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## Towards an understanding of the shallow-water holothuroid (Echinodermata: Holothuroidea) fauna of the western Indian Ocean

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**Source:** Summary of PhD

The study of the Holothuroidea, commonly known as sea cucumbers, started some 23 centuries ago when Aristotle defined them as "a kind of motionless marine organisms". Only in the mid sixteenth century were holothuroids recognised as animals *per se*. Nowadays, Holothuroidea is firmly recognised as one of the five extant classes of echinoderms. Currently some 1600 species are described; these occur from the intertidal to the deep ocean trenches and from the polar to the tropical regions.

The ultimate aim of this dissertation is to understand the shallow-water holothuroid biodiversity of the western Indian Ocean, the area stretching from Suez to Cape Town and from the East African coast (Red Sea and Persian Gulf included) to 65 degrees East. To attain this goal, several expeditions to two contrasting regions of the western Indian Ocean (the tropical coast of Kenya together with Pemba Island in northern Tanzania and the subtropical coast of KwaZulu-Natal in the northeast of the Republic of South Africa) were undertaken. The purpose of these was to assemble a representative collection of species.

An extensive part of this dissertation is concerned with the construction of a reliable and up-to date faunistic list of the holothuroid fauna of these two case areas. The faunistics of the rest of the western Indian Ocean was filled in with the aid of important collections from the Seychelles and Inhaca (which were deposited as largely unidentified material in the collection of the Royal Africa Museum, Tervuren, Belgium), with detailed study of virtually all the available literature as well as with numerous

loans of specimens from museums worldwide. In the course of the construction of this species inventory, my colleagues and I discovered several species and one genus new to science. We, however, did not blindly follow the standing biological classifications when describing our findings. *Au contraire*, while constructing the species lists we took great care to critically question the employed classifications. This attitude resulted in the taxonomic revision of the *Holothuria* subgenus *Mertensiothuria* and of the holothuriid genus *Labidodemas*. Interestingly, the type species of the latter genus revealed itself as a cryptic species. We were rewarded with the discovery of two additional new species.

Throughout this process, the historical opinions towards holothuroid taxonomy and systematics were never denied. This approach enabled us to revive methodically ignored characters such as the ossicles from the musculature. These characters proved not only diagnostic in the recognition of taxa, but also were informative in terms of recovery of phylogenies. By using our rejuvenated insights into such (and other) characters we were able to construct a large, morphology-based dataset, which allowed the recovery of the phylogeny of the Holothuriidae, the family best represented in this work. This cladistic analysis not only revealed that *Labidodemas* was indeed monophyletic (as suggested by our earlier systematic revision), but also allowed us to state that it has arisen from within the (now paraphyletic) genus *Holothuria*. As such, *Labidodemas* is evolutionary much younger than generally assumed. Our phylogenetic studies further suggest a close relationship between the genera *Actinopyga*, *Bohadschia*

and *Pearsonothuria*, but unfortunately the recovered support proved low. Future studies (see also annex on CD Rom) will have to decide whether a new classification of the Holothuriidae is desired.

Taking all these caveats into account, we finally succeeded to construct the wanted faunistic list of the Holothuroidea from the western Indian Ocean. This list was then further used to analyse the patterns of

biodiversity by means of cluster analysis on several b-diversity coefficients and parsimony analyses of endemism. These analyses showed that the investigated holothuroid fauna of the western Indian Ocean is non-homogeneous and best split into several biogeographic units. These can be explained with (i) species' dispersion ability, (ii) the prevalent current patterns and (iii) the recent geological history.

### Phylogeny of the Holothuriidae (Echinodermata: Holothuroidea) inferred from morphology

W. Appeltans

Source: MSc thesis, Vrije Universiteit Brussel. 94 p. 2002

Despite the efforts of numerous notable taxonomists, the taxonomy and systematics of the grand family Holothuriidae (Holothuroidea: Aspidochirotida) remain vague for some groups. As such, a cladistically substantiated phylogeny based upon clearly defined morphological characters can bring insights into the evolution of the holothuriids. Thus, here I present a phylogeny of the group based on morphological characters. Cladistic analysis was performed by an heuristic search under the maximum-parsimony optimality criterion. 27 ingroup taxa, representing all currently recognised genera and subgenera and 6 outgroup taxa, belonging to the closely related Stichopodidae and deep-sea Synallactidae, were scored for a total of 68 characters concerning gross external and internal morphology and ossicle assemblage in the body wall, the tentacles and the

tube feet. Moreover, often ignored body parts such as longitudinal muscles, cloacal muscles and anal papillae were examined for the presence of ossicles. Preliminary analysis revealed that for the synallactid genus *Mesothuria*, the species used as outgroup (e.g. *M. sufflava*) clustered in the ingroup. As it is here argued that this is the result of erroneous identification, *Mesothuria* was omitted as outgroup taxon. On the other hand, two genera of the Stichopodidae successfully lent themselves as outgroup. For the Holothuriidae, the monophyly of the genera *Actinopyga*, *Bohadschia* and *Labidodemas* was confirmed. *Labidodemas*, however, clustered in the paraphyletic lineage *Holothuria*. The enigmatic genus *Pearsonothuria* turned out to be the sister group of *Actinopyga*. From the resulting phylogeny, inferences on character evolution are made.

### Contribution to the study of the relations between fish of the family of Carapidae and their holothurian hosts

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Source: PhD Thesis, Université de Liège, Laboratoire de Morphologie Fonctionnelle et Évolutive, 4000 Liège Belgium

A remarkable example of association between animals is this one of Carapidae fish (Ophidiiformes) and different invertebrates. These fish are known as pearlfish. The origin of this name would be the discovery of dead carapid fish, paralysed and completely covered in mother of pearl in the inner face of the valves of the shell of certain oysters (Ballard 1991). The fish belonging to the genus *Onuxodon*, *Carapus* and *Encheliophis* are capable of penetrating and residing inside different invertebrates such as sea cucumbers, sea stars, bivalve molluscs and ascideans.

In this thesis, a multidisciplinary approach was realised to understand the biology of the Carapini (*Carapus* and *Encheliophis*) fish living inside holothurians and to highlight the various factors which make these associations possible.

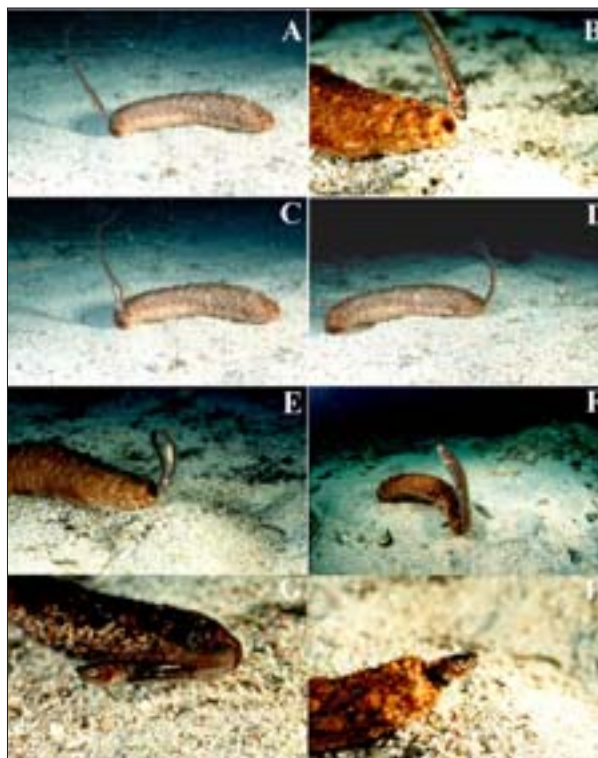
The morphofunctional study of the bucco-pharyngeal apparatus, the stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ), the stomach content determinations and the study of otolith increments allow to define clearly the commensal behaviour of *Carapus* and the parasitic behaviour of *Encheliophis*, the latter eating the gonads and the respiratory trees.

At the tenuis stage, the *Carapus* larvae leave the pelagic area and may enter an holothurian host for the first time. Once inside the sea cucumber, the fish undergo a drastic metamorphosis with a spectacular 50-60 per cent reduction of the body length. The study of the larval development (axial skeleton and otoliths) reveals that the association between the fish and the host is obligatory as the fish needs its host to initiate its metamorphosis. However, the

host is used as a black box and not as a source of food.

Three Carapini fish (*C. boraborensis*, *C. homei* and *E. gracilis*) have also show the ability to emit sounds and the structures (skeletal, muscular and of the swim bladder) responsible were identified and described. In the framework of the present study, sounds are not likely to be used by the fish to identify the presence of an eventual congener in the holothurian before penetration but are produced in the presence of the congener, inside the holothurian. Indeed, no sound has been recorded while the fish were approaching the potential host or while penetrating the unoccupied holothurian.

The sea cucumber cloaca is located close to the current of water exhaled, coming from the respiratory trees. Two strategies have then been observed. (1) The fish can penetrate into the host head first, propelling itself by violent strokes of the tail; (2) it places its head at the entry to the host anus and brings its thin caudal extremity forwards, alongside the fish body, at the level of the lateral line. This position allows the fish to use its body as a guide to rapidly bring the tail towards the cloaca and have it penetrate when the exhalant current is emitted by the host. Once the caudal extremity of the fish is inside the sea-cucumber, the Carapidae redresses itself and enters the host with backwards movements. The penetration head first is realised when the cloaca is wide-opened and the penetration tail first is used when the cloaca is closed. Because the host needs to breathe, it can not avoid the fish entrance.



*A pearl fish (Carapus acus) penetrates a sea cucumber*  
Photo: Daniel Bay (Capri, Mediterranean Sea, 1997)

The sticking principle of the Cuvierian tubules does not have an effect on pearlfish and the latter shows a better resistance to the sea cucumber toxins than other reef fish. The system of defence, however, is still unknown.

### Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific

S. Uthicke and J.A.H. Benzie

Source: *Molecular Ecology* 12(10):2635–2648

The sea cucumber, *Holothuria nobilis*, has a long-lived planktotrophic larvae, and previous allozyme surveys have suggested that high dispersal is realised. In contrast, recent ecological studies indicate that dispersal is low. To reconcile these data, and to investigate the evolution of this Indo-Pacific species, we screened geographical variation in 559 bp of a mitochondrial gene (*COI*) in 360 samples from the Australasian region and La Réunion. Sequences from La Réunion differed by > 7% from others and may constitute another species. Haplotype diversity in other samples was high (0.942, SD = 0.007), but haplotypes were closely related (mean nucleotide diversity: 0.0075, SD = 0.0041). Anova, pairwise *F<sub>ST</sub>* values and exact tests did not detect significant population structure. Nested clade analysis showed that

one of two main clades was over-represented in west Australia, whereas the other was more common in the northern Great Barrier Reef. Isolation-by-distance was identified as the main determinant of population structure at several clade levels. Contiguous range expansion was inferred for evolutionary older clade levels and this may correspond to a late Pleistocene (88,000–193,000 years ago) population expansion inferred from haplotype mismatch distributions. Thus, the population genetic structures detected are likely to be formed prior to the last ice age, with some indications for high dispersal on shorter time scales.

## Evaluation of the resource following the sea cucumber fishery of Saipan, Northern Mariana Islands

M. S. Trianni

**Source:** Proceedings 9th International Coral Reef Symposium, Bali, Indonesia 23–27 October 2000, Vol.2; 829–835.

A sea cucumber fishery targeting the surf redfish, *Actinopyga mauritiana* and the black teatfish, *Holothuria whitmaei*, occurred on the island of Saipan in the Commonwealth of the Northern Mariana Islands (CNMI) during 1996 and 1997. A pre-harvest stock assessment was not conducted and the fishery was managed based on catch-effort statistics. The fishery was temporarily halted in early 1997 due to declining CPUE. A subsequent analysis of catch-effort statistics was conducted using three depletion models; the Leslie, DeLury, and an unbiased likelihood estimator derived from the Leslie, termed the Akamine model. These models indicated that the remaining population numbers in the fishery man-

agement units were considerably harvested, with 78 to 90 per cent of the initial population sizes taken. The fishery was subsequently shut down and a post-harvest survey conducted by the CNMI Division of Fish and Wildlife supported the depletion model analysis results. Results from the depletion models varied, with the Leslie and DeLury failing to produce valid results for all management units. The Akamine model was preferred for any future depletion estimation analyses, although a pre-harvest stock assessment along with the collection of harvest statistics was concluded essential for coherent management.

## The genus *Stichopus* (Echinodermata: Holothuroidea) from the Johore Marine Park (Malaysia) with the description of two new species

C. Massin, Y. Zulfigar, A. Tan Shau Hwai and S.Z. Rizal Boss

**Source:** Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie, 72: 73–99, 2002

The study of the holothurian genus *Stichopus* from the Johore Marine Park (Malaysia) revealed at least six different species, two being new to science: *Stichopus ocellatus* n. sp. and *Stichopus rubermaculosus* n. sp. Each species is fully described and illustrated. For the known species a list of records and the geo-

graphic distribution are given. A seventh species, *Stichopus monotuberculatus*, not yet recorded from the Johore Marine Park, but likely to be found there, is included in an identification key based on ossicle characters, colour patterns and behaviour.

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**ABSTRACTS FROM THE 6th INTERNATIONAL ECHINODERM CONFERENCE**

The following abstracts of oral communications and posters related to holothurians have kindly been provided by Pr Thomas Heinzeller: [heinzeller@anat.med.uni-muenchen.de](mailto:heinzeller@anat.med.uni-muenchen.de) [Ed.: Abstracts have been reproduced as is]

**Behavioural-ecological, morphological, and mtDNA evidence to resurrect *Bohadschia bivittata* (Mitsukuri) from the *B. marmorata* (Jaeger) species complex**

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Behaviour, habitat selection, body size, colour, spicule complexity and mtDNA were used to examine taxonomic relationships among specimens from the *Bohadschia marmorata* species complex. Spicules were more highly branched, perforated, and spiked in *bivittata* specimens than those of the classic *marmorata* type. Moreover, spicule complexity did not correlate with body size. Phylogenetic analyses of partial nucleotide sequences of 16s and 12s ribosomal genes were done with the hypothesis that cladistic groupings would reflect morphological similarity instead of geographic proximity. This hy-

pothesis was supported by the clustering of *bivittata*-looking specimens despite being from different Micronesian islands. The resulting tree (*Pearsonothuria graeffei* (*B. marmorata*) (*Bohadschia argus* (*B. bivittata*))) indicated that *marmorata* and *bivittata* are not even sister species, with *bivittata* more closely related to *argus*. These results were corroborated by colour and spicule examinations made of specimens from this complex in the NMNH (Washington, DC, USA) collection and by behavioural observations made in the field.

**A study of the biology for fishery in two populations of *Cucumaria frondosa*: in the Gulf of Maine (USA) and in the Barents Sea (Russia)**

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*Cucumaria frondosa* has been involved in the commercial fishery in some localities: the East coast of Canada, the Gulf of Maine (USA), the Barents Sea (Russia). Despite some series of the investigations in the biology and ecology of *C. frondosa*, we still know very little that to evaluate the extent to which the population can withstand sustained fishery pressure. Impacts of the fishery to the sea cucumber resources and effectiveness of the current regulation remain unknown.

To prevent overfishing, a rational management is essential for long-term harvesting of *C. frondosa* populations. The rational management of valuable re-

sources means not just sustainable fishery approach but it must be coupled with complete utilisation, and both of them are based on thorough research. In fact, a sustainable fishery is based on three foundation: biology, ecology and fishery management. Hence, certain information on the biology and ecology of the sea cucumber is needed for developing sound stock assessment models.

Results of the recent research in the taxonomy, biology, ecology, fishery and utilisation of *C. frondosa* in the Barents Sea (Russia) and the State of Maine (USA) will be presented and discussed.

## Evolutionary diversification of Holothuroid ecologies on coral reefs

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An organism's potential to adapt to a novel environment depends in part upon its history of evolving features needed in previous environs. Aspidochirote and apodan holothuroids are diverse and ubiquitous groups of echinoderms inhabiting a derived habitat, scleractinian coral reefs. These two groups provide replicate radiations into an identical ecosystem, allowing one to test the question: How has the evolutionary history of organisms prior to their expansion in the tropics constrained or facilitated their diversification onto coral reefs? Ancestral character reconstructions using ecological data and a new phylogeny based on morphology and several

molecular markers suggest that aspidochirote coral-reef sea cucumbers evolved from deepwater epibenthic ancestors, while reef apodans arose from littoral infaunal forms. Much ecological novelty evolved in situ as species expanded into the numerous new microhabitats afforded by coral reefs. Associations between habitat choice and diel patterns of activity have evolved multiple times. Finally, despite high local species diversity in the tropics, expansion onto coral reefs does not appear to have been an adaptive radiation facilitated by the evolution of a "key innovation," as diversification was not accompanied by an increase in net speciation rates.

## Phylogeny of Holothuroidea based on 18S and histone 3 DNA sequences

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We present a phylogeny of 30 species of Holothuroidea based on PCR-amplified complete 18S-like ribosomal and partial histone-3 gene sequences. Estimated maximum parsimony and maximum likelihood topologies using POY direct optimisation strategies are largely congruent with a previous phylogeny based on morphological characters. For example, as first hypothesised by Carl Semper in 1868, Apodida (as currently defined) is sister to all other holothuroids. Molpadida is sister

to Dendrochirotida. The new estimates also differed in several important ways. Synallactidae, a morphologically diverse group, appears to be paraphyletic. Also, surprisingly, the deepwater order Elaspodida appears polyphyletic with the deimatids grouping within synallactids at the base of the aspidochirote clade. Points of contention are defined by short internal branches co-eval with a rapid radiation of holothuroids and other marine organisms in the early Mesozoic.

## Development of an apodous holothuroid, *Oestergrenia variabilis* (Theel, 1886)

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Holothuroids consist of about 1250 species; 215 belong to the order Apodida. Larval development has been reported in less than 10 apodous holothurians. Both indirect and direct development occur, the former with an auricularia larva and the latter with a doliolaria. Among apodids with the doliolaria, brooding is known.

Spawning of an apodous holothuroid in the laboratory occurred on the morning after collection from about 25 m depth in Tsukumo Bay, Ishikawa on 11 December 1991. This holothuroid which has been identified by D. Pawson as *Oestergrenia variabilis*

(Family Synaptidae) is hermaphroditic and natural self-fertilisation takes place. Ova are orange in color and 200 µm in average diameter. Four and a half hours after spawning the 8-cell stage is reached at 15°C. Cleavage is radial and equal and the resulting blastula is holoblastic. Gastrulation begins by invagination approximately 10 hr after spawning. Embryos develop into doliolaria larvae with 4 transverse ciliary bands 2 days after spawning. The larvae are ca. 400 µm in length and 250 µm in width. One day later, formation of the calcareous ring is apparent. Five primary tentacles are visible, confirming the onset of the pentactula stage. At this stage

ciliary bands begin to degenerate. At about 15 days, pentactulae stop swimming and sink to the bottom, signalling the completion of metamorphosis. Metamorphosed juveniles move on the substratum

by using the tentacles. They are about 400 µm in length and 180 µm in diameter. Present study shows that development of this species is direct and non-brooding.

### The pattern of Vietnamese holothurians symbionts interactions

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Shallow-water stichopodids and holothurids very often are inhabited by a lot of animals from different taxa. That implies the appearance of various kind of intra- and interspecific interactions among symbionts. To reveal the pattern of these interactions the diets, injuries, distributions on hosts and locations on the holothurians body were studied in crabs *Lissocarcinus orbicularis*, polychaetes *Gastrolepidia clavigera*, fishes *Carapus homei* and *C. mourlani* and shrimp *Periclimenes imperator*. The regular distribution of symbionts and the traumas suggests the territoriality in these species. The crabs feed on the free-living bottom organisms and were considered as commensals. The polychaetes feed on the host tissues and associated crustaceans, so their relation-

ships with holothurians are parasitic ones. The main food items of fishes were free-living crustaceans and carapids juveniles indicating on commensal interactions with hosts. Symbionts occupy the different parts of holothurians bodies. Crabs were found mainly in cloaca or among oral arms, polychaetes and shrimps were located on the surface of holothurians and fishes inside the hosts. Thus, symbionts diverge to the different trophic niches and locations. The frequency of co-occurrence of symbionts from different taxa didn't differ significantly from the random. It means that the competition for food and space between different symbionts species is minimal. The interactions among symbionts could be characterized as neutralism.

### Feeding rate and impact of sediment reworking by two deposit feeders *Holothuria leucospilota* and *Holothuria atra* on a fringing reef (Reunion Island, Indian Ocean)

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In order to investigate the quality and the quantity of sediment fed by deposit-feeders holothurians, experiments were conducted on the common species *Holothuria atra* and *Holothuria leucospilota* at two stations of Reunion fringing reefs. A relationship has been established between enrichment level of the area and holothurians densities: holothurians are abundant (densities up to 3 ind m<sup>-2</sup>) in dystrophic areas whereas low densities characterise oligotrophic areas. During field observations, *H. atra* and *H. leucospilota* consumed an average quantity of sediment of 79.7 g dw ind<sup>-1</sup> d<sup>-1</sup> and 88.8 g dw ind<sup>-1</sup> d<sup>-1</sup> respectively in both stations. We showed that a mixed population of both species in a dystrophic area can rework 82 kg dw m<sup>-2</sup> y<sup>-1</sup>, whereas in the oligotrophic area 3 kg m<sup>-2</sup> are reworked. There was no significant difference in sediment organic matter between stations. Nevertheless, high densities

found in dystrophic area indicate biomass storage of benthic production. Gut content analysis showed that the organic matter ingested from the sediment was used with 10 per cent efficiency for both species. C/N ratio decreased along the gut, showing organic matter degradation. It may show the ability of these holothurians to transform organic matter, and make it easily available for other organisms from the sediment. In both species, organic carbon and chlorophyll-a content in the oesophagus were significantly higher than the concentrations in the sediment sampled directly in front of the individuals, suggesting selectivity by both species. The bioturbation activity of these species displays two aspects: on one hand, the nutrition activity will have an impact on bacterial and diatoms populations; on the other hand, perpetual reworking of the sediment will forbid endofauna settlement.

## Gut formation during development and regeneration in the holothurian *Eupentacta fraudatrix* (Holothuroidea, Dendrochirota)

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It seems to be of great importance to compare morphogenetic processes involved in embryonic development and regeneration in the same animal species. Regeneration often results in the formation of the body part, which is very similar or identical to that formed during embryonic development. If the result is the same, one could ask whether the morphogenetic mechanisms underlying both these processes are also the same. In order to throw some light on this problem, we investigated the formation of the gut during embryonic development and regeneration in juveniles and adults of the holothurian *Eupentacta fraudatrix*. Our results may be summarised as follows. 1) The digestive epithelium (even the cuticular lining of the anterior gut parts) is

endodermal in its origin. 2) In regenerating gut of the transversely bisected juveniles, the digestive epithelium and mesothelium are restored at the expense of their respective cells. The most important morphogenetic events are proliferation of the differentiated cells in the digestive epithelium, and dedifferentiation followed by mitotic division, migration and redifferentiation in the mesothelium. 3) In the eviscerated adult specimens of *E. fraudatrix*, the digestive epithelium regenerates from the mesothelium, which covers the thickening at the torn edge of the mesentery. Thus, there are clear differences between the development and regeneration of the gut in *E. fraudatrix*.

## Temporal change in density and biomass of five aspidochirotid holothurians species (Holothuroidea: Echinodermata) inhabiting the *Posidonia oceanica* meadow of the Sidi Fredj peninsula (Algeria)

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The "deposit feeders" aspidochirotid holothurians species represent the major component of the *Posidonia oceanica* ecosystem in the Mediterranean sea. Few studies have been undertaken on their life cycle and the factors influencing their density and biomass.

The purpose of this work was to evaluate and follow monthly (from March 1995 to February 1996) the variations of the densities and biomasses of five holothurians species namely, *Holothuria* (*Holothuria*) *tubulosa*; *Holothuria* (*Lessonothuria*) *polii*; *Holothuria* (*Holothuria*) *stellati*; *Holothuria* (*Panningothuria*) *forskali* and *Holothuria* (*Platyperona*) *sanctori* occurring in one shallow water area characterised by an homogenous *Posidonia oceanica* meadow. The studied area is about 500 m<sup>2</sup> and present a depth of 3–9 m. This evaluation is justified by the part played by these species in recycling of the organic matter by ingestion of the sediment layer and/or the bottom wreck.

The sampling was done by mean of scuba diving and consisted of counting and measuring individuals of each species using the Quadrat method. The parallel analysis of the size structures and the densi-

ties/ biomasses cycles showed that the densities and the biomasses evolve in the same way for all the studied species. The two parameters present a spring peak, generally represented by visible individuals of big size, which arrives close to the surface for the reproduction. A reduction of the mean values of density and biomass in summer period would seem to correspond to the migration in depth and by the escapement of young individuals from the studied area. The minimal values observed in fall and winter corresponds to the species migration in depth (where the hydrodynamism is weak) and to a notable mortality caused by the high intensity of hydrodynamism in the area.



## Effect of delayed metamorphosis and settlement on the survival and size structure of cultured juvenile *Holothuria scabra* (Holothuroidea:Aspidochirotida)

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The settlement to early juvenile stage of the cultured sea cucumber *Holothuria scabra* is an interesting phase of its life cycle. As in many marine invertebrates with complex life cycle, this is a critical phase when along with developmental changes, shifts in lifestyle and habitat take place. Considerable mortality usually happens at this transition from planktonic to early benthic stage with consequence on the abundance, growth rate and size-structure of the benthic population. In the context of the culture of this species, events in these stages of its life cycle could have implications in the quantity and quality of the yield.

We looked closely at the effect of the timing of addition of the metamorphic and settlement cue on the survival, recruitment to visible size and size structure of juvenile yield from a batch of cultured *H.*

*scabra*. The timing of addition of the cue was critical to post larval survival and recruitment rate. A "9-day delay" in cue addition (at Day 20) gave low juvenile yield (6% + 10) and low recruitment rate. Interestingly, more visible recruits were seen in this treatment early in the monitoring period of recruitment but this was followed by mass mortality. Recruitment to visible size was sustained in both "no-delay" (addition at Day 11) and "4-day delay" (addition at Day 15) treatments. The recruitment rate was higher when cue addition was not delayed while juvenile yield were nearly equally high in both "no-delay" (21% + 6) and "4-day delay" (23% + 20) treatments. However, the size structure in the "no-delay" treatment was more skewed towards the larger sized juveniles compared to the "4-day delay" treatment indicating post-larval performance differences other than mortality.

## Zoogeography of the shallow-water holothuroids of the western Indian Ocean

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Understanding patterns of biodiversity always involves gross abstractions because these patterns are subject to a correlate of causal parameters that operate at different scales. At the local-scale, ecologists advocate that the assembly of faunas is shaped by biological mechanisms such as migration, recruitment, predation, competition, extinction and speciation, while at the macro- or geographical-scale biogeographers call mostly upon climate or historical explanations to explain biodiversity patterns. The present study provides the first attempt to describe and understand the biodiversity of the shallow-water holothuroids (as defined by the 50-m isobaths), at the geographical-scale of the western Indian Ocean (here defined as the area ranging from Suez to Cape Town and from the coastline of East Africa up to 65° East). Cluster analysis on several biodiversity coefficients and parsimony analyses of en-

demicity revealed that the western Indian Ocean is best split in (at least) three biogeographic realms: the Red Sea and associated Arab Basin, an asymmetrical circumtropical region stretching from the horn of Africa to southern Mozambique and the temperate Cape province. The subtropical Natal Basin acts as the bleeding zone between the tropical and temperate provinces. The zoogeography of western Indian Ocean holothuroids is here explained with (i) species' dispersion ability, (ii) the prevalent current patterns and associated up-and downwellings, and (iii) recent geological history. As the ghost of over-harvesting has in recent years also reached the East African Coast, the here proposed zoogeographic scenario will aid in the recognition of local regions that are important to the generation and maintenance of biodiversity.

## Revision of the genus *Synallactes* (Holothuroidea: Synallactidae)

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The holothurian family Synallactidae Ludwig, 1894 holds mostly deep-sea forms and it is the least-studied large taxa among the deep-sea cucumbers. The synallactids are one of the most characteristic animals of the deep ocean. They appear repeatedly in photographic collections of abyssal megafauna. Many of these photographs show their characteristic tracks and faecal remains providing evidence of their important role in modifying the sediment landscape and in structuring the communities that live within it. The Synallactidae, as presently recognised, comprises approximately one hundred and thirty one species currently named.

The genus *Synallactes* Ludwig, 1894, embraces approximately twenty-two species. As far as we know, five of these species occur in the Atlantic Ocean, the rest inhabit the Pacific (11 species), Indian Ocean (4 species) and Antarctic Ocean (2 species).

The systematic status of certain species within the genus remains somewhat confused. The purpose of this work is to describe a new species of *Synallactes* from the Southeast Atlantic and clarify some taxonomic problems within the genus.

## Comparative analysis of the spicular shape of the six species of the cucumariids (Echinodermata: Holothurioidea)

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We worked out calculus of approximations for quantitative estimation of the spicular shape of cucumariids (Stepanov, Gaidaeu and Levin 2001). To calculate quantitative characteristics, a computer programme was created that digitize the ossicle graphic image, create digital data file and estimate necessary parameters before stocking them into the database. We carried out computer analysis of the spicular shape of the six species cucumariids: *Cucumaria frondosa*, *C. japonica*, *C. savelijevae*, *C. djakonovi*, *C. conicospermium* and *C. levini*. Two of them *C. conicospermium* and *C. levini* were described in 2002 (Levin and Stepanov 2002; Stepanov and Pil'ganchuk 2002). We calculated quantitative characteristics of spicular shape using different features as: oblongness (factor Rilay sphericity  $K_{sr}$ , parameter of the shape  $K_{sh}$ , parameter of elliptic  $K_e$ ), ridgi-

ness of edge (index of circularity  $K_c$ , factor of ridginess  $K_r$ ), and relative area of holes  $Q_{rel}$  (Shvanov 1969; Victorov 1986; Gudimova 1991, 1999; Stepanov, Gaidaeu and Levin 2001). For each quantitative characteristics of spicular shape, the minimum, maximum, mean, standard error of mean, mode and standard deviation were estimated. All quantitative characteristics were tested on normalcy of distribution using the Kolmogorov-Smirnov one-sample test. After this, the difference between the quantitative characteristics of all species was estimated by Kolmogorov-Smirnov two-sample test and t-test for independent samples. The analysis displayed differences among the examining species on characteristics of the spicular shape as oblongness, ridginess of edge and relative area of holes.

## Shallow-water Holothuroid biodiversity and biogeography of the subtropical east coast of South Africa

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Because of market demands for beche-de-mer, holothurian stocks of the south-western Indian Ocean, notably Madagascar, the Mascarene Islands, Mozambique and the subtropical east coast of South Africa, have all become vulnerable. Of the approximately 150 shallow-water species of holothuroids recorded from these four regions, about 50 species occur on the east coast of South Africa, as far as Port

St. Johns (ca. 31.5°S). An attempt is made here to compare the holothuroid faunas of the latter region with that of Mozambique and West Madagascar on the one hand and Mascarene Islands and East Madagascar on the other, in order to determine the degree of shared similarities, using several analyses. It is concluded that the holothuroid fauna of the South African east coast, at least as far south as Port

St Johns, has a mixed origin, being derived from migrating tropical Indo-West Pacific species from east Madagascar and the Mascarene Islands, under the influence of the East Madagascar current and from Mozambique and West Madagascar, under the influence of the Mozambique Current; some warm temperate species from the south, due to the influence of the northward-bound counter-current; and a fairly high level of local endemics (ca. 15%). If southern Mozambique (south of 20°S) is rightly in-

cluded in the southern African subtropical region, the level of local endemism increases to ca. 20%, making the southern African east coast region a definite zoogeographical province. Despite a high percentage of Indo-West Pacific species (ca. 70%), the shallow inshore aspidochirotid fauna of the east coast of South Africa is poorly established, a situation, in combination with the establishment of marine reserves and strict ordinances, makes harvesting not only uneconomical but well nigh impossible.

### DNA fingerprinting and repeated surveys suggest very slow growth and recovery in over-fished holothurians (*Holothuria nobilis*) on the Great Barrier Reef

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To investigate the potential for recovery of over-fished beche-de-mer stocks on the Great Barrier Reef (GBR), field surveys were conducted on reefs before and after fishery closure. Total virgin biomass was modelled and compared to the total amount fished. In addition, individual growth rates were estimated using a novel technique involving individual recognition by DNA fingerprinting. Initial surveys on over 60 reefs of the GBR indicated that densities of *H. nobilis* were reduced in the sectors fished for beche-de-mer. This trend was not apparent in other species observed on the reef flats. Two years after fishery-closure for *H. nobilis*, no recovery of stocks on reefs previously open to fishing was observed. Densities on reefs protected from fishing since the onset of the fishery in the mid 1980s remained on a level several times higher than on fished reefs. It was calculated that the virgin biomass

(in the main fished area between 12° and 19° S) was about 5500 t, which is now reduced to about 2500 t. The reduction is in the same order than the total amount fished between 1987 and 1999 (2000 to 2500 t). DNA analysis of repeated samples on three locations indicated high recapture rates of fingerprinted and released individuals of *H. nobilis*. Fitting growth curves with Francis's growth function indicated that medium sized individuals (1 kg) grew at 35–533 g yr<sup>-1</sup>, whereas large animals (2.5 kg) consistently shrank and no recruitment was observed. In combination, these data indicate that production of *H. nobilis* stocks is very low, presumably with low mortality, low recruitment and slow individual growth rates. Consistent with anecdotal evidence, recovery of GBR *H. nobilis* stocks may take several decades and a highly conservative management is suggested.

## ABSTRACTS OF POSTERS FROM THE 6th INTERNATIONAL ECHINODERM CONFERENCE

### The white spot disease in cultivated juveniles of *Holothuria scabra* (Echinodermata)

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It is frequently reported that cultivated holothuroids can suffer from a disease affecting their integument. We report here on a disease of juvenile *Holothuria scabra*, the most marketed edible sea cucumber, reared in the Aqua-Lab hatchery of Toliara, Madagascar. This disease, which has been called the white spot disease, is very contagious and is due to a severe bacterial infection that causes death within three days. The first sign of the infection is a white spot that appears on the integument of individuals, close to the cloacal aperture. The spot extends quickly

onto the whole integument leading to the death of individuals. Microscopic (histology and S.E.M.) and biomolecular (D.G.G.E. and sequencing) techniques have been used to describe the lesions and to investigate the infecting microbial communities. The white spot lesions consist in a zone where the epidermis is totally destroyed and where collagen fibres and ossicles are exposed to the external medium. This zone is surrounded by a border line where degrading epidermis is mixed with the connective tissue. White spot lesions include three bac-

terial morphotypes: rod-shaped bacteria, rough ovoid bacteria, and smooth ovoid bacteria. The last morphotype is the only one found on the ossicles and is assumed to be responsible of their degradation. Three species of bacteria have been put in evidence in the white spot lesions thanks to biomolec-

ular analyses: *Vibrio* sp., *Bacteroides* sp., and an a-Proteobacterium. Infection assays of healthy holothuroids have been performed from white spot lesions and from bacterial cultures but the etiologic agent (i.e., the initiator of the disease) has not been identified.

### **On two rare abyssal Myriotrochidae (Echinodermata: Holothuroidea: Apodida) new to the South Atlantic: *Siniotrochus myriodontus* Gage and Billett, 1986 and *Lepidotrochus* cf. *parvidiscus* Belyaev and Mironov, 1980**

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In the course of the DIVA I expedition (RV *Meteor*, cruise M48/1) to the Angola Basin (South Atlantic) two rare Myriotrochidae were brought up from abyssal depths — *Siniotrochus myriodontus* Gage and Billett, 1986 and *Lepidotrochus* cf. *parvidiscus* Belyaev and Mironov, 1980. Until now only few specimens of *S. myriodontus* are known from the northeast

Atlantic. The only specimen of *L. parvidiscus* is a posterior fragment from the southern Indian Ocean. These new records for the South Atlantic Ocean indicate a wide geographical distribution of both taxa. The species are described and detailed parameters for the wheel deposits are given.

### **Remarks on some Holothuroidea described by Heller from the eastern Adriatic Sea**

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In 1868 Camil Heller described five new Holothuroidea from the eastern Adriatic Sea. Two of these species – *Thyonidium ehlersi* Heller, 1868 and *Synapta hispida* Heller, 1868 – are still only known from the original description. Recently, some of Heller's specimens were rediscovered in the collection of the Institute of Zoology and Limnology in

Innsbruck. From these specimens *Thyonidium ehlersi* Heller, *Thyone inermis* Heller, *Stereoderma kirchsbergii* (Heller) and *Synapta hispida* Heller are redescribed and their current taxonomic status is discussed. Furthermore, a lectotype is designated for *S. kirchsbergii* and a neotype for *T. inermis*.

### **Population biology of Raine Island and Moulter Cay echinoderms**

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Raine Island and Moulter Cay support a diversity of echinoderms with the most abundant being the ophiuroids and holothuroids. These cays support a high density of the commercially important sea cucumber (beche-de-mer), the black teatfish *Holothuria nobilis* (= *H. whitmaei*). Surveys of the sea cucumber populations were done through an extensive series of manta tows. Belt transects were also undertaken in the seagrass habitat. The standing stock of *H. nobilis* on Raine Island and Moulter Cay is higher than that recorded from many Green Zones elsewhere on the Great Barrier Reef. Manta tows of Raine Island and Moulter Cay indicated that the mean densities

of this species were 36.74 ha<sup>-1</sup> (SE = 9.08) and 19.25 ha<sup>-1</sup> (SE = 7.61). The seagrass habitat showed an extremely high density of *H. nobilis*, average 120.8 ha<sup>-1</sup> (SE = 29.5). Weight measurement revealed that *H. nobilis* in the seagrass habitat were smaller than those in the lagoon, supporting the suggestion that this habitat is an important settlement area for holothuroids. In comparison to fished and unfished reefs elsewhere on the GBR, the data from Raine Island represents the highest densities recorded for *H. nobilis*. A detailed survey of the ophiuroid population from rubble zone on the reef flat of Raine Island was also undertaken.

## An efficient way to evaluate volume and biomass of sea cucumbers through diving surveys

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Biomass can be estimated from organisms wet or dry weight per unit area or their volume per unit area. Estimating sea cucumbers biomass through wet weight represents a problem as animals retain a fair amount of water even after dump drying. Sea cucumbers need to be brought to the surface in order to be weighed or to have their volume estimated. Often, researchers are limited by the number of animals they can bring to the surface, and biomass can be underestimated only by using the weight of a few animals collected at any time. Through a pilot study in Ilha Grande Bay, Rio de Janeiro State, seven reference areas were identified as good sites for working on *Isostichopus badionotus*. A total area of 2200 m<sup>2</sup> was surveyed using SCUBA. The total density of *I. badionotus* was estimated as 0.15 ind. m<sup>-2</sup> (n = 319), but this varied from 0.03 to 0.47 ind. m<sup>-2</sup> (n = 18 to 93 respectively) in the different reference areas. As sea cucumbers are touched,

their first reaction is to contract for a few seconds, enough time for any diver to take length and circumference at the largest ambit measurements. Supposing the shape of a sea cucumber resembles that of a cylinder with two semi-spheres at both ends, the following formula was applied using the length and ambit taken under water:  $V = \frac{\pi}{6} r^2 h - \frac{2}{3} \pi r^3$ , where V = body volume, r = circumference radius, and h = body length. A correlation between wet weight (g) of animals brought to the surface and volume (cm<sup>3</sup>) was then used to validate the above-mentioned volume measurement ( $y = 0.3946x + 140.57$ ;  $r^2 = 0.6926$ ; n = 131). The total biomass for the study area was then estimated as 68.11 cm<sup>3</sup> m<sup>-2</sup>. This method could be applied to other sea cucumber species, causes less stress to animals, and it is a much more efficient way of measuring their volume and biomass.

## Ultrastructural organisation of the radial nerve cord in the holothurian *Eupentacta fraudatrix* (Holothuroidea, Dendrochirotia)

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Most of the available data on the holothurian nervous system comes from the studies of visceral organs. There has been very few information concerning the fine structure of the radial nerve cord and peripharyngeal nerve ring in these animals. The aim of our study was to the ultrastructure of the radial nerve cord in the holothurian *Eupentacta fraudatrix*.

The radial nerve cord is composed of an outer ectoneural part and a smaller inner hyponeural part. The outer and inner surfaces of the cord are surrounded by the epineural and hyponeural sinuses, respectively. The ectoneural and hyponeural parts are separated from the connective tissue by the common basal lamina, which forms deep narrow folds

on both sides of the cord separating the two parts. The separation is incomplete, allowing bundles of nerve processes run from one part to another. The ectoneural and hyponeural parts are similar in their structure: both are neuroepithelia composed of supporting cells and nerve cells. Supporting cells are genuine epithelial cells. Their characteristic feature is the presence of thick bundles of tonofilaments in the basal cell processes. The nerve cells are ciliated. Those neurons, whose cell bodies face the lumen of the hypo- or ectoneural sinuses, are probably receptor cells. Nerve processes are mostly longitudinal, but there are also processes running in other directions. Synapse-like structures were occasionally observed.

## Dexamethasone-induced apoptosis of holothurian *Eupentacta fraudatrix* phagocytes: Role of catalase

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Phagocytes of holothurian *Eupentacta fraudatrix* were isolated by using ficoll-verographine discontinuous gradient. These cells had high levels of activities of such antioxidant enzymes as superoxide

dismutase (SOD) and catalase compared to those in mammals' monocytes. The results of analysing DNA fragmentation by agarose gel electrophoresis have shown apoptosis to be low in the intact phago-

cytes. Dexamethasone at concentrations of  $10^{-7}$ – $10^{-4}$  M, but not of  $10^{-8}$  M, induced apoptosis in 18 hr of incubation. In 72 hr, apoptosis was increased at all concentrations of dexamethasone studied. In 18 hr, dexamethasone ( $10^{-8}$ – $10^{-4}$  M) also activated SOD of phagocytes and inhibited catalase activity in a concentration-dependent manner (maximum 467% and 50% of control, correspondingly, at a dexamethasone concentration of  $10^{-4}$  M). The data about de-

creased level of catalase activity in 18 hr of incubation of the cells with dexamethasone indicate the possible participation of catalase in the mechanisms of hormone-induced apoptosis in holothurian phagocytes. This idea was supported by the fact that apoptosis was reduced in phagocytes treated with hormone ( $10^{-4}$  M) and commercially available catalase ( $0.7 \text{ mkg ml}^{-1}$ ) in 18 hr.

### Functional morphology of the tentacles in the apodid holothuroid *Synapta maculata*

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*Synapta maculata* is a long snake-like holothuroid inhabiting seagrass beds of the Indo-West Pacific. It has 15–16 pinnate tentacles, each comprising a central stem bearing 30–40 pairs of pinnules which are located in a plane tangential to the mouth. The tentacle inner tissues are organised in such a way that the stem and each pinnules can move independently from the others. This makes the tentacles exceedingly prehensile, being able to wrap around seagrass leaves or press against a flat surface. The internal surface of the tentacles (i.e., facing the mouth) is smooth while the external surface is covered with bulges. It is this surface that is applied on the substratum during feeding. The external epidermis is mainly made up of a typical echinoderm duogland adhesive system involved in the catching of

food particles. An additional cell type may also be observed in the tentacular epidermis: the vesicular cells. These cells, packed with large vesicles each containing one spherule, are conspicuous in the tentacle epidermis, being present on both the inner and outer surfaces, but preferentially located on the tentacle margin. It is proposed that vesicular cells could be defensive cells containing a toxic material. The tentacles of *S. maculata* appear therefore to be well adapted for efficient deposit feeding: their large adhesive outer surface and great mobility allow a maximal capture of food particles while the presence in their epidermis of defensive cells would deter tentacle predators and thus allow the animal to optimise its feeding time.

### Estimation of the evolution of the Cuvierian tubules, defense organs in the family Holothuriidae, by the character mapping method and by ultrastructural analyses

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Cuvierian tubules are specialised defense organs occurring exclusively in some holothuroid species from the family Holothuriidae. Within the family, these organs differ greatly in terms of their morphology and their mode of functioning. The goal of this work was to determine the evolutionary path of Cuvierian tubules by the character mapping method and by ultrastructural analyses. A fragment of the mitochondrial genome corresponding to two genes was first sequenced for 20 species of Holothuriidae (3 *Actinopyga*, 3 *Bohadschia*, 12 *Holothuria*, *Labidodermas semperianum* and *Pearsonothuria graeffei*) and the relationships between these species were estimated from the molecular data obtained. The methods used to reconstruct those relationships were the neighbour joining, the maximum parsimony and the maximum likelihood. The consensus phylogenetic tree indicates that: (1) the genus *Actinopyga* is

monophyletic and was the first to diverge from the rest of the family, (2) the second diverging group was a clade comprising the 3 *Bohadschia*, *P. graeffei* and 4 *Holothuria*, (3) within this clade the genus *Bohadschia* is monophyletic, (4) the remaining clade comprises the other species of *Holothuria* and *L. semperianum*, (5) the genus *Holothuria* is paraphyletic. The analysis of the different characteristics of Cuvierian tubules from the viewpoint of this phylogenetic tree strongly suggests that the common ancestor of the Holothuriidae had Cuvierian tubules and that those tubules were ramified, non-adhesive, non-expellable and non-stretchable; that those tubules have evolved to give the non-ramified, adhesive, expellable and stretchable tubules; and that the loss of Cuvierian tubules has occurred several times independently during evolution.

## Reproductive biology displayed by two symbiotic copepods of holothuroids

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Nearly half of the species of copepods are symbionts. These copepods have developed a wide range of morphofunctional and ecological adaptations. More particularly, the life cycle and the reproductive strategies are indicial of the degree of specificity of the symbiosis. This work investigates on a comparative basis two species of symbiotic copepods whose biology is contrasted, namely, (1) *Synaptiphilus luteus*, an ectocommensal living on the tegument of the burrowing synaptid *Leptosynapta inhaerens* and (2) *Allantogynus delamarei*, an endoparasite of the coelomic cavity of the epifaunal aspidochirote *Holothuria tubulosa*. Analysis of the life-

cycle of *S. luteus* enabled the identification of two nauplii and four copepodite stages, it also revealed, by the seasonality of sex-ratio and fecundity, that a complex mating behaviour could have evolved in that species. In *A. delamarei*, whose cycle remains largely undescribed, the discovery of a potential male specimen, unknown so far, and the observation of a peculiar sensory apparatus on the first copepodite, bring new hypothesis regarding the life-cycle of this highly modified species. Moreover, the morphological comparison of mature females leads to reinterpret the structural organisation of the ovigerous sac in the two species.

## Parasitic turbellaria in *Holothuria tubulosa*, *Holothuria forskali* and *Cucumaria planci* in the northern Adriatic Sea

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The infestation by parasitic turbellaria of the coelom and the intestine of *Holothuria forskali*, *Holothuria tubulosa* and *Cucumaria planci* was investigated. Animals were collected by dredging in the bay of Rovinj (northern Adriatic Sea, Croatia). *H. tubulosa*, the only resident species in shallow water in Val Saline 4 km north of Rovinj was collected by snorkelling.

*Umagilla forskalensis* and *Monticellina longituba* live in the intestine of *H. tubulosa*. The body cavity of *H. tubulosa* and *H. forskali* is infested by *Anoploidium* sp., *Anoploidera voluta* and *U. forskalensis*. *Umagilla forskalensis* was the only species found in the intestine of *H. forskali*. In *H. tubulosa* from Val Saline only *M. longituba* was found in the intestine and *Anoploidium* sp. in the coelom.

In the bay of Rovinj all specimen of *H. tubulosa* are infested with turbellarians (7.3 flatworms per sea cucumber). 80 per cent of *H. forskali* are infested (3.7 turbellaria per sea cucumber). 87 per cent of *H. tubulosa* from Val Saline were inhabited by parasites but the degree of infestation with 2.4 parasites per sea cucumber was lower.

*H. tubulosa* and *H. forskali* are deposit feeders. The different particle size of the sediment ingested might be one reason for the different degree of infestation of the two species collected from the same site. The way of infestation is supposed to be via egg capsules in the sediment. The suspension feeding *C. planci* was never infested by parasitic flatworms in our study material.

## Allee effects and species of g. *Cucumaria*

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Echinoderm species with planktonic larvae may exhibit Allee effects (AE) in reproduction or recruitment. Holothurian as most of echinoderm populations consist of isolated subpopulations of relatively sessile adults.

We may assume that species of g. *Cucumaria* as well as other benthic species broadcasting gametes are subjected to AE due to depressed fertilisation success at low population densities. So, increasing harvesting pressure on the populations of the sea cucumbers may result in the densities below to insure

the adequate recruitment. However there was only one experimental evolution of the AE and survival as function of density for g. *Cucumaria*.

On the other hand, AE can play an important role in the adaptation and evolution of the free-spawning species, and the sea cucumbers, in particular. Fertilisation success is high within a small distance from a sperm source and spatial relationship between spawning organisms influence fertilisation. Since metapopulations of marine invertebrates, e.g. *Cucumaria frondosa/japonica* re relatively isolated from each other and connected by low levels of dis-

posal, they can be subjected to morphological changes as adaptation to the local environment. As a result of this process the appearance of new species of g. *Cucumaria* might be expected. Precipitous changes are particularly likely if the populations display AE. An example of metapopulation model can be the population of *C. japonica* on the Far East (in the Sea of Japan, Okhotsk Sea) with recently described species *C. conicospermium*, *C. levini*, *C. okhotensis*. Some taxonomic changes appear to have evolved in the history of these species and others as the subsequent diversification of larval and adult morphology caused by AE.

### Parental investment in *Synaptula hydriformis* (Holothuroidea: Apoda)

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*Synaptula hydriformis* (Lesueur, 1824) is a matrotrophic holothuroid that supplies its young with nutrients other than yolk during their development. This work aimed to establish the relationship between this parental investment and the survival rate of the released young in laboratory and the size they attained on their first stages of development. Adult specimens were reared in isolation in laboratory, and were measured once a week. The size corresponds to the distance between the oral ring and the posterior end of the body. The young of the first generation released in the laboratory measured between 0.15 and 3.51 mm and were distributed in 12 size classes. The highest mortality rate was observed on the first eight weeks after the young were released, falling sharply after that. This period was considered as a "critic period" for young survival on

laboratory conditions. Therefore, data refers to survival to this critical period. It was observed that more than 50% of the dead individuals belonged to the three first size classes. Less than 20% of the survivors belonged to these same classes, and the remaining 80% measured between 1.21 and 3.6 mm. Survival of the young reached 100% among the largest animals, with sizes between 3.0 and 3.6 mm. It was also observed that the size of the young when liberated is related to the size attained at the eighth week of life outside the parental body. These results show that the young released with sizes larger than 1.2 mm have a survival expectancy three times higher than the smaller ones. The size of the young when liberated from the parental body is related to the size they will achieve at the end of the critical period of eight weeks.

### Changes in dermal ossicles of *Chiridota rotifera* and *Synaptula hydriformis* (Echinodermata: Holothuroidea)

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Echinoderms usually have a rigid body wall with an elaborate magnesium-rich calcite endoskeleton. The integumental skeleton of the Holothuroidea contains many microscopic ossicles formed within multinucleated sclerocytes' syncytia situated in the dermal layer of the body wall. The sequence of ossicle formation of two apodid holothurians, *Chiridota rotifera* (Pourtalès 1851) and *Synaptula hydriformis* (Lesueur 1824), was observed using light microscopy, scanning and confocal electron microscopy. The first species has clusters of calcareous structures shaped like wheels, gathered inside convex white papillae in the body wall. Those ossicles

begin to form as little stars with six or more rays, which develop to form wheels with six rays also, measuring from 40 to 150 µm long. Ossicles aggregate in the papillae by addition of new ones at the star stage in its outer layer. The oldest ossicles, in the wheel stage, concentrate in the middle of the papillae. In the second species, the ossicles have two parts, a plate and an anchor, distributed all over the skin. Each anchor measures about 120 µm long and is attached to a plate. The anchor begins to form as a small baton. The plate is formed later and measures 95–130 µm. In both species ossicle formation occurs during all the life span of the animals.



## Lower Jurassic 'worm holothurians': First Liassic body fossils and/or intestinally transformed individuals

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Holothurian spicule morphotypes of *Calclamna*, *Binoculites*, and *Achistrum* are very common in European Lower Jurassic marine clays. Besides some aggregates of associations of such types as possible relicts of original body wall deposits, no complete fossils were known before. Meanwhile, during recent years dozens of worm-like holothurians consisting of those morphotype associations have been extracted from an almost 2-m thick and several metres laterally extended portion within a monotonous 50-m succession of Lower Liassic silty clays (at Göttingen, Germany). A few 'worm holothurians' contained up to ten ossicles of the calcareous ring showing different 2 dorsal / 3 ventral radials as typical in certain synaptids.

However, some observations may cast doubt on the supposed nature as body fossils, e.g.: (1) apparently non-anatomical positions of ring elements; (2) thin "worms" of densely crowded spicules obviously without a hypothetic space left for an original body cavity; (3) extremely narrow and irregular three-dimensional meanders of thin specimens seemingly too acrobatic (even in rigor mortis if compared with recent synaptids). Hence, similarities with the typical Solnhofen fossil *Lumbricaria* may not be excluded which would suggest a coprolitic (fecal) origin of the 'worm holothurians'. In this case, however, biostratigraphic observations would suggest that the supposed holothurian eater can only have fed on one individual until defecation thus transforming it by intestinal passage.

## Distribution and abundance of starfish, sea urchins, and sea cucumbers in Galapagos

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Although the starfish, sea urchins, and sea cucumbers that occur in Galapagos are well known, reports of the distribution and abundance are few. This information is essential for understanding their ecology and biology. We documented the occurrence of species at seven sites with distinct habitats in Galapagos. Eight species of sea urchins, four species of sea cucumbers, and five species of starfish were found. Only one or two individuals of some species and low densities  $<0.5$  ind.  $m^{-2}$  of most species were found. Only a few species, all sea urchins, had densities  $>0.5$  ind.  $m^{-2}$ . Only *Eucidaris galapagensis* was found with densities  $>0.5$  ind.  $m^{-2}$  at Caamaño ( $15.6 \pm 6.1$ ) and at Punta Estrada ( $10.1 \pm$

$4.8$  ind.  $m^{-2}$ ). *Eucidaris galapagensis* and *Lytechinus semituberculatus* exceeded this threshold at Academy Bay ( $1.5 \pm 1.2$  and  $4.6 \pm 8.9$ , respectively) and at Muelle, Santa Cruz ( $0.7 \pm 0.8$  and  $2.0 \pm 3.4$ , respectively). The most diverse assemblage was at Itabaca Channel with *E. galapagensis* ( $0.7 \pm 0.8$ ), *L. semituberculatus* ( $2.6 \pm 2.2$ ), *Tripneustes depressus* ( $2.0 \pm 1.9$ ), and *Diadema mexicana* ( $0.5 \pm 1.3$ ). Of these dominant sea urchins, *E. galapagensis* was most ubiquitous, followed by *L. semituberculatus*. Variation in the distribution and abundance of the echinoderms in these different habitats has implications for both the characteristics of the habitats and the specificity of habitat requirements of the echinoderms.

## Fossil Holothuroidea (Echinodermata): an overview

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The taxonomy and biostratigraphy of fossil holothurians have been reviewed including new records from the Ordovician and Silurian of the Baltic realm as well as Mesozoic of Europe and Africa. These new data suggest that the earliest unequivocal sea cucumber, according to ossicles and calcareous ring elements, is from Lower Ordovician (Arenigian; 470 Myr old) sediments. Currently nearly 800 paraspecies and species of the fossil Holothuroidea have been reported. 778 paraspecies

/16 species (body fossils and sclerite associations) versus 282 paraspecies/4 species ("Treatise on invertebrate paleontology" 1966) are known today. The predominant part ( $>460$ ) of the fossil paraspecies/species comes from the Triassic and Jurassic period; only slightly more than 100 taxa are from Cretaceous respectively Paleogene sediments. Specimens from Neogene sediments are insufficiently known ( $<50$ ). Compared to their post-Palaeozoic counterparts, the fossil record of

Palaeozoic sea cucumbers are very poor, reported as follows: Ordovician (<5), Silurian (1), Devonian (<50), Carboniferous (<70), and Permian (<20). Localities yielding well-preserved complete fossil holothurians are very rare in the world. Previously, less than 10 such localities (Fossilagerstätten) have been reported in the literature. Non-European and

Non-North-American records of fossil holothurians are hitherto rare (e.g. Africa, Australia, South America), or undescribed up to now. A better knowledge of fossil Holothuroidea is strongly necessary for understanding the early evolution of the Eleutherozoa as well as evolution of Holothuroidea.

### **Late Cretaceous holothurians (Echinodermata): An overview**

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The Late Cretaceous holothurians have been barely studied for over a century, and it is normally assumed that holothurian fossils are so rare and non-diverse as to be of little importance. This poster introduces Late Cretaceous holothurian faunas from several Late Cretaceous successions of Europe (>50 000 holothurian ossicles and pieces from nearly 500 samples), that contradict this view. The taxonomy, palaeoecology, biostratigraphy, and palaeogeography of Late Cretaceous holothurians have been reviewed including new records from the Turonian, Campanian and Maastrichtian of Germany, Denmark, Netherlands, Poland, France, Austria, and the U.K. Currently slightly more than 70 paraspecies and species of Holothuroidea from Late Cretaceous sediments (99 – 65 Myr) are known. All

paraspecies have been assigned orthotaxonomical to Recent holothurian orders and families — all modern holothurian orders (Dendrochirotida, Dactylochirotida, Aspidochirotida, Elasipoda, Molpadiida, and Apodida) are present. Compared to the other Upper Cretaceous stages, the knowledge of sea cucumbers from the Coniacian, Santonian, and Campanian periods are very poor, reported as follows: Cenomanian (13 paraspecies), Turonian (12), Coniacian (1), Santonian (1), Campanian (6), and Maastrichtian (45). Localities yielding body fossil of holothurians are very rare in the world. Previously, only one such Late Cretaceous Fossilagerstätte have been reported — the Late Cenomanian lithographic limestone of Lebanon.

### **Holothurians from the Late Cretaceous "Fish Shale" of Lebanon**

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Localities yielding nearly complete fossil holothurians are very rare in the world. Previously, less than ten such localities (Fossilagerstätten) have been reported in the literature; only two are Cretaceous (144–65 Myr old). The lithographic limestones of the Lebanon comprise Fossil Lagerstätten of different Late Cretaceous ages and faunal composition. They have been renowned for a rich fish fauna as well as soft tissue preservation (Coleoidea, Annelida etc). A Late Cenomanian (94 Myr old) age is assigned to the Haqel and Hjoula localities as well as to the Nammoura locality, whereas the beds at Sahel Alma

are dated as Late Santonian. The Hjoula sites, from which my material comes, are located about 10 km inland from Jbail (Byblos). Hjoula have yielded nearly 50 specimens of complete fossil holothurians. All specimens consist of more or less good preserved calcareous ring (diameter 0.5–4.0 cm), and sometimes body outline. Systematically nearly all specimens can be assigned to the Aspidochirotida (Holothuriidae and Stichopodidae). In addition, the results of micropalaeontological investigations are meagre: some poor preserved ossicles indicate also apodid sea cucumbers (Chiridotidae).

### **Aspidochirote holothurians from the Middle Triassic of southern Germany**

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Localities yielding well-preserved fossil holothurians are very rare in the world. Previously, less than ten such localities (Fossilagerstätten) have been reported in the literature – only three are Triassic (e. g. Cherbonnier 1978, Smith and Gallemí 1991).

Exceptionally preserved holothurians of Middle Triassic age were found in the Upper Muschelkalk (spinosus zone; 234 Myr old) of Baden-Wuerttemberg, southern Germany (Hagdorn 1993). All new investigated specimens from Nitzenhausen

consist of good preserved calcareous rings (diameter 0.5–1.0 cm), and poor preserved remains of the whole body. Systematically nearly all specimens can be assigned to the aspidochirotes (family Holothuriidae). One new genus and species (*Palaeoholothuria hagdorni*) is represented. The calcareous rings are very similar to the Recent subgen-

era *Holothuria* (*Panningothuria*), *H. (Platyperona)*, *H. (Stauropora)* as well as *H. (Mertensiothuria)*. Additionally, some micropalaeontological investigations (Ockert 1993, Hagdorn unpublished) have yielded comparable disarticulated radialia and interradialia – such a wider distribution in the Muschelkalk of the Germanic Basin is assumed.

### **A study of *Holothuria scabra* (Jaeger) on different types of sediment at Bolinao, Pangasinan (Philippines)**

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The most obvious environmental impact of aquaculture is eutrophication, caused by excessive feeding and decrease in water circulation. If the sea cucumber can efficiently consume the sediment from aquaculture, the deterioration of the water might be lessened and the growth of the sea cucumber might be enhanced by the aquaculture. For this study, juvenile *Holothuria scabra* was placed in cages at three different locations, two with coral sand in a sea grass bed, and one with silty sand near an area of intensive fish farming. Two different batches of laboratory bred animals were used (11 and 4 months after fertilisation).

The organic content in the sediment was not as expected, showing higher values for coral sand than for fish pen silty sand. This was unexpected, and difficult to explain. The animals grew faster (measured as biomass) at the fish pen site than on coral sand. Mortality was higher at the fish pen site, but the total biomass at the end of the experiment was still higher there, than at the other sites. This indicates that a grow-out culture of *H. scabra* juveniles would benefit from being located near fish pen areas.

### **Variations in the form of the spicules from different parts of the introvert of dendrochirotid holothuroids**

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It is well known that the form of the spicules of the body wall and other organs play an important part in the identification and classification of not only species but also genera among holothuroid echinoderms. It is also well known that tentacle spicules often vary in form both in the tentacle shaft and the branches but variations have thus far been overlooked in the introvert of dendrochirotid holothuroids, although many writers have shown that introvert deposits play an important part in the identification of species, especially within the very large "super-genus" *Thyone*. An examination of several species within this genus and *Stolus*, among phylloporid dendrochirotid, has shown that introvert

spicules may also vary along the length of the introvert of a single specimen. For example a new, still undescribed species of *Stolus*, possesses only small-noduled buttons and smooth, perforated plates in the anterior part of the retracted introvert and elongated, perforated rods and rosettes in the more posterior part, while all four types of deposits occur in the mid-introvert. Hence, it is advised that in dendrochirotid holothuroids, especially those belonging to the family Phylloporidae, spicules be studied from all regions of the introvert to prevent erroneous identifications and eliminate, as far as is possible, the duplication of species.

## An enzymatic method for examining calcareous ossicles from holothurians

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The different shapes of ossicles are characters of paramount importance in holothurian taxonomy. The method that has been used to isolate these ossicles is to dissolve a small piece of tissue with a small quantity of hypochlorite solution. However, this bleach solution can corrode the calcareous pieces and modify the original shape and dimensions of the ossicles. A new method using a proteolytic enzyme (Pancreatin NF), instead of hypochlorite solution, to dissolve holothurian tissues is proposed. This method was developed by using fragments of

body tissues from three species from three different orders: *Holothuria grisea* Selenka, 1867 (Aspidochiro-tida), *Duasmodyctyla seguroensis* (Deichman, 1930) (Dendrochiro-tida) and *Synaptula secreta* Lopez, 1957 (Apoda). Observations with light and scanning electronic microscopy (SEM) of material prepared using the traditional and the proposed methods was made in order to compare the results. The SEM images show the striking differences in the surface of the ossicles obtained by each method.

## Tubulin expression and the regeneration of the enteric nervous system in the sea cucumber *Holothuria glaberrima*

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The sea cucumber *Holothuria glaberrima* has the capacity to regenerate its internal organs, including the digestive tract with its enteric nervous systems (ENS). Tubulin, the main constituent of microtubules, has been used extensively as a marker to study regeneration in many organisms. We have identified a 2.1 kb  $\alpha$ -tubulin clone (Hgtub) from a regenerating intestine library of *H. glaberrima*. Northern blots of intestinal tissue done with a probe from the conserved coding region identified five bands, suggesting the presence of different  $\alpha$ -tubulin isoforms. Moreover, when an Hgtub 3'UTR probe was used, a single band was obtained. This band up-regulates during late regenerative stages, concomitant with ENS regeneration. In fact, im-

munochemical studies using  $\alpha$ - and  $\beta$ -tubulin antibodies and other ENS markers demonstrated: (1) an increase in nerve fiber density throughout the intestinal serosa and muscle layers as regeneration advances and (2) the existence of at least two populations of fibers, an extrinsic population that enters the regenerating intestine from the mesentery and that probably originates within the mesentery or body wall and an intrinsic population that appears to originate from neurons within the serosa that distributes homogeneously along the regenerating tissue. These results should provide a clear understanding on the regeneration of the digestive tract and in particular of its associated ENS.

## Sea cucumber diversity and resources in shallow waters of Brunei, Borneo Island

D.J.W. Lane

Fisheries for tropical Indo-Pacific sea cucumbers are typically unregulated, overexploited and have historically operated on boom and bust cycles. Concerns range from loss of biodiversity and fishery productivity, to the possible deleterious ecological effects of sea cucumber biomass-depletion on reefs and associated marine sediments. In some localities many highly prized (and priced) species are very rare — so much so that they have been suggested as possible candidates for CITES listing. In contrast to most Indo-Pacific reef habitats, shallow shelf waters off the Brunei sector of the NW Borneo coastline are, in terms of sea cucumbers, unexplored and are relatively unexploited, at least in recent years. There are no licenses issued for beche-de-mer

harvesting in Brunei but the relatively undisturbed status of holothurian resources may also be a consequence of offshore oil and gas facilities placing many large reef areas and shallow sediments off limits for fishing. Although there is some evidence of illegal harvesting, both amateur and professional, survey transects, carried out in 2003/2004, indicate a rich holothurian diversity and a wide range of commercial species in significant numbers. Given the limited domain of reef sites in Brunei territorial waters, stocks of commercial species are probably not sufficient to support a viable national fishery but the resource of high-value commercial species is of potential importance as a source of broodstock for mariculture.