orientation. (b) Anterior view of head. (c, d) *R. aciculata* specimen (NENH Pinned Insect Collection #1142). (c) Left lateral view. (d) Anterior view of head, showing where mandible was sectioned (areas in white). Due to mandible curvature, not all of the structure was sectioned [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 1b). They may reflect a foraging or scavenging ecology (Shattuck, 2000).

Odontomachus simillimus (Figure 1c) is a predatory, trap-jaw ant from north Australia that hunts singularly and feeds on invertebrates (Shattuck, 2000). Elongated mandibles with 2 to 3 large teeth at the distal gnathal edge benefit the known predatory behaviour (Figure 1d) (Larabee & Suarez, 2015). Mandibles can open to 180° and close on prey at up to 60 ms^{-1} , producing forces in excess of 300 times their body weight (Larabee et al., 2016; Larabee & Suarez, 2015; Manting et al., 2015).

Iridomyrmex purpureus (Figure 2a)—“meat ants”—are distributed across most Australian states. They are generalised, polydomous scavengers that forage for honeydew and plant secretions and build nests close to new food sources to limit transport time (Shattuck, 2000; Van Wilgenburg & Elgar, 2007). Their mandibles are short, sturdy so potentially effective in combat (Figure 2b).

Rhytidoponera aciculata (Figure 2c) inhabit the Australian east coast, especially urban areas. They are myrmecochorous, foraging for seeds of native plants (Majer, Gove, Sochacki, Searle, & Portlock, 2011; Shattuck, 2000). Their mandibles

are hooked and allow individuals in the genus to manoeuvre seeds up to eight times their body mass (Figure 2d) (Nielsen, 2001).

By comparing these different genera, we explore whether cuticular microstructure is similar across the four taxa. We present the null hypothesis that mandibles will be microstructurally similar, despite the varied life modes. Confirming this hypothesis allows us to explore the conserved nature of cuticle construction across varied morphologies. This work builds on the use of transverse cuticular sections to examine internal features (Brito et al., 2017; Cribb et al., 2008; Edwards, Fawke, McClements, Smith, & Wyeth, 1993); a method applied recently to other extant and fossil arthropod taxa (Bicknell, Paterson, Caron, & Skovsted, 2018).

2 | MATERIAL AND METHODS

2.1 | Specimens

One specimen per species (*C. nigriceps*, *I. purpureus*, *O. similimus* and *R. aciculata*) was sampled from the University of New England (UNE), Natural History Museum, Entomology spirit collection (NENH Spirit Collection). The left mandible of *C. nigriceps* and *O. similimus* and the right mandible of *I. purpureus* and *R. aciculata* were extracted for the study. The right mandible was used when the left was damaged in removal. Housing specimens in ethanol had dehydrated them thoroughly. Mandibles were embedded in Leco epoxy to make an epoxy block (a ratio of 1:7 hardener to resin). The posterior side of embedded mandibles was ground through with fine sandpaper and polished with a Mecatech 334 polisher and 1, 3 and 6 μm diamond suspension liquid to produce sections through the mandibles. Ideally, dorsal–ventral planes were produced for mandibles, but the success of this approach varied depending on mandible curvature. Sectioned surfaces of epoxy blocks were polished and then photographed under normal (fibre optic) lighting conditions using a SZX-ZB7 Olympus stereomicroscope, with a SC50 5MP colour camera at UNE. Blocks were later gold coated and imaged using the JEOL JSM-6010LA scanning electron microscope (SEM) at UNE. An accelerating voltage of 5 kV was used to acquire secondary electron images of mandibles. The blocks are housed at the UNE Natural History Museum. The chosen method contrasts serial sectioning and follows Bicknell et al. (2018) who effectively resolved minute cuticular microstructures. Furthermore, serial sectioning would have destroyed the specimens, precluding further use.

To show overall species morphology, where the studied mandibles are located in life position, and approximate planes of sectioning, one more specimen of all studied taxa

was documented. They were taken from the UNE Natural History Museum, Entomology pinned insect collection (NENH Pinned Insect Collection). They were photographed with Leica MZ16A microscope with a DFC 320 camera. The photos were stacked and montaged with LAS Core software.

2.2 | Terminology

The following terminology is used:

Cuticular microstructure—Features identified either by examining cuticular surfaces or by making sections through cuticle (Waugh, Feldmann, & Schweitzer, 2009). Here, cuticular microstructures are the features observed after sectioning the mandible. These include procuticle divisions, pore canals and cuticle indentations.

Epicuticle—The thin, dark, sclerotised outer layer of cuticle (Dalingwater, 1975; Muzzarelli, 2011). This feature was not confidently resolved here.

Procuticle—Major portion of cuticle beneath the epicuticle. Includes exocuticle and endocuticle (Dalingwater, 1975; Muzzarelli, 2011).

Endocuticle—Part of procuticle located beneath the exocuticle, softer and more elastic than exocuticle (Dalingwater, 1975; Richards, 1951).

Exocuticle—Upper procuticle, located above the endocuticle and sclerotised (Dalingwater, 1975; Richards, 1951).

Laminate cuticle—Cuticle constructed from the deposition of laminae, found within procuticle. Appears here as unidirectional, sometimes fluctuating, bands (Bicknell et al., 2018; Dalingwater, 1975; Richards, 1951).

Laminae unit—Pairs of cuticle bands; darker, narrow and dense laminae next to lighter, wider and less dense laminae (Dalingwater, 1985; Richards, 1951).

Transverse section—Removal of outer cuticle to document internal cuticular structures (*sensu* Bicknell et al., 2018).

3 | RESULTS

3.1 | *Camponotus nigriceps*

Image under normal light shows that the appendage centre is surrounded by thick cuticle (Figure 3a). Epicuticle is not resolved definitively, and procuticle thickness varies around the mandible (50–200 μm). Cuticle is thinnest proximally. Short hairs, potentially tactile hairs, extend from the gnathal edge. SEM images (Figure 3b–d) show a laminate procuticle consisting of laminae units that have a maximum thickness of ca. 4 μm . Endo-exocuticle boundary is not observed, and there is no obvious epicuticle. Pore canals are present in the procuticle (Figure 3c) (Dalingwater, 1973; Richards, 1951).

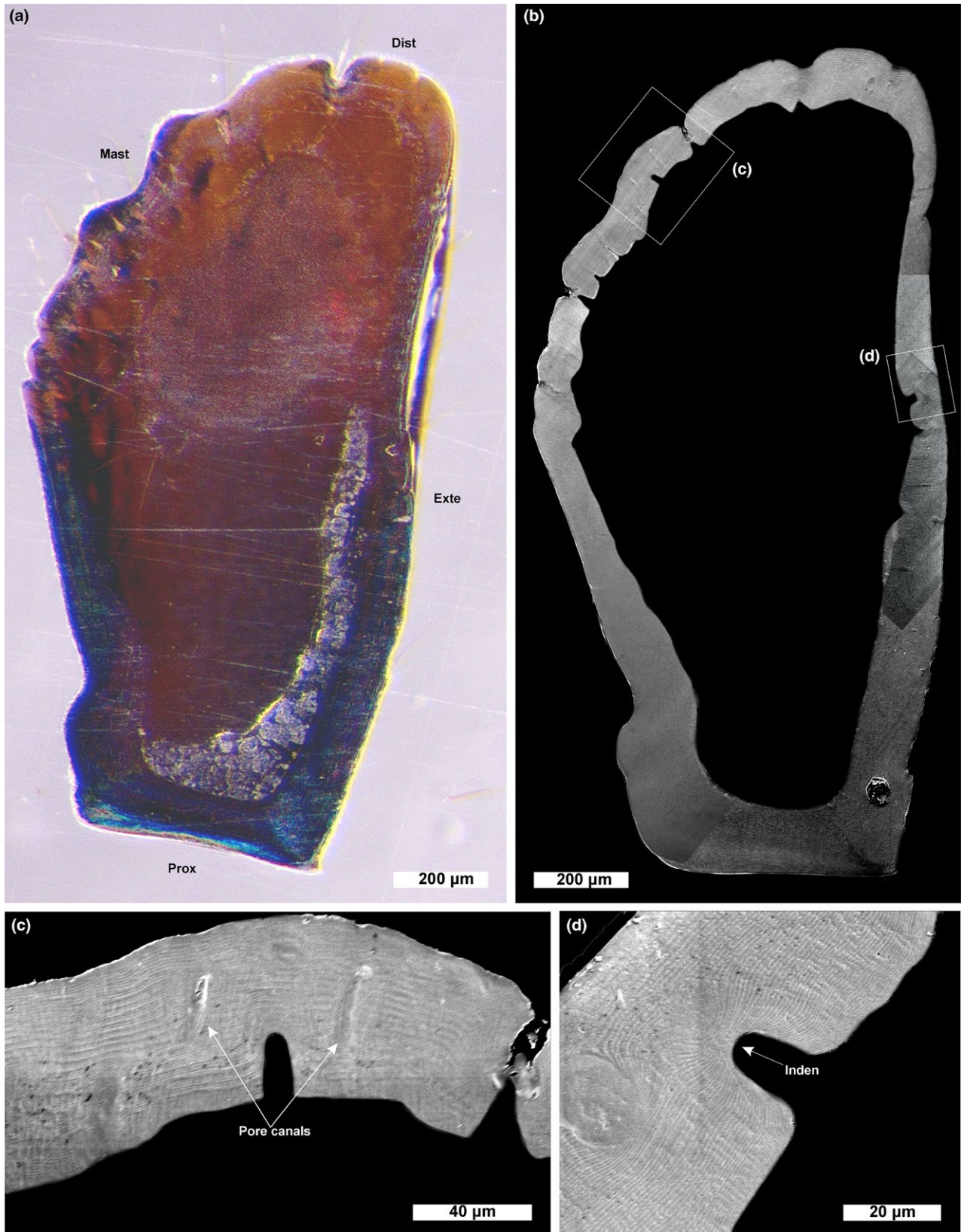


FIGURE 3 Dorsal–ventral section of *Camponotus nigriceps* from forager caste (NENH Spirit Collection 00065, left mandible). (a) Mandible photographed under normal light conditions. (b) Image of sectioned mandible taken under SEM. Boxes are expanded in c and d. (c) Close-up of mandible spines showing laminate procuticle and pore canals. (d) Close-up of right mandible side showing laminae structure and indentations. Dist: Distal area; Exte: External margin; Indet: Indentation; Mast: Masticatory margin; Prox: Proximal area [Colour figure can be viewed at wileyonlinelibrary.com]



FIGURE 4 Dorsal–ventral section of *Odontomachus simillimus* (NENH Spirit Collection 00057, left mandible). (a) Left mandible photographed under normal light, showing mandible spines. (b) Image of sectioned mandible taken under SEM. Boxes are expanded in c, d and e. (c) Close-up of mandible tip showing endo-exocuticle boundary. (d) Close-up of left mandible side showing laminae units and an indentation. (e) Close-up of mandible spines showing pore canals and more thinly laminate cuticle. Dist: Distal area; Endo: Endocuticle; Exo: Exocuticle; Exte: External margin; Indet: Indentation; Mast: Masticatory margin; Prox: Proximal area; Tac: Tactile hair [Colour figure can be viewed at wileyonlinelibrary.com]

Indentations in mandible cuticle extending into laminae layers are noted (Figure 3d).

3.2 | *Odontomachus simillimus*

Image under normal light shows that appendage is long, thin and has small tactile hairs extruding from gnathal edge (Figure 4a). Hairs extend into the cuticle and disrupt the cuticular deposition. Cuticular divisions and epicuticle are not noted. SEM images (Figure 4b–e) show lamina units up to 2–3 μm thick. Most proximal gnathal spines were bisected and are constructed from thinly (1 μm) laminate cuticle (Figure 4e). An endo-exocuticle boundary was identified in SEM images (Figure 4c). Pore canals cross-cutting laminae are noted (Figure 4e). Indentations in the cuticle are also noted and only extend 10 μm into the cuticle (Figure 4d).

3.3 | *Iridomyrmex purpureus*

Image under normal light shows a curved mandible ending in an apical tooth. No evidence of the epicuticle or cuticular boundaries was noted (Figure 5a). Mandible curvature precluded dorsal–ventral sectioning of mandible. SEM images show that laminae units were up to 6 μm thick. No pore canals and indentations were identified (Figure 5b–e). Cuticle is mostly continuous, unidirectional laminae that deform only slightly about the gnathal edge. Endo-exocuticle boundary is observed (Figure 5c, e).

3.4 | *Rhytidoponera aciculata*

Image under normal light shows gnathal spines along the distal end of the mandible and no obvious epicuticle (Figure 6a). Mandible curvature precluded complete bisection of the

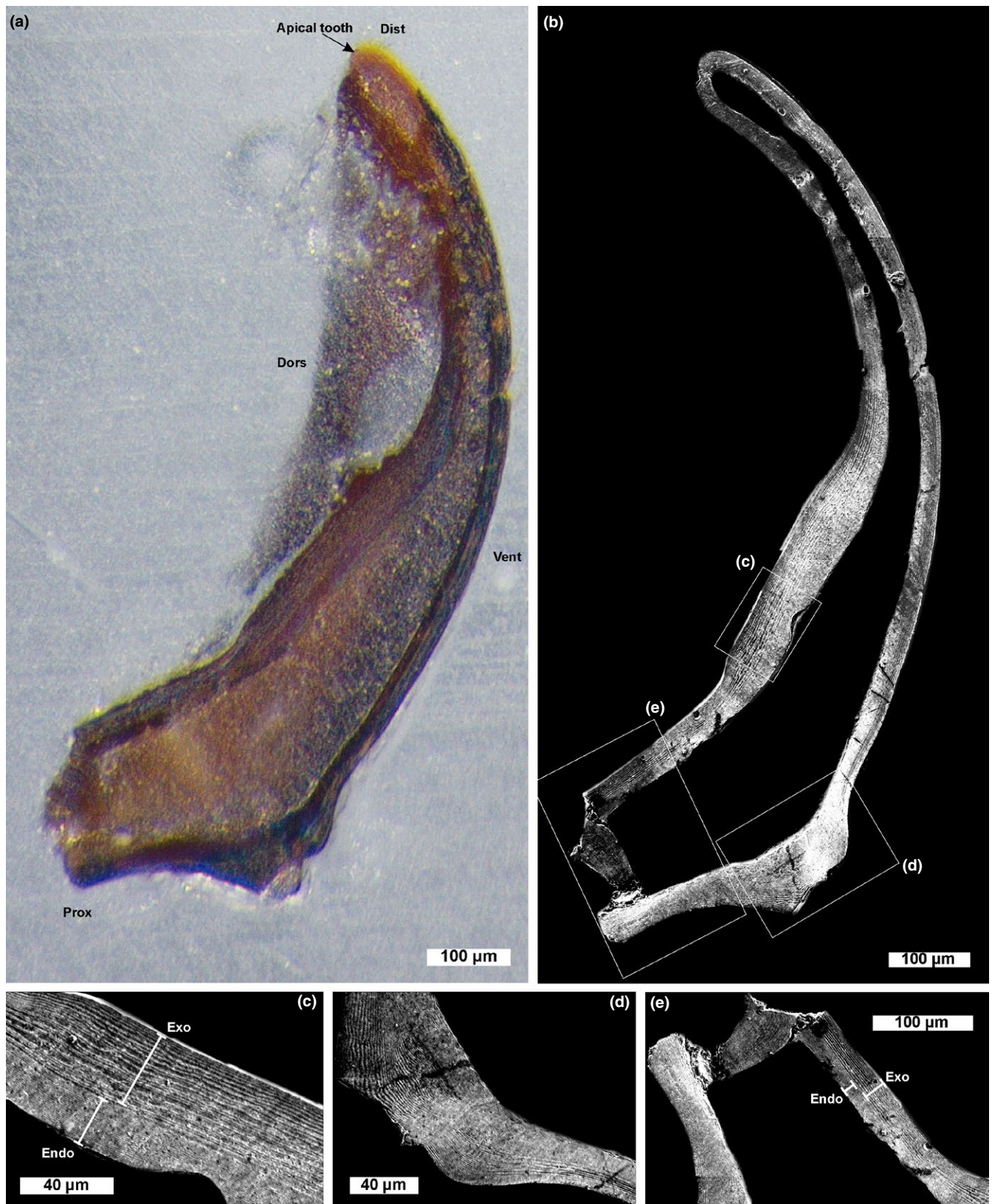


FIGURE 5 Oblique, almost longitudinal section *Iridomyrmex purpureus* (NENH Spirit Collection 00005, right mandible). (a) Image of sectioned mandible taken under normal light conditions, showing sclerotised cuticle. (b) Image of sectioned mandible taken under SEM. Boxes are expanded in c, d and e. (c) Close-up image of procuticle showing laminae of higher and lower density and the endo-exocuticle boundary. (d) Close-up of procuticle showing continuous wavy laminae units. (e) Close-up of basal end of mandible showing endo-exocuticle boundary. Dist: Distal area; Dors: Dorsal side; Endo: Endocuticle; Exo: Exocuticle; Prox: Proximal area; Vent: Ventral side [Colour figure can be viewed at wileyonlinelibrary.com]

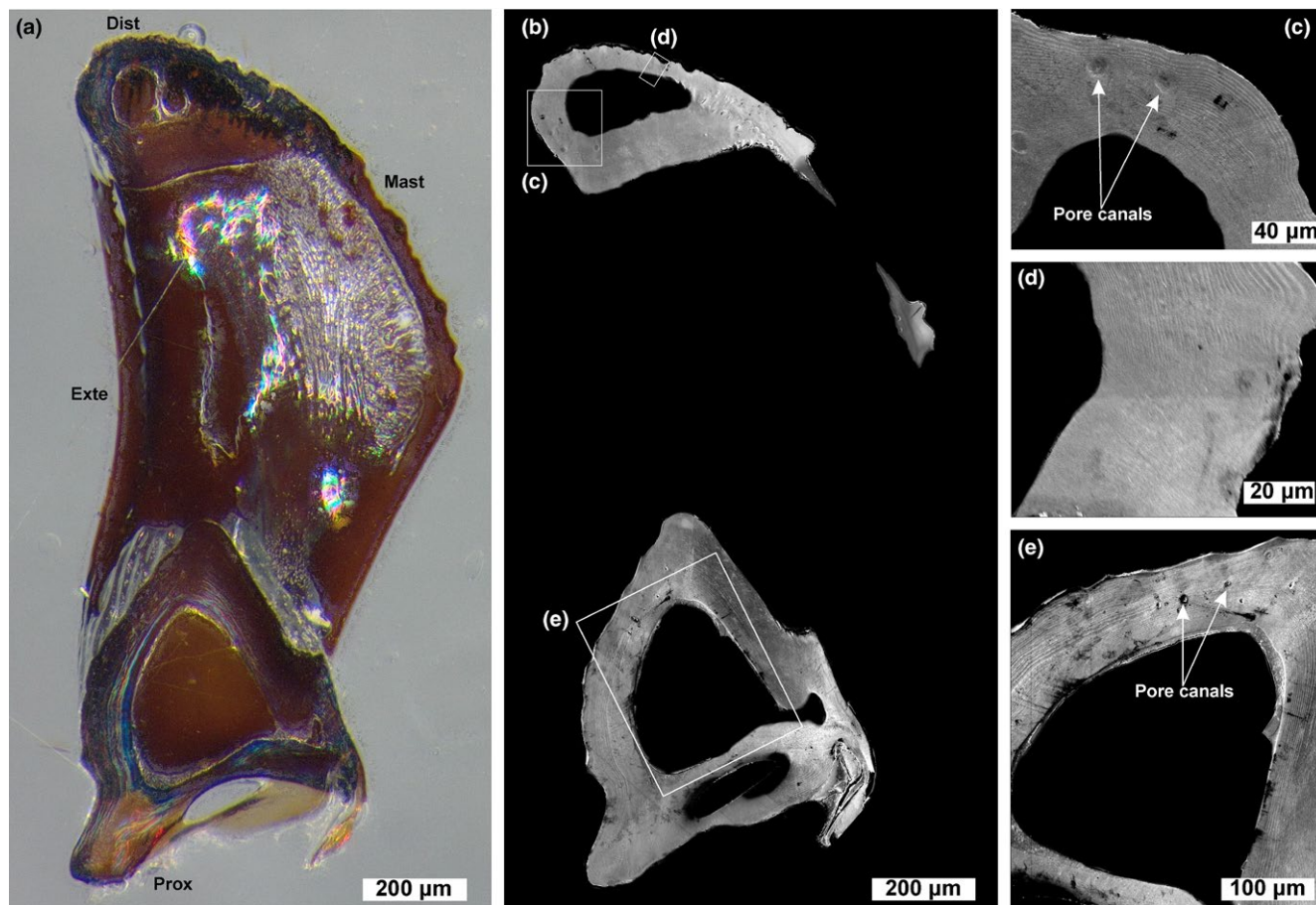


FIGURE 6 Dorsal–ventral section of *Rhytidoponera aciculata* (NENH Spirit Collection 00050, right mandible). (a) Sectioned mandible photographed under normal light showing sclerotised gnathal edge. (b) Image of sectioned mandible taken under SEM. Note not all of the mandible was uncovered during sectioning. Boxes are expanded in c, d and e. (c) Close-up of mandible tip showing pore canals and cuticle deforming about the canals. (d) Close-up of mandible spines, showing laminae structure of the procuticle. (e) Close-up of proximal mandible section showing laminate cuticle deforming about pore canals. Dist: Distal area; Exte: External margin; Mast: Masticatory margin; Prox: Proximal area [Colour figure can be viewed at wileyonlinelibrary.com]

mandible (Figure 6b). Under SEM, the cuticle has lamina units that are 2 µm thick (Figure 6c–e). An endo-exocuticle boundary is observed, but no epicuticle. Pore canals throughout the cuticle are noted and are unevenly distributed.

4 | DISCUSSION

Environmental pressures, dietary needs and niche partitioning are the drivers behind the formicidan mandible diversity (Manting et al., 2015; Morehead & Feener, 1998; Smith & Capinera, 2005). To augment the few studies of internal mandible structures and to illustrate mandible morphology more completely, we documented internal anatomy of four formicidan taxa. We confirm our original null hypothesis that mandible microstructure of these four disparate species was broadly similar. These results illustrate that mandibles are morphologically plastic at a macroscopic level, but the microstructural construction

thereof is highly conserved. It is therefore worth considering why ant mandibles are so conserved, despite the varied life modes. Similar mandible conservation is known to the highly diverse Lepidoptera genus *Hypsmocoma* (Rubinoff, 2008; Williams, 2013). Conserved structures are thought to reflect functional effectiveness of the microstructure across ecologies (Williams, 2013). Formicidae mandible microstructure likely mirrors this concept. Furthermore, from a palaeontological and evolutionary perspective, it might also be that an effective *Bauplan* for ant mandible construction arose in the Cretaceous (Nel, Perrault, & Néraudeau, 2004) or was retained from the ancestral hexapod stock that gave rise to modern Formicidae (see discussion in Kukalová-Peck, 2008). Ant mandible microstructure therefore potentially represents an example of stasis and conserved evolution at the microscopic scale (Eldredge et al., 2005). Examining mandibles of fossil hexapods, especially Formicidae, represent an avenue worth exploring to confirm this thesis.

4.1 | Cuticle

“Stacking” of laminae units reflects rhythmic cuticle growth: darker stripes are denser, protein-rich layers while the protein-poor layers are lighter (Dalingwater, 1973; Dennell, 1978; Richards, 1951). This feature was observed in all species: an expected outcome for cuticular features (see discussion in Mani, 1973; Neville, 1975 and Richards, 1951). However, the endo-exocuticle boundary was only found in *I. purpureus* and *O. simillimus* and the division was less clear than other extant and extinct groups (Bicknell et al., 2018; Dalingwater, 1975; Gorb, 1999). This lack of division is known to weevils and illustrates that the endo- and exocuticle are connected cuticular zones (van de Kamp, Riedel, & Greven, 2016). Homogenous procuticle may reflect complete sclerotisation of the appendages, but more research into the sclerotisation is needed to confirm this idea. *O. simillimus* specifically is worth exploring further as the taxon has highly modified mandibles for high-impact, spring-loaded attacks (Larabee & Suarez, 2015). The gnathal edge is constructed to facilitate these attacks and associated wear (Figure 4b). There are more laminae units along the gnathal edge ($n > 30$) than on external margin sections of similar thickness ($n \geq 20$). More cuticle is therefore deposited on the gnathal edge to allow more wear. Furthermore, this species has the most prominent tactile hairs (Figure 4d) that develop from the gnathal edge. These hairs are embedded in the mandible and disrupt where laminae units develop. Finally, the lack of epicuticle is curious, as this feature is resolved in articles such as Scholz, Baumgartner, and Federle (2008) and Noh, Muthukrishnan, Kramer, and Arakane (2016). Curiously, Bicknell et al. (2018) were unable to resolve the feature in their sample of *Limulus polyphemus*. The lack of epicuticle may reflect imbedding specimens in epoxy or limited magnification.

4.2 | Pore canals

This feature is often documented in arthropod cuticle (Bresciani, 1986; Dalingwater, 1985; Selden, 1981) as pore canals transport nutrients, excrement, wax and metals through the cuticle (Brito et al., 2017; Chen et al., 2002; Cribb et al., 2008; Mekhanikova et al., 2012; Neville, 1975). Formicidan taxa impregnate metal in mandible cuticle to increase the strength and durability thereof (Cribb et al., 2008; Edwards et al., 1993; Schofield, Nesson, & Richardson, 2002). Here, pore canals were observed in three of the four studied species. Undoubtedly, these canals have the same function of mandible fortification in all species.

4.3 | Limitations

The primary aim of this work was to determine whether the four taxa had similar mandible construction. However, two

major caveats arose in only assessing one specimen. These limitations should be avoided if this method is to be used for taxonomy or phylogenetics. The first issue was that the single specimen limited the degree of interpretation we could present and the likelihood of identifying more features. Future applications would benefit from sectioning multiple mandibles in different orientations. This approach will allow more data to be gathered, especially as cuticle is deposited in three dimensions (Dalingwater, 1987). Exploring multiple sections will also avoid the other issue experienced here: mandible curvature. *I. purpureus* mandibles are highly curved, so we only able to report an oblique, almost longitudinal section (Figure 2a). This contrasts the dorsal–ventral sections achieved for the other taxa. This has slightly limited the scope of our comparative results. Nonetheless, the data presented here represent a useful and informative step towards completely understanding these iconic arthropods.

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