Observations on *Dactylocephala madagascariensis* (Vayssière, 1892), a temnocephalan with twelve tentacles from Madagascar

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ABSTRACT
The 12-tentacled Madagascan temnocephalan *Dactylocephala madagascariensis* is reported on from the first new collections for over 70 years. Previously known from the crayfish *Astacoides madagascarensis*, it is now reported from *A. granulimanus* and a further unidentified *Astacoides* sp. Worms were found in several locations in Madagascar on the cephalothorax, claws and abdomen of the crayfish as well as on pereopods 4 and 5. Using more recent techniques such as de Faure's mounting fluid, we have photographed the cirrus and shown it to possess a slightly inflated terminal introvert armed with many rows of tiny spines. Furthermore, scanning electron microscopy has revealed an epidermal mosaic unlike any other temnocephalan, *viz.*, one with a dorsal syncytium which covers the tentacles, a ventral syncytium which includes the peduncle of the posterior attachment organ as well as the lateral excretory pores, a unique anterior ventral syncytium just posterior to the base of the tentacles which has a textured surface, and, typical of other temnocephalans, an adhesive field syncytium. The anterior ventral syncytium may be analogous to the post-tentacular (and presumed osmoregulatory) syncytium of higher temnocephalans.

KEY WORDS
Platyhelminthes,
Temnocephalida,
Temnocephalidae,
Temnocephala,
Dactylocephala,
Madagascar,
cirrus,
epidermal mosaic,
parasitology.

RÉSUMÉ

Observations sur Dactylocephala madagascariensis *(Vayssière, 1892), un temnocéphale de Madagascar à douze tentacules*.

Le temnocéphale de Madagascar à 12 tentacules, *Dactylocephala madagascariensis,* est étudié à partir des premières récoltes effectuées depuis plus de 70 ans. Connu précédemment de l'écrevisse *Astacoides madagascarensis*, on le signale maintenant de *A. granulimanus* et d'une autre espèce non identifiée d'*Astacoides*. Les temnocéphales ont été trouvés dans plusieurs localités de Madagascar et ont été récoltés sur le céphalothorax, les pinces et l'abdomen des écrevisses ainsi que sur les péréopodes 4 et 5. En utilisant des techniques modernes comme le liquide de montage fluide de Faure, nous avons photographié le cirre et montré qu'il possède un introvert légèrement dilaté, armé de nombreuses rangées de petites épines. De plus, la microscopie électronique à balayage a montré une mosaïque épidermique différente des autres temnocéphales, c'est-à-dire un syncytium dorsal qui couvre les tentacules, un syncytium ventral qui recouvre le pédoncule de l'organe postérieur d'attachement et les pores excrétoires latéraux, un syncytium antéro-ventral original postérieur à la base des tentacules et qui a une surface texturée et, comme les autres temnocéphales, un syncytium du champ adhésif. Le syncytium antéro-ventral pourrait être analogue au syncytium post-tentaculaire des temnocéphales les plus évolués, dont le rôle dans l'osmorégulation a été suggéré.

MOTS CLÉS
Platyhelminthes,
Temnocephalida,
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Temnocephala,
Dactylocephala,
Madagascar,
cirre,
mosaïque épidermique,
parasitologie.

INTRODUCTION

Dactylocephala madagascariensis (Vayssière, 1892) was originally described from specimens taken from the large crayfish *Astacoides madagascarensis* (Milne-Edwards & Audouin, 1839) collected in the vicinity of Tananarive, Madagascar and sent to France. The worms are unique in having 12 anterior tentacles and six pairs of testes rather than the customary five tentacles and two pairs of testes found in species of *Temnocephala* Blanchard, 1849 and related genera. Furthermore, it has a trailing terminal sucker, not one tucked ventrally on a peduncle as is characteristic of *Temnocephala* spp. These characters prompted Monticelli (1899) to erect a new genus for it, *Dactylocephala* Monticelli, 1899. Later, Baer (1929) made a further study of the worms, in part from material which Vayssière had deposited in the Muséum national d'Histoire naturelle (MNHN) in Paris, but including specimens recovered from crayfish held in the collections of the Natural History Museum in Berlin.

Although André (1937) reported on the presence of the worms on crayfish, the only accounts of the worms are those of Vayssière (1892) and Baer (1929). These present observations result from an opportunity to examine with more modern techniques some recently acquired specimens.

ABBREVIATIONS USED

af	adhesive field syncytium;
av	anterior ventral syncytium;
deF	de Faure's fluid;
dt	dorsal trunk syncytium;
i	introvert;
mo	mouth;
MNHN	Muséum national d'Histoire naturelle, Paris;
pts	post-tentacular syncytium;
s	shaft;
SEM	scanning electron microscopy;
ss	serial sections;
vt	ventral trunk syncytium;
wm	whole mount.

MATERIAL AND METHODS

Table 1 provides details of the collections in Madagascar made by Dr J.-M. Élouard (IRD, formerly ORSTOM). All worms were identified by us as *Dactylocephala madagascariensis*. They were collected from *A. madagascarensis* and *A. granulimanus* Monod & Petit, 1929 and an unidentified crayfish (*Astacoides* sp.).

Material examined is deposited in the MNHN, Paris and in the Queensland Museum, Brisbane. It is as follows (MHNH collection-original tube number/slide number, followed by other data where available): 634 HF/HI 117, ex *Astacoides madagascarensis*; 635 HF/HI 118 (plus wet specimens), ex *Astacoides madagascarensis*; 636 HF/HI 119-24 ex *Astacoides madagascarensis*, field number PO 151, 17.X.1993, Bassin Mangoro, Ambatoloana; 637 HF/HI 125 (plus wet specimens) Ambatoloana; 638 HF/HI 126 (plus wet specimens), ex *Astacoides* sp., field number PO 485, 18.X.1995, Bassin Betsiboka, Amobasy; 639 HF/HI 127, ex *Astacoides granulimanus*, field number PO 425, 24.V.1995, Bassin Namorona, Sahavondrona; 640 HF/HI 128 (plus wet specimens), ex *Astacoides granulimanus*, field number PO 158, 14.XI.93, Bassin Namorona, Ranomafana; plus slides HI 129-130 and wet specimens 641 HF and H 66 and those labelled sp. 4, 5, 7 and 8 all ex *A. madagascarensis*. (Queensland Museum collection): G215193 (wm), ex *Astacoides madagascarensis* on pereopods 4 and 5 of specimen #3, female 13 cm, 90 g; G215194 (ss) (same data); G215195 (wm, deF), (same data); G215196 (wm, deF), ex *Astacoides madagascarensis* on pereopods 4 and 5 of specimen #4, female 13 cm, 100 g; G216006 (wm), ex *Astacoides granulimanus*, Ranomafana, 14.XI.1993; G216007 (wm), ex *Astacoides madagascarensis*, Amobasy, 18.X.1995; G216008 (wm) (same data); G217455 (wm, deF), ex *Astacoides madagascarensis*, on cephalothorax of specimen #4, female, 13 cm, 100 g; G217456 (wm, deF), ex *Astacoides madagascarensis*, on claws and abdomen of specimen #7, female, 10.5 cm, 60 g.

TABLE 1. — Records of *Dactylocephala madagascariensis* prevalence.

Host	Collection date	Locality	Prevalence
Astacoides madagascarensis	22.II.1998	Ankerana	
	"	Ambatolaona	+
	22.XII.1990	Ranomafana	-
	"	"	+
	"	Ambatolaona	-
	"	"	-
	"	"	-
	"	"	+
	03.II.1995	"	-
	"	"	-
	22.III.1992	"	-
	17.IV.1994	"	-
	26.XI.1993	"	-
	15.XI.1993	Namorona	-
A. granulimanus	24.XI.1993	Andringitra	-
	14.XI.1993	Ranomafana	+
	20.XI.1993	Andringitra	+
	28.IV.1994	Ambatovaky	-
	24.V.1995	Namorona	+
	18.IV.1994	"	-
A. crosnieri	15.XI.1993	"	-
A. petiti	20.XI.1995	Ivondro	-
	24.XI.1995	Betoreo	-
A. caldwelli	21.III.1998	Ankaratra	-
	14.XI.1993	Namorona	-
A. betsileoensis	"	"	-
Astacoides sp.	17.IV.1994	Ranomafana	-
	18.X.1995	Betsiboka	+
	25.X.1995	"	-
	14.XI.1993	Namorona	-
	17.X.1993	Mandraka	-

For anatomical study, worms were first fixed with 10% Formalin. Anatomical terminology essentially follows Cannon & Sewell (1995) and updated in Sewell & Cannon (1998a). Cirrus morphology of the worms was revealed after mounting in de Faure's fluid (distilled water 50 ml, chloral hydrate 50 g, glycerol 20 ml and gum arabic 30 g) and measurements are in microns (µm), obtained using a camera lucida, and presented, following the specimen number, as: shaft (length × width at proximal opening); introvert (length × width).

For scanning electron microscopy (SEM), specimens were dehydrated in ethanol, then critical point dried, mounted on stubs, coated with gold,

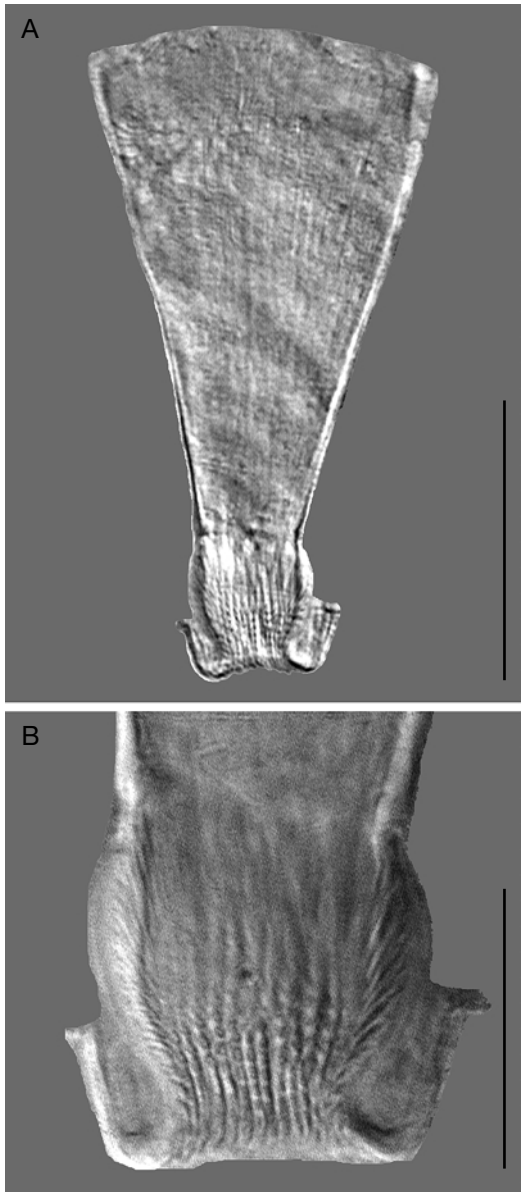

FIG. 1. — *Dactylocephala madagascariensis*; **A**, whole cirrus; **B**, Introvert partly everted. Scale bars: A, 50 µm; B, 20 µm.

and examined with an Hitachi S-530 operating at 25 kV with a Robinson backscatter detector to reduce charging.

The photographs and drawings were scanned, edited and prepared using Adobe Photoshop® and Illustrator®.

OBSERVATIONS

Dactylocephala madagascariensis were collected from only two of the sampled species of crayfish, *Astacoides madagascarensis* and *A. granulimanus,* both found in montane stream habitats and some specimens obtained from an unidentified *Astacoides* sp. The worms were not found on the more distantly related and more plesiomorphic *A. crosnieri* Hobbs, 1987 or *A. petiti* Hobbs, 1987, also known from rocky creeks and riffles, nor from *A. caldwelli* (Bate, 1865) or *A. betsileoensis* Petit, 1923 which are found in swamps and streams (Hobbs 1987).

The male copulatory organ (Fig. 1) is typical of the Temnocephalida. It has a thin-walled conical proximal shaft and a flexible cirrus introvert lined with fine spines: G215196: s (75 × 55), i (36 × 16); G217455: s (89 × 64), i (35 × 18); G217456: s (89 × 72), i (35 × 16).

SEM shows clearly the 12 short anterior tentacles and the anterior position of the mouth (Fig. 2A). Also, a mosaic of syncytial plates is revealed. At the base of the tentacles on the ventral side is a narrow transverse syncytium, the anterior ventral syncytium (av) (Fig. 2B). The body is cupped with the ventral side concave and the lateral edges of the body reveal the border between the dorsal trunk (dt) and ventral trunk (vt) syncytia (Fig. 2C, arrows). This junction loops dorsally, on each side, around the lateral excretory pore just behind the tentacles, leaving the excretory pores wholly within the ventral trunk syncytium (Fig. 2D). Posteriorly, the junction loops to pass dorsally across the base of the terminal trailing body (here greatly contracted) (Fig. 2E), leaving the posterior adhesive organ wholly within the more ventral trunk syncytium. Fig. 2F shows the junction marking the border between the ventral trunk syncytium and the adhesive field syncytium (af) of the sucker.

A diagrammatic reconstruction of the syncytial mosaic of *Dactylocephala madagascariensis* as determined solely from these SEM observations is presented in Fig. 3. Four syncytial plates are seen: 1) a dorsal trunk syncytium (dt) which covers the tentacles and extends posteriorly to

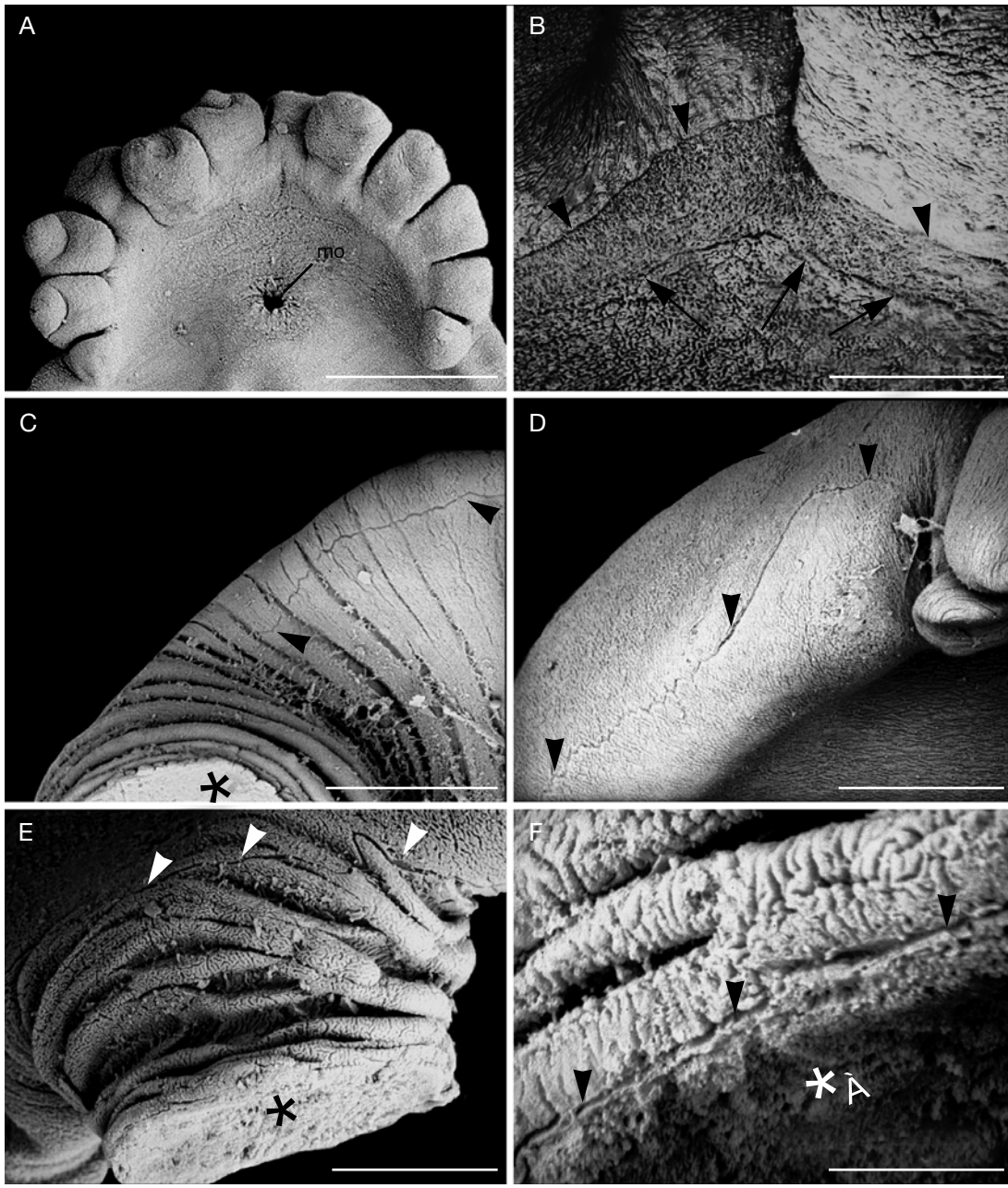

FIG. 2. — *Dactylocephala madagascariensis*, SEM micrographs of the epidermal mosaic; **A**, anterior ventral surface showing mouth (**mo**) and the 12 tentacles; **B**, anterior ventral syncytium immediately behind the tentacles, arrows show the syncytial junctions (tailed arrows are posterior); **C**, posterior lateral body showing the syncytial junction between the dorsal and the ventral trunk syncytia (arrows), * indicates the adhesive disk syncytium; **D**, anterior lateral body showing the syncytial junction between dorsal and ventral trunk syncytia (arrows), and the ventral plate curving to the dorsal body to enclose the lateral excretory pore; **E**, posterior dorsal body showing the much contracted stalk, * indicates the adhesive surface and white arrows the junction between dorsal and ventral trunk syncytia curving across the dorsal body; **F**, border of the ventral trunk and adhesive disk syncytia (arrows), * indicates the adhesive surface. Scale bars: A, C-E, 500 µm; B, 100 µm; F, 50 µm.

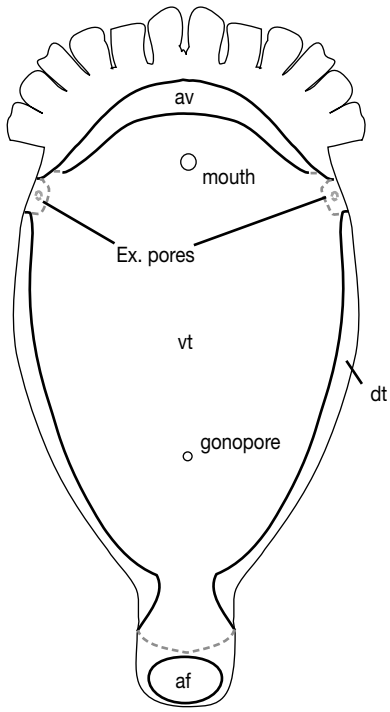

FIG. 3. — *Dactylocephala madagascariensis*, reconstruction of the epidermal mosaic based on SEM observations, unresolved borders dotted. Abbreviations: **av**, anterior ventral syncytium; **dt**, dorsal trunk syncytium; **vt**, ventral trunk syncytium; **af**, adhesive disk syncytium.

just before the base of the posterior stalk; 2) a narrow, anterior ventral syncytium (av) which extends across the ventral body just behind the base of the tentacles; 3) a ventral trunk syncytium (vt) which extends posteriorly from the av and incorporates small lateral lappets which extend dorsally to enclose the lateral excretory pores and also extends dorsally at the posterior to enclose most of the terminal stalk; 4) an adhesive field syncytium (af). The junction between the dt and vt syncytia runs along most of the lateral edges of the body.

DISCUSSION

Despite examination of a variety of hosts from several habitats, only a single species of worm was identified. Nevertheless, the possibility of other temnocephalans in Madagascar cannot be ruled out given the diversity of temnocephalans found in several niches on many species of Australian crayfishes. In particular Cannon & Joffe (2001) suggest it would not be surprising if examination of the branchial chamber of *Astacoides* spp. from Madagascar revealed the presence of species of *Didymorchis* Haswell, 1900.

Vayssière (1892) and Baer (1929) provided a full account of the general anatomy of *D. madagascariensis*; we do not wish to treat this further.

The cirrus of *D. madagascariensis,* not previously photographed, is typical of the Temnocephalida. As mentioned earlier (Cannon & Sewell 1995; Sewell & Cannon 1998a, b; Damborenea & Cannon 2001), the cirrus is of value at a specific, but not higher level since the amount of variation is considerable across species, while cirri of distantly related genera are often similar.

Use of silver nitrate on living material is the easiest and most reliable way to determine if a syncytial mosaic exists and its pattern. Lacking living material, SEM can reveal an epidermal syncytial mosaic (a characteristic of all the Temnocephalida). *D. madagascariensis* has a mosaic although the pattern is not immediately referable to those already known mainly because of the following characters: 1) the presence of an anterior ventral syncytium (av) which has a highly developed surface; 2) possession of basically dorsal and ventral plates (dt and vt) defined by lateral intersyncytial junctions.

The epidermal mosaic of *Dactylocephala madagascariensis* presented here is based purely on an SEM study of fixed material that leaves some regions partially unresolved, particularly those adjacent to the posterior adhesive disc and laterally near the excretory pores. The mosaic, however, is unlike any known from other temnocephalans (Joffe *et al.* 1998). The most intriguing plate is the av which has the surface, size and anterior location similar to the post-tentacular syncytium (pts) of higher Temnocephalidae, but is ventral, not dorsal as is customary, although the pts does extend ventrally in *Notodactylus handschini* (Baer, 1945). This plate is believed to be osmoregulatory (Joffe *et al.* 1996).

The only temnocephalans with a narrow, ventral plate anterior to the mouth are the species of *Didymorchis*. Could the avi syncytium found in *Didymorchis* spp. be homologous with the av of *D. madagascariensis*? Are these plates osmoregulatory?

The relatively simple mosaic seen in *D. madagascariensis* may be compared with the more complex, basal plan seen in species of *Didymorchis* by considering fusion of ventral, ciliated plates together with migration of the excretory pores to the lateral body. *D. madagascariensis*, however, is not particularly close to Didymorchidae. It belongs in Temnocephalidae and has the apomorphies of "true" tentacles and a prominent bundle of axial muscle in the posterior adhesive organ (Joffe *et al.* 1998). By comparison of the mosaic of *D. madagascariensis* with higher Temnocephalidae, we see from a study of *Temnocephala dendyi* Haswell, 1893 that during ontogenesis the mosaic is formed by fusion of syncytia (Joffe & Cannon 1998) and that sometimes the adult can secondarily return to a "plesiomorphic" state. Such may be the case with *D. madagascariensis.*

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