

Abstracts and new publications...

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The fossil record, diversity and evolution of holothurians (Echinodermata)

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The palaeobiology and evolutionary history of holothurians are relatively poorly understood. Currently, there are only 800 fossil holothurian paraspecies and species, ranging from the Early Palaeozoic to the Quaternary, in comparison to more than 1,450 recent species. In part, this is due to their disjunct endoskeleton with ossicles and calcareous ring elements, which are released following decomposition of the surrounding tissue. However, under favourable conditions, holothurian body fossils may be preserved in various fossil lagerstätten. The evolution and diversification of sea cucumbers have been reviewed and reinterpreted, including new records of Early Palaeozoic (Ordovician and Silurian) and Mesozoic (Jurassic and Cretaceous) material (calcareous rings and ossicles). Holothurians have a Phanerozoic history extending back more than 466 million years, ranging from the early Middle Ordovician to the present time. The maximum level of morphological diversification appears to have been reached in the Mesozoic, but this is in all likelihood due to a lack of research in other geological strata. A revised hypothesis of higher-level relationships within the Holothuroidea is presented. A more detailed interpretation of the fossil record requires a better understanding of the skeletal morphology of modern holothurian families, e.g. three-dimensional characters, stereom structure etc. of calcareous ring elements and body wall ossicles. There is also recent diversity that is hidden because detailed investigations are missing or sparse.

Morphological and molecular systematic data of elasipodid species (Echinodermata: Holothuroidea) from New Zealand's International Polar Year — Census of Antarctic Marine Life (IPY-CAML) 2008 NZ survey of the Ross Sea and Scott and Admiralty seamounts, Antarctica

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An extensive biodiversity survey of the Ross Sea and Scott/Admiralty seamounts was carried out by New Zealand in February/March 2008. Biological sampling using bottom trawls and coarse and fine mesh epibenthic sleds collected 900 holothuroid specimens. These specimens have been identified using traditional morphological systematics. Some molecular systematic analysis using the cytochrome oxidase-1 (CO1) gene has been completed by Gustav Paulay at the University of Florida. A total of 20 species of elasipodids were identified from the survey. The biogeographical relationships of these species are discussed in relation to the known ranges of Antarctic elasipodid biogeography. Distribution and depth records have been extended. Some new species or variations of known species were found. Five of the elasipodid species — *Pannychia* sp. cf. *moseleyi*, *Benthodytes sanguinolenta*, *Laetmogone wyvillethomsoni*, *Peniagone affinis* and *Peniagone wiltoni* — are discussed in relation to circumpolar distribution and occurrence north of the Antarctic Convergence. There is some degree of congruence between morphological systematic data and molecular phylogenetics, as well as evidence of sister species north and south of the Antarctic Convergence. Genetic data thus suggest a review of some morphological systematic conclusions.

Holothurian settlement in two protected reefs at Cozumel, Mexico

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We analysed the intensity of settlement of holothurians in two reefs from the Arrecifes de Cozumel marine park using a series of suspended collector systems (Witham type). The collectors were composed of squares of Astroturf mat (50 x 50 cm) attached to plastic frames supported by buoys and weights to keep them straight. Three collectors were deployed at each site in two reefs (Palancar and Tormentos) (total 10 sites) from May 2007 to June 2008. A total of 191 individuals of *Holothuria arenicola* and 114 of *Euaпта lappa* were counted during the study period. The smallest specimen belonged to *H. arenicola* (0.9 cm) and the largest (10 cm) to *E. lappa*, both found in November 2007 at Tormentos reef. The size of youngsters was not statistically different between months or between reefs. There were differences in settlement amongst reefs but not between months, suggesting that reproduction may be continuous throughout the year. Palancar showed the lowest number of recruits. These differences may be explained by the strong currents that influence Palancar reef and by the lack of hard substrate; this reef is located at southern boundary of the park and is dominated by sea grass and high levels of turbidity, associated with anthropogenic activities. Tormentos reef is located in the north of the park and is dominated by coral reef and characterised by clear waters. We concluded that both species settled most of the year and that settlement seemed to be strongly influenced by currents and water conditions.

Epifauna associated with the sea cucumber *Holothuria mexicana* in Puerto Rico

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The epifauna of two populations of the shallow water sea cucumber *Holothuria mexicana*, inhabiting sea grass beds in Guillian Island y Caribe Keys (Puerto Rico), were analysed. We found that all individuals are covered by a whitish mucilage, probably secreted by them or by microorganisms, to which organic debris (shells, vegetative litter, and coral fragments) and nonorganic debris (gravels, sand, and plastic and metallic fragments) adhere. The epidermis of 122 specimens was gently scrubbed *in situ* with a spatula for approximately 15 seconds while washing the animal to collect the epidermic material. Individuals were returned to their habitat. The collected material was preserved in a 75 per cent ethanol solution. In the lab, samples were filtered with a set of meshes to separate the debris. Using a stereo microscope the debris was separated and organisms were collected and identified. The liquid sample was examined with a compound microscope to identify smaller organisms. A photographic inventory was developed. We found that 100 per cent of samples contained macro- and micro-invertebrates, which were classified as polychaetes (39.0 per cent), mollusks (29.5 per cent), crustaceans (isopods, copepods, ostracods, stomatopods, amphipods, decapods) (28.0 per cent) and others (zoanths, ophiuroids, porifers) (3.5 per cent). Statistical analyses revealed that the presence of mollusks and crustaceans was significantly different in the two localities (ANOVA $F = 3.097^*$ and 12.542^{**} , respectively). We do not know if the existence of this epifauna occurs at random or is a byproduct of commensalisms or symbiosis, or if the mucilage provides a food or shelter to them. This sea cucumber species secretes holothurin A and B, a toxin for many potential predators. These preliminary findings constitute baseline data for future projects aiming to assess the ecological niche of *Holothuria mexicana* and its epifauna.

Aspidochirote sea cucumber diversity and status of stocks in the Bunaken National Marine Park (BNMP), North Sulawesi, Indonesia

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The species richness of aspidochirote sea cucumbers associated with reefs of the BNMP is very high, suggesting, along with data for other faunal groups, that this archipelagic region, including other neighbouring islands at the tip of North Sulawesi, may constitute a diversity 'hot spot' within the Indo-Malay zone of maximum marine biodiversity. For some forms, abundances are high but the animals are patchily distributed compared to other areas in the Indo-Pacific, a phenomenon which may reflect localised concentration

of settling recruits in areas of eddy currents. Yet in general, abundances of commercial sea cucumber species are low. Prior to initial designation of this area as a National Marine Park in 1991 and the commencement of protection measures, the reefs and reef slopes around the Bunaken Islands were heavily fished for commercial sea cucumber species. No reliable data exist for this fishery but recent preliminary findings of sea cucumber surveys indicate that over the last two decades recovery of populations has not occurred.

Molecular phylogeny of symbiotic pearlfishes

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Carapid fishes are certainly among the most remarkable organisms living in symbiosis with echinoderms. These fishes, known as pearlfishes, belong to the family of Ophidiiform, which is divided in two sub-families, the Pyramodontinae and the Carapinae. Symbiotic pearlfishes belong to the latter sub-family. They are found inside bivalves, ascidians, asteroids and holothuroids. Their behaviour and morphology are extremely adapted to their symbiotic way of life. Numerous aspects of the biology of these fishes have been recently elucidated, though not from an evolutionary perspective. We present here the first molecular phylogeny of the symbiotic pearlfishes, including *Echiodon*, *Onuxodon*, *Carapus* and *Encheliophis* genera. The phylogenetic relationships of 20 specimens from eight species coming from the Mediterranean, the Indian Ocean and the Pacific Ocean have been estimated. Five mitochondrial fragments (3,645 base pairs) have been sequenced and the phylogenetic trees were obtained via Maximum Parsimony, Maximum Likelihood and Bayesian analyses. The analyses suggest the paraphyly of the *Carapus* group in regard with the monophyletic *Encheliophis*. It also suggested that *C. boraborensis* is the sister group of all the other sequenced pearlfishes and that *C. bermudensis* and *C. acus* are grouped within a clade. Various characters have been mapped on the trees to estimate the history of these symbiotic fishes.

Mitochondrial markers reveal many species complexes and non-monophyly in aspidochirotid holothurians

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Despite their striking presence on coral reefs and the multi-million dollar industry targeting them, aspidochirotid holothurians have received relatively little taxonomic attention. Heavy reliance on ossicles has led to a confused taxonomy and masks substantial cryptic diversity. As part of a revisionary effort on holothurians, we are sequencing multiple specimens of available species to test for species limits and construct phylogenetic hypotheses. We are using a bottom-up approach, sequencing fast-evolving mitochondrial markers (16S and COI) first, as these are mostly informative at lower taxonomic levels. We have unraveled many species complexes and identified several undescribed species. For instance, our analyses revealed that the circumtropical 'species' *Holothuria impatiens* consists of a dozen reciprocally monophyletic, well-defined, evolutionary significant units (ESUs). Broad overlap in the range of some, in combination with recent divergence, indicates the rapid evolution of reproductive isolating barriers among these ESUs. Such rapid evolution to sympatric coexistence is also found in several other species complexes we identified, and contrasts with most other marine invertebrates. At a higher taxonomic level, preliminary results show that non-monophyly of currently recognised taxa is prevalent. Stichopodids emerge from paraphyletic synallactids. While holothuriids appear monophyletic, *Holothuria* is not, as several subgenera in that genus are deeply divergent. We present the latest phylogenetic hypotheses, which further our understanding of speciation and trait evolution in sea cucumbers.

Some molecular phylogenetic data for holothuroid species from Antarctica, Australia and New Zealand (Echinodermata: Holothuroidea)

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In collaboration with Gustav Paulay in the University of Florida, DNA phylogenetic data (COI gene) are becoming available for holothuroids from Antarctica, New Zealand and Australia. Data for some dendro-

chirotid and molpadiid and synallactid are reported. There is some good congruence for traditional morphological systematic conclusions and molecular phylogenetic data. These data also indicate a need for review of some morphological systematic conclusions in terms of synonymies and the existence of cryptic species. For example CO1 gene data indicate: *Molpadia musculus* Risso appears not to be cosmopolitan, but comprises at least four discrete species; the Antarctic *Psolus arnaudi* Cherbonnier and *Psolus cherbonnieri* Carriol and Féral appear to be junior synonyms of *Psolus dubiosus* Ludwig and Heding; specimens of *Molpadiodemas morbillus* O'Loughlin and Ahearn from the South Sandwich Trench (5452 m), New Zealand (2500 m) and southeast Australia (1993 m) show no genetic variations.

Diversity and distribution of holothuroid species south of the Antarctic Convergence (Echinodermata: Holothuroidea)

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A morphological systematic overview of current knowledge of the diversity and distribution of holothuroid echinoderm species from all depths south of the Antarctic Convergence is presented. There are 155 species, with 38 undescribed. Species occurrences south of the Convergence at Bouvet Island, Heard and Kerguelen Islands, Prydz Bay, Ross Sea, Bellingshausen Sea, Antarctic Peninsula and Weddell Sea, and north of the Convergence in the Magellanic region, are compared. Based on morphological systematics there is typically a circumpolar distribution south of the Convergence. However, most holothuroid species on the Heard/Kerguelen Plateau do not show a circumpolar distribution. The Convergence is a significant barrier to gene flow north from the Antarctic Ocean.

Observations of reproductive strategies for some dendrochirotid holothuroid species (Echinodermata: Holothuroidea: Dendrochirotida)

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Some recently observed reproductive strategies by dendrochirotid holothuroid species are reported and illustrated: fissiparity by *Cucuvitrum rowei* O'Loughlin and O'Hara, from southeast Australia; evidence of intra-coelomic brood fissiparity in *Staurothyone inconspicua* (Bell), from southeast Australia; intra-coelomic brood protection by an undescribed species of *Parathyonidium* Heding, from Antarctica; evidence of intra-coelomic brood auto-ingestion by *Neoamphicyclus materiae* O'Loughlin, from southeast Australia; brood protection in a longitudinal dorsal invaginated marsupium in small specimens of *Cladodactyla crocea* (Lesson), from the Falkland Islands. An analysis is presented of brood protection in interradial anterior marsupia by species of the *Cucumaria georgiana* (Lampert) group (including *Cucumaria acuta* Massin and *Cucumaria attenuata* Vaney), *Microchoerus splendidus* Gutt, *Psolidiella mollis* (Ludwig and Heding) and *Psolus charcoti* Vaney, all from Antarctica. The report and illustration of anterior interradial marsupial brood protection by *Psolus koehlerii* Vaney, from Antarctica, is rejected.

Quantitative analysis of morphological characters in Stichopodidae (Holothuroidea, Aspidochirotida)

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Gross morphology and ossicles from the body wall provide the basic characters used in holothurian taxonomy. However, the plasticity of soft tissue morphology in both living and preserved specimens has focused taxonomic discrimination on the calcareous ossicles. Differentiation between ossicles is typically based on size and qualitative description of form. Variation in ossicle form exists, even within named forms (e.g. button, rosette, tack) within a single individual, and can challenge comparison of study specimens with published descriptions. While ossicles have been recorded from tissues other than the body wall, they have only been irregularly applied to taxonomy. By evaluating ossicles from tissues other than the body wall within the Stichopodidae and by applying statistical analysis (regression and multi-variate analysis) to ossicle size and presence-absence observations, this study makes available additional quantitative characters for differentiating between holothurian taxa.

Molecular diversity and body distribution of saponins in the sea cucumber *Holothuria forskali*

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Sea cucumbers contain triterpene glycoside toxins called saponins. We investigated the complex saponin mixture extracted from the common Mediterranean species *Holothuria forskali*. Two different body components were analysed separately: the body wall (which protects the animal and is moreover the most important organ in terms of weight) and the Cuvierian tubules (a defensive organ that can be expelled on predators in response to an attack). Mass spectrometry (MALDI-MS and MALDIMS/MS) was used to detect saponins and describe their molecular structures. As isomers were found in the Cuvierian tubules, a preliminary chromatographic separation (LC-MS and LC-MS/MS) was performed to identify each saponin separately. A quantitative study was also conducted to compare the amount of toxin in both body components. Twelve saponins were detected in the body wall and 26 in the Cuvierian tubules. All the saponins from the body wall are also present in the Cuvierian tubules but the latter also contain 14 specific saponins. The presence of isomeric saponins complicated elucidation of the structure of the whole set of toxins but 16 saponins have been characterised through their fragmentation pattern. Among these, three had already been reported in the literature as holothurinosides A and C, and desholothurin A. Molecular structures have been suggested for the 13 others which, in the present work, have been provisionally named holothurinosides E, F, G, H, I, A1, C1, E1, F1, G1, H1 and I1 and desholothurin A1.

Vertical sediment displacement caused by *Holothuria scabra* using fluorescent tracers

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This study investigated vertical sediment movement (mixing) caused by feeding and burying behaviours of the sea cucumber *Holothuria scabra* (sandfish). Animals were kept in aquaria with constant flow-through filtered seawater for six weeks. The entire sediment matrix (12 cm) was marked with three different coloured tracer particles. A sampling grid was established and sediment cores were randomly extracted after 1, 10 and 56 days. Cores were later cut in 2 cm sections and tracers were counted in subsamples from each section under a fluorescent microscope. Results were used to calculate instantaneous mixing rates of the surface layer as well as general sediment displacement throughout the entire sediment matrix. The study showed that sandfish did not affect sediment deeper than about 6 cm. After 56 days approximately 14 per cent of surface sediment was displaced to deeper layers with an instantaneous mixing rate of 0.25 per cent d⁻¹, meaning sandfish (at a natural density of 0.48 individuals m⁻²) can displace the top 2 cm every 400 days. Furthermore, bioturbation figures were extrapolated to the sea cucumber population in Moreton Bay, showing that 33.4 tonnes of sediment would be displaced by the sandfish population (3.6 million) in the bay each day under summer conditions. Sandfish turned over sediment by a combination of two mechanisms: smearing (diffusion) and upward conveyor belt movements. Thus, based on existing functional groups of bioturbators, a new mode is suggested: the 'conveyor diffusors'. Holothurians have an important ecological role within subtropical seagrass habitats; however, they are quantitatively only half as effective as other important bioturbators such as polychaetes.

Daily and seasonal patterns in behaviour of the commercially important sea cucumber *Holothuria scabra*

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This study monitored and modelled long-term daily and seasonal patterns in behaviour of adult sea cucumber *Holothuria scabra* in Moreton Bay, Australia. Animals were kept in outdoor tanks for two years and behaviour was recorded each month for a 24-hour period by means of time-lapse video. Behaviour was classified into eight categories and a series of nested conditional, binomial models (generalised linear models) were applied to describe the probabilities of key behaviours occurring. Active behaviours, such as feeding and searching, were negatively correlated to water temperature and were approximately five times more common during summer (approximately 16 hours day⁻¹) than during winter (approximately 3 hours day⁻¹). Animals were less likely to bury themselves during summer (December–February), with at least one month

where they did not bury themselves at all. There was an 80 per cent probability of animals being inactive during the early hours of the morning (around 5:00 a.m.), irrespective of the time of year; and a 50 per cent probability of animals being fully buried during mid-winter (July/August), irrespective of the time of day. Searching behaviour showed a bimodal pattern, where animals spent more time searching during autumn and spring (approximately 2 hours day⁻¹) than during summer (approximately 1 hour day⁻¹) or winter (approximately 20 minutes day⁻¹). Describing patterns in holothurian behaviour, especially producing a probability matrix of active behaviour and burying frequencies, is crucial for designing sustainable fisheries management strategies and aquaculture projects. The key findings of this study provide information about optimal timeframes to conduct population surveys, and can be applied to the ecosystem function of tropical holothurians overall.

Concurring evidence of rapid sea cucumber overfishing in the Sultanate of Oman

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A fishery for *Holothuria scabra* recently developed in a small area of the eastern coast of the Sultanate of Oman. The area covered by the fishery is limited to a single shallow embayment covering only 320 km² in Mahout Bay and involves approximately 400 fishers, around 50 per cent of whom are women. The 2004–2005 fishing season (October to May) was the first officially on record. However, anecdotal evidence suggests a low level of exploitation since the early 1970s, although catch and export data for this period are unavailable. Average size of individuals collected in 2005 varied between 170 and 200 mm in length. The total biomass at the time was estimated at 1500 t (fresh weight). The following year at least 14.5 t of processed *H. scabra* were exported to the United Arab Emirates, corresponding to approximately 145 t or around 10 per cent of the recorded biomass. Interviews with fishermen and traders revealed that the CPUE for sandfish was around 100 individuals hour⁻¹ in 2005. However, the CPUE had declined to 10–20 individuals hour⁻¹ by 2007, indicating significant pressure on the resource. Over the same timeframe, the value of an average sized *H. scabra*, which was OMR 0.1 (USD 0.25) in 2005, increased to OMR 1.5 (USD 3.75) and is still climbing. Concomitantly fishers have begun targeting the less valuable *H. atra* in large numbers. This species commands market prices of OMR 0.2 (USD 0.5). Finally, an examination of the processed specimens for sale showed the presence of a significant number of very small individuals (<5 cm processed, corresponding to around 10 cm live length). This concurring evidence suggests a rapid decline of the population of *H. scabra* in Mahout Bay while indicating building pressure on *H. atra*. Accordingly, the Ministry of Fisheries Wealth has commenced a number of projects aimed at monitoring the sea cucumber fishery with the ultimate objective of providing a regulatory framework to ensure the sustainability of the resource. Projects also include an evaluation of enhancement and ranching techniques.

Gametogenic synchronicity with respect to time, depth and location in three deep-sea echinoderms

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This study examined the prevalence of inter-individual synchrony in the gamete synthesis of three deep-sea echinoderms, *Phormosoma placenta* (Echinoidea), *Zygothuria lactea* (Holothuroidea) and *Hippasteria phrygiana* (Asteroidea) collected along the continental slope off the coast of Newfoundland and Labrador (eastern Canada). Analysis of gonad development using histology and gonad indices revealed diverse degrees of asynchrony at the scales examined (within trawls, between trawls over similar or different periods, as well as between depths and locations over the same period). Annual and seasonal patterns were therefore largely masked by heterogeneousness in most samples. These data suggest that determination of so called 'continuous' reproductive cycles in many deep-sea species may in fact reflect sampling inadequacies inherent to most deep-sea studies. Assessment of true reproductive patterns and periodicities may require much tighter collection designs as these species are likely to rely on fine-scale cohesion and inter-individual exchanges (i.e. aggregation, chemical communication) to synchronise their breeding activities.

Evaluation of tagging techniques for *Australostichopus (Stichopus) mollis* (Hutton, 1872) for potential ranching studies

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The sea cucumber *Stichopus mollis* is a common benthic resident in New Zealand coastal waters and is often found in abundance under mussel farms. Since *S. mollis* has potential as a lucrative export commodity for Asian markets, ranching cultured sea cucumbers under farms presents an attractive investment opportunity. The first step in assessing the potential for ranching is to determine whether cultured sea cucumbers once placed under a farm remain in residence for later harvesting. To determine residence times under farms we applied a release-recapture design, which required the effective tagging of sea cucumbers. Because of the nature of their body wall, holothurians are not easy to tag, so we investigated a number of different tagging methods to find the one with the least potential for tag loss. Of the six methods trialled in the laboratory (freeze branding, micro-sand blasting, oxy-tetracycline, pit-tagging, T-bars and visible implant fluorescent elastomer [VIFE]), T-tags and VIFE had the best results after four weeks in tanks (87–93% tag retention). These two methods were then selected to assess whether tag retention was the same in the field as in the laboratory. Fifteen tagged sea cucumbers were placed in each of four tanks with either sand or mussel shell substrate in the laboratory. Cage experiments in the field with 15 tagged sea cucumbers each ran simultaneously on either sand or mussel shell substrate. Different substrata were tested because we hypothesised that substrate has an influence on sea cucumber movement, which in turn influences tag retention. Results showed that sea cucumbers in the field cages lost tags much faster than those in the laboratory tanks. Further, the rougher mussel shell substrate appeared to cause slightly higher tag loss for both tag types. The implication of these results for our release-recapture design to assess residence times of sea cucumbers under mussel farms is discussed.

Development of the sea cucumber *Holothuria leucospilota*

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Development of the tropical sea cucumber *Holothuria leucospilota* was studied from November to December 2007. Five adult individuals were collected from the northern part of Okinawa Island and maintained in an aerated aquarium. Spawning occurred spontaneously without any stimulation. The fertilised eggs reached the blastula stage 7 hours after fertilisation at 25°C. The early gastrula stage was reached at 20 hours. Hatching occurred at 22 hours. Early auricularia were formed after 48 hours. The diatom *Chaetoceros gracillius* was added every two days during the auricularia stage. By 6 days, the larvae reached the mid-auricularia stage with pronounced pre-oral and post-oral lobes, several pairs of lateral processes, and an ossicle at the posterior end. The auricularia continued to grow during the next two weeks. At this stage, they began to accumulate hyaline spheres at the tip of the posterior projection and lateral processes. The late-auricularia stage was reached at 19 to 21 days; animals in that stage had left and right stomatocoels, as well as a hydrocoel. The transition period from late-auricularia to doliolaria, which was marked by total resorption of all lateral processes, lasted several days. The larvae reached the barrel-shaped doliolaria stage at 23 to 25 days. To induce metamorphosis, the doliolaria were transferred to a Petri dish covered with a biological film. By 28 days, the doliolaria transformed into early pentactula possessing five primary tentacles and a single podium. Like other congeneric species, *H. leucospilota* has planktotrophic development through the auricularia, doliolaria and pentactula larval stages.

Intracellular pathways involved in oocyte maturation induced by dithiothreitol (DTT) and by a new maturation inducing substance (MIS) in sea cucumbers

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In sea cucumbers, oocyte maturation begins in ovaries but is stopped in prophase I of meiosis. In natural conditions, the blockage is removed during the laying by an unknown mechanism. When oocytes are

taken by dissection, the meiotic release can only be induced by an artificial inductor: dithiothreitol (DTT). We recently discovered a new maturation inducing substance (MIS) and compared its effects to DTT. DTT induces the maturation of 91% of sea cucumber oocytes but the rate of fertilisation never exceeds 40%. The new MIS induces the maturation of more than 90% of oocytes and all are fertilisable. To identify the intracellular pathway mediated by DTT and the new MIS, oocytes of sea cucumbers were incubated in the presence or absence of DTT or MIS after being treated with various modulators of cAMP, an important regulator of hormone-induced maturation in general. The use of these modulators (forskolin, isobutylmethylxanthine [IBMX] and hypoxanthine) in presence of DTT shows a decrease in cAMP during meiotic release. On the other hand, results strongly suggest that the way the new MIS acts is cAMP-independent. Whatever the maturation inducer used (either DTT or MIS), oocyte maturation always requires the synthesis of new proteins and the activation of protein serine/threonine kinase.

Sea cucumbers of Australia

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The Australian sea cucumber fauna comprises about 15 families, 69 genera and 211 species. Research was conducted at the National Museum of Natural History, Smithsonian Institution. The museum collection includes about 68 species of holothurians, each of which we studied. We began with bibliographic research to start getting familiar with Australian sea cucumbers. Then each specimen was photographed using a digital camera, and the ossicles were observed under a microscope and photographed. Using a scanning electron microscope, it was possible to obtain more detailed and precise photographs of ossicles from seven species. Data obtained from each specimen included the region, type of substrate, depth, etc. This work could be the foundation for a future taxonomic catalogue of sea cucumbers of Australia, with their respective description, photographs of specimens and ossicles, distribution, habitat, etc.

Taxonomic revision of the genus *Euapta* (Holothuroidea: Synaptidae)

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Integrative approaches help clarify many previously difficult problems in taxonomy. The sea cucumber family Synaptidae has many such problems, because it is relatively character poor and different researchers have often not agreed which characters are informative about species limits. Synaptids lack tube feet, respiratory trees, and enlarged haemal system, and they have thin, simple body walls and only a few types of ossicles. Three large-bodied genera are common on reefs: *Synapta*, *Euapta*, and *Opheodesoma*. Six nominal species of synaptids have been assigned to *Euapta*, three from the West Atlantic: *E. lappa*, *E. polii*, and *E. tobagoensis*, and three from the Indo-west Pacific: *E. godeffroyi*, *E. magna*, and *E. tahitensis*. Heding (1928), a notorious splitter, recognised all (five — *E. tahitensis* was described subsequently) and described two of these species. However most authors (Fisher, Clark, Deichmann, Rowe, Massin, Samyn, Pawson) over the past century have recognised only one in each biogeographic region: *E. lappa* and *E. godeffroyi*. We encountered two color morphs of *Euapta* in the Marshall Islands, which raised the issue of whether the current view of a single Pacific species is accurate, and led us to reflect on the differentiation of these and related forms. We show that there are at least two sympatric Indo-west Pacific species of *Euapta*, recognisable by color, ossicle, and DNA sequence characters. We also evaluated the genetic status of the East Pacific population and the divergence of *Euapta* across the Isthmus of Panama. Sequence data also support the recognition of *Synapta*, *Euapta*, and *Opheodesoma* as distinct, deeply divergent, monophyletic genera.

Conserved role of ARIS (acrosome reaction-inducing substance) in the echinodermata

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In many species acrosome reaction (AR) is a key process in fertilisation. Sperms specifically approach homologous eggs and undergo an exocytosis of the acrosomal vesicle in their head. This is followed by morphological change; this exocytosis enables sperm to pass through the egg coat by chemical and physical processes. In the starfish *Asterias amurensis*, the AR inducer has been purified from the egg coat and is a large proteoglycan-like molecule known as ARIS (AR-inducing substance). The part of its sugar chain part called Fr.1 is responsible for this activity; however its protein components are still unknown. In this study we make clear that ARIS consists of three dependent glycoproteins modified by Fr.1, named ARIS1, ARIS2 and ARIS3. These three ARIS proteins have similar structures and two of them conserved novel domains. The egg coats of other starfishes, *Distolasteras nippon* (Forcipulatida) or *Asterina pectinifera* (Asteroidea) share the ARIS protein structure as *DnARIS1*, *DnARIS2*, *DnARIS3* and *ApARIS1*, *ApARIS2*, *ApARIS3* respectively. Thereby the BLAST search results of ARISs were highly similar to those obtained with DY635177 and DY625100 in the sea cucumber *Apostichopus japonicus*. In the sea lily *Oxycomanthus japonica* we suggested the presence of ARIS-like glycoproteins in the egg coat, although a similar sequence for ARIS couldn't be detected in the sea urchins. Moreover, among Cephalochordata *Branchiostoma floridae*, BW770556, BW773889 and BW706662 are similar to ARIS1, ARIS2 and ARIS3 respectively. As in ARIS molecules, the protein is conserved in starfishes, sea cucumbers, sea lilies and Amphioxus; the ARIS protein may serve a significant role in the acrosome reaction evolutionally.

Characterisation and quantification of the intradigestive bacterial microflora in *Holothuria scabra* (Jaeger, 1833) (Holothuroidea), major macrodeposit feeder of recifal intertidal zones

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For a long time, sea cucumbers were considered nonselective deposit feeders, but recent research suggests that they absorb nutrients from only part of the sediment. The sediment is a complex environment, a mix of mineral material and living or dead organic fragments (i.e. microorganisms like bacteria, fungi, protists and meiofauna). The aims of the present study are to characterise and quantify the intestinal microbial community in *H. scabra* and that of the substrate on which it lives. To investigate the intradigestive microflora three methods were used: (i) gut bacterial cultures followed by 16S rDNA sequencing; (ii) denaturing gradient gel electrophoresis (DGGE) and (iii) cloning. The bacterial count was obtained by DAPI coloration (4',6-diamino-2-phenylindole) and the size of the substrate particles was analysed by sieving. The results suggest that *H. scabra* does not select particles by size. However, the number of bacteria is high in the foregut of the animal, indicating that bacteria are either selected from the substrate or cultured in the foregut. It is suggested that *H. scabra* is a nonselective deposit feeder (i.e. it ingests all particles of the sediment without selection) but that the nutrients absorbed by the sea cucumber come mostly from the living fraction of the sediment.

A nearly articulated aspidochirote holothurian from the Late Cretaceous (Santonian) of England

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With the exception of the Maastrichtian, only scarce data exist on fossil holothurians from the Upper Cretaceous, especially the Santonian (84 million years ago). Until now, only two records have been cited worldwide: one from the Munsterland area, Germany, and one from the Montsec area, Spain; both referred to the Apodida and Dendrochirotida. Here I report the discovery of a nearly articulated holothuroid specimen from the 'Upper Chalk' (Santonian; BMNH E 48764) of England (unfortunately the exact locality is unknown). The unique specimen shows two different types of ossicles: (A) tables and (B) 'buttons', demonstrating aspidochirote affinities, very likely to the family Holothuriidae. Concerning the morphology of the buttons, the new find shares characteristics with tube feet and papillae ossicles of *Holothuria* (*Mertensiothuria*), *H. (Microthela)*, and *H. (Semperothuria)* and *H. (Thelothuria)*. According to the decomposition of the surrounding tissue after the death of the animal, only a part of the former body skeleton is preserved; the anterior part of the skeleton (calcareous ring) is missing. Due to the well-preserved body-wall ossicles and the formation of the ossicles of the new find, I can exclude digestion and excretion of the sea cucumber by any predator. After comparison with modern members of the Holothuriidae family, we can speculate that the body of our new Santonian species was probably also cylindrical, elongate and scattered with numerous podia over the entire body, dorsally as papillae and ventrally as cylindrical tube feet. A wider distribution

of the new genus / species can also be assumed, because comparable long button-shaped sclerites were also known from Early and Late Maastrichtian chalk sediments of the islands of Møn (Denmark) and Rugen (northeast Germany) as well as the Baltic Sea. Considering how important aspidochirote sea cucumbers are today (nearly 1/4 of all known modern species), their fossil record is meagre and poorly understood. The phylogeny of the family Holothuriidae was recently investigated by Kerr et al. and Samyn et al., unfortunately without detailed consideration to the fossil record. The origin and early diversification of the Holothuriidae can be located in the Late Palaeozoic. Some of the diversity of the Mesozoic is still hidden as detailed investigations are missing or sparse.

Unusual holothurians (Echinodermata) from the Late Ordovician of Sweden

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Compared to other modern echinoderms, the early evolutionary history of sea cucumbers is poorly understood. In part, this is due to their disjunct endoskeleton with ossicles and calcareous ring elements, which are released following decomposition of the surrounding tissue. Newly sampled öjlemyr flints from the northwestern part of Gotland, Sweden, yield well-preserved echinozoan echinoderms, including holothurian ossicles. The studied material is Ashgill (upper Pirgu stage; 447 million years ago) in age and reveals the presence of several new or poorly known taxa of elasipodid, aspidochirote, and apodid holothurians or stem group representatives. The minute ossicles, around 70–400 µm in length / diameter, exhibit an impressive morphology with arms, spires, perforations, teeth etc. The new material differs from previously described Palaeozoic and Mesozoic material and most of the modern material by its unusual symmetry. This study shows that the Holothuroidea diversified significantly through Late Ordovician times.

Glacial persistence of North Atlantic populations of *Cucumaria frondosa* (Holothuroidea: Echinodermata)

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In the upper latitudes of the Northern Hemisphere shallow water invertebrates were vastly impacted by glaciations during the Last Glacial Maximum (LGM) in the Late Pleistocene. Many marine taxa show a genetic signature of extirpation in the Western Atlantic followed by recolonisation by Eastern Atlantic populations after glaciers receded. *Cucumaria frondosa*, the most abundant and widely distributed holothuroid in the North Atlantic, was used as a model to test whether its present phylogenetic structure reflects a history of persistence or extirpation and recolonisation with respect to Pleistocene glaciations. Mitochondrial DNA (mtDNA) was extracted and sequenced from a total of 334 specimens collected from 20 locations (7 to 5900 km apart) throughout the North Atlantic. Distribution of shared haplotypes indicated groups sampled were part of a large panmictic population with high gene flow between regions. In contrast, exact test for population differentiation was overall significant ($p < 0.001$) and showed differentiation of several pairs of populations irrespective of their geographic distance, signifying genetic patchiness at the local level. High connectivity between regions and estimates that the approximate time of expansion was the Late Pleistocene suggested persistence through the LGM. Haplotype diversity indicated the western Atlantic was recolonised by populations residing in ice-free areas east of Newfoundland and Labrador. Long larval times (ca. 6 weeks) and ability to tolerate arctic conditions enabled *C. frondosa* to survive on the fringes of glaciated regions and colonise areas as glaciers receded.

Resistance of pearlfishes to saponins

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Carapid fishes, known as pearlfishes, are anguilliformes fishes that enter and live in some invertebrates, especially in some echinoderms such as holothuroids and seastars. However, these echinoderms contain a strong concentration of saponins, a secondary metabolite which acts as a predator deterrent. These sa-

ponins, based on a triterpene glycosides structure, show a wide range of biological activities including haemolytic damage and ichthyotoxicity. The present work aims at analysing the effects of saponins on the gills of fishes including pearlfishes. Saponins were extracted from the sea cucumber *Bohadschia argus* (Tuléar, Madagascar) which is a natural host of some pearlfishes. Five carapid species (*Carapus acus*, *C. boraborensis*, *C. homei*, *C. mourlani* and *Encheliophis gracilis*) and five coral reef fish species (*Amphiprion akallopisos*, *Dascyllus aruanus*, *D. flavicaudus*, *D. trimaculatus*, *Neoniphon sammara*) were investigated. Animals were kept in tanks during two hours and exposed to various concentrations of saponin. At the end of each test, a sample of gill was taken and fixed in order to realise histological and scanning electron microscope analyses. Coral reef fishes exposed to saponin concentrations of 0.2–0.5 $\mu\text{l ml}^{-1}$ died within two hours and exhibited pathological alterations of gill filaments. The pearlfishes proved to be resistant and had no gill damage.

Genetic barcoding of commercial beche-de-mer species

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A recent review conducted by the Food and Agriculture Organization of the United Nations (FAO) identified 47 species of holothurians used for bêche-de-mer production. With three exceptions from the dendrochirotidés, all bêche-de-mer species are aspidochirotidés. Identifying many of these species is difficult. To improve this situation, we conducted a review of available genetic information and obtained additional data to test if genetic barcoding can be used as a tool for bêche-de-mer species identification. We focussed on the mitochondrial COI gene, as have other barcoding projects. Although some genetic information was available for about 50 per cent of all bêche-de-mer species, sufficient information and within-species replication was only available for 6 species. We obtained 96 new COI sequences, extending the existing database to cover most common bêche-de-mer species. COI unambiguously identified bêche-de-mer species in most cases and therefore provides excellent genetic barcodes. However, since this marker did not work for two of the most valuable species, development of additional methods will be required. In addition to species identification in adults, COI sequences were useful in juvenile identification, and sequences demonstrated that large (deep) and small (shallow) specimens of *Holothuria atra* belong to the same species. Our study has also demonstrated that further genetic and taxonomic work in this group is essential: work conducted here provided evidence for at least three further species that are currently undescribed (e.g. one *Bohadschia* species), or species that are likely to constitute separate species in the Indian and Pacific Oceans (e.g. *H. fuscogilva*).

Saponin diversity and body distribution in five tropical species of sea cucumbers from the family Holothuriidae

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Sea cucumbers lack structural defences because of their reduced skeleton. To face predation holothuroids contain feeding deterrent molecules — the saponins — in their body wall and viscera. Saponins are secondary metabolites based structurally on a triterpene glycoside structure. The aim of this study is to analyse and compare the saponin mixtures of five sea cucumber species from the Indian Ocean: *Actinopyga echinites*, *Bohadschia subrubra*, *Holothuria atra*, *H. leucospilota* and *Pearsonothuria graffei*. Mass spectrometry (MALDI-MS and MALDI-MS/MS) was used to detect saponins and describe their molecular structures. LC-MS and LC-MS/MS were also used to separate saponins and, in some case, to identify isomers. Two different body components were analysed separately — the body wall (which protects the animal and is moreover the largest organ) and the Cuvierian tubules (a defensive organ that can be expelled on predators in response to an attack) — for all species except *H. atra*, which lacks Cuvierian tubules. Holothuriid saponins are usually classified into two categories: non-sulfated in the genus *Bohadschia* and sulfated in all other genera. Our results do not completely corroborate this distribution. Indeed, although *B. subrubra* has only non-sulfated saponins and *A. echinites* and *H. atra* have only sulfated saponins, *H. leucospilota* and *P. graffei* contain both saponin types. The number of saponins in a mixture varies between species but also between the body components of the same species. For each species, some saponins are common to both body components while others are specific to one organ.