

Anopheles (An.) *pilinotum*, A New Species Name in the *aitkenii*
Complex for An. *insulaeflorum* from the Philippines
and Eastern Indonesia (Diptera: Culicidae)¹

by

Bruce A. Harrison² and John E. Scanlon³

ABSTRACT

Anopheles insulaeflorum (Swellengrebel and Swellengrebel de Graaf) as previously recognized, involves 2 species which can be differentiated by larval, pupal and male genitalia characters. Accordingly, the distribution of *insulaeflorum* is redefined and the second species, *pilinotum* n. sp., is described, illustrated and compared morphologically with *insulaeflorum* and other members of the *aitkenii* species complex.

INTRODUCTION

We recently noted that the male genitalia of *Anopheles* (*Anopheles*) *insulaeflorum* Swellengrebel and Swellengrebel de Graaf) from Thailand do not agree with existing illustrations of this species. All previous illustrations of the male genitalia for this species are from specimens originating in eastern Indonesia or the Philippines. Only one male genitalia description (without illustration) made by Puri (1930) from Indian specimens agrees with the Thailand *insulaeflorum* specimens. Reid (1965, 1968) commented on the discrepancy between Puri's description and the other descriptions, but, since Reid only had males from the Philippines he could not pursue it further; however, he did note 2 differences between Indian-Malayan and Philippine larvae. Using these facts we initiated a further study based on the literature, the specimens in the U. S. National Museum (Natural History) (USNM) and the British Museum (Natural History) (BMNH) labeled *insulaeflorum*, and the identity of topotypic *insulaeflorum*. We have now determined that *insulaeflorum*, as previously recognized, involves 2 species which can be differentiated by larval, pupal and male genitalia characters. There are no synonyms known for *insulaeflorum*, thus, the second species is described here as new, and the distribution of *insulaeflorum* is redefined. The name for the new species was selected because of the numerous small scutal setae on the caudal portion of the prescutellar space. This space is bare on most other species in the *aitkenii* species complex.

¹This work was supported, in part, by Research Contract No. DA-49-193-MD-2672 from the U. S. Army Medical Research and Development Command, Office of the Surgeon General, Washington, D. C. This paper is contribution number 1250 from the Army Research Program on Malaria.

²Department of Entomology, Walter Reed Army Institute of Research, Washington, D. C. 20012

³School of Public Health, University of Texas Health Science Center at Houston, P. O. Box 20186, Houston, Texas 77025.

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 1974		2. REPORT TYPE		3. DATES COVERED 00-00-1974 to 00-00-1974	
4. TITLE AND SUBTITLE Anopheles (An.) pilinotum, A New Species Name in the aitkenii Complex for An. insulaeflorum from the Philippines and Eastern Indonesia (Diptera: Culicidae)				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Walter Reed Army Institute of Research, Department of Entomology, Washington, DC, 20012				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT see report					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 9	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

Anopheles (Anopheles) pilinotum New Species
(Figures 1 and 2)

Stethomyia aitkenii var. *insulaeflorum* of Swellengrebel and Swellengrebel de Graaf 1920, Bull. Ent. Res. 11: 81 (distribution in part); Swellengrebel and Swellengrebel de Graaf 1920, Geneesk. Tijdschr. Ned.-Indie 60: 34 (distribution in part); Swellengrebel and Swellengrebel de Graaf 1920, Tijdschr. Ent. 63: 98 (distribution in part); Swellengrebel 1921, Tijdschr. Ent. 64: 40 (♂*).

Anopheles (Anopheles) aitkenii var. *insulaeflorum* of Christophers 1924, Indian Med. Res. Mem. 3: 19 (distribution in part); Strickland and Choudhury 1927, Key Anopheline Larvae, p. 26 (distribution in part); Swellengrebel and Rodenwaldt 1932, Anophelen Niederl.-Ost., p. 111 (♂*, L*, distribution in part).

Anopheles (Anopheles) insulaeflorum of Holt and Russell 1932, Philipp. J. Sci. 49: 329 (distribution); Edwards 1932, Gen. Insect. 194: 38 (distribution in part); Christophers 1933, Fauna Brit. India, Diptera 4: 111 (♂, L, distribution in part); Russell and Baisas 1934, Philipp. J. Sci. 55: 299 (larval bionomics); Russell and Baisas 1934, Philipp. J. Sci. 55: 322 (L*); Russell and Baisas 1936, Philipp. J. Sci. 59: 29 (♂*, ♀*); Baisas 1938, Mon. Bull. Bur. Hlth. 18: 220 (P*); Simmons and Aitken 1942, Army Med. Bull. 59: 128 (distribution in part); Russell, Rozeboom and Stone 1943, Anopheline Mosq. World, p. 115 (distribution in part); Lee and Woodhill 1944, Monogr. Dept. Zool. Univ. Sidney 2: 97 (♂*, ♀*, distribution in part); Knight, Bohart and Bohart 1944, Nat. Res. Council Div. Med. Sci., p. 7 (distribution in part); Bohart 1945, U. S. NavMed. 580: 15 (♂*, L, distribution in part); Puri in Boyd [Ed.] 1949, Malariaology 1: 485 (distribution in part); Bonne-Wepster and Swellengrebel 1953, Anopheline Mosq. Indo-Austr. Reg., p. 97 (♂*, L*, distribution in part); Stone, Knight and Starcke 1959, Thomas Say Found., Ent. Soc. Amer. 6: 20 (distribution in part); Reid 1965, Ann. Trop. Med. Parasit. 59: 119 (♂*, P*, distribution in part); Baisas and Dowell 1965, PACAF Tech. Rep. 13-65: 1 (L*); Reid 1968, Stud. Inst. Med. Res. Malaysia 3: 248 (♂*, P*, distribution in part); Cagampang-Ramos and Darsie 1970, PACAF Tech. Rep. 70-1; 3 (L*); Basio 1971, Monogr. Natn. Mus. Philipp. 4: 36 (distribution in part).

FEMALE (Fig. 2). Unicolorous, light brown. *Head*. Vertex with very narrow erect scales slightly expanded and notched at tip; interocular space narrow, with short white scales and long tan frontal setae; clypeus without scales; antennal pedicel and flagellomeres without scales; antennal whorl setae 3-5 times as long as segments; ratio fore femur/proboscis (sample 10 females), range 0.98 - 1.16, mean 1.04; palpus very slender, slightly shorter than proboscis, 2 apical segments slightly swollen, scales decumbent. *Thorax*. Anterior promontory without scales; scutal integument tan, often with paler orange longitudinal line on each side of slightly darker median (acrostichal) longitudinal line; scutum without scales, with mixed long and short brown setae in anterior promontory, acrostichal, dorsocentral, lateral prescutal, fossal,

antealar and supraalar groups; prescutellar space with small fine setae extending back to just cephalad of scutellum; scutellum with long and short dark brown setae, without scales; anterior pronotum without scales, with long sparsely scattered setae; pleural area pale tan; pleural setae number 1-4 propleural, 1-7 spiracular, 1-2 prealar, 1-4 upper and 3-6 lower sternopleural, 2-7 upper and 0 lower mesepimeral. *Wing*. Unicolorous with narrow dark scales; humeral cross vein without scales; cell M_2 one half to equal length of stem (from R-M cross vein). *Halter*. With dark scales on knob. *Legs*. Coxae without scales, upper mid coxa with 2-3 setae; femora, tibiae and tarsomeres long, slender and entirely dark scaled. *Abdomen*. Mottled gray-brown with long brown setae, without scales.

MALE (Fig. 1). Like female except antenna more strongly plumose; palpus with 2 apical segments distinctly flattened and club-like. *Genitalia*. Basimere without scales, with 2 parabasal spines on tubercle; ventro-mesal spine inserted near distimere on distal 1/4 of basimere; claspette with 2 lobes; dorsal lobe with 2-4 (usually 3) separate, flattened outer setae; inner club-like structure on dorsal lobe formed from 2 separate flattened setae, longer than outer dorsal setae and often appearing as hood-like structure over them; ventral lobe of claspette with 3-5 setae shorter than those on dorsal lobe; distal end of aedeagus with numerous spines, usually sharp, often split.

PUPA (Fig. 1). Light tan to light brown. *Cephalothorax*. Seta 7 with 2-4 branches; seta 10 long, stout and usually simple. *Trumpet*. Simple, with deep meatal cleft, meatus less than 1/3 as long as trumpet. *Abdomen*. Seta 0 on II-VIII with 2-6 (usually 3-5) branches; seta 1 on III-VIII well developed; 1-III with numerous branches, stem as stout as that of 3-III; 1-IV with 5-13 branches, as well developed as 5-IV; 1-VI with 5-6 branches; 1-VII with 3-5 branches; seta 5 on V-VII strongly developed, 1/2 to 2/3 as long as segment; 5-I well developed with 2-3 branches, much longer than 2 and 3-I; 5-IV with 6-11 branches; 5-VII with 9-12 branches; 6-II with 3-8 branches, 1/2 to 2/3 as long as segment II; 6-III with 3-6 branches, 1/2 to 2/3 as long as segment III; 7-I with 3-5 branches, 1/2 to 2/3 as long as 6-I; 7 on VI-VII, 2/3 or less length of following segments; setae 9 moderately long, without long tapering tip, usually pale; 9-IV, 4-7 times as long as 9-III; 9-VII, 1/5 to 1/4 as long as segment VIII; 10 on IV-V approximately 1/2 as long as following segments. *Paddle*. Refractile margin short, 2/5 to 1/2 as long as paddle; fringe hairs approximately 1/2 length of 1-P; outer margin straight or nearly so on distal half; 1-P long, simple and tip may be curved; 2-P with 3-6 branches.

LARVA (Fig. 2). Unicolorous gray or brown. *Head*. Antenna base slightly wider than tip; antenna with long spicules, particularly on mesal and dorsal aspects; 1-A with 4-10 branches, inserted on basal 1/6 to 1/5 of antenna; 2 and 3-A (sabre pieces) with one edge serrate; 4-A with 5-7 branches; setae 2-C with bases very close together, much closer than distance between bases of 2-C and 3-C on one side; 2-C long, stout and simple; 3-C simple or bifid, rarely trifid, 1/4 to 1/3 as long as 2-C; setae 4-C slightly wider apart than setae 3-C, with 2-6 branches arising from near base; 5, 6 and 7-C long, well developed and plumose; 8-C with 2-6 branches; 9-C with 4-9 branches; 11-C approximately equal to length of antenna, with many branches particularly at

apex. *Thorax*. Seta 1-P with 7-13 branches, approximately 1/2 length of 2-P, without sclerotized base; 2-P with 8-14 branches, arising from sclerotized tubercle; 2-P tubercle with prominent apical spur or tooth; 3-P simple or bifid, about same length as 1-P, much closer to 2-P than 1-P is to 2-P; 4-P with stout stem and numerous short lateral and apical branches, slightly longer than 2-P; 11-P short with 2-5 distal branches, rarely single on one side; prothoracic pleural tubercle with slender spine; long pleural thoracic setae (9 and 10) simple on all 3 thoracic segments; 13-P with 3-7 branches; 14-P with 3-6 branches; 4-M small with 2-4 branches from base, inserted caudally to long simple 3 and 5-M; 12-M often bifid or trifid; 3-T with slightly pigmented flattened leaflets without filaments; 12-T bifid or trifid. *Abdomen*. Anterior tergal plates on I-VII, 1/4 to 1/3 width of segment; posterior tergal plates usually present on segments III-VII, that on VII nearly twice as large as others; seta 0 on II-VIII with 3-6 branches; 1-I with lightly pigmented flattened leaflets without filaments; seta 1 on II-VII with pigmented flattened leaflets with filaments; 5-II with 4-II branches; 6-III with 3-11 branches; 6 on IV-V with 3-4 branches from near base, approximately 1/2 as long as 6-III; 6-VI less than 1/3 as long as 6 on IV-V, with 3-10 branches; 11-II long and simple; 13 on I-VII with 5 or more branches; stigmal knob on anterior plate of spiracular apparatus unpigmented, nearly transparent; pecten plate with 11-15 teeth; 1-X with 2-4 distal branches, as long as saddle.

EGG. Unknown.

TYPE-DATA. The holotype male is deposited in the USNM, along with a slide bearing its associated immature skins and a slide with its genitalia. The holotype is in excellent condition except the right hind leg is missing, and has the following label data: (1st label)- "Lot 323.3, W. V. King"; (2nd label)- "Baong, N. Ecija, Luzon, P. I., III-13-'32"; (3rd label)- "SEAMP Acc. No. Prep 72/24♂"; and (4th label)- "*Anopheles* (An.) *pilinotum* Harrison and Scanlon, Holotype♂." The allotype female, with associated immature skins on a slide, is also deposited in the USNM. The allotype is in excellent condition except the left hind leg is missing, and has the following label data: (1st label)- "Lot 322.12, W. V. King"; (2nd label)- as for the holotype; and (3rd label)- "*Anopheles* (An.) *pilinotum* Harrison and Scanlon, Allotype♀." Two male and 2 female paratypes with slides of associated immature skins, from the above locality and with the numbers, lot 322.3 (♂), 323.12 (♂), 322.4 (♀) and 323.22 (♀), have been sent to the BMNH. An additional 5 male and 5 female paratypes with associated immature skins and numbered, 322.1 (♂), 322.2 (♀), 322.5 (♂), 322.6 (♀), 322.10 (♀), 322.11 (♂), 322.14 (♀), 323.7 (♂), 323.16 (♀) and 323.17 (♂) are deposited in the USNM. Five slides with whole larvae, in the USNM, have also been designated as paratypes, these are labeled lot 322 (3 slides), 323a and 323b. All specimens and slides belonging to the type-series have been clearly labeled because there are other specimens from lots 322 and 323 that have not been designated as types.

DISTRIBUTION. *Anopheles pilinotum* apparently has a distribution similar to that of *Anopheles pseudobarbistrostris* Ludlow, *An. vagus limosus* King and *An. vanus* Walker. These species all range from Luzon Island in the northern Philippines down through the eastern islands of Indonesia. Specimens of *pilinotum* (38♂, 39♀, 28 larvae and 143 larval and pupal skins) in the USNM and the BMNH were examined from: INDONESIA (Ambon and Ceram) and the PHILIPPINES (Luzon, Mindanao, Negros and Palawan). There are a number of records

of *insulaeflorum* from other eastern Indonesian islands (e.g., Misool off the western tip of New Guinea, and the Moluccas) that probably apply to *pilinotum*, but, these must be confirmed. Only further surveys in Indonesia will determine where or if the distributions of *insulaeflorum* and *pilinotum* overlap. Neither species is recorded from Borneo, and since Java is currently the eastern-most confirmed record for *insulaeflorum*, the identity of the species occurring on the islands between Java and Ambon-Ceram is uncertain.

BIOLOGY. This is a true forest species, with the immature stages found in clear, shallow and cool water seeps or streams under partial to heavy shade, in forested foot hill and mountainous areas. Holt and Russell (1932) record this species (as *insulaeflorum*) from mostly shaded streams and rivers. Records for the Philippine specimens in the USNM indicate immatures were collected in mostly shaded seepage areas on banks of rivers and streams, stream margins, large stream pools, rock pools and from undercut banks with masses of roots in the water. Collection sites were usually water eddy areas where there was only slight water movement and accumulations of debris such as dead leaves, sticks, roots and grass. Occasional collections were made from sites with green algae or from around stones. Only one elevation record (lot 322) of 500 feet was available for the Philippine specimens. Collection and isolation records for the USNM specimens indicate *pilinotum* has been collected in association with larvae of *Anopheles bengalensis* Puri, *An. filipinae* Manalang, *An. kolambuganensis* Baisas, *An. lindesayi benguetensis* King, *An. ludlowae* (Theobald), *An. maculatus* Theobald, *An. mangyanus* (Banks), *An. minimus flavirostris* (Ludlow), *An. riparis* King and Baisas, *An. vanus* Walker, an undetermined species of the *barbirostris* complex and *Uranotaenia* species.

Prior to this publication adult females of *pilinotum* (as *insulaeflorum*) in the Philippines were inseparable from other *aitkenii* complex females. Thus, there are no published records of females of this species having been collected by methods other than reared immatures. For this reason the suggestion (Basio 1971) that *pilinotum* (as *insulaeflorum*) is a serious pest in the jungle is groundless.

DISCUSSION

Anopheles insulaeflorum was described from larvae collected on Noesa Kembangan (now Nusa Kambangan), an island just off the south coast of Java; however, the location of the type-specimen is unknown. There are 2 whole larvae in the USNM from Pangandaran, Java, only 15 kilometers from the type-locality, and they have a character combination like that found on Indian, Malaysian and Thailand larvae (seta 11-P simple, seta 0 with 2-3 branches on segments II-VI and seta 1-X simple). Since we are considering these 2 larvae as "topotypic" representatives of *insulaeflorum*, we here assign this name to the mainland, Sumatra and Java specimens, while those from the Philippines and the eastern Indonesian islands of Ambon and Ceram are described here as *pilinotum*. *Anopheles insulaeflorum* will be described more fully and illustrated in a forthcoming monograph on the Subgenus *Anopheles* in Thailand.

The adults of *insulaeflorum* and *pilinotum* are currently inseparable except by the male genitalia, but these 2 species can be differentiated from the other members of the *aitkenii* complex by having short scutal setae on the caudal portion of the prescutellar space. The distinctive male genitalia of

pilinotum are the primary reason for distinguishing this species from *insulaeflorum*. The ventral lobe of the claspette on *pilinotum* has 3-5 small setae that are much shorter than those on the dorsal lobe, while the ventral lobe on *insulaeflorum* has 3-4 long stout setae, the most mesal being longer than the setae on the dorsal lobe. The aedeagus of *pilinotum* has obvious spines around the distal end, with a sharp tip that often appears split. The aedeagus of *insulaeflorum* has spines, but they are normally much smaller and lateral only, and the tip is rounded. The only other species in this complex that has spines on the aedeagus is *An. tigertti* Scanlon and Peyton, an uncommonly collected crab-hole species from Thailand that has very distinct larval and pupal characters.

The pupa of *pilinotum* is very similar to that of *insulaeflorum*, but can be differentiated from it by having: seta 0 on II-VIII with 2-6 (usually 3-5) branches; 6-II with 3-8 branches and approximately $1/2$ to $2/3$ as long as segment II; 6-III with 3-6 branches and approximately $1/2$ to $2/3$ as long as segment III; seta 10 on IV-V approximately $1/2$ as long as the following segments; paddle refractile margin short, only $2/5$ to $1/2$ as long as paddle; and outer margin of paddle straight or nearly straight on distal half. The pupa of *insulaeflorum* has: seta 0 on II-VIII simple (infrequently bifid); 6-II simple or bifid and as long as segment II; 6-III simple and slightly less than length of segment III; seta 10 on IV-V, $2/3$ or more as long as the following segments; paddle with long refractile margin, $1/2$ to $2/3$ as long as paddle; and outer margin of paddle evenly convex on distal half.

The larvae of *pilinotum* and *insulaeflorum* are very similar, but can be separated by the former having seta 1-X with 2-4 branches, seta 11-P with 2-5 distal branches and seta 0 on II-VI with 3-6 branches, while the latter has 1-X simple, 11-P simple (rarely bifid on one side) and 0 on II-VI simple or with 2 (rarely 3) branches.

We currently recognize only 3 other members of the *aitkenii* complex in the Philippines, i.e., *An. acaci* Baisas, *An. bengalensis* Puri and *An. fragilis* (Theobald). Adults of *pilinotum* are easily differentiated from these species by having short scutal setae on the caudal portion of the prescutellar space. The male of *pilinotum* is the only known member of this complex in the Philippines that has spines on the aedeagus, thus, it is easily distinguished from the other 3 species.

The pupa of *pilinotum* can be separated from those of *bengalensis* and *fragilis* by the last 2 species having very long lateral spines (seta 9), seta 0 on II-VIII small and simple and the refractile margin on the paddle over half as long as the paddle. The pupae of *acaci* and *pilinotum* are more difficult to separate. The pupa of *acaci* has seta 5-I short and approximately as long as 2-I, seta 0 on II-VIII simple or bifid and seta 1 on IV-VII small and weakly developed. The pupa of *pilinotum* has 5-I much longer than 2-I, 0 on II-VIII with 3-6 branches and abdominal seta 1 on IV-VII well developed.

The larva of *pilinotum* is easily separated from those of *acaci*, *bengalensis* and *fragilis* by the different development of seta 2-C. Setae 2-C on larvae of *pilinotum* are simple and very close together, much closer together than the distance between setae 2 and 3-C on one side. Larvae of the other 3

species either have seta 2-C branched or frayed, and setae 2-C are approximately as far apart as the distance between setae 2 and 3-C on one side. These 3 species also have 18 or more branches on seta 6-III, while *pilinetum* has 3-11 branches on that seta.

Two other members of this complex, *An. palmatus* (Rodenwaldt) and *An. stricklandi* Reid, have larvae with a head setal arrangement and development nearly identical to that of *pilinetum*, but, these 2 species are not currently known from the Philippines and only *palmatus* is known from the eastern Indonesian islands. Larvae of *palmatus* are unique in this complex because they have extremely large abdominal anterior tergal plates that are 2/3 to 3/4 as wide as the segments. Larvae of *stricklandi* can be separated from those of *pilinetum* by the same characters that differentiate *insulaeflorum* from *pilinetum*.

ACKNOWLEDGMENTS

We are very grateful to Mr. E. L. Petyon, Acting Principal Investigator, Southeast Asia Mosquito Project (SEAMP), and LTC. Bruce F. Eldridge, Chief, MAJ John F. Reinert and Dr. Ronald A. Ward, Department of Entomology, Walter Reed Army Institute of Research (WRAIR), for critically reviewing the manuscript. We are also grateful to Miss Gloria Gordon, SEAMP, for preparing the illustrations.

REFERENCES

- Basio, R. G. 1971. The mosquito fauna of the Philippines (Diptera Culicidae). Monogr. Natn. Mus. Philipp. 4:1-198.
- Holt, R. L. and P. F. Russell. 1932. Malaria and Anopheles reconnaissance in the Philippines. Philipp. J. Sci. 49: 305-371.
- Puri, I. M. 1930. A note on two species of Indian anopheline mosquitoes-*A. insulaeflorum* Swellengrebel and *A. aitkenii* James, with its variety *bengalensis* nov. var. Indian J. Med. Res. 18: 953-956.
- Reid, J. A. 1965. A revision of the *Anopheles aitkenii* group in Malaya and Borneo. Ann. Trop. Med. Parasit. 59: 106-125.
- Reid, J. A. 1968. Anopheline mosquitoes of Malaya and Borneo. Stud. Inst. Med. Res. Malaysia 31: 1-520.

Fig. 1

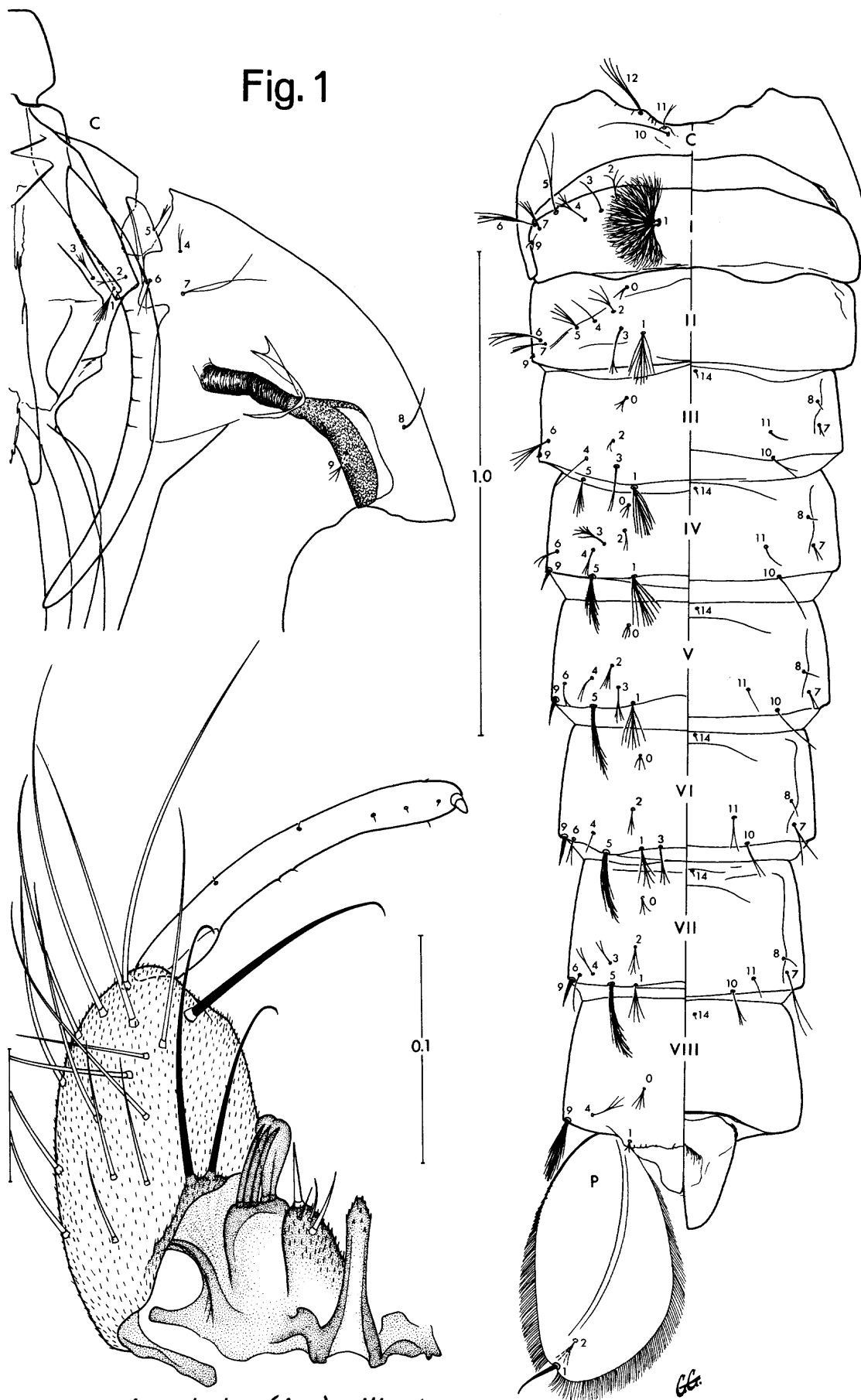
*Anopheles (An.) pilinotum*

Fig.2

