

SEM studies on the *area rugosa* of two species of *Brumptaemilius* Dollfus, 1952 (Rhigonematida: Carnoyidae)

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Summary. SEM studies of the *area rugosa* of two undescribed species of *Brumptaemilius* Dollfus, 1952 collected from the gut of diplopods from Nigeria and South Africa are compared and contrasted and the value of the *area rugosa* as a taxonomic character in the genus discussed. The disposition and form of the copulatory papillae and the form of the spicules are also commented upon. A table of the known distribution of the genus in Africa is provided.

Key words: Africa, *Brumptaemilius*, distribution, morphology, Nigeria, SEM, South Africa.

The genus *Brumptaemilius* Dollfus, 1952 contains 17 nominal species, all described from the gut of diplopods from the African region (Table 1). One of their most distinctive characters is the development of an *area rugosa* in the male, a complex feature that appears to assist in copulation and which is located in the adcloacal region of the body, often being subventrally extended anteriorly for a considerable proportion of the body length. The majority of nominal species of *Brumptaemilius* were described by Dollfus (1952, 1964) and are usually poorly characterized and known only from the male. Consequently, ten of Dollfus's species were regarded as *species inquirendae* by Van Waerebeke (1984). Descriptions made subsequent to Dollfus by Adamson (1983, 1987), Adamson & Anderson (1985) and Van Waerebeke (1984) are much more comprehensive and have clearly demonstrated the importance of the detailed structure of the *area rugosa* in the taxonomy of the genus.

The *area rugosa* typically comprises three parts: a postcloacal area of cuticular spines or bosses; fields of minute cuticular rugosities arranged along the striae in transverse rows and extending, mostly subventrally, on either side of the cloacal aperture and two to four irregular rows of subventral papilla-like formations extending anterior to the cloaca. In addition, the disposition and form of the copulatory papillae is of importance as is the form of the spicules which are paired and usually isometric and may have the distal region of each shaft twisted and wrapped around one another, either once or twice. *Brumptae-*

milius species bear faint scale-like ornamentation (tessellation) in the central third or more of the spicule shaft. The gubernaculum is elongate, boat-shaped and has a prominent dorsal hole which may be obscure in some species.

The detailed structure of the components of the *area rugosa* has not been previously studied with the aid of the SEM, although Adamson & Zaman (1984) did use the SEM to study the metastomal projections of the male of *Brumptaemilius gabonensis* Adamson, 1983. This species and *B. venardi* Van Waerebeke, 1984 probably do not belong to *Brumptaemilius*, *sensu stricto* as, amongst other atypical characters, the *area rugosa* is weakly developed and lacks the subventral rows of papilla-like formations.

MATERIALS AND METHODS

During the last few years colleagues have provided us with diplopod species from various parts of Africa. Not all of these were well preserved, but two diplopod species, one from the Krüger National Park, South Africa (leg. J. Bridge, 1995) and one from IITA, Ibadan, Nigeria (leg. N. Price, 1995) arrived alive and, on killing by decapitation and dissection of the gut, were found to contain a number of *Brumptaemilius* specimens representing two, probably undescribed, species. Specimens were killed by gentle heat and fixed in TAF. Material of both *Brumptaemilius* species was used for SEM study and the results obtained are presented here. Specimens for SEM study were postfixed overnight in 1% osmium

Table 1. Distribution of the genus *Brumptaemilius* Dollfus, 1952 by species and country in the African region.

Species and authority	Status	Country
<i>B. biinflatus</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. caudabrevis</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. congolensis</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. decimus</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. filifer</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. gabonensis</i> Adamson, 1983	valid	Gabon
<i>B. gubcurvatus</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. gubercristatus</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. jolivetii</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. justini</i> Adamson & Anderson, 1985	valid	Malawi
<i>B. longicauda</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. monsarratae</i> Van Waerebeke, 1984	valid	Ivory Coast
<i>B. oschei</i> Dollfus, 1964	valid	Tanzania (?)
<i>B. sclerophorus</i> Dollfus, 1952	valid	Guinea
<i>B. seychellarum</i> Adamson, 1987	valid	Seychelles
<i>B. tuzetae</i> Dollfus, 1964	<i>species inquirenda</i>	Zaire/Congo
<i>B. venardi</i> Van Waerebeke, 1984	valid	Ivory Coast
<i>B. sp. aff. justini</i> (Hunt, unpublished)	undescribed	South Africa
<i>B. sp. aff. monsarratae</i> (Hunt, unpublished)	undescribed	Nigeria
<i>B. sp.</i> (Hunt, unpublished)	undescribed	Ghana
<i>B. sp.</i> (Hunt, unpublished)	undescribed	Uganda
<i>B. sp.</i> (Hunt, unpublished)	undescribed	Zimbabwe

tetraoxide, dehydrated through a graded series of ethanol, critical point dried with CO₂ and sputter coated with gold. The specimens were examined at an accelerating voltage of 10 kv.

RESULTS AND DISCUSSION

Micrographs obtained in this study are compared and contrasted in Figs. 1-3 to demonstrate the complexity and variability of the structures forming the *area rugosa*. Only a few specimens were available for SEM study and unfortunately several of these had features partially obscured by substantial plaques, presumably formed from bacteria and fungi, adhering to the cuticle surface.

The Nigerian species (Figs. 1 & 2) has affinities with *B. monsarratae* Van Waerebeke, 1984, a species based on a small number of specimens from Ivory Coast, but differs mainly in the slightly more anterior location of the precloacal pair of sublateral papillae and in having the *area rugosa* extending ventrally on to the tail spike, a feature not recorded in either of the two males described by Van Waerebeke (1984). The *Brumptaemilius* species from South Africa (Fig. 3) is similar to *B. justini* Adamson & Anderson, 1985, a species described from a spirostreptid from Malawi, but differs mainly in having a longer, more filiform, tail appendage; the posterior pair of sublateral papillae being located slightly more anterior to the cloaca

and fewer papilla-like formations between the pre-cloacal pairs of papillae. Both species examined in our study have the *area rugosa* forming a ventral band of rugosities just prior to the anterior cloacal lip, a feature not described for either *B. justini* or *B. monsarratae* and possibly of specific importance.

Area rugosa. The *area rugosa* is one of the most important morphological characters in the male and essentially comprises three parts:

(i) a postcloacal area of cuticular spines or bosses, the shape and disposition of which are significant. The spines in the Nigerian species are more or less upright and arranged as two irregular rows of small, well spaced, conical spines immediately posterior to the cloaca and two rows of larger spines marking the periphery of the posterior cloacal area. The central region of the field is spineless, apart from a small central cluster of conical spines (Figs. 1A, B, D & 2E). In the species from South Africa, however, the entire field is covered by virtually contiguous rows of flatter spines which are smaller towards the lateral peripheries (Fig. 3A & B). The spines are retorse and frequently overlap one another, thus appearing intricate.

(ii) fields of minute cuticular rugosities arranged along the striae in transverse rows and extending, mostly subventrally, on either side of the cloacal aperture. These fields may extend anteriorly for a

considerable distance or be confined to the cloacal region. The extremities of the rugosities are usually simple. In the South African species the rugosities are numerous, rounded and almost contiguous. They have simple tips and extend to the lateral region from the area of the posterior cloacal lip. They neither extend on to the tail spike nor on to the base of the last pair of prominent subventral papillae (Fig. 3A & B). Anteriorly, the rugosities are absent in the vicinity of the three papillae straddling the cloacal lip, but then form a broad ventral band which almost immediately bifurcates and extends anteriorly, passing beyond the level of the sublateral papillae for some distance as two irregular rugose areas on either side of the midventral line, eventually petering-out before the precloacal pair of subventral papillae. The precloacal fusion of the two bands as seen in our specimens was not described for *B. justini* by Adamson & Anderson (1985), but it is possible that the authors overlooked the feature as the rugosities in that region appear to be less prominent. The Nigerian species also has rugosities with simple tips, but they are less numerous and more widely separated. The rugose area in this species extends further posteriorly, partially extending along the basal portion of the subventral papillae and continuing on to the ventral region of the tail spike (Fig. 1B, C, D; 2A, B). The rugose area extends anteriorly on either side of the cloaca and, as in the South African species, the area bearing the three papillae on the anterior cloacal lip lacks rugosities (Fig. 2B). A ventral rugose band precedes the cloacal lip (also not described in the original description of *B. monsarratae*) and bifurcates at about the level of the sublateral papillae, extending anteriorly as two irregular subventral fields, the rugosities becoming smaller and more widely dispersed until they eventually disappear (Figs. 1A, C & 2B). The sublateral precloacal papillae immediately anterior to the cloaca are also basally rugose (Fig. 2C), the rugosity extending posteriorly to about cloacal level and being distinctly weaker than that of the major fields (Figs. 1C & 2B).

(iii) two to four irregular rows of subventral papilla-like formations extending anterior to the cloaca. These structures were originally referred to as 'papilles scléreuses' by Dollfus (1952, 1964) but, according to Van Waerebeke (1984), nerve endings have not been demonstrated in the so-called 'sclerotised papillae' and he preferred the term 'papilla-like formations' instead. The form of these structures varies from simple to rosette-like, the distal tip bearing up to twelve rounded peripheral processes and a central lobe. In the South African species there are two irregular subventral rows of these papilla-like structures, each having a broad base tapering into a

short shaft bearing four to six distal lobes plus a central lobe which appears to have a terminal nerve opening (Fig. 3C & D). This minute central pore is not always clear, but its presence lends credence to the idea that the structures may be derived from development of the ventrosubmedian rows of somatic pores. The papilla-like formations extend anteriorly for a considerable distance with about 50 protuberances in each series. The Nigerian species also has two subventral rows of papilla-like formations which have areolate bases and become increasingly irregularly arranged anteriorly (Figs. 1A & 2A, B) so that the formations appear to zigzag, thus giving the impression of four rows. These papilla-like structures are of different appearance, considerably shorter and less well developed than those of the South African species, the six or seven terminal protuberances being slender and pointed rather than broad and rounded (cf. Figs. 1C and 2A & B with Fig. 3C & D), but with a central, rounded-conoid, lobe. In contrast to the South African species, no nerve ending was visible on the terminal lobe. The protuberances are situated towards the ventral margin of the rugose areas. The form of these protuberances is very similar to those described for *B. monsarratae* by Van Waerebeke (1984), who referred to them as being in the form of a crown ('en forme de couronne').

Copulatory papillae. In addition to the *area rugosa*, there are 15 copulatory papillae, some pairs of which may be areolate in appearance - the 'rosette papillae' of Adamson (1983). There are three post-cloacal pairs: one subventral pair on the posterior cloacal lip; one hypertrophied, pedunculate, subventral pair near the tail spike; one subdorsal pair near the base of, or on, the tail appendage, and four precloacal pairs situated as: one pair on the anterior cloacal lip, a sublateral pair followed by a subventral pair and then a more sublateral pair anteriormost plus a single papilla located midventrally on the anterior cloacal lip. The rosette papillae can be clearly seen on the Nigerian species, the areolate appearance being particularly prominent on the two subventral pairs of postcloacal papillae, the pair on the anterior cloacal lip and the first pair of precloacal sublateral papillae (Figs. 1A, C, D & 2A, C). The original description of the male of *B. monsarratae* was based on two specimens (Van Waerebeke, 1984) and shows the adcloacal pair of sublateral papillae as being closer to the cloaca than is evident in our specimens from Nigeria (Fig. 2B). The papillae on the South African species, with the exception of the sublateral pair immediately anterior to the cloaca, are not areolate (Fig. 3A & B). In addition to the enlarged copulatory papillae, there are also a number of innervated somatic papillae, some of which are

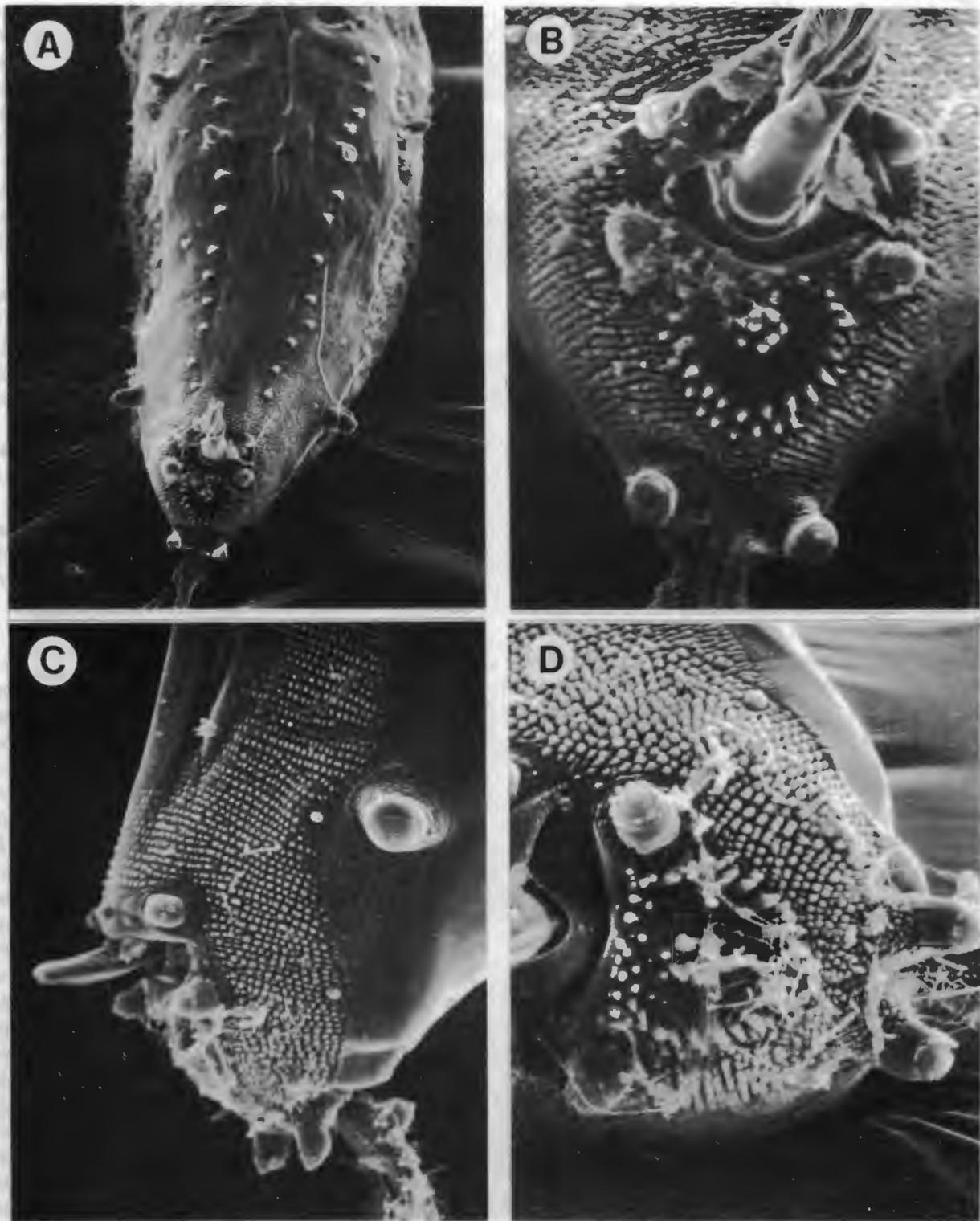


Fig. 1. *Brumptaemilius* sp. (ex. Nigeria). A: Posterior region, ventral view, showing papillae and area rugosa; B, D: Cloacal region, ventral view. Note the formation of larger conical projections on the posterior cloacal lip; C: Cloacal region, lateral view.

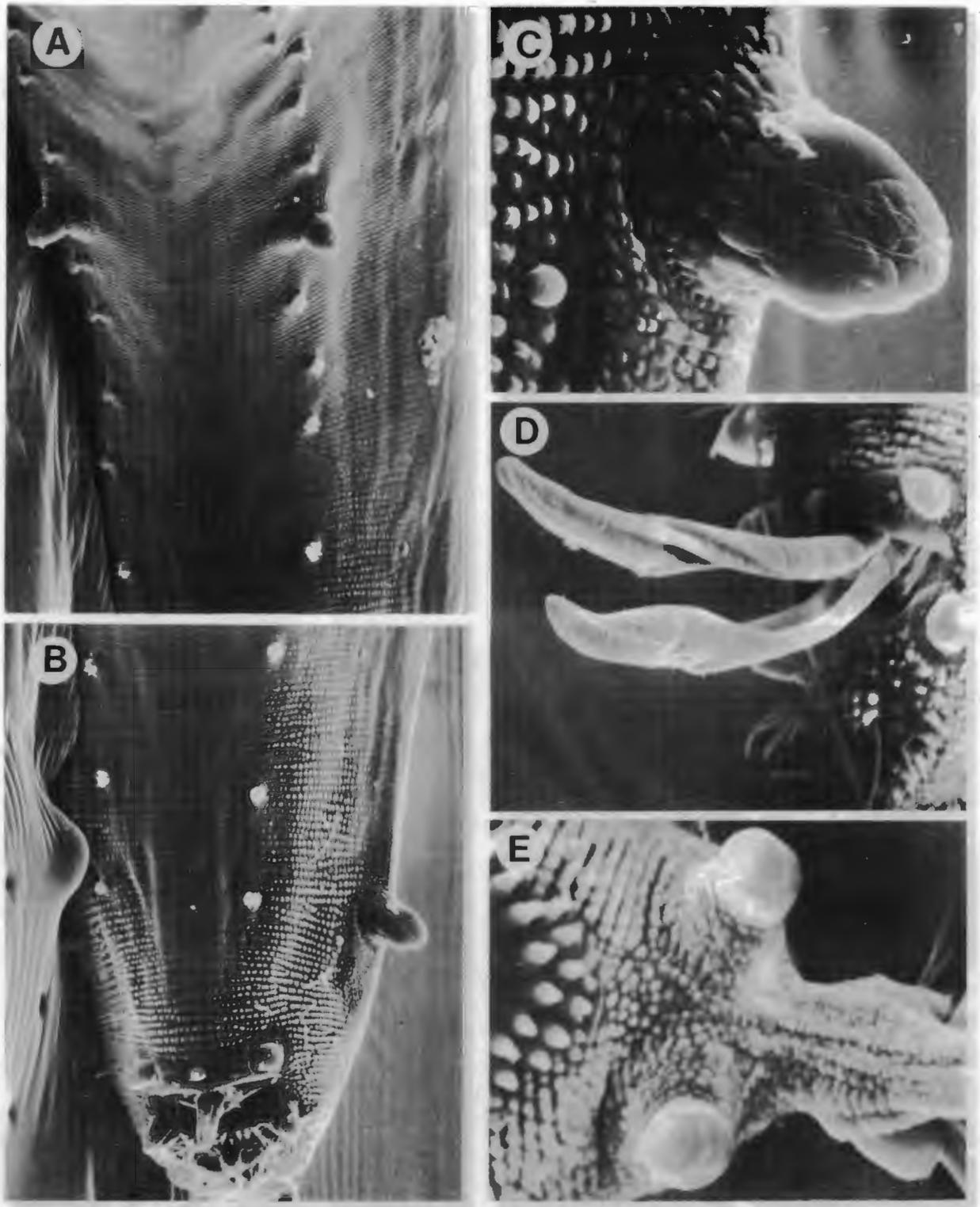


Fig. 2. *Brumptaemilius* sp. (ex. Nigeria). A,B: Posterior region, ventral view, showing papillae and elements of the *area rugosa* (the posteriormost papilla-like formations in A match with the anteriormost formations in B); C: Sublateral rosette-like papilla slightly anterior to cloaca; D: Spicules, showing twisting and scale-like tessellation; E: Tail region, ventral view, showing ventrally directed hypertrophied, pedunculate, subventral papillae and rugosities extending on to tail spike.

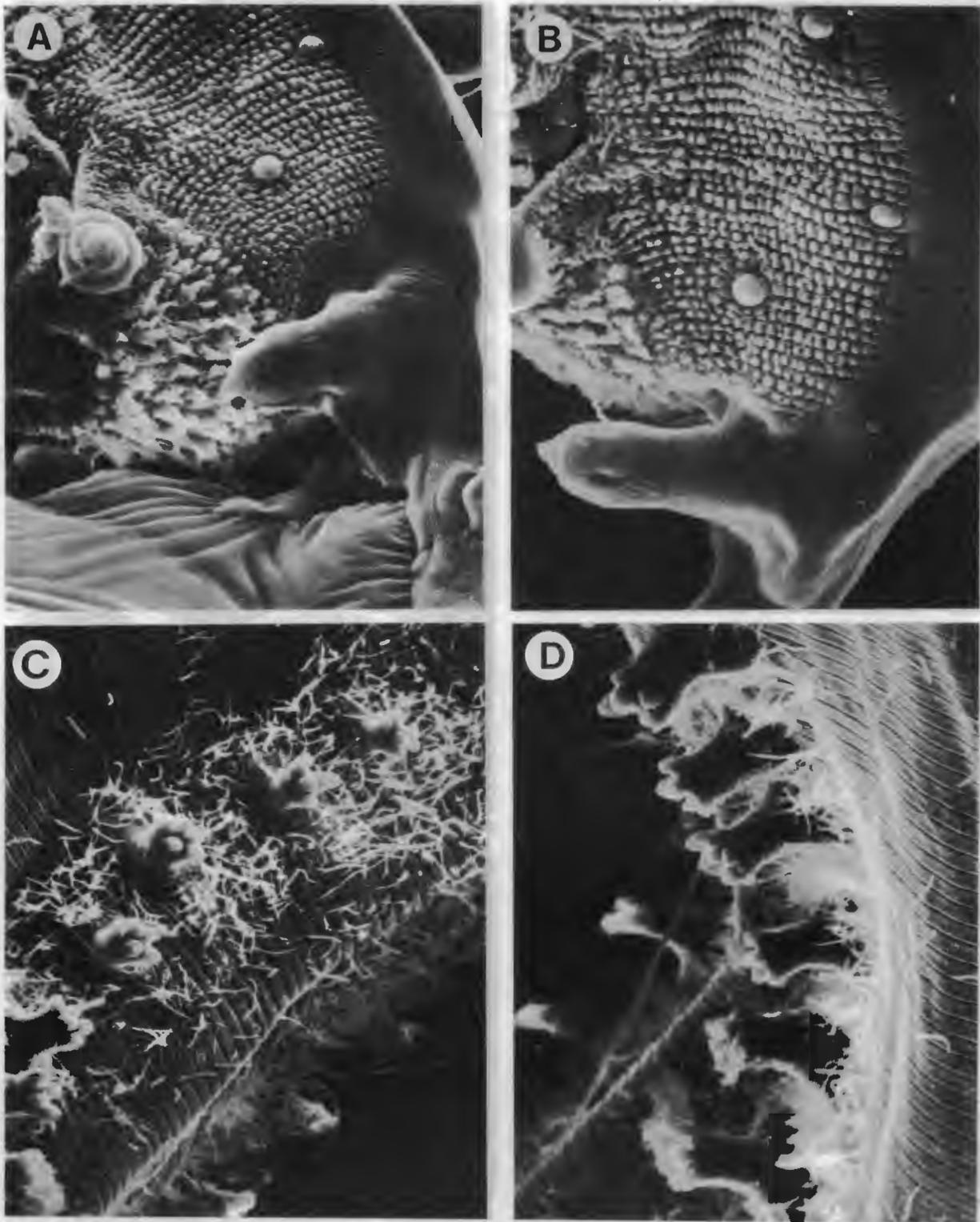


Fig. 3. *Brumptaemilius* sp. (ex. South Africa). A: Cloacal region, ventrolateral view, showing rugosities and imbricate conical projections on the posterior cloacal lip; B: Cloacal region, lateral view. The rugose area does not extend beyond the last pair of pedunculate subventral papillae; C, D: Papilla-like formations of the *area rugosa*. Note the lobed tips.

located in the fields of rugosities in the cloacal region (Fig. 1C & D and Figs. 3A & B).

Spicules. The spicules were only seen in the protruded state in a specimen from the Nigerian species. Faint tessellation in the middle section of the shaft and distal twisting of the spicule is clearly evident (Fig. 2C).

CONCLUSION

It is evident from this study that the potential morphological diversity of the *area rugosa* in the taxonomy of the genus is substantial, but to fulfill this potential it is essential that species are described with appropriate attention to detail to facilitate precise and meaningful comparisons. Consequently, it is desirable that ventral studies of the male posterior region, if possible supplemented by SEMs, are done when describing new species of *Brumptaemilius*. Although SEM is a valuable tool for examining such cuticular features, particularly the form and disposition of the rugosities, the appropriate level of detail can still be achieved by classical methods as is evident in the descriptions provided by Adamson (1983, 1987), Adamson & Anderson (1985) and Van Waerebeke (1984). Many of the species proposed by Dollfus (1952, 1964), however, will continue to cause problems because of the paucity of detail in the original descriptions and apparent lack of type material. Following the example of Van Waerebeke (1984) in regarding the majority as *species inquirendae* may seem over critical, but is probably the only practical solution to establish the taxonomy of *Brumptaemilius* on a sound footing, many of the original descriptions by Dollfus (1964) being too impoverished to allow unequivocal reidentification of the species.

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REFERENCES

- Adamson, M.L. 1983.** *Brumptaemilius gabonensis* n. sp. (Ransomnematinae, Rhigonematidae, Nematoda) from *Pachybolus* sp. (Spirobolida, Diplopoda) from Gabon with comments on the Ransomnematinae. *Bulletin du Muséum national d'Histoire naturelle, 4e série* 5: 759-766.
- Adamson, M.L. 1987.** Rhigonematid (Rhigonematida; Nematoda) parasites of *Scaphiostreptus seychellarum* (Spirostreptida; Diplopoda) in the Seychelles with comments on the ovejector structure in *Rhigonema* Cobb, 1898. *Canadian Journal of Zoology* 65: 1889-1897.
- Adamson, M.L. & Anderson, N.H. 1985.** Rhigonematid (Nematoda) parasites of *Archispirostreptus tumuliporus* (Spirostreptida; Diplopoda) in Malawi. *Canadian Journal of Zoology* 63: 916-923.
- Adamson, M.L. & Zaman, V. 1984.** Metastomal projections in *Brumptaemilius gabonensis* Adamson, 1983 (Ransomnematinae; Nematoda): their distribution in the subfamily and possible function. *Journal of Parasitology* 70: 239-242.
- Dollfus, R.Ph. 1952.** Quelques Oxyuroidea de Myriapodes. *Annales de Parasitologie humaine et comparée* 27: 143-236.
- Dollfus, R.Ph. 1964.** Nématodes de Myriapodes du Congo Belge. *Mémoires du Muséum national d'Histoire naturelle, Paris, série A* 32: 109-169.
- Van Waerebeke, D. 1984.** *Brumptaemilius monsarratae* n. sp. et *Brumptaemilius venardi* n. sp. (Rhigonematidae, Nematoda), parasites de *Pachybolus laminatus* Cook (Spirobolida, Diplopoda) en Côte d'Ivoire. *Bulletin du Muséum national d'Histoire naturelle, 4e série* 6: 323-334.

Hunt D. J., Sheldon J. Исследование *area rugosa* двух видов *Brumptaemillus* Dollfus, 1952 (Rhigonematida: Carnoyidae) в сканирующем электронном микроскопе.

Резюме. Проведено сравнение морфологии *area rugosa* у двух новых неописанных видов *Brumptaemillus* Dollfus, 1952 из кишки диплопод из Нигерии и Южной Африки. Проведена оценка строения *area rugosa* как признака в таксономии рода. Анализируется таксономическая значимость расположения и формы генитальных папилл и формы спикул. Дана таблица распространения видов рода *Brumptaemillus* в Африке.
