Definition of the number of instars, description of the second instar and redescription of the last instar and pupa of *Pantophthalmus kerteszianus* (Enderlein, 1914) (Diptera: Pantophthalmidae)

Definição do número de ínstares, descrição do segundo ínstar e redescrição do último ínstar e pupa de *Pantophthalmus kerteszianus* (Enderlein, 1914) (Diptera: Pantophthalmidae)

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Abstract: The total number of larval instars of *Pantophthalmus kerteszianus* was determined through morphological and biometric studies. The second larval instar of *P. kerteszianus* is described, the last larval instar in detail and the pupa are redescribed. Larvae were collected from *paricá* trees (*Schizolobium parahyba* var. *amazonicum*) in the Paragominas county, Pará, Brazil. Upon collection, larvae and pupae were killed in boiling water, preserved in 70% alcohol, identified, measured and morphologically studied. The total number of larval instars was determined by techniques based on Dyar's rule (1890), supported by measurements of the several characters: body length, maximum body width, maximum diameter of the prothoracic spiracle, maximum diameter of the anal spiracle, distance from the margin of the prothoracic spiracle to the lower bristle, distance from the margin of the prothoracic spiracle to the prothorax and distance between the anal spiracles. Through these measurements it was possible to establish that *P. kerteszianus* has seven larval instars; this species is, up to now, the only one having the number of its larval instars determined. Photographs and drawings of the complete larvae and pupae, as well as of the most important structures discussed in the descriptions, are provided.

Keywords: Pantophthalmus kerteszianus. Timber flies. Immatures. Instar number.

Resumo: O número total de ínstares larvais de *Pantophthalmus kerteszianus* foi determinado através de estudos morfológicos e biométricos. O segundo ínstar larval de *P. kerteszianus* é descrito, o último ínstar larval e a pupa são redescritos. As larvas foram coletadas em árvores de paricá (*Schizolobium parahyba* var. *amazonicum*) no município de Paragominas, Pará, Brasil. Depois de coletadas, as larvas e pupas foram mortas em água fervente, conservadas em álcool 70%, identificadas, medidas e estudadas morfologicamente. O número total de ínstares larvais foi determinado por técnicas baseadas na regra de Dyar (1890), apoiadas em medidas dos seguintes caracteres: comprimento do corpo, largura máxima do corpo, diâmetro máximo do espiráculo protorácico, diâmetro máximo do espiráculo protorácico até a cerda inferior, distância da margem do espiráculo protorácico até a margem protorácica, largura da faixa mediana do protórax e distância entre os espiráculos anais. Através dessas medidas foi possível estabelecer que *P. kerteszianus* possui sete ínstares larvais; esta espécie é, até o momento, a única que tem o número de ínstares larvais determinado. São fornecidas fotografias e desenhos das larvas e pupas, bem como das estruturas mais importantes tratadas nas descrições.

Palavras-chave: Pantophthalmus kerteszianus. Mosca da madeira. Imaturos. Número de ínstares.

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INTRODUCTION

Pantophthalmidae is a small family of the order Diptera, suborder Brachycera, exclusive to Neotropics and geographically distributed primarily in South and Central America. A few species occur in Mexico and the Antilles (Carrera & D'Andretta, 1957; Val, 1976).

The larvae are wood-borers, that attack various species of both native and introduced trees (list in Papavero, 2009a). Lunz et al. (2010) recorded larvae *Pantophthalmus* spp. in paricá (*Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby) in the state of Pará, Brazil. Rapp (2011) describes the immatures of *P. bellardii* (Bigot) and *P. kerteszianus* (Enderlein).

Twenty species of Pantophthalmidae are known, belonging to two genera: *Opetiops* Enderlein 1921, with only one species, *O. alienus* (Hermann, 1916), and Pantophthalmus Thunberg 1819, with nineteen species (Val, 1976). Nine species of Pantophthalmidae occur in the Brazilian Amazon, all in the genus *Pantophthalmus* (Papavero, 2009b). Of the twenty existing species of Pantophthalmidae, only five have described larvae, so it is important to understand the immature stages of the other species.

This work describes the second instar larvae for the first time and redescribes the last instar larva and pupa of *Pantophthalmus kerteszianus* (Enderlein, 1914). It also determines, for the first time, the number of larval instars of a species of the family Pantophthalmidae, based on morphological and biometric characteristics. Variations in the structures measured in the instars and differences between the larvae and pupae of *P. kerteszianus* compared to *P. tabaninus* (Greene and Urich, 1931) and *P. palniventris* (Wiedemann, 1821) are presented.

The work presents photos, drawings and a diagram of the body division of *P. kerteszianus* that facilitate the visualization of the structures described.

MATERIAL AND METHODS

Pantophthalmus. kerteszianus larvae were collected in trunks of paricá in August 2011 at the Paragominas, Pará,

Brazil, in coordinates 03° 38′ 976″ S, 48° 50′ 449″ W. The collected larvae and pupae were killed in boiling water, preserved in 70% alcohol for subsequent identification, measurement, and morphological analysis to determine larval instars and describe the immature stages.

The following characters were analyzed and measured: body length, greatest body width, greatest diameter of the prothoracic spiracle, greatest diameter of the abdominal spiracle, distance from the margin of the prothoracic spiracle to the upper bristle, distance the margin of the prothoracic spiracle to the lower bristle, distance from the margin of the prothoracic spiracle to the margin of the prothorax, width of the median band of the prothorax and distance between the anal spiracles (Table 1 and Figures 1A-1H). These structures were selected because less subject to variations arising from feeding, for being are more robust due to sclerotization and contain fewer membranous areas. Only a single second instar larva was collected, and for that reason it was not included in the calculation. No first instar larvae were collected.

Mathematical average values and standard deviations were calculated, allowing the construction of graphs with the analyzed structures. By examining the frequency intervals, the stages of *P. kerteszianus* was defined. These procedures were based on Dyar's rule (Dyar, 1890) and the methodology described by Parra and Haddad (1989).

The identification of the larvae was based on the adults obtained in laboratory from the pupae collected in the *paricá* trunks. They were identified with assistance of Val's revision (Val, 1976). Morphological terminology was based on the works of Greene and Urich (1931), Peterson (1960), Teskey (1981) and Stehr (1987).

The specimens are deposited in the entomological collection of the *Museu Paraense Emílio Goeldi*, Belém, Pará, Brazil.

Drawings of the immatures and measurements were made with a Zeiss SV11 stereoscopic microscope with camera lucida and a micrometric lens. Digital photographs of larvae and pupae of *P. kerteszianus* were captured with a Leica MZ16 camera adapted to a Leica DFC 420

Table 1. Average and standard deviation of measurements of the body structures of larval instars II-VII of *Pantophthalmus kerteszianus*. Legend: * = measurements of the structures of the second larval instar were taken from a single specimen collected. The lower bristle of the prothoracic spiracle and the bands of the prothorax do not exist in the second instar; the anal spiracles were not measured, in order not to damage the specimen.

Measured structures (mm)	Larval instars					
	II*	III (n = 22)	IV (n = 32)	V (n = 61)	VI (n = 22)	VII (n = 7)
Body length	12	12.72 ± 2.62	22.90 ± 1.69	29.59 ± 2.47	41.37 ± 3.16	55.33 ± 3.16
Body width	1.21	3.87 ± 0.53	6.73 ± 0.46	8.05 ± 0.28	9.02 ± 0.32	10.53 ± 0.44
Diameter of the prothoracic spiracle	0.16	0.35 ± 0.03	0.51 ± 0.04	0.58 ± 0.01	0.73 ± 0.08	0.92 ± 0.05
Distance from the prothoracic spiracle to the upper bristle	0.28	0.13 ± 0.02	0.24 ± 0.02	0.32 ± 0.02	0.40 ± 0.03	0.55 ± 0.03
Distance from the prothoracic spiracle to the lower bristle	-	0.15 ± 0.03	0.22 ± 0.01	0.29 ± 0.03	0.38 ± 0.01	0.46 ± 0.02
Distance from the spiracle to the prothoracic margin	0.34	0.49 ± 0.10	0.85 ± 0.06	1.15 ± 0.12	1.58 ± 0.07	1.8 ± 0.07
Width of the median band of the prothorax	-	0.64 ± 0.11	1.24 ± 0.12	1.58 ± 0.07	1.84 ± 0.11	2.39 ± 0.02
Diameter of the anal spiracle	-	0.41 ± 0.03	0.63 ± 0.05	0.80 ± 0.05	1.00 ± 0.08	1.31 ± 0.06
Distance between the anal spiracles	-	0.29 ± 0.06	0.64 ± 0.07	0.81 ± 0.03	0.95 ± 0.05	1.47 ± 0.25

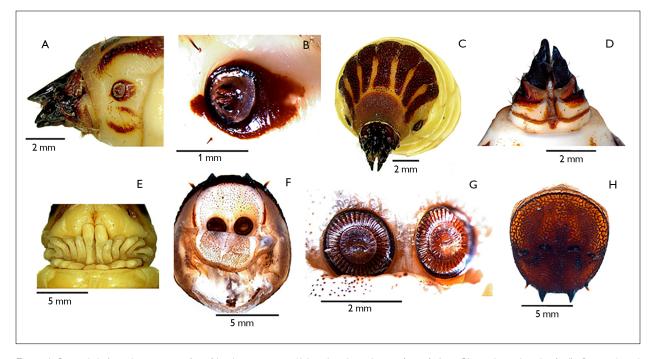


Figure 1. *Pantophthalmus kerteszianus*, larval body structures: A) head and prothorax, lateral view; B) prothoracic spiracle (in figures A and B may be seen the spiracle); C) head and prothorax, frontal view; D) head, ventral view; E) abdominal segments 7 and 8, ventral view; F) 8th abdominal segment, posterior view, with detail of opening of the anal spiracles; G) anal spiracles; H) sclerotized plate of 8th abdominal segment, posterior view. Images: I. S. Gorayeb (2014).

stereoscopic microscope, operated using Leica IM50 software, version 5.03.0061, release 247, and the photo editor software Auto MontagePro, version 5.

RESULTS AND DISCUSSION

DETERMINATION OF THE NUMBER OF LARVAL INSTARS OF *P. KERTESZIANUS*

The number of larval instars was determined through biometric studies and confirmed by the several measured structures, based on Dyar's rule (Dyar, 1890). Averages and standard deviations of the nine analyzed structures are shown in Table 1. Measurements were taken from a total of 144 larvae of various sizes. Data of each measured structure were applied in frequency intervals where peaks and gaps allowed the determination of the larval instars. Averages and standard deviations of the specimens within each interval of frequency were then calculated; and these data were used to generate graphs displaying the trend line of tendency, the function and the coefficient of determination (R²) (Table 1, Figures 2A-2I).

The calculi made it possible to determine that *P. kerteszianus* shows seven larval instars, as proven by the measurements of all the structures chosen for analysis, some of them presenting less variation.

VARIATION OF THE STRUCTURES MEASURED IN THE INSTARS

In general, the measured structures exhibited relatively little variation, as evinced their standard deviations; indicating that all could be effectively used to distinguish the larval instars of *P. kerteszianus* (Table 1 and Figures 2A-2I). Nonetheless, as expected, structures with membranous connections between segments and those that are less sclerotized displayed greater variability. Specifically, body length and width showed the highest variation, whereas the distance from the margin of the prothoracic spiracle to the lower bristle and the width of the median band of the prothorax demonstrated relatively lower variation.

No significant difference was observed among the analyzed characters between instars III and VII – except for body length. The second instar exhibited many differences as compared to the remaining instars which is why it is described in detail. No specimens from the first instar were available.

The descriptions below are presented in the following order: last (seventh) instar, second instar larva and pupa.

REDESCRIPTION OF THE LAST LARVAL (SEVENTH) INSTAR OF PANTOPHTHALMUS KERTESZIANUS

The last (seventh) larval instar of *P. kerteszianus* is cylindrical, with an average body length of 55,33mm and a width of 10,53 mm (Table 1; Figures 3A-3C).

Head: Hemicephalic, strongly sclerotized, and black, with a transition to brown toward the dorsolateral proximal margin; the ventral and lower lateral areas are white, bearing brown spots near the mandible and on the central area. The ventral surface exhibits a brown line fringed with densely set bristles. The frontoclypeus and labrum form a strongly chitinized, dorsal snout located in a sulcus near the dorsal region, which bears pale bristles; centrally, a larger, pale bristle is present in a depression, and on the proximal third of the margin of the frontoclypeal suture, another pale bristle is observed. The integument of the gena is yellowish-brown, with an irregular surface featuring raised areas, depressions, and black transverse grooves resembling cracks; additionally, an upper bristle is located near the margin of the frontoclypeal suture and three bristles on the proximal ventral area. The mandible is strongly sclerotized and black. At the site where the antenna is implanted, a plaque is present, located anteriorly to the gena, between the snout and the base of the mandible. The antenna is centered on a white area and contains a small. grayish cone; the mandible is sclerotized, black, with a dorsal tooth; below this tooth, within a sulcus, there is a large bristle (the largest one in the head). Along the internal margin of the mandible is the prostheca, with bears a tuft of yellowish bristles. More distally the maxilla is situated, with the maxillary palpus located on the external margin; the palpus, similarly to

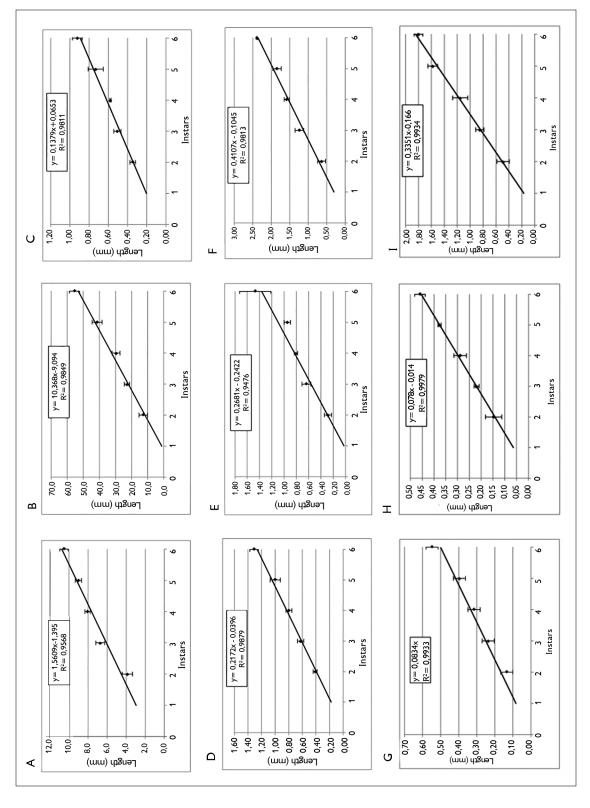


Figure 2. Averages and standard deviations of the measured structures of *Pantophthalmus tabaninus* larval instars, with lines of tendency, functions (y) and coefficients of determination (R₂): A) body width; B) body length; C) diameter of prothoracic spiracle; D) diameter of anal spiracle; E) distance between abdominal spiracles; F) width of median band of prothorax; G) distance from the prothoracic spiracle to the prothorax. Graphics: L. E. C. Cardoso (2014).

the antenna, the palpus is inserted on a white area with a grey cone; the ventral integument of the mandible presents about 15 transversal grooves (Figures 1A and 1D).

Thorax: The prothorax, along with the last abdominal segment, exhibits the greatest concentration of dark and sclerotized structures, in addition to the spiracle and setae. Its dorsolateral area displays brown spots arranged in seven longitudinal bands, with a longer central one and six lateral ones (three on each side); among the bands, on the intervening white area, there is a pair of bristles - one basal, the other distal (Figure 1C); the integument of the brown bands is composed by small, circular, inflated, dark-brown areas and clearer oval depressions. At the distal margin of the prothorax there is a spot that nearly encircles the entire segment. This spot it is formed by small, circular, inflated similar to those observed in the longitudinal bands; however, it decreases in size in the lateral areas, reducing to micropilosity; while the ventral area remains bare. The prothoracic spiracle with an internal diameter of 0.92 mm in average (Table 1), is laterally positioned and features two ovoid internal structures parallelly placed anteroposteriorly, situated from the middle towards the proximal margin of the spiracle and are light brown in the center and darker in the margin; the remaining white central area opens towards the body; the borders are well sclerotized and black; around the spiracle there is an irregular brown spot; two lateral, brown spots below the spiracle and a bristle situated above the spiracle (at a distance of 0.55 mm, in average) and a bristle below the spiracle (at a distance of 0.46 mm, in average) are present (Table 1). Two more bristles are present further down on the lateroventral area of the prothorax (Figures 1A and 1B).

Lateral stripes of the body: The larval body is laterally marked, from the mesothorax to the 6th abdominal segment, by two distinct stripes (Figures 3A and 4). These consist of punctiform tegumentary spots or small traits within tegumentary sulci. The median area between the stripes is slightly more inflated and whitish. The pattern of the spots forming the stripes is similar in the meso- and metathorax. On the upper abdominal segments, the

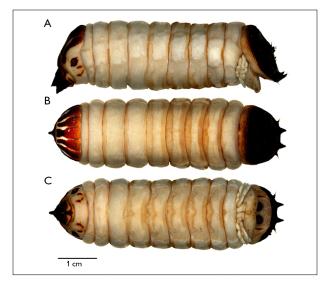


Figure 3. Larva of *Pantophthalmus kerteszianus*: A) lateral view; B) dorsal view; C) ventral view. Images: I. S. Gorayeb (2014).

stripe is formed by a single continuous sequence of points, whereas the lower stripe is broken up into two sequences; on the abdominal segments 2-6, the rear sequences of the anterior stripe curves upward from the preceding section. In contrast, the 7th segment does not show well defined stripes as observed in the previous segments, only two sinuous lines formed by tegumentary spots. Additionally, the inferior surface of the mesothorax, of the methatorax and of the abdominal segments 1-7, below the lower stripe, is slightly more yellowish than the surrounding areas.

Mesothorax: White. The segment is white and represents the narrowest portion of the body. Its anterior margin is irregular, with a more pronounced invagination in the lateral regions. Rows of small, faint tegumentary spots are present on both the posterodorsal and ventral regions, and two transversal lateral sulci bear small spots that form I lateral stripes. Bristles: On the dorsal area above the transversal stripe, near the anterior margin, three bristles are arranged in a row. In the median area, between the stripes, four bristles are present, near the margin. Below the inferior transversal stripe five bristles are found, two of these have fused bases and inserted medially and anteroposteriorly; two additional bristles are positioned more anteriorly, also in an anteroposterior

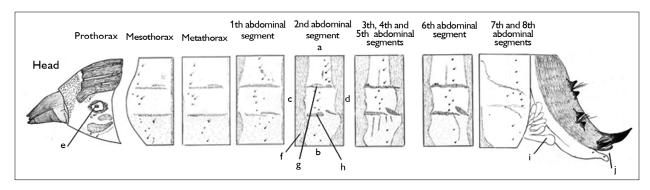


Figure 4. Schematic drawing of the segments of the *Pantophthalmus kerteszianus* larva. Legends: a = dorsal margin; b = ventral margin; c = anterior margin; d = posterior margin; e = prothoracic spiracle; f = tegumentary spots; g = upper lateral stripe of body; h = lower lateral stripe of body; i = branchiae; j = opening of anal spiracles. Illustration: I. S. Gorayeb (2014).

orientation; and a fifth bristle is located ventrally, inserted anteriorly. <u>Tegumentary spots</u>: A narrow stripe of spots is present along the posterior margin (Figures 3 and 4).

Metathorax: The structure is similar to that of the mesothorax but wider, with regular margins; and the ventral bristles are positioned anteriorly. <u>Bristles</u>: They display the same arrangement as those of the mesothorax, except for the lower ventral bristle, which is inserted medially in an anteroposterior direction. <u>Tegumentary spots</u>: A weak, narrow stripe is present along the posterior margin (Figures 3 and 4).

<u>Abdomen</u>: Segments 1-7, display a longitudinal whitish line along the dorsal and lateral surfaces, with the lateral line being slightly more pronounced. On the ventral side, there are patterned, stained areas defined by series of small spots in the anterior and posterior regions of each segment, which are less distinct in the first segment (Figures 3 and 4).

First abdominal segment: In the upper area above the lateral stripe, there are six small circular dark spots. Some of these spots bear bristles arranged dorsoventrally in the central area in the anteroposterior orientation. Two of these points nearest the upper transverse stripe are aligned parallel in the anteroposterior direction. In the median area between the transverse stripes, there are six points, some have bristles arranged in a dorsoventral queue, with three points aligned parallel in a anteroposterior direction. In the ventral area below the transverse stripe, seven points are observed, some stronger and with bristles.

<u>Tegumentary spots</u>: The spots along the anterior margin are elongated in both the dorsal and ventral areas, while those located between the transverse strips and along the entire posterior edge are very narrow (Figures 3 and 4).

Second abdominal segment. Bristles: In the upper area above the transverse strip five bristles are arranged in a dorsoventral row, with their orientation positioned posteriorly in an anteroposterior direction. In the median area between the transverse strips, five bristles are arranged medially in the anteroposterior direction, among these, two in the upper part and two in the lower part are almost parallel in an anteroposterior orientation. In the lower area below the transverse strip, there are three bristles. Tegumentary spots: The anterior and posterior margins exhibit wide bands of tegumentary spots (Figures 3 and 4).

Third, fourth and fifth abdominal segments: The number and position of the bristles and tegumentary spots are equal in abdominal segments 3, 4 and 5. <u>Bristles</u>: In the upper area above the transversal stripe, three bristles are arranged vertically, positioned relatively far from the center to the posterior margin. In the median area between the transverse stripes, five bristles are aligned centrally, two of which are oriented parallel to the anteroposterior axis. In the lower area, below the transversal strip, two bristles are arranged vertically, situated slightly off-center toward the posterior margin, arranged in a distinctive pattern. Tegumentary spots: The spots along the anterior and

posterior margins are broad. Above the transverse stripes, there is a narrow median line running in a dorsoventral direction toward the upper stripe. In the ventral area below the lower transverse stripe, four narrow lines, also oriented dorsoventrally, are present, with the anterior line being longer than the others (Figures 3 and 4).

Sixth abdominal segment. Bristles: The number and arrangement of bristles in the sixth abdominal segment are the same as those in segments 3 to 5. Tegumentary spots: The tegumentary spot along the posterior margin is narrower than that in segments 3–5 but wider than in segments 1–2. In the upper area, a narrow line—similar to the one in segments 3–5—is present, whereas in the lower area there is a single, longer curved line extending nearly to ventral bristle (Figures 3 and 4).

Seventh abdominal segment. Bristles: in the upper area, three bristles, are arranged in a row, similar to those in abdominal segments 3-6. In the median area between the transverse stripes, five bristles are present, arranged in a pattern that differs from the other segments. In the lower area, two bristles are arranged in a row, as in abdominal segments 3-6. Tegumentary spots: the upper and lower sidebands of the spots are absent, there are two sinuous rows of spots—one in the upper area and another in the median ventral area. The first row starts at the center and extends toward the ventral side, with no additional markings beyond this (Figures 3 and 4).

Respiratory organ: In the ventral median area between segments 7 and 8 there is a milky-white structure with 22 lobes (11 on each side) that are shaped like slightly clavate fingers. This structure functions as a respiratory organ, likely serving as gills that allow the larvae to breathe when the galleries in the tree trunk are filled with water or sap (Figures 1E, 3A, 3C and 4).

<u>Eighth abdominal segment</u>: The morphology of the eighth abdominal segment is quite different from other parts of the body. The fourth former is milky white, similar to the preceding segments. Subsequent three-quarters are dark brown and black, sclerotized with wrinkled tegument,

depressions and protrusions. In the median area features two pairs of strong spines black sclerotized; among the thorns of each pair there is a tuft of setae on the ventral region of this plate sclerotized; there is a pair of black thorns sclerotized, a smaller side, with a tuft of bristles at its base medial and another three to four times larger (Figure 1H). In the posterior ventral region there is a cavity where the annals respiratory spiracles are. These spiracles are positioned on the dorsal surface of this cavity (Figures 1F, 1G, 3A and 3C), the distal edge is more sclerotized distally and brown, basally enlarged. In basal edge there is tegumentary spot. At the distal edge of the spiracles, which are separated by bare areas, there are three groups of dark structures that seem sclerotized thorns. The ventral 'language' of the anal opening has edges with weak tegumentary spots, there are three points of bristles on each side of the edge. The internal groove is milky white and has two groups of sclerotized dots that seem spikes separated by a median bare area containing a median line of points. At the distal edge of each dark spot, there is a large bristle. In the outer distal edge of the 'language' there are four stages and one more at the proximal edge. Moreover, tegumentary spots appear on the outer distal edge of the midline and another circular spot with a central point in the lateral median area.

Anal respiratory spiracles: The anal respiratory spiracles have an average internal diameter of 1.31 mm and are separated by an average distance of 1.47 mm (Table 1). Each spiracle features a darker gray outer ring and an inner ring with several radial grooves. Within this inner ring is a darker gray circular area that displays an irregular, linear groove oriented laterally (Figures 1F and 1G).

DESCRIPTION OF THE SECOND LARVAL INSTAR OF PANTOPHTHALMUS KERTESZIANUS

Cylindrical; length 12 mm; width 1.20 mm. The head, prothorax and the 8th abdominal segment are more heavily sclerotized than the remaining parts, appearing white, with brown spots formed by tegumentary rugosities. A light milky-white lateral stripe, slightly more inflated than the others on other parts of the body, may be seen from the

mesothorax up to the 7^{th} abdominal segment. The head is sclerotized, dark grey. Prothorax sclerotized, brown, covered by rugosities; its spiracle placed on a sclerotized, smooth, dark-brown area (Figures 5 e 6).

Head: Hemicephalic, sclerotized, and uniformly gray, with dark brown coloration along the proximal margin and ventral area. In the dorsolateral area of the head capsule an anteroposterior oval depression is present, bearing two strong brown bristles, one at the anterior end of the depression and another on the back. Along the side of the capsule, a long bristle is observed, accompanied by a shorter one on the ventral side. The front clypeus and labrum form a snout, also gray; front clypeus has a lateral bristle. The antenna is a circular brown area with a whitish brown central point. In lateroventral head region there is an area not sclerotized milky-white that looks like an invagination

3 mm

Figure 5. Second instar larva of *Pantophthalmus kerteszianus*. Illustration: I. S. Gorayeb (2014).

prothorax in head capsule. Ventrally, between these two milky white areas, there is a sclerotized reddish-brown plate. The mandibles and the associated mouthparts, which are attached to this ventral plate and the dorsal snout, together form a brown complex.

Thorax: In addition to the head, prothorax and the last abdominal segment are the most sclerotized. The prothorax integument is slightly sclerotized light brown, covered with coarse rugosities formed by relatively large circular wrinkles compared to other wrinkles' body. Unlike last instar, there are no rough integument dark brown sclerotized bands. Above the spiracle, dorsal area, there are two strong bristles and three other longer more queued previously. On the side there is an oval area not sclerotized, yellowish white, in the center of this area there is a smooth dark brown sclerotized spot; anterodorsal area in this spot is the spiracle round with the previous darker handle, light center two structures in the form of rods abutting the rear end, which constitute a structure in the form of 'V.' The ventral region features an unsclerotized, smooth, milky-white area lacking rugosities;

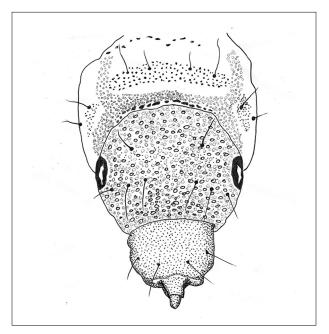


Figure 6. Head, prothorax and mesothorax of second instar larva of *Pantophthalmus kerteszianus*, dorsal view. Illustration: I. S. Gorayeb (2014).

along its upper lateral margin, a small bristle is present. Additionally, along the lateral and anterodorsal edge—where this area contacts the head capsule—there is a row of oval, dark brown sclerotized.

Mesothorax: The segment has a brown, sclerotized ring along its entire anterior margin. On each side of the body, two lateral stripes (upper and lower) are visible. In the median area between these stripes, the anterior quarter shows two areas of fine grooves and two bristles—one in the upper part and one centrally located in a dorsoventral orientation—while the remainder of the median area is milky-white. The area between the lateral stripes is slightly more swollen than other parts of milky-white segment. The upper lateral strip is sclerotized, brown, continuous, although it does not extend to the segment's edges. In contrast, the lower lateral strip is discontinuous and consists of four oval brown spots. Dorsally between the upper lateral strips on both sides of the body, mesothorax have a rugosities pattern configured by: brown areas with small circular ridges covering the leading edge and the margins of the upper strips; in the one-third previous there is an area with fine rugosities brown dark, where there are two groups of three bristles queued in the transverse direction of the segment, the central bristle in each group is greater; the posterior third there is a series of oval spots isolated, dark brown in irregular positions; later there is a finer rugosities to the posterior margin. The lower lateral area of the segment from the lower lateral strip to the venter, is milky-white and bears a bristle in the upper median area.

Metathorax: The sclerotized ring along the anterior margin is less robust than of the mesothorax. Dorsally between the lateral stripes, on each side of the body, there are no ridges, which are restricted to the anterior ring. Additionally, the upper lateral strip is weaker than the mesothorax, consisting of spots in the anterior third, while the spots on dorsoposterior are also faint. The sets of three bristles on each side of the anterodorsally area are present, the two outer bristles are closest than the mesothorax and the next middle one is smaller. In the

lateral area between the upper and lower lateral strips is more whitish than the rest of the segment, in this area tegumentary weak and thin spots are present anterior and ventrally, two anteroposterior bristles are present, but the bristle is positioned more posteriorly than the lower. The lateroventral area is white-yellowish and has a brown bristle near the center anteroposterior of the lateral stripe.

First abdominal segment: It is yellowish white, with the area between the lateral stripes appearing whiter and slightly inflated. The sclerotized dark areas include: two rows of tegumentary spots stains off small, just above the upper lateral strip, a anterior, small, like a dorsoventral line formed by about ten points and another later with about eight points; tegumentary spots below the lower strip in an inflated area, where is a row of brown tegumentary points on form of inverted 'C,' structure that looks like a false leg. Bristles: Two bristles above the tegumentary line (a larger anterior and a smaller posterior) on the upper side area above the upper strip. Two bristles on the side of the strips (one upper and one lower) between spots positioned at the height of the posterior third. Two bristles in the lower lateral area below the 'false leg.'

Second to sixth abdominal segments: In these segments the color pattern, tegumentary spots and bristles are similar to those described in the 1st abdominal segment. The 5th and 6th segments have a well-defined upper lateral strip formed by 3-4 brown traces sclerotized separately in the rear area after posterior tegumentary spot; these segments there are also a number of traces sclerotized forming two lines that run along the lateral area anterior up, more evident on the 6th segment.

Seventh abdominal segment: The seventh abdominal segment appears to merge with the eighth segment, as their separation is less distinct and articulated than that observed between the other segments. Together, they form a single sclerotized structure, with a less pronounced division between them. Additionally, the upper lateral band is a fold in the integument that extends into the eighth segment, reaching its most distal point. The lower lateral stripe does not have a regular shape, it consists of 12 irregularly

arranged integumentary points. The proximal third of the segment is yellowish white similar to adjacent segments, and it gradually becomes more sclerotized toward the distal margin, where the sclerotized areas are composed of circular spots. Dorsally, the distal two-thirds of the segment are sclerotized. The area between the upper and lower bands is sclerotized along its superior and posterior portions. Bristles: Pairs of are present in the three regions defined by the lateral bands—a laterodorsal pair, a median pair, and a lateroventral pair. The lateroventral area is entirely whitish except for the posterior ring.

Eighth abdominal segment: The segment is almost entirely sclerotized, except for the proximal and lateroventral areas, where sclerotization is present only as dots. The dorsum and the remaining areas are continuously sclerotized and brown, becoming darker toward the distal end. Bristles: The bristles are yellowish-brown and longer than those on the other segments: one is located dorsally, another—larger—latero-posteriorly on the lateral stripe, one is on the distal vertex, and two smaller ones are present in the middle of the lower lateral area. The anal spiracles were not described because de 'chamber' in which they are located is closed, and the larva was not dissected, since it was the only entire and preserved specimen.

REDESCRIPTION OF THE EXUVIA OF THE PUPA OF PANTOPHTHALMUS KERTESZIANUS Cylindrical, body length 32 mm, wide 8 mm (Figures 7A-7C).

The anterior capsule, formed by the union of the head with the prothorax, has the anterior surface strongly chitinized and black, with a coarse rugosity. One of the dorsal wrinkles is larger and more elevated. At the anteroventral end there is a black prolongation with spines on its tip (where internally the head should be accommodated). Laterally, on the submedian and ventral region, two spines may be seen. Ventrally, on the median region there is a V-shaped structure, showing a spine on its anterior tip. The posterior area of the anterior capsule is sclerotized, smooth and shining, and

brown; its margin, between the interior rugose area and this smooth area is irregular. A line separates this anterior capsule from the other segments of the thorax. In the lateroanterior region of these fused segments the thoracic spiracle is situated, being the largest one of the bodies. Dorsally and laterally, the segment exhibits several transversal grooves, oriented in a dorsoventral direction. Ventrolaterally are the wing thecae, which reach up to the anterior half of the 2nd abdominal segment. Ventrally, between the wing thecae, some tubular structures may be seen, from the anterior capsule to the 2nd abdominal segment; these structures must accommodate internally the legs and the mouthparts. The abdominal segments milky-white, and less sclerotized, showing, especially on the dorsal region, tegumentary spots. A lateral stripe is present on abdominal segments 1-7, formed by a more invaginated integument, marked by distinct upper and lower sulci. In the middle of that stripe there is a line of paler integument anteroposteriorly oriented, which is more accentuated on segments 2-5. The spiracles are clearly visible on segments 1-4, gradually decreasing in size towards the last segment. Abdominal segments 6 and 7 with rows of strong bristles on their posterior margin.

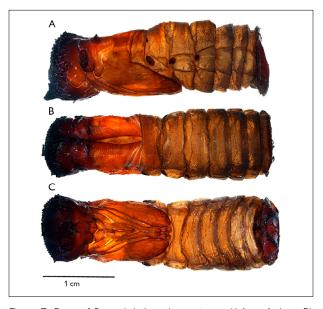


Figure 7. Pupa of *Pantophthalmus kerteszianus*: A) lateral view; B) dorsal view; C) ventral view. Images: I. S. Gorayeb (2014).

Segment 8 has a sclerotized, brown posterior plate, with two sutures dorsoventrally oriented, dividing this plate into three parts. The median part exhibits an inflated, dorsoventral region, with sclerotized rugosities. Dorsally to this structure there exists a plate shaped like a half-moon. Between this latter and the margin of the posterior plate there are rows of strong and short bristles. The anterolateral and anteroventral areas of the 8th segment display the same type of integument and color as the remaining regions, and the two abdominal spiracles are visible ventrally (Figures 7A-7C).

DIFFERENCES BETWEEN THE LARVAE OF PANTOPHTHALMUS TABANINUS AND P. KERTESZIANUS

The first instar of *P. tabaninus* is 3 mm long and 1 mm wide; the last one is 27-32 mm long and 7-10 mm wide. The last instar of *P. kerteszianus* is on average 55.33 mm long, over twice the length of that of *P. tabaninus*. The width of the larva of both species is very similar (ca. 10 mm), but the larva of *P. tabaninus* is shorter.

The morphological differences between the last instar larvae of *P. tabaninus* (Greene and Urich, 1931) and *P. kerteszianus* are shown in Table 2.

DIFFERENCES BETWEEN THE KNOWN PANTOPHTHALMUS PUPAE

The morphological differences between the pupae of *P. tabaninus* and *P. kerteszianus* may be seen as in Table 3.

Rapp (2007) provided a detailed description of the first instar larva of *P. planiventris*, figured in lateral and dorsal views. However, he did not specify the thoracic division nor the prothoracic spiracle. He treated the head proper together with the prothorax, that is why his drawings present, erroneously, in addition to head, 12 segments. Moreover, he mistakenly considered the prothoracic spiracles to be simple eyes. He also furnished a description of the last larval instar and the pupa, which are differentiated from those of *P. kerteszianus* by the characters presented in Tables 4 and 5 below, respectively.

Table 2. Morphological differences between the last instar larvae of Pantophthalmus tabaninus and P. kerteszianus.

Characters	P. tabaninus	P. kerteszianus	
Prothorax Position of head Drawings on the spiracle	Prothorax pronouncedly curved ventrad, so that the head is downwardly directed (Plate VIII - Greene & Urich, 1931); head and prothorax with long bristles; spiracles with evident sinuous drawings	The prothorax is less curved ventrally, so that the head is directed forward. Both the head and prothorax bear shorter bristles, and the spiracles exhibit different internal pattern (Figure 1B)	
Metathorax	With only one large lateroventral bristle	Meso- and metathorax with several short bristles (Figure 4)	
Bristles on abdominal segments 1-7	Absent	Present and short (Figure 4)	
Transversal stripes	The upper transverse stripe very evident on the mesothorax, metathorax and abdominal segments 1-7; lower transversal stripe absent	Both upper and lower transversal stripes very evident on mesothorax, metathorax and abdominal segments 1-7	
Tegumentary spots	Not described, but according to the segments show darker margins and lateroventral areas	Very evident, as shown in Figure 4	
Respiratory organ	Large, occupying a wide ventral area between segments 7 and 8	Small	
Hair tufts at the base of the strong spines at the chitinized plate on the last segment	Not mentioned, except for the more ventral spine	Present	

Table 3. Morphological differences between the pupae of *Pantophthalmus tabaninus* and *P. kerteszianus*.

Characters	P. tabaninus	P. kerteszianus
Sclerotized wrinckles of anterior capsule	Small	Larger, one of them, on the dorsal area, more projected, looking like a small horn
Spiracles	They are very visible on the thorax and abdominal segments 1-7, their internal pattern showing characteristic sinuous drawings	Visible only up to the 4th abdominal segment; different internal pattern
Fringe on segments 7 and 8	More evident, with long bristles	More discrete, with short bristles

Table 4. Morphological differences between the last instar larvae of Pantophthalmus planiventris and P. kerteszianus.

Characters	P. planiventris	P. kerteszianus	
Maximum size of the larva	Length 48 mm; width 10 mm	Length 58,50 mm; width 11,0 mm	
Inclination of head and prothorax in relation to the longitudinal body axis	More accentuated	Less accentuated	
Shape of the body, from the mesothorax to the 7 th abdominal segment	Sinuous, with a distinct waist between segments 4 and 5. From the mesothorax to the 2nd abdominal segment the segments are wider dorsocentrally. From the 3rd to the 5th the abdominal segments become gradually narrower. 6th and 7th segments respectively wider. The 5th segment is the narrowest one	Body straight, without a waist. Abdominal segments more uniform as to width	
Angle formed by the sclerotized plate of the 8th abdominal segment in relation to the longitudinal axis of the body	Small	Large	

Table 5. Morphological differences between the pupae of *Pantophthalmus planiventris* and *P. kerteszianus*.

Characters	P. planiventris	P. kerteszianus	
Thoracic spiracle	Over twice as long as broad, not inflated. Length 2,1 mm; width 0,8 mm	Large, dark brown, noticeably inflated. Length 2,5 mm; width 1,2 mm	
Abdominal spiracles	Very evident on segments 1-5	Visible only in segments 1-3. Reddish-brown. Width over twice its length	
'Crown'	Proximal dorsolateral region of head with a 'crown' with a row of 11-12 sclerotized, bean- like projections, smooth and shining	'Crown' not evident. The bean-like structures reddish-brown as the integument of the thorax and not much projected	
Pair of acute, black, sclerotized projections on the dorsum of the prothorax	Absent	Present	
Black, sclerotized rostrum on anterolateral region	Large and protruded forwards and upwards	Smaller, less protruded	
Wing thecae	Reaching the beginning of 2 nd abdominal segment	Reaching the middle of 2nd abdominal segment	

CONCLUSION

The second immature stages of *Pantophthalmus kerteszianus* are described for the first time, and the last larval instar and pupa are redescribed, being the only species of the family, up to now, to have the number of its larval instars determined. The descriptions are more detailed than those made for the other described immatures of the genus.

Biometric analyses revealed that *P. kerteszianus*, exhibits seven larval instars during its development, as evidenced by measurements of nine selected morphological structures. The greatest variation was observed in the body's length and width, while the smallest variation was found in the distance from the prothoracic spiracle to the lower bristle and the width of the median band of the prothorax.

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AUTHORS' CONTRIBUTION

L. E. C. Cardoso contributed to project administration, methodology, investigation, formal analysis, data curation and writing (original draft); and I. S. Gorayeb contributed to formal analysis, conceptualization, data curation, supervision, visualization and writing (review and editing).