

# Redescription of larva, pupa and adult of *Anopheles (Anopheles) annulipalpis* (Diptera: Culicidae) and the removal of the specie of the Cyclolepteron Series

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**ABSTRACT.** The redescription of male, female, pupa and larva of *Anopheles (Anopheles) annulipalpis* Lynch Arribálzaga, 1878 is presented. The species is included in the Cyclolepteron Series of *Anopheles*, although several authors consider that this is artificial. The redescription of *Anopheles annulipalpis* demonstrate that pupae have laticorn trumpet. By this fact is compared with *An. grabhamii* Theobald, 1901 and similar species of the Arribalzagia Series and Myzorhynchus Series. The laticorn type of trumpet joined the other differences and similarities found are sufficient to remove *An. annulipalpis* of Cyclolepteron Series and included as an unplaced member of the Angusticorn Section.

**KEYWORDS.** Anophelini, mosquitoes, Neotropical, taxonomy, Argentina.

*Anopheles annulipalpis* Lynch Arribálzaga, 1878 is a Neotropical species. The description of adult, pupa and larva are incomplete for the current status of Anophelinae. LYNCH ARRIBÁLZAGA (1878) described the species based on a female. BRÉTHES (1912) made a brief description of the male, later in 1916 he published a drawing of the male genitalia. SHANNON & DEL PONTE (1928) commented on this matter, and redescribed the female. DYAR (1928) described the female. ALVARADO & DEL PONTE (1938) described the larva with specimens collected in Mendoza province, more than 850 km W of the type locality. DEL PONTE (1940) presented a new description of the male genitalia with specimens of the Delta del Paraná in Entre Ríos province. GARCÍA & CASAL (1964) described the pupa of *An. annulipalpis* from the same area. ROMEO VIAMONTE & CASTRO (1951) described the cibarial armature of female. EDWARDS (1932) and LANE (1953) includes *An. annulipalpis* in the Cyclolepteron Series of the Angusticorn Section of *Anopheles (Anopheles)*. Subsequently, REID & KNAB (1961) proposed only two species in the Cyclolepteron Series (*An. grabhamii* Theobald, 1901 and *An. annulipalpis*). This classification adopted by WILKERSON & PEYTON (1990) and HARBACH (1994). Later, COLLUCCI & SALLUM (2007) and HARBACH & KITCHING (2015) demonstrated the Section Angusticorn and the Series Cyclolepteron to be artificial groups within the subgenus *Anopheles*.

The objective of this study is the redescrbe the male, female, pupa and fourth-instar larva of *An. annulipalpis*, update the nomenclature and compared it with the most

similar species as *An. grabhamii*, *An. punctimacula* Dyar & Knab, 1906, *An. shannoni* Davis, 1931 (Arribalzagia Series), *An. coustani* Laveran, 1900, *An. tenebrosus* Donitz, 1902 and *An. sinensis* Wiedemann, 1828 (Series Myzorhynchus) for being the most similar species of *Anopheles*.

## MATERIAL AND METHODS

Morphological structures were examined in the adult, pupa, and fourth-instar larval stages. Diagnostic and differential characters were confirmed in all specimens listed in the Material examined section. The morphological nomenclature follows HARBACH & KNIGHT (1980, 1982). Abbreviations of generic level are taken from REINERT (2009).

Morphological data of species used in the comparison were taken from BELKIN *et al.* (1970) (*An. grabhamii*), WILKERSON *et al.* (1997) (*An. shannoni*), WILKERSON (1990) (*An. punctimacula*), GILLIES & DE MEILLON (1968) (*An. coustani*, *An. tenebrosus*), HARRISON & SCANLON (1975) (*An. sinensis*), and the data set of HARBACH & KITCHING (2005) and COLLUCCI & SALLUM (2007).

Life stages are indicated by the symbols ♂ (male), ♀ (female), L (fourth-instars larva), Le (larval exuviae), and Pe (pupal exuviae), and male and female genitalia are denoted by the letter G (genitalia) used in combination with the respective sex symbol. A total of 76 specimens were examined. Measurements are in millimeters, the minimum and the maximum values followed by the mean between brackets; counts and ratios are similarly formatted. Adults

were pin mounted, genitalia, head and immature were mounted between slide and coverslip with Canada balsam. Voucher specimens were deposited in La Plata Museum (MLP), Argentina. Latitude and longitude data of the collecting locations were taken from Google Earth.

Specimens that were examined are the following: 7 males, 4 male genitalia, 54 females, 2 female genitalia, 7 pupal exuviae (reared to adult), 2 larval exuviae (reared to adult), 6 larvae, 3 female wing. All specimens are deposited in División Entomología, Museo de La Plata.

Material examined. ARGENTINA, **Salta**: Tartagal (23°31'S, 63°46'W), IX.01.1963, ♀, Hepper, H. coll.; **Misiones**: Iguazú, Wanda (25°58'S, 54°36'W), VIII.01.1972, 3♀, MLP coll.; **Mendoza**: Uspallata, Potrerillos, río Mendoza (32°56'59"S, 69°11'49"W, 1370 masl), II.27.2002, 4♀, Scheibler, E. coll.; **Entre Ríos**: Concepción del Uruguay (32°30'S, 58°14'W), V.01.1969, ♀, MLP coll.; **Buenos Aires**: Berisso, Los Talas (34°53'17"S, 57°49'31"W), II.10.1988, ♀, García, J. J. coll.; idem, VIII.18.1988, ♀, Spinelli, G. coll.; idem, IX.06.1988, ♀, Marino, H. coll.; XI/15/1988, ♀, Marino, H. coll.; La Plata, Observatorio Astronómico (34°54'27"S, 57°55'57"W), IX.10.1991, ♀, Maciá, Campos coll.; Magdalena Arroyo Zapata y RP 11 (35°3'38"S, 57°37'41"W), IX.06.1988, 2♀, Armesto, Marino coll.; idem, XI.29.1988, 2♀, Marino, H. coll.; Ensenada, Punta Lara, Reserva (34°47'23"S, 58°0'01"W), XI.11.1985, 2♀, Balseiro, E. coll., R. Ronderos det.; idem, VIII.08.1989, ♀, Maciá, Campos coll. and det.; idem, VIII.14.1989, ♀, Rossi, Maciá coll.; idem, VIII.29.1989, ♀, Maciá, Campos coll. and det.; idem, IX.25.1989, 3♀, ♀ wing, 1 L, García, Rossi, Maciá, Campos coll.; idem, X.23.1989, ♀, Maciá, Campos coll.; idem, XI.06.1989, 2♂, 2♀, ♀ wing, Maciá, Campos coll.; idem, V.01.1990, 7♀, ♀ wing, Maciá, Campos coll.; idem, VIII/21.1990, 2♀, ♀G, Maciá, Campos coll.; Ensenada, Punta Lara, Boca Cerrada (34°47'8"S, 58°0'57"W); idem, XII.09.1996, 2♂, 2♂G, ♀, 3 Pe, 2 Le, 2 L, García, J. J. coll.; idem, X.01.1998, 9♀, ♀G, Micieli, M. V. coll.; idem, XI.26.2002, 1 L, Micieli, Marti coll.; Ensenada, Punta Lara, Provincial Route 19, 5.5 km from Boca Cerrada (34°49'2"S, 58°03'11"W); idem, V.01.1989, 3♀, Rossi, coll.; idem, IV.01.1991, ♀, Campos, Maciá, coll. and det.; Brandsen, Provincial Route 215, Brandsen - La Plata (35°05'25"S, 58°09'17"W), IV.18.1998, 3♂, 2♂G, 2 Pe, 2 L, García, Rossi coll.; idem, IV.25.1989, ♀, 2 Pe, García, Campos coll.; Zárate (34°06'45"S, 58°59'53"W), XI.20.1998, ♀, Marino, P. coll.

## RESULTS AND DISCUSSION

### *Anopheles* (*Anopheles*) *annulipalpis* Lynch Arribálzaga, 1878

*Anopheles annulipalpis* LYNCH ARRIBÁLZAGA, 1878:149. BRÈTHES, 1912:13M; 1916:199M\*; ALVARADO & DEL PONTE, 1938:388L\*; DEL PONTE, 1940:302MG\*; GARCÍA & CASAL, 1964:5P\*; CAMPOS, 1997:31 (biology). Further details about the synonymy are in LANE (1953).

Female. Integument light to dark brown with grayish pollinosity. *Head*. Interocular space with 8–14 long, and

whitish setae and lateral row of small, narrow, appressed white to light cream scales; vertex and occiput with whitish erect scales, dark scales posteriorly, upper portion of postgena with erect, truncate black scales; head with 6–8 long, dark ocular setae; postgena with long black setae ventrally. Clypeus bare. Pedicel of antenna with several small, narrow to broad, grayish white spatulate scales dorsolaterally; flagellomere 1 with several narrow to broad whitish scales on lateral surface and a few whitish scales on inner surface; flagelomeres 2–7 with patch of whitish scales on lateroapical surface. Scales of maxillary palpus slender, spatulate, dark brown with intermixed dark brown setae; palpomere 2, covered with erect dark and whitish erect scales intermixed in inner side of dorsal surface of basal half and at apex, palpomere 3–5 decreasing in size, dark scales, with whitish basal ring, 3–4 with intermixed whitish scales in dorsal and outer sides, 5 dark-scaled, sometime with whitish scales at apex, sometimes with whitish scales on middle of outer side of palpomere; length of maxillary palpus 2.40–2.85 mm (mean = 2.59 mm); palpus longer than forefemur length. Proboscis dark-scaled, base with long erect scales and setae; proboscis length 2.40–2.95 mm (mean = 2.74 mm). *Thorax*. Scutum grayish brown, with three longitudinal pruinose lines, covered with small whitish scattered scales, two bare areas widen gradually posteriorly from posterior scutal fossa to scutellum. Acrosticals, anterior and posterior dorsocentral bristles dark brown. Anterior promontory, lateral scutal fossa ante- and supralar areas with spatular white scales. Scutellum with 16 (13–18) long dark brown setae, about 40 short, light brown setae, these with golden reflections and whitish spatular scales. Anteprepronotum with dense patch of dark brown spatulate scales and long, strong brown setae and a patch of whitish scales on lower. Anteprepronotum Pleural vestiture as follows: all scales whitish translucent, spatulate. Proepisternum sometimes with 2,3 scales; upper proepisternum with 4–6 setae of different length; prespiracular area with 2,3 setae, rarely with 1,2 scales; prealar area with 3–7 setae; upper mesokatepisternum with 1 (1,2) setae, 4–6 (n = 10) translucent scales; lower mesokatepisternum with 3–6 setae, 5–9 scales; upper mesepimeron with 5–9 setae, scales absent. Legs. Forecoxa with upper patch of black scales on anterior surface, and a patch of long, erect whitish scales posterolaterally; foretrochanter mostly with small appressed white scales posteriorly, midcoxa with three patches of white scales, hindcoxa with one patch of whitish scales, mid- and hind trochanters with patches of whitish scales. Extent and number of pale spots on femur and tibia variable. Forefemur rather whitish scaled ventrally; mid- and hindfemur with ventral stripe of whitish scales with well demarcated borders, extending on almost entire length of femur; foretibia dark-scaled, with white spots extending to ventral and dorsal surfaces, midtibia with ventral longitudinal stripe of yellow scales extending to 0.7 from base, anterior, posterior and dorsal surfaces with spots of white scales, sometimes encircling the tibia, hindtibia dark, with white spots forming a strip from middle to apex internally; foretarsomere I dark-scaled with spots of white scales on anterior and dorsal surfaces, fore-

and midtarsomere 2,3 dark scaled, with spot of white scales anterolaterally at apex, sometimes with one or two whitish spots; foretarsomeres 4 and 5 dark-scaled; hindtarsomeres 1 dark-scaled with spots of white scales on anterior and dorsal surfaces, hindtarsomere 2 dark-scaled with prebasal and apical lateral spots of white scales, hindtarsomere 3 with apical spots of white scales, hindtarsomeres 4 and 5 entirely white-scaled. Forefemur length 2.15–2.40 mm (mean = 2.28 mm). Wing. Length (measured from humeral crossvein to tip of wing, not including fringe) 4.70–5.70 mm (mean = 5.21 mm). Dark scales brown, pale wing scales whitish. Vein C dark except by the presence of apical pale spot; humeral cross-vein dark-scaled dorsally and ventrally. Vein SC dark-scaled, vein  $R_1$  with pale and dark spots very similar to those of the Arribalzagia Series (*sensu* WILKERSON & PEYTON 1990). Posteriors veins with intermixed brownish, whitish, and dark scales. Vein  $R_{4+5}$  with basal spot of white scales, a small white spot, 6–8 dark scales, remaining of vein pale-scaled, with 2–12 dark and white spots at apex (in some specimens apex with or without dark scales), vein M mostly whitish-scaled intermixed with only dark scales, preapical end with two brownish spots and one dark spot that continues on proximal veins  $M_1$  and  $M_2$ , veins  $M_{3+4}$  pale-scaled with four and two spots of dark scales respectively, CuA with pale whitish scales intermixed with dark scales and two patches of dark scales near base and middle of vein, vein 1A mainly dark, with mixture of whitish and dark scales at base, alternating with five small, white spots. Fringe brownish, with whitish spots apically at  $M_{3+4}$ . Halter. Scabellum with whitish integument, pedicel and capitellum with dark brown integument, without scales. *Abdomen*. Integument brown to dark brown with some grayish pollinosity. Terga with numerous long yellowish setae; terga without posterolateral scales; tergum VIII with long, yellowish setae and several white, spatulate scales. Sterna with scattered yellowish setae; sternum I with elongated patch of white spatulate scales; sterna II–VII without scales.

*Genitalia* (Fig. 1): Tergum IX narrow, with small finger-like lobe on each side of median straight line; cerci elongate, somewhat elliptical in outline with dark scales and with numerous long brownish setae; postgenital lobe weakly sclerotized, with small posterior bridge with minute spicules, caudal margin rounded with two strong setae at apex; indistinct insular setae upper and lower vaginal lips indistinct; upper vaginal sclerite well-sclerotized.

Male. As in female except for the following sexual differences. Maxillary palpus 2.43–2.71 mm (mean 2.65 mm), apex of palpomere 3, and all palpomere 4 and 5 enlarged. Maxillary palpi with dark brown and yellowish scales; base of palpomere 2 with erect scales, basal 0.5 with patch of yellowish scales, a few yellowish scales at apex of dorsal surface; palpomere 3 with patch of pale yellow scales at base and apex and on 0.2 of dorsal surface; palpomere 4 mostly pale yellow, a spot white scales at base; palpomere 5 usually yellowish; setae at tip of palpomeres 4 and 5 mostly yellowish. Proboscis length 2.29–2.40 mm (mean 2.35 mm), with small, decumbent, dark brown scales and ventrobasal patch of long, erect, dark scales.

*Genitalia* (Figs 2–9): ninth tergal lobes (Fig. 9) short, somewhat triangular in outline, widely separated. Dorsal surface of gonocoxite (Fig. 6) with a few scattered, short and moderately long setae, longer on apicolateral area; lateral surface with several slender, fusiform scales; ventral surface with short to moderately long setae, and 3,4 long setae on apical 0.3 and scales on all length. Parabasal setae arising from a protuberance or distinct tubercle, located near 0.30 from base of gonocoxite; most mesal parabasal spine stouter, rodlike with wide sinuous, hooked tip, borne on strong tubercle, the second parabasal seta longer, slender than mesal seta, straight at apex, near 20 short setae in vicinity of parabasal setae on medial side of gonocoxite. Gonostylus (Fig. 6) widened at both ends, with 4–7 setae on dorsal side and row of small setae in the apical 0.7 of lateral side, gonostylar claw short, spiniform and blunt. Claspette (Figs 2, 3, 7, 8). Dorsal lobe of claspette long, with three closely appressed setae of increasing length, tip of most lateral seta with apex enlarged and forming a small hook; ventral lobe of claspette less sclerotized, with three apical or subapical setae, most apical longest, the shorter median; remainder of ventral lobe and area between it and dorsal lobe with many evenly spaced prominent short spicules. Aedeagus (Figs 4, 5): Aedeagal leaflets usually 4 (4–5) per side, most mesal leaflets longest and broadest with small serrations on the internal edge, the rest of decreasing size.

Larva (Figs 10–17). Position and development of setae as figured; range and modal number of branches in Tab. I. *Head* (Fig. 11). Antennal length 0.30–0.35 mm (mean = 0.33 mm), tapered toward apex (Fig. 15), 5.88–6.25 (mean = 6.0) longer than wide, with spicules longer and more numerous in vicinity of seta 1-A; ventral surface with sparse, short spicules; seta 1-A with 12–23 branches, inserted 0.31–0.38 (mean = 0.35) distant from base of antenna; seta 2-A pointed; 3-A truncate, fringed at apex (not illustrated); 4-A plumose on about apical 0.8, shorter than 2-A or 3-A. Seta 2-C single with 2–7 branches arising from 0.3 apical, length 1.0–1.25 (mean = 1.08) length of 3-C, seta 2-C close to mate of opposite side, distance between bases 1.5–2.0 (mean = 1.75) width base of single seta; 3-C 0.80–1.0 length of 2-C, with 5–10 branches, clypeal index (distance between bases of 2-C and 3-C (Fig. 16) on one side/distance between bases of 2-C) 2.50–3.67 (mean = 3.07). Mentum (Fig. 14) usually with four irregular teeth at the sides of the central but larger tooth. *Thorax* (Fig. 10). Seta 1-P not palmate, always single; 9, 10, 12-P single, 11-P single or double; 9–12-M single, 12-M 0.3 length of 9, 10-M, 11-M very short; 3-T weakly developed, hyaline, palmate; 11-T very short, ~0.25 length of 12-T, 12-T moderately developed. *Abdomen* (Fig. 10): Integument hyaline, with minute spicules on ventral surface, more evident on central portion of each segment; setae I–I–VII palmate (Fig. 17), I–I, II weakly developed, hyaline (not illustrated), leaflets of developed setae broad with jagged margins, apices weakly pigmented. Segment X (Fig. 12): Seta 1-X not inserted on saddle. Saddle with minute, sparse spicules on lateral surface, caudally present a prominent median dorsal caudal process of saddle. Integument of posterior margin of segment X



Figs 1–9, *Anopheles (Anopheles) annulipalpis* Lynch Arribálzaga, 1878. 1, Female genitalia; 2–9, male genitalia: 2, dorsal lobe of Claspette; 3, setae of dorsal lobe of Claspette; 4, aedeagus; 5, lateral view of aedeagus; 6, gonocoxite and gonostylus dorsal aspect (prerotation sense); 7, ventral lobe of Claspette; 8, detail of ventral lobe of Claspette; 9, IX-Tergum. Scales in mm, except when indicated.

Tab. I. Number of branches for setae of the fourth-instar larva of *Anopheles (Anopheles) annulipalpis* Lynch Arribálzaga, 1878<sup>a</sup>.

	Head	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
0		1				3–10 (3)	3–9 (5)	2–6 (4)	4–6 (4)	4,5 (4)	3–5 (4)	1	
1	1	1,2 (1) <sup>b</sup>	17–29 (23)	1–3 (1)	10–16 (13)	10–16 (13)	17–26 (24)	12–24 (16)	16–21 (16)	15–24 (16)	3–6 (5)	1	1
2	1	8–14 (13)	1,2 (1)	1–3 (1)	6–9 (8)	5–8 (5)	5–10 (9)	2–6 (2)	2–5 (2)	2–6 (6)	4–6 (4)	4–7 (4)	8–12 (11)
3	6–14 (10)	1	1	5–13 (12)	1–3 (2)	1,2 (1)	1	1	1,2 (1)	1–3 (1)	1–6 (1)	5–10 (7)	3–7 (5)
4	2–6 (3)	>11	2–4 (2)	1–4 (2)	6–9 (7)	4–6 (5)	2–5 (4)	2,3 (2)	2–4 (3)	1–5 (1)	1,2 (1)	1	9
5	11–17 (13)	>22	1	>15	3–8 (5)	9–12 (9)	5–10 (6)	3–7 (4)	3–6 (5)	3–8 (7)	4–9 (8)	4–6 (5)	
6	14–19 (15)	1	1	1,2 (1)	1	1	1	1–3 (1)	1,2 (1)	5–6 (5)	6–10 (7)		Siphon
7	9–17 (14)	1	2–5 (3)	>20	1	1	4–6 (4)	3–7 (4)	2–6 (3)	3–7 (3)	2–6 (6)		1-S: 3–5 (4)
8	6–10 (7)	7–15 (8)	8–15 (11)	>18		1–4 (2)	1–4 (2)	2,3 (2)	2,3 (2)	2–4 (2)	1–4 (4)		2-S: 4–6 (5)
9	3–12 (7)	1	1	1	3–7 (4)	5–8 (8)	3–8 (5)	4–6 (5)	2–7 (6)	5–8 (7)	2–6 (3)		6-S: 1,3 (1)
10	2–7 (5)	1	1	1–3 (1)	1	2,3 (3)	1,3 (1)	1,2 (1)	1,2 (1)	1,2 (1)	2–4 (2)		7-S: 1
11	>35	1	1	1	3	– (1)	1–4 (2)	1–3 (2)	1–3 (2)	2–5 (3)	1		8-S: 2–4 (3)
12	3–7 (5)	1	1	1	1,2 (1)	1,2 (1)	1–4 (1)	1–2 (2)	1–3 (2)	1,2 (2)	4–8 (4)		9-S: 3–5 (3)
13	2–9 (6)	6–11 (9)	3–7 (7)	1–4 (3)	7–11 (8)	8–11 (10)	7–11 (7)	2–6 (3)	2–5 (4)	6–11 (7)	4–6 (4)		
14	1–4 (3)	10–15 (14)	9–12 (10)				1	2–4 (3)	2–3 (3)	2–4 (3)	3	1–2 (1)	
15	3–7 (3)												

<sup>a</sup>: based on nine specimens.<sup>b</sup>: range (mode)

with several, strongly developed, dark spicules. Spiracular apparatus (Fig. 12). Pecten plate (Fig. 13) with 15–19 teeth; arrangement of teeth alternating long and short, with 4–6 long and 9–13 short; long spines 2.93–4.47 (mean = 4.07) about twice length of short spines.

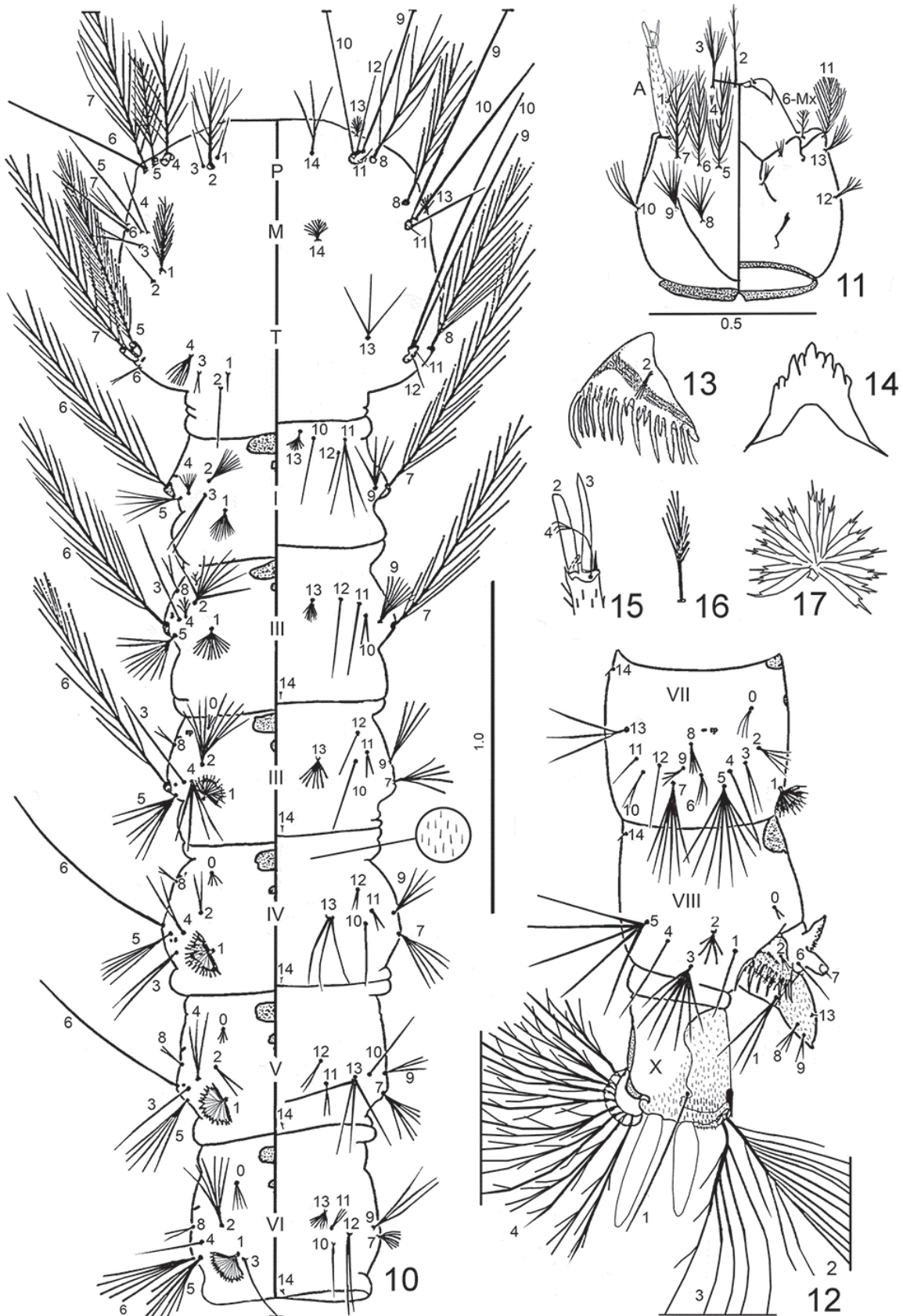
Pupa (Figs 18–20). Position and development of setae as figured; range and modal number of branches in Tab. II. Integument weakly pigmented, with variably pigmented pattern of dark areas and bars on wing case, slightly darker on leg cases, antennal case with dark pigmentation at flagellomere joints; mesothoracic wings light brown with dark areas; abdominal segments weakly pigmented, usually dark at anterior middle. Cephalothorax (Fig. 18): trumpet (Fig. 20) laticorn tragus well developed, elongate; secondary cleft present. Setae 1-3-C single, 1, 3 shorter than 2-CT, setae 4, 6, 7-CT single, 5, 6-CT longer than 4, 7-CT, 5 double, 8, 9-CT single, short. Metanotum (Fig. 19): Setae 10, 11, 12 increasing in size, single, 11-CT usually triple. Abdomen (Fig. 19) with terga and sterna II-VII mostly covered with minute spicules. Seta 6-II single, sometimes double. Terga V-VII with small spicules on caudal border. Genital lobe 0.25–0.28 mm in male and near 0.17 mm in female. Abdominal segments III-VII with ventral fold line. Setae 0-II-VII near 0.07–0.20 mm, 0-VIII near 0.04–0.06 mm. Setae 0-IV-V 0.80 length of 2-IV-V. Setae 9-II-VIII peg-like to long and blunt, 9-II minute, unpigmented, 9-III-VII stout, increasing in size caudally, 9-VIII peg-like, branched (2–9), shorter than 4-VIII. Paddle weakly pigmented, external buttress, base of midrib and base of paddle usually darker, length 0.76–0.95 mm (mean = 0.82

mm), width 0.53–0.79 mm (mean = 0.62 mm), somewhat rounded in outline; length of marginal spicules 0.03–0.07. Seta 1-Pa usually triple, 2-Pa single, 0.5 length of 1-Pa.

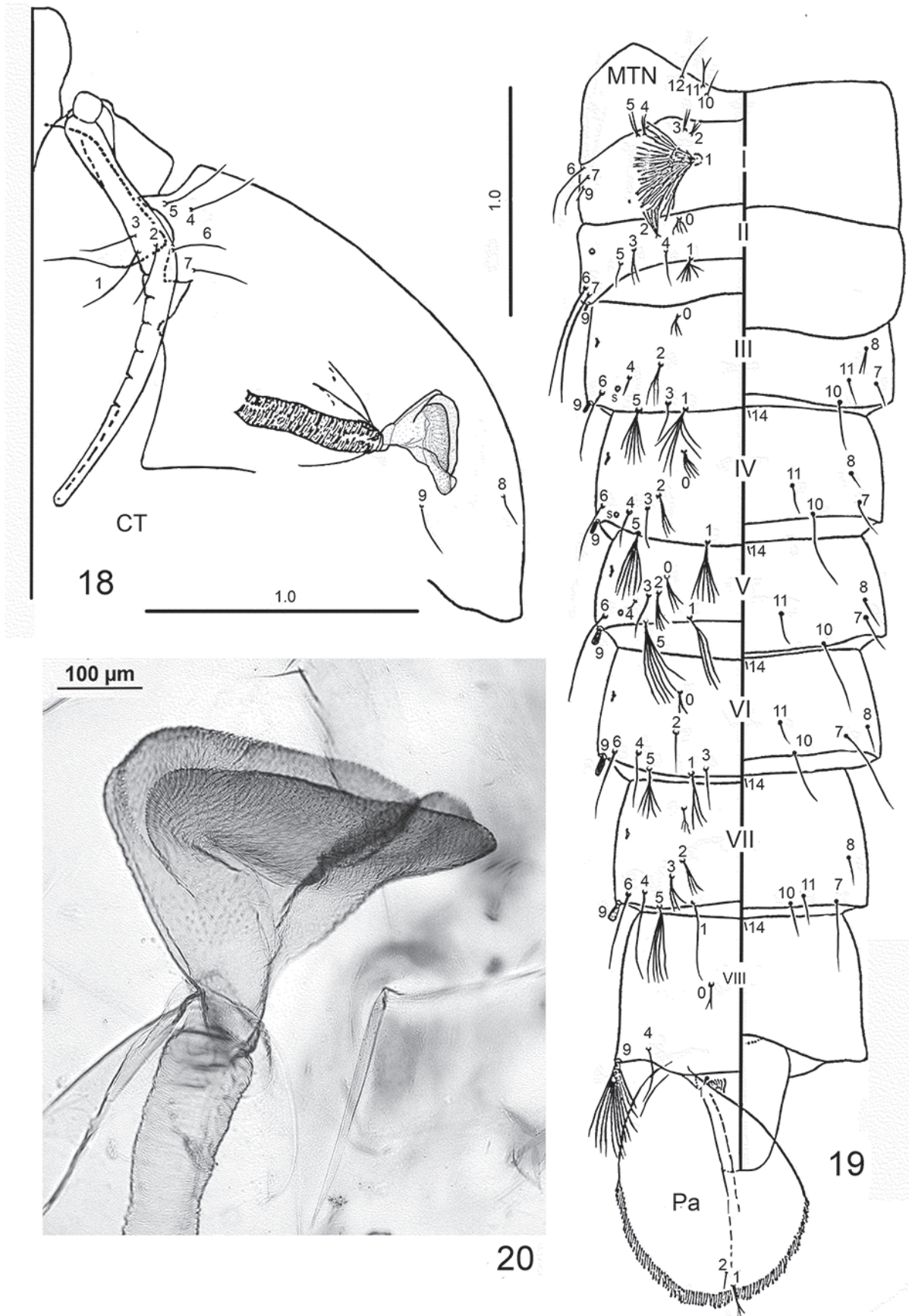
Bionomics. Larvae and pupae of *An. annulipalpis* from Buenos Aires province were collected in large swamp-marshy depressions with grasses, sunlight, pH between 5.50 and 6.79, together with larvae of *An. albitarsis* Lynch Arribálzaga, *Aedeomyia squamipennis* (Lynch Arribálzaga), *Culex idottus* Dyar, *Cx. brethesi* Dyar and species of *Mansonia*, *Psorophora* and *Uranotaenia* (CAMPOS, 1997). Mendoza adults were reared from larvae collected near puddles or pools with water from the derivations of the river, which were scarcely covered with grass and aquatic macrophyta or with no vegetation. Larvae were found in association with *Cx. cuyanus* Duret and other macroinvertebrates such as Aeshnidae (Odonata), Chironomidae (Diptera), Corixidae (Hemiptera), Hydrophilidae (Coleoptera) and Hyallellidae (Crustacea). The pools have a maximum depth of 0.5 m, the water temperature was 16 °C (April) and 20.7 °C (February), pH between 6.77 and 7.57 (ROSSI *et al.*, 2006).

The adults of *An. annulipalpis* are known principally by the descriptions of LYNCH ARRIBÁLZAGA (1878, 1891) and DEL PONTE (1940), the larva by a brief description (ALVARADO & DEL PONTE, 1938) and the pupa by the description of one exuvia by GARCÍA & CASAL (1964).

Based on this redescription is observed that certain morphological characters of *An. annulipalpis* separate it from *An. grabhamii* – both included in the Cyclolepteron Series (Tab. III) – and it should be included on one of the Series of



Figs 10–17, *Anopheles (Anopheles) annulipalpis* Lynch Arribálzaga, 1878, larva: 10, pro- (P), meso- (M) and metathorax (T), and abdominal segments I–VI (left side dorsal, right side ventral); 11, head, left dorsal side, right ventral side; 12, abdominal segments VII–X lateral view; 13, pecten plate; 14, dorsosentum; 15, detail of antenna; 16, seta 3-C alternative; 17, seta 1-III. Scales in mm.



Figs 18–20, *Anopheles (Anopheles) annulipalpis* Lynch Arribálzaga, 1878, pupa: 18, CT (cephalothorax); 19, MT (metathorax) and abdominal segments I–VIII (left side dorsal, right side ventral); 20, pupa trumpet; Pa. (paddle). Scales in mm except, when indicated.

Tab. II. Number of branches for setae of the pupa of *Anopheles* (*Anopheles*) *annulipalpis*.<sup>a</sup>

Seta no.	CT	I	II	III	IV	V	VI	VII	VIII	X	P
0			1-4 (2) <sup>b</sup>	2-7 (3)	4	4-6 (5)	2-4 (4)	2-4 (3)	2-4 (2)		
1	1	12-16 (14)	5-9 (6)	4-6 (6)	4-6 (5)	3-5 (3)	1-3 (3)	1-3 (2)		1	1-3 (3)
2	1-2 (1)	2-3 (2)	3-7 (4)	3-4 (3)	2-4 (3)	1-4 (3)	3	1-3 (3)			1
3	1-2 (1)	2-3 (3)	1	1	1	1	1	2-3 (2)			
4	1	1-4 (2)	1-4 (3)	1-4 (2)	3-4 (4)	1-2 (1)	1	1	1		
5	1-3 (1)	1-2 (2)	1-4 (2)	6-7 (6)	3-8(5)	6-8 (7)	3-6 (5)	2-5 (3)			
6	1	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)	1-2 (1)			
7	1	1-2 (1)	1-2 (1)	1-4 (2)	3-7 (6)	1-4 (1)	1-2 (1)	1-2 (1)			
8	1			1-2 (2)	1-2 (1)	1-3 (1)	1-2 (1)	1-2 (1)			
9	1	1	1	1	1	1	1	1	3-9 (8)		
10	1		1	1	1	1	1	1			
11	2-4 (3)		1	1	1	1	1	1			
12	1										
13											

<sup>a</sup> Based on counts made on all setae available of 8 specimens; P: prothorax; M: mesothorax; T: metathorax<sup>b</sup> Range (mode).Tab. III. Morphological comparison between *An. annulipalpis*, *An. grabhamii*, *An. punctimacula*, *An. shannoni*, *An. coustani*, *An. tenebrosus* and *An. sinensis*.

Character	Cyclolepteron Series		Arribalzagia Series		Myzorhynchus Series		
	<i>An. annulipalpis</i>	<i>An. grabhamii</i>	<i>An. punctimacula</i>	<i>An. shannoni</i>	<i>An. coustani</i>	<i>An. tenebrosus</i>	<i>An. sinensis</i>
Adults							
Antenna, scales on flagellomere 1	present	absent	absent	absent	present	absent	present
Anteprenotum, ventral scales	present	absent	present	present	absent	absent	absent
Upper mesokatepisternal scales	absent	present	present	present	absent	absent	absent
Upper mesepimeral scales	absent	present	absent	present	present	absent	absent
Vein R2+3 long versus cell R2	aproximately equal or longer	shorter	shorter	shorter	equal or longer	aproximately equal or longer	shorter
Sternum VII, presence of scales (female)	present	absent	present	present	present	present	present
Hindtarsomere 3	two or three and apical pale ring	inconspicuous pale basal ring	one or two pale bands	inconspicuous pale basal ring	conspicuous pale apical ring	conspicuous pale apical ring	inconspicuous pale basal ring
Hindtarsomeres 4 and 5	totally pale	inconspicuous pale basal ring	inconspicuous pale basal ring	inconspicuous pale basal ring	totally pale	totally pale	inconspicuous pale basal ring
Genitalia, margin of distal aedeagal leaflet (male)	with serrations or denticles on one or both edges	with serrations or denticles on one or both edges	smooth	smooth	with serrations or denticles on one or both edges	with serrations or denticles on one or both edges	with serrations or denticles on one or both edges
Larva							
Seta 4-C, development	weakly developed	weakly developed	weakly developed	weakly developed	strongly developed	strongly developed	strongly developed
Seta 11-C, development	branched, not plumose	branched, plumose	branched, plumose	branched, plumose	branched, not plumose	branched, not plumose	branched, not plumose
Seta 1-A, placement	inserted dorsomesally	inserted dorsally	inserted dorsomesally	inserted dorsomesally	inserted dorsomesally	inserted dorsomesally	inserted dorsomesally
Abdominal segment VIII, anterior dorsomedian sclerotized plate	moderately developed	small	small	small	moderately developed	moderately developed	moderately developed
Pecten spines	long and short spines with smooth edges	long spines smooth, short spines with spicules on dorsal edge	long and short spines with smooth edges	long spines smooth, short spines with spicules on dorsal edge	long spines smooth, short spines with spicules on dorsal edge	long spines smooth, short spines with spicules on dorsal edge	long spines smooth, short spines with spicules on dorsal edge
Pupa							
Trumpet, development	laticorn, tragus absent	angusticorn	laticorn, tragus absent	laticorn, tragus present	laticorn, tragus absent	laticorn, tragus absent	laticorn, tragus absent
Trumpet, meatal cleft(s)	present	absent	present	present	present	present	absent
Seta 5-V, development	similar to seta 1-V	similar to seta 1-V	similar to seta 1-V	less developed than seta 1-V	similar to seta 1-V	similar to seta 1-V	similar to seta 1-V



subgenus *Anopheles*. For this reason it is compared with *An. grabhamii* (Cyclolepteron Series), *An. punctimacula* Dyar & Knab, 1906, *An. shannoni* Davis, 1931 –Arribalzagia Series–, *An. coustani* Laveran, 1900, *An. sinensis* Wiedemann, 1828 and *An. tenebrosus* Donitz, 1902 of Myzorhynchus Series. *Anopheles annulipalpis* can be recognized in all its stages by combinations of certain morphological characters that separate species with which it was compared (Tab. III).

The subgenus *Anopheles s. stricto* includes 182 nominal species (HARBACH, 2014). EDWARDS (1932) proposed the first infrasubgeneric classification of *Anopheles (Anopheles)* and divided the subgenus into groups and series. REID & KNIGHT (1961) adopted EDWARDS' (1932) classification and divided the subgenus into two sections, Angusticorn and Laticorn, and six series. The Laticorn Section includes the Arribalzagia, Christya, and Myzorhynchus Series, and the Angusticorn Section includes members of the *Anopheles*, Cyclolepteron, and Lophoscelomyia Series. The presence of a funnel-shaped pupal trumpet occurs on the members of the laticorn section, whereas those of the angusticorn possess a tubular pupal trumpet. Except for minor changes, the internal classification of the subgenus *Anopheles s. stricto* remains as it was proposed by REID & KNIGHT (1961).

In his molecular study SALLUM *et al.* (2002) conclude that the relationship among the Myzorhynchus, Arribalzagia and *Anopheles* Series remain unresolved because the present data do not provide unambiguous support for any relationships. Moreover, the exact placement of subgenus *Anopheles* within genus *Anopheles* remains unresolved as a result of poor resolution of basal relationships within trees. HARBACH & KICHTING (2005) found that the largest clade to branch from the main stem of the ordinal *Anopheles* consists of the Cyclolepteron Series in a sister-group relationship with the Arribalzagia plus Myzorhynchus Series. COLLUCCI & SALLUM (2007) mentioned that *An. annulipalpis* and *An. grabhamii* of the Cyclolepteron Series did not group together, suggesting that the Series is not monophyletic. They considered the Myzorhynchus Series to be a paraphyletic assemblage, because it excluded species of the Arribalzagia and Christya Series, and *An. annulipalpis* of the Cyclolepteron Series.

HARBACH & KICHTING (2015) considered that the Angusticorn and Laticorn Sections, the *Anopheles* and Cyclolepteron Series of the former and the Myzorhynchus Series of the latter are not monophyletic. The two species of the Cyclolepteron Series are unrelated, with *An. annulipalpis* sister to a clade comprised of the Coustani + Hyrcanus Groups.

Moreover, it is noteworthy that *An. annulipalpis* was not included in the phylogenetic analyses of the Anophelinae conducted by SALLUM *et al.* (2002) and by HARBACH & KICHTING (2005). In COLLUCCI & SALLUM (2007) *An. annulipalpis* is included in the data set, although there are differences in the morphological characteristics of the data set and the ones observed here. Consequently, its position within *Anopheles* is uncertain. Based on the above and the presence of a laticorn trumpet, *An. annulipalpis* is removed from the Cyclolepteron

series, however based on current cladistic analysis, *An. annulipalpis* and *An. grabhamii* are clearly unrelated and the Cyclolepteron Series only includes *An. grabhamii*. According to HARBACH & KICHTING (2015) it is not possible to assign *An. annulipalpis* to an existing group, and they propose that the species must be retained in the Angusticorn Section as an unplaced species. Evidently, a complete re-assessment of the anopheline phylogeny is necessary.

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