

Culex (Culex) pipiens pipiens Linnaeus and *Culex (Culex) pipiens quinquefasciatus* Say in South Africa: Morphological and Reproductive Evidence in Favour of Their Status as Two Species

by

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ABSTRACT. The study showed that both larvae and adult males of *C. pipiens pipiens* and *C. pipiens quinquefasciatus* from Johannesburg, Transvaal, can be distinguished morphologically. Males were separable either by the structure of their genitalia or according to the proportion of the shaft of the maxillary palp which bears hairs. Ninety six percent of adult females were distinguishable by a quantitative character in the wing venation. Laboratory colonization showed that *quinquefasciatus* was stenogamous and *pipiens* eurygamous which suggests that reproductive isolation is due to an ethological premating mechanism. In 9 crossing experiments there was no cross-insemination between the 2 taxa except in one experiment where 2 *quinquefasciatus* females were inseminated by male *pipiens*. The $F_1 + F_2$ hybrids subsequently reared were vigorous and fertile indicating the absence of postmating isolating mechanisms. After study of these laboratory hybrids a search was made for natural hybrids among mixed field collections from Johannesburg and from Bethulie, Orange Free State. Not one such hybrid was found. From this evidence that *pipiens* and *quinquefasciatus* are not interbreeding and because of their considerable morphological difference it is concluded that the 2 taxa in South Africa should be classified as separate species.

INTRODUCTION

No definite agreement has yet been reached concerning the taxonomic status of the various members of the *Culex pipiens* complex. In the latest reviews written on the systematics of the complex (Mattingly, 1967; Laven, 1967) it was concluded that the complex should be treated as a single polytypic species embracing six named taxa; these were *C. pipiens pipiens* Linnaeus, *C. pipiens quinquefasciatus* Say*, *C. pipiens australicus* Dobrotworksy and Drummond, *C. pipiens* var. *molestus* Forskal, *C. pipiens* var. *pallens* Coquillet, *C. pipiens* var. *comitatus* Dyar and Knab. *C. australicus* was raised to specific rank by Belkin (1962) and Knight and Stone (1977) upheld this view in their recently revised "Catalogue of mosquitoes of the world". However, some of these supposedly infraspecific

*The name *quinquefasciatus* Say 1823 is regarded here as a senior synonym to *fatigans* Wiedemann, 1828 (see White (1975), Belkin (1977), Knight and Stone (1977), Sirivanakarn and White (1978)).

taxa have certain natural populations displaying different morphological, ecological, behavioural and physiological characters, which raises the possibility that all or some might exist as distinct species (Rozeboom, 1951; Paterson and James, 1973). The difficulty is to show that 2 sympatric forms are not interbreeding in nature - the critical evidence for distinguishing 2 species. In a recent study of sympatric populations of members of the complex in Australia, Miles (1976) tackled this problem by using isoenzymes as genetic markers and showed that not only *C. globocoxitus* but *C. p. australicus* and *C. p. quinquefasciatus* were coexisting at Perth without interbreeding.

In South Africa there are 2 members of the complex, *C. p. pipiens* and *C. p. quinquefasciatus*, which differ in morphology and other ways suggesting that these taxa are also specifically distinct. *C. quinquefasciatus* is an important nuisance mosquito which occurs throughout the country, particularly in urban environments, although it has occasionally been collected in rural areas in the vicinity of farmsteads in the Orange Free State province (Jupp *et al* - in preparation). *C. pipiens*, on the other hand, occurs more frequently in rural than in urban and peri-urban habitats, although both taxa often occur sympatrically. Both are ornithophilic, particularly *pipiens*, but while *quinquefasciatus* also enters houses and bites man readily, *pipiens* is poorly anthropophilic (Jupp 1973). The larvae of *quinquefasciatus* are more commonly encountered in artificial containers whereas larvae of *pipiens*, although also occurring in this habitat, prefer natural ground pools, vleis, river margins and irrigation furrows (Jupp, 1967, and unpublished observations). Laboratory colonies of the 2 taxa also have certain differences.

This paper describes morphological and reproductive studies undertaken on *pipiens* and *quinquefasciatus* in South Africa in an attempt to establish whether they are specifically distinct. The extent of morphological difference observed between them constitutes evidence for the existence of 2 taxa and the question of whether these were biological species was approached in 2 ways. Firstly evidence for reproductive isolation was sought through a study of laboratory mating behaviour and by carrying out cross-mating experiments. Secondly, after making a study of laboratory hybrids and gaining a picture of hybrid morphology it was possible to search for naturally occurring hybrids.

MATERIALS AND METHODS

Mosquitoes studied morphologically

Field specimens originated from in or near Johannesburg, Transvaal province, except where indicated otherwise. All the *pipiens* and some of the *quinquefasciatus* examined were the progeny reared from wild-caught gravid females. Part of the egg raft deposited by each such wild-caught female was reared out and a larva, pupa, adult male and adult female studied in each case. The remaining *quinquefasciatus* specimens collected were larvae which were identified and reared in isolation to obtain a series of associated immature and imago specimens. Four F_1 larvae arising from a cross-mating experiment were preserved from each of 3 egg rafts. The remaining larvae were then pooled for rearing F_1 adults, a sample of which was killed for examination. Eighteen F_2 rafts

were obtained, each of which was reared in isolation, so that a larva, adult male and adult female could be kept for examination. Genitalia were also examined from males reared from larvae of the 2 taxa collected together in the same water at Johannesburg and at Bethulie in the southern part of the Orange Free State province.

Morphological characters

Two larval and 3 adult characters were studied as shown below. Terminology used is that recommended by Knight and Laffoon (1970, 1971 a,b).

1. Larva; siphonal Index. The siphonal index was calculated, that is the length of the siphon (excluding the valves and acus) divided by its greatest width at the basal ring.
2. Larva; siphonal setae. The number of branches borne by the siphonal setae 1a-S and 1b-S was counted. These subventral tufts of setae are the first 2 proximal pairs on the siphon and both in each pair were examined.
3. Adult; cross vein index in wing. The length of each cross vein (c & d) and their distance apart (a) were measured in the wings of male and female mosquitoes, as shown in fig 1, so that a "cross vein index" could be calculated. This was the ratio of the combined lengths of the cross veins to their distance apart.
4. Maxillary palp hair index in adult male. In the male maxillary palp the length of the shaft (segments 2 + 3) was measured and the proportion of its length bearing hairs (figure 2). A "hair index" was calculated which was the ratio of total shaft length to length with hairs.
5. DV/D index in male genitalia. The DV/D index (Sundararaman, 1949) was calculated from measurements made on the lateral plate of the aedeagus (figure 3).

Siphonal setae on the larva were studied by floating each specimen in a small dish containing a 70% ethanol solution with glycerol added to 5% of volume, while to measure the siphon the larva was transferred, in the same solution, to a hollow ground microscope slide. Examinations were made under a stereomicroscope at a magnification of 50 x (siphonal index) or 100 x (setae). Measurement of the maxillary palp was made after removing the mosquito's antennae. Each such male mosquito was examined on a hollow ground slide in ethanol-glycerol under a stereomicroscope at a magnification of 50 x. For measuring the cross veins, adult wings were mounted permanently on slides in polyvinyl lactophenol; and permanent euparal mounts were made of male abdomens with aedeagus still intact for measurements on the aedeagus. Slides of both wings and genitalia were examined under a compound microscope at a magnification of 80 x (wings) or 500 x (genitalia).

Mating behaviour in the laboratory.

Various attempts to establish laboratory colonies of *quinquefasciatus* and *picipiens* were carried out, starting with adults reared from gravid females collected in or near Johannesburg. This work produced information on the laboratory mating behaviour of each taxon.

Crossing experiments.

As a laboratory colony of *pipiens* was unavailable at the time the crossing experiments were performed, individuals of *pipiens* used were the progeny reared from gravid females collected near Johannesburg. Pupae harvested from among such *pipiens* progeny and from *quinquefasciatus* colonies were reared individually and on emergence the adult mosquitoes were separated according to sex and placed in cages. Exceptions to this were some of the male *quinquefasciatus*, which were taken directly from the colonies.

Nine experiments were carried out in which the 2 taxa were mixed together in 35 cm³ cages. This was done because any cross-matings would reveal whether postmating isolating mechanisms existed between the 2 taxa. In 6 experiments *pipiens* females were mixed with *quinquefasciatus* males and in 3 experiments the reciprocal cross was attempted. From 50 to 100 mosquitoes of each sex, usually 1-20 days old, were used. Temperature was 25 - 26^o C and relative humidity 75 - 80%. The daily light cycle, provided by controlled artificial lighting, consisted of a day period of 12 hours, a night period of 10 hours and twilight and dawn periods of 70 minutes each when the light intensity gradually decreased or increased respectively. An interval of 1 week or more elapsed before mosquitoes were fed on pigeons or young chickens. Five days or more after feeding, gravid mosquitoes were confined individually in "laying tubes" (Jupp 1967) for oviposition. Rafts deposited were regarded as infertile if they had not hatched after 3 days.

RESULTS

1. MORPHOLOGICAL DISTINCTION BETWEEN *C. PIPIENS* AND *C. QUINQUEFASCIATUS* FROM JOHANNESBURG.

Examination of eggs and pupae under the light microscope revealed no differences between the 2 taxa. Morphological differences found in larvae and adults are shown in table 1.

Siphonal index of larva. The larvae of *pipiens* usually had a longer, more slender siphon than those of *quinquefasciatus*. Out of a total sample of 100 (50 *pipiens* and 50 *quinquefasciatus*) specimens only 2 had siphonal indices in the area of overlapping distribution of values.

Siphonal setae of larva. Setae 1a-S and 1b-S both had 1 - 3 branches in the specimens of *pipiens*, while seta 1a-S had 4 - 11 and seta 1b-S 4 - 10 branches in the specimens of *quinquefasciatus*.

Cross vein index in wing. Five different ratios were calculated from preliminary measurements made on the wings of adult females as follows: -

$$\frac{x, a, x, a,}{y b a c} \quad \frac{a + b}{c}$$

The meaning for all these symbols are shown in fig. 1. The values for all these ratios determined on samples of *pipiens* and *quinquefasciatus* overlapped

considerably. However, when values for the cross vein index $(c + d)$ were determined there was overlap in only 5 out of 98 (4%) of female *a* specimens as shown in table 1, although in males the proportion was bigger with 9 out of 91 (10%) overlapping.

Male maxillary palp hair index. It was found that in the case of *pipiens* hairs extend about halfway down the shaft of the palp from its distal end (index of 2.3 - 3.1) while in *quinquefasciatus* they only occur at the tip of the shaft (index 3.2 - 8.7). Edwards (1941) described this difference although he did not express it quantitatively.

Male DV/D index. Fig 3 shows drawings of the inner division of the lateral aedeagal plates. In *pipiens* the ventral arm never overlapped the dorsal arm so that the DV/D index remained zero, while in *quinquefasciatus* there was always overlap so that the values of the index were 0.6 - 0.9. The tip of the dorsal arm also differed in the 2 forms of mosquito, being blunt-ended in *pipiens* but pointed in *quinquefasciatus*.

2. REPRODUCTIVE ISOLATION IN THE LABORATORY

Mating Behaviour.

C. quinquefasciatus mated readily in cages as small as 16 cm³ which showed that the species was stenogamous. Three colonies were thus established easily in the laboratory. No special lighting regime was necessary and 2 of these colonies provided *quinquefasciatus* for the crossing experiments.

Colonization of *pipiens* was much more difficult. Previously the author had attempted to colonize this species in 35 cm³ cages exposed to lighting which represented a 12 hour day - 12 hour night and subsequently in a larger cage 75 x 50 cm in cross-section and 150 cm high, exposed to illumination simulating a 12 hr day, 1 hr dusk of constant intensity and 11 hr night. However, the mosquitoes did not mate because of the eurygamous nature of this species until the method described for *C. univittatus* (Jupp and Brown, 1967) was used with a walk-in cage measuring 3.0 metres high, 2.9 m long and 1.8 m wide and with lighting incorporating a gradual increase in the morning and decrease in the evening. With this environment a high raft viability index of about 80% was attained by the 8th generation which indicated a similarly high frequency of matings.

At the 11th generation this colony was transferred to small 35 cm³ cages kept in identical conditions. The percentage viability indices for F₁₂ - F₂₂ rafts were 3, 2, 9, 22, 9, 8, 8, 30, 14, 33 and 49 but none of 228 F₂₃ rafts hatched so that the colony was then lost. The sudden cessation of mating by the F₂₂ adults was probably due to these adults being moved out of the main insectory room with its special illumination for a period while this room was being repainted.

Crossing experiments

Altogether 136 egg rafts were deposited in the 9 crossing experiments. These all failed to hatch except in one experiment where 100 *pipiens* males had

been mixed with 45 *quinquefasciatus* females. In this experiment 2 rafts hatched out of 13 and one of the females which had deposited one of these viable rafts was re-fed and a second raft was laid. Hence the F_1 hybrid generation originated from 3 viable rafts in each of which the proportion of eggs which hatched was normal and the numbers of each sex equal. Vigorous F_1 adults were reared, given a blood-meal and they subsequently deposited 30 F_2 rafts, 25 of which hatched. These in turn lead to vigorous F_2 adults. No attempt was made to rear a third generation although it is unlikely that any difficulty would have been encountered in doing this.

Morphology of hybrids

Table 1 also shows the results of a study of the morphology of the F_1 and F_2 hybrids and how they compare with the morphology of *pipiens* and *quinquefasciatus*. It can be seen that in both the F_1 and F_2 larvae the range of values for both the siphonal index and the number of branches on setae la-S and lb-S overlaps the ranges of both *pipiens* and *quinquefasciatus*. Similarly the range of values for the female wing cross vein index and the male maxillary palp hair index determined on adult F_1 and F_2 hybrids showed the same kind of overlap. The DV/D indices determined on the genitalia of 45 F_1 hybrids were all intermediate between the zero index of *pipiens* and the range of indices obtained for *quinquefasciatus*. This was also the case in 11 out of 12 of the F_2 hybrids, the twelfth mosquito having an index of 0.6 which just falls in the *quinquefasciatus* range. Drawings of the lateral aedeagal plates in the genitalia of *pipiens*, *quinquefasciatus* and hybrid males are shown in fig. 4 from which it is clear that those of *pipiens* and *quinquefasciatus* are readily distinguishable. Furthermore, most hybrid genitalia would probably be recognizable without measurement.

3. SEARCH FOR HYBRIDS AMONG FIELD SPECIMENS

It was thought that the newly gained knowledge of the morphology of laboratory hybrids described above should prove useful for recognition of hybrids if they existed in nature. Accordingly certain slides of male genitalia prepared from mosquitoes previously collected were re-examined. These mosquitoes had been reared from collections of larvae which had comprised both *pipiens* and *quinquefasciatus* co-existing in the same water.

Table 2 shows the DV/D indices determined on samples originating from Bethulie and Johannesburg respectively. The indices determined for the F_1 and F_2 laboratory hybrids are included for comparative purposes. As can be seen none of the resulting indices corresponded to one determined on a laboratory hybrid if the solitary F_2 hybrid with a ratio of 0.6 is discounted. Rather the indications are that the field samples consisted of distinct *pipiens* and *quinquefasciatus* only, as indicated in table 2. In addition to the specimens shown in table 2 for which the DV/D index was determined, a further 86 males from Bethulie and 112 males from Johannesburg were identified as *pipiens* or *quinquefasciatus* from their male genitalia without any measurement.

DISCUSSION

It is evident from the morphological study that it is possible to distinguish Johannesburg populations of *pipiens* and *quinquefasciatus* by examination of the larva and adult male. The number of branches in the first 2 pairs of siphonal setae is diagnostic for differentiating larvae while the value for the siphonal index is almost diagnostic. Adult males can either be distinguished by examination of the lateral aedeagal plate in their genitalia or according to the proportion of the shaft of the maxillary palp which bears hairs. The second character is easier to use than the genitalia as slide mounts are unnecessary. The adult females cannot be differentiated with complete certainty, although the majority of specimens (96%) can be identified by means of the cross vein index in the wing. There are other characters which are helpful in recognizing females of the 2 taxa. *C. quinquefasciatus* usually has a buff-coloured scutum and well marked white tergal bands as compared to *pipiens*, which tends to have a darker rust brown scutum and narrower tergal bands. The considerable morphological difference between *pipiens* and *quinquefasciatus* suggests that they are 2 separate species, as they were formerly regarded by Edwards (1941).

Previously the author attempted to cross the 2 taxa by the artificial mating technique; this was unsuccessful, which prevented the study of a large sample of cross-inseminated females. However, the insemination of 2 *quinquefasciatus* females by *pipiens* males in one of the present crosses, with deposition of viable egg rafts and subsequent production of vigorous F_1 and F_2 mosquitoes, shows that a postmating isolating mechanism does not exist.¹ It is likely that the absence of interbreeding between *pipiens* and *quinquefasciatus* in South Africa is due to an ethological premating isolating mechanism and that this explains why there were only 2 successful inseminations in the crossing experiment.

Laboratory colonization of the 2 taxa showed very clearly that *quinquefasciatus* was stenogamous and *pipiens* eurygamous although by selection over a number of generations the latter became stenogamous. This difference indicates that *quinquefasciatus* probably has a quite different requirement for swarm formation in nature than *pipiens* and that this is probably the basis for the ethological isolation. In Australia isolation between 2 members of the *C. pipiens* complex, *C. globocoxitus* and *C. australicus* seems to have a similar basis (Patterson and James, 1973).

The search for naturally occurring hybrids among male mosquitoes reared from mixed larval collections did not reveal a single hybrid. The samples of such males on which the DV/D index was measured were small, but the genitalia of many more potential hybrid males were examined from the field without detecting any intermediates. This is thought to indicate that hybridization either does not occur between *pipiens* and *quinquefasciatus* at Johannesburg and Bethulie and probably throughout South Africa, or that it occurs only very rarely.

The evidence that the 2 taxa are not interbreeding, together with their considerable morphological difference means that their present taxonomic status needs to be re-evaluated. There are now grounds for referring to the 2 taxa in South Africa as separate species.

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TABLE 1. Morphological comparison between *C. pipiens*, *C. quinquefasciatus* and laboratory-reared hybrids.

	Character	<i>C. pipiens</i>	<i>C. quinquefasciatus</i>	F ₁ hybrids	F ₂ hybrids
Larva	Siphonal index	4.8-6.1 (48) 4.3-4.4 (2)	3.5-4.7 (50)	4.1-4.9 (12)	3.9-5.0 (18)
	No. branches on seta 1a-S; 1b-S	1.3; 1-3 (50)	4-11; 4-10 (50)	3-6; 2-5 (12)	2-6; 2-5 (18)
Adult female	Wing; cross-vein index	1.4-5.0 (47) 1.2 (1)	0.7-1.3 (51) 1.4-1.7 (4)	1.0-1.9 (50)	0.9-1.5 (18)
Adult male	Maxillary palp; hair index. Genitalia; lateral aedeagal plate - DV/D index	2.3-3.1 (50) 0.0 (32)	3.2-8.7 (50) 0.6-0.9 (31)	2.4-4.6 (50) 0.2-0.4 (45)	2.6-4.9 (18) 0.2-0.6 (12)

Numbers in parenthesis are the numbers of specimens

TABLE 2. DV/D indices for adult males reared from collections of larvae which each contained both *C. pipiens* (C.P.) and *C. quinquefasciatus* (C.Q.) as compared to indices for laboratory hybrid males.

Locality	No. specimens	DV/D index	Identity
Bethulie (Orange Free State)	19	0.0	C.P.
	13	0.6-1.1	C.Q.
Johannesburg (Transvaal)	16	0.0	C.P.
	14	0.6-1.7	C.Q.
Laboratory specimens	45	0.2-0.4	F ₁ hybrids
	11	0.2-0.4	F ₁ "
	1	0.6	F ₂ "

- Fig. 1. Diagram of portion of wing to show various measurements made on the venation including those used for the cross-vein index $\frac{c + d}{a}$.
- Fig. 2. Maxillary palp of male mosquito to show the two measurements made for calculation of the palp hair index.
- Fig. 3. Diagram of the inner division of the lateral aedeagal plate to show ventral arm (VA), dorsal arm (DOA) and measurements made for the DV/D ratio (after Sundararaman 1949).
- Fig. 4. Camera lucida drawings of the lateral aedeagal plates in *C. pipiens*(a), *C. quinquefasciatus* (b), F₁ laboratory hybrid (c) and F₂ hybrid (d).

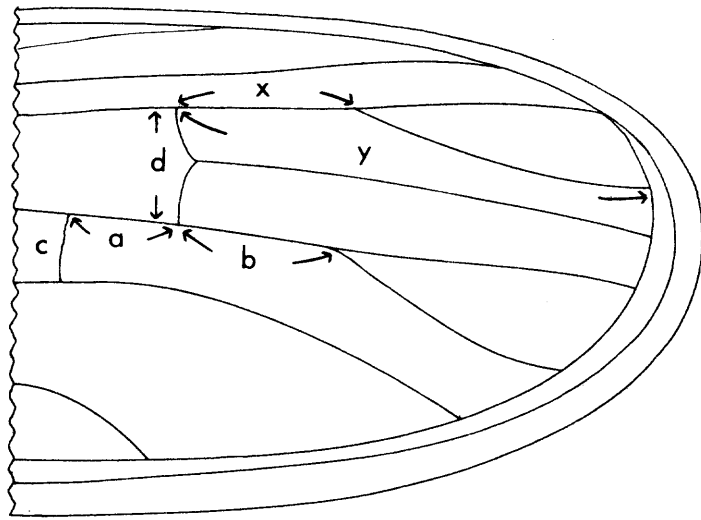


Fig. 1

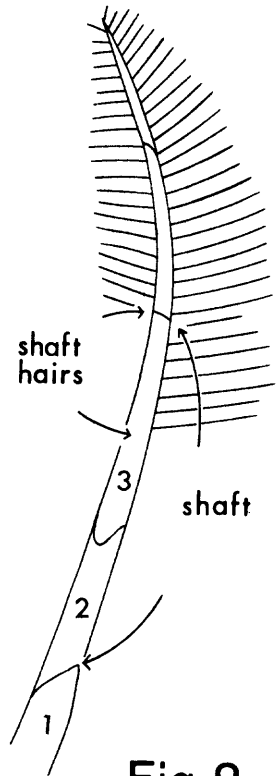


Fig. 2

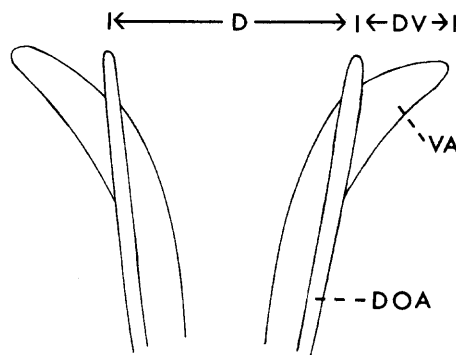


Fig. 3

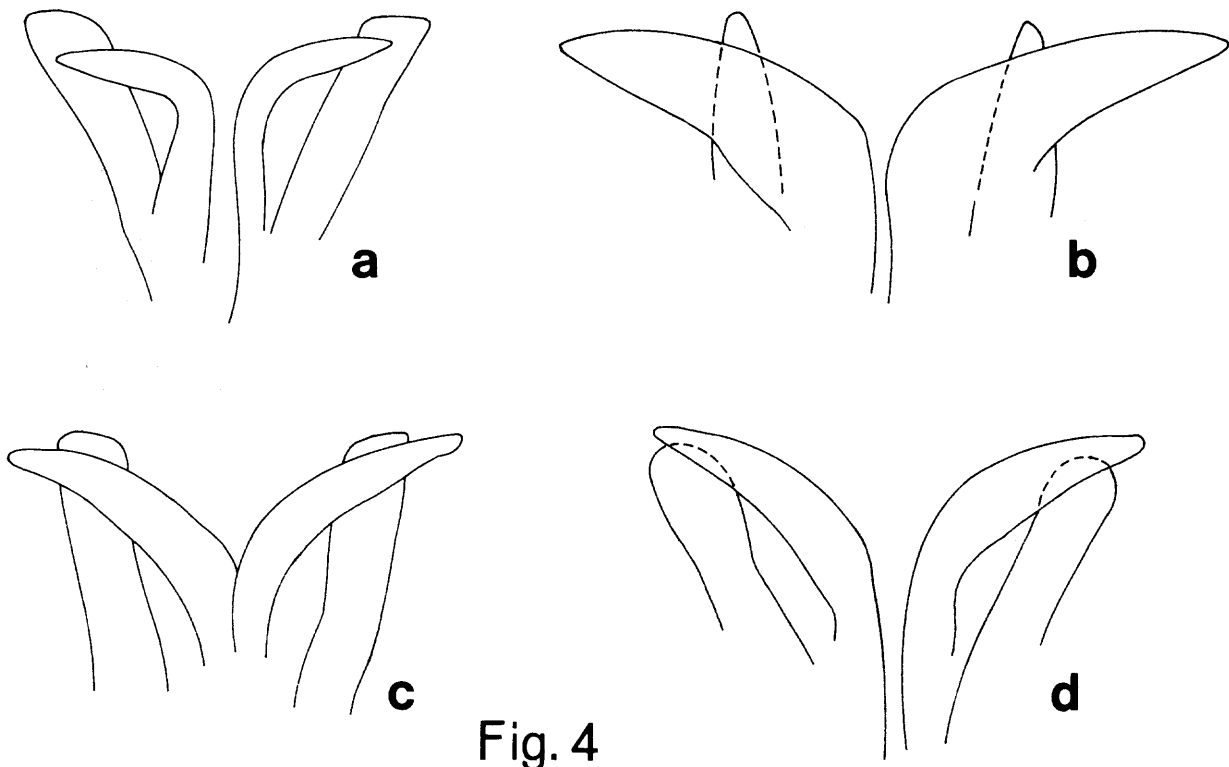


Fig. 4