Belg. J. Zool., 131 (Supplement 1): 167-171 April 2001

The mosaic of the epidermal syncytia in *Didymorchis* sp. (Didymorchidae, Temnocephalida) from South America

Maria Cristina Damborenea[1] **and Lester R.G. Cannon**[2]

[1] **CONICET. Depto. Cient. Zool. Inv., Fac. Cs. Nat. y Museo, UNLP. Paseo del Bosque, 1900 La Plata, Argentina**
[2] **Queensland Museum, P.O. Box 3300, South Brisbane 4101, Australia**

ABSTRACT. Two species of *Didymorchis* (Turbellaria, Temnocephalida) from Lago Nahuel Huapi, Argentina were studied using a scanning electron microscope and silver nitrate staining to demonstrate the presence of syncytial plates and map the epidermal mosaic. The *Didymorchis* species studied have twenty syncytia, more than are present in the Australian and New Zealand species. Otherwise, the same six morphological groups found in the other species can be recognised. The syncytial plate topography of the South American species of *Didymorchis* has the following distinguishing characteristics: the presence of ventro lateral posterior syncytia, the absence of outer intermediate syncytia, the higher number of plates than present on Australian and New Zealand species, including the replications, and the position of the nephridiopores. The results presented provide data relevant to the origin and relationships of the Didymorchidae within the Temnocephalida, and also their relationships with their hosts.

KEY WORDS: Platyhelminthes, *Didymorchis*, Temnocephalida, South America, syncytial epidermis.

INTRODUCTION

Only four species of *Didymorchis* Haswell, 1900 have been described: one from New Zealand (*D. paranephropis* Haswell, 1900), two from Australia (*D. astacopsidis* Haswell, 1915 and *D. cherapsis* Haswell, 1915), and one from Uruguay, South America (*D. haswelli* Mañe Garzon, 1960). A variety of the last species (*D. haswelli* var. *australis* Dioni, 1972) has been described from Argentina and three undescribed species were reported from Australia (JOFFE et al, 1995a). All these species are commensal on parastacid crayfish and, in South America, they are also found in the branchial chamber of crabs, *Aegla neuquensis* Schmidt, 1942 (Crustacea Anomura). The systematic position and the relationships of these species were uncertain because some authors linked them with the dalyelliids and others with the temnocephalids.

WILLIAMS (1979, 1986) demonstrated the multisyncytial plates of the epidermis of the temnocephalids. Further studies showed that this characteristic is a synapomorphy that distinguishes Temnocephalida from the dalyelliids (JOFFE et al. 1995b). JOFFE et al. (1995a) demonstrated that the epidermis of the Australian species of *Didymorchis* has several syncytial plates and later SEWELL & CANNON (1998) mapped the epidermal mosaic of the type species of the genus, *Didymorchis paranephropis*. *Didymorchis* is now considered an early derived group of the Temnocephalida.

The South American species have been little studied: the presence or the topography of the epidermal plates remains unknown. The aim of this work is to demonstrate the syncytial plates of South American species of *Didymorchis*, to map their topography and compare this with the epidermis of the other species of the genus.

MATERIAL AND METHODS

Two species of *Didymorchis* (*Didymorchis* sp. A and *Didymorchis* sp. B) were found in the branchial chamber of *Aegla neuquensis* Schmitt, 1942 (Crustacea Anomura Aeglidae) from Lago Nahuel Huapi, 2 km west to San Carlos de Bariloche city, Río Negro Province, Argentina (41º07'54.3 S - 71º19'51.5 W), collected by Martín García Asorey (Universidad Nacional del Comahue) and

Corresponding author: C. Damborenea, e-mail: cdambor@way.com.ar; cdambor@museo.fcnym.unlp.edu.ar

transported alive to the laboratory. The worms could be easily differentiated by the structure of the male copulatory organ, but a full description of these species will be presented only after a more detailed morphological and histological study.

Worms were removed from the host with the aid of a stereo-microscope and observed alive under a microscope and then fixed.

The method of JOFFE & CANNON (1998) was used to study the syncytial plates. First, worms were killed with 5% hot (ca. 60º C) silver nitrate, then exposed to cold light for about 5 minutes, and finally washed in distilled water, dehydrated and mounted in Eukitt.

For Scanning Electron Microscope (SEM) observation, the worms were fixed in hot 10% phosphate buffered formalin. They were then dehydrated in ethanol, subjected to critical point drying, mounted and examined with a Jeol SEM.

The terminology used for the syncytial plates and the functional groups follows JOFFE et al. (1995a) and SEWELL & CANNON (1998).

Figures and Photographs were scanned and edited using Adobe Photoshop.

RESULTS

Both, silver nitrate staining and SEM revealed the epidermal mosaic of both *Didymorchis* spp. Twenty syncytial plates can be recognised (Fig.1) with clear borders separating the plates. The mouth and gonopore are midventral in the anterior and posterior quarters of the body respectively, and the nephridiopores are lateral, somewhat dorsal in the middle of the body.

The ventral surface is covered by (from anterior to posterior) several ciliated plates: the ventral frontal syncytium (VF) (Fig. 2a-b), an anterior ventral intermediate syncytium (AVI) (Fig. 2c), two ventro-lateral frontal syncytia (VLF), a preoral syncytium (PrO), four (two side by side pairs) post oral syncytia (PtO), six (three longitudinal pairs) ventral trunk syncytia (VT), and a single posterior intermediate (PI) syncytium. Posteriorly is the unciliated adhesive field syncytium (AD) (Fig. 2d).

The dorsal trunk syncytium (DT) (Fig. 2a) and two ventro-lateral syncytia (VLP) cover the dorsal and lateral surfaces of the animal (Fig. 3a and e). The VLP are elongated plates that contact with the ventral trunk ones lying behind the level of the nephridiopores (Fig. 3c).

Both DT and VLP are similar, unciliated plates. Numerous ciliated receptors can be observed in this surface. On the VLP plates a row a receptors can be recognised (Fig. 3c).

The borders between both the DT and the VLP syncytia with those of the VT syncytia are very clear (Fig. 3d). The ventral region has a covering of dense locomotory cilia. The ventral region is composed of six VT syncytia, all alike. The borders between these syncytia are evident with nitrate staining, but very difficult to see under SEM, because of the numerous, long cilia.

There is a single posterior intermediate syncytium (PI), which is ciliated and looks similar to the VT syncytia. It bears the gonopore. In two specimens it was split longitudinally in two. Behind this lies the AD syncytium. In general, it is a single plate with a horse-shoe shape, but in

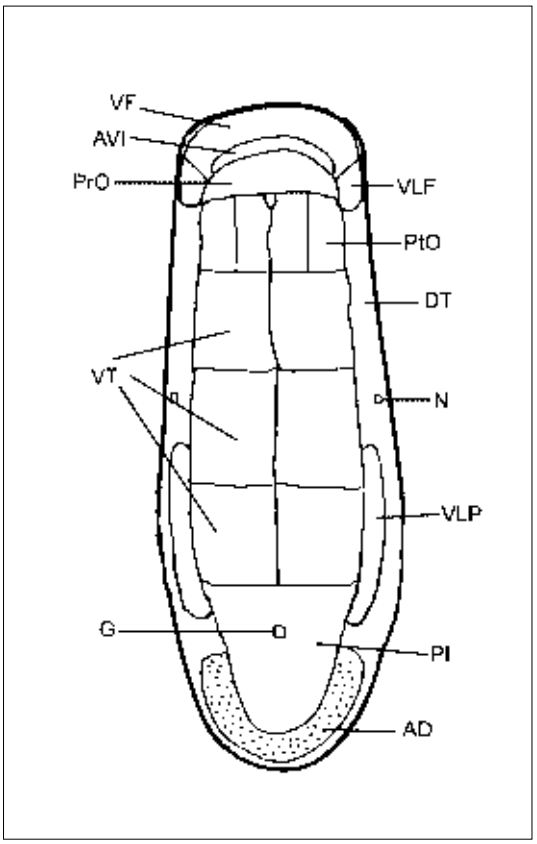
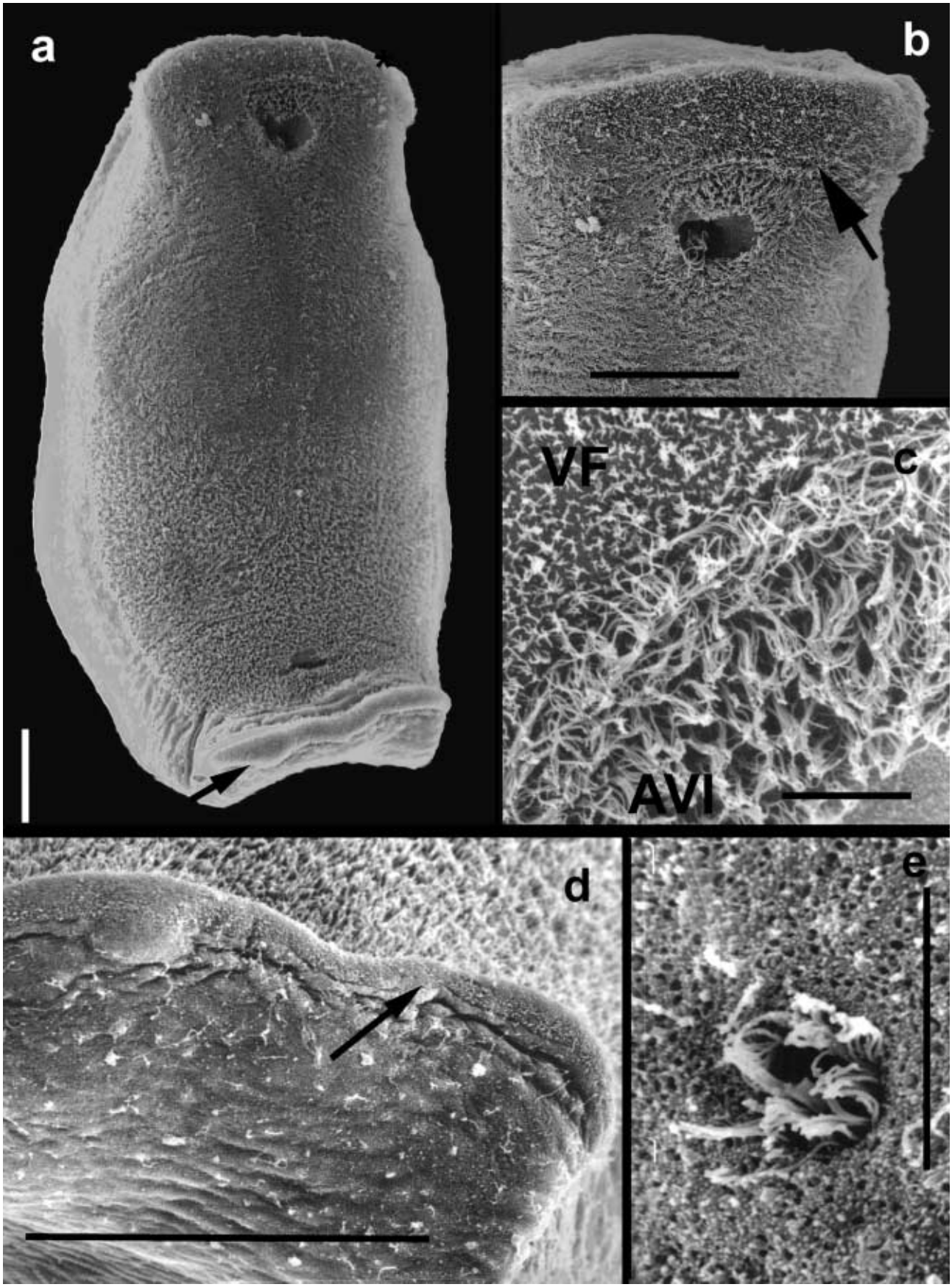


Fig. 1. – Mosaic of epidermal syncitia for *Didymorchis* sp. B. Syncytia: AD, adhesive field; AVI, anterior ventral intermediate; DT, dorsal trunk; PI, posterior intermediate; PrO, preoral; PtO, postoral; VLF, ventral lateral frontal; VLP, ventral lateral posterior; VF, ventral frontal; VT, ventral trunk. G: gonopore; N, nephridiopore (terminology after JOFFE et al., 1995a).

Legend to the figure (see opposite page)

Fig. 2. – SEM of *Didymorchis* sp. B.

a. ventral view of whole specimen showing the ventral cilia and dorsal unciliated surface. Arrow indicates border between AD and DT. Scale: 100 µm.
b. VF syncytium. Arrow indicates the border between VF and AVI. Scale 100 µm.
c. Detail of the VF and AVI. Scale: 10 µm.
d. Posterior end. The border between the AD and DT is indicated by the arrow. Scale 100 µm.
e. Nephridiopore. Scale: 10 µm.


a
b
VF
c
AVI
d
e

some specimens it is split into two small, ovoid plates. The AD abuts anteriorly with the PI and posteriorly and laterally with the DT (Fig. 2d).

In the anterior ventral region, several ciliated syncytia can be recognised. The ventral frontal syncytium (VF) has few cilia (Fig. 2c). The AVI is a narrow structure with longer cilia than the more anterior VF (Fig. 2c) and behind is the PrO syncytium just before the mouth. Immediately behind the mouth lie four syncytia side by side (PTO), which bear dense cilia, similar to those of the VT.

Two other syncytia, the VLF, are also evident. They are anterior and lateral, joined to the DT and to the VF. The cilia are short and few in number.

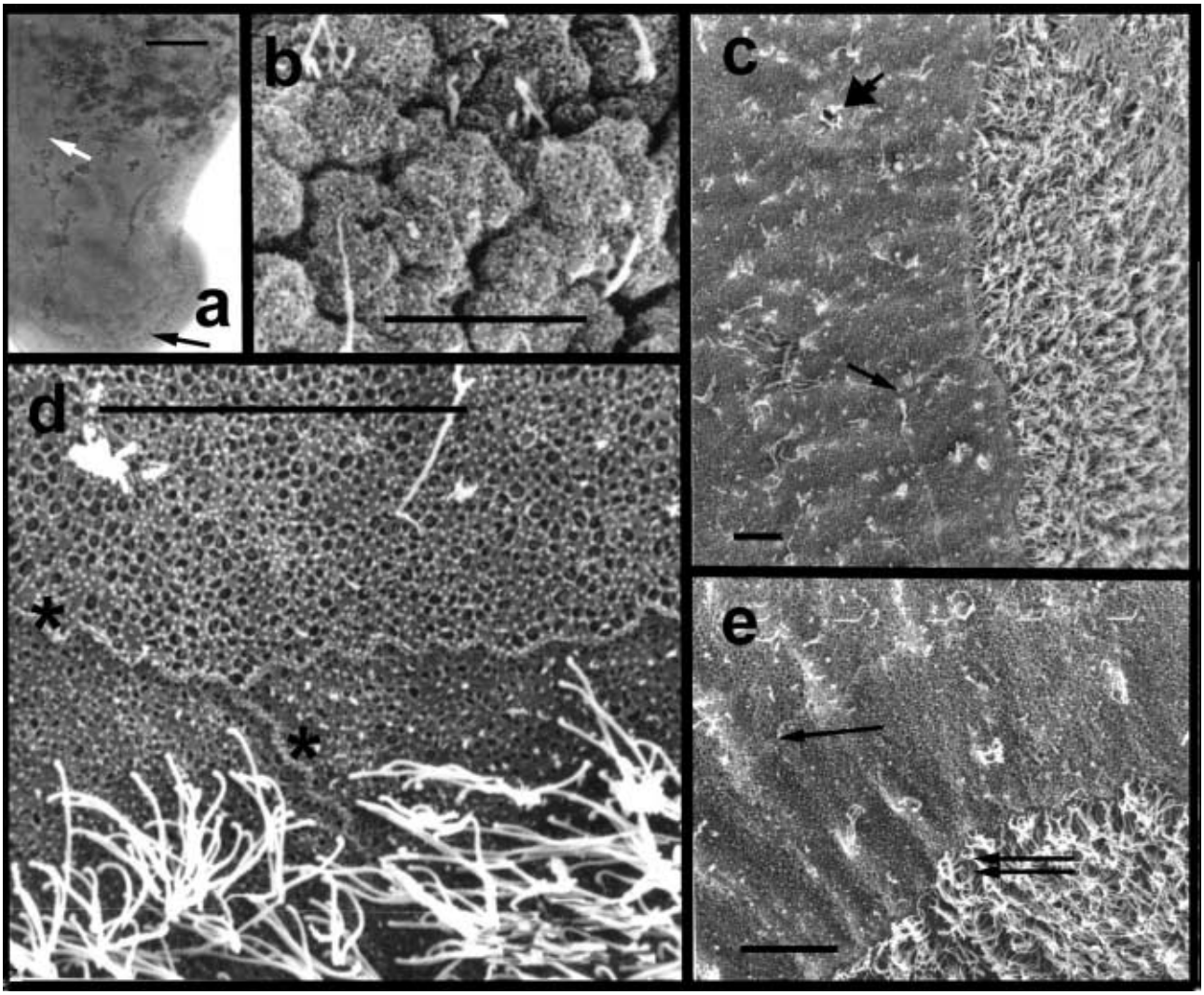


Fig. 3. – a. Lateral view of *Didymorchis* sp. B stained with silver nitrate. White arrow: the border between the VLP and PI; black arrow: the border between the AD and PI. Scale: 50 µm.
b-e. SEM of *Didymorchis* sp.
b. Dorsal surface. Scale: 10 µm.
c. Lateral view showing the ciliated VT, DT and VLP. Small arrow the border between DT and VLP; big arrow the nephridiopore. Scale: 100 µm.
d. Detail of the border between DT and VT and between two VT, *show the borders. Scale: 10 µm.
e. Detail of the VLP, border between DT and VLP single arrow; border between VT and VLP double arrow. Scale: 10 µm.

DISCUSSION

The species of *Didymorchis* are now placed in the Temnocephalida, due to the observed mosaic syncytial plate pattern. The South American species studied here share this characteristic, so they are clearly Temnocephalida.

The *Didymorchis* species studied from Argentina have numerous syncytia, more than the Australian and New Zealand species. Otherwise, the same six morphological groups found in the other species can be recognised. These groups were described for Australian species by Joffe et al. (1995a) and observed again in the New Zealand species (Sewell & Cannon, 1998).

The ventro-lateral posterior plates found in the South American worms, however, have not been found in any other species of the genus. They have the same morphology as the dorsal trunk plate and could be considered as a division of the same functional group.

The morphology of some plates shows variability. Variability, as already noted by JOFFE & CANNON (1998), seems characteristic of the Didymorchidae and although some minor differences are observed in plate topography within the other Temnocephalida it is much less obvious. The most striking variation was found in the adhesive field. Some specimens have this plate split into two. This has been observed by JOFFE et al. (1995a) for the Australian species. Another notable, but less frequent split, was observed in the posterior internal (PI) syncytium.

Both New Zealand and Australian species have the nephridiopores opening on the ventral trunk syncytia. In the South American specimens they open on the dorsal trunk syncytium where they are more anterior and somewhat dorsal.

The position of the gonopore is on the posterior intermediate syncytium, as is the case also with the New Zealand species. In contrast, the Australian specimens have the gonopore on the single ventral trunk syncytium, and the inner posterior intermediate plate is very small. Since the South American species lack the outer intermediate syncytium of the Australian species we call this single plate the posterior intermediate syncytium.

The ventro-lateral frontal syncytia are present both in Australian and South American species, but are absent in the New Zealand one.

If the topography of the syncytial plates is considered alone, we can affirm that the South American species are close to the New Zealand ones, because of the high number of syncytia, the split of the ventral trunk syncytia, the dense cilia of the posterior intermediate plate and the position of the gonopore in the posterior intermediate plate. The South American species are similar to the Australian ones, however, they differ in the presence of the ventro-lateral frontal syncytia and in the form and variation of the adhesive field. Furthermore, the plate topography of the South American species of *Didymorchis* is unique in having the following characteristics: (1) the presence of the ventro-lateral posterior syncytia, (2) the absence of the outer intermediate syncytia, present in all other species studied until now, (3) the higher number of plates, including the replications (laterally of the PRO and longitudinally with the VT) and (4) the position of the nephridiopores. Such differences may prove to be worthy of generic redesignation (see DAMBORENEA & CANNON, 2001).

The genus has a Gondwanan distribution and shares crustacean hosts (Astacidea and Anomura). The results presented here provide valuable data relevant to the origin and relationships of the Didymorchidae within the Temnocephalida, and also their relationships with their hosts.

ACKNOWLEDGEMENTS

We thank the Administración de Parques Nacionales, regional Bariloche, Argentina, for allowing collection of the Crustacean host species. We especially wish to acknowledge Martín García Asorey, Faculty of Science of Universidad Nacional del Comahue who caught the crustaceans for this study.

REFERENCES

DAMBORENEA, M.C. & L.R.G. CANNON (2001). On Neotropical temnocephalans. *J. Nat. Hist.* (in press).

JOFFE, B.I. & L.R.G. CANNON (1998). The organisation and evolution of the mosaic of the epidermal syncytia in the Temnocephalida (Platyhelminthes: Neodermata). *Zool. Anz*., 237: 1-14.

JOFFE, B.I., I.V. SOLOVEI & L.R.G. CANNON. (1995a). The structure of the epidermis in *Didymorchis* (Temnocephalida: Platyhelminthes). *Aust. J. Zool.*, 43: 631-641.

JOFFE, B.I., I.V. SOLOVEI, K.B. SEWEL & L.R.G. CANNON (1995b). The organisation of the epidermal syncytial mosaic in *Diceratocephala boschmai* (Temnocephalida, Platyhelminthes). *Aust. J. Zool*., 43: 509-518.

SEWELL K.B. & L.R.G. CANNON (1998). The taxonomic status of the ectosymbiotic flatworm *Didymorchis paranephropis* Haswell. *Mem. Queensland Mus.*, 42(2): 585-595.

WILLIAMS, J.B. (1979). Studies on the epidermis of the *Temnocephala* IV. Observations on the ultrastructure of the epidermis of *Temnocephala dendyi*, with notes on the role of the Golgi apparatus in autolysis. *Aust. J. Zool.*, 27: 483-499.

WILLIAMS, J.B. (1986). Phylogenetic relationships of the Temnocephaloidea (Platyhelminthes). *Hydrobiologia*, 132: 59-67.