

# Seasonal Polyphenism and Behavioral Variations of *Ceroplastes glomeratus* Peronti (Hemiptera: Coccidae)

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## Abstract

The wax scale *Ceroplastes glomeratus* Peronti shows seasonal behavioral and phenotypic differences. Individuals develop gregariously during autumn, whereas solitarily in spring. Besides such behavioral differences, spring (“isolated form”) and autumn (“aggregated form”) will also produce different morphotypes. We provide additional data on the morphological data of the species based on the microscopic and macroscopic characteristics of adult females and of first-instars of the two adult morphotypes of *C. glomeratus*.

## Introduction

Polyphenism is a plastic response of a genotype to internal and/or external elicitors, such as environmental stress conditions (nutrition, temperature, crowding, among others), and this developmental plasticity is related to the fitness success of several species of insects (Mayr 1963, Greene 1989, Smith 1991, Strübing & Drosopoulus 2005). Polyphenism in insects may be presented in several ways, but caste and seasonal polyphenisms are among the most common (Simpson *et al* 2011). Seasonal polyphenism is a good example of how insects may benefit from their developmental plasticity and is defined as “an annually repeating pattern of changing phenotypic ratios in successive generations, under some kind of environmental control” (Shapiro 1976). Moreover, the occurrence of different morphological types may lead to species misidentification because these are not recognized as a polyphenic response, particularly for

species in which different morphological types do not overlap during their development (Grella *et al* 2015).

Among Coccoidea, the few examples of polyphenism are found in Diaspididae. Lupo (1943) demonstrated the armored scale *Mytilococcus ficifoliae* (Berlese) was in fact the summer morph of *Mytilococcus conchiformis* (Gmelin). Morphological differences observed for these seasonal morphs were also related to the tissue of the host plant to which each morphotype was associated with. The *ficifoliae* morph was found to feed on leaves of fig trees, while the *conchiformis* morph on branches. *Mytilococcus ficifoliae* was synonymized with *M. conchiformis* by Lupo (1943) and is currently placed in the genus *Lepidosaphes* Shimer (García *et al* 2016). Phenotypic variation in diaspidids in response to their association with different tissues of the host plant is known to occur in a number of species (Takahashi 1953, Stannard 1965, Takagi & Kawai 1967, Knipscher *et al* 1976, Liu *et al* 1989), and polyphenism induced by the association

with different host plant tissues has been referred as “host-site-induced polymorphism” (Liu et al 1989). But different morphotypes associated with the same host plant tissue in different seasons of the year have also been described (Takagi 2012).

Seasonal polyphenism among Coccidae are extremely rare, but we were able to demonstrate in this study the existence of two morphological types in *Ceroplastes glomeratus* Peronti (Hemiptera: Coccidae: Ceroplastinae) under daily natural laboratory conditions. *Ceroplastes glomeratus* is a Neotropical species, known only from Brazil. Ceroplastinae are commonly named wax scales because adult females have a thick waxy test covering the whole of the dorsum. *Ceroplastes* Gray is the largest genus of Ceroplastinae with 145 species described worldwide (García et al 2016). *Ceroplastes glomeratus* was described occurring on Fabaceae but also recorded on Asteraceae and Anacardiaceae, presenting only the agglomerated form (Peronti et al 2008).

Herein, we also provide additional morphological and behavioral data on *C. glomeratus*, report on the occurrence of its seasonal polyphenism, and provide a comparative morphological analysis to characterize each morphotype.

## Material and Methods

Mature, ovipositing females of the agglomerated morphotype (form) of *C. glomeratus*, identified based on the description by Peronti et al (2008), were collected on *Inga* sp. (Fabaceae) at the municipality of São Carlos (22°01' S–47°53'W), state of São Paulo, Brazil, in July 2012. Insects were placed in glass tubes, closed with cotton until hatching. Part of the newly hatched first-instars was slide-mounted, and the remaining was placed on seedlings of *Inga* sp. for rearing at room temperature at the Laboratory of Entomology, Department of Ecology and Evolutionary Biology, Federal University of São Carlos, São Carlos, Brazil, for two generations. The two morphotypes produced from each generation were subjected to molecular and morphological analyses.

### Molecular analysis

Adults (2) of each morphotype were individually subjected to nondestructive DNA extraction following Gilbert et al (2007), as modified by Dossi et al (2014). The insect cuticle was removed from the digestion buffer, stored in 70% ethanol, and slide-mounted according to Granara de Willink (1989) for voucher species identification.

The obtained DNA was subjected to PCR amplification of the mitochondrial cytochrome c oxidase subunit I (COI), using the set of primers C1-1554F (5'-CAGGAATAATAGGA

ACATCAATAAG-3') and C1-2342R (5'-ATCAATGTCTAATC CGATAGTAAATA-3') (Deng et al 2012), and the expansion segment (D2) of the 28S rDNA, using the primers D2-3566F (5'-TGCAGCTCTAAGTTGGTGGT-3') and D2-4068R (5'-TTGG TCCGTGTTCAAGACGGG-3') (Campbell et al 1993). PCR reactions contained 1 µL of DNA template, 5 µL of 5x Green Reaction Buffer, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.32 µM of each primer, and 1 unit of Taq DNA polymerase in a total reaction volume of 25 µL. Thermocycling conditions were 3 min at 95°C (1x), followed by 35 cycles at 94°C for 30 s, 52°C for 50 s, and 72°C for 1 min, followed by final extension of 10 min at 72°C. Amplicons were resolved by gel electrophoresis in a 1% agarose gel slab, using TAE buffer with ethidium bromide, at a constant voltage (100 V). Amplicons were visualized using a transilluminator.

PCR products were purified following precipitation with 80% ethanol (Davis et al 1994) and subjected to ExoSAP (Fermentas) treatment following the manufacturer's recommendations for clean-up prior to sequencing. Samples were sequenced bidirectionally using the original set of primers at the “Centro de Estudos do Genoma Humano (CEGH-USP)” (<http://genoma.ib.usp.br/>). Sequences obtained were analyzed, edited, and aligned using the Sequencher version 4.2 (Gene Codes Corporation, Ann Arbor, MI, USA). The resulting sequences were also compared to sequences deposited at the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>) using the Blastn search algorithm for putative species identification. All sequences obtained were deposited at the NCBI database (GenBank accession numbers KX650805–KX650808 and KX670819–KX670822).

### Morphological analysis

Adults and first-instars of each generation were slide-mounted according to Granara de Willink (1989) and studied under an optical microscope Olympus CBA; morphological descriptions were based on five adults and six first-instars of each generation, following the terminology of Hodgson & Peronti (2012) for adults and of Rosa et al (2011) for nymphs. Digital images were acquired with a Nikon D3200 coupled to a stereomicroscope Olympus SZ40.

All slide-mounted specimens used for morphological and molecular analyses were deposited in the Entomological Collection of the Laboratory of Economic Entomology, Department of Ecology and Evolutionary Biology, Federal University of São Carlos.

## Results

*Ceroplastes glomeratus* had two generations between July 2012 and July 2013 under laboratory rearing. Adult

females of the first generation were collected for analysis in November 29, 2012 (late spring), and those of the second generation in May 20, 2013 (late autumn), both collected on twigs of *Inga* sp. (Fabaceae). The adults of the spring generation presented morphological and behavioral differences from their parentals. Adult females sampled in springer did not show the aggregation behavior such as their parents, and thus, their morphotype was named as “isolated form” (Fig 1 (a–d)). The adult females of the autumn generation returned to the agglomerated behavior of the field-collected adults. Autumn individuals were densely aggregated, forming a mass of wax that made very difficult to distinguish the limits of each specimen. The morphotype of these specimens was named as “agglomerated form” (Fig 1 (e–g)). Although the adult females of the two morphotypes displayed behavioral and morphological differences, the first-instars obtained from each of the generations of *C. glomeratus* under laboratory conditions did not show any detected differences.

#### Molecular analysis

Molecular analyses of the partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) and the expansion segment (D2) of the 28S rDNA for both morphotypes resulted in 100% similarity between them, confirming that they belong to the same species.

#### Morphological description

##### *First-instar* (Figs 1 (i) and 2)

##### *Unmounted material* (Fig 1 (i))

Wax thin, glassy, translucent, but may look yellow because of the color of the scale tegument, detachable, covering whole dorsum, with marginal expansions little evident. Dry-wax filaments and expansions medially on the dorsum absent. Stigmatic bands are visible only at the stigmatic regions.

**Body.** Elongate oval, 370–430  $\mu\text{m}$  long; 200–225  $\mu\text{m}$  wide, yellowish in color. Caudal process absent.

##### *Mounted material* (Fig 2)

**Dorsum.** Derm entirely membranous, dorsal clear areas absent. Dorsal simple pores not detected. With one pair of pores near head apex, each about 2.5–3.0  $\mu\text{m}$  in diameter with three loculi and two pairs of sharply conical dorsal setae, longer than width of basal socket, each 2.5  $\mu\text{m}$  long, basal socket width 2.0  $\mu\text{m}$ , present on median dorsal area of the head. Preopercular pores absent. Anal plates each with a rather diagonal anterior margin and rounded posterior margin, length of plates 42–49  $\mu\text{m}$ , width of both plates combined 55–56  $\mu\text{m}$ ; single plate 26–28  $\mu\text{m}$ ; each with one

ventral seta 17–20  $\mu\text{m}$  long and with four dorsal setae on posterior half of each plate: apical seta longest, 230–260  $\mu\text{m}$  long, with three smaller setae: inner margin seta 6–9  $\mu\text{m}$  long, and two near apex, each 20–30  $\mu\text{m}$  long. Anterior margin of anogenital fold with one pair of setae. Anal tube shorter than size to length of anal plate. Anal ring with four pairs of setae: two well-developed setae, each 50–58  $\mu\text{m}$  long; one pair of shorter seta 35–45  $\mu\text{m}$  long; plus one pair of very short setae, 6–10  $\mu\text{m}$  long.

**Margin.** Marginal setae flagellate, each about 5–9  $\mu\text{m}$  long, with a broad basal-socket; with six anteriorly between eyespots, and, on each side, two between eyespots and anterior stigmatic area, two laterally between stigmatic areas, and seven between posterior stigmatic area and anal lobe; each anal lobe with one slightly thicker seta, 10–11  $\mu\text{m}$  long. Stigmatic clefts shallow, each with three stigmatic setae with rounded apex (knob like); central seta 4–5  $\mu\text{m}$  tall and 4–6  $\mu\text{m}$  wide, basal socket 4–5  $\mu\text{m}$  wide, slightly onto dorsum and lateral setae with rounded apex, 3–4  $\mu\text{m}$  tall and 3–4  $\mu\text{m}$  wide, basal socket 3.5–4  $\mu\text{m}$  wide. Eyespots set slightly onto dorsum; width of each lens 11–12  $\mu\text{m}$ .

**Venter.** Derm entirely membranous. Pregenital disc-pores absent. Spiracular disc-pores each with 5 (rarely 4 and 7) loculi, and 2.5–3.5  $\mu\text{m}$  in diameter; with 2–3 in a single line in each spiracular pore band. Ventral tubular ducts absent. Ventral microducts with a cruciform pore, each 2–3  $\mu\text{m}$  wide; with 9–10 microducts submarginally on each side of body; absent in rest of venter. Submarginal setae bristle-like, each about 2–4  $\mu\text{m}$  long, with two between eyespots and, on each side: one between stigmatic clefts and seven between each posterior furrow and anal cleft. Ventral setae similar to submarginal setae but slightly longer, with seven in longitudinal submedian row on each side, each 3–7  $\mu\text{m}$  long; with one pair of interantennal setae, each 25–35  $\mu\text{m}$  long, plus one pair of pregenital setae, each 25–30  $\mu\text{m}$  long.

Antennae each six segmented, total length 115–126  $\mu\text{m}$  long; segment lengths ( $\mu\text{m}$ ): I: 16–20; II: 14–15; III: 29–30; IV: 13–14; V: 17–18; VI: 28–30; antennal setae: segment I: 3 flagellate setae; II: 2 flagellate setae; III: 3 flagellate setae; IV: 1 fleshy seta; V: 1 fleshy seta and 1 flagellate seta; VI: 6–7 flagellate setae and 4 fleshy setae. Clypeolabral shield about 95–107  $\mu\text{m}$  long, with one pair of setae on clypeus plus four pairs of setae on labium. Spiracles with muscle plate longer than diameter of peritreme: diameter of peritreme 6–7  $\mu\text{m}$ ; length of muscle plate 8–13  $\mu\text{m}$ . Legs well-developed, without a tibio-tarsal sclerosis; each claw with a denticle; tarsal digitules unequal in length and thickness, one shorter and more slender than other, each 27–30 and 37–40  $\mu\text{m}$  long, respectively, but both knobbed at apex; claw digitules unequal, one broader than other, each 19–23  $\mu\text{m}$  long; dimensions of metathoracic legs ( $\mu\text{m}$ ): coxa 35–40; trochanter +



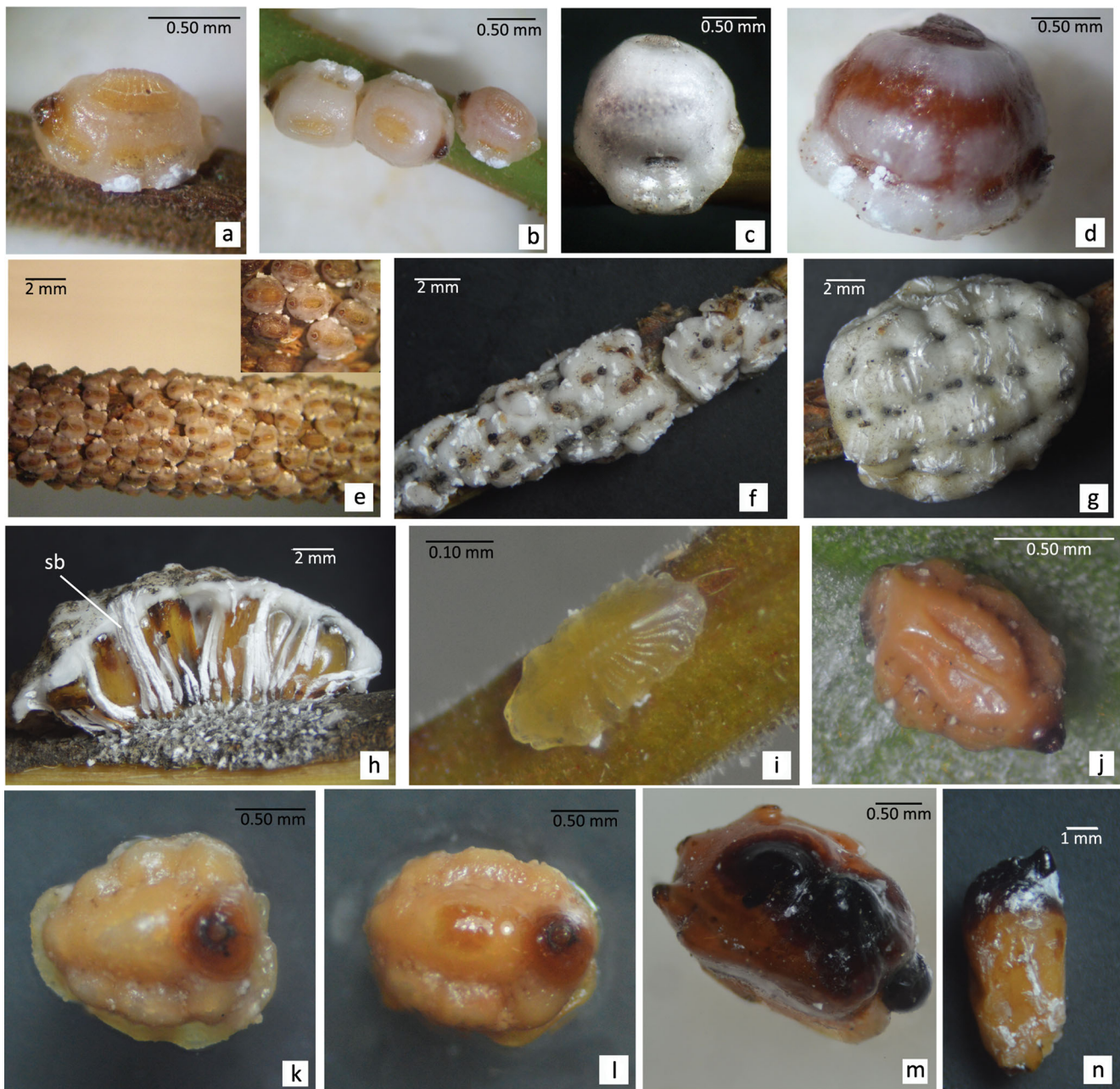


Fig 1 *Ceroplastes glomeratus* Peronti: (a, b) wax test of young adult females (isolated form); (c, d) wax test of old adult females (isolated form); (e) wax test of young adult female (agglomerated form); (f, g) wax test of old adult female (agglomerated form); (h) lateral view of old adults females with lateral wax removed (agglomerated form), where sb = stigmatic band; (i) first-instar with glassy test; (j) dorsal view of adult female (isolated form) with wax test removed, showing small caudal process; (k–m) dorsal view of adult females (agglomerated form) with wax test removed, showing development of dorsal sclerotization and caudal process; (n) lateral view of mature specimen with wax removed (agglomerated form), with strong sclerotization of caudal process on dorsum.

femur 65–73; tibia 45–50, tarsus 32–38, and claw 15–16; with three setae on tibia and four on tarsus.

#### Adult Females

*Isolated form* (Figs 1 (a–d, j) and 3)

#### Unmounted Material

Young adult female: more or less oval in shape; with a white wet waxy test covering dorsal surface of the body, except for an area around anal plates plus a mediodorsal region and seven submarginal nuclei, which lack wet wax and are filled with

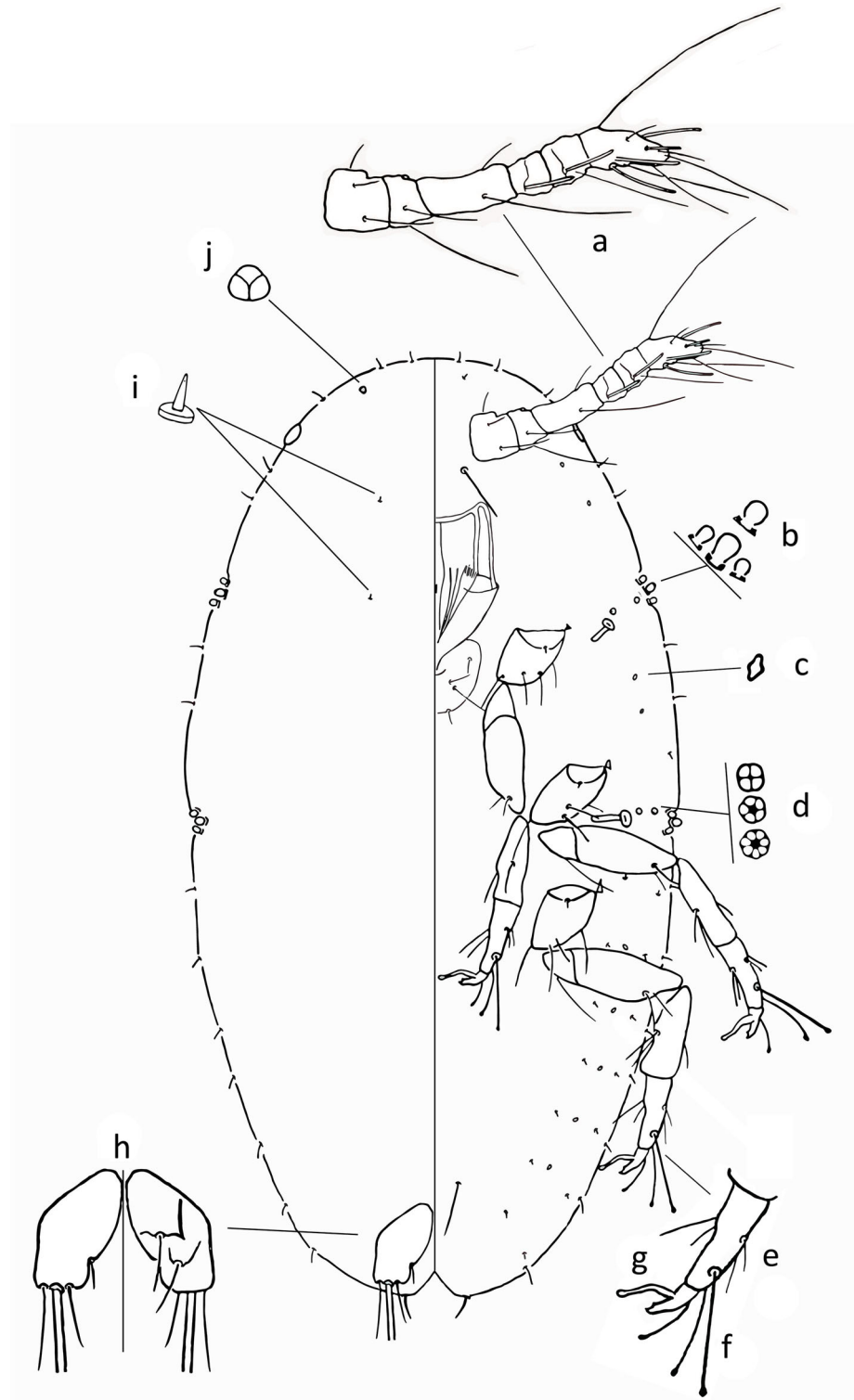


Fig 2 *Ceroplastes glomeratus* Peronti. First-instar, where *a* = antenna; *b* = stigmatic setae; *c* = ventral microducts; *d* = spiracular disc-pores; *e* = tarsal segment; *f* = tarsal digitules; *g* = claw digitules; *h* = anal plates, dorsal, and ventral aspect; *i* = dorsal seta; *j* = dorsal pore.

translucent glassy wax test; wet wax thick, with complete absence of division of test into wax plates; clear white stigmatic wax bands broad, ill-developed and visible on wet wax ventral marginal region and confined to stigmatic areas (Fig 1 (a, b)).

Old adult female: somewhat hemispherical in shape, dorsal derm more sclerotized and white wet wax becoming

thinner on dorsum, sometimes allowing visualization of sclerotized derm (Fig 1 (c, d)).

**Body.** Length 0.9–1.6 mm long and 0.6–1.3 mm wide, light brown in color. Small sclerotized caudal process (Fig 1 (j)).

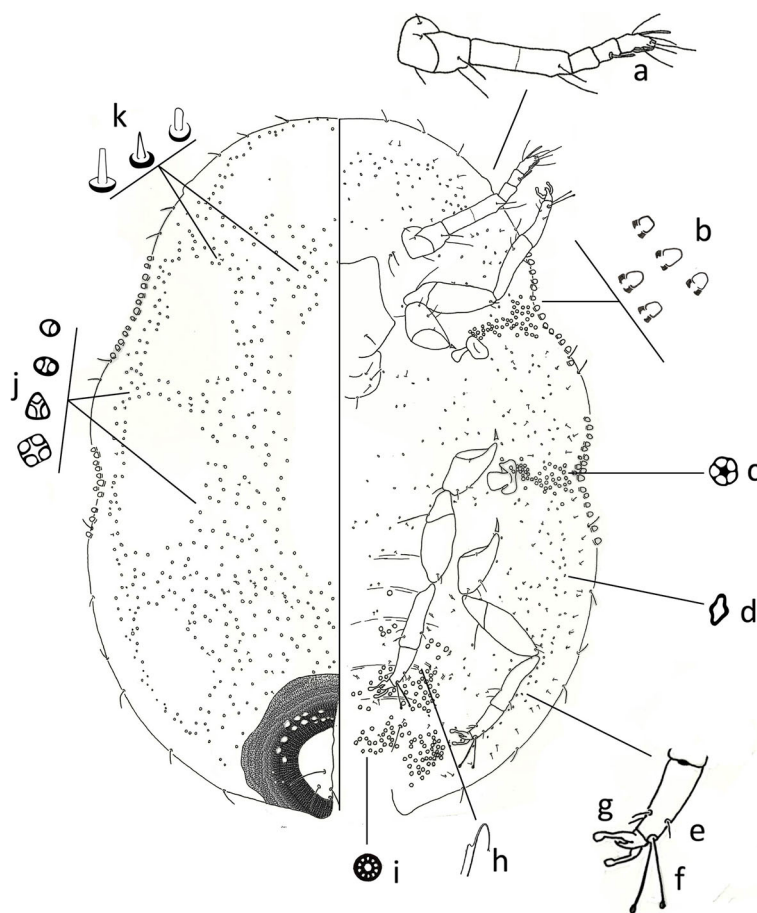


Fig 3 *Ceroplastes glomeratus* Peronti. Adult female (isolated form), where *a* = antenna; *b* = stigmatic setae; *c* = spiracular disc-pores; *d* = ventral microducts; *e* = tarsal segment; *f* = tarsal digitules; *g* = claw; *h* = ventral tubular ducts; *i* = pregenital disc-pores; *j* = dorsal pores; *k* = dorsal setae.

#### Mounted material (Fig 3)

**Dorsum.** Derm entirely membranous when young, except for sclerotized caudal process; derm becoming more sclerotized on oldest individuals. Caudal process little wider than long, about 0.35–0.5 mm wide and 0.3–0.4 mm long. Derm with eight clear areas, distributed as: one cephalic, one mediodorsal, and three pairs of lateral clear areas, without pores or setae, but a pair of setae can be present in cephalic area. Dorsal setae of variable shapes and sizes: (i) spinose, length 5–6  $\mu\text{m}$ , about 1.6–2.0 $\times$  longer than width of basal socket; basal socket width 3  $\mu\text{m}$ ; and (ii) with parallel-sided or slightly convergent sides and a truncate, rounded or laterally truncate apex, length 2.0–4.5  $\mu\text{m}$ , 0.6–1.5 $\times$  longer than width of basal socket; basal socket width 3  $\mu\text{m}$ ; quite frequent throughout but absent from clear areas apart from up to 2 short setae in cephalic clear areas. Dorsal pores: (i) loculate microducts, each with 1–4 satellite loculi, mainly with two satellite loculi; each pore 2–4  $\mu\text{m}$  wide, abundant throughout but absent from clear areas; and (ii) simple microducts not detected. Preopercular pores present and clearly visible; with about 20–30 in a narrow band 1–2 pores wide, present on derm around entire anterior margin of anal plates, extending to each corner; each pore about 4  $\mu\text{m}$

wide. Anal plates with rounded outer margin; length of plates 87–107  $\mu\text{m}$ , width of both plates combined 87–108  $\mu\text{m}$ , each plate with 3 long dorsal setae 35–50  $\mu\text{m}$  long, in a triangle plus a short subapical seta 10–15  $\mu\text{m}$  long. Anogenital fold with four pairs of setae on anterior margin. Anal tube short, apparently shorter than length of anal plates.

**Margin.** Marginal setae setose; each about 15–21  $\mu\text{m}$  long; with six anteriorly between eyespots, and (on each side) two between eyespots and anterior stigmatic area, two between stigmatic areas, and seven on each side of abdomen; each anal lobe with one seta. Stigmatic clefts shallow, with about 35–42 stigmatic setae; each group wider than long, with two to four irregular rows of stigmatic setae; each group of stigmatic setae extending some distance along margins but not meeting between clefts; stigmatic setae each roundly conical or cylindrical with a slightly flattened apex; each setae 5–12  $\mu\text{m}$  wide at base and 6–10  $\mu\text{m}$  long. Eyespots 15–19  $\mu\text{m}$  wide.

**Venter.** Derm entirely membranous. Pregenital disc-pores abundant around vulva and in segment VI, and with 4 medially and 4–6 submedially on each side in segment V; plus 0–1



medially in segment IV. Spiracular disc-pores present in fairly broad bands of about 40–47 pores, each band about 1.0–1.5x width of peritremes near margin, with few pores extending up to area of peritremes. Ventral microducts each about 3  $\mu\text{m}$  wide, present throughout venter and abundant in a submarginal band. Ventral tubular ducts each with a narrow inner ductule without an obvious terminal gland; absent anteriorly between antennae, sparse submedially on abdominal segments III–VI. Submarginal setae more frequent than marginal setae, each 5–8  $\mu\text{m}$  long.

Antennae each with 7 segments; total length 202–222  $\mu\text{m}$  long. Clypeolabral shield about 162–225  $\mu\text{m}$  long. Spiracles: width of peritremes 35–40  $\mu\text{m}$ . Legs well-developed, each with a tibio-tarsal articulatory sclerosis; each claw with a distinct denticle; claw digitules both broad and shorter than tarsal digitules; dimensions of metathoracic legs ( $\mu\text{m}$ ): coxa 80–90; trochanter + femur 120–135; tibia 85–95; tarsus 55–57, and claw 20–21.

#### Agglomerated form (Figs 1 (e–h, k–n), 4, and 5)

*Unmounted material.* Young adult female: densely aggregated around the twig, but their wax often touching neighbor individuals but not fused; more or less oval in shape; covered with a white wet wax, with a complete absence of divisions of the test

into wax plates. Mediodorsal nucleus plus seven submarginal nuclei and an area around anal plate lacking wet wax, but filled with a translucent glassy wax test. Clear white stigmatic wax bands broad, ill-developed and visible on wet wax on ventral marginal region and confined to stigmatic areas (Fig 1 (e)).

Old adult female: Wax test thicker with wax of adjacent individuals completely fused, forming a mass of wax around twig (Fig 1 (f, g)); stigmatic wax bands well developed, broad and extending onto dorsum (Fig 1 (h)); body growing vertically (taller than wide), with an elongation toward the dorsum (Fig 1 (h, n)); caudal process fused with dorsal sclerotization on the center of dorsum, like a dark spot on dorsum (Fig 1 (m, n)).

*Body.* Length of the body approximately 1.4–3.5 mm long and 1.1–2.5 mm wide, light brown in color. When young, small sclerotized caudal process, but with age, with an area of sclerotization on mid dorsum extending from caudal process to head, covering most of median part of dorsum on older individuals (Fig 1 (k–n)).

#### Mounted material (Figs 4 and 5)

*Dorsum.* Derm entirely membranous when young, except for a heavily sclerotized caudal process. Caudal process about 0.45–1.5 mm wide and 0.3–2.0 mm long, developing over

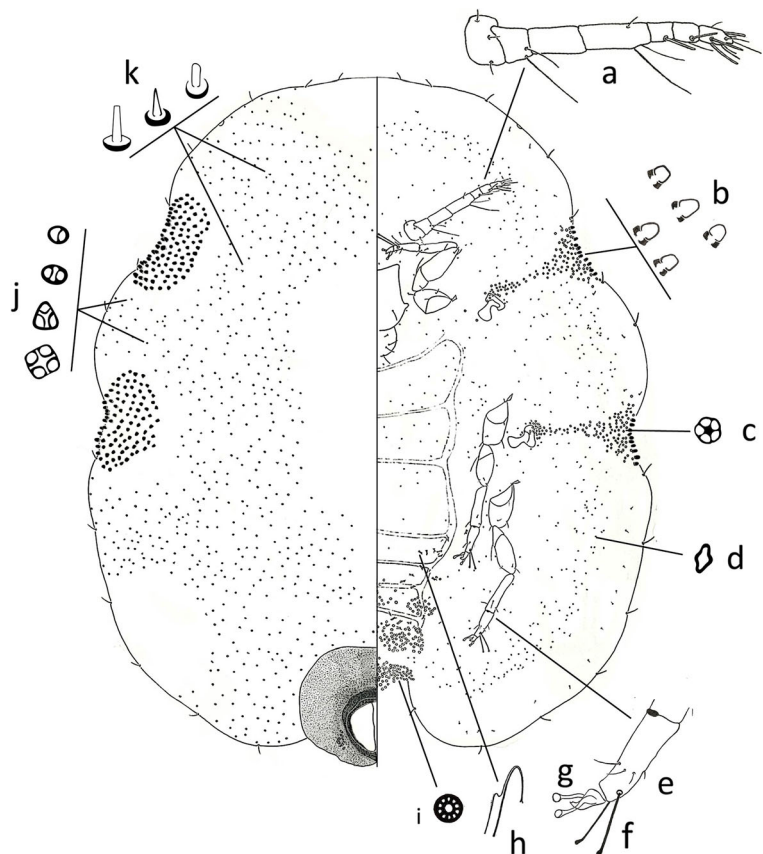


Fig 4 *Ceroplastes glomeratus* Peronti. Young adult female (agglomerated form), where a = antenna; b = stigmatic setae; c = spiracular disc-pores; d = ventral microducts; e = tarsal segment; f = tarsal digitules; g = claw digitules; h = ventral tubular ducts; i = pregenital disc-pores; j = dorsal pores; k = dorsal setae.

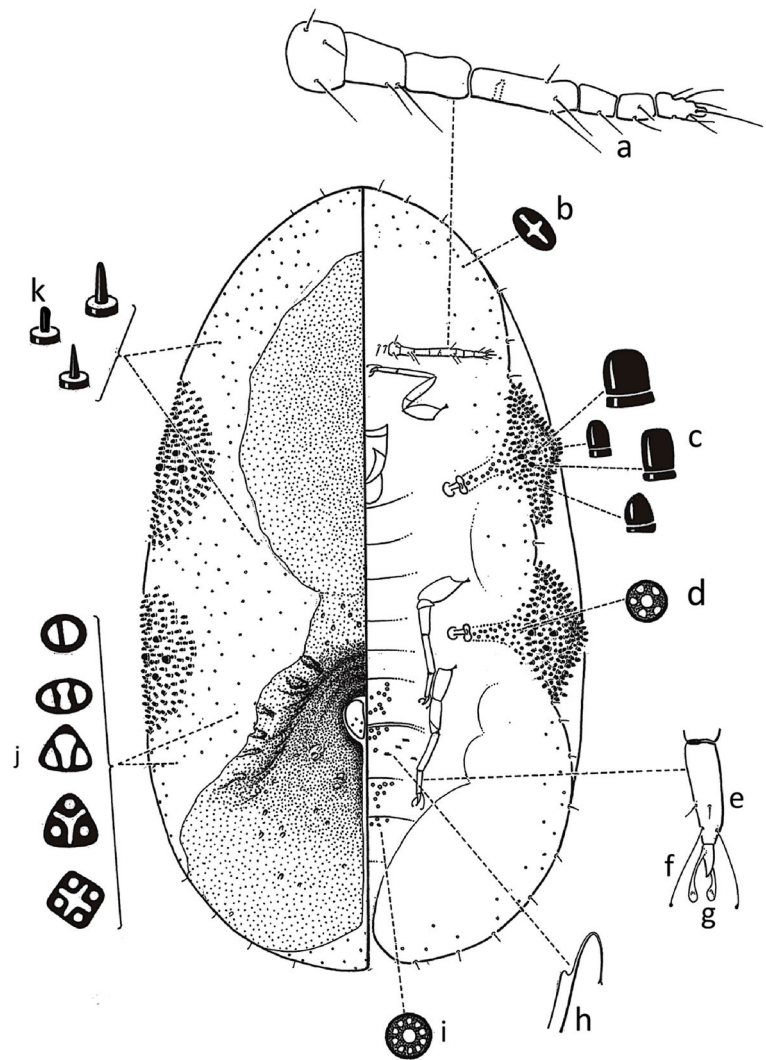


Fig 5 *Ceroplastes glomeratus* Peronti. Old adult female (agglomerated form), where *a* = antenna; *b* = ventral microducts; *c* = stigmatic setae; *d* = spiracular disc-pores; *e* = tarsal segment; *f* = tarsal digitules; *g* = claw digitules; *h* = ventral tubular ducts; *i* = pregenital disc-pores; *j* = dorsal pores; *k* = dorsal setae (modified after Peronti et al 2008).

the dorsum. Derm with 8 clear areas, distributed as: one cephalic, one mediodorsal (visible only on younger individuals), and three pairs of lateral, without pores or setae. Dorsal setae of variable shapes and sizes: (i) spinose, length 3–5  $\mu\text{m}$ , about 1.0–1.7 $\times$  longer than width of basal socket; basal socket width 3  $\mu\text{m}$ , and (ii) with parallel-sided or slightly convergent sides and a truncate, rounded or laterally truncate apex, length 4–6  $\mu\text{m}$ , about 1.0–1.5 $\times$  longer than width of basal socket; basal socket width 3–4  $\mu\text{m}$ ; frequent throughout. Dorsal pores: (i) loculate microducts, each with one to four satellite loculi, mainly with two satellite loculi; each pore about 3–5  $\mu\text{m}$  wide, abundant throughout but absent from clear areas; and (ii) simple microducts not detected. Preopercular pores: about six present in a transverse band along anterior margin of anal plates, difficult to see; each pore 2–3  $\mu\text{m}$  wide. Anal plates rather elongate, with rounded outer margin and a pointed apex; length of plates 130–140  $\mu\text{m}$ , width of both plates combined 125–130  $\mu\text{m}$ , each plate with 3 long dorsal setae about 40–50  $\mu\text{m}$  long,

in a triangle plus a short subapical seta about 17–25  $\mu\text{m}$  long. Anogenital fold with four pairs of setae on anterior margin. Anal tube short, apparently shorter than length of anal plates.

**Margin.** Marginal setae setose; each about 20–23  $\mu\text{m}$  long; with six anteriorly between eyespots, and (on each side) two between eyespots and anterior stigmatic area, two between stigmatic areas, and six to seven on each side of abdomen; each anal lobe with one seta. Stigmatic clefts quite distinct; with about 110–125 stigmatic setae; each group wider than long, with eight to ten irregular rows of stigmatic setae, extended dorsally; stigmatic setae each roundly, conical 5–7  $\mu\text{m}$  wide and 5–10  $\mu\text{m}$  long or cylindrical with a rounded or slightly flattened apex 7–9  $\mu\text{m}$  wide and 6–7  $\mu\text{m}$  long; each setae 5–9  $\mu\text{m}$  wide at base and 6–10  $\mu\text{m}$  long. Eyespots about 15–17  $\mu\text{m}$  wide each.



**Venter.** Derm entirely membranous. Pregenital disc-pores abundant around vulva and in segment IV–VI, and with nine medially and three to five submedially on each side in segment III; plus three to four medially in segment II. Spiracular disc-pores present in fairly broad bands of about 100+ pores, each band about as wide as peritremes medially, with few extending to the peritremes; but about 3.0× width of peritremes near margin. Ventral microducts each about 3 µm wide, present throughout venter and abundant in a submarginal band. Ventral tubular ducts each with a narrow inner ductule without an obvious terminal gland; absent anteriorly between antennae, sparse submedially on abdominal segments II–V. Submarginal setae more frequent than marginal setae, each 10–13 µm long.

Antennae each with seven segments; total length 245–335 µm long. Clypeolabral shield about 225–285 µm long. Spiracles: width of peritremes 55–65 µm. Legs well-developed, each with a tibio-tarsal articulatory sclerosis; each claw with a distinct denticle; claw digitules both broad and short than tarsal digitules; dimensions of metathoracic legs (µm): coxa 110–150; trochanter + femur 130–160; tibia 100–135; tarsus 60–80, and claw 20–25.

## Discussion

Adult females of *C. glomeratus* have two different adult morphotypes (phenotypes) during the year as confirmed by molecular analysis and laboratory rearing. In addition to laboratory observations, these morphotypes have also been found in the field. *Ceroplastes glomeratus* showed two different morphotypes, herein defined as agglomerated form and isolated form, which occur, respectively, in autumn and spring; both morphotypes feed on twigs of *Inga* sp. In addition to the behavioral differences, major morphological differences are found between the two morphotypes. The agglomerated form differs from the isolated form as follows (character states of the isolated form in parenthesis): (i) with about 110–125 stigmatic setae (35–42 stigmatic setae), (ii) about 100+ spiracular disk pores (about 40–47 pores), (iii) preopercular pores about 6, present in a transverse band along anterior margin of anal plates and difficult to see (preopercular pores totaling about 20–30, present in a narrow band 1–2 pores wide, present on derm around entire anterior margin of anal plates and clearly visible), and iv) with a large and heavily sclerotized area extending from the caudal process to the head, covering most of the median part of dorsum of older specimens (with a small caudal process, regardless of age). In contrast, first-instars from the agglomerated and isolated forms were similar.

Elucidating the reasons for the behavioral and morphological changes and when the environmental variation is noticed, whether by the parents or during the development

of the offspring, were not a subject of study in this paper. However, in other insect species, polyphenic development has been reported as being induced by several environmental factors such as temperature, humidity, and photoperiod (Smith 1991, Roskam & Brakefield 1996, Yamamoto *et al* 2011). Therefore, adequate experiments should be designed to investigate the factors inducing such morphological plasticity in the development of *C. glomeratus*, and the fitness benefits associated with each morphotype. Furthermore, the occurrence of morphotypes should be expanded to other species, such as *C. bruneri* Cockerell & Cockerell, *C. lahillei* Cockerell, *C. caesalpiniae* Reyne, and *C. madagascariensis* Targioni Tozzetti, which according to Peronti *et al* (2008) also produce individuals that densely aggregate, forming a mass of wax.

Species that have more than one phenotype (with macroscopic and microscopic differences in morphological traits) may be difficult to identify with contrasting morphotypes being identified as different species. In cases where biological studies and laboratory rearing cannot be developed to confirm different morphological types are due to polyphenism, such as in *C. glomeratus*, errors in species identification can occur if rearing experiments or molecular tools are not used. Species misidentification can lead to misinformation on ecological traits of organisms and have implications in natural and agricultural resource management (Bortolus 2008, Shea *et al* 2011, Bin *et al* 2012)

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