

## Annexure-II

The influences of different salinities and temperatures on the larval development of the mangrove sesarmid crabs *Neosarmatium indicum*, *N. trispinosum* and *Neoepisesarma lafondi* were examined under laboratory conditions, at salinities 0 to 35‰ and temperatures of 25.5 to 27.5°C, 20 to 30°C, and 25 to 30°C, respectively. The larval development consists of five zoeal stages and a megalopa in *N. indicum* and *N. trispinosum*, and four zoeal stages and a megalopa in *N. lafondi*. Larvae survived to the first crab stage at salinities between 5 and 35‰, 15 and 35‰, and 10 and 35‰ with different percentages in *N. indicum*, *N. trispinosum* and *N. lafondi* respectively. At 0, 5 and 10‰, the larvae died within 12 to 18 hours without moulting to subsequent stages in all the species. The highest survival rate with shortest development durations to the first crab stage for each larval stage of these species was recorded in 20 to 25‰ at 25 to 30°C. Results of the combined effects of salinity and temperature suggest that the larvae of these species develop in estuarine water and recruit to the mangrove swamp at the megalopa stage, where they spend the rest of their lives.

A study was conducted to determine the egg number and size of the sesarmid crabs, *Neosarmatium indicum* and *N. trispinosum*, from the mangrove swamp of the Okinawa Island. The egg number was 14208 and 20081, where the estimated egg size was 0.42 and 0.38 mm in *N. indicum* and *N. trispinosum* respectively. The mean egg number of *N. indicum* was lower than *N. trispinosum*, while the egg size was bigger than *N. trispinosum*. Therefore, smaller egg size indicated higher number of eggs. Simple regression detected a relation between the egg number and the egg size.

The larvae of the mangrove sesarmid crab *Neosarmatium indicum*, *N. trispinosum* and *N. fourmanoiri* were reared in the laboratory for morphological studies. Five zoeal stages and one megalopa of both species were described in detailed and illustrated. Morphologically, the first zoeae of these species are similar to those of other species of *Neosarmatium* as well as species of the family Sesarmidae, in that they share most of the following characters: no lateral carapace spines; 1+5, 5, and 0+1+6 setae on the endopod of the maxillule, the maxilla and the second maxillipeds respectively; uniform setal formula on the basis of the first (2,2,3,3) and the second (1,1,1,1) maxilliped. In the megalopa stage, four terminal setae on the distal segment of the mandibular palp of these species. The morphological differences between the first zoeae and the megalopae of these species, and those of their related sesarmid species are discussed briefly.

Form 3

### Abstract of Thesis

Thesis Title: Isolation and Structure Elucidation of New Metabolites from Coral Reef Invertebrates

This thesis describes the chemical investigations of two species of ascidian and a sponge, resulting in the isolation of ten new metabolites; floresolides A-C (35-37), tricyclic alkaloids A (70) and B (71) and five furano sesterterpenoids (95, 97, 99-101). The structures have been assigned using a combination of spectroscopic and chemical methods.

Floresolides A-C (35-37), isolated from an ascidian, *Aplidium* sp., are new cyclofarnesylated hydroquinones possessing an  $\epsilon$ -lactone bridging the aromatic ring and a [10]metacyclophane system. These compounds are unique members of the class of compounds known as longithorones and longithorols characterized by the presence of paracyclophane and/or metacyclophane system built in a farnesyl quinone or hydroquinone system. The structures were determined by spectroscopic and X-ray diffraction analysis.

Tricyclic alkaloids 70 and 71 have been isolated from an unidentified ascidian and their structures determined by extensive application of spectroscopic methods. These alkaloids are closely related to the known polycyclic alkaloids cylindricines A (55) and B (56) but lacks C-4 oxygenation found in cylindricines and have butyl instead of hexyl appendages.

New cytotoxic furanosesterterpenes (95, 97, 99-101) have been isolated from a lipophilic extract of a sponge, *Ircinia* sp., and their structures elucidated by spectroscopic and chemical methods. The absolute configurations of two metabolites (95 and 97) were established by chemical degradation. These compounds showed moderate cytotoxicity against KB cells.

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
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## 学位（博士）論文審査及び最終試験の終了報告書

学位（博士）の申請に対し、学位論文の審査及び最終試験を終了したので、下記のとおり報告します。

（記）

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成績評価	学位論文	<input checked="" type="radio"/> 合格 <input type="radio"/> 不合格	最終試験 <input checked="" type="radio"/> 合格 <input type="radio"/> 不合格
論文題目	Isolation and Structure Elucidation of New Metabolites from Coral Reef Invertebrates		
審査要旨（2000字以内）	本論文の本論は3部から成り、3種のサンゴ礁生物を素材に10種の新規二次代謝物質を単離して、それぞれの構造決定を行っている。		
	インドネシアのフローレス島で採集したマンジュウホヤの一種 <i>Aplidium</i> sp. の脂溶性		
	分画から細胞毒性を示す floresolide A-C と名付けた3つの新規メロセスキテルペン類		

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審査要旨

を見いだした。これらの化学構造は、それぞれのNMR等のスペクトルデータの解析、および floresolide C の X 線結晶回折によって絶対配置まで決定した。その結果、fioresolide 類はメロセスキテルペンが環化した *m*-cyclophane 構造の中に  $\epsilon$ -lactone が含まれているユニークな構造と判明した。Aplidium 属からは *m*-および *p*-cyclophane タイプの longithorol 類が見いだされてきているが、fioresolide 類のような rigid な構造がどのように生成したのか興味深い。これらの化合物は 10  $\mu$ g/mL で細胞毒性を示した。

2つ目の生物はフローレス島で採集した未同定の群体ホヤで、これから2つの新規アルカロイドを見いだしている。NMR 等での機器分析の結果、これらの構造は、3級アミンを含むユニークな環構造を含む新規物質と推定された。相対立体配置についても NOE 測定等により決定している。

3番目の試料は沖縄県内で採集した海綿 *Ircinia* sp. より得たセスタテルペン類である。細胞毒性を示した脂溶性分画を分離することにより、合計5つの一連の新規セスタテルペン類を得ている。これらの構造については、スペクトル測定による構造推定  
の他に、各種誘導体を作成してその比旋光度の比較、Brewster's rule の適用、NOE 測定などを通して相対、あるいは絶対立体配置を決定している。また、これらの新規セスタテルペン類も細胞毒性を示した。

以上の内容は、すでに2つの論文として国際誌に発表されており、当研究科の要件を満たしていることから学位論文として要求される水準に達していると判断された。