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Authors: Da Silva Wengrat, Ana Paula Gonçalves, Matesco, Viviana Cauduro, Barão, Kim Ribeiro, Grazia, Jocelia, and Pietrowski, Vanda

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# External morphology of the immature stages of *Vatiga manihotae* (Hemiptera: Tingidae) with comments on ontogenesis

Ana Paula Gonçalves Da Silva Wengrat<sup>1</sup>\*, Viviana Cauduro Matesco<sup>2</sup>,  
Kim Ribeiro Barão<sup>2</sup>, Jocelia Grazia<sup>2</sup>, and Vanda Pietrowski<sup>1</sup>

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## Abstract

*Vatiga* (Hemiptera: Tingidae) species show preference for Cassava plants (Euphorbiaceae: *Manihot*), an important food source in the American tropics. Infestations of Cassava by *Vatiga* can cause serious harvest losses. Information on immature stages morphology can aid in early identifications of crop pests and provide data for use in phylogenetic analyses; thus, we describe and illustrate the external morphology of all life stages of *Vatiga manihotae* using optical and scanning electron microscopies. Eggs are laid inserted in the leaf blade, are whitish and oblong, with smooth chorion. In the larvae, the tegument is covered by hemispherical projections, which remain the same and become denser through ontogenesis. Dorsal surface of head and lateral margins of body with tubercles; from the second through the fifth instars the cephalic and lateral tubercles maintain the same structure, only adding setae along its surfaces. The cephalic tubercles of *V. manihotae* nymphs are more complex than those of the adults, as on the latter the cephalic tubercles lack setae. As suggested by literature, our discoveries fit the intermediate clades scenario of the ontogenetic pathway in which larvae have outgrowths and adults are simple, suggesting that brood protection is absent and larval protection by secretions may be present.

Key Words: lace bug; *Manihot esculenta*; nymph; *Vatiga*

## Resumen

Las especies de *Vatiga* (Hemiptera: Tingidae) muestran preferencia por plantas de yuca (Euphorbiaceae: *Manihot*), una importante fuente de alimento en los trópicos americanos. Las infestaciones en yuca por *Vatiga* pueden causar pérdidas importantes de cosechas. La información sobre la morfología de estados inmaduros puede ayudar en las identificaciones tempranas de plagas de los cultivos y proporcionar datos para su uso en análisis filogenéticos, por lo que describimos e ilustramos la morfología externa de todos los estadios de vida de *Vatiga manihotae* mediante microscopía óptica y electrónica de barrido. Los huevos son inseridos en la lámina foliar, blanquecinos y oblongos, con corion liso. En las larvas, el tegumento está cubierto por proyecciones hemisféricas, que se mantienen y se vuelven más densos a través de la ontogénesis. La superficie dorsal de la cabeza y las márgenes laterales del cuerpo poseen tubérculos; desde el segundo hasta el quinto instar los tubérculos cefálicos y laterales mantienen la misma estructura, sólo se suman setas a lo largo de sus superficies. Los tubérculos cefálicos de las ninfas de *V. manihotae* tienen una mayor complejidad que los de los adultos, ya que en este último los tubérculos cefálicos son desprovistos de pelos. Según lo sugerido por la literatura, se adapta a un escenario de clado intermediario ontogenético, donde las larvas tienen excrecencias y adultos son más simples, lo que sugiere que la protección mecánica en larvas está ausente y la protección por las secreciones puede estarse presentando.

Palabras Clave: tingidos; *Manihot esculenta*; ninfa; *Vatiga*

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Tingidae (Hemiptera: Heteroptera: Cimicomorpha) includes about 2,600 species, which in part are easily recognized by their lacy pronotum, scutellum, and hemelytra (Guilbert 2001). The taxonomy of the family is based mostly on external morphological structures (Guilbert 2004a), and adults and nymphs may show strikingly elaborated tegumental ornamentations (Guilbert 2001, 2004b).

Tingid nymphs already have been considered in the systematics of the family (Guilbert 2001), but the knowledge of nymph morphology still is poor. Most nymphal descriptions focus only on the fifth instar (Guilbert & Montemayor 2010; Montemayor 2010; Montemayor *et al.*

2011; Guidoti & Montemayor 2014), and few provide descriptions of all instars (e.g., Livingstone 1976; Montemayor 2009; Montemayor & Dellapé 2010; Guidoti & Barcellos 2013). Fewer papers focus on immature stages of Neotropical species (e.g., Montemayor 2009; Guilbert & Montemayor 2010).

The taxonomic and systematic knowledge on the Neotropical *Vatiga* Drake & Hambleton is limited. Froeschner (1993) revised the genus, proposed new synonymies, and established the current genus composition: *Vatiga cassiae* (Drake & Hambleton), *V. illudens* (Drake), *V. manihotae* (Drake), *V. pauxillae* (Drake & Poor), and *V. varianta* (Drake).

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<sup>1</sup>Programa de Pós-Graduação em Agronomia, Universidade Estadual do Oeste do Paraná, Rua Pernambuco, 1777, CEP 85960-000, Marechal Cândido Rondon, PR, Brazil

<sup>2</sup>Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, Prédio 43435, CEP 91501-970, Porto Alegre, RS, Brazil

Corresponding author; E-mail: anawengrat@gmail.com

Information on the morphology of *Vatiga* species is scattered in the literature and mostly is restricted to the species' original descriptions; also, the immature stages still are unknown.

*Vatiga* species show preference for *Manihot* Miller (Euphorbiaceae) plants, commonly known as "cassava". Cassava plants are an important food source in the American tropics, and infestations by *Vatiga*, especially *V. manihotae*, can cause serious harvest losses (Bellotti et al. 1999).

The aim of this article is to describe and illustrate all immature stages of *V. manihotae*, as a help to identify early developmental stages of *V. manihotae* and to provide morphological data on *Vatiga* immatures for future use in phylogenetic analysis.

# Material and Methods

Adult and immature specimens of *V. manihotae* were collected manually on leaves of *Manihot esculenta* Crantz (Euphorbiaceae), in the Brazilian states of Bahia, Mato Grosso do Sul, Paraná, and Santa Catarina, from 2009 to 2013. Eggs were obtained by dissecting fresh leaves. Specimens were preserved in 70% ethanol.

The adults were identified to species level based on Froeschner (1993). Drawings were made with a camera lucida coupled to a stereomicroscope, digitalized, and edited with a vectorial image processor. For observation of the dorsal abdominal scent glands, nymphs were cleared in 10% KOH and stained with Congo Red.

In addition, all stages were observed by scanning electron microscopy (SEM) at the Centro de Microscopia Eletrônica (CME) of UFRGS. For SEM analysis, specimens were kept submersed in contact lens solution for 24 h, then washed three times in distilled water, and dehydrated in increasing concentrations of acetone. Samples were dried further in a critical point dryer, mounted on stubs, metalized with carbon and gold in a Baltec SCD050 sputter coater, and analyzed with a JSM6060 scanning electron microscope.

Measurements (mean  $\pm$  standard deviation; 15 nymphs of each instar), given in millimeters, include: total length (TTL), from head apex to abdomen apex, along longitudinal mid-line, not including the antero-median tubercle; total width (TTW), corresponding to the largest abdominal width (at posterior margin of the urosternite V, not including the lateral tubercle); head length (HL), along longitudinal mid-line, not including the antero-median tubercle; head width (HW), at mid-level of eyes; interocular distance (ID), at mid-level of eyes; length of antennal segments I, II, III, and IV; rostrum length (RL); thorax length (TL), along longitudinal mid-line; thorax width (TW), at pronotum not in-

cluding the tubercles; and wing pad length (WL), from 3<sup>rd</sup> to 5<sup>th</sup> instars, corresponding to the greatest length, at a paramedian line (Table 1).

Terminology follows Southwood (1956) and Baker & Brown (1994) for eggs, and Guilbert (2005) and Guidoti & Barcellos (2013) for nymphs. Voucher specimens are deposited in the Coleção Entomológica de Departamento de Zoologia of Universidade Federal do Rio Grande do Sul and in the Laboratório de Entomologia of Universidade Estadual do Oeste do Paraná, Brazil.

# Results

## EGG

Eggs deposited singly, deeply inserted in the leaf blade, only operculum visible at leaf outer surface (Figs. 1, 3). Egg whitish; brownish to reddish near eclosion. Egg oblong (Fig. 2), slightly tapered towards opercular end. Chorion smooth (Figs. 2, 4). Cap rounded, convex, with reticulated surface (Figs. 1, 3). Cap easily drops when manipulated, leaving the rim and the vitelline covering exposed (Figs. 2, 4).

## FIRST INSTAR

Body elongated, slightly convex dorsally (Fig. 6), whitish; eyes red; apical half of antennal segment IV and apex of labial segment IV pale brown; legs whitish, tarsi pale brown.

Head compressed, subquadrate (Fig. 11); armed with three slender setae, one medioapical and 1+1 occipital (Figs. 11, 16-17). Medioapical seta almost reaching apex of clypeus (Fig. 16). A pair of setae, one of each side of the midline, anterior to medioapical seta (Fig. 16). Occipital setae not surpassing lateral margins of eyes (Fig. 17). Clypeus prominent, with rounded apex, bearing two setae inserted basally (Fig. 11). Labium tapering toward apex; distally reaching metacoxae. Antennal segment III larger than the other three segments combined (Fig. 6); tegument covered by scattered setae (Fig. 22); apex of last antennal segment with higher setal density, with three distinct ampulla-like setae (Fig. 24).

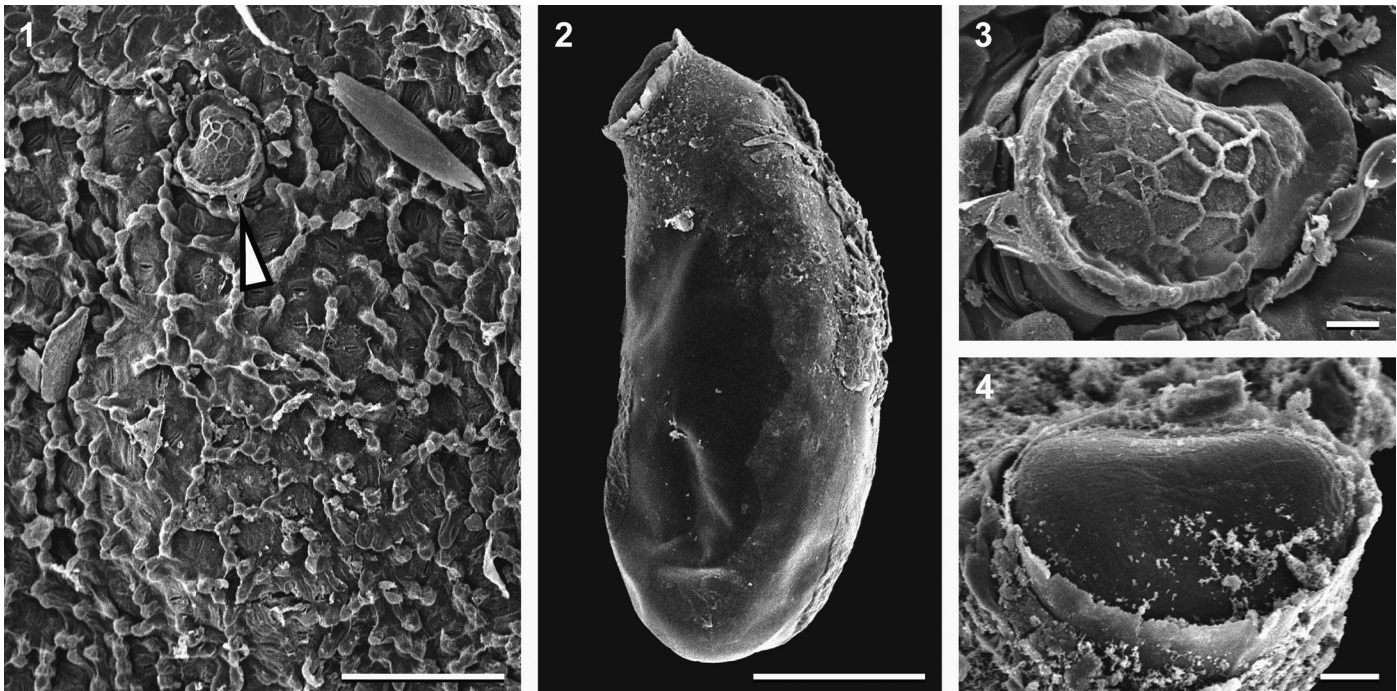
Pronotum, mesonotum, and metanotum rectangular; each bearing 1+1 tubercles near posterior margins laterally (Fig. 26). Legs bearing scattered setae; inner and outer surfaces of tibial apex bearing a set of 3-6 stout setae. Tarsi two-segmented. Pretarsi with paired and symmetrical claws, heavily sclerotized; paired parempodial sclerites, with minute, stout seta each.

Each abdominal segment (AB) with 1+1 tubercles with setae in the postero-lateral margin. AB2 with two setae, resting in a sclerotized

**Table 1.** Morphometric parameters (mean  $\pm$  standard deviation), in millimeters, of the *Vatiga manihotae* nymphs ( $n = 15$ , for each instar).

Measure	1st instar	2nd instar	3rd instar	4th instar	5th instar
TTL	0.65 $\pm$ 0.04	0.89 $\pm$ 0.04	1.18 $\pm$ 0.04	1.62 $\pm$ 0.04	2.34 $\pm$ 0.05
TTW	0.17 $\pm$ 0.03	0.27 $\pm$ 0.02	0.35 $\pm$ 0.02	0.48 $\pm$ 0.03	0.66 $\pm$ 0.05
HL	0.15 $\pm$ 0.02	0.18 $\pm$ 0.01	0.23 $\pm$ 0.02	0.28 $\pm$ 0.02	0.33 $\pm$ 0.05
HW	0.16 $\pm$ 0.01	0.22 $\pm$ 0.01	0.27 $\pm$ 0.01	0.34 $\pm$ 0.01	0.42 $\pm$ 0.02
ID	0.11 $\pm$ 0.01	0.15 $\pm$ 0.01	0.17 $\pm$ 0.01	0.23 $\pm$ 0.01	0.25 $\pm$ 0.02
I	0.03 $\pm$ 0.01	0.06 $\pm$ 0.01	0.09 $\pm$ 0.00	0.13 $\pm$ 0.01	0.22 $\pm$ 0.01
II	0.04 $\pm$ 0.00	0.04 $\pm$ 0.01	0.07 $\pm$ 0.00	0.08 $\pm$ 0.01	0.11 $\pm$ 0.01
III	0.16 $\pm$ 0.02	0.35 $\pm$ 0.02	0.52 $\pm$ 0.03	0.82 $\pm$ 0.04	1.23 $\pm$ 0.04
IV	0.14 $\pm$ 0.02	0.22 $\pm$ 0.01	0.29 $\pm$ 0.01	0.39 $\pm$ 0.02	0.52 $\pm$ 0.04
RL	0.23 $\pm$ 0.03	0.30 $\pm$ 0.01	0.36 $\pm$ 0.02	0.44 $\pm$ 0.02	0.58 $\pm$ 0.03
TL	0.17 $\pm$ 0.01	0.25 $\pm$ 0.02	0.29 $\pm$ 0.02	0.41 $\pm$ 0.02	0.52 $\pm$ 0.03
TW	0.19 $\pm$ 0.01	0.28 $\pm$ 0.02	0.36 $\pm$ 0.01	0.50 $\pm$ 0.01	0.71 $\pm$ 0.05
WL	—	—	0.14 $\pm$ 0.01	0.33 $\pm$ 0.01	0.89 $\pm$ 0.02

HL, head length; HW, head width; ID, interocular distance; RL, rostrum length; TL, thorax length; TTL, total length; TTW, total width; TW, thorax width; WL, wing pads length; I, II, III, IV, length of antennal segments.



**Figs. 1-4.** Egg of *Vatiga manihotae*. **1.** Egg inserted on the abaxial side of host plant leaf, only the operculum (white arrow) appearing. **2.** Egg, in lateral view, after removal from host plant leaf. **3.** Detail of cap while inserted in the host plant leaf. **4.** Egg after removal from host plant leaf, showing the vitelline covering and rim. Scale bars = 100, 100, 10, and 10  $\mu$ m, respectively.

area in the midline dorsally. Tergum of AB6 and AB8 bearing a median-dorsal tubercle; median-dorsal tubercle of AB8 longer than the median-dorsal tubercle of AB6. AB10 bearing, medially, a pair of setae (Fig. 31). Abdominal scent efferent system opening dorsally on the posterior margin of AB4 and AB5 (Figs. 6, 38).

Body tegument sculptured by hemispheric structures (Figs. 39-41); dorsally, sculptures undivided, covered with scale-like secretions (Fig. 39); laterally, sculptures often divided (Fig. 40); ventrally, sculptures entirely hemispheric, surrounded by smooth tegument (Fig. 41).

## SECOND INSTAR

Body elongated (Fig. 7), yellow-whitish; antennae whitish, antennal segments I and II bearing lateral pale-brown stripe, apical half of antennal segment IV pale-brown; labium whitish, apex of labial segment IV pale brown; legs whitish, tarsi pale-brown.

Head subquadrate, armed with three slender, long tubercles, one medioapical and 1+1 occipital (Fig. 12). Medioapical tubercle surpassing apex of clypeus (Fig. 18). Occipital spines not surpassing lateral margins of eyes (Fig. 12). Apex of labium laying between mesocoxae and metacoxae. Lateral margins of thorax and abdomen bearing 1+1 tubercles, more robust than in the first instar (Figs. 27, 32). Tergum of AB6 and AB8 bearing a median-dorsal tubercle; median-dorsal tubercle of AB8 longer than the median-dorsal tubercle of AB6; median-dorsal tubercles more produced than in the first instar. Apex of tubercles bearing an ampulla-like seta (Fig. 36). Sculptures covering body surface denser. Other characteristics as described in the first instar.

## THIRD INSTAR

Body elongated (Fig. 8), yellow-whitish; antennae whitish, antennal segments I-III bearing lateral pale-brown stripe, apical half of antennal segment IV pale-brown; labium whitish, apex of labial segment IV pale brown; legs whitish, tarsi pale-brown.

Head subquadrate. Medioapical tubercle surpassing apex of clypeus (Figs. 13, 19). Occipital tubercles surpassing lateral margins of eyes (Fig. 13). Labium may surpass mesocoxae, but does not reach metacoxae.

Pronotum rectangular, lateral margins armed with minute claviform-like setae (Fig. 37), and, distally, with 1+1 tubercles directed laterally (Figs. 8, 28, 33). Mesonotum subrectangular, lateral margins armed with minute claviform-like setae, and distally with 1+1 tubercles directed backwards. Metanotum rectangular, lateral surfaces smooth; distally with 1+1 tubercles directed backwards.

Lateral margins of abdomen bearing 1+1 tubercles, increasing in length towards AB9 (Figs. 8, 33). Abdominal tubercles bearing claviform-like setae. Abdominal tergum as in previous instars, but median-dorsal tubercles more produced than in the anterior instars. Sculptures covering body surface denser. Other characteristics as described in the first instar.

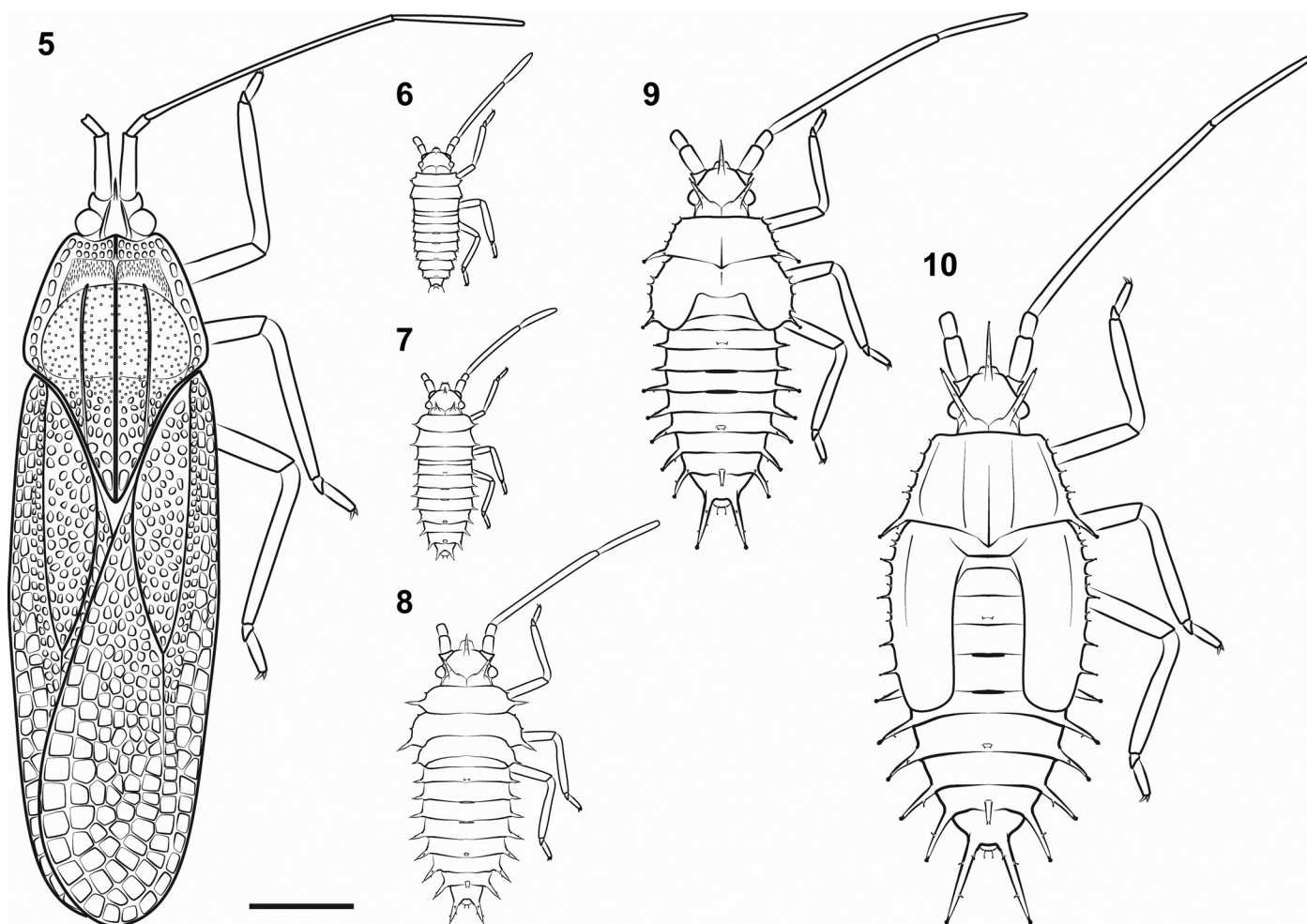
## FOURTH INSTAR

Body elongated (Fig. 9), yellow-whitish; antennae yellow-whitish, basal third of antennal segment III and apical half of antennal segments IV pale-brown; labium whitish, apex of labial segment IV pale brown; legs whitish, tarsi pale-brown; spines of AB9 pale-brown.

Head tubercles surpassing the head margins by at least one third of their lengths (Figs. 14, 20). Labium may surpass mesocoxae.

Pronotum trapezoidal; lateral margins armed with small tubercles bearing an ampulla-like seta apically; at the postero-lateral angle, having a long, stout tubercle projected backwards, with claviform-like setae along its length, and apically with an ampulla-like seta (Fig. 29). Posterior margin of pronotum projected apically at the midline; median carina present. Mesonotal wing pads reaching AB2; lateral margins armed with a series of tubercles and armed with 1+1 tubercles directed backwards distally; all tubercles with an ampulla-like seta at the apex.





**Figs. 5-10.** Habitus of *Vatiga manihotae* in dorsal view. Left antennae and legs were digitally removed. **5.** Adult; **6.** First instar; **7.** Second instar; **8.** Third instar; **9.** Fourth instar; **10.** Fifth instar. Scale bar = 0.5 mm.

Lateral margins of abdomen, tubercles (Fig. 34), and sculpturing of tegument as described in the third instar. Other characteristics as described in the first instar.

#### FIFTH INSTAR

Body elongated (Fig. 10), yellow-whitish. Antennal segments I and II whitish; antennal segment III pale-brown, except for whitish stripes in its base and apex; antennal segment IV pale-brown, with base whitish. Labium whitish, apex of labial segment IV pale-brown; legs whitish, tarsi pale-brown; spines of AB9 pale-brown.

Head with medioapical tubercle as long as antennal segment I, surpassing clypeal apex (Figs. 10, 15, 21). Occipital tubercles surpassing the margins of head at least by half of their lengths (Fig. 15). Labium may surpass mesocoxae.

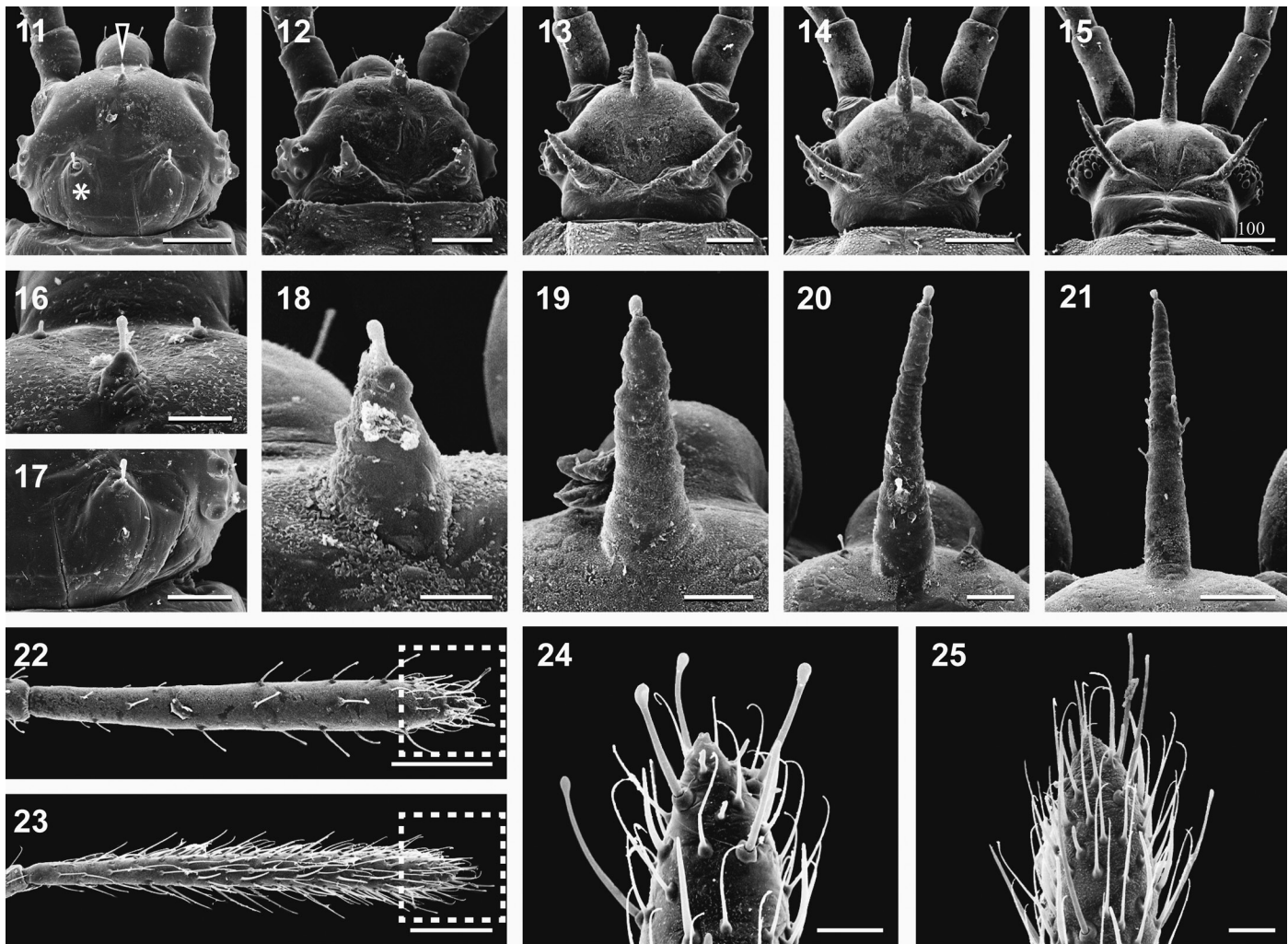
Pronotum trapezoidal, expanded laterally; lateral margins as described in the fourth instar (Figs. 10, 30); posterior margin of pronotum projected posteriorly at the midline, bearing a median carina and 1+1 less-developed, lateral carinae. Mesonotal wing pads reaching AB5; lateral margins armed as in the fourth instar. AB2-3 lacking lateral tubercles.

Lateral margins of abdomen, tubercles (Fig. 35), and sculpturing of tegument as described in the third instar. Other characteristics as described in the first instar.

#### Discussion

Immatures of tingids occasionally have been described, particularly fifth instars, probably because of accidental collection. They exhibit particular outgrowths distributed on the dorsum of head, thorax, and abdomen, that can be lost in the adults (Guilbert 2005). Studies concerning all tingid preimaginal life stages are rare (Livingstone 1976; May 1977; Guidoti & Barcellos 2013), thus, ontogenetic pathways of nymph body outgrowths are still incipient. Nevertheless, if first larvae show tubercles, there is a tendency of increasing complexity of the cephalic and marginal tubercles through ontogenesis (Livingstone 1976; May 1977; Montemayor & Dellapé 2010; Guidoti & Barcellos 2013; but see Guidoti & Montemayor 2014 for exceptions). In some point of ontogenesis, there may occur losses of tubercles, which can be replaced by translocation (Štusák & Štys 1959). Independently, tegumental sculpturing may increase in density through ontogenesis (Livingstone 1976; May 1977; Guilbert 2004a; Guidoti & Barcellos 2013).

Our findings on *V. manihotae* agree, in part, with the trends described above. The sculpturing of tegument also retains the same pattern and become denser through ontogenesis. From the second through the fifth instars the cephalic and lateral tubercles maintain the same structure, only adding setae along its surfaces. In the fifth instar, the tubercles on abdominal segments II and III are lost, probably due to the development of the wing pads; and the costal mar-



**Figs. 11-25.** Ontogenetic changes in the head, cephalic tubercles, and antenna of *Vatiga manihotae*, in dorsal view. **11.** 1st instar head; **12.** 2nd instar head; **13.** 3rd instar head; **14.** 4th instar head; **15.** 5th instar head; **16.** Anterior region of 1st instar head with the presence of three setae; **17.** Occipital region of 1st instar head; **18.** Anterior tubercle of 2nd instar head; **19.** Anterior tubercle of 3rd instar head; **20.** Anterior tubercle of 4th instar head; **21.** Anterior tubercle of 5th instar head; **22.** 4th antenna segment of 5th instar nymph; **23.** 4th antenna segment of adult; **24.** Apex of 4th antenna segment of 5th instar nymph; **25.** Apex of 4th antenna segment of adult. Scale bars = 50, 50, 50, 100, 100, 10, 10, 10, 20, 20, 50, 100, 100, 20, and 20  $\mu$ m, respectively.

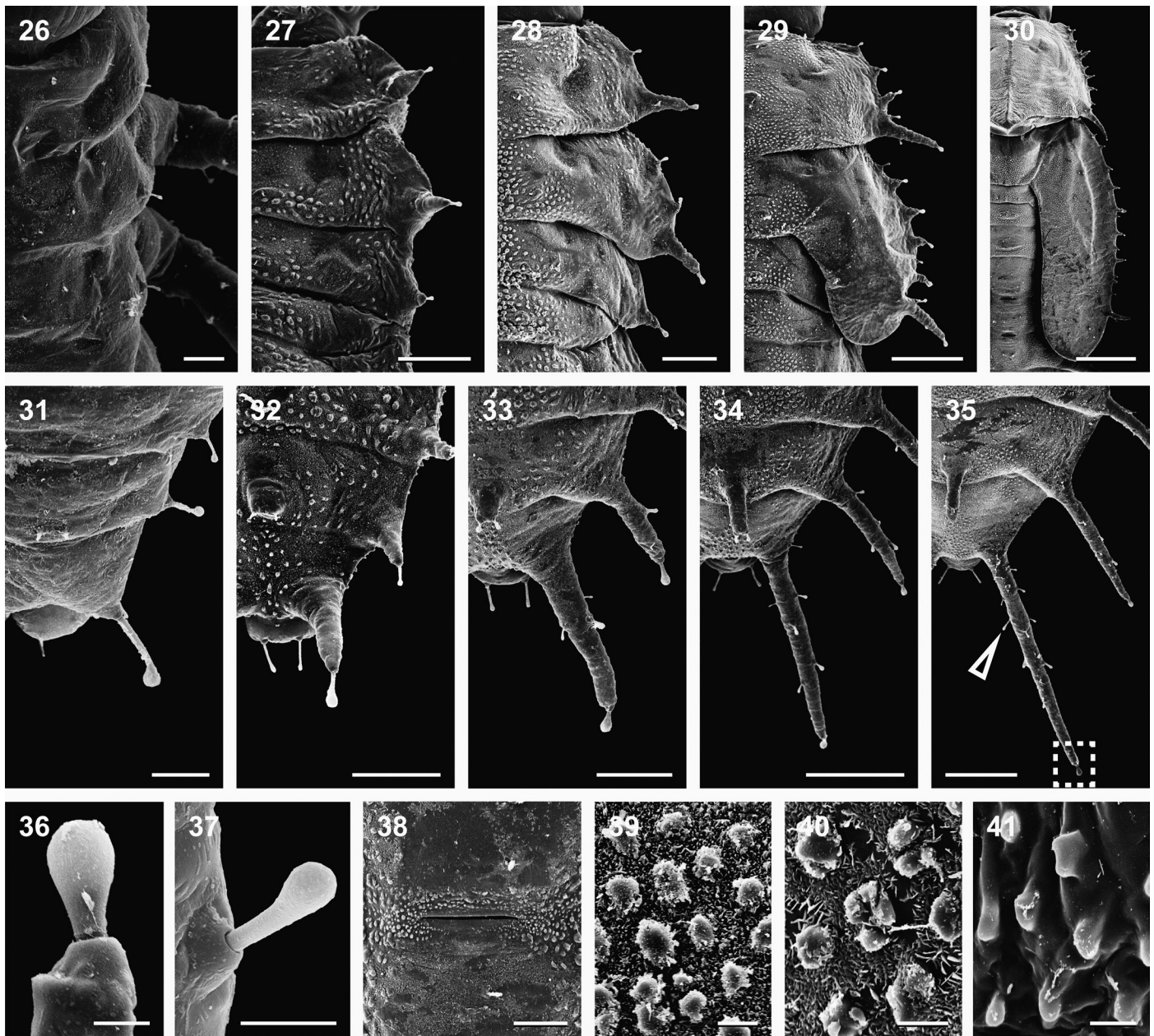
gin of wing pads becomes more armed, probably by translocation, as suggested by Štusák & Štys (1959). The cephalic tubercles of *V. manihotae* nymphs are more complex than those of the adults, as on the latter the cephalic tubercles lack setae. Where known, in tingid ontogenesis, the cephalic tubercles are always simple in the adult when complex in the larvae (Guilbert 2005). *Vatiga manihotae* fits Guilbert's second pattern of tubercle development (Guilbert 2004a), as the number of marginal tubercles remains the same, mostly by marginal tubercle translocation. Besides the main differences in adult and nymphs morphology, such as the full development of adult structures and lack of thoracic and abdominal outgrowths, the composition of setae of the adult antennae also changes, lacking ampulla-like setae (Figs. 23, 25).

*Vatiga manihotae* nymphs are similar to the nymphs of, e.g., *Gargaphia bergi* Monte, *Leptopharsa firma* Drake & Hambleton, and *Nobarnus pilosus* Guilbert in having slender, long cephalic and marginal tubercles, and in lacking complex tegumental projections (Guilbert 2004a; Guilbert & Montemayor 2010; Montemayor & Dellapé 2010). In recent phylogenetic studies of Tingidae (Guilbert 2001, 2004b), the lack of pronotal and hemelytral expansions in

adults, as well as the lack of outgrowths in larvae, were hypothesized as plesiomorphic conditions. On the other hand, complex outgrowths on larvae and adults were hypothesized as apomorphic, and probably are related to predator protection and brood care (Guilbert 2004b). In our study, we did not find any evidence of brood protection by adults of *V. manihotae* during fieldwork. The *Vatiga* phylogenetic position and relationships have not been investigated yet, but the *V. manihotae* nymph and adult morphology fits the scenario hypothesized by Guilbert (2004b) for 'intermediate clades', where larvae have outgrowths and adults are simple, suggesting that brood protection is absent and larval protection by secretions may be present.

The findings on the eggs of *V. manihotae* largely agree with the information available in the literature. The eggs usually are laid inserted in plant tissues, with different levels of insertion (Southwood 1956; Baker & Brown 1994). The chorion of *V. manihotae* is smooth, as is the chorion of *Corythucha arcuata* (Say). This differs from the finding of Southwood (1956), that the chorion is covered by hexagonal sculpturation. In *V. manihotae* and *C. arcuata*, the only area covered by such polygonal ornamentation is the dorsal surface of





**Figs. 26-41.** Ontogenetic changes in the thorax and abdomen of *Vatiga manihotae*, in dorsal view, and tegument structures. **26.** 1st instar thorax; **27.** 2nd instar thorax; **28.** 3rd instar thorax; **29.** 4th instar thorax; **30.** 5th instar thorax; **31.** Abdominal segments 8-10 of the 1st instar; **32.** Abdominal segments 8-10 of the 2nd instar; **33.** Abdominal segments 8-10 of the 3rd instar; **34.** Abdominal segments 8-10 of the 4th instar; **35.** Abdominal segments 8-10 of the 5th instar; **36.** Apex of posterolateral tubercle (area marked by a dashed square in Figure 31); **37.** Seta of posterolateral tubercle (as indicated by a white arrow in Figure 31); **38.** Gland on the 4th abdominal segment; **39.** Dorsal tegument sculpture; **40.** Dorsolateral tegument sculpture; **41.** Ventral tegument sculpture. Scale bars = 20, 50, 50, 100, 200, 20, 50, 50, 100, 100, 5, 10, 20, 5, 5, and 5  $\mu\text{m}$ , respectively.

the cap. The rim around the opercular end seems to show some morphological variability (Baker & Brown 1994), the function of which is not known.

Larvae outgrowths are remarkable by their unique morphology, being a potential source for character sampling in phylogenetic analyses (Guilbert 2004b). There are few species whose fifth instar is described, and fewer to whose complete larval ontogenetic development is known. As an important source of character information, comprehensive taxonomic descriptions for as many species as possible are needed (Guidoti & Barcellos 2013) in order to provide data for phylogenetic analysis.

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