BULLETIN DE L'INSTITUT ROYAL DES SCIENCES NATURELLES DE BELGIQUE, BIOLOGIE, 66 suppl. : 103-108, 1996
BULLETIN VAN HET KONINKLIJK BELGISCH INSTITUUT VOOR NATUURWETENSCHAPPEN, BIOLOGIE, 66 suppl. : 103-108, 1996

Cymbastela hooperi sp. nov. (Halichondrida : Axinellidae) from the Great Barrier Reef, Australia

by Rob W.M. VAN SOEST, Ruth DESQUEYROUX-FAÚNDEZ, Anthony D. WRIGHT & Gabriele M. KÖNIG

Abstract

Cymbastela hooperi sp. nov. is described from the Great Barrier Reef, Australia. This species belongs to the recently erected genus of stalked sponges, *Cymbastela* HOOPER & BERGQUIST, 1992. The new species is the eighth member of this Australasian genus.
Cymbastela hooperi sp. nov. is distinguished from other *Cymbastela* species, by its unique growth form (stalkless and flattened on substrate), its yellow to brown colour, its loose skeletal morphology, the often telescoped spicule shape and its chemistry. The new species produces a large number of structurally related diterpene isonitrile derivatives which demonstrate significant *in vitro* antimalarial activity. One of these compounds, di-isocyano-adociane, is also found in *Amphimedon terpenensis* FROMONT, 1993. In addition both species present similar skeletal characters and spicule size. Considering this, a new combination is proposed : *Cymbastela terpenensis* (from *Amphimedon*).
Keywords : Demospongiae, Halichondrida, diterpenes, isonitrile derivatives, secondary metabolites.

Resumen

Se describe *Cymbastela hooperi* sp. nov. de la Gran Barrera, Australia. Esta especie pertenece al recientemente creado género de esponjas pedunculadas, *Cymbastela* HOOPER & BERGQUIST, 1992. La nueva especie incrementa a ocho el número de especies descritas de este género, todas ellas endémicas de la region Australasia.
Cymbastela hooperi sp. nov. se diferencia de otras especies de *Cymbastela* por su forma única de crecimiento apegada al substrato, sin pedúnculo; de color amarillento a café, con esqueleto laxo y espículas, a menudo con extremos en forma de telescopio, se diferencia además por la estructura química de sus metabolitos secundarios. La nueva especie produce un gran número de diterpeno derivados isonitrilos, estructuralment semejantes que muestran *in vitro*, importante actividad antimalaria. Uno de estos compuestos, di-isocyano-adociano se encuentra también en *Amphimedon terpenensis*, FROMONT 1993. Ambas especies presentan también, caracteres esqueléticos y tamaño de las espículas, similares. Considerando esto, una nueva combinacion es aquí propuesta : *Cymbastela terpenensis* (de *Amphimedon*).
Palabras claves : Demospongiae, Halichondrida, diterpenos, derivados isonitrilos, metabolitos secundarios.

Introduction

The recently erected axinellid genus *Cymbastela* HOOPER & BERGQUIST, 1992 is endemic to Australia and New Caledonia. It presents a skeleton of exclusively small to medium sized oxeas that is reminiscent of a haplosclerid skeleton. However, a tangential surface skeleton is absent and in many species a more or less distinct axial and extra-axial skeletal differentiation is found. The chemistry of the secondary metabolites of one of the species of the genus, *C. cantharella* (LÉVI, 1983) is similar to that known of Axinellidae (cf. DE NANTEUIL *et al.*, 1985; AHOND *et al.*, 1988; BRAEKMAN *et al.*, 1992), not to that of Haplosclerida. The genus so far contains seven species differentiated principally by their skeletal reticulation, by the development of their axial skeleton and by the sizes of their oxeas. One species has a specialised ectosomal skeleton (HOOPER & BERGQUIST, 1992). We report here the existence of an eighth species : *C. hooperi* sp. nov. distinguished from the other *Cymbastela* species by its morphology and by the presence of secondary metabolites observed for the first time.

Material and methods

Studies of the new species and other comparative material were carried out from thick sections, light microscopy of spicule slides and SEM stubs mounted with fragments and spicules, prepared in the usual way (cf. HAJDU & DESQUEYROUX-FAÚNDEZ, 1994).
The study of secondary metabolite chemistry of *Cymbastela hooperi* sp. nov. was performed from a dichloromethane soluble fraction obtained from sponge tissue that was subjected first to normal phase vacuum liquid column chromatography for pre-fractionation. Final purification step was performed by high pressure liquid chromatography (HPLC) (G. KÖNIG, unpubl.). Separations yielded 19 pure natural products, the structures of which will be published elsewhere.

Abbreviations for institutions cited in the text
BMNH : Natural History Museum, London.
JCUA : James Cook University, Queensland.
MHNG : Muséum d'histoire naturelle, Geneva
QMA : Queensland Museum, South Brisbane.
ZMA : Zoölogisch Museum, Amsterdam.

Systematics

Order **Halichondrida** VOSMAER, 1887

Definition : Demospongiae with a plumoreticulate skeletal architecture built of interchangeable styles and oxeas and intermediate spicules of widely diverging sizes and not functionally localised (VAN SOEST *et al.*, 1990).

Family **Axinellidae** RIDLEY & DENDY, 1887

Definition : Halichondrida with axially condensed and extra-axially plumoreticulate choanosomal skeletons (VAN SOEST *et al.*, 1990).

Genus ***Cymbastela*** HOOPER & BERGQUIST, 1992

Type species : *Pseudaxinyssa stipitata* BERGQUIST & TIZARD, 1967, by original designation.
Diagnosis : Typically cup-shaped, lamellate sponges. With or without specialised surface skeleton of small oxea. Choanosomal axial skeleton compressed or not. Extra-axial skeleton radial, reticulate or plumose (modified from HOOPER & BERGQUIST, 1992).

Cymbastela hooperi sp. nov.

Material studied : Holotype MHNG 18990, schizotype fragment ZMA POR. 11008, field number CT293V, Kelso Reef Australia, Queensland (18°25'S 147°02'E), 03. 1993, G. A. D. WRIGHT, col., 6-9 m.

DESCRIPTION

Known from a single specimen (Figs 1, 2).
Irregularly spreading over the substrate, stalkless, partly simulating an encrusting shape, but is in fact a modified cup-shaped, thickly lamellate sponge, with uneven, undulating incurved margins. Basal flattened part large, about 30 cm diameter. Thickness of the lamella about 10-30 mm.

Both surfaces are similar. They are rough, slightly micro-hispid, from single protruding terminal spicules. They bear characteristic rounded elevations, and are covered overall by a translucent collagenous membrane. Numerous and evenly distributed pores are visible on both surfaces. Oscules are small, 1.5 to 2.0 mm diameter, irregularly distributed, usually placed on the top of the rounded elevations or on surface ridges present on both surfaces of the sponge.

Consistency
Hard, stiff, not flexible.

Colour
Alive colour was yellowish to brown. Beige in alcohol.

Substrate
Dead coral rock, 6-9 m.

Skeleton
The skeletal structure is different from most other *Cymbastela* species in being predominantly loose. There is an extensive system of aquiferous canals in the centre of the lamella.
Ectosomal skeleton : no specialised surface skeleton, but terminal oxeas from the principal skeleton protrude through the surface membrane (Figs 3, 4), isolated or in divergent, close-set terminal bundles (Fig. 5).
Choanosomal skeleton : Axial skeleton slightly compressed into axial tracts or bundles, longitudinally distributed. Tracts are wavy, vaguely plumose, of irregular width. In cross section 6 to 8 spicules are visible in strongly developed fibers (Fig. 6), which have a thickness of about 80 µm. Distance between tracts 400 µm. Interconnecting tracts are weak, uni or paucispicular (1 - 3 spicules) and 1 or 2 spicules long (Fig. 7). Interstitial free oxeas are very abundant (Fig. 8). Aquiferous canals in the centre of the fan have a diameter of 600 - 700 µm.

Spicules (Table 1)
Oxeas are long, curved at the central part or straight, often with telescoped ends (Figs 9, 10), or with one of the apices blunt (Fig. 11) and sometimes a mucron. Many of them present a wide axial canal.

Ecology and distribution
Known only from the type locality, a shallow-water reef habitat, in the middle section of the Great Barrier Reef.

▷

Figs 1-11. - *Cymbastela hooperi* sp. nov. - 1, 2. External morphology. - 3, 4. Oxeas protruding through the surface membrane. - 5. Terminal bundles of the axial skeleton. - 6. Cross section of a longitudinal tract. - 7. Interconnecting tracts. - 8. Free oxeas abundant. - 9, 10. Telescoped apices of oxeas. - 11. blunt apices of oxeas.
Scale bars : Figs 1 & 2 = 0.5 cm; Figs 3, 4 & 7 = 50 µm; Fig. 5 = 100 µm; Fig. 6 = 20 µm; Fig. 8 = 200 µm; Figs 9 & 11 = 5 µm; Fig. 10 = 2 µm.

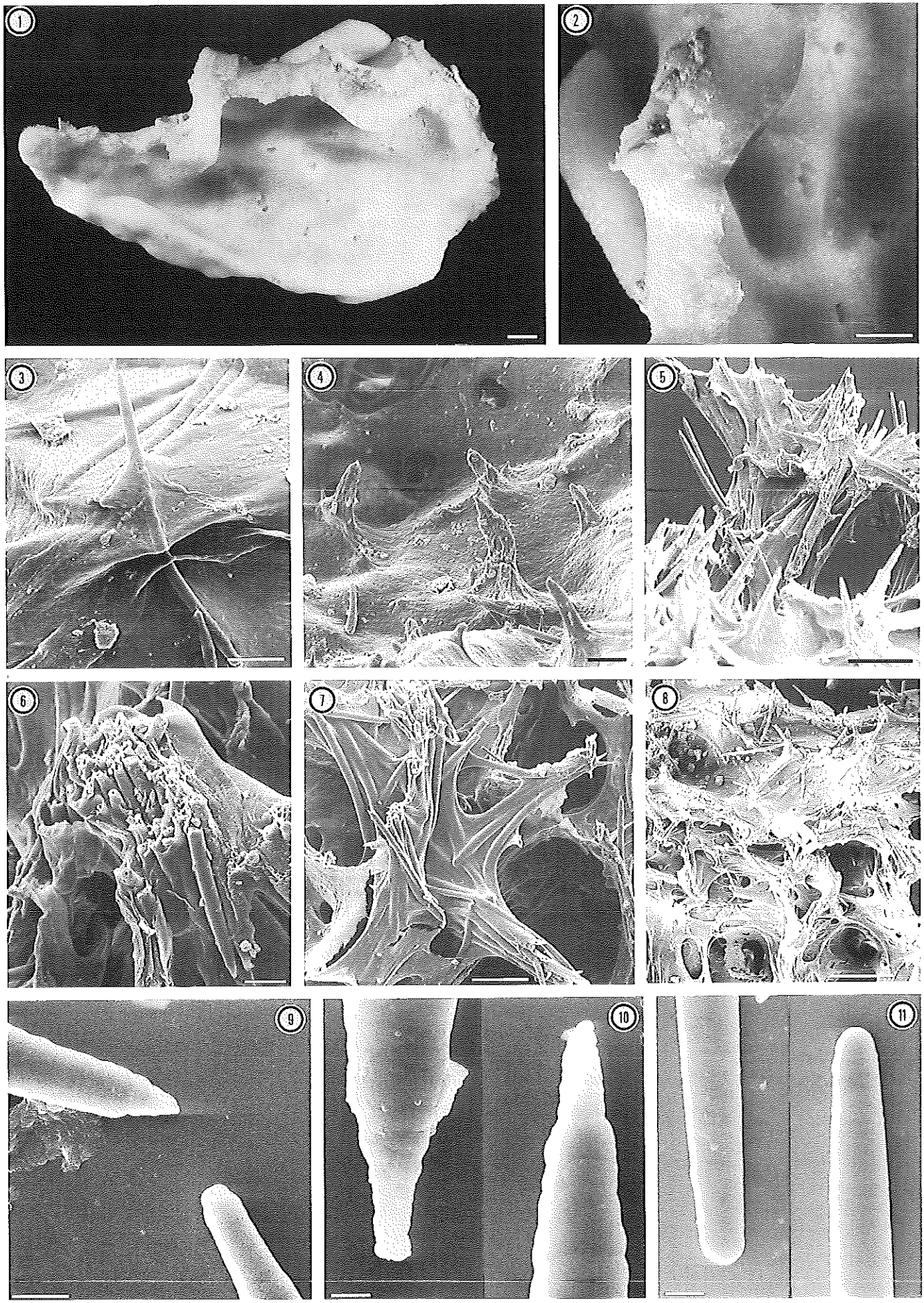
1
2
3
4
5
6
7
8
9
10
11

Table 1.
Spicule measurements of the holotype of *C. hooperi* sp. nov., compared with measurements of the holotypes of described species of *Cymbastela* : in HOOPER & BERGQUIST (1992); *LÉVI (1983) & **FROMONT (1993). Measurements in μm, expressed as minimum - mean - maximum. *C. hooperi* sp. nov. N = 25 spicules.

Species	Length	Diameter
C. cantharella	187 - 215.5 - 232	4.1 - 8.1 - 10.0
**C. cantharella*	200 - 250	8.0 - 10.0
C. concentrica	172 - 239.6 - 305	2.5 - 9.5 - 16.0
C. coralliophila	236 - 318.8 - 357	6.5 - 14.4 - 21.7
C. marshae	124 - 156.8 - 192	2.0 - 5.4 - 9.0
C. notiaina	49 - 77.7 - 98	1.5 - 3.2 - 4.5
C. stipitata	155 - 259.1 - 344	2.7 - 8.6 - 16.0
C. vespertina	167 - 252.2 - 270	5.2 - 11.9 - 15.0
***"A". terpenensis*	223 - 256 - 292	4.7 - 6.1 - 7.7
C. hooperi sp. nov.	163 - 232 - 269	3 - 6.4 - 10.0

Chemistry
The new species produces a large number of secondary metabolites which are either sesqui- or diterpenes. Almost all of the diterpenes were distinguished by being isonitrile derivatives. The most abundant compound, present as 0.33% of the dry sponge tissue, was the known compound di-isocyano-adociane (Fig. 12). This particular compound is identical to the major compound isolated from *"Amphimedon" terpenensis* by GARSON (1986).

Etymology
We dedicate this species to John N.A. HOOPER in recognition of his admirable efforts to bridge the enormous gap between existing and described Australian sponge biodiversity.

COMPARISON WITH OTHER *CYMBASTELA* SPECIES

We compared the new species with a member of the genus *Cymbastela*, viz. *C. vespertina* (specimens ZMA POR. 11006, from a coral reef, 10 m, East Point, Darwin, Northern Territory, Australia, collected & donated by J.N.A. HOOPER, 29.02.1987). Comparison of the new species with this material and with the descriptions and figures provided by HOOPER & BERGQUIST (1992) suggested its affinity to *Cymbastela* because it shares lamellate shape, tough consistency, differentiation into axial and extra-axial skeleton, spicule type, size and telescoped endings, with most *Cymbastela* species. The definition of the genus needs to be widened only slightly to include forms that are not precisely cup-shaped but more generally lamellate. Differences and similarities with various species of *Cymbastela* are the following:

Growth form
The new species differs from all other *Cymbastela* species in the lamellate-flattened-encrusting habit. Also the oscules on elevations are unique. Spicule lengths are similar to those of most other *Cymbastela* species, excepting *C. marshae* and *C. notiaina* which have much smaller oxeas (HOOPER & BERGQUIST, 1992). The thickness of the oxeas is relatively low, lower than most *Cymbastela* species, and close to that of *C. marshae*. Like most *Cymbastela*, excepting *C. coralliophila*, there is only a single oxea size category.

Skeletal structure
C. hooperi sp. nov. differs from most other *Cymbastela* species in having a relatively loose skeleton and an extensive system of aquiferous canals. The distance of the fibers exceeds that of all other *Cymbastela* species, with the possible exception of *C. cantharella*. Interconnecting tracts relatively weak.

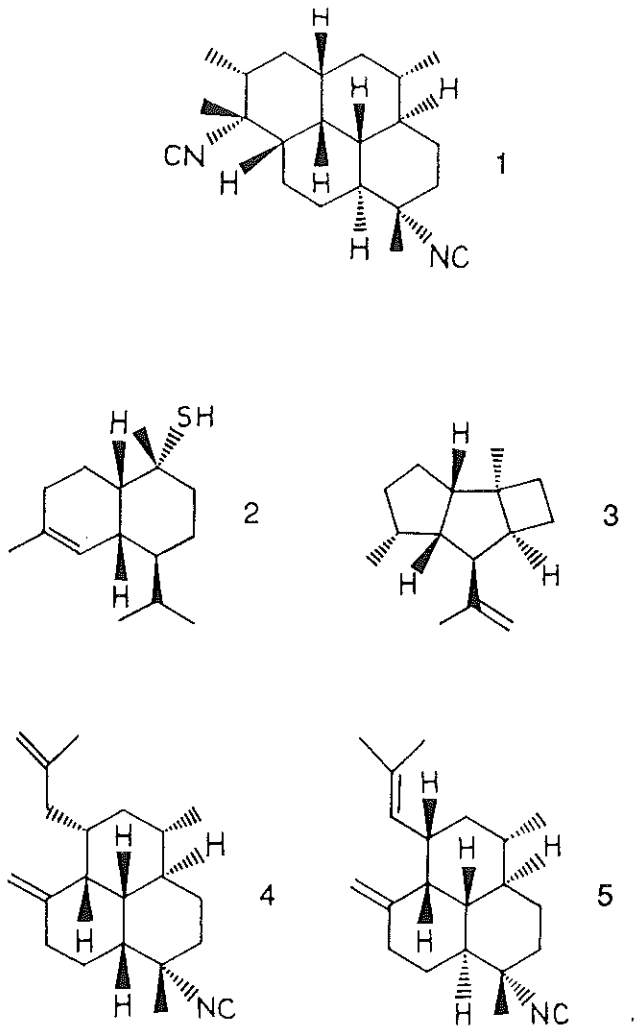

Fig. 12. - Natural products from *Cymbastela hooperi* sp. nov. - 1. Di-isocyane-adociane. - 2 & 3. are new products. - 4 & 5. known products.

COMPARISON WITH *AMPHIMEDON TERPENENSIS*

The skeletal similarity of certain axinellid genera such as *Cymbastela* with Haplosclerida, especially family Niphatidae, noted by HOOPER & BERGQUIST (1992) is indeed striking and has probably led to at least one misjudged ordinal assignment.

FROMONT (1993) employed the definition of the niphatid genus *Amphimedon* (by VAN SOEST, 1980) loosely to include at least one species without a proper ectosomal tangential reticulation. Her drawings and photos do not show evidence of an ectosomal tangential skeleton, and the specimen she very kindly sent us (ZMA POR. 10896, from John Brewer Reef, Great Barrier Reef, Queensland, Australia, collected & donated by J. FROMONT, 29.08.1986; this specimen does not belong to the type, but was collected at the same area and is, in all respects, similar to the type) also did not reveal a surface reticulation. The type species of *Amphimedon* and other close related *Amphimedon* species all have a clearly developed tangential surface reticulation.

The skeletal structure of *"A". terpenensis* is very loose and irregular, not at all as in true *Amphimedon*, nor, it must be conceded, true *Cymbastela*. However, the relatively loose structure described above for *C. hooperi* n.sp. connects these extreme skeletal developments.

Spicule sizes (210 - 335 x 5.9 - 12.6 µm) are similar to those of most *Cymbastela* and considerably longer than true *Amphimedon* species (70 - 180 x 1-12 µm).

The shape of *"A". terpenensis* is thickly, irregularly lamellate. Consistency is elastic (FROMONT, 1993), rather easily compressible (ZMA POR 10896), a clear difference with most *Cymbastela*, caused no doubt by the loose skeletal arrangement. Colour is red-brown, partly caused by a cyanobacterial symbiont, which is a common feature of *Cymbastela* (cf. HOOPER & BERGQUIST, 1992).

Apart from *"Amphimedon" terpenensis* no other Haplosclerid is recorded as a source of isonitriles. The fact that the largest secondary metabolite fractions of *"A". terpenensis* and *C. hooperi* n.sp. have identical chemical structure is strong evidence for close phylogenetic relationship. Other isonitrile derivatives are exclusively produced by axinellid (*Axinella, Acanthella*) and halichondrid (*Hymeniacidon, Ciocalypta*) sponges (BRAEKMAN *et al.*, 1992).

Further evidence that *"A." terpenensis* does not belong in *Amphimedon* are chemical studies made of the fatty acids of *"A." terpenensis*. They are qualified as widely different from those of true *Amphimedon* such as *A. compressa* (type species of *Amphimedon*) and *A. complanata* (GARSON *et al.*, 1994). In view of this and of the close similarity in skeleton structure and size and form of the spicules we introduce here the new combination : *Cymbastela terpenensis* (from *Amphimedon*).

However, its specific characters make it likely that it occupies an isolated position among the known species of *Cymbastela*.

The occurrence in a single genus of two unrelated structural chemical types, viz. isonitriles (*"A". terpenensis,* cf. GARSON, 1986; *C. hooperi,* cf. present study) and pyrrole-derivatives (*C. cantharella* cf. DE NANTEUIL *et al.*, 1985; AHOND *et al.*, 1988) is perhaps odd. However, it is not unprecedented, because isonitriles are found in the Mediterranean *Axinella cannabina* (cf. e.g. ADINOLFI *et al.*, 1977), while former compounds are recorded from the Mediterranean *Axinella verrucosa* (cf. CIMINO *et al.*, 1982). These compounds form part of a more extensive problem of distribution of these chemical types over Axinellidae and Halichondriidae, already

outlined in BRAEKMAN *et al.* (1992). Although the chemical types have a limited distribution (iso-nitriles : Axinellidae, Halichondriidae; pyrrole-derivatives : Agelasidae, Axinellidae, Halichondriidae) their precise significance for chemotaxonomy is still not clear.

GEOGRAPHIC DISTRIBUTION OF *CYMBASTELA*

C. hooperi sp. nov. and *C. terpenensis* are recorded from lat 14°-18°S, long 145-147°E. Thus, their sympatric distributions are added to the two overlapping sympatric species *C. coralliophila* and *C. concentrica* found on the Great Barrier Reef between 11°S and 22°S. Other species of *Cymbastela* occur allopatrically or only slightly overlapping in Western and Southern Australia, and in New Caledonia (HOOPER & BERGQUIST, 1992). All *Cymbastela* species are reef sponges occurring from the intertidal to 30 m.

Acknowledgements

We are indebted to the following persons : J.N.A. HOOPER (QMA), J. FROMONT (JCUA), C. VALENTINE (BMNH), for the loan or donation of specimens. J.WÜEST (SEM), C. RATTON (photo), I. JURIENS (MHNG) assisted with the preparation of microscopical material. J.N.A. HOOPER is thanked for his constructive advice. Two anonymous reviewers improved our work.

References

ADINOLFI, M., DE NAPOLI, L., DI BLASIO, B., IENGO, A., PEDONE, C. & SANCTACROCE, C., 1977. The absolute configuration of the axane sesquiterpenes from the sponge *Axinella cannabina*. *Tetrahedron Letters*, 1977 : 2815-2816.

AHOND, A., ZURITA, M.B., COLIN, M., FIZAMES, C., LABOUTE, P., LAVELLE, F., LAURENT, D., POUPAT, C., PUSSET, J., PUSSET, M. & THOISON, D., 1988. A new antitumoural compound extracted from the sponge *Pseudaxinyssa cantharella* sp. nov. (Axinellidae). *Comptes Rendus de l'Académie des Sciences*, Série II, 307 : 145-148.

BRAEKMAN, J.C., DALOZE, D., STOLLER, C. & SOEST, R.W.M., VAN, 1992. Chemotaxonomy of *Agelas* (Porifera : Demospongiae). *Biochemical Systematics and Ecology*, 20 : 417-431.

CIMINO, G., DE ROSA, S., DE STEFANO, S., MAZZARELLA, L., PULITI, R. & SODANO, G., 1982. Isolation and X-ray crystal structure of a novel bromocompound from the sponges *Axinella verrucosa* and *Acanthella aurantiaca*. *Tetrahedron Letters*, 23 : 767-768.

DE NANTEUIL, G., AHOND, A., GUILHEM, J., POUPAT, C., TRAN HUU DAU, E., POTIER, P., PUSSET, J. & LABOUTE, P., 1985. Isolation and identification of nine pyrrolic metabolites from the sponge *Pseudaxinyssa cantharella*. *Tetrahedron*, 41 : 6019-6033.

FOOKES, C.J.R., GARSON, M.J., MACLEOD, J.K., SKELTON, B.W. & WHITE, A. H., 1988. Biosynthesis of di-isocyano-adociane, a novel diterpene from the marine sponge *Amphimedon* sp. Crystal structure of a monoamide derivative. *Journal of the Chemical Society Perkin Transactions*, 1 : 1003-1011.

FROMONT, J., 1993. Descriptions of species of the Haplosclerida (Porifera : Demospongiae) occurring in tropical waters of the Great Barrier Reef. *The Beagle, Records of the Northern Territory Museum of Arts and Sciences*, 10 (1) : 7-40.

GARSON, M.J., 1986. Biosynthesis of the novel diterpene isonitrile di-isocyano-adociane by a marine sponge of the *Amphimedon* genus : incorporation studies with sodium [^{14}C] cyanide and sodium [2-^{14}C] acetate. *Journal of the Chemical Society, Chemical Communications*. 1986 : 35-36.

GARSON, M.J., 1994. The biosynthesis of sponge secondary metabolites : Why it is important. *In* : VAN SOEST, R.W.M., VAN KEMPEN, Th.M.G. & BRAEKMAN, J.C. (Eds), Sponges in Time and Space. Proceedings of the IVth International Porifera Congress. A.A. Balkema, Rotterdam, pp. 427-440.

GARSON, M.J., ZIMMERMANN, M.P., BATTERSHILL, C., HOLDEN, N., & MURPHY, P.T., 1994. The distribution of brominated long-chain fatty acids in sponge and symbiont cell types from the tropical marine sponge *Amphimedon terpenensis*. *Lipids*, 29 (7) : 509-516.

HAJDU, E. & DESQUEYROUX-FAÚNDEZ, R., 1994. A synopsis of South American *Mycale* (*Mycale*) (Poecilosclerida, Demospongiae), with description of three species and a cladistic analysis of Mycalidae. *Revue suisse de zoologie*, 100 (3) : 563-600.

HOOPER, J.N.A. & BERGQUIST, P.R., 1992. *Cymbastela*, a new genus of lamellate coral reef sponges. *Memoirs of the Queensland Museum*, 32 (1) : 99-137.

LÉVI, C., 1983. *Pseudaxinyssa cantharella* n. sp., Démosponge Axinellidae du lagon de Nouméa (Nouvelle-Calédonie). *Bulletin du Muséum national d'histoire naturelle*, 4^{e} sér., 5, section A, (3) : 719-722.

SOEST, R.W.M. VAN, DIAZ, M.C. & POMPONI, S.A., 1990. Phylogenetic classification of the halichondrids (Porifera, Demospongiae). *Beaufortia*, 40 (2) : 15-62.

Rob W. M. VAN SOEST
Institute for Systematics and Population Biology
(Zoölogish Museum)
University of Amsterdam
PO Box 94766, 1090 Amsterdam, The Netherlands

Ruth DESQUEYROUX-FAUNDEZ
Muséum d'Histoire naturelle, CP 6434
CH-1211 Geneva 6, Switzerland

Anthony D. WRIGHT
Department of Pharmacy
Swiss Federal Institute of Technology Zurich
CH-8057 Zurich, Switzerland

Gabriele M. KÖNIG
Institut für Pharmazeutische Biologie
Technische Universität Braunschweig
D-38106 Braunschweig, Germany